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Amino acid derivatives, VIII [1]: synthesis and antimicrobial evaluation of α-amino acid esters bearing an indole side chain

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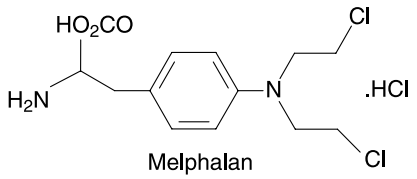
Abstract A series of peptide and dipeptide derivatives conjugated with an indole residue were synthesized. The prepared compounds were tested for antimicrobial activity against two different bacterial and one antifungal species displaying different degrees of antimicrobial activities or inhibitory actions.

Keywords Indole derivatives; Amino acids; Dipeptides; Antimicrobial activity.

Introduction

Indole derivatives are of great significance because of their occurrence in Nature as part of the structure of a large number of alkaloids [2–4] and their wide-ranging biological activity [5, 6], and for this reason indole synthesis is a very active field [7, 8]. On the other hand, several α-amino acids conjugated heterocyles reported as potential antitumor agents, such as 4-toluenesulfonylureido derivatives of amines, amino acids, dipeptides [9], and 2-(4-aminophenyl)benzothiazoles [10]. Some alkylating agents bearing amino acid residues showed high cytotoxic activity against various cancer cell lines, such as melphalan (L-phenylalanine Mustard hydrochloride) [11]. In connection with synthesis of new α-amino acid derivatives [12] and due to the pharmacological properties of indoles and amino acid derivatives we were prompted to prepare new indole bearing amino acid derivatives to study their antimicrobial activity.



Melphalan

Results and discussion

Chemistry

Indole derivatives **1a**–**1c** were treated with ethyl chloroacetate in dry acetone containing anhydrous K_2CO_3 at reflux temperature to afford 1-(ethoxycarbonylmethyl)-1*H*-indoles **2a**–**2c** in 74–80% yields. The ^{1}H NMR spectra showed a triplet at $\delta = 1.21$–1.28 ppm and a quartet at $\delta = 4.15$–4.24 ppm corresponds to O*Et*. The singlet at $\delta = 5.11$–5.22 ppm corresponds to N^1-CH_2, while the signals of H-3 and H-2 appeared as two doublets at $\delta = 6.40$–6.45 and 6.90–6.99 ppm. Nitration of **2a**–**2c** using acetyl nitrate afforded the corresponding 3-nitroindole derivatives **3a**–**3c** in 94–98% yields. The ^{1}H NMR spectra showed a singlet at $\delta = 7.70$–7.75 ppm

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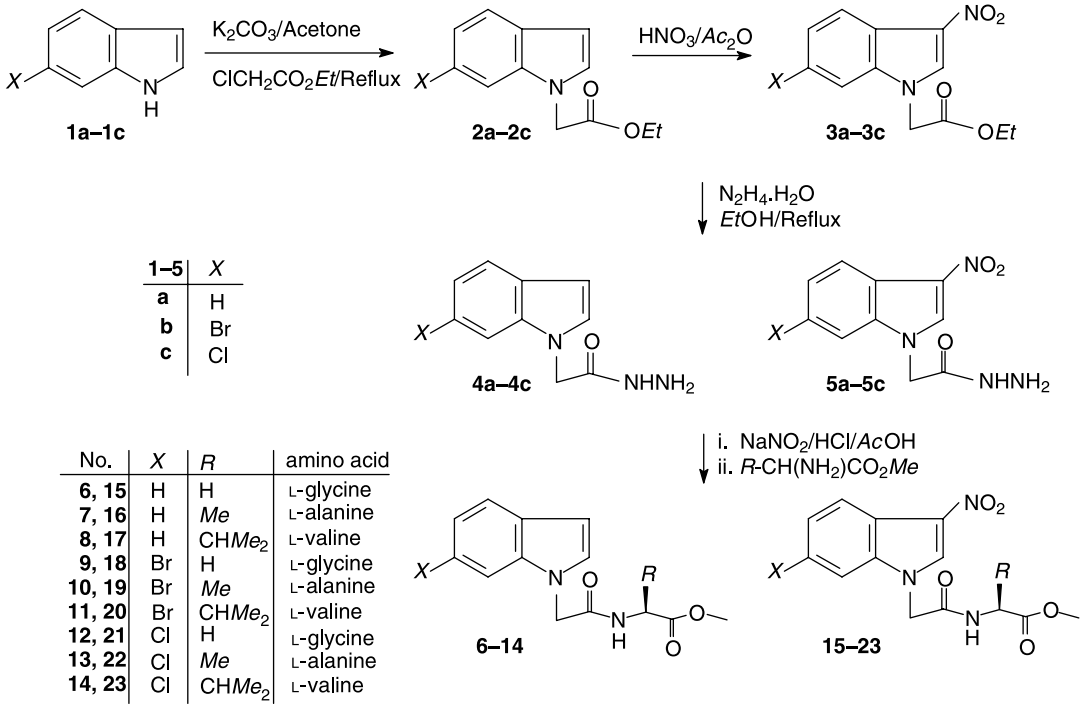
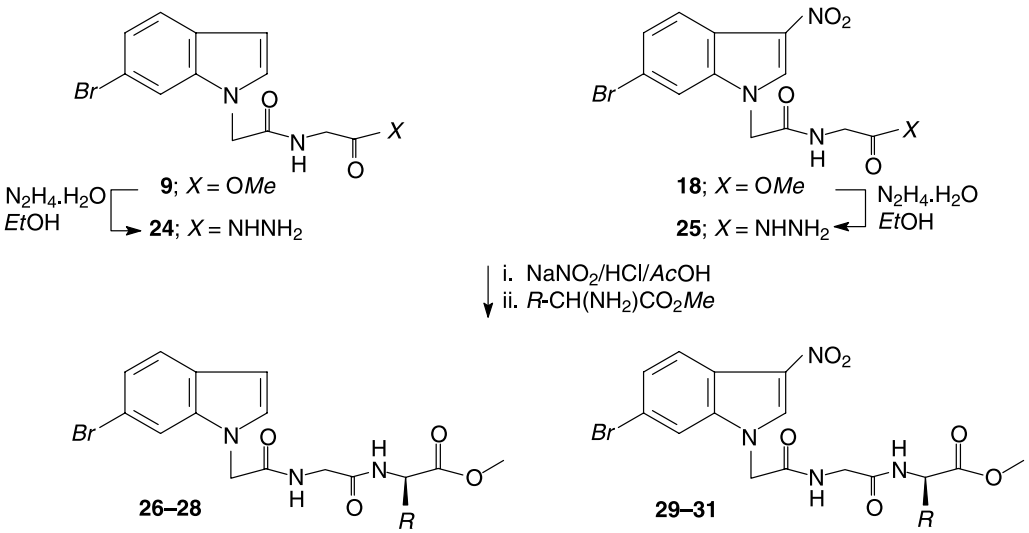
corresponding to H-2. Treatment of **2a**–**2c** or **3a**–**3c** with hydrazine hydrate in ethanol gave the corresponding hydrazide derivatives **4a**–**4c** in 92–95% or **5a**–**5c** in 91–94% yields. These hydrazides were selected as starting materials for the coupling reaction with the appropriate acylated amino acides, *via* the azide-coupling method [13]. Thus, treatment of **4a**–**4c** or **5a**–**5c** at −5°C in *Ac*OH and 1 *N* HCl with



