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Biarylmethoxy 2-nitroimidazooxazine antituberculosis agents: Effects of proximal ring substitution and linker reversal on metabolism and efficacy

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ABSTRACT

Certain biaryl analogues of antitubercular drug PA-824 displayed enhanced in vivo efficacies yet retained some susceptibility towards oxidative metabolism; therefore, two new strategies were explored to address this. *Ortho*-substitution of the proximal aryl ring with larger electron-withdrawing substituents maintained or improved compound stability but reduced aerobic potency; however, fluoro and cyano were well tolerated. In vivo, only 2′- or 3′-fluoro mono-substitution preserved high efficacy against acute infection, although one example was twofold more effective than delamanid against chronic infection. Reversal of the 6-oxymethylene linkage also permitted high potency and improved stability towards human liver microsomes, albeit, in vivo results were inferior. These novel findings provide further insight into the preferred structural features for lead candidates in this important drug class.

Despite some significant progress in the 20 years since the World Health Organisation declared tuberculosis (TB) to be a global public health emergency, today's TB epidemic still afflicts more than 11 million people worldwide and kills more than one person every 25 s (1.5 million in 2013).[1] Current lengthy and complex treatments are simply inadequate to contain and defeat particularly multidrug resistant (MDR) forms of TB, leading to a revitalised search for both better agents and shorter-acting, safe regimens. The nitroimidazooxazine derivative PA-824 (pretomanid; **1**, see Fig. 1) is a new TB drug with a unique bioreductive mechanism of action (generating nitric oxide)[2] and is currently being assessed in a Phase III clinical study, as part of a novel combination (with moxifloxacin and pyrazinamide) that has the potential to significantly shorten and simplify both TB and MDR-TB treatment.[3]

In previous SAR studies on the wider nitroimidazooxazine class, we have shown that more lipophilic biphenyl analogues of **1** (e.g., **2**) have superior potency, and that the lower aqueous solubility of these compounds can be ameliorated by incorporation of aza atoms at suitable positions in the benzene rings (e.g., **3**), resulting in improved pharmacokinetic properties and excellent in vivo efficacy.[4,5] Further work focused on modifications to the 6-oxymethylene linker group was in part designed to address the potential for cleavage of the aromatic side chain via oxidative metabolism, which was thought to be associated with an increased toxicity risk (based on positive Ames results for some simple 6-nitroimidazooxazole analogues such as CGI-17341, **4**).[6] Thus, although **1** itself is not mutagenic (both in vitro and in vivo)[7] and has shown excellent safety in clinical trials,[3,8] we were interested in developing a backup to **1** with an enhanced metabolic stability profile. Initial investigations found that α-methyl substitution on the benzylic methylene of **1**, a modification reported to suppress oxidative metabolism of other benzyl ethers,[9] instead decreased microsomal stability compared to **1**; moreover, a more stable α-methyl biphenyl analogue (**5**) was unexpectedly toxic in vivo.[10] Removal of the benzylic methylene (**6**) did increase stability but significantly reduced in vivo efficacy relative to **2**,[10] suggesting a non-optimal linker length. Alternative amide, urea, and carbamate linkers also generally provided high microsomal stability and good in vitro activity but gave only moderate results in vivo,[11] again pointing to the original linkage as being most preferred.

Recently, Cherian et al.[12] reported an SAR study of 6-amino-linked analogues of **1** which revealed that additional substitution in the benzyl ring at either the 2- or 3-positions could

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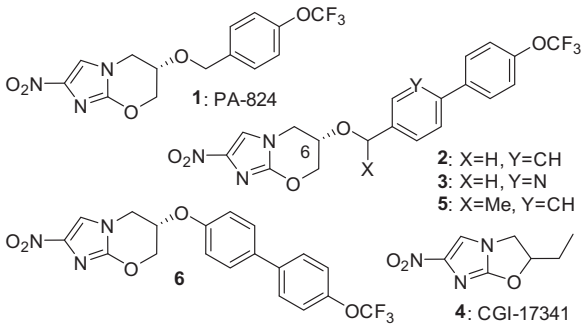


