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Structure-Activity Relationships of Antitubercular Nitroimidazoles. II. Determinants of aerobic activity and quantitative structure-activity relationships

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Abstract

The (*S*)-2-nitro-6-substituted 6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazines have been extensively explored for their potential use as new antituberculars based on their excellent bactericidal properties on aerobic whole cells of *Mycobacterium tuberculosis*. An oxygen atom at the 2-position of the imidazole ring is required for aerobic activity. Here we show that substitution of this oxygen by either nitrogen or sulfur yielded equipotent analogs. Acylating the amino series, oxidizing the thioether, or replacing the ether oxygen with carbon significantly reduced the potency of the compounds. Replacement of the benzylic oxygen at the 6-position by nitrogen slightly improved potency and facilitated exploration of the SAR in the more soluble 6-amino series. Significant improvements in potency were realized by extending the linker region between the 6-(*S*) position and the terminal hydrophobic aromatic substituent. A simple 4-feature QSAR model was derived to rationalize MIC results in this series of bicyclic nitroimidazoles.

Introduction

The recent emergence of extensively drug-resistant (XDR) strains of *Mycobacterium tuberculosis* (Mtb[a]) has spurred an urgent effort to identify new agents to treat an otherwise incurable disease.[1] The antitubercular nitroimidazoles, including two classes of new bicyclic agents with either fused oxazole or oxazine rings, are one of the most exciting recent developments in the field of antituberculosis chemotherapy and two candidates are already in human clinical trials for the treatment of both drug-susceptible, and drug-resistant disease.[2] The aerobic antitubercular activity of the 2,3-dihydro-6-nitroimidazo[2,1-*b*]oxazole class of

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[a]Abbreviations: FGD1, F_{420}-dependent glucose-6-phosphate dehydrogenase; MAC, minimum anaerobicidal concentration; MIC, minimum inhibitory concentration; MBP, maltose-binding protein; Mtb, *Mycobacterium tuberculosis*; Mtz, metronidazole; SAR, structure-activity relationship; TB, tuberculosis; TBS or TBDMS, *tert*-butyldimethylsilyl; THP, tetrahydro-2*H*-pyran; TBAI, tetrabutylammonium iodide; MsCl, methanesulfonyl chloride; DIPEA, *N*,*N*-diispropylethylamine; *m*CPBA, *meta*-chloroperoxybenzoic acid; DTAD, di-*tert*-butyl azodicarboxylate; DIBAL-H, diisobutylaluminum hydride.

molecules was discovered in the late 1980s.[3] Although the series was dropped at that time for suspected problems with mutagenicity, the related 2,3-dihydro-6-nitroimidazo[2,1-*b*]oxazine class was discovered shortly thereafter.[4] This oxazine class produced one candidate molecule ((*S*)-2-nitro-6-(4-(trifluoromethoxy)benzyloxy)-6,7-dihydro-5H-imidazo[2,1-*b*][1,3]oxazine, PA-824, **1**) currently in Phase II clinical trials.[2] In parallel the oxazole class has been further elaborated at Otsuka Pharmaceuticals and has given rise to a second candidate currently in clinical trials, (*R*)-2-methyl-6-nitro-2-((4-(4-(4-(trifluoromethoxy)phenoxy)piperidin-1-yl) phenoxy)methyl)-2,3-dihydroimidazo[2,1-*b*]oxazole (OPC-67683, **2**).[5] In all three of these programs a whole cell-based lead optimization strategy was applied, prioritizing compounds on their *in vitro* potency and their *in vivo* efficacy in mice.

Both PA-824 and **2** suffer from extremely low solubility, complicating formulations and potentially limiting ultimate use. In addition, both series were optimized for activity on aerobic bacilli, while a potentially useful anaerobic effect was not the focus of these projects. We have examined the mechanistic details underlying the biological activity of these compounds with the aim of designing nitroimidazoles with more potent activity. Both the oxazine and the oxazole classes of bicyclic nitroimidazoles are pro-drugs that require reduction by Ddn, a coenzyme F_{420}-dependent nitroreductase that we have recently purified and characterized.[6] Ddn displays extremely high substrate specificity for this series of compounds. The kinetic properties (for example, k_{cat}/K_m) of specific members of this family as substrates for Ddn are important determinants of aerobic activity against the whole organism. However, the detailed structural requirements of Ddn for this reduction are still poorly understood. In our previous work[7] we have explored the SARs of 5-nitroimidazoles (*e.g*., metronidazole) and 4-nitroimidazoles (*e.g*., PA-824) as they relate to aerobic and anaerobic potency across these two classes of compounds and found that the 2-position oxygen was an essential distinguishing feature of aerobic potency. In this work we describe the synthesis and evaluation of a number of 2- and 6-substituted 2,3-dihydro-6-nitroimidazo[2,1-*b*]oxazines (Figure 1) and report here the SAR governing the aerobic activities of the 4-nitroimidazoles in depth.

CHEMISTRY

Substitution on the 2-position of the imidazole ring

The preparation of the 2N-substituted analogs of PA-824 is shown in Scheme 1. Synthesis began with TBS-protected alcohol **3**.[7] Alcohol **3** was alkylated with 4-trifluoromethoxybenzyl bromide, NaH, and TBAI to give **4** in 49% yield. TBS-deprotection of **4** in the presence of TBAF afforded primary alcohol **5** in quantitative yield. The treatment of **5** with MsCl and DIPEA provided mesylate **6** in 91% yield.[8] Azidation of mesylate **6** with NaN_3 gave compound **7** in 85% yield.[9] Reduction of the azido group in the presence of $SH(CH_2)_3SH$ and Et_3N[10] furnished amine **8** in 78% yield. Cyclization of **8** was facilitated under microwave conditions at 150°C to afford 2N-824 (**9**) in 32% yield. Conventional solution phase synthesis of a similar reaction has been reported,[11] however, we found that use of microwave conditions improved reaction yield. *N*-formylation of **9** in the presence of HCO_2H and Ac_2O afforded 2N-CHO-824 (**10**, 33%).[12] The treatment of **9** with AcCl and Et_3N furnished 2N-Ac-824 (**11**) in 23% yield.[13] Functional group transformation of mesylate **6** with NaI gave iodo compound **12** in 91% yield.[14] Cyclization of **12** with $MeNH_2$ under microwave conditions at 200°C afforded 2N-Me-824 (**13**, 52%) in addition to the dichloro compound (**14**) as a by-product.

To introduce a sulfur atom in the 6-membered ring, mesylate **6** was treated with KSAc in DMF[15] to give rise to thioester **15** in 95% yield (Scheme 2). Tandem saponification and cyclization[16] of **15** using 3M NaOH (aq) in MeOH afforded the bicyclic compound 2S-824 (**16**) in 26% yield. Oxidation of **16** with *m*CPBA[17] in CH_2Cl_2 furnished both 2SO-824 (**17**; 26%) and $2SO_2$-824 (**18**; 59%). Enantiomer **19** was prepared in an analogous manner (see **Supporting Information**).

Substitution of the benzylic ether to produce secondary amino derivatives

The preparation of compounds **24–28**, where the benzyl ether oxygen atom of PA-824 is replaced with a nitrogen atom, is shown in Scheme 3–Scheme 8. Mesylation of alcohol **20**[18] using MsCl and Et_3N furnished **21** in 80% yield. Treatment of **21** with NaN_3 gave azide **22** in 46% yield. Alternatively, a Mitsunobu reaction on **20** using DTAD, PPh_3, and diphenylphosphoryl azide also afforded **22** in 67% yield.[19] Reduction of azide **22** either under catalytic hydrogenation (10% Pd/C)[20] conditions or using 1,3-propanedithiol in Et_3N[21] gave rise to amine **23**. Amine **23** was found to be unstable to purification, therefore subsequent reactions were performed with the crude amine mixture generated from **22** without further purification. Tandem reduction and reductive amination[22] using the appropriate aldehydes furnished desired products **25** (69%), **26** (31%), **27** (46%), and **28** (77%). Amido-compound **24** was prepared starting from azide **22** by tandem catalytic hydrogenation and acylation using 4-trifluoromethoxyphenoxyacetyl chloride in 61% yield.

In Scheme 4, alcohol **29** was oxidized using Dess-Martin periodinane to give **30**[23] in 82% yield. The aminoethyl compound **31** was prepared by reductive amination of **30** and **23** in the presence of $NaBH_3CN$ and AcOH in 58% yield. Similar chemistry was used to prepare the aminopropyl compound **37** as shown in Scheme 5. Heck reaction[24] of **32** using methyl acrylate, $Pd(OAc)_2$, PPh_3, and Et_3N gave conjugated ester **33** in 91% yield. Ester reduction using DIBAL-H in CH_2Cl_2 delivered allylic alcohol **34**[25] in 84% yield. Catalytic hydrogenation of **34** in the presence of Pd/C and H_2 (g) furnished alcohol **35** in 88% yield. Oxidation of **35** with Dess-Martin periodinane and $NaHCO_3$ gave rise to aldehyde **36** in 86% yield. The aminopropyl compound **37** was prepared by reductive amination of **36** and **23** in the presence of $NaBH_3CN$ and AcOH in 40% yield.

Scheme 6 shows the preparation of the aminobutyl compound **41**. A Horner-Wadsworth-Emmons (HWE) reaction[26] of **30** using triethyl phosphonoacetate gave conjugated ester **38** in 73% yield. Catalytic hydrogenation of **38** under Pd/C and H_2 (g) furnished saturated ester **39** in quantitative yield. Ester **39** was reduced to give aldehyde **40** (76%) using DIBAL-H in CH_2Cl_2 at −78°C. Reductive amination of **40** and **23** in the presence of $NaBH_3CN$ and AcOH afforded **41** in 58% yield. In Scheme 7, allylic alcohol **34** was oxidized using Dess-Martin periodinane and $NaHCO_3$ to give aldehyde **42** in 88% yield. A HWE reaction of **42**[27] with triethyl phosphonoacetate delivered conjugated ester **43**[28] in 64% yield. Catalytic hydrogenation of **43** using Pd/C and H_2 (g) furnished ester **44** in 94% yield. Reduction of **44** in the presence of DIBAL-H in CH_2Cl_2 afforded aldehyde **45** in 83% yield. Reductive amination of **45** and **23** in the presence of $NaBH_3CN$ and AcOH afforded pentylamine **46** in 55% yield. In Scheme 8, alkylation of 4-hydroxybenzaldehyde (**47**) using 4-trifluoromethoxybenzyl bromide and NaH gave rise to compound **48** in quantitative yield. Subsequent reductive amination of **48** and **23** in the presence of $NaBH_3CN$ and AcOH afforded compound **49** in 67% yield.

RESULTS AND DISCUSSION

Replacement of the 2-position oxygen

In our previous work describing the SAR of 4- and 5-nitroimidazoles related to PA-824 and metronidazole,[7] we observed that the 2-position oxygen of the oxazine ring of PA-824 was required for aerobic activity against Mtb.[7] The corresponding 2-carba analog of PA-824 had 30-fold less aerobic activity than the oxazine parent. To explore this further we replaced the 2-position oxygen with two other electron-donating atoms, nitrogen and sulfur, resulting in compounds **9** and **16**, respectively. Surprisingly, these two substitutions had no effect on the aerobic potency of the resulting analogs with measured MICs of 0.8 μM, identical to PA-824 (Table 1). As with PA-824, inverting the stereochemistry at the 6-position of the oxazine ring

in the 2-S-analog **19** resulted in a 50-fold loss of activity, suggesting that these compounds were also activated by Ddn. The catalytic efficiency of these molecules as substrates for Ddn, expressed as the ratio of k_{cat}/K_m for reoxidation of reduced F_{420}, was also comparable to that of PA-824 (Table 1). Both the 2-amino (**9**) and the 2-thio (**16**) analogs show reduced potency under anaerobic conditions (reported as Minimum Anaerobicidal Concentration, MAC), greater than 10-fold and 2 – 4-fold, respectively.

Lowering the electron-donating nature of the 2-position substituent significantly reduces the potency of the analogs in both the amino and thio series. Formylation or acetylation of the amino group to give **10** or **11** results in a 4–8 fold decrease in MIC and a concomitant 2–3 fold reduction in k_{cat}/K_m for Ddn. Likewise, oxidation of **16** to the sulfoxide or the sulfone (**17** or **18**) abolished aerobic activity, reduced anaerobic activity and resulted in analogs that are poor substrates for Ddn (Table 1). Simple methylation of the 2-amino analog (**9**) to give **13** reduced both the aerobic and the anaerobic activity by about 8-fold, yet has a comparatively small effect on the efficiency of this molecule as a Ddn substrate with k_{cat}/K_m of 0.061.

6(S)-amino-derivatives of PA-824

The lipophilic tail of the 6(*S*)-oxazine series has been the most intensively investigated part of the molecule, with several dozen molecules described in the two published patents.[18, 29] One curious observation from the molecules described in these patents was that highly active molecules could have either an aromatic hydrophobic group 2–3 atoms from the 6-position of the oxazine ring or two aromatic groups at roughly 2–3 and 9 atoms' distance. This was highlighted by molecules such as PA-647 (the ether analog of **27**) with a 4-benzyloxybenzyl substituent which was reported to have an MIC more than 10-fold lower than PA-824.[29] This implied that perhaps there were additional hydrophobic pockets on Ddn adjacent to the binding site of PA-824. We wanted to confirm and refine this observation by making a series of molecules to more systematically explore this hypothesis.

One significant problem with accurate measurements of kinetic constants and other activities for all of these compounds has been solubility, so we sought to initially improve this by replacement of the ether oxygen with an amine. Amino-824 (**25**) proved to be slightly more active aerobically than the ether parent and gratifyingly had significant improvements in solubility that will be described elsewhere. To confirm what had been reported in the patent literature for the corresponding 6-position ether derivatives with longer hydrophobic substituents, we synthesized two 6-amino derivatives, with either one (**26**) or two (**27**) aromatic substituents in the lipophilic tail. Similar to the findings previously reported in the patent literature, **26** was equal to, and **27** significantly better than, **25** in terms of their aerobic MIC values.

We next tested a series of derivatives of **25** in which we increased the length of the carbon chain connecting the 6-position amine with the aromatic hydrophobe one carbon at a time so that the spacing from the amino linkage increased from two to five carbons. Aminoethyl-824 (**31**) was 4-fold better in MIC than PA-824 while there was little change in the k_{cat}/K_m (0.110 and 0.155, respectively). Aminopropyl-824 (**37**) was a full log better in MIC (0.08 μM) and showed substantial improvement in k_{cat}/K_m for Ddn to 0.307. Aminobutyl-824 (**41**) had the best MIC (0.04 μM) with continued improvement in k_{cat}/K_m to 0.514. Aminopentyl-824 (**46**) appeared slightly worse in MIC (0.08 μM), the same as aminopropyl-824. Despite this, k_{cat}/K_m for Ddn improved for the pentyl derivative (0.910) compared to the corresponding butyl derivative (0.514). This series of compounds nicely demonstrates the completely different SAR for aerobic and anaerobic activity. Despite an overall 50-fold improvement in MIC, the MAC of aminobutyl-824 (**41**) was the same as the parent compound.

Substitutions at the para position of the second hydrophobic site

The molecules discussed in the previous paragraph (**31, 37, 41** and **46**) were all based on the scaffold of PA-824 and therefore contained the *p*-trifluoromethoxy substituent. The potent activity of these compounds suggests that both extension into the second aromatic site in Ddn and the presence of the *p*-trifluoromethoxy substituent are beneficial. It was therefore a logical extension of the SAR to incorporate a *p*-substituent into amino-647 (**27**). Hence compound **28**, which has a *para*-fluoro substituent, and compound **49**, which has a *para*-trifluoromethoxy substituent, were synthesized and evaluated. To our disappointment, compound **28** showed a significant improvement in k_{cat}/K_m, but no improvement in aerobic MIC, while **49** was inferior as a substrate for Ddn and was equivalent to **27** in aerobic MIC.

A predictive QSAR model for whole cell activity of antitubercular nitroimidazoles

We employed the Catalyst algorithm as implemented in Discovery Studios to search for pharmacophore features within this compound series that best predicted activity. Because of the limited range of k_{cat}/K_m values for these compounds as substrates of Ddn (not quite 3 orders of magnitude) as well as the added mechanistic complexity that this kinetic parameter is only reflective of F_{420} reoxidation and does not translate directly into whole cell activity, we used MIC as the primary activity for developing this model. Twenty-one oxazine analogs were used to develop the model. Highly active molecules **41, 24, 27**, and **49** were used to enumerate potential features and 17 additional molecules were used during the subtractive and optimization phases of the program. Catalyst successfully generated ten robust hypotheses for a QSAR model in four discrete clusters. Seven of these ten were significant using the Fischer validation test at 90% confidence level. The best of these models had a correlation coefficient for the training set of 0.96. Next, 22 additional oxazines (previously unseen by the algorithm) were used to construct a test set that was run against all ten of the hypotheses generated. We then examined both correlation with the training set and the predicted MICs for the test set.

Because all ten hypotheses showed significant correlations using the Fisher validation algorithm in which the input activities are scrambled using the same structures, we explored the differences between them in detail. These hypotheses clustered into four groups that contained many of the same elements. There were two primary elements that differentiated the four groups of QSAR hypotheses: the elements used to position the nitroimidazo-oxazine and the distance of hydrophobic areas from this nucleus. The ten different hypotheses anchored the nitroimidazo-oxazine ring using either two pairs of hydrogen bond acceptors positioned nearly identically (7 of the ten models) or a hydrogen bond donor (nitro group) -acceptor (oxazine oxygen) pair (3 of the ten models). These two donor/acceptor atoms are complemented by a hydrogen bond donor positioned at the 6-position substituent of the oxazine nucleus. An example of one of these models is shown in Figure 2 (hydrogen bond acceptors are shown as green spheres, the donor as purple).

The second main element that distinguished all ten of the top QSAR models was the distance of a hydrophobe (blue sphere) from the imidazo-oxazine nucleus. Four of the ten models actually converged on the presence of two different hydrophobes, one relatively close (2–3 Å) and one more distant (5–6 Å). The remaining six models were split and predicted either a single hydrophobe that was close in space with the ligands binding to it in a U-shaped conformation, or they predicted ligand binding in an extended conformation to access a more distant hydrophobe. One interpretation of these results is that both hydrophobic areas exist but few substrate molecules within the currently available spectrum of compounds are able to take advantage of them. This is, of course, a testable hypothesis that can be further explored.

CONCLUSIONS

This work explores two areas of the nitroimidazo-oxazine pharmacophore: the 2-position atom and the nature of the substituent at the 6-position on the oxazine ring. First, the data further highlights the critical importance of the nature of the 2-position imidazole substituent in determining the aerobic potency of this compound series, a trend we initially observed in earlier work.[7] We show that the oxygen normally occupying this position can be substituted with either nitrogen or sulfur without affecting aerobic potency, but that reducing the electron-donating nature of this substituent by either acylation (in the case of the 2-aza series) or oxidation (in the case of the 2-thia series), leads to decreased aerobic potency. The loss of potency seen with alkylation of the 2-position amine in **9** is difficult to understand based solely on the inductive effect of a simple methyl substituent. The QSAR model suggests that the orientation of the 2-position of the oxazine ring with respect to the nitro group on the imidazole is a critical determinant of activity. The 2-N-methyl group may prevent formation of a critical hydrogen bond with an active site residue in Ddn, preventing correct alignment of the entire nucleus.

Secondly, examination of imidazo-oxazines having hydrophobic regions at varying lengths from the oxazine nucleus has led to an increased understanding of our knowledge of the Ddn active site. Molecules such as **41** and **46** may indicate that optimization of this portion of the molecular series remains to be done. More importantly, these compounds contributed to the QSAR model indicating the presence and location of the Ddn hydrophobic pocket.

