Antimalarial Properties of Drugs in the Triazolopyrazine Series

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Abstract:

This study reported the synthesis of four compounds as part of the Triazolopyrazine Series, identified as having some potential antimalarial properties. These four compounds were then sent to the University of Sydney for further synthesis and eventual testing for potency, toxicity and effectiveness against the malaria parasite. We began with the synthesis of the core unit 2-chloro-6-hydrazinylpyrazine, and then created four separate compounds in the second stage of the synthesis, finishing with a ring-closing reaction to form the four products desired in the study. Thin Layer Chromatography (TLC) was performed on the spot to determine the effectiveness of the procedure. Afterwards, the results were confirmed by ^{1}H NMR spectroscopy, allowing us to show that the compounds we intended to produce were indeed present in the final product.

Introduction:

Malaria is a life-threatening disease caused by *plasmodium* parasites transmitted by infected mosquito bites, causing fever, headache, chills, vomiting, severe anaemia, respiratory distress and/or cerebral malaria.[1] In 2013, there were an estimated 198 million cases of malaria, with an estimated 584,000 deaths, 90% of which were in Africa.[1] Progress is being made throughout the world to help combat malaria in less developed regions; for example, the mortality rate of malaria has decreased by 47% since 2000;[1] however, the need for a cure for malaria is still high. No class of antimalarials have been introduced since 1996 into clinical practice,[2] a major restraint to further eradication of the deadly disease from the world. The purpose of our study was to help contribute to the worldwide research into antimalarial drugs which could help save the lives of people affected if it becomes readily available by creating four core compounds (5-chloro-3-(pyridin-2-yl)-[1,2,4]triazolo[4,3-a]pyrazine, 4-(5-chloro-[1,2,4]triazolo[4,3-a]pyrazin-3-yl)benzonitrile, 5-chloro-3-(pyridine-3-yl)-[1,2,4]triazolo[4,3-a]pyrazine and 5-chloro-3-(pyridine-4-yl)-[1,2,4]triazolo[4,3-a]pyrazine) in the Triazolopyrazine Series. In 2010, GlaxoSmithKline made the chemical structures of over 13,533 compounds which were confirmed to inhibit parasite growth by at least [illegible]ion known to the public to help find a cure,[2] which led to the beginning of the Open Source Malaria Project. Within this worldwide effort, the Triazolopyrazine Series (Series 4) makes up a large number of compounds – some compounds have useful physiochemical properties such as being non-toxic, and two compounds have been proven to be potent *in vitro*.[3] We performed the first 3 steps in the synthesis of these compounds, first starting with the synthesis of the core unit 2-chloro-6-hydrazinylpyrazine. Then, we synthesised four separate compounds in the second stage of the synthesis, finishing with a ring-closing reaction to form the four products desired in the study (see Figure 1.). The specific part of the compound we focussed on was the triazolopyrazine group and the adjacent benzene ring, the other groups on the other side of the triazolopyrazine group would be added on later by the University of Sydney, producing the final products of the project (see Figure 2.).

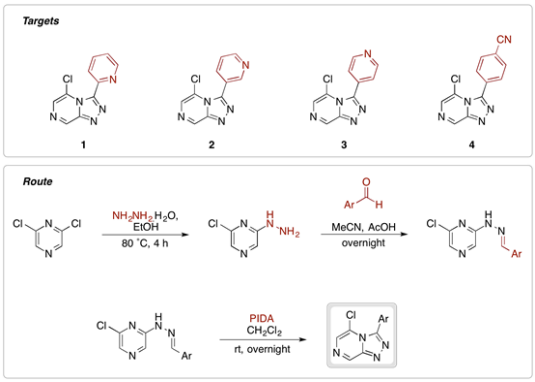


Figure 1. The Target Compounds and the Proposed Synthetic Route.

