6-Nitro-2,3-dihydroimidazo[2,1-*b*][1,3]thiazoles: facile synthesis and comparative appraisal against tuberculosis and neglected tropical diseases

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As part of a quest for backups to the antitubercular drug pretomanid (PA-824), we investigated the unexplored 6-nitro-2,3-dihydroimidazo[2,1-*b*][1,3]-thiazoles and related -oxazoles. The nitroimidazothiazoles were prepared in high yield from 2-bromo-4-nitroimidazole via heating with substituted thiiranes and diisopropylethylamine. Equivalent examples of these two structural classes provided broadly comparable MICs, with 2-methyl substitution and extended aryloxymethyl side chains preferred; albeit, *S*-oxidised thiazoles were ineffective for tuberculosis. Favourable microsomal stability data for a biaryl thiazole (**45**) led to its assessment in an acute *Mycobacterium tuberculosis* mouse model, alongside the corresponding oxazole (**48**), but the latter proved to be more efficacious. *In vitro* screening against kinetoplastid diseases revealed that nitroimidazothiazoles were inactive versus leishmaniasis but showed interesting activity, superior to that of the nitroimidazooxazoles, against Chagas disease. Overall, “thio-delamanid” (**49**) is regarded as the best lead.

More than 2 billion people are latently infected with *Mycobacterium tuberculosis* (*M. tb*) and up to 10% of these will develop active tuberculosis (TB).[1] While renewed efforts over the last two decades have reduced the TB mortality rate in 1990 by an impressive 45%, an enormous global disease burden remains (10.4 million new cases in 2015, with 1.4 million deaths).[2] Synergy with HIV/AIDS and the ongoing spread of often undiagnosed multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB undergird this major epidemic.[2-4] Current treatment regimens are complex, lengthy and toxic, yet for almost half a century no new therapies were developed.[4-6] Very recently though, two new highly lipophilic drugs have been approved for treating MDR-TB: the diarylquinoline bedaquiline and delamanid (**1**, see Fig. 1).[6,7] Pretomanid (PA-824, **2**) has also progressed to phase II/III clinical trials within various new drug regimens having the potential to shorten and simplify therapy for all forms of TB.[2,7]

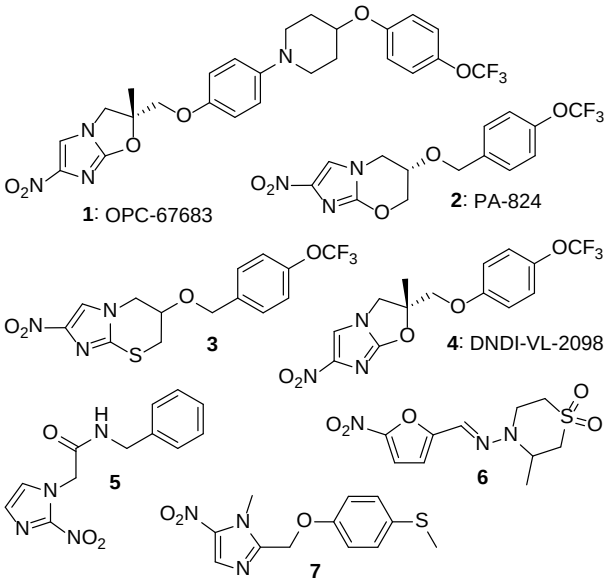


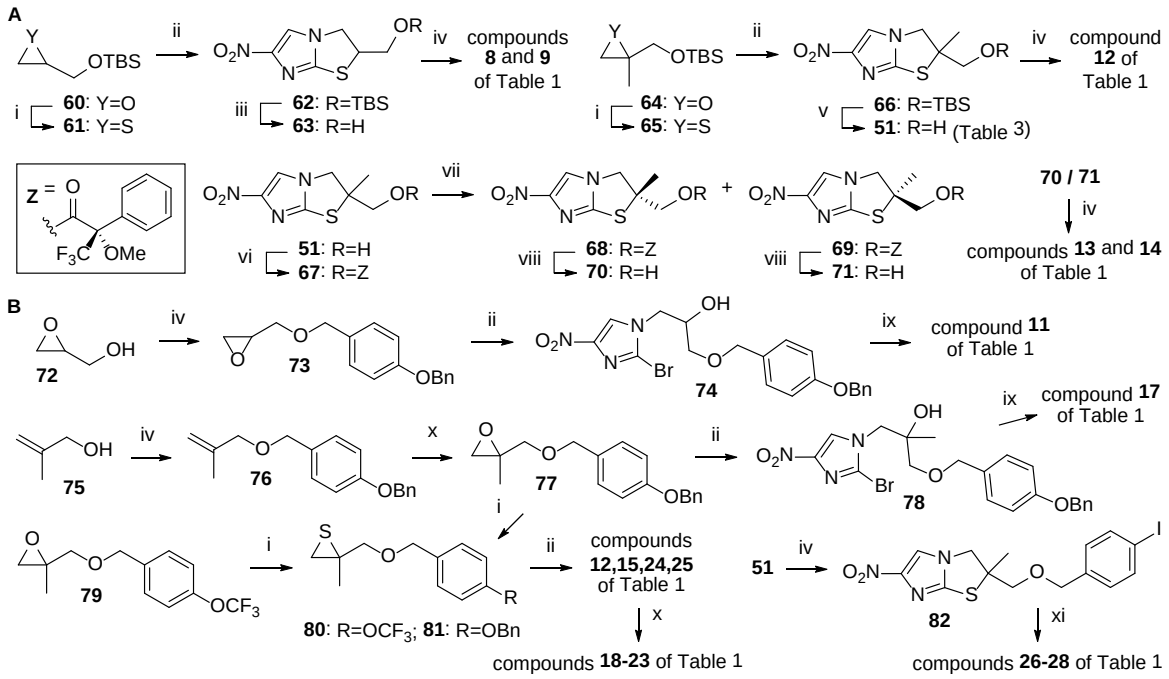
Figure 1. Nitro drugs/leads for TB, leishmaniasis and Chagas disease

