Synthesis and antimicrobial activities of novel peptide deformylase inhibitors

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Abstract
A new series of N-formylhydroxylamine compounds were designed, optimized with the AutoDock 4.0.1 to investigate the interactions between the target compounds and the amino acid residues of the *Escherichia coli* PDF•Ni enzyme, and then synthesized through multi-step sequence starting from diethyl malonate. The structures of the compounds were characterized on the basis of spectral (FT-IR, 1H NMR and mass) analysis. All the synthesized compounds have been screened for their antimicrobial activities. It was found that the compounds **11c**, **11d**, **11f** and **11g** exhibited potent inhibitory activity against *S. aureus in vitro*.

Keywords: Peptide deformylase, inhibitors, N-formylhydroxylamine compounds, antibacterial activity

Introduction

In recent years, with the changing of environment, bacterial infections emerged rapidly and have become a serious threat to respiratory and skin infections.[1-2] There is, therefore, an urgent need to identify new antibiotics to combat infectious diseases. One of the new targets receiving widespread interest from both academic and industrial researcher groups is Peptide deformylase

(PDF),[3-5] which is an iron containing metalloenzyme responsible for the removal of the *N*-formyl group from the terminal methionine residue following protein synthesis in bacteria,[6] and this enzyme is a high priority target for antibiotic design.[7] There are several kinds of compounds which have inhibitory activity against PDF as reported.[8-9] The *N*-formyl hydroxylamine BB-3497 is an effective inhibitor (IC_{50} = 7nM) of the *Escherichia coli* PDF•Ni enzyme, exhibiting potent antibacterial activity both *in vitro* and *in vivo.*[10]

In order to reduce the peptide structural features to find more active compounds, we referred to the structure of the BB-3497, which was reported in the literature,[11] and designed a new series of *N*-formylhydroxylamine derivatives. Our intention is to synthesize new active compounds and detect their antibacterial activities by introducing one benzimidazole ring[12-13] to serve as a hydrogen bond donor or accepter. At the meantime, in order to investigate the interactions between our designed compounds and the amino acid residues of the *Escherichia coli* PDF•Ni enzyme, a molecular docking study was also performed. After the optimization of the designed compounds, they were synthesized with our own designed multi-step reaction route and their antibiotic activities were tested *in vitro* also.

Results and Discussion

Molecular docking study

The molecular docking study was performed using the AutoDock 4.0.1 software.[14] The crystallographic structure of *Escherichia coli* PDF•Ni enzyme which is retrieved from the RSCB Protein Data Bank (PDB code 1G2A) serves as docking receptor,[15] and all the synthesized compounds are selected as ligand molecules. The 50 docking runs for each ligand were clustered on the basis of root mean square deviation (RMSD = 1.0 Å) between the Cartesian coordinates of the ligand atoms and were ranked according to the binding free energy. The structure with the lowest binding free energy and the most cluster members was chosen for the optimum docking conformation.

Docking results show that all the designed molecules have similar orientations in the binding pocket of PDF enzyme, except that the terminal substituents on the benzimidazole ring have relatively large conformational differences because of their diversity on atomic composition and chemical property. The functional group HCO-NOH in all molecules are chelated to Ni^{2+} ion and can effectively form hydrogen bonds with Gln50, Leu91 and Glu133, which is very similar to inhibitor BB-3497.[16] The binding modes of BB-3497 and the synthesized compound **11c** bound to active site of PDF•Ni enzyme are shown in Figure 1. It should be noted that on the benzimidazole portion of **11c**, the N^3 atom is hydrogen bonded to the amine hydrogen of Gly89 and the bonding length is 1.96 Å, and another hydrogen bond which located at the O atom of methoxyl and residue Arg97 also forms, with a bond length of 1.84 Å. These strong hydrogen-bonding interactions are concomitant with the introduction of the benzimidazole ring,

which means that this portion can increase the binding affinity between the target molecule and the *Escherichia coli* PDF•Ni enzyme.