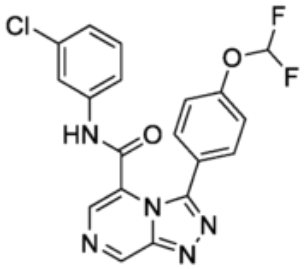


Figure 2. One of the Final Products of the Triazolopyrazine Series

Experimental:

Synthesis of 2-chloro-6-hydrazinylpyrazine (SGS 1-3)

4th May 2015 @ 22:31

2,6-dichloropyrazine (40.7 g, 270 mmol) was dissolved in 100 mL EtOH and hydrazine hydrate (27.8 mL, 540

mmol) was added (see Figure 3.). The mixture was stirred under reflux for 7 h. At this time TLC (DCM) showed the presence of a new material (R_f = 0.21). The cooled, crude mixture was allowed to stand overnight. The solvent was then removed from the crude mixture *in vacuo*, giving an orange mixture of oil and solid to which EtOAc (200 mL) and water (150 mL) was added. The aqueous layer was removed and washed with EtOAc (3x 100 mL). The combined organic layers were concentrated *in vacuo* to produce an orange crystalline solid. 11.84 g of crude was produced (see Appendix 1.). TLC (in EtOAc) showed the presence of a new material (R_f =0.6) (see Appendix 2.).

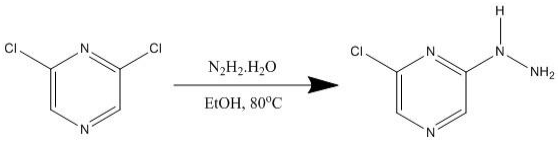


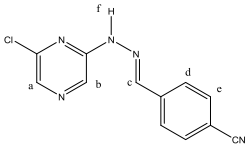
Figure 3. Synthesis of 2-chloro-6-hydrazinylpyrazine (SGS 1-3).

Synthesis of (*E*)-4-((2-(6-chloropyrazin-2-yl)hydrazono)methyl)benzonitrile (SGS 2-2)

1st June 2015 @ 23:01

SGS 1-3 (crude, 4.1g, 2.84 mmol) was stirred into MeCN (50 mL) along with 4-formylbenzonitrile (3.737 g, 2.85 mmol) (see Figure 4.). The mixture (a light brown suspension) was stirred for 72 h at room temperature. TLC (in DCM) showed the presence of a new material (R_f = 0.72), and a reduction in the amount of starting material (see Appendix 3.). NMR was then conducted in $CDCl_3$ (see Appendix 12.), with the following possible allocation of peaks:

^{1}H NMR ($CDCl_3$): 8.64 (c) (1H, s), 8.52 (b)(1H, s), 8.00 (e) (2H, d, J_{HH} 2.6 Hz), 7.95 (a) (1H, s), 7.85 (d) (2H, d, J_{HH} 2.7 Hz). (f) is likely the peak at ~10.1 ppm, exact shift unknown.



SGS 2

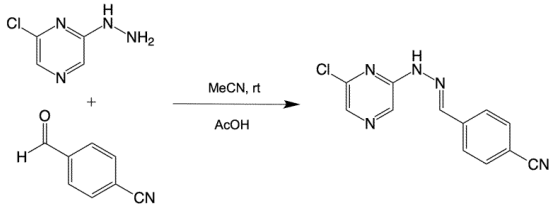


Figure 4. Synthesis of (*E*)-4-((2-(6-chloropyrazin-2-yl)hydrazono)methyl)benzonitrile (SGS 2-2).

Synthesis of (*E*)-2-chloro-6-(2-(pyridin-3-ylmethylene)hydrazinyl)pyrazine (SGS 3-2)

4th June 2015 @ 23:31

SGS 1-3 (crude, 9.15g, 6.33 mmol) was stirred into MeCN (120 mL) along with 3-pyridinecarboxaldehyde (6.787 g, 6.34 mmol) (see Figure 5.). The mixture (a green suspension) was stirred for 72 h at room temperature. Volatiles were removed *in vacuo* to produce a pale brown solid which still smelt of AcOH. To remove the AcOH, the pale brown solid was dissolved in DCM, washed twice with aqueous $NaHCO_3$, then dried over $MgSO_4$, and filtered. The solvent was removed *in vacuo* to produce a pale brown solid. 11.724 g of crude was produced. TLC showed no clear evidence of new products produced (see Appendix 4.).

