Design, synthesis and antibacterial activity of novel N-formylhydroxylamine derivatives as PDF inhibitors

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A new series of N-formylhydroxylamines 11a-i have been synthesized through a multi-step protocol starting from diethyl malonate. These compounds have been structurally characterized by IR, \textsuperscript{1}H NMR and HRMS. All the synthesized compounds 11a-i have been screened for antibacterial activities. All the compounds are found to exhibit potent \textit{in vitro} inhibitory activity against \textit{Staphylococcus aureus} and relatively weak antibacterial activity against \textit{Klebsiella pneumoniae}.

Keywords: Peptide deformylase (PDF), N-formylhydroxylamine derivatives, antibacterial activity

In recent years, with the changing environment, bacterial infections spread rapidly and become a serious threat to respiratory organs and skin tissues\textsuperscript{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 research groups are Peptide deformylase (PDF)\textsuperscript{3-5}. This enzyme catalyzes the removal of the N-terminal formyl group of proteins and is essential for protein maturation, growth and survival of bacteria\textsuperscript{6}. PDF is essential in a variety of pathogenic bacteria but it is not required for cytoplasmic protein synthesis in eukaryotes, which makes this enzyme an attractive target for developing novel antibiotics\textsuperscript{7}. Several compounds have been reported\textsuperscript{8,9} to exhibit inhibitory activity against PDF. N-Formyl hydroxylamine BB-3497 is an effective inhibitor (IC\textsubscript{50} = 7 nM) against \textit{Escherichia coli} PDF•Ni enzyme, exhibiting potent antibacterial activity both \textit{in vitro} and \textit{in vivo}\textsuperscript{10}. Bearing a substituted aniline moiety in place of amide fragment of BB-3497 to work as an amide isostere. Several anilines were chosen for incorporation into 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 (Figure 1). These were synthesized by novel multi-step reaction route (outlined in Scheme I and II) and their \textit{in vitro} antibiotic activities studied.

Results and Discussion

The synthetic route to compounds 11a-i is shown in Scheme I and Scheme II.

The key step to the synthesis of intermediate 4 consisted of Michael addition of 3 to 2. Several catalysts have been found suitable for the Michael addition, such as CeCl\textsubscript{3}-NaI (Ref 12), LiClO\textsubscript{4} (Ref 13) and PMe\textsubscript{3} (Ref 14). However, due to the presence of the amide functionality in 3, DBU was found to be the most a suitable reagent to effect this reaction. In the presence of a catalytic amount of copper (I), 7 \textit{in situ} generated nitrile oxide underwent a stepwise addition to copper (I) acetylenide under mild reaction conditions. This method was found suitable for application to the large scale preparation of 3,5-disubstituted isoxazole 8a-i in high yields, and for one regioisomer to be obtained in each case without tedious chromatographic purification (Scheme II)\textsuperscript{11}.
The primary amines 9 were obtained in good yields from primary alcohols 8 by a well-established three-step procedure. Thus, primary alcohols 8 were treated in turn with methanesulfonyl chloride, sodium azide and zinc to furnish the primary amines 9 (Ref 15).

Two kinds of condensing agents were utilized for the condensation of amines 9 and carboxylic acids 5, viz., DCC and CDMT/NMM. However, CDMT/NMM was preferred in view of its producing high yield up to 85%.

Hydrogenation of 10 with 10%Pd/C removed the benzyl group and concomitantly opened the isoxazole ring to give 11 (Ref 16). The structures of 11 were confirmed by IR, 1H NMR and HRMS.

All the synthesized compounds were evaluated for their antibacterial activity with Linezolid as the positive control. The results which emanated from this study are summarized in Table I. Compounds 11a-i exhibited weak inhibitory activity against Klebsiella pneumoniae (CMCC46117), and good antibacterial activity against Staphylococcus aureus (CMCC26112).

**Experimental Section**

All the melting points were determined on an XT-4 microscopic melting-point apparatus and are uncorrected. IR spectra were measured on a Thermo Nicolet Avatar FT370 FT spectrophotometer as KBr pellets. Thin-layer chromatography (TLC) was performed on Silica Gel F254 plates with visualization by UV or iodine vapor. 1H NMR spectra were recorded on a Bruker Avance DPX300 spectrometer with CDCl3 or DMSO-d6 as the solvent and TMS as the internal standard. The high resolution mass spectra were obtained with an Agilent 6510 Q-TOF spectrometer.
Table I — The antibacterial activity of the target compounds (MIC, µg/mL)

<table>
<thead>
<tr>
<th>Compd</th>
<th>S. aureus</th>
<th>K. pneumoniae</th>
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<tbody>
<tr>
<td>11a</td>
<td>8</td>
<td>&gt;32</td>
</tr>
<tr>
<td>11b</td>
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<td>16</td>
</tr>
<tr>
<td>11c</td>
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<tr>
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<td>&lt;1</td>
<td>16</td>
</tr>
<tr>
<td>Linezolid</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

All the solvents were obtained from commercial suppliers and used without further purification.

