Scientific paper

A Facile *One-pot* Synthesis and Antimicrobial Activity of Pyrido [2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidin-5-ones

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Abstract

A series of pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidin-5-ones (**8**) has been synthesised *via* reaction of 5-substituted-2-thioxo-2,3-dihydro-1*H*-pyrido[2,3-*d*]pyrimidin-4-one (**3**) or its methylthio derivative **4** with hydrazonoyl chlorides **5**. Alternative syntheses of products **8** were carried out either by reaction of enaminone **1** with 7-amino-1,3-disubstituted[1,2,4]triazolo[4,3-*a*]pyrimidin-5-one (**10**) or *via* the Japp-Klingemann reaction of compound **13**. Both conventional thermal and microwave irradiation techniques were used for synthesis of the target products **8** and a comparative study of these techniques using triethylamine or chitosan, as basic catalysts, was carried out. The mechanisms of the reactions under investigation are discussed. In addition, the antimicrobial activity of the newly synthesized products was evaluated.

Keywords: Enaminone, 2-thioxo-2,3-dihydro-1*H*-pyrido[2,3-*d*]pyrimidin-4-one, hydrazonoyl chlorides, microwave irradiation, chitosan, antimicrobial activity.

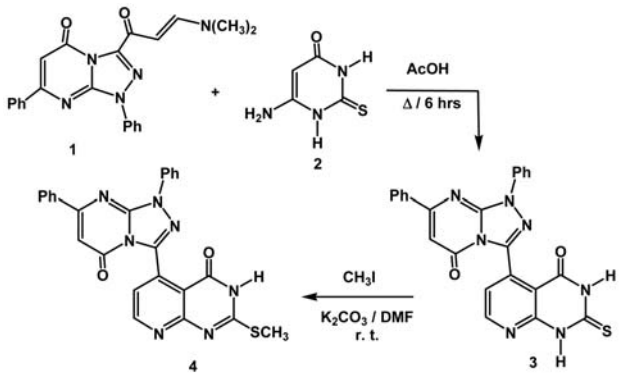
1. Introduction

[1,2,4]Triazolo[4,3-*a*]pyrimidines are pharmacological scaffolds displaying a wide range of biological activities such as antitumor,[1] human A3 adenosine receptor,[2] antibacterial,[3] CNS depressant,[4] antiallergy,[5] and anti-inflammatory.[6] In the field of coordination chemistry, [1,2,4]triazolo[4,3-*a*]pyrimidine can be used as a bridging ligand.[7] On the other hand, pyridopyrimidines, another class of heterocyclic compounds, can be used as active modulators of cannabinoid-1 receptor (CB1R).[8] Fusion of [1,2,4]triazole ring to pyridopyrimidines tends to expose novel biological activities. Based on these findings and as a part of our research program aiming at synthesis of heterocyclic systems with biological and pharmacological activities,[9–15] we became interested in extending the scope of this approach for synthesis of novel pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*] pyrimidin-5-ones incorporating the [1,2,4]triazolo [4,3-*a*]pyrimidine moiety. Also, in this context the antimicrobial evaluation of the newly synthesized compounds was performed.

2. Results and Discussion

The starting reactant **3**, namely 5-substituted-2-thioxo-2,3-dihydro-1*H*-pyrido[2,3-*d*]pyrimidin-4-one which has not previously been reported, was prepared by reaction of 3-[3-(dimethylamino)-2-propenoyl]-1,7-diphenyl-1,5-dihydro[1,2,4]triazolo[4,3-*a*]pyrimidin-5-one (**1**)[16] with 6-amino-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one (**2**)[17] under reflux in acetic acid (Scheme 1). Also, the new compound, 2-methylthio derivative **4** was prepared by reaction of **3** with methyl iodide in dimethylformamide in the presence of anhydrous potassium carbonate at room temperature (Scheme 1).

