Synthesis of chiral N-(2-(1-oxophthalazin-2(1H)-yl) ethanoyl)-α-amino acid derivatives as antitumor agents

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Abstract

A series of chiral α-amino acid derivatives conjugated with 1-oxophthalazine moiety 5a-e were synthesized by coupling of various L-acylated amino acid derivatives with 2-(1-oxophthalazin-2(1H)-yl)acetic acid in the presence of 1-hydroxybenzotriazole (HOBt) and N,N'-dicyclohexylcarbodiimide (DCC) as coupling reagent. Alternatively, compounds 5a-e were prepared by the reaction of the corresponding azide 4, via the azide-coupling method, with L-acylated amino acid derivatives. The peptide esters 5a-d were converted into their corresponding amides 6a-d by treating with methanolic ammonia. Moreover, 5a was boiled with hydrazine hydrate to afford the corresponding hydrazide 7. Finally, the dipeptides 8a-c were prepared by coupling of 5a with the appropriate L-amino acid methyl ester. The synthesized compounds were tested against Caucasian breast adenocarcinoma MCF7 cell line.

Keywords: 1-Oxophthalazine, α-amino acids, antitumor agents

Introduction

In the past few decades, the synthesis of new heterocyclic compounds has been a subject of great interest due to their wide applicability. Heterocyclic compounds are widely occurring in nature and are significantly essential to life. Among a large variety of heterocyclic nuclei is phthalazine (I, HAV3C II) which is interesting because its derivatives show various pharmacological and biological activities (Figure 1).\textsuperscript{1-3} Phthalazine derivatives were reported to possess anticonvulsant,\textsuperscript{4} cardiotonic,\textsuperscript{5} and vasorelaxant activities.\textsuperscript{6} Therefore, a number of methods have been reported for the synthesis of phthalazine derivatives.\textsuperscript{4,7-13} On the other hand, several α-amino acids-conjugated heterocycles were reported as potential antitumor agents, for instance, 4-toluenesulfonylureido derivatives of amines, amino acids, dipeptides,\textsuperscript{14} and 2-(4-
aminophenyl)benzothiazoles. Some alkylating agents bearing amino acid residues showed high cytotoxic activity against various cancer cell lines, such as melphalan (L-phenylalanine mustard hydrochloride, Figure 1). Furthermore, amino acids could also improve the cell uptake of antitumor agents. Therefore, a possible strategy for increasing the transport of lipophilic compounds of biological interest across cell membrane was the conjugation with amino acids.

This motivated us to synthesize 1-oxophthalazine moiety conjugated with flexible amino acids as side chain hoping that the produced compounds will have improved biological activity.

Guided by the above observations, and in continuation of our previous work in the direction of the synthesis of bioactive compounds, we report here a convenient synthesis of new α-amino acid-1-oxophthalazine conjugates to study their antitumor activity.

![Figure 1. Structures of some reported biologically active compounds.](image)

### Results and Discussion

Commercially available phthalazin-1(2H)-one (1) was treated with ethyl chloroacetate in dry acetone containing anhydrous K$_2$CO$_3$ at reflux temperature to afford the known compound, ethyl 2-(1-oxophthalazin-2(1H)-yl)acetate (2a) in 85% yield. Subsequently, the corresponding acid 2b was obtained by saponification of ester 2a. A suitable coupling method was employed for the formation of peptides by the reaction of the carboxylic acid group with the L-amino acid methyl ester hydrochloride, using 1-hydroxybenzotriazole (HOBT) and $N,N'$-
dicyclohexylcarbodiimide (DCC)$^{29}$ as coupling reagent. Currently, HOBt is used very frequently as activating agent for the coupling of carboxylic acid group and amino group, not only because the coupling process is fast, but also it suppresses racemization, especially in the presence of DCC.$^{30-33}$ Thus, the peptides 5a-e were prepared by coupling of 2b with the appropriate L-amino acid methyl ester hydrochloride in the presence of HOBt and DCC as coupling reagents in 81-87% yields.

