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Antiprotozoal and Cytotoxicity Evaluation of Sulfonamide and Urea Analogues of Quinacrine

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Abstract—Sulfonamide and urea derivatives of quinacrine with varying methylene spacer lengths were synthesised and tested for inhibition of trypanothione reductase (TryR) and for activity in vitro against strains of the parasitic protozoa *Trypanosoma*, *Leishmania*, and *Plasmodium*. These derivatives are superior inhibitors of TryR relative to quinacrine with the best compound being 40 times more potent. Urea derivatives generally displayed good in vitro activity against all parasites.

We have become interested in utilising the acridine moiety of the antiprotozoal drug quinacrine **1** (also known as mepacrine) as a potential template for the design and discovery of antileishmanial, antigiardial, antimalarial, and antitrypanosomal agents. The use of **1** in the treatment of cutaneous leishmaniasis and its in vitro activity against *Trypanosoma cruzi* are known.[1,2] It is noteworthy that acridine derivatives have been considered for the treatment of several protozoan infections.[3] Recently, a number of 9-anilinoacridines and bis(9-amino-6-chloro-2-methoxyacridines) with antimalarial, antileishmanial and antitrypanosomal activities have been reported.[4–6] Various modes of action of quinacrine and derivatives have previously been proposed, including inhibition of trypanothione reductase (TryR),[7] intercalation,[8] binding to haem,[9] and inhibition of the enzyme topoisomerase II.[6]

Within the context of inhibition of TryR by quinacrine, two binding modes for **1** have been predicted.[10] The tricyclic moiety of quinacrine binds in two hydrophobic pockets of the enzyme. We became interested in testing this model by incorporating methylene spacers between the tricyclic moiety of **1** and a second hydrophobic aromatic moiety. This led to the preliminary design of sulfonamides **2** and ureas **3** (Fig. 1). The sulfonamide

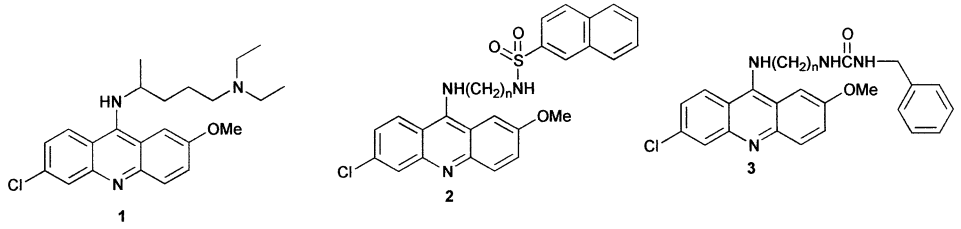

Figure 1. Chemical structures of quinacrine **1** and sulfonamide **2**, and urea derivatives **3**.

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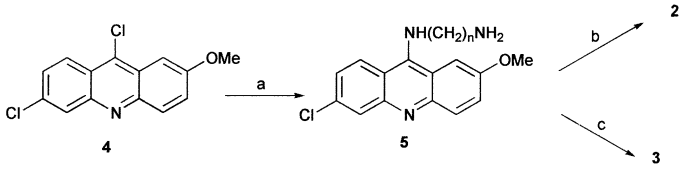

Scheme 1. Reagents and conditions: (a) 32.0 equiv of $H_2N(CH_2)_nNH_2$, phenol, 90 °C, 4 h, 90–95%; (b) 1.2 equiv of 2-naphthylsulfonyl chloride, 2.0 equiv of Et_3N, DMF, 43–94%; (c) 1.1 equiv of benzyl isocyanate, CH_2Cl_2, 25 °C, h, 60–96%.

and urea moieties were incorporated with a view to improving solubility properties of the molecules. We were also interested in the in vitro antiparasitic activities of these compounds in view of previous aforementioned literature reports on related compounds.[4–6]

The synthesis of the target molecules was simple and straightforward and is depicted in Scheme 1. The amine scaffolds **5** were synthesised by reacting 6,9-dichloro-2-methoxyacridine **4** with a large excess of diamines in phenol. The resulting products were converted to the respective sulfonamide and urea products by standard reaction with the respective acid chloride and isocyanate. All new compounds gave ^{1}H NMR, FABMS and, in relevant cases, microanalysis data consistent with their structures.

The enzyme assays were performed essentially as described before.[11] Recombinant *T. cruzi* TryR (128 mU) was assayed using a Beckman DU640 spectrophotometer in 40 mM HEPES, pH 7.5, 1 mM EDTA and 200 μM NADPH at 25 °C followed by the addition of 100 μM Try[SH]2. Human glutathione reductase, purified from human erythrocytes (42.3 mU), was analysed in a similar manner and under identical conditions followed by addition of glutathione disulfide (100 μM). Enzyme mixtures were preincubated with NADPH (10 min at 25 °C) before the addition of varying concentrations of inhibitor added in DMSO (1% v/v final concentration).

