Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(10):130-140

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Design, Synthesis and Antimicrobial Evaluation of Novel Benzoxazole Derivatives

Wei Zhang[1,2], Jingbao Liu[1], Jocelyn M Macho[2], Xizhen Jiang[1], Dongsheng Xie[1], Faqin Jiang[1], Wenlu Liu[1] and Lei Fu[1*]

[1]School of Pharmacy, Shanghai Jiao Tong University, Shanghai, China
[2]Department of Chemistry, University of Florida, Gainesville, USA

ABSTRACT

The synthesis of (S)-2-(4-tert-butylphenoxy)-3-(benzoxazol-5-yl) propanoic acid derivatives (2a-k) were described and their in vitro antibacterial activities were determined against Gram-negative and -positive bacteria. These compounds were found to exert a broad spectrum of activity against the screened bacteria, but poor MIC values were found for Candida albicans fungi. Compound 2b bearing a hydrophobic aromatic tie was the most active derivative against all bacteria studied with MIC values ranging from 0.098 to 0.78 μg/mL. The activity of 2b against B. subtilis was 2-fold higher than Penicillin, and 8- to 510-fold higher than other control antibiotics.

Keywords: Antibacterial activity; Minimum inhibitory concentration; Benzoxazole; Propanoic acid

---

INTRODUCTION

Benzoxazole derivatives were widely reported as a class of biologically active compounds with anti-bacterial and anti-tumor activities [1-5]. In the early 1980s, Hisano et al. reported the first synthetic compounds with benzoxazole skeletons that displayed potential antimicrobial activities [6]. Over the past twenty years, a large library of benzoxazole derivatives has been developed, some of which have exerted good minimum inhibitory concentration (MIC) values [7-15]. In addition, some benzoxazole-containing natural products were also proven to have antimicrobial abilities, for example calcimycin, routeennocin and cezomycin separated from *Streptomyces chartreusis* [16-18], and pseudopteroxazole from *Pseudopterogorgia elisabethae* [19].

Previously, we reported the synthesis and antibacterial study of a series of (*S*)-2-(substituted-hydroxyl)-3-(benzoxazol-5-yl)propanoic acid derivatives (Fig. 1) [20]. This family of compounds was designed by an integration of four fragments: an aromatic tie, a linker, a benzoxazole skeleton and a polar acidic head. In our previous report, we investigated the effect of the chiral side chain on anti-bacterial activity; our preliminary structure-activity study revealed that the hydrophobic substitutes, 4-tert-butyl (1a), 4-phenyl (1b) and 4-benzyloxyl (1c) on the phenoxyl side chain displayed best activities against all Gram-negative and Gram-positive bacteria studied with MIC values between 1.56 and 6.25 μg/mL.

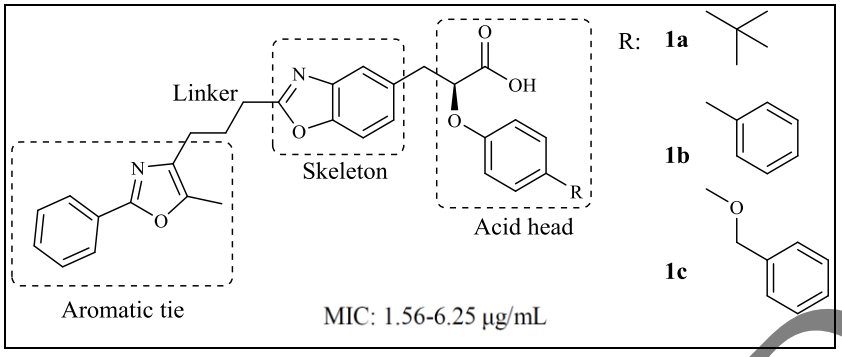


Figure 1: (*S*)-2-(substituted-hydroxyl)-3-(benzoxazol-5-ly)propanoic acid derivatives 1a-c

As we continue efforts in search for more active benzoxazole-containing compounds while gaining a better understanding of the mechanism of action, herein, we focused on further structural modification of the aromatic tie of 1a, and examined their anti-bacterial activities (Fig. 2). Our previous study suggested that the hydrophobicity of agents may alter the functional biology activities significantly [20-23]. Specifically, in this study the modification of the aromatic tie (R group), with either hydrophobic or hydrophilic groups, resulted in a new family of (*S*)-2-(*tert*-butylphenoxy)-3-(benzoxazol-5-yl)propanoic acid derivatives.

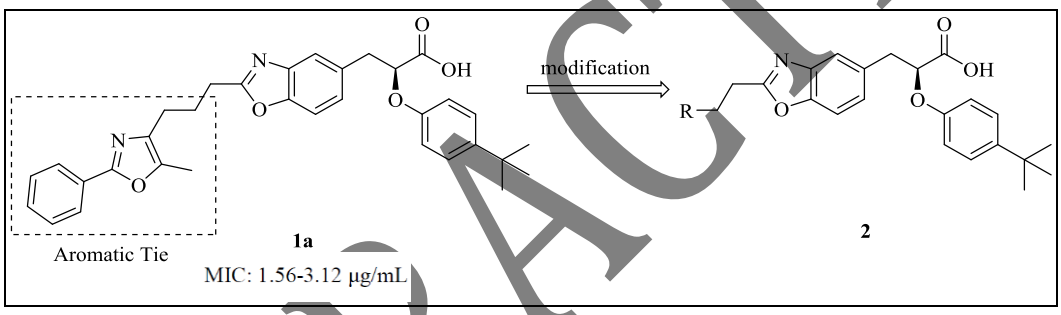


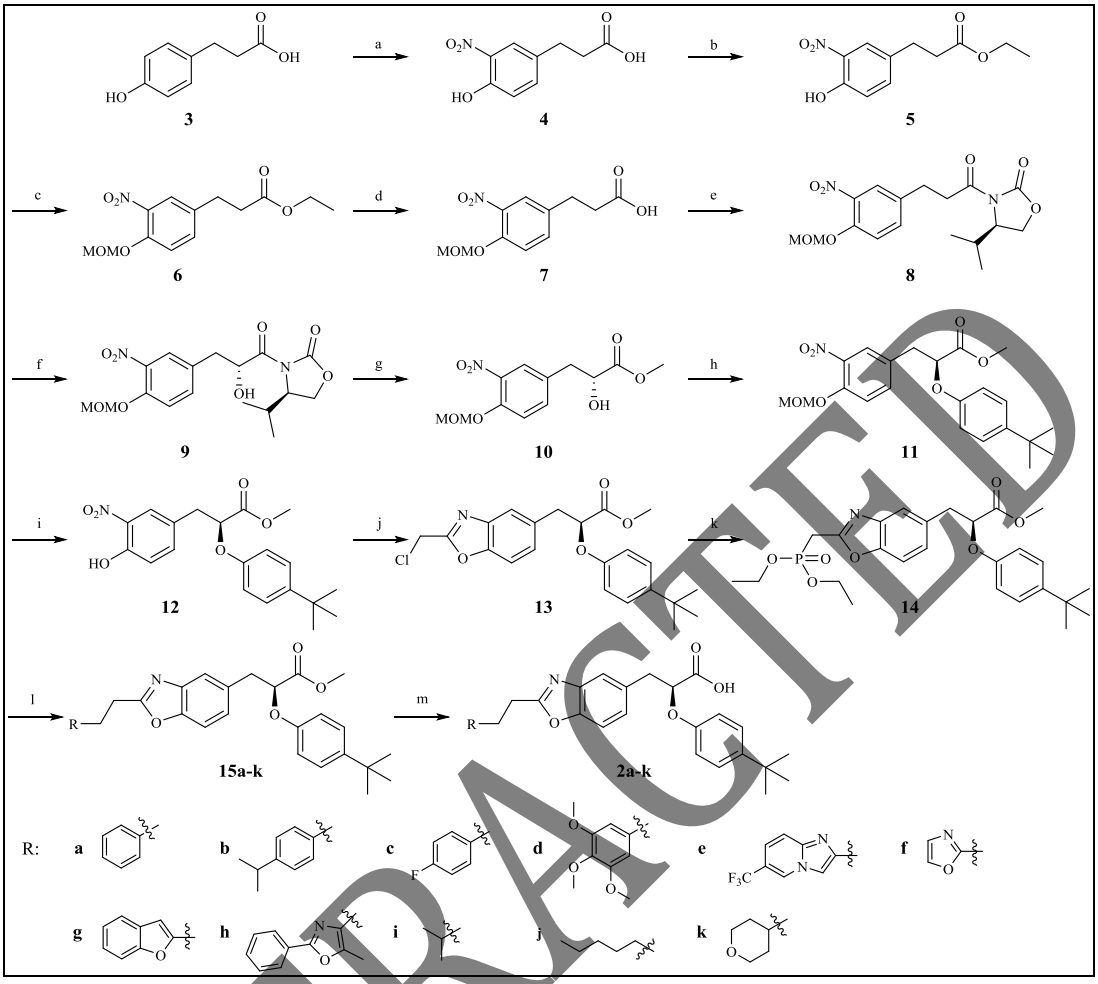
Figure 2: Structural modification of compound 1a