Our results show that the correlation between simple catalytic efficiency as a Ddn substrate and MIC, at least as reflected by k_{cat}/K_m for reoxidation of the deazaflavin cofactor, remains somewhat imperfect. While improvements in catalytic efficiency for Ddn can generally be said to correlate with MIC, the results suggests the interplay of a more subtle mechanistic complexity. This complexity probably results from the two possible courses of reduction of the nitroimidazole ring as we have previously argued, one course resulting from reduction at C-5 of the imidazole followed by release of a reactive nitrogen species, and the other course resulting from reduction of the nitro group. While so far we have not found a parameter (*e.g.*, metabolite composition, catalytic efficiency on Ddn, etc.) that perfectly correlates with the aerobic MIC,[6] it is likely that further study of this enzyme will provide data that can be used to more accurately predict which mechanistic course correlates most directly with aerobic potency. Although aerobic activity can be roughly explained by the efficiency of these molecules as substrates of Ddn, anaerobic activity is considerably more complex. Compounds that are susceptible to conjugate reduction on the imidazole ring suffer a subsequent elimination of nitrous acid producing reactive nitrogen species within the bacterial cell thereby poisoning bacterial respiration.

Despite this complexity, our systematic exploration of the features required for aerobic activity of 4-nitroimidazoles, such as PA-824 as compared to 5-nitroimidazoles such as metronidazole[7] and those described here, has allowed us to build a simple predictive QSAR model that enhances our ability to develop improved analogs of PA-824. This model is robust, testable and provides a clear tool for lead optimization of compounds in this series.

EXPERIMENTALS

Anhydrous solvents and reagents were purchased from Sigma-Aldrich and used as received unless otherwise stated. Melting points were obtained on an Electrothermal 9100 apparatus and are uncorrected. LC-MS analysis was conducted on an Agilent 1100 series HPLC with attached Agilent quadrupole mass analyzer model G1946 D SL with electrospray ionization in positive ion mode. LC chromatography used a Phenomenex Luna $C_{18}(2)$ column (2 × 50 mm, 3 μm) with a water/acetonitrile (each with 0.1% (v/v) formic acid) gradient using a flow

rate of 0.3 mL/min. UV detection was with an Agilent Diode Array Detector model G1315A spectrometer at 270 and 310 nm. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded at 300 and 75.5 MHz, respectively, on a Varian Gemini spectrometer, using TMS or the solvent peak as an internal standard. Column chromatography was conducted using either silica gel (Geduran, 60, mesh 40–63 μm) or prepacked RediSep columns (Teledyne Isco, Inc., Lincoln, NE USA) on an Isco CombiFlash Optix10 instrument. Elemental analyses were performed by Atlantic Microlab, Inc. (Norcross, GA USA). HRMS analyses were performed at the W.M. Keck Foundation Biotechnology Resource Laboratory (Yale University, New Haven, CT USA) or at the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), NIH. Isoniazid, rifampicin, metronidazole and methylene blue were obtained from Sigma-Aldrich. PA-824 was prepared as described in US patent 5,668,127. All stocks were made in 20 mM DMSO.

(*S*)-1-(3-(*tert*-butyldimethylsilyloxy)-2-(4-(trifluoromethoxy)benzyloxy)propyl)-2-methyl-4-nitro-1*H*-imidazole (4)

A mixture of **3**[7] (6.84 g, 20.4 mmol), 4-trifluoromethoxybenzyl bromide (6.23 g, 24.4 mmol), and TBAI (753 mg, 2.04 mmol) in DMF (50 mL) was prepared under an argon atmosphere. To this mixture, 60% NaH (980 mg, 24.4 mmol) was added at −78°C. The reaction mixture was stirred at 0°C for 30 min and then at rt for 14 h, quenched with H_2O, and extracted with EtOAc (2x). The combined organic layers were dried with $MgSO_4$, filtered, and concentrated. Chromatography on silica gel (EtOAc:Hx, 1:4) afforded **4** as a slightly yellow oil (5.14 g, 10.1 mmol, 49%). $[\alpha]_D^{20} = -19.7$ (*c* 1.03, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.06 (d, *J* = 1.2 Hz, 6H), 0.89 (s, 9H), 3.59 (dd, *J* = 9.9, 6.0 Hz, 1H), 3.63–3.77 (m, 2H), 4.06 (dd, *J* = 14.4, 7.5 Hz, 1H), 4.30 (dd, *J* = 14.7, 3.0 Hz, 1H), 4.42 (d, *J* = 11.7 Hz, 1H), 4.58 (d, *J* = 11.7 Hz, 1H), 7.13 (d, *J* = 8.7 Hz, 2H), 7.20 (d, *J* = 8.7 Hz, 2H), 7.79 (s, 1H); ^{13}C NMR ($CDCl_3$) δ −5.6, 18.1, 25.5, 25.6, 48.9, 61.4, 71.5, 77.3, 118.6, 121.0, 122.0, 125.4, 129.1, 132.5, 135.7, 145.4, 148.9; HRMS (ESMS) calcd for $C_{20}H_{28}ClF_3N_3O_5Si$ [M + H^+] 510.1439, found 510.1427.

(*S*)-2-(4-(trifluoromethoxy)benzyloxy)-3-(2-chloro-4-nitro-1*H*-imidazol-1-yl)propan-1-ol (5)

To a solution of **4** (3.48 g, 6.82 mmol) in THF (50 mL) was added 1.0 M TBAF in THF (8.2 mL, 8.2 mmol) at rt. The reaction mixture was stirred at rt for 30 min and concentrated. Chromatography on silica gel (MeOH:CH_2Cl_2, 1:20) afforded **5** as a yellow oil (2.70 g, 6.82 mmol, 100%). $[\alpha]_D^{20} = -21.9$ (*c* 0.52, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.82 (s, br, 1H), 3.71–3.80 (m, 3H), 4.15–4.30 (m, 2H), 4.43 (d, *J* = 12.0 Hz, 1H), 4.61 (d, *J* = 12.0 Hz, 1H), 7.11 (d, *J* = 8.1 Hz, 2H), 7.20 (d, *J* = 8.7 Hz, 2H), 7.83 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 48.6, 60.4, 71.2, 76.7, 118.5, 120.9, 122.0, 122.2, 129.1, 132.7, 135.5, 145.2, 148.8; HRMS (ESMS) calcd for $C_{14}H_{14}ClF_3N_3O_5$ [M + H^+] 396.0574, found 396.0566.

(*S*)-2-(4-(trifluoromethoxy)benzyloxy)-3-(2-chloro-4-nitro-1*H*-imidazol-1-yl)propyl methanesulfonate (6)

To a solution of **5** (1.56 g, 3.94 mmol) in CH_2Cl_2 (30 mL) was added DIPEA (1.5 mL, 8.6 mmol) and MsCl (0.45 mL, 5.8 mmol) at rt. The reaction mixture was stirred at rt for 30 min, quenched with H_2O and concentrated. The organic layer was separated and aqueous layers were extracted with CH_2Cl_2 (1x). The combined organic layers were dried with anhydrous $MgSO_4$, filtered, and concentrated. Chromatography on silica gel (EtOAc:Hx, 1:1–2:1) afforded **6** as a yellow oil (1.70 g, 3.59 mmol, 91%). $[\alpha]_D^{20} = -30.6$ (*c* 0.80, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 3.10 (s, 3H), 4.21–4.38 (m, 3H), 4.48 (dd, *J* = 11.4, 4.2 Hz, 2H), 4.72 (d, *J* = 11.4 Hz, 1H), 7.16 (d, *J* = 8.7 Hz, 2H), 7.26 (d, *J* = 8.7 Hz, 2H), 7.91 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 37.2, 48.2, 66.6, 71.3, 118.3, 120.7, 121.8, 122.1, 129.3, 132.5, 134.9, 145.2, 148.70, 148.72; HRMS (ESMS) calcd for $C_{15}H_{16}ClF_3N_3O_7S$ [M + H^+] 474.0350, found 474.0349.

1-((*S*)-2-(4-(trifluoromethoxy)benzyloxy)-3-azidopropyl)-2-chloro-4-nitro-1*H*-imidazole (7)

A mixture of **6** (850 mg, 1.79 mmol) and NaN_3 (233 mg, 3.59 mmol) in DMF (10 mL) was heated at 55°C for 14 h. The reaction mixture was concentrated under vacuum. Column chromatography on silica gel (EtOAc:Hx, 1:2-1:1) afforded **7** as a slightly yellow oil (640 mg, 1.52 mmol, 85%). $[\alpha]_D^{20} = -26.7$ (*c* 0.67, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 3.45 (dd, *J* = 13.2, 4.2 Hz, 1H), 3.62–3.68 (m, 1H), 3.91–3.98 (m, 1H), 4.18–4.31 (m, 2H), 4.48 (d, *J* = 11.7 Hz, 1H), 4.71 (d, *J* = 11.7 Hz, 1H), 7.17 (d, *J* = 9.0 Hz, 2H), 7.27 (d, *J* = 8.4 Hz, 2H), 7.87 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 48.8, 50.4, 71.2, 75.3, 118.4, 120.7, 121.8, 122.0, 129.1, 132.4, 135.1, 145.1, 148.64, 148.67; HRMS (ESMS) calcd for $C_{14}H_{13}ClF_3N_6O_4$ [M + H^+] 421.0639, found 421.0649.

(*R*)-2-(4-(trifluoromethoxy)benzyloxy)-3-(2-chloro-4-nitro-1*H*-imidazol-1-yl)propan-1-amine (8)

To a solution of 7 (780 mg, 1.85 mmol) in MeOH (8 mL) was added 1,3-propanedithiol (0.91 mL, 9.04 mmol) and Et_3N (1.26 mL, 9.04 mmol) sequentially at rt. The reaction mixture was stirred at rt for 2 h and was concentrated under vacuum. Column chromatography on silica gel (MeOH:CH_2Cl_2, 1:15) afforded **8** as a yellow oil (568 mg, 1.44 mmol, 78%). $[\alpha]_D^{20} = -37.5$ (*c* 5.0, MeOH); ^1H NMR ($CDCl_3$) δ 1.44 (s, br, 2H), 2.90 (dd, *J* = 13.4, 4.4 Hz, 1H), 3.03 (dd, *J* = 13.5, 5.4 Hz, 1H), 3.72–3.79 (m, 1H), 4.22–4.37 (m, 2H), 4.45 (d, *J* = 11.7 Hz, 1H), 4.64 (d, *J* = 12.0 Hz, 1H), 7.16 (d, *J* = 8.7 Hz, 2H), 7.26 (d, *J* = 8.7 Hz, 2H), 7.92 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 41.4, 48.9, 70.8, 118.4, 120.6, 121.8, 122.1, 128.9, 132.4, 135.7, 145.0, 148.48, 148.50; HRMS (ESMS) calcd for $C_{15}H_{15}ClF_3N_4O_6$ [M + $HCOO^-$] 439.0632, found 439.0615.

(*R*)-6-(4-(trifluoromethoxy)benzyloxy)-5,6,7,8-tetrahydro-2-nitroimidazo[1,2-*a*]pyrimidine (9)

A mixture of **8** (300 mg, 0.76 mmol) and DIPEA (0.15 mL, 0.86 mmol) in DMF (3 mL) was heated under microwave conditions at 150°C for 30min. The reaction mixture was concentrated under vacuum. Chromatography on preparative TLC (MeOH:CH_2Cl_2, 1:9) afforded **9** as a yellow solid (85 mg, 0.24 mmol, 32%). $[\alpha]_D^{20} = -12.9$ (*c* 0.39, $CHCl_3$); Mp = 86–88°C; ^1H NMR ($CDCl_3$) δ 3.56 (d, *J* = 12.9 Hz, 1H), 3.75–3.87 (m, 1H), 3.97–4.11 (m, 3H), 4.57 (d, *J* = 12.3 Hz, 1H), 4.72 (d, *J* = 12.0 Hz, 1H), 7.17 (d, *J* = 8.7 Hz, 2H), 7.34 (d, *J* = 8.7 Hz, 2H), 8.34 (s, 1H); HRMS (ESMS) calcd for $C_{14}H_{14}F_3N_4O_4$ [M + H^+] 359.0967, found 359.0964.

(*R*)-6-(4-(trifluoromethoxy)benzyloxy)-6,7-dihydro-2-nitroimidazo[1,2-*a*]pyrimidine-8(5*H*)-carbaldehyde (10)

A mixture of HCO_2H (0.50mL, 13 mmol) and Ac_2O (1.0 mL, 11 mmol) was heated at 80°C for 30min. The reaction mixture was cooled down to rt. A solution of **9** (17 mg, 0.048 mmol) in CH_2Cl_2 (3 mL) was added to the reaction mixture at rt. The reaction mixture was stirred at rt for 14 h and then concentrated under vacuum. Chromatography on preparative TLC (EtOAc:Hx, 5:1) afforded **10** as a yellow oil (6.2 mg, 0.016 mmol, 33%). $[\alpha]_D^{20} = +16.6$ (*c* 0.98, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 3.41 (d, *J* = 13.2 Hz, 1H), 4.12–4.32 (m, 3H), 4.52 (d, *J* = 12.0 Hz, 1H), 4.65 (d, *J* = 12.0 Hz, 1H), 4.66–4.76 (m, 1H), 7.18 (d, *J* = 8.4 Hz, 2H), 7.29 (d, *J* = 8.4 Hz, 2H), 7.63 (s, 1H), 9.37 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 77.0, 39.4, 48.5, 66.2, 70.1, 117.2, 118.6, 121.2, 122.0, 129.2, 134.9, 139.2, 145.3, 149.1, 159.8; HRMS (ESMS) calcd for $C_{15}H_{14}F_3N_4O_5$ [M + H^+] 387.0916, found 387.0912.

1-((*R*)-6-(4-(trifluoromethoxy)benzyloxy)-6,7-dihydro-2-nitroimidazo[1,2-*a*]pyrimidin-8(5*H*)-yl)ethanone (11)

To a solution of **9** (50 mg, 0.14 mmol) and Et_3N (0.030 mL, 0.22 mmol) in CH_2Cl_2 was added AcCl (0.015 mL, 0.21 mmol) at 0°C. The reaction mixture was stirred at rt for 2 h and was concentrated under vacuum. Chromatography on preparative TLC (EtOAc:Hx, 5:1) afforded **11** as a yellow oil (13 mg, 0.032 mmol, 23%). $[\alpha]_D^{20} = -25.7$ (*c* 0.65, $CHCl_3$); ^1H NMR

($CDCl_3$) δ 2.70 (s, 3H), 3.41 (d, *J* = 14.1 Hz, 1H), 4.13–4.21 (m, 3H), 4.50 (d, *J* = 11.9 Hz, 1H), 4.66 (d, *J* = 11.9 Hz, 1H), 4.97 (dd, *J* = 14.1, 3.9 Hz, 1H), 7.19 (d, *J* = 8.4 Hz, 2H), 7.30 (d, *J* = 8.4 Hz, 2H), 7.60 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 25.0, 41.7, 49.7, 67.1, 70.1, 116.9, 118.7, 121.2, 122.1, 129.2, 135.2, 140.1, 145.0, 149.1, 170.4; LC-MS (ESMS) 401.0 [M + H^+], 423.1 [M + Na^+].

1-((*S*)-2-(4-(trifluoromethoxy)benzyloxy)-3-iodopropyl)-2-chloro-4-nitro-1*H*-imidazole (12)

A solution of **6** (850 mg, 1.79 mmol) and NaI (1.18 g, 7.87 mmol) in acetone (10 mL) was refluxed for 6 h. The reaction mixture was filtered and concentrated under vacuum. Chromatography on silica gel (EtOAc:hexanes, 2:1) afforded **12** as a colorless oil (824 mg, 1.63 mmol, 91%). $[\alpha]_D^{20}$ = −20.7 (*c* 0.80, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 3.23 (dd, *J* = 10.8, 7.2 Hz, 1H), 3.36 (dd, *J* = 10.8, 3.6 Hz, 1H), 3.62–3.69 (m, 1H), 4.10 (dd, *J* = 14.4, 8.4 Hz, 1H), 4.34 (d, *J* = 11.7 Hz, 1H), 4.38 (dd, *J* = 14.4, 3.0 Hz, 1H), 4.65 (d, *J* = 11.7 Hz, 1H), 7.16 (d, *J* = 8.7 Hz, 2H), 7.22 (d, *J* = 8.7 Hz, 2H), 7.79 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 2.7, 51.4, 71.2, 75.7, 118.6, 121.1, 121.7, 122.0, 129.4, 132.6, 134.8, 145.6, 149.17, 149.20; LC-MS (ESMS) 506.2 [M + H^+].

(*R*)-6-(4-(trifluoromethoxy)benzyloxy)-5,6,7,8-tetrahydro-8-methyl-2-nitroimidazo[1,2-*a*] pyrimidine (13) and 1-((*S*)-2-(4-(trifluoromethoxy)benzyloxy)-3-chloropropyl)-2-chloro-4-nitro-1*H*-imidazole (14)

A mixture of **12** (245 mg, 0.485 mmol), 2.0 M $MeNH_2$ (1.5 mL, 3.0 mmol) in THF, and DIPEA (0.10 mL, 0.57 mmol) in DMF (2 mL) was heated under microwave conditions at 200°C for 20 min. The reaction mixture was concentrated under vacuum. Chromatography on preparative TLC (MeOH:CH_2Cl_2, 1:15) afforded **13** as a yellow solid (94 mg, 0.25 mmol, 52%) in addition to **14** (72 mg, 0.17 mmol, 35%) as a colorless oil. **13:** $[\alpha]_D^{20}$ = −35.3 (*c* 1.12, MeOH); Mp = 116.0–117.3°C; ^{1}H NMR ($CDCl_3$) δ 3.06 (s, 3H), 3.32–3.47 (m, 2H), 3.96–4.13 (m, 3H), 4.62 (s, 2H), 7.17 (d, *J* = 8.1 Hz, 2H), 7.31 (d, *J* = 8.7 Hz, 2H), 7.27 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 37.2, 47.5, 50.0, 68.0, 70.1, 116.5, 118.6, 121.0, 122.0, 128.8, 135.8, 145.2, 147.0, 148.8; HRMS (ESMS) calcd for $C_{15}H_{16}F_3N_4O_4$ [M + H^+] 373.1124, found 373.1118. **14:** $[\alpha]_D^{20}$ = −26.8 (*c* 0.52, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 3.58 (dd, *J* = 12.0, 6.9 Hz, 1H), 3.68 (dd, *J* = 12.0, 3.9 Hz, 1H), 3.88–3.95 (m, 1H), 4.16 (dd, *J* = 14.6, 8.0 Hz, 1H), 4.33–4.43 (m, 2H), 4.66 (d, *J* = 11.7 Hz, 1H), 7.15 (d, *J* = 8.4 Hz, 2H), 7.21 (d, *J* = 8.4 Hz, 2H), 7.80 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 41.7, 49.2, 71.5, 76.2, 118.6, 121.1, 121.8, 122.0, 129.3, 132.6, 134.9, 145.6, 149.1; HRMS (ESMS) calcd for $C_{14}H_{12}Cl_2F_3N_3O_4$ [M^-] 413.0157, found 413.0159.

S-(*S*)-2-(4-(trifluoromethoxy)benzyloxy)-3-(2-chloro-4-nitro-1*H*-imidazol-1-yl)propyl ethanethioate (15)

To a solution of **6** (850 mg, 1.79 mmol) in DMF (15 mL) was added KSAc (410 mg, 3.59 mmol) at rt. The reaction mixture was stirred at 45°C for 14 h and quenched with H_2O. The crude mixture was extracted with EtOAc (2x). The combined organic layers were dried with anhydrous $MgSO_4$, filtered, and concentrated. Chromatography on silica gel (EtOAc:Hx, 2:1) afforded **15** as a white solid (768 mg, 1.69 mmol, 95%). $[\alpha]_D^{20}$ = −34.1 (*c* 1.27, $CHCl_3$); Mp = 84.2–84.5°C; ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 3H), 2.95 (dd, *J* = 14.1, 7.2 Hz, 1H), 3.24 (dd, *J* = 14.1, 3.9 Hz, 1H), 3.68–3.78 (m, 1H), 3.99 (dd, *J* = 14.4, 8.4 Hz, 1H), 4.17 (dd, *J* = 14.4, 3.09 Hz, 1H), 4.38 (d, *J* = 11.7 Hz, 1H), 4.64 (d, *J* = 11.7 Hz, 1H), 7.10 (d, *J* = 8.7 Hz, 2H), 7.18 (d, *J* = 8.7 Hz, 2H), 7.75 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 29.2, 30.4, 50.1, 71.0, 118.5, 120.8, 121.86, 121.92, 129.4, 132.4, 135.2, 145.3, 148.8, 148.9, 194.5; HRMS (ESMS) calcd for $C_{16}H_{16}ClF_3N_3O_5S$ [M + H^+] 454.0451, found 454.0454.