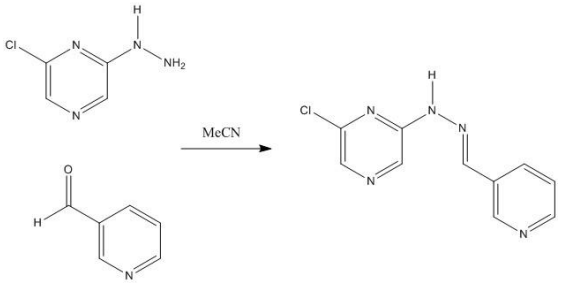


Figure 5. Synthesis of (*E*)-2-chloro-6-(2-(pyridin-3-ylmethylene)hydrazinyl)pyrazine (SGS 3-2).

Resynthesis of (*E*)-2-chloro-6-(2-(pyridin-2-ylmethylene)hydrazinyl)pyrazine (SGS 4-2)

12th March 2015 @ 20:43

SGS 1-2 (crude, 1.007 g, 7.1 mmol) was stirred into MeCN (12 ml). 2-Pyridinecarboxaldehyde (0.74 g, 6.9 mmol, density 1.137 g/mL) was added and the reaction mixture (light brown suspension) was observed (see Figure 6.). The reaction mixture was stirred at room temperature for 7 days. TLCs did not yield conclusive results in methanol and 1:1 DCM and hexane mixture; in pure hexane and in pure DCM it showed no clear evidence of new compounds being created (see Appendix 5.).

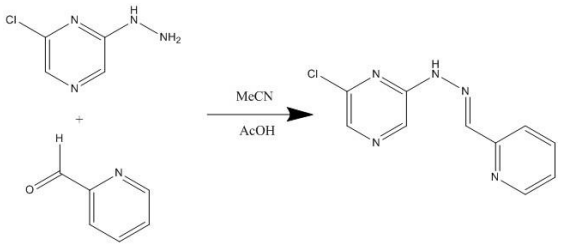


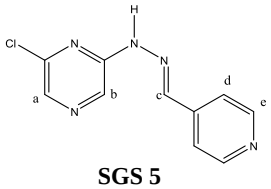
Figure 6. Synthesis of (*E*)-2-chloro-6-(2-(pyridin-2-ylmethylene)hydrazinyl)pyrazine (SGS 4-2).

Synthesis of (*E*)-2-chloro-6-(2-(pyridin-3-ylmethylene)hydrazinyl)pyrazine (SGS 5-2)

28th May 2015 @ 22:41

SGS 1-3 (crude, 3.02 g, 21 mmol) was stirred into MeCN (36 mL). 4-Pyridinecarboxaldehyde (2.25 g, 21 mmol, density 1.137 g/mL) was added and the reaction mixture (terracotta) was stirred at room temperature for 72 h (see Figure 7.). Solvent was removed *in vacuo* to produce a pale orange solid. 3.82 g of crude was produced. TLC (in DCM) suggested that there was a reaction, but no new patches were observed (see Appendix 6.). NMR was then conducted in DMSO (see Appendix 12.), with the following possible allocation of peaks:

^{1}H NMR (d_6-DMSO): 8.69 (c) (1H, s), 8.63 (e) (2H, d, J_{HH} 3.2 Hz), 8.17 (b)(1H, s), 8.06 (a) (1H, s), 7.73 (d) (2H, d, J_{HH} 3.0 Hz). Hydrogen (f) is probably peak at ~11.9 ppm, but it hasn't been identified for value.



SGS 5

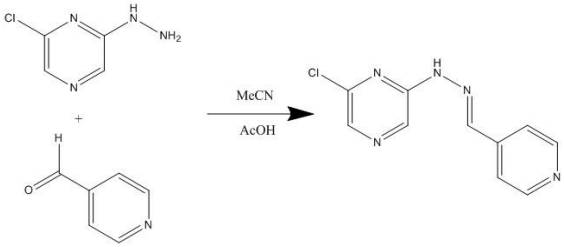


Figure 7. Synthesis of (*E*)-2-chloro-6-(2-(pyridin-3-ylmethylene)hydrazinyl)pyrazine (SGS 5-2).

