# Synthesis of novel inhibitors of CDK 4 / Cyclin D1 based on the natural marine sponge pigment fascaplysin.

**Thesis submitted for the degree of Doctor of Philosophy at the University of Leicester**

**by**

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**September 2008**

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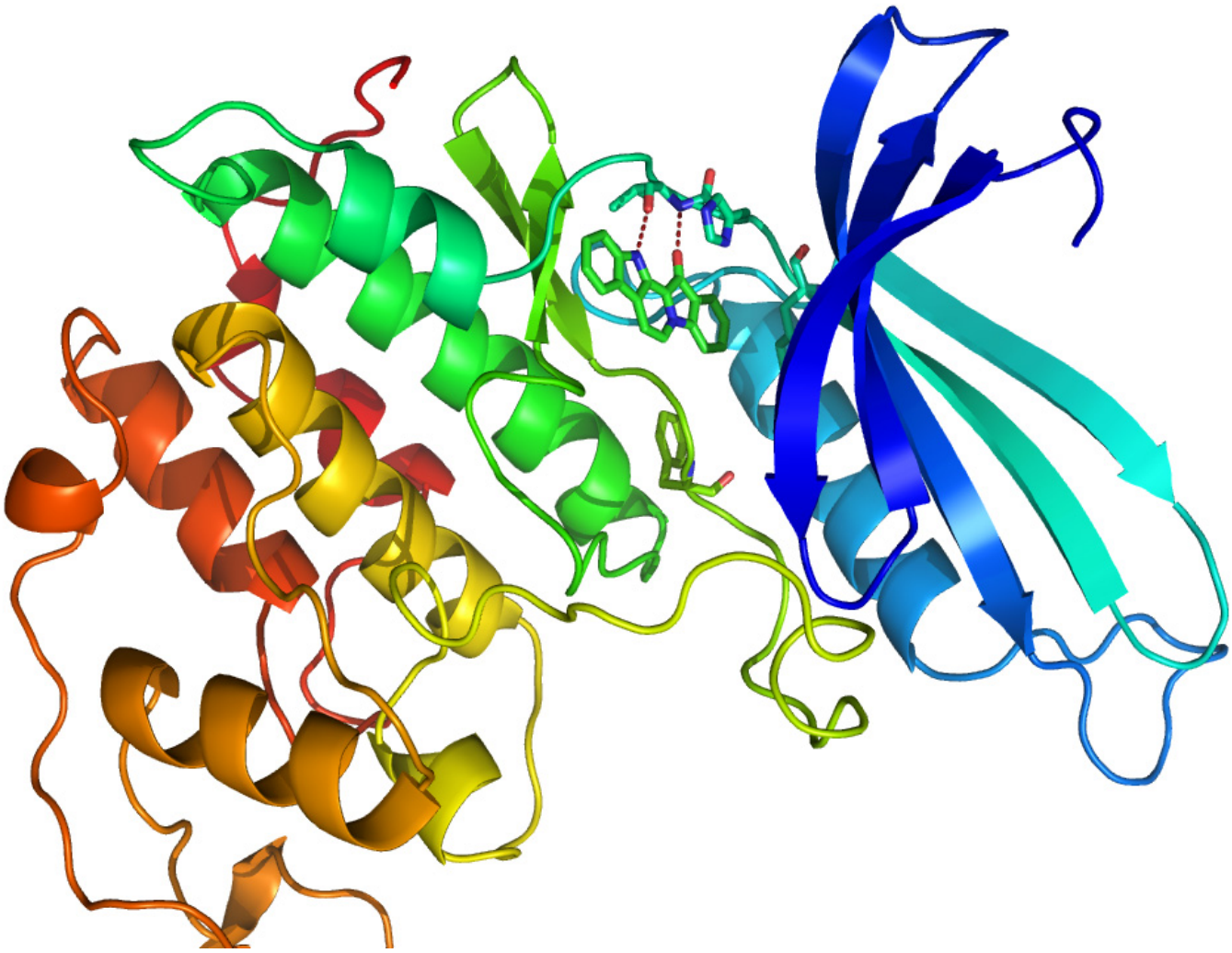

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*"Publish and be damned"*

*Arthur Wellesley, 1st Duke of Wellington*

**Statement**

**This thesis is the result of work conducted by the author mainly during the period October 2004 to September 2007 in the Department of Chemistry at the University of Leicester. None of this work has been submitted for any other degree at this or any other institution. All the work contained within this thesis is original except where indicated otherwise, aspects of this work have been published as indicated below, see appendix 2.**

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**Signed……..** **Date……..**

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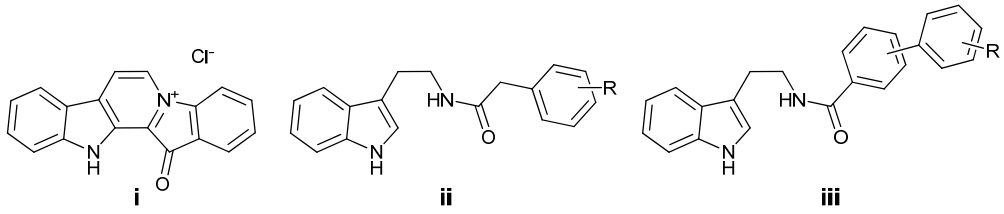
# Abstract

**Anthony James Wilson**

**Synthesis of novel inhibitors of CDK 4 / Cyclin D1 based on the natural marine sponge pigment fascaplysin**

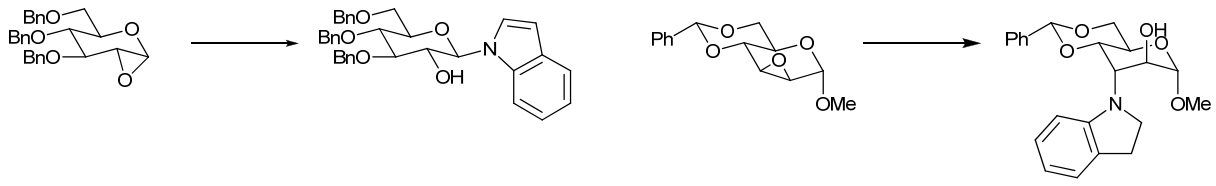
This thesis describes an investigation into the design and synthesis of novel inhibitors of the cell-cycle regulatory enzyme CDK4/Cyclin D1 utilising the structure of the natural product fascaplysin as a lead compound.

The structure activity relationship of fascaplysin (**i**) has been investigated and its structure simplified and rationalised to give novel inhibitor pharmacophores based on structures (**ii**) and (**iii**). Inhibitors of structure (**ii**) were found to possess activity against CDK4/Cyclin D1 of $IC_{50}$ 51-176 μM and to be approximately 15-20 fold selective over CDK2/Cyclin A. The second generation compounds of structure (**iii**) were synthesised to explore a suspected π-stacking pocket in the active site of CDK4 around the Phe93 residue. These compounds were active against CDK4/Cyclin D1 with $IC_{50}$ values in the range 7-50 μM and to be 20-100 fold selective over CDK2/Cyclin A.




**i** **ii** **iii**

New methodology towards the synthesis of un-natural products bearing a carbohydrate subunit connected via the anomeric 1-position and the 2- and 3- positions of the sugar was also explored. Indole was successfully glycosylated with a glucose subunit at the anomeric position (**v**) using the anhydrosugar (**iv**) and indoline was glycosylated with a glucose sugar derivative at the 3-position (**viii**) using the manno-epoxide (**vi**).




**iv** **v** **vi** **viii**

## Definitions and Abbreviations used in this thesis

| | |
|---|---|
| $^{o}C$ | Degrees Celsius |
| $^{1}H$ | Hydrogen-1 (Proton) |
| $^{13}C$ | Carbon-13 |
| aq. | Aqueous |
| Asp | Aspartic acid |
| ATR | Attenuated total reflection |
| Bu | Butyl |
| $cm^{-1}$ | Reciprocal centimetres or wave numbers |
| CML | Chronic myeloid leukaemia |
| DCM | Dichloromethane, $CH_2Cl_2$ |
| DMAP | *N,N*-Dimethyl-4-aminopyridine, $(CH_3)_2N(C_5H_4N)$ |
| DMF | *N,N*-Dimethylformamide, $(CH_3)_2NC(O)H$ |
| DMSO | Dimethyl sulfoxide, $CH_3S(O)CH_3$ |
| E2F | A genetic transcription factor |
| EI | Electron impact |
| eq. | Equivalents |
| FAB | Fast atom bombardment |
| g | Grams |
| GC | Gas chromatography |
| GCMS | Gas chromatography mass spectrometry |
| Gln | Glutamine |
| Glu | Glutamic acid |
| h | Hour(s) |
| HPLC | High performance liquid chromatography |
| Hz | Hertz |
| $IC_{50}$ | Concentration of a given inhibitor which results in 50% inhibition of an enzyme *in vitro* |
| IR | Infrared |
| *J* | Coupling constant |
| Leu | Leucine |
| M | Molar |
| $M^{+}$ | Molecular ion |
| Me | Methyl ($CH_3$-) |
| mg | Milligram(s) |
| mL | Millilitre(s) |
| mmol | Millimoles, mol x $10^{-3}$ |
| MS | Mass spectrometry |
| Ms | Methyl sulphonyl |
| NMR | Nuclear magnetic resonance (spectroscopy) |
| oxone® | Potassium peroxymonosulfate, $2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$ |
| p53 | The p53 protein |
| pdb | Protein Data Bank, online database of x-ray and NMR structures. |
| pdb xxxx | Protein data bank code, where xxxx is a four character alpha-numeric code denoting a specific structure stored in the protein data bank. |
| Ph | Phenyl ($C_6H_5$-) |
| ppm | Parts per million |
| ppt | Precipitate |
| pRb | The retinoblastoma protein, a product of the Rb gene |
| Rb | The retinoblastoma gene |
| rbf | Round-bottomed flask |

| | |
|---|---|
| RT | Room temperature |
| sat. | Saturated (solution) |
| SM | Starting material |
| $^{t}$BuOH | *tert*-butanol, 2-methyl-2-propanol, *tert*-butyl alcohol, $(CH_3)_3COH$ |
| Tetralin | 1,2,3,4-tetrahydronaphthalene |
| THF | Tetrahydrofuran |
| TLC | Thin layer chromatography |
| TMS | Tetramethylsilane, $Me_4Si$ |
| Ts | Tosyl (*p*-toluene sulphonyl) |
| UV | Ultra violet |
| VIS | Visible |

# Contents

# Chapter One Introduction

*"Where a cell arises, there must be a previous cell, just as animals can only arise from animals and plants from plants."*

*Rudolf Virchow 1858*

## 1.1 Cell division

### *1.1.1 Introduction*

It is thought that life first arose on the earth around 4 billion years ago; [1-3] its continued survival is dependent on the process of reproduction, the ability of one living organism to create another. The most basic task that any organism can perform is to pass on its genetic information to future generations. This process, by which a single mother cell is able to create a copy of itself by splitting into two identical daughter cells, is known as cell division.

For the first 2.5 billion years all life existed as organisms consisting of one single cell. In these unicellular organisms only one single cycle of cell division is required to create a new organism. Over time life slowly evolved to include multi-cellular, or eukaryotic organisms.[3-5] The length of time taken for this change to take place is thought in part to be due to the need to develop the necessary signalling pathways required to coordinate the activity of two or more cells together for the overall good of the organism as a whole. As multi-cellular organisms became ever more complex, [6] different cell types, with different functions and specialities, evolved to perform specific tasks within the organism. Inter cell communication became ever more complex and important to the successful functioning of an organism. [7]

Cell division also became a more complex process, in multi-cellular organisms cell-division is required both to maintain the parent organism and to produce new organisms through the process of reproduction. [8] For the successful maintenance of a eukaryotic organism, dead or damaged cells must be continually replaced to ensure continued survival. In the average adult human body millions of cell divisions must take place every second simply to replace existing cells. If all cell division within a human being were halted, the body would die within a matter of days. Reproduction would now require long complex sequences of cell divisions producing the correct number of the different specialist cell types, in the correct positions and connected correctly, in order to produce offspring. [9]

Whilst being critical to life the division of cells is also deeply involved with the death of an organism, when a body loses the ability to repair the damage that is naturally accumulated during the course of repeated cycles of cell division, then that organism will die. [7]

*1.1.2 The Human body*

Within an organism as complex as the human body there are approximately 210 different types of cells, all of which are daughter cells of the one original zygote cell formed from the union of one egg cell (an ovum) from the female parent and one sperm cell (a spermatozoon) from the male parent. The original zygote cell must then divide and produce the correct number of each of the different types of cell that are required for a fully developed human being. The process of duplication of cells is known as cell-division and the process by which one 'master' cell becomes a specialist cell, such as a liver cell, is known as differentiation. Cells which have become specialised cells within the body are referred to as being terminally differentiated, they cannot normally either; divide to produce new cells or change speciality to become another type of cell.

Cells which have retained the ability to divide and differentiate are referred to as stem cells, of which there are several types. Embryonic stem-cells are found in a developing embryo, they are able to divide and differentiate into any type of cell found within the organism and they are referred to as being pluripotent. [10] Adult stem cells are found within the tissues of both children and adults, they retain the ability to divide and differentiate into specific types of cells to repair damaged tissues and dead cells. They are specific to a certain tissue type, for example hematopoietic stem cells which are found in the bone marrow are able to divide and differentiate into any of the different types of blood cells, they are multipotent. The final type of stem cells are the cord blood stem cells, these consist mainly of different types of multipotent stem cells which are found in high levels in blood from the umbilical cord and placenta.

Those cells which have retained the ability to divide must be tightly controlled at all times, only proliferating as and when required. Inappropriate cell division and growth is both wasteful of resources and potentially harmful to an organism, tight regulation over cell proliferation is a built in feature of the process of cell division known as the cell cycle. [11]

## 1.2 The eukaryotic cell cycle

The cell cycle in eukaryotic organisms is broken down into a number of phases, the $G_0$, $G_1$, S, $G_2$ and M phases. [7] (Figure 1.1) Collectively the $G_1$, $G_2$ and S phases are known as interphase. A typical human cell, growen in culture, will take approximately 24 hours to traverse the entire cell cycle, approximately 23 hours of this cycle will be spent in interphase with the remaining one hour being spent in the M or mitosis phase.

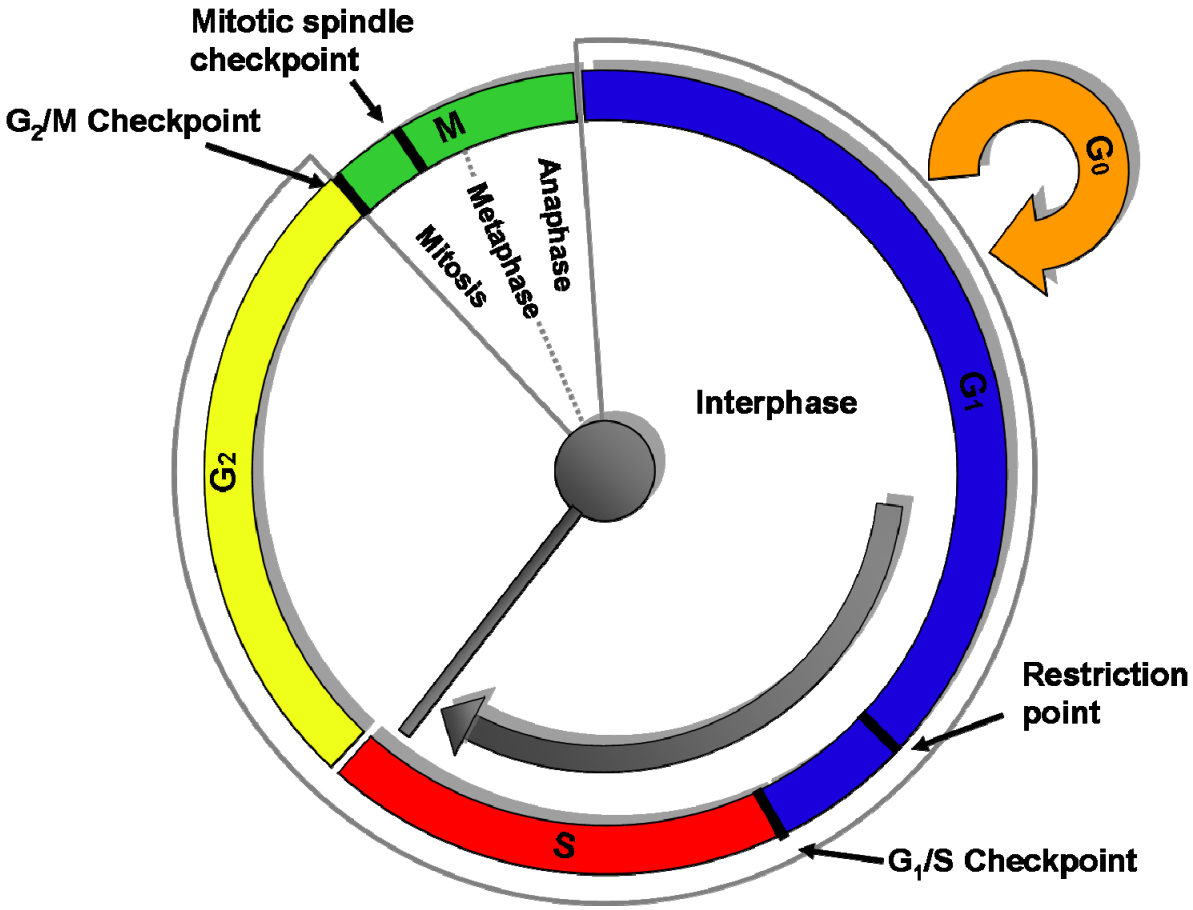



**Figure 1.1** The Eukaryotic cell cycle.

### *1.2.1 The $G_O$ phase*

The $G_0$ phase of the cell cycle is a sub-phase of the $G_1$ phase, this resting phase is often referred to as quiescence. [7] It is here that a cell spends most of its life time performing its tissue specific functions. The length of time a cell spends in this phase can vary from minutes to several years; whilst it is possible for $G_0$ phase cells to re-enter the $G_1$ phase and the cell cycle, many do not.

*1.2.2 The S Phase*

The S or synthesis phase, is where the replication of genetic material takes place, this phase of the cell cycle will take approximately half the length of the cell cycle, typically between 10 and 12 hours. [7] In a normal resting cell the genetic material or DNA is stored in a series of tightly compacted structures known as chromosomes; a cell from a normal human being will contain 23 pairs of chromosomes. For DNA synthesis to take place the chromosomes must be disassembled, exposing the double stranded DNA enabling transcriptase enzymes access to the information stored in the base pairs of DNA. This genetic material must be copied and re-compacted into two identical sets of chromosomes, these 'new' chromosomes are still joined to each other by a region called the centromere and together they are known as the sister chromatids.

*1.2.3 The M Phase*

The M or mitosis phase is where the separation of the duplicated chromatids into two complete sets of chromosomes [12] and the division of the cell takes place. [13] This process takes approximately one hour and contains a series of key events. There are two distinct parts to the M phase; the division of the nucleus which is known as mitosis and the division of the newly enlarged cytoplasm and cell wall into two smaller daughters, which is known as cytokinesis. There are a number of distinct steps in mitosis, [7] firstly the individual chromatids condense in preparation for separation, the nuclear envelope is then broken down and the sister chromatids become attached to a collection of microtubules which together form a structure known as the mitotic spindle. A brief pause, known as metaphase, then occurs as the individual chromosomes become aligned at the equator of this mitotic spindle, the cell then moves into anaphase. The sister chromatids are then separated by the mitotic spindle and the two new sets of chromosomes moved to opposite ends of the cell. The chromosomes are then decondensed back to their 'resting' state and two new nuclear envelopes are constructed, this results in a single cell with two nuclei and double the quantity of cytoplasm and other structures that are found in a normal cell. The cell then enters into cytokinesis; the formation of a contractile ring pinches together the cell wall between the two separate nuclei, resulting in the splitting of the large mother cell into the two daughter cells. [7]

*1.2.4 The Gap Phases*

The S and M phases of the cell cycle are divided by two 'gap' phases, the $G_1$ and $G_2$ phases. Throughout interphase the cell duplicates the various organelles, proteins and other constituents of the cytoplasm which will be needed to support two daughter cells as well as proteins needed for future stages of the cell-cycle. The two gap phases allow extra time for this duplication process to occur but they also allow chance for the cell-cycle control mechanism to monitor the internal and external environments and decide if conditions are favourable to continued division, the $G_1$ phase is particularly important to this 'decision' process. The length of the gap phases can therefore vary enormously, if extra-cellular signals are unfavourable to cell division then cells can delay progress through $G_1$ or even re-enter the $G_0$ state, possibly remaining there for days, weeks or even years. If signals which are favourable to growth and division are present early in either the $G_1$ or $G_0$ phase, then dividing cells may progress through a 'commitment' point, this point is referred to as the 'start' point in yeast, or the 'restriction' point in mammalian cells, after passing this point a cell is committed to either traversing the entire cell-cycle producing two daughter cells or to exiting the cell cycle by cell-death or apoptosis.

*1.2.5 Cell signalling*

The decision over when to initiate a new phase of cell-division is controlled in part by various cell-signalling pathways, [14, 15] there are four different types of intercellular signals which operate within the human body and these are:

1. Contact dependent signalling pathways, which operating with membrane bound signalling molecules.
2. Paracrine signalling pathways, signalling molecules which when released by one cell act on nearby cells only.
3. Synaptic signalling pathways, signals transmitted electrically along neurons but which utilise signalling molecules to cross the gap or synapse between two adjacent neurons.
4. Endocrine signalling pathways, these utilise the release of a hormone from a specific area of the body, the signal is then carried throughout the body by these hormones in the blood stream.

One of the most important signalling pathways in the context of cell division and cancer concerns the so called, 'contact inhibition of growth' whereby cells will often stop dividing when in close proximity to other cells, for example the cell division required to heal a wound to the skin will cease when the two edges of the wound are reunited and the damage is repaired.

## 1.3 Cell cycle regulation

For a cell within a human body to successfully divide into two daughter cells it must successfully transverse through this cell cycle in the correct order and without error. [16] A complex system of regulatory proteins has evolved to achieve the necessary control; known as the cell-cycle control system together they control and regulate progression through the cell cycle. This system consists of a series of 'biochemical switches' which trigger the main events, such as DNA replication or the segregation of duplicated chromosomes, but also contains mechanisms which allow the control system to respond to other events both internal and external to the dividing cell. As well as the most basic process of copying the genetic material, a dividing cell must possess processes to replicate the various cellular components needed by both daughter cells, things such as organelles, proteins and other macromolecules. This additional activity must be coordinated with the actual division process however the precise mechanism by which this coordination is achieved is not well understood.

When a cell begins to traverse the cell cycle it must pass through each of the different phases successfully and in turn, in order for the division of the mother cell into two daughter cells to be achieved. Any errors which may occur during this process must either be corrected before proceeding to the next stage of the cycle or the cell must be destroyed to prevent the accumulation of such 'damage' in future daughter cells.
Control over progression through the cell-cycle is tightly regulated within an organism by a cell-cycle control system, the structure and function of which first appeared around one billion years ago and is highly conserved between different eukaryotic organisms.

The control system must provide a number of key mechanisms, there must be some form of clock which can trigger specific events at specific times, as well as a mechanism for triggering events in the correct order. There should be a mechanism to ensure that specific

events occur only once, for example if DNA synthesis were to occur twice then each daughter cell would have double the genetic material of the mother cell, with disastrous consequences. There must also be a series of switches which can trigger events in an irreversible fashion as well as a range of backup systems with multiply redundant mechanisms.

Above all the control system should be versatile and adaptive in order to suit a range of different cell types and environments within an organism. The cell-cycle control system has the ability to arrest the cell cycle at a number of specific points, for example there exists a mechanism for detecting the completion of DNA synthesis, if this is not detected then the cell cycle will be temporarily halted to allow extra time for synthesis to be completed. The positions where the cell cycle may be halted are know as 'check-points', [17] the cell cycle can be halted at these check points either as a response to signalling pathways within the cell, such as the detection of DNA damage [18] or can be regulated by external factors such as the restriction point in $G_1$. These check points operate through the action of negative signals which arrest the cell cycle rather than the removal of positive signals which normally trigger the next phase of the cycle. There are several checkpoints, [19] the $G_1$/S checkpoint, often referred to as the restriction point, the $G_2$/M check point and the mitotic spindle checkpoint. [20, 21]

### *1.3.1 Cyclin dependent kinases*

The cell-cycle control mechanism is based around a family of cyclically activated protein kinases collectively known as the cyclin dependent kinases or CDK's. [22, 23] The catalytic activity of these CDK's rises and falls during the different phases of the cell cycle. [24] The CDK's act to phosphorylate other intracellular proteins which initiate or regulate major events, their activity is controlled by a complex array of enzymes and proteins, but most importantly by the binding of smaller partner proteins known as cyclins to an allosteric site on the surface of the CDK. The concentrations of CDK's found in a cell remain relatively constant throughout a cells life time, but they are only catalytically active when bound to their corresponding cyclin partners; it is the rising and falling levels of cyclins within a cell which regulate the activity of the CDK's [25] by binding to and activating them. (Figure 1.2) Currently there are ten known separate cyclin dependent kinases; only four of these are

involved in the human cell-cycle, different CDK/Cyclin complexes are associated with the different phases of the cell-cycle.

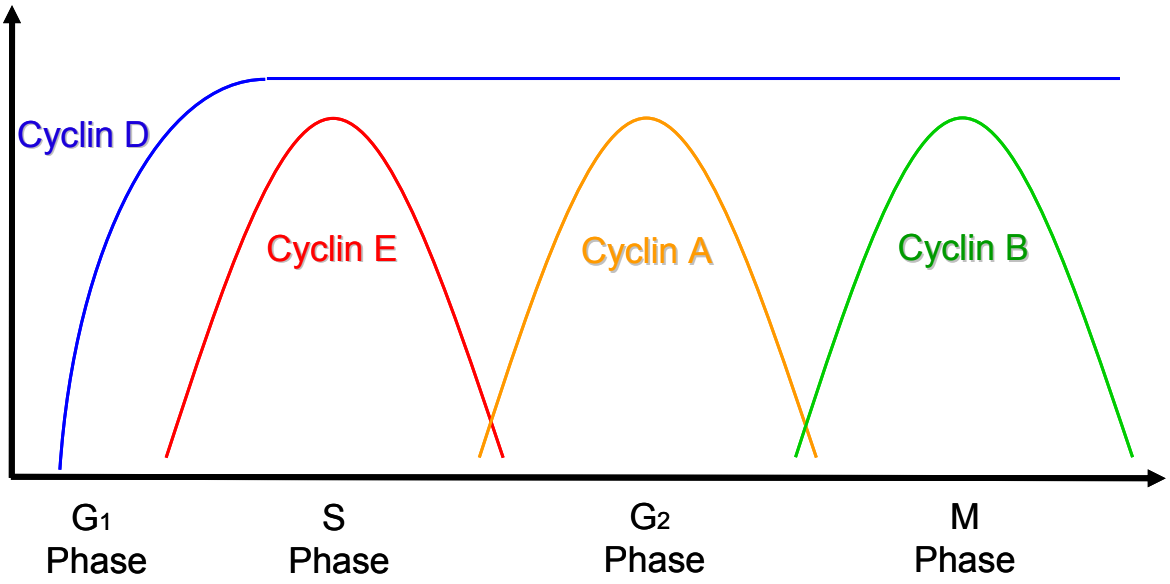



**Figure 1.2** Changing levels of cyclins found during the cell-cycle.

*1.3.2 Cyclins*

There are four classes of cyclins found in human cells and they are classified by the phase of the cell-cycle during which they are expressed and bind to CDK's. [26] The $G_1$ phase cyclins are cyclins D1, D2 and D3 which bind to and activate CDK's 4 and 6; active $G_1$ phase CDK/Cyclin complexes promote progression through the restriction point and into S phase. [26]

Cyclin E, which is expressed during S phase, binds to and activates CDK2 which initiates DNA replication. The M phase cyclin, cyclin B, is expressed only during M phase; it binds to and activates CDK1, active CDK1/Cyclin B promotes various mitotic events. The fourth class of cyclins are known as the $G_1$/S phase cyclins; principally cyclin E which activates CDK2, this CDK/Cyclin complex is active late in the $G_1$ phase and is responsible for committing the cell to DNA synthesis. [26]

Each active CDK/Cyclin complex is able to phosphorylate specific amino acid residues on other cellular proteins, but in the absence of a bound cyclin partner the active site of a CDK is partially obscured, renderings the lone CDK inactive. Cyclin binding confers

partial catalytic activity to the complex but full activity is only achieved after a series of phosphorylations and dephosphorylations by CDK activating kinases such as CKI and dephosphorylation kinases such as wee1. The net result of these processes is phosphorylation of a single amino acid residue near to the active site of the CDK; this induces a small conformational change to the active site which results in full catalytic activity. CDK activity is further regulated by additional phosphorylations at other sites, which are involved principally in the regulation of M phase CDK/cyclin complexes and by the action of CDK inhibiting proteins, CKI's, which regulate $G_1$ and S phase CDK's.

*1.3.3 The $G_1$/S phase check point*

The active CDK4/Cyclin D1 complex plays a key regulatory role at the $G_1$/S check point, [26] the active complex phosphorylates tyrosine residues on the inhibitory 'retinoblastoma' protein, pRb. The retinoblastoma protein, pRb, is itself an inhibitor of the transcription factor E2F, [27] pRb binds to E2F and prevents it driving expression of genes required for the S-phase of the cell cycle.

Phosphorylation by active CDK4/Cyclin D1 renders pRb inactive, such that it no longer binds to and inactivates E2F. E2F, which is bound to a 'promoter' site on the surface of DNA, is then free to drive expression of S-phase genes. When expressed these genes code for proteins, such as cyclins E and A, which are required for S-phase and whose presence triggers DNA synthesis (Figure 1.3).

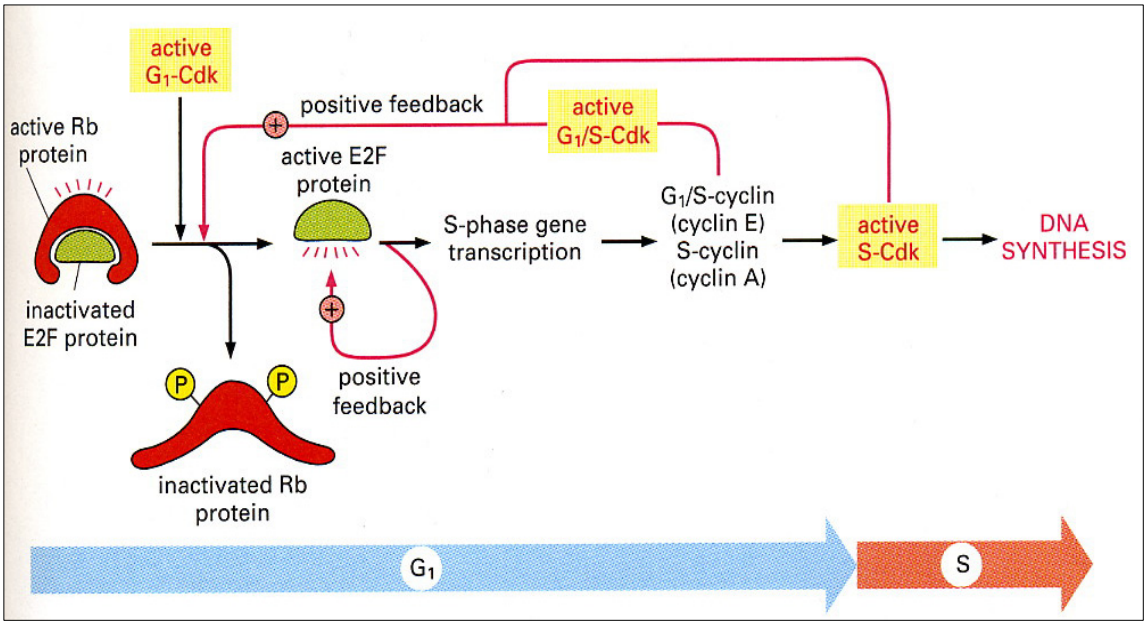



**Figure 1.3** The $G_1$/S phase check point. *(Molecular Biology of The Cell, Alberts et al., p.1005)*

The overall sequence of events is detailed above (Figure 1.4 A), the protein p16 is an inhibitor of active CDK4; it binds to CDK4 and prevents cyclin binding. Active CDK4/Cyclin D1 phosphorylates the Rb protein rendering it inactive. The Rb protein is an inhibitor of the E2F transcription factor, it binds to the surface of E2F and blocks its activity. Active E2F drives transcription of genes required for the S-phase of the cell cycle.

The situation in a non-proliferating cell is detailed in Figure 1.4, B. The catalytic activity of CDK4 is blocked by the presence of p16 a CDK inhibitory protein or CKI. This small protein binds to CDK4 and prevents the binding of cyclin D1 which renders the CDK enzyme inactive, pRb is therefore not phosphorylated and remains bound to the surface of E2F, S-phase genes therefore remain un-expressed and the cell remains in $G_1$ phase.

In a proliferating cell (Figure 1.4 C) p16 is either absent or itself inhibited. Cyclin D1 is therefore able to bind to and activate CDK4. This active CDK/Cyclin complex is then free to phosphorylate and inhibit the Rb protein. The phosphorylated Rb protein, ppRB, then no longer binds to and inhibits E2F. E2F is free to drive expression of genes which code for proteins required for the S-phase of the cell cycle and the cell moves from $G_1$ into S phase.
26

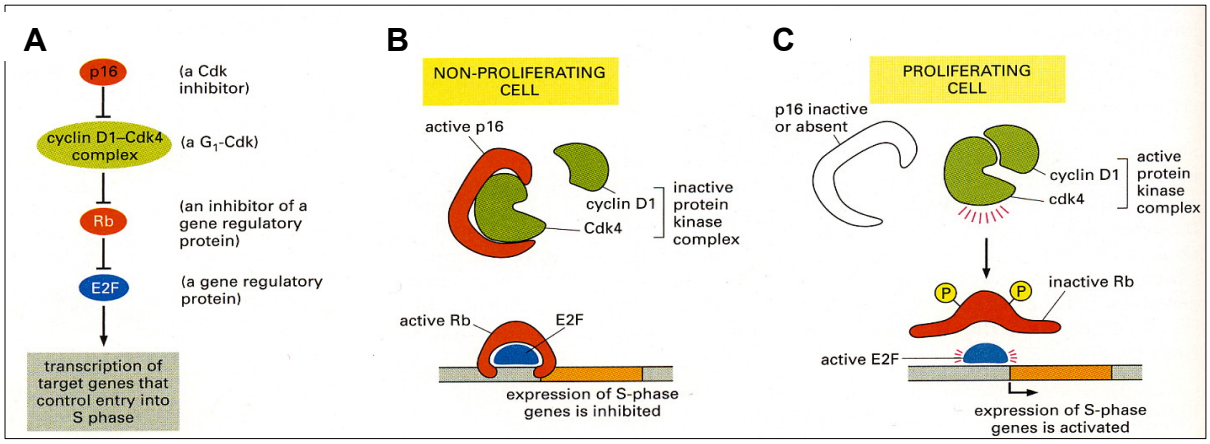



**Figure 1.4** The role of CDK4/Cyclin D1 in the cell cycle. *(Molecular Biology of The Cell, Alberts et al., p.1344)*

## 1.4 Cell death

### *1.4.1 Cell death by apoptosis*

Eukaryotic organisms regulate the number of cells that they contain by maintaining a tight control over the process of cell division. However they also regulate the number of cells they contain by controlling the rate of cell-death. If an individual cell is surplus to requirements then a process of programmed cell death or cell suicide is activated. This process is known as apoptosis. [28-30] Apoptosis may be carried out under conditions of starvation, damage to DNA or through infection with a virus. The result is the removal of the cell from the organism, either simply to remove the need to maintain a damaged cell or to prevent the spread of mutated DNA arising either from viral infection or from damage caused by radiation or chemical mutagens. It can be triggered by the damaged cell, cells of the immune system or by signals from neighbouring cells.

During apoptosis various processes take place within a cell; the cell shrinks in size, the cytoskeleton collapses, the nuclear envelope is dismantled and the DNA is broken down into its components. The cell surface is also modified before any leakage of the cell content takes place, this avoids any spread of cellular material which could affect neighbouring cells but also allows the reuse of cellular components. Apoptosis is performed by a specific group of proteases known as the caspases, [31] they contain a cysteine residue in their active sites and cleave their target proteins at aspartic acids. Entry into cell-death is an irreversible process and is tightly regulated like entry into cell division, key regulatory proteins associated with apoptosis include members of the bcl-2 family [32, 33] of proteins.

### *1.4.2 Cell death by necrosis*

In contrast to the process of programmed cell death, apoptosis, cells that have been damaged by physical trauma typically swell is size and burst, spreading their contents throughout the surrounding environment often triggering a local inflammatory response. This contrasting process of cell death is known as necrosis.

## 1.5 Changes in cell behaviour in cancer

Loss of control over some aspect of this process of cell-division can prove fatal to an organism; [34] if the ability to repair damage is lost an organism will die, if proliferation is able to take place in an uncontrolled fashion, unwanted cells will begin to accumulate resulting in the growth of a neoplasm or tumour.

*"Cancer is a collection of diseases characterised by unregulated cell growth leading to invasion of surrounding tissues and spread (metastasis) to other parts of the body"*

*Roger J. B. King, Cancer Biology 2000*

Cancer cells break the 'rules' of cell division at every opportunity: [35] they divide despite the normal controls and restraints placed on cell division by the cell-cycle control mechanism [16, 36] and they invade and 'colonize' the tissues normally occupied by other cell types. The process by which a normal cell becomes cancerous and spreads throughout the body is known as carcinogenesis, the generation of cancer.

The way this is achieved is through the acquisition of errors or mutations in the genetic material contained within the cell nucleus however, a single mutation in one gene is not normally enough to cause the growth of a tumour, normally at least ten separate mutations in a specific group of 'cancer-critical' genes, is required for a cell to turn cancerous. These 'cancer-critical' [37] genes are often referred to as 'cancer-genes', it is not the case that simply possessing one of these genes leads to the development of cancer, it is the mutation or over expression of these genes which leads to the development of cancer. However, many of these genes were first discovered through be examination of their involvement in cancer, some of the cellular proteins that play a pivotal role in the growth, spread and death of cancerous cells within the body are summarised in figure 1.5. For example the retinoblastoma gene is a tumour suppressor gene [38] which, when mutated leads to the development of a particular type of eye tumour known as a retinoblastoma, the gene takes its name from the cancer.

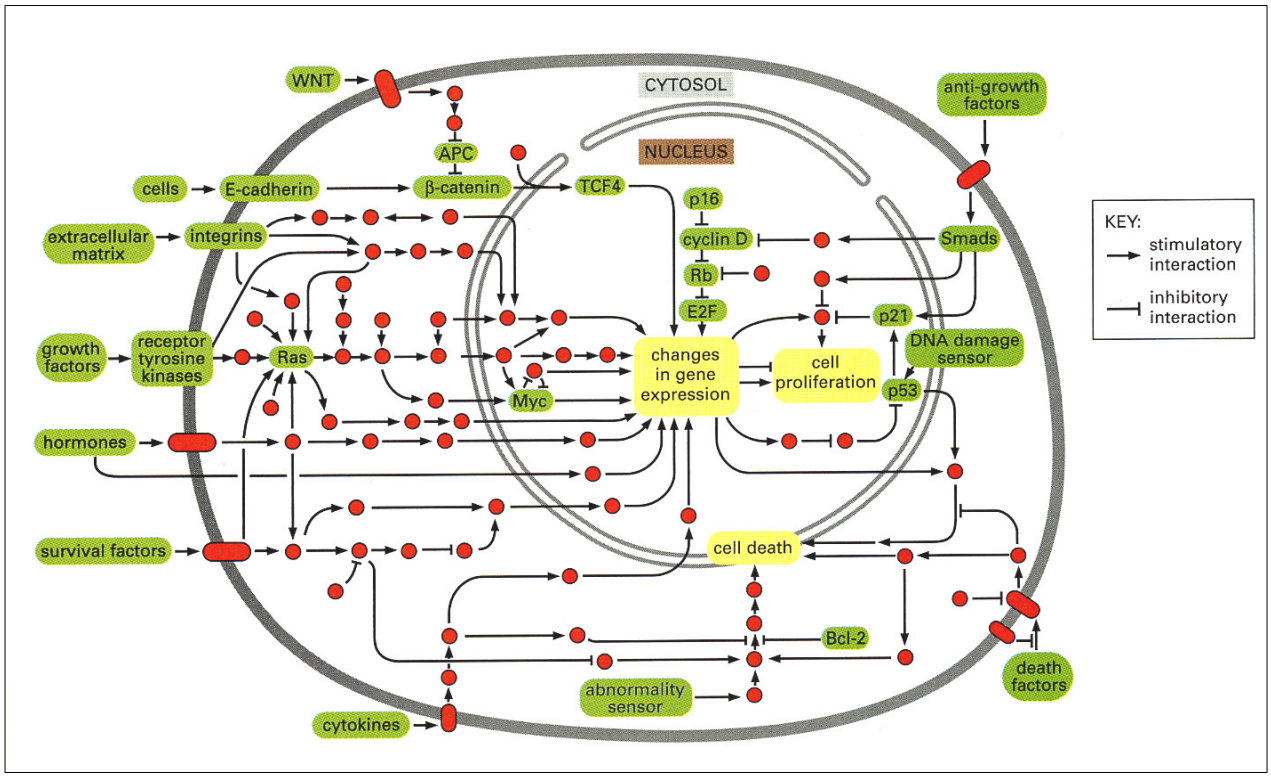



**Figure 1.5** Cellular Proteins with a role in cancer. *(Molecular Biology of The Cell, Alberts et al., p.1343)*

Cancerous growth of tumours is nearly always due to defective control of the division, death or differentiation of a single cell, the vast majority of tumours are formed from one single defective cell. Cancer cells are generally thought of as being:

1. Able to disregard the internal and external signalling pathways that normally govern cell proliferation.
2. Able to avoid programmed cell death by apoptosis. [39]
3. Able to circumvent the natural limitations to proliferation avoiding replicative senescense and terminal differentiation.
4. Capable of growing outside there parent tissues.
5. Able to spread and proliferate at distant sites, metastasis. [40]
6. Genetically unstable. [41]

Cancer as a disease has been known since Ancient Egyptian times but only relatively recently has an understanding of the molecular processes involved with the initiation, progression and spread of cancers become known. [35] Over ten thousand billion billion cell division processes, $1 \times 10^{22}$, take place during a human body over the course of an average

lifespan, random sporadic errors or mutations occur during the synthesis of DNA at the rate of approximately $1 \times 10^{-6}$ mutations per gene per division cycle. During an average life span each separate gene, of which there are over 30,000 in the human genome, is likely to have suffered random point mutations on at least $1 \times 10^{10}$ separate occasions; it is the fact that cells are able to repair such damage that spares an organism from developing many hundreds of separate tumours from a very early stage in its life. The rational treatment of cancers both now and in the future depends absolutely on an understanding of the properties and processes occurring within cancerous cells.

The human body is a complicated piece of machinery with many thousands of different proteins functioning together in a very precise way; the cellular changes which take place in cancerous cells are similarly complex. There are currently over one hundred known 'cancer-critical' genes, specific mutations within these genes can lead to the development of a cancer. These genes play some role in various control processes, either inhibitory or stimulatory, which take place within a cell, most code for proteins in regulatory pathways which govern the behaviour of cell within the body. Some of these 'cancer-critical' genes act to disrupt the cell-cycle control machinery, damaged or defective cell-cycle control pathways are very commonly encountered in cancerous cells. (Figure 1.5)

There are several processes which are important in the development and spread of a tumour within the body, these include: The ability of a cell to break away from a tumour and spread throughout the body colonising distant sites and causing secondary tumours, this process is known as metastasis. The process by which a developing tumour constructs a blood supply with which to recruit the nutrients and oxygen necessary for further growth, this process is known as angiogenesis.

One of the more important such pathways is the Rb pathway, (Figure 1.4) a key event during the cell-cycle where cells commit to the duplication of DNA and move into S-phase, the Rb protein, a product of the Rb gene, acts to restrict entry into S-phase by inhibiting the action of the expression factor E2F. [42] Normally this restriction is removed at the appropriate time by the action of the CDK4/Cyclin D1 complex in phosphorylating the Rb protein, some cancers overcome this restriction point via the loss of the Rb gene others by mutations in other components of the Rb pathway. Certain forms of human breast cancer and glioblastomas possess elevated levels of CDK4 or cyclin D1, with the result that Rb is permanently phosphorylated and control over entry into S-phase is lost. Another

very common feature of human cancers is inactivating mutations in the structure of, or deletion of gene coding for the cyclin-dependant kinase inhibitor p16.

## 1.6 Current Cancer Therapies

Traditional methods for the treatment for cancer have involved three techniques; surgical removal of individual tumours, chemotherapeutic drugs and radiotherapy, however these traditional techniques are fundamentally limited. Surgical removal of a tumour is ineffective as a cure if a tumour has spread or 'metastasised' to other sites within the body, whilst chemotherapy and radiotherapy are also toxic to normal non-cancerous cells.

### *1.6.1 Principles of Cancer Treatment*

Most anticancer treatments, whether by design or accident, exploit some feature which distinguishes cancerous from normal cells; chemotherapy and radiotherapy for example rely on the higher rates of cell division taking place within a tumour when compared to normal cells. When the whole body is exposed to a toxic chemical or radiation the rapidly dividing cancer cells are damaged but continue to divide whereas the normal cells halt their cell cycles to repair the damaged caused. The damaged cancer cells continue to divide and suffer further damage, this accumulation of damage eventually renders the cancer cells non viable. The side effects of radio and chemotherapy are often noticed first in areas of the body which naturally have a higher rate of cell division than other tissues, for example hair follicles, the lining of the stomach and gut and cells in the testes or ovaries.
The clinical treatment of human cancer is a diverse and complex field however, there are several important underlying features, namely, treatment is required as soon as possible after the initial diagnosis and may be required at later stages if the cancer has spread. Initial surgical removal of as many cancerous cells as possible is important, however if metastasis has occurred then chemotherapy and radiotherapy are likely to be required in addition to surgery. The fewer cancerous cells that are present when treatment is begun the more likely it is that a cure is possible. The objective of cancer treatments are two fold; to prevent proliferation of cancerous cells, which is known as cytostatic effects and to kill cancerous cells, cytotoxic effects.

It is known that the sensitivity of cells to treatment varies depending on which stage of the cell-cycle they are in, cells in the $G_0$ phase are resistant to the effects of cytotoxic drugs. However, current chemotherapeutic agents disrupt DNA synthesis and cell division in all cells in the body, this is the cause of most of the side effects associated with current drugs, hence optimal drug treatment requires finding the correct balance between desired effects on cancerous cells and unwanted side effects on normal cells. Advanced cancers which have spread throughout the body are normally treated with drug therapy alone and are often fatal to the organism.

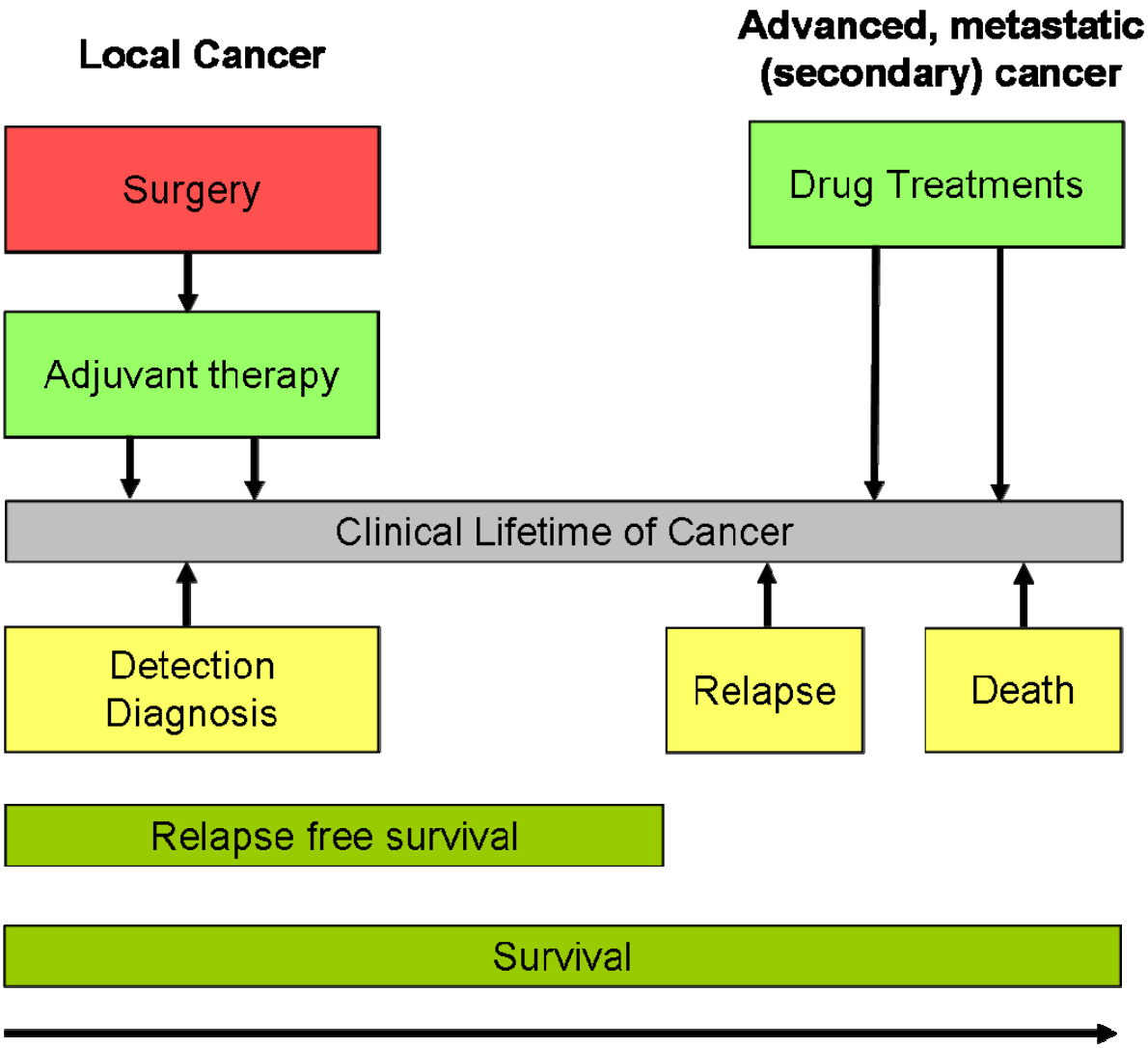



**Figure 1.6** Clinical lifetime of cancer.

*1.6.2 Current Chemotherapy*

There are many different chemotherapeutic agents in current use, involving several different classes which have different effects on cells including; alkylating agents which damage DNA, antimetabolites which disrupt nucleic acid synthesis or natural products which can have various different effects. A typical clinical approach to the treatment of cancer will include; surgery to remove the bulk of the cancer if possible together with treatment with a number (typically 2 to 4) chemotherapeutic agents with complementary effects. Using different drugs in combination has been found to be more effective than any one drug is isolation, also the development of resistance to a particular drug is less likely.

*1.6.3 Alkylating agents*

Chlorambucil **1** (Leukeran®) is a chemotheratpeuic drug often administered in the treatment of chronic lymphocytic leukaemia, low grade non-Hodgkin lymphoma and Hodgkin lymphoma. Chlorambucil **1** belongs to a group of compounds known collectively as the nitrogen mustard alkylating agents and was developed from mustine **2**, the first ever drug to be used as a chemotherapeutic agent in 1942. Mustine **2** itself was a development of the original mustard gas used as a chemical weapon during the first world war. The nitrogen mustard alkylating agents work by intramolecular displacement of chloride to form a highly electrophilic aziridinium ion which is then able to react with nucleophilic groups such as the lone pair on the nitrogen atom of guanine. Two such reactions results in a cross linking of two DNA bases, either between two complementary strands or two bases on the same strand. This cross linking prevents DNA replication. Mustine is no longer in common medical use due to its side effects, but other less reactive nitrogen mustards, such as compounds which bear a deactivating aromatic ring on the nitrogen atom e.g. melphalan **3** are still is use today. Other refinements have been made to the structure of the nitrogen mustards aimed at localising their effects within the body, uramustine **4** or uracil mustard contains a nucleic acid base, has a certain level of selectivity for tumour cells over normal cells due to the fact that rapidly dividing tumour cells require more nucleotide bases for their growth than normal cells.

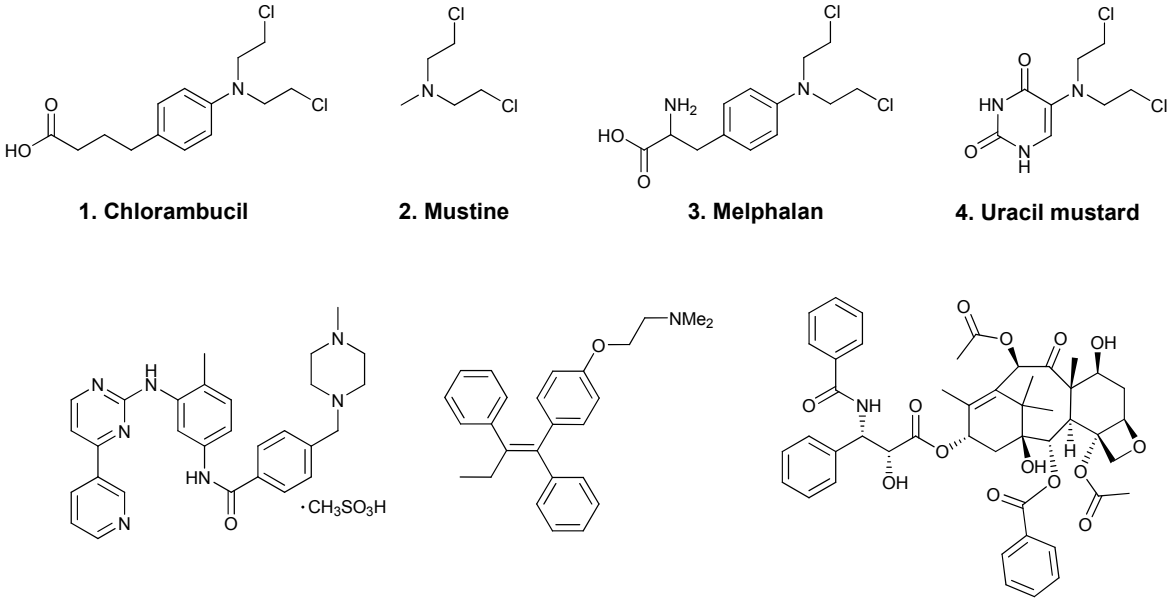



**Figure 1.7** Chemotherapeutic agents.

The side effects of chlorambucil **1** include; lowered resistance to infection due to a decrease in the number of white blood cells produced in the bone marrow, bruising or bleeding caused by reduced production of platelets, anaemia, lowered number of red blood cells, which can cause tiredness and breathlessness, also nausea, vomiting and loss of appetite. Rarer side effects include changes to lung tissue causing further breathlessness, changes to the skin, liver function, mouth ulcers, diarrhoea and changes in the perception of tastes. The nitrogen mustards also interfere with fertility and can stop sperm production in men. They can also trigger other forms of cancer many years after successful treatment of a primary cancer.

*1.6.4 Protein kinase inhibitors*

The first synthetic protein kinase inhibitor to be used successfully in the treatment of cancer was imatinib or glivec **5**. [43, 44] Produced by Novartis it was originally approved for treatment of chronic myeloid leukaemia (CML). CML is characterised by a mutant chromosome, the Philadelphia chromosome, formed from the fusion of chromosomes 9 and 22. This mutant chromosome contains a new gene, the bcr-abl gene, which encodes for a mutant tyrosine-kinase protein. This mutant protein contains a continuously active tyrosine kinase which is inhibited by imatinib **5**, along with several other tyrosine-kinase

proteins. The use of imatinib **5** and has proved highly effective in the treatment of bcr-abl positive CML. [45] Glivec was the result of a rational drug discovery program and its success in the treatment of cancer has encouraged further research in the field of targeted intervention. [46, 47]

### *1.6.5 Hormone therapies*

Some types of cancer require the presence of specific hormones for continued growth, for example some forms of breast cancer require the presence of the female sex hormone oestrogen for survival. Later stage breast cancer often mutates to remove this reliance on oestrogen. Agents which either inhibit hormone binding or hormones which have the opposite effect to the natural hormone can have therapeutic benefit in those types of cancer that are still hormone sensitive. Since hormone receptors are found in relatively few cell types throughout the body, agents which act against hormone receptors have some degree of specificity. Oestrogens can be used in the treatment of prostate cancer as they inhibit the bio-synthesis of testosterone in the male body, similarly progestins can be utilised in the treatment of endometrial carcinoma or advanced breast cancer. However, androgens or male sex hormones, are used less often in the treatment of female cancers due to their masculinising effect on the female body. Antioestrogens [48] such as tamoxifen **6**, are synthetic oestrogenic antagonists, [49] they compete with oestrogen for binding to the oestrogenic receptor and are routinely used for the treatment of hormone-dependent breast cancers.

### *1.6.6 Inhibitors of tubulin depolymerisation*

Tubulin is a cellular protein that, when polymerised, forms microtubules that make up the structure of the mitotic spindle which is critical to separating the two sister chromatids during mitosis. Polymerisation of tubulin leads to the construction of the mitotic spindle while de-polymerisation causes deconstruction of the spindle which must take place before the division of the dividing cell into two daughter cells can take place.

Taxol **7** is a natural product originally isolated from the bark of the pacific yew tree *Taxus brevifola*. It is now made commercially on a large scale using plant cell fermentation (PCF) technology. Taxol binds to tubulin and accelerates polymerisation by stabilising the

resulting microtubules. Thus depolymerisation is inhibited and cell-death via apoptosis is triggered. [50] Taxol is used to great success in the treatment of breast, ovarian cancers, [51] leukaemia and testicular cancer. However Taxol is toxic to bone marrow and neural tissue and some cancers are able to develop drug resistance.

### *1.6.7 Monoclonal antibodies*

Monoclonal antibodies are antibodies produced from cells cloned from a single cell from the immune system. Monoclonal antibodies can be raised which bind to almost any substance, chemical or protein. In the field of cancer treatment it is theoretically possible to raise monoclonal antibodies for any over expressed protein, treatment with the antibody would then act to raise an immune response to the cell over expressing that particular protein, [52] thus undoing the effect of a specific mutation in the genome of that cancer. This type of therapy is often referred to as the 'magic bullet' approach. [53] The best example of a monoclonal antibody in current use is trastuzumab or herceptin. This humanised monoclonal antibody binds to a receptor known as HER2/neu (or erbB2) that is expressed in elevated levels in some forms of breast cancer.

Clearly side effects and lack of selectivity towards cancerous cells over normal cells limits the levels of these drugs that the human body can tolerate, current research efforts are moving towards exploiting our knowledge of the molecular basis of cancers and utilising this knowledge to devise new treatments which target other features unique to cancer cells.

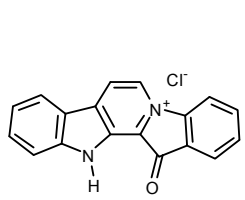
## 1.7 Potential of cell-cycle inhibitors as cancer treatments

A major area of research is on inhibitors of the protein kinases, [54] the family of enzymes to which the cyclin dependent kinases belong. The CDK's are an attractive target because of their central role in the cell cycle but also because the actual structure of the CDK's themselves is never mutated in cancer cells. A potent selective inhibitor of specific CDK/Cyclin complexes would allow us to counter and even reverse the consequences of mutations in specific parts of the cell cycle control machinery; recent research in this area has concentrated on identifying, understanding and developing naturally occurring inhibitors of the CDK's. Of particular interest are the changes in cell-cycle regulation

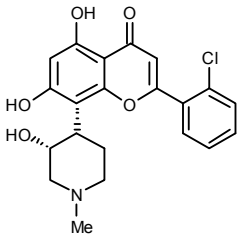
associated with the various check points in the cell cycle, e.g. the $G_1$/S checkpoint. [55] cancerous cells must acquire mechanisms of defeating the systems which would ordinarily halt cell-cycle progression and either instigate genetic repair or apoptosis. Increasingly, inhibitors of the CDK's are being found to have great potential for the treatment of other diseases including, malaria. [56]

## 1.8 Small molecule inhibitors of the cell cycle

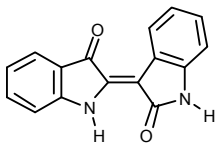
There are a large number of 'small-molecule' inhibitors of the CDK's, which are a current area of intense research, [57, 58] several of which have progressed to the early stages of clinical trials. [59] Such 'small-molecules', with molecular weights below 500, are generally perceived to be well tolerated by the human body, as such they are thought of as good lead compounds for the development of new drugs.



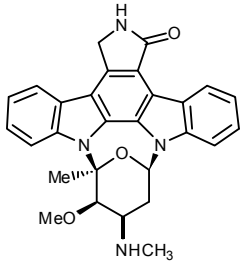

**8. Fascaplysin**



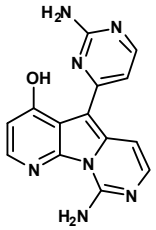

**9. Flavopiridol**



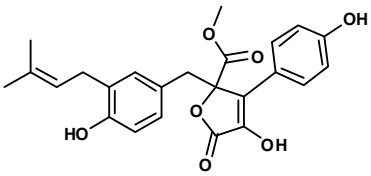

**10. Indirubin**




**11. Staurosporine**




**12. Variolin**




**13. Butyrolactone I**

**Figure 1.8** Small molecule inhibitors of Cyclin Dependent Kinases

*1.8.1 Fascaplysin*

The red penta-cyclic pigment fascaplysin **8**, [60] isolated from the marine sponge *Fascaplysinopsis berquist*, was originally identified as a novel antibiotic, its ability to selectively inhibit CDK4/Cyclin D1 ($IC_{50}$ 0.35 μM) and CDK6/Cyclin D1 ($IC_{50}$ 3.4 μM) over CDK1/Cyclin B ($IC_{50}$ >100 μM) was discovered later. [61] Fascaplysin **8** inhibits CDK4/Cyclin D1 in the sub micro-molar range, it shows selectivity over the bound cyclin partner as it does not inhibit CDK4/Cyclin D2 ($IC_{50}$ >100 μM). Neither does it inhibit unrelated protein-tyrosine kinases such as *v*-ABL, c-met, IGF-1R and insulin-R. Fascaplysin has also been shown to interchelate DNA, [62] a feature which goes some way to explaining its toxicity. [63]

*1.8.2 Flavopiridol*

Flavopiridol **9** belongs to a class of compounds known as the flavones and is an analogue of the natural alkaloid rohitukine which is obtained from an Indian plant *Dysoxylum binectariferum*. Flavopiridol **9** was originally identified as an inhibitor of the receptor tyrosine kinases, [64, 65] receptor-associated kinases, [66] and cytosolic signal transduction kinases. [64, 65, 67] It selective inhibits CDK4/Cyclin D ($IC_{50}$ 20-40 nM), CDK6/Cyclin D ($IC_{50}$ 60 nM), CDK1/Cyclin B ($IC_{50}$ 30-40 nM) over CDK2/Cyclin A, CDK2/Cyclin E ($IC_{50}$ 100 nM) and CDK7/Cyclin H ($IC_{50}$ 100-300 nM). [64, 66] It is also able to inhibit the evolutionarily related glycogen synthase 3β (GSK-3β, $IC_{50}$ 450nM) [68] and is known to induce cell-cycle arrest at the $G_1$ or $G_2$/M checkpoints. [69] More recently flavopiridol has also been shown to be the most potent inhibitor of CDK9/Cyclin T1 discovered to date. [70] This makes flavopiridol a good candidate for the clinical treatment of HIV and molecular modelling has been carried out on the complex to determine the binding interactions present. [71] Flavopiridol was the first inhibitor of CDK's to enter into clinical trials, [72-74] it showed great promise because of its ability to inhibit cyclin-dependent kinases but also due to the fact that it can inhibits the growth of a range of human tumours *in vivo* and *in vitro*. [67, 72, 75, 76] Flavopiridol **9** is also known to kill tumour cells not involved in the process of cell division [77, 78] and can trigger apoptosis in human cancer cells both *in vivo* and *in vitro*. [76-83] It has also been shown to cause tumour regression in some human cases. [73] However, it is also toxic to non-cancerous cells and has been shown to bind to DNA. [84] Flavopiridol has been used extensively as a lead compound in many drug discovery programs. [85, 86]

*1.8.3 Indirubin*

Another family of inhibitors is the oxoindoles, for example indirubin **10**, identified as the active ingredient from a mixture of plants known as Danggui Longhui Wan, utilised in traditional Chinese medicine as a treatment for Leukaemia and other chronic diseases. [87] It shows selective inhibition of the CDK's over other protein kinases, CDK1/Cyclin B ($IC_{50}$ 10 μM), CDK2/Cyclin A ($IC_{50}$ 2.2 μM), CDK2/Cyclin E ($IC_{50}$ 7.5 μM), CDK4/Cyclin D ($IC_{50}$ 12 μM), CDK5/p53 ($IC_{50}$ 5.5 μM) and GSK-3β ($IC_{50}$ 0.6 μM). X-ray crystallographic studies on indirubin sulphate and CDK2 show that inhibition occurs via an ATP competitive mechanism.

*1.8.4 Staurosporin*

Staurosporin **11**, a metabolite produced by Streptomyces sp., is another rare example of a naturally occurring ATP competitive inhibitor, originally identified for its ability to inhibit protein kinase C (PKC) [88] it has since been shown to be an unselective inhibitor of CDK's. [89] The lack of selectivity is thought to be due to the structure of staurosporin **11** being too close a match for that of ATP, as shown by X-ray crystallography. [90-92]

*1.8.5 Variolin B*

The marine natural product variolin B **12** was originally isolated from the Antarctic sponge *Kirkpatrickia variolosa*. [93] It has been shown to inhibit CDK2/Cyclin A, CDK2/Cyclin E, CDK1/Cyclin B and to a lesser extent CDK7/Cyclin H. It also shows slight inhibition of CDK4/Cyclin D, [94] variolin B blocks cell-cycle progression from $G_1$ to S phase and also triggers an accumulation of cells in $G_2$. Variolin B can also trigger apoptosis in cancerous cell lines, independent of the presence of p53. However it has also been shown to interchelate DNA, although without causing DNA strand breaks.

*1.8.6 Butyrolactone I*

Butyrolactone I **13** was first derived from *Aspergillus terreus* var. *africanus* and then from *Aspergillus* strain IFO 8835 in 1977. [95] It was identified as causing cell-cycle arrest during $G_1$ and $G_2$ in cultured cells [96] and has been shown to inhibit the cdc2 family of kinases.[97]

*1.8.7 Fascaplysin as a lead compound*

Fascaplysin **8** shows the highest specificity for inhibition of CDK4/Cyclin D1 over other CDK4/cyclin complexes (e.g. CDK4/Cyclin D2) and other CDK's. Hence it potentially the most promising lead compound for intervention at the $G_1$/S check point, as this selectivity should reduce unwanted side effects and allow administration of higher concentrations of drug. Fascaplysin **8** itself has limited potential for use as an anti-cancer drug as it is highly toxic to living organisms, the most common explanation for these side effects is that the planar pentacyclic structure is able to intercalate between the base pairs of double strand DNA [62] and either prevent transcription or cause breaks in the DNA backbone.

The Jenkins research group at the University of Leicester has been working for a number of years on the synthesis of analogues of fascaplysin. The primary aim of this thesis is to examine the rational design, synthesis and testing of novel non-toxic inhibitors of CDK4/cyclin D1, that have potential for therapeutic use in the treatment of cancer. There have been several previous studies examining the design of ATP competitive inhibitors of the cyclin dependent kinases in general,[98] and CDK4 specifically. [99]

| Inhibitor | Cdk1/B | Cdk2/A | Cdk2/E | Cdk4/D | Cdk4/D1 | Cdk4/D2 | Cdk4/D3 | Cdk5/pX | Cdk6/D1 | Cdk6/D2 | GSK-3β |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Fascaplysin **8** | >100 | >50 | >50 | - | 0.35 | >100 | >100 | 20§ | 3.4 | >50 | |
| Flavopiridol **9** | 10 | 0.1 | - | - | 0.02-0.04 | - | - | - | - | - | |
| Indirubin **10** | 10 | 2.2 | 7.5 | 12 | - | - | - | 5.5§ | - | - | |
| Staurosporin **11** | 3.2 nm | | | | | | | | | | |

**Table 1.9** $IC_{50}$ values for natural product inhibitors and derivatives on cyclin dependent kinases and related enzymes. §Cdk5/p53, ‡Cdk5/p25.

## 1.9 Rational design of CDK inhibitors.

### *1.9.1 CDK2*

The design of inhibitors of CDK2 has been greatly aided by the availability of a number of x-ray crystallographic structures of CDK2 and CDK2 bound to various inhibitors, [100] for example staurosporin **11**, [101] which unspecifically inhibits many different kinases, the X-ray crystal structure of staurosporin **11** bound to CDK2 is shown below, this structure and others has facilitated the mode of action of many kinase inhibitors.

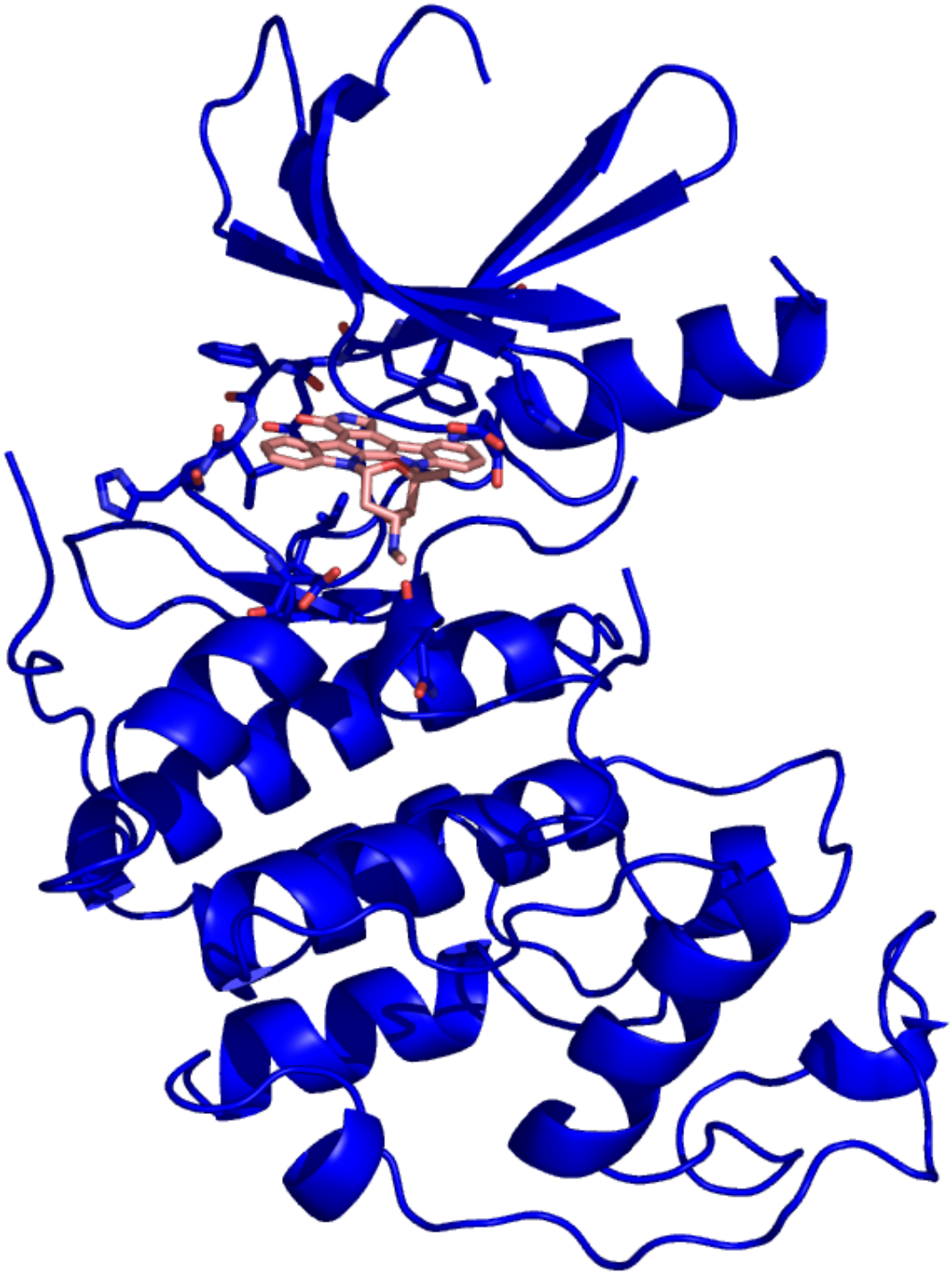


**Figure 1.9** X-ray crystal structure of human CDK2 in complex with the inhibitor Staurosporin **11**. PDB 1aq1.[102]

A number of CDK2 inhibitors have entered clinical trials, however success has been limited by side effects. For example the natural product Roscovitine **12** which inhibits CDK2-cyclin X with $IC_{50}$ 80 ± 20 nM, [103] also inhibits other CDK's not involved with the cell-cycle, [104] other protein kinases and non-kinase targets. [105] A great deal of work has been undertaken on the structure and mechanism of inhibition of CDK2, this has lead to a

fair understanding of the binding interactions that take place within the ATP binding site. [98]

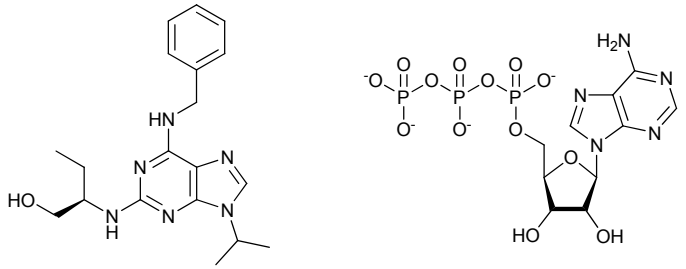



**Figure 1.10** Roscovitine a potent inhibitor of CDK2 and the structure of ATP.

The ATP binding site is can be thought of as containing four main binding interactions; [98] the hinge region, the Phe 80 pocket, the ribose/phosphate site and surface specificity interactions. The hinge region, residues 81-84, contains a number of hydrogen bond donor and acceptor sites that are considered important to inhibitor binding. The adenine ring of ATP forms two hydrogen bonds, N1 accepts a hydrogen bond from Leu 83 and N6 is a hydrogen bond donor to Glu 81. Every known CDK2 inhibitor forms a hydrogen bond with Leu 83 and the vast majority also form a hydrogen bond with Glu 81. The Phe80 pocket is a shallow cleft towards the back of the ATP binding region, while ATP itself does not bind to this region of the active site several inhibitors including indirubin **10** and staurosporine **11** do. Many other kinases have a similar pocket, however the size of the pocket has been found to vary considerably and many kinases have a much larger pocket present, a feature which has often been exploited to improve selectivity.

The ribose/phosphate site is the area around residues 11-18; this region undergoes a great amount of conformational change upon both cyclin binding and phosphorylation, Gln 131 and Asp 86 are both within hydrogen bonding range of bound staurosporine **11**.

*1.9.2 CDK4*

Unlike CDK2 the x-ray crystal structure of CDK4/cyclin D1 is not known, therefore any attempt at rational inhibitor design is based on the known structures of related enzymes. The amino acid sequence of CDK4 is known and is similar to other related CDK's. Previous research within the Jenkins research group has produced a homology model of

CDK4/Cyclin D1 [106] based on the known x-ray crystal structures of the closely related enzymes CDK2 and CDK6, (Figure 1.11) this was produced using the computer program ESPRIPT. [107] Areas with identical or similar amino acid sequence are assumed to have similar tertiary structure.

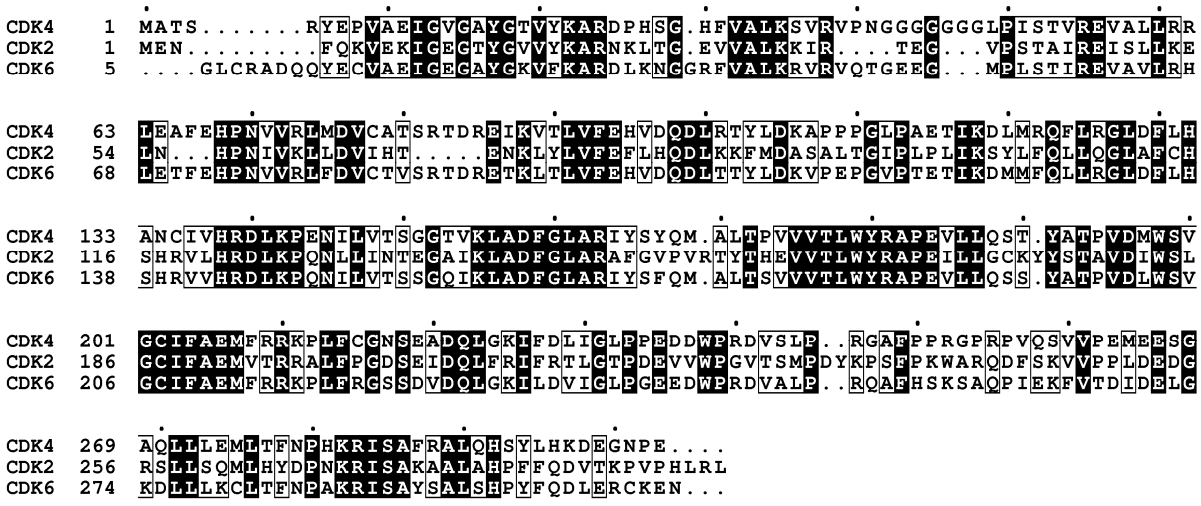



**Figure 1.11** Sequence alignment used to generate the homology model of CDK4 based on the known structures of CDK2 and CDK6. Identical residues in all three sequences are shown on a black background similar residues are boxed. [106]

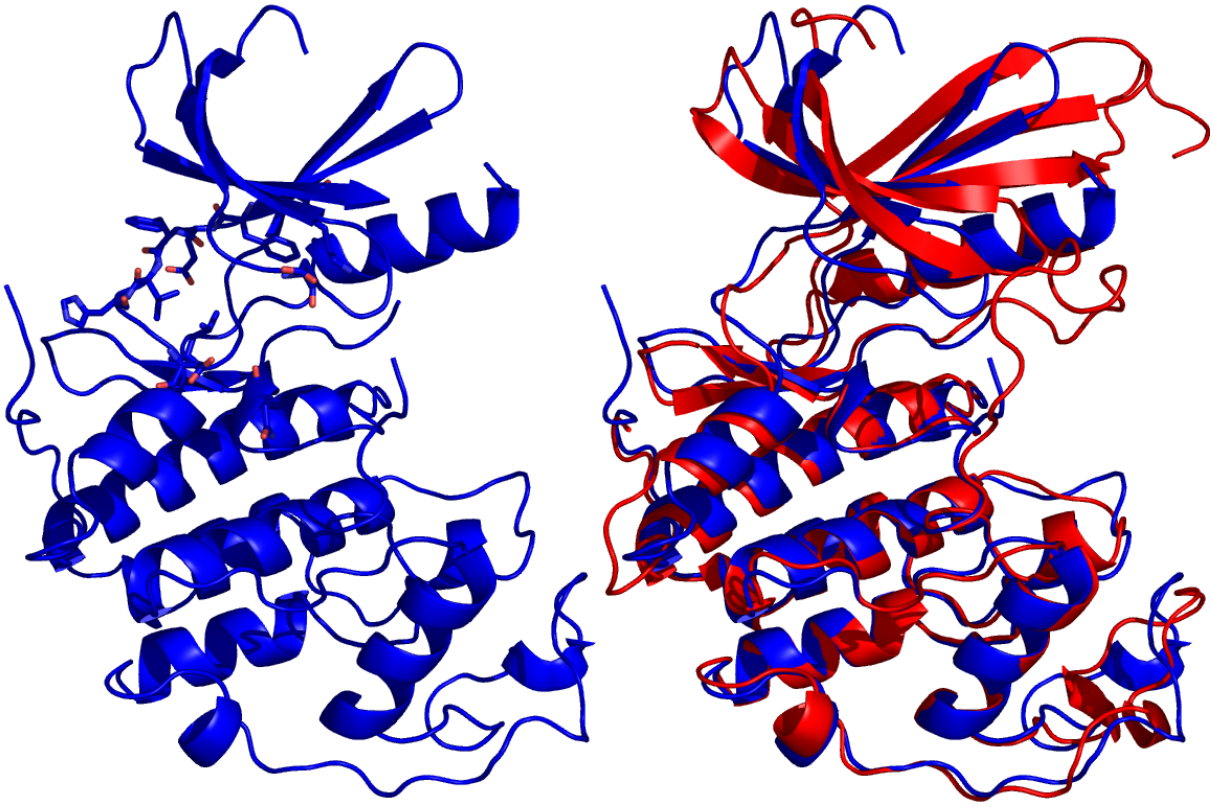


**Figure 1.12** X-Ray crystal structure of human CDK2, pdb code 1AQ1[91] (left, blue) and CDK2 overlaid with CDK4 homology model (right, red).

The program GOLD v2.0 [108] (Genetic Optimisation of Ligand Docking) has been used to produce a 'best-fit' approximation as to the localisation of inhibitor molecules within the active site of this homology model, GOLD is also capable of outputting an overall 'Goldscore' fitness function [109] as well as highlighting important interactions such as hydrogen bonding and Van der Waals interactions within the inhibitor and between inhibitor and active site.

The docking of this fascaplysin **8** into this homology model produces a possible mode of binding, which takes place when fascaplysin **8** inhibits CDK4/cyclin D1. Specifically, a hydrogen bond between the indole N and the ketone O of fascaplysin **8** and the valine 96 amino acid residue of CDK4, this 'double-hydrogen bond' is proposed to locate fascaplysin within the active site, (Figure 1.13) in an analogous manor to the 'hinge region' of CDK2. [98] We can also see a hydrophobic pocket present between residues Phe 93 and Phe 159, again possible analogous to the Phe 80 pocket in CDK2.

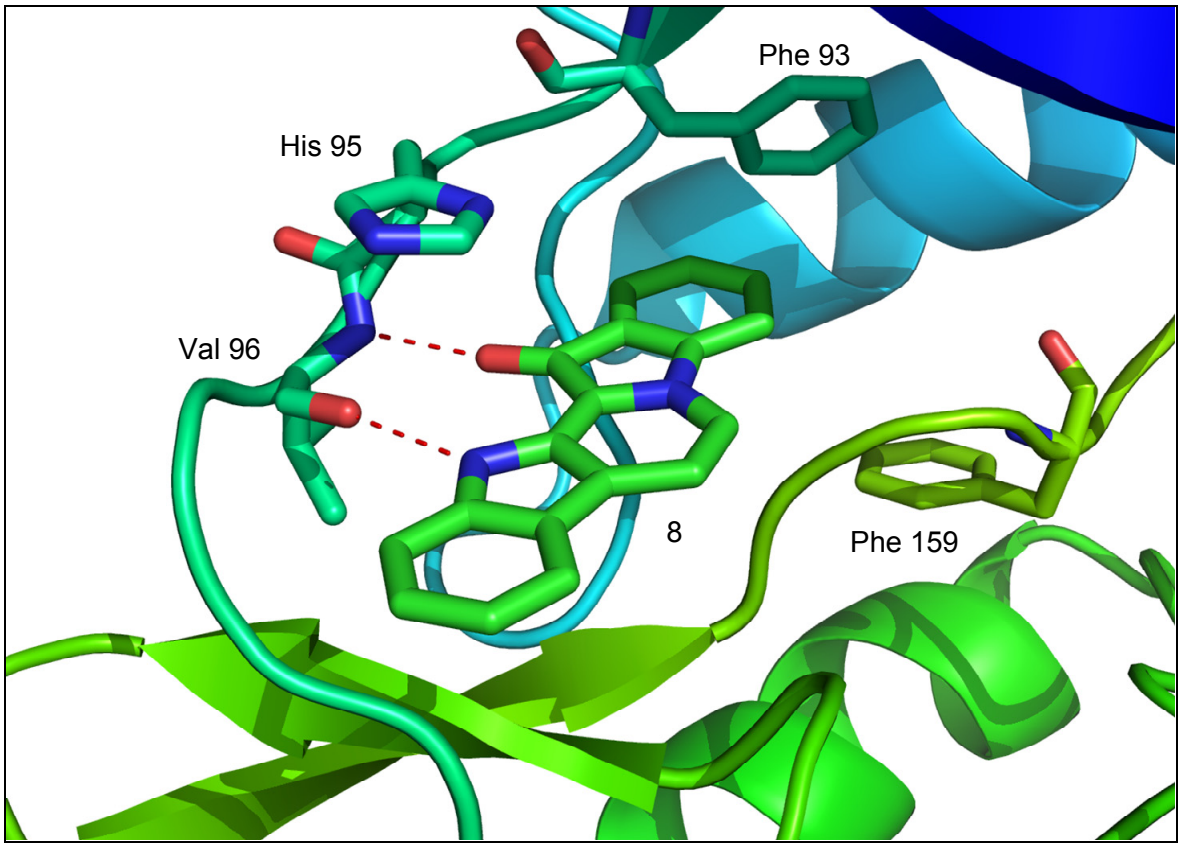
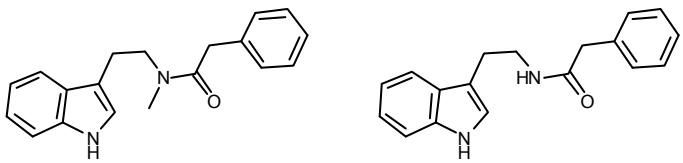



**Figure 1.13** Fascaplysin **8** docked into the active site of the CDK4 homology model using the program GOLD. [106] The hinge region comprising residues 94-97, the gate keeper residue Phe93 and the Phe93-Phe159 hydrophobic pocket are shown. Dotted red lines indicate proposed hydrogen bonding interactions.

## 1.10 Research Proposal

Previous work by the Jenkins research group has produced a series of computational dockings of **16a** and **16b** into the homology model of CDK4, which has suggested that compounds with the same general structure occupy a similar region of the active site to fascaplysin **8**. (Figure 1.14) [106]




**16a** **16b**

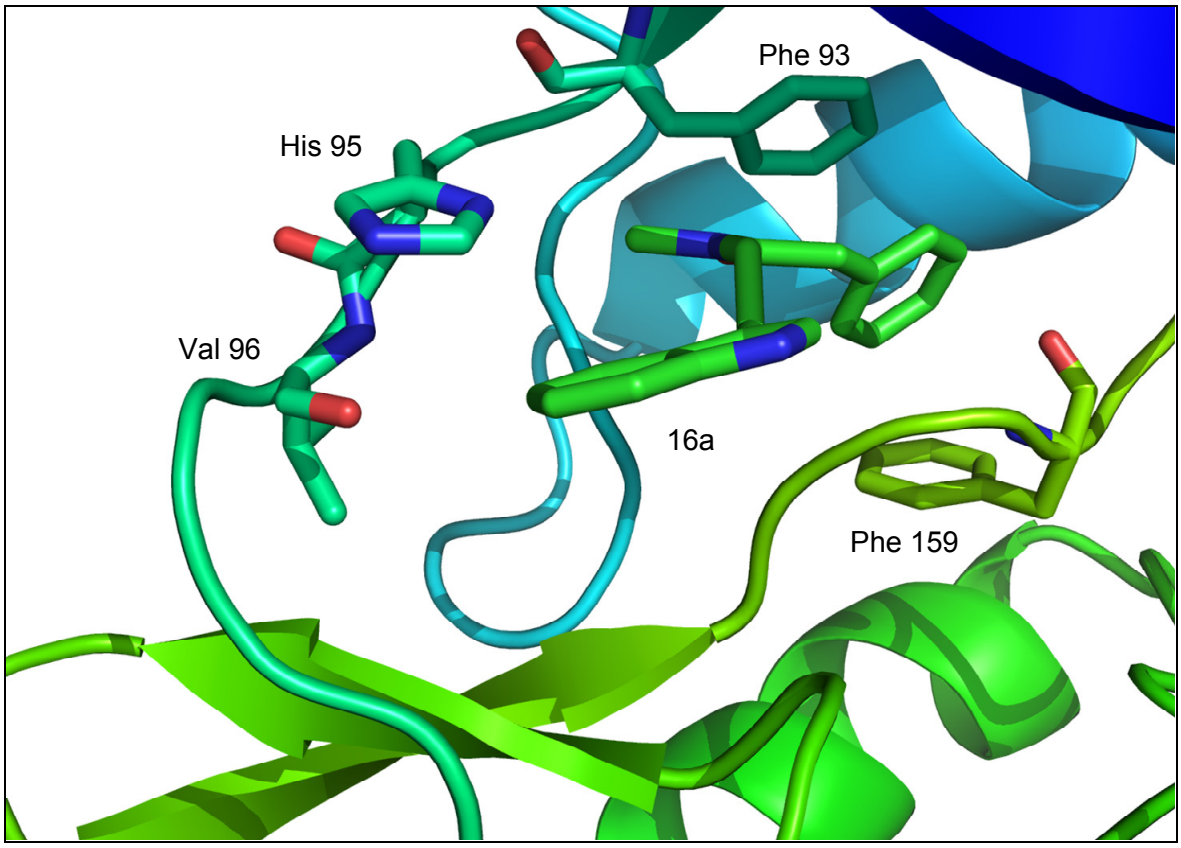



**Figure 1.14** Structure **16a** docked to the active site of a CDK4 homology model. [106] The hinge region comprising residues 94-97, the gate keeper residue Phe93 and the Phe93-Phe159 hydrophobic pocket are shown.

There are indications of π-π stacking interactions taking place between the phenyl ring of **14a** and phenylalanine residues 93 and 159 of the CDK4 homology model, a previously unseen interaction but one which is commonly encountered in other enzyme substrate interactions. [110-112]

Previous studies have suggested that the nature of aromatic interactions is governed by electrostatic and dispersion forces, [113-115] in particular interactions between quadrupole moments in aromatic π-electron clouds. [116] To probe the nature and importance of this interaction a series of analogues of **16a** bearing substitution on the phenyl ring were synthesised. Introduction of substituent groups onto the phenyl ring changes both the quadrupole moment [117] but also the steric properties of the benzene ring.

The first biologically active non-planar analogues of Fascaplysin **8** have been synthesised in previous work by Dr Carine Aubry at the University of Leicester, compounds 1**7a-c**. (Figure 1.15) [118]

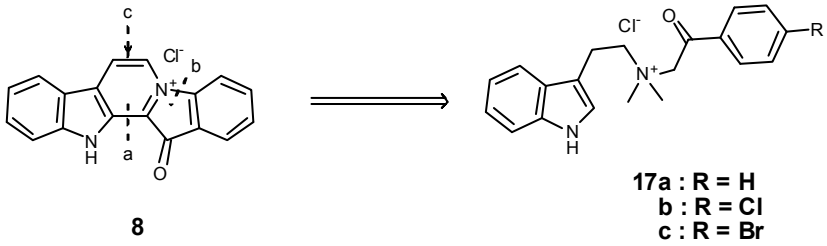



**Figure 1.15** Rationalisation of the first non-planar analogues of fascaplysin **8**. [118]

These *N*-ω-dimethyl-tryptamine salts were rationalised from fascaplysin **8** by cleavage of bonds a and b, and the reduction of bond c to a single bond, these compounds were found to selectively inhibit CDK4/Cyclin D1 over CDK2/Cyclin A (table 1.2).

| | $IC_{50}$ in vitro inhibition (μM) | |
|---|---|---|
| Structure | CDK4/Cyclin D1 | CDK2/Cyclin A |
| **17a** | 68 ± 7.5 | >500 |
| **17b** | 50 ± 7.0 | >500 |
| **17c** | 50 ± 7.0 | >500 |

**Table 1.2** Activity of structures 1**7a-c**. [106]

The first step in the process of designing a new family of analogues is to investigate the structure activity relationship between fascaplysin **8** and the active site of CDK4/cyclin D1, to accomplish this a series of compounds structurally similar to fascaplysin **8** were synthesised, beginning with a repeated total synthesis of fascaplysin to produce a series of 'intermediate compounds' each containing one or more of the structural features of fascaplysin **8**. Testing these 'intermediate compounds' should reveal which features of the molecule are essential for activity and at what point the molecule becomes toxic. This will then be used as a route for designing further generations of compounds based initially on each of these intermediates.

# Chapter Two Synthesis of fascaplysin analogues

*"Intelligence is quickness to apprehend as distinct from ability, which is capacity to act wisely on the thing apprehended."*

*Alfred North Whitehead*

## 2.1 Activity of fascaplysin

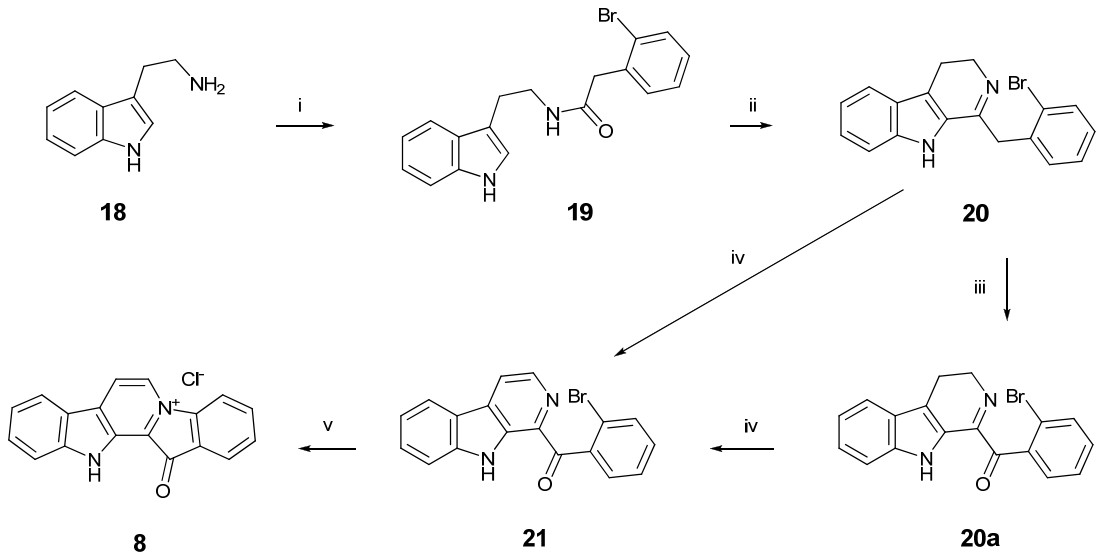
As detailed previously[61] fascaplysin **8** inhibits CDK4/Cyclin D1 ($IC_{50}$ 0.35 μM) and CDK6/Cyclin D1 ($IC_{50}$ 3.4 μM) selectively over CDK1/Cyclin B ($IC_{50}$ >100 μM), but very little is known about what structural features of fascaplysin are required for activity. Previous results published by the Jenkins group have shown that compounds with the same structural fragments as fascaplysin retained selectivity towards CDK4 over CDK2. [118]

## 2.2 Synthetic Routes to Fascaplysin

There are a number of synthetic routes to fascaplysin **8** reported in the literature, [119-121] the route described by Novikov *et al.,* [119] (Scheme 1) is a good starting point for examining the structure activity relationship as each of the intermediate compounds **21**, **20**, **20a** and **19** are progressively simpler compounds, each lacking one or more structural features of fascaplysin. While each of these compounds has been previously described in the literature, full characterisation has not been reported. By repeating the synthesis of these intermediate compounds and screening them against CDK4/Cyclin D1, it may be possible to reveal which structural features of fascaplysin are essential to activity. Any precursor molecules exhibiting interesting biological properties can then be investigated further.

