5-chloro-[1,2,4]triazolo[4,3-a]pyrazine series 4 of Open Source Malaria synthesis, purification and characterisation

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Abstract Malaria is one of the deadliest diseases and effects up to 660,000 people in Africa each year (2010). The Open Source Malaria (OSM) project has been working towards antimalarial drug discovery with from different universities around the world. The efforts of this group are focused around a series of triazolopyrazines which require the precursor 5-chloro-[1,2,4]triazolo[4,3-a]pyrazine. The current method of synthesis has a final yield of 34.6% (after three steps) and therefore was investigated in this report. Optimisation through divergent synthesis was explored. Purification methods such as liquid/liquid extraction and semi-preparative HPLC was also investigated. As well as monitoring and characterisation techniques. A new method using trimethyl orthoformate was determined to have a 91.5% purity via GC-FID compared to the previous 4.2% crude product (via FID) or 42% yield. Methods for characterisation and preparation were explored and resulted in new techniques including GC characterisation and HPLC Semi-preparation purification. These methods will provide members of OSM with more resources to further efforts in antimalarial research allowing a greater number of options for characterisation and monitoring of these common synthetic steps.

Introduction

Open Source Malaria is a project started by the University of Sydney. Using 13,500 compounds released from GlaxoSmithKlein to take an open-source approach to drug discovery which should reduce duplication of work and have shared research. [1] The group is currently focused on obtaining SAR data from a series of triazolopyrazines and has not optimised the pathway to its common precursor. The current reported method has a approx. yield of 34.6%, in 3 steps and has not been changed since 2014.[2] This investigation is directed to increase the efficiency of the synthetic pathway through divergent synthesis [3] and consider characterisation and purification techniques.

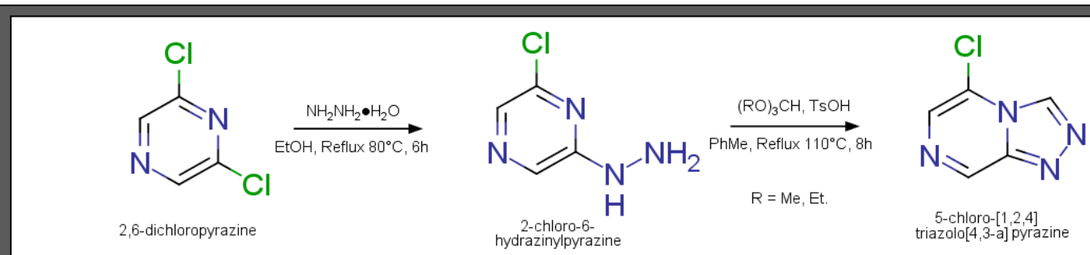


Figure 1: Reaction Scheme of 5-chloro-[1,2,4]triazolo[4,3-a]pyrazine

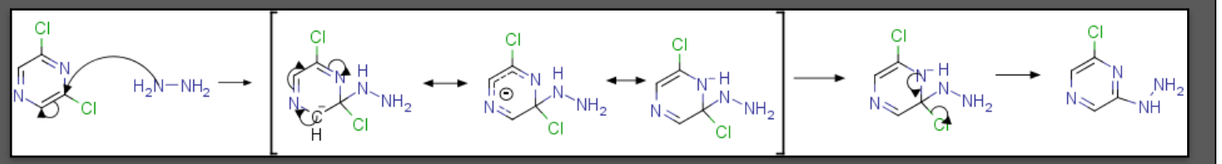


Figure 2: Mechanism of reaction, 2-6dicholorpyrazine to 2-chloro-6-hydrazinylpyrazine