EXPERIMENTAL SECTION

Chemistry

All commercial chemicals and solvents were grade and were used without further purification unless otherwise specified. All reactions expect those in aqueous media were carried out with the use of standard techniques for the exclusion of moisture. Reactions were monitored by TLC under 254 nm UV. ^{1}H NMR was recorded on either a Brucker 300 MHz Avance DPX or a Brucker 400 MHz Avance DPX instrument. Chemical shifts are reported in parts per million (δ) and coupling constants (*J*) in hertz (Hz). Mass spectroscopy analyses were obtained by using Shimadzu Biotech LCMS-2010EV mass spectrometer system. High resolution mass spectroscopy was conducted using Micromass LCT system.

Similar to our previously reported procedures [20], the synthesis of compounds 2a-k was achieved in thirteen steps as shown in Scheme 1. Intermediate 5 was easily obtained from 3-(4-hydroxyphenyl) propanoic acid 3 by nitration and esterification. The hydroxyl-protected 6 was produced by using CH_3OCH_2Cl. After hydrolysis of 6, the resulting acid 7 was coupled with (*R*)-4-isopropyloxazolidin-2-one to give Evans amide 8. Davis asymmetric oxidation conditions converted 8 to a chiral hydroxyl compound 9. The chiral accessory (*R*)-4-isopropyloxazolidin-2-one was removed by using magnesium methoxide to produce methyl (*R*)-α-hydroxyl-propanoic acid 10. (*S*)-11 was obtained by treating 10 with 4-tert-butylphenol under Mitsunobu conditions [24]. Treatment of 11 with HCl/MeOH yielded 12, quantitatively. The benzoxazole skeleton 13 was obtained from 12 by sequential treatment with Pd/C and acetimidate [25]. 14 was produced from 13 by using $P(EtO)_3$. Compound 15 was obtained from 14 by treating with respective acetones and aldehydes via Horner-Wadsworth-Emmons olefination and platinum dioxide hydrogenation conditions successively. The final propanoic acids 2a-k were produced by treatment of 15a-k with LiOH.



Scheme 1: Synthesis of compounds 2[a]

[a] Reagents and conditions: (a) 60 % HNO_3, HOAc, 15 ^oC; (b) EtOH, H_2SO_4, reflux, 83 % two steps; (c) CH_3OCH_2Cl, DIPEA, CH_3CN, 0 ^oC, 99 %; (d) NaOH, EtOH, H_2O, rt, 91 %; (e) 1) pivaloylchloride, Et_3N, THF, -78 ^oC, 30 min, 2) lithium (*R*)-4-isopropyloxazolidin-2-one, THF, -78 ^oC, 30 min, 97 %; (f) 1) 1.0 M KHMDS/THF, THF, -78 ^oC, 2) 3-phenyl-2-(phenylsulfonyl)-1,2-oxaziridine, THF, -78 ^oC, 3) AcOH, THF, -78 ^oC, 65 %; (g) 2.5 M EtMgBr/Ether, MeOH, 85 %; (h) 4-*t*-Bu-Phenol, DIAD, Ph_3P, toluene, 0 ^oC then rt, 69 %; (i) trace cont. HCl, MeOH, 60 ^oC, 30 min, 99 %; (j) 10 % Pd/C, H_2, MeOH, 1 atm; 2) $ClCH_2C(=NH)OCH_3 \cdot HCl$, MeOH, reflux, 51 %; (k) $P(OEt)_3$, DMF, 150 ^oC, 88 %; (l) 1) acetone or aldehyde, NaH, THF, -10 ^oC; 2) $PtO_2 \cdot 3H_2O$, MeOH, 3 atm, H_2, 38-97 %; (m) $LiOH \cdot H_2O$, EtOH, H_2O, rt, 70-97 %.

Ethyl 3-(4-hydroxy-3-nitrophenyl)propanoate (5):

A mixture of 60 % HNO_3 (9.8 mL, 0.2 mmol) and acetic acid (20 mL) was added drop-wise into a solution of 3-(4-hydroxyphenyl)propanoic acid 3 (20.0 g, 0.1 mmol) in acetic acid (150 mL) at 15 ^oC over 1 hour. The resulting mixture was stirred for 30 min, and then it was poured into 300 mL cool water. The water layer was extracted with 200 mL CH_2Cl_2. The organic layer was washed with brine and dried with anhydrous Na_2SO_4. The solvent was removed *in vacuo* to give a yellow solid 4 (24.6 g). The crude yellow solid 4 was dissolved in a mixture of EtOH (300 mL)/H_2SO_4 (30 mL), and the mixture was heated at reflux for 12 hours. The reaction was cooled to room temperature, the solvent was then removed, and the residue was poured into ice-water and then extracted with ether (300 mL). The ether layer was washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed to give a solid residue, which was filtered through a short silica column eluting with petroleum ether/ethyl acetate (3:1) to give compound 5 (22.5 g, 83 %) as a bright yellow solid; mp: 41-43 ^oC; ^1H NMR (300 MHz, $CDCl_3$) δ 10.41 (s, 1H, OH), 7.90 (d, 1H, ArH, *J* = 2.4 Hz), 7.42 (dd, 1H, ArH, *J* = 8.4 Hz, 1.2 Hz), 7.04 (d, 1H, ArH, *J* = 8.7 Hz), 4.04

(q, 2H, CH_2, *J* = 7.2Hz), 2.90 (t, 2H, CH_2, *J* = 7.5 Hz), 2.61 (t, 2H, CH_2, *J* = 7.7 Hz), 1.21 (t, 3H, CH_3, *J* = 7.2 H_Z); MS (ESI *m/z*): 240 $(M+H)^+$.

Ethyl 3-(4-(methoxymethoxy)-3-nitrophenyl)propanoate (6):
To a mixture of compound 5 (2.0 g, 8.3 mmol) and *N*, *N*-di*iso*propylethylamine (2.3 mL, 12.5 mmol) in 40 mL acetonitrile, was added dropwise CH_3OCH_2Cl (0.75 mL, 10.0 mmol) at 0 ^oC. The resulting mixture was stirred for 2 hours at 0 ^oC, and then it was quenched with 10 mL water. Acetonitrile was removed *in vacuo*, and the residue was diluted with 20 mL ethyl acetate, washed with brine, and dried with anhydrous Na_2SO_4. The solvent was removed to give compound 6 (2.3 g, 99 %) as a yellow oil; ^1H NMR (300 MHz, $CDCl_3$) δ 7.67 (d, 1H, ArH, *J* = 2.1 Hz), 7.37 (dd, 1H, ArH, *J* = 8.1 Hz, 2.1Hz), 7.23 (d, 1H, ArH, *J* = 8.4 Hz), 5.27 (s, 2H, CH_2), 4.13 (q, 2H, CH_2, *J* = 7.0 Hz), 3.52 (s, 3H, CH_3), 2.96 (t, 2H, CH_2, *J* = 7.5 Hz), 2.62 (t, 2H, CH_2, *J* = 7.5 Hz), 1.25 (t, 3H, CH_3, *J* = 7.2 Hz); HRMS-ESI^+: $C_{13}H_{18}NO_6$ calcd $[M+H]^+$: 284.1134, found 284.1130.

3-(4-(Methoxymethoxy)-3-nitrophenyl)propanoic acid (7):
Aqueous solution of NaOH (7 mL, 10.6 mmol) was added to a solution of compound 6 (1.0 g, 3.5 mmol) in 15 mL EtOH. The resulting mixture was stirred for 2 hours at room temperature. EtOH was evaporated and the water residue was acidified with 1M HCl to pH = 4, and then it was extracted with 20 mL ethyl acetate. The organic phase was washed with brine and dried with anhydrous Na_2SO_4. The solvent was concentrated to give compound 7 (0.8 g, 91 %) as a white solid; mp: 55-57 ^oC; ^1H NMR (300 MHz, $CDCl_3$) δ 7.67 (d, 1H, ArH, *J* = 1.8 Hz), 7.34 (dd, 1H, ArH, *J* = 8.7 Hz, 2.1 Hz), 7.23 (d, 1H, ArH, *J* = 9.0 Hz), 5.26 (s, 2H, CH_2), 3.51 (s, 3H, CH_3O), 2.93 (t, 2H, CH_2, *J* = 7.2 Hz), 2.68 (t, 2H, CH_2, *J* = 7.8 Hz); HRMS-ESI^-: $C_{11}H_{11}NO_6$ calcd $[M-H]^-$: 253.0586, found 253.0577.

