Using Chemistry and Social Media to find a cure for Malaria

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Abstract

The aim of the project was to synthesise a target triazolopyrazine. A side project was undertaken when the yields from the chosen synthetic route were consistently low. Literature determined the synthetic route chosen for synthesising a Triazolopyrazine core. The core was successfully synthesised and the desired functional groups were added to the core in the correct positions. Purification of the final product has yet to be done; however this would be completed using a flash column and could be a starting point for future projects. The yields of the reaction were extremely low and the oxidative cyclisation step was identified as being the step responsible for these low yields. Using NMR the underlying cause of this was determined; the oxidative cyclisation reaction was E isomer selective. On determining this, several different reaction conditions were suggested and undertaken in an attempt to redeem this problem. Despite the yields of the reaction increasing slightly, this was not a result of the Z isomer reacting and further conditions were suggested for future groups to try.

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Introduction

An Introduction to Malaria

Malaria is a disease caused by a parasite, genus *Plasmodium*, which is carried and transmitted by the females of the Anopheles mosquito, as only the females are able to bite. Once bitten it travels to the liver, *via* the circulatory system, where it is able to mature and reproduce asexually [1]. It is endemic to countries in Tropical and Subtropical regions forming a large broad band around the equator as these countries, with their heavy rainfall, consistent warm temperatures and stagnant waters make ideal habitats for the continuous breeding of mosquitos [2]. 3.3 billion people, just under half the world's population, are at risk from malaria every year. There are five different strains of the parasite that can infect humans; the most fatal by far is *Plasmodium falciparum*. In 2010 *P.falciparum* accounted for 91% of the deaths caused by malaria [3]. In 2010 malaria was responsible for the deaths of 660,000 people, the majority of these living in Sub-Saharan Africa (statistics taken from the WHO World Report 2010).

Antimalarials and the problems associated with current therapies

Malaria is treated using Antimalarials and currently there are several established drugs used to treat the disease. Due to parasite strain varying between countries, the type of antimalarial used is determined by location; the majority of these therapies of these are derived from two molecules, Quinine and Artemisinin [4]. As the majority of therapies are derived from just two drugs this results in their mechanisms of action all being very similar, and once the parasite has gained resistance to one of the drugs, it can become resistant to whole families of drugs within a very short space of time.

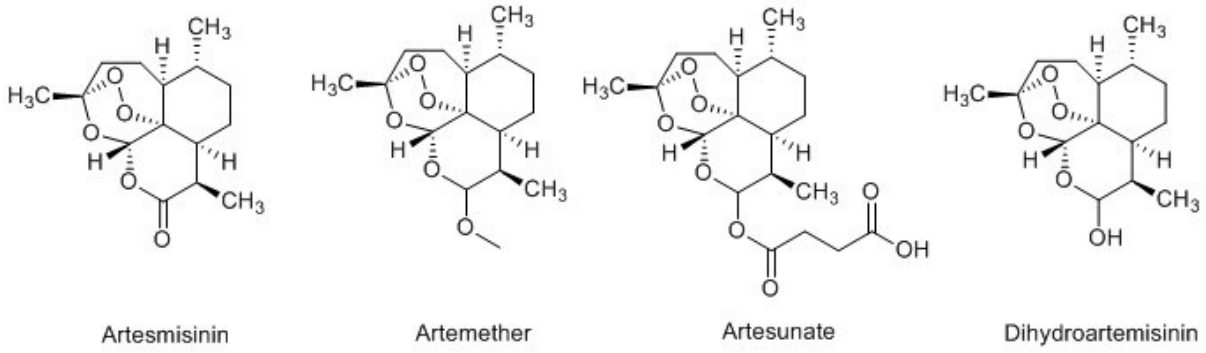


Figure 1 | Molecular Structures of Artemisinin and its semi synthetic derivatives. All the derivatives contain the Endoperoxide Bridge motif essential for its mechanism of action against the malaria parasite. There is very little difference between the four molecules apart from the change in functional group attached to the C=O in artemisinin.

Due to rapid development of resistance to these drugs, there is an urgent need for the development of new drugs acting through novel mechanisms. Resistance to previous drugs has developed when the malaria parasite acquires a mutation in the sequence that the drug targets, allowing the protein to still function however the parasite is no longer inhibited by the drug, rendering the drug ineffective [5]. This has happened for a number of drugs derived from Quinine in locations such as the Cambodian Thai border and resulting in these drugs no longer being suitable therapies in the treatments of malaria. This location has a history of generating antimalarial resistance and in recent years this has been proven true with the confirmation of the first cases of Artemisinin resistance. This has had a detrimental effect on the WHO aims of trying to eradicate malaria worldwide, as worldwide eradication is only possible when the drugs used have had no cases of resistance reported [6]. Due to the ease in which the parasite seems to become resistant to drugs, one of the future aims for antimalarials is that they contain sequences that target several of the parasites proteins – therefore making it difficult for the parasite to acquire full resistance to the drug without

changing the sequence for several proteins. Changing several proteins would have a profound effect on the structure and shape of the protein, which could render it useless (protein shape is usually essential for its function). With no new chemical class of antimalarials introduced to the market since 1996, and the number of parasites being resistant to drugs increasing, there is a need more than ever to produce a new class of drugs with novel mechanisms [7].

Problems associated with finding new antimalarials

The biggest problem associated with Malaria research is the lack of funding. It is a non-profitable disease and the pharmaceutical industry has more interest and need in spending money on research for more profitable causes. In 2010 almost four times more money went into research and surgical procedures regarding Male Patent Balding than went into antimalarial research. So in an attempt encourage research, in 2009, 2 million compounds from GlaskoSmithKine's chemical library were screened for inhibitors of *P.falciparum*. 13,533 compounds were confirmed to inhibit the parasites growth by at least 80% at 2μM concentration and at least 8,000 of these showed potent activity against a multidrug resistant strain of the parasite. The chemical structures and associated data of these compounds were then made public, hoping to generate research outside of GSK [7].

In 2011, Open Source Malaria (OSM) was started with the aim of taking these public domain compounds and to try and resynthesize them and confirm there activity against the malaria parasite. Those which showed activity were then modified to improve any unfavourable properties that the compound may possess, such as problems with solubility or metabolic stability, with the end goal of discovering a compound that can enter Phase I clinical trials.