2-Methylenec Hexanoic acid, 1

Compound 1 was prepared according to reported methods. 2-Butylmalonic acid (24.0 g, 150.0 mmol) was added to anhydrous ethanol (250 mL), then formaldehyde solution (13.3 mol/L, 55.4 mL) and piperidine (18.5 mL) were added into the mixture successively. After refluxing about 12 hr, the reaction mixture was concentrated to remove ethanol and then was extracted with ethyl acetate (3 x 50 mL). The combined extracts were washed with 1 mol/L HCl(aq) and brine successively and then dried over anhydrous MgSO₄ followed by evaporation. The crude product was distilled under reduced pressure to obtain 1 in the form of a colourless oil. Yield: 94.3%; ¹H NMR (300 MHz, CDCl₃): δ 0.88-0.97 (t, 3H, CH₃), 1.28-1.54 (m, 4H, CH₂), 2.26-2.36 (t, 2H, CH₂), 5.65 (s, 1H, CH), 6.28 (s, 1H, CH); HRMS (ESI-Q-TOF): Calcd for [M + H]⁺ C₇H₁₂O₂: m/z 129.0915. Found 129.0922.

Ethyl 2-methylenec hexanoate, 2

Compound 1 (10.0 g, 78.1 mmol) and oil of vitriol (1 mL) were added to anhydrous ethanol (50 mL), and then refluxed until no water was produced. The solvent was evaporated and the aqueous phase was basified to pH > 7 with NaHCO₃ solution. The
aqueous solution was then extracted with ethyl acetate (3 × 30 mL) and washed with brine, and the combined extracts were dried over anhydrous MgSO₄ and concentrated under reduced pressure to afford a colourless oil. Yield: 80%.

**N-(Benzyloxy)formamide, 3**

Sodium hydroxide (5.0 g, 125.0 mmol) and ethyl formate (23.2 g, 313.5 mmol) were added to a stirred mixture of O-benzylhydroxylamine (10.0 g, 62.7 mmol) in anhydrous methanol (120 mL), and the resulting mixture was stirred at RT for 16 hr. The reaction mixture was concentrated to remove ethanol and then was extracted with ether (3 × 40 mL), and the combined extracts were washed with water, 2 mol/L HCl(aq) and brine successively and then dried over anhydrous MgSO₄ and concentrated to afford a residue, which was purified by 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%; ¹H NMR (300 MHz, CDCl₃): δ 0.85-0.89 (t, 3H, CH₃), 1.26-1.60 (m, 6H, CH₂), 2.39 (s, 3H, CH₃), 4.70-4.97 (m, 2H, CH₂), 7.35-7.47 (m, 2H, Ar-H), 7.99, 8.16 (br, 1H, CHO), 10.91 (brs, 1H, COOH).

**General procedure for the synthesis of compounds, 7a-i**

Compound 6 (40 mmol) was stirred in 60 mL of methanol/water (1:2, v:v) until the solid dissolved. Hydroxylamine hydrochloride (44 mmol) and Na₂CO₃ (22 mmol) were added successively, and then the solution was stirred for 4 hr at RT. The reaction mixture was diluted with water (150 mL), and then was extracted with dichloromethane (4 × 30 mL), and the extracts were dried over anhydrous Na₂SO₄. After 0.5 hr, the solvent was concentrated.

**Benzenaldehyde oxime, 7a:** White solid; yield: 97%; m.p. 128-30°C; ¹H NMR (300 MHz, CDCl₃): δ 7.40-7.50 (m, 3H, Ar-H), 7.58-7.65 (m, 2H, Ar-H), 8.24 (s, 1H, OH).

**4-Methylbenzaldehyde oxime, 7b:** White solid; yield: 90%; m.p. 75-76°C; ¹H NMR (300 MHz, CDCl₃): δ 7.40-7.50 (m, 3H, Ar-H), 7.58-7.65 (m, 2H, Ar-H), 8.24 (s, 1H, OH).