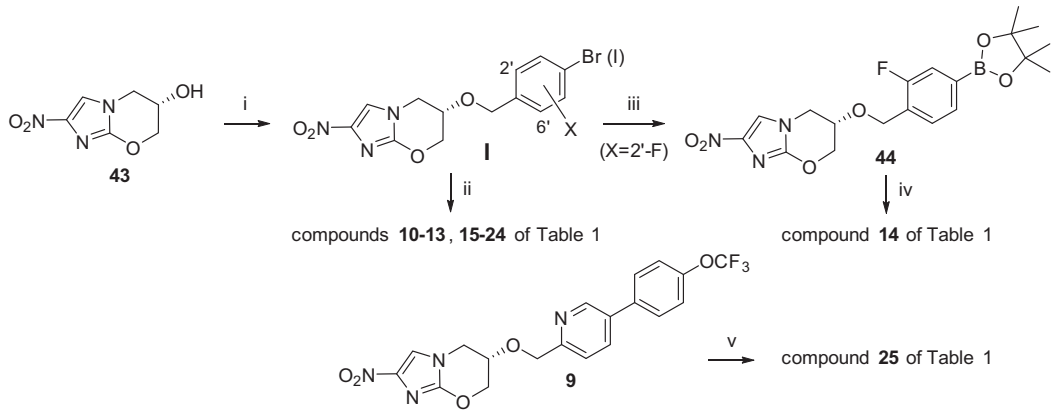
Figure 1. Structures of antituberculosis leads.

significantly improve in vitro potency. We hypothesised that similar substitution in the first benzene ring (proximal to the ether linkage) of biaryl compounds such as **2**, or related aza analogues, might also lead to a decreased rate of metabolism (e.g., via steric or electronic means) and/or a greater level of in vivo activity. We have previously reported wide variations in both microsomal stability and in vivo efficacy across a range of biaryl derivatives substituted in the terminal benzene ring.[4,5] Fluorine and fluorinated substituents (e.g., CF_3, OCF_3) are well known to influence the rate and extent of drug metabolism, sometimes even at sites distal to the position of metabolic attack.[13,14] In looking towards these objectives, another important final strategy to examine was reversal of the 6-oxymethylene side chain linkage, based on the better microsomal stability of **6**.[10] The findings from these studies are now described here.

