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Short communication

Design, synthesis and antibacterial activity of novel actinonin derivatives containing benzimidazole heterocycles

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Abstract

A series of novel actinonin derivatives containing a benzimidazole heterocycle linked as amide isostere have been designed and synthesized. The structures of all the synthesized compounds were confirmed by analytical and spectroscopic methods. All the compounds were evaluated in vitro against *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Sarcina lutea*. Among them, compound **1a** with unsubstituted benzimidazole ring exhibited potent antibacterial activities.

Keywords: Actinonin derivatives; Benzimidazole; Synthesis; Antibacterial activity

1. Introduction

The increase in bacterial resistance has attracted considerable interest in the discovery and development of new classes of antibacterial agents [1]. The new agents should preferably consist of chemical characteristics that clearly differ from those of existing agents. Actinonin was first isolated from a Malayan strain of *Actinomyces* and found to show a weak inhibitory activity against Gram-positive and Gram-negative bacteria [2,3]. However, recently actinonin has been proven to have antiproliferative effects on human tumor cells [4]. The action mechanism of actinonin is believed to be the inhibition of the peptide deformylase that is a new class of metalloenzyme which is essential for bacterial survival [5,6]. The hydroxamate group of actinonin, which can complex with the metal ion in the active pocket of the peptide deformylase, is necessary for its activity [7]. Nevertheless, actinonin lacks in vivo efficacy, due to the poor bioavailability [7,8]. A lot of actinonin analogues have been developed to test their bioactivities [9,10], and the general structure of these compounds consists of a metal-chelating group and various amidation fragments. Given the apparent hydrolyzable amide bonds, we decided to investigate a series of compounds bearing a heterocyclic moiety in place of amide fragment of actinonin molecule to work as an amide isostere. Several benzimidazole heterocycles were chosen to be incorporated into our designed new compounds in view of their ability to act as both hydrogen bond acceptor and donor while maintaining the proper orientation of the side chain (Fig. 1). Such a peptidomimetic modification has led to the discovery of potent, competitive, and reversible inhibitors of tyrosine phosphatase 1B (PTP1B) with improved caco-2 permeability [11]. In this paper, we describe the synthesis of the title compounds with seven different substituents on the benzimidazole ring. The antibacterial activities were also evaluated.

2. Chemistry

The general strategy for the synthesis of **1** is illustrated in Scheme 1. The chiral intermediate (*R*)-2-butylbutanedioic acid-4-*tert*-butyl ester was prepared in high ee value according

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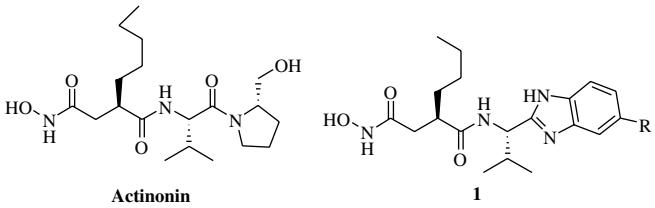
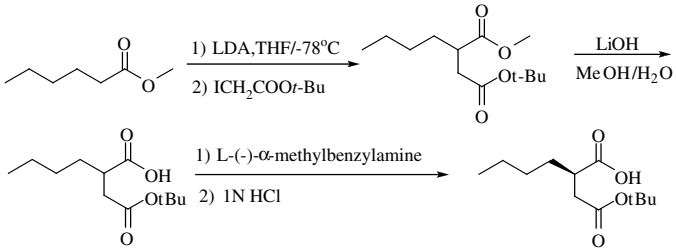


Fig. 1. Peptidomimetic modification of actinonin.



Scheme 2. Synthesis of (*R*)-2-butylbutanedioic acid-4-*tert*-butyl ester.

to an alkylation procedure on methyl hexanoate followed by resolution of the racemic 2-*n*-butylbutanedioic acid-4-*tert*-butyl monoester (Scheme 2) [12]. Benzimidazole intermediates **2** were deprotected with hydrogen catalyzed by Pd/C and then coupled with the chiral succinate with the acceleration of 1-chloro-3,5-dimethoxy-2,4,6-triazine (CDMT) and *N*-methyl morpholine [13]. After removal of the *tert*-butyl group and formation of new methyl ester **4**, the hydroxamates were obtained through a cyanide-catalyzed hydroxylamination of the esters [14]. Actually, the cyanide-catalyzed hydroxylamination procedure with aqueous hydroxylamine solution simplified the operation and gave consistently modest to good yield.

Preparation of benzimidazole intermediates **2a**–**2c** was achieved using a procedure similar to that described by Chen et al. [15]. Coupling of **5** with Cbz-protected L-valine, followed by the intramolecular cyclization in acetic acid yielded benzimidazole intermediates **2a**–**2c**. Amidation of **6** with appropriate amines and then condensing with Cbz-L-valine afforded the precursor amides **7**. Heating the amides in acetic acid gave the desired cyclization compounds **2d**–**2g** (Scheme 3).

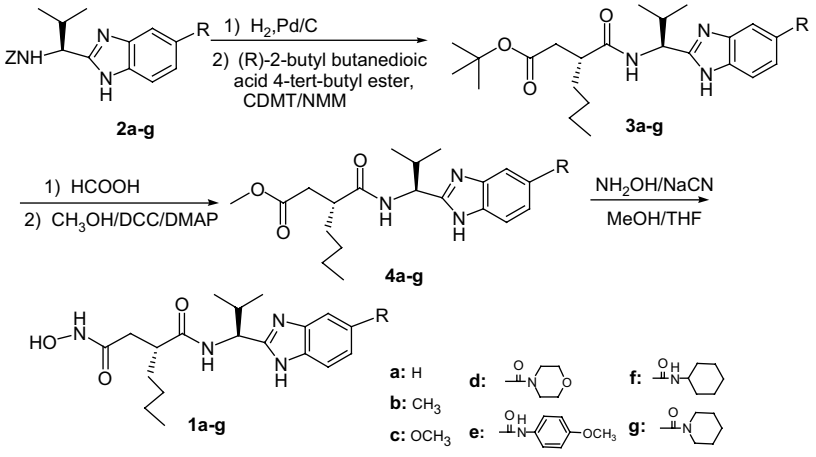
Some characteristics of the synthesized target compounds are outlined in Table 1. Analytical and spectral data (^{1}H NMR, ^{13}C NMR, ESI-MS, IR, and elemental analysis) confirmed the structures of the new compounds.

