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Amino acid derivatives, IX [1]: synthesis and antimicrobial evaluation of α-amino acid esters bearing a tryptophane side chain

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Abstract A series of peptide and dipeptide derivatives conjugated with a tryptophane residue were synthesized. The prepared compounds were tested for antimicrobial activity against four different bacterial species displaying different degrees of antibacterial activities or inhibitory actions.

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

Introduction

Tryptophane is considered as one of the most important nutritionally essential amino acids and represents an exceptional component in its diversity of biological functions. It contributes importantly to normal growth and protein synthesis in a number of tissues [2] and regulates numerous physiological mechanisms. For example, tryptophane is the precursor of the neurotransmitter serotonine (5-hydroxytryptamine) and therefore is important in brain function [3]. It can influence sleep in man [4] and the aging process of rats [5]. Tryptophane and some of its derivatives also alter behavior [6], particularly the regulation of the intake of food and drink [7]. Tryptophane also serves as the *in vivo* precursor for the vitamin niacin [8], stimulates insulin and growth hormone secretion, and prevents the development of cortisone-induced hypertension [9], interacts with carbohydrates [10] and mycotoxins [11], and participates in the formation of enzyme-enzyme inhibitor complexes [12]. Furthermore, the essential amino acid L-tryptophane is one limiting factor in protein biosynthesis and is involved in biosynthesis of nicotinic acid derivatives. It is also well known that, alkaloids derived from the indole containing amino acid, tryptophane, are found widely in nature [13] and exhibit a variety of pharmacologically important effects [14]. These include the human 5-hydroxytryptamine (5-HT) hormones, serotonine, which is involved in regulation of the nervous system including neurotransmission, and melatonin, which regulates circadian rhythms and sleep processes. Wide interest for the natural occurrence of tryptophane glycoconjugates as a new group of tryptophane derivatives was raised by reports on enzymatically glycosylated tryptophane residues in proteins [15]. In connection with our work in synthesis of new α-amino acid derivatives [16] and due to the pharmacological properties of tryptophane and amino acid derivatives prompted us to prepare new tryptophane bearing amino acid derivatives to study their antimicrobial activity.

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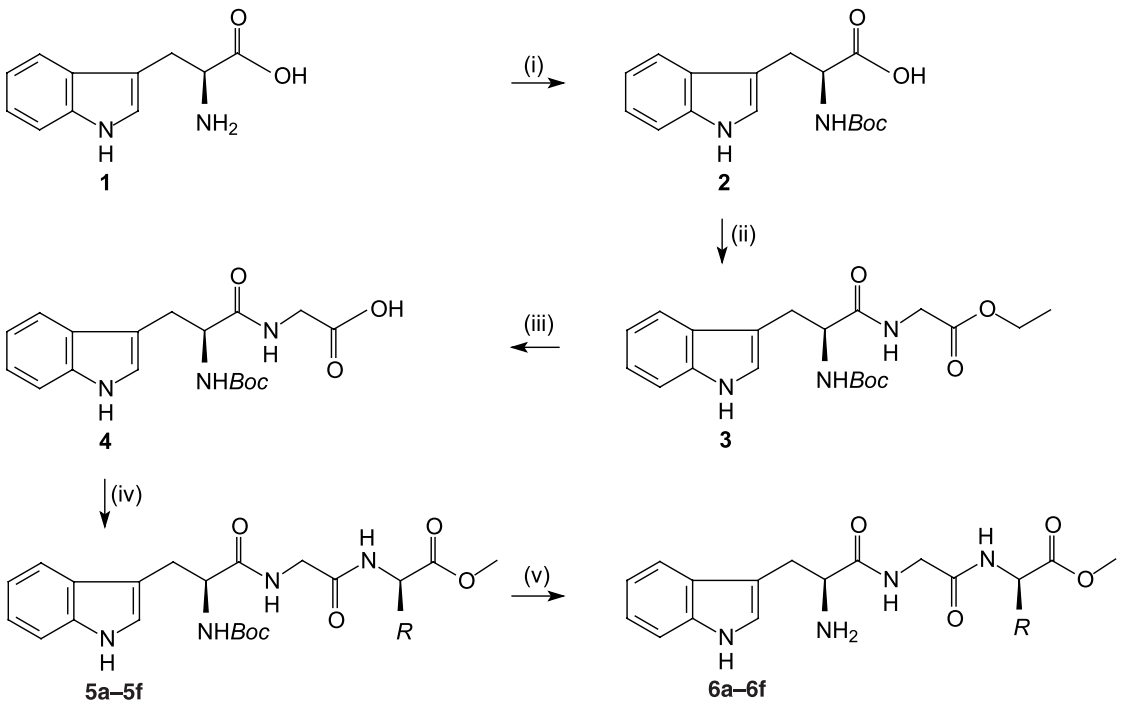
Results and discussion