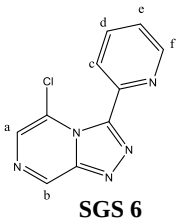
Synthesis of 5-chloro-3-(pyridin-2-yl)-[1,2,4]triazolo[4,3-a]pyrazine (SGS 6-2)

15th June 2015 @ 23:13

SGS 4-3 (crude, 1.73 g, 7.4 mmol) was stirred into DCM (100 mL) along with PIDA (2.38 g, 7.4 mmol) (see Figure 8.). The mixture (a dark green suspension) was stirred for 24 h at room temperature. The crude product was washed with saturated sodium hydrogencarbonate with the organic layer collected, and solvent was removed by evaporation. TLC (in DCM) showed the presence of new material(s), with three new spots (R_f = 0.94, 0.83 and 0.62) (see Appendix 7.).

In NMR analysis, SGS 6-2 was fully dissolved by adding ethyl acetate (~300 mL), dichloromethane (~10 mL), methanol (~10 mL), and washed with water (~30 mL) then brine (~70 mL). The organic layer was collected and dried with sodium sulfate and the solvent was removed. The crude product was dissolved in dichloromethane (~100 mL), ethyl acetate (~50 mL), and methanol (~1 mL). Silica was added and the solvent was removed under vacuum and the SGS 6-2 product was separated by column chromatography. Fractions were combined based on TLC character into 8 larger fractions. The NMR was then conducted in $CDCl_3$ (see Appendix 13.), with the following possible allocation of peaks:

^{1}H NMR ($CDCl_3$): 9.34 (a) (1H, s), 8.78 (f) (1H, D, J_{HH} 2.4 Hz), 7.91 (d, e, c) (3H, m), 7.49 (e) (1H, dd, J_{HH} 2.3, 6.9 Hz).



SGS 6

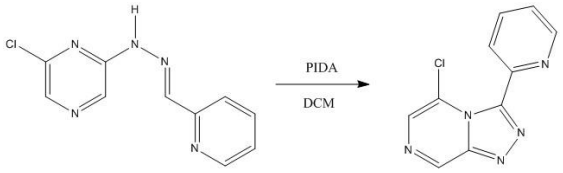


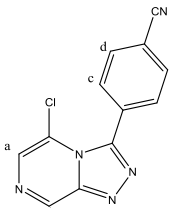
Figure 8. Synthesis of 5-chloro-3-(pyridin-2-yl)-[1,2,4]triazolo[4,3-a]pyrazine (SGS 6-2).

Synthesis of 4-(5-chloro-[1,2,4]triazolo[4,3-a]pyrazin-3-yl)benzonitrile (SGS 7-1)

8th May 2015 @ 04:35

SGS 2-1 (1.43 g, 5.6 mmol) was dissolved in DCM (70 mL) and stirred with PIDA (1.77 g, 5.5 mmol) overnight. Solvent was removed *in vacuo*. After 24 h, the solution had turned dark orange and an initial TLC was performed in DCM showing a new material (R_f = 0.7), and a subsequent TLC was then run in EtOAc with 3% MeOH confirming the presence of a new material (R_f = 0.75) (see Appendix 8.). NMR was then conducted in $CDCl_3$ (see Appendix 14.), with the following possible allocation of peaks:

^{1}H NMR ($CDCl_3$): 9.40 (a) (1H, s), 8.01(b) (1H, s), 8.00 (c) (2H, d, J_{HH} 2.6 Hz), 7.82 (d) (2H, d, J_{HH} 2.6 Hz).



SGS 7

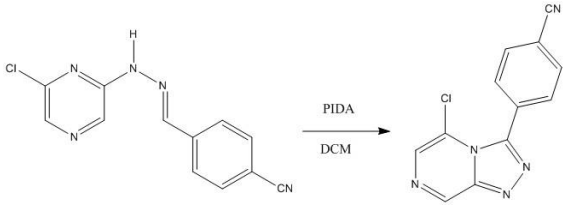
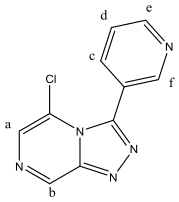


Figure 9. Synthesis of 4-(5-chloro-[1,2,4]triazolo[4,3-a]pyrazin-3-yl)benzonitrile (SGS 7-1).

