Improving synthesis of the ether-linked triazolopyrazine compounds

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Malaria is one of the world's most severe diseases, affecting over 198 million people each year, many of whom are amongst the poorest in the world. The purpose of our research was to improve synthesis of the ether-linked compounds of the triazolopyrazine series, and increase both purity and yield of the reactions performed. Of the nine compounds produced, all were derivatives of 2-chloro-6-hydrazinylpyrazine, and were concentrated either through washing or *in vacuo*. Once completed, TLC's were taken, and samples sent for NMR analysis to determine the composition and purity of the mixtures formed. Results were largely positive, although some compounds were found to be slightly impure. These indicate that while the desired compounds were produced, further work is needed to increase purity.

Introduction

In 2013, 198 million cases of malaria were reported, leading to an estimated total of 584,000 deaths worldwide. The infectious disease is most prevalent in the world's poorest regions, with over 90% of deaths attributed to malaria occurring in the African Region. The child mortality rate associated with malaria is also high, with 78% of malaria deaths being young children under the age of 5.[1] However, there is currently no effective licensed vaccine for the disease, and no new class of antimalarials has been introduced into clinical practice since 1996. This is due largely to the difficulties in developing new drugs, but also a lack of research funding and commitment towards a disease that affects mainly the poorest in the world.[2-3]

Currently, the most effective available class of antimalarial drugs are Artemisinin-based combination therapies (ACTs), which are used for the treatment of the most common strain of malaria, *Plasmodium falciparum*.[4] The efficacy of ACTs is threatened, however, by the rise of the *P. vivax* strain, as well as *P. falciparum* strains displaying reduced clinical response to the drug. In 2010, GlaxoSmithKline (GSK) and other companies took the unprecedented step of releasing thousands of antimalarial compound codes into the public domain. Since then several projects have used the data released to begin work on synthesising new series of compounds, and improve the potency and efficacy of antimalarial drugs. Series 4, the triazolopyrazines (TP), in particular appears positive, with most compounds displaying high potency.

In this work, the focus was on improving the synthesis of the ether-linked compounds of the TP series originally released in 2010. The aim was to improve solubility and stability of the compounds, while also developing a method of synthesis that produced a higher percentage yield. Due to the project's small scale, however, work involved only synthesis of the partly functionalized pyrazine core of the ether series. Nine compounds were synthesised in total (SGS 1 - SGS 9), and their purity analysed through TLC and NMR analysis.

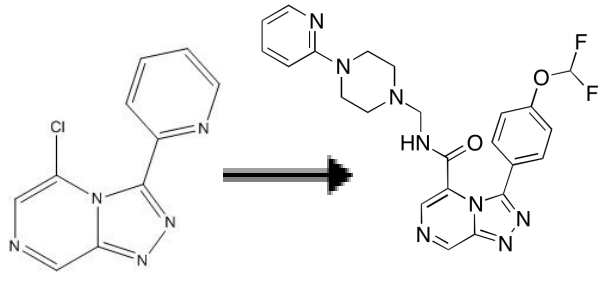


Figure 1: the partly functionalized pyrazine core (SGS 6), left, and example of a fully functionalized ether compound, right.

While this work was limited to the ether series, the issue of improving potency is one that has worldwide importance. Although ACTs are still the most widely used class of antimalarials, increasing resistance threatens the efficacy of malaria treatment that is often vital in the world's poorest regions. The goal of our work, therefore, was to potentially contribute to the development of an effective drug to limit the perpetually high mortality rates associated with malaria.

Experimental

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

2,6-dichloropyrazine (40.2 g, 270 mmol) was dissolved in 120 mL EtOH and hydrazine hydrate (27.2 mL, 540 mmol) was added. The mixture was stirred under reflux for 7 h. 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 (200mL) and water (150mL) was added. The aqueous layer was removed and washed with EtOAc (3x 100mL). The combined organic layers were concentrated *in vacuo* to produce an orange crystalline solid.

