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Structure-Activity Relationship of New Antituberculosis Agents Derived from Oxazoline and Oxazole Esters

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Abstract

During the syntheses and studies of natural iron chelators (mycobactins), we serendipitously discovered that a simple, small molecule, oxazoline-containing intermediate **3** displayed surprising anti-tuberculosis activity (MIC of 7.7 μ M, average). Herein we report elaboration of SAR around this hit as well as the syntheses and evaluation of a hundred oxazoline- and oxazole-containing compounds derived from an efficient three step process: 1) formation of β -hydroxy amides with serine or threonine; 2) cyclization to afford oxazolines; and 3) dehydration to give the corresponding oxazoles. A number of compounds prepared by this method were shown to possess impressive activity against *M. tuberculosis*, extremely low toxicity and therefore high therapeutic indexes, as well as activity against even the more recalcitrant non-replicating form of *M. tuberculosis*. The uniqueness of their structures and their simplicity should allow them to be further optimized to meet ADME (absorption, distribution, metabolism, excretion) requirements. The syntheses of eight of the most potent *in vitro* compounds were scaled up and the compounds were tested in an *in vivo* mouse infection model to evaluate their efficacy before engaging upon more elaborate compound design and optimization.

Keywords

Aryl oxazoline; Aryl oxazole; Benzyl ester; Tuberculosis agents; Structure-activity relationships; Mycobactins

Introduction

The battle against tuberculosis (TB, caused by *Mycobacterium tuberculosis*) has raged for millennia. [1,2] This disease has claimed the lives of over one billion people and currently

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Supporting Information Available. Analytical data for the following compounds (**3**, **4**, **12** – **111**) can be found. This material is available free of charge via the Internet at <http://www.sciencedirect.com>

infects *one third* of the world's population. With 1.7 million deaths in 2006, TB as a single causative agent is the leading killer among infectious diseases [3]. The spread of TB was temporarily interrupted with the advent of several chemotherapeutic agents [2]. However, since the 1980s TB has been on the rise. Presently, over 9 million new cases are reported annually [3]. The rise in TB/HIV co-infection incidence and the spread of strains of TB that are resistant to at least two frontline anti-TB drugs (multidrug-resistant TB or MDR-TB) [1–7] has compounded the challenge for treating new cases. Today only sustained aggressive chemotherapy treatment with a combination of drugs can stop the disease [8]. This increase in infection has become so alarming that in 1993 the World Health Organization (WHO) [9] declared TB a global emergency. The development of multidrug resistant (MDR) and, more recently, **extensively drug resistant (XDR) forms of TB** have further stimulated research efforts globally [10]. More than ever, there is an urgent need to develop new anti-TB drugs to combat the spread of TB, particularly in its hard-to-kill multidrug-resistant, persistent and latent forms.

One particular strategy to combat TB involves exploiting its need for iron as illustrated by our design, syntheses and studies of siderophore analogs and siderophore-drug conjugates [11–15]. For example, we have demonstrated that synthetic mycobactin S (**1**), with an (*S*)-methyl group configuration in the butyrate linker region, inhibits the growth of *Mycobacterium tuberculosis* H₃₇Rv while mycobactin T, with an (*R*)-methyl group configuration, promotes growth (Figure 1) and substitution of the butyrate linker of the natural mycobactins with a α -Boc-protected α,β -diaminopropionate linker (**2**) resulted in potent inhibition of TB growth.

While effective, this approach involves the syntheses of the structurally complex mycobactin-family [16–18] of siderophores in order to exploit TB's essential iron assimilation processes. Throughout the syntheses of these siderophore natural products, protected and non-protected intermediates were routinely tested for anti-TB activity. This led to the discovery of ND-005859, **3**, a simple, non-iron binding dibenzyl-protected oxazoline, which is a precursor in the syntheses of Mycobactin S and T, as well as the diaminopropionate siderophore analog (**2**, Figure 1). When tested *in vitro*, the structurally simple compound **3** displayed notable anti-TB activity (MIC of 7.7 μ M [19]), and furthermore was non-toxic (>128 μ M) for VERO cells (green monkey kidney cell line). Indeed the potency of this serendipitous “hit” (compound **3**) compared favorably to the natural *M. smegmatis* siderophore Mycobactin S [20] (**1**) or the amide analog **2** which boast MIC's of 3.1 μ M and 0.2 μ M, respectively [21]. However, after benzyl deprotection of compound **3**, the resulting iron binding analog **4** (ND-005862) lost all activity. This suggested that iron assimilation might not be involved in the anti-TB mode of action of this core structure, though it is possible that the benzyl ester of **3** could serve as a TB membrane permeable “prodrug” of the corresponding carboxylic acid which, in turn, might competitively inhibit siderophore biosynthesis [22–25]. While the mechanism of action is currently under investigation, the initial goal of this research was to explore structure-activity-relationships of this structurally simple lead compound in order to improve activity while also providing additional compounds for future mode of action studies.

Given the simplicity of the lead compound, its effectiveness against TB *in vitro*, and a possibly new mechanism of action, we sought to explore further the potential of compounds of this class as anti-TB agents. Here we report results from the syntheses, characterization, and screening of over 100 compounds against TB. A series of eight compounds were further examined for their toxicity and efficacy in an *in vivo* mouse model of TB. These simple compounds show promise for future development as a new class of anti-TB agents.

Results and discussion

The goal of this study was to first produce a series of derivatives of compound **3** (Figure 2, Series 1) to explore structure-activity-relationships (SAR) around the head group of the oxazoline core followed by conversion of all active oxazoline analogs to the corresponding oxazoles and comparison of their activity.

Initially, focus was placed on modifications of the head group region (“series 1” analogs, Figure 2) in order to determine possible SAR trends, increase overall potency and identify an appropriate scaffold for eventual further elaboration of SAR by “tail” region (series 2) modifications. The ultimate goal is to combine the most potent head groups, as described in this document, with the most potent tail groups of the series 2 compounds (work to be reported in a future publication) and produce potentially the most potent and metabolically stable, “drug like” series 3 compounds. Thus, a relatively small set of series 1 compounds was prepared while also considering drug-like parameters [molecular weights under 500, a predicted LogP between 2.5 to 4.5, <5 hydrogen bond donors and <10 hydrogen bond acceptors]. It should be noted that this was not a combinatorial approach. Rather, it was intended to be a methodical parallel approach to create a precise, well characterized set of compounds with properties intended to fall within the calculated range of the molecular physiochemical profile of the currently used anti-TB drugs [26]. Syntheses of all compounds were easily scaled up for *in vivo* testing due to a short straight forward synthetic plan that involved an EDC mediated coupling of the desired benzoic acid **5** with L-serine benzyl ester and then dehydrative cyclization of the β -hydroxy amide **7** with bis(2-methoxyethyl)amino-sulfur trifluoride (DAST) as reported by Wipf et. al. [27] to form the oxazoline core **8** as products **12** – **56** (Scheme 1). Additionally, analog **57** was easily prepared by reduction of nitro analog **28** with indium, ammonium chloride and ethanol at 100°C. Using this effective procedure, over 45 oxazolines (**8**) were prepared in overall good yields (as described in the experimental section).

Additionally, when an oxazoline compound displayed some activity against TB, it was immediately converted to the corresponding oxazole by a mild BrCCl₃/DBU oxidation [28] procedure (Scheme 2). Overall, the oxazole analogs, **58** – **100**, were, on average, more potent than the corresponding oxazolines despite having a simple, “unoptimized”, benzyl ester tail moiety. Additionally, when scale up was required for *in vivo* testing the initial amide coupling was performed with an easily prepared or purchased acid chloride **6** resulting in fast, efficient production of multi-gram quantities of **15**, **29** and others.

To evaluate the importance of substitution on the oxazoline and oxazole cores a small set of threonine derived analogs were prepared utilizing the EDC coupling of the desired benzoic acid **5** with threonine benzyl ester. Then cyclization of general structure **9** with DAST gave the methyl substituted oxazolines **10** as analogs **101** – **105**. Lastly, the corresponding oxazoles (**11**) were produced by the BrCCl₃/DBU oxidation to give final compounds **106** – **111** (Scheme 3).

To determine structure-activity-relationships (SAR) around our hit compound **3**, the previously mentioned rational design approach was carried out with variation of the head group to produce 47 oxazoline compounds which were tested for inhibition of *M. tuberculosis* H₃₇Rv (Table 1) in two different culture media, GAS and GAST. GAST contains Tween 80 and is a more iron deficient medium than the GAS (which has added iron but lacks the Tween 80). Surprisingly, these different media had statistically relevant affects upon the MIC of our compounds as well as on the positive control rifampin (MIC of 0.11 μ M and <0.01 μ M in the GAS to GAST assays, respectively). Interpretation of the anti-TB data along with the VERO cytotoxicity assay data assisted in our selection of compounds to advance for testing *in vivo*.

The SAR of the oxazoline compounds showed an overwhelming increase in potency when the phenyl ring had a 3-chloro substitution (**17**) compared to the 2-chloro (**16**) and 4-chloro (**18**) analogs (1.80 μM , 15.2 μM and 14.9 μM in the GAST medium, respectively). However, analogs with other electron withdrawing groups like the 3-fluoro (**25**), 3-bromo (**54**), and 3-cyano (**53**) were all less active (6.86, 52.5, and 75.1 μM in the GAST medium, respectively) than those with the 3-chloro substitution (**17**, 1.80 μM in the GAST medium). Incorporation of the electron donating, 3-methoxy group (**52**, 42.5 μM GAST medium) significantly decreased activity relative to the 3-chloro analog (**17**, 1.80 μM in GAST medium). Similarly, analog **57** with the 4-amino group (26.8 μM in the GAST medium) was considerably less active than 4-nitro analog **28** (7.59 μM in the GAST medium). It was also evident that the benzyl ester was important for activity since the corresponding methyl ester analogs **12**, **19**, and **27** were considerably less active. Similarly, homologation between the oxazoline core and the phenyl head group was not tolerated as shown by diminished activity of analogs **43** and **44**. The most potent oxazoline compounds contained two substituents on the phenyl ring. For instance, 4-fluoro, 3-nitro analog **29** was very active with an MIC of 0.95 μM in the GAST medium! Compound **30**, with a reversal of the substituents relative to **29**, was 3-fold less active (3.7 μM in the GAST medium) and further displacement, as in analogs **32** and **33**, resulted in additional reduction of activity (40.4 and 8.7 μM in the GAST medium, respectively). It should also be noted that compounds **29** and **30** showed moderate activity (26 μM and 59 μM , respectively) in the LORA assay. This assay was specifically designed to simulate non-replicating *M. tuberculosis* during persistence and/or latency. Analog **51** with the 4-benzyl and 3-fluoro substituents was the most active in this series with MIC's of 0.77 μM and 1.4 μM in the GAS and GAST assays. Additionally, to validate the importance of the heterocyclic core, all oxazoline precursors of general structure **7** (non-cyclized L-serine amides) were screened and the overwhelming majority of compounds were found to be inactive ($>128 \mu\text{M}$).

Next, since many of the oxazoline analogs showed relatively potent anti-TB activity, the corresponding oxazole derivatives were produced to test what effects a planar, aromatic heterocyclic core would have on anti-TB activity (Table 2). Remarkably, the majority of oxazoles synthesized had improved *in vitro* activity, with *seven* compounds **59**, **65**, **66**, **68**, **70**, **78** and **89** having sub micromolar ($<1 \mu\text{M}$) MIC's! Interestingly, there was little difference in activity of the ortho-, meta-, and para-methoxy substituted analogs (**93**, **94**, and **60**) and the corresponding chloro derivatives (**68**, **59**, and **87**), though the ortho- and meta-chloro compounds were the most active. Of these, most compounds tested were active in the LORA non-replicating assay and were non-toxic in the VERO cytotoxicity assay ($>121 \mu\text{M}$). Replacement of the phenyl ring with a pyridyl substituent gave oxazoles with significant activity (**76**, **77**, **79** as the 2-pyridyl, 3-pyridyl, 4-pyridyl, respectively) with MIC's between 1 – 4 μM in the GAS assay, whereas the analogous oxazoline compounds (**38**, **39**, and **40**) had weak activities at best (MIC's $>50 \mu\text{M}$ in the GAS medium). In contrast, oxazoline **51** (MIC's $\sim 1 \mu\text{M}$ in both assays) was three-fold more active than the corresponding oxazole, **72** (MIC $\sim 3 \mu\text{M}$ in both assays). As with the xazoline core, removal of the benzyl ester or transesterification to the methyl, ethyl or t-butyl esters resulted in nearly complete loss of activity (data not shown). This loss of activity after transesterification as was surprising considering that the ethyl ester was key to the potency in a series of related 3,5-disubstituted isoxazolines reported by Lee et. al. [29] and the quinoline-isoxazole series described by Kozikowski et. al. [30].

Next, in an attempt to determine whether simple methyl substitution on the oxazoline (Table 3) and oxazole (Table 4) cores would affect anti-TB activity, a small focused set of threonine-derived compounds was prepared and tested. Preliminary results indicated that methyl substituted oxazoline compounds were significantly less active compared to the analogous oxazoline parents. For instance, compounds **102** and **103** were completely inactive ($>128 \mu\text{M}$) relative to compounds **3** and **15** (7.69 μM and 4.42 μM in the GAST assay, respectively). The

threonine-derived oxazole compounds also had greatly diminished activity, as seen, for example, by comparison of compound **108** (>128 μ M) to compound **3** (7.69 μ M in the GAST assay). In fact, the more potent head group analogs, **109**, **110**, and **111** all had diminished anti-TB activity as methyl substituted oxazole analogs compared to the non-methyl bearing oxazole analogs (**65**, **60**, **66**). Furthermore, compound **109** had greatly increased toxicity in the VERO assay. In short, even simple methyl substitution on the oxazoline and oxazole cores resulted in diminished potency and often increased toxicity toward the VERO cells.

Encouraged by the anti-TB data, several compounds were subsequently tested against a diverse panel of nontuberculosis mycobacteria and other bacteria (Gram positive and negative) as well as select strains of yeast. This panel included *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* SG 511, *Mycobacterium luteus* ATCC 10240, *E. coli* SG 458, *Proteus vulgaris* OX 19, *Mycobacterium smegmatis* SG 987, *Mycobacterium aurum* SB 66, *Mycobacterium aurum* DSM 43536, *Mycobacterium vaccae* 10670, *Mycobacterium fortuitum* B, *Sporobolomyces salmonicolor* SBUG 549, *Candida albicans* C.A., *Glomerella*, and *Mycobacterium avium* strains MAC 100, 101, 108, 109, 116. In all cases, compounds **1**, **15**, **29**, **60**, **63**, **65**, **66**, and **68** were inactive (MIC's equal to negative control) despite showing some zones of inhibition in the agar diffusion assay against *Mycobacterium vaccae* 10670 and *Mycobacterium fortuitum* B. This remarkably selective inhibition of TB suggests a unique mode of action for the slower growing *M. tuberculosis*. A microarray experiment [31] with compound **3** revealed up regulation of the Mtb genes (responsible for mycobacin/exochelin synthesis) coupled with the down regulation of the bacterioferritin (bfr) genes (data not shown). These results suggested a condition of iron stress, possibly due to inhibition of the mycobacterial siderophore biosynthesis pathway and in turn interference of iron trafficking [22–25]. Further mode of action studies are ongoing and will be reported in due course.

Animal Testing

In vivo studies to assess toxicity were performed using compounds **3**, **15**, **29**, **60**, **63**, **65**, **66**, and **68**. Mice were given 100 mg/kg po once a day in 100 μ L of 0.5% carboxymethylcellulose (CMC) for five days, followed by 2 days of rest and then 200 mg/kg for five days. All compounds were well tolerated with respect to body weight, gross appearance and behavior with the exception of compound **66** where the average weight loss was 12% at the end of the two week period (from 21 g to 18.5 g). Subsequently, all compounds were tested for efficacy in BALB/c mice infected with *M. tuberculosis*. At days 5, 60 and 85 following a loose dose aerosol infection with *M. tuberculosis* Erdman, mean cfu (SD) in mouse lungs of untreated controls were $2.3 (2.5) \times 10^2$, $1.8 (1.0) \times 10^6$ and $4.7 (2.3) \times 10^6$, respectively. Beginning on day 60 post-infection, groups of 8 mice received a single oral dose at 200 mg/kg (except compound **66** which was dosed at 50 mg/kg due to a 10% loss in body weight during tolerance testing) in 200 μ L of 0.5% CMC 5 days per week for three weeks. Untreated controls received the CMC vehicle only and the positive drug control consisted of rifampin (RMP) at 10 mg/kg dosed in an identical manner and vehicle as the test compounds. After two days of no treatment (in order to reduce the probability of carry over of lung tissue-associated compound on the plating medium), on day 85 post-infection mice were sacrificed and lung tissue homogenates plated to determine bacterial load. Whereas the rifampin control effected a reduction in bacterial load of greater than 2 logs versus the untreated control, none of the test compounds showed efficacy with this dosing regimen and mode of administration due potentially to the metabolic liability of the benzyl ester moiety. The stability of the benzyl ester was then evaluated *in vitro* in simulated gastric fluid and rat liver microsomes. Not surprisingly, the benzyl esters were rapidly cleaved to their corresponding inactive carboxylic acids in simulated gastric fluid assay (Table 5).

Furthermore, these compounds were metabolically unstable (Table 5). The *in vitro* metabolism of **15** was further studied and the major metabolite was the corresponding carboxylic acid, providing an additional reason why these fairly potent *in vitro* compounds failed to show *in vivo* efficacy. As such, plans are now underway to improve the pharmacokinetic properties of the lead compounds through the use of more metabolically stable replacements of the simple benzyl esters to hopefully impart enhanced *in vivo* stability and possibly efficacy. The experimental section profiles the syntheses and characterization of these eight compounds evaluated *in vivo*, as well as by microsomal and simulated gastric fluid assays.

Conclusion

On a planet in which over 1/3 of the population is infected with tuberculosis, it is more than ever prudent to evaluate new anti-tuberculosis agents *in vitro* and *in vivo*. Herein we described simple serine derived oxazoline and oxazole templates with highly selective anti-TB activity. SAR studies demonstrate that, in general, oxazoles are more potent than their oxazoline precursors and that alkyl substitution on either the oxazoline or oxazole diminishes anti-TB activity. Activity can also be influenced by the type and position of substituents on the pendant phenyl group, with electron withdrawing chloro and combination of chloro and nitro groups being somewhat advantageous though clear correlations require more study. The corresponding acids and simple alkyl esters are substantially less active than the corresponding benzyl esters. While the metabolic lability of the benzyl esters precludes their use as orally active agents, many of these agents like compound **65** (with an MIC of $<0.5 \mu\text{M}$ against replicating *Mycobacterium tuberculosis* H₃₇Rv) can be made on multiple gram scale in just three steps, thus further studies to extend both SAR and ADME properties are merited.