Alternatively, 2a was boiled with hydrazine hydrate in ethanol to afford the hydrazide 3 which subsequently converted into the new azide 4 by treatment with NaNO$_2$/HCl mixture. The $^1$H NMR spectrum of azide 4 revealed a characteristic singlet at $\delta = 4.91$ ppm due to $\text{NCH}_2\text{C}=\text{O}$ protons, which was confirmed by its $^{13}$C NMR signals at $\delta = 55.17$ ppm and 175.59 ppm. This azide 4 was selected as starting material for the coupling reaction with the appropriate amino acid methyl ester hydrochloride via the azide-coupling method. The azide compound was then treated with the appropriate L-amino acid methyl esters in ethyl acetate containing Et$_3$N at 0 °C to give, after neutralization, the desired peptides 5a-e in 73–79% yields (Scheme 1). The structures of peptides 5a-e were assigned on the basis of their elemental analyses and spectral data. Complete $^1$H and $^{13}$C-NMR assignments of compound 5b were shown in Figure 2 taken as an example. It is noteworthy to mention that likewise the above mentioned procedure; this alternative synthetic protocol also suppressed racemization and could be useful for synthesizing peptides without jeopardizing the absolute configuration of the asymmetric center of the produced peptide.
NH
N
O
1
ClCH₂COOEt, K₂CO₃, acetone

N
N
O
COOEt
2a
NaOH, heat
N
N
O
COOH
2b

NH₂NH₂·H₂O

N
N
O
CONH₂NH₂
3
HCl, NaNO₂, AcOH, -5 °C, 24 h

N
N
O
CON₃
4
amino acid methyl ester, ethyl acetate, Et₃N, -5 °C

O
R
NH
O
R
NH
O
R
NH
O
R
NH

R | Amino acid
---|------------------
a | H | Glycine
b | Me | L-alanine
c | CH₂CH(Me)₂ | L-leucine
d | CH₂CH₂SMe | L-methionine
e | Ph | L-phenylglycine

Scheme 1
**Figure 2.** Complete $^1$H and $^{13}$C-NMR assignments of compound 5b.

Following this synthetic protocol, the methyl esters of peptides 5a-d were converted into their corresponding amides 6a-d by treating with methanolic ammonia solution (Scheme 2).

![Chemical structures](image)

**Scheme 2**

Furthermore, peptide 5a was boiled with hydrazine hydrate in absolute ethanol to afford the corresponding hydrazide 7 (Scheme 3). Finally the dipeptides 8a-c were prepared by coupling of 5a with the appropriate L-amino acid methyl ester hydrochloride in the presence of HOBt and DCC as coupling reagents in 80-85% yields.
Scheme 3

Antitumor activity
The synthesized compounds 5a-e, 6a-d, 7 and 8a-c were tested for their in vitro cytotoxicity towards human Caucasian breast adenocarcinoma MCF7 cell line. Cytotoxic activity was determined using the method reported by Thabrew et al.\(^3\) Only the amide compounds 6a-d showed cytotoxicity with LC\(_{50}\) ranging from 3.7 to 6.8 and LC\(_{90}\) ranging from 6.4 to 18.3 (µg/mL). The most active compound against this cell line was 6d, which was 2-fold more cytotoxic than 6c which showed the least cytotoxicity. Compounds 6a and 6b showed moderate cytotoxicity relative to 6c and 6d. On the contrary, 5a-e, 7 and 8a-c were found to be completely inactive against the same human tumor cell line. The reason for the difference in cytotoxicity amongst the amides 6a-d could be attributed to the neighboring chiral atom substituents. However, the remarkable cytotoxicity of compounds 6a-d compared with 5a-e and 7 and 8a-c could be possibly referred to the presence of terminal free amide group.