64.3 g of orange crude was produced (≈45% yield)

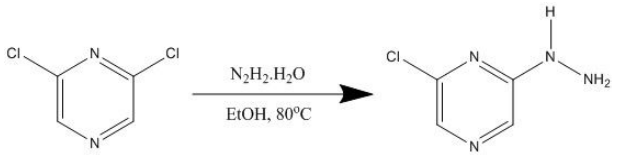
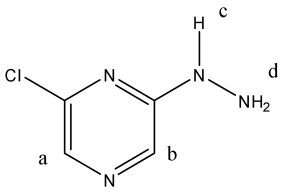


Figure 2: reaction for the synthesis of SGS 1



^{1}H NMR (d_6-DMSO): 8.87 (c)(1H, s), 8.44 (b) (1H, s), 8.06 (a) (1H, s), 4.39 (d) (2H, s)

Above, figure 3: NMR peak allocation for SGS 1

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

SGS 1 (crude, 4.1g, 2.84 mmol) was stirred into MeCN (50 mL) along with 4-formylbenzonitrile (3.737 g, 2.85 mmol). The mixture (a light brown suspension) was stirred for 72 h at room temperature.

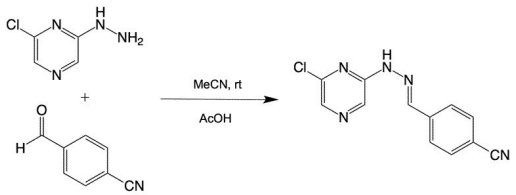
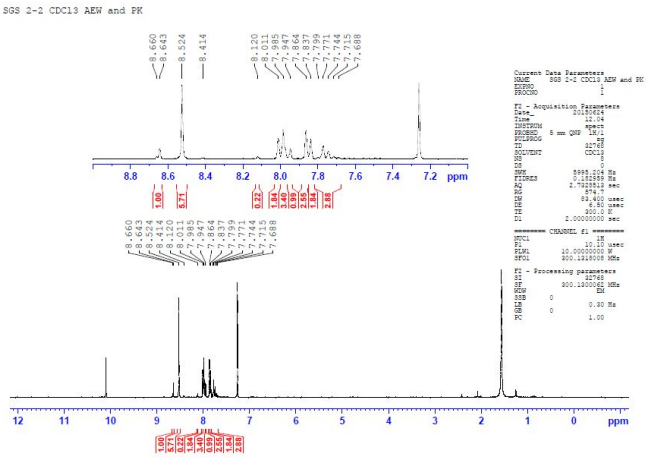
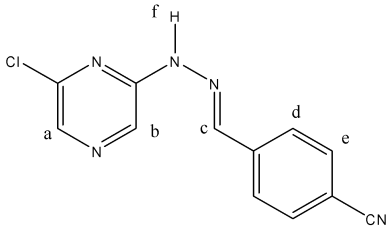


Figure 4: reaction for the synthesis of SGS 2



^{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).



Top, figure 5: NMR data for SGS 2
Above, figure 6: NMR peak allocation for SGS 2

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

SGS 1 (1.01 g, 6.99 mmol) was added to 0.756 g (7.06 mmol) of 3-pyridinecarboxaldehyde with 0.45 mL of glacial acetic acid and 12 mL of MeCN solvent. This was stirred at room temperature for 48 h. 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 (200 mL), 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.

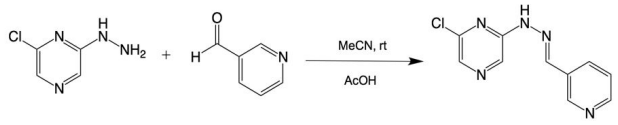


Figure 7: reaction for the synthesis of SGS 3

Synthesis of (E)-2-chloro-6-(2-(pyridine-2-yl methylene)hydrazinyl)pyrazine (SGS 4)

SGS 1 (1.06 g, 7.33 mmol) was stirred into MeCN (15 mL). 2-Pyridinecarboxaldehyde (0.77 g, 7.2 mmol) and AcOH (0.4 mL) were added to the mixture. This reaction mixture was then stirred at room temperature overnight.

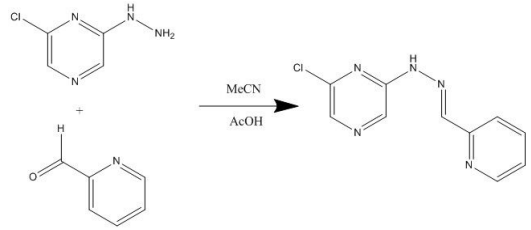


Figure 8: reaction for the synthesis of SGS 4

Synthesis of (E)-2-chloro-6-(2-(pyridin-3-yl methylene)hydrazinyl)pyrazine (SGS 5)SGS 1 (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. Solvent was removed *in vacuo* to produce a pale orange solid.

