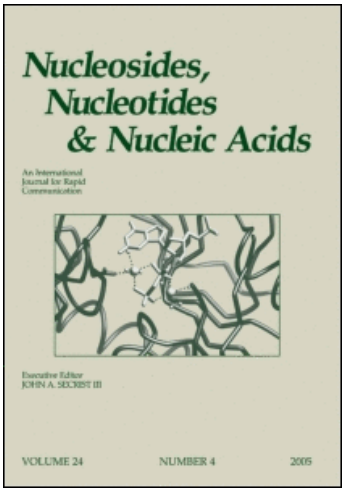


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Selective Transport of a New Class of Purine Antimetabolites by the Protozoan Parasite *Trypanosoma brucei*

L. J. M. Wallace[a]; D. Candlish[a]; A. Hagos[b]; K. L. Seley[bc]; H. P. de Koning[a]
[a] Institute of Biomedical and Life Sciences, Division of Infection and Immunity, University of Glasgow, Glasgow, UK [b] School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, Georgia, USA [c] University of Maryland, Baltimore, Maryland, USA

Online publication date: 27 October 2004

To cite this Article Wallace, L. J. M. , Candlish, D. , Hagos, A. , Seley, K. L. and de Koning, H. P.(2004) 'Selective Transport of a New Class of Purine Antimetabolites by the Protozoan Parasite *Trypanosoma brucei* ', Nucleosides, Nucleotides and Nucleic Acids, 23: 8, 1441 — 1444
To link to this Article: DOI: 10.1081/NCN-200027660
URL: http://dx.doi.org/10.1081/NCN-200027660

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 23, Nos. 8 & 9, pp. 1441–1444, 2004

Selective Transport of a New Class of Purine Antimetabolites by the Protozoan Parasite *Trypanosoma brucei*[#]

L. J. M. Wallace,[1] D. Candlish,[1] A. Hagos,[2] K. L. Seley,[2,†] and H. P. de Koning[1,*]

[1]Institute of Biomedical and Life Sciences, Division of Infection and Immunity, University of Glasgow, Glasgow, UK
[2]School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, Georgia, USA

ABSTRACT

Purine antimetabolites have been very successful therapeutic agents against a host of infectious diseases and malignancies. Success of the treatment relies as much on the efficient accumulation by the target cell or organism as it does on selective action on a vital biochemical pathway of the target cell. Here we compare the ability of a new class of tricyclic purine antimetabolites to interact with transporters from human erythrocytes or *Trypanosoma brucei*. We show that these compounds display a remarkable selectivity for the parasite's transporters. The adenine analogue showed greater trypanocidal activity than the hypoxanthine or guanine analogues in vitro.

Key Words: Trypanosoma brucei; Purine antimetabolite; Purine transporter; Drug uptake; Drug selectivity.

[#]This work was funded by the Wellcome Trust.
[†]Current Address: University of Maryland, Baltimore County, Baltimore, MD, USA.
[*]Correspondence: H. P. de Koning, Institute of Biomedical and Life Sciences, Division of Infection and Immunity, University of Glasgow, Glasgow G12 8QQ, UK.

DOI: 10.1081/NCN-200027660 1525-7770 (Print); 1532-2335 (Online)

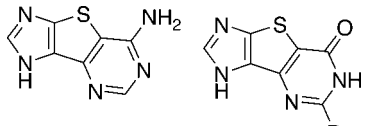
INTRODUCTION

Purine analogues have long been a rich source of chemotherapeutic agents. Modified purine nucleosides, as well as their corresponding purine heterobase moieties have provided a plethora of potent antiviral, anticancer and antiparasitic drugs. In the case of parasites, energy-dependent, highly selective membrane transporters exist which allow the purine analogues to specifically accumulate to high levels within the parasite.[1,2] The fact that nucleobase transporters are found at the blood brain barrier[3] is equally important, since trypanosomes infect the brain. The purine nucleobases offer a distinct advantage over their nucleoside counterparts since they are readily incorporated into the nucleotide pool by phosphoribosyltransferases while, in contrast, nucleosides may be rapidly hydrolyzed in the bloodstream forms of *T. brucei*. Currently, allopurinol is the only purine analogue used clinically against a member of the Trypanosomatidae, but our laboratories have recently developed a series of tricyclic purine analogues that display at least equal in vitro activity against *T. brucei* and appear to be selectively accumulated by the parasite. These tricyclic analogues represent a significant lead against human African trypanosomiasis.