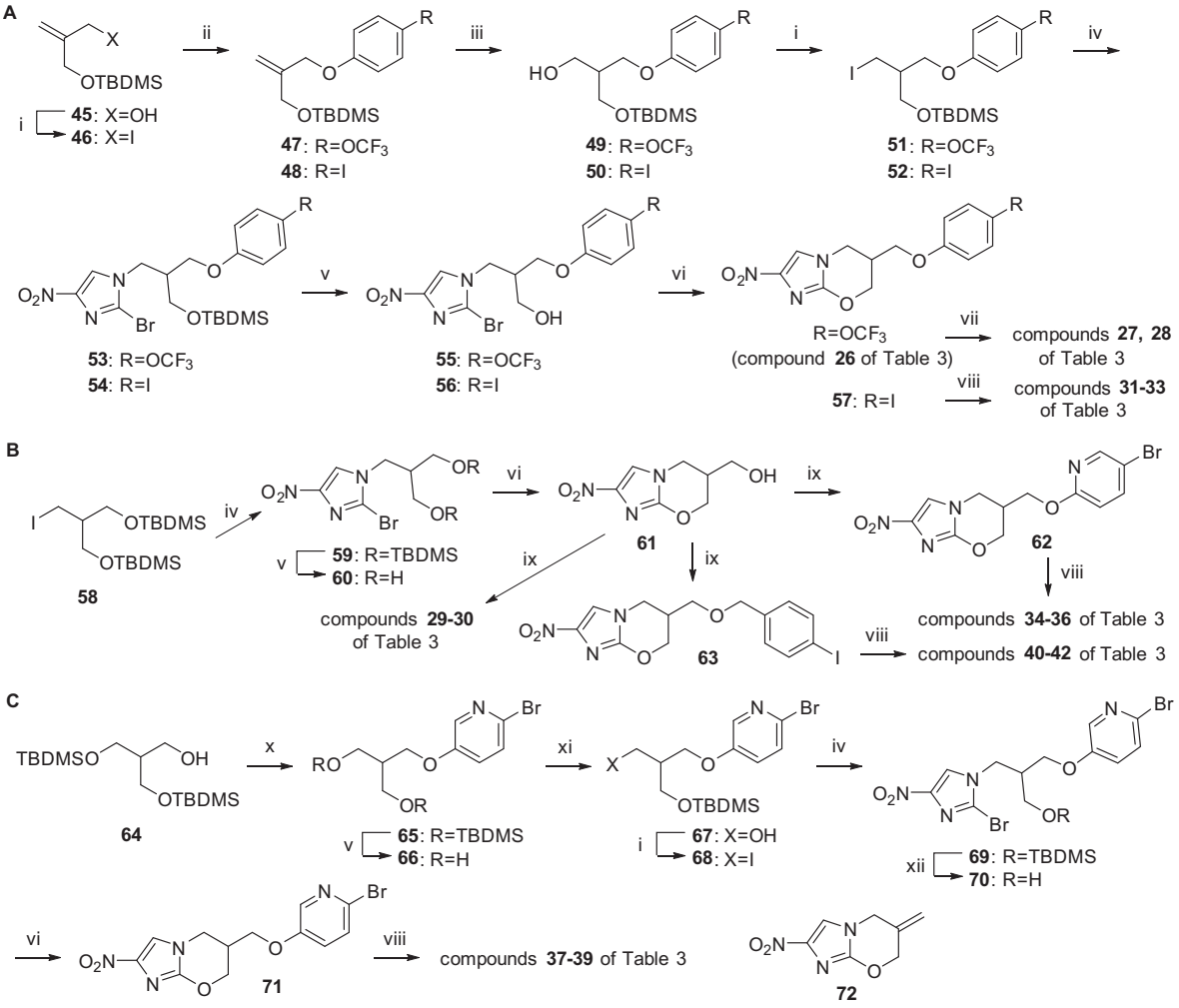
The synthetic methods employed for the proximal ring substituted biaryl analogues (**10**–**25**) are outlined in Scheme 1. The required 4-halobenzyl ether precursors (**I**) were prepared by NaH-promoted alkylation of the known[15] chiral alcohol **43** using appropriately-substituted benzyl bromides (or benzyl iodide in the case of **18**). The benzyl halides were readily available, either commercially, or via standard methods (e.g., bromination of known[16] or commercial alcohols with HBr/AcOH, or iodination using I_2/PPh_3/imidazole). The halobenzyl ethers (**I**) were typically Suzuki-coupled directly with arylboronic acids to give the final products but in one example (**14**) it was necessary to first transform the 4-bromide into the pinacol boronate ester derivative (**44**) and then reverse couple this with the corresponding chloropyridine. The novel N-oxide derivative of pyridine **9** (**25**) was also obtained in moderate yield via a buffered oxidation with 3-chloroperbenzoic acid.

The more complex chemical syntheses of the reversed linker analogues (**26**–**42**) are described in Scheme 2. Thus, alkylation of both 4-trifluoromethoxy- and 4-iodo-phenol with the known[17] iodide **46** (conveniently obtained from commercial 2-methy lene-1,3-propanediol via alcohol **45**[18]), followed by hydroboration[19] of the terminal double bond in each product, led to the intermediate alcohols **49** and **50** (Scheme 2A). Alkylation of 2-bromo-4-nitroimidazole with their derived iodides **51** and **52**, acid-catalysed desilylation, and NaH-assisted ring closure of the resulting alcohols (**55** and **56**) then gave the target compound **26** and iodo analogue **57**, respectively. The latter was further elaborated (via Suzuki couplings) to the biaryl derivatives **31-33**, while **26** was separated into its two enantiomers via preparative chiral HPLC (ChiralPak IA, 27% EtOH/hexane). Benzyl ether analogues of these were derived from the key alcohol **61** (Scheme 2B), obtained by alkylation of 2-bromo-4-nitroimidazole with the known[20] bis-silyl ether protected iodide **58**, followed by desilylation and ring closure of the resulting diol **60** using excess NaH (3.5 equiv). Reaction of alcohol **61** with 5-bromo-2-fluoropyridine (NaH/DMF), followed by Suzuki couplings (in the presence of DMF to improve solubility), also led to the 2-pyridine derivatives **34**–**36**. Finally, a higher-yielding third route (Scheme 2C) was developed to access the 3-pyridine derivatives **37**–**39**. Mitsunobu reaction of the known[20] bis-silyl protected triol **64** with 6-bromo-3-pyridinol and desilylation of the product gave the diol **66**. Selective monosilylation[21] of this diol gave alcohol **67**, which was then converted into iodide **68** and further elaborated via similar chemistry to give the desired products. Unfortunately, various attempts to transform the key alcohol **61** into phenyl ether **26** (via Mitsunobu reactions or mesylation, followed by reaction with the sodium salt of the required phenol) all gave predominantly elimination to the terminal alkene **72**, necessitating the alternative synthetic approaches described. All final compounds were characterised by ^{1}H NMR, MS, melting point, and combustion analysis.