(R)-4-Isopropyl-3-(3-(4-(methoxymethoxy)-3-nitrophenyl) propanoyl)oxazolidin-2-one (8):
Pivaloyl chloride (1.5 mL, 12.3 mmol) was added over 30 min to a mixture of compound 7 (3.0 g, 11.7 mmol) and triethylamine (2.0 mL, 14.0 mmol) in dry tetrahydrofuran (60 mL) at -78 ^oC. Sequentially, the resulting solution was stirred at 0 ^oC for 30 min and re-cooled to -78 ^oC. A -78 ^oC solution of lithium (*R*)-4-*iso*propyl-2-oxooxazolidin-3-ide (prepared from (*R*)-4-isopropyloxazo lidin-2-one (1.7 g, 12.9 mmol) and ^{n}BuLi (7.8 mL 1.6 M/hexane, 12.9 mmol) in THF at -78 ^oC) was transferred through a cannula to the mixture above, and the resulting mixture was warmed to room temperature. After another 30 min stirring, the reaction was quenched with saturated NH_4Cl aqueous solution (100 mL), and then extracted with ethyl acetate (100 mL). The organic layer was washed with brine and dried over anhydrous Na_2SO_4; the concentrated residue was purified over silica gel column eluting with petroleum ether/ethyl acetate (3:1) to give compound 8 (4.2 g, 97 %) as a yellow oil; ^1H NMR (300 MHz, $CDCl_3$) δ 7.69 (d, 1H, ArH, *J* = 2.4 Hz), 7.41 (dd, 1H, ArH, *J* = 8.4 Hz, 2.0 Hz), 7.23 (d, 1H, ArH, *J* = 8.7 Hz), 5.27 (s, 2H, CH_2), 4.44-4.40 (m, 1H, CH), 4.31-4.20 (m, 2H, CH_2), 3.52 (s, 3H, CH_3O), 3.52-3.23 (m, 2H, CH_2), 2.99 (t, 2H, CH_2, *J* = 7.0 Hz), 2.35-2.34 (m, 1H, $CH(CH_3)_2$), 0.91 (d, 3H, CH_3, *J* = 7.2 Hz), 0.84 (d, 3H, CH_3, *J* = 6.6 Hz); HRMS-ESI^+: $C_{17}H_{23}N_2O_7$ calcd $[M+H]^+$: 367.1505, found 367.1512.

(R)-3-((R)-2-Hydroxy-3-(4-(methoxymethoxy)-3-nitrophenyl) propanoyl)-4-isopropyloxazolidin-2-one (9):
A solution of KHMDS (5.7 mL 0.5 M/Toluene, 2.8 mmol) was added dropwise into a solution of compound 8 (0.7 g, 1.8 mmol) in dry THF (10 mL) at -78 ^oC and stirred for 1.0 hour. A -78 ^oC solution of 3-phenyl-2-(phenylsulfonyl)-1,2-oxaziridine (0.8 g, 2.8 mmol) in 10 mL THF was transferred into the solution above through a cannula, and stirred for 1 hour. The reaction was quenched with a solution of acetic acid (1 mL) in 2 mL dry THF at -78 ^oC, and then the solvent was removed *in vacuo*. The residue was diluted with 50 mL ethyl acetate, washed with brine, and dried over anhydrous Na_2SO_4. The solvent was removed and the crude product was purified over silica column eluting with petroleum ether/ethyl acetate (2:1) to give compound 9 (0.5 g, 65 %) as a white solid; mp: 120-123 ^oC; ^1H NMR (300 MHz, $CDCl_3$) δ 7.72 (d, 1H, ArH, *J* = 1.8 Hz), 7.47 (dd, 1H, ArH, *J* = 8.7 Hz, 2.1 Hz), 7.26 (d, 1H, ArH, *J* = 8.7 Hz), 5.27 (s, 2H, OCH_2O), 5.17-5.13 (m, 1H, CONCH), 4.42-4.32 (m, 3H, CH, CH_2), 3.68 (d, 1H, OH, *J* = 7.5 Hz), 3.52 (s, 3H, CH_3O), 3.15 (dd, 1H, CH, *J* = 14.1 Hz, 4.2 Hz), 2.83 (dd, 1H, CH, *J* = 14.1 Hz, 8.4 Hz), 2.44-2.43 (m, 1H, CH), 0.93 (d, 3H, CH_3, *J* = 6.9 Hz), 0.91 (d, 3H, CH_3, *J* = 6.6 Hz); HRMS-ESI^+: $C_{17}H_{23}N_2O_8$ calcd $[M+H]^+$: 383.1454, found 383.1460.

(R)-methyl 2-hydroxy-3-(4-(methoxymethoxy)-3-nitrophenyl) propanoate (10):
A 2.5M EtMgBr/ether solution (5.2 mL, 13.0 mmol) was added slowly into 40 mL dry MeOH at -10 ^oC. After addition of EtMgBr, a solution of compound 9 (2.5 g, 6.5 mmol) in dry MeOH (20 mL) was added in one portion. The mixture was stirred for 10 min then quenched with a saturated NH_4Cl aqueous solution (30 mL). MeOH was removed *in vacuo* and the residue water layer was extracted with 50 mL ethyl acetate. The organic layer was dried

over anhydrous Na_2SO_4, and the crude product was purified over silica column eluting with petroleum ether/ethyl acetate (2.5:1) to give compound 10 (1.8 g, 85 %) as a sticky colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68 (s, 1H, ArH), 7.39 (dd, 1H, ArH, *J* = 8.1 Hz, 1.7 Hz), 7.24 (d, 1H, ArH, *J* = 8.5 Hz), 5.27 (s, 2H, CH_2), 4.45-4.42 (m, 1H, CH), 3.81 (s, 3H, CH_3), 3.52 (s, 3H, CH_3), 3.03 (dd, dd, 2H, CH_2, *J* = 14.2 Hz, 7.2 Hz, 4.0 Hz), 2.85 (d, 1H, OH, *J* = 5.4 Hz); HRMS-ESI$^+$: $C_{12}H_{15}NNaO_7$ calcd $[M+Na]^+$: 308.0746, found 308.0743.

(S)-methyl 2-(4-tert-butylphenoxy)-3-(4-(methoxymethoxy)-3-nitrophenyl)propanoate (11):

To a solution of compound 10 (84.0 mg, 0.3 mmol) in 2 mL toluene, Ph_3P (85.0 mg, 0.3 mmol) and 4-tert-butylphenol (48.0 mg, 0.3 mmol) were added. The resulting mixture was cooled to 0 ^{o}C and a solution of diisopropyl azodicarboxylate (DIAD) (64.0 mg, 0.3 mmol) in 1 mL toluene was added dropwise. Sequentially, the mixture was stirred for 24 hours. Toluene was then removed *in vacuo*, and the residue was purified over silica gel column eluting with petroleum ether/ethyl acetate (10: 1) to give compound 11 (72.0 mg, 69%) as a colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (d, 1H, ArH, *J* = 2.4 Hz), 7.47 (dd, 1H, ArH, *J* = 8.4 Hz, 2.1 Hz), 7.30-7.24 (m, 3H, ArH), 6.84 (d, 2H, ArH, *J* = 8.7 Hz), 5.28 (s, 2H, CH_2), 4.80 (dd, 1H, CH, *J* = 6.9 Hz), 3.77 (s, 3H, CH_3), 3.53 (s, 3H, CH_3), 3.25 (d, 2H, CH_2, *J* = 6.6 Hz), 1.31 (s, 9H, $3CH_3$); HRMS-ESI$^+$: $C_{22}H_{27}NNaO_7$ calcd $[M+Na]^+$: 440.1685, found 440.1680.