Chemistry

L-Tryptophane (**1**) was treated with di-*tert*-butylcarbonate in 10% NaOH to afford the corresponding NH-*Boc* derivative **2** in 67% yield. The ^{1}H NMR spectrum showed a singlet at $\delta = 1.43$ ppm for the three methyl of *Boc* group. Treatment of **2** with glycine ethyl ester hydrochloride in the presence of $Et_3N/TBTU$ gave **3** in 78% yield. The ^{1}H NMR spectrum showed a triplet and quartet at $\delta = 1.23$ and 4.12 ppm for the OCH_2CH_3 group. The ethyl acetate derivative **3** was hydrolyzed in 80% yield to the corresponding carboxylic acid **4** using 2*N* KOH in *Me*OH. A suitable coupling method [17] was employed for the formation of peptides by reaction of the carboxylic acid group with an acylated amino acid using 1-hydroxybenzotriazole (HO*Bt*) [18], and *N*,*N*′-dicyclohexylcarbodiimide (*DCC*) [19] as coupling reagents. HO*Bt* is currently the most frequently used activating agent for the carboxyl group of amino acids. The procedure is fast and suppresses racemization, especially in the presence of *DCC* [20]. Amides **5a**–**5f** were prepared by coupling of **4** with the appropriate acylated amino acids in the presence of HO*Bt* and *DCC* to yield **5a**–**5f** in 71–80% yields after chromatography. The structures of the newly synthesized compounds were determined from their ^{1}H NMR and mass spectra. The ^{1}H NMR spectra



(i) Boc_2O/dioxane/NaOH; (ii) glycine ethyl ester/*DCC*/HO*Bt*/*Me*CN;
(iii) KOH/*Me*OH; (iv) AA methyl ester/*DCC*/HO*Bt*/*Me*CN; (v) *TFA*/CH_2Cl_2

5, 6	*R*	Amino acid
a	H	Glycine
b	*Me*	L-Alanine
c	$CH(Me)_2$	L-Valine
d	$CH_2CH(Me)_2$	L-Leucine
e	CH_2CH_2SMe	L-Methionine
f	*Ph*	L-Phenylglycine

Scheme 1

showed a singlet at $\delta = 3.60$–3.69 ppm for the O*Me*. The protons of the amino acid residues were fully assigned. The free amino derivatives **6a**–**6f** were obtained in a quantitative yields after the deprotection of **5a**–**5f** using 50% *TFA* in CH_2Cl_2 at room temperature. The structures of the deprotected derivatives were confirmed by their ^{1}H NMR and mass spectra, which showed the disappearance of the *Boc*-group in all cases (Scheme 1).

Antimicrobial activity

The new α-amino acid derivatives bearing a tryptophane side chain were preliminary 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 [21, 22] were measured. Cefotaxim was used as antibacterial reference, while nystatin was used as antifungal references. The highest degrees of inhibition were recorded for compounds **6a**–**6d** and **6f** followed by **6a** and **6e**, while the lowest degree of inhibition was recorded for compounds **5a**–**5f** (Table 1).

Table 1 Antimicrobial activity of the newly synthesized compounds **5**–**6**

Compd No.	*S. aureus*	*E. coli*	*C. albicans*
DMF	−	+	+
Cefotaxim	++	+	−
Nystatin	−	−	+
5a	++	+	+
5b	++	++	−
5c	+	++	+
5d	++	+	++
5e	+	+	+
5f	++	+	+
6a	+++	++	++
6b	++++	+++	+++
6c	++++	++++	++
6d	++++	+++	++
6e	+++	+	+
6f	++++	+++	+++

− No antimicrobial effect
+ Low antimicrobial effect (4 mm)
++ Moderate antimicrobial effect (8–10 mm)
+ ++ High antimicrobial effect (15–18 mm)
++++ Complete antimicrobial effect (20–22 mm)

Conclusions

New α-amino acid derivatives bearing a tryptophane 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 free amino derivatives 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 F254 (E. Merck, layer thickness 0.2 mm). NMR spectra were recorded on a Bruker AC 300 FT NMR spectrometer at 300 MHz for ^{1}H NMR with *TMS* as an internal standard. ESI 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 favorably 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.

