Development of a divergent synthesis of potent antimalarial series

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Abstract

With increased reports of drug resistance being a major concern for malaria prevention, there is a growing need for new and effective anti-malarial drugs. The latest Open Source Malaria (OSM) series of compounds, developed from work by Pfizer and Medicines for Malaria Venture (MMV), has been shown to contain many potent drugs. As well as high activity, potential drug molecules must meet requirements for solubility and metabolic clearance. This clearly demonstrates the ongoing need to develop new analogues to meet these criteria. Due to limitations in the current linear synthesis of the series, the capability to achieve diversification is restricted. This work focuses on the development of a divergent synthetic strategy using a previously proposed triazolopyrazine starting material and assessing its ability to access functionalities not yet explored by the group. Here, a successful synthesis of the 3-Bromo-5-chloro heterocycle was completed, with characterisation of the major by-product of the reaction being the key notable result. Nucleophilic displacement of the starting material was explored and found to be limited by its regioselectivity. An alternative synthesis which removes the selectivity issues whilst still allowing diversification was also designed and attempted.

Introduction and Background

Open Source Malaria is a project dedicated to accelerating the discovery and refinement of antimalarial drugs using open source methodology. Making the research public allows a vast network of collaborating parties to pool resources and insight to aid in the achievement of the project's aims. Important information related to the

group is shared via the website GitHub,[1] and the raw data of the synthetic chemistry is made available as Electronic Lab Notebooks (ELNs)[2] which are open to the public.

Malaria is a mosquito-borne disease caused by an infectious *Plasmodium* type parasite.[3] It can be introduced into an organism's bloodstream through the mosquito's bite, resulting in fever, vomiting and, in severe cases, death. In 2015, the World Health Organisation (WHO) estimated 212 million new cases of malaria resulting in 429 000 deaths in their annual World Malaria Report.[4] Malaria has the biggest impact in Africa which accounts for 92% of global malaria deaths[4]. It is a future aim of the project that the OSM group can work in collaboration with researchers from the countries most affected by the disease. The current most effective antimalarial drug is artemisinin,[5] the discovery of which by Tu Youyou earned her a share in the 2015 Nobel Prize in Physiology or Medicine.[6] While artemisinin-based combination therapies have slowed the development of resistance it continues to be the major issue with the drug. New reports of more resistant species[7] clearly demonstrates the need for new and effective drugs that can be widely distributed to the areas most affected.

The current set of potential drug molecules that the OSM project has been developing are known as the Series 4 compounds, and are all linked by a triazolopyrazine (TP) core (Figure 1). Variation of the substituents on the **3** and **5** positions has provided the group with a wide range of potent compounds. From extensive bioassay testing, the mode of action for these drug types is thought to be through inhibition of the *Pf*ATP4 sodium ion pump leading to termination of the parasite.[8] The current strategy to access the Series 4 compounds involves a linear synthesis in which the **3**-position substituent is linked in an early stage to a pyrazine precursor. Cyclisation and the subsequent displacement of the chlorine at the **5**-position yields the desired product. This use of a prefunctionalised starting material limits the synthesis to the production of a single targeted compound and is therefore an inefficient method for generating a library of potential drug molecules.

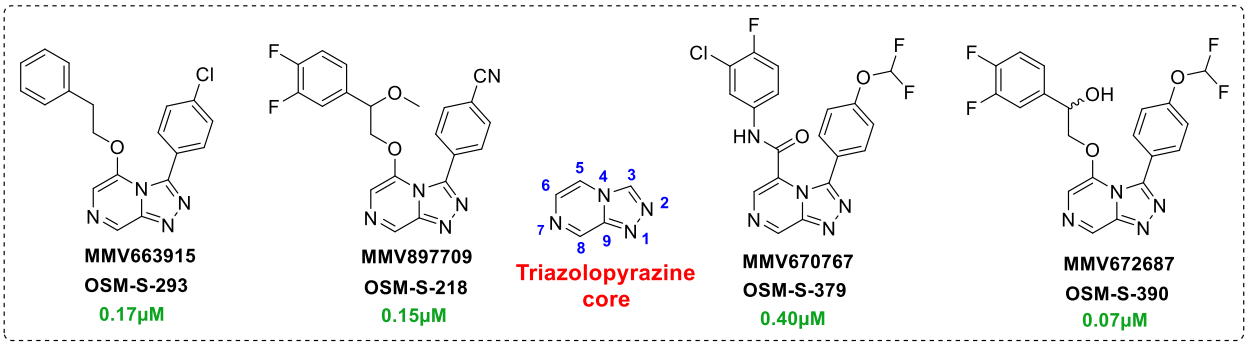


Figure 1: Examples of Series 4 frontrunners and their *in vivo* potency data (shown in green). TP core ring numbering shown in centre.

It has been demonstrated that altering the nature of either the pyrazine or triazole rings results in a dramatic loss in potency.[9] This makes a difunctionalised TP core a logical starting point for a divergent synthesis (Figure 2). By sequential addition of the two desired substituents, this common starting material could be employed to generate the Series 4 compounds as well as new derivatives in an efficient and cost-effective manner. The strategy was initially proposed by Thomas MacDonald[10] and in 2016 a synthesis was attempted by Haochuan Mao with limited success.[11] The target of this divergent synthesis was the 'Norcross' compounds, proposed by Neil Norcross of The University of Dundee.[12] These **3**-position secondary amine derivatives of the Series 4 compounds are unavailable to the current synthetic method. With additional sites of protonation and by deplanarising the substituent on the triazole ring, it is expected that these compounds will have increased *in vivo* solubility and hence be better distributed around the body. Using this kind of divergent strategy will increase the scope of the series and therefore improve the probability of finding a compound with the optimal activity, rate of metabolism and solubility.