**4-Methoxybenzaldehyde oxime, 7c:** White solid; yield: 95%; m.p. 49-51°C; ¹H NMR (300 MHz, CDCl₃): δ 7.18-7.20 (m, 3H, Ar-H), 7.35 (d, J = 8.3 Hz, 2H, Ar-H), 7.46 (d, J = 8.3 Hz, 2H, Ar-H), 8.13 (s, 1H, OH).

**4-Methoxybenzaldehyde oxime, 7e:** White solid; yield: 95%; m.p. 75-76°C; ¹H NMR (300 MHz, CDCl₃): δ 7.40-7.50 (m, 3H, Ar-H), 7.58-7.65 (m, 2H, Ar-H), 8.24 (s, 1H, OH).

**2-Chlorobenzaldehyde oxime, 7d:** White solid; yield: 85%; m.p. 72.5-73°C; ¹H NMR (300 MHz, CDCl₃): δ 7.18-7.41 (m, 2H, Ar-H), 7.76-7.85 (m, 2H, Ar-H), 8.59 (s, 1H, OH).

**4-Chlorobenzaldehyde oxime, 7e:** White solid; yield: 85%; m.p. 109-10°C; ¹H NMR (300 MHz, CDCl₃): δ 7.35 (d, J = 8.3 Hz, 2H, Ar-H), 7.50 (d, J = 8.2 Hz, 2H, Ar-H), 8.10 (s, 1H, OH).

**Dichlorobenzaldehyde oxime, 7f:** White solid; yield: 82%; m.p. 115-16°C; ¹H NMR (300 MHz, CDCl₃): δ 7.50 (m, 3H, Ar-H), 7.58-7.65 (m, 2H, Ar-H), 8.24 (s, 1H, OH).
General procedure for the synthesis of compounds, 8a-i

Compound 7 (41.3 mmol) was stirred in 20 mL N,N-dimethylformamide, and then N-chlorosuccinimide (9.1 mmol) was added to the solution. The mixture was heated until N-chlorosuccinimide dissolved, and was stirred at RT for 20 min. Into the above solution was added N-chlorosuccinimide (36.4 mmol) in batches under 35°C. After 3 hr, propargyl alcohol (49.6 mmol) was added, then a saturated solution of CuSO$_4$·5H$_2$O (2.48 mmol) and L-ascorbic acid (9.92 mmol) were also added. The solution of K$_2$CO$_3$ (45.5 mmol) was added to the mixture and the mixture was stirred for 1 hr. The reaction mixture was diluted with the saturated solution of ethylenediaminetetraacetic acid, and then was extracted with dichloromethane (3 × 50 mL), and the extracts were dried over anhydrous Na$_2$SO$_4$. The solution was concentrated to afford a residue, which was purified over a silica-gel column (using petroleum ether / ethyl acetate, 3:1 by volume as eluant).

(3-Phenylisoxazol-5-yl)methanol, 8a: White solid; yield: 61.7%; m.p. 52-54°C; $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 2.45 (brs, 1H, OH), 4.82 (s, 2H, CH$_2$), 6.57 (s, 1H, CH), 7.43-7.46 (m, 3H, Ar-H), 7.78-7.80 (m, 2H, Ar-H).

(3-p-Tolylisoxazol-5-yl)methanol, 8b: White solid; yield: 74%; m.p. 73-74°C; $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 1.88 (s, 1H, OH), 2.36 (s, 3H, CH$_3$), 4.84 (s, 2H, CH$_2$), 6.50 (s, 1H, CH), 7.07 (d, $J$ = 7.8 Hz, 2H, Ar-H), 7.49 (d, $J$ = 8.4 Hz, 2H, Ar-H).

[3-(4-Methoxyphenyl)isoxazol-5-yl]methanol, 8c: White solid; yield: 72%; m.p. 89-90°C; $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 1.89 (brs, 1H, OH), 3.85 (s, 3H, CH$_3$), 4.81 (s, 2H, CH$_2$), 6.51 (s, 1H, CH), 7.02 (d, $J$ = 8.4 Hz, 2H, Ar-H), 7.39 (d, $J$ = 8.7 Hz, 2H, Ar-H).

[3-(2-Chlorophenyl)isoxazol-5-yl]methanol, 8d: White solid; yield: 61.7%; m.p. 115-16°C; $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 2.47 (brs, 1H, OH), 4.82 (s, 2H, CH$_2$), 6.69 (s, 1H, CH), 7.42-7.43 (m, 1H, Ar-H), 7.57 (d, $J$ = 2.4 Hz, 1H, Ar-H), 7.69 (d, $J$ = 8.1 Hz, 1H, Ar-H).

