Antitubercular Nitroimidazoles Revisited: Synthesis and Activity of the Authentic 3-Nitro Isomer of Pretomanid

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Supporting Information

ABSTRACT: A published study of structural features associated with the aerobic and anaerobic activities of 4- and 5-nitroimidazoles had found that the 3-nitro isomer of pretomanid, 8, displayed interesting potencies, including against nitroreductase mutant Mycobacterium tuberculosis. However, recent nuclear magnetic resonance analyses of two trace byproducts, isolated from early process optimization studies toward a large-scale synthesis of pretomanid, raised structural assignment queries, particularly for 8, stimulating further investigation. Following our discovery that the reported compound was a 6-nitroimidazooxazole derivative, we developed a de novo synthesis of authentic 8 via nitration of the chiral des-nitro imidazooxazine alcohol 26 in trifluoroacetic or acetic anhydride, and verified its identity through an X-ray crystal structure. Unfortunately, 8 displayed no antitubercular activity (MICs > 128 μM), whereas the second byproduct (3′-methyl pretomanid) was eight-fold more potent than pretomanid in the aerobic assay. These findings further clarify target specificities for bicyclic nitroimidazoles, which may become important in the event of any future clinical resistance.

KEYWORDS: Tuberculosis, pretomanid, drug resistance, nitroimidazole, nitration, silyl migration

Tuberculosis (TB) currently ranks alongside HIV/AIDS as one of the leading causes of global mortality, claiming about 1.4 million lives every year. The spread of multidrug- and extensively drug-resistant (MDR/XDR) TB is widely regarded as a burgeoning health crisis, placing great strain on limited healthcare resources for only modest treatment outcomes (cure rates of 20–50% following complex therapy for up to 30 months with several more costly and toxic agents). The recent emergence of programmatically incurable tuberculosis has led to many patients being discharged back into the community, further threatening control efforts. In this context, the conditional approval of two new MDR-TB drugs, delamanid (OPC-67683, 1; see Figure 1) and bedaquiline (TMC-207, 2), is a tremendous advance, although access to these and repurposed agents such as linezolid (3) remains very limited in many countries. To further address this urgent need, the TB Alliance has been developing shorter acting novel regimens involving the nitroimidazooxazine pretomanid (PA-824, 4). Initial phase III clinical results for the combination of 2, 3, and 4 (Nix-TB) in XDR-TB patients look highly encouraging, with 29 of 31 patients who completed the treatment and 6 month follow-up being cured. Another regimen that combines 2 and 4 with pyrazinamide (5) and moxifloxacin (6) is also demonstrating excellent bactericidal efficacy against both drug-sensitive- and MDR-TB.

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The mechanism of action of 4 involves bioreductive activation by a deazaflavin (F420)-dependent nitroreductase (Ddn), leading to several metabolites arising from reduction of the imidazole ring at C-3, including a des-nitro derivative. Formation of the latter correlates with the release of nitric oxide, a crucial element in the antibacterial activity of M. tuberculosis (M. tb) by this isomeric compound. This result suggested a different biological target. This result could be of particular significance in the event of future resistance to 4. Nevertheless, in the related nitroimidazoazole class (cf. 1), we discovered that relocating the nitro group from C6 to C5 reduced the aerobic MIC against M. tb by at least 2 orders of magnitude. During initial attempts to optimize a large-scale synthesis of 4, two trace byproducts were isolated (<0.5%), and one of these was postulated to be the 3-nitro isomer, although its nuclear magnetic resonance (NMR) data did not match that of the expected 4-nitroimidazole derivative. To establish its identity, we sought to make this 3-nitroimidazooxazine analogue of 4 via an unambiguous chiral synthesis. Concurrently, we also targeted the second compound, thought to be a 3′-methyl derivative of 4. We now report the intriguing findings from this study, including some preliminary biological assessments.

