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Synthesis and anti-tubercular activity of 2-nitroimidazooxazines with modification at the C-7 position as PA-824 analogs

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ABSTRACT

Tuberculosis (TB) is a major global health problem, and new drug targets and scaffolds need to be identified to combat the emergence of drug resistant TB.

The nitroimidazooxazine PA-824 represents a new class of bio-reductive drug to treat TB. In this study we report a 2-nitroimidazooxazine derivative with modification at the C-7 position that exhibited better activity than PA-824 against *Mycobacterium tuberculosis* (*Mtb*) H37Rv strain in vitro. From **7a** as a key intermediate, we functionalized with benzyl ether (**8**), phenyl ether (**9**), benzyl carbonate (**10**) and benzyl carbamate (**11**). Among the 23 compounds produced, **8a-*R*** (MIC = 0.078 μM) with trifluoromethoxy benzyl group was 5-fold more potent than PA-824 (MIC = 0.390 μM) in the in vitro assays against the wild-type *Mtb*, and the phenyl ether compound **9g-*R*** (MIC = 0.050 μM) exhibited the most potent antimycobacterial activity.

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Tuberculosis (TB) is a major global health problem, and there is an urgent need to discover new agents with improved efficacy and safety and shorter treatment duration. According to the world health organization (WHO), the global incidence of TB in 2013 approached 9 million cases, of which 500,000 were multidrug-resistant (MDR).[1] Since 2013, the situation has worsened due to the emergence of drug resistant TB, HIV-TB coinfection and ineffectiveness of the BCG vaccine against adult pulmonary TB. A number of studies have identified various scaffolds that are currently in clinical trials. Bedaquiline (TMC207) has recently been approved by the FDA for the treatment of MDR-TB.[2] In addition, novel scaffolds such as nitroimidazooxazines (PA-824 and OPC-67683), oxazolidinones (eperesol and linezolid) and ethylenediamines (SQ109)[3] are being tested in clinical trials.

PA-824 ((6*S*)-2-nitro-6-{[4-(trifluoromethoxy)benzyl]oxy}-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazine has a 4-(trifluoromethoxy)benzyloxy side chain with an *S*-configuration at the C-6 position of the 2-nitrodihydro-5*H*-imidazo[2,1-*b*][1,3]oxazine ring and is currently in phase II clinical trials for TB.[4] As another biologically property of PA-824, the enantiomer of PA-824(*R*-PA-824, EC_{50} = 0.16 μM)) exhibited a six-fold increase in potency over PA-824 (EC_{50} = 0.9 μM) in parasiticidal activity against *Leishmania donovani*, the causative agent of visceral leishmaniasis (VL).[5] In *Mtb*, PA-824 is a prodrug that is activated by a deazaflavin-dependent nitro reductase (Rv3547) in a F420-dependent manner, resulting information of the desnitro derivative as a major metabolite. The releasing of NO upon activation of PA-824 seems to be responsible for its anaerobic activity.[6] The major drawbacks of PA-824 are its poor aqueous solubility and propensity to bind to human plasma proteins.[6] Synthesis of nitroimidazooxazine derivatives have attempted to address these shortcomings, including biphenyl analogs that replace benzyl ether with urea, carbamate or amide linkers and the hybridization of PA-824 with oxazolidinone at the C-6 position, among others. When the side-chains of (hetero)biaryl ether were introduced instead of the benzyl ether of PA-824, these scaffolds exhibit better in vitro and in vivo ADME properties as well as potency compared to their parent compounds.[7–11] In the PA-824 derivatives containing (hetero)biaryl side-chains, TBA-354, ((*S*)-2-nitro-6-((6-(4-(trifluoromethoxy)phenyl)pyridin-3-yl)methoxy)-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazine), is currently in clinical trials.[11] Furthermore, the introduction of benzyloxymethyl, biphenyl or (hetero)biaryl groups at the C-7 position of the 2-nitrodihydro-5*H*-imidazo[2,1-*b*][1,3]oxazine ring has been reported.[12,13] As a part of the ongoing the modification at the C-7 position of PA-824 in our laboratory,[14] we

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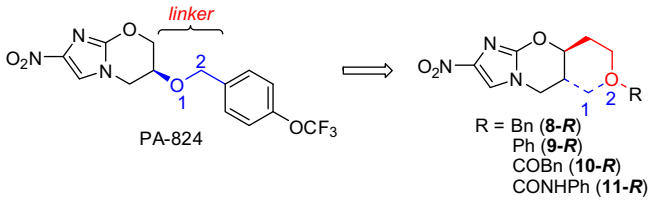


Figure 1. Structure of PA-824 and synthetic targets.

