Journal of Medicinal Chemistry

Article

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J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.5b01699 • Publication Date (Web): 22 Feb 2016

ACS Publications

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Repositioning antitubercular 6-nitro-2,3-dihydroimidazo[2,1-*b*][1,3]oxazoles for neglected tropical diseases: structure-activity studies on a preclinical candidate for visceral leishmaniasis

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Abstract

6-Nitro-2,3-dihydroimidazo[2,1-*b*][1,3]oxazole derivatives were initially studied for tuberculosis within a backup program for the clinical trial agent pretomanid (PA-824). Phenotypic screening of representative examples against kinetoplastid diseases unexpectedly led to the identification of DNDI-VL-2098 as a potential first-in-class drug candidate for visceral leishmaniasis (VL). Additional work was then conducted to delineate its essential structural features, aiming to improve solubility and safety without compromising activity against VL. While the 4-nitroimidazole portion was specifically required, several modifications to the aryloxy side chain were well tolerated e.g., exchange of the linking oxygen for nitrogen (or piperazine), biaryl extension, and replacement of phenyl rings by pyridine. Several less lipophilic analogues displayed improved aqueous solubility, particularly at low pH, although stability towards liver microsomes was highly variable. Upon evaluation in a mouse model of acute *Leishmania donovani* infection, one phenylpyridine derivative (**37**) stood out, providing efficacy surpassing that of the original preclinical lead.

INTRODUCTION

Neglected tropical diseases (NTDs) affect in excess of one billion people, predominantly in the most impoverished areas of the world, causing more than half a million deaths each year, as well as much physical suffering and social stigma.[1,2] Of the 17 major NTDs, the kinetoplastid parasite diseases visceral leishmaniasis (VL), Chagas disease, and human African trypanosomiasis (HAT) are considered amongst the most challenging, due to their high mortality rates and limited treatment options, with little economic incentive for this to change.[2] VL is caused by *Leishmania donovani* (*L. don*) and *Leishmania infantum* (*L. inf*), transmitted by female sand flies; these parasites first multiply inside host macrophages before spreading to and destroying other tissues (e.g., spleen, liver, and bone marrow).[3,4] Annual incidence of VL is now estimated at 300000, with infection occurring mainly in rural areas of East Africa, India, Bangladesh and Brazil. Most of the ~35000 deaths each year pass unrecognised, and in East Africa, lethal epidemics take place frequently.[5] However, the current treatments for VL are unsatisfactory.[3,4] Pentavalent antimonials have been the standard first-line remedy since the 1940s, but these are cardiotoxic and require painful parenteral administration over 1 month; drug resistance has now severely limited their utility in India. A less toxic liposomal form of the antifungal agent, amphotericin B (**1**; see Figure 1), has proven widely effective but is limited by high cost. The cheaper aminoglycoside antibiotic, paromomycin (**2**), has variable efficacy, both within and between regions. Finally, the alkyl phospholipid miltefosine (**3**), a long-acting anticancer drug repurposed for VL, is the sole oral agent; nevertheless, this also suffers from several disadvantages, including cost, teratogenicity, and treatment failures. Hence, there remains an urgent need for more effective, safe, and affordable oral treatments for VL. In the last decade of tuberculosis (TB) and NTD drug discovery, there has been major interest in exploiting potential development shortcuts, via the repurposing of approved drugs, rescue of "failed" clinical agents, or repositioning of

early or late stage drug candidates.[6-8] Indeed, for organizations such as the Global Alliance for TB Drug Development (TB Alliance) and the Drugs for Neglected Diseases *initiative* (DND*i*), phenotypic screening is still viewed as a highly useful, cost-effective strategy to identify "low hanging fruit".[9-11] Recent cooperation between these two organizations in this regard actually provided the foundation for this current study.[12]

In early collaborative work with TB Alliance (2005-6) we investigated[13] several nitroheterobicyclic ring analogues of the bioreductive TB agent pretomanid (PA-824, **4**), now in phase III clinical evaluation.[14] However, our efforts in the known[15] 6-nitroimidazooxazole class were curbed following disclosure of the recently approved[16] MDR-TB drug delamanid (OPC-67683, **5**),[17,18] turning our focus for a backup toward the efficacious biaryl analogues of **4**.[19-21] Both **4** and **5** are activated by an intracellular deazaflavin dependent nitroreductase (Ddn) in *Mycobacterium tuberculosis* (*M. tb*), resulting in inhibition of cell wall synthesis as well as respiratory poisoning (via nitric oxide release).[22] This mode of action is distinct from the postulated *nucleophilic* nitro reduction (e.g., by thiolate) of other new nitroaromatic TB agents, such as PBTZ169 (**6**) and nitrobenzamides, to form reactive nitroso intermediates, which trigger suicide inhibition of an enzyme (DprE1) involved in cell wall biosynthesis.[23,24]

In late 2009, follow-up interest from DND*i* in some promising antileishmanial screening data for several of our earlier nitroimidazole derivatives (above) then led to our nomination of three unoptimised racemic hits, including two from the most potent 6-nitroimidazooxazole class (**7** and **8**), for proof-of-concept *in vivo* assessment in a mouse model of VL. In parallel, taking into account the preliminary SAR findings, a further 72 compounds were screened against *L. don* in a macrophage-based luciferase assay at the Central Drug Research Institute (CDRI, India),[25] and 10 of these were later tested in the same mouse model. However, the outstanding *in vivo* efficacy of **7** was not surpassed by any of the other candidates, and following our synthesis of its enantiomers and ensuing head-to-head assessments of these, the

R enantiomer **9** (DNDI-VL-2098)[18] was chosen for preclinical evaluation as a potential first-in-class drug candidate for VL.[25,26] In this report, we initially detail results relevant to the selection of **9**. We then describe the findings of more recent work aimed at discovering backups to **9** having an improved physicochemical/ pharmacological profile and better safety, providing wider SAR conclusions for the nitroimidazooxazole class against TB and three kinetoplastid NTDs, together with a first *in vivo* appraisal of the best new leads for VL.

CHEMISTRY

Several earlier investigators[15,27] prepared 6-nitro-2,3-dihydroimidazo[2,1-*b*][1,3]oxazoles ("oxazoles") by condensing epoxides with the explosive intermediate 2,4-dinitroimidazole. In the interests of improving safety, we first sought alternative conditions using 2-bromo-4-nitroimidazole (**75**; see Scheme 1A/1B).[13] Satisfactory results for both 2-H and 2-methyl glycidyl ethers were obtained by neat reaction with **75** in the presence of a suitable base (DIPEA, 105-108 °C) and the resulting (uncyclised) alcohol intermediates were readily transformed into the desired oxazoles **7** and **10**-**12** in high yield (85-90%) upon treatment with sodium hydride (DMF, 0 °C). Minor quantities (1-3%) of 5-nitroimidazooxazoles were also generated in the first step, with two examples (**15** and **87**) being isolated for comparison. The requisite epoxides (**74**, **78**, **82**,[28] and **86**) were acquired via standard methods, starting with (4-OCF_3) benzylations of glycidol (**73**) or 2-methylprop-2-en-1-ol (**77**), or alkylations of 4-(trifluoromethoxy)phenol with epibromohydrin (**81**) or 3-chloro-2-methylprop-1-ene (**84**).

To synthesise the enantiomers of **12** and **15** (**13**, **14**, **16**, and **17**), we employed cesium fluoride promoted reactions[29] between 4-(trifluoromethoxy)phenol and the chiral glycidyl nosylates **89** and **93** (Scheme 1C). The ether products (**90** and **94**) were then alternatively coupled with the more accessible 2-chloro-4-nitroimidazole (**91**), where the use of toluene as cosolvent (DIPEA, 80 °C) enabled better isolated yields of the more thermally labile 5-nitro

compounds **16** and **17** (15-16%). In the final step, sodium hydride induced cyclisation of the intermediate alcohols (**92** and **95**) was completed by warming to 20 °C, giving both oxazoles **13** and **14** in high yield (86-87%) and excellent ee (100%).

Reaction of the known[18,30,31] chiral epoxides **96** and **97** with 4-(trifluoromethoxy)phenol or 4'-fluorobiphenyl-4-ol in the presence of sodium hydride (Scheme 2A) also provided a direct route to the enantiomers of early VL leads **7** and **8**, as recorded for **9**.[18] These epoxides were conveniently obtained by the reaction of 2-chloro-4-nitroimidazole (**91**) with (*R*) or (*S*)-2-methylglycidyl 4-nitrobenzenesulfonate (derived from the Sharpless epoxidation of 2-methylprop-2-en-1-ol, **77**),[31] although a multistep approach[18] based on the 4-nitrobenzoate ester derivative of (*S*)-2-methylglycidol reportedly achieved a slightly better ee for **96** (98.8% vs. 95.9%).[30] While isolated yields of the final products **9**, **18**, **44**, and **45** were moderate (33-44%), these important entities were rapidly produced in excellent chiral purities (>98% ee), and a superior kilogram scale process synthesis of **9** has recently been developed.[32]

For more efficient generation of additional analogues of **7**, we then turned to the known[33] racemic epoxide **98** (Scheme 2B). Heating this epoxide with various phenols and sodium hydride furnished the oxazole products **19**-**23** directly, albeit in variable yields (12-45%), depending on the conditions. Alternatively, reaction of **98** with 4-(trifluoromethoxy)aniline (or its N-methyl derivative) in the presence of anhydrous cobaltous chloride[34] gave uncyclised β-anilino alcohols (**99** and **100**), which could be transformed into the novel oxazoles **24** and **25** by careful treatment with sodium hydride (1.3-1.4 equiv).

Next, in order to access larger quantities of the known[30] alcohol **26** for the preparation of heteroaryl ethers, we devised an improved plan, comprising TIPS monoprotection of known[30] diol **102**, ring closure, and desilylation under acidic conditions (Scheme 2C). Diol **102** was obtained from 2-chloro-4-nitroimidazole (**91**) in two steps (85%), via dihydroxylation of the new alkene **101**, and elaborated to the oxazole **104** as planned (92% from **102**; two steps).

However, attempted removal of the TIPS group using a reported acidic method (1% HCl in 95% EtOH)[35] required prolonged heating at 45 °C (4.5 days) and was not clean, leading to a suboptimal yield of alcohol **26** (53%). Fortunately, treatment of **104** with hydrofluoric acid in acetonitrile[36] gave a much better result (95% yield), and **26** was easily converted into various heteroaryl ether targets (**27**, **29**-**31**) via sodium hydride-catalysed S_NAr reactions on the haloheterocycles **105**-**108**.[37] The isomeric pyridine **28** was also obtained in moderate yield (25%) by reaction of 6-(trifluoromethyl)pyridin-3-ol and sodium hydride with the known[30] epoxide **109** (Scheme 2D). By way of comparison, epoxides **98** and **109** each afforded comparable yields (55% and 51%) for scale-up of racemic VL lead **7**.

In the 2*H* oxazole series, the synthesis of biphenyl analogues (**32**-**34**) was achieved in a straightforward manner (Scheme 3A), starting from epibromohydrin (**81**), and using methodology described above (except that DMF was employed in the final step to improve solubility). We then envisaged the assembly of related phenylpyridine side chains via Suzuki couplings on bromopyridinyl ether derivatives (e.g., **119** in Scheme 3B). To this end, the known[27] alcohol **117** was prepared in three steps from TBS-protected glycidol (**113**), via condensation with **91**, ring closure of alcohol **115**, and acid-catalysed desilylation (Scheme 3B). Unfortunately, reaction of **117** with 5-bromo-2-fluoropyridine (**118**) under the optimised conditions developed for the 2-methyl congener **144** (Scheme 4B) gave a poor yield of ether **119** (9%), and attempted Suzuki couplings on this substrate (even with weak bases) resulted only in decomposition. In an attempt to obtain at least one or two examples featuring this isomer pattern, we next considered reversing the order of these final two steps. Here, the 5-aryl-2-fluoropyridines **120** and **121** were quantitatively obtained from bromide **118** but the final alkylations involving alcohol **117** gave very poor yields (<1%) of the authentic products **35** and **36**, precluding synthesis of the less electron deficient 4-fluorophenyl analogue.

To circumvent any similar complications in the preparation of the 3-pyridyl targets (**37**, **40** and **41**), a modified strategy was adopted (Scheme 3C). Here, 6-bromopyridin-3-ol (**122**) was first protected (as the ethoxymethyl ether, **123**) and then Suzuki-coupled with arylboronic acids. Following acidic deprotection, the 6-arylpyridin-3-ols (**125**,[38] **129**, and **133**) were alkylated with epibromohydrin (**81**) and the resulting epoxides were then elaborated to the final products (**37**, **40** and **41**), as above, in consistently good yields (56-59% over the last 2 steps). For the enantiomers of **37** (**38** and **39**), we varied this route by reacting the (NaH-generated) anion of arylpyridinol **125**[38] with the chiral glycidyl nosylates **89** and **93** (Scheme 3D). The resulting ether products (**136** and **138**) were again coupled with 2-chloro-4-nitroimidazole (**91**), using toluene as cosolvent (DIPEA, 80 °C) in order to obtain better quantities of the 5-nitro isomers **42** and **43** (10-12% yield). Alcohols **137** and **139** were then ring closed to the oxazoles **38** and **39** in excellent yields (87-89%), as above.

Methods for the preparation of biaryl analogues and some soluble bioisosteres in the 2-methyl oxazole series are outlined in Scheme 4. Epoxide **141** (sourced directly[39] from the commercial chloride **140**) was converted in two steps to the 4-iodophenoxy precursor **143**, with Suzuki couplings then delivering the biphenyl analogues **8**, **46**, and **47** (Scheme 4A). The bromopyridinyl ethers **144** and **147** (obtained from alcohol **26**, and epoxide **98**, respectively) also proved to be effective Suzuki substrates for creating phenylpyridine and bipyridine derivatives (**48**-**56**; Scheme 4B/4C), although for bipyridines **55** and **56** it was best to use a weaker base ($KHCO_3$). Furthermore, in the case of bipyridine **54**, it was necessary to employ a copper(I) additive,[40] in order to circumvent facile protodeboronation of the required 2-pyridylpinacol boronate **145** (Scheme 4B). Arylated cyclic amine targets (**57** and **58**) were readily achieved by reaction of epoxide **109** with the commercial amines (**148** and **150**), followed by (NaH-induced) ring closure of the resulting alcohols (**149** and **151**; Scheme 4D).

Lastly, the *O*-carbamate **59** was made in high yield (86%) by chloroformylation of alcohol **26** and one-pot coupling with arylpiperazine **148** (Scheme 4E).[41]

To conclude this SAR study, several heterocyclic ring A analogues of **7** were synthesized from epoxide **86** (Scheme 5). Thus, similar condensations (DIPEA, 104-105 °C) of **86** with 2-nitroimidazole (**152**), 5-bromo-3-nitro-1,2,4-triazole (**157**), or 3,5-dinitropyrazole (**158**) each provided heterobicyclic products directly, although **152** gave equivalent yields of oxazole **61** and its barely separable alcohol precursor **153** (Scheme 5A). Treatment of the latter with sodium hydride (DMF, 0-20 °C) readily cyclised this to **61**, in agreement with a recent report.[42] In the case of 5-bromo-3-nitro-1,2,4-triazole (**157**), a separable mixture of nitro- and bromo-substituted products (**70** and **71**) was obtained in a ratio of 3:2 (Scheme 5D), consistent with initial alkylation at the 1- or 2-positions of the triazole, followed by intramolecular substitution of the adjacent bromine or nitro group by the intermediate alcohol.[43] Reaction of epoxide **86** with 3,5-dinitropyrazole (**158**) yielded mainly oxazole **72** (66%), together with a small amount of alcohol **159** (Scheme 5E).

Finally, alternative condensation of epoxide **86** with 2,4-dibromoimidazole (**154**) (DIPEA, toluene, 109 °C) produced a resolvable mixture of two uncyclised alcohols (**155** and **156**, in a 2.4:1 ratio; Scheme 5B), which were transformed into the isomeric oxazoles **62** and **67** upon mild heating of their preformed anions (NaH, DMF, 45-55 °C). Bromide **62** was converted into the desired methyl sulfone **66** (regarded as a potential bioisostere of **7**) via lithiation and quenching with methyl disulfide (to give thioether **65**), followed by oxidation (*m*-CPBA), after an attempted copper(I) iodide/L-proline sodium salt-induced coupling of **62** with sodium methanesulfinate[44] proved unsatisfactory (7% yield of crude **66**). Similar lithiation of **62** and quenching with DMF also gave the aldehyde **63**, which was easily reduced ($NaBH_4$) to alcohol **64**. Methyl sulfone **66** had earlier been sought via displacement of the nitro group in **7** by methanethiol, based on previous observations. Here, treatment of **7** with methanethiol

and triethylamine (10 equiv in MeOH, 0-10 °C) furnished only one major product, **68**, which upon oxidation gave sulfone **69** (Scheme 5C). A survey of the literature[45-47] had suggested that *cine*- or *ipso*-substitution was possible; therefore, we studied compounds **7**, **61**, **62**, **65**, **67**-**69**, and a 5-nitro intermediate (**114**) by 2D NMR experiments (e.g., NOESY, ROESY, HSQC, and HMBC). The NOE evidence and ^{13}C NMR chemical shift data unambiguously established the assigned structures (see Supporting Information), although an absence of any three bond correlations between H-5 and C-3 in the HMBC experiments (when four bond correlations between H-6 and C-3 were apparent) was initially misleading. Weakly observed or missing long range correlations (due to the wide range of $^{n}J_{CH}$ spin coupling constants, 1 to 50 Hz)[48] constitute a well-known deficiency of the standard HMBC experiment, but recent HMBC variants also possess some disadvantages (e.g., reduced signal to noise).[49] Thus, it was verified that thioether **68** was the product of *cine*-substitution,[47] in a fashion reminiscent of the Ddn-catalysed addition of hydride to the unsubstituted imidazole carbon atoms of **4** and **5**, leading to loss of the adjacent nitro group (and production of nitric oxide in *M. tb*).[22,23]

RESULTS AND DISCUSSION

Tables 1 and 2 present *in vitro* antiparasitic and antitubercular data for 67 compounds (6 known) from mainly the nitroimidazooxazole class, prepared in two collaborative projects. For the TB studies, compounds were screened in two *M. tb* (strain H37Rv) growth inhibition assays, MABA and LORA, respectively conducted under either aerobic (replicating) or hypoxic (non-replicating) conditions, as described.[50,51] Given that both **4** and **5** exhibit at least two distinct mechanisms of action against TB, depending on the oxygenation environment,[22] the LORA assay was regarded as a useful starting tool to identify analogues that may be more efficient at killing persistent subpopulations of *M. tb in vivo*.[51,52] Recorded MIC values (for a ≥90% growth inhibitory effect) are the mean of at least two independent determinations.