Conclusions
In conclusion, different novel oxopthalazinone-amino acid conjugates 5a-e were synthesized according to standard protocol. The same protocol was applied to synthesize the dipeptides 8a-c.
Compounds 5a-d were transformed into their corresponding amides 6a-d by treating with methanolic ammonia solution. The reaction between 5a and hydrazine hydrate afforded 7. The synthesized compounds 5a-e, 6a-d, 7 and 8a-c were evaluated for their in vitro antitumor activity against MCF7 cell line. Only the amides 6a-d exhibited cytotoxic activity whereas compounds 5a-e, 7 and 8a-c showed no cytotoxicity towards the same cell line.

**Experimental Section**

**General.** All chemicals were purchased from commercial suppliers and used without further purification. Melting points were determined on a Gallenkamp melting point apparatus. $^1$H and $^{13}$C NMR spectra were recorded on a Jeol EX-Spectrometer, respectively, at 270 and 62.5 MHz using DMSO-d$_6$ and CDCl$_3$ as solvents and TMS as the internal standard. Mass spectra were recorded on a Finnigan mat. SSQ-7000 GC-MS spectrometer. Microanalyses were performed at the Microanalytical Center at Cairo University and their results were found to be in good agreement with calculated values (±0.2%). Analytical thin-layer chromatography (TLC) was carried out using Merck 60 F$_{254}$ aluminum sheets and visualized by UV light (254 nm).

**2-(2-Azido-2-oxoethyl)phthalazin-1(2H)-one (4).** To hydrazide 3 (0.218 g, 1.0 mmol) cooled to -5 °C in AcOH (6 mL), 1 N HCl (3 mL), and water (25 mL) was added NaNO$_2$ (0.069 g, 1.0 mmol) in cold water (3 mL). After stirring at -5 °C for 15 min, the yellow syrup was formed. The crude product was extracted with cold ethyl acetate (30 mL), washed with cold 3% NaHCO$_3$, then water and finally dried over anhydrous Na$_2$SO$_4$ to afford 4. Yield: 0.20 g (87%) of white crystals. mp 200-201 °C. $^1$H NMR (270 MHz, CDCl$_3$) δ = 4.91 (s, 2H, NCH$_2$), 7.65-7.76 (m, 3H, H-5, H-6, H-7), 8.10 (s, 1H, H-4), 8.33 (d, 1H, $J = 7.0$ Hz H-8). $^{13}$C NMR (62.5 MHz, CDCl$_3$): δ = 55.17 (CH$_2$), 126.92 (C-8), 127.26 (C-8'), 128.01 (C-5'), 130.43 (C-7), 132.55 (C-5), 134.17 (C-6), 139.22 (C-4), 160.07 (C-1), 175.59 (C=O) ppm. Anal. Calcd. for C$_{10}$H$_7$N$_5$O$_2$ (229): C, 52.40; H, 3.08; N, 30.56 Found: C, 52.33; H, 3.04; N, 30.13.

**General procedure for the preparation of chiral α-amino acid derivatives conjugated with phthalazin-1(2H)-one (5a-e)**

**Method A.** A solution of the appropriate amino acid methyl ester hydrochloride derivatives (1 mmol) in MeCN (5 mL) was cooled to -5 °C, then 2b, (0.204 g, 1 mmol), HOBT (0.14 g, 1 mmol), and DCC (0.21 g, 1 mmol) were added successively. The reaction mixture was stirred at -5 °C for 2 h, and then at room temperature for 16 h. Dicyclohexylurea (DCU) was filtered off and the filtrate was evaporated to dryness. The residue was dissolved in EtOAc (20 mL) and extracted successively with brine (10 mL), 5% NaHCO$_3$ (10 mL) solution, 1 N HCl (10 mL), followed by brine (10 mL), and finally with H$_2$O (10 mL). The organic layer was dried (Na$_2$SO$_4$), filtered, and evaporated to dryness. The residue was purified by silica gel column chromatography using 5% MeOH in CH$_2$Cl$_2$ to give 5a-e in 81–87% yields.
Method B. To a solution of the appropriate amino acid esters hydrochloride (1.0 mmol) in ethyl acetate (20 mL) containing Et₃N (0.2 mL) was added the azide 4. The mixture was kept at -5 °C for 24 h, then at 25 °C for another 24 h, followed by washing with 0.5 N HCl, water, 3% solution of NaHCO₃ and finally dried over anhydrous Na₂SO₄. The solution was evaporated to dryness, and the residue was recrystallized from petroleum ether/ethyl acetate to give the desired product 5a-e in 73-79% yields.