[3-(2-Bromo-4,5-dimethoxyphenyl)isoxazol-5-yl]methanol, 8e: White solid; yield: 79%; m.p. 113-15°C; $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 2.47 (brs, 1H, OH), 4.82 (s, 2H, CH$_2$), 6.69 (s, 1H, CH), 7.42-7.43 (m, 1H, Ar-H), 7.57 (d, $J$ = 2.4 Hz, 1H, Ar-H), 7.69 (d, $J$ = 8.1 Hz, 1H, Ar-H).

[3-(3,4-Dichlorophenyl)isoxazol-5-yl]methanol, 8f: White solid; yield: 73%; m.p. 110-115°C; $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 2.42 (brs, 1H, OH), 3.45 (s, 3H, CH$_3$), 3.68 (s, 3H, CH$_3$), 4.83 (s, 2H, CH$_2$), 6.71 (s, 1H, CH), 7.18 (s, 1H, Ar-H), 7.27 (s, 1H, Ar-H).

[3-(2-Chloro-4,5-dimethoxyphenyl)isoxazol-5-yl]methanol, 8g: White solid; yield: 79%; m.p. 111-113°C; $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 2.45 (brs, 1H, OH), 4.81 (s, 2H, CH$_2$), 6.51 (s, 1H, CH), 7.53 (d, $J$ = 8.4 Hz, 1H, Ar-H), 7.64 (dd, $J$ = 2.1, 2.1 Hz, 1H, Ar-H), 7.91 (d, $J$ = 2.4 Hz, 1H, Ar-H).

General procedure for the synthesis of compounds, 9a-i

Compound 8 (50 mmol) was stirred in 90 mL dichloromethane, and then triethylamine (60 mmol) was added to the solution. Methanesulfonyl chloride (60 mmol) in 30 mL dichloromethane was added to the above solution in ice bath. The mixture was stirred at < 5°C for 1 hr and then at RT for 5 hr. The mixture was washed successively with 20% Na$_2$CO$_3$(aq) and water. The organic phase was dried and evaporated to give a slightly yellow solid.

The yellow solid (50 mmol) was stirred in 20 mL anhydrous N,N-dimethylformamide, and then sodium
azide (90 mmol) was added into the solution. The reaction was stirred at 55°C for 8 hr. The reaction mixture was diluted with 300 mL water and then was extracted with ether (4 × 60 mL), and the extracts were dried over anhydrous Na₂SO₄. The solution was concentrated to afford a slightly yellow solid. The yellow solid (50 mmol) was stirred in 36 mL of ethanol/water 5:1 (by volume) until the solid dissolved. Zinc powder (65 mmol) and NH₄Cl (86 mmol) were added into the solution, and then the mixture was refluxed for 0.5 hr. A solution of 15% NaOH (100 mL) and ethyl acetate (100 mL) were added into the mixture, and then the organic phase was concentrated to afford a residue, which was purified over a silica-gel column (using petroleum ether / ethyl acetate / dichloromethane, 3:1:1 by volume as eluant).

(3-Phenylisoxazol-5-yl)methanamine, 9a: Slightly yellow solid; yield: 78%; m.p. 46-48°C; ¹H NMR (300 MHz, CDCl₃): δ 1.75 (brs, 2H, NH₂), 4.04 (s, 2H, CH₂), 6.46 (s, 1H, CH), 7.44-7.47 (m, 3H, Ar-H), 7.78-7.81 (m, 2H, Ar-H).

(3-p-Tolylisoxazol-5-yl)methanamine, 9b: Slightly yellow solid; yield: 74%; m.p. 55-57°C; ¹H NMR (300 MHz, CDCl₃): δ 1.76 (brs, 2H, NH₂), 2.39 (s, 3H, CH₃), 4.01 (s, 2H, CH₂), 6.42 (s, 1H, CH), 7.24 (d, J = 7.8 Hz, 2H, Ar-H), 7.67 (d, J = 8.4 Hz, 2H, Ar-H).

(3-(4-Methoxyphenyl)isoxazol-5-yl)methanamine, 9c: Slightly yellow solid; yield: 68%; m.p. 61-63°C; ¹H NMR (300 MHz, CDCl₃): δ 1.64 (brs, 2H, NH₂), 3.85 (s, 3H, CH₃), 4.00 (s, 2H, CH₂), 6.39 (s, 1H, CH), 6.95 (d, J = 8.7 Hz, 2H, Ar-H), 7.71 (d, J = 9.0 Hz, 2H, Ar-H).