Experimental Section

All anhydrous solvents, reagent grade solvents for chromatography and starting materials were purchased from either Aldrich Chemical Co. (Milwaukee, WI) or Fisher Scientific (Suwanee, GA). Water was distilled and purified through a Milli-Q water system (Millipore Corp., Bedford, MA). L-Glutathione reduced form (GSH) and nicotinamide adenine dinucleotide phosphate reduced form (NADPH) were obtained from Sigma-Aldrich (St. Louis, MO). Male Sprague-Dawley rat liver microsomes were purchased from BD Gentest (Woburn, MA). General methods of purification of compounds involved the use of silica cartridges purchased from AnaLogix, Inc. (Burlington, WI; www.ana-logix.com) and/or recrystallization. The reactions were monitored by thin-layer chromatography (TLC) on precoated Merck 60 F₂₅₄ silica gel plates and visualized using UV light (254 nm) reported in R_f values. All compounds were analyzed for purity and characterized by ¹H and ¹³C NMR using Varian 300MHz NMR and/or Varian 500MHz NMR spectrometers. Chemical shifts are reported in ppm (δ) relative to the residual solvent peak in the corresponding spectra; chloroform 7.26 ppm and 77.23 ppm, methanol 3.31 ppm and 49.00 ppm, methylene chloride 5.32 ppm and 54.00 ppm and coupling constants (*J*) are reported in hertz (Hz) (where, s = singlet, bs = broad singlet, d = doublet, dd = double doublet, bd = broad doublet, ddd = double doublet of doublet, t = triplet, tt = triple triplet, q = quartet, m = multiplet) and analyzed using MestReC NMR data processing. High resolution mass spectra ("HRMS") by fast atom bombardment ("FAB") were taken on a JEOL AX505HA magnetic sector mass spectrometer and values are reported as *m/z*. Melting points ("mp") were measured on a Thomas-Hoover capillary melting point apparatus and are uncorrected. FTIR ("IR") spectra were obtained using a Thermo-Nicolet IR200 spectrometer and absorption frequencies were reported in reciprocal centimeters (cm⁻¹). The high pressure liquid chromatography ("HPLC") analyses were carried out on a Waters 2695 instrument consisting of a photodiode array detector 996, using the Waters symmetry C₁₈ 5 μm reverse phase column (Waters, Milford, MA, www.waters.com). Mobile phases: (a) 50% HPLC grade acetonitrile in Millipore purified water at a flow rate of 1.0 mL min⁻¹ and UV detection at 254

nm. (b) 60% HPLC grade acetonitrile in Millipore purified water at a flow rate of 1.0 mL min⁻¹ and UV detection at 254 nm. A typical run time was 20 minutes. The liquid chromatography mass spectrum ("LC/MS") analyses were carried out on Waters ZQ instrument consisting of chromatography module Alliance HT, photodiode array detector 2996, and mass spectrometer Micromass ZQ, using a 3 × 50 mm Pro C18 YMC reverse phase column. Mobile phases: 10 mM ammonium acetate in HPLC grade water (A) and HPLC grade acetonitrile (B). A gradient was formed from 5% to 80% of B in 10 minutes at 0.7 mL/min. The MS electrospray source operated at capillary voltage 3.5 kV and a desolvation temperature 300°C. Elemental analyses were performed by Midwest Microlabs, LLC (Indianapolis, IN). All reactions were conducted under argon unless otherwise noted. Solvents were removed *in vacuo* on a rotary evaporator. Abbreviations: DCM = dichloromethane; DMF = dimethylformamide; ACN = acetonitrile; EtOAc = ethyl acetate; HOAc = acetic acid; DAST = Bis(2-methoxyethyl)amino-sulfur trifluoride; EDC = *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride.

General Procedure for Preparation of Serine Amides (7, open L-Serine form)

Method 1—Benzoic acid (1 g, 8.2 mmol, 1.1 equiv) and L-serine benzyl ester hydrochloride (1.7 g, 7.4 mmol, 1 equiv) in 30 mL of acetonitrile (ACN) were treated with *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC-HCl, 2.9 g, 14.9 mmol, 2 equiv) and *N,N*-dimethylaminopyridine (DMAP, 2.3 g, 18.6 mmol, 2.5 equiv). The resulting solution was stirred for 12 h at room temperature. The reaction mixture was diluted with ethyl acetate (EtOAc), washed with 0.5 N citric acid (2×), water and 10% aqueous NaHCO₃ (2×). The organic phase was collected and dried over sodium sulfate (Na₂SO₄), filtered and then concentrated *in vacuo*. Crude material obtained was purified by silica gel column chromatography with a 1:10 to 1:1 ethyl acetate : dichloromethane (DCM) gradient solvent system.

Method 2—Benzoyl chloride (4.15 g, 3.4 mL, 28.6 mmol, 1 equiv) was dissolved in 120 mL of dichloromethane (DCM, anhydrous) and then L-serine benzyl ester hydrochloride (7.2 g, 30.1 mmol, 1.05 equiv) and diisopropylamine (DIPEA, 9.3 g, 12.5 mL, 71.6 mmol, 2.5 equiv) was added very slowly to the chilled reaction mixture (0°C, ice bath temperature). The reaction was warmed to room temperature and then heated to reflux, 50°C (bath temperature) for 14 h. The reaction mixture was cooled, washed with water and then 10% aqueous NaHCO₃ solution (2×). The organic phase was collected and dried over sodium sulfate (Na₂SO₄), filtered and then concentrated *in vacuo*. Crude material obtained was purified by silica gel column chromatography with a 1:10 to 1:1 ethyl acetate : DCM gradient solvent system.

General Procedure for Preparation of Oxazoline Products (compounds 3, 4, 8, 12 – 57)

L-serine amide **7** (204 mg, 0.68 mmol, 1 equiv) was dissolved in DCM (5 mL) and cooled to -78°C (bath temp, dry ice-acetone). Bis(2-methoxyethyl)amino-sulfur trifluoride (DAST, 0.1 mL, 0.78 mmol, 1.15 equiv) was added slowly to the cold mixture under argon and the reaction was stirred for 30 min. Next, K₂CO₃ (254 mg, 1.8 mmol, 2.7 equiv) was added and then the reaction was allowed to warm to room temperature. Once the reaction appeared to be complete by TLC analysis, the reaction mixture was poured into 10% aqueous NaHCO₃ solution and was extracted with DCM (2×). The organic layer washed again with the NaHCO₃ solution, brine, dried over Na₂SO₄, filtered and then concentrated *in vacuo*. Crude material obtained was purified by silica gel column chromatography with a 1:10 to 1:1 ethyl acetate : DCM gradient solvent system.

(S)-Benzyl 2-(2-(benzyloxy)phenyl)-4,5-dihydrooxazole-4-carboxylate (3)—See our previous studies.^{17,18}

2-(Benzyloxy)benzoyl chloride (13.3 g, 52.3 mmol) was dissolved in 250 mL of DCM and then L-serine benzyl ester hydrochloride (13.1 g, 54.9 mmol) and diisopropylethylamine (22.8 mL, 130.7 mmol) were added while the reaction was chilled in an ice bath (0°C bath temperature). The reaction mixture was warmed to room temperature and then heated to reflux, 50°C (bath temperature) for 14 h. The reaction mixture was cooled, washed with 10% aqueous NaHCO₃ solution (2×), water, then washed with 0.5 N citric acid (2×) and then brine. The organic phase was collected and dried over sodium sulfate (Na₂SO₄), filtered and then concentrated *in vacuo* to give 21.2 g (99%) of (S)-benzyl 2-(2-(benzyloxy)benzamido)-3-hydroxypropanoate as an oil which became an off white solid upon standing. ¹H NMR (300 MHz, CDCl₃) δ 8.79 (1 H, d, *J* = 7.1 Hz), 8.20 (1 H, dd, *J* = 7.8, 1.8 Hz), 7.52-7.27 (10 H, m), 7.07 (2 H, dd, *J* = 15.9, 8.0 Hz), 5.23-5.14 (4 H, m), 4.93-4.85 (1 H, m), 3.92 (2 H, dd, *J* = 3.9, 1.6 Hz). (S)-Benzyl 2-(2-(benzyloxy)benzamido)-3-hydroxypropanoate (21.2 g, 54 mmol) was dissolved in DCM (450 mL) and then cooled to -78°C (bath temp, dry ice-acetone). Bis(2-methoxyethyl)amino-sulfur trifluoride (DAST, 8.2 mL, 62.1 mmol) was added slowly to the cold mixture under argon where it was stirred for 30 mins. Then K₂CO₃ (20.2 g, 145.8 mmol) was added and the reaction mixture was allowed to warm to room temperature. Once the reaction appeared complete by TLC analysis, the reaction mixture was poured into 10% aqueous NaHCO₃ solution and was extracted with DCM (2×). The organic layer washed again with the NaHCO₃ solution, brine washed, and was dried over Na₂SO₄, filtered and then concentrated *in vacuo*. Crude material obtained was purified by silica gel column chromatography with 1:10 to 1:1 ethyl acetate : DCM gradient solvent system to give 13.7 g (65%) of **3** as a white solid. *R*_f = 0.44 (DCM); mp 69–70°C; IR (neat) 1740 (C=O), 1638 (C=N), 1498, 1450, 1257, 1177 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) 7.82 (1 H, dd, *J* = 8.0, 1.8 Hz), 7.50-7.47 (2H, m), 7.42-7.26 (9H, m), 6.99 (2 H, dd, *J* = 7.7, 6.2 Hz), 5.25 (4 H, dd, *J* = 25.1, 11.8 Hz), 5.01 (1 H, dd, *J* = 10.6, 8.0 Hz), 4.61 (2 H, m); ¹³C NMR (126 MHz, CDCl₃) δ 171.03, 165.65, 157.56, 136.79, 135.40, 132.64, 131.62, 128.50, 128.36, 128.29, 128.25, 127.53, 126.69, 120.69, 120.67, 117.12, 113.75, 70.55, 69.16, 68.77, 67.12; FABMS: 388 (M + 1); HRMS calcd. for C₂₄H₂₁NO₄ 387.1471, found 387.1458. Elemental calcd. for C₂₄H₂₁NO₄ C, 74.40; H, 5.46; N, 3.62; found C, 74.35; H, 5.32; N, 3.60. [α]_D²⁵ +1.02 (c = 1.545 g/100 mL CHCl₃).

(S)-Benzyl 4,5-dihydro-2-phenyloxazole-4-carboxylate (15)—Benzoyl chloride (4.15 g, 3.4 mL, 28.6 mmol) was dissolved in 120 mL of dichloromethane (DCM, anhydrous) and then L-serine benzyl ester hydrochloride (7.2 g, 30.1 mmol) and diisopropylamine (DIPEA, 9.3 g, 12.5 mL, 71.6 mmol) was added very slowly to the chilled reaction mixture (0°C, ice bath temperature). The reaction mixture was warmed to room temperature and then heated to reflux, 50°C (bath temperature) for 14 h. The reaction mixture was cooled, washed with 10% aqueous NaHCO₃ solution (2×), water, then washed with 0.5 N citric acid (2×) and then brine. The organic phase was collected and dried over sodium sulfate (Na₂SO₄), filtered and then concentrated *in vacuo* to give 7.15 g (83%) of (S)-benzyl 2-(benzamido)-3-hydroxypropanoate as an oil which became an off white solid upon standing. ¹H NMR (300 MHz, CDCl₃) δ 7.85-7.79 (2 H, m), 7.56-7.30 (7 H, m), 7.14 (1 H, d, *J* = 7.1 Hz), 5.25 (2 H, s), 4.91 (1 H, td, *J* = 7.1, 3.5, 3.5 Hz), 4.15-4.01 (2 H, m).

(S)-Benzyl 2-(benzamido)-3-hydroxypropanoate (7.15 g, 28.5 mmol) was dissolved in DCM (240 mL) and cooled to -78°C (bath temp, dry ice-acetone). Bis(2-methoxyethyl)amino-sulfur trifluoride (DAST, 4.4 g, 3.6 mL, 27.5 mmol) was added slowly to the cold mixture under argon where it stirred for 30 min. Then K₂CO₃ (8.9 g, 64.4 mmol) was added and the reaction mixture was allowed to warm to room temperature. Once the reaction appeared complete by TLC analysis, the reaction mixture was poured into 10% aqueous NaHCO₃ solution and extracted with DCM (2×). The organic layer was washed again with the NaHCO₃ solution, brine, and then dried over Na₂SO₄, filtered and concentrated *in vacuo*. Crude material obtained was purified by silica gel column chromatography with a 1:10 to 1:1 ethyl acetate : DCM

gradient solvent system to give 5.7 g (85%) of **15** as a white solid. $R_f = 0.25$ (DCM); mp 51–52°C; IR (neat) 1741 (C=O), 1643 (C=N), 1188 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ 8.04–7.92 (2 H, m), 7.57–7.29 (8 H, m), 5.26 (2 H, dd, $J = 20.9, 12.3$ Hz), 4.99 (1 H, dd, $J = 10.6, 7.9$ Hz), 4.80–4.42 (2 H, m). ^{13}C NMR (126 MHz, CDCl_3) δ 170.42, 163.45, 158.40, 156.25, 135.56, 135.48, 135.14, 128.67, 128.61, 128.49, 126.70, 124.19, 118.88, 118.71, 70.31, 68.83, 67.60. Elemental calcd. for $\text{C}_{17}\text{H}_{15}\text{NO}_3$: C, 72.58; H, 5.37; N, 4.98; found C, 72.44; H, 5.40; N, 4.95; $[\alpha]_D^{25} +1.14$ ($c = 1.54$ g/100 mL CHCl_3).

(S)-Benzyl 2-(4-fluoro-3-nitrophenyl)-4,5-dihydrooxazole-4-carboxylate (29)—4-Fluoro-3-nitrobenzoic acid (6.65 g, 35.2 mmol) was dissolved in 75 mL of anhydrous DCM and then oxalyl chloride (6.2 mL, 70.4 mmol) was added drop wise followed by the addition of 50 μL of dimethylformamide (DMF) to catalyze the reaction. The reaction mixture bubbled and was stirred at room temperature for four h. During this time it became a homogeneous solution. The reaction mixture was concentrated to give a yellow paste which was subsequently concentrated *in vacuo* from toluene to give 4-fluoro-3-nitrobenzoyl chloride as a crude yellow solid (7.2 g, 94%) which is used immediately in the subsequent reaction. $R_f = 0.5$ (EtOAc, red streak) ^1H NMR (300 MHz, CDCl_3) δ 8.85 (1H, dd, $J = 7.0, 2.3$ Hz), 8.40 (1H, ddd, $J = 8.8, 4.0, 2.4$ Hz), 7.57–7.43 (1H, m).

4-Fluoro-3-nitrobenzoyl chloride (7.2 g, 34.1 mmol) was dissolved in 150 mL of DCM and then L-serine benzyl ester hydrochloride (8.56 g, 35.8 mmol) and diisopropylethylamine (14.9 mL, 85.3 mmol) were added while the reaction was chilled in an ice bath (0°C bath temperature). The reaction mixture was warmed to room temperature and then heated to reflux, 50°C (bath temperature) for 14 h. The reaction mixture was cooled, washed with 10% aqueous NaHCO_3 solution (2 \times), water, then washed with 0.5 N citric acid (2 \times) and then brine. The organic phase was collected and dried over Na_2SO_4 , filtered and then concentrated *in vacuo*. The crude material obtained was purified using silica gel column chromatography with 5% to 50% ethyl acetate : DCM as the solvent system to give (S)-benzyl 2-(4-fluoro-3-nitrobenzamido)-3-hydroxypropanoate (11.1 g, 90%) as a yellow oil which became a yellow solid upon standing. R_f product = 0.1 (DCM). ^1H NMR (300 MHz, CDCl_3) δ 8.54 (1 H, dd, $J = 7.0, 2.2$ Hz), 8.13 (1 H, ddd, $J = 8.6, 4.1, 2.3$ Hz), 7.44–7.30 (5 H, m), 7.19 (1 H, d, $J = 6.86$ Hz), 5.33–5.20 (2 H, m), 4.96–4.87 (1 H, m), 4.10 (2 H, ddd, $J = 35.3, 11.2, 3.1$ Hz).

(S)-Benzyl 2-(4-fluoro-3-nitrobenzamido)-3-hydroxypropanoate (5.9 g, 16.3 mmol) was dissolved in DCM (160 mL) and cooled to –78°C (bath temp, dry ice-acetone). DAST (2.45 mL, 18.7 mmol) was added slowly to the cold mixture under argon and the reaction was stirred for 30 min. Then K_2CO_3 (6.1 g, 43.9 mmol) was added and the reaction was allowed to warm to room temperature. Once the reaction appeared complete by TLC analysis, the mixture was poured into 10% aqueous NaHCO_3 and extracted with DCM (2 \times). The organic layer washed again with the NaHCO_3 solution, brine, and then dried over Na_2SO_4 , filtered and concentrated *in vacuo*. Crude material obtained was purified by silica gel column chromatography with a 1:10 to 1:1 ethyl acetate : DCM gradient solvent system to give 4.9 g (87%) of **29** as a yellow solid upon standing. $R_f = 0.18$ (DCM); mp 77–78°C; IR (neat) 1741 (C=O), 1651, 1541 (C=O), 1350, 1190 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ 8.68 (1 H, dd, $J = 7.2, 2.2$ Hz), 8.28 (1 H, ddd, $J = 8.7, 4.2, 2.2$ Hz), 7.45–7.31 (6 H, m), 5.26 (2 H, dd, $J = 20.8, 12.2$ Hz), 5.02 (1 H, dd, $J = 10.6, 8.0$ Hz), 4.78–4.61 (2 H, m). ^{13}C NMR (126 MHz, CDCl_3) δ 170.42, 163.45, 158.40, 156.25, 135.56, 135.48, 135.14, 128.67, 128.61, 128.49, 126.69, 124.19, 118.88, 118.71, 70.32, 68.83, 67.60. HRMS calcd. For $\text{C}_{17}\text{H}_{13}\text{ClFNO}_3$ 345.0887, found 345.0901. Elemental calcd. for $\text{C}_{17}\text{H}_{13}\text{ClFNO}_3$: C, 59.30; H, 3.81; N, 8.14; found C, 59.20; H, 3.77; N, 8.13; $[\alpha]_D^{25} +0.94$ ($c = 1.52$ g/100 mL CHCl_3).

General Procedure for Preparation of Oxazole Products (compounds 58 – 100)

Oxazoline **8** (165 mg, 0.5 mmol, 1 equiv) was dissolved in 2 mL of dry DCM and chilled in an ice bath (0°C bath temperature). DBU (0.22 mL, 1.5 mmol, 3 equiv) was added followed by slow addition of bromotrichloromethane (0.15 mL, 1.5 mmol, 3 equiv). The reaction was stirred for 1 h cold and then allowed to warm to room temperature. Once the reaction appeared complete by TLC analysis, the reaction mixture was poured into 10% aqueous NaHCO₃ solution and extracted with DCM (2×). The organic layer washed again with the NaHCO₃ solution, brine, dried over Na₂SO₄, filtered and then concentrated *in vacuo*. Crude oily material obtained was purified by silica gel column chromatography with a 1:10 to 1:1 ethyl acetate : DCM gradient solvent system to give solid upon standing.