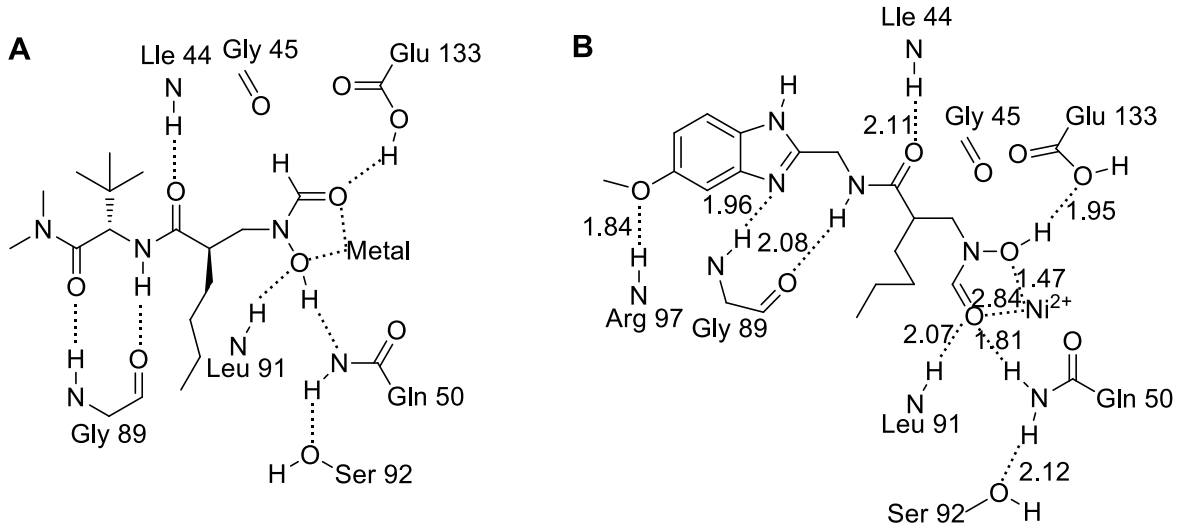
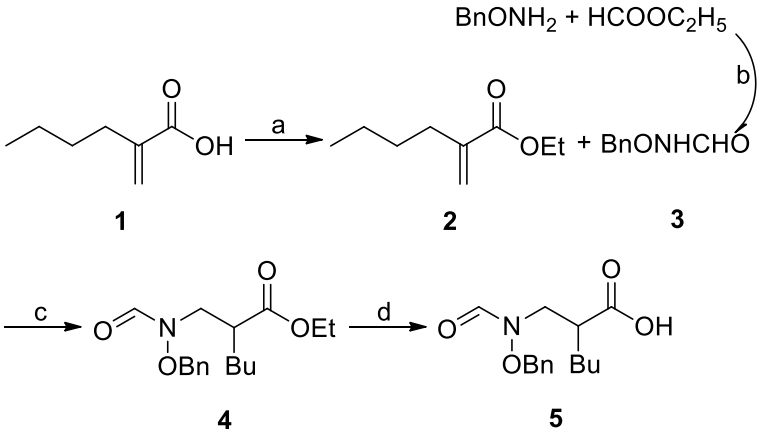


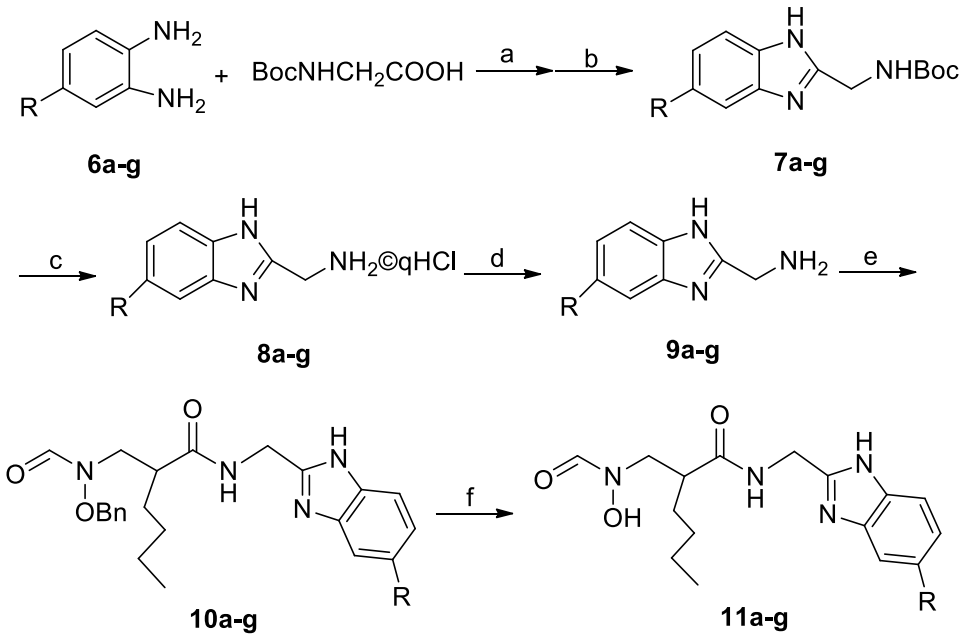
Figure 1. The binding conformation of inhibitor BB-3497[16] and **11c** with *E. coli* PDF, shown in part A and B respectively.