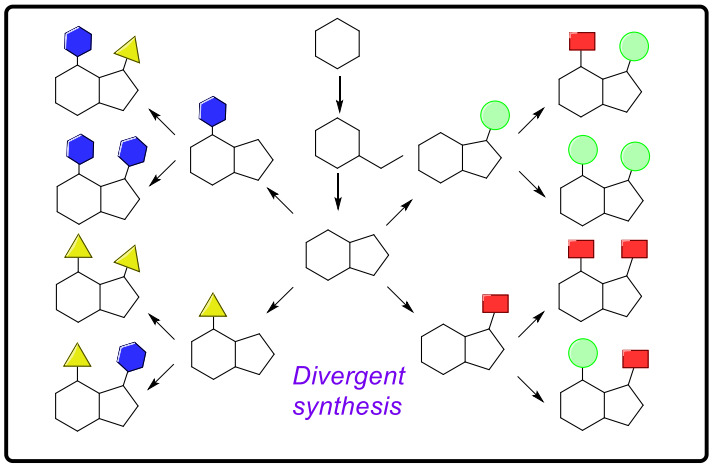


Figure 2: Divergent synthetic strategy from a difunctionalised TP starting material (centre), synthesised in a two step reaction. A diverse library of compounds can subsequently be generated via coupling of the desired substituents.

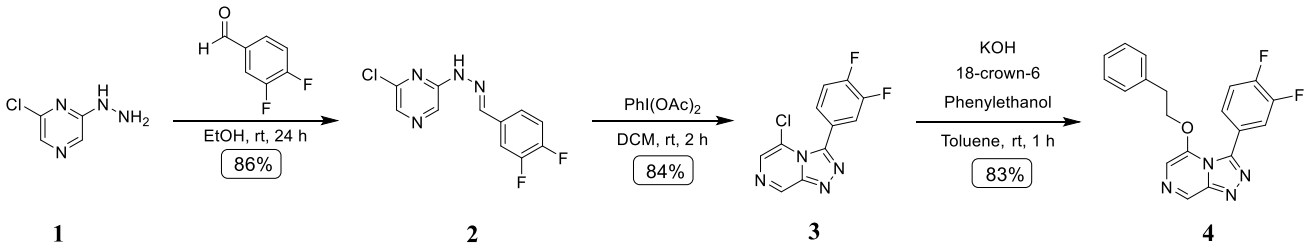
Aims of the Project

The aims of the project can be summarised in three points. Firstly, to develop the synthetic route to the difunctionalised core whilst optimising the reactions and analysing their viability for large scale synthesis. Secondly, to explore possible methods for accessing the 'Norcross' compounds to demonstrate the benefits of a divergent synthesis. And finally, to conduct all research using open source methodology, making all data available for the public.

Results & Discussion Part 1:

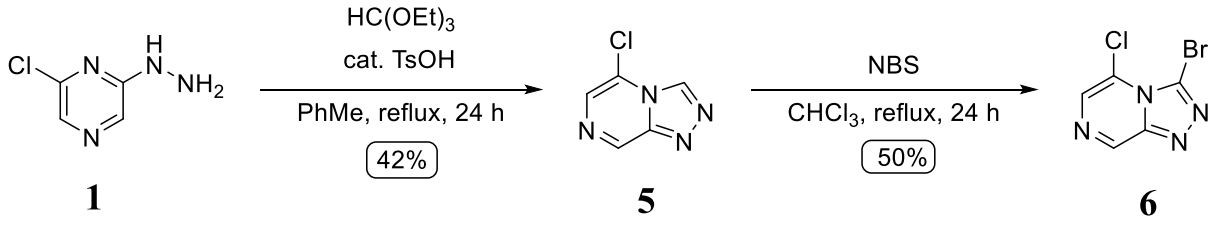
Synthesis and optimisation of a difunctionalised TP core

To assess the capabilities of the current linear synthesis, the frontrunner compound 5-Chloro-3-(3,4-difluorophenyl)-[1,2,4]triazolo[4,3-a]pyrazine (**4**) was synthesised using the method as previously stated with an overall yield of 59%. Using commercially available 2,6-Dichloropyrazine, the starting material 2-Chloro-6-hydrazinylpyrazine (**1**) was synthesised on a > 20 g scale with an 85-90% yield. This route is readily performed and allows potent drug molecules to be synthesised under mild conditions with relatively high yields (Scheme 1). Despite its benefits the traditional synthesis of the Series 4 compounds suffers from serious limitations. Most significantly, the linearity of the chemistry necessitates an early stage decision on the substituents at the **3**-position. Additionally, the cyclisation step can only proceed with a stabilised hydrazone which inherently places a carbon-carbon bond at the position **3**. This restricts the types of functional groups that may be employed at this position, and as a result, variation of the substituent at the **3-**position has been poorly explored.



Scheme 1: Synthetic route used to demonstrate current linear synthesis with isolated yields.

