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Synthesis, Characterization and Antimicrobial Activity of Novel Pharmacophores Incorporating Imidazoline-Oxazoline Scaffold

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In this work, synthesis, characterization and antimicrobial activity of series of imidazolines-oxazolines scaffolds **5a-f** and **10a-d** have been investigated. All the imidazolines-oxazolines derivatives were prepared from acid derivatives **1** and **6a-c**, and enantiomerically pure (*S*)-2-amino-3-methyl-1-butanol in four steps with excellent optical purity. The structures of all newly synthesized compounds have been elucidated by ^{1}H, ^{13}C NMR, GCMS, and IR spectrometry. Their purity was confirmed using elemental analysis. Some newly synthesized compounds were examined to *in-vitro* antimicrobial activity. Among the prepared products **10d** was found to exhibits the most active against all tested bacteria and fungi with minimal inhibitory concentration (MIC) ranged between 21.9 and 42.6 μg/mL.

Key Words : Imidazoline, Oxazoline, Antimicrobial activity

Introduction

Imidazolines and oxazolines scaffolds are important five membered heterocycles.[1] Among the imidazolines and oxazolines derivatives, the importance of 2-imidazolines and 2-oxazolines is the highest, for their wide applications in different fields of natural product chemistry, pharmaceutical chemistry, organic synthesis, coordination chemistry, and homogeneous catalysis. Since the discovery of the imidazoline receptor (imidazoline binding site, IBS) in 1984,[2] many bioactive 2-imidazoline-containing molecules have been synthesized and isolated. Among, the marine alkaloid 4,5-dihydro-6'-deoxybromotopsentin was isolated from a sponge which was identified as *Spongosorites* sp. with high cytotoxicity,[3] Imidazofurin,[4] Nutlin-3,[5] Benazoline, Cirazoline and Idazoxan.[6] In addition, 2-imidazolines have also been investigated as anti-inflammatory, antihyperglycemic, antihypercholesterolemic, antihypertensive, and antidepressant reagents.[7] On the other hand, 2-oxazolines and its analogs constitute the active class of compounds possessing wide spectrum of biological activities. Among of 2-oxazolines scaffold is Deflazacort which have been reported to anti-inflammatory agent, which at present in the lunch phase under trade name Calcort®.[8] Synthetic 2-oxazolines and 2-imidazolines are not only useful in biological applications, but also have found use as ligands in coordination chemistry.[9-14] The incidences of drug resistance of microorganisms to antibacterial agents were constantly reported for the past few years. Subsequently, there is an urgent need for the development of new drug molecules with newer targets, more potent, selective non-traditional antimicrobial agents and with an alternative mechanism of action.[15]

On the basis of the above considerations, we reasoned that coupling of imidazoline ring with oxazoline nucleus could result in compounds afford multifunctional system for biological and pharmacological evaluation.

Experimental

General Remarks. Glassware was oven-dried overnight at 120 ºC before use. Reactions were performed under an inert atmosphere using an argon filled glove box and standard Schlenk-line techniques. All the reactions were monitored by TLC analysis using Merck Silica Gel 60 F-254 thin layer plates. Column chromatography was performed on silica gel 100-200 mesh.

Materials: Petroleum ether (PE), hexane and ethyl acetate for column chromatography were distilled prior to use. CH_2Cl_2, EtOH were distilled from P_2O_5 and Mg respectively and stored on 4 Å molecular sieves. Diethyl ether, tetrahydrofuran, benzene, toluene were distilled from sodium benzophenone ketyl. Acetonitrile and dimethyformamide were dried by distillation over calcium hydride. Triethylamine and diisopropylamine were dried over sodium hydroxide. (*S*)-(+)-2-amino-3-methyl-1-butanol, aromatic amines, oxalyl chloride and indole were commercially obtained and used as purchased without further purification. Acid **6a-c** were prepared according to procedures reported in the literature.[20,21,23]

Instrumentation: NMR spectra were recorded with a Jeol

spectrometer at 400 MHz (^{1}H-NMR) and 100 MHz (^{13}C-NMR). The chemical shifts (δ in ppm) were reported down field from tetramethylsilane (TMS, δ scale) with the deuterated solvent resonance referenced as internal standard. Specific optical rotations were measured on a highly sensitive automatic 'A. KRÜSS OPTRONOCS' polarimeter using sodium light (D line 589 nm). Elemental analyses were performed on a Perkin Elmer 2400 Elemental Analyzer. IR spectra were obtained using Perkin Elmer FTIR-800 Model. Mass spectrometric analysis was conducted by using ESI mode on AGILENT Technologies 6410-triple quad LC/MS instrument.

General Method for Preparation of Compounds Derivatives (2 & 7a-c)(GP1). In a 100 mL round bottom flask compound **1** or **6a-c** (5.00 g, 31.3 mmol) was suspended in dry CH_2Cl_2 (75 mL) and a catalytic amount of DMF (3 drops) was added. Then oxalyl chloride (11.9 g, 93.8 mmol) was added dropwise to the reaction mixture at room temperature. The reaction mixture was stirred at ambient temperature for 1.5 h and gave a light yellow solution of acid chloride. Then the solvent was removed under reduced pressure to afford the crude acid chloride (6.15 g, ~100%) (crude). IR (cm^{-1}): 1802 cm^{-1} (C=O str.), (absence of OH str. Frequency at 3451 cm^{-1}). Then the solution of acid chloride (6.15 g, 31.3 mmol) in CH_2Cl_2 (60 mL), was added slowly to a solution of amine (6.77 g, 65.6 mmol, 2.1 eq.) and diisopropylamine (19.0, 26.5 mL, 187 mmol, 6 eq.) at 0 to 5 °C. Then the reaction mixture was allowed to stir at ambient temperature for 4 h. TLC analyses (10% MeOH/CH_2Cl_2) showed complete consumption of the starting material. The reaction mixture was then quenched with a saturated aqueous solution of ammonium chloride (50 mL) and extracted with chloroform (5 × 100 mL). The combined organic layers were washed with brine (100 mL) and dried over anhydrous magnesium sulfate. The solvent was removed under reduced pressure to afford the crude product which was washed with diethyl ether to afford pure amide **2 & 7a-c**.