(S)-methyl 2-(4-tert-butylphenoxy)-3-(4-hydroxy-3-nitro phenyl)propanoate (12):

To a solution of compound 11 (0.7 g, 1.7 mmol) in 10 mL methanol, concentrated HCl (0.3 mL) was added, and the resulting mixture was heated at 60 ^{o}C for 30 min, then concentrated. The residue was diluted with 10 mL CH_2Cl_2 and the organic layer was washed with water, brine and dried over anhydrous Na_2SO_4. The solvent was removed to give a compound 12 (0.6 g, 99 %) as pale yellow oil without further purification; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (d, 1H, ArH, *J* = 1.8 Hz), 7.48 (dd, 1H, ArH, *J* = 8.7 Hz, 2.1 Hz), 7.28-7.23 (m, 3H, ArH), 6.77 (d, 2H, ArH, *J* = 9.0 Hz), 4.76-4.72 (m, 1H, CH), 3.76 (s, 3H, CH_3), 3.23 (d, 2H, CH_2, *J* = 7.2 Hz), 1.27 (s, 9H, $3CH_3$); HRMS-ESI$^+$: $C_{20}H_{24}NO_6$ calcd $[M+H]^+$: 374.1604, found 374.1598.

(S)-methyl 2-(4-tert-butylphenoxy)-3-(2-(chloromethyl) benzo[d]oxazol-5-yl)propanoate (13):

A mixture of compound 12 (1.1 g, 3.0 mmol) and Pd/C (10 %, 0.2 g) was stirred in 10 mL MeOH under hydrogen (1 atm) for 1 hour. The solution was filtered to give a white solid and it was mixed with methyl 2-chloroacetimidate hydrochloride (0.5 g, 3.7 mmol) in 25 mL MeOH. The resulting mixture was heated at reflux for 2 hours, then it was cooled to room temperature and solvent was removed *in vacuo*. The residue was diluted with 50 mL ethyl acetate and washed with 20 mL saturated $NaHCO_3$ aqueous solution, then brine and dried over anhydrous Na_2SO_4. The solvent was removed to give a crude product, which was over silica gel column eluting with petroleum ether/ethyl acetate (3:1) to give compound 13 (6.4 g, 51 %) as a colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68 (s, 1H, ArH), 7.47 (d, 1H, ArH, *J* = 8.4 Hz), 7.33 (dd, 1H, ArH, *J* = 8.1 Hz, 1.5 Hz), 7.23 (d, 2H, ArH, *J* = 9.3 Hz), 6.74 (d, 2H, ArH, *J* = 8.7 Hz), 4.81-4.77 (m, 1H, CH), 4.73 (s, 2H, CH_2), 3.74 (s, 3H, CH_3), 3.35 (d, 2H, CH_2, *J* = 5.4 Hz), 1.25 (s, 9H, $3CH_3$); HRMS-ESI$^+$: $C_{22}H_{25}ClNO_4$ calcd $[M+H]^+$: 402.1472, found 402.1471.

(S)-methyl2-(4-tert-butylphenoxy)-3-(2-((diethoxyphosphoryl)methyl)benzo[d]oxazol-5-yl) propanoate (14):

Compound 13 (0.3 g, 0.9 mmol) and triethyl phosphite (0.3 g, 1.7 mmol) were dissolved in 1 mL DMF and stirred at 150 ^{o}C for 4 hours. The solvent was removed *in vacuo*, and the residue was purified over silica gel column eluting with petroleum ether/ethyl acetate (1:2) to give compound 14 (0.4 g, 88 %) as a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62 (s, 1H, ArH), 7.42 (d, 1H, ArH, *J* = 8.4 Hz), 7.26 (d, 1H, ArH, *J* = 7.8 Hz), 7.23 (d, 2H, ArH, *J* = 9.3 Hz), 6.74 (d, 2H, ArH, *J* = 8.7 Hz), 4.80-4.76 (m, 2H, CH_2), 4.22-4.08 (m, 4H, CH_2, CH_2), 3.73 (s, 3H, CH_3), 3.57 (s, 1H, CH), 3.50 (s, 1H, CH), 3.32 (d, 1H, CH, *J* = 5.4 Hz), 1.32 (t, 6H, $2CH_3$, *J* = 6.9 Hz), 1.24 (s, 9H, $3CH_3$); HRMS-ESI$^+$: $C_{26}H_{35}NO_7P$ calcd $[M+H]^+$: 504.2151, found 504.2148.

General synthesis of compounds 15a-k:

A solution of compound 14 (80.0 mg, 0.2 mmol) and acetone or aldehyde (0.2 mmol) in 2 mL dry THF was added NaH (60 %, 7.0 mg, 0.2 mmol) in one portion at -10 ^{o}C, and was stirred for 5 min at this temperature. The mixture was quenched with 10 mL saturated NH_4Cl aqueous solution. The water layer was sequentially extracted with ethyl acetate, washed with brine and dried with anhydrous Na_2SO_4. An orange residue was afforded, and it was stirred with $PtO_2 \cdot 3H_2O$ (5.5 mg, 0.0079 mmol) in 5 mL methanol for 12 hours under hydrogen at 1.0 atm. Sequentially, the mixture was filtered and purified over silica gel column eluting with petroleum ether/ethyl acetate (10:1) to give compound 15a-k.

(S)-Methyl 2-(4-tert-butylphenoxy)-3-(2-phenethylbenzo[d] oxazol-5-yl)propanoate (15a): Compound 15a (51.0 mg, 75 %) as a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61 (s, 1H, ArH), 7.40 (d, 1H, ArH, *J* = 8.7 Hz), 7.31 (d, 1H, ArH, *J* = 8.4 Hz), 7.27-7.22 (m, 7H, ArH), 6.77 (d, 2H, ArH, *J* = 9.0 Hz), 4.81-4.77 (m, 1H, CH), 3.73 (s, 3H, CH_3), 3.33 (d, 2H, CH_2, *J* = 5.1 Hz), 3.21 (s, 4H, $2CH_2$), 1.25 (s, 9H, $3CH_3$); HRMS-ESI$^+$: $C_{29}H_{32}NO_4$ calcd $[M+H]^+$: 458.2331, found 458.2335.

(S)-Methyl 2-(4-tert-butylphenoxy)-3-(2-(4-isopropylphen ethyl)benzo[d]oxazol-5-yl)propanoate (15b): Compound 15b (59.0 mg, 75 %) as a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61 (s, 1H, ArH), 7.41 (d, 1H, ArH, *J* = 8.7 Hz), 7.26-7.22 (m, 3H, ArH), 7.17 (s, 4H, ArH), 6.77 (d, 2H, ArH, *J* = 9.0 Hz), 4.81-4.79 (m, 1H, CH), 3.73 (s, 3H, CH_3), 3.34-3.32 (m, 2H, CH_2), 3.25-3.19 (m, 4H, $2CH_2$), 2.92 (qui, 1H, CH, *J* = 6.8 Hz), 1.25 (s, 9H, $3CH_3$), 1.23 (d, 6H, $2CH_3$, *J* = 6.9 Hz); HRMS-ESI$^+$: $C_{32}H_{38}NO_4$ calcd $[M+H]^+$: 500.2801, found 500.2800.

(S)-Methyl 2-(4-tert-butylphenoxy)-3-(2-(4-fluorophenethyl) benzo[d]oxazol-5-yl)propanoate (15c): Compound 15c (54.0 mg, 74 %) as a colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72 (s, 1H, ArH), 7.52 (d, 1H, ArH, *J* = 8.1 Hz), 7.38-7.28 (m, 5H, ArH), 7.11 (t, 2H, ArH, *J* = 8.4 Hz), 6.88 (d, 2H, ArH, *J* = 8.4 Hz), 4.93-4.88 (m, 1H, CH), 3.85 (s, 3H, CH_3O), 3.46-3.43 (m, 2H, CH_2), 3.30 (s, 4H, CH_2, CH_2), 1.37 (s, 9H, $3CH_3$); HRMS-ESI$^+$: $C_{29}H_{31}FNO_4$ calcd $[M+H]^+$: 476.2237, found 476.2242.

(S)-Methyl 2-(4-tert-butylphenoxy)-3-(2-(3,4,5-trimethoxy phenethyl)benzo[d]oxazol-5-yl)propanoate (15d): Compound 15d (42.0 mg, 47 %) as a colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72 (s, 1H, ArH), 7.51 (d, 1H, ArH), 7.37-7.33 (m, 4H, ArH), 6.86 (d, 2H, ArH, *J* = 8.7 Hz), 6.54 (s, 2H, ArH), 4.92-4.88 (m, 1H, CH_2), 3.92 (s, 3H, CH_3O), 3.88 (s, 6H, CH_3O), 3.84 (s, 3H, CH_3O), 3.45 (d, 2H, CH_2, *J* = 5.4 Hz), 3.35-3.30 (m, 2H, CH_2), 3.27-3.22 (m, 2H, CH_2), 1.36 (s, 9H, $3CH_3$); HRMS-ESI$^+$: $C_{32}H_{38}NO_7$ calcd $[M+H]^+$: 548.2648, found 548.2641.

