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Amino Acid Derivatives, VI [1]: Synthesis, Antiviral, and Antimicrobial Evaluation of α-Amino Acid Esters Bearing a 1,2,3-Triazolo[4,5-*d*]pyrimidinedione Side Chain

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Summary. A series of peptide and dipeptide derivatives conjugated with a 1,2,3-triazolo[4,5-*d*]pyrimidinedione residue were synthesized. The new compounds were evaluated *in vitro* for cytotoxicity against HAV-27 and *HS*V-1 and showed moderate activity. The prepared compounds were tested for antimicrobial activity against four different bacterial species, and they displayed different degrees of antibacterial activities or inhibitory actions.

Keywords. Triazolopyrimidines; Amino acids; Dipeptides; Antiviral activity; Antimicrobial activity.

Introduction

Much attention has been paid to the synthesis of various triazolopyrimidines due to their CNS depressant [2], antibacterial [3–6], antimicrobial [7], anti-inflammatory [8–11], antiallergy [12], and herbicidal activities [13–16]. On the other hand, a new target for the development of anti-HIV and antitumor therapies has been reported by the use, *in vivo* and *in vitro*, of amino acid derived heterocycles. Such compounds are the lysyl amide prodrug of 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole [17], amino acid derivatives of paclitaxel [18], cysteine-modifying agents [19], and isoquinoline carboxylic acid derivatives as building blocks for HIV protease inhibitors [20]. In connection with our strategy in synthesis of new α-amino acid derivatives [21–24] and due to the pharmacological properties of triazolopyrimidines and amino acid derivatives we were prompted to prepare new 1,2,3-triazolo[4,5-*d*]pyrimidinediones bearing amino acid derivatives as potential antiviral and antimicrobial candidates.

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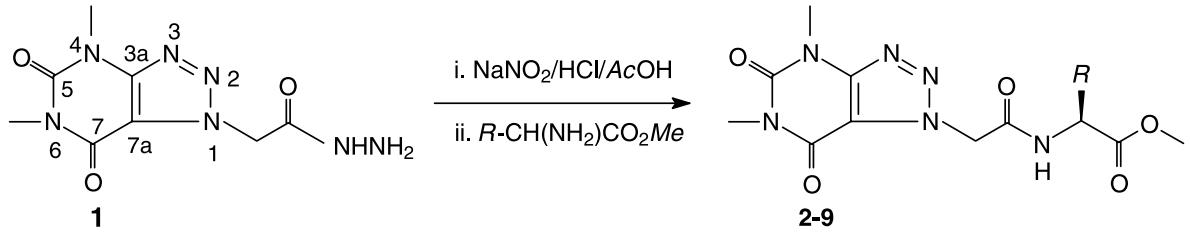
Results and Discussion

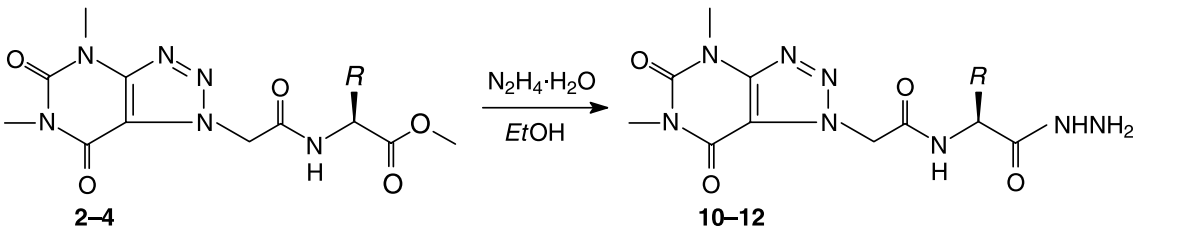
Chemistry

The starting material 1-acetylhydrazine-4,6-dimethyl-1*H*-[1,2,3]triazolo[4,5-*d*]pyrimidine-5,7(4*H*,6*H*)-dione (**1**) [25] was synthesized by refluxing 1-carbethoxymethyl-4,6-dimethyl-1*H*-[1,2,3]triazolo-[4,5-*d*]pyrimidine-5,7(4*H*,6*H*)-dione [25, 26] with hydrazine hydrate in ethanol. This hydrazide was selected as a starting material for the coupling reaction with the appropriate acylated amino acides, *via* the azide-coupling method [27]. Thus, treatment of **1** at −5°C in *Ac*OH and 1 *N* HCl with $NaNO_2$ 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 **2–9** in 75–80% yields. The structures of **2–9** were assigned from their ^1H, ^{13}C NMR, and mass spectra. The ^1H NMR spectra showed a broad singlet at $\delta = 9.70$–7.15 ppm corresponding to NH. The two protons of N^1–CH_2 appeared as a singlet at $\delta = 5.57$–5.43 ppm, while

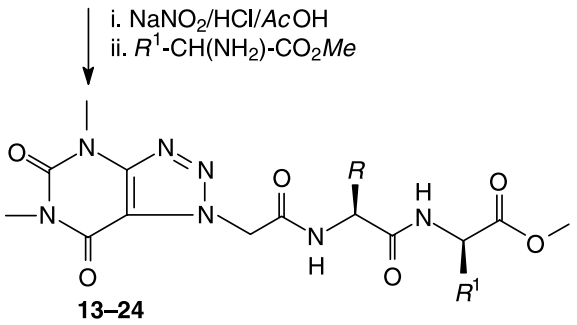


No.	*R*	amino acids
2	H	L-glycine
3	*Me*	L-alanine
4	CH_2OH	L-serine
5	$CHMe_2$	L-valine
6	CH_2CHMe_2	L-leucine
7	CH_2CH_2SMe	L-methionine
8	*Ph*	L-phenylglycine
9	*Bn*	L-phenylalanine