Benzyl 2-(4-methoxyphenyl)oxazole-4-carboxylate (60)—4-Methoxybenzoyl chloride (4.11 g, 23.4 mmol) was dissolved in 120 mL of DCM and then L-serine benzyl ester hydrochloride (5.86 g, 24.5 mmol) and diisopropylethylamine (10.2 mL, 58.4 mmol) were added while the reaction was chilled in an ice bath (0°C bath temperature). The reaction mixture was warmed to room temperature and then heated to reflux, 50°C (bath temperature) for 14 h. The reaction mixture was cooled, washed with 10% aqueous NaHCO₃ solution (2×), water, with 0.5 N citric acid (2×) and then brine. The organic phase was collected dried over sodium sulfate (Na₂SO₄), filtered and then concentrated *in vacuo*. The crude material obtained was purified using silica gel column chromatography with 5% to 50% ethyl acetate : DCM as the solvent system to give (S)-benzyl 2-(4-methoxybenzamido)-3-hydroxypropanoate (7.65 g, 99%) as a yellow oil which solidified upon standing. *R*_f = 0.31 (DCM). ¹H NMR (300 MHz, CDCl₃) δ 1H NMR (300 MHz, CDCl₃) δ ppm 7.83-7.74 (2 H, m), 7.44-7.30 (4 H, m), 7.07 (1 H, d, *J* = 7.0 Hz), 6.95-6.86 (2 H, m), 5.24 (2 H, s), 4.89 (1 H, td, *J* = 7.1, 3.6, 3.6 Hz), 4.05 (2 H, dd, *J* = 3.6, 1.9 Hz), 3.84 (3 H, s).

(S)-Benzyl 2-(4-methoxybenzamido)-3-hydroxypropanoate (7.65 g, 23.3 mmol) was dissolved in DCM (240 mL) and cooled to -78°C (bath temp, dry ice-acetone). DAST (3.6 mL, 4.3 mmol) was added slowly to cold mixture under argon where it stirred for 30 min. Then K₂CO₃ (8.73 g, 63.1 mmol) was added and the reaction was allowed to warm to room temperature. Once the reaction appeared complete by TLC analysis the mixture was poured into 10% aqueous NaHCO₃ solution and was extracted with DCM (2×). The organic layer washed again with the NaHCO₃ solution, brine, dried over Na₂SO₄, filtered and then concentrated *in vacuo*. Crude material obtained was purified by silica gel column chromatography with a 1:10 to 1:1 ethyl acetate : DCM gradient solvent system to give 6.74 g (93%) of (S)-benzyl 2-(4-methoxyphenyl)-4,5-dihydrooxazole-4-carboxylate, **21**, as a white solid upon standing. *R*_f = 0.40 (DCM). ¹H NMR (300 MHz, CDCl₃) δ 7.96-7.89 (2 H, m), 7.44-7.31 (5 H, m), 6.94-6.87 (2 H, m), 5.25 (2 H, q, *J* = 12.3, 12.3, 12.3 Hz), 4.95 (1 H, dd, *J* = 10.5, 7.8 Hz), 4.69-4.50 (2 H, m), 3.84 (3 H, s).

(S)-benzyl 2-(4-methoxyphenyl)-4,5-dihydrooxazole-4-carboxylate (**21**, 6.74 g, 21.6 mmol) was dissolved in 75 mL of dry DCM and chilled in an ice bath (0°C bath temperature). DBU (9.7 mL, 64.9 mmol) was added followed by slow addition of bromotrichloromethane (6.5 mL, 64.9 mmol). The reaction was stirred for 1 h cold and then allowed to warm to room temperature. Once the reaction appeared complete by TLC analysis, the reaction mixture was poured into 10% aqueous NaHCO₃ solution and extracted with DCM (2×). The combined organic layers were washed again with the NaHCO₃ solution and brine then dried over Na₂SO₄, filtered and concentrated *in vacuo*. Crude material obtained was purified by silica gel column chromatography with a 1:10 to 1:1 ethyl acetate : DCM gradient solvent system to give 5.0 g (75%) of **60** as a white solid. *R*_f = 0.55 (DCM); mp 111–112°C; IR (neat) 1716 (C=O), 1313, 1263, 1138, 1117 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 8.23 (1 H, s), 8.10-8.01 (2 H, m), 7.51-7.30 (5 H, m), 7.02-6.94 (2 H, m), 5.40 (2 H, s), 3.86 (2 H, s). ¹³C NMR

(126 MHz, CDCl₃) δ 162.86, 162.17, 161.56, 143.65, 135.80, 134.39, 128.88, 128.83, 128.76, 128.66, 119.32, 114.45, 67.00, 55.65. HRMS calcd. For C₁₈H₁₅NO₄, 310.1079 found 310.1071. Elemental calcd. for C₁₈H₁₅NO₄: C, 69.89; H, 4.89; N, 4.53; found C, 69.67; H, 4.92; N, 4.46.

Benzyl 2-(2-(benzyloxy)phenyl)oxazole-4-carboxylate (63)—(S)-Benzyl 2-(2-(benzyloxy)phenyl)-4,5-dihydrooxazole-4-carboxylate (**3**, 5.88 g, 15.2 mmol) was dissolved in 100 mL of dry DCM and chilled in an ice bath (0°C bath temperature). Next, DBU (6.8 mL, 45.5 mmol) was added followed by slow addition of bromotrichloromethane (4.5 mL, 45.5 mmol). The reaction was stirred for 1 h cold and then allowed to warm to room temperature. Once the reaction appeared complete by TLC analysis, the reaction mixture was poured into 10% aqueous NaHCO₃ solution and extracted with DCM (2×). The combined organic layers were washed again with the NaHCO₃ solution, brine, dried over Na₂SO₄, filtered and then concentrated *in vacuo*. Crude material obtained was purified by silica gel column chromatography with a 1:10 to 1:1 ethyl acetate : DCM gradient solvent system to give 4.7 g (81%) of **63** as a white solid. *R*_f = 0.6 (DCM); mp 74–75°C; IR (neat) 1740 (C=O), 1453, 1319, 1145, 1121 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 8.31 (1 H, d, *J* = 2.4 Hz), 8.06 (1 H, dd, *J* = 8.1, 1.7 Hz), 7.57–7.27 (11 H, m), 7.11–7.03 (2 H, m), 5.41 (2 H, s), 5.22 (2 H, s). ¹³C NMR (126 MHz, CDCl₃) δ 161.32, 156.80, 143.92, 136.55, 135.59, 133.89, 132.44, 131.10, 128.57, 128.50, 128.49, 128.36, 127.72, 126.77, 120.98, 116.17, 113.47, 70.48, 66.68. HRMS calcd. For C₂₄H₁₉NO₄, 386.1392 found 386.1385. Elemental calcd. for C₂₄H₁₉NO₄: C, 74.79; H, 4.97; N, 3.63; found C, 74.59; H, 4.97; N, 3.66.

Benzyl 2-phenyloxazole-4-carboxylate (65)—(S)-Benzyl 4,5-dihydro-2-phenyloxazole-4-carboxylate (**15**, 6.27 g, 22.3 mmol) was dissolved in 75 mL of dry DCM and chilled in an ice bath (0°C bath temperature). DBU (10 mL, 66.9 mmol) was added followed by slow addition of bromo-trichloromethane (6.6 mL, 66.9 mmol). The reaction was stirred for 1 h cold and then allowed to warm to room temperature. Once the reaction appeared complete by TLC analysis, the reaction mixture was poured into 10% aqueous NaHCO₃ solution and extracted with DCM (2×). The organic layer washed again with the NaHCO₃ solution, brine, dried over Na₂SO₄, filtered and then concentrated *in vacuo*. Crude material obtained was purified by silica gel column chromatography with a 1:10 to 1:1 ethyl acetate : DCM gradient solvent system to give 5.64 g (91%) of **65** as a white solid. *R*_f = 0.37 (DCM); mp 94–95°C; IR (neat) 1741 (C=O), 1323, 1144, 1114 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 8.28 (1 H, s), 8.12 (2 H, dt, *J* = 4.9, 4.7, 3.1 Hz), 7.52–7.31 (8 H, m), 5.41 (2 H, s). ¹³C NMR (126 MHz, CDCl₃) δ 162.49, 161.13, 143.84, 135.47, 134.32, 131.15, 128.96, 128.78, 128.57, 128.50, 128.46, 128.41, 126.86, 126.35, 66.79. HRMS calcd. For C₁₇H₁₃NO₃, 280.0974 found 280.0973. Elemental calcd. for C₁₇H₁₃NO₃: C, 73.11; H, 4.69; N, 5.02; found C, 72.82; H, 4.67; N, 4.96.

Benzyl 2-(4-fluoro-3-nitrophenyl)oxazole-4-carboxylate (66)—(S)-4-Fluoro-3-nitrobenzyl 2-(benzamido)-3-hydroxypropanoate (**29**, 5.10 g, 14.8 mmol) was dissolved in 200 mL of dry DCM and chilled in an ice bath (0°C bath temperature). DBU (6.6 mL, 44.4 mmol) was added followed by slow addition of bromo-trichloromethane (4.4 mL, 44.4 mmol). The reaction was stirred for 1 h cold and then allowed to warm to room temperature. Once the reaction appeared complete by TLC analysis, the reaction mixture was poured into 10% aqueous NaHCO₃ solution and extracted with DCM (2×). The combined organic layers were washed again with NaHCO₃, brine dried over Na₂SO₄, filtered and then concentrated *in vacuo*. Crude material obtained was purified by silica gel column chromatography with a 1:10 to 1:1 ethyl acetate : DCM gradient solvent system to give 3.1 g (60%) of **66** as a light yellow solid upon standing. *R*_f = 0.29 (DCM); mp 120–121°C; IR (neat) 1732 (C=O), 1534 (C=O), 1349, 1322, 1151 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 8.79 (1 H, dd, *J* = 7.0, 2.2 Hz), 8.40

(1 H, ddd, $J = 8.8, 4.2, 2.3$ Hz), 8.33 (1 H, d, $J = 2.6$ Hz), 7.50-7.31 (6 H, m), 5.41 (2 H, s). ^{13}C NMR (126 MHz, CDCl_3) δ 160.62, 159.33, 157.89, 155.74, 144.60, 135.17, 134.85, 133.59, 128.67, 128.62, 124.70, 124.68, 123.53, 123.50, 119.57, 119.40, 67.15. For $\text{C}_{17}\text{H}_{11}\text{FN}_2\text{O}_5$, 343.0730 found 343.0751. Elemental calcd. for $\text{C}_{17}\text{H}_{11}\text{FN}_2\text{O}_5$: C, 59.65; H, 3.24; F, 5.55; N, 8.18; found C, 59.83; H, 3.29; N, 8.00.

Benzyl 2-(2-chlorophenyl)oxazole-4-carboxylate (68)—2-Chlorobenzoyl chloride (5.39 g, 29.9 mmol) was dissolved in 150 mL of DCM and then L-serine benzyl ester hydrochloride (7.49 g, 31.4 mmol) and diisopropylethylamine (13 mL, 74.7 mmol) were added while the reaction was chilled in an ice bath (0°C bath temperature). The reaction mixture was warmed to room temperature and then heated to reflux, 50°C (bath temperature) for 14 h. The reaction mixture was cooled, washed with 10% aqueous NaHCO_3 solution (2 \times), water, 0.5 N citric acid (2 \times) and then brine. The organic phase was collected and dried over sodium sulfate (Na_2SO_4), filtered and then concentrated *in vacuo*. The crude material obtained was purified using silica gel column chromatography with 5% to 50% ethyl acetate : DCM as the solvent system to give (S)-benzyl 2-(2-chlorobenzamido)-3-hydroxypropanoate (9.9 g, 99%) as a yellow oil which solidified upon standing. R_f product = 0.16 (DCM). ^1H NMR (300 MHz, CDCl_3) δ 7.72-7.64 (1 H, m), 7.46-7.18 (8 H, m), 5.28 (2 H, d, $J = 12.2$ Hz), 4.97-4.86 (1 H, m), 4.10 (2 H, s).

(S)-Benzyl 2-(2-chlorobenzamido)-3-hydroxypropanoate (5.9 g, 16.3 mmol) was dissolved in DCM (160 mL) and cooled to -78°C (bath temp, dry ice-acetone). DAST (2.4 mL, 18.7 mmol) was added slowly to the cold mixture under argon where it stirred for 30 min. K_2CO_3 (6.0 g, 43.9 mmol) was added and the reaction was allowed to warm to room temperature. Once the reaction appeared complete by TLC analysis, the reaction mixture was poured into 10% aqueous NaHCO_3 and extracted with DCM (2 \times). The organic layer washed again with the NaHCO_3 solution, brine, dried over Na_2SO_4 , filtered and then concentrated *in vacuo*. Crude material obtained was purified by silica gel column chromatography with a 1:10 to 1:1 ethyl acetate : DCM gradient solvent system to give 4.9 g (87%) of (S)-benzyl 2-(2-chlorophenyl)-4,5-dihydrooxazole-4-carboxylate, **16**, as a white solid. R_f product = 0.36 (DCM). ^1H NMR (300 MHz, CDCl_3) δ 7.82 (1 H, dd, $J = 7.6, 1.7$ Hz), 7.49-7.27 (8 H, m), 5.33-5.19 (2 H, m), 5.04 (1 H, dd, $J = 10.6, 7.7$ Hz), 4.77-4.58 (2 H, m).

(S)-Benzyl 2-(2-chlorophenyl)-4,5-dihydrooxazole-4-carboxylate (**16**, 5.88 g, 15.2 mmol) was dissolved in 100 mL of dry DCM and chilled in an ice bath (0°C bath temperature). DBU (6.8 mL, 45.5 mmol) was added followed by slow addition of bromo-trichloromethane (4.5 mL, 45.5 mmol). The reaction was stirred for 1 h cold and then allowed to warm to room temperature. Once the reaction appeared complete by TLC analysis, the reaction mixture was poured into 10% aqueous NaHCO_3 solution and extracted with DCM (2 \times). The organic layer washed again with the NaHCO_3 solution, brine, and dried over Na_2SO_4 , filtered and then concentrated *in vacuo*. Crude material obtained was purified by silica gel column chromatography with a 1:10 to 1:1 ethyl acetate : DCM gradient solvent system to give 4.7 g (81%) of **68** as a white solid. $R_f = 0.59$ (DCM); mp $62-63^\circ\text{C}$ IR (neat) 1741 (C=O), 1314, 1145, 1118 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 8.36 (1 H, d, $J = 1.2$ Hz), 8.09-7.99 (1 H, m), 7.56-7.21 (8 H, m), 5.41 (2 H, s). ^{13}C NMR (126 MHz, CDCl_3) δ 160.94, 160.67, 144.32, 135.44, 134.14, 132.88, 131.86, 131.64, 131.07, 128.59, 128.53, 128.44, 126.85, 125.51, 66.85. For $\text{C}_{17}\text{H}_{12}\text{ClNO}_3$, 314.0584 found 314.0571. Elemental calcd. for $\text{C}_{17}\text{H}_{12}\text{ClNO}_3$: C, 59.65; H, 3.24; F, 5.55; N, 8.18; found C, 65.08; H, 3.86; N, 4.46; found C, 65.07; H, 3.93; N, 4.42.

General Procedure for Preparation of Threonine Amides (9, open L-Threonine form)

Benzoic acid (414 mg, 3.3 mmol, 1 equiv) and L-threonine benzyl ester hydrochloride (874 mg, 3.5 mmol, 1.05 equiv) were dissolved in 7 mL of acetonitrile (ACN) and treated with *N*-

(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC-HCl, 1 g, 6.6 mmol, 2 equiv) and *N,N*-dimethylaminopyridine (DMAP, 1.2 g, 9.9 mmol, 3 equiv). The resulting solution was stirred for 12 h at room temperature. The reaction mixture was diluted with EtOAc, washed with 0.5 N citric acid (2×), water, 10% aqueous NaHCO₃ solution (2×). The organic phase was collected, dried over sodium sulfate (Na₂SO₄), filtered and then concentrated *in vacuo*. Crude material obtained was purified by silica gel column chromatography with a 1:10 to 1:1 ethyl acetate : dichloromethane (DCM) gradient solvent system.

Method 2—Benzoyl chloride (449 mg, 0.4 mL, 3.1 mmol, 0.95 equiv) was dissolved in 6 mL of DCM and then L-threonine benzyl ester hydrochloride (711 mg, 3.3 mmol, 1 equiv) and triethylamine (1 g, 1.4 mL, 9.9 mmol, 3 equiv) were added. The reaction mixture was warmed to room temperature and then heated to reflux, 50°C (bath temperature) for 14 h. The reaction mixture was cooled, washed with 10% aqueous NaHCO₃ solution (2×), water, 0.5 N citric acid (2×) and then brine. The organic phase was collected and dried over Na₂SO₄, filtered and then concentrated *in vacuo*. The crude material obtained was purified by silica gel column chromatography with a 1:10 to 1:1 ethyl acetate : DCM gradient solvent system.

General Procedure for Preparation of Threonine Derived Oxazoline Products (compounds 10, 101 – 105)

L-Threonine amide **9** (330 mg, 1.1 mmol, 1 equiv) was dissolved in DCM (5 mL) and cooled to –78°C (bath temp, dry ice-acetone). DAST (0.16 mL, 1.3 mmol, 1.2 equiv) was added slowly to the cold mixture under argon and the reaction was stirred for 30 min. Then K₂CO₃ (393 mg, 2.8 mmol, 2.7 equiv) was added and the reaction was allowed to warm to room temperature. Once the reaction appeared complete by TLC analysis, the reaction mixture was poured into 10% aqueous NaHCO₃ solution and extracted with DCM (2×). The combined organic layers were washed with the NaHCO₃ solution, brine, dried over Na₂SO₄, filtered and then concentrated *in vacuo*. Crude material obtained was purified by silica gel column chromatography with a 1:10 to 1:1 ethyl acetate : DCM as the gradient solvent system.

General Procedure for Preparation of Threonine Derived Oxazoles (compounds 11, 106 – 111)

Threonine derived oxazoline **10** (195 mg, 0.66 mmol, 1 equiv) was dissolved in 2 mL of dry DCM and chilled in an ice bath (0°C bath temperature). DBU (0.3 mL, 2 mmol, 3 equiv) was added followed by slow addition of bromotrichloromethane (0.2 mL, 2 mmol, 3 equiv). The reaction was stirred for 1 h cold and then allowed to warm to room temperature. Once the reaction appeared complete by TLC analysis, the reaction mixture was poured into 10% aqueous NaHCO₃ solution and extracted with DCM (2×). The combined organic layers were washed again with the NaHCO₃ solution, brine, dried over Na₂SO₄, filtered and then concentrated *in vacuo*. Crude material obtained was purified by silica gel column chromatography with a 1:10 to 1:1 ethyl acetate : DCM gradient solvent system.

Anti-tuberculosis and Cytotoxicity Assays

Activity against replicating *Mycobacterium tuberculosis* H₃₇Rv (ATCC 27294, American Type Culture Collection, Rockville, MD) was determined using a fluorescence readout in the Microplate Alamar Blue Assay (MABA) [32] following incubation for one week with test compounds in glycerol-alanine-salts medium (GAS) as well as in medium without added iron but with Tween 80 (GAST) [33]. The MIC was defined as the minimum concentration inhibiting fluorescence by 90% relative to bacteria-only controls. Activity against non-replicating *M. tuberculosis* was determined using the Low Oxygen Recovery Assay (LORA) [34] by exposing low oxygen-adapted *M. tuberculosis* containing a luciferase gene to test compounds under anaerobic conditions for 10 days. After a 28 hour recovery in air, the

luciferase signal was measured. MIC was defined as the lowest concentration inhibiting recovery of luciferase signal by $\geq 90\%$ relative to bacteria-only controls. Toxicity to African green monkey kidney cells (VERO) was determined using a colorimetric assay as previously described [32].

