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AN APPROACH TO USE AN UNUSUAL ADENOSINE TRANSPORTER TO SELECTIVELY DELIVER POLYAMINE ANALOGUES TO TRYPANOSOMES

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Abstract: In this paper we describe an approach to selectively deliver compounds to trypanosomes using an adenosine transporter which is unique to the trypanosome. Various polyamine analogues have been attached to known substrates of this adenosine transporter. The compounds prepared interact specifically with the adenosine transporter, some with a similar efficiency to berenil, a known substrate.

Introduction

African trypanosomes cause the disease Sleeping Sickness in humans and a number of related diseases in domestic livestock. The parasites live free in the mammalian bloodstream, invading the central nervous system late in infection. Chemotherapy remains the only way of controlling the disease at the host-parasite level. There is an urgent need for new drugs for the treatment of African trypanosomiasis. The current drugs have very bad side effects, show poor clinical efficacy and require hospitalisation for treatment. The search for new improved chemotherapeutics centres around differences in biochemistry or metabolism between the human host and the causative organisms of African trypanosomiasis, *Trypansoma brucei rhodesiense* and *T. brucei gambiense*.

Carter and Fairlamb[1] have recently characterised a unique adenosine transporter in *T. brucei* which they have called the P2 transporter. Fairlamb *et al.* have screened a large number of (186) compounds[2] to determine which structural features are required for the uptake of molecules through the P2 transporter. A summary of their findings is shown in Figure 1. Compounds of Type **1** (adenine derivatives), Type **2** (benzamidine derivatives) and Type **3** (melamine derivatives) are taken up by the P2 transporter. Compounds of Types **2** and **3** are not substrates of mammalian adenosine transporters[1] and hence may provide a means of selective targeting of compounds to trypanosomes. The P2 transporter may account for the anti-trypanosomal activity of melarsoprol and pentamidine, by selectively accumulating these compounds in the trypanosome: melarsoprol is a compound of type **3** and pentamidine of Type **2**. In support of this it has been demonstrated that pentamidine (and possibly other diamidines) are also taken up on the P2 transporter being accumulated over a thousand fold within an hour when parasites were incubated with 0.5 micromolar drug.[3]

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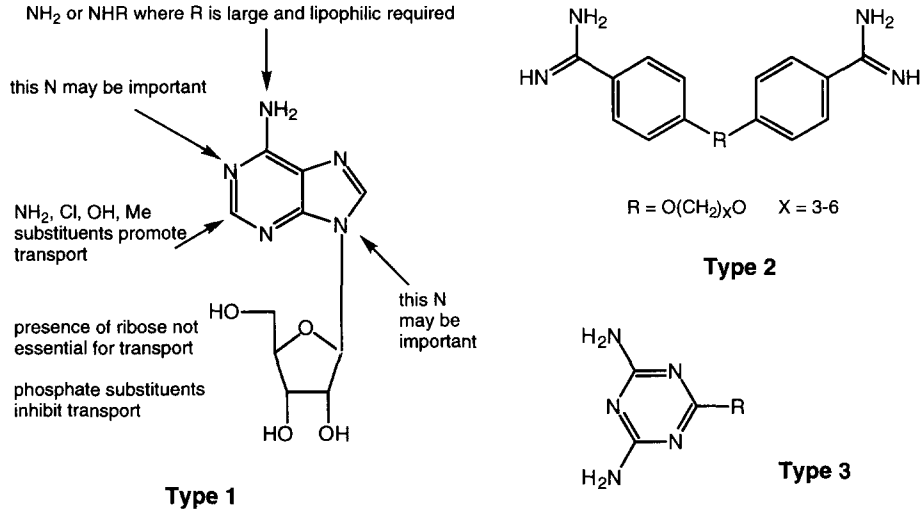


Figure 1

We propose to use the P2 transporter to selectively deliver cytotoxic compounds to the trypanosomes. Initial work has centred around the selective delivery of polyamine analogues to trypanosomes. Polyamine metabolism is known to be a drug target in African trypanosomes as inhibition of the key polyamine biosynthesis enzyme, ornithine decarboxylase, by D,L α-difluoromethylornithine is toxic to these cells.[4]

Various benzyl polyamines have been shown to have activity against *Plasmodium falciparum*,[5] *T. brucei*,[6] *Leishmania donovani*[7] and *T. cruzi*.[8] An example is the Merrell Dow compound MDL 27695 (**4**, Figure 2). These compounds may act by binding to DNA as inhibition of both DNA and RNA synthesis was detected following administration of MDL 27695 to *P. falicaprum*.[5] These compounds may also inhibit enzymes in the polyamine biosynthetic pathway such as ornithine decarboxylase and adenosyl-methionine decarboxylase.[9,10,11,12] By attaching these polyamine analogues to a substrate of the P2 transporter it should be possible to selectively deliver them to the trypanosome.