1–5	X
a	H
b	Br
c	Cl

No.	X	R	amino acid
6, 15	H	H	L-glycine
7, 16	H	*Me*	L-alanine
8, 17	H	$CHMe_2$	L-valine
9, 18	Br	H	L-glycine
10, 19	Br	*Me*	L-alanine
11, 20	Br	$CHMe_2$	L-valine
12, 21	Cl	H	L-glycine
13, 22	Cl	*Me*	L-alanine
14, 23	Cl	$CHMe_2$	L-valine

Scheme 1



No.	R	dipeptides
26, 29	H	L-glycyl-L-glycine
27, 30	*Me*	L-glycyl-L-alanine
28, 31	$CHMe_2$	L-glycyl-L-valine

Scheme 2

$NaNO_2$ afforded the inseparable azide derivative. The yellow syrupy azide compound was then treated, *in situ*, with the appropriate amino acid methyl esters in ethyl acetate containing Et_3N at 0°C to give, after neutralization, the desired peptides **6–14** in 73–79% or **15–23** in 70–78% yields. The structures of **6–19** were assigned from their ^1H NMR and mass spectra (Scheme 1).

Treating of **9** or **18** with $N_2H_4 \cdot H_2O$ in ethanol at reflux temperature afforded the corresponding hydrazides **24** or **25** in 91–93% yields. Treatment of **24** or **25** at −5°C in *Ac*OH and 1 *N* HCl with $NaNO_2$ afforded the inseparable azide derivatives. The yellow syrupy azide compounds were treated, as mentioned above, with the appropriate amino acid methyl esters in ethyl acetate containing Et_3N at 0°C to afford **26–28** in 72–75% and **29–31** in 70–72% yields. The structures of the dipeptide derivatives were confirmed by their ^1H NMR and mass spectra (Scheme 2).

Table 1 Antimicrobial activity of the newly synthesized α-amino acid derivatives **6–23** and **26–31**

Compound No.	*S. aureus*	*E. coli*	*C. albicans*
DMF	–	+	+
Cefotaxim	++	+	–
Nystatin	–	–	+
6	+	+	+
7	++	+	+
8	+	+	+
9	+++	+++	++
10	++++	+++	++
11	++++	+++	+
12	+++	+++	+
13	++++	+++	++
14	+++	++	+
15	++	++	++
16	++++	++	++
17	++	++	++
18	++++	+++	++
19	++++	+++	++
20	++++	+++	++
21	+++	++	+
22	+++	++	+
23	+++	++	+
26	+++	++	+
27	++++	++	+
28	+++	++	+
29	++++	++	+
30	++++	++	+
31	++++	++	+

–: no effect, +: low effect, ++: moderate effect, +++: high effect, and ++++: complete effect

Antimicrobial activity

New α-amino acid derivatives bearing an indole side chain were preliminarily evaluated for their *in vitro* antibacterial activity against two representative types of bacteria, *Staphylococcus aureus* as Gram-positive bacteria and *Escherichia coli* as Gram-negative bacteria. The last compounds were also evaluated for their *in vitro* antifungal activity against *C. albicans*. Their inhibition zones using the agar cup diffusion technique [14, 15] were measured. Cefotaxim was used as antibacterial reference, while nystatin was used as antifungal reference. The highest degrees of inhibition were recorded for compounds **10**, **11**, **13**, **16**, **18–20**, **27**, and **29–31** followed by **9**, **12**, **14**, **21–23**, **26**, and **28**, while the lowest degree of inhibition was recorded for compounds **6–8**, **15**, and **17** (Table 1).

Conclusions

New α-amino acid derivatives bearing an indole side chain were synthesized in order to increase the number of tested compounds screened for antimicrobial activity. The data recorded in Table 1 revealed that the 6-haloindoles as well as 6-halo-3-nitroindoles were the most active compounds.

