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Synthesis and antitubercular evaluation of aryl substituted 2-oxazolines from L-amino acids

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Abstract: This paper describes the synthesis and the *in vitro* antibacterial activity of a series of twelve substituted aryl-2-oxazolines against *Mycobacterium tuberculosis*. Seven compounds showed activity and two compounds exhibited a minimal inhibitory concentration (MIC) of 25 μg/mL were not cytotoxic for the host cells in cell viability assay. These results could be a good starting point for the development of new antitubercular lead series based on this family of compounds.

Keywords: 2-oxazolines, tuberculosis, antimycobacterial activity, L-amino acids.

Introduction

The 2-oxazoline nucleus characterizes an important class of heterocyclic compound with a wide range of applications including in polymeric materials[1], as building blocks[2], in combinatorial chemistry[3] and in medicinal chemistry[4]. However, in despite of its versatility very few studies have been made in drug discovery against tuberculosis (TB) [5]. Nowadays, this disease is the most important infectious cause of death worldwide and several problems were responsible for TB resurgence such as, the lack of new anti-TB drugs and the coinfection with HIV/AIDS[6]. Among these problems, the emergence of drug-resistant TB is especially alarming, and it can be mentioned the advent of extensively drug-resistant TB (XDR-TB), which is commonly defined as MDR-TB (strains resistant to isoniazid and rifampicin) plus resistance to any fluorquinolone and to, at least, one of the three injectable second-line anti-TB drugs used in TB treatment (capreomycin, kanamycin, and amikacin). Unfortunately, between 2006 and 2009 the first news isolated cases were reported in patients, which were resistant to all anti-TB drugs tested defined as "totally drug-resistant TB"[7] (TDR-TB).

Due to the emergence of new bacterial strains resistant to the majority of anti TB drugs, we urgently need to develop new drugs and strategies to fight this disease. In this context, in our continuous program in the search for new candidates to antitubercular agents, we proposed the synthesis of various aryl substituted 2-oxazolines, as well its evaluation against *Mycobacterium tuberculosis*.

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Results and Discussion

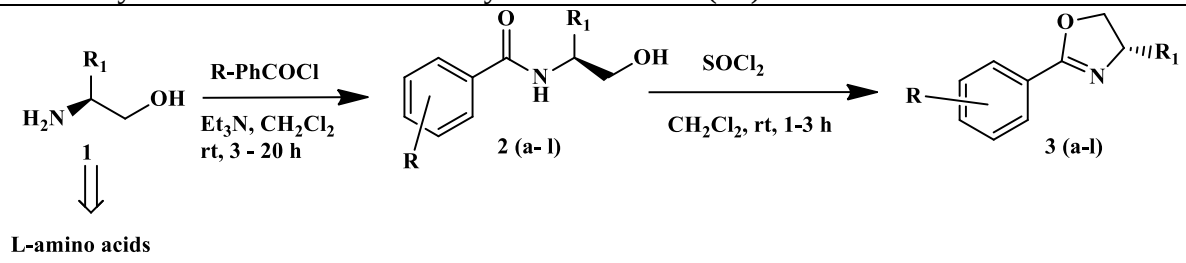
Synthesis

Several methodologies have been described for the preparation of 2-oxazolines, and the majority of them uses amino alcohols as start materials[8], which are usually prepared by the reduction of the respective amino acids.

The synthesis of the aryl-2-oxazolines compounds **3 (a-l)** involved the use of appropriated amino acids as starting material (Table 1) which were converted in good yields primarily, for the substituted amino alcohols **1** in according with procedures described in the literature[9]. The amino alcohols reacted with an appropriated acyl chloride in dichloromethane in the presence of triethylamine as base at 0 ^{o}C[10] to provide the respective amides **2 (a-n)** selectively in appreciable yields (30-99%), with traces of the ester in some cases. The amides were converted to the desired aryl-2-oxazolines products **3 (a-l)** in a cyclization procedure employing thionyl chloride in dichloromethane at 0 ^{o}C, in variable yields (18-97%), which not lead to racemization in according with described papers[11]. No success was obtained in the cyclization of the amides derived from the amino acid methionine, for the respective oxazolines.

All the compounds were identified by spectral data. In general IR spectra of the oxazolines showed a peak at 1645-1647 cm^{-1}, relative to the C=N bond. The ^{1}H NMR spectrum of the oxazolines showed the endocyclic methylene (–O-C<u>H</u>$_2$-) signals at lowest field and more complex than the methylene of the amide, with scrolling of the signals.

Table 1: Synthesis of the substituted aryl-2-oxazolines 3(a-l) from L-amino acids.



Product	R	R_1	MIC (μg/mL)
3a	H	H	>100
3b	*p*-Cl	H	>100
3c	H	-CH(CH$_3$)$_2$	>100
3d	*p*-Cl	-CH(CH$_3$)$_2$	>100
3e	H	-Bn	25
3f	*p*-Cl	-Bn	50
3g	*p*-OCH$_3$	-Bn	25
3h	*m*-OCH$_3$	-Bn	100
3i	*o*-OCH$_3$	-Bn	>100
3j	*m*,*m*-OCH$_3$	-Bn	100
3k	*p*-NO$_2$	-Bn	>100
3l	H	-Ph	100

Biological evaluation

In the biological evaluation of the series of the aryl-2-oxazolines **3 (a-l)** compounds **3e** and **3g** exhibited the highest activity with a MIC of 25 μg/mL. The derivative **3f** exhibited a MIC of 50 μg/mL while compounds **3j** and **3l** showed a MIC of 100 μg/mL against *M. tuberculosis*. All the others derivatives were inactive in this assay. The absence of biological activity in concentrations lower or equal than 100 μg/mL for the oxazolines **3a**, **3b**, **3c**, **3d** indicated that the lateral hydrophobic aromatic chain in C4 of the oxazoline nucleus is critical for biological activity. Furthermore, the presence of an electron withdrawing group on the phenyl ring in C2, as in **3k**, leads to loss of activity. The more active compounds of the series, **3e** and **3g**, were not cytotoxic in the cell viability assay (Table 2), as well as the compounds **3f**, **3h** and **3j**. Despite this, the compound **3l**, which do not have the methylene benzilic in the lateral chain at C4, was cytotoxic at a low concentration (10nM; Table 2).

