Supplementary Material 1

AN EFFICIENT SYNTHETIC ROUTE TOWARDS BIOLOGICALLY ACTIVE γ -CARBOLINE DERIVATIVES

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General

All reactions were carried out under an inert atmosphere with dry solvents, unless otherwise stated. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (Kieselgel 60 F₂₅₄, Merck). Visualization of the spots on TLC plates was achieved either by UV light or by staining the plates in 2, 4-dinitrophenylhydrazine/ anisaldehyde and charring on hot plate. All products were characterized by ¹H NMR and ¹³C NMR, IR. ¹H NMR and ¹³C NMR were recorded on Varian Mercury 300 MHz spectrometer. Chemical shifts are expressed in ppm values and ¹H NMR spectrum are referenced to 0.00 ppm for Me₄Si (TMS) and ¹³C NMR spectrum are referenced to 77.00 and 39.5 ppm for CDCl₃ and DMSO respectively. Peak multiplicities are designated by the following abbreviations: s, singlet; bs, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet; *J*, coupling constant in Hz. IR spectrum were obtained on a Bruker – Tensor 37 with samples loaded as neat or with CH₂Cl₂ as indicated. HRMS spectrum were analyzed using ESI TOF. Melting points recorded are uncorrected. Column chromatography on silica gel (100-200 mesh) was performed with reagent grade ethyl acetate and hexane as an eluent.

General Procedure for the synthesis of Compound 6, 7 and 8

To a stirred solution of LDA (34.8 mmol) was added a solution of 1-phenyl sulphonyl indole (17.4 mmol) in THF (25 ml) at 0°C. A solution of acetone/propionaldehyde/ cyclopentanone (35 mmol) in THF (15 mL) was added after 30 min at -78°C. The reaction mixture was gradually warmed to room temperature and kept under stirring for 4 hours. The mixture was poured into aqueous NH₄Cl solution and extracted with ethyl acetate. The extract was washed with water, dried over Na₂SO₄, and evaporated. The crude product was chromatographed using silica gel with 8% ethyl acetate-hexane as an eluent system to furnish corresponding products.

***N*-(benzenesulfonyl)-2-(propan-2-ol) indole (6):** Yield:85%; M.p.60-62°C; ^1H NMR (300 MHz, CDCl_3) δ : 1.85 (s, 6H), 5.0 (s, 1H), 6.7 (s, 1H), 7.2 (m, 2H), 7.3-7.5 (m, 4H), 7.82 (d, J = 7.5Hz, 2H), 8.0 (d, J = 8.1Hz, 1H); ^{13}C NMR (75 MHz, CDCl_3) δ : 30.96, 69.24, 111.38, 115.43, 121.04, 124.07, 125.03, 126.43, 128.94, 133.67, 137.70, 137.90, 148.26. FT-IR (KBr cm^{-1}) 3549, 2980, 1449, 1343, 1180; HR-MS (EI/GCMS) m/z calcd for $\text{C}_{17}\text{H}_{17}\text{O}_3\text{NS}$ ($\text{M}+\text{Na}$) $^+$ 338.0817 found 338.0836

***N*-(benzenesulfonyl)- 2-(propan-1-ol) indole (7):** Yield: 82%; M.p.89-92°C; ^1H NMR (300 MHz, CDCl_3) δ : 1.0 (t, J = 7.6 Hz, 3H), 1.9-2.1 (m, 2H), 3.17 (d, J = 5.3Hz, 1H), 5.2 (m, 1H), 6.6 (s, 1H), 7.2-7.3 (m, 2H), 7.35-7.55 (m, 4H), 7.8 (m, 2H), 8.1 (d, J = 8.6Hz, 1H); ^{13}C NMR (75 MHz, CDCl_3) δ : 10.69, 28.69, 68.20, 109.32, 114.79, 121.14, 123.87, 124.92, 126.26, 129.24, 133.84, 137.24, 138.66, 144.03. FT-IR (KBr cm^{-1}) 3570, 2986, 1574, 1450, 1163, 748.