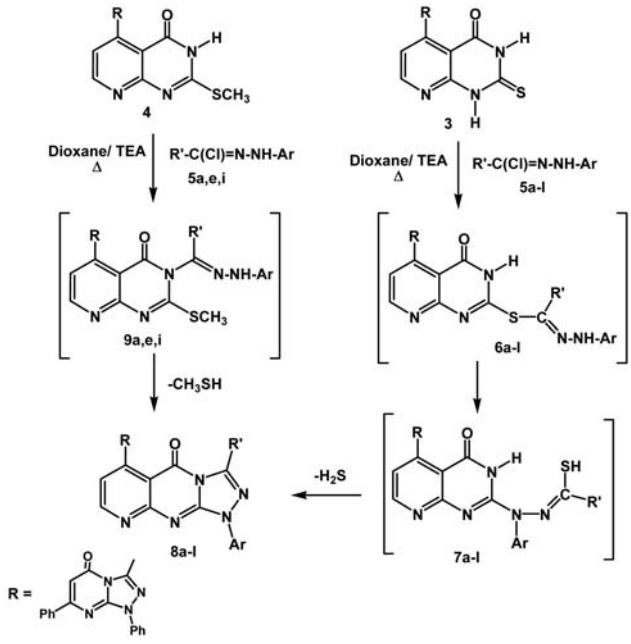
The reaction of **3** with the appropriate hydrazonoyl chlorides **5a-l** was carried out in dioxane in the presence of triethylamine under reflux until all hydrogen sulfide gas ceased to evolve (Scheme 2). After work up the reaction gave in each case, only one isolable product as evidenced by TLC analysis of the crude product. The assigned structure **8** for the isolated products is based on microanalysis and spectral (IR, ^{1}H NMR, MS) data. For example, the IR spectra of products **8** revealed in each ca-



Scheme 1

se an absorption band at υ (1751–1693) cm^{-1} due to the carbonyl group of the substituent at position-3. ^1H NMR spectra showed no signals assignable to the 2 NH groups in compound **3** and instead revealed the appearance of signals due to the protons of the acetyl, ester, and anilide substituents at position-3 (see Experimental). The mass spectra revealed in each case a molecular ion peak at the expected *m/z* value.

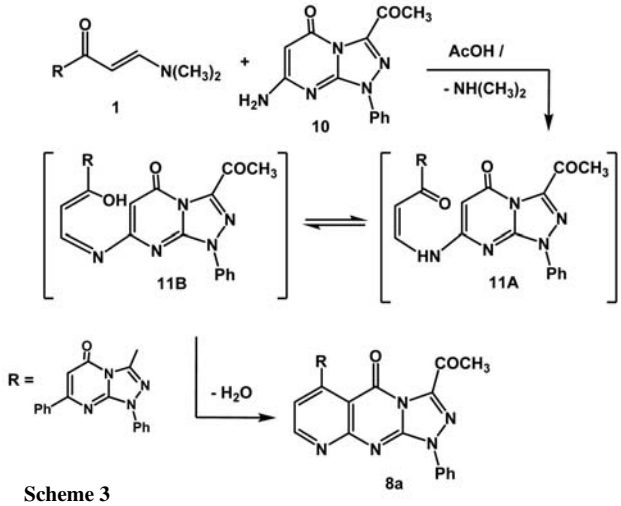
To account for the direct formation of products **8a-l** from reaction of **3** with **5a-l**, the mechanism outlined in Scheme 2 is proposed. The reaction starts with the formation of thiohydrazonates **6a-l** which undergo Smiles rearrangement[18,19] to yield the corresponding thiohydrazides **7a-l**. The latter compounds undergo *in situ* cyclization with elimination of hydrogen sulfide gas to give **8a-l** as the end products (Scheme 2). The regioselective cyclization of the thiohydrazides **7a-l** was further evidenced by an alternative synthesis of **8** *via* reaction of **4** with hydrazonoyl chlorides **5a,e,i** under the same conditions. Thus, treatment of **4** with **5a,e,i** in dioxane in the presence of triethylamine under reflux led to formation of products identical in all respects (mp, mixed mp, IR) with products **8a,e,i** (Scheme 2). The reaction of **4** with **5a,e,i** proceeds *via* the formation of the amidrazones **9a,e,i**, which in turn, undergo cyclization with elimination of methanethiol to give **8a,e,i** as end products (Scheme 2). The intermediates **6a-l**, **7a-l**, and **9a,e,i** could not be isolated in any case, which indicates that such intermediates are consumed *in situ* under the reaction conditions employed.



Compd. no.	R'	Ar	Compd. no.	R'	Ar	Compd. no.	R'	Ar
5a, 8a	CH_3CO	C_6H_5	5e, 8e	CO_2Et	C_6H_5	5i, 8i	PhNHCO	C_6H_5
5b, 8b	CH_3CO	4-$CH_3C_6H_4$	5f, 8f	CO_2Et	4-$CH_3C_6H_4$	5j, 8j	PhNHCO	4-