Table 2: Cell viability of the bioactive compounds

Compound	**% Cell viability / concentration**					
	1nM	**10nM**	**100nM**	**1μM**	**10μM**	**100μM**
3e	100	100	100	100	100	100
3f	100	100	100	100	100	100
3g	100	100	98.3	97.3	98.3	98.9
3h	100	100	100	99.4	96.2	95.5
3j	100	100	100	100	100	100
3l	95.7	81.3	78.7	70.9	71.7	77.4

Conclusion

A series of twelve aryl substituted 4,5-dihydrooxazoles **3a-3l** were synthesized and evaluated for their *in vitro* antibacterial activity against *Mycobacterium tuberculosis*. The compounds **3e** and **3g** exhibited the highest activity (MIC) at 25 μg/mL and were not cytotoxic for the macrophages cells. The absence of biological activity in concentrations lower or equal than 100 μg/mL for the oxazolines **3a**, **3b**, **3c**, **3d** indicated that the lateral hydrophobic aromatic chain in C4 of the oxazoline nucleus is critical for biological activity. More information about structure-activity relationship is necessary, but this findings anticipate the importance the of the oxazoline nucleus associated with a lateral hydrophobic chain for anti-TB activity.

Acknowledgments

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Experimental Section

Melting point was determined with a Fisatom 130 apparatus and is uncorrected. Proton magnetic resonance (^{1}H NMR) was determined in deuterated solvents, as showed below with Brucker AC spectrometers at 400 MHz or 500 MHz.. Splitting patterns are as follows: s, singlet; d, duplet; t, triplet; dd, duple duplet; m, multiplet; sl, broad signal. Carbon magnetic resonance (^{13}C NMR) was determined at 100 MHz or 125 MHz. Infrared spectra was performed in a Perkin-Elmer 467 FTIR spectrometer by using potassium bromide plates. The mass spectra (MS) were recorded on Agilent 122 5532 GC/MS column by electron impact. Elemental Analysis was performed on Perkin-Elmer CHN 2400. The progress of all reactions was monitored by thin-layer chromatography (tlc) which was performed on 2.0 cm X 6.0 cm aluminum sheets (silica gel 60, HF-254, Merck) to a thickness of 0.25 mm. The developed of chromatograms were viewed under an ultraviolet light. For column chromatography Merck silica gel (230-400 mesh) was used. Solvents and reagents were used generally used without previous purification.

Aminoalcohol were prepared from corresponding amino acids as previously described[9] and used without purification. Ethanolamine was obtained from source commercial.

Synthesis of the amides 2a - 2n

To a solution of the amino alcohol **1** (1.0 - 5.0 mmol) in CH_2Cl_2 (15 - 20 mL) and Et_3N (3 eq.) at 0 ^oC were added 1.5 equivalents of respective benzoil chloride slowly. The mixture was stirred at room temperature until tlc indicate the terminus of the reaction. After this time the solvents were removed in the rotovap. The residue was suspended in 20 mL of solution aqueous of NaOH 1M at 0 ^oC and briefly agitated. The phases were separated and the phase aqueous was extracted with ethyl acetate (2x). The combined organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (230-400 mesh, MeOH/$CHCl_3$ 10%) to afford the desired amides **2** (a-l) as showed below.

***N*-(2-Hydroxyethyl)benzamide 2a** Yield: 99%. m.p.: 50-53 ^oC (lit.[12] 60-62 ^oC) ^{1}H-NMR (MeOD, 400 MHz): 7.82 (2H, dd, $ArH_{1,5}$, J = 5.2 and 7.1 Hz), 7.52 (1H, m, ArH_3), 7.44 (2H, m, $ArH_{2,4}$), 3.71 (2H, t,-C$\underline{H}_2$-OH, J = 5.9 Hz), 2.42 (2H, t, -C$\underline{H}_2$-NHCO-, J = 5.9 Hz). ^{13}C-NMR (MeOD, 100 MHz): 43.70 (NH-$\underline{C}H_2$-), 61.79 (-$\underline{C}H_2$-OH), 128.43, 129.68, 132.78 ($\underline{C}$H arom.), 135.81($\underline{C}$-C=N- arom.), 170.69 ($\underline{C}$=O). IR (KBr): 3300 (N-H), 1633 (CO amide), 694 (CH arom.); GC/MS (100%) m/z: 165 (M^+).

4-Chloro-*N*-(2-hydroxyethyl)benzamide 2b Yield: 59%. m.p.: 115-118 ^oC (lit. [13]: 116-118^oC) ^{1}H-NMR (MeOD, 400 MHz): 7.82 (2H, d, $ArH_{1,5}$, J = 8.6 Hz), 7.46 (2H, d, $ArH_{2,4}$), 3.70 (2H, t,-C$\underline{H}_2$-OH, J = 5.8 Hz), 3.49 (2H, t, -C$\underline{H}_2$-NHCO-, J = 5.8 Hz). ^{13}C-NMR (MeOD, 100 MHz): 43.66 (-NH$\underline{C}H_2$-), 61.61 (-$\underline{C}H_2$-OH), 128.43, 129.74 (C$\underline{H}$ arom.), 134.40 ($\underline{C}$-CO arom.), 138.75 ($\underline{C}$-Cl), 169.37 ($\underline{C}$=O). IR (KBr): 3294 (N-H), 1643 (C=O amide), 646 (CH arom.). GC/MS (100%) m/z: 199 (M^+).