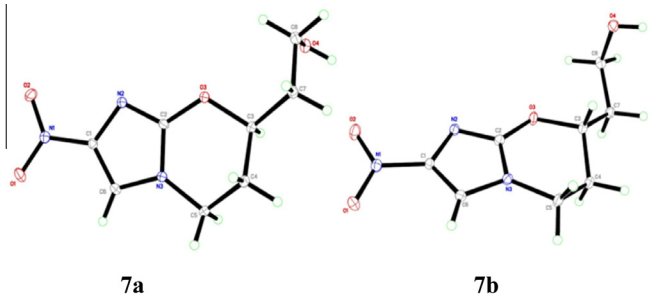
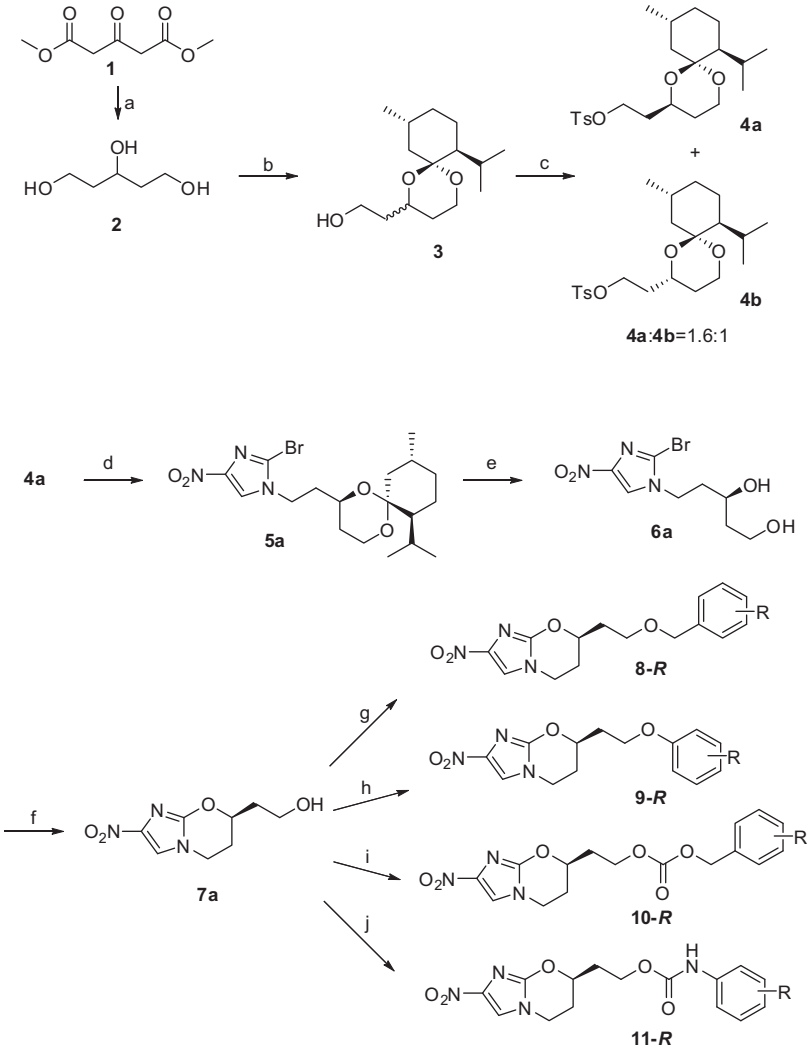


Figure 2. The X-ray crystal structure of **7a** and **7b**.[22]

synthesized and investigated the antimycobacterial properties of new PA-824 analogs that contain benzyloxyethyl **8**, phenoxyethyl **9**, ethyl benzyl carbonate **10** and ethyl phenyl carbamate **11** groups at the C-7 position of the 2-nitrodihydro-5*H*-imidazo[2,1-*b*][1,3] oxazine ring (Fig. 1). We expected that an ethyloxy linker would increase the conformational mobility of the 4-trifuoromethoxy-benzyl group, which will affect solubility through inhibition of the π–π stacking of aromatics in molecules that differ from the tight packing of PA-824 in the crystal structure.[15]