(3-(2-Chlorophenyl)isoxazol-5-yl)methanamine, 9d: Slightly yellow solid; yield: 71%; m.p. 52-54°C; ¹H NMR (300 MHz, CDCl₃): δ 1.64 (brs, 2H, NH₂), 4.04 (s, 2H, CH₂), 6.60 (s, 1H, CH), 7.32-7.41 (m, 2H, Ar-H), 7.47-7.50 (m, 1H, Ar-H), 7.70-7.73 (m, 1H, Ar-H).

(3-(4-Chlorophenyl)isoxazol-5-yl)methanamine, 9e: Slightly yellow solid; yield: 73%; m.p. 57-58°C; ¹H NMR (300 MHz, CDCl₃): δ 1.71 (brs, 2H, NH₂), 4.03 (s, 2H, CH₂), 6.44 (s, 1H, CH), 7.41 (d, J = 8.4 Hz, 2H, Ar-H), 7.71 (d, J = 8.7 Hz, 2H, Ar-H).

(3-(2,4-Dichlorophenyl)isoxazol-5-yl)methanamine, 9f: Slightly yellow solid; yield: 77%; m.p. 65-67°C; ¹H NMR (300 MHz, CDCl₃): δ 1.83 (br, 2H, NH₂), 4.05 (s, 2H, CH₂), 6.60 (s, 1H, CH), 7.32-7.37 (m, 1H, Ar-H), 7.50 (d, J = 2.1 Hz, 1H, Ar-H), 7.66 (d, J = 8.4 Hz, 1H, Ar-H).

(3-(3,4-Dichlorophenyl)isoxazol-5-yl)methanamine, 9g: Slightly yellow solid; yield: 64%; m.p. 62-64°C; ¹H NMR (300 MHz, CDCl₃): δ 1.64 (brs, 2H, NH₂), 4.04 (s, 2H, CH₂), 6.44 (s, 1H, CH), 7.51 (d, J = 8.4 Hz, 1H, Ar-H), 7.61 (dd, J = 1.8, 2.1 Hz, 1H, Ar-H), 7.88 (d, J = 2.1 Hz, 1H, Ar-H).

(3-(2-Bromo-4,5-dimethoxyphenyl)isoxazol-5-yl)methanamine, 9h: Slightly yellow solid; yield: 69%; m.p. 73-74°C; ¹H NMR (300 MHz, CDCl₃): δ 1.63 (brs, 2H, NH₂), 3.89 (s, 3H, CH₃), 3.91 (s, 3H, CH₃), 4.04 (s, 2H, CH₂), 6.63 (s, 1H, CH), 7.11 (s, 1H, Ar-H), 7.19 (s, 1H, Ar-H).

(3-(4,5-Dimethoxy-2-nitrophenyl)isoxazol-5-yl)methanamine, 9i: Slightly yellow solid; yield: 62%; m.p. 76-77°C; ¹H NMR (300 MHz, CDCl₃): δ 1.25 (brs, 2H, NH₂), 3.83 (s, 3H, CH₃), 3.86 (s, 3H, CH₃), 4.01 (s, 2H, CH₂), 6.31 (s, 1H, CH), 6.42 (s, 1H, Ar-H), 6.90 (s, 1H, Ar-H).

General procedure for the synthesis of compounds, 10a-i:

To a stirred solution of 4,6-dimethoxy-2-chloro-1,3,5-triazine (CDMT, 7.88 mmol) and 2-[(N-hydroxyformamido)methyl]hexanoic acid 5 (7.16 mmol) in dry CH₂Cl₂ (30 mL). N-methylmorpholine (NMM, 8.60 mmol) was added dropwise at 0°C and was continuously stirred at 0°C for 4 hr. The crude solution of 9 (7.16 mmol) was added into CH₂Cl₂ (5 mL) at 0°C and stirred for 2 hr. The mixture was then left overnight at RT. The solvent was evaporated and the residue was diluted with CH₂Cl₂. The suspension was washed successively with 0.5 mol/L HCl(aq), saturated NaHCO₃(aq), and brine. The organic phase was dried and concentrated to give a white solid. The crude product was purified over a silica-gel column (using dichloromethane / methanol, 40:1 by volume as eluant).