(*S*)-*N*-(1-Hydroxy-3-methylbutan-2-yl)benzamide 2c Yield:70%. m.p.: 98-99 ^oC (li.[14]: 99^oC). ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.80 (2H, d, $ArH_{1,5}$, J = 8.0 Hz), 7.50 (3H, m, $ArH_{2,3,4}$), 3.95 (1H, sl, -CONH-C$\underline{H}$-), 3.82 (2H, sl, -C$\underline{H}_2$-OH), 2.05 (1H, sex, -C$\underline{H}$(Me)$_2$, J = 7.0 Hz), 1.05 (6H, t, 2-C$\underline{H}_3$, J = 7.0 Hz). ^{13}C-NMR ($CDCl_3$, 100 MHz): 19.43, 20.28 (2-$\underline{C}H_3$), 30.45 (-$\underline{C}$H-), 58.95 (-NH$\underline{C}$H-), 63.34 (-$\underline{C}H_2$OH), 128.49, 129.62, 132.62 ($\underline{C}$H arom.), 136.35

(C-C=O arom.), 170.86 (C=O). IR (KBr): 3309 (N-H), 1631 (C=O amide), 700 (CH arom.). GC/MS (100%) m/z: 207 (M^+).

(*S*)-4-Chloro-*N*-(1-hydroxy-3-methylbutan-2-yl)benzamide 2d Yield: 28%. oil (lit.[15] not found). ^{1}H-NMR (MeOD, 400 MHz): 7.82 (2H, d, $ArH_{1,5}$, *J* = 7.2 Hz), 7.46 (2H, d, $ArH_{1,5}$, *J* = 7.2 Hz), 3.90 (1H, sl, -CONH-CH-), 3.70 (2H, sl, -CH_2-OH) 1.98 (1H, sex, -CH$(Me)_2$, *J* = 7.1 Hz), 1.00 (6H, t, 2-CH_3, *J* = 7.1 Hz). ^{13}C-NMR (MeOD, 100 MHz): 19.43, 20.25 (2-CH_3), 30.45 (-CH-), 59.19 (-NHCH-), 63.29 (-CH_2OH), 129.78, 130.25 (CH arom.), 135.02 (C-C=O arom.), 138.67 (C-Cl), 169.72 (C=O). IR (KBr): 3296 (N-H), 1633 (C=O amide), 846 (CH arom.). GC/MS (100% area) m/z: 241 (M^+).

(*S*)-*N*-(1-Hydroxy-3-phenylpropan-2-yl)benzamide 2e Yield: 50%. m.p.: 138-140 °C (lit.[16]: 171-173 °C). ^{1}H-NMR (MeOD, 500 MHz): 7.71 (2H, dd, $ArH_{1,5,}$ *J* = 6.7 Hz, 1.0 Hz), 7.47 (1H, tt, ArH_3, *J* = 6.7 Hz, 1.0 Hz), 7.40 (2H, t, $ArH_{2,4}$, *J* = 6.7 Hz), 7.27 (4H, m, $ArH'_{1,2,4,5}$), 7.23 (1H, m, ArH'_3), 4.33 (1H, sl, -CONH-CH-), 3.64 (2H, m, -CH_2-OH), 2.90 (2H, 2dd, -CH_2Ph, *J* = 6.1 Hz, 7.6Hz). ^{13}C-NMR (MeOD, 125 MHz): 38.13 (-CH_2Ph), 55.09, 55.18 (NH-CH-), 64.44 (-CH_2-OH), 127.48, 128.42, 129.56, 130.49, 132.63 (-CH arom.), 136.19 (-O=C-C- arom.), 140.11 (CH_2-C- arom.), 170.48, 170.56 (-C=O); IR (KBr): 3365 (-OH), 3309 (N-H), 1639 (C=O amide), 746, 698 (CH arom.); GC/MS (100% area) m/z: 254 (M^+).

(*S*)-4-Chloro-*N*-(1-hydroxy-3-phenylpropan-2-yl)benzamide 2f Yield: 46%. m.p.: 169-171 °C (lit.[17]: 168-169 °C). ^{1}H-NMR (MeOD, 500 MHz): 7.75 (2H, dd, $ArH_{1,5,}$ *J* = 7.1 Hz), 7.45 (2H, d, $ArH_{2,4}$, *J* = 7.1 Hz), 7.25 (4H, m, $ArH'_{1,2,4,5}$), 7.23 (1H, m, ArH'_3), 4.38 (1H, m, -CONH-CH-), 3.65 (2H, m, -CH_2-OH), 2.80-3.15 (2H, m, -CH_2Ph). ^{13}C-NMR (MeOD, 125 MHz): 38.15 (CH_2Ph), 55.28 (CH-NH-), 64.42 (-CH_2-OH), 127.51, 129.52, 129.73, 130.14, 130.47 (-CH arom.), 134.85 (O=C-C-), 138.68 (-C-Cl), 140.06 (CH_2-C- arom.), 169.34 (-C=O); IR (KBr): 3296 (N-H), 1639 (CO amide), 748, 842 (CH arom.). GC/MS (100% area) m/z: 289 (M^+).