2,2-Diethyl-*N*^{1},*N*^{3}-bis((*S*)-1-hydroxy-3-methylbutan-2-yl)malonamide (2): 2 was prepared according to method GP1, as a white solid; (9.5 g, 92%); mp 76-78 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 0.70 (t, 6H, *J* = 3.6 Hz, CH_3CH_2), 0.81-0.87 (m, 12H, $(CH_3)_2CH$), 1.74-1.87 (m, 6H, CH_3CH_2, & $(CH_3)_2CH$), 3.35-3.39 (m, 4H, $CHCH_2OH$), 3.65-3.67 (m, 2H, CH_2OH), 4.55-4.65 (m, 2H, $NHCH(CH_2OH)$), 8.26 (d, 2H, *J* = 8.8 Hz, CONH). The other analytical data are in accordance with the literature.[16]

(*Z*)-N1-((*R*)-1-Hydroxy-3-methylbutan-2-yl)-N3-((*S*)-1-hydroxy-3-methylbutan-2-yl)-2-(4-methylbenzylidene)-malonamide (7a): 7a was prepared according to method GP1, as oil liquid; (82%); IR ν_{max} (KBr) 3445.3, 3360.19, 3275.55, 1626.53 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.52 (s, 1H, CH), 7.34 (d, 2H, *J* = 8.08 Hz, Ph), 7.28 (d, *J* = 6.5 Hz, NH), 7.09 (d, 2H, *J* = 8.08 Hz, Ph), 3.97 (m, 2H, CH), 3.71-3.49 (m, 4H, CH_2OH), 2.31 (s, 3H, CH_3), 1.80 (m, $CH(CH_3)_2$), 0.99-0.89 (m, 3H, $2CH_3$); ^{13}C-NMR δ 18.7, 19.5, 19.6, 29.0, 29.5, 53.5, 57.3, 57.7, 63.2, 63.6, 129.3, 129.4, 130.6, 140.1, 165.2, 169.6; MS *m/z* (%): 377.21 (M^+, 100); Anal. for $C_{21}H_{32}N_2O_4$ (376.49) calcd; C, 66.99; H, 8.57; N, 7.44; Found: C, 67.02; H, 8.56; N, 7.48.

(*Z*)-N1-((*R*)-1-Hydroxy-3-methylbutan-2-yl)-N3-((*S*)-1-hydroxy-3-methylbutan-2-yl)-2-(4-methoxybenzylidene)-malonamide (7b): 7b was prepared according to method GP1, as oil liquid; (80%); IR ν_{max} (KBr) 3444.3, 3361.19, 3274.55, 1623.53 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.45 (S, 1H, CH), 7.41 (d, 2H, *J* = 8.8 Hz, Ph), 6.80 (d, 2H, *J* = 8.8 Hz, Ph), 3.80 (S, 6H, $2CH_3$), 3.83 (m, 2H, CH), 3.74-3.49 (m, 4H, CH_2OH), 1.75 (m, $CH(CH_3)_2$), 0.90-0.83 (m, 6H, $2CH_3$); ^{13}C-NMR δ 18.7, 19.5, 19.6, 57.4, 57.6, 63.2, 63.3, 114.0, 125.9, 129.3, 131.6, 137.8, 160.8, 165.5, 169.7; MS *m/z* (%): 393.41 (M^+, 100); Anal. for $C_{21}H_{32}N_2O_5$ (392.49) calcd; C, 64.26; H, 8.22; N, 7.14; Found: C, 64.27; H, 8.23; N, 7.15.

(*Z*)-N1-((*R*)-1-Hydroxy-3-methylbutan-2-yl)-N3-((*S*)-1-hydroxy-3-methylbutan-2-yl)-2-(thiophen-2-ylmethylene)-malonamide (7c): 7c was prepared according to method GP1, as oil liquid; (75%); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.57 (s, 1H, CH), 7.25 (d, 1H, *J* = 5.16 Hz, thiophene), 7.20 (d, 1H, *J* = 2.92 Hz, thiophene), 6.96 (t, 1H, *J* = 4.4 Hz, thiophene), 3.73 (m, 2H, CH), 3.69-3.34 (m, 4H, CH_2OH), 1.91 (m, $CH(CH_3)_2$), 0.96-92 (m, 6H, $2CH_3$). The other analytical data are in accordance with the literature.[17-19]

General Procedure for the Synthesis of Oxazoline-imidazoline Derivatives (5a-f & 10a-d) (GP2). Bis-amido alcohol **2 &7a-c** (2 mmol) was treated with thionylchloride (6 mL) at reflux for 2 h. Excess thionylchloride was removed under reduced pressure and the residue was dissolved in diethylether (20 mL). To this solution triethylamine (1.6 mL, 12 mmol) and RNH_2 (3 mmol) were added. The reaction mixture was then stirred for 4 hours at room temperature. 10% aqueous NaOH solution (8.3 mL) was then added and the reaction mixture was stirred for further 6 hours. Then the aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL) and the combined organic phases were washed with brine (25 mL) and dried over anhydrous magnesium sulfate. Then the organic part was concentrated under reduced pressure to afford the crude product mainly containing imidazoline-amidochloride with some expected oxazoline-imidazoline product (Intermediate **4a-f** & **9a-d**). The imidazoline-amidochloride was cyclized to oxazoline by treating with 10% NaOH (160 mg, 4 mmol) in MeOH: THF (3 mL/12 mL) at reflux for 12 h. The solvent was then removed and ligands were isolated by column chromatography using 100-200 mesh silica gel (EtOAc/Pet Ether/Et_3N = 1:1:0.02) afford **5a-f** &**10a-d**.