## 2.3 Fascaplysin precursors

Attempts were made to repeat the synthesis of fascaplysin **8** as reported by Novikov *et al.*, [119] following the procedure described in Scheme 2.1. However the published material lacks full experimental conditions and the first step, the azeotropic distillation of tryptamine **18** and 2-bromophenylacetic acid in toluene and tetralin producing amide **19**, could not be reproduced.

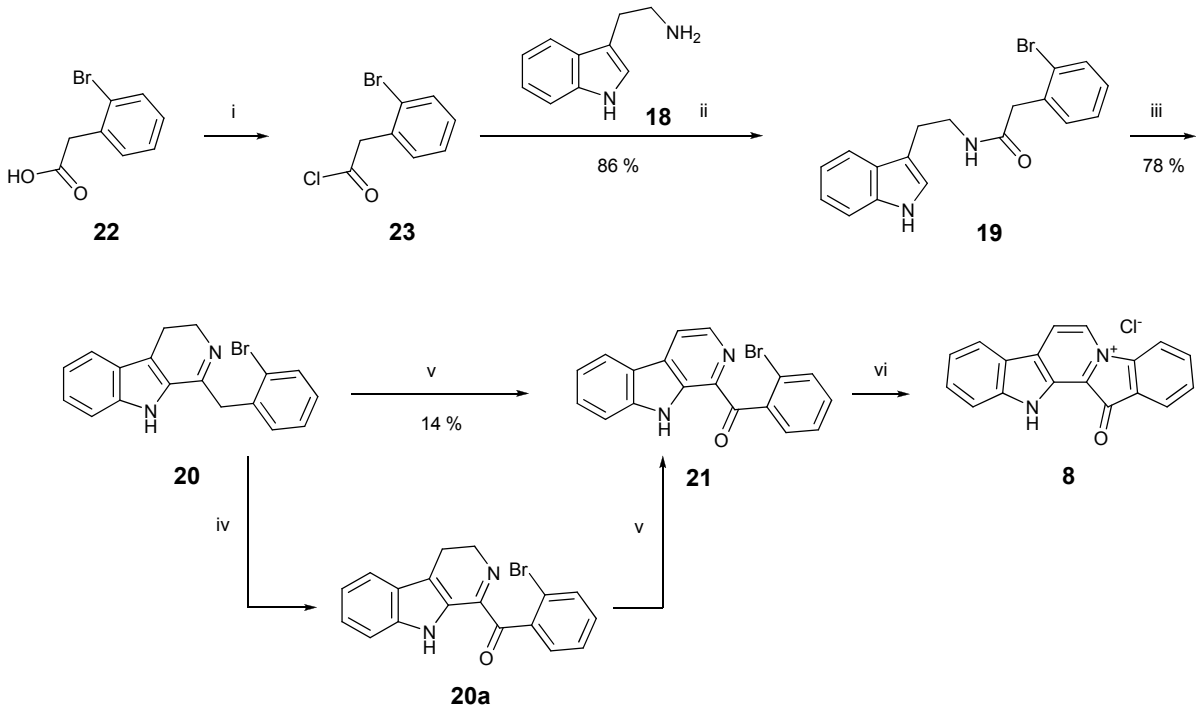



**Scheme 2.1** Literature synthesis of fascaplysin **8**.[119]
*Reagents and conditions*: (i) *o*-Bromophenylacetic acid, tetralin, benzene, azeotropic distillation, 40 min; (ii) $POCl_3$, PhH, reflux, 30 min; (iii) $MnO_2$, $CHCl_3$, 3h; (iv) $MnO_2$, $CHCl_3$, reflux, 3h; (v) 220°C, 20 min then HCl/MeOH

The reaction was repeated a number of times with differing ratios of toluene and tetralin, but no trace of products was observed. As neither author could be contacted, the published route was abandoned and an adapted synthetic route used in its place.

### *2.3.1 Revised route to the amide precursor*

An alternative synthetic route to 2-(2-Bromo-phenyl-*N*-[2-(1*H*-indol-3-yl)-ethyl]-acetamide **19** was proposed utilising the coupling of an acid chloride, 2-bromophenyl acetyl chloride **23**, with tryptamine. The acid chloride **23** was prepared by refluxing 2-bromophenyl acetic acid **22** in thionyl chloride for 30 minutes; the thionyl chloride was removed under vacuum and the crude acid chloride used without further purification.




**Scheme 2.2** Revised route to fascaplysin **8** and intermediate compounds.
*Reagents and conditions*: (i) $SOCl_2$, reflux, 30 min; (ii) tryptamine, DCM, NaOH $_{(aq)}$, r.t., 3 hrs; (iii) $POCl_3$, PhH, reflux, 30 min; (iv) $MnO_2$, $CHCl_3$, 3h; (v) $MnO_2$, $CHCl_3$, reflux, 3h; (vi) 220$^{o}$C, 20 min; then HCl/MeOH.

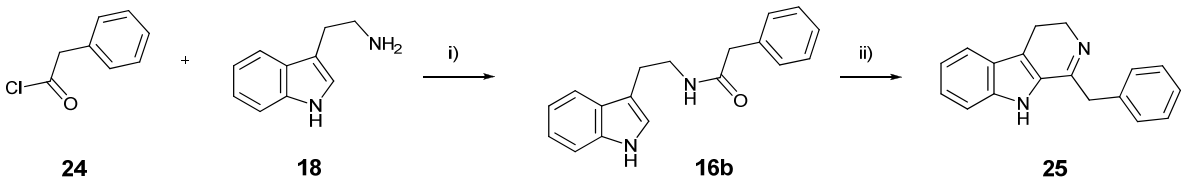
The reaction of 2-bromophenyl acetyl chloride **23** with tryptamine **18** under basic conditions (Scheme 2.1) proceeded in 86 % yield to give the desired amide intermediate **19** as an off white powdery solid. Full characterisation was achieved using MS and NMR.

*2.3.2 Tetrahydro-β-carboline type compounds*

The next step in the published synthetic route to fascaplysin utilises the Bischler-Napieralski cyclisation reaction[122, 123] of amide **19** to give the tetrahydro-β-carboline derivative **20**. Following the general procedure as outlined by Novikov *et al.,*[119] treatment of the amide intermediate **19** with $POCl_3$ in refluxing benzene afforded compound **20** as a crude oil. The isolation and purification of which proved to be non-trivial; the original literature is described as a simple synthesis of fascaplysin **8** without the need for column chromatography and no attention is given to the isolation or purification of any of the intermediate compounds.

A model system was utilised to investigate the best method for the isolation and purification of these tetra-hydro-β-carboline type compounds, replacing 2-bromophenyl acetyl chloride **23**

with the cheaper commercially available phenyl acetic acid in the reaction gives rapid access to the simpler, unsubstituted **25**. The larger volumes of the crude product readily available allowed rapid development and testing of an isolation and purification procedure.




**24** **18** **16b** **25**

**Scheme 2.3** Synthesis of model compound **25**.
*Reagents and conditions*: (i) DCM, NaOH $_{(aq)}$, r.t., 3 hrs; ii) $POCl_3$, PhH, reflux, 30 min.

Column chromatography proved extremely demanding, the crude oil being only sparsely soluble in most common solvents, this necessitated very large column volumes for isolation of only a very small quantity of product. The most successful method for the isolation of **25** was to dissolve the crude oil in dilute acetic acid, cooling this solution to 0 $^{o}$C followed by the gradual addition of aqueous ammonia solution (35%) caused precipitation of a pale yellow powder which could be filtered off and dried under vacuum to give **25** in 63 % yield. Identical treatment of the brominated **20** furnished yielded the desired compound as a powdery pale yellow solid, 78 %.

### *2.3.3 The Bischler-Napieralski reaction*

The conversion of amide **16b** to dihydro-β-carboline **25** is an example of the Bischler-Napieralski reaction, [122, 124] which is the dehydration of an *N*-acyl-β-arylethyl amine into the corresponding heterocycle. The reaction mechanism proceeds as outlined in figure 2.1. [123]

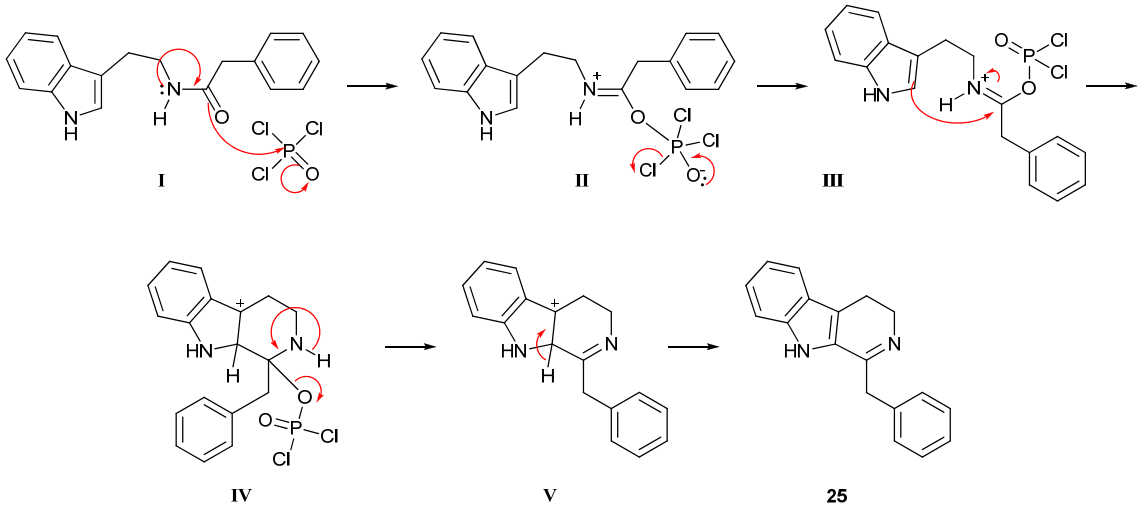
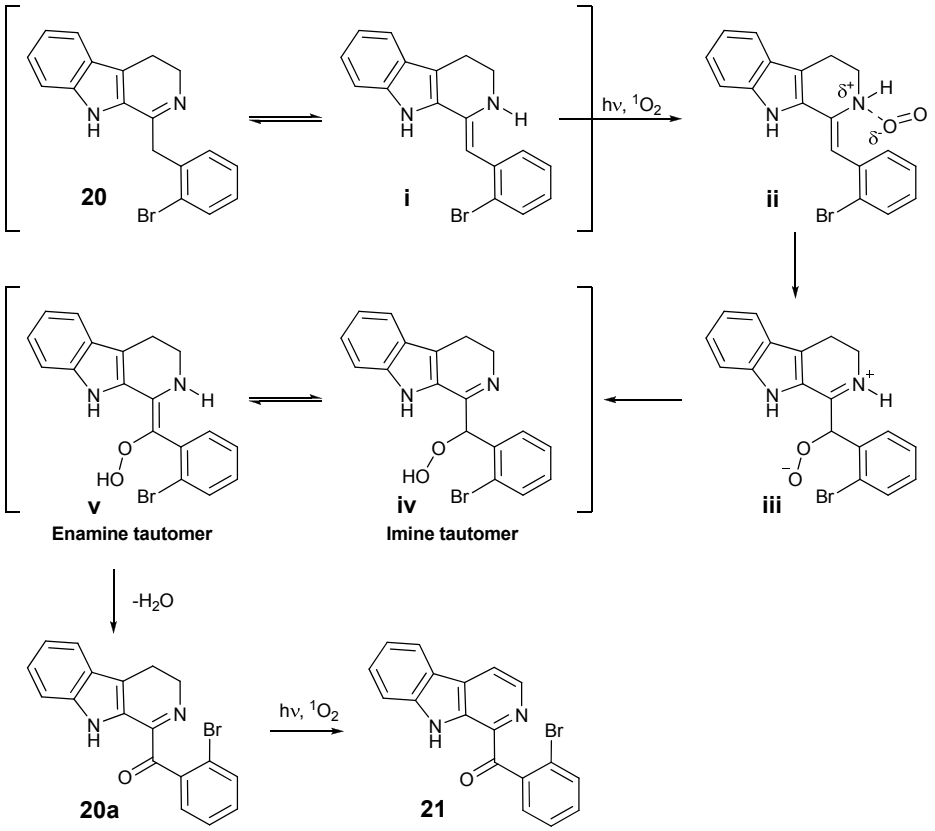


**Figure 2.1** Reaction mechanism of Bischler-Napieralski cyclisation reaction.

*2.2.3 Carbonyl compounds*

At this point the original published work [119] described two possibilities; treatment of **20** with $MnO_2$ in $CHCl_3$ at room temperature affords compound **20a** whilst use of the same reagent and solvent but at reflux produces compound **21**. This observation suggests that there are two different oxidation reactions taking place; the low activation energy partial oxidation of **20** to **20a**, and the higher energy oxidation of **20a** to **21**. However, during the synthesis of compounds **20** and **25**, it was noted that both compounds were slightly unstable. After being stored in air at room temperature an NMR sample would accumulate traces of another compounds. Further work by another member of the Jenkins research group, Dr Marcos García, has identified a photo-oxidation reaction taking place, whereby **20** was spontaneously oxidising to **20a** and **21** without the addition of $MnO_2$. The proposed mechanism for this novel regioselective photo-oxidation is shown in scheme 2.4. [125] By careful control of temperature during a photo-oxidation reaction, achieved simply by modifying the distance between the light source and the reaction vessel, Dr García has shown it possible to isolate either **20a** or **21** from a solution of **20** in toluene.




**Scheme 2.4** Proposed mechanism for the self-sensitized photo-oxidation of **20**. [125]

The proposed mechanism for the photo-oxidation reaction is outlined in scheme 2.4. Initially a transfer of charge from the enamine-tautomer **i** to singlet oxygen forms a charge transfer complex **ii**, this would rapidly collapse to the zwitter ionic peroxide **iii**. Intramolecular proton transfer would then lead to the formation of the tautomeric peroxides i**v** and **v**, dehydration would then take place to yield the iminoketone **20a**. A final photodehydrogenation would then give the aromatic **21**. The final photodehydrogenation step has a higher activation energy than the oxidation process, hence the ability to isolate virtually pure **20a** by maintaining the reaction mixture at room temperature.

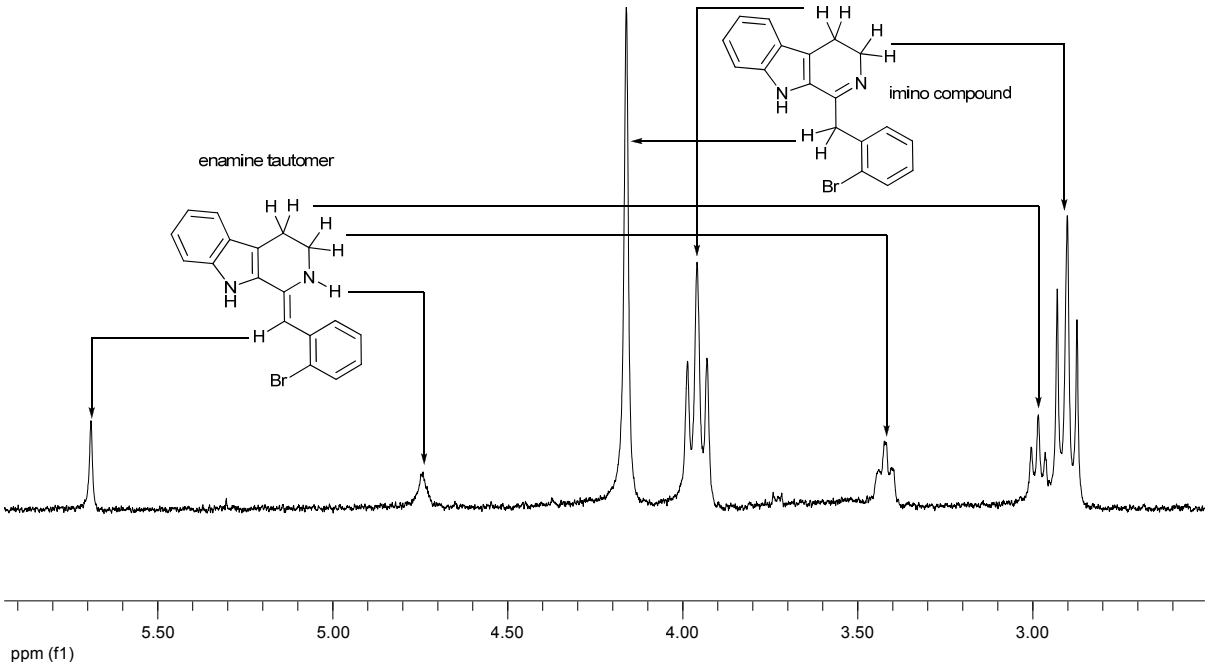



**Figure 2.2** Aliphatic region of the $^{1}$H NMR of **20**, (300 MHz; $CDCl_3$; TMS) [125]

Figure 2.2 shows the aliphatic region of the $^{1}$H NMR of compound **20**. From the integrals we can see that the imino compound and enamine tautomer exist in a ratio of 1:0.3.

This tautomerisation is temperature dependent, variable temperature $^{1}$H NMR in DMSO (300 – 373 K) shows an increasing proportion of the enamine tautomer with increasing temperature, at 300 K the ratio of imino compound to enamine tautomer is 1.0:0.35, at 373 K the proportion of the two forms has increased to 1:0.68. Moving to $CDCl_3$ and low temperature (218 – 300 K) also shows an increasing proportion of the enamine tautomer at higher temperatures. At 300 K, in either solvent system, approximately 20-25 % of the molecules of **20** will exist as the enamine tautomer, this raises to approximately 25-30 % at 60 $^{o}$C, the temperature required for the second stage of the reaction.

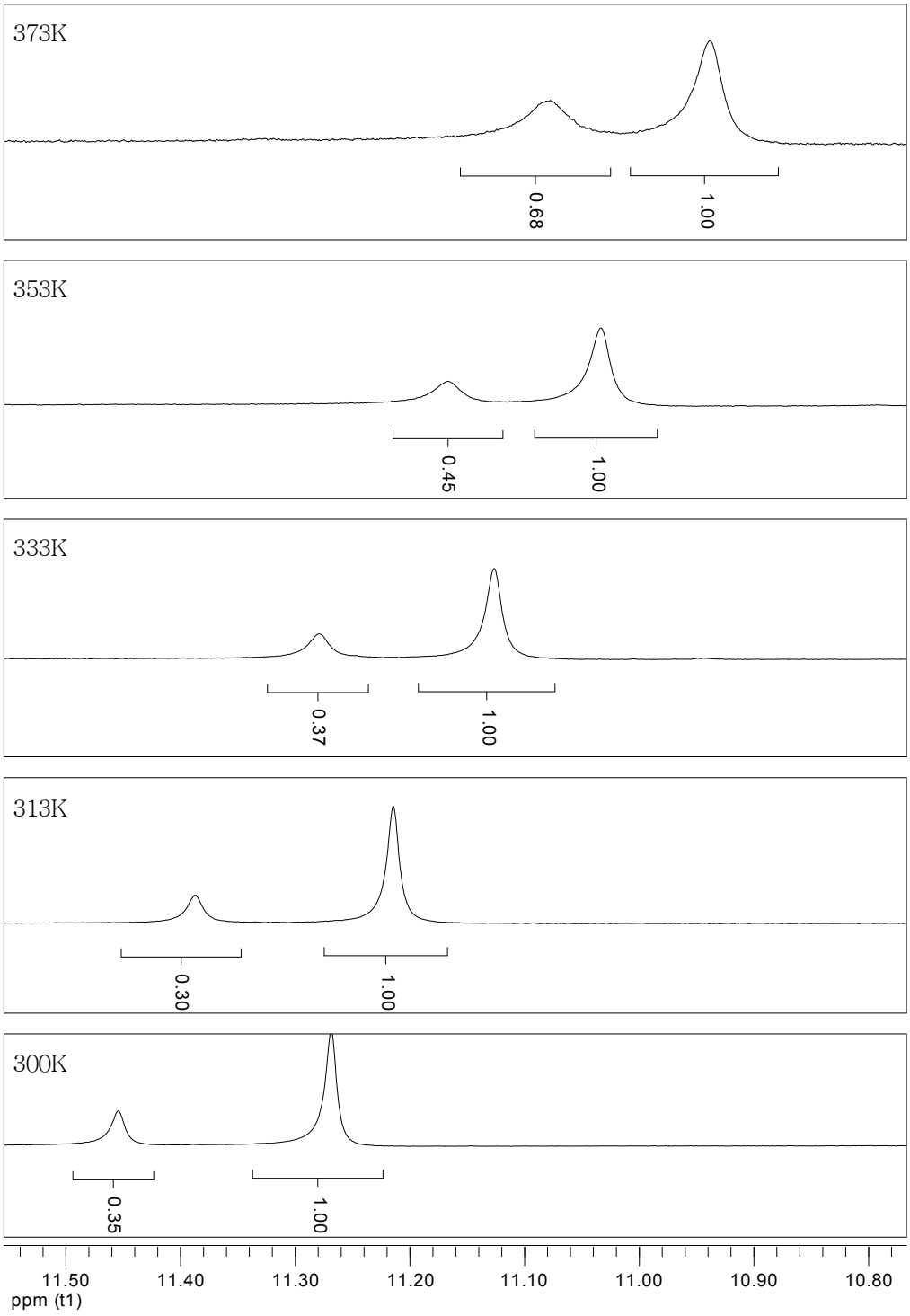



**Figure 2.3** Compound **20** portion of the $^{1}$H NMR spectrum showing the indole NH signals at variable temperature, (400 MHz, DMSO). Spectra courtesy of Dr Marcos Garcia.

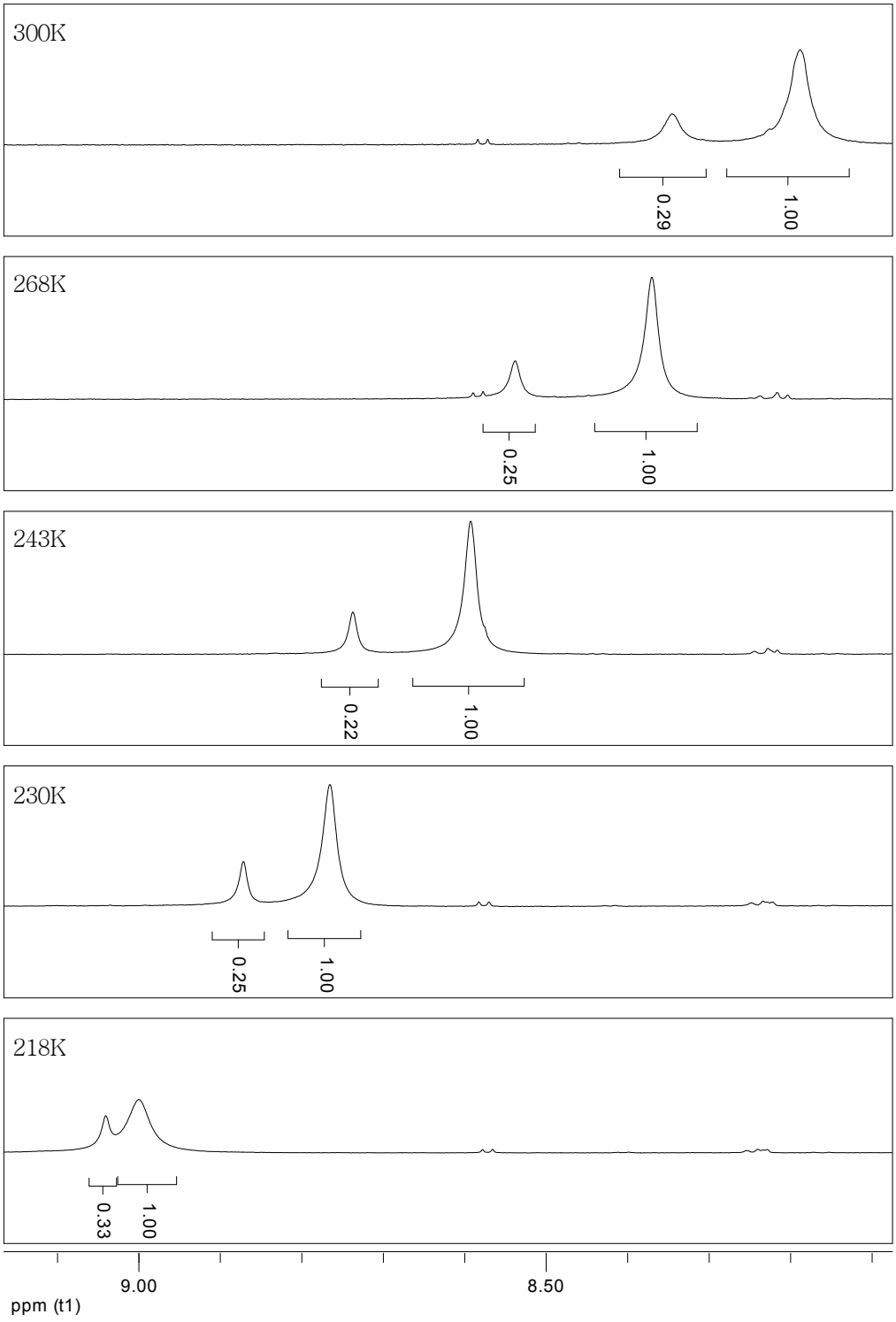
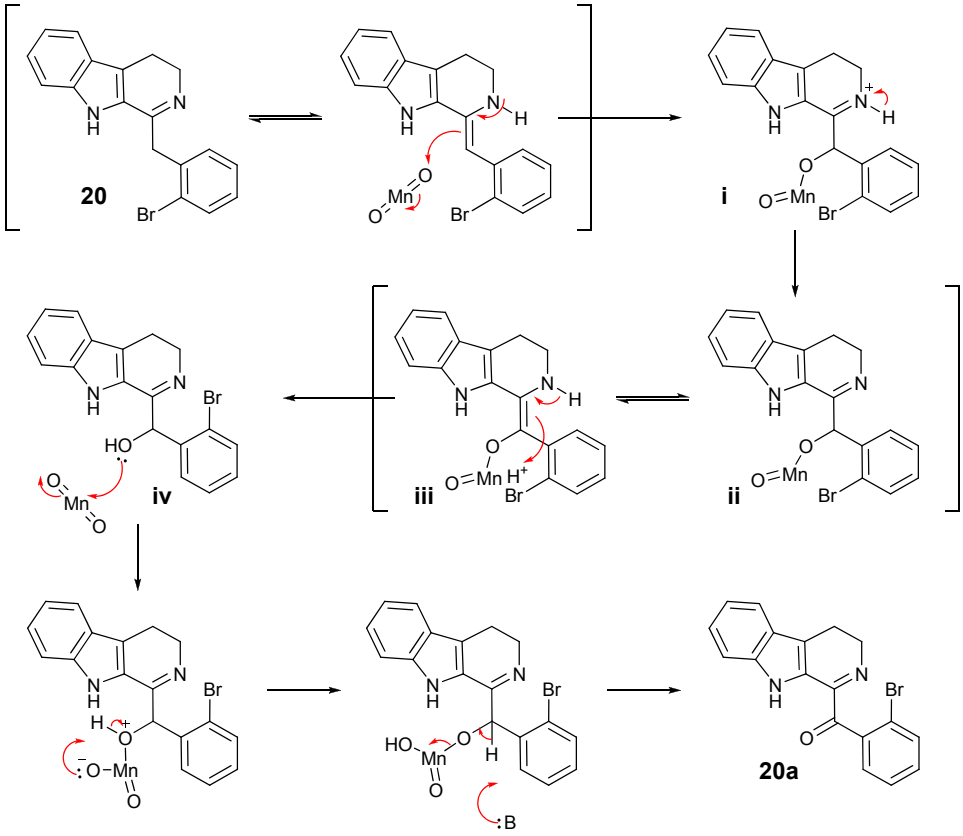



**Figure 2.4** Compound **20** portion of the $^{1}H$ NMR spectrum showing the indole NH signals at variable temperature, (400 MHz, $CDCl_3$). Spectra courtesy of Dr Marcos Garcia.

The mechanism for the $MnO_2$ oxidation of **20** to **20a** is most likely to follow a similar route although without the formation of a charge transfer complex (Scheme 2.5). $MnO_2$ attacks the enamine tautomer of **20** to form the protonated intermediate **i** which upon deprotonation forms **ii** which can again undergo tautomerisation. The enamine tautomer **iii** can then undergo intramolecular proton transfer to form the intermediate alcohol **iv**. A second oxidation process with another molecule of $MnO_2$ then takes the alcohol through to the ketone **20a**.




**Scheme 2.5** Proposed mechanisms for the two step $MnO_2$ oxidation of **20**.

A third equivalent of $MnO_2$ is then required to de-hydrogenate **20a** to the aromatic **21**. Both compounds **20** and **20a** were prepared as outlined above and purified by flash column chromatography prior to biological evaluation.

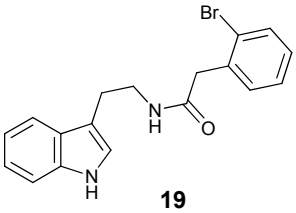
*2.2.4 Fascaplysin*

The final step in the published synthesis of fascaplysin **8** requires the precursor molecule **21** to be heated to 220 $^{o}$C for 20 minutes; this high temperature initiates an intra-molecular aromatic substitution mechanism that displaces the bromine atom as bromide. The bromide is captured as the counter-ion to the positive charge formed on the quaternary nitrogen, this must be exchanged for chloride to give the natural product fascaplysin **8**.

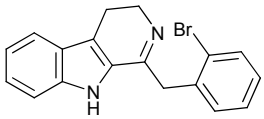
Initially the reaction was trialled on a very small scale using a melting point apparatus. For larger quantities **21** was added to a pear-shaped flask fitted with an air condenser, which was then lowered into a pre-heated oil bath. The condenser was necessary to trap a small quantity of **21** which sublimed before the melting point was reached. Ion exchange with HCl in dry MeOH then produced a crude sample of fascaplysin **8**, however purification of this crude product proved extremely difficult. A better method is to take the crude sample before ion exchange, which contains fascaplysin with a bromine counterion, and wash the sample with warm dry DCM, unreacted **21** is sparingly soluble in warm DCM whilst fascaplysin is not. The DCM is decanted and the procedure repeated until the DCM becomes clear. The crude fascaplysin is then dissolved in anhydrous MeOH and perfused with $HCl_{(g)}$ for two minutes, the MeOH can then be removed to give a dark red solid film, upon trituration with hexane this becomes a dark red solid, fascaplysin **8**. Confirmation of the structure was by MS.

*2.2.5 Activity of Fascaplysin precursor molecules*

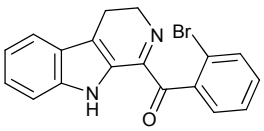
Compounds **19**, **20**, **20a** and **21** were tested for activity against CDK4/Cyclin D1 and CDK2/Cyclin A (Table 2.1). See section 5.2.1 for a detailed description of the assay.



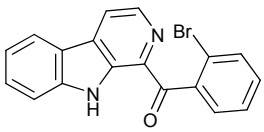

**19**




**20**




**20a**




**21**

| | $IC_{50}$ in vitro inhibition (μM) | |
|---|---|---|
| Structure | CDK4/Cyclin D1 | CDK2/Cyclin A |
| **19** | 150 ± 9 | 1210 ± 15 |
| **20** | 230 ± 10 | 1030 ± 16 |
| **20a** | 11 ± 2 | 818 ± 16 |
| **21** | 14 ± 1 | 940 ± 12 |

**Table 2.1** CDK4/Cyclin D1 vs. CDK2/Cyclin A activity of compounds **19**, **20**, **20a** and **21**.

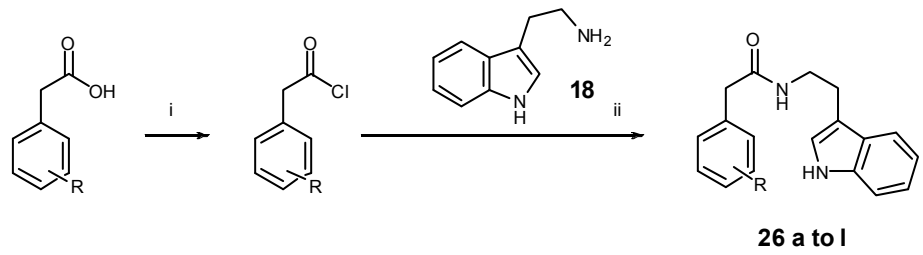
β-Carboline based compounds **20a** and **21** show activity against CDK4/Cyclin D1 of 11 and 14 μM respectively, they are both approximately 20 fold less active than fascaplysin itself ($IC_{50}$ CDK4/Cyclin D1 0.35 μM), yet still retain selectivity over CDK2/Cyclin A. Compound **20**, which lacks the ketone functionality of fascaplysin and compounds **20a** and **21**, shows greatly reduced activity ($IC_{50}$ CDK4/Cyclin D1 230 μM).

Interestingly the simpler tryptamine amide derivative **19** shows increased activity of 150 μM when compared to **20**. Potentially the oxygen atom in the amide can engage in hydrogen bonding interaction that was not available in compound **20**.

## 2.3 Tryptamine derivatives

The increased activity of amide **19** is perhaps to be expected, due to its structural similarity to the previously reported inhibitors **16a** and **16b**. To investigate the activity of structures of this type a series of analogues based on the fascaplysin intermediate amide **19** were synthesised. Previous molecular modelling had suggested that the unsubstituted **16** would adopt a similar orientation in the active site of CDK4/Cyclin D1 to that observed when fascaplysin itself was docked into the model, so compound **16** was also synthesised as part of the sequence.

### *2.3.1 Synthesis of tryptamine amides*



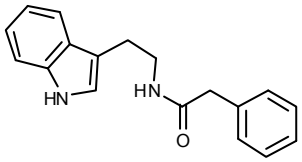

**Scheme 2.3** Synthesis of a range of tryptamine based analogues of fascaplysin **8**.
R = *o, m* and *p* – F, Br, Cl.
*Reagents and conditions*: (i) $SOCl_2$, reflux, 30 minutes; (ii) DCM, $NaOH_{(aq)}$, r.t., 3 hrs.

A range of commercially available phenyl acetic acids were converted to their corresponding acid chlorides by treatment with thionyl chloride, these were then reacted with tryptamine under basic conditions to furnish compounds **26a-l**. (Scheme 2.3) Column chromatography from hexanes and ethyl acetate followed by recrystalisation from ethanol afforded the compounds **26a-l** in yields of 48-83 %.

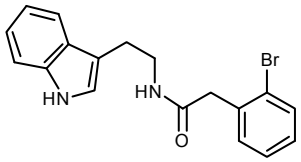
### *2.3.2 Biological activity of the tryptamine amide analogues*

All the compounds **16**, **19** and **26a-h** show activity against CDK4/Cyclin D1 and maintain selectivity over CDK2/Cyclin A. The unsubstituted **16b** shows similar activity to many of the

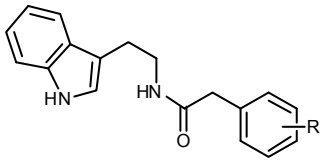
other compounds with $IC_{50}$ 140-170 μM, however compounds **26b** and **26f** stand out as being more active than the rest with $IC_{50}$ values of 65 μM and 51 μM respectively.




**16b**




**19**



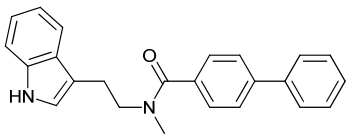

**26 a to l**

| Compound | R group | Yield | $IC_{50}$ in vitro inhibition (μM) | |
|---|---|---|---|---|
| | | | CDK4/Cyclin D1 | CDK2/Cyclin A |
| **16b** | H | 60 | 164 ± 6 | 975 ± 12 |
| **26a** | *o*-F | 48 | 176 ± 11 | 1025 ± 17 |
| **26b** | *m*-F | 72 | 65 ± 4 | 936 ± 13 |
| **26c** | *p*-F | 61 | 108 ± 5 | 1250 ± 18 |
| **26d** | *o*-Cl | 83 | 170 ± 9 | 1225 ± 19 |
| **26e** | *m*-Cl | 73 | 142 ± 5 | 1124 ± 20 |
| **26f** | *p*-Cl | 60 | 51 ± 3 | 937 ± 5 |
| **19** | *o*-Br | 49 | 150 ± 9 | 1210 ± 15 |
| **26g** | *p*-Br | 79 | 148 ± 8 | 1070 ± 15 |

**Table 2.2** Activity of compounds **16**, **19** and **26a-g**, $IC_{50}$ values in μM.

The *meta*-fluoro substituted compound **26b** has an $IC_{50}$ of 65 μM and the *para*-chloro compound **26f** has an $IC_{50}$ of 51 μM, both compounds are significantly more active than the parent unsubstituted compound **16**. This effect is unlikely to be due solely to the electronic properties of the substituent atom as the position of substitution appears critical, the *ortho*-fluoro **26a** and *para*-fluoro **26c** both have lower activity than the *meta*-fluoro **26b**. Neither can the effect be due simply to the steric effects, as the position of the substituent producing the highest activity varies. One possibility is that the addition of a substituent atom to the phenyl ring of **16b** is altering the lowest energy conformation of the molecule and making a favourable binding interaction more likely.

Parallel work in the Jenkins group, by Dr Carine Aubry, had been examining a series of compounds following on from the simplification of fascaplysin **8.** This work which had earlier produced the first non-planar analogues of fascaplysin, compounds **15a-c**, then produced a closely related series of compounds, including compound **27** which had an $IC_{50}$ for the inhibition of CDK4/Cyclin D1 of 6.2 μmol. [106]




**27**

As this new compound shared many of the structural features of the tryptamine amide inhibitors **16**, **19** and **26a-g**, but had the lowest $IC_{50}$ of any compound produced within the group to date, it was decided to move the focus of this work more in line with that of Dr Aubrey, who was due to leave the group at that time.

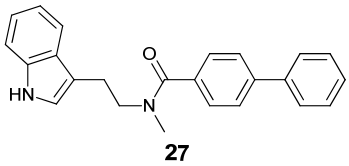
# Chapter Three Biphenyl Inhibitors

*"Could the search for ultimate truth really have revealed so hideous and visceral looking an object?"*

*Max Perutz 1964*

## 3.1 Bi-phenyl lead compound CA224

Related work in the group by Dr Carine Aubry [106] had identified compound **27** (Published as compound CA-224) as a potent selective inhibitor of CDK4/Cyclin D1 ($IC_{50}$ 6.2 μM) over CDK2/Cyclin A ($IC_{50}$ 521 μM), [106] the observed $IC_{50}$ value produced being nearly an order of magnitude lower than any other compound produced by the Jenkins group to date. Molecular modelling by Dr Aubry has suggested that **27** to bind to the same region of the active site as Fascaplysin **8** and compounds **16a** and **16b**. (Figure 3.1)




**27**

**Figure 3.1** Compound CA224 **27**.

Compound **27** only differs from **16a** by the addition of one extra phenyl ring and the removal of a $CH_2$ space, yet the $IC_{50}$ value for the inhibition of CDK4/Cyclin D1 has improved by more than one order of magnitude from 68 μM to 6.2 μM. If we examine the molecular modelling, there are two potential contributory factors. The addition of the second phenyl ring may probe deeper into the proposed π-π stacking pocket of Phe 93 and Phe 159 and that the improved inhibitory activity is due to the second hydrogen bond which has formed between **27** and the backbone of CDK4. Homology model docking of fascaplysin **8** shows two hydrogen bonds to Val 96, there is no such observed interaction for **16a**; however it is again present in compound **27**.

In order to better understand and potentially improve on this activity it was decided to synthesise a range of analogues based on the bi-phenyl structure of **27** by changing the connection of the two phenyl rings from *para* to *ortho* and *meta* and also by introducing substitution onto the second phenyl ring. Similar modifications to compound **16a** had already produced a small series of inhibitors **19** and **26a-h**, some of which showed improved activity when compared to the parent compound. Compounds bearing these modifications would be synthesised both with and without the *N*-ω-methyl group as there is no discernable preference for either.

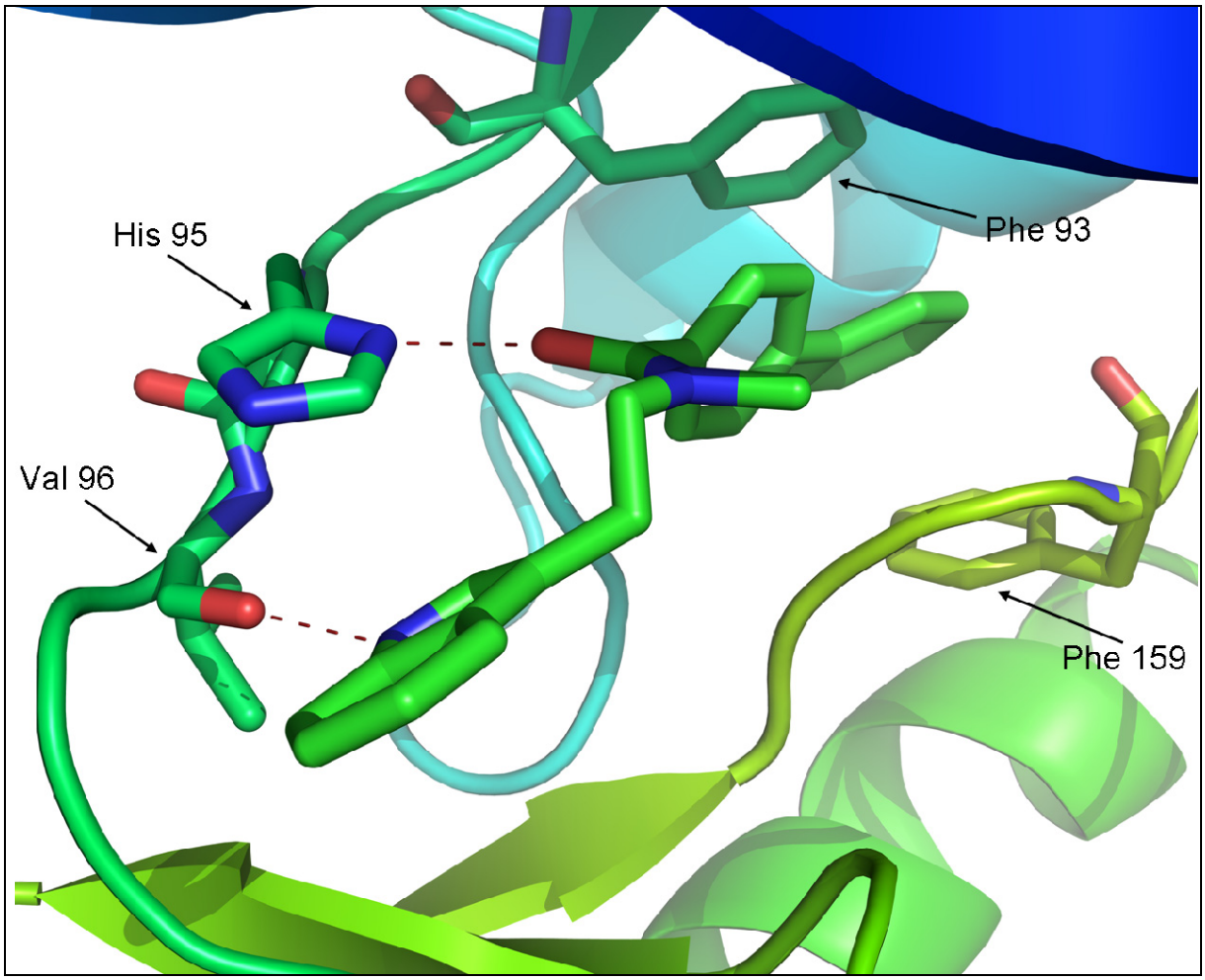
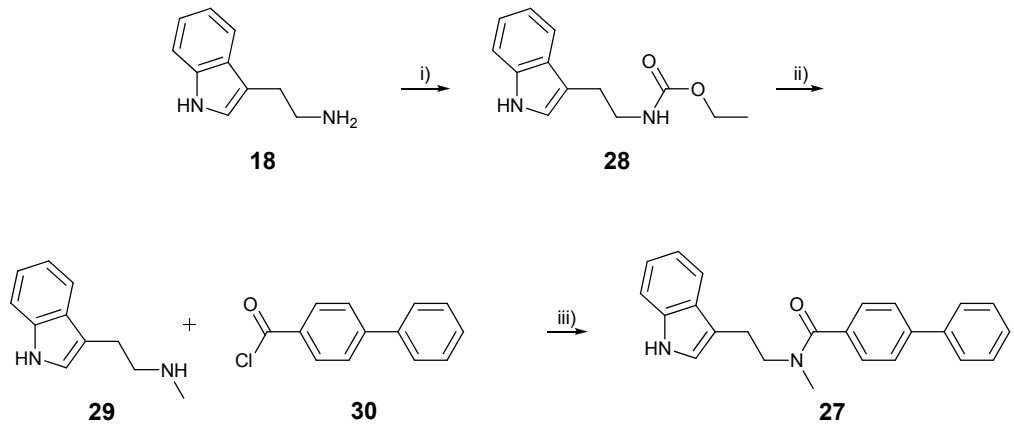



**Figure 3.2** Compound CA-224 **27** docked to the active site of the CDK4/Cyclin D1 homology model. [106] Likely hydrogen bonding interactions shown as red dotted lines.

### *3.1.1 Original synthetic route to CA224*

The previously utilised synthetic route to **27**, which utilised the reaction of methyl tryptamine **29** with 4-biphenyl carbonyl chloride **30** (Scheme 3.1) was not a suitable route to substituted analogues, as no substituted biphenyl carbonyl chlorides were commercially available.




**Scheme 3.1** Original synthetic route to CA-224 **27**.
i) $CH_3CH_2OCOCl$, DCM, $NaOH_{(aq)}$, $N_2$, 3hrs. ii) $LiAlH_4$, THF, $Et_2O$, iii) DCM, $NaOH_{(aq)}$, $N_2$.

## 3.2 Alternate route to CA224 utilising the Suzuki Coupling Reaction

Retro-synthetic analysis of CA-224 **27** (Figure 3.3) proposed a synthetic route utilising a 'Suzuki-coupling' [110] between a brominated intermediate **31**, which could be prepared easily using the tryptamine and acid-chloride reaction exploited in previous sections, and a commercially available boronic acid **33**.

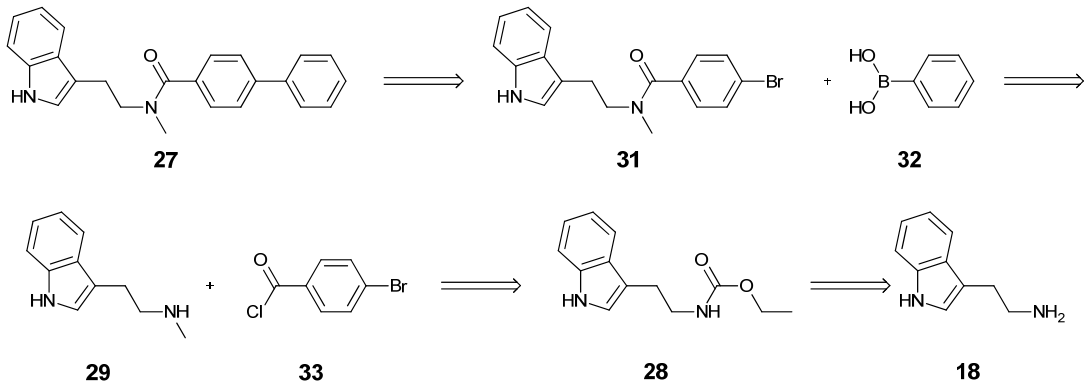



**Figure 3.3** Retro-synthetic analysis of **27**

The principal advantage of this route over the originally published route being that a common brominated intermediate **31** could be prepared in bulk and then treated with a large range of different commercial boronic acids to produce different analogues. Compound **31** has previously been synthesised, using this route, within the research group and is itself an inhibitor of CDK4/Cyclin D1. [106]

*3.1.1 Scope of the Suzuki reaction.*

The Suzuki coupling reaction [126-128] is an incredibly versatile reaction that creates a new carbon carbon bond between an organoborane [129] and an organic halide using a palladium catalyst [130] a base and optionally a co-ligand. [131, 132] Typically the organic halide is either a bromide or chloride, [133] although reactions using iodides have also been reported, [134] pseudo halides such as triflates have also been extensively used. Originally the organoborane species was a boronic acid however the scope of the reaction has been increased to also utilise boronate esters, organoboranes and organotrifluoroborates. [135] There have been many reviews [136-138] of the reaction and its application to the synthesis of a range of complex natural products, antibiotics [139] and other targets of interest.

Suzuki-coupling reactions can be performed in a wide range of organic solvents and water, many different bases have been used and the reaction has also utilised microwave heating. [140] The reaction is not limited to the synthesis of simple by-phenyl compounds, it is also applicable to the synthesis of alkene and alkane chains.

*3.1.2 Mechanism of the Suzuki-Coupling reaction.*

The mechanism of the Suzuki-coupling reaction is represented in figure 3.4, it comprises of three main steps; oxidative addition of an organic halide to palladium (0), transmetallation and the reductive elimination which forms the product and reforms the active catalyst.

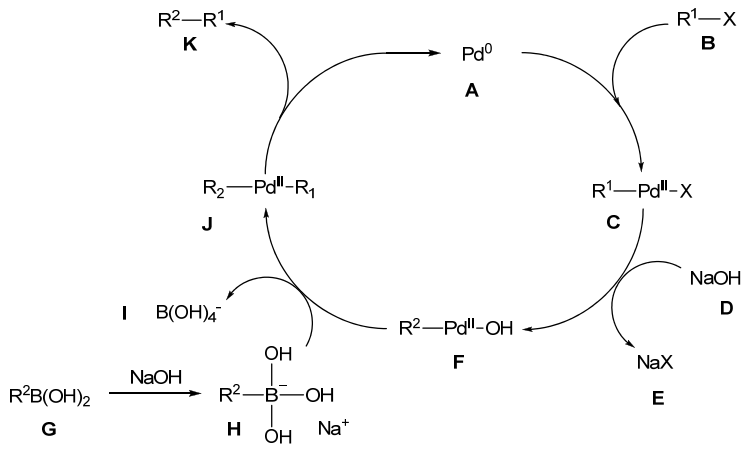



**Figure 3.4** Mechanism of the Suzuki-Coupling reaction.

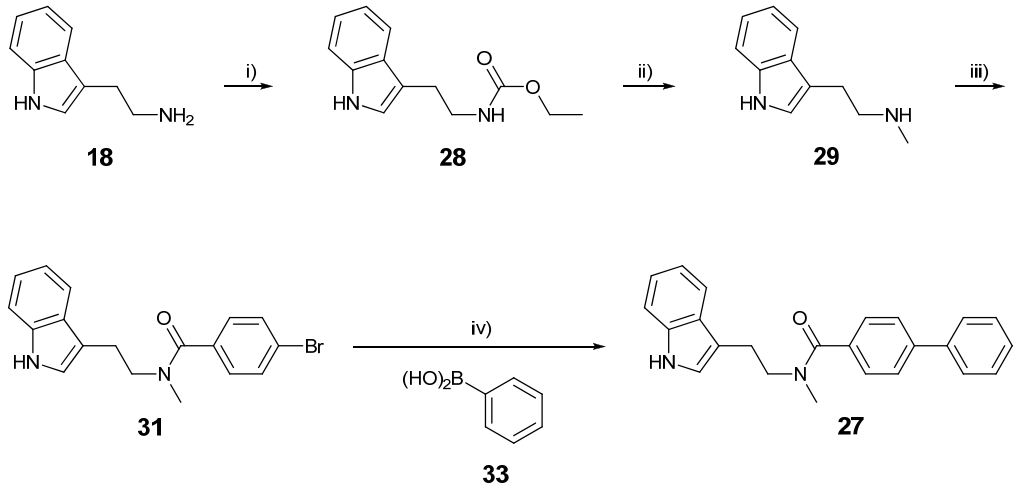
Oxidative addition [141, 142] of the aryl halide **B** to $Pd^0$ **A** generates a *trans*-σ-palladium $^{(II)}$ complex **C** which is stable and has been isolated. Oxidative addition is often the rate determining step in many catalytic cycles and the relative rate of reaction depends on the nature of the halide (or pseudo halide), in general: I > OTf > Br >> Cl.

Reaction of **C** with bases then gives intermediate **F** which is able to undergo transmetallation [143] with the boronate complex **H**. The major difference between the Suzuki-coupling and other similar reactions such as the Stille coupling is that in the Suzuki-coupling the boronic acid **G** must first be activated, for example by base, this activation of the boron atom increases the polarisation of the organic ligand **H**, which drives the transmetallation step.

Following the transmetallation [144] step the final process is one of reductive elimination which generates the desired product **K** and reforms the active catalyst **A**.

### *3.1.3 Method Development: Resynthesis of CA224*

Tryptamine **18** was reacted with ethylchloroformate to produce *N*-ω-ethylcarbayl-tryptamine **28** in 95 % yield, which was then reduced with lithium aluminium hydride to produce *N*-ω-methyl tryptamine **29** in 89 % yield. Treatment with *para*-bromobenzoyl chloride **33,** gave the key brominated intermediate **31** in 73 % yield. [106]



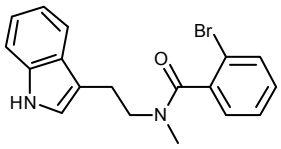

**Scheme 3.2** Alternative synthesis of **27** via a 'Suzuki-coupling' route.
i) $ClCO_2C_2H_5$, DCM, $NaOH_{(aq)}$, $N_2$, 3hrs. ii) $LiAlH_4$, THF, $Et_2O$, iii) *p*-$BrC_6H_4COCl$, DCM, $NaOH_{(aq)}$, $N_2$. iv) $Pd(PPh_3)_4$, toluene, EtOH, $K_2CO_{3(aq)}$.

A Suzuki coupling reaction with phenyl boronic acid **33**, carried out in toluene and ethanol under an atmosphere of $N_2$ using 5 mol % $Pd(PPh_3)_4$ as the catalyst and aqueous potassium carbonate as the base yielded the desired product **27** in 47 % yield.

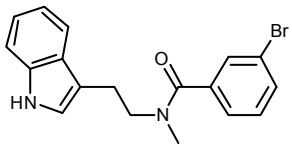
The alternate use of 2- and 3-bromobenzoyl chloride in place of 4-bromobenzoyl chloride in step iii) (Scheme 3.2) should allow the second phenyl ring to be connected *ortho*, *meta* or *para* relative to the tryptamine fragment, as observed previously the pattern of connection of a substituent to a phenyl ring in this region of the active site can have an impact on the overall activity of the inhibitor.

## 3.3 Simple Bi-phenyl compounds

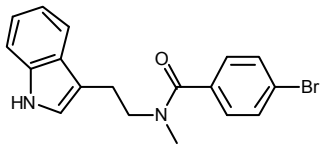
A range of brominated compounds, both with, **31**, **35**, **35** and without, **36**, **37**, **38**, the *N*-ω-methyl group were synthesised from either tryptamine **18** or *N*-ω-methyl tryptamine **28**. Compounds **31**, **34** and **35** have been previously reported by the Jenkins group and are known inhibitors of CDK4/Cyclin D1. Their activities against CDK4/CyclinD1 are reproduced below as a comparison.



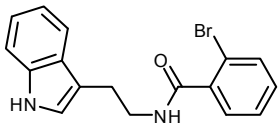

**34**



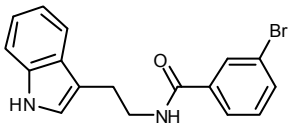

**35**



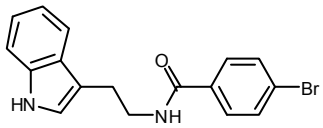

**31**




**36**




**37**

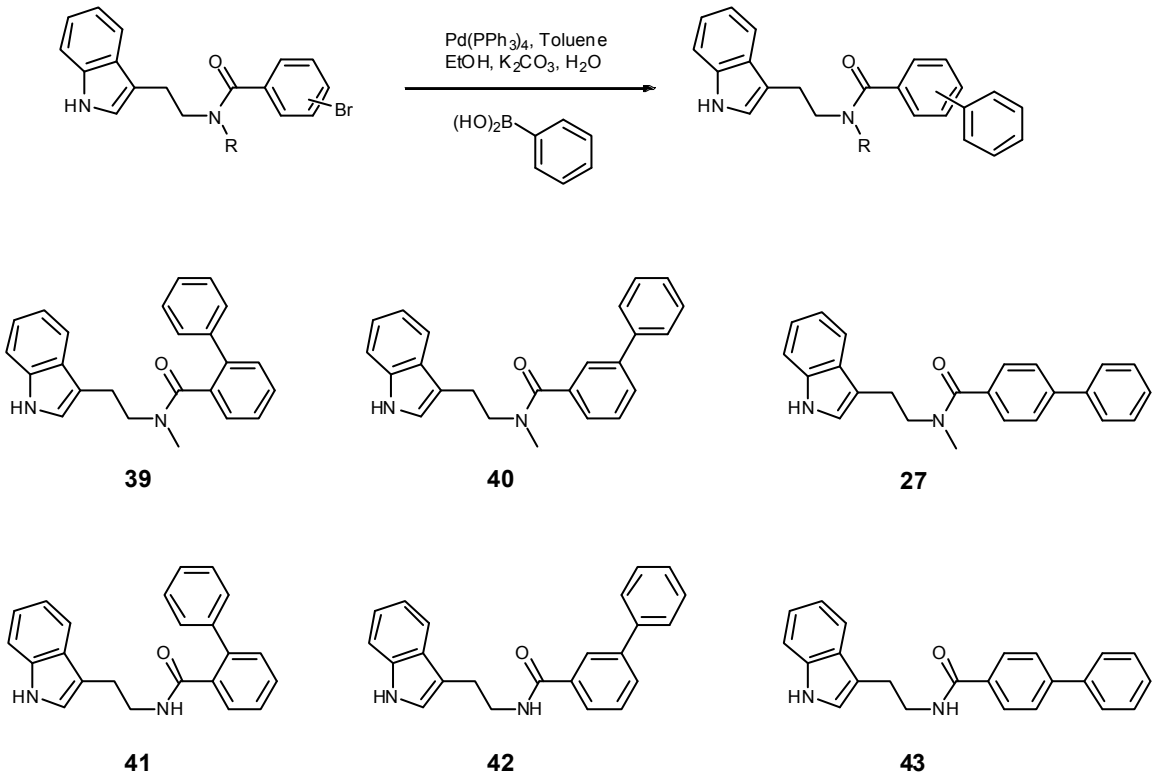



**38**

| Compound | $IC_{50}$ in vitro inhibition (μM) | |
|---|---|---|
| | CDK4/Cyclin D1 | CDK2/Cyclin A |
| **34** | 74 ± 7 | 635 ± 40 |
| **35** | 95 ± 7 | 584 ± 24 |
| **31** | 37 ± 5 | 580 ± 38 |
| **38** | 91 ± 5 | 912 ± 1 |

**Figure 3.5** Biological activity of compounds **34**, **35**, **31** and **38**.

A 'Suzuki-coupling' reaction between each compound and phenyl boronic acid yielded five analogues of **27**. Two isomers with varying geometry at the bi-phenyl link, the *ortho* linked **39** and meta linked **40** and three compounds with the *N*-ω-methyl group replaced by a proton, **41-43**.




**39** **40** **27**

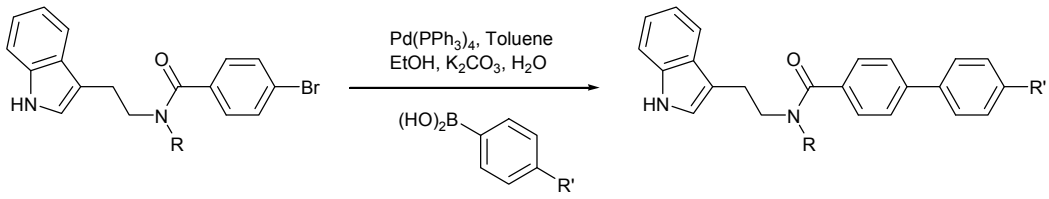
**41** **42** **43**

| Compound | $IC_{50}$ in vitro inhibition (μM) | |
|---|---|---|
| | CDK4/Cyclin D1 | CDK2/Cyclin A |
| **39** | 22 ± 2.7 | 842 ± 11 |
| **41** | 7 ± 7.1 | 775 ± 13.5 |
| **42** | 86 ± 4 | 1002 ± 13 |
| **43** | 16 ± 2.1 | 855 ± 7.5 |

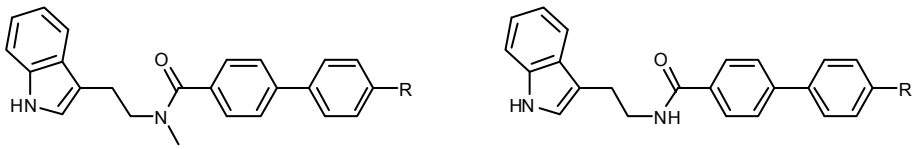
**Scheme 3.3** Synthesis and activity of first generation biphenyl analogues of CA-224 **27**.

## 3.4 Substituted Bi-phenyl compounds

A second series of compounds was synthesised by the reaction of a range of substituted phenyl boronic acids with the brominated intermediates **31** and **38**.




Where R = H or $CH_3$ and R' = $CH_3$, tBu, F or OMe




**44 : R=F**
**45 : R=OMe**
**46 : R=$^t$Bu**
**47: R=$CH_3$**

**48 : R=F**
**49 : R=OMe**
**50 : R=$^t$Bu**
**51 : R=$CH_3$**

| Compound | R | $IC_{50}$ in vitro inhibition (μM) | |
|---|---|---|---|
| | | CDK4/Cyclin D1 | CDK2/Cyclin A |
| **44** | F | 21 ± 3 | 875 ± 9 |
| **45** | OMe | 49 ± 2 | 289 ± 10 |
| **46** | $^t$Bu | 11 ± 1.3 | 745 ± 20 |
| **47** | Me | 24 ± 1.5 | 70 ± 4 |
| **48** | F | 15 ± 1.5 | 764 ± 14 |
| **49** | OMe | 48 ± 3 | 1085 ± 17 |
| **50** | $^t$Bu | 9 ± 0.8 | 790 ± 18 |
| **51** | Me | 18 ± 1.9 | 816 ± 12 |

**Scheme 3.4** Synthesis and biological activity of substituted analogues of lead compound CA224 **27**.

From the biological activities of these eight compounds we can draw the following conclusions. A *para*-methoxy group causes a drop in activity against CDK4/Cyclin 1, whilst the addition of a *tert*-butyl group causes an increase in activity against CDK4/Cyclin D1. This suggests that the Phe-93 pocket of CDK4 is relatively large, in being able to accommodate $^t$Bu, however the introduction of an oxygen atom into this region causes a loss of activity, as would be expected with a hydrophobic pocket. The introduction of an electronegative fluorine atom into this pocket, again does not show a significant improvement in activity, this further supports the existence of a hydrophobic Phe-93 pocket in CDK4, as is observed in CDK2, this hydrophobic pocket may be a valuble structural feature in the design of future inhibitors of CDK4. The change from a tertiary amide in structures **44-47** to a secondary amide in structures **48-51** does not seem to make any significant difference to the activity of the inhibitor.

## 3.5 The Valine 96 'Hinge Region'

By examining the trends observed in the inhibitors detailed above, in conjunction with the wider research undertaken within the Jenkins group at the time, [145] it is possible to start to draw some conclusions and move towards an understanding of the the key binding interactions within the active site of CDK4/Cyclin D1.

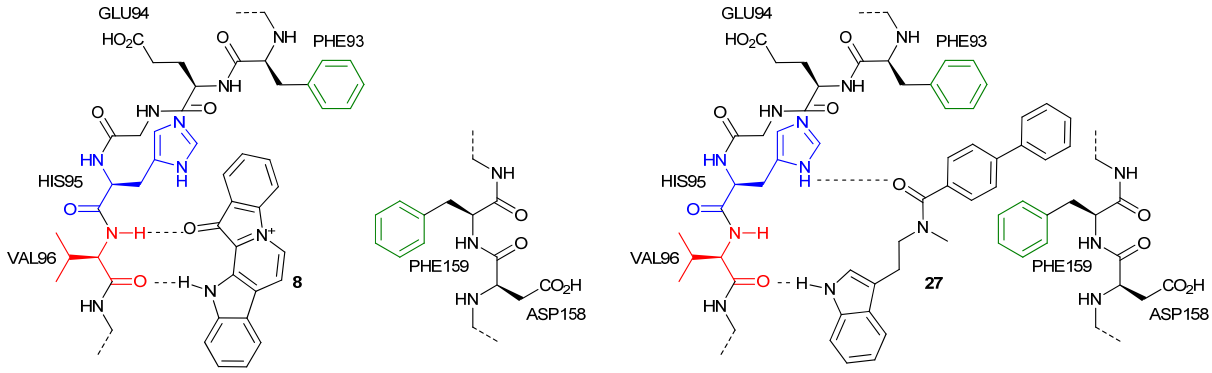



**Figure 3.5** Schematic representation of fascaplysin **8** and CA224 **27** bound to the active site of CDK4/Cyclin D1. Valine 96 in red, histidine 95 in blue, proposed π stacking pocket in green, hydrogen bonds represented by dashed lines.

Earlier molecular modelling studies within the group have postulated the existence of a number of hydrogen bonding interactions involving the valine 96 residue of the enzyme. In the case of fascaplysin **8** the homology model suggests this takes the form of two hydrogen bonds; one bond between the backbone carbonyl atom of valine 96 and the indole NH of fascaplysin and a second hydrogen bond between the carbonyl group of fascaplysin and the backbone NH of valine 96. In the case of compound CA224 **27** the first of these bonds, between the indole NH of the inhibitor and the valine 96 C=O, is retained. However the second hydrogen, bond between the inhibitor carbonyl oxygen atom and the valine 96 backbone NH, has migrated to the histidine aromatic NH group, which is close in space.

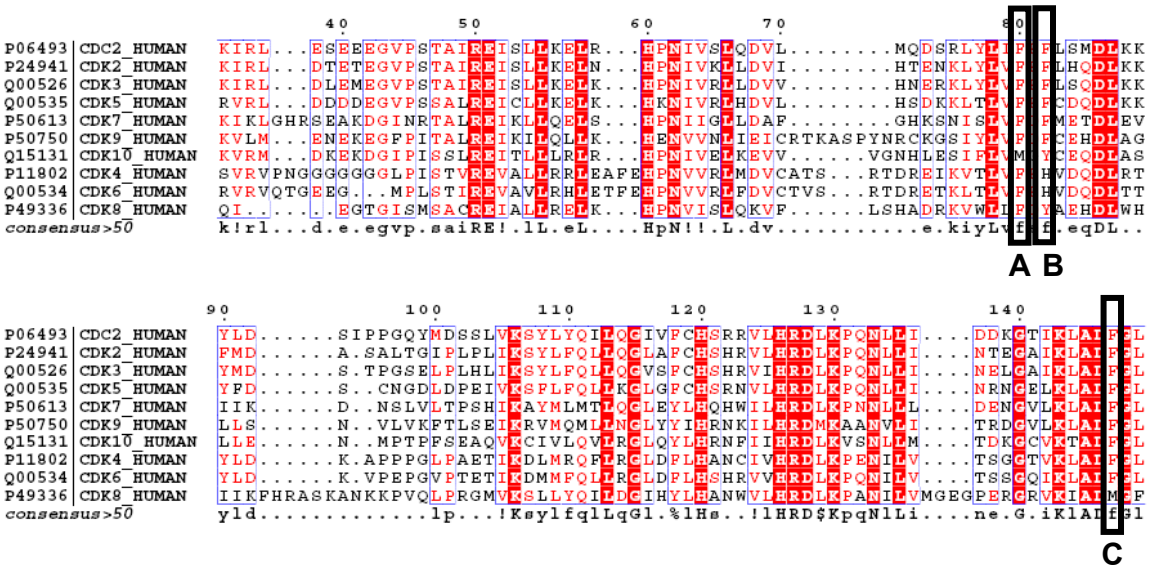
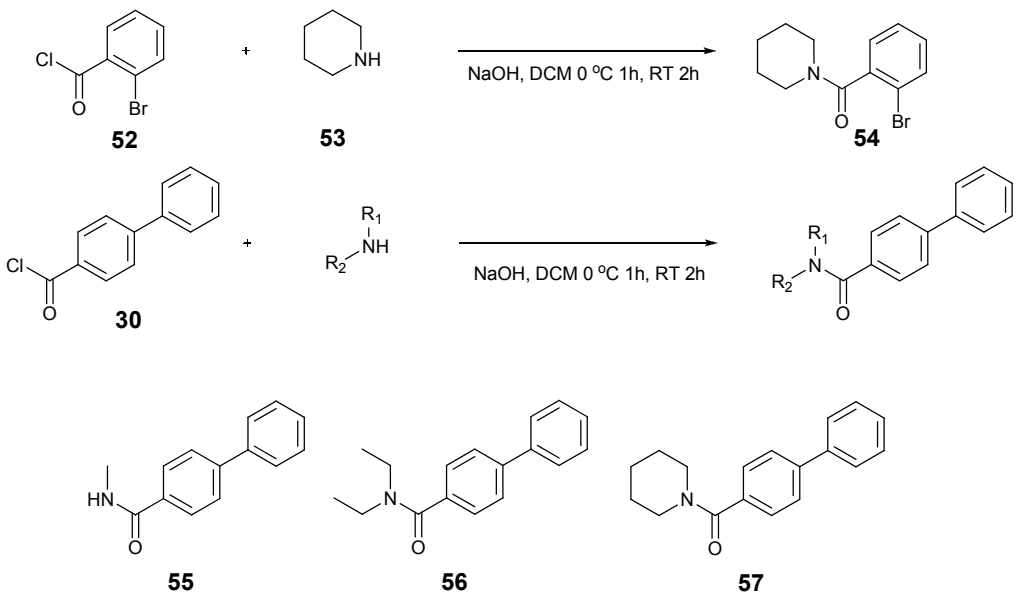



**Figure 3.6** Section of the aligned sequences of human CDKs 1-10. Highlighted areas are the amino acids corresponding to the following residues in CDK4. **A** Phe93, **B** His95, **C** Phe159. See Appendix for full sequence alignment. Produced using MultAlin [146] and ESPRipt 2.2.

Interactions with this hinge region are well documented for the case of cyclin dependent kinase 2. [98] The hinge region of CDK2 is defined as the residues 81-84 (Corresponding to residues 94-97 in CDK4), two hydrogen bonds are formed between the adenosine ring of ATP and CDK2, N1 accepts a hydrogen bond from the backbone nitrogen of Leu83 and N6 donates a hydrogen bond to the backbone carbonyl of Glu81. [147] The Leu83 of CDK2 corresponds to Val96 in CDK4, while the Glu81 of CDK2 is preserved in CDK4 as Glu94.

All CDK2-Inhibitor complex structures solved to date preserve this hydrogen bond to Leu 83, it is therefore likely that a hydrogen bond to Val 96 in CDK4 is equally vital.

To validate the 'requirement' of hydrogen bonding to the hinge region a small number of compounds lacking the required indole ring, but retaining the bi-phenyl motif were synthesised. These compounds were synthesised by reacting biphenyl-4-carbonyl chloride with; methylamine, diethylamine and piperidine. Compound **32** is a simplification of previous inhibitor **24c**, lacking the indole ring and one $CH_2$ group, whilst compounds **31** and **30** more closely mimic the structures of CA224 and a related series of β-carboline based bi-phenyl inhibitors of CDK4 synthesised within the Jenkins group. [145]




52 53 54 30 55 56 57

**Scheme 3.5** Synthesis of a series of inhibitors lacking the tryptamine/indole fragment.

These compounds were tested for inhibition of CDK4 and CDK2 and essentially found to be inactive, no activity was observed for compounds **55-58** over the range of concentrations screened in the biological assay. The inactivity of these compounds adds further evidence to the requirement to maintain the vitally important hydrogen bonding interaction to Val96.

| Compound | Yield | $IC_{50}$ in vitro inhibition (μM) | |
|---|---|---|---|
| | | CDK4/Cyclin D1 | CDK2/Cyclin A |
| **54** | 68.5 % | > 150 | > 1000 |
| **55** | 59.4 % | > 150 | > 1000 |
| **56** | 69.4 % | > 150 | > 1000 |
| **57** | 99.0 % | 145 ± 9 | 950 ± 25 |

**Table 3.1** $IC_{50}$ inhibition values for compounds lacking the indole subunit.

## 3.6 The CDK4 Phe 93 Pocket

Our previous work used a homology model of CDK4/Cyclin D1, which postulated the existence of a π-π stacking interaction between the terminal phenyl ring of CA224 27 and two phenylalanine residues (Phe 93 and Phe 159) within the active site of CDK4/Cyclin D1.

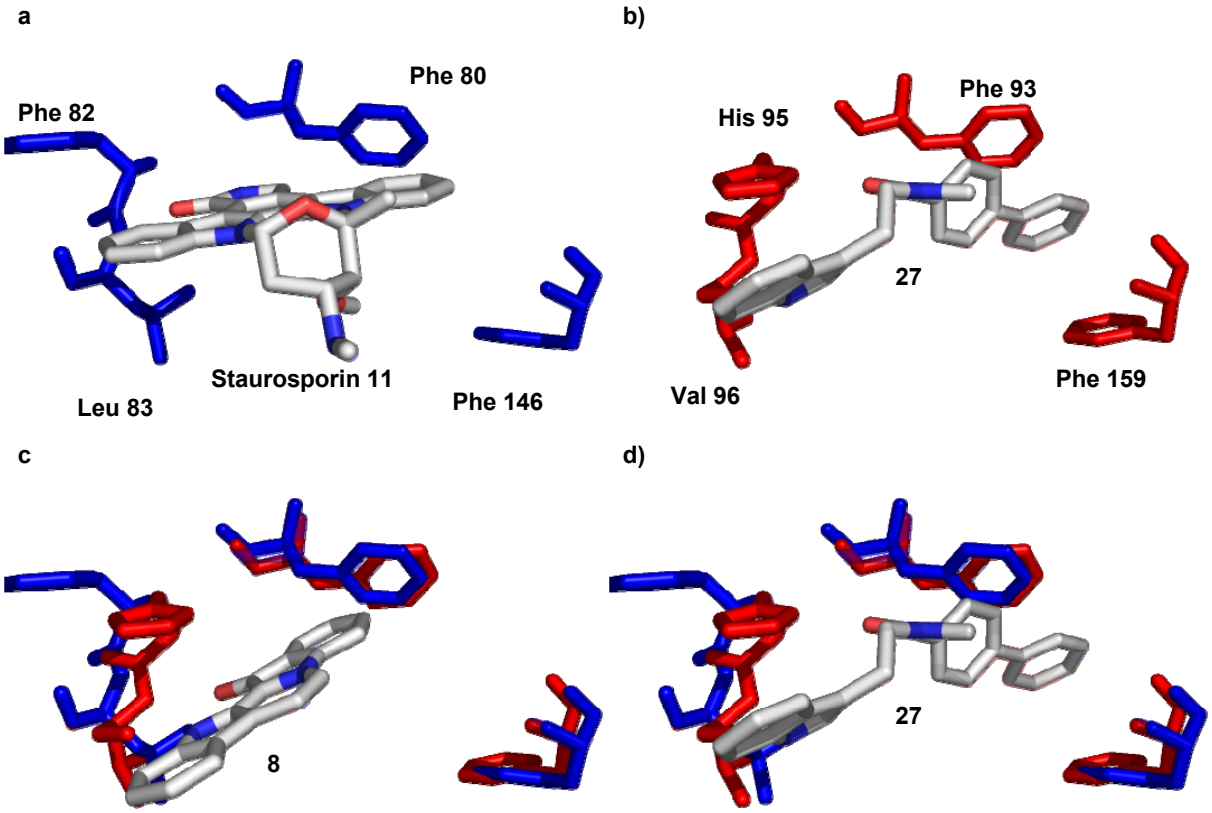



**Figure 3.7** a) X-Ray Crystal structure of CDK2 with bound staurosporine **11**, pdb: 1aq1 [102] b) Homology model of CDK4 with docked CA224. [106] c) Homology model of CDK4 (Red) with docked Fascaplysin **8** overlaid with the structure of CDK2, d) Homology model of CDK4 (Red) with docked CA224 **8** overlaid with the structure of CDK2.

CDK2 is known to contain a shallow cavity towards the back of its ATP binding pocket around the Phe80 residue. [98] The natural substrate ATP does not form any binding interactions with this region of the active site, however many inhibitors of CDK2, including staurosporine **11** and indirubin **10** do. Staurosporine in particular presents the edge of a phenyl ring towards the Phe80 residue.

Figure 3.7 shows: a) the x-ray crystal structure of staurosporine **11** bound to CDK2, [102] one ring of staurosporine can clearly be seen to be packed into the Phe80 pocket; b) the homology

model docking of CA224 into CDK4, [106] with the terminal ring pushed deeper into the corresponding Phe93 pocket; c) and d) show dockings of fascaplysin **8** and CA224 **27** into CDK4 (Red) with the overlaid structure of CDK2. Protein alignment carried out using the 'align' function of PyMol. The Phe80, or gatekeeper residue, in CDK2 is reported to play a role in inhibitor specificity by controlling access to a larger hydrophobic pocket deeper inside the active site. [148, 149]

## 3.7 Further probing the Phe93 Pocket

So far all the synthesised inhibitors which are suspected of exploiting the Phe93 π-stacking or hydrophobic pocket of CDK4 have contained a biphenyl ring system, the outer ring of which is suspected of interacting with the two phenylalanine residues 93 and 159. To explore the depth and specificity of this potential new binding pocket two new pharmacaphores were devised.

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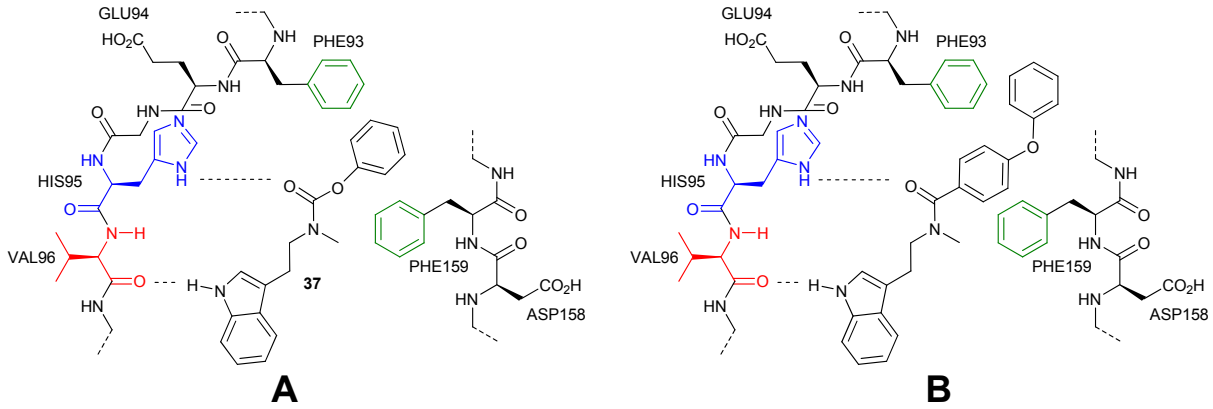
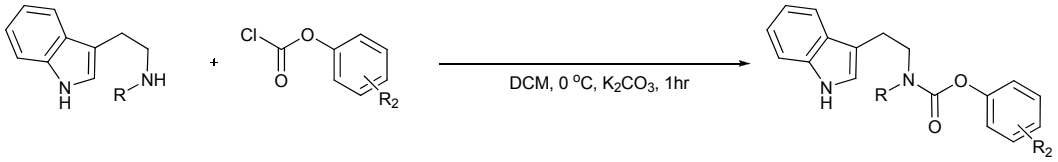
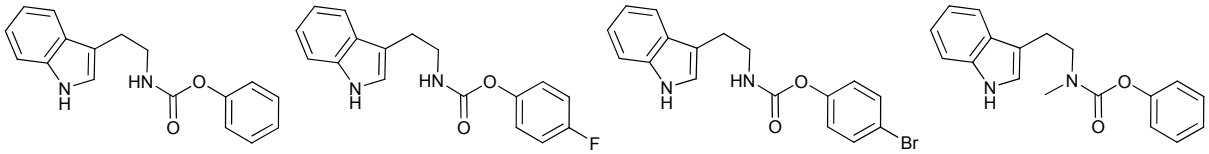



**Figure 3.8** Rational for new structures to probe the Phe93 pocket of CDK4.

The first new pharmacophore (Figure 3.8 **A**) results from substituting a oxygen atom in place of the $CH_2$ group linking the amide and phenyl regions of compounds **16a**, **19** and **26a-l**, the effect being to alter the position of the phenyl ring relative to the amide C=O group. This retains both the indole NH group and amide oxygen atom which are required to maintain the double hydrogen bond to Val96 and His95.

These compounds were synthesised by the reaction of either tryptamine **18** or methyl tryptamine **28** with 1.2 equivalents of substituted phenyl chloroformate in DCM at 0 $^{o}$C for one hour.



**58** **59** **60** **61**

| Compound | Yield | $IC_{50}$ in vitro inhibition (μM) | |
|---|---|---|---|
| | | CDK4/Cyclin D1 | CDK2/Cyclin A |
| **58** | 79.2 % | 76 ± 4.5 | 700 ± 2 |
| **59** | 88.8 % | 61 ± 3.5 | 540 ± 20 |
| **60** | 92.4 % | 54 ± 4.0 | 765 ± 15 |
| **60** | 59.8 % | 72 ± 6.0 | 590 ± 14 |

**Scheme 3.6** Synthesis and biological activity of Urethane based inhibitors.

Comparing the activity of these compounds with the related compounds **26a-l** we see that they have similar activity against CDK4/Cyclin D1 (**26a-l** $IC_{50}$ in the range 51-176 μM) but marginally increased activity towards CDK2/Cyclin A (**26a-l** $IC_{50}$ in the range 936-1250 μM). The compounds retain approximately ten fold selectivity for CDK4/Cyclin D1 over CDK2/Cyclin A, but do not show the increased activity observed in the bi-phenyl series of inhibitors, the most likely explanation for this being that the phenyl ring does not approach the area of the Phe93 pocket.

## 3.8 Biaryl ethers and biaryl amines

The second new pharmacophore (Figure 3.8 **B**) was rationalised by the insertion of a linker group between the two rings of the bi-phenyl unit, which will the position of the outer ring relative to the rest of the molecule. The two linker groups chosen were O and NH, as these should be relatively simple to introduce utilising palladium catalysed coupling reactions.

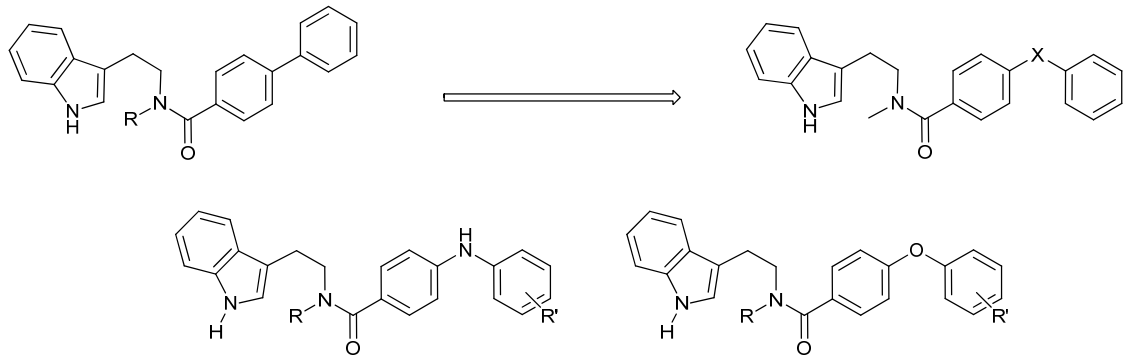



**Figure 3.8** Rationalisation of a second new pharmacophore to probe the Phe93 pocket.

*3.8.1 Bi-aryl amines*

The Hartwig-Buchwald reaction involves the reaction of an aryl halide and an amine in the presence of a palladium catalyst and a base, during the reaction a new carbon-nitrogen bond is formed. The advantage of this method of synthesising bi-aryl amines is that the required starting materials have already been prepared, the six brominated compounds **31**, **34**, **35**, **36**, **37** and **38**, previously synthesized can be subjected to a Hartwig-Buchwald coupling with anilines to produce a new series of compounds.

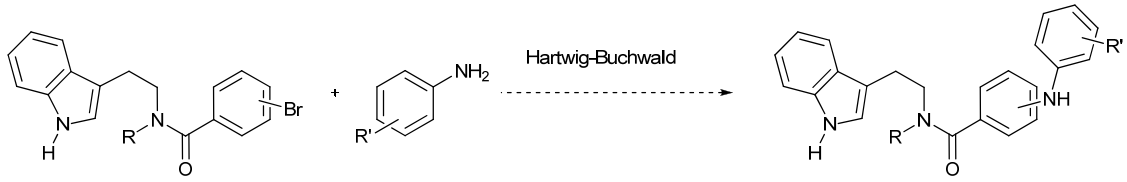



**Figure 3.9** Synthesis of new bi-aryl amine inhibitors

The reaction mechanism of the Hartwig-Buchwald reaction is similar to that of the Suzuki coupling reaction. The palladium catalyst **A** is reduced to the active $Pd^0$ species **B** which is stabilised by a ligand, typically this is a phosphine ligand. The di-ligand palladium complex **B** is in equilibrium with the mono-ligand complex **C**, it is this mono-ligand complex to which an aryl halide **D** coordinates via oxidative addition to give species **E** which exists in equilibrium with its dimmer **E'**, one of the halides **X** is replaced by the nitrogen atom of amine **F**, to give the complex **G**. A strong base **H** then extracts a proton from the amine to give complex **I**, reductive elimination then yields the desired bi-aryl amine **J**.

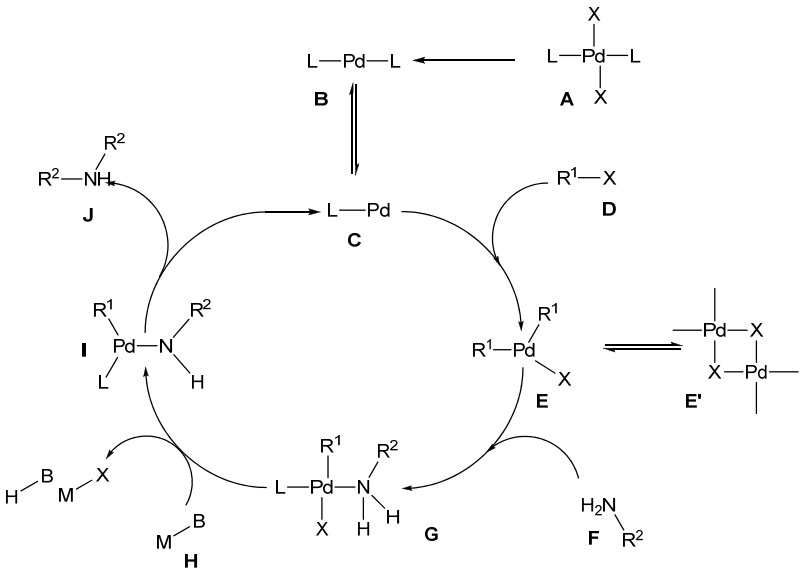
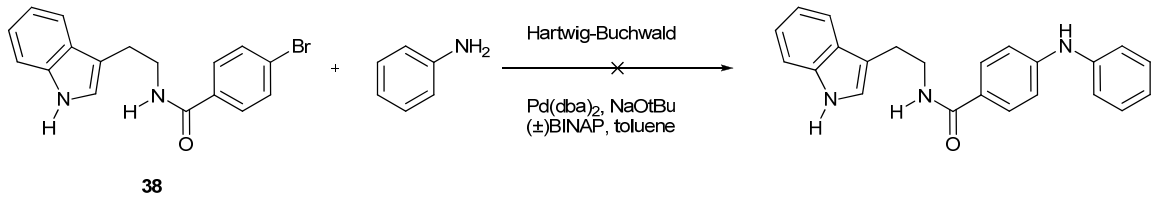



**Figure 3.10** Mechanism of the Hartwig-Buchwald coupling reaction

Typical reaction conditions would consist of $Pd(dba)_2$ as the initial palladium source, sodium tert-butoxide as the base, (±)BINAP as the ligand with toluene as the solvent. Compound **22c** the *para*-brominated intermediate was selected as the starting material for the synthesis of the first bi-aryl amines, the anticipated product should have the least steric hindrance about the newly created bi-aryl amine region and the $^1H$ NMR spectrum should be the easiest to interpret due to the lack of rotamers about the amine bond and the *para*-disubstituted nature of the amine phenyl ring.

Compound **22c** was suspended in anhydrous toluene along with 0.5 mol % of $Pd(dba)_2$, 1 mol % of (±)BINAP and 1.4 equivalents of sodium *tert*-butoxide, 1.1 equivalents of aniline was introduced and the reaction mixture was heated to 80 $^oC$ under an atmosphere of argon. Aliquots were removed at 24 and 48 hours and analysed by mass spectrometry for any trace

of the anticipated product, no peak of the expected *m/z* ratio was observed, additional Pd(dba)2 and (±)BINAP (1 and 2 mol % respectively) were injected to the reaction mixture and heating continued, after 69 hours of heating a peak in the ES+ MS of the anticipated *m/z* ratio was observed, however TLC showed no additional spots. Heating was continued for a total of 9 days but no additional spots were observed on the TLC of the reaction mixture. The reaction mixture was worked up and a NMR recorded of the crude product, but no evidence of the existence of the desired product was observed.

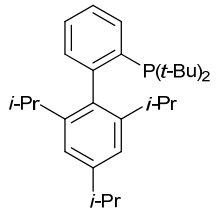



**Scheme 3.7** Attempted synthesis of bi-aryl amine.

The reaction was repeated at a higher catalytic loading of 5 mol % $Pd(dba)_2$ and 15 mol % (±)BINAP and a higher temperature of 100 $^{o}$C, again the only evidence as to the synthesis of the product was a peak in the $ES^{+}$ MS. There was no evidence in the $^{1}$H NMR or TLC of the product, an extensive investigation using HPLC again failed to show the location of any product.

*3.8.2 Bi-aryl ethers: Hartwig-Buchwald Route*

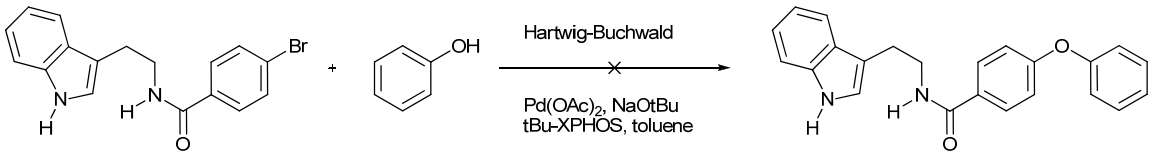
Synthesis of bi-aryl ethers using the Hartwig-Buchwald mechanism was also attempted concurrently to the work of bi-aryl ethers. The research group of Stephen Buchwald has conducted extensive research into the best conditions for the synthesis of such compounds, this research has principally focussed on the synthesis of improved ligands specific to the synthesis of bi-aryl amines and bi-aryl ethers. The current state of the art ligand for the synthesis of ethers is a phosphine ligand known as $^{t}$Bu-XPHOS **62**(Figure 3.XM)




tBuXPHOS **62**

**Figure 3.11** Structure of the ligand $^{t}$BuXPHOS **62**.

Using a similar procedure for the attempted synthesis of the bi-aryl ethers above, the brominated starting material **38** was dissolved in anhydrous toluene along with 5 mol % $Pd(OAc)_2$, 15 mol % $^{t}$BuXPHOS and 1.5 equivalents of sodium *tert*-butoxide, 1.2 equivalents of phenol in toluene were injected and the reaction mixture heated to 80 $^{o}$C. The reaction was monitored by $ES^{+}$ MS, HPLC and end point NMR but without trace of the expected product **64**. An alternative route towards these bi-aryl ethers was then sought, the most obvious choice being a version of the Ullmann reaction.




**38** **63** **64**

**Figure 3.12** Attempted synthesis of compound **64** using a Hartwig-Buchwald coupling reaction.

*3.8.3 Bi-aryl ethers: Ullmann Route*

The Ullmann reaction was first described in 1901 as a convenient method for the creation of aryl-aryl bonds using stoichiometric quantities of copper. [150] A useful development of the standard Ullmann reaction is the 'Chan-Evans-Lam modified Ullmann reaction' or 'Chan-Lam coupling' [151] which facilitates the creation of aryl carbon-heteroatom bonds via oxidative coupling of aryl boronic acids. [152-154](Figure 3.13) The reaction has advantages over the similar Buchwald-Hartwig cross coupling reaction in that it may be carried out in air at room temperature. The reaction is often performed under an atmosphere of air or oxygen as reductive elimination from a copper (III) species is faster than from a copper (II) species. [151]

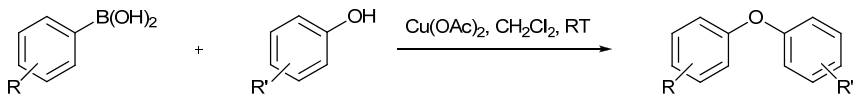



**Figure 3.13** Chan-Evans-Lam modified Ullmann reaction.

The mechanism of the reaction is outlined below (Figure 3.14). The aryl alcohol **A** is deprotonated by the base and co-ordinates to the copper (II) centre to give species **C**, transmetalation then occurs to insert boronic acid **D** onto the copper centre to give intermediate **E**. Reductive elimination of species **E** then occurs to give the desired bi-aryl ether product **G**. If the reaction is carried out in the presence of oxygen the copper (II) species **E** oxidises to the copper (III) species **F** which undergoes reductive elimination at a faster rate than the copper (II) species. The reaction is sensitive to the presence of water so must be carried out under scrupulously dry conditions, but in the presence of oxygen. This is often achieved by using an open reaction vessel fitted with a drying tube and by adding powdered 4Å molecular sieves to the reaction mixture to eliminate any water produced by the hydrolysis of the boronic acid.

