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Synthesis and Preliminary Antibacterial Evaluation of 2-Butyl Succinate-based Hydroxamate Derivatives Containing Isoxazole Rings

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Two series of novel 2-butyl succinate-based Hydroxamate derivatives containing isoxazole rings were synthesized, characterized and evaluated for antibacterial activity. The synthesized compounds were found to exhibit weak to moderate inhibitory activity against *Staphytlococcus aureu* and *Klebsiellar pneumonia in vitro*. All the compounds synthesized were found to be more effective against *Klebsiellar pneumonia* compared to *Staphytlococcus aureu*.

Key words: 2-Butyl succinate-based hydroxamate derivatives, Isoxazole, Synthesis, Antibacterial activity

INTRODUCTION

Emergence of bacterial resistance to all known classes of antibiotics is a serious threat to humans, and continued discovery of new antibiotics with novel modes of action is urgent to overcoming resistant bacteria (Davies, 1994; Spratt, 1994; Aarons et al., 1997). Some δ-alkyl substituted succinate derivatives have been found to show inhibitory activity against Gram positive or Gram negative bacteria, such as actinonin, Sch 382582, VRC3375, macrocyclic inhibitor, etc (Chen et al., 2000, 2004; Chu et al., 2001; Shen et al., 2008) (Fig. 1). The action mechanism of these compounds is believed to inhibit the peptide deformylase. Peptide deformylase (PDF) is a ferrous-containing metalloprotease, which catalyzes the removal of a formyl group from the N-termini of polypeptides biosynthesized in prokaryotes (Yuan et al., 2001). This step in bacterial protein synthesis is essential for bacterial proliferation (Giglione et al., 2000). The PDF inhibitors mentioned above share a common scaffold of δ-alkyl substituted succinate and the hydroxamate or the carboxylic group can complex the metal ion in the active pocket of the peptide deformylase, which is necessary to maintain the enzyme's activity (Clements et al., 2001; Madison et al., 2002).

Recent studies from several research groups have shown that PDF inhibitors may act as broad-spectrum antibacterial agents (Lofland et al., 2004; Jain et al., 2005). However, these compounds often suffer from weak *in vivo* activity, poor pharmacokinetic properties or selectivity for their apparent peptidic characteristics (Broughton et al., 1975; Clements et al., 2001; Lee et al., 2004; Shen et al., 2008). Electron-rich heterocyclics play an important role in diverse biological activities. To remove the influence of the hydrolyzable peptidic structures, we designed two series of conformationally restricted compounds in which isoxazole heterocycle was incorporated to replace the amide fragment of PDF inhibitors to work as an amide isostere (Fig. 2). Such peptidomimetic modification has led to the discovery of potent and selective $\alpha_v\beta_3$ receptor antagonist with good pharmacokinetic properties (Penning et al., 2006). This strategy has also been employed to develop potent HIV protease inhibitors (Chung et al., 1995). The hydroxamate group was

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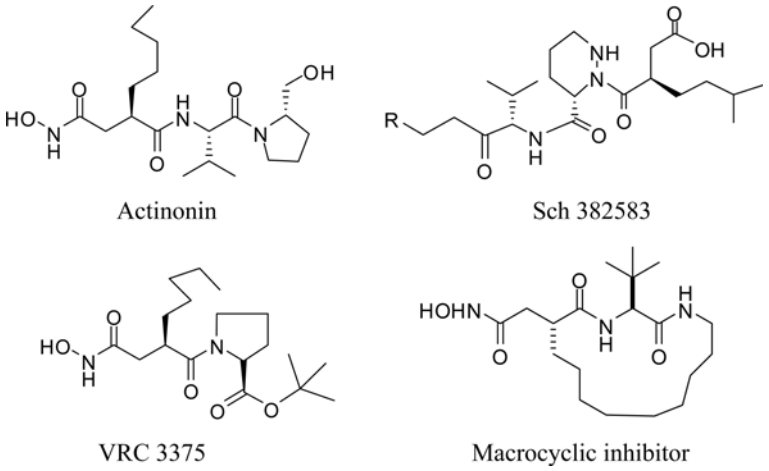


Fig. 1. Natural and synthetic δ-alkyl substituted succinate derivatives

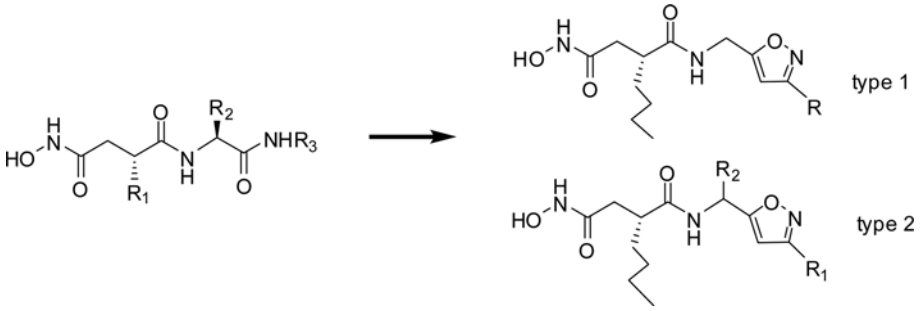


Fig. 2. Design strategy

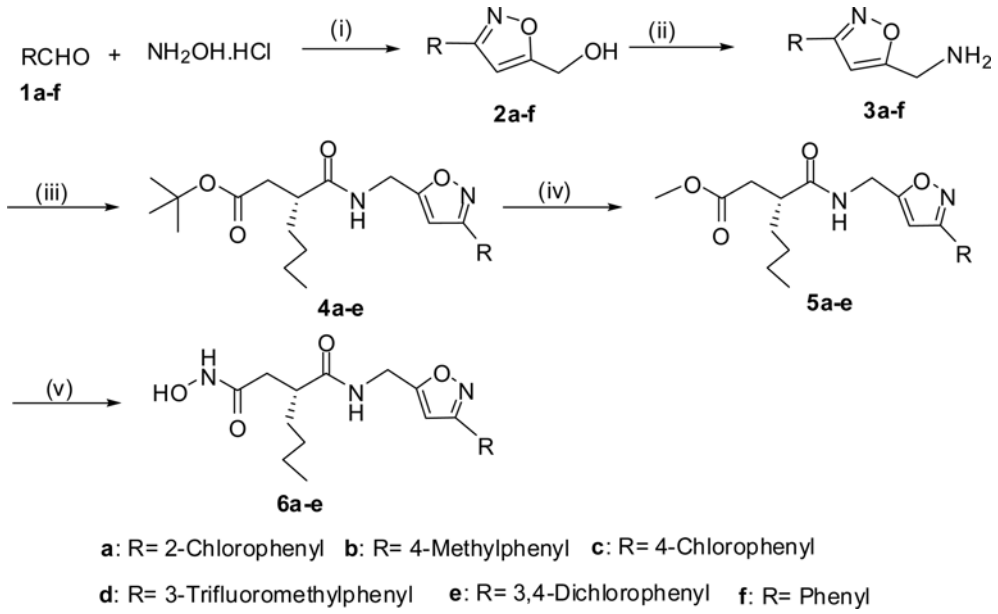
retained to fulfill the demand of a common structural feature of a "chelator + peptidomimetic" scaffold of PDF inhibitors (Guilloteau et al., 2002; Smith et al., 2002). For type 2 compounds, the second chiral center was constructed to model the actual steric orientation of the corresponding moiety of PDF inhibitors. In this paper, we describe the synthesis and preliminary antibacterial activity evaluation of some new isoxazole-containing 2-alkyl substituted succinate hydroxamate derivatives.