From 2010-2012, in collaboration with DND*i*, new analogues of lead oxazoles **7** and **8** were screened against *L. don* in a macrophage-based luciferase assay[25] at CDRI, albeit only single determinations of the IC_{50} values were obtained, limiting reliable SAR interpretation. Therefore, a wider set of compounds was later evaluated in at least duplicate *in vitro* assays against three tropical protozoan parasites (*L. inf*, *T. cruzi*, and *T. brucei*) at the University of Antwerp (LMPH).[53] Here, the *L. inf* assay employed primary peritoneal mouse macrophages as the host cell for the amastigotes, whereas the *T. cruzi* assay utilised human lung fibroblasts (MRC-5 cells); compounds having mean IC_{50} values below 1 μM were considered to be highly active. Cytotoxicity on MRC-5 cells was also measured and almost all of the 67 compounds tested could be regarded as non-toxic (IC_{50}s >30 μM, most >64 μM), in broad agreement with assessments on VERO cells (data not shown).[50]

In the design of new analogues of **7** and **8**, two strategies were employed to enhance aqueous solubility while minimising the risk of major potency loss.[20,21] First, we focused on reducing lipophilicity (probed using CLogP predictions derived from ACD LogP/LogD software, version 12.0; Advanced Chemistry Development Inc., Toronto, Canada) via the replacement of benzene rings by pyridine, or closely related heterocycles (*viz*. pyrimidine, pyrazine, and pyridazine). This approach enabled CLogP reductions of up to 2.3 units (typically ~1.1 units for a single pyridine substitution, although the predicted data displayed some variation between isomers). Second, we evaluated the removal of full aromaticity from the side chain by the incorporation of a saturated heterocycle (e.g., piperazine or piperidine) in combination with a terminal aromatic ring. Arylated cyclic amines are known bioisosteres for biaryl moieties[21] and can provide improved solubility via salt formation, as well as by the disruption of molecular planarity and symmetry.[54] Kinetic aqueous solubility values at pH=7 were determined for selected active compounds, using dry powder forms, while analogous

data at pH=1 were measured for examples containing basic functionalities that could form hydrochloride salts (based on calculated pK_a estimates derived from similar ACD software).

1. Initial studies relevant to preclinical candidate selection

The investigations began in late 2009 with the proof-of-concept *in vivo* assessment of oxazoles **7** and **8** in a mouse model of VL at the London School of Hygiene and Tropical Medicine (LSHTM). The selection of these two candidates was based on a combination of their promising activities in an *L. don*-infected macrophage assay conducted at the Swiss Tropical Institute (later confirmed at CDRI) and reasonable conjecture for their better metabolic stabilities in comparison to other similarly active analogues (e.g., **11**). In the event, both **7** and **8** provided outstanding efficacy in this *L. don* model at 50 mg/kg (99.9 to 100% reduction of the liver parasite burden, after oral dosing once daily for 5 days) and subsequent dose-response experiments (Table 3) established that **7** was the most effective lead (ED_{50}s were 3.0 and 4.8 mg/kg for **7** and **8**, respectively). This result was consistent with **7** having superior mouse microsomal stability (79% vs. 57% parent remaining after 30 min, Table 3), better aqueous solubility (16-fold), and a more favourable mouse pharmacokinetic profile (lower clearance, greater exposure, and higher oral bioavailability; Table 4 and Supporting Information, Figure S1) in comparison to **8**.

The more stringent chronic infection hamster model of VL is considered the gold standard *in vivo* assay due to its similar pathology to human disease.[55] Here, 5 and 10 day assessments of **7** and **8** at CDRI led to opposing conclusions on which oxazole was better (Supporting Information, Table S3); therefore, the enantiomers of both compounds were prepared. In the mouse model, the *R* enantiomer of **7** (**9**) was greatly superior to the *S* enantiomer (**18**) and was slightly better than **7** itself (83%, 8%, and 64% inhibition at 3.13 mg/kg, respectively; Table 3). This was in good agreement with the microsomal stability results (Table 3) and *L. inf* IC_{50} data (Table 1) showing a 4.5-fold potency difference between **9** and **18**. In contrast,

although the *R* enantiomer of **8** (**44**) was also better than the *S* enantiomer (**45**) in this model, it was less active than **8** itself (36% vs. 68% at 6.25 mg/kg; Table 3), indicating reduced oral exposure (**44** and **45** gave sparing aqueous solubilities of ~0.07 μg/mL). A comparative appraisal of **7**, **9**, and **18** in the CDRI hamster model (Supporting Information, Table S3) confirmed the preeminent activity of the *R* enantiomer (**9**) over the *S* form (**18**) and **7** itself, at all dose levels.[25] Furthermore, dose-response evaluations of **9** and the TB drug delamanid (**5**) in a similar *L. inf* infected hamster model at LMPH also corroborated the excellent *in vivo* efficacy of **9** (>99% inhibition in 3 target organs at 25 mg/kg; Table 5), whereas **5** displayed a weak effect (22-66% at 50 mg/kg), consistent with its poor *in vitro* potency (IC_{50} 7.1 μM). In view of these findings, the decision was made to proceed with **9** as a lead candidate for VL.

While the initial *in vivo* work was in progress, the oxazole analogues in hand (**7**, **8**, **10**-**12**, **46**, and **47**) were screened against *L. don* in the macrophage-based luciferase assay at CDRI.[25] Based on these results (Table 1) and some encouraging microsomal stability data (Table 3), two additional compounds were identified as worthy of investigation in the VL mouse model. These were the (6-fold) more potent des-methyl analogue of **7** (**12**: IC_{50} 0.005 μM) and the 4-trifluoromethoxy analogue of **8** (**47**: IC_{50} 0.05 μM). However, counterintuitively to our previous work,[19] **47** was markedly inferior to **8** for VL (only 42% inhibition at 25 mg/kg), while the more potent lead **12** also displayed slightly lower efficacy than **7** (89% inhibition at 6.25 mg/kg; Table 3). To confirm the latter result, we examined the enantiomers of **12** (**13** and **14**), but surprisingly, while the *R* form (**13**) was preferred *in vitro* (Table 1), both stereoisomers had an equivalent effect *in vivo* (~75% at 6.25 mg/kg; Table 3).

Examination of the pharmacokinetic properties of selected compounds afforded further insight (Table 4), revealing that **12** had a higher rate of clearance than **7**, gave a lower exposure level, and had a concomitantly reduced oral bioavailability (52% vs. 79%), likely due in part to its 4-fold lower aqueous solubility (Table 3). Furthermore, while **47** did show

better metabolic stability than **8** (decreased clearance and longer half-life; Table 4), it also suffered from poor oral absorption (C_{max} 0.29 μg/mL; see Supporting Information, Figure S1), resulting in modest oral bioavailability (18% *cf.* 63% for **8**). Again, this was consistent with the 11-fold lower aqueous solubility of **47** compared to **8** (Table 3) and suggests the need for stronger oral formulations when evaluating such sparingly soluble compounds. Thus, the early selection of **9** for advanced development[12] was validated by the discovery of inferior *in vivo* results for the few alternative candidates that were assessed at the time.

Following the demonstration of suitable *in vivo* efficacy and *in vivo* pharmacokinetics, drug discovery for leishmaniasis typically requires several additional ADME and safety studies.[56] For example, many nitroheterocyclic compounds provide positive results in the Ames test for mutagenicity; indeed, the development of an early 6-nitroimidazooxazole lead for TB (CGI-17341) was halted following such a discovery.[15] Extensive investigation by Otsuka Pharmaceutical Co. established that the introduction of heteroatoms and larger side chain functionality (e.g., **5**) overcame this effect[18,57] and both **8** and **9**[26] were Ames negative. Other safety factors (e.g., hERG, CYP inhibition, potential for drug-drug interactions) were also broadly acceptable for **9**,[26] which additionally satisfied such criteria as high stability, low cost of synthesis and suitability for once-per-day oral administration noted in the proposed target product profile for a VL drug.[58] Oral treatment for VL offers many important advantages, including convenience and improved patient compliance; it also enables direct delivery of the absorbed drug to the liver, a major parasite population site.[26] Therefore, the repositioning of **9** for VL may represent a significant medical breakthrough for this highly neglected disease.

2. SAR of nitroimidazooxazoles for VL

In an attempt to better understand the SAR for VL (with a view to further improving drug-like features, e.g., solubility and safety), additional oxazoles were prepared and screened, first at CDRI, and then at LMPH. The latter testing confirmed (Table 1) that this class had no

obvious potential for treating HAT (*T. brucei* IC_{50}s >1 μM, most >64 μM), nor any clear utility for Chagas disease (*T. cruzi* IC_{50}s mostly 1-3 μM, and no new leads were better than **5** or **8**), so the major focus on VL was justified. Nevertheless, in light of the recently reported efficacy of fexinidazole (a 5-nitroimidazole derivative) in a mouse model of VL,[59] one fundamental question was whether 5- or 6-nitroimidazooxazoles were preferred. However, an inspection of the data for **15**-**17** (5-nitro isomers of **12**-**14** above) immediately showed that the 5-nitro analogues were more than two orders of magnitude less effective against *L. inf*.

Having established this, compounds **19**-**23** initially examined the influence of the phenyl ring substituent. Interestingly, while the single determination CDRI data (*L. don*) appeared to indicate a clear advantage for 4-trifluoromethoxy (**7**), this was not supported by the mean IC_{50} results vs. *L. inf*, which pinpointed only the 4-phenoxy analogue (**23**) as being significantly less active, with the remaining compounds (including **7**) falling within a ca. 2-fold potency range. Replacement of the side chain linking oxygen atom in **7** by nitrogen (**24** and **25**) was also very well tolerated (*L. inf* IC_{50}s ≤1.5-fold apart), and, in the case of **24**, this allowed a 6-fold solubility improvement (albeit, this compound seemed to be less stable than **7**). In contrast, removal of the aryl moiety (alcohol **26**; ΔCLogP -3.4 units over **7**) led to a 55-fold loss in VL activity, suggesting that an aryl-based side chain was necessary (as for TB).

Therefore, we next investigated a small set of trifluoromethyl-substituted heteroaryl ethers (**27**-**31**). This design concept was based on some partial success with employing trifluoromethylpyridine as a more soluble substitute for trifluoromethoxyphenyl in our previous TB studies.[20,21,37] Pleasingly, pyridine **27** (*L. inf* IC_{50} 0.24 μM) was slightly more potent than both **7** and the direct phenyl equivalent **22**, and was 3-fold more soluble than **7** (11 μg/mL). However, less lipophilic analogues of **27** (**28**-**31**) gave 2- to 6-fold poorer IC_{50} values against the parasite, despite in two cases (**28**, **30**) providing superior solubility.

The SAR focus then switched to targets with phenylpyridine side chains, which could be regarded[20] as more soluble mimics of the biphenyl lead **8**. For completeness, the relatively insoluble 2*H* biphenyls **32**-**34** were first evaluated and found to be 2- to 6-fold more active than their 2-methyl counterparts (**8**, **46**, and **47**), similar to the 2-fold higher *L. inf* potency of **12** vs. **7**. The 2*H* phenyl-2-pyridines **35** and **36** had comparable or up to 6-fold reduced activities compared to the biphenyls **33** and **34**, respectively. Conversely, 2*H* phenyl-3-pyridine derivatives **37**, **40** and **41** were noteworthy for the exceptional potency of the 4-fluoro compound **37** (*L. inf* IC_{50} 0.03 μM), which was 11-fold better than **7** and 25-fold superior to the 4-trifluoromethoxy analogue **41**.

Similar SAR trends were revealed in the 2-methyl series where, for phenyl-3-pyridine derivatives (**51**-**53**), the 4-fluoro compound **51** (*L. inf* IC_{50} 0.13 μM) was overwhelmingly more active than congeners **52** and **53** (by 40- to 85-fold). By contrast, in the less soluble and more lipophilic (ΔCLogP +0.6 units) isomeric phenyl-2-pyridine series (**48**-**50**), the 4-fluoro compound **48** (*L. inf* IC_{50} 2.9 μM) was no better than biphenyl lead **8** or close analogue **49**, although the 4-trifluoromethoxy derivative **50** was inferior. It was interesting to observe that neither **37** nor **51** showed enhanced potencies against the other parasites (or TB) and that both compounds displayed ~5-fold better aqueous solubility than **8** at neutral pH, in harmony with their decreased lipophilicities (ΔCLogP -1.1 to -1.5 units). This solubility differential over **8** was considerably larger at low pH (>2000-fold; Table 3), suggesting further promise. Therefore the enantiomers of **37** (**38** and **39**) were also prepared and assessed, together with their 5-nitro isomers (**42** and **43**). Here, unexpectedly, the *S* enantiomer **39** was the most active (2-fold over **38**), while the 5-nitro analogues were 3 orders of magnitude less effective.

Overall, given the superior *in vivo* efficacy of **8** compared to **47** and the much higher *in vitro* potencies of **37** and **51**, it did appear that a 4-fluoro substituent in the terminal ring was preferred for VL. Therefore, in the remaining compounds (**54**-**59**) we retained this element

and probed further modifications expected to improve solubility. Commencing with the bipyridines **54**-**56**, the most synthetically challenging isomer **54** was found to be the best (*L. inf* IC_{50} 0.14 μM), having >20-fold higher potencies than the related phenylpyridine **48** and the biphenyl congener **8**, and 13- to 16-fold greater aqueous solubility than these compounds at neutral pH (in accordance with its reduced lipophilicity, ΔCLogP -1.1 to -1.6 units). Importantly, more structurally diverse phenylpiperazine and phenylpiperidine analogues (**57** and **58**) also retained excellent activities (*L. inf* IC_{50}s 0.20 and 0.23 μM). As noted above, phenylpiperazine and phenylpiperidine are soluble bioisosteres for biphenyl and these moieties are now appearing in many new drug candidates.[60,61] Here, **57** and **58** were respectively 61-fold and 11-fold more soluble than **8** at neutral pH and formed nicely soluble hydrochloride salts at low pH (19-23 mg/mL), in line with their calculated pKa values of 5.9 and 7.3. Finally, based on previous success with an arylpiperazine carbamate analogue of **4** for TB,[41] carbamate **59** was studied, but the additional carboxy group proved detrimental for VL (8.5-fold potency loss). There was also a poor tolerance for longer side chains (**60** and **5**).

With these SAR results in hand, the next stage of this work was to assess microsomal stability and *in vivo* efficacy for the best new leads. Six racemic compounds (pyridine **27**, phenylpyridines **37** and **51**, bipyridines **54** and **56**, and phenylpiperazine **57**) were selected as representative of both potency and solubility optimisations for the new side chain classes investigated here. Unsurprisingly, these diverse compounds demonstrated a wide range of metabolic stabilities (Table 3) following 1 h exposures to human and mouse liver microsomes (HLM and MLM). While all compounds showed broadly acceptable stabilities towards HLM (~50% or more parent remaining), phenylpyridine **51** and phenylpiperazine **57** displayed low stabilities toward MLM (14-21% parent), with the others having moderate MLM stabilities (47-69% parent). The most stable compounds were pyridine **27** and bipyridine **56**, and a 2-H substituent (**37**) appeared to reduce the rate of MLM metabolism (*cf.* 2-methyl derivative **51**).

Nevertheless, upon *in vivo* evaluation in the mouse model of VL (dosing orally at 25 mg/kg for 5 consecutive days), all candidates except the least potent one (bipyridine **56**) provided essentially total parasite clearance (>99.5%), similar to **7**, **8**, and **12**. At 6.25 mg/kg, the more rapidly metabolised phenylpiperazine **57** demonstrated poor efficacy (16% inhibition), whereas pyridine **27** (83%) was very similar to the des-methyl phenyl ether **12** (89%), bipyridine **54** was highly effective (96%), and phenylpyridines **37** and **51** both achieved a 100% cure (Figure 2 and Table 3). From further dose reductions on **37**, **51**, and **54**, it was subsequently established that the highly potent 2*H* phenylpyridine **37** was the most active lead (98% inhibition at 1.56 mg/kg, *cf.* <50% for **9**, **51**, and **54**), and a final head-to-head assessment of its enantiomers determined that the *S* form was marginally preferred (ED_{50}s 1.3 and 1.1 mg/kg for **38** and **39**), despite demonstrating inferior MLM stability.

As discussed, a key objective of this work was to identify backups to **9** having an improved physicochemical/pharmacological profile. From our study of nitroimidazooxazoles, we have discovered several new racemic analogues (e.g., bipyridine **54**, and phenylpyridines **37** and **51**) with very high *in vivo* efficacies in a mouse model of VL, broadly comparable to **9**. However, **54** could not be regarded as a good candidate for further development due to its high synthetic difficulty, while the low metabolic stability of phenylpyridine **51** toward MLM would not bode well for its assessment in the more stringent hamster model (where faster rates of metabolism were often observed). Thus, the best new racemic lead identified here was the phenylpyridine **37**, and the most active enantiomer of **37** was **39**. With improved aqueous solubility under acidic conditions (a calculated pK_a value of 3.73), acceptable metabolic stability toward HLM, and excellent *in vivo* efficacy in the mouse model of VL, this compound may warrant further investigation as a potential new antileishmanial agent.

3. SAR of new heterocyclic analogues for NTDs

The mechanism of action of these nitroimidazooxazoles against VL is currently unknown. It has recently been disclosed that the parasite *L. don* does not possess a homologue of the *M. tb* nitroreductase Ddn and that compounds such as **4** are not activated by the same type I nitroreductase that operates on fexinidazole.[62] In addition to this, *L. don* reportedly does not possess the mycolic acid biosynthetic pathway that is present in *M. tb* and inhibited by **4** and **5** under aerobic conditions.[59] Therefore, as an extension to the current SAR study, we were interested in ascertaining which elements of the nitroheterocyclic ring system of **7** were essential for the antiparasitic activities observed (*L. inf*, *T. cruzi*) and whether or not any new ring A analogues could become useful leads. The des-nitro congener of **7** (**61**) was inactive against VL but unexpectedly retained similar potency to **7** against *T. cruzi* (IC_{50} 2.1 μM; Table 2); therefore, a variety of alternative imidazole ring substituents (e.g., Br, CHO, CH_2OH, SMe, SO_2Me) were evaluated (**62**-**69**). As expected, such compounds showed almost no utility for VL, but the 5-bromo (**67**) and 5-methylthio (**68**) analogues provided enhanced antitrypanosomal potencies (*T. cruzi* IC_{50}s 0.13 and 0.58 μM; 6-substituted congeners were notably inferior). It is attractive to speculate that the inactivity of **61**-**69** against *L. inf* may point to a specific requirement for the 6-nitro group of **7** in the mechanism of action, beyond simply electronic or protein binding effects. In this work, we have shown that the 6-nitro group of **7** can readily undergo *cine*-substitution by thiolate. Since it has been established that nitric oxide is cidal to *Leishmania*,[63] the known capacity of these compounds to produce nitric oxide (via loss of the nitro group as nitrous acid) might still be a factor in their VL activity if specific *nucleophilic* activation (e.g., by an active site cysteine thiolate or hydride)[23] could occur via some other enzyme system within the parasite.