Experimental

General

Melting points were determined using a *Kofler* block instrument. TLC was performed on plastic plates Silica Gel 60 F_{254} (E. Merck, layer thickness 0.2 mm). NMR spectra were recorded on a Bruker AC 250 FT NMR spectrometer at 250 MHz for ^1H NMR with *TMS* as an internal standard. ES mass spectra were obtained from an Esquire 3000plus iontrap mass spectrometer from Bruker Daltonics. The microanalyses were performed at the microanalytical unit, Cairo University, Egypt, and were found to agree favourably with the calculated values. Antimicrobial activity of the synthesized compounds was conducted at the Botany Department, Faculty of Science, Menoufia University, Shebin El-Koam, Egypt.

General procedure for the preparation of 1-(ethoxycarbonylmethyl)-1H-indoles ***2a–2c***

A mixture of **1a–1c** (0.1 mol), 14.7 g ethyl chloroacetate (0.12 mol), and 13.8 g anhydrous K_2CO_3 (0.1 mol) in 36 cm^3 dry acetone was refluxed for 5 h (TLC). The solvent was removed *in vacuo* and the residue was diluted with H_2O and extracted with CH_2Cl_2 (3 × 30 cm). The combined organic layers were dried (Na_2SO_4) and the solvent was evaporated. The residue was purified by silica gel column chromatography using (petroleum *ether:Et*OAc = 7:1) to afford 12.24 g **2** (60%) and 6.12 g **3** (30%).

Ethyl 2-(1H-indol-1-yl)acetate (**2a**, $C_{12}H_{13}NO_2$)
White powder (80%); mp 145–147°C; ^{1}H NMR ($CDCl_3$, 250 MHz): $\delta = 1.21$ (t, $J = 6.1$ Hz, CH_3CH_2O), 4.15 (q, $J = 6.1$ Hz, CH_3CH_2O), 5.11 (s, N^1-CH_2), 6.40 (d, $J = 7.0$ Hz, H-3), 6.90 (d, $J = 7.0$ Hz, H-2), 7.48–7.66 (m, *Ar*–H) ppm; MS (ESI): $m/z = 226$ [$M^+ + Na$].

Ethyl 2-(6-bromo-1H-indol-1-yl)acetate (**2b**, $C_{12}H_{12}BrNO_2$)
White powder (78%); mp 188–189°C; ^{1}H NMR ($CDCl_3$, 250 MHz): $\delta = 1.27$ (t, $J = 6.1$ Hz, CH_3CH_2O), 4.20 (q, $J = 6.1$ Hz, CH_3CH_2O), 5.18 (s, N^1-CH_2), 6.44 (d, $J = 7.0$ Hz, H-3), 6.97 (d, $J = 7.0$ Hz, H-2), 7.00 (d, $J = 7.5$ Hz, H-4), 7.25 (d, $J = 7.5$ Hz, H-5), 7.66 (s, H-7) ppm; MS (ESI): $m/z = 304/306$ [$M^+ + Na$].

Ethyl 2-(6-chloro-1H-indol-1-yl)acetate (**2c**, $C_{12}H_{12}ClNO_2$)
White powder (74%); mp 122–124°C; ^{1}H NMR ($CDCl_3$, 250 MHz): $\delta = 1.28$ (t, $J = 6.1$ Hz, CH_3CH_2O), 4.24 (q, $J = 6.1$ Hz, CH_3CH_2O), 5.22 (s, N^1-CH_2), 6.45 (d, $J = 7.0$ Hz, H-3), 6.99 (d, $J = 7.0$ Hz, H-2), 7.09 (d, $J = 7.5$ Hz, H-4), 7.33 (d, $J = 7.5$ Hz, H-5), 7.71 (s, H-7) ppm; MS (ESI): $m/z = 260$ [$M^+ + Na$].

General procedure for the preparation of 1-(ethoxycarbonylmethyl)-3-nitro-1H-indoles ***3a–3c***
Acetyl nitrate was generated by the dropwise addition of 1.35 cm^3 neat yellow 90% HNO_3 (30 mmol) to 20 cm^3 cooled (0°C) Ac_2O followed by standing at room temperature for 10 min and was used immediately. To a stirred solution of **2a–2c** (1 mmol) in 2 cm^3 Ac_2O at −70°C was added a solution of the acetyl nitrate dropwise *via* addition funnel over 30 min. The mixture was then allowed to warm to room temperature with stirring overnight. The reaction mixture was poured on crushed ice and then extracted with 100 cm^3 CH_2C1_2. The solvent was dried over Na_2SO_4, evaporated, and coevaporated with toluene to afford a yellow solid which was purified by column chromatography (*EtOAc*:*n*-hexane = 1:9) to give **3a–3c** in 94–98% yields.

Ethyl 2-(3-nitro-1H-indol-1-yl)acetate (**3a**, $C_{12}H_{12}N_2O_4$)
Yellow foam (98%); ^{1}H NMR ($CDCl_3$, 250 MHz): $\delta = 1.25$ (t, J = 6.1 Hz, CH_3CH_2O), 4.12 (q, $J = 6.1$ Hz, CH_3CH_2O), 5.00 (s, N^1-CH_2), 7.34–7.40 (m, *Ar*–H), 7.70 (s, H-2), 7.90–7.97 (m, *Ar*–H) ppm; MS (ESI): $m/z = 271$ [$M^+ + Na$].

Ethyl 2-(6-bromo-3-nitro-1H-indol-1-yl)acetate
(**3b**, $C_{12}H_{11}BrN_2O_4$)
Yellow foam (95%); ^{1}H NMR ($CDCl_3$, 250 MHz): $\delta = 1.27$ (t, $J = 6.1$ Hz, CH_3CH_2O), 4.16 (q, $J = 6.1$ Hz, CH_3CH_2O), 4.98 (s, N^1-CH_2), 7.60–7.69 (m, *Ar*–H), 7.75 (s, H-2), 8.05 (s, H-7) ppm; MS (ESI): $m/z = 349/351$ [$M^+ + Na$].

