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Disciplines
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Antimalarial Activity of 2,4-Diaminopyrimidines

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ABSTRACT

A series of 2,4- and 4,6-diaminopyrimidines were prepared and evaluated for their *in vitro* antimalarial activity. Of the 12 compounds tested 7 showed reasonable activity with 1 having a sub-micromolar IC_{50}.

KEYWORDS

Antimalarial, diaminopyrimidine, structure-activity relationship

INTRODUCTION

Malaria is a growing disease that is a major health threat in over 100 countries world wide [1] infecting more than 500 million people per year with 2.7 million associated deaths [2]. Of the four parasitic strains that infect humans *Plasmodium falciparum* is the most virulent [3]. Over the past 3 decades drug resistant strains of *P. falciparum* have been increasing at such rate that in 1998 this parasite showed reduced susceptibility to at least one drug in almost all cases [4]. The increase in drug resistant strains has resulted in a clear requirement for new drugs with novel modes of action to be discovered.

RESULTS

Recent work in our laboratory has focused upon the synthesis and biological evaluation of a series of diaminopyrimidines as potential antimalarial agents. There are numerous examples of pyrimidine-based structures showing antimalarial activity including pyrimidine-2,4-dione derivatives (**1**) [5], 2,4,6-trisubstituted pyrimidines (**2**) [6], and the drug pyrimethamine (**3**) [6] Fig. (**1**). The mechanism of

action of pyrimethamine is known to be inhibition of dihydrofolate reductase (DHFR), which prevents DNA biosynthesis leading to cell death [6].

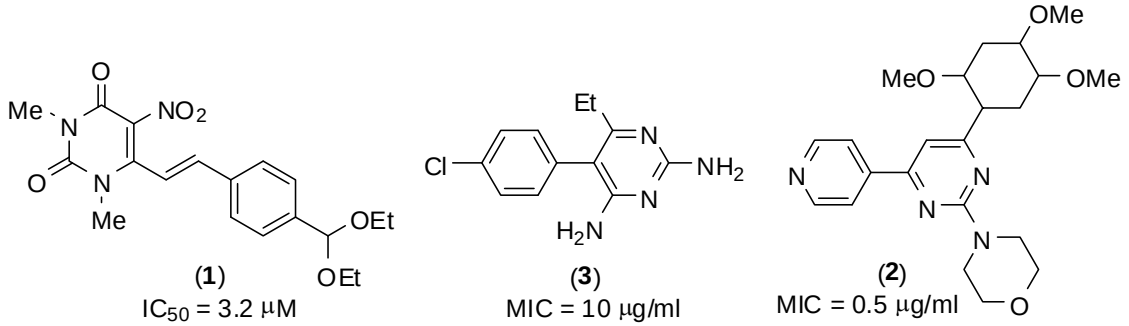
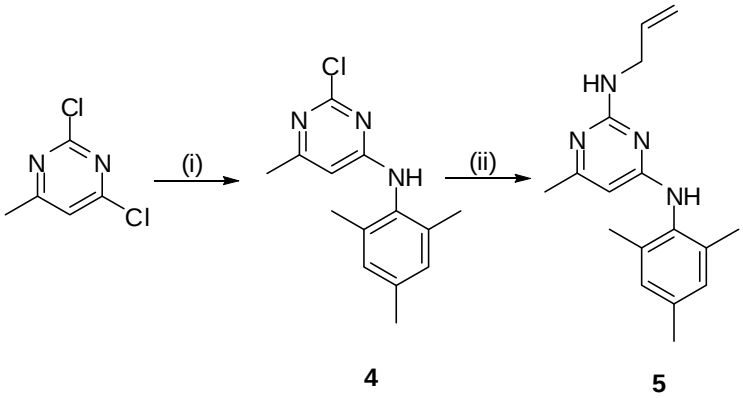


Figure 1. Known pyrimidine containing anti-malarial agents including drug pyrimethamine 3 [5,6]

The 2,4- and 4,6-diaminopyrimidine compounds were prepared *via* two sequential substitution reactions of dichloropyrimidine with substituted amines; an example reaction is outlined in Scheme 1 [7].