MATERIALS AND METHODS

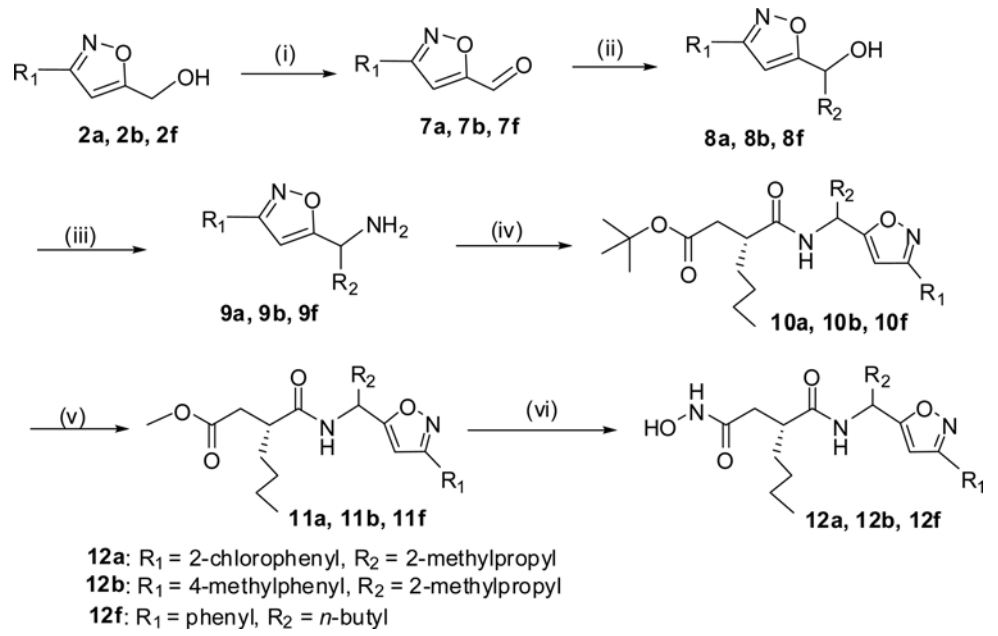
Chemistry

Melting points were determined by using an XT-4 microscopic apparatus and are uncorrected. Thin-layer chromatography (TLC) was performed on Silica Gel F_{254} plates with visualization by UV or iodine vapor. ^{1}H NMR spectra of $CDCl_3$/DMSO-d_6 solutions (TMS as internal standard) were recorded at Bruker DPX300 and DPX400 spectrometers, respectively. The IR spectra were measured on a Bruker Vector FT-IR spectrophotometer as KBr pellets or film. Mass spectra were obtained with an API4000 spectrometer. Elemental analyses were conducted on a Perkin-Elmer 2400 analyzer. Optical rotations were measured with a WZZ-2 polarimeter. All reagents were used as purchased from commercial suppliers without further purification.

The synthetic chemical routes employed for the synthesis of **6a-e, 12a, 12b** and **12f** is outlined in Scheme 1 and Scheme 2. 3-arylisoxazole was chosen to be incorporated into the target compounds. The commercially available compounds **1a-f** was treated with hydroxylamine hydrochloride to give the aromatic oximes, which underwent a 3+2 dipolar cycloaddition with propargyl alcohol, in the presence of N-chlorosuccinimide and triethylamine, to provide the 3-aryl-5-hydroxy- methylisoxazoles **2a-f**. The hydroxy group of the primary alcohols was transformed into amine group via methylsulfonylation, azidation and reduction in turn. The chiral intermediate (R)-2-butylbutanedioic acid 4-tert-butyl ester was prepared in high ee value according to the literature method (Zhang et al., 2006). 5-aminomethyl-3-arylisoxazoles **3a-e** was coupled with the chiral succinate with the acceleration of 1-chloro-3, 5-dimethoxy-2, 4, 6-triazine (CDMT) and N-methyl morpholine (NMM) (Kamiski, 1987). After deprotection and esterification, the methyl esters **5a-e**



Scheme 1. Synthetic route of type 1 compounds. (i) Na_2CO_3, CH_3OH/H_2O; propargyl alcohol, NCS/Et_3N/CH_2Cl_2 (ii) MsCl/NEt_3; NaN_3/DMF; Zn/NH_4Cl (iii) (R)-2-butylbutanedioic acid 4-tert-butyl ester, CDMT/NMM/CH_2Cl_2 (iv) HCOOH; CH_3OH/DCC/DMAP (v) NH_2OH, NaCN, CH_3OH/THF



Scheme 2. Synthetic route of type 2 compounds. (i) PCC, CH_2Cl_2 (ii) R_2MgBr, THF (iii) MsCl/NEt_3; NaN_3/DMF; Zn/NH_4Cl (iv) (R)-2-butylbutanedioic acid 4-tert-butyl ester, CDMT, NMM, CH_2Cl_2 (v) HCOOH; CH_3OH/DCC/DMAP (vi) NH_2OH, NaCN, CH_3OH/THF

were transformed into hydroxamates via a cyanide-catalyzed hydroxylamination procedure (Ho et al., 2005).

Oxidation of 3-aryl-5-hydroxymethylisoxazoles **3a**, **3b** and **3f** with PCC produced 3-arylisoxazole-5-carbaldehyde, which reacted with the Grignard reagents to afford the secondary alcohols. The hydroxyl group of compounds **8a**, **8b** and **8f** was transformed into amine via the three-step reaction sequence. The synthesis of target compounds **12a**, **12b** and **12f** was achieved using procedures similar to that depicted in Scheme 1.

General procedure for the synthesis of compounds (2a-f)

A mixture of $NH_2OH \cdot HCl$ (8.34 g, 0.12 mol), aromatic aldehyde (0.1 mol), methanol (50 mL) and water (150 mL) was stirred at room temperature until the solution turned clear. Sodium carbonate (6.36 g, 0.06 mol) was added slowly to the solution and then the mixture was stirred for additional 4 h. 200 mL of water was added and the resulting aqueous solution was extracted with dichloromethane. The combined organic phase was washed with water, brine and dried over sodium sulfate. The solvent was removed to afford the desired aromatic oximes.

N-chlorosuccinimide (3.2 g, 24.06 mmol) was added portionwise to a solution of substituted benzaldoxime (20 mmol) in dry dichloromethane (30 mL). After stirred at room temperature for 40 min, propargyl alcohol (1.12 g, 20.00 mmol) was added to the solution followed by triethylamine (2.20 g, 21.78 mmol). The mixture was heated to reflux for 4 h and then cooled to room temperature. The reaction mixture was washed with brine (10 mL), dried over sodium sulfate and concentrated. The residue was purified on silica gel column, eluting with petroleum ether/EtOAc (3:1) to provide compounds **2a-f**.

3-*o*-Chlorophenyl-5-hydroxymethylisoxazole (2a)
Slightly yellow solid, yield 75%, mp 73.5-74°C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.82 (s, 2H), 6.72 (s, 1H), 7.37 (m, 2H), 7.49 (m, 1H), 7.72 (m, 1H).

5-Hydroxymethyl-3-*p*-methylphenylisoxazole (2b)
Slightly yellow solid, yield 71%, mp 73-74°C. ^{1}H NMR ($CDCl_3$, 90 MHz) δ: 4.75 (s, 2H), 6.49 (s, 1H), 7.21 (d, J = 7.8 Hz, 2H), 7.63 (d, J = 7.8 Hz, 2H).

3-*p*-Chlorophenyl-5-hydroxymethylisoxazole (2c)
Slightly yellow solid, yield 75%, mp 99.5-100°C. ^{1}H NMR ($CDCl_3$, 90 MHz) δ: 4.79 (s, 2H), 6.51 (s, 1H), 7.39 (d, J = 9 Hz, 2H), 7.69 (d, J = 9 Hz, 2H).

5-Hydroxymethyl-3-*m*-trifluoromethylphenylisoxazole (2d)
Slightly yellow liquid, yield 70%. ^{1}H NMR ($CDCl_3$, 90 MHz) δ: 4.85 (s, 2H), 6.59 (s, 1H), 7.45-7.71 (m, 2H), 7.89-8.01 (m, 2H).

3-(3,4-Dichlorophenyl)-5-hydroxymethylisoxazole (2e)
Slightly yellow solid, yield 73%, mp 106-108°C. ^{1}H NMR ($CDCl_3$, 90 MHz) δ: 4.81 (s, 2H), 6.51 (s, 1H), 7.42-7.65 (m, 2H), 7.82 (d, J = 1.5 Hz, 1H).

5-Hydroxymethyl-3-phenylisoxazole (2f)
Slightly yellow solid, yield 61.7%, mp 52-53.5°C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.75 (s, 2H), 6.40 (s, 1H), 7.32-7.35 (m, 3H), 7.72-7.75 (m, 2H).