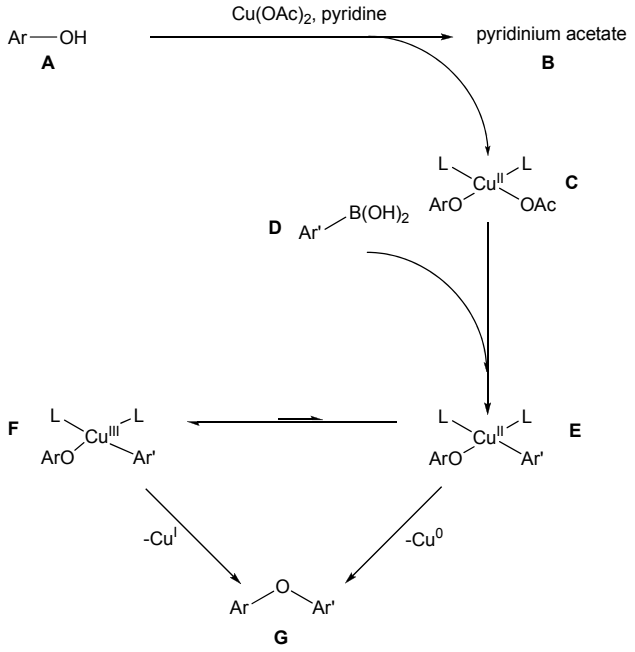
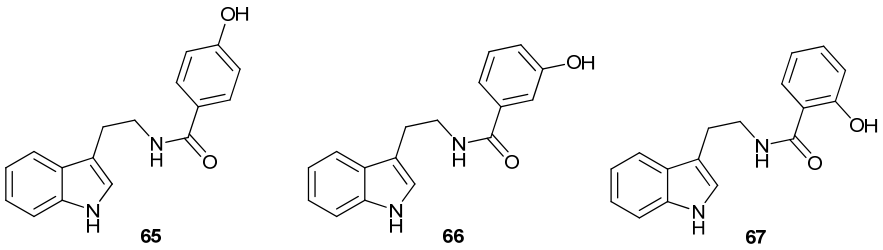
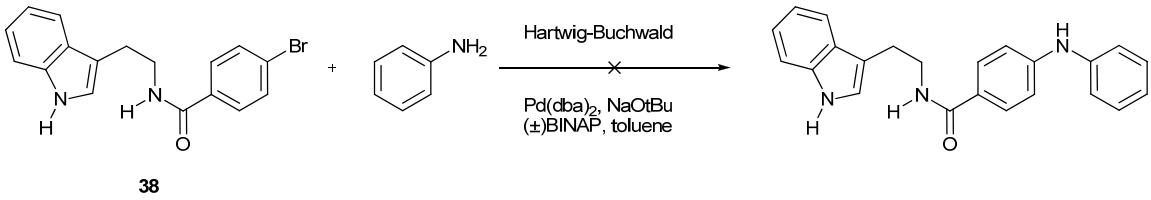



**Figure 3.14** Mechanism of the Chan-Evans-Lam modified Ullmann reaction

The required starting materials for the synthesis of bi-aryl ether inhibitors of CDK4/Cyclin D1 are phenols **65**, **66** and **67**, initially alcohol **65** was synthesised to devise a method of synthesising the corresponding bi-aryl ether **D**.

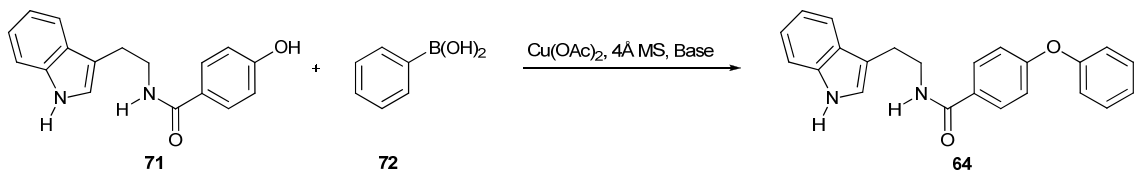



4-Benzyloxybenzoic acid **D** was converted to the corresponding acid chloride by refluxing in $SOCl_2$ for 60 minutes, the crude acid chloride was then reacted with tryptamine in DCM in the presence of aqueous NaOH to yield the protected compound **70** in 89 % yield. The desired compound **71** was obtained in 44 % yield.



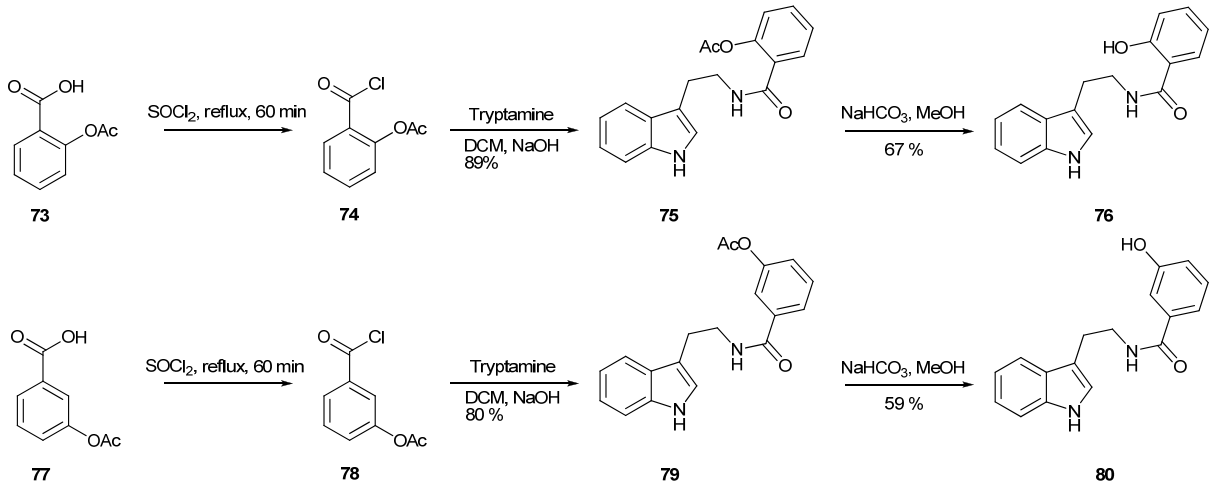

**Scheme 3.8** Synthesis of **71**.

Following the established best reaction conditions [151] alcohol **71** was dissolved in anhydrous DCM in a round bottomed flask fitted with a drying tube containing anhydrous potassium carbonate, oven dried powdered 4Å molecular sieves were added to the reaction mixture along with 2 equivalents of copper (II) acetate, 1.5 equivalents of phenyl boronic acid **72** and 3 equivalents of DMAP. The reaction mixture was stirred at room temperature for 24 hours, at which time TLC showed the reaction had halted. The desired product **64** was isolated by column chromatography in 16 % yield.



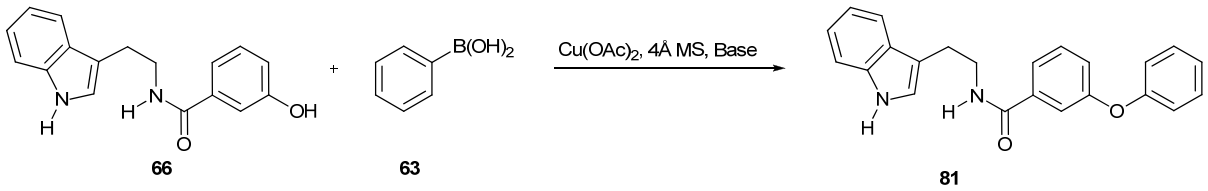

**Scheme 3.9** Synthesis of bi-aryl inhibitor **64** with three different bases.

The synthesis of the corresponding *ortho* and *meta* substituted bi-aryl ethers required the synthesis of the corresponding starting materials **76** and **80**. Since 3-benzyloxybenzoic acid and 2-benzyloxy benzoic acid were not commercially available at the time a different method was employed in their synthesis. 2-Acetoxybenzoic acid (aspirin) **73** and 3-acetoxybenzoic acid **77** were converted to the corresponding acid chlorides by refluxing in thionyl chloride, the acid chlorides **74** and **78** were then reacted with tryptamine to yield the acetyl protected compounds **75** and **79** in 89 and 80 % respectively. The acetyl protecting group were then removed by treatment with sat. aqueous $NaHCO_3$ in MeOH to afford the desired alcohols **76** amd **80** in 67 and 59 % respectively.




**Scheme 3.10** Synthesis of alcohols **76** and **80**.

Alcohol **80** was converted into the corresponding ether in 56 % yield using pyridine as the base, triethylamine and DMAP were also used as bases but both produced lower yields. When alcohol **76** was reacted with phenyl boronic acid under identical conditions to trace of product was detected. The most likely explanation is either steric hindrance around the copper centre or chelation.




| Base | Yield |
|---|---|
| $Et_3N$ | 36 % |
| DMAP | 16 % |
| Pyridine | 56 % |

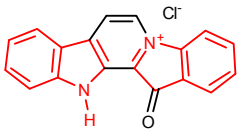
**Scheme 3.11** Synthesis and yield of bi-aryl inhibitor **81** with three different bases.

Compound **81** was tested for biological activity against CDK4/Cyclin D1 and found to be active, $IC_{50}$ CDK4/Cyclin D1 70±5 μM, CDK2/Cyclin A 860 ±25 μM, which represents a slight drop in activity compared to the parent bi-phenyl structures. Unfortunately it was not possible to arrange for the testing of all compounds, as by this stage in the project the CRUK project providing the screening facility was coming to an end. Only a final batch of 30 compounds could be screened, it was decided to only send the one biphenyl ether for testing to leave room to test other compounds from the research group. If compound **81** had shown higher activity a further range of compounds could then be synthesised and commercial screening sources investigated.

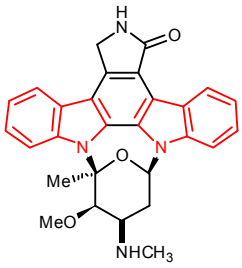
## 3.9 Conclusions and future work

The results outlined above provide further evidence [106] for the existence of a hydrophobic π-stacking pocket in the active site of CDK4, as is observed in many other protein kinases. [98] The introduction of a second phenyl ring that can probe deeper into this hydrophobic pocket in CDK4 has proved to be a versatile and robust method for increased activity and specificity of inhibition of CDK4 over CDK2. The introduction of substituent groups and atoms onto this second phenyl ring has demonstrated the robustness of this binding interaction; however none of the synthesised inhibitors has greatly increased the inhibitory activity over and above that of the lead compound **27**. [106] To move forwards with the development of novel inhibitors of CDK4/Cyclin D1 it will now be necessary to look towards other binding interaction at the hinge region and elsewhere in the active site. In particular there has been little attention paid to the indole portion of our inhibitor families.

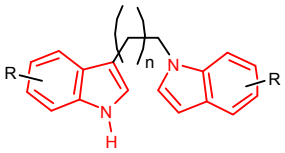
Parallel work within the group has looked at the synthesis of a range of inhibitors of CDK4 based on linked di-indole structures, two indole rings connected together via means of a linking alkyl chain in either a 1,3- or 3,3- fashion. [155] Further unpublished work has looked at the effects of substitution on the indole rings and on varying the chain length.



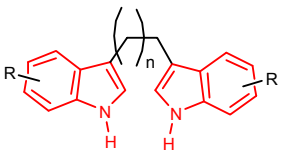

**8. Fascaplysin**




**11. Staurosporine**




**1,3-linked di-indoles**




**3,3-linked di-indoles**

Investigating the effects of introducing substitution onto the indole rings of the bi-phenyl type inhibitors and looking at the structure of the 'left hand side' of the pharmacophore is one obvious way to improve on the activity of these compounds.

# Chapter Four Sugar Motifs

*"Who are we? The answer to this question is not only one of the tasks but the task of science."*

*Erwin Schrodinger 1953*

## 4.1 Indolocarbazole Natural Products

The indolocarbazole family of natural products are structurally related to fascaplysin since both contain two indole rings fused together by a six-membered ring. There are five distinct possibilities for the arrangement of one indole and one carbazoles ring system which makes up an indolocarbazole; indolo[2,3-*a*]carbazole **82**, indolo[2,3-*b*]carbazole **83**, indolo[2,3-*c*]carbazole **84**, indolo[3,2-*a*]carbazole **85** and indolo[3,2-*b*]carbazole **86**. (Figure 5.1) Of these five possibilities virtually all of the indolocarbazoles isolated from natural sources are indolo[2,3-*a*]carbazoles. Derivatives of bisindolylmaleimide (arcyriarubin A) **87** are also widely considered to be members of the indolocarbazole family.

Fascaplysin **8** also consists of two indole rings joined by a six-membered ring; however unlike the indolocarbazoles this linking six membered ring (Ring C, figure 5.1) incorporates one of the indole nitrogen atoms as a quaternary hydrochloride salt. In the indolocarbazole family itself neither of the two NH groups are incorporated into the linking 'C' ring.

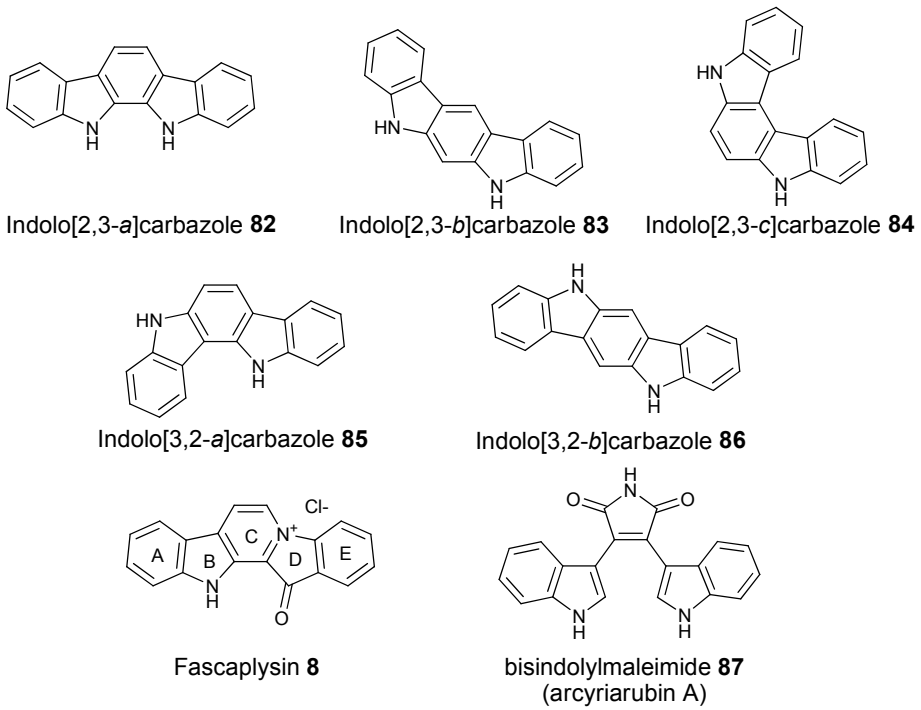



**Figure 4.1** The five ring system arrangements observed in the indolocarbazole structure family **82-86**, fascaplysin **8** and bisindolylmaleimide **87**.

The first known indolocarbazole, staurosporin **11**, was first isolated from *Streptomyces* staurosporeus in 1977, [88] since then over one hundred examples of the family have been discovered, with the topic receiving frequent attention in the literature. [156] Indolocarbazoles have been isolated from slime moulds, marine invertebrates and have been cultivated in a range of microorganisms including cyanobacteria and the actinomycetes. Structurally staurosporine consists of a pyrrol derivative of indolo[2,3-*a*]carbazoles which also bears an amino pyranose linked to the two NH groups of the carbazoles back bone.

## 4.2 Intention

Previous sections of this work have concentrated on understanding and attempting to improve upon the binding interactions between fascaplysin **8** and CDK4/Cyclin D1, with a specific focus on the Phe 93 π-stacking pocket and the valine 96 hinge region. As detailed previously this has produced a series of analogues of fascaplysin which have retained the desired selectivity and removed the toxicity observed in fascaplysin [157] but have not improved on the activity of the parent compound.

In an attempt to improve on the binding affinity of fascaplysin it was decided to investigate the possibility of introducing a sugar motif into the pharmacophore developed thus far. The majority of work conducted within the Jenkins group at this time has focused on the development of inhibitors of CDK4/Cyclin D1 which bear one or two linked indole rings. [106, 118, 125, 145, 155] However there is also a vast experience of carbohydrate chemistry within the group and as detailed above sugar units are often incorporated into natural product with anti-cancer and kinase inhibition properties. It is hoped that by expanding the focus of our work to include these two fields of indole and carbohydrate chemistry it will be possible to further improve the activity of our kinase inhibitors.

## 4.3 Rebeccamycin

This new research effort required a starting point to bring together our work on bis-indole inhibitors and carbonhydrate chemistry. The natural product rebeccamycin **88** offers an ideal starting point as it incorporates an indolo[2,3-*a*]carbazole structure, which contains two linked indole rings, but also bears a linked sugar ring.

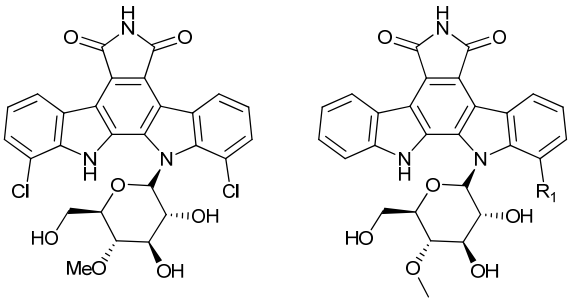
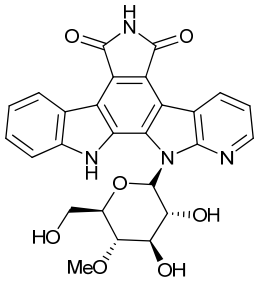



**Figure 4.2** Rebeccamycin **88** and analogues **89-91**.

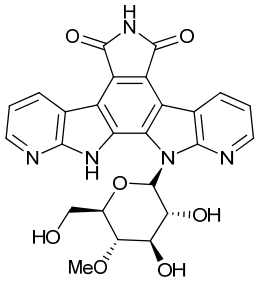
Rebeccamycin **88** is an antimicrobial metabolite first isolated from fermentations of *Nocardia aerocoligenen* in 1985. [158, 159] (The organism which produces rebeccamycin has subsequently been renamed as *Saccharothrix aerocolonigenes* [159, 160] and then again as *Lechevalieria aerocolonigenes*). [161] Additional compounds were also isolated from *Lechevalieria aerocolonigenes* specifically 11-dechlororebeccamycin **89**, [162] 1,11-dischloro-rebeccamycin **90** and 4-*O*-demethoxy-1,11-dideschloro-rebeccamycin (BMY-41219) **91**. These compounds all showed antitumour activity, with **89** also exhibiting antibacterial activity. Rebeccamycin **88** has a number of interesting biological properties, it has shown activity against a number of cancers including P388 leukemia, L1210 leukemia and B16 melanoma, it has also been shown to cause single strand breaks in the DNA of human lung adenocarcinoma. Rebeccamycin is also an inhibitor of topoisomerase I [158, 159]

Research into rebeccamycin has created a large number of analogues, variations to the parent structure have included substituting an azaindole ring for one or more of the indole rings to give the 7-azaindole containing analogues **92** and **93.** [163-166] Other work has examined creating more water soluble analogues, compounds incorporating the indolo[2,3-c]carbazole

framework, [167] de-chlorinated derivatives 'Dechlororebeccamycin' [168] and analogues which more closely resemble staurosporine. [169]



**92** 7-Aza-indole rebeccamycin

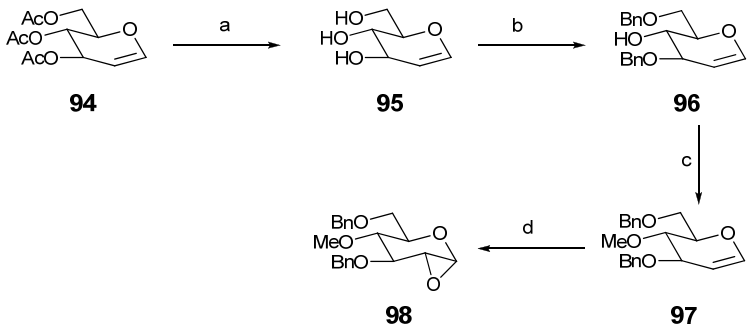


**93**

## 4.2 Synthesis of Rebeccamycin

There are several published routes to the synthesis of rebeccamycin, the route which offers the greatest flexibility for the proposed work is one published by Danishefsky. [170] The synthesis follows a combinatorial pathway, the heterocyclic unit is derived from a dichloro-bisindolylmaleimide which is then coupled with an epoxide, global deprotection then yields the natural product rebeccamycin. The advantage of this method is that it allows the substitution of a range of different indole backbone structures at a late stage in the synthesis.

### *4.2.1 Epoxide synthesis*

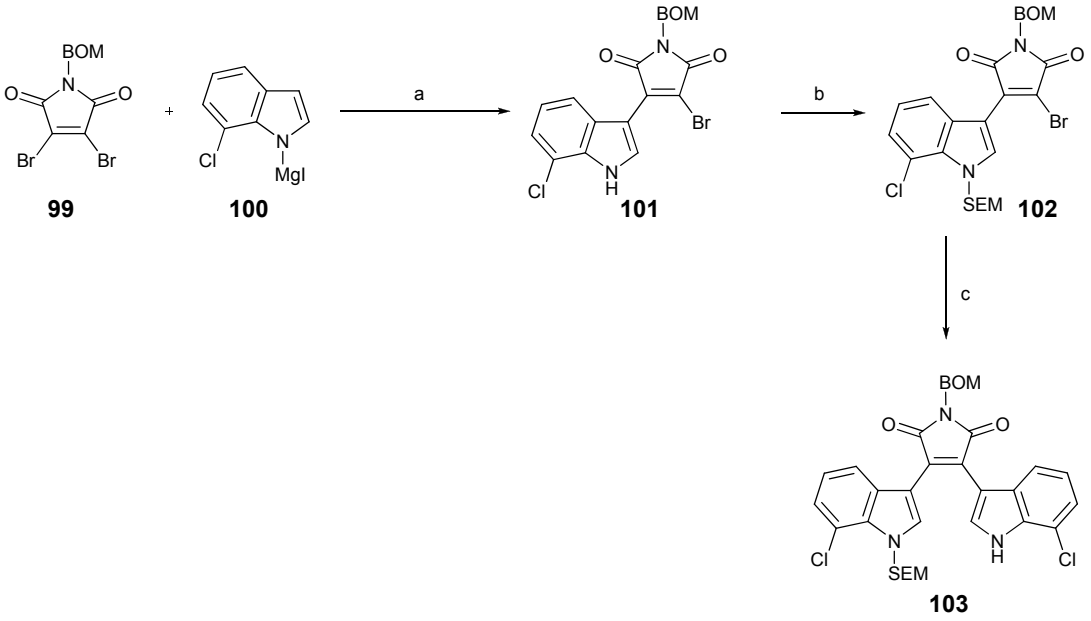


**Scheme 4.1** Synthesis of the 'Danishefsky' epoxide. [170]
*Reagents and conditions*: a) cat. MeONa, MeOH, 98 %; b) (i) $Bu_2SnO$ (1.0 eq), MeOH, (ii) BnBr (2 eq), $Bu_4NBr$ (2 eq), PhH, reflux, 10 h, 47 %; c) NaH, MeI, DMF, 85 %; d) DMDO (1.1 eq), 0 $^oC$, $CH_2Cl_2$, quantitative.

The synthesis of the required 3,6-di-*O*-benzyl-4-*O*-methyl-D-glucal **97** is outlined above (Scheme 5.1), the synthetically most demanding steps are required to differentially methylate the 4-hydroxyl group, as required in the end product rebeccamycin **88**. This was achieved by Danishefsky *et al.* by dibenzylating D-glucal **95** regiospecifically at the 3 and 6 positions using stannylene methodology. [171] The remaining free 4-hydroxyl group was then methylated with methyl iodide and the double bond epoxidised with dimethyldioxirane (DMDO), this yielded the desired α-1,2-anhydrosugar **98** as the major product (15:1 ratio of the α/β) in good yield. [170]

*4.2.2 Indole unit synthesis*

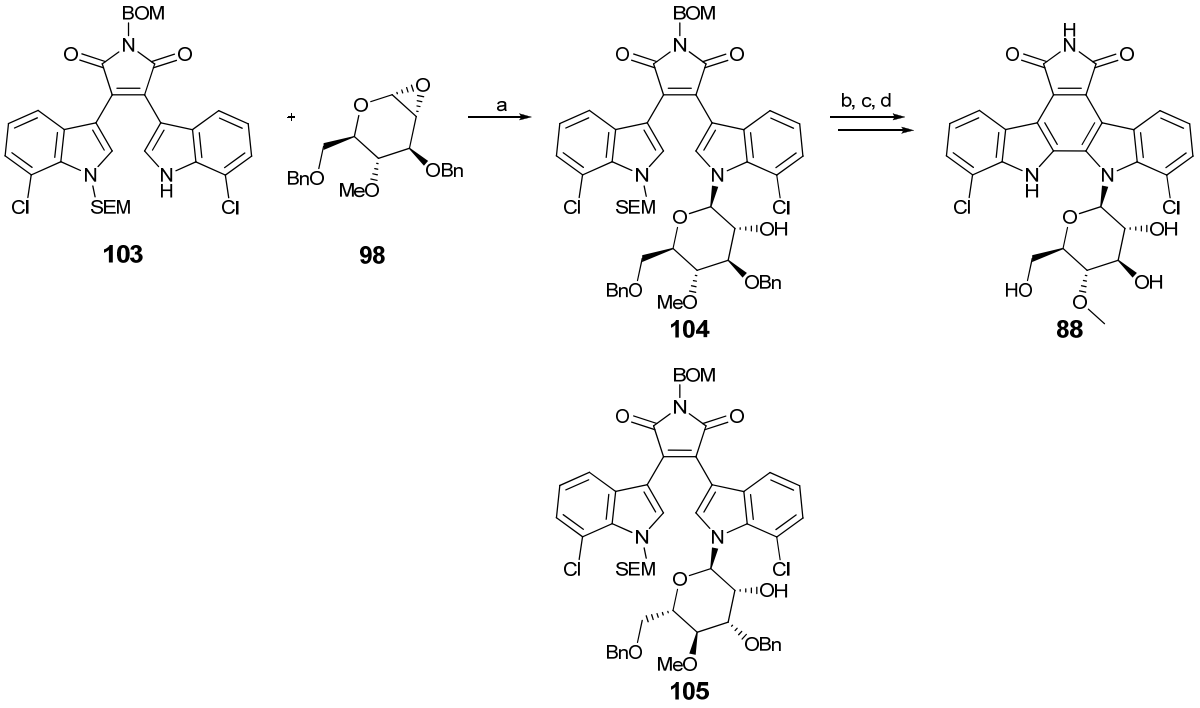
The carbazoles fragment of rebeccamycin is built up by sequential addition of indole Grignard **100** onto the protected dibromomaleimide **98** with protection of the free indole NH of the intermediate **101** with a (2-(trimethylsilyl)ethoxy)methyl group gave the desired secaglycon **103**.




**Scheme 4.2** Synthesis of the carbazoles fragment
*Reagents and conditions*: a) PhH, 25 $^{o}$C, 12 h, 91 %. b) i) NaH, THF, 25 $^{o}$C, 15 min, ii) SEM-Cl, PhH, 25 $^{o}$C, 30 min, 94 % yield. c) PhH, 25 $^{o}$C, 12 h, 77 % yield.

*4.2.3 Glycosidation*

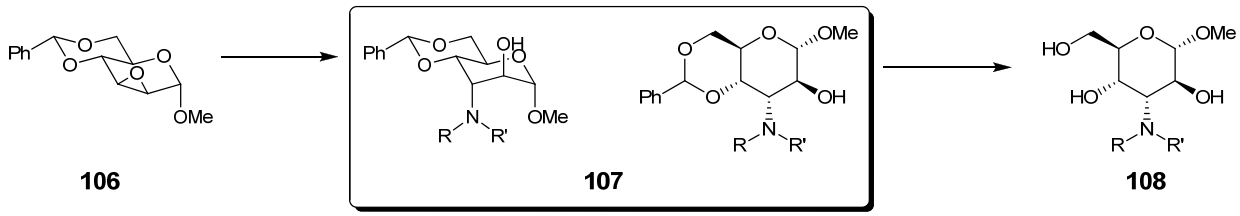
Glycosidation of secoaglycon **103** was reported to require an excess of the epoxide **98**. Two equivalents of the desired epoxide **98** were prepared by treating the D-glucal **97** with dimethyldioxirane, this was added to the sodium salt of secoaglycon **103**, obtained by treatment with 1.1 equivalents of NaH. Heating the mixture to reflux produced the desired β-glucopyranoside **104** in 42 % yield along with 8 % of the undesired α-mannopyranoside **105**. The SEM protecting group was removed with tetrabutylammonium fluoride in 63 % yield and the 2,2'-bisindole bond was created by photocyclisation in 74 % yield. Deprotection of the two benzyl groups was accomplished via hydrogenation with Pearlman's catalyst followed by ammonolysis, this was achieved in 72 % yield. Danishefsky also reported hydrogenolysis of the C-Cl bonds with prolonged hydrogenation over metal catalysts, using Pearlman's catalyst this was reduced to 14 %, this observation will be important in the later stages of synthesising novel sugar bearing natural product analogues as many of the bis-indole compounds synthesised by the Jenkins group contain carbon-halogen bonds.




**Scheme 4.3** Coupling of the carbazoles and carbohydrate units of rebeccamycin **507**. *Reagents and conditions*: a) THF, reflux 8 h; b) TBAF (2 eq), 4Å powdered molecular sieves, reflux 2 h; c) hν Hg lamp, 450 W, cat. $I_2$, air, PhH, 8 h, 74 % yield; d) i) $Pd(OH)_2$, $H_2$, EtOH/EtOAc (4:1), 5 h, ii) sat. $NH_3$ in THF, 25 $^oC$, 2 h, 72 % yield.

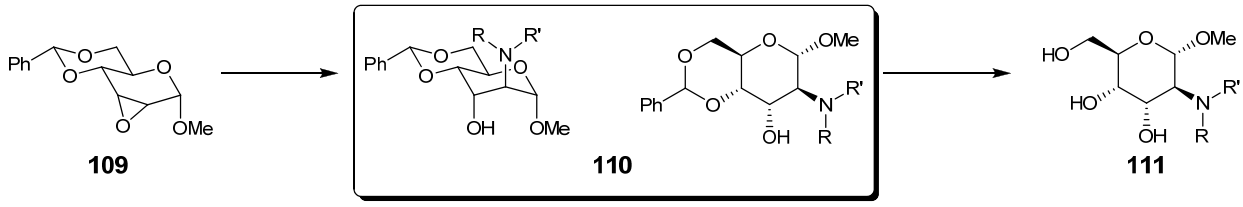
## 4.3 Synthesis of other glycosylamines

Other work conducted in the Jenkins group by Dr Dominic Laventine has demonstrated the versatility of using two different epoxides for the creation of a range of 3- and 4- amino pyranosides. When treated with an amine the manno epoxide methyl-α-D-2,3-anhydro-(*R*)-4,6-*O*-benzylidene-mannopyranoside **106** undergoes regioselective trans-diaxial ring opening to give the 3-amino substituted amino pyranoside **108**.




**Scheme 4.4** Manno epoxide ring opening

The allo epoxide methyl-2,3-anhydro-(*R*)-4,6-*O*-benzylidene-α-D-allopyranoside **109** undergoes ring opening at the 2 position to give the corresponding 2-amino derivatives **110** and the deprotected **111**. (Figure 5.3)




**Scheme 4.5** Allo epoxide ring opening.

By adapting the synthetic routes devised by Danishefsky for the synthesis of rebeccamycin and staurosporine by substituting a bis-indole compound for the carbazole fragment and by incorporating these two epoxides **106** and **109** in place of **98** it should be possible to synthesis a new range of bis-indole derivatives incorporating a sugar ring linked at the anomeric position, as in many natural products, but also at the 2- and 3- positions. (Figure 5.6)

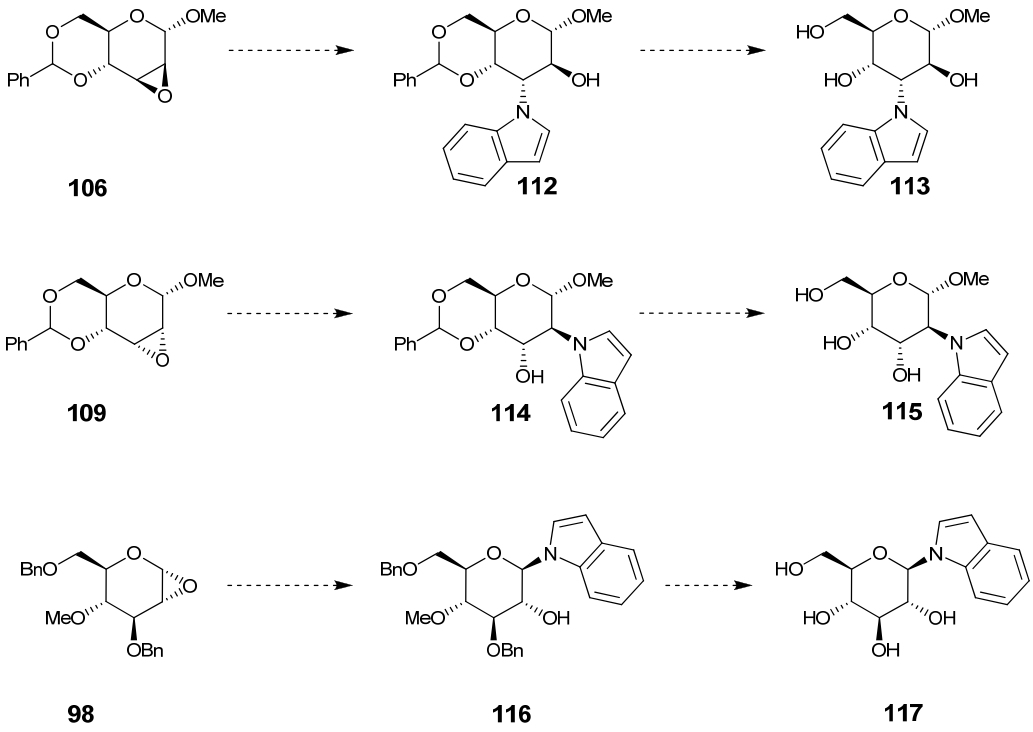
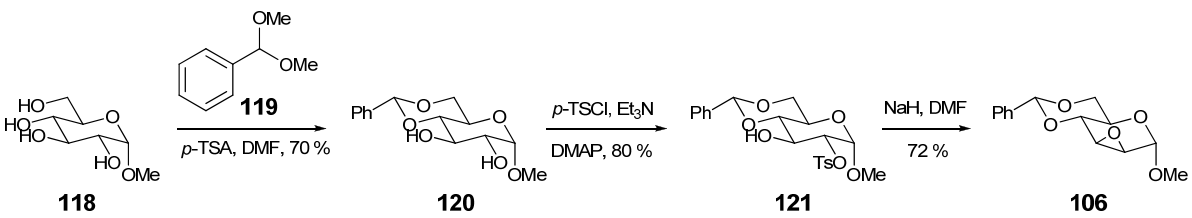



**Figure 4.3** Summary of the three possible substitution patterns of indole-sugars.

## 4.4 Synthesis of epoxide starting materials

The starting material for the synthesis of the mano and allo epoxides **106** and **109** is α-D-methyl glucopyranoside **118**, a cheap commercially available analogue of glucose. [172] The anomeric position of the sugar is already protected as a methyl acetal which reduces the number of protection steps required in the synthesis of the epoxide and renders the molecule more stable to hydrolysis.

### *4.4.1 Manno epoxide synthesis*

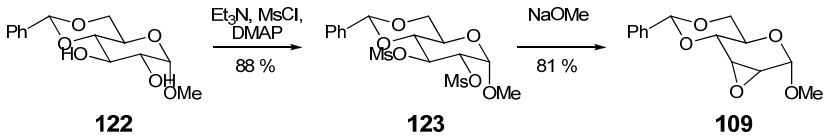



**Scheme 4.6** Manno epoxide synthesis.

Initially the 4- and 6- position hydroxyl groups of **118** were protected as an acetal. There are a number of possible choices of acetal; the most often encountered is the benzylidine acetal which has a number of advantages over alternatives such as *iso*-propylidine acetal, primarily that the protons introduced into the molecule are aromatic and thus do not overlap with other proton signals in the $^{1}$H NMR spectra.

Benzaldehyde dimethyl acetal **119** is reacted with α-D-methyl glucopyranoside **118** in anhydrous DMF in the presence of a catalytic quantity of *para*-toluene sulphonic acid monohydrate to give methyl-α-D-(*R*)-4,6-*O*-benzylidene-glucopyranoside **120** in 70 % yield. Regioselective protection of the 2-hydroxyl group is then carried out by treatment with one equivalent of para-tolylsulphonyl chloride in triethylamine and a catalytic quantity of DMAP to give the tosylate **121** in 80 % yield. [173] The desired manno epoxide is then obtained by a intramolecular nucleophilic substitution reaction, treatment of the tosylate **121** with sodium hydride creates an oxide anion at C-3 which displaces the tosylate group at C-2. [174] Recrystalisation from propan-2-ol affords the desired methyl-α-D-2,3-anhydro-(*R*)-4,6-*O*-benzylidene-mannopyranoside **106** in 72 % yield.

*4.4.2 Allo epoxide*



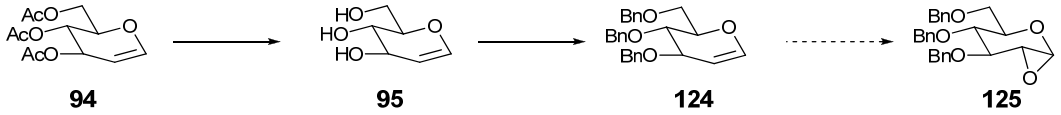

**122** **123** **109**

**Scheme 4.7** Allo epoxide synthesis.

The allo epoxide **109** may be synthesised according to the published procedure [174] starting from methyl-α-D-(*R*)-4,6-*O*-benzylidene-glucopyranoside **122** produced in the synthesis of the manno epoxide **123**. Methyl-α-D-(*R*)-4,6-*O*-benzylidene-glucopyranoside **122** is dissolved in anhydrous DCM and treated with methyl sulphonyl chloride in the presence of triethylamine and a catalytic quantity of DMAP. This gives the desired di-mesylate, methyl-(R)-4,6-*O*-benzylidene-2,3-di-*O*-methylsulphonyl-α-D-glucopyranoside **123** in 96 % yield. An x-ray crystal structure of this di-mesylate has been previously obtained within the group. The desired allo-epoxide **109** may then be obtained in 86 % yield by treatment of the di-mesylate with an excess of sodium methoxide, produced in situ by the addition of sodium metal to a

solution of **123** in methanol. A large quantity of the epoxide **109** was already available from previous work within the group and it was not necessary to re-synthesise this material.

*4.4.3 Synthesis of a simplified Danishefsky epoxide.*




**94** **95** **124** **125**

**Scheme 4.8** Synthesis of a simplified Danishefsky epoxide.

The epoxide described in the Danishefsky synthesis of rebeccamycin is outline above. (Scheme 5.1) The sugar ring of rebeccamycin bears a methyl group on the 4-hydroxy group, consequently the literature procedure details the steps required to differentially protect the three hydroxyl groups of the starting glucal **94**. [170] However for this work it was not necessary to have a differentially protected sugar, therefore the synthesis was adapted to prepare the tri-benzylated glucal **124** which could then be converted to the simplified epoxide **125** as required.

Commercially available 3,6-di-*O*-methyl-D-glucal **94** was deprotected with catalytic sodium methoxide in methanol to yield D-glucal **95** in 92 % yield. Confirmation of the structure of **95** was by analysis of $^{1}$H NMR, an X-ray crystal structure was also obtained; it is believed that this is the first time an X-ray structure has been obtained for D-glucal. (Figure 5.4)

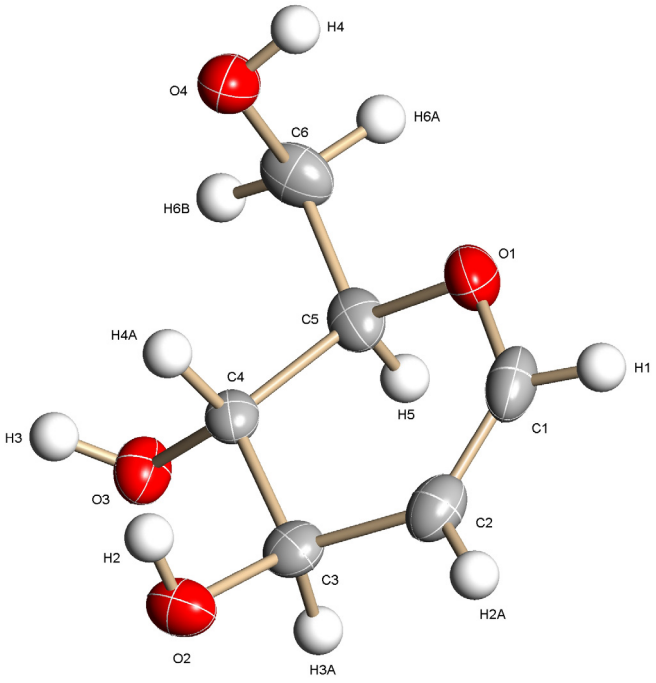



**Figure 4.4** X-Ray crystal structure of D-glucal **95** with 50% displacement ellipsoids.

Deprotonation of D-glucal **95** with NaH (1.3 eq) in THF/DMF (4:1) followed by the addition of benzylbromide (1.3 eq) and heating to 60 $^{o}$C for 4 hours afforded the desired tri-*O*-benzyl-D-glucal **124** in 76 % yield after chromatography. An X-ray crystal structure was obtained for this compound. (Figure 4.5) This was stored at -18 $^{o}$C prior to use; the epoxide **123** was prepared *in situ* prior to each coupling reaction with indole.

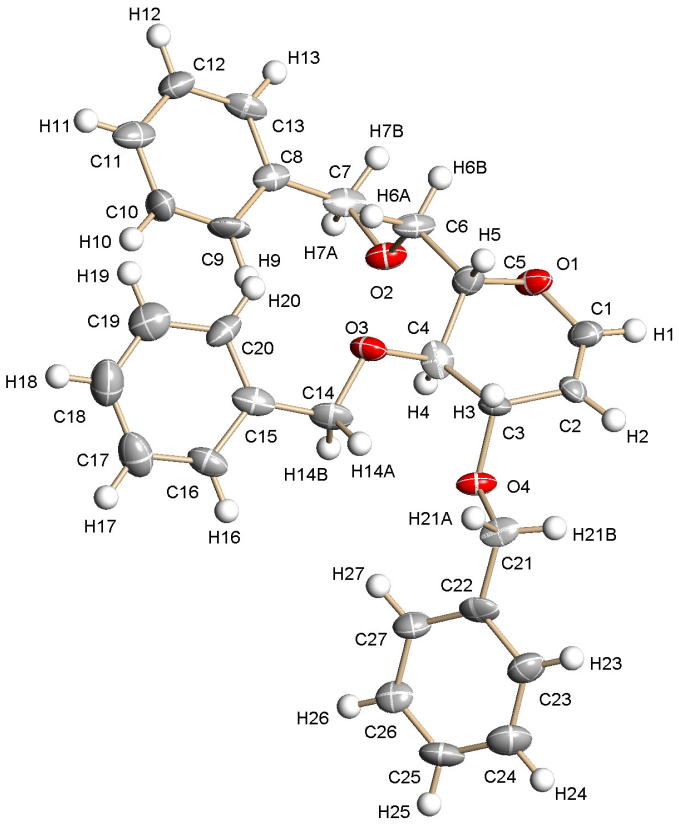



**Figure 4.5** X-Ray crystal structure for tri-O-benzyl-D-glucal **124** with 50% displacement ellipsoids.

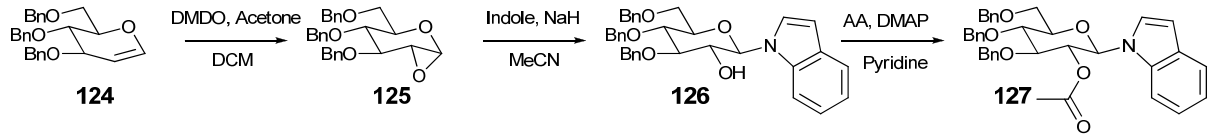
## 4.5 Reactions of epoxides with indole

To investigate the feasibility of creating a large number of new natural product derivatives bearing the three sugars discussed above it was decided to take the three epoxides **125**, **106** and **109**, and then test their reactivity towards indole. If a simple series of three glycosylated indoles can be prepared in good yield then the method can be applied to the library of bis-indole inhibitors already synthesised by the Jenkins group.

### *4.5.1 Anomeric indolo-sugar*

Tri-*O*-benzyl-D-glucal **124** was dissolved in anhydrous DCM and treated with a freshly prepared solution of DMDO in acetone, the reaction was monitored by TLC with additional DMDO solution being added until the glucal component was consumed. The crude epoxide **125** was isolated under vacuum as a white solid; this was immediately added to a stirred solution of the sodium salt of indole, prepared by treatment of indole with 1.2 equivalents of

NaH. Stirring for 30 minutes at R.T. followed by heating to 50 $^{o}$C for 24 hours afforded the indolo-sugar **126** in 42 % yield after chromatography.

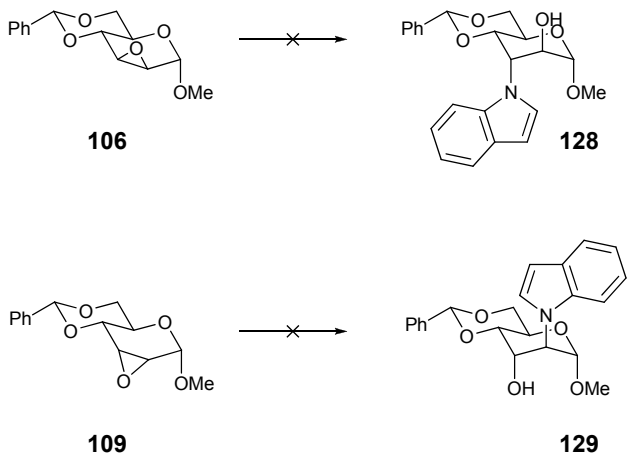



**Scheme 4.9** Synthesis of anomeric indolo-sugar **126** and the acetyl derivative **127**.

Isolation and purification of the indolo-sugar proved much easier if the crude reaction mixture was evaporated to dryness and acetylated with acetic anhydride (AA), catalytic DMAP and pyridine as the solvent. The protected indolo-sugar **127** was then achieved in 35 % overall yield from glucal **124**, the acetyl derivative **127** also exhibited a much cleaner $^{1}$H NMR spectrum. In Danishefsky's original publication [170] the desired product was present as the major isomer of a 15:1 mixture of the α and β substituted products, the ratio of these two products being determined by the acetylation of the crude mixture followed by chromatographic separation. Although there was no visible $^{1}$H NMR evidence of any of the undesired β-substituted sugar, the spectrum of the acetylated product **127** was much cleaner after chromatography.

*4.5.2 2- and 3- indolo-sugars*

The epoxides **106** and **109** were reacted with solutions of the sodium salt of indole, a wide range of solvents, bases and conditions were used but neither epoxide could be made to react with the deprotonated indole in any significant quantity. Some conditions produced a peak in the mass-spectrum of the crude reaction mixture corresponding to the anticipated *m/z* ratio for the product. But in no example was any evidence of the existence of either compound observed in the 300 MHz $^{1}$H NMR spectrum.




**Scheme 4.10** Attempted synthesis of 2- and 3- indolo-sugars.

Since the reactivity of both epoxides towards simpler nucleophiles has been well documented [175] it must be assumed that either; indole is too bulky to approach the epoxide or that it is not sufficiently neucleophillic. Danishefsky's original paper reported unsuccessful reactions between the more reactive 1α,2α-anhydrosugars and indoles under Lewis acid catalysed conditions, to determine which of these factors is determining the lack of reactivity of epoxides **106** and **109** towards indole, the reactions were repeated replacing indole with indoline.

*4.5.3 Indoline based sugars*

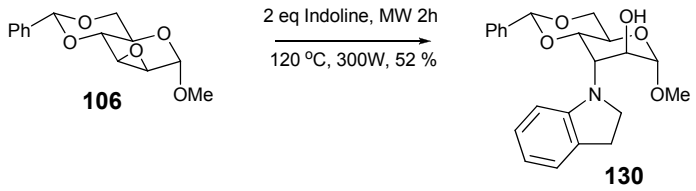
Duplication of the reaction conditions used above for the attempted synthesis of indolo-sugars, with indoline substituted for indole did not produce any detectable trace of the desired product. A range of conditions were screened rapidly to determine the best possible technique, microwave irradiation was used in addition to simply refluxing in toluene and MeOH. (Table 5.1) For the reactions using conventional heating techniques, mass spectra were taken of each reaction at 1 hour, 24 hours and 72 hours. For the microwave reactions, MS were obtained after 1, 2 and three cycles of heating. Each reaction tube contained 0.5 mmol of the manno epoxide **106**, 2 equivalents of indoline, 1 mL of solvent in the case of the microwave reactions and 5 mL for the conventionally heated reactions and 2 equivalents of any additive.

| Solvent | Heating | Additive |
|---|---|---|
| Toluene | 150 oC | - |
| Toluene | 120 $^{o}$C, 10 min, MW | - |
| Toluene | 120 $^{o}$C, 10 min, MW | LiCl |
| Toluene | 120 $^{o}$C, 10 min, MW | $NaBF_4$ |
| Toluene | 120 $^{o}$C, 10 min, MW | $LaCl_3$ |
| Toluene | 120 $^{o}$C, 10 min, MW | $TeCl_4$ |
| Toluene | 120 $^{o}$C, 10 min, MW | $CrCl_3{\cdot}6H_2O$ |
| Toluene | 120 $^{o}$C, 10 min, MW | cat NaOMe |
| Toluene | 120 $^{o}$C, 10 min, MW | cat $K_2CO_3$ |
| MeOH | 120 $^{o}$C, 10 min, MW | - |
| MeOH | 120 $^{o}$C, 10 min, MW | cat NaOMe |
| Toluene | Reflux | - |
| Toluene | Reflux | LiCl |
| Toluene | Reflux | $NaBF_4$ |
| Toluene | Reflux | $LaCl_3$ |
| Toluene | Reflux | $TeCl_4$ |
| Toluene | Reflux | $CrCl_3{\cdot}6H_2O$ |
| Toluene | Reflux | cat NaOMe |
| Toluene | Reflux | cat $K_2CO_3$ |
| Toluene | Reflux | $FeCl_3$ |
| Toluene | Reflux | BFDE |
| Toluene | Reflux | $ZnCl_2$ |
| Toluene | Reflux | $SnCl_2$ |

**Table 4.1** Trialled conditions for the opening of manno epoxide **106**. BFDE = Boron trifluoride diethyl etherate

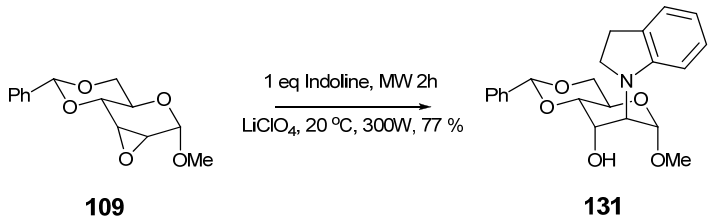
The microwave reaction containing $NaBF_4$ as an additive produced the only/largest *m/z* peak of the anticipated product, the reaction was repeated using double the quantities of reactants. The reaction mixture was then dissolved in the minimum of DCM and an aqueous work up performed. (Washings with $H_2O$, 1M citric acid and sat. aq. $NaHCO_3$) Column chromatography isolated the desired product **128** in 4.5 % yield.

Some success was also encountered using lithium perchlorate as an additive to catalyse the opening of these epoxides, however the use of $LiClO_4$ is extremely hazardous, there is a serious risk of explosion when heating this material in the microwave reactor, DO NOT attempt this reaction on anything other than a small scale, more than approximately 1 mmol of epoxide and 1 mL of solvent.




**Scheme 4.12** Synthesis of 3-indoline sugar **130**.

The best reaction conditions found were longer heating times under microwave irradiation with $LiClO_4$ as an aditive; after two hours of microwave irradiation of manno epoxide **106** with 2 equivalents of indoline as the solvent and one equivalent of $LiClO_4$, the desired indoline-sugar **130** was obtained in 75 % yield after chromatography, the corresponding 2-indoline sugar **131** was also synthesised using the same procedure in 77 % yield.




**Scheme 4.11** Synthesis of 2-indoline sugar **131**.

## 4.6 Future directions

It was hoped that the three epoxides **125**, **109** and **109** could be reacted with the library of bis-indole compounds previously synthesised by the Jenkins group. However this preliminary investigation suggests that epoxides **106** and **109** are not sufficiently reactive towards indole. The alternative route, of first opening each epoxide with the more reactive indoline, followed by the sequential building up of each bis-indole backbone, as in the original synthesis of these bis-indoles would need to be followed. (Figures 4.6 and 4.7) This is a much longer synthetic route and as such falls outside the scope of this project.

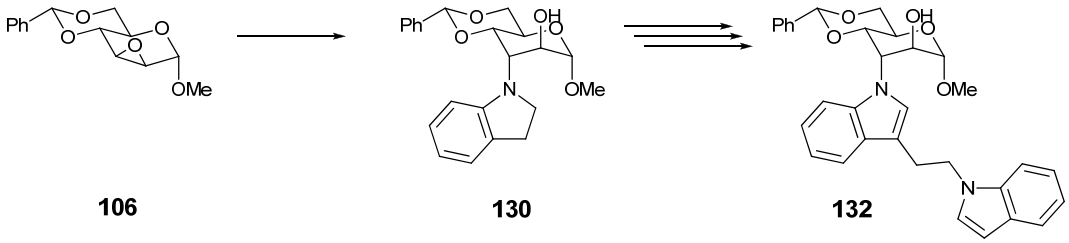



**Figure 4.6** Manno epoxide opening

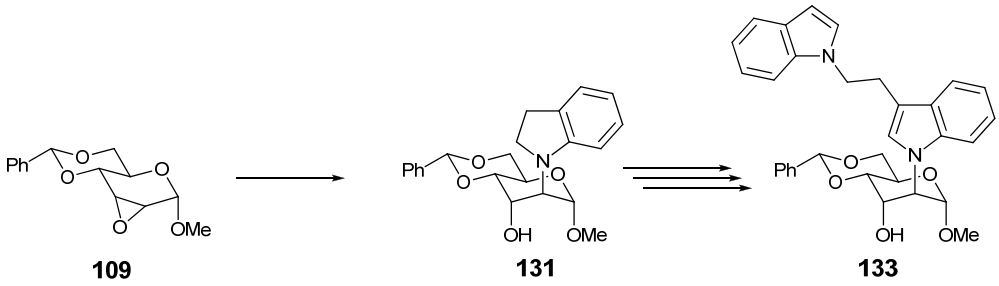



**Figure 4.7** Allo epoxide opening.

# Chapter Five Experimental Data

*"I often say that when you can measure what you are speaking about, and express it in numbers, you know something about it; but when you cannot express it in numbers, your knowledge is of a meagre and unsatisfactory kind; it may be the beginning of knowledge, but you have scarcely, in your thoughts, advanced to the stage of Science, whatever the matter may be"*

*Attributed to*

*William Thomson, 1st Baron Kelvin*

## 5.1 Materials and Equipment

Melting points were recorded on a Reichert kofler hot stage melting point apparatus and are uncorrected. NMR spectra were recorded on Bruker DPX 300 ($^{1}$H, 300.13 MHz ; $^{13}$C, 75.47 MHz; $^{19}$F, 282.39 MHz) or Brucker DPX 400 ($^{1}$H, 400.13 MHz; $^{13}$C, 100.61 MHz) spectrometers as indicated, chemicals shifts were measured relative to chloroform ($^{1}$H $\delta$ 7.26, $^{13}$C $\delta$ 77.0) or dimethylsulfoxide ($^{1}$H $\delta$ 2.50, $^{13}$C $\delta$ 39.43) and are expressed in ppm. Coupling constants *J* are expressed in hertz and the measured values are corrected to; $^{1}$H two decimal places, $^{13}$C one decimal place. $^{19}$F and $^{13}$C spectra are proton decoupled, $^{13}$C signals not denoted as either ($CH_3$), ($CH_2$) or (CH) are quaternary carbon centres.

Fast atom bombardment (FAB) mass spectra were recorded on a Kratos Concept 1H using xenon and *m*-nitrobenzyl alcohol as the matrix, electrospray (ES) mass spectra were recorded on a Micromass Quattro LC spectrometer. Accurate mass were measured on a Kratos Concept 1H spectrometer using peak matching to stable reference peak. Infra red spectra were recorded on a PerkinElmer System 100 with a Universal ATR attachment accessory.

Flash column chromatography was carried out using Merk Kiesegel 60 (230-400 mesh). Dry acetonitrile, dichloromethane, toluene, tetrahydrofuran, diethyl ether and hexane were obtained using a PURE SOLV$^{TM}$ system of Innovative Technology Inc. Dry benzene was obtained by drying commercial, thiophen free benzene over calcium hydride, followed by distillation[176]. Dry acetone was obtained by drying over and then distillation from calcium sulphate.[176] Absolute methanol was obtained by distillation,[176] *tert*-butanol was dried over anhydrous magnesium sulphate then refluxed and distilled over magnesium activated with iodine.[176] Anhydrous DMF was purchased from the Aldrich chemical company.

HPLC was performed using a PerkinElmer Series 200 fitted with a Series 200 diode array detector (DAD); reverse phase seperations were performed using gradient elutions of $H_2O$:MeCN on a Phenomenex 125 Å Aqua C18 column (150 × 4.6 mm, 5μm particle size), normal phase separations were performed on a Varian Polaris Si-A column (150 × 4.6 mm, 10 μm particle size).

## 5.2 Biological screening procedures

Biological screening against CDK4/Cyclin D1 and CDK2/Cyclin A were carried out by the research group of Prof. Bhabatosh Chaudhuri at the School of Pharmacy, De Montfort University, Leicester. A brief description of the procedure used is included below.

### *5.2.1 CDK4/Cyclin D1 and CDK2/Cyclin A assay*

Expression and purification of CDK4/GST-CyclinD1, CDK2/GST-CyclinA and GST-RB152. Fusion proteins of human cyclins A and D1, covalently linked to glutathione S-transferase (GST), were co-expressed with the catalytic subunits CDK2 and CDK4 in Sf-9 insect cells as described previously. [177-179] Active enzyme complexes, containing a catalytic subunit bound to GST-Cyclin, were bound to glutathione-agarose columns (Sigma, Cat. No. G3907) and were eluted from the columns with reduced glutathione. The reduced glutathione was removed by dialysing the enzymes in 10,000 MCO dialysis cassettes (Pierce, Cat No. 66830) with two buffer changes. The GST-RB152 fusion construct was transformed into the *Escherichia coli* strain BL21(DE3)pLysS (Novagen Cat. No 69451-4). For expression of GST-RB152, the cells were induced in the presence of a final concentration of 4 mM isopropyl-β-thiogalactopyranoside (IPTG, Invitrogen Cat. No. 15529-091) and were allowed to grow for 4 h in a shaking incubator at 37º C and 220 rpm. Purification of the GST-RB152 protein was carried out as described previously. [179] Protein estimation was performed using the Bradford protein assay (Bio-Rad Laboratories) with bovine serum albumin (BSA) as the standard and the purity of the fusion protein was assessed by SDS-PAGE analysis. Proteins were stained with Coomassie blue for visualisation.

Kinase assays and $IC_{50}$ determination. The assay measures the depletion in ATP concentration as a result of phosphorylation of retinoblastoma (GST-RB152) and Histone H1 (Upstate Biotech Cat. No. 14-155) by CDK4 and CDK2, respectively. The assay was run in a 96 well format and all steps in one assay were carried out in a single white polystyrene plate (Sarstedt, Catalogue No. DPS-134-050A). The compounds were dissolved in DMSO as 10 mM stock solutions. Compounds were further serially diluted in kinase buffer (40 mM Tris(pH 7.5), 20 mM $MgCl_2$, 0.1mg/ml BSA) in order to obtain the desired concentrations. The kinase assay was performed in 50 μl of kinase buffer containing 2 μg of purified GST-RB152 (in case of Cdk4/GST-cyclin D1) or 3 μg of Histone H1 (in case of Cdk2/GST-cyclin A) and 6 μM ATP.

The phosphatase and protease inhibitor cocktail containing β-glycerophophate, sodium fluoride and sodium orthovanadate in the presence of reducing agent dithiothreitol was added at the final concentrations of 10 mM, 0.1 mM, 0.1 mM and 1 mM, respectively. The assay was initiated by adding 200 ng of active enzyme complexes and the plate was incubated for 30 min at 30ºC in a humidified incubator. The reaction was stopped by addition of equal volume of the Kinase Glow Reagent™ (Promega Cat. No. V6711). The luminescence was measured using the Packard Luminometer (Fusion 3.50) and the rate of ATP depletion (rate of reaction) in the control blank reactions (i.e. without substrate or enzyme) was calculated and used to determine the $IC_{50}$ concentrations of compounds. In case of CDK4/cyclin D1 assay, the two compounds fascaplysin and flavopiridol with known $IC_{50}$ values were used to validate the assay. For the CDK2/cyclin A assay, roscovitine and flavopiridol were used as standards for the assay.

## 5.3 Experimental Procedures

*5.3.1 General procedure for the conversion of substituted phenyl ethanoic acids to substituted phenyl ethanyl chlorides : Procedure A.*

One equivalent of substituted phenyl ethanoic acid was refluxed in thionyl chloride (1 mL $mmol^{-1}$) for 60 minutes, the substituted phenyl ethanoic chloride was then isolated by removal of thionyl chloride by rotary evaporation, dissolved in $CH_2Cl_2$ (2 mL $mmol^{-1}$) and cooled to $0^oC$. The acid chloride solution was then added drop wise to a stirred solution of tryptamine (1.5 eq.) and NaOH (4M aq., 1.5 eq.) in $CH_2Cl_2$ (20mL $mmol^{-1}$) under nitrogen at 0 $^oC$. The reaction mixture was then stirred for 60 minutes at 0 $^oC$ and a further 60 minutes at room RT before being washed with water (3 x 10 mL $mmol^{-1}$), dried over anhydrous magnesium sulphate and evaporated to dryness.

*5.3.2 General procedure for the synthesis of tryptamine-benzamides: Procedure B*

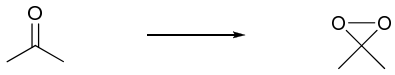
To a stirred solution of tryptamine **18** (or *N*-ω-methyl tryptamine **29** as appropriate) (1.2 mmol) in DCM (3 mL) at 0 $^oC$ was added slowly an aqueous solution of sodium hydroxide 4 M (1.2 mmol). After 5 min stirring at 0 $^oC$ was added dropwise the benzoyl chloride derivative (1.2 mmol). The mixture was stirred for 5 min at 0 $^oC$ and then for 3 h at room temperature. $H_2O$ (20 mL) was added and the two layers were separated, the aqueous phase

was extracted with dichloromethane (3× 20 mL). The combined organic phases were dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by flash column chromatography on silica gel from; ethyl acetate/petroleum ether (40-60 $^oC$) to give the title compound.

*5.3.3 General procedure for the Suzuki-Coupling reactions: Procedure* C

A suspension of the appropriate brominated starting material (1 mmol), $Pd(PPh_3)_4$ (0.0347g, 0.03 mmol) and a solution of $K_2CO_3$ (1ml, 2M aqueous, 2mmol) was prepared in toluene (5ml) under nitrogen, this was stirred for 15 minutes at R.T.. To this was added a solution of the appropriately substituted phenyl boronic acid (1.2 mmol) in ethanol (15 mL), the reaction mixture was then heated to 90 $^oC$ for 24 hrs, before being cooled to room temperature. Residual boronic acid was then oxidised by addition of $H_2O_2$ (1 mL, 30% aqueous) followed by stirring for 60 minutes. The reaction mixture was then extracted into $CHCl_3$ (15 mL), washed with saturated brine solution (2 x 10mL) and water (10 mL), the aqueous phases were extracted with $CHCl_3$ (3 x 10 mL). The combined organic phases were then dried over anhydrous magnesium sulphate and evaporated to dryness, the crude product was purified by column chromatography on silica from ethyl acetate and petroleum ether (40-60 oC).

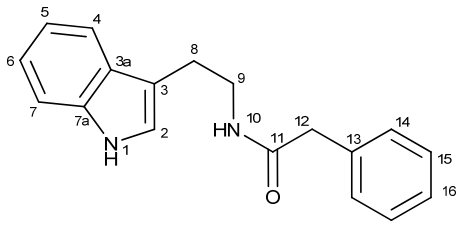
*5.3.4 Synthesis of dimethyldioxirane : Procedure D* [180]



A 3 L three-necked round bottom flask was fitted with an overhead mechanical stirrer, a powder addition funnel and connected, by means of a glass tube, to a cold finger receiver trap cooled to -78 $^oC$ by a dry ice/acetone bath, which was in turn connected to a vacuum pump. The reaction flask was charged with $H_2O$ (75 mL), reagent grade acetone (60 mL) and $NaHCO_3$ (17.4 g, 208 mmol), then cooled to 5 – 10 $^oC$ by a $H_2O$/ice bath. With vigorous overhead stirring oxone (36.6 g, 59.6 mmol) was added in five equal portions at three minute intervals, three minutes after the last addition the powder funnel and the cooling bath were removed from the round bottomed flask and a vacuum applied to the system (110 mbar). A dilute solution of dimethyldioxirane in acetone was collected in the receiver flask; this was dried over anhydrous $K_2CO_3$ and stored at -18 $^oC$, over 4Å molecular sieve until needed.

## 5.4 Experimental Data

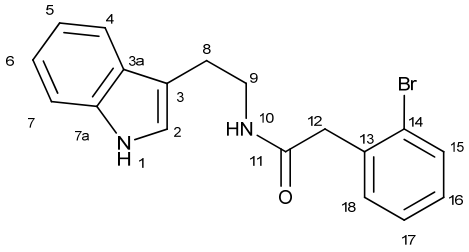
**_N_-[2-(1_H_-Indol-3-yl)-ethyl]-2-phenyl-acetamide 16b.**




Procedure A was followed (phenylacetic acid, 1.36 g, 10 mmol).

Off white solid; 60 % yield; mp. 133-134 $^{o}$C; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 2.8 (2 H, t, 7.3, C(8)H), 3.3 (2H, t, 6.8, C(9)H), 3.4 (2H, s, C(12)H), 7.0 (1H, dt, 8.0, 0.8, C(5)H), 7.1 (1H, dt, 8.0, 0.8, C(6)H), 7.1 (1H, d, 2.3, C(2)H), 7.2 (1H, d, 8, C(4)H), 7.2-7.3 (4H, m, (14+15)H), 7.4 (1H, d, 8, C(12)H), 7.5 (1H, d, 7.8, C(16)H), 8.1 (1H, t, 5.5, N(10)H), 10.8 (1H, s , C(2)H); $\delta_C$(75 MHz; $(CD_3)_2SO$) 28.18, 39.64, 42.47, 111.34, 111.75, 118.20, 120.876, 122.65, 126.24, 127.19, 128.14, 128.97, 136.23, 136.50, 169.96; *m/z* (FAB) 279.14971 ($M+H^+$) $C_{18}H_{19}N_2$ requires 279.14981); (Found: C, 77.58; H, 6.48; N, 9.93. $C_{18}H_{18}N_2O$ requires C, 77.67; H, 6.52; N, 10.06 %).

**2-(2-Bromo-phenyl-_N_-[2-(1_H_-indol-3-yl)-ethyl]-acetamide 19.**



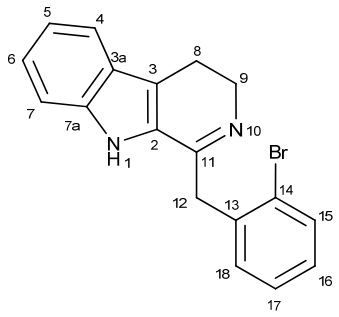

Known compound. [119]

Procedure A was followed (2-bromophenylacetic acid, 2.15 g, 10 mmol).

Light brown solid; yield 86 %; mp. 114-116 $^{o}$C; $\upsilon_{max}$ (ATR) cm$^{-1}$ 3386 (indole NH), 3320, 3273, 1613 and 1559 (amide CO), 739; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 2.90 (2H, t, 7.4, C(8)H), 3.43 (2H, td, 7.4, 5.6, C(9)H), 3.63 (2H, s, C(12)H), 7.02 (1H, td, 7.1, 1.1, C(6)H), 7.11 (1H, td, 7.1, 1.1 C(6)H), 7.20 (1H, d, 2.2, C(2)H), 7.20-7.25 (1H, m, C(17)H), 7.35-7.46 (2H, m, C(16, 4)H), 7.38 (1H, d, 8.0, C(18)H), 7.58 (1H, br-d, C(7)H), 7.62 (1H, d, 8.0, C(15)H), 8.16 (1H, t, 5.6, N(10)H), 10.85 (1H, br-s, N(1)H); $\delta_C$ (75 MHz; $(CD_3)_2SO$) 25.2, 39.7, 42.4, 111.3,

111.7, 118.2, 118.2, 120.9, 122.7, 124.5, 127.2, 127.5, 128.5, 131.8, 132.2, 136.1, 136.2, 168.6; (Found: C, 60.51; H, 4.75; N, 7.94; $C_{18}H_{17}N_2O$ requires C, 60.52; H, 4.80; N, 7.84.); *m/z* (ES) ES+ 357, 359.

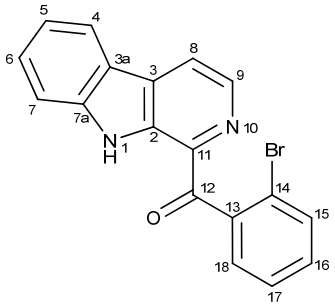
**1-(2-Bromo-benzyl)-4,9-dihydro-3*H*-β-carboline 20.**




Known compound. [119]

2-(2-Bromo-phenyl-*N*-[2-(1*H*-indol-3-yl)-ethyl]-acetamide **19** (1.0861 g, 3 mmol) was refluxed, under nitrogen for 60 minutes, in toluene (30 mL) and $POCl_2$ (3 mL). Toluene and $POCl_2$ were removed under vacuum and the resulting brown residue stirred with dilute acetic acid (60 mL, 50% aqueous) and ethanol (20 mL) for 20 minutes before being filtered. The solution was then cooled to 0 $^oC$ followed by slow addition of $NH_3$ (50 mL, 30% aqueous solution), solid product was isolated by filtration to give 1-(2-Bromo-benzyl)-4,9-dihydro-3*H*-β-carboline (0.7928 g, 78.0 %) as a bright yellow solid; mp. 129-130 $^oC$; IR $\nu_{max}$ 3412, 1672 $cm^{-1}$; *m/z* (ES) ES+ 339, 341; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 2.83 (2H, br-d), 3.30 (2H, br-d, partially obscured by water), 5.81 (2H, br-s), 6.97-7.70 (8H, m), 11.28 (1H, br-s); $\delta_C$ (75 MHz; $(CD_3)_2SO$) 21.1 ($CH_2$), 41.3 ($CH_2$), 47.8 ($CH_2$), 93.0, 111.3, 111.8, 118.5, 118.8, 122.4, 122.7, 125.8, 126.3, 127.6, 128.8, 130.5, 132.7, 137.0, 137.3; *m/z* ($ES^+$) 339 & 341 ($MH^+$); *m/z* (FAB) 339.0499 ($M+H^+$ $C_{18}H_{16}N_2Br$ requires 339.0497); (Found: C, 63.68; H, 4.47; N, 8.19; $C_{18}H_{15}N_2OBr$ requires C, 63.73; H, 4.46; N, 8.26.).

**(2-Bromo-phenyl)-(9*H*-β-carbolin-1-yl)-methanone 21.**

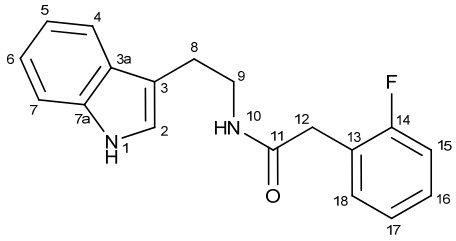


Known compound. [119, 181]

2-(2-Bromo-phenyl-*N*-[2-(1*H*-indol-3-yl)-ethyl]-acetamide **20** was refluxed in $CHCl_3$ (25 mL $mmol^{-1}$) with $MnO_2$ (4-10 fold excess) for 3 hours, the reaction mixture was filtered through celite and the crude product purified by flash column chromatography from ethyl acetate 20%, petroleum ether (40-60 $^oC$) 80% to give (2-Bromo-phenyl)-(9*H*-β-carbolin-1-yl)-methanone as a bright yellow solid; yield 4%; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 7.4 (1H, t, 7.1), 7.4-7.7 (4H, m), 7.8 (1H, dd, 7.8, 1.0), 7.9 (1H, d, 7.9, C(8)H), 8.4 (1H, d, 7.9, C(9)H), 8.5 (2H, s), 12.2 (1H, s, N(1)H), $\delta_C$ (75 MHz; $(CD_3)_2SO$) 113.1, 118.9, 119.6, 119.9, 120.5, 121.9, 127.2, 129.1, 129.4, 131.1, 131.3, 132.3, 135.1, 135.3, 137.8, 141.4, 141.9, 196.8; *m/z* ($ES^+$) 353 & 355 ($MH^+$).

A crystal structure of this compound was previously obtained within the group. [181]

**2-(2-Fluoro-phenyl)-*N*-[2-(1*H*-indol-3-yl)-ethyl]-acetamide 26a.**

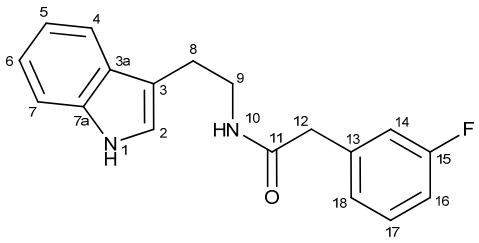


Procedure A was followed (2-flurophenylacetic acid, 0.77 g, 5 mmol).

Off white solid; yield 48 %; mp. 108-109 $^oC$; $\upsilon_{max}$(ATR)/$cm^{-1}$ 3285, 1612 and 1564 (amide CO), 740; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 2.85 (2H, t, 7.3, C(8)H), 3.37 (2H, br, C(9)H), 3.49 (2H, s), 6.98 (1H, t, 7.7, C(5)H), 7.07 (1H, t, 7.1, C(6)H), 7.10-7.18 (3H, m, C(18,17,2)H), 7.20-7.36 (2H, m, C(15,16)H), 7.35 (1H, d, 8.0, C(4)H), 7.54 (1H, d, 8.0, C(7)H), 8.17 (1H, t, 5.5,

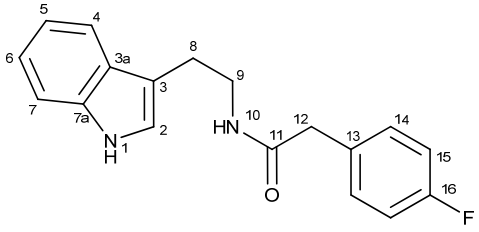
N(10)H), 10.82 (1H, s, N(1)H), $\delta_F$ (282 MHz; ($(CD_3)_2SO$) -118.16; $\delta_C$ (75 MHz; $(CD_3)_2SO$) 23.4, 36.8, 41.2, 112.4, 113.2, 115.8, 116.1, 119.4, 119.6, 122.2, 123.6, 125.1, 125.2, 128.6, 129.6, 129.7, 132.8, 132.8, 137.8, 170.6, 207.0; *m/z* (FAB) 297.14028 ($M+H^+$ $C_{18}H_{18}N_2OF$ requires 297.14039); (Found: C, 72.99; N, 9.39; H, 5.65. $C_{18}H_{17}N_2OF$ requires C, 72.96; N, 9.45; H, 5.78.).

**2-(3-Fluoro-phenyl)-*N*-[2[(1*H*-indol-3-yl)-ethyl]-acetamide 26b.**




Procedure A was followed (4-flurophenylacetic acid, 0.77 g, 5 mmol)..
Off white solid; yield 72 %; mp. 96 $^oC$; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 2.88 (2H, t, 7.2, C(8)H), 3.42 (2H, td, 7.2, 5.4, C(9)H), 7.02 (1H, t, 7.9, C(5)H), 7.11 (1H, t, 7.9 , C(6)H), 7.1-7.2( 4H, m, C(14, 17, 18)H), 7.33 (1H, dt, 6.3, 8.0, C(16)H), 7.39 (1H, d, 8.0, C(4)H), 7.58 (1H, d, 7.4, C(7)H), 8.23 (1H, t, 5.4, N(10)H), 10.86 (1H, s, N(1)H); $\delta_F$ (282 MHz, $(CD_3)_2SO$) -115.2; $\delta_C$ (75 MHz; $(CD_3)_2SO$) 26.3, 40.9, 43.4, 112.2, 113.3,113.9, 114.1, 116.7, 117.0, 119.3, 119.4, 122.1, 123.4, 126.1, 126.1, 128.6, 130.8 (d, $J_{CF}$ 8.3), 206.4; *m/z* (FAB) 297.14025 ($M+H^+$. $C_{18}H_{18}N_2OF$ ($M+H^+$) requires 297.14043); Found: C, 72.91; N, 5.78; H, 9.45; $C_{18}H_{17}N_2OF$ requires C, 72.96; N, 5.78; H,9.45.

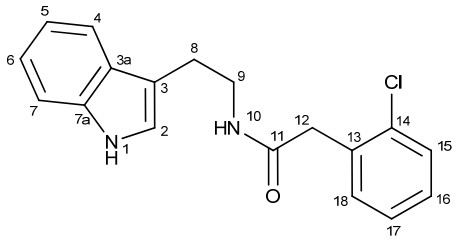
**2-(4-Fluoro-phenyl)-*N*-[2[(1H-indol-3-yl)-ethyl]-acetamide 26c.**




Procedure A was followed (4-flurophenylacetic acid, 0.77 g, 5 mmol)..

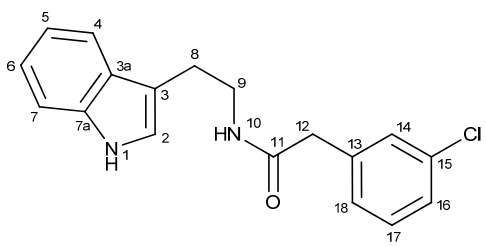
Off white solid; yield 61 %; mp. 133 $^{o}$C; $\delta_H$(300 MHz; $(CD_3)_2SO$) 2.85 (2H, t, 7.0, C(8)H), 3.41 (2H, t, 7.0, 5.4, C(9)H), 3.4 (2H, s, C(12)H), 6.98 (1H, dt, 7.4, 1.0, C(5)H), 7.07 (1H, dt, 8.0, 1.1, C(6)H), 7.1-7.2 (m, 3H, C(2)H), 7.27 (2H, dd, 5.6 $J_{HF}$, 4.4, C(15)H), 7.36 (1H, d, 8, C(4)H), 7.52 (1H, d, 8, C(7)H), 8.14 (1H, t, 5.4, N(10)H), 10.81 (s, N(1)H); $\delta_F$ (282 MHz; $(CD_3)_2SO$) -118.24; $\delta_C$ (75 MHz; $(CD_3)_2SO$) 26.3, 40.9, 42.9, 112.2, 113.3, 115.6, 115.9, 119.3, 119.4, 122.1, 123.4, 128.6, *131.8, 131.9*(d, $J_{CF}$) 133.5, 137.7, 206.4; *m/z* (FAB) 297.14030 (M+H+) $C_{18}H_{18}N_2OF$ requires 297.14039); Found: C, 71.85; H, 4.99; N, 8.97; $C_{18}H_{17}N_2OF$ requires C, 72.96; H, 5.78; N, 9.45).

**2-(2-Chloro-phenyl)-*N*-[2-(1*H*-indol-3-yl)-ethyl]-acetamide 26d.**



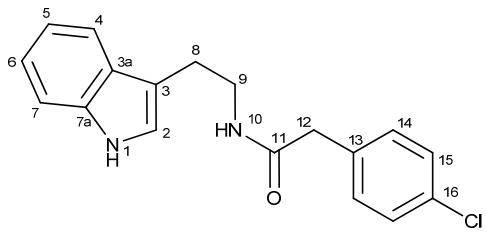

Procedure A was followed (2-chlorophenyl acetic acid, 0.85 g, 5 mmol).
Off white solid; yield 83 %; mp. 126-127 $^{o}$C; $\upsilon_{max}$ (ATR) $cm^{-1}$ 3319, 3275, 1612 and 1563 (amide CO), 740; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 2.89 (2H, t, 7.4, C(8)H), 3.42 (2H, td, 7.4, 5.5, C(9)H), 3.63 (2H, s, C(12)H), 7.02 (1H, td, 8.0, 1.2 C(5)H), 7.11 (1H, dt, 8.0, 8.0, 1.2, C(6)H), 7.18 (1H, d, 2.3, C(2)H), 7.29-7.47 (5H, m, C(4, 15, 16, 17)H), 7.58 (1H, d, 8.0, C(7)H), 8.18 (1H, t, 5.5, N(10)H), 10.85 (1H, br-s, N(2)H); $\delta_C$ (75 MHz; $(CD_3)_2SO$) 25.2, 39.7, 39.9, 111.3, 111.7, 118.2, 118.2, 120.9, 122.7, 126.9, 127.2, 128.3, 128.9, 131.8, 133.6, 134.3, 136.2, 168.7; *m/z* (FAB) 313.11077 ($MH^+$ $C_{18}H_{18}N_2OCl$ requires 313.11084); Found: C, 69.06; N, 8.88; H, 5.41. $C_{18}H_{17}N_2OCl$ requires C, 69.12; N, 8.96; H, 5.48.

**2-(3-Chloro-phenyl)-*N*-[2-(1*H*-indol-3-yl)-ethyl]-acetamide 26e.**



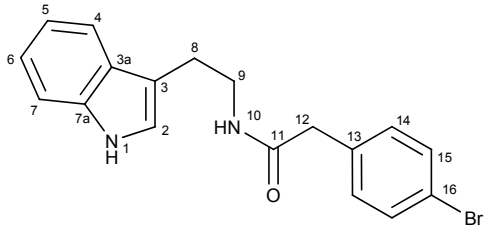
Procedure A was followed (3-chlorophenyl acetic acid, 0.85 g, 5 mmol).
Off white solid; yield 73 %; mp. 62-65 $^{o}$C; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 2.85 (2H, t, 7.3, C(8)H), 3.36 (2H, dt, 6.0, C(9)H), 3.41 (2H, s, C(12)H), 7.00 (1H, dt, 1.1, 8.0, C(5)H), 7.07 (1H, dt, 1.1, 8.0, C(6)H), 7.14 (1H, d, 2.3, C(2)H), 7.25 (1H, d, 7.8, C(4)H), 7.3-7.4 (4H, m, C(16, 16, 17, 18)H), 7.54 (1H, d, 7.8, C(4)H), 8.14 (1H, t, 5.5, N(10)H), 10.81 (1H, s, N(1)H), $\delta_C$ (75 MHz; $(CD_3)_2SO$) 25.1, 39.8, 41.8, 111.4, 111.7, 118.2 (2C), 120.9, 122.6, 126.3, 127.2, 127.7, 128.9, 129.9, 132.7, 136.2, 138.9, 169.4; *m/z* (FAB) 313.11077 ($M+H^+$ $C_{18}H_{18}N_2OCl$ requires 313.11084); Found: C, 68.91; N, 8.81; H, 5.59. $C_{18}H_{17}N_2OCl$ requires C, 69.12; N, 8.96; H, 5.48.

**2-(4-Chloro-phenyl)-*N*-[2-(1*H*-indol-3-yl)-ethyl]-acetamide 26f.**



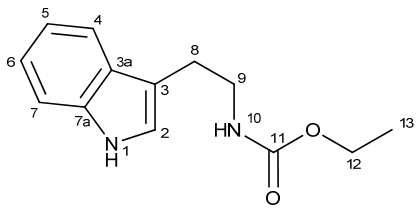

Procedure A was followed (4-chlorophenyl acetic acid, 0.85 g, 5 mmol).
Off white solid; yield 60 %; mp. 133-135 $^{o}$C; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 2.85 (2H, t, 7.2, C(8)H), 3.37 (2H, q, 7.0, C(9)H), 3.47 (2H, s, C(12)H), 6.99 (1H, dt, 0.8, 7.4, C(5)H), 7.08 (1H, dt, 0.8, 7.1, C(6)H), 7.13 (1H, d, 2.1, C(2)H), 7.25 (2H, d, 8.5, C(15)H), 7.34 (2H, d, 8.5, C(14)H), 7.37 (1H, d, 7.7, C(4)H), 7.53 (2H, d, 7.7, C(7)H), 8.21 (1H, t, 5.5, N(10)H), 10.85 (1H, s, N(1)H); $\delta_C$ (75 MHz; $(CD_3)_2SO$) 25.1, 39.7, 41.6, 111.4, 111.7, 118.2, 120.9, 122.7, 127.2, 128.1, 130.8, 131..0, 135.5, 136.3, 169.7; *m/z* (FAB) 313.11085 (M+H+ $C_{18}H_{18}N_2OCl$ requires 313.11084); Found: C, 68.38; N, 8.88, H, 3.61. $C_{18}H_{17}N_2OCl$ requires C, 69.12; N, 8.96; H, 5.48.

**2-(4-Bromo-phenyl)-*N*-[2-(1*H*-indol-3-yl)-ethyl]-acetamide 26g.**




Procedure A was followed (4-bromophenylacetic acid, 1.08 g, 5 mmol).
Off white solid; yield 79 %; mp. 141-142 $^{o}$C; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 2.85 (2H, t, 7.3, C(8)H), 3.38 (2H, td, 7.3, 5.6,C(9)H), 3.41 (2H, s, C(12)H), 6.99 (1H, dt, 1.0, 7.0, C(5)H), 7.09 (1H, dt, 1.0, 8.0, C(6)H), 7.13 (1H, d, 2.2, C(2)H), 7.20 (2H, d, 8.4, C(14)H), 7.37 (1H, d, 8.0, C(4)H), 7.48 (2H, d, 8.4, C(15)H), 7.54 (1H, d, 7.5, C(7)H), 8.18 (1H, t, 5.6, N(10)H), 10.83 (1H, s, N(1)H), $\delta_C$ (75 MHz; $(CD_3)_2SO$) 25.2, 39.7, 41.7, 111.4, 111.7, 118.2 (2C), 119.5, 120.9, 122.7, 127.2, 131.0, 131.2, 135.9, 136.2, 169.5; *m/z* (ES) ES+ 357, 359.

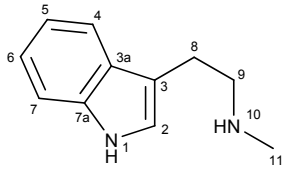
**[2-(1*H*-Indol-3-yl)-ethyl]-carbamin acid ethyl ester 28.** [106, 182]




Known compound.
To a solution of tryptamine **18** (10.00 g, 62.4 mmol) in chloroform (156 mL) at 0°C was added ethylchloroformate (5.97 mL, 62.4mmol) and an aqueous solution of NaOH (4M, 15.60 mL, 62.4 mmol). The mixture was then stirred for 3h at R.T, and then washed with water (150 mL). The two layers were separated and the aqueous phase was extracted into dichloromethane (2 × 150 mL). The combined organic phases were dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to give an orange oil. No purification was required. The oil was dried in vaccuo to give the title compound **28** (13.78 g, 95%). $\delta_H$ (300 MHz; $CDCl_3$) 1.33 (3H, t, 7.0), 3.05 (2H, t, 6.5), 3.60 (2H, q, 6.5), 4.24 (2H, q, 7.0), 5.12 (1H, br s), 6.99 (1H, s), 7.23 (1H, t, 6), 7.30 (1H, td, 6, 1.2), 7.42 (1H, d, 7.7), 7.71 (1H, d, 7.7), 8.75 (1H, s). $\delta_C$ (75 MHz, $CDCl_3$) 14.57 ($CH_3$), 25.64 ($CH_2$), 41.21 ($CH_2$), 60.72 ($CH_2$),

111.33 (CH), 112.33, 118.54 (CH), 119.11 (CH), 121.83 (CH), 122.26 (CH), 127.18, 136.39, 156.93; *m/z* (FAB$^+$) 232.12126 (M+H)$^+$, 465 (2M+H)$^+$ $C_{13}H_{16}N_2O_2$ requires (MH) 232.12118.

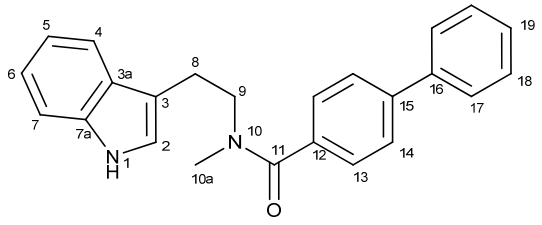
**[2-(1*H*-Indol-3-yl)-ethyl]-methyl-amide. (*N*-ω-Methyl tryptamine) 29.** [106, 183]




Known compound.

To a solution of **28** (13.78 g, 59.4 mmol) in dry THF (110 mL) under $N_2$ at 0 ºC was added LiAlH (6.76 g, 178 mmol). After the addition was complete the mixture was heated to reflux for 1h. The reaction was then cooled to 0 ºC and excess LiAlH was hydrolysed by the addition of $H_2O$ (13.25 mL), 15% aqueous solution of NaOH (13.25 mL) and water (3×13.25 mL) successively. During these steps it was necessary to add THF (100 mL) to avoid the mixture becoming too viscous for efficient stirring. The suspension was filtered and the white solid, made up of LiOH and $Al(OH)_3$, was washed with THF (30 mL). The organic layer was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to give the title compound **29** (9.24 g, 89 %) as a beige solid; mp. 82 $^oC$; $\delta_H$ (300MHz, $CDCl_3$) 1.47 (1H, s), 2.35 (3H, s), 2.81-2.86 (2H, m), 2.89-2.94 (2H, m), 6.80 (1H, s), 7.03 (1H, td, 7.4, 1.2), 7.10 (1H, td, 7.4, 1.2), 7.19 (1H, d, 7.6), 7.54 (1H, d, 7.6), 9.52 (1H, s); $\delta_C$ (75MHz, $CDCl_3$) 25.42 ($CH_2$), 35.99 ($CH_3$), 51.82 ($CH_2$), 111.32 (CH), 112.91, 118.65 (CH), 118.85 (CH), 121.59 (CH), 122.45 (CH), 127.30, 136.53; *m/z* (FAB+) 175.12354 (M+H)$^+$ $C_{11}H_{15}N_2$ requires MH , 175.12352; Found : C, 75.74 ; H, 8.04 ; N, 16.00 ; $C_{11}H_{15}N_2$ requires C, 75.82 ; H, 8.10 ; N, 16.08 %.

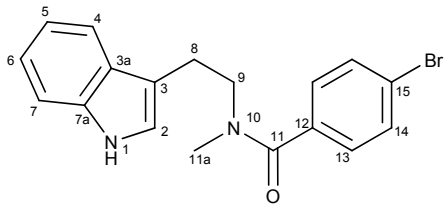
**N-(2-(1H-indol-3-yl)ethyl)-N-methylbiphenyl-4-carboxamide 27.**




Procedure C was followed (phenylboronic acid, 0.15 g, 1.2 mmol).

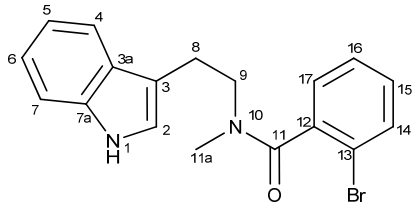
Beige solid; yield 58%; mp 178 °C; $\delta_H$ (300MHz, $CDCl_3$) Rotamers 1:1.5 (from the duplicated triplet signal (1H) at 3.59 and 3.89 ppm). *δ* (major rotamer) 2.95 (2H, distorted t, 6.8), 3.18 (3H, s), 3.59 (2H, t, 6.8), 6.84–7.55 (14H, m), 8.42 (1H, br-s). *δ* (distinct peaks for minor rotamer) 2.91 (3H, br s), 3.89 (2H, m), 7.72 (1H, br d, 6.9); $\delta_C$ (100MHz, $CDCl_3$) *δ* (major rotamer) 24.4 ($CH_2$), 33.1 ($CH_3$), 51.9 ($CH_2$), 111.33 (2×CH), 111.6, 118.2 (CH), 119.3 (2×CH), 121.96 (2×CH), 127.01 (2×CH), 127.2 (2×CH), 127.5, 127.7 (CH), 128.9 (2×CH), 135.2, 136.3, 140.5, 142.0, 172.4, *δ* (distinct peaks for minor rotamer) 23.0 ($CH_2$), 38.3 ($CH_3$), 48.6 ($CH_2$), 112.9, 118.7 (CH), 122.3 (CH), 135.5, 142.3, 171.3; *m*/*z* (ES+) 355 ($MH^+$); ($ES^-$) 353 $(M-H)^-$; *m*/*z* ($FAB^+$) 355.18103 ($MH^+$) $C_{24}H_{22}N_2O$ requires (MH)+ 355.18104; Found: C, 81.34; H, 6.07; N, 7.84 %; $C_{24}H_{22}N_2O$ requires C, 81.26; H, 6.26; N, 7.90 %.

**4-Bromo-*N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-methylbenzamide 31.** [106]




Procedure B was followed (4-bromobenzoyl chloride, 0.26 g, 1.2 mmol).
White solid; yield 73%; mp 160 °C; $\delta_H$ (300MHz, $CDCl_3$) Rotamers 1:1.5 (from the duplicated triplet signal (1H) at 3.45 and 3.77 ppm). *δ* (major rotamer) 2.83 (2H, t, 6.3), 3.07 (3H, s), 3.45 (2H, t, 6.3), 6.71–7.62 (9H, m), 8.41 (1H, br-s). *δ* (distinct peaks for minor rotamer) 2.76 (3H, s), 3.77 (2H, t, 7.1); $\delta_C$ (100MHz, $CDCl_3$) *δ* (major rotamer) 24.2 ($CH_2$), 33.0 ($CH_3$), 51.7 ($CH_2$), 111.4 (CH), 112.7, 118.0 (CH), 119.4 (CH), 122.1 (CH), 122.4 (CH), 123.3, 127.0, 128.1 (2×CH), 131.3 (2×CH), 135.0, 136.3; 171.4. *δ* (distinct peaks for minor rotamer) 22.89 ($CH_2$), 38.2 ($CH_3$), 48.6 ($CH_2$), 118.7 (CH), 123.7, 127.5, 128.7 (CH), 131.6 (CH), 135.5, 170.4; *m*/*z* (ES+) 358 ($MH^+$); ($ES^-$) 356 $(M-H)^-$.

