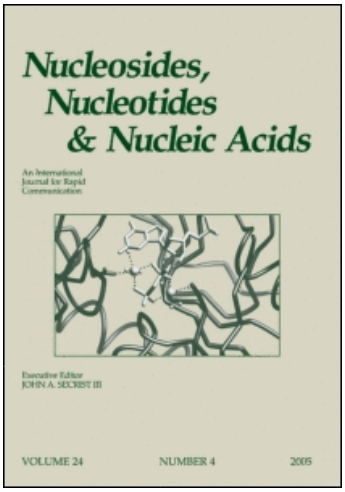

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

In Vitro Anti-Malarial Activity Of N⁶-Modified Purine Analogs

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To cite this Article Too, Kathleen , Brown, Daniel M. , Loakes, David , Bongard, Emily and Vivas, Livia(2007) 'In Vitro Anti-Malarial Activity Of N⁶-Modified Purine Analogs', Nucleosides, Nucleotides and Nucleic Acids, 26: 6, 579 — 583
To link to this Article: DOI: 10.1080/15257770701490134
URL: http://dx.doi.org/10.1080/15257770701490134

Nucleosides, Nucleotides, and Nucleic Acids, 26:579–583, 2007

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770701490134

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IN VITRO ANTI-MALARIAL ACTIVITY OF N^6-MODIFIED PURINE ANALOGS

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□ *A library of* N^6*-hydroxy-, methoxy-, or amino-adenosine analogs was prepared and screened for anti-malarial properties. We found three compounds that possess anti-plasmodial activity in the low micromolar range against the multi-drug resistant* Plasmodium falciparum *VS1 strain, namely* N^6*-hydroxy-9*H*-purin-6-amine (*IC_{50} *5.57* μ*M), 2-amino-*N^6*-amino-adenosine (*IC_{50} *12.2* μ*M), and 2-amino-*N^6*-amino-*N^6*-methyladenosine (*IC_{50} *0.29* μ*M). More importantly, the compounds were non-toxic, with 2-amino-*N^6*-amino-*N^6*-methyladenosine showing a selectivity index of 5008.*

Keywords Purines; malaria; nucleosides; tautomers; *Plasmodium faciparum*; parasites

INTRODUCTION

Plasmodium falciparum causes the deadliest form of malaria, and with the emergence and spread of drug resistance, currently available drugs such as chloroquine and pyrimethamine/sulfadoxine are becoming ineffective. It is imperative to develop new active compounds of different chemical types and novel mechanisms of action. It is well documented that *P. falciparum* parasites are unable to synthesise purines de novo.[1] As a result, they have systems to internalize and metabolize the required substrates such as adenosine, inosine, and hypoxanthine, and these provide the building blocks for inter alia DNA, RNA, and cofactor synthesis. Therefore the selective inhibition of purine transporters,[1,2] of which there may be one or several in the plasmodia parasite, or inhibition of metabolic enzymes along

The authors would like to thank Dr. Harry de Koning at the University of Glasgow for helpful discussions regarding purine nucleobase/nucleoside transporters. They also wish to acknowledge the use of the EPSRC's Chemical Database Service at Daresbury. Finally, they are grateful to the Medical Research Council Technology (MRCT) Development GAP fund for financial support.

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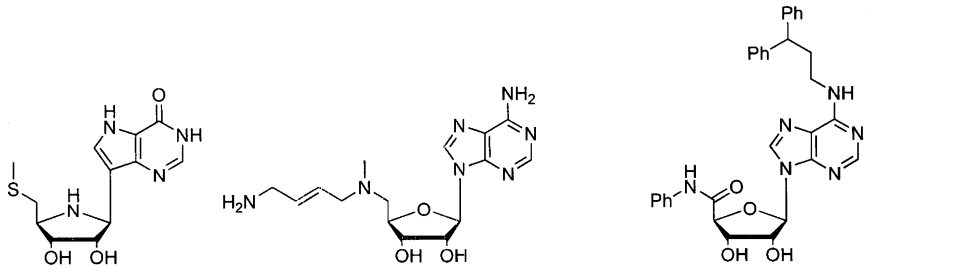
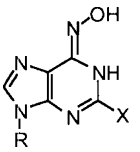
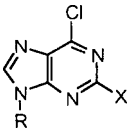
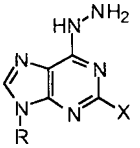
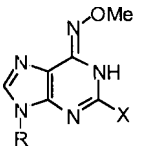
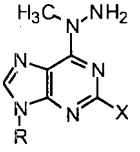