Synthesis of 5-chloro-3-(pyridine-3-yl)-[1,2,4]triazolo[4,3-a]pyrazine (SGS 8-1)

7th May 2015 @ 22:50

SGS 3-1 (0.164 g, 0.702 mmol) was dissolved in DCM (10 mL) and stirred with PIDA (0.2238 g, 0.695 mmol) overnight (see Figure 10.). Solvent was removed *in vacuo*. After 24 h, the solution had turned deep red and an initial TLC was conducted in DCM showing no evidence of new compounds created (although there was an unidentified spot with $R_f = 1$), and a subsequent TLC was then run in EtOAc with 3% MeOH showing the presence of a new material ($R_f = 0.33$) (see Appendix 9.). NMR was then conducted in $CDCl_3$ (see Appendix 15.), with the following possible allocation of peaks:



SGS 8

^{1}H NMR (CDCl3): 9.38 (a) (1H, s), 8.91 (f) (1H, s), 8.84 (e)(1H, d, JHH 4.9 Hz), 8.05 (c) (1H, d, JHH 7.9 Hz), 7.93 (b) (1H, s), 7.53 (d) (1H, dd, JHH 5.0, 7.8 Hz).

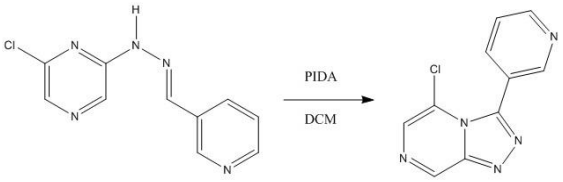
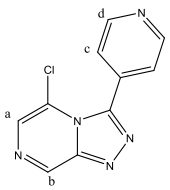


Figure 10. Synthesis of 5-chloro-3-(pyridine-3-yl)-[1,2,4]triazolo[4,3-a]pyrazine (SGS 8-1).

Synthesis of 5-chloro-3-(pyridine-4-yl)-[1,2,4]triazolo[4,3-a]pyrazine (SGS 9-1)

11th June 2015 @ 23:05

SGS 5-2 (3.017 g, 12.91 mmol) was dissolved into DCM (150 mL) and stirred with PIDA (4.133 g, 12.83 mmol) overnight (see Figure 11). The reaction was stopped and the organic layer washed with saturated $NaHCO_3$ solution (100 mL). The inorganic layer was extracted with CH_2Cl_2, (2 x 150 mL). The combined organic layers were filtered and the solvent removed by rotary evaporation before the crude product was dried *in vacuo*. TLC (in DCM) was inconclusive, some new spots developed but R_f was unable to be obtained (see Appendix 10.). NMR was conducted in d_6-DMSO (see Appendix 16.), with the following possible allocation of peaks:



SGS 9

^{1}H NMR (d_6-DMSO): 8.82 (a)(1H, s), 8.74 (b) (1H, s), 7.90 (c)(1H, d, J_{HH} 3.0 Hz), 7.80 (d) (1H, d, J_{HH} 2.8 Hz).

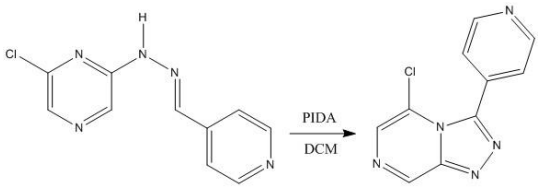


Figure 11. Synthesis of 5-chloro-3-(pyridine-4-yl)-[1,2,4]triazolo[4,3-a]pyrazine (SGS 9-1).

Results and Discussion:

The TLC and ^{1}H NMR analysis showed evidence that we made all of the required compounds, albeit with some starting material left over. Most of the TLCs performed showed evidence of a mixture of starting material and a new material in each of the samples. However, the results from the H[1] NMR highly suggested that the materials desired are indeed present in the final samples (see above). It is important to the further synthesis of new compounds that there is a large amount of these four compounds available with relatively high purity so the reactions can be undertaken without issue, and the NMR data shows that the compounds are all present in the final samples.

There were a number of issues we encountered along the way, such as seemingly unwanted side-products which hampered the process. For example, in a number of the later compounds, a black tar-like substance formed, making it difficult to transfer from one vessel to the other. This was a challenge s previous experience in using these compounds. In addition, some of the reactants began to react even before the solvent was added, possibly giving us unwanted products. Finally, because we didn't purif substances means that it is highly probable that starting material was left unreacted in the final sample.