To prepare the 3-nitro isomer of 4, we first investigated the method of Kim22 (Scheme 1A). Reaction of 2-chloro-4-nitroimidazole (10) with the TBS ether derivative of R-glycidol (11) gave two products in a ratio of ~3:1, with the major one being the expected 4-nitroimidazole derivative 12, as described. However, after low-temperature crystallization of the more polar oily minor product (reportedly the 5-nitroimidazole isomer of 4) with 1276000, MeOH, 0–20 °C, 1.5 h; then aq. citric acid; (xii) NaBH4, MeOH, 0–20 °C, 1.5 h; (xiii) HBr, AcOH, 20 °C, 13 h.

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During initial attempts to optimize a large-scale synthesis of 4, two trace byproducts were isolated (<0.5%), and one of these was postulated to be the 3-nitro isomer, although its nuclear magnetic resonance (NMR) data did not match that provided by Kim et al.22 To establish its identity, we sought to make this 3-nitroimidazooxazine analogue of 4 via an unambiguous chiral synthesis. Concurrently, we also targeted the second compound, thought to be a 3′-methyl derivative of 4. We now report the intriguing findings from this study, including some preliminary biological assessments.

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experiment revealed a strong NOE effect between the imidazole proton resonance and the proton resonances from the directly attached N-methylene. HMBC correlations between H-5 and this NCH₃ carbon and between the NCH₂ protons and both C-2 and C-5 (Figure 2) confirmed that the correct structure of this minor product was the 4-nitroimidazole 16. This result is in good accordance with the known tendency of a TBS group to migrate under basic conditions, as employed here.⁴⁴

A critical consequence of this structural assignment error is that subsequent THP protection of the primary hydroxyl of 16 and treatment with TBAF (as reported) would induce cyclization to a 6-nitroimidazo[1,2-a]pyridin-6-ol in acetic anhydride,¹⁹ as claimed by Kim et al. as the correct structure of the compound, as verified by their identical ¹H and ¹³C NMR data. We note that while our compound had been prepared by an independent and unequivocal method, we have rigorously confirmed its structure by 2D NMR, including a NOESY experiment, where an NOE effect was observed between the imidazole proton resonance (H-5) and resonances from the adjacent methylene protons at the 3-position in the oxazole ring. This revised structure (20) for the compound claimed by Kim et al. as 8 is also consistent with the much smaller than expected optical rotation value (α_D^20 +7.4, cf. −44.7 for 4 in MeOH) as well as the moderate antitubercular potency recorded (vide infra), as S enantiomers are known to be an order of magnitude less effective than R forms in the 2H-nitroimidazooxazole class.²³

In an alternate approach to the 3-nitro isomer of 4, we explored the novel nitration of known¹¹ imidazooxazino alcohol 26 (Scheme 1B). This strategy was based on previous success in our laboratory with the nitration of a related 5,6,7,8-tetrahydroimidazo[1,2-a]pyridin-6-ol in acetic anhydride,¹⁹ as well as literature precedence for the regioselective C-5 nitration of both 1-methyl-2-(methylsulfanyl)-1H-imidazolo[2,3-b][1,3]thiazole.²⁰ During our initial synthesis of 26, we again encountered a problem with migration of the TBS group when glycidyl ether 11 was reacted with 2-nitroimidazole (21).²² Fortunately, this was easily overcome by switching the protecting group to the more stable TIPS protection (22).²³,²⁴,²⁵ We also made improvements to the subsequent THP protection and deprotection steps by adopting methods from Orita et al.,²⁵ enabling the synthesis of alcohol 26 in 65% yield over four steps. Surprisingly, attempted preparation of its acetate ester (30) using acetyl chloride in pyridine gave only ring opened N-acetyl imidazol-2-one products, 27 and 28 (having a distinctive ¹H NMR methyl resonance at δ_H 2.64 ppm); this was largely circumvented by employing acetic anhydride.