Chemistry

The target compounds were prepared using the reaction sequence as shown in Scheme 1, 2. The chemical structures of the synthesized compounds were confirmed by means of their IR, ^{1}H NMR and mass spectral analysis.



Scheme 1. Syntheses of compounds **1-5.** Reagents and conditions: (a) EtOH, 98% H_2SO_4, reflux; (b) KOH, MeOH, rt; (c) DBU, acetonitrile, N_2, reflux; (d) LiOH•H_2O, MeOH/H_2O, rt.



a: R=H; b: R=CH_3; c: R=OCH_3; d: R=OC_2H_5; e: R=CN; f: R=$COOCH_3$; g: R=Cl

Scheme 2. Syntheses of compounds **11a-g.** Reagents and conditions: (a) DCC, THF, rt; (b) HOAc, 72 °C; (c) HCl, MeOH, rt; (d) Et_3N, CH_2Cl_2; (e) **5**, CDMT, NMM, CH_2Cl_2, rt; (f) H_2, 10%Pd/C, EtOH, rt.

Antibacterial activity

All the synthesized compounds were evaluated for their antimicrobial activities. The results are summarized in Table 1. All the target compounds exhibit weak inhibitory activity against *K. pneumonia*. Compounds **11a**, **11b** and **11e** show weak to moderate antibacterial effect against *S. aureus*, while compounds **11c**, **11d**, **11f** and **11g** show high activity comparable to that of the positive drug. The presence of electron-withdrawing groups on the benzene ring in generally increased the antimicrobial activity against *S. aureus* compared to compounds with electron-donating groups. Based upon the results, it is necessary to optimize the target molecule by substituting a series of electron-withdrawing groups on the benzene ring. Taken together, these results confirmed the mode of action of the target compounds and directed our design in the future.

Table 1. The antibacterial activity of the target compounds (MIC)

Sr. No.	R	Molecular Formula	Drug Units	*S. aureus*	*K. pneumoniae*
11a	H	$C_{16}H_{22}N_4O_3$	μg/ml	16	>32
11b	5-CH_3	$C_{17}H_{24}N_4O_3$	μg/ml	32	>32
11c	5-OCH_3	$C_{17}H_{24}N_4O_4$	μg/ml	<1	>32
11d	5-OC_2H_5	$C_{18}H_{26}N_4O_4$	μg/ml	<1	>32
11e	5-CN	$C_{17}H_{21}N_5O_3$	μg/ml	32	>32
11f	5-COOC	$C_{18}H_{24}N_4O_5$	μg/ml	<1	>32
11g	H_3	$C_{16}H_{21}ClN_4O_3$	μg/ml	<1	>32
Linezolid	5-Cl		μg/ml	1	1

Experimental Section

General. All solvents used were of analytical grade. Melting points were determined in open capillary tubes and were uncorrected. The ^{1}H NMR spectra were recorded on a Bruker Avance DPX300 spectrometers with $CDCl_3$ as the solvent and TMS as the internal standard. The IR spectra were measured on a Bruker Vector FTIR spectrophotometer with KBr pellets or film. The mass spectra were obtained with an Agilent 6510 Q-TOF spectrometer.