3. Biological activity

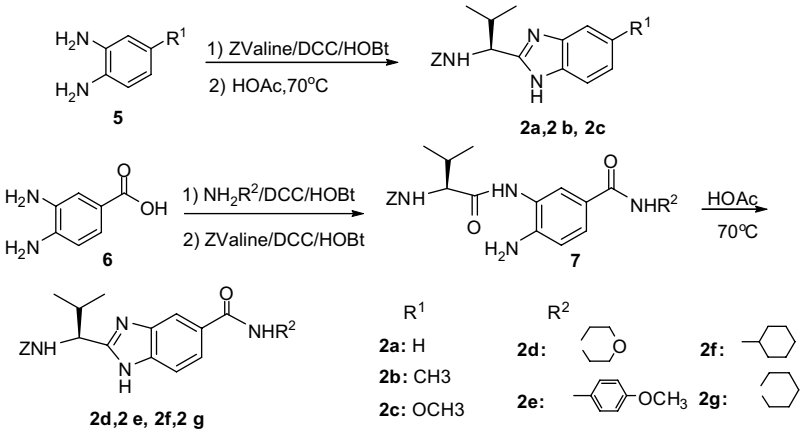
Antimicrobial activities of synthesized compounds were tested using microbroth dilution method [16,17]. Tested microorganism strains were *Staphylococcus aureus* (CMCC26112), *Klebsiella pneumoniae* (CMCC46117), and *Sarcina lutea* (CMCC28001). Cefoperazone was used as the standard drug. The results of the antimicrobial studies of the compounds and standard drug are given in Table 2.

4. Results and discussion

A series of seven new compounds were synthesized. We obtained benzimidazole intermediates (**2a**–**2g**) through an intramolecular cyclization after the coupling of Cbz-L-valine with two kinds of substituted *o*-phenylenediamines. The condensation of the chiral succinate with the benzimidazole moiety was accelerated by 1-chloro-3,5-dimethoxy-2,4,6-triazine (CDMT) and *N*-methyl morpholine. With the combination of the two reagents, high-yield target compounds were obtained and the work-up procedures were simplified compared with the conventional DCC/HOBt method. The deprotection procedure of the *tert*-butyl group with trifluoroacetic acid in dichloromethane described by Chen et al. [15] gave a sticky liquid difficult to deal with. Then 98% formic acid was employed and the free acid obtained could be used without further purification. The methods of one-step condensation of free carboxylic acid with hydroxylamine hydrochloride have been reported [18,19]. However, all the attempts with our benzimidazole-containing succinic acid derivatives failed to afford the desired hydroxamic acids. The reaction of hydroxylamine with ester (**4a**–**4g**) which was carried out in the presence of large amount of KOH [20] led to complex products



Scheme 1. Synthetic route to the title compounds.



Scheme 3. Synthesis of benzimidazole intermediates **2a**–**2g**.

and tedious purification steps. The cyanide-catalyzed hydroxylamination procedures of ester (**4a**–**4g**) with 50% aqueous hydroxylamine solution were proven to be simple and efficient among these reported methods.

In the IR spectrum of these compounds, a broad absorption band around 3250 cm^{-1} indicates the presence of active hydrogen group in the compounds. The amide carbonyl stretching frequency was observed at about 1646 cm^{-1}. The other prominent absorption bands observed in the IR spectrum are 3035 (Ar–H), 2924 (C–H) and 1540 (C=N) cm^{-1}.

^{1}H NMR spectrum of **1a** showed a triplet at δ 0.65 due to the CH_3 protons at the terminal of the butyl group. The two CH_3 protons of the isopropyl appeared as two doublets centered at δ 0.80 and δ 0.94 with a vicinal coupling constant $J = 6.7$ Hz. The six methylene protons of the butyl group resonated as complex multiplets at δ 1.01–1.42. One of the methylene protons of the succinate moiety appeared as a double doublet centered at δ 2.06 ($J = 7.4$, 14.5 Hz). The other methylene protons, mixed with the tertiary hydrogen signal of the isopropyl, resonated as multiplets at δ 2.21–2.30. The appearance of a double doublet at δ 2.06 clearly reveals the magnetic nonequivalence of the two protons of CH_2 group adjacent to a chiral center. The chiral proton of succinate was observed as multiplet between δ 2.70 and 2.75. A triplet at δ 4.87 ($J = 8.4$ Hz) integrating for one proton was attributable to the chiral proton adjacent to the benzimidazole ring. All the other aromatic and active hydrogen were observed at expected regions. ^{13}C NMR chemical shift values of the carbon atoms at δ 35.10–35.31 (C3), 41.60–41.84 (C2), 167.50–167.79 (CONHOH) and 173.93–174.28 (CONH) corroborated the succinamide character of these compounds deduced from the ^{1}H NMR. 2-Methylpropyl moiety was confirmed by the carbon atoms observed at δ 18.77–18.89 (CH_3), 19.31–19.51 (CH_3), 31.65–32.03 ($-CH(CH_3)_2$) and 53.07–53.22 (NH–CH–Ar).

Further evidence for the formation of hydroxamates **1a**–**1g** was obtained by recording the mass spectra. The mass spectra showed the molecular ion peak corresponding to its molecular

Table 1
Physical data for compounds **1a**–**1g**

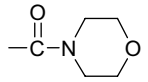
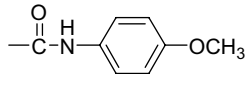
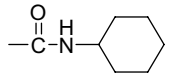
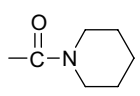
Compound	R =	Mol. formula	M. wt	m.p. (°C)	Yield (%)
1a	–H	$C_{19}H_{28}N_4O_3$	360.45	132–134	76
1b	$-CH_3$	$C_{20}H_{30}N_4O_3$	374.48	134–135	65
1c	$-OCH_3$	$C_{20}H_{30}N_4O_4$	390.48	123–125	75
1d		$C_{24}H_{35}N_5O_5$	473.57	142–144	80
1e		$C_{27}H_{35}N_5O_5$	509.6	201–203	70
1f		$C_{26}H_{39}N_5O_4$	485.62	220–222	73
1g		$C_{25}H_{37}N_5O_4$	471.59	138–140	77

Table 2
Antibacterial activities of **1a–1g** (MIC, μg/mL)

Compound	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Sarcina lutea*
1a	2	0.5	4
1b	8	4	8
1c	8	8	8
1d	16	8	16
1e	32	16	16
1f	64	32	64
1g	>64	8	>64
Standard	0.25	0.25	0.25

Standard: cefoperazone.

weight. The elemental analyses showed that all the newly synthesized compounds were having proper purity.

All target compounds **1a–1g** were evaluated for their antibacterial activities against *S. aureus*, *K. pneumoniae* and *S. lutea*. 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 0.5–64 μg/mL against all evaluated strains.

On comparing their MIC values with the standard, compounds **1a–1c** were effective against all evaluated strains. Compound **1a** exhibited a good inhibitory effect on these bacteria. Compound **1a** especially showed high activity against *K. pneumoniae*. Compounds **1d–1g** showed moderate activity. The investigation on the structure–activity relationship revealed that the compounds with aliphatic substituents on the benzene ring of the benzimidazole gave better result than those with the amidation groups. Introduction of the amidation groups into the 5-position of the benzimidazole led to the reduced inhibitory activity. Compounds **1f** and **1g** with saturated cyclic hydrocarbon substituents at the amidation group showed less activity when compared with compounds **1d** and **1e**.