The remaining compounds in Table 2 (**70**-**72**) investigated replacement of the imidazole ring by triazole or pyrazole. The direct analogues of **7** (**70** and **72**) were both completely inactive against *L. inf*, suggesting that in addition to the 6-nitro group, this imidazole ring is

also essential for VL activity (mimicking the structural requirements for TB activity[13,64]). Based on measurements in the oxazine series,[13] the nitro reduction potentials of **72** and **7** should be very similar, but **70** and **72** could not undergo the unique ring reduction chemistry and nitric oxide release exhibited by **4**. Interestingly, the nitrotriazole **70** was more active than **7** against *T. cruzi* (IC_{50}s 0.59 and 1.8 μM, respectively) whereas the bromotriazole **71** and nitropyrazole **72** were less active. Thus, while the 4-nitroimidazole ring of **7** appeared to be essential for retaining utility against VL, a much greater degree of structural variation was tolerated for Chagas disease, suggesting a different mechanism of action. The results from this investigation may therefore stimulate interest in developing new agents for Chagas disease from these rather novel leads.

4. SAR of nitroimidazooxazoles for TB

While the major focus of these efforts was directed towards VL, we also examined the utility of the new oxazoles against TB, using the *in vitro* assays described above (Table 1). Generally, it appeared that pyridine, phenylpyridine, bipyridine, phenylpiperazine, phenylpiperidine, and phenylpiperazine carbamate were all useful side chains for TB, consistent with findings in the nitroimidazooxazine series and fairly scant disclosures from Otsuka Pharmaceutical Co.[31,57] It was particularly fascinating to observe that, except for **12**, **14**, and **39**, all of the most active VL leads (including **7**, **8**, and **9**, but also **27**, **37**, **51**, and **54**) possessed notably high antitubercular potencies in the MABA assay (MICs <0.08 μM, comparable to **5**), despite exhibiting only modest growth inhibitory effects under low oxygen conditions (LORA). A closer examination of monoheterocyclic side chains revealed that the 2-pyridine **27** (MABA MIC 0.038 μM) was indeed optimal (as for VL), but replacement of the oxygen linkage in **7** by nitrogen (**24**, **25**) reduced MABA potency by 5-fold. For the new biaryl side chain derivatives, phenylpyridines such as **37**, **38**, **40**, **41**, and **48**-**53**, as well as bipyridine **54**, uniformly displayed the highest aerobic activities (MICs ≤0.05 μM), similar or

slightly better than the original biphenyl analogues (**8**, **46**, and **47**). However, the elusive 2*H* phenyl-2-pyridines **35** and **36** showed dramatically lower potencies in this assay (MICs of 58 and 5.3 μM), possibly indicating reduced stability. More soluble bioisosteres containing a cyclic amine (**57**-**59**) also provided slightly inferior results (MABA MICs 0.13-0.29 μM).

Because of the early promise of **7** and **8** for VL, it was considered worthwhile to evaluate the enantiomers of **7** (**9** and **18**) and the *R* enantiomer of **8** (**44**) alongside pretomanid (**4**) in a mouse model of acute TB infection, dosing orally for 3 weeks (daily for 5 days each week).[19,50] In this assay, both **9** and **44** (100 mg/kg) showed efficacies similar to rifampicin (15 mg/kg), respectively 9.3- and 7.6-fold superior to **4**, whereas the *S* enantiomer of **7** (**18**) was slightly less efficacious than **4** (at 100 mg/kg; Figure 3). Nevertheless, the VL lead **9** was reportedly much less active than delamanid (**5**) in a comparable *M. tb* infection model.[18]

CONCLUSIONS

This study began with discovery of the extraordinary utility of several antitubercular oxazole analogues against the neglected kinetoplastid disease VL. Having first identified **9** as a preferred lead for preclinical development, we later investigated its SAR, with the objective of improving aqueous solubility and other drug-like features without compromising *in vivo* efficacy. All modifications to the nitroheterocycle (*viz.* removal of the nitro group or switching its position, and replacement of the imidazole ring by pyrazole or triazole) resulted in a total loss of activity against VL (but not Chagas disease), whereas many aryl-, heteroaryl-, and heterobiaryl-based side chains were well tolerated *in vitro*. Surprisingly, there were several significant similarities in these respects to the SAR for TB, while the discovery of facile *cine*-substitution of the nitro group of **7** by thiolate was intriguing.

Overall, the most effective strategies to enhance aqueous solubility while maintaining or increasing potency were the replacement of phenyl rings by pyridine and the use of

arylpiperazine and arylpiperidine as bioisosteres for a biaryl moiety. These strategies enabled solubility improvements over **7** of 3- to 14-fold at pH = 7 and up to 6400-fold at pH = 1. Assessments of stability toward HLM gave broadly acceptable results, although two compounds (phenylpyridine **51** and phenylpiperazine **57**) had high MLM metabolism rates. Further appraisal of six candidates in the acute infection mouse model of VL revealed three racemic compounds (phenylpyridines **37** and **51**, and bipyridine **54**) that displayed efficacies at least as strong as **7**. Of these, one compound (**37**) was notably superior, in line with its excellent *in vitro* profile, and subsequent synthesis and evaluation of its enantiomers has pinpointed the *S* form **39** as being the slightly preferred VL lead for further investigation. Findings from this work may also inspire interest in these promising compounds for other NTDs.

EXPERIMENTAL SECTION

Combustion analyses were performed by the Campbell Microanalytical Laboratory, University of Otago, Dunedin, New Zealand. Melting points were determined using an Electrothermal IA9100 melting point apparatus, and are as read. NMR spectra were measured on a Bruker Avance 400 spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C and are referenced to Me_4Si or solvent resonances. Chemical shifts and coupling constants are recorded in units of ppm and hertz, respectively. High-resolution chemical ionisation (HRCIMS) and fast atom bombardment (HRFABMS) mass spectra were determined on a VG-70SE mass spectrometer at nominal 5000 resolution. High-resolution electrospray ionisation (HRESIMS) mass spectra were determined on a Bruker micrOTOF-Q II mass spectrometer. Low-resolution atmospheric pressure chemical ionisation (APCI) mass spectra were obtained for organic solutions using a ThermoFinnigan Surveyor MSQ mass spectrometer, connected to a Gilson autosampler. Optical rotations were measured on a Schmidt + Haensch Polartronic

NH8 polarimeter. Column chromatography was performed on silica gel (Merck 230-400 mesh). Thin-layer chromatography was carried out on aluminium-backed silica gel plates (Merck 60 F_{254}), with visualization of components by UV light (254 nm), I_2, or $KMnO_4$ staining. Tested compounds (including batches screened *in vivo*) were ≥ 95% pure, as determined by combustion analysis (results within 0.4% of theoretical values) and/or by HPLC conducted on an Agilent 1100 system, using a 150 mm x 3.2 mm Altima 5 µm reversed phase C18 column with diode array detection. Finally, chiral purity was assessed by HPLC carried out on a Gilson Unipoint system (322-H pump, 156 UV/vis detector), employing a 250 mm x 4.6 mm CHIRALCEL OD 10 µm analytical column, or 250 mm x 4.6 mm CHIRALPAK IA or CHIRALPAK AD-H 5.0 µm analytical columns.

Compounds of Table 1. The following section details the syntheses of compounds **10**-**13**, **15**, **16**, **18**, **23**, **24**, **35**, **37**, **48**, **55**, and **57** of Table 1, via representative procedures and key intermediates, as described in Schemes 1-4. For the syntheses of all of the other compounds in Table 1, please refer to Supporting Information.

Synthesis of 10 (Scheme 1A):

Procedure A: 2-({[4-(Trifluoromethoxy)benzyl]oxy}methyl)oxirane (74). A solution of glycidol (**73**) (303 mg, 4.09 mmol) and 4-(trifluoromethoxy)benzyl bromide (0.81 mL, 5.06 mmol) in anhydrous DMF (6 mL) under N_2 at 0 °C was treated with 60% NaH (246 mg, 6.15 mmol), then quickly degassed and resealed under N_2. The resulting mixture was stirred at 20 °C for 7 h and then cooled (CO_2/acetone), quenched with ice/aqueous $NaHCO_3$ (100 mL), and extracted with EtOAc (4 x 100 mL). The extracts were washed with brine (100 mL) and then evaporated to dryness under reduced pressure (at 30 °C), and the residue was chromatographed on silica gel. Elution with 0-5% Et_2O/petroleum ether first gave foreruns, and then further elution with 5-10% Et_2O/petroleum ether gave **74** (625 mg, 62%) as a

colourless oil; ^{1}H NMR ($CDCl_3$) δ 7.38 (br d, *J* = 8.7 Hz, 2 H), 7.20 (br d, *J* = 8.7 Hz, 2 H), 4.62 (d, *J* = 12.0 Hz, 1 H), 4.56 (d, *J* = 12.0 Hz, 1 H), 3.82 (dd, *J* = 11.5, 2.8 Hz, 1 H), 3.43 (dd, *J* = 11.5, 6.0 Hz, 1 H), 3.24-3.18 (m, 1 H), 2.82 (dd, *J* = 4.9, 4.2 Hz, 1 H), 2.63 (dd, *J* = 5.0, 2.7 Hz, 1 H); HRESIMS calcd for $C_{11}H_{11}F_3NaO_3$ *m*/*z* $[M + Na]^+$ 271.0552, found 271.0557.

Procedure B: 1-(2-Bromo-4-nitro-1*H*-imidazol-1-yl)-3-{[4-(trifluoromethoxy)benzyl]oxy}propan-2-ol (76). A mixture of epoxide **74** (500 mg, 2.01 mmol), 2-bromo-4-nitro-1*H*-imidazole (**75**) (390 mg, 2.03 mmol), and DIPEA (1.75 mL, 10.0 mmol) in a sealed vial was stirred at 107 °C for 13 h. The resulting cooled mixture was dissolved in CH_2Cl_2 and added to ice/aqueous $NaHCO_3$ (50 mL), then extracted with CH_2Cl_2 (5 x 50 mL). The extracts were evaporated to dryness under reduced pressure and the residue was chromatographed on silica gel. Elution with 0-1% EtOAc/CH_2Cl_2 first gave foreruns, and then further elution with 1-2% EtOAc/CH_2Cl_2 gave **76** (538 mg, 61%) as a cream solid: mp (CH_2Cl_2/pentane) 80-81 °C; ^{1}H NMR ($CDCl_3$) δ 7.95 (s, 1 H), 7.35 (br d, *J* = 8.7 Hz, 2 H), 7.23 (br d, *J* = 7.9 Hz, 2 H), 4.57 (s, 2 H), 4.20 (dd, *J* = 13.6, 2.9 Hz, 1 H), 4.18-4.10 (m, 1 H), 4.07 (dd, *J* = 13.4, 7.1 Hz, 1 H), 3.59 (dd, *J* = 9.6, 4.2 Hz, 1 H), 3.46 (dd, *J* = 9.6, 5.3 Hz, 1 H), 2.61 (d, *J* = 5.0 Hz, 1 H); HRESIMS calcd for $C_{14}H_{14}BrF_3N_3O_5$ *m*/*z* $[M + H]^+$ 442.0044, 440.0063, found 442.0044, 440.0061.

Procedure C: 6-Nitro-2-({[4-(trifluoromethoxy)benzyl]oxy}methyl)-2,3-dihydroimidazo[2,1-*b*][1,3]oxazole (10). A solution of alcohol **76** (524 mg, 1.19 mmol) in anhydrous DMF (12 mL) under N_2 at 0 °C was treated with 60% NaH (74 mg, 1.85 mmol), then quickly degassed and resealed under N_2. The resulting mixture was stirred at 0 °C for 65 min and then cooled (CO_2/acetone), quenched with ice/aqueous $NaHCO_3$ (20 mL), added to brine (100 mL), and extracted with CH_2Cl_2 (6 x 100 mL). The combined extracts were evaporated to dryness under reduced pressure (at 30 °C) and the residue was

chromatographed on silica gel. Elution with CH_2Cl_2 first gave foreruns, and then further elution with 0-2% EtOAc/CH_2Cl_2 gave **10** (383 mg, 90%) as a cream solid: mp (CH_2Cl_2/hexane) 134-135 °C; ^{1}H NMR ($CDCl_3$) δ 7.53 (s, 1 H), 7.29 (br d, *J* = 8.7 Hz, 2 H), 7.20 (br d, *J* = 8.0 Hz, 2 H), 5.46-5.38 (m, 1 H), 4.60 (s, 2 H), 4.32 (dd, *J* = 10.0, 8.6 Hz, 1 H), 4.26 (dd, *J* = 10.0, 6.5 Hz, 1 H), 3.89 (dd, *J* = 11.3, 3.9 Hz, 1 H), 3.78 (dd, *J* = 11.3, 3.5 Hz, 1 H). Anal. ($C_{14}H_{12}F_3N_3O_5$) C, H, N.

Synthesis of 11 (Scheme 1A):

1-{[(2-Methylprop-2-en-1-yl)oxy]methyl}-4-(trifluoromethoxy)benzene (78). A solution of 2-methylprop-2-en-1-ol (**77**) (2.34 mL, 27.8 mmol) in anhydrous DMF (10 mL, then 2 x 2 mL to rinse) was added dropwise to a stirred suspension of 60% NaH (1.32 g, 33.0 mmol) in anhydrous DMF (10 mL) under N_2 at 0 °C. After 30 min, 4-(trifluoromethoxy)benzyl bromide (5.1 mL, 31.9 mmol) was added, and the mixture was stirred at 20 °C for 21 h. The resulting mixture was added to ice/aqueous $NaHCO_3$ (200 mL) and extracted with 25% EtOAc/petroleum ether (2 x 200 mL) and 50% EtOAc/petroleum ether (3 x 200 mL). The extracts were washed with water (200 mL) and then concentrated under reduced pressure, and the remaining oil was chromatographed on silica gel. Elution with petroleum ether first gave foreruns, and then further elution with 0-5% CH_2Cl_2/petroleum ether gave **78** (6.57 g, 96%) as a colourless oil, which was used directly in the next step; ^{1}H NMR ($CDCl_3$) δ 7.37 (br d, *J* = 8.7 Hz, 2 H), 7.19 (br d, *J* = 8.0 Hz, 2 H), 5.00 (m, 1 H), 4.94 (m, 1 H), 4.48 (s, 2 H), 3.94 (br s, 2 H), 1.77 (s, 3 H).

Procedure D: 2-Methyl-2-({[4-(trifluoromethoxy)benzyl]oxy}methyl)oxirane (79). 3-Chloroperoxybenzoic acid (19.5 g of 50%, 56.5 mmol) was added to a mixture of alkene **78** (6.57 g, 26.7 mmol) and disodium hydrogen phosphate (9.87 g, 69.5 mmol) in CH_2Cl_2 (250 mL) at 0 °C. The mixture was stirred at 20 °C for 3 h and then added to an ice-cold aqueous solution of sodium sulphite (400 mL of 10%) and extracted with CH_2Cl_2 (3 x 400 mL). The

extracts were sequentially washed with ice-cold aqueous sodium sulphite solution (400 mL of 10%), aqueous $NaHCO_3$ (400 mL), and brine (300 mL), and then the aqueous solutions were back-extracted with CH_2Cl_2 (200 mL). The combined extracts were concentrated under reduced pressure and the remaining oil was chromatographed on silica gel. Elution with 0-15% CH_2Cl_2/petroleum ether first gave foreruns, and then further elution with 15% CH_2Cl_2/petroleum ether and CH_2Cl_2 gave **79** (6.53 g, 93%) as a colourless oil; ^{1}H NMR ($CDCl_3$) δ 7.37 (br d, *J* = 8.7 Hz, 2 H), 7.19 (br d, *J* = 7.9 Hz, 2 H), 4.59 (d, *J* = 12.1 Hz, 1 H), 4.54 (d, *J* = 12.1 Hz, 1 H), 3.61 (d, *J* = 11.1 Hz, 1 H), 3.44 (d, *J* = 11.1 Hz, 1 H), 2.75 (d, *J* = 4.9 Hz, 1 H), 2.64 (d, *J* = 4.9 Hz, 1 H), 1.40 (s, 3 H); HRCIMS (NH_3) calcd for $C_{12}H_{17}F_3NO_3$ *m*/*z* $[M + NH_3 + H]^+$ 280.1161, found 280.1144.

1-(2-Bromo-4-nitro-1*H*-imidazol-1-yl)-2-methyl-3-{[4-(trifluoromethoxy)benzyl]oxy}-propan-2-ol (80). Reaction of epoxide **79** with 2-bromo-4-nitro-1*H*-imidazole (**75**) (1.1 equiv) and DIPEA (7.6 equiv), using procedure B at 108 °C for 15 h, followed by chromatography of the product on silica gel, eluting with CH_2Cl_2, gave **80** (94%) as a pale yellow oil; ^{1}H NMR ($CDCl_3$) δ 8.00 (s, 1 H), 7.33 (br d, *J* = 8.6 Hz, 2 H), 7.22 (br d, *J* = 8.0 Hz, 2 H), 4.56 (s, 2 H), 4.15 (d, *J* = 14.8 Hz, 1 H), 4.04 (d, *J* = 14.5 Hz, 1 H), 3.39 (s, 2 H), 2.51 (s, 1 H), 1.22 (s, 3 H); HRESIMS calcd for $C_{15}H_{16}BrF_3N_3O_5$ *m*/*z* $[M + H]^+$ 456.0200, 454.0220, found 456.0197, 454.0221.

2-Methyl-6-nitro-2-({[4-(trifluoromethoxy)benzyl]oxy}methyl)-2,3-dihydroimidazo[2,1-*b*][1,3]oxazole (11). Reaction of alcohol **80** with NaH (1.4 equiv), using procedure C for 85 min, followed by chromatography of the product on silica gel, eluting with CH_2Cl_2 (foreruns) and then with 0-1% EtOAc/CH_2Cl_2, gave **11** (87%) as a pale yellow solid: mp (CH_2Cl_2/hexane) 110-111 °C; ^{1}H NMR ($CDCl_3$) δ 7.50 (s, 1 H), 7.26 (br d, *J* = 8.4 Hz, 2 H), 7.19 (br d, *J* = 8.3 Hz, 2 H), 4.59 (d, *J* = 12.3 Hz, 1 H), 4.56 (d, *J* = 12.3 Hz, 1 H),

4.36 (d, *J* = 10.0 Hz, 1 H), 3.91 (d, *J* = 10.0 Hz, 1 H), 3.72 (d, *J* = 10.7 Hz, 1 H), 3.59 (d, *J* = 10.6 Hz, 1 H), 1.65 (s, 3 H). Anal. ($C_{15}H_{14}F_3N_3O_5$) C, H, N.