3.82g of crude was produced (≈11% yield)

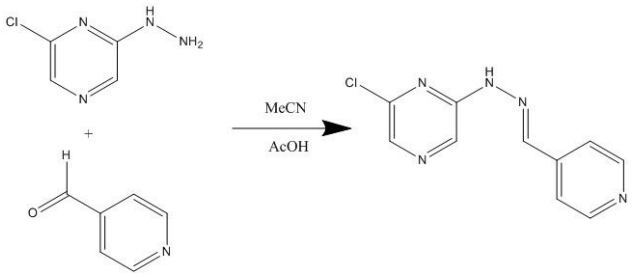
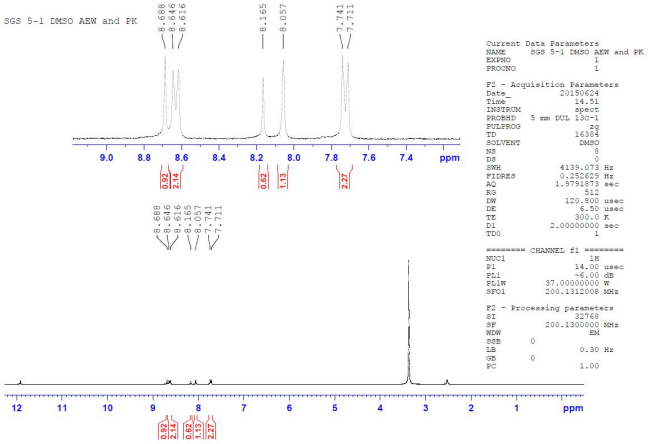
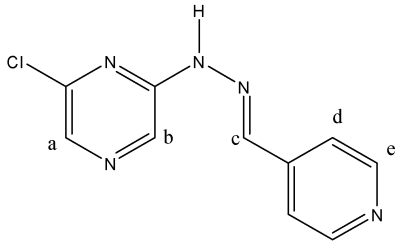


Figure 9: reaction for the synthesis of SGS 5



^{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).



Top, figure 10: NMR data for SGS 5
Above, figure 11: NMR peak allocation for SGS 5

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

SGS 4 (crude, 1.73 g, 7.4 mmol) was stirred into DCM (100 mL) along with PIDA (2.38 g, 7.4 mmol). The mixture (a dark green suspension) was stirred for 24 h at room temperature.

The reaction mixture was washed with saturated sodium hydrogencarbonate and then the organic layer was collected and solvent was removed by evaporation.

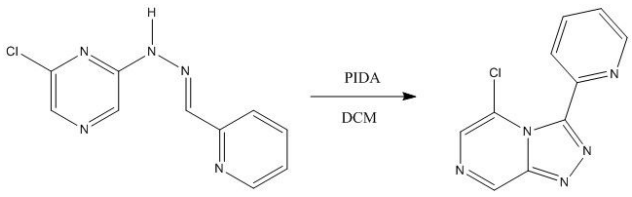
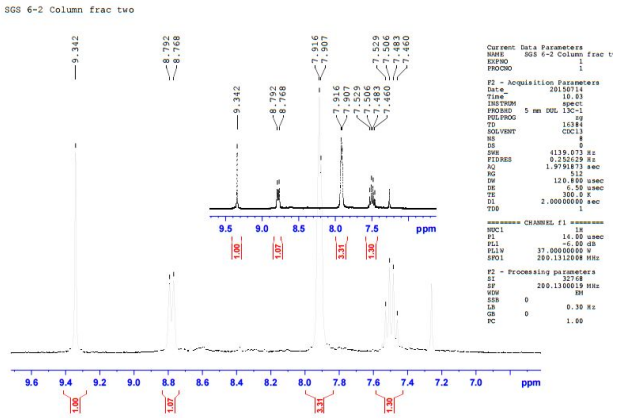
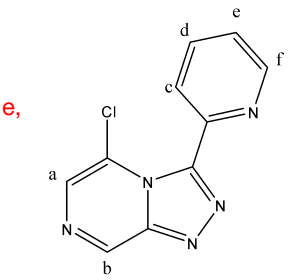