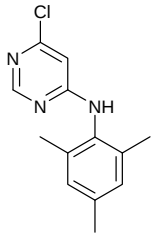
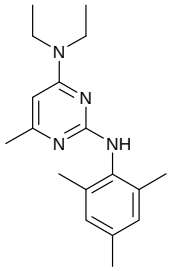
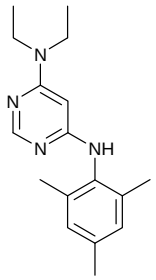
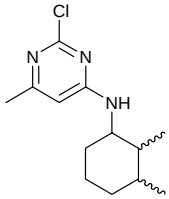
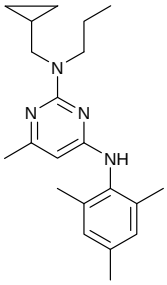
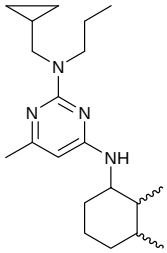
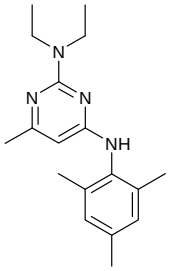
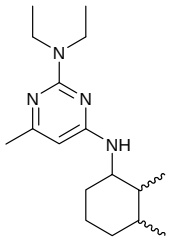
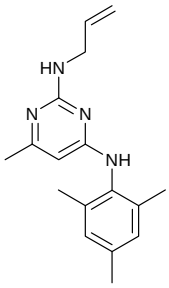
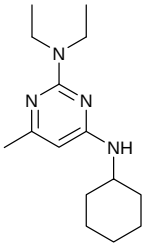


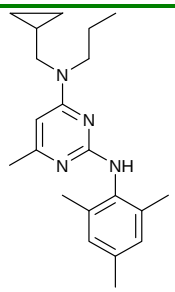
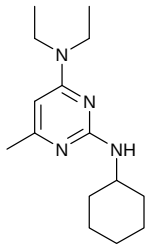
(i) 2,4,6-trimethylaniline, H_2O, Δ, 35%; (ii) allylamine, Δ, 88%

Scheme 1. Synthetic pathway for the preparation of 2,4-diaminopyrimidine

Twelve of these diaminopyrimidine derivatives were evaluated for antimalarial activity against *P. falciparum*, with results summarized in Table 1. Of the 12 diaminopyrimidine derivatives 7 have shown IC_{50} values of less than 15 μM and two of these have an IC_{50} of 1 μM (0.3 μg/ml) or less which is 30 times more potent than pyrimethamine (**3**).

Table 1. The structure and antimalarial activity (IC_{50}, μg/ml) of a range of aminopyrimidine derivatives.

Comp	Structure	IC_{50} (μg/ml)	IC_{50} (μM)	Comp	Structure	IC_{50} (μg/ml)	IC_{50} (μM)
6		inactive		**11**		2.8	9.4
7		inactive		**12**		inactive	
8		inactive		**13**		0.3	0.9
9		inactive		**14**		0.3	1.0
5		3.4	12.0	**15**		2.6	9.9

10		4.2	12.4	**16**		0.4	1.5

Using this biological data a preliminary structure-activity relationship (SAR) can be examined Fig. (**2**). The incorporation of cyclohexylamine instead of a substituted aniline [as in compounds (**13**)-(**16**)] results in a significant increase in activity. This suggests that associated potential hydrophobic interactions may require flexibility of the substrate. Increased substitution of the cyclohexyl group also results in an increase in activity. The favoured positions for the nitrogen atoms of the pyrimidine ring is either side of the disubstituted amino moiety (positions 1 and 3). However, due to the preliminary nature of the SAR study it not possible to determine the preferred substitution at positions 4 or 6.

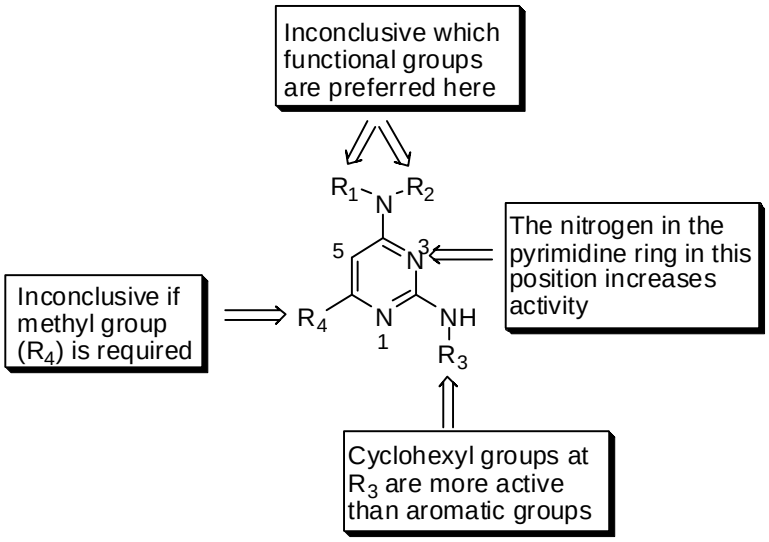


Figure 2. Preliminary structure-activity relationship study on 2,4-diaminopyrimidine compounds possessing antimalarial activity.