An Introduction to Open Science

OSM basis their research on Open Science, and follow six principles when carrying out their work (these six laws were taken from the OSM wiki page):

1. First law: All data are open and all ideas are shared
2. Second Law: Anyone can take part at any level of the project
3. Third Law: There will be no patents
4. Fourth Law: Suggestions are the best form of criticism
5. Fifth Law: Public discussion is much more valuable than private email
6. Sixth Law: The project is bigger than, and is not owned by, any given lab. The aim is to find a good drug for malaria, by whatever means, as quickly as possible.

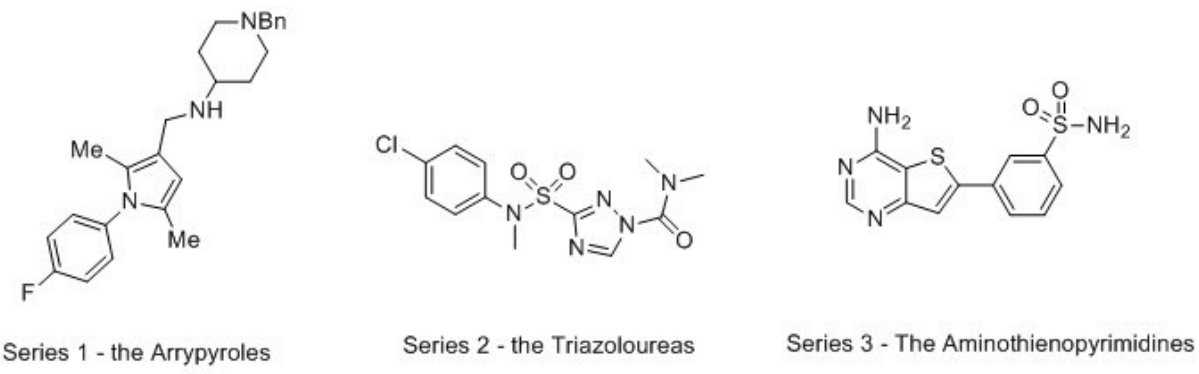
Following these rules means that any experiment performed, whether incorrect or the outcome was a negative result, still had to be documented online in our electronic lab books. This lab books are accessible to the public, they require no log in details or membership fees in order to view them allowing scientists from outside the group to be able to have an input and help improve the synthesis of these compounds. It also allows the team to post problems that have arisen from the synthesis, sometimes involving the solvent, purification or problems arising from racemic mixtures, online and again allow scientists from outside our group to voice their opinions or possible solutions.

Benefits of conducting projects following the open science set of rules usually include quicker results due to involvement of people from a wide range of backgrounds and abilities, input from experts who have had no previous contact with the team, the results produced are usually more reliable and more reproducible. Due to constant peer review and the publication of both positive and negative results means that there will be a reduction in

time wasted on unnecessary repeating of research [8]. These benefits were highlighted in the first Open Source project which focused on improving the synthesis of the chiral drug praziquantel (PZQ) as a single enantiomer, a drug used to treat schistosomiasis [9]. The project started in 2006, but a funded effort began in 2010 and inputs from outside the research group changed the direction and focus of the research. In 2011 a paper was published addressing a solution to the problem and a suitable scale up – a relatively short space of time compared to many drugs on the market.

Due to the success of PZQ, it opened the door to the possibility of open science being used for drug discovery – not just improving previous synthetic methods [9]. This, along with GSK releasing the documents of 2 million compounds, allowed for the OSM group to form. By the time I joined the group in September 2013 the group had already been established for 2 years and was on their fourth series of interest.

The fourth series, the Triazolopyrazines, is the newest of the Open Source Malaria (OSM) series. This series was chosen as the compounds within the series along with significant quantity of data have a suggested mechanism of action against parasite – which differs than previous series. Previous series included Series 1, the Arrypyroles, Series 2, the triazoloureas and Series 3, the aminothienopyrimidines, shown in Figure 2.



Series 1 - the Arrypyroles

Series 2 - the Triazoloureas

Series 3 - The Aminothienopyrimidines

Figure 2 | Representative compounds of the previous 3 OSM Series

The Triazolopyrazines differ from previous series as there was previous evidence from parasite ion regulations assays that the compounds in this series could be potential pfATP4 inhibitors, prior series showed no experimental evidence for this mechanism of action. Series 4 also had promising pharmacokinetics such as good potency against the parasite and they also appeared to have good in vitro human liver microsome (HLM) stability. HLM are used to determine the *in vitro* clearance of a compound as the liver is the most important organ in terms of drug metabolism and clearance from the human body. The series also showed little polypharmacology (little interaction with other targets) or cytotoxity (toxicity to cells other than the target cell) [10].

There were some problems associated with series 4. There was some concern over the metabolic stability of the series as despite having good HLM stability, RLM (rat liver microsomes) remained high, this was particular more evident for the more potent members of the series, which has led to short half-life's in rats. Previous studies have also shown weaker results for the Triazolopyrazines against resistant strains of the parasite. The series also shows very little (>>1uM) activity against the parasites gametocytes, and no activity against Winzeler's Pb liver stage.

Aims for the Series

On joining the group in September, the main aims of the OSM group was to try and optimise the synthetic routes of the triazolopyrazines – especially the synthetic route taken to synthesise the core. Other projects were working on trying to improve the solubility and metabolic stability of the compounds, whilst maintaining potency (this was proving to be difficult as changing the side groups often resulted in reduced potency). Aside from synthesising and modifying compounds, there were also groups working on confirming the

series did inhibit *Pf*ATP4. *Pf*ATP4 is a Na^+ATPase that are located in the red blood cell plasma membrane when the cell is infected with the malaria parasite. It imports Na^+ and H^+ ions out of the parasite and into the infected red blood cell and it is thought if a drug could inhibit this activity, this would lead to the parasite being unable to import the ions into the red blood cell. This would result in a disruption to the parasites Na^+ homeostasis, eventually resulting in the parasites death [11]. There are currently no therapies on the market that has this mechanism of action – hence why it is important to confirm this series could be PfATP4 inhibitors.

The research surrounding series 4 was being focused on two main targets – the ether linked compounds, ones with similar structure to the molecule being synthesised in this project, and the amide linked compound, see Figure 3. A lot of the active compounds in the series contain the ether motif, however the biggest problem surrounding these compounds is the separation of the enantiomers, which would allow us to determine and purify the active stereoisomer. There were fewer compounds with the amide motif and most of them showed lower levels of potency; however they possessed other pharmacokinetic properties that were desirable, such as good stability in RLM.