5. Conclusion

Based on structure–activity relationship and the peptidomimetic idea, the benzimidazole ring was incorporated into the actinonin molecule as an amide isostere and the hydroxamate group was retained. A series of seven new hydroxamates bearing benzimidazole ring have been synthesized. The investigation on antibacterial screening data reveals that compound **1a** showed good bacterial inhibition. Varying the substituents on the 5-position of the benzimidazole with different groups significantly influenced the antibacterial activity. The compound with unsubstituted benzimidazole exhibited potent inhibitory activity. In conclusion, these preliminary results are promising and are beneficial for further studies in developing new lead compounds.

6. Experimental

All reagents were used as purchased from commercial suppliers without further purification. 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 and ^{13}C NMR spectra of $CDCl_3$/DMSO-d_6 solutions (TMS as an internal standard) were recorded on 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 performed on a Perkin–Elmer 2400 analyzer. Optical rotations were measured with a WZZ-2 polarimeter.

6.1. General procedure for the synthesis of compounds *2a–2c*

To a solution of *Z*-valine (5.5 mmol) in anhydrous tetrahydrofuran (10 mL) 1-hydroxybenzotriazole (6.05 mmol) was added. After cooling to 0 °C, dicyclohexylcarbodiimide (5.8 mmol) was added in portions to the reaction mixture and the resulting solution was stirred for 3 h with gradual warming to room temperature. The precipitate obtained was removed by suction filtration and compound **5** (5 mmol) was introduced to the filtrate. The stirring was continued for 7 h. After filtration, the filtrate was concentrated in an evaporator and the residue was purified on a silica gel column with methylene dichloride/methanol (20:1).

The solution of the intermediates above in acetic acid (8 mL) was heated at 70–75 °C for 7 h. After removal of the solvent under vacuum, the crude product was purified on silica gel with petroleum ether/EtOAc (1:1).

6.1.1. *N*-Benzyloxycarbonyl-(1*S*)-(1-benzimidazol-2-yl)-2-methylpropylamine (*2a*)

Slightly yellow solid, yield: 71%; m.p. 182–184 °C; $[\alpha]_D^{25} = -58.5$ (*c* 1, MeOH). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.93 (d, $J = 6.7$ Hz, 3H), 1.05 (d, $J = 6.5$ Hz, 3H), 2.45–2.55 (m, 1H), 4.66 (t, $J = 8$ Hz, 1H), 4.98 (d, $J = 12$ Hz, 1H), 5.10 (d, $J = 12$ Hz, 1H), 6.09 (d, $J = 7.8$ Hz, 1H), 7.19–7.28 (m, 7H), 7.45–7.53 (m, 2H). IR (KBr) ν/cm^{-1}: 3375, 3309, 1690, 1540, 1257.

6.1.2. *N*-Benzyloxycarbonyl-(1*S*)-[1-(5-methylbenzimidazol-2-yl)]-2-methylpropylamine (*2b*)

White-off solid, yield: 80%; m.p. 139–141 °C; $[\alpha]_D^{25} = -63.0$ (*c* 1, MeOH). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.91 (d, $J = 6.7$ Hz, 3H), 1.03 (d, $J = 6.5$ Hz, 3H), 2.42 (s, 3H), 2.43–2.55 (m, 1H), 4.63 (t, $J = 8$ Hz, 1H), 4.98 (d, $J = 12.2$ Hz, 1H), 5.10 (d, $J = 12.2$ Hz, 1H), 6.09 (d, $J = 7.8$ Hz, 1H), 7.03 (d, $J = 8$ Hz, 1H), 7.19–7.28 (m, 6H), 7.41–7.50 (m, 1H). IR (KBr) ν/cm^{-1}: 3335, 3030, 1686, 1524, 1234.

6.1.3. *N*-Benzyloxycarbonyl-(1*S*)-[1-(5-methoxyl benzimidazol-2-yl)]-2-methylpropylamine (*2c*)

Slightly yellow solid, yield: 58%; m.p. 60–61 °C; $[\alpha]_D^{25} = -52.5$ (*c* 1, MeOH). ^{1}H NMR ($CDCl_3$, 400 MHz) δ:

0.92 (d, $J = 6.7$ Hz, 3H), 1.03 (d, $J = 6.6$ Hz, 3H), 2.43–2.54 (m, 1H), 3.79 (s, 3H), 4.62 (t, $J = 8$ Hz, 1H), 4.98 (d, $J = 12.2$ Hz, 1H), 5.10 (d, $J = 12.2$ Hz, 1H), 6.06 (d, $J = 7.8$ Hz, 1H), 6.85 (dd, $J = 2.4$, 8.8 Hz, 1H), 6.90–6.98 (m, 1H), 7.25–7.32 (m, 5H), 7.38–7.45 (m, 1H). IR (KBr) ν/cm^{-1}: 3288, 3032, 1695, 1538, 1267.

6.2. General procedure for the synthesis of compounds **7** and **2d–2g**

1-Hydroxybenzotriazole (12 mmol) was added under N_2 to a solution of 3,4-diaminobenzoic acid (10 mmol) and triethylamine (11 mmol) in dry dimethylformamide/methylene dichloride (14 mL, 1:1). After cooling to 0 °C, dicyclohexylcarbodiimide (1.1 mmol) was added in portions to the reaction mixture followed by various amines (15 mmol) and the resulting solution was stirred for 12 h with gradual warming to room temperature. The solution was filtered and evaporated under vacuum. The residue was purified on a short silica gel column with methylene dichloride/methanol (20:1). The solid obtained above was dissolved in dry tetrahydrofuran (50 mL) and *Z*-L-valine (5.5 mmol) and 1-hydroxybenzotriazole (6.05 mmol) were added under N_2. After cooling to 0 °C, dicyclohexylcarbodiimide (5.8 mmol) was added in portions to the reaction mixture and the resulting solution was stirred for 12 h with gradual warming to room temperature. The solution was filtered and evaporated under vacuum. The residue was purified on a short silica gel column with methylene dichloride/methanol (20:1) to afford compound **7**.

The solution of compound **7** in acetic acid (10 mL) was heated at 70–75 °C for 7 h. After removal of the solvent under vacuum, the crude product was purified on silica gel with methylene dichloride/methanol (20:1) to produce **2d–2g**.

6.2.1. 2-[1-(*S*)-Benzyloxycarbonylamino-2-methyl]propyl-5-(1-morpholin)carbonylbenzimidazole (**2d**)

White-off solid, yield: 70%; m.p. 95–97 °C; $[\alpha]_D^{25} = -37.5$ (*c* 1, MeOH). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 0.84 (d, $J = 6.6$ Hz, 3H), 0.96 (d, $J = 6.6$ Hz, 3H), 2.23–2.34 (m, 1H), 3.31–3.60 (m, 8H), 4.64 (t, $J = 7.8$ Hz, 1H), 5.01 (d, $J = 12.6$ Hz, 1H), 5.08 (d, $J = 12.6$ Hz, 1H), 7.19–7.63 (m, 8H), 7.81 (d, $J = 8.4$ Hz, 1H), 12.47 (s, 1H). IR (KBr) ν/cm^{-1}: 3310, 3193, 3032, 1718, 1612, 1526, 1239.