(*S*)-4-Methoxy-*N*-(1-hydroxy-3-phenylpropan-2-yl)benzamide 2g Yield: 60%. m.p.: 142-144 °C (lit.[17]:142-143 °C). ^{1}H-NMR (MeOD, 400 MHz): 7.71 (2H, d, $ArH_{1,5,}$ *J* = 6.8 Hz), 7.24 (4H, m, $ArH'_{1,2,4,5}$), 7.16 (1H, m, ArH'_3), 6.93 (2H, d, $ArH_{2,4}$, *J* = 6.8 Hz), 4.38 (1H, m, -CONH-CH-), 3.83 (3H, s, -OCH_3), 3.63 (2H, m, -CH_2-OH), 2.80-3.35 (2H, m, -CH_2Ph). ^{13}C-NMR (MeOD, 125 MHz): 38.16 (CH_2Ph), 54.99, 56.03 (-O-CH_3 or NH-CH), 64.46 (-CH_2-OH), 114.76 , 127.45, 129.50, 129.73, 130.28, 130.47 (CH arom.), 134.85 (O=C-C- arom.), 140.17 (-CH_2-C- arom.), 163.97 (C-OCH_3 arom.), 169.46 (C=O). IR (KBr): 3304 (N-H), 1633 (C=O amide), 700 (CH arom.).
MS m/z: 285 (M^+).

(S)-3-Methoxy-*N*-(1-hydroxy-3-phenylpropan-2-yl)benzamide 2h Yield: 65%. m.p.: 89-90 °C (lit.[23] not found). ^{1}H-NMR (MeOD, 400 MHz): 7.17-7.35 (8H, m, $ArH_{1,4,5,}$ ArH'_{1-5}), 7.04 (1H, ddd, ArH_3 J = 4.2, 1.7 Hz), 4.33 (1H, m, -CONH-CH-), 3.81 (3H, s, -OCH_3), 3.64 (2H, d, -CH_2-OH, *J* = 5.5 Hz), 2.80-3.05 (2H, m, -CH_2Ph). ^{13}C-NMR (MeOD, 100 MHz): 38.00 (-CH_2Ph), 55.02, 55.88 (CH_3-O- or NHCH-), 64.34 (-CH_2OH), 113.61, 118.38, 120.43, 127.39, 129.41, 130.39, 130.55 (CH arom.), 137.44 (O=C-C arom.), 140.00 (-CH_2-C arom.), 161.19 (C-OCH_3), 170.22 (C=O). IR (KBr): 3298 (N-H), 1637 (C=O amide), 700 (CH arom.).
MS m/z: 285 (M^+).

(*S*)-2-Methoxy-*N*-(1-hydroxy-3-phenylpropan-2-yl)benzamide 2i Yield: 44%. oil (lit.[18]: oil). ^{1}H-NMR (MeOD, 400 MHz): 7.87 (1H, dd, , ArH_5 *J* = 1.7, 7.6 Hz), 7.00-7.50 (8H, m, $ArH_{2,3,4}$, ArH'_{1-5}), 4.33 (1H, m, -CONH-CH-), 3.89 (3H, s, -OCH_3), 3.61

(2H, m, -C<u>H</u>$_2$-OH), 2.95 (2H, m, -C<u>H</u>$_2$Ph). ^{13}C-NMR (MeOD, 100 MHz): 38.10 (-<u>C</u>H$_2$Ph), 54.44, 54.53, 56.68 (<u>C</u>H$_3$-O- or NH<u>C</u>H-), 63.64 (-<u>C</u>H$_2$OH), 113.10, 122.09, 127.59, 129.56, 130.65, 132.28 (<u>C</u>H arom.), 134.29 (<u>C</u>-CO arom.), 139.80 (<u>C</u>-CH$_2$- arom.), 159.29 (<u>C</u>-OCH$_3$ arom.), 167.95 (<u>C</u>=O). MS m/z: 285 (M^+).

(*S*)-3,5-Dimethoxy-*N*-(1-hydroxy-3-phenylpropan-2-yl)benzamide 2j Yield: 61%. m.p.: 104-105 ^{o}C (Lit.[15] not found) ^{1}H-NMR (MeOD, 400 MHz): 7.15-7.30 (5H, m, , ArH'$_{1-5}$), 6.86 (2H, d, ArH$_{1,5}$,*J* = 2.2 Hz), 6.58 (1H, t, ArH$_3$ *J* = 2.2 Hz), 4.33 (1H, m, -CONH-C<u>H</u>-), 3.78 (6H, s, -OCH$_3$), 3.64 (2H, d, -C<u>H</u>$_2$-OH, *J* = 5.6 Hz), 2.80-3.05 (2H, m, -C<u>H</u>$_2$Ph). ^{13}C-NMR (MeOD, 100 MHz): 38.08 (-<u>C</u>H$_2$Ph), 55.12, 56.10 (<u>C</u>H$_3$O- or –NH<u>C</u>H-), 64.45 (-<u>C</u>H$_2$OH), 104.58, 106.35, 127.49, 129.51, 130.50 (-<u>C</u>H arom.), 138.19, 140.10 ((<u>C</u>-CO arom. or <u>C</u>-CH$_2$- arom.), 162.40 (2-<u>C</u>-OCH$_3$ arom.), 170.23 (<u>C</u>=O). IR (KBr): 3298 (N-H), 1637 (C=O amide), 700 (CH arom.); MS (ES): 314 (M-1).

(*S*)-4-Nitro-*N*-(1-hydroxy-3-phenylpropan-2-yl)benzamide 2k Yield: 50%. m.p.: 140-143 ^{o}C (lit.[17]: 139-141 ^{o}C). ^{1}H-NMR (MeOD, 500 MHz): 8.25 (2H, d, ArH$_{2,4,}$ *J* = 11.1 Hz), 7.90 (2H, d, ArH$_3$, *J* = 11.1 Hz), 7.10-7.30 (5H, m, ArH'), 4.36 (1H, m, -CONH-C<u>H</u>-), 3.65 (2H, m, -C<u>H</u>$_2$-OH), 2.80-3.06 (2H, m, -C<u>H</u>$_2$Ph). ^{13}C-NMR (MeOD, 100 MHz): 38.10 (<u>C</u>H$_2$Ph), 55.38 (-NH<u>C</u>H-), 64.39 (-CH$_2$OH), 124.64, 127.56, 129.54, 130.46 (-<u>C</u>H arom.), 139.94 (-<u>C</u>-CH$_2$-), 141.98 (-<u>C</u>-CO arom.), 151.04 (-<u>C</u>-NO$_2$ arom.), 168.42 (-<u>C</u>=O). IR (KBr): 3365 (-OH), 3336 (N-H), 1598 (C=O amide), 1313 (NO$_2$), 837 (CH arom.).