***N*-(benzenesulfonyl)-2-(1-hydroxycyclopentyl)indole (8):** Yield: 76%; M.p.104-106°C; ^1H NMR (300 MHz, CDCl_3) δ : 1.75(m, 2H), 1.98 (m, 2H), 2.14-2.42 (m, 4H), 4.48 (bs, 1H), 6.72 (s, 1H), 7.18 (bs, 2H), 7.38 (m, 4H), 7.8 (d, J = 5.5Hz, 2H), 7.94 (d, J = 6.1 Hz, 1H); ^{13}C NMR (75 MHz, CDCl_3) δ : 23.15, 40.65, 79.10, 110.62, 115.19, 121.06, 123.98, 124.92, 126.40, 129.0, 129.20, 133.64, 137.68, 138.12, 146.94. FT-IR (KBr cm^{-1}) : 3565, 1356

General Procedure for the synthesis of Compound 9, 10, 11 and 12

A mixture of 6 / 7/ 8 (0.01 mole) were dissolved in 20 ml THF. TBAF (0.015 mole) 1. 0 M solution in THF was added in the reaction mixture, the reaction mass was refluxed for 2 hours. After completion of reaction, reaction mixture was evaporated on rota-evaporator. Water was added to the residue; the mixture was extracted with ethyl acetate (25 ml x 3), dried over sodium sulphate and evaporated on rota-evaporator. The crude product thus obtained was purified by column chromatography by using hexane.

2-(Prop-1-enyl)-2H-indole (9) ^[1,2]: Yield: 78%; M.p. 117-119 °C (Lit M.p. 116-118 °C)

2-(Prop-1-enyl)-1H-indole (10) ^[3]: Yield: 76%, Oily compound.

1-Methyl-2-vinyl-1H-indole (11) ^[4]: Yield: 80%; M.p. 82-84 °C (Lit M.p. 84-85 °C)

2-Cyclopentenyl-1H-indole (12): Yield: 82%; M.p. 129-130°C; (Lit^[3] M.p. 129.3-130.6)

¹H NMR (300 MHz, CDCl₃) δ : 2.02 (m, 2H), 2.49-2.74 (m, 4H), 5.90 (t, *J* = 1.92Hz, 1H), 6.40 (s, 1H), 7.0-7.26 (m, 3H), 7.54 (d, *J* = 7.7 Hz, 1H), 7.98 (s, 1H); ¹³C NMR (75 MHz, CDCl₃) δ : 23.12, 32.96, 33.30, 100.66, 110.40, 119.75, 120.44, 122.16, 124.58, 128.80, 134.76, 135.34, 136.42. FT-IR (KBr cm⁻¹) 3419, 1620; GC-MS (DIP) = 183 (M⁺); calcd for C₁₃H₁₃N: C, 85.21; H, 7.15; N, 7.64; Found : C, 85.10; H, 7.35; N, 7.42.

General Procedure for the synthesis of Compound 13, 14, 15 and 16

A mixture of DMF (52.60 mmol) and POCl₃ (23.80 mmol) was stirred at 25°C for 30 min. To this solution was added a solution of 9/10/11/12 (12.73mmol) in DMF (10mL). The mixture was stirred for 1 hour and monitored by TLC. After the completion, reaction was poured into ice and reaction mixture was made basic with 10% NaOH to give yellowish precipitate, which was extracted in ethyl acetate to give crude product. The crude product was purified by column chromatography using ethyl acetate - hexane (15%).

2-(Prop-1-en-2-yl)-1H-indole-3-carbaldehyde (13): Yield: 90%; M.p. 168-170°C (decomposed); ¹H NMR (300 MHz, CDCl₃) δ : 2.29 (s, 3H), 5.58 (d, *J* = 1.5Hz, 2H), 7.2-7.31 (m, 2H), 7.32-7.4 (m, 1H), 8.40 (m, 1H), 8.48 (bs, 1H), 10.17 (s, 1H); ¹³C NMR (75 MHz, CDCl₃) δ : 22.51, 111.06, 122.14, 123.09, 124.34, 133.62, 186.36. FT-IR (KBr cm⁻¹) 3179, 2724, 1628; HR-MS (EI/GCMS) *m/z* calcd for C₁₂H₁₁NO (M+H)⁺ is 186.0919 found 186.0927.