Methyl 2-(2-(1-oxophthalazin-2(1H)-yl)acetamido)acetate (5a). Yield: 0.24 g (87%) of white crystals; mp 161-163 °C. ¹H NMR (270 MHz, CDCl₃) δ = 3.64 (s, 3H, CH₃), 3.96 (s, 2H, CH₂), 4.92 (s, 2H, NCH₂), 6.92 (br s, 1H, NH), 7.66-7.78 (m, 3H, H-5, H-6, H-7), 8.20 (s, 1H, H-4), 8.36 (d, J = 7.0 Hz, 1H, H-8). ¹³C NMR (62.5 MHz, CDCl₃): δ = 41.10 (CH₃), 52.17 (CH₂), 54.54 (CH₂), 126.15 (C-8), 126.62 (C-8`), 127.45 (C-5`), 129.44 (C-7), 131.83 (C-5), 133.39 (C-6), 138.67 (C-4), 159.94 (C-5`, C-6), 169.94 (C=O, ester). Anal. Calcd. for C₁₃H₁₃N₃O₄ (275): C, 56.72; H, 4.76; N, 15.27. Found: C, 56.78; H, 4.79; N, 15.23.

Methyl 2-(2-(1-oxophthalazin-2(1H)-yl)acetamido)propanoate (5b). Yield: 0.25 g (86%) of white crystals; mp 233 °C. ¹H NMR (270 MHz, CDCl₃) δ = 1.33 (d, J = 2.3 Hz, 3H, CH₃), 3.62 (s, 3H, CH₃), 4.54 (m, 1H, *CH), 4.88 (s, 2H, NCH₂), 6.99 (br s, 1H, NH), 7.60-7.74 (m, 3H, H-5, H-6, H-7), 8.20 (s, 1H, H-4), 8.33 (d, J = 7.2 Hz, 1H, H-8). ¹³C NMR (62.5 MHz, CDCl₃): δ = 19.12 (CH₃), 42.54 (CH₃), 51.16 (CH₂), 54.54 (CH), 126.18 (C-8), 126.78 (C-8`), 127.41 (C-5`), 127.98 (C-7), 131.96 (C-5), 133.47 (C-6), 138.69 (C-4), 159.69 (C-1), 168.56 (C=O, ester), 169.92 (C=O). Anal. Calcd. for C₁₄H₁₅N₃O₄ (289): C, 58.13; H, 5.23; N, 14.53. Found: C, 58.17; H, 5.20; N, 14.26.

Methyl 2-(2-(1-oxophthalazin-2(1H)-yl)acetamido)-4-methylpentanoate (5c). Yield: 0.27 g (81%) of white crystals; mp 119-122 °C. ¹H NMR (270 MHz, CDCl₃): δ = 0.84-0.86 (dd, J = 2.3, 2.6 Hz, 6H, 2CH₃), 1.54-1.56 (m, 3H, CH, CH₂), 3.62 (s, 3H, CH₃), 4.62 (m, 1H, *CH), 4.92 (s, 2H, NCH₂), 6.71 (br s, 1H, NH), 7.68-7.78 (m, 3H, H-5, H-6, H-7), 8.17 (s, 1H, H-4), 8.36 (d, J = 7.3 Hz, 1H, H-8). ¹³C NMR (62.5 MHz, CDCl₃): δ = 22.03 (CH₃), 22.72 (CH₃), 24.83 (CH), 41.61 (CH₂), 50.92 (CH₂, CH₃), 52.26 (CH), 126.32 (C-8), 126.84 (C-8`), 127.68 (C-5`), 127.89 (C-7), 131.96 (C-5), 133.47 (C-6), 138.73 (C-4), 159.82 (C-1), 167.16 (C=O, ester). Anal. Calcd. for C₁₇H₂₁N₃O₄ (331): C, 61.62; H, 6.39; N, 12.68; O, 19.31. Found C, 61.12; H, 6.23; N, 12.41; O, 19.63.