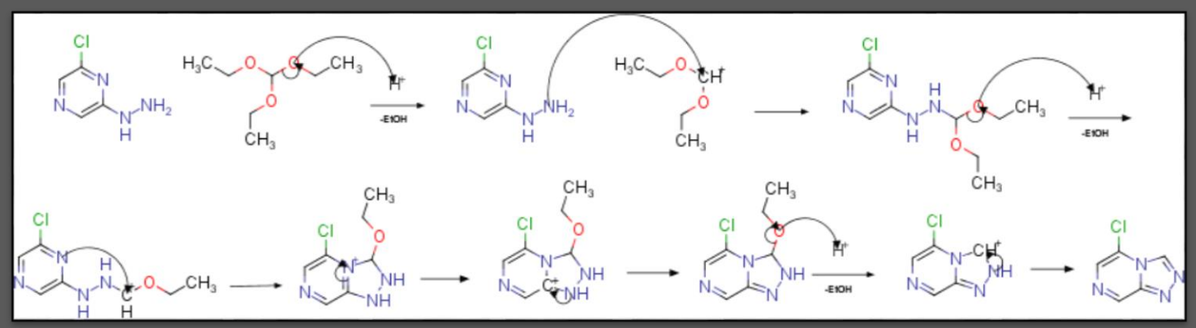


Figure 3: Mechanism of reaction, 2-chloro-6-hydrazinylpyrazine to 5-chloro-[1,2,4]triazolo[4,3-a]pyrazine

Experimental Methods

Entry	2,6-dichloropyrazine	EtOH	Hydrazine Hydrate	Reflux Time	Crude Yield
1†	0.0273mol, 1eq	50ml	0.0547 mol, 2eq	6h	17.4%*
2	0.0274mol, 1eq	20ml	0.0548 mol, 2eq	5.16h	71.9%
3	0.0405 mol, 1eq	35ml	0.0811 mol, 2eq	6h	103%‡

Table 1: 2,6-dichloropyrazine to 2-chloro-6-hydrazinylpyrazine, synthesis

Entry	2-chloro-6-hydrazinylpyrazine	From reaction	Triethyl orthoformate	TsOH	PhMe	Time	Crude Yield
4	0.0031 mol, 1eq	2	0.0062 mol, 2eq	0.05eq*	13ml	25h	NA**
5	0.0071 mol, 1eq	2	0.0141 mol, 2eq	0.1eq	20ml	6h	113%
6	0.0056 mol, 1eq	1/2	0.0113 mol, 2eq	0.5eq†	20ml	40h‡	152%
7	0.0251 mol, 1eq	3	0.0500 mol, 2eq	0.1eq	80ml	7.2h	112%

Table 2: 2-chloro-6-hydrazinylpyrazine to 5-chloro-[1,2,4]triazolo[4,3-a]pyrazine, syntheis

Entry	Tag	Chloro-hydrazinylpyrazine	Orthoformate*	Orthoformate Volume	Solvent	pTsOH	Crude Yield
8	1ET	1.38mmol	Triethyl	2eq	PhMe(4ml)	NA	116%
9	2EN	1.39mmol	Triethyl	Neat (2ml)	NA	NA	105%
10	3MTp	1.38mmol	Trimethyl	2eq	PhMe (4ml)	0.1eq	84%
11	4MT	1.38mmol	Trimethyl	2eq	NA	NA	114%
12	5MN	1.38mmol	Trimethyl	Neat (2ml)	NA	NA	113%

Table 3: Divergent synthesis of 2-chloro-6-hydrazinylpyrazine to 5-chloro-[1,2,4]triazolo[4,3-a]pyrazine

*Table 1: * Yields reduced due to manual handling error, † No brine washes, ‡ Most likely due to scale error, Table 2: * The Thomas MacDonald method stated 0.1eq but used 0.05eq which was followed in 3.4 but changed to 0.1eq hereafter. ** Crude yield was not recorded† A higher catalyst ratio was used to increase the rate of the reaction to be completed in 4 hours, however it did not do so. Table 3: *Triethyl orthoformate or trimethyl orthoformate were used.*

Divergent Synthesis

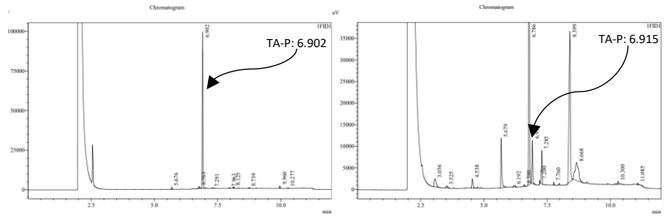


Figure X: Chromatogram of Crude 10 (left) vs Crude 7 (right)

