Assessment of a pretomanid analogue library for African trypanosomiasis: Hit-to-lead studies on 6-substituted 2-nitro-6,7-dihydro-5H-imidazo[2,1-b][1,3]thiazine 8-oxides

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A 900 compound nitroimidazole-based library derived from our pretomanid backup program with TB Alliance was screened for utility against human African trypanosomiasis (HAT) by the Drugs for Neglected Diseases initiative. Potent hits included 2-nitro-6,7-dihydro-5H-imidazo[2,1-b][1,3]thiazine 8-oxides, which surprisingly displayed good metabolic stability and excellent cell permeability. Following comprehensive mouse pharmacokinetic assessments on four hits and determination of the most active chiral form, a thiazine oxide counterpart of pretomanid (24) was identified as the best lead. With once daily oral dosing, this compound delivered complete cures in an acute infection mouse model of HAT and increased survival times in a stage 2 model, implying the need for more prolonged CNS exposure. In preliminary SAR findings, antitrypanosomal activity was reduced by removal of the benzylic methylene but enhanced through a phenylpyridine-based side chain, providing important direction for future studies.

Human African trypanosomiasis (HAT, also known as sleeping sickness) is a particularly lethal neglected tropical disease that is endemic in remote sub-Saharan Africa.1 HAT arises from infection by two subspecies of the kinetoplastid parasite Trypanosoma brucei (T. b. gambiense and T. b. rhodesiense), which are transmitted through the bite of tsetse flies.2 Because symptoms of the initial bloodstream stage are fairly mild and non-specific (e.g., headache, fever, weakness), the disease often progresses to the potentially fatal CNS stage characterised by neurological and psychiatric disorders before treatment is sought.1,3 However, there are pitifully few available drugs for late stage HAT, and all require hospitalization.3–5

The nitroimidazooxazine pretomanid (PA-824, 6) has demonstrated excellent bactericidal efficacy in phase II clinical studies for tuberculosis (TB), stimulating its further appraisal in new drug combination trials.10 Within a comprehensive backup program, in...
collaboration with the TB Alliance, we generated a library of more than 1000 compounds, whose assessment led to the advancement of a second generation TB candidate (TBA-354, 7) into phase I clinical evaluation. We recently disclosed that phenotypic screening of a second generation TB candidate (TBA-354, 7) for Neglected Diseases initiative (DNDi) unexpectedly enabled the discovery of DNDI-VL-2098 (8) as a preclinical lead for visceral leishmaniasis. Unfortunately, exhibited poor potency against T. b. brucei (IC[50] 53 μM) and it was reported that 6 also had weak activity versus this parasite (IC[50] 38 μM). However, unlike fenixinazole (4), 6 did not display cross-resistance to nifurtimox (3), indicating that it is not activated by the same type 1 nitroreductase employed by 3 and 4 (implying a different mechanism of action). Therefore, as part of a wider search for improved development candidates for HAT, ~900 analogues of 6 were screened by DNDI and several promising hits were unearthed. Herein, we reveal initial in vitro/in vivo profiling data on these hits, and findings from a preliminary SAR study of a nitroimidazothiazine oxide lead.

Medium-throughput screening and follow-up IC[50] testing at Scynexis[14] identified 48 active hits (IC[50] < 3 μg/mL), of which 19 were initially considered to be of potential interest (mean IC[50] < 1 μg/mL, with selectivity index > 10). Intriguingly, the most active compounds (9–12; Table 1) were either 2-nitroimidazothiazine oxides[15] or 6-nitroimidazothiazole oxides,[16] but a wide variety of other structures, including extended side chain analogues of 6, featured in this set. Since good CNS penetration is a critical requirement for the effective treatment of stage 2 HAT,[9] the 10 most potent hits were first evaluated for cell permeability in the MDCK-MDR1 assay. In this test system, apparent permeability (Papp) values >150 nm/s are indicative of high brain penetration potential provided that the transport is not affected by P-gp inhibition[17] (necessitating an absorption quotient in the range ~0.1 to 0.1). Unsurprisingly, the compound with a triaryl side chain (13, MW > 500) lacked any significant permeability (Papp < 0.8 nm/s), while four others (10, 14, 16, and 18)[15,18,19] gave only modest permeability values (Papp < 150 nm/s) and were suggested to be P-gp substrates (absolute AQs ≥ 0.3).[20] On the basis of results from this training set, additional hits were selected for assessment (19–23)[15,16,19] and, pleasingly, all of these demonstrated a high propensity to cross the blood-brain barrier.