Scheme 1



	R
2, 10	H
3, 11	*Me*
4, 12	CH_2OH



No.	*R*	R^1	dipeptides
13	H	H	L-glycyl-L-glycine
14	H	*Me*	L-glycyl-L-alanine
15	H	CH_2OH	L-glycyl-L-serine
16	H	$CHMe_2$	L-glycyl-L-valine
17	H	CH_2CHMe_2	L-glycyl-L-leucine
18	H	CH_2CH_2SMe	L-glycyl-L-methionine
19	*Me*	H	L-alanyl-L-glycine
20	*Me*	CH_2OH	L-alanyl-L-serine
21	*Me*	CH_2CH_2SMe	L-alanyl-L-methionine
22	CH_2OH	H	L-seryl-L-glycine
23	CH_2OH	CH_2OH	L-seryl-L-serine
24	CH_2OH	CH_2CH_2SMe	L-seryl-L-methionine

Scheme 2

OCH_3 group appeared as a singlet at $\delta = 3.88$–3.63 ppm. The protons of the side chain were fully analyzed as well as the remaining protons. The ^{13}C NMR spectra of **2**–**9** were fully analyzed (Experimental section) (Scheme 1).

Treating of **2**–**4** with $N_2H_4 \cdot H_2O$ in ethanol at reflux temperature afforded the corresponding hydrazides **10**–**12** in 96% yield. Treatment of **10**–**12** 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 **13**–**24** in 78–85% yields. The structures of the dipeptide derivatives were confirmed by their ^1H, ^{13}C NMR, and mass spectra. Compound **21** was selected for the ^1H and ^{13}C NMR analysis. The ^1H NMR spectrum showed a broad signal at $\delta = 9.02$ ppm, characterized by D_2O exchange as NH group. The multiplet at $\delta = 4.61$–4.50 ppm was identified as two CH groups, while the two singlets at $\delta = 4.50$ and 3.65 ppm were attributed to N^1–CH_2 and OCH_3. The multiplet at $\delta = 2.50$–2.22 ppm was identified as two CH_2 and SCH_3. Finally, the doublet at $\delta = 1.39$ ppm is corresponding to CH_3. The ^{13}C NMR spectrum demonstrated three higher field signals at $\delta = 176.0$, 175.0, and 163.0 ppm which were assigned to three C=O groups in the side chain, while the two C=O groups in the pyrimidine ring (C-7) and (C-5) were assigned at 154.2 and 150.7. The resonances at $\delta = 152.2$ and 111.9 ppm were assigned to the two fused carbons ($C\text{-}3_a$) and ($C\text{-}7_a$). The methyl of the carboxylate group and N^1–CH_2 were resonated at $\delta = 52.0$ and 51.1 ppm. The two signals at $\delta = 49.9$ and 45.7 ppm were identified as two CH groups. The two methylene groups, N^4–CH_3 and N^6–CH_3, were resonated at $\delta = 31.0$, 29.9, 29.3, and 27.7 ppm, while the lower field signals at $\delta = 17.7$ and 14.7 ppm were assigned as CH_3 and SCH_3 group (Scheme 2).

Antiviral Activity

The plaque infectivity assay [28] was carried out to test the prepared compounds for antiviral activity. The test was performed to include three possibilities for antiviral activity, virucidal effect, virus adsorption, and effect on virus replication for both HAV-27 and *HS*V-1.

For the antiviral activity against HAV-27 it was noticed that, at both concentrations 10 and 20 $\mu g/10^5$ cells, compounds **9**, **18**, **23**, and **24** revealed the highest antiviral activity in this series of compounds and compounds **7** and **17** revealed high activity at 10 $\mu g/10^5$ cells using amantadine (C*) as a control. Compounds **3**, **6**, **8**, **14**, **16**, and **18**–**21** showed moderate activity, while at concentration of 20 $\mu g/10^5$ cells, compounds **2**, **4**, **5**, **13**, **15**, and **22** revealed little antiviral activity.

For the antiviral activity against *Herpes Simplex* virus-1 (*HS*V-1) the results revealed that compounds **7**–**9** and **18**–**24** showed the highest effect on *HS*V-1 at concentration 10 $\mu g/10^5$ cells, while compounds **2**–**6** and **13**–**17** showed moderate activity.

Antimicrobial Activity

The newly synthesized compounds were tested for their antimicrobial action [29, 30] against four different bacterial species namely, *Pseudomonas* sp. (*Gram* negative bacterium), *Bacillus subtilis* (*Gram*

Table 1. Antimicrobial activity of the newly synthesized compounds **2**–**9** and **13**–**24**

Compd no.	*Pseudomonas* sp.	*Bacillus subtilis*	*Bacillus cereus*	*Streptomyces* sp.
Amoxicillin (Penicillin)	−	++	+++	+
2	+	+++	+++	++
3	+	++++	+++	+
4	+	+++	+++	+
5	+	++	+	−
6	+	+	+	+
7	+	++++	+	−
8	+	++	++	+
9	+	+++	+++	+
13	+	++	++	+
14	+	++++	++++	++
15	+	++++	++++	++
16	+	++++	++++	++
17	+	++++	++++	+
18	+	++++	+++	+
19	+	++++	++++	+
20	+	+++	+++	+
21	+	+++	+++	++
22	+	+++	+++	+
23	+	++	++	+
24	+	++	++	+

− No antibacterial effect
+ Low antibacterial effect
++ Moderate antibacterial effect
+++ High antibacterial effect
++++ Complete antibacterial effect

positive bacterium), *Bacillus cereus* (*Gram* positive bacterium), and *Streptomyces* sp. (one of the important actinomycetes). All the tested compounds exhibited different degrees of antibacterial activities or inhibitory actions. The most susceptible organisms were the two *Gram* positive bacteria (*Bacillus subtilis* and *Bacillus cereus*) followed by *Streptomyces* sp., while the lowest inhibitory effect was encountered in the case of *Pseudomonas* sp. The highest degrees of inhibition were recorded for compounds **3**, **7**, and **14–19** followed by **2**, **4**, **9**, and **20–22**, while the lowest degree of inhibition was recorded for the compounds **5**, **6**, **8**, **13**, **23**, and **24** (Table 1). The results were compared to amoxicillin (penicillin) as a reference drug.