Methyl-2-(2-(1-oxophthalazin-2(1H)-yl)acetamido)-4-(methylthio) butanoate (5d). Yield: 0.28 g (80%) of white crystals; mp 158 °C. ¹H NMR (270 MHz, CDCl₃) δ = 1.88 (s, 3H, S(CH₃), 2.02-2.08 (m, 2H, CH₂), 2.42 (t, 2H, SCH₂), 3.59 (s, 3H, CH₃), 4.65 (m, 1H, *CH), 4.89 (s, 2H, NCH₂), 7.12 (br s, 1H, NH), 7.66-7.76 (m, 3H, H-5, H-6, H-7), 8.12 (s, 1H, H-4), 8.32 (d, J = 6.3 Hz, 1H, H-8). ¹³C NMR (62.5 MHz, CDCl₃): δ = 14.96 (S(CH₃), 29.51 (CH₂), 31.03 (CH₂), 51.25 (CH₃), 52.08 (CH), 54.26 (CH₂), 125.93 (C-8), 126.29 (C-8`), 127.19 (C-5`), 129.38 (C-7), 131.52 (C-5), 133.11 (C-6), 138.34 (C-4), 159.32 (C-1), 166.16 (C=O, ester). Anal. Calcd. for C₁₆H₁₉N₃O₄S (349): C, 55.00; H, 5.48; N, 12.03. Found C, 55.23; H, 5.21; N, 11.97.
Methyl 2-(2-(1-oxophthalazin-2(1H)-yl)acetamido)-2-phenylacetate (5e). Yield: 0.29 g (83%) of white crystals; mp 165-166 °C. \(^1\)H NMR (270 MHz, CDCl\(_3\)) \(\delta = 3.64\) (s, 3H, CH\(_3\)), 4.92 (s, 2H, NCH\(_2\)), 5.54 (m, 1H, *CH), 6.23 (br s, 1H, NH), 7.35 (m, 5H, Ph), 7.69-7.79 (m, 3H, H-5, H-6, H-7), 8.21 (s, 1H, H-4), 8.42 (d, \(J = 7.5\) Hz, 1H, H-8). \(^1\)C NMR (62.5 MHz, CDCl\(_3\)): \(\delta = 48.03\) (CH\(_3\)), 52.24 (CH\(_2\)), 54.34 (CH), 126.12 (3C, C-8, 2C of Ph), 126.56 (3C, C-8’, 2C of Ph), 127.41 (C-5’), 129.61 (C-7), 131.72 (2C, C-5, 2C of Ph), 133.31 (2C, C-6, 2C of Ph), 138.53 (C-4), 159.57 (C-1), 166.79 (C=O, ester), 173.05 (C=O). Anal. Calcd. for C\(_{19}\)H\(_{17}\)N\(_3\)O\(_4\) (351): C, 64.95; H, 4.88; N, 11.96. Found C, 64.82; H, 4.72; N, 11.74.

General procedure for the preparation of 6a-d from the corresponding 5a-d
The ester was dissolved in methanolic ammonia (10 mL, 33%, w/v) and left for 4 h with continuous stirring. After the reaction completion (followed by TLC), the solution was evaporated in vacuo and the residue was coevaporated twice with chloroform (3 × 25 mL). The residue was taken up into CH\(_2\)Cl\(_2\) (25 mL) and the solution was washed with water (3 × 25 mL) and brine (25 mL), dried over anhydrous MgSO\(_4\), and the solvent was removed in vacuo to yield the amides 6a-d.

