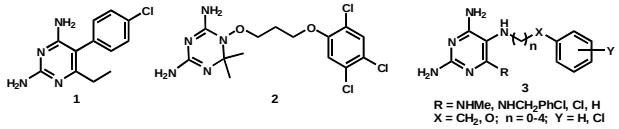
Pyrimidines In Antimalarial Drug Design

S S Moleele[1], D Gravestock[1], A L Rousseau[1], R L Van Zyl[2]

[1]Discovery Chemistry, CSIR, Biosciences, Private Bag X2, Modderfontein, 1645, South Africa; SMoleele@csir.co.za
[2]Department of Pharmacy and Pharmacology, University of the Witwatersrand, 7 York Road, Parktown, 2193, South Africa

Introduction

Malaria causes the death of 2-3 million people annually, most of these children under 5 years of age. Approximately 300 million cases of acute malaria are reported each year, 90% of these in Africa. Until recently, folate metabolism has been successfully targeted in both prophylaxis and treatment of malaria. *Plasmodium falciparum* dihydrofolate reductase-thymidylate synthase (PfDHFR-TS, Figure 1a) is a well defined and validated target for malaria chemotherapy. Unfortunately, resistance to the most commonly used anti-DHFR drugs, including pyrimethamine **1** now limits the clinical usefulness of these drugs.



Resistance to **1**, which is a rigid molecule, is thought to arise from steric clashes with mutant amino acid residues within the PfDHFR active site. In comparison, WR99210 **2**, which contains a flexible linker between the two rings, maintains binding affinity to all mutant forms of PfDHFR. The flexibility imparted by this linker enables **2** to avoid unfavourable contacts with mutant amino acid residues.

Using molecular modeling as a tool, we designed a series of flexible pyrimethamine analogues **3** that would theoretically be less susceptible to steric clashes in the active site of drug resistant *P. falciparum* strains, and embarked on the synthesis of these analogues.

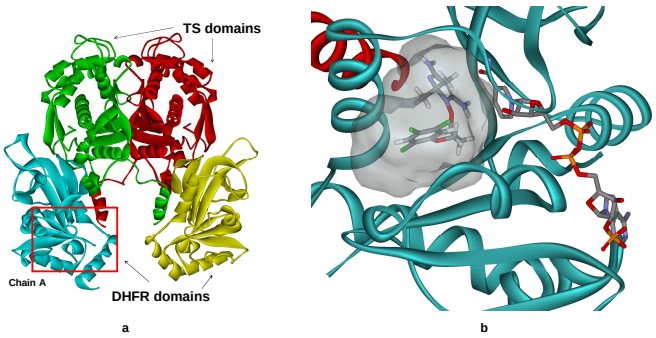
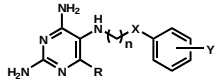


Figure 1 a) Crystal structure of bifunctional PfDHFR-TS, with TS domains shown in green and red, and DHFR domains shown in blue and yellow; b) Close-up view of DHFR active site with WR99210 **2** bound and the co-factor NADPH shown

Molecular Modelling

The crystal structure selected for *in silico* screening was the quadruple mutant 1J3K bearing the following mutations: Asn51Ile, Cys59Arg, Ser108Asn, Ile164Leu, complexed with WR99210, NADPH and dUMP (at the TS site). The active site was defined at chain A of the DHFR region with the co-factor NADPH bound (Figure 1b), using Accelrys Discovery Studio 2.0.

Of the initial series of pyrimethamine analogues **3** screened *in silico*, **3a-c** scored well in the docking experiments (Figure 2), with the majority of the compounds scoring better than pyrimethamine **1**. In each case, the flexible linker allows for the aromatic residue to bend away from the Ser108Asn mutation.