Conclusions

New α-amino acid derivatives bearing a 1,2,3-triazolo[4,5-*d*]pyrimidine-5,7(4*H*,6*H*)-dione residue were synthesized in order to increase the number of potential compounds screened for antiviral and antimicrobial activity.

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 ^{1}H NMR and 62.9 MHz for ^{13}C NMR with *TMS* as an internal standard. MALDI-MS were measured with a KRATOS Analytical Compact, using 2,5-dihydroxybenzoic acid (*DHB*) as matrix. The $(M+Na)^+$ and $(M+K)^+$ ions were peak-matched using ions derived from the 2,5-dihydroxybenzoic acid matrix. The microanalyses were performed at the microanalytical unit, Tokyo University, Japan, and were found to agree favorably with the calculated values. Viral screening against HAV and *HS*V was conducted at the Environmental Virology Lab., Department of Water Pollution Research, National Research Centre, Cairo, Egypt. 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 1,2,3-Triazolo[4,5-d]pyrimidinedione Bearing Amino Acid Esters 2–9

A solution of 1.0 g **1** (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). $NaNO_2$ (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* HCl, 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:ethyl acetate = 5:1 to afford **2–9** in 75–80% yields.

1-{4,6-Dimethyl-1H-[1,2,3]triazolo[4,5-d]pyrimidine-5,7(4H,6H)-dione}acetyl L-glycine methyl ester
(**2**, $C_{11}H_{14}N_6O_5$)
White foam (75%); ^{1}H NMR (*DMSO*-d_6, 250 MHz): $\delta = 3.40$ (s, N^6–CH_3), 3.60 (s, N^4–CH_3), 3.65 (s, OCH_3), 4.15 (s, CH_2), 5.50 (s, N^1–CH_2, 7.20 (br, s, NH) ppm; ^{13}C NMR (*DMSO*-d_6, 62.5 MHz): $\delta = 27.8$ (N^6–CH_3), 29.8 (N^4–CH_3), 51.9 (N^1–CH_2), 52.0 (CH_2), 53.5 (OCH_3), 111.5 (C-7_a), 150.5 (C-5), 152.9 (C-3_a), 154.8 (C-7), 167.3 (C=O), 172.9 (C=O) ppm; MS (MALDI, positive mode, Matrix: *DHB*): m/z (%) = 333 (22) $[M+Na]^+$, 349 (45) $[M+K]^+$.

1-{4,6-Dimethyl-1H-[1,2,3]triazolo[4,5-d]pyrimidine-5,7(4H,6H)-dione}acetyl L-alanine methyl ester (**3**, $C_{12}H_{16}N_6O_5$)
White foam (76%); ^{1}H NMR (*DMSO*-d_6, 250 MHz): $\delta = 1.48$ (d, $J = 5.0$ Hz, CH_3), 3.44 (s, N^6–CH_3), 3.65 (s, N^4–CH_3), 3.88 (s, OCH_3), 4.66 (q, $J = 5.0$ Hz, CH), 5.48 (s, N^1–CH_2), 7.22 (br, s, NH) ppm; ^{13}C NMR (*DMSO*-d_6, 62.5 MHz): $\delta = 17.3$ (CH_3), 28.1 (N^6–CH_3), 29.5 (N^4–CH_3), 47.3 (CH), 52.5 (N^1–CH_2), 54.3 (OCH_3), 111.7 (C-7_a), 151.3 (C-5), 153.5 (C-3_a), 154.5 (C-7), 167.7 (C=O), 173.7 (C=O) ppm; MS (MALDI, positive mode, Matrix: *DHB*): m/z (%) = 347 (33) $[M+Na]^+$, 363 (51) $[M+K]^+$.

1-{4,6-Dimethyl-1H-[1,2,3]triazolo[4,5-d]pyrimidine-5,7(4H,6H)-dione}acetyl L-serine methyl ester
(**4**, $C_{12}H_{16}N_6O_6$)
White powder (79%); mp 180–181°C; ^{1}H NMR (*DMSO*-d_6, 250 MHz): $\delta = 3.36$–3.40 (m, CH_2) 3.45 (s, N^6–CH_3), 3.63 (s, N^4–CH_3), 3.71 (s, OCH_3), 4.45–4.50 (m, CH), 5.48 (s, N^1–CH_2), 6.66 (br, s, OH), 7.15 (br, s, NH) ppm; ^{13}C NMR (*DMSO*-d_6, 62.5 MHz): $\delta = 28.3$ (N^6–CH_3), 29.9 (N^4–CH_3), 52.8 (N^1–CH_2), 53.4 (OCH_3), 54.7 (CH), 72.0 (OCH_2), 112.1 (C-7_a), 151.2 (C-5), 153.6 (C-3_a), 155.3 (C-7), 167.6 (C=O), 173.9 (C=O) ppm; MS (MALDI, positive mode, Matrix: *DHB*): m/z (%) = 363 (34) $[M+Na]^+$, 379 (21) $[M+K]^+$.