2-(2-(1-Oxophthalazin-2(1H)-yl)acetamido)acetamide (6a). Yield: 0.22 g (85%) of white crystals; mp 198 °C. \(^1\)H NMR (270 MHz, DMSO-d\(_6\)) \(\delta = 3.65\) (s, 2H, CH\(_2\)), 4.82 (s, 2H, NCH\(_2\)), 7.12-7.28 (2 × s, 2H, NH\(_2\)), 7.74-7.96 (m, 3H, H-5, H-6, H-7), 8.25 (s, 1H, H-4), 8.42 (d, \(J = 7.2\) Hz, 1H, H-8), 8.45 (br s, 1H, NH). \(^1\)C NMR (62.5 MHz, DMSO-d\(_6\)): \(\delta = 42.86\) (CH\(_2\)), 53.69 (CH\(_2\)), 125.64 (C-8), 126.81 (C-8’), 126.52 (C-8”), 127.02 (C-5’), 131.99 (C-7), 133.59 (C-6), 138.08 (C-4), 158.72 (C-1), 167.06 (C=O), 170.64 (C=O). Anal. Calcd. for C\(_{12}\)H\(_{12}\)N\(_4\)O\(_3\) (260): C, 55.38; H, 4.65; N, 21.53. Found: C, 55.43; H, 4.56; N, 21.62.

2-(2-(1-Oxophthalazin-2(1H)-yl)acetamido)propanamide (6b). Yield: 0.21 g (80%) of white crystals; mp 186-187 °C. \(^1\)H NMR (270 MHz, DMSO-d\(_6\)) \(\delta = 1.23\) (d, \(J = 2.4\) Hz, 3H, CH\(_3\)), 4.25 (m, 1H, *CH), 4.79 (s, 1H, NH), 7.06-7.32 (2 × s, 2H, NH\(_2\)), 7.84-8.06 (m, 3H, H-5, H-6, H-7), 8.26 (d, \(J = 2.7\) Hz, 1H, H-8), 8.41 (br s, 1H, NH), 8.48 (s, 1H, H-4). \(^1\)C NMR (62.5 MHz, DMSO-d\(_6\)): \(\delta = 18.22\) (CH\(_3\)), 47.99 (CH\(_2\)), 53.47 (CH), 125.61 (C-8), 126.76 (C-8’), 126.93 (C-5’), 129.53 (C-7), 131.95 (C-5), 133.53 (C-6), 137.85 (C-4), 158.63 (C-1), 166.30 (C=O), 173.92 (C=O). Anal. Calcd. for C\(_{13}\)H\(_{14}\)N\(_4\)O\(_3\) (274): C, 56.93; H, 5.14; N, 20.43. Found: C, 56.97; H, 5.12; N, 20.40.

Methyl 2-(2-(1-oxophthalazin-2(1H)-yl)acetamido)-4-methylpentanamide (6c). Yield: 0.27 g (86%) of white crystals; mp 204-206 °C. \(^1\)H NMR (270 MHz, DMSO-d\(_6\)) \(\delta = 0.86-0.92\) (dd, \(J = 2.3, 2.7\) Hz, 6H, 2CH\(_3\)) 1.42-1.65 (m, 3H, CH, CH\(_2\)), 4.26 (m, 1H, *CH), 4.84 (s, 2H, NCH\(_2\)), 7.04, 7.32 (2 × s, 2H, NH\(_2\)), 7.81-7.96 (m, 3H, H-5, H-6, H-7), 8.20 (d, \(J = 2.4\) Hz, 1H, H-8), 8.31 (br s, 1H, NH), 8.42 (s, 1H, H-4). \(^1\)C NMR (62.5 MHz, DMSO-d\(_6\)): \(\delta = 22.51\) (CH\(_3\)), 22.94 (CH\(_3\)), 24.15 (CH), 40.88 (CH\(_2\)), 50.87 (CH\(_2\)), 53.47 (CH), 125.61 (C-8), 126.76 (C-8’), 126.95 (C-5’), 129.54 (C-7), 131.92 (C-5), 133.50 (C-6), 137.84 (C-4), 158.63 (C-1), 166.53 (C=O), 173.85 (C=O). Anal. Calcd. for C\(_{16}\)H\(_{20}\)N\(_4\)O\(_3\) (316): C, 60.75; H, 6.37; N, 17.71. Found C, 60.79; H, 6.34; N, 17.69.
Methyl 2-(2-(1-oxophthalazin-2(1H)-yl)acetamido)-4-(methylthio)butanamide (6d). Yield: 0.27 g (81%) of white crystals; mp 69-72 °C. 1H NMR (270 MHz, DMSO-d6) δ = 1.8 (m, 2H, CH2), 2.04 (s, 3H, SCH3), 2.44 (t, 2H, SCH2), 4.34 (m, 1H, *CH), 4.82 (s, 2H, NCH2), 7.14, 7.41 (2 × s, 2H, NH2), 7.82–7.94 (m, 3H, H-5, H-6, H-7), 8. 25 (d, 1H, J = 6.8 Hz, H-8), 8.38 (br s, 1H, NH), 8.41 (s, 1H, H-4). 13C NMR (62.5 MHz, DMSO-d6): δ = 14.53 (SCH3), 29.56 (CH2), 31.66 (CH2), 51.66 (CH2), 53.67 (CH), 125.59 (C-8), 126.74 (C-8’), 126.94 (C-5’), 129.53 (C-7), 131.92 (C-5), 133.48 (C-6), 137.92 (C-4), 158.64 (C-1), 166.81 (C=O), 172.88 (C=O). Anal. Calcd. for C15H18N4O3S (334): C, 53.88; H, 5.43; N, 16.75. Found: C, 53.83; H, 5.46; N, 16.71.