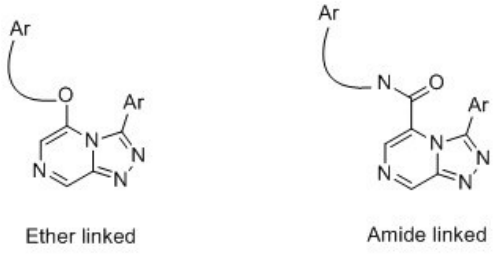


Figure 3 | Diagram showing representative compounds for the two different directions taken in the project – the ether and amide linked triazolopyrazine cores.

Series 4 has previously showed good *in vivo* efficacy and potency against P*f*NF54 and the main problem associated with both the ether and amide compounds was to do with metabolic stability. Possible synthetic reactions could revolve around modifying the side chains or the core in an attempt to balance both potency and metabolic stability. Changing the substituents allows for modification of the physical properties of the compounds i.e. can change its solubility or lipophilicity. Rate of absorption of a drug depends on two physical properties – lipophilicity and solubility. The membrane of the GI epithelial cells is composed of tightly packed phospholipids interspersed with proteins. Therefore the drugs need to be able to penetrate this phospholipid barrier, which is dependent on the lipophilicity of the drug. Drugs with poor lipophilicity will be poorly absorbed when taken the drug orally. However this is counteracted by drugs that have too high a lipophilicity are often associated with rapid metabolism (such as the Triazolopyrazines)– trying to improve metabolic stability without affecting the potency or further lowering the solubility of the compounds was proving to be difficult.

This was all based on Lipinkski's rule of five – a set of rules that describe molecular properties of a drug that would result in the correct pharmacokinetics suitable for human use. This includes absorption, distribution, metabolism and excretion of the drug. Drugs who keep to these rules often have lower attrition rates when being trialled and therefore usually have an increased chance of being introduced onto the market.

Previous attempts at trying to lower the lipophilicity of the TP series where done by replacing the triazole aryl substituent with a cyclo(hetero)aliphatic group. If needed this could be linked with a heteroatom. This lowered the lipophilicity but the potency of the tested compound against PfNF54 was also lowered.

As modifying the substituents was proving to be unsuccessful the group then looked at how modifying the core triazolopyrazine ring could affect the molecules properties. Changing the core was based on the assumption that the pyrazine template would undergo aldehyde oxidase (AO) metabolism at positions alpha to the nitrogen – therefore several compounds were made with different R groups – Cl, Me, NH2 etc. AO is capable of oxidising drugs in the liver and has wide substrate specificity – allowing it to oxidise a wide range of compounds. It helps contribute to the hepatic clearance of drugs. By adding an R group alpha to the nitrogen, the pyrazine motif coulfd undergo a big enough change so it would no longer under oxidation by AO and therefore would have increased metabolic stability. However with addition of an R group on the core, the potency of the drugs was lowered. The projects future aim was to continue modifying the substituents but focusing mostly on the ether and amide groups.

The aim of this project was to synthesize and purify the following compound, which is part of the series.

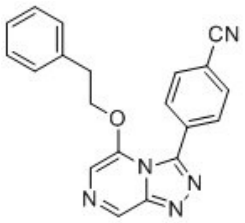


Figure 4 | The target compound

A side project also formed during the synthesis and this was to try and improve the yield of this compound and therefore this would require modifying the current method of synthesis.

Methods

Part 1 – Synthesizing the Triazolopyrazine Core.

The synthetic steps below are the most effective way determined to make the compound. Part 2 shows where what was changed in order to try and Improve the yield.

Synthesis of 2-chloro-6-hydrazinylpyrazine

2,6-Dichloropyrazine (1 g, 6.7mmol) was vigorously stirred in ethanol (10 mL) and hydrazine hydrate (0.33 mL, 340 mg, 6.7mmol) was added. The resulting mixture was heated at 100 ˚C for 16 hours. After completion solvent was removed under vacuum to yield a crude orange solid (1.1012 mg, 6.5mmol, 110% crude yield). An NMR of the product was taken, and suggested that there was significant amount of starting materials still present. Hydrazine hydrate (0.33 mL, 340 mg, 6.7mmol) and ethanol (10 mL) was added, and the mixture was heated at 100˚C for a further 21 hours. Once completed solvent was removed under vacuum to yield a crude orange solid (1.37904 mg, 6.5mmol, 138% yield) and an NMR was taken.

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

DS 1-1 (1.23 g, 13.2mmol) was stirred in AcOH (0.41mL) in MeCN (12.29mL) and 4-formylbenzonitrile (1.11 g, 13.2mmol) was added to the orange suspension and the resulting reaction mixture stirred at room temperature for 2 hours. The solvent was removed under vacuum to yield an orange/brown solid.

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

To a well stirred solution of 4-((2-(6-Chloropyrazin-2-yl)hydrazono)methyl)benzonitrile (100 mg, 0.38mmol), 2-methyltetrahydrofuran (6 mL) and Chloramine-T (123 mg, 0.54 mmol) was

added and the resulting reaction mixture was stirred at 75^{o}C for 1.5h. The resulting solution was then left to cool to room temperature and a small sample was used to perform a TLC (50% EtOAc in hexane) and NMR. DCM (20ml) was added to the reaction mixture.

The organic layer was then washed with 10 wt% aq. sodium sulphite solution (10mL), 2M sodium hydroxide (3 x 10 mL) and then evaporated to dryness. An NMR (NMR 5-2) was taken of the resulting solid.

A silica column was set up to purify the collected product. The column was ran with 50% EtOAc/Hexane and the first spot was collected and an NMR taken, which confirmed that this was not the product. The column was then ran with 100% EtOAc and another spot was collected and an NMR was also taken. This NMR confirmed that our product had been purified.

Alkoxide Displacement reaction

4-(5-chloro-[1,2,4]triazolo[4,3-a]pyrazin-3-yl) benzonitrile (100mg, 0.39mmols), 2-dimethylaminoethanol (0.03ml. 30mmols), potassium tert-butoxide (60mg) and THF (0.5ml) where all added to a RBF and stirred for 2h at room temperature. After completion, this was determined doing a TLC in 100%EtOAc, water (1ml) was added dropwise to the solution, whilst still being stirred. The mixture was transferred to a separating funnel where washed 3 times with DCM (20ml) and water (20ml) until the remaining water layer is clear.