(S)-3-(1H-Indol-3-yl)-2-(t-butoxycarbonylamino)propanoic acid (**2**, $C_{16}H_{20}N_2O_4$)

To a solution of 2.0 g **1** (9.8 mmol) in 120 cm^3 dioxane, an aqueous solution of 10 cm^3 10% NaOH was added. The resulting solution was stirred at room temperature and 3.41 g di-*tert*-butylcarbonate (19.6 mmol) were added with constant stirring for overnight. The solvent was evaporated under reduced pressure and the residue was dissolved in 100 cm^3 H_2O, acidified with conc. HCl, and extracted with 3×100 cm^3 *Et*O*Ac*. The organic phase was washed with 100 cm^3 H_2O, 100 cm^3 brine, and dried over Na_2SO_4. The solvent was evaporated and the residue was purified by silica gel column chromatography using (CH_2Cl_2:*Et*O*Ac*, 9:1) to afford 2.0 g **2** (67%) as a white powder, mp 178–180°C. ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 1.43$ (s, $3 \times CH_3$), 3.28 (d, $J = 3.5$ Hz, CH_2), 4.90 (m, CH), 7.00–7.20 (m, *Ar*–H), 7.40–7.54 (m, *Ar*–H), 8.00 (br, s, NH), 10.90 (br, s, NH), 12.30 (br, s, OH) ppm; MS (ESI): $m/z = 327$ [$M^+ + Na$].

Ethyl [(2S)-3-(1H-indol-3-yl)-2-(t-butoxycarbonylamino)-propanoylamino]acetate (**3**, $C_{20}H_{27}N_3O_5$)

To a solution of 0.91 g **2** (3 mmol) in 10 cm^3 dry *DMF*, 1.26 cm^3 Et_3N (9 mmol) was added. Glycine ethyl ester hydrochloride (0.42 g, 3 mmol) and 1.06 g *TBTU* (3.3 mmol) were added with stirring at room temperature for 2 h. The reaction mixture was acidified with 2 *N* HCl and extracted with 3×100 cm^3 *Et*O*Ac*. The organic layer was washed with 100 cm^3 H_2O, 100 cm^3 brine, and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using (hexane:*Et*O*Ac*, 8:2) to afford 0.90 g **3** (77.6%) as a white foam. ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 1.23$ (t, $J = 4.5$ Hz,

C*H*$_3$CH$_2$), 1.41 (s, 3 × CH$_3$), 3.09 (d, $J = 3.5$ Hz, CH$_2$), 4.12 (q, $J = 3.5$ Hz, CH$_3$C*H*$_2$), 4.20 (s, CH$_2$), 4.92 (m, CH), 7.09–7.25 (m, *Ar*–H), 7.43–7.59 (m, *Ar*–H), 8.00 (br, s, NH), 9.08 (br, s, NH), 10.90 (br, s, NH) ppm; MS (ESI): $m/z = 412$ [M$^+$ + Na].

[(2S)-3-(1H-Indol-3-yl)-2-(t-butoxycarbonylamino)-propanoylamino]acetic acid (**4**, $C_{18}H_{23}N_3O_5$)
A mixture of 0.72 g **3** (1.87 mmol), 10 cm^3 *Me*OH, and 5 cm^3 2 *N* KOH was stirred for 3 h. The reaction mixture was acidified with HCl and evaporated *in vacuo*. The residue was purified by silica gel column chromatography (petroleum ether:*Et*O*Ac*, 2:1) to give a white powder (0.53 g, 80%), mp 211–213°C. ^{1}H NMR (CDCl$_3$, 300 MHz): $\delta = 1.40$ (s, 3 × CH$_3$), 2.98 (d, $J = 3.5$ Hz, CH$_2$), 4.13 (s, CH$_2$), 4.88 (m, CH), 7.03–7.30 (m, *Ar*–H), 7.40–7.50 (m, *Ar*–H), 8.04 (br, s, NH), 9.00 (br, s, NH), 10.50 (br, s, NH), 12.20 (br, s, OH) ppm; MS (ESI): $m/z = 384$ [M$^+$ + Na].

