

Interaction of Monobenzamidine-Linked Trypanocides with the *Trypanosoma brucei* P2 Aminopurine Transporter

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Single benzamidine group-carrying compounds were shown to interact with the *Trypanosoma brucei* P2 aminopurine transporter. Replacement of the amidine with a guanidine group decreased affinity. Trypanocidal activity was evident, but compounds were equally toxic against trypanosomes lacking the P2 transporter, which indicates additional uptake routes for monobenzamidine-derived compounds.

Human African trypanosomiasis is endemic in sub-Saharan Africa. Around 300,000 individuals are afflicted (3). Current drugs have problems including accessibility, administration, and disease stage specificity; most cause side effects of variable severity (3). The development of new trypanotoxic compounds is vital.

We are developing an approach to deliver toxins to trypanosomes via a specific plasma membrane transporter, P2, that is usually involved in aminopurine accumulation (6, 15). The substrate structural motif recognized by the P2 transporter has been characterized (Fig. 1) (4, 8, 10); it recognizes, binds, and internalizes compounds carrying a recognition motif in the form of a benzamidine or melamine residue. This includes the arsenical and diamidine classes of drugs in common use today (4).

Previous studies, adding melamine-based transport units to polyamine (14, 19) or nitroheterocyclic (1, 18) toxic residues, have given rise to trypanocidal compounds. Interestingly these studies indicate that transporters in addition to P2 might also accumulate such compounds; hence, resistance through loss of this transporter need not be an issue. Two other transporters, HAPT1 and LAPT1, have been shown to mediate pentamidine transport in *Trypanosoma brucei* (9), although the full comple-

ment of transporters capable of mediating uptake of melamine- or benzamidine-bearing compounds has yet to be established. In principle, monobenzamidine moieties would be predicted to interact with the P2 transporter, but to date studies have focused on diamidine type compounds (2, 7, 11, 16).

A series of compounds (5) synthesized originally to mimic peptide interactions involving the human immunodeficiency virus gp120 protein carry the P2 recognition motif in the form of a monobenzamidine moiety. It was of interest to determine how they interacted with the P2 transporter and also to determine whether they had any capacity to kill trypanosomes. The ability of these compounds to interact with the P2 transporter was assessed using the well-established oil-stop method to measure tritiated adenosine (0.05 μ M) uptake via that transporter in the presence of a range of concentrations (0.1 mM to 100 nM) of each inhibitor. In general the compounds were found to have high affinities for the P2 transporter (Table 1), of the same magnitude as the transporter's natural substrate adenosine, and generally around an order of magnitude higher than the affinities of a series of novel melamine-carrying compounds (1, 14, 18, 19). This could indicate a structural preference for the benzamidine group in P2 interactions, and, this being the case, future compounds developed as potential P2-

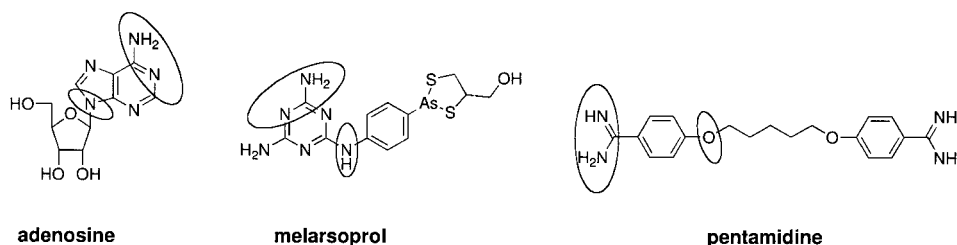


FIG. 1. Substrates for the P2 transporter. The P2 recognition motif is circled: an amidine moiety, an aromatic ring, and a heteroatom.

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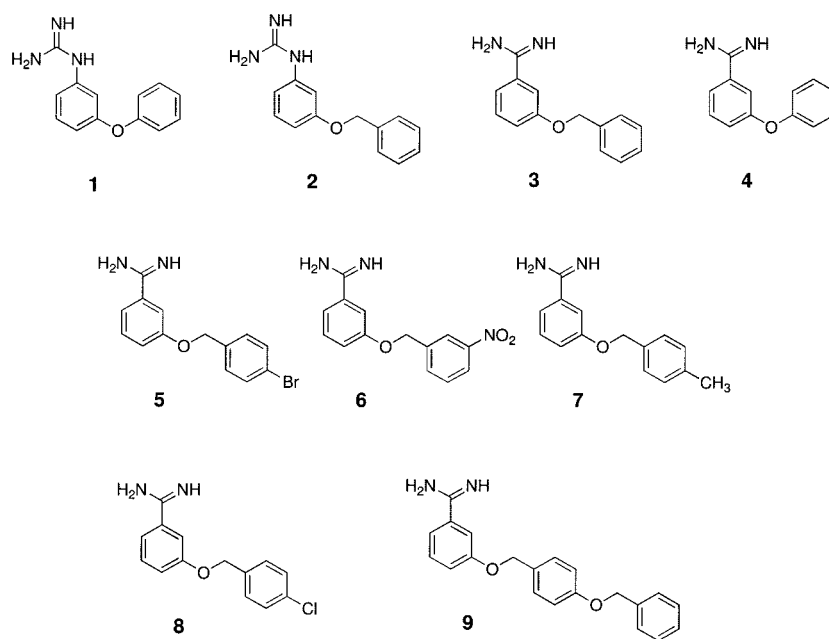


FIG. 2. Structures of the compounds evaluated.

selective trypanocides could benefit from carrying a benzamidine residue.