**2-Bromo-N-[2-(1H-indol-3-yl)-ethyl]-N-methyl-benzamide 34.** [106]

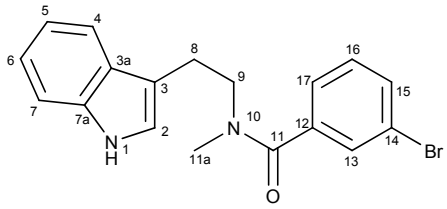



Procedure B was followed (2-bromobenzoyl chloride, 0.26 g, 1.2 mmol)..

Known compound. [106]

Pale yellow solid; yield 48 %; mp. 205 °C; Rotamers 1/1.2 (from the duplicated triplet signal ($^1$H) at 3.34 and 3.79 ppm). $\delta_H$ (400 MHz, DMSO) δ *(major rotamer)* 2.92 (2H, t, 7.3, C(8)H), 3.10 (3H, s, C(11a)H), 3.34 (2H, t, 7.3, C(19)H), 6.90-7.10 (3H, m), 7.19-7.44 (5H, m), 7.63 (1H, br-t, 9.4), 10.61 (1H, br-s, N(1)H); (distinct peaks for *minor rotamer)* 2.77 (3H, s), 3.79 (2H, t, 7.6), 6.84 (1H, t, 7.4). $\delta_C$ (75MHz, DMSO) *(major rotamer)* 24.3, 32.4, 51.3, 110.7, 111.9, 118.1, 118.7, 118.8, 121.4, 123.5, 127.7, 128.1, 128.5, 130.7, 132.7, 136.6, 138.8, 168.3; (distinct peaks for *minor rotamer)* 22.9, 36.5, 47.7, 111.6, 118.7, 118.9, 121.4, 123.4, 127.3, 130.9, 132.9, 136.8, 139.2, 168.2; *m/z* ($ES^+$) 358, ($ES^-$) 356 $(M-H)^-$.

**3-Bromo-*N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-methyl-benzamide 35.** [106]

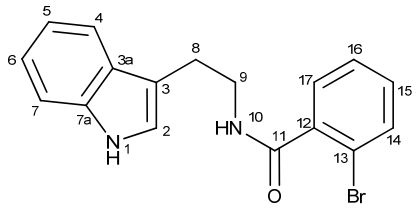



Procedure B was followed (3-bromobenzoyl chloride, 0.26 g, 1.2 mmol).

Known compound. [106]

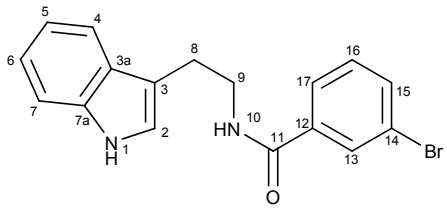
Off white solid; yield 76 %; mp. 166 °C; Rotamers 1/1.4 (from the duplicated triplet signal ($^1$H) at 3.46 and 3.78 ppm); $\delta_H$ (300 MHz, $CDCl_3$) *(major rotamer)* 2.87 (2H, t, 6.9, C(10)H), 3.09 (3H, s, C(11a)H), 3.46 (2H, t, 6.9, C(19)H), 6.76-7.61 (9H, m), 8.25 (1H, br s, N(1)H); (distinct peaks for *minor rotamer)* 2.76 (3H, s), 3.78 (2H, t, *J* 6.9); $\delta_C$ (75MHz, $CDCl_3$) *(major rotamer)* 24.2, 33.1, 51.9, 111.4, 112.7, 118.0, 119.4, 122.1, 122.3, 125.0, 127.0, 129.8, 129.9, 132.1, 133.0, 136.2, 138.2, 170.7; (distinct peaks for *minor rotamer)* 22.9, 38.2, 48.61, 118.7, 125.5, 127.5, 129.5, 130.1, 132.5, 136.0, 138.6, 169.7; *m/z* ($ES^+$) 357 $M^+$, 358 $MH^+$, ($ES^-$) 356 $(M-H)^-$.

**2-Bromo-*N*-[2-(1*H*-indol-3-yl)-ethyl]-benzamide 36.** [106]



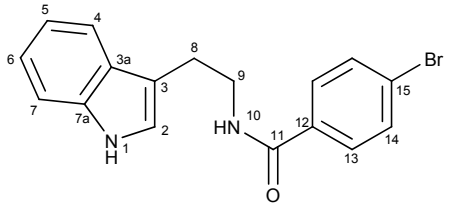

Procedure B was followed (2-bromobenzoyl chloride, 0.26 g, 1.2 mmol).
Off white solid; yield 86 %, mp. 144-145 $^{o}$C; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 3.0 (2H, t, 7.7, C(8)H), 3.5 (2H, td, 7.7, 5.7, C(9)H), 7.0 (1H, dt, 1.2, 7.9, C(5)H), 7.1 (1H, dt, 1.2, 6.9, C(6)H), 7.2 (1H, d, 2.2, C(2)H), 7.3-7.5 (4H, m, C(4, 15, 16, 17)H), 7.6 (1H, d, 7.7, C(7)H), 8.6 (1H, t, 5.7, N(10)H), 10.9 (1H, s, N(1)H), $\delta_C$ (75 MHz; $(CD_3)_2SO$) 25.5, 40.4, 111.9, 112.1, 118.7 (2C), 119.5, 121.4, 123.3, 127.7, 128.0,129.2, 131.2, 133.1, 136.7, 139.8, 167.7; *m/z* (FAB) ($MH^+$) 343.04427 ($C_{17}H_{16}BrN_2O$ requires 343.04460).

**3-Bromo-*N*-[2-(1*H*-indol-3-yl)-ethyl]-benzamide 37.**




Procedure B was followed (3-bromobenzoyl chloride, 0.26 g, 1.2 mmol).
Off white solid; yield 79 %; mp. 132-135 $^{o}$C; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 3.0 (2H, t, 7.6, C(8)H), 3.6 (1H, br-t, 7.6, C(9)H), 7.0 (1H, t, 7.0, C(5)H), 7.1 (1H, t, 7.0, C(6)H), 7.2 (1H, d, 2.1, C(2)H), 7.4 (1H, t, 7.9), 7.6 (1H, d, 8.9), 7.7 (1H, d, 8.9), 7.9 (1H, d, 7.9), 8.1 (1H, N(10)H), 10.8 (1H, s, N(1)H), $\delta_C$ (75 MHz; $(CD_3)_2SO$) 25.0 ($CH_2$), 40.3 ($CH_2$), 111.4, 111.8, 118.2 (2C), 120.9, 121.6, 122.6, 126.3, 127.3, 129.9, 130.5, 133.8, 136.2, 136.9, 164.6; *m/z* ($ES^+$) 343 & 345 $MH^+$, ($ES^-$) 341 & 343 $M\text{-}H^-$, *m/z* (FAB) ($MH^+$) 343.04427 ($C_{17}H_{16}BrN_2O$ requires 343.04460).

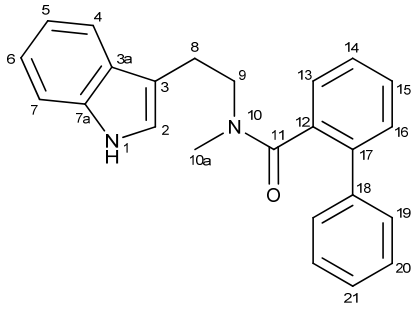
**4-Bromo-*N*-[2-(1*H*-indol-3-yl)-ethyl]-benzamide 38.**




Procedure B was followed (4-bromobenzoyl chloride, 0.26 g, 1.2 mmol).

Pale yellow solid; yield 59%; mp. 148-149 $^{o}$C; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 3.0 (2 H, t, 7.4, C(8)H), 3.6 (2 H, q, 6.9, C(9)H), 7.0 (1 H, t, 7.4, C(5)H), 7.1 (1 H, t, 7.4, C (6)H), 7.2 (1 H, d, 1.5, C(2)H), 7.4 (1 H, d, 8.1, C(4)H), 7.6 (1 H, d, 7.8, C(7)H), 7.7 (2 H, d, 8.7, C(14)H), 7.8 (2 H, d, 8.4, C(13)H), 8.7 (1 H, t, 5.4, N(10)H), 10.8 (1 H, s, N(1)H); $\delta_C$(75 MHz; $(CD_3)_2SO$) 25.1, 40.3 (under solvent peak), 111.4, 111.8, 118.2, 120.9, 122.7, 126.2, 127.2, 128.1, 129.0, 136.2, 136.5, 170.0; *m/z* (FAB) 343.04453 ($M+H^+$ $C_{17}H_{16}N_2OBr$ requires 343.04467); Found: C, 59.38; N, 8.16; H, 4.35 %; $C_{17}H_{15}N_2OBr$ requires C, 59.49; N, 8.16; H, 4.40 %.

**Biphenyl-2-carboxylic acid [2-(1H-indol-3-yl)-ethyl]-methyl-amide 39**

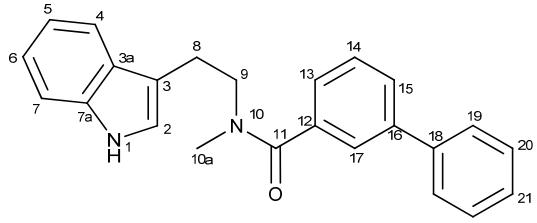



Procedure C was followed (phenylboronic acid, 0.15 g, 1.2 mmol).

Off white solid; 35 % yield; $\delta_H$ (300 MHz; $(CD_3)_2SO$) Overlapping rotamers 1:2.1 (from the duplicated triplet signal ($^1$H) at 2.48 and 2.83 ppm) (distinct peaks from major rotomer) 2.48 (3H, br), 2.65 (2H, br), 10.78 (1H, br-s) (distinct peaks from minor rotomer) 2.83 (3H, br), 10.26 (1H, br-s) (indistinct peaks) 2.62 (2H, br), 3.25 (2H, br) 3.31 (2H, br) 6.70-7.55 (14H, m); $\delta_C$ (75 MHz; $(CD_3)_2SO$) (distinct peaks from major rotomer) 21.9 ($CH_2$), 36.0 ($CH_3$), 50.5 ($CH_2$,) 169.9, (distinct peaks from minor rotomer) 23.6 ($CH_2$), 31.8 ($CH_3$), 47.1 ($CH_2$), (indistinct peaks) 110.3, 111.2 (CH), 111.4 (CH), 117.6 (CH), 117.8 (CH), 118.3 (CH), 120.9 (CH), 120.9 (CH), 122.6 (CH), 122.8 (CH), 126.8 (CH), 127.1 (CH), 127.3 (CH), 127.5 (CH), 128.1 (CH), 128.4 (CH), 128.9 (CH), 129.1 (CH), 129.2 (CH), 129.4 (CH), 132.2, 135.8,

136.1, 136.2, 137.5, 138.0, 139.6; Found: C,76.59; N,7.72; H,6.00; $C_{24}H_{22}N_2O$ requires C,81.33; N,7.90; H,6.26); *m/z* ($ES^+$) 355 ($MH^+$).

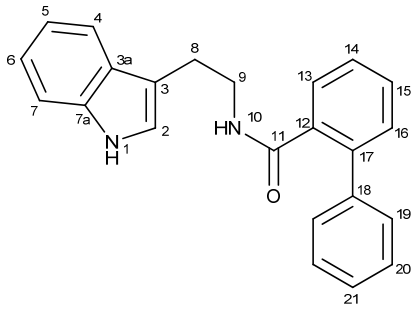
**Biphenyl-3-carboxylic acid-[2-(1*H*-indol-3-yl)-ethyl]-methyl-amide 40.**



Procedure C was followed (phenylboronic acid, 0.15 g, 1.2 mmol).

Off white solid; 82 % yield; indiscrete melting point; $\delta_H$ (300 MHz; $(CD_3)_2SO$) Overlapping rotamers 1/1.72 (from the duplicated triplet signal ($^1H$) at 2.92 and 3.77 ppm) (distinct peaks from major rotomer) 2.87 (2H, m), 3.12 (2H, m), 3.49 (2H, m), 3.75 (1H, t, 7.2), 10.84 (1H, br-s), (distinct peaks from minor rotomer) 3.12 (2H, m), 3.10 (2H, m), 3.77 (2H, m), 10.92 (1H, br-s), (indistinct peaks) 6.93-7.71 (14H, m); $\delta_C$ (75 MHz; $(CD_3)_2SO$) 24.0 (distinct peaks from major rotomer) 24.0 ($CH_2$), 23.4 ($CH_2$), 51.6 ($CH_2$), 126.7 (2×CH), 128.9 (2×CH), 170.6 (distinct peaks from minor rotomer) 20.9 ($CH_2$), 37.2 ($CH_2$), 47.9 ($CH_2$), 120.9 (2×CH), 123.0 (2×CH), 169.7 (indistinct peaks) 110.4, 114.4, 114.4, 117.8, 118.2, 118.3, 124.4, 124.8, 125.4, 125.7, 126.9, 127.1, 127.4, 127.7, 136.1, 137.6, 139.4, 140.1, 140.2; *m/z* ($ES^+$) 355 ($MH^+$); *m/z* (FAB) 355.18092 ($M+H^+$ $C_{24}H_{23}N_2O$ requires 355.18115).

**Biphenyl-2-carboxylic acid[2-(1*H*-indol-3-yl)-ethyl]-amide 41.**

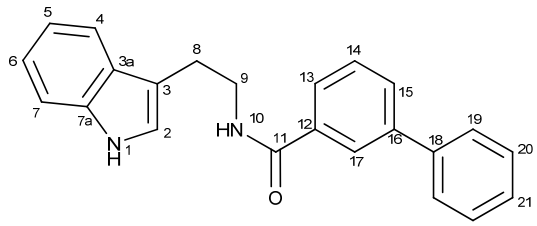


Procedure C was followed (phenylboronic acid, 0.15 g, 1.2 mmol).

Off white solid; yield 86 %; mp. 67 $^oC$; $\upsilon_{max}$(ATR)/$cm^{-1}$ 3402 (w, indole NH), 3267 and 3056 (amide NH), 1634 and 1520 (amide CO), 739, 699, 661; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 2.75 (2H, t, 7.6, C(8)H), 3.40 (2H, td, 7.6, 5.7, C(9)H), 7.00 (1H, td, 7.4, 1.1), 7.07-7.12 (2H, m), 7.33-7.54 (11H, m), 8.27 (1H, t, 5.7, N(10)H), 10.82 (1H, br-s, N(1)H); $\delta_{13}$ (75 MHz; $(CD_3)_2SO$)

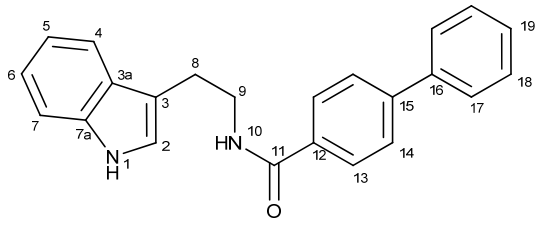
24.6 ($CH_2$), 39.7 ($CH_2$), 111.3 (CH), 111.6, 118.1 (2C, CH), 120.8 (CH), 122.4 (CH), 126.9 (CH), 127.0 (CH), 127.5 (CH), 128.0 (2C, CH), 128.3 (2C, CH), 129.2 (CH), 129.7 (CH), 136.1, 137.4, 138.9, 140.1, 169.0; *m/z* ($ES^+$) 341 ($MH^+$), ($ES^-$) 339 ($M-H^-$); *m/z* (FAB) 341.16529 ($M+H^+$ $C_{23}H_{21}N_2O$ requires 341.16539); Found: C, 81.07; N, 8.12; H, 6.03%; $C_{23}H_{20}N_2O$ requires C, 81.15; N, 8.23; H, 5.92 %.

**Biphenyl-3-carboxylic acid [2-(1H-indol-3-yl)-ethyl]-amide 42.**




Procedure C was followed (phenylboronic acid, 0.15 g, 1.2 mmol).
Off white solid; yield 62 %; mp 59–69 $^oC$; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 2.98 (2H, t, 7.5), 3.58 (2H, td, 7.5, 5.5), 6.98 (1H, td, 7.5, 1.1), 7.07 (1H, td, 7.5, 1.1), 7.20 (1H, d, 2.2), 7.35 (1H, br-d, 8.0), 7.42 (1H, br-d, 7.3), 7.50 (2H, t, 7.9), 7.57 (1H, d, 7.9, 7.9), 7.60 (1H, d, 7.9), 7.73 (2H, dd, 7.9, 1.4), 7.80–7.86 (2H, m), 8.12 (1H, t, 1.5), 8.74
(1H, t, 5.5), 10.82 (1H, br s); $\delta_C$ (75 MHz; $(CD_3)_2SO$) 25.1 ($CH_2$), 40.3 ($CH_2$), 111.3 (CH), 111.8, 118.2 (CH), 118.2 (CH), 120.8 (CH), 122.6 (CH), 125.2 (CH), 126.3 (CH), 126.8 (CH, 2×C), 127.2, 127.7 (CH), 128.9 (CH, 2C), 129.1 (CH), 135.3, 136.2, 139.5, 140.1, 165.9; *m/z* (FAB) 341.16544 (M+H+ $C_{23}H_{21}N_2O$ requires 341.16539). Found: C, 77.39; N, 7.89; H, 5.78 %. $C_{23}H_{20}N_2O$ requires C, 81.15; N, 8.23; H, 5.92 %.

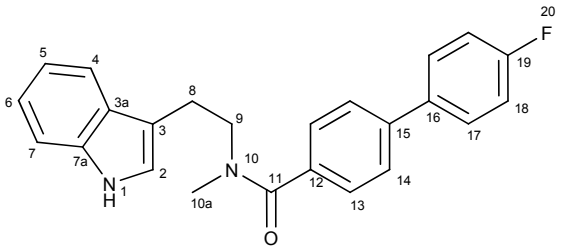
**Biphenyl-4-carboxylic acid [2-(1*H*-indol-3-yl)-ethyl]-amide 43.**




Procedure C was followed (phenylboronic acid, 0.15 g, 1.2 mmol).

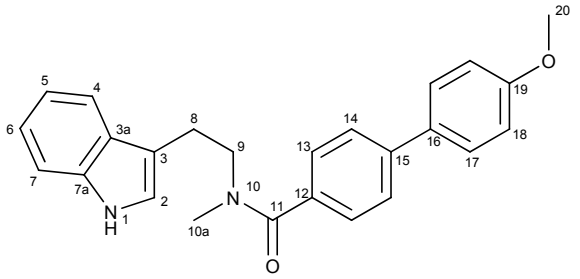
Off white solid; yield 63 %; mp 183-185 $^{o}$C; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 3.02 (2H, t, 7.5, C(8)H), 3.62 (2H, dt, 7.5, 5.6, C(9)H), 7.03 (1H, td, 7.5, 1.1, C(5)H), 7.11 (1H, td, 7.5, 1.1, C(6)H), 7.23 (1H, d, 2.2, C(2)H), 7.39 (1H, d, 7.5, C(4)H), 7.44 (1H, dd, 7.4, 1.1, C(19)H), 7.51 (2H, t, 7.4, C(18)H), 7.64 (1H, d, 7.4, C(7)H), 7.76 (2H, d, 7.4, C(17)H), 7.80 (2H, d, 8.4, C(14)H), 8.00 (2H, d, 8.4, C(13)H), 8.71 (1H, t, 5.6, N(10)H), 10.86 (1H, br, N(1)H); $\delta_{13}$ (75 MHz; $(CD_3)_2SO$) 25.2, 40.3, 111.4, 111.9, 118.2, 118.3, 120.9, 122.6, 126.4, 126.8, 127.3, 127.8, 128.0, 129.0, 133.5, 136.2, 139.2, 142.5, 165.8; *m/z* (FAB) 341.16532 ($M+H^+$ $C_{23}H_{21}N_2O$ requires 341.16546); Found: C, 81.09; N, 8.18; H, 5.95%. $C_{23}H_{20}N_2O$ requires C, 81.15; N, 8.23; H, 5.92 %.

**4'-Fluoro-biphenyl-4-carboxylic acid [2-(1H-indol-3-yl)-ethyl]-methyl-amide 44.**[145]



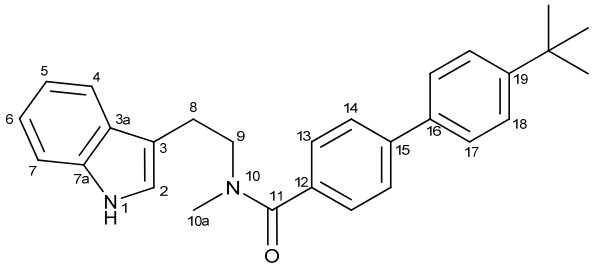

Procedure C was followed (4-fluorophenylboronic acid, 0.17 g, 1.2 mmol).
Pale yellow solid; yield 65%; mp. 181-183 $^{o}$C; $\upsilon_{max}$(ATR) cm$^{-1}$ 3217, 2924, 1604 (amide CO), 1230, 825, 739; $\delta_H$ (300 MHz; $(CD_3)_2SO$) Rotamers 1/1.25 (from the duplicated triplet signal ($^1$H) at 3.47 and 3.74 ppm); 2.91 (3H, br, C(20a)H), 3.08 (2H, br, C(9)H), 3.47 (2H, br, C(8)H), 6.72 (distorted t, C96)H), 6.93-7.06 (4H, m), 7.19-7.34 (7H, m), 7.45 (1H, br-d, 7.2), 7.54 (2H, br-d, 7.6), 7.70 (5H, m), 10.83 (1H, br-s, N(1)H); (distinct peaks for *minor rotamer)* 3.74 (2H, br, C(8)H), 2.91 (3H, br, C(9)H), 3.08 (2H, br, C(20a)H), 10.87 (1H, br-s, N(1)H); $\delta_C$ (75 MHz; $(CD_3)_2SO$) 24.4 ($CH_2$), 32.8 ($CH_3$), 51.9 ($CH_2$) 110.9, 111.9 (CH), 116.3 (d, $^2J_{CF}$, 21, CH), 118.3 (CH), 118.7 (CH), 121.4 (CH), 123.7 (CH), 126.8 (CH), 127.4 (CH), 128.0, 129.2 (d, $^3J_{CF}$ 8, CH), 136.2, 136.4, 136.7, 140.0, 162.5 (d, $^1J_{CF}$, 245), 171.0; (distinct peaks for *minor rotamer)* 23.1 ($CH_2$), 37.9 ($CH_3$), 48.4 ($CH_2$), 123.3 (CH), 128.0, 170.0; Found: C, 77.32; N, 7.42; H, 5.60 %; $C_{24}H_{21}N_2OF$ requires C, 77.40; N, 7.52; H ,5.68 %.

**4'-Methoxy-biphenyl-4-carboxylic acid-[2-(1*H*-indol-3-yl)-ethyl]-methyl-amide 45.** [145]



Procedure C was followed (4-methoxyphenylboronic acid, 0.18 g, 1.2 mmol).
Off white solid; yield 46%; mp 201–202 $^{o}$C; $\delta_H$ (300 MHz; $(CD_3)_2SO$) Rotomers 1/1.8 (from the duplicated triplet signal (1H) at 3.47 and 3.73 ppm), 2.91 (3H, br), 3.07 (2H, br), 3.46 (2H, br), 3.80 (3H, s), 6.71 (1H, m), 6.92–7.05 (4H, m), 7.18–7.20 (2H, m), 7.29–7.31 (1H, m), 7.41–7.53 (2H, m), 7.62 (3H, br m), 10.81 (1H, br s); d (distinct peaks for minor rotamer) 3.07 (3H, br), 2.91 (2H, br), 3.73 (2H, br), $\delta_C$ (75 MHz; $(CD_3)_2SO$) 23.9 ($CH_2$), 32.3 ($CH_3$), 51.4 ($CH_2$), 55.1 ($CH_3$), 110.3, 111.3 (CH), 114.3 (CH, 4C), 117.8 (CH), 118.0 (CH) 120.8 (CH), 123.0 (CH), 125.8 (CH, 2C), 127.7 (2×CH), 131.7, 134.9, 136.1, 140.2, 140.5, 159.1, 170.5; d (distinct peaks for minor rotamer) 22.5 ($CH_2$), 37.4 ($CH_3$), 47.8 ($CH_2$), 122.7 (CH), 126.8 (CH), 127.4 (CH); $\delta_H$ (300 MHz; $(CD_3)_2SO$, 353 K) 22.9 ($CH_2$), 54.8 ($CH_3$), 110.7, 10.8 (CH), 114.1 (CH), 117.5 (CH), 117.7 (CH), 120.4 (CH), 122.3 (CH), 125.3 (CH), 126.6 (CH), 126.8,127.3 (CH), 131.5, 134.8, 135.9, 140.0, 158.9, 169.8; *m/z* ($ES^+$) 385 ($MH^+$); *m/z* (FAB) 385.19154 ($C_{25}H_{25}N_2O_2$ requires 385.19160).

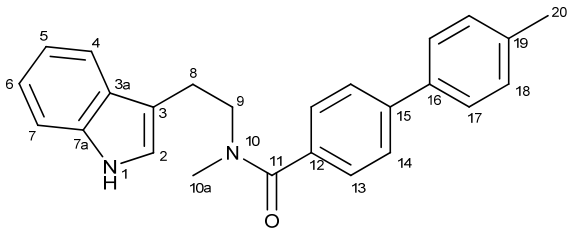
**N-(2-(1H-indol-3-yl)ethyl)-4'-tert-butyl-N-methylbiphenyl-4-carboxamide 46.** [145]



Procedure C was followed (4-*tert*-butylphenylboronic acid, 0.21 g, 1.2 mmol).
Off white solid; yield 51 %; mp 178–180 $^{o}$C; $\delta_H$ (300 MHz; $(CD_3)_2SO$) Overlaping rotomers 1.36 (9H, s, $C(CH_3)3$), 2.86 (3H, br s), 3.04–3.14 (2H, br), 3.60–3.82 (2H, br), 7.08–7.62 (13H, br), 10.05 (1H, br s); $\delta_C$ (75 MHz; $(CD_3)_2SO$) 22.6 ($CH_2$), 23.9 ($CH_2$), 31.0 ($CH_3$), 32.3 ($CH_3$), 34.2 (C), 37.4 ($CH_3$), 47.9 ($CH_2$), 51.4 ($CH_2$), 110.4, 111.4 (CH), 117.9 (CH), 120.9

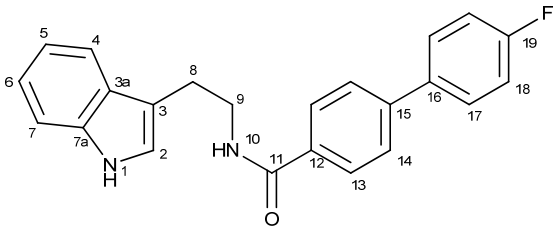
(CH), 123.0 (CH), 125.7 (CH), 126.2 (CH), 126.4 (CH), 126.9 (CH), 127.5 (CH), 135.5, 136.2, 140.5, 140.8, 150.2, 169.6, 170.6; *m/z* ($ES_+$) 411 $(MH)^+$, 433 $(M+Na)^+$, ($ES^-$) 409 ($M-H^-$); *m/z* (FAB) 411.24347 ($MH^+$ $C_{28}H_{31}N_2O$ requires 411.24364); Found: C, 81.81; N, 6.72; H, 7.27 %. $C_{28}H_{30}N_2O$ requires C, 81.91; N, 6.82; H, 7.36 %.

**N-(2-(1H-indol-3-yl)ethyl)-N,4'-dimethylbiphenyl-4-carboxamide 47.** [145]




Procedure C was followed (4-methylphenylboronic acid, 0.16 g, 1.2 mmol).
Off white solid; yield 44%; mp. 215-217 $^oC$; Rotomers 1/2.1 (from the duplicated triplet signal ($^1H$) at 3.46 and 3.73 ppm); $\delta_H$ (300 MHz; $(CD_3)_2SO$) 2.91 (3H, br, C(20a)H), 3.07 (2H, br, C(9)H), 3.46 (2H, br, C(8)H), 6.70 (1H, br, C(5)H), 6.91-7.05 (3H, m), 7.19-7.30 (4H, m), 7.42-7.44 (1H, m), 7.56-7.67 (4H, m), 10.8 (1H, br-s, N(1)H); (distinct peaks for *minor rotamer)* 3.73 (2H, br, C(8)H), 10.83 (1H, br-s, N(1)H); $\delta_H$ (400 MHz; $(CD_3)_2SO$; 353K) 2.38 (3H, s, C(20)H), 2.80-2.95 (5H, m, C(9&20a)H), 3.64 (2H, br-s, C(8)H), 6.89 (1H, br-s), 7.05 (1H, t, 7.5), 7.10 (1H, br-s), 7.23-7.36 (3H, m), 7.56-7.58 (2H, m), 7.61 (5H, d, 8.0), 10.60 (1H, s, N(1)H), $\delta_C$ (75 MHz; $(CD_3)_2SO$) 20.6 ($CH_3$), 23.9 ($CH_2$), 32.3 ($CH_3$), 51.4 ($CH_2$), 110.3, 111.3 (CH), 117.8 (CH), 118.2 (CH), 120.8 (CH), 123.0 (CH), 126.1 (CH, 2C), 126.5 (CH, 2C), 129.5 (CH, 4C), 135.4, 136.1, 136.5, 137.1, 140.4, 140.7, 170.5; (distinct peaks for *minor rotamer)* 22.5 ($CH_2$), 37.3 ($CH_3$), 47.8 ($CH_2$), 126.9 (CH), 127.4 (CH); *m/z* (FAB) 369.19669 ($C_{25}H_{24}N_2O$ requires 369.19654).

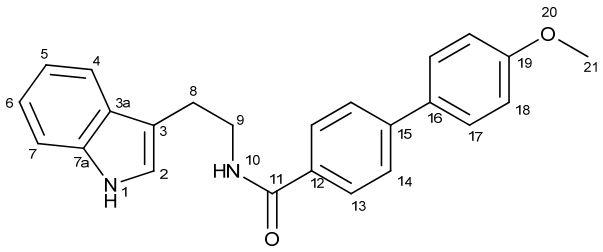
**N-(2-(1H-indol-3-yl)ethyl)-4'-fluorobiphenyl-4-carboxamide 48.** [145]




Procedure C was followed (4-fluorophenylboronic acid, 0.17 g, 1.2 mmol).

Off white solid; yield 59 %; mp 181–183 $^{o}$C; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 3.03 (2H, t, 7.4), 3.62 (2H, td, 7.4, 5.6), 7.04 (1H, td, 7.4, 1.1), 7.13 (1H, td, 7.4, 1.1), 7.25 (1H, d, 2.2), 7.34–7.41 (3H, m), 7.65 (1H, d, 7.7), 7.79–7.86 (2H, m), 7.81 (2H, d, 8.4), 8.00 (2H, d, 8.4), 8.72 (1H, t, 5.6), 10.87 (1H, br s); $\delta_C$ (75 MHz; $(CD_3)_2SO$) 25.7 ($CH_2$), 40.7 ($CH_2$), 111.9 (CH), 112.4, 116.3 (d, $^{2}$JCF, 21, CH), 118.7 (CH), 121.4 (CH), 123.1 (CH), 126.9 (CH, 2C), 127.8, 128.3 (CH, 2C), 129.4 (d, $^{3}$JCF, 8, CH), 136.2 (d, $^{4}$JCF, 3), 136.7 (CH), 142.0, 162.7 (d, $^{1}$JCF, 245) 166.2; *m/z* (FAB) 359.15593 (MH+ $C_{23}H_{20}N_2OF$ requires 359.15597); Found: C, 76.97; N, 7.75; H, 5.51 %. $C_{23}H_{19}N_2OF$ requires C, 77.08; N, 7.82; H, 5.34 .

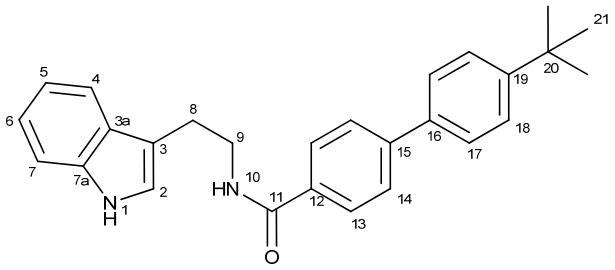
**4'-Methoxy-biphenyl-4-carboxylic acid-[2-(1*H*-indol-3-yl)-ethyl]-amide 49.** [145]




Procedure C was followed (phenylboronic acid, 0.15 g, 1.2 mmol).

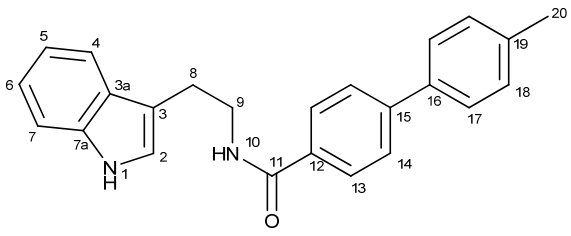
Off white solid; yield 45 %; mp. 200-205 $^{o}$C; $\upsilon_{max}$ (ATR) cm$^{-1}$ 3430 (indole NH), 3241 and 3054 (amide NH), 2931, 2837, 1621 (amide CO), 1606 (amide NH), 833, 740, 721; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 2.99 (2H, t, 7.4, C(8)H), 3.59 (2H, td, 7.4, 5.6, C(10)H), 3.81 (3H, s, C(21)H), 7.00 (1H, td, 7.4, 1.1, C(5)H), 7.05 (2H, d, 8.8, C(18)H), 7.09 (1H, td, 7.4, 1.1, C(6)H), 7.21 (1H, d, 2.2, C(2)H), 7.36 (1H, d, 7.4, C(4)H), 7.62 (2H, d, 7.4, C(7)H), 7.69 (2H, d, 8.8, C(17)H), 7.72 (2H, d, 8.5, C(14)H), 7.94 (2H, d, 8.5, C(13)H), 8.65 (1H, t, 5.6, N(10)H), 10.83 (1H, br-s, N(1)H); $\delta_C$ (75 MHz; $(CD_3)_2SO$) 25.7 ($CH_2$), 40.7 ($CH_2$), 55.7 ($CH_3$),111.9 (CH), 112.4, 114.9 (CH), 118.7 (CH), 118.8 (CH), 121.4 (CH), 123.1 (CH), 126.3 (CH), 127.8, 128.3 (CH), 128.5 (CH), 129.2 (CH), 129.3 (CH), 131.9 (CH), 132.5 (CH), 133.2, 136.7, 159.8, 166.3; Found: C, 77.92; N, 7.41; H, 5.89 %; $C_{24}H_{22}N_2O_2$ requires C, 77.81; N, 7.56; H, 5.99 %; *m/z* (ES$^-$) 371 (M-H$^-$); *m/z* (FAB), 371.17595 ($C_{24}H_{22}N_2O_2$ requires 371.17607).

**4'-tert-Butyl-biphenyl-4-carboxylic acid [2-(1H-indol-3-yl)-ethyl]-amide 50.** [145]



Procedure C was followed (4-*tert*-butylphenylboronic acid, 0.21 g, 1.2 mmol).
Off white solid; yield 61 %; mp. 201-203 $^{o}$C; $\upsilon_{max}$(ATR) cm$^{-1}$ 3422 (indole NH), 3254 and 3037 (amide NH), 2960, 1619, 1542, 833, 740; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 1.37 (9H, s, $^{t}$Bu(21)H), 3.03 (2H, t, 7.3, C(8)H), 3.63 (2H, td, 7.3, 5.5, C(9)H), 7.04 (1H, br-t, 7.5, C(5)H), 7.13 (1H, br-t, 7.5, C(6)H), 7.25 (1H, d, 1.6, C(2)H), 7.40 (1H, d, 7.5, C(4)H), 7.55 (2H, d, 8.4, C(18)H), 7.66 (1H, d, 7.5, C(7)H), 7.71 (2H, d, 8.4, C(17)H), 7.80 (2H, d, 8.3, C(14)H), 7.99 (2H, d, 8.3, C(13)H), 8.70 (1H, t, 5.5, N(10)H), 10.87 (1H, br-s, N(1)H); $\delta_C$ (75 MHz; $(CD_3)_2SO$) 25.7 ($CH_2$), 31.6 ($CH_3$, 3C), 34.8, 40.7 ($CH_2$), 111.9, 112.4 (CH), 118.7 (CH), 118.8 (CH), 121.4 (CH), 123.1 (CH), 126.3 (CH, 2C), 126.7 (CH, 2C), 127.0 (CH, 2C), 127.8, 128.3 (CH, 2C), 133.7, 136.7, 136.8, 142.9, 151.0, 166.3; *m/z* (FAB) 397.22798 ($M+H^+$) $C_{27}H_{29}N_2O$ requires 397.22799); Found: C, 81.76; N, 6.97; H, 7.03 %. $C_{27}H_{28}N_2O$ requires C, 81.78; N, 7.06; H, 7.12 %.

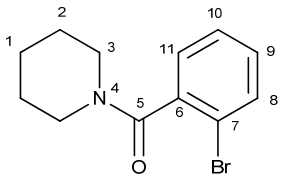
**N-(2-(1H-indol-3-yl)ethyl)-4'-methylbiphenyl-4-carboxamide 51.** [145]



Procedure C was followed (4-methylphenylboronic acid, 0.16 g, 1.2 mmol).
Off white solid; yield 42 %; mp. 204-206 $^{o}$C; $\upsilon_{max}$(ATR) cm$^{-1}$ 3420 (indole NH), 3240 and 3039 (amide NH), 1609, 1494, 821, 738; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 2.39 (3H, s, C(20)H), 3.02 (2H, t, 7.5, C(8)H), 3.62 (2H, dt, 7.5, 5.6, C(9)H), 7.03 (1H, td, 7.4, 1.1, C(5)H), 7.12 (1H, td, 7.4, 1.1, C(6)H), 7.24 (1H, d, 2.2, C(2)H), 7.33 (2H, d, 8.1, C(18)H), 7.39 (1H, d, 8.1, C(4)H), 7.35 (1H, d, 8.1, C(7)H), 7.66 (2H, d, 8.1, C(14)H), 7.78 (2H, d, 8.4, C(14)H), 7.98

(2H, d, 8.4, C(13)H), 8.69 (1H, t, 5.6, C(10)H), 10.86 (1H, br, N(1)H); $\delta_C$ (75 MHz; $(CD_3)_2SO$) 21.2 ($CH_2$), 25.7 ($CH_3$), 40.7 ($CH_2$), 111.9 (CH), 112.4, 118.7 (CH), 118.8 (CH), 121.4 (CH), 123.1 (CH), 126.6 (CH, 2C), 127.1 (CH, 2C), 127.8, 128.3 (CH, 2C), 130.1 (CH, 2C), 133.7, 136.8 (2C), 137.9, 143.0, 166.3; Found: C, 79.20; N, 7.40; H, 5.84 %. $C_{24}H_{22}N_2O$ requires C, 81.33; N, 7.90; H, 6.26 %; *m/z* (FAB) 355.18110 ($M+H^+$) $C_{24}H_{23}N_2O$ requires 355.18104).

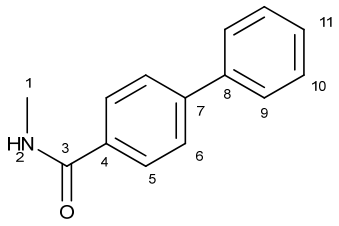
**(2-Bromophenyl)(piperidin-1-yl)methanone 54.**




Known compound, CAS 61153-35-3.

2-Bromobenzoylchloride (130 µL, 1 mmol) was added to a stirred solution of piperidine (100 µL, 1 mmol) and NaOH (0.5 mL, 4 M aqueous, 2 mmol) in DCM (10 mL) at 0 oC. The reaction mixture was stirred for 60 minutes at 0 oC and then a further two hours at R.T.. DCM (10 mL) was added and the reaction mixture was washed with $H_2O$ (2 × 20 mL). The aqueous washings were extracted with DCM (20 mL) and the combined organic phases were dried over anhydrous sodium sulphate, filtered and evaporated to dryness to yield (2-bromophenyl)(piperidin-1-yl)methanone as a clear oil; 98 % yield; $\delta_H$ (300 MHz; $CDCl_3$) 1.36-1.65 (6H, m), 3.04-3.19 (2H, m), 3.61-3.75 (2H, m), 7.12-7.18 (2H, m), 7.25-7.30 (1H, m), 7.48-7.51 (1H, m); *m/z* (ES+) 268 & 270 ($MH^+$); *m/z* (FAB) 268.03370 & 270.03178 ($C_{12}H_{15}NOBr$ requires 268.03382 & 270.03177).

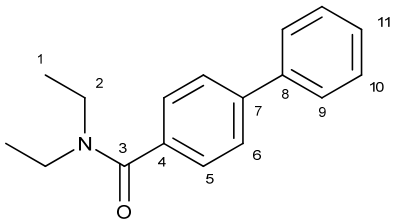
***N*-Methylbiphenyl-4-carboxamide 55.**




Known compound, [184] CAS 3815-23-4.

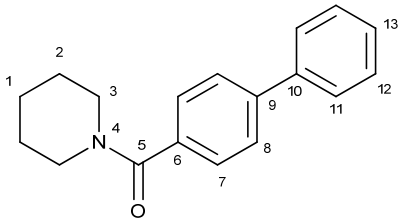
Methylamine gas was bubbled through a stirred suspension of 4-biphenylcarbonyl chloride (0.28 g, 1.3 mmol) in DCM (10 mL) at 0 $^{o}$C, the R.M. was then stirred for 60 minutes at 0 $^{o}$C and then washed with $H_2O$ (3 × 20 mL). The aqueous washings were extracted with DCM (3 × 20 mL), the combined organic phases were then dried over anhydrous sodium sulphate, filtered and evaporated to dryness to yield *N*-methylbiphenyl-4-carboxamide (0.19 g, 0.89 mmol) as an off white oil; 69 % yield; *m/z* (FAB), 212.10754 ($C_{14}H_{13}NO$ requires 212.10765).

**_N,N_-Diethylbiphenyl-4-carboxamide 56.**




Known compound, [185] CAS 204706-70-7.
Diethylamine (110 µL, 1.0 mmol) was dissolved in DCM (5 mL) added to a stirred solution of 4-biphenylcarbonyl chloride (0.22 g, 1 mmol) and NaOH (0.5 mL, 2 M aqueous, 2 mmol) in DCM (5 mL) at 0 $^{o}$C, the reaction mixture was stirred for 60 minutes at 0 $^{o}$C and then for a further 2 hours at R.T, DCM (30 mL) was added and the reaction mixture was washed with H2O (3 × 30mL), the aqueous washings were then extracted with DCM (20 mL) and the combined organic phases dried over anhydrous sodium sulphate, filtered and evaporated to dryness to give *N,N*-diethylbiphenyl-4-carboxamide (0.15 g, 0.6 mmol) as an off white oil; 59 % yield; $\delta_H$ (300 MHz; $CDCl_3$) 1.21 (6H, br), 3.45 (4H, br), 7.37 (1H, m), 7.43-7.48 (4H, m), 7.58-7.64 (4H, m); *m/z* ($ES^+$) 254 ($MH^+$); *m/z* (FAB), 254.15446 ($C_{17}H_{20}NO$ requires 254.1546).

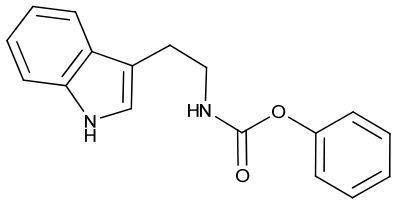
**Biphenyl-4-yl(piperidin-1-yl)methanone 56.**



Known compound. [186, 187]

4-Biphenylcarbonyl chloride (0.22 g, 1.0 mmol) was dissolved in DCM (4 mL) and added to a stirred solution of piperidine (100 μL, 1.0 mmol) and NaOH (0.5 mL, 4 M aqueous, 2 mmol) in DCM (10 mL) at 0 $^{o}$C. The reaction mixtures was stirred for 60 minutes at 0 $^{o}$C and then a further two hours at R.T., DCM (10 mL) was added and the reaction mixture washed with H2O (2 × 20 mL), the aqueous washings were extracted with DCM (20 mL) and the combined organic phases dried over anhydrous sodium sulphate, filtered and evaporated to dryness. The crude product was purified by flash column chromatography on silica from ethyl acetate/petroleum ether (40-60 $^{o}$C) (1:1) to give biphenyl-4-yl(piperidin-1-yl)methanone as a colourless oil; 73 % yield; $\delta_H$ (300 MHz; $CDCl_3$) 1.57-1.72 (6H, br-m), 3.42-3.72 (4H, br-m), 7.37 (1H, m), 7.42-7.49 (4H, m), 7.57-7.64 (4H, m); $\delta_C$ (75 MHz; $CDCl_3$) 24.6, 25.6-26.6 (b), 43.1-43.2 (b), 44.8-44.9 (b), 127.1 (CH), 127.4 (CH), 127.6 (CH), 128.8 (CH), 135.2, 140.3, 142.2; *m/z* ($ES^+$) 266 ($MH^+$).

**Phenyl 2-(1H-indol-3-yl)ethylcarbamate 58.**

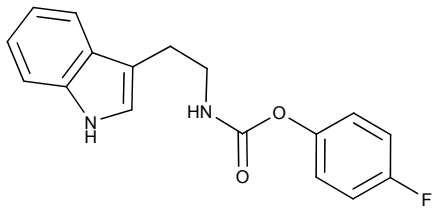



Known compound, CAS 32539-42-7.

Procedure B was followed (phenyl chloroformate, 0.19 g, 1.2 mmol).

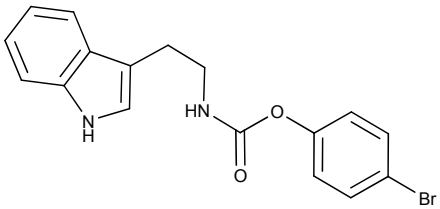
Clear oil; 79 % yield; $\delta_H$ (75 MHz; $CDCl_3$) 3.06 (2H, t, 6.9), 3.61 (2H, q, 6.6), 5.14 (1H, br-s), 7.05-7.39 (9H, m), 7.65 (1H, d, 7.8), 8.18 (1H, s); $\delta_C$ (75 MHz; $CDCl_3$) 25.6 ($CH_2$), 41.4 ($CH_2$), 111.3 (CH), 112.6, 118.7 (CH), 119.5 (CH), 121.6 (CH), 122.1 (2×CH), 122.2 (CH), 125.2 (CH), 127.2, 129.2 (2×CH), 136.4, 151.0, 154.6; *m/z* ($ES_+$) 281 ($MH^+$); *m/z* (FAB), 281.12900 ($C_{17}H_{17}N_2O_2$ requires 281.12912).

**4-Fluorophenyl 2-(1H-indol-3-yl)ethylcarbamate 59.**



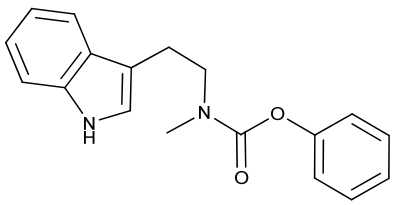
Procedure B was followed (4-fluorophenyl chloroformate, 0.21 g, 1.2 mmol).
White oily oil; 89 % yield; $\delta_H$ (300 MHz; $CDCl_3$) 2.97 (2H, t, 6.9), 3.52 (2H, q, 6.9), 5.03 (1H, br-s), 6.91-6.99 (5H, br), 7.07 (1H, td, 7.3, 0.9), 7.15 (1H, td, 7.3, 0.9), 7.30 (1H, br-d, 8.1), 7.56 (1H, br-d, 8.1), 8.03 (1H, br-s); $\delta_F$ (300 MHz; $CDCl_3$) -117.80; *m/z* ($ES^+$) 299 ($MH^+$); *m/z* (FAB) 298.11168 ($C_{17}H_{15}N_2O_2F$ requires 298.11176).

**4-Bromophenyl 2-(1H-indol-3-yl)ethylcarbamate 60.**



Procedure B was followed (4-bromophenylchloroformate, 0.28 g, 1.2 mmol).
Off white oil; 93 % yield; $\delta_H$ (300 MHz; $CDCl_3$) 3.06 (2H, t, 6.6), 3.61 (2H, t, 6.6), 5.11 (1H, br-s), 6.98 (2H, d, 9.0), 7.07 (1H, d, 2.4), 7.13 (1H, td, 7.8, 2.4), 7.24 (1H, td, 7.8, 2.4), 7.38-7.40 (1H, m), 7.44 (2H, d, 9.0), 7.64 (1H, br-d, 7.8), 8.09 (1H, br-s); $\delta_C$ (75 MHz; $CDCl_3$) 25.5 ($CH_2$), 41.5 ($CH_2$), 111.3 (CH), 112.6, 118.2, 118.7 (CH), 119.6 (CH), 122.1 (CH), 122.3 (CH), 123.4 (CH), 127.2, 132.2 (CH), 136.4, 150.1, 154.1; m/z (ES+) 359 & 361 ($MH^+$).

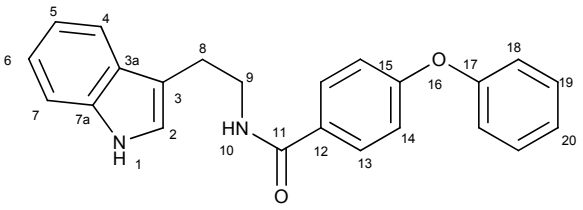
**Phenyl 2-(1H-indol-3-yl)ethyl(methyl)carbamate 61.**



Procedure B was followed (phenyl chloroformate, 0.19 g, 1.2 mmol).
Off white solid; 60 % yield; $\delta_H$ (300 MHz; $CDCl_3$) (complex overlapping rotomers) (distinct peaks from major rotomer) 3.02 (3H, br-s), (distinct peaks from minor rotomers) 3.08 (3H, br-s), (indistinct peaks) 3.13 (2H, m), 3.66-3.78 (2H, m), 6.91 (1H, d, 7.8), 7.02 (1H, br-d), 7.10-

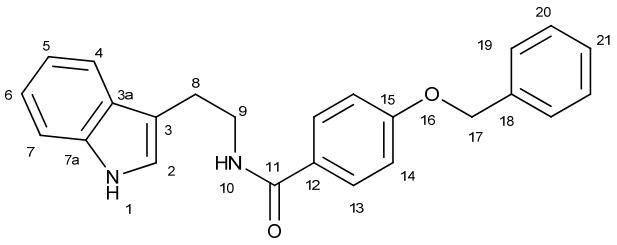
7.38 (9H, br-m), 8.12 (1H, br-s); *m/z* (ES+) 295 (MH+); *m/z* (FAB) 294.13681 $M^+$ ($C_{17}H_{17}N_2O_2$ requires 294.13683).

**N-(2-(1H-indol-3-yl)ethyl)-4-phenoxybenzamide 64.**



A schlenk tube was charged with *N*-(2-(1H-indol-3-yl)ethyl)-4-hydroxybenzamide **65** (0.09 g, 0.3 mmol), $Cu(OAc)_2$ (0.11 g, 0.6 mmol), phenyl boronic acid (0.05 g, 0.5 mmol), $Et_3N$ (0.13 mL, 0.9 mmol), powdered 4Å molecular sieves (0.03 g) and anhydrous DCM (12 mL). The tube was sealed and heated to 50 $_oC$ for 25 hours. The reaction mixture was allowed to cool to room temperature, filtered through celite and evaporated to dryness. The crude material was then purified by flash column chromatography on silica from ethyl acetate/petroleum ether (40-60 oC) 6:4, to N-(2-(1H-indol-3-yl)ethyl)-4-phenoxybenzamide as a clear oil; 16 % yield; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 2.97 (2H, t, 7.2, C(8)H), 3.55 (2H, dt, 7.2, 5.7, C(9)H), 6.95-7.10 (6H, m), 7.18-7.23 (2H, m), 7.34 (1H, br-d, 7.8, C(2)H), 7.43 (1H, t, 7.8), 7.58 (1H, br-d, 7.8), 7.88 (2H, d, 8.4), 8.56 (1H, t, 5.7, N(10)H), 10.81 (1H, s, N(1)H); $\delta_H$ (300 MHz; $(CD_3)_2SO$) 25.2 ($CH_2$), 40.2 ($CH_2$), 111.3 (CH), 111.9, 117.4 (CH), 118.2 (CH), 118.3 (CH), 119.4 (CH) 120.9 (CH), 122.6 (CH), 124.2 (CH), 127.2, 129.3 (2×CH), 129.4, 130.2 (2×CH), 136.2, 155.7, 159.2, 165.4; *m/z* ($ES^+$) 357 ($MH^+$).

**N-(2-(1H-indol-3-yl)ethyl)-4-(benzyloxy)benzamide 70.**

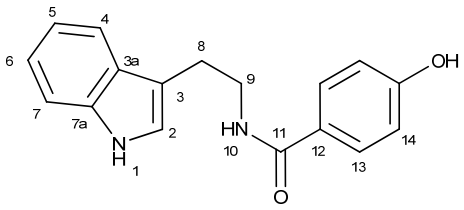


Procedure B was followed.

Oily white semi solid; 89 % yield; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 2.93 (2H, t, 7.5, C(8)H), 3.52 (2H, dt, 7.5, 5.6, C(9)H), 5.17 (2H, s, C(17)H), 6.98 (1H, td, 6.9, 1.0, C(5)H), 7.06 (1H, td,

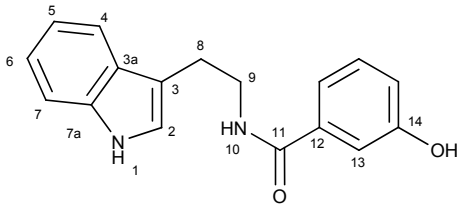
6.9, 6.9, 1.2, C(6)H), 7.07 (2H, d, 8.9, C(13)H), 7.17 (1H, d, 2.2, C(2)H), 7.32-7.38 (6H, m, C(4,19,20,21)H), 7.58 (1H, d, 6.9, C(7)H), 7.83 (2H, d, 8.9, C914)H), 8.45 (1H, t, 5.6, N(10)H), 10.80 (1H, br-s, N(1)H); $\delta_C$ (75 MHz; $(CD_3)_2SO$) 25.2 ($CH_2$), 40.1 ($CH_2$), 69.2 ($CH_2$), 111.3 (CH), 111.9, 114.2 (CH, 2C), 118.1 (CH), 118.2 (CH), 120.8 (CH), 122.5 (CH), 127.1, 127.2, 127.7 (CH, 2C), 127.8 (CH), 128.4 (CH, 2C), 128.8 (CH, 2C), 136.2, 136.6, 160.4, 165.5; *m/z* ($ES^+$) 371 ($MH^+$).

**_N_-(2-(1H-indol-3-yl)ethyl)-4-hydroxybenzamide 65.**




N-(2-(1H-indol-3-yl)ethyl)-4-(benzyloxy)benzamide **70** (0.41 g, 1.1 mmol) and Pd/C (5%, 0.06 g) were stirred in MeOH (30 mL) under an atmosphere of $H_2$ gas for four hours, the reaction mixture was then filtered through a plug of celite and evaporated to dryness. The crude product was purified by flash column chromatography on silica from ethyl acetate/petroleum ether (40-60 oC) 6:4 to yield *N*-(2-(1H-indol-3-yl)ethyl)-4-hydroxybenzamide as a light yellow solid; 32 % yield; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 2.91 (2H, 7.2, C(7)H), 3.49 (2H, br-d, C(8)H), 6.78 (2H, br-d, 8.7, C(13)H), 6.97 (1H, t, 7.4, C(5)H)), 7.06 (1H, t, 7.4, C(6)H), 7.16 (1H, br-s, C(2)H), 7.32 (1H, br-d, 7.4), 7.56 (1H, br-d, 7.4), 7.70(2H, br-d. 8.7, C(14)H), 8.33 (1H, br-s, N(10)H), 9.93 (1H, br, O(16)H), 10.79 (1H, br-s, N(1)H), *m/z* ($ES^+$) 281 ($MH^+$); *m/z* (FAB), 281.12900 ($C_{17}H_{17}N_2O_2$ requires 281.12912).

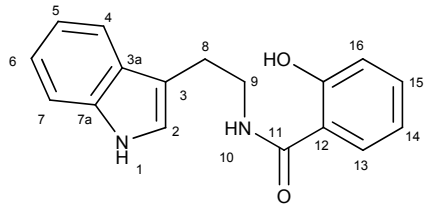
**_N_-(2-(1H-indol-3-yl)ethyl)-3-hydroxybenzamide 66**



Known compound CAS 939386-91-1

Light yellow solid; 58 % yield; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 2.93 (2H, t, 7.5, C(8)H), 3.51 (2H, dt, 7.5, 5.5, C(9)H), 6.88-6.92 (1H, m), 6.98 (1H, t, 7.4, C(5)H), 7.07 (1H, t, 7.4, C(6)H), 7.18 (1H, d, 1.9, C(2)H), 7.21-7.27 (3H, m), 7.34 (1H, d, 7.9), 7.59 (1H, d, 7.9), 8.53 (1H, t, 5.5, N(10)H), 9.67 (1H, s, O(18)H), 10.84 (1H, br-s, N(1)H); $\delta_C$ (75 MHz; $(CD_3)_2SO$) 25.2 ($CH_2$), 40.1 ($CH_2$), 111.4 (CH), 111.9, 114.2 (CH), 117.6 (CH), 117.9 (CH), 118.2 (CH), 118.3 (CH), 120.9 (CH), 122.6 (CH), 127.2, 129.2, 136.2, 157.3, 166.1; *m/z* ($ES^+$) 281 ($MH^+$); *m/z* (FAB), 281.12900 ($C_{17}H_{17}N_2O$ requires 281.12912).

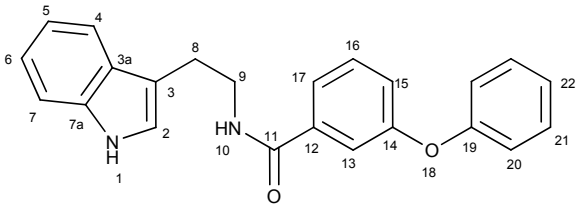
**N-(2-(1H-indol-3-yl)ethyl)-2-hydroxybenzamide 67.**



Known compound, [188] CAS 31384-98-2.

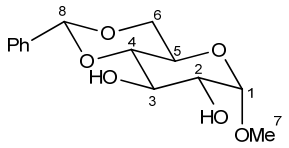
Light yellow solid; 67 % yield; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 2.91 (2H, t, 7.2, C(8)H), 3.42 (2H, br-td, C(9)H), 6.99 (1H, t, 7.2, C(6)H), 7.07 (1H, t, 7.2, C(5)H), 7.16-7.19 (2H, m), 7.29-7.36 (2H, m), 7.47-7.58 (3H, m), 8.40 (1H, t, 5.7), 10.84 (1H, br-s, N(1)H); *m/z* ($ES^+$) 281 ($MH^+$).

***N*-(2-(1H-Indol-3-yl)ethyl)-3-phenoxybenzamide 81.**



A schlenk tube was charged with *N*-(2-(1H-indol-3-yl)ethyl)-3-hydroxybenzamide **66** (0.14 g, 0.5 mmol), $Cu(OAc)_2$ (0.19 g, 1.0 mmol), phenyl boronic acid (0.10 g, 0.8 mmol), $Et_3N$ (0.21 mL, 1.5 mmol), powdered 4Å molecular sieves (0.05 g) and anhydrous DCM (20 mL). The tube was sealed and heated to 50 $^oC$ for 20 hours. The reaction mixture was allowed to cool to room temperature, filtered through celite and evaporated to dryness. The crude material was then purified by flash column chromatography on silica from ethyl acetate/petroleum ether (40-60 oC) 4:6, to give N-(2-(1H-indol-3-yl)ethyl)-3-phenoxybenzamide as a white amorphous solid; 51 % yield; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 2.99 (2H, t, 7.2), 3.57 (2H, td, 7.2, 6.0), 7.02 (1H, m), 7.09-7.14 (3H, m), 7.21-7.26 (3H, m), 7.39 (1H, d, 8.1), 7.45-7.56 (4H, m), 7.62 (1H, d, 7.8), 7.69 (1H, d, 7.8), 8.74 (1H, t, 6.0, N(10)H), 10.88 (1H, s, N(1)H); $\delta_C$ (100 MHz; $(CD_3)_2SO$) 25.0 ($CH_2$), 40.2 ($CH_2$), 111.3 (CH), 111.8, 117.2 (CH), 118.2 (CH), 118.2 (CH), 118.7 (2×CH), 120.9 (CH), 121.2 (CH), 122.0 (CH), 122.5 (CH), 123.7 (CH), 127.2, 129.9 (CH), 130.1 (2×CH), 136.2, 136.6, 156.3, 156.6, 165.2; *m/z* (ES) 357 ($MH^+$) 355 ($M-H^-$); *m/z* (FAB) 357.16046 ($C_{23}H_{21}N_4O_2$ requires 357.16030).

**Methyl-α-D-(R)-4,6-*O*-benzylidene-glucopyranoside 120.**

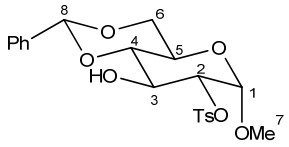


Know compound. [173]

Oven dried (overnight, 90 $^oC$) methyl-α-D-glucopyranoside (13.3 g, 84 mmol), benzaldehyde-dimethylacetal (12.6 mL, 84 mmol) and *p*-toluene sulphonic acid (0.05 g, 0.26 mmol) were refluxed in anhydrous DMF (50 mL) under reduced pressure (70 $^oC$, 33 mbar) for 17 hours. The DMF was removed by rotary evaporation and the resulting white solid dispersed by refluxing in aqueous $NaHCO_3$ solution (0.3 M, 100 mL). The reaction mixture was then filtered and washed with cold $H_2O$ (3 × 100 mL), the white solid was dried in a vacuum desiccator for 24 hours over $P_2O_5$ before being recrystalised from propan-2-ol and pyridine (60:1) to yield methyl-α-D-(R)-4,6-*O*-benzylidene-glucopyranoside as an off white solid; 71 % yield; mp. 163-166 $^oC$; $\delta_H$ (250 MHz; $CDCl_3$) 2.85 (1H, d. 8.7), 3.42 (3H, s), 3.44 (1H, t,

9.1), 3.45 (1H, d, 2.5), 3.56 (1H, td, 9.0, 3.9,), 3.69 (1H, t, 10.6), 3.76 (1H, td, 10.1, 4.1), 3.89 (1H, td, 9.2, 2.5), 4.26 (1H, dd, 10.3, 3.9), 4.72 (1H, d, 3.9), 5.48 (1H, s), 7.32-7.52 (5H, m).

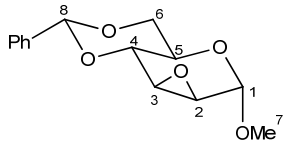
**Methyl-α-D-(R)-4,6-*O*-benzylidene-2-*O*-tosyl-glucopyranoside 121.** [189]




Known compound.

Methyl-α-D-(R)-4,6-*O*-benzylidene-glucopyranoside (16.6 g, 58.7 mmol), DMAP (1.4 g, 11.8 mmol) and Et3N (25 mL, 176 mmol) were dissolved in anhydrous $CH_2Cl_2$ (100 mL) at 0 $^oC$. *p*-Toluene sulphonyl chloride (11.3 g, 59.3 mmol) was added portion wise over 15 minutes and the reaction was stirred for 2 h at RT. $H_2O$ (100 mL) was added and the product was extracted into $CH_2Cl_2$ (2 × 100 mL). The combined organic phases were dried over anhydrous $MgSO_4$ and evaporated to dryness. The resulting yellow foam was repeatedly triturated with hot propan-2-ol followed by rotary evaporation, until white crystals were formed. These were collected by filtration and washed with cold propan-2-ol to give methyl-α-D-(R)-4,6-*O*-benzylidene-2-*O*-tosyl-glucopyranoside; 78 % yield; mp. 152-156 $^oC$; $\delta_H$ (250 MHz; ($CDCl_3$) 2.41 (3H, s), 2.98 (1H, br-s), 3.40 (3H, s), 3.53 (1H, t, 9.3), 3.70 (1H, t, 10.2), 3.80 (1H, td, 10.2, 4.8), 4.32 (1H, t, 9.2), 4.28 (1H, dd, 9.8, 4.2), 4.38 (1H, dd, 9.4, 3.9), 4.84 (1H, d, 3.7), 5.52 (1H, s), 7.4-7.6 (5H, m), 7.6-7.7 (2H, m), 8.09 (2H, d).

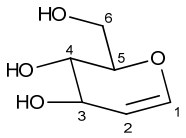
**Methyl-α-D-2,3-anhydro-(R)-4,6-*O*-benzylidene-mannopyranoside 106.** [174]




Methyl-α-D-(R)-4,6-*O*-benzylidene-2-*O*-tosyl-glucopyranoside (19.8 g, 45.3 mmol) was dissolved in anhydrous DMF (160 mL) and cooled to 0 $^oC$. NaH (60 % dispersion in mineral oil, 2.0 g, 50.3 mmol) was added portion wise over 20 minutes and the reaction mixture was stirred at RT for 2 h. EtOH (17 mL) was added slowly and the resulting brown suspension was poured onto $H_2O$-ice (82 mL), the precipitate formed was collected by vacuum filtration and recrystalised from propan-2-ol to afford methyl-α-D-2,3-anhydro-(R)-4,6-*O*-benzylidene-

mannopyranoside as a white solid; 65 % yield; $\delta_H$ (300 MHz; ($CDCl_3$) 3.17 (1H, d, 3.6, C(2)H), 3.47 (3H, s, C(7)H), 3.48-3.50 (1H, m, C(4)H), 3.68-3.75 (3H, m, C(3,5,6ax)H), 4.26 (1H, dd, 5.7, 9.9, C(6eq)H), 4.90 (1H, s, C(1)H), 5.57 (1H, s, C(8)H), 7.36-7.41 (3H, m), 7.45-7.52 (2H, m); $\delta_C$ (75 MHz; ($CDCl_3$) 50.5 (CH), 53.8 (CH), 55.7 (CH), 61.6 (CH), 69.4 (CH), 74.8 (CH), 96.8 (CH), 102.4 (CH), 126.1 (CH), 128.3 (CH), 129.2 (CH), 137.0.

**2-(hydroxymethyl)-3,4-dihydro-2H-pyran-3,4-diol. (D-Glucal) 95.**

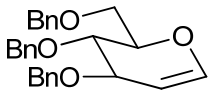



Known compound CAS [13265-84-4]

A solution of NaOMe (0.5 mL, 2M methanolic, 1 mmol) was added to a stirred solution of tri-*O*-acetyl-D-glucal (10.0 g, 36.8 mmol) in anhydrous methanol (50 mL) which was then stirred at R.T. for 3 hours. The methanol was removed under vacuum and the residue passed through a short column of silica, elution with acetone:diethyl ether (3:7). The solution was dried over anhydrous magnesium sulphate, filtered and evaporated to dryness to yield D-glucal as an off white solid; yield 92 %; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 3.37 (1H, m), 3.55-3.61 (2H, m), 3.68-3.72 (1H, m), 3.93-3.95 (1H, m), 4.55-4.59 (2H, m), 4.85 (1H, d, 5.4), 5.08 (1H, d, 5.4).

An X-ray crystal structure was obtained see section 5.5.1.

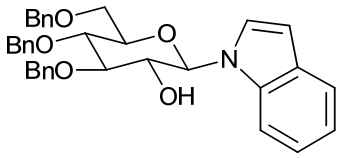
**(2R,3S,4S)-3,4-bis(benzyloxy)-2-(benzyloxymethyl)-3,4-dihydro-2H-pyran 124.**




D-Glucal (1.2 g, 8.0 mmol) was added to a stirred suspension of NaH (1.3 g, 60 % disp. In mineral oil, 32 mmol) in anhydrous THF/DMF (60 mL, 4:1) at 0 $^oC$. Benzyl bromide (3.8 mL, 32 mmol) was then added and the R.M. heated to 60 $^oC$ for 4 hours and then cooled to R.T. Diethyl ether (20 mL) and H2O (5 mL) were added and $CO_2$ bubbled through the solution until pH neautral by UI paper. The R.M. was then washed with saturated brine (50 mL), dried over anhydrous magnesium sulphate, filtered and evaporated to dryness. The resulting yellow oil was dried overnight under high vacuum and then purified by flash column chromatography on silica from 5 % ethyl acetate, 95 % petroleum ether (40-60 $^oC$) to yield tri-*O*-benzyl-D-glucal as a white solid; yield 76.4 %; m.p. 53-54 $^oC$; store at -

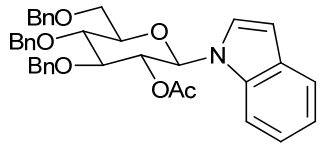
18 °C; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 3.74-3.86 (3H, m), 4.02-4.05 (1H, m), 4.18-4.20 (1H, m), 4.50-4.63 (5H, m), 4.79-4.86 (2H, m), 6.40 (1H, br-s, 6.3), 7.21-7.31 (15H, m); $\delta_C$ (100 MHz; $(CD_3)_2SO$) (many operlapping signals in the aromatic region, all $CH_2$ by DEPT) 68.5 ($CH_2$), 70.4 ($CH_2$), 73.5 ($CH_2$), 73.7 ($CH_2$), 74.4 (CH), 75.7 (CH), 76.7 (CH), 99.9 (CH), 127.6 (CH), 127.7 (CH), 127.7 (CH), 127.9 (CH), 128.4 (CH), 138.0, 138.1, 138.3, 144.7.

**(2R,3R,4R,5R,6R)-4,5-bis(benzyloxy)-6-(benzyloxymethyl)-2-(1H-indol-1-yl)tetrahydro-2H-pyran-3-ol 126.**



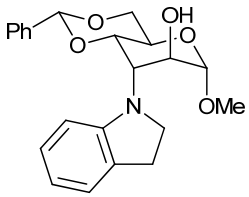

Tri-*O*-benzyl-D-glucal **124** (0.21 g, 0.5 mmol) was dissolved in anhydrous DCM (5 mL) under an atmosphere of $N_2$ and cooled to 0 °C. A solution of DMDO (6 mL, approx 1.5 eq) in acetone (Procedure D) was added wia syringe and the reaction mixture stirred at 0 °C for 50 minutes, additional DMDO solution was added until TLC showed consumption of the starting material, the crude epoxide was then isolated under reduced pressure as an oily white solid which was then dissolved in anhydrous MeCN (3 mL) and added via canula to a stirred solution of indole (0.12 g, 1.0 mmol) and NaH (0.05 g, 1.05 mmol) in anhydrous MeCN (2 mL) at 0 °C under $N_2$. The reaction mixture was then stirred at room temperature for 15 minutes and then heated to 50 °C for 3 hours then cooled to R.T. EtOAC (60 mL) was added and the crude product was absorbed onto silica and purified by flash column chromatography on silica from ethyl acetate:petroleum ether (40-60 oC) 15:85 to give (2R,3R,4R,5R,6R)-4,5-bis(benzyloxy)-6-(benzyloxymethyl)-2-(1H-indol-1-yl)tetrahydro-2H-pyran-3-ol as a clear oil; 42 % yield; $^1$H NMR showed a mixture of products for the single spot; *m/z* ($ES^+$) 549 & 551, this product was used without purification.

**(2R,3R,4S,5R,6R)-4,5-bis(benzyloxy)-6-(benzyloxymethyl)-2-(1H-indol-1-yl)tetrahydro-2H-pyran-3-yl acetate 127.**




(2R,3R,4R,5R,6R)-4,5-bis(benzyloxy)-6-(benzyloxymethyl)-2-(1H-indol-1-yl)tetrahydro-2H-pyran-3-ol **126** (0.10 g, 0.18 mmol) was dissolved in pyridine (5 mL) and cooled to 0 oC, acetic anhydride (1 mL) was added along with a catalytic quantity of DMAP, the R.M. was then stirred at 0 $^{o}$C for 45 minutes and then at R.T. for two hours. The R.M. was then poured onto sat. aqueous $NaHCO_3$ (35 mL). The product was then extracted into ethyl acetate (2 × 35 mL), the aqueous phase was then washed with sat. aqueous $NaHCO_3$ (35 mL), $H_2O$ (30 mL), citric acid (aqueous 1M, 30 mL) and $H_2O$ (30 mL), dried over anhydrous sodium sulphate, filtered and evaporated to dryness. The crude product was purified by flash column chromatography on silica from ethyl acetate/petroleum ether (40-60 oC) 1:9 to yield (2R,3R,4S,5R,6R)-4,5-bis(benzyloxy)-6-(benzyloxymethyl)-2-(1H-indol-1-yl)tetrahydro-2H-pyran-3-yl acetate as a clear oil; 43 % yield; $\delta_H$ (400 MHz; $CDCl_3$) 3.78-3.85 (3H, m), 3.90-4.01 (3H, m), 4.52 (1H, br-d, 12.0), 4.61 (1H, br-d, 12.0), 4.68-4.76 (2H, m), 4.88 (1H, br-d, 2.4), 4.91 (1H, m), 5.50-5.57 (2H, m), 6.57 (1H, d, 3.2), 7.15 (1H, m), 7.21-7.38 (18H, m), 7.45 (1H, br-d, 8.0), 7.62 (1H, br-d, 7.6); $\delta_C$ (100 MHz; $CDCl_3$) 20.3 ($CH_3$), 68.5 ($CH_2$), 72.4 (CH), 73.6 ($CH_2$), 75.2 ($CH_2$), 75.3 ($CH_2$), 77.8 (CH), 78.1 (CH), 83.5 (CH), 83.6 (CH), 103.7 (CH), 109.8 (CH), 120.4 (CH), 121.1 (CH), 122.0 (CH), 124.9 (CH), 127.6-128.5 (multiple overlapping CH and quaternary C signals), 129.1, 136.3, 137.9, 138.0, 168.9; m/z (ES+) 592 ($MH^+$).

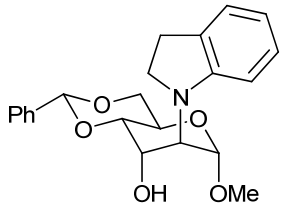
**(2R,4aR,7S,8S,8aS)-8-(indolin-1-yl)-6-methoxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-7-ol 130.**




Methyl-α-D-2,3-anhydro-(R)-4,6-*O*-benzylidene-mannopyranoside (0.14 g, 0.51 mmol), $LiClO_4$ (Caution explosion risk, 0.06 g, 0.5 mmol) and indoline (56 µL, 0.5 mmol) were suspended in anhydrous MeCN (0.5 mL) and heated to 120 oC for 2 hours by microwave

irradiation, the reaction mixture was then diluted with DCM (20 mL) and washed with H2O (2 × 20 mL), the aqueous washings were extracted with DCM (20 mL) and the combined organic phases dried over anhydrous sodium sulphate, filtered and evaporated to dryness. The crude product was purified by flash column chromatography on silica from ethyl acetate/petroleum ether (40-60 oC) 2:8 to yield (2R,4aR,7S,8S,8aS)-8-(indolin-1-yl)-6-methoxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-7-ol as a clear oil; 75 % yield; $\delta_H$ (300 MHz; $CDCl_3$) 2.72 (1H, br-s), 3.02 (2H, br-t, indoline $CH_2$), 3.40 (3H, s, C(7)H), 3.79-3.82 (1H, m, C(4)H), 3.93-3.81 (3H, m), 4.10-4.12 (1H, m) 4.21-4.25 (2H, m), 4.35-4.40 (1H, m, C(5)H), 4.59 (1H, d, 1.2, C(3)H), 5.59 (1H, s, C(8)H), 6.33 (1H, d, 8.1), 6.58 (1H, td, 7.2, 0.9), 7.03 (2H, ov-m), 7.32-7.35 (3H, m), 7.37-7.41 (2H, m); $\delta_C$ (100 MHz; $CDCl_3$) 28.9 ($CH_2$), 49.3 ($CH_2$), 55.3 ($CH_3$), 60.5 (CH), 68.9 (CH), 70.1 ($CH_2$), 74.9 (CH), 101.1 (CH), 102.6 (CH), 104.8 (CH), 116.4 (CH), 124.4 (CH), 126.2 (2 × CH), 127.3 (CH), 128.3 (2 × CH), 129.1 (CH), 137.4, 151.5, 171.5; *m/z* (FAB) 383.17321 ($C_{22}H_{25}NO_5$ requires 383.17327).

**(2R,4aR,7S,8S,8aS)-7-(indolin-1-yl)-6-methoxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-8-ol 131.**

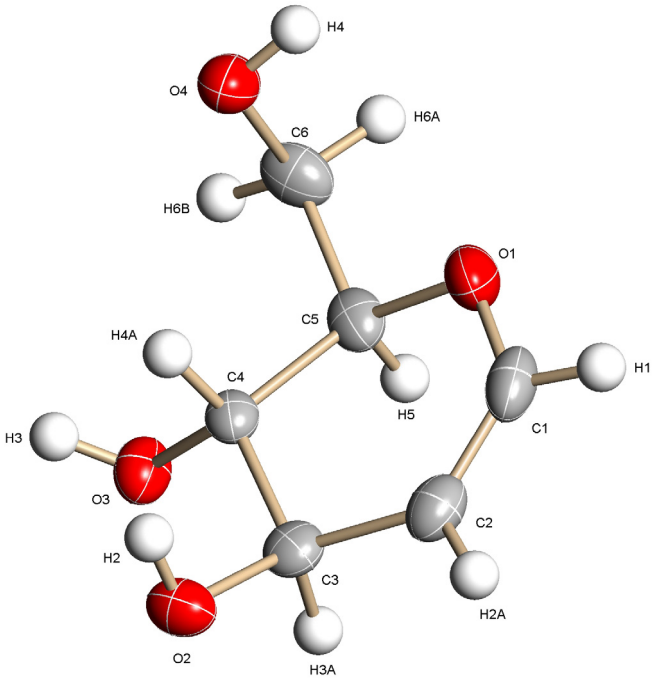



Methyl-2,3-anhydro-(*R*)-4,6-*O*-benzylidene-α-D-allopyranoside (0.13 g, 0.5 mmol), indoline (56 µL, 0.5 mmol) and $LiClO_4$ (Caution explosion risk, 0.06 g, 0.5 mmol) were suspended in anhydrous MeCN (0.5 mL) and heated to 120 oC for 2 hours by microwave irradiation, the reaction mixture was then diluted with DCM (20 mL) and washed with H2O (2 × 20 mL), the aqueous washings were extracted with DCM (20 mL) and the combined organic phases dried over anhydrous sodium sulphate, filtered and evaporated to dryness. The crude product was purified by flash column chromatography on silica from ethyl acetate/petroleum ether (40-60 oC) 2:8 to yield (2R,4aR,7S,8S,8aS)-7-(indolin-1-yl)-6-methoxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-8-ol as a clear oil; 77 % yield; $\delta_H$ (400 MHz;

$CDCl_3$) 2.89 (1H, br-s, O(3)H), 3.02 (2H, t, 8.8, Indoline C(3)H), 3.46 (3H, s, C(7)H), 3.53-3.69 (2H, m, Indoline C(2)H), 3.83 (1H, t, 10.4, C(6ax)H), 4.24 (1H, br-s, C(3)H), 4.28-4.34 (1H, m, C(5)H), 4.38-4.42 (1H, dd, 4.8, 10.4, C(6eq)H), 4.88 (1H, s, C(1)H), 5.64 (1H, s, C(8)H), 6.50 (1H, d, 8.0, Indoline C(7)H), 6.68 (1H, Indoline C(6)H), 7.07-7.11 (2H, m, Indoline C(4,5)H), 7.34-7.39 (3H, m), 7.46-7.50 (2H, m); $\delta_C$ (100 MHz; $CDCl_3$) 28.8 ($CH_2$), 50.5 ($CH_2$), 55.6 ($CH_3$), 57.8 (CH), 59.1 (CH), 67.0 (CH), 69.4 ($CH_2$), 77.8 (CH), 100.1 (CH), 102.3 (CH), 106.2 (CH), 117.9 (CH), 124.5 (CH), 126.2 (2 × CH), 127.7 (CH), 128.3 (2 × CH), 129.2 (CH), 137.2, 150.4, 169.5 (By HMBC); *m/z* (FAB) 383.17321 ($C_{22}H_{25}NO_5$ requires 383.17327).

## 5.5 X-Ray Structure Data

*5.5.1 Crystal structure data for D-glucal 95.*




Figures show 50% displacement ellipsoids. There is intermolecular hydrogen bonding;

Hydrogen bonds with H..A < r (A) + 2.000 Angstroms and <DHA > 110 deg.

| D-H | d(D-H) | d(H..A) | <DHA | d(D..A) | A |
|---|---|---|---|---|---|
| O2-H2 | 0.840 | 1.983 | 172.99 | 2.819 | O3 [x-1, y, z] |
| O3-H3 | 0.840 | 1.800 | 179.29 | 2.640 | O4 [-x+1, y+1/2, -z+1/2] |
| O4-H4 | 0.840 | 1.895 | 178.27 | 2.735 | O2 [x, y-1, z] |

Table 1. Crystal data and structure refinement for 07020.

| | | |
|---|---|---|
| Identification code | 07020 | |
| Empirical formula | C6 H10 O4 | |
| Formula weight | 146.14 | |
| Temperature | 150(2) K | |
| Wavelength | 0.71073 Å | |
| Crystal system | Orthorhombic | |
| Space group | P2(1)2(1)2(1) | |
| Unit cell dimensions | a = 4.6893(10) Å | α= 90°. |
| | b = 6.7229(15) Å | β= 90°. |
| | c = 22.108(5) Å | γ = 90°. |
| Volume | 697.0(3) $Å^3$ | |
| Z | 4 | |
| Density (calculated) | 1.393 $Mg/m^3$ | |
| Absorption coefficient | 0.118 $mm^{-1}$ | |

| | |
|---|---|
| F(000) | 312 |
| Crystal size | 0.20 x 0.15 x 0.08 $mm^3$ |
| Theta range for data collection | 1.84 to 25.98°. |
| Index ranges | -5<=h<=5, -8<=k<=8, -27<=l<=27 |
| Reflections collected | 5445 |
| Independent reflections | 1369 [R(int) = 0.0491] |
| Completeness to theta = 25.98° | 100.0 % |
| Absorption correction | None |
| Refinement method | Full-matrix least-squares on $F^2$ |
| Data / restraints / parameters | 1369 / 0 / 94 |
| Goodness-of-fit on $F^2$ | 0.980 |
| Final R indices [I>2sigma(I)] | R1 = 0.0344, wR2 = 0.0677 |
| R indices (all data) | R1 = 0.0419, wR2 = 0.0703 |
| Absolute structure parameter | 0.1(14) |
| Largest diff. peak and hole | 0.167 and -0.164 e.$Å^{-3}$ |

Table 2. Atomic coordinates ( x $10^4$) and equivalent isotropic displacement parameters ($Å^2$x $10^3$) for 07020. U(eq) is defined as one third of the trace of the orthogonalized $U^{ij}$ tensor.

| | x | y | z | U(eq) |
|---|---|---|---|---|
| O(1) | 4360(3) | 8052(2) | 782(1) | 37(1) |
| O(2) | 1835(2) | 13639(2) | 1337(1) | 33(1) |
| O(3) | 7138(2) | 11691(2) | 1868(1) | 32(1) |
| O(4) | 4302(3) | 6663(2) | 1977(1) | 35(1) |
| C(1) | 2867(4) | 9332(3) | 429(1) | 36(1) |
| C(2) | 2542(4) | 11240(3) | 530(1) | 33(1) |
| C(3) | 3809(4) | 12276(3) | 1063(1) | 28(1) |
| C(4) | 4914(4) | 10795(2) | 1522(1) | 24(1) |
| C(5) | 6189(4) | 8945(2) | 1231(1) | 30(1) |
| C(6) | 6826(4) | 7330(3) | 1683(1) | 36(1) |

Table 3. Bond lengths [Å] and angles [°] for 07020.

| | |
|---|---|
| O(1)-C(1) | 1.356(2) |
| O(1)-C(5) | 1.4429(19) |
| O(2)-C(3) | 1.436(2) |
| O(2)-H(2) | 0.8400 |
| O(3)-C(4) | 1.4264(19) |
| O(3)-H(3) | 0.8400 |
| O(4)-C(6) | 1.423(2) |
| O(4)-H(4) | 0.8400 |
| C(1)-C(2) | 1.310(3) |
| C(1)-H(1) | 0.9500 |
| C(2)-C(3) | 1.493(2) |
| C(2)-H(2A) | 0.9500 |
| C(3)-C(4) | 1.514(2) |
| C(3)-H(3A) | 1.0000 |
| C(4)-C(5) | 1.523(2) |
| C(4)-H(4A) | 1.0000 |
| C(5)-C(6) | 1.505(2) |
| C(5)-H(5) | 1.0000 |
| C(6)-H(6A) | 0.9900 |
| C(6)-H(6B) | 0.9900 |
| | |
| C(1)-O(1)-C(5) | 116.01(13) |
| C(3)-O(2)-H(2) | 109.5 |
| C(4)-O(3)-H(3) | 109.5 |
| C(6)-O(4)-H(4) | 109.5 |
| C(2)-C(1)-O(1) | 125.69(17) |
| C(2)-C(1)-H(1) | 117.2 |
| O(1)-C(1)-H(1) | 117.2 |
| C(1)-C(2)-C(3) | 123.00(17) |
| C(1)-C(2)-H(2A) | 118.5 |
| C(3)-C(2)-H(2A) | 118.5 |
| O(2)-C(3)-C(2) | 111.96(14) |
| O(2)-C(3)-C(4) | 110.95(13) |
| C(2)-C(3)-C(4) | 111.03(14) |
| O(2)-C(3)-H(3A) | 107.6 |
| C(2)-C(3)-H(3A) | 107.6 |
| C(4)-C(3)-H(3A) | 107.6 |
| O(3)-C(4)-C(3) | 109.39(13) |

| | |
|---|---|
| O(3)-C(4)-C(5) | 106.52(14) |
| C(3)-C(4)-C(5) | 112.82(13) |
| O(3)-C(4)-H(4A) | 109.3 |
| C(3)-C(4)-H(4A) | 109.3 |
| C(5)-C(4)-H(4A) | 109.3 |
| O(1)-C(5)-C(6) | 105.93(13) |
| O(1)-C(5)-C(4) | 113.42(14) |
| C(6)-C(5)-C(4) | 112.71(14) |
| O(1)-C(5)-H(5) | 108.2 |
| C(6)-C(5)-H(5) | 108.2 |
| C(4)-C(5)-H(5) | 108.2 |
| O(4)-C(6)-C(5) | 111.46(14) |
| O(4)-C(6)-H(6A) | 109.3 |
| C(5)-C(6)-H(6A) | 109.3 |
| O(4)-C(6)-H(6B) | 109.3 |
| C(5)-C(6)-H(6B) | 109.3 |
| H(6A)-C(6)-H(6B) | 108.0 |

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for 07020. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ...\ + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

| | $U^{11}$ | $U^{22}$ | $U^{33}$ | $U^{23}$ | $U^{13}$ | $U^{12}$ |
|---|---|---|---|---|---|---|
| O(1) | 49(1) | 34(1) | 30(1) | -8(1) | 3(1) | -6(1) |
| O(2) | 28(1) | 28(1) | 43(1) | 1(1) | 5(1) | 0(1) |
| O(3) | 27(1) | 41(1) | 28(1) | -3(1) | -1(1) | -8(1) |
| O(4) | 41(1) | 32(1) | 32(1) | 0(1) | 0(1) | -5(1) |
| C(1) | 34(1) | 53(1) | 21(1) | -6(1) | 4(1) | -13(1) |
| C(2) | 27(1) | 45(1) | 26(1) | 4(1) | 1(1) | -6(1) |
| C(3) | 22(1) | 31(1) | 30(1) | 2(1) | 5(1) | -5(1) |
| C(4) | 21(1) | 26(1) | 25(1) | -2(1) | 3(1) | -6(1) |
| C(5) | 23(1) | 34(1) | 33(1) | -6(1) | 7(1) | -4(1) |
| C(6) | 28(1) | 31(1) | 51(1) | -4(1) | 2(1) | 4(1) |

Table 5. Hydrogen coordinates ( x $10^4$) and isotropic displacement parameters ($Å^2$x $10^{-3}$) for 07020.

| | x | y | z | U(eq) |
|---|---|---|---|---|
| H(2) | 537 | 12998 | 1511 | 49 |
| H(3) | 6694 | 11679 | 2236 | 48 |
| H(4) | 3554 | 5747 | 1774 | 52 |
| H(1) | 1980 | 8799 | 78 | 43 |
| H(2A) | 1450 | 11997 | 251 | 39 |
| H(3A) | 5475 | 13068 | 915 | 33 |
| H(4A) | 3325 | 10395 | 1798 | 29 |
| H(5) | 8018 | 9332 | 1030 | 36 |
| H(6A) | 7740 | 6196 | 1474 | 44 |
| H(6B) | 8183 | 7841 | 1989 | 44 |

Table 6. Torsion angles [°] for 07020.

| | |
|---|---|
| C(5)-O(1)-C(1)-C(2) | -13.2(2) |
| O(1)-C(1)-C(2)-C(3) | -0.1(3) |
| C(1)-C(2)-C(3)-O(2) | -136.82(17) |
| C(1)-C(2)-C(3)-C(4) | -12.2(2) |
| O(2)-C(3)-C(4)-O(3) | -80.69(16) |
| C(2)-C(3)-C(4)-O(3) | 154.12(13) |
| O(2)-C(3)-C(4)-C(5) | 160.94(12) |
| C(2)-C(3)-C(4)-C(5) | 35.7(2) |
| C(1)-O(1)-C(5)-C(6) | 161.90(14) |
| C(1)-O(1)-C(5)-C(4) | 37.76(18) |
| O(3)-C(4)-C(5)-O(1) | -169.87(12) |
| C(3)-C(4)-C(5)-O(1) | -49.83(19) |
| O(3)-C(4)-C(5)-C(6) | 69.76(17) |
| C(3)-C(4)-C(5)-C(6) | -170.20(15) |
| O(1)-C(5)-C(6)-O(4) | -62.65(17) |
| C(4)-C(5)-C(6)-O(4) | 61.94(19) |

Symmetry transformations used to generate equivalent atoms:

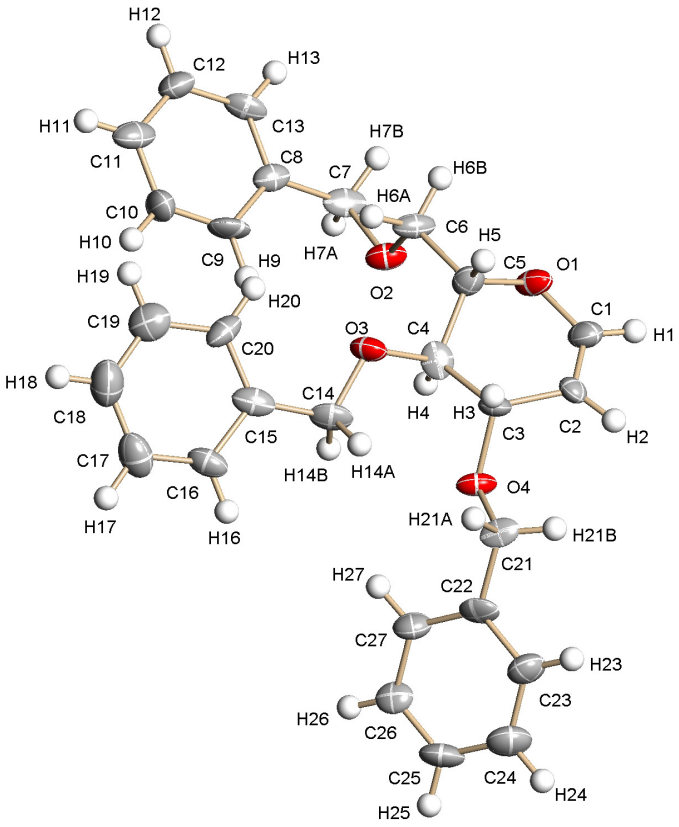
Table 7. Hydrogen bonds for 07020 [Å and °].

| D-H...A | d(D-H) | d(H...A) | d(D...A) | <(DHA) |
|---|---|---|---|---|
| O(2)-H(2)...O(3)#1 | 0.84 | 1.98 | 2.8190(17) | 173.0 |
| O(3)-H(3)...O(4)#2 | 0.84 | 1.80 | 2.6403(17) | 179.3 |
| O(4)-H(4)...O(2)#3 | 0.84 | 1.90 | 2.7350(17) | 178.3 |

Symmetry transformations used to generate equivalent atoms:

#1 x-1,y,z #2 -x+1,y+1/2,-z+1/2 #3 x,y-1,z

*5.5.2 Crystal data for tri-O-benzyl-D-glucal 124.*




Figures show 50% displacement ellipsoids. The hydrogen atoms have been included. R1 = 0.1166, wR2 = 0.2069.

