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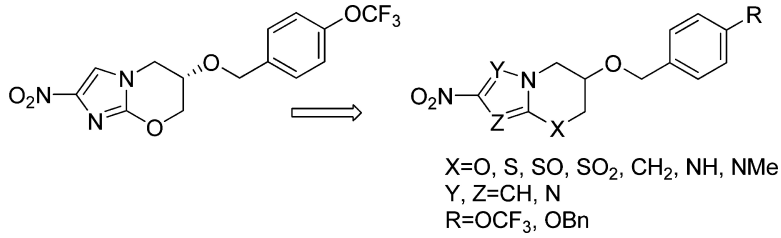

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Synthesis, Reduction Potentials, and Antitubercular Activity of Ring A/B Analogues of the Bioreductive Drug (6*S*)-2-Nitro-6-{[4-(trifluoromethoxy)benzyl]oxy}-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazine (PA-824)

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The nitroimidazooxazine *S*-**1** (PA-824) is a new class of bioreductive drug for tuberculosis. A series of related bicyclic nitroheterocycles was synthesized, designed to have a wide range of one-electron reduction potentials $E(1)$ (from −570 to −338 mV, compared with −534 mV for *S*-**1**). The observed $E(1)$ values closely correlated with the σ_m values of the heteroatom at the 4/8-position of the adjacent six-membered ring. Although the compounds spanned a range of $E(1)$ values around that of *S*-**1**, only the nitroimidazothiazines showed significant antitubercular activity (at a similar level of potency), suggesting that $E(1)$ is not the main driver of efficacy. Furthermore, there was a correlation between activity and the formation of imidazole ring-reduced products at the two-electron level, pointing to the potential importance of this reduction pathway, which is determined by the nature of the substituent at the 2-position of the 4-nitroimidazole ring.

Introduction

Tuberculosis (TB[a]) remains a leading infectious cause of death worldwide, but very few new drugs have been approved for TB treatment in the past 35 years, despite recent efforts.[1−3] The current drug therapy for TB is long and complex, involving multidrug combinations (usually isoniazid, rifampin, ethambutol, and pyrazinamide for the initial 2 months and rifampin and isoniazid for an additional 4 months).[4] The need for such lengthy treatment is largely because the drugs are relatively ineffective against the persistent form of the disease.[5] The recent introduction of the nitroimidazooxazine *S*-**1** (PA-824)[4] (Figure 1) to clinical trial by the Global Alliance for TB Drug Development is thus of potential significance, since this compound shows good in vitro and in vivo activity against *Mycobacterium tuberculosis* (*M. tb*) in both its active and persistent forms.[4−8]

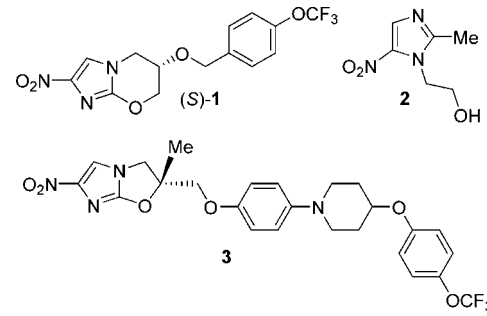

Figure 1. Structures of nitroheterocyclic tuberculosis drugs.

The mechanism of action of *S*-**1** is thought[9] to involve reduction of the nitro group, in a process dependent on the bacterial glucose-6-phosphate dehydrogenase (FGD1) and its cofactor F_{420}. More recent studies[10] on mutant strains showed that a 151-amino acid (17.37 kDa) protein of unknown function, Rv3547, also appears to be critical for this activation. Equivalent genes are present in *M. bovis* and *M. avium*.

Initial cyclic voltammetry studies[11] on *S*-**1** in aqueous solution suggested that it had a one-electron reduction potential [$E(1)$] broadly similar to that of metronidazole (**2**), which has an $E(1)$ of −486 mV[12] and undergoes a similar four-electron reduction. However, later voltammetry work in aprotic media indicated that the reduction peak potential of *S*-**1** was about 200 mV more negative than that of **2**, with the nitro radical anion being correspondingly 50- to 100-fold less stable.[13]

Many side chain analogues of *S*-**1** have been synthesized, although scarcely any biological assay data were reported on these (particularly against *M. tb*).[9,14,15] Very recently, the synthesis and antitubercular activity of 7-methyl derivatives of *S*-**1** were also described,[16] but there has been very little study of fundamental chromophore variations that preserve the respective ring sizes of the nitroheterocyclic core.[15] However, related 6-nitroimidazo[2,1-*b*]oxazoles have been known for almost 2

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[a] Abbreviations: TB, tuberculosis; *M. tb*, *Mycobacterium tuberculosis*; FGD1, F_{420} dependent glucose-6-phosphate dehydrogenase; $E(1)$, one-electron reduction potential; TBDMS, *tert*-butyldimethylsilyl; THP, tetrahydropyranyl; TBAF, tetra-*n*-butylammonium fluoride; STIPS, triisopropylsilanethiol; *m*-CPBA, 3-chloroperbenzoic acid; NOESY, nuclear Overhauser enhancement spectroscopy; BOC, *tert*-butyl carbamate; NHE, normal hydrogen electrode; MIC, minimum inhibitory concentration; COSY, correlation spectroscopy; HSQC, heteronuclear single quantum coherence; HMBC, heteronuclear multiple bond correlation; HREIMS, high resolution electron impact mass spectrometry; HRFABMS, high resolution fast atom bombardment mass spectrometry; HRESMS, high resolution atmospheric pressure electrospray mass spectrometry; HRCIMS, high resolution chemical ionization mass spectrometry; DMF, *N*,*N*-dimethylformamide; THF, tetrahydrofuran; MsOH, methanesulfonic acid; ND, not determined; NP, no discernible peak; DIPEA, *N*,*N*-diisopropylethylamine; PPTS, pyridinium *p*-toluenesulfonate.

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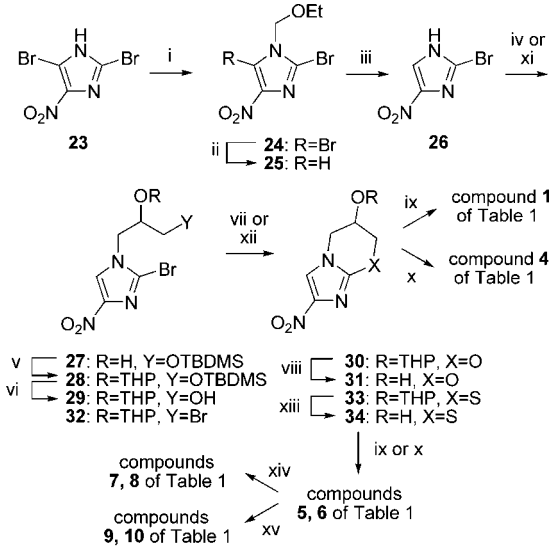
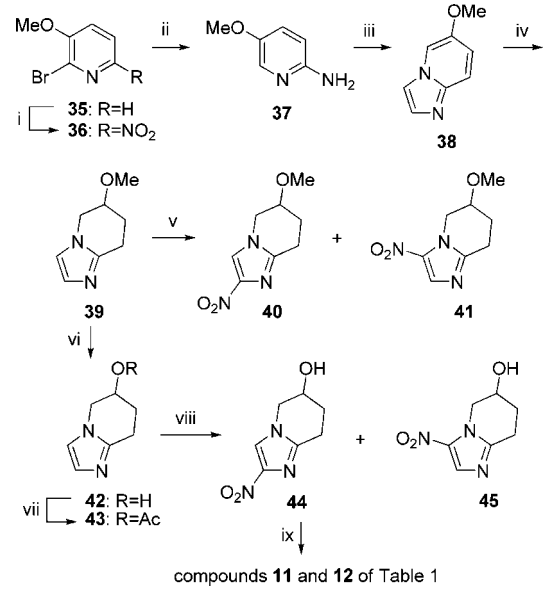
Scheme 1[a]



[a] Reagents and conditions: (i) $ClCH_2OEt$, NaH, DMF, 0−20 °C, 4 h; (ii) aqueous Na_2SO_3, DMF, 20 °C, 29 h; (iii) 5 N HCl, MeOH, 69 °C, 3 h; (iv) TBDMS glycidyl ether, DIPEA, toluene, 70 °C, 18 h; (v) dihydropyran, PPTS, CH_2Cl_2, 20 °C, 16 h; (vi) 1 M TBAF, THF, 20 °C, 1 h; (vii) NaH, DMF, 20 °C, 3 h; (viii) cat. MsOH, MeOH, 20 °C, 3 h; (ix) 4-F_3COBnBr, NaH, DMF, 20 °C, 24 h; (x) 4-BnOBnCl, NaH, DMF, 20 °C, 16−24 h; (xi) $BrCH_2CH(OTHP)CH_2Br$, K_2CO_3, DMF, 80 °C, 18 h; (xii) LiSTIPS, THF, −78 to 20 °C, 18 h, then 1 M TBAF, THF, 20 °C, 1 h; (xiii) 2 N HCl, MeOH, 20 °C, 3 h; (xiv) Davis reagent, CH_2Cl_2, 20 °C, 4 days; (xv) *m*-CPBA, CH_2Cl_2, 20 °C, 72 h.