2-[(N-Benzoylformamido)methyl]-N-[(3-phenylisoxazol-5-yl)methyl]hexanamide, 10a: White solid; yield: 85%; m.p. 63-65°C; IR (KBr): 3300, 2956, 2931, 1677, 1544, 1443, 1361, 1218, 972, 913, 740, 697, 510 cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): δ 0.80-0.84 (t, 3H, J = 6.9 Hz, CH₃), 1.21-1.41 (brm, 6H, CH₂), 2.68 (m, 1H, CH), 3.46-3.74 (brm, 2H, CH₂), 3.67-3.69 (m, 1H, CH₂), 4.44 (m, 2H, CH₂), 4.88-4.91 (m, 2H, CH₂), 6.71 (brs, 1H, CH), 7.35-7.43 (m, 5H, Ar-H), 7.47-7.52 (m, 3H, Ar-H), 7.80 (m, 3H, Ar-H), 7.81, 8.18 (br, 1H, CHO); HRMS (ESI-Q-TOF): m/z Calcd for [M + H]+ C₂₅H₂₅N₅O₄; 436.2236. Found 436.2242.
2955, 2928, 1676, 1541, 1359, 1214, 911, 821, 774, 700, 512 cm$^{-1}$; $^1$H NMR (300 MHz, DMSO-$d_6$): δ 0.80-0.84 (t, 3H, $J = 6.6$ Hz, CH$_3$), 1.20-1.41 (brm, 6H, CH$_2$), 2.35 (s, 3H, CH$_3$), 2.69 (m, 1H, CH), 3.34-3.74 (brm, 2H, CH$_2$), 3.67-3.69 (m, 1H, CH$_2$), 4.43 (m, 2H, CH$_2$), 4.88-4.91 (m, 2H, CH$_2$), 6.67 (brs, 1H, CH), 7.24-7.43 (m, 7H, Ar-H), 7.69-7.71 (d, 2H, $J = 7.8$ Hz, Ar-H), 7.89, 8.18 (br, 1H, CHO); HRMS (ESI-Q-TOF): m/z Calcd for [M + H]$^+$ C$_{28}$H$_{31}$N$_3$O$_3$: 450.2393. Found 450.2394.

2-[[N-(Benzylxoy)formamido](methyl)]-N-[[3-(4-methoxyphenyl)isoxazol-5-yl](methyl)hexanamide, 10c: Yellow solid; yield: 78%; m.p. 92-94°C; IR (KBr): 3300, 2956, 2831, 1667, 1530, 1433, 1361, 1254, 1179, 1029, 911, 838, 749, 701, 531 cm$^{-1}$; $^1$H NMR (300 MHz, DMSO-$d_6$): δ 0.80-0.84 (t, 3H, $J = 6.3$ Hz, CH$_3$), 1.20-1.44 (brm, 6H, CH$_2$), 2.69 (m, 1H, CH), 3.36-3.75 (brm, 2H, CH$_2$), 3.67-3.70 (m, 1H, CH$_2$), 3.81 (s, 3H, CH$_3$), 4.42 (m, 2H, CH$_2$), 4.89 (m, 2H, CH$_2$), 6.65 (brs, 1H, CH), 7.03-7.06 (m, 2H, Ar-H), 7.36-7.81 (m, 5H, Ar-H), 7.74-7.77 (m, 2H, Ar-H), 7.90, 8.19 (br, 1H, CHO); HRMS (ESI-Q-TOF): m/z Calcd for [M + H]$^+$ C$_{28}$H$_{31}$N$_3$O$_3$: 466.2342. Found 466.2341.

2-[[N-(Benzylxoy)formamido](methyl)]-N-[[3-(2-chlorophenyl)isoxazol-5-yl](methyl)hexanamide, 10d: Yellow solid; yield: 79%; m.p. 71-72°C; IR (KBr): 3313, 2958, 2931, 1676, 1540, 1452, 1360, 1265, 1049, 913, 738, 703, 458 cm$^{-1}$; $^1$H NMR (300 MHz, DMSO-$d_6$): δ 0.80-0.84 (t, 3H, $J = 6.6$ Hz, CH$_3$), 1.20-1.44 (brm, 6H, CH$_2$), 2.67 (m, 1H, CH), 3.34-3.74 (brm, 2H, CH$_2$), 3.66-3.69 (m, 1H, CH), 4.46-4.52 (m, 2H, CH$_2$), 4.87-4.91 (m, 2H, CH$_2$), 6.65 (brs, 1H, CH), 7.35-7.67 (m, 9H, Ar-H), 7.91, 8.18 (br, 1H, CHO); HRMS (ESI-Q-TOF): m/z Calcd for [M + H]$^+$ C$_{25}$H$_{23}$Cl$_2$N$_3$O$_3$: 504.1509. Found 504.1509.