Several reagent systems were then explored for the nitration of 26 or 30 (Table 1). Neither heating in aqueous nitric acid nor treatment with acetyl nitrate in acetic anhydride gave the desired product, while a reaction of 30 with nitronium tetrafluoroborate was low-yielding and accompanied by minor side products. Overall, the best conditions were 70% nitric acid in trifluoroacetic anhydride²⁹ or a mixture of concentrated nitric and sulfuric acids in acetic anhydride, which enabled yields of ∼25–30% of the 3-nitro derivatives, 7 and 33, respectively. Intriguingly, only one nitro isomer was formed in trifluoroacetic anhydride, but this reaction proved to be more capricious than the acetic anhydride alternative, where the isomer ratio was ∼9:1 in favor of nitration at C-3 over C-2 (for confirmation, known²⁵ 2-nitro acetate ester 32 was prepared from alcohol 31).²⁵ Isomers 32 and 33 were separable by careful column chromatography, and cleavage of acetate 33 to alcohol 7 was cleanly achieved through the use of a mild base (NaHCO₃).

Importantly, all of the successful nitration methods gave a complete retention of stereochemistry (100% ee by chiral HPLC analysis, using the analogously synthesized racemic form of 7 as a reference standard). This indicated that the in situ formed trifluoroacetate or acetate esters were sufficiently stable to protect the chiral alcohol from potential racemization, e.g., through the formation of a chiral nitrate ester and subsequent hydrolysis, as this can induce inversion of configuration.³¹ Alkylation of pure 7 with 4-(trifluoromethoxy)benzyl bromide (NaH/DMF) then gave the desired target 8 in excellent yield (95%), and gratifyingly, this proved to be identical (by NMR, mp, HPLC, and optical rotation) to the first byproduct derived from optimization studies for a large-scale process route to 4. As expected from findings in the nitromidazooxazole class, the rotation value for 8 (α_D^24 −150.7 (c 1.002, CHCl₃)) was indeed much larger than the one recorded for 4.²⁵ Conclusive structural proof was gained through a single-crystal X-ray diagram.

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**Table 1. Summary of Nitration Methods Explored for the Synthesis of Alcohol 7 or Acetate 33**

| compd | solvent | reagents (equiv) | temp range (°C) | time/temp | products (% yield) |
|-------|---------|-----------------|-----------------|-----------|-------------------|
| 26    | H₂O     | 3 M HNO₃ (9)    | 80 to 97        | 3 h/97°C  |                   |
| 26    | H₂O     | 50% HNO₃ (>100) | 90              | 2 h/90°C  |                   |
| 26    | Ac₂O    | 70% HNO₃ (3.1)²⁶| −15 to 20       | 16 h/20°C |                   |
| 26    | Ac₂O    | 96% H₂SO₄ (1.7), 100% HNO₃ (3.0) | −55 to 0 | 60 min/0 °C | 32 (<3), 33 (20)³² |
| 26    | Ac₂O    | 96% H₂SO₄ (1.7), 70% HNO₃ (3.0) | −50 to 0 | 75 min/0 °C | 32 (<3), 33 (26)³² |
| 26    | Ac₂O    | 96% H₂SO₄ (2.5), KNO₃ (1.7) | −35 to −30 | 20 min/−30 °C | 32 (<2), 33 (14)³² |
| 26    | TFAA    | 70% HNO₃ (2.5−2.8) | −50 to 20 | 2−3 h/20 °C | 7 (12−35) |
| 30    | CH₃CN   | NO₂BF₄ (1.5)   | −48 to 20       | 90 min/20 °C | 30 (15), 32 (1), 33 (14)³² |
| 30    | Ac₂O    | 96% H₂SO₄ (1.9), 70% HNO₃ (3.2) | −50 to 0 | 30 min/0 °C | 32 (<3), 33 (28)³² |

²⁶Acetylated or trifluoroacetylated in situ, prior to nitration. ³²Preformed acetyl nitrate. ³³Yields after chromatography and crystallization.
structure (Figure 3); of note, the benzoxyl side chain adopted a pronounced pseudoaxial conformation at C6, the same as that observed in the previously reported crystal structure of 4.21

Figure 3. X-ray crystal structure of compound 8.