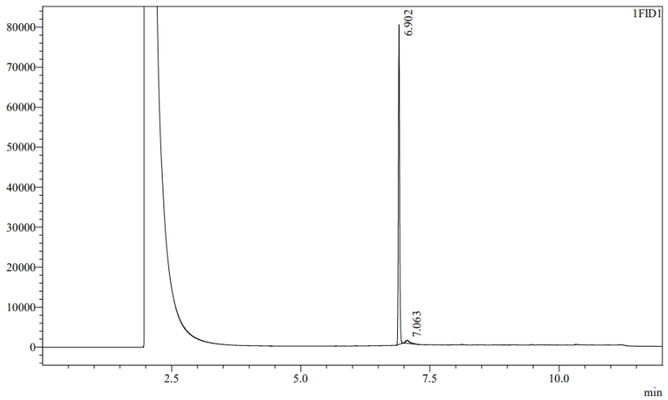
Crude 10: 91.5% Area vs Crude 7: 4.226% Area

Liquid/Liquid Extraction

Table X: Liquid/Liquid Extraction on Crude 7, GC-FID

	Peak (Ret. Time)	Area	Percentage Area	ID
MeOH	6.786	225882	46.305%	
	9.915	20819	4.268%	TA-P
	8.399	127839	26.206%	
Water (Filtered)	6.780	75439	9.270%	
	6.897	6195	0.761%	TA-P
	8.366	63201	7.766%	
Acetate/Water, Ethyl Acetate Sample	6.778	150848	29.440%	
	6.905	8095	1.580%	TA-P
	8.416	262721	51.274%	
Ethyl Acetate/Water, Water Sample	6.796	352501	66.812%	
	6.928	41298	7.827%	TA-P
	8.382	99453	18.850%	
Hexane/Water, Hexane Sample	6.887	4309	20.229	TA-P

Characterisation



Peak Table CH_OSM_170921_1_Semi Prep 2nd Peak MTp_02.gcd

FID1

Peak#	Ret. Time	Height	Height%	Area	Area%	Area/Height	Name
1	6.902	78257	98.993	135227	96.707	1.728	TA-P
2	7.063	796	1.007	4604	3.293	5.786	
Total		79053	100.000	139831	100.000		

Figure X: GC-FID of pool fraction at R_t 6.862 min (254nm) Semi-prep of Reaction 10

Semi-Prep Purification

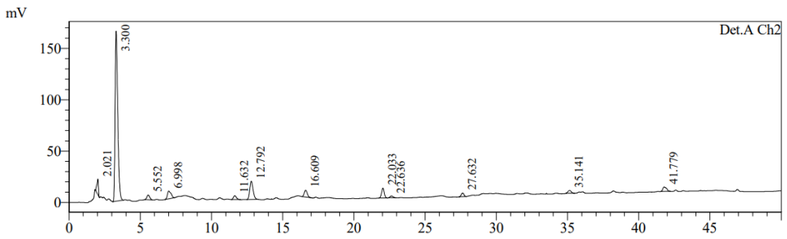


Figure X: Analytic HPLC of reaction mixture 10 (table 3), and 254nm.

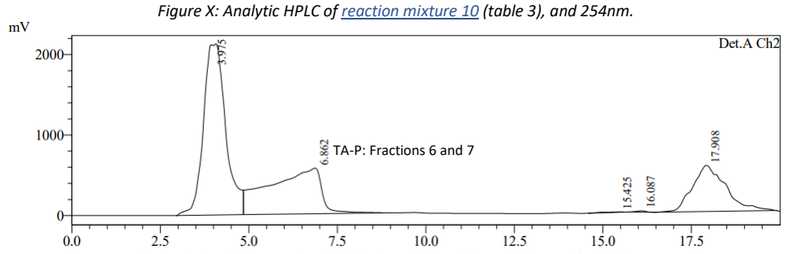


Figure X: Semi-preparative HPLC of reaction mixture 10 (table 3) at 254nm.