1-{4,6-Dimethyl-1H-[1,2,3]triazolo[4,5-d]pyrimidine-5,7(4H,6H)-dione}acetyl L-valine methyl ester
(**5**, $C_{14}H_{20}N_6O_5$)
White foam (77%); ^{1}H NMR (*DMSO*-d_6, 250 MHz): $\delta = 0.94$ (dd, $J = 1.9$, 7.2 Hz, $2CH_3$), 2.18–2.26 (m, CH), 3.41 (s, N^6–CH_3), 3.57 (s, N^4–CH_3), 3.63 (s, OCH_3), 4.32–4.40 (m, CH),

5.52 (s, N^1–CH_2), 8.44 (br, s, NH) ppm; ^{13}C NMR (*DMSO*-d_6, 62.5 MHz): $\delta = 16.8$ (*C*H_3), 18.8 (CH_3), 27.8 (N^6–CH_3), 29.8 (N^4–CH_3), 30.9 (CH), 51.8 (CH), 52.6 (N^1–CH_2), 57.5 (OCH_3), 111.8 (C-7_a), 150.8 (C-5), 152.4 (C-3_a), 154.9 (C-7), 167.9 (C=O), 175.4 (C=O) ppm; MS (MALDI, positive mode, Matrix: *DHB*): m/z (%) = 375 (40) $[M + Na]^+$, 391 (18) $[M + K]^+$.

1-{4,6-Dimethyl-1H-[1,2,3]triazolo[4,5-d]pyrimidine-5,7(4H,6H)-dione}acetyl L-leucine methyl ester
(**6**, $C_{15}H_{22}N_6O_5$)
White foam (78%); ^{1}H NMR (*DMSO*-d_6, 250 MHz): $\delta = 0.89$ (dd, $J = 1.9$, 7.2 Hz, $2CH_3$), 1.44–1.70 (m, CH_2, CH), 3.39 (s, N^6–CH_3), 3.52 (s, N^4–CH_3), 3.64 (s, OCH_3), 4.17–4.24 (m, CH), 5.50 (s, N^1–C*H*$_2$), 8.17 (br, s, NH) ppm; ^{13}C NMR (*DMSO*-d_6, 62.5 MHz): $\delta = 21.3$ ($2CH_3$), 24.6 (CH), 27.4 (N^6–CH_3), 29.2 (N^4–CH_3), 39.7 (CH_2), 51.0 (CH), 52.9 (N^1–CH_2), 53.4 (OCH_3), 112.3 (C-7_a), 151.0 (C-5), 153.5 (C-3_a), 155.3 (C-7), 168.8 (C=O), 172.4 (C=O) ppm; MS (MALDI, positive mode, Matrix: *DHB*): m/z (%) = 389 (32) $[M + Na]^+$, 405 (15) $[M + K]^+$.

1-{4,6-Dimethyl-1H-[1,2,3]triazolo[4,5-d]pyrimidine-5,7(4H,6H)-dione}acetyl L-methionine methyl ester
(**7**, $C_{14}H_{20}N_6O_5S$)
Pale yellow foam (75%); ^{1}H NMR (*DMSO*-d_6, 250 MHz): $\delta = 2.18$–2.51 (m, $2CH_2$, SCH_3), 3.44 (s, N^6–CH_3), 3.62 (s, N^4–CH_3), 3.67 (s, OCH_3), 4.43–4.50 (m, CH), 5.43 (s, N^1–CH_2), 8.11 (br, s, NH) ppm; ^{13}C NMR (*DMSO*-d_6, 62.5 MHz): $\delta = 14.6$ (SCH_3), 27.4 (N^6–CH_3), 29.4 (CH_2), 29.7 (N^4–CH_3), 30.6 (CH_2), 46.4 (CH), 52.7 (N^1–CH_2), 53.0 (OCH_3), 111.2 (C-7_a), 151.5 (C-5), 152.7 (C-3_a), 154.3 (C-7), 171.1 (C=O), 177.2 (C=O) ppm; MS (MALDI, positive mode, Matrix: *DHB*): m/z (%) = 407 (15) $[M + Na]^+$, 423 (19) $[M + K]^+$.

1-{4,6-Dimethyl-1H-[1,2,3]triazolo[4,5-d]pyrimidine-5,7(4H,6H)-dione}acetyl L-phenylglycine methyl ester
(**8**, $C_{17}H_{18}N_6O_5$)
White powder (79%); mp 211–213°C; ^{1}H NMR (*DMSO*-d_6, 250 MHz): $\delta = 3.48$ (s, N^6–CH_3), 3.64 (s, N^4–CH_3), 3.75 (s, OCH_3), 5.57 (s, N^1–CH_2), 4.77 (s, CH), 7.20–7.27 (m, *Ph*–H), 7.33–7.44 (m, *Ph*–H), 9.10 (br, s, NH) ppm; ^{13}C NMR (*DMSO*-d_6, 62.5 MHz): $\delta = 27.9$ (N^6–CH_3), 29.6 (N^4–CH_3), 52.7 (N^1–CH_2), 53.5 (OCH_3), 56.7 (CH), 111.9 (C-7_a), 125.5, 129.9, 131.1, 137.7 (*Ph*–C), 151.7 (C-5), 152.4 (C-3_a), 154.3 (C-7), 167.6 (C=O), 173.0 (C=O) ppm; MS (MALDI, positive mode, Matrix: *DHB*): m/z (%) = 409 (23) $[M + Na]^+$, 425 (13) $[M + K]^+$.