Synthesis of 12 and 15 (Scheme 1B):

Procedure E: 2-{[4-(Trifluoromethoxy)phenoxy]methyl}oxirane (82). A mixture of epibromohydrin (**81**) (0.30 mL, 3.51 mmol), 4-(trifluoromethoxy)phenol (0.152 mL, 1.17 mmol), and powdered K_2CO_3 (260 mg, 1.88 mmol) in acetone (3 mL) in a sealed vial was stirred at 59 °C for 36 h. The resulting cooled mixture was filtered, washing with CH_2Cl_2, and then the filtrate was concentrated under reduced pressure and the remaining oil was chromatographed on silica gel. Elution with 0-15% CH_2Cl_2/pentane first gave foreruns, and then further elution with 20-25% CH_2Cl_2/pentane gave **82**[28] (260 mg, 95%) as a colourless oil; ^{1}H NMR ($CDCl_3$) δ 7.14 (br d, *J* = 9.0 Hz, 2 H), 6.91 (br d, *J* = 9.1 Hz, 2 H), 4.23 (dd, *J* = 11.1, 3.1 Hz, 1 H), 3.94 (dd, *J* = 11.1, 5.7 Hz, 1 H), 3.37-3.31 (m, 1 H), 2.91 (dd, *J* = 4.8, 4.2 Hz, 1 H), 2.75 (dd, *J* = 4.9, 2.6 Hz, 1 H); HRFABMS calcd for $C_{10}H_9F_3O_3$ *m*/*z* (M^+) 234.0504, found 234.0508.

5-Nitro-2-{[4-(trifluoromethoxy)phenoxy]methyl}-2,3-dihydroimidazo[2,1-*b*][1,3]oxazole (15) and 1-(2-bromo-4-nitro-1*H*-imidazol-1-yl)-3-[4-(trifluoromethoxy)phenoxy]propan-2-ol (83). Reaction of epoxide **82** with 2-bromo-4-nitro-1*H*-imidazole (**75**) (1.1 equiv) and DIPEA, using procedure B at 105 °C for 6.5 h, followed by chromatography of the product on silica gel, eluting with CH_2Cl_2, first gave a mixture of oxazole isomers, which was further purified by chromatography on silica gel. Elution with 3:1 and 4:1 CH_2Cl_2/petroleum ether first gave foreruns, and then further elution with 4:1 CH_2Cl_2/petroleum ether and CH_2Cl_2 gave **15** (2.5%) as a white solid: mp (CH_2Cl_2/pentane) 120-122 °C; ^{1}H NMR ($CDCl_3$) δ 7.68 (s, 1 H), 7.17 (br d, *J* = 8.5 Hz, 2 H), 6.90 (br d, *J* = 9.1 Hz, 2 H), 5.77-5.69 (m, 1 H), 4.71 (dd, *J* = 10.7, 8.9 Hz, 1 H), 4.61 (dd, *J*

= 10.7, 6.9 Hz, 1 H), 4.41 (dd, *J* = 10.9, 4.1 Hz, 1 H), 4.31 (dd, *J* = 10.9, 3.8 Hz, 1 H). Anal. ($C_{13}H_{10}F_3N_3O_5$) C, H, N.

Further elution of this second column above with 1% EtOAc/CH_2Cl_2 gave **12** (2%) as a white solid (see below). Further elution of the original column above with 0-1% EtOAc/CH_2Cl_2 gave **83** (70%) as a white solid: mp (MeOH/CH_2Cl_2/hexane) 139-141 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.52 (s, 1 H), 7.30 (br d, *J* = 9.1 Hz, 2 H), 7.05 (br d, *J* = 9.2 Hz, 2 H), 5.66 (br s, 1 H), 4.28 (dd, *J* = 13.3, 3.3 Hz, 1 H), 4.25-4.17 (m, 1 H), 4.13 (dd, *J* = 13.3, 8.0 Hz, 1 H), 4.01 (d, *J* = 5.0 Hz, 2 H). Anal. ($C_{13}H_{11}BrF_3N_3O_5$) C, H, N.

6-Nitro-2-{[4-(trifluoromethoxy)phenoxy]methyl}-2,3-dihydroimidazo[2,1-*b*][1,3]oxazole (12). Reaction of alcohol **83** with NaH, using procedure C for 40 min, followed by chromatography of the product on silica gel, eluting with CH_2Cl_2, gave **12** (85%) as a cream solid: mp (MeOH/CH_2Cl_2/hexane) 170-172 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.16 (s, 1 H), 7.31 (br d, *J* = 9.1 Hz, 2 H), 7.05 (br d, *J* = 9.2 Hz, 2 H), 5.78-5.70 (m, 1 H), 4.50 (dd, *J* = 10.8, 8.9 Hz, 1 H), 4.46 (dd, *J* = 11.5, 2.8 Hz, 1 H), 4.39 (dd, *J* = 11.5, 5.2 Hz, 1 H), 4.22 (dd, *J* = 10.8, 6.5 Hz, 1 H); ^{13}C NMR [$(CD_3)_2SO$] δ 156.8, 156.1, 145.7, 142.2 (q, J_{C-F} = 1.5 Hz), 122.6 (2 C), 120.1 (q, J_{C-F} = 255.2 Hz), 116.0 (3 C), 85.3, 68.3, 45.1. Anal. ($C_{13}H_{10}F_3N_3O_5$) C, H, N.

Synthesis of 13 and 16 (Scheme 1C):

Procedure F: (2*R*)-2-{[4-(Trifluoromethoxy)phenoxy]methyl}oxirane (90). 4-(Trifluoromethoxy)phenol (0.22 mL, 1.70 mmol) was added to a suspension of cesium fluoride (0.80 g, 5.27 mmol) in anhydrous DMF (1.4 mL) under N_2 and the mixture was stirred at 20 °C for 1 h. (2*R*)-Oxiran-2-ylmethyl 3-nitrobenzenesulfonate (**89**) (446 mg, 1.72 mmol) was added and the reaction mixture was briefly degassed and resealed under N_2 and then stirred at 20 °C for 35 h. The resulting mixture was added to water (50 mL) and

extracted with 50% Et_2O/petroleum ether (3 x 50 mL). The extracts were concentrated under reduced pressure (at 25 °C) and the remaining oil was chromatographed on silica gel. Elution with 0-15% CH_2Cl_2/pentane first gave foreruns, and then further elution with 15% CH_2Cl_2/pentane gave **90** (313 mg, 79%) as a colourless oil; ^{1}H NMR ($CDCl_3$) δ 7.14 (br d, *J* = 9.2 Hz, 2 H), 6.91 (br d, *J* = 9.2 Hz, 2 H), 4.24 (dd, *J* = 11.0, 3.0 Hz, 1 H), 3.94 (dd, *J* = 11.0, 5.8 Hz, 1 H), 3.39-3.32 (m, 1 H), 2.92 (dd, *J* = 4.8, 4.2 Hz, 1 H), 2.76 (dd, *J* = 4.9, 2.7 Hz, 1 H); $[\alpha]^{25}_D$ -4.0 (*c* 2.00, $CHCl_3$); HRESIMS calcd for $C_{10}H_9F_3NaO_3$ *m*/*z* $[M + Na]^+$ 257.0396, found 257.0399.

Procedure G: (2*R*)-5-Nitro-2-{[4-(trifluoromethoxy)phenoxy]methyl}-2,3-dihydroimidazo[2,1-*b*][1,3]oxazole (16) and (2*R*)-1-(2-chloro-4-nitro-1*H*-imidazol-1-yl)-3-[4-(trifluoromethoxy)phenoxy]propan-2-ol (92). A mixture of epoxide **90** (202 mg, 0.863 mmol), 2-chloro-4-nitro-1*H*-imidazole (**91**) (141 mg, 0.956 mmol), and DIPEA (0.170 mL, 0.976 mmol) in anhydrous toluene (1.0 mL) in a sealed vial was stirred at 80 °C for 24 h. The resulting cooled mixture was transferred to a flask (in CH_2Cl_2) and evaporated to dryness under reduced pressure (at 30 °C) and the residue was chromatographed on silica gel. Elution with CH_2Cl_2 first gave foreruns, and then further elution with 0.5% EtOAc/CH_2Cl_2 gave **16** (47 mg, 16%) as a white solid: mp (CH_2Cl_2/pentane) 151-152 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 7.87 (s, 1 H), 7.32 (br d, *J* = 9.0 Hz, 2 H), 7.07 (br d, *J* = 9.2 Hz, 2 H), 5.93-5.84 (m, 1 H), 4.68 (dd, *J* = 10.1, 9.4 Hz, 1 H), 4.48 (dd, *J* = 11.6, 2.9 Hz, 1 H), 4.43 (dd, *J* = 11.6, 5.4 Hz, 1 H), 4.40 (dd, *J* = 10.1, 6.8 Hz, 1 H); ^{13}C NMR [$(CD_3)_2SO$] δ 160.0, 156.7, 142.2 (q, J_{C-F} = 2.0 Hz), 134.7, 133.5, 122.6 (2 C), 120.1 (q, J_{C-F} = 255.2 Hz), 116.0 (2 C), 87.5, 68.3, 46.3; $[\alpha]^{24}_D$ -239.0 (*c* 1.004, DMF). Anal. ($C_{13}H_{10}F_3N_3O_5$) C, H, N.

Further elution of the above column with 0.5-1% EtOAc/CH_2Cl_2 gave **92** (222 mg, 67%) as a white solid: mp (CH_2Cl_2/pentane) 110-111 °C; ^{1}H NMR ($CDCl_3$) δ 7.96 (s, 1 H), 7.19 (br d, *J* = 9.1 Hz, 2 H), 6.90 (br d, *J* = 9.1 Hz, 2 H), 4.42-4.30 (m, 2 H), 4.21 (dd, *J* = 14.6, 7.8 Hz,

1 H), 4.06 (dd, *J* = 9.5, 4.7 Hz, 1 H), 3.98 (dd, *J* = 9.5, 5.5 Hz, 1 H), 2.67 (d, *J* = 5.6 Hz, 1 H); $[\alpha]^{27}_{D}$ 19.4 (*c* 2.010, DMF). Anal. ($C_{13}H_{11}ClF_3N_3O_5$) C, H, N.

(2*R*)-6-Nitro-2-{[4-(trifluoromethoxy)phenoxy]methyl}-2,3-dihydroimidazo[2,1-*b*][1,3]oxazole (13). Reaction of alcohol **92** with NaH, using procedure C at 0 °C for 1 h and then at 20 °C for 1 h, followed by chromatography of the product on silica gel, eluting with CH_2Cl_2, gave **13** (87%) as a cream solid: mp (CH_2Cl_2/*i*Pr_2O) 179-180 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.17 (s, 1 H), 7.31 (br d, *J* = 9.0 Hz, 2 H), 7.05 (br d, *J* = 9.2 Hz, 2 H), 5.78-5.70 (m, 1 H), 4.50 (dd, *J* = 10.8, 9.0 Hz, 1 H), 4.45 (dd, *J* = 11.5, 2.7 Hz, 1 H), 4.39 (dd, *J* = 11.5, 5.2 Hz, 1 H), 4.21 (dd, *J* = 10.8, 6.5 Hz, 1 H); $[\alpha]^{25}_{D}$ -24.9 (*c* 1.005, DMF). Anal. ($C_{13}H_{10}F_3N_3O_5$) C, H, N. HPLC purity: 100%.

Chiral HPLC (using a CHIRALPAK IA analytical column and eluting with 15% EtOH/hexane at 1 mL/min) determined that the ee of **13** was 100%.

Synthesis of 18 (Scheme 2A):

Procedure H: (2*S*)-2-Methyl-6-nitro-2-{[4-(trifluoromethoxy)phenoxy]methyl}-2,3-dihydroimidazo[2,1-*b*][1,3]oxazole (18). 4-(Trifluoromethoxy)phenol (2.21 g, 12.4 mmol) was added slowly to a suspension of 60% NaH (0.542 g, 13.6 mmol) in anhydrous DMF (6 mL) under N_2, and the mixture was stirred at 20 °C for 10 min, and then added via cannula to a solution of 2-chloro-1-{[(2*S*)-2-methyloxiran-2-yl]methyl}-4-nitro-1*H*-imidazole[18] (**97**) (2.46 g, 11.3 mmol) in anhydrous DMF (6 mL) at 0 °C under N_2. The resulting mixture was stirred at 20 °C for 15 min and at 80 °C for 15 min, then cooled, and quenched with water (200 mL). Following salt saturation, the mixture was extracted with EtOAc (2 x 200 mL), the extracts were evaporated to dryness under reduced pressure, and the residue was chromatographed on silica gel. Elution with 0-2% MeOH/CH_2Cl_2 gave the crude product, which was recrystallized from a mixture of CH_2Cl_2 and *i*Pr_2O, to give **18** (1.35 g, 33%) as a

pale yellow solid: mp 170-171 °C; ^{1}H NMR ($CDCl_3$) δ 7.56 (s, 1 H), 7.16 (br d, *J* = 8.7 Hz, 2 H), 6.85 (br d, *J* = 9.1 Hz, 2 H), 4.48 (d, *J* = 10.2 Hz, 1 H), 4.23 (d, *J* = 10.0 Hz, 1 H), 4.09 (d, *J* = 10.1 Hz, 1 H), 4.05 (d, *J* = 10.2 Hz, 1 H), 1.79 (s, 3 H); $[\alpha]^{25}_D$ -9.0 (*c* 1.002, $CHCl_3$). Anal. ($C_{14}H_{12}F_3N_3O_5$) C, H, N. HPLC purity: 99.6%.

Chiral HPLC (using a CHIRALPAK AD-H analytical column and eluting with 15% EtOH/hexane at 1 mL/min) determined that the ee of **18** was 98.4%.

Syntheses of 23 and 24 (Scheme 2B):

Procedure I: 2-Methyl-6-nitro-2-[(4-phenoxyphenoxy)methyl]-2,3-dihydroimidazo[2,1-*b*][1,3]oxazole (23). A solution of 2-bromo-1-[(2-methyloxiran-2-yl)methyl]-4-nitro-1*H*-imidazole[33] (**98**) (151 mg, 0.576 mmol) and 4-phenoxyphenol (115 mg, 0.618 mmol) in anhydrous DMF (2 mL) under N_2 at 0 °C was treated with 60% NaH (30 mg, 0.75 mmol), then quickly degassed and resealed under N_2. The mixture was stirred at 20 °C for 10 min and then at 54 °C for 3 h. The resulting mixture was cooled (CO_2/acetone) and quenched with ice/aqueous $NaHCO_3$ (5 mL), then added to brine (50 mL) and extracted with CH_2Cl_2 (6 x 50 mL). The combined extracts were evaporated to dryness under reduced pressure (at 30 °C) and the residue was chromatographed on silica gel. Elution with 25-33% EtOAc/petroleum ether first gave foreruns, and then further elution with 33-67% EtOAc/petroleum ether gave the crude product, which was further chromatographed on silica gel, eluting with CH_2Cl_2, to give **23** (96 mg, 45%) as a pale yellow solid: mp (CH_2Cl_2/pentane) 127-129 °C; ^{1}H NMR ($CDCl_3$) δ 7.56 (s, 1 H), 7.31 (br dd, *J* = 8.6, 7.4 Hz, 2 H), 7.06 (tt, *J* = 7.4, 1.1 Hz, 1 H), 6.97 (br d, *J* = 9.1 Hz, 2 H), 6.94 (br dd, *J* = 8.7, 1.0 Hz, 2 H), 6.83 (br d, *J* = 9.1 Hz, 2 H), 4.51 (d, *J* = 10.2 Hz, 1 H), 4.22 (d, *J* = 10.1 Hz, 1 H), 4.08 (d, *J* = 10.1 Hz, 1 H), 4.04 (d, *J* = 10.2 Hz, 1 H), 1.79 (s, 3 H). Anal. ($C_{19}H_{17}N_3O_5$) C, H, N.

Procedure J: 1-(2-Bromo-4-nitro-1*H*-imidazol-1-yl)-2-methyl-3-{[4-(trifluoromethoxy)phenyl]amino}propan-2-ol (99). A mixture of epoxide **98**[33] (100 mg,

0.382 mmol), 4-(trifluoromethoxy)aniline (70 μL, 0.522 mmol), and anhydrous cobalt(II) chloride (20.4 mg, 0.157 mmol) in anhydrous CH_3CN (2.2 mL) under N_2 was stirred at 63-65 °C for 52 h. The resulting cooled mixture was added to water (50 mL) and extracted with 10% MeOH/CH_2Cl_2 (3 x 50 mL) and CH_2Cl_2 (2 x 50 mL). The combined extracts were concentrated under reduced pressure and the residue was chromatographed on silica gel. Elution with 0-0.5% MeOH/CH_2Cl_2 first gave foreruns and then further elution with 0.5-1% MeOH/CH_2Cl_2 gave **99** (140 mg, 83%) as a yellow solid: mp (MeOH/CH_2Cl_2/hexane) 158-160 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.34 (s, 1 H), 7.06 (br d, *J* = 8.3 Hz, 2 H), 6.72 (br d, *J* = 9.1 Hz, 2 H), 5.81 (br t, *J* = 6.2 Hz, 1 H), 5.19 (s, 1 H), 4.13 (d, *J* = 14.3 Hz, 1 H), 4.09 (d, *J* = 14.3 Hz, 1 H), 3.09 (d, *J* = 6.2 Hz, 2 H), 1.10 (s, 3 H); HRESIMS calcd for $C_{14}H_{14}BrF_3N_4NaO_4$ *m*/*z* $[M + Na]^+$ 463.0024, 461.0043, found 463.0018, 461.0033.

***N*-[(2-Methyl-6-nitro-2,3-dihydroimidazo[2,1-*b*][1,3]oxazol-2-yl)methyl]-4-(trifluoromethoxy)aniline (24).** Reaction of alcohol **99** with NaH (1.3 equiv), using procedure C at 20 °C for 75 min, followed by chromatography of the product on silica gel, eluting with CH_2Cl_2, gave **24** (47%) as a light yellow solid: mp (CH_2Cl_2/pentane) 120-121 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.11 (s, 1 H), 7.05 (br d, *J* = 8.9 Hz, 2 H), 6.74 (br d, *J* = 9.1 Hz, 2 H), 6.19 (br t, *J* = 6.6 Hz, 1 H), 4.20 (d, *J* = 10.9 Hz, 1 H), 4.10 (d, *J* = 10.9 Hz, 1 H), 3.53 (dd, *J* = 15.4, 6.7 Hz, 1 H), 3.49 (dd, *J* = 15.2, 6.6 Hz, 1 H), 1.62 (s, 3 H); ^{13}C NMR [$(CD_3)_2SO$] δ 155.4, 148.1, 145.6, 138.8, 121.9 (2 C), 120.3 (q, J_{C-F} = 254.0 Hz), 116.1, 112.8 (2 C), 96.6, 51.3, 49.9, 23.1. Anal. ($C_{14}H_{13}F_3N_4O_4$) C, H, N.