6.2.2. 2-[1-(*S*)-Benzyloxycarbonylamino-2-methyl]propyl-5-(4-methoxyanilino)carbonylbenzimidazole (**2e**)

White solid, yield: 65%; m.p. 100–103 °C; $[\alpha]_D^{25} = -34.5$ (*c* 1, MeOH). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 0.83 (d, $J = 6.6$ Hz, 3H), 0.96 (d, $J = 6.6$ Hz, 3H), 2.25–2.34 (m, 1H), 3.75 (s, 3H), 4.64 (t, $J = 8.1$ Hz, 1H), 5.02 (d, $J = 12.6$ Hz, 1H), 5.08 (d, $J = 12.6$ Hz, 1H), 6.93 (d, $J = 8.4$ Hz, 2H), 7.23–7.42 (m, 5H), 7.53–7.84 (m, 5H), 10.09 (s, 1H), 12.57 (s, 1H). IR (KBr) ν/cm^{-1}: 3288, 3032, 1703, 1645, 1511, 1244.

6.2.3. N-[2-Amino-5-(cyclohexylamino)carbonyl]phenyl-3-methyl-2-(*S*)-benzyloxycarbonylaminobutamide (**2f**)

White foam, yield: 60%; $[\alpha]_D^{25} = -34.0$ (*c* 1, MeOH). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 0.83 (d, $J = 6.9$ Hz, 3H), 0.94 (d, $J = 6.9$ Hz, 3H), 1.14–1.35 (m, 6H), 1.59–1.82 (m, 4H), 2.22–2.28 (m, 1H), 3.73–3.78 (m, 1H), 4.62 (dd, $J = 7.8$ Hz, 1H), 4.97 (d, $J = 8.7$ Hz, 1H), 5.04 (d, $J = 8.7$ Hz, 1H), 7.31–7.36 (m, 5H), 7.48–7.82 (m, 3H), 8.08–8.15 (m, 2H), 12.47 (s, 1H). IR (KBr) ν/cm^{-1}: 3324, 3219, 3033, 1686, 1620, 1551, 1287.

6.2.4. N-[2-Amino-5-(piperidino)carbonyl]phenyl-3-methyl-2-(*S*)-benzyloxycarbonylaminobutamide (**2g**)

White-off solid, yield: 55%; m.p. 95–100 °C; $[\alpha]_D^{25} = -36.5$ (*c* 1, MeOH). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 0.83 (d, $J = 6.9$ Hz, 3H), 0.95 (d, $J = 6.9$ Hz, 3H), 1.43–1.62 (m, 6H), 2.22–2.32 (m, 1H), 3.35–3.48 (m, 4H), 4.62 (t, $J = 7.8$ Hz, 1H), 5.02 (d, $J = 12.6$ Hz, 1H), 5.08 (d, $J = 12.6$ Hz, 1H), 7.14–7.61 (m, 8H), 7.79 (d, $J = 8.4$ Hz, 6H), 12.43 (s, 1H). IR (KBr) ν/cm^{-1}: 3303, 3029, 1717, 1603, 1524, 1233.

6.3. General procedure for the synthesis of compounds **3a–3g**

A solution of compound **2** (1.65 mmol) in 15 mL of methanol was hydrogenated over 5% Pd/C (145 mg) using an H_2 balloon. After 3 h, the reaction mixture was filtered and evaporated to give a white solid which was used without further purification.

4,6-Dimethoxy-2-chloro-1,3,5-triazine (CDMT) (1.82 mmol) and (*R*)-(+)-2-butylbutanedioic acid-4-*tert*-butyl monoester (1.65 mmol) were dissolved in dry methylene dichloride (10 mL). *N*-Methyl morpholine (1.98 mmol) was added at −5 to 0 °C. After 4 h, the white solid obtained above 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 filtrate was 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 (30:1).

6.3.1. N-[1-(*S*)-(Benzimidazol-2-yl)-2-methyl]propyl-(*R*)-2-tert-butoxycarbonylmethylhexanamide(**3a**)

White solid, yield: 90%; m.p. 70–72 °C; $[\alpha]_D^{25} = -85.5$ (*c* 1, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.65 (t, $J = 6.8$ Hz, 3H), 0.80 (d, $J = 6.7$ Hz, 3H), 0.96 (d, $J = 6.6$ Hz, 3H), 1.00–1.30 (m, 6H), 1.37 (s, 9H), 2.18–2.25 (m, 2H), 2.42 (dd, $J = 9$, 16 Hz, 1H), 2.71–2.76 (m, 1H), 4.87 (t, $J = 8.5$ Hz, 1H), 7.11–7.18 (m, 2H), 7.44 (d, $J = 6.8$ Hz, 1H), 7.55 (d, $J = 6.8$ Hz, 1H), 8.30 (d, $J = 8.8$ Hz, 1H), 12.19 (s, 1H). IR (KBr) ν/cm^{-1}: 3288, 3035, 1732, 1644, 1565, 1116. ESI-MS *m*/*z*: 402 $[M + H]^+$.

*6.3.2. N-[1-(S)-(5-Methylbenzimidazol-2-yl)-2-methyl] propyl-(R)-2-tert-butoxycarbonylmethylhexanamide (**3b**)*

White solid, yield: 91%; m.p. 193–194 °C; $[\alpha]_D^{25} = -104.5$ (*c* 1, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.65 (t, $J = 6.8$ Hz, 3H), 0.80 (d, $J = 6.7$ Hz, 3H), 0.96 (d, $J = 6.6$ Hz, 3H), 1.02–1.20 (m, 4H), 1.25–1.39 (m, 11H), 2.18–2.25 (m, 1H), 2.32–2.38 (m, 4H), 2.54 (dd, $J = 9.2$, 16 Hz, 1H), 2.73–2.78 (m, 1H), 4.86 (t, $J = 8.5$ Hz, 1H), 6.95 (d, $J = 8$ Hz, 1H), 7.24–7.39 (m, 2H), 8.31 (d, $J = 9$ Hz, 1H), 12.15 (s, 1H). IR (KBr) ν/cm^{-1}: 3269, 3035, 1733, 1638, 1549, 1155. ESI-MS *m/z*: 416 $[M + H]^+$.