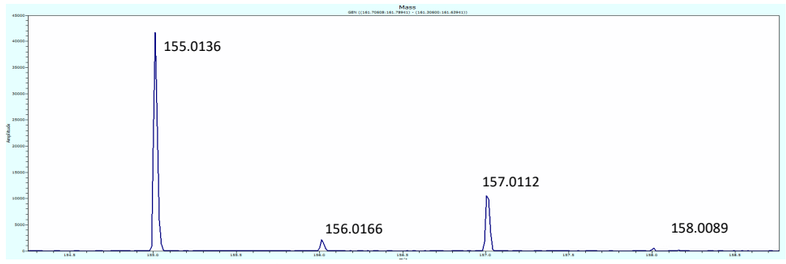


Figure X: TOF-MS of reaction mixture 10, semi-prep R_t 6.862 min (254nm).

Results and Discussion

Divergent synthesis was undertaken with the process in table 3. The purity of reaction 10 was found to be 91.5% via GC-FID. Reaction mixture (4-7) when added to water formed a solid. It was deemed that most of liquid/liquid trials had a negative effect on the purity (via GC-FID) other than the water sample of ethyl acetate. This could be investigated further. HLPC Semi-prep was used to investigate purification. GC-FID was used to development a method of triazolopyrazine characterisation. The method must be confirmed through physical yields but was successful in determine retention times of the desire compound. TOF-MS was used to determine accurate mass and isotopic ratios. UV/Vis and FTIR data was obtained.

All reagents were used as purchased from Sigma Aldrich unless otherwise stated and temperatures recorded from oil bath. Reactions were analysed via gas chromatography on a Shimadzu© GC-2010 Plus using Labsolutions and Rxi-1MS Column 30m, 0.25mmID, 0.25µm df, with either method below (Table X). All liquid chromatography UV/Vis and Semi-preparative work occurred on a Shimadzu© LC-10ADVP using Labsolutions and analytic column Alltech Platinum (C18) 100A, 5µ and Semi-preparative column Supelco Ascentis C18, 15cm, 21.2mm, 5µm, according to the methods below (Table X). Gas Chromatography – Mass Spectrum was obtained using Shimadzu© GC-2010 Plus and Shimadzu© GCMS-TQ8030 with Agilent GC Column 122-5532UI 30m, 0.25mm ID and 0.25 µm df. Mass Spectrum data was acquired via WATERS Micromass ZQ using MassLynx and MS-TOF Perkin Elmer AxION 2 TOF using TOF MS Driver. NMR data was acquired on either Varian 400MHz Unity INOVA spectrometer in d^6-dmso (acquired from Cambridge Isotope Laboratories Inc.) solvent at 298K. 1H and 13C{1H} NMR chemical shifts are referenced to solvent residuals, taken as δ2.49ppm and δ39.5ppm respectively. 2D gradient filtered correlation spectroscopy (gCOSY), heteronuclear single quantum correlation (gHSQC) and gradient filtered heteronuclear multiple bond correlations (gHMBC) were acquired using the standard sequences implicit in the VNMRJ 2.1b software package. Spectra were processed using the MestreNova 11.0 software package. Full Spectrum UV/Vis data was obtained via Agilent 8453.