Synthesis of 35 (Scheme 3B):

Procedure K: 2-{[(5-Bromopyridin-2-yl)oxy]methyl}-6-nitro-2,3-dihydroimidazo[2,1-*b*][1,3]oxazole (119). A solution of (6-nitro-2,3-dihydroimidazo[2,1-*b*][1,3]oxazol-2-yl)methanol[27] (**117**) (382 mg, 2.06 mmol) and 5-bromo-2-fluoropyridine (**118**) (0.70 mL,

6.80 mmol) in anhydrous DMF (7.5 mL) under N_2 at 0 °C was treated with 60% NaH (128 mg, 3.20 mmol), then quickly degassed and resealed under N_2. Further **118** (0.70 mL, 6.80 mmol) was added and the mixture was stirred at 20 °C for 3 h. The resulting mixture was cooled (CO_2/acetone), quenched with ice/aqueous $NaHCO_3$ (20 mL), then added to brine (100 mL), and extracted with CH_2Cl_2 (8 x 100 mL). The combined extracts were evaporated to dryness under reduced pressure (at 30 °C) and the residue was chromatographed on silica gel. Elution with 0-2% EtOAc/CH_2Cl_2 first gave foreruns, and then further elution with 2% EtOAc/CH_2Cl_2 gave **119** (63 mg, 9%) as a cream solid: mp (MeOH/CH_2Cl_2/hexane) 191-194 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.30 (br d, *J* = 2.6 Hz, 1 H), 8.17 (s, 1 H), 7.93 (dd, *J* = 8.8, 2.6 Hz, 1 H), 6.85 (br d, *J* = 8.8 Hz, 1 H), 5.79-5.70 (m, 1 H), 4.67 (dd, *J* = 12.5, 2.8 Hz, 1 H), 4.60 (dd, *J* = 12.5, 5.6 Hz, 1 H), 4.48 (dd, *J* = 10.7, 8.9 Hz, 1 H), 4.21 (dd, *J* = 10.8, 6.7 Hz, 1 H); HRESIMS calcd for $C_{11}H_{10}BrN_4O_4$ *m/z* $[M + H]^+$ 342.9860, 340.9880, found 342.9861, 340.9882.

Procedure L: 2-Fluoro-5-[4-(trifluoromethyl)phenyl]pyridine (120). A stirred mixture of 4-(trifluoromethyl)phenylboronic acid (1.43 g, 7.53 mmol) and Pd(dppf)Cl_2 (370 mg, 0.506 mmol) in toluene (50 mL) and EtOH (25 mL) was degassed for 15 min (vacuum pump) and then N_2 was added. An aqueous solution of Na_2CO_3 (12.5 mL of 2 M, 25.0 mmol) was added by syringe and the stirred mixture was again degassed for 15 min, and then N_2 was added, followed by 5-bromo-2-fluoropyridine (**118**) (0.520 mL, 5.05 mmol). The resulting mixture was stirred at 85-88 °C for 3 h, and then cooled, diluted with aqueous $NaHCO_3$ (100 mL), and extracted with CH_2Cl_2 (5 x 100 mL). The extracts were concentrated under reduced pressure (at 30 °C) and the residue was chromatographed on silica gel. Elution with 0-10% CH_2Cl_2/petroleum ether first gave foreruns, and then further elution with 10-20% CH_2Cl_2/petroleum ether gave **120** (1.22 g, 100%) as a cream solid: mp (CH_2Cl_2/petroleum ether) 55-57 °C; ^{1}H NMR ($CDCl_3$) δ 8.45 (br d, *J* = 2.6 Hz, 1 H), 8.00 (ddd, *J* = 8.5, 7.5, 2.6

Hz, 1 H), 7.75 (br d, J = 8.2 Hz, 2 H), 7.66 (br d, J = 8.1 Hz, 2 H), 7.06 (dd, J = 8.5, 3.0 Hz, 1 H); HRESIMS calcd for $C_{12}H_8F_4N$ m/z $[M + H]^+$ 242.0587, found 242.0585.

6-Nitro-2-[({5-[4-(trifluoromethyl)phenyl]pyridin-2-yl}oxy)methyl]-2,3-dihydroimidazo[2,1-*b*][1,3]oxazole (35). A solution of alcohol **117**[27] (900 mg, 4.86 mmol) and fluoropyridine **120** (1.20 g, 4.98 mmol) in anhydrous DMF (4.5 mL) under N_2 was treated with 60% NaH (280 mg, 7.00 mmol), then quickly degassed and resealed under N_2. The resulting mixture was stirred at 20 °C for 220 min and then cooled (CO_2/acetone), quenched with ice/aqueous $NaHCO_3$ (10 mL), added to brine (100 mL), and then filtered through Celite, washing with CH_2Cl_2 (100 mL). The aqueous portion was further extracted with CH_2Cl_2 (5 x 100 mL) and then the combined organic portions were evaporated to dryness under reduced pressure (at 30 °C), and the residue was chromatographed on silica gel. Elution with 0-12% CH_2Cl_2/petroleum ether first gave foreruns, and then further elution with 20% CH_2Cl_2/petroleum ether gave recovered **120** (1.16 g). Elution with 0-0.33% MeOH/CH_2Cl_2 gave foreruns, and then further elution with 0.5% MeOH/CH_2Cl_2 gave **35** (18 mg, 0.9%) as a cream solid: mp (MeOH/CH_2Cl_2/pentane) 165-168 °C (dec.); ^{1}H NMR [$(CD_3)_2SO$] δ 8.58 (br d, J = 2.6 Hz, 1 H), 8.18 (s, 1 H), 8.14 (dd, J = 8.7, 2.6 Hz, 1 H), 7.91 (br d, J = 8.2 Hz, 2 H), 7.82 (br d, J = 8.4 Hz, 2 H), 6.98 (br d, J = 8.7 Hz, 1 H), 5.83-5.74 (m, 1 H), 4.77 (dd, J = 12.5, 2.8 Hz, 1 H), 4.70 (dd, J = 12.5, 5.5 Hz, 1 H), 4.52 (dd, J = 10.7, 8.9 Hz, 1 H), 4.25 (dd, J = 10.8, 6.7 Hz, 1 H); ^{13}C NMR [$(CD_3)_2SO$] δ 162.6, 156.2, 145.7, 145.1, 140.8, 138.3, 128.5, 127.9 (q, $J_{C\text{-}F}$ = 31.9 Hz), 127.1 (2 C), 125.8 (q, $J_{C\text{-}F}$ = 3.7 Hz, 2 C), 124.3 (q, $J_{C\text{-}F}$ = 271.8 Hz), 116.0, 111.0, 85.4, 65.7, 45.2; HRESIMS calcd for $C_{18}H_{14}F_3N_4O_4$ m/z $[M + H]^+$ 407.0962, found 407.0961. HPLC purity: 98.8%.

Synthesis of 37 (Scheme 3C):

2-Bromo-5-(ethoxymethoxy)pyridine (123). Chloromethyl ethyl ether (4.00 mL, 43.1 mmol) was added dropwise to a cooled mixture of 6-bromopyridin-3-ol (**122**) (5.00 g, 28.7

mmol) and K_2CO_3 (7.97 g, 57.7 mmol) in anhydrous DMF (20 mL) under N_2 at 0 °C, and then the mixture was stirred at 0-20 °C for 24 h. The resulting mixture was added to ice-water (180 mL) and extracted with 50% Et_2O/petroleum ether (5 x 80 mL). The extracts were washed with brine (100 mL) and then concentrated under reduced pressure to give an oil, which was chromatographed on silica gel. Elution with 0-2% Et_2O/petroleum ether first gave foreruns, and then further elution with 2.5-4% Et_2O/petroleum ether gave **123** (5.25 g, 79%) as a colourless oil; ^{1}H NMR ($CDCl_3$) δ 8.18 (br d, *J* = 3.0 Hz, 1 H), 7.38 (br d, *J* = 8.7 Hz, 1 H), 7.28 (dd, *J* = 8.7, 3.0 Hz, 1 H), 5.23 (s, 2 H), 3.72 (q, *J* = 7.1 Hz, 2 H), 1.22 (t, *J* = 7.1 Hz, 3 H); HRESIMS calcd for $C_8H_{11}BrNO_2$ *m/z* $[M + H]^+$ 233.9947, 231.9968, found 233.9940, 231.9962.

5-(Ethoxymethoxy)-2-(4-fluorophenyl)pyridine (124). Reaction of bromide **123** with 4-fluorophenylboronic acid (1.8 equiv) and Pd(dppf)Cl_2 (0.25 equiv), using procedure L for 4.5 h, followed by chromatography of the product on silica gel, eluting with 0-2% Et_2O/petroleum ether (foreruns) and then with 4% Et_2O/petroleum ether, gave **124** (97%) as a colourless oil; ^{1}H NMR ($CDCl_3$) δ 8.46 (br d, *J* = 2.9 Hz, 1 H), 7.91 (br dd, *J* = 9.0, 5.4 Hz, 2 H), 7.61 (br d, *J* = 8.7 Hz, 1 H), 7.45 (dd, *J* = 8.7, 2.9 Hz, 1 H), 7.13 (br t, *J* = 8.8 Hz, 2 H), 5.28 (s, 2 H), 3.76 (q, *J* = 7.1 Hz, 2 H), 1.24 (t, *J* = 7.1 Hz, 3 H); HRESIMS calcd for $C_{14}H_{15}FNO_2$ *m/z* $[M + H]^+$ 248.1081, found 248.1084.

Procedure M: 6-(4-Fluorophenyl)pyridin-3-ol (125). Ethoxymethyl ether **124** (719 mg, 2.91 mmol) was treated with a solution of HCl in MeOH (20 mL of 1.25 M, 25 mmol) and the mixture was stirred at 54 °C for 5 h. The resulting cooled solution was added to ice-water (80 mL) and neutralised with Na_2CO_3 (to pH=6) to give a cream precipitate, which was filtered, washing with water and pentane, to give some product **125**[38] (361 mg, 66%). The filtrate was saturated with salt and extracted with CH_2Cl_2 (4 x 100 mL) and EtOAc (3 x 100 mL), then the combined extracts were concentrated to dryness under reduced pressure. The residue was

suspended in CH_2Cl_2 and filtered to remove salt, and then the filtrate was concentrated to dryness under reduced pressure and triturated in petroleum ether to give further **125**[38] (160 mg, 29%) as a cream solid: mp 142-144 °C (lit.[38] mp 145-147 °C); ^{1}H NMR [$(CD_3)_2SO$] δ 10.03 (s, 1 H), 8.20 (br d, *J* = 2.9 Hz, 1 H), 8.00 (br dd, *J* = 9.0, 5.6 Hz, 2 H), 7.78 (br d, *J* = 8.7 Hz, 1 H), 7.29-7.21 (m, 3 H); HRESIMS calcd for $C_{11}H_9FNO$ *m/z* $[M + H]^+$ 190.0663, found 190.0659.

Procedure N: 2-(4-Fluorophenyl)-5-(oxiran-2-ylmethoxy)pyridine (126). A mixture of epibromohydrin (**81**) (0.24 mL, 2.80 mmol), pyridinol **125** (250 mg, 1.32 mmol), and powdered K_2CO_3 (550 mg, 3.98 mmol) in MEK (5.0 mL) in a sealed vial was stirred at 65 °C for 49 h. The resulting cooled mixture was filtered, washing with CH_2Cl_2, and then the filtrate was concentrated under reduced pressure and the residue was chromatographed on silica gel. Elution with CH_2Cl_2 first gave foreruns, and then further elution with 0-2% Et_2O/CH_2Cl_2 gave **126** (274 mg, 85%) as a white solid: mp (CH_2Cl_2/pentane) 80-81 °C; ^{1}H NMR ($CDCl_3$) δ 8.39 (br d, *J* = 2.9 Hz, 1 H), 7.90 (br dd, *J* = 8.9, 5.4 Hz, 2 H), 7.62 (br d, *J* = 8.7 Hz, 1 H), 7.31 (dd, *J* = 8.7, 3.0 Hz, 1 H), 7.13 (br t, *J* = 8.7 Hz, 2 H), 4.36 (dd, *J* = 11.1, 2.9 Hz, 1 H), 4.03 (dd, *J* = 11.1, 5.8 Hz, 1 H), 3.42-3.37 (m, 1 H), 2.95 (dd, *J* = 4.8, 4.2 Hz, 1 H), 2.80 (dd, *J* = 4.9, 2.6 Hz, 1 H); HRESIMS calcd for $C_{14}H_{13}FNO_2$ *m/z* $[M + H]^+$ 246.0925, found 246.0931.

1-(2-Chloro-4-nitro-1*H*-imidazol-1-yl)-3-{[6-(4-fluorophenyl)pyridin-3-yl]oxy}propan-2-ol (127). Reaction of epoxide **126** with 2-chloro-4-nitro-1*H*-imidazole (**91**) (1.1 equiv) and DIPEA (10 equiv), using procedure B at 100 °C for 12 h, followed by chromatography of the product on silica gel, eluting with 0-1% MeOH/CH_2Cl_2 (foreruns) and then with 1-1.5% MeOH/CH_2Cl_2, gave **127** (69%) as a pale yellow solid: mp (CH_2Cl_2/pentane) 181-182 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.54 (s, 1 H), 8.38 (br d, *J* = 2.7 Hz, 1 H), 8.06 (br dd, *J* = 9.0, 5.5 Hz, 2 H), 7.92 (br d, *J* = 8.6 Hz, 1 H), 7.50 (dd, *J* = 8.8, 3.0 Hz, 1 H), 7.28 (br t, *J* = 8.9 Hz, 2 H),

5.73 (d, J = 5.2 Hz, 1 H), 4.32 (dd, J = 13.2, 3.0 Hz, 1 H), 4.29-4.20 (m, 1 H), 4.18 (dd, J = 13.2, 7.9 Hz, 1 H), 4.13 (br d, J = 4.9 Hz, 2 H); HRESIMS calcd for $C_{17}H_{15}ClFN_4O_4$ m/z $[M + H]^+$ 395.0732, 393.0760, found 395.0739, 393.0762.

2-({[6-(4-Fluorophenyl)pyridin-3-yl]oxy}methyl)-6-nitro-2,3-dihydroimidazo[2,1-*b*][1,3]oxazole (37). Reaction of alcohol **127** with NaH, using procedure C at 0 °C for 1 h and then at 20 °C for 40 min, followed by chromatography of the product on silica gel, eluting with 0-0.5% MeOH/CH_2Cl_2 (foreruns) and then with 1% MeOH/CH_2Cl_2, gave **37** (85%) as a cream solid: mp (MeOH/CH_2Cl_2/hexane) 211-213 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.37 (br d, J = 2.7 Hz, 1 H), 8.18 (s, 1 H), 8.06 (br dd, J = 9.0, 5.6 Hz, 2 H), 7.93 (br d, J = 8.6 Hz, 1 H), 7.52 (dd, J = 8.8, 3.0 Hz, 1 H), 7.28 (br t, J = 8.9 Hz, 2 H), 5.82-5.74 (m, 1 H), 4.58 (dd, J = 11.7, 2.7 Hz, 1 H), 4.52 (dd, J = 10.7, 8.9 Hz, 1 H), 4.51 (dd, J = 11.7, 5.3 Hz, 1 H), 4.24 (dd, J = 10.8, 6.6 Hz, 1 H); ^{13}C NMR [$(CD_3)_2SO$] δ 162.4 (d, J_{C-F} = 245.2 Hz), 156.1, 153.5, 148.3, 145.7, 137.5, 134.8 (d, J_{C-F} = 2.7 Hz), 128.1 (d, J_{C-F} = 8.2 Hz, 2 C), 122.6, 120.6, 116.0, 115.5 (d, J_{C-F} = 21.4 Hz, 2 C), 85.4, 68.3, 45.0. Anal. ($C_{17}H_{13}FN_4O_4$) C, H, N.

Syntheses of 48 and 55 (Scheme 4B):

2-{[(5-Bromopyridin-2-yl)oxy]methyl}-2-methyl-6-nitro-2,3-dihydroimidazo[2,1-*b*][1,3]oxazole (144). Reaction of alcohol **26**[30] with 5-bromo-2-fluoropyridine (**118**) and NaH, using procedure K for 2.5 h, followed by chromatography of the product on silica gel, eluting with 0-1.5% EtOAc/CH_2Cl_2 (foreruns) and then with 1.5-2% EtOAc/CH_2Cl_2, gave **144** (46%) as a cream solid: mp (CH_2Cl_2/hexane) 151-153 °C; ^{1}H NMR ($CDCl_3$) δ 8.17 (br d, J = 2.5 Hz, 1 H), 7.68 (dd, J = 8.8, 2.5 Hz, 1 H), 7.52 (s, 1 H), 6.60 (br d, J = 8.7 Hz, 1 H), 4.58 (d, J = 12.0 Hz, 1 H), 4.50 (d, J = 12.0 Hz, 1 H), 4.41 (d, J = 10.2 Hz, 1 H), 4.01 (d, J = 10.2 Hz, 1 H), 1.76 (s, 3 H). Anal. ($C_{12}H_{11}BrN_4O_4$) C, H, N.

Procedure O: 2-({[5-(4-Fluorophenyl)pyridin-2-yl]oxy}methyl)-2-methyl-6-nitro-2,3-dihydroimidazo[2,1-*b*][1,3]oxazole (48). A stirred mixture of bromide **144** (77.2 mg, 0.217

mmol), 4-fluorophenylboronic acid (58.0 mg, 0.415 mmol) and Pd(dppf)Cl_2 (43.5 mg, 0.059 mmol) in DMF (2.3 mL), toluene (1.6 mL) and EtOH (1.1 mL) was degassed for 9 min (vacuum pump) and then N_2 was added. An aqueous solution of Na_2CO_3 (0.55 mL of 2 M, 1.1 mmol) was added by syringe and the stirred mixture was again degassed for 9 min, and then N_2 was added. The resulting mixture was stirred at 90 °C for 3 h, and then cooled, diluted with aqueous $NaHCO_3$ (50 mL), and extracted with CH_2Cl_2 (6 x 50 mL). The extracts were evaporated to dryness under reduced pressure (at 30 °C) and the residue was chromatographed on silica gel. Elution with 0-1% EtOAc/CH_2Cl_2 first gave foreruns, and then further elution with 1-2% EtOAc/CH_2Cl_2 gave **48** (60 mg, 75%) as a cream solid: mp (CH_2Cl_2/hexane) 162-164 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.45 (br d, *J* = 2.6 Hz, 1 H), 8.16 (s, 1 H), 8.01 (dd, *J* = 8.6, 2.6 Hz, 1 H), 7.70 (br dd, *J* = 8.9, 5.4 Hz, 2 H), 7.29 (br t, *J* = 8.9 Hz, 2 H), 6.84 (br d, *J* = 8.6 Hz, 1 H), 4.64 (d, *J* = 12.1 Hz, 1 H), 4.61 (d, *J* = 12.1 Hz, 1 H), 4.40 (d, *J* = 11.0 Hz, 1 H), 4.21 (d, *J* = 11.0 Hz, 1 H), 1.71 (s, 3 H); ^{13}C NMR [$(CD_3)_2SO$] δ 162.0, 161.9 (d, $J_{C\text{-}F}$ = 244.5 Hz), 155.5, 145.6, 144.4, 138.0, 133.2 (d, $J_{C\text{-}F}$ = 3.1 Hz), 129.1, 128.4 (d, $J_{C\text{-}F}$ = 8.2 Hz, 2 C), 116.0, 115.8 (d, $J_{C\text{-}F}$ = 21.4 Hz, 2 C), 110.7, 94.2, 69.0, 51.0, 22.0. Anal. ($C_{18}H_{15}FN_4O_4$) C, H, N.