In order to determine the suitability of the more permeable hits for in vivo efficacy studies, we first measured their aqueous solubilities, and their tendencies to metabolise, following a 1 h incubation with CD-1 mouse liver S9 subcellular fractions. Here, the most poorly soluble compounds (11, 15, 20, and 23) were also found to be the least stable, displaying half-lives of less than 70 min. Overall, the 2-nitroimidazothiazine oxides 9, 12 and 19, together with the 6-amino-linked analogue of 6 (17),[21] provided the best balance of potency, stability, aqueous solubility and CNS penetration potential. This led us to probe their in vivo pharmacokinetic (PK) profiles in mice, examining concentration levels in plasma, whole blood and brain tissue following both intravenous and oral administration (Table 2; for further experimental details, see the Supporting Information).

The most potent hit (9) exhibited an unacceptable PK profile, giving inadequate oral exposure and poor oral bioavailability (<1.5%), consistent with both its low solubility (causing unsatisfactory absorption) and more rapid metabolism. This was unsurprising, as the 4-benzylxoybenzyl analogue of 6 was known to be markedly inferior to 6 against Mycobacterium tuberculosis in vivo, despite being an order of magnitude more potent than 6 in vitro, due to similar PK issues.[22] In contrast, the 4-trifluoromethoxybenzyl congener of 9 (12) demonstrated the slowest rate of clearance of the four, and a prolonged, high exposure level above the MIC following oral dosing (Fig. 2), with good oral bioavailability (52–55%) at all three sampling sites. Moreover, the high brain/plasma concentration ratio (~3:2) presented by 12 was encouraging for CNS uptake, as required in the treatment of stage 2 HAT.[14] The sulfone derivative of 12 (19), which was produced to a significant extent in PK samples from the analysis of 12, showed reduced oral exposure, in accordance with its inferior solubility and faster rate of clearance. Given its weaker potency (5.6-fold vs 12), these results for 19 were not predictive of good in vivo activity, thus in situ oxidation of 12 should have a minimal contribution to efficacy. Finally, the most soluble hit 17 (the 6-amino analogue of 6) was notable for having the best oral bioavailability, with excellent concentration levels observed in brain tissue (2- to 3-fold higher than in plasma). However, this compound also suffered from a high rate of clearance and a rather short oral half-life (1.2–1.5 h), leading to inadequate exposure above the MIC beyond ~2 h. These latter results mirrored findings from a recently reported PK-PD study of analogues of 6 against TB, in which 17 displayed a 1.3 h oral half-life in mouse lung tissue (in comparison to 4.8 h for 6), effectively precluding useful in vivo activity. Hence, of the four most promising hits, only the 2-nitroimidazothiazine oxide 12 proved to be suitable for efficacy assessment in the acute infection mouse model of HAT.

One remaining matter to resolve with racemic hit 12 was which of the four possible stereoisomers was the most active chiral form. This issue was partially clarified through a better optimised resynthesis of 12 (Scheme 1). Following side chain attachment to the racemic alcohol 42[13] (93% yield), careful oxidation of thiazine 30 with fresh m-CPBA (1.01 equiv) led to a separable mixture of 12 (75%) and a previously unidentified more polar racemic diastereomer 38 (20%) (for experimental details, see the Supporting Information).