In collaboration with the TB Alliance, we initially investigated new nitroheterobicyclic ring analogues of **2**, seeking a novel active scaffold upon which to develop a backup series.[8] However, amongst the fused 5/6 ring systems examined, only nitroimidazothiazines (e.g., **3**) retained significant potency, pointing to the critical importance of the nitroimidazole moiety and its interesting ring reduction chemistry.[8,9] While **3** was metabolised into inactive oxides too rapidly to be a useful lead (*vide infra*), we postulated that novel nitroimidazothiazole analogues of the known nitroimidazooxazole class[10] (*cf.* **1**) might be significantly more stable due to steric factors, and therefore worthy of assessment, as described herein. For comparative purposes, we also elected to evaluate corresponding nitroimidazooxazoles. Our broad strategy was to explore both monoaryl and biaryl side chains, due to the markedly better potency and *in vivo* efficacy of biaryl analogues of **2** (which ultimately became our major focus for backup studies).[11,12] Since this work was performed, Mugunthan[13] reported the synthesis of two sugar-derived 6-nitro-2,3-dihydroimidazo[2,1-*b*][1,3]thiazoles and their oxazole counterparts as anti-TB agents, but these compounds had poor activities in comparison to **2**, and no further examples have been described to date.

Neglected tropical diseases (NTDs) caused by pathogens such as viruses, bacteria, protozoa and helminths affect one-sixth of the world's population, resulting in enormous suffering and more than half a million deaths annually, yet there is an extreme paucity of safe, effective and

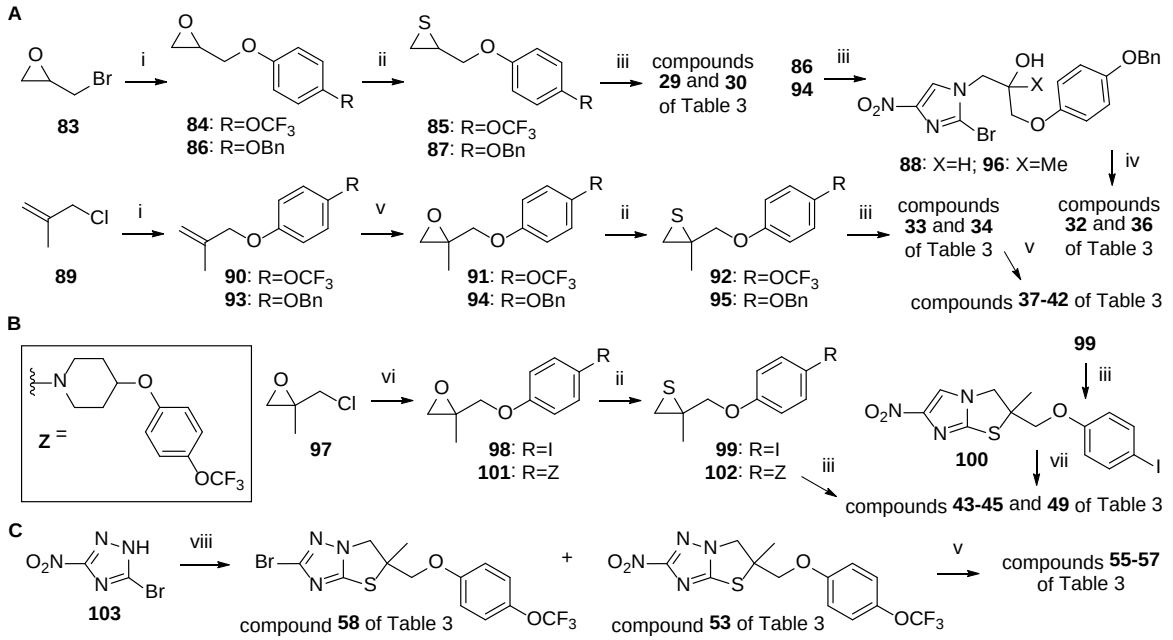
affordable medications for their treatment, and the drug pipelines are almost empty.[14,15] We have recently recounted[16] that phenotypic screening of selected nitroimidazole derivatives (above) against kinetoplastid diseases by the Drugs for Neglected Diseases *initiative* (DND*i*) unexpectedly led to discovery of the outstanding activity of **4** in mouse and hamster models of visceral leishmaniasis (VL) and then to its selection as a preclinical candidate. As part of our backup studies with DND*i*, we elucidated SARs for nitroimidazooxazoles against 3 NTDs, identifying efficacious new leads for VL, as well as novel hits for Chagas disease.[16] Current therapeutic options for Chagas disease are limited to two drugs (benznidazole **5** and nifurtimox **6**) that can cause serious side effects and have reduced efficacy in chronic cases.[17] A phase II clinical study of fexinidazole (**7**: an investigational drug for human African trypanosomiasis[18]) in chronic Chagas patients was interrupted due to safety and tolerability issues,[19] reinforcing the urgent need for new treatments.[15] Therefore, as an extension to the present study, we tested our new analogues in several anti-parasitic assays, seeking innovative leads for the development of oral agents to combat these NTDs.

