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3-(Oxazolo[4,5-*b*]pyridin-2-yl)anilides as a novel class of potent inhibitors for the kinetoplastid *Trypanosoma brucei*, the causative agent for human African trypanosomiasis

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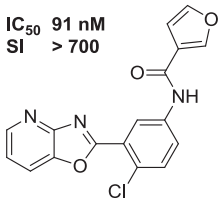
ABSTRACT

A whole organism high-throughput screen of approximately 87,000 compounds against *Trypanosoma brucei brucei* led to the recent discovery of several novel compound classes with low micromolar activity against this organism and without appreciable cytotoxicity to mammalian cells. Herein we report a structure–activity relationship (SAR) investigation around one of these hit classes, the 3-(oxazolo[4,5-*b*]pyridin-2-yl)anilides. Sharp SAR is revealed, with our most active compound (**5**) exhibiting an IC_{50} of 91 nM against the human pathogenic strain *T.b. rhodesiense* and being more than 700 times less toxic towards the L6 mammalian cell line. Physicochemical properties are attractive for many compounds in this series. For the most potent representatives, we show that solubility and metabolic stability are key parameters to target during future optimisation.



(**5**)

Abbreviations: BBB, blood–brain barrier; cPPB, chromatographic plasma protein binding; cytotox., cytotoxicity; DCM, dichloromethane; DMAP, *N*,*N*-dimethylaminopyridine; DMEM, Dulbecco's modified Eagle's medium; DMF, *N*,*N*-dimethylformamide; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; E_H, hepatic extraction; FCS, foetal calf serum; HAT, human African trypanosomiasis; HBTU, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; STPHI, Swiss Tropical and Public Health Institute; TBAB, tetrabutylammonium bromide; *L. donovani*, *Leishmania Donovani*; *P. falciparum*, *Plasmodium falciparum*; *T.b. brucei*, *Trypanosoma brucei brucei*; *T.b. gambiense*, *Trypanosoma brucei gambiense*; *T.b. rhodesiense*, *Trypanosoma brucei rhodesiense*; WHO, World Health Organisation.

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1. Introduction

Human African trypanosomiasis (HAT), more commonly known as sleeping sickness, is a vector-borne parasitic disease caused by infection of the host with either *Trypanosoma brucei gambiense* or *Trypanosoma brucei rhodesiense* [1]. The World Health Organisation (WHO) currently estimates that there are approximately 30,000 cases of HAT in Africa, which has a significant socioeconomic impact [2]. HAT is largely confined to the sub-Saharan continent where the vector, parasite and animal reservoirs co-exist [1]. The disease consists of two stages; the first involves the invasion of the haemolymphatic system by the parasite, and the second is associated with the transmission of the parasites across the blood–brain barrier (BBB) and into the central nervous system (CNS) [1]. Infection of the CNS leads to a number of symptoms including mental impairment, severe headaches, fever, chronic encephalopathy and eventual death. This stage of the disease is particularly in need of improved therapies [1].

There are a number of treatment options currently available for HAT, though none of them are ideal. Pentamidine and suramin are used to treat the first stage of HAT where *T.b. gambiense* and *T.b. rhodesiense* are the causative agents, respectively. However, neither of these drugs are able to cross the BBB making both ineffective against the second stage of HAT [3–5]. In addition, both treatments have significant side effects. Suramin has been linked to exfoliative dermatitis and renal failure [4], whilst pentamidine use is associated with diabetes mellitus and nephrotoxicity [6]. Melarsoprol is the primary treatment option available for second stage HAT being effective against both subspecies of trypanosome [6,7]. However, high failure rates have been reported even though resistance has not been proven [7]. Alternatively, eflornithine can also be used to treat second stage HAT [7]. It is a safer option but it is not effective against *T.b. rhodesiense* and the high costs may make it prohibitive [7]. Furthermore, administration requires four intravenous infusions daily for 14 days which is impractical in rural African facilities [8]. Recently nifurtimox has been introduced as a combination therapy with eflornithine (NECT) [8]. The combination therapy has the advantage of having a shorter and simplified regimen which has made it the current first line treatment for second stage HAT caused by *T.b. gambiense* [8]. Orally bioavailable oxaborole 6-carboxamides and orally active benzoxaboroles were identified and selected to enter pre-clinical studies in 2009 [9]. Also, fexinidazole has been progressed through phase 1 clinical trials and is currently recruiting for a phase 2 clinical trial [10]. However, there is still a great need for new trypanosomacidal compounds given the current chemotherapeutic options available, particularly for the CNS-resident second stage of this disease.