Procedure P: 2-{[(6'-Fluoro-[3,3'-bipyridin]-6-yl)oxy]methyl}-2-methyl-6-nitro-2,3-dihydroimidazo[2,1-*b*][1,3]oxazole (55). A stirred mixture of bromide **144** (80.3 mg, 0.226 mmol), (6-fluoropyridin-3-yl)boronic acid (57.4 mg, 0.407 mmol) and Pd(dppf)Cl_2 (50.1 mg, 0.068 mmol) in DMF (2.3 mL), toluene (1.5 mL) and EtOH (1.1 mL) was degassed for 8 min (vacuum pump) and then N_2 was added. An aqueous solution of $KHCO_3$ (0.55 mL of 2 M, 1.1 mmol) was added by syringe and the stirred mixture was again degassed for 10 min, and then N_2 was added. The resulting mixture was stirred at 82 °C for 3 h, and then cooled, diluted with aqueous $NaHCO_3$ (50 mL), and extracted with CH_2Cl_2 (6 x 50 mL). The extracts were evaporated to dryness under reduced pressure (at 30 °C) and the residue was

chromatographed on silica gel. Elution with 0-0.4% MeOH/CH_2Cl_2 first gave foreruns, and then further elution with 0.4-0.5% MeOH/CH_2Cl_2 gave **55** (67 mg, 80%) as a pale brown solid: mp (CH_2Cl_2/hexane) 161-163 °C; ^{1}H NMR ($CDCl_3$) δ 8.36 (br d, *J* = 2.5 Hz, 1 H), 8.29 (br d, *J* = 2.5 Hz, 1 H), 7.91 (ddd, *J* = 8.5, 7.5, 2.6 Hz, 1 H), 7.76 (dd, *J* = 8.6, 2.6 Hz, 1 H), 7.55 (s, 1 H), 7.04 (br dd, *J* = 8.5, 3.0 Hz, 1 H), 6.79 (br d, *J* = 8.6 Hz, 1 H), 4.70 (d, *J* = 12.0 Hz, 1 H), 4.58 (d, *J* = 12.0 Hz, 1 H), 4.47 (d, *J* = 10.2 Hz, 1 H), 4.05 (d, *J* = 10.2 Hz, 1 H), 1.80 (s, 3 H). Anal. ($C_{17}H_{14}FN_5O_4$) C, H, N.

Synthesis of 57 (Scheme 4D):

Procedure Q: 1-(2-Chloro-4-nitro-1*H*-imidazol-1-yl)-3-[4-(4-fluorophenyl)piperazin-1-yl]-2-methylpropan-2-ol (149). A mixture of 2-chloro-1-[(2-methyloxiran-2-yl)methyl]-4-nitro-1*H*-imidazole[30] (**109**) (130 mg, 0.597 mmol) and 1-(4-fluorophenyl)piperazine (**148**) (151 mg, 0.838 mmol) in MEK (2.5 mL) in a sealed vial was stirred at 65 °C for 3 d. The resulting cooled mixture was transferred to a flask (in CH_2Cl_2) and evaporated to dryness under reduced pressure (at 30 °C), and then the residue was chromatographed on silica gel. Elution with 0-33% EtOAc/petroleum ether first gave foreruns, and then further elution with 33-40% EtOAc/petroleum ether gave **149** (208 mg, 88%) as a yellow solid: mp (CH_2Cl_2/pentane) 158-160 °C; ^{1}H NMR ($CDCl_3$) δ 8.06 (s, 1 H), 6.97 (br dd, *J* = 9.2, 8.3 Hz, 2 H), 6.86 (br dd, *J* = 9.2, 4.6 Hz, 2 H), 4.02 (s, 2 H), 3.42 (br s, 1 H), 3.12 (t, *J* = 4.8 Hz, 4 H), 2.87 (dt, *J* = 11.2, 4.9 Hz, 2 H), 2.74 (dt, *J* = 11.4, 4.9 Hz, 2 H), 2.55 (d, *J* = 13.9 Hz, 1 H), 2.42 (d, *J* = 13.9 Hz, 1 H), 1.16 (s, 3 H); HRESIMS calcd for $C_{17}H_{22}ClFN_5O_3$ *m*/*z* [M + H]$^+$ 400.1366, 398.1390, found 400.1368, 398.1390.

2-{[4-(4-Fluorophenyl)piperazin-1-yl]methyl}-2-methyl-6-nitro-2,3-dihydroimidazo[2,1-*b*][1,3]oxazole (57). Reaction of alcohol **149** with NaH, using procedure C, followed by chromatography of the product on silica gel, eluting with 50% EtOAc/petroleum ether (foreruns) and then with 0-2% MeOH/CH_2Cl_2 (saturated with NH_3),

gave **57** (75%) as a light yellow solid: mp 160-161 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.12 (s, 1 H), 7.00 (br dd, *J* = 9.1, 8.7 Hz, 2 H), 6.88 (br dd, *J* = 9.3, 4.7 Hz, 2 H), 4.26 (d, *J* = 10.7 Hz, 1 H), 4.07 (d, *J* = 10.7 Hz, 1 H), 2.96 (dt, *J* = 11.7, 4.9 Hz, 2 H), 2.88 (dt, *J* = 11.6, 4.8 Hz, 2 H), 2.81 (d, *J* = 14.8 Hz, 1 H), 2.76 (d, *J* = 14.7 Hz, 1 H), 2.72-2.61 (m, 4 H), 1.56 (s, 3 H); ^{13}C NMR [$(CD_3)_2SO$] δ 155.9 (d, J_{C-F} = 235.4 Hz), 155.5, 147.8, 145.5, 116.9 (d, J_{C-F} = 7.7 Hz, 2 C), 115.9, 115.2 (d, J_{C-F} = 21.9 Hz, 2 C), 97.2, 63.3, 54.2 (2 C), 51.6, 49.1 (2 C), 23.3. Anal. ($C_{17}H_{20}FN_5O_3$) C, H, N.

Compounds of Table 2. The following section details the syntheses of compounds **62** and **65** of Table 2, as described in Scheme 5. For the syntheses of all of the other compounds in Table 2, please refer to Supporting Information.

Syntheses of 62 and 65 (Scheme 5B):

1-(2,4-Dibromo-1*H*-imidazol-1-yl)-2-methyl-3-[4-(trifluoromethoxy)phenoxy]propan-2-ol (155) and 1-(2,5-dibromo-1*H*-imidazol-1-yl)-2-methyl-3-[4-(trifluoromethoxy)-phenoxy]propan-2-ol (156). Reaction of epoxide **86** (see Supporting Information) with 2,4-dibromo-1*H*-imidazole (**154**) (1.05 equiv) and DIPEA in anhydrous toluene, using procedure G at 109 °C for 12 h, followed by chromatography of the product on silica gel, eluting with CH_2Cl_2 (foreruns) and then with additional CH_2Cl_2, first gave the crude major product (**155**). This was further purified by chromatography on silica gel, eluting with CH_2Cl_2 (to remove a minor higher running impurity) and then with 0-1% Et_2O/CH_2Cl_2 (to give the product), and by additional chromatography on silica gel, eluting with 0-25% Et_2O/petroleum ether (foreruns, including excess **154**) and then with CH_2Cl_2, to give **155** (69%) as a colourless oil; ^{1}H NMR ($CDCl_3$) δ 7.18 (s, 1 H), 7.17 (br d, *J* = 8.9 Hz, 2 H), 6.88 (br d, *J* = 9.2 Hz, 2 H), 4.16 (d, *J* = 14.6 Hz, 1 H), 4.08 (d, *J* = 14.6 Hz, 1 H), 3.86 (d, *J* = 9.0 Hz, 1 H), 3.81 (d, *J* =

9.1 Hz, 1 H), 2.38 (s, 1 H), 1.34 (s, 3 H); HRESIMS calcd for $C_{14}H_{14}Br_2F_3N_2O_3$ *m/z* $[M + H]^+$ 476.9280, 474.9298, 472.9318, found 476.9276, 474.9302, 472.9326.

Further elution of the original column above with 3-5% Et_2O/CH_2Cl_2 gave the crude minor product (**156**), which was further purified by chromatography on silica gel, eluting with 0-30% Et_2O/petroleum ether (foreruns, including excess **154**) and then with 0-3% Et_2O/CH_2Cl_2, to give **156** (29%) as a cream solid: mp (CH_2Cl_2/pentane) 129-131 °C; ^{1}H NMR ($CDCl_3$) δ 7.17 (br d, *J* = 9.1 Hz, 2 H), 7.05 (s, 1 H), 6.90 (br d, *J* = 9.2 Hz, 2 H), 4.33 (d, *J* = 15.0 Hz, 1 H), 4.19 (d, *J* = 15.1 Hz, 1 H), 3.94 (d, *J* = 9.0 Hz, 1 H), 3.91 (d, *J* = 9.0 Hz, 1 H), 2.51 (s, 1 H), 1.38 (s, 3 H); HRESIMS calcd for $C_{14}H_{14}Br_2F_3N_2O_3$ *m/z* $[M + H]^+$ 476.9280, 474.9298, 472.9318, found 476.9279, 474.9303, 472.9313.

6-Bromo-2-methyl-2-{[4-(trifluoromethoxy)phenoxy]methyl}-2,3-dihydroimidazo[2,1-*b*][1,3]oxazole (62). Reaction of alcohol **155** with NaH (1.25 equiv), using procedure C at 20 °C for 20 min and then at 55 °C for 4 h, followed by chromatography of the product on silica gel, eluting with 33-50% CH_2Cl_2/petroleum ether (foreruns) and then with 50-75% CH_2Cl_2/petroleum ether, gave **62** (87%) as a cream solid (following trituration in cold pentane): mp 55-57 °C; ^{1}H NMR ($CDCl_3$) δ 7.14 (br d, *J* = 9.1 Hz, 2 H), 6.87 (br d, *J* = 9.2 Hz, 2 H), 6.60 (s, 1 H), 4.30 (d, *J* = 9.5 Hz, 1 H), 4.16 (d, *J* = 9.8 Hz, 1 H), 4.04 (d, *J* = 9.8 Hz, 1 H), 3.90 (d, *J* = 9.5 Hz, 1 H), 1.73 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 157.3 (C-7a), 156.6 (C-1'), 143.8 (C-4'), 122.8 (2 C, C-3',5'), 120.7 (q, $J_{C\text{-}F}$ = 256.5 Hz, 4'-OCF_3), 115.8 (2 C, C-2',6'), 114.0 (C-6), 110.1 (C-5), 92.1 (C-2), 72.0 (2-CH_2O), 52.1 (C-3), 23.5 (2-CH_3). Anal. ($C_{14}H_{12}BrF_3N_2O_3$) C, H, N.

2-Methyl-6-(methylsulfanyl)-2-{[4-(trifluoromethoxy)phenoxy]methyl}-2,3-dihydroimidazo[2,1-*b*][1,3]oxazole (65). *n*-Butyllithium (0.58 mL of a 2.0 M solution in cyclohexane, 1.16 mmol) was added dropwise to a stirred solution of bromide **62** (303 mg, 0.771 mmol) in anhydrous freshly distilled THF (7 mL) under N_2 at -78 °C. After 80 min

at -78 °C, methyl disulfide (0.28 mL, 3.16 mmol) was added dropwise, and the mixture was stirred at -78 °C for 3.5 h, slowly warmed to -20 °C (over 1 h), stored at -20 °C for 10 h, and finally stirred at 0 °C for 3 h. The reaction was then quenched with ice/aqueous citric acid (20 mL), and the resulting mixture was added to water (30 mL), and extracted with CH_2Cl_2 (6 x 50 mL). The combined extracts were evaporated to dryness under reduced pressure and the residue was chromatographed on silica gel. Elution with 0-1% Et_2O/CH_2Cl_2 first gave foreruns, and then further elution with 5% Et_2O/CH_2Cl_2 gave the crude product (0.12 g) as an 8:1 mixture of isomers, which was crystallised from CH_2Cl_2/pentane, to give pure **65** (93 mg, 33%) as a cream solid: mp 104-106 °C; ^{1}H NMR ($CDCl_3$) δ 7.15 (br d, J = 9.1 Hz, 2 H), 6.87 (br d, J = 9.2 Hz, 2 H), 6.61 (s, 1 H), 4.26 (d, J = 9.5 Hz, 1 H), 4.15 (d, J = 9.6 Hz, 1 H), 4.04 (d, J = 9.7 Hz, 1 H), 3.86 (d, J = 9.6 Hz, 1 H), 2.41 (s, 3 H), 1.73 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 158.7 (C-7a), 156.7 (C-1'), 143.7 (q, $J_{C\text{-}F}$ = 2.1 Hz, C-4'), 135.7 (C-6), 122.8 (2 C, C-3',5'), 120.7 (q, $J_{C\text{-}F}$ = 256.4 Hz, 4'-OCF_3), 115.8 (2 C, C-2',6'), 111.9 (C-5), 91.6 (C-2), 72.1 (2-CH_2O), 51.8 (C-3), 23.5 (2-CH_3), 18.7 (SCH_3). Anal. ($C_{15}H_{15}F_3N_2O_3S$) C, H, N.

Minimum Inhibitory Concentration Assays (MABA and LORA). These were carried out according to the published protocols.[50,51]

***In Vitro* Parasite Growth Inhibition Assays.** The activity of test compounds against the amastigote stage of the *L. don* parasite was assessed at CDRI using a mouse macrophage-based luciferase assay, carried out according to the reported procedures.[25] Additional assays measuring the growth inhibitory action of compounds against *L. inf*, *T. cruzi*, and *T. brucei*, and determining any cytotoxic effects on human lung fibroblasts (MRC-5 cells), were conducted at the University of Antwerp (LMPH), as described in a recent report.[53]

Solubility Determinations. The solid compound sample was mixed with water or 0.1 M HCl (enough to make a 2 mM solution) in an Eppendorf tube, and the suspension was sonicated for 15 min and then centrifuged at 13000 rpm for 6 min. An aliquot of the clear

supernatant was diluted 2-fold with water (or 0.1 M HCl), and then HPLC was conducted. The solubility was calculated by comparing the peak area obtained with that from a standard solution of the compound in DMSO (after allowing for varying dilution factors and injection volumes).

Microsomal Stability Assays. Studies on compounds **7**-**9**, **12**, **18**, and **47** (Table 3) were performed by Advinus Therapeutics Ltd., 21 & 22 Phase II, Peenya Industrial Area, Bangalore 560058, India, using a published protocol[26] in which the compound concentration was 0.5 μM and the incubation time was 30 min. Additional analyses on compounds **5**, **9**, **27**, **37**-**39**, **51**, **54**, **56**, and **57** (Table 3) were carried out by WuXi AppTec (Shanghai) Co., Ltd, 288 FuTe ZhongLu, WaiGaoQiao Free Trade Zone, Shanghai 200131, China. Here, test compounds (at 1 μM) were incubated at 37 °C with liver microsomes from human, CD-1 mouse or hamster species in the presence of a NADPH regenerating system and phosphate buffer (100 mM, pH 7.4) at 0.5 mg/mL microsomal protein (the positive controls were testosterone, propafenone and diclofenac). Samples were removed at time intervals of 0, 5, 10, 20, 30, and 60 mins and immediately mixed with cold CH_3CN (containing 0.1 μg/mL of tolbutamide as an internal standard), then centrifuged prior to analysis by LCMS/MS.

***In Vivo* Experiments.** All animal experiments were performed according to institutional ethical guidelines for animal care. Antitubercular efficacy studies in mice were approved by the UIC IACUC (UIC AWA no. A3460-01; ACC application no. 12-183). For VL, mouse model studies (LSHTM) were conducted under license from the UK Home Office (license no. PIL 70/6997), hamster studies at CDRI were approved by the CSIR-CDRI animal ethics committee (license no. 19/2009/PARA/IAEC), and hamster studies at LMPH were approved by the ethical committee of the University of Antwerp (UA-ECD 2010-17).

Acute TB Infection Assay. Each compound (including **4**, which was used as an internal reference standard) was administered orally to a group of 7 *M. tb*-infected BALB/c mice at

100 mg/kg daily for 5 days a week for 3 weeks, beginning on day 11 postinfection, in accordance with published protocols.[19,50] The results are recorded as the ratio of the average reduction in colony forming units (CFUs) in the compound-treated mice /the average CFU reduction in the mice treated with **4**. In this assay, **4** caused 2.5-3 log reductions in CFUs.

Acute VL Infection Assay (Mouse Model, LSHTM). Test compounds were orally dosed once per day for 5 consecutive days to groups of 5 female BALB/c mice infected with 2 x 10^7 *L. donovani* amastigotes, with treatment commencing one week postinfection, as reported.[25] Miltefosine (**3**) and AmBisome were positive controls and parasite burdens were determined from impression smears of liver sections. Efficacy was expressed as the mean percentage reduction in parasite load for treated mice in comparison to untreated (vehicle-only) controls.

Chronic VL Infection Assay 1 (Hamster Model, CDRI). Golden hamsters (weighing 40-45 g) were infected intracardially with 1 x 10^7 *L. donovani* amastigotes and then, 15 days later, all animals were subjected to splenic biopsy to assess the level of infection. Groups of hamsters having an appropriate infection grading (5-15 amastigotes/100 spleen cell nuclei) were treated with test compounds, starting on day 17 and dosing orally once per day for either 5 or 10 consecutive days, according to the published procedure.[25] Post-treatment splenic biopsies performed 12 days after the first dose were employed to determine the intensity of infection, as previously described.[25]

Chronic VL Infection Assay 2 (Hamster Model, LMPH). Golden hamsters (weighing 75-80 g) were infected with 2 x 10^7 *L. infantum* amastigotes and, 21 days post-infection, treatment groups of 6 animals each were dosed orally once per day with test compounds (formulated in PEG_{400}) for 5 consecutive days. Parasite burdens in three target organs (liver, spleen, and bone-marrow) were determined by microscopic evaluation of impression smears (stained with Giemsa) and efficacy was expessed as the mean percentage load reduction for

treated hamsters in comparison to untreated (vehicle-only) controls. Miltefosine (**3**) was included as a reference drug in all experiments.

Mouse Pharmacokinetics. The study was conducted by Advinus Therapeutics Ltd., 21 & 22 Phase II, Peenya Industrial Area, Bangalore 560058, India, according to a published protocol.[26] Briefly, compounds were administered to groups of male Swiss Albino mice (30-40 g); intravenous dosing (at 1 mg/kg) employed a solution vehicle comprising 20% NMP and 40% PEG-400 in 100mM citrate buffer pH 3, while oral dosing (at 12.5 or 25 mg/kg) was as a suspension in 0.08% Tween 80/0.5% carboxymethylcellulose in water. Samples derived from blood plasma (at 0.083 for iv only, 0.25, 0.5, 1, 2, 4, 6, 8, 10, 24 and 48 h) were centrifuged prior to analysis by LC-MS/MS and the pharmacokinetic parameters were determined using WinNonlin software (Version 5.2).