The novel 6-nitro-2,3-dihydroimidazo[2,1-*b*][1,3]thiazoles ("thiazoles") were readily accessible in a single step, via the base-assisted solventless reaction (DIPEA, 108 °C) of 2-bromo-4-nitroimidazole with appropriately substituted thiiranes (Scheme 1). The latter were conveniently obtained in generally high yield from the corresponding epoxides, following treatment with thiourea on silica gel.[20] Hence, compounds with benzyl ether side chains (**8**, **9**, and **12**, *cf*. **2**) were initially achieved by starting from TBS-protected glycidol (**60**), or its 2-methyl derivative (**64**)[21] and elaborating to the thiazole alcohols **63** and **51** (Scheme 1A).



Scheme 1. Reagents and conditions: (i) thiourea/silica gel, CH_2Cl_2, 20 °C, 2.5-3 h (37-94%); (ii) 2-bromo-4-nitroimidazole, DIPEA, 108-109 °C, 12-21 h (73-95%); (iii) 1% HCl in 95% EtOH, 20 °C, 7 h (100%); (iv) 4-OCF_3BnBr or 4-BnOBnI or 4-BnOBnCl or 4-IBnBr, NaH, DMF, 0-20 °C, 0.7-16 h (32-93%); (v) TBAF, THF, 20 °C, 4 h (96%); (vi) (*R*)-(-)-MTPA-Cl, DMAP, pyridine, 20 °C, 6 h (99%); (vii) preparative chiral HPLC (CHIRALCEL OD, 40% *i*PrOH/hexane) (40-42%); (viii) K_2CO_3, aq MeOH, 20 °C, 3-3.5 h (100%); (ix) NaH, DMF, 0 °C, 80 min (88-93%); (x) *m*-CPBA, Na_2HPO_4, CH_2Cl_2, 0-20 °C, 2.5-22 h (10-97%); (xi) $ArB(OH)_2$, toluene, EtOH, 2 M Na_2CO_3, $Pd(dppf)Cl_2$ under N_2, 90 °C, 1 h (67-74%).

The enantiomers of **12** were also regarded as useful early TB targets, but we were not persuaded that any of the reported methods for thiirane synthesis could ensure products of sufficiently high optical purity.[20] Therefore, racemic alcohol **51** was derivatized to **67** (using Mosher's acid chloride, *R* form), and then the diastereomeric esters (**68** and **69**) were separated by chiral preparative HPLC and hydrolysed, leading to **13** and **14**. The absolute configurations of these were deduced from their assay results against *M. tb* in comparison to data for known nitroimidazooxazoles,[16] as assignment based on the ^{1}H NMR spectra of Mosher esters derived from β-chiral primary alcohols is not regarded as definitive.[22] For the larger scale preparation of **12** and **15**, benzyl ether substituted thiiranes **80** and **81** were synthesised (Scheme 1B). Reaction of these with 2-bromo-4-nitroimidazole also gave small amounts (2%) of 5-nitro isomers **24** and **25**, while reaction of 2-bromo-4-nitroimidazole with related epoxides, followed by NaH-assisted ring closure of the alcohol intermediates at 0 °C,[16] provided the nitroimidazooxazoles (**10**, **11**, **16**, and **17**). *S*-Oxidation of **12** and **15** with *m*-CPBA (3-4 equiv) yielded mixtures of the sulfoxide and sulfone derivatives, which were separated by chromatography on silica gel to give **18**-**23**. Finally, analogues with a biaryl side chain (**26**-**28**) were readily obtained via Suzuki couplings on the iodobenzyl ether **82**.