Preparation of the second byproduct from the synthesis of 4 was straightforward (Scheme 1C). Lithiation of bromotoluene 34 and quenching with DMF gave the required aldehyde 35, which was easily reduced (NaBH₄) and brominated to give 37. Alkylation of chiral alcohol 31 with bromide 37 then provided the expected compound 9, which was also identical to that derived from the nonoptimized process chemistry route for 4. This byproduct (<0.2%) was postulated to arise from traces of 37 in the bulk commercial 4-(trifluoromethoxy)benzyl bromide. Full experimental procedures and characterization data for all compounds have been provided in the Supporting Information.

The comparative data for 7–9 and several reference drugs against M. tb (strain H37Rv) were assessed in two in vitro assays, MABA13 and LORA,14 conducted under aerobic and hypoxic conditions, respectively. The latter assay employed bacteria preadapted to low oxygen conditions and represented a targeted screen for the identification of agents with better sterilizing ability against nonreplicating persistent bacteria. Recorded minimum inhibitory concentrations (MICs) corresponding to growth inhibitions of ≥90% (Table 2) were mean values obtained from replicate measurements (± standard deviation). Compounds 7–9 were also evaluated for cytotoxicity against mammalian cells (VERO) in a 72 h assay2 and found to be nontoxic (IC₅₀ > 128 μM).

Table 2. In Vitro Activities of 7–9 versus Other TB Drugs

| compd | MIC₉₀ (μM) | VERO IC₅₀ (μM) |
|-------|------------|---------------|
| 2     | 0.070 ± 0.018 | >10           |
| 3     | 2.9 ± 1.2   | >128          |
| 4'    | 0.30 ± 0.30 | >128          |
| 6     | 0.42 ± 0.13 | >128          |
| 7     | >128        | >128          |
| 8     | >128        | >128          |
| 9     | 0.065 ± 0.003 | 1.0 ± 0.1    |
| MET   | >512        | 79 ± 40       |
| RMP   | 0.049 ± 0.027 | 0.64 ± 0.35  |
| INH   | 0.34 ± 0.18 | >128          |

“Minimum inhibitory concentration against M. tb, determined under aerobic (MABA)13 or hypoxic (LORA)14 conditions. Each value is the mean of ≥2 independent determinations (7–9 were tested three times). The controls were metronidazole (MET), rifampicin (RMP), and isoniazid (INH). IC₅₀ values for cytotoxicity toward VERO cells.

As expected from our studies in the nitroimidazoazole class,23 3-nitro compounds 7 and 8 were completely inactive in both M. tb assays (MICs > 128 μM; Table 2). For 9, this indicates a >256-fold loss in activity in comparison to its 2-nitro isomer. Conversely, the 1′-methyl derivative of 4 (9) was seven- to eight-fold more active than both 4 and moxifloxacin (6) in MABA (similar to rifampicin and bedaquiline 2) and about three-fold better than both 4 and linezolid (3) in LORA (comparable to rifampicin). These results for 9 were in accordance with the findings reported by Cherian et al.16 for the 1′-methoxy derivative of 4, which was five-fold superior to 4 in the aerobic assay and two-fold more effective than 4 under hypoxic conditions.

In their original investigation, Kim et al.22 reported an aerobic MIC₉₀ value of 4–8 μM and weak anaerobic activity (MIC₉₀ 31 μM) for the compound they believed to be 8 (shown here to be the 2H-nitroimidazoazole 20). Intriguingly, the same compound also yielded an anaerobic MIC₉₀ value of 62.5–125 μM against M. tb having mutations in the nitroreductase Ddn, suggesting the participation of a different biological target. While delamanid (1) was shown to be primarily triggered by Ddn, several Ddn homologues have been implicated in the cellular activation of simple 2H-nitroimidazoazoxazines (e.g., 2-Et, 2-Ph).34 However, in the case of 20, the lack of significant potency against this Ddn mutant under aerobic conditions (MIC₉₀ > 100 μM) renders this explanation unsatisfactory. Overall, the results of the current investigation support findings from previous studies of resistance to 4, that the activation of nitroimidazoazoxazines relies exclusively on Ddn.35 In this class, relocation of the nitro group from C-2 to C-3 destroys all antitubercular activity, implying that, like the R-enantiomer of 4,34 8 is not a substrate for Ddn or its homologues and does not release nitric oxide.