(*S*)-6-(4-(trifluoromethoxy)benzyloxy)-6,7-dihydro-2-nitro-5*H*-imidazo[2,1-*b*][1,3]thiazine (16)

To a solution of **15** (300 mg, 0.661 mmol) in MeOH (50 mL) was added 3M NaOH (aq) (0.30 mL, 0.90 mmol) at 0°C. The reaction mixture was stirred at 0°C for 30 min and at rt for 17 h, and then concentrated under vacuum. Column chromatography on silica gel (EtOAc:Hx, 1:1–2:1) afforded **16** as a yellow oil (65 mg, 0.17 mmol, 26%). $[\alpha]_D^{20} = -62.7$ (*c* 1.00, MeOH); ^{1}H NMR ($CDCl_3$) δ 3.27–3.38 (m, 2H), 4.19 (d, *J* = 3.6 Hz, 2H), 4.30 (m, 1H), 4.59 (d, *J* = 6.0 Hz, 1H), 4.72 (d, *J* = 6.0 Hz, 1H), 7.17 (d, *J* = 8. Hz, 2H), 7.34 (d, *J* = 8.4 Hz, 2H), 7.67 (s, 1H); ^{13}C NMR (MeOH-*d*$_4$) δ 28.7, 49.9, 67.4, 70.0, 118.6, 121.0, 122.0, 129.1, 129.2, 135.4, 139.0, 147.2, 148.9; HRMS (ESMS) calcd for $C_{14}H_{13}F_3N_3O_4S$ [M + H$^+$] 376.0579, found 376.0575.

17 and 18

To a solution of **16** (60 mg, 0.16 mmol) in CH_2Cl_2 (6 mL) was added 77% *m*CPBA (107 mg, 0.48 mmol) at 0°C. The reaction mixture was stirred at 0°C for 30 min and at rt for 1 h, and then concentrated under vacuum. Column chromatography on silica gel (EtOAc:Hx, 5:1) afforded **17** as a colorless oil (16 mg, 0.042 mmol, 26%) and **18** as a white solid (38 mg, 0.094 mmol, 59%). **17**: $[\alpha]_D^{20} = -112.9$ (*c* 0.82, MeOH); ^{1}H NMR (MeOH-*d*$_4$) δ 3.66–3.71 (m, 1H), 3.81–3.91 (m, 1H), 4.27–4.35 (m, 1H), 4.66–4.82 (m, 4H), 7.26 (d, *J* = 8.1 Hz, 2H), 7.46 (d, *J* = 8.7 Hz, 2H), 8.30 (s, 1H); ^{13}C NMR (MeOH-*d*$_4$) δ 51.3, 51.6, 68.8, 71.8, 120.4, 122.3, 123.2, 124.2, 130.7, 138.1, 143.8, 149.2, 150.3; HRMS (ESMS) calcd for $C_{14}H_{13}F_3N_3O_5S$ [M + H$^+$] 392.0528, found 392.0522. **18**: $[\alpha]_D^{20} = -112.9$ (*c* 0.82, MeOH); Mp = 71.5–72.7° C; ^{1}H NMR (MeOH-*d*$_4$) δ 4.04 (dd, *J* = 14.7, 2.1 Hz, 1H), 4.25 (dd, *J* = 14.7, 6.6 Hz, 1H), 4.58–4.72 (m, 5H), 7.20 (d, *J* = 8.7 Hz, 2H), 7.43 (d, *J* = 8.7 Hz, 2H), 8.24 (s, 1H), ^{13}C NMR (MeOH-*d*$_4$) δ 52.0, 55.3, 71.3, 71.7, 120.3, 122.1, 123.2, 123.7, 130.9, 137., 143.5, 148.0, 150.28, 150.30; HRMS (ESMS) calcd for $C_{14}H_{13}F_3N_3O_6S$ [M + H$^+$] 408.0477, found 408.0471.

(*R*)-6,7-dihydro-2-nitro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-yl methanesulfonate (21)

MsCl (0.78 mL, 10.0 mmol) was added to a stirred solution of **20** (0.93 g, 5.0 mmol) and Et_3N (2.1 mL, 15.0 mmol) in DMF (40 mL) at 0°C. The reaction mixture was then further stirred at 0°C for 1 h. The solvent and excess reagents were removed under reduced pressure. H_2O (50 mL) was added to the light brown residue. The mixture was then filtered, and the solid was washed with H_2O (50 mL) to give **21** as a yellow/white solid (1.05 g, 80%). Mp = 213–214°C; ^{1}H NMR (acetone-*d*$_6$) δ 3.30 (s, 1 H), 4.58 (br d, *J* = 14.1 Hz, 1 H), 4.69 (dd, *J* = 14.1, 3.3 Hz, 1 H), 4.77–4.78 (m, 2 H), 5.56–5.60 (m, 1 H), 7.86 (s, 1 H); ^{13}C NMR (DMSO-*d*$_6$) δ 37.9, 47.6, 68.6, 69.1, 117.9, 142.2, 146.5.

(*S*)-6-azido-6,7-dihydro-2-nitro-5*H*-imidazo[2,1-*b*][1,3]oxazine (22)

Method A: A mixture of **21** (1.68 g, 6.3 mmol), NaN_3 (5 g, 76 mmol) in DMF (20 mL) was heated at 70°C under an inert atmosphere for 48 h. The solvent was removed under reduced pressure. H_2O (100 mL) was added to the residue. The mixture was extracted with ethyl acetate (3 × 80 mL). The organic extracts were combined, washed with brine (200 mL), and dried over $MgSO_4$. The solvent was then removed under reduced pressure to give a brown solid, which was purified via column chromatography (MeOH:CH_2Cl_2, 0–5%) to give **22** as a yellow solid (0.60 g, 46%). Mp = 152.0–152.4°C (lit=157°C [29]); ^{1}H NMR (acetone-*d*$_6$) δ 4.36 (dt, *J* = 13.5, 2.4 Hz, 1 H), 4.58 (dd, *J* = 13.5, 3.6 Hz, 1 H), 4.62–4.74 (m, 3 H), 7.83 (s, 1 H). **Method B:** Di-*tert*-butyl azodicarboxylate (DTAD, 0.34 g, 1.5 mmol) in THF (4.0 mL) was added dropwise to a stirred mixture of **20** (185 mg, 1.0 mmol), triphenylphosphine (0.39 g, 1.5 mmol), and diphenylphosphoryl azide (0.33 mL, 1.5 mmol) in anhydrous THF (10 mL) at 0°C. The reaction mixture was stirred under argon at rt for 18 h. The solution was decanted (white precipitate discarded) and evaporated *in vacuo*. The residue was purified via preparative TLC

(6 % MeOH in $CHCl_3$) to give **22** as a yellow crystalline solid (145 mg, 67 %). ^{1}H NMR (acetone-d_6) δ 4.35 (dt, *J* = 13.7, 2.2 Hz, 1 H), 4.56 (dd, *J* = 13.7, 3.8 Hz, 1 H), 4.65–4.73 (m, 3 H), 7.82 (s, 1 H).

(*S*)-6,7-dihydro-2-nitro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (23)

Method A: A mixture of **22** (40 mg, 0.19 mmol) and Pd/C (10 % palladium on activated carbon, 38 mg) in EtOAc (8 mL) was stirred under a hydrogen atmosphere (balloon) at rt for 2 h. TLC (5 % MeOH in $CHCl_3$) showed that the starting material was consumed. The same TLC was further developed in 25 % MeOH in $CHCl_3$ to show one spot at R_f = 0.20. The mixture was filtered, and the solution was concentrated under reduced pressure to give **23** as a pale brown film (17 mg, 50%). ^{1}H NMR (MeOH-d_4) δ 3.54–3.60 (m, 1 H), 3.88 (ddd, *J* = 12.8, 5.5, 1.4 Hz, 1 H), 4.23–4.30 (m, 2 H), 4.47 (ddd, *J* = 12.8, 2.7, 1.6 Hz, 1 H), 7.79 (s, 1 H). **Method B:** To a stirred solution of **22** (0.10 g, 0.48 mmol) in MeOH (2.5 mL) was added propane-1,3-dithiol (0.24 mL, 2.38 mmol) and triethylamine (0.36 mL, 2.38 mmol). The reaction mixture was stirred at room temperature for 5 min during which time the reaction solution turned clear. The solvent was removed and the remaining thiol was removed by washing with 10% methylene chloride in hexane solution to get crude **23** as a pale yellow solid (89 mg, 100%). This material was used in subsequent reactions without further purification.

(*S*)-*N*-(2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-yl)-2-(4-(trifluoromethoxy) phenoxy)acetamide (24)

4-(Trifluoromethoxy)phenoxyacetyl chloride (1.2 g, 4.6 mmol) was added to a stirred solution of crude amine **23** (crude amine from Method A, ~1.1 mmol) and triethylamine (1.1 mL, 8.0 mmol) in DMF (20 mL) at room temperature. The reaction mixture was further stirred at rt for 18 h. The solvent and excess reagents were removed under reduced pressure. H_2O (50 mL) was added. And the mixture was extracted with CH_2Cl_2 (3 × 50 mL). The organic extracts were combined, washed with H_2O (2 × 100 mL), and dried over $MgSO_4$. The solvent was then removed under reduced pressure to give a brown gum, which was purified by preparative TLC (5% MeOH in CH_2Cl_2) to give **24** as a yellow solid (0.27 g, 61% from azide). Mp = 158.0–159.5°C; ^{1}H NMR ($CDCl_3$) δ 4.18 (br d, *J* = 13.2 Hz, 1 H), 4.29 (dd, *J* = 13.2, 4.7 Hz, 1 H), 4.43 (dd, *J* = 11.7, 1.7 Hz, 1 H), 4.53–4.62 (m, 3 H), 4.78–4.88 (m, 1 H), 6.82–7.14 (m, 1,4-disubstitued pattern, 4 H), 7.28 (s, 1 H), 8.20 (br d, *J* = 7.8 Hz, 1 H). Anal. Calcd for $C_{15}H_{13}F_3N_4O_6$: C, 44.78; H, 3.26; N, 13.93. Found: C, 44.89; H 3.25; N 13.75.

(*S*)-*N*-(4-(trifluoromethoxy)benzyl)-6,7-dihydro-2-nitro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (25)

4-(Trifluoromethoxy)benzaldehyde (475 mg, 2.5 mmol) was added to a stirred solution of **23** (crude amine from Method A, ~1.2 mmol) in DMF at rt, followed by addition of glacial acetic acid (143 μL, 2.5 mmol). After 30 min, $NaBH_3CN$ (223 mg, 3.1 mmol) was added. The reaction mixture was further stirred at rt for 18 h. The solvent was removed under reduced pressure. H_2O (50 mL) was added to the residue. The mixture was extracted with CH_2Cl_2 (3 × 50 mL). The organic extracts were combined, washed with H_2O (100 mL), and dried over $MgSO_4$. The solvent was then removed under reduced pressure to give a brown gum, which was purified via preparative TLC (5 % MeOH in CH_2Cl_2) to give **25** as a yellow solid (0.29 g, 69% from the azide). Mp = 118–119°C; ^{1}H NMR (acetone-d_6) δ 3.47–3.53 (m, 1 H), 4.00 (s, 2 H), 4.19 (ddd, *J* = 12.6, 4.5, 1.5 Hz, 1 H), 4.38 (dd, *J* = 12.6, 4.2 Hz, 1 H), 4.47 (ddd, *J* = 11.1, 5.4, 1.5 Hz,, 1 H), 4.55 (dd, *J* = 11.1, 5.4 Hz, 1 H), 7.24–7.53 (m, 1,4-disubstituted pattern, 4 H), 7.73 (s, 1 H); ^{13}C NMR (acetone-d_6) δ 48.3, 49.0, 50.5, 70.3, 117.6, 121.8, 130.6, 140.9, 148.8. Anal. Calcd for $C_{14}H_{13}F_3N_4O_4$: C, 46.93; H, 3.66; N, 15.64. Found: C, 46.94; H, 3.60; N, 15.85.

(*S*)-*N*-(4-butylbenzyl)-2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (26)

To a stirred solution of **23** (87 mg, 0.48 mmol) and 4-butylbenzaldehyde (0.16 mL, 0.96 mmol) in DMF (5 mL) was added AcOH (55.0 μL, 0.96 mmol) at room temperature. The reaction was stirred for 30 minutes and $NaBH_3CN$ (9.1 mg, 1.44 mmol) was added to the reaction mixture. The reaction was further stirred for 18 hours. The reaction was quenched with MeOH (0.5 mL) and the solvent was removed. Saturated NH_4Cl solution (10 mL) was added and the mixture was extracted with methylene chloride (3 × 10 mL). The organic layer was washed with brine (5 mL), dried ($MgSO_4$) and evaporated. The crude residue was purified by preparative TLC (CH_2Cl_2:MeOH, 20:1) to give **26** as a white solid (48.3mg, 31%). ^{1}H-NMR (acetone-d_6) δ 0.91 (t, *J* = 7.5 Hz, 6.9 Hz, 3H), 1.29 –1.37 (m, 2H), 1.55–1.60 (m, 2H), 2.59 (t, *J* = 7.8 Hz, 7.5 Hz, 2H), 3.93 – 4.10 (m, 1H), 4.36 (dd, *J* = 12.9 Hz, 2.1 Hz, 1H), 4.57 – 4.70 (m, 5H), 7.14 (d, *J* = 7.8 Hz, 2H), 7.25 (d, *J* = 7.8 Hz, 2H), 7.83 (s, 1H); MS (ESI) m/e 331 $[M + H]^+$; HRMS m/e calcd. for $C_{17}H_{23}N_4O_3$ $[M + H^+]$ 331.1770, found 331.1760.

(*S*)-*N*-(4-(benzyloxy)benzyl)-2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (27)

To a stirred solution of **23** (87.6 mg, 0.48 mmol) and 4-benzyloxybenzaldehyde (0.203g, 0.96 mmol) in DMF (5 mL) was added AcOH (55.4 μL, 0.96 mmol) at room temperature. The reaction was stirred for 30 minutes and $NaBH_3CN$ (9.1 mg, 1.44 mmol) was added to the reaction mixture. The reaction was further stirred for 18 hours. The reaction was quenched with MeOH (0.5 mL) and the solvent was removed. Saturated NH_4Cl solution (10 mL) was added and the mixture was extracted with methylene chloride (3 × 10 mL). The organic layer was washed with brine (5 mL), dried ($MgSO_4$) and evaporated. The crude residue was purified by preparative TLC (CH_2Cl_2:MeOH, 20:1) to give **27** as a white solid (82.8 mg, 46%). ^{1}H-NMR (acetone-d_6) δ 3.42 – 3.47 (m, 1H), 3.87 (m, 1H), 4.14 (ddd, *J* = 12.6 Hz, 4.5 Hz, 1.8Hz, 1H), 4.33 (dd, *J* = 12.9 Hz, 4.5 Hz, 1H), 4.43 (ddd, *J* = 11.4 Hz, 5.1 Hz, 1.8 Hz, 1H), 4.52 (dd, *J* = 11.4 Hz, 2.1 Hz, 1H), 4.63–4.73 (m, 1H), 5.10 (s, 2H), 6.96 (d, *J* = 8.4 Hz, 2H), 7.27–7.48 (m, 7H), 7.84 (s, 1H); MS (ESI) m/e 381 $[M + H]^+$; HRMS m/e calcd. for $C_{22}H_{20}N_4O_4$ $[M + H^+]$ 381.1563, found 381.1551.

(*S*)-*N*-(4-(4-fluorobenzyloxy)benzyl)-6,7-dihydro-2-nitro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (28)

4-(4-Fluorobenzyloxy)benzaldehyde (0.22 g, 0.94 mmol) was added to a stirred solution of **23** (0.47 mmol) in DMF (5 mL), followed by addition of glacial acetic acid (54 μL, 0.94 mmol) at rt. The reaction was stirred at rt for 30 min. $NaBH_3CN$ (88 mg, 1.41 mmol) was added to the reaction mixture, and it was further stirred at rt for 18 h. The reaction was quenched with MeOH (0.5 mL). The solvent was removed under reduced pressure. Saturated aqueous NH_4Cl solution (50 mL) was added to the residue. The mixture was extracted with CH_2Cl_2 (3 × 30 mL). The organic extracts were combined, washed with brine (100 mL), and dried over $MgSO_4$. The solvent was then removed under reduced pressure. The residue was purified via preparative TLC (5% MeOH in CH_2Cl_2) to give **28** as a yellow solid (0.14 g, 77%). Mp = 169–170°C; ^{1}H NMR (DMSO-d_6) δ 3.14–3.20 (m, 1 H), 3.70 (s, 2 H), 3.97 (dd, *J* = 12.6, 3.6 Hz, 1 H), 4.13 (dd, *J* = 12.6, 3.8 Hz, 1 H), 4.32–4.42 (m, 2 H), 5.05 (s, 2 H), 6.92–7.50 (m, 8 H), 8.00 (s, 1 H); ^{13}C NMR (acetone-d_6) δ 47.4, 47.9, 50.0, 68.9, 69.4, 114.7, 115.0, 115.3, 116.6, 129.3, 129.6, 129.7, 132.8, 148.6, 158.8, 164.9. HRMS m/e calcd. for $C_{20}H_{20}FN_4O_4$ $[M + H^+]$ 399.1469, found 399.1471.

(4-Trifluoromethoxy-phenyl)-acetaldehyde (30)

2-[4-(Trifluoromethoxy)phenyl]ethanol (**29**, 1.00 g, 4.9 mmol) was dissolved in methylene chloride (100 mL) and treated sequentially with solid $NaHCO_3$ (4.07 g, 48.5 mmol) and Dess-Martin periodinane (4.11 g, 9.70 mmol) at 0°C, and the resulting solution was further stirred for 3 h. The reaction mixture was filtered to remove salts and washed with saturated

$NaHCO_3$ (aq) solution (2 × 30 mL) and brine (30 mL). The organic layers were dried ($MgSO_4$), filtered and concentrated to give crude **30** as a viscous oil (0.80 g, 82%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.74 (d, *J* = 2.1 Hz, 2H), 7.22–7.29 (m, 4H), 9.78–9.79 (m, 1H); GC-MS (EI) *m/e* 204 [M^+], 175 [M^+ - CHO].

(*S*)-2-nitro-N-(4-(trifluoromethoxy)phenethyl)-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (31)

To a stirred solution of **23** (0.050 g, 0.27 mmol) and **30** (0.083 g, 0.41 mmol) in DMF (5 mL) was added $NaBH_3CN$ (26 mg, 0.41 mmol). To this mixture AcOH was added to reach pH 5 at 0°C. The reaction mixture was warmed to rt and further stirred for 4 h. The solvent was removed, and then the saturated $NaHCO_3$ (aq) solution (10 mL). The mixture was extracted with methylene chloride (2 × 10 mL). The organic layers were washed with brine (15 mL), dried ($MgSO_4$), and evaporated. The crude residue was purified by preparative TLC (CH_2Cl_2:MeOH, 20:1) to give **31** as a white solid (56mg, 58%). $[\alpha]_D^{20} = -5.62$ (*c*, 0.87, $CHCl_3$); Mp = 54.3–55.6°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.79 (t, *J* = 6.6 Hz, 2H), 2.93–3.03 (m, 2H), 3.40–3.42 (m, 1H), 3.89 (dd, *J* = 12.0, 3.9 Hz, 1H), 4.14 (dd, *J* = 12.6, 4.5 Hz, 1H), 4.32–4.44 (m, 2H), 7.06–7.14 (m, 2H), 7.19–7.22 (m, 2H), 7.37 (s, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 35.9, 47.9, 48.5, 48.8, 69.5, 115.3, 118.9, 121.3, 122.3, 130.1, 138.1, 143.8, 147.5, 148.2; HRMS (ESMS) calcd for $C_{15}H_{16}N_4O_4F_3$ [M + H^+] 373.1124, found 373.1140.