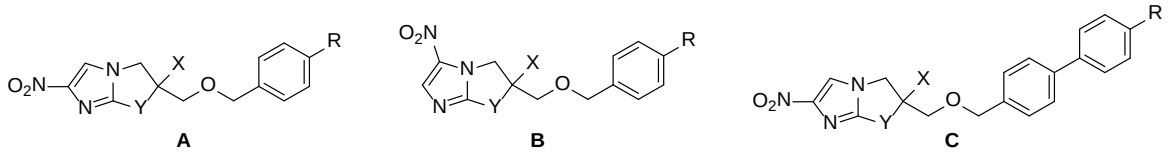


Scheme 2. Reagents and conditions: (i) RPhOH, K_2CO_3, acetone, 58-60 °C, 24-41 h (68-95%); (ii) thiourea/silica gel, CH_2Cl_2, 20 °C, 2-5 h (64-89%); (iii) 2-bromo-4-nitroimidazole, DIPEA, 105-108 °C, 3-16 h (65-91%); (iv) NaH, DMF, 0 °C, 40-50 min (92-94%); (v) *m*-CPBA, Na_2HPO_4, CH_2Cl_2, 0-20 °C, 3.5-70 h (19-93%); (vi) RPhOH, K_2CO_3, NaI, DMF, 72-82 °C, 1-2.5 d (49-73%); (vii) $ArB(OH)_2$, toluene, EtOH, 2 M Na_2CO_3, $Pd(dppf)Cl_2$ under N_2, 90 °C, 1.3-3 h (52-67%); (viii) **92**, DIPEA, 105 °C, 14 h (17-50%).

Phenyl ether substituted thiazoles (and oxazole congeners) were similarly accessed from appropriately substituted epoxides[16,23,24] (Scheme 2A). The latter approach became necessary when attempts to form **29** and **33** from the alcohols **63** and **51** via Mitsunobu reactions (or from the mesylate derivative of **51** by reaction with the phenol in K_2CO_3/DMF) proved too low-yielding, due to either facile elimination to an exocyclic alkene (in the case of **63**) or the competitive formation of other close-running side products (for **51**). Of note, a more direct route to the epoxides **98** and **101** (Scheme 2B), starting from commercial 2-(chloromethyl)-2-methyloxirane (**97**), allowed a critical non-oxidative synthesis of the latter (which contained a

sensitive tertiary amine). Unexpectedly, Suzuki couplings on thiazole **100** (to give **43**-**45**) proved to be particularly difficult, requiring higher catalyst loadings and longer reaction times for completion (consistent with catalyst poisoning by sulfur); however, it is unclear why this complication was more evident with this shorter side chain length. Lastly, an alternative base-assisted reaction of thiirane **92** with 5-bromo-3-nitro-1,2,4-triazole (**103**) (DIPEA, 105 °C) resulted in a separable 3:1 mixture of nitro and bromo products (**53** and **58**) and oxidation of **53** with *m*-CPBA (1.8 equiv) provided the remaining targets (**55**-**57**). All new compounds were characterised by ^{1}H NMR, MS, melting point, and combustion analysis (or HRMS and HPLC); full synthetic procedures and characterisation data for several representative examples have been provided in the Supporting Information.

Table 1. *In vitro* antitubercular and antiparasitic activities of benzyloxymethyl-substituted nitroimidazothiazoles and nitroimidazooxazoles



A B C

					MIC[a,b] (μM)			IC$_{50}$[c,b] (μM)			
compd	Fm	X	Y	R	MABA	LORA	VERO	*L. inf*	*T. cruzi*	*T. bruc*	MRC-5
8	A	H	S	OCF_3	4.4	15	>128	64	0.87	>64	>64
9	A	H	S	OBn	4.4	3.4	>128	48	0.62	6.2	>64
10[d]	A	H	O	OCF_3	14	43	>128	0.11	0.60	>64	>64
11	A	H	O	OBn	11	63	>128	1.5	6.9	61	>64
12	A	Me	S	OCF_3	0.24	7.9	NT	57	0.67	56	>64
13	A[e]	Me	S	OCF_3	0.20	5.8	24	>64	0.57	>64	43
14	A[f]	Me	S	OCF_3	6.1	51	52	>64	1.6	>64	>64
15	A	Me	S	OBn	3.2	12	NT	56	0.28	31	64
16[d]	A	Me	O	OCF_3	0.24	34	>128	0.28	0.42	60	60
17	A	Me	O	OBn	0.30	50	>128	7.3	3.2	60	>64
18	A[g]	Me	SO	OCF_3	>128	>128	NT	>64	1.6	>64	>64
19	A[h]	Me	SO	OCF_3	>128	>128	NT	>64	3.0	>64	>64
20	A[g]	Me	SO	OBn	>128	>128	NT	56	0.08	0.33	>64
21	A[h]	Me	SO	OBn	>128	>128	NT	>64	0.49	2.3	>64
22	A	Me	SO_2	OCF_3	>128	21	NT	>64	0.97	>64	>64
23	A	Me	SO_2	OBn	>128	>128	NT	56	0.09	0.98	62
24	B	Me	S	OCF_3	55	59	45	>64	7.3	22	47
25	B	Me	S	OBn	>128	>128	NT	55	19	53	>64
26	C	Me	S	F	1.5	>64	NT	>64	0.15	>64	>64
27	C	Me	S	CF_3	1.1	>64	NT	56	0.04	1.1	>64
28	C	Me	S	OCF_3	1.4	>64	NT	56	0.14	2.8	>64