We recently screened approximately 87,000 compounds against *T.b. brucei* using an Alamar Blue® based 384-well viability assay [11]. Being the same species but just a different sub-species that is non-pathogenic to humans, *T.b. brucei* has a long history of useful and relevant application as a surrogate for *T.b. rhodesiense* and *T.b. gambiense* for early HAT drug discovery projects and comprises the mainstay of most primary animal models for HAT [12]. Indeed, *T.b. brucei* and *T.b. rhodesiense* differ in one gene – the serum resistance gene that allows *T.b. brucei* to survive in human serum – and there is recent strong evidence that *T.b. rhodesiense* is only a phenotypic variant of *T.b. brucei* [13]. Eight structurally unique compound classes were identified with IC_{50} values of less than 10 μM against *T.b. brucei*, that were more than ten times selective for *T.b. brucei* over the mammalian cell line HEK293, and had favourable physicochemical properties. One compound of particular interest was an oxazolopyridine derivative with an IC_{50} against *T.b. brucei* of 0.22 μM, shown in Fig. 1. This compound (**1**) had a moderately low molecular weight of 339.5, a reasonably low polar surface area of

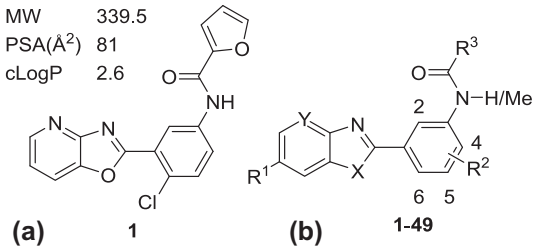

Fig. 1. (a) Structure and physicochemical profile of an initial screening hit; (b) numbering used herein.

81 Å^2 that could have potential for CNS penetration to treat stage two HAT [14], and an attractively low cLogP of 2.6.

Furthermore, testing against a wider panel of parasites confirmed potent activity not only against *T.b. rhodesiense* (IC_{50} 0.59 μM), but also against *Trypanosoma cruzi* (IC_{50} 0.23 μM). In both cases **1** was highly selective for these kinetoplastids compared with the L6 mammalian cell line, with respective selectivity indices of 39 and 99 (Table 1). Activity was significantly weaker against the unrelated protozoan *Plasmodium falciparum* (IC_{50} 7.8 μM), a major causative agent of malaria [15].

As such, **1** was of interest as a starting point for drug development resulting in the initiation of an SAR study. Herein we report our discovery of a new class of compounds that are potent and specific against *T.b. brucei* and *T.b. rhodesiense*, as well as *T. cruzi*, the causative agent of Chagas' disease [16].

2. Chemistry

After mining our database for analogues, it was determined that directed synthesis was required to establish a focused SAR investigation. We have recently discussed the database mining results elsewhere [11]. Investigations initially centred around the "northern" heterocyclic group. As shown in Scheme 1, the synthesis was relatively straightforward requiring a one-step cyclocondensation reaction between 2-amino-3-hydroxypyridine and a substituted 3-aminobenzoic acid, mediated by PPA, followed by amide bond formation between the amino group and the relevant heteroaryl carboxylic acid using EDCI and DMAP. For selected compounds, amide *N*-methylation (c) was undertaken. This yielded the target compounds, which are listed in Table 2 along with their IC_{50} values against *T.b. brucei*.

3. Results and discussion

Introduction of a 3-methyl group (**2**) into the furan ring leads to a slight loss of activity with an IC_{50} of 0.65 μM relative to an IC_{50} of 0.22 μM for **1** (Table 2). An isoxazole is less tolerated and **3** is more than 10-fold less active than **1** while the oxazole (**4**) is only slightly better than this with an IC_{50} of 1.5 μM. On the other hand, a 3-furyl group (**5**) is well tolerated with an IC_{50} of 0.3 μM. The thiazole (**6**) is about 10-fold less active than **1** while the corresponding pyrazole

Table 1
Biological activity[a] profile of **1** against a number of parasites.

Parasite	IC_{50} (μM)	SI
T.b. brucei	0.22	>345[b]
T.b. rhodesiense	0.59	39[c]
T. cruzi	0.23	99[c]
P. falciparum	7.8	–

[a] Values are the mean of 3 experiments, <±50%.
[b] Selectivity relative to HEK293 cells.
[c] Selectivity relative to L6 (rat skeletal myoblast) cells.