3a: R=H, n=0, X=CH$_2$, Y=4-Cl
3b: R=NMe, n=1, X=CH$_2$, Y=3,4-diCl
3c: R=NMe, n=2, X=O, Y=4-Cl

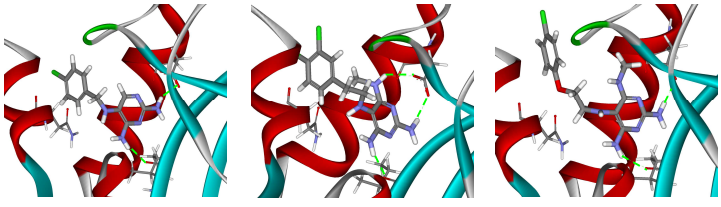
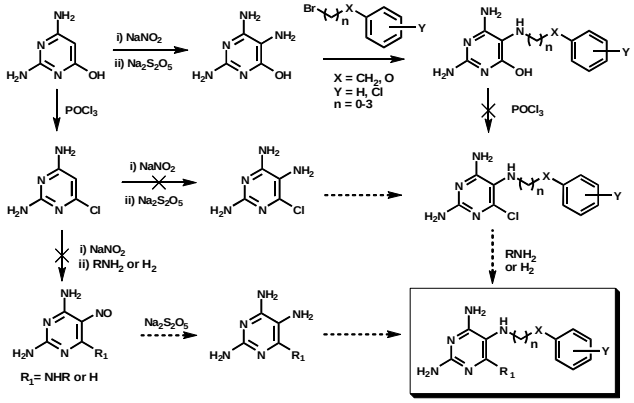


Figure 2 Crystal structure of pyrimethamine analogues **3** bound in the PfDHFR active site with hydrogen bonds to key residues shown. a) **3a**; b) **3b**; c) **3c**

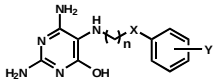
Synthesis

Synthesis of the target pyrimethamine analogues proved to be problematic. Although numerous routes were attempted, we were often unable to accomplish the last step in the synthesis, or were unable to isolate highly polar key intermediates from aqueous reaction mixtures. Some of the routes attempted are shown in Scheme 1.



Scheme 1 Synthetic approaches to pyrimethamine analogues **3**

Advanced intermediates **3d-j** were subjected to *in silico* screening, and compounds **3f**, **3g**, **3h** and **3i** showed promising hydrogen bonding interactions within the PfDHFR active site (Figure 3). All the intermediates prepared were screened for antimalarial activity.



3d: X=CH$_2$, n=0, Y=4-Cl
3e: X=CH$_2$, n=0, Y=3,4-diCl
3f: X=CH$_2$, n=1, Y=4-Cl
3g: X=CH$_2$, n=1, Y=3,4-diCl
3h: X=O, n=2, Y=4-Cl
3i: X=O, n=2, Y=3,4-diCl
3j: X=O, n=3, Y=4-Cl
3k: X=CH$_2$, n=0, Y=H

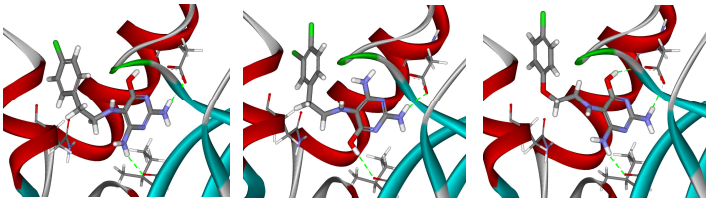


Figure 3 Crystal structure of pyrimethamine analogues **3** bound in the PfDHFR active site with hydrogen bonds to key residues shown. a) **3f**; b) **3g**; c) **3h**

Biological Screening

The antimalarial activity of the pyrimethamine analogues were assessed *in vitro* using the tritiated hypoxanthine incorporation assay against the chloroquine-resistant FCR-3 strain of *Plasmodium falciparum*. Pyrimethamine was used as the positive control. The concentration required to inhibit 50% parasite growth was determined from a log sigmoid dose response curve.

Unfortunately, all of the intermediates screened were significantly less active than the control, pyrimethamine. The most active compound of this series was **3g** with an IC$_{50}$ value of 26.8µM.

Table 1 Whole cell antimalarial activity

Compound	Antimalarial activity IC$_{50}$ value ± s.d. (µM)
3d	73.11 ± 5.67
3e	59.99 ± 8.58
3f	59.23 ± 3.84
3g	26.82 ± 7.85
3h	66.82 ± 8.99
3i	81.73 ± 8.52
3j	62.62 ± 4.25
3k	30.90 ± 6.62
Pyrimethamine	0.096± 0.01

Conclusion

The hydroxyl substitution in the pyrimethamine analogues significantly decreased the *in vitro* antimalarial activity, regardless of the interaction of this group within the active site of PfDHFR predicted by molecular modelling.