

Multicomponent synthesis in water of 7-unsubstituted 4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidines and their antimicrobial and antifungal activity

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Abstract

Simple, efficient and eco-friendly method for preparation of 7-unsubstituted 4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidines was elaborated, which presumes the three-component reaction of 3-amino-1,2,4-triazole with paraformaldehyde and different 1,3-dicarbonyl compounds in hot-water by either conventional heating or microwave irradiation without any catalyst. The target compounds showed antimicrobial and antifungal activity.

Keywords: Multicomponent reaction, heterocycle, 1,4-dihydroazine, hot-water organic synthesis, microwave-assisted synthesis, green chemistry

Introduction

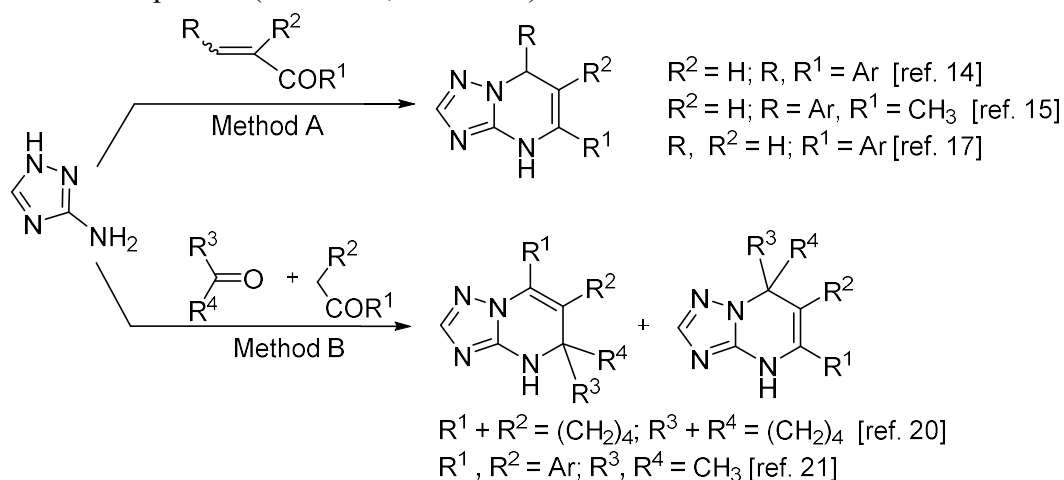
One of the principles of green chemistry presumes use of safe solvent for preparation of new substances¹. Surprisingly, water, which seems to be the safest possible solvent, was not considered to be a very suitable reaction medium for organic synthesis during a long time, largely on account of its non-inert properties and low solubility of most organic compounds in it. Recently the investigation of organic reactions in water gained some impact. Latest reviews^{2,3} and monographs^{4,5} showed that use of water as a reaction medium can be useful for carrying out very different kinds of organic reactions; among them are aldol reaction⁵ and Knoevenagel reaction⁶, which is especially important because these reactions could be key steps in the multicomponent synthesis of six-membered heterocycles, such as pyridines⁷ and pyrimidines⁸. Although the limited

solubility of most organic compounds in water leads to synthetic limitations, this makes sometimes the isolation of final compound easier, which can be great advantage in some cases.

The main subject of our study are partially hydrogenated azolopyrimidines, compounds which possess interesting biological properties.⁹⁻¹² Among them, 4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidines are well-studied representatives, the synthetic availability of which is the most developed.

The general method for the preparation of 6,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidines¹³ includes cyclocondensation reaction of 3-amino-1,2,4-triazole with either α,β -unsaturated carbonyl compound (for example, chalcone derivatives,¹⁴ arylideneacetones,¹⁵ arylideneacetoacetates¹⁶), or with Mannich base of substituted acetophenone as synthetic precursor of α,β -unsaturated carbonyl compound (**Scheme 1**, Method A).¹⁷

On the other hand, use of multicomponent reactions is a powerful and efficient tool of modern organic synthesis, which often provides great advantages in comparison with sequential procedures¹⁸. Therefore, another efficient synthetic approach to 4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidines¹⁹ is a multicomponent treatment of 3-amino-1,2,4-triazole with aldehyde and methylene-active carbonyl compound. It is presumed, that in such multicomponent reactions the formation of α,β -unsaturated carbonyl compound may occur in the reaction medium. However, some publications have shown that similar treatments proceeded in other ways giving different types of final compounds (**Scheme 1**, Method B).²⁰⁻²³



Scheme 1. Two general methods for synthesis of dihydro-1,2,4-triazolo[1,5-*a*]pyrimidines.