The [1H] NMR spectra of 12 and 38 showed pronounced chemical shift differences for the H-6 resonance in particular, which was ~0.4 ppm further downfield in the spectrum of 12. The sulfoxide oxygen in six-membered rings is known to exhibit an axial preference, such that the deshielding effect of the sulfoxide group on axial α-hydrogen atoms has been used to assign relative stereochemistry.[24] Hence, 12 is postulated to have the sulfoxide oxygen and H-6 in a pseudo-diaxial orientation, placing the (4-OCF3)ben-
zyloxy side chain at C-6 in a 1.3-trans relationship to the sulfoxide oxygen. This assignment is supported by the diastereomer ratio (3.5:1) in favour of 12, which might be rationalised by an expected preference for the C-6 side chain to adopt a pseudoaxial conformation in the thiazine precursor 30 (based on the crystal structure of 6), providing a steric disincentive to formation of the cis sulfoxide 38.

Preparative chiral SFC separation of the enantiomers of 12 (24 and 26) and 38 (25 and 27) facilitated the assessment of all four stereoisomers (Table 3). The C-6 configuration of 12 (24 and 26) was later firmly established via a known chiral synthesis. The most active (6S) form 24 (IC_{50} 0.07 µg/mL) was 40-fold more potent than cis isomer 25, and more than 70-fold more potent than its (6R) enantiomer 26. This level of potency compared well with data reported for 5 (IC_{50} 0.29 µg/mL vs T. b. brucei 427) in the same Scynexis assay. Compound 24 also displayed an improved selectivity index (>143), good aqueous solubility (106 µg/mL), and excellent metabolic stability following a 1 h exposure to human and mouse liver microsomes (respectively, 82% and 96% parent remaining).

Therefore, 24 was examined in a stage 1 HAT mouse model. Briefly, dosing was orally once daily for four days, starting 24 h postinfection, and parasitemia was assessed weekly via tail vein blood smears (see the Supporting Information). Excellent activity was observed (Table 4), with 24 providing complete cures (i.e. parasite free blood smears after >30 days) to all mice at doses as low as 5 mg/kg, similar to the control drug pentamidine (given i.p. at 2 mg/kg), whereas the vehicle only mice died on day 7. The efficacy seen with 24 in this model was equivalent to the level of activity reported for 5 and ~20-fold superior to the results described for fexinidazole (4), stimulating further evaluation of this lead in a stage 2 HAT mouse model. Here, oral dosing of 24 (at 12.5 to...
50 mg/kg once daily for seven days from day 21 postinfection) led to significant increases in survival times in comparison to untreated controls (66–70 days vs 31 days; Table 5), although cure rates were inadequate (0–20%). In contrast, 5 was 100% curative in the same CNS model at a dosage of 25 mg/kg once daily for 7 days. 9 while 4 gave an 88% cure rate in a comparable model when administered orally at 200 mg/kg once daily for 5 days. 8

Table 2

Mouse pharmacokinetic parameters for selected compounds.

| Compd | Intravenous (0.5–3 mg/kg) | Oral (50–80 mg/kg) |
|-------|---------------------------|-------------------|
|       | CL (L/h/kg) | Vdss (L/kg) | t1/2 (h) | AUCint (µg h/mL) | Cmax (µg/mL) | Tmax (h) | t1/2 (h) | AUCint (µg h/mL) | F (%) |
| 9     | 0.97 | 0.59 | 0.43 | 0.505 | 0.20 | 2 | 3.7 | 0.869 | 1.4 |
| 12    | 0.52 | 1.6 | 2.5 | 2.09 | 9.3 | 4 | 6.9 | 51.9 | 55 |
| 17    | 6.8  | 4.7 | 0.48 | 0.418 | 12 | 1 | 1.2 | 20.5 | 100 |
| 19    | 1.0  | 3.6 | 2.5 | 1.80 | 2.3 | 2 | 2.7 | 18.7 | 42 |
| Plasma |              |                  |          |          |          |       |       |         |       |
| Whole blood |              |                  |          |          |          |       |       |         |       |
| Brain  |              |                  |          |          |          |       |       |         |       |
| 9     | 0.87 | 0.33 | 0.42 | 0.489 | 0.11 | 2 | 5.3 | 0.494 | 0.8 |
| 12    | 0.39 | 1.7 | 9.3 | 2.70 | 8.6 | 4 | 5.8 | 63.5 | 52 |
| 17    | 2.4  | 8.1 | 9.3 | 1.10 | 26 | 0.5 | 1.5 | 42.2 | 100 |
| 19    | 0.69 | 2.8 | 5.9 | 2.60 | 3.0 | 2 | 3.3 | 23.7 | 37 |
| a The corrected intravenous doses for 9, 12, 17 and 19 were 0.5, 1.1, 2.9 and 2.0 mg/kg, respectively, and the corresponding oral doses were 62, 50, 78 and 49 mg/kg, respectively.
| b Oral bioavailability, determined using dose normalised AUCint values.