2-[[N-(Benzylxoy)formamido](methyl)]-N-[[3-(2-bromo-4,5-dimethoxyphenyl)isoxazol-5-yl](methyl)hexanamide, 10g: White solid; yield: 77%; m.p. 105-106°C; IR (KBr): 3306, 2957, 2934, 1677, 1521, 1432, 1359, 1256, 1013, 872, 788, 736, 701, 650, 512 cm$^{-1}$; $^1$H NMR (300 MHz, DMSO-$d_6$): δ 0.80-0.84 (t, 3H, $J = 6.6$ Hz, CH$_3$), 1.21-1.44 (brm, 6H, CH$_2$), 2.69 (m, 1H, CH), 3.33-3.69 (brm, 2H, CH$_2$), 3.66-3.69 (m, 1H, CH), 3.78 (s, 3H, CH$_3$), 3.84 (s, 3H, CH$_3$), 4.39-4.62 (m, 2H, CH$_2$), 4.87-4.90 (m, 2H, CH$_2$), 6.60 (brs, 1H, CH), 7.11 (s, 1H, Ar-H), 7.29 (s, 1H, Ar-H), 7.35-7.41 (m, 5H, Ar-H), 7.88, 8.17 (br, 1H, CHO); HRMS (ESI-Q-TOF): m/z Calcd for [M + H]$^+$ C$_{27}$H$_{25}$Br$_2$N$_3$O$_6$: 574.1552. Found 574.1545.

2-[[N-(Benzylxoy)formamido](methyl)]-N-[[3-(4,5-dimethoxy-2-nitrophenyl)isoxazol-5-yl](methyl)hexanamide, 10i: Yellow solid; yield: 84%; m.p. 117-19°C; IR (KBr): 3306, 2956, 2932, 1680, 1536, 1453, 1385, 1215, 1136, 1053, 1002, 873, 747, 701 cm$^{-1}$; $^1$H NMR (300 MHz, DMSO-$d_6$): δ 0.80-0.84 (t, 3H, $J = 6.6$ Hz, CH$_3$), 1.15-1.56 (brm, 6H, CH$_2$), 2.72 (m, 1H, CH), 3.33-3.70 (brm, 2H, CH$_2$), 3.66-3.70 (m, 1H, CH$_2$), 3.75 (s, 3H, CH$_3$), 3.81 (s, 3H, CH$_3$), 4.43 (m, 2H, CH$_2$), 4.88-4.93 (m, 2H, CH$_2$), 6.75 (brs, 1H, CH), 7.04-7.41 (m, 8H, Ar-H), 7.89, 8.19 (br, 1H, CHO); HRMS (ESI-Q-TOF): m/z Calcd for [M + H]$^+$ C$_{28}$H$_{26}$N$_5$O$_4$: 504.1543. Found 504.1557.

2-Hydroxy-1-(benzylxoy)formamidomethyl)-N-[[3-(2,4-dichlorophenyl)isoxazol-5-yl](methyl)hexanamide, 10f: White solid; yield: 82%; m.p. 113-15°C; IR (KBr): 3272, 2954, 2925, 1685, 1553, 1436, 1348, 1214, 1104, 1044, 934, 813, 753, 701, 576, 519, 415 cm$^{-1}$; $^1$H NMR (300 MHz, DMSO-$d_6$): δ 0.80-0.84 (t, 3H, $J = 6.6$ Hz, CH$_3$), 1.18-1.45 (brm, 6H, CH$_2$), 2.69 (m, 1H, CH), 3.36-3.70 (brm, 2H, CH$_2$), 4.24-4.57 (m, 2H, CH$_2$), 4.87 (m, 2H, CH$_2$), 6.67 (brs, 1H, CH), 7.32-7.42 (m, 5H, Ar-H), 7.49-7.58 (m, 1H, Ar-H), 7.67-7.72 (m, 1H, Ar-H), 7.80-7.82 (m, 1H, Ar-H), 7.88, 8.18 (br, 1H, CHO); HRMS (ESI-Q-TOF): m/z Calcd for [M + H]$^+$ C$_{28}$H$_{32}$Cl$_2$N$_3$O$_4$: 504.1442.
General procedure for the synthesis of compounds 11a-i

10% Pd/C (0.20 g, containing 65.8% water) was added to a stirred mixture of compound 10 (1.96 mmol) and anhydrous ethanol (20 mL) under H₂ at RT for 3 hr. The organic phase was dried and concentrated to give the product.