*6.3.3. N-[1-(S)-(5-Methoxybenzimidazol-2-yl)-2-methyl] propyl-(R)-2-tert-butoxycarbonylmethylhexanamide (**3c**)*

White solid, yield: 90%; m.p. 68.5–70.5 °C; $[\alpha]_D^{25} = -80.5$ (*c* 1, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.67 (t, $J = 6.7$ Hz, 3H), 0.78 (d, $J = 6.7$ Hz, 3H), 0.93 (d, $J = 6.6$ Hz, 3H), 1.02–1.20 (m, 4H), 1.25–1.52 (m, 11H), 2.18–2.25 (m, 2H), 2.44 (dd, $J = 9$, 16 Hz, 1H), 2.69–2.73 (m, 1H), 3.73 (s, 3H), 4.82 (t, $J = 8.3$ Hz, 1H), 6.73–6.78 (m, 1H), 6.94 (d, $J = 1.7$ Hz, 1H), 7.41 (d, $J = 8.7$ Hz, 1H), 8.26 (d, $J = 9$ Hz, 1H), 12.00 (s, 1H). IR (KBr) ν/cm^{-1}: 3270, 3068, 1733, 1642, 1558, 1157. ESI-MS *m/z*: 390 $[M + H]^+$.

*6.3.4. N-[1-(S)-[5-(Morpholinocarbonyl) benzimidazol-2-yl]-2-methyl]propyl-(R)-2-tert-butoxycarbonylmethylhexanamide (**3d**)*

White solid, yield: 95%; m.p. 178–180 °C; $[\alpha]_D^{25} = -68.5$ (*c* 1, MeOH). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 0.74 (t, $J = 6.4$ Hz, 3H), 0.92 (d, $J = 6.3$ Hz, 3H), 1.05 (d, $J = 6.4$ Hz, 3H), 1.04–1.30 (m, 6H), 1.37 (s, 9H), 2.25–2.27 (m, 2H), 2.44 (dd, $J = 9$, 16 Hz, 1H), 2.69–2.73 (m, 1H), 3.45–3.65 (m, 8H), 4.96 (t, $J = 8.1$ Hz, 1H), 7.27–7.33 (m, 1H), 7.58–7.70 (m, 2H), 8.44 (d, $J = 8.7$ Hz, 1H), 12.52 (s, 1H). IR (KBr) ν/cm^{-1}: 3278, 3035, 1732, 1643, 1543, 1154. ESI-MS *m/z*: 515 $[M + H]^+$, 537 $[M + Na]^+$.

*6.3.5. N-[1-(S)-[[5-(4-Methoxyanilino)carbonyl] benzimidazol-2-yl]-2-methyl]propyl-(R)-2-tert-butoxycarbonylmethylhexanamide (**3e**)*

White solid, yield: 90%; m.p. 168–169.5 °C; $[\alpha]_D^{25} = -74.0$ (*c* 1, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.66 (t, $J = 6.8$ Hz, 3H), 0.81 (d, $J = 6.5$ Hz, 3H), 0.96 (d, $J = 6.7$ Hz, 3H), 1.04–1.21 (m, 4H), 1.29–1.37 (m, 11H), 2.21–2.27 (m, 2H), 2.45 (dd, $J = 9$, 16 Hz, 1H), 2.68–2.76 (m, 1H), 3.75 (s, 3H), 4.89 (t, $J = 8.4$ Hz, 1H), 6.92 (d, $J = 9$ Hz, 2H), 7.54–7.63 (m, 1H), 7.69 (d, $J = 9$ Hz, 2H), 7.76–7.81 (m, 1H), 8.19–8.33 (m, 2H), 10.05 (s, 1H), 12.52 (s, 1H). IR (KBr) ν/cm^{-1}: 3301, 3035, 1730, 1645, 1512, 1154. ESI-MS *m/z*: 551 $[M + H]^+$.

*6.3.6. N-[1-(S)-[5-(Cyclohexylaminocarbonyl) benzimidazol-2-yl]-2-methyl]propyl-(R)-2-tert-butoxycarbonylmethylhexanamide (**3f**)*

White solid, yield: 90%; m.p. 156–158 °C; $[\alpha]_D^{25} = -67.0$ (*c* 1, MeOH). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 0.63 (t, $J = 6.7$ Hz, 3H), 0.80 (d, $J = 6.6$ Hz, 3H), 0.96 (d, $J = 6.6$ Hz, 3H), 1.00–1.42 (m, 21H), 1.70–1.83 (m, 4H), 2.25–2.35 (m, 2H), 2.54 (dd, $J = 9$, 16 Hz, 1H), 2.71–2.76 (m, 1H), 3.73–3.78 (m, 1H), 4.87 (t, $J = 8.4$ Hz, 1H), 7.45–7.55 (m, 1H), 7.67 (d, $J = 8$ Hz, 1H), 7.92–8.13 (m, 2H), 8.31 (d, $J = 8$ Hz, 1H), 12.41 (s, 1H). IR (KBr) ν/cm^{-1}: 3335, 3271, 3035, 1732, 1645, 1547, 1152. ESI-MS *m/z*: 527 $[M + H]^+$.

*6.3.7. N-[1-(S)-[5-(Piperidinocarbonyl) benzimidazol-2-yl]-2-methyl]propyl-(R)-2-tert-butoxycarbonylmethylhexanamide (**3g**)*

White solid, yield: 85%; m.p. 70–72 °C; $[\alpha]_D^{25} = -72.0$ (*c* 1, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.63 (t, $J = 6.4$ Hz, 3H), 0.80 (d, $J = 6.6$ Hz, 3H), 0.96 (d, $J = 6.6$ Hz, 3H), 1.00–1.35 (m, 6H), 1.37 (s, 9H), 1.45–1.63 (m, 6H), 2.25–2.35 (m, 2H), 2.42 (dd, $J = 9$, 16 Hz, 1H), 2.71–2.76 (m, 1H), 3.35–3.46 (m, 4H), 4.85 (t, $J = 8.4$ Hz, 1H), 7.12–7.18 (m, 1H), 7.45–7.58 (m, 2H), 8.33 (d, $J = 8.4$ Hz, 1H), 12.41 (s, 1H). IR (KBr) ν/cm^{-1}: 3275, 3035, 1731, 1643, 1539, 1153. ESI-MS *m/z*: 513 $[M + H]^+$.

*6.4. General procedure for the synthesis of compounds **4a–4g***

A solution of compound **3** in formic acid (98%, 10 mL) was stirred at room temperature for 10 h. Removal of the solvent under vacuum gave a white solid. The white solid was added to CH_2Cl_2 (10 mL), followed by methanol (1.5 mL) and 4-dimethylaminopyridine (0.155 mmol). After cooling to 0 °C, DCC (1.63 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 (1:1).