Scheme 2[a]



[a] Reagents and conditions: (i) fuming HNO_3−conc H_2SO_4, 2−55 °C, 1 h; (ii) H_2, 5% Pd−C, KOAc, EtOAc−MeOH, 20 °C, 5 h; (iii) $ClCH_2CHO$, $NaHCO_3$, aq MeOH, reflux, 2 h; (iv) H_2, 10% Pd−C, AcOH, 20 °C, 6 h; (v) fuming HNO_3−conc H_2SO_4, 5−20 °C, 2 h; (vi) BBr_3, CH_2Cl_2, 20 °C, 3 h; (vii) Ac_2O, 20 °C, 15 min; (viii) fuming HNO_3−conc H_2SO_4, Ac_2O, −10 to 10 °C, 2 h, then 3 N KOH, MeOH, 20 °C, 15 min; (ix) 4-F_3COBnBr or 4-BnOBnCl, NaH, DMF, 20 °C, 2.5−6 h.

decades to possess potent antitubercular activity[17] and include a recent clinical candidate, **3** (OPC-67683).[18] Considering the apparent importance of nitro reduction in the activation of *S*-**1** and the variation in nitro reduction potentials and electron affinity that can be effected by changing the electronics of the nitroheterocycle, we report here the synthesis, $E(1)$ values, and preliminary biological evaluation of ring A/B analogues of *S*-**1**, together with a brief investigation of the radical reduction chemistry of selected compounds.

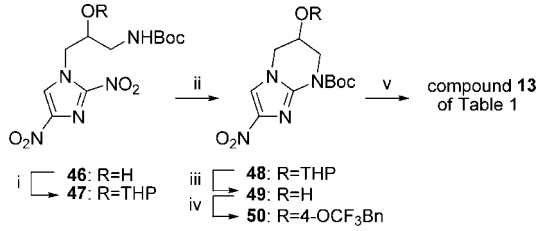
Results and Discussion

Compound Synthesis. Both the original synthesis[15] of *S*-**1** and some recently reported synthetic improvements[19] employed the explosive intermediate 2,4-dinitroimidazole for the initial ring-opening reaction of a glycidyl silyl ether. In the interests of improving safety, an alternative synthesis of racemic **1** was developed from 2-bromo-4-nitroimidazole, based partly on work reported by Goto[20] (Scheme 1). 2-Bromo-4(5)-nitroimidazole (**26**) was prepared as reported[20] from 2,5-dibromo-4-nitroimidazole[21] (**23**) except that ethoxymethyl was used as the N-protecting group (intermediate **24**). Reduction of **24** with sodium sulfite to **25** followed by cleavage of the protecting group with 5 N HCl gave **26** in very good overall yield from **23** (increased from 48%[20] to 76%). Reaction of **26** with the TBDMS ether of racemic glycidol gave predominantly the 4-nitroimidazole **27**, together with a trace of its 5-nitro isomer. THP protection of the secondary hydroxyl group in **27** gave the known[20] ether **28**, which was treated with excess TBAF at room temperature to give the primary alcohol[20] **29**. The latter was partly converted into the oxazine **30** during the desilylation reaction, and treatment of the crude product with sodium hydride at room temperature[20] completed the cyclization, giving **30** in 55% overall yield from **28**. Deprotection of the THP ether followed by reaction of the resulting alcohol **31** with sodium hydride and the appropriate benzyl bromide gave racemic **1**. Similar reaction of **31** with 4-benzyloxybenzyl chloride gave the racemic ether **4**.

The sulfur-linked analogues **5**−**10** were also prepared from 2-bromo-4-nitroimidazole (**26**) (Scheme 1). Reaction of **26** with excess THP-protected 1,3-dibromo-2-propanol[22] at 80 °C gave the dibromide **32** in moderate yield, with little formation of dimeric material or the 5-nitro isomer. Reaction of **32** with the lithium salt of triisopropylsilanethiol (STIPS) gave a product with a mass consistent with substitution of bromide by STIPS, which ^{1}H NMR spectroscopy indicated was a mixture resulting from displacement of the 2-bromo group or the side chain bromine. Treatment of this crude product with TBAF gave the sulfide **33** in good yield, which was deprotected to the alcohol **34** and then converted into the benzyl ethers **5** and **6** as above. Oxidation of **5** and **6** with Davis' oxaziridine reagent was slow but eventually afforded the sulfoxides **7** and **8**. Alternatively, oxidation of **5** and **6** with *m*-CPBA gave the corresponding sulfones **9** and **10**.

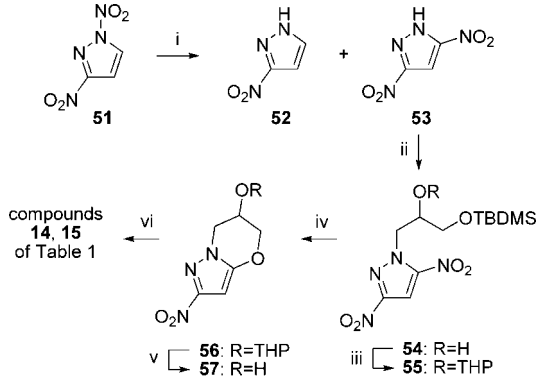
The carbon-linked compounds were prepared as shown in Scheme 2. The 2-aminopyridine **37** reacted with chloroacetaldehyde to give imidazopyridine **38** in good yield. Several conditions were studied for hydrogenation of the pyridine ring of **38** to give **39**. Reduction in acetic acid using PtO_2 as catalyst led to concomitant hydrogenolysis of the methyl ether group, giving a product that was contaminated with the alcohol analogue. However, changing the catalyst to Pd−C suppressed this, giving the desired product in quantitative yield. Nitration of **39** could be achieved using fuming HNO_3 and concentrated H_2SO_4 at room temperature to give a 29% yield of a 1:1 mixture of the 2- and 3-nitro derivatives (**40** and **41**, respectively), which could be separated by preparative reversed phase HPLC. A variety of reagents were investigated for demethylation of the mixture, largely without success. However, the precursor imidazole **39** without a nitro group was demethylated rapidly and cleanly with BBr_3, giving alcohol **42** in good yield, together with a small amount of the corresponding secondary bromide.

Scheme 3[a]



[a] Reagents and conditions: (i) dihydropyran, PPTS, CH_2Cl_2, 20 °C, 18 h; (ii) NaH, DMF, 20 °C, 3 h; (iii) cat. MsOH, MeOH, 20 °C, 3 h; (iv) 4-F_3COBnBr, NaH, DMF, 20 °C, 1 h; (v) 1 M LiOMe, MeOH, 20 °C, 20 min.

Scheme 4[a]



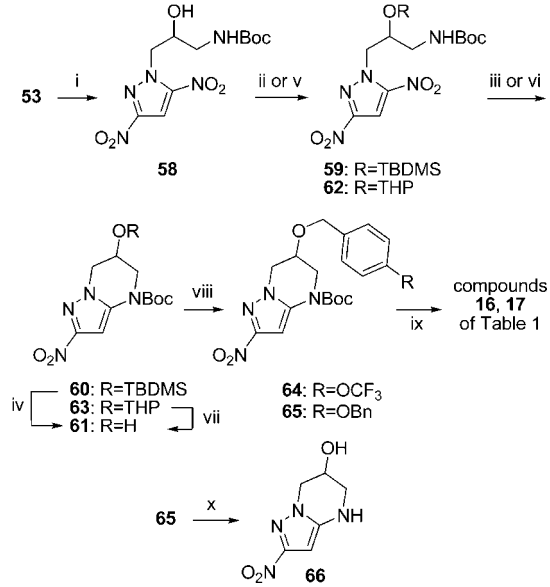
[a] Reagents and conditions: (i) anisole, 160 °C, 60 h; (ii) TBDMS glycidyl ether, DIPEA, toluene, 68 °C, 28 h; (iii) dihydropyran, PPTS, CH_2Cl_2, 20 °C, 1 day; (iv) 1 M TBAF, THF, 20 °C, 13 h; (v) 10% HCl, MeOH, 20 °C, 3 h; (vi) 4-F_3COBnBr or 4-BnOBnCl, NaH, DMF, 0−20 °C, 16−24 h.