3-(4-Trifluoromethoxy-phenyl)-acrylic acid methyl ester (33)

To a stirred solution of **32** (0.50 g, 1.74 mmol) in anhydrous MeCN (10 mL) was added methyl acrylate (0.23 mL, 2.60 mmol), $Pd(OAc)_2$ (7.80 mg, 0.035 mol), triphenylphosphine (11.0 mg, 0.042 mmol) and triethylamine (0.49 mL, 3.48 mmol) at room temperature. The resulting mixture was heated to 100°C and further stirred for 3 h. The reaction mixture was filtered and the solvent was removed. The crude residue was purified by column chromatography (hexane:EtOAc, 30:1) to give **33** as a white solid (0.40 g, 93%): Mp = 46.3–47.2°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.92 (s, 3H), 6.49 (d, *J* = 15.9 Hz, 1H), 7.32–7.35 (m, 2H), 7.64–7.67 (m, 2H), 7.75 (d, *J* = 15.9 Hz, 1H); GC-MS (EI) *m/e* 246 [M^+], 215 [M^+ - OCH_3], 187 [M^+ - OCH_2CH_3].

3-(4-Trifluoromethoxy-phenyl)-prop-2-en-1-ol (34)

To a stirred solution of **33** (0.28 g, 1.14 mmol) in methylene chloride (5 mL) was added DIBAL-H (1M solution in methylene chloride, 5.68 mL, 5.68 mmol) at −78°C and the resulting mixture was further stirred for an hour. 1 M HCl (15 mL) was added slowly at room temperature and the aqueous layer was extracted with methylene chloride (2 × 15 mL). The organic layer was washed with brine (15 mL), dried ($MgSO_4$) and evaporated. The crude residue was purified by column chromatography (hexane:EtOAc, 5:1) to give **34** as a colorless oil (0.21 g, 84%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23 (br, s, 1H), 4.32–4.35 (m, 2H), 6.29 (dt, *J* = 16.2, 5.4 Hz, 1H), 6.58 (d, *J* = 16.2 Hz, 1H), 7.15–7.18 (m, 2H), 7.38–7.41 (m, 2H); GC-MS (EI) *m/e* 218 [M^+], 133 [M^+ - OCF_3].

3-(4-Trifluoromethoxy-phenyl)-propan-1-ol (35)

A mixture of **34** (0.24 g, 1.10 mmol) and palladium on activated carbon (0.030 g) was stirred under a H_2 atmosphere (balloon) at room temperature for 4h. The reaction mixture was filtered and the solvent was removed to give **35** as a colorless oil (0.21g, 88%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.83–1.93 (m, 2H), 2.69 (t, *J* = 7.8 Hz, 2H), 3.65 (t, *J* = 6.6 Hz, 2H), 7.11–7.14 (m, 2H), 7.19–7.22 (m, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 31.5, 34.3, 62.1, 119.1, 121.1, 122.4, 129.8, 140.8, 147.6; GC-MS (EI) *m/e* 220 [M^+], 135 [M^+ - OCF_3].

3-(4-Trifluoromethoxy-phenyl)-propionaldehyde (36)

35 (0.21 g, 0.95 mmol) was dissolved in methylene chloride (10 mL) and treated sequentially with solid $NaHCO_3$ (0.80 g, 9.54 mmol) and Dess-Martin periodinane (0.81 g, 1.91 mmol) at 0 °C and the resulting solution was further stirred for 3 h. The reaction mixture was filtered to remove salt and washed with saturated $NaHCO_3$ solution (2 × 30 mL) and brine (30 mL). The organic layer was dried ($MgSO_4$), filtered and evaporated to give crude **36** as a colorless, viscous oil (0.18 g, 86%). **36** was used for the next reaction without further purification. ^1H NMR (300 MHz, $CDCl_3$) δ 2.76–2.81 (m, 2H), 2.94 (t, *J* = 7.5 Hz, 2H), 7.12–7.15 (m, 2H), 7.19–7.23 (m, 2H), 9.81–9.82 (m, 1H); GC-MS (EI) *m/e* 218 [M^+], 175 [M^+ - CH_2CHO], 135 [M^+ - OCF_3].

(*S*)-2-Nitro-*N*-(3-(4-(trifluoromethoxy)phenyl)propyl)-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3] oxazin-6-amine (37)

To a stirred solution of **23** (0.10 g, 0.54 mmol) and **36** (0.15 g, 0.71 mmol) in DMF (10 mL) was added $NaBH_3CN$ (51 mg, 0.82 mmol) and AcOH was added to reach pH 5 at 0°C. The reaction temperature was raised room temperature and the reaction mixture was further stirred for 4 h. The solvent was removed, saturated $NaHCO_3$ solution (20 mL) was added and the mixture was extracted with methylene chloride (2 × 20 mL). The organic layer was washed with brine (15 mL), dried ($MgSO_4$) and evaporated. The crude residue was purified by column chromatography (CH_2Cl_2:MeOH, 50:1) to give **37** as a pale yellow solid (83 mg, 40%): $[\alpha]_D^{20} = -5.63$ (*c*, 0.75, $CHCl_3$); Mp = 101.7–103.1°C; ^1H NMR (300 MHz, $CDCl_3$) δ 1.76–1.85 (m, 2H), 2.65–2.79 (m, 4H), 3.32–3.76 (m, 1H), 3.86–3.92 (m, 1H), 4.14 (dd, *J* = 12.3 Hz, 4.2 Hz, 1H), 4.29–4.35 (m, 1H), 4.39 (dd, *J* = 11.7, 2.4 Hz, 1H), 7.10–7.18 (m, 4H), 7.40 (s, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 31.6, 32.6, 46.5, 48.0, 48.8, 69.6, 115.4, 118.9, 121.2, 122.4, 129.7, 140.4, 144.0, 147.5, 147.6; HRMS (ESMS) calcd for $C_{16}H_{18}N_4O_4F_3$ [M + H^+] 387.1280, found 387.1280.

4-(4-Trifluoromethoxy-phenyl)-but-2-enoic acid ethyl ester (38)

To a stirred suspension of LiCl (0.062 g, 1.47 mmol) in dry MeCN (4 mL) was added sequentially triethyl phosphonoacetate (0.29 mL, 1.47 mmol), DBU (0.18 mL, 1.22 mmol) and **30** (0.25 g, 1.22 mmol). The reaction mixture was stirred at room temperature for 3 h. The solvent was removed, 1M HCl solution (15 mL) was added, and then the mixture was extracted with EtOAc (2 × 15 mL). The organic layer was washed with brine (15 mL), dried ($MgSO_4$), filtered and evaporated. The resulting residue was purified by column (hexane:EtOAc, 30:1) to give **38** as a white solid (0.24 g, 73%): Mp = 39.8–41.2°C; ^1H NMR (300 MHz, $CDCl_3$) δ1.26 (t, *J* = 7.2 Hz, 3H), 3.23 (d, *J* = 7.8 Hz, 2H), 4.14 (q, *J* = 7.2 Hz, 2H), 6.23–6.34 (m, 1H), 6.45 (d, *J* = 15.9 Hz, 1H), 7.14–7.19 (m, 2H), 7.36–7.40 (m, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 14.41, 38.56, 61.07, 118.98, 121.25, 122.39, 123.25, 127.72, 130.34, 132.13, 135.88, 171.57; HRMS (ESMS) calcd for $C_{13}H_{14}O_3F_3$ [M + H^+] 275.0895, found 275.0900.

4-(4-Trifluoromethoxy-phenyl)-butyric acid ethyl ester (39)

A mixture of **38** (0.21 g, 0.77 mmol) and palladium on activated carbon (0.040 g) was stirred under a H_2 atmosphere (balloon) at room temperature for 3 h. The reaction mixture was filtered and the solvent was removed to give **39** as a colorless oil (0.21 g, 100%): ^1H NMR (300 MHz, $CDCl_3$) δ 1.23 (t, *J* = 7.5 Hz, 3H), 1.89 – 1.99 (m, 2H), 2.29 (t, *J* = 7.5 Hz, 2H), 2.63 (t, *J* = 8.1 Hz, 2H), 4.09 (q, *J* = 7.5 Hz, 2H), 7.11–7.14 (m, 2H), 7.18–7.25 (m, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 14.29, 26.50, 33.60, 33.49, 60.43, 118.87, 121.04, 122.27, 129.77, 140.25, 147.57, 173.39; HRMS (ESMS) calcd for $C_{13}H_{16}O_3F_3$ [M + H^+] 277.1052, found 277.1067.

4-(4-Trifluoromethoxy-phenyl)-butyraldehyde (40)

To a stirred solution of **39** (0.20 g, 0.72 mmol) in methylene chloride (3 mL) was added DIBAL-H (1M solution in methylene chloride, 1.09 mL, 1.09 mmol) at −78°C and the resulting mixture was further stirred for 30 min. 1M HCl (15 mL) was added slowly at room temperature and the aqueous layer was extracted with methylene chloride (2 × 10 mL). The organic layer was washed with brine (15 mL), dried ($MgSO_4$) and evaporated to give crude **40** as a colorless oil (0.13 g, 76%). This material was used for the next reaction without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.90–2.00 (m, 2H), 2.44 (td, *J* = 7.2, 1.5 Hz, 2H), 2.63 (t, *J* = 7.8 Hz, 2H), 7.11–7.20 (m, 4H), 9.77–9.78 (m, 1H).

(*S*)-2-nitro-*N*-(4-(4-(trifluoromethoxy)phenyl)butyl)-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3] oxazin-6-amine (41)

To a stirred solution of **23** (0.050 g, 0.27 mmol) and **40** (96 mg, 0.41 mmol) in DMF (5 mL) was added $NaBH_3CN$ (26 mg, 0.41 mmol). AcOH was added to reach pH 5 at 0°C. The reaction temperature was warmed to rt and the reaction mixture was further stirred for 4 h. The solvent was removed, saturated $NaHCO_3$ solution (20 mL) was added and the mixture was extracted with methylene chloride (2 × 20 mL). The organic layer was washed with brine (15 mL), dried ($MgSO_4$) and evaporated. The crude residue was purified by column chromatography (CH_2Cl_2:MeOH, 50:1) to give **41** as a pale yellow oil (48 mg, 48%): $[\alpha]_D^{20} = -5.67$ (*c*, 0.89, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.46–1.70 (m, 4H), 2.59–2.77 (m, 4H), 3.32–3.38 (m, 1H), 3.87 (ddd, *J* = 12.6, 4.5, 1.5 Hz, 1H), 4.14 (dd, *J* = 12.6, 4.5 Hz, 1H), 4.28 (ddd, *J* = 11.4, 5.1, 1.5 Hz, 1H), 4.39 (dd, *J* = 11.4, 2.7 Hz, 1H), 7.10–7.18 (m, 4H), 7.40 (s, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 28.7, 29.7, 34.9, 47.0, 47.9, 48.7, 69.3, 115.0, 119.1, 120.9, 121.1, 129.5, 140.7, 143.9, 147.3, 147.4; HRMS (ESMS) calcd for $C_{17}H_{20}N_4O_4F_3$ [M + H$^+$] 401.1437, found 401.1447.

3-(4-Trifluoromethoxy-phenyl)-propenal (42)

34 (0.50 g, 2.29 mmol) was dissolved in methylene chloride (15 mL) and treated sequentially with solid $NaHCO_3$ (1.93 g, 0.023 mol) and Dess-Martin periodinane (2.92 g, 6.87 mmol) at 0°C. The resulting solution was allowed to stir for 3 h. The reaction mixture was filtered and washed with saturated $NaHCO_3$ (2 × 30 mL) and brine (30 mL). The organic layer was dried ($MgSO_4$), filtered and evaporated. The crude residue was purified by column chromatography (hexane:EtOAc, 30:1) to give **42** as a white solid which melted at rt (0.43 g, 88%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.63 (dd, *J* = 15.8, 7.4 Hz, 1H), 7.26–7.30 (m, 2H), 7.42 (d, *J* = 15.8 Hz, 1H), 7.5–7.63 (m, 2H), 9.70 (d, *J* = 7.4 Hz, 1H); GC-MS (EI) *m/e* 216 [M$^+$], 131 [M$^+$ - OCF_3].

5-(4-Trifluoromethoxy-phenyl)-penta-2,4-dienoic acid ethyl ester (43)

To a stirred suspension of LiCl (0.094 g, 2.22 mmol) in dry MeCN (5 mL) was added sequentially triethyl phosphonoacetate (0.44 mL, 2.22 mmol), DBU (0.28 mL, 1.85 mmol) and **42** (0.40 g, 1.85 mmol). The reaction mixture was allowed to stir at rt for 3 h. The solvent was removed, 1M HCl solution (15 mL) was added, and then the mixture was extracted with EtOAc (2 × 15 mL). The organic layer was washed with brine (15 mL), dried ($MgSO_4$), filtered and evaporated. The resulting residue was purified by silica filter (hexane:EtOAc, 30:1) to give **43** as a pale yellow solid (0.34 g, 64%): Mp = 40.7–41.7°C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.28 (t, *J* = 7.4 Hz, 3H), 4.18 (q, *J* = 7.4 Hz, 2H), 5.97 (d, *J* = 15.2 Hz, 1H), 6.83–6.86 (m, 1H), 7.18 (d, *J* = 8.2 Hz, 1H), 7.36–7.50 (m, 1H); GC-MS (EI) *m/e* 286 [M$^+$], 241 [M$^+$ - OCH_2CH_3], 213 [M$^+$ - COOEt].

5-(4-Trifluoromethoxy-phenyl)-pentanoic acid ethyl ester (44)

A mixture of **43** (0.32 g, 1.12 mmol) and palladium on activated carbon (10%, 0.040 g) was stirred under a H_2 atmosphere (balloon) at rt for 4 h. The reaction mixture was filtered and the solvent was removed to give **44** as a colorless oil (0.30 g, 94%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22 (t, *J* = 7.2 Hz, 3H), 1.64–1.67 (m, 4H), 2.29 (t, *J* = 6.9 Hz, 2H), 2.61 (t, *J* = 6.9 Hz, 2H), 4.09 (q, *J* = 7.2 Hz, 2H), 7.09–7.26 (m, 4H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 14.3, 24.5, 30.8, 34.1, 34.9, 60.3, 118.9, 121.0, 122.3, 129.6, 140.9, 147.4, 173.6; HRMS (ESMS) calcd for $C_{14}H_{18}O_3F_3$ [M + H^+] 291.1208, found 291.1222.

5-(4-Trifluoromethoxy-phenyl)-pentanal (45)

To a stirred solution of **44** (0.28 g, 0.97 mmol) in methylene chloride (5 mL) was added DIBAL-H (1M solution in methylene chloride, 1.45 mL, 1.45 mmol) at −78°C. The reaction mixture was allowed to stir for 1 h. 1 M HCl (15 mL) was added slowly at room temperature and the aqueous layer was extracted with methylene chloride (2 × 15 mL). The organic layer was washed with brine (15 mL), dried ($MgSO_4$) and evaporated. The crude residue was purified by column chromatography (hexane:EtOAc, 20:1) to give **45** as a viscous oil (0.20 g, 83%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.63–1.68 (m, 4H), 2.43–2.49 (m, 2H), 2.61–2.67 (m, 2H), 7.10–7.19 (m, 4H), 9.75 (t, *J* = 1.8 Hz, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 21.6, 30.8, 35.0, 43.7, 118.9, 121.0, 122.3, 129.6, 140.7, 147.5, 202.4; HRMS (ESMS) calcd for $C_{17}H_{13}NO_3Cl_2$ [M + H^+] 349.0272, found 349.0278.

(*S*)-2-Nitro-*N*-(5-(4-(trifluoromethoxy)phenyl)pentyl)-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3] oxazin-6-amine (46)

To a stirred solution of **23** (0.050 g, 0.27 mmol) and **45** (100 mg, 0.41 mmol) in DMF (5 mL) was added $NaBH_3CN$ (26 mg, 0.41 mmol). AcOH was added to reach pH 5 at 0°C. The reaction mixture was warmed to rt and stirring continued for 4 h. The solvent was removed, saturated $NaHCO_3$ solution (20 mL) was added and the mixture was extracted with methylene chloride (2 × 20 mL). The organic layer was washed with brine (15 mL), dried ($MgSO_4$) and evaporated. The crude residue was purified by preparative TLC (CH_2Cl_2:MeOH, 20:1) to give **46** as a pale yellow, viscous oil (61 mg, 55%): $[\alpha]_D^{20}$ = −5.65 (*c*, 0.93, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32–1.40 (m, 2H), 1.46–1.66 (m, 4H), 2.58–2.75 (m, 4H), 3.33–3.39 (m, 1H), 3.87 (dd, *J* = 12.0, 4.8 Hz, 1H), 4.14 (dd, *J* = 12.0, 4.5 Hz, 1H), 4.29 (dd, *J* = 11.1, 4.8 Hz, 1H), 4.40 (dd, *J* = 11.1, 2.4 Hz, 1H), 7.10–7.18 (m, 4H), 7.40 (s, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 26.8, 30.1, 31.3, 35.3, 47.3, 48.0, 48.8, 69.6, 115.4, 119.0, 121.0, 122.4, 129.7, 141.3, 143.8, 147.5, 147.6; HRMS (ESMS) calcd for $C_{18}H_{22}N_4O_4F_3$ [M + H^+] 415.1593, found 415.1607.

4-(4-Trifluoromethoxy-benzyloxy)-benzaldehyde (48)

NaH (60% dispersion in mineral oil, 0.20 g, 5.32 mmol) was added to stirred solution of 4-hydroxybenzaldehyde (**47,** 0.50 g, 4.09 mmol) and 4-(trifluoromethoxy)benzyl bromide (1.57 g, 6.14 mmol) in DMF (15 mL) at 0°C. The resulting mixture was further stirred for 1.5 h. The reaction mixture was quenched with 1M HCl (5 mL) and the solvent was removed. The resulting oil was dissolved with EtOAc (30 mL) and the organic layer was washed with 1M HCl (20 mL) and brine (20mL). The organic phase was dried ($MgSO_4$) and evaporated to give crude **48** as a viscous oil (1.2 g, 100%). This material was used for the next reaction without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.14 (s, 2H), 7.06–7.09 (m, 2H), 7.24–7.27 (m, 2H), 7.44–7.48 (m, 2H), 7.83–7.87 (m, 2H), 9.89 (s, 1H); GC-MS (EI) *m/e* 296 [M^+], 175 [M^+ - OPhCHO].

(*S*)-2-Nitro-*N*-(4-(4-(trifluoromethoxy)benzyloxy)benzyl)-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (49)

To a stirred solution of **23** (0.025 g, 0.13 mmol) and **48** (0.060 g, 0.20 mmol) in DMF (5 mL) was added $NaBH_3CN$ (13 mg, 0.20 mmol). AcOH was added to reach pH 5 at 0°C. The reaction temperature was warmed to rt and the reaction mixture was allowed to stir for 4 h. The solvent was removed, saturated $NaHCO_3$ solution (15 mL) was added and the mixture was extracted with methylene chloride (2 × 15 mL). The organic layer was washed with brine (15 mL), dried ($MgSO_4$) and evaporated. The crude residue was purified by preparative TLC (CH_2Cl_2:MeOH, 40:1) to give **49** as a white solid (42 mg, 67%): $[\alpha]_D^{20} = 7.22$ (*c*, 0.82, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.38–3.41 (m, 1H), 3.81 (d, *J* = 13.2 Hz, 1H), 3.87 (d, *J* = 13.2 Hz, 1H), 3.89–3.94 (m, 1H), 4.10 (dd, *J* = 12.3, 4.5 Hz, 1H), 4.31–4.37 (m, 1H), 4.39 (dd, *J* = 11.7, 2.4 Hz, 1H), 5.05 (s, 2H), 6.91–6.95 (m, 2H), 7.21–7.24 (m, 2H), 7.35 (s, 1H), 7.45–7.48 (m, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 47.9, 48.0, 50.6, 69.3, 69.6, 115.2, 115.4, 119.2, 121.3, 122.5, 129.0, 129.4, 130.7, 131.7, 135.8, 143.6, 147.5, 148.9, 158.2; HRMS (ESMS) calcd for $C_{21}H_{20}N_4O_5F_3$ [M + H^+] 465.1386, found 465.1391.