In summary, we set out to establish the identities of two novel byproducts from optimization studies around a manufacturing route to 4 through a combination of 2D NMR analysis and de novo chiral synthesis. In the case of 3-nitro isomer 8, this entailed the development of an innovative nitration route, following our discovery of a critical structural assignment error in the published method, and we obtained an X-ray crystal structure of this compound for final confirmation. Preliminary in vitro assessments indicated that, whereas the 3′-methyl derivative of 4 (9) was markedly more effective than 4, both 8 and its alcohol precursor 7 were completely inactive, overturning previous misconceptions regarding their aerobic and anaerobic activities and the suggested involvement of another target facilitating their activity against Ddn mutant M. tb. These results provide further clarity of fundamental SARs for pretomanid and of the structural features of antitubercular nitroimidazoles that are more likely to overcome any future clinical resistance to 4.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.7b00356.

Further background, scheme for preparation of racemic 7, experimental procedures and characterizations for compounds, combustion analytical data, packing diagram for X-ray structure of 8, crystallographic data, NMR...
s spectra for key compounds, chiral HPLC trace for 7 (PDF)

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Notes
The authors declare no competing financial interest.

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■ REFERENCES
TB, tuberculosis; *M. tb* Mycobacterium tuberculosis; MDR, multidrug-resistant; XDR, extensively drug-resistant; Ddn, deazafavlin-dependent nitroreductase