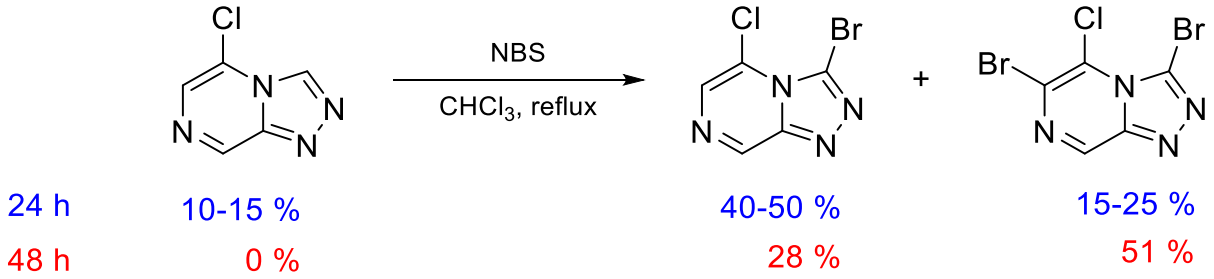
An alternative method, in which a 3-Bromo-5-chloro TP core (**6**) is used as a common starting material, will allow the synthesis to achieve rapid diversification as sequential displacement reactions can be performed in any order. The new method would also increase the scope of functionality that can occupy the **3**-position. To validate the use of the divergent synthesis for the Series 4 compounds using **6** as a universal starting point, a potential scheme to synthesise the core was investigated (Scheme 2). The first step in this process involved cyclisation of **1** to give the monochlorinated TP core (**5**). This method was developed from the work of Nelson and Potts[13] who reported cyclisation of pyrazines via a reaction with orthoesters. When refluxed in acidic conditions with triethylorthoformate, **2** was obtained with a yield of 42%. Given the low cost of the reagents, this yield was deemed adequate to proceed, despite the issue of low conversion rates. In previous work on the project, full cyclisation was discovered to be prevented due to a highly stable ethoxyhydrazone intermediate of the reaction.[10]



Scheme 2: Synthetic route used to synthesise compound **6** with isolated yields.

It was predicted that electrophilic attack of a halogenation agent would selectively displace the **3**-position hydrogen, due to the higher associated electron density of the triazole ring compared to the pyrazine ring. This assumption was justified using previous ^{13}C-NMR data from this project.[10] n-Bromosuccinimide (NBS) was a logical choice of brominating reagent as it is well reported to undergo H for Br substitution on aromatic heterocycles.[14] After refluxing **5** for 24 hours, it was observed that the reaction had not gone to completion. Due to time restraints, the reaction was stopped and purified by column chromatography to yield compound **6**. Spectroscopic analysis of the compound showed that the product had been isolated to a higher level of purity than any previous attempts made by the group.

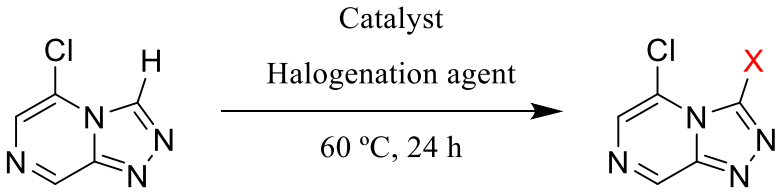
As well as starting material, a by-product was observed in the reaction mixture. This was first observed in the TLC of the reaction and isolated using column chromatography. The molecular formula was deduced using low resolution mass spectrometry and found to be the doubly brominated TP core (Scheme 3). Initial ^{1}H-NMR analysis supported this result as it showed a single aromatic peak. This signal was located between the chemical shifts of the two possible hydrogen environments of the starting material, so the identity of the by-product could not be initially concluded. To determine the site of secondary bromination, spectroscopic data of **6** from the original synthesis of this reaction was gathered. Using a combination of previous HSQC and HMBC 2D-NMR spectra,[10] it was found that the **6**-position was the most likely site of secondary bromination. The **6**-position carbon displayed a lower chemical shift (in the ^{13}C-NMR of **6**) than the **8**-position making this the site with the greater electron density and hence the more reactive to electrophiles. It is still possible that a mix of isomers was obtained as NMR prediction software shows there is only a small difference in the electronics of the two possible products. Although not the desired product, the functionalisation of this new position has not been well explored by the group and opens the possibility of a new area of diversification. By allowing all starting material to fully react a significant increase in yield of the doubly brominated core is observed, as shown in Scheme 3. It seems that reaction time strongly influences the selectivity of the reaction.



Scheme 3: The effect of varying reaction time with respect to the formation of the two major brominated products in the synthesis of **6**.

Due to the nature of the products formed, this reaction was an obvious candidate for optimisation. In theory, a reagent that directs substitution exclusively to the **3-**position could increase the yield by a substantial amount and in turn improve the overall efficiency of the synthetic route. In an attempt to increase the yield of the reaction, a series of homogenous catalysts was screened (Table 1). The compounds assessed were selected due to literature reports of their ability to either activate NBS towards electrophilic addition or increase the lability of the aromatic C-H bond.[12-16]

Table 1: Evaluation of halogenation catalysts[a].