M. tuberculosis MIC Assay

The inhibitors were dissolved in DMSO to make stock solutions of 50 μmol/mL. Inhibitors were diluted into 7H9-based medium (Middlebrook 7H9 broth (Difco) supplemented with 0.05% Tween 80, 0.2% glycerol and albumin/NaCl/glucose (ADC) complex) and 100 μL added to the first row of wells of a 96-well plate containing 50 μL / well 7H9-based medium in all other rows. After pipette mixing and using a multi-channel pipette, 50 μL was removed from each well in the first row and added to the second row. 2-Fold dilution in this manner was carried out to give 11 dilutions of each inhibitor (200 μM-0.19 μM). A stock culture of *M. tuberculosis* H37Rv (ATCC 27294) was grown to OD_{650nm} 0.2 in 7H9-based medium. The culture was diluted 1:1000 in 7H9-based medium before aliquoting 50 μL into each well of the 96-well plate containing the drug dilutions. The plates were incubated for 2 weeks at 37° C and the MIC_{99} values were read macroscopically using an inverted plate reader. Each measurement was made three independent times.

M. tuberculosis MAC Assay

The minimum anaerobic cidal activity of the inhibitors was measured using anaerobically adapted cultures of Mtb H37Rv.[30] These were generated by culturing cells from an initial OD_{650nm} of 0.005 in glycerol-free Dubos-based broth medium in 19.5 × 145 mm glass tubes at a head-space ratio of 0.5 with a magnetic stirrer that were sealed with Teflon-lined caps and subsequently with paraplast. In these cultures, methylene blue, an indicator of dissolved oxygen, decolorizes after approximately 17 days incubation. After three weeks incubation at 37°C on a magnetic stirrer, the tubes were opened in a Vinyl anaerobic chamber (Coy Laboratories, Michigan) fitted with a Coy Model 10 gas analyzer and a vacuum air lock chamber and maintained under 90% nitrogen and 10% hydrogen. Culture was transferred (100 μL / well) to 96-well microtiter plates containing inhibitor in DMSO giving a final DMSO concentration of 2%. Isoniazid and metronidazole were used as negative and positive control drugs, respectively. The plates were incubated at 37°C in Type A Bio-bag anaerobic chamber (Becton Dickinson, Maryland) along with an oxygen indicator strip for 7 days. The cells were washed three times with fresh Dubos broth and serially-diluted by two-fold dilutions. 5 μL of cell suspension from each well was spotted in a single well of a 7H11 Middlebrook agar-containing 96-well plate. After 3 weeks of incubation at 37°C, the minimal anaerobicidal concentration (MAC) was read as the concentration of drug that caused a 90% reduction in visible growth. The MAC values were corroborated by determining the number of surviving bacterial by colony forming unit enumeration. For this, 1mL volumes of 3-week anaerobically adapted Mtb culture were treated with various concentrations of the compounds in 24-well

plates whereas control cultures were treated with an equivalent amount of DMSO solvent. Isoniazid (73 μM) and metronidazole (50 and 100 μM) were always included as negative and positive controls, respectively. The plates were sealed in anaerobic bags and incubated for 7 days at 37°C. Serial dilutions were subsequently plated in triplicate on 7H11 Middlebrook agar to monitor bacterial survival.

Nitroimidazole reduction assay

F_{420} was purified from *Mycobacterium smegmatis* as described earlier.[31] Reduced F_{420} (H_2F_{420}) was prepared using a recombinant Mtb F_{420} dependent glucose-6-phosphate dehydrogenase as described,[32] followed by heat inactivation of FGD1 at 65°C water bath for 10 minutes and ultra-filtration (Centricon 10 kDa). Reduction of nitroimidazoles by Rv3547 was monitored by oxidation of H_2F_{420} by florescence (Ex_{400} nm / Em_{470} nm) using Infinite M1000 spectrophotometer at 25°C. The reaction was carried out in buffer containing 200 mM Tris (pH 8.0), 0.01% TritonX-100 and 20 RM H_2F_{420} in 30 μl volume in a 96-well plate. Initial rates were calculated based on the florescence standard curve with purified F_{420}. Apparent steady-state kinetic parameters, K_m and V_{max}, were obtained by fitting initial rate data to the Michaelis-Menton equation.

QSAR modeling

Modeling was performed using Discovery Studios release 2.0 (Accelrys Software, Inc). The following molecules were used in the training set: **9, 10, 11, 13, 16, 18, 24, 26, 27, 31, 41, 49** (from this work); **3, 11, 12, 15, 20, 35** (reference [7]); the 7(*S*)-methyl derivative of PA-824 we have described previously[33]; PA-824 and PA-1147 (*i.e.*, 6(*R*)-PA-824).[29] The following molecules were used in the test set: **17, 19, 23, 25, 28, 37, 46** (from this work); **10a, 10b, 14, 16, 17, 27a, 27b, 30, 34** (reference [7]); the 7(*R*)-methyl derivative of PA-824 we have described previously[33] and PA-1282, PA-1298, PA-1343, and PA-653.[29] For both sets, conformer generation was performed using the CAESAR algorithm[34] and a maximum of 255 conformers was considered for each molecule. Catalyst HypoGen was run using four potential pharmacophore features (hydrogen-bond acceptor, donor, hydrophobe and hydrophobe-aromatic) with a minimum interfeature distance of 2.0 Å. Variable tolerances were allowed during pharmacophore elaboration and significance was assessed based upon a cost analysis (all ten of the reported hypotheses cost 40–60 bits less than the cost of the null hypothesis which was 70 bits higher than the fixed cost, therefore these hypotheses are considered statistically significant) and based upon scrambling the activity data for the same training set structures and assessing correlation (Fisher Test).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Matteelli A, Migliori GB, Cirillo D, Centis R, Girard E, Raviglion M. Multidrug-resistant and extensively drug-resistant Mycobacterium tuberculosis: epidemiology and control. Expert Rev Anti Infect Ther 2007;5(5):857–871. [PubMed: 17914919]

2. http://www.stoptb.org/wg/new_drugs/assets/documents/2007GlobalPipeline.pdf

3. Nagarajan K, Shankar RG, Rajappa S, Shenoy SJ, Costa-Pereira R. Nitroimidazoles XXI 2,3-dihydro-6-nitroimidazo [2,1-b] oxazoles with antitubercular activity. European Journal of Medicinal Chemistry 1989;24(6):631–633.
4. Stover CK, Warrener P, VanDevanter DR, Sherman DR, Arain TM, Langhorne MH, Anderson SW, Towell JA, Yuan Y, McMurray DN, Kreiswirth BN, Barry CE, Baker WR. A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. Nature 2000;405(6789):962–966. [PubMed: 10879539]
5. Matsumoto M, Hashizume H, Tomishige T, Kawasaki M, Tsubouchi H, Sasaki H, Shimokawa Y, Komatsu M. OPC-67683, a nitro-dihydro-imidazooxazole derivative with promising action against tuberculosis in vitro and in mice. PLoS Med 2006;3(11):2131–2144.
6. Singh R, Manjunatha U, Boshoff H, Ha Y, Niyomrattanakit P, Ledwidge R, Dowd C, Lee I, Kim P, Zhang L, Kang S, Keller T, Jiricek J, Barry C. PA-824 Kills Nonreplicating *Mycobacterium tuberculosis* by intracellular NO release. Science 2008;322:1392–1395. [PubMed: 19039139]
7. Kim P, Zhang L, Manjunatha U, Singh R, Patel S, Jiricek J, Keller T, Boshoff H, Barry C, Dowd C. Structure-activity relationships of antitubercular nitroimidazoles. I. Structural features associated with aerobic and anaerobic activities of 4- and 5-nitroimidazoles. J Med Chem. 2008(submitted)
8. Li HY, Wang Y, Heap CR, King CH, Mundla SR, Voss M, Clawson DK, Yan L, Campbell RM, Anderson BD, Wagner JR, Britt K, Lu KX, McMillen WT, Yingling JM. Dihydropyrrolopyrazole transforming growth factor-beta type I receptor kinase domain inhibitors: a novel benzimidazole series with selectivity versus transforming growth factor-beta type II receptor kinase and mixed lineage kinase-7. J Med Chem 2006;49(6):2138–2142. [PubMed: 16539403]
9. Sessler JL, Magda D, Furuta H. Synthesis and binding properties of monomeric and dimeric guanine and cytosine amine derivatives. J. Org. Chem 1992;57(3):818–826.
10. Broutin PE, Colobert F. Efficient stereochemical controllers in biaryl Suzuki coupling reactions: benzylic carbinols bearing in beta-position thioether, dimethylamino, or sulfoxide groups. Org Lett 2005;7(17):3737–3740. [PubMed: 16092863]
11. Dreier A, Haller R. Optically active tetrahydrodiazixanopurines. Archiv der Pharmazie 1987;320(10): 1004–1009.
12. Smid P, Coolen HK, Keizer HG, van Hes R, de Moes JP, den Hartog AP, Stork B, Plekkenpol RH, Niemann LC, Stroomer CN, Tulp MT, van Stuivenberg HH, McCreary AC, Hesselink MB, Herremans AH, Kruse CG. Synthesis, structure-activity relationships, and biological properties of 1-heteroaryl-4-[omega-(1H-indol-3-yl)alkyl]piperazines, novel potential antipsychotics combining potent dopamine D2 receptor antagonism with potent serotonin reuptake inhibition. J Med Chem 2005;48(22):6855–6869. [PubMed: 16250644]
13. Ohyabu N, Nishikawa T, Isobe M. First asymmetric total synthesis of tetrodotoxin. J Am Chem Soc 2003;125(29):8798–8805. [PubMed: 12862474]
14. Hay MP, Lee HH, Wilson WR, Roberts PB, Denny WA. Hypoxia-Selective Antitumor Agents. 10. Bis(nitroimidazoles) and Related Bis(nitroheterocycles): Development of Derivatives with Higher Rates of Metabolic Activation under Hypoxia and Improved Aqueous Solubility. J Med Chem 1995;38(11):1928–1941. [PubMed: 7783125]
15. Ulgar V, Lopez O, Maya I, Fernandez-Bolanos JG, Bols M. Synthesis of furan 4′-thio-C-nucleosides, their methylsulfonium and sulfoxide derivatives. Evaluation as glycosidase inhibitors. Tetrahedron 2003;59(16):2801–2809.
16. Kumagai T, Tamai S, Abe T, Matsunaga H, Hayashi K, Kishi I, Shiro M, Nagao Y. New straightforward synthesis and characterization of a unique 1 beta-methylcarbapenem antibiotic biapenem bearing a sigma-symmetric bicyclotriazoliumthio group as the pendant moiety. Journal of Organic Chemistry 1998;63(23):8145–8149.
17. Padayatti PS, Sheri A, Totir MA, Helfand MS, Carey MP, Anderson VE, Carey PR, Bethel CR, Bonomo RA, Buynak JD, van den Akker F. Rational design of a beta-lactamase inhibitor achieved via stabilization of the trans-enamine intermediate: 1.28 A crystal structure of wt SHV-1 complex with a penam sulfone. J Am Chem Soc 2006;128(40):13235–13242. [PubMed: 17017804]
18. Baker, WRS.; C; Keeler, EL. Nitroimidazole antibacterial compounds and methods of use thereof. US Patent 5,668,127. 1997.

19. Chen JH, Forsyth CJ. Total synthesis of apratoxin A. Journal of the American Chemical Society 2003;125(29):8734–8735. [PubMed: 12862462]
20. Evans DA, Aye Y, Wu J. Asymmetric, anti-selective scandium-catalyzed Sakurai additions to glyoxyamide. Applications to the syntheses of N-Boc D-alloisoleucine and D-isoleucine. Organic Letters 2006;8(10):2071–2073. [PubMed: 16671784]
21. Bayley H, Standring DN, Knowles JR. Propane-1,3-Dithiol – Selective Reagent for Efficient Reduction of Alkyl and Aryl Azides to Amines. Tetrahedron Letters 1978;(39):3633–3634.
22. Lau J, Behrens C, Sidelmann UG, Knudsen LB, Lundt B, Sams C, Ynddal L, Brand CL, Pridal L, Ling A, Kiel D, Plewe M, Shi SU, Madsen P. New beta-alanine derivatives are orally available glucagon receptor antagonists. Journal of Medicinal Chemistry 2007;50(1):113–128. [PubMed: 17201415]
23. Hoelderich W, Goetz N, Recher HG, Smuda H. Method for preparation of phenylacetaldehydes as insecticide intermediates. European Patent Application 282902. 1988(282902)
24. Karig G, Moon MT, Thasana N, Gallagher T. C–H activation and palladium migration within biaryls under Heck reaction conditions. Organic Letters 2002;4(18):3115–3118. [PubMed: 12201730]
25. Kim, CY.; Mahaney, PE.; Trybulski, EJ.; Zhang, P.; Terefenko, EA.; Mccomas, CC.; Marella, MA.; Coghlan, RD.; Heffernan, GD.; Cohn, ST.; Vu, AT.; Sabatucci, JP.; Ye, F. Preparation of nitrogen-heterocycle-containing phenylaminopropanol derivatives and methods of their use to prevent and treat conditions ameliorated by monoamine reuptake. U.S. Pat. Appl. Publ. 2005222148. 2005.
26. Taber DF, Storck PH. Synthesis of (−)-tetrodotoxin: Preparation of an advanced cyclohexenone intermediate. Journal of Organic Chemistry 2003;68(20):7768–7771. [PubMed: 14510553]
27. Oida S, Tanaka T, Tajima W, Kounosu T, Somaki A, Miyaoka T, Yasuda H. Triazole derivatives as antimycotic agents. Jpn. Kokai Tokkyo Koho 10158167. 1998
28. Oida S, Tajima K, Yasuda H. Preparation of 2-alkenyl-5-(1,2,4-triazol-1-yl)methyl-1,3-oxathiolane derivatives as fungicides for humans and animals. Jpn. Kokai Tokkyo Koho 08333367. 1996
29. Baker, WRS.; C; Keeler, EL. Nitro-[2,1-b]imidazopyran compounds and antibacterial uses thereof. US Patent 6,087,358. 2000.
30. Wayne, LG. Mycobacterium tuberculosis Protocols. Parish, T.; Stoker, NG., editors. New Jersey: Humana Press; 2001. p. 247-270.
31. Isabelle D, Simpson DR, Daniels L. Large-scale production of coenzyme F-420-5,6 by using Mycobacterium smegmatis. Applied and Environmental Microbiology 2002;68(11):5750–5755. [PubMed: 12406775]
32. Manjunatha UH, Boshoff H, Dowd CS, Zhang L, Albert TJ, Norton JE, Daniels L, Dick T, Pang SS, Barry CE 3rd. Identification of a nitroimidazo-oxazine-specific protein involved in PA-824 resistance in Mycobacterium tuberculosis. Proc Natl Acad Sci U S A 2006;103(2):431–436. [PubMed: 16387854]
33. Li X, Manjunatha UH, Goodwin MB, Knox JE, Lipinski CA, Keller TH, Barry CE 3rd, Dowd CS. Synthesis and antitubercular activity of 7-(R)-and 7-(S)-methyl-2-nitro-6-(S)-(4-(trifluoromethoxy) benzyloxy)-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazines, analogues of PA-824. Bioorg Med Chem Lett 2008;18(7):2256–2262. [PubMed: 18358721]
34. Li J, Ehlers T, Sutter J, Varma-O'Brien S, Kirchmair J. CAESAR: A new conformer generation algorithm based on recursive buildup and local rotational symmetry consideration. Journal of Chemical Information and Modeling 2007;47(5):1923–1932. [PubMed: 17691764]

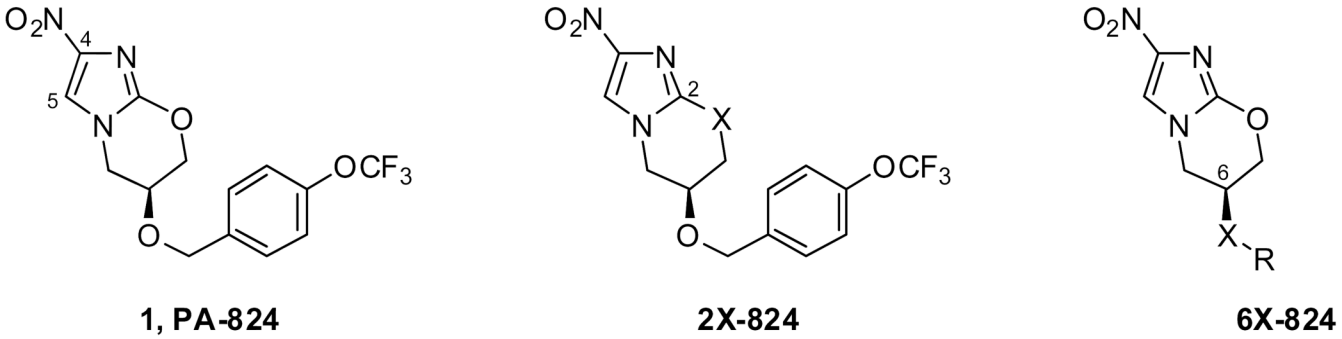


Figure 1.

PA-824 (**1**) and the derivatives examined in the present study. When referring to the imidazole ring, the numbering shown for PA-824 is used for consistency with previous work. Derivatives at the 2-position of the imidazole (2X-824) and at the 6-position of the imidazo-oxazine (6X-824) are investigated here.

