Synthesis of chiral *N*-(2-(1-oxophthalazin-2(1*H*)-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(1*H*)-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 aizde **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

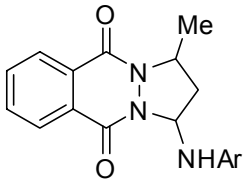
Introduction

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).[1-3] Phthalazine derivatives were reported to possess anticonvulsant,[4] cardiotonic,[5] and vasorelaxant activities.[6] Therefore, a number of methods have been reported for the synthesis of phthalazine derivatives.[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,[14] and 2-(4-

aminophenyl)benzothiazoles.[15] 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).[16] Furthermore, amino acids could also improve the cell uptake of antitumor agents.[17] Therefore, a possible strategy for increasing the transport of lipophilic compounds of biological interest across cell membrane was the conjugation with amino acids.[18]

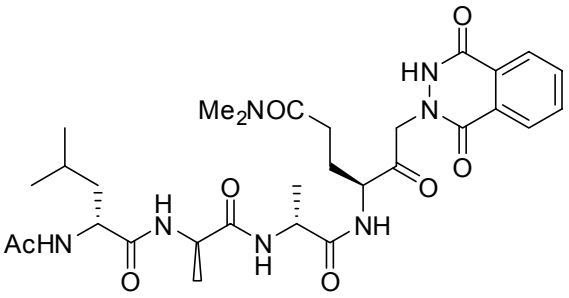
This motivated us to synthesize 1-oxophthalazine moiety conjugated with flexible amino acids as side chain hoping that the produced compounds will have improved biological activity.

Guided by the above observations, and in continuation of our previous work in the direction of the synthesis of bioactive compounds,[19-23] we report here a convenient synthesis of new α-amino acid-1-oxophthalazine conjugates to study their antitumor activity.



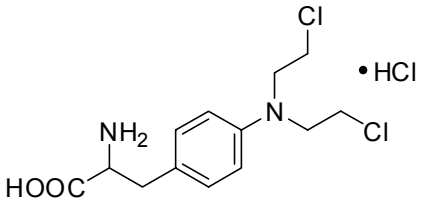
I

Antihypoxic and antipyretic agent



II

HAV 3C inhibitor



Melphalan

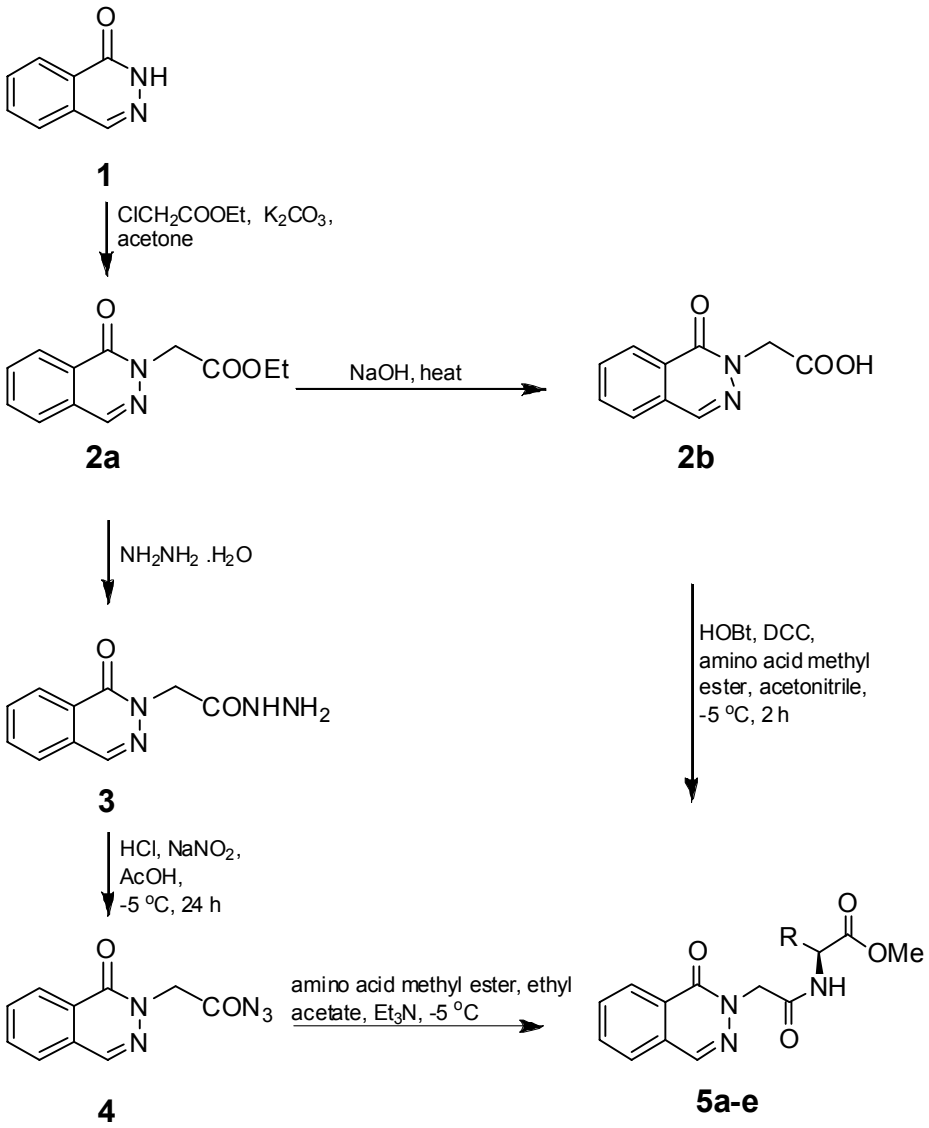
Figure 1. Structures of some reported biologically active compounds.