(S)-Methyl 2-(4-tert-butylphenoxy)-3-(2-(2-(6-(trifluoromethyl)imidazo[1,2-a]pyridin-2-yl)ethyl)benzo[d] oxazol-5-yl)propanoate (15e): Compound 15e (77.0 mg, 77 %) as a brown oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.51 (s, 1H, ArH), 7.78 (d, 1H, ArH, *J* = 9.0 Hz), 7.71 (s, 1H, ArH), 7.51 (d, 1H, ArH, *J* = 8.1 Hz), 7.41-7.33 (m, 5H, ArH), 6.87 (d, 2H, ArH, *J* = 9.0 Hz), 4.92-4.87 (m, 1H, CH), 3.84 (s, 3H, CH_3O), 3.53 (s, 4H, CH_2, CH_2), 3.44-3.42 (m, 2H, CH_2), 1.36 (s, 9H, $3CH_3$); HRMS-ESI$^+$: $C_{31}H_{31}F_3N_3O_4$ calcd $[M+H]^+$: 566.2267, found 566.2271.

(S)-Methyl 2-(4-tert-butylphenoxy)-3-(2-(2-(oxazol-2-yl) ethyl)benzo[d]oxazol-5-yl)propanoate (15f): Compound 15f (46.0 mg, 65 %) as a colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61 (d, 2H, ArH, *J* = 7.8 Hz), 7.40 (d, 1H, ArH, *J* = 8.4 Hz), 7.26 (s, 1H, ArH), 7.25 (d, 2H, ArH, *J* = 8.7 Hz), 7.03 (s, 1H, ArH), 6.76 (d, 2H, ArH, *J* = 8.7 Hz), 4.81-4.77 (m, 1H, CH), 3.73 (s, 3H, CH_3), 3.43-3.37 (m, 4H, $2CH_2$), 3.33 (d, 2H, CH_2, *J* = 5.7 Hz), 1.25 (s, 9H, $3CH_3$); HRMS-ESI$^+$: $C_{26}H_{29}N_2O_5$ calcd $[M+H]^+$: 449.2076, found 449.2080.

(S)-Methyl 3-(2-(2-(benzofuran-2-yl)ethyl)benzo[d]oxazol-5-yl)-2-(4-tert-butylphenoxy)propanoate (15g): Compound 15g (52.0 mg, 66 %) as a colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64 (s, 1H, ArH), 7.43-7.37 (m, 2H, ArH), 7.34-7.26 (m, 2H, ArH), 7.19-7.09 (m, 4H, ArH), 6.70 (d, 2H, ArH, *J* = 9.0 Hz), 6.45 (s, 1H, ArH), 4.76-4.72 (m, 1H, CH), 3.68 (s, 3H, CH_3), 3.42-3.36 (m, 4H, $2CH_2$), 3.29 (d, 2H, CH_2, *J* = 6.3 Hz), 1.19 (s, 9H, $3CH_3$); HRMS-ESI$^+$: $C_{31}H_{32}NO_5$ calcd $[M+H]^+$: 498.2280, found 498.2283.

(S)-Methyl 2-(4-tert-butylphenoxy)-3-(2-(2-(5-methyl-2-phenyloxazol-4-yl)ethyl)benzo[d]oxazol-5-yl) propanoate (15h): Compound 15h (32.0 mg, 38 %) as a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08-8.05 (m, 2H, ArH), 7.71 (s, 1H, ArH), 7.54-7.49 (m, 4H, ArH), 7.37-7.33 (m, 3H, ArH), 6.88 (d, 2H, ArH, *J* = 8.7 Hz), 4.92-4.88 (m, 1H, CH), 3.84 (s, 3H, CH_3O), 3.44-3.39 (m, 4H, CH_2, CH_2), 3.22-3.17 (m, 2H, CH_2), 2.39 (s, 3H, CH_3), 1.36 (s, 9H, $3CH_3$); HRMS-ESI$^+$: $C_{33}H_{35}N_2O_5$ calcd $[M+H]^+$: 539.2546, found 539.2540.

(S)-Methyl 2-(4-tert-butylphenoxy)-3-(2-isobutylbenzo[d] oxazol-5-yl)propanoate (15i): Compound 15i (49.0 mg, 76 %) as a colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62 (s, 1H, ArH), 7.40 (d, 1H, ArH, *J* = 8.7 Hz), 7.25 (d, 1H, ArH, *J* = 8.4 Hz), 7.23 (d, 2H, ArH, *J* = 8.7 Hz), 6.74 (d, 2H, ArH, *J* = 8.7 Hz), 4.81-4.77 (m, 1H, CH), 3.73 (s, 3H, CH_3), 3.33 (d, 2H, CH_2, *J* = 5.4 Hz), 2.95 (t, 2H, CH_2, *J* = 7.8 Hz), 1.82-1.74 (m, 2H, CH_2), 1.71-1.62 (m, 1H, CH), 1.25 (s, 9H, $3CH_3$), 0.96 (d, 6H, $3CH_3$, *J* = 6.3 Hz); HRMS-ESI$^+$: $C_{25}H_{32}NO_4$ calcd $[M+H]^+$: 410.2331, found 410.2327.

(S)-Methyl 2-(4-tert-butylphenoxy)-3-(2-heptylbenzo[d] oxazol-5-yl)propanoate (15j): Compound 15j (60.0 mg, 84 %) as a brown oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (s, 1H, ArH), 7.40 (d, 1H, ArH, *J* = 8.1 Hz), 7.26-7.21 (m, 3H, ArH), 6.74 (d, 2H, ArH, *J* = 8.7 Hz), 4.81-4.76 (m, 1H, CH), 3.73 (s, 3H, CH_3), 3.32 (d, 2H, CH_2, *J* = 5.4 Hz), 2.93 (t, 2H, CH_2, *J* = 6.2 Hz), 1.45-1.25 (m, 13H, $5CH_2$, CH_3), 1.25 (s, 9H, $3CH_3$); HRMS-ESI$^+$: $C_{28}H_{38}NO_4$ calcd $[M+H]^+$: 452.2801, found 452.2807.

(S)-Methyl 2-(4-tert-butylphenoxy)-3-(2-((tetrahydro-2H-pyran-4-yl)methyl)benzo[d]oxazol-5-yl)propanoate (15k): Compound 15k (83.0 mg, 97 %) as a colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 (s, 1H, ArH), 7.40 (d, 1H, ArH, *J* = 8.1 Hz), 7.24-7.21 (m, 3H, ArH), 6.75 (d, 2H, ArH, *J* = 9.0 Hz), 4.80-4.76 (m, 1H, CH), 3.98-3.93 (m, 2H, CH_2), 3.74 (s, 3H, CH_3), 3.44-3.36 (m, 2H, CH_2), 3.33 (d, 2H, CH_2, *J* = 5.7 Hz), 2.86 (d, 2H, CH_2, *J* = 7.2 Hz), 1.70-1.66 (m, 2H, CH_2), 1.52-1.40 (m, 3H, CH_3), 1.25 (s, 9H, $3CH_3$); HRMS-ESI$^+$: $C_{27}H_{34}NO_5$ calcd $[M+H]^+$: 452.2437, found 452.2432.

General synthesis of compounds 2a-k:

Compounds 15 (0.05 mmol) were dissolved in 1 mL ethanol, and then aqueous solution $LiOH{\cdot}H_2O$ (0.3 mmol, 1 mL) was added. The mixture was stirred for 2 hours at room temperature. The ethanol was removed *in vacuo* and the water residue was acidified with 1M HCl to pH = 4, and extracted with ethyl acetate (5 mL). The organic layer was dried over anhydrous Na_2SO_4, the solvent was concentrated, and the crude product was purified over a silica gel column eluting with CH_2Cl_2/MeOH (10: 1) to give final compounds 2a-k.