Entry	Halogenation agent	Catalyst	Solvent	Yield (%)[b]
1	NBS	none	$CHCl_3$	50, (24)[c]
2	NCS	none	$CHCl_3$	0
3	NBS	$AuCl_3$ (0.01 equiv.)	DCE	0, (37)[c]
4	NBS	$Pd(OAc)_2$ (0.01 equiv.)[d]	PhMe	25
5	$CuCl_2$[e]	none	MeCN	0
6	HBr	DMSO (1 equiv.)	EtOAc	0
7	TBAB	Selectfluor (2 equiv.)[f]	PhMe	31

[a]Reactions were performed on 50 mg of **5** with addition of 1 equiv. of halogenation agent unless stated otherwise. [b]Percentage yield obtained after purification by column chromatography. [c]Isolated yield of doubly brominated core. [d]Reaction performed with PTSA (1 equiv.). [e]Reaction performed with $CuCl_2$ (1.5 equiv.). [f]Reaction performed with $NaHCO_3$ (1 equiv.).

Although selectivity was achieved for Entry **4** and **7**, the isolated yields of the product were still lower than that obtained without a catalyst (Entry **1**). NCS was

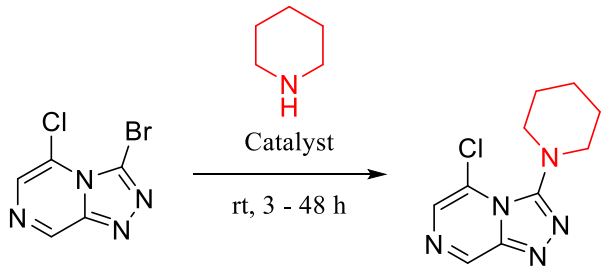
completely inactive towards compound **2,** possibly as a consequence of the stronger and less labile carbon-chlorine bond. An unusual outcome was observed in Entry **3** as the catalyst seemed to selectively favour formation of the doubly brominated core with no evidence for the formation of compound **6**. This is a notable result and may be considered as a possible catalyst for unselective diversification, despite not yielding the desired product.

Results & Discussion Part 2:

Assessment of the diversification strategies

With the proposed synthetic route being vindicated by allowing large scale production of compound **6**, the sequential coupling reactions were accessed. The **3**-position was targeted for amine coupling as the lability of the carbon-bromine bond was expected to allow selective displacement in the presence of the chlorine at carbon **5**. Piperidine was selected as the secondary amine as its Series 4 analogue is the simplest of the 'Norcross' compounds. Although simple nucleophilic aromatic substitution is expected to readily proceed for this system, previous attempts for similar reactions yielded only unwanted side products. To address this issue a series of 5 reaction conditions were screened in parallel and monitored closely with TLC (Table 2). To assess the effectiveness of nucleophilic substitution, two different metal catalysed cross-coupling reactions were screened in conjugation. Traditionally, primary and secondary amines have been linked to aryl halides using Buchwald-Hartwig cross coupling via a palladium catalyst.[20] In more recent years, Ullman type reactions have been increasingly reported as a cheaper and more effective form of metal catalysed amine coupling.[21]

Table 2. Evaluation of amine coupling conditions[a]



Entry	Time (h)	Catalyst	Solvent	Overall yield (%)[b]
1	48	5% $Pd(dba)_2$, 7% BINAP[c]	Toluene	25
2	20	No catalyst	"	30
3[d]	3	No catalyst	"	34
4	20	No catalyst	Dioxane	27
5	24	5% CuI, 20% (1,2)-Diaminocyclohexane[e]	"	31

[a]Reactions performed on 10 mg of **6** with piperidine (1.2 equiv.) and [t]BuONa (1.5 equiv.) [b]Isolated yield after purification using flash column chromatography. [c]Reaction was performed under argon atmosphere. [d]Reaction was performed with 3.6 equiv. of piperidine. [e]Reaction was performed under argon atmosphere with K_2CO_3 (3 equiv.).

After 2 hours, all reactions showed conversion to a lower polarity compound by TLC. Although no by-products were detected from column chromatography, the isolated yields were relatively low. This occurred as transferring, loading of the column and isolation of the product resulted in significant mass loss as a consequence of the small scale of reaction. ^{1}H-NMR analysis of Entry's **3** and **5**, showed evidence of all expected product peaks. Broad and low intensity signals were seen, presumably due to the low concentrations of the samples. Unexpectedly, low resolution mass spectrometry showed that all samples contained a mixture of products (Figure 3).