Figure 12: reaction for the synthesis of SGS 6





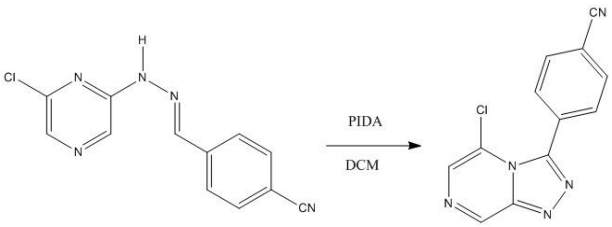
^{1}H NMR ($CDCl_3$): 9.34 (a) (1H, s), 8.78 (f) (1H, D, J_{HH} 2.4 Hz), 7.91 (d, c?) (3H, m), 7.49 (e) (1H, dd, J_{HH} 2.3, 6.9 Hz).

Top, figure 13: NMR data for SGS 6

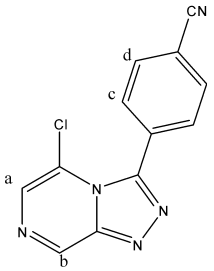
Above, figure 14: Possible NMR peak allocation for SGS 6

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

SGS 2 (1.43 g, 5.6 mmol) was dissolved in DCM (70 mL) and stirred with PIDA (1.77 g, 5.5 mmol) overnight. After 24 h, the solution had turned dark orange. Solvent was removed *in vacuo*.



Above, figure 15: reaction for the synthesis of SGS 7



^{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).

Left, figure 16: NMR peak allocation for SGS 7

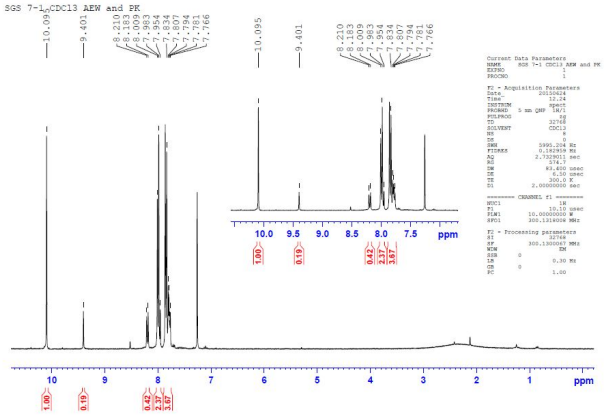


Figure 17: NMR data for SGS 7

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

SGS 3 (0.164 g, 0.702 mmol) was dissolved in DCM (10 mL) and stirred with PIDA (0.2238 g, 0.695 mmol) overnight. After 24 h, the solution had turned deep red. Solvent was removed *in vacuo*.

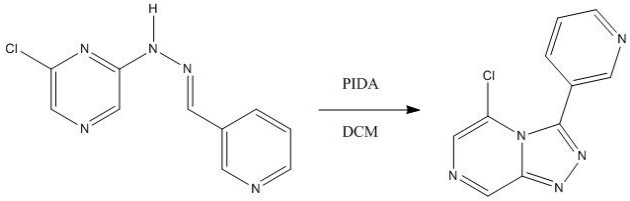
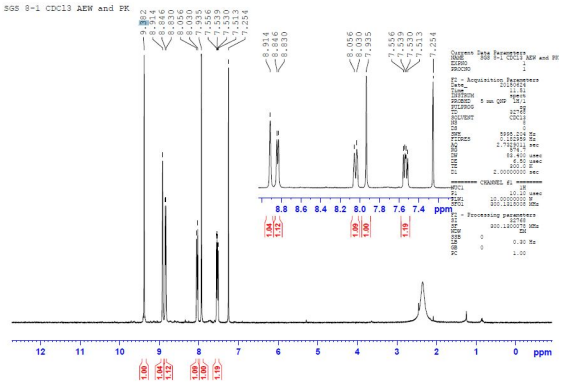
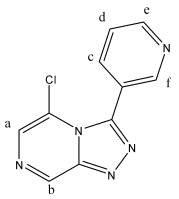


Figure 18: reaction for the synthesis of SGS 8





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

Top, figure 19: NMR data for SGS 8

Above, figure 20: NMR peak allocation for SGS 8

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

SGS 5 (3.017 g, 12.91 mmol) was dissolved into DCM (150 mL) and stirred with PIDA (4.133 g, 12.83 mmol) overnight.