Synthesis of *N*-(benzyloxy)formamide (3). Sodium hydroxide (5.00 g, 125.0 mmol) and ethyl formate (23.19 g, 313.5 mmol) were added to a stirred mixture of *O*-benzylhydroxylamine (10.00 g, 62.7 mmol) in anhydrous methanol (120 ml), and the resulting mixture was stirred at room temperature for 16 h. The reaction mixture was concentrated to remove methanol and then was extracted with dichloromethane (3 × 40 ml), and the combined extracts were washed with water, 2 mol/L HCl and brine successively and then dried over anhydrous $MgSO_4$ followed by evaporation. The crude product was distilled under the reduced pressure to result in compound **3** in the form of a colorless oil. Yield: 70%.

Synthesis of 2-methylenehexanoic acid (1). Compound **1** was prepared according to the described method.[11]

Synthesis of ethyl 2-methylenehexanoate (2). Compound **1** (10.00 g, 78.1 mmol) and 98% H_2SO_4 (1 ml) were added to anhydrous ethanol (50 ml), and then refluxed for 6 h. The solvent was evaporated and the residue was basified with $NaHCO_3$ solution. The solution was then extracted with ethyl acetate (3 × 30 ml) and washed with brine, and the combined extracts were dried over anhydrous $MgSO_4$ and evaporated to afford a colorless oil. Yield: 80%.

Ethyl 2-{[*N*-(benzyloxy)formamido]methyl}hexanoate (4). Compound **3** (10.00 g, 66.2 mmol), compound **2** (15.51 g, 99.3 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)(10.08 g,

66.2 mmol) were stirred in 50 ml of acetonitrile under N_2,[17-18] and then was refluxed for 8 h. The solvent was evaporated and then the residue was extracted with ethyl acetate (3 × 50 ml), and the combined extracts were washed with 2 mol/L HCl and brine successively and then dried over anhydrous $MgSO_4$ and evaporated to afford a residue, which was purified by a silica-gel column chromatography (petroleum ether / ethyl acetate / dichloromethane, 6:1:1 by volume) to yield compound **4** in the form of a colorless oil. Yield: 85%. ^{1}H NMR (300MHz, $CDCl_3$, δ ppm): 0.86-0.90 (t, 3H , *J* = 6.9 Hz, CH_3), 1.20-1.24 (t, 3H, CH_3), 1.26-1.60 (m, 6H, CH_2), 2.70 (m, 1H, CH), 3.24-3.81 (m, 2H, CH_2), 4.02-4.19 (m, 2H, CH_2), 4.70-4.97 (m, 2H, CH_2), 7.38 (m, 5H, Ar-H), 7.94 (brs, 0.32H, CHO), 8.15 (brs, 0.68H, CHO).

Synthesis of 2-{[*N*-(benzyloxy)formamido]methyl}hexanoic acid (5). Compound **4** (5.01 g, 16.3 mmol) and $LiOH·H_2O$ (0.79 g, 17.9 mmol) were stirred in 75 ml of 2:1 (volume ratio) methanol/water at room temperature for 24 h. The reaction mixture was concentrated to remove methanol, and the residue was diluted with water (10 ml). The mixture was then extracted with ethyl acetate (3 × 20 ml), and the aqueous phase was acidified to pH < 3 with concentrated HCl. The acidic aqueous solution was then extracted with ethyl acetate (3 × 30 ml), and the extracts were dried over anhydrous $MgSO_4$ and evaporated to afford a residue, which was purified by a silica-gel column chromatography (petroleum ether / ethyl acetate / dichloromethane, 6:1:1 by volume, containing some formyl acid) to give compound **5** in the form of a light yellow oil. Yield: 75%. ^{1}H NMR (300MHz, $CDCl_3$, δ ppm): 0.85-0.89 (t, 3H, *J* = 6.6 Hz, CH_3), 1.30-1.61 (m, 6H, CH_2), 2.74 (m, 1H, CH), 3.24-3.85 (m, 2H, CH_2), 4.75-4.97 (m, 2H, CH_2), 7.35-7.41 (m, 5H, Ar-H), 7.99 (brs, 0.35H, CHO), 8.16 (brs, 0.65H, CHO), 10.91 (brs, 1H, COOH).