Results and Discussion

Commercially available phthalazin-1(*2H*)-one (**1**) was treated with ethyl chloroacetate in dry acetone containing anhydrous K_2CO_3 at reflux temperature to afford the known compound, ethyl 2-(1-oxophthalazin-2(*1H*)-yl)acetate (**2a**)[24] in 85% yield. Subsequently, the corresponding acid **2b**[25] was obtained by saponification of ester **2a**. A suitable coupling method[26] 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)[27,28] 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**[34] 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 δ = 4.91 ppm due to –N$\underline{CH_2}$C=O protons, which was confirmed by its ^{13}C NMR signals at δ = 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_3N 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.



5	R	Amino acid
a	H	Glycine
b	Me	L-alanine
c	$CH_2CH(Me)_2$	L-leucine
d	CH_2CH_2SMe	L-methionine
e	Ph	L-phenylglycine

Scheme 1

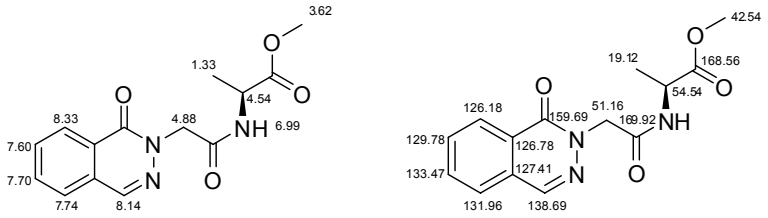
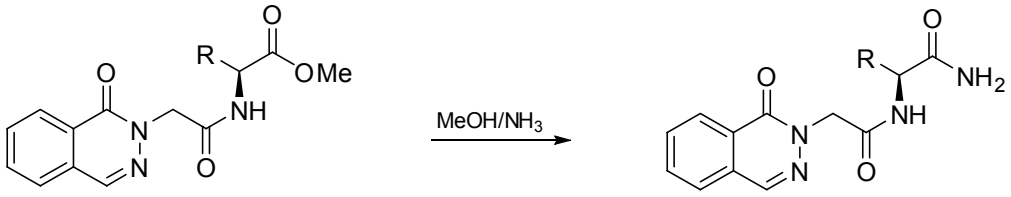


Figure 2. Complete ^{1}H and ^{13}C-NMR assignments of compound **5b**.

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).