*6.4.1. N-[1-(S)-(Benzimidazol-2-yl)-2-methyl]propyl-(R)-2-methoxycarbonylmethylhexanamide (**4a**)*

White solid, yield: 70%; m.p. 165–168 °C; $[\alpha]_D^{25} = -92.0$ (*c* 0.5, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.64 (t, $J = 6.8$ Hz, 3H), 0.80 (d, $J = 6.7$ Hz, 3H), 0.94 (d, $J = 6.7$ Hz, 3H), 1.00–1.42 (m, 6H), 2.19–2.28 (m, 1H), 2.36 (dd, $J = 5.2$, 16 Hz, 1H), 2.54 (dd, $J = 9.3$, 16 Hz, 1H), 2.72–2.80 (m, 1H), 3.55 (s, 3H), 4.86 (t, $J = 8.5$ Hz, 1H), 7.10–7.16 (m, 2H), 7.44 (dd, $J = 2.1$, 6.4 Hz, 1H), 7.55 (dd, $J = 1.7$, 6.8 Hz, 1H), 8.34 (d, $J = 9.2$ Hz, 1H), 12.20 (s, 1H). IR (KBr) ν/cm^{-1}: 3327, 3024, 1626, 1575. ESI-MS *m/z*: 360 $[M + H]^+$.

*6.4.2. N-[1-(S)-(5-Methylbenzimidazol-2-yl)-2-methyl] propyl-(R)-2-methoxycarbonylmethylhexanamide (**4b**)*

White solid, yield: 85%; m.p. 162–165 °C; $[\alpha]_D^{25} = -110.0$ (*c* 0.5, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.66 (t, $J = 6.8$ Hz, 3H), 0.78 (d, $J = 6.7$ Hz, 3H), 0.92 (d, $J = 6.7$ Hz, 3H), 1.02–1.39 (m, 6H), 2.18–2.24 (m, 1H),

2.32–2.39 (m, 1H), 2.54 (dd, $J = 9.3$, 16 Hz, 1H), 2.73–2.78 (m, 1H), 3.55 (s, 3H), 4.85 (t, $J = 8.5$ Hz, 1H), 6.95 (d, $J = 8$ Hz, 1H), 7.24–7.39 (m, 2H), 8.31 (d, $J = 9$ Hz, 1H), 12.05 (s, 1H). IR (KBr) ν/cm^{-1}: 3271, 3035, 1744, 1642, 1550, 1168. ESI-MS *m/z*: 374 $[M + H]^+$.

*6.4.3. N-[1-(S)-(5-Methoxybenzimidazol-2-yl)-2-methyl] propyl-(R)-2-methoxycarbonylmethylhexanamide (**4c**)*

White solid, yield: 70%; m.p. 151–153 °C; $[\alpha]_D^{25} = -94.0$ (*c* 0.5, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.66 (t, $J = 6.7$ Hz, 3H), 0.79 (d, $J = 6.7$ Hz, 3H), 0.93 (d, $J = 6.6$ Hz, 3H), 1.02–1.39 (m, 6H), 2.18–2.25 (m, 1H), 2.35 (dd, $J = 5.2$, 16 Hz, 1H), 2.54 (dd, $J = 9.3$, 16 Hz, 1H), 2.73–2.77 (m, 1H), 3.55 (s, 1H), 3.76 (s, 3H), 4.82 (t, $J = 8.5$ Hz, 1H), 6.74–6.76 (m, 1H), 6.94 (d, $J = 1.7$ Hz, 1H), 7.42 (d, $J = 8.5$ Hz, 1H), 8.29 (d, $J = 9$ Hz, 1H), 12.00 (s, 1H). IR (KBr) ν/cm^{-1}: 3325, 3272, 3068, 1740, 1642, 1541, 1158. ESI-MS *m/z*: 390 $[M + H]^+$.

*6.4.4. N-[1-(S)-[5-(Morpholinocarbonyl)benzimidazol-2-yl]-2-methyl]propyl-(R)-2-methoxycarbonylmethylhexanamide (**4d**)*

White solid, yield: 75%; m.p. 74–76 °C, $[\alpha]_D^{25} = -77.0$ (*c* 0.5, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.63 (t, $J = 6.4$ Hz, 3H), 0.82 (d, $J = 6.6$ Hz, 3H), 0.95 (d, $J = 6.7$ Hz, 3H), 1.03–1.40 (m, 6H), 2.20–2.31 (m, 1H), 2.36 (dd, $J = 9$, 16 Hz, 1H), 2.55–2.65 (m, 1H), 2.75–2.85 (m, 1H), 3.41–3.65 (m, 11H), 4.86 (t, $J = 8.4$ Hz, 1H), 7.17–7.23 (m, 1H), 7.48–7.59 (m, 2H), 8.38 (d, $J = 8.8$ Hz, 1H), 12.42 (s, 1H). IR (KBr) ν/cm^{-1}: 3275, 3035, 1738, 1644, 1541, 1115. ESI-MS *m/z*: 473 $[M + H]^+$.

*6.4.5. N-[1-(S)-[[5-(4-Methoxyanilino)carbonyl] benzimidazol-2-yl]-2-methyl]propyl-(R)-2-methoxycarbonylmethylhexanamide (**4e**)*

White solid, yield: 70%; m.p. 95–96 °C; $[\alpha]_D^{25} = -71.0$ (*c* 0.5, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.66 (t, $J = 6.8$ Hz, 3H), 0.81 (d, $J = 6.6$ Hz, 3H), 0.96 (d, $J = 6.7$ Hz, 3H), 1.01–1.21 (m, 4H), 1.29–1.42 (m, 2H), 2.24–2.28 (m, 1H), 2.36 (dd, $J = 5.2$, 16 Hz, 1H), 2.55 (dd, $J = 9.3$, 16 Hz, 1H), 2.75–2.79 (m, 1H), 3.56 (s, 3H), 3.75 (s, 3H), 4.90 (t, $J = 8.5$ Hz, 1H), 6.92 (d, $J = 9$ Hz, 2H), 7.54–7.63 (m, 1H), 7.70 (d, $J = 9$ Hz, 2H), 7.78–7.81 (m, 1H), 8.07–8.21 (m, 1H), 8.37 (d, $J = 8.8$ Hz), 10.05 (s, 1H), 12.46 (s, 1H). IR (KBr) ν/cm^{-1}: 3324, 3035, 1739, 1643, 1511, 1171. ESI-MS *m/z*: 509 $[M + H]^+$.

*6.4.6. N-[1-(S)-[5-(Cyclohexylaminocarbonyl) benzimidazol-2-yl]-2-methyl]propyl-(R)-2-methoxycarbonylmethylhexanamide (**4f**)*

White solid, yield: 70%; m.p. 92–94 °C; $[\alpha]_D^{25} = -76.0$ (*c* 0.5, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.65 (t, $J = 7.0$ Hz, 3H), 0.79 (d, $J = 6.6$ Hz, 3H), 0.95 (d, $J = 6.6$ Hz, 3H), 1.01–1.42 (m, 12H), 1.70–1.83 (m, 4H), 2.22–2.27 (m, 1H), 2.36 (dd, $J = 5.2$, 16 Hz, 1H), 2.54 (dd, $J = 9.3$, 16 Hz, 1H), 2.73–2.78 (m, 1H), 3.55 (s, 3H), 3.73–3.84 (m, 1H), 4.88 (t, $J = 8.4$ Hz, 1H), 7.48–7.54 (m, 1H), 7.68 (d, $J = 8$ Hz, 1H), 7.92–8.10 (m, 2H), 8.35 (d, $J = 8.6$ Hz, 1H), 12.41 (s, 1H). IR (KBr) ν/cm^{-1}: 3290, 3035, 1741, 1644, 1542, 1170. ESI-MS *m/z*: 485 $[M + H]^+$.