Synthesis of *tert*-butyl (1*H*-benzo[*d*]imidazol-2-yl)methylcarbamate (7a). Typical procedure Benzene-1, 2-diamine (1.05 g, 9.72 mmol) and *N*-(*tert*-butoxycarbonyl)glycine (1.71 g, 9.72 mmol) were dissolved in 30 ml of THF and cooled to 0 °C. Into the above solution was added *N*, *N'*-dicyclohexylcarbodiimide (2.41 g, 11.7 mmol) in batches and the mixture was stirred at 0 °C for half an hour and then at room temperature overnight. The reaction mixture was filtrated and evaporated to afford a brown oil, which was purified by a silica-gel column chromatography (dichloromethane / methanol, 25:1 by volume) to get a yellow solid (2.24 g). The solid was dissolved in 20 ml of acetic acid and the solution was stirred at 72 °C for 8 h. The acetic acid was removed under reduced pressure and the crude compound was purified by a silica-gel column chromatography (dichloromethane / methanol, 25:1 by volume) to afford a white solid. Yield: 82%. m.p. 181-183 °C. IR (KBr) cm^{-1}: 3343 (-NH-), 3058, 2980, 2940 (CH, aliphatic), 1686 (>C=O), 1529 (-C=C-), 736 (-Ar-); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.45 (s, 9H, CH_3), 4.53-4.55 (d, 2H, *J* = 6.3 Hz, CH_2), 6.12 (brs, 1H, NH), 7.22-7.25 (m, 2H, Ar-H), 7.54-7.60 (m, 2H, Ar-H); HRMS (ESI): $[M + H]^+$ calcd *m/z* 248.1400, found 248.1397.

***tert*-Butyl (5-methyl-1*H*-benzo[*d*]imidazol-2-yl)methylcarbamate (7b).** Compound **7b** was obtained as a white solid. Yield: 81%. m.p. 171-172 °C. IR (KBr) cm^{-1}: 3336 (-NH-), 2988, 2911 (CH, aliphatic), 1679 (>C=O), 1521 (-C=C-); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.46 (s, 9H, CH_3), 2.46 (s, 3H, CH_3), 4.48-4.50 (d, 2H, *J* = 6.3 Hz, CH_2), 5.64 (brs, 1H, NH), 7.09 (m, H,

Ar-H), 7.34 (m, H, Ar-H), 7.45 (m, H, Ar-H); HRMS (ESI): $[M + H]^+$ calcd *m/z* 262.1556, found 262.1546.

_N_-[(1_H_-Benzo[_d_]imidazol-2-yl)methyl]-2-{[_N_-(benzyloxy)formamido]methyl}hexanamide (10a). Typical procedure

Compound **7a** (1.72 g, 4.21 mmol) was added to a stirred mixture of 2 mol/L HCl in methanol (20 ml) at room temperature for 8 h. The reaction mixture was concentrated, and then was washed with ether to yield a white solid **8a** (1.16 g). To a stirred solution of 4,6-dimethoxy-2-chloro-1,3,5-triazine (CDMT) (1.00 g, 5.72 mmol) and 2-[(*N*-hydroxyformamido)methyl]hexanoic acid (1.45 g, 5.20 mmol) in dry CH_2Cl_2 (30 ml). *N*-methylmorpholine (NMM, 683 mg, 6.76 mmol) was added dropwise at 0 °C and was continuously stirred at 0 °C for 4 h. To the crude solution of (1*H*-benzo[*d*]imidazol-2-yl) methanamine hydrochloride (764 mg, 5.20 mmol) was added triethylamine (526 mg, 5.20 mmol) in CH_2Cl_2 (5 ml) at 0 °C. Stirring was continued for 2 h and then the mixture was left overnight at room temperature. The solvent was evaporated and the residue was diluted with CH_2Cl_2. The suspension was washed successively with H_2O, 0.5 mol/L HCl, saturated $NaHCO_3$, and brine. The organic phase was dried and evaporated to give a white solid. The crude product was purified by a silica-gel column chromatography (dichloromethane / methanol, 30:1 by volume) to get a white solid. Yield: 89%. m.p. 114-117 °C. IR (KBr) cm^{-1}: 3298 (-NH-), 2958, 2930 (CH, aliphatic), 1679 (>C=O), 1554 (-C=C-), 1384 (-CHO), 748 (-Ar-); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 0.80-0.84 (t, 3H, *J* = 6.9 Hz, CH_3), 1.25-1.63 (brm, 6H, CH_2), 2.64 (m, 1H, CH), 3.20-3.90 (brm, 2H, CH_2), 4.36-4.42 (dd, 1H, *J* = 4.5, 4.5 Hz, CH_2), 4.71 (brm, 3H, CH_2), 7.21-7.24 (m, 2H, Ar-H), 7.26-7.30 (m, 5H, Ar-H), 7.51 (m, 2H, Ar-H), 7.96 (brs, 1H, CHO); HRMS (ESI): $[M + H]^+$ calcd *m/z* 409.2240, found 409.2244.