2-(2-(1-oxophthalazin-2(1H)-yl)acetamido)acetohydrazide (7)

A solution of methyl 2-(2-(1-oxophthalazin-2(1H)-yl)acetamido)acetate (5a) (0.27 g, 1.0 mmol) in absolute ethanol (10 mL) was heated under reflux with hydrazine hydrate (0.5 mL). After the reaction completion (TLC, 4 h), the reaction mixture was left overnight at room temperature. The white crystals separated was collected by filtration. It was washed with methanol, dried and recrystallized from methanol to afford 7. Yield: 0.24 g (88%) of white crystals; mp 145-146 °C. 1H NMR (270 MHz, DMSO-d6) δ = 3.67 (d, J = 2.1 Hz, 2H, CH2), 4.22 (br s, 2H, NH2) 4.81 (s, 2H, NCH2), 7.28 (br s, 1H, NH), 7.81-7.94 (m, 3H, H-5, H-6, H-7), 8.21 (s, 1H, H-4), 8.40 (d, 1H, J = 7.1 Hz, H-8), 8.96 (s, 1H, NH). Anal. Calcd. For C12H13N5O3 (275): C, 52.36; H, 4.76; N, 25.44. Found C, 52.39; H, 4.74; N, 25.41.

General procedure for the preparation of dipeptides 8a–c

A solution of methyl 2-(2-(1-oxophthalazin-2(1H)-yl)acetamido)acetate (5a) (0.27 g, 1.0 mmol) in methanol (30 mL) and 2N NaOH (5 ml) was stirred for 3 h. The reaction mixture was acidified with HCl and evaporated in vacuo. To the residue obtained was dissolved in MeCN (5 mL), cooled to -5 °C and to it was successively added a solution of the appropriate amino acid methyl ester hydrochloride derivatives (1 mmol), HOBt (0.14 g, 1 mmol), and DCC (0.21 g, 1 mmol). The reaction mixture was stirred at -5 °C for 2 h, and then at room temperature for 16 h. Dicyclohexylurea (DCU) was filtered off and the filtrate was evaporated to dryness. The residue was dissolved in EtOAc (20 mL) and extracted successively with brine (10 mL), 5% NaHCO3 (10 mL) solution, 1N HCl (10 mL), followed by brine (10 mL), and finally with H2O (10 mL). The organic layer was dried (Na2SO4), filtered, and evaporated to dryness. The residue was purified by silica gel column chromatography using 5% MeOH in CH2Cl2 to give 8a–c in 80-85% yields.