Fig. 2. Time vs concentration curves for 12, following administration to male CD-1 mice (at 50 mg/kg po and 1.1 mg/kg iv). The horizontal line represents the MIC for complete inhibition of visible parasite growth in vitro.

Scheme 1. Reagents and conditions: (i) 4-OCF3Br, NaH, DMF, 20 °C, 160 min (93%); (ii) m-CF3Br, NaH, THF, 0 °C, 2 h (73%, 2 steps); (iii) preparative chiral SFC (see text).

Scheme 2. Mitsunobu coupling of the orthogonally diprotected triol 41 with 4-(trifluoromethyl)phenol and conversion of the product 45 to iodide 47 (via successive hydrolysis of the benzyl ether and iodination using I2/PPh3/imidazole) set the stage for the preparation of phenyl ether 28 (Scheme 2A). Thus, base-assisted alkylation of 2-chloro-4-nitromidazole with iodide 47, followed by desilylation (TBAF), provided the key alcohol 49 (73%, 2 steps). Then, reaction of the tosylate derivative of 49 with the lithium salt of triisopropylsilanethiol and treatment of the crude product with TBAF enabled cyclisation to thiazine 28 (31%). Finally, careful oxidation of 28 with fresh m-CF3Br (1.2
detailed in vivo studies in the benzoaborole 6-carboxamide class have revealed that efficacy in the CNS model is heavily dependent upon the maintenance of drug concentrations in the brain for at least 15 h at levels above the MIC (defined as the lowest compound concentration that completely inhibits visible parasite growth in vitro after a 72 h incubation). Thus, a more potent analogue of 5 without the gem-dimethyl group (SCYX-6759) required an oral dosing regimen of 50 mg/kg twice daily (b.i.d.) in order to obtain an 83% cure rate of the CNS infection, due to the shorter time that its brain concentration level was at or above the MIC (∼12 h vs ∼24 h for 5 at 25 mg/kg). These findings imply that a similar oral dosing regimen of 50 mg/kg b.i.d. might be required to achieve useful efficacy for 24 in the stage 2 HAT model (via more prolonged CNS exposure). Nevertheless, these initial in vivo results with 24 were still regarded as encouraging, and indicated that 2-nitro-6,7-dihydro-5H-imidazo[2,1-b][1,3]thiazine 8-oxides merited further investigation as potential treatments for HAT. Specifically, as illustrated with benzoaboroles, we considered the possibility of designing new analogues of 24 having improved potency and extended CNS exposure. On the basis of the results above and insights from previous SAR studies directed at developing a backup TB candidate to the structurally related nitroimidazooxazine 6, 19,29 we devised two preliminary strategies for optimisation of the side chain of 24: a) removal of the benzylic methylene group and b) insertion of a proximal pyridine ring (cf. 7). Notably, both strategies had the potential to improve metabolic stability, leading to longer in vivo half-lives and better exposure levels. Furthermore, to mitigate any reduction in solubility with the first approach, we also proposed the preparation of a trifluoromethylpyridinyl ether analogue (cf. 21 and 22).