2-((_N_-(Benzyloxy)formamido)methyl)-_N_-((5-methyl-1_H_-benzo[_d_]imidazol-2-yl)methyl)hexanamide(10b). Compound **10b** was obtained as a white solid. Yield: 82%. m.p. 60-62 °C. IR (KBr) m^{-1}: 3277 (-NH-), 2948, 2930 (CH, aliphatic), 1677 (>C=O), 1546 (-C=C-),1382 (-CHO); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 0.79-0.84 (t, 3H, *J* = 7.2 Hz, CH_3), 1.24-1.63 (m, 6H, CH_2), 2.44 (s, 3H, CH_3), 2.66 (m, 1H, CH), 3.17-3.88 (m, 2H, CH_2), 4.31-4.37 (dd, 1H, *J* = 4.8, 4.8 Hz, CH_2), 4.61-4.69 (m, 3H, CH_2), 7.02-7.05 (d, 1H, *J* = 8.1 Hz, Ar-H), 7.30 (m, 5H, Ar-H), 7.39 (m, 1H, Ar-H), 7.75 (m, 1H, Ar-H), 7.95 (brs, 1H, CHO); HRMS (ESI): $[M + H]^+$ calcd *m/z* 423.2397, found 423.2390.

2-[(_N_-Hydroxyformamido)methyl]-_N_-[(5-substituted-1_H_-benzo[_d_]imidazol-2-yl)methyl]hexanamide (11a-g). General procedure

10% Pd/C (0.20 g, containing 65.8% water) was added to a stirred mixture of compound **10a** (0.80 g, 1.96 mmol) and anhydrous ethanol (20 ml) under H_2 at room temperature for 3 h. The filtrate was evaporated to yield a white solid, which was purified by a silica-gel column chromatography (dichloromethane / methanol, 20:1 by volume).

***N*-[(1*H*-Benzo[*d*]imidazol-2-yl)methyl]-2-[(*N*-hydroxyformamido)methyl]hexanamide (11a).** Compound **11a** was obtained as a white solid. Yield: 92%. m.p. 85-86 °C. IR (KBr) cm^{-1}: 3279 (-NH-), 2956, 2930 (CH, aliphatic), 1655 (>C=O), 1540 (-C=C-), 1384 (-CHO), 743 (-Ar-); ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 0.82-0.83 (t, 3H, CH_3), 1.23-1.44 (m, 6H, CH_2), 2.64-2.73 (m, 1H, CH), 3.39-3.80 (m, 2H, CH_2), 4.29-4.76 (m, 2H, CH_2), 7.14-7.19 (m, 2H, Ar-H), 7.51 (m, 2H, Ar-H), 7.87 (brs, 0.49H, CHO), 8.30 (brs, 0.51H, CHO); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 14.34, 22.61, 22.67, 29.14, 29.28, 29.55, 36.97, 37.27, 44.10, 44.39, 49.76, 52.13, 122.11, 152.62, 153.23, 157.97, 162.46, 173.62, 173.84; HRMS (ESI): $[M + H]^+$ calcd *m/z* 319.1771, found 319.1761.