Ethyl 2-(6-chloro-3-nitro-1H-indol-1-yl)acetate
(**3c**, $C_{12}H_{11}ClN_2O_4$)
Yellow foam (94%); ^{1}H NMR ($CDCl_3$, 250 MHz): $\delta = 1.26$ (t, $J = 6.1$ Hz, CH_3CH_2O), 4.13 (q, $J = 6.1$ Hz, CH_3CH_2O), 4.99 (s, N^1-CH_2), 7.75 (d, $J = 7.5$ Hz, H-4), 7.98 (s, H-7), 8.06 (s, H-2), 8.19 (d, $J = 7.5$ H-5) ppm; MS (ESI): $m/z = 305$ [$M^+ + Na$].

General procedure for the preparation of 1-acetylhydrazine-1H-indoles ***4a–4c*** *and* ***5a–5c***
A mixture of **2a–2c** or **3a–3c** (10 mmol) and 1.25 g $N_2H_4 \cdot H_2O$ (25 mmol) in 30 cm^3 ethanol was heated under reflux for 2 h. The excess of ethanol was removed under reduced pressure and the resulting precipitate was filtered off, washed with ethanol, and recrystallized from ethanol to give **4** (93%) and **5** (90%).

2-(lH-Indol-l-yl)acetohydrazide (**4a**, $C_{10}H_{11}N_3O$)
White powder (95%); mp 220–222°C; ^{1}H NMR (*DMSO*-d_6, 250 MHz): $\delta = 4.20$ (br, s, NHNH_2), 5.06 (s, N^1-CH_2), 6.41 (d, $J = 7.0$ Hz, H-3), 7.42 (d, $J = 7.0$ Hz, H-2), 7.70–7.94 (m, *Ar*–H), 9.50 (br, s, N*H*NH_2) ppm; MS (ESI): $m/z = 212$ [$M^+ + Na$].

2-(6-Bromo-1H-indol-1-yl)acetohydrazide
(**4b**, $C_{10}H_{10}BrN_3O$)
Pale yellow powder (93%); mp 187–189°C; ^{1}H NMR (*DMSO*-d_6, 250 MHz): $\delta = 4.66$ (br, s, NHNH_2), 5.14 (s, N^1-CH_2), 6.45 (d, $J = 7.0$ Hz, H-3), 7.08 (d, $J = 7.5$ Hz, H-4), 7.19 (d, $J = 7.5$ Hz, H-5), 7.53 (d, $J = 7.0$ Hz, H-2), 7.66 (s, H-7), 9.45 (br, s, N*H*NH_2) ppm; MS (ESI): $m/z = 290/292$ [$M^+ + Na$].

2-(6-Chloro-1H-indol-1-yl)acetohydrazide
(**4c**, $C_{10}H_{10}ClN_3O$)
White powder (92%); mp 236–238°C; ^{1}H NMR (*DMSO*-d_6, 250 MHz): $\delta = 4.43$ (br, s, NHNH_2), 5.10 (s, N^1-CH_2), 6.75 (d, $J = 7.0$ Hz, H-3), 7.02 (d, $J = 7.5$ Hz, H-4), 7.14 (d, $J = 7.5$ Hz, H-5), 7.55 (s, H-7), 7.73 (d, $J = 7.0$ Hz, H-2), 9.49 (br, s, N*H*NH_2) ppm; MS (ESI): $m/z = 246$ [$M^+ + Na$].

2-(3-Nitro-1H-indol-1-yl)acetohydrazide (**5a**, $C_{10}H_{10}N_4O_3$)
Pale yellow powder (94%); mp 163–165°C; ^{1}H NMR (*DMSO*-d_6, 250 MHz): $\delta = 4.35$ (br, s, NHNH_2), 4.99 (s, N^1-CH_2), 7.30–7.50 (m, *Ar*–H), 7.72 (s, H-2), 9.20 (br, s, N*H*NH_2) ppm; MS (ESI): $m/z = 257$ [$M^+ + Na$].

2-(6-Bromo-3-nitro-1H-indol-1-yl)acetohydrazide
(**5b**, $C_{10}H_9BrN_4O_3$)
Pale yellow powder (93%); mp 199–201°C; ^{1}H NMR (*DMSO*-d_6, 250 MHz): $\delta = 4.60$ (br, s, NHNH_2), 5.06 (s, N^1-CH_2), 7.55 (m, *Ar*–H), 7.77 (s, H-2), 7.96 (s, H-7), 9.32 (br, s, N*H*NH_2) ppm; MS (ESI): $m/z = 335/337$ [$M^+ + Na$].

2-(6-Chloro-3-nitro-1H-indol-1-yl)acetohydrazide
(**5c**, $C_{10}H_9ClN_4O_3$)
Pale yellow powder (91%); mp 229–231°C; ^{1}H NMR (*DMSO*-d_6, 250 MHz): $\delta = 4.50$ (br, s, NHNH_2), 5.02 (s, N^1-CH_2), 7.65 (d, $J = 7.5$ Hz, H-4), 7.90 (s, H-7), 8.00 (d, $J = 7.5$ Hz, H-5), 8.06 (s, H-2), 9.45 (br, s, N*H*NH_2) ppm; MS (ESI): $m/z = 291$ [$M^+ + Na$].