Simulated Gastric Fluid Stability

Compounds were incubated in simulated gastric fluid [35] (consisting of 3.2 mg/mL pepsin in 0.03 M sodium chloride at pH 1.2) at 37°C at a concentration of 50 μM . Aliquots (500 μL) were taken at 0 hr and 2 hr, 500 μL of 10% sodium bicarbonate containing internal standard was added, followed by centrifugation for 5 min at 12,000 rpm. Aliquots of the supernatants were analyzed by reversed-phase HPLC with UV detection at 254 nm. The mobile phase consisted of elution on a YMC 5 μ -basic 4.6 mm i.d. \times 15 cm column at 1 mL/min with 5-min isocratic elution with 10% acetonitrile/90% water, 20-min linear gradient from 10% acetonitrile to 90% acetonitrile, and 10-min isocratic elution with 90% acetonitrile/10% water. The peak area ratio relative to internal standard was measured and compared to that at time 0 and reported as percent remaining.

Metabolic Stability

Compounds (20 μM) were mixed with NADPH (0.5 mM) in potassium phosphate buffer (50 mM, pH 7.4) in a total volume of 0.5 mL and preincubated at 37 °C. The reaction was started by adding pooled male Sprague-Dawley liver microsomes (0.2 mg protein). After 15-min incubation, the reaction was quenched with 0.5 mL acetonitrile containing internal standard and centrifuged to remove protein. A second incubation was terminated at time 0. An aliquot of the supernatant was analyzed by reversed-phase HPLC with UV detection at 254 nm. The chromatographic conditions were the same as for the gastric fluid stability. The peak area ratio relative to internal standard was measured and compared relative to that at time 0 and reported as percent remaining.

HPLC chromatography—The HPLC system consisted of a Perkin-Elmer series 200 quaternary pump (Perkin-Elmer Corp., Norwalk, CT), a Perkin-Elmer UV detector series 200, and a Perkin-Elmer auto sampler series 200. Samples were analyzed on a YMC 5 μm basic 4.6 mm i.d. \times 15 cm column (YMC Inc., Wilmington, NC) connected to a YMC 5 μm basic 4.0 mm i.d. \times 2 cm guard cartridge.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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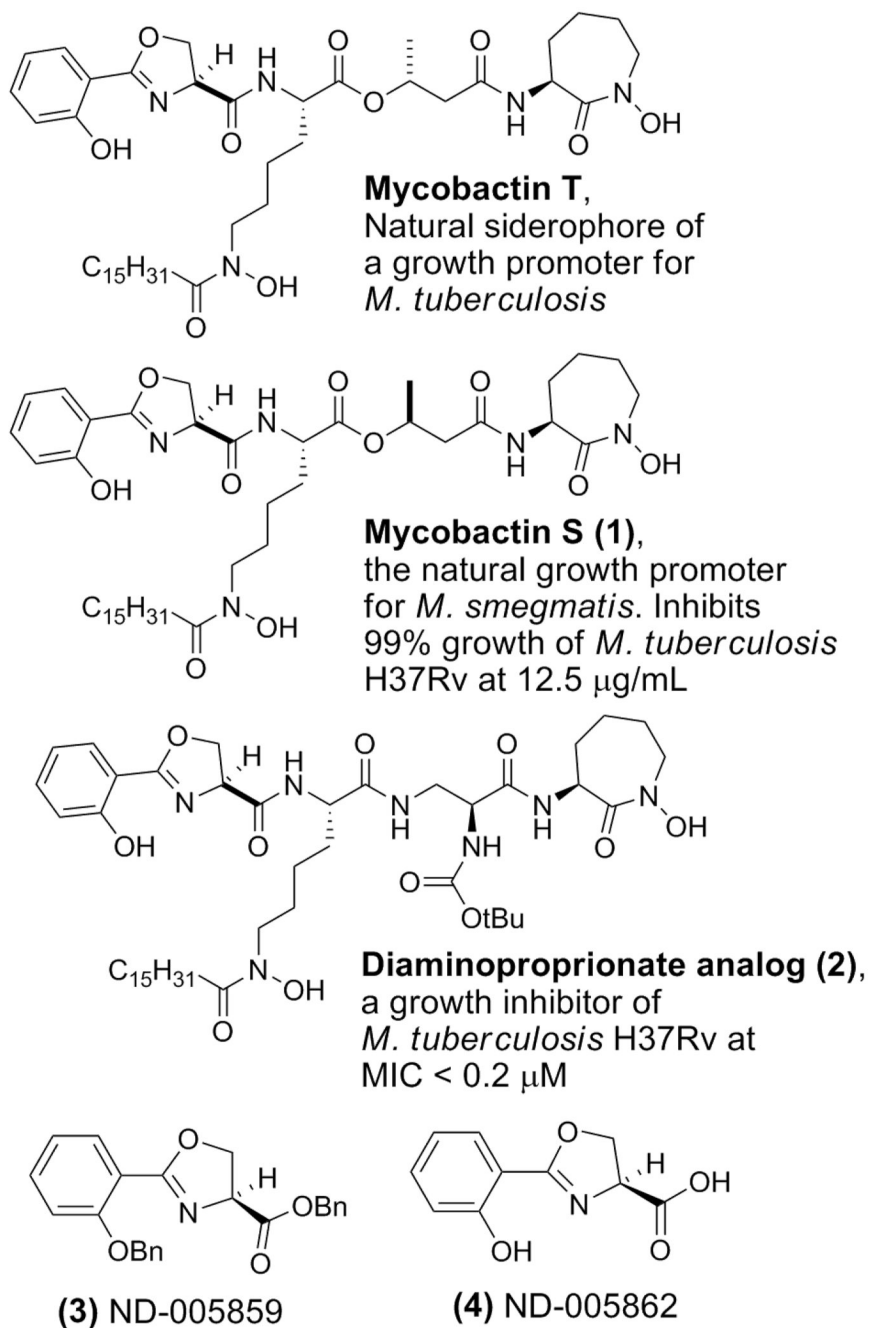


Figure 1.
Mycobactins T and S and the synthetic anti-TB analogs (diaminopropionate siderophore, ND-005859, and ND-005862).

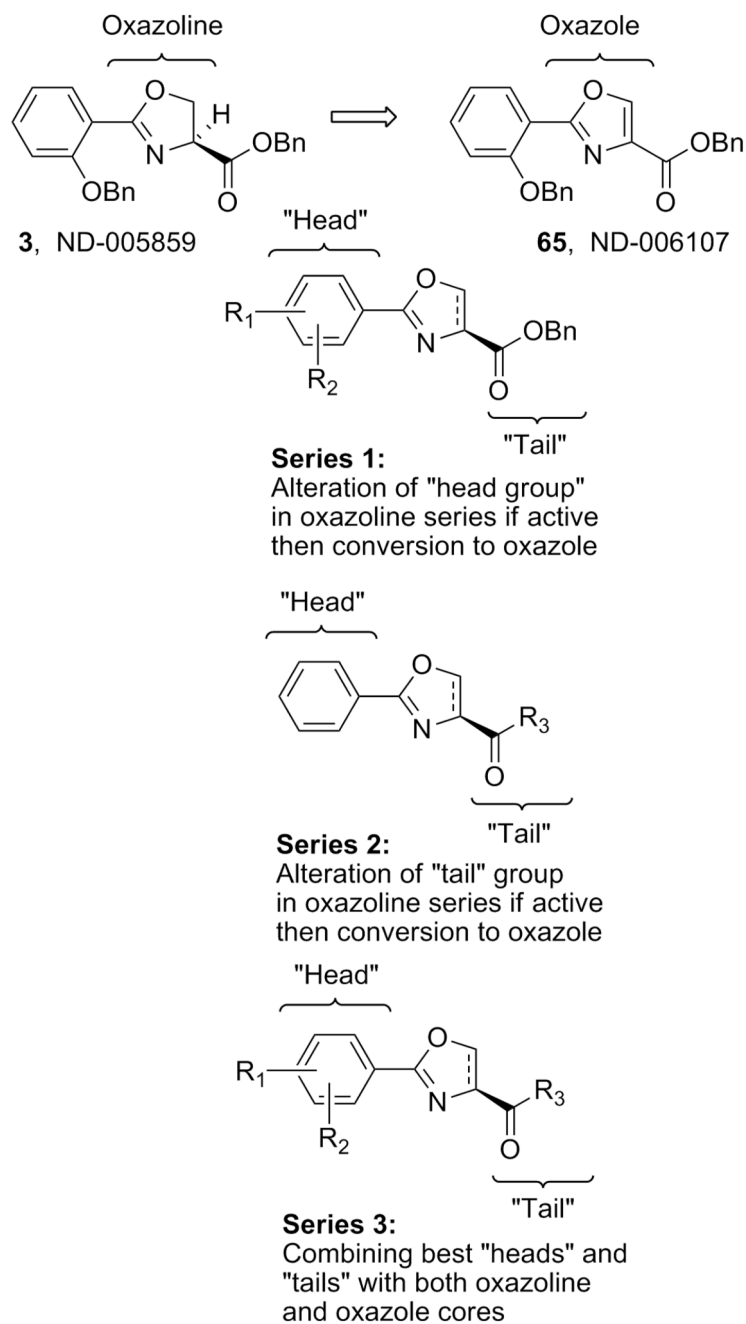
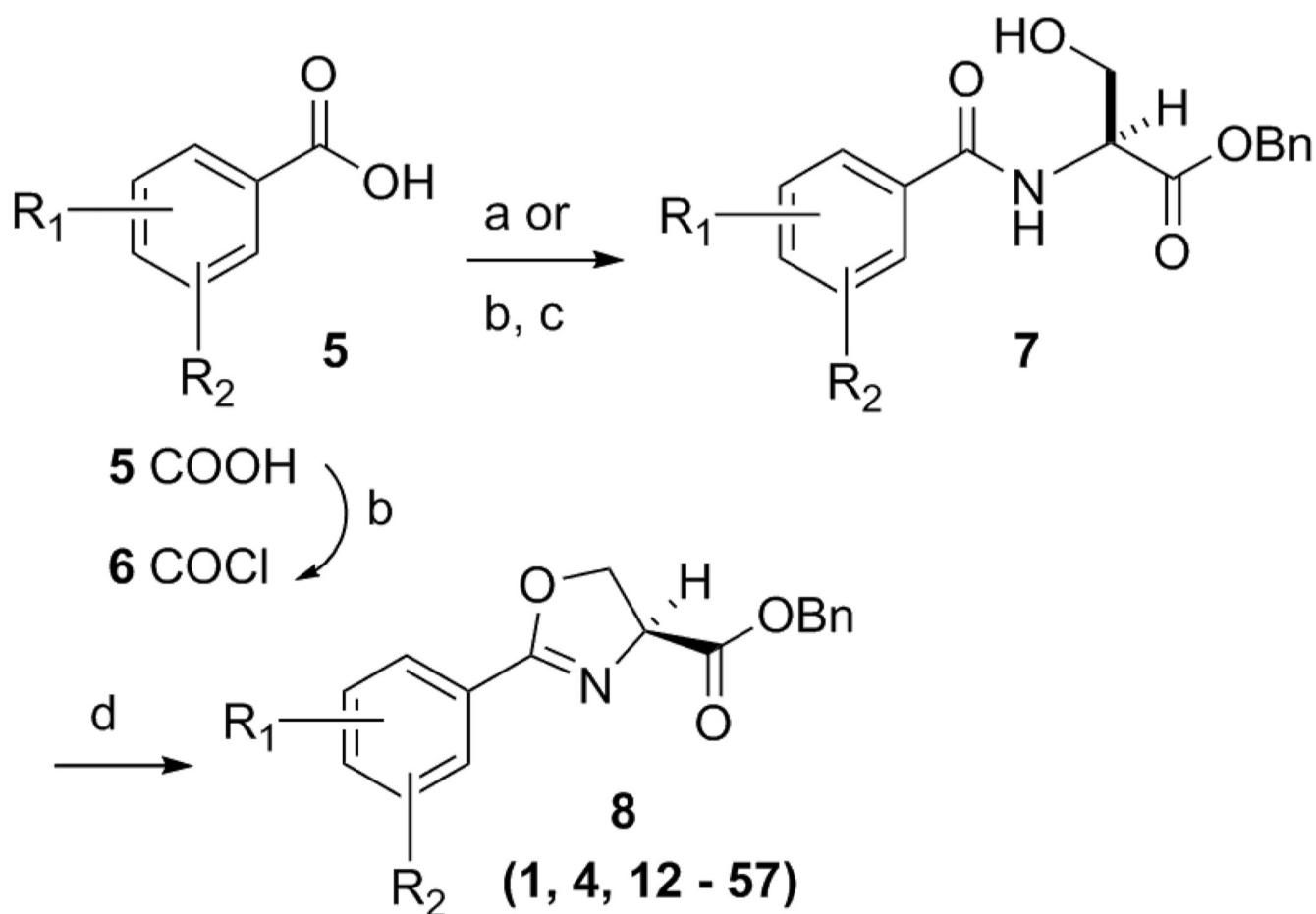
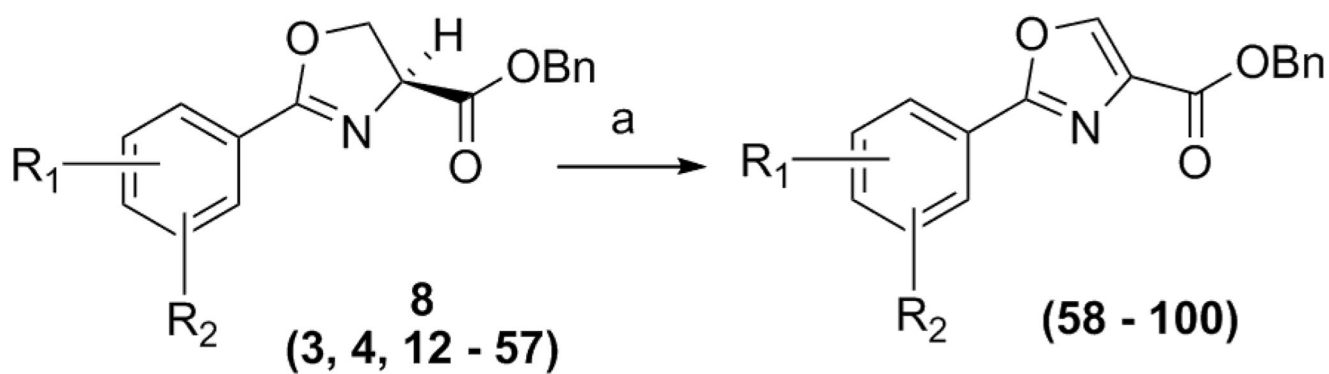


Figure 2.
Derivatization strategy of **3**, an anti-TB "hit" compound.

**Scheme 1a.**

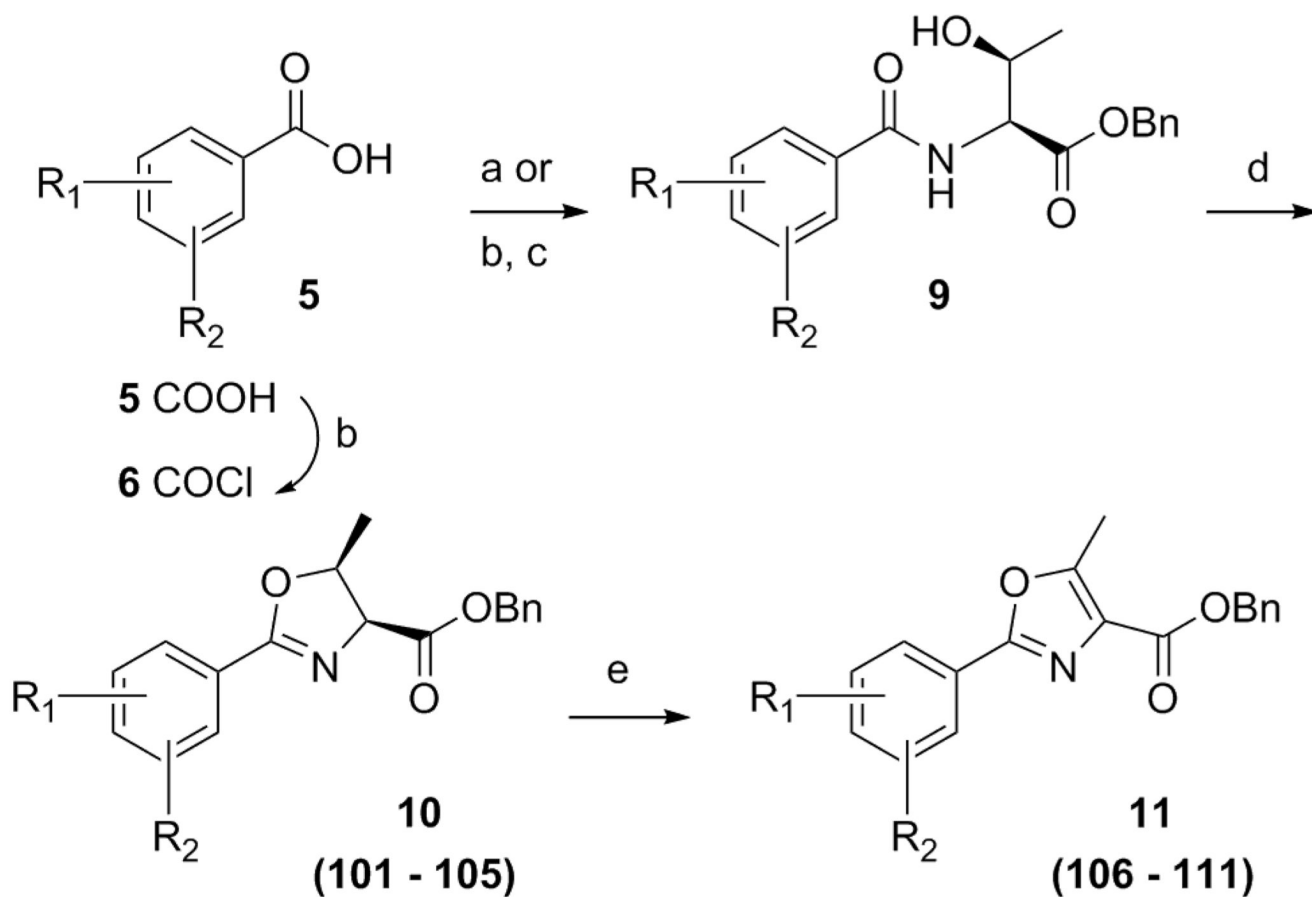
Synthesis of oxazoline analogs.