In conclusion, the first three steps of the synthesis of compounds in the Triazolopyrazine Series were completed, with the four products (5-chloro-3-(pyridin-2-yl)-[1,2,4]triazolo[4,3-a]pyrazine, 4-(5-chloro-[1,2,4]triazolo[4,3-a]pyrazin-3-yl)benzonitrile, 5-chloro-3-pyridine-3-yl)-[1,2,4]triazolo[4,3-a]pyrazine and 5-chloro-3-(pyridine-4-yl)-[1,2,4]triazolo[4,3-a]pyrazine) present albeit with impurities. However, they are present in high enough quantity to allow the further synthesis of materials in the Triazolopyrazine series and the eventual testing of these compounds for antimalarial properties.

Acknowledgements:

We are grateful to Alice Williamson and Matthew Todd (University of Sydney) for allowing us to help them partake in the OSM project and for conducting the H[1] NMR data on the samples created. We would like to acknowledge the headmaster of Sydney Grammar

School, Dr John Vallance, for his support. In addition, we would like to thank Sydney Grammar School for its support and supply of the labs and equipment, and the Open Source Malaria Project as a whole for allowing us to gain experience in chemistry and research. Most of all we acknowledge Mr Trent Wallis for his support as Head of Chemistry and for supervising us, and Dr Erin Sheridan for all the time she has put in helping us with the reactions and aiding us with our reports.

References:

1. World Health Organisation: Media Centre: Malaria. http://www.who.int/mediacentre/factsheets/fs094/en/ (accessed 2 August 2015)

2. Gamo, F.; Sanz, L. M.; Vidal, J.; de Cozar, C.; Alvares, E.; Lavandera, J.; Vanderwall, D. E.; Green, D. V. S.; Kumar, V.; Hasan, S.; Brown, J. R.; Peishoff, C. E.; Cardon, L. R.; Carcia-Bustos, J. F.; Thousands of Chemical Starting Points for Antimalarial Lead Identification.

3. Open Source Malaria. *Open Source Malaria Series 4: The Triazolopyrazine (TP) Series*. http://openwetware.org/wiki/OpenSourceMalaria:Triazolopyrazine_%28TP%29_Series (accessed 2 August 2015)

Appendices:

Appendix 1. Pictures from the Synthesis of SGS 1-2.

20 s after hydrazine addition

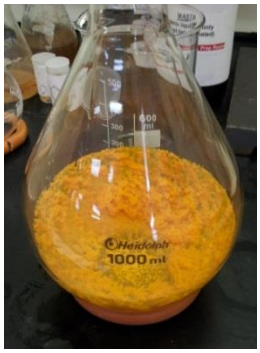
30 min after hydrazine addition

55 min after hydrazine addition

17.5 h after hydrazine addition

Product mixture after removing the ethanol

Crude product after water wash, extraction into EtOAc and removal of EtOAc



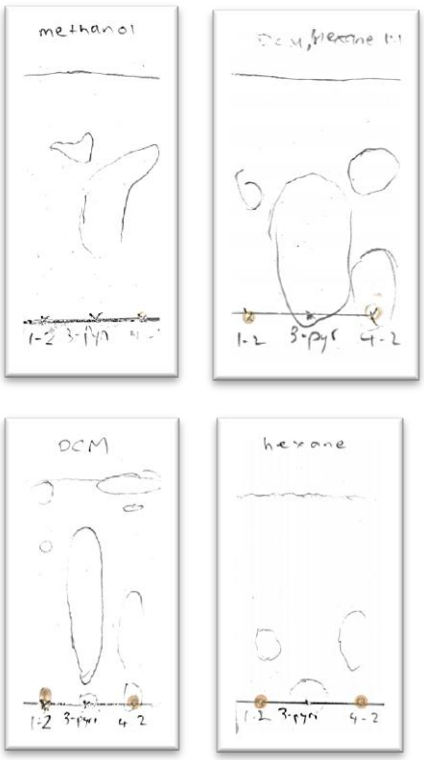
Recrystallised product

Appendix 2. TLC of SGS 1-3 (right) against Starting Material (2,6-dichloropyrazine) (left) in EtOAc.

Appendix 3. TLC of SGS 2-2 (right) against SGS 1-3 (left) in DCM (performed on 5/6/15).