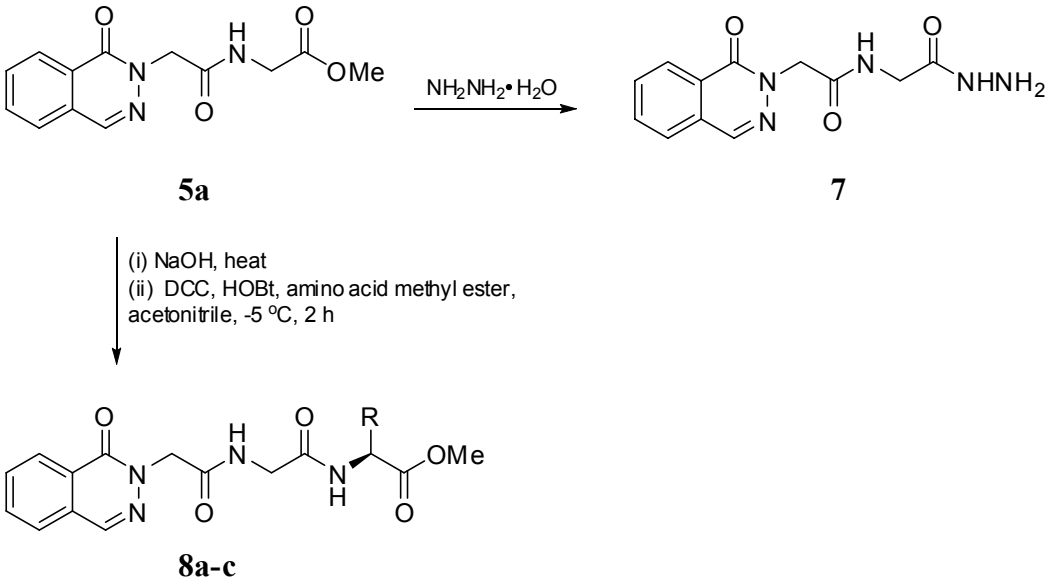


5a-d **6a-d**

6	R	Amino acids
a	H	glycine
b	Me	L-alanine
c	$CH_2CH(Me)_2$	L-leucine
d	CH_2CH_2SMe	L-methionine

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.