1-{4,6-Dimethyl-1H-[1,2,3]triazolo[4,5-d]pyrimidine-5,7(4H,6H)-dione}acetyl L-phenylalanine methyl ester
(**9**, $C_{18}H_{20}N_6O_5$)
White powder (80%); mp 195–197°C; ^{1}H NMR (*DMSO*-d_6, 250 MHz): $\delta = 3.09$–3.24 (m, CH_2), 3.41 (s, N^6–CH_3), 3.62 (s, N^4–CH_3), 3.69 (s, OCH_3), 4.85 (m, CH), 5.53 (s, N^1–CH_2), 7.21–7.29 (m, *Ph*–H), 7.36–7.45 (m, *Ph*–H), 9.70 (br, s, NH) ppm; ^{13}C NMR (*DMSO*-d_6, 62.5 MHz): $\delta = 27.5$ (N^6–CH_3), 29.6 (N^4–CH_3), 37.5 (CH_2), 51.6 (N^1–CH_2), 52.6 (CH), 53.3 (OCH_3), 111.8 (C-7_a), 126.5 128.1, 128.9, 135.4 (*Ph*–C), 150.3 (C-5), 152.2 (C-3_a), 154.0 (C-7), 167.6 (C=O), 171.9 (C=O) ppm; MS (MALDI, positive mode, Matrix: *DHB*): m/z (%) = 423 (20) $[M + Na]^+$, 439 (28) $[M + K]^+$.

General Procedure for the Preparation of the Hydrazides ***10–12***
A mixture of **2–4** (10 mmol) and 1.25 g $N_2H_4 \cdot H_2O$ (25 mmol) in 30 cm^3 *Et*OH was heated under reflux for 3 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 **10–12** in 96% yields.

1-{4,6-Dimethyl-1H-[1,2,3]triazolo[4,5-d]pyrimidine-5,7(4H,6H)-dione}acetyl L-glycine hydrazide
(**10**, $C_{10}H_{14}N_8O_4$)
White powder (96%); mp 250–252°C; ^{1}H NMR (*DMSO*-d_6, 250 MHz): $\delta = 3.49$ (s, N^6–CH_3), 3.53 (s, CH_2), 3.67 (s, N^4–CH_3), 4.82 (br, s, NHN*H*$_2$), 5.34 (s, N^1–CH_2), 7.51 (br, s, NH), 9.45 (br, s, N*H*NH$_2$) ppm; ^{13}C NMR (*DMSO*-d_6, 62.5 MHz): $\delta = 27.8$ (N^6–CH_3), 29.9 (N^4–CH_3), 40.4 (CH_2), 51.1 (N^1–CH_2), 112.6 (C-7_a), 151.6 (C-5), 153.1 (C-3_a), 154.7 (C-7), 164.3 (C=O), 169.5 (C=O) ppm; MS (MALDI, positive mode, Matrix: *DHB*): m/z (%) = 333 (35) $[M + Na]^+$, 349 (52) $[M + K]^+$.

1-{4,6-Dimethyl-1H-[1,2,3]triazolo[4,5-d]pyrimidine-5,7(4H,6H)-dione}acetyl L-alanine hydrazide
(**11**, $C_{11}H_{16}N_8O_4$)
White powder (96%); mp 266–268°C. ^{1}H NMR (*DMSO*-d_6, 250 MHz): $\delta = 1.40$ (d, $J = 5.0$ Hz, CH_3), 3.44 (s, N^6–CH_3), 3.64 (s, N^4–CH_3), 4.18 (q, $J = 5.0$ Hz, CH), 4.77 (br, s, NHN*H*$_2$), 5.42 (s, N^1–CH_2), 7.49 (br, s, NH), 9.34 (br, s, N*H*NH$_2$) ppm; ^{13}C NMR (*DMSO*-d_6, 62.5 MHz): $\delta = 8.6$ (CH_3), 27.3 (N^6–CH_3), 129.7 (N^4–CH_3), 46.2 (CH), 51.5 (N^1–CH_2), 111.9 (C-7_a), 151.2 (C-5), 153.0 (C-3_a), 154.6 (C-7), 163.1 (C=O), 170.0 (C=O) ppm; MS (MALDI, positive mode, Matrix: *DHB*): m/z (%) = 347 (30) $[M + Na]^+$, 363 (16) $[M + K]^+$.

1-{4,6-Dimethyl-1H-[1,2,3]triazolo[4,5-d]pyrimidine-5,7(4H,6H)-dione}acetyl L-serine hydrazide
(**12**, $C_{11}H_{16}N_8O_5$)
White powder (96%); mp 279–281°C. ^{1}H NMR (*DMSO*-d_6, 250 MHz): $\delta = 3.41$ (s, N^6–C*H*$_3$) 3.62 (s, N^4–C*H*$_3$), 3.70–3.88 (m, CH_2), 4.30–4.42 (m, CH), 4.70 (br, s, NHN*H*$_2$), 5.32 (s, N^1–C*H*$_2$), 6.52 (br, s, OH), 7.31 (br, s, NH), 9.30 (br, s, N*H*NH$_2$) ppm; ^{13}C NMR (*DMSO*-d_6, 62.5 MHz): $\delta = 29.5$ (N^4–CH_3), 50.3 (CH), 51.9 (N^1–CH_2), 61.6 (OCH_2), 111.8 (C-7_a), 151.0 (C-5), 152.7 (C-3_a), 154.7 (C-7), 162.8 (C=O), 169.0 (C=O) ppm; MS (MALDI, positive mode, Matrix: *DHB*): m/z (%) = 363 (65) $[M + Na]^+$, 379 (43) $[M + K]^+$.

General Procedure for the Preparation of Dipeptides ***13–24***
A solution of **10–12** (0.80 mmol) in 6 cm^3 HO*Ac*, 3 cm^3 1 *N* HCl, and 25 cm^3 H_2O was cooled in an ice-bath

(−5°C). $NaNO_2$ (0.87 g, 12.60 mmol) in 3 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 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 (0.90 mmol) in 20 cm^3 ethyl acetate containing 0.2 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 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:ethylacetate = 5:1 to afford **13**–**24** in 78–85% yields.