These analogs **8**–**11** were prepared through functionalization of chiral 2-(2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-7-yl)ethanol (**7**) as a key intermediate (Fig. 2). For the synthesis of **7a**, the ketone group of dimethyl 3-oxoglutarate (**1**) was preferentially reduced with sodium borohydride followed by reduction of the ester with borane-dimethyl sulfide (BMS) to yield 1,3,5-pentanetriol (**2**) in 69% over two steps.[16] After preparing (−)-menthone spiroketal **3** from 1,3,5-pentanetriol (**2**) according to known procedures, tosylation of the alcohol in **3** yielded **4a** and **4b** at 60% yield with a 1.6:1 diastereomeric ratio after purification by column chromatography.[17–20] Chiral **4a** was coupled with 2-bromo-4-nitro-1*H*-imidazole, which was prepared from 4-nitroimidazoles through regioselective reduction of



Scheme 1. Reagents and conditions: (a) (i) $NaBH_4$ (0.85 equiv), MeOH, 0 °C–rt, 5 h, 94%; (ii) BH_3Me_2S (3.0 equiv), THF, 0 °C to reflux, 2 days, 73%; (b) (i) (−)-menthone (1.1 equiv), cat. *p*-TsOH, $CH(OMe)_3$ (4.0 equiv), CH_2Cl_2, rt, 24 h, (ii) 1 N NaOH, MeOH, 0 °C–rt, 12 h, 82%; (c) TsCl (1.1 equiv), DMAP (1.3 equiv), CH_2Cl_2, 0 °C–rt, 3 h, 60%; (d) 2-bromo-4-nitro-1*H*-imidazole (1.0 equiv), K_2CO_3 (1.2 equiv), cat. TBAI, DMF, 120 °C, 3 h, 65%; (e) Dowex® 50wx8 resin, MeOH, rt, overnight 87%; (f) Cs_2CO_3 (2.0 equiv), DMF, 75 °C, overnight, 74%; (g) BnBr (10.0 equiv), NaH (2.0 equiv), DMF, −60 °C–rt, 1 h, 12–41%; (h) PhOH (1.0 equiv), PPh_3 (1.3 equiv), DIAD (1.3 equiv) DMF, 0 °C–rt, >4 h, 31–41%; (i) BnBr (3.0 equiv), Ag_2CO_3 (3.0 equiv), cat. TBAI, DMF, 75 °C, overnight, 30–40%; (j) PhNCO (1.2 equiv), cat. CuCl, DMF, rt, overnight, 22–25%.

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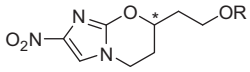
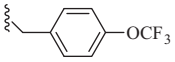
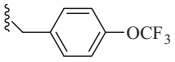
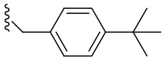
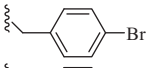
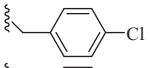
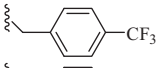
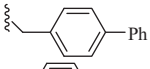
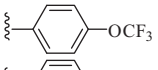
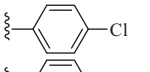
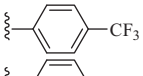
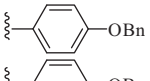
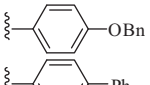
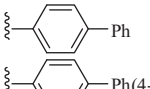
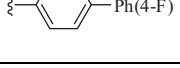
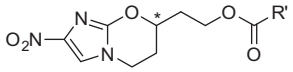
Table 1
Biological activities (H37Rv) and physicochemical properties of **8** and **9**

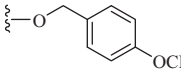
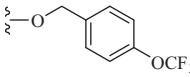
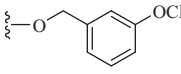
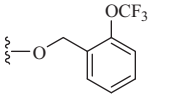
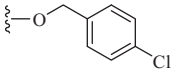
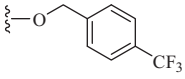
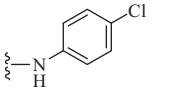
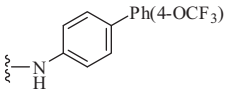