General procedure for the synthesis of compounds (3a-e)

To a solution of substituted 5-hydroxymethyl-3-phenylisoxazole (17.18 mmol) and triethylamine (2.08 g, 20.57 mmol) in dichloromethane (30 mL) was added a solution of mesyl chloride (2.36 g, 20.57 mmol) in dichloromethane (10 mL) at 0°C. The mixture was stirred under 5°C for 1 h and then stirred at room temperature for 10 h. The resulting mixture was washed with 20% sodium carbonate solution (10 mL), brine (10 mL) and dried over sodium sulfate. The solvent was removed to afford the mesylate intermediates as white solid**.**

The white solid obtained was dissolved in dry DMF (20 mL) and sodium azide (1.98 g, 30.47 mmol) was added slowly to the solution. The reaction mixture was stirred at 55°C for 8 h. 250 mL of water was added and the resulting aqueous solution was extracted with ethyl ether (4 × 60 mL). The combined organic phase was washed with water (60 mL), brine (60 mL) and dried over sodium sulfate. The organic solvent was removed to afford the azide intermediates as yellow solid.

To a solution of azide intermediates obtained above in ethyl alcohol (30 mL) and water (7 mL) was added Zinc powder (1.44 g, 22.18 mmol) and ammonium chloride (1.58 g, 29.56 mmol). The mixture was stirred at reflux for 0.5 h and then cooled to room temperature. Ethyl acetate (100 mL) and 15% sodium hydroxide aqueous solution (100 mL) was introduced to the reaction mixture. The organic phase was separated, washed with brine (20 mL) and dried with sodium sulfate. Removal of the solvent provide compound **3a-e**.

5-Aminomethyl-3-*o*-chlorophenylisoxazole (3a)
Slightly yellow liquid, yield 55.6%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.60 (s, 2H), 6.24 (s, 1H), 6.89-6.96 (m, 2H), 7.05-7.07 (m, 1H), 7.32-7.34 (m, 1H); IR (KBr, ν/cm^{-1}) 3311, 3374, 3065; ESI-MS m/z 209 $[M+H]^+$.

5-Aminomethyl-3-*p*-methylphenylisoxazole (3b)
White solid, yield 75%, mp 63.3-64.5°C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.00 (s, 2H), 6.41 (s, 1H), 7.25 (d, J = 8 Hz, 2H), 7.68 (d, J = 8 Hz, 2H); IR (KBr, ν/cm^{-1}) 3359, 3385, 3017; ESI-MS m/z 189 $[M+H]^+$, 211 $[M+Na]^+$.

5-Aminomethyl-3-*p*-chlorophenylisoxazole (3c)
White solid, yield 75%, mp 78.8-79.4°C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.02 (s, 2H), 6.42 (s, 1H), 7.42 (d, *J* = 8 Hz, 2H), 7.72 (d, *J* = 8 Hz, 2H); IR (KBr, ν/cm^{-1}) 3356, 3484, 3044; ESI-MS *m/z* 209 $[M+H]^+$.

5-Aminomethyl-3-*m*-trifluoromethylphenylisoxazole (3d)
White solid, yield 70%, mp 43.5-44.5°C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.03 (s, 2H), 6.49 (s, 1H), 7.46-7.74 (m, 2H), 7.93 (d, *J* = 8.3 Hz, 2H); IR (KBr, ν/cm^{-1}) 3330, 1327; ESI-MS *m/z* 243 $[M+H]^+$, 265 $[M+Na]^+$.

5-Aminomethyl-3-(3,4-dichlorophenyl)isoxazole (3e)
White solid, yield 73%, mp 96.1-96.6°C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.03 (s, 2H), 6.43 (s, 1H), 7.51 (d, *J* = 8 Hz, 1H), 7.62 (dd, *J* = 2, 8 Hz, 1H), 7.88 (d, *J* = 2 Hz, 1H); IR (KBr, ν/cm^{-1}) 3321, 3384, 3082; ESI-MS *m/z* 243 $[M+H]^+$.

General procedure for the synthesis of compounds (4a-e)

4, 6-Dimethoxy-2-chloro-1, 3, 5-triazine (CDMT) (424 mg, 2.42 mmol) and (R)-(+)-2- butylbutanedioic acid-4-tert-butyl monoester (506 mg, 2.2 mmol) were dissolved in dry methylene dichloride (15 mL). N-methylmorpholine (267 mg, 2.64 mmol) was added at -5-0°C. After 4 h, substituted 5-aminomethyl-3-phenylisoxazole (**3a-e**) (2.31 mmol) was added to the reaction mixture. The mixture was stirred at the same temperature for 2 h, then overnight at room temperature. The precipitate produced was removed by suction filtration and washed with a small amount of methylene dichloride. The filtrate was washed with water, 0.5 N HCl, saturated $NaHCO_3$, brine successively and dried with sodium sulfate. The solvent was concentrated, and the residue was purified on silica gel with petroleum ether/EtOAc (1:1).

N-[(3-*o*-chlorophenyisoxazol-5-yl)-methyl]-(R)-2-tert-butoxycarbonylmethylhexanamide (4a)
Colorless liquid, yield 80%, $[\delta]^D_{20}$ = + 18.5 (*c* 1, $CHCl_3$). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.82 (t, *J* = 7.1 Hz, 3H), 1.19-1.60 (m, 6H), 1.33 (s, 9H), 2.26 (dd, *J* = 5.3, 16 Hz, 1H), 2.46 (dd, *J* = 9.2, 16 Hz, 1H), 2.58-2.71 (m, 1H), 4.48 (d, *J* = 5.8 Hz, 2H), 6.68 (s, 1H), 7.47-7.55 (m, 2H), 7.61-7.67 (m, 2H); IR (KBr, ν/cm^{-1}) 3300, 3065, 2958, 2931, 2861, 1727, 1655, 1546, 1154; ESI-MS *m/z* 421 $[M+H]^+$.

N-[(3-*p*-methylphenylisoxazol-5-yl)-methyl]-(R)-2-tert-butoxycarbonylmethylhexanamide (4b)
White solid, yield 85%, mp 101.5-103.5°C, $[\delta]^D_{20}$ = + 35.5 (*c* 1, $CHCl_3$). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.82 (t, *J* = 7.1 Hz, 3H), 1.19-1.60 (m, 6H), 1.35 (s, 9H), 2.26 (dd, *J* = 5.4, 16 Hz, 1H), 2.35 (s, 3H), 2.46 (dd, *J* = 8.4, 16 Hz, 1H), 2.58-2.71 (m, 1H), 4.42 (t, *J* = 5.7 Hz, 2H), 6.75 (s, 1H), 7.31 (d, *J* = 8 Hz, 2H), 7.70 (d, *J* = 8 Hz, 2H); IR (KBr, ν/cm^{-1}) 3303, 3055, 2958, 5931, 2861, 1727, 1654, 1545, 1154; ESI-MS *m/z* 401 $[M+H]^+$.

N-[(3-*p*-chlorophenylisoxazol-5-yl)-methyl]-(R)-2-tert-butoxycarbonylmethylhexanamide (4c)
White solid, yield 81%, mp 115-117°C, $[\delta]^D_{20}$ = + 43.5 (*c* 1, $CHCl_3$). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.82 (t, *J* = 7.1 Hz, 3H), 1.19-1.60 (m, 6H), 1.35 (s, 9H), 2.26 (dd, *J* = 5.5, 16 Hz, 1H), 2.46 (dd, *J* = 8.9, 16 Hz, 1H), 2.56-2.71 (m, 1H), 4.42 (t, *J* = 5.7 Hz, 2H), 6.83 (s, 1H), 7.58 (d, *J* = 6.8 Hz, 2H), 7.83 (d, *J* = 6.8 Hz, 2H); IR (KBr, ν/cm^{-1}) 3319, 3090, 2976, 2931, 2862, 1732, 1655, 1552, 1155; ESI-MS *m/z* 421$[M+H]^+$.