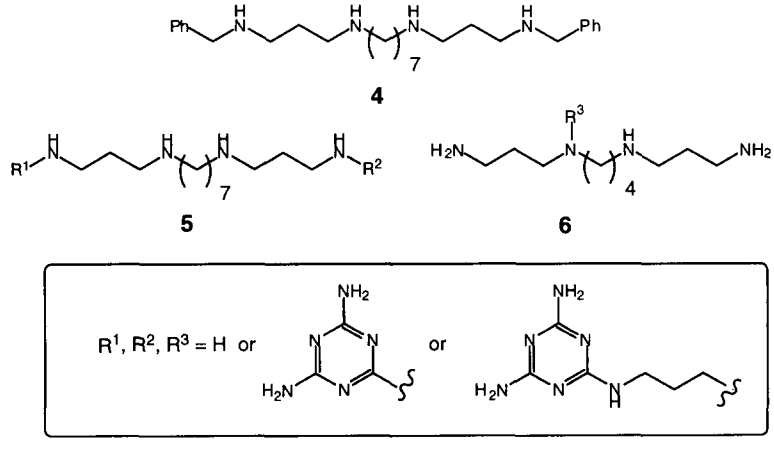


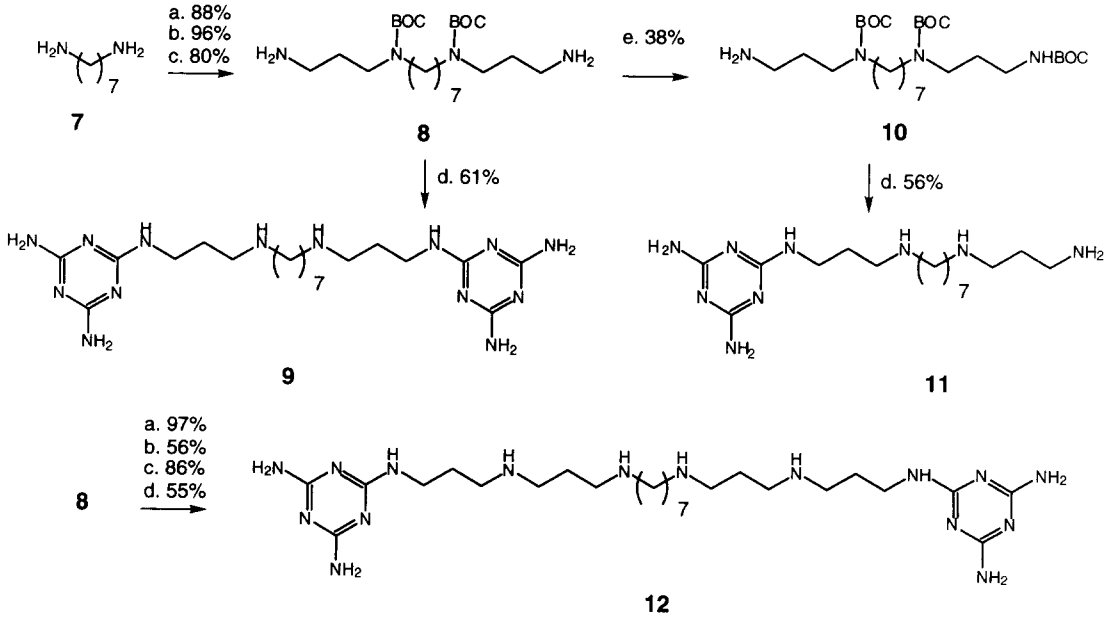
Figure 2

In a first series of compounds (**5**) we replaced the benzyl moiety with a melamine group, attaching the carrier or targeting compound to the terminal ends of the polyamine analogues. In the second series of compounds the transporter was attached to the middle of the polyamine (**6**), either directly or via a spacer. These compounds should all be specifically recognised by the P2 transporter of trypanosomes and may therefore be selectively accumulated by the parasites.