Tables 1 and 3 summarise the structures and in vitro antitubercular potencies of the 33 new PA-824 analogues studied, together with relevant microsomal stability and in vivo efficacy data for selected compounds (some published data were also included for comparison). Briefly, minimum inhibitory concentrations (MICs, for a growth inhibition of >90%) were determined against *Mycobacterium tuberculosis* (*M. tb*, strain H37Rv) under both aerobic and hypoxic conditions, using either an 8 day microplate-based assay with an Alamar blue readout or an 11 day assay (involving bacteria pre-adapted to low oxygen conditions) with a luminescence readout, respectively.[22,23] Screening



Scheme 1. Reagents and conditions: (i) $ArCH_2Br$ (or $ArCH_2I$), NaH, DMF, 0–20 °C, 2–4 h (73–93%); (ii) $ArB(OH)_2$, toluene, EtOH, 2 M Na_2CO_3, $Pd(dppf)Cl_2$ under N_2, 90 °C, 0.5–6 h (34–93%); (iii) bis(pinacolato)diboron, KOAc, DMSO, $Pd(dppf)Cl_2$ under N_2, 89 °C, 5 h (66%); (iv) 2-Cl-5-CF_3pyridine, toluene, EtOH, 2 M Na_2CO_3, $Pd(dppf)Cl_2$ under N_2, 90 °C, 2 h (83%); (v) *m*-CPBA, Na_2HPO_4, CH_2Cl_2, 20 °C, 32 h (38%).



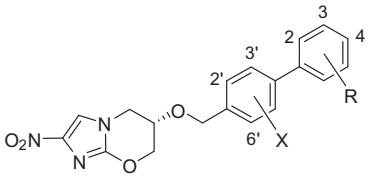
Scheme 2. Reagents and conditions: (i) I_2, PPh_3, imidazole, CH_2Cl_2, 5 °C, 6 h (for **46**), or 20 °C, 12–18 h (92–100%); (ii) RPhOH, K_2CO_3, acetone, 50 °C, 6–11 h (58–94%); (iii) I_2, $NaBH_4$, THF, 0 °C, 3–4 h, then 20 °C, 13 h, then 30% H_2O_2, 3 N NaOH, 0–20 °C, 3 h (61–71%); (iv) 2-bromo-4-nitroimidazole, K_2CO_3, DMF, 82–87 °C, 24–42 h (73–95%); (v) 1% HCl in 95% EtOH, 20 °C, 4–13 h (85–94%); (vi) NaH, DMF, 0–20 °C, 3.3–5 h (67–78%); (vii) preparative chiral HPLC (CHIRALPAK IA, 27% EtOH/hexane) (86%); (viii) $ArB(OH)_2$, 2 M Na_2CO_3, toluene, EtOH, (DMF), $Pd(dppf)Cl_2$ under N_2, 90 °C, 15–150 min (69–95%); (ix) $ArCH_2Br$ or 4-BnOBnCl or 5-Br-2-Fpyridine, NaH, DMF, 0–20 °C, 3 h (24–67%); (x) 6-Br-3-pyridinol, DEAD, PPh_3, THF, 0 °C, 1 h, then 20 °C, 41 h (97%); (xi) NaH, THF, 20 °C, 1 h, then TBDMSCl, 20 °C, 100 min (79%); (xii) TBAF, THF, 20 °C, 4 h (100%).

under low oxygen conditions is a recommended first step toward identifying agents with a better ability to kill persistent bacilli;[24] recorded values are the mean of at least two independent determinations (± standard deviation). Cytotoxicity against mammalian cells (VERO) was also checked in a 72 h exposure;[22] the compounds were generally non-toxic (IC_{50}s >128 μM, except for **19**, **29**, **30**, **41** and **42**, which had IC_{50}s of 43, 46, 113, 38 and 24 μM, respectively).