FIGURE 1 Purine analogs that have been shown to possess anti-malarial properties.

the parasite purine salvage pathway[3,4] remain attractive targets for anti-malarial chemotherapy.

Purine analogs have been shown to be potent, versatile small molecule inhibitors and modulators of key biological targets.[5] However, currently, there are very few such analogs that have been identified as having

TABLE 1 Library of analogs synthesized from 6-chloropurine/riboside or 2-amino-6-chloropurine/riboside

Entry	Starting Material	Amine	Product	R	% Yield[a] X=H	% Yield[a] X=NH_2
1		NH_2OH		Ribosyl	77	69
					1a	**1b**
				H	87[b]	78
					1c	**1d**
2		NH_2NH_2		Ridosyl	84	90
					2a	**2b**
				H	**2c**[12]	**2d**[12]
3		NH_2OMe		Ribosyl	55	63
					3a	**3b**
				H	85	90
					3c	**3d**
4		$NHMeNH_2$		Ribosyl	85	83
					4a	**4b**
				H	87	**4d**[13]
					4c	

[a]Chloropurine ribosides were reacted with the corresponding amine at 40–60°C for 3–24 h. In cases where hydrochloride salts were used, 1 eq. triethylamine or diisopropylethylamine was used as the base.

[b]Reaction was conducted at 60°C for 30 min.

anti-malarial properties. Some examples include 5′-methylthio-immucillin-H (MT-ImmH) (Figure 1) as a selective inhibitor of *P. falciparum* purine nucleoside phosphorylase (PfPNP) (K_d 2.7 nM),[6] MDL73811, which has an IC_{50} of 2–3 μM against both chloroquine-sensitive and chloroquine-resistant *P. falciparum* strains by inhibiting *S*-adenosylmethioninedecarboxylase[7] and N^6-diphenylethyl-5′-phenylcarboxamidoadenosine, which possesses an IC_{50} value of 1.8 μM against chloroquine-resistant *P. falciparum.*[8]

Our strategy is to design purine-related compounds that retain the structure of the native purines but which could be inhibitors of protein transporters or metabolic enzymes along the purine salvage system. Herein, we present their syntheses and in vitro activity.

RESULTS AND DISCUSSION

Natural nucleobases adenine and cytosine have tautomeric constants (K_T) in the order of 10^5 favoring the amino form, whereas hypoxanthine, guanine, and uracil have K_T values of the same order but favoring the keto form.[9,10] It was previously shown that the addition of an electronegative atom to the exocyclic amino groups can significantly alter tautomeric ratios[9,10]. Similarly, the presence of an electronegative substituent at position 6 of 2,6-diaminopurine 2′-deoxyriboside shifts K_T so that the two tautomeric forms, amino and imino, become energetically very similar and may thus potentially provide different hydrogen bonding opportunities in the active site of protein transporters or purine metabolic enzymes.[11]

A library of N^6-substituted adenosine and guanosine analogs and their corresponding nucleobases were synthesized by substituting either 6-chloropurine/riboside or 2-amino-6-chloropurine/riboside with various hydroxylamine and hydrazine derivatives. Generally, the reactions were carried out at 60°C for 3-24 h (monitoring by TLC), in a 1:1 mixture of ethanol and water to afford the corresponding analogs (Table 1). All the compounds in this library were screened for anti-malarial activity.