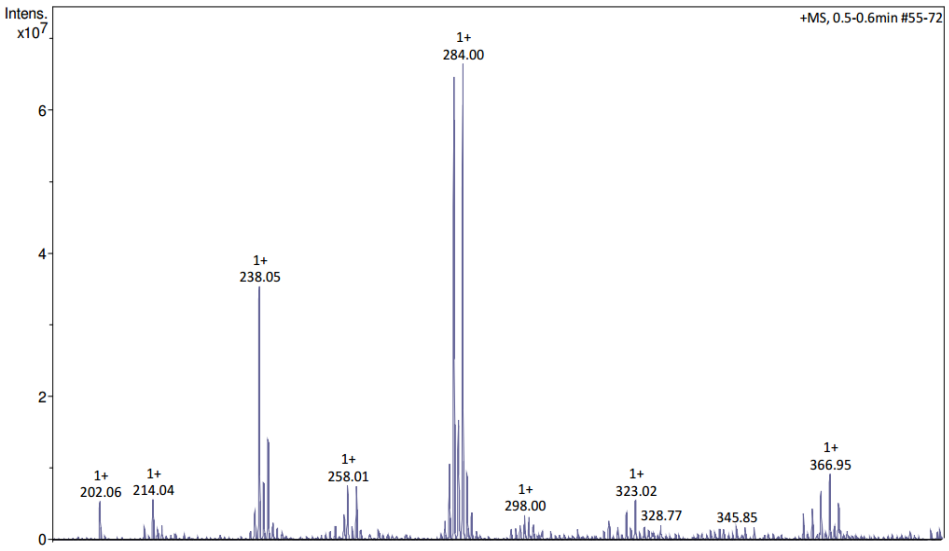
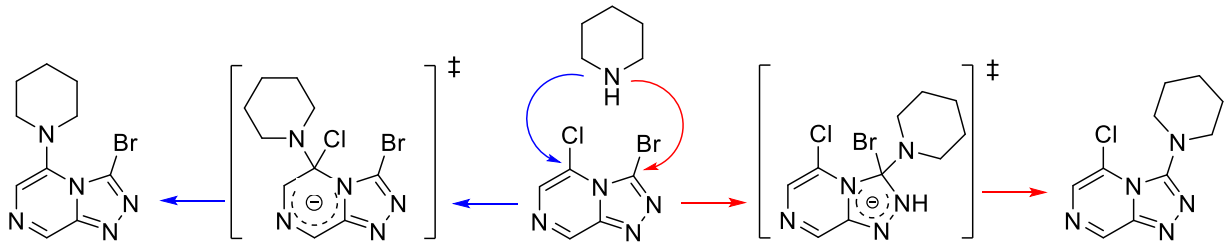


Figure 3: Mass spectrometry analysis of Entry **3**, representative of all amine coupling conditions.

The desired product was clearly present (238.05 m/z) but a peak of similar intensity was also observed (284.00 m/z). The identity of the by-product was estimated using the isotopic peak patterns, as the two signals show a 3:1 and 1:1 peak intensity, diagnostic of chlorine and bromine containing compounds. It was therefore proposed that this was the result of the piperidine selectively displacing the chlorine (Scheme 4). This was confirmed when the calculated mass of the suggested by-product correlated with that from mass spectrometry. Another interesting result of the mass

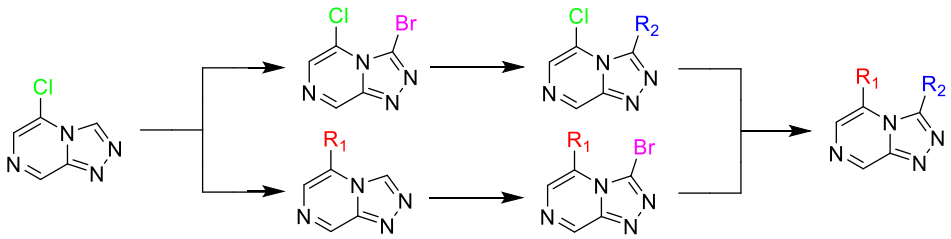
spectrometry analysis was that no evidence of the double displaced compound was observed.



Scheme 4: Competing amine displacement reactions showing transition states.

Assuming that the relative stabilities of the two molecular ions are similar, it can be said that the mass spectrum shows there is a slight preference for chlorine displacement. This is supported by the ^{13}C-NMR of compound **6** which when fully assigned shows that the **5**-position carbon has the greatest chemical shift making it the most electron deficient position and therefore more reactive to nucleophiles. With this information, the chlorine displacement reaction was attempted on **6** to test its selectivity. Using the alcohol coupling conditions used in the linear synthesis, the reaction was found to displace both positions simultaneously. Although the product was isolated with a 12% yield, the poor selectivity of reaction resulted in the diphenethoxy compound being the major product.

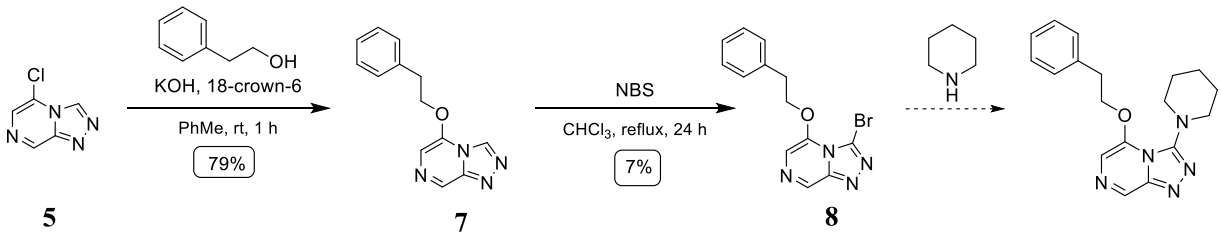
As non-selectivity of compound **6** was found to be an obstacle, a secondary diversification route was proposed that eliminated the possibility of competing reactions. This method involves displacement of the monochlorinated core (**5**) and then subsequent bromination and coupling of the **3**-position (Scheme 5).



Scheme 5: Comparison of divergent strategies to access the Series 4 compounds. Top: Primary diversification using compound **6** as a common starting material. Bottom: Secondary diversification strategy involving displacement of **5** before bromination of **3**-position.