ASSOCIATED CONTENT

Supporting Information

Additional biological assay data, graphs of pharmacokinetic data, experimental procedures and characterisations for compounds, combustion analytical data, representative NMR spectra, and discussion of 2D NMR results.

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ACKNOWLEDGMENTS

We thank the Global Alliance for TB Drug Development and the Drugs for Neglected Diseases initiative [specific funding from UK Department for International Development, Dutch Ministry of Foreign Affairs, Federal Ministry of Education and Research (BMBF) through KfW/GERMANY and part of the EDCTP2 programme supported by the European

Union, the Bill & Melinda Gates Foundation, Médecins Sans Frontières and an anonymous donor] for financial support through collaborative research agreements. The authors also thank Drs Jakir Pinjari and Rao Mukkavilli (Advinus Therapeutics Ltd., Bangalore, India) for the pharmacokinetic data and Sisira Kumara (ACSRC) for the solubility measurements.

ABBREVIATIONS USED

NTD, neglected tropical disease; VL, visceral leishmaniasis; HAT, human African trypanosomiasis; *L. don*, *Leishmania donovani*; *L. inf*, *Leishmania infantum*; TB, tuberculosis; Ddn, deazaflavin-dependent nitroreductase; *M. tb*, *Mycobacterium tuberculosis*; DIPEA, *N*,*N*-diisopropylethylamine; vs., versus; *T. cruzi*, *Trypanosoma cruzi*; *T. brucei*, *Trypanosoma brucei*; HLM, human liver microsomes; MLM, mouse liver microsomes; HRCIMS, high resolution chemical ionization mass spectrometry; HRFABMS, high resolution fast atom bombardment mass spectrometry; HRESIMS, high resolution electrospray ionization mass spectrometry; APCI MS, atmospheric pressure chemical ionization mass spectrometry; MEK, methyl ethyl ketone (2-butanone); CFU, colony forming unit; SD, standard deviation.

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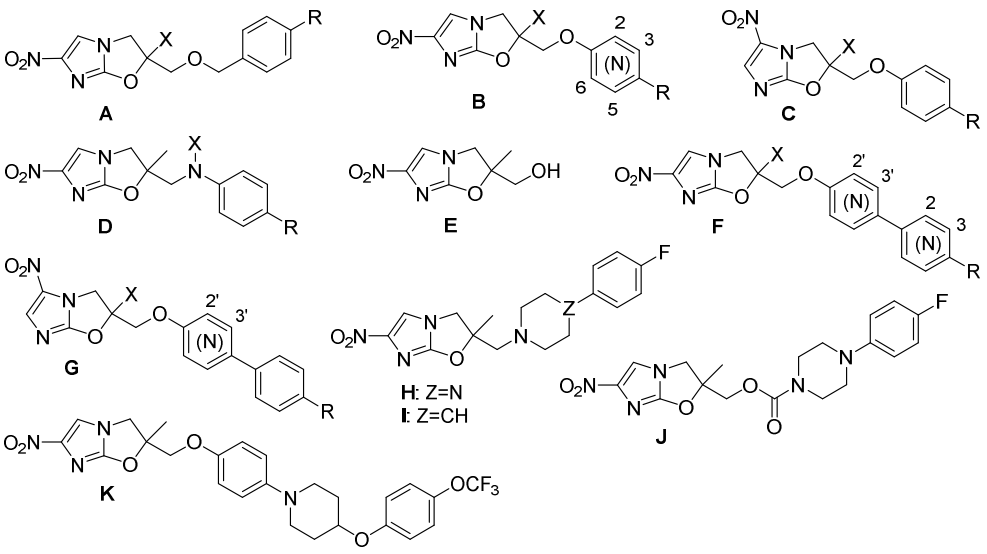
Table 1. *In vitro* antiparasitic and antitubercular activities of nitroimidazooxazoles



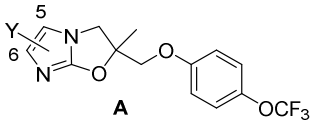
					IC_{50}[a,b] (μM)					MIC[c,b] (μM)	
compd	Fm	X	aza	R	*L. don*	*L. inf*	*T. cruzi*	*T. bruc*	MRC-5	MABA	LORA
10	A	H		OCF_3	0.03	0.11	0.60	>64	>64	14	43
11	A	Me		OCF_3	0.08	0.28	0.42	60	60	0.24	34
12	B	H		OCF_3	0.005	0.15	1.5	>64	>64	2.0	73
13	B[d]	H		OCF_3	0.02	0.33	4.7	>64	>64	0.32	70
14	B[e]	H		OCF_3	0.05	0.50	2.6	>64	>64	3.1	>128
15	C	H		OCF_3		48	9.8	33	31	>128	>128
16	C[d]	H		OCF_3		>64	>64	>64	>64	56	61
17	C[e]	H		OCF_3		>64	>64	>64	>64	>128	>128
7[f]	B	Me		OCF_3	0.03	0.33	1.8	>64	>64	0.077	55
9[g]	B[d]	Me		OCF_3	0.03	0.17	2.6	53	>64	0.046	5.9
18	B[e]	Me		OCF_3	0.03	0.77	1.6	38	>64	2.5	27
19[g]	B	Me		H	0.14	0.30	1.8	>64	>64	0.16	22
20	B	Me		F	0.12	0.17	1.8	>64	>64	0.11	15
21	B	Me		Cl	0.12	0.20	2.4	57	>64	0.038	41
22	B	Me		CF_3	0.28	0.41	2.5	>64	>64	0.058	46

23	B	Me		OPh	0.25	2.2	4.5	8.9	>64	0.11	48
24	D	H		OCF_3	0.06	0.32	7.1	50	>64	0.36	24
25	D	Me		OCF_3	0.08	0.50	0.90	12	>64	0.40	9.7
26[f]	E					18	52	>64	>64	16	116
27	B	Me	2	CF_3	0.21	0.24	1.7	>64	>64	0.038	28
28	B	Me	3	CF_3	0.30	0.55	2.9	>64	>64	0.48	45
29	B	Me	2,3	CF_3		1.4	2.2	>64	>64	0.91	106
30	B	Me	2,5	CF_3		0.43	1.4	>64	>64	0.14	70
31	B	Me	2,6	CF_3		0.50	1.5	>64	>64	0.47	>128
32	F	H		F	0.02	1.8	2.8	>64	>64	0.025	>128
33	F	H		CF_3	0.11	3.8	2.8	>64	>64	0.040	>128
34	F	H		OCF_3	0.01	0.83	1.4	3.1	>64	0.039	>128
35	F	H	2’	CF_3		2.4	19	2.2	45	58	57
36	F	H	2’	OCF_3	2.1	5.0	10	5.2	47	5.3	48
37	F	H	3’	F	0.07	0.03	1.3	>64	>64	0.049	42
38	F[d]	H	3’	F		0.06	7.1	>64	>64	0.037	2.7
39	F[e]	H	3’	F		0.03	0.95	>64	>64	0.46	3.8
40	F	H	3’	CF_3	0.01	1.5	0.94	>64	>64	0.024	>128
41	F	H	3’	OCF_3	0.01	0.76	1.4	4.2	>64	0.037	>128
42	G[d]	H	3’	F		48	34	>64	54	>128	>128
43	G[e]	H	3’	F		49	22	>64	55	84	>128
8	F	Me		F	0.06	3.3	0.52	9.2	>64	0.043	11
44	F[d]	Me		F	0.06	2.3	2.8	>64	>64	0.046	4.0
45	F[e]	Me		F	0.35	4.2	0.58	>64	>64	0.080	>128
46	F	Me		CF_3	0.06	11	1.5	1.0	>64	0.081	>128
47	F	Me		OCF_3	0.05	5.3	0.89	0.85	>64	0.088	64
48	F	Me	2’	F	0.20	2.9	0.65	>64	>64	0.024	35
49	F	Me	2’	CF_3	0.11	3.6	0.85	16	>64	0.018	4.5
50	F	Me	2’	OCF_3	0.07	26	2.4	8.9	>64	0.018	19

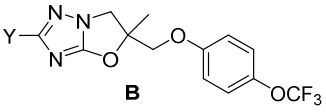
51	F	Me	3’	F	0.04	0.13	0.99	>64	>64	0.036	35
52	F	Me	3’	CF_3	0.06	5.2	2.5	>64	>64	0.023	>128
53	F	Me	3’	OCF_3	0.03	11	1.6	3.9	>64	0.025	>128
54	F	Me	2’,2	F	0.15	0.14	1.2	40	>64	0.053	24
55	F	Me	2’,3	F	0.34	0.31	5.6	52	>64	0.091	14
56	F	Me	3’,3	F	0.09	0.57	7.9	>64	>64	0.37	39
57	H				0.16	0.20	1.1	>64	>64	0.13	41
58	I				0.63	0.23	1.1	12	>64	0.29	60
59	J				0.81	1.7	3.6	>64	>64	0.23	29
60[f]	K					13	0.40	3.4	>64	0.051	26
5[g]	K[d]					7.1	0.43	1.7	>64	0.033	2.3

[a]IC_{50} values for inhibition of the growth of *Leishmania donovani*, *Leishmania infantum*, *Trypanosoma cruzi*, and *Trypanosoma brucei*, or for cytotoxicity toward human lung fibroblasts (MRC-5 cells). [b]Each value (except the single test *L. don* data) is the mean of at least two independent determinations. For complete results (mean ± SD) please refer to Supporting Information. [c]Minimum inhibitory concentration against *M. tb*, determined under aerobic (MABA)[50] or hypoxic (LORA)[51] conditions. [d]*R* enantiomer. [e]*S* enantiomer. [f]Ref 30. [g]Ref 18.

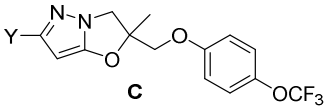
Table 2. *In vitro* antiparasitic and antitubercular activities of ring A analogues of **7**



A



B



C

			IC_{50}[a,b] (μM)				MIC[c,b] (μM)	
compd	Fm	Y	*L. inf*	*T. cruzi*	*T. bruc*	MRC-5	MABA	LORA
7	A	6-NO_2	0.33	1.8	>64	>64	0.077	55
61	A	6-H	>64	2.1	>64	>64	81	>128
62	A	6-Br	>64	22	33	34	55	51
63	A	6-CHO	>64	33	>64	>64	>128	>128
64	A	6-CH_2OH	>64	>64	>64	>64	>128	>128
65	A	6-SMe	57	9.4	>64	38	54	66
66	A	6-SO_2Me	57	>64	>64	>64	>128	>128
67	A	5-Br	30	0.13	33	26	61	61
68	A	5-SMe	>64	0.58	33	25	27	70
69	A	5-SO_2Me	>64	20	>64	>64	>128	>128
70	B	NO_2	>64	0.59	20	>64	>128	>128
71	B	Br	>64	13	60	43	46	>128
72	C	NO_2	>64	4.7	34	>64	52	>128

[a]IC_{50} values for inhibition of the growth of *Leishmania infantum*, *Trypanosoma cruzi*, and *Trypanosoma brucei*, or for cytotoxicity toward human lung fibroblasts (MRC-5 cells). [b]Each value is the mean of at least two independent determinations. For complete results (mean ± SD) please refer to Supporting Information. [c]Minimum inhibitory concentration against *M. tb*, determined under aerobic (MABA)[50] or hypoxic (LORA)[51] conditions.

Table 3. Aqueous solubility, microsomal stability, and *in vivo* antileishmanial efficacy data for selected analogues

	aq solubility[a] (μg/mL)		microsomal stability[b] [% remaining at 1 (0.5) h]			*in vivo* efficacy against *L. don* (mouse) (% inhibition at dose in mg/kg)[c]						
compd	pH=7	pH=1	H	M	Ham	25	12.5	6.25	3.13	1.56	0.78	0.39
12	0.91		(99)	(76)	(54)	>99		89				
13	0.96							74				
14	1.2							76				
7	3.6		(86)	(79)	(49)	100	>99	99	64	31	7	
9	2.4		(92)	(89)	18 (54)		>99	>99	83	49	11	
18	2.6		(84)	(70)	(64)		84	19	8			
24	23	11[d]										
27	11	11[d]	84	69		>99		83				
28	50											
30	27											
31	4.2											
32	0.059											
37	1.3	526	60	47		>99		100	>99	98		
38	1.1	260	57	54						52	45	36
39	1.4	299	61	41						59	46	13
8	0.23		(88)	(57)	(42)	>99	93	68				
44	0.075							36				
45	0.068							6				
47	0.021		(91)	(100)	(79)	42						
48	0.19	2.7										
51	1.1	634	58	21		>99		100	93	34		
54	3.1	46	71	52		>99		96	94	47		

55	3.3	19					
56	10		82	62		91	
57	14	19100	49	14		>99	16
58	2.6	23200					
5	0.31	116			55		

[a]Solubility (μg/mL) in water (pH = 7) or 0.1 M HCl (pH = 1) at 20 °C, determined by HPLC (see Experimental Section). [b]Pooled human (H), CD-1 mouse (M), or hamster (Ham) liver microsomes; data in brackets are the percentage parent compound remaining following a 30 min incubation. [c]Dosing was orally, once daily for 5 consecutive days; data are the mean percentage reduction of parasite burden in the liver. [d]Unstable under assay conditions.

Table 4. Pharmacokinetic parameters for selected compounds in male Swiss Albino mice

	intravenous (1 mg/kg)					oral (12.5 or 25 mg/kg)[a]			
compd	C_0 (μg/mL)	CL (mL/kg/min)	Vdss (L/kg)	$t_{1/2}$ (h)	AUC_{last} (μg·h/mL)	C_{max} (μg/mL)	T_{max} (h)	AUC_{last} (μg·h/mL)	F[b] (%)
12	0.61	13	2.2	3.2	1.14	1.8	1.0	14.8	52
7	0.88	9.5	1.7	2.2	1.69	4.1	4.0	33.5	79
9	0.36	11	2.6	2.7	1.39	1.3	2.0	15.8	91
8	0.30	28	7.5	3.8	0.48	0.70	6.0	7.58	63
47	0.34	7.6	5.7	11	2.13	0.29	6.0	9.81	18

[a]The oral dose for **9** was 12.5 mg/kg; the remainder were dosed at 25 mg/kg. [b]Oral bioavailability, determined using dose normalised AUC_{last} values.

Table 5. *In vivo* efficacy of **5** and **9** against *L. infantum* in the early curative hamster model (LMPH)

	dosage [a]	% inhibition in target organs		
compd	(mg/kg)	liver	spleen	bone marrow
3	20	81.8	92.0	84.7
5	50	65.7	51.3	21.7
	25	53.9	42.9	37.3
	12.5	32.5	24.8	23.7
9	50	100	100	100
	25	100	99.9	99.7
	12.5	99.0	98.7	94.0

[a]All compounds were dosed orally once daily for 5 consecutive days.

Figure 1. Structures of various antileishmanial or antitubercular agents

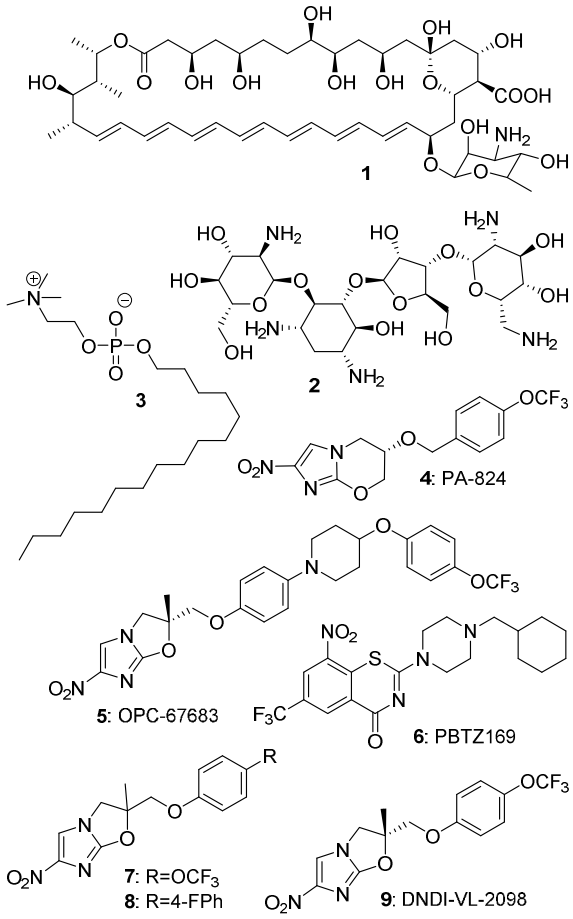


Figure 2. Comparative *in vivo* efficacy in the mouse VL model: a) 6.25 mg/kg; b) 1.56 mg/kg