MATERIALS AND METHODS

The synthesis of TRI-B-001, TRI-B-002 and TRI-B-003 (structures see Table 1) has been described previously.[4,5] Transport of [^{3}H]-hypoxanthine (Amersham, 32.0 Ci/mmol) by *T. b. brucei* s427 bloodstream forms grown in rats[1,2] and of [^{3}H]adenine (PerkinElmer Life Sciences, 24 Ci/mmol) by human erythrocytes[1] was performed exactly as described, and performed at 0.1 μM radiolabel. Assuming competitive inhibition, K_i values were calculated from $K_i = IC_{50}/(1 + ([L] + K_m))$ in which IC_{50} is the concentration giving 50% inhibition of permeant transport and L the permeant. The Alamar Blue™ (Trek Diagnostics, UK) assay[6] for trypanocidal action was performed as described,[1] using 10^4 procyclics/well in HMI9 medium with 20% Fetal Bovine Serum (Gibco).

Table 1. K_i and EC_{50} values of tricyclic nucleobases for TbH2 and hFNT1 (μM).



I II

	Structure	R	K_i for TbH2	K_i for hFNT1	S.I.	EC_{50}
TRI-B-001	I	–	0.38 ± 0.04	0.47 ± 0.21	1.2	155
TRI-B-002	II	H	1.6 ± 0.6	~1000	~1000	450
TRI-B-003	II	NH_2	0.70 ± 0.24	64 ± 11	91	243

All transport values are the average of 3–4 experiments and S.E.'s; Trypanocidal activity was the average of two very similar experiments. S.I., selectivity index, the fold higher affinity for TbH2 over hFNT1. EC_{50}, 50% effective concentration.

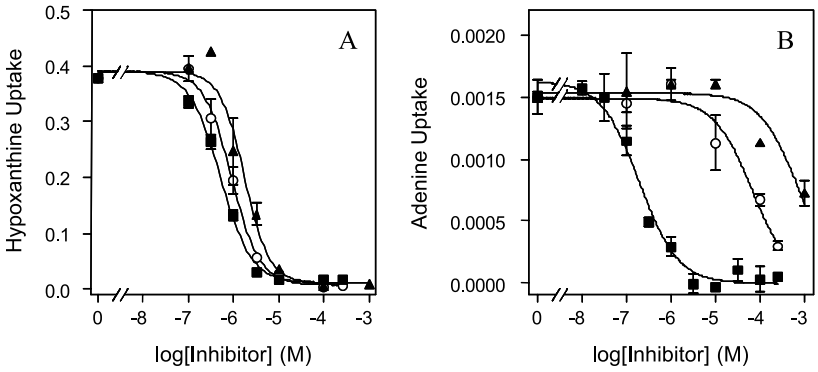


Figure 1. Inhibition of (A) transport of 0.1 μM [^{3}H]hypoxanthine by *T. brucei* bloodstream forms or (B) 0.1 μM [^{3}H]adenine by human erythrocytes by TRI-B-001 (■), TRI-B-002 (▲) or TRI-B-003 (○). When inhibition was incomplete due to the limits of compound solubility in aqueous buffer, curves were calculated assuming a Hill coefficient of −1 and an eventual 100% inhibition. Transport was expressed as pmol(10^7 cells)$^{-1}$s^{-1}.

RESULTS

The thieno-separated tricyclic nucleobases TRI-B-001, TRI-B-002 and TRI-B-003 all potently inhibited transport of 0.1 μM [^{3}H]hypoxanthine in *T. b. brucei* bloodstream forms (Fig. 1A). At this concentration of radiolabel, >90% of hypoxanthine transport is mediated by the H2 nucleobase transporter in these cells.[2] K_i values were typically between 0.2 and 2 μM (Table 1). Even so, the affinity of the tricyclic hypoxanthine and guanine analogues for the H2 transporter were less than for their naturally occurring counterparts,[2] by 13-and 2-fold, respectively. In contrast, the adenine analogue displayed >8-fold higher affinity for the transporter than adenine itself.