General procedure for the preparation of tryptophane bearing amino acid esters ***5a–5f***
A solution of amino acid methyl ester hydrochloride derivatives (1 mmol) in 5 cm^3 *Me*CN was cooled to −5°C. 0.361 g **4** (1 mmol), 0.14 g HO*Bt* (1 mmol), and 0.21 g *DCC* (1 mmol) were added successively. The reaction mixture was stirred at 0–5°C for 2 h, and at room temperature for 16 h. Dicyclohexylurea (*DCU*) was filtered off and the filtrate was evaporated to dryness. The residue was dissolved in 20 cm^3 *Et*O*Ac* and extracted successively with 10 cm^3 brine, 10 cm^3 5% NaHCO$_3$ solution, 10 cm^3 1 *N* HCl, followed by 10 cm^3 brine, and finally with 10 cm^3 H$_2$O. 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% *Me*OH in CH$_2$Cl$_2$ to give **5a**–**5f** in 71–80% yields.

Methyl {[(2S)-3-(1H-indol-3-yl)-2-(t-butoxycarbonylamino)-propanoylamino]acetylamino}acetate (**5a**, $C_{21}H_{28}N_4O_6$)
White foam (70%); ^{1}H NMR (CDCl$_3$, 300 MHz): $\delta = 1.42$ (s, 3 × CH$_3$), 2.85 (d, $J = 3.5$ Hz, CH$_2$), 3.65 (s, OCH$_3$), 4.05 (s, CH$_2$), 4.17 (s, CH$_2$), 4.93 (m, CH), 7.05–7.25 (m, *Ar*–H), 7.33–7.45 (m, *Ar*–H), 8.00 (br, s, NH), 9.07 (br, s, 2 × NH), 10.80 (br, s, NH) ppm; MS (ESI): $m/z = 455$ [M$^+$ + Na].

Methyl {(2R)-2-(2S)-3-(1H-indol-3-yl)-2-[(t-butoxy-carbonylamino)propanoylamino]acetylamino}propanoate (**5b**, $C_{22}H_{30}N_4O_6$)
White foam (80%); ^{1}H NMR (CDCl$_3$, 300 MHz): $\delta = 1.40$ (s, 3 × CH$_3$), 1.50 (d, $J = 2.5$ Hz, CH$_3$), 2.88 (d, $J = 3.5$ Hz, CH$_2$), 3.67 (s, OCH$_3$), 4.09 (s, CH$_2$), 4.66 (q, $J = 2.5$ Hz, CH), 4.92 (m, CH), 7.01–7.27 (m, *Ar*–H), 7.30–7.40 (m, *Ar*–H), 8.00 (br, s, NH), 8.50 (br, s, NH), 9.07 (br, s, NH), 10.80 (br, s, NH) ppm; MS (ESI): $m/z = 469$ [M$^+$ + Na].

Methyl {(2R)-2-(2S)-3-(1H-indol-3-yl)-2-[(t-butoxycarbonyl-amino)propanoylamino]acetylamino}-3-methylbutanoate (**5c**, $C_{24}H_{34}N_4O_6$)
White foam (78%); ^{1}H NMR (CDCl$_3$, 300 MHz): $\delta = 1.00$ (d, $J = 2.0$ Hz, 2 × CH$_3$), 1.43 (s, 3 × CH$_3$), 2.80 (d, $J = 3.5$ Hz, CH$_2$), 3.00 (m, CH), 3.60 (s, OCH$_3$), 4.01 (s, CH$_2$), 4.46 (m, CH), 4.90 (m, CH), 7.00–7.30 (m, *Ar*–H), 7.38–7.50 (m, *Ar*–H), 8.09 (br, s, NH), 8.45 (br, s, NH), 9.12 (br, s, NH), 10.85 (br, s, NH) ppm; MS (ESI): $m/z = 497$ [M$^+$ + Na].

Methyl {(2R)-2-(2S)-3-(1H-indol-3-yl)-2-[(t-butoxycarbonyl-amino)propanoylamino]acetylamino}-4-methylpentanoate (**5d**, $C_{25}H_{36}N_4O_6$)
White foam (71%); ^{1}H NMR (CDCl$_3$, 300 MHz): $\delta = 1.07$ (d, $J = 2.0$ Hz, 2 × CH$_3$), 1.40 (s, 3 × CH$_3$), 1.80 (m, CH), 1.90 (m, CH$_2$), 2.89 (d, $J = 3.5$ Hz, CH$_2$), 3.65 (s, OCH$_3$), 4.08 (s, CH$_2$), 4.41 (m, CH), 4.94 (m, CH), 7.00–7.20 (m, *Ar*–H), 7.28–7.40 (m, *Ar*–H), 8.05 (br, s, NH), 8.33 (br, s, NH), 9.10 (br, s, NH), 10.80 (br, s, NH) ppm; MS (ESI): $m/z = 511$ [M$^+$ + Na].