In Table 1, several derivatives of early biphenyl lead **2** and promising aza analogues **7**, **8** and **9** were evaluated, featuring either 2′ or 3′ or 2′,6′ substituents of varying size, electronic and lipophilic character in the first aryl ring (proximal to the ether linkage). For derivatives of **2**, excellent potency in the aerobic (MABA) assay was maintained with small, H-bond accepting, electron -withdrawing substituents (2′-F, 3′-F, 2′,6′-diF, and 2′-CN); the latter result was intriguing as CN is reportedly 8 times smaller than a methyl group, polar (lowering compound lipophilicity), and capable of reducing susceptibility to oxidative metabolism.[25] However, other 2′ substituents (Cl, CF_3, OCF_3, OCH_3) significantly diminished aerobic potency compared to **2** (particularly the larger groups), consistent with the moderate activity previously observed for the α-methyl biphenyl analogue (**5**).[10] The attenuated activity of the 2′,6′-diCl derivative (**12**) compared to the 2′-Cl derivative (**10**) was also in accordance with this SAR trend. Substitution of aza analogue **8** with fluoro and cyano groups in the proximal ring (**15**, **17**, **19**, **21**) was surprisingly less effective in the MABA assay but the 2′-F derivative of **7** (**14**) was equipotent. The N-oxide derivative of **9** (**25**) also retained similar aerobic activity to **9** itself. In contrast to these findings, almost all of the compounds displayed very good potencies in the hypoxic (LORA) assay, with some (e.g., **15**, **16**, **17**, **20** and **21**) being slightly better than the parent 2′-H compounds and only **12** (2′,6′-diCl), **23** (2′-OCF_3), **24** (2′-OCH_3), and the N-oxide **25** giving notably inferior results.

Most of the compounds in Table 1 were assessed for stability toward mouse and human liver microsomes. Unsurprisingly, the most metabolically stable compounds were generally those with larger electron-withdrawing substitution (e.g., 2′-Cl, 2′,6′-diCl, 2′,6′-diF, 2′-CF_3, 2′-OCF_3); other substituents (e.g., 2′-F, 2′-CN, 2′-OCH_3) resulted in slightly lower stabilities than the parent compounds but still seemed quite acceptable (>75% parent remaining after a 1 h incubation with mouse microsomes). Accordingly, a small subset of analogues providing high potency in the MABA assay was selected for efficacy studies in a mouse model of acute *M. tb* infection. In this assay, compounds were administered orally using a once daily dose of 100 mg/kg for 5 days a week for 3 weeks, beginning on day 11 post-infection.[4,22] For more meaningful

Table 1
In vitro and in vivo results for proximal ring-substituted biaryl analogues of **1**



Compd	X	R	MIC[a] (μM)		Microsomes[b] (% remaining at 1 h)		In vivo efficacy[c]
			MABA	LORA	H	M	(ratio vs **1**)
5[d]			0.19 ± 0.03	1.5 ± 0.4	100	92	toxic
2[e]	H	4-OCF_3	0.035 ± 0.015	1.3 ± 0.1	97	96	>205
7[f]	H	2-aza, 4-CF_3	0.06 ± 0.03	1.0 ± 0.2	93	90	33
8[f]	H	3-aza, 4-CF_3	0.03 ± 0	2.1 ± 0.2	86	91	15
9[f]	2′-aza	4-OCF_3	0.065 ± 0.038	3.7 ± 1.2	97	97	27
3[f]	3′-aza	4-OCF_3	0.05 ± 0.01	0.54 ± 0.24	83	87	>89
10	2′-Cl	4-OCF_3	0.17 ± 0.04	1.6 ± 0.1	99	100	2.1
11	3′-Cl	4-OCF_3	0.21 ± 0.10	1.5 ± 0.5			
12	2′,6′-diCl	4-OCF_3	0.62 ± 0.14	11 ± 3	98	93	
13	2′-F	4-OCF_3	0.017 ± 0.005	1.2 ± 0.4	93	85	33
14	2′-F	2-aza, 4-CF_3	0.055 ± 0.025	2.3 ± 1.0	90	77	51
15	2′-F	3-aza, 4-CF_3	0.13 ± 0.01	0.68 ± 0.17	96	78	13
16	3′-F	4-OCF_3	0.055 ± 0.015	0.51 ± 0.15	97	91	>933
17	3′-F	3-aza, 4-CF_3	0.25 ± 0.10	0.90 ± 0.23			
18	2′,6′-diF	4-OCF_3	0.06 ± 0.03	1.5 ± 0.2	95	94	0.98
19	2′,6′-diF	3-aza, 4-CF_3	0.35 ± 0.13	2.9 ± 0.6			
20	2′-CN	4-OCF_3	0.053 ± 0.021	0.45 ± 0.05	91	88	2.9
21	2′-CN	3-aza, 4-CF_3	0.28 ± 0.04	1.1 ± 0.2			
22	2′-CF_3	4-OCF_3	0.65 ± 0.23	2.4 ± 0.5	100	96	
23	2′-OCF_3	4-OCF_3	0.45 ± 0.15	7.5 ± 4.3	97	100	
24	2′-OCH_3	4-OCF_3	0.64 ± 0.20	5.5 ± 2.4	88	87	
25	2′-aza-O[g]	4-OCF_3	0.05 ± 0	32 ± 6			