(S)-2-(4-tert-butylphenoxy)-3-(2-phenethylbenzo[d]oxazol-5-yl)propanoic acid (2a): Compound 2a (17.0 mg, 76 %) as a white solid; mp: 80-82 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.59 (d, 1H, ArH, *J* = 1.2 Hz), 7.54 (d, 1H, ArH, *J* = 8.0 Hz), 7.28 (dd, 1H, ArH, *J* = 8.4 Hz, 1.6 Hz), 7.24-7.23 (m, 4H, ArH), 7.21 (d, 2H, ArH, *J* = 8.4 Hz), 7.20-7.15 (m, 1H, ArH), 6.74 (d, 2H, ArH, *J* = 8.8 Hz), 4.90-4.87 (m, 1H, CH), 3.28 (dd, 1H, CH, *J* = 14.0 Hz, 4.4 Hz), 3.25-3.19 (m, 3H, CH, CH_2), 3.10 (t, 2H, CH_2, *J* = 7.2 Hz), 1.19 (s, 9H, $3CH_3$); HRMS-ESI$^-$: $C_{28}H_{28}NO_4$ calcd $[M-H]^-$: 442.2018, found 442.2010.

(S)-2-(4-tert-butylphenoxy)-3-(2-(4-isopropylphenethyl) benzo[d]oxazol-5-yl)propanoic acid (2b): Compound 2b (20.0 mg, 82 %) as a white solid; mp: 65-68 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.59 (s, 1H, ArH), 7.53 (d, 1H, ArH, *J* = 8.0 Hz), 7.28 (d, 1H, ArH, *J* = 8.0 Hz), 7.21 (d, 2H, ArH, *J* = 8.8 Hz), 7.15 (d, 2H, ArH, *J* = 8.0 Hz), 7.10 (d, 2H, ArH, *J* = 8.0 Hz), 6.73 (d, 2H, ArH, *J* = 8.8 Hz), 4.90-4.87 (m, 1H, CH), 3.30-3.21 (m, 2H, CH_2), 3.17 (t, 2H, CH_2, *J* = 6.8 Hz), 3.05 (t, 2H, CH_2, *J* = 7.2 Hz), 2.82-2.75 (m, 1H, CH), 1.19 (s, 9H, $3CH_3$), 1.13 (d, 6H, $2CH_3$, *J* = 6.8 Hz); HRMS-ESI$^-$: $C_{31}H_{34}NO_4$ calcd $[M-H]^-$: 484.2488, found 484.2491.

(S)-2-(4-tert-butylphenoxy)-3-(2-(4-fluorophenethyl) benzo[d]oxazol-5-yl)propanoic acid (2c): Compound 2c (19.0 mg, 85 %) as a white solid; mp: 72-74 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.59 (s, 1H, ArH), 7.54 (d, 1H, ArH, *J* = 8.8 Hz), 7.29-7.26 (m, 4H, ArH), 7.21 (d, 1H, ArH, *J* = 8.8 Hz), 7.06 (t, 2H, ArH, *J* = 9.0 Hz), 6.72 (d, 2H, ArH, *J* = 8.4 Hz), 4.90-4.87 (m, 1H, CH), 3.30-3.23 (m, 2H, CH_2), 3.19 (t, 2H, CH_2, *J* = 7.8 Hz), 3.09 (t, 2H, CH_2, *J* = 7.4 Hz), 1.19 (s, 9H, $3CH_3$); HRMS-ESI$^-$: $C_{28}H_{27}FNO_4$ calcd $[M-H]^-$: 460.1924, found 460.1918.

(S)-2-(4-tert-butylphenoxy)-3-(2-(3,4,5-trimethoxyphenethyl) benzo[d]oxazol-5-yl)propanoic acid (2d): Compound 2d (23.0 mg, 87 %) as a white solid; mp: 85-87 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.61-7.60 (m, 1H, ArH), 7.77-7.53 (m, 1H, ArH), 7.29-7.27 (m, 1H, ArH), 7.29-7.27 (m, 1H, ArH), 7.23-7.19 (m, 1H, ArH), 6.74-6.70 (m, 2H, ArH), 6.53-6.51 (m, 2H, ArH), 5.05-5.02 (m, 1H, CH), 3.64-3.63 (m, 6H, $2CH_3$), 3.58-3.56 (m, 3H, CH_3), 3.34-3.19 (m, 4H, $2CH_2$), 3.03-3.01 (m, 2H, CH_2), 1.18 (s, 9H, $3CH_3$); HRMS-ESI$^-$: $C_{31}H_{34}NO_7$ calcd $[M-H]^-$: 532.2335, found 532.2344.

(S)-2-(4-tert-butylphenoxy)-3-(2-(2-(6-(trifluoromethyl) imidazo[1,2-a]pyridin-2-yl)ethyl)benzo[d]oxazol-5-yl)propanoic acid (2e): Compound 2e (24.0 mg, 88 %) as a white solid; mp: 90-92 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.12 (s, 1H, ArH), 7.86 (s, 1H, ArH), 7.63 (d, 1H, ArH, *J* = 9.2 Hz), 7.58 (s, 1H, ArH), 7.58 (t, 1H, ArH, *J* = 8.4 Hz), 7.38 (dd, 1H, ArH, *J* = 9.2 Hz, 2.0 Hz), 7.28 (dd, 1H, ArH, *J* = 8.4 Hz, 1.6 Hz), 7.21 (d, 2H, ArH, *J* = 8.4 Hz), 6.71 (d, 2H, ArH, *J* = 8.8 Hz), 4.90-4.86 (m, 1H, CH), 3.39 (t, 2H, CH_2, *J* = 7.5 Hz), 3.30 (t, 2H, CH_2, *J* = 7.5 Hz), 3.28-3.21 (m, 2H, CH_2), 1.18 (s, 9H, $3CH_3$); HRMS-ESI$^-$: $C_{30}H_{27}F_3N_3O_4$ calcd $[M-H]^-$: 550.1954, found 550.1946.

(S)-2-(4-tert-butylphenoxy)-3-(2-(2-(oxazol-2-yl)ethyl)benzo [d]oxazol-5-yl)propanoic acid (2f): Compound 2f (18.0 mg, 85 %) as a white sold; mp: 89-91 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.96 (s, 1H, ArH), 7.58 (s, 1H, ArH), 7.54 (d, 1H, ArH, *J* = 8.0 Hz), 7.28 (dd, 1H, ArH, *J* = 8.4 Hz, 1.6 Hz), 7.20 (d, 2H, ArH, *J* = 8.8 Hz), 7.05 (s, 1H, ArH), 6.72 (d, 2H, ArH, *J* = 8.8 Hz), 4.87-4.84 (m, 1H, CH), 3.41-3.39 (m, 2H, CH_2), 3.37-3.30 (m, 2H, CH_2), 3.30-3.22 (m, 2H, CH_2), 1.18 (s, 9H, $3CH_3$); HRMS-ESI$^-$: $C_{25}H_{25}N_2O_5$ calcd [M-H]$^-$: 433.1763, found 433.1770.

(S)-3-(2-(2-(benzofuran-2-yl)ethyl)benzo[d]oxazol-5-yl)-2-(4-tert-butylphenoxy)propanoic acid (2g): Compound 2g (23.0 mg, 97 %) as a white solid; mp: 78-80 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.60 (s, 1H, ArH), 7.55 (d, 1H, ArH, *J* = 8.4 Hz), 7.50 (d, 1H, ArH, *J* = 7.6 Hz), 7.45 (d, 1H, ArH, *J* = 8.0 Hz), 7.29 (dd, 1H, ArH, *J* = 8.4 Hz, 1.6 Hz), 7.23-7.20 (m, 3H, ArH), 7.18-7.15 (m, 1H, ArH), 6.72 (d, 2H, ArH, *J* = 8.8 Hz), 6.63 (s, 1H, ArH), 4.89-4.86 (m, 1H, CH), 3.39-3.28 (m, 4H, CH, CH_2), 3.26-3.18 (m, 2H, CH_2), 1.19 (s, 9H, $3CH_3$); HRMS-ESI$^-$: $C_{30}H_{28}NO_5$ calcd [M-H]$^-$: 482.1967, found 482.1971.

(S)-2-(4-tert-butylphenoxy)-3-(2-(2-(5-methyl-2-phenyl oxazol-4-yl)ethyl)benzo[d]oxazol-5-yl)propanoic acid (2h): Compound 2h (22.0 mg, 86 %) as a white solid; mp: 72-73 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.85-7.82 (m, 2H, ArH), 7.59 (s, 1H, ArH), 7.55 (d, 1H, ArH), 7.47-7.43 (m, 3H, ArH), 7.29 (d, 1H, ArH, *J* = 8.8 Hz), 7.21 (d, 2H, ArH, *J* = 9.2 Hz), 6.72 (d, 2H, ArH, *J* = 8.4 Hz), 4.89-4.86 (m, 1H, CH), 3.30-3.19 (m, 4H, $2CH_2$), 2.98 (t, 2H, CH_2, *J* = 7.2 Hz), 2.26 (s, 3H, CH_3), 1.18 (s, 9H, $3CH_3$); HRMS-ESI$^-$: $C_{32}H_{31}N_2O_4$ calcd [M-H]$^-$: 523.2233, found 523.2226.