Num	R	MIC (μM)[a]	TC_{50} (μM)[b]	*C*log*P*[c]
8a-*R*		0.078	>100	3.21
8a-*S*		0.390	>100	3.21
8b-*R*		0.390	>100	4.00
8c-*R*		0.390	>100	3.04
8d-*R*		0.390	>100	2.89
8e-*R*		0.195	>100	3.06
8f-*R*		0.195	>100	4.07
9a-*R*		0.098	>100	3.52
9b-*R*		3.125	>100	2.32
9c-*R*		0.390	>100	3.17
9d-*R*		0.195	>100	3.45
9e-*R*		0.078	>100	4.18
9e-*S*		0.078	>100	4.18
9f-*R*		0.078	>100	4.21
9g-*R*		0.050	>100	4.38
PA-824		0.390	>100	2.79

[a] MIC (μM) = minimum inhibitory concentration.
[b] TC_{50} (μM) = toxic concentration.
[c] *C*log*P* values were calculated by ChemBioDraw Ultra, version 11.0.1.

Table 2
Biological activities (H37Rv) and physicochemical properties of **10** and **11**



Num	R′	MIC(μM)[a]	TC_{50}(μM)[b]	*C*log*P*[c]
10a-*R*		0.078	>100	3.27
10a-*S*		1.560	>100	3.27
10b-*R*		0.780	>100	3.27
10c-*R*		0.390	>100	3.27
10d-*R*		0.195	>100	2.95
10e-*R*		0.390	>100	3.12
11a-*R*		0.195	>100	4.88
11b-*R*		0.195	>100	4.88
PA-824		0.390	>100	2.79

[a] MIC (μM) = minimum inhibitory concentration.
[b] TC_{50} (μM) = toxic concentration.
[c] *C*log*P* values were calculated by ChemBioDraw Ultra, version 11.0.1.

2,5-dibromo-4-nitro-1*H*-imidazole in 77% yield,[21] to obtain the spiroketal menthonyl nitroimidazole **5a**.[17,18] Hydrolysis of **5a** with Dowex ® 50wx8 resin in MeOH and subsequent cyclization of **6a** produced the 2-nitrodihydro-5*H*-imidazo[2,1-*b*][1,3]oxazine ring **7a**. Different bases (K_2CO_3, Na_2CO_3, NaH, DBU, Ag_2CO_3 or Cs_2CO_3) and solvents (DMF, DMSO or CH_3CN) were evaluated to optimize cyclization conditions, and the best yield (74% for **7a**) was obtained when **6a** was heated with two equivalents of Cs_2CO_3 in DMF at 75 °C overnight. Preparation of the **7b**, enantiomer of **7a**, started with **4b** for the synthesis of **8-*S***, **9-*S*** and **10-*S*** with (*S*)-configuration under the same reaction conditions as in Scheme 1.

^{1}H NMR data of compounds **4a** and **4b** matched with data of a literature[17] among known literatures. So the structures **7a** and **7b** were confirmed by X-ray analysis of single crystals to assign stereochemistry to the C-7 position on the 2-nitrodihydro-5*H*-imidazo[2,1-*b*][1,3]oxazine ring of **7a** and **7b** starting from each **4a** and **4b**. Further proof for the chiral structure assignment of **7a**, the mesylation of **7a** afforded (*R*)-2-(2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-7-yl)ethyl methane-sulfonate, which was also confirmed by X-ray crystallographic data and ORTEP plots (Fig. S3 in Supplement data).

As PA-824 analogs, new nitroimidazooxazine benzyl ethers **8a–f** were synthesized from **7** by reaction with substituted benzyl halides. The synthesis of phenyl ether **9-*R*** was accomplished under Mitsunobu conditions.[23] To our knowledge, the carbonate linkage between the nitroimidazooxazine ring and benzyl group in PA-824 analogs has not been published, but linkages have been introduced, such as amides, carbamates, thiourea or urea.[7–11] We attempted to obtain carbonate analog **10-*R*** from **7a** with a substituted benzyl halide and Ag_2CO_3 in DMF with catalytic amounts of tetrabutylammonium iodide. The carbamate analog **11-*R*** was prepared from **7a** with isocyanate by CuI-catalyzed condensation. Synthetic procedures for **8–11** are summarized in Supplementary data.