[a] Minimum inhibitory concentration, determined under aerobic (MABA)[22] or hypoxic (LORA)[23] conditions. Each value is the mean of at least two independent determinations ± standard deviation.
[b] Pooled human (H) or CD-1 mouse (M) liver microsomes.
[c] Fold reduction in lung colony forming units (CFUs) for compound compared with the fold CFU reduction for **1** in a mouse model of acute TB infection.
[d] Ref. 10.
[e] Ref. 4.
[f] Ref. 5.
[g] N-oxide.

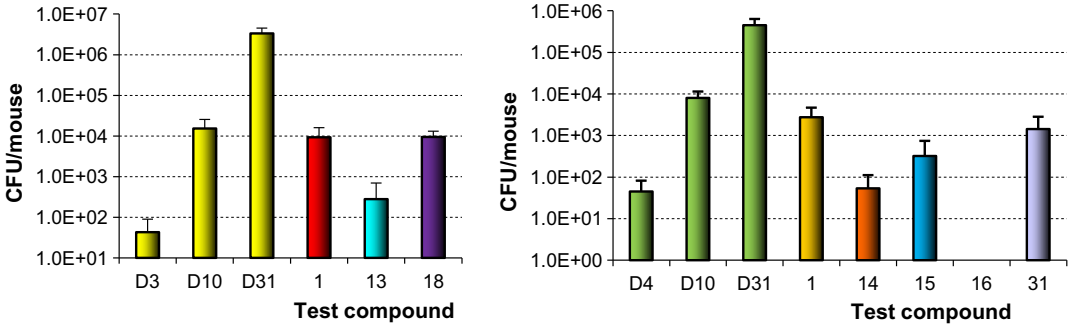


Figure 2. Comparative efficacies of some analogues of **1** in the acute infection mouse model.

interexperiment comparisons, compound activity (being the fold decrease in colony-forming units recovered from the lungs) was expressed in relation to the activity of **1**, which was routinely included as an internal reference standard in each in vivo experiment.

The most active compound (Table 1 and Fig. 2) was the 3′-F compound **16**, whose efficacy at this dosage could not be clearly distinguished from that of **2**. While the low efficacy of the 2′-Cl analogue (**10**) could possibly be rationalised by its moderate in vitro potency and high crystallinity (giving poor oral

Table 2
Pharmacokinetic parameters for **2** and **13** in CD-1 mice (dosing po at 40 mg/kg[10])

Compd	Plasma			Lung			AUC ratio[b]
	AUC_{0-inf}[a] (μg h/mL)	C_{max} (μg/mL)	$t_{1/2}$ (h)	AUC_{0-inf}[a] (μg h/mL)	C_{max} (μg/mL)	$t_{1/2}$ (h)	
2	198	7.4	14.4	218	9.0	12.8	1.1
13	202	6.7	22.3	549	12.1	28.1	2.7

[a] Area under the curve, extrapolated to infinity.
[b] Lung AUC/plasma AUC.