General procedure for the preparation of N^1-indole bearing amino acid esters ***6–23***

A solution of 1.90 g **4** or **5** (4 mmol) in 30 cm^3 HO*Ac*, 15 cm^3 1 *N* HCl, and 125 cm^3 H_2O was cooled in an ice-bath (−5°C). Sodium nitrite (4.35 g, 63 mmol) in 15 cm^3 cold H_2O was added with stirring. After stirring at −5°C for 15 min, the yellow syrup was formed. The azide was taken in 150 cm^3 cold ethyl acetate, washed with 150 cm^3 $NaHCO_3$ (3%), 150 cm^3 H_2O, and dried (Na_2SO_4). A solution of the appropriate amino acid methyl ester hydrochloride (4.5 mmol) in 100 cm^3 ethyl acetate containing 1.0 cm^3 Et_3N was stirred at 0°C for 20 min, filtered, and the filtrate was added to the azide solution. The mixture was kept at −5°C for 12 h, then at room temperature for another 12 h, followed by washing with 150 cm^3 0.5 *N* HC1, 150 cm^3 $NaHCO_3$ (3%), 150 cm^3 H_2O, and dried (Na_2SO_4). The filtrate was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography using petroleum *ether:Et*O*Ac* = 7:1 to afford **6–14** in 73–79% or **15–23** in 70–79% yields.

Methyl 2-[2-(1H-indol-1 -yl)acetamido]acetate (**6**, $C_{13}H_{14}N_2O_3$)

White foam (77%); ^{1}H NMR ($CDCl_3$, 250 MHz): $\delta = 3.60$ (s, OCH_3), 4.00 (s, CH_2), 5.43 (s, N^1-CH_2), 6.53 (d, $J = 7.0$ Hz, H-3), 7.59 (d, $J = 7.0$ Hz, H-2), 7.74–7.81 (m, *Ar*–H), 8.40 (br, s, NH) ppm; MS (ESI): $m/z = 269$ [$M^+ + Na$].

(S)-Methyl 2-[2-(1H-indol-1-yl)acetamido]propanoate (**7**, $C_{14}H_{16}N_2O_3$)

White foam (79%); ^{1}H NMR ($CDCl_3$, 50 MHz): $\delta = 1.49$ (d, $J = 5.0$ Hz, CH_3), 3.50 (s, OCH_3), 4.50 (q, $J = 5.0$ Hz, CH), 5.50 (s, N^1-CH_2), 6.60 (d, $J = 7.0$ Hz, H-3), 7.55 (d, $J = 7.0$ Hz, H-2), 7.70–7.85 (m, *Ar*–H), 8.40 (br, s, NH) ppm; (MS (ESI): $m/z = 283$ [$M^+ + Na$].

(S)-Methyl 2-[2-(1H-indol-1 -yl)acetamido]-3-methylbutanoate (**8**, $C_{16}H_{20}N_2O_3$)

White foam (78%); ^{1}H NMR ($CDC1_3$, 250 MHz): $\delta = 0.99$ (dd, $J = 1.9$, 7.2 Hz, $2 \times CH_3$), 2.30–2.37 (m, CH), 3.60 (s, OCH_3), 4.30–4.40 (m, CH), 5.51 (s, N^1-CH_2), 6.63 (d, $J = 7.0$ Hz, H-3), 7.52 (d, $J = 7.0$ Hz, H-2), 7.69–7.80 (m, *Ar*–H), 8.40 (br, s, NH) ppm; MS (ESI): $m/z = 311$ [$M^+ + Na$].

Methyl 2-[2-(6-bromo-1H-indol-1-yl)acetamido]acetate (**9**, $C_{13}H_{13}BrN_2O_3$)

Pale yellow foam (75%); ^{1}H NMR ($CDCl_3$, 250 MHz): $\delta = 3.57$ (s, OCH_3), 4.09 (s, CH_2), 5.55 (s, N^1-CH_2), 6.52 (d, $J = 7.0$ Hz, H-3), 7.00 (d, $J = 7.5$ Hz, H-4), 7.22 (d, $J = 7.5$ Hz, H-5), 7.48 (d, $J = 7.0$ Hz, H-2), 7.60 (s, H-7), 8.30 (br, s, NH) ppm; MS (ESI): $m/z = 347/349$ [$M^+ + Na$].

(S)-Methyl 2-[2-(6-bromo-1H-indol-1-yl)acetamido]-propanoate (**10**, $C_{14}H_{15}BrN_2O_3$)

Pale yellow foam (74%); ^{1}H NMR ($CDCl_3$, 250 MHz): $\delta = 1.45$ (d, $J = 5.0$ Hz, CH_3), 3.42 (s, OCH_3), 4.39 (q, $J = 5.0$ Hz, CH), 5.44 (s, N^1-CH_2), 6.52 (d, $J = 7.0$ Hz, H-3), 7.11 (d, $J = 7.5$ Hz, H-4), 7.16 (d, $J = 7.5$ Hz, H-5), 7.55 (d, $J = 7.0$ Hz, H-2), 7.68 (s, H-7), 8.33 (br, s, NH) ppm; MS (ESI): $m/z = 361/363$ [$M^+ + Na$].

(S)-Methyl 2-[2-(6-bromo-1H-indol-1-yl)acetamido]-3-methyl-butanoate (**11**, $C_{16}H_{19}BrN_2O_3$)

Pale yellow foam (73%); ^{1}H NMR ($CDCl_3$, 250 MHz): $\delta = 0.96$ (dd, $J = 1.9$, 7.2 Hz, $2 \times CH_3$), 2.30–2.40 (m, CH), 3.50 (s, OCH_3), 4.26–4.38 (m, CH), 5.48 (s, N^1-CH_2), 6.49 (d, $J = 7.0$ Hz, H-3), 7.06 (d, $J = 7.5$ Hz, H-4), 7.13 (d, $J = 7.5$ Hz, H-5), 7.58 (d, $J = 7.0$ Hz, H-2), 7.69 (s, H-7), 8.37 (br, s, NH) ppm; MS (ESI): $m/z = 389/391$ [$M^+ + Na$].