There are some examples of multicomponent synthesis of dihydroazolopyrimidine derivatives in water described²⁴⁻²⁶.

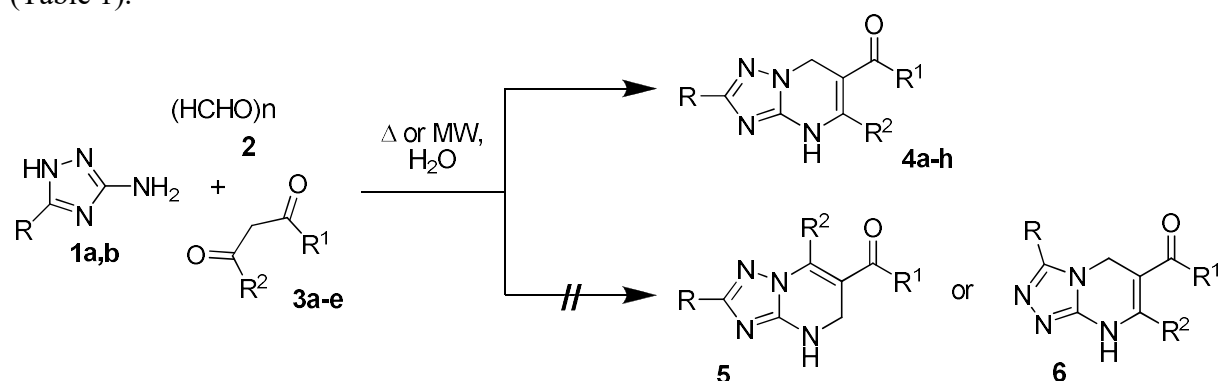
However, in most cases cited above, aryl- or diaryl-substituted derivatives of azoloazines were obtained, and, there are relatively few literature references which describe the preparation of 4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidines containing no bulky substituent. Unexpectedly, there are only two examples of application of the formaldehyde in such kind of three-component reactions.^{27,28}

The presence of aryl substituent in the molecule of 4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine allows to stabilize the structure of heterocycle, lowers the solubility of compound and makes the isolation process easier. On the other hand, obtaining the azolopyrimidines with lower molecule masses, especially without any aryl ring, can open much wider possibilities for search of new biologically active compounds. It should be noted, that such azolopyrimidines could be used as building-blocks for their chemical modification in order to develop new approaches to wide ranges of compounds based on dihydroazolopyrimidine moiety and with molecular mass of final compound lower than 500 a.u., which is important, taking into account Lipinski principles for biologically active compounds.²⁹

Thus, the main goal of current research was to elaborate efficient and eco-friendly synthetic route for the preparation of novel 7-unsubstituted 4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidines with no aryl substituent by the three-component reaction of 3-amino-1,2,4-triazoles, paraformaldehyde and 1,3-dicarbonyl compounds, and study of biological activity of target compounds.

Results and Discussion

We have established, that the heating an equimolar mixture of 3-amino-1,2,4-triazoles (**1a,b**), paraformaldehyde (**2**) and 1,3-dicarbonyl compounds **3a-e** at 100 °C in water without any catalyst leads to the selective formation of 7-unsubstituted 4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidines **4a-h** in good yields (**Scheme 2**, Table 1). In case of compound **4b**, its isolation was possible only after cooling the reaction medium, while in other cases the precipitation of target compounds **4a,c-f** was observed even during the heating process. In order to increase yields, we attempted to apply microwave irradiation to activate the current reaction. According to our previous experience³⁰⁻³⁵, microwave-assisted multicomponent reactions are usually more efficient in terms of reaction times, yields and purity in comparison with processes under classical conditions. Surprisingly, our attempts in this case gave the results being similar to those obtained for conventional heating (Table 1).



Scheme 2. Reaction of 3-amino-1,2,4-triazoles **1** with paraformaldehyde (**2**) and 1,3-dicarbonyl compounds **3**.