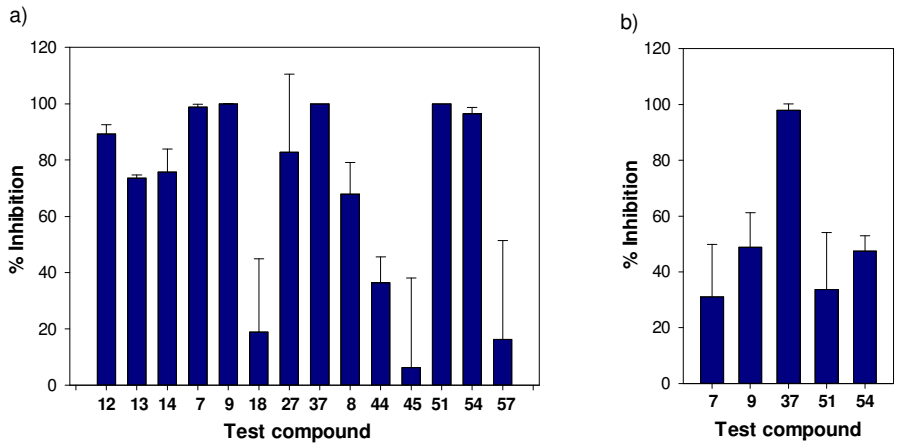
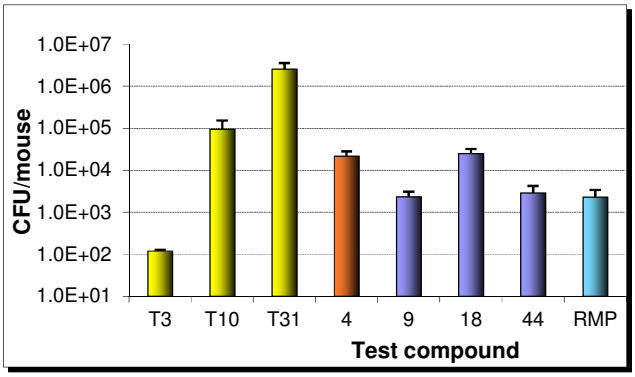
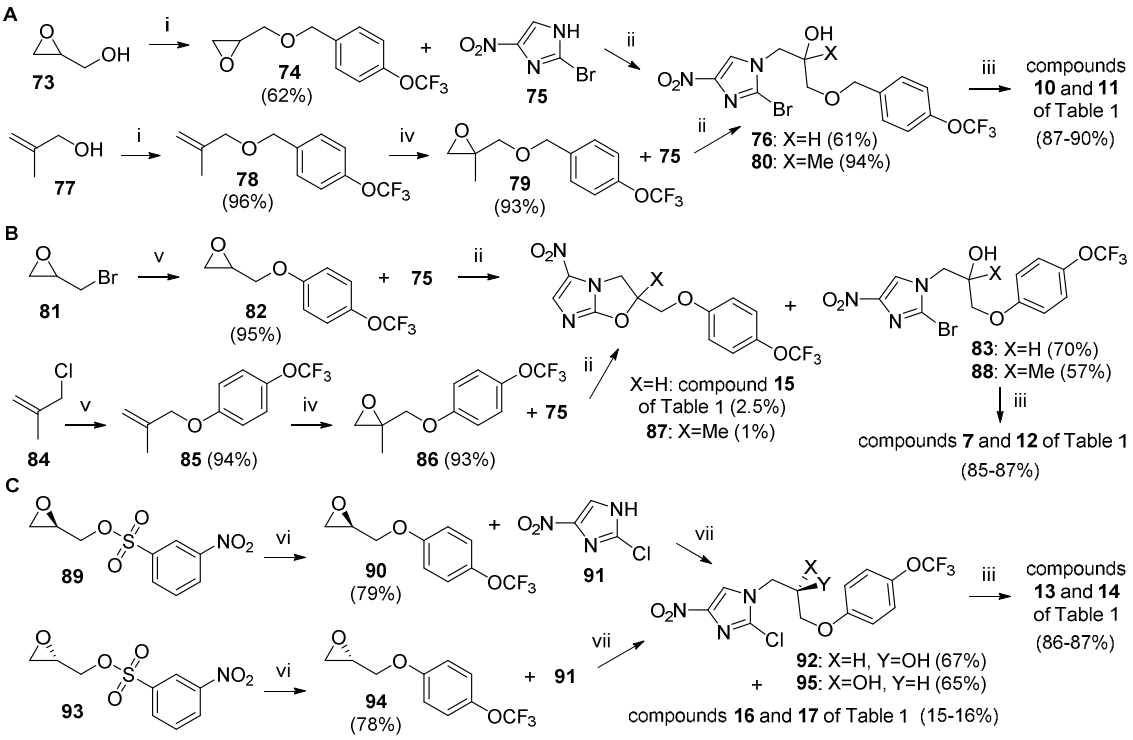


Figure 3. Comparison of **4**, **9**, **18**, and **44** with rifampicin in the acute TB infection mouse model

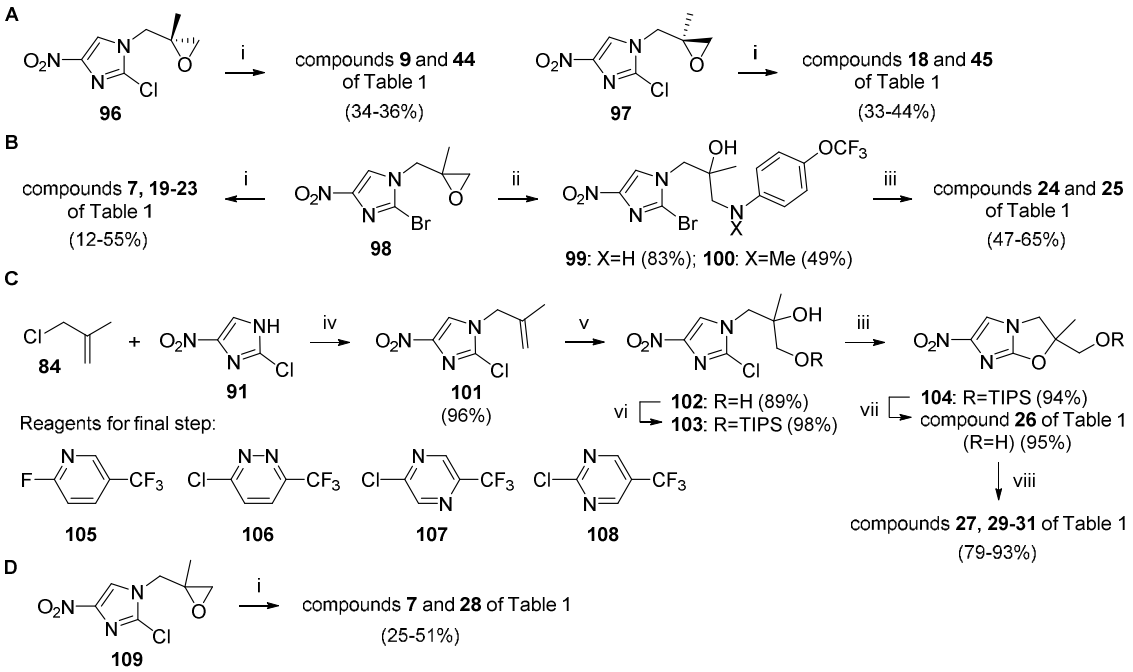


Scheme 1[a]



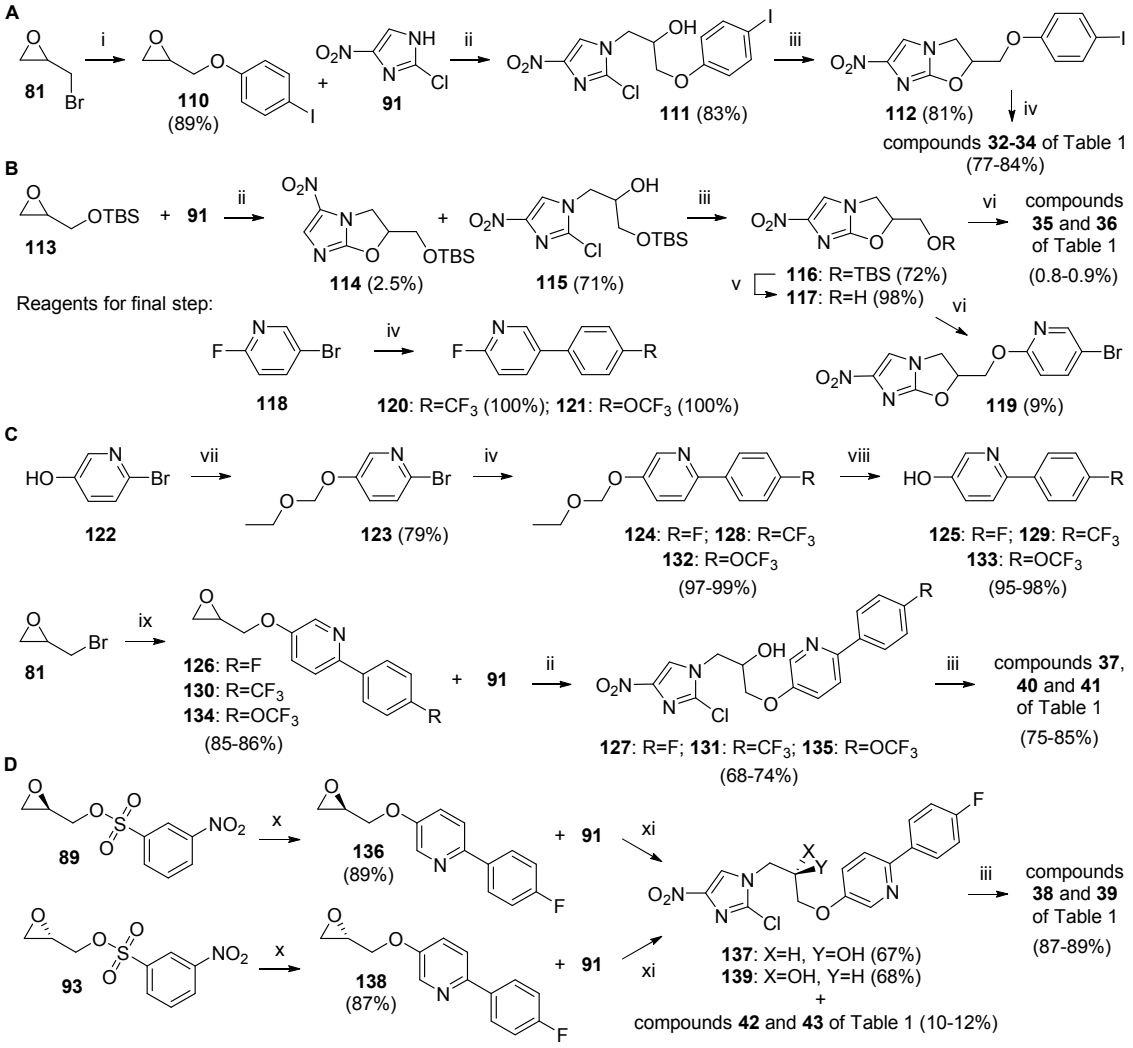
[a] Reagents and conditions: (i) 4-OCF_3BnBr, NaH, DMF, 0-20 °C, 7-21 h; (ii) DIPEA, 105-108 °C, 6.5-15 h; (iii) NaH, DMF, 0 °C, 0.7-1.4 h or 0-20 °C, 2-3 h; (iv) *m*-CPBA, Na_2HPO_4, CH_2Cl_2, 0-20 °C, 3-3.5 h; (v) 4-OCF_3PhOH, K_2CO_3, acetone, 59 °C, 36-41 h; (vi) 4-OCF_3PhOH, CsF, DMF, 20 °C, 35-45 h; (vii) DIPEA, toluene, 79-80 °C, 24 h.

Scheme 2[a]



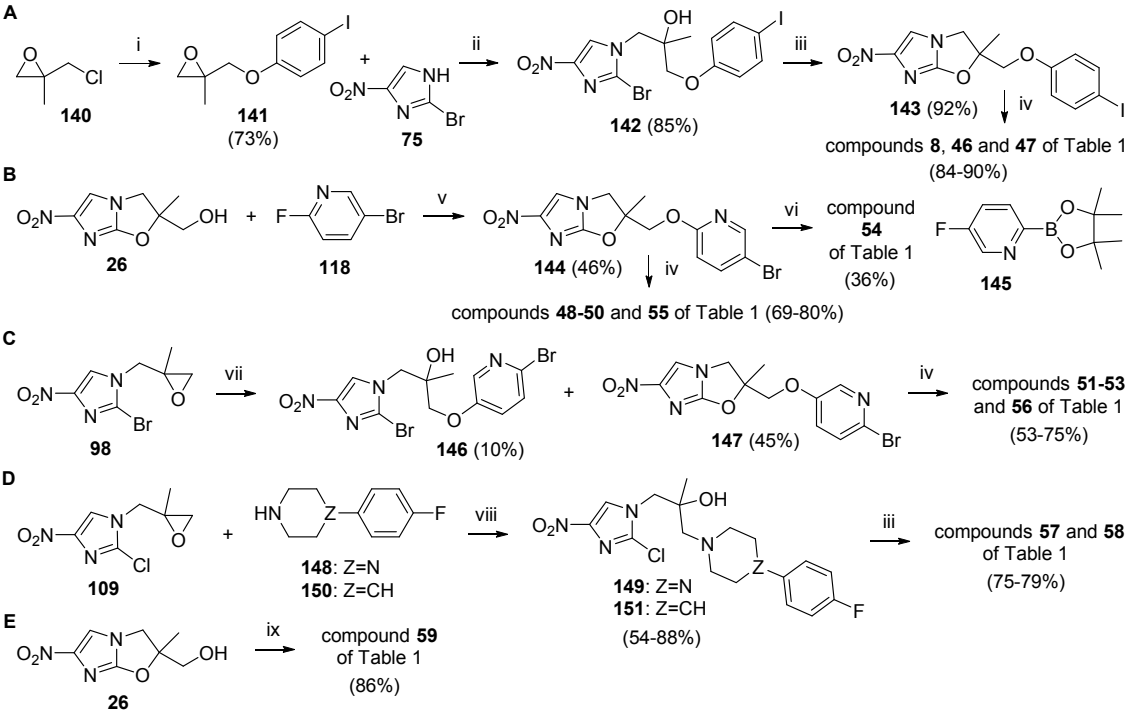
[a] Reagents and conditions: (i) ArOH, NaH, DMF, 0-55 °C, 2-3 h, or 0-80 °C, 5-30 min; (ii) 4-OCF_3PhNH_2 or 4-$OCF_3PhNHMe$, $CoCl_2$, CH_3CN, 63-65 °C, 24-52 h; (iii) NaH, DMF, 0-20 °C, 1.3-2.8 h (or 0 °C, 2 h); (iv) K_2CO_3, DMF, 65 °C, 33 h; (v) OsO_4, NMO, CH_2Cl_2, 20 °C, 8 h; (vi) TIPSCl, imidazole, DMF, 20 °C, 6.5 d; (vii) 40% HF, CH_3CN, 20 °C, 11 h; (viii) ArX (**105**, **106**, **107**, or **108**), NaH, DMF, 0-20 °C, 1-2.5 h.

Scheme 3[a]



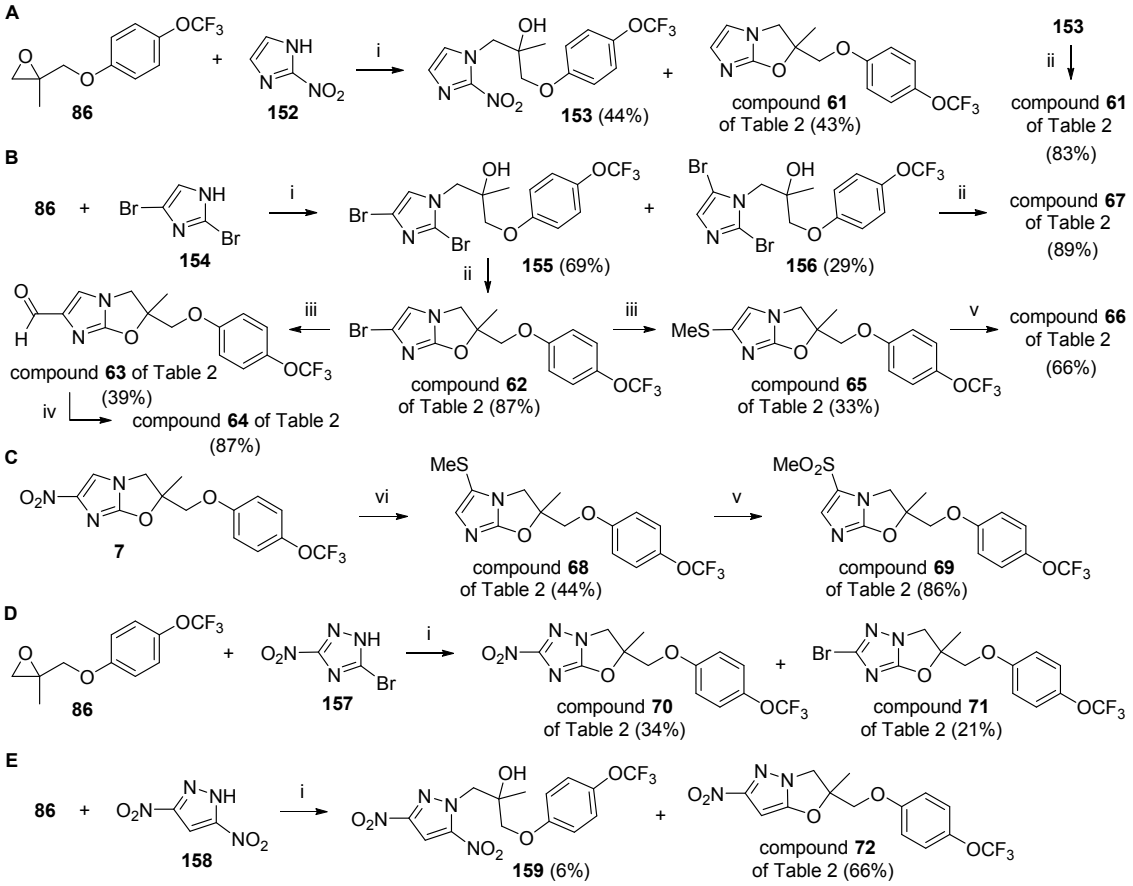
[a] Reagents and conditions: (i) 4-IPhOH, K_2CO_3, acetone, 59 °C, 52 h; (ii) DIPEA, 100-105 °C, 6-13 h; (iii) NaH, DMF, 0-20 °C, 1.7-4 h; (iv) $ArB(OH)_2$, (DMF), toluene, EtOH, 2 M Na_2CO_3, $Pd(dppf)Cl_2$ under N_2, 85-90 °C, 0.8-4.5 h; (v) 1% HCl in 95% EtOH, 20 °C, 6.5 h; (vi) ArF (**118**, **120**, or **121**), NaH, DMF, 0-20 °C, 2.5-3.7 h; (vii) $EtOCH_2Cl$, K_2CO_3, DMF, 0-20 °C, 24 h; (viii) 1.25 M HCl in MeOH, 54-55 °C, 5 h; (ix) ArOH (**125**, **129**, or **133**), K_2CO_3, MEK, 65 °C, 39-49 h; (x) **125**, NaH, DMF, 20 °C, 40 h; (xi) DIPEA, toluene, 80 °C, 24 h.

Scheme 4[a]



[a] Reagents and conditions: (i) 4-IPhOH, K_2CO_3, NaI, DMF, 72 °C, 24 h; (ii) DIPEA, 108 °C, 14.5 h; (iii) NaH, DMF, 0 °C, 1-1.5 h; (iv) $ArB(OH)_2$, (DMF), toluene, EtOH, 2 M Na_2CO_3 (or 2 M $KHCO_3$), $Pd(dppf)Cl_2$ under N_2, 78-92 °C, 0.75-3 h; (v) NaH, DMF, 0-20 °C, 2.5 h; (vi) **145**, Cs_2CO_3, CuCl, dppf, DMF, $Pd(dppf)Cl_2$ under N_2, 85 °C, 4.3 h; (vii) 6-Br-pyridin-3-ol, NaH, DMF, 0-50 °C, 4 h; (viii) MEK, ($CHCl_3$), 65-80 °C, 18-72 h; (ix) triphosgene, Et_3N, THF, 0-20 °C, 1.7 h, then **148**, THF, 20 °C, 3.5 h.

Scheme 5[a]



[a] Reagents and conditions: (i) DIPEA, (toluene), 104-109 °C, 12-16 h; (ii) NaH, DMF, 0-20 °C, 80 min (for **61**), or 55 °C, 4 h (for **62**), or 45 °C, 2.5 h (for **67**); (iii) *n*BuLi, THF, -78 °C, 80 min, then DMF or $(MeS)_2$, -78 to 20 °C, 4 h (for **63**), or -78 to 0 °C, 18 h (for **65**), then aq citric acid; (iv) $NaBH_4$, EtOH, 0 °C, 1 h; (v) *m*-CPBA, Na_2HPO_4, CH_2Cl_2, 0-20 °C, 18-26 h; (vi) MeSH, Et_3N, MeOH, 0-10 °C, 3 h.

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