Methyl {(2R)-2-(2S)-3-(1H-indol-3-yl)-2-[(t-utoxycarbonyl-amino)propanoylamino]acetylamino}-4-methylthiobutanoate (**5e**, $C_{24}H_{34}N_4O_6S$)
Pale yellow foam (78%); ^{1}H NMR (CDCl$_3$, 300 MHz): $\delta =$ 1.42 (s, 3 × CH$_3$), 2.11 (s, SCH$_3$), 2.30 (m, CH$_2$), 2.50 (m, CH$_2$), 2.81 (d, $J = 3.5$ Hz, CH$_2$), 3.69 (s, OCH$_3$), 4.09 (s, CH$_2$), 4.48 (m, CH), 4.99 (m, CH), 7.03–7.22 (m, *Ar*–H), 7.29–7.44 (m, *Ar*–H), 8.08 (br, s, NH), 8.38 (br, s, NH), 9.12 (br, s, NH), 10.89 (br, s, NH) ppm; MS (ESI): $m/z = 529$ [M$^+$ + Na].

Methyl {(2R)-2-(2S)-3-(1H-indol-3-yl)-2-[(t-butoxycarbonyl-amino)propanoylamino]acetylamino}-2-phenylacetate (**5f**, $C_{27}H_{32}N_4O_6$)
White foam (80%); ^{1}H NMR (CDCl$_3$, 300 MHz): $\delta = 1.42$ (s, 3 × CH$_3$), 2.80 (d, $J = 3.5$ Hz, CH$_2$), 3.68 (s, OCH$_3$), 4.12 (s, CH$_2$), 4.90 (m, CH), 5.80 (s, CH), 7.00–7.23 (m, *Ar*–H), 7.28–7.40 (m, *Ar*–H), 7.45–7.58 (m, *Ar*–H), 8.00 (br, s, NH), 8.53 (br, s, NH), 9.00 (br, s, NH), 10.70 (br, s, NH) ppm; MS (ESI): $m/z = 531$ [M$^+$ + Na].

General procedure for the preparation of free peptides ***6a–6f***
A solution of **5a**–**5f** (1 equiv) was dissolved in 4 cm^3 50% *TFA* in CH$_2$Cl$_2$ and was stirred for 3 h at room temperature. The solvent was evaporated under reduced pressure and the crude oil was washed with cold ether. The precipitate was formed in a quantitative yield.

Methyl {(2S)-3-(1H-indol-3-yl)-2-[(aminopropanoyl)amino]-acetylamino}acetate (**6a**, $C_{16}H_{20}N_4O_4$)
White powder, mp 166–168°C; ^{1}H NMR (*DMSO*-d$_6$, 300 MHz): $\delta = 2.77$ (d, $J = 3.5$ Hz, CH$_2$), 3.65 (s, OCH$_3$), 4.00 (s, CH$_2$), 4.10 (s, CH$_2$), 4.87 (m, CH), 7.00–7.25 (m, *Ar*–H), 7.30–7.52 (m, *Ar*–H), 8.00 (br, s, NH), 8.80 (br, s, NH$_2$), 9.00 (br, s, NH), 10.80 (br, s, NH) ppm; MS (ESI): $m/z = 355$ [M$^+$ + Na].

Methyl {(2R)-2-(2S)-3-(1H-indol-3-yl)-2-[(aminopropanoyl)-amino]acetylamino}propanoate (**6b**, $C_{17}H_{22}N_4O_4$)
White powder, mp 177–179°C; ^{1}H NMR (*DMSO*-d$_6$, 300 MHz): $\delta = 1.44$ (d, $J = 2.5$ Hz, CH$_3$), 2.75 (d, $J =$ 3.5 Hz, CH$_2$), 3.64 (s, OCH$_3$), 4.09 (s, CH$_2$), 4.59 (q, $J =$ 2.5 Hz, CH), 4.88 (m, CH), 7.00–7.27 (m, *Ar*–H), 7.30–7.44 (m, *Ar*–H), 8.00 (br, s, NH), 8.76 (br, s, NH$_2$), 9.16 (br, s, NH), 10.67 (br, s, NH) ppm; MS (ESI): $m/z = 369$ [M$^+$ + Na].