The solvent was then removed under vacuum and an NMR of the sample was taken, NMR 11-3.

Part 2 – Modification of the synthetic route

The synthesis of 4-(5-chloro-[1,2,4]triazolo[4,3-a]pyrazin-3-yl) benzonitrile was originally carried out using PIDA (125 mg, 1.94 mmol) as the oxidising agent instead of Chloramine T. Below is a list of other modifications to the synthetic route taken to try and improve the yield of the final product. There was a total of 6 different reaction conditions for the oxidative cyclisation step were tried and listed below.

1. To a well stirred solution of 4-((2-(6-Chloropyrazin-2-yl)hydrazono)methyl)benzonitrile (100 mg, 0.39 mmol), CH_2Cl_2 (3 mL) and $PhI(OAc)_2$ (125 mg, 0.38 mmol) was added and the resulting reaction mixture was stirred at room temperature for 48h. 40ml dichloromethane, 5ml methanol and 40ml sodium bicarbonate were added to the resulting mixture, and the layers seperated. The product was then dried using magnesium sulphate, which was removed via vacuum filtration, and the resulting solution was dried under vaccum. An NMR of the remaining solid was taken.
2. To a well stirred solution of 4-((2-(6-Chloropyrazin-2-yl)hydrazono)methyl)benzonitrile (100 mg, 1.94 mmol), CH_2Cl_2 (3 mL) and $PhI(OAc)_2$ (125 mg, 0.38 mmol) was added and the resulting reaction mixture was stirred at room temperature for 1h. The solvent was then removed using a rotary evaporator and the crude residue was purified by column chromatography (using silica gel 40-63µm and gradient elution of 100% EtOAc). The solvent was then removed using a rotary evaporator. An NMR of the remaining solid was taken.
3. To a well stirred solution of 4-((2-(6-Chloropyrazin-2-yl)hydrazono)methyl)benzonitrile (100 mg, 1.94 mmol), CH_2Cl_2 (3 mL) and DMP (165

mg, 0.38 mmol) was added and the resulting reaction mixture was stirred at room temperature for 1h. The solvent was then removed using a rotary evaporator and the crude residue was purified by column chromatography (using silica gel 40-63µm and gradient elution of 100% EtOAc). The solvent was then removed using a rotary evaporator. An NMR of the remaining solid was taken.

4. To a well stirred solution of 4-((2-(6-Chloropyrazin-2-yl)hydrazono)methyl)benzonitrile (100 mg, 1.94 mmol), CH_2Cl_2 (3 mL) and DMP (165 mg, 0.38mmol) was added and the resulting reaction mixture was stirred at room temperature for 24h. The solvent was then removed using a rotary evaporator and the crude residue was purified by column chromatography (using silica gel 40-63µm and gradient elution of 100% EtOAc). The solvent was then removed using a rotary evaporator. An NMR of the remaining solid was taken.
5. To a well stirred solution of of 4-((2-(6-Chloropyrazin-2-yl)hydrazono)methyl)benzonitrile (100 mg, 0.38 mmol), 2-methyltetrahydrofuran (6 mL) and Chloramine-T (123 mg, 0.44 mmol) was added and the resulting reaction mixture was stirred at 75oC for 1.5h. The organic layer was then washed with 10 wt% aq. sodium sulphite solution (10mL), 2M sodium hydroxide (3 x 10 mL) and then evaporated to dryness. An NMR was taken of the resulting solid.
6. To a well stirred solution of 4-((2-(6-Chloropyrazin-2-yl)hydrazono)methyl)benzonitrile (100 mg, 0.38 mmol), 2-methyltetrahydrofuran (6 mL) and Chloramine-T (123 mg, 0.44 mmol) was added and the resulting reaction mixture was stirred at 60^oC for 24h. The reaction mixture was then allowed to cool to room temperature, and the organic phase washed with 10 wt% aq. sodium

sulphite solution (10mL) and 2M dilute sodium hydroxide (3 x 10 mL) and then evaporated to dryness. An NMR of the product was taken.

7. To a well stirred solution of 4-((2-(6-Chloropyrazin-2-yl)hydrazono)methyl)benzonitrile (100 mg, 0.38 mmol), 2-methyltetrahydrofuran (6 mL), Chloramine-T (123 mg, 0.44 mmol) and one drop of glacial acetic acid was added and the resulting reaction mixture was stirred at 75oC for 1.5h. The reaction mixture was then allowed to cool to room temperature, and the organic phase washed with 10 wt% aq. sodium sulphite solution (10mL) and 2M dilute sodium hydroxide (3 x 10 mL) and then evaporated to dryness. An NMR of the product was taken.
8. To a well stirred solution of 4-((2-(6-Chloropyrazin-2-yl)hydrazono)methyl)benzonitrile (100 mg, 0.38 mmol), 2-methyltetrahydrofuran (6 mL), Chloramine-T (123 mg, 0.44 mmol) and TFA (one drop)was added and the resulting reaction mixture was stirred at 75^{o}C for 1.5h.

   The reaction mixture was then allowed to cool to room temperature, and the organic phase washed with 10 wt% aq. sodium sulphite solution (10mL) and 2M dilute sodium hydroxide (3 x 10 mL) and then evaporated to dryness. An NMR of the product was taken.

Results

Part 1 – Synthesising the compound

Throughout the multistep synthesis, NMR and TLC was used to determine whether the product had been made or the reaction was completed and no product has formed i.e. starting material spot had disappeared but no product spot had formed on the TLC. Determining the products in the reaction mixture was done my comparing NMR's to previous experiments or those conducted by the research groups in Sydney.

NMR's can be found in the Appendix at the back of the report.

Part 2 – Modifying reaction conditions

Figure 5 is a table displaying all the outcomes for the second half of the experiment. Due to time constraints, yields weren't always properly calculated and were estimates were taken from the NMR spectra.