^aReagents: (a) L-Serine-OBn, EDC, DMAP, ACN, room temp, 14 h; (b) Oxalyl chloride, DCM, DMF (catalytic), 4 h, room temp; (c) L-Serine-OBn, DIPEA, DCM, 14 h, 50°C; (d) DAST, K₂CO₃, -78°C, 1 h.

**Scheme 2a.**

Conversion of oxazoline analogs to oxazoles.

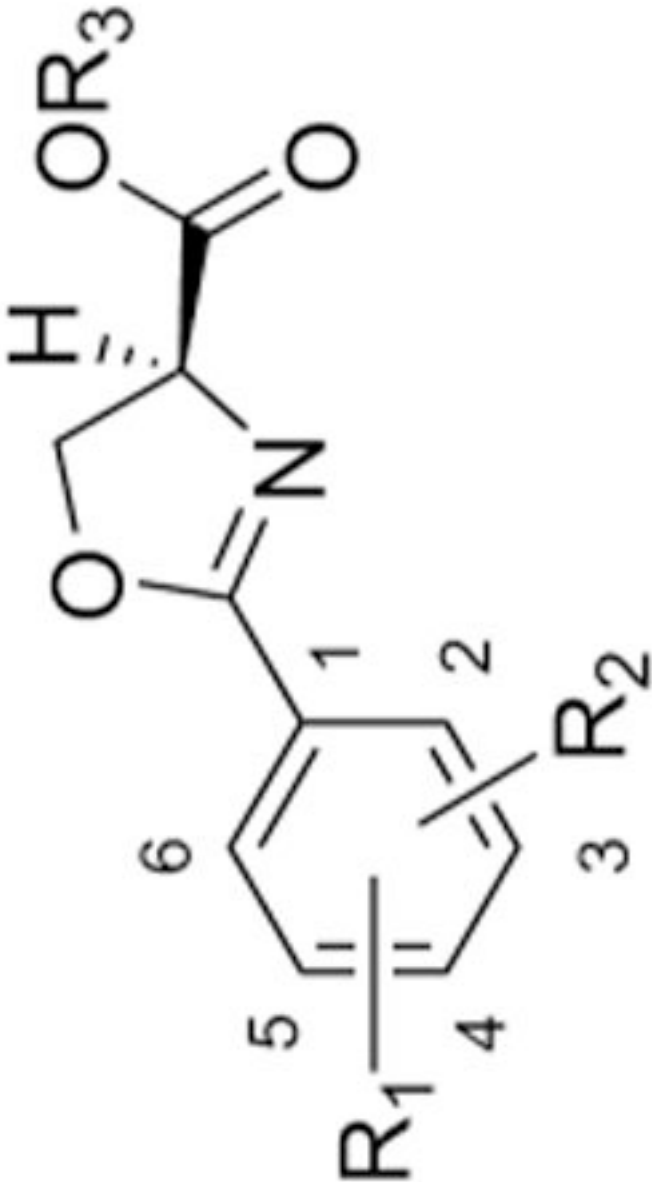
^aReagents: (a) DBU, BrCCl₃, DCM, 0°C to RT, 1 h to 14 h.

**Scheme 3a.**

Preparation of threonine derived oxazoline and oxazole analogs.

^aReagents: (a) L-Threonine-OBn, EDC, DMAP, ACN, room temp, 14 h; (b) Oxalyl chloride, DCM, DMF (catalytic), 4 h, room temp; (c) L-Threonine-OBn, DIPEA, DCM, 14 h, 50°C (d) DAST, K₂CO₃, -78°C, 1 h. (e) DBU, BrCCl₃, DCM, 0°C to RT, 1 h.

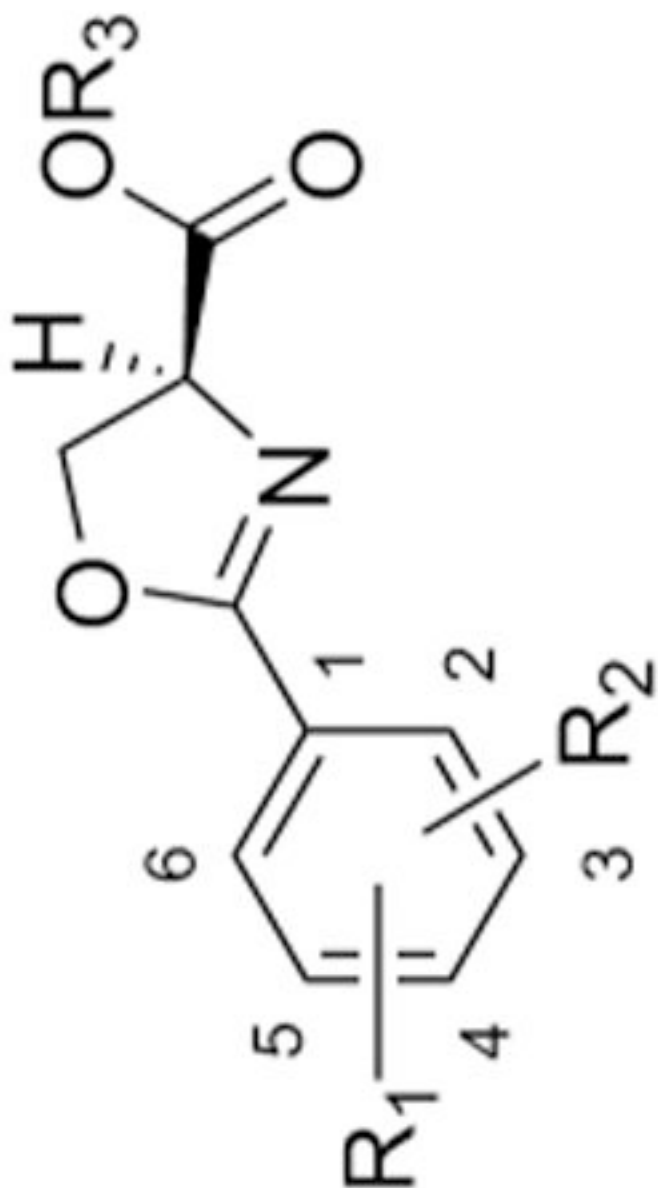
Table 1



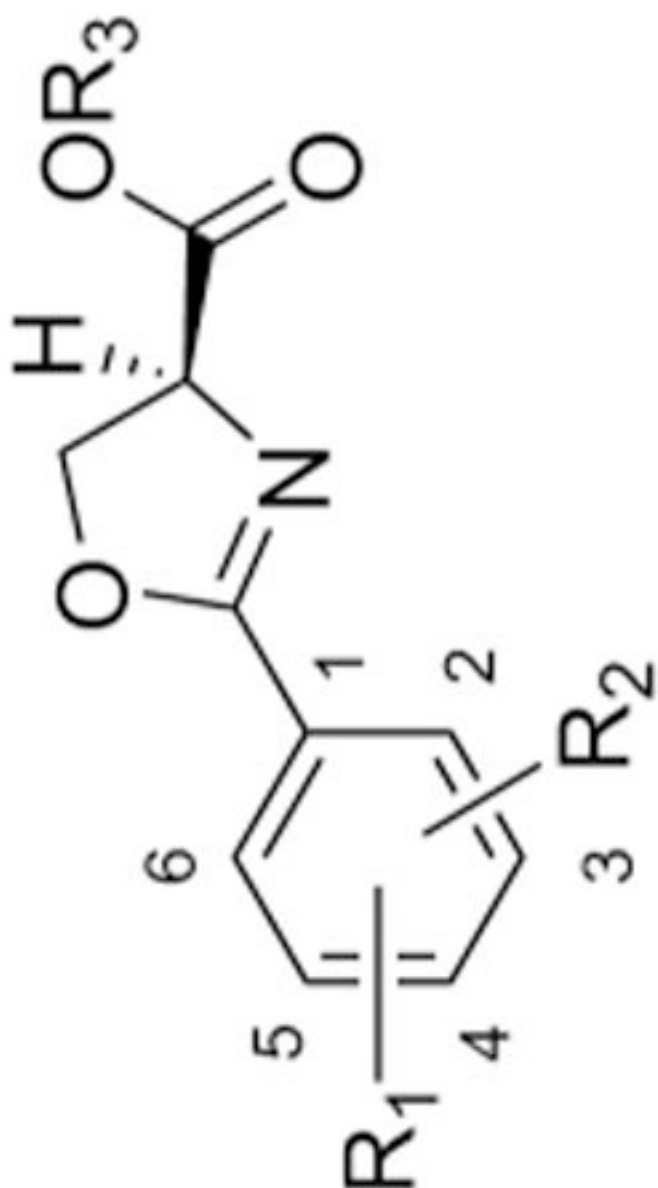
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R ¹	R ²	R ³	MolWt	ClogP ^a	GAS (μM)	GAST (μM)	VERO Cells: IC50 (μM)	NRP-TB: LORA (μM)
H	2-OBn	Bn	387.44	5.77	12.6 [*]	7.69 [*]	>128	67.3
H	2-OH	H	207.19	4.06	>128	>128	>128	
H	2-OBn	Me	311.34	4.06	118	>128	>128	
H	H	H	191.19	1.93	>128	>128	>128	
H	2-OH	Me	221.21	1.71	>128	>128	>128	
H	H	Bn	281.31	4.08	7.20 [*]	4.42 [*]	>128	63
H	2-Cl	Bn	315.75	4.79	49.9	15.2		
H	3-Cl	Bn	315.76	4.79	11.6	1.80	>128	
4-Cl	H	Bn	315.75	4.79	61.0	14.9		

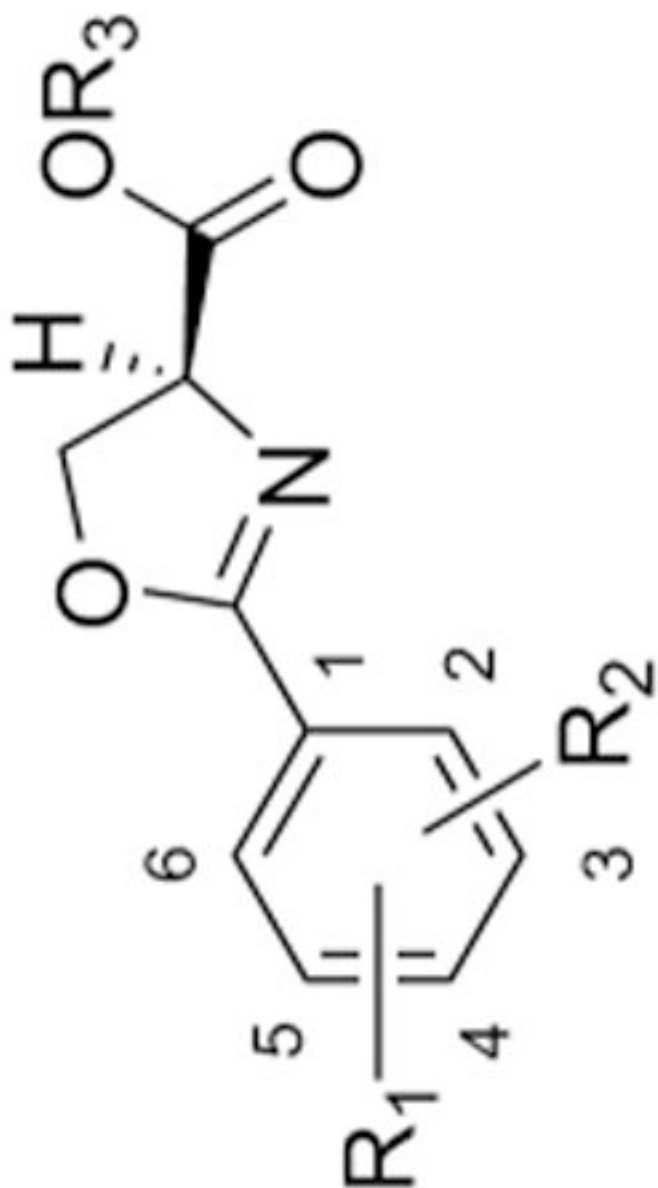
1) compounds.



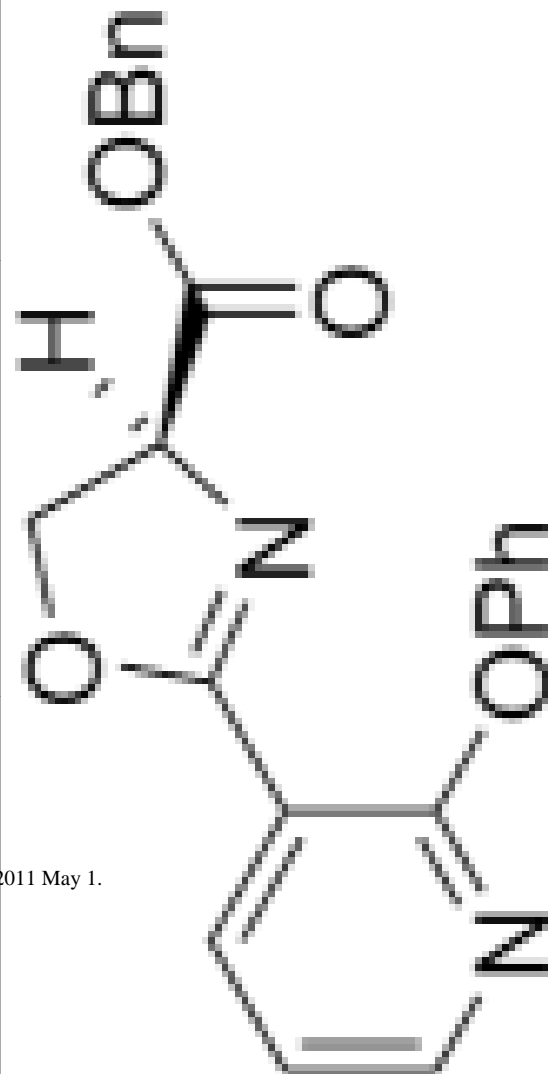
R ¹	R ²	R ³	MolWt	ClogP ^a	GAS (μM)	GAST (μM)	VERO Cells: IC50 (μM)	NRP-TB: LORA (μM)
H	3-Cl	Me	239.66	3.09	>128	68.7		
H	3-Cl	H	225.63	3.09	>128	106		
4-OMe	H	Bn	311.34	4.00	26.1	2.29	>128	
4-OEt	H	Bn	325.36	4.53	60.6	30.5		
4-OMe	3-Cl	Bn	345.78	4.62	60.7	7.58		
4-OMe	3-F	Bn	329.32	4.08	50.5	3.91		
H	3-F	Bn	299.3	4.22	60.7	6.86		
4-Cl	3-F	Bn	333.74	4.94	52.0	6.49		
4-NO2	H	Me	250.21	2.12	>128	>128		
4-NO2	H	Bn	326.31	3.82	30.6	7.59	>128	
4-F	3-NO2	Bn	344.29	3.67	6.24	0.95	>128	26
4-NO2	3-F	Bn	344.29	3.67	30.2	3.68	>128	59

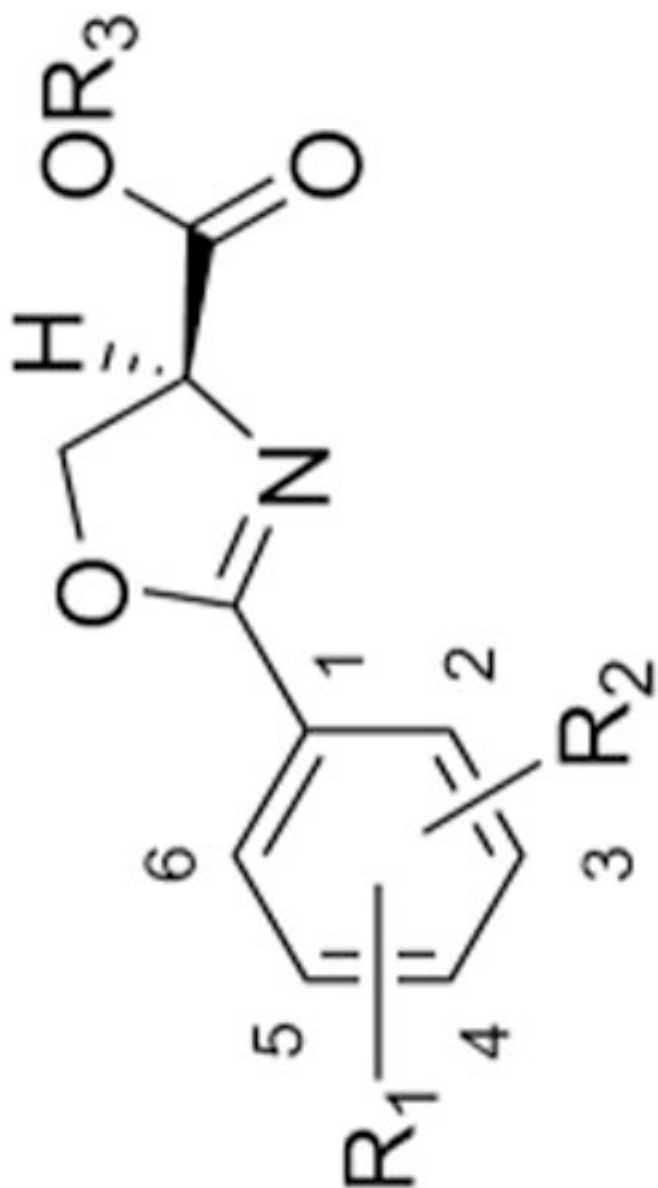


R ¹	R ²	R ³	MolWt	ClogP ^a	GAS (μM)	GAST (μM)	VERO Cells: IC50 (μM)	NRP-TB: LORA (μM)
3-Cl	2-OBn	Bn	421.87	6.39	6.30	34.0		
4-F	2-NO2	Bn	344.29	3.97	30.7	40.4		
4-NO2	2-F	Bn	344.29	3.97	13.0	8.70		
			390.45	5.50	17.8	13.4		