Table 1. Crystal data and structure refinement for 07022.

| | | |
|---|---|---|
| Identification code | 07022 | |
| Empirical formula | C27 H28 O4 | |
| Formula weight | 416.49 | |
| Temperature | 150(2) K | |
| Wavelength | 0.71073 Å | |
| Crystal system | Monoclinic | |
| Space group | P2(1) | |
| Unit cell dimensions | a = 11.885(17) Å | α= 90°. |
| | b = 4.629(6) Å | β= 90.97(3)°. |
| | c = 19.47(3) Å | γ = 90°. |
| Volume | 1071(3) $Å^3$ | |
| Z | 2 | |
| Density (calculated) | 1.291 $Mg/m^3$ | |
| Absorption coefficient | 0.086 $mm^{-1}$ | |
| F(000) | 444 | |
| Crystal size | 0.25 x 0.23 x 0.04 $mm^3$ | |
| Theta range for data collection | 1.71 to 24.99°. | |
| Index ranges | -14<=h<=13, -5<=k<=5, -23<=l<=23 | |
| Reflections collected | 7780 | |
| Independent reflections | 3749 [R(int) = 0.2499] | |
| Completeness to theta = 24.99° | 100.0 % | |
| Absorption correction | None | |
| Refinement method | Full-matrix least-squares on $F^2$ | |
| Data / restraints / parameters | 3749 / 1 / 280 | |
| Goodness-of-fit on $F^2$ | 0.996 | |
| Final R indices [I>2sigma(I)] | R1 = 0.1166, wR2 = 0.2069 | |
| R indices (all data) | R1 = 0.2259, wR2 = 0.2608 | |
| Absolute structure parameter | -6(4) | |
| Largest diff. peak and hole | 0.359 and -0.321 $e.Å^{-3}$ | |

Table 2. Atomic coordinates ( x $10^4$) and equivalent isotropic displacement parameters ($Å^2$x $10^3$) for 07022. U(eq) is defined as one third of the trace of the orthogonalized $U^{ij}$ tensor.

| | x | y | z | U(eq) |
|---|---|---|---|---|
| O(1) | 8473(5) | -1281(13) | 568(3) | 38(2) |
| O(2) | 6237(5) | -1774(13) | 1076(3) | 37(2) |
| O(3) | 7720(5) | 2024(13) | 2183(3) | 35(2) |
| O(4) | 9864(5) | -1637(12) | 2467(3) | 32(2) |
| C(1) | 9580(8) | -1850(20) | 684(5) | 38(2) |
| C(2) | 10154(8) | -1141(18) | 1246(4) | 27(2) |
| C(4) | 8325(8) | -38(19) | 1778(5) | 32(2) |
| C(5) | 8005(7) | 750(20) | 1031(5) | 34(2) |
| C(6) | 6756(7) | 895(19) | 876(5) | 35(3) |
| C(7) | 5094(7) | -1982(19) | 838(5) | 35(2) |
| C(8) | 4297(7) | 156(19) | 1161(5) | 31(2) |
| C(9) | 4453(8) | 940(20) | 1843(5) | 45(3) |
| C(10) | 3685(8) | 2780(20) | 2139(5) | 45(3) |
| C(11) | 2772(8) | 3750(20) | 1774(5) | 44(3) |
| C(12) | 2625(8) | 2980(20) | 1098(5) | 39(3) |
| C(13) | 3392(8) | 1170(20) | 794(5) | 35(3) |
| C(14) | 7912(8) | 1670(20) | 2897(4) | 39(3) |
| C(15) | 7093(8) | 3390(20) | 3292(5) | 37(3) |
| C(16) | 7129(8) | 3170(20) | 4004(4) | 38(2) |
| C(17) | 6431(9) | 4720(30) | 4420(6) | 52(3) |
| C(18) | 5629(9) | 6550(30) | 4115(6) | 59(3) |
| C(19) | 5544(9) | 6740(20) | 3416(6) | 49(3) |
| C(20) | 6281(7) | 5170(20) | 3000(5) | 37(3) |
| C(21) | 10874(7) | -589(17) | 2813(5) | 30(2) |
| C(22) | 11086(8) | -2580(20) | 3408(5) | 37(3) |
| C(23) | 12174(8) | -3320(20) | 3584(5) | 42(3) |
| C(24) | 12396(9) | -5140(20) | 4129(5) | 52(3) |
| C(25) | 11505(9) | -6220(20) | 4513(5) | 48(3) |
| C(26) | 10425(8) | -5461(18) | 4343(5) | 35(3) |
| C(27) | 10218(8) | -3620(20) | 3801(5) | 37(3) |
| C(3) | 9575(7) | 120(20) | 1863(4) | 35(2) |

Table 3. Bond lengths [Å] and angles [°] for 07022.

| | |
|---|---|
| O(1)-C(1) | 1.358(10) |
| O(1)-C(5) | 1.423(10) |
| O(2)-C(7) | 1.430(9) |
| O(2)-C(6) | 1.438(10) |
| O(3)-C(14) | 1.413(9) |
| O(3)-C(4) | 1.439(10) |
| O(4)-C(21) | 1.449(9) |
| O(4)-C(3) | 1.466(9) |
| C(1)-C(2) | 1.322(11) |
| C(1)-H(1) | 0.9500 |
| C(2)-C(3) | 1.514(12) |
| C(2)-H(2) | 0.9500 |
| C(4)-C(3) | 1.493(12) |
| C(4)-C(5) | 1.541(12) |
| C(4)-H(4) | 1.0000 |
| C(5)-C(6) | 1.511(11) |
| C(5)-H(5) | 1.0000 |
| C(6)-H(6A) | 0.9900 |
| C(6)-H(6B) | 0.9900 |
| C(7)-C(8) | 1.514(13) |
| C(7)-H(7A) | 0.9900 |
| C(7)-H(7B) | 0.9900 |
| C(8)-C(13) | 1.364(11) |
| C(8)-C(9) | 1.387(12) |
| C(9)-C(10) | 1.380(13) |
| C(9)-H(9) | 0.9500 |
| C(10)-C(11) | 1.365(11) |
| C(10)-H(10) | 0.9500 |
| C(11)-C(12) | 1.373(13) |
| C(11)-H(11) | 0.9500 |
| C(12)-C(13) | 1.379(13) |
| C(12)-H(12) | 0.9500 |
| C(13)-H(13) | 0.9500 |
| C(14)-C(15) | 1.483(13) |
| C(14)-H(14A) | 0.9900 |
| C(14)-H(14B) | 0.9900 |
| C(15)-C(20) | 1.383(12) |
| C(15)-C(16) | 1.389(12) |

| | |
|---|---|
| C(16)-C(17) | 1.373(13) |
| C(16)-H(16) | 0.9500 |
| C(17)-C(18) | 1.401(14) |
| C(17)-H(17) | 0.9500 |
| C(18)-C(19) | 1.365(13) |
| C(18)-H(18) | 0.9500 |
| C(19)-C(20) | 1.407(13) |
| C(19)-H(19) | 0.9500 |
| C(20)-H(20) | 0.9500 |
| C(21)-C(22) | 1.499(12) |
| C(21)-H(21A) | 0.9900 |
| C(21)-H(21B) | 0.9900 |
| C(22)-C(23) | 1.375(11) |
| C(22)-C(27) | 1.383(12) |
| C(23)-C(24) | 1.378(13) |
| C(23)-H(23) | 0.9500 |
| C(24)-C(25) | 1.397(14) |
| C(24)-H(24) | 0.9500 |
| C(25)-C(26) | 1.366(13) |
| C(25)-H(25) | 0.9500 |
| C(26)-C(27) | 1.375(12) |
| C(26)-H(26) | 0.9500 |
| C(27)-H(27) | 0.9500 |
| C(3)-H(3) | 1.0000 |

| | |
|---|---|
| C(1)-O(1)-C(5) | 114.4(7) |
| C(7)-O(2)-C(6) | 112.3(6) |
| C(14)-O(3)-C(4) | 112.9(7) |
| C(21)-O(4)-C(3) | 111.5(7) |
| C(2)-C(1)-O(1) | 125.0(9) |
| C(2)-C(1)-H(1) | 117.5 |
| O(1)-C(1)-H(1) | 117.5 |
| C(1)-C(2)-C(3) | 121.3(9) |
| C(1)-C(2)-H(2) | 119.4 |
| C(3)-C(2)-H(2) | 119.4 |
| O(3)-C(4)-C(3) | 114.2(7) |
| O(3)-C(4)-C(5) | 104.0(7) |
| C(3)-C(4)-C(5) | 108.8(7) |
| O(3)-C(4)-H(4) | 109.9 |
| C(3)-C(4)-H(4) | 109.9 |

| | |
|---|---|
| C(5)-C(4)-H(4) | 109.9 |
| O(1)-C(5)-C(6) | 107.1(7) |
| O(1)-C(5)-C(4) | 110.3(7) |
| C(6)-C(5)-C(4) | 115.2(8) |
| O(1)-C(5)-H(5) | 108.0 |
| C(6)-C(5)-H(5) | 108.0 |
| C(4)-C(5)-H(5) | 108.0 |
| O(2)-C(6)-C(5) | 109.4(7) |
| O(2)-C(6)-H(6A) | 109.8 |
| C(5)-C(6)-H(6A) | 109.8 |
| O(2)-C(6)-H(6B) | 109.8 |
| C(5)-C(6)-H(6B) | 109.8 |
| H(6A)-C(6)-H(6B) | 108.2 |
| O(2)-C(7)-C(8) | 114.7(7) |
| O(2)-C(7)-H(7A) | 108.6 |
| C(8)-C(7)-H(7A) | 108.6 |
| O(2)-C(7)-H(7B) | 108.6 |
| C(8)-C(7)-H(7B) | 108.6 |
| H(7A)-C(7)-H(7B) | 107.6 |
| C(13)-C(8)-C(9) | 120.2(9) |
| C(13)-C(8)-C(7) | 120.1(8) |
| C(9)-C(8)-C(7) | 119.6(8) |
| C(10)-C(9)-C(8) | 118.9(9) |
| C(10)-C(9)-H(9) | 120.6 |
| C(8)-C(9)-H(9) | 120.6 |
| C(11)-C(10)-C(9) | 120.8(10) |
| C(11)-C(10)-H(10) | 119.6 |
| C(9)-C(10)-H(10) | 119.6 |
| C(10)-C(11)-C(12) | 120.0(10) |
| C(10)-C(11)-H(11) | 120.0 |
| C(12)-C(11)-H(11) | 120.0 |
| C(11)-C(12)-C(13) | 119.7(9) |
| C(11)-C(12)-H(12) | 120.1 |
| C(13)-C(12)-H(12) | 120.1 |
| C(8)-C(13)-C(12) | 120.4(9) |
| C(8)-C(13)-H(13) | 119.8 |
| C(12)-C(13)-H(13) | 119.8 |
| O(3)-C(14)-C(15) | 110.6(8) |
| O(3)-C(14)-H(14A) | 109.5 |
| C(15)-C(14)-H(14A) | 109.5 |

| | |
|---|---|
| O(3)-C(14)-H(14B) | 109.5 |
| C(15)-C(14)-H(14B) | 109.5 |
| H(14A)-C(14)-H(14B) | 108.1 |
| C(20)-C(15)-C(16) | 117.7(10) |
| C(20)-C(15)-C(14) | 124.4(9) |
| C(16)-C(15)-C(14) | 118.0(9) |
| C(17)-C(16)-C(15) | 122.8(10) |
| C(17)-C(16)-H(16) | 118.6 |
| C(15)-C(16)-H(16) | 118.6 |
| C(16)-C(17)-C(18) | 118.6(10) |
| C(16)-C(17)-H(17) | 120.7 |
| C(18)-C(17)-H(17) | 120.7 |
| C(19)-C(18)-C(17) | 120.0(11) |
| C(19)-C(18)-H(18) | 120.0 |
| C(17)-C(18)-H(18) | 120.0 |
| C(18)-C(19)-C(20) | 120.3(11) |
| C(18)-C(19)-H(19) | 119.8 |
| C(20)-C(19)-H(19) | 119.8 |
| C(15)-C(20)-C(19) | 120.5(10) |
| C(15)-C(20)-H(20) | 119.8 |
| C(19)-C(20)-H(20) | 119.8 |
| O(4)-C(21)-C(22) | 106.3(7) |
| O(4)-C(21)-H(21A) | 110.5 |
| C(22)-C(21)-H(21A) | 110.5 |
| O(4)-C(21)-H(21B) | 110.5 |
| C(22)-C(21)-H(21B) | 110.5 |
| H(21A)-C(21)-H(21B) | 108.7 |
| C(23)-C(22)-C(27) | 118.8(9) |
| C(23)-C(22)-C(21) | 119.5(10) |
| C(27)-C(22)-C(21) | 121.7(8) |
| C(22)-C(23)-C(24) | 120.8(10) |
| C(22)-C(23)-H(23) | 119.6 |
| C(24)-C(23)-H(23) | 119.6 |
| C(23)-C(24)-C(25) | 119.5(10) |
| C(23)-C(24)-H(24) | 120.2 |
| C(25)-C(24)-H(24) | 120.2 |
| C(26)-C(25)-C(24) | 119.8(9) |
| C(26)-C(25)-H(25) | 120.1 |
| C(24)-C(25)-H(25) | 120.1 |
| C(25)-C(26)-C(27) | 120.0(10) |

| | |
|---|---|
| C(25)-C(26)-H(26) | 120.0 |
| C(27)-C(26)-H(26) | 120.0 |
| C(26)-C(27)-C(22) | 121.0(9) |
| C(26)-C(27)-H(27) | 119.5 |
| C(22)-C(27)-H(27) | 119.5 |
| O(4)-C(3)-C(4) | 106.3(7) |
| O(4)-C(3)-C(2) | 108.6(7) |
| C(4)-C(3)-C(2) | 110.9(8) |
| O(4)-C(3)-H(3) | 110.3 |
| C(4)-C(3)-H(3) | 110.3 |
| C(2)-C(3)-H(3) | 110.3 |

---

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for 07022. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ...\ + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

| | $U^{11}$ | $U^{22}$ | $U^{33}$ | $U^{23}$ | $U^{13}$ | $U^{12}$ |
|---|---|---|---|---|---|---|
| O(1) | 46(4) | 35(4) | 31(4) | -8(3) | -14(3) | 15(4) |
| O(2) | 41(4) | 28(4) | 41(4) | 2(3) | -17(3) | -1(4) |
| O(3) | 48(4) | 34(4) | 23(4) | 0(3) | -10(3) | 6(3) |
| O(4) | 37(4) | 22(3) | 35(4) | 4(3) | -18(3) | -4(3) |
| C(1) | 42(6) | 39(6) | 34(6) | 2(5) | -6(5) | 13(6) |
| C(2) | 35(5) | 27(5) | 21(5) | 6(4) | 4(4) | 1(5) |
| C(4) | 38(6) | 18(5) | 41(6) | -9(5) | 4(5) | -16(5) |
| C(5) | 29(6) | 40(6) | 32(6) | -7(5) | -7(5) | 2(5) |
| C(6) | 34(6) | 27(5) | 43(6) | 11(5) | -18(5) | -11(5) |
| C(7) | 38(6) | 21(5) | 45(6) | 12(5) | -14(5) | -19(5) |
| C(8) | 28(6) | 25(6) | 41(6) | 6(5) | -9(5) | -8(5) |
| C(9) | 42(7) | 64(8) | 29(6) | 13(6) | -21(5) | -2(6) |
| C(10) | 44(6) | 65(8) | 26(6) | -4(6) | 3(5) | 17(6) |
| C(11) | 40(6) | 42(7) | 50(7) | 20(6) | -12(5) | -6(5) |
| C(12) | 34(6) | 40(6) | 43(6) | 4(6) | -8(5) | 10(5) |
| C(13) | 50(7) | 31(6) | 25(5) | 1(5) | -17(5) | -17(5) |
| C(14) | 48(7) | 37(6) | 31(6) | 0(5) | -21(5) | -9(6) |
| C(15) | 50(6) | 27(5) | 33(6) | 4(5) | -14(5) | -15(6) |
| C(16) | 57(7) | 32(5) | 25(5) | 0(5) | -9(5) | -6(6) |
| C(17) | 51(7) | 67(8) | 38(7) | -16(6) | 6(6) | -14(7) |
| C(18) | 46(8) | 75(9) | 56(8) | -17(7) | 10(6) | -1(7) |
| C(19) | 55(7) | 38(6) | 55(8) | -9(6) | -8(6) | 0(6) |
| C(20) | 25(6) | 27(5) | 59(7) | 7(5) | -3(5) | 4(5) |
| C(21) | 38(6) | 10(4) | 43(6) | -8(4) | -14(5) | -3(4) |
| C(22) | 50(7) | 40(7) | 21(5) | -9(5) | -19(5) | 5(5) |
| C(23) | 34(7) | 48(7) | 44(7) | -1(6) | -12(5) | 4(5) |
| C(24) | 48(7) | 59(7) | 47(7) | 4(6) | -21(6) | 0(6) |
| C(25) | 59(7) | 50(7) | 34(6) | 11(6) | -20(6) | 3(6) |
| C(26) | 45(7) | 18(5) | 41(6) | -4(4) | -6(5) | 1(5) |
| C(27) | 40(6) | 29(6) | 41(6) | 7(5) | -9(5) | 4(5) |
| C(3) | 35(6) | 43(6) | 25(5) | 13(5) | -5(4) | 8(5) |

Table 5. Hydrogen coordinates ( x $10^4$) and isotropic displacement parameters ($Å^2$x $10^{3}$) for 07022.

| | x | y | z | U(eq) |
|---|---|---|---|---|
| H(1) | 9972 | -2846 | 334 | 46 |
| H(2) | 10946 | -1427 | 1262 | 33 |
| H(4) | 8060 | -2038 | 1883 | 39 |
| H(5) | 8335 | 2690 | 929 | 40 |
| H(6A) | 6418 | 2523 | 1130 | 42 |
| H(6B) | 6627 | 1227 | 378 | 42 |
| H(7A) | 4819 | -3961 | 930 | 42 |
| H(7B) | 5075 | -1693 | 334 | 42 |
| H(9) | 5077 | 225 | 2103 | 54 |
| H(10) | 3794 | 3365 | 2603 | 54 |
| H(11) | 2238 | 4972 | 1988 | 53 |
| H(12) | 1997 | 3686 | 841 | 47 |
| H(13) | 3289 | 626 | 327 | 42 |
| H(14A) | 8686 | 2302 | 3018 | 47 |
| H(14B) | 7841 | -394 | 3019 | 47 |
| H(16) | 7661 | 1897 | 4211 | 46 |
| H(17) | 6490 | 4555 | 4906 | 62 |
| H(18) | 5145 | 7668 | 4393 | 71 |
| H(19) | 4984 | 7938 | 3210 | 59 |
| H(20) | 6220 | 5323 | 2514 | 44 |
| H(21A) | 10764 | 1415 | 2976 | 36 |
| H(21B) | 11518 | -619 | 2497 | 36 |
| H(23) | 12779 | -2574 | 3326 | 51 |
| H(24) | 13149 | -5665 | 4244 | 62 |
| H(25) | 11650 | -7469 | 4891 | 57 |
| H(26) | 9817 | -6207 | 4598 | 42 |
| H(27) | 9466 | -3052 | 3696 | 44 |
| H(3) | 9817 | 2169 | 1938 | 42 |

Table 6. Torsion angles [°] for 07022.

| | |
|---|---|
| C(5)-O(1)-C(1)-C(2) | 13.7(13) |
| O(1)-C(1)-C(2)-C(3) | 7.2(15) |
| C(14)-O(3)-C(4)-C(3) | -61.0(10) |
| C(14)-O(3)-C(4)-C(5) | -179.5(7) |
| C(1)-O(1)-C(5)-C(6) | -174.3(8) |
| C(1)-O(1)-C(5)-C(4) | -48.2(10) |
| O(3)-C(4)-C(5)-O(1) | -175.1(7) |
| C(3)-C(4)-C(5)-O(1) | 62.7(9) |
| O(3)-C(4)-C(5)-C(6) | -53.7(9) |
| C(3)-C(4)-C(5)-C(6) | -175.8(8) |
| C(7)-O(2)-C(6)-C(5) | -170.0(7) |
| O(1)-C(5)-C(6)-O(2) | 69.2(9) |
| C(4)-C(5)-C(6)-O(2) | -54.0(11) |
| C(6)-O(2)-C(7)-C(8) | -66.7(9) |
| O(2)-C(7)-C(8)-C(13) | 148.9(8) |
| O(2)-C(7)-C(8)-C(9) | -35.1(12) |
| C(13)-C(8)-C(9)-C(10) | -0.7(14) |
| C(7)-C(8)-C(9)-C(10) | -176.7(9) |
| C(8)-C(9)-C(10)-C(11) | 1.6(15) |
| C(9)-C(10)-C(11)-C(12) | -1.8(15) |
| C(10)-C(11)-C(12)-C(13) | 1.1(15) |
| C(9)-C(8)-C(13)-C(12) | 0.0(13) |
| C(7)-C(8)-C(13)-C(12) | 176.0(9) |
| C(11)-C(12)-C(13)-C(8) | -0.2(14) |
| C(4)-O(3)-C(14)-C(15) | -167.6(7) |
| O(3)-C(14)-C(15)-C(20) | -2.1(13) |
| O(3)-C(14)-C(15)-C(16) | 176.9(8) |
| C(20)-C(15)-C(16)-C(17) | -2.7(14) |
| C(14)-C(15)-C(16)-C(17) | 178.3(9) |
| C(15)-C(16)-C(17)-C(18) | 1.4(16) |
| C(16)-C(17)-C(18)-C(19) | 1.0(16) |
| C(17)-C(18)-C(19)-C(20) | -2.0(16) |
| C(16)-C(15)-C(20)-C(19) | 1.6(13) |
| C(14)-C(15)-C(20)-C(19) | -179.4(9) |
| C(18)-C(19)-C(20)-C(15) | 0.6(15) |
| C(3)-O(4)-C(21)-C(22) | -179.1(7) |
| O(4)-C(21)-C(22)-C(23) | 142.3(8) |
| O(4)-C(21)-C(22)-C(27) | -39.9(11) |

| | |
|---|---|
| C(27)-C(22)-C(23)-C(24) | 2.0(15) |
| C(21)-C(22)-C(23)-C(24) | 179.8(9) |
| C(22)-C(23)-C(24)-C(25) | -0.8(15) |
| C(23)-C(24)-C(25)-C(26) | 0.2(16) |
| C(24)-C(25)-C(26)-C(27) | -0.8(15) |
| C(25)-C(26)-C(27)-C(22) | 2.1(14) |
| C(23)-C(22)-C(27)-C(26) | -2.7(14) |
| C(21)-C(22)-C(27)-C(26) | 179.5(8) |
| C(21)-O(4)-C(3)-C(4) | -150.8(7) |
| C(21)-O(4)-C(3)-C(2) | 89.8(9) |
| O(3)-C(4)-C(3)-O(4) | 85.1(9) |
| C(5)-C(4)-C(3)-O(4) | -159.1(7) |
| O(3)-C(4)-C(3)-C(2) | -157.0(7) |
| C(5)-C(4)-C(3)-C(2) | -41.3(10) |
| C(1)-C(2)-C(3)-O(4) | 125.5(9) |
| C(1)-C(2)-C(3)-C(4) | 9.0(12) |

Symmetry transformations used to generate equivalent atoms:

# Appendix One References

## A1.0 References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# Appendix Two Publications

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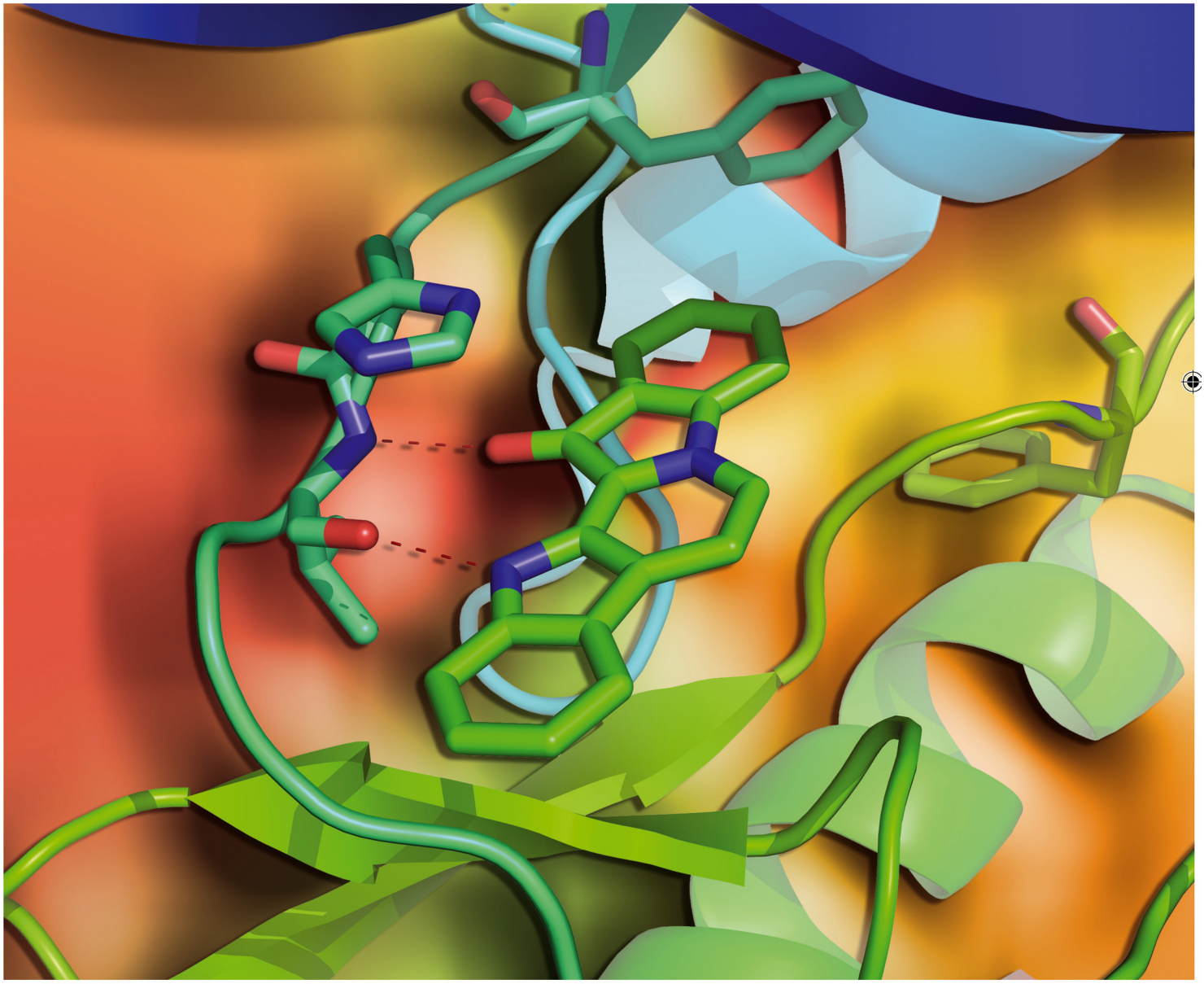

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# Design, synthesis and biological activity of new CDK4-specific inhibitors, based on fascaplysin

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We present the design, synthesis, and biological activity of three classes of tryptamine derivatives, which are non-planar analogues of the toxic anti-cancer agent fascaplysin. We show these compounds to be selective inhibitors of CDK4 over CDK2, the most active compound **9q** has an $IC_{50}$ for the inhibition of CDK4 of 6 μM.

## Introduction

Fascaplysin **1** (Fig. 1), is a pentacyclic quaternary salt originally isolated from the Fijian sponge *Fascaplysinopsis* Bergquist sp.,[1] which inhibits the growth of several microbes, including *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Saccharomyces cerevisiae*, and suppresses the proliferation of mouse leukemia cells L-1210 with $ED_{50} = 0.2$ μm $mL^{-1}$. Fascaplysin has also been reported to specifically inhibit CDK4, causing $G_1$ arrest of tumour (U2-OS, HCT-116) and normal (MRC-5) cells.[2]

The inhibition of cyclin-dependent kinases, CDKs, by small molecules is an area of major current interest in the anti-cancer field.[3] CDKs are a vital component of the check-points in the various phases of the cell division cycle,[4] they are required for healthy cell growth and proliferation. CDK4 has a very specific function in the $G_0$–$G_1$ phase of the cell division cycle—the CDK4–cyclin D1 complex phosphorylates the protein retinoblastoma (pRB), an active repressor of the E2F family of transcription factors. CDK4-mediated hyperphosphorylation of pRB facilitates liberation of E2F proteins and allows them to carry out their transcriptional activation roles. This enables the cell to pass through the restriction point, an early $G_1$ checkpoint, where the cell commits itself to complete one cell division cycle.[5] Inhibition of CDK4 is therefore a vital factor in controlling the rate of cell proliferation. In normal cells this is carried out by CDK4-specific cyclin-dependent kinase inhibitors (CKIs) such as p16; in tumour cells, inactivating mutations which result in the underproduction of p16 are common.[6] Moreover, the activating partner cyclin D1 and the catalytic subunit CDK4 are often either overproduced or hyper-activated in many tumour cells. A small-molecule inhibitor of CDK4 would result in early $G_1$ arrest of the cell cycle and thus prevent uncontrolled cell growth, the hallmark of all tumour cells. Given that CDK4 is a kinase, the most obvious structures to act as inhibitors of CDK4 are analogues of ATP.[7] Additionally, it is known that structures totally unrelated to ATP, such as staurosporine and flavopiridol, are also effective inhibitors of CDK4.[3]

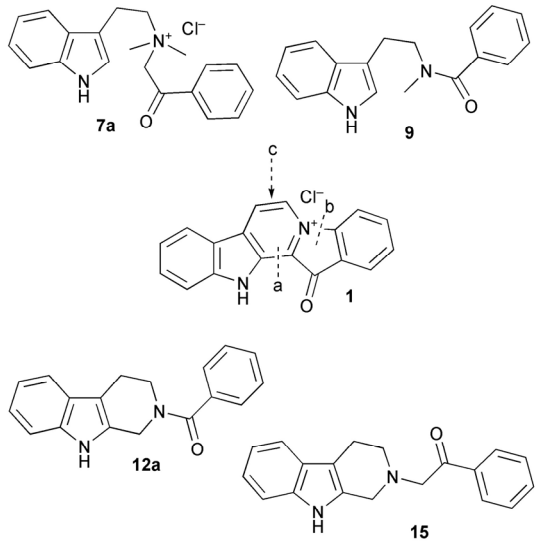


**Fig. 1** Strategy used to produce the non-planar tryptamine derivatives **7a**, **9**, **12a** and **15** from fascaplysin **1**.

Fascaplysin **1** cannot be used as an anti-cancer drug because it is highly toxic—the potential for its planar structure to intercalate with DNA has been suggested as a possible explanation.[8] The aim of the current study is therefore to devise a potent, non-toxic (non-planar) CDK4 inhibitor based on the structure of fascaplysin.

## Results and discussion

The general approach we adopt for generating non-toxic inhibitors of CDK4 involves the synthesis of non-planar analogues of

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fascaplysin, thus avoiding their interchelation with DNA. To enhance the chances of success, these compounds are designed to maintain most of the key interactions thought to occur between fascaplysin and CDK4. Since the 3-dimensional structure of CDK4 has not been determined, structural information is obtained by molecular modelling. Thus, prior to embarking on the synthesis of potential inhibitors, we first carried out *in silico* studies based on our homology model of CDK4, produced using the known crystal structures of CDK2 and CDK6 as templates (Fig. 3). Our dockings of ATP and fascaplysin suggest that inhibition of CDK4 activity by fascaplysin arises from binding to the same site as ATP (Fig. 2a). This suggests that a key feature of fascaplysin binding is the double hydrogen bond to Val 96[7] (Fig. 2b).

In this study, the general strategy we adopt for removing toxicity[10] (Fig. 1) comprises releasing bonds a and b in fascaplysin **1** and changing double bond c into a single bond, leading to the tryptamine derivative **7a**. This compound contains the structural components of fascaplysin, but it is non-planar and is therefore not likely to intercalate with DNA. This was our first generation compound, which had a great deal of flexibility due to rotation around six bonds. Herein we also describe the second generation compounds **9a–q** which have a shorter chain between the indole and the benzene ring and an amide bond leading to fewer degrees of rotational freedom in the molecules. In third generation compounds **12a–q** and **16** we have reduced still further the rotational freedom of the structure by incorporating the tetrahydro β-carboline structure. Hence, compounds **5a–c**, **7a–c**, **9a–q**, **10**, **12a–q**, and **13–16** were synthesised (Schemes 1–3). These compounds are not planar and so intercalation in DNA is very unlikely.

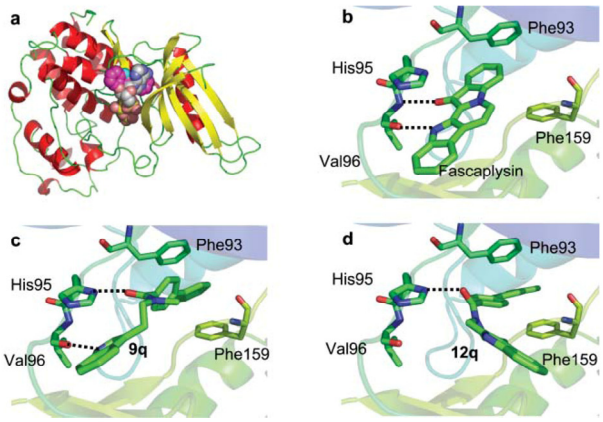
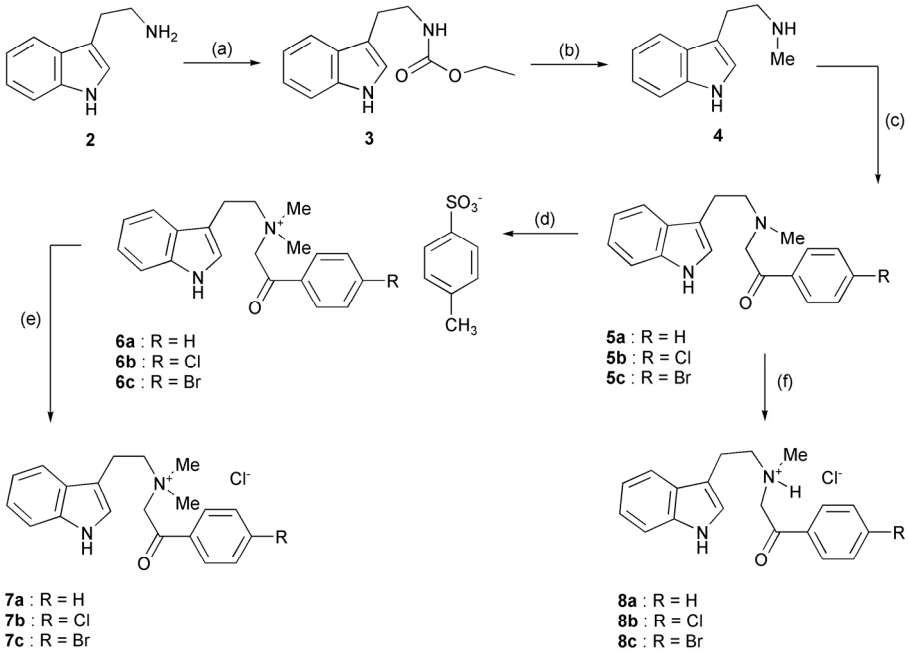



**Fig. 2** The active site of our CDK4 model. (a) Cartoon representation of the overall structure of the CDK4, with the positions of ATP–$Mg^{2+}$ (C in grey, N in slate, O in salmon, P in yellow) and fascaplysin (C in magenta, N in cyan, O in red) shown. (b) Predicted binding mode of fascaplysin. (c) Predicted binding mode of the most potent compound, **9q**; note the π–π interactions of the benzoid ring with Phe 93 and Phe 159. (d) Predicted binding mode of **12q**—this weaker inhibitor is structurally similar to, but more conformationally constrained than, **9q**. Hydrogen bonds are shown as dashed lines. Figures produced using PyMol.[9]

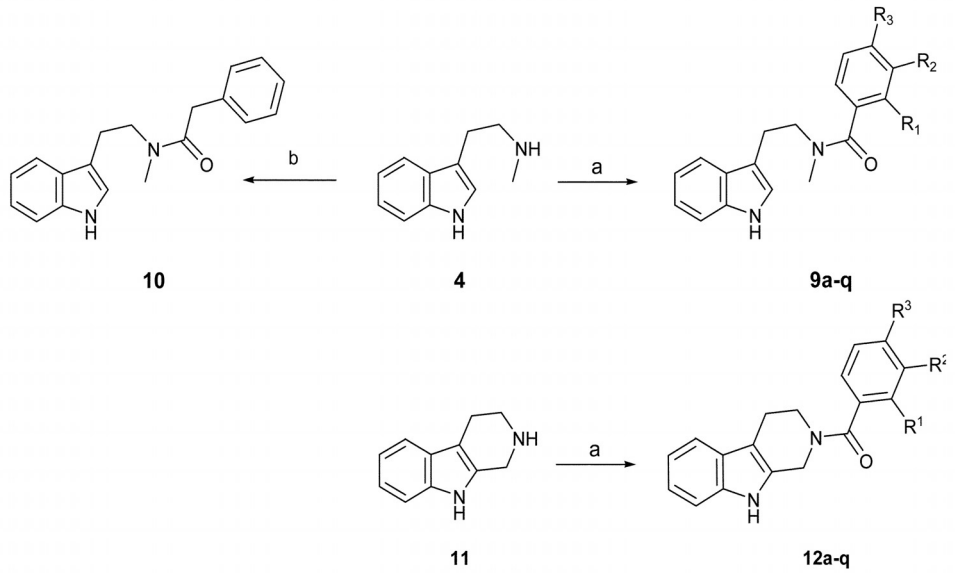
### First generation compounds

The synthesis of **7a** is shown in Scheme 1. Tryptamine **2** was reacted with ethyl chloroformate to give the urethane **3** in 95% yield.[11] Reduction with lithium aluminium hydride produced




**Scheme 1** *Reagents and conditions*. (a) Ethyl chloroformate, NaOH 4 M, $CHCl_3$, 3 h, 95%; (b) $LiAlH_4$, THF, $N_2$, reflux, 1 h, 89%; (c) $BrCH_2COC_6H_4R$, toluene, $N_2$, $NaHCO_3$, $Na_2SO_4$, $H_2O$, 4 h, 49–61%; (d) methyl-*p*-toluenesulfonate, acetonitrile, reflux, 4 h; (e) Dowex® $Cl^-$ 1 × 8–400 ion exchange resin, overnight stirring and column, 46–53%; (f) HCl gas, $Et_2O$ or $CH_2Cl_2$, <1 min, 46–88%.




| | $R^1$ | $R^2$ | $R^3$ | | | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|---|---|---|---|---|
| **9a & 12a** | H | H | H | | **9j & 12j** | H | Br | H |
| **9b & 12b** | H | H | $^t$Bu | | **9k & 12k** | H | H | Br |
| **9c & 12c** | F | H | H | | **9l & 12l** | OMe | H | H |
| **9d & 12d** | H | F | H | | **9m& 12m** | H | OMe | H |
| **9e & 12e** | H | H | F | | **9n & 12n** | Me | H | H |
| **9f & 12f** | Cl | H | H | | **9o & 12o** | H | Me | H |
| **9g & 12g** | H | Cl | H | | **9p & 12p** | H | H | Me |
| **9h & 12h** | H | H | Cl | | **9q & 12q** | H | H | Ph |
| **9i & 12i** | Br | H | H | | | | | |

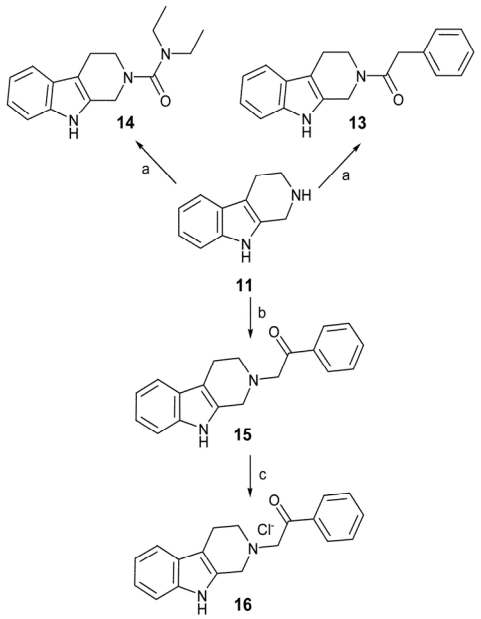
**Scheme 2** *Reagents and conditions*. (a) $ClCOC_6H_4R$, $NaOH_{(aq)}$ 4 M, $CH_2Cl_2$, 0 °C, 15 min then RT, 3 h, 37–99%; (b) $ClCOCH_2C_6H_5$, $NaOH_{(aq)}$ 4 M, $CH_2Cl_2$, 0 °C, 15 min then RT, 3 h, 37–99%.

*N*-methyltryptamine **4** in 89% yield.[11] This intermediate **4** was then reacted with different 4-substituted bromoacetophenone derivatives to afford the compounds **5a–c** in yields between 49% and 61% after column chromatography.[12] The tertiary amines **5a–c** were unstable after a week when stored at −25 °C, as indicated by NMR. These materials were reacted separately with methyl-*p*-toluene sulfonate to furnish the tosylate salts **7a–c**. Ion exchange using Dowex® $Cl^-$ 1 × 8–400 ion exchange resin produced the chloride salts **7a–c** in yields between 46% and 53%.[13]

The CDK4 activities of compounds **7a–c** and **5a–c** have been tested for some of the biochemical features that would identify a CDK4 inhibitor. In accord with our predictions, the $IC_{50}$ values (Table 1) show that all compounds are CDK4 active. Furthermore, the CDK2 activities of compounds **7a–c** and **5a–c**, measured in terms of $IC_{50}$ values, reveal that all twelve tryptamine derivatives are CDK4 selective compared to CDK2 (Table 1). Representative compounds (**7a–c**), like fascaplysin, block growth of cancer cells in the $G_1$ phase of the cell division cycle and they also maintain the $G_1$ block of serum-starved cells. However, unlike fascaplysin, the compounds do not intercalate DNA or inhibit topoisomerase I which we believe contributes to the general toxicity of fascaplysin (manuscript in preparation).

### Second generation compounds

As mentioned above the second generation compounds **9a–q** and **10** are amide derivatives of *N*-methyl tryptamine. The series of compounds were readily synthesised as shown in Scheme 2 and the results for the inhibition of CDK4 and CDK2 are shown in Table 1. The first clear trend from the results is the selectivity for inhibition of CDK4 compared to CDK2. The parent compound **9a** shows a modest $IC_{50}$ of 88 μM; this activity changes when



**Scheme 3** *Reagents and conditions*. (a) $ClCOC_6H_4R$, $NaOH_{(aq)}$ 4 M, $CH_2Cl_2$, 0 °C, 15 min then RT, 3 h, 63–91%; (b) $BrCH_2COC_6H_5$, toluene, $N_2$, $NaHCO_3$, $Na_2SO_4$, $H_2O$, 4 h, 61%; (c) HCl gas, $CH_2Cl_2$, 3 min, 95%.

halogen substituents are present on the benzenoid ring and shows a consistent improvement for *para* substituted derivatives (**9e**, *p*-F, $IC_{50}$ 59; **9h**, *p*-Cl, $IC_{50}$ 38 and **9k**, *p*-Br, $IC_{50}$ 37 μM). The trend of improved activity, lower $IC_{50}$, for *para* substituted compounds is continued with **9p**, *p*-Me, $IC_{50}$ 63; **9b**, *p*-$^t$Bu, $IC_{50}$ 49 μM and the best result **9q**, *p*-Ph, $IC_{50}$ 6 μM. The phenyl substitution in the *para* position appears responsible for enhanced inhibition of the series **9** compounds.

### Third generation compounds

In the third generation compounds **12a–q** and **13–16** we have investigated the effect of a tetrahydro β-carboline structure, which further reduced the degree of conformational flexibility of the inhibitor. The series of compounds **12a–q**, **13** and **14** were readily synthesised by the same method used for **9a–q** as shown in Schemes 2 and 3. The β-carboline **11** was reacted with 2-bromoacetophenone in the presence of sodium hydrogen carbonate and sodium sulfate to produce **15** in 61% yield. The HCl salt **16** was then formed by bubbling HCl gas into a solution of **15** in dichloromethane. Surprisingly the resultant salt was soluble in dichloromethane, as no precipitate was formed, so the mixture was evaporated off to give the HCl salt **16** with 95% yield which was then characterized by NMR and mass spectroscopy. NMR clearly showed two NH signals (10.98 and 11.23 ppm) and the expected mass was recorded in a FAB experiment.

The results for the inhibition of CDK4 and CDK2 are shown in Table 1. Again we see that all compounds tested are selective inhibitors of CDK4 compared to CDK2. The parent structure **12a** has a modest $IC_{50}$ of 92 μM, which is improved by halogen substituents of which **12e**, *p*-F, $IC_{50}$ 55; **12g**, *m*-Cl, $IC_{50}$ 56 and **12j**, *m*-Br, $IC_{50}$ 32 μM are the most active. It is not clear at this stage why the *m*-Cl, **12g**, and *m*-Br, **12j**, compounds are better inhibitors in this series compared with *p*-Cl, **9h**, and *p*-Br, **9k**. The *meta* methyl

**Table 1** CDK4 activity *versus* CDK2 activity

| Compound | [a]CDK4 measured $IC_{50}$/μM | [b]CDK2 measured $IC_{50}$/μM |
|---|---|---|
| Fascaplysin **1** | 0.55[10] | 500 |
| **7a** | 68 ± 8 | 720 ± 16 |
| **7b** | 50 ± 7 | 605 ± 10 |
| **7c** | 50 ± 6 | 665 ± 18 |
| **5a** | 212 ± 16 (91 ± 7)[c] | 1310 ± 21 (860 ± 14)[c] |
| **5b** | 90 ± 11 (110 ± 9)[c] | 910 ± 7 (911 ± 24)[c] |
| **5c** | 80 ± 11.5 (84 ± 9)[c] | 921 ± 16 (932 ± 20)[c] |
| **9a** | 88 ± 6 | 1523 ± 38 |
| **9b** | 49 ± 7 | 658 ± 23 |
| **9c** | 103 ± 9 | 1230 ± 29 |
| **9d** | 88 ± 9 | 765 ± 27 |
| **9e** | 59 ± 7 | 850 ± 34 |
| **9f** | 46 ± 4 | 784 ± 20 |
| **9g** | 109 ± 8 | 1120 ± 38 |
| **9h** | 38 ± 6 | 731 ± 26 |
| **9i** | 74 ± 7 | 635 ± 40 |
| **9j** | 95 ± 7 | 584 ± 24 |
| **9k** | 37 ± 5 | 580 ± 38 |
| **9l** | 79 ± 9 | 849 ± 30 |
| **9m** | 81 ± 4 | 938 ± 47 |
| **9n** | 113 ± 8 | 1125 ± 40 |
| **9o** | 78 ± 8 | 830 ± 31 |
| **9p** | 63 ± 6 | 790 ± 27 |
| **9q** | 6 ± 1 | 521 ± 12 |
| **10** | 28 ± 4 | 959 ± 18 |
| **12a** | 92 ± 10 | 490 ± 40 |
| **12b** | 44 ± 9 | 720 ± 24 |
| **12c** | 61 ± 7 | 800 ± 22 |
| **12d** | 72 ± 5 | 680 ± 20 |
| **12e** | 55 ± 6 | 851 ± 22 |
| **12f** | 98 ± 10 | 1197 ± 30 |
| **12g** | 56 ± 6 | 873 ± 38 |
| **12h** | 76 ± 10 | 674 ± 25 |
| **12i** | 44 ± 5 | 679 ± 31 |
| **12j** | 32 ± 6 | 675 ± 30 |
| **12k** | 51 ± 4 | 875 ± 37 |
| **12l** | 26 ± 6 | 861 ± 42 |
| **12m** | 24 ± 4 | 766 ± 33 |
| **12n** | 68 ± 8 | 750 ± 31 |
| **12o** | 27 ± 5 | 818 ± 35 |
| **12p** | 45 ± 6 | 751 ± 34 |
| **12q** | 64 ± 9 | 829 ± 28 |
| **13** | 95 ± 11 | 1450 ± 42 |
| **14** | 54 ± 5 | 926 ± 35 |
| **15** | 62 ± 7 | 1301 ± 21 |
| **16** | 34 ± 4 | 1467 ± 39 |

[a] CDK4–cyclin D1 assay, using GST-RB152 fusion protein as the substrate. [b] CDK2–cyclin A assay using histone H1 as the substrate. [c] The values in parentheses () indicate the results of the HCl salts of **5a–c**, *e.g.* **8a–c**.

derivative **12o** has an $IC_{50}$ of 27 μM making it the best of the three methyl derivatives, while *p*-$^t$Bu, **12b**, $IC_{50}$ 44 and *p*-phenyl, **12q**, $IC_{50}$ 64 μM are better inhibitors than the parent compound **12a**. The *meta*-methoxy compound **12m**, $IC_{50}$ 24 μM, is the best result of this series and follows the trend of *meta* compounds being most active.

### Biphenyl-4-carboxylic acid [2-(1*H*-indol-3-yl)-ethyl]-methylamide 9q, the most potent inhibitor

The interactions of the most potent inhibitor, **9q**, were rationalised by *in silico* modelling. When docking our full set of compounds into the CDK4 model, only **9q** and **12q** (Chemscore values of the respective best solutions 32.8 kJ $mol^{-1}$ and 32.2 kJ $mol^{-1}$) show similar Chemscore values to that observed for fascaplysin

**1** (Chemscore 30.0 kJ $mol^{-1}$). All the other compounds are predicted to be weaker binders with Chemscore values in the range of 20 to 25 kJ $mol^{-1}$. The binding mode of **9q** with the best Chemscore value (Fig. 2c) clearly overlaps with the best binding mode of fascaplysin (Fig. 2b). In both complexes, the indole group occupies a similar position and the interaction between the quinole nitrogen and the backbone of Val 96 is maintained. With fascaplysin **1**, an additional hydrogen bond is predicted between the carbonyl of the ligand and the backbone amide of His 95; in **9q** this is predicted to be replaced by an interaction with the side chain N of His 95. The protein–ligand interactions elsewhere on the ligand differ more substantially. The larger aromatic moiety of **9q** is predicted to form a new π–π interaction with the side chain phenyl rings of both Phe 93 and Phe 159.

The binding mode of **12q** is noteworthy for different reasons. This structurally similar, but more conformationally constrained, compound is a much weaker inhibitor. Analysis of the docking solutions for **12q** with the best Chemscore values predicts two main clusters. In the cluster with the best Chemscore, **12q** is predicted not to bind in the ATP binding site of CDK4 but instead in a distinctly different part of the binding site that would not necessarily inhibit ATP binding. In the second cluster of solutions **12q** is located in the ATP binding site in a similar orientation to that predicted for fascaplysin and **9q** (Fig. 2d). This mode, like that predicted for **9q**, has a strong lipophylic component and the π–π interaction with Phe 93 and Phe 159 is maintained. In contrast, the position predicted to be occupied by the indole moiety is substantially different to that observed for both fascaplysin and **9q**. In particular, the position of the ring is incompatible with the double interaction with the backbone of His 95/Val 96. We therefore suggest that the good binding affinity of **9q** is facilitated both by retaining a double hydrogen bond with the backbone of His 95/Val 96 and introducing a new π interaction[14] from sandwiching the phenyl moiety of the ligand between the side chains of Phe 93 and Phe 159.

In conclusion, we have used a homology model of CDK4 to design specific inhibitors based on fascaplysin. The most potent compound, **9q**, has an $IC_{50}$ value for the inhibition of CDK4 of 6 μM. Three additional compounds—**12l**, **12m** and **12o**—have $IC_{50}$ values below 30 μM. The molecular basis of the affinity of **9q** is suggested to be due to the presence of a double hydrogen bond of the ligand with the backbone of His 95/Val 96 coupled with a π–π interaction—not present with fascaplysin—with the side chains of Phe 93 and Phe 159. This predicted new stabilising interaction will be studied elsewhere and will serve as a basis for the development of further potential inhibitors of CDK4.

## Experimental

### Modelling

Our homology model of CDK4 was produced using Modeller[15] with CDK2 (PDB[16] accession code 1HCK[14]; containing ATP–$Mg^{2+}$ and sharing 40% sequence identity with CDK4) and CDK6 (1BLX[17]; sharing 70% sequence identity with CDK4) as templates (Fig. 3; sequence alignment produced using ClustalX[18] combined with structural alignment). Docking studies were then performed to generate 10 solutions for each of the compounds **7a–c**, **5a–c**, **9a–q**, **10**, **12a–q** and **13–16**, using GOLD v3.0[19] with the Chemscore fitness function.[20]

### Bio assays

**Expression and purification of CDK4–GST–cyclin D1, CDK2–GST–cyclin A and GST–RB152.** Fusion proteins of human cyclins A and D1, covalently linked to glutathione S-transferase (GST), were co-expressed with the catalytic subunits CDK2 and CDK4 in Sf-9 insect cells as described previously.[22–24]

Active enzyme complexes, containing a catalytic subunit bound to GST–cyclin, were bound to glutathione–agarose columns (Sigma, Cat. No. G3907) and were eluted from the columns with reduced glutathione. The reduced glutathione was removed by dialysing the enzymes in 10 000 MCO dialysis cassettes (Pierce, Cat. No. 66830) with two buffer changes.

The GST–RB152 fusion construct was transformed into the *Escherichia coli* strain BL21(DE3)pLysS (Novagen Cat. No. 69451-4). For expression of GST–RB152, the cells were induced in the presence of a final concentration of 4 mM isopropyl-β-thiogalactopyranoside (IPTG, Invitrogen Cat. No. 15529-091) and were allowed to grow for 4 h in a shaking incubator at 37 °C and 220 rpm. Purification of the GST–RB152 protein was carried out

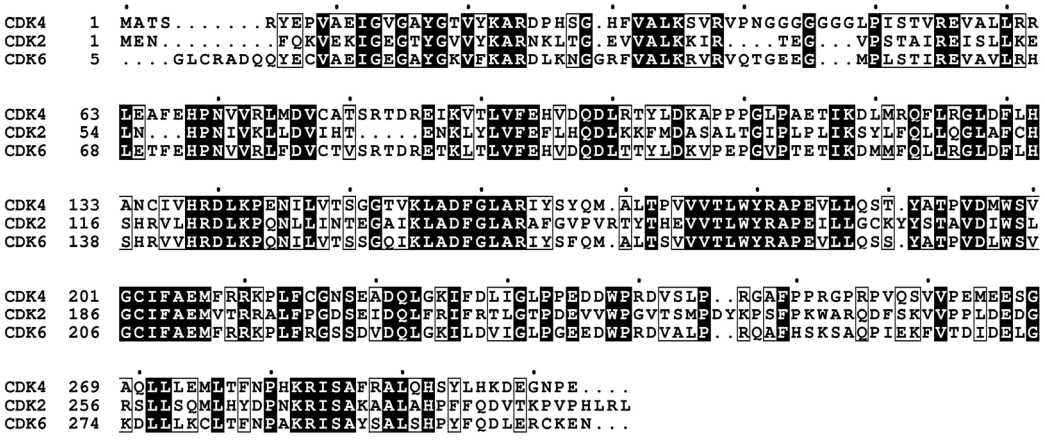



**Fig. 3** Sequence alignment used to generate the homology model of CDK4 based on the structures of CDK2 and CDK6. Residues that are identical in all three sequences are shown on a black background, and residues that are similar are boxed. (Figure produced using ESPRIPT.[21])

as described previously.[24] Protein estimation was performed using the Bradford protein assay (Bio-Rad Laboratories) with bovine serum albumin (BSA) as the standard and the purity of the fusion protein was assessed by SDS-PAGE analysis. Proteins were stained with Coomassie blue for visualisation.

**Kinase assays and $IC_{50}$ determination.** The assay measures the depletion in ATP concentration as a result of phosphorylation of retinoblastoma (GST–RB152) and histone H1 (Upstate Biotech Cat. No. 14–155) by CDK4 and CDK2, respectively. The assay was run in a 96 well format and all steps in one assay were carried out in a single white polystyrene plate (Sarstedt, Catalogue No. DPS-134-050A). The compounds were dissolved in DMSO as 10 mM stock solutions. Compounds were further serially diluted in kinase buffer (40 mM Tris (pH 7.5), 20 mM $MgCl_2$, 0.1 mg $ml^{-1}$ BSA) in order to obtain the desired concentrations. The kinase assay was performed in 50 µl of kinase buffer containing 2 µg of purified GST–RB152 (in case of CDK4–GST–cyclin D1) or 3 µg of histone H1 (in case of CDK2–GST–cyclin A) and 6 µM ATP. The phosphatase and protease inhibitor cocktail containing β-glycerophosphate, sodium fluoride and sodium orthovanadate in the presence of reducing agent dithiothreitol was added at the final concentrations of 10 mM, 0.1 mM, 0.1 mM and 1 mM, respectively. The assay was initiated by adding 200 ng of active enzyme complexes and the plate was incubated for 30 min at 30 °C in a humidified incubator. The reaction was stopped by the addition of an equal volume of the Kinase Glow Reagent™ (Promega Cat. No. V6711). The luminescence was measured using the Packard Luminometer (Fusion 3.50) and the rate of ATP depletion (rate of reaction) in the control blank reactions (*i.e.* without substrate or enzyme) was calculated and used to determine the $IC_{50}$ concentrations of compounds. In the case of the CDK4–cyclin D1 assay, the two compounds fascaplysin and flavopiridol with known $IC_{50}$ values were used to validate the assay. For the CDK2–cyclin A assay, roscovitine and flavopiridol were used as standards for the assay.

## Chemistry

### General

NMR spectra were recorded on a Bruker DPX 300 ($^1H$, 300.13 MHz; $^{13}C$, 75.47 MHz) spectrometer. Chemicals shifts were measured relative to chloroform ($^{13}C$ $\delta$ 77.0) or dimethylsulfoxide ($^{13}C$ $\delta$ 39.5) and are expressed in ppm. Coupling constants $J$ are expressed in Hertz and the measured values are corrected to one decimal place. Fast Atom Bombardment (FAB) mass spectra were recorded on a Kratos Concept 1H using xenon and *m*-nitrobenzyl alcohol as the matrix. Electrospray (ES) mass spectra were recorded on a Micromass Quattro LC spectrometer. Accurate mass was measured on a Kratos Concept 1H spectrometer using peak matching to a stable reference peak. Flash column chromatography was carried out using Merck Kieselgel 60 (230–400 mesh). Dry solvents were provided by the system PURE SOLV™, Innovative Technology Inc.

**[2-(1*H*-Indol-3-yl)-ethyl]-carbamic acid ethyl ester 3[13].** To a solution of tryptamine **2** (10.00 g, 62.4 mmol) in chloroform (156 mL) at 0 °C was added ethylchloroformate (5.97 mL, 62.4 mmol) and an aqueous solution of NaOH 4 M (15.60 mL, 62.4 mmol). After addition, the mixture was stirred for 3 h at room temperature, and then washed with water (150 mL). The two layers were separated and the aqueous phase was extracted with dichloromethane (2 × 150 mL). The chloroform and dichloromethane layers were combined, dried ($MgSO_4$), and evaporated under reduced pressure to give an orange oil. No purification was needed. The oil was dried *in vacuo* to give the title compound **3** (13.78 g, 95%). $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 1.33 (3H, t, $J$ 7.0), 3.05 (2H, t, $J$ 6.5), 3.60 (2H, q, $J$ 6.5), 4.24 (2H, q, $J$ 7.0), 5.12 (1H, br s), 6.99 (1H, s), 7.23 (1H, t, $J$ 6.0), 7.30 (1H, td, $J$ 6.0 and 1.2), 7.42 (1H, d, $J$ 7.7), 7.71 (1H, d, $J$ 7.7), 8.75 (1H, s). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 14.57 ($CH_3$), 25.64 ($CH_2$), 41.21 ($CH_2$), 60.72 ($CH_2$), 111.33 (CH), 112.33 (Cq), 118.54 (CH), 119.11 (CH), 121.83 (CH), 122.26 (CH), 127.18 (Cq), 136.39 (Cq), 156.93 (Cq). *R*f (ethyl acetate–$NH_{3(aq)}$ 100 : 5) 0.76. $m/z$ ($FAB^+$) 232 ($M^+$), 233 $(M + H)^+$, 465 $(2M + H)^+$ (found: $M^+$, 232.12126. $C_{13}H_{16}N_2O_2$ requires M, 232.12118).

**[2-(1*H*-Indol-3-yl)-ethyl]-methylamine 4[25].** To a solution of **3** (13.78 g, 59.4 mmol) in dry THF (110 mL) under $N_2$ flux at 0 °C was added portionwise LAH (6.76 g, 178 mmol). After the addition was complete the mixture was heated under reflux for 1 h. The reaction was then cooled to 0 °C and the excess of LAH was hydrolysed by adding successively and very carefully: water (13.25 mL), 15% aqueous solution of NaOH (13.25 mL) and water (3 × 13.25 mL). During these steps it was necessary to add THF (100 mL) to avoid the mixture becoming very thick. The suspen sion was filtered and the white solid, made up of LiOH and $Al(OH)_3$, was washed with THF (30 mL). The organic layer was dried ($MgSO_4$) and evaporated under reduced pressure to give the title compound **4** (9.24 g, 89%) as a beige solid. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 1.47 (1H, s), 2.35 (3H, s), 2.81–2.86 (2H, m), 2.89–2.94 (2H, m), 6.80 (1H, s), 7.03 (1H, td, $J$ 7.4 and 1.2), 7.10 (1H, td, $J$ 7.4 and 1.2), 7.19 (1H, d, $J$ 7.6), 7.54 (1H, d, $J$ 7.6), 9.52 (1H, s). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 25.42 ($CH_2$), 35.99 ($CH_3$), 51.82 ($CH_2$), 111.32 (CH), 112.91 (Cq), 118.65 (CH), 118.85 (CH), 121.59 (CH), 122.45 (CH), 127.30 (Cq), 136.53 (Cq). *R*f (ethyl acetate–MeOH–$NH_{3(aq)}$ 9 : 0.5 : 0.5) 0.4. Mp 82 °C. $m/z$ ($FAB^+$) 175 $(M + H)^+$ (found: C, 75.74; H, 8.04; N, 16.00; $MH^+$, 175.12354. $C_{11}H_{15}N_2$ requires C, 75.82; H, 8.10; N, 16.08%; MH, 175.12352).

### General procedure for the preparation of {[2-(1*H*-indol-3-yl)-ethyl]-methylamino}-ethanone derivatives

**1-(4-Bromophenyl)-2-{[2-(1*H*-indol-3-yl)-ethyl]-methylamino}-ethanone 5c.** To a solution of **4** (1.00 g, 5.74 mmol) in toluene (28 mL) was added a solution of $NaHCO_3$ (1.12 g, 13.3 mmol) and $Na_2SO_3$ (0.56 g, 4.48 mmol) in water (11.2 mL). The mixture was stirred under a $N_2$ atmosphere and 2,4′-dibromoacetophenone (1.59 g, 5.74 mmol) was added. The stirring was maintained for 4 h at room temperature, and the reaction mixture was washed with a saturated aqueous solution of $Na_2HPO_4$ (28 mL). The organic layer was dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by chromatography on silica gel. Elution was made successively with: ethyl acetate–cyclohexane 7 : 3; ethyl acetate; ethyl acetate–methanol 1 : 1, to give the title compound **6c** as a hard and sticky paste (1.09 g, 51%). The identical procedure using as starting material 2-bromo-4′-chloroacetophenone and 2-bromoacetophenone was

used toward the synthesis of **5b** and **5a** in yields of 61% and 49%, respectively. Note: these compounds can be kept for up to one week at −23 °C.

$^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 2.49 (3H, s), 2.89–2.94 (2H, m), 2.98–3.03 (2H, m), 3.87 (2H, s), 6.94 (1H, d, *J* 2.0), 7.08 (1H, td, *J* 7.4 and 1.1), 7.16 (1H, td, *J* 7.4 and 1.1), 7.31 (1H, br d, *J* 7.9), 7.47 (2H, dt, *J* 8.6 and 2.0, *para* benzene), 7.56 (1H, d, *J* 7.6), 7.75 (2H, dt, *J* 8.6 and 2.0, *para* benzene), 8.35 (1H, s). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 23.12 ($CH_2$), 42.42 ($CH_3$), 58.07 ($CH_2$), 63.60 ($CH_2$), 111.69 (CH), 113.91 (Cq), 119.09 (CH), 119.64 (CH), 122.34 (2CH), 127.76 (Cq), 128.82 (Cq), 130.20 (2CH), 132.17 (2CH), 134.84 (Cq), 136.98 (Cq), 196.38 (Cq). *R*f (ethyl acetate–cyclohexane 7 : 3) 0.58. *m/z* (FAB$^+$) 371 ($M^+$) (found: $M^+$, 371.07600. $C_{19}H_{19}BrN_2O$ requires M, 371.07590).

**1-(4-Chlorophenyl)-2-{[2-(1*H*-indol-3-yl)-ethyl]-methylamino}-ethanone 5b.** $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 2.44 (3H, s), 2.84–2.90 (2H, m), 2.95–3.00 (2H, m), 3.81 (2H, s), 6.93 (1H, d, *J* 2.3), 7.08 (1H, td, *J* 7.8 and 1.2), 7.15 (1H, td, *J* 7.8 and 1.2), 7.30 (3H, 2 br d, *J* 7.8 and 7.8), 7.56 (1H, br d, *J* 7.8), 7.83 (2H, dt, *J* 7.8 and 2.3), 8.40 (1H, br s). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 23.25 ($CH_2$), 42.58 ($CH_3$), 58.34 ($CH_2$), 64.08 ($CH_2$), 111.32 (CH), 113.64 (Cq), 118.71 (CH), 119.17 (CH), 121.87 (CH), 121.97 (CH), 127.42 (Cq), 128.74 (2CH), 129.77 (2CH), 134.15 (Cq), 136.30 (Cq), 139.52 (Cq), 196.58 (Cq). *R*f (ethyl acetate–cyclohexane 7 : 3) 0.68. *m/z* (FAB$^+$) 327 $(M + H)^+$ (found $MH^+$, 327.12635. $C_{19}H_{20}ClN_2O$ requires MH, 327.12642).

**2-{[2-(1*H*-Indol-3-yl)-ethyl]-methylamino}-1-phenylethanone 5a.** $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 2.36 (3H, s), 2.75–2.81 (2H, m), 2.86–2.92 (2H, m), 3.78 (2H, s), 6.82 (1H, d, *J* 2.1), 6.94–7.06 (2H, m), 7.18 (1H, d, *J* 7.8), 7.26 (2H, apparent t, *J* 7.5), 7.39 (1H, tt, *J* 7.8 and 1.8), 7.47 (1H, d, *J* 7.5), 7.82 (2H, d, *J* 7.2), 8.51 (1H, s). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 23.34 ($CH_2$), 42.75 ($CH_3$), 58.49 ($CH_2$), 63.79 ($CH_2$), 111.44 (CH), 113.63 (Cq), 118.77 (CH), 119.15 (CH), 121.84 (CH), 122.13 (CH), 127.54 (Cq), 128.23 (2CH), 128.63 (2CH), 133.33 (CH), 136.07 (Cq), 136.42 (Cq), 197.65 (Cq). *R*f (ethyl acetate cyclohexane 7 : 3) 0.37. *m/z* (FAB$^+$) 293 $(M + H)^+$ (found: $MH^+$, 293.16544. $C_{19}H_{21}N_2O$ requires MH, 293.16539).

### General procedure for the formation of 2-(1*H*-indol-3-yl)-ethyl hydrochloride salts

**[2-(4-Bromophenyl)-2-oxoethyl]-[2-(1*H*-indol-3-yl)-ethyl]-methyl-ammonium chloride 8c.** To a solution of **5c** in a minimum of diethyl ether (also for compound **5b**), or dichloromethane (for compound **5a**) was flushed HCl gas. After a few seconds a precipitate was formed, the colour generally white at the beginning and changing to tan afterwards. The mixture was filtered and the solid dried under vacuum to give the title compound **8c** (0.61 g, 77%). The identical procedure using as starting material **5b** and **5a** was used toward the synthesis of **8b** and **8a** in yields of 46% and 88%, respectively. $^1$H NMR showed traces of ether in these products which could not be removed by treatment at high vacuum for several days.

$^1$H NMR (300 MHz, DMSO) $\delta$ 3.00 (3H, s), 3.23 (2H, t, *J* 7.8), 3.40 (2H, signal obscured by the water in solvent), 5.09 (1H, d, *J* 16.5), 5.18 (1H, d, *J* 16.5), 7.02 (1H, td, *J* 7.7 and 0.9), 7.10 (1H, td, *J* 7.7 and 0.9), 7.24 (1H, d, *J* 2.1), 7.37 (1H, d, *J* 7.7), 7.65 (1H, d, *J* 7.7), 7.85 (2H, dt, *J* 8.7 and 1.8), 7.94 (2H, dt, *J* 8.7 and 1.8), 10.34 (1H, s), 11.02 (1H, s). $^{13}$C NMR (75 MHz, DMSO) $\delta$ 20.31 ($CH_2$), 41.68 ($CH_3$), 57.09 ($CH_2$), 60.87 ($CH_2$), 109.32 (CH), 112.05 (Cq), 118.75 (CH), 118.95 (CH), 121.72 (CH), 123.76 (CH), 127.14 (Cq), 129.34 (Cq), 130.61 (2CH), 132.57 (2CH), 133.32 (Cq), 136.71 (Cq), 191.66 (Cq). *m/z* (FAB$^+$) 371 $(M - Cl^-)^+$ (found: C, 55.88; H, 4.87; N, 6.76; $(M - Cl^-)^+$, 371.07600. $C_{19}H_{20}BrN_2O$ requires C, 55.97; H, 4.94; N, 6.87%; $(M - Cl^-)$, 371.07590).

**[2-(4-Chlorophenyl)-2-oxoethyl]-[2-(1*H*-indol-3-yl)-ethyl]-methyl-ammonium chloride 8b.** $^1$H NMR (300 MHz, DMSO) $\delta$ 3.00 (3H, d, *J* 3.9), 3.23 (2H, t, *J* 7.8), 3.50 (2H, signal obscured by the water in the solvent), 5.09 (1H, dd, *J* 18 and 4.8), 5.19 (1H, dd, *J* 18 and 2.7), 7.02 (1H, td, *J* 7.3 and 0.9), 7.10 (1H, td, *J* 7.3 and 0.9), 7.24 (1H, d, *J* 2.4), 7.37 (1H, d, *J* 7.3), 7.65 (1H, d, *J* 7.3), 7.71 (2H, br d, *J* 8.7), 8.03 (2H, br d, *J* 8.7), 10.35 (1H, s), 11.02 (1H, s). $^{13}$C NMR (75 MHz, DMSO) $\delta$ 20.31 ($CH_2$), 41.68 ($CH_3$), 57.10 ($CH_2$), 60.88 ($CH_2$), 109.32 (CH), 112.05 (Cq), 118.76 (CH), 118.94 (CH), 121.72 (CH), 123.76 (CH), 127.14 (Cq), 129.62 (2CH), 130.58 (2CH), 133.02 (Cq), 136.72 (Cq), 140.05 (Cq), 191.44 (Cq). *m/z* (FAB$^+$) 327 $(M - Cl^-)^+$ (found: C, 62.84; H, 5.47; N, 7.63; $(M - Cl^-)^+$, 327.12649. $C_{19}H_{20}Cl_2N_2O$ requires C, 62.82; H, 5.55; N, 7.71%; $(M - Cl^-)$, 327.12642).

**[2-(1*H*-Indol-3-yl)-ethyl]-methyl-(2-oxo-2-phenylethyl)-ammonium chloride 8a.** $^1$H NMR (300 MHz, DMSO) $\delta$ 3.02 (3H, d, *J* 4.5), 3.21 3.29 (2H, m), 3.42 3.47 (1H, m), 3.49 3.54 (1H, m), 5.16 (1H, dd, *J* 18.3 and 5.4), 5.24 (1H, dd, *J* 18.3 and 4.2), 7.25 (1H, d, *J* 2.1), 7.38 (1H, d, *J* 6.9), 7.60 (1H, t, *J* 7.6), 7.62 (1H, d, *J* 7.8), 7.68 (1H, d, *J* 7.8), 7.75 (1H, tt, *J* 7.2 and 1.5), 8.02 (2H, d, *J* 7.2), 10.66 (1H, s), 11.14 (1H, s). $^{13}$C NMR (75 MHz, DMSO) $\delta$ 20.38 ($CH_2$), 41.51 ($CH_3$), 57.00 ($CH_2$), 60.74 ($CH_2$), 109.40 (CH), 112.06 (Cq), 118.80 (CH), 118.90 (CH), 121.66 (CH), 123.75 (CH), 127.18 (Cq), 128.67 (2CH), 129.45 (2CH), 134.36 (Cq), 135.10 (CH), 136.73 (Cq), 192.30 (Cq). *m/z* (FAB$^+$) 293 $(M - Cl^-)^+$, 621 $(2M - Cl^-)^+$ (found: $(M - Cl^-)^+$, 293.16534. $C_{19}H_{21}ClN_2O$ requires $(M - Cl^-)$, 293.16539).

### General procedure for the formation of 2-(1*H*-indol-3-yl)-ethyl ammonium tosylate salts

**[2-(4-Bromophenyl)-2-oxoethyl]-[2-(1*H*-indol-3-yl)-ethyl]-dimethylammonium toluene-4-sulfonate 6c.** To a solution of the **5c** (1.07 g, 2.89 mmol) in acetonitrile (14.5 mL) was added methyl-*p*-toluenesulfonate (0.54 g, 2.89 mmol). The mixture was heated under reflux for 4 h. After cooling at room temperature, the solvent was removed to give the title compound as a crude yellow powder **6c** (1.62 g, >100%). Further purification was not attempted according to the nature of the compound. NMR showed the presence of the starting material methyl-*p*-toluenesulfonate which was conveniently removed in the subsequent ion exchange step, this explains why the yield is more than 100%. The identical procedure using as starting material **5b** and **5a** was used toward the synthesis of **6b** and **6a** in quantitative yields for both.

$^1$H NMR (300 MHz, DMSO) of the expected compound **6c** $\delta$ 2.29 (3H, s), 3.23–3.26 (2H, m), 3.39 (6H, s), 3.83–3.88 (2H, m), 5.35 (2H, s), 7.03–7.13 (4H, m), 7.27 (1H, d, *J* 2.3), 7.37 (1H, d, *J* 8.0), 7.48 (2H, d, *J* 8.0), 7.59 (1H, d, *J* 7.7), 7.85 (2H, d, *J* 8.7), 7.93 (2H, d, *J* 8.7), 10.98 (1H, s). $^{13}$C NMR (75 MHz, DMSO) $\delta$ 19.04 ($CH_2$), 21.24 ($CH_3$), 51.86 ($2CH_3$), 65.23 ($CH_2$), 65.38 ($CH_2$),

108.52 (CH), 112.10 (Cq), 118.63 (CH), 119.05 (CH), 121.81 (CH), 124.13 (CH), 125.96 (2CH), 127.02 (Cq), 128.50 (2CH), 129.39 (Cq), 130.47 (2CH), 132.52 (2CH), 133.90 (Cq), 136.73 (Cq), 138.06 (Cq), 146.22 (Cq), 191.24 (Cq). *m/z* (FAB$^+$) 386 (M − $CH_3C_6H_4SO_3^-$)$^+$, 943 (2(M − $CH_3C_6H_4SO_3^-$)$^+$ + $CH_3C_6H_4SO_3^-$)$^+$ (found: (M − $CH_3C_6H_4SO_3^-$)$^+$, 385.09152. $C_{20}H_{22}BrN_2O$ requires (M − $CH_3C_6H_4SO_3^-$), 385.09155).

**[2-(4-Chlorophenyl)-2-oxoethyl]-[2-(1*H*-indol-3-yl)-ethyl]-dimethylammonium toluene-4-sulfonate 6b.** $^1$H NMR (300 MHz, DMSO) of the expected compound **6b** $\delta$ 2.28 (3H, s), 3.21–3.26 (2H, m), 3.40 (6H, s), 3.83–3.89 (2H, m), 5.37 (2H, s), 7.00–7.13 (4H, m), 7.28 (1H, d, *J* 2.2), 7.37 (1H, d, *J* 8.0), 7.48 (2H, d, *J* 8.0), 7.60 (1H, d, *J* 7.7), 7.71 (2H, d, *J* 8.6), 8.02 (2H, d, *J* 8.6), 10.99 (1H, s). $^{13}$C NMR (75 MHz, DMSO) $\delta$ 19.04 ($CH_2$), 21.24 ($CH_3$), 51.85 ($2CH_3$), 65.25 ($CH_2$), 65.41 ($CH_2$), 108.52 (CH), 112.11 (Cq), 118.64 (CH), 119.04 (CH), 121.80 (CH), 124.14 (CH), 125.96 (2CH), 127.03 (Cq), 128.50 (2CH), 129.57 (2CH), 130.45 (2CH), 133.60 (Cq), 136.73 (Cq), 138.03 (Cq), 140.09 (Cq), 146.27 (Cq), 191.04 (Cq). *m/z* (FAB$^+$) 341 (M − $CH_3C_6H_4SO_3^-$)$^+$, 854 (2(M − $CH_3C_6H_4SO_3^-$)$^+$ + $CH_3C_6H_4SO_3^-$)$^+$ (found: (M − $CH_3C_6H_4SO_3^-$)$^+$, 341.14202. $C_{20}H_{22}ClN_2O$ requires (M − $CH_3C_6H_4SO_3^-$), 341.14207).

**[2-(1*H*-Indol-3-yl)-ethyl]-dimethyl-(2-oxo-2-phenylethyl)-ammonium toluene-4-sulfonate 6a.** $^1$H NMR (300 MHz, DMSO) of the expected compound **6a** $\delta$ 2.28 (3H, s), 3.21 3.36 (2H, m), 3.41 (6H, s), 3.84–3.90 (2H, m), 5.39 (2H, s), 7.03–7.14 (4H, m), 7.28 (1H, d, *J* 2.3), 7.38 (1H, d, *J* 8.0), 7.48 (2H, d, *J* 8.0), 7.60 (1H, d, *J* 6.9), 7.63 (2H, t, *J* 7.6), 7.78 (1H, t, *J* 7.6), 8.02 (2H, d, *J* 7.2), 10.99 (1H, s). $^{13}$C NMR (75 MHz, DMSO) $\delta$ 19.06 ($CH_2$), 21.24 ($CH_3$), 51.82 ($2CH_3$), 65.22 ($CH_2$), 65.47 ($CH_2$), 108.52 (CH), 112.11 (Cq), 118.62 (CH), 119.05 (CH), 121.81 (CH), 124.12 (CH), 125.96 (2CH), 127.03 (Cq), 128.49 (2CH), 128.53 (2CH), 129.47 (2CH), 134.89 (Cq), 135.21 (CH), 136.73 (Cq), 138.00 (Cq), 146.30 (Cq), 192.00 (Cq). *m/z* (FAB$^+$) 307 (M − $CH_3C_6H_4SO_3^-$)$^+$, 786 (2(M − $CH_3C_6H_4SO_3^-$)$^+$ + $CH_3C_6H_4SO_3^-$)$^+$ (found: (M − $CH_3C_6H_4SO_3^-$)$^+$ 307.18104. $C_{20}H_{23}N_2O$ requires (M − $CH_3C_6H_4SO_3^-$), 307.18104).

### General procedure for the ion exchange of tosylate salts to chloride salts

**[2-(4-Bromophenyl)-2-oxoethyl]-[2-(1*H*-indol-3-yl)-ethyl]-dimethylammonium chloride 7c.** To a solution of the crude tosylate salt **6c** (0.96 g, 1.73 mmol) in acetonitrile–water 2.3 : 5 (19.7 mL), was added Dowex® Cl$^-$ 1 × 8–400 ion exchange resin (3.60 g). The suspension was stirred overnight at room temperature. A column was packed with Dowex® Cl$^-$ 1 × 8–400 ion exchange resin (10 g) with the same solvent mixture. The suspension was poured onto the column and eluted with acetonitrile–water 2.3 : 5 (150 mL) and acetonitrile (100 mL). The combined eluate was evaporated under reduced pressure, an aqueous solution of HCl 2 N (3.2 mL) was added, and removed after a few minutes to give the title compound **7c** as a tan solid (0.33 g, 46%). The identical procedure using as starting material **6b** and **6a** was used toward the synthesis of **7b** and **7a** in yields of 50% and 53%, respectively.

$^1$H NMR (300 MHz, DMSO) of the expected compound **2c** $\delta$ 3.25–3.32 (2H, m), 3.51 (6H, s), 3.92–3.97 (2H, m), 5.54 (2H, s), 7.09 (1H, td, *J* 7.4 and 1.0), 7.17 (1H, td, *J* 7.4 and 1.0), 7.34 (1H, d, *J* 2.3), 7.43 (1H, d, *J* 7.8), 7.66 (1H, d, *J* 7.8), 7.91 (2H, d, *J* 8.6), 8.02 (2H, d, *J* 8.6), 11.13 (1H, s). $^{13}$C NMR (75 MHz, DMSO) $\delta$ 19.09 ($CH_2$), 51.74 ($2CH_3$), 65.04 ($CH_2$), 65.49 ($CH_2$), 108.53 (CH), 112.11 (Cq), 118.66 (CH), 118.99 (CH), 121.73 (CH), 124.13 (CH), 127.03 (Cq), 129.35 (Cq), 130.55 (2CH), 132.50 (2CH), 133.96 (Cq), 136.72 (Cq), 191.50 (Cq). *m/z* (FAB$^+$) 385 (M − Cl$^-$)$^+$, 807 (2(M − Cl$^-$)$^+$ + Cl$^-$)$^+$ (found: C, 57.00; H, 5.27; N, 6.62; (M − Cl$^-$)$^+$, 385.09152. $C_{20}H_{22}BrN_2O$ requires C, 56.96; H, 5.26; N, 6.64%; (M − Cl$^-$), 385.09155).

**[2-(4-Chlorophenyl)-2-oxoethyl]-[2-(1*H*-indol-3-yl)-ethyl]-dimethylammonium chloride 7b.** $^1$H NMR (300 MHz, DMSO) of the expected compound **7b** $\delta$ 3.20–3.27 (2H, m), 3.50 (6H, s), 3.87–3.93 (2H, m), 5.53 (2H, s), 7.02 (1H, td, *J* 6.8 and 1.0), 7.11 (1H, td, *J* 6.8 and 1.0), 7.29 (1H, d, *J* 2.3), 7.37 (1H, d, *J* 7.8), 7.60 (1H, d, *J* 7.8), 7.70 (2H, d, *J* 8.6), 8.05 (2H, d, *J* 8.6), 11.10 (1H, s). $^{13}$C NMR (75 MHz, DMSO) $\delta$ 19.08 ($CH_2$), 51.76 ($2CH_3$), 65.08 ($CH_2$), 65.49 ($CH_2$), 108.53 (CH), 112.10 (Cq), 118.65 (CH), 118.99 (CH), 121.74 (CH), 124.13 (CH), 127.03 (Cq), 129.55 (2CH), 130.50 (2CH), 133.65 (Cq), 136.70 (Cq), 140.05 (Cq), 191.23 (Cq). *m/z* (FAB$^+$) 341 (M − Cl$^-$)$^+$, 717 (2(M − Cl$^-$) + Cl$^-$)$^+$ (found: C, 63.57; H, 5.67; N, 7.32; (M − Cl$^-$)$^+$, 341.14209. $C_{20}H_{22}ClN_2O$ requires C, 63.67; H, 5.88; N, 7.42%; (M − Cl$^-$), 341.14207).

**[2-(1*H*-Indol-3-yl)-ethyl]-dimethyl-(2-oxo-2-phenylethyl)-ammonium chloride 7a.** $^1$H NMR (300 MHz, DMSO) $\delta$ 3.19–3.27 (2H, m), 3.48 (6H, s), 3.86–3.92 (2H, m), 5.48 (2H, s), 7.03 (1H, td, *J* 7.0 and 1.2), 7.11 (1H, td, *J* 8.2 and 1.2), 7.29 (1H, d, *J* 2.4), 7.38 (1H, dt, *J* 7.9 and 0.9), 7.58–7.65 (3H, m), 7.77 (1H, tt, *J* 7.4 and 1.3), 8.00–8.06 (2H, m), 11.06 (1H, s). $^{13}$C NMR (75 MHz, DMSO) $\delta$ 18.57 ($CH_2$), 51.25 ($2CH_3$), 64.61 ($CH_2$), 65.00 ($CH_2$), 108.01 (CH), 111.61 (Cq), 118.12 (CH), 118.52 (CH), 121.27 (CH), 123.60 (CH), 126.52 (Cq), 128.06 (2CH), 128.95 (2CH), 134.40 (Cq), 134.68 (CH), 136.21 (Cq), 191.61 (Cq). *m/z* (FAB$^+$) 307 ((M − Cl$^-$) + H)$^+$, 649 (2(M − Cl$^-$) + Cl$^-$)$^+$ (found: (M − Cl$^-$)$^+$, 307.18103. $C_{20}H_{23}N_2O$ requires (M − Cl$^-$), 307.18104).

***N*-[2-(1*H*-Indol-3-yl)-ethyl]-*N*-methylbenzamide 9a§.** Yellow paste. Yield 37%. *Rf* (ethyl acetate–petroleum ether 50 : 50) 0.15. Rotamers 1 : 1.4 (from the duplicated triplet signal ($^1$H) at 3.48 and 3.81 ppm). $^1$H NMR (300 MHz, DMSO) $\delta$ (major rotamer) 2.89 (2H, t, *J* 7.2), 3.11 (3H, br s), 3.48 (2H, t, *J* 7.2), 6.81–7.29 (10H, m), 8.01 (1H, br s). $\delta$ (distinct peaks for minor rotamer) 2.80 (3H, s), 3.81 (2H, t, *J* 6.9), 7.65 (1H, d, *J* 6.9). $^{13}$C NMR (75 MHz, DMSO) $\delta$ (major rotamer) 24.33 ($CH_2$), 32.78 ($CH_3$), 51.89 ($CH_2$), 110.91 (Cq), 111.82 (CH), 118.68 (CH), 121.39 (CH), 123.26 (CH), 123.43 (CH), 126.70 (CH), 127.38 (Cq), 128.67 (3CH), 129.26 (CH), 137.36 (2Cq), 171.17 (Cq). $\delta$ (distinct peaks for minor rotamer) 23.01 ($CH_2$), 37.83 ($CH_3$), 48.24 ($CH_2$), 118.32 (CH), 127.16 (CH), 127.76 (Cq), 129.66 (CH), 136.62 (Cq), 170.33 (Cq). *m/z* (ES$^+$) 279 (MH$^+$), 557 (2M + H)$^+$; (ES$^-$) 277 (M − H)$^-$, 555 (2M − H)$^-$; *m/z* (FAB$^+$) 279 MH$^+$ (found: MH$^+$, 279.14977. $C_{18}H_{18}N_2O$ requires MH, 279.14974).

**4-*tert*-Butyl-*N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-methylbenzamide 9b.** White solid. Yield 57%. Mp 194 °C. Rotamers 1 : 1.6 (from

§ The general procedure for the synthesis of compounds **9a–q** and **10** is the same as the general procedure for the synthesis of compounds **12a–q**, except that methyltryptamine **4** was used as a starting material and not **11**.

the duplicated triplet signal ($^1$H) at 3.47 and 3.79 ppm). *R*f (ethyl acetate–petroleum ether, 50 : 50) 0.20. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ (major rotamer) 1.23 (9H, s), 2.86 (2H, distorted t, *J* 6.8), 3.09 (3H, s), 3.47 (2H, t, *J* 6.8), 6.75–7.31 (9H, m), 8.43 (1H, br s). $\delta$ (distinct peaks for minor rotamer) 2.81 (3H, s), 3.79 (2H, br t, *J* 7.1), 7.62 (1H, d, *J* 6.3). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ (major rotamer) 24.49 ($CH_2$), 31.27 ($3CH_3$), 33.06 ($CH_3$), 34.74 (Cq), 51.99 ($CH_2$), 111.32 (CH), 111.60 (Cq), 118.15 (CH), 119.21 (CH), 121.86 (2CH), 125.22 (3CH), 126.20 (CH), 127.10 (Cq), 133.52 (Cq), 136.34 (Cq), 152.23 (Cq), 172.76 (Cq). $\delta$ (distinct peaks for minor rotamer) 22.97 ($CH_2$), 38.29 ($CH_3$), 48.51 ($CH_2$), 112.86 (Cq), 118.70 (CH), 122.28 (2CH), 126.89 (CH), 127.57 (Cq), 133.72 (Cq), 152.55 (Cq), 171.62 (Cq). *m/z* ($ES^+$) 335 ($MH^+$); ($ES^-$) 333 ($M - H)^-$; *m/z* ($FAB^+$) 335 ($MH^+$), 670 ($2M + 2H)^+$ (found: C, 78.92; H, 7.88; N, 8.25; $MH^+$, 335.21232. $C_{22}H_{26}N_2O$ requires C, 79.01; H, 7.84; N, 8.38%; MH, 335.21234).

**2-Fluoro-*N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-methylbenzamide 9c.** Tan solid. Yield 59%. Mp 125 °C. Rotamers 1 : 1.4 (from the duplicated triplet signal ($^1$H) at 3.40 and 3.79 ppm). *R*f (ethyl acetate–petroleum ether, 50 : 50) 0.27. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ (major rotamer) 2.84 (2H, t, *J* 7.4), 3.10 (3H, s), 3.40 (2H, t, *J* 7.4), 6.73–7.29 (9H, m), 8.39 (1H, br s). $\delta$ (distinct peaks for minor rotamer) 2.73 (3H, s), 3.05 (2H, distorted t, *J* 7.4), 3.79 (2H, t, *J* 7.4), 7.62 (1H, d, *J* 7.5). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ (major rotamer) 24.29 ($CH_2$), 32.92 ($CH_3$), 48.42 ($CH_2$), 111.33 (CH), 111.43 (Cq), 112.62 (Cq), 115.83 (CH, d, *J* 15.5), 117.96 (CH), 118.66 (CH), 119.25 (CH), 121.90 (CH), 124.43 (CH, d, *J* 6.3), 127.24 (Cq, d, *J* 32.5), 128.64 (CH, d, *J* 4.6), 130.76 (CH, d, *J* 7.7), 136.35 (Cq), 158.00 (Cq, d, *J* 224.8), 166.78 (Cq). $\delta$ (distinct peaks for minor rotamer) 23.05 ($CH_2$), 37.01 ($CH_3$), 51.53 ($CH_2$), 115.55 (CH, d, *J* 15.6), 122.34 (CH), 124.66 (CH, d, *J* 7.4), 128.92 (CH, d, *J* 3.2), 131.13 (CH, d, *J* 8.0), 136.35 (Cq), 157.78 (Cq, d, *J* 302.2), 167.21 (Cq). $^{19}$F NMR (282 MHz, $CDCl_3$) $\delta$ (major rotamer) −115.55. $\delta$ (minor rotamer) −115.15. *m/z* ($ES^+$) 297 ($MH^+$), 593 ($2M + H)^+$; ($ES^-$) 295 ($M - H)^-$, 591 ($2M - H)^-$; *m/z* ($FAB^+$) 297 ($MH^+$), 594 ($2M + 2H)^+$ (found: C, 72.89; H, 5.70; N, 9.39; $MH^+$, 297.14027. $C_{18}H_{17}FN_2O$ requires C, 72.96; H, 5.78; N, 9.45%; MH, 297.14032).

**3-Fluoro-*N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-methylbenzamide 9d.** Tan solid. Yield 54%. Mp 144 °C. Rotamers 1 : 1.4 (from the duplicated triplet signal ($^1$H) at 3.44 and 3.76 ppm). *R*f (ethyl acetate–petroleum ether 50 : 50) 0.20. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ (major rotamer) 2.83 (2H, t, *J* 7.0), 3.07 (3H, s), 3.44 (2H, t, *J* 7.0), 6.63–7.22 (9H, m), 8.54 (1H, br s). $\delta$ (distinct peaks for minor rotamer) 2.73 (3H, s), 3.76 (2H, t, *J* 7.1), 7.60 (1H, d, *J* 7.2). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ (major rotamer) 24.20 ($CH_2$), 33.03 ($CH_3$), 51.81 ($CH_2$), 111.22 (Cq), 111.42 (CH), 112.52 (Cq), 113.75 (CH, d, *J* 22.8), 116.06 (CH, d, *J* 21.0), 117.88 (CH), 118.62 (CH), 119.28 (CH), 121.95 (CH), 122.19 (CH, d, *J* 10.6), 127.01 (Cq), 130.02 (CH, d, *J* 7.6), 136.33 (Cq), 138.26 (Cq, d, *J* 6.8), 162.28 (Cq, d, *J* 246.2), 170.96 (Cq). $\delta$ (distinct peaks for minor rotamer) 22.92 ($CH_2$), 30.95 ($CH_3$), 48.62 ($CH_2$), 114.16 (CH, d, *J* 23.7), 116.48 (CH, d, *J* 20.9), 122.41 (CH), 122.51 (CH, d, *J* 15.1), 127.51 (Cq), 130.28 (CH, d, *J* 11.3), 138.75 (Cq, d, *J* 10.4), 162.50 (Cq, d, *J* 225.0), 169.99 (Cq). $^{19}$F NMR (282 MHz, $CDCl_3$) $\delta$ (major rotamer) −111.84. $\delta$ (minor rotamer) −111.87. *m/z* ($ES^+$) 297 ($MH^+$), 593 ($2M + H)^+$; ($ES^-$) 295 ($M - H)^-$, 591 ($2M - H)^-$; *m/z* ($FAB^+$) 297 ($MH^+$), 593 ($2M + H)^+$ (found: C, 72.85; H, 5.70; N, 9.35; $MH^+$, 297.14034. $C_{18}H_{17}FN_2O$ requires C, 72.96; H, 5.78; N, 9.45%; MH, 297.14032).

**4-Fluoro-*N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-methylbenzamide 9e.** White solid. Yield 58%. Mp 180 °C. Rotamers 1 : 1.5 (from the duplicated singlet signal ($^{19}$F) observed at room temperature at −112.22 and −111.49 ppm). *R*f (ethyl acetate–petroleum ether 50 : 50) 0.14. $^1$H NMR (400 MHz, DMSO, 363 K) $\delta$ 2.98 (2H, distorted t, *J* 5.7), 3.05 (3H, s), 3.61 (2H, t, *J* 5.7), 6.94 (1H, t, *J* 6.5), 7.05–7.14 (6H, m), 7.27 (1H, br t, *J* 7.0), 7.35 (1H, d, *J* 8.4), 10.54 (1H, br s). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ (major rotamer) 24.15 ($CH_2$), 32.85 ($CH_3$), 51.88 ($CH_2$), 110.86 (Cq), 111.85 (CH), 115.41 (2CH, br d, *J* 20.0), 118.64 (2CH), 121.40 (CH), 123.57 (CH), 127.42 (Cq), 129.13 (2CH, d, *J* 11.0), 133.63 (Cq), 136.68 (Cq), 162.50 (Cq, d, *J* 259.6), 170.31 (Cq). $\delta$ (distinct peaks for minor rotamer) 23.01 ($CH_2$), 37.91 ($CH_3$), 48.40 ($CH_2$), 115.48 (CH, d, *J* 29.8), 115.68 (CH, d, *J* 19.9), 118.24 (CH), 123.29 (CH), 127.74 (Cq), 129.80 (CH, d, *J* 9.2), 169.43 (Cq). $^{13}$C NMR (100 MHz, 363 K, DMSO) $\delta$ 23.75 ($CH_2$), 111.69 (Cq), 111.84 (CH), 115.29 (CH), 115.51 (CH), 118.45 (CH), 118.73 (CH), 121.40 (CH), 123.32 (CH), 127.77 (Cq), 129.34 (CH), 129.43 (CH), 133.93 (Cq), 136.96 (Cq), 162.77 (Cq, d, *J* 245.0), 170.06 (Cq). At this temperature, one $CH_2$ and one $CH_3$ were not observed. $^{19}$F NMR (282 MHz, $CDCl_3$) $\delta$ (major rotamer) −112.22. $\delta$ (minor rotamer) −111.49. *m/z* ($ES^+$) 297 ($MH^+$), 593 ($2M + H)^+$; ($ES^-$) 295 ($M - H)^-$, 591 ($2M - H)^-$; *m/z* ($FAB^+$) 297 ($MH^+$), 593 ($2M + H)^+$ (found: C, 72.85; H, 5.93; N, 9.52; $MH^+$, 297.14035. $C_{18}H_{17}FN_2O$ requires C, 72.96; H, 5.78; N, 9.45%; MH, 297.14032).