Compounds 1 and 2 are identical to compounds 4 and 3, respectively, other than that they carry a guanidine group in place of the amidine (Fig. 2). This factor alone apparently accounts for 24-fold and 21-fold differences in apparent affinity for the P2 transporter. An obvious explanation for this is not apparent; however, in the guanidine derivatives, the relative orientation of the P2 recognition motifs may be suboptimal, due to extra nitrogen of the guanidine holding this guanidine moiety at an angle. Furthermore the charge on the guanidine is more delocalized.

Compounds 5 and 8 have an additional halogen attached to the end benzene ring, and this appears to slightly reduce their apparent affinities for P2. Compound 9 also has a lower apparent affinity for P2. With three benzene rings in this compound, it may be the bulk of this compound that is interfering with substrate/transporter interactions.

Compounds were also tested against the bloodstream form *T. brucei brucei* (strain 427), a *T. brucei* line lacking the P2 transporter (*tbat1*^{-/-}) and its wild-type parent line (16), against *T. brucei rhodesiense*, and also against mammalian L6 cells as previously reported (14). Cells were cultivated in HMI-9 medium containing 20% fetal calf serum (13) at 37°C in

TABLE 1. Inhibition of adenosine transport and toxicity of benzamidine-derived compounds

Compound	Inhibition of adenosine transport (μM) ^a	Toxicity in vitro (IC_{50} [μM])			Resistance factor ^b	IC_{50} (μM) for:	
		427	TbAT1	ΔTbat1		<i>T. brucei rhodesiense</i>	L6 cells
1	9.3*	57	52	79	1.52	15.9	140
2	8.05*	109	111	128	1.15	81	71
3	0.38*	54	33	44	1.33	12.8	167
4	0.38*	56	37	60	1.62	31.1	203
5	0.81*	10	6	7	1.06	6.2	16.3
6	0.33*	66	58	71	1.22	49.8	129
7	0.21*	14	10	28	2.80	11.2	52
8	1.01*	13	11	14	1.27	8.4	46
9	1.57*	7	8	12	1.50	11.1	14.7
Adenosine	0.92†	ND ^f	ND	ND	ND	ND	ND
Melarsoprol	0.54‡	0.13 ^c	0.053 ^d	0.12 ^d	2.3	0.006 ^e	7.8 ^e
Pentamidine	0.43‡	0.04 ^c	0.011 ^d	0.026 ^d	2.36	0.00135	2.53
Diminazene aceturate	2.36‡	0.99 ^c	0.30 ^d	5.77 ^d	19.23	0.0088	93.5

^a *, IC_{50} ; †, K_m (6); ‡, K_i (10).

^b IC_{50} measured against ΔTbat1 (the P2 knockout line)/ IC_{50} measured for the wild-type line.

^c From reference 12.

^d From reference 16.

^e From reference 1.

^f ND, not determined.

a humidified CO₂ environment. The Alamar Blue assay (17) was used to determine 50% inhibitory concentration (IC₅₀) values.

All compounds displayed some toxicity towards *T. brucei* 427 and *T. brucei rhodesiense* (STIB900). There was good correlation in the growth inhibition of both species of trypanosomes. Compounds were also evaluated against L6 cells as a measure of toxicity. Compounds showed up to a 10-fold selectivity for the parasite compared to the mammalian cells.

The P2 knockout line (tbat1^{-/-}) showed at most a 2.6-fold resistance factor; most experiments yielded under-1.5-fold-reduced activity compared with the wild type. This indicates that the P2 transporter is not the only route into trypanosomes for these compounds. There may be other transporters involved, for example, the high- and low-affinity pentamidine transporters (HAPT1 and LAPT1), or the compounds may enter the cells by diffusion or other transporters. An alternative explanation could be that interaction with cell surface targets is responsible for activity.

Two compounds in particular, 3 and 6, show similar affinity for P2 as adenosine; however, they demonstrate very little trypanotoxicity. Clearly, uptake alone is not sufficient for trypanocidal activity. Two further compounds, 5 and 8, have affinities for P2 of the same magnitude as adenosine and are active against *T. brucei* in vitro (Table 1). The increase in toxicity in compounds 5 and 8 may be associated with the addition of a halogen group in the *para* position of the benzyl ring. This could relate to a specific interaction of these drugs with a target molecule, and these observations will be central to the design of new compounds.

The addition of monobenzamidine moieties to potential toxophiles can clearly be employed in the design of new trypanocidal agents, and future work will focus on the coupling of other potentially toxic moieties to this head group with the aim of selectively targeting the drugs to trypanosomes.

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