N-[(3-*m*-trifluoromethylphenylisoxazol-5-yl)-methyl]-(R)-2-tert-butoxycarbonylmethylhexanamide (4d)
White solid, yield 90%, mp 51-53°C, $[\delta]^D_{20}$ = + 44.0 (*c* 1, $CHCl_3$). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.83 (t, *J* = 7.1 Hz, 3H), 1.19-1.60 (m, 6H), 1.35 (s, 9H), 2.26 (dd, *J* = 5.4, 16 Hz, 1H), 2.50 (dd, *J* = 9.1, 16 Hz, 1H), 2.58-2.65 (m, 1H), 4.43 (dd, *J* = 5.6, 16 Hz, 1H), 4.50 (dd, *J* = 5.9, 16 Hz, 1H), 6.97 (s, 1H), 7.77 (t, *J* = 7.8 Hz, 1H), 7.88 (d, *J* = 7.9Hz, 1H), 8.10 (s, 1H), 8.15 (d, *J* = 7.8 Hz, 1H); IR (KBr, ν/cm^{-1}) 3296, 3075, 2960, 2932, 2862, 1725, 1655, 1543, 1156; ESI-MS *m/z* 455 $[M+H]^+$.

N-[[[3-(3,4-dichlorophenyl)isoxazol]-5-yl]methyl]-(R)-2-tert-butoxycarbonylmethylhexanemide (4e)
White solid, yield 92%, mp 99-101°C, $[\delta]^D_{20}$ = + 49.5 (*c* 1, $CHCl_3$). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.83 (t, *J* = 6.8 Hz, 3H), 1.19-1.60 (m, 6H), 1.35 (s, 9H), 2.26 (dd, *J* = 5.5, 16 Hz, 1H), 2.49 (dd, *J* = 8.8, 16 Hz, 1H), 2.58-2.65 (m, 1H), 4.42 (m, 2H), 6.92 (s, 1H), 7.78 (d, *J* = 8.4 Hz, 1H), 7.83 (dd, *J* = 1.9, 8.4 Hz, 1H), 8.06 (d, *J* = 1.9 Hz, 1H); IR (KBr, ν/cm^{-1}) 3300, 3086, 2931, 2860, 1731, 1653, 1556, 1155; ESI-MS *m/z* 455 $[M+H]^+$.

General procedure for the synthesis of compounds (5a-e)

A solution of compound **4a-e** (1.66 mmol) in formic acid (98%, 8 mL) was stirred at room temperature for 7 h. Removal of the solvent under vacuum gave a white solid. The white solid was added to CH_2Cl_2 (12 mL), followed by methanol (3 mL) and 4-dimethylaminopyridine (0.158 mmol). After cooled to 0°C, DCC (342 mg, 1.66 mmol) was added and the solution was

stirred at the same temperature for 0.5 h. Then the reaction was continued at room temperature for 8 h. The precipitate produced was removed by suction filtration. The solvent was concentrated, and the residue was purified on silica gel with petroleum ether/EtOAc (3:1).

N-[(3-*o*-chlorophenyisoxazol-5-yl)-methyl]-(R)-2-methoxycarbonylmethylhexanamide (5a)
White solid, yield 78.9%, mp 80.5-82.5°C, $[\delta]_{20}^{D}$ = + 32.5 (*c* 1, $CHCl_3$). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.82 (t, *J* = 7.2 Hz, 3H), 1.18-1.50 (m, 6H), 2.38 (dd, *J* = 5.1, 16 Hz, 1H), 2.58 (dd, *J* = 9.2, 16 Hz, 1H), 2.65-2.69 (m, 1H), 3.54 (s, 3H), 4.48 (d, *J* = 5.8 Hz, 2H), 6.67 (s, 1H), 7.78-7.54 (m, 2H), 7.62-7.68 (m, 2H); IR (KBr, ν/cm^{-1}) 3302, 3067, 2955, 2930, 2859, 1737, 1654, 1603, 1542, 1171; ESI-MS *m/z* 380 $[M+H]^+$.

N-[(3-*p*-methylphenylisoxazol-5-yl)-methyl]-(R)-2-methoxycarbonylmethylhexanamide (5b)
White solid, yield 75%, mp 109.5-111.5°C, $[\delta]_{20}^{D}$ = + 43.5 (*c* 1, $CHCl_3$). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.82 (t, *J* = 7.2 Hz, 3H), 1.18-1.50 (m, 6H), 2.35 (s, 3H), 2.38 (dd, *J* = 5.2, 16 Hz, 1H), 2.59 (dd, *J* = 9, 16 Hz, 1H), 2.65-2.69 (m, 1H), 3.56 (s, 3H), 4.42 (d, *J* = 5.8 Hz, 2H), 6.73 (s, 1H), 7.31 (d, *J* = 8 Hz, 2H), 7.70 (d, *J* = 8 Hz, 2H); IR (KBr, ν/cm^{-1}) 3277, 3076, 2955, 2928, 2859, 1730, 1645, 1550, 1171; ESI-MS *m/z* 360 $[M+H]^+$.

N-[(3-*p*-chlorophenylisoxazol-5-yl)-methyl]-(R)-2-methoxycarbonylmethylhexanamide (5c)
White solid, yield 88%, mp 105.5-107.5°C, $[\delta]_{20}^{D}$ = + 45.5 (*c* 1, $CHCl_3$). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.82 (t, *J* = 7.2 Hz, 3H), 1.18-1.51 (m, 6H), 2.39 (dd, *J* = 5.2, 16 Hz, 1H), 2.59 (dd, *J* = 9, 16 Hz, 1H), 2.65-2.69 (m, 1H), 3.56 (s, 3H), 4.44 (d, *J* = 5.8 Hz, 2H), 6.81 (s, 1H), 7.58 (d, *J* = 8.5 Hz, 2H), 7.70 (d, *J* = 8.5 Hz, 2H); IR (KBr, ν/cm^{-1}) 3277, 3068, 2959, 2928, 2857, 1731, 1641, 1541, 1174; ESI-MS *m/z* 380 $[M+H]^+$.

N-[(3-*m*-trifluoromethylphenylisoxazol-5-yl)methyl]-(R)-2-methoxycarbonylmethylhexanemide (5d)
White solid, yield 78.5%, mp 104-106°C, $[\delta]_{20}^{D}$ = + 50.5 (*c* 1, $CHCl_3$). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.82 (t, *J* = 7.2 Hz, 3H), 1.18-1.51 (m, 6H), 2.38 (dd, *J* = 5.2, 16 Hz, 1H), 2.59 (dd, *J* = 9, 16 Hz, 1H), 2.65-2.69 (m, 1H), 3.56 (s, 3H), 4.46 (d, *J* = 5.7 Hz, 2H), 6.96 (s, 1H), 7.71 (t, *J* = 7.8 Hz, 1H), 7.88 (d, *J* = 7.8 Hz, 1H), 8.12 (s, 1H), 8.16 (d, *J* = 7.8 Hz, 1H); IR (KBr, ν/cm^{-1}) 3296, 3068, 2960, 2928, 2856, 1739, 1645, 1546, 1327, 1124; ESI-MS *m/z* 414$[M+H]^+$.

N-[[[3-(3,4-dichlorophenyl)isoxazol]-5-yl]-methyl]-(R)-2-methoxycarbonylmethylhexanamide (5e)
White solid, yield 82%, mp 136-138°C, $[\delta]_{20}^{D}$ = + 45.0 (*c* 1, $CHCl_3$). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.82 (t, *J* = 7.2 Hz, 3H), 1.18-1.51 (m, 6H), 2.38 (dd, *J* = 5.2, 16 Hz, 1H), 2.59 (dd, *J* = 8.9, 16 Hz, 1H), 2.65-2.69 (m, 1H), 3.56 (s, 3H), 4.45 (d, *J* = 5.7 Hz, 2H), 6.93 (s, 1H), 7.79 (d, *J* = 8.4 Hz, 1H), 7.84 (dd, *J* =1.9, 8.4 Hz, 1H), 8.08 (d, *J* = 1.9 Hz, 1H), 8.67 (t, *J* = 5.7 Hz, 1H); IR (KBr, ν/cm^{-1}) 3269, 3051, 1733, 1640, 1541, 1179; ESI-MS *m/z* 414 $[M+H]^+$.

General procedure for the synthesis of compounds (6a-e)

To a methanol/tetrahydrofuran (4 mL, 1:1) solution of compound **5a-e** (1.06 mmol) was added 50% aqueous hydroxylamine (10.6 mmol), followed by NaCN (40 mg). After 5 h, several drops of acetic acid were added to adjust the pH of the reaction mixture to 6. The solution was concentrated and the residue was purified on silica gel with methylene dichloride/methanol (20:1).