(*S*)-*N*-(2-Hydroxy-1-phenylethyl)benzamide 2l Yield: 80%. m.p.: 171-172 ^{o}C (lit.[19]: 152-154 ^{o}C).^{1}H-NMR (MeOD, 500 MHz): 7.20-7.50 (10H, m, ArH) 5.20 (1H, t, -CONH-C<u>H</u>-, *J* = 6.6 Hz), 3.85 (2H, d, -C<u>H</u>$_2$-OH, *J* = 6.6 Hz) ^{13}C-NMR (MeOD, 100 MHz): 54.56, 57.94 (-NH<u>C</u>H-), 66.22, 67.80 (-<u>C</u>H$_2$OH), 127.19, 128.57, 129.67, 130.74, 132.91 (<u>C</u>H arom.), 136.03 (O=C-<u>C</u> arom.), 141.57 (CH-<u>C</u> arom.), 170.49 (-<u>C</u>=O), IR (KBr): 3317 (N-H), 1633 (C=O amide), 700 (CH arom.). MS m/z: 241 (M^+).

Synthesis of the oxazolines 3 (a – l)

To a solution of the amides **2** (a - l; 1 - 3 mmol) in CH_2Cl_2 (20 - 30 mL) cooled at 0 ^{o}C was added a solution of $SOCl_2$ (20 eq.) in CH_2Cl_2 (20 mL) slowly over 30 minutes and the mixture was allowed to stir for 1 to 3 h, when tlc indicated the terminus of the reaction. Then aqueous $NaHCO_3$ was added slowly at 0 ^{o}C and the organic phase separated. The aqueous phase was extracted with ethyl acetate. The combined organic phases was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The obtained oil was purified by column chromatography on silica gel (230-400 mesh, AcOEt/Hexane 50%) to afford the desired oxazoles **3** (a - l) as showed below.

2-Phenyl-4,5-dihydrooxazole 3a Yield: 92%. oil (lit.[20]: oil). ^{1}H-NMR (MeOD, 400 MHz): 7.90 (2H, dd, ArH$_{1,5}$, *J* = 1.0, 8.0 Hz), 7.52 (1H, m, ArH$_3$), 7.44 (2H, m, ArH$_{2,4}$), 4.50 (1H, t,-C<u>H</u>$_2$-O-C$_{sp}$-, *J* = 9.6 Hz), 4.02 (1H, t, -C<u>H</u>$_2$-O-C$_{sp}$-, *J* = 9.6 Hz). ^{13}C-NMR ($CDCl_3$, 100 MHz): 55.25 (-<u>C</u>H$_2$-N=), 69.26 (<u>C</u>H$_2$-O-), 128.43, 129.68, 132.78 (<u>C</u>H arom.), 134.84 (<u>C</u>-C=N arom.), 167.21 (<u>C</u>=O). IR (KBr): 1647 (-C=N-), 696 (CH arom.). GC/MS (100% area) m/z: 147 (M^+).

2-(4-Chlorophenyl)-4,5-dihydrooxazole 3b Yield: 62%. m.p.: 70 - 71 ^{o}C (lit.[8e]: 77-79 ^{o}C). ^{1}H-NMR (MeOD, 400 MHz): 7.90 (2H, d, ArH$_{1,5}$, *J* = 8.6 Hz), 7.47 (2H, ArH$_{2,4}$, *J* = 8.6 Hz), 4.50 (1H, t,-C<u>H</u>$_2$-O-C$_{sp}$-, *J* = 9.6 Hz), 4.03 (1H, t, -C<u>H</u>$_2$-O-C$_{sp}$-, *J* = 9.6 Hz). ^{13}C-NMR ($CDCl_3$, 100 MHz): 55.26 (-<u>C</u>H$_2$-N=), 69.34 (-<u>C</u>H$_2$-O-), 127.25, 129.89 (<u>C</u>H arom.), 130.79 (<u>C</u>-C=N arom.), 139.00 (<u>C</u>-Cl arom.), 166.02 (<u>C</u>=O). IR (KBr): 1647 (-C=N-),

667 (CH arom.). GC/MS (100% area) m/z: 181 (M^+).

(*S*)-4-Isopropyl-2-phenyl-4,5-dihydrooxazole 3c Yield: 95%. oil (Lit.[21] not found) ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.96 (2H, d, $ArH_{1,5}$, *J*=8.0 Hz), 7.45 (3H, m, $ArH_{2,3,4}$), 4.42 (1H, m, =N-C<u>H</u>-), 4.15 (2H, m, -C<u>H</u>$_2$-O-C$_{sp}$-), 1.85 (1H, sex, -C<u>H</u>(Me)$_2$, *J* = 7.0 Hz), 1.00 (6H, 2d, 2-C<u>H</u>$_3$, *J* = 7.0 Hz). ^{13}C-NMR ($CDCl_3$, 100 MHz): 18.26, 19.13 (2-<u>C</u>H$_3$), 33.02 (-<u>C</u>H-), 70.26 (-<u>C</u>H-N=), 72.79(-<u>C</u>H$_2$-O-), 128.16, 128.44 (<u>C</u>H arom.), 131.34 (<u>C</u>H arom., -<u>C</u>-C=N- arom.), 163.54 (-O-<u>C</u>=N-). IR (KBr): 1649 (-C=N-), 694 (CH arom.). GC/MS (100% area) m/z:189 (M^+).