CONCLUSIONS

Seven compounds with antimalarial activity have been identified and utilized to develop a preliminary SAR. These compounds will be useful leads for further optimization in the quest to find new antimalarial agents.

BIOLOGICAL TESTING

The parasite *P. falciparum* (K1, multidrug-resistant strain) was cultured continuously according to the method of Trager and Jensen [8]. Quantitative assessment of anti-plasmodial activity *in vitro* was undertaken by means of the microculture radioisotope technique based upon the method described by Desjardins *et al* [9]. Inhibition concentration (IC_{50}) represents the concentration which causes 50% reduction in parasite growth as indicated by the uptake of [^{3}H]-hypoxanthine by *P. falciparum*.

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[7] A general synthetic procedure for the preparation of 2,4-diaminopyrimidines is outlined here. To a suspension of 2,4-dichloro-6-methylpyrimidine (2.01 g, 12.3 mmol) in distilled water (50 ml) was added 2,4,6-trimethylaniline (1.8 ml, 17.3 mmol) and the resulting mixture was heated at reflux for 72 hours. The cooled reaction mixture was extracted with DCM (4 x 30 ml), the combined organic layers dried ($MgSO_4$) and concentrated *in vacuo*. The crude residue was subjected to gravity silica column chromatography and elution with 5% ethyl acetate:hexane gave 2-chloro-6-methyl-4-(2,4,6-trimethylanilino)pyrimidine **4** (1.14 g, 4.4 mmol, 35%) as a white powder, mp. 208 °C. MS (CI); *m/z* 264 (32%) $[M+H\ ^{37}Cl]^+$; 262 (100) $[M+H\ ^{35}Cl]^+$; 203 (18); 138 (93); HRMS (CI) for $C_{14}H_{17}N_3{}^{35}Cl$, calculated 262.1113, found 262.1113; ^{1}H NMR δ 6.98, s, 2H, ArH3′,5′; 6.73, bs, 1H, NH; 5.65, s, 1H, ArH5; 2.33, s, 3H, Ar6-CH_3; 2.25, s, 3H, Ar4′-CH_3; 2.17, s, 6H, Ar2′,6′-CH_3; ^{13}C NMR δ 168.9, ArC4; 164.5, ArC1′; 160.2, ArC2; 138.1, ArC6; 136.6, ArC4′; 131.5, ArC2′,6′; 129.6, ArC3′,5′; 99.5, ArC5; 24.1, Ar6-CH_3; 21.5, Ar4′-CH_3; 18.3, Ar2′,6′-CH_3. A solution of **4** (0.34 g, 1.29 mmol) in allylamine (2.0 ml, 26.6 mmol) was flushed with nitrogen, and stirred in a sealed tube at 105 °C for 72 hours. The reaction mixture was concentrated *in vacuo*. The crude residue was subjected to gravity silica column chromatography and elution with 25% ethyl acetate:hexane gave 6-methyl-2-*N*-(3-prop-1-enyl)4-(2,4,6-trimethylanilino)aminopyrimidine **5** (0.32 g, 1.13 mmol, 88%) as a white powder, mp. 155 °C. MS (CI); *m/z* 283 (94%) $[M+H]^+$; 81 (100); HRMS (CI) for $C_{17}H_{23}N_4$, calculated 283.1923, found

283.1933; ^{1}H NMR δ 6.94, s, 2H, ArH3′,5′; 6.17, bs, 1H, Ar-NH; 5.95, m, 1H, $CH_2C\underline{H}=CH_2$; 5.27, s, 1H, ArH5; 5.12, m, 2H, $CH_2CH=C\underline{H_2}$; 4.88, bs, 1H, $CH_2N\underline{H}$; 4.04, m, 2H, $C\underline{H_2}CH=CH_2$; 2.31, s, 3H, Ar6-CH_3; 2.17, s, 6H, Ar2′,6′-CH_3; 2.09, s, 3H, Ar4′-CH_3; ^{13}C NMR δ 166.8, ArC4; 163.2, ArC2; 162.0, ArC1′; 136.9, ArC4′; 136.6, ArC6; 135.5, $CH_2\underline{C}H=CH_2$; 132.3, ArC2′,6′; 129.0, ArC3′,5′; 115.3, $CH_2CH=\underline{C}H_2$; 91.7, ArC5; 43.9, $\underline{C}H_2CH=CH_2$; 24.1, Ar6-CH_3; 21.0, Ar4′-CH_3; 18.3, Ar2′,6′-CH_3.

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