(R)-2-Butyl-N^4-hydroxy-N^1-[(3-*o*-chlorophenyisoxazol-5-yl)-methyl]succinamide (6a)
White solid, yield 66.8%, mp 132-134.5°C, $[\delta]_{20}^{D}$ = + 18.5 (*c* 0.5, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.79 (t, *J* = 7.1 Hz, 3H), 1.15-1.46 (m, 6H), 2.01-2.29 (m, 2H), 2.45-2.68 (m, 1H), 4.36-4.52 (m, 2H), 6.69 (s, 1H), 7.47 (t, *J* = 7.4 Hz, 1H), 7.53 (t, *J* = 7.5 Hz, 1H), 7.63 (d, *J* = 7.8 Hz, 1H), 7.67 (d, *J* = 7.5 Hz, 1H); IR (KBr, ν/cm^{-1}) 3296, 3062, 2958, 2923, 2855, 1624, 1543; ESI-MS *m/z* 380 $[M+H]^+$; Anal. Calcd for $C_{18}H_{22}ClN_3O_4$: C, 56.92; H, 5.84; N, 11.06; found C, 57.04; H, 5.90; N, 10.93.

(R)-2-Butyl-N^4-hydroxy-N^1-[(3-*p*-methylphenyisoxazol-5-yl)-methyl]succinamide (6b)
White solid, yield 51.5%, mp 143-145°C, $[\delta]_{20}^{D}$ = + 20.0 (*c* 0.5, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.82 (t, *J* = 7.2 Hz, 3H), 1.15-1.51 (m, 6H), 2.01-2.41 (m, 2H), 2.36 (s, 3H), 2.47-2.68 (m, 1H), 4.41 (d, *J* = 5.6 Hz, 2H), 6.76 (s, 1H), 7.30 (d, *J* = 8 Hz, 2H), 7.73 (d, *J* = 8 Hz, 2H); IR (KBr, ν/cm^{-1}) 3276, 3070, 2960, 2926, 2855, 1628, 1542; ESI-MS *m/z* 360 $[M+H]^+$; Anal. Calcd for $C_{19}H_{25}N_3O_4$: C, 63.49; H, 7.01; N, 11.69; found C, 63.58; H, 6.95; N, 11.71.

(R)-2-Butyl-N^4-hydroxy-N^1-[(3-*p*-chlorophenyisoxazol-5-yl)-methyl]succinamide (6c)
White solid, yield 45%, mp 146-148°C, $[\delta]_{20}^{D}$ = + 21.0 (*c* 0.6, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.82 (t, *J* = 7.1 Hz, 3H), 1.17-1.51 (m, 6H), 2.06 (dd, *J* = 6.9, 14.4 Hz, 1H), 2.24 (dd, *J* = 7.1, 14.4 Hz, 1H), 2.65-2.72 (m, 1H), 4.37-4.49 (m, 2H), 6.84 (s, 1H), 7.57 (d, *J* =

8.4 Hz, 2H), 7.87 (d, J = 8.4 Hz, 2H); IR (KBr, ν/cm^{-1}) 3298, 3216, 3068, 2958, 2927, 2857, 1649, 1555; ESI-MS m/z 380 [M+H]$^+$; Anal. Calcd for $C_{18}H_{22}ClN_3O_4$: C, 56.92; H, 5.84; N, 11.06; found C, 56.84; H, 5.87; N, 11.12.

(R)-2-Butyl-N^4-hydroxy-N^1-[(3-*m*-trifluoromethyl-phenyisoxazol-5-yl)-methyl]succinamide (6d)
White solid, yield 55%, mp 140.5-142.5°C, $[\delta]_{20}^{D}$ = + 28.1 (*c* 0.82, MeOH). ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ: 0.82 (t, J = 7.2 Hz, 3H), 1.19-1.48 (m, 6H), 2.01-2.45 (m, 2H), 2.50-2.70 (m, 1H), 4.44-4.47 (m, 2H), 6.97 (s, 1H), 7.76 (t, J = 7.7 Hz, 1H), 7.86 (d, J = 7.7 Hz, 1H), 8.15-8.19 (m, 1H); IR (KBr, ν/cm^{-1}) 3310, 3224, 3076, 2952, 2930, 2861, 1628, 1555; ESI-MS m/z 414 [M+H]$^+$; Anal. Calcd for $C_{19}H_{22}F_3N_3O_4$: C, 55.20; H, 5.36; N, 10.16; found C, 55.30; H, 5.29; N, 10.18.

(R)-2-Butyl-N^4-hydroxy-N^1-[[[3-(3,4-dichlorophenyl) isoxazol]-5-yl]-methyl]succinamide (6e)
White solid, yield 85%, mp 145-147.5°C, $[\delta]_{20}^{D}$ = + 13.6 (*c* 0.85, MeOH). ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ: 0.82 (t, J = 7.1 Hz, 3H), 1.15-1.45 (m, 6H), 2.01-2.42 (m, 2H), 2.45-2.69 (m, 1H), 4.37-4.48 (m, 2H), 6.91 (s, 1H), 7.77 (d, J = 8 Hz, 1H), 7.86 (d, J = 8 Hz, 1H), 8.11 (s, 1H); IR (KBr, ν/cm^{-1}) 3290, 3179, 3029, 2957, 2930, 2859, 1649, 1558; ESI-MS m/z 414 [M+H]$^+$; Anal. Calcd for $C_{18}H_{21}Cl_2N_3O_4$: C, 52.19; H, 5.11; N, 10.14; found C, 52.17; H, 5.05; N, 10.21.

General procedure for the synthesis of compounds (7a, 7b, and 7f)

To a solution of PCC (3.23 g, 15 mmol) in CH_2Cl_2 (20 mL) was added substituted 5-Hydroxymethyl-3-phenylisoxazole (10 mmol). The mixture was stirred at room temperature for 3 h and monitored by TLC. After the end of reaction, the reaction mixture was filtered through a pad of silica gel and washed with petroleum ether/EtOAc(9:1). The solvent was concentrated, and the residue was purified on silica gel with petroleum ether/EtOAc (9:1).

3-*o*-Chlorophenylisoxazole-5-carbaldehyde (7a)
Slightly yellow liquid, yield 60%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.36-7.47 (m, 2H), 7.49 (s, 1H), 7.50-7.53 (m, 1H), 7.77-7.80 (m, 1H), 10.03 (s, 1H); IR (KBr, ν/cm^{-1}) 3051, 2856, 1703; ESI-MS m/z 208 [M+H]$^+$.

3-*p*-Methylphenylisoxazole-5-carbaldehyde (7b)
White solid, yield 55%, mp 77-79°C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.44 (s, 3H), 7.28 (s, 1H), 7.33 (d, J = 7.8 Hz, 2H), 7.77 (d, J = 7.8 Hz, 2H), 10.04 (s, 1H); IR (KBr, ν/cm^{-1}) 3045, 2857, 1704; ESI-MS m/z 188 [M+H]$^+$.

3-Phenylisoxazole-5-carbaldehyde (7f)
Slightly yellow solid, yield 65%, mp 67-69°C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.28 (s, 1H), 7.49-7.51 (m, 3H), 7.83-7.86 (m, 2H), 10.03 (s, 1H); IR (KBr, ν/cm^{-1}) 3048, 2857, 1704; ESI-MS m/z 174 [M+H]$^+$.