[a]Minimum inhibitory concentration against *M. tb*, measured under aerobic (MABA)[26] or hypoxic (LORA)[27] conditions. [b]Each value (except the single test VERO data) is the mean of ≥2 independent determinations (NT means not tested). For complete results (mean ± SD) please refer to the Supporting Information. [c]IC$_{50}$ values for inhibition of growth of the parasites *Leishmania infantum*, *Trypanosoma cruzi*, and *Trypanosoma brucei*, or for cytotoxicity toward human lung fibroblasts (MRC-5 cells) and VERO cells. [d]Ref. 16. [e](*R*)-Enantiomer. [f](*S*)-Enantiomer. [g]Less polar diastereoisomer (TLC). [h]More polar diastereoisomer (TLC).

The structures and *in vitro* biological assay data for the 41 new and 11 known[16,25] target compounds are summarised in Tables 1 and 3. For the TB studies, MIC values describing growth inhibitions of ≥90% against *M. tb* (strain H37Rv) were measured under both aerobic and hypoxic conditions (MABA[26] and LORA[27] assays, respectively). This recognised the finding that **2** displays unique modes of action under these differing oxygenation states,[7] and that testing compounds against bacteria exposed to one or more host-relevant stresses is postulated to assist the discovery of drugs with greater sterilizing ability against persistent bacteria.[28] Cytotoxicity against mammalian cells (VERO) was also tested in a 72 h assay.[26] In the NTD work, 50% inhibitory concentrations provided quantification of the levels of activity against three protozoan parasites (*L. infantum*, *T. cruzi*, and *T. brucei*) or cytotoxic effects on human lung fibroblasts (MRC-5 cells).[29] In all cases (except for VERO), recorded data are mean values derived from two or more independent experiments.

In line with our original studies on fused 5/6 ring nitroheterobicycles,[8] our first side chain targets in the new nitroimidazothiazole series were simple benzyl ethers having either a 4-trifluoromethoxy or 4-benzyloxy substituent (**8**, **9**, **12**, and **15**; Table 1). This choice reflected both the excellent *in vivo* antitubercular effects of **2** and the superior *in vitro* potency of its 4-benzyloxybenzyl analogue (an order of magnitude better than **2**,[30] as also shown for the racemates[8]). Although the latter compound was reportedly inferior to **2** *in vivo* (due to less optimal pharmacokinetic properties),[31] we had elected to employ this 4-benzyloxyaryl side chain in several studies[8,30] as an SAR tool to probe the potential to enhance *in vitro* potency by increasing lipophilicity.[11] Such effects may be derived from hydrophobic binding to specific aromatic residues within the N terminus of the known nitroreductase Ddn.[32]

Table 2. Reduction potentials, solubility values, microsomal stabilities, and *in vivo* antitubercular efficacy data for selected analogues

		Solubility[b] (μg/mL)		Microsomes[c] (% remaining at 1 h)		*In vivo* efficacy against *M. tb*
compd	*E*(1)[a] (mV)	pH 7	pH 1	H	M	(ratio vs **2**)[d]
1		0.31	116	71	87	68
2	-534 ± 7	19		82	94	1.00
3[e]	-534 ± 5			42	0.7	
4[f]		2.4				9.3
12	-537 ± 9	2.4				
13				29	0	
16		11		68	30	0.025
19	-492 ± 6					
22	-519 ± 7					
45		0.25		84	75	5.7
48		0.41		82	81	115
49		0.18	13			
51	-527 ± 5					
52	-526 ± 6					