1-{4,6-Dimethyl-1H-[1,2,3]triazolo[4,5-d]pyrimidine-5,7(4H,6H)-dione}acetyl L-glycyl-L-glycine methyl ester
(**13**, $C_{13}H_{17}N_7O_6$)
White foam (78%); ^{1}H NMR (*DMSO*-d_6, 250 MHz): δ = 3.40 (s, N^6–CH_3), 3.50 (s, OCH_3), 3.60 (s, N^4–CH_3), 3.71 (s, CH_2), 3.97 (s, CH_2), 5.40 (s, N^1–CH_2), 8.62 (br, s, NH) ppm; ^{13}C NMR (*DMSO*-d_6, 62.5 MHz): δ = 27.6 (N^6–CH_3), 29.5 (N^4–CH_3), 41.7 (CH_2), 42.9 (CH_2), 51.1 (N^1–CH_2), 53.4 (OCH_3), 111.7 (C-7_a), 150.2 (C-5), 152.3 (C-3_a), 154.0 (C-7), 163.2 (C=O), 170.0 (C=O), 171.0 (C=O) ppm; MS (MALDI, positive mode, Matrix: *DHB*): *m*/*z* (%) = 390 (43) $[M + Na]^+$, 406 (13) $[M + K]^+$.

1-{4,6-Dimethyl-1H-[1,2,3]triazolo[4,5-d]pyrimidine-5,7(4H,6H)-dione}acetyl L-glycyl-L-alanine methyl ester
(**14**, $C_{14}H_{19}N_7O_6$)
White foam (79%); ^{1}H NMR (*DMSO*-d_6, 250 MHz): δ = 1.35 (d, *J* = 5.0 Hz, CH_3), 3.44 (s, N^6–CH_3), 3.61 (s, N^4–CH_3), 3.71 (s, CH_2), 3.80 (s, OCH_3), 4.47 (q, *J* = 5.0 Hz, CH), 5.38 (s, N^1–CH_2), 9.03 (br, s, NH) ppm; ^{13}C NMR (*DMSO*-d_6, 62.5 MHz): δ = 18.4 (CH_3), 27.7 (N^6–CH_3), 29.8 (N^4–CH_3), 45.6 (CH_2), 48.0 (CH), 51.1 (N^1–CH_2), 52.6 (OCH_3), 112.9 (C-7_a), 150.4 (C-5), 152.8 (C-3_a), 154.9 (C-7), 162.5 (C=O), 168.8 (C=O), 173.3 (C=O) ppm; MS (MALDI, positive mode, Matrix: *DHB*): *m*/*z* (%) = 404 (25) $[M + Na]^+$, 420 (11) $[M + K]^+$.

1-{4,6-Dimethyl-1H-[1,2,3]triazolo[4,5-d]pyrimidine-5,7(4H,6H)-dione}acetyl L-glycyl-L-serine methyl ester
(**15**, $C_{14}H_{19}N_7O_7$)
White powder (85%); mp 155–157°C; ^{1}H NMR (*DMSO*-d_6, 250 MHz): δ = 3.40 (s, N^6–CH_3), 3.45–3.51 (m, OCH_2), 3.60 (s, N^4–CH_3), 3.68 (s, OCH_3), 3.70 (s, CH_2), 4.45–4.50 (m, CH), 5.42 (s, N^1–CH_2), 6.50 (br, s, OH), 7.88 (br, s, NH) ppm; ^{13}C NMR (*DMSO*-d_6, 62.5 MHz): δ = 27.7 (N^6–CH_3) 29.3 (N^4–CH_3), 41.7 (CH_2), 43.7 (CH_2), 51.3 (N^1–CH_2), 53.5 (OCH_3), 54.5 (CH), 72.0 (OCH_2), 111.6 (C-7_a), 150.9 (C-5), 152.7 (C-3_a), 154.8 (C-7), 162.6 (C=O), 167.7 (C=O), 174.0 (C=O) ppm; MS (MALDI, positive mode, Matrix: *DHB*): *m*/*z* (%) = 420 (22) $[M + Na]^+$, 436 (10) $[M + K]^+$.

1-{4,6-Dimethyl-1H-[1,2,3]triazolo[4,5-d]pyrimidine-5,7(4H,6H)-dione}acetyl L-glycyl-L-valine methyl ester
(**16**, $C_{16}H_{23}N_7O_6$)
White foam (84%); ^{1}H NMR (*DMSO*-d_6, 250 MHz): δ = 0.95 (dd, *J* = 1.9, 7.3 Hz, $2CH_3$), 2.22–2.28 (m, CH), 3.41 (s, N^6–CH_3), 3.45 (s, OCH_3), 3.66 (s, N^4–CH_3), 3.71 (s, CH_2), 3.77 (s, CH_2), 4.36–4.41 (m, CH), 5.47 (s, N^1–CH_2), 9.02 (br, s, NH) ppm; ^{13}C NMR (*DMSO*-d_6, 62.5 MHz): δ = 17.1, 19.0 ($2CH_3$) 27.7 (N^6–CH_3), 29.5 (N^4–CH_3), 32.7 (CH), 43.6 (CH_2), 51.0 (N^1–CH_2), 51.4 (CH), 56.7 (OCH_3), 112.7 (C-7_a), 150.7 (C-5), 152.8 (C-3_a), 154.9 (C-7), 162.9 (C=O), 168.5 (C=O), 175.3 (C=O) ppm; MS (MALDI, positive mode, Matrix: *DHB*): *m*/*z* (%) = 432 (40) $[M + Na]^+$, 448 (15) $[M + K]^+$.