General procedure for the synthesis of compounds (8a, 8b, and 8f)

0.23 g of magnesium powder was covered with 5 mL of tetrahydrofuran, and an iodine crystal was added. A solution of n-bromobutane (1.0 g, 7.3 mmol) in 2 mL of tetrahydrofuran was added dropwise under N_2 to keep the reaction mixture boiling slightly. When the addition was complete, the mixture was refluxed for further 1 h and then cooled to room temperature. The Grignard reagent obtained was introduced slowly to a solution of substituted 3-phenylisoxazole-5-carbaldehyde (2.6 mmol) in 20 mL of tetrahydrofuran in an ice bath. After the addition, the stirring was continued for 3 h and then for additional 10 h at room temperature. 20 mL of 10% aqueous ammonium chloride was added and the mixture was extracted by CH_2Cl_2 (20 mL × 3). The organic phase was dried over Na_2SO_4 and concentrated at reduced pressure. The residue was purified on silica gel with petroleum ether/EtOAc (9:1)

1-[3-(*o*-Chlorophenyl)isoxazol-5-yl]-3-methylbutan-1-ol (8a)
Light yellow sticky liquid, yield 75%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.97 (d, J = 8 Hz, 3H), 0.99 (d, J = 8 Hz, 3H), 1.77-1.90 (m, 3H), 4.98-5.01 (m, 1H), 6.66 (s, 1H), 7.35 (dt, J = 7.2, 1.6 Hz, 1H), 7.38 (dt, J = 7.6, 2 Hz, 1H), 7.48 (dd, J = 7.6, 2 Hz, 1H), 7.72 (dd, J = 7.2, 2 Hz, 1H); IR (KBr, ν/cm^{-1}) 3364, 3036, 1605; ESI-MS m/z 265 [M+H]$^+$.

1-[3-(*p*-Methylphenyl)isoxazol-5-yl]-3-methylbutan-1-ol (8b)
Light yellow sticky liquid, yield 70%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.96 (d, J = 8 Hz, 3H), 0.98 (d, J = 8 Hz, 3H), 1.72-1.76 (m, 3H), 2.40 (s, 3H), 4.95-4.98 (m, 1H), 6.49 (s, 1H), 7.26 (d, J = 8 Hz, 2H), 7.69 (d, J = 8 Hz, 2H); IR (KBr, ν/cm^{-1}) 3367, 3065, 1607; ESI-MS m/z 246 [M+H]$^+$.

1-(3-Phenylisoxazol-5-yl)pentan-1-ol (8f)
Light yellow sticky liquid, yield 72%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.93 (t, J = 5.2 Hz, 3H), 1.35-1.50 (m, 4H), 1.88-1.96 (m, 2H), 4.88-4.91 (m, 1H), 6.51 (s, 1H), 7.43-7.47 (m, 3H), 7.79-7.81 (m, 2H); IR (KBr, ν/cm^{-1}) 3353, 3045, 1608; ESI-MS m/z 232 [M+H]$^+$.

General procedure for the synthesis of compounds (9a, 9b, and 9f)

To an ice-cooled mixture of compounds **8** (2 mmol) and triethylamine (0.25 g, 2.5 mmol) in CH_2Cl_2 (25 mL) was added Mesyl chloride (0.26 g, 2.3 mmol) in CH_2Cl_2 (5 mL). The reaction mixture was stirred at 0 to 5°C for 1 h and then for additional 5 h at room temperature. The mixture was washed with 20% aqueous Na_2CO_3 solution and water, dried over Na_2SO_4, and evaporated under vacuum to afford a light yellow sticky liquid.

DMF (15 mL) was added to dissolve the syrupy residue, followed by NaN_3 (0.13 g, 1.94 mmol) and the reaction mixture was stirred at room temperature for 5 h. Upon completion of the reaction, as monitored by TLC, 50 mL of water was added and the mixture was extracted with diethyl ether (30 mL × 3). The combined organic phase was washed with water, dried over Na_2SO_4, and evaporated under vacuum. The obtained light-yellow syrup was dissolved in a mixture of ethanol (25 mL) and water (8 mL), Zinc powder (0.13g, 1.95 mmol) and NH_4Cl (0.17 g, 3.14 mmol) were added, and the mixture was heated to reflux for 0.5 h. After cooling to room temperature, 70 mL of ethyl acetate and 10 mL of 15% aqueous NaOH solution were added to the mixture. The organic phase was separated, dried and evaporated. The residue was purified on silica gel with petroleum ether/EtOAc (2:1)

1-[3-(*o*-Chlorophenyl)isoxazol-5-yl]-3-methylbutan-1-amine (9a)

Light yellow sticky liquid, yield 57.5%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.96 (d, J = 8 Hz, 3H), 0.98 (d, J = 8 Hz, 3H), 1.65-1.80 (m, 3H), 1.87 (brs, 2H), 4.18-4.21 (m, 1H), 6.57 (s, 1H), 7.34 (dt, J = 7.2, 1.6 Hz, 1H), 7.37 (dt, J = 7.6, 2 Hz, 1H), 7.47 (dd, J = 7.6, 2 Hz, 1H), 7.72 (dd, J = 7.2, 2 Hz, 1H); IR (KBr, ν/cm^{-1}) 3304, 3377, 3063, 1605; ESI-MS m/z 265 $[M+H]^+$.

3-Methyl-1-[3-(*p*-methylphenyl)isoxazol-5-yl)butan-1-amine (9b)

Light yellow sticky liquid, yield 55%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.95 (d, J = 8 Hz, 3H), 0.97 (d, J = 8 Hz, 3H), 1.62-1.76 (m, 3H), 1.81 (brs, 2H), 2.39 (s, 3H), 4.14-4.18 (m, 1H), 6.39 (s, 1H), 7.25 (d, J = 8 Hz, 2H), 7.68 (d, J = 8 Hz, 2H); IR (KBr, ν/cm^{-1}) 3304, 3374, 3021, 1606; ESI-MS m/z 245 $[M+H]^+$.

1-(3-Phenylisoxazol-5-yl)pentan-1-amine (9f)

Light yellow sticky liquid, yield 53%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.91 (t, J = 5.2 Hz, 3H), 1.35-1.42 (m, 4H), 1.75-1.89 (m, 4H), 4.08-4.11 (m, 1H), 6.42 (s, 1H), 7.42-7.45 (m, 3H), 7.78-7.80 (m, 2H); IR (KBr, ν/cm^{-1}) 3305, 3371, 3035,1607; ESI-MS m/z 231 $[M+H]^+$.

General procedure for the synthesis of compounds (10a, 10b, and 10f)

4, 6-Dimethoxy-2-chloro-1, 3, 5-triazine (CDMT) (193 mg, 1.1 mmol) and (R)-(+)-2- butylbutanedioic acid-4-tert-butyl monoester (230 mg, 1 mmol) were dissolved in dry methylene dichloride (7 mL). N-methylmorpholine (1.98 mmol) was added at -5-0°C. After 4 h, compound **9** (1.05 mmol) was added to the reaction mixture. The mixture was stirred at the same temperature for 2 h, then overnight at room temperature. The precipitate produced was removed by suction filtration and washed with a small amount of methylene dichloride. The combined washing and filtrate were washed with water, 0.5 N HCl, saturated $NaHCO_3$, saturated saline successively and dried with sodium sulfate. The solvent was concentrated, and the residue was purified on silica gel with methylene dichloride/methanol (3:1).

N-[1-[3-(*o*-chlorophenyl)isoxazol-5-yl]-3-methyl]butyl-(R)-2-tert-butoxycarbonylmethylhexanamide (R, R/S, R mixture) (10a)

White solid, yield 93%, mp 92-94°C, $[\delta]_{20}^{D}$ = + 13.0 (*c* 1, $CHCl_3$). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.76-0.95 (m, 9H), 1.11-1.48 (m, 15H), 1.63-1.82 (m, 3H), 2.25-2.27 (m, 1H), 2.46-2.51 (m, 1H), 2.60-2.69 (m, 1H), 5.12-5.20 (m, 1H), 6.68/6.74 (s/s, 1H), 7.45-7.55 (m, 2H), 7.61-7.67 (m, 2H); IR (KBr, ν/cm^{-1}) 3291, 3057, 2959, 2932, 2870, 1730, 1645, 1550,1154; ESI-MS m/z 477 $[M+H]^+$.

N-[1-[3-(*p*-methylphenyl)isoxazol-5-yl]-3-methyl]butyl-(R)-2-tert-butoxycarbonylmethylhexanamide (R, R/S, R mixture) (10b)

White solid, yield 85%, mp 102-104°C, $[\delta]_{20}^{D}$ = + 22.5 (*c* 1, $CHCl_3$). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.76-0.95 (m, 9H), 1.13-1.48 (m, 15H), 1.65-1.82 (m, 3H), 2.25 (dd, J = 5.4, 16 Hz, 1H), 2.35 (s, 3H), 2.46 (dd, J = 9, 16 Hz, 1H), 2.60-2.69 (m, 1H), 5.06-5.17 (m, 1H), 6.73/6.76 (s/s, 1H), 7.30 (dd, J = 1.8, 8 Hz, 2H), 7.70 (dd, J =3.7, 8 Hz, 2H); IR (KBr, ν/cm^{-1}) 3297, 3059, 1730, 1645, 1547, 1154; ESI-MS m/z 457 $[M+H]^+$.