The synthetic methods employed to prepare the new nitroimidazothiazine derivatives 28, 29, 32–37, 39–41 are outlined in Scheme 2. Mitsunobu coupling of the orthogonally diprotected triol 41 with 4-(trifluoromethyl)phenol and conversion of the product 45 to iodide 47 (via successive hydrolysis of the benzyl ether and iodination using I2/PPh3/imidazole) set the stage for the preparation of phenyl ether 28 (Scheme 2A). Thus, base-assisted alkylation of 2-chloro-4-nitromidazole with iodide 47, followed by desilylation (TBAF), provided the key alcohol 49 (73%, 2 steps). Then, reaction of the tosylate derivative of 49 with the lithium salt of triisopropylsilanethiol and treatment of the crude product with TBAF enabled cyclisation to thiazine 28 (31%). Finally, careful oxidation of 28 with fresh m-CF3Br (1.2
equiv) led to a separable mixture of sulfone 40 (11%) and the diastereomeric sulfoxides 33 and 36 (82% and 2%), where the sizeable diastereomer ratio (dr/)C24 34:1) was in accordance with the greater steric hindrance induced by this phenoxy side chain. Thiazine pyridinyl ether 29 was more directly accessed via a sodium hydride-induced SNAr reaction of thiazine alcohol 42 with 2-chloro-5-trifluoromethylpyridine (52) (69%; Scheme 2B), while alternative alkylation of 42 with 5-bromo-2-(bromomethyl)pyridine (53), followed by Suzuki coupling with 4-(trifluoromethoxy)phenylboronic acid, furnished the extended side chain thiazine 32 (35% over 2 steps; Scheme 2C). However, whereas m-CPBA oxidation of 29 proved straightforward, similar oxidation of 32 was complicated by the formation of smaller amounts of pyridine N-oxide derivatives, such that only the sulfoxides 35 and 39 (55% and 8%) could be obtained. All new compounds (Table 6) were characterised by 1H NMR, MS, melting point, and combustion analysis (or HRMS and HPLC); full synthetic procedures and characterisation data have been provided in the Supporting Information.

Table 3

| Compd | IC50 (μg/mL) | Microsomesb (% remaining at 1 h) |
|-------|-------------|----------------------------------|
|       | T. b. brucei | L929 | Human | Mouse |
| 24    | 0.070 ± 0.005 | >10 | 82 | 96 |
| 25    | 2.8 ± 0.4     | >10 | 93 | 93 |
| 26    | >5           | >10 | 91 | 26 |
| 27    | >5           | >10 | 88 | 89 |

a IC50 values for inhibition of the growth of T. b. brucei 427 or for cytotoxicity toward L929 mouse fibroblasts. Each value is the mean of 2 independent determinations ± standard deviation.
b Pooled human or CD-1 mouse liver microsomes.

Table 4

| Compd | Dosagea (mg/kg) | Mean survival (days) | Cured/Total Cured (%) |
|-------|----------------|----------------------|-----------------------|
| 24    | 50             | >30                  | 5/5 (100)             |
| 24    | 25             | >30                  | 5/5 (100)             |
| 24    | 12.5           | >30                  | 5/5 (100)             |
| 24    | 5              | >30                  | 4/4 (100)             |
| 24    | 2.5            | >30                  | 1/4 (25)              |
| Pentamidine | 2            | >30                  | 7 (0/3)              |

a Dosing of 24 was orally, once daily for 4 days consecutively, while pentamidine was dosed i.p. once daily for the same period.
b Vehicle for 24: 0.8% CMC, 0.1% SDS in water.

Table 5

| Compd | Dosagea (mg/kg) | Mean relapse time (days) | Cured/Total Cured (%) |
|-------|----------------|--------------------------|-----------------------|
| 24    | 50             | 66                       | 0/10                 |
| 24    | 25             | 70                       | 2/10 (20)            |
| 24    | 12.5           | 45                       | 0/8 (0)              |
| 24    | 2.5            | 13                       | 1/4 (25)             |
| 24    | 1.25           | 7.5                      | 0/4 (0)              |
| 24    | 1.25           | 7.5                      | 0/4 (0)              |
| 24    | 1.25           | 7.5                      | 0/4 (0)              |
| Berenil | 10 (D4)     | 5/5                      | 100%                |
| Berenil | 10 (D21)    | 41                       | 0/5 (0)              |
| Vehicle | 31            | 5/5                      | 100%                |
| Vehicle | 31            | 5/5                      | 100%                |