**2-Chloro-*N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-methylbenzamide 9f.** Tan solid. Yield 55%. Mp 176 °C. Rotamers 1 : 1.2 (from the duplicated singlet signal ($^1$H) at 2.75 and 3.09 ppm). *R*f (ethyl acetate–petroleum ether 50 : 50) 0.26. $^1$H NMR (300 MHz, DMSO) $\delta$ (major rotamer) 2.85–2.93 (2H, m), 3.09 (3H, s), 3.26–3.33 (2H, m), 6.77–7.81 (9H, m), 10.82 (1H, br s). $\delta$ (distinct peaks for minor rotamer) 2.75 (3H, s), 3.01–3.07 (2H, m), 3.75 (2H, br s), 10.86 (1H, s). $^{13}$C NMR (75 MHz, DMSO) $\delta$ (major rotamer) 24.26 ($CH_2$), 32.38 ($CH_3$), 51.25 ($CH_2$), 110.71 (Cq), 111.86 (CH), 118.09 (CH), 118.77 (CH), 121.39 (CH), 123.52 (CH), 127.33 (Cq), 127.69 (CH), 128.44 (CH), 129.43 (Cq), 129.63 (CH), 130.56 (CH), 131.08 (Cq), 136.63 (Cq), 167.48 (Cq). $\delta$ (distinct peaks for minor rotamer) 22.99 ($CH_2$), 36.39 ($CH_3$), 47.66 ($CH_2$), 111.64 (Cq), 118.68 (CH), 121.41 (CH), 123.40 (CH), 128.06 (CH), 128.16 (CH), 129.83 (CH), 130.76 (CH), 137.04 (Cq), 172.00 (Cq). *m/z* ($ES^+$) 313 ($MH^+$), 625 ($2M + H)^+$; ($ES^-$) 311 ($M - H)^-$, 623 ($2M - H)^-$; *m/z* ($FAB^+$) 313 ($MH^+$), (found: C, 68.95; H, 5.57; N, 8.83; $MH^+$, 313.11071. $C_{18}H_{17}ClN_2O$ requires C, 69.12; H, 5.48; N, 8.96%; MH, 313.11077).

**3-Chloro-*N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-methylbenzamide 9g.** Beige solid. Yield 52%. Mp 152 °C. Rotamers 1 : 1.4 (from the duplicated triplet signal ($^1$H) at 3.55 and 3.86 ppm). *R*f (ethyl acetate–petroleum ether 50 : 50) 0.23. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ (major rotamer) 2.96 (2H, t, *J* 7.1), 3.17 (3H, s), 3.55 (2H, t, *J* 7.1), 6.89–7.38 (9H, m), 8.11 (1H, br s). $\delta$ (distinct peaks for minor rotamer) 2.85 (3H, s), 3.86 (2H, t, *J* 7.1), 7.71 (1H, d, *J* 7.5). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ (major rotamer) 24.20 ($CH_2$), 33.04 ($CH_3$), 51.89 ($CH_2$), 111.35 (CH), 111.49 (Cq), 117.97 (CH), 119.44 (CH), 122.14 (2CH), 124.58 (CH), 126.64

(CH), 126.94 (Cq), 129.56 (2CH), 134.22 (Cq), 136.22 (Cq), 137.96 (Cq), 170.76 (Cq). $\delta$ (distinct peaks for minor rotamer) 22.89 ($CH_2$), 38.15 ($CH_3$), 48.57 ($CH_2$), 112.78 (Cq), 118.69 (CH), 122.26 (CH), 125.02 (CH), 127.11 (CH), 127.51 (Cq), 129.16 (CH), 129.79 (CH), 134.40 (Cq), 138.40 (Cq), 169.83 (Cq). *m/z* ($ES^+$) 313 ($MH^+$), 625 ($2M + H)^+$; ($ES^-$) 311 ($M - H)^-$, 623 ($2M - H)^-$; *m/z* ($FAB^+$) 313 ($MH^+$), 625 ($2M + H)^+$ (found: C, 69.00; H, 5.54; N, 8.88; $MH^+$, 313.11073. $C_{18}H_{17}ClN_2O$ requires C, 69.12; H, 5.48; N, 8.96%; MH, 313.11077).

**4-Chloro-*N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-methylbenzamide 9h.** Beige solid. Yield 62%. Mp 160 °C. Rotamers 1 : 1.4 (from the duplicated triplet signal ($^1H$) at 3.55 and 3.86 ppm). *R*f (ethyl acetate–petroleum ether 50 : 50) 0.17. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ (major rotamer) 2.94 (2H, t, *J* 6.4), 3.17 (3H, s), 3.55 (2H, t, *J* 6.4), 6.87–7.42 (9H, m), 8.16 (1H, br s). $\delta$ (distinct peaks for minor rotamer) 2.86 (3H, s), 3.86 (2H, distorted t, *J* 5.4), 7.70 (1H, d, *J* 6.9). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ (major rotamer) 24.17 ($CH_2$), 33.01 ($CH_3$), 51.74 ($CH_2$), 111.31 (CH), 112.77 (Cq), 118.01 (CH), 119.42 (CH), 122.12 (2CH), 127.03 (Cq), 127.89 (2CH), 128.38 (2CH), 135.02 (Cq), 136.27 (2Cq), 171.41 (Cq). $\delta$ (distinct peaks for minor rotamer) 22.88 ($CH_2$), 38.20 ($CH_3$), 48.59 ($CH_2$), 118.69 (CH), 122.31 (CH), 127.53 (Cq), 128.51 (CH), 128.64 (CH), 134.55 (Cq), 135.50 (Cq). *m/z* ($ES^+$) 313 ($MH^+$), 625 ($2M + H)^+$; ($ES^-$) 311 ($M - H)^-$, 623 ($2M - H)^-$; *m/z* ($FAB^+$) 313 ($MH^+$), 625 ($2M + H)^+$ (found: C, 68.99; H, 5.54; N, 8.87; $MH^+$, 313.11072. $C_{18}H_{17}ClN_2O$ requires C, 69.12; H, 5.48; N, 8.96%; MH, 313.11077).

**2-Bromo-*N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-methylbenzamide 9i.** Tan solid. Yield 48%. Mp 205 °C. Rotamers 1 : 1.2 (from the duplicated triplet signal ($^1H$) at 3.34 and 3.79 ppm). *R*f (ethyl acetate–petroleum ether 50 : 50) 0.31. $^1H$ NMR (400 MHz, 363 K, DMSO) $\delta$ (major rotamer) 2.92 (2H, t, *J* 7.3), 3.10 (3H, s), 3.34 (2H, t, *J* 7.3), 6.90–7.10 (3H, m), 7.19–7.44 (5H, m), 7.63 (1H, br t, estimated *J* 9.4), 10.61 (1H, br s). $\delta$ (distinct peaks for minor rotamer) 2.77 (3H, s), 3.79 (2H, t, *J* 7.6), 6.84 (1H, t, *J* 7.4). $^{13}C$ NMR (75 MHz, DMSO) $\delta$ (major rotamer) 24.27 ($CH_2$), 32.37 ($CH_3$), 51.31 ($CH_2$), 110.69 (Cq), 111.89 (CH), 118.10 (CH), 118.74 (CH), 118.82 (Cq), 121.36 (CH), 123.54 (CH), 127.66 (Cq), 128.14 (CH), 128.49 (CH), 130.68 (CH), 132.72 (CH), 136.63 (Cq), 138.79 (Cq), 168.31 (Cq). $\delta$ (distinct peaks for minor rotamer) 22.94 ($CH_2$), 36.50 ($CH_3$), 47.67 ($CH_2$), 111.61 (Cq), 118.65 (CH), 118.94 (Cq), 121.42 (CH), 123.43 (CH), 127.32 (Cq), 130.87 (CH), 132.94 (CH), 136.77 (Cq), 139.16 (Cq), 168.15 (Cq). *m/z* ($ES^+$) 358; ($ES^-$) 356 ($M - H)^-$; *m/z* ($FAB^+$) 357 ($M^+$) (found: C, 60.38; H, 4.81; N, 7.81; $M^+$, 357.06015. $C_{18}H_{17}BrN_2O$ requires C, 60.52; H, 4.80; N, 7.84%; M, 357.06025).

**3-Bromo-*N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-methylbenzamide 9j.** White solid. Yield 76%. Mp 166 °C. Rotamers 1 : 1.4 (from the duplicated triplet signal ($^1H$) at 3.46 and 3.78 ppm). *R*f (ethyl acetate–petroleum ether 50 : 50) 0.35. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ (major rotamer) 2.87 (2H, t, *J* 6.9), 3.09 (3H, s), 3.46 (2H, t, *J* 6.9), 6.76–7.61 (9H, m), 8.25 (1H, br s). $\delta$ (distinct peaks for minor rotamer) 2.76 (3H, s), 3.78 (2H, t, *J* 6.9). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ (major rotamer) 24.20 ($CH_2$), 33.08 ($CH_3$), 51.94 ($CH_2$), 111.39 (CH), 112.72 (Cq), 117.97 (CH), 119.44 (CH), 122.13 (CH), 122.30 (CH), 125.04 (CH), 126.98 (Cq), 129.79 (CH), 129.94 (CH), 132.10 (CH), 132.99 (Cq), 136.22 (Cq), 138.15 (Cq), 170.65 (Cq). $\delta$ (distinct peaks for minor rotamer) 22.88 ($CH_2$), 38.18 ($CH_3$), 48.61 ($CH_2$), 118.66 (CH), 125.45 (CH), 127.52 (Cq), 129.45 (CH), 130.06 (CH), 132.51 (CH), 135.95 (Cq), 138.59 (Cq), 169.73 (Cq). *m/z* ($ES^+$) 357 ($M^+$), 358 ($MH^+$); ($ES^-$) 356 ($M - H)^-$; *m/z* ($FAB^+$) 357 ($M^+$) (found: C, 60.38; H, 4.80; N, 7.73; $M^+$, 357.06030. $C_{18}H_{17}BrN_2O$ requires C, 60.52; H, 4.80; N, 7.84%; M, 357.06025).

**4-Bromo-*N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-methylbenzamide 9k.** White solid. Yield 73%. Mp 160 °C. Rotamers 1 : 1.5 (from the duplicated triplet signal ($^1H$) at 3.45 and 3.77 ppm). *R*f (ethyl acetate–petroleum ether 50 : 50) 0.23. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ (major rotamer) 2.83 (2H, t, *J* 6.3), 3.07 (3H, s), 3.45 (2H, t, *J* 6.3), 6.71–7.62 (9H, m), 8.41 (1H, br s). $\delta$ (distinct peaks for minor rotamer) 2.76 (3H, s), 3.77 (2H, distorted t, *J* 7.1). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ (major rotamer) 24.17 ($CH_2$), 32.99 ($CH_3$), 51.72 ($CH_2$), 111.35 (CH), 112.65 (Cq), 117.98 (CH), 119.39 (CH), 122.05 (CH), 122.38 (CH), 123.27 (Cq), 127.04 (Cq), 128.09 (2CH), 131.33 (2CH), 135.02 (Cq), 136.31 (Cq), 171.44 (Cq). $\delta$ (distinct peaks for minor rotamer) 22.89 ($CH_2$), 38.18 ($CH_3$), 48.62 ($CH_2$), 118.65 (CH), 123.74 (Cq), 127.54 (Cq), 128.69 (CH), 131.60 (CH), 135.51 (Cq), 170.40 (Cq). *m/z* ($ES^+$) 358 ($MH^+$); ($ES^-$) 356 ($M - H)^-$; *m/z* ($FAB^+$) 357 ($M^+$) (found: C, 60.58; H, 4.96; N, 7.76; $M^+$, 357.06027. $C_{18}H_{17}BrN_2O$ requires C, 60.52; H, 4.80; N, 7.84%; M, 357.06025).

***N*-[2-(1*H*-Indol-3-yl)-ethyl]-2-methoxy-*N*-methylbenzamide 9l.** Beige solid. Yield 57%. Mp 203 °C. Rotamers 1 : 1.3 (from the multiplet and triplet signals ($^1H$) at 3.38–3.46 and 3.88 ppm). *R*f (ethyl acetate–petroleum ether, 50 : 50) 0.09. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ (major rotamer) 2.81–2.95 (2H, m), 3.17 (3H, s), 3.38–3.46 (2H, m), 3.77 (3H, s), 7.11–7.38 (9H, m), 8.31 (1H, br s). $\delta$ (distinct peaks for minor rotamer) 2.77 (3H, s), 3.11–3.14 (2H, m), 2.78 (3H, s), 3.88 (2H, distorted t, *J* 7.5), 7.71 (1H, d, *J* 7.5), 8.34 (1H, br s). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ (major rotamer) 24.51 ($CH_2$), 32.80 ($CH_3$), 51.57 ($CH_2$), 55.51 ($CH_3$), 110.96 (CH), 111.23 (CH), 112.05 (Cq), 118.24 (CH), 119.20 (CH), 120.83 (CH), 121.90 (CH), 122.04 (CH), 126.30 (Cq), 127.11 (Cq), 127.72 (CH), 130.06 (CH), 136.26 (Cq), 155.17 (Cq), 169.69 (Cq). $\delta$ (distinct peaks for minor rotamer) 23.00 ($CH_2$), 36.70 ($CH_3$), 47.94 ($CH_2$), 55.56 ($CH_3$), 112.98 (Cq), 118.74 (CH), 120.88 (CH), 121.84 (CH), 122.26 (CH), 126.72 (Cq), 127.56 (Cq), 127.78 (CH), 130.21 (CH), 136.32 (Cq), 155.27 (Cq). *m/z* ($ES^+$) 309 ($MH^+$), 617 ($2M + H)^+$; ($ES^-$) 307 ($M - H)^-$, 615 ($2M - H)^-$; *m/z* ($FAB^+$) 309 ($MH^+$), 618 ($2M + 2H)^+$ (found: C, 73.97; H, 6.49; N, 9.02; $MH^+$, 309.16027. $C_{19}H_{20}N_2O_2$ requires C, 74.00; H, 6.54; N, 9.08%; MH, 309.16030).

***N*-[2-(1*H*-Indol-3-yl)-ethyl]-3-methoxy-*N*-methylbenzamide 9m.** Tan solid. Yield 54%. Mp 175 °C. Rotamers 1 : 1.2 (from the duplicated triplet signal ($^1H$) at 3.48 and 3.78 ppm). *R*f (ethyl acetate–petroleum ether 50 : 50) 0.17. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ (major rotamer) 2.89 (2H, br t, *J* 7.2), 3.10 (3H, br s), 3.48 (2H, t, *J* 7.2), 3.63 (3H, br s), 6.65–7.28 (9H, m), 8.20 (1H, br s). $\delta$ (distinct peaks for minor rotamer) 2.79 (3H, s), 3.70 (3H, s), 3.78 (2H, distorted t, *J* 7.1), 7.64 (1H, d, *J* 7.2). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ (major rotamer) 24.48 ($CH_2$), 33.03 ($CH_3$), 51.91 ($CH_2$), 55.25 ($CH_3$), 111.24 (CH), 111.85 (CH), 112.92 (Cq), 115.08 (CH), 118.63 (CH), 118.70 (CH), 119.33 (CH), 122.00 (CH), 122.12 (CH), 127.12 (Cq), 129.46 (CH), 136.26 (Cq), 137.78 (Cq), 159.50 (Cq), 172.02 (Cq). $\delta$ (distinct peaks for minor rotamer) 22.90

($CH_2$), 38.13 ($CH_3$), 48.41 ($CH_2$), 112.14 (CH), 115.39 (CH), 118.24 (CH), 119.03 (CH), 127.26 (Cq). *m/z* ($ES^+$) 309 ($MH^+$), 617 ($2M + H)^+$; ($ES^-$) 307 ($M - H)^-$, 615 ($2M - H)^-$; *m/z* ($FAB^+$) 309 $MH^+$ (found: C, 73.87; H, 6.44; N, 9.01; $MH^+$, 309.16031. $C_{19}H_{20}N_2O_2$ requires C, 74.00; H, 6.54; N, 9.08%; MH, 309.16030).

***N*-[2-(1*H*-Indol-3-yl)-ethyl]-2,*N*-dimethylbenzamide 9n.** Tan solid. Yield 63%. Mp 188 °C. Rotamers 1 : 1.4 (from the duplicated triplet signal ($^1H$) at 3.40 and 3.91 ppm). *R*f (ethyl acetate–petroleum ether 50 : 50) 0.24. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ (major rotamer) 2.19 (3H, s), 2.90 (2H, t, *J* 7.7), 3.20 (3H, s), 3.40 (2H, t, *J* 7.7), 6.90–7.34 (9H, m), 8.33 (1H, br s). $\delta$ (distinct peaks for minor rotamer) 2.24 (3H, s), 2.75 (3H, s), 3.16 (2H, apparent t, estimated *J* 7.5), 3.91 (2H, td, *J* 7.5), 7.70 (1H, d, *J* 7.5), 8.35 (1H, br s). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ (major rotamer) 18.91 ($CH_3$), 24.51 ($CH_2$), 32.52 ($CH_3$), 51.38 ($CH_2$), 111.24 (CH), 111.67 (Cq), 118.22 (CH), 119.28 (CH), 121.84 (CH), 121.98 (CH), 125.80 (CH), 125.92 (CH), 127.01 (Cq), 128.67 (CH), 130.30 (CH), 133.97 (Cq), 136.28 (Cq), 136.50 (Cq), 171.86 (Cq). $\delta$ (distinct peaks for minor rotamer) 18.81 ($CH_3$), 23.04 ($CH_2$), 36.80 ($CH_3$), 47.53 ($CH_2$), 111.28 (CH), 112.72 (Cq), 118.69 (CH), 122.09 (CH), 122.21 (CH), 125.71 (CH), 127.50 (Cq), 130.32 (CH), 133.88 (Cq), 136.36 (Cq), 137.00 (Cq), 171.40 (Cq). *m/z* ($ES^+$) 293 ($MH^+$), 585 ($2M + H)^+$; ($ES^-$) 291 ($M - H)^-$, 583 ($2M - H)^-$; *m/z* ($FAB^+$) 293 ($MH^+$) (found: C, 78.08; H, 6.73; N, 9.51; $MH^+$, 293.16541. $C_{19}H_{20}N_2O$ requires C, 78.05; H, 6.89; N, 9.58%; MH, 293.16539).

***N*-[2-(1*H*-Indol-3-yl)-ethyl]-3,*N*-dimethylbenzamide 9o.** White solid. Yield 56%. Mp 140 °C. Rotamers 1 : 1.3 (from the duplicated triplet signal ($^1H$) at 3.47 and 3.80 ppm). *R*f (ethyl acetate–petroleum ether 50 : 50) 0.20. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ (major rotamer) 2.15 (3H, s), 2.89 (2H, t, *J* 6.9), 3.10 (3H, br s), 3.47 (2H, t, *J* 6.9), 6.81–7.30 (9H, m), 8.07 (1H, br s). $\delta$ (distinct peaks for minor rotamer) 2.27 (3H, s), 2.80 (3H, s), 3.80 (2H, apparent t, estimated *J* 6.3), 7.65 (1H, d, *J* 7.2). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ (major rotamer) 21.34 ($CH_3$), 24.39 ($CH_2$), 33.01 ($CH_3$), 51.93 ($CH_2$), 111.32 (CH), 111.61 (Cq), 118.15 (CH), 119.24 (CH), 121.92 (2CH), 122.06 (Cq), 123.42 (CH), 127.07 (CH), 128.19 (CH), 129.81 (CH), 136.30 (Cq), 136.47 (Cq), 138.22 (Cq), 172.64 (Cq). $\delta$ (distinct peaks for minor rotamer) 22.96 ($CH_2$), 38.18 ($CH_3$), 48.47 ($CH_2$), 112.83 (Cq), 118.71 (CH), 122.25 (2CH), 123.86 (CH), 127.53 (CH), 130.13 (CH), 136.71 (Cq), 171.54 (Cq). *m/z* ($ES^+$) 293 ($MH^+$), 585 ($2M + H)^+$; ($ES^-$) 291 ($M - H)^-$; *m/z* ($FAB^+$) 293 ($MH^+$) (found: C, 77.88; H, 6.81; N, 9.42; $MH^+$, 293.16542. $C_{19}H_{20}N_2O$ requires C, 78.05; H, 6.89; N, 9.58%; MH, 293.16539).

***N*-[2-(1*H*-Indol-3-yl)-ethyl]-4,*N*-dimethylbenzamide 9p.** White solid. Yield 77%. Mp 178 °C. Rotamers 1 : 1.3 (from the duplicated broad singlet signals ($^1H$) at 3.46 and 3.77 ppm). *R*f (ethyl acetate–petroleum ether 50 : 50) 0.24. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ (major rotamer) 2.24 (3H, br s), 2.84 (2H, br s), 3.07 (3H, br s), 3.46 (2H, br s), 6.70–7.15 (9H, m), 8.51 (1H, br s). $\delta$ (distinct peaks for minor rotamer) 2.79 (3H, br s), 3.77 (2H, br s), 7.60 (1H, br s). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ (major rotamer) 21.35 ($CH_3$), 24.47 ($CH_2$), 33.14 ($CH_2$), 52.00 ($CH_3$), 111.36 (CH), 111.53 (Cq), 118.20 (CH), 119.11 (CH), 121.82 (2CH), 126.51 (CH), 127.08 (Cq), 128.94 (3CH), 133.56 (Cq), 136.36 (Cq), 139.15 (Cq), 172.65 (Cq). $\delta$ (distinct peaks for minor rotamer) 22.98 ($CH_2$), 38.26 ($CH_2$), 48.57 ($CH_3$), 112.74 (Cq), 118.67 (CH), 122.26 (2CH), 127.08 (CH), 127.55 (Cq), 133.71 (Cq), 139.54 (Cq), 171.63 (Cq). *m/z* ($ES^+$) 293 ($MH^+$), 585 ($2M + H)^+$; ($ES^-$) 291 ($M - H)^-$; *m/z* ($FAB^+$) 293 ($MH^+$) (found: C, 77.91; H, 6.81; N, 9.62; $MH^+$, 293.16543. $C_{19}H_{20}N_2O$ requires C, 78.05; H, 6.89; N, 9.58%; MH, 293.16539).

**Biphenyl-4-carboxylic acid [2-(1*H*-indol-3-yl)-ethyl]-methyl-amide 9q.** Beige solid. Yield 58%. Mp 178 °C. Rotamers 1 : 1.5 (from the duplicated triplet signal ($^1H$) at 3.59 and 3.89 ppm). *R*f (ethyl acetate–petroleum ether 50 : 50) 0.16. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ (major rotamer) 2.95 (2H, distorted t, *J* 6.8), 3.18 (3H, s), 3.59 (2H, t, *J* 6.8), 6.84–7.55 (14H, m), 8.42 (1H, br s). $\delta$ (distinct peaks for minor rotamer) 2.91 (3H, br s), 3.89 (2H, apparent t, estimated *J* 6.9), 7.72 (1H, br d, *J* 6.9). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ (major rotamer) 24.41 ($CH_2$), 33.06 ($CH_3$), 51.92 ($CH_2$), 111.33 (2CH), 111.57 (Cq), 118.15 (CH), 119.31 (2CH), 121.96 (2CH), 127.01 (2CH), 127.16 (2CH), 127.54 (Cq), 127.66 (CH), 128.87 (2CH), 135.21 (Cq), 136.34 (Cq), 140.45 (Cq), 141.95 (Cq), 172.35 (Cq). $\delta$ (distinct peaks for minor rotamer) 22.98 ($CH_2$), 38.28 ($CH_3$), 48.55 ($CH_2$), 112.86 (Cq), 118.73 (CH), 122.30 (CH), 135.47 (Cq), 142.34 (Cq), 171.27 (Cq). *m/z* ($ES^+$) 355 ($MH^+$); ($ES^-$) 353 ($M - H)^-$; *m/z* ($FAB^+$) 355 ($MH^+$) (found: C, 81.34; H, 6.07; N, 7.84; $MH^+$, 355.18103. $C_{24}H_{22}N_2O$ requires C, 81.26; H, 6.26; N, 7.90%; MH, 355.18104).

***N*-[2-(1*H*-Indol-3-yl)-ethyl]-*N*-methyl-2-phenylacetamide 10§.** Yellow paste. Yield 55%. Rotamers 1 : 1.2 (from the duplicated doublet signal ($^1H$) at 7.58 and 7.69 ppm). *R*f (ethyl acetate–petroleum ether 50 : 50) 0.27. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ (major rotamer) 2.98 (2H, t, *J* 7.1), 3.08 (3H, s), 3.62 (2H, t, *J* 7.1), 3.76 (2H, s), 6.84 (1H, d, *J* 2.4), 7.09–7.39 (8H, m), 7.58 (1H, d, *J* 7.8), 8.92 (1H, br s). $\delta$ (distinct peaks for minor rotamer) 2.94 (3H, s), 3.07 (2H, apparent t, estimated *J* 7.5), 3.44 (2H, s), 3.76 (2H, apparent t, estimated *J* 7.5), 6.91 (1H, d, *J* 2.4), 7.69 (1H, d, *J* 7.5), 8.70 (1H, br s). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ (major rotamer) 24.19 ($CH_2$), 33.68 ($CH_3$), 40.33 ($CH_2$), 50.88 ($CH_2$), 111.76 (CH), 112.58 (Cq), 118.09 (CH), 118.64 (CH), 119.43 (CH), 122.05 (CH), 122.40 (CH), 122.78 (CH), 126.71 (CH), 127.49 (Cq), 128.60 (CH), 128.89 (CH), 135.39 (Cq), 136.52 (Cq), 171.59 (Cq). $\delta$ (distinct peaks for minor rotamer) 23.22 ($CH_2$), 36.50 ($CH_3$), 41.28 ($CH_2$), 49.25 ($CH_2$), 111.44 (CH + Cq), 119.16 (CH), 121.77 (CH), 126.80 (CH), 127.05 (Cq), 128.73 (CH), 128.94 (CH), 135.12 (Cq), 136.42 (Cq), 171.14 (Cq). *m/z* ($FAB^+$) 293 ($MH^+$) (found: $MH^+$, 293.16532. $C_{19}H_{20}N_2O$ requires MH, 293.16539).

**General procedure for the synthesis of phenyl-(1,3,4,9-tetrahydro-β-carbolin-2-yl)-methanone and all derivatives 12a–q, 13 and 14.** To a suspension of carboline (1.2 mmol) in dichloromethane (3 mL) at 0 °C was added slowly an aqueous solution of sodium hydroxide 4 M (1.2 mmol). After 5 min stirring at 0 °C, the benzoyl chloride derivative (1.2 mmol) was added dropwise. The mixture was stirred for 5 min at 0 °C and 3 h at room temperature. Water (20 mL) was added. The two layers were separated and the aqueous phase was extracted with dichloromethane (3 × 20 mL). The organic layers were dried ($MgSO_4$), and evaporated under reduced pressure. The crude product was purified by chromatography on silica gel. Elution was made successively with: ethyl acetate–petroleum ether 50 : 50 and ethyl acetate, to give the expected compound.

**Phenyl-(1,3,4,9-tetrahydro-β-carbolin-2-yl)-methanone 12a.** Yellow solid. Yield 82%. Mp 78 °C. Rotamers 1 : 3 (from the duplicated broad singlet signal ($^1$H) at 8.71 and 9.23 ppm). *R*f (ethyl acetate–petroleum ether 50 : 50) 0.27. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ (major rotamer) 2.88 (2H, br s), 3.75 (2H, br s), 4.99 (2H, br s), 7.16–7.30 (4H, m), 7.54–7.56 (5H, m), 9.23 (1H, br s). $\delta$ (distinct peaks for minor rotamer) 2.97 (2H, br s), 4.16 (2H, br s), 4.54 (2H, br s), 8.71 (1H, br s). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 22.21 ($CH_2$), 41.28 ($CH_2$), 46.18 ($CH_2$), 107.51 (Cq), 111.41 (CH), 117.86 (CH), 119.44 (CH), 121.68 (CH), 126.86 (Cq), 126.99 (CH), 128.81 (2CH), 130.15 (2CH), 130.28 (Cq), 136.14 (Cq), 136.53 (Cq), 171.80 (Cq). No rotamers observed in $^{13}$C at RT. *m/z* (FAB$^+$) 276 ($M^+$), 277 ($MH^+$) (found: $M^+$, 276.12624. $C_{18}H_{16}N_2O$ requires M, 276.12626).

**(4-*tert*-Butylphenyl)-(1,3,4,9-tetrahydro-β-carbolin-2-yl)-methanone 12b.** White solid. Yield 90%. Mp 217 °C. Rotamers 1 : 2 (from the duplicated broad singlet signal ($^1$H) at room temperature at 10.66 and 10.94 ppm). *R*f (ethyl acetate–petroleum ether 50 : 50) 0.34. $^1$H NMR (400 MHz, 373 K, DMSO) $\delta$ 1.36 (9H, s), 2.81 (2H, t, *J* 5.6), 3.83 (2H, t, *J* 5.6), 4.79 (2H, s), 7.01 (1H, td, *J* 7.4 and 1.1), 7.08 (1H, td, *J* 7.4 and 1.1), 7.34 (1H, d, *J* 8.0), 7.41–7.43 (3H, m), 7.51 (2H, d, *J* 8.0), 10.53 (1H, br s). $^{13}$C NMR (100 MHz, 373 K, DMSO) $\delta$ 21.80 ($CH_2$), 31.48 ($3CH_3$), 34.95 (Cq), 43.15 ($CH_2$), 44.10 ($CH_2$), 107.53 (Cq), 111.51 (CH), 117.92 (CH), 119.04 (CH), 121.30 (CH), 125.55 (2CH), 127.17 (2CH), 127.35 (Cq), 131.42 (Cq), 134.20 (Cq), 136.90 (Cq), 152.96 (Cq), 170.64 (Cq). No rotamers observed in $^{13}$C at RT. *m/z* (FAB$^+$) 333 ($MH^+$) (found: C, 79.56; H, 7.28; N, 8.40; $MH^+$, 333.19665. $C_{22}H_{24}N_2O$ requires C, 79.48; H, 7.28; N, 8.43%; MH, 333.19669).

**(2-Fluorophenyl)-(1,3,4,9-tetrahydro-β-carbolin-2-yl)-methanone 12c.** Yellow solid. Yield 95%. Mp 79 °C. Rotamers 1 : 2.6 (from the duplicated broad singlet signal ($^1$H) at 4.35 and 4.82 ppm). *R*f (ethyl acetate–petroleum ether 50 : 50) 0.29. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ (major rotamer) 2.66 (2H, br s), 3.51 (2H, t, *J* 5.4), 4.82 (2H, s), 6.92–7.37 (8H, 2m), 8.69 (1H, br s). $\delta$ (distinct peaks for minor rotamer) 2.77 (2H, br s), 4.35 (2H, br s), 8.19 (1H, br s). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ (major rotamer) 21.96 ($CH_2$), 40.85 ($CH_2$), 45.65 ($CH_2$), 107.60 (Cq), 111.30 (CH), 116.10 (CH, d, *J* 21.1), 117.82 (CH), 119.42 (CH), 121.71 (CH), 124.30 (Cq, d, *J* 17.7), 124.83 (CH, d, *J* 3.0), 126.71 (Cq), 128.83 (CH, d, *J* 3.0), 129.80 (Cq), 131.56 (CH, d, *J* 8.0), 136.45 (Cq), 158.44 (Cq, d, *J* 246.5), 166.63 (Cq). $\delta$ (distinct peaks for minor rotamer) 21.04 ($CH_2$), 45.11 ($CH_2$), 109.03 (Cq), 111.09 (CH), 115.89 (CH, d, *J* 18.1), 118.13 (CH), 119.59 (CH), 121.89 (CH), 126.81 (Cq), 129.36 (Cq), 136.33 (Cq), 158.39 (Cq, d, *J* 241.1), 166.08 (Cq). $^{19}$F NMR (282 MHz, $CDCl_3$) $\delta$ (major rotamer) −114.86. $\delta$ (minor rotamer) −114.47. *m/z* (ES$^+$) 295 ($MH^+$), 589 ($2M + H)^+$; (ES$^-$) 293 $(M - H)^-$. *m/z* (FAB$^+$) 294 ($M^+$), 295 ($MH^+$) (found: $MH^+$, 295.12473. $C_{18}H_{15}FN_2O$ requires MH, 295.12467).

**(3-Fluorophenyl)-(1,3,4,9-tetrahydro-β-carbolin-2-yl)-methanone 12d.** Pale yellow solid. Yield 99%. Mp 65 °C. Rotamers 1 : 5 (from the duplicated triplet signal ($^1$H) at 2.81 and 3.42 ppm). *R*f (ethyl acetate–petroleum ether 50 : 50) 0.35. $^1$H NMR (400 MHz, 373 K, DMSO) $\delta$ (major rotamer) 2.81 (2H, t, *J* 5.6), 3.81 (2H, br s), 4.78 (2H, s), 7.00 (1H, td, *J* 7.5 and 1.0), 7.08 (1H, td, *J* 7.5 and 1.0), 7.27–7.35 (3H, m), 7.42 (1H, d, *J* 8.0), 7.50–7.55 (2H, m), 10.52 (1H, br s). $\delta$ (distinct peaks for minor rotamer) 3.42 (2H, t, *J* 7.0), 7.65 (1H, br d, *J* 8.8), 7.83 (1H, br d, *J* 8.2), 11.36 (1H, br s). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 22.10 ($CH_2$), 41.22 ($CH_2$), 46.07 ($CH_2$), 107.57 (Cq), 111.26 (CH), 114.24 (CH, d, *J* 22.6), 117.11 (CH, d, *J* 20.9), 117.88 (CH), 119.56 (CH), 121.83 (CH), 122.60 (CH), 126.76 (Cq), 129.85 (Cq), 130.61 (CH, d, *J* 7.7), 136.40 (Cq), 138.05 (Cq, d, *J* 6.6), 162.99 (Cq, d, *J* 246.7), 170.16 (Cq). No rotamers observed in $^{13}$C at RT. $^{19}$F NMR (282 MHz, $CDCl_3$) $\delta$ (major rotamer) −111.15, $\delta$ (minor rotamer) −112.77. *m/z* (FAB$^+$) 294 ($M^+$), 295 ($MH^+$) (found: $MH^+$, 295.12470. $C_{18}H_{15}FN_2O$ requires MH, 295.12467).

**(4-Fluorophenyl)-(1,3,4,9-tetrahydro-β-carbolin-2-yl)-methanone 12e.** Yellow solid. Yield 97%. Mp 76 °C. Rotamers 1 : 4 (from the duplicated broad singlet signal ($^1$H) at 4.43 and 4.75 ppm). *R*f (ethyl acetate–petroleum ether 50 : 50) 0.33. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ (major rotamer) 2.70 (2H, br s), 3.56 (2H, br s), 4.75 (2H, br s), 6.63–7.35 (8H, 2m), 8.75 (1H, br s). $\delta$ (distinct peaks for minor rotamer) 4.00 (2H, br s), 4.43 (2H, br s), 8.69 (1H, br s). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 22.13 ($CH_2$), 41.41 ($CH_2$), 46.26 ($CH_2$), 107.57 (Cq), 111.24 (CH), 115.81 (2CH, d, *J* 21.7), 117.89 (CH), 119.55 (CH), 121.81 (CH), 126.78 (Cq), 129.31 (2CH, d, *J* 7.9), 130.00 (Cq), 132.00 (Cq, d, *J* 3.2), 136.41 (Cq), 163.63 (Cq, d, *J* 248.6), 170.80 (Cq). No rotamers observed in $^{13}$C at RT. $^{19}$F NMR (282 MHz, $CDCl_3$) $\delta$ −109.52. *m/z* (FAB$^+$) 294 ($M^+$) (found: $MH^+$, 295.12470. $C_{18}H_{15}FN_2O$ requires MH, 295.12467).

**(2-Chlorophenyl)-(1,3,4,9-tetrahydro-β-carbolin-2-yl)-methanone 12f.** White solid. Yield 81%. Mp 97 °C. Rotamers 1 : 3 (from the duplicated doublet signal ($^1$H) at 4.56 and 5.18 ppm). *R*f (ethyl acetate–petroleum ether 50 : 50) 0.39. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ (major rotamer) 2.75 (1H, t, *J* 6.3), 2.84 (1H, t, *J* 5.1), 3.63 (2H, t, *J* 5.7), 4.92 (1H, d, *J* 16.8), 5.18 (1H, d, *J* 16.8), 7.10–7.36 (8H, m), 8.27 (1H, br s). $\delta$ (distinct peaks for minor rotamer) 2.73 (1H, apparent t, estimated *J* 6.0), 2.90 (1H, t, *J* 6.0), 2.98 (2H, t, *J* 5.7), 4.40 (1H, d, *J* 16.5), 4.56 (1H, d, *J* 16.5), 7.78 (1H, br s). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ (major rotamer) 21.88 ($CH_2$), 40.33 ($CH_2$), 45.13 ($CH_2$), 108.01 (Cq), 111.08 (CH), 117.88 (CH), 119.66 (CH), 121.94 (CH), 126.74 (Cq), 127.27 (CH), 127.65 (CH), 129.59 (Cq), 129.82 (CH), 130.34 (CH), 130.57 (Cq), 136.02 (Cq), 136.29 (Cq), 168.04 (Cq). $\delta$ (distinct peaks for minor rotamer) 21.00 ($CH_2$), 44.71 ($CH_2$), 110.88 (CH), 118.23 (CH), 119.83 (CH), 122.11 (CH), 127.39 (CH), 127.95 (CH), 129.71 (CH). *m/z* (ES$^+$) 311 ($MH^+$), 621 ($2M + H)^+$; (ES$^-$) 309 $(M - H)^-$. *m/z* (FAB$^+$) 311 ($MH^+$) (found: $MH^+$, 311.09503. $C_{18}H_{15}ClN_2O$ requires MH, 311.09512).

**(3-Chlorophenyl)-(1,3,4,9-tetrahydro-β-carbolin-2-yl)-methanone 12g.** Pale yellow solid. Yield 73%. Mp 72 °C. Rotamers 1 : 3.3 (from the duplicated broad singlet signal ($^1$H) at room temperature at 4.46 and 4.80 ppm). *R*f (ethyl acetate–petroleum ether 50 : 50) 0.44. $^1$H NMR (400 MHz, 373 K, DMSO) $\delta$ 2.81 (2H, t, *J* 5.9), 3.81 (2H, br s), 4.78 (2H, s), 7.00 (1H, td, *J* 7.5 and 1.2), 7.08 (1H, td, *J* 7.5 and 1.2), 7.34 (1H, d, *J* 8.0), 7.42 (2H, apparent dd, estimated *J* 7.2 and 1.6), 7.50–7.54 (3H, m), 10.52 (1H, br s). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 22.10 ($CH_2$), 41.17 ($CH_2$), 46.08 ($CH_2$), 107.69 (Cq), 111.18 (CH), 117.88 (CH), 119.63 (CH), 121.92 (CH), 124.97 (CH), 126.74 (Cq), 127.12 (CH), 129.71 (Cq), 130.10 (2CH), 134.76 (Cq), 136.32 (Cq), 137.68 (Cq), 170.03 (Cq). No rotamers observed in $^{13}$C at RT.

*m/z* (FAB$^+$) 311 (MH$^+$) (found: MH$^+$, 311.09512. $C_{18}H_{15}ClN_2O$ requires MH, 311.09512).

**(4-Chlorophenyl)-(1,3,4,9-tetrahydro-β-carbolin-2-yl)-methanone 12h.** White solid. Yield 83%. Mp 196 °C. Rotamers 1 : 2 (from the duplicated broad singlet signal ($^1$H) at room temperature at 4.60 and 4.83 ppm). *R*f (ethyl acetate–petroleum ether 50 : 50) 0.41. $^1$H NMR (400 MHz, 373 K, DMSO) $\delta$ 2.81 (2H, t, *J* 5.5), 3.81 (2H, br t, *J* 5.5), 4.78 (2H, s), 7.01 (1H, td, *J* 7.5 and 0.9), 7.08 (1H, td, *J* 7.5 and 0.9), 7.35 (1H, d, *J* 8.0), 7.42 (2H, d, *J* 7.6), 7.48–7.53 (3H, m), 10.52 (1H, br s). $^{13}$C NMR (100 MHz, 373 K, DMSO) $\delta$ 21.74 ($CH_2$), 43.09 ($CH_2$), 44.17 ($CH_2$), 107.50 (Cq), 111.54 (CH), 117.94 (CH), 119.10 (CH), 121.37 (CH), 127.31 (Cq), 129.01 (2CH), 129.15 (2CH), 131.18 (Cq), 134.96 (Cq), 135.81 (Cq), 136.91 (Cq), 169.50 (Cq). No rotamers observed in $^{13}$C at RT. *m/z* (ES$^+$) 311 (MH$^+$), 621 (2M + H)$^+$; (ES$^-$) 309 (M − H)$^-$. *m/z* (FAB$^+$) 311 (MH$^+$) (found: C, 69.58; H, 4.54; N, 8.94; MH$^+$, 311.09522. $C_{18}H_{15}ClN_2O$ requires C, 69.57; H, 4.86; N, 9.01%; MH, 311.09512).

**(2-Bromophenyl)-(1,3,4,9-tetrahydro-β-carbolin-2-yl)-methanone 12i.** White solid. Yield 64%. Mp 190 °C. Rotamers 1 : 3.3 (from the duplicated broad singlet signal ($^1$H) at 8.03 and 8.59 ppm). *R*f (ethyl acetate–petroleum ether 50 : 50) 0.35. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ (major rotamer) 2.66 (1H, t, *J* 6.5), 2.73 (1H, t, *J* 5.0), 3.49 (2H, t, *J* 5.7), 4.77 (1H, d, *J* 16.8), 5.06 (1H, d, *J* 16.8), 6.93–7.56 (8H, m), 8.59 (1H, br s). $\delta$ (distinct peaks for minor rotamer) 2.61 (1H, apparent t, estimated *J* 6.3), 2.79 (1H, t, *J* 5.0), 2.85 (2H, t, *J* 5.7), 4.24 (1H, d, *J* 16.1), 4.38 (1H, d, *J* 16.1), 8.03 (1H, br s). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ (major rotamer) 21.87 ($CH_2$), 40.47 ($CH_2$), 45.24 ($CH_2$), 107.63 (Cq), 111.28 (CH), 117.81 (CH), 119.34 (Cq), 119.48 (CH), 121.77 (CH), 126.70 (Cq), 127.60 (CH), 127.86 (CH), 129.68 (Cq), 130.52 (CH), 133.00 (CH), 136.39 (Cq), 138.18 (Cq), 168.93 (Cq). $\delta$ (distinct peaks for minor rotamer) 21.01 ($CH_2$), 44.87 ($CH_2$), 109.18 (Cq), 111.03 (CH), 118.15 (CH), 119.10 (Cq), 119.66 (CH), 121.93 (CH), 126.81 (Cq), 129.15 (Cq), 132.87 (CH), 136.28 (Cq), 138.27 (Cq), 168.44 (Cq). *m/z* (ES$^+$) 355 (MH$^+$); (ES$^-$) 354 (M)$^-$. *m/z* (FAB$^+$) 355 (MH$^+$) (found: C, 60.75; H, 4.17; N, 7.92; M$^+$, 354.03678. $C_{18}H_{15}BrN_2O$ requires C, 60.86; H, 4.26; N, 7.89%; M, 354.03677).

**(3-Bromophenyl)-(1,3,4,9-tetrahydro-β-carbolin-2-yl)-methanone 12j.** White solid. Yield 91%. Mp 207 °C. Rotamers 1 : 1.6 (from the duplicated broad singlet signal ($^1$H) at room temperature at 4.60 and 4.84 ppm). *R*f (ethyl acetate–petroleum ether 50 : 50) 0.41. $^1$H NMR (400 MHz, 373 K, DMSO) $\delta$ 2.80 (2H, t, *J* 5.8), 3.81 (2H, br s), 4.77 (2H, s), 7.00 (1H, td, *J* 7.5 and 1.2), 7.08 (1H, td, *J* 7.5 and 1.2), 7.34 (1H, d, *J* 7.6), 7.41–7.48 (3H, m), 7.64–7.69 (2H, m), 10.52 (1H, br s). $^{13}$C NMR (100 MHz, 373 K, DMSO) $\delta$ 21.70 ($CH_2$), 43.06 ($CH_2$), 44.09 ($CH_2$), 107.49 (Cq), 111.54 (CH), 117.96 (CH), 119.09 (CH), 121.37 (CH), 122.24 (Cq), 126.12 (CH), 127.29 (Cq), 129.93 (CH), 131.09 (CH + Cq), 132.84 (CH), 136.90 (Cq), 139.33 (Cq), 168.80 (Cq). No rotamers observed in $^{13}$C at RT. *m/z* (ES$^+$) 355 (MH$^+$); (ES$^-$) 353 (M − H)$^-$. *m/z* (FAB$^+$) 355 (MH$^+$) (found: C, 60.94; H, 4.17; N, 7.93; MH$^+$, 355.04478. $C_{18}H_{15}BrN_2O$ requires C, 60.86; H, 4.26; N, 7.87%; MH, 355.04460).

**(4-Bromophenyl)-(1,3,4,9-tetrahydro-β-carbolin-2-yl)-methanone 12k.** White solid. Yield 62%. Mp 217 °C. Rotamers 1 : 1.7 (from the duplicated broad singlet signal ($^1$H) at room temperature at 4.59 and 4.83 ppm). *R*f (ethyl acetate–petroleum ether 50 : 50) 0.45. $^1$H NMR (400 MHz, DMSO, 363 K) $\delta$ 2.79 (2H, t, *J* 5.8), 3.79 (2H, br s), 4.75 (2H, s), 6.99 (1H, td, *J* 7.4 and 0.8), 7.07 (1H, td, *J* 7.6 and 1.2), 7.32 (1H, d, *J* 8.0), 7.40–7.44 (4H, m), 7.67 (1H, dt, *J* 8.8 and 2.4), 10.55 (1H, br s). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 22.03 ($CH_2$), 40.94 ($CH_2$), 45.94 ($CH_2$), 107.05 (Cq), 111.55 (CH), 118.02 (CH), 119.06 (CH), 121.38 (CH), 123.53 (Cq), 126.97 (Cq), 129.47 (2CH), 131.01 (Cq), 132.03 (2CH), 135.84 (Cq), 136.46 (Cq), 169.56 (Cq). No rotamers observed in $^{13}$C at RT. *m/z* (ES$^+$) 356 (M + 2H)$^+$; (ES$^-$) 353 (M − H)$^-$. *m/z* (FAB$^+$) 355 (MH$^+$) (found: C, 60.87; H, 4.20; N, 7.70; M$^+$, 354.03674. $C_{18}H_{15}BrN_2O$ requires C, 60.86; H, 4.26; N, 7.89%; M, 354.03677).

**(2-Methoxyphenyl)-(1,3,4,9-tetrahydro-β-carbolin-2-yl)-methanone 12l.** White solid. Yield 83%. Mp 92–93 °C. Rotamers 1 : 3.2 (from the duplicated broad singlet signals ($^1$H) at 2.62 and 2.78 ppm). *R*f (ethyl acetate–petroleum ether 50 : 50) 0.24. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ (major rotamer) 2.62 (2H, br s), 3.47 (2H, t, *J* 5.4), 3.69 (3H, s), 4.80 (1H, d, *J* 16.7), 4.92 (1H, d, *J* 16.7), 6.75–7.37 (8H, m), 8.89 (1H, br s). $\delta$ (distinct peaks for minor rotamer) 2.78 (2H, br s), 3.58 (3H, s), 4.25–4.31 (2H, m), 8.20 (1H, br s). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ (major rotamer) 21.95 ($CH_2$), 40.56 ($CH_2$), 45.38 ($CH_2$), 55.56 ($CH_3$), 107.62 (Cq), 111.14 (CH), 111.32 (CH), 117.69 (CH), 119.23 (CH), 121.02 (CH), 121.47 (CH), 125.94 (Cq), 126.75 (Cq), 127.83 (CH), 130.29 (Cq), 130.66 (CH), 136.47 (Cq), 155.56 (Cq), 169.20 (Cq). $\delta$ (distinct peaks for minor rotamer) 21.15 ($CH_2$), 44.85 ($CH_2$), 108.92 (Cq), 111.04 (CH), 111.14 (CH), 118.01 (CH), 119.44 (CH), 121.02 (CH), 121.63 (CH), 126.89 (Cq), 128.13 (CH), 129.98 (Cq), 130.74 (CH), 136.30 (Cq), 168.69 (Cq). *m/z* (ES$^+$) 307 (MH$^+$), 613 (2M + H)$^+$; (ES$^-$) 611 (2M − H)$^-$. *m/z* (FAB$^+$) 307 (MH$^+$) (found: C, 74.53; H, 5.91; N, 9.04; MH$^+$, 307.14463. $C_{19}H_{18}N_2O_2$ requires C, 74.49; H, 5.92; N, 9.14%; MH 307.14465).

**(3-Methoxyphenyl)-(1,3,4,9-tetrahydro-β-carbolin-2-yl)-methanone 12m.** Yellow solid. Yield 98%. Mp 75–78 °C. Rotamers 1: 2.7 (from the duplicated broad singlet signal ($^1$H) at 4.48 and 4.81 ppm). *R*f (ethyl acetate–petroleum ether 50 : 50) 0.34. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ (major rotamer) 2.73 (2H, br s), 3.68 (2H, br s), 3.73 (3H, s), 4.81 (2H, br s), 6.80–7.43 (8H, m), 8.60 (1H, br s). $\delta$ (distinct peaks for minor rotamer) 4.48 (2H, br s). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 22.15 ($CH_2$), 41.13 ($CH_2$), 46.06 ($CH_2$), 55.42 ($CH_3$), 107.72 (Cq), 111.19 (CH), 112.21 (CH), 115.94 (CH), 117.83 (CH), 118.97 (CH), 119.52 (CH), 121.77 (CH), 126.79 (Cq), 129.84 (CH), 130.07 (Cq), 136.34 (Cq), 137.32 (Cq), 159.73 (Cq), 171.40 (Cq). No rotamers observed in $^{13}$C at RT. *m/z* (ES$^+$) 307 (MH$^+$), 613 (2M + H)$^+$. *m/z* (FAB$^+$) 307 (MH$^+$) (found: MH$^+$, 307.14460. $C_{19}H_{18}N_2O_2$ requires MH 307.14465).

**(1,3,4,9-Tetrahydro-β-carbolin-2-yl)-*o*-tolylmethanone 12n.** White solid. Yield 83%. Mp 186 °C. Rotamers 1 : 2.8 (from the duplicated singlet signal ($^1$H) at 2.24 and 2.39 ppm). *R*f (ethyl acetate–petroleum ether 50 : 50) 0.32. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ (major rotamer) 2.39 (3H, s), 2.78 (2H, apparent d, *J* 4.8), 3.62 (2H, apparent q, *J* 5.7), 4.93 (1H, d, *J* 16.2), 5.18 (1H, d, *J* 16.2), 7.10–7.57 (8H, m), 8.98 (1H, br s). $\delta$ (distinct peaks for minor rotamer) 2.24 (3H, s), 2.97 (2H, apparent t, estimated *J* 5.1), 4.35 (2H, br s), 8.24 (1H, br s). $^{13}$C NMR (75 MHz, $CDCl_3$)

$\delta$ (major rotamer) 19.13 ($CH_3$), 22.01 ($CH_2$), 40.38 ($CH_2$), 45.22 ($CH_2$), 107.52 (Cq), 111.27 (CH), 117.77 (CH), 119.42 (CH), 121.70 (CH), 125.72 (CH), 126.11 (CH), 126.74 (Cq), 129.16 (CH), 130.08 (Cq), 130.65 (CH), 134.39 (Cq), 136.34 (Cq), 136.42 (Cq), 171.33 (Cq). $\delta$ (distinct peaks for minor rotamer) 18.87 ($CH_3$), 21.14 ($CH_2$), 45.10 ($CH_2$), 109.05 (Cq), 111.06 (CH), 118.15 (CH), 119.62 (CH), 121.90 (CH), 126.86 (Cq), 129.52 (Cq), 130.56 (CH), 134.31 (Cq), 136.51 (Cq), 170.72 (Cq). *m/z* ($FAB^+$) 291 ($MH^+$) (found: C, 78.48; H, 6.35; N, 9.77; $MH^+$, 291.14969. $C_{19}H_{18}N_2O$ requires C, 78.59; H, 6.25; N, 9.65%; MH, 291.14974).

**(1,3,4,9-Tetrahydro-β-carbolin-2-yl)-*m*-tolylmethanone 12o.** White solid. Yield 76%. Mp 170 °C. Rotamers 1 : 3.5 (from the duplicated broad singlet signal ($^1H$) at room temperature at 4.44 and 4.82 ppm). *R*f (ethyl acetate–petroleum ether 50 : 50) 0.40. $^1H$ NMR (400 MHz, 373 K, DMSO) $\delta$ 2.39 (3H, s), 2.79 (2H, t, *J* 5.5), 3.81 (2H, t, *J* 5.5), 4.77 (2H, s), 7.00 (1H, td, *J* 7.5 and 1.1), 7.08 (1H, td, *J* 7.5 and 1.1), 7.24–7.38 (5H, m), 7.42 (1H, d, *J* 8.0), 10.52 (1H, br s). $^{13}C$ NMR (100 MHz, 373 K, DMSO) $\delta$ 21.23 ($CH_2$), 21.76 ($CH_3$), 43.04 ($CH_2$), 44.13 ($CH_2$), 107.51 (Cq), 111.51 (CH), 117.93 (CH), 119.05 (CH), 121.31 (CH), 124.18 (CH), 127.34 (Cq), 127.64 (CH), 128.73 (CH), 130.50 (CH), 131.38 (Cq), 136.89 (Cq), 137.15 (Cq), 138.37 (Cq), 170.66 (Cq). *m/z* ($ES^+$) 291 ($MH^+$), 581 $(2M + H)^+$; ($ES^-$) 289 $(M - H)^-$. *m/z* ($FAB^+$) 291 ($MH^+$) (found: C, 78.78; H, 6.06; N, 9.42; $MH^+$, 291.14968. $C_{19}H_{18}N_2O$ requires C, 78.59; H, 6.25; N, 9.65%; MH, 291.14974).

**(1,3,4,9-Tetrahydro-β-carbolin-2-yl)-*p*-tolylmethanone 12p.** White solid. Yield 90%. Mp 186 °C. *R*f (ethyl acetate–petroleum ether 50 : 50) 0.57. $^1H$ NMR (400 MHz, 373 K, DMSO) $\delta$ 2.39 (3H, s), 2.80 (2H, br t, *J* 5.6), 3.82 (2H, t, *J* 5.6), 4.78 (2H, s), 7.01 (1H, td, *J* 7.3 and 0.8), 7.08 (1H, td, *J* 7.3 and 1.1), 7.28 (2H, br d, *J* 7.6), 7.34 (1H, br d, *J* 8.0), 7.37 (2H, br d, *J* 8.0), 7.42 (1H, br d, *J* 7.6), 10.52 (1H, br s). $^{13}C$ NMR (100 MHz, 373 K, DMSO) $\delta$ 21.23 ($CH_2$), 21.78 ($CH_3$), 43.12 ($CH_2$), 44.17 ($CH_2$), 107.53 (Cq), 111.52 (CH), 117.93 (CH), 119.05 (CH), 121.31 (CH), 127.28 (2CH), 127.36 (Cq), 129.36 (2CH), 131.44 (Cq), 134.24 (Cq), 136.90 (Cq), 139.70 (Cq), 170.69 (Cq). No rotamers observed at RT neither from the $^1H$ nor from the $^{13}C$ NMR. *m/z* ($FAB^+$) 291 ($MH^+$) (found: C, 78.76; H, 6.12; N, 9.63; $MH^+$, 291.14978. $C_{19}H_{18}N_2O$ requires C, 78.59; H, 6.25; N, 9.65%; MH, 291.14974).

**Biphenyl-4-yl-(1,3,4,9-tetrahydro-β-carbolin-2-yl)-methanone 12q.** White solid. Yield 47%. Mp 239 °C. *R*f (ethyl acetate–petroleum ether 50 : 50) 0.63. $^1H$ NMR (400 MHz, 373 K, DMSO) $\delta$ 2.83 (2H, t, *J* 5.6), 3.86 (2H, t, *J* 5.6), 4.80 (2H, s), 6.99 (1H, td, *J* 7.6 and 0.7), 7.07 (1H, td, *J* 7.6 and 1.1), 7.33 (1H, br d, *J* 8.0), 7.42 (2H, br d, *J* 7.2), 7.50 (2H, br t, *J* 6.8), 7.56 (2H, dt, *J* 5.2 and 1.6), 7.72 (2H, dt, *J* 8.0 and 2.0), 7.77 (2H, dt, *J* 6.0 and 2.1), 10.54 (1H, br s). $^{13}C$ NMR (100 MHz, 373 K, DMSO) $\delta$ 21.79 ($CH_2$), 107.51 (Cq), 111.52 (CH), 117.93 (CH), 119.05 (CH), 121.32 (CH), 127.17 (2CH), 127.21 (2CH), 127.32 (Cq), 127.90 (2CH), 128.23 (CH), 129.38 (2CH), 131.35 (Cq), 135.97 (Cq), 136.88 (Cq), 140.03 (Cq), 142.02 (Cq), 170.35 (Cq). Two $CH_2$ are missing from the $^{13}C$ NMR at 373 K. The two signals were observed at 75 MHz, at RT, in DMSO at 41.11 and 45.49 ppm. No rotamers observed at RT neither from the $^1H$ nor from the $^{13}C$ NMR. *m/z* ($ES^+$) 353 ($MH^+$); ($ES^-$) 351 $(M - H)^-$. *m/z* ($FAB^+$) 353 ($MH^+$) (found: C, 81.86; H, 5.62; N, 7.98; $MH^+$, 353.16532. $C_{24}H_{20}N_2O$ requires C, 81.79; H, 5.72; N, 7.95%; MH, 353.16539).

**2-Phenyl-1-(1,3,4,9-tetrahydro-β-carbolin-2-yl)-ethanone 13.** White solid. Yield 63%. Mp 188–190 °C. Rotamers 1 : 4 (from the duplicated singlet signal ($^1H$) at 4.52 and 4.77 ppm). *R*f (ethyl acetate–petroleum ether 50 : 50) 0.36. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ (major rotamer) 2.56 (2H, t, *J* 5.7), 3.70 (2H, t, *J* 5.7), 3.81 (2H, s), 4.77 (2H, s), 6.99 (1H, td, *J* 7.3 and 1.2), 7.06 (1H, td, *J* 7.3 and 1.3), 7.13–7.29 (6H, m), 7.33 (1H, d, *J* 7.5), 8.32 (1H, br s). $\delta$ (distinct peaks for minor rotamer) 2.75 (2H, t, *J* 5.7), 3.75 (2H, s), 3.92 (2H, t, *J* 5.7), 4.52 (2H, s), 7.41 (1H, d, *J* 7.8), 7.80 (1H, br s). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 21.78 ($CH_2$), 40.74 ($CH_2$), 41.36 ($CH_2$), 44.52 ($CH_2$), 107.76 (Cq), 111.07 (CH), 117.77 (CH), 119.48 (CH), 121.71 (CH), 126.94 (CH), 128.71 (2CH), 128.83 (2CH), 130.27 (Cq), 134.96 (Cq), 136.22 (Cq), 170.59 (Cq). One $^{13}C$ quaternary is not observed. No rotamers observed in $^{13}C$ at RT. *m/z* ($ES^+$) 291 ($MH^+$), 581 $(2M + H)^+$. *m/z* ($ES^-$) 289 $(M - H)^-$, 579 $(2M - H)^-$. *m/z* ($FAB^+$) 290 ($M^+$), 291 ($MH^+$) (found: $M^+$, 290.14192. $C_{19}H_{18}N_2O$ requires M, 290.14191).

**1,3,4,9-Tetrahydro-β-carboline-2-carboxylic acid diethylamide 14.** White solid. Yield 91%. Mp 102 °C. *R*f (ethyl acetate–petroleum ether 50 : 50) 0.22. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 1.09 (6H, t, *J* 7.1), 2.77 (2H, t, *J* 5.4), 3.22 (4H, q, *J* 7.1), 3.45 (2H, t, *J* 5.4), 4.37 (2H, s), 7.00 (2H, apparent quintet, estimated *J* 7.3), 7.20 (1H, d, *J* 7.5), 7.37 (1H, d, *J* 7.2), 9.11 (1H, br s). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 13.35 ($2CH_3$), 21.78 ($CH_2$), 42.18 ($2CH_2$), 44.69 ($CH_2$), 47.06 ($CH_2$), 107.94 (Cq), 111.13 (CH), 117.78 (CH), 119.17 (CH), 121.31 (CH), 126.99 (Cq), 131.79 (Cq), 136.42 (Cq), 165.03 (Cq). No rotamers observed at RT neither from the $^1H$ nor from the $^{13}C$ NMR. *m/z* ($FAB^+$) 271 ($M^+$), 272 ($MH^+$) (found: C, 70.93; H, 7.50; N, 15.36; $M^+$, 271.16850. $C_{16}H_{21}N_3O$ requires C, 70.82; H, 7.80; N, 15.49%; M, 271.16846).

**1-Phenyl-2-(1,3,4,9-tetrahydro-β-carbolin-2-yl)-ethanone 15.** To a solution of 1,2,3,4-tetrahydro-β-carboline (0.40 g, 2.32 mmol) in toluene (26 mL) was added a solution of $NaHCO_3$ (0.45 g, 5.39 mmol) and $Na_2SO_3$ (0.23 g, 1.81 mmol) in water (8.0 mL). The mixture was stirred under $N_2$ atmosphere and 2-bromoacetophenone (0.23 g, 1.16 mmol) was added. The stirring was maintained for 4 h at room temperature. The crude mixture was evaporated under reduced pressure and the resulting crude product was purified directly by column chromatography on silica gel. Elution was made successively with: ethyl acetate–cyclohexane 50 : 50 and ethyl acetate to give the title compound **19** as an orange solid (0.21 g, 61%). Mp 152–153 °C. *R*f (ethyl acetate–petroleum ether 50 : 50) 0.37. $^1H$ NMR (300 MHz, $CDCl_3$) 2.78 (2H, t, *J* 5.7), 2.98 (2H, t, *J* 5.7), 3.78 (2H, s), 3.97 (2H, s), 7.01 (1H, t, *J* 3.2), 7.01 (1H, ddd, *J* 10.5 and 6.9 and 1.8), 7.15–7.19 (1H, m), 7.34–7.40 (3H, m), 7.48 (1H, tt, *J* 7.4 and 1.7), 7.90–7.93 (3H, m). $^{13}C$ NMR (75 MHz, $CDCl_3$) 20.64 ($CH_2$), 50.08 ($CH_2$), 51.13 ($CH_2$), 62.70 ($CH_2$), 107.95 (Cq), 110.87 (CH), 117.92 (CH), 119.34 (CH), 121.41 (CH), 127.17 (Cq), 128.19 (2CH), 128.63 (2CH), 131.25 (Cq), 133.39 (CH), 135.91 (Cq), 136.06 (Cq), 196.80 (Cq). *m/z* ($ES^+$) 291 ($MH^+$), 581 $(2M + H)^+$. *m/z* ($ES^-$) 289 $(M - H)^-$, 579 $(2M - H)^-$. *m/z* ($FAB^+$) 291 ($MH^+$) (found: C, 78.67; H, 6.30; N, 9.71; $M^+$, 291.14975. $C_{19}H_{18}N_2O$ requires C, 78.59; H, 6.25; N, 9.65%; M, 291.14974).

**2-(2-Oxo-2-phenylethyl)-2,3,4,9-tetrahydro-1*H*-β-carbolin-2-ium chloride 16.** To a solution of **15** (0.20 g, 0.07 mmol) in a minimum of dichloromethane was flushed HCl gas. After around 3 min the mixture was evaporated under reduced pressure to give the title compound **16** (0.21 g, 95%) as a yellow solid, which was dried under vacuum for a few days. Mp 158–159 °C. $^{1}$H NMR (300 MHz, DMSO) 3.17 (2H, t, *J* 5.3), 3.74 (2H, br s), 4.70 (2H, br s), 5.31 (2H, s), 7.10 (1H, td, *J* 7.6 and 1.0), 7.18 (1H, td, *J* 7.6 and 1.0), 7.44 (1H, d, *J* 7.8), 7.55 (1H, d, *J* 7.5), 7.67 (2H, apparent t, estimated *J* 7.7), 7.82 (1H, t, *J* 7.4), 8.10 (2H, apparent d, estimated *J* 7.2), 10.98 (1H, br s), 11.23 (1H, br s). $^{13}$C NMR (75 MHz, DMSO) 18.29 ($CH_2$), 49.79 ($CH_2$), 51.67 ($CH_2$), 60.00 ($CH_2$), 105.43 (Cq), 111.87 (CH), 118.47 (CH), 119.47 (CH), 122.18 (CH), 126.21 (Cq), 126.32 (Cq), 128.70 (2CH), 129.49 (2CH), 134.26 (Cq), 135.20 (CH), 136.74 (Cq), 192.19 (Cq). *m/z* ($ES^+$) 291 $(M - Cl)^+$, 582 $(2(M - Cl))^+$. *m/z* ($ES^-$) 325 $(M - H)^-$, 615 $(2(M - H) - Cl)^-$. *m/z* ($FAB^+$) 291 $(M - Cl)^+$ (found: $(M - Cl)^+$, 291.14970. $C_{19}H_{19}ClN_2O$ requires (M − Cl), 291.14974).

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# Regioselective photo-oxidation of 1-benzyl-4,9-dihydro-3*H*-β-carbolines†

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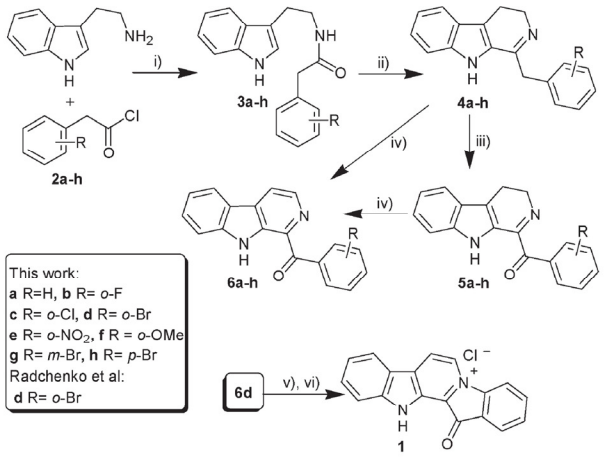
**The synthesis of a series of β-carboline-based analogues of the natural product fascaplysin is presented; the compounds were produced using a novel photo-oxidation reaction of 1-benzyl-4,9-dihydro-3*H*-β-carbolines as the key step.**

The natural product fascaplysin **1**, originally isolated from the Fijian sponge *Fascaplynosis* Bergquist sp,[1] inhibits the growth of several microbes including *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Saccharomyces cerevisiae*, and suppresses the proliferation of mouse leukaemia cells L-1210 with $ED_{50}$ = 0.2 μm $mL^{-1}$. Fascaplysin **1** has also been reported to specifically inhibit CDK4 ($IC_{50}$ = 0.55 μM) causing $G_1$ arrest in U2-OS and HCT-116 tumour cells, as well as normal (MRC-5) cells.[2] Fascaplysin **1** itself cannot be used as an anticancer drug because of its high toxicity, thought to be due to the fact that its flat structure can act as a DNA intercalator.[3] We have recently investigated the design, synthesis and biological evaluation of non-toxic (non-planar) analogues of fascaplysin **1** as potential specific CDK4/Cyclin D1 inhibitors.[4]

As part of this programme of research we planned to use the Radchenko approach to fascaplysin **1** (Scheme 1, R = *o*-Br)[5] for the synthesis of derivatives of the natural product.[4]




**Scheme 1** Synthetic plan based on the Radchenko approach to fascaplysin **1** (R = *o*-Br). *Reagents and conditions*: i) DCM, NaOH, 0 °C to r.t., 1 h. ii) $POCl_3$, toluene, reflux, 30 min. iii) $MnO_2$, $CH_2Cl_2$, r.t., 3 h. iv. $MnO_2$, $CH_2Cl_2$, reflux, 3 h. v. 220 °C, 20 min. vi) HCl/MeOH.

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† Electronic supplementary information (ESI) available: Physical data for compounds **5a–h** and **6a–h**. See DOI: 10.1039/b604922b

In this method, the acetamide derivative **3d** is subjected to a Bischler–Napieralski reaction with $POCl_3$ in refluxing toluene; the 1-benzyl-β-carboline **4d** obtained is oxidized by $MnO_2$ in $CH_2Cl_2$ at room temperature or at reflux to afford, respectively, compounds **5d** and **6d**. Heating of compound **6d** at 220 °C for 20 minutes, followed by anion interchange with dry HCl in MeOH, leads to fascaplysin **1** with an overall 44% yield from tryptamine.

In a first attempt at reproducing the original sequence it was found that after cyclisation of the acetamide **3d**, basic work-up and column chromatography, the expected compound **4d** was isolated (58% yield) accompanied by a small amount of the oxidized product **6d** (4%).[6] The $^1$H-NMR of compound **4d** showed two different sets of signals in a ratio of about 10 : 1; this fact was attributed to imine–enamine tautomerism (Fig. 1).

The aliphatic region in the $^1$H-NMR spectrum of compound **4d** (Fig. 1) clearly shows the corresponding signals of the imino compound [$\delta_H$ 2.89 (t, 2H, *J* 8.5 Hz, 3-C$H_2$), 3.95 (t, 2H, *J* 8.5 Hz, 4-C$H_2$), 4.14 (s, 2H, partially exchangeable with $D_2O$, -C$H_2$-Ar)] and the peaks of the enamino tautomer [$\delta_H$ 2.97 (t, 2H, *J* 6.1 Hz, 3-C$H_2$), 3.41 (dt, 2H, *J* 2 and 6.1 Hz), 4.73 (br s, 1H, $D_2O$ exch, 2-N*H*), 5.69 (s, 1H, partially exchangeable with $D_2O$, -C*H*-Ph)]. In the more complex aromatic region (not shown), singlet signals for the indole NHs of the imino tautomer [$\delta_H$ 8.11 (br s, $D_2O$ exch)] and of the enamino partner [$\delta_H$ 8.29 (br s, $D_2O$ exch)] are observed. The $D_2O$ partial exchange of the benzylic protons (being a faster process than the auto-oxidation) confirmed the suspicion of an imine–enamine tautomerism in solution for compound **4d**. Another fact that supports this hypothesis is that the UV/Vis spectrum of the compound shows an absorption maximum near 355 nm [UV/Vis (toluene) 348 nm (log ε 1.062)], which is the typical value for the absorption of the α-aminostilbene

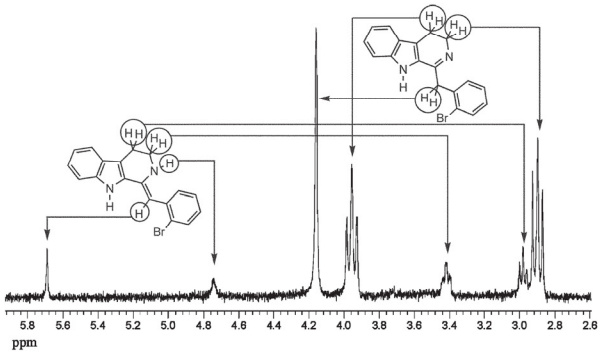



**Fig. 1** Aliphatic region of the $^1$H-NMR (400 MHz; $CDCl_3$; $Me_4Si$) of isolated compound **4d**. Aromatic region not shown for clarity.

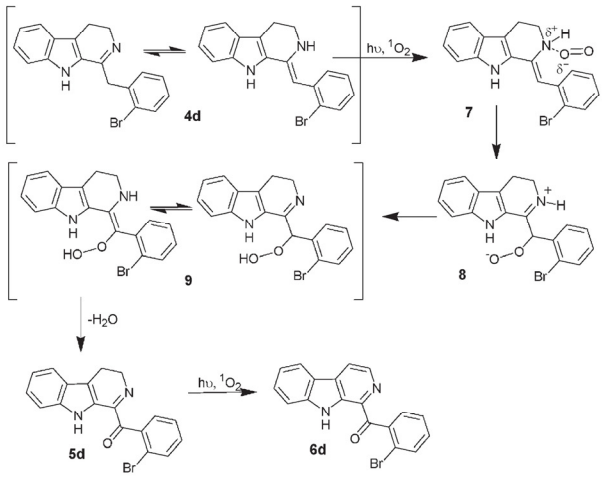
chromophore.[7] Moreover, **4d** was found to be unstable in solution; when the $^1$H-NMR of the initial sample was recorded again two weeks later using the same sample, the spectrum showed substantial amounts of the oxidation products **5d** and **6d**.

These observations were in agreement with previous results reported by Martin *et al.*[7] who found that related 1-benzyl-3,4-dihydroisoquinolines were converted into the corresponding 1-benzoyl derivatives by means of a self-sensitized photo-oxygenation of the α-aminostilbene moiety.

The proposed mechanism of the photo-oxidation is outlined for compound **4d** (Scheme 2). A transfer of charge from the enamine-tautomer nitrogen of **4d** to singlet oxygen would form a *charge-transfer* complex **7**, which could collapse to the zwitterionic peroxide **8**, and then, by intramolecular proton transfer, to the tautomeric peroxides **9**. This intermediate could undergo dehydration to the iminoketone **5d**.[8] Finally, **5d** could undergo further photodehydrogenation to the aromatized β-carboline **6d**. As related aromatizations of dihydro-β-carbolines sensitized by methylene blue have been reported,[9] in our case the most probable explanation is that compound **5d** acts as a self-sensitizer.[10]

In an initial experiment, we tried to induce the auto-oxidation by irradiating an oxygen-bubbled solution of the purified compound **4d** in toluene with a 500 W halogen lamp (290–300 nm frequency cut off). After 16 hours at reflux, TLC analysis showed complete conversion of the initial material to a single product. Recrystallisation of the crude product yielded the compound **6d** in almost pure form (84% yield). Qualitative determination of the relative amounts of compounds **4d/5d** and **6d** during the reaction was achieved by means of $^1$H-NMR (Fig. 2).

After 4 hours most of the starting material had been consumed. From this point the reaction comprised the transformation of the iminoketone **5d** into the fully aromatized compound **6d**. Taking these observations into account, a slight change in the reaction conditions, avoiding reflux by changing the distance between the lamp and the reaction flask (d = 5 cm → reflux, d = 25 cm → measured temperature 30 °C), allows us to obtain **5d** regioselectively in almost quantitative yield and purity after 16 hours of reaction. As expected, when **5d** was irradiated at reflux with $O_2$ bubbling (d = 5 cm) for 16 hours, compound **6d** was obtained after recrystallisation of the crude product with a 90% yield.




**Scheme 2** Proposed mechanism for the self-sensitized photo-oxidation of **4d**.

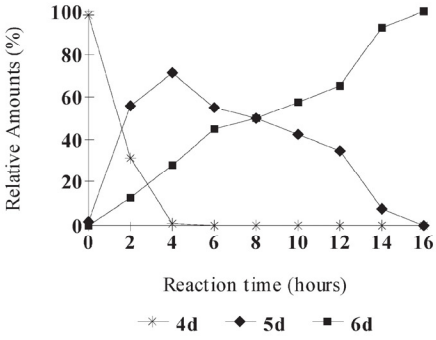
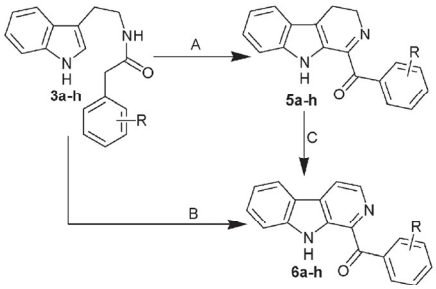



**Fig. 2** Relative amounts of compounds **4d/5d** and **6d** during the irradiation of **4d** in toluene at reflux. The relative ratios were directly measured from the $^1$H-NMR spectra; the peaks chosen for this purpose were $\delta_H$ = 4.14 ppm (-C*H*$_2$-Ar, **4d**), $\delta_H$ = 9.48 ppm (indolyl N*H*, **5d**) and $\delta_H$ = 10.43 ppm (indolyl N*H*, **6d**).

The synthetic scope of this mild, green and selective oxidation for the synthesis of compounds **5a–h** and **6a–h** was investigated; the results are summarized in Table 1.

**Table 1** Results for the conversion of **3a–h** into **5a–h/6a–h** and **5a–h** into **6a–h**[a]




| Entry | R | Method | [b]Time/h | [c]Product | [d]Yield (%) |
|---|---|---|---|---|---|
| 1 | H | A | 16 | **5a** | 50 |
| 2 | *o*-F | A | 16 | **5b** | 57 |
| 3 | *o*-Cl | A | 16 | **5c** | 59 |
| 4 | *o*-Br | A | 16 | **5d** | 48 |
| 5 | *o*-$NO_2$ | A | 16 | — | decomposition |
| 6 | *o*-OMe | A | 16 | **5f/6f**: 1/0.25 | 31/10 |
| 7 | *m*-Br | A | 16 | **5g** | 46 |
| 8 | *p*-Br | A | 16 | **5h** | 44 |
| 9 | *o*-F | B | 16 | **6b** | 11 |
| 10 | *o*-Cl | B | 32 | **6c** | 29 |
| 11 | *o*-Br | B | 16 | **6d** | 49 |
| 12 | *o*-OMe | B | 16 | **6f** | 55 |
| 13 | *p*-Br | B | 72 | **6h** | 44 |
| 14 | H | C | 48 | **6a** | 99 |
| 15 | *o*-F | C | 16 | **6b** | 62 |
| 16 | *o*-Cl | C | 32 | **6c** | 99 |
| 17 | *o*-Br | C | 16 | **6d** | 99 |
| 18 | *m*-Br | C | 48 | **6g** | 99 |

[a] *Reagents and conditions* (see footnote[11] for experimental details): A: i) $POCl_3$, toluene, $N_2$, reflux. ii) basic work-up. iii) *hν*, $O_2$, toluene, 30 °C; B: i) $POCl_3$, toluene, $N_2$, reflux. ii) basic work-up. iii) *hν*, $O_2$, toluene, reflux; C: i) *hν*, $O_2$, toluene, reflux. [b] Reactions monitored by $^1$H-NMR. [c] Isolated compounds after purification of crude reaction mixture. [d] Yields of the purified products from the tryptamine derivatives **3** (entries 1–13) or ketoimines **5** (entries 14–18).

To circumvent the intrinsic instability of compounds **4a–h**, purification after cyclisation of the corresponding acetamide derivatives **3a–h** was avoided, so the crude reaction product after basic work-up of the Bischler–Napieralski reaction was irradiated under the conditions explained in Table 1.

Using the method A (entries 1–8), regioselectivity in the oxidation was achieved in most of the cases, yielding the dihydro-β-carbolines **5a–h** with satisfactory yields from the corresponding tryptamine derivatives **3a–h**. When d = 5 cm [method B, entries 9–13] reflux of the toluene solution occurred and, consequently, the fully aromatic β-carbolines **6a–h** were isolated with acceptable yields from **3a–h**. On other occasions the compounds **6a–h** were obtained in good yields by irradiation (d = 5 cm) of the isolated ketoimines **5a–h** [method C, entries 14–18].

Finally, a one-pot sequence for the synthesis of the target compounds was also explored. Tryptamine was reacted with 2-bromophenylacetic chloride **2d** (1.1 eq.) in toluene at reflux for 15 minutes; $POCl_3$ (2.5 eq.) was then added to the mixture. Once TLC analysis of a worked-up aliquot showed complete reaction, the crude mixture was irradiated with the 500 W halogen lamp and oxygen bubbled throughout the solution. After 24 hours at reflux, the only isolated product was **4d** in a 48% yield from tryptamine. One probable explanation for the inhibition of the oxidation is that the product of the Bischler–Napieralski reaction before the basic work-up should be the hydrochloride of compound **4d**. In such a situation the imine–enamine tautomerism is inhibited, so the charge transfer between the enamine NH and singlet oxygen required for the oxidation does not occur.

In conclusion, we have investigated the spontaneous photo-oxidation of the 1-(2-bromobenzyl)-4,9-dihydro-3*H*-β-carboline; this has led to the development of a mild, regioselective and practical protocol for the preparation of dihydro-β-carbolines of type **5** and β-carbolines of type **6** from tryptamine derivatives **4** by a sequential cyclisation-induced photo-oxidation of the non-isolated 1-benzyl-4,9-dihydro-3*H*-β-carboline derivatives.

A separate investigation into the biological activity of compounds **5/6**, and their optimization as potential inhibitors of CDK4/Cyclin D1 is currently underway.

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## Notes and references


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8 We propose that the photo-oxidation proceeds *via* a charge-transfer complex. The precedent for this comes from the work of Martins *et al.*[7] who proposed a similar mechanism in a related isoquinoline case. The alternative concerted ene mechanism was discounted on the basis of structure activity studies.

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10 That is in agreement with the known fact that the natural product xestomanzamine B was gradually converted (at 21 °C, for 20 days) presumably *via* air-oxidation to xestomanzamine A. M. Kobayashi, C. Yin-Ju, S. Aoki, Y. In, T. Ishida and I. Kitawaga, *Tetrahedron*, 1995, **51**, 3727.

11 Examples of general procedures A, B and C (Table 1): **Method A: synthesis of 1-(2-bromobenzoyl)-3,4-dihydro-β-carboline 5d from 2-(2-bromophenyl)-*N*-[2-(1*H*-indol-3-yl)ethyl]acetamide 3d**. The acetamide derivative **3d** (1 mmol, 357 mg) was heated in toluene until complete dissolution was achieved. $POCl_3$ (10 mmol, 0.93 mL) was added and the resulting mixture refluxed for 1 hour until TLC analysis showed completion. Afterwards, toluene was removed and the residue dissolved in 20 mL of a 2 : 1 mixture of $CH_2Cl_2$/$NaHCO_3$. The biphasic mixture was then cooled and basified with aqueous $NH_3$ to pH 9. The organic phase was separated and the aqueous layer extracted with $CH_2Cl_2$ (2 × 25 mL). Then, the combined organic phases were dried over sodium sulfate and the solvent evaporated. The crude product **4d** obtained was suspended in toluene with vigorous stirring; oxygen was bubbled through the solution and the mixture was irradiated with a 500 W halogen lamp located at 25 cm from the reaction flask (measured temperature of the suspension *ca.* 30° C). The reaction was monitored by $^1$H-NMR until completion. Then, the toluene was evaporated under reduced pressure and the crude product obtained (410 mg) filtrated over a short pad of $SiO_2$ (2 g of $SiO_2$) eluting with $CH_2Cl_2$ to give **5d** as an orange solid (170 mg, 48%); mp 136 137 °C (from EtOH); Found: C, 61.14; H, 3.72; N, 7.88%; $C_{18}H_{13}BrN_2O$ requires: C, 61.21; H, 3.71; N, 7.93%; $\nu_{max}$/cm$^{-1}$ 3453, 1660, 1585, 1539, 1437, 1295, 1222, 1143, 732; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ 3.0 (2H, t, *J* 8.9 Hz), 4.13 (2H, t, *J* 8.9 Hz), 7.14 7.19 (1H, m), 7.30 7.53 (4H, m), 7.61 (1H, d, *J* 1.2 Hz), 7.61 (1H, d, *J* 1.2 Hz), 7.64 (1H, d, *J* 1.2 Hz) and 9.48 (1H, br s, $D_2O$ exch, NH) ppm; $^{13}$C-NMR (75 MHz, $CDCl_3$) $\delta$ 18.8 ($CH_2$), 49.7 ($CH_2$), 112.3 (CH), 118.2 (Cq), 120.0 (CH), 120.3 (Cq), 120.4 (CH), 124.7 (Cq), 125.2 (CH), 126.1 (Cq), 127.0 (CH), 129.8 (CH), 131.9 (CH), 133.1 (CH), 137.1 (Cq), 139.1 (Cq), 155.5 (Cq) and 196.5 (CO) ppm; *m/z* (ES$^+$) 353 (MH$^+$); *m/z* (FAB$^+$) 353 (MH$^+$) (found: MH$^+$, 353.02897; $C_{18}H_{14}BrN_2O$ requires 353). **Method B: synthesis of 1-(2-bromobenzoyl)-β-carboline 6d from 2-(2-bromophenyl)-*N*-[2-(1*H*-indol-3-yl)ethyl]acetamide 3d**. The crude product **4d** obtained from the acetamide derivative **3d** (1 mmol, 357 mg) as described in method A was irradiated following the protocol described in method A with the 500 W halogen lamp located at 5 cm from the reaction flask so reflux was achieved. The mixture was refluxed until completion (monitored by $^1$H-NMR). The crude product (430 mg) obtained after evaporation of the toluene was purified as in method A yielding **6d** (172 mg, 49%) as an orange solid; mp 215 216 °C (from EtOH/$H_2O$); Found: C, 61.41; H, 3.09; N 7.92%; $C_{18}H_{11}N_2OBr$ requires: C, 61.56; H, 3.16; N, 7.98%; $\nu_{max}$/cm$^{-1}$ 3447, 1654, 1581, 1186, 1174, 950, 792, 775, 746; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ 7.35 7.42 (2H, m), 7.47 (1H, dt, *J* 1.3 and 7.3 Hz), 7.56 (1H, dd, *J* 1.7 and 7.3 Hz), 7.63 (1H, d, *J* 0.9 Hz), 7.64 (1H, q, *J* 0.9 Hz), 7.69 (1H, dd, *J* 1.0 and 8.0 Hz), 8.16 8.20 (2H, m), 8.56 (1H, d, *J* 5 Hz) and 10.43 (1H, br s, $D_2O$ exch, NH) ppm; $^{13}$C-NMR (75 MHz, $CDCl_3$) $\delta$ 112.1 (CH), 119.2 (CH), 120.1 (Cq), 120.7 (Cq), 121.0 (CH), 121.9 (CH), 126.9 (CH), 129.5 (CH), 129.8 (CH), 131.2 (CH), 131.9 (Cq), 133.1 (CH), 135.2 (Cq), 136.9 (Cq), 138.8 (CH), 140.4 (Cq), 141.2 (Cq) and 198.2 (CO) ppm; *m/z* (ES$^+$) 351 (MH$^+$); *m/z* (FAB$^+$) 351 (MH$^+$) (found: MH$^+$, 351.01321; $C_{18}H_{12}BrN_2O$ requires 351). **Method C: synthesis of 1-(2-bromobenzoyl)-β-carboline 6d from 1-(2-bromobenzoyl)-3,4-dihydro-β-carboline 5d**. Compound **5d** (176 mg, 0.5 mmol) obtained as described in method A was irradiated with the 500 W halogen lamp as in method B. The reaction was monitored by $^1$H-NMR until completion and the resulting crude product obtained was purified as described to yield **6d** (350 mg, 99%), which displayed spectroscopic features identical to those described above.


 

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# CA224, a non-planar analogue of fascaplysin, inhibits Cdk4 but not Cdk2 and arrests cells at $G_0/G_1$ inhibiting pRB phosphorylation

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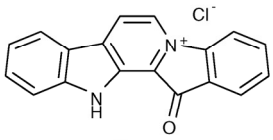
**Abstract**—Tryptamine derivatives, non-planar and potentially less toxic analogues of the anti-cancer agent fascaplysin, have been synthesised. They specifically inhibit Cdk4-D1 vis a vis Cdk2-A but, unlike fascaplysin, do not bind or intercalate DNA. CA224 is the most potent compound identified (Cdk4-D1 $IC_{50} \sim 5.5\ \mu M$). As would be expected of a Cdk4 inhibitor that does not inhibit Cdk2, it maintains a $G_0/G_1$ block in synchronised cancer cells and inhibits Cdk4-specific phosphorylation of the retinoblastoma protein.


Fascaplysin (I), originally isolated from the Fijian sponge *Fascaplysinopsis* Bergquist sp.,[1] has been recently shown to block growth of cancer cells presumably through inhibition of cyclin-dependent kinase 4 (Cdk4), an early cell cycle enzyme misregulated in most cancers.[2]




(I)

Inhibition of Cdk4 concomitantly arrests cancer cells at the $G_0/G_1$ phase of the cell cycle and prevents phosphorylation of the retinoblastoma protein, pRB.[2] Hypophosphorylated pRB bound to the E2F family of transcription factors actively represses E2F-mediated gene transcription. When Cdk4 phosphorylates pRB, the hyperphosphorylated form dissociates so that E2Fs are free to induce a set of proteins that allow entry into the DNA synthesis (S) phase of the cell cycle. Thus, Cdk4 inhibition prevents entry of a cell into the S phase and thereby blocks its ability to multiply.[3]

Cdk4 is activated by D-type cyclins and negatively regulated by inhibitory proteins, one of them being $p16^{INK4A}$ (often referred to as p16). In most cancers, it is either that cyclin D1 is overproduced or that p16 is inactive. Sometimes, hyperactivating mutations in the catalytic part, Cdk4, are seen. Recent studies have provided compelling evidence that misregulation of Cdk4 activity can cause cancer and suggest that Cdk4-specific inhibition would be important for cancer therapy.[4–6] In contrast, it seems that Cdk2 which has often been proposed as a cancer target may not be at all suitable.[7] Hence, there is a great need for finding Cdk4-specific inhibitors that do not inhibit Cdk2.

Fascaplysin is one of the very few known Cdk4-specific inhibitors[8] that has shown efficacy in the NCI panel of 60 cancer cell lines[9] and it is being currently considered for therapeutic trials.[10] However, fascaplysin is quite toxic to normal cells, most likely because it binds and intercalates DNA.[11]

In order to identify non-toxic analogues of fascaplysin, we aimed to separate fascaplysin's ability to inhibit Cdk4 from its property of binding and intercalating

*Keywords*: Cyclin-dependent kinases; $G_0/G_1$ arrest; pRB; Cell cycle; Cell growth inhibition; DNA intercalation.
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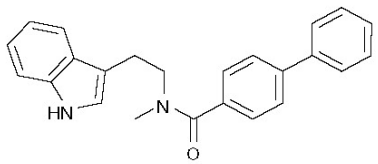
DNA. Therefore, we decided to synthesise three classes of non-planar tryptamine derivatives that would be structurally analogous to fascaplysin but would avoid intercalation with DNA.[12–14] Since the 3-D structure of Cdk4 is yet to be determined, the Cdk4 ATP-binding site was modelled on the basis of the known crystal structures of the homologous Cdk2 and Cdk6 enzymes.[12–14] Using this homology model, in silico studies suggested that inhibition of Cdk4 activity by fascaplysin arises from binding to the same amino acid residues to which ATP binds.[12–14] Non-planar compounds were then sought that maintained most of the key interactions thought to occur between fascaplysin and Cdk4. Preliminary SAR studies using Cdk4 and Cdk2 in vitro enzyme assays have corroborated that the predicted molecules inhibit Cdk4 and not Cdk2.[12–14] Here we describe more extensive studies on one class of tryptamine derivatives which prove that these fascaplysin analogues not only inhibit Cdk4 specifically but also fail to bind or intercalate DNA. The most potent compound in this series, CA224, was chosen to confirm that it also acts as a Cdk4 inhibitor in cancer cells.

*Inhibition of Cdk4/Cdk2 kinases.* The chemical syntheses of these fascaplysin analogues (Table 1) have been described earlier.[14] They were initially screened in the Cdk4 and Cdk2 enzyme assays based on chemiluminescence detection rather than radioactivity. The kinase assays measure the $IC_{50}$s of the compounds (i.e., concentrations at which 50% enzyme activity is inhibited) through the depletion in ATP concentrations occurring as a result of phosphorylation by Cdks of GST-pRB152 (a substrate for both Cdk4-cyclin D1 and Cdk2-cyclin A).[2,14] The $IC_{50}$s of all compounds in this series in the Cdk4-cyclin D1 and Cdk2-cyclin A assays are shown in Table 1.

CA224 was identified as the most active compound that inhibits Cdk4-cyclin D1 with an $IC_{50}$ of approximately 5.5 μM (Table 1). CA224 showed approximately 100-fold greater specificity towards Cdk4-cyclin D1 than the Cdk2-cyclin A enzyme. All results in Table 1 show means and standard deviations from three independent experiments.




CA224

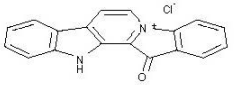
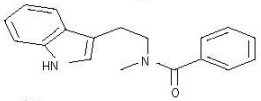
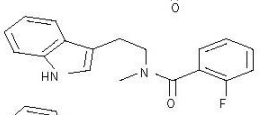
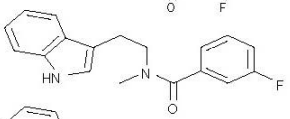
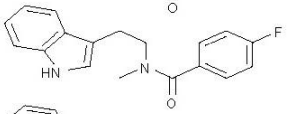
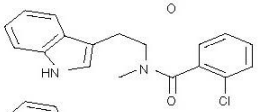
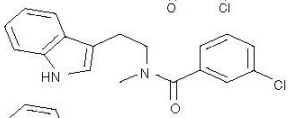
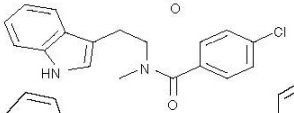
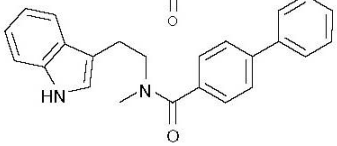
*Inhibition of cell proliferation.* All the non-planar analogues of fascaplysin were tested for their ability to inhibit cancer cell growth. The four cancer cell lines that were used are the non-small cell lung carcinoma lines A549 ($pRB^+$, $p53^+$), Calu-1 ($pRB^+$, p53-null), colon carcinoma LS174T ($pRB^+$, $p53^+$) and the prostate carcinoma PC3 ($pRB^+$, p53-null) lines. All lines were chosen for their relative resistance to chemotherapeutic agents. The genotypes within brackets indicate the status of the tumour suppressor proteins pRB and p53. The cell lines were maintained at 37 °C in 5% $CO_2$ in RPMI-1640 medium, supplemented with 10% fetal calf serum and 100 μg/ml Normocin™.