N-[1-(3-phenylisoxazol-5-yl)]pentyl-(R)-2-tert-butoxycarbonylmethylhexanamide (R, R/S, R mixture) (10f)

White solid, yield 85%, mp 91-93°C, $[\delta]_{20}^{D}$ = + 21.5 (*c* 1, CHCl3). ^{1}H NMR (DMSO-d6, 400 MHz) δ: 0.76-0.89 (m, 9H), 1.13-1.48 (m, 17H), 1.65-1.82 (m, 2H), 2.25 (dd, J = 5.2, 16 Hz, 1H), 2.46 (dd, J = 6.5, 16 Hz, 1H), 2.60-2.69 (m, 1H), 5.03-5.07 (m, 1H), 6.80/6.87 (s/s,

1H), 7.48-7.52 (m, 3H), 7.80-7.83 (m, 2H); IR (KBr, ν/cm^{-1}) 3278, 3068, 2958, 2931, 2861, 1728, 1646, 1548, 1154; ESI-MS *m/z* 443 $[M+H]^+$.

General procedure for the synthesis of compounds (11a, 11b, and 11f)

A solution of compound **10** (0.66 mmol) in formic acid (98%, 4 mL) was stirred at room temperature for 7 h. Removal of the solvent under vacuum gave a white solid. The white solid was added to CH_2Cl_2 (5 mL), followed by methanol (1 mL) and 4, 4'-dimethylaminopyridine (8 mg, 0.0656 mmol). After cooled to 0°C, DCC (142 mg, 0.689 mmol) was added and the solution was stirred at the same temperature for 0.5 h. Then the reaction was continued at room temperature for 12 h. The precipitate produced was removed by suction filtration. The solvent was concentrated, and the residue was purified on silica gel with petroleum ether/EtOAc (3:1).

N-[1-[3-(*o*-chlorophenyl)isoxazol-5-yl]-3-methyl] butyl-(R)-2-methoxycarbonylmethylhexanamide (R, R/S, R mixture) (11a)

White solid, yield 80%, mp 83.5- 85.5°C, $[\delta]_{20}^{D}$ = + 17.0 (*c* 1, $CHCl_3$). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.76-0.94 (m, 9H), 1.18-1.50 (m, 6H), 1.68-1.77 (m, 3H), 2.37 (dd, *J* = 5, 16 Hz, 1H), 2.56 (dd, *J* = 9.3, 16Hz, 1H), 2.63-2.72 (m, 1H), 3.50/3.56 (s/s, 3H), 5.12-5.16 (m, 1H), 6.67/6.72 (s/s, 1H), 7.47 (dt, *J* = 1.2, 7.4 Hz, 1H), 7.53 (dt, *J* = 1.8, 8 Hz, 1H), 7.63 (dd, *J* = 1.1, 8 Hz, 1H), 7.67 (dd, *J* = 1.5, 8 Hz, 1H); IR (KBr, ν/cm-1) 3288, 3065, 2958, 2930, 2870,1739, 1647, 1545, 1173; ESI-MS *m/z* 435 $[M+H]^+$.

N-[1-[3-(*p*-methylphenyl)isoxazol-5-yl]-3-methyl] butyl-(R)-2-methoxycarbonylmethylhexanamide (R, R/S, R mixture) (11b)

White solid, yield 83%, mp 123-125°C, $[\delta]_{20}^{D}$ = + 25.0 (*c* 1, $CHCl_3$). 1H NMR (DMSO-d_6, 400 MHz) δ: 0.75-0.94 (m, 9H), 1.13-1.48 (m, 6H), 1.65-1.82 (m, 3H), 2.35 (s, 3H), 2.38-2.40 (m, 1H), 2.52-2.60 (m, 1H), 2.60-2.69 (m, 1H), 3.52/3.56 (s/s, 3H), 5.05-5.16 (m, 1H), 6.76/6.80 (s/s, 1H), 7.31 (dd, *J* = 2.8, 8.1 Hz, 2H), 7.71 (dd, *J* = 2.2, 8.1 Hz, 2H); IR (KBr, ν/cm^{-1}) 3304, 3108, 2955, 2932, 2870, 1736, 1652, 1540, 1172; ESI-MS *m/z* 415 $[M+H]^+$.

N-[1-(3-phenylisoxazol-5-yl)]pentyl-(R)-2-methoxycarbonylmethylhexanamide (R, R/S, R mixture) (11f)

White solid, yield 95%, mp 61-63°C, $[\delta]_{20}^{D}$ = + 24.0 (*c* 1, $CHCl_3$). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.76-0.89 (m, 6H), 1.23-1.50 (m, 10H), 1.75-1.89 (m, 2H), 2.38 (dd, *J* =5, 16 Hz, 1H), 2.54-2.62 (m, 1H), 2.64-2.72 (m, 1H), 3.53/3.57 (s/s, 3H), 5.01-5.07 (m, 1H), 6.80/6.84 (s/s, 1H), 7.47-7.54 (m, 3H), 7.82-7.84 (m, 2H); IR (KBr, ν/cm^{-1}) 3286, 3066, 2955, 2931, 2860, 1737, 1648, 1543, 1173; ESI-MS *m/z* 401 $[M+H]^+$.

General procedure for the synthesis of compounds (12a, 12b, and 12f)

To a methanol/tetrahydrofuran (2 mL, 1:1) solution of compound **11** (0.449 mmol) was added 50% aqueous hydroxylamine (0.27 mL, 4.49 mmol), followed by NaCN (20 mg). After 5 h, several drops of acetic acid were added to adjust the pH of the reaction mixture to 6. The solution was concentrated and the residue was purified on silica gel with methylene dichloride/methanol (20:1).

(R)-2-Butyl-N^4-hydroxy-N^1-[1-[3-(*o*-chlorophenyl) isoxazol-5-yl]-3-methyl]butylsuccinamide (R, S/R, R mixture) (12a)

White solid, yield 61%, mp 97-99°C, $[\delta]_{20}^{D}$ = + 11.0 (*c* 0.68, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.75-0.94 (m, 9H), 1.18-1.50 (m, 6H), 1.68-1.78 (m, 3H), 2.01-2.06 (m, 1H), 2.15-2.21 (m, 1H), 2.65-2.72 (m, 1H), 5.11-5.18 (m, 1H), 6.67/6.76 (s/s, 1H), 7.45-7.55 (m, 1H), 7.61-7.69 (m, 1H); IR (KBr, ν/cm^{-1}) 3268, 3062, 2957, 2930, 2870, 1645, 1616, 1540; ESI-MS *m/z* 436 $[M+H]^+$; Anal. Calcd for $C_{22}H_{30}ClN_3O_4$: C, 60.61; H, 6.94; N, 9.64; found C, 60.51; H, 6.85; N, 9.87.

(R)-2-Butyl-N^4-hydroxy-N^1-[1-[3-(*p*-methylphenyl) isoxazol-5-yl]-3-methyl]butylsuccinamide (R, S/R, R mixture) (12b)

White solid, yield 68%, mp 135-137°C, $[\delta]_{20}^{D}$ = + 20.3 (*c* 0.91, $CHCl_3$). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.74-0.93 (m, 9H), 1.13-1.48 (m, 6H), 1.65-1.82 (m, 3H), 2.02-2.10 (m, 1H), 2.18-2.26 (m, 1H), 2.35 (s, 3H), 2.62-2.70 (m, 1H), 5.05-5.16 (m, 1H), 6.75/6.84 (s/s, 1H), 7.30 (m, 2H), 7.72 (m, 2H); IR (KBr, ν/cm^{-1}) 3276, 3073, 2956, 2929, 2870, 1644, 1616, 1543; ESI-MS *m/z* 416 $[M+H]^+$; Anal. Calcd for $C_{23}H_{33}N_3O_4$: C, 66.48; H, 8.00; N, 10.11; found C, 66.28; H, 7.90; N, 10.32.