Methyl 2-[2-(6-chloro-1H-indol-1-yl)acetamido]acetate (**12**, $C_{13}H_{13}ClN_2O_3$)

Colorless syrup (75%); ^{1}H NMR ($CDCl_3$, 250 MHz): $\delta = 3.50$ (s, OCH_3), 4.11 (s, CH_2), 5.47 (s, N^1-CH_2), 6.79 (d, $J = 7.0$ Hz, H-3), 7.09 (d, $J = 7.5$ Hz, H-4), 7.17 (d, $J = 7.5$ Hz, H-5), 7.50 (s, H-7), 7.73 (d, $J = 7.0$ Hz, H-2), 8.40 (br, s, NH) ppm; MS (ESI): $m/z = 303$ [$M^+ + Na$].

(S)-Methyl 2-[2-(6-chloro-1H-indol-1-yl)acetamido]-propanoate (**13**, $C_{14}H_{15}ClN_2O_3$)

Colorless syrup (74%); ^{1}H NMR ($CDCl_3$, 250 MHz): $\delta = 1.53$ (d, $J = 5.0$ Hz, CH_3), 3.53 (s, OCH_3), 4.57 (q, $J = 5.0$ Hz, CH), 5.50 (s, N^1-CH_2), 6.74 (d, $J = 7.0$ Hz, H-3), 7.06 (d, $J = 7.5$ Hz, H-4), 7.14 (d, $J = 7.5$ Hz, H-5), 7.53 (s, H-7), 7.76 (d, $J = 7.0$ Hz, H-2), 8.42 (br, s, NH) ppm; MS (ESI): $m/z = 217$ [$M^+ + Na$].

(S)-Methyl 2-[2-(6-chloro-1H-indol-1-yl)acetamido]-3-methyl-butanoate (**14**, $C_{16}H_{19}ClN_2O_3$)

Colorless syrup (73%); ^{1}H NMR ($CDCl_3$, 250 MHz): $\delta = 0.99$ (dd, $J = 1.9$, 7.2 Hz, $2 \times CH_3$), 2.30–2.44 (m, CH), 3.55 (s, OCH_3), 4.30–4.46 (m, CH), 5.45 (s, N^1-CH_2), 6.70 (d, $J = 7.0$ Hz, H-3), 7.03 (d, $J = 7.5$ Hz, H-4), 7.19 (d, $J = 7.5$ Hz, H-5), 7.57 (s, H-7), 7.80 (d, $J = 7.0$ Hz, H-2), 8.44 (br, s, NH) ppm; MS (ESI): $m/z = 345$ [$M^+ + Na$].

Methyl 2-[2-(3-nitro-1H-indol-1-yl)acetamido]acetate (**15**, $C_{13}H_{13}N_3O_5$)

Pale yellow foam (78%); ^{1}H NMR ($CDCl_3$, 250 MHz): $\delta = 3.62$ (s, OCH_3), 4.06 (s, CH_2), 5.48 (s, N^1-CH_2), 7.30–7.48 (m, *Ar*–H), 7.70 (s, H-2), 8.30 (br, s, NH) ppm; MS (ESI): $m/z = 314$ [$M^+ + Na$].

(S)-Methyl 2-[2-(3-nitro-1H-indol-1-yl)acetamido]-propanoate (**16**, $C_{14}H_{15}N_3O_5$)

Pale yellow foam (78%); ^{1}H NMR ($CDCl_3$, 250 MHz): $\delta = 1.52$ (d, $J = 5.0$ Hz, CH_3), 3.56 (s, OCH3), 4.50 (q, $J = 5.0$ Hz, CH), 5.50 (s, N^1-CH_2), 7.30–7.53 (m, *Ar*–H), 7.77 (s, H-2), 8.37 (br, s, NH) ppm; MS (ESI): $m/z = 328$ [$M^+ + Na$].

(S)-Methyl 2-[2-(3-nitro-1H-indol-1-yl)acetamido]-3-methyl-butanoate (**17**, $C_{16}H_{19}N_3O_5$)

Pale yellow foam (77%); ^{1}H NMR ($CDCl_3$, 250 MHz): $\delta = 0.96$ (dd, $J = 1.9$, 7.2 Hz, $2 \times CH_3$), 2.34–2.44 (m, CH),

3.50 (s, OCH_3), 4.30–4.38 (m, CH), 5.50 (s, N^1-CH_2), 7.30–7.45 (m, *Ar*–H), 7.71 (s, H-2), 8.36 (br, s, NH) ppm; MS (ESI): $m/z = 356$ [$M^+ + Na$].

Methyl 2-[2-(6-bromo-3-nitro-1H-indol-1-yl)acetamido]-acetate (**18**, $C_{13}H_{12}BrN_3O_5$)
Pale yellow syrup (76%); ^{1}H NMR ($CDCl_3$, 250 MHz): $\delta = 3.58$ (s, OCH_3), 4.00 (s, CH_2), 5.47 (s, N^1-CH_2), 7.55 (m, *Ar*–H), 7.77 (s, H-2), 7.97 (s, H-7), 8.34 (br, s, NH) ppm; MS (ESI): $m/z = 392/394$ [$M^+ + Na$].