Five thousand to 10,000 cells were seeded in 96-well plates in 180 μl of complete growth medium and incubated for 24 h. Ten millimolar stock solutions of drug compounds in DMSO were serially diluted in medium without serum. Twenty microlitres of 10× concentrated compounds was added into the wells in triplicate, while equivalent amount of DMSO was added to the control wells. The contents of the wells were mixed gently and incubated further for 48 h. After exposure to compound, 50 μl of 2 mg/ml MTT (Sigma) was added and the plates were incubated for 2–3 h at 37 °C in the dark. The medium containing MTT was removed, the blue-coloured formazan that formed was dissolved in 150 μl DMSO per well. The absorbance was measured at 540 nm. The $IC_{50}$s of the compounds were calculated as the concentrations at which 50% of cell growth was inhibited as compared to the control wells which did not contain any drug. The results are depicted in Table 1 and indicate that CA224 is also most potent at the cellular level in inhibiting the growth of cancer cells at low micromolar concentrations (3–12 μM). The tryptamine derivatives CA225 and CA223 which are moderately active in the Cdk4 assay inhibit cell growth in the range of 10–20 and 20–40 μM, respectively.

*Displacement of ethidium bromide from DNA.* The DNA-binding affinities of fascaplysin and the new tryptamine derivatives, structurally analogous to fascaplysin, were then investigated using an ethidium bromide fluorescence quenching assay. It measures a compound's ability to displace the DNA intercalating agent ethidium bromide from closed circular plasmid DNA.[15,16] Ten microlitres of 10× concentrated serially diluted stock solutions of compounds (dissolved in DMSO) was added to 90 μl of a reaction mix that contains 6 μg of purified pBlue-Script DNA (Stratagene) and 1.3 μM ethidium bromide in a buffer (20 mM NaCl, 2 mM Hepes and 10 μM EDTA, pH 7.4). Equivalent amounts of DMSO were added to the vehicle controls. The decrease in fluorescence is monitored ($\lambda_{excit}$ = 260 nM, $\lambda_{emiss}$ = 600 nM) and recorded after a 1 min equilibration time. Fascaplysin and actinomycin D, which are known to intercalate double-stranded DNA molecules, were used as controls in the assay.[11] The results show that none of the analogues displace bound ethidium bromide from double-stranded DNA (Table 1). Results of the representative compound CA224 are shown graphically in Fig. 1. As expected, both actinomycin D and fascaplysin dislodge ethidium bromide bound to DNA ($IC_{50}$ = 35 and 5 μM, respectively), but CA224 is incapable of doing so; less than 5% displacement of bound ethidium bromide is observed even at 100 μM concentration of CA224.

*Inhibition of DNA unwinding initiated by topoisomerase I.* The ability of fascaplysin and its analogues to intercalate plasmid DNA was determined by a topoisomerase I

**Table 1.** Activity of fascaplysin analogues in different in vitro assays and their chemical structures ($IC_{50}$ values are in μM)

| Compound | Structures | Cdk4-cyclin D1 | Cdk2-cyclin A | EtBr displacement from DNA | Cell growth inhibition | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | LS174T | A549 | Calu-1 | PC3 |
| Fascaplysin |  | 0.41 ± 0.04 | >250 | 5 ± 0.4 | 0.88 ± 0.04 | 0.69 ± 0.03 | 1.3 ± 0.1 | 0.92 ± 0.06 |
| CA192 |  | 88 ± 6.1 | 1523 ± 27 | Does not displace | 83 ± 3 | 95 ± 2.5 | 104 ± 5 | 86 ± 3.5 |
| CA218 |  | 103 ± 8.5 | 1230 ± 28 | Does not displace | 110 ± 4.5 | 103 ± 4 | 145 ± 5 | 108 ± 7 |
| CA219 |  | 88 ± 9 | 765 ± 26 | Does not displace | 97 ± 4 | 106 ± 2.5 | 136 ± 3.4 | 98 ± 5.5 |
| CA220 |  | 59 ± 7 | 850 ± 34 | Does not displace | 87 ± 2 | 102 ± 6 | 141 ± 4.5 | 94 ± 3.6 |
| CA221 |  | 46 ± 3.5 | 784 ± 20 | Does not displace | 99 ± 4 | 96 ± 7 | 147 ± 8.5 | 92 ± 6.2 |
| CA222 |  | 109 ± 7.5 | 1120 ± 38 | Does not displace | 118 ± 5 | 95 ± 4.2 | 123 ± 5.9 | 94 ± 4.5 |
| CA223 |  | 38 ± 6 | 731 ± 26 | Does not displace | 42 ± 2.5 | 27 ± 2.5 | 78 ± 3 | 47 ± 3 |
| CA224 |  | 6.2 ± 0.9 | 521 ± 11.5 | Does not displace | 3.5 ± 0.9 | 3.5 ± 0.6 | 11.5 ± 2.5 | 6.2 ± 1.1 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| CA225 |  | 49 ± 6.5 | 658 ± 23 | Does not displace | 18 ± 1 | 12 ± 1.8 | 52 ± 3.5 | 15 ± 1.5 |
| CA226 |  | 79 ± 9 | 849 ± 30 | Does not displace | 106 ± 4 | 103 ± 9 | 146 ± 8 | 89 ± 5 |
| CA228 |  | 81 ± 4 | 938 ± 25 | Does not displace | 109 ± 6.4 | 95 ± 5.7 | 139 ± 7 | 85 ± 5 |
| CA229 |  | 113 ± 7.5 | 1125 ± 23 | Does not displace | 105 ± 6.5 | 110 ± 8 | 141 ± 11 | 92 ± 4 |
| CA230 |  | 78 ± 8 | 830 ± 31 | Does not displace | 88 ± 3.8 | 96 ± 5.7 | 136 ± 9.5 | 81 ± 3.2 |
| CA233 |  | 63 ± 6 | 790 ± 27 | Does not displace | 82 ± 4.3 | 92 ± 2.5 | 103 ± 4.8 | 55 ± 3 |
| CA234 |  | 95 ± 7 | 584 ± 24 | Does not displace | 76 ± 4 | 95 ± 6.2 | 122 ± 4.4 | 59 ± 4 |
| CA237 |  | 74 ± 6.5 | 635 ± 21 | Does not displace | 89 ± 3.8 | 91 ± 4.6 | 144 ± 7 | 66 ± 3.5 |
| CA238 |  | 37 ± 5 | 580 ± 18 | Does not displace | 52 ± 2.9 | 55 ± 3.2 | 64 ± 4.1 | 36 ± 2.2 |

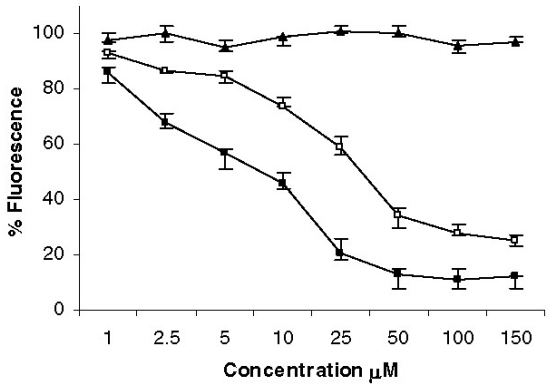



**Figure 1.** CA224, like all tryptamine derivatives structurally analogous to fascaplysin, does not displace ethidium bromide from the minor groove of double-stranded DNA. The assay was performed with increasing concentrations of fascaplysin (filled squares), actinomycin D (unfilled squares) and CA224 (filled triangles) to see if the compounds could displace ethidium bromide from the minor groove of double-stranded DNA. The results represent means and standard deviations from three independent experiments.

unwinding assay.[17] Reaction mixtures contained 5 nM supercoiled pBlueScript (Stratagene) plasmid DNA and 10 units of topoisomerase I (Invitrogen) that allows relaxation of supercoiled DNA. Assays were performed in the presence or absence of compounds in 40 μl of DNA unwinding buffer (50 mM Tris–HCl, pH 7.5, 50 mM KCl, 10 mM $MgCl_2$, 0.5 mM dithiothreitol, 0.1 mM EDTA, and 30 μg/ml bovine serum albumin). Following a 15-min incubation at 37 °C, reaction mixtures were treated with 3 μl of 250 mM EDTA and the DNA was extracted with phenol/chloroform. Aqueous samples (20 μl) were treated with 2 μl of 2.5% SDS, mixed with 2.5 μl agarose gel-loading buffer (10×) and subjected to electrophoresis on a 1% Tris-acetate (pH 7.4)–agarose gel. DNA bands were stained with 1 μg/ml ethidium bromide and visualised using a UV illuminator.

Figure 2 compares CA224's ability to intercalate DNA molecules with camptothecin (a known intercalator of DNA, as control) and fascaplysin. Fascaplysin shows inhibition of DNA relaxation catalysed by the enzyme topoisomerase I indicating its intercalating nature, possibly manifested because of its planar structure. The non-planar compound CA224 did not show any inhibition of DNA relaxation even at a high concentration of 100 μM. To ensure that these results truly reflected a lack of DNA intercalation rather than an inhibition of topoisomerase I, a second set of experiments (data not shown) were performed using relaxed DNA as substrate (prepared first by treating supercoiled pBlueScript plasmid DNA with topoisomerase I). The DNA remained relaxed after treatment with 100 μM CA224, confirming the non-intercalative nature of the compound which may indicate that other compounds in this series are also likely to behave in the same way.

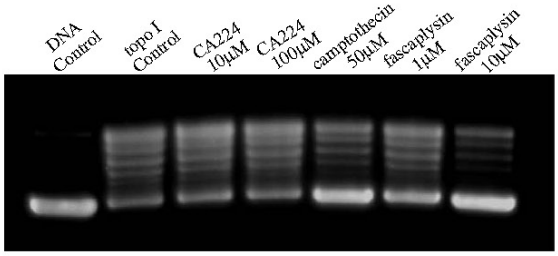



**Figure 2.** CA224 does not intercalate DNA. The ability of fascaplysin's structural analogue CA224 to intercalate DNA was investigated after DNA was unwound/relaxed using topoisomerase I and the results were compared with that from fascaplysin and camptothecin. Lane 1, control supercoiled form of pBlueScript plasmid DNA. Lane 2, supercoiled pBlueScript plasmid DNA unwound/relaxed with topoisomerase I enzyme, in the absence of any compound. Lanes 3 7, pBlueScript plasmid DNA unwound/relaxed by topoisomerase I in the presence of CA224, camptothecin and fascaplysin at the concentrations indicated.

The results from the ethidium bromide displacement and topoisomerase I catalysed DNA unwinding assays indicate that CA224 neither interacts nor intercalates with the minor groove of double-stranded DNA molecules (Figs. 1 and 2).

*Flow cytometric analysis*. The in vitro enzyme assays had confirmed that the fascaplysin analogue CA224 inhibits Cdk4-cyclin D1 and not Cdk2-cyclin A. In proliferative cells, Cdk4 is activated at the $G_0/G_1$ phase of the cell cycle. Therefore, we queried if CA224 would maintain a $G_0/G_1$ block induced by serum starvation. Calu-1 (p53-null) cells were used to test the effect of CA224 on the cell cycle.

The untreated (control) and treated (with compounds) Calu-1 cells were harvested by trypsinization, washed once with PBS and then fixed in 70% chilled (−20 °C) ethanol for 1 h. After the fixation step, cells were centrifuged for 5 min at 3000*g* at room temperature and the pellet was suspended in PBS containing 50 μg/ml propidium iodide (Sigma) and 0.5 mg/ml DNase-free Ribonuclease (Sigma). The cells were stained for 1 h in dark at 4 °C. Cell cycle analysis was performed on the Beckman-Coulter (Epics® Altra™) fluorescence-activated cell sorter (FACS). In order to gate all events representing single cells, and not cell doublets or cell clumps, cytograms of propidium iodide fluorescence peak signals versus integrated or linear fluorescence signals were plotted. All data points on the straight line were isolated in a single gate and the gated data were used for plotting a histogram that represents a complete cell cycle. The total number of events was not allowed to exceed 200 events/s. Data acquisition was stopped after a minimum of 10,000 events had been collected.

Calu-1 cells were starved of serum for 24 h using 0.1% FBS. When these $G_0/G_1$ synchronised cells were released in the presence of [$IC_{70}$] of CA224, the $G_0/G_1$ block was fully maintained (Fig. 3a) indicating that CA224 most likely inhibits cellular Cdk4. A higher $G_0/G_1$:S ratio is observed in cells released from serum starvation, in the presence of CA224, compared to serum-starved cells. This is because nearly all cells in the S phase, present during serum starvation, enter the $G_2$/M phase after release, while cells in $G_0/G_1$ phase are prevented from

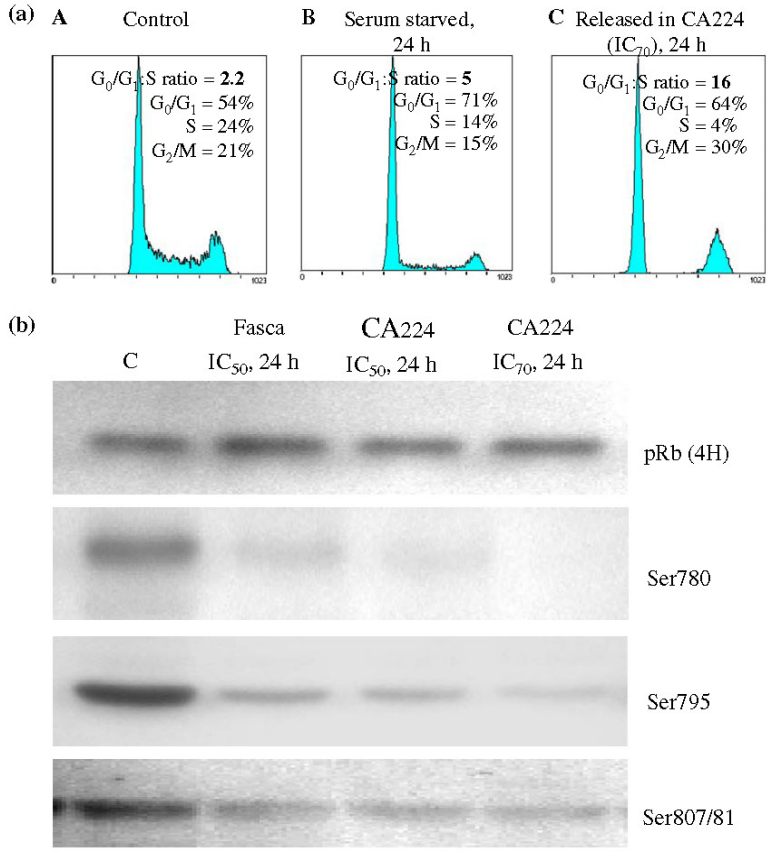



**Figure 3.** (a) FACS analysis of serum-starved Calu-1 cells released in the presence of CA224. (A) Untreated or control cells; (B) cells starved of serum for 24 h; (C) serum-starved cells released in the presence of CA224 at $IC_{70}$ concentration for 24 h. (b) Western blotting of proteins obtained from asynchronous Calu-1 cells treated with CA224. Asynchronous Calu-1 cells were treated with CA224 for 24 h. The antibodies to Ser780, Ser795 and Ser807/811 detect pRB proteins phosphorylated at Ser780, Ser795 and Ser807/811, respectively, while pRb (4H) detects both phosphorylated and unphosphorylated forms of the pRB protein. The lane 'C' indicates proteins from untreated cells; 'fasca' is an abbreviation of fascaplysin.

entering into the S phase of the cell cycle (Fig. 3a, B and C) suggesting inhibition of Cdk4 enzyme at the cellular level.

*Western blot analysis*. Cdk4 phosphorylates pRB at specific serine residues, Ser780, Ser795 and Ser807/811, when cells progress from $G_0/G_1$ to $G_1/S$ phases of the cell cycle. If CA224 were to be a cellular Cdk4 inhibitor, it should prevent phosphorylation at these serine residues when asynchronously growing cells are treated with CA224. Calu-1 cells were seeded in tissue culture flasks. When cells reached 40–50% confluency, they were treated with $IC_{50}$ and $IC_{70}$ concentrations of CA224, or $IC_{50}$ concentration of fascaplysin for 24 h. After treatment, the cells were harvested by trypsinization, washed in ice-cold PBS and then lysed in a buffer that contains a cocktail of protease inhibitors. The lysates were centrifuged at 14,000*g* for 10 min at 4 °C and the amounts of proteins in the clear supernatant were estimated using the Bradford method (Bio-Rad). Fifty micrograms of protein from each sample was subjected to SDS–PAGE separation. The proteins were transferred to a PVDF membrane (Millipore) and blocked with 5% milk. Membranes were probed with polyclonal antibodies raised against the full-length pRB protein, and the phospho-specific pRB epitopes, pRB (Ser780-P), pRB (Ser795-P) and pRB (Ser807/811-P) (New England Biolabs). After overnight incubation at 4 °C, membranes were exposed to appropriate HRP-conjugated secondary antibodies at room temperature for 1 h. Immuno-reactivity was visualised with the enhanced chemiluminescence Western blot detection reagents (GE-Amersham).

The Western blot analyses (Fig. 3b) show that, after treatment of Calu-1 cells with CA224 ($IC_{50}$ and $IC_{70}$) for 24 h, pRB remains unphosphorylated at Ser780, Ser795 and Ser807/811 which are specifically phosphorylated by the Cdk4 enzyme. The total pRB levels in CA224-treated cells remain unchanged. As reported earlier,[2] it is observed that fascaplysin treatment of cancer cells also prevents pRB phosphorylation at the same Cdk4-specific serine residues.

In conclusion, novel tryptamine derivatives, which were designed on the basis of the fascaplysin structure, have been shown to be inhibiting Cdk4 selectively vis a vis Cdk2 and their cellular relevance has been confirmed using CA224, the most potent compound of this series. With this proof of concept in hand, we think it would be possible to generate more potent CA224 analogues

using our fascaplysin structure-based chemical biological approach.

**Acknowledgment**

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# Synthesis, crystal structure and biological activity of β-carboline based selective CDK4-cyclin D1 inhibitors†

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The design, synthesis and biological activity of a series of non-planar dihydro-β-carboline and β-carboline-based derivatives of the toxic anticancer agent fascaplysin is presented. We show these compounds to be selective inhibitors of CDK4 over CDK2 with an $IC_{50}$ (CDK4-cyclin D1) = 11 μmol for the best compound in the series **4d**. The crystallographic analysis of some of the compounds synthesised (**3b/d** and **4a–d**) was carried out, in an effort to estimate the structural similarities between the designed inhibitors and the model compound fascaplysin.

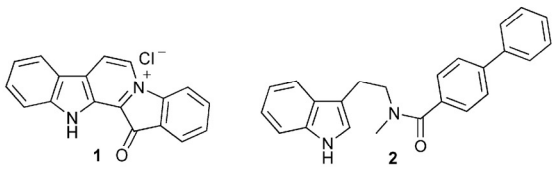
## Introduction

The cyclin-dependent kinases (CDKs) are a set of proteins that play a vital role in the regulation of the cell cycle checkpoints, controlling the transition between the different phases of the process.[1] As a wide variety of diseases (cancer in particular) are characterised by a deregulation in the cell cycle causing uncontrolled cell proliferation, the inhibition of CDKs by small molecules is one of the most active fields in current anti-cancer research.[2]

In this context, the specific inhibition of the CDK4-cyclin D1 complex has arisen as an interesting anticancer target.[3] CDK4 is one of the key players in the transition between $G_1$ and S phases of the cell cycle and is constitutively activated in many human cancers. Similarly, cyclin D1 is often over expressed, whereas the CDK4 inhibitor (p16) is deleted in a variety of human tumours.

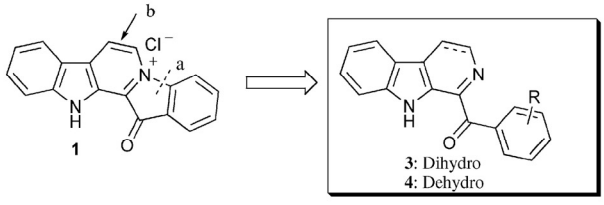
In recent times our group has been interested in the design, modelling, synthesis and biological evaluation of novel, specific CDK4-cyclin D1 inhibitors based on the structure of the pentacyclic quaternary salt fascaplysin **1**.[4–6] This natural product, originally isolated from the Fijian sponge *Fascaplynosis* Bergquist sp,[7] inhibits the growth of several microbes including *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Saccharomyces cerevisiae*, and suppresses the proliferation of mouse leukemia cells (L-1210) with $ED_{50} = 0.2\ \mu g\ mL^{-1}$. Fascaplysin has been also reported to specifically inhibit CDK4-cyclin D1 ($IC_{50} = 0.55\ \mu M$), causing $G_1$ arrest of both tumour (U2-OS, HCT-116) and normal (MRC-5) cells.[8] The use of fascaplysin **1** as an anticancer drug is limited due to its high toxicity; because of its planar structure it can act as a DNA intercalator.[9] The aim of the present study consists of the design of non-toxic (non-planar) analogues of the natural product with increased activity as CDK4-cyclin D1 inhibitors.

It has been shown, using a computational approach, that one of the most important features of the predicted binding mode of fascaplysin **1** in the ATP binding site of a CDK4 homology model is a double hydrogen bonding to Val 96.[6,10] It is also noteworthy that our most active compound so far, the tryptamine based compound **2** ($IC_{50}$ = 6 μm), was predicted to be located in the ATP binding site of the CDK4 homology model in a similar fashion to fascaplysin **1** but with the double hydrogen-bonding interaction being with the backbone of His 95/Val 96 and an extra stabilization arising from a π–π stacking interaction between the biphenyl moiety of the ligand with the side chains of Phe 93 and Phe 159 (Scheme 1).[6]




**Scheme 1** Structures of fascaplysin **1** and the tryptamine based analogue **2**.

Taking these facts into account we designed a series of non-planar β-carboline-based analogues of the natural product fascaplysin **1** of general type **3** and **4** with different substituents in the benzenoid ring by removing bond *a* in the original structure (Scheme 2) and in some cases converting double bond *b* into a single bond. As four of the five rings of fascaplysin **1**, and the relative disposition of the H-bond acceptors/donors needed for the double interaction with Val 96 (*i.e.* the carbonyl and the




**Scheme 2** Strategy used to produce the non-planar β-carboline derivatives **3** and **4** from fascaplysin **1**.

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† Electronic supplementary information (ESI) available: Colour versions of Fig. 1–3. See DOI: 10.1039/b613861f

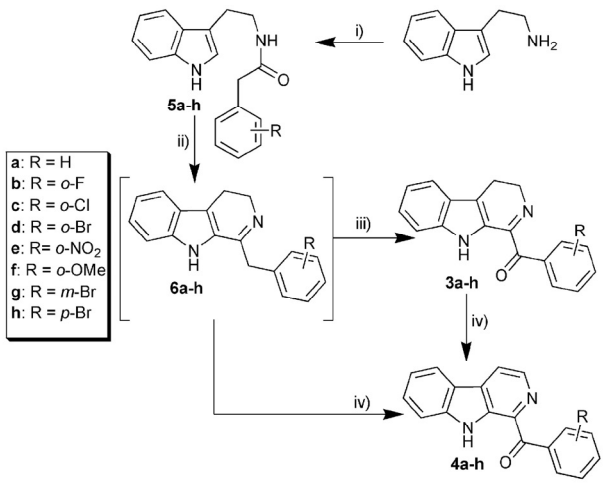
indolyl NH group in **3/4**), is nearly the same as in fascaplysin **1**, by inference, the binding mode and hence the CDK4 inhibition activity was expected to be retained.

Moreover, the introduction of different aromatic substituents in the benzenoid ring into the parent compounds of type **3** and **4**, was also planned in an attempt to explore the presence or not of stabilizing π–π interactions as for compound **2** leading to increased activities as CDK4-cyclin D1 specific inhibitors.

## Results and discussion

### β-Carboline and dihydro β-carboline compounds

In a previous communication,[11] the investigation of the spontaneous photo-oxidation of the 1-(2-bromo-benzyl)-4,9-dihydro-3*H*-β-carboline, led us to the development of a mild, green, regioselective and practical protocol for the preparation of a series of dihydro-β-carbolines of type-**3** and β-carbolines of type-**4** from tryptamine derivatives **5** by a sequential Bischler–Napieralski cyclisation followed by induced photo-oxidation of the non-isolated 1-benzyl-4,9-dihydro-3*H*-β-carboline derivatives **6** (Scheme 3).




**Scheme 3** *Reagents and conditions:* i) $RC_6H_4CH_2COCl$, $CH_2Cl_2$, $NaOH_{(aq)}$. ii) $POCl_3$, toluene, $N_2$, reflux, basic work-up. iii) hυ, $O_2$, toluene, 30 °C. iv) hυ, $O_2$, toluene, reflux.

To circumvent the intrinsic instability of type-**6** compounds, purification after cyclisation of the corresponding acetamide derivatives **5** was avoided, so the crude reaction product after basic work-up of the Bischler–Napieralski reaction was irradiated under the conditions detailed in Scheme 3.

Irradiation of **6a–h** in toluene with a 500 Watt halogen lamp (290–300 nm frequency cut-off) at 30 °C led, regioselectively in most cases, to the dihydro-β-carbolines (**3a–d**, **3g–h**) with satisfactory yields (44–59%) from the corresponding tryptamine derivative **5**. Irradiation under these conditions of crude **6e** led to decomposition of the starting material to a complex mixture, and irradiation of **6f** yielded a mixture of the dihydro-β-carboline **3f** (31% isolated yield) and the β-carboline **4f** (10% isolated yield). A slight modification of the irradiation method, inducing reflux of the toluene solution, produced the fully aromatic β-carbolines **4b–d**, **4f** and **4h** with modest to acceptable yields from **5a–h** (11–55%). On other occasions, the fully aromatic compounds **4a–e** were obtained in good yields (62–99%) by irradiation in refluxing toluene of the isolated ketoimines **3a–e**.

### Solid state structure

As we wished to maintain the double H-bond to Val 96 as seen in fascaplysin **1**, X-ray structure analysis was carried out for some of the compounds **3/4** synthesised (**3b/d** and **4a–d**).

The conformation of type **4** compounds can be described by means of the torsion angles $\tau_1$ (C11–C1–C12–O1) and $\tau_2$ (C1–C12–C13–C14) (Fig. 1). Whilst $\tau_1$ gives a qualitative idea of the deviation of the carbonyl group from the plane of the heterocyclic moiety, $\tau_2$ is connected to the relative disposition of the β-carboline substructure and the benzenoid ring. For compounds of type **3** an extra degree of conformational freedom arises from the non-planar (non-aromatic) cyclic imine substructure in the β-carboline so a third torsion angle $\tau_3$ (C4–C3–C2–N1) was included. In both cases, for type-**3** and type-**4** compounds, these defined torsion angles are interrelated because of the geometry of the molecules.

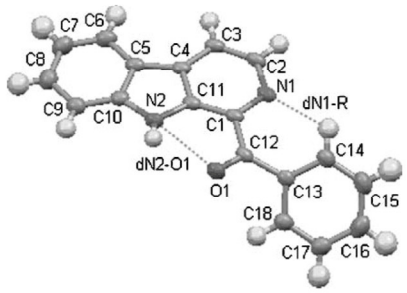


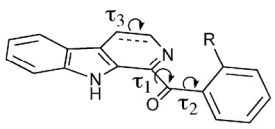

**Fig. 1** MERCURY projection (displacement ellipsoids, 50% probability) of one of the two conformers of **4a** in the minimal asymmetric unit of the crystal, with the arbitrary labelling used in the study.†

As exemplified in Fig. 1 for compound **4a**, we found all the structures **3/4** displaying a strong N2–H···O1 intramolecular H-bond as in the model compound. The geometrical features of this interaction are shown in Table 1.

This intramolecular hydrogen bond is forced by the molecular geometry, with the carbonyl group located nearly in the same plane as the β-carboline moiety (the same situation occurs for fascaplysin), so the free rotation around the C1–C12 bond (characterized by the torsion angle $\tau_1$) in compounds **4** is restricted. Once this geometry is constrained, the phenyl ring in **4** acquires a tilted-T shape relative to the planar heterocyclic substructure characterized by the torsion angle $\tau_2$. For type-**3** molecules the non-planar configuration of the dihydro-β-carboline substructure (characterized by $\tau_3$), causes the carbonyl group to be more out of the plane of the heterocyclic moiety than for type-**4** molecules, increasing $\tau_1$ as exemplified in Fig. 2 for compounds **3d** and **4d**.

Moreover, this nearly planar configuration of the intramolecular donor/acceptors allows the molecule to form intermolecular cyclic dimers by a double hydrogen bonding as exemplified in Fig. 3 for **4a**. The β-carboline substructures of the two molecules are arranged in parallel planes separated by a perpendicular distance di (see Table 1 for geometrical details of the intermolecular hydrogen bonding).

**Table 1** Geometrical data discussed in the text†




**3b** dihydro R = F
**3d** dihydro R = Br
**4a** dehydro R = H
**4b** dehydro R = F
**4c** dehydro R = Cl
**4d** dehydro R = Br

| | Monomers | | | | | | Dimers | | |
|---|---|---|---|---|---|---|---|---|---|
| Structure | $d$(N2 ··· O1)/Å | ∠N2HO1/° | $\tau_1$/° | $\tau_2$/° | $\tau_3$/° | $d$(N1 ··· R)/Å | $d$(N2′ ··· O1)/Å | ∠N2HO1/° | di/Å[c] |
| **4a**[b] | 2.744 | 117.2 | 2.13 | 50.05 | — | 2.603 | 2.945 | 154.9 | 0.616 |
| | 2.767 | 115.7 | 11.78 | −49.70 | — | 2.567 | 2.944 | 154.2 | 0.616 |
| **4b** | 2.889 | 113.4 | −12.54 | −63.18 | — | 3.099 | 3.056 | 144.4 | 1.095 |
| **4c** | 2.808 | 113.9 | −5.70 | −70.49 | — | 3.262 | 2.996 | 145.6 | 0.275 |
| **4d** | 2.813 | 114.2 | −4.11 | −68.10 | — | 3.351 | 3.013 | 145.0 | 0.000 |
| **3b** | 2.837 | 120.5 | 19.90 | 50.34 | −45.53 | 2.939 | — | — | — |
| **3d** | 2.935 | 116.6 | −24.94 | −58.96 | −17.30 | 3.361 | 3.088 | 153.8 | 0.770 |

[a] Data measured using the program Mercury. [b] Two different conformers are presented in the asymmetric unit cell for compound **4a**. [c] Perpendicular distance between the parallel planes containing the β-carboline (type **4** compounds) or indolyl (type **3**) moieties of the molecules involved in the double hydrogen bonding.

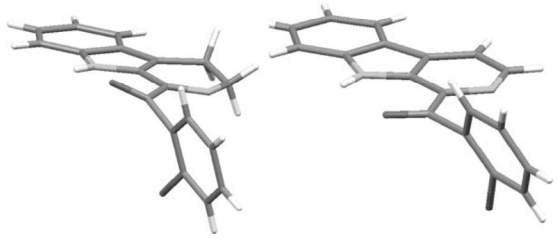


**Fig. 2** MERCURY capped sticks representation of the minimal asymmetric unit in the crystalline structure for compound **3d** (left) and **4d** (right).†

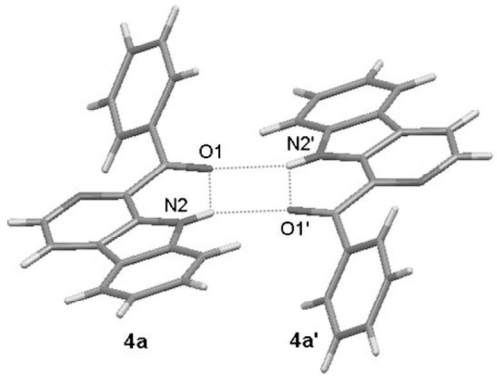



**Fig. 3** MERCURY capped sticks representation of the cyclic symmetric dimer formed by two molecules of **4a** (symmetry codes $x,y,z$ and $-x+1, -y, -z$). Intramolecular (N2H1 ··· O1, N2′H2′ ··· O1) and intermolecular (N2H ··· O1′, N2′H′ ··· O1). Hydrogen bonds shown as dashed blue lines.†

This finding modifies one of Etters rules for hydrogen bond priorities[12] which asserts that: "six-membered-ring intramolecular hydrogen bonds form in preference to intermolecular hydrogen bonds", showing that the two situations can coexist for the same pair of donor/acceptors.

One interesting consequence of the rigidity of the molecules studied is the presence in most of the cases of short contacts between the pyridine-type nitrogen in **3**/**4** and the *ortho*-R group linked to C14 in the benzenoid ring. These non-covalent interactions are characterized by a $d$(N1–R) slightly lower (see Table 1 for details) than the corresponding sum of the van der Waals radii of the atoms $r$ ($r_F$ 1.47 Å, $r_{Cl}$ 1.75 Å, $r_{Br}$ 1.85 Å, $r_N$ 1.55 Å[13] and $r_H$ 1.09 Å).[14] Although these could be considered as halogen bonding for the compounds **4c**/**d** and **3b**/**d**, and non-classic hydrogen bonding for the two conformers found in the unit cell for **4a**, these interactions could be artefacts imposed by the constrained molecular geometry.‡

It is also probable that in solution, as in the solid state, these compounds could present a preferred rigid conformation arising from the inter/intramolecular H-bonding. For instance, in the $^1$H-NMR spectra ($CDCl_3$, 300 MHz) of compounds **6d**, **3d** and **4d** the $\delta NH_{indol}$ signal is respectively 8.00, 9.48 and 10.43 ppm; depending on the environment of the NH group as well as its ability to form intra and intermolecular hydrogen bondings.

### Bi/triphenyl compounds

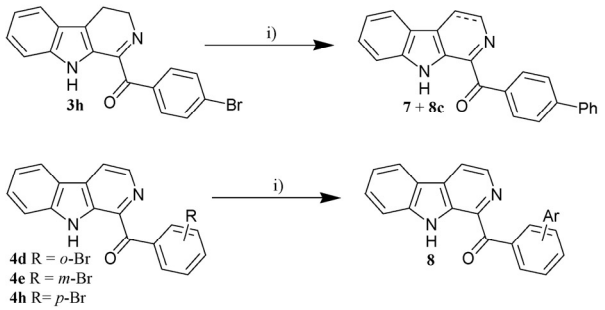
Based on the good biological activity of compound **2**, our group has explored the design, synthesis and biological activity of a wide range of biphenyl derivatives of **2** demonstrating the existence of a π-stacking region in the active site of CDK4,[17] that could be analogous to the "Phe 80 pocket" of CDK2.[18]

In an effort to evaluate the importance of this π-stacking interaction compared to the double hydrogen bonding presented for fascaplysin, we planned a further functionalization of the parent dehydro and dihydro-β-carboline derivatives using the Suzuki–Miyaura cross-coupling reaction to prepare a library of bis/triphenyl compounds related to **3** and **4** (Scheme 4).

In a first attempt to use this approach, the *para*-brominated dihydro-β-carboline **3h** was used as starting material for the coupling reaction. This compound was reacted with phenylboronic

‡ The expected priority order for the halogen bond interactions C–X ··· B is B = S < N < O for the Lewis base and X = Cl < Br < I for the halogen,[15] the contact with X = F being rarely reported and quite controversial.[16] In our case, with the carbonyl group involved in two hydrogen bonds, the pyridine-like nitrogen is the only available acceptor for the intramolecular interaction.




| Compound | Ar | Compound | Ar | Compound | Ar |
|---|---|---|---|---|---|
| **8a** | *o*-Ph | **8b** | *m*-Ph | **8c** | *p*-Ph |
| **8d** | *o*-4-Me-Ph | **8e** | *m*-4-Me-Ph | **8f** | *p*-4-Me-Ph |
| **8g** | *o*-4-F-Ph | **8h** | *m*-4-F-Ph | **8i** | *p*-4-F-Ph |
| **8j** | *o*-4-$^t$Bu-Ph | **8k** | *m*-4-$^t$Bu-Ph | **8l** | *p*-4-$^t$Bu-Ph |
| **8m** | *o*-4-Ph-Ph | **8n** | *m*-4-Ph-Ph | **8o** | *p*-4-Ph-Ph |

**Scheme 4** *Reagents and conditions:* i) $Pd(PPh_3)_{4(cat)}$, $K_2CO_{3(aq)}$, $ArB(OH)_2$, toluene–EtOH, 90 °C.

acid in a 1 : 1 mixture of toluene–EtOH at 90 °C using $Pd(PPh_3)_4$ as catalyst and aqueous $K_2CO_3$ as base. This produced an unresolved mixture of the desired dihydro-β-carboline **7** and the fully aromatic β-carboline **8d** in a 10 : 1 ratio.

Using the same reaction conditions with the corresponding *ortho*/*meta*/*para* brominated β-carbolines **4d**, **4e** and **4h** as starting materials, the synthesis of the target compounds **8a–o** was achieved in a straightforward fashion with high yields (82–99%, see experimental part for further details).

### Biological results

The CDK4 and CDK2 inhibitory activities of compounds **3a–d**, **3g–h**, **4a–d**, **4f–h**, **7**, and **8a–o** were measured in terms of $IC_{50}$ using standard methods[6] and the results are shown in Table 2. As expected, all the compounds are active as CDK4 inhibitors showing a clear selectivity for this kinase compared to CDK2.

The first series of compounds discussed above, the dihydro-β-carboline derivatives, shows, in general, better activities compared to the corresponding fully aromatic compounds. In both series the introduction of a halogen in the *ortho*-position of the benzenoid ring increases the activity, the greater the electronegativity of the halogen the lesser the effect. Furthermore, taking the *ortho*-brominated derivatives **3d** and **4d** as reference, the activity of these compounds is decreased if the halogen is located in the *meta* or *para*-positions on the aromatic ring. For these two series the best hits correspond to the *ortho*-brominated compounds **3d** ($IC_{50}$ = 11 μm) and **4d** ($IC_{50}$ = 14 μm).

As the crystallographic analysis of compounds of type **3** and **4** has shown, these should exist in a rigid conformation, differing from fascaplysin only in the relative disposition of the outer phenyl group (characterised by $\tau_2$), so a similar binding mode in the ATP site of the enzyme is expected.

Concerning the bi/triphenyl derivatives, the introduction of aromatic ring or rings in the initial structure decreases the activity with respect to the parent brominated compounds, the exception being **8f** (more active than **4h**) and **8k** (more active than **4g**).

For these series of compounds, instead of a synergic effect between the two interactions (*i.e.* the fascaplysin-like double hydrogen bonding and the π-stacking present in compound **2**), in our case, the decreased activities for the coupling products can be rationalized in terms of a competition between the two binding modes. Once the molecule is located in the ATP binding site in a similar fashion as for fascaplysin **1**, the bulky introduced bis/tris aromatic moieties are possibly not oriented properly in the direction of the π-stacking pocket.

**Table 2** CDK4 activity *versus* CDK2 activity

| Compound | CDK4 measured $IC_{50}$/μM[a] | CDK2 measured $IC_{50}$/μM[b] |
|---|---|---|
| Fascaplysin | 0.55 | 500 |
| **1** | | |
| **2** | 6 ± 1 | 521 ± 12 |
| **3a** | 45 ± 4 | 515 ± 11 |
| **3b** | 16 ± 2 | 812 ± 8 |
| **3c** | 12 ± 1.8 | 630 ± 15 |
| **3d** | 11 ± 2 | 818 ± 16 |
| **3g** | 78 ± 6 | 1209 ± 15 |
| **3h** | 34 ± 2 | 538 ± 9 |
| **4a** | 39 ± 3 | 913 ± 8 |
| **4b** | 24 ± 2.5 | 974 ± 11 |
| **4c** | 22 ± 2 | 855 ± 10 |
| **4d** | 14 ± 1 | 940 ± 12 |
| **4f** | 65 ± 3.5 | 512 ± 7 |
| **4g** | 32 ± 3 | 868 ± 10 |
| **4h** | 34 ± 2 | 538 ± 9 |
| (**7**+**8a**)[c] | 61 ± 4 | 712 ± 7 |
| **8a** | 72 ± 5 | 1216 ± 11 |
| **8b** | 67 ± 3 | 920 ± 20 |
| **8c** | 77 ± 4 | 1135 ± 18 |
| **8d** | 75 ± 4 | 496 ± 9 |
| **8e** | 59 ± 5 | 384 ± 15 |
| **8f** | 30 ± 1 | 983 ± 17 |
| **8g** | 65 ± 3 | 865 ± 13 |
| **8h** | 54 ± 5 | 367 ± 8 |
| **8i** | 42 ± 2 | 350 ± 8 |
| **8j** | 48 ± 2.5 | 423 ± 15 |
| **8k** | 25 ± 2 | 971 ± 11 |
| **8l** | 58 ± 3 | 800 ± 15 |
| **8m** | 77 ± 4 | 526 ± 14 |
| **8n** | 58 ± 2.5 | 437 ± 10 |
| **8o** | 61 ± 3.5 | 566 ± 12 |

[a] CDK4-cyclin D1 assay, using GST-RB152 fusion protein as the substrate. [b] CDK2-cyclin A assay using histone H1 as the substrate. [c] Tested as an unresolved mixture of **7** and **8a** in a 10 : 1 ratio.

## Conclusions

A series of dehydro-β-carboline and β-carboline-based compounds related to the toxic anticancer agent fascaplysin **1** were synthesised and their biological activities as CDK4-cyclin D1 specific inhibitors measured.

Compounds **3a–d**, **3g–h**, **4a–d** and **4f–h** were designed to retain the double hydrogen bonding to Val 96 and prepared with acceptable yields by a novel synthetic methodology previously reported.

Compounds **7** and **8a–o** were produced using the well established Suzuki–Miyaura coupling methodology in an effort to increase the activities of the parent compounds by interaction with a proposed π-stacking pocket (Phe 93–159).

The structural similarity between compounds of type **3**/**4**, and fascaplysin **1** was estimated by means of the solid state structure analysis of some of the compounds synthesised (**3b**/**d** and **4a–d**).

This analysis showed the compounds have a rigid conformation, keeping the carbonyl group and the indolyl NH approximately in the same plane as for fascaplysin **1** caused by a strong intramolecular hydrogen bonding. As predicted, the compounds showed a good activity as CDK4-cyclin D1 inhibitors with an $IC_{50}$ for the best compound of 11 μm.

The second series (compounds **7** and **8a–o**), showed in general a decreased activity compared to the parent compounds. This was attributed to a competition between the two proposed binding modes.

## Experimental

### General

All reactions were performed under an atmosphere of nitrogen (unless otherwise stated in the text) and solvent extractions dried with anhydrous sodium sulfate. NMR spectra were recorded on a Bruker DPX 300 ($^1$H, 300.13 MHz; $^{13}$C, 75.47 MHz) spectrometer. Chemical shifts were measured relative to chloroform ($^{13}$C $\delta$ 77.0) or dimethylsulfoxide ($^{13}$C $\delta$ 39.5) and are expressed in ppm. Coupling constants $J$ are expressed in Hertz and the measured values are rounded to one decimal place. Fast atom bombardment (FAB) mass spectra were recorded on a Kratos Concept 1H using xenon and *m*-nitrobenzyl alcohol as the matrix. Electrospray (ES) mass spectra were recorded on a Micromass Quattro LC spectrometer. Accurate mass was measured on a Kratos Concept 1H spectrometer using peak matching to a stable reference peak. Flash column chromatography was carried out using Merck Kieselgel 60 (230–400 mesh). Melting points were recorded on a Reichert Kofler thermopan and are uncorrected.

### Crystal structure determinations§

Single crystals of compounds **3b/d** and **4a-d** suitable for X-ray diffractometry were obtained by dissolving crystals of the corresponding pure compound in the minimum quantity of cold EtOH in an open vial that was then placed in a larger container with a little $H_2O$ in its bottom; the container was closed, and after a few days in a cool, dark place free from vibrations, afforded the desired single crystals. These were mounted in inert oil and transferred to the cold gas stream of the diffractometer.

### General method for the Suzuki coupling reaction

To a stirred solution of the corresponding brominated intermediate (**3h**, **4d/e/h**, 1 mmol) in toluene (10 mL), under nitrogen, was added $K_2CO_3$ (1 mmol, 2M aqueous solution) and $Pd(PPh_3)_4$ (5 mol%, 0.05 mmol). The solution was stirred for 20 minutes at room temperature before the addition of a solution of the appropriately substituted phenylboronic acid (1.2 mmol) in EtOH (10 mL), the reaction mixture was then heated to 90 °C for 24 h and allowed to cool to room temperature before the addition of $H_2O_2$ (30%, 1 mL). The reaction mixture was then stirred for a further hour, and extracted into $CHCl_3$, washed with saturated brine solution (2 × 25 mL) and $H_2O$ (2 × 25 mL), aqueous washings being re-extracted with $CH_2Cl_2$ (3 × 50 mL). The combined organic phases were then dried over anhydrous sodium or magnesium sulfate, filtered and isolated under reduced pressure. The crude products were then purified by flash column chromatography on silica from $CH_2Cl_2$.

**Suzuki coupling of compound 3h.** The reaction between the brominated derivative **3h** and phenylboronic acid in the conditions explained above yielded, after column chromatography ($CH_2Cl_2$), an unresolved mixture of **7** + **8a** in a 10 : 1 ratio (isolated overall yield 67%). The spectroscopic data for the major compound is described below.

**Biphenyl-4-yl(4,9-dihydro-3*H*-pyrido[3,4-*b*]indol-1-yl)-methanone 7.** 67% (Mixture), yellow sticky solid, $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ 3.05 (2H, t, $J$ 8.8), 4.21 (2H, t, $J$ 8.8), 7.18 (1H, ddd, $J$ 8.0, 7.0, 1.0), 7.33 (1H, ddd, $J$ 8.3, 7.0, 1.2), 7.42–7.52 (4H, m), 7.63–7.66 (3H, m), 7.71 (2H, d, $J$ 8.6), 8.28 (2H, d, $J$ 8.6) and 9.51 (1H, br s) ppm; $^{13}$C-NMR (75 MHz, $CDCl_3$) $\delta$ 19.1 ($CH_2$), 49.3 ($CH_2$), 112.3 (CH), 118.0 (Cq), 119.9 (CH), 120.3 (CH), 124.8 (Cq), 125.1 (CH), 126.6 (Cq), 126.9 (CH), 127.3 (CH), 128.2 (CH), 128.9 (CH), 131.6 (CH), 134.0 (Cq), 136.9 (Cq), 140.0 (Cq), 146.2 (Cq), 155.9 (Cq) and 192.8 (Cq); $m/z$ (ES$^+$) 351 (MH$^+$).

**Biphenyl-2-yl-(9*H*-β-carbolin-1-yl)-methanone 8a.** 99% Yield, yellow solid; mp 194–195 °C (from EtOH); Found: C, 82.83; H, 4.70; N 7.84%; $C_{24}H_{16}N_2O$ requires: C, 82.74; H, 4.63; N, 8.04%; $\nu_{max}$/cm$^{-1}$ 3366, 3058, 1635, 1425, 1315, 1210, 1117, 969, 748, 737, 701; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ 7.06–7.17 (3H, m), 7.30–7.35 (3H, m), 7.48–7.64 (5H, m), 7.72 (1H, dd, $J$ 1.2 and 7.4), 8.00 (1H, d, $J$ 4.9), 8.11 (1H, d, $J$ 8.0 Hz), 8.42 (1H, d, $J$ 4.9) and 10.28 (1H, br s, $D_2O$ exch, NH) ppm; $^{13}$C-NMR (75 MHz, $CDCl_3$) $\delta$ 112.0 (CH), 115.4 (CH), 118.5 (CH), 120.1 (CH), 120.7 (Cq), 120.8 (CH), 121.8 (CH), 126.8 (CH), 126.9 (CH), 128.1 (CH), 128.9 (CH), 129.2 (CH), 129.5 (CH), 130.2 (CH), 130.6 (CH), 131.4 (Cq), 136.4 (Cq), 136.6 (Cq), 138.5 (CH), 138.6 (Cq), 141.1 (Cq), 142.01 (Cq) and 200.6 (CO) ppm; $m/z$ (ES$^+$) 349 (MH$^+$); $m/z$ (FAB$^+$) 351 (MH$^+$) (found: MH$^+$, 349.13407; $C_{24}H_{17}N_2O$ requires 349.13409).

**Biphenyl-3-yl-(9*H*-β-carbolin-1-yl)-methanone 8b.** 93% Yield, yellow solid; mp 151–152 °C (from EtOH); Found: C, 82.83; H, 4.56; N 7.99%; $C_{24}H_{16}N_2O$ requires: C, 82.74; H, 4.63; N, 8.04%; $\nu_{max}$/cm$^{-1}$ 3436, 3057, 1614, 1425, 1316, 1203, 748, 722, 693; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ 7.34–7.41 (2H, m), 7.48 (2H, t, $J$ 7.4), 7.59–7.70 (5H, m), 7.85 (1H, dt, $J$ 1.4 and 7.8), 8.18–8.21 (2H, m), 8.29 (1H, dt, $J$ 7.7 and 1.3), 8.52 (1H, t, $J$ 1.7), 8.62 (1H, d, $J$ 4.9) and 10.46 (1H, br s, $D_2O$ exch, NH) ppm; $^{13}$C-NMR (75 MHz, $CDCl_3$) $\delta$ 110.9 (CH), 117.5 (CH), 119.7 (CH), 119.7 (Cq), 120.7 (CH), 126.2 (CH), 126.5 (CH), 127.3 (CH), 127.8 (CH), 128.2 (CH), 128.4 (CH), 128.7 (CH), 129.1 (CH), 129.9 (Cq), 129.9 (CH), 130.6 (Cq), 135.1 (Cq), 136.2 (Cq), 137.0 (CH), 139.5 (Cq), 140.0 (2 × Cq) and 194.5 (CO) ppm; $m/z$ (ES$^+$) 349 (MH$^+$); $m/z$ (FAB$^+$) 351 (MH$^+$) (found: MH$^+$, 349.13404; $C_{24}H_{17}N_2O$ requires 349.13409).

**Biphenyl-4-yl-(9*H*-β-carbolin-1-yl)-methanone 8c.** 99% Yield, yellow solid; mp 183–184 °C (from EtOH); Found: C, 82.63; H, 4.59; N 7.91%; $C_{24}H_{16}N_2O$ requires: C, 82.74; H, 4.63; N, 8.04%; $\nu_{max}$/cm$^{-1}$ 3377, 3055, 1641, 1425, 1318, 1205, 742, 728, 709, 688; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ 7.33–7.43 (2H, m), 7.49 (2H, t, $J$ 7.3), 7.62–7.64 (2H, m), 7.66–7.60 (2H, m), 7.76 (2H, dt, $J$ 1.8

§ CCDC reference numbers 621742–621747. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b613861f

and 8.4), 8.18–8.21 (2H, m), 8.43 (2H, dt, *J* 1.7 and 8.4), 8.63 (1H, d, 4.9 Hz) and 10.48 (1H, br s, $D_2O$ exch, NH) ppm; $^{13}$C-NMR (75 MHz, $CDCl_3$) $\delta$ 112.0 (CH), 118.5 (CH), 120.7 (CH), 120.8 (Cq), 121.8 (CH), 126.7 (CH), 127.3 (CH), 128.0 (CH), 128.9 (CH), 129.2 (CH), 131.6 (Cq), 131.8 (CH), 136.3 (Cq), 136.4 (Cq), 137.3 (Cq), 138.0 (CH), 140.3 (Cq), 141.0 (Cq), 145.0 (Cq) and 194.8 (CO) ppm; *m/z* (ES$^+$) 349 (MH$^+$); *m/z* (FAB$^+$) 351 (MH$^+$) (found: MH$^+$, 349.13416; $C_{24}H_{17}N_2O$ requires 349.13409).

**(9*H*-β-Carbolin-1-yl)-(4′-methyl-biphenyl-2-yl)-methanone 8d.** 99% Yield, yellow solid; mp 185–186 °C (from EtOH); Found: C, 82.95; H, 4.98; N 7.65%; $C_{25}H_{18}N_2O$ requires: C, 82.85; H, 5.01; N, 7.73%; $\nu_{max}$/cm$^{-1}$ 3407, 3045, 1641, 1463, 1315, 1207, 1114, 972, 766, 750, 728, 711; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ 2.20 (3H, s), 6.96 (2H, d, *J* 7.9), 7.20 (2H, d, *J* 8.0), 7.34 (1H, t, *J* 7.3), 7.45–7.63 (5H, m), 7.69 (1H, dd, *J* 1.0 and 7.6), 8.05 (1H, d, *J* 4.9), 8.13 (1H, d, *J* 7.8), 8.45 (1H, d, *J* 4.9) and 10.31 (1H, br s, $D_2O$ exch, NH) ppm; $^{13}$C-NMR (75 MHz, $CDCl_3$) $\delta$ 21.01 ($CH_3$), 111.9 (CH), 118.5 (CH), 120.7 (CH), 120.7 (Cq), 121.7 (CH), 126.4 (CH), 128.7 (CH), 128.9 (CH), 129.2 (CH), 129.5 (CH), 130.2 (CH), 130.4 (CH), 131.4 (Cq), 131.6 (Cq), 136.3 (Cq), 136.6 (2 × Cq), 138.1 (Cq), 138.4 (CH), 141.0 (Cq), 141.9 (Cq) and 200.6 (CO) ppm; *m/z* (ES$^+$) 363 (MH$^+$); *m/z* (FAB$^+$) 363 (MH$^+$) (found: MH$^+$, 363.14973; $C_{25}H_{18}N_2O$ requires 363.14975).

**(9*H*-β-Carbolin-1-yl)-(4′-methyl-biphenyl-3-yl)-methanone 8e.** 94% Yield, yellow solid; mp 159–160 °C (from EtOH); Found: C, 82.75; H, 5.11; N 7.63%; $C_{25}H_{18}N_2O$ requires: C, 82.85; H, 5.01; N, 7.73%; $\nu_{max}$/cm$^{-1}$ 3437, 3060, 1615, 1425, 1316, 1249, 1204, 978, 785, 750, 734, 723; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ 2.41 (3H, s), 7.27 (2H, d, *J* 8.0), 7.36 (1H, quintet, *J* 4.0), 7.57–7.64 (5H, m), 7.81 (1H, dt, *J* 1.7 and 7.7), 8.18–8.21 (2H, m), 8.28 (1H, dt, *J* 1.3 and 7.7), 8.50 (1H, t, *J* 1.6), 8.62 (1H, d, *J* 4.9) and 10.46 (1H, br s, $D_2O$ exch, NH) ppm; $^{13}$C-NMR (75 MHz, $CDCl_3$) $\delta$ 21.2 ($CH_3$), 112.0 (CH), 118.6 (CH), 120.8 (CH), 120.9 (Cq), 121.8 (CH), 127.1 (CH), 128.4 (CH), 129.3 (CH), 129.6 (CH), 130.0 (CH), 130.9 (CH), 131.70 (Cq), 136.3 (Cq), 137.3 (Cq), 137.4 (2xCq), 137.8 (Cq), 138.1 (Cq), 138.2 (CH), 141.0 (Cq) and 195.7 (CO) ppm; *m/z* (ES$^+$) 363 (MH$^+$); *m/z* (FAB$^+$) 363 (MH$^+$) (found: MH$^+$, 363.14976; $C_{25}H_{18}N_2O$ requires 363.14975).

**(9*H*-β-Carbolin-1-yl)-(4′-methyl-biphenyl-4-yl)-methanone 8f.** 97% Yield, yellow solid; mp 209–210 °C (from EtOH); Found: C, 82.77; H, 4.96; N 7.61%; $C_{25}H_{18}N_2O$ requires: C, 82.85; H, 5.01; N, 7.73%; $\nu_{max}$/cm$^{-1}$ 3398, 3056, 1640, 1424, 1317, 1214, 1204, 970, 793, 737, 727, 707; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ 2.42 (3H, s), 7.31 (2H, d, *J* 8.0 Hz), 7.36 (1H, quintet, *J* 4.0), 7.57–7.63 (4H, m), 7.74 (2H, dt, *J* 1.7 and 8.4), 8.17–8.20 (2H, m), 8.28 (2H, dt, *J* 1.3 and 7.7 Hz), 8.64 (1H, d, *J* 4.9 Hz) and 10.48 (1H, br s, $D_2O$ exch, NH) ppm; $^{13}$C-NMR (75MHz, $CDCl_3$) $\delta$ 21.2 ($CH_3$), 112.0 (CH), 118.5 (CH), 120.7 (CH), 120.8 (Cq), 121.8 (CH), 126.5 (CH), 127.18 (CH), 129.3 (CH), 129.6 (CH), 131.7 (Cq), 131.8 (CH), 136.0 (Cq), 136.5 (Cq), 137.3 (Cq), 137.4 (Cq), 138.0 (Cq), 138.1 (CH), 141.0 (Cq), 141.0 (Cq) and 194.9 (CO) ppm; *m/z* (ES$^+$) 363 (MH$^+$); *m/z* (FAB$^+$) 363 (MH$^+$) (found: MH$^+$, 363.14979; $C_{25}H_{18}N_2O$ requires 363.14975).

**(9*H*-β-Carbolin-1-yl)-(4′-fluoro-biphenyl-2-yl)-methanone 8g.** 97% Yield, yellow solid; mp 245–246 °C (from EtOH); Found: C, 78.52; H, 4.07; N 7.58%; $C_{24}H_{15}FN_2O$ requires: C, 78.68; H, 4.13; N, 7.65%; $\nu_{max}$/cm$^{-1}$ 3390, 3059, 1634, 1624, 1426, 1316, 1209, 1117, 970, 859, 750, 734; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ 6.84 (2H, t, *J* 8.6), 7.27–7.36 (3H, m), 7.48–7.62 (5H, m), 7.71 (1H, d, *J* 7.2), 8.03 (1H, d, *J* 4.9), 8.13 (1H, d, *J* 8.0), 8.41 (1H, d, *J* 4.9) and 10.28 (1H, br s, $D_2O$ exch, NH) ppm; $^{13}$C-NMR (75 MHz, DMSO) $\delta$ 113.0 (CH), 114.7 (CH, d, $^2J_{CF}$ 21.5), 119.0 (CH), 119.9 (Cq), 120.2 (CH), 121.8 (CH), 126.7 (CH), 128.9 (CH), 129.1 (CH), 129.6 (CH), 130.0 (CH), 130.2 (CH, d, $^3J_{CF}$ 8.1), 130.8 (Cq), 135.1 (Cq), 136.2 (Cq), 137.0 (Cq), 137.4 (CH), 139.3 (Cq), 139.6 (Cq), 141.8 (Cq), 161.2 (Cq, d, $^1J_{CF}$ 243.6) and 198.8 (CO) ppm; *m/z* (ES$^+$) 367 (MH$^+$); *m/z* (FAB$^+$) 367 (MH$^+$) (found: MH$^+$, 367.12476; $C_{24}H_{15}FN_2O$ requires 367.12468).

**(9*H*-β-Carbolin-1-yl)-(4′-fluoro-biphenyl-3-yl)-methanone 8h.** 97% Yield, yellow solid; mp 188–189 °C (from EtOH); Found: C, 78.59; H, 4.04; N 7.51%; $C_{24}H_{15}FN_2O$ requires: C, 78.68; H, 4.13; N, 7.65%; $\nu_{max}$/cm$^{-1}$ 3437, 3053, 1615, 1593, 1425, 1317, 1204, 1162, 979, 840, 751, 736; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ 7.16 (2H, t, *J* 8.7), 7.37 (1H, quintet, *J* 4.0), 7.58–7.66 (5H, m), 7.78 (1H, dt, *J* 1.4 and 7.7), 8.18–8.21 (2H, m), 8.30 (1H, dt, *J* 1.3 and 7.7), 8.47 (1H, t, *J* 1.6), 8.3 (1H, d, 4.9) and 10.46 (1H, br s, $D_2O$ exch, NH) ppm; $^{13}$C-NMR (75 MHz, $CDCl_3$) $\delta$ 113.0 (CH), 115.8 (CH, d, $^2J_{CF}$ 21.4), 118.9 (CH), 120.0 (Cq), 120.2 (CH), 121.8 (CH), 128.6 (CH), 128.8 (CH), 128.9 (CH), 130.0 (CH), 129.6 (CH), 130.2 (CH), 131.1 (Cq), 135.9 (Cq), 136.0 (Cq), 136.1 (Cq), 137.2 (CH), 138.3 (Cq), 138.7 (Cq), 141.7 (Cq), 162.0 (Cq, d, $^1J_{CF}$ 244.6) and 193.9 (CO) ppm; *m/z* (ES$^+$) 367 (MH$^+$); *m/z* (FAB$^+$) 367 (MH$^+$) (found: MH$^+$, 367.12462; $C_{24}H_{15}FN_2O$ requires 367.12468).

**(9*H*-β-Carbolin-1-yl)-(4′-fluoro-biphenyl-4-yl)-methanone 8i.** 95% Yield, yellow solid; mp 221–222 °C (from EtOH); Found: C, 78.53; H, 4.05; N 7.54%; $C_{24}H_{15}FN_2O$ requires: C, 78.68; H, 4.13; N, 7.65%; $\nu_{max}$/cm$^{-1}$ 3431, 3041, 1601, 1426, 1317, 1215, 1164, 970, 837, 794, 736, 714; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ 7.17 (2H, t, *J* 8.7), 7.36 (1H, quintet, *J* 4.0), 7.62–7.66 (4H, m), 7.71 (1H, d, *J* 8.4), 8.18–8.20 (2H, m), 8.42 (2H, d, *J* 8.4) and 10.48 (1H, br s, $D_2O$ exch, NH) ppm; $^{13}$C-NMR (75 MHz, $CDCl_3$) $\delta$ 113.0 (CH), 115.8 (CH, d, $^2J_{CF}$ 21.5), 118.8 (CH), 120.0 (Cq), 120.2 (CH), 121.8 (CH), 126.1 (CH), 128.1 (CH), 128.9 (CH), 131.0 (Cq), 131.5 (CH), 135.6 (Cq), 135.7 (Cq), 135.8 (Cq), 136.2 (Cq), 136.3 (Cq), 137.1 (CH), 141.7 (Cq), 142.6 (Cq), 162.3 (Cq, d, $^1J_{CF}$ 245.3) and 193.0 (CO) ppm; *m/z* (ES$^+$) 367 (MH$^+$); *m/z* (FAB$^+$) 367 (MH$^+$) (found: MH$^+$, 367.12471; $C_{24}H_{15}FN_2O$ requires 367.12468).

**(4′-*tert*-Butyl-biphenyl-2-yl)-(9*H*-β-carbolin-1-yl)-methanone 8j.** 96% Yield, yellow solid; mp 176–177 °C (from EtOH); Found: C, 83.02; H, 5.86; N 6.83%; $C_{28}H_{24}N_2O$ requires: C, 83.14; H, 5.98; N, 6.93%; $\nu_{max}$/cm$^{-1}$ 3421, 2921, 1651, 1429, 1315, 1245, 1206, 969, 835, 762, 752, 737, 725; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ 1.12 (9H, s), 7.11 (2H, d, *J* 8.4 Hz), 7.22 (2H, d, *J* 8.4 Hz), 7.32 (1H, t, *J* 6.5 Hz), 7.46–7.63 (5H, m), 7.71 (1H, dd, *J* 0.8 and 7.4 Hz), 7.98 (1H, d, *J* 5.0 Hz), 8.12 (1H, d, *J* 7.9 Hz), 8.40 (1H, d, *J* 5.0 Hz) and 10.23 (1H, br s, $D_2O$ exch, NH) ppm; $^{13}$C-NMR (75 MHz, $CDCl_3$) $\delta$ 31.1 ($CH_3$), 31.3 (Cq), 112.0 (CH), 118.3 (CH), 120.7 (CH), 120.7 (Cq), 121.8 (CH), 125.0 (CH), 126.5 (CH), 128.5 (CH), 129.2 (CH), 129.4 (CH), 130.2 (CH), 130.6 (CH), 131.3 (Cq), 136.5 (Cq), 136.7 (Cq), 138.0 (Cq), 138.4 (CH), 138.7 (Cq), 141.1 (Cq), 142.0 (Cq), 149.7 (Cq) and 194.9 (CO) ppm; *m/z* (ES$^+$) 405 (MH$^+$); *m/z*

(FAB$^+$) 405 (MH$^+$) (found: MH$^+$,405.19661; $C_{28}H_{24}N_2O$ requires 405.19670).

**(4′-*tert*-Butyl-biphenyl-3-yl)-(9*H*-β-carbolin-1-yl)-methanone 8k.** 99% Yield, yellow solid; mp 173–174 °C (from EtOH); Found: C, 83.06; H, 5.89; N 6.83%; $C_{28}H_{24}N_2O$ requires: C, 83.14; H, 5.98; N, 6.93%; $\nu_{max}$/cm$^{-1}$ 3423, 2962, 1621, 1427, 1317, 1248, 1207, 979, 841, 791, 754, 728; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ 1.38 (9H, s), 7.36 (1H, quintet, *J* 4.0 Hz), 7.50 (2H, d, *J* 8.4 Hz), 7.60–7.64 (5H, m), 7.83 (1H, d, *J* 7.8 Hz), 8.18–8.21 (2H, m), 8.28 (1H, d, *J* 7.7 Hz), 8.52 (1H, br t), 8.63 (1H, d, *J* 4.9 Hz) and 10.47 (1H, br s, $D_2O$ exch, NH) ppm; $^{13}$C-NMR (75 MHz, $CDCl_3$) $\delta$ 31.3 ($CH_3$), 34.5 (Cq), 112.0 (CH), 118.5 (CH), 120.7 (CH), 120.8 (Cq), 121.8 (CH), 125.8 (CH), 126.9 (CH), 128.3 (CH), 129.2 (CH), 129.7 (CH), 129.9 (CH), 130.9 (CH), 131.6 (Cq), 136.3 (Cq), 137.3 (Cq), 137.7 (Cq), 138.0 (Cq), 138.1 (CH), 140.9 (Cq), 141.0 (Cq), 150.6 (Cq) and 195.6 (CO) ppm; *m/z* (ES$^+$) 405 (MH$^+$); *m/z* (FAB$^+$) 405 (MH$^+$) (found: MH$^+$, 405.19660; $C_{28}H_{24}N_2O$ requires 405.19670).

**(4′-*tert*-Butyl-biphenyl-4-yl)-(9*H*-β-carbolin-1-yl)-methanone 8l.** 99% Yield, yellow solid; mp 245–246 °C (from EtOH); Found: C, 83.21; H, 5.88; N 6.82%; $C_{28}H_{24}N_2O$ requires: C, 83.14; H, 5.98; N, 6.93%; $\nu_{max}$/cm$^{-1}$ 3420, 2964, 1649, 1604, 1424, 1309, 1242, 1203, 1182, 1118, 964, 827, 796, 747, 732; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ 1.38 (9H, s), 7.36 (1H, quintet, *J* 4.0 Hz), 7.52 (2H, d, *J* 7.5 Hz), 7.62–7.65 (4H, m), 7.77 (2H, d, *J* 8.3 Hz), 8.18–8.21 (2H, m), 8.28 (1H, d, *J* 7.7 Hz), 8.43 (2H, d, *J* 8.3 Hz), 8.64 (1H, d, *J* 4.9 Hz) and 10.48 (1H, br s, $D_2O$ exch, NH) ppm; $^{13}$C-NMR (75 MHz, $CDCl_3$) $\delta$ 31.4 ($CH_3$), 34.6 (Cq), 112.0 (CH), 118.5 (CH), 120.8 (CH), 120.9 (Cq), 121.8 (CH), 125.9 (CH), 126.6 (CH), 127.0 (CH), 129.3 (CH), 131.7 (Cq), 131.9 (CH), 136.0 (Cq), 136.5 (Cq), 137.3 (Cq), 137.4 (Cq), 138.1 (CH), 141.0 (Cq), 145.0 (Cq), 151.2 (Cq) and 194.9 (CO) ppm; *m/z* (ES$^+$) 405 (MH$^+$); *m/z* (FAB$^+$) 405 (MH$^+$) (found: MH$^+$, 405.19663; $C_{28}H_{24}N_2O$ requires 405.19670).

**(9*H*-β-Carbolin-1-yl)-[1,1′;4′,1″]terphenyl-2-yl-methanone 8m.** 98% Yield, yellow solid; mp 216–217 °C (from EtOH); Found: C, 84.93; H, 4.56; N 6.55%; $C_{30}H_{20}N_2O$ requires: C, 84.88; H, 4.75; N, 6.60%; $\nu_{max}$/cm$^{-1}$ 3324, 3027, 1644, 1425, 1313, 1205, 966, 755, 721; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ 7.22–7.42 (10H, m), 7.48–7.64 (5H, m), 7.73 (1H, dd, *J* 0.7 and 7.5), 7.98 (1H, d, *J* 4.9), 8.07 (1H, d, *J* 7.9), 8.42 (1H, d, *J* 4.9) and 10.32 (1H, br s, $D_2O$ exch, NH) ppm; $^{13}$C-NMR (75 MHz, $CDCl_3$) $\delta$ 112.0 (CH), 118.5 (CH), 120.7 (Cq), 120.8 (CH), 121.8 (CH), 126.8 (CH), 126.9 (CH), 127.1 (CH), 128.6 (CH), 129.2 (CH), 129.6 (CH), 130.2 (CH), 130.6 (CH), 131.4 (Cq), 136.3 (Cq), 136.6 (Cq), 138.5 (CH), 138.5 (Cq), 139.6 (Cq), 140.1 (Cq), 140.5 (Cq), 141.1 (Cq), 141.5 (Cq) and 200.4 (CO) ppm; *m/z* (ES$^+$) 425 (MH$^+$); *m/z* (FAB$^+$) 425 (MH$^+$) (found: MH$^+$, 425.16543; $C_{30}H_{20}N_2O$ requires 425.16540).

**(9*H*-β-Carbolin-1-yl)-[1,1′;4′,1″]terphenyl-3-yl-methanone 8n.** 97% Yield, yellow solid; mp 216–217 °C (from EtOH); Found: C, 84.80; H, 4.80; N 6.58%; $C_{30}H_{20}N_2O$ requires: C, 84.88; H, 4.75; N, 6.60%; $\nu_{max}$/cm$^{-1}$ 3424, 3034, 1619, 1425, 1315, 1203, 977, 757, 733, 717; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ 7.37 (2H, quintet, *J* 3.8), 7.47 (2H, t, *J* 7.5), 7.61–7.67 (5H, m), 7.73 (4H, q, *J* 8.3 and 17.5), 7.89 (1H, d, *J* 7.8), 8.19–8.22 (2H, m), 8.30 (1H, d, *J* 7.7), 8.57 (1H, br t), 8.65 (1H, d, *J* 4.9) and 10.48 (1H, br s, $D_2O$ exch, NH) ppm; $^{13}$C-NMR (75 MHz, $CDCl_3$) $\delta$ 112.0 (CH), 118.6 (CH), 120.8 (Cq), 120.8 (CH), 121.8 (CH), 127.1 (CH), 127.4 (CH), 127.6 (CH), 127.7 (CH), 128.5 (CH), 128.8 (CH), 129.3 (CH), 129.7 (CH), 130.3 (CH), 130.9 (CH), 131.7 (Cq), 136.3 (Cq), 137.2 (Cq), 137.4 (Cq), 138.2 (CH), 139.5 (Cq), 140.4 (Cq), 140.5 (Cq), 140.6 (Cq), 140.5 (Cq), 141.1 (Cq) and 195.6 (CO) ppm; *m/z* (ES$^+$) 425 (MH$^+$); *m/z* (FAB$^+$) 425 (MH$^+$) (found: MH$^+$, 425.16536; $C_{30}H_{20}N_2O$ requires 425.16540).

**(9*H*-β-Carbolin-1-yl)-[1,1′;4′,1″]terphenyl-4-yl-methanone 8o.** 82% Yield, yellow solid; mp 255–256 °C (from EtOH); $\nu_{max}$/cm$^{-1}$ 3387, 3053, 3034, 1640, 1426, 1316, 1247, 1206, 1117, 970, 758, 735; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ 7.34–7.40 (2H, m), 7.48 (2H, t, *J* 7.6), 7.63–7.84 (10H, m), 8.19–8.21 (2H, m), 8.44 (2H, d, *J* 8.2), 8.66 (1H, d, *J* 4.9) and 10.48 (1H, br s, $D_2O$ exch, NH) ppm; $^{13}$C-NMR (75 MHz, DMSO) $\delta$ 113.5 (CH), 116.2 (CH), 119.4 (CH), 120.6 (CH), 120.7 (Cq), 122.3 (CH), 126.4 (CH), 127.8 (CH), 128.0 (CH), 128.2 (CH), 128.4 (CH), 129.2 (CH), 129.5 (Cq), 131.6 (CH), 136.3 (Cq), 136.8 (Cq), 136.9 (Cq), 137.7 (CH), 138.6 (Cq), 139.9 (Cq), 140.5 (Cq), 142.2 (Cq), 143.6 (Cq) and 193.6 (CO) ppm; *m/z* (ES$^+$) 425 (MH$^+$); *m/z* (FAB$^+$) 425 (MH$^+$) (found: MH$^+$, 425.16538; $C_{30}H_{20}N_2O$ requires 425.16540).

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# Design, synthesis and biological evaluation of new tryptamine and tetrahydro-β-carboline-based selective inhibitors of CDK4

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This paper is dedicated to the memory of Prof. Yoshihiko Ito of Kyoto University, Japan.

*Keywords:*
CDK4 inhibitors
Suzuki–Miyaura reaction
Anti-cancer
Fascaplysin

ABSTRACT

We present the design, synthesis and biological activity of a library of substituted (biphenylcarbonyl)-tryptamine and (biphenylcarbonyl)-tetrahydro-β-carboline compounds related to the natural product fascaplysin, as novel inhibitors of CDK4/cyclin D1. We show all these molecules, prepared using the Suzuki–Miyaura reaction, being selective inhibitors of CDK4 over CDK2. The most active compounds have a CDK4 $IC_{50}$ in the range 9–11 μM, three of them containing the *para*-biphenyl plus *para*-substituents supporting the existence of a π-stacking pocket within the active site of CDK4.



## 1. Introduction

The development of new inhibitors of the cyclin-dependent kinases is an ongoing area of research in the anti-cancer field.[1] The importance of the development of new inhibitors could be exemplified by the recent validation of CDK4/cyclinD1 as an anti-cancer drug target in MCF-7 breast cancer cells.[2]

The natural pigment fascaplysin (**1**, Fig. 1) was first isolated from the marine sponge *Fascaplysinopsis* Bergquist sp.[3] in 1988 and has a range of interesting properties.[4–6] It inhibits the growth of several organisms including *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Saccharomyces cerevisiae*, it is capable of suppressing the proliferation of mouse leukaemia cells and is a potent inhibitor of cyclin-dependant kinase 4 (CDK4), causing cell cycle arrest at the G1 phase of the cell cycle in both normal and tumour cell lines.[7] Fascaplysin (**1**) itself has limited potential as an anti-cancer drug due to its toxic side effects, these are thought to arise largely from the ability of its planar structure to intercalate into the structure of DNA.[8]

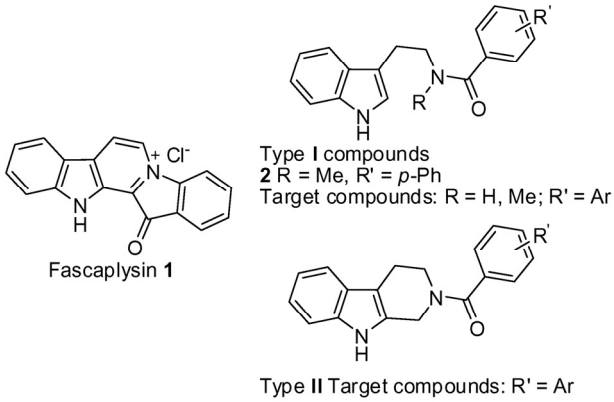


**Figure. 1.** Fascaplysin **1** and type **I**/**II** target compounds.

We have previously reported a systematic study on the synthesis and biological activity of non-planar analogues of fascaplysin.[9] To devise a potent, non-toxic (non-planar) CDK4 inhibitor based

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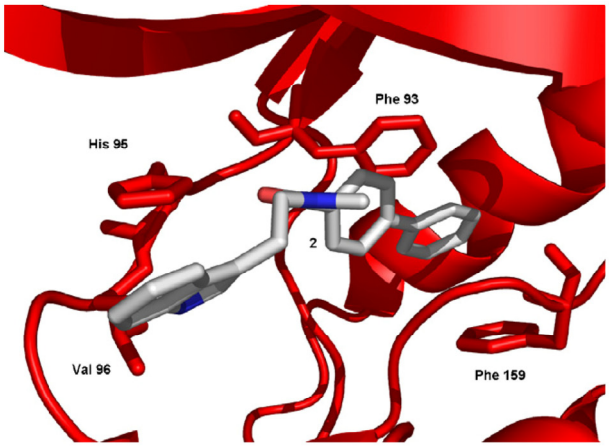



**Figure. 2.** Compound **2** docked with the CDK4 homology model.[9b]

on fascaplysin (**1**), we designed a series of tryptamine-based analogues of type **I** (Fig. 1).[9a,9b] Moreover, a series of related tetrahydro-β-carbolines of type **II** (Fig. 1), that have less conformational freedom, were synthesised.[9b] The most active compound reported in these series was biphenyl-4-carboxylic acid [2-(1*H*-indol-3-yl)-ethyl]-methyl-amide (**2**, Fig. 1), which has an $IC_{50}$ of 6 μM for the inhibition of CDK4/cyclin D1 and 521 μM for the inhibition of CDK2/cyclin A.[10]

These results, coupled with in silico studies using our homology model of CDK4 postulated the existence of a strong π-stacking interaction between the terminal phenyl ring of compound **2** and two phenylalanine residues (Phe 93 and Phe 159) within the active site of CDK4 (Fig. 2),[9b] possibly analogous to the 'Phe 80 pocket' of CDK2,[11] where the amino acid residue Phe 93 of CDK4 would be equivalent to Phe 80 in CDK2.

In this paper, taking compound **2** as a lead, a series of bi- and tri-phenyl derivatives of type **I** and **II** (Fig. 1) were synthesised using the Suzuki–Miyaura reaction in an effort to investigate the nature and scope of the π-stacking interaction in the proposed 'Phe 93 pocket' of CDK4 as a possible target for the development of future inhibitors of this enzyme.

## 2. Results and discussion

### 2.1. Synthesis

Compound **2** was originally synthesised from *N*-ω-methyl tryptamine and biphenyl carbonyl chloride;[9a,9b] however, this synthetic route does not lend itself to the synthesis of the target compounds of type **I**/**II**, so a new strategy was designed using the Suzuki–Miyaura coupling reaction as the key step as exemplified in Scheme 1 in the retro-synthetic analysis of compound **2**. Hence, a library of analogues of type **I**/**II** bearing substitution on the terminal phenyl ring of the biphenyl system, differing groups on the chain nitrogen and alterations to the tryptamine backbone structure, were efficiently synthesised using this strategy (Schemes 2, 3 and Fig. 3).

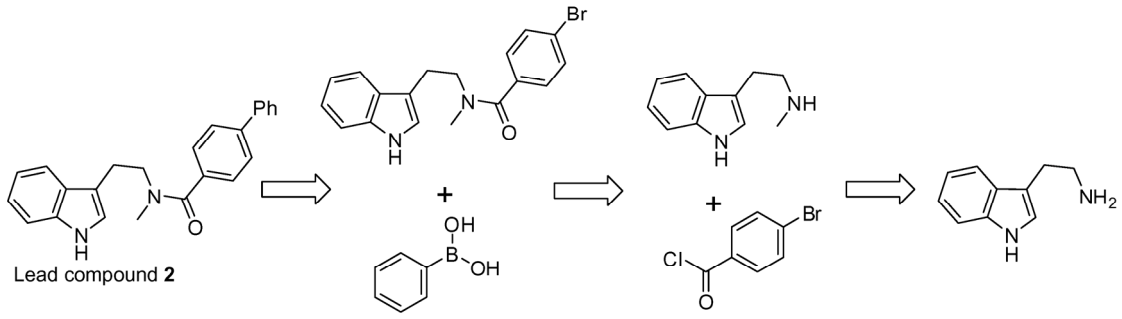
The Suzuki–Miyaura reaction is now a well-established procedure for the coupling of sp2 centres.[12–14] An aromatic halide, often an aromatic bromide, is coupled to an aromatic boronic acid using $Pd^0$ as a catalyst. The reaction is easily applicable to a wide range of substrates and generally proceeds in high yield, side products, and starting materials being easily separated by flash column chromatography. Even though the Suzuki–Miyaura reaction on indole substrates has been reported,[15] the closest example to the chemistry described in this paper is the conversion of a tryptamine sulfonamide to a biphenyl structure.[16] The results shown below further illustrate the versatility of this cross-coupling methodology for the synthesis of unsubstituted indole compounds.

Tryptamine (**3**) and *N*-ω-methyl tryptamine (**4**) (Scheme 2) were reacted with the corresponding *o*/*m*/*p*-benzoyl chlorides to produce key intermediates **5a–f** in moderate to good yields. Two series of type **I** *para*-biphenyl derivatives (**6a–k**) in which the terminal aromatic ring contained different substituents in the 4-position were prepared by Suzuki–Miyaura coupling of **5a** and **5d** with a range of 4-substituted boronic acids in yields of 42–65% (Scheme 1).

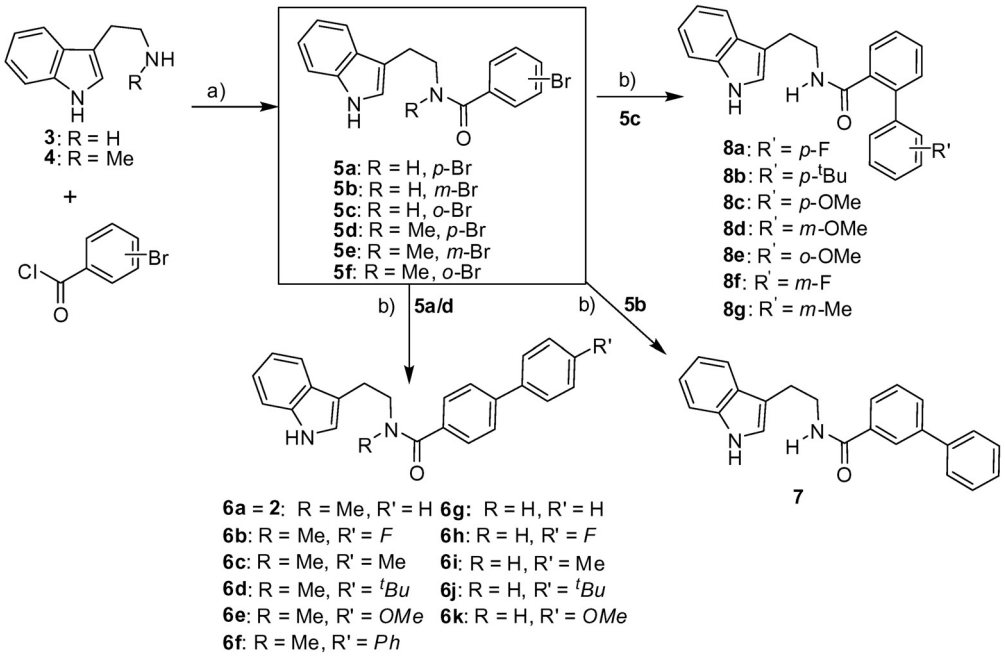
The biphenyl derivatives were also synthesised in the 1,2 form (*ortho*-biphenyl) by reaction of key intermediate **5c** with the appropriate phenylboronic acid to provide the series **8a–e** (Scheme 1). One example of 1,3-derivative (*meta*-biphenyl) was produced from the intermediate **5b** to give **7** (Scheme 1). As compound **7** was found to have a significantly lower $IC_{50}$ value than compounds in the *ortho*/*para*-biphenyl type **I** series in an initial screen, no more compounds with the *meta*-biphenyl moiety were prepared for this study.

A small library of 1,2- 1,3- and 1,4-biphenyl inhibitors of type **II** was produced from the three tetrahydro-β-carboline derivatives **10a–c** (Scheme 3). The *ortho*-biphenyls **11a–d** (in 56–82% yields), *meta*-biphenyls **12a–h** (in 61–88% yields) and the *para*-biphenyl derivatives **13a–h** (in 66–91% yields).

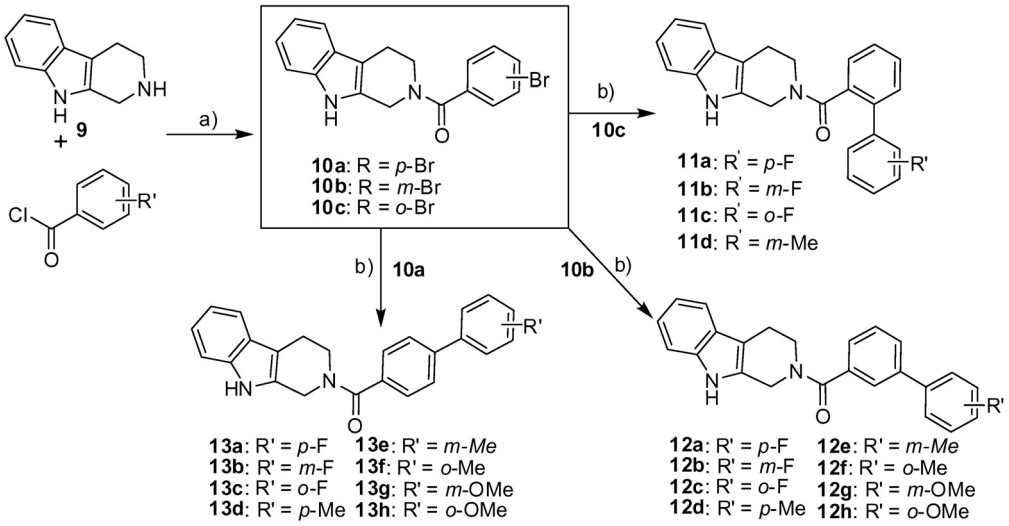
In some cases, the terminal phenyl ring of the biphenyl unit in type **I** and **II** compounds was replaced by a pyridine ring. Pyridine boronic acids have been used extensively in the Suzuki–Miyaura reaction; however, only very few examples involving indole and tryptamine substrates[17,16] have been reported. The compounds shown in Figure 3 further illustrate the range of structures that can be prepared using the highly versatile Suzuki–Miyaura reaction. For this purpose, key intermediates **5b**, **5d** and **10c** were




**Scheme 1.** Retrosynthetic analysis for lead compound **2**.




**Scheme 2.** Synthetic route to type **I** target compounds. Reagents and conditions: (a) $CH_2Cl_2$, NaOH (4 M, aq, 1 equiv), 0 °C 15 min then rt. 3 h; (b) $Pd(PPh_3)_4$, toluene, EtOH, $(HO)_2BC_6H_4R'$, $K_2CO_3$ (2 M, aq), 90 C, 24 h.




**Scheme 3.** Synthetic route to type **II** target compounds (for reagents and conditions see Scheme 2).

reacted with 3- and 4-pyridineboronic acids to produce compounds **14**, **15** and **16a–b** (54–80% yields).

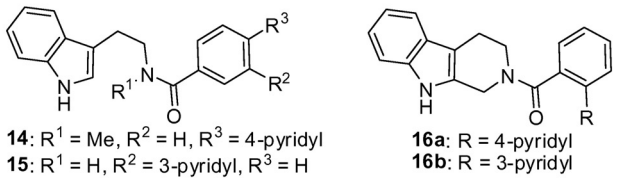



**Figure. 3.** Type **I**/**II** pyridinyl substituted inhibitors.

## 2.2. Biological evaluation

The *para*-biphenyl compound **6g**, Table 1 has an $IC_{50}$ for CDK4 inhibition of 16 μM. The addition of a *para*-methyl group in **6i** leads to a small increase in the $IC_{50}$ to 18 μM. However, with a *tert*-butyl group in **6j** the $IC_{50}$ falls to 9.2 μM. One possible explanation for this is the existence of a synergy between the proposed π-stacking interaction and a strong lipophilic component in the binding mode of the inhibitors in the proposed 'Phe 93 pocket' of CDK4. A *para*-fluoro substituent in **6h** gives an $IC_{50}$ of 15 μM, which is the same as the parent compound **6g**, within experimental error. A *para*-electron donating group such as OMe in **6k** leads to a substantially higher $IC_{50}$ of 48 μM.

**Table 1**
CDK4 activity versus CDK2 activity

| Compound | CDK4 inhibition[a] $IC_{50}$/μM | CDK2 inhibition[b] ($IC_{50}$/μM) |
|---|---|---|
| **1** Fascaplysin | 0.55 | 500 |
| **2 (6a)** | 6 ± 1 | 521 ± 12 |
| **6b** | 21 ± 3 | 875 ± 9 |
| **6c** | 24 ± 1.5 | 70 ± 4 |
| **6d** | 11.2 ± 1.3 | 745 ± 20 |
| **6e** | 49 ± 2 | 289 ± 10 |
| **6f** | 35 ± 2.5 | 91 ± 6 |
| **6g** | 16 ± 2.1 | 855 ± 7.5 |
| **6h** | 15 ± 1.5 | 764 ± 14 |
| **6i** | 18 ± 1.9 | 816 ± 12 |
| **6j** | 9.2 ± 0.8 | 790 ± 18 |
| **6k** | 48 ± 3.1 | 1085 ± 17 |
| **7** | 86 ± 4 | 1002 ± 13 |
| **8a** | 38 ± 2.6 | 1216 ± 19 |
| **8b** | 38 ± 2.5 | 262 ± 9 |
| **8c** | 79 ± 4 | 921 ± 12 |
| **8d** | 53 ± 1.8 | 926 ± 17 |
| **8e** | 26 ± 2 | 375 ± 10 |
| **8f** | 20 ± 1.5 | 417 ± 9 |
| **8g** | 15 ± 2 | 406 ± 12 |
| **11a** | 23 ± 1.5 | 357 ± 10 |
| **11b** | 35 ± 3 | 480 ± 10 |
| **11c** | 19 ± 2 | 434 ± 13 |
| **11e** | 26 ± 2 | 386 ± 11 |
| **12a** | 25 ± 1 | 318 ± 11 |
| **12b** | 27 ± 1 | 611 ± 12 |
| **12c** | 18 ± 2.5 | 335 ± 12 |
| **12d** | 24 ± 2 | 466 ± 10 |
| **12e** | 11 ± 1 | 465 ± 16 |
| **12f** | 75 ± 3 | 665 ± 13 |
| **12g** | 33 ± 3 | 362 ± 9 |
| **12h** | 79 ± 2.4 | 1109 ± 9 |
| **13a** | 12 ± 1.2 | 438 ± 12 |
| **13b** | 21 ± 1.5 | 296 ± 9 |
| **13c** | 17 ± 2 | 310 ± 15 |
| **13d** | 9 ± 0.8 | 736 ± 14 |
| **13e** | 25 ± 3 | 405 ± 12 |
| **13f** | 32 ± 2 | 340 ± 18 |
| **13g** | 28 ± 2 | 424 ± 12 |
| **13h** | 22 ± 1 | 321 ± 13 |
| **14** | 26 ± 2.5 | 92 ± 3 |
| **15** | 30 ± 2.5 | 150 ± 3.5 |
| **16a** | 39 ± 3 | 354 ± 5 |
| **16b** | 36 ± 4 | 312 ± 7 |

[a] CDK4-cyclin D1 assay, using GST-RB152 fusion protein as the substrate.
[b] CDK2-cyclin A assay using histone H1 as the substrate.

The trend described above for structures **6g–k** is also exhibited by derivatives of **2** (**6a**), **6b–f**. Compound **2** (**6a**) shows an $IC_{50}$ for inhibition of CDK4 of 6 μM. The *para*-methyl group in compound **6c** leads to a higher $IC_{50}$ of 24 μM, the figure then falls in the case of the *para*(*tert*-butyl) group in **6d** $IC_{50}$ 11.2 μM and the *para*-phenyl of **6f** leads to an $IC_{50}$ of 35 μM. In this series, the *para*-fluoro compound **6b** has a higher $IC_{50}$ of 21 μM compared to the parent compound **2** (**6a**). Compound **6e** with a *para*-OMe group is a weaker inhibitor, $IC_{50}$ 49 μM, a similar figure to **6k**.

Conversion to an *ortho*-biphenyl structure leads to compounds **8a–g**, where the $IC_{50}$ values are in general higher than parent compounds **6g–k**. The most active compounds in the *ortho*-biphenyl series are **8e** *ortho*-OMe $IC_{50}$ 26 μM, *meta*-fluoro **8f** 20 μM and finally the best compound is **8g** *meta*-Me $IC_{50}$ 15 μM.

The overall conclusion of the results of type **I** compounds is that the best $IC_{50}$ is obtained for the *para*-biphenyl compounds **6d** and **6j** (both with a *tert*-butyl group in the *para*-position of the outer phenyl ring) with $IC_{50}$ values in the same range as for the lead compound **2** (**6a**).

Type **II** tetrahydro-β-carboline inhibitors **11a–d**, **12a–h** and **13a–h** show a range of $IC_{50}$ values from 70 to 9 μM. In the *para*-biphenyl series, **13a–h,** three have $IC_{50}$ less than 20 μM, the best compound **13d**, with a *para*-Me group, has an $IC_{50}$ of 9 μM, *ortho*- and *para*-fluoro derivatives **13c** and **13a** show $IC_{50}$ of 17 and 12 μM, respectively. The best *meta*-biphenyl substituent is the *meta*-methyl compound **12e** ($IC_{50}$ 11 μM) and the best *ortho*-biphenyl compound is the *ortho*-fluoro compound **11c** ($IC_{50}$ 19 μM). Following the trend of the type **I** compounds, the *para*-biphenyl molecules showed, in general, higher $IC_{50}$ values than the *ortho*/*meta*-biphenyl series.

Inclusion of a pyridine moiety in the outer phenyl ring of type **I** and **II** target compounds (Fig. 3) leads to decreased CDK4 inhibition activities with respect to the lead compound **2**.

## 3. Conclusions

We have synthesised a library of biphenyl derivatives of tryptamine and tetra-hydro-β-carboline of type **I**/**II** using the Suzuki–Miyaura reaction, clearly showing the versatility of this important process.

Most of the compounds exhibit a clear CDK4/cyclin D1 selectivity (compared with CDK2/cyclin A). Additionally, *para*-biphenyl tryptamine and tetrahydro-β-carboline based compounds showed, in general, better biological results than the *ortho*- and *meta*-biphenyl analogues, pointing out the preferred orientation of the biphenyl moiety of the inhibitor within the proposed 'Phe 93 pocket' of the enzyme.

Three of the most active compounds **6d**, **6j** and **13d** ($IC_{50}$ = 9.2, 11.2 and 9 μM, respectively) are *para*-substituted biphenyls with *tert*-butyl or Me in the *para*-position. This suggests that a strong lipophylic component in the binding mode of these compounds, as well as the proposed π-stacking interaction of the inhibitors within the 'Phe 93 pocket', could be responsible for the observed CDK4/cyclin D1 inhibition results.