2-[(*N*-Hydroxyformamido)methyl]-*N*-[(5-methyl-1*H*-benzo[*d*]imidazol-2-yl)methyl]hexanamide (11b). Compound **11b** was obtained as a white solid. Yield: 91%. m.p. 93-95 °C. IR (KBr) cm^{-1}: 3271 (-NH-), 2957, 2929 (CH, aliphatic), 1656 (>C=O), 1543 (-C=C-), 1384 (-CHO); ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 0.82-0.84 (t, 3H, CH_3), 1.23-1.48 (m, 6H, CH_2), 2.39 (s, 3H, CH_3), 2.61-2.72 (m, 1H, CH), 3.38-3.80 (m, 2H, CH_2), 4.24-4.76 (m, 2H, CH_2), 6.96-7.01 (t, 1H, *J* = 8.1 Hz, Ar-H), 7.29 (s, 1H, Ar-H), 7.36-7.39 (d, 1H, *J* = 8.1 Hz, Ar-H), 7.87 (brs, 0.49H, CHO), 8.30 (brs, 0.51H, CHO); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 14.33, 21.69, 22.59, 22.65, 29.15, 29.29, 29.53, 36.90, 37.23, 44.21, 44.47, 49.88, 52.18, 123.47, 123.75, 152.25, 152.89, 157.97, 162.44, 173.59, 173.82; HRMS (ESI): $[M + H]^+$ calcd *m/z* 333.1927, found 333.1931.

2-[(*N*-hydroxyformamido)methyl]-*N*-[(5-methoxy-1*H*-benzo[*d*]imidazol-2-yl)methyl]hexanamide (11c). Compound **11c** was obtained as a white solid. Yield: 89%. m.p. 92-94 °C. IR (KBr) cm^{-1}: 3282 (-NH-), 2956, 2930 (CH, aliphatic), 1655 (>C=O), 1541 (-C=C-), 1384 (-CHO), 1031 (-C-O-C-); ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 0.84 (t, 3H, CH_3), 1.23-1.47 (m, 6H, CH_2), 2.58-2.72 (m, 1H, CH), 3.39-3.77 (m, 4H, CH_2), 4.24-4.73 (m, 2H, CH_2), 6.77-6.81 (t, 1H, *J* = 6.3 Hz, Ar-H), 6.99 (s, 1H, Ar-H), 7.37-7.40 (d, 1H, *J* = 8.7 Hz, Ar-H), 7.87 (brs, 0.50H, CHO), 8.30 (brs, 0.50H, CHO); HRMS (ESI): $[M + H]^+$ calcd *m/z* 349.1877, found 349.1861.

***N*-[(5-ethoxy-1*H*-benzo[*d*]imidazol-2-yl)methyl]-2-[(*N*-hydroxyformamido)methyl]hexanamide (11d).** Compound **11d** was obtained as a white solid. Yield: 85%. m.p. 80-83 °C. IR (KBr) cm^{-1}: 3277 (-NH-), 2957, 2929 (CH, aliphatic), 1655 (>C=O), 1541 (-C=C-), 1384 (-CHO), 1044 (-C-O-C-); ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 0.84 (t, 3H, CH_3), 1.23 (m, 5H, CH_2), 1.31-1.36 (t, 3H, *J* = 6.9 Hz, CH_3), 1.44 (m, 1H, CH_2), 2.62-2.72 (m, 1H, CH), 3.39-3.79 (m, 2H, CH_2), 4.00-4.02 (m, 2H, CH_2), 4.24-4.73 (m, 2H, CH_2), 6.78 (s, 1H, Ar-H), 6.98 (s, 1H, Ar-H), 7.36-7.39 (d, 1H, *J* = 8.1 Hz, Ar-H), 7.87 (brs, 0.49H, CHO), 8.30 (brs, 0.51H, CHO); HRMS (ESI): $[M + H]^+$ calcd *m/z* 363.2033, found 363.2039.