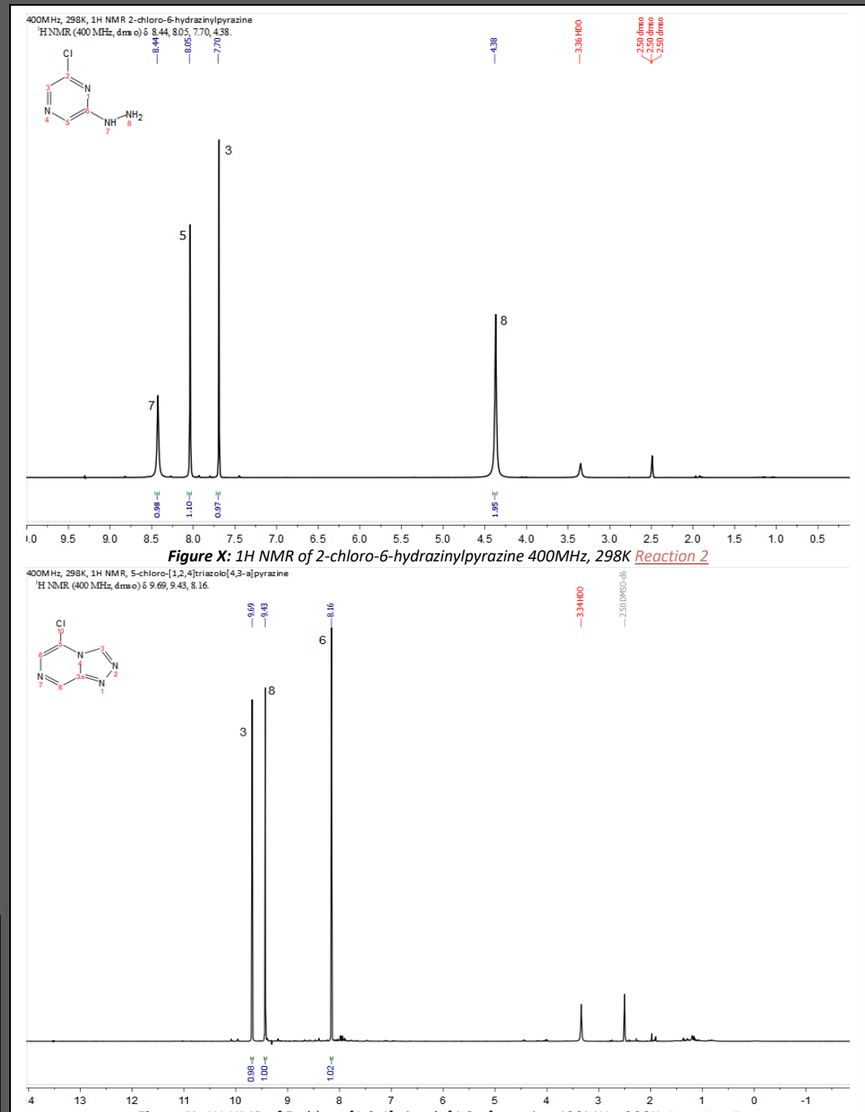


Figure X: *1H NMR of 2-chloro-6-hydrazinylpyrazine 400MHz, 298K Reaction 2*

Figure X: *1H NMR of 5-chloro-[1,2,4]triazolo[4,3-a]pyrazine 400MHz, 298K, Reaction 7*

Conclusion

The aim of the project was to optimise and investigate the common synthetic reaction for the Open Source Malaria project. The current method of synthesis for the 5-chloro-[1,2,4]triazolo[4,3-a]pyrazine is low yield with the reaction step having 42% yield. Work on trimethyl orthoformate reaction should continue as the yield of the synthetic pathway may increase due to its high purity and should be investigated in future. The development of characterisation techniques should allow the Open Source Malaria members to access a wider range of methods for further reactions. In future the trimethyl orthoformate reaction (10) should be repeated and worked up. Further investigation into simple purification on the crude should also be considered.

Acknowledgements I would like to acknowledge and thank A/Prof Mark Coster for supervising and instructing the project. Dr Fanny Lombard for providing insight and patience. And Alan White for allowing me to use the teaching laboratories, sharing his knowledge on instrumental techniques and for his tolerance.

1. Todd, M., 2016. *Open Source Malaria.* [Online] Available at: http://malaria.ourexperiment.org/the_osm_blog [Accessed 23 09 2017]. 2. Tyler, J., 2017. *Development of a Divergent Synthesis of Potent Antimalarial Series.* [Online] Available at: https://goo.gl/gZLhne [Accessed 24 09 2017]. 3. Dooseop Kim, Liping Wang, Maria Beconi, George J. Eiermann, Michael H. Fisher, Huaibing He, Gerard J. Hickey, Jennifer E. Kowalchick, Barbara Leiting, Kathryn Lyons, Frank Marsilio, Margaret E. McCann, Reshma A. Pate, § Aleksandr Petrov, Giovanna Scapin, 2005. (2R)-4-Oxo-4-[3-(Trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine: A Potent, Orally Active Dipeptidyl Peptidase IV Inhibitor for the Treatment of Type 2 Diabetes. *J. Med. Chem,* Volume 48, pp. 141-151.