Chemistry

The synthetic routes to the compounds are shown in Schemes 1 and 2. The final compounds were all isolated as hydrochloride salts using ion exchange chromatography.[13] Series 1 compounds are direct analogues of MDL 27695 (**4**) with the melamine moiety attached to the terminal ends of the polyamine. In series 2 compounds the melamine moiety is attached to the secondary amines in the centre of the polyamine.

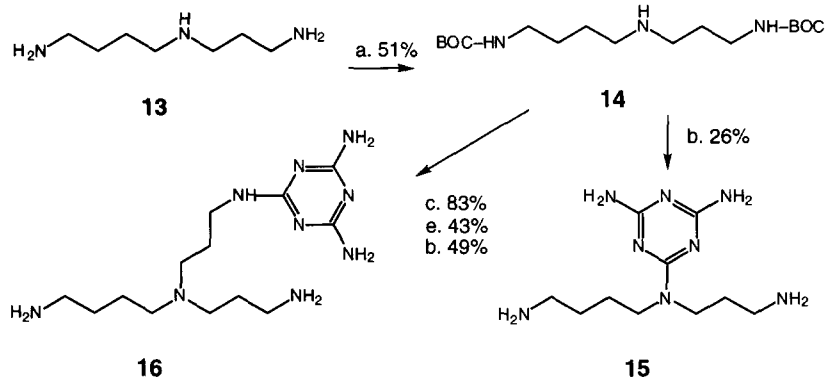
The direct analogue of MDL 27695 (**4**) is compound **9**. Compounds **11** and **12** were also prepared to probe two additional factors. Firstly the pKa of the nitrogen adjacent to the melamine ring will be reduced so that the nitrogen may not be protonated at physiological pH and secondly the proximity of the bulky melamine ring adjacent to the polyamine analogue may interfere when the polyamine analogue interacts with the intracellular target(s). Compound **11** is just protonated at one end and in compound **12** the melamine moieties are attached via spacers. Similarly for compounds of series 2 (Scheme 2) the melamine moiety was attached directly to the polyamine analogue (**15** and **20**) and through a spacer (**16** and **19**).



(**a**). CH_2=CH_2CN, EtOH, rt; (**b**). $(BOC)_2O$, Et_3N, MeOH, rt; (**c**). Raney Ni, H_2, EtOH, NaOH, rt; (**d**). (i) 2-chloro-4,6-diamino-1,3,5-triazine, $NaHCO_3$, H_2O, 80°C, (ii) CF_3CO_2H, anisole, rt; (**e**). BOC-ON (1 equiv), THF, 0°C.

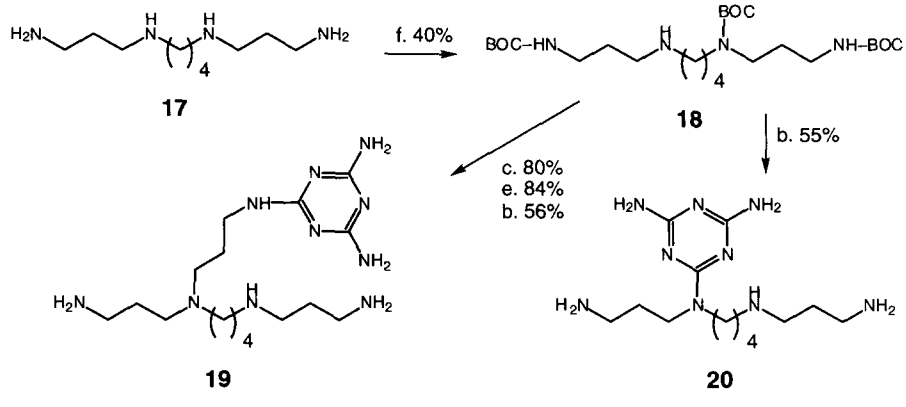
Scheme 1

Spermidine Series



13 14 16 15

Spermine Series



17 18 19 20

(**a**). BOC-ON (2 equiv), THF, 0°C; (**b**). (i) 2-chloro-4,6-diamino-1,3,5-triazine, $NaHCO_3$, H_2O, 80°C, (ii) CF_3CO_2H, anisole, rt; (**c**). $CH_2{=}CH_2CN$, EtOH, rt; (**d**). $(BOC)_2O$, Et_3N, MeOH, rt; (**e**). Raney Ni, H_2, EtOH, NaOH, rt; (**f**). (i) BOC-ON (2 equiv), THF, 0°C, (ii) BOC-ON (1 equiv), THF, rt.