***N*-[(5-Cyano-1*H*-benzo[*d*]imidazol-2-yl)methyl]-2-[(*N*-hydroxyformamido)methyl)hexanamide (11e).** Compound **11e** was obtained as a white solid. Yield: 86%. m.p. 74-77 °C. IR (KBr) cm^{-1}: 3281 (-NH-), 2953, 2929 (CH, aliphatic), 2224 (-CN), 1655 (>C=O), 1539 (-C=C-), 1384 (-CHO); ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 0.79-0.83 (t, 3H, *J* = 6.9 Hz, CH_3), 1.21-1.38 (m, 6H, CH_2), 2.66-2.74 (m, 1H, CH), 3.38-3.77 (m, 2H, CH_2), 4.41-4.67 (m, 2H, CH_2), 7.55-7.58 (d, 1H, *J* = 8.1 Hz, Ar-H), 7.66-7.69 (d, 1H, *J* = 8.4 Hz, Ar-H), 8.05 (s, 1H, Ar-H), 7.86

(brs, 0.51H, CHO), 8.30 (brs, 0.49H, CHO); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 14.32, 22.64, 29.08, 29.60, 37.30, 37.42, 43.77, 43.97, 49.08, 51.97, 104.00, 104.16, 120.48, 125.74, 125.86, 156.20, 156.53, 157.89, 162.55, 173.80, 173.93; HRMS (ESI): $[M + H]^+$ calcd *m/z* 344.1723, found 344.1728.

Methyl 2-{{2-[(*N*-hydroxyformamido)methyl]hexanamido}methyl} 1*H*-benzo[*d*] imidazole-5-carboxylate (11f). Compound **11f** was obtained as a white solid. Yield: 84%. m.p. 86-88 °C. IR (KBr) cm^{-1}: 3287 (-NH-), 2955, 2930 (CH, aliphatic), 1718, 1659 (>C=O), 1541 (-C=C-), 1384 (-CHO); ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 0.80-0.82 (t, 3H, CH_3), 1.22-1.44 (m, 6H, CH_2), 2.66-2.74 (m, 1H, CH), 3.41-3.80 (m, 2H, CH_2), 3.86 (s, 3H, CH_3), 4.38-4.71 (m, 2H, CH_2), 7.59-7.61 (d, 1H, *J* = 7.8 Hz, Ar-H), 7.82-7.84 (d, 1H, *J* = 8.1 Hz, Ar-H), 8.12 (s, 1H, Ar-H), 7.87 (brs, 0.55H, CHO), 8.31 (brs, 0.45H, CHO); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 14.32, 22.64, 29.12, 29.21, 29.59, 37.22, 37.35, 43.98, 44.16, 49.44, 52.06, 52.44, 111.88, 113.60, 118.69, 120.49, 123.71, 138.33, 142.82, 146.72, 155.11, 156.09, 157.95, 162.48, 167.19, 167.25, 173.75, 173.93; HRMS (ESI): $[M + H]^+$ calcd *m/z* 377.1826, found 377.1825.

***N*-[(5-Chloro-1*H*-benzo[*d*]imidazol-2-yl)methyl]-2-[(*N*-hydroxyformamido)methyl]hexana mide (11g).** Compound **11g** was obtained as a white solid. Yield: 79%. m.p. 96-98 °C. IR (KBr) cm^{-1}: 3276 (-NH-), 2957, 2930 (CH, aliphatic), 1656 (>C=O), 1541 (-C=C-), 706 (-C-Cl-); ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 0.79-0.82 (t, 3H, CH_3), 1.22-1.43 (m, 6H, CH_2), 2.64-2.73 (m, 1H, CH), 3.39-3.78 (m, 2H, CH_2), 4.33-4.68 (m, 2H, CH_2), 7.19 (s, 1H, Ar-H), 7.50-7.56 (m, 2H, Ar-H), 7.86 (brs, 0.51H, CHO), 8.30 (brs, 0.49H, CHO); HRMS (ESI): $[M(^{35}Cl) + H]^+$ calcd *m/z* 353.1381, found 353.1380.

Antibacterial activity

The compounds **11a-g** were screened *in vitro* for their antibacterial activity against *S. aureus* and *K. pneumoniae* using Linezolid as standard and DMSO as solvent control. The method of micro-dilution was performed using Muller-Hinton agar (Hi-medium) to test the seven new compounds of their inhibitory activity. After 18h of incubation at 37 °C, minimum inhibitory concentration (MIC) of the synthesized compounds were observed and shown in Table 1.

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