Table 1 presents the minimum inhibitory concentrations (MIC) against the wild-type *M. tuberculosis* strain (H37Rv), toxic concentrations and physicochemical data for compounds **8** and **9**. When we compare **8a-*R***[24] and PA-824 containing same 4-trifuoromethoxybenzyl substituents, the activity of **8a-*R*** (MIC = 0.078 μM) was approximately 5-fold higher than PA-824 (MIC = 0.390 μM). Compound **8a-*S*** (MIC = 0.390 μM), an enantiomer of **8a-*R***, exhibited similar potency to PA-824. Among substituents at the *para*-position of the benzyl group, CF_3 (**8e-*R***; MIC = 0.195 μM) and the phenyl group (**8f-*R***; MIC = 0.195 μM) displayed a 2-fold increase in inhibition compared to PA-824, which was different from halogens (Br or Cl) or a *t*-butyl group (**8b-*R***, **8c-*R*** and **8d-*R***; MIC = 0.390 μM). In the *para*-substituted phenyl ether **9**, OCF_3 (**9a-*R***), Cl (**9c-*R***), and CF_3 (**9d-*R***) also showed similar potencies (MIC = 0.078–0.098 μM) to **8-*R*** with the same substituent. The non-substituted phenyl ether **9b-*R*** showed weak inhibition activity (MIC = 3.125 μM). Compound **9e-*R*** and its enantiomer **9e-*S*** had the same activity (MIC = 0.078 μM), but (*R*)-PA-

824 is known to be 9-fold less potent in vitro than PA-824 with (*S*)-configuration.[25] With respect to *para* substituents on the phenyl side chain, lipophilic **9g**-***R*** (*C*log*P* = 4.38) was the most active (MIC = 0.050 μM) in the biaryl compound series (**9e**–**9g**).

While benzyl ether **8a**-***R***, **8c**-***R*** and **8d**-***R*** did generally followed the same trend of increase potency with higher *C*log*P* that has also been reported in literlatures,[9–11] compound **8b**-***R*** and **8f**-***R***, which had *C*log*P* values of 4.00 and 4.07, respectively, showed reduced activities than **8a**-***R*** (*C*log*P* = 3.21) despite having higher lipophilicites compare with **8a**-***R***. The observed inhibition activities of **9** correlated well with *C*log*P* compared to **8**.

In the carbonyl containing linkers in Table 2, *para*-trifluoromethoxy benzyl carbonate (**10a**) was an unknown linker in the PA-824 analogs. The (*R*)-configuration **10a**-***R*** (MIC = 0.078 μM) had noticeably greater inhibitory activity than the (*S*)-configuration **10a**-***S*** (MIC = 1.560 μM). Changing the position of the trifluoromethoxy group from the *para* to the *meta* and *ortho* positions altered the activity order as follows: *para* (**10a**-***R***; MIC = 0.078 μM) = *meta* (**10b**-***R***; MIC = 0.078 μM) > *ortho* (**10c**-***R***; MIC = 1.560 μM). *para*-Trifluoromethyl carbonate (**10e**-***R***; MIC = 0.390 μM) had equipotent values to PA-824. With respect to the *O*-carbamate linker, **11a**-***R*** (MIC = 0.195 μM) and **11b**-***R*** (MIC = 0.195 μM) gave inferior results similar to the carbonate linker of oxazolidinone at the C-6 position.[25]

In summary, we have synthesized a novel series of 2-nitroimidazooxazines with modifications including a benzyl ether (**8**), phenyl ether (**9**), benzyl carbonate (**10**) and phenyl carbamate (**11**) at the C-7 position of 2-nitroimidazooxazines as PA-824 analogs. Among the 23 compounds screened for their in vitro activity against *Mtb*, most of the compounds exhibited less than micromolar potency and low toxicity (TC_{50} > 100 μM). **9g**-***R*** exhibited excellent values with MIC = 0.050 μM. In addition, chiral **7a** will be used as a key intermediate in the synthesis of new derivatives. Anti-mycobacterial activities of 2-nitrodihydro-5*H*-imidazo[2,1-*b*][1,3]oxazine chromophores synthesized from **7a** with novel linkers and heterocyclic side chains will be published in due course.

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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.2015.06.060. These data include MOL files and InChiKeys of the most important compounds described in this article.

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