(S)-2-(4-tert-butylphenoxy)-3-(2-isobutylbenzo[d]oxazol-5-yl)propanoic acid (2i): Compound 2i (18.0 mg, 90 %) as a white solid; mp: 51-52 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.58 (s, 1H, ArH), 7.54 (d, 1H, ArH, *J* = 8.8 Hz), 7.27 (d, 1H, ArH, *J* = 8.4 Hz), 7.21 (d, 2H, ArH, *J* = 8.8 Hz), 6.73 (d, 2H, ArH, *J* = 9.2 Hz), 4.98-4.87 (m, 1H, CH), 3.36-3.18 (m, 2H, CH_2), 2.88 (d, 2H, CH_2, *J* = 7.4 Hz), 1.61-1.55 (m, 1H, CH), 1.19 (s, 9H, $3CH_3$), 0.88 (d, 6H, $2CH_3$, *J* = 6.0 Hz); HRMS-ESI$^-$: $C_{24}H_{28}NO_4$ calcd [M-H]$^-$: 394.2018, found 394.2020.

(S)-2-(4-tert-butylphenoxy)-3-(2-heptylbenzo[d]oxazol-5-yl)propanoic acid (2j): Compound 2j (19.0 mg, 90 %) as a white solid; mp: 63-66 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.59 (d, 1H, ArH, *J* = 1.6 Hz), 7.53 (d, 1H, ArH, *J* = 8.4 Hz), 7.27 (dd, 1H, ArH, *J* = 8.0 Hz, 1.2 Hz), 7.21 (d, 2H, ArH, *J* = 8.8 Hz), 6.72 (d, 2H, ArH, *J* = 9.2 Hz), 4.88 (dd, 1H, CH, *J* = 8.0 Hz, 4.4 Hz), 3.26-3.18 (m, 2H, CH_2), 2.87 (t, 2H, CH_2, *J* = 7.2 Hz), 1.74 (qui, 2H, CH_2, *J* = 7.2 Hz), 1.34-1.21 (m, 8H, $4CH_2$), 1.19 (s, 9H, $3CH_3$), 0.82 (t, 3H, CH_3, *J* = 6.8 Hz); HRMS-ESI$^-$: $C_{27}H_{34}NO_4$ calcd [M-H]$^-$: 436.2488, found 436.2480.

(S)-2-(4-tert-butylphenoxy)-3-(2-((tetrahydro-2H-pyran-4-yl)methyl)benzo[d]oxazol-5-yl)propanoic acid (2k): Compound 2k (15.0 mg, 70 %) as a white solid; mp: 102-103 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.61 (s, 1H, ArH), 7.54 (d, 1H, ArH, *J* = 8.4 Hz), 7.28 (d, 1H, ArH, *J* = 8.4 Hz), 7.20 (d, 2H, ArH, *J* = 8.8 Hz), 6.73 (d, 2H, ArH, *J* = 8.8 Hz), 4.90-4.87 (m, 1H, CH), 3.80-3.78 (m, 2H, CH_2), 3.28-3.19 (m, 4H, $2CH_2$), 2.83-2.81 (m, 2H, CH_2), 2.07-2.06 (m, 1H, CH), 1.59-1.56 (m, 2H, 2CH), 1.33-1.27 (m, 2H, CH_2), 1.19 (s, 9H, $3CH_3$); HRMS-ESI$^-$: $C_{26}H_{30}NO_5$ calcd [M-H]$^-$: 436.2124, found 436.2119.

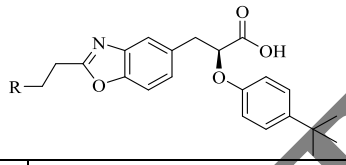
Antimicrobial Activity Evaluations

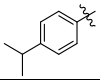
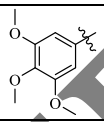
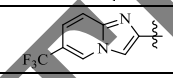
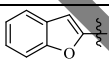
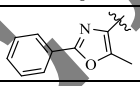
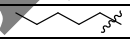
According to the National Committee for Clinical Laboratory Standards, we adopted a 96-well microtiter broth dilution method to determinate MIC values [26, 27]. Activities of the newly synthesized compounds were determined against fungus *Candida albicans ATCC 10231*, Gram-negative bacteria *Escherichia coli ATCC 11303* and Gram-positive bacteria *Staphylococcus aureus ATCC 10832*, Methicillin-resistant *Staphylococcus aureus ATCC 700699*, *Bacillus subtilis ATCC 33712*. The MIC value is defined as the lowest antibiotic concentration that resulted in visible growth after incubation at 37 ^{o}C for 24 h. Compound 1a, Ceftazidime, Cefotaxime, Cefradine, Sodium Penicillin, Miconazole nitrate, and Ketoconazole were used as reference or control drugs.

All the compounds and control drugs were dissolved in DMSO (4 mg/mL), and then diluted with Mueller-Hinton broth bacteria (10^5 CFU/mL) or Sabouraud dextrose broth fungus (10^4 CFU/mL) to the sample concentration (4 mg/mL). Using the multipipettor, 10 μL of a 4 mg/mL compound solution were dispensed into the wells in column 2. 100 μL of the broth bacteria or fungus were dispensed into column 2 through column 12 and another 100 μL dispensed bacteria into column 2. Using the multipipettor set at 100 μL, the compound solution was mixed in the wells in column 2 by sucking up and down 6-8 times. 100 μL were withdrawn from column 2 and added to column 3; this makes column 3 a twofold dilution of column 2. Mix up and down 6-8 times. 100 μL were transferred to

column 4. The procedure was repeated down to column 11 only and 100 µL were discarded from column 11. The final concentrations from column 2 to 11 were 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 and 0.39 µg/mL. 100 µL of sterile medium were pipetted into column 1, and the column 12 as control groups. Compounds with antibacterial concentration less than 0.39 µg/mL were diluted again to 0.195, 0.098 and 0.049 µg/mL as above. After incubation for 24 h at 37 ^{o}C, we streaked the bacterial culture on plates to check their clarity. MIC values were taken as the lowest concentration of drug that made the well clean. In order to ensure that the solvent DMSO had no effect on bacterial growth, a control test was also performed containing inoculated broth supplemented with only DMSO at the same dilutions used in our experiments and found inactive in culture medium. The antimicrobial activity data of the compounds and the control drugs are given as MIC (µg/mL) values in Table 1.

Table 1: The *in vitro* antimicrobial activity of compounds 2a-k with the control drugs, [a]E. coli-Escherichia coli; S. aureus-Staphylococcus aureus; MRSA-methicillin-resistant Staphylococcus aureus; B. subtilis-Bacillus subtilis; C. albicans-Candida albicans; [b] The sign (-) referred to that compounds not tested



Compound	R	MIC (µg/mL)[a, b]				
		E. coli	*S.aureus*	*MRSA*	*B.subtilis*	*C.albicans*
2a		3.12	1.56	1.56	0.39	>200
2b		0.78	0.39	0.195	0.098	>200
2c		1.56	1.56	1.56	0.39	>200
2d		200	100	100	100	>200
2e		>200	50	25	50	>200
2f		>200	>200	200	200	>200
2g		0.78	0.78	0.78	0.39	>200
2h		1.56	1.56	0.78	0.78	>200
2i		3.12	1.56	1.56	1.56	>200
2j		1.56	0.78	1.56	0.78	>200
2k		>200	100	100	200	>200
1a		3.12	1.56	1.56	3.12	>200
Ceftazidime		200	0.78	12.5	6.25	-
Cefotaxime		200	3.12	3.12	0.78	-
Cefradine		25	25	50	50	-
Sodium		0.78	3.12	3.12	0.195	-
Penicillin		-	-	-	-	1.56
Miconazole		-	-	-	-	<0.39
Nitrate		3.12	1.56	1.56	0.39	>200
Ketoconazole		0.78	0.39	0.195	0.098	>200

RESULTS AND DISCUSSION

Table 1 summarized the results of the minimum inhibitory concentration (MIC) assays of eleven compounds synthesized. Compared to the lead compound 1a, general improvement of anti-bacterial activity was observed, while none for fungus.