As can be seen from the data in Table 1, compounds **2** and **3** were generally superior to quinacrine in inhibiting TryR. Sulfonamides **2** were generally superior to ureas **3**. This may be due to a more hydrophobic naphthalene moiety in **2** compared to the benzyl moiety in **3**. However, despite the superior inhibitory activities of the derivatives, selectivity against the equivalent human enzyme, glutathione reductase, was lost. The loss of a positive charge (from the terminal tertiary amino group) in moving from quinacrine to the new derivatives could explain this loss in selectivity.[12] The three- and four-carbon methylene spacer length appears optimum for inhibition of TryR within this series of compounds.

Compounds were tested in vitro for activity against amastigote forms of *Leishmania donovani* (cultured in murine macrophages), amastigote forms of *Trypanosoma cruzi* (cultured in murine macrophages), and the bloodstream form trypomastigote *Trypanosoma brucei rhodesiense*. Experimental details have previously been described.[13] For *Plasmodium falciparum*, all compounds were tested against the chloroquine-sensitive strain (3-D7) and the most potent also tested against a chloroquine-resistant strain (K1). The whole cell inhibition assay of *Plasmodium falciparum* growth in human red blood cells was carried out in a 48 h [^{3}H]-hypoxanthine incorporation assay.[14,15] Potential toxicity of the quinacrine derivatives was determined against the KB cell line in comparison to podophyllotoxin. The data are presented in Table 2. Data for standard control drugs are included for comparative purposes.

Compounds generally showed significant activity against *L. donovani* with compounds **2b**, **3b**, and **3c** being the most superior. These data correlate well with the data for inhibition of TryR in Table 1. With the exception of urea compounds **3c** and **3d**, which showed good activity against *T. cruzi*, the rest of the compounds displayed weak to no activity at the test concentrations. Like with inhibition of TryR and activity against *L. donovani*, compounds **2b**, **3b,** and **3c** showed the best activity against *T. brucei*. The same picture is more or less true for *P. falciparum* where the compounds showed the greatest activity, compound **3b** being most noteworthy. This compound was 4 and 10 times more active than chloroquine against 3D7 and K1 strains, respectively. The fact that **3b** is less active against the K1 than the 3D7 strain suggests haem polymerization as a target, like the 4-aminoquinolines.[9] This, in turn, suggests cross-resistance with chloroquine. The compounds that were active against *L. donovani* and *T. cruzi* were also toxic to

Table 1. Inhibition of trypanothione reductase and glutathione reductase by quinacrine and analogues

Compound	Number of methylene groups (*n*)	Trypanothione reductase IC_{50} (μM)	Glutathione reductase IC_{50} (μM)	Selectivity index[a]
Quinacrine **1**	N/A	133±11	>1000	>7.5
2a	2	5.9±0.6	9.9±0.4	1.7
2b	3	3.3±0.3	27.2±0.6	8.2
2c	4	5.0±0.2	13.9±1.3	2.8
3a	2	19.3±1.0	27.2±0.6	1.4
3b	3	13.1±0.7	44.7±4.4	3.4
3c	4	15.5±0.8	55.8± 2.6	3.6
3d	6	11.4±0.7	17.1± 3.1	1.5

[a]Ratio IC_{50} GR/IC_{50} TryR.

Table 2. In vitro sensitivity of parasites to quinacrine analogues **2** and **3**

Compound	ED_{50} (μg/mL)					Toxicity ED_{50} (μg/mL)
	L. donovani	*T. cruzi*	*T. brucei*	*P. falciparum* 3D7[a]	*P. falciparum* K1[b]	
Podophyllotoxin						0.008
Pentostam	8.9					
Benznidazole		12.4				
Pentamidine			0.0002			
Chloroquine				0.002	0.15	
2a	5.8	>30	0.47	0.025	nd[c]	2.7
2b	1.9	23.9	0.078	0.010	nd	0.4
2c	3.3	>30	0.12	0.033	nd	0.4
3a	10.7	>30	0.42	0.069	nd	4.1
3b	1.9	22.0	0.083	0.0005	0.015	0.7
3c	1.9	<1	0.043	0.0013	nd	0.8
3d	5.8	6.8	0.46	0.14	nd	4.3

[a]Chloroquine-sensitive strain.
[b]Chloroquine-resistant strain.
[c]Not determined.

KB cells at the same concentrations, indicating a lack of selectivity against these parasites.

A series of sulfonamide and urea analogues of quinacrine generally show superior activity (relative to quinacrine) against TryR. Although sulfonamide derivatives were more active than ureas in inhibiting TryR, this trend did not correlate with the in vitro activities against *L. donovani*, *T. cruzi*, and *T. brucei*. Urea compounds in particular showed significant activity in vitro against all four parasites. Although *T. cruzi* appears to be the least sensitive comparared to *L. donovani*, it is noteworthy that compounds display activity against these intracellular parasites where the drug must cross the macrophage to reach the amastigote. The mode of action of these compounds is not clear and merits further investigation. Clearly, if a common mechanism were operative, then inhibition of TryR[7] and polymerisation of haem into haemozoin[9] can be excluded as mechanisms, since malaria parasites do not possess TryR and kinetoplastids do not polymerise haem.

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