(1) Global Tuberculosis Report 2016; World Health Organization: Geneva, Switzerland, 2016.
(2) Alffenaar, J.-W. C.; Akkerman, O. W.; Anthony, R. M.; Tiberi, S.; Heyssels, S.; Grobusch, M. P.; Cobelens, F. G.; van Soolingen, D. Individualizing management of extensively drug-resistant tuberculosis: diagnostics, treatment, and biomarkers. Expert Rev. Anti-Infect. Ther. 2017, 15, 11–21.
(3) Dheda, K.; Gumbo, T.; Gandhi, N. R.; Murray, M.; Theron, G.; Udawadia, Z.; Migliori, G. B.; Warren, R. Global control of tuberculosis: from extensively drug-resistant to treatable tuberculosis. Lancet Respir. Med. 2014, 2, 321–338.
(4) Dheda, K.; Limbers, J. D.; Pietersen, E.; Phelan, J.; Esmail, A.; Lesosky, M.; Fennelly, K. P.; te Riele, J.; Mastrapa, B.; Streicher, E. M.; Dolby, T.; Abdallah, A. M.; Ben-Rached, F.; Simpson, J.; Smith, L.; Lesosky, M.; Fennelly, K. P.; te Riele, J.; Mastrapa, B.; Streicher, E. M.; Gandhi, N. R.; Gandhi, N. R.; M. Transmission of extensively drug-resistant tuberculosis in South Africa. N. Engl. J. Med. 2017, 376, 243–253.
(5) Shah, N. S.; Auld, S. C.; Brust, J. C. M.; Mathema, B.; Ismail, N.; Moodley, P.; Mislan, K.; Allana, S.; Campbell, A.; Mihayane, T.; Morris, N.; Mpangase, P.; van der Meulen, H.; Omar, S. V.; Brown, T. S.; Nahid, A.; Shankina, E.; Kapa, T.; Kreiswirth, B.; Gandhi, N. R.; Gandhi, N. R.; N. Transmission of extensively drug-resistant tuberculosis in South Africa. N. Engl. J. Med. 2017, 376, 243–253.
(6) Gualano, G.; Capone, S.; Matteelli, A.; Palmieri, F. New antituberculosis drugs: from clinical trial to programmatic use. Infect. Dis. Rep. 2016, 8, 6569.
(7) Dawson, R.; Dacor, A. H.; Everitt, D.; van Niekerk, C.; Donald, P. R.; Burger, D. A.; Schall, R.; Spigelman, M.; Conradie, A.; Eisenach, K.; Venter, A.; Ise, P.; Page, Shipp, L.; Varela, E.; Reither, K.; K. Nitinay, E. N.; Dym, A.; von Groote-Bijldingmaier, F.; Mendel, C. M. Efficiency and safety of the combination of moxifloxacin, pretomanid (PA-824), and pyrazinamide during the first 8 weeks of antituberculosis treatment: a phase 2b, open-label, partly randomised trial in patients with drug-susceptible or drug-resistant pulmonary tuberculosis. Lancet 2015, 385, 1738–1747.
(8) Murray, S.; Mendel, C.; Spigelman, M. TB Alliance region development for multidrug-resistant tuberculosis. Int. J. Tuberc. Lung Dis. 2016, 20 (Suppl.1), S38–S41.
(9) Alcorn, K. Nine-TB trial: Good results for three-drug regimen against XDR-TB (BPaL). www.tbonline.info/posts/2017/2/20/good-results-three-drug-regimen-against-xdr-tb/(accessed 30 April, 2017).
(10) Dawson, R.; Harris, K.; Conradie, A.; Burger, D.; Murray, S.; Mendel, C.; Spigelman, M. Efficacy of bedaquiline, pretomanid, moxifloxacin & PZA (BPAZM) against DS- & MDR-TB. In Abstracts of Papers for Conference on Retroviruses and Opportunistic Infections (CROI 2017), Seattle, WA, February 13–16, 2017; 724LB. http://www.croiconference.org/sessions/effects-bedaquiline-pretomanid-moxifloxacin-pza-bpamz-against-ds-mdr-tb/(accessed 30 April, 2017).
(11) Singh, R.; Manjunatha, U.; Boshoff, H. I. M.; Ha, Y. H.; Niyomrattanakit, P.; Ledwidge, R.; Dowd, C. S.; Lee, I. Y.; Kim, P.; Zhang, L.; Kang, S.; Keller, T. H.; Jiricek, J.; Barry, C. E. PA-824 kills nonreplicating Mycobacterium tuberculosis by intracellular NO release. Science 2008, 322, 1392–1395.