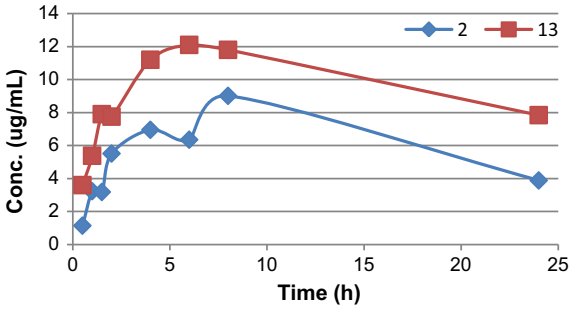


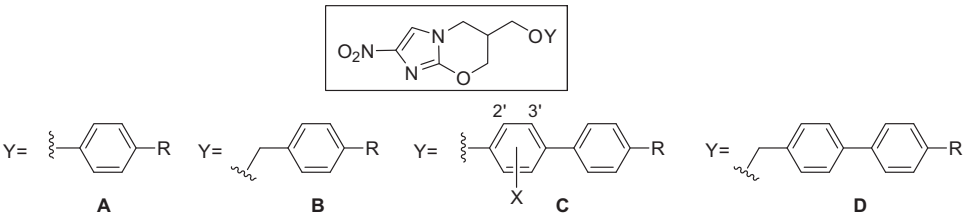
Figure 3. Concentration–time profiles for **2** and **13** in lung tissue of CD-1 mice.

bioavailability), the inferior results for the more potent 2′,6′-diF (**18**) and 2′-CN (**20**) congeners were surprising. Although not as effective as **16** and **2** in this assay, the 2′-F derivative (**13**) was still much better than **1** (33-fold) so the 2′-F derivatives of aza analogues **7** and **8** (**14** and **15**, respectively) were also assessed. These two compounds had similar in vivo activities to the original leads **7** and **8**, again suggesting no clear utility for this additional ring substitution. However, compound **13** was finally compared with **1**, **2** and the MDR-TB drug delamanid (OPC-67683)[26] in a more stringent mouse model of chronic *M. tb* infection, where dosing (orally, once daily at 100 mg/kg, 5 days a week for 3 weeks) was initiated 70 days post infection, and bacteria were in a well-established plateau phase of growth. In this assay, **13** was found to be superior to delamanid (2.2-fold), **2** (2.6-fold) and **1** (16-fold). A potential explanation for this unexpected result was revealed following comparison of the pharmacokinetic data for **2** and **13** in CD-1 mice (Table 2); these data showed that **13** had a significantly longer half-life than **2** in both plasma and lung and provided a much greater exposure in lung tissue (Fig. 3). This is supported by evidence from a recently reported pharmacoki netic–pharmacodynamic study of further analogues of **1** (tested in a comparable mouse model of TB) which indicated that in vivo activity correlated best with the time during which total lung concentrations were greater than the in vitro MIC.[27] However, it remains unclear why **13** demonstrated this therapeutic advantage over **2** only in the more demanding chronic TB infection model.

While the in vivo efficacy results for **13** and **16** were encouraging, it was clear that the original concept of improving the metabolic stability of biaryl analogues via proximal ring substitution had achieved only limited success, due to the poor tolerance for larger *ortho* substituents (in vitro and, particularly, in vivo). In Table 3, we therefore summarise the results of an alternative stabilisation strategy, namely, reversal of the 6-oxymethylene linkage.

Compared to *racemic* **1** (which has MICs of 1.1 and 4.4 μM in MABA and LORA, respectively),[28] the reversed linker analogue **26** was almost twice as active in the aerobic MIC assay and approximately fourfold less active in the hypoxic assay. However, unlike the original 6-*O*-benzyl series where the *S*-enantiomer form was about 2 orders of magnitude more potent than the *R*-enantiomer form,[29] the enantiomers of **26** (**27**, **28**; obtained in 100% enantiomeric excess by chiral HPLC—for experimental data, see Supporting information) showed fairly comparable activities (only a twofold difference in MABA and a fivefold difference in LORA), much like the enantiomers of simple 6-nitroimidazooxazoles such as **4**.[29] Benzyl ether congeners (**29**, **30**) were less effective, again