a Dosing of 24 was orally, once daily for 7 d consecutively, starting on day 21 postinfection.
b Single i.p. dose on day 4 or day 21.
c Vehicle for 24: 0.8% CMC, 0.1% SDS in water.
The new compounds and relevant comparators were screened at the University of Antwerp against a panel of four protozoan parasites (T. b. brucei, T. b. rhodesiense, T. cruzi, and L. infantum); cytotoxic effects on human lung fibroblasts (MRC-5 cells, the host for T. cruzi) were also assessed. In all cases, recorded data (Table 6) are mean values derived from two or more independent experiments. For the parent thiazines (28–32), antitrypanosomal potency was enhanced by an order of magnitude with biaryl side chains (d and e), and this SAR pattern was maintained for the considerably less lipophilic major sulfoxide disastereoisomers (Ba-e), where was the most impressive new HAT lead (T. b. brucei IC₅₀ 0.030 µM). This lead was also highly effective against Chagas disease (T. cruzi IC₅₀ 0.067 µM) and was the only compound to display submicromolar antileishmanial activity (L. inf IC₅₀ 0.41 µM). In contrast, shorter linked aryl ether sulfoxides 33 and 34 were 4- to 6-fold less potent than the initial hit 12 against T. b. brucei, while their sulfone derivatives (40 and 41) were an order of magnitude inferior to sulfone 19, indicating that the original (OCH₂) linkage was best. In comparison to 12 (the racemic form of lead 24), racemic sulfoxide 35 displayed a 9-fold greater potency against T. b. brucei, an 11-fold higher potency against T. b. rhodesiense, and a 2.4-fold better selectivity index (MRC-5 IC₅₀ > 500 times larger than the HAT IC₅₀). Compound 35 also demonstrated acceptable aqueous solubility (9.9 µg/mL at pH 7 and 1260 µg/mL at pH 1), high permeability potential without P-gp mediated efflux (MDCK-MDR1 cell Pₐₙₕ A/B 117/182 nm/s cf. Pₐₙₕ A/B of 197 nm/s for the CNS positive drug propranolol in the same assay) and very good stability toward human and mouse liver microsomes (respectively, 78% and 72% parent remaining after 1 h). While we have not yet had the opportunity to evaluate 35 beyond this stage, these promising results certainly point to the viability of this SAR approach to provide useful new HAT leads.

In summary, this investigation set out to evaluate a nitroimidazole-based compound library related to pretomanid for possible utility against HAT. Although the hit rate was low (~2%), several compounds displayed good metabolic stability, adequate solubility, and excellent CNS penetration potential. Comprehensive mouse pharmacokinetic studies of three oxidised nitroimidazothiazines and a 6-amino-linked analogue of pretomanid identified the racemic thiazine oxide 12 as a suitable candidate for in vivo efficacy studies. The most potent stereoisomer of 12 (24) was indeed highly efficacious in the stage 1 HAT mouse model with once daily oral dosing (similar to oxaborole 5), but was less effective in a stage 2 model. While it seemed reasonable to speculate that more frequent dosing with 24 should achieve better outcomes in this latter model, we also envisaged the generation of new analogues with higher potency and longer half-lives. In preliminary SAR work, we noted that removal of the benzylic methylene was...
disfavoured but that adding a proximal pyridine ring (35) enhanced potency while broadly retaining other essential properties. These additional findings are very encouraging and provide a rational foundation for further development of this interesting class of antitrypanosomal agents.

Acknowledgments

The authors thank the Drugs for Neglected Diseases initiative for financial support through a collaborative research agreement. For this project, DNDi received financial support from the following donors: Department for International Development (DFID), UK; Federal Ministry of Education and Research (BMBF), through KfW Germany; Directorate-General for International Cooperation (DGIS), The Netherlands; Bill & Melinda Gates Foundation (BMGF), USA; Médecins Sans Frontières (MSF), International. The donors had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The authors also thank Dr Bakela Nare and Ms Tana Bowling (Scynexis) for IC50 data, Sisira Kumara (ACSRC) for some solubility measurements, and Donna Sarno, Elena Mejia and Wendy Becker (Pace University) for technical assistance.

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmcl.2017.10.067. These data include MOL files and InChiKeys of the most important compounds described in this article.

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