1-{4,6-Dimethyl-1H-[1,2,3]triazolo[4,5-d]pyrimidine-5,7(4H,6H)-dione}acetyl L-glycyl-L-leucine methyl ester
(**17**, $C_{17}H_{25}N_7O_6$)
White foam (79%); ^{1}H NMR (*DMSO*-d_6, 250 MHz): δ = 0.89 (dd, *J* = 1.8, 7.3 Hz, $2CH_3$), 1.42–1.62 (m, CH_2, CH), 3.45 (s, N^6–CH_3), 3.59 (s, OCH_3), 3.64 (s, N^4–CH_3), 3.70 (s, CH_2), 3.72 (s, CH_2), 4.16–4.23 (m, CH), 5.39 (s, N^1–CH_2), 9.32 (br, s, NH) ppm; ^{13}C NMR (*DMSO*-d_6, 62.5 MHz): δ = 21.9 ($2CH_3$), 24.4 (CH), 27.6 (N^6–CH_3), 29.5 (N^4–CH_3), 39.6 (CH_2), 49.4 (CH_2), 50.9 (CH), 51.0 (N^1–CH_2), 52.0 (OCH_3), 111.9 (C-7_a), 150.9 (C-5), 152.7 (C-3_a), 154.0 (C-7), 163.0 (C=O), 169.1 (C=O), 172.8 (C=O) ppm; MS (MALDI, positive mode, Matrix: *DHB*): *m*/*z* (%) = 446 (12) $[M + Na]^+$, 462 (17) $[M + K]^+$.

1-{4,6-Dimethyl-1H-[1,2,3]triazolo[4,5-d]pyrimidine-5,7(4H,6H)-dione}acetyl L-glycyl-L-methionine methyl ester
(**18**, $C_{16}H_{23}N_7O_6S$)
Yellow foam (78%); ^{1}H NMR (*DMSO*-d_6, 250 MHz): δ = 2.19–2.48 (m, SCH_3, $2CH_2$), 3.42 (s, N^6–CH_3), 3.52 (s, OCH_3), 3.63 (s, N^4–CH_3), 3.77 (s, CH_2), 4.40–4.48 (m, CH), 5.41 (s, N^1–CH_2), 8.69 (br, s, NH) ppm; ^{13}C NMR (*DMSO*-d_6, 62.5 MHz): δ = 14.6 (SCH_3), 27.9 (N^6–CH_3), 29.0 (CH_2), 29.5 (N^4–CH_3), 30.6 (CH_2), 43.4 (CH_2), 46.5 (CH), 51.2 (N^1–CH_2), 53.2 (OCH_3), 111.7 (C-7_a), 150.2 (C-5), 152.3 (C-3_a), 154.0 (C-7), 163.2 (C=O), 171.6 (C=O), 175.0 (C=O) ppm; MS (MALDI, positive mode, Matrix: *DHB*): *m*/*z* (%) = 464 (19) $[M + Na]^+$, 480 (11) $[M + K]^+$.

1-{4,6-Dimethyl-1H-[1,2,3]triazolo[4,5-d]pyrimidine-5,7(4H,6H)-dione}acetyl L-alanyl-L-glycine methyl ester
(**19**, $C_{14}H_{19}N_7O_6$)
White foam (79%); ^{1}H NMR (*DMSO*-d_6, 250 MHz): δ = 1.39 (d, *J* = 5.0 Hz, CH_3), 3.46 (s, N^6–CH_3), 3.63 (s, N^4–CH_3), 3.72 (s, OCH_3), 3.94 (s, CH_2), 4.64 (q, *J* = 5.1 Hz, CH), 5.40 (s, N^1–CH_2), 8.82 (br, s, NH) ppm; ^{13}C NMR (*DMSO*-d_6, 62.5 MHz): δ = 17.6 (CH_3), 27.6 (N^6–CH_3), 29.5 (N^4–CH_3), 41.9 (CH_2), 44.6 (CH), 51.5 (N^1–CH_2), 53.7 (OCH_3), 111.6 (C-7_a), 150.2 (C-5), 152.3 (C-3_a), 154.0 (C-7), 163.0 (C=O), 172.3 (C=O), 173.7 (C=O) ppm; MS (MALDI, positive mode, Matrix: *DHB*): *m*/*z* (%) = 404 (21) $[M + Na]^+$, 420 (15) $[M + K]^+$.

1-{4,6-Dimethyl-1H-[1,2,3]triazolo[4,5-d]pyrimidine-5,7(4H,6H)-dione}acetyl L-alanyl-L-serine methyl ester (**20**, $C_{15}H_{21}N_7O_7$)
White foam (80%); ^{1}H NMR (*DMSO*-d_6, 250 MHz): $\delta = 1.39$ (d, $J = 5.0$ Hz, CH_3), 3.42 (s, N^6–CH_3), 3.48–3.53 (m, CH_2), 3.64 (s, N^4–CH_3), 3.69 (s, OCH_3), 4.50–4.59 (m, 2CH), 5.39 (s, N^1–CH_2), 7.06 (br, s, OH), 8.12 (br, s, NH) ppm; ^{13}C NMR (*DMSO*-d_6, 62.5 MHz): $\delta = 17.5$ (CH_3), 27.5 (N^6–CH_3), 29.9 (N^4–CH_3), 44.3 (CH), 51.7 (N^1–CH_2), 53.7 (OCH_3), 58.2 (CH), 72.2 (OCH_2), 111.5 (C-7_a), 150.2 (C-5), 152.3 (C-3_a), 154.0 (C-7), 163.0 (C=O), 172.2 (C=O), 173.0 (C=O) ppm; MS (MALDI, positive mode, Matrix: *DHB*): m/z (%) = 434 (32) $[M + Na]^+$, 450 (12) $[M + K]^+$.