(*S*)-2-(4-Chlorophenyl)-4-isopropyl-4,5-dihydrooxazole 3d Yield: 95%. m.p.: 51-52 ^{o}C (Lit.[15] not found). ^{1}H-NMR (MeOD, 400 MHz): 7.90 (2H, d, $ArH_{1,5}$, *J* = 6.7 Hz), 7.46 (2H, d, $ArH_{2,4}$,*J* = 6.7 Hz), 4.20 - 4.50 (2H, m, -C<u>H</u>$_2$-O-C$_{sp}$-), 4.15 (1H, m, =N-C<u>H</u>-), 1.86 (1H, sex, -C<u>H</u>(Me)$_2$, *J* = 6.5 Hz), 0.97 (6H, 2d, 2-C<u>H</u>$_3$, *J* = 6.5 Hz). ^{13}C-NMR ($CDCl_3$, 100 MHz): 18.28, 18.96 (2-<u>C</u>H$_3$), 33.94 (-<u>C</u>H-), 72.70, 73.37 (-<u>C</u>H$_2$-O-), 127.48, 130.00, 131.01 (<u>C</u>H arom.), 139.14 (<u>C</u>-C=N-arom.), 165.25 (-O-<u>C</u>=N-). IR (KBr): 1649 (-C=N-), 694 (CH arom.). GC/MS (100% area) m/z: 223 (M^+).

(*S*)-4-Benzyl-2-phenyl-4,5-dihydrooxazole 3e Yield: 97 %. oil (Lit.[8c]: 61-63 ^{o}C). ^{1}H-NMR (MeOD, 400 MHz): 7.88 (2H, d, $ArH_{1,5}$, *J* = 7.1 Hz), 7.53 (1H, t, ArH_3, *J* = 7.6 Hz), 7.44 (2H, t, $ArH_{2,4}$, *J* = 7.6 Hz), 7.15-7.31 (5H, m, ArH'$_{1-5}$), 4.60 (1H, m, =N-C<u>H</u>-), 4.43 (1H, t, -C<u>H</u>$_2$-O-C$_{sp}$-, *J* = 8.6 Hz), 4.23 (1H, dd, -C<u>H</u>$_2$-O-C$_{sp}$-, *J* = 8.6 Hz), 3.1 (1H, dd, -C<u>H</u>$_2$Ph, *J* = 13.6 Hz), 2.83 (1H, dd, -C<u>H</u>$_2$Ph, *J* = 13.6 Hz). ^{13}C-NMR (MeOD, 125 MHz): 42.49 (-<u>C</u>H$_2$Ph), 68.46 (=N-<u>C</u>H-), 73.19 (-<u>C</u>H$_2$O-), 127.74, 128.68, 129.35, 129.43, 129.65, 129.71, 130.66 (<u>C</u>H arom.), 133.07 (-<u>C</u>-C=N- arom.), 138.95 (-<u>C</u>-CH$_2$- arom.), 166.58 (-O-<u>C</u>=N-). IR (KBr): 1647 (-C=N-), 696 (CH arom.). GC/MS (100% area) m/z: 237 (M^+).

(*S*)-4-Benzyl-2-(4-chlorophenyl)-4,5-dihydrooxazole 3f Yield: 18 %. m.p.: 45-46 ^{o}C (lit.[8c]: 128-130 ^{o}C). ^{1}H-NMR (MeOD, 400 MHz): 7.85 (2H, d, $ArH_{1,5,}$ *J* = 8.6 Hz), 7.45 (2H, d, $ArH_{2,4}$, *J* = 8.6 Hz), 7.15-7.31 (5H, m, ArH'$_{1-5}$), 4.60 (1H, m, =N-C<u>H</u>-), 4.43 (1H, t, -C<u>H</u>$_2$-O-C$_{sp}$-, *J* = 8.6 Hz), 4.23 (1H, dd, -C<u>H</u>$_2$-O-C$_{sp}$-, *J* = 8.6 Hz), 3.07 (1H, dd, -C<u>H</u>$_2$Ph, *J* = 13.7 Hz), 2.83 (1H, dd, -C<u>H</u>$_2$Ph, *J* = 13.7 Hz). ^{13}C-NMR (MeOD, 125 MHz): 42.44 (-<u>C</u>H$_2$Ph), 68.63 (=N-<u>C</u>H-), 73.41 (-<u>C</u>H$_2$-O-), 127.44, 127.77, 129.66, 130.00, 130.68 (<u>C</u>H arom.), 130.99 (<u>C</u>-C=N- arom.), 138.92, 139.19 (<u>C</u>-Cl arom. or <u>C</u>-CH$_2$ arom.), 165.59 (-O-<u>C</u>=N-). IR (KBr): 1647 (-C=N-), 700, 671 (CH arom.). GC/MS (100% area) m/z: 271 (M^+).

(*S*)-4-Benzyl-2-(4-methoxyphenyl)-4,5-dihydrooxazole 3g Yield: 97 %. oil (Lit.[22]: 41-43 ^{o}C). ^{1}H-NMR (MeOD, 400 MHz): 7.82 (2H, d, $ArH_{1,5,}$ *J* = 6.9 Hz), 7.15-7.30 (5H, m, ArH'$_{1-5}$), 6.96 (2H, d, $ArH_{2,4}$, *J* = 6.9 Hz), 4.55 (1H, m, =N-C<u>H</u>-), 4.39 (1H, t, -C<u>H</u>$_2$-O-C$_{sp}$-, *J* = 8.5 Hz), 4.20 (1H, dd, -C<u>H</u>$_2$-O-C$_{sp}$-, *J* = 8.5 Hz), 3.84 (3H, s, -OC<u>H</u>$_3$), 3.08 (1H, dd, -C<u>H</u>$_2$Ph, *J* = 13.7 Hz), 2.80 (1H, dd, -C<u>H</u>$_2$Ph, *J* = 13.7 Hz). ^{13}C-NMR (MeOD, 125 MHz): 42.44 (-<u>C</u>H$_2$Ph), 55.98 (<u>C</u>H$_3$O-), 68.03 (=N-<u>C</u>H-), 73.09 (-<u>C</u>H$_2$-O-), 114.98, 120.56, 127.64, 129.56, 130.55 (<u>C</u>H arom.), 131.23 (<u>C</u>-C=N- arom.), 138.87 (<u>C</u>-CH$_2$- arom.), 164.26 (<u>C</u>-OCH$_3$ arom.), 166,61 (-O-<u>C</u>=N-). IR (KBr): 1647 (-C=N-), 702 (CH arom.). GC/MS (100% area) m/z: 267 (M^+).