*6.4.7. N-[1-(S)-[5-(Piperidinocarbonyl)benzimidazol-2-yl] -2-methyl]propyl-(R)-2-methoxycarbonylmethylhexanamide (**4g**)*

White solid, yield: 65%; m.p. 75–77 °C; $[\alpha]_D^{25} = -74.0$ (*c* 0.5, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.62 (t, $J = 6.9$ Hz, 3H), 0.81 (d, $J = 6.6$ Hz, 3H), 0.96 (d, $J = 6.6$ Hz, 3H), 1.03–1.38 (m, 6H), 1.45–1.63 (m, 6H), 2.23–2.28 (m, 1H), 2.35 (dd, $J = 5.2$, 16 Hz, 1H), 2.55 (dd, $J = 9.3$, 16 Hz, 1H), 2.71–2.76 (m, 1H), 3.33–3.60 (m, 7H), 4.87 (t, $J = 8.5$ Hz, 1H), 7.16 (m, 1H), 7.50 (br s, 2H), 8.34 (d, $J = 8.8$ Hz, 1H), 12.38 (s, 1H). IR (KBr) ν/cm^{-1}: 3326, 3136, 3035, 1734, 1625, 1573, 1176. ESI-MS *m/z*: 471 $[M + H]^+$.

*6.5. General procedure for the synthesis of compounds **1a–1g***

To a methanol/tetrahydrofuran (6 mL, 1:1) solution of compound **4** (1 mmol) was added 50% aqueous hydroxylamine (10 mmol), followed by NaCN (50 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).

*6.5.1. (R)-2-Butyl-N^4-hydroxy-N^1-[1-(S)-(benzimidazol-2-yl)-2-methyl]propylsuccinamide (**1a**)*

White solid, yield: 76%; m.p. 132–134 °C, $[\alpha]_D^{25} = -86.7$ (*c* 0.3, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.65 (t, $J = 6.9$ Hz, 3H), 0.80 (d, $J = 6.7$ Hz, 3H), 0.94 (d, $J = 6.7$ Hz, 3H), 1.01–1.42 (m, 6H), 2.06 (dd, $J = 7.4$, 14.5 Hz, 1H), 2.21–2.30 (m, 2H), 2.70–2.75 (m, 1H), 4.87 (t, $J = 8.4$ Hz, 1H), 7.12–7.14 (m, 2H), 7.50 (br s, 2H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 13.71, 18.78, 19.31, 22.10, 28.65, 31.80, 31.94, 35.15, 41.68, 53.07, 111.10, 121.61, 134.15, 154.83, 167.64, 173.93. IR (KBr) ν/cm^{-1}: 3243, 3057, 2924, 1649, 1539. ESI-MS *m/z* (%): 384 ($[M + Na]^+$, 20), 361 ($[M + H]^+$, 100%), 328 (28). Anal. Calcd for $C_{19}H_{28}N_4O_3$: C, 63.30; H, 7.83; N, 15.54. Found: C, 63.41; H, 7.79; N, 15.37.

*6.5.2. (R)-2-Butyl-N^4-hydroxy-N^1-[1-(S)-(5-methyl benzimidazol-2-yl)-2-methyl]propylsuccinamide (**1b**)*

White solid, yield: 65%; m.p. 134–135 °C; $[\alpha]_D^{20} = -101.7$ (*c* 0.3, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.66 (t, $J = 6.8$ Hz, 3H), 0.80 (d, $J = 6.7$ Hz, 3H), 0.93 (d, $J = 6.7$ Hz, 3H), 1.05–1.40 (m, 6H), 2.06 (dd, $J = 7.2$, 14.4 Hz, 1H), 2.19–2.27 (m, 2H), 2.39 (s, 3H), 2.72–2.75 (m, 1H), 4.85 (t, $J = 8.4$ Hz, 1H), 6.95 (d, $J = 7.8$ Hz, 1H), 7.24–7.38 (m, 2H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 13.83, 18.89, 19.51, 21.32, 22.20, 28.77, 31.66, 32.03, 35.31, 41.84, 53.15, 111.35, 118.25, 123.15, 131.07, 134.05, 141.23, 154.53, 167.79, 174.00. IR (KBr) ν/cm^{-1}: 3229,

3051, 2925, 1648, 1538. ESI-MS *m/z* (%): 375 ($[M+H]^+$, 100%), 342 (40). Anal. Calcd for $C_{20}H_{30}N_4O_3$: C, 64.15; H, 8.07; N, 14.96. Found: C, 64.01; H, 7.98; N, 15.11.

6.5.3. (R)-2-Butyl-N^4-hydroxy-N^1-[1-(S)-(5-methoxy benzimidazol-2-yl)-2-methyl]propylsuccinamide (**1c**)

White solid, yield: 75%; m.p. 123–125 °C; $[\alpha]_D^{25} = -85.0$ (*c* 0.3, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.66 (t, $J = 6.9$ Hz, 3H), 0.79 (d, $J = 6.7$ Hz, 3H), 0.93 (d, $J = 6.6$ Hz, 3H), 1.04–1.38 (m, 6H), 2.05 (dd, $J = 7.4$, 14.5 Hz, 1H), 2.19–2.26 (m, 2H), 2.71–2.74 (m, 1H), 3.76 (s, 3H), 4.83 (t, $J = 8.4$ Hz, 1H), 6.77 (s, 1H), 6.94 (br s, 1H), 7.40 (br s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 13.67, 18.77, 19.36, 22.06, 28.67, 31.86, 31.94, 35.19, 41.79, 53.10, 55.40, 110.70, 126.37, 126.56, 127.97, 142.44, 154.40, 155.32, 167.77, 173.95. IR (KBr) ν/cm^{-1}: 3242, 3068, 2925, 1646, 1540. ESI-MS *m/z* (%): 391 ($[M+H]^+$, 100%), 358 (2). Anal. Calcd for $C_{20}H_{30}N_4O_4$: C, 61.52; H, 7.74; N, 14.35. Found: C, 61.65; H, 7.79; N, 14.24.