Scheme 2

Biological Assays

Compounds were assayed for their ability to inhibit uptake of adenosine through the P2 transporter of blood stream *Trypanosoma brucei brucei* trypomastigotes. Inhibition of uptake via the P2 adenosine transporter was determined by measuring uptake of 1 μM [2-^{3}H] adenosine.[1] Three concentrations of each compound were tested (Table), along with known P2 substrates adenine and berenil. During the assay uptake through the P1 transporter (another adenosine transporter)[1] was blocked by the addition of 1 mM inosine, ensuring that only uptake through the P2 transporter was measured. All compounds showed dose dependent inhibition of adenosine uptake via the P2 transporter. Some of the compounds showed inhibition not much less than that of berenil, a known substrate.

Compounds were also assayed for their *in vitro* activity against the trypomastigote form[14] (the clinically relevant form of the parasite found in the human blood stream) of *T. brucei* (Table).[15]

Table: Percentage Inhibition of Adenosine Uptake via the P2 Transporter and *In Vitro* Activity Against Bloodstream Form Trypomastigotes

Competitor/ Compound	Inhibition of Adenosine Uptake via the P2 Transporter			*In Vitro* Activity Against Trypomastigotes
	Concentration (μg/ml)	Molarity (μM)	% Inhibition of Adenosine uptake via the P2 transporter	IC_{50} (μM)
Adenine	1	7.4	88	
	10	74	89	
	100	740	99	
Berenil	1	3.5	67	0.003
	10	35	84	
	100	350	99	
9	1	1.4	36	110
	10	14	63	
	100	140	93	
11	1	1.7	12	21
	10	17	32	
	100	170	77	
12	1	1.1	0	24
	10	11	18	
	100	110	88	
15	1	2.7	29	>270
	10	27	47	
	100	270	73	
16	1	2.0	7	>200
	10	20	5	
	100	200	61	
19	1	1.6	2	24
	10	16	17	
	100	160	37	
20	1	2.1	24	12
	10	21	44	
	100	210	60	

Discussion

Polyamine metabolism is known to be essential to African trypanosomes and the P2 recognition motif has been coupled to a number of polyamines and polyamine analogues. All of the compounds show a dose dependent inhibition of adenosine uptake by the P2 transporter; some compounds inhibit within the same order of efficiency as the known P2 transporter berenil. This data shows that these types of compound interact specifically with the parasites, although it does not confirm that they are actually taken up through the P2 transporter, nor does it preclude alternative routes of entry for these compounds into the cell. In the first series of compounds (**9**, **11**, **12**), removing one of the melamine groups (**11**) or placing a spacer between the polyamine and the melamine groups (**12**) seems to cause a small decrease in inhibition of adenosine transport. In the second series of compounds (**15**, **16**, **19**, **20**), those compounds where the melamine is directly attached to the polyamine (**15**, **20**) show slightly greater inhibition than those where the melamine is attached via a linker (**16**, **19**).

Some compounds also showed *in vitro* anti-parasitic activity, in particular compounds **11, 12, 19** and **20**. There is no correlation between the efficiency with which the compounds interact with the transporter and their anti-parasitic activity, suggesting that interaction with the P2 transporter is not the rate limiting step in trypanocidal action. The interaction with intracellular targets once inside the cell is more likely to be critical in determining toxicity. The first series of compounds (**9, 11, 12**) are analogues of MDL 27695 (**4**). The mode of action of this compound (**4**) in rat hepatoma cells is by debenzylation to the free 3-7-3 polyamine and then inhibition of ornithine decarboxylase and adenosyl-methionine decarboylase.[12] It is acting as a prodrug. If the same mode of action is maintained in trypanosomes, the relatively weak *in vitro* activity of compound **9** (110μM) may be due to less rapid metabolism compared to MDL 27695 (**4**) (12-15μM). Another possible explanation for the relatively weak *in vitro* activity of compound **9** is that it is binding to the P2 transporter but not being taken up into the trypanosome. However, the fact that other compounds show stronger *in vitro* activity suggests this is not the case. In the second series of compounds the spermine analogues (**19, 20**) show greater activity than spermidine analogues (**15, 16**).

To summarise, we have prepared a number of compounds which interact specifically with the P2 transporter with a similar efficiency to berenil, a known P2 substrate. Some of these compounds then showed *in vitro* activity against African trypanosomes. Further work is planned to carry out structure activity studies and probe the mechanism of action of these compounds.

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