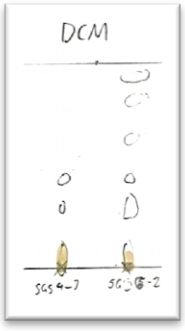
Appendix 4. TLC of SGS 3-2 (right) against SGS 1-3 (left) in DCM (performed on 9/6/15).

Appendix 5. TLCs of SGS 4-2 (right in each) against SGS 1-2 (left in each) and 2-pyridinecarboxaldehyde (centre in each, label incorrect) in methanol (top left), 1:°DCM and hexane mixture (top right), DCM (bottom left) and hexane (bottom right).

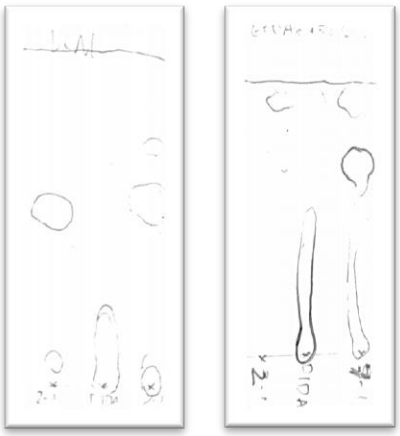


Appendix 6. TLC of SGS 5-2 (right) against SGS 1-3 (left) in DCM (performed on 5/6/15).

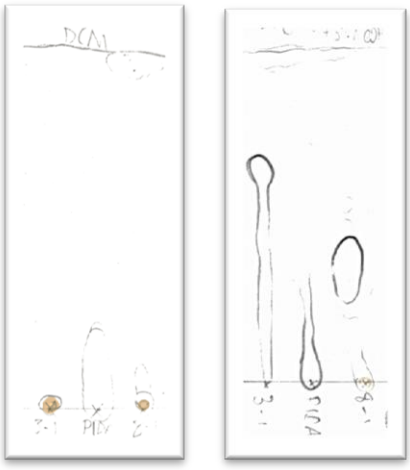
Appendix 7. TLC of SGS 6-2 (right) against SGS 4-2 (left) in DCM.



Appendix 8. TLCs of SGS 7-1 (right in each) against SGS 2-1 (left in each) and PIDA (centre in each) in DCM (left) and 97:3 EtOAc and MeOH (right).

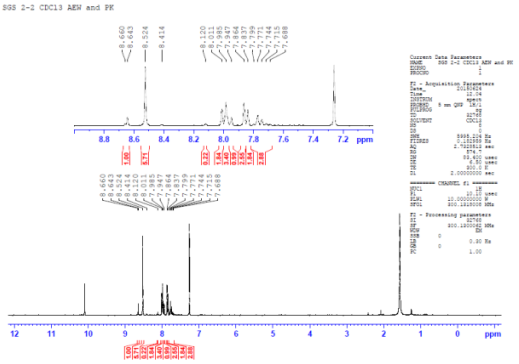


Appendix 9. TLC of SGS 8-1 (right in each) against SGS 3-1 (left in each) and PIDA (centre in each) in DCM (left) and 97:3 EtOAc and MeOH (right).



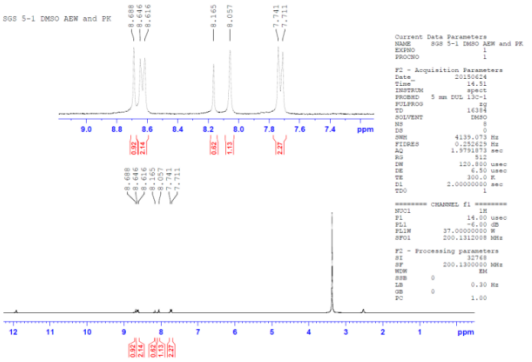
Appendix 10. TLC of SGS 9-1 (right) against SGS 5-2 (left) in DCM (performed on 15/6/15).

Appendix 11. ^{1}H NMR data of SGS 2.