Figure 5 | Table showing results modifying reaction conditions on yields

Reaction Conditions	**Conv (NMR)**	**Remaining e isomer**	**Remaining z isomer**
PIDA, 48h, rt, DCM	14%	36%	50%
PIDA, 1h, rt, DCM	38%	ND	ND
DMP, 1h, rt, DCM	0%	0%	0%
DMP, 24h, rt, DCM	0%	0%	0%
Chloramine T, 1.5h, 75oC, 2-MeTHF	~40%		~50%
Chloramine T, 24h, 75oC, 2-MeTHF	~40%		~50%
Chloramine T + acetic acid (1 equiv), 1.5h, 75oC, 2-MeTHF	~40%		~50%
Chloramine T + TFA (1 equiv), 1.5h, 75oC, 2-MeTHF	0%	0%	0%

Discussion

The synthesis of the triazolopyrazine core began with a nucleophilic aromatic substitution reaction between 2,6-dichloropyrazine **1** with hydrazine hydrate to give molecule **2**. This was an S_N1 reaction, with the carbocation produced stabilising the aromatic ring. This was then reacted with an aromatic aldehyde, in this case 4-formylbenzonitrile, in a subsequent condensation to give hydrazone **3**. Treatment with the oxidant phenyliodonium diacetate, or PIDA, causes **3** to undergo oxidative cyclisation to generate the triazolopyrazine core **4**. The final step was to replace the Chloride with a alkoxide group and this was done using 2-phenyl ethanol and potassium tert-butoxide in a nucleophilic aromatic substitution reaction. Due to the synthetic route used to make the core, it was easiest to leave the displacement of the chloride by the alkoxide until the final step. However this worked to our advantage as maintaining a –Cl side chain offered the possibility of introducing further substituents. This could be achieved using palladium-catalysed coupling reactions, an area of research that could be explored by future groups [12].

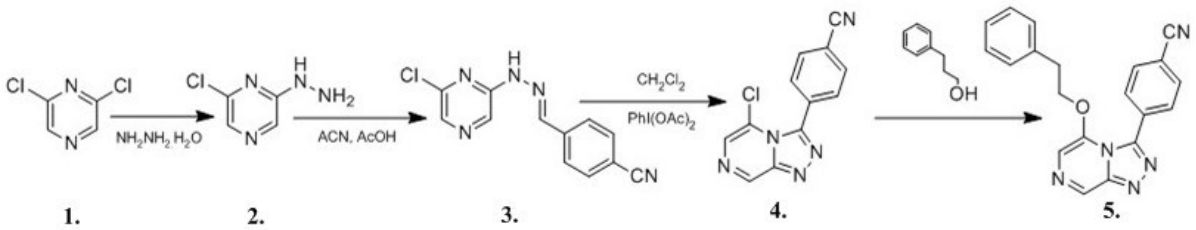


Figure 6 | Overall Reaction scheme to make desired product 5.

Synthesizing the triazolopyrazine core was chosen as hydrazones are often used to synthesise heterocyclic compounds, as they possess an ability to react with both nucleophiles and electrophiles. This is due to the structure of hydrazones; they contain two connected nitrogens, by a single bond, and a carbon-nitrogen double bond that is

conjugated with a lone pair on the terminal nitrogen atom [13]. These motifs are responsible for the hydrazones chemical and physical properties, as both the nitrogen atoms are nucleophilic (the amino nitrogen is slightly more nucleophilic, hence when reacting with the oxidising agent the reaction occurs at this nitrogen) and as both possess a lone pair of electrons, and undergo lone pair resonance, this results in the carbon atom present in the CN double bond to possess both nucleophilic and electrophilic character [14,15].

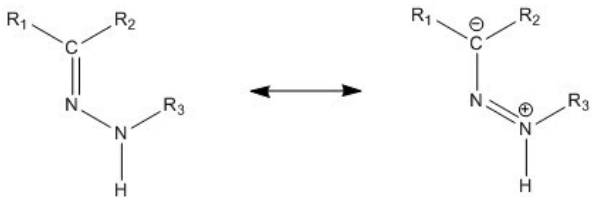


Figure 7 | Diagram showing lone pair resonance of the Nitrogen atoms, resulting in the Carbon atom possessing both nucleophilic and electrophilic character.

Depending on the R groups, there can also be an acidic hydrogen bound to the amino nitgrogen, allowing for hydrogen bonding to other molecules [16].

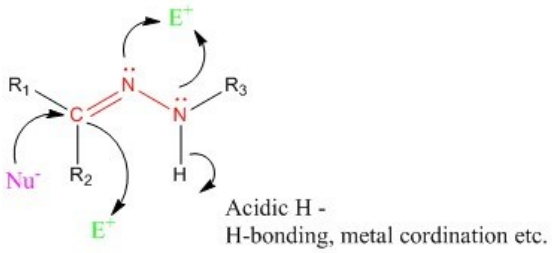


Figure 8 | Diagram showing hydrazone structure, which is highlighted in red. It also shows areas of attack from reagents. Electrophiles are highlighted in green and Nucleophiles in pink. Note that the C in the hydrazone structure is prone to attack from both nucleophiles and electrophiles.

The final part of the reaction, the alkoxide displacement (reaction 4 to 5), was a nucleophilic aromatic substitution reaction. A nucleophilic aromatic substitution reaction is a substitution reaction that involves a nucleophile, in our case the deprotonated 2 – phenyl ethanol, displacing a good leaving group, which is the chloride. Halogens are known to be good leaving groups, the main reason it was essential to maintain a halogen as a substituent on the aromatic ring, as leaving groups are needed to be able to stabilize the additional electron density acquired during bond heterolysis [17]. Good leaving groups also give rise to faster reactions as, by transition state theory, these reactions will have lower activation energies leading to relatively stable transition states.

This reaction is also favoured as there is an electron withdrawing group positioned ortho to the chloride leaving group, which activates the ring towards nucleophilic attack as electrons are withdrawn from reaction centre making it more susceptible to attack from electron rich nucleophile i.e. the alkoxide.

The reaction is a S_N1 reaction; therefore the rate-determining step is unimolecular. The reaction cannot be an S_N2 reaction as Aryl halides cannot undergo this kind of reaction. This is because the C-Cl bond is in the same plane as the aromatic ring as the carbon attached to the chloride is trigonal. Therefore, for the nucleophile to attack from the back, it would have to appear from inside the ring and somehow invert the carbon atom – which is not possible [18].