(*S*)-2-(3-((*S*)-1-(4-Chlorophenyl)-4-isopropyl-4,5-dihydro-1*H*-imidazol-2-yl)pentan-3-yl)-4-isopropyl-4,5-dihydro-oxazole (5a): 5a was prepared from bis-amidoalcohol **2** (660 mg, 2.0 mmol) and *p*-chloroaniline (381 mg, 3.0 mmol) according to **GP2** as described above. Light yellow colored oily product **5a** (392 mg, 48.6%, over 3 steps) was isolated after purification by silica gel chromatography. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.54-1.03 (m, 18H, CH_3CH_2 & $(CH_3)_2CH$), 1.48-1.63 (m, 1H, $(CH_3)_2CH$, imidazoline), 1.63-1.82 (m, 1H, $(CH_3)_2CH$, oxazoline), 1.82-2.10 (m, 4H, CH_3CH_2),

3.30-3.42 (m, 1H, $NCH_{2(a)}CH$, imidazoline), 3.42-3.51 (m, 1H, $NCH_{2(b)}CH$, imidazoline), 3.61-3.71 (m, 1H, $OCH_{2(a)}CH$, oxazoline), 3.71-3.80 (m, 1H, $OCH_{2(b)}CH$, oxazoline), 3.62-3.98 (m, 1H, $OCH_2CH(CH_3)_2$, oxazoline), 4.01-4.13 (m, 1H, $NCH_2CH(CH_3)_2$, imidazoline), 7.08 (d, 2H, J = 8.8 Hz, ArH), 7.26 (d, 2H, J = 8.8 Hz, ArH); The other analytical data are in accordance with the literature.[16]

(*S*)-4-Isopropyl-2-(3-((*S*)-4-isopropyl-1-(4-methoxyphenyl)-4,5-dihydro-1*H*-imidazol-2-yl)pentan-3-yl)-4,5-dihydrooxazole (5b): 5b was prepared from bis-amidoalcohol **2** (660 mg, 2.00 mmol) and *p*-anisidine (369 mg, 3.00 mmol) according to **GP2** as described above. Light yellow colored oily product **5b** (470 mg, 58.9%, over 3 steps) was isolated after purification by silica gel chromatography. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.71-0.99 (m, 18H, CH_3CH_2 & $(CH_3)_2CH$), 1.59-1.72 (m, 1H, $(CH_3)_2CH$, imidazoline), 1.73-1.85 (m, 1H, $(CH_3)_2CH$, oxazoline), 1.85-2.10 (m, 4H, CH_3CH_2), 3.38-3.50 (m, 2H, NCH_2CH, imidazoline), 3.58-3.72 (m, 1H, $OCH_{2(a)}CH$, oxazoline), 3.72-3.77 (m, 1H, $OCH_{2(b)}CH$, oxazoline), 3.79 (s, 3H, $ArOCH_3$), 3.87-3.96 (m, 1H, $OCH_2CH(CH_3)_2$, oxazoline), 4.06-4.19 (m, 1H, $NCH_2CH(CH_3)_2$, imidazoline), 6.80 (d, 2H, J = 8.8 Hz, ArH), 7.06 (d, 2H, J = 8.8 Hz, ArH); The other analytical data are in accordance with the literature. [16]

(*S*)-4-Isopropyl-2-(3-((*S*)-4-isopropyl-1-(quinolin-3-yl)-4,5-dihydro-1*H*-imidazol-2-yl)pentan-3-yl)-4,5-dihydrooxazole (5c): 5c was prepared from bis-amidoalcohol **2** (660 mg, 2.00 mmol) and quinolin-3-amine (432 mg, 3.00 mmol) according to **GP2** as described above. Light yellow colored oily product **5c** (410 mg, 48.8%, over 3 steps) was isolated after purification by silica gel chromatography. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.58-0.79 (m, 6H, CH_3CH_2), 0.79-1.06 (m, 12H, $(CH_3)_2CH$), 1.52-1.71 (m, 2H, $(CH_3)_2CH$, oxazoline & imidazoline), 1.72-2.11 (m, 4H, CH_3CH_2), 3.11-3.16 (m, 1H, $NCH_{2(a)}CH$, imidazoline), 3.16-3.34 (m, 1H, $NCH_{2(b)}$-CH, imidazoline), 3.57-3.67 (m, 1H, $OCH_{2(a)}CH$, oxazoline), 3.67-3.71 (m, 1H, $OCH_{2(b)}CH$, oxazoline), 3.72-3.83 (m, 1H, OCH_2CH, oxazoline), 4.03-4.12 (m, 1H, NCH_2CH, imidazoline), 7.25 (s, 1H, ArH), 7.54-8.05 (m, 4H, ArH),7.50-7.59 (m, 1H, ArH), 7.64-7.79 (m, 2H, ArH), 8.06 (d, J = 7.4 Hz, 1H, ArH), 8.70 (s, 1H, ArH); The other analytical data are in accordance with the literature. [16]

(*S*)-4-Isopropyl-2-(3-((*S*)-4-isopropyl-1-(*p*-tolyl)-4,5-dihydro-1*H*-imidazol-2-yl)pentan-3-yl)-4,5-dihydrooxazole (5d): 5d was prepared from bis-amidoalcohol **2** (660 mg, 2.00 mmol) and *p*-toluidine (321 mg, 3.00 mmol) according to the described procedure **GP2**. Light yellow colored oily product **5d** (345 mg, 45%, over 3 steps) was isolated after purification by silica gel chromatography. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.65-0.10 (m, 18H, CH_3CH_2 & $(CH_3)_2CH$), 1.69-2.07 (m, 6H, CH_3CH_2, & $(CH_3)_2CH$), 2.30 (s, 3H, $ArCH_3$), 3.32-3.43 (m, 1H, $NCH_{2(a)}CH$, imidazoline), 3.43-3.53 (m, 1H, $NCH_{2(b)}CH$, imidazoline), 3.62-3.71 (m, 1H, $OCH_{2(a)}CH$, oxazoline), 3.71-3.79 (m, 1H, $OCH_{2(b)}CH$, oxazoline), 3.79-3.98 (m, 1H, $OCH_2CH(CH_3)_2$, oxazoline), 4.01-4.15 (m, 1H, $NCH_2CH(CH_3)_2$, imidazoline), 7.03 (d, 2H, J = 8.0 Hz, ArH), 7.09 (d, 2H, J = 8.0 Hz, ArH); The other analytical data are in accordance with the literature.[16]