(S)-Methyl 2-[2-(6-bromo-3-nitro-1H-indol-1-yl)-acetamido]propanoate (**19**, $C_{14}H_{14}BrN_3O_5$)
Pale yellow foam (75%); ^{1}H NMR ($CDCl_3$, $\delta = 250$ MHz): $\delta = 1.45$ (d, $J = 5.0$ Hz, CH_3), 3.48 (s, OCH_3), 4.52 (q, $J = 5.0$ Hz, CH), 5.60 (s, N^1-CH_2), 7.57 (m, *Ar*–H), 7.79 (s, H-2), 7.96 (s, H-7), 8.32 (br, s, NH) ppm; MS (ESI): $m/z = 406/408$ [$M^+ + Na$].

(S)-Methyl 2-[2-(6-bromo-3-nitro-1H-indol-1-yl)acetamido]-3-methylbutanoate (**20**, $C_{16}H_{18}BrN_3O_5$)
Pale yellow foam (74%); ^{1}H NMR ($CDCl_3$, 250 MHz): $\delta = 0.97$ (dd, $J = 1.9$, 7.2 Hz, $2 \times CH_3$), 2.30–2.39 (m, CH), 3.60 (s, OCH_3), 4.30–4.40 (m, CH), 5.50 (s, N^1-CH_2), 7.50 (m, *Ar*–H), 7.75 (s, H-2), 7.97 (s, H-7), 8.30 (br, s, NH) ppm; MS (ESI): $m/z = 434/436$ [$M^+ + Na$].

Methyl 2-[2-(6-chloro-3-nitro-1H-indol-1-yl)acetamido]-acetate (**21**, $C_{13}H_{12}ClN_3O_5$)
Colorless syrup (73%); ^{1}H NMR ($CDCl_3$, 250 MHz): $\delta = 3.50$ (s, OCH_3), 3.98 (s, CH_2), 5.42 (s, N^1-CH_2), 7.60 (d, $J = 7.5$ Hz, H-4), 7.90 (s, H-7), 8.02 (d, $J = 7.5$ Hz, H-5), 8.11 (s, H-2), 8.35 (br, s, NH) ppm; MS (ESI): $m/z = 348$ [$M^+ + Na$].

(S)-Methyl 2-[2-(6-chloro-3-nitro-1H-indol-1-yl)-acetamido]propanoate (**22**, $C_{14}H_{14}ClN_3O_5$)
Colorless syrup (72%); ^{1}H NMR ($CDCl_3$, 250 MHz): $\delta = 1.43$ (d, $J = 5.0$ Hz, CH_3), 3.50 (s, OCH_3), 4.47 (q, $J = 5.0$ Hz, CH), 5.56 (s, N^1-CH_2), 7.64 (d, $J = 7.5$ Hz, H-4), 7.80 (s, H-7), 8.06 (d, $J = 7.5$ Hz, H-5), 8.09 (s, H-2), 8.30 (br, s, NH) ppm; MS (ESI): $m/z = 362$ [$M^+ + Na$].

(S)-Methyl 2-[2-(6-chloro-3-nitro-1H-indol-1-yl)acetamido]-3-methylbutanoate (**23**, $C_{16}H_{18}ClN_3O_5$)
Colorless syrup (70%); ^{1}H NMR ($CDC1_3$, 250 MHz): $\delta = 0.98$ (dd, $J = 1.9$, 7.2 Hz, $2 \times CH_3$), 2.33–2.42 (m, CH), 3.63 (s, OCH_3), 4.30–4.44 (m, CH), 5.55 (s, N^1-CH_2), 7.63 (d, $J = 7.5$ Hz, H-4), 7.86 (s, H-7), 8.02 (d, $J = 7.5$ Hz, H-5), 8.11 (s, H-2), 8.42 (br, s, NH) ppm; MS (ESI): $m/z = 390$ [$M^+ + Na$].

*General procedure for the preparation of the hydrazides **24** and **25***
A mixture of **9** or **18** (5 mmol) and 0.63 g $N_2H_4 \cdot H_2O$ (12.5 mmol) in 15 cm^3 *Et*OH was heated under reflux for 4 h. The excess of *Et*OH was removed under reduced pressure and the resulting precipitate was filtered off and recrystallized from *Et*OH to give **24** and **25** in 91–93% yields.

2-(6-Bromo-1H-indol-1-yl)-N-(2-hydrazinyl-2-oxoethyl)-acetamide (**24**, $C_{12}H_{13}BrN_4O_2$)
White powder (93%); mp 188–189°C; ^{1}H NMR (*DMSO*-d_6, 250 MHz): $\delta = 3.53$ (s, CH_2), 4.90 (br, s, NHN*H*$_2$), 5.22 (s, N^1-CH_2), 7.50–7.55 (m, *Ar*–H), 7.72–7.79 (m, *Ar*–H), 7.92 (s, H-7), 9.32 (br, s, N*H*NH$_2$) ppm; MS (ESI): $m/z = 347/349$ [$M^+ + Na$].

2-(6-Bromo-3-nitro-1H-indol-1-yl)-N-(2-hydrazinyl-2-oxoethyl)acetamide (**25**, $C_{12}H_{12}BrN_5O_4$)
White powder (91%); mp 212–214°C; ^{1}H NMR (*DMSO*-d_6, 250 MHz): $\delta = 3.57$ (s, CH_2), 5.03 (br, s, NHN*H*$_2$), 5.19 (s, N^1-CH_2), 7.45–7.59 (m, *Ar*–H), 7.78 (s, H-2), 7.99 (s, H-7), 9.40 (br, s, N*H*NH$_2$) ppm; MS (ESI): $m/z = 392/394$ [$M^+ + Na$].