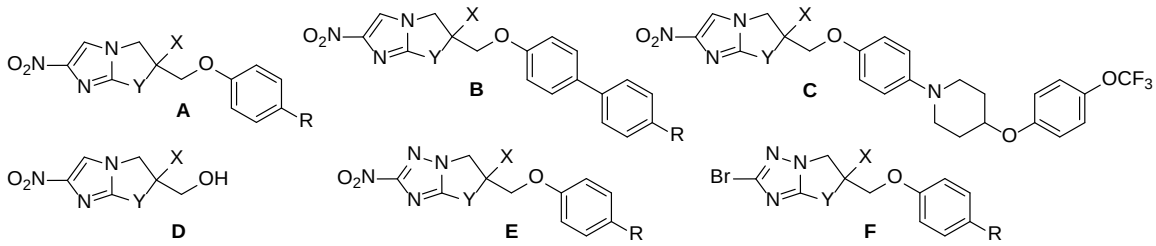
[a]One-electron reduction potentials, determined[8] by pulse radiolysis. [b]Solubility in water (pH 7) or 0.1 M HCl (pH 1) at 20 °C, measured[16] by HPLC. [c]Pooled human (H) or CD-1 mouse (M) liver microsomes. [d]Fold reduction in lung colony forming units (CFUs) for compound compared with the fold CFU reduction for **2** in a mouse model[26] of acute TB infection. [e]Ref. 8. [f]Ref. 16.

Of these initial targets, the 2-methyl thiazole **12** appeared to be the most promising for TB (MABA MIC 0.24 μM), being fairly comparable to **2** (MABA MIC 0.50 μM, LORA MIC 2.6 μM)[11] and slightly superior to **3** (MABA MIC 1.1 μM, LORA MIC 13 μM).[8] Thiazole **12** also had equivalent solubility to **4** and a one-electron reduction potential very similar to **2** and **3** (Table 2). The *R* enantiomer of **12** (**13**) was 9- to 31-fold more potent than the *S* enantiomer **14** in the two MIC assays, consistent with the recorded 63-fold difference in aerobic activity for the enantiomers of a phenoxy analogue in the nitroimidazooxazole series.[33] However, the *S*-oxidised derivatives **18**-**23** were generally devoid of any antitubercular activity (despite **19** and **22** respectively having 16 and 31 mV lower one-electron reduction potentials than corresponding nitroimidazothiazine oxide analogues;[8] Table 2), while the 5-nitro isomer of **12** (**24**) was more than 200-fold less active in the MABA assay. Moreover, unlike the SAR for nitroimidazooxazines (e.g., **2**),[11,12] the formation of biaryl analogues of **12** (**26**-**28**) actually resulted in a decreased level of potency.

These findings led us to investigate the shorter chain length phenyl ethers (**29**, **30**, **33**, **34**, and **37**-**42**) and their biaryl derivatives (**43**-**45**; Table 3). Here, a 2-methyl substituent on the thiazole ring was again favoured, but **33** (MABA MIC 0.49 μM) was not superior to **12**, and *S*-oxidation (**37**-**42**) still conferred a 29- to >260-fold potency loss (MABA). Nevertheless, the aerobic potencies of the biaryl derivatives **43**-**45** were notably improved (up to 8-fold over **33**), although activity in the hypoxic assay was poor. Interestingly, the racemic thiazole analogue of **1** (**49**) was equivalent in potency to the biaryl compound **45**, while the simple alcohol **51** and the nitrotriazole analogue **53** were relatively ineffective, in line with SAR for the oxazine series.[8] However, in order to better understand the significance of these data, it was necessary to consider oxazole congeners having the same side chains. The oxazole benzyl ethers (**10**, **11**, **16**, **17**) were all less active than related thiazoles in the LORA assay (3- to 19-fold), whereas in the MABA assay, **10** and **11** were less active than **8** and **9** but **16** was equipotent to **12** and **17** was 11-fold better than **15**. Similarly, oxazole phenyl ether **35** was superior to **33** under aerobic conditions only (6-fold better in the MABA assay but 6-fold worse in the LORA assay). For the biaryl oxazole derivatives (**46**-**48**), the fluoro analogue **46** was more effective in both TB assays than thiazole **43** but there was little variance between the other pairs and racemic **1** (**50**) was only marginally better than **49**. Therefore, apart from a couple of exceptions, the nitroimidazothiazoles and nitroimidazooxazoles were fairly comparable in terms of their overall *in vitro* antitubercular activity.

To determine whether the nitroimidazothiazole class could be developed any further, it was important to assess the potential for metabolism (e.g., to the inactive oxides), as this was an irreparable flaw with the nitroimidazothiazine series, based on the microsomal stability data obtained for **3** (Table 2). The (2*R*) benzyl ether **13** was indeed metabolised very rapidly, particularly by mouse liver microsomes, and was clearly less stable than the related oxazole **16**. Nevertheless, results for the more lipophilic and shorter linked biaryl derivative **45** were more encouraging, with an approximately 80% recovery of the parent compound following a 1 h exposure to either mouse or human liver microsomes, similar to data for the equivalent oxazole **48**. Therefore, compounds **45** and **48** were further evaluated head-to-head (together with **2**) in a mouse model of acute TB infection, dosing orally for 3 weeks (daily at 100 mg/kg, for 5 days each week).[11,26] Here, in comparison to **2** (Table 2), oxazole **48** gave a 115-fold greater reduction in colony-forming units (CFUs) in the lung, whereas thiazole **45** was significantly less efficacious (5.7-fold better than **2**, 20-fold less than **48**). Both delamanid **1** and the VL lead **4** also demonstrated better activities than **45** in this model, although benzyl ether **16** was inferior (consistent with its low microsomal stability). Based on these findings, we elected to discontinue development of the nitroimidazothiazole class for TB.