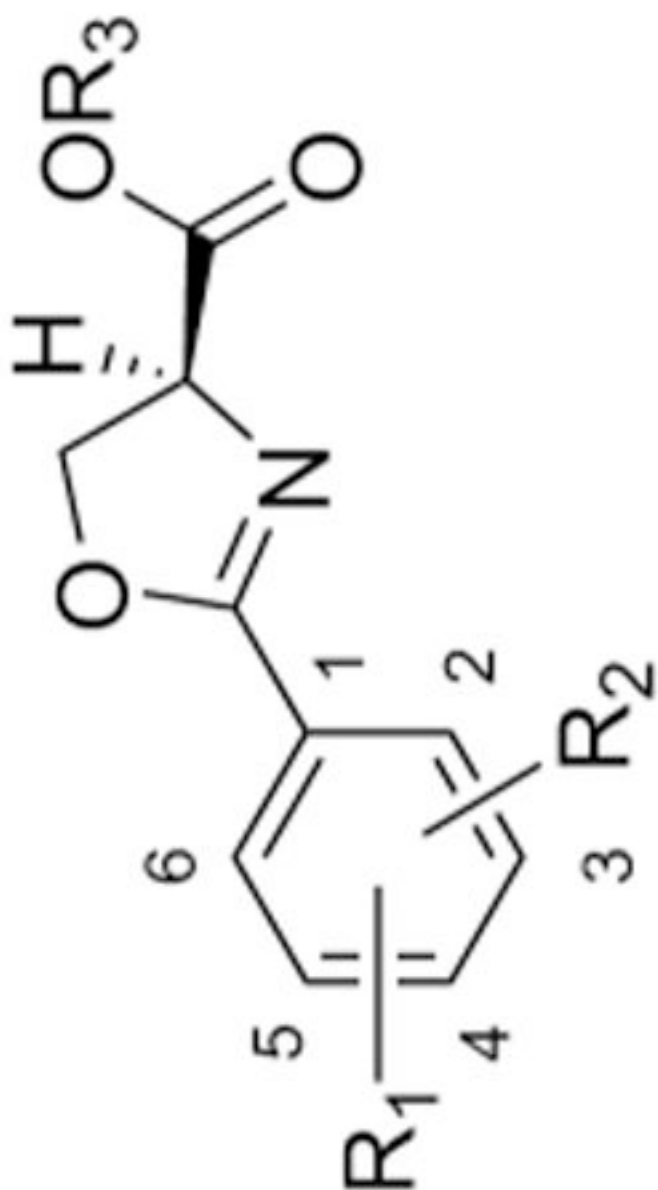


R ¹	R ²	R ³	MolWt	ClogP ^a	GAS (μM)	GAST (μM)	VERO Cells: IC50 (μM)	NRP-TB: LORA (μM)
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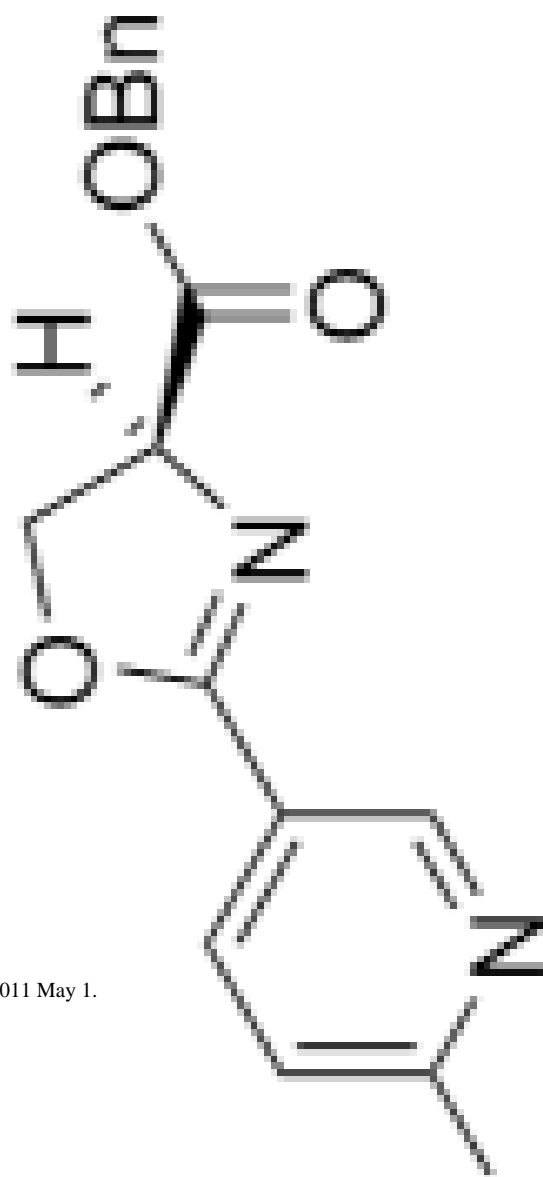


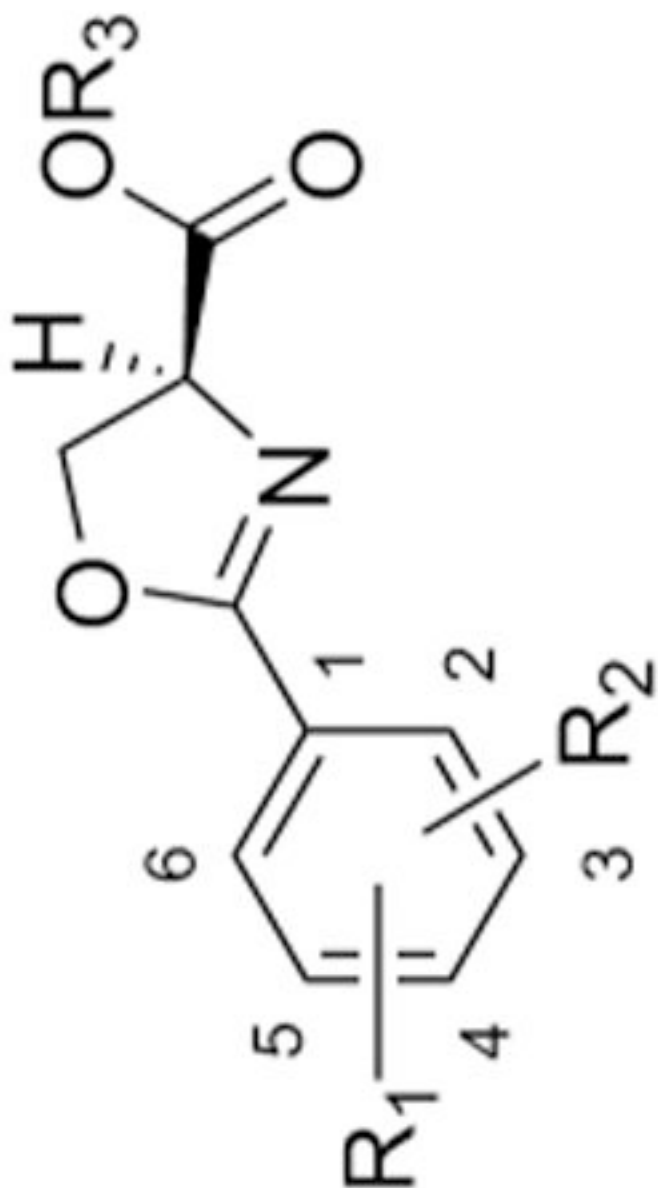


R ¹	R ²	R ³	MolWt	ClogP ^a	GAS (μM)	GAST (μM)	VERO Cells: IC50 (μM)	NRP-TB: LORA (μM)
			361.19	3.53	27.6	26.6		

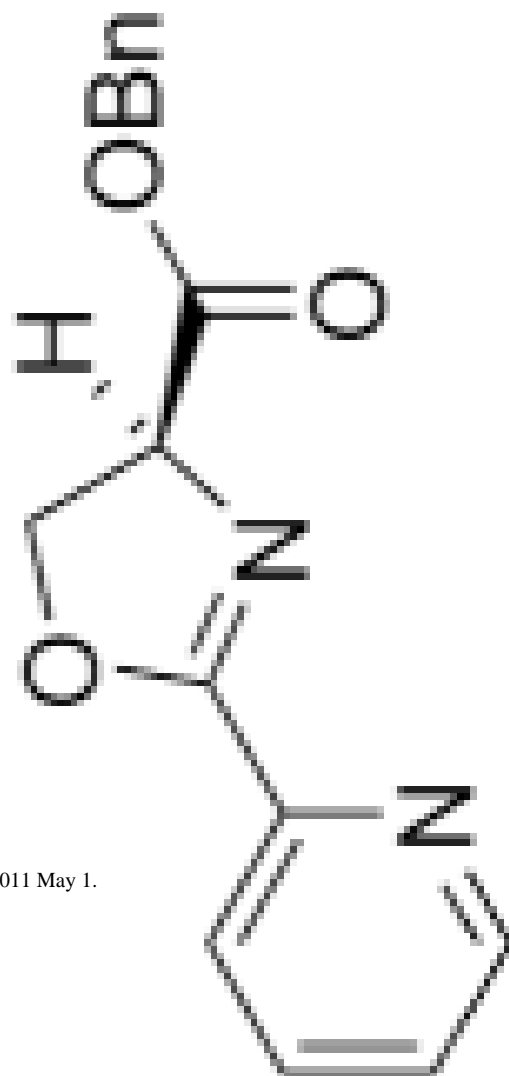


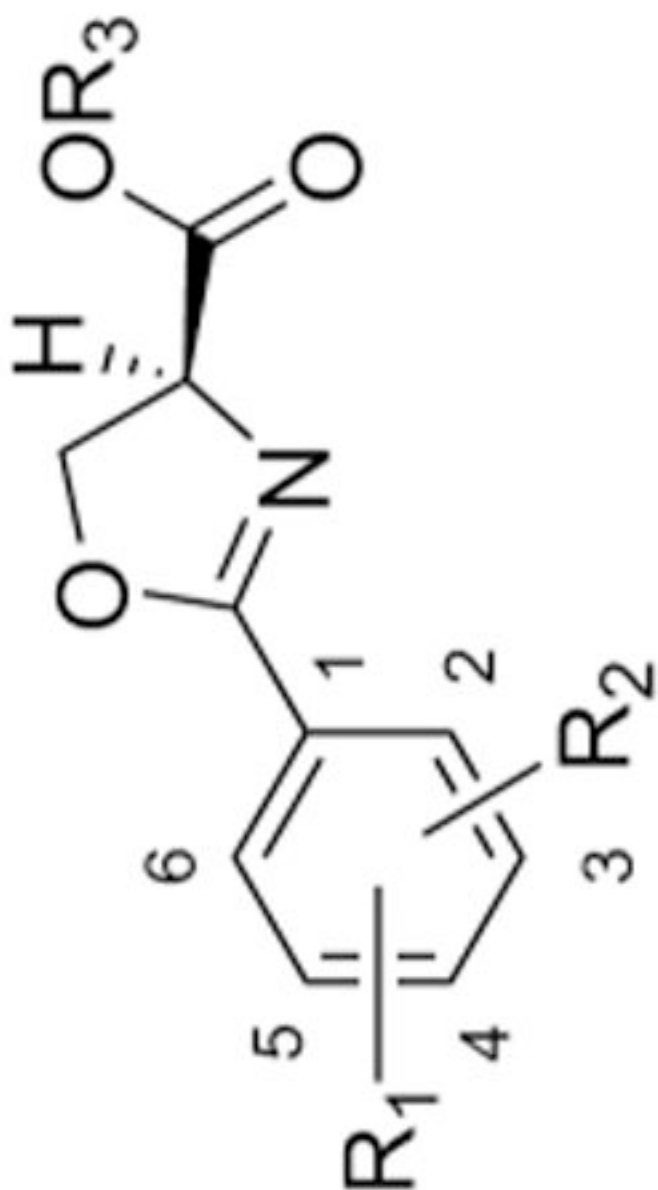
R ¹	R ²	R ³	MolWt	ClogP ^a	GAS (μ M)	GAST (μ M)	VERO Cells: IC50 (μ M)	NRP-TB: LORA (μ M)
			361.19	3.53	22.9	21.1		



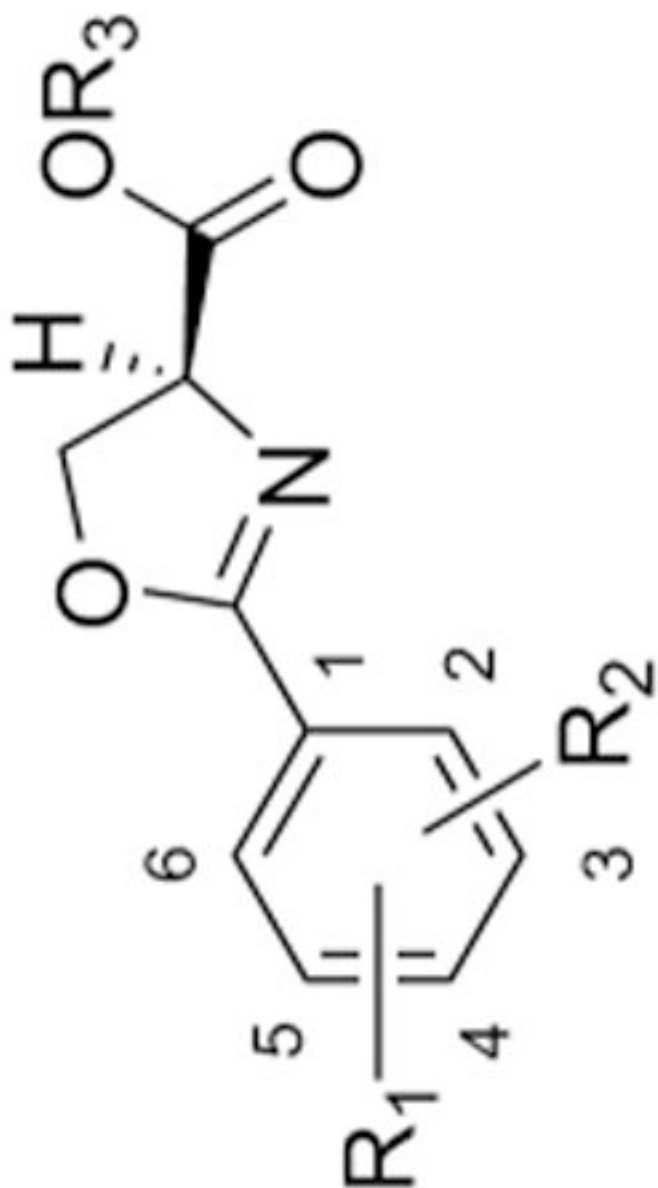


R ¹	R ²	R ³	MolWt	ClogP ^a	GAS (μM)	GAST (μM)	VERO Cells: IC50 (μM)	NRP-TB: LORA (μM)
			282.29	2.58	110	127		

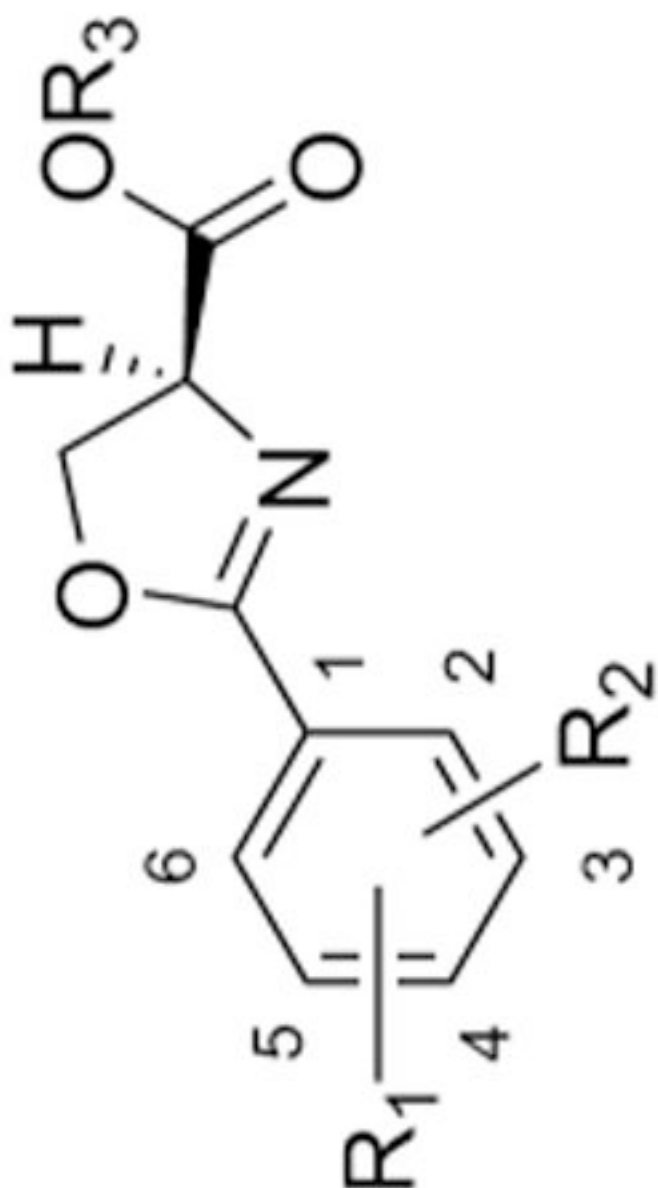


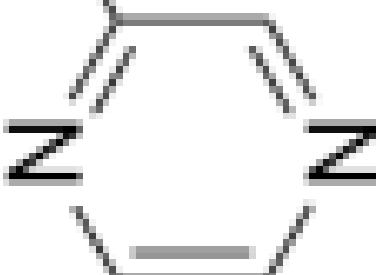
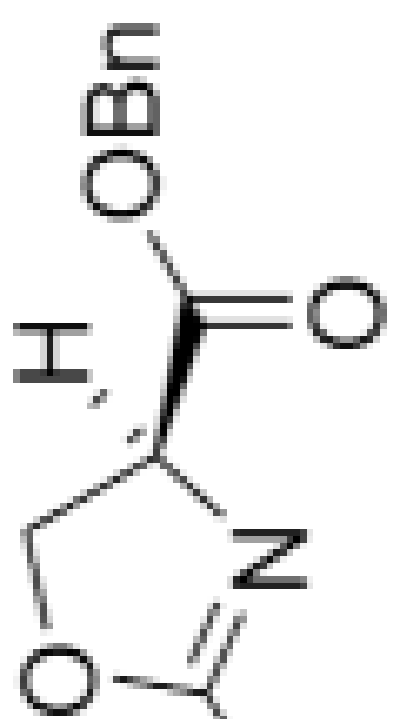


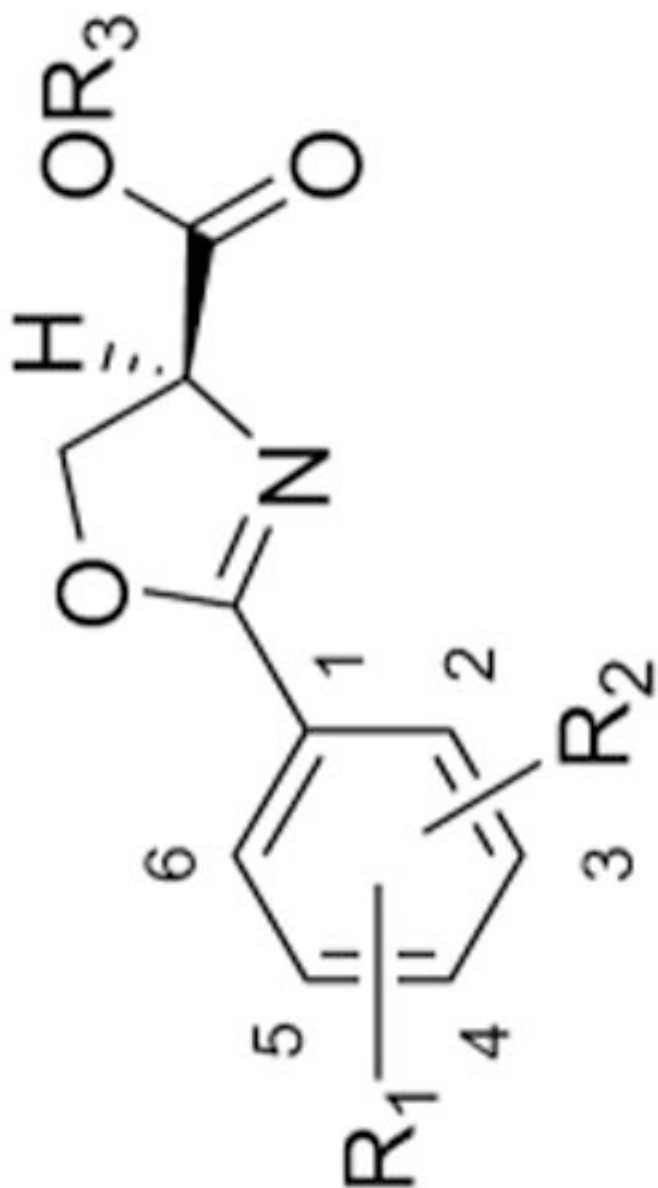
R ¹	R ²	R ³	MolWt	Clog _P ^a	GAS (μM)	GAST (μM)	VERO Cells: IC50 (μM)	NRP-TB: LORA (μM)
			282.29	2.58	53.3	92.9		