Displacement of **5** with phenylethanol was successfully performed with a 79% isolated yield from column chromatography (Scheme 6). Although isolation of **8** was achieved and the identity verified with mass spectrometry, the reaction was low yielding. As well as column chromatography, preparative TLC was required to fully

separate out the by-products which presumably form due to the poor selectivity of the brominating agent. However, with the product having been identified and characterised, optimisation of the conditions and the subsequent amine coupling can now be performed.



Scheme 6: Attempted synthesis of 5-Phenethoxy-3-(piperidin-1-yl) 'Norcross' compound using secondary diversification strategy with isolated yields.

Conclusions and Future Work

After identifying the restrictions of the current linear synthesis of the Series 4 compounds, possible diversification strategies using a functionalised TP core starting material were analysed. Using the proposed 3 step synthesis (Scheme 2), compound **6** was successfully isolated and characterised. The major side product of the reaction was identified as a doubly brominated triazolopyrazine analogue. The most likely site of secondary bromination was estimated using a combination of NMR data from this study with that from previous work. Preliminary screening of catalytic conditions to allow selective bromination achieved selectivity in certain cases (see **Table 1** Entries **4** and **7**) but was unable to improve the yield. From the attempted amine coupling to **6**, it was shown that nucleophilic displacement of the **3**-position bromine is possible although the reaction is non-selective. Non-selectivity was also the key concern of the attempted chlorine displacement reaction. With selectivity being the major drawback to the synthesis, a secondary diversification strategy was explored which removed this issue. Difficulty in the bromination reaction limited the effectiveness of this route, however it has been shown this method is capable of obtaining the desired compounds so can now be optimised to improve yields. It has been demonstrated that a divergent synthesis is a viable method to access the 'Norcross' compounds and hence be used to explore new types of functionalities for the Series 4 drugs.

With the aims of the project being largely achieved, the future directions of the work can be considered. Improvement to the selectivity of bromination would not only allow a higher yielding method for the synthesis of **6** but also greatly increase the effectiveness of the secondary diversification strategy. The biggest weakness of this synthesis was the difficulty in brominating compound **7**, arising from by-product formation. Selectivity may be achieved through the use of a more reactive brominating

agent like BDSB[22] or by using newly reported halogenation catalysts that acts as a Lewis base (e.g. triphenylphosphine sulfide).[23] Far from being a side product of synthesis of **6**, the doubly brominated compound could be a useful tool for protein binding studies. By having a tertiary functionalised site that is independent of the substituents at the **3** and **5** positions, the Series 4 compounds could be tethered to solid support particles and used analytically to measure drug-protein binding interactions. Any proteins that interact strongly with the drug will stay on the solid support and hence be easily separated and identified. This may lead to an elucidation of the specific mode of activity for the series of compounds, which is still unknown. As well as this, fluorescent tags may also be attached at this position to determine where the molecules accumulate in the cell using fluorescence imaging techniques.

Achieving selectivity would also be a future target for the chlorine and bromine displacement reactions of compound **6** as none of the amine displacement conditions that were screened were able to prevent chlorine displacement. Once the reactions for the divergent synthesis have been optimised, these methods can be fully validated by synthesising the library of Series 4 compounds as well as derivatives containing new functionalities and hence increase the probability of finding potent anti-malarial drugs.

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Experimental section

General procedure 1 for halogenation catalyst screening

5 (50 mg, 0.323 mmol, 1 equiv.), the halogenation agent and the chosen catalyst were stirred at 60 ºC in the appropriate solvent for 24 h. Reactions were monitored with TLC to detect product conversion. The reactions were worked up according to relevant literature procedure and purified by automatic FCC (25-100% EtOAc in hexanes).

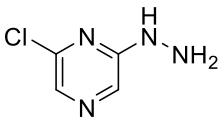
General procedure 2 for bromination reactions

Triazolopyrazine (1.0 equiv.) and NBS (1.7 equiv.) were refluxed in chloroform for 24 h. The reaction mixture was diluted with chloroform, washed with sodium carbonate solution, and the aqueous layer re-extracted with chloroform. The combined organic portions were dried with $NaSO_4$ and concentrated under reduced pressure. The crude product was purified by automatic FCC (25-100% EtOAc in hexanes).

General procedure 3 for piperidine coupling reactions

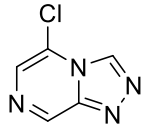
6 (10.0 mg, 1.07 mmol, 1.0 equiv.), piperadine (1.2-3.6 equiv.), base (1.5 equiv.) and chosen catalyst was placed in 1 ml of solvent and stirred at rt until the reaction was complete. The solvent was removed under reduced pressure and purified by automatic FCC (18-100% EtOAc in hexanes).

2-Chloro-6-hydrazinylpyrazine 1



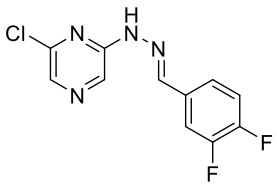
A solution of 2,6-dichloropyrazine (23.4g, 157 mmol, 1 equiv.) and hydrazine hydrate (15.4 mL, 314 mmol, 2 equiv.) in EtOH (280 mL) was stirred at reflux for 3 h. The solvent was removed under reduced pressure to give a pale yellow solid. Water (200 mL) and EtOAc (250 mL) were added and the aqueous layer was extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine (50 mL) and dried with $MgSO_4$ before being concentrated under reduced pressure to give **1** as a yellow solid (19.4 g, 134 mmol 85%); δ_H (200 MHz, $CDCl_3$) 8.12 (s, 1H), 7.89 (s, 1H), 6.23 (s, 1H), 3.86 (s, 2H).