2-((E)-prop-1-enyl)-1H-indole-3-carbaldehyde (14): Yield: 86%; M.p. 177-180°C (decomposed); ¹H NMR (300 MHz, CDCl₃) δ : 2.0 (d, *J* = 6.7Hz, 3H), 6.3-6.5 (m, 1H), 7.0

(d, $J = 16.7\text{Hz}$, 1H), 7.2-7.3 (m, 3H), 8.26 (m, 1H), 8.61 (bs, 1H), 10.29 (s, 1H); ^{13}C NMR (75 MHz, CDCl_3) δ : 19.0, 110.65, 118.76, 121.53, 122.85, 124.33, 132.49, 184.59. FT-IR (KBr cm^{-1}) 3188, 2800, 1630; HR-MS (EI/GCMS) m/z calcd for $\text{C}_{12}\text{H}_{11}\text{NO}$ ($\text{M}+\text{H}$) $^{+}$ is 186.0919 found 186.0927.

1-Methyl-2-vinyl-1H-indole-3-carbaldehyde (15): Yield: 92%; M.p. 185-187°C (decomposed); ^1H NMR (300 MHz, CDCl_3) δ : 3.77 (s, 3H), 5.83 (dd, $J = 0.6\text{Hz}$, 17.1Hz 2H), 6.92 (dd, $J = 11.4\text{Hz}$, 17.6Hz, 1H), 7.3 (m, 3H), 8.3 (m, 1H), 10.08 (s, 1H); ^{13}C NMR (75 MHz, CDCl_3) δ : 30.60, 109.44, 109.99, 115.18, 122.07, 123.12, 123.93, 124.10, 125.50, 126.80, 185.58. FT-IR (KBr cm^{-1}) 2939, 2747, 2348, 1633, 1454, 1374; HRMS (EI/GCMS) m/z calcd for $\text{C}_{12}\text{H}_{11}\text{NO}$ ($\text{M}+\text{H}$) $^{+}$ is 185.0841 found 185.0835.

2-(Cyclopent-1-en-1-yl)-1H-indole-3-carbaldehyde (16): Yield, 88%; Off white crystalline solid; M.p 233-234°C; ^1H NMR (300 MHz, $\text{CDCl}_3 + \text{DMSO}-d_6$): 2.12 (m, 3H), 2.88 (m, 3H), 6.50 (bs, 1H), 7.18 (m, 2H), 7.4 (d, $J = 7.42\text{ Hz}$, 1H), 8.2 (d, $J = 7.42\text{ Hz}$, 1H), 10.22 (s, 1H), 11.60 (s, 1H); ^{13}C NMR (75 MHz, $\text{CDCl}_3 + \text{DMSO}-d_6$): 22.90, 32.9, 34.30, 110.98, 114.12, 120.86, 121.64, 123.02, 125.62, 132.62, 135.48, 135.87, 144.96, 184.78.; IR (Nujol): ν 1710, 2730, 3174 cm^{-1} ; GC-MS(DIP) = 211(M^{+}); calcd for $\text{C}_{14}\text{H}_{13}\text{NO}$: C, 79.62; H, 6.16; N, 6.63; Found : C, 79.52; H, 6.10; N, 6.42.

Cytotoxicity assessment by MTT assay ^[5]

The cells were trypsinized using TPVG solution. 1 ml of 1×10^5 cells/ml of medium and dilutions of concentration 5, 10, 15, 20 and 25 M was added in 96 well plates and kept in the CO₂ incubator for 24, 28, 72 and 96 hours. The compounds were dissolved in 20% DMSO to obtain a solution of 1 mM concentration each. These samples were then filter sterilized using a 0.22 m filter using syringe filter. All experiments were carried out in Laminar flow hoods, Laminar Flow Ultraclean Air Unit, Microfilt, India. The cells were visualized using an Inverted Microscope, Olympus.