(*S*)-2-(3-((*S*)-1-Cyclohexyl-4-isopropyl-4,5-dihydro-1*H*-imidazol-2-yl)pentan-3-yl)-4-isopropyl-4,5-dihydrooxazole (5e): 5e was prepared from bis-amidoalcohol **2** (660 mg, 2.00 mmol) and cyclohexyl amine (298 mg, 3.00 mmol) according to the described procedure **GP2**. Light yellow colored oily product **5e** (586 mg, 78%, over 3 steps) was isolated after purification by silica gel chromatography. $[\alpha]_{25}^{D}$ = 85.7° (*c* 0.5%, $CHCl_3$); IR (cm^{-1}): 2959 (s), 2874 (s), 1660 (s), 1596 (s), 1515 (s), 1466 (s), 1382(s), 1236 (s), 1110(s), 1035(s), 985 (s), 824(s), 751(s); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.85-0.91 (m, 18H, CH_3CH_2 & $(CH_3)_2CH$), 1.62-2.07 (m, 6H, CH_3CH_2, & $(CH_3)_2CH$ & Cyclohexyl), 3.49-4.30 (m, 6H, $NCH_2CH(CH_3)_2$ & $OCH_2CH(CH_3)_2$); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 8.22 (**C**H_3CH_2), 16.0 (($\mathbf{C}H_3)_2CH$, Imidazoline), 17.9 (($\mathbf{C}H_3)_2CH$, Oxazoline), 19.6 ($CH_3\mathbf{C}H_2$), 25.5 (Cyclohexyl), 31.2 (($CH_3)_2\mathbf{C}H$, Imidazoline), 33.48 (($CH_3)_2\mathbf{C}H$, Oxazoline), 47.09 (**C**($CH_2CH_3)_2$), 55.71 (N**C**H_2, Imidazoline), 60.1 (O**C**H_2, Oxazoline), 71.22 (($NCH_2\mathbf{C}H$-$(CH_3)_2$, Imidazoline)), 72.9 ($OCH_2\mathbf{C}H(CH_3)_2$, Oxazoline), 165.2 (N**C**N), 166.9 (O**C**N); Anal. Calcd. for $C_{23}H_{41}N_3O$: C, 73.55; H, 11.00; N, 11.19; Found: C, 73.59; H, 11.03; N, 11.20; LC/MS (ESI): *m/z* = 376.46 $[M]^+$.

(*S*)-2-(3-((*S*)-1-(*tert*-Butyl)-4-isopropyl-4,5-dihydro-1*H*-imidazol-2-yl)pentan-3-yl)-4-isopropyl-4,5-dihydrooxazole (5f): 5f was prepared from bis-amidoalcohol **2** (660 mg, 2.00 mmol) and *tert*-butyl amine (220 mg, 3.00 mmol) according to the described procedure **GP2**. Light yellow colored oily product **5f** (574 mg, 82%, over 3 steps) was isolated after purification by silica gel chromatography. $[\alpha]_{25}^{D}$ = 65.7° (*c* 0.5%, $CHCl_3$); IR (cm^{-1}): 2959 (s), 2874 (s), 1660 (s), 1596 (s), 1515 (s), 1466 (s), 1382(s), 1236 (s), 1110 (s), 1035 (s), 985 (s), 824 (s), 751 (s); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.65-0.95 (m, 18H, CH_3CH_2 & $(CH_3)_2CH$), 1.20 (s, 9H, $3CH_3$), 1.65-2.33 (m, 6H, CH_3CH_2, & $(CH_3)_2CH$), 3.25-4.21 (m, 6H, $NCH_2CH(CH_3)_2$ & $OCH_2CH(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 7.9 (CH_3CH_2), 8.0 (CH_3CH_2), 18.1 (($CH_3)_2CH$, imidazoline), 19.4 (($CH_3)_2CH$, oxazoline), 25.3 (CH_3CH_2), 25.9 (CH_3CH_2), 29.1 (($CH_3)_3C$), 32.3 ($CH_3)_2CH$, imidazoline), 32.9 ($CH_3)_2CH$, oxazoline), 46.7 ((NCH_2, imidazoline), 59.1 (OCH_2, oxazoline), 66.8 (($CH_3)_3C$), 70.1 (NCH_2CH, imidazoline), 72.8 ((OCH_2CH, oxazole), 162.8 (NCN), 166.9 (OCN); Anal. Calcd. for $C_{21}H_{39}N_3O$: C, 72.16; H, 11.25; N, 12.02 Found: C, 72.21; H, 11.29; N, 12.08; LC/MS (ESI): *m/z* = 350.52 $[M]^+$.

(*R*)-4-Isopropyl-2-((*Z*)-1-((*R*)-4-isopropyl-1-(4-methoxyphenyl)-4,5-dihydro-1*H*-imidazol-2-yl)-2-(*p*-tolyl)vinyl)-4,5-dihydrooxazole (10a): 10a was prepared from bis-amidoalcohol **7a** (752 mg, 2.00 mmol) and *p*-anisidine (369 mg, 3.00 mmol) according to the described procedure **GP2**. Light yellow colored oily product **10a** (1030 mg, 77%, over 3 steps) was isolated after purification by silica gel chromatography. $[\alpha]_{25}^{D}$ = 123.5° (*c* 0.5%, $CHCl_3$); IR (cm^{-1}): 2959 (s), 2874 (s), 1660 (s), 1596 (s), 1515 (s), 1466 (s), 1382 (s), 1236 (s), 1110 (s), 1035 (s), 985 (s), 824 (s), 751 (s); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.85-0.96 (m, 18H, CH_3CH_2 & $(CH_3)_2CH$), 1.11-1.88 (m, 6H, CH_3CH_2, & $(CH_3)_2CH$), 3.81