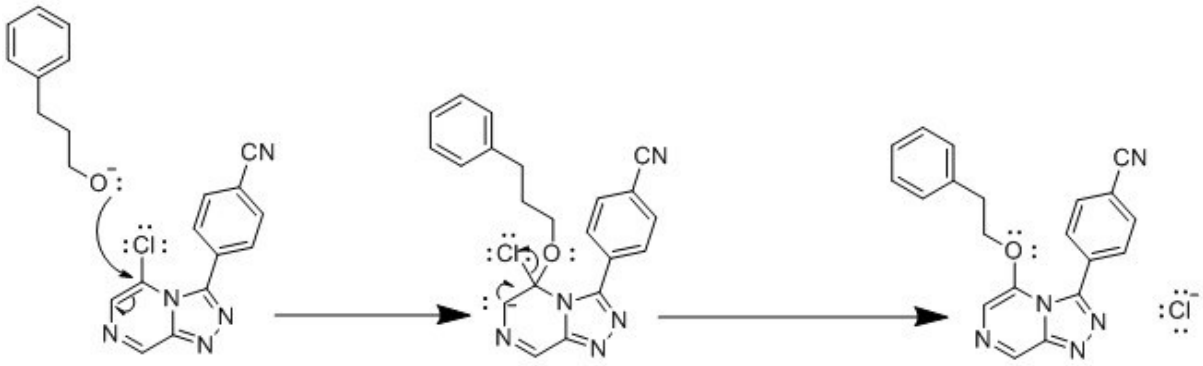


Figure 9 | Alkoxide displacement mechanism

As previously mentioned in the abstract, the main problem arising from the synthesis of the molecule was the low yields that were achieved (yields were consistently around 10-20% and never over 50%) with using PIDA as the oxidising agent. This, along with NMR evidence, suggested that the oxidative cyclisation was in fact an E-selective reaction. The nature of the C=N bond within the hydrazone results in configurational isomerisation allowing the hydrazone to exist as two isomers, E/Z, in solutions [16]. NMR showed 2 peaks for the hydrazone GIVE VALUES, corresponding to the E/Z isomers and the final product had one of these peaks remaining suggesting that one of the isomers hadn't reacted. Due to sterics, it was determined that the E stereoisomer was the isomer that was reacting as, due to steric clashes with the Z isomer, the E isomer is usually the major product [16]. Below is a diagram of what occurs during the cyclisation reaction, note that this diagram shows if the reaction with the E isomer was 100% efficient, in reality this did not occur and there would still be some of the E isomer present in the reaction product.

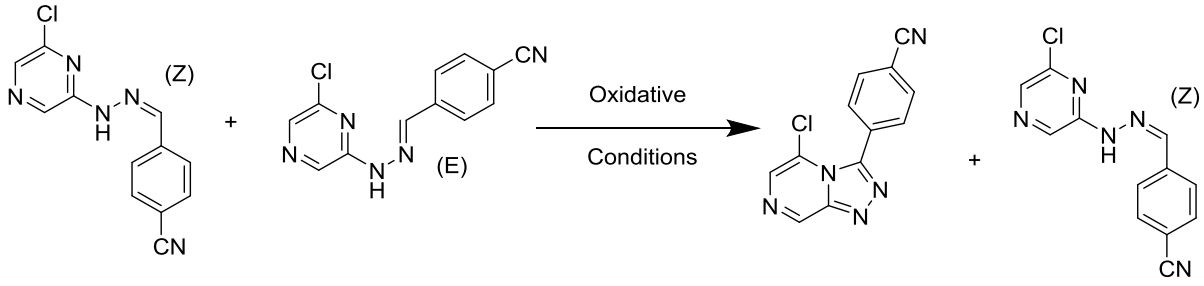


Figure 10 | Simplified diagram showing what is occuring in our reaction mixture.

Below is the mechanism when using PIDA as the oxidising agent.

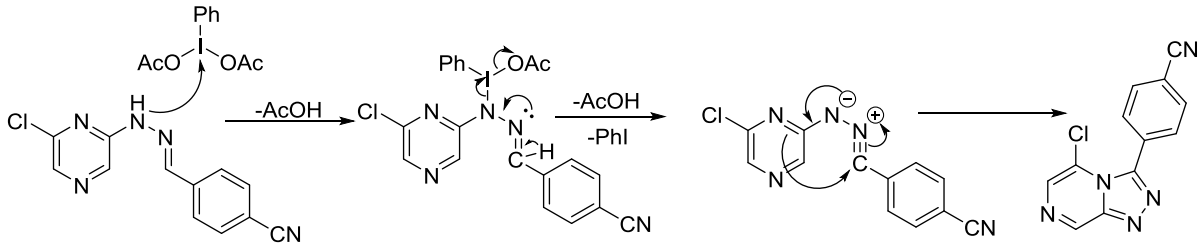


Figure 11 | Oxidative cyclisation using PIDA mechanism

As the low yields were caused by the oxidative cyclisation reaction with PIDA being E isomer selective, the reaction conditions needed to be modified in order for the Z isomer to react and increase the yield. The first modification was to allow the reaction with PIDA to go for 48 hours instead of 1 hour, hoping the Z isomer was just slower to react and the longer reaction time would improve yield. NMR evidence, the Z isomer peak was still present in the spectra, suggested this was not the case and again the Z isomer had not reacted with longer reaction time.

The next step was to change the oxidising agent used in the reaction. PIDA is a periodinane, a group of chemical compounds containing hypervalent iodine. These compounds are called hypervalent as the iodine present has more than 8 electrons within its valence shell. Many of these compounds are organic reagents used as oxidising agents, therefore changing the oxidising agent to a related compound of PIDA seemed logical – as the reaction with the E

isomer was successful with PIDA. Dess-Martin Periodinane (or DMP) is usually used to oxidise primary alcohols to form aldehydes and secondary alcohols to form ketones. It is known for being reactive at mild conditions, such as room temperature and neutral pH, and to have shorter reaction times and higher yields compared to other periodinane oxidants [19]. In the case of reaction mixtures that are sensitive to epimerisation, DMP allowed oxidation with almost no loss of enantiomeric excess. It was based on iodoxybenzoic acid (or IBX) another periodinane used for oxidising alcohols however DMP is much more reactive and soluble in organic solvents due to DMP's acetate groups being attached to the central iodine atom.