In situ acetylation of **42** followed by nitration using acetic anhydride as the solvent and then base hydrolysis of the acetate group gave a ∼1:1 mixture of the 2- and 3-nitro isomers **44** and **45** in 29% yield. The isomers could be separated with difficulty by chromatography on silica, and assignment of the regiochemistry of the nitro groups was confirmed by 2D NMR experiments, which established a correlation between the imidazole ring proton and the NCH_2 methylene group of **44** in a NOESY spectrum. Reaction of a mixture of **44** and **45** with 4-(trifluoromethoxy)benzyl bromide and sodium hydride, followed by purification of the product using preparative reversed phase HPLC, gave the desired 2-nitro isomer **11** in poor yield, together with a trace of the corresponding 3-nitro isomer. Similar reaction of the alcohol mixture with 4-benzyloxybenzyl chloride and NaH gave the analogous benzyloxybenzyl ether derivative **12**.

The (NH) alcohol precursor to imidazopyrimidine **13** has been described previously,[15] but in that report the NH was then methylated prior to coupling of the benzyloxybenzyl side chain. To prepare **13**, we elected to form the THP ether **47** from known[15] BOC-protected aminoalcohol **46** and to cyclize this to give the fully protected precursor **48** (Scheme 3). Selective removal of the THP group with methanesulfonic acid and alkylation of the resulting alcohol **49** with 4-(trifluoromethoxy-)benzyl bromide and sodium hydride yielded the BOC-protected imidazopyrimidine **50**, which could be deprotected using lithium methoxide. The last two steps (alkylation and BOC deprotection) gave disappointingly low yields but provided **13** in sufficient quantity for characterization and biological testing.

Synthesis of the novel 2-nitropyrazolo[5,1-*b*][1,3]oxazine scaffold is described in Scheme 4. The preparation of the known[23] 3,5-dinitropyrazole (**53**) by thermal rearrangement of

Scheme 5[a]

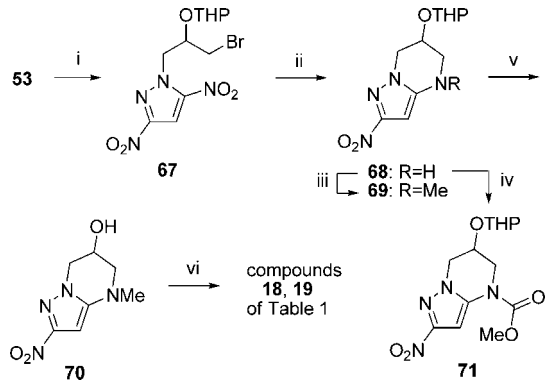


[a] Reagents and conditions: (i) glycidyl-NHBOC, 2,6-lutidine, toluene, 65 °C, 14 h; (ii) TBDMSCl, imidazole, DMF, 20 °C, 46 h; (iii) NaH, DMF, 0−20 °C, 40 min; (iv) 1 M TBAF, THF, 20 °C, 3.5 h; (v) dihydropyran, PPTS, CH_2Cl_2, 20 °C, 6 h; (vi) NaH, DMF, 0−20 °C, 20 min; (vii) PPTS, EtOH, 55 °C, 20 h; (viii) 4-F_3COBnBr or 4-BnOBnI, NaH, DMF, 0−20 °C, 40−60 min; (ix) 1 M LiOMe, MeOH, 54 °C, 15−23 h; (x) 1.25 M HCl, MeOH, 20−42 °C, 1−4 days.

1,3-dinitropyrazole (**51**) was conveniently achieved in anisole at 160 °C (rather than the higher boiling benzonitrile as described[23]) to give **53** without changing the proportion of 3-nitropyrazole (**52**) obtained as an impurity in the crude product material (10−12%). The latter was removed from **53** by pH-dependent extraction, based on the large difference in their pK_a values[24] (9.8 for **52** and 3.1 for **53**). The next step of the synthesis required an unprecedented intramolecular nitro displacement reaction on an N-alkylated derivative of **53** (by analogy with the synthesis of 2-nitroimidazo[2,1-*b*][1,3]-oxazines[15] and 6-nitroimidazo[2,1-*b*]oxazoles[17,25] from 2,4-dinitroimidazole). Thus, base catalyzed condensation of **53** with TBDMS-glycidyl ether gave the alcohol **54** and treatment of the corresponding THP derivative **55** with TBAF at room temperature resulted in spontaneous formation of the desired oxazine scaffold **56** in very high yield (92%). This was then deprotected to give alcohol **57**, which was elaborated to the required products (**14**, **15**) by alkylation reactions, as above.

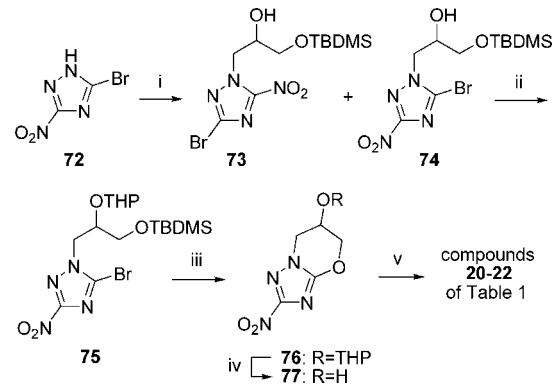
The novel 2-nitropyrazolo[1,5-*a*][1,3]pyrimidine scaffold was prepared in a similar manner (again, by analogy with the synthesis of 2-nitroimidazo[1,2-*a*]pyrimidines[15] from 2,4-dinitroimidazole). Thus, condensation of 3,5-dinitropyrazole **53** with the epoxide derived from *N*-BOC allylamine, followed by protection of the resultant alcohol **58** as the TBDMS ether, gave **59** (Scheme 5). Despite extensive attempts at optimization, the best yield of the product **60** from the following base catalyzed ring closure step was only 15% (substantial decomposition occurred). Alternative protection of the alcohol as the THP ether (**62**) resulted in an improved yield for this ring closure step (a 43% yield of the corresponding THP-protected product **63**), and the THP protecting group was then selectively removed in the presence of the *N*-BOC group using pyridinium *p*-toluenesulfonate in ethanol to give alcohol **61**. To minimize further compound losses due to the potential base lability of the BOC group of **61** (as suggested by low yields in the preceding ring closure reactions), the more reactive 4-benzyloxybenzyl iodide[26]

Scheme 6[a]



[a] Reagents and conditions: (i) $BrCH_2CH(OTHP)CH_2Br$, K_2CO_3, DMF, 85 °C, 22 h; (ii) LiSTIPS, THF, −78 to 20 °C, 19 h, then 1 M TBAF, THF, 20 °C, 4 h; (iii) MeI, NaH, DMF, 0−20 °C, 10 min; (iv) MeI, K_2CO_3, DMF, 20 °C, 49 h; (v) 10% HCl, MeOH, 20 °C, 3 h; (vi) 4-F_3COBnBr or 4-BnOBnCl, NaH, DMF, 0−20 °C, 5−6 h.

Scheme 7[a]



[a] Reagents and conditions: (i) TBDMS glycidyl ether, DIPEA, toluene, 70 °C, 24 h; (ii) dihydropyran, PPTS, CH_2Cl_2, 20 °C, 24 h; (iii) 1 M TBAF, THF, 20 °C, 3 h; (iv) 2 N HCl, MeOH, 20 °C, 2 h; (v) 4-F_3COBnBr or 4-BnOBnCl, NaH, DMF, 20 °C, 16−18 h.

was employed in the synthesis of **65**. Selective cleavage of the *N*-BOC group was then investigated. However, treatment of **65** with HCl in methanol (at 20 or 42 °C) resulted in the slow loss of both the 4-benzyloxybenzyl ether side chain and the *N*-BOC group to give alcohol **66**. Therefore, a rare base catalyzed cleavage of the *N*-BOC group was employed for both **65** and analogue **64** (lithium methoxide in methanol at 54 °C) to give the required products (**16**, **17**) in very good yield (73−76%).