(*S*)-4-Benzyl-2-(3-methoxyphenyl)-4,5-dihydrooxazole 3h Yield: 70 %. oil (lit.[23] not found). ^{1}H-NMR (MeOD, 400 MHz): 7.46 (2H, m, $ArH_{1,5}$), 7.33 (1H, t, ArH_4, *J* = 8.0 Hz), 7.19-7.29 (5H, m, ArH'$_{1-5}$), 7.08 (1H, m, ArH_3), 4.58 (1H, m, =N-C<u>H</u>-), 4.40 (1H, t, -C<u>H</u>$_2$-O-C$_{sp}$-, *J* = 8.6 Hz), 4.20 (1H, dd, -C<u>H</u>$_2$-O-C$_{sp}$-, *J* = 8.6 Hz), 3.82 (3H, s, -OC<u>H</u>$_3$),

3.09 (1H, dd, -C<u>H</u>$_2$Ph, *J* = 13.7 Hz), 2.81 (1H, dd, -C<u>H</u>$_2$Ph, *J* = 13.7 Hz). ^{13}C-NMR (MeOD, 100 MHz): 42.38 (-<u>C</u>H$_2$Ph), 55.89 (<u>C</u>H$_3$O-), 68.34 (=N-<u>C</u>H-), 73.13 (-<u>C</u>H$_2$-O-), 114.27, 119.01, 121.67, 127.65, 129.57, 130.56 (<u>C</u>H arom.), 130.72 (<u>C</u>-C=N- arom.), 138.84 (<u>C</u>-CH$_2$), 161.17 (<u>C</u>-OCH$_3$ arom.), 166.45 (-O-<u>C</u>=N-). IR (KBr): 1647 (-C=N-), 704 (CH arom.). GC/MS (100% area) m/z: 267 (M^+).

(*S*)-4-Benzyl-2-(2-methoxyphenyl)-4,5-dihydrooxazole 3i Yield: 74 %. oil. (Lit.[24] not found) ^{1}H-NMR (MeOD, 400 MHz): 6.90-7.60 (9H, m, ArH,H'), 4.59 (1H, m, =N-C<u>H</u>-), 4.34 (1H, t, -C<u>H</u>$_2$-O-C$_{sp}$-, *J* = 8.6 Hz), 4.16 (1H, dd, -C<u>H</u>$_2$-O-C$_{sp}$-, *J* = 8.6 Hz), 3.87 (3H, s, -OCH$_3$), 3.09 (1H, dd, -C<u>H</u>$_2$Ph, *J* = 13.7 Hz), 2.83 (1H, dd, -C<u>H</u>$_2$Ph, *J* = 13.7 Hz). ^{13}C-NMR (MeOD, 100 MHz): 42.39 (-<u>C</u>H$_2$Ph), 56.31 (<u>C</u>H$_3$O-), 68.71 (=N-<u>C</u>H-), 72.33 (-<u>C</u>H$_2$-O-), 113.07, 118.00, 121.47, 122.11, 127.74, 129.62, 130.81, 132.09 (<u>C</u>H arom.), 134.05 (<u>C</u>-C=N- arom.), 138.98 (<u>C</u>-CH$_2$- arom.), 160.11 (<u>C</u>-OCH$_3$ arom.), 165.44 (-O-<u>C</u>=N-). IR (KBr): 1647 (-C=N-), 704 (CH arom.). GC/MS (98% area) m/z: 267 (M^+).

(*S*)-4-Benzyl-2-(3,5-dimethoxyphenyl)-4,5-dihydrooxazole 3j Yield: 78 %. oil, ^{1}H-NMR (MeOD, 400 MHz): 7.15-7.30 (5H, m, ArH'$_{1-5}$), 7.03 (2H, d, ArH$_{1,5}$, *J* = 2.32), 6.64 (1H, t, ArH$_3$, *J* = 2.32 Hz), 4.59 (1H, m, =N-C<u>H</u>-), 4.41 (1H, t, -C<u>H</u>$_2$-O-C$_{sp}$-, *J* = 8.6 Hz), 4.21 (1H, dd, -C<u>H</u>$_2$-O-C$_{sp}$-, *J* = 8.6 Hz), 3.80 (6H, s, -OC<u>H</u>$_3$), 3.09 (1H, dd, -C<u>H</u>$_2$Ph, *J* = 13.7 Hz), 2.81 (1H, dd, -C<u>H</u>$_2$Ph, *J* = 13.7 Hz). ^{13}C-NMR (MeOD, 100 MHz): 42.46 (-<u>C</u>H$_2$Ph), 56.12 (2 <u>C</u>H$_3$O-), 68.44 (=N-<u>C</u>H-), 73.26 (-<u>C</u>H$_2$-O-), 105.25, 107.26, 127.78, 129.70, 130.34 (<u>C</u>H arom.), 130.65 (<u>C</u>-C=N- arom.), 138.94 (<u>C</u>-CH$_2$- arom.), 162.46 (2 <u>C</u>-OCH$_3$ arom.), 166.55 (-O-<u>C</u>=N-). IR (KBr): 1647 (-C=N-), 703 (CH arom.). Elemental Analysis calculated for $C_{18}H_{19}NO_3.1{,}5H_2O$: C, 66.65; H, 6.84; N, 4.32; Found: C, 66.46; H, 6.59; N, 4.30