(s, 6H, $2CH_3$), 3.74-3.99 (m, 6H, $NCH_2CH(CH_3)_2$ & $OCH_2CH(CH_3)_2$) 7.25 (s, 1H, CH), 6.94 (d, 2H, *J* = 8.08 Hz, Ph), 6.74 (d, 2H, *J* = 8.08 Hz, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 16.3 (($\mathbf{C}H_3)_2CH$, Imidazoline), 18.1 (($\mathbf{C}H_3)_2CH$, Oxazoline), 19.6 ($CH_3\mathbf{C}H_2$), 29.7 (Cyclohexyl), 31.2 (($CH_3)_2$-**C**H, Imidazoline), 33.48(($CH_3)_2\mathbf{C}H$, Oxazoline), 53.12 ($\mathbf{C}(CH_2CH_3)_2$), 55.51 ($N\mathbf{C}H_2$, Imidazoline), 60.3 ($O\mathbf{C}H_2$, Oxazoline), 71.22 (($NCH_2\mathbf{C}H(CH_3)_2$, Imidazoline)), 72.9 ($OCH_2\mathbf{C}H(CH_3)_2$, Oxazoline), 114.6 (Ph), 127.1 (ph), 131.7 (Ph), 158.5 (N**C**N), 160.4 (O**C**N); Anal. Calcd. for $C_{28}H_{35}N_3O_2$: C, 75.47; H, 7.92; N, 9.43; Found: C, 75.49; H, 7.94; N, 9.45; LC/MS (ESI): *m/z* = 446.48[M]$^+$.

(*R*)-2-((*Z*)-1-((*R*)-1-Cyclohexyl-4-isopropyl-4,5-dihydro-1*H*-imidazol-2-yl)-2-(p-tolyl)vinyl)-4-isopropyl-4,5-dihydrooxazole (10b): 10b was prepared from bis-amidoalcohol **7a** (752 mg, 2.00 mmol) and cyclohexyl amine (298 mg, 3.00 mmol) according to the described procedure **GP2**. Light yellow colored oily product **10b** (708 mg, 84%, over 3 steps) was isolated after purification by silica gel chromatography. $[\alpha]_{25}^{D}$ = 125.6° (*c* 0.5%, $CHCl_3$); IR (cm^{-1}): 2945 (s), 2870 (s), 1662 (s), 1596 (s), 1515 (s), 1466 (s), 1382 (s), 1236 (s), 1110 (s), 1035 (s), 985 (s), 824 (s), 751 (s); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.87-0.91 (m, 18H, CH_3CH_2 & $(CH_3)_2CH$), 1.01-1.83 (m, 6H, CH_3CH_2, & $(CH_3)_2CH$ & Cyclohexyl), 2.33 (s, 3H, CH_3), 3.45-3.75 (m, 6H, $NCH_2CH(CH_3)_2$ & $OCH_2CH(CH_3)_2$) 7.34 (s, 1H, CH), 7.25 (d, 2H, *J* = 8.08 Hz, Ph), 7.13 (d, *J* = 6.5 Hz, NH), 7.09 (d, 2H, *J* = 8.08 Hz, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 8.22 ($\mathbf{C}H_3CH_2$), 16.0 (($\mathbf{C}H_3)_2CH$, Imidazoline), 17.9 (($\mathbf{C}H_3)_2CH$, Oxazoline), 19.6 ($CH_3\mathbf{C}H_2$), 25.5 (Cyclohexyl), 31.2 (($CH_3)_2\mathbf{C}H$, Imidazoline), 33.48(($CH_3)_2\mathbf{C}H$, Oxazoline), 47.09 ($\mathbf{C}(CH_2CH_3)_2$), 55.71 ($N\mathbf{C}H_2$, Imidazoline), 60.1 ($O\mathbf{C}H_2$, Oxazoline), 71.22 (($NCH_2\mathbf{C}H(CH_3)_2$, Imidazoline)), 72.9 ($OCH_2\mathbf{C}H(CH_3)_2$, Oxazoline), 165.2 (N**C**N), 166.9 (O**C**N); Anal. Calcd. for $C_{27}H_{39}N_3O$: C, 76.92; H, 9.32; N, 9.97; Found: C, 76.90; H, 9.31; N, 10.01; LC/MS (ESI): *m/z* = 422.70 [M]$^+$.