Table 1. Used building-blocks **1**, **3** and obtained reaction products **4**, their yields and reaction conditions

En-try	Building-blocks					Reaction product		
	3-amino-1,2,4-triazole		1,3-Dicarbonyl compound			Yield, %		
	R		R ¹	R ²	Δ (time, min)			
1	1a	H	3a	OC ₂ H ₅	CH ₃	4a	72 (15)	75 (15; 100)
2	1a	H	3b	CH ₃	CH ₃	4b	66 (20)	68 (15; 100)
3	1a	H	3c	OCH ₃	CH ₃	4c	70 (12)	70 (10; 100)
4	1b	COOCH ₃	3a	OC ₂ H ₅	CH ₃	4d	75 (15)	75 (15; 100)
5	1a	H	3d	OC ₂ H ₄ OCH ₃	CH ₃	4e	72 (15)	75 (15; 100)
6	1a	H	3e	OC ₂ H ₅	<i>n</i> -C ₃ H ₇	4f	75 (17)	75 (15; 100)
7	1b	COOCH ₃	3b	CH ₃	CH ₃	4g	72 (15)	75 (13; 100)
8	1b	COOCH ₃	3e	OC ₂ H ₅	<i>n</i> -C ₃ H ₇	4h	68 (20)	70 (20; 100)

The purity and structures of compounds **4a-h** were confirmed by their elemental analysis and spectral data (¹H and ¹³C NMR, IR and MS). Typical ¹H NMR spectra of all the compounds synthesized showed signals for 6-H methylene protons at 4.79 - 4.95 ppm, broad singlet for NH protons of dihydropyrimidine ring at 10.40 - 10.84 ppm and sharp singlet for 2-H proton of triazole moiety at 7.68-7.71 ppm (for **4a-c,e,f**) and other signals for terminal substituents. The ¹³C NMR and mass spectra were also in good agreement with the structure proposed. However, it is known,^{22,23,36,37} that the three-component reactions between aminoazoles, carbonyl compounds and CH-acids in some cases can lead to the formation of several isomeric compounds with very similar spectral characteristics. Therefore, in the reaction studied, the isolation of three different heterocyclic compounds may be expected with a certain degree of probability: 4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine (**4**), 4,5-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine (**5**) or 5,8-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine (**6**). It was shown²⁰ that the simplest criterion for assignment of the reaction product either to 4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine or to 4,5-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine (structures **4** and **5** respectively) is the chemical shift of NH proton in the ¹H NMR spectrum, which is about 6.9-7.1 for 4,7-dihydro structure and 9.9-10.0 for 4,5-dihydro structure. Therefore, according to our ¹H NMR data, all the products **4a-f** had structures of 4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine (**4**), as shown on **Scheme 1**. Additionally, in the 1D NOE experiment of **4a**, the irradiation of NH proton showed enhancement of CH₃ proton and no enhancement of CH₂ protons; in the NOESY experiment no cross-peaks were observed between CH₂ and NH protons and between CH₂ and CH proton of triazole moiety, which allowed to reject structures **5** and **6**, and therefore, structure of **4** was finally confirmed.

After evaporation of mother liquor no additional amount of **4a** was detected; the ^1H NMR of the residue did not allow to identify signals of heterocycles **4**, **5** or **6** and showed only decomposition products.

Obviously, when water is used as a solvent, there is no need in acidic catalyst for the formation of current dihydroazolopyrimidine derivatives, therefore, the mechanism of dihydroazolopyrimidine formation may be different from the classical mechanism of Biginelli reaction³⁸ and presumes the previous formation of α,β -unsaturated compound in the Knoevenagel reaction on the first step and followed by heterocyclisation according to **Scheme 1 Method A**) which includes the 1,4-addition of endocyclic nitrogen to the enone system of unsaturated carbonyl compound. However, it is known³⁹ that the preparative formation of such unsaturated carbonyl compound from formaldehyde and acetylacetone is not possible, therefore, the three-component synthesis of azolopyrimidines **4** seems to be the single way here.

Compounds **4** are easily soluble in most organic solvents, like ethanol, and slightly soluble in water, in contrast to their analogs with aromatic rings¹⁶, therefore, their isolation from reaction media by use other organic solvents can cause some problems.