Table 3
In vitro and in vivo results for reversed linker analogues of **1**



Compd	Form	X	R	MIC[a] (μM)		Microsomes[b] (% remaining at 1 h)		In vivo efficacy[c]
				MABA	LORA	H	M	(ratio vs **1**)
1				0.50 ± 0.30	2.6 ± 1.4	82	94	1.0
26	A		OCF_3	0.63 ± 0.15	16 ± 5			
27	A[d]		OCF_3	0.23 ± 0.12	1.9 ± 0.1	91	80	0.70
28	A[e]		OCF_3	0.50 ± 0.23	9.0 ± 3.9	90	72	0.41
29	B		OCF_3	2.4 ± 0.5	7.9 ± 1.2	71	41	
30	B		OBn	3.1 ± 0.3	35 ± 20			
31	C	H	OCF_3	0.14 ± 0.06	>128	100	93	2.0
32	C	H	CF_3	0.035 ± 0.015	100 ± 10			
33	C	H	F	0.03 ± 0.01	108 ± 8			
34	C	2′-aza	OCF_3	0.045 ± 0.025	100 ± 4			
35	C	2′-aza	CF_3	0.04 ± 0.02	109 ± 5			
36	C	2′-aza	F	0.03 ± 0.01	25 ± 3			
37	C	3′-aza	OCF_3	0.15 ± 0.09	6.9 ± 0.6			
38	C	3′-aza	CF_3	0.22 ± 0.01	1.3 ± 0.5			
39	C	3′-aza	F	0.15 ± 0.09	2.0 ± 0.8			
40	D		OCF_3	0.30 ± 0.16	>128			
41	D		CF_3	0.49 ± 0.25	>128			
42	D		F	0.47 ± 0.30	7.9 ± 4.3			

[a] Minimum inhibitory concentration, determined under aerobic (MABA)[22] or hypoxic (LORA)[23] conditions. Each value is the mean of at least two independent determinations ± standard deviation.
[b] Pooled human (H) or CD-1 mouse (M) liver microsomes.
[c] Fold reduction in lung colony forming units (CFUs) for compound compared with the fold CFU reduction for **1** in a mouse model of acute TB infection.
[d] *R* enantiomer.
[e] *S* enantiomer.

paralleling results for 6-nitroimidazooxazoles.[30] Of particular interest for this study were the biaryl analogues (**31**–**39**). The biphenyl compounds (**31**–**33**) and their 2′-aza derivatives (**34**–**36**) generally showed excellent aerobic potencies, similar to their original series counterparts (e.g., **2**, **9**), but were surprisingly ineffective in the LORA assay. Conversely, the 3′-aza derivatives (**37**–**39**) showed more moderate activities in both assays. Finally, although phenylbenzyl analogues (**40**–**42**) provided better MABA data than the benzyl ethers (**29**, **30**), they were still less impressive than the biphenyl compounds (**31**–**33**).

In an initial examination of in vitro metabolism (Table 3), the enantiomers of phenyl ether **26** (**27** and **28**) both exhibited higher stabilities than **1** toward human liver microsomes but correspondingly lower stabilities than **1** toward mouse liver microsomes. An early benzyl ether derivative (**29**) predictably showed only modest stability but the reversed linker analogue of **2** (**31**) displayed excellent stability (>90% in both species). The enantiomers of **26**, together with biaryl analogue **31**, were then evaluated in the acute mouse model discussed previously. Here, **27** and **28** were both only slightly less effective than **1** (1.4- to 2.4-fold), consistent with their slightly faster rates of metabolism in this species. However, in sharp contrast to **2**, compound **31** was only marginally more efficacious than **1** (twofold; see Fig. 2) and therefore no additional reversed linker biaryl analogues were assessed in vivo.

In this paper, we have described the results of two final studies in our search for an advanced backup to **1**, which were primarily aimed at reducing metabolism of the side chain ether linkage. Substitution of the proximal aryl ring of leading biaryl candidates with larger electron-withdrawing substituents was found to maintain or improve compound stability but, in contrast to reported data for the 6-benzylamino series, was unfortunately counter-productive in reducing aerobic potency by an order of magnitude. While addition of one or two small substituents such as fluoro and cyano appeared to be acceptable in vitro, only the former (2′-F or 3′-F) preserved a markedly superior level of in vivo efficacy in comparison to **1**, suggesting fairly limited utility. Reversal of the 6-oxymethylene linkage initially appeared to be a more promising approach, with generally high in vitro potencies being manifested, together with an overall improvement in stabilities toward human liver microsomes. However, as found with previous attempts to modify the original ether linkage, the direct analogue of **2** (**31**) did not provide a sufficiently high level of efficacy in vivo, despite being apparently able to access a similar extended side chain conformation. These accumulated findings now suggest that previous lead compounds such as **2** and **3** (and two related acetylene-extended analogues[10,31]) are already ideally positioned to maximise binding interactions with the known deazaflavin-dependent nitroreductase Ddn.[29] This, in conjunction with their more suitable pharmacokinetic properties, facilitates essentially optimal in vivo activity, such that various alternative SAR directions in the 6-substituted 2-nitroimidazooxazine class are evidently unable to yield either comparable or superior results.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.07.084. These data include MOL files and InChiKeys of the most important compounds described in this article.

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