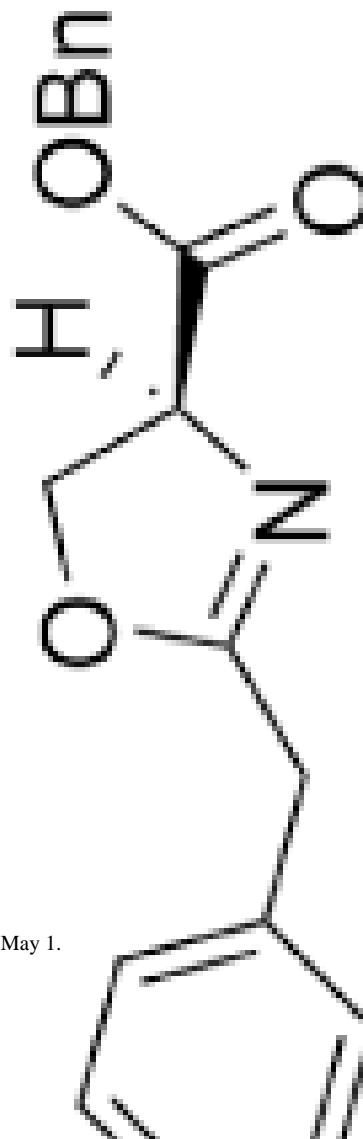
R ¹	R ²	R ³	MolWt	ClogP ^a	GAS (μM)	GAST (μM)	VERO Cells: IC50 (μM)	NRP-TB: LORA (μM)
			282.29	2.58	55.6	81.3		

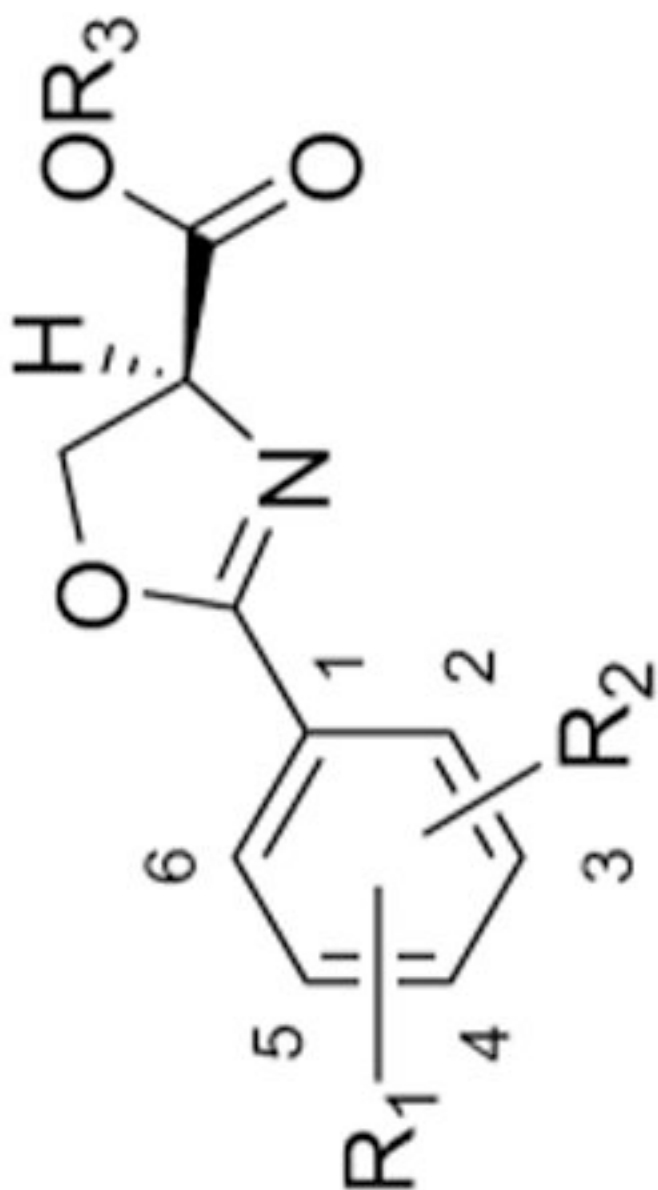


R ¹	R ²	R ³	MolWt	ClogP ^a	GAS (μM)	GAST (μM)	VERO Cells: IC50 (μM)	NRP-TB: LORA (μM)
			283.28	1.63	61.2	>128		

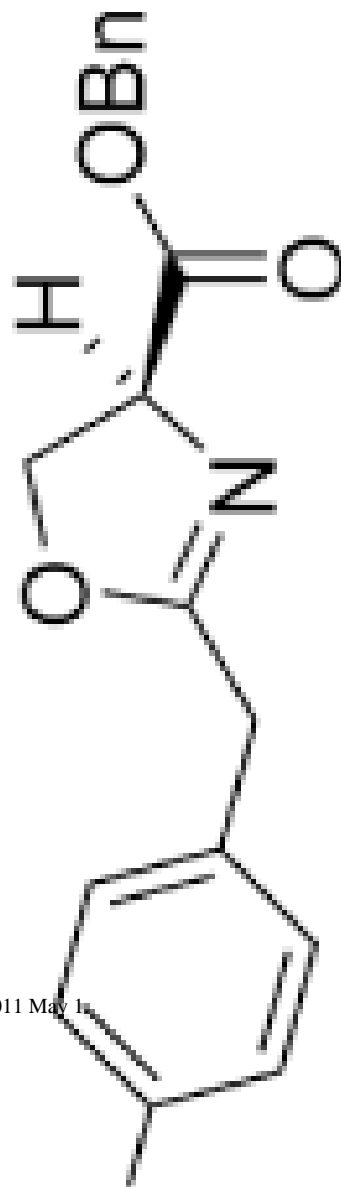


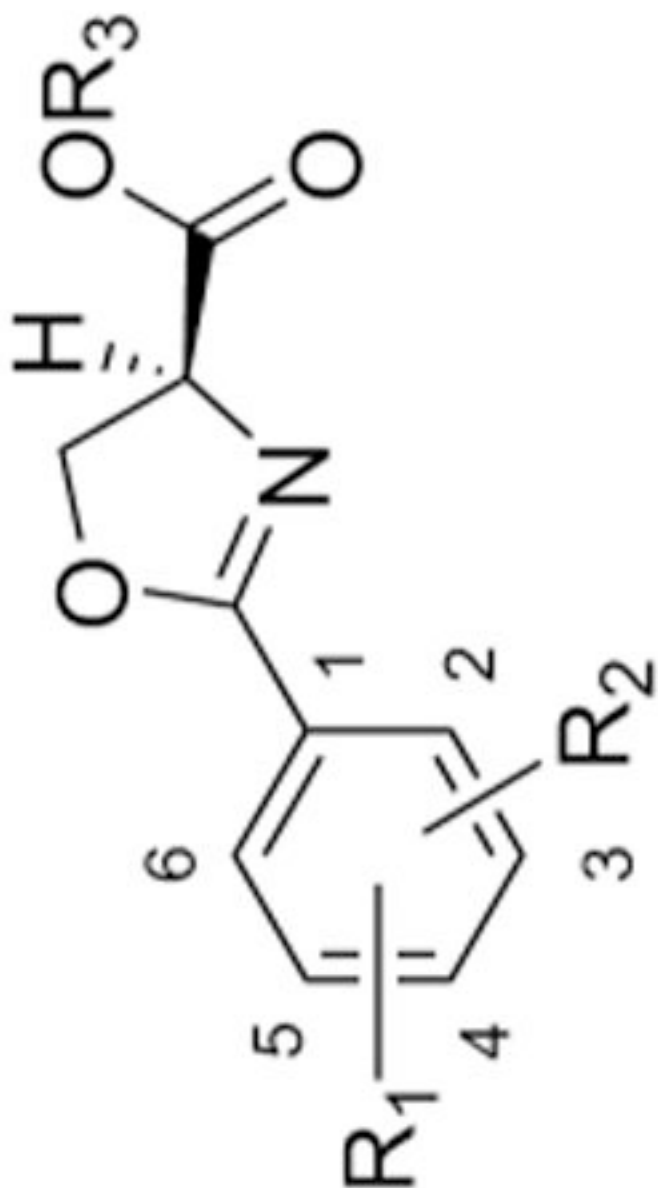
R ¹	R ²	R ³	MolWt	ClogP ^a	GAS (μM)	GAST (μM)	VERO Cells: IC50 (μM)	NRP-TB: LORA (μM)
4-OMe	3-OMe	Bn	341.36	3.74	39.0	21.6		
			295.33	3.26	>128	>128		



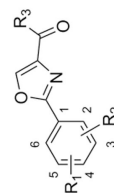


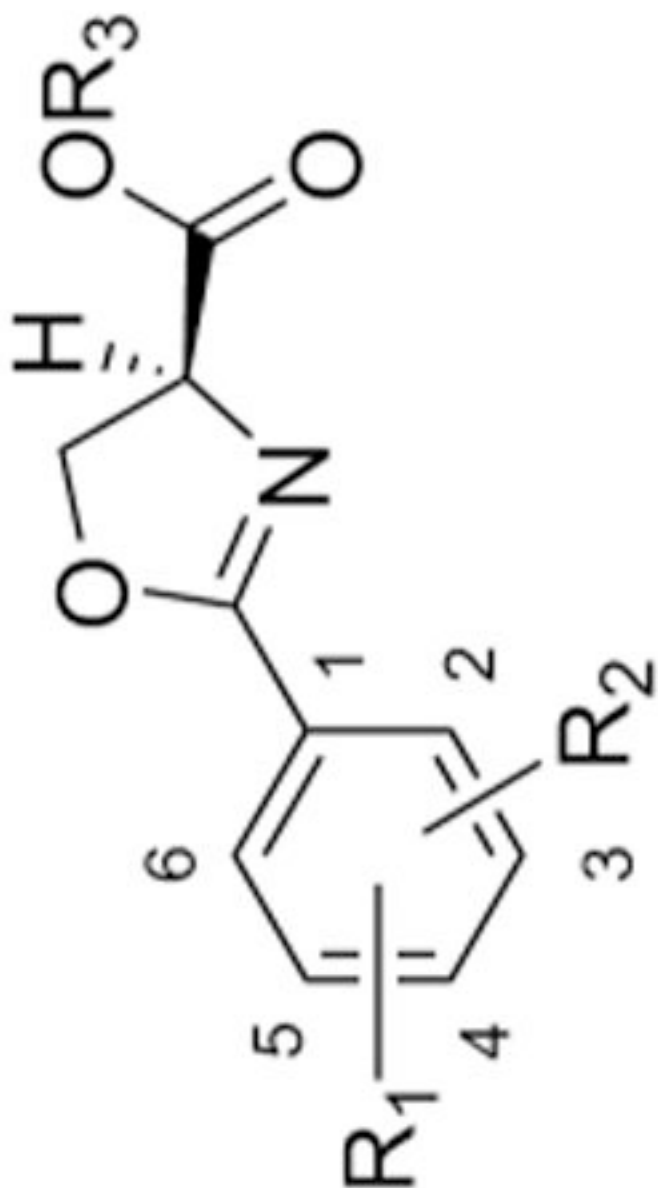
R ¹	R ²	R ³	MolWt	ClogP ^a	GAS (μM)	GAST (μM)	VERO Cells: IC50 (μM)	NRP-TB: LORA (μM)
H	2-OMe	Bn	311.33	4.00	37.0	29.2		
4-OPr	H	Bn	339.39	5.06	2.97	22.4		
			325.36	4.01	>128	>128		





R ¹	R ²	R ³	MolWt	ClogP ^a	GAS (μM)	GAST (μM)	VERO Cells: IC50 (μM)	NRP-TB: LORA (μM)
4-OiPr	H	Bn	339.39	4.84	3.2	14.9		
4-OBu	H	Bn	353.41	5.59	2.72	5.11		
4-CH3	H	Bn	295.33	4.58	15.5	27.3		
4-NO2	2-OBn	Bn	432.43	5.81	15.6	4.86		
4-OBn	3-F	Bn	405.42	5.85	0.77	1.42	>128	
H	3-OMe	Bn	311.33	4.00	24.8	42.5		
H	3-CN	Bn	306.32	3.51	47.2	75.1		
H	3-Br	Bn	360.20	4.94	24.2	52.5		
			296.32	3.08	73.0	122		





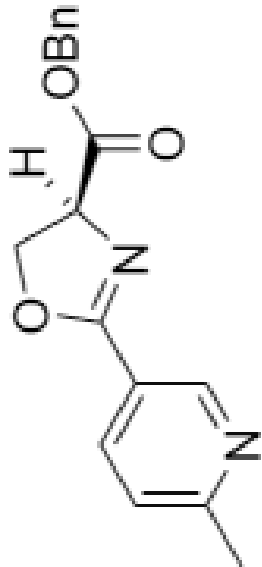
Eur J Med Chem. Author manuscript; available in PMC 2011 May 1.

R ¹	R ²	R ³	MolWt	ClogP ^a	GAS (μ M)	GAST (μ M)	VERO Cells: IC50 (μ M)	NRP-TB: LORA (μ M)
4-NH2		H	358.39	4.47	12.5	29.8		
Rifampin (control)		Bn	296.32	2.85	25.4	26.8		
			822.94		0.06*	0.01*	125*	

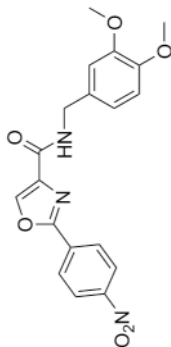
Cambridge Soft ChemDraw Ultra, v 8.0 software. Activity against non-replicating *M. tuberculosis* was determined using the Low Oxygen Recovery Assay (LORA).

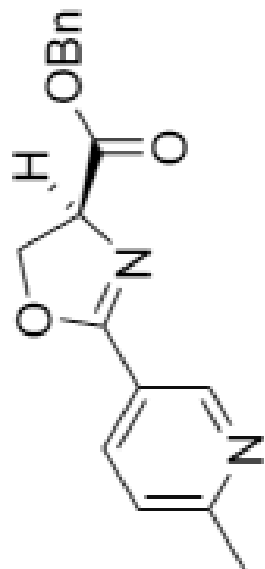
Table 2

le compounds.

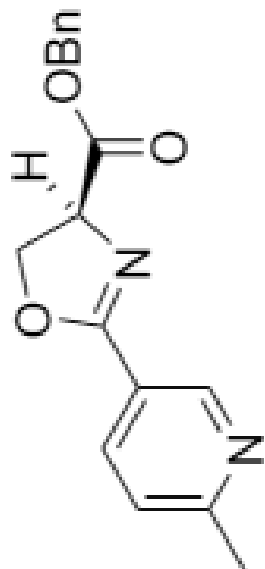


R ¹	R ²	R ³	MolWt	ClogP ^a	GAS (μM)	GAST (μM)	VERO Cells: ICS0 (μM)	NRP-TB: LORA (μM)
4-NO ₂	H	OBn	324.29	3.49	1.79	42.3		
H	3-Cl	OBn	313.74	4.19	1.25	0.99	>128	
4-OMe	H	OBn	309.32	3.49	1.72	1.80	>128	
			383.36	3.13	35.8	49.5	>128	

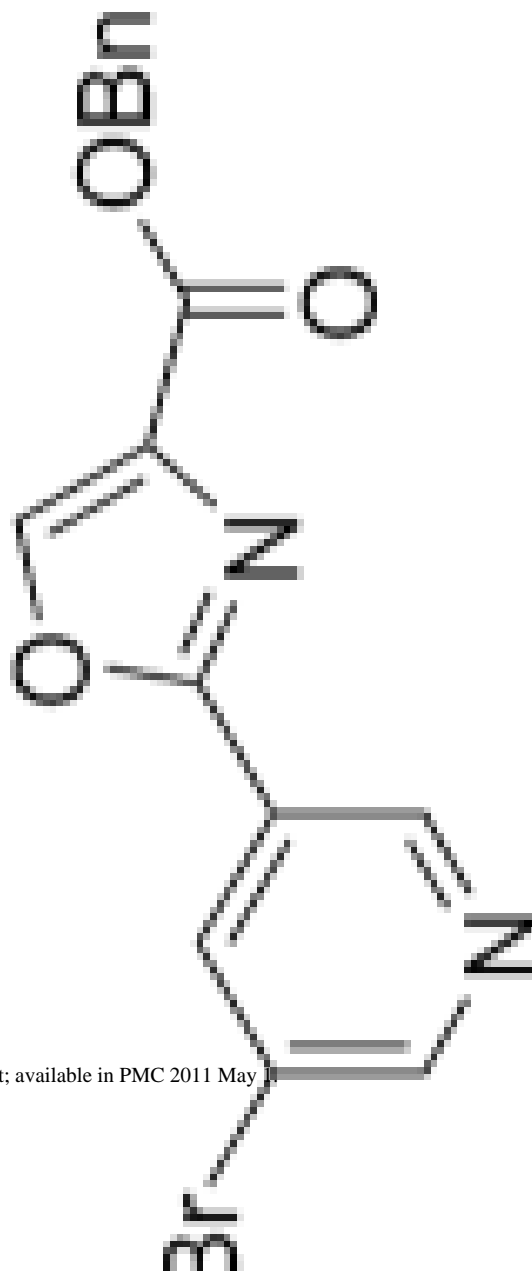


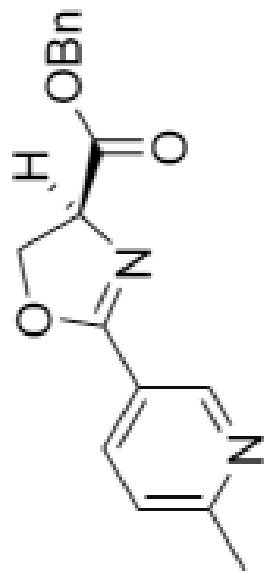


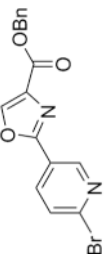
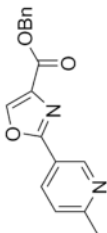
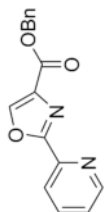
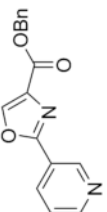
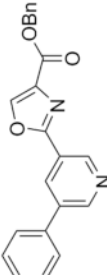
R ¹	R ²	R ³	MolWt	ClogP ^{aa}	GAS (μM)	GAST (μM)	VERO Cells: IC50 (μM)	NRP-TB: LORA (μM)
			372.8	4.09	118	48.1		
H	2-OBn	OBn	385.41	4.69	62.6	10.3	>128	
3-Cl	2-OBn	OBn	419.86	5.26	5.40	>128		
H	H	OBn	279.29	3.45	0.47	0.49	121	3.80
4-F	3-NO2	OBn	342.28	3.34	1.10	0.48	>128	31.9