A similar pattern was apparent when the ability to inhibit the human facilitative nucleobase transporter (hFNT1) was assessed. The hypoxanthine and guanine tricyclic analogues were less efficient in inhibiting transport of 0.1 μM [^{3}H]adenine by human

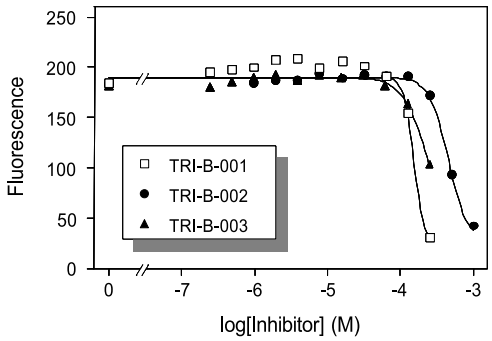


Figure 2. Alamar Blue assay showing trypanocidal action of tricyclic purine analogues.

erythrocytes than the parent compounds, whereas TRI-B-001 was a very potent inhibitor with a K_i value of 0.47 ± 0.21 μM (Fig. 1B). To the authors' knowledge this is by far the highest affinity reported to date for hFNT1 and compares with reported K_m values for adenine of 13 ± 1[7] or 16 ± 4[1] μM.

All three tricyclic bases dose-dependently inhibited the growth of bloodstream *T. b. brucei* in vitro (Fig. 2), with EC_{50} values between 150 and 450 μM (Table 1). These values are comparable to values obtained with the antiprotozoan hypoxanthine analogue allopurinol.[8]

DISCUSSION

Purine analogues have great potential as much-needed drugs against protozoan infections. Here we investigate whether tricyclic purine bases could be selectively accumulated by the parasite and display any trypanocidal action. We show that not only do all tricyclic bases tested display high affinity for the main *T. b. brucei* nucleobase transporter (indicating probable efficient transport) but that the affinity for the parasite transporter is up to 1000-fold higher than for the corresponding human transporter in erythrocytes. The level of trypanocidal activity is similar to that of allopurinol against *T. b. gambiense* and *T. b. rhodesiense*, but less than that of allopurinol against *Leishmania* species.[8] It is anticipated, however, that this activity can be increased while still retaining the selective uptake by fine-tuning various structural features on the tricyclic scaffold.

REFERENCES

1. Wallace, L.J.M.; Candlish, D.; De Koning, H.P. Different substrate recognition motifs of human and trypanosome nucleobase transporters: selective uptake of purine antimetabolites. J. Biol. Chem. **2002**, *277*, 26149–26156.
2. De Koning, H.P.; Jarvis, S.M. Purine nucleobase transport in bloodstream forms of *Trypanosoma brucei brucei* is mediated by two novel transporters. Mol. Biochem. Parasitol. **1997**, *89*, 245–258.
3. Washington, C.B.; Giacommini, K.M. Mechanisms of nucleobase transport in rabbit choroids plexus. J. Biol. Chem. **1995**, *270*, 22816–22819.
4. Seley, K.L.; Januszcyk, P.; Hagos, A.; Zhang, L.; Dransfield, D.T. Synthesis and antitumor activity of thieno-separated tricyclic purines. J. Med. Chem. **2000**, *43*, 4877–4883.
5. Seley, K.L. Tricyclic nucleosides revisited. In *Recent Advances in Nucleosides: Chemistry and Chemotherapy*; Chu, C.K., Ed.; Elsevier Science, 2002; 299–326.
6. Räz, B.; Iten, Y.; Grether-Bühler, R.; Brun, R. The Alamar Blue® assay to determine drug sensitivity of African trypanosomes (*T. b. rhodesiense* and *T. b. gambiense*) in vitro. Acta Trop. **1997**, *68*, 139–147.
7. Domin, B.A.; Mahoney, W.B.; Zimmerman, T.P. Purine nucleobase transport in human erythrocytes; reinvestigation with a novel ''inhibitor-stop'' assay. J. Biol. Chem. **1988**, *263*, 9276–9284.
8. Marr, J.J.; Berens, R.L. Pyrazolopyrimidine metabolism in the pathogenic trypanosomatidae. Mol. Biochem. Parasitol. **1983**, *7*, 339–356.