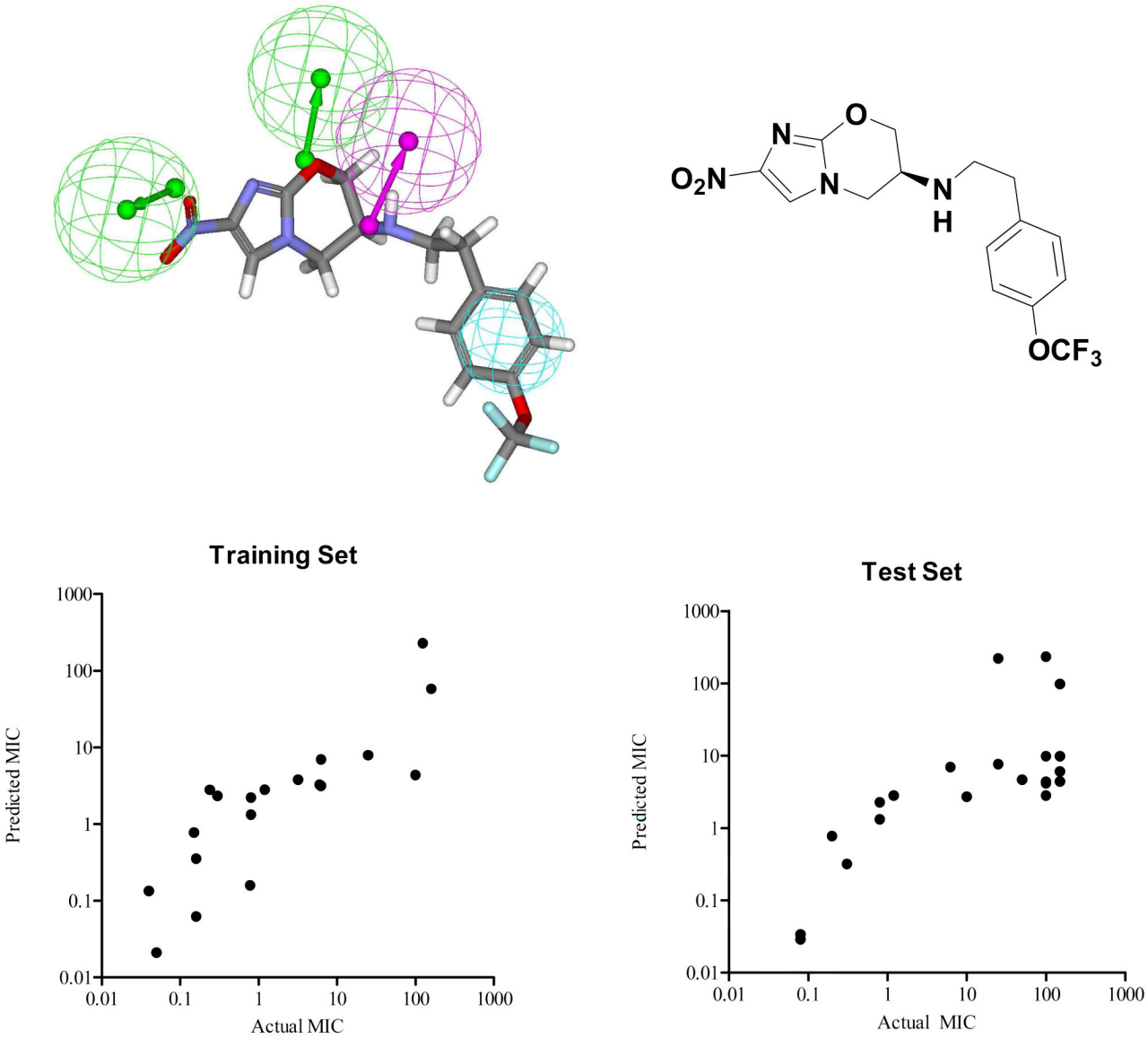
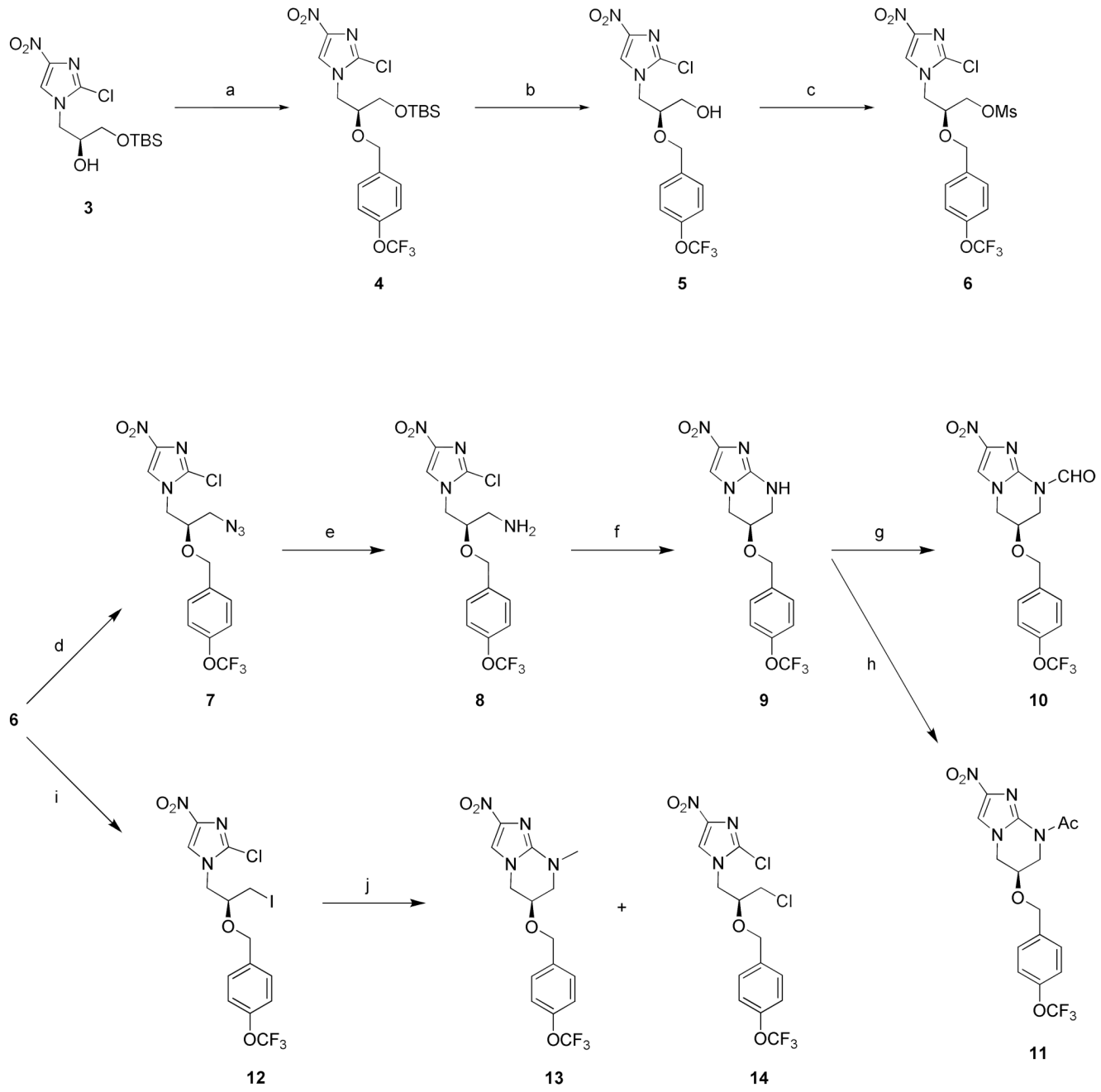


Figure 2.

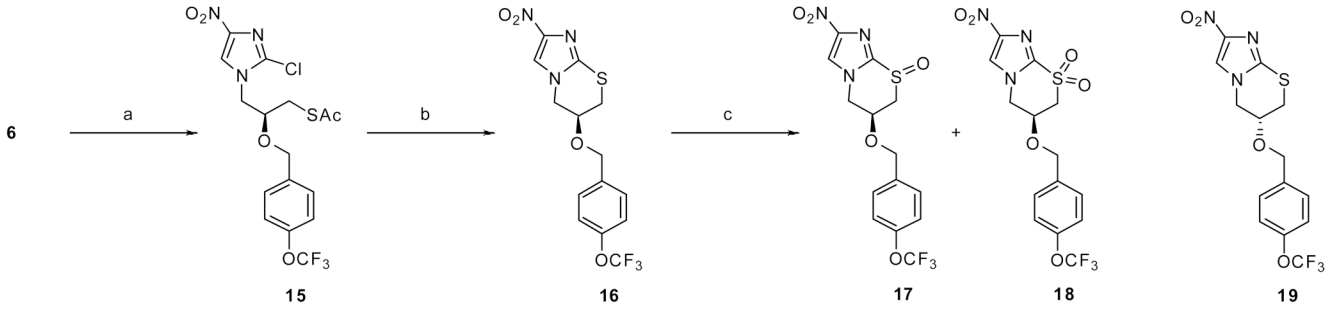
3D-QSAR model for antitubercular nitroimidazoles. Compound **31** is shown aligned to a pharmacophore hypothesis featuring two hydrogen bond acceptors (green), one hydrogen bond donor (purple) and one hydrophobe (aqua). The predictive ability of this model for the 21 molecules in the training set and the 22 molecules in the test set is shown with the actual activity (MIC in μM) on the x-axis compared with the predicted activity on the y-axis.



Scheme 1.

Synthesis of the 2N-824 series of compounds.a

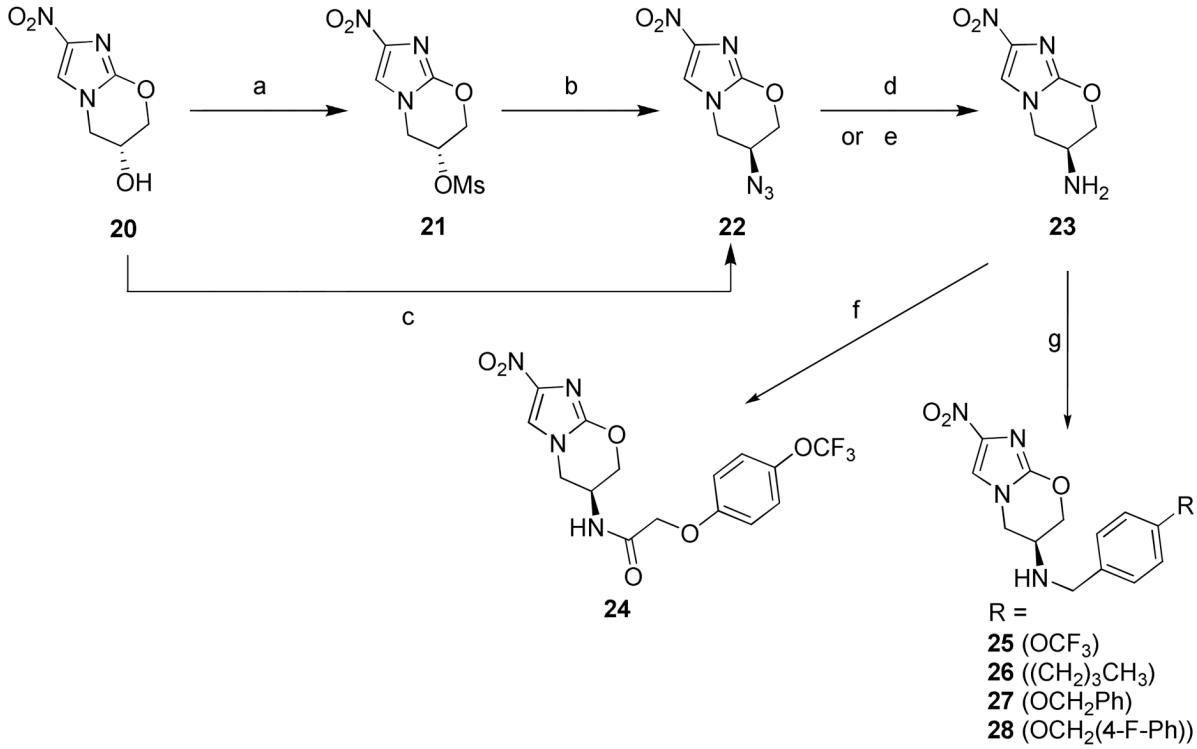
[a]Reagents and conditions: (a) 4-trifluoromethoxybenzyl bromide, NaH, TBAI, DMF, −78°C to rt, 14 h; (b) TBAF, THF, rt, 30 min; (c) MsCl, DIPEA, CH_2Cl_2, rt, 30 min; (d) NaN_3, DMF, 55°C, 14 h; (e) $SH(CH_2)_3SH$, Et_3N, rt, 2 h; (f) DIPEA, DMF, μW, 150°C, 30min; (g) HCO_2H, Ac_2O, CH_2Cl_2, rt, 14 h; (h) AcCl, Et_3N, CH_2Cl_2, 0 °C to rt, 2 h; (i) NaI, acetone, reflux, 6 h; (j) 2M $MeNH_2$ in THF, DIPEA, DMF, μW, 200°C, 20 min.



Scheme 2.

Synthesis of 2S-824, 2SO-824, and $2SO_2$-824.a

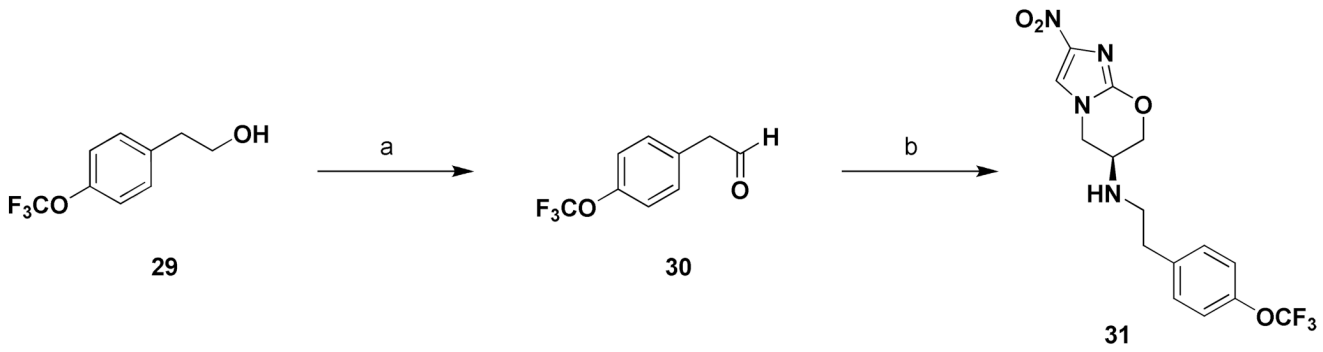
[a]Reagents and conditions: (a) KSAc, DMF, 45°C, 14 h; (b) 3M NaOH (aq), MeOH, 0°C to rt, 17 h; (c) *m*CPBA, CH_2Cl_2, 0°C to rt, 1 h.



Scheme 3.

Synthesis of compounds **24–28**.a

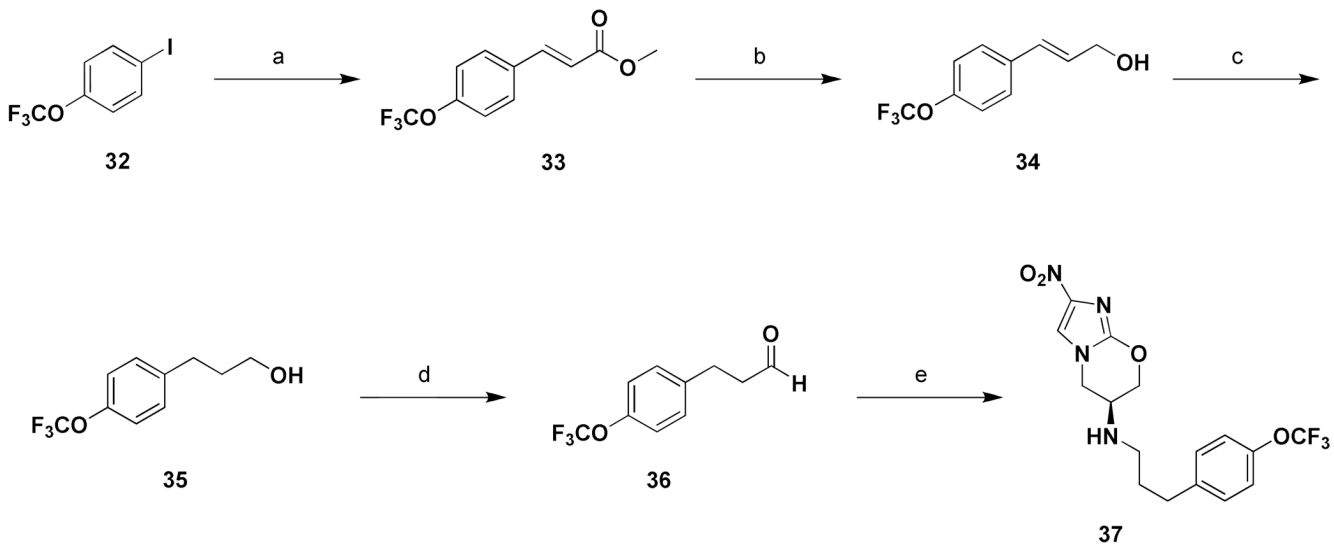
[a]Reagents and conditions: (a) MsCl, Et_3N, DMF, 0°C, 1 h; (b) NaN_3, DMF, 70°C, 2 h; (c) DTAD, PPh_3, $(PhO)_2P(=O)N_3$, THF, 0°C to rt, 18 h; (d) 10% Pd/C, H_2 (g), EtOAc, rt, 2 h; (e) $SH(CH_2)_3SH$, Et_3N, rt, 5 min; (f) 4-trifluoromethoxyphenoxyacetyl chloride, Et_3N, rt, 18 h; (g) aldehyde, AcOH, $NaBH_3CN$, DMF, rt, 2 h.



Scheme 4.

Synthesis of compound **31**.a

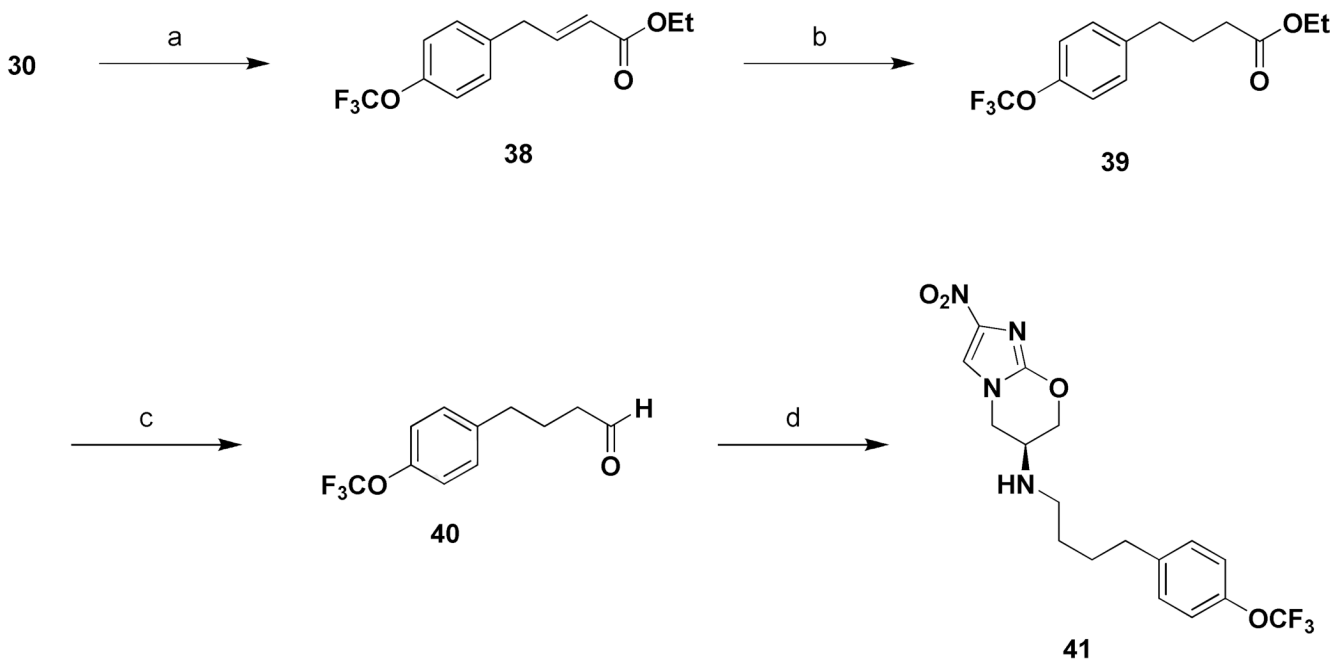
[a]Reagents and conditions: (a) Dess-Martin periodinane, $NaHCO_3$, CH_2Cl_2, 0°C to rt, 3 h; (b) **23**, $NaBH_3CN$, AcOH, DMF, 0°C to rt, 4 h.



Scheme 5.