BIOLOGICAL ACTIVITY

The analogs were tested against three strains of *P. falciparum,* namely 3D7 strain which is a standard drug sensitive laboratory clone of the NF54 isolate; K1 strain (Thailand) which is chloroquine, pyrimethamine, and cycloguanil resistant; and the VS1 strain (Vietnam) which is highly chloroquine, pyrimethamine, and cycloguanil resistant. Three of the analogs showed activity in the low micromolar range (Table 2) with **4b** possessing the highest potency (IC_{50} 0.29 μM) against the highly multi-drug resistant VS1 strain. More importantly, **4b** was also non-toxic in KB cells (CC_{50} 1476 μM) giving it a selectivity index (SI) of about 5000. Other interesting

TABLE 2 In vitro anti-malarial activity of purine analogs

	IC_{50} [μ M]			CC_{50} [μ M]	Selectivity index (CC_{50}/IC_{50})		
Compound	3D7	K1	VS1	KB Cells	3D7	K1	VS1
la	11.61	6.90	8.56	44.30	3.8	6.4	5.2
lb	16.03	10.83	13.33	280.7	17.5	25.9	21.1
Ic	**7.48**	**10.21**	**5.57**	**1485.8**	**199**	**146**	**267**
Id	27.77	9.03	16.79	45.01	1.6	5.0	2.7
2a	13.26	7.48	7.92	2.26	<1	<1	<1
2b	**10.16**	**10.00**	**12.22**	**1364.00**	**134**	**136**	**112**
2c	23.08	22.08	18.77	49.28	2.1	2.2	2.6
2d	188.95	108.87	89.32	1000.74	5.3	9.2	11.2
3a	11.42	20.57	9.87	6.16	<1	<1	<1
3b	231.94	184.45	224.78	784.16	3.4	4.3	3.5
3c	98.90	353.61	>606	1257.6	12.7	3.6	2.1
3d	428.06	435.35	>555	n.d.	n.d.	n.d.	n.d.
4a	72.71	35.40	22.47	**101.00**	1.4	2.9	4.5
4b	**2.27**	**3.60**	**0.29**	**1476.0**	**533**	**410**	**5008**
4c	507.89	478.01	418.51	n.d.	n.d.	n.d.	n.d.
4d	>558	>558	>558	n.d.	n.d.	n.d.	n.d.
Chloroquine	0.0024	0.32	0.86	187	77917	584	217
Podophylotoxin	—	—	—	0.048	—	—	—

Tested against 3 different strains of *plasmodium falciparum.*
3D7:Drug sensitive.
K1: Chloroquine and pyrimethamine resistant.
VS1: Highly chloroquine, pyrimethamine, and cycloguanil resistant.
n.d. = not determined.

compounds in the series include **2b** and **1c** which have SI values of 112 and 267 against the VS1 highly resistant strain, 136 and 146 against the K1 resistant strain. There was no indication of a correlation between sensitivity to the analogs and the resistant phenotypes, clearly showing that they could be utilized against multi-drug resistant strains of *P. falciparum* from endemic areas in which current anti-malarials are already ineffective.

There are several hypotheses to try to explain the mechanism of action of the compounds. Currently studies are underway to try to investigate whether they are inhibiting either one or more of the metabolic enzymes such as the hypoxanthine guanine phosphoribosyltransferase or adenosine deaminase on the purine salvage pathway or whether they are inhibiting the purine transporters PfNT1 or PfENT1.

CONCLUSIONS

We show here that the three most active compounds were N^6-hydroxy-9*H*-purin-6-amine (**1c**), 2-amino-N^6-amino-adenosine (**2b**), and 2-amino-N^6-amino-N^6-methyladenosine (**4b**) with the latter possessing a selectivity index of over 5000 with an IC_{50} 0.29 μM. The active compounds were also non-toxic against KB cells in vitro. Experiments are currently underway to

measure the affinity of these purine analogs for the purine transporters and the enzymes on the purine salvage pathway and to determine whether the anti-malarial activity is maintained in vivo.

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