The number of viable cells remaining after appropriate treatment was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma Chemical Co.) assay. After 24, 48, 72h, and 96hrs MTT was added to each well at a final volume of 0.5 mg/mL and the microplates were incubated at 37°C for 3h. After the supernatant was removed, the formazan salt resulting from the reduction of MTT was solubilized in dimethyl sulfoxide (DMSO, Sigma Chemical Co.) and the absorbance was read at 570 nm using an automatic plate reader (Thermo Corporation). The cell viability was extrapolated from optical density (OD) 570 nm values and expressed as percent survival as compared to the untreated cells. The CC50: 50% cytotoxic concentration, based on the reduction of viable cell number is expressed in the graphs.

Fluorescence Microscopic studies were carried out on a Carl Zeiss Fluorescence Microscopes.

Cell imaging

For cell morphology cell imaging was carried out in a 6-well plate. Cells were grown on coverslips for 48 hrs. The media was aspirated and the cells were washed with 1 ml of PBS for 1min. Then the cells were treated with 4% paraformaldehyde for 5mins after which they were washed with 1 ml of PBS for 1min. Coverslips were washed with PBS to remove excess stain. The images were taken in a Carl Zeiss Axio Scope A1 fluorescent microscope.

Phase contrast microscopic images of 4 on HeLa and HL-60 cancer cells.

HeLa Cells

(Control)

5 μ M

10 μ M

15 μ M

20 μ M

25 μ M

HL-60 cells

5 μ M

10 μ M

15 μ M

20 μ M

25 μ M

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Supplementary Material 2

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DERIVATIVES**

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¹H-NMR and ¹³C-NMR spectra of compounds **6, 13, 1, 7, 14, 2, 15, 3, 8, 12, 16, 4**

^1H and ^{13}C NMR spectrum of products

Figure S1. ^1H -NMR spectrum of **6** (300MHz, CDCl_3)

Figure S2. ^{13}C -NMR spectrum of **6** (75MHz, CDCl_3)

Figure S3. ^1H -NMR spectrum of **13** (300MHz, CDCl_3)

Figure S4. ^{13}C -NMR spectrum of **13** (75MHz, CDCl_3)

Figure S5. ^1H -NMR spectrum of **1** (300MHz, $\text{DMSO}-d_6$)

Figure S6. ^{13}C -NMR spectrum of **1** (75 MHz, $\text{DMSO-}d_6$)

Figure S7. ^1H -NMR spectrum of **7** (300MHz, CDCl_3)

Figure S8. ^{13}C -NMR spectrum of **7** (75MHz, CDCl_3)

Figure S9. ^1H -NMR spectrum of **14** (300MHz, CDCl_3)

Figure S10. ^{13}C -NMR spectrum of **14** (75MHz, CDCl_3)

Figure S11. ^1H -NMR spectrum of **2** (300MHz, $\text{DMSO}-d_6$)

Figure S12. ^{13}C -NMR spectrum of **2** (75MHz, $\text{DMSO-}d_6$)

Figure S13. ^1H -NMR spectrum of **15** (300MHz, CDCl_3)

Figure S14. ^{13}C -NMR spectrum of **15** (75MHz, CDCl_3)

Figure S15. ^1H -NMR spectrum of **3** (300MHz, $\text{DMSO}-d_6$)

Figure S16. ^{13}C -NMR spectrum of **3** (75MHz, $\text{DMSO-}d_6$)

Figure S17. ^1H -NMR spectrum of **8** (300MHz, CDCl_3)

Figure S18. ^{13}C -NMR spectrum of **8** NMR (75MHz, CDCl_3)

Figure S19. ^1H -NMR spectrum of **12** (300MHz, CDCl_3)

Figure S20. ^{13}C -NMR spectrum of **12** (75MHz, CDCl_3)

Figure S21. ^1H -NMR spectrum of **16** (300MHz, $\text{CDCl}_3 + \text{DMSO}-d_6$)

Figure S22. ^{13}C -NMR spectrum of **16** (75MHz, $\text{CDCl}_3 + \text{DMSO}-d_6$)

Figure S23. ^1H -NMR spectrum of **4** (300 MHz, $\text{DMSO}-d_6$)

Figure S 24. ^{13}C -NMR spectrum of **4** (75MHz, $\text{DMSO-}d_6$)