6.5.4. (R)-2-Butyl-N^4-hydroxy-N^1-[1-(S)-[5-(morpholinocarbonyl)benzimidazol-2-yl]-2-methyl]propylsuccinamide (**1d**)

White solid, yield: 80%; m.p. 142–144 °C; $[\alpha]_D^{20} = -70.0$ (*c* 0.3, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.63 (t, $J = 6.5$ Hz, 3H), 0.81 (d, $J = 6.6$ Hz, 3H), 0.95 (d, $J = 6.6$ Hz, 3H), 1.00–1.40 (m, 6H), 2.05 (dd, $J = 7.4$, 14 Hz, 1H), 2.20–2.31 (m, 2H), 2.72–2.75 (m, 1H), 3.51–3.60 (m, 8H), 4.86 (t, $J = 8.3$ Hz, 1H), 7.20 (d, $J = 7.8$ Hz, 1H), 7.55 (br s, 2H), 8.34 (d, $J = 8.7$ Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 13 .75, 18.86, 19.43, 22.11, 28.66, 31.60, 31.65, 35.11, 41.60, 53.18, 62.85, 66.12, 110.11, 121.27, 126.38, 126.59, 128, 142.37, 156.70, 167.63, 169.92, 174.04. IR (KBr) ν/cm^{-1}: 3243, 3035, 2920, 1646, 1540. ESI-MS *m/z* (%): 474 ($[M+H]^+$, 100%), 441 (58), 413(12). Anal. Calcd for $C_{24}H_{35}N_5O_5$: C, 60.87; H, 7.45; N, 14.79. Found: C, 60.97; H, 7.56; N, 14.57.

6.5.5. (R)-2-Butyl-N^4-hydroxy-N^1-[1-(S)-[[5-(4-methoxyanilino)carbonyl]benzimidazol-2-yl]-2-methyl]propylsuccinamide (**1e**)

White solid, yield: 70%; m.p. 201–203 °C; $[\alpha]_D^{20} = -78.3$ (*c* 0.3, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.65 (t, $J = 6.4$ Hz, 3H), 0.82 (d, $J = 6.0$ Hz, 3H), 0.96 (d, $J = 6.5$ Hz, 3H), 1.04–1.37 (m, 6H), 2.06 (dd, $J = 6.8$, 14.4 Hz, 1H), 2.21–2.41 (m, 2H), 2.73–2.76 (m, 1H), 3.75 (s, 3H), 4.89 (t, $J = 8.2$ Hz, 1H), 6.93 (d, $J = 8.8$ Hz, 2H), 7.54 (d, $J = 8$ Hz, 1H), 7.70 (d, $J = 8$ Hz, 2H), 7.78–7.80 (m, 1H), 8.23 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 13.74, 18.80, 19.38, 22.06, 28.63, 31.52, 31.75, 35.10, 41.68, 53.18, 55.15, 110.08, 113.66, 117.80, 120.80, 121.86, 127.86, 132.56, 135.78, 142.80, 155.33, 157.15, 165.60, 167.61, 174.04. IR (KBr) ν/cm^{-1}: 3299, 3035, 2918, 1641, 1621, 1530. ESI-MS *m/z* (%): 510 ($[M+H]^+$, 100%), 477 (39), 449 (1). Anal. Calcd for $C_{27}H_{35}N_5O_5$: C, 63.64; H, 6.92; N, 13.74. Found: C, 63.75; H, 7.02; N, 13.51.

6.5.6. (R)-2-Butyl-N^4-hydroxy-N^1-[1-(S)-[[5-(cyclohexylamino)carbonyl]benzimidazol-2-yl]-2-methyl]propylsuccinamide (**1f**)

White solid, yield: 73%; m.p. 220–222 °C; $[\alpha]_D^{20} = -80.0$ (*c* 0.3, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.65 (t, $J = 6.6$ Hz, 3H), 0.80 (d, $J = 6.5$ Hz, 3H), 0.95 (d, $J = 6.6$ Hz, 3H), 1.03–1.34 (m, 12H), 1.74–1.89 (m, 4H), 2.05 (dd, $J = 7.3$, 14.6 Hz, 1H), 2.22–2.32 (m, 2H), 2.72–2.75 (m, 1H), 3.73–3.80 (m, 1H), 4.87 (t, $J = 8.2$ Hz, 1H), 7.47–7.55 (m, 1H), 7.68 (s, 1H), 7.90–8.12 (m, 2H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 137.72, 18.77, 19.36, 22.04, 24.97, 25.28, 28.61, 31.51, 31.65, 32.49, 35.11, 41.70, 48.31, 53.14, 110.09, 118.08, 121.15, 128.83, 135.30, 142.25, 156.50, 165.87, 167.63, 174.00. IR (KBr) ν/cm^{-1}: 3336, 3280, 3035, 2920, 1644, 1617, 1543. ESI-MS *m/z* (%): 486 ($[M+H]^+$, 100%), 453 (32). Anal. Calcd for $C_{26}H_{39}N_5O_4$: C, 64.31; H, 8.09; N, 14.42. Found: C, 64.22; H, 7.85; N, 14.66.

6.5.7. (R)-2-Butyl-N^4-hydroxy-N^1-[1-(S)-[(5-piperidinocarbonyl)benzimidazol-2-yl]-2-methyl]propylsuccinamide (**1g**)

White solid, yield: 77%; m.p. 138–140 °C; $[\alpha]_D^{20} = -78.3$ (*c* 0.3, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.62 (t, $J = 6.7$ Hz, 3H), 0.81 (d, $J = 6.6$ Hz, 3H), 0.96 (d, $J = 6.6$ Hz, 3H), 1.03–1.38 (m, 6H), 1.50–1.61 (m, 6H), 2.05 (dd, $J = 7.4$, 14.5 Hz, 1H), 2.19–2.32 (m, 2H), 2.70–2.77 (m, 1H), 3.33–3.60 (m, 4H), 4.87 (t, $J = 8.4$ Hz, 1H), 7.15 (br s, 1H), 7.46–7.56 (m, 2H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 13.66, 18.80, 19.37, 22.06, 24.06, 25.63, 28.63, 31.58, 31.78, 35.10, 41.69, 53.22, 62.87, 112.25, 126.36, 126.56, 127.96, 129.08, 142.41, 156.39, 167.72, 169.71, 174.07. IR (KBr) ν/cm^{-1}: 3201, 3035, 2921, 1646, 1607, 1540. ESI-MS *m/z* (%): 472 ($[M+H]^+$, 100%), 439 (71), 411 (1). Anal. Calcd for $C_{25}H_{37}N_5O_4$: C, 63.67; H, 7.91; N, 14.85. Found: C, 63.58; H, 7.79; N, 14.98.

6.6. In vitro antibacterial activity evaluation

The newly synthesized compounds were screened for their antibacterial activity against *S. aureus* (CMCC26112), *K. pneumoniae* (CMCC46117), and *S. lutea* (CMCC28001) using broth microdilution method [16,17]. Each compound was tested over a range of doubling dilution concentrations. The MIC of a compound was determined according to the lowest concentration that prevented visible growth of bacteria after incubation at 37 °C for 24 h. Muller–Hinton broth was used as the test medium. 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.

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