5-Chloro-[1,2,4]triazolo[4,3-a]pyrazine 5



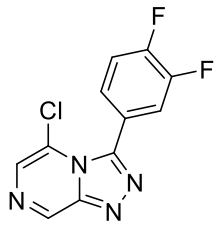
1 (1.03 g, 7.12 mmol, 1 equiv.), triethyl orthoformate (2.40 ml, 13.4 mmol, 2 equiv.) and TsOH (238 mg, 1.38 mmol, 0.2 equiv.) were refluxed for 24 h in toluene (20 mL). The solvent was removed under reduced pressure, and the crude product purified by automatic FCC (25-100% EtOAc in hexanes) to give **5** as a yellow powder (0.450 g, 2.91 mmol, 42%); mp 170-172 ºC; ν_{max} (film) /cm^{-1} 3078, 2963, 2920, 1494, 1347, 1237; δ_H (200 MHz, $CDCl_3$) 9.34 (s, 1H), 9.06 (s, 1H), 7.95 (s, 1H); *m/z* (ESI) 177, 274, 353, 381 ([M + Na]$^+$); HRMS (ESI) 155.01182 ([M + H]$^+$) calcd. for $C_{11}H_4N_4Cl^+$ 155.1190.

2-Chloro-6-(2-(3,4-difluorobenzylidene)hydrazineyl)pyrazine 2



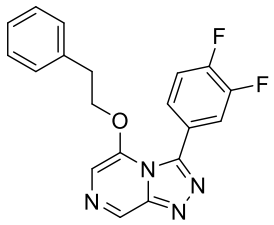
1 (4.92 g, 34.6 mmol, 1 equiv.) and 3,4-difluorobenzaldehyde (3.6 mL, 34.0 mmol, 1 equiv.) were stirred in ethanol at rt for 24 h. The solvent was removed under reduced pressure and the pale-yellow solid was recrystallised from EtOAc to yield **2** as a white solid (7.92 g, 29.5 mmol, 86%); δ_H (400 MHz, DMSO-d^6) 11.65 (s, 1H), 8.65 (s, 1H), 8.08 (s, 1H), 8.03 (s, 1H), 7.91-7.85 (m, 1H), 7.60-7.55 (m, 1H), 7.52-7.44 (m, 1H) ; *m/z* (ESI) 267 ([M - H]$^-$); HRMS (ESI) 269.03994 ([M + H]$^+$) calcd. for $C_{11}H_6N_4ClF_2^-$ 269.04001.

5-Chloro-3-(3,4-difluorophenyl)-[1,2,4]triazolo[4,3-a]pyrazine 3



A solution of **2** (7.92 g, 29.4 mmol, 1 equiv.) and $PhI(OAc)_2$ (7.22 g, 22.4 mmol, 1 equiv.) in DCM (200 mL) was stirred at rt for 2 h. The reaction was diluted with DCM (50 mL) and quenched using sat. $NaHCO_3$ (150 mL). The aqueous layer was extracted with DCM (2 x 100 mL) and the combined organic layers were washed with a sat. $NaHCO_3$ (150 mL) and brine (100 mL). The organic layer was dried with $MgSO_4$ and concentrated under reduced pressure. The crude product was recrystallised from EtOAc to yield a yellow solid (5.01 g, 18.8 mmol, 84%); mp 146-148 ºC; ν_{max} (film) /cm^{-1} 3088, 3058, 1473, 1359, 1276; δ_H (400 MHz, $CDCl_3$) 9.33 (s, 1H), 7.91 (s, 1H), 7.51-7.28 (m, 3H); δ_C (125 MHz, $CDCl_3$) 153.36, 150.83, 148.34, 147.18, 143.00, 129.97, 128.19, 123.41, 121.66,120.88, 117.20; *m/z* (ESI) 289, 353, 620 ([M + Na]$^+$); HRMS (ESI) 267.02415 ([M + H]$^+$) calcd. for $C_{11}H_6N_4ClF_2^+$ 267.02436.

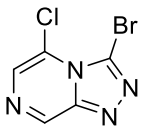
3-(3,4-Difluorophenyl)-5-phenethoxy-[1,2,4]triazolo[4,3-a]pyrazine 4



3 (200 mg, 1.29 mmol, 1.0 equiv.), phenylethanol (128 mg, 1.29 mmol, 1.0 equiv.), potassium hydroxide (240 mg, 4.27 mmol, 3.3 equiv.) and 18-Crown-6 (27.3 mg, 0.10 mmol, 0.080 equiv.) were added to toluene (10 mL) and stirred at rt for 1 h. The reaction was diluted with water (20 mL), then extracted with EtOAc (3 x 15 mL). The organic layers were washed with water (2 x 20 mL) until the