Methyl 2-(2-(1-oxophthalazin-2(1H)-yl)acetamido)acetamido)propanoate (8a). Yield: 0.29 g, (84%) of white crystals; mp 213 °C. 1H NMR (270 MHz, CDCl3) δ 1.39 (d, 3H, J = 2.5 Hz, CH3), 3.68 (s, 3H, CH3), 4.51 (m, 1H, *CH), 4.82 (s, 2H, NCH2), 5.58 (s, 2H, NCH2), 6.42 (br s, 1H, NH), 7.04 (br s, 1H, NH), 7.60–7.74 (m, 3H, H-5, H-6, H-7), 8.14 (s, 1H, H-4), 8.33 (d, 1H, J = 7.3 Hz, H-8). Anal. Calcd. for C16H18N4O5 (346): C, 55.49; H, 5.24; N, 16.18. Found: C, 55.44; H, 5.26; N, 16.15.
Methyl 2-(2-(1-oxophthalazin-2(1H)-yl)acetamido)acetamido)-4-methyl pentanoate (8b).
Yield: 0.31 g (80%) of white crystals; mp 139-141 °C. $^1$H NMR (270 MHz, CDCl$_3$) δ 0.81-0.85 (2 × d, 6H, $^1$J = 2.3, 2.4 Hz, 2CH$_3$) 1.40-1.52 (m, 3H, CH, CH$_2$), 3.60 (s, 3H, CH$_3$), 4.52 (m, 1H, *CH), 4.90 (s, 2H, NCH$_2$), 5.80 (s, 2H, NCH$_2$), 6.65 (br, s, 1H, NH), 7.24 (br, s, 1H, NH), 7.68-7.78 (m, 3H, H-5, H-6, H-7), 8.17 (s, 1H, H-4), 8.36 (d, 1H, $^1$J = 7.3 Hz, H-8). $^{13}$C NMR (62.5 MHz, CDCl$_3$): δ 22.08 (CH$_3$), 22.92 (CH$_3$), 24.88 (CH), 41.62 (CH$_2$), 50.90 (CH$_2$), 51.40 (CH$_2$), 52.20 (CH), 126.36 (C-8), 126.82 (C-8`), 127.64 (C-5`), 129.82 (C-7), 131.94 (C-5), 133.52 (C-6), 138.71 (C-4), 159.80 (C-1), 167.32 (C=O), 171.24 (C=O, ester). Anal. Calcd. for C$_{19}$H$_{24}$N$_4$O$_5$ (388): C, 58.75; H, 6.23; N, 14.42. Found C, 58.79; H, 6.21; N, 14.40.

Methyl (2-(2-(1-oxophthalazin-2(1H)-yl)acetamido)acetamido)phenylacetate (8c).
Yield: 0.35 g (85%) of white crystals; mp 168-169 °C. $^1$H NMR (270 MHz, CDCl$_3$) δ 3.66 (s, 3H, CH$_3$), 4.54 (m, 1H, *CH), 4.94 (s, 2H, NCH$_2$), 5.49 (s, 2H, NCH$_2$), 6.24 (br, s, 1H, NH), 7.38 (m, 6H, Ph + NH), 7.66-7.78 (m, 3H, H-5, H-6, H-7), 8.24 (s, 1H, H-4), 8.41 (d, 1H, $^1$J = 6.9 Hz, H-8). $^{13}$C NMR (62.5 MHz, CDCl$_3$): δ 48.12 (CH$_3$), 52.20 (CH$_2$), 52.46 (CH$_2$), 54.31 (CH), 126.14 (3C, C-8, 2C of Ph ), 126.46 (3C, C-8`,2C of Ph), 127.44 (C-5`), 129.62 (C-7), 131.75 (2C, C-5, 2C of Ph), 133.34 (2C, C-6, 2C of Ph), 138.52 (C-4), 159.58 (C-1), 166.72 (C=O, ester), 171.04 (C=O), 172.02 (C=O). Anal. Calcd. for C$_{21}$H$_{20}$N$_4$O$_5$ (408): C, 61.76; H, 4.94; N, 13.72. Found C, 61.73; H, 4.96; N, 13.70.

References