Table 3. *In vitro* antitubercular and antiparasitic activities of (phenoxy or hydroxy)methyl-substituted nitroimidazothiazoles, nitroimidazooxazoles, and triazole-based analogues



					MIC[a,b] (μM)			IC_{50}[c,b] (μM)			
compd	Fm	X	Y	R	MABA	LORA	VERO	*L. inf*	*T. cruzi*	*T. bruc*	MRC-5
29	A	H	S	OCF_3	4.5	8.2	>128	>64	0.85	>64	>64
30	A	H	S	OBn	>128	29	>128	56	1.6	11	56
31[d]	A	H	O	OCF_3	2.0	73	115	0.15	1.5	>64	>64
32	A	H	O	OBn	0.96	>128	>128	3.4	3.1	>64	19
33	A	Me	S	OCF_3	0.49	10	>128	>64	0.38	>64	>64
34	A	Me	S	OBn	0.76	5.3	>128	>64	0.14	>64	>64
35[d,e]	A	Me	O	OCF_3	0.077	55	NT	0.33	1.8	>64	>64
36	A	Me	O	OBn	0.79	24	NT	1.3	1.9	28	>64
37	A[f]	Me	SO	OCF_3	>128	>128	>128	>64	2.3	>64	>64
38	A[g]	Me	SO	OCF_3	26	58	>128	>64	1.7	41	>64
39	A[f]	Me	SO	OBn	>128	>128	>128	55	0.19	0.36	53
40	A[g]	Me	SO	OBn	>128	25	>128	42	0.75	0.98	51
41	A	Me	SO_2	OCF_3	14	15	>128	>64	1.8	>64	>64
42	A	Me	SO_2	OBn	>128	>128	>128	55	0.46	3.8	45
43	B	Me	S	F	0.21	>64	>128	>64	0.21	>64	>64
44	B	Me	S	CF_3	0.12	46	>128	>64	0.035	52	>64
45	B	Me	S	OCF_3	0.063	>128	>128	45	0.085	>64	>64
46[d]	B	Me	O	F	0.043	11	>128	3.3	0.52	9.2	>64
47[d]	B	Me	O	CF_3	0.081	>128	>128	11	1.5	1.0	>64
48[d]	B	Me	O	OCF_3	0.088	64	>128	5.3	0.89	0.85	>64
49	C	Me	S		0.073	71	>128	53	0.055	59	>64
50[d,e]	C	Me	O		0.051	26	>128	13	0.40	3.4	>64
51	D	Me	S		10	>128	NT	NT	NT	NT	NT
52[d,e]	D	Me	O		16	116	>128	18	52	>64	>64
53	E	Me	S	OCF_3	21	>128	104	53	0.49	10	>64
54[d]	E	Me	O	OCF_3	>128	>128	>128	>64	0.59	20	>64
55	E[f]	Me	SO	OCF_3	6.4	61	50	16	0.60	7.1	1.2
56	E[g]	Me	SO	OCF_3	9.3	90	98	0.90	1.2	3.0	0.74
57	E	Me	SO_2	OCF_3	2.2	27	46	1.3	1.3	2.1	0.42
58	F	Me	S	OCF_3	24	>128	89	>64	6.2	34	42
59[d]	F	Me	O	OCF_3	46	>128	60	>64	13	60	43

[a]Minimum inhibitory concentration against *M. tb*, measured under aerobic (MABA)[26] or hypoxic (LORA)[27] conditions. [b]Each value (except the single test VERO data) is the mean of ≥2 independent determinations (NT means not tested). For complete results (mean ± SD) please refer to the Supporting Information. [c]IC_{50} values for inhibition of growth of the parasites *Leishmania infantum*, *Trypanosoma cruzi*, and *Trypanosoma brucei*, or for cytotoxicity toward human lung fibroblasts (MRC-5 cells) and VERO cells. [d]Ref. 16. [e]Ref. 25. [f]Less polar diastereoisomer (TLC). [g]More polar diastereoisomer (TLC).

Following our discovery that nitroimidazooxazoles such as **4** were highly efficacious against VL, we screened these thiazole analogues for possible utility against NTDs (Tables 1 and 3). However, *none* of the nitroimidazothiazoles showed any significant activity against VL (*L. inf* IC_{50}s >40 µM). A novel nitroreductase (NTR2) has recently been identified as the enzyme responsible for the activation of **4** in *Leishmania* parasites.[34] In our previous SAR study of **4**,[16] we had shown that both the position of the nitro group (at C-6) and the imidazole ring itself were essential for the retention of VL potency, broadly mimicking the structural requirements for TB activity (involving substrate binding to the nitroreductase Ddn[32]). The one-electron reduction potential of thiazole alcohol **51** (-527 ± 5 mV; Table 2) was equivalent to that obtained for the corresponding oxazole alcohol **52** (and similar to data measured for **2**, **3**, and **12**). Therefore, the observed lack of activity against VL for nitroimidazothiazoles cannot be accounted for by any differences in reduction potential, which may point instead to a highly critical role for the ring oxygen atom of **4** in binding to NTR2, in order for successful activation of the nitroimidazole "warhead" to occur.