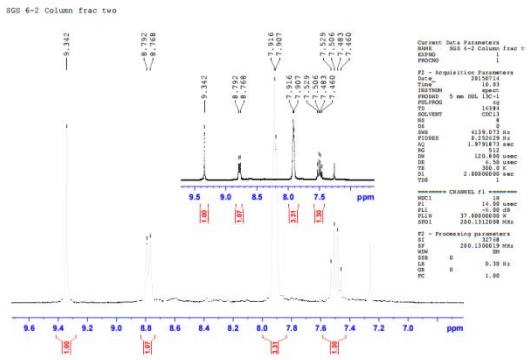
^{1}H NMR ($CDCl_3$): 8.64 (1H, s), 8.52 (1H, s), 8.00 (2H, d, J_{HH} 2.6 Hz), 7.95 (1H, s), 7.85 (2H, d, J_{HH} 2.7 Hz).

Appendix 12. ^{1}H NMR data of SGS 5.



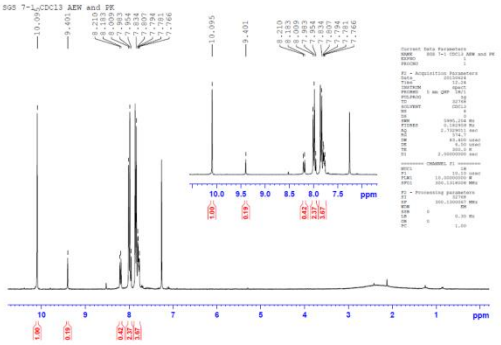
^{1}H NMR (d_6-DMSO): 8.69 (1H, s), 8.63 (2H, d, J_{HH} 3.2 Hz), 8.17 (1H, s), 8.06 (1H, s), 7.73 (2H, d, J_{HH} 3.0 Hz).

Appendix 13. ^{1}H NMR data of SGS 6.



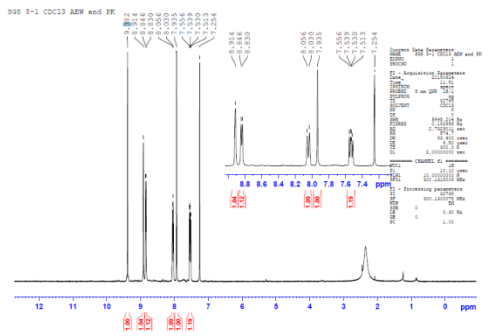
^{1}H NMR ($CDCl_3$): 9.34 (1H, s), 8.78 (1H, D, J_{HH} 2.4 Hz), 7.91 (3H, m), 7.49 (1H, dd, J_{HH} 2.3, 6.9 Hz).

Appendix 14. ^{1}H NMR data of SGS 7.



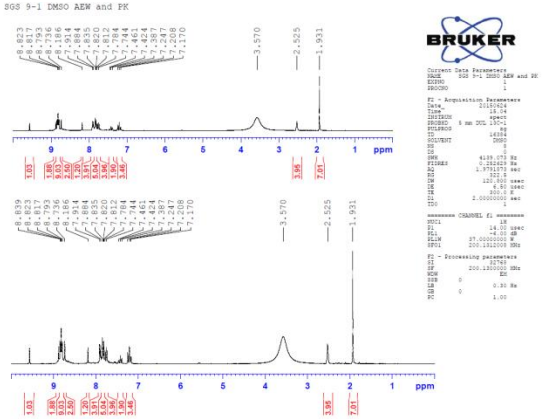
^{1}H NMR ($CDCl_3$): 9.40 (1H, s), 8.01(1H, s), 8.00 (2H, d, J_{HH} 2.6 Hz), 7.82 (2H, d, J_{HH} 2.6 Hz).

Appendix 15. ^{1}H NMR data of SGS 8.



^{1}H NMR ($CDCl_3$): 9.38 (1H, s), 8.91 (1H, s), 8.84 (1H, d, J_{HH} 4.9 Hz), 8.05 (1H, d, J_{HH} 7.9 Hz), 7.93 (1H, s), 7.53 (1H, dd, J_{HH} 5.0, 7.8 Hz).

Appendix 16. ^{1}H NMR data of SGS 9.



^{1}H NMR (d_6-DMSO): 8.82 (1H, s), 8.74 (1H, s), 7.90 (1H, d, J_{HH} 3.0 Hz), 7.80 (1H, d, J_{HH} 2.8 Hz).