The compounds **4a,b,d,g,f** were tested as substances having antimicrobial and antifungal activity (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 885-653 strains); as control substances, ampicillin antibiotic and nystatin as antifungal drug were used. The obtained data of minimum inhibitory concentration (MIC), minimum bactericidal or fungicidal concentrations (MBC and MFC respectively) are given in Table 2.

Table 2. Data of antimicrobial and antifungal activity test for **4a,b,d,f,g**

Code name	E.Coli		S.aureus		P.aeruginosa		C.albicans	
	MIC $\mu\text{g/mL}$	MBC $\mu\text{g/mL}$	MIC $\mu\text{g/mL}$	MBC $\mu\text{g/mL}$	MIC $\mu\text{g/mL}$	MBC $\mu\text{g/mL}$	MIC $\mu\text{g/mL}$	MFC $\mu\text{g/mL}$
4a	100	200	25	50	50	100	50	100
4b	100	200	50	100	100	200	50	50
4c	100	100	50	100	50	100	12.5	25
4d	100	200	25	50	100	200	100	200
4e	100	200	50	100	100	200	50	100
4f	100	200	50	100	100	200	100	200
4g	100	200	25	50	100	200	50	50
Ampicillin	0,78	0,78	0,1	0,195	50	200		
Nystatin	100	>200	200	>200	100	>200	0,78	1,56

Our results indicate that all tested compounds exhibit activity against a pathogenic microflora at high MIC, MBC, MFC concentrations in comparison with the control preparation. One of the best results was obtained on compound **4a**; ethoxycarbonyl derivative **4a** showed a bit higher antimicrobial activity, than acetyl derivative **4b**, whereas antifungal activity for **4a** and **4b** is similar.

Experimental Section

General. The melting points of all compounds synthesized were determined with a Gallenkamp melting point apparatus. The NMR spectra were recorded at 400 MHz (100 MHz for ^{13}C) and at 200 MHz (50 MHz for ^{13}C) with a Varian MR-400 spectrometer. The EI MS spectra were measured on a GC-MS Varian 1200L (ionizing voltage 70 eV, direct input of the sample) instrument. Elemental analysis was realized on EuroVector EA-3000. Analytical samples of the compounds were obtained by their crystallization in water and further drying at room temperature. Microwave experiments were performed using the Emrys Creator EXP from Biotage AB (Uppsala, Sweden) possessing a single-mode microwave cavity producing controlled irradiation at 2.45 GHz. Solvents, all reagents were commercially available and used without additional purification.

The antimicrobial and antifungal activity was carried out on standard *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 885-653 strains obtained in bacteriological laboratory of Zaporizhia Regional Laboratory Center of the State Epidemiological Service of Ukraine.

Basic solution was prepared by dissolving 1 mg of investigated substance in 1 mL of dimethylsulfoxide. The 4 mL of Muller-Hinton broth⁴⁰ (for 25923 *S. aureus* ATCC, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853) or Sabouraud broth⁴⁰ (for *C. albicans* ATCC 885-653) were added to basic solution to obtain the dilution with the concentration of substance 200 $\mu\text{g} / \text{mL}$. After series of two-fold dilutions, three additional solutions were prepared with concentrations 100, 50, 25 $\mu\text{g} / \text{mL}$ respectively, each in volume of 1 mL. The suspension of microbial culture on amount of 0.1 mL was added into each tube. The test tubes with crops of *S. aureus*, *E. coli*, *P. aeruginosa* were incubated at $37 \pm 1 \text{ }^\circ\text{C}$ for 16-24 hours, with crops of *C. albicans* - at $28 \pm 1 \text{ }^\circ\text{C}$ for 44-48 hours. MIC was determined on visual absence of bacterial growth in the test tube with minimal concentration of substance investigated.

For determination of MBC and MFC, the content of test tube after MIC determination with no bacterial growth was put into 0.1 mL of Muller-Hinton agar⁴⁰ (for *S. aureus*, *E. coli*, *P. aeruginosa*) or into 0.1 mL of Sabouraud agar⁴⁰ (for *C. albicans*); cups were incubated at $37 \pm 1 \text{ }^\circ\text{C}$ for 16-24 hours and at $28 \pm 1 \text{ }^\circ\text{C}$ for 44-48 hours respectively. Values of MBC and MFC were detected by visual absence of bacterial growth of cultures.