(R)-2-Butyl-N^4-hydroxy-N^1-[1-(3-phenylisoxazol-5-yl)]pentylsuccinamide (R, S/R, R mixture) (12f)

White solid, yield 62%, mp 153.5-155.5°C, $[\delta]_{20}^{D}$ = + 15.5 (*c* 0.5, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.75-0.89 (m, 6H), 1.15-1.45 (m, 10H), 1.75-1.88 (m, 2H), 2.01-2.07 (m, 1H), 2.18-2.23 (m, 1H), 2.65-2.74 (m, 1H), 5.01-5.06 (m, 1H), 6.80/6.88 (s/s, 1H), 7.48-7.54 (m, 3H), 7.81-7.88 (m, 2H); IR (KBr, ν/cm^{-1}) 3270, 3079, 2957, 2929, 2870, 1646, 1619, 1543; ESI-MS *m/z* 402 $[M+H]^+$; Anal. Calcd for $C_{22}H_{31}N_3O_4$: C, 65.81;

H, 7.78; N, 10.47; found C, 65.92; H, 7.85; N, 10.35.

In vitro antibacterial activity evaluation

Microdilution broth susceptibility assay was used for the antibacterial evaluation of the new compounds (Koneman et al., 1997; NCCLS documents, 2003). Muller-Hinton broth was used as the test medium. Each compound was tested over a range of doubling dilution concentrations. Tested microorganism strains were *Staphylococcus aureus* (CMCC26112) and *Klebsiellar pneumonia* (CMCC46117). The minimum inhibitory concentratio ns (MIC) was determined according to the lowest concentration that prevented visible growth of bacteria after incubation at 37°C for 24 h. Cefoperazone was used as a standard drug (positive control). Bacterial strains and broth were bought from National Institute for the Control of Pharmaceutical and Biological Products.

RESULTS AND DISCUSSION

Chemistry

Eight novel 2-butyl succinate-based hydroxamate derivatives containing isoxazole rings were synthesized. 3, 5-disubstituted isoxazoles **2a-f** were obtained utilizing a 3+2 dipolar cycloaddition reaction. To create the second chiral center in type 2 compounds, 3,5-disubstituted isoxazoles **3a**, **3b** and **3f** were oxidized with PCC to form 3-arylisoxazole-5-carbaldehyde, which reacted with Grignard reagents to afford the secondary alcohols **8a**, **8b** and **8f**. The hydroxy group of these 3-aryl-5-(hydroxymethyl)isoxazoles derivatives were transformed to amino group via a convenient three-step reaction procedure in modest yield. The condensation of the chiral succinate with the eight 5-aminomethyl-3-arylisoxazoles derivatives (**3a-e**, **9a**, **9b** and **9f)** was accelerated by 1-chloro-3, 5-dimethoxy-2, 4, 6-triazine and *N*-methyl morpholine. With the combination of the two reagents, high-yield target compounds were obtained and the work-up procedures were simple. The free acids, obtained after removal of the tert-butyl group in 98% formic acid, were transformed to methyl esters. The reaction of the methyl esters with 50% aqueous hydroxylamine solution catalyzed by cyanide led to the target hydroxamates in better yield and purity compared with these reported methods (Levy et al., 1998; Reddy et al., 2000). Structures of all the newly synthesized compounds were supported by IR, MS, NMR spectral data analysis and elemental analysis.

In the IR spectrum of these compounds, a broad absorption band around 3270 cm^{-1} indicates the presence of active hydrogen group in the compounds. The amide carbonyl stretching frequency was observed at about 1640 cm^{-1}. The other prominent absorption bands observed in the IR spectrum are 3060 (Ar-H) and 1550 (C=N) cm^{-1}.

^{1}H NMR spectrum of **6a** showed a triplet at δ 0.79 due to the CH_3 protons at the terminal of the butyl group. The six methylene protons of the butyl group resonated as complex multiplets at δ 1.15-1.46. The two methylene protons of the succinate moiety appeared as multiplets at δ 2.01-2.29 with a total integral of two. The chiral proton of succinate was observed as multiplet between δ 2.45-2.68. A multiplet at δ 4.36-4.52 integrating for two protons was attributable to the methylene protons adjacent to the isoxazole ring. The proton on the isoxazole resonated as singlet at δ 6.69. ^{1}H NMR spectrum of **12a** showed nine more protons at δ 0.75-1.78 than that of **6a**. The tertiary proton adjacent to the isoxazole ring appeared as multiplet at δ 5.14. The two singlets at δ 6.67 and 6.76 having a total integral of one were assigned as the proton on the isoxazole. The integration values of the two singlets were approximately 1:1, which meant that the compound was composed of a pair of diastereoisomers in equal quantities.

Biological activity

All target compounds (**6a-e**, **12a**, **12b** and **12f**) were evaluated for their antibacterial activities against *Staphylococcus aureus* and *Klebsiella pneumoniae* using microbroth dilution method (Koneman et al., 1997; NCCLS documents, 2003). MICs were recorded as the minimum concentration of a compound that inhibits the growth of tested microorganisms. The MIC values of all the new compounds are generally within the range of 8-64 μg/mL against the evaluated strains. The observed data on the antimicrobial activity of the compounds and control drug are given in Table I.

The antibacterial screening data revealed that all the tested compounds showed weak to moderate bacterial inhibition. Compounds **6b** and **6e** exhibited moderate activity against the two bacteria. Compound **6c** was effective against *K. pneumoniae* (MIC value of 8 μg/mL), whereas it was less active against *S. aureus* (MIC > 64 μg/mL). Compounds **12a**, **12b** and **12f** showed weak activity against both *S. aureus* and *K. pneumoniae*. To the two tested bacteria, all the compounds were found to exhibit better activity against *K. pneumoniae*.

The SAR investigation of the synthesized compounds revealed that variation of the substituents on the benzene attached at position 3 of isoxazole affected the activity significantly. As can be seen that com-

Table I. Antimicrobial activities of compounds **6a-e, 12a, 12b** and **12f** (MIC[a], μg/mL)

Compound	*Staphylococcus aureus*	*Klebsiella pneumoniae*
6a	32	16
6b	16	8
6c	>64	8
6d	64	32
6e	16	8
12a	>64	32
12b	>64	64
12f	>64	64
Standard[b]	0.25	0.25

[a]The MIC (μg/mL) values of reported compounds against Staphylococcus aureus were: 8-16 (Actinonin) and 1-4 (VRC3375) (Chen et al., 2000, 2004).
[b]Standard: Cefoperazone

pounds **6b**, **6c** and **6e**, which had substituents situated in the para-positon to the isoxazole ring on the benzene, were more active than those having substituents at the ortho or meta position. Opposite to our expectation, creation of the second chiral center in the molecules did not cause an increase in the activity. As a result, compounds **12a**, **12b** and **12f** exhibited decreased activity when compared with the compounds having only one chiral center (**12a** *versus* **6a**, **12b** *versus* **6b**). Different alkyl chains, e. g. isobutyl or n-butyl, have been introduced to the methylene adjacent to the isoxazole ring. However, none of the modification produced improved activity. The isoxazole was incorporated to model the amide moiety in PDF inhibitors as hydrogen bond donor, which has been demonstrated to be important to inhibitors' activities (Clements et al., 2001; Chen et al., 2004; East et al., 2004). The decrease of the inhibitory activity should be attributable to the introduction of the alkyl chain, which influenced the steric orientation of the heteroatom of the isoxazole ring.

In conclusion, two series of novel hydroxamates bearing aryl substituted isoxazole ring have been designed based on the peptidomemic idea and then synthesized. The synthesized compounds were found to exhibit weak to moderate inhibitory activity against *Staphytlococcus aureu* and *Klebsiellar pneumonia in vitro*. Compound **6b** and **6e** exhibited moderate bacterial inhibition. All of the compounds were more effective against *Klebsiellar pneumonia* compared to *Staphytlococcus aureu*. Varying the substituents on the benzene at position 3 of the isoxazole with different groups significantly influenced the antibacterial activity. Construction of the second chiral center in the molecules did not improve the antibacterial activity. It seems the steric changes at this region have a slight influence on the activity. These preliminary results are beneficial for further lead optimization.

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