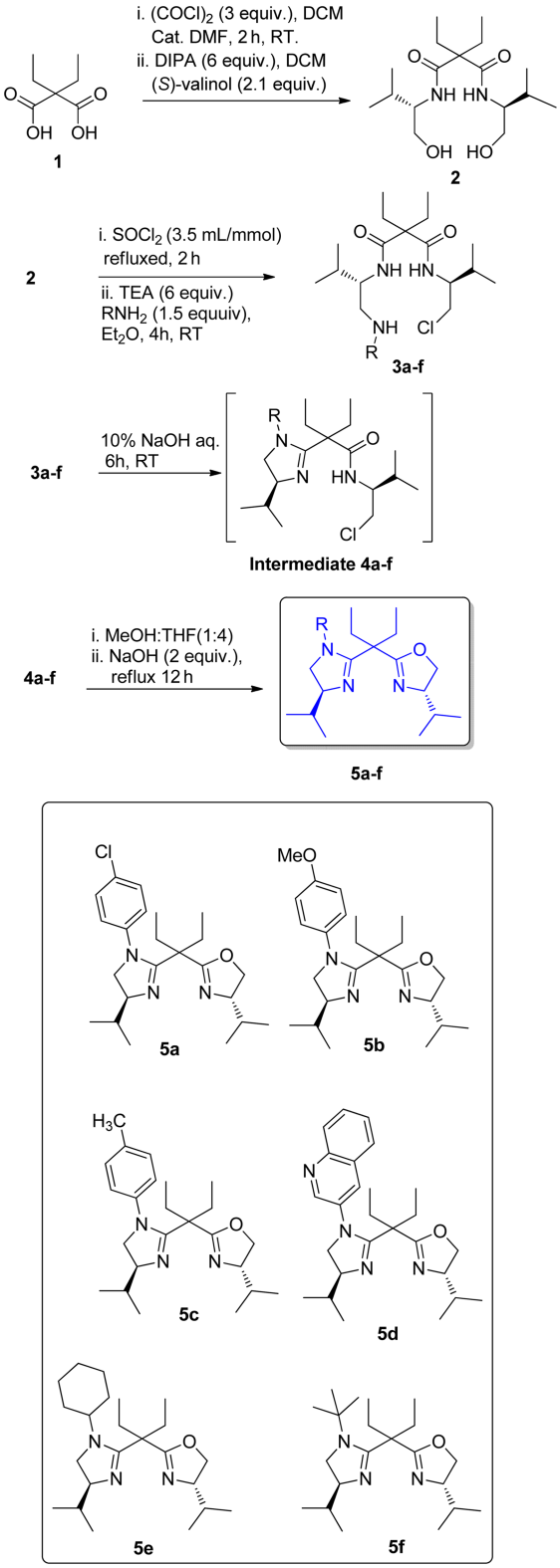
(*R*)-4-Isopropyl-2-((*Z*)-1-((*R*)-4-isopropyl-1-(4-methoxyphenyl)-4,5-dihydro-1*H*-imidazol-2-yl)-2-(4-methoxyphenyl)vinyl)-4,5-dihydrooxazole (10c): 10c was prepared from bis-amidoalcohol **7b** (785 mg, 2.00 mmol) and *p*-anisidine (369 mg, 3.00 mmol) according to the described procedure **GP2**. Light yellow colored oily product **10c** (750 mg, 81%, over 3 steps) was isolated after purification by silica gel chromatography. $[\alpha]_{25}^{D}$ = 128.5° (*c* 0.5%, $CHCl_3$); IR (cm^{-1}): 2962 (s), 2876 (s), 1660 (s), 1596 (s), 1515 (s), 1466 (s), 1382 (s), 1236 (s), 1110 (s), 1035 (s), 985 (s), 824 (s), 751 (s); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.85-0.96 (m, 18H, CH_3CH_2 & $(CH_3)_2CH$), 1.11-1.88 (m, 6H, CH_3CH_2, & $(CH_3)_2CH$), 3.81 (s, 6H, $2CH_3$), 3.74-3.99 (m, 6H, $NCH_2CH(CH_3)_2$ & $OCH_2CH(CH_3)_2$) 7.25 (s, 1H, CH), 6.94 (d, 2H, *J* = 8.08 Hz, Ph), 6.74 (d, 2H, *J* = 8.08 Hz, Ph); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 16.3 (($\mathbf{C}H_3)_2CH$, Imidazoline), 18.1 (($\mathbf{C}H_3)_2CH$, Oxazoline), 19.6 ($CH_3\mathbf{C}H_2$), 29.7 (Cyclohexyl), 31.2 (($CH_3)_2$-**C**H, Imidazoline), 33.48(($CH_3)_2\mathbf{C}H$, Oxazoline), 53.12 ($\mathbf{C}(CH_2CH_3)_2$), 55.51 ($N\mathbf{C}H_2$, Imidazoline), 60.3 ($O\mathbf{C}H_2$, Oxazoline), 71.22 (($NCH_2\mathbf{C}H(CH_3)_2$, Imidazoline)), 72.9 ($OCH_2\mathbf{C}H(CH_3)_2$, Oxazoline), 114.6 (Ph), 127.1 (ph), 131.7 (Ph), 158.5 (N**C**N), 160.4 (O**C**N); Anal. Calcd. for $C_{28}H_{35}N_3O_3$: C, 72.86; H, 7.64; N, 9.10; Found: C, 72.86; H, 7.64; N, 9.10; LC/MS (ESI): *m/z* = 463.51 [M]$^+$.

(*R*)-2-((*Z*)-1-((*R*)-1-Cyclohexyl-4-isopropyl-4,5-dihydro-1*H*-imidazol-2-yl)-2-(thiophen-2-yl)vinyl)-4-isopropyl-4,5-dihydrooxazole (10d): 10d was prepared from bis-amidoalcohol **7c** (734 mg, 2.00 mmol) and cyclohexylamine (298 mg, 3.00 mmol) according to the described procedure **GP2**. Light yellow colored oily product **10d** (705 mg, 85%, over 3 steps) was isolated after purification by silica gel chromatography. $[\alpha]_{25}^{D}$ = 125.6° (*c* 0.5%, $CHCl_3$); IR (cm^{-1}): 2959 (s), 2874 (s), 1660 (s), 1596 (s), 1515 (s), 1466 (s), 1382 (s), 1236 (s), 1110 (s), 1035 (s), 985 (s), 824 (s), 751 (s); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.81-0.96 (m, 18H, CH_3CH_2 & $(CH_3)_2CH$), 1.01-1.28 (m, 6H, CH_3CH_2, & $(CH_3)_2CH$ & Cyclohexyl), 3.96-3.12 (m, 6H, $NCH_2CH(CH_3)_2$ & OCH_2-$CH(CH_3)_2$) 7.34-7.61 (s, 1H, CH), 7.54 (d, 1H, *J* = 5.16 Hz, thiophene), 7. 22 (d, 1H, *J* = 2.92 Hz, thiophene), 7.07 (t, 1H, *J* = 4.4 Hz, thiophene); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 8.7 ($\mathbf{C}H_3CH_2$), 17.0 (($\mathbf{C}H_3)_2CH$, Imidazoline), 17.9 (($\mathbf{C}H_3)_2CH$, Oxazoline), 19.6 ($CH_3\mathbf{C}H_2$), 24.5 (Cyclohexyl), 31.2 (($CH_3)_2$-**C**H, Imidazoline), 33.48 (($CH_3)_2\mathbf{C}H$, Oxazoline), 46.09 ($\mathbf{C}(CH_2CH_3)_2$), 50.9 ($N\mathbf{C}H_2$, Imidazoline), 62.1 ($O\mathbf{C}H_2$, Oxazoline), 71.22 (($NCH_2\mathbf{C}H(CH_3)_2$, Imidazoline)), 72.9 ($OCH_2\mathbf{C}H(CH_3)_2$, Oxazoline), 165.2 (N**C**N), 166.9 (O**C**N); Anal. Calcd. for $C_{24}H_{35}N_3O_S$: C, 69.69; H, 8.53; N, 10.16; S, 7.75; Found: C, 69.73; H, 8.55; N, 10.18; S, 7.74; LC/MS (ESI): *m/z* = 415.22 [M]$^+$.