Methyl {(2R)-2-(2S)-3-(1H-indol-3-yl)-2-[(aminopropanoyl)-amino]acetylamino}-3-methylbutanoate (**6c**, $C_{19}H_{26}N_4O_4$)
White powder, mp 158–160°C; ^{1}H NMR (*DMSO*-d_6, 300 MHz): $\delta = 1.07$ (d, $J = 2.0$ Hz, $2 \times CH_3$), 2.72 (d, $J = 3.5$ Hz, CH_2), 3.11 (m, CH), 3.63 (s, OCH_3), 3.98 (s, CH_2), 4.40 (m, CH), 4.85 (m, CH), 7.00–7.30 (m, *Ar*–H), 7.39–7.47 (m, *Ar*–H), 8.14 (br, s, NH), 8.80 (br, s, NH_2), 9.10 (br, s, NH), 10.70 (br, s, NH) ppm; MS (ESI): $m/z = 397$ [$M^+ + Na$].

Methyl {(2R)-2-(2S)-3-(1H-indol-3-yl)-2-[(aminopropanoyl)-amino]acetylamino}-4-methylpentanoate (**6d**, $C_{20}H_{28}N_4O_4$)
White powder, mp 189–191°C; ^{1}H NMR (*DMSO*-d_6, 300 MHz): $\delta = 1.09$ (d, $J = 2.0$ Hz, $2 \times CH_3$), 1.79 (m, CH), 1.95 (m, CH_2), 2.80 (d, $J = 3.5$ Hz, CH_2), 3.68 (s, OCH_3), 4.11 (s, CH_2), 4.48 (m, CH), 4.88 (m, CH), 7.00–7.30 (m, *Ar*–H), 7.38–7.48 (m, *Ar*–H), 8.09 (br, s, NH), 8.80 (br, s, NH_2), 9.19 (br, s, NH), 10.67 (br, s, NH) ppm; MS (ESI): $m/z = 411$ [$M^+ + Na$].

Methyl {(2R)-2-(2S)-3-(1H-indol-3-yl)-2-[(aminopropanoyl)-amino]acetylamino}-4-methylthiobutanoate
(**6e**, $C_{19}H_{26}N_4O_4S$)
Pale yellow powder, mp 134–136°C; ^{1}H NMR (*DMSO*-d_6, 300 MHz): $\delta = 2.20$ (s, SCH_3), 2.36–2.44 (m, $2 \times CH_2$), 2.88 (d, $J = 3.5$ Hz, CH_2), 3.66 (s, OCH_3), 4.16 (s, CH_2), 4.55 (m, CH), 4.92 (m, CH), 7.03–7.29 (m, *Ar*–H), 7.38–7.49 (m, *Ar*–H), 8.00 (br, s, NH), 8.71 (br, s, NH_2), 9.00 (br, s, NH), 10.70 (br, s, NH) ppm; MS (ESI): $m/z = 429$ [$M^+ + Na$].

Methyl {(2R)-2-(2S)-3-(1H-indol-3-yl)-2-[(aminopropanoyl)-amino]acetylamino}-2-phenylacetate (**6f**, $C_{22}H_{24}N_4O_4$)
White powder, mp 215–217°C; ^{1}H NMR (*DMSO*-d_6, 300 MHz): $\delta = 2.72$ (d, $J = 3.5$ Hz, CH_2), 3.62 (s, OCH_3), 4.05 (s, CH_2), 4.91 (m, CH), 5.70 (s, CH), 7.00–7.30 (m, *Ar*–H), 7.40–7.50 (m, *Ar*–H), 7.55–7.60 (m, *Ar*–H), 7.79 (br, s, NH), 8.66 (br, s, NH_2), 8.89 (br, s, NH), 10.66 (br, s, NH) ppm; MS (ESI): $m/z = 431$ [$M^+ + Na$].

Antimicrobial testing
The hole plate method was the most suitable technique in investigating the antibacterial activities of the different compounds. Nutritive agar plates seeded with the test organisms (three plates for each organism) were allowed to solidify, and then 5 mm diameter holes were formed in the plates using a cork borer. Each hole was filled with one drop of the ethanolic solution of the tested compound, while the hole in the center of the plate filled with one drop of ethanol. Plates were separately incubated at the optimum temperature for each test organism for 24 h. Inhibition zones (zones with no growth) around the holes were measured as an indicator for the antibacterial action.

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