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*.

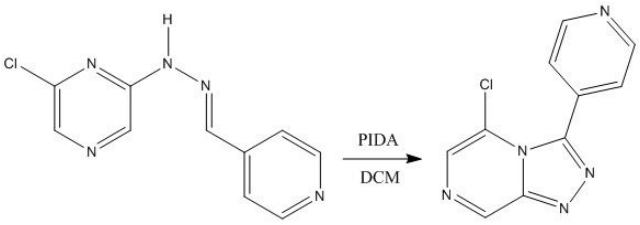


Figure 21: reaction for the synthesis of SGS 9

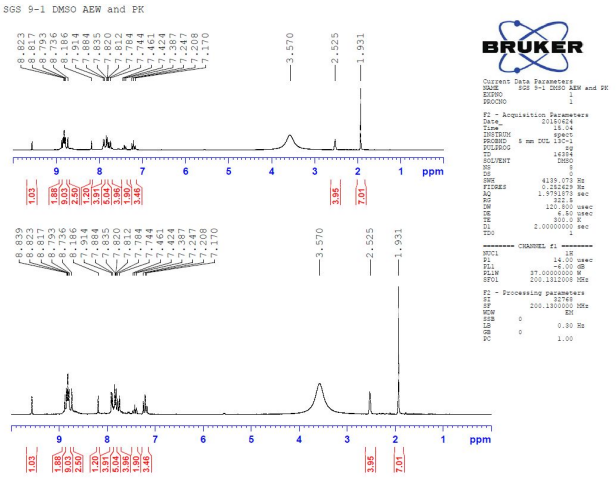
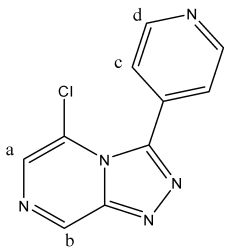


Figure 22: NMR data for SGS 9



^{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).

Above, figure 23: NMR peak allocation for SGS 9

Results and Discussion

All raw experimental data, as well as TLC (thin-layer chromatography) analyses for many of the compounds, were recorded in full on the online Open Source Malaria (OSM) Lab Notebook. Once the project was complete, samples of all compounds were sent for further purification and NMR (nuclear magnetic resonance) analysis at OSM labs.

SGS 1

Synthesis of SGS 1 was successful, with TLC analysis (appendix 1) comparing starting material to products showing the presence of a new material (at R_f =0.6). NMR data supports this, with peak allocation matching results to the compound's structure.

SGS 2

Results for SGS 2 were also positive. The TLC (appendix 2) indicates a change from SGS 1, and is supported by data from the NMR. Apart from the peak at 1.5, most likely water, all expected peak changes have taken place, indicating the reaction to be successful. There is no evidence of the starting material, SGS 1, while peak allocation matches with what is expected for SGS 2.

SGS 3

Data from SGS 3 appeared to be less positive. The reaction produced a dark green suspension, an unexpected result in the context of the experiment. TLC analysis (appendix 3), run in dichloromethane (DCM), was also inconclusive, providing little evidence that the reaction from SGS 1 was successful. NMR analysis was not conducted for SGS 3.

SGS 4

Although limited, data for SGS 4 indicates the reaction was successful, producing a new compound from SGS 1. This is supported by TLC analysis (appendix 4) in both DCM and ethyl acetate (EtOAc), although NMR was not conducted.

SGS 5

Following from SGS 2, the results for SGS 5 appeared positive. While TLC analysis was not conducted, NMR data was conclusive. There was some evidence of water at the peak at 3.3, while the peak at 2.5 is

most likely DMSO. Apart from these, though, peak allocation suggests the successful synthesis of SGS 5.

SGS 6

In the absence of NMR data for SGS 4, the basis for SGS 6, it was difficult to determine the purity of any compound produced. While TLC data (appendix 5) seems to suggest the synthesis of a new substance, NMR peak allocation is less conclusive, and unable to certify the presence of SGS 6. Thus, with the limited available data for this compound string, both the success of the reaction and purity of any compound produced are in doubt**.**

SGS 7

Analysis of SGS 7 presented several problems. While TLC analysis (appendix 6) suggests the presence of a new material following the reaction of SGS 2, NMR data is less conclusive. Although the peak at 8.65 has disappeared, the persistence of the peak at 10.1 indicates the presence of starting material, SGS 2. This means that any SGS 7 produced is slightly impure, and therefore has a lower yield percentage. Despite not being ideal, both NMR and TLC data ultimately show the desired result - the production of SGS 7.