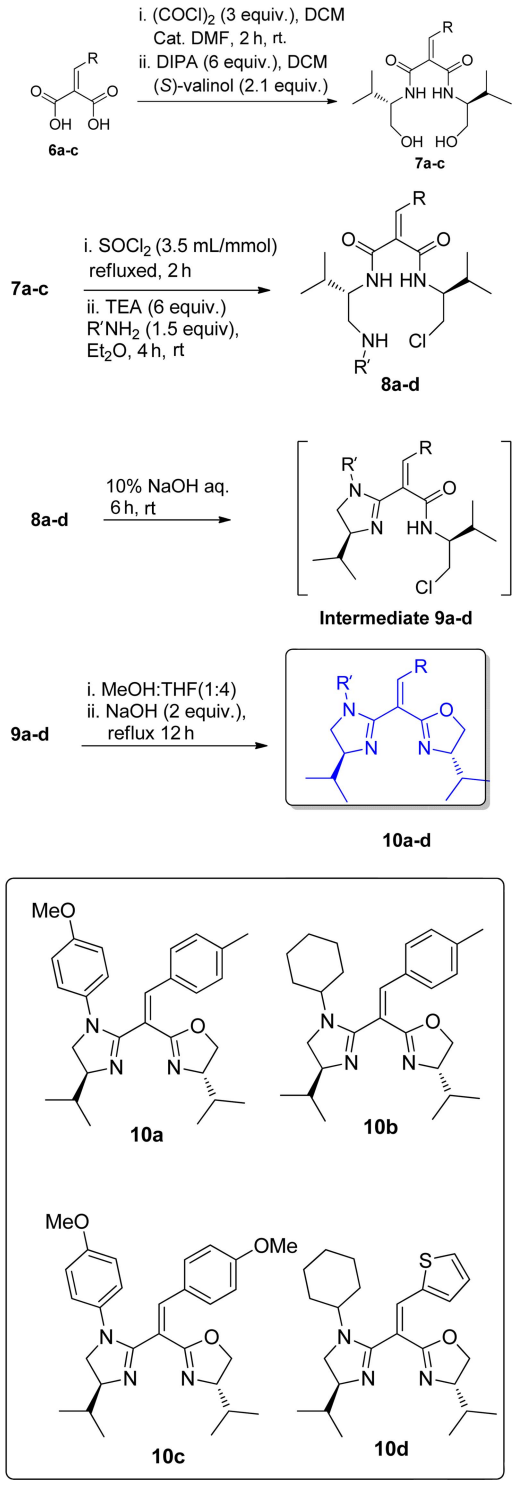
Antimicrobial Activity. Chemical compounds were individually tested against a panel of gram positive and negative bacterial pathogens. Antimicrobial tests were carried out by the agar well diffusion method[22] using 100 mL of suspension containing 1×10^8 CFU/mL of pathological tested bacteria, 1×10^6 CFU/mL of yeast and 1×10^4 spore/mL of fungi spread on nutrient agar (NA), Sabourand dextrose agar (SDA), and potato dextrose agar (PDA) medium respectively. After the media had cooled and solidified, wells (10 mm in diameter) were made in the solidified agar and loaded with 100 mL of tested compound solution prepared by dissolving 100 mg of the chemical compound in one mL of dimethyl sulfoxide (DMSO). The inculcated plates were then incubated for 24 h at 37 °C for bacteria and 48 h at 28 °C for fungi. Negative controls were prepared using DMSO employed for dissolving the tested compound. Ciprofloxacin (50 mg/mL) and Ketoconazole (50 mg/mL) were used as standard for antibacterial and antifungal activity respectively. After incubation time, antimicrobial activitywas evaluated by measuring the zone of inhibition against the test organisms and compared with that of the standard. The observed zone of inhibition is presented in Table 1. Antimicrobial activities were expressed as inhibition diameter zones in millimeters (mm) as follows: N.A. (no activity) ≤ 4 mm; + (weak) = 5-9 mm; ++ (moderate) = 10-15 mm; +++ (strong) = 16-20 mm and ++++ (very strong) ≥ 21 mm. The experiment was carried out in triplicate and the average zone of inhibition was calculated.

Minimal Inhibitory Concentration (MIC) measurement.

The bacteriostatic activity of the active compounds (having inhibition zones (IZ) ≥ 16 mm) was then evaluated using the two fold serial dilution technique.[22] Two fold serial dilutions of the tested compounds solutions were prepared using the proper nutrient broth. The final concentration of the solutions was 250; 175; 87.5; 43.8 and 21.9 mg/mL. The tubes were then inoculated with the test organisms, grown in their suitable broth at 37 °C for 24 h for bacteria (about 1×10^8 CFU/mL), each 5 mL received 0.1 mL of the above inoculum and incubated at 37 °C for 24 h. MIC is defined as the lowest concentration of the drug that kills or inhibits visible growth of microorganism.



Scheme 1. Synthesis of imidazoline-oxazoline derivatives **5a-f** from diethylmalonic acid **1**.



Scheme 2. Synthesis of oxazoline-imidazoline derivatives **10a-d** from acid derivatives **6a-c**.