In other screening results, the thiazoles were generally ineffective against human African trypanosomiasis (*T. brucei* IC_{50}s mostly >10 µM), except for two sulfoxide derivatives (**20** and **39**), which displayed moderate potencies (IC_{50}s ~0.35 µM). However, while the latter results were intriguing, the lack of activity shown by the parent thiazoles (**15** and **34**) and the (3- to 11-fold) weaker potencies observed for the more polar sulfoxide diastereomer forms (**21** and **40**) and their sulfone analogues (**23** and **42**) discouraged further assessment of these hits. In contrast, many thiazole compounds, including **15** and **34** and (notably) their oxidised derivatives (**20**, **21**, **23**, **39**, **40**, and **42**), as well as biaryls such as **26**-**28** and **43**-**45**, and the thiazole analogue of **1** (**49**), all showed interesting activity (IC_{50}s of 0.035-0.8 µM) against *T. cruzi*, the protozoan parasite responsible for Chagas disease. For the 6-nitroimidazothiazoles, potency tended to increase with lipophilicity (Fig. 2; CLogP data from ChemDraw v. 15.0). Interestingly, the nitrotriazole-based congener **53** also showed reasonable potency in the same assay (IC_{50} 0.49 µM) but its *S*-oxidised derivatives (**55**-**57**) were cytotoxic. Overall, where oxazole comparators were available, the thiazoles were superior in 11/13 cases (and by at least 5-fold for most of these), suggesting a general advantage for this latter class. Previous studies directed at finding a more stable backup to **2** had verified the faster metabolism of its 4-benzyloxybenzyl analogue and the improved stability achieved by removal of benzylic methylenes.[30,35] Therefore, we were unsurprised by results from a first assessment of sulfone **23** revealing a fairly short (25 min) half-life in human liver microsomes. Taken together, these data point to biaryls **43**-**45** and especially the delamanid counterpart **49** (*T. cruzi* IC_{50} 0.055 µM; IC_{90} 0.19 µM) as the most useful new antitrypanosomal leads.

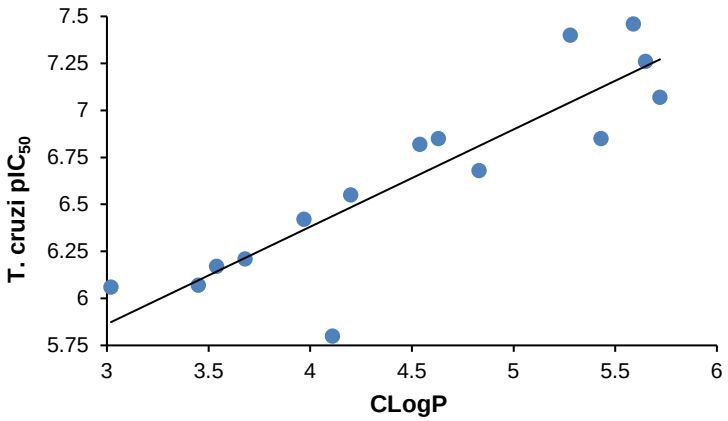


Figure 2. Effect of lipophilicity on potency against *T. cruzi* for 15 6-nitroimidazothiazoles

To summarise, we first set out to synthesise and assess the utility of novel nitroimidazothiazole analogues of the known nitroimidazooxazole class as potential antitubercular agents. The ring system was easily constructed in one high yielding step, providing simple access to the target compounds. Based on their promising MIC data, comparable to those of the corresponding oxazoles, we then examined the microsomal stabilities of two of the most potent examples, benzyl ether **13** and biaryl **45**. However, while the latter compound demonstrated a low susceptibility toward *in vitro* metabolism, equivalent to results for the oxazole congener **48**, it was found to be 20-fold less effective than **48** in a mouse model of acute TB infection. The unexpected identification of racemic oxazole **35**, then **4**, as potential development candidates for VL triggered more extensive SAR studies and the discovery of novel leads for Chagas disease, including the interesting triazole **54**. Despite their close structural similarity to nitroimidazooxazoles and equivalent reduction potentials, nitroimidazothiazoles surprisingly lacked any utility for VL but showed superior activity against Chagas disease. Importantly, the latter activity was not ablated by oxidation of the thiazole sulfur, indicating that the potent "thio-delamanid" analogue **49** may represent a useful new lead that is worthy of further assessment for this highly neglected disease.

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