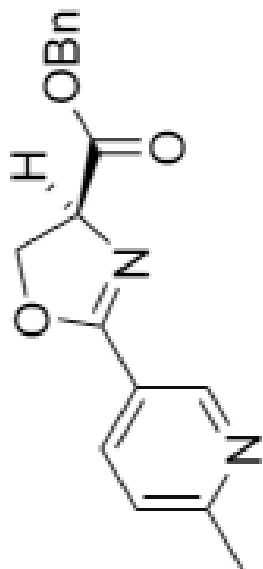


R ¹	R ²	R ³	MolWt	ClogP ^a	GAS (μM)	GAST (μM)	VERO Cells: IC50 (μM)	NRP-TB: LORA (μM)
4-NO2	3-F	OBn	342.28	3.34	35.4	2.66	>128	
H	2-Cl	OBn	313.74	3.94	0.73	1.69	>128	11.4
4-F	2-NO2	OBn	342.28	3.64	2.40	9.90	>128	
4-NO2	2-F	OBn	342.28	3.64	0.70	1.65	>128	11.6
4-NO2	2-OBn	OBn	430.41	4.68	>128	>128		
4-OBn	3-F	OBn	403.4	5.27	3.03	3.86		
			359.17	2.96	2.37	2.24	>128	>128

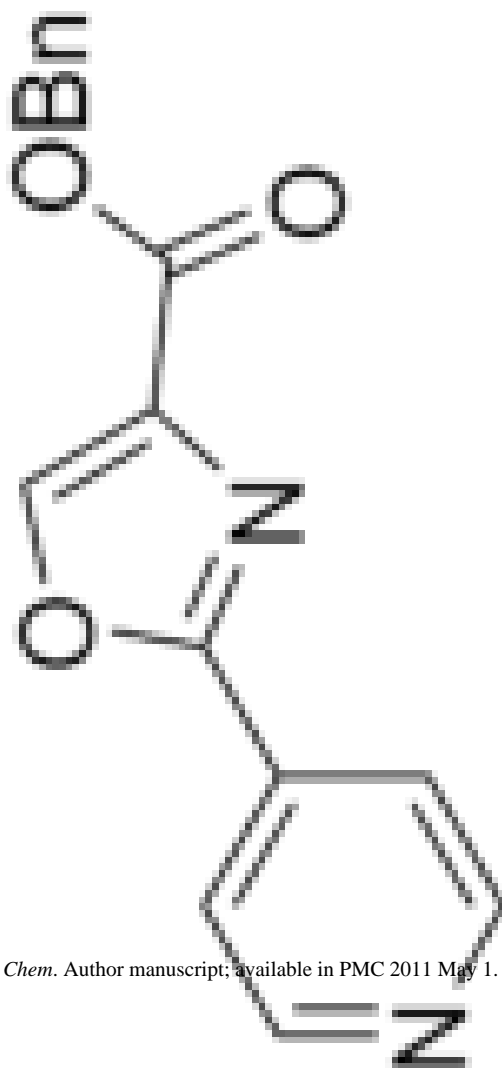


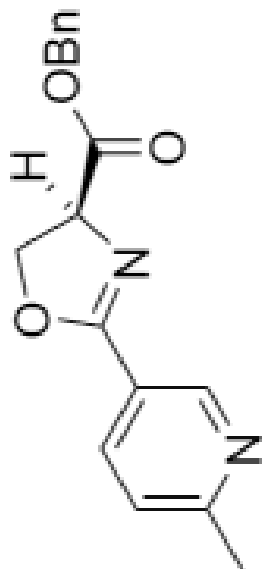


R ¹	R ²	R ³	MolWt	ClogP ^a	GAS (μM)	GAST (μM)	VERO Cells: IC50 (μM)	NRP-TB: LORA (μM)
			359.17	2.96	2.54	1.84	>128	25.1
			294.30	2.55	3.01	10.2		
			280.28	2.25	2.00	2.47		
			280.28	2.04	3.82	6.20		
			356.37	3.93	1.49	0.98	>128	

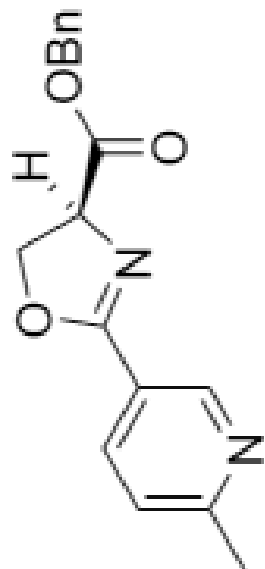


R ¹	R ²	R ³	MolWt	ClogP ^{aa}	GAS (μM)	GAST (μM)	VERO Cells: IC50 (μM)	NRP-TB: LORA (μM)
			280.28	2.04	1.42	6.10		

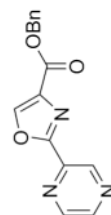
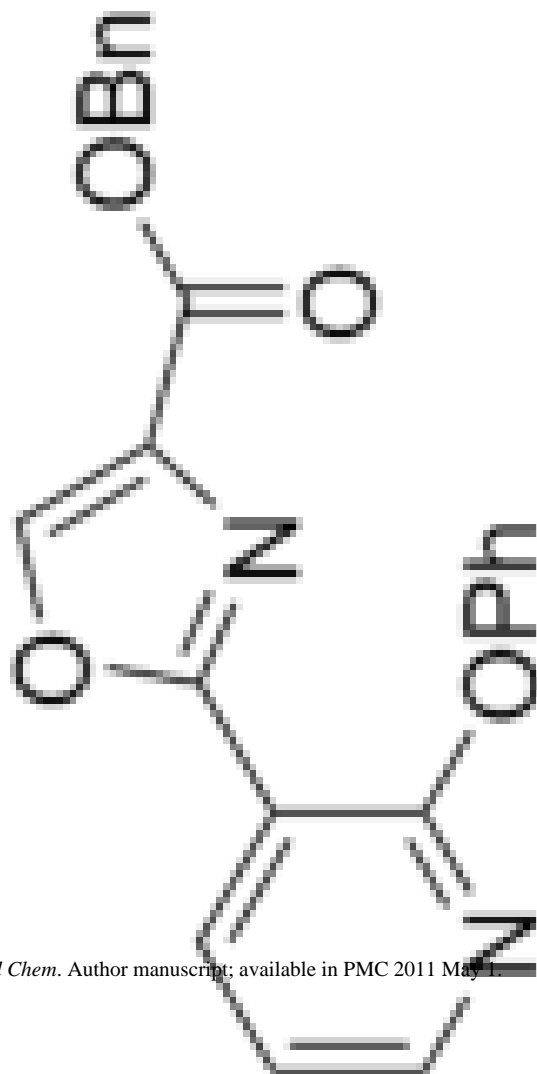




R ¹	R ²	R ³	MolWt	Clogp ^{aa}	GAS (μM)	GAST (μM)	VERO Cells: IC50 (μM)	NRP-TB: LORA (μM)
			420.44	3.74	66.2	>128		
			388.44	4.98	8.00	>128		



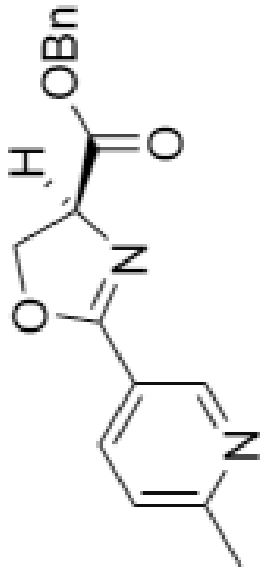
R ¹	R ²	R ³	MolWt	ClogP ^{aa}	GAS (μM)	GAST (μM)	VERO Cells: IC50 (μM)	NRP-TB: LORA (μM)
			389.44	3.59	6.86	91.4		
			281.27	1.29	7.32	22.5		
			479.86	4.22	61.8	61.5		
			339.34	3.20	16.0	17.5		
			323.34	4.02	15.5	1.97	>128	29.7
			313.74	3.99	18.9	7.77	>128	56.2



H
4-OMe
4-OEt
4-Cl

2-PO(Ph)₂
3-OMe
H
H

OBn
OBn
OBn
OBn



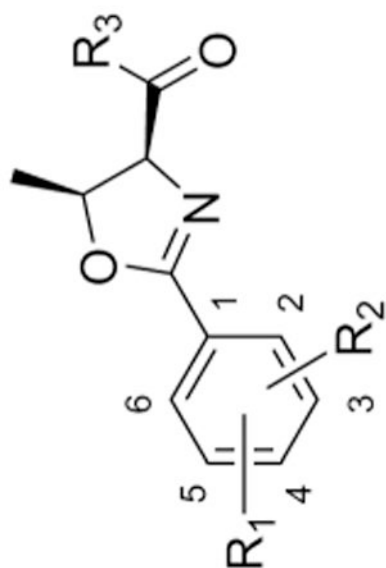
R ¹	R ²	R ³	MolWt	ClogP ^a	GAS (μM)	GAST (μM)	VERO Cells: IC50 (μM)	NRP-TB: LORA (μM)
4-NH2	H	OBn	294.3	2.63	1.98	3.67	>128	68.6
H	3-F	OBn	297.28	3.59	0.91	1.42	>128	
4-OMe	3-Cl	OBn	343.76	3.87	2.35	5.33	>128	
4-Cl	3-F	OBn	331.73	4.15	2.87	22.4		
4-OMe	3-F	OBn	327.31	3.47	5.55	6.99	>128	
H	2-OMe	OBn	309.32	3.49	3.88	7.54		
H	3-OMe	OBn	309.32	3.49	6.04	3.76		
H	3-CN	OBn	304.3	2.92	25.2	60.5		
H	3-Br	OBn	358.19	4.34	1.93	6.08		
4-CH3	H	OBn	293.32	3.97	12.84	7.68		
4-OPr	H	OBn	337.37	4.54	3.48	1.94		
4-OiPr	H	OBn	337.37	4.32	1.95	3.21		
4-OBu	H	OBn	351.40	5.07	1.76	3.83		
Rifampin (control)			822.94		0.06 [*]	0.01 [*]	125 [*]	

repeats.

ated using the Cambridge Soft ChemDraw Ultra, v 8.0 software. Activity against non-replicating *M. tuberculosis* was determined using the Low Oxygen Recovery Assay (LORA).

Table 3

SAR of threonine-derived oxazoline.



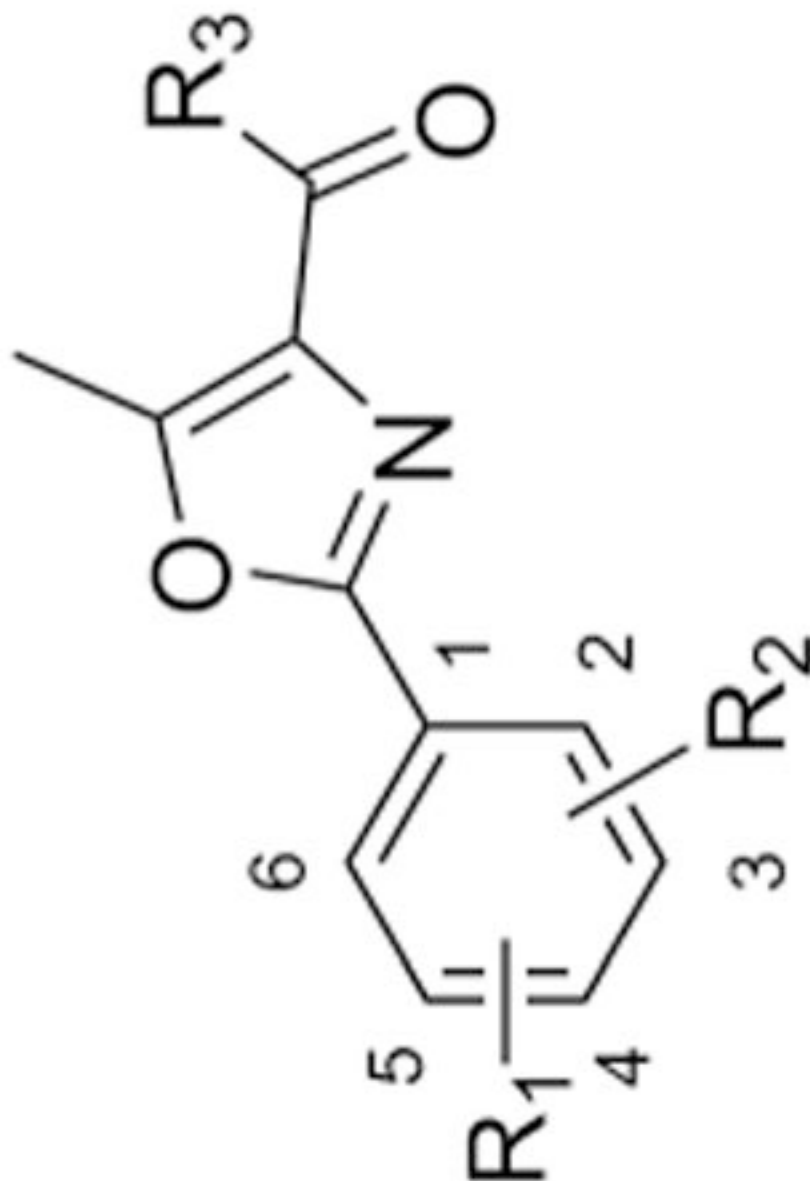
Compd	R ¹	R ²	R ³	MolWt	ClogP ^a	GAS (μM)	GAST (μM)	VERO Cells: IC ₅₀ (μM)	NRP-TB: LORA (μM)
101	H	H	OH	205.21	2.44	>128	>128		
102	H	H	OMe	219.24	2.89	>128	>128		
103	H	2-OBn	OBn	401.45	6.29	>128	>128		
104	4-OMe	H	OBn	325.35	4.51	54.1	37.8		
105	4-F	2-NO ₂	OBn	358.32	4.18	12.5	4.11	38.1	
	Rifampin (control)			822.94		0.06*	0.01*	125*	

* Average of assay repeats.

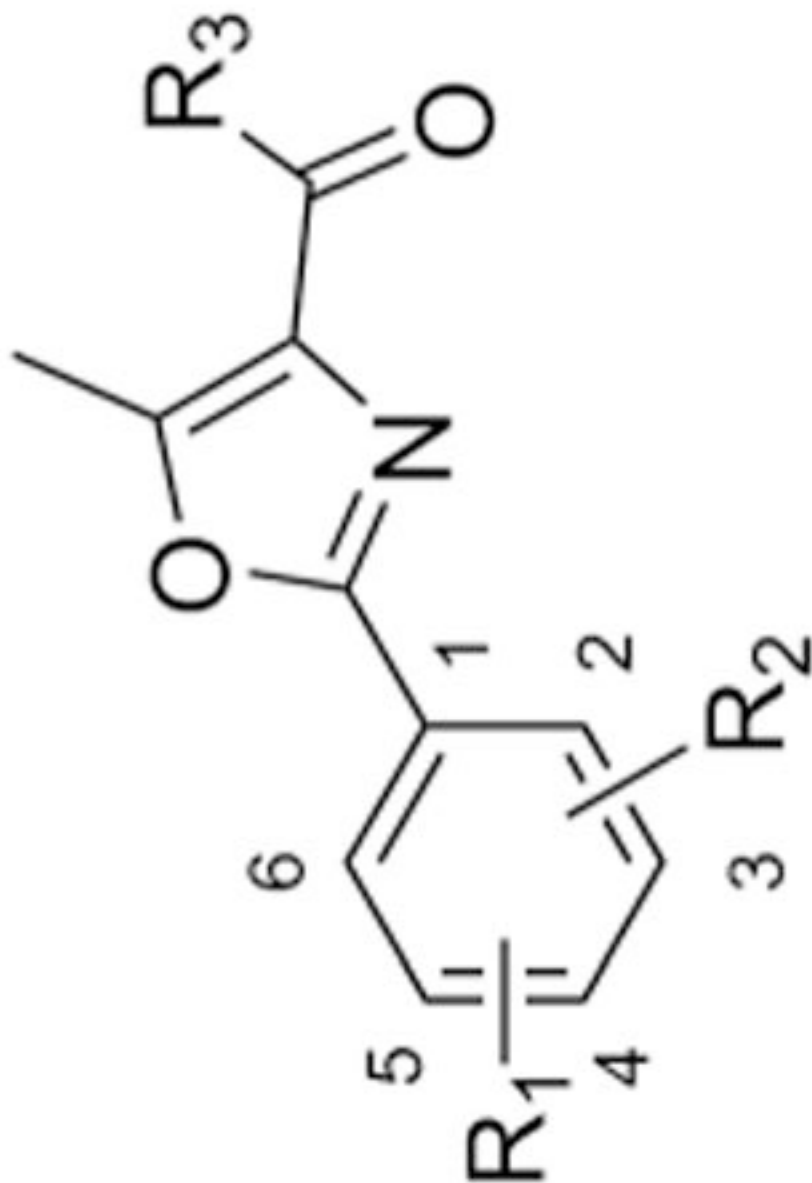
^a CLogP was calculated using the Cambridge Soft ChemDraw Ultra, v 8.0 software. Activity against non-replicating *M. tuberculosis* was determined using the Low Oxygen Recovery Assay (LORA).

Table 4

SAR of threonine-derived oxazole.



Compd	R ¹	R ²	R ³	MolWt	ClogP ^{ad}	GAS (μM)	GAST (μM)	VERO Cells; IC50 (μM)	NRP-TB: LORA (μM)
106	H	H	OH	203.2	2.46	>128	>128		
107	H	2-OH	OH	219.2	1.49	>128	>128		
108	H	2-OBn	OBn	399.45	4.94	28.3	>128	110	
109	H	H	OBn	293.32	3.74	3.92	15.2	35.0	>128



Compd	R ¹	R ²	R ³	MolWt	ClogP ^a	GAS (μM)	GAST (μM)	VERO Cells: IC50 (μM)	NRP-TB: LORA (μM)
110	4-OMe	H	OBn	323.34	3.76	47.4	15.8	>128	>128
111	4-F	2-NO2	OBn	356.3	3.34	15.0	4.62	>128	>128
	Rifampin (control)			822.94		0.06*	0.01*	125*	

* Average of assay repeats.

^aClogP was calculated using the Cambridge Soft ChemDraw Ultra, v 8.0 software. Activity against non-replicating *M. tuberculosis* was determined using the Low Oxygen Recovery Assay (LORA).

Table 5

Compound stability to simulated gastric juices and microsomes.

Compound	Gastric Stability	Microsomes
	% Remaining (2 h.)	% Remaining (15 min.)
3	1.4	8.4
15	0.4	5.7
29	2.2	13
60	0.4	14
63	4.2	13
65	0.5	15
66	2.1	8.1
68	4.2	8.8