SGS 8

Just as with SGS 6, it is difficult to assess whether the reaction of SGS 3 to form SGS 8 was successful, largely due to the lack of NMR data for SGS 3. Although NMR peak allocation appears to match the expected structure of SGS 8, results from TLC's (appendix 7) run in both DCM and EtOAc are inconclusive, and do not confirm the presence of a new substance. In addition, doubts over the synthesis and purity of SGS 3 mean that the results for SGS 8 are most likely unreliable.

SGS 9

Analysis of SGS 9 presented similar problems to SGS 7, with NMR data appearing to indicate the existence of impurities in the sample. The peak at 2.5 is DMSO, while the peak at 1.93 is ethyl acetate. The peak at 3.5, however, is unaccounted for - too downfield for water, it seems to match diglyme, although none was used in the reaction. While peak allocation seems to match the desired compound, and suggests SGS 9 has been produced, the signs of diglyme indicate only a low level of purity and the presence of contamination.

Overall, the results of the project were mixed, with several of the compounds created showing signs of contamination or impurities. In addition, many of the compounds had a low yield, making analysis more difficult. We were thus unable to fully complete our aim of improving the purity and yield in synthesising the ether-linked compounds of the TP series. Despite this, though, the project had several positives. While purity was low for some compounds, and some NMR data was inconclusive, many of the reactions produced the desired result. SGS 1, SGS 2 and SGS 5 were all produced in high yield and with high purity, an encouraging sign in the continued search for a new class of antimalarials. With further work, it is possible that both purity and yield could be improved. Therefore, although many results proved inconclusive, the project was ultimately a success in contributing to the worldwide effort to reduce the prevalence and mortality rates of malaria.

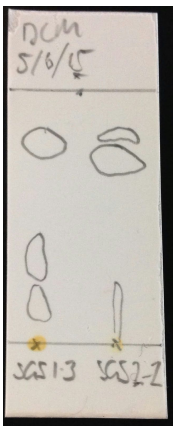
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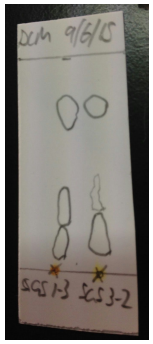
Many thanks to Dr Sheridan, for her continued help and guidance with the project. Thanks are also extended to Dr Williamson and Mr Wallis, for making the project possible. Finally, thanks to OSM, Sydney Grammar School, the Todd Research Group and the ARC for financial and technical support.

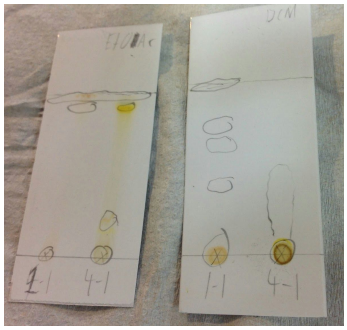
Appendices

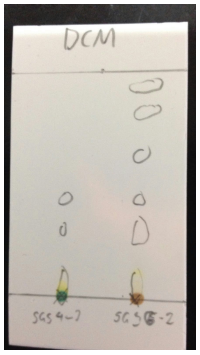
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5. TLC for SGS 6
6. TLC for SGS 7
7. TLC for SGS 8

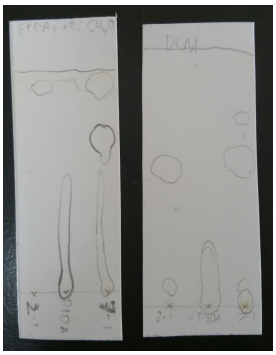
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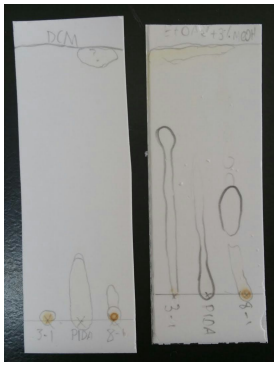
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References

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