Synthesis of compound **37**.a

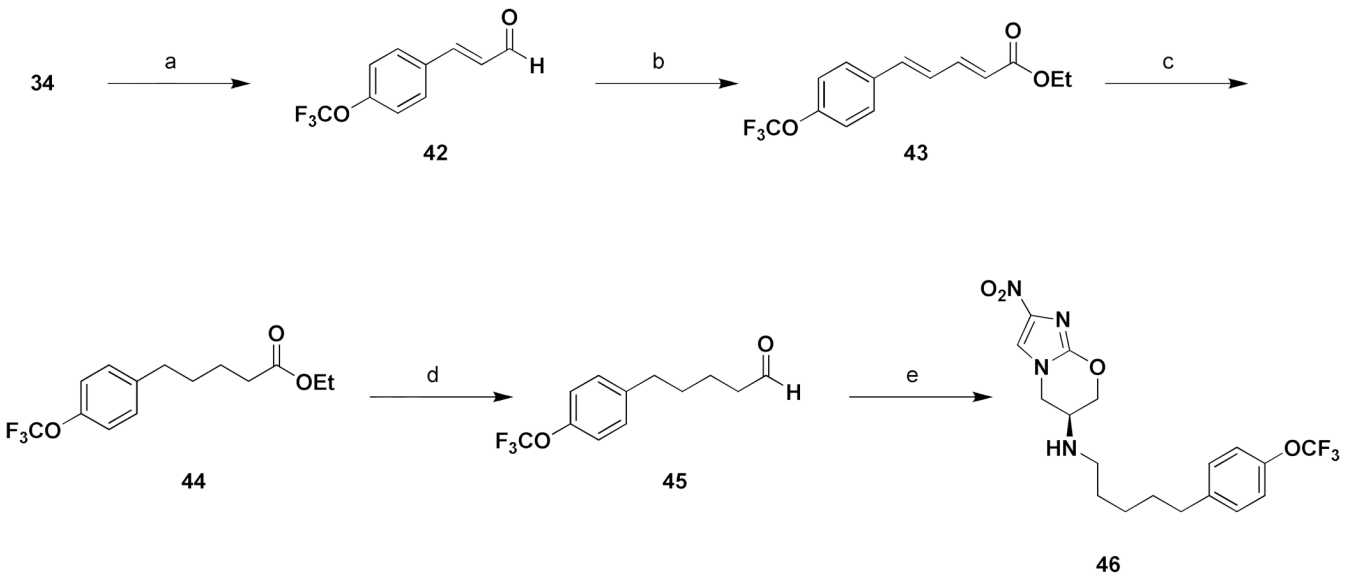
[a]Reagents and conditions: (a) methyl acrylate, $Pd(OAc)_2$, PPh_3, Et_3N, CH_3CN, rt to 100°C, 3 h; (b) i) DIBAL-H, CH_2Cl_2, −78°C, 1 h; ii) 1M HCl (aq); (c) Pd/C, H_2 (g), rt, 4 h; (d) Dess-Martin periodinane, $NaHCO_3$, CH_2Cl_2, 0°C, 3 h; (e) **23**, $NaBH_3CN$, AcOH, DMF, 0°C to rt, 4 h.



Scheme 6.

Synthesis of compound **41**.a

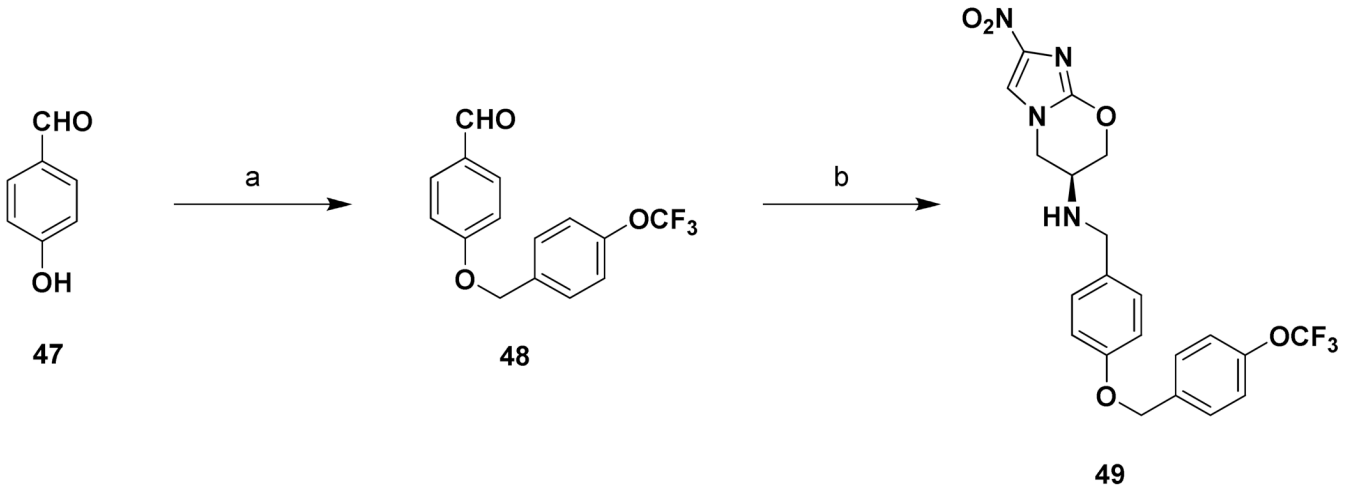
[a]Reagents and conditions: (a) triethyl phosphonoacetate, LiCl, DBU, CH_3CN, rt, 3 h; (b) Pd/C, H_2 (g), rt, 3 h; (c) i) DIBAL-H, CH_2Cl_2, −78°C, 30 min; ii) 1M HCl (aq); (d) **23**, $NaBH_3CN$, AcOH, DMF, 0°C to rt, 4 h.



Scheme 7.

Synthesis of compound **46**.a

[a]Reagents and conditions: (a) Dess-Martin periodinane, $NaHCO_3$, CH_2Cl_2, 0°C, 3 h; (b) triethyl phosphonoacetate, LiCl, DBU, CH_3CN, rt, 3 h; (c) Pd/C, H_2 (g), rt, 4 h; (d) i) DIBAL-H, CH_2Cl_2, −78°C, 1 h; ii) 1M HCl (aq); (e) **23**, $NaBH_3CN$, AcOH, DMF, 0°C to rt, 4 h.



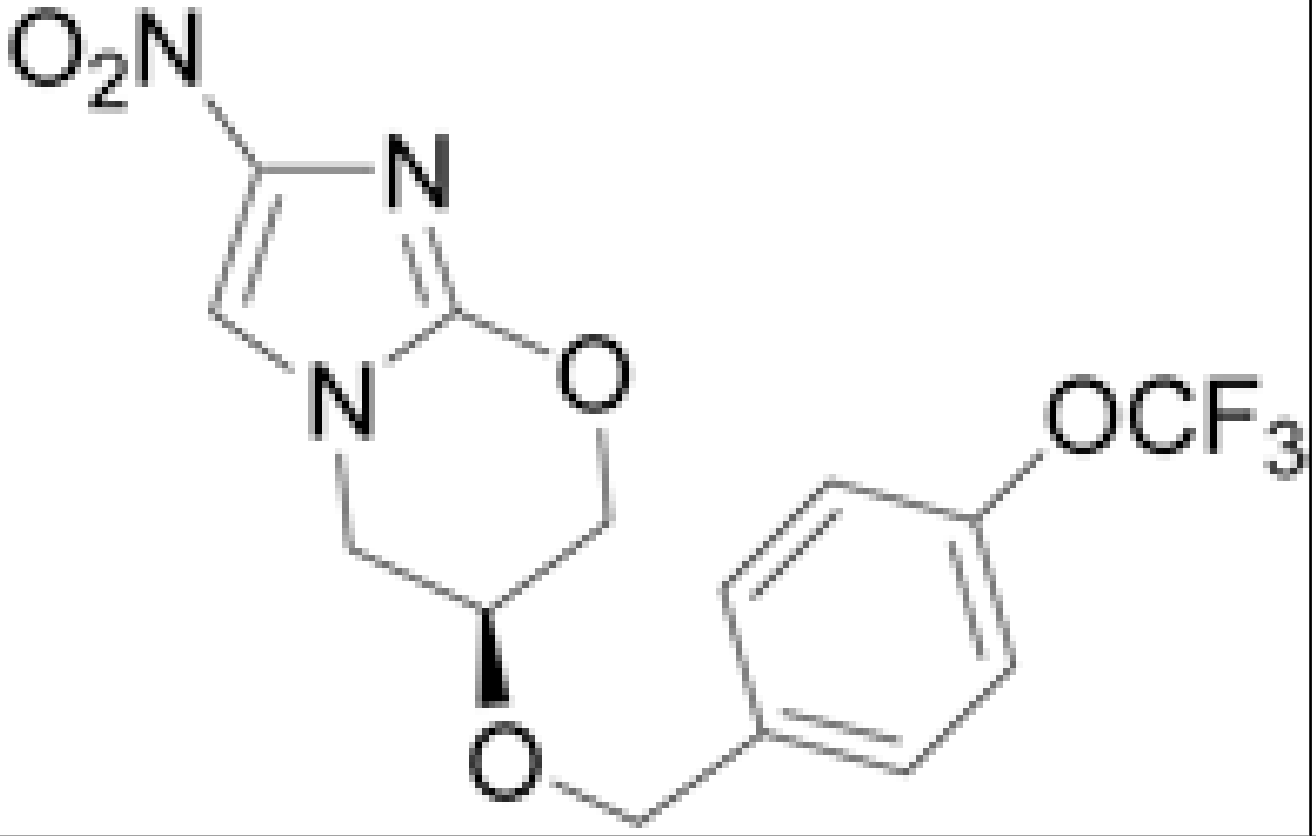
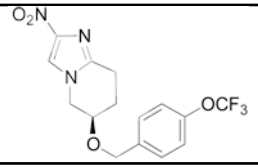
Scheme 8.

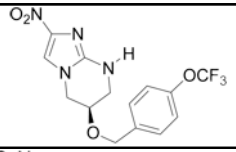
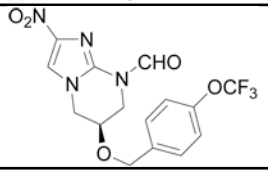
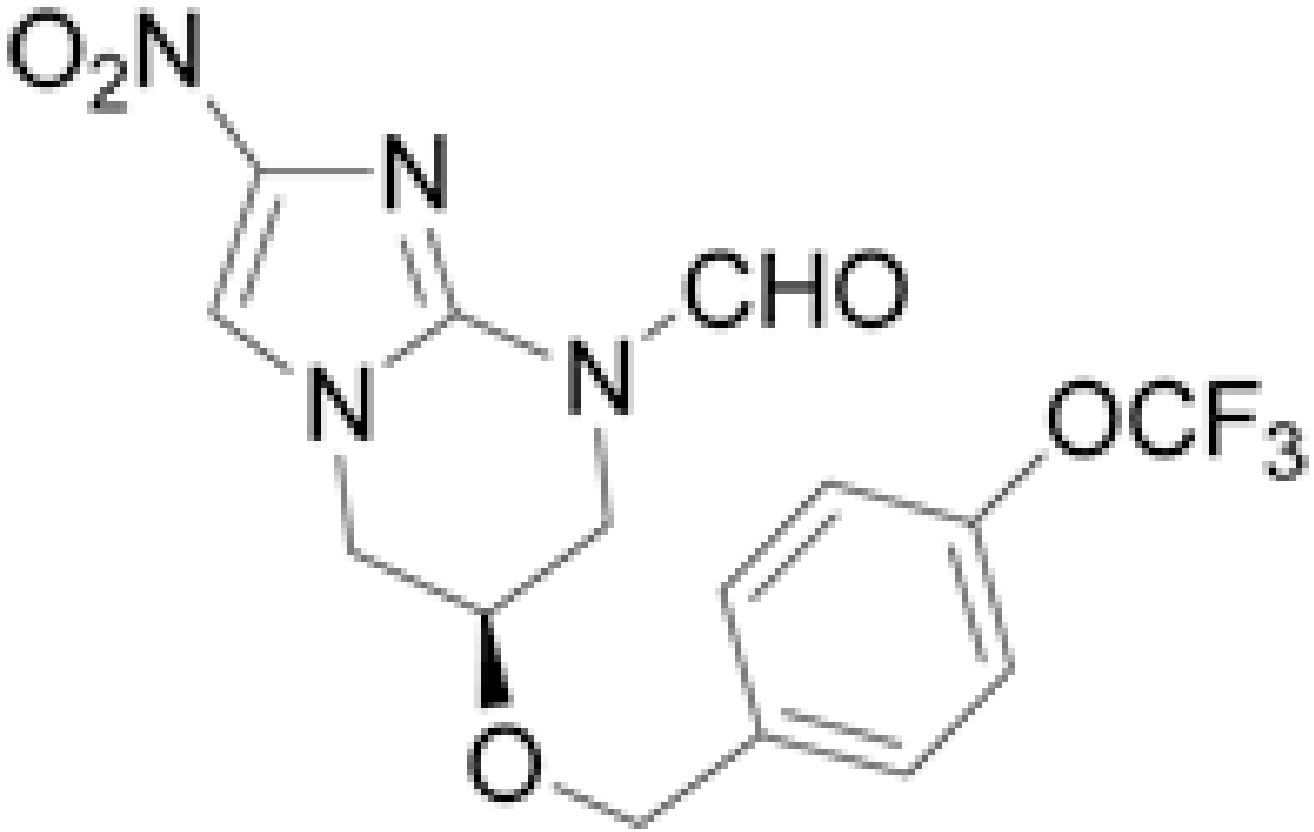
Synthesis of compound **49**.a

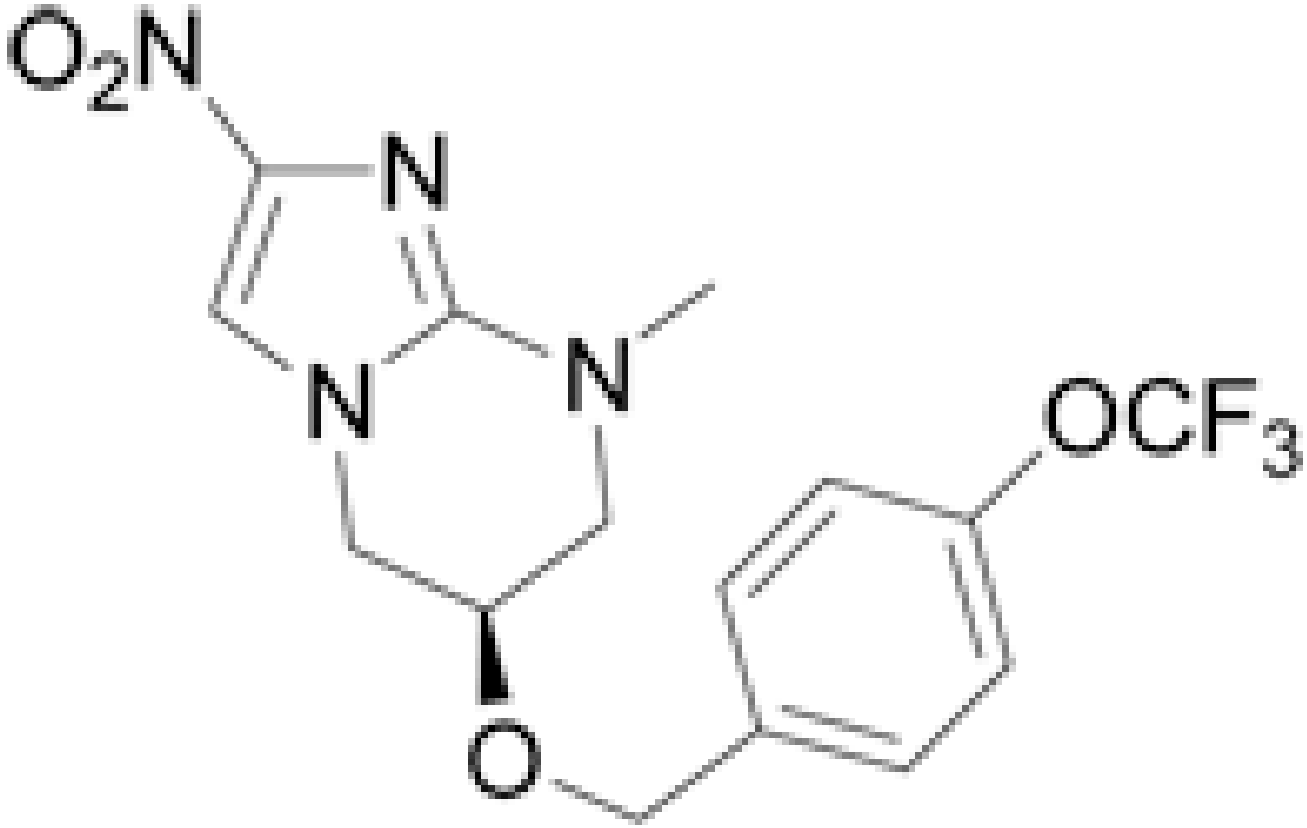
[a]Reagents and conditions: (a) 4-trifluoromethoxybenzyl bromide, NaH, DMF, 0°C, 1.5 h; (b) **23**, $NaBH_3CN$, AcOH, DMF, 0°C to rt, 4 h.

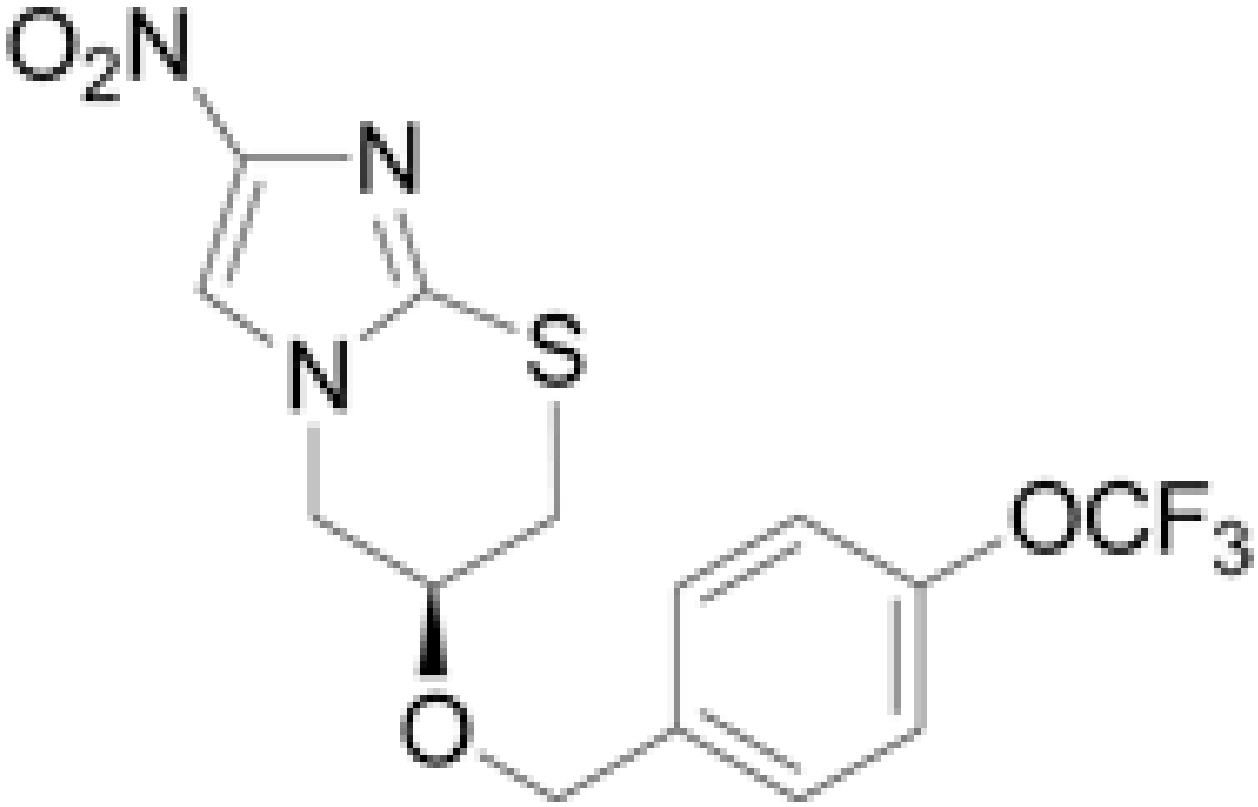
Table 1

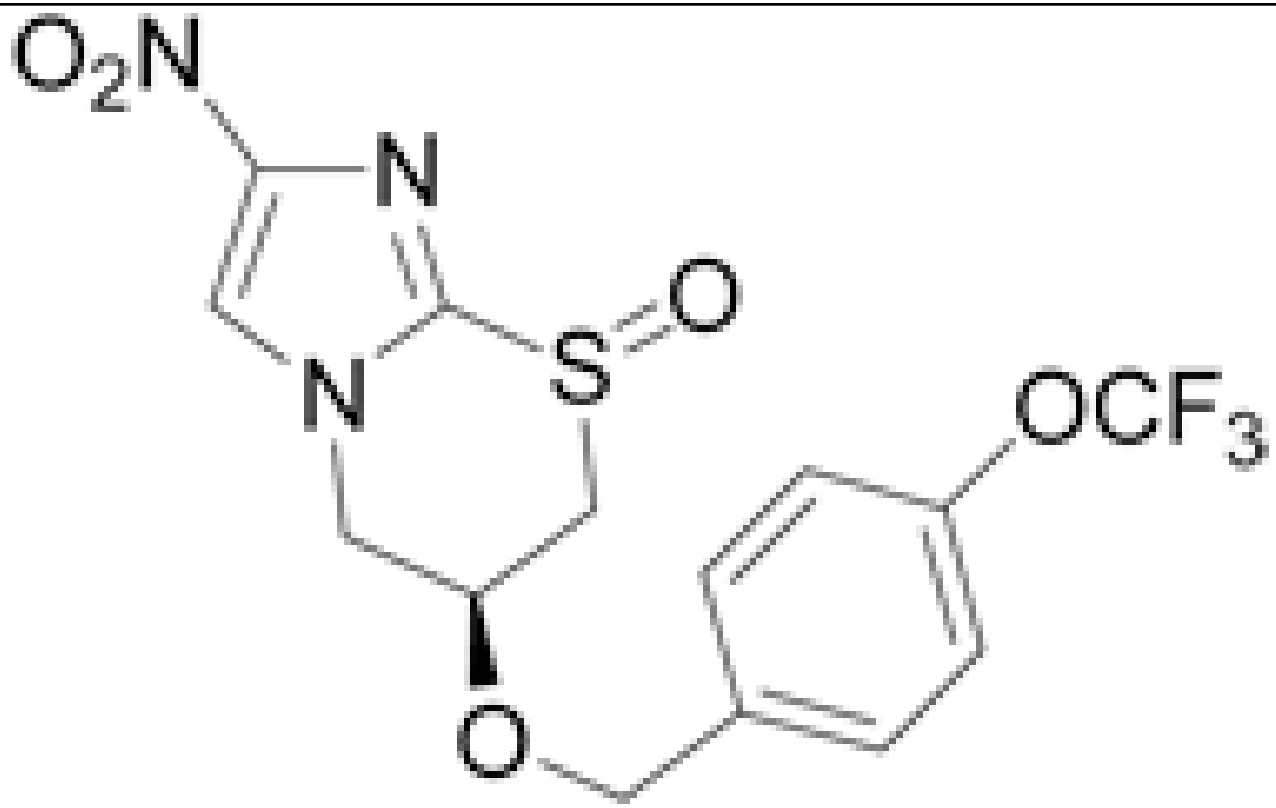
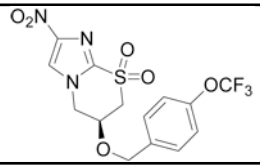
Biological Activities of 2-position analogs of PA-824.