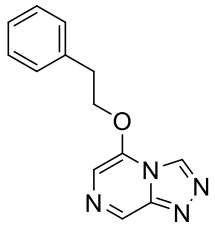
aqueous layer became neutral, followed by brine (15 mL), dried with Mg_2SO_4, filtered and concentrated under reduced pressure to give the crude product as a brown solid. The crude product was purified by automated FCC (25-100% EtOAc in hexanes) to give **4** (1.095 g, 3.11 mmol, 83%). δ_H (400 MHz, $CDCl_3$) 9.03 (s, 1H), 7.36 (s, 1H), 7.57-7.52 (m, 1H), 7.44-7.40 (m,1H), 7.32-7.26 (s, 1H), 7.23 (s, 1H), 6.99-6.97 (m, 2H), 4.53 (t, $J = 6.6$, 2H), 3.04 (t, $J = 6.6$, 2H); δ_C (125 MHz, $CDCl_3$) 152.79, 150.73, 150.37, 148.25, 147.79, 145.28, 143.74, 136.38, 135.89, 128.74, 128.38, 127.13, 127.41, 124.41, 120.10, 116.85, 108.56, 71.15, 34.46; *m/z* (ESI) 375, 727 ($[M + Na]^+$); HRMS (ESI) 375.10289 ($[M + H]^+$) calcd. for $C_{19}H_{14}N_4F_2O^+$ 267.02436.

3-Bromo-5-chloro-[1,2,4]triazolo[4,3-a]pyrazine 6



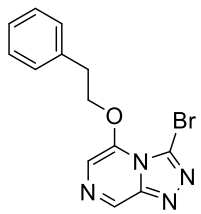
Prepared from **5** (100 mg, 0.502 mmol, 1 equiv.) according to general procedure **2** to give **6** as a yellow powder (59.0 mg, 0.253 mmol, 50%); δ_H (400 MHz, $CDCl_3$) 9.24 (s, 1H), 7.87 (s, 1H); *m/z* (APCI) 235, 279, 367 ($[M + H]^+$); HRMS (ESI) 232.9223 ($[M+H]^+$) calcd. for $C_5H_3BrClN_4^+$ 232.92241.

5-Phenethoxy-[1,2,4]triazolo[4,3-a]pyrazine 7



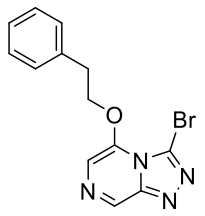
5 (200 mg, 1.29 mmol, 1.0 equiv.), phenylethanol (128 mg, 1.29 mmol, 1.0 equiv.), potassium hydroxide (240 mg, 4.27 mmol, 3.3 equiv.) and 18-crown-6 (27.3 mg, 0.10 mmol, 0.080 equiv.) were stirred in toluene (10 mL) at rt for 20 h. The reaction was diluted with water (20 mL) and extracted with EtOAc (3 x 15 mL). The organic layers were washed with water (2 x 20 mL) until the aqueous layer became neutral, followed by brine (15 mL), dried with Na_2SO_4, filtered and concentrated under reduced pressure to give the crude product as a yellow solid. The crude product was purified by automatic FCC (25-100% EtOAc in hexanes) to give **7** as a beige powder (246 mg, 1.02 mmol, 79%); δ_H (500 MHz, $CDCl_3$) 9.01 (s, 1H), 8.89 (s, 1H), 7.38-7.28 (m, 6H), 4.58 (t, $J = 7.5$ Hz, 2H), 3.27 (t, $J = 7.5$ Hz, 2H); δ_C (125 MHz, $CDCl_3$) 146.25, 142.50, 136.23, 135.60, 132.37, 128.94, 128.89, 127.34, 107.81, 71.22, 29.70; *m/z* (ESI) 263, 503 ($[M + Na]^+$); HRMS (ESI) 263.09049 ($[M + H]^+$) calcd. for $C_{13}H_{12}N_4ONa^+$ 263.09033.

3-Bromo-5-phenethoxy-[1,2,4]triazolo[4,3-a]pyrazine 8



Performed on **7** (100 mg, 0.416 mmol, 1 equiv.) according to general procedure **2**. Further purified by preparative TLC to yield **8** (4.90 mg, 0.0154 mmol, 7%). δ_H (200 MHz, $CDCl_3$) 9.04 (s, 1H), 8.91 (s, 1H), 7.59-7.30 (s, 5H), 4.55 (t, $J = 6.7$, 2H), 3,33 (t, $J = 6.7$, 2H); *m/z* (ESI) 275, 319, 429 ($[M + H]^+$);

3-Bromo-5-(phenethoxy)-[1,2,4]triazolo[4,3-a]pyrazine 8



6 (100 mg, 0.428 mmol, 1.0 equiv.), phenylethanol (52.3 mg, 0.428 mmol, 1.0 equiv.), potassium hydroxide (79.0 mg, 1.41 mmol, 3.3 equiv.) and 18-crown-6 (29.06 mg, 0.0343 mmol, 0.080 equiv.) were stirred in toluene (6 mL) at rt for 2 h. The reaction was diluted with water (20 mL) and extracted with EtOAc (3 x 15 mL). The organic layers were washed with water (2 x 20 mL) until the aqueous layer became neutral, followed by brine (15 mL), dried with Na_2SO_4, filtered and concentrated under reduced pressure to give the crude product as a yellow solid. The crude product was purified by automatic FCC (25-100% EtOAc in hexanes) to give **8** (17.0 mg, 0.0533 mmol, 12%); δ_H (200 MHz, $CDCl_3$) 9.03 (s, 1H), 8.89 (s, 1H), 7.63-7.31 (s, 5H), 4.54 (t, 2H), 3,33 (t, 2H); *m/z* (ESI) 341, 343, 361.