An expeditious alternative route to the related *N*-methyl derivatives (**18**, **19**) was discovered during attempts to synthesize the novel 2-nitropyrazolo[5,1-*b*][1,3]thiazine scaffold (Scheme 6). Alkylation of **53** with 2-[2-bromo-1-(bromomethyl)ethoxy]tetrahydropyran gave **67** in modest yield, likely due to the reported propensity of this reagent to undergo base-catalyzed elimination.[22] Treatment of **67** with the lithium salt of triisopropylsilanethiol, followed by desilylation in situ, gave the 2-nitropyrazolo[1,5-*a*]pyrimidine **68** as the only isolable product. Formation of **68** requires a selective sulfide reduction of the nitro group adjacent to the side chain, followed by intramolecular alkylation, prior to sulfide displacement (the selective reduction of 1-methyl-3,5-dinitropyrazole with sodium hydrosulfide has been reported[27]). Surprisingly, methylation of **68** using iodomethane with potassium carbonate as the base in dimethylformamide gave methyl carbamate **71** as the sole product (67% yield). This was avoided by the use of sodium hydride, which gave the desired **69** quantitatively. This was then elaborated (via the alcohol **70**) to the required products (**18**, **19**) using standard methods.

The three compounds in the triazole series were prepared using the chemistry outlined in Scheme 7. Bromonitrotriazole **72** reacted with racemic TBDMS-protected glycidol under basic conditions to give approximately 1:1 mixture of regioisomers **73** and **74**, which were readily separated by chromatography. Their structures were assigned following detailed NMR studies, which established a three-bond correlation between the carbon atom bearing the nitro group in **73** and the side chain methylene proton atoms. The desired isomer (**74**) was elaborated to racemic alcohol **77** by successive THP protection and desilylation (with ring closure) reactions, and from this the benzyl ethers **20**−**22** were prepared by standard chemistry. Optical resolution of the racemic (trifluoromethoxy)benzyl ether to give **20** and **21** was achieved by preparative chiral HPLC using a Chiralcel OD column. The absolute configuration of these enantiomers was assigned by comparison of the sign of their optical rotations with those of the enantiomers of **1**.

One-Electron Reduction Potentials. Nitroheterocycles have been studied as potential antituberculosis agents for many years, but the nitroimidazooxazine *S*-**1** has stood out in recent times for its high in vivo activity and its ability to target both the active and persistent forms of the disease.[4,6−8] While selective bioreduction is likely to be the main mechanism for this, and much work is being invested in identification of the enzyme(s) responsible,[9,10] less work has been reported on the radical chemistry of *S*-**1**.

Table 1 reports thermodynamic reversible one-electron reduction potentials [*E*(1) vs NHE, in 5 mM phosphate buffer, pH 7.0] for racemic **1** and a series of related novel bicyclic nitroheterocycles. The compound set was designed to span a range of reduction potentials in order to evaluate the effects of this parameter on biological activity. Most of the compounds in Table 1 are racemic, for ease of synthesis, and are compared biologically with racemic **1**. While the *S*-enantiomers of nitroimidazooxazines such as **1** are reportedly at least 10-fold more potent than the *R*-enantiomers,[9] the racemates are only 2-fold less active. Since the 4-benzyloxybenzyl ether analogue *S*-**4** is known to be up to 9-fold more potent in vitro than *S*-**1** itself,[15] the benzyl ether side chains of both **1** and **4** were added to most of the new heterocycles.

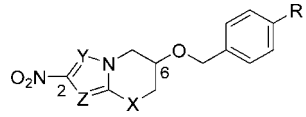
As shown in Table 1, we have recently reported[28] that the one-electron reduction potential [*E*(1)] of racemic **1** is −534 ± 7 mV, a value typical of that found for 4-nitroimidazoles[29] and distinctly lower than the value (−486 mV)[12] for the 5-nitroimidazole metronidazole (**2**) to which it is often compared. As expected, variation in the 6-substituent had little effect; the *E*(1) of the 6-OH analogue **31** was −532 ± 6 mV, and that of the corresponding 6-OMe analogue[28] was −527 ± 6 mV. This allowed the use with confidence of related more soluble analogues in place of other members of the series (**11**, **13**) which were too insoluble for *E*(1) values to be determined (see Table 1).

Within the imidazo series, the measured *E*(1) values of compounds with heteroatoms of varying electronegativity in the 8-position of the adjacent six-membered fused ring (compounds **1**, **5**, **7**, **9**, **11**, **13**) varied by nearly 100 mV. The measured potentials correlated very well with the σ_m values of the heteroatom (eq 1), whereas the use of σ_p values gave a much poorer correlation ($r = 0.761$).

$$E(1)\ (\text{mV}) = (110 \pm 16)\sigma_m - (547 \pm 10) \qquad (1)$$

$$n = 6\ r = 0.958\ s = 12.5\ F = 45.1$$

Table 1. Reduction Potentials, in Vitro Inhibitory Activity, and Selectivity of Various Chromophore Analogues of *S*-**1** (All Compounds except **20** and **21** Are Racemic)



compd	X	Y	Z	R	$E(1)^a$ (mV)	MIC^b (μM) MABA aerobic	MIC^b (μM) LORA anaerobic	$IC_{50}{}^c$ (μM), VERO
					Imidazole			
1	O	CH	N	OCF_3	-534 ± 7^d	1.1	4.4	>128
4	O	CH	N	OCH_2Ph		0.11	2.7	>128
5	S	CH	N	OCF_3	-534 ± 5	1.1	13	>128
6	S	CH	N	OCH_2Ph		1.1	11	>128
7	SO	CH	N	OCF_3	-476 ± 7	>128 (87)	92	>128
8	SO	CH	N	OCH_2Ph		>128 (23)	>128 (20)	63
9	SO_2	CH	N	OCF_3	-488 ± 7	>128 (89)	89	>128
10	SO_2	CH	N	OCH_2Ph		>128 (61)	>128 (79)	>128
11	CH_2	CH	N	OCF_3	$(-570 \pm 9)^e$	126	>128 (75)	ND
12	CH_2	CH	N	OCH_2Ph		>128 (5)	>128 (49)	ND
13	NH	CH	N	OCF_3	$(-568 \pm 6)^f$	>128 (63)	>128 (64)	ND
					Pyrazole			
14	O	N	CH	OCF_3	-500 ± 9	>128 (89)	>128 (86)	88
15	O	N	CH	OCH_2Ph		>128 (38)	>128 (19)	82
16	NH	N	CH	OCF_3	-517 ± 9	>128 (61)	111	ND
17	NH	N	CH	OCH_2Ph		>128 (55)	>128 (41)	ND
18	NMe	N	CH	OCF_3	-522 ± 10	>128 (61)	>128 (37)	>128
19	NMe	N	CH	OCH_2Ph		>128 (58)	>128 (3)	>128
					Triazole			
20	O	N	N	$OCF_3{}^g$		>128 (81)	112	>128
21	O	N	N	$OCF_3{}^h$	-338 ± 10	>128 (84)	120	>128
22	O	N	N	OCH_2Ph	(26)	>128 (18)	>128	

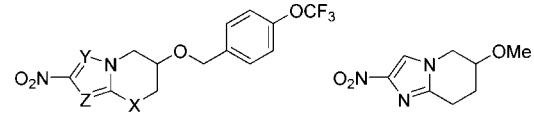
[a] $E(1)$: one-electron reduction potentials, determined by pulse radiolysis (see text). [b] MIC: minimum inhibitory concentration, determined under aerobic (MABA)[33] or anaerobic (LORA)[32] conditions. Numbers in parentheses are the % inhibition at 128 μM. [c] Cytotoxicity assay against VERO cells. ND means not determined. [d] Reference 28. [e] $E(1)$ data for 6-OMe analogue (**40**, Scheme 2). [f] $E(1)$ data for 6-OH analogue (example 11 of ref 15). [g] *S* enantiomer. [h] *R* enantiomer.

This suggests that good control over the reduction potential of the imidazo compounds can be achieved by the selection of this heteroatom.

The pyrazole derivatives (compounds **14**−**19**) showed the same trend but with a lesser degree of dependence of $E(1)$ values on the adjacent heteroatom, with a range of only 22 mV (there were insufficient examples for statistical analysis). Finally, the triazole compounds (**20**−**22**) had much higher $E(1)$ values, as expected[30] for this chromophore.