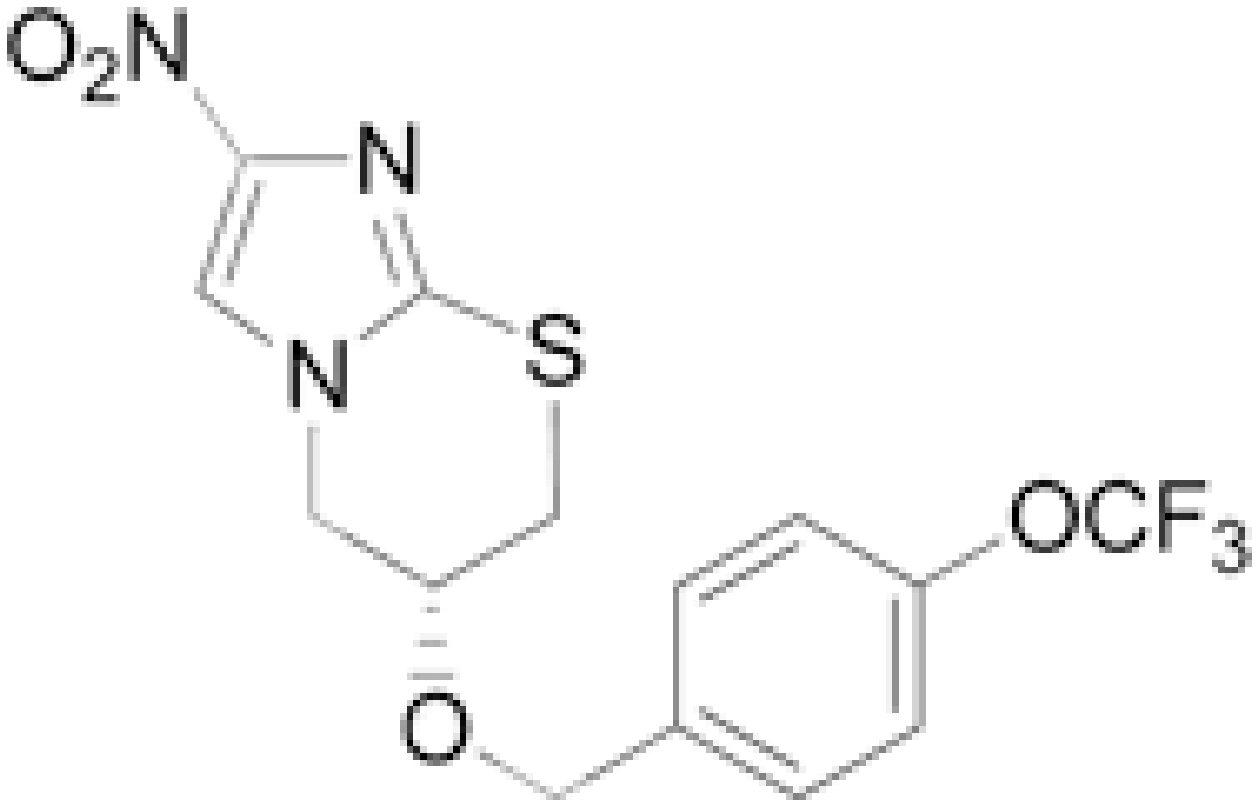
Number	Name	Structure	H37Rv MIC (μM)	H37Rv MAC (μM)	Rv3547[b] k_{cat}/K_m ($min^{-1}\mu M^{-1}$)
1	PA-824		0.8	6.25 – 12.5	0.155
	2C-824[a]		25	250	0.024

Number	Name	Structure	H37Rv MIC (μM)	H37Rv MAC (μM)	Rv3547[b] k_{cat}/K_m ($min^{-1}\mu M^{-1}$)
9	2-amino-824		0.8	125	0.243
10	2-N-formyl-824		3.2	25	0.065
11	2-acetamido-824		6.25	31.25	nd

Number	Name	Structure	H37Rv MIC (μM)	H37Rv MAC (μM)	Rv3547[b] k_{cat}/K_m ($min^{-1}\mu M^{-1}$)
12	2-N-Me-824		6.25	≥100	0.061

Number	Name	Structure	H37Rv MIC (μM)	H37Rv MAC (μM)	Rv3547[b] k_{cat}/K_m ($min^{-1}\mu M^{-1}$)
16	(*R*)-2S-824		0.8–1.6	25	0.146

Number	Name	Structure	H37Rv MIC (μM)	H37Rv MAC (μM)	Rv3547[b] k_{cat}/K_m ($min^{-1}\mu M^{-1}$)
17	2-SO-824		>100	50–100	ns
18	2-SO_2-824		>100	25	nd

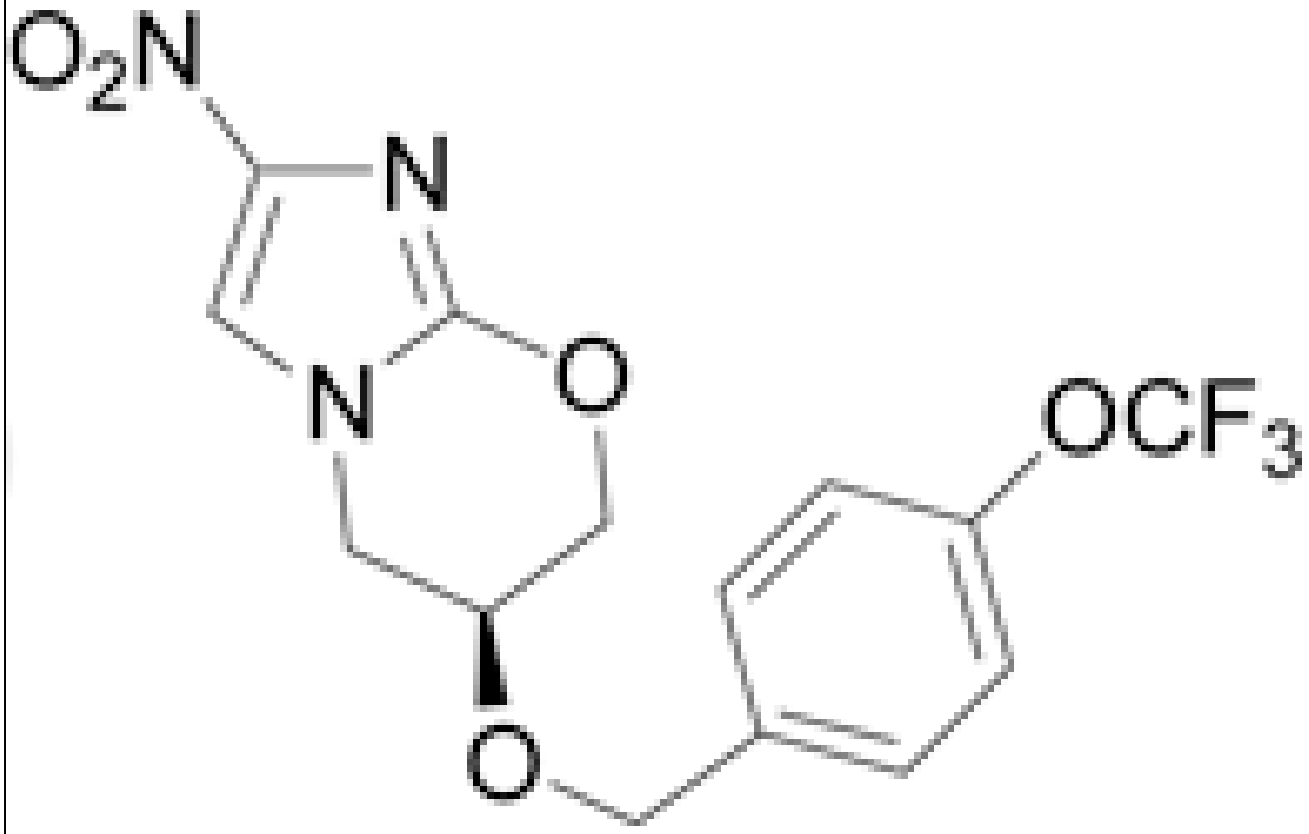
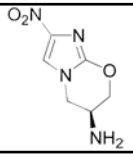
Number	Name	Structure	H37Rv MIC (μM)	H37Rv MAC (μM)	Rv3547[b] k_{cat}/K_m ($min^{-1}\mu M^{-1}$)
19	(*S*)-2S-824		50	50 – 100	ns

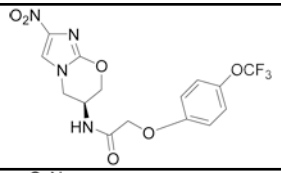
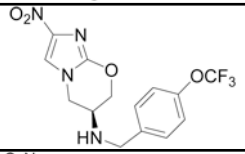
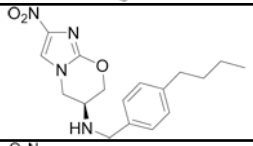
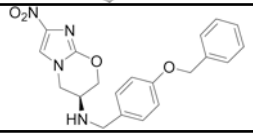
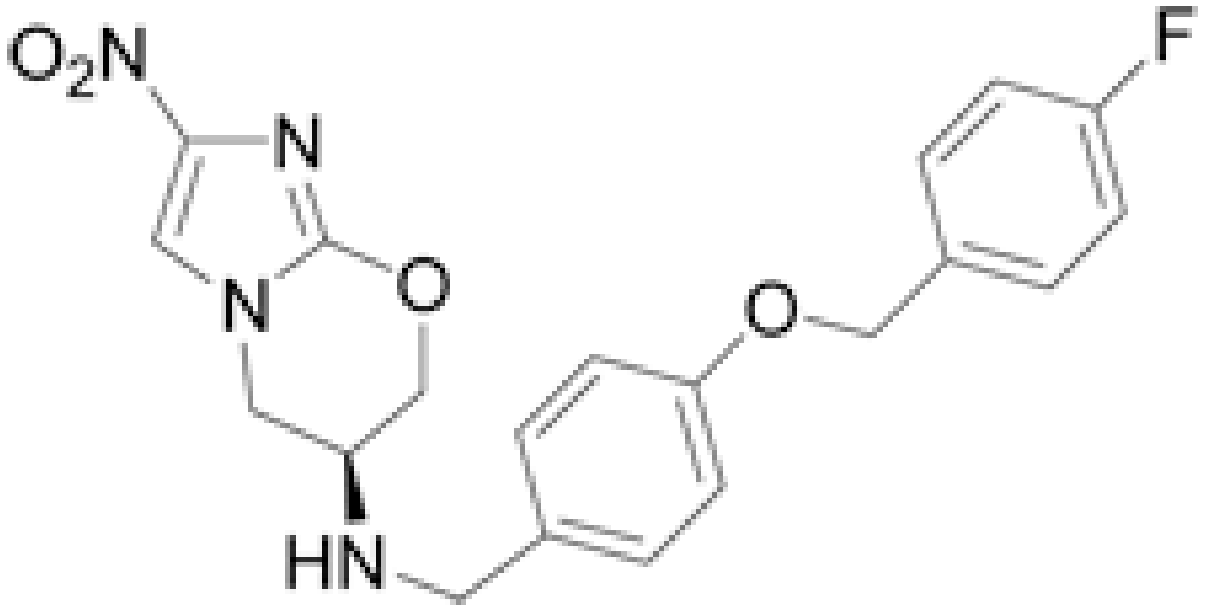
[a] reference [7].

[b] ns = not a substrate, nd = not determined.

Table 2

Biological Activities of amino-substituted analogs of PA-824.

Number	Name	Structure	H37Rv MIC (μM)	H37Rv MAC (μM)	Rv3547[a] k_{cat}/K_m ($min^{-1}\mu M^{-1}$)
1	**PA-824**		0.8	6.25 – 12.5	0.155
23	**Amine**		>100	>500	ns

Number	Name	Structure	H37Rv MIC (μM)	H37Rv MAC (μM)	Rv3547[a] k_{cat}/K_m ($min^{-1}\mu M^{-1}$)
24	**Amido-824**		0.05	62.5	0.017
25	**Amino-824**		0.31	12.5	0.172
26	**Amino-822**		0.78	50 – 100	0.054
27	**Amino-647**		0.15	6.25	0.207
28	**Amino-F-647**		0.2	2–4	0.452

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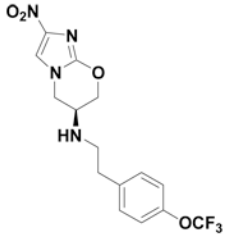
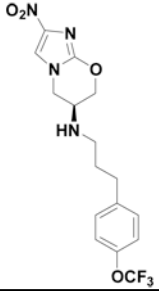
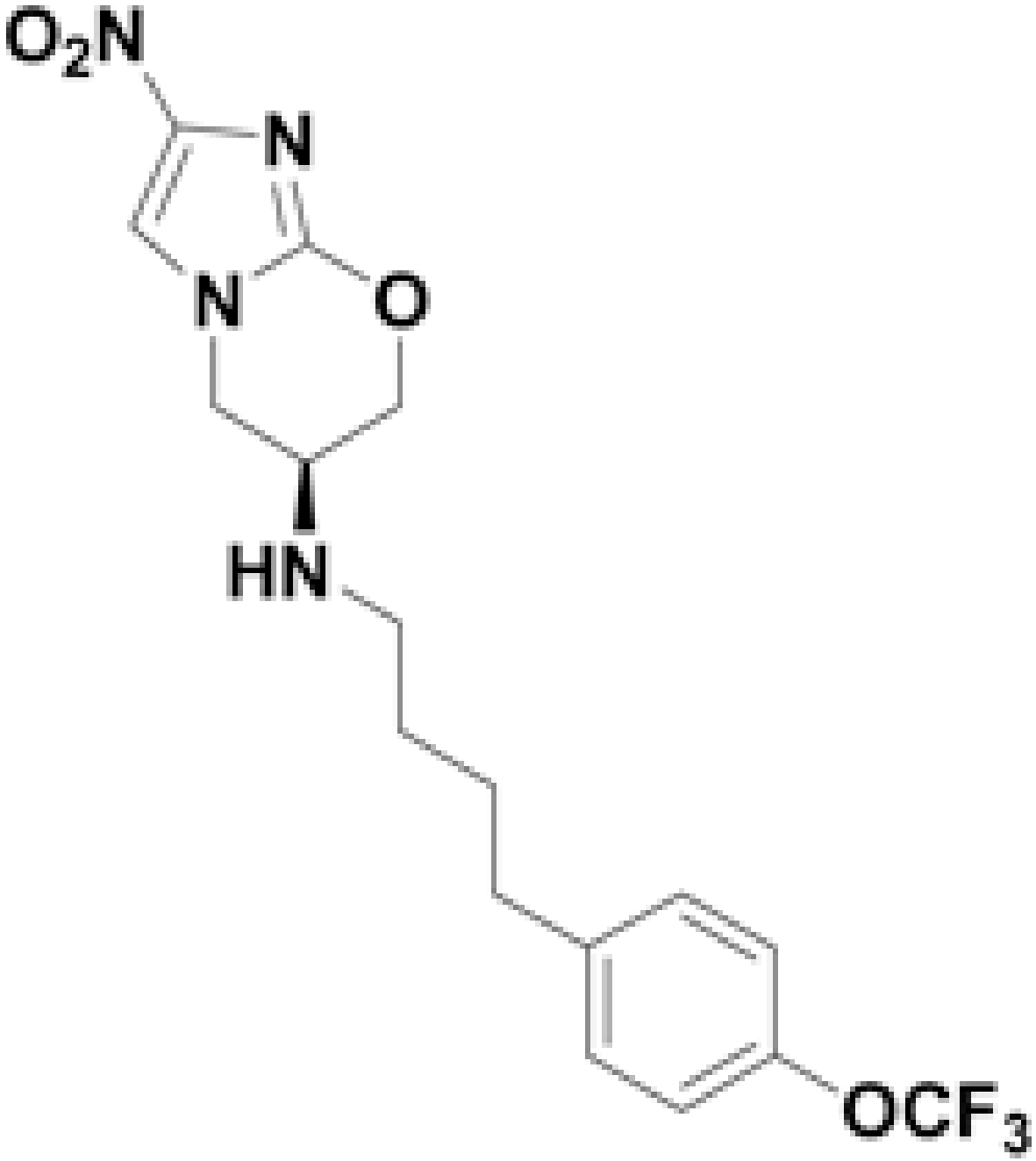
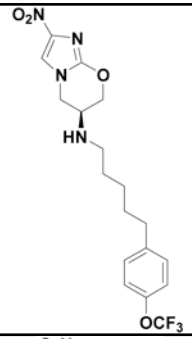
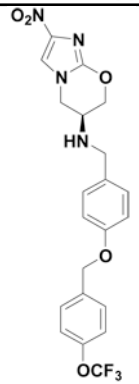
Number	Name	Structure	H37Rv MIC (μM)	H37Rv MAC (μM)	Rv3547[a] k_{cat}/K_m ($min^{-1}\mu M^{-1}$)
31	**AminoEthyl-824**		0.16	6.25	0.110
37	**AminoPropyl-824**		0.078	3.125	0.307

Number	Name	Structure	H37Rv MIC (μM)	H37Rv MAC (μM)	Rv3547[a] k_{cat}/K_m ($min^{-1}\mu M^{-1}$)
41	**AminoButyl-824**		0.039	3.13–12.5	0.514

Number	Name	Structure	H37Rv MIC (μM)	H37Rv MAC (μM)	Rv3547[a] k_{cat}/K_m ($min^{-1}\mu M^{-1}$)
46	**AminoPentyl-824**		0.078	6.25	0.910
49	**AminoOCF3-647**		0.16	1.56 – 3.13	0.074

ns = not a substrate