Results and Discussion

Chemistry. The oxazoline-imidazoline analogue **5a-f** was obtained following the method described by Xin-Qi Hao *et al.*[14] and Barakat *et al.*[16] from commercially available 2,2-diethylmalonic acid **1** and (*S*)-valinol. At the outset, 2,2-diethylmalonic acid **1** was converted into acid chloride, *via* oxalyl chloride in the presence of catalytic amount of dimethylformamide (DMF), followed by subsequent reaction with (*S*)-valinol in the presence of excess diisopropylamine (DIPA) afforded compound bis-amidoalcohol **2** with overall 92% yield. This compound bis-amidoalcohol **2** was first converted into the bisamidochloride, (2 mmol) by treatment with thionylchloride under reflux for 2 h, then coupled to commercial amines (*p*-chloroaniline, *p*-methoxyaniline, 3-quinoline, *p*-toludine, cyclohexylamine, and *tert*-butylamine) in presence of TEA furnished the intermediates **3a-f**, subsequently ring closure by using 10% aqueous solution of sodium hydroxide to resulted in imidazoline ring afford crude product **4a-f**, mainly containing imidazoline-amidochloride with some expected imidazoline-oxazoline product (intermediates **4a-f**, Scheme 1).

The oxazoline analogues were obtained by ring closer by refluxing the crude intermediates **4a-f** with NaOH in THF: MeOH mixture (4:1) for 12 h.

Our study is now extended to include the synthesis of oxazoline-imidazoline analogues **10a-d** following the same method described above from synthetically acid derivatives **6a-c** with (*S*)-valinol. Elucidations of the chemical structures of **10a-d** are inferred from their spectroscopic and analytical data.

Antimicrobial Activity. A sample of some synthesized compounds have been subjected for antimicrobial activity studies including Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*), and fungi (*Candida albicans*). Antimicrobial tests were carried out by the agar well diffusion method. When compared to the standard drug Ciprofloxacin it was seen that compound **10d** with frame structure of oxazoline-imidazoline with thiophene backbone showed a potent inhibitory effect against *S. aureus* ATCC 29213, *Bacillus subtilis* ATCC 6633, *E. coli* ATCC 25922, *P.*

Table 1. Antimicrobial activity of the newly synthesized compounds against the pathological strains based on well diffusion assay[a]

Comp. No.	Gram-postive Bacteria		Gram-negative Bacteria		Fungi
	Staphylococcus aureus ATTCC 25923	*Bacilils subtilis* ATTCC 6633	*Escherichia coli* ATTCC 25922	*Pseudomonas aeruginosa* ATTCC 27853	*Candida albicans* ATTCC 10231
5a	+++	N.A.	N.A.	N.A.	++
5b	N.A.	N.A.	N.A.	N.A.	N.A.
5c	+++	N.A.	N.A.	N.A.	N.A.
5d	++	N.A.	N.A.	N.A.	N.A.
5e	N.A.	N.A.	N.A.	N.A.	N.A.
5f	N.A.	N.A.	N.A.	N.A.	N.A.
10a	+++	N.A.	N.A.	++	+++
10b	+++	N.A.	N.A.	++	+++
10c	+++	N.A.	N.A.	N.A.	++
10d	++++	+++	++++	+++	++++
Ciprofloxacin	+++	++++	++++	++++	N.A.
Ketoconazole	N.A.	N.A.	N.A.	N.A.	++++

[a]Antimicrobial activities were expressed as inhibition diameter zones in millimeters (mm) as follows: N.A. (no activity) ≤ 4 mm; + (weak) = 5-9 mm; ++ (moderate) = 10-15 mm; +++ (strong) = 16-20 mm and ++++ (very strong) ≥ 21 mm. The experiment was carried out in triplicate and the average zone of inhibition was calculated.

Table 2. Minimum inhibitory concentration (mg/mL) against the pathological strains based on two fold serial dilution technique

Comp. No.	Gram-postive Bacteria		Gram-negative Bacteria		Fungi
	Staphylococcus aureus ATTCC 25923	*Bacilils subtilis* ATTCC 6633	*Escherichia coli* ATTCC 25922	*Pseudomonas aeruginosa* ATTCC 27853	*Candida albicans* ATTCC 10231
5a	43.8	-	-	-	87.5
5c	43.8	-	-	-	-
5d	87.5	-	-	-	-
10a	43.8	-	-	87.5	43.8
10b	43.8	-	-	87.5	43.8
10c	43.8	-	-	-	87.5
10d	21.9	21.9	21.9	42.6	21.9
Ciprofloxacin	21.9	10.9	21.9	22.9	-
Ketoconazole	-	-	-	-	21.9

aeruginosa ATCC 27853, and *Candida albicans* ATCC 10231 with minimial inhibitory concentration (MIC) ranged between 21.9 and 42.6 μg/mL (Table 2). Never less, **10a** and **10b** showed a moderate effect against *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853, and *Candida albicans* with minimial inhibitory concentration (MIC) ranged between 43.8 and 87.5 μg/mL. On the other hand, **5a** and **10c** showed effect against *S. aureus* ATCC 29213, and *Candida albicans* with minimial inhibitory concentration (MIC) ranged between 43.8 and 87.5 μg/mL. Compounds **5c** and **5d** showed moderate activity and have zone of inhibition (43.8 and 87.5 μg/mL respectively) comparable to that of the standard ciprofloxacin (21.9 μg/mL) against *S. aureus* ATCC 29213. The other series of imidazoline-oxazoline **5b** and **5e-f** no activity was observed. The results indicate that compounds **10d** has tremendous biological potential and deserve to be further investigated. The results obtained are summarized in Tables 1 & 2.

Conclusion

In conclusion, we have successfully accessed biologically relevant imidazoline-oxazoline from acid **1** & **6a-c**. This method offers a distinct alternative to access **4a-f** & **9a-d** intermediates *in situ*, which can in turn be subjected to the reaction with various nucleophiles to provide functionalized imidazoline-oxazoline. Broad scope and ease of the reaction highlight this method. Compound **10d** showed the inhibitory effect against Gram-negative bacteria, Gram-positive bacteria, and anti-fungal activity against. Future work is focused on the further evaluation of analogues of compound **10d** and the identification of its biological target.

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