1-{4,6-Dimethyl-1H-[1,2,3]triazolo[4,5-d]pyrimidine-5,7(4H,6H)-dione}acetyl L-alanyl-L-methionine methyl ester (**21**, $C_{17}H_{25}N_7O_6S$)
Yellow foam (81%); ^{1}H NMR (*DMSO*-d_6, 250 MHz): $\delta = 1.39$ (d, $J = 5.1$ Hz, CH_3), 2.22–2.50 (m, $2CH_2$, SCH_3), 3.42 (s, N^6–CH_3), 3.61 (s, N^4–CH_3), 3.65 (s, OCH_3), 4.50–4.61 (m, 2CH), 5.40 (s, N^1–CH_2), 9.02 (br, s, NH) ppm; ^{13}C NMR (*DMSO*-d_6, 62.5 MHz): $\delta = 14.7$ (SCH_3), 17.7 (CH_3), 27.7 (N^6–CH_3), 29.3 (CH_2), 29.9 (N^4–CH_3), 31.0 (CH_2), 45.7 (CH), 49.9 (CH), 51.1 (N^1–CH_2), 52.0 (OCH_3), 111.9 (C-7_a), 150.7 (C-5), 152.2 (C-3_a), 154.2 (C-7), 163.0 (C=O), 175.0 (C=O), 176.0 (C=O) ppm; MS (MALDI, positive mode, Matrix: *DHB*): m/z (%) = 478 (16) $[M + Na]^+$, 494 (12) $[M + K]^+$.

1-{4,6-Dimethyl-1H-[1,2,3]triazolo[4,5-d]pyrimidine-5,7(4H,6H)-dione}acetyl L-seryl-L-glycine methyl ester (**22**, $C_{14}H_{19}N_7O_7$)
White powder (82%); mp 167–169°C. ^{1}H NMR (*DMSO*-d_6, 250 MHz): $\delta = 3.45$ (s, N^6–CH_3), 3.66 (s, N^4–CH_3), 3.68–3.75 (m, OCH_2, OCH_3), 3.93 (s, CH_2), 4.50–4.55 (m, CH), 5.34 (s, N^1–CH_2), 7.20 (br, s, OH), 8.69 (br, s, NH) ppm; ^{13}C NMR (*DMSO*-d_6, 62.5 MHz): $\delta = 27.5$ (N^6–CH_3), 29.7 (N^4–CH_3), 41.9 (CH_2), 49.9 (CH), 51.2 (N^1–CH_2), 53.3 (OCH_3), 61.4 (OCH_2), 112.2 (C-7_a), 150.5 (C-5), 152.3 (C-3_a), 154.0 (C-7), 162.7 (C=O), 1710.8 (C=O), 172.4 (C=O) ppm; MS (MALDI, positive mode, Matrix: *DHB*): m/z (%) = 420 (18) $[M + Na]^+$, 436 (11) $[M + K]^+$.

1-{4,6-Dimethyl-1H-[1,2,3]triazolo[4,5-d]pyrimidine-5,7(4H,6H)-dione}acetyl L-seryl-L-serine methyl ester (**23**, $C_{15}H_{21}N_7O_8$)
White powder (85%); mp 180–182°C; ^{1}H NMR (*DMSO*-d_6, 250 MHz): $\delta = 3.42$ (s, N^6–CH_3), 3.51–3.55 (m, OCH_2), 3.62 (s, N^4–CH_3), 3.66–3.77 (m, OCH_2, OCH_3), 4.48–4.56 (m, 2CH), 5.36 (s, N^1–CH_2), 6.33 (br, s, 2OH), 8.60 (br, s, NH) ppm; ^{13}C NMR (*DMSO*-d_6, 62.5 MHz): $\delta = 27.8$ (N^6–CH_3), 29.8 (N^4–CH_3), 49.7 (CH), 51.7 (N^1–CH_2), 53.9 (OCH_3), 56.7 (CH), 61.3 (OCH_2), 72.4 (OCH_2), 111.9 (C-7_a), 150.7 (C-5), 152.9 (C-3_a), 154.9 (C-7), 162.8 (C=O), 172.2 (C=O), 173.9 (C=O) ppm; MS (MALDI, positive mode, Matrix: *DHB*): m/z (%) = 450 (20) $[M + Na]^+$, 466 (13) $[M + K]^+$.

1-{4,6-Dimethyl-1H-[1,2,3]triazolo[4,5-d]pyrimidine-5,7(4H,6H)-dione}acetyl L-seryl-L-methionine methyl ester (**24**, $C_{17}H_{25}N_7O_7S$)
Yellow foam (83%); ^{1}H NMR (*DMSO*-d_6, 250 MHz): $\delta =$ 2.23–2.50 (m, $2CH_2$, SCH_3), 3.44 (s, N^6–CH_3), 3.60 (s, OCH_3), 3.67 (s, N^4–CH_3), 3.70–3.77 (m, CH_2), 4.50–4.57 (m, 2CH), 5.36 (s, N^1–CH_2), 7.21 (br, s, OH), 8.33 (br, s, NH) ppm; ^{13}C NMR (*DMSO*-d_6, 62.5 MHz): $\delta = 14.8$ (SCH_3), 27.8 (N^6–CH_3), 29.3 (CH_2), 29.8 (N^4–CH_3), 30.9 (CH_2), 48.4 (CH), 51.0 (CH), 51.8 (N^1–CH_2), 53.2 (OCH_3), 61.3 (OCH_2), 111.8 (C-7_a), 150.7 (C-5), 152.6 (C-3_a), 154.1 (C-7), 162.9 (C=O), 174.1 (C=O), 175.0 (C=O) ppm; MS (MALDI, positive mode, Matrix: *DHB*): m/z (%) = 494 (15) $[M + Na]^+$, 510 (8) $[M + K]^+$.

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