General procedure for synthesis of 4a-h

Conventional heating. A solution of 3-amino-1,2,4-triazole derivative **1** (0.0012 mol), 0.04 g of paraformaldehyde (**2**) (0.0013 mol) and 1,3-dicarbonyl compound **3** (0.0012 mol) in water (4,5 mL) was refluxed during the time given in the Table 1. The resulting precipitate was filtered off, recrystallized from water and air-dried.

Microwave activation. An equimolar mixture (0.0012 mol) of **1**, **2** and **3** in water (4 mL) was irradiated in MW reactor at 100 °C during the time given it table 1. The crystalline product started to separate out either during the reaction or just after cooling. The precipitate formed was filtered off, washed with water and air-dried.

Ethyl 5-methyl-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylate (4a). White solid, yield 75%, mp 189-190 °C (from water) (Lit.²⁸ 194-197°C from ethanol); IR (v, cm⁻¹): 1711 (C=O), 1665 (C=C). ¹H NMR (400 MHz, DMSO-*d*₆): δ 1.21 (3H, t, ³J_{HH} 7.2 Hz, CH₃), 2.29 (3H, s, CH₃), 4.09 (2H, q, CH₂), 4.80 (2H, s, CH₂), 7.70 (1H, s, 2-H), 10.43 (1H, s, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 13.9, 17.7, 45.2, 59.1, 92.1, 146.6, 147.0, 149.4, 165.0. MS (EI, 70 eV): *m/z* (%) 208 (44) [M⁺], 180 (19), 179 (100), 163 (10). Anal. Calcd for C₉H₁₂N₄O₂ (208.22): C, 51.92; H, 5.81; N, 26.91%. Found: C, 51.77; H, 5.56; N, 26.63%.

1-(5-Methyl-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidin-6-yl)-ethanone (4b). White solid, yield 68 %, mp 233-234 °C (from water); ¹H NMR (400 MHz, DMSO-*d*₆): δ 2.21 (3H, s, CH₃), 2.29 (3H, s, CH₃), 4.91 (2H, s, CH₂), 7.72 (1H, s, 2-H), 10.43 (1H, s, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 19.2, 30.2, 46.0, 102.5, 145.9, 146.9, 149.8, 194.6. MS (EI, 70 eV): *m/z* (%) 178 (41) [M⁺], 177 (16), 163 (100), 135 (22), 109 (13). Anal. Calcd for C₈H₁₀N₄O (178.19): C, 53.92; H, 5.66; N, 31.44%. Found: C, 54.17; H, 5.90; N, 31.43%.

Methyl 5-methyl-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylate (4c). White solid, yield 70%, mp 241-242 °C (from water); IR (v, cm⁻¹): 1678 (C=O), 1649 (C=C). ¹H NMR (400 MHz, DMSO-*d*₆): δ 2.30 (3H, s, 5-CH₃), 3.64 (3H, s, OCH₃), 4.81 (2H, s, CH₂), 7.71 (1H, s, 2-H), 10.47 (1H, s, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 18.1, 45.4, 51.0, 91.9, 147.1, 147.2, 149.7, 165.7. MS (EI, 70 eV): *m/z* (%) 194 (28) [M⁺], 179 (100), 161 (25), 163 (12). Anal. Calcd for C₈H₁₀N₄O₂ (194.19): C, 49.48; H, 5.19; N, 28.85%. Found: C, 49.62; H, 5.18; N, 28.67%.

6-Ethyl 2-methyl 5-methyl-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-2,6-dicarboxylate (4d). White solid, yield 75%, 0.35 g, mp 244-245 °C (from water); IR (v, cm⁻¹): 1740, 1715 (2C=O), 1738 (C=C). ¹H NMR (400 MHz, DMSO-*d*₆): δ 1.21 (3H, t, ³J_{HH} 7.2 Hz, CH₃), 2.30 (3H, s, 5-CH₃), 3.81 (3H, s, OCH₃), 4.11 (2H, q, CH₂), 4.86 (2H, s, CH₂), 10.59 (1H, s, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 14.2, 18.1, 45.8, 52.1, 59.6, 92.5, 146.7, 148.1, 151.5, 159.9, 165.1. MS (EI, 70 eV): *m/z* (%) 266 (25) [M⁺], 238 (29), 237 (100), 221 (42), 193 (19), 189 (20), 179 (11), 161 (25). Anal. Calcd for C₁₁H₁₄N₄O₄ (266.25): C, 49.62; H, 5.30; N, 21.04%. Found: C, 49.69; H, 5.30; N, 21.11%.