## 4. Experimental

### 4.1. Bio assays

Expression and purification of CDK4/GST-cyclinD1, CDK2/GST-cyclinA and GST-RB152. Fusion proteins of human cyclins A and D1, covalently linked to glutathione *S*-transferase (GST), were co-expressed with the catalytic subunits CDK2 and CDK4 in Sf-9 insect cells as described previously.[18–21]

Active enzyme complexes, containing a catalytic subunit bound to GST-Cyclin, were bound to glutathione–agarose columns (Sigma, Catalogue No. G3907) and were eluted from the columns with reduced glutathione. The reduced glutathione was removed by dialysing the enzymes in 10,000 MCO dialysis cassettes (Pierce, Catalogue No. 66830) with two buffer changes.

The GST-RB152 fusion construct was transformed into the *Escherichia coli* strain BL21(DE3)pLysS (Novagen Catalogue No. 69451-4). For expression of GST-RB152, the cells were induced in the presence of a final concentration of 4 mM isopropyl-β-thiogalactopyranoside (IPTG, Invitrogen Catalogue No. 15529-091) and were allowed to grow for 4 h in a shaking incubator at 37 °C and 220 rpm. Purification of the GST-RB152 protein was carried out as described previously.[21] Protein estimation was performed using the Bradford protein assay (Bio-Rad Laboratories) with bovine serum albumin (BSA) as the standard and the purity of the fusion protein was assessed by SDS–PAGE analysis. Proteins were stained with Coomassie blue for visualisation.

Kinase assays and $IC_{50}$ determination. The assay measures the depletion in ATP concentration as a result of phosphorylation of

retinoblastoma (GST-RB152) and Histone H1 (Upstate Biotech Catalogue No. 14-155) by CDK4 and CDK2, respectively. The assay was run in a 96-well format and all steps in one assay were carried out in a single white polystyrene plate (Sarstedt, Catalogue No. DPS-134-050A). The compounds were dissolved in DMSO as 10 mM stock solutions. Compounds were further serially diluted in kinase buffer (40 mM Tris (pH 7.5), 20 mM $MgCl_2$ and 0.1 mg/mL BSA) in order to obtain the desired concentrations. The kinase assay was performed in 50 µl of kinase buffer containing 2 µg of purified GST-RB152 (in case of Cdk4/GST-cyclin D1) or 3 µg of Histone H1 (in case of Cdk2/GST-cyclin A) and 6 µM ATP. The phosphatase and protease inhibitor cocktail containing β-glycerophosphate, sodium fluoride and sodium orthovanadate in the presence of reducing agent dithiothreitol was added at the final concentrations of 10, 0.1, 0.1 and 1, respectively. The assay was initiated by adding 200 ng of active enzyme complexes, and the plate was incubated for 30 min at 30 °C in a humidified incubator. The reaction was stopped by addition of equal volume of the Kinase Glow Reagent™ (Promega Catalogue No. V6711). The luminescence was measured using the Packard Luminometer (Fusion 3.50) and the rate of ATP depletion (rate of reaction) in the control blank reactions (i.e., without substrate or enzyme) was calculated and used to determine the $IC_{50}$ concentrations of compounds. In case of CDK4/cyclin D1 assay, the two compounds fascaplysin and flavopiridol with known $IC_{50}$ values were used to validate the assay. For the CDK2/cyclin A assay, roscovitine and flavopiridol were used as standards for the assay.

### 4.2. Chemistry

NMR spectra were recorded on Bruker DPX 300 ($^1$H, 300.13 MHz ; $^{13}$C, 75.47 MHz; $^{19}$F 282.39 MHz) or DPX 400 ($^1$H, 400.13 MHz; $^{13}$C, 100.61 MHz) spectrometers as indicated. Chemical shifts were measured relative to chloroform ($^1$H $\delta$ 7.26, $^{13}$C $\delta$ 77.0) or dimethylsulfoxide ($^1$H $\delta$ 2.50, $^{13}$C $\delta$ 39.43) and are expressed in ppm. Coupling constants *J* are expressed in Hertz and the measured values are corrected to one decimal place. Fast atom bombardment (FAB) mass spectra were recorded on a Kratos Concept 1H using xenon and *m*-nitrobenzyl alcohol as the matrix. Electrospray (ES) mass spectra were recorded on a Micromass Quattro LC spectrometer. Accurate mass was measured on a Kratos Concept 1H spectrometer using peak matching to stable reference peak. Flash column chromatography was carried out using Merck Kiesegel 60 (230–400 mesh). Dry solvents were provided by a PURE SOLV™ system from Innovative Technology Inc.

#### 4.2.1. [2-(1*H*-Indol-3-yl)-ethyl]-carbamic acid ethyl ester[9b,22]

To a solution of tryptamine **5** (10.00 g, 62.4 mmol) in $CHCl_3$ (156 mL) at 0 °C was added ethylchloroformate (5.97 mL, 62.4 mmol) and an aqueous solution of NaOH (15.60 mL, 4 M, 62.4 mmol). After addition, the mixture was stirred for 3 h at room temperature and then washed with water (150 mL), the aqueous phase was extracted with dichloromethane (2× 150 mL), and the combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure to give an orange oil, no purification was necessary. The oil was dried in vacuo to give the title compound **3** (13.78 g, 95%); $\delta_H$ (300 MHz, $CDCl_3$) 1.33 (3H, t, *J* 7.0), 3.05 (2H, t, *J* 6.5), 3.60 (2H, q, *J* 6.5), 4.24 (2H, q, *J* 7.0), 5.12 (1H, br s), 6.99 (1H, s), 7.23 (1H, t, *J* 6), 7.30 (1H, td, *J* 6 and 1.2), 7.42 (1H, d, *J* 7.7), 7.71 (1H, d, *J* 7.7), 8.75 (1H, s); $\delta_C$ (75 MHz, $CDCl_3$) 14.57 ($CH_3$), 25.64 ($CH_2$), 41.21 ($CH_2$), 60.72 ($CH_2$), 111.33 (CH), 112.33, 118.54 (CH), 119.11 (CH), 121.83 (CH), 122.26 (CH), 127.18, 136.39, 156.93; *m/z* (FAB$^+$) 232 M$^+$, 233 (M+H)$^+$, 465 (2M+H)$^+$. Found: M$^+$, 232.12126. $C_{13}H_{16}N_2O_2$ requires M, 232.12118.

#### 4.2.2. [2-(1*H*-Indol-3-yl)-ethyl]-methyl-amine 4[9b,22]

To a solution of [2-(1*H*-indol-3-yl)-ethyl]-carbamic acid ethyl ester (13.78 g, 59.4 mmol) in dry THF (110 mL) under $N_2$ at 0 °C was added portionwise LiAlH (6.76 g, 178 mmol). After the addition was complete, the mixture was heated under reflux for 1 h. The reaction was then cooled to 0 °C and the excess of $LiAlH_4$ was hydrolysed by adding successively and very carefully; water (13.25 mL), 15% aqueous solution of NaOH (13.25 mL) and water (3× 13.25 mL). During these steps, it was necessary to add THF (100 mL) to avoid the mixture becoming very thick. The suspension was filtered and the white solid, made up of LiOH and $Al(OH)_3$, was washed with THF (30 mL). The organic layer was dried ($MgSO_4$) and evaporated under reduced pressure to give the title compound **4** (9.24 g, 89%) as a beige solid; mp 82 °C; $\delta_H$ (300 MHz, $CDCl_3$) 1.47 (1H, s), 2.35 (3H, s), 2.81–2.86 (2H, m), 2.89–2.94 (2H, m), 6.80 (1H, s), 7.03 (1H, td, *J* 7.4 and 1.2), 7.10 (1H, td, *J* 7.4 and 1.2), 7.19 (1H, d, *J* 7.6), 7.54 (1H, d, *J* 7.6), 9.52 (1H, s); $\delta_C$ (75 MHz, $CDCl_3$) 25.4 ($CH_2$), 36.0 ($CH_3$), 51.8 ($CH_2$), 111.3 (CH), 112.9, 118.7 (CH), 118.9 (CH), 121.6 (CH), 122.5 (CH), 127.3, 136.5; *m/z* (FAB) 175.12354 (M+H$^+$ $C_{11}H_{15}N_2$ requires 175.12352). Found: C, 75.74; H, 8.04; N, 16.00. $C_{11}H_{15}N_2$ requires C, 75.82; H, 8.10; N, 16.08.

### 4.3. General procedure for the synthesis of 5a–c

To a stirred solution of tryptamine **3** (1.2 mmol) in dichloromethane (3 mL) at 0 °C was added slowly an aqueous solution of sodium hydroxide 4 M (1.2 mmol). After 5 min stirring at 0 °C was added dropwise the benzoyl chloride derivative (1.2 mmol). The mixture was stirred for 5 min at 0 °C and then for 3 h at room temperature. $H_2O$ (20 mL) was added. The two layers were separated and the aqueous phase was extracted with dichloromethane (3× 20 mL). The organic layers were dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by flash column chromatography on silica gel. Elution was made successively with; ethyl acetate/petroleum ether 50:50 and ethyl acetate, to give the title compound.

#### 4.3.1. 4-Bromo-*N*-[2-(1*H*-indol-3-yl)-ethyl]-benzamide (5a)

Pale yellow solid; yield 67%; mp 148–149 °C; $\delta_H$ (300 MHz, $(CD_3)_2SO$) 3.0 (2H, t, 7.4) 3.6 (2H, q, 6.9), 7.0 (1H, t, 7.4), 7.1 (1H, t, 7.4), 7.2 (1H, d, 1.5), 7.4 (1H, d, 8.1), 7.6 (1H, d, 7.8), 7.7 (2H, d, 8.7), 7.8 (2H, d, 8.4), 8.7 (1H, t, 5.4), 10.8 (1H, s); $\delta_C$ (75 MHz; $(CD_3)_2SO$) 25.1 ($CH_2$), 40.3 (under solvent peak, $CH_2$), 111.4 (CH), 111.8, 118.2 (CH, d), 120.9 (CH), 122.7 (CH), 126.2, 127.2, 128.1 (CH, 2C), 129.0 (CH, 2C), 136.2, 136.5, 167.0; *m/z* (FAB) 343.04453 (M+H$^+$ $C_{17}H_{16}N_2OBr$ requires 343.04467). Found: C,59.38; N, 8.16; H,4.35. $C_{17}H_{15}N_2OBr$ requires C, 59.49; N, 8.16; H, 4.40.

#### 4.3.2. 3-Bromo-*N*-[2-(1*H*-indol-3-yl)-ethyl]-benzamide (5b)

Off white solid; yield 79%; mp 132–135 °C, $\delta_H$ (300 MHz; $(CD_3)_2SO$) 3.0 (2H, t, 7.6,), 3.6 (1H, aq, 7.0), 7.0 (1H, t, 7.0), 7.1 (1H, t, 7.0), 7.2 (1H, d, 2.1), 7.4 (1H, t, 7.9), 7.6 (1H, d, 8.9), 7.7 (1H, d, 8.9), 7.9 (1H, d, 7.9), 8.1 (1H, s), 10.8 (1H, s), $\delta_C$ (75 MHz; $(CD_3)_2SO$) 25.0 ($CH_2$), 40.3 ($CH_2$), 111.4, 111.8, 118.2, 120.9, 121.6, 122.6, 126.3, 127.3, 129.9, 130.5, 133.8, 136.2, 136.9, 164.6. *m/z* (FAB) 343.04427 (M+H$^+$ $C_{17}H_{16}BrN_2O$ requires 343.04460).

#### 4.3.3. 2-Bromo-*N*-[2-(1*H*-indol-3-yl)-ethyl]-benzamide (5c)

Off white solid; yield 86%; mp 144–145 °C; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 3.0 (2H, t, 7.7), 3.5 (2H, aq, 6.9), 7.0 (1H, dt, 1.2, 7.9), 7.1 (1H, dt, 1.2, 6.9), 7.2 (1H, d, 2.2), 7.3–7.5 (4H, br m), 7.6 (1H, d, 7.7), 8.6 (1H, t, 5.7), 10.9 (1H, s); $\delta_C$ (75 MHz; $(CD_3)_2SO$) 25.5 ($CH_2$), 40.4 ($CH_2$), 111.9 (CH), 112.1, 118.7 (CH) (2C), 119.5, 121.4

(CH), 123.3 (CH), 127.7, 128.0 (CH), 129.2 (CH), 131.2 (CH), 133.1 (CH), 136.7, 139.8, 167.7. *m/z* (FAB) 343.04417 ($M+H^+$ $C_{17}H_{16}BrN_2O$ requires 343.04460).

### 4.4. General procedure for the synthesis of 5d/e

To a stirred solution of 2-(1*H*-indol-3-yl)-ethyl]-methyl-amine **4** (1.2 mmol) in dichloromethane (3 mL) at 0 °C was added slowly an aqueous solution of sodium hydroxide 4 M (1.2 mmol). After 5 min stirring at 0 °C was added dropwise the appropriate benzoyl chloride derivative (1.2 mmol). The mixture was stirred for 5 min at 0 °C and for 3 h at room temperature. Water (20 mL) was added and the two layers were separated, the aqueous phase was extracted with dichloromethane (3× 20 mL). The organic layers were then dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by flash column chromatography on silica gel. Elution was made successively with; ethyl acetate/petroleum ether 50:50 and ethyl acetate, to give the expected compound.

#### 4.4.1. 4-Bromo-*N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-methyl-benzamide (5d)[9b]

White solid; yield 73%; mp 160 °C; rotamers 1/1.5 (from the duplicated triplet signal ($^1H$) at 3.45 and 3.77 ppm); $\delta_H$ (300 MHz, $CDCl_3$) $\delta$ (*major rotamer*) 2.83 (2H, t, *J* 6.3), 3.07 (3H, s), 3.45 (2H, t, *J* 6.3), 6.71–7.62 (9H, m), 8.41 (1H, br s); $\delta$ (distinct peaks for *minor rotamer*) 2.76 (3H, s), 3.77 (2H, distorted t, *J* 7.1); $\delta_C$ (75 MHz, $CDCl_3$) $\delta$ (*major rotamer*) 24.2 ($CH_2$), 33.0 ($CH_3$), 51.7 ($CH_2$), 111.4 (CH), 112.7, 118.0 (CH), 119.4 (CH), 122.1 (CH), 122.4 (CH), 123.3, 127.0, 128.1 (2CH), 131.3 (2× CH), 135.0, 136.3, 171.4; $\delta$ (distinct peaks for *minor rotamer*) 22.9 ($CH_2$), 38.2 ($CH_3$), 48.6 ($CH_2$), 118.7 (CH), 123.7, 127.5, 128.7 (CH), 131.6 (CH), 135.5, 170.4; *m/z* ($ES^+$) 358 $MH^{+;}$ ($ES^-$) 356 $(M-H)^-$; *m/z* (FAB) $M^+$, 357.06027 ($M^+$ $C_{18}H_{17}BrN_2O$ requires 357.06025). Found: C, 60.58; H, 4.96; N, 7.76. $C_{18}H_{17}BrN_2O$ requires C, 60.52; H, 4.80; N, 7.84.

#### 4.4.2. 3-Bromo-*N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-methyl-benzamide (5e)[9b]

White solid; yield 76%; mp 166 °C; rotamers 1/1.4 (from the duplicated triplet signal ($^1H$) at 3.46 and 3.78 ppm); $\delta_H$ (300 MHz, $CDCl_3$) $\delta$ (*major rotamer*) 2.87 (2H, t, *J* 6.9), 3.09 (3H, s), 3.46 (2H, t, *J* 6.9), 6.76–7.61 (9H, m), 8.25 (1H, br s); $\delta$ (distinct peaks for *minor rotamer*) 2.76 (3H, s), 3.78 (2H, t, *J* 6.9); $\delta_C$ (75 MHz, $CDCl_3$) $\delta$ (*major rotamer*) 24.2 ($CH_2$), 33.1 ($CH_3$), 51.9 ($CH_2$), 111.4 (CH), 112.7, 118.0 (CH), 119.4 (CH), 122.1 (CH), 122.3 (CH), 125.0 (CH), 127.0, 129.8 (CH), 129.9 (CH), 132.1 (CH), 133.0, 136.2, 138.2, 170.7; $\delta$ (distinct peaks for *minor rotamer*) 22.9 ($CH_2$), 38.2 ($CH_3$), 48.6 ($CH_2$), 118.7 (CH), 125.5 (CH), 127.5, 129.5 (CH), 130.1 (CH), 132.5 (CH), 136.0, 138.6, 169.7; *m/z* ($ES^+$) 357 $M^+$, 358 $MH^{+;}$ ($ES^-$) 356 $(M-H)^{-;}$ *m/z* (FAB) 357.06030 ($M^+$ $C_{18}H_{17}BrN_2O$ requires 357.06025). Found: C, 60.38; H, 4.80; N, 7.73. $C_{18}H_{17}BrN_2O$ requires C, 60.52; H, 4.80; N, 7.84.

#### 4.4.3. 2-Bromo-*N*-[2-(1*H*-indol-3-yl)-ethyl]-*N*-methyl-benzamide (5f)[9b]

Tan solid; yield 48%; mp 205 °C; rotamers 1/1.2 (from the duplicated triplet signal ($^1$H) at 3.34 and 3.79 ppm); $\delta_H$ (400 MHz, 363 K, $(CD_3)_2SO$) $\delta$ (*major rotamer*) 2.92 (2H, t, *J* 7.3), 3.10 (3H, s), 3.34 (2H, t, *J* 7.3), 6.90–7.10 (3H, m), 7.19–7.44 (5H, m), 7.63 (1H, br t, estimated *J* 9.4), 10.61 (1H, br s); $\delta$ (distinct peaks for *minor rotamer*) 2.77 (3H, s), 3.79 (2H, t, *J* 7.6), 6.84 (1H, t, *J* 7.4); $\delta_C$ (75 MHz, $(CD_3)_2SO$) $\delta$ (*major rotamer*) 24.3 ($CH_2$), 32.4 ($CH_3$), 51.3 ($CH_2$), 110.7, 111.9 (CH), 118.1 (CH), 118.7 (CH), 118.8, 121.4 (CH), 123.5 (CH), 127.7, 128.2 (CH), 128.5 (CH), 130.7 (CH), 132.7 (CH), 136.6, 138.8, 168.3; $\delta$ (distinct peaks for *minor rotamer*) 22.9 ($CH_2$), 36.5 ($CH_3$), 47.7 ($CH_2$), 111.6, 118.7 (CH), 118.9, 121.4 (CH), 123.4 (CH), 127.3, 130.9 (CH), 132.9 (CH), 136.8, 139.2, 168.2; *m/z* ($ES^+$) 358; ($ES^-$) 356 $(M-H)^-$; *m/z* ($FAB^+$) 357.06015 ($M^+$ $C_{18}H_{17}BrN_2O$ requires 357.06025). Found: C, 60.38; H, 4.81; N, 7.81. $C_{18}H_{17}BrN_2O$ requires C, 60.52; H, 4.80; N, 7.84.

### 4.5. General procedure for the preparation of compounds 2 (6a), 6b–k, 7, 8a–g, 11a–d, 12a–h, 13a–h, 14, 15 and 16a–b: The Suzuki coupling reaction

To a stirred solution of the corresponding brominated intermediate (**5a–f**, **10a–c**, 1 mmol) in toluene (10 mL), under nitrogen, were added $K_2CO_3$ (1 mmol, 2 M aqueous) and $Pd(PPh_3)_4$ (5 mol%, 0.05 mmol). The solution was stirred for 20 min at room temperature before the addition of a solution of the appropriately substituted boronic acid, (1.2 mmol) in EtOH (10 mL). The reaction mixture was heated to 90 °C for 24 h, then allowed to cool to room temperature before the addition of $H_2O_2$ (30%, 1 mL), the reaction mixture was then stirred for a further 1 h. The desired product was extracted into $CHCl_3$, washed with saturated brine solution (2× 25 mL) and $H_2O$ (2× 25 mL), aqueous washings being re-extracted with $CH_2Cl_2$ (3× 50 mL), the combined organic phases were then dried over anhydrous sodium or magnesium sulfate, filtered and isolated under reduced pressure. The crude product was then purified by flash column chromatography on silica from ethyl acetate–petroleum ether (40–60 °C).

#### 4.5.1. *N*-(2-(1*H*-Indol-3-yl)ethyl)-4′-fluoro-*N*-methylbiphenyl-4-carboxamide (6b)

Pale yellow solid; yield 65%; mp 181–183 °C; $\delta_H$ (300 MHz; $(CD_3)_2SO$) rotamers 1/1.25 (from the duplicated triplet signal ($^1H$) at 3.47 and 3.74 ppm); 2.91 (3H, br), 3.08 (2H, br), 3.47 (2H, br), 6.72 (distorted t), 6.93–7.06 (4H, m), 7.19–7.34 (7H, m), 7.45 (1H, br d, 7.2), 7.54 (2H, br d, 7.6), 7.70 (5H, m), 10.83 (1H, br s); $\delta$ (*distinct peaks for minor rotamer*) 3.74 (2H, br), 2.91 (3H, br), 3.08 (2H, br), 10.87 (1H, br s); $\delta_C$ (75 MHz; $(CD_3)_2SO$) 24.4 ($CH_2$), 32.8 ($CH_3$), 51.9 ($CH_2$) 110.9, 111.9 (CH), 116.3 (d, $^2J_{CF}$, 21, CH), 118.3 (CH), 118.7 (CH), 121.4 (CH), 123.7 (CH), 126.8 (CH), 127.4 (CH), 128.0, 129.2 (d, $^3J_{CF}$ 8, CH), 136.2, 136.4, 136.7, 140.0, 162.5 (d, $^1J_{CF}$, 245), 171.0; (*distinct peaks for minor rotamer*) 23.1 ($CH_2$), 37.9 ($CH_3$), 48.4 ($CH_2$), 123.3 (CH), 128.0, 170.0. Found: C, 77.32; N, 7.42; H, 5.60. $C_{24}H_{21}N_2OF$ requires C, 77.40; N, 7.52; H, 5.68.

#### 4.5.2. *N*-(2-(1*H*-Indol-3-yl)ethyl)-*N*,4′-dimethylbiphenyl-4-carboxamide (6c)

Off white solid; yield 44%; mp 215–217 °C; $\delta_H$ (300 MHz; $(CD_3)_2SO$) Rotomers 1/2.1 (from the duplicated triplet signal ($^1H$) at 3.46 and 3.73 ppm), 2.91 (3H, br), 3.07 (2H, br), 3.46 (2H, br), 6.70 (1H, br), 6.91–7.05 (3H, m), 7.19–7.30 (4H, m), 7.42–7.44 (1H, m), 7.56–7.67 (4H, m), 10.8 (1H, br s); $\delta$ (*distinct peaks for minor rotamer*) 3.73 (2H, br), 10.83 (1H, br s); $\delta_H$ (400 MHz; $(CD_3)_2SO$; 353 K) 2.38 (3H, s), 2.99 (5H, m), 3.64 (2H, br s), 6.89 (1H, br s), 7.05 (1H, t, 7.5), 7.10 (1H, br s), 7.23–7.36 (3H, m), 7.56–7.58 (2H, m), 7.61 (5H, d, 8.0), 10.60 (1H, s); $\delta_C$ (75 MHz; $(CD_3)_2SO$) 20.6 ($CH_3$), 23.9 ($CH_2$), 32.3 ($CH_3$), 51.4 ($CH_2$), 110.3, 111.3 (CH), 117.8 (CH), 118.2 (CH), 120.8 (CH), 123.0 (CH), 126.1 (CH, 2C), 126.5 (CH, 2C), 129.5 (CH, 4C), 135.4, 136.1, 136.5, 137.1, 140.4, 140.7, 170.5; $\delta$ (*distinct peaks for minor rotamer*) 22.5 ($CH_2$), 37.3 ($CH_3$), 47.8 ($CH_2$), 126.9 (CH), 127.4 (CH).

#### 4.5.3. *N*-(2-(1*H*-Indol-3-yl)ethyl)-4′-*tert*-butyl-*N*-methyl biphenyl-4-carboxamide (6d)

Off white solid; yield 51%; mp 178–180 °C, overlaping rotomers; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 1.36 (9H, s, $C(CH_3)_3$), 2.86 (3H, br s), 3.04–3.14 (2H, br), 3.60–3.82 (2H, br), 7.08–7.62 (13H, br), 10.05 (1H, br s); $\delta_C$ (75 MHz; $(CD_3)_2SO$) 22.6 ($CH_2$), 23.9 ($CH_2$), 31.0

($CH_3$), 32.3 ($CH_3$), 34.2 (C), 37.4 ($CH_3$), 47.9 ($CH_2$), 51.4 ($CH_2$), 110.4 (C), 111.4 (CH), 117.9 (CH), 120.9 (CH), 123.0 (CH), 125.7 (CH), 126.2 (CH), 126.4 (CH), 126.9 (CH), 127.5 (CH), 135.5 (C), 136.2 (C), 140.5 (C), 140.8 (C), 150.2 (C), 169.6 (C), 170.6 (C). Found: C, 81.81; N, 6.72; H, 7.27. $C_{28}H_{30}N_2O$ requires C, 81.91; N, 6.82; H, 7.36; *m/z* ($ES^+$) 411 [$M+H^+$], 433 [$M+Na^+$], ($ES^-$) 409 [$M-H^-$]; *m/z* (FAB) 411.24347 ($M+H^+$ $C_{28}H_{31}N_2O$ requires 411.24364).

#### 4.5.4. *N*-(2-(1*H*-Indol-3-yl)ethyl)-4′-methoxy-*N*-methylbiphenyl-4-carboxamide (6e)

Off white solid; yield 46%; mp 201–202 °C; $\delta_H$ (300 MHz; $(CD_3)_2SO$) Rotomers 1/1.8 (from the duplicated triplet signal ($^1H$) at 3.47 and 3.73 ppm), 2.91 (3H, br), 3.07 (2H, br), 3.46 (2H, br), 3.80 (3H, s), 6.71 (1H, m), 6.92–7.05 (4H, m), 7.18–7.20 (2H, m), 7.29–7.31 (1H, m), 7.41–7.53 (2H, m), 7.62 (3H, br m), 10.81 (1H, br s); $\delta$ (*distinct peaks for minor rotamer*) 3.07 (3H, br), 2.91 (2H, br), 3.73 (2H, br), $\delta_C$ (75 MHz; $(CD_3)_2SO$) 23.9 ($CH_2$), 32.3 ($CH_3$), 51.4 ($CH_2$), 55.1 ($CH_3$), 110.3, 111.3 (CH), 114.3 (CH, 4C), 117.8 (CH), 118.0 (CH) 120.8 (CH), 123.0 (CH), 125.8 (CH, 2C), 127.7 (CH, 2C), 131.7, 134.9, 136.1, 140.2, 140.5, 159.1, 170.5; $\delta$ (*distinct peaks for minor rotamer*) 22.5 ($CH_2$), 37.4 ($CH_3$), 47.8 ($CH_2$), 122.7 (CH), 126.8 (CH), 127.4 (CH); $\delta_C$ (100 MHz; $(CD_3)_2SO$, 353 K) 22.9 (CH2), 54.8 (CH3), 110.7, 110.8 (CH), 114.1 (CH), 117.5 (CH), 117.7 (CH), 120.4 (CH), 122.3 (CH), 125.3 (CH), 126.6 (CH), 126.8, 127.3 (CH), 131.5, 134.8, 135.9, 140.0, 158.9, 169.8.

#### 4.5.5. *N*-(2-(1*H*-Indol-3-yl)ethyl)-4′-phenyl-*N*-methylbiphenyl-4-carboxamide (6f)

Off white solid; yield 52%; mp 230–234 °C; $\delta_H$ (300 MHz; $(CD_3)_2SO$) rotamers 1/1.58 (from the duplicated triplet signal ($^1H$) at 3.49 and 3.75 ppm) 2.93 (3H, br), 3.09 (2H, br), 3.49 (2H, br), 6.74 (1H, br t, 7.0), 6.95–7.07 (2H, m), 7.23 (2H, br d, 7.4), 7.32 (1H, br d, 7.9), 7.37–7.42 (2H, m), 7.47–7.52 (3H, m), 7.63 (1H, d, 7.5), 7.74 (2H, br d, 7.4), 7.79 (4H, br s), 10.83 (1H, br s), $\delta$ (*distinct peaks for minor rotamer*) 3.75 (2H, br), 10.86 (1H, br s); $\delta_C$ (100 MHz; $(CD_3)_2SO$, 363 K) (*some peaks not observed due relaxation time*) 23.9, 111.9, 118.5, 118.7, 121.4, 123.3, 126.8, 127.0, 127.7, 128.0, 129.4, 136.6, 137.0, 139.0, 140.2, 140.2, 140.8, 170.7; *m/z* (FAB) 431.21230 ($M+H^+$ $C_{30}H_{27}N_2O$ requires 431.21234). Found: C, 3.56; N, 6.42; H, 5.94. $C_{30}H_{26}N_2O$ requires C, 83.69; N, 6.51; H, 6.09.

#### 4.5.6. *N*-(2-(1*H*-Indol-3-yl)ethyl)biphenyl-4-carboxamide (6g)

Off white solid; yield 63%; mp 183–185 °C; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 3.02 (2H, t, 7.5), 3.62 (2H, *ap*-q, 7.5, 5.6), 7.03 (1H, ddd, 7.5, 7.5, 1.1), 7.11 (1H, ddd, 7.5, 7.5, 1.1), 7.23 (1H, d, 2.2), 7.39 (1H, d, 7.5), 7.44 (1H, dd, 7.4, 1.1), 7.51 (2H, dd, 7.4, 7.4), 7.64 (1H, d, 7.4), 7.76 (2H, d, 7.4), 7.80 (2H, d, 8.4), 8.00 (2H, d, 8.4), 8.71 (1H, t, 5.6), 10.86 (1H, br s); $\delta_{13}$ (75 MHz; $(CD_3)_2SO$) 25.2, 40.3, 111.4, 111.9, 118.2, 118.3, 120.9, 122.6, 126.4, 126.8, 127.3, 127.8, 128.0, 129.0, 133.5, 136.2, 139.2, 142.5, 165.8; *m/z* (FAB) 341.16532 ($M+H^+$ $C_{23}H_{21}N_2O$ requires 341.16546). Found: C, 81.09; N, 8.18; H, 5.95. $C_{23}H_{20}N_2O$ requires C, 81.15; N, 8.23; H, 5.92.

#### 4.5.7. *N*-(2-(1*H*-Indol-3-yl)ethyl)-4′-fluorobiphenyl-4-carboxamide (6h)

Off white solid; yield 59%; mp 181–183 °C; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 3.03 (2H, dd, 7.4, 7.4), 3.62 (2H, td, 7.4, 5.6), 7.04 (1H, td, 7.4, 1.1), 7.13 (1H, ddd, 7.4, 7.4, 1.1), 7.25 (1H, d, 2.2), 7.34–7.41 (3H, m), 7.65 (1H, d, 7.7), 7.79–7.86 (2H, m), 7.81 (2H, d, 8.4), 8.00 (2H, d, 8.4), 8.72 (1H, t, 5.6), 10.87 (1H, br s); $\delta_C$ (75 MHz; $(CD_3)_2SO$) 25.7 ($CH_2$), 40.7 ($CH_2$), 111.9 (CH), 112.4, 116.3 (d, $^2J_{CF}$, 21, CH), 118.7 (CH), 121.4 (CH), 123.1 (CH), 126.9 (CH, 2C), 127.8, 128.3 (CH, 2C), 129.4 (d, $^3J_{CF}$, 8, CH), 136.2 (d, $^4J_{CF}$, 3), 136.7 (CH), 142.0, 162.7 (d, $^1J_{CF}$, 245) 166.2; *m/z* (FAB) 359.15593 ($M+H^+$ $C_{23}H_{20}N_2OF$ requires 359.15597). Found: C, 76.97; N, 7.75; H, 5.51. $C_{23}H_{19}N_2OF$ requires C, 77.08; N, 7.82; H, 5.34.

#### 4.5.8. *N*-(2-(1*H*-Indol-3-yl)ethyl)-4′-methylbiphenyl-4-carboxamide (6i)

Off white solid; yield 42%; mp 204–206 °C; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 2.39 (3H, s), 3.02 (2H, t, 7.5), 3.62 (2H, dt, 7.5, 5.6), 7.03 (1H, ddd, 7.4, 7.4, 1.1), 7.12 (1H, ddd, 7.4, 7.4, 1.1), 7.24 (1H, d, 2.2), 7.33 (2H, d, 8.1), 7.39 (1H, d, 8.1), 7.35 (1H, d, 8.1), 7.66 (2H, d, 8.1), 7.78 (2H, d, 8.4), 7.98 (2H, d, 8.4), 8.69 (1H, t, 5.6), 10.86 (1H, br); $\delta_C$ (75 MHz; $(CD_3)_2SO$) 21.2 ($CH_2$), 25.7 ($CH_3$), 40.7 ($CH_2$), 111.9 (CH), 112.4, 118.7 (CH), 118.8 (CH), 121.4 (CH), 123.1 (CH), 126.6 (CH, 2C), 127.1 (CH, 2C), 127.8, 128.3 (CH, 2C), 130.1 (CH, 2C), 133.7, 136.8 (2C), 137.9, 143.0, 166.3; *m/z* (FAB) 355.18110 ($M+H^+$ $C_{24}H_{23}N_2O$ requires 355.18104). Found: C, 79.20; N, 7.40; H, 5.84. $C_{24}H_{22}N_2O$ requires C, 81.33; N, 7.90; H, 6.26.

#### 4.5.9. *N*-(2-(1*H*-Indol-3-yl)ethyl)-4′-*tert*-butylbiphenyl-4-carboxamide (6j)

Off white solid; yield 61%; mp 201–203 °C; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 1.37 (9H, s), 3.03 (2H, t, 7.3), 3.63 (2H, td, 7.3, 5.5), 7.04 (1H, br dd, 7.5, 7.5), 7.13 (1H, br dd, 7.5, 7.5), 7.25 (1H, d, 1.6), 7.40 (1H, d, 7.5), 7.55 (2H, d, 8.4), 7.66 (1H, d, 7.5), 7.71 (2H, d, 8.4), 7.80 (2H, d, 8.3), 7.99 (2H, d, 8.3), 8.70 (1H, dd, 5.5), 10.87 (1H, br s); $\delta_C$ (75 MHz; $(CD_3)_2SO$) 25.7 ($CH_2$), 31.6 ($CH_3$, 3C), 34.8, 40.7 ($CH_2$), 111.9, 112.4 (CH), 118.7 (CH), 118.8 (CH), 121.4 (CH), 123.1 (CH), 126.3 (CH, 2C), 126.7 (CH, 2C), 127.0 (CH, 2C), 127.8, 128.3 (CH, 2C), 133.7, 136.7, 136.8, 142.9, 151.0, 166.3; *m/z* (FAB) 397.22798 ($M+H^+$ $C_{27}H_{29}N_2O$ requires 397.22799). Found: C, 81.76; N, 6.97; H, 7.03. $C_{27}H_{28}N_2O$ requires C, 81.78; N, 7.06; H, 7.12.

#### 4.5.10. *N*-(2-(1*H*-Indol-3-yl)ethyl)-4′-methoxybiphenyl-4-carboxamide (6k)

Off white solid; yield 45%; mp 200–205 °C; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 2.99 (2H, t, 7.4), 3.59 (2H, td, 7.4, 5.6), 3.81 (3H, s), 7.00 (1H, ddd, 7.4, 7.4, 1.1), 7.05 (2H, d, 8.8), 7.09 (1H, ddd, 7.4, 7.4, 1.1), 7.21 (1H, d, 2.2), 7.36 (1H, d, 7.4), 7.62 (2H, d, 7.4), 7.69 (2H, d, 8.8), 7.72 (2H, d, 8.5), 7.94 (2H, d, 8.5), 8.65 (1H, t, 5.6), 10.83 (1H, br s); $\delta_C$ (75 MHz; $(CD_3)_2SO$) 25.7 ($CH_2$), 40.7 ($CH_2$), 55.7 ($CH_3$),111.9 (CH), 112.4, 114.9 (CH), 118.7 (CH), 118.8 (CH), 121.4 (CH), 123.1 (CH), 126.3 (CH), 127.8, 128.3 (CH), 128.5 (CH), 129.2 (CH), 129.3 (CH), 131.9 (CH), 132.5 (CH), 133.2, 136.7, 159.8, 166.3. Found: C, 77.92; N, 7.41; H, 5.89. $C_{24}H_{22}N_2O_2$ requires C, 77.81; N, 7.56; H, 5.99.

#### 4.5.11. *N*-(2-(1*H*-Indol-3-yl)ethyl)biphenyl-3-carboxamide (7)

Off white solid; yield 62%; mp 59–69 °C; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 2.98 (2H, t, 7.5), 3.58 (2H, td, 7.5, 5.5), 6.98 (1H, ddd, 7.5, 7.5, 1.1), 7.07 (1H, ddd, 7.5, 7.5, 1.1), 7.20 (1H, d, 2.2), 7.35 (1H, br d, 8.0), 7.42 (1H, br d, 7.3), 7.50 (2H, dd, 7.9, 7.9), 7.57 (1H, d, 7.9, 7.9), 7.60 (1H, d, 7.9), 7.73 (2H, dd, 7.9, 1.4), 7.80–7.86 (2H, m), 8.12 (1H, dd, 1.5, 1.5), 8.74 (1H, dd, 5.5, 5.5), 10.82 (1H, br s); $\delta_C$ (75 MHz; $(CD_3)_2SO$) 25.1 ($CH_2$), 40.3 ($CH_2$), 111.3 (CH), 111.8, 118.2 (CH), 118.2 (CH), 120.8 (CH), 122.6 (CH), 125.2 (CH), 126.3 (CH), 126.8 (CH, 2C), 127.2, 127.7 (CH), 128.9 (CH, 2C), 129.1 (CH), 135.3, 136.2, 139.5, 140.1, 165.9; *m/z* (FAB) 341.16544 ($M+H^+$ $C_{23}H_{21}N_2O$ requires 341.16539). Found: C, 77.39; N, 7.89; H, 5.78. $C_{23}H_{20}N_2O$ requires C, 81.15; N, 8.23; H, 5.92.

#### 4.5.12. *N*-(2-(1H-Indol-3-yl)ethyl)-4′-fluorobiphenyl-2-carboxamide (8a)

White solid; yield 28%; mp 157–159 °C; $\delta_H$ (300 MHz; $(CD_3)_2SO$) 2.78 (2H, t, 7.3), 7.04 (1H, t, 6.9), 7.12 (1H, t, 6.9), 7.16 (1H, d, 2.3), 7.19

(1H, t, 8.9), 7.43 (2H, d, 5.3), 7.44–7.47 δ (6H, m), 7.55 (1H, d, 7.4), 8.34 (1H, t, 5.7), 10.86 (1H, br s); $\delta_F$ (282 MHz, $CDCl_3$) −114.37 δ; $\delta_C$ (75 MHz; $(CD_3)_2SO$) 24.6 (CH2), 39.6 (CH2), 111.3 (CH), 111.6, 114.8 (d, $^2J_{CF}$, 21, CH, 2C), 118.1 (CH, 2C), 120.8 (CH), 122.3, 122.5 (CH), 127.0 (CH), 127.5 (CH), 129.2 (CH), 129.7 (CH), 130.2 (d, $^3J_{CF}$, 8, CH, 2C), 136.1, 136.5 (d, $^4J_{CF}$, 3), 137.3, 137.9, 161.5 (d, $^1J_{CF}$, 243), 168.8;); *m/z* ($ES^-$) 357 (100%, $M-H^-$) ($ES^+$) 359 (100% $M+H^+$); *m/z* (FAB) 359.1559 ($M+H^+$ $C_{23}H_{19}N_2OF$ requires 279.14981). Found: C, 74.99; N, 7.35; H, 4.62. $C_{23}H_{19}N_2OF$ requires C, 77.08; N, 7.82; H, 5.34.

#### 4.5.13. *N*-(2-(1*H*-Indol-3-yl)ethyl)-4′-*tert*-butylbiphenyl-2-carboxamide (8b)

White solid; yield 16%; mp 149–151 °C; $\delta_H$ (300 MHz; $CDCl_3$) 1.35 (9H, s) 2.62 (2H, t, 6.8) 3.50 (2H, td, 6.8, 5.4) 5.30 (1H, t, 5.4), 6.55 (1H, d, 2.0), 7.05 (1H, dd, 6.9, 6.9), 7.15 (1H, dd, 6.9, 6.9), 7.31 (1H, d, 8.1), 7.31–7.41 (4H, m), 7.37 (2H, d, 8.5), 7.45 (2H, d, 8.5), 7.64 (1H, d, 7.5), 7.87 (1H, br s); $\delta_C$ (75 MHz; $CDCl_3$) 24.5 ($CH_2$), 31.0 ($CH_3$, 3C), 34.1, 36.9 ($CH_2$), 111.3 (CH), 111.6, 118.0 (CH, 2C), 120.8 (CH), 122.4 (CH), 124.8 (CH, 2C), 126.7 (CH), 127.0, 127.5 (CH), 127.9 (CH, 2C), 129.1 (CH), 129.6 (CH), 136.1, 137.1, 137.3, 138.7, 149.4, 169.0; *m/z* (FAB) 397.2280 ($M+H^+$ $C_{27}N_2OH_{29}$ requires 397.22799).

#### 4.5.14. *N*-(2-(1*H*-Indol-3-yl)ethyl)-4′-methoxybiphenyl-2-carboxamide (8c)

White solid; yield 34%; mp 155–157 °C; $\delta_H$ (300 MHz; $CDCl_3$) 2.72 (2H, t, 6.8), 3.55 (2H, dt, 6.8), 3.80 (3H, s), 5.34 (1H, t, 4.8), 6.66 (1H, d, 2.3), 6.89 (2H, d, 8.8), 7.07 (1H, t, 6.9), 7.16 (1H, t, 6.9), 7.28–7.32 (2H, m), 7.30 (2H, d, 8.7), 7.36 (1H, d, 7.4), 7.41 (1H, d, 7.4), 7.48 (1H, d, 7.8), 7.64 (1H, d, 7.6), 7.99 (1H, br s); $\delta_C$ (75 MHz; $CDCl_3$) 24.6 ($CH_2$), 39.6 ($CH_2$), 55.0 ($CH_3$), 111.2 (CH), 111.6, 113.5 (CH, 2C), 118.1 (CH, 2C), 120.8 (CH), 122.5 (CH), 126.4 (CH), 127.1, 127.5 (CH), 129.1 (CH), 129.4 (CH, 2C), 129.5 (CH), 132.4, 136.1, 137.2, 138.5, 158.5, 169.1; *m/z* (FAB) 371.1759 ($M+H^+$ $C_{24}N_2O_2H_{23}$ requires 371.17595).

#### 4.5.15. *N*-(2-(1*H*-Indol-3-yl)ethyl)-3′-methoxybiphenyl-2-carboxamide (8d)

Pale orange solid; yield 38%; mp 156–158 °C; $\delta_H$ NMR (300 MHz, $(CD_3)_2SO$) 2.76 (2H, t, 7.3), 3.84 (3H, s), 6.99 (1H, d, 7.7), 7.04 (1H, t, 7.1), 7.07 (1H, s), 7.14 (1H, t, 7.1), 7.18 (1H, d, 1.6), 7.35 (1H, d, 7.9), 7.40–7.45 (2H, m), 7.42 (1H, d, 7.9), 7.50–7.55 (2H, m), 7.50 (1H, d, 6.9), 7.57 (2H, d, 7.2), 8.34 (1H, t, 5.7), 10.89 (1H, br s); $\delta_C$ (75 MHz; $CDCl_3$) 24.6 ($CH_2$), 39.7 ($CH_2$), 54.9 ($CH_3$), 111.3 (CH), 111.6, 112.6 (CH), 114.0 (CH), 118.1 (CH), 118.1 (CH), 120.7 (CH), 120.8 (CH), 122.4 (CH), 127.0 (CH), 127.1, 127.5 (CH), 129.1(CH, 2C), 129.6 (CH), 136.1, 137.4, 138.8, 141.5, 158.9, 169.0; *m/z* (FAB) 371.17598 ($M+H^+$ $C_{24}H_{23}N_2O_2$ requires 371.17595).

#### 4.5.16. *N*-(2-(1*H*-Indol-3-yl)ethyl)-2′-methoxybiphenyl-2-carboxamide (8e)

Off white solid; yield 72%; mp 137–139 °C; $\delta_H$ NMR (300 MHz, $CDCl_3$) 2.57 (2H, t, 7.0), 3.42 (2H, q, 6.9), 3.51 (3H, s), 5.58 (1H, t, 4.2), 6.64 (1H, d, 2.0), 6.71 (1H, d, 8.1), 6.88 (1H, t, 7.4), 6.99 (1H, t, 7.0), 7.06–7.11 (m, 2H), 7.15–7.20 (m, 2H), 7.24 (1H, d, 8.2), 7.32 (1H, d, 7.5), 7.36 (1H, d, 7.3), 7.40 (1H, d, 7.4), 7.68 (1H, d, 7.4), 8.10 (br s); $\delta_C$ (75 MHz; $(CD_3)_2SO$) 24.8 ($CH_2$), 39.6 ($CH_2$), 54.9 ($CH_3$), 110.7 (CH), 111.3 (CH), 111.6, 118.1 (CH, 2C), 120.1 (CH), 120.8 (CH), 122.4 (CH), 126.7 (CH), 127.1, 127.1 (CH), 128.6 (CH), 129.0 (CH), 129.3, 130.2 (CH), 130.8 (CH), 136.1, 136.3, 137.7, 155.9, 168.5; *m/z* (FAB) 371.17602 ($M+H^+$ $C_{24}H_{23}N_2O_2$ requires 371.17595).

#### 4.5.17. *N*-(2-(1*H*-Indol-3-yl)ethyl)-3′-fluorobiphenyl-2-carboxamide (8f)

Pale beige solid; yield 33%; mp 96–99 °C; $\delta_H$ NMR (300 MHz, $(CD_3)_2SO$) 2.67 (2H, t, 7.4), 6.96 (1H, t, 7.0), 7.05 (1H, t, 7.0), 7.10 (1H, d, 1.7), 7.14–7.24 (2H, m), 7.21 (1H, d, 5.5), 7.33 (1H, d, 7.9), 7.38–7.51 (4H, m), 7.43 (1H, d, 6.4), 7.50 (1H, d, 6.0), 8.36 (1H, t, 5.7), 10.81 (1H, br s); $\delta_C$ (75 MHz; $CDCl_3$) 24.6 ($CH_2$), 39.7 ($CH_2$), 111.3 (CH), 111.5, 113.8 (d, $^2J_{CF}$, 21, CH), 115.1 (d, $^2J_{CF}$, 22, CH), 118.0 (CH), 118.1 (CH), 120.8 (CH), 122.4 (CH), 124.5 (d, $^4J_{CF}$, 2, CH), 127.1, 127.5 (CH), 127.5 (CH), 129.3 (CH), 129.6 (CH), 130.0 (d, $^3J_{CF}$, 8, CH), 136.1, 137.4, 137.6 (d, $^4J_{CF}$, 2), 142.5 (d, $^3J_{CF}$, 8), 161.8 (d, $^1J_{CF}$, 243), 167.8; $\delta_F$ (282 MHz, $CDCl_3$) −112.66; *m/z* (FAB) 359.15607 ($M+H^+$ $C_{23}H_{20}FN_2O$ requires 359.15597).

#### 4.5.18. *N*-(2-(1*H*-Indol-3-yl)ethyl)-3′-methylbiphenyl-2-carboxamide (8g)

Pale yellow solid; yield 52%; mp 121–122 °C; $\delta_H$ NMR (300 MHz, $(CD_3)_2SO$) 2.62 (2H, t, 7.2), 2.37 (3H, s), 6.96 (1H, t, 7.8), 7.03 (1H, t, 7.8), 7.07 (1H, d, 2.3), 7.13 (1H, d, 7.0), 7.18–7.25 (3H, m), 7.24 (1H, s), 7.34 (1H, d, 8.0), 7.37–7.42 (2H, m), 7.46 (1H, d, 7.3), 7.48 (1H, d, 7.3), 8.21 (1H, t, 5.7), 10.78 (1H, br s); $\delta_C$ (75 MHz; $(CD_3)_2SO$) 21.0 ($CH_3$), 24.7 ($CH_2$), 39.7 ($CH_2$), 111.3 (CH), 111.6, 118.0 (CH), 118.1 (CH), 120.8 (CH), 122.4 (CH), 125.4 (CH), 126.8 (CH), 127.1, 127.5 (CH), 127.7 (CH), 128.0 (CH), 128.9 (CH), 129.1 (CH), 129.6 (CH), 136.1, 137.0, 137.4, 139.0, 140.1, 169.0; *m/z* (FAB) 355.18098 ($M+H^+$ $C_{24}H_{23}N_2O$ requires 355.18103).

### 4.6. General procedure for the synthesis of phenyl-(1,3,4,9-tetrahydro-β-carbolin-2-yl)-methanone derivatives 10a–c

To a suspension of carboline **9** (1.2 mmol) in $CH_2Cl_2$ (3 mL) at 0 °C was added slowly an aqueous solution of sodium hydroxide 4 M (1.2 mmol). After 5 min stirring at 0 °C the appropriate benzoyl chloride derivative (1.2 mmol) was added dropwise. The mixture was stirred for 5 min at 0 °C and for a further 3 h at room temperature. $H_2O$ (20 mL) was added. The two layers were separated and the aqueous phase was extracted with dichloromethane (3× 20 mL). The organic layer was dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by flash column chromatography on silica. Elution was made successively with; ethyl acetate/petroleum ether (40–60 °C) 50:50 and ethyl acetate, to give the expected compound.

#### 4.6.1. (4-Bromo-phenyl)-(1,3,4,9-tetrahydro-β-carbolin-2-yl)-methanone (10a)

White solid; yield 62%; mp 217 °C; rotamers 1/1.7 (from the duplicated singlet signal ($^1H$) at room temperature at 4.59 and 4.83 ppm); $\delta_H$ (400 MHz, $(CD_3)_2SO$, 363 K) 2.79 (2H, t, *J* 5.8), 3.79 (2H, br s), 4.75 (2H, s), 6.99 (1H, td, *J* 7.4 and 0.8), 7.07 (1H, td, *J* 7.6 and 1.2), 7.32 (1H, d, *J* 8.0), 7.40–7.44 (4H, m), 7.67 (1H, dt, *J* 8.8 and 2.4), 10.55 (1H, br s); $\delta_C$ (75 MHz, $CDCl_3$) δ 22.0 ($CH_2$), 40.9 ($CH_2$), 45.9 ($CH_2$), 107.1, 111.6 (CH), 118.0 (CH), 119.1 (CH), 121.4 (CH), 123.5, 127.0, 129.5 (2CH), 131.0, 132.0 (2CH), 135.8, 136.5, 169.6; *m/z* ($ES^+$) 356 $(M+2H)^+$; ($ES^-$) 353 $(M-H)^-$. *m/z* (FAB) 354.03677 ($M+H^+$ $C_{18}H_{15}BrN_2O$ requires 354.03677). Found: C, 60.87; H, 4.20; N, 7.70. $C_{18}H_{15}BrN_2O$ requires C, 60.86; H, 4.26; N, 7.89.

#### 4.6.2. (3-Bromo-phenyl)-(1,3,4,9-tetrahydro-β-carbolin-2-yl)-methanone (10b)

White solid; yield 91%; mp 207 °C; rotamers 1/1.6 (from the duplicated broad singlet signal ($^1H$) at room temperature at 4.60 and 4.84 ppm); $\delta_H$ (400 MHz, $(CD_3)_2SO$), 373 K) 2.80 (2H, t, 5.8), 3.81 (2H, br s), 4.77 (2H, s), 7.00 (1H, td, 7.5, 1.2), 7.08 (1H, td, 7.5, 1.2), 7.34 (1H, d, 7.6), 7.41–7.48 (3H, m), 7.64–7.69 (2H, m), 10.52 (1H, br s); $\delta_C$ (100 MHz; $(CD_3)_2SO$); 373 K) 21.7 ($CH_2$), 43.1 ($CH_2$), 44.1 ($CH_2$), 107.5, 111.5 (CH), 118.0 (CH), 119.1 (CH), 121.4 (CH), 122.2, 126.1 (CH), 127.3, 129.9 (CH), 131.1 (CH), 131.1, 132.8 (CH), 136.9, 139.3, 168.8; *m/z* ($ES^+$) 355 $MH^+$; ($ES^-$)

353 $(M-H)^-$;. *m/z* (FAB) 355.04478 ($M+H^+$ $C_{18}H_{15}BrN_2O$ requires 355.04460). Found: C, 60.94; H, 4.17; N, 7.93. $C_{18}H_{15}BrN_2O$ requires C, 60.86; H, 4.26; N, 7.87.

#### 4.6.3. (2-Bromo-phenyl)-(1,3,4,9-tetrahydro-β-carbolin-2-yl)-methanone (10c)

White solid; yield 64%; mp 190 °C; rotamers 1/3.3 (from the duplicated broad singlet signal ($^1H$) at 8.03 and 8.59 ppm); $\delta_H$ (300 MHz, $CDCl_3$) (*major rotamer*) 2.66 (1H, t, 6.5), 2.73 (1H, t, 5.0), 3.49 (2H, t, 5.7), 4.77 (1H, d, 16.8), 5.06 (1H, d, 16.8), 6.93–7.56 (8H, m), 8.59 (1H, br s); $\delta$ (distinct peaks for *minor rotamer*) 2.61 (1H, apparent t, *J* 6.3), 2.79 (1H, t, 5.0), 2.85 (2H, t, 5.7), 4.24 (1H, d, 16.1), 4.38 (1H, d, 16.1), 8.03 (1H, br s); $\delta_C$ (75 MHz, $CDCl_3$) $\delta$ (*major rotamer*) 21.9 ($CH_2$), 40.5 ($CH_2$), 45.2 ($CH_2$), 107.6, 111.3 (CH), 117.8 (CH), 119.3, 119.5 (CH), 121.8 (CH), 126.7, 127.6 (CH), 127.9 (CH), 129.7, 130.5 (CH), 133.0 (CH), 136.4, 138.2, 168.9; $\delta$ (distinct peaks for *minor rotamer*) 21.0 ($CH_2$), 44.9 ($CH_2$), 109.2, 111.0 (CH), 118.2 (CH), 119.1, 119.7 (CH), 121.9 (CH), 126.8, 129.2, 132.9, 136.3, 138.3, 168.4; *m/z* ($ES^+$) 355 $MH^+$; ($ES^-$) 354 $(M)^-$; *m/z* (FAB) 354.03678 ($M^+$ $C_{18}H_{15}BrN_2O$ requires 354.03677). Found: C, 60.75; H, 4.17; N, 7.92. $C_{18}H_{15}BrN_2O$ requires C, 60.86; H, 4.26; N, 7.89.

#### 4.6.4. (3,4-Dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)(4′-fluorobiphenyl-2-yl)methanone (11a)

Off White amorphous solid; 63% yield; mp 136–139 °C; 2.2:1 mixture of rotamers; $\delta_H$ (300 MHz, $CDCl_3$) 2.14–2.21 (1H, m), 2.42–2.52 (1H, m), 2.89–2.96 (1H, m), 3.35–3.42 (1H, m), 4.23 (1H, $d_{AB}$, *J* 17.0), 5.03 (1H, $d_{AB}$, *J* 17.0), 6.62 (1H, at, *J* 8.7), 6.87–7.13 (5H, m), 7.20–7.43 (6H, m), 8.70 (1H, s), *distinct signals for minor rotamer*: 2.58–2.65 (1H, m), 3.64–3.73 (1H, m), 3.82 (1H, $d_{AB}$, *J* 16.0), 3.82–3.90 (1H, m), 4.03 (1H, $d_{AB}$, *J* 16.1), 7.94 (1H, s); $\delta_C$ (75.5 MHz, $CDCl_3$) 21.4 ($CH_2$), 40.2 ($CH_2$), 44.8 ($CH_2$), 107.4, 111.0 (CH), 115.4 (CH, d, $^2J_{CF}$ 21.5), 117.6 (CH), 119.2 (CH), 121.5 (CH), 126.4, 127.1 (CH), 127.8 (CH), 129.4, 129.6 (CH), 129.6 (CH), 130.1 (CH, d, $^3J_{CF}$ 8.4), 135.4, 135.7 (d, $^4J_{CF}$, 3.6), 136.2, 137.7, 162.4 (d, $^2J_{CF}$ 247), 170.1; (*distinct signals for minor rotamer*) 20.6 ($CH_2$), 40.2 ($CH_2$), 44.7 ($CH_2$), 108.7, 110.6 (CH), 115.0 (CH, d, $^2J_{CF}$ 21.5), 117.9 (CH), 119.4 (CH), 121.7 (CH), 126.7, 127.1 (CH), 127.7 (CH), 128.8, 129.4 (CH), 129.5 (CH), 129.8 (CH, d, $^3J_{CF}$ 8.4), 135.1 (d, $^4J_{CF}$, 3.6), 135.5, 136.0, 137.8, 162.2 (d, $^2J_{CF}$ 248), 170.9; *m/z* ($ES^+$) 371 ($[M+H]^+$, 100%); *m/z* (FAB) 370.14821 ($M^+$ $C_{24}H_{19}N_2OF$ requires 370.14814).

#### 4.6.5. (3,4-Dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)(3′-fluorobiphenyl-2-yl)methanone (11b)

Off white amorphous solid; 56% yield; mp 203–207 °C; 2:1 mixture of rotamers; $\delta_H$ (300 MHz, $CDCl_3$) 2.55–2.76 (2H, m, $CH_2$), 3.09 (1H, ddd, *J* 13.2, 8.5, 4.7), 3.52 (1H, dat, 13.2, 4.8), 4.41 (1H, $d_{AB}$, *J* 17.0), 5.14 (1H, $d_{AB}$, *J* 17.0), 6.95–7.17 (12H, m, ArH), 8.59 (1H, s, ArH), (*distinct signals for minor rotamer*) 2.25–2.30 (2H, m, $CH_2$), 3.80 (1H, ddd, *J* 12.6, 7.4, 5.2, $CH_2$), 3.99 (1H, $d_{AB}$, *J* 16.3, $CH_2$), 4.07 (1H, m, $CH_2$), 4.19 (1H, $d_{AB}$, *J* 16.3, $CH_2$), 7.88 (1H, s, NH); $\delta_C$ (75.5 MHz, $CDCl_3$) 21.4 ($CH_2$), 40.2 ($CH_2$), 44.8 ($CH_2$), 107.5, 111.0 (CH), 114.6 (CH, d, $^2J_{CF}$ 21.0), 115.4 (CH, d, $^2J_{CF}$ 22.0), 117.7 (CH), 119.3 (CH), 121.6 (CH), 124.3 (CH, d, $^4J_{CF}$ 2.7), 126.5, 127.3 (CH), 128.2 (CH), 129.3 (CH), 129.4, 129.6 (CH, d, $^3J_{CF}$ 6.6), 129.6 (CH), 135.5, 136.2, 137.5, 141.8 (d, $^3J_{CF}$ 7.8), 162.9 (Cq, d, $^1J_{CF}$ 247), 170.7; (*distinct signals for minor rotamer*) 20.5 ($CH_2$), 40.3 ($CH_2$), 44.8 ($CH_2$), 108.9, 110.7 (CH), 114.4 (CH, d, $^2J_{CF}$ 20.9), 115.1 (CH, d, $^2J_{CF}$ 22.0), 117.9 (CH), 119.4 (CH), 121.7 (CH), 124.0 (CH, d, $^4J_{CF}$ 2.7), 126.8, 127.4 (CH), 128.2 (CH), 128.8, 129.7 (CH), 130.0 (CH, d, $^3J_{CF}$ 8.4), 135.5, 136.0, 137.5, 141.2 (Cq, d, $^3J_{CF}$ 7.8), 162.4 (Cq, d, $^1J_{CF}$ 247), 169.9; *m/z* ($ES^+$) 371 ($[M+H]^+$, 100%); *m/z* (FAB) 370.14815 ($M^+$ $C_{24}H_{19}FN_2O$ requires 370.14814).

#### 4.6.6. (3,4-Dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)(2′-fluorobiphenyl-2-yl)methanone (11c)

Off white amorphous solid; 82% yield; mp 188–190 °C; 3:2 mixture of rotamers; $\delta_H$ (300 MHz, $CDCl_3$) 2.36 (1H, br s), 2.56 (1H, br s), 3.13 (1H, br s), 3.57 (1H, br s), 4.21 (1H, $d_{AB}$, *J* 16.7), 4.99 (1H, $d_{AB}$ *J* 16.4), 6.40–7.40 (12H, m, ArH), 8.62 (1H, br s); (*distinct signals for minor rotamer*) 2.32 (1H, br s), 2.52 (1H, br s), 3.44 (1H, br s), 4.00–4.09 (3H, m), 7.82 (1H, br s, NH); $\delta_C$(75.5 MHz, $CDCl_3$) 21.5 ($CH_2$), 40.4 ($CH_2$), 45.1 ($CH_2$), 107.5, 111.1 (CH), 115.6 (CH, d, $^2J_{CF}$ 22.3), 117.6 (CH), 119.2 (CH), 121.4 (CH), 124.1 (CH, d, $^3J_{CF}$ 3.7), 126.5, 126.9 (Cq, d, $^2J_{CF}$ 16.8) 127.0 (CH), 128.2 (CH), 129.2 (CH), 129.6, 129.7 (CH, d, $^3J_{CF}$ 8.2), 131.0 (CH, d, $^4J_{CF}$ 1.9), 131.4 (CH, d, $^4J_{CF}$ 3.0), 133.2, 136.2, 136.2, 159.4 (d, $^1J_{CF}$ 246), 170.6; (*distinct signals for minor rotamer*) 20.6 ($CH_2$), 40.3 ($CH_2$), 44.8 ($CH_2$), 108.7, 110.7 (CH), 115.0 (CH, d, $^2J_{CF}$ 22.3), 117.8 (CH), 119.3 (CH), 121.5 (CH), 123.7 (CH, d, $^3J_{CF}$ 3.6), 126.3 (Cq, d, $^2J_{CF}$ 14.8), 127.1 (CH), 127.3, 128.3 (CH), 128.9 (CH), 129.0, 129.5 (CH, d, $^3J_{CF}$ 8.2), 130.7 (CH, d, $^4J_{CF}$ 2.1), 131.2 (CH, d, $^4J_{CF}$ 2.8), 132.6 (136.1, 136.4, 159.0 (d, $^1J_{CF}$ 245), 169.6; *m/z* ($ES^+$) 371 ($[M+H]^+$, 100%); *m/z* (FAB) 370.14821 ($M^+$ $C_{24}H_{19}FN_2O$ requires 370.14814).

#### 4.6.7. (3,4-Dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)(3′-methylbiphenyl-2-yl)methanone (11d)

Off white amorphous solid; yield 97%; mp 187–191 °C; 1.7:1 mixture of rotamers; $\delta_H$ (300 MHz, $CDCl_3$) 2.04–2.19 (1H, m, $CH_2$), 2.14 (3H, s, $CH_3$), 2.38–2.50 (1H, m, $CH_2$), 2.91 (1H, ddd, *J* 13.0, 8.5, 4.7, $CH_2$), 3.39 (1H, dat, *J* 13.3, 5.0, $CH_2$), 4.27 (1H, $d_{AB}$, pseudo *J* 17.0, $CH_2$), 5.01 (1H, $d_{AB,}$ pseudo *J* 17.0, $CH_2$), 6.84–7.40 (12H, m), 8.84 (1H, m, $CH_2$); (*distinct signals for minor rotamer*) 1.89 (3H, s, $CH_3$), 2.38–2.50 (1H, m, $CH_2$), 2.54–2.62 (1H, m, $CH_2$), 3.56 (1H, ddd, *J* 12.6, 7.8, 4.8, $CH_2$), 3.03 (1H, $d_{AB}$, pseudo *J* 16.4, $CH_2$), 3.97 (1H, $d_{AB}$, *J* 16.4, $CH_2$), 3.94–4.01 (1H, m, $CH_2$), 8.00 (1H, s, NH); $\delta_C$(75.5 MHz, $CDCl_3$), 21.1 ($CH_2$), 21.3 ($CH_3$), 40.2 ($CH_2$), 44.7 ($CH_2$), 107.2, 111.0 (CH), 117.5 (CH), 119.0 (CH), 121.3 (CH), 125.4 (CH), 126.4, 127.2 (CH), 127.6 (CH), 128.3 (CH), 128.4 (CH), 129.0 (CH), 129.4, 129.5 (CH), 129.5 (CH), 135.2, 136.2, 138.0, 138.9, 139.5, 171.1; (*distinct signals for minor rotamer*) 20.5 ($CH_2$), 21.0 ($CH_3$), 40.2 ($CH_2$), 44.7 ($CH_2$), 108.5, 110.7 (CH), 117.7 (CH), 119.1 (CH), 121.3 (CH), 125.3 (CH), 126.7, 127.0 (CH), 127.5 (CH), 127.9 (CH), 128.2 (CH), 128.5 (CH), 129.0, 129.4 (CH), 129.4 (CH), 135.3, 136.0, 137.7, 138.9, 139.1, 170.3; *m/z* (ES+) 367 ($[M+H]^+$ 100%); *m/z* (FAB) 366.17324 ($M^+$ $C_{25}H_{22}N_2O$ requires 366.17321).

#### 4.6.8. (3,4-Dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)(4′-fluorobiphenyl-3-yl)methanone (12a)

Off white amorphous solid; 83% yield; mp 132–135 °C; 5:1 mixture of rotamers; $\delta_H$ (300 MHz, $CDCl_3$) 2.81 (2H, br s), 2.72 (2H, br s), 4.93 (2H, br s), 7.05–7.64 (12H, m, Ar), 8.75 (1H, br s); (*distinct signals for minor rotamer*) 2.90 (2H, br s), 4.10 (2H, br s), 4.58 (2H, br s), 8.10 (1H, br s); $\delta_C$ (75.5 MHz, $CDCl_3$) 22.1 ($CH_2$), 44.1 ($CH_2$), 46.0 ($CH_2$), 107.6, 111.1 (CH), 115.8 (CH, d, $^2J_{CF}$ 21.4), 117.7 (CH), 119.4 (CH), 121.7 (CH), 125.4 (CH), 125.5 (CH), 126.7, 128.4 (CH), 128.7 (CH, d, $^3J_{CF}$ 8.1), 129.1 (CH), 129.9, 136.2 (d, $^5J_{CF}$ 1.3), 136.2 (d, $^4J_{CF}$ 4.8), 136.6, 140.7, 162.1 (Cq, d, $^1J_{CF}$, 247), 171.3; *m/z* (ES+) 371 ($[M+H]^+$, 100%); *m/z* (FAB) 370.14822 ($M^+$ $C_{24}H_{19}FN_2O$ requires 370.14814).

#### 4.6.9. (3,4-Dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)(3′-fluorobiphenyl-3-yl)methanone (12b)

Off white amorphous solid; 72% yield; mp 134–138 °C; 5:1 mixture of rotamers; $\delta_H$ (300 MHz, $CDCl_3$) 2.85 (2H, br s, $CH_2$), 3.76 (2H, br s, $CH_2$), 4.97 (2H, br s, $CH_2$), 7.07–7.72 (12H, m, ArH), 8.90 (1H, br s); (*distinct signals for minor rotamer*) 2.95 (2H, br s, $CH_2$), 4.14 (2H, br s, $CH_2$), 4.62 (2H, br s, $CH_2$), 8.28 (1H, br s); $\delta_C$

(75.5 MHz, $CDCl_3$), 22.0 ($CH_2$), 41.2 ($CH_2$), 4.60 ($CH_2$), 107.4, 111.1 (CH), 113.9 (CH, d, $^2J_{CF}$ 22.1), 114.5 (CH, d, $^2J_{CF}$ 20.9), 117.7 (CH), 119.4 (CH), 121.6 (CH), 122.7 (CH, d, $^4J_{CF}$ 2.7), 125.4 (CH), 126.1 (CH), 126.6, 128.5 (CH), 129.2 (CH), 129.9, 130.3 (CH, d, $^3J_{CF}$, 8.4), 136.2, 136.6, 140.3 (d, $^4J_{CF}$ 1.2), 142.2 (d, $^3J_{CF}$ 7.8), 163.1 (d, $^1J_{CF}$ 246), 171.2; *m/z* ($ES^+$) 371 ($[M+H]^+$, 100%); *m/z* (FAB) 370.14806 ($M^+$ $C_{24}H_{19}FN_2O$ requires 370.14814).

#### 4.6.10. (3,4-Dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)(2′-fluorobiphenyl-3-yl)methanone (12c)

Off white amorphous solid; 83% yield; mp 125–127 °C; 5:1 mixture of rotamers; $\delta_H$ (300 MHz, $CDCl_3$) 2.71 (2H, br s), 3.52–3.63 (2H, m), 4.76-4.81 (2H, m), 6.96–7.56 (12H, m), 8.80 (1H, br s); (*distinct signals for minor rotame):* 2.76 (2H, br s), 3.96 (2H, br s), 4.36–4.47 (2H, m), 8.13 (1H, br s); $\delta_C$ (100 MHz, $CDCl_3$) 22.0 ($CH_2$), 41.1 ($CH_2$), 46.1 ($CH_2$), 107.5, 111.1 (CH), 116.1 (CH, d, $^2J_{CF}$ 22.7), 117.7 (CH), 119.3 (CH), 121.5 (CH), 124.5 (CH, d, $^3J_{CF}$ 3.6), 126.1 (CH), 126.6, 127.4 (CH), 127.8, 128.8 (CH), 129.4 (CH, d, $^3J_{CF}$ 8.4), 129.9, 130.4 (CH, d, $^4J_{CF}$ 3.0), 130.6 (CH, d, $^4J_{CF}$ 3.3), 136.1, 136.1 (d, $^2J_{CF}$ 13.5), 137.8, 159.6 (d, $^1J_{CF}$ 248), 171.2.

#### 4.6.11. (3,4-Dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)(4′-methylbiphenyl-3-yl)methanone (12d)

Off white amorphous solid; yield 61%; mp 166–168 °C; 5:1 mixture of rotamers; $\delta_H$ (300 MHz, $CDCl_3$) 2.27 (3H, br s), 2.67 (2H, br s), 3.58 (2H, br s), 4.80 (2H, br s), 6.93–7.57 (12H, m), 8.80 (1H, br s); (*distinct signals for the minor rotamer*) 2.78 (2H, br s), 3.96 (2H, br s), 4.43 (2H, br s), 8.20 (1H, br s); $\delta_C$ (75.5 MHz, $CDCl_3$) 21.0 ($CH_3$), 22.0 ($CH_2$), 41.0 ($CH_2$), 46.0 ($CH_2$), 107.4, 111.1 (CH), 117.6 (CH), 119.2 (CH), 121.5 (CH), 125.1 (CH), 125.2 (CH), 126.5, 126.8 (CH), 128.3 (CH), 129.0 (CH), 129.5 (CH), 129.9, 136.1, 136.3, 137.1, 137.5, 141.4, 171.5; *m/z* ($ES^+$) 367 ($[M+H]^+$ 100%); *m/z* (FAB) 366.17309 ($M^+$ $C_{25}H_{22}N_2O$ requires 366.17321).

#### 4.6.12. (3,4-Dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)(3′-methylbiphenyl-3-yl)methanone (12e)

Off white amorphous solid; yie;d 88%; 5:1 mixture of rotamers; $\delta_H$ (300 MHz, $CDCl_3$) 2.29 (3H, br s), 2.69 (2H, br s), 3.60 (2H, br s), 4.38 (2H, br s), 6.94–7.59 (12H, m), 8.79 (1H, br s); (*distinct signals for the minor rotamer*) 2.79, (2H, br s), 3.98 (2H, br s), 4.45 (2H, br s), 8.17 (1H, br s); $\delta_C$ (75.5 MHz, $CDCl_3$) 21.4 ($CH_3$), 22.0 ($CH_2$), 41.1 ($CH_2$), 46.0 ($CH_2$), 107.4, 111.1 (CH), 117.6 (CH), 119.3 (CH), 121.5 (CH), 124.1 (CH), 125.4 (CH), 126.6, 127.8 (CH), 128.4 (CH), 128.6 (CH), 128.7 (CH), 128.7 (CH), 129.0 (CH), 129.9, 136.2, 136.3, 138.5, 140.0, 141.7, 171.5.

#### 4.6.13. (3,4-Dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)(2′-methylbiphenyl-3-yl)methanone (12f)

Off white amorphous solid; yield 80%; mp 139–141 °C; 3:1 mixture of rotamers; $\delta_H$ (300 MHz, $CDCl_3$) 2.15 (3H, br s), 2.67 (2H, br s), 3.60 (2H, br s), 4.79 (2H, br s), 6.60–6.65 (1H, m), 6.80–7.58 (11H, m), 8.74 (1H, br s); $\delta$ (distinct signals for *minor rotamer*) 2.75 (2H, br s), 3.94 (2H, br s), 4.40 (2H, br s), 8.24 (1H, br s); $\delta_C$ (75.5 MHz, $CDCl_3$) 20.5 ($CH_3$), 22.1 ($CH_2$), 41.1 ($CH_2$), 46.1 ($CH_2$), 107.4, 111.0 (CH), 117.8 (CH), 119.6 (CH), 121.8 (CH), 125.4 (CH), 125.9 (CH), 126.8, 127.5 (CH), 127.6 (CH), 128.5 (CH), 129.8 (CH), 130.4 (CH), 130.4, 130.7 (CH), 135.2, 135.9, 136.2, 140.8, 142.2, 171.5; *m/z* ($ES^+$) 367 ($[M+H]^+$ 100%); *m/z* (FAB) 366.17322 ($M^+$ $C_{25}H_{22}N_2O$ requires 366.17321).

#### 4.6.14. (3,4-Dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)(3′-methoxybiphenyl-3-yl)methanone (12g)

Off white amorphous solid; yield 74%; 4:1 mixture of rotamers; $\delta_H$ (300 MHz, $CDCl_3$) 2.85 (2H, br s), 3.77 (2H, br s), 3.87 (3H, br s) 4.97 (2H, br s), 6.95–7.75 (12H, m), 8.88 (1H, br s); $\delta$ (distinct signals for *minor rotamer*) 2.95 (2H, br s), 4.13 (2H, br s), 4.61 (2H, br s), 8.30 (1H, br s); $\delta_H$ (75.5 MHz, $CDCl_3$) 22.0 ($CH_2$), 41.0 ($CH_2$), 46.0 ($CH_2$), 55.2 ($CH_3$), 107.5, 111.1 (CH), 112.7 (CH), 113.2 (CH), 117.7 (CH), 119.3 (CH), 119.5 (CH), 121.6 (CH), 125.5 (CH), 125.7 (CH), 126.6, 128.6 (CH), 129.0 (CH), 129.9 (CH), 136.2, 135.5, 141.5, 141.5, 159.9, 171.4 (C=O); *m/z* (ES+) 383 ($[M+H]^+$ 100%); *m/z* (FAB) 382.16820 ($M^+$ $C_{25}H_{22}N_2O_2$ requires 382.16813).

#### 4.6.15. Biphenyl-3-yl(3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)methanone (12h)

Beige solid; 92% yield; mp 104–106 °C; 3.6:1 mixture of rotamers; $\delta_H$ (300 MHz, $CDCl_3$) 2.71 (2H, br s), 3.63 (2H, br s), 4.84 (2H, br s), 6.95–7.60 (13H, m, Ar), 8.67 (1H, br s); $\delta$ *(distinct signals for minor rotamer*) 2.80 (2H, br s), 3.99 (2H, br s), 4.47 (2H, br s), 8.04 (1H, br s); $\delta_C$ (75.5 MHz, $CDCl_3$) 22.1 ($CH_2$), 41.1 ($CH_2$), 46.1 ($CH_2$), 107.6, 111.1 (CH), 117.7 (CH), 119.4 (CH), 121.7 (CH), 125.5 (CH), 125.6 (CH), 126.7, 127.1 (CH), 127.7 (CH), 128.6 (CH), 128.9 (CH), 129.1 (CH), 129.9, 136.3, 136.5, 140.0, 143.6, 171.5; *m/z* (ES+) 353 ($[M+H]^+$, 100%).

#### 4.6.16. (3,4-Dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)(4′-fluorobiphenyl-4-yl)methanone (13a)

Off white amorphous solid; 80% yield; mp 227−229 °C; 5:1 mixture of rotamers; $\delta_H$ (300 MHz, $CDCl_3$) 2.88 (2H, br s), 3.79 (2H, br s), 4.97 (2H, br s), 7.09 (12H, m, ArH), 8.34 (1H, br s, NH); $\delta$ *(distinct signals for minor rotamer*) 2.95 (2H, br s), 4.13 (2H, br s), 4.69 (2H, br s), 7.83 (1H br s); $\delta_C$ (100 MHz, $(CD_3)_2SO$) 21.5 ($CH_2$), 45.4 ($CH_2$), 54.8 ($CH_2$), 106.5, 111.0 (CH), 115.7 (CH, d, $^2J_{CF}$ 21.6), 117.4 (CH), 118.5 (CH), 120.8 (CH), 126.4, 126.6 (CH), 127.4 (CH), 128.7 (CH, d, $^3J_{CF}$ 8.4), 130.6, 135.0, 135.7 (d, $^5J_{CF}$ 2.4), 135.9 (Cq, d, $^4J_{CF}$ 2.8), 140.2, 162.0 (d, $^1J_{CF}$ 245), 169.7; *m/z* ($ES^+$) 371 ($[M+H]^+$, 100%); *m/z* (FAB) 370.14821 ($M^+$ $C_{24}H_{19}FN_2O$ requires 370.14814).

#### 4.6.17. (3,4-Dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)(3′-fluorobiphenyl-4-yl)methanone (13b)

Pale yellow amorphous solid; 84% yield; mp 212–216 °C; 5:1 mixture of rotamers; $\delta_H$ (300 MHz, $CDCl_3$) 2.77 (2H, br s, $CH_2$), 3.67 (2H, br s, $CH_2$), 4.86 (2H, br s, $CH_2$), 6.97–7.56 (12H, m, Ar), 8.57 (1H, br s); $\delta$ *(distinct signals for minor rotamer*) 4.02 (2H, br s, $CH_2$), 4.56 (2H, br s, $CH_2$), 7.97 (1H, br s); $\delta_C$ (75.5 MHz, $CDCl_3$), 22.1 ($CH_2$), 41.2 ($CH_2$), 46.1 ($CH_2$), 107.7, 111.1 (CH), 114.0 (CH, d, $^2J_{CF}$ 22.1), 114.6 (CH, d, $^2J_{CF}$ 20.9), 117.8 (CH), 119.5 (CH), 121.8 (CH), 122.8 (CH, d, $^4J_{CF}$ 2.7), 126.7, 127.3 (CH), 127.6 (CH), 129.9, 130.4 (CH, d, $^3J_{CF}$ 8.4), 135.3, 136.3, 141.5 (d, $^4J_{CF}$ 1.5), 142.4 (d, $^3J_{CF}$ 7.5), 163.2 (d, $^1J_{CF}$ 246), 171.2; *m/z* (ES+) 371 ($[M+H]^+$, 100%); *m/z* (FAB) 370.14819 ($M^+$ $C_{24}H_{19}FN_2O$ requires 370.14814).

#### 4.6.18. (3,4-Dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)(2′-fluorobiphenyl-4-yl)methanone (13c)

Pale yellow amorphous solid; 79% yield; mp 197–200 °C; 5:1 mixture of rotamers; $\delta_H$ (300 MHz, $CDCl_3$) 2.89 (2H, br s, $CH_2$), 3.81 (2H, m, $CH_2$), 4.96 (2H, br s, $CH_2$), 7.01–7.80 (12H, m, ArH), 8.36 (1H, br s, NH); $\delta$ *(distinct signal for minor rotamer*) 4.10 (2H, m, $CH_2$), 4.69 (2H, m, $CH_2$), 9.08 (1H, br s); $\delta_C$ (100 MHz, $CDCl_3$) 21.5 ($CH_2$), 40.3 ($CH_2$), 45.5 ($CH_2$), 106.5, 111.0 (CH), 116.1 (CH, d, $^2J_{CF}$ 22.5), 117.4 (CH), 118.5 (CH), 120.8 (CH), 125.0 (CH, d, $^3J_{CF}$ 3.5), 126.4, 127.0 (CH), 127.4 (d, $^2J_{CF}$ 13.1), 128.8 (CH, d, $^4J_{CF}$ 3.0), 129.9 (CH, d, $^3J_{CF}$ 8.3), 130.6, 130.7 (CH, d, $^4J_{CF}$ 3.2), 135.5, 135.9, 136.2, 159.0 (d, $^1J_{CF}$ 246), 169.5; *m/z* ($ES^+$) 371 ($[M+H]^+$, 100%); *m/z* (FAB) 370.14821 ($M^+$ $C_{24}H_{19}FN_2O$ requires 370.14814).

#### 4.6.19. (3,4-Dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)(4′-methylbiphenyl-4-yl)methanone (13d)

Off white amorphous solid; yield 66%; mp 223–226 °C; 4:1 mixture of rotamers; $\delta_H$ (300 MHz, $CDCl_3$) 2.32 (3H, br s), 2.78 (2H, br s), 3.68 (2H, br s), 4.84 (2H, br s), 6.99–7.61 (12H, m), 8.53 (1H, br s); $\delta$ *(distinct signals for minor rotamer*) 1.81 (3H, br s), 3.99 (2H, br

s), 4.57 (2H, br s), 8.11 (1H, br s); $\delta_C$ (75.5 MHz, $CDCl_3$) 21.1 ($CH_3$), 22.1 ($CH_2$), 41.1 ($CH_2$), 46.1 ($CH_2$), 107.8, 111.1 (CH), 117.8 (CH), 119.5 (CH), 121.7 (CH), 126.8, 126.9 (CH), 127.0 (CH), 127.5 (CH), 129.6 (CH), 134.4, 136.3, 137.2, 137.7, 142.7, 171.4 (C=O), *δ (distinct signals for minor rotamer)* 128.4 (CH), 128.6 (CH), 132.0 (CH), 132.0 (CH), 132.1 (CH); *m/z* ($ES^+$) 367 ($[M+H]^+$ 100%); *m/z* (FAB) 366.17324 ($M^+$ $C_{25}H_{22}N_2O$ requires 366.17321).

#### 4.6.20. (3,4-Dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)(3′-methylbiphenyl-4-yl)methanone (13e)

Pale beige amorphous solid; yield 91%; mp 158–161 °C; 5:1 mixture of rotamers; $\delta_H$ (300 MHz, $CDCl_3$) 2.31 (2H, br s, $CH_3$), 2.72 (2H, br s, $CH_2$), 3.62 (2H, br s, $CH_2$), 4.81 (2H, br s, $CH_2$), 6.96–7.54 (12H, m, Ar), 8.77 (1H, br s); *δ (distinct signals for minor rotamer)* 2.75 (2H, br s, $CH_2$), 3.96 (2H, br s, $CH_2$), 4.51 (2H, br s, $CH_2$), 8.24 (1H, br s); $\delta_C$ (300 MHz, $CDCl_3$) 21.5 ($CH_3$), 22.0 ($CH_2$), 41.1 ($CH_2$), 46.1 ($CH_2$), 107.4, 111.1 (CH), 117.7 (CH), 119.3 (CH), 121.5 (CH), 124.2 (CH), 126.6, 127.2 (CH), 127.4 (CH), 127.8 (CH), 128.5 (CH), 128.7 (CH), 130.0, 134.4, 136.2, 138.5, 140.0, 142.9, 171.5; *m/z* (ES+) 367 ($[M+H]^+$ 100%); *m/z* (FAB) 366.17320 ($M^+$ $C_{25}H_{22}N_2O$ requires 366.17321).

#### 4.6.21. (3,4-Dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)(2′-methylbiphenyl-4-yl)methanone (13f)

Off white amorphous solid; yield 69%; mp 185–188 °C; 6:1 mixture of rotamers; $\delta_H$ (300 MHz, $CDCl_3$) 2.19 (3H, br s), 2.77 (2H, br s), 4.85 (2H, br s), 6.96–7.54 (12H, m), 8.74 (1H, br s); *δ (distinct signals for minor rotamer)* 2.70 (2H, br s), 3.55 (2H, br s), 4.80 (2H, br s), 8.07 (1H, br s); $\delta_C$ (75.5 MHz, $CDCl_3$) 20.4 ($CH_3$), 22.1 ($CH_2$), 41.2 ($CH_2$), 46.1 ($CH_2$), 107.6, 111.1 (CH), 117.7 (CH), 119.4 (CH), 121.6 (CH), 125.8 (CH), 126.7, 126.8 (CH), 127.6 (CH), 129.4 (CH), 129.6 (CH), 130.0, 130.4 (CH), 134.3, 135.2, 136.3, 140.8, 143.7, 171.5; *m/z* ($ES^+$) 367 ($[M+H]^+$ 100%); *m/z* (FAB) 366.17316 ($M^+$ $C_{25}H_{22}N_2O$ requires 366.17321).

#### 4.6.22. (3,4-Dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)(3′-methoxybiphenyl-4-yl)methanone (13g)

Pale yellow amorphous solid; yield 68%; mp 182–185 °C; 4:1 mixture of rotamers; $\delta_H$ (300 MHz, $CDCl_3$) 2.71 (2H, br s), 3.62 (2H, br s), 3.74 (3H, br s), 4.80 (2H, br s), 6.83–7.53 (12H, m), 8.78 (1H, br s); *δ (distinct signals for minor rotamer)* 3.97 (2H, br s), 4.49 (2H, br s), 8.25 (1H, br s); $\delta_C$ (75.5 MHz, $CDCl_3$) 22.0 ($CH_2$), 41.1 ($CH_2$), 46.0 ($CH_2$), 55.2 ($CH_3$), 107.8, 111.1 (CH), 112.8 (CH), 113.1 (CH), 117.7 (CH), 119.3 (CH), 119.5 (CH), 121.6 (CH), 126.7, 127.3 (CH), 127.4 (CH), 129.9 (CH), 130.0, 134.8, 136.2, 141.5, 142.6, 159.9, 171.3 (C=O); *m/z* ($ES^+$) 383 ($[M+H]^+$ 100%); *m/z* (FAB) 382.16821 ($M^+$ $C_{25}H_{22}N_2O_2$ requires 382.16813).

#### 4.6.23. (3,4-Dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)(2′-methoxybiphenyl-4-yl)methanone (13h)

Off white amorphous solid; yield 96%; mp 167–171 °C; 3:1 mixture of rotamers; $\delta_H$ (300 MHz, $CDCl_3$) 2.89 (2H, br s, $CH_2$), 3.81–3.85 (2H, m, $CH_2$), 5.01 (2H, br s, $CH_2$), 7.05–7.71 (12H, m, ArH), 9.14 (1H, br s); *δ (distinct signals for minor rotamer)* 2.96 (2H, br s, $CH_2$), 4.15 (2H, br s, $CH_2$), 4.60 (2H, br s $CH_2$), 8.42 (1H, br s); $\delta_C$ (75.5 MHz, $CDCl_3$), 22.0 ($CH_2$), 41.1 ($CH_2$), 46.0 ($CH_2$), 55.3 ($OCH_3$), 107.3, 111.1 (CH), 117.6 (CH), 119.1 (CH), 120.8 (CH), 121.4 (CH), 126.5 (CH), 126.6, 126.7 (CH), 129.1 (CH), 129.3, 129.6 (CH), 130.0, 130.7 (CH), 134.1, 136.2, 140.2, 156.2, 171.5; *m/z* ($ES^+$) 383 ($[M+H]^+$ 100%); *m/z* (FAB) 382.16814 ($M^+$ $C_{25}H_{22}N_2O_2$ requires 382.16813).

#### 4.6.24. *N*-(2-(1*H*-Indol-3-yl)ethyl)-*N*-methyl-4-(pyridin-4-yl)benzamide (14)

White solid; yield 68%; mp 195 °C; rotomers 1.6/1 (from the duplicated triplet signal at 3.47 and 3.75 ppm); $\delta_H$ (400 MHz, 363 K, $(CD_3)_2SO$) 2.97 (3H, s), 3.00 (2H, t, 6.7), 3.64 (2H, dd, 6.7, 6.7), 6.88 (1H, t, 7.4), 7.05 (1H, t, 7.4), 7.09 (1H, s), 7.35 (2H, d, 8.0), 7.37 (2H, d, 6.0), 7.67 (2H, d, 6.0), 7.73 (2H, d, 8.0), 8.66 (2H, d, 6.0), 10.55 (1H, br s); $\delta_C$ (75 MHz, $CD_2Cl_2$) *δ (major rotamer)* 24.5 ($CH_2$), 33.0 ($CH_3$), 52.1 ($CH_2$), 111.7 (CH), 111.9, 113.2, 118.4 (CH), 119.4 (CH), 122.2 (CH, 2C), 122.9 (CH), 127.1 (CH, 2C), 127.5 (CH, 2C), 128.1 (CH), 136.8, 137.7, 138.7, 147.9, 150.4 (CH, 2C), 171.8; *δ* (distinct peaks for *minor rotamer*) 23.3 ($CH_2$), 38.2 ($CH_3$), 48.8 ($CH_2$), 119.0 (CH), 122.8 (CH, 2C), 127.4 (CH, 2C); *m/z* ($ES^+$) 356 ($M+H^+$), ($ES^-$) 354 ($M-H^-$); *m/z* (FAB) 356.17627 ($M+H^+$ $C_{23}H_{22}N_3O$ requires 356.17629). Found: C, 77.80; H, 5.86; N, 11.90. $C_{23}H_{21}N_3O$ requires C, 77.72; H, 5.96; N, 11.82%.

#### 4.6.25. *N*-(2-(1*H*-Indol-3-yl)ethyl)-3-(pyridin-3-yl)benzamide (15)

White solid; yield 80%; mp 59 °C; $\delta_H$ (300 MHz, $(CD_3)_2SO$) 3.04 (2H, t, 7.2), 3.64 (2H, t, 7.2), 7.04 (1H, td, 7.9, 1.2), 7.13 (1H, td, 7.0, 1.2), 7.25 (1H, s), 7.41 (1H, d, 7.9), 7.57–7.72 (3H, m), 7.94 (2H, dd, 7.9, 2.0), 8.17 (2H, dd, 7.9, 2.0), 8.66 (1H, s), 8.87 (1H, t, 6.0), 8.99 (1H, br s), 10.87 (1H, br s); $\delta_C$ (75 MHz, 363K, $CDCl_3$) 25.2 (CH2), 40.7 (CH2), 111.5 (CH), 112.7, 118.7, 119.4 (CH), 122.1 (CH), 125.9 (CH), 126.5 (CH), 127.4, 128.5 (CH), 129.3 (CH), 129.8 (CH), 132.0 (CH), 134.5 (CH), 135.7, 136.6, 138.0, 148.0 (CH), 148.6 (CH), 167.3; *m/z* ($ES^+$) 342 ($M+H^+$), ($ES^-$) 340 ($M-H^-$); *m/z* (FAB) 342.16061 ($M+H^+$ $C_{22}H_{20}N_3O$ requires 342.16064). Found: C, 74.33; H, 5.28; N, 11.70. $C_{22}H_{19}N_3O$ requires C, 77.40; H, 5.61; N, 12.31%.

#### 4.6.26. (3,4-Dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)(2-(pyridin-4-yl)phenyl)methanone (16a)

Off white amorphous solid; 73% yield; mp 135–138 °C; 2.0:1.0 mixture of rotamers; $\delta_H$ (300 MHz, $CDCl_3$) 2.14–2.21 (1H, m), 2.61–2.64 (1H, m) 3.11 (1H, br s), 3.51 (1H, br s), 4.35 (1H, $d_{AB}$, *J* 15.8), 5.12 (1H, $d_{AB}$, *J* 16.2), 7.02–7.70 (10 H, m), 8.59 (2H, br s), *δ (distinct signals for minor rotamer)* 2.68 (1H, br s), 2.76 (1H, br s), 3.90–3.93 (2H, m), 3.98–4.04 (1H, m), 4.20–4.25 (1H, m), 8.33 (2H, br s); $\delta_C$ (75.5 MHz, $CDCl_3$) 21.5 ($CH_2$), 40.2 ($CH_2$), 44.9 ($CH_2$), 107.1, 111.0 (CH), 117.6 (CH), 119.2 (CH), 121.5 (CH), 123.3 (CH), 126.4, 127.2 (CH), 128.4 (CH), 129.1 (CH), 129.3, 129.4 (CH), 129.7 (CH), 132.0 (CH), 135.5, 135.9, 136.3, 147.6, 149.6 (CH), 170.0; *δ (distinct signals for minor rotamer)* 20.5 (CH), 40.4 (CH), 45.0 (CH), 108.8, 110.7 (CH), 118.0 (CH), 119.5 (CH), 121.8 (CH), 123.0 (CH), 126.6, 127.2 (CH), 128.6 (CH), 128.6, 129.0 (CH), 129.2 (CH), 129.7 (CH), 131.9 (CH), 135.5, 136.0, 136.1, 147.2, 149.1 (CH), 169.4; *m/z* (ES+) 354 ($[M+H]^+$, 100%); *m/z* (FAB) 354.16054 ($M+H^+$ $C_{23}H_{20}N_3O$ requires 354.16064).

#### 4.6.27. (3,4-Dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)(2-(pyridin-3-yl)phenyl)methanone (16b)

Off white amorphous solid; 54% yield; 2:1 mixture of rotamers; $\delta_H$ (300 MHz, $CDCl_3$) 2.15–2.20 (1H, m), 2.50–2.55 (1H, m), 3.04–3.10 (1H, m), 3.40–3.45 (1H, m), 4.36 (1H, $d_{AB}$, *J* 16.8), 5.02 (1H, $d_{AB}$, *J* 16.8), 6.88–7.61 (10 H, m), 8.42–8.44 (2H, m), 8.66–8,67 (1H, m), 9.08 (1H, br s), *δ (distinct signals for minor rotamer)* 2.62–2.66 (1H, m), 2.53–2.54 (1H, m), 3.69–3.76 (1H, m), 3.86–3.97 (2H, m), 4.09–4.14 (1H, m), 8.07–8.08 (1H, m, Ar), 8.46 (1H, br s), 8.53 (1H, br s); $\delta_C$ (75.5 MHz, $CDCl_3$) 21.4 ($CH_2$), 40.3 ($CH_2$), 44.9 ($CH_2$), 107.3, 108.6, 111.0 (CH), 117.7 (CH), 119.2 (CH), 121.5 (CH), 123.3 (CH), 126.5, 127.3 (CH), 128.6 (CH), 129.4, 129.7 (CH), 131.9 (CH), 135.1, 135.5, 135.7, 136.0 (CH), 148.7 (CH), 148.9 (CH), 170.2; *δ (distinct signals for minor rotamer)* 20.6 ($CH_2$), 40.4 ($CH_2$), 44.8 ($CH_2$), 107.3, 108.6, 110.9 (CH), 117.8 (CH), 119.3 (CH), 121.7 (CH), 122.9 (CH), 126.7, 127.5 (CH), 128.4 (CH), 129.4 (CH), 129.7 (CH), 129.4, 132.0 (CH), 134.8, 136.1, 136.3, 148.0 (CH), 148.9 (CH), 169.5; *m/z* (ES+) 354 ($[M+H]^+$, 100%); *m/z* (FAB) 354.16066 ($M+H^+$ $C_{23}H_{20}N_3O$ requires 354.16064).

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