(12) Tyagi, S.; Nierementer, E.; Yoshimatsu, T.; Williams, K.; Rosenthal, I.; Lounis, N.; Bishai, W.; Grosset, J. Bacterial activity of the nitroimidazopyrnan PA-824 in a murine model of tuberculosis. Antimicrob. Agents Chemother. 2005, 49, 2289–2293.
(13) Manjunatha, U.; Boshoff, H. I. M.; Barry, C. E. The mechanism of action of PA-824: novel insights from transcriptional profiling. Commun. Integr. Biol. 2009, 2, 215–218.
(14) Carroll, M. W.; Jeon, D.; Mounta, J. M.; Lee, J. D.; Jeong, Y. J.; Zia, N.; Lee, M.; Lee, J.; Via, L. E.; Lee, S.; Eum, S.-Y.; Lee, S.; Goldfeld, L. C.; Cai, Y.; Jin, B.; Kim, Y.; Oh, T.; Chen, R. Y.; Dodd, L. E.; Gu, D.; Dartois, V.; Park, S.-K.; Kim, C. T.; Barry, C. E., et al. Efficacy and safety of metronidazole for pulmonary multidrug-resistant tuberculosis. Antimicrob. Agents Chemother. 2013, 57, 3903–3909.
(15) Palmer, B. D.; Sutherland, H. S.; Blaser, A.; Kmentova, I.; Franzbau, S. G.; Wan, B.; Wang, Y.; Ma; Z.; Denny, W. A.; Thompson, A. M. Synthesis and structure-activity relationships for extended side chain analogues of the antitubercular drug (6S)-2-nitro-6-[[4-(trifluoromethoxy)benzyl][oxy]-6,7-dihydro-SH-imidazol[1,5-b][1,3]oxazine (PA-824). J. Med. Chem. 2015, 58, 3036–3059.
(16) Cherian, J.; Choi, I.; Nayar, A.; Manjunatha, U. H.; Mukherjee, T.; Lee, Y. S.; Boshoff, H. I. L.; Singh, R.; Ha, Y. H.; Goodwin, M.; Lakshminarayana, S. B.; Niyomrattanakit; P.; Jiricek, J.; Ravindran, S.; Dick, T.; Keller, T. H.; Barry, C. E.; III Structure-activity relationships of antitubercular nitroimidazoles. 3. Exploration of the linker and lipophilic tail of (5S)-2-nitro-6,7-dihydro-SH-imidazol[1,5-b][1,3]oxazin-6-yl-(4-trifluoromethylbenzyl)amine (6-amino PA-824). J. Med. Chem. 2011, 54, 5639–5659.
(17) Upton, A. M.; Cho, S.; Yang, T. J.; Kim, Y.; Wang, Y.; Lu, Y.; Wang, B.; Xu, J.; Mdluli, K.; Ma, Z.; Franzbau, S. G. In vitro and in vivo activities of the nitroimidazole TBA-354 against Mycobacterium tuberculosis. Antimicrob. Agents Chemother. 2015, 59, 136–144.
(18) Thompson, A. M.; Blaser, A.; Palmer, B. D.; Franzbau, S. G.; Wan, B.; Wang, Y.; Ma, Z.; Denny, W. A. Biazyloxymethoxy-2-nitroimidazoxazine antituberculosis agents: effects of proximal ring substitution and linker reversal on metabolism and efficacy. Bioorg. Med. Chem. Lett. 2015, 25, 3804–3809.
(19) Thompson, A. M.; Blaser, A.; Anderson, R. F.; Shinde, S. S.; Franzbau, S. G.; Ma, Z.; Denny, W. A.; Palmer, B. D. Synthesis, reduction potentials, and antitubicular activity of ring A/B analogues of the bioreductive drug (6S)-2-nitro-6-[[4-(trifluoromethoxy)-benzyl][oxy]-6,7-dihydro-SH-imidazol[1,5-b][1,3]oxazine (PA-824). J. Med. Chem. 2009, 52, 637–645.
(20) Kim, P.; Kang, S.; Boshoff, H. I.; Jiricek, J.; Collins, M.; Singh, R.; Manjunatha, U. H.; Niyomrattanakit, P.; Zhang, L.; Goodwin, M.; Dick, T.; Keller, T. H.; Dowd, C. S.; Barry, C. E.; III Structure-activity relationships of antitubercular nitroimidazoles. 2. Determinants of aerobic activity and quantitative structure-activity relationships. J. Med. Chem. 2009, 52, 1329–1344.
(21) Li, X.; Manjunatha, U. H.; Goodwin, M. B.; Knox, J. E.; Lipinski, C. A.; Keller, T. H.; Barry, C. E.; Dowd, C. S. Synthesis and
antitubercular activity of 7-(R)- and 7-(S)-methyl-2-nitro-6-(S)-(4-
(trifluoromethoxy)benzyloxy)-6,7-dihydro-5H-imidazo[2,1-b][1,3]-
oxazines, analogues of PA-824. Bioorg. Med. Chem. Lett. 2008, 18,
2256–2262.

(22) Kim, P.; Zhang, L.; Manjunatha, U. H.; Singh, R.; Patel, S.;
Jiricek, J.; Keller, T. H.; Boshoff, H. I.; Barry, C. E.; Dowd, C. S.
Structure-activity relationships of antitubercular nitroimidazoles. 1.
Structural features associated with aerobic and anaerobic activities of 4-
and 5-nitroimidazoles. J. Med. Chem. 2009, 52, 1317–1328.

(23) Thompson, A. M.; O’Connor, P. D.; Blaser, A.; Yardley, V.;
Maes, L.; Gupta, S.; Launay, D.; Martin, D.; Franzblau, S. G.; Wan, B;
Wang, Y.; Ma, Z.; Denny, W. A. Repositioning antitubercular 6-nitro-
2,3-dihydroimidazo[2,1-b][1,3]oxazoles for neglected tropical dis-
eases: structure-activity studies on a preclinical candidate for visceral
leishmaniasis. J. Med. Chem. 2016, 59, 2530–2550.

(24) Wuts, P. G. M.; Greene, T. W. Greene’s Protective Groups in
Organic Synthesis, 4th ed.; Wiley-Interscience: New Jersey, 2007; pp
166–171.

(25) Orita, A.; Miwa, K.; Uehara, G.; Otera, J. Integration of
solventless reaction in a multi-step process: application to an efficient
synthesis of PA-824. Adv. Synth. Catal. 2007, 349, 2136–2144.

(26) Chauvière, G.; Bouteille, B.; Enanga, B.; de Albuquerque, C.;
Croft, S. L.; Dumas, M.; Périé, J. Synthesis and biological activity of
nitro heterocycles analogous to megazol, a trypanocidal lead. J. Med.
Chem. 2003, 46, 427–440.

(27) Stratford, I. J.; Adams, G. E.; Hardy, C.; Hoe, S.; O’Neill, P.;
Sheldon, P. W. Thiol reactive nitroimidazoles: radiosensitization
studies in vitro and in vivo. Int. J. Radiat. Biol. Relat. Stud. Phys., Chem.
Med. 1984, 46, 731–745.

(28) Mloston, G.; Celeda, M.; Prakash, G. K. S.; Olah, G. A.;
Heimgartner, H. Synthesis of imidazole derivatives using 2-
unsubstituted 1H-imidazole 3-oxides. Helv. Chim. Acta 2000, 83,
728–738.

(29) Katritzky, A. R.; Scriven, E. F. V.; Majumder, S.; Akhmedova, R.
G.; Akhmedov, N. G.; Vakulenko, A. V. Direct nitration of five
membered heterocycles. ARKIVOC 2005, No. iii, 179–191.

(30) Baker, W. R.; Shaopei, C.; Keeler, E. L. Nitro-[2,1-b]imidazo-
opyran compounds and antibacterial uses thereof. U.S. Patent
6,087,358, 2000.

(31) Boschan, R.; Merrow, R. T.; van Dolah, R. W. The chemistry of
nitrate esters. Chem. Rev. 1955, 55, 485–510.

(32) Falzari, K.; Zhu, Z.; Pan, D.; Liu, H.; Hongmanee, P.; Franzblau,
S. G. In vitro and in vivo activities of macrolide derivatives against
Mycobacterium tuberculosis. Antimicrob. Agents Chemother. 2005, 49,
1447–1454.

(33) Cho, S. H.; Warit, S.; Wan, B.; Hwang, C. H.; Pauli, G. F.;
Franzblau, S. G. Low-oxygen-recovery assay for high-throughput
screening of compounds against nonreplicating Mycobacterium tuber-
culosis. Antimicrob. Agents Chemother. 2007, 51, 1380–1385.

(34) Gurumurthy, M.; Mukherjee, T.; Dowd, C. S.; Singh, R.;
Niyomrattanakit, P.; Tay, J. A.; Nayyar, A.; Lee, Y. S.; Cherian, J.;
Boshoff, H. I.; Dick, T.; Barry, C. E., III; Manjunatha, U. H. Substrate
specificity of the deazaflavin-dependent nitroreductase from
Mycobacterium tuberculosis responsible for the bioreductive activation of
bicyclic nitroimidazoles. FEBS J. 2012, 279, 113–125.

(35) Manjunatha, U. H.; Boshoff, H.; Dowd, C. S.; Zhang, L.; Albert,
T. J.; Norton, J. E.; Daniels, L.; Dick, T.; Pang, S. S.; Barry, C. E.
Identification of a nitroimidazo-oxazine-specific protein involved in
PA-824 resistance in Mycobacterium tuberculosis. Proc. Natl. Acad. Sci.
U. S. A. 2006, 103, 431–436.