*General procedure for the preparation of dipeptides **26–31***
A solution of **24** or **25** (1 mmol) in 7 cm^3 HO*Ac*, 4 cm^3 1 *N* HCl, and 30 cm^3 H_2O was cooled in an ice-bath (−5°C). Sodium nitrite (1 g, 14.31 mmol) in 4 cm cold H_2O was added with stirring. After stirring at −5°C for 15 min, the yellow syrup was formed. The azide was taken in 30 cm^3 cold ethyl acetate, washed with 30 cm^3 $NaHCO_3$ (3%), 30 cm^3 H_2O, and dried (Na_2SO_4). A solution of the appropriate amino acid methyl ester hydrochloride (1 mmol) in 22 cm^3 ethyl acetate containing 0.3 cm^3 of Et_3N was stirred at 0°C for 20 min, filtered, and the filtrate was added to the azide solution. The mixture was kept at −5°C for 12 h, then at room temperature for another 12 h, followed by washing with 30 cm^3 0.5 *N* HCl, 30 cm^3 $NaHCO_3$ (3%), 30 cm^3 H_2O, and dried (Na_2SO_4). The filtrate was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography using petroleum ether:*Et*O*Ac* = 5:1 to afford **26–28** in 75–78% and **29–31** in 70–71% yields.

Methyl 2-{2-[2-(6-bromo-1H-indol-1-yl)acetamido]-acetamido}acetate (**26**, $C_{15}H_{16}BrN_3O_4$)
Pale yellow foam (75%); ^{1}H NMR ($CDCl_3$, 250 MHz): $\delta = 3.50$ (s, OCH_3), 3.60 (s, CH_2), 3.80 (s, CH_2), 5.10 (s, N^1-CH_2), 7.50–7.60 (m, *Ar*–H), 7.70–7.77 (m, *Ar*–H), 7.90 (s, H-7), 8.67 (br, s, NH) ppm; MS (ESI): $m/z = 404/406$ [$M^+ + Na$].

(S)-Methyl 2-{2-[2-(6-bromo-1H-indol-1-yl)acetamido]-acetamido}propanoate (**27**, $C_{16}H_{18}BrN_3O_4$)
Pale yellow foam (72%); ^{1}H NMR ($CDCl_3$, 250 MHz): $\delta = 1.37$ (d, $J = 5.0$ Hz, CH_3), 3.60 (s, CH_2), 3.70 (s, OCH_3), 4.50 (q, $J = 5.0$ Hz, CH), 5.22 (s, N^1-CH_2), 7.50–7.56 (m, *Ar*–H), 7.72–7.79 (m, *Ar*–H), 7.88 (s, H-7), 9.01 (br, s, NH) ppm; MS (ESI): $m/z = 418/420$ [$M^+ + Na$].

(S)-Methyl 2-{2-[2-(6-bromo-1H-indol-1-yl)acetamido]-acetamido}-3-methylbutanoate (**28**, $C_{18}H_{22}BrN_3O_4$)
Pale yellow foam (72%); ^{1}H NMR ($CDCl_3$, 250 MHz): $\delta = 0.92$ (dd, $J = 1.9$, 7.3 Hz, $2 \times CH_3$), 2.25–2.38 (m, CH),

3.55 (s, OCH_3), 3.70 (s, CH_2), 3.77 (s, CH_2), 4.35–4.49 (m, CH), 5.19 (s, N^1-CH_2), 7.50–7.55 (m, *Ar*–H), 7.70–7.80 (m, *Ar*–H), 7.90 (s, H-7), 8.89 (br, s, NH) ppm; MS (ESI): $m/z = 446/448$ [$M^+ + Na$].

Methyl 2-{2-[2-(6-bromo-3-nitro-1H-indol-1-yl)-acetamido]acetamido}acetate (**29**, $C_{15}H_{15}BrN_4O_6$)
Yellow foam (72%); ^{1}H NMR ($CDCl_3$, 250 MHz): $\delta = 3.55$ (s, OCH_3), 3.72 (s, CH_2), 3.87 (s, CH_2), 5.21 (s, N^1-CH_2), 7.45–7.57 (m, *Ar*–H), 7.75 (s, H-2), 7.95 (s, H-7), 8.72 (br, s, NH) ppm; MS (ESI): $m/z = 449/451$ [$M^+ + Na$].

(S)-Methyl 2-{2-[2-(6-bromo-3-nitro-1H-indol-1-yl)-acetamido]acetamido}propanoate (**30**, $C_{16}H_{16}BrN_4O_6$)
Yellow foam (71%); ^{1}H NMR ($CDCl_3$, 250 MHz): $\delta = 1.37$ (d, $J = 5.0$ Hz, CH_3), 3.63 (s, CH_2), 3.70 (s, OCH_3), 4.54 (q, $J = 5.0$ Hz, CH), 5.16 (s, N^1-CH_2), 7.45–7.60 (m, *Ar*–H), 7.79 (s, H-2), 7.96 (s, H-7), 8.88 (br, s, NH) ppm; MS (ESI): $m/z = 463/465$ [$M^+ + Na$].

(S)-Methyl 2-{2-[2-(6-bromo-3-nitro-1H-indol-1-yl)-acetamido]acetamido}-3-methylbutanoate
(**31**, $C_{18}H_{21}BrN_4O_6$)
Yellow foam (70%); ^{1}H NMR ($CDCl_3$, 250 MHz): $\delta = 0.98$ (dd, $J = 1.9, 7.3$ Hz, $2 \times CH_3$), 2.25–2.35 (m, CH), 3.66 (s, OCH_3), 3.76 (s, CH_2), 3.83 (s, CH_2), 4.46–4.55 (m, CH), 5.16 (s, N^1-CH_2), 7.45–7.56 (m, *Ar*–H), 7.75 (s, H-2), 7.90 (s, H-7), 8.94 (br, s, NH) ppm; MS (ESI): $m/z = 491/493$ [$M^+ + Na$].

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