N-(4-Amino-2-oxo-4-phenylbutyl)-2-[(N-hydroxyformamido)methyl]hexanamide, 11a: Yellow solid; yield: 92%; m.p. 103-105°C; IR (KBr): 362.2079, 1163, 1030, 878, 835, 732, 514, 421 cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): δ 0.81-0.83 (t, 3H, J = 6.6 Hz, CH₂), 1.12-1.23 (m, 6H, CH₂), 2.75 (m, 1H, CH), 3.35-3.39 (m, 4H, CH₂), 5.46 (s, 1H, CH), 7.38-7.53 (m, 4H, Ar-H), 7.78, 8.20 (br, 1H, CHO); HRMS (ESI-Q-TOF): m/z Calcd for [M + H]⁺ C₁₀H₁₂N₂O₄: 282.1530. Found 282.1532.

N-[4-Amino-4-(4-chlorophenyl)-2-oxobutyl]-2-[(N-hydroxyformamido)methyl]hexanamide, 11e: White solid; yield: 91%; m.p. 105-106°C; IR (KBr): 3352, 2955, 2930, 1666, 1537, 1498, 1409, 1384, 1142, 1025, 878, 836, 719, 497, 435 cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): δ 0.80-0.82 (t, 3H, J = 6.6 Hz, CH₂), 1.23-1.35 (m, 6H, CH₂), 2.73 (m, 1H, CH), 3.30-3.39 (m, 4H, CH₂), 5.47 (s, 1H, CH), 7.36-7.44 (m, 2H, Ar-H), 7.49-7.51 (m, 2H, Ar-H), 7.81, 8.20 (br, 1H, CHO); HRMS (ESI-Q-TOF): m/z Calcd for [M + H]⁺ C₁₈H₁₈Cl₂N₂O₄: 382.1533. Found 382.1535.

N-[4-Amino-4-(2,4-dichlorophenyl)-2-oxobutyl]-2-[(N-hydroxyformamido)methyl]hexanamide, 11f: Yellow solid; yield: 87%; m.p. 95-97°C; IR (KBr): 3352, 2957, 2931, 1668, 1535, 1497, 1384, 1243, 1161, 1024, 878, 812, 742, 506, 397 cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): δ 0.80-0.83 (t, 3H, J = 6.6 Hz, CH₂), 1.23-1.34 (m, 6H, CH₂), 2.73 (m, 1H, CH), 3.36-3.39 (m, 4H, CH₂), 5.46 (s, 1H, CH), 7.39-7.41 (m, 2H, Ar-H), 7.49-7.51 (m, 1H, Ar-H), 7.83, 8.44 (br, 1H, CHO); HRMS (ESI-Q-TOF): m/z Calcd for [M + H]⁺ C₁₈H₁₆Cl₂N₂O₄: 381.1414. Found 381.1415.

N-[4-Amino-4-(3,4-dichlorophenyl)-2-oxobutyl]-2-[(N-hydroxyformamido)methyl]hexanamide, 11g: White solid; yield: 83%; m.p. 106-108°C; IR (KBr): 3356, 2955, 2929, 1663, 1534, 1497, 1384, 1264, 1175, 1024, 878, 798, 748, 517, 431 cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): δ 0.80-0.82 (t, 3H, J = 6.6 Hz, CH₂), 1.23-1.35 (m, 6H, CH₂), 2.73 (m, 1H, CH), 3.38-3.41 (m, 4H, CH₂), 5.47 (s, 1H, CH), 7.36-7.43 (m, 2H, Ar-H), 7.49-7.59 (m, 1H, Ar-H), 7.88, 8.24 (br, 1H, CHO); HRMS (ESI-Q-TOF): m/z Calcd for [M + H]⁺ C₁₈H₁₆Cl₂N₂O₄: 416.1144. Found 416.1145.

N-[4-Amino-4-(2-bromo-4,5-dimethoxyphenyl)-2-oxobutyl]-2-[(N-hydroxyformamido)methyl]hexanamide, 11h: Yellow solid; yield: 80%; m.p. 97-98°C; IR (KBr): 3354, 2957, 2930, 1667, 1538, 1496, 1384, 1256, 1164, 1021, 878, 788, 754, 498, 415 cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): δ 0.82-0.84 (t, 3H, J = 6.6 Hz, CH₂), 1.23-1.38 (m, 6H, CH₂), 2.73 (m, 1H, CH), 3.35-3.60 (m, 2H, CH₂), 3.81-3.83 (m, 6H, CH₃), 4.16-4.18 (m, 4H, CH₂), 6.6-6.7 (m, 2H, Ar-H), 7.67-7.78 (m, 2H, Ar-H), 7.83, 8.24 (br, 1H, CHO); HRMS (ESI-Q-TOF): m/z Calcd for [M + H]⁺ C₁₉H₁₉Br₂Cl₂N₂O₄: 441.1142. Found 441.1142.
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