(*S*)-4-Benzyl-2-(4-nitrophenyl)-4,5-dihydrooxazole 3k Yield: 84 %. m.p.: 91-92 ^{o}C (lit.[22]: 101-102 ^{o}C). ^{1}H-NMR (MeOD, 400 MHz): 8.30 (2H, d, ArH$_{2,4}$ *J* = 6.9 Hz), 8.11 (2H, d, ArH$_{1,5}$, *J* = 6.9 Hz), 7.25-7.30 (5H, m, ArH'$_{1-5}$), 4.66 (1H, m, =N-C<u>H</u>-), 4.50 (1H, t, -C<u>H</u>$_2$-O-C$_{sp}$-, *J* = 8.6 Hz), 4.28 (1H, dd, -C<u>H</u>$_2$-O-C$_{sp}$-, *J* = 8.6 Hz), 3.09 (1H, dd, -C<u>H</u>$_2$Ph, *J* = 13.7 Hz), 2.88 (1H, dd, -C<u>H</u>$_2$Ph, *J* = 13.7 Hz). ^{13}C NMR (MeOD, 100 MHz): 42.32 (-<u>C</u>H$_2$Ph), 69.00 (=N-<u>C</u>H-), 73.71 (-<u>C</u>H$_2$-O-), 124.81, 127.80, 129.65, 130.62, 130.72 (<u>C</u>H arom.), 134.55 (<u>C</u>-CH$_2$- arom.), 138.84 (<u>C</u>-C=N- arom.), 151.34 (<u>C</u>-NO$_2$ arom.), 164.57 (-O-<u>C</u>=N-). GC/MS (99% area) m/z: 282 (M^+).

(*S*)-2,4-Diphenyl-4,5-dihydrooxazole 3l Yield: 45%. m.p.: 66-67 ^{o}C (lit.[25]: oil). ^{1}H-NMR (MeOD, 400 MHz): 8.00 (2H, m, ArH$_{1,5}$) 7.25-7.60 (8H, m, ArH$_{2,3,4}$, ArH'$_{1-5}$), 5.39 (1H, t, -C<u>H</u>$_2$-O-C$_{sp}$-, *J* = 8.1 Hz), 4.87 (1H, dd, -C<u>H</u>$_2$-O-C$_{sp}$-, *J* = 8.1 Hz), 4.29 (1H, t, =N-C<u>H</u>-, *J* = 8.1 Hz), 3.09 (1H, dd, -C<u>H</u>$_2$Ph, *J* = 13.7 Hz), 2.81 (1H, dd, -C<u>H</u>$_2$Ph, *J* = 13.7 Hz). ^{13}C-NMR (MeOD, 100 MHz): 70.93 (=N-<u>C</u>H-), 76.58 (-<u>C</u>H$_2$-O-), 128.00, 128.44, 129.04, 129.63, 129.85, 130.03, 130.09 (<u>C</u>H arom.), 133.35 (<u>C</u>-C=N arom.), 143.59 (<u>C</u>-CH- arom.), 167.33 (-O-<u>C</u>=N-). IR (KBr): 1645 (-C=N-), 696 (CH arom.). GC/MS (100% area) m/z: 213 (M^+).

Biological assay

The antimycobacterial activities of synthesized compounds were assessed against *M. tuberculosis* ATTC 27294, using the micro plate Alamar Blue assay (MABA) [26] (Table 1). This methodology is nontoxic, uses thermally–stable reagent and shows good correlation with proportional and BACTEC radiometric methods[27]. Briefly, sterile deionized water (200 μL) was added to all outer-perimeter wells of sterile 96 well plates (falcon, 3072: Becton Dickinson, Lincoln Park NJ) to minimize evaporation of the medium in the test wells during incubation. The 96 plates received 100 μL of the Middlebrook 7H9 broth (Difco laboratories,

Detroit, MI, USA) and a serial dilution of the compounds **3a** a **3l** was made directly on the plate. The final drug concentrations tested was 6.25 μg/mL. Plates were covered and sealed with parafilm and incubated at 37 ^{o}C for five days. After this time, 25 μL of a freshly prepared 1:1 mixture of Alamar Blue (Accumed International, Westlake Ohio) reagent and 10% tween 80 was added to the plate and incubated for 24 h. A blue color in the well was interpreted as no bacterial growth; and a pink color was scored as growth. The MIC (Minimal Inhibition Concentration) was defined as the lowest drug concentration, which prevented a color change from blue to pink.

Cell Viability Assay

Cellular viability in the presence and absence of test compounds was determined by Mosmans´s MTT (3-(4,5-dimethylthiazol-2yl)-2,5-phenyltetrazolium bromide; Merck) microcultured tetrazolium assay[28]. The cells were plated in flat bottom 96-well plates (2.5 × 106 cells/mL) cultured for 1 h in a controlled atmosphere (CO_2 5% at 37 ^{o}C), and non-adherent cells were washed by gentle flushing with RPMI1640. Adherent cells were cultured in the presence of medium alone, tween 20 (3%) (live and dead controls, respectively) or different concentrations of compounds (Table 1) in a triplicate assay. After 18 h, stock MTT solution (5 mg/mL of saline; 20 μL/well) was added to the culture and 4 h later, supernatant was discharged and DMSO (100 μL/well) was added for formazan crystals solubilization, and the absorbance was read at 540 nm in a plate reader (Biorad-450).

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