When the aromatic tail of 1a was substituted with phenyl (2a), 4-*iso*propyl-phenyl (2b) and 4-fluorophenyl (2c), the antibacterial activities were increased by 8-32 fold. 4-*Iso*propyl phenyl (2b) especially exerted the best activities against all Gram-positive and -negative bacteria with MIC value between 0.098-0.78 μg/mL. The activity of 2b against *B. subtilis* was 2-fold higher than sodium penicillin, and 8- to 510-fold higher than other antibiotics. Compound 2h, a short linker homologue of 1a, showed 2- to 4-fold higher activities than 1a on some bacteria (*E. coli* and *MRSA*).

From the MIC values of compounds 2a-2d and 2h, we found that the hydrophobicity of tail fragments impacted the anti-bacterial activities dramatically. The law of anti-bacterial activity was in full accordance with the order of calculated logP value (clogP): 2b (clogP 7.98) > 2c (6.90) > 2h (6.79) > 2a (6.74) > 2d (6.36), and corresponded with our previous study, namely hydrophobicity-activity relationship which indicates that hydrophobicity can increase the antibacterial activities [20]. Evidently, the hydrophilic aromatic tie 3,4,5-trimethoxyphenyl (2d) produced a negative effect on the inhibition of bacterial proliferation, with MIC values only at 100-200μg/mL. Furthermore, the polar heterocyclic ties (e.g. imidazo[1,2-a]pyridine-2-yl (2e) and oxazol-5-yl (2f)) were also proved to decrease the antibacterial activity dramatically.

Benzofuran-2-yl (2g) (clogP 6.27) displayed a unique broad-spectrum activity against all screened bacteria without following the hydrophobicity-activity relationship. We reason that benzofuran moiety might possess special characteristics against bacteria as it was reported in our previous studies [28, 29].

When the tail fragment of compound 1a was converted to long-chain heptyl (2j), similar anti-bacterial activities were retained and the *iso*-propyl substituted derivative (2i) exhibited 1-fold increasing activity. However, when the alkyl tail contained an oxygen atom, tetrahydropyran-4-yl (2k), weak inhibitory effects were observed. These results were also fully consistent with the contribution of hydrophobicity on the structure-activity relationship (SAR): 2j (clog P 7.22) > 2i (6.30) > 2k (4.96).

CONCLUSIONS

Based on the structure of the lead compound (1a), a novel series of (*S*)-2-(4-*tert*-butylphenoxy)-3-(benzoxazl-5-yl)propanoic acid derivatives 2 were designed and synthesized by structural modification with different lipophilic and hydrophilic fragments, which were introduced into the tail fragment of the leading. The newly designed compounds displayed a broad spectrum of *in vitro* activities against Gram-positive bacteria such as *S. aureus*, *MRSA*, *B. subtilis*, and Gram-negative bacteria *E. coli* as well.

Furthermore, we have demonstrated that hydrophobic tails, like phenyl (2a), 4-*iso*propyl-phenyl (2b) and 4-fluorophenyl (2c), *iso*-propyl (2i), heptyl (2j) showed the best prominent antibacterial activities in all screened assays, and were even better than the leading compound (1a) and positive control antibiotics examined in this study.

The introduction of the hydrophobic groups on the tail fragment is believed to produce a positive effect on the anti-bacterial activity, and the structure-activity relationship (SAR) presented is manifested in this work.

ACKNOWLDGEMENT

We thank the National Comprehensive Technology Platforms for Innovative Drug R&D, 2009ZX09301-007 for the financial support. We thank Professor Fengsheng Zhao (Shanghai Jiao Tong University) for the biological support of this research.

REFERENCES

[1] B Gong; F Hong; C Kohm; L Bonham; P Klein. Bioorg. *Med. Chem. Lett.*, **2004**, 14(6), 1455-1459.

[2] O Temiz-Arpaci; B Tekiner-Gulbas; I Yildiz; E Aki-Sener; I Yalcin. *Bioorg. Med. Chem.*, **2005**, 13(23), 6354-6359.

[3] BB Wang; N Maghami; VL Goodlin; PJ Smith. *Bioorg. Med. Chem. Lett.*, **2004**, 14(12), 3221-3226.

[4] SM Rida; FA Ashour; SAM El-Hawash; MM El-Semary; MH Badr; MA Shalaby. *Eur. J. Med. Chem.*, **2005**, 40(9), 949-959.

[5] J Jiang; X Tang; W Dou; H Zhang; W Liu; C Wang; J Zheng. *J. Inorg. Biochem.*, **2010**, 104(5), 583-591.
[6] T Hisano; M Ichikawa; K Tsumoto; M Tasaki. *Chem. Pharm. Bull.*, **1982**, 30(8), 2996-3004.
[7] O Temiz; I Oren; E Sener; I Yalcin; N Ucarturk. *Il Farmaco*, **1998**, 53(5), 337-341.
[8] I Yalcin; I Oren; A Akin; N Ucarturk. *Eur. J. Med. Chem.*, **1992**, 27(4), 401-406.
[9] I Oren; O Temiz; I Yalcin; E Sener; N Altanlar. *Eur. J. Pharm. Sci.*, **1998**, 7(2), 153-160.
[10] I Yildiz-Oren; I Yalcin; E Aki-Sener; N Ucarturk. *Eur. J. Med. Chem.*, **2004**, 39(3), 291-298.
[11] OT Arpaci; I Oren; N Altanlar. *Il Farmaco*, **2002**, 57(3), 175-181.
[12] S Alper-Hayta; M Arisoy; O Temiz-Arpaci; I Yildiz; E Aki; S Özkan; F Kaynak. *Eur. J. Med. Chem.*, **2008**, 43(11), 2568-2578.
[13] O Temiz-Arpaci; I Yildiz; S Ozkan; F Kaynak; E Aki-Sener; I Yalcin. *Eur. J. Med. Chem.*, **2008**, 43(7), 1423-1431.
[14] OT Arpaci; EA Sener; I Yalcin; N Altanlar. *Il Farmaco*, **2002**, 57(9), 771-775.
[15] B Tekiner-Gulbas; O Temiz-Arpaci; I Yildiz; N Altanlar. *Eur. J. Med. Chem.*, **2007**, 42(10), 1293-1299.
[16] M Prudhomme; J Guyot; G Jeminet. *J. Antibiot.*, **1986**, 39(7), 934-937.
[17] RK Boeckman; AB Charette; T Asberom; BH Johnston. *J. Am. Chem. Soc.*, **1991**, 113(14), 5337-5353.
[18] GD Smith; WL Duax. *J. Am. Chem. Soc.*, **1976**, 98(6), 1578-1580.
[19] AD Rodriguez; C Ramirez; II Rodriguez; E Gonzalez. *Org. Lett.*, **1999**, 1(3), 527-530.
[20] W Zhang; W Liu; X Jiang; F Jiang; H Zhuang; L Fu. *Eur. J. Med. Chem.*, **2011**, 46(9), 3639-3650.
[21] J Liu; F Jiang; Y Jin; Y Zhang; J Liu; W Liu; L Fu. *Eur. J. Med. Chem.*, **2012**, 57, 10-20.
[22] J Liu; X Deng; Y Jin; B Xu; W Liu; F Jiang; L Fu. *Pharmazie*, **2015**, 70(7), 446-451.
[23] X Deng; J Liu; Y Jin; Y Zhang; W He; J Bao; W Liu; F Jiang; L Fu. *Pharmazie*, **2015**, 70(12), 777-783.
[24] W Zhang; C Chen; R Cao; L Maurmann; P Li. *ChemBioChem*, **2015**, 16 (1), 156-166.
[25] W Zhang; W Liu; X Jiang; F Jiang; L Fu. *Syn. Commun.*, **2012**, 42 (18), 2772-2779.
[26] National Committee for Clinical Laboratory Standard. Methods for dilution antimicrobial, tests for bacteria that grow aerobically, 5th ed. Approved standard, M7-A6. Wayne, PA: National Committee for Clinical Laboratory Standards. **2003**.
[27] National Committee for Clinical Laboratory Standard. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Conidium-forming Filamentous Fungi. Proposed Standard M38-P. Wayne, PA: National Committee for Clinical Laboratory Standards. **1998**.
[28] X Jiang; W Liu; W Zhang; F Jiang; Z Gao; H Zhuang; L Fu. *Eur. J. Med. Chem.*, **2011**, 46(8), 3526-3530.
[29] J Liu; F Jiang; X Jiang; W Zhang; J Liu; W Liu; L Fu. *Eur. J. Med. Chem.*, **2012**, 54, 879-886.