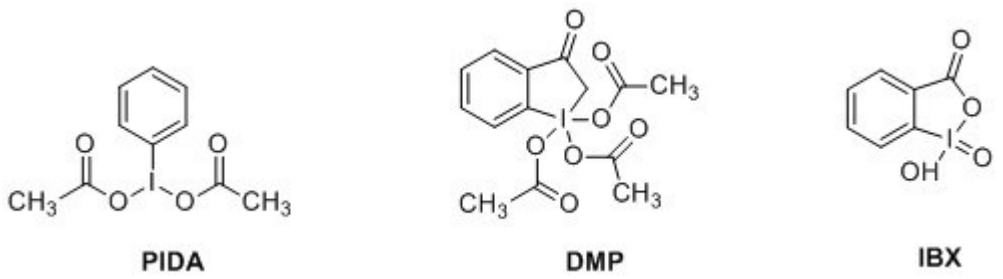


Figure 12 | Comparison of structures of PIDA, DMP and IBX. All these structures contain hypervalant iodine atoms.

DMP is known to mediate the intramolecular cyclization of phenolic azomethines at room temperature leading to substituted benzoxazoles as well as oxidative cyclisation of N-acylhydrazones when used in excess in mild conditions [20,21]. Based on this information, DMP was used as the oxidising agent in the reaction. The first reaction with DMP was done for 1 hour at room temperature. The NMR showed that there was no product formed in the reaction and therefore reaction time was increased to 24 hours, based on the same principle as before. However, NMR evidence again showed that no product peak had been

formed and therefore DMP was not a suitable oxidising agent in the oxidative cyclisation reaction.

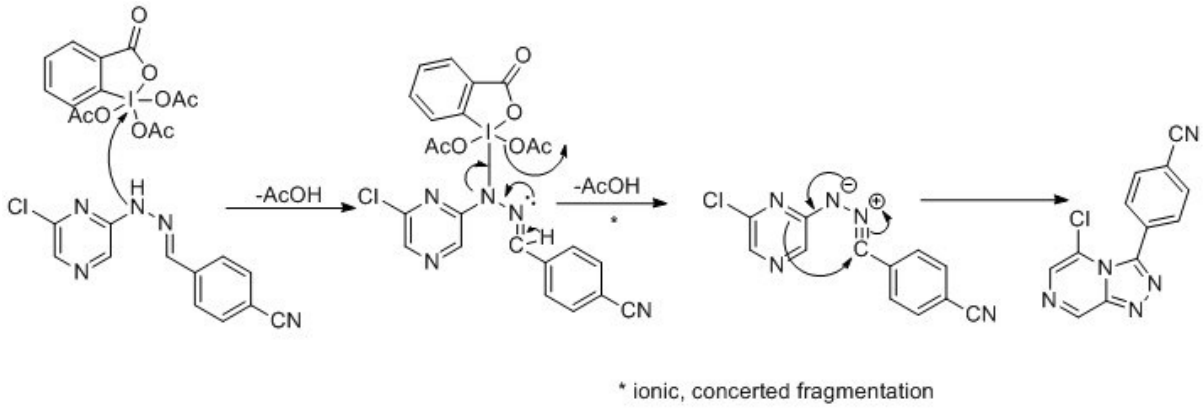


Figure 13 | Proposed mechanism for DMP based on literature, NMR evidence proved this didn't happen.

As the reaction didn't work using DMP and PIDA as oxidising agents, when changing the oxidising agent again it was important not to choose any other periodinanes. Using a stronger oxidising agent for the reaction was desired however it was noted that using too strong an oxidising agent could result in the promotion of other reactions within the molecule, and not the desired oxidative cyclisation. Literature suggested the use of Chloramine T in 2-methyltetrahydrofuran [12]. 2-methyltetrahydrofuran was used in preference to THF as the immiscibility of the solvent allowed for the removal of the by-product to be easily removed with a basic-aqueous workup. When using THF this was not possible and removal of the side products would be much more difficult [12].

Figure 14 | Structure of Chloramine T

Chloramine T structure is based on a *N*-chlorinated and *N*-deprotonated sulphonamide. This results in the chlorine present being electrophilic. Chloramine T possesses a high redox potential (E_{red} = 1.138V at pH0.65) and this allows it to be a good oxidising agent in both acidic and basic solutions (which was important for the reactions when acetic acid and TFA were added) [22]. Also increasing the ionic strength of the reaction mixture had little effect on the reaction rate of oxidation by Chloramine T, again important for later reactions [23].

When using Chloramine T as the oxidising agent, the yield of the reaction slightly increased, to around 38%, however NMR evidence showed that this cyclisation was again E-selective – the higher yields were because the reaction with the E isomer and the chloramine T was a cleaner more efficient reaction, so more of the E isomer was reacting and producing more product. The Z isomer had no effect on the increasing yield, hence why the yield never got to over 50%.

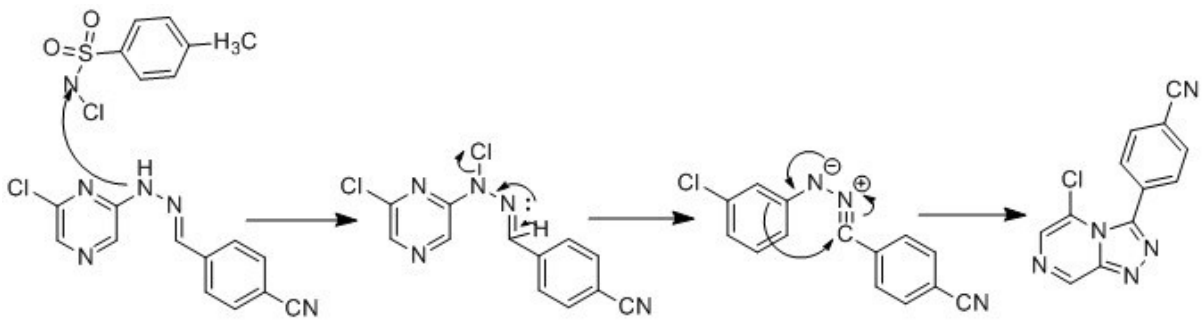


Figure 15 | Mechanism for oxidative cyclisation using Chloramine T as an oxidising agent.