2-Methoxyethyl 5-methyl-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylate (4e). White solid, yield 75%, mp 170-171°C (from water); IR (v, cm⁻¹): 1700 (C=O), 1645 (C=C). ¹H NMR (400 MHz, DMSO-*d*₆): δ 2.30 (3H, s, 5-CH₃), 3.27 (3H, s, CH₃), 3.57 (2H, t, CH₂), 4.18

(2H, t, CH₂), 4.81 (2H, s, CH₂), 7.72 (1H, s, 2-H), 10.50 (1H, s, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 18.1, 45.3, 58.1, 62.6, 69.9, 91.8, 147.1, 147.4, 149.7, 165.2. MS (EI, 70 eV): *m/z* (%) 238 (9) [M⁺], 179 (54), 163 (43), 161 (13), 135 (22), 134 (100). Anal. Calcd for C₁₀H₁₄N₄O₃ (238.24): C, 50.41; H, 5.92; N, 23.52%. Found: C, 50.49; H, 6.01; N, 23.41%.

Ethyl 5-propyl-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylate (4f). White solid, yield 75%, mp 190-191°C (from water); IR (ν, cm⁻¹): 1705 (C=O). ¹H NMR (400 MHz, DMSO-*d*₆): δ 0.91 (3H, t, ³J_{HH} 7.2 Hz, OCH₂CH₃), 1.21 (3H, t, CH₃), 1.56 (2H, m, CH₂), 2.69 (2H, t, CH₂), 4.10 (2H, q, OCH₂CH₃), 4.81 (2H, s, CH₂), 7.71 (1H, s, 2-H), 10.43 (1H, s, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 13.7, 14.1, 21.7, 32.6, 45.4, 59.4, 91.7, 147.2, 149.7, 151.1, 165.0. MS (EI, 70 eV) : *m/z* (%) 236 (19) [M⁺], 208 (19), 207 (100), 149 (11), 134 (19). Anal. Calcd for C₁₁H₁₆N₄O₂ (236.27): C, 55.92; H, 6.83; N, 23.71%. Found: C, 55.78; H, 6.72; N, 23.84%.

Methyl 6-acetyl-5-methyl-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-2-carboxylate (4g). White solid, yield 75%, mp 248-249°C (from water); ¹H NMR (400 MHz, DMSO-*d*₆): δ 2.22 (3H, s, CH₃), 2.30 (3H, s, CH₃), 3.81 (3H, s, CH₃), 4.96 (2H, s, CH₂), 10.57 (1H, s, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 19.2, 30.3, 46.4, 52.2, 103.0, 145.6, 148.0, 151.6, 159.9, 194.6. MS (EI, 70 eV): *m/z* (%) 236 (48) [M⁺], 221 (99), 177 (20). Anal. Calcd for C₁₀H₁₂N₄O₃ (236.23): C, 50.84; H, 5.12; N, 23.72%. Found: C, 50.98; H, 5.26; N, 23.59%.

6-Ethyl 2-methyl 5-propyl-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-2,6-dicarboxylate (4h). White solid, yield 70%, 0.35 g, mp 191-192°C (from water); ¹H NMR (400 MHz, DMSO-*d*₆): δ 0.92 (3H, t, CH₃), 1.22 (3H, t, ³J_{HH} 7.2 Hz, OCH₂CH₃), 1.58 (2H, m, CH₂), 2.69 (2H, t, CH₂), 3.81 (3H, s, OCH₃), 4.12 (2H, q, OCH₂CH₃), 4.88 (2H, s, CH₂), 10.56 (1H, s, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 13.6, 14.1, 21.6, 32.6, 45.8, 52.1, 59.6, 92.3, 148.2, 150.7, 151.5, 159.9, 164.8. MS (EI, 70 eV): *m/z* (%) 294 (9) [M⁺], 238 (29), 265 (99), 221 (42). Anal. Calcd for C₁₃H₁₈N₄O₄ (294.31): C, 53.05; H, 6.16; N, 19.04%. Found: C, 53.1; H, 6.11; N, 19.09%.

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