5a **7**

8a-c

8	R	Amino acids
a	Me	L-alanine
b	$CH_2CH(Me)_2$	L-leucine
c	Ph	L-phenylglycine

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*.[35] Only the amide compounds **6a-d** showed cytotoxicity with LC_{50} ranging from 3.7 to 6.8 and LC90 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 oxophthalazinone-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 ^{o}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 ^{o}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_2SO_4 to afford **4**. Yield: 0.20 g (87%) of white crystals. mp 200-201 ^{o}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 $C_{10}H_7N_5O_2$ (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 ^{o}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 ^{o}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_2O (10 mL). The organic layer was dried (Na_2SO_4), filtered, and evaporated to dryness. The residue was purified by silica gel column chromatography using 5% MeOH in CH_2Cl_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 Et_3N (0.2 mL) was added the azide **4**. The mixture was kept at -5 ^oC for 24 h, then at 25 ^oC for another 24 h, followed by washing with 0.5 N HCl, water, 3% solution of $NaHCO_3$ and finally dried over anhydrous Na_2SO_4. 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, $CDCl_3$) δ = 3.64 (s, 3H, CH_3), 3.96 (s, 2H, CH_2), 4.92 (s, 2H, NCH_2), 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). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ = 41.10 (CH_3), 52.17 (CH_2), 54.54 (CH_2), 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), 159.67 (C-1), 167.45 (C=O, ester), 169.94 (C=O). Anal. Calcd. for $C_{13}H_{13}N_3O_4$ (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, $CDCl_3$) δ = 1.33 (d, *J* = 2.3 Hz, 3H, CH_3), 3.62 (s, 3H, CH_3), 4.54 (m, 1H, *CH), 4.88 (s, 2H, NCH_2), 6.99 (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, *J* = 7.2 Hz, 1H, H-8). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ = 19.12 (CH_3), 42.54 (CH_3), 51.16 (CH_2), 54.54 (CH), 126.18 (C-8), 126.78 (C-8`), 127.41 (C-5`), 129.78 (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 $C_{14}H_{15}N_3O_4$ (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 ^oC. ^1H NMR (270 MHz, $CDCl_3$): δ = 0.84-0.86 (dd, *J* = 2.3, 2.6 Hz, 6H, $2CH_3$) 1.54-1.56 (m, 3H, CH, CH_2), 3.62 (s, 3H, CH_3), 4.62 (m, 1H, *CH), 4.92 (s, 2H, NCH_2), 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). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ = 22.03 (CH_3), 22.72 (CH_3), 24.83 (CH), 41.61 (CH_2), 50.92 (CH_2, CH_3), 52.26 (CH), 126.32 (C-8), 126.84 (C-8`), 127.68 (C-5`), 129.84 (C-7), 131.96 (C-5), 133.54 (C-6), 138.73 (C-4), 159.82 (C-1), 167.16 (C=O), 173.21 (C=O, ester). Anal. Calcd. for $C_{17}H_{21}N_3O_4$ (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 ^oC. ^1H NMR (270 MHz, $CDCl_3$) δ = 1.88 (s, 3H, SCH_3) 2.02-2.08 (m, 2H, CH_2), 2.42 (t, 2H, SCH_2), 3.59 (s, 3H, CH_3), 4.65 (m, 1H, *CH), 4.89 (s, 2H, NCH_2), 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). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ = 14.96 (SCH_3), 29.51 (CH_2), 31.03 (CH_2), 51.25 (CH_3), 52.08 (CH), 54.26 (CH_2), 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), 171.77 (C=O, ester). Anal. Calcd. for $C_{16}H_{19}N_3O_4S$ (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, $CDCl_3$) δ = 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). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ = 48.03 (CH_3), 52.24 (CH_2), 54.3 4 (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 $C_{19}H_{17}N_3O_4$ (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_2Cl_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) δ = 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). ^{13}C NMR (62.5 MHz, DMSO-d_6): δ = 42.86 (CH_2), 53.69 (CH_2), 125.64 (C-8), 126.81 (C-8`), 127.02 (C-5`), 129.61 (C-7), 131.99 (C-5), 133.59 (C-6), 138.08 (C-4), 158.72 (C-1), 167.06 (C=O), 170.64 (C=O). Anal. Calcd. for $C_{12}H_{12}N_4O_3$ (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) δ = 1.23 (d, *J* = 2.4 Hz, 3H, CH_3), 4.25 (m, 1H, *CH), 4.84 (s, 2H, NCH_2), 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). ^{13}C NMR (62.5 MHz, DMSO-d_6): δ = 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 $C_{13}H_{14}N_4O_3$ (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) δ = 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). ^{13}C NMR (62.5 MHz, DMSO-d_6): δ = 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 $C_{16}H_{20}N_4O_3$ (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. ^{1}H NMR (270 MHz, DMSO-d_6) δ = 1.8 (m, 2H, CH_2), 2.04 (s, 3H, SCH_3), 2.44 (t, 2H, SCH_2), 4.34 (m, 1H, *CH), 4.82 (s, 2H, NCH_2), 7.14, 7.41 (2 × s, 2H, NH_2), 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). ^{13}C NMR (62.5 MHz, DMSO-d_6): δ = 14.53 (SCH_3), 29.56 (CH_2), 31.66 (CH_2), 51.66 (CH_2), 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 $C_{15}H_{18}N_4O_3S$ (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. ^{1}H NMR (270 MHz, DMSO-d_6) δ = 3.67 (d, *J* = 2.1 Hz, 2H, CH_2), 4.22 (br s, 2H, NH_2) 4.81 (s, 2H, NCH_2), 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 $C_{12}H_{13}N_5O_3$ (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% $NaHCO_3$ (10 mL) solution, 1N HCl (10 mL), followed by brine (10 mL), and finally with H_2O (10 mL). The organic layer was dried (Na_2SO_4), filtered, and evaporated to dryness. The residue was purified by silica gel column chromatography using 5% MeOH in CH_2Cl_2 to give **8a-c** in 80-85% yields.

Methyl 2-(2-(2-(1-oxophthalazin-2(1H)-yl)acetamido)acetamido)propanoate (8a). Yield: 0.29 g, (84%) of white crystals; mp 213 °C. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.39 (d, 3H, *J* = 2.5 Hz, CH_3), 3.68 (s, 3H, CH_3), 4.51 (m, 1H, *CH), 4.82 (s, 2H, NCH_2), 5.58 (s, 2H, NCH_2), 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). [1] Anal. Calcd. for $C_{16}H_{18}N_4O_5$ (346): C, 55.49; H, 5.24; N, 16.18. Found: C, 55.44; H, 5.26; N, 16.15**.**

Methyl 2-(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, *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, *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), 167.12 (C=O), 167.32 (C=O), 171.24 (C=O, ester). Anal. Calcd. for $C_{19}H_{24}N_4O_5$ (388): C**,** 58.75; H, 6.23; N, 14.42. Found C, 58.79; H, 6.213; N, 14.40.

Methyl (2-(2-(1-oxophthalazin-2(1*H*)-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, *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 $C_{21}H_{20}N_4O_5$ (408): C, 61.76; H, 4.94; N, 13.72. Found C, 61.73; H, 4.96; N, 13.70.

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