As the Z isomer was still not reacting, something other than the oxidising agent needed to be changed, as increasing the oxidising agent anymore would result in other competing side reactions being favoured and the product not being formed at all. Exploring this could be another area for future research. As changing the oxidising agent was having little success in promoting the Z isomer to react, the next step was to look at adding a reagent to the reaction mixture that would make the Z isomer react without decreasing the reaction with the E isomer. Literature suggested that the addition of an acid might increase the yield for both isomers. Acid addition results in the double bond being equilibrated, allowing the Z isomer to isomerise to the E isomer allowing for higher yields. Acetic acid was the weaker of the two acids, pka = 4.76, so when this reaction was unsuccessful (confirmed by NMR spectra) it was thought that a stronger acid might be able to equilibrate the CN bond. TFA, pka = 0.23, was a stronger acid used in the reaction however not only did the Z isomer not react, the E isomer did not form the product either. The acid addition was thought to not have worked, as one of the auxiliaries in the literature was that the molecules possess a basic centre, which none of our hydrazones possessed.

Another reason for using TFA was to try and coerce the hydrazone to epimerise and therefore give us a form of dynamic kinetic resolution – we thought TFA might be capable of this as literature states that stronger acids have better catalytic activity in the conversion of isomers (reference acid-catalysed Kejun Cheng).

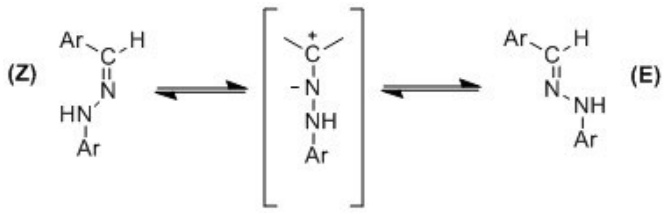


Figure 16 | Diagram showing the possible transition state between isomers with the addition of the acid [24].

A dynamic kinetic resolution is able to form when the starting solution is a mix of stereoisomers that can epimerise easily – literature states that this is possible with hydrazones as the activation barrier between the E and Z isomer is low. This allows for the starting racemic mixture to contain compounds at all stages of the reaction, which allows the enantiomer with the lowest activation barrier, in this case the e isomer, to be formed in yields of up to 100%, in theory. This differs from if the reaction was a standard kinetic mixture as the enantiomers are unable to epimerise and therefore would remain in the ratios determined at the start of the reaction. The ability for one enantiomer to be formed with yields up to 100% means that dynamic kinetic resolutions are very useful compounds for synthetic organic chemists. The dynamics of this reaction are based on the Curtin-Hammett principle – this states that the barrier for the reaction for both enantiomers must be higher than the barrier to epimerisation, which will result in a kinetic well containing the racemic mixture. The reaction converting the stereoisomers will be working both way, so some E isomer will be converted to its Z form, however this reaction will be much slower than the reverse reaction and therefore will have little effect on the overall reaction. We were hoping that the addition of TFA would protonate the nitrogen, which would lead to a N-H bond breaking and rotation around the C-N bond (which due to electron transportation would now exhibit single bond character) would occur resulting in the Z isomer being

formed [25]. As there was no product peak in the NMR, TFA was unable to epimerise the Z isomer and the E isomer also didn't react and cyclise correctly. However this was thought to be an area for further study as some literature suggests that this reaction could happen using stoichiometric amounts of Lead Tetraacetate. Lead Tetraacetate can be fatal is ingested, inhaled or absorbed through the skin, so using this method would not be suitable in drug production, however for research methods only gathering results regarding the Z isomer yield when using a strong oxidising agent such as Lead tetraacetate, could be potentially helpful for future groups regarding synthesising the core.

As the yield was not improved by changing reaction conditions, future projects could focus on how we could use this to our own advantage. Instead of focusing on getting the Z isomer to react, the focus could be shifted on making the reaction with the E isomer almost 100% and therefore the yield would be about 50%.

Determining reaction completion was done using TLC, comparing starting material spots with those of the product – sometimes the product spot was compared to material being added to the starting material as the starting material was added in excess.

The final part of the reaction, the alkoxide displacement, was the easiest reaction to determine whether the product had been formed as this was done using UV and fluorescence. Aromatic rings have the ability to possess fluorescent properties, as they posses a highly polarizable electron cloud in the form of a pi-conjugated system, especially when one of the substituents on the ring is an electron-withdrawing group and another is an electron-donating group. This is known as push-pull fluorescence. The product we were making contained an –OH group, which is strong electron donating group. Electron donating groups donates some of its electron density into a conjugated pi system, such as a benzene

ring, thus making the system more nucleophilic. The other substituent on the ring, the aromatic nitrogen containing five membered ring, is an electron withdrawing group which removes electron density from the conjugated pi system, making the system more electrophilic. Having these groups' para to each other on the ring enhances the polarizability and hyperpolarizability of the electron cloud, resulting in the molecule exhibiting fluorescence – which can be monitored using TLC and the spectrometer [26].

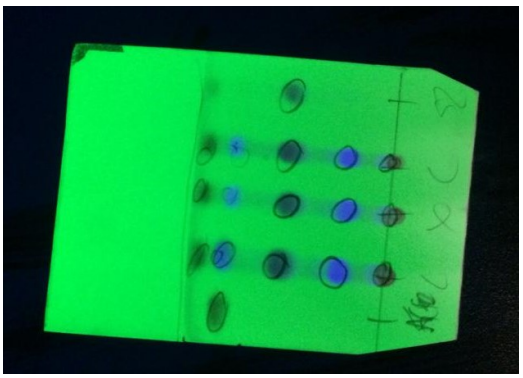


Figure 17 | Photo of TLC plate showing the violet coloured fluorescence of the final produc

Conclusion

The stubbornness of the Z isomer to react was a little unexpected, as literature suggested that the energetic barrier for E/Z isomers for hydrazones is usually low enough to make it difficult to isolate isomers. It also suggests that making the Z isomer isomerise could be done with relative ease and not having to resort to reactions using lead and other harmful reactants. This could be due to sterics, as the group attached to the Z isomer is very large and could increase the energetic barrier (sterics is the reason why the E isomer is usually the major project formed in reactions, or the isomer that is more likely to react).

To summarize, the desired triazolopyrazine was successfully synthesised, but not purified, and the synthetic route taken to make the core was modified from using PIDA as the oxidising agent to using Chloramine T, due to a more efficient reaction with the E isomer. The Z isomer remained unreacted in all of the modified synthetic routes, and further areas of research and reaction conditions were suggested for future groups.

Acknowledgements

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