Radical Reduction Chemistry. The stepwise reduction of **1**, by its reaction with radiolytically produced $CO_2^{\bullet-}$ radicals, has revealed that the imidazole ring is reduced before the nitro group.[28] This order is atypical for nitroimidazoles, where reduction of the nitro group commonly precedes ring reduction. The initial product formed upon reduction of **1** under mildly acidic conditions (pH 4) has been shown by MS and NMR analyses to be consistent with the formation of a dihydroimidazole, leading to a $-NCH_2CHNO_2-$ system, which would result from the two-electron reduction of the C2−C3 bond of the imidazole ring.[28] Products obtained radiolytically under mildly acidic conditions at the two-electron reduced level may well represent the products formed upon a hydride ion transfer from the essential deazaflavin cofactor of F_{420}-dependent glucose-6-phosphate dehydrogenase present in the bacterium. The same methodology was used here to test for formation of the [M + 2] increased mass (dihydro) product in a subgroup of compounds, spanning a range of $E(1)$ values, in the imidazole class (**5**, **9**, **40**), as well as examples of both a pyrazole (**14**) and a triazole (**21**) (see Table 2). Only the thiazine derivative, **5**, formed such a product similar to that observed for **1** (although a very minor amount of a [M + 2] increased mass product was also seen for the alkyl derivative **40**, a more soluble analogue of **11**).

Table 2. Formation of Products after Reduction of Compounds to the Two-Electron Reduced Level by the $CO_2^{\bullet-}$ Radical at pH 4.0 in Aqueous Solution



A **B**

compd	X	λ_{max} of parent (nm)	ion mass of parent (g mol^{-1})	λ_{max} of product(s) (nm)	ion mass of product(s)[a] (g mol^{-1})
1 (**A**, Y = CH, Z = N)	O	355	360.6	262	362.6[b]
5 (**A**,Y = CH, Z = N)	S	349	376.6	275	378.7 (362.6) (360.6)
9 (**A**,Y = CH, Z = N)	SO_2	284	408.8	250	408.8 394.7
40 (**B**)		313	198.4	320[c]	198.5 (200.5)
14 (**A**,Y = N, Z = CH)	O	267	360.7	NP[d]	(360.7) 346.7 344.6
21 (**A**,Y = N, Z = N)	O	283	361.6	NP[d]	361.6 347.6

[a] Data in parentheses are for minor products. [b] Data from ref 28. [c] Product absorbs less than parent compound at this wavelength. [d] NP: no discernible peak in the UV−visible region.

Biological Activity. The compounds **1** and **4**−**22** were evaluated for their ability to inhibit *M. tb* in two assays (Table 1). The compounds were evaluated for activity (minimum inhibitory concentrations, MICs) against replicating *M. tb* in an 8 day microplate-based assay using Alamar blue reagent (added on day 7) for determination of growth (MABA).[31] The lowest drug concentration effecting an inhibition of >90% was considered the MIC. Screening for the activity of the compounds against bacteria in the nonreplicating state that models clinical persistence used an 11 day high-throughput, luminescence-based low-oxygen-recovery assay (LORA), where *M. tb* containing a plasmid with an acetamidase promoter driving a bacterial luciferase gene was first adapted to low oxygen conditions by extended culture.[32] Mammalian cytotoxicity was also assessed[33] against VERO cells (CCL-81, American Type Culture Collection) in a 72 h exposure, using a tetrazolium dye assay.

Compared to the racemic imidazooxazine **1**, the corresponding imidazothiazine analogue **5** showed the same or slightly (3-fold) reduced potencies in the MABA and LORA assays, respectively. However, unlike the imidazooxazine series (**4** versus **1**), the switch to a benzyloxybenzyl ether side chain did not significantly enhance the potencies of imidazothiazine **6** over those of analogue **5**. Oxidation of the sulfur atom in **5** and **6** (compounds **7**−**10**) markedly reduced activity, with the best analogues (**7** and **9**) showing a >100-fold potency loss in the MABA assay and a 7-fold potency loss in the LORA assay, compared to **5**. The imidazopyridine **11** displayed similarly weak activity (MABA MIC of 126 μM, 115-fold less than **1**), but again, the benzyloxybenzyl ether analogue (**12**) was even less active, as was the imidazopyrimidine **13**. Replacement of the imidazole ring of **1** by pyrazole (compound **14**) or triazole (compound **20**) also led to compounds with marginal activity (MICs close to 128 μM), although **14** was weakly cytotoxic. None of the pyrazolopyrimidine analogues **16**−**19** were significantly better than pyrazolooxazine **14**, despite having reduction potentials closer to that of *S*-**1**. Finally, two simple nitroimidazooxazine derivatives (compound **31** and its methyl ether derivative[28]) which lacked the lipophilic benzyl ether side chains of **1** and **4** were also tested in both MIC assays and found to be at least 90-fold less active than racemic **1** (MICs greater than 100 μM; data not shown in Table 1). This demonstrates the importance of both the nitroheterocyclic core and the lipophilic side chain in the activities of compounds **1**, **4**, **5**, and **6**.

Conclusions

This work provides new synthetic routes to a series of chromophore analogues (imidazoles, pyrazoles, and triazoles with varying heteroatoms in the 4/8-position of the adjacent fused ring) of the nitroimidazooxazine tuberculosis drug *S*-**1**. These compounds span a wide range of one-electron reduction potentials $E(1)$, from −570 to −338 mV, compared with −534 mV for *S*-**1**, which are closely correlated with the σ_m values of the adjacent heteroatom. Although many of the different nitroheterocyclic compounds have $E(1)$ values close to that of *S*-**1**, only the nitroimidazothiazines showed significant in vitro antitubercular activity (at a similar level of potency to the nitroimidazooxazines). This suggests that their absolute $E(1)$ values are not the major determinant of their activity, which may instead depend on their fit to the reducing enzyme and/or the nature of the reactive species formed and its subsequent reactions. The results are consistent with the nitroimidazooxazine chromophore having particular utility in tuberculosis chemotherapy, with specific substitution at the 2-position of the 4-nitroimidazole ring being a major determinant of activity. Furthermore, the present study points to a possible correlation between the formation of a dihydroimidazole ring-reduced product at the two-electron level and activity. This scenario is in line with the known involvement of a deazaflavin cofactor, a two-electron biological reductant, in the activation of *S*-**1**. While the lipophilic side chains of *S*-**1** and its analogues may well be a determinant for the fit of these compounds to the reducing enzyme, the anomalous reduction of the imidazo ring may also be an important factor in their activity against the *M. tb* bacterium.

Experimental Section

Analyses were carried out in the Microchemical Laboratory, University of Otago, Dunedin, New Zealand. Melting points were determined on an Electrothermal 2300 melting point apparatus. NMR spectra were obtained on a Bruker Avance 400 spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C spectra and are referenced to Me_4Si. Chemical shifts and coupling constants are recorded in units of ppm and Hz, respectively. Assignments were verified using COSY, HSQC, HMBC, and NOESY two-dimensional experiments, as required. High resolution electron impact (HREIMS) and fast atom bombardment (HRFABMS) mass spectra were determined on a VG-70SE mass spectrometer using an ionizing potential of 70 eV, at nominal resolutions of 3000, 5000, or 10 000 as appropriate. High resolution atmospheric pressure electrospray (HRESMS) and chemical ionization (HRCIMS) mass spectra were measured for methanol solutions on a ThermoFinnigan Quantum TSQ triple quadrupole mass spectrometer, operating in enhanced resolution mode; values are the average of five independent determinations. Thin-layer chromatography was carried out on aluminum-backed silica gel plates (Merck 60 F_{254}) with visualization of components by UV light (254 nm) or exposure to I_2. Column chromatography was carried out on silica gel (Merck 230−400 mesh). Preparative reversed phase HPLC and preparative chiral HPLC were carried out using a Gilson Unipoint system (322-H pump, 156 UV/visible detector) and a 250 mm × 21 mm Synergi-Max 4 μm C12 column or a 250 mm × 20 mm Chiralcel OD 10 μm column, respectively.

2,5-Dibromo-1-(ethoxymethyl)-4-nitro-1*H*-imidazole (24) (Scheme 1). A solution of 2,5-dibromo-4-nitro-1*H*-imidazole[21] (**23**) (26.49 g, 97.8 mmol) in dry DMF (130 mL) under N_2 at 0 °C was treated with 60% NaH (4.83 g, 121 mmol), then quickly degassed and resealed under N_2. After the mixture was stirred for 10 min at 0 °C, chloromethyl ethyl ether (10.0 mL, 117 mmol) was added dropwise, and the mixture was stirred at room temperature for 4 h. The resulting solution was poured into aqueous $NaHCO_3$ (1 L) and extracted with EtOAc (3 × 500 mL). The extracts were washed with brine (400 mL) and evaporated to dryness. The residue was triturated in CH_2Cl_2 (100 mL) and slowly diluted with petroleum ether (400 mL) to precipitate crude **24** (26.77 g, 83%) as a cream solid, which was used directly. Evaporation of the mother liquors to dryness and chromatography of the residue on silica gel, eluting with CH_2Cl_2/petroleum ether (1:2 and 2:3), followed by crystallization gave additional pure **24** (2.63 g, 8%) as a creamy white solid: mp (CH_2Cl_2/light petroleum) 98−100 °C; ^{1}H NMR ($CDCl_3$) δ 5.48 (s, 2 H), 3.64 (q, J = 7.0 Hz, 2 H), 1.25 (t, J = 7.0 Hz, 3 H). Anal. ($C_6H_7Br_2N_3O_3$) C, H, N.

2-Bromo-1-(ethoxymethyl)-4-nitro-1*H*-imidazole (25). A solution of **24** (28.27 g, 85.9 mmol) in DMF (166 mL) was treated with a solution of Na_2SO_3 (22.8 g, 181 mmol) in water (83 mL), and then the mixture was stirred at room temperature for 18 h. Further Na_2SO_3 (6.36 g, 50.5 mmol) was added, and then the mixture was stirred at room temperature for 11 h. The resulting mixture was poured into ice/aqueous $NaHCO_3$ (800 mL) and extracted with EtOAc (4 × 600 mL). The extracts were washed with brine (600 mL) and evaporated to dryness to give **25** (18.94 g, 88%) as a pale-yellow solid: mp (CH_2Cl_2/pentane) 62−63 °C; ^{1}H NMR ($CDCl_3$) δ 7.91 (s, 1 H), 5.37 (s, 2 H), 3.59 (q, J = 7.0 Hz, 2 H), 1.25 (t, J = 7.0 Hz, 3 H). Anal. ($C_6H_8BrN_3O_3$) C, H, N.

2-Bromo-4-nitro-1*H*-imidazole (26). A suspension of **25** (18.36 g, 73.4 mmol) in MeOH (16.5 mL) and 5 N HCl (100 mL) was stirred at 69 °C for 3 h, then cooled and stored at −20 °C for 3 h. The solid was collected by filtration, washed with petroleum ether, and dried at 50 °C under vacuum to give **26** (10.92 g, 77%) as a cream solid: mp (MeOH/H_2O) 238−240 °C (lit.[34] mp 238−239 °C); ^{1}H NMR [$(CD_3)_2SO$] δ 14.10 (br s, 1 H), 8.41 (s, 1 H). Evaporation of the filtrate to dryness and chromatography of the residue on silica gel, eluting with CH_2Cl_2, then 5% MeOH/CH_2Cl_2, gave a further 2.55 g (18%) of **26**.

1-(2-Bromo-4-nitro-1*H*-imidazol-1-yl)-3-{[*tert*-butyl(dimethyl)silyl]oxy}-2-propanol (27). A mixture of **26** (2.00 g, 0.010 mol), *tert*-butyl(dimethyl)(2-oxiranylmethoxy)silane (2.15 g, 0.011 mol, 1.1 equiv), and *N*,*N*-diisopropylethylamine (0.91 mL, 5.21 mmol, 0.50 equiv) in toluene (60 mL) was warmed at 70 °C for 18 h. The solution was concentrated to dryness and the residue triturated with petroleum ether to leave the crude product as an oily solid. This material was adsorbed onto silica and chromatographed. Elution with EtOAc/petroleum ether (1:3) gave foreruns, and then further elution with EtOAc/petroleum ether (1:1) gave **27** (2.66 g, 67%): mp (EtOAc/petroleum ether) 101 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.44 (s, 1 H), 5.31 (d, J = 5.4 Hz, 1 H, exchanged with D_2O), 4.18 (dd, J = 13.9, 3.4 Hz, 1 H), 3.96 (dd, J = 13.9, 8.7 Hz, 1 H), 3.89−3.80 (m, 1 H), 3.64 (dd, J = 10.5, 4.7 Hz, 1 H), 3.50 (dd, J = 10.5, 6.7 Hz, 1 H), 0.88 (s, 9 H), 0.06 (s, 6 H); HRCIMS calcd for $C_{12}H_{23}BrN_3O_4Si$ m/z ($M + H^+$) 382.0621, 380.0641; found 382.0619, 380.0637.

2-Bromo-1-[3-{[*tert*-butyl(dimethyl)silyl]oxy}-2-(tetrahydro-2*H*-pyran-2-yloxy)propyl]-4-nitro-1*H*-imidazole (28). A solution of **27** (2.65 g, 6.97 mmol), 3,4-dihydro-2*H*-pyran (6.50 mL, 0.07 mol, 10.0 equiv), and pyridinium *p*-toluenesulfonate (1.78 g, 1.0 equiv) in CH_2Cl_2 (50 mL) was stirred at room temperature for 16 h. After being washed with water and saturated aqueous $NaHCO_3$, the solution was dried and concentrated to give **28**[20] (3.18 g, 98%) as a yellow oil, a mixture of diastereomers. This material was not characterized further and was used directly in the next step.

2-Nitro-6-(tetrahydro-2*H*-pyran-2-yloxy)-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazine (30). Tetra-*n*-butylammonium fluoride (19.3 mL of a 1 M solution in THF, 0.019 mol, 3.0 equiv) was added under N_2 to a solution of **28** (3.00 g, 6.45 mmol) in THF (60 mL), and the solution was stirred at room temperature for 1 h. After dilution with EtOAc, the solution was washed with brine, saturated aqueous $NaHCO_3$, and water. After the mixture was dried, the extract was evaporated onto silica and chromatographed. Elution with EtOAc/petroleum ether (1:4) gave silicon-containing residues, and then further elution with EtOAc gave a mixture of **29**[20] and **30**[20] as a glassy solid (1.68 g). This product was dissolved in dry DMF (15 mL), NaH (0.13 g, 5.41 mmol) was added, and the mixture was stirred at room temperature for 3 h. Brine was added and the mixture was extracted into EtOAc, washed well with brine, and evaporated to give **30**[20] (0.95 g, 55%) as a viscous oil, a mixture of diastereomers (lit.[15] for *S*-**30**, mp 138−139 °C, and for *R*-**30**, mp 145−146 °C); ^{1}H NMR [$(CD_3)_2SO$] δ 8.06, 8.00 (2s, 1 H total, diastereomers), 4.89, 4.84 (2m, 1 H total), 4.60−4.15 (m, 5 H), 3.79, 3.68, 3.47 (3m, 2 H total), 1.65−1.37 (m, 6 H).

2-Nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-ol (31). Methanesulfonic acid (5 drops) was added to a solution of **30** (0.95 g, 0.014 mol) in MeOH (60 mL), and the solution was stirred at room temperature for 3 h and then concentrated to a small volume under reduced pressure. The residue was treated with excess saturated aqueous $NaHCO_3$ solution and extracted into EtOAc (6 times). The combined extracts were evaporated to give (racemic) alcohol **31** (0.65 g, ∼100%) as a cream solid. A portion was crystallized from EtOAc/petroleum ether to give an off-white powder: mp 206−209 °C (lit.[15] for *S*-**31**, mp 220 °C dec, and for *R*-**31**, mp 208 °C dec); ^{1}H NMR [$(CD_3)_2SO$] δ 8.05 (s, 1 H), 5.65 (br s, 1 H, exchanged with D_2O), 4.41 (dd, J = 11.3, 1.2 Hz, 1 H), 4.31 (ddd, J = 11.3, 2.6, 2.6 Hz, 1 H), 4.29−4.24 (br, 1 H), 4.19 (dd, J = 12.9, 3.3 Hz, 1 H), 3.96 (ddd, J = 12.9, 2.6, 2.6 Hz, 1 H); ^{13}C NMR δ 147.05, 142.02, 117.90, 70.64, 58.94, 49.12; HREIMS calcd for $C_6H_7N_3O_4$ m/z (M^+) 185.0437, found 185.0434. Anal. ($C_6H_7N_3O_4$) H, N. C: calcd, 38.92; found, C, 39.38.

6-{[4-(Trifluoromethoxy)benzyl]oxy}-2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazine (1). NaH (21 mg of a 60% dispersion in mineral oil, 0.52 mmol) was added at room temperature to a solution of **31** (87.2 mg, 0.47 mmol) and 4-trifluoromethoxybenzyl bromide (83 μL, 0.52 mmol) in dry DMF (3 mL), and the mixture was stirred at room temperature for 24 h. After dilution with brine and extraction with EtOAc, the organic solution was evaporated and chromatographed on silica. Elution with EtOAc/petroleum ether (1:1) gave foreruns, then elution with EtOAc gave the product, which was triturated with Et_2O (with addition of petroleum ether when crystallization began) to give (racemic) **1** (0.11 g, 68%) as a white powder: mp 121−123 °C (lit.[15] for *S*-**1**, mp 149−150 °C); ^{1}H NMR [$(CD_3)_2SO$] δ 8.02 (s, 1 H), 7.44 (d, J = 8.7 Hz, 2 H), 7.34 (d, J = 8.7 Hz, 2 H), 4.70 (d, J = 12.2 Hz, 1 H), 4.69−4.63 (m, 1 H), 4.66 (d, J = 12.2 Hz, 1 H), 4.47 (d, J = 11.9 Hz, 1 H), 4.31−4.21 (m, 3 H). Anal. ($C_{14}H_{12}F_3N_3O_5$) C, H, N.

6-{[4-(Benzyloxy)benzyl]oxy}-2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazine (4). NaH (33 mg, 0.84 mmol) was added in one portion to a solution of **31** (0.10 g, 0.56 mmol) and 4-benzyloxybenzyl chloride (0.19 g, 0.84 mmol) in dry DMF (4 mL), and the mixture was stirred at room temperature for 16 h. Water was added. The mixture was extracted with EtOAc, and the extract was washed well with brine, then evaporated to give an oil that was chromatographed on silica. Elution with EtOAc/petroleum ether (1:5) gave foreruns, and then elution with EtOAc gave **4** (56 mg, 30%), which crystallized from CH_2Cl_2/petroleum ether as pale-yellow cubes: mp 158−160 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.00 (s, 1 H), 7.44−7.29 (m, 5 H), 7.24 (d, J = 8.7 Hz, 2 H), 6.98 (d, J = 8.7 Hz, 2 H), 5.09 (s, 2 H), 4.64−4.60 (m, 1 H), 4.58 (d, J = 11.5 Hz, 1 H), 4.53 (d, J = 11.5 Hz, 1 H), 4.45 (d, J = 11.9 Hz, 1 H), 4.22−4.16 (m, 3 H). Anal. ($C_{20}H_{19}N_3O_5$) C, H, N.

2-Bromo-1-[3-bromo-2-(tetrahydro-2*H*-pyran-2-yloxy)propyl]-4-nitro-1*H*-imidazole (32) (Scheme 1). A mixture of **26** (3.32 g, 0.017 mol), 2-[2-bromo-1-(bromomethyl)ethoxy]tetrahydro-2*H*-pyran[22] (7.76 g, 0.024 mol), and K_2CO_3 (3.58 g, 0.024 mol) in dry DMF (50 mL) was stirred at 80 °C for 18 h. After dilution with brine, the mixture was extracted with EtOAc and the extract was evaporated and chromatographed on silica. Elution with EtOAc/petroleum ether (1:9) gave foreruns, and then elution with EtOAc/petroleum ether (3:7) gave **32** (2.22 g, 31%) as a viscous oil, a mixture of diastereomers: ^{1}H NMR ($CDCl_3$) δ 8.00, 7.90 (2s, 1 H), 4.70−3.30 (m, 8 H), 1.80−1.38 (m, 6 H); HRFABMS calcd for $C_{11}H_{14}Br_2N_3O_4$ m/z ($M - H^+$) 411.9508, found 411.9504.

2-Nitro-6-(tetrahydro-2*H*-pyran-2-yloxy)-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]thiazine (33). *n*-Butyllithium (2.13 mL of a 2.5 M solution in hexanes, 5.34 mmol) was added dropwise under N_2 to a stirred solution of triispropylsilanethiol (1.14 mL, 5.34 mmol) in dry THF (50 mL) at 5 °C. After 5 min, the resulting solution was added dropwise to a solution of **32** (2.22 g, 5.34 mmol) in dry THF (50 mL) which had been cooled to −78 °C under N_2. After 5 min, the cooling bath was removed and the solution was allowed to warm to room temperature, when stirring was continued for 18 h. The solution was diluted with water and extracted with EtOAc and the extract was dried and evaporated to give an orange oil (2.97 g). This was immediately dissolved in THF (60 mL). Tetra-*n*-butylammonium fluoride (17.0 mL of a 1 M solution in THF, 0.017 mol) was added, and the solution was stirred at room temperature for 1 h. Brine was added, the mixture was extracted into EtOAc, and the extract was washed with saturated aqueous $NaHCO_3$ and evaporated to give an oil, which was chromatographed on silica. Elution with EtOAc/petroleum ether (1:1) gave foreruns, and then elution with EtOAc/petroleum ether (3:1) gave an oily solid, which was triturated with MeOH to give **33** (1.09 g, 71%) as a yellow solid, a mixture of diastereomers: mp 129−131 °C; ^{1}H NMR ($CDCl_3$) δ 7.68, 7.67 (2s, 1 H), 4.85−4.80 (m, 1 H), 4.54−4.43 (m, 1 H), 4.27−4.05 (m, 2 H), 3.96−3.89 (m, 0.5 H), 3.80−3.73 (m, 0.5 H), 3.61−3.51 (m, 1H), 3.45−3.39 (m, 0.5 H), 3.36−3.30 (m, 0.5 H), 3.26 (d, J = 4.9 Hz, 1 H), 1.83−1.40 (m, 6 H). Anal. ($C_{11}H_{15}N_3O_4S \cdot {}^1/_4H_2O$) C, H, N.

2-Nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]thiazin-6-ol (34). HCl (2 N, 20 mL) was added to a solution of **33** (1.09 g, 3.82 mmol) in MeOH (200 mL), and the solution was stirred at room temperature for 3 h. Water was added, the MeOH was removed under reduced pressure, and the residue was extracted into EtOAc (5 times). The combined extracts were dried and concentrated to dryness and the residue was triturated with Et_2O to give **34** (0.56 g, 73%) as a yellow powder: mp 240−242 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.37 (s, 1 H), 5.71 (d, J = 3.9 Hz, 1 H), 4.42−4.36 (m, 1 H), 4.16 (dd, J = 13.3, 3.0 Hz, 1 H), 4.05 (dd, J = 13.3, 1.2 Hz, 1 H), 3.38 (dd, J = 12.6, 2.0 Hz, 1 H), 3.17 (ddd, J = 12.6, 6.3, 1.3 Hz, 1 H). Anal. ($C_6H_7N_3O_3S$) C, H, N, S.

2-Nitro-6-{[4-(trifluoromethoxy)benzyl]oxy}-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]thiazine (5). NaH (0.13 g of a 60% dispersion in mineral oil, 2.95 mmol) was added in one portion to a solution of **34** (0.54 g, 2.68 mmol) and 4-(trifluoromethoxy)benzyl bromide (0.58 mL, 2.95 mmol) in dry DMF (15 mL), and the mixture was stirred at room temperature for 24 h. Brine was added, the mixture was extracted into EtOAc, and the extract was dried and evaporated to give an oil, which was chromatographed on silica. Elution with EtOAc/petroleum ether (1:9) gave foreruns, and then elution with EtOAc/petroleum ether (7:3) gave **5** (0.76 g, 75%) as a light-yellow powder: mp 68−70 °C; ^{1}H NMR ($CDCl_3$) δ 7.65 (s, 1 H), 7.36 (d, J = 8.6 Hz, 2 H), 7.21 (d, J = 8.6 Hz, 2 H), 4.76 (d, J = 11.8 Hz, 1 H), 4.59 (d, J = 11.8 Hz, 1 H), 4.31−4.26 (m, 1 H), 4.21−4.11 (m, 2 H), 3.38−3.29 (m, 2 H). Anal. ($C_{14}H_{12}F_3N_3O_4S\cdot{}^1/_3$-EtOAc) C, H, N, S.

6-{[4-(Benzyloxy)benzyl]oxy}-2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]thiazine (6). Similar reaction of **34** with NaH and 4-(benzyloxy)benzyl chloride in DMF as described above for the preparation of **5** gave **6** (55%) as tan cubes: mp (CH_2Cl_2/petroleum ether) 139−140 °C; ^{1}H NMR ($CDCl_3$) δ 7.59 (s, 1 H), 7.44−7.30 (m, 5 H), 7.25 (d, J = 9.9 Hz, 2 H), 6.96 (d, J = 9.9 Hz, 2 H), 5.07 (s, 2 H), 4.68 (d, J = 11.6 Hz, 1 H), 4.52 (d, J = 11.6 Hz, 1 H), 4.25−4.19 (m, 1 H), 4.12−4.02 (m, 2 H), 3.29−3.20 (m, 2 H). Anal. ($C_{20}H_{19}N_3O_4S\cdot{}^1/_4H_2O$) C, H, N, S.

2-Nitro-6-{[4-(trifluoromethoxy)benzyl]oxy}-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]thiazine 8-Oxide (7). A solution of 3-phenyl-2-(phenylsulfonyl)-1,2-oxaziridine (0.18 g, 0.68 mmol) in CH_2Cl_2 (5 mL) was added to a solution of **5** (0.25 g, 0.68 mmol) in CH_2Cl_2 (20 mL), and stirring was continued at room temperature for 4 days. The reaction product was adsorbed directly onto silica by concentration and chromatographed on silica. Elution with EtOAc/petroleum ether (1:1) gave foreruns, and then elution with EtOAc gave **7** (0.17 g, 65%) as a sticky gum: ^{1}H NMR ($CDCl_3$) δ 7.83 (s, 1 H), 7.37 (d, J = 8.6 Hz, 2 H), 7.24 (d, J = 8.6 Hz, 2 H), 4.86−4.79 (m, 1 H), 4.75 (d, J = 11.8 Hz, 1 H), 4.65 (d, J = 11.8 Hz, 1 H), 4.56 (dd, J = 13.3, 4.6 Hz, 1 H), 4.16 (dd, J = 13.3, 8.5 Hz, 1 H), 3.65 (dd, J = 13.9, 2.1 Hz, 1 H), 3.29 (dd, J = 13.9, 9.8 Hz, 1 H). Anal. ($C_{14}H_{12}F_3N_3O_5S$) C, H, N, S.

6-{[4-(Benzyloxy)benzyl]oxy}-2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]thiazine 8-Oxide (8). Similar oxidation of **6** with 3-phenyl-2-(phenylsulfonyl)-1,2-oxaziridine in CH_2Cl_2 as described above for the preparation of **7** gave **8** (38%) as a white solid: mp (CH_2Cl_2/petroleum ether) 178−180 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.57 (s, 1 H), 7.45−7.30 (m, 5 H), 7.25 (d, J = 8.6 Hz, 2 H), 6.99 (d, J = 8.6 Hz, 2 H), 5.09 (s, 2 H), 4.66−4.54 (m, 4 H), 4.23 (dd, J = 13.0, 6.5 Hz, 1 H), 3.94 (dd, J = 13.4, 8.6 Hz, 1 H), 3.63 (dd, J = 13.4, 1.5 Hz, 1 H). Anal. ($C_{20}H_{19}N_3O_5S$) C, H, N, S.

2-Nitro-6-{[4-(trifluoromethoxy)benzyl]oxy}-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]thiazine 8,8-Dioxide (9). A solution of **5** (0.34 g, 0.91 mmol) and 3-chloroperbenzoic acid (0.78 g of 50% purity, 2.25 mmol) in CH_2Cl_2 (40 mL) was stirred at room temperature for 72 h. After dilution with CH_2Cl_2, the solution was washed sequentially with aqueous solutions of Na_2SO_3 and $NaHCO_3$ and evaporated to give an oil, which was chromatographed on silica. Elution with EtOAc/petroleum ether (3:1) gave **9** (0.27 g, 73%) as a white powder: mp (CH_2Cl_2/petroleum ether) 161 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.60 (s, 1 H), 7.44 (d, J = 8.6 Hz, 2 H), 7.33 (d, J = 8.6 Hz, 2 H), 4.78 (d, J = 11.7 Hz, 1 H), 4.66−4.61 (m, 1 H), 4.61 (d, J = 11.7 Hz, 1 H), 4.58−4.50 (m, 2 H), 4.43 (dd, J = 14.4, 6.4 Hz, 1 H), 4.16 (dd, J = 14.4, 1.9 Hz, 1 H). Anal. ($C_{14}H_{12}F_3N_3O_6S$) C, H, N, S.

6-{[4-(Benzyloxy)benzyl]oxy}-2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]thiazine 8,8-Dioxide (10). Similar oxidation of **6** with 3-chloroperbenzoic acid as described above for the preparation of **9** gave **10** (64%) as a white powder: mp (CH_2Cl_2/petroleum ether) 172−175 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.58 (s, 1 H), 7.44−7.29 (m, 5 H), 7.25−7.22 (d, J = 8.6 Hz, 2 H), 6.98 (d, J = 8.6 Hz, 2 H), 5.08 (s, 2 H), 4.66 (d, J = 11.1 Hz, 1 H), 4.58−4.44 (m, 4 H), 4.38 (dd, J = 14.2, 6.5 Hz, 1 H), 4.13 (dd, J = 14.2, 1.9 Hz, 1 H). Anal. ($C_{20}H_{19}N_3O_6S$) C, H, N, S.

Radical Chemistry. Steady-state radiolysis of the compounds in aqueous solution was performed using a ^{60}Co γ-source delivering a dose rate of 7.5 Gy min^{-1}. The methods of solution preparation, observation of the stepwise reduction of the compounds to products, and the determination of their molecular masses have been described.[28] Pulse radiolysis studies were carried out at room temperature (22 ± 2 °C) using The University of Auckland's linear accelerator and radical detection facility.[35] One electron reduction of the compounds (C) was selectively carried out by either (i) scavenging the e_{aq}^-, produced upon the pulse radiolysis of solvent water (2.5 Gy in 200 ns) with 4 MeV electrons while at the same time scavenging the oxidizing radicals with 2-methylpropan-2-ol (0.2 M) to form an inert radical or (ii) saturating solutions containing sodium formate (50 mM) and sodium phosphate (5 mM, adjusted to pH 4 with perchloric acid) with N_2O gas to convert the e_{aq}^- species into $^\bullet$OH radicals, which in turn are scavenged (along with the H-atoms) to produce the reducing $CO_2^{\bullet-}$ radicals at a concentration of 0.66 μmol Gy^{1-}.[36]

$$H_2O \rightsquigarrow e_{aq}^- + {}^\bullet OH + H^\bullet + H_2 + H_2O_2 + H_3O^+$$

$$e_{aq}^- + C \rightarrow C^{\bullet-}$$

$$^\bullet OH(H^\bullet) + (CH_3)_3COH \rightarrow {}^\bullet CH_2(CH_3)_2COH + H_2O(H_2)$$

$$e_{aq}^- + N_2O \rightarrow {}^\bullet OH + OH^- + N_2$$

$$^\bullet OH(H^\bullet) + HCOO^- \rightarrow CO_2^{\bullet-} + H_2O(H_2)$$

$$CO_2^{\bullet-} + C \rightarrow C^{\bullet-} + CO_2$$

The one-electron reduction potentials of the compounds, E(C/C$^{\bullet-}$) vs NHE, were determined at pH 7.0 (5 mM phosphate buffer) by establishing redox equilibria between three mixtures of the one-electron reduced compounds and the reference viologen compounds; tetraquat [E(TeQ^{2+}/TeQ$^{+\bullet}$) = −635 ± 5 mV][37] or methylviologen [E(MV^{2+}/MV$^{+\bullet}$) = −447 ± 7 mV].[38]

$$MV^{+\bullet}/TeQ^{+\bullet} + C \overset{K_e}{\rightleftharpoons} MV^{2+}/TeQ^{2+} + C^{\bullet-}$$

The ΔE values were calculated using the Nernst equation from the equilibrium constants, K_e (established within 50 μs), as described in the literature.[29]

Biology. MABA and LORA Tuberculosis Assays. These were carried out according to the published protocols.[32,33]

Cytotoxicity Assay. Cytotoxicity was assessed[33] against VERO cells (CCL-81, American Type Culture Collection) by exposing monolayers in 96-well plates to 3-fold dilutions of test compounds for 72 h. Cell viability was measured using the CellTiter96 aqueous nonradioactive cell proliferation assay (Promega Corp, Madison, WI), which determines the extent of reduction of a tetrazolium dye by measuring the absorbance of the product at 490 nm. Untreated cells and cells lysed with sodium dodecyl sulfate were used to determine 0% and 100% inhibition, respectively.

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Supporting Information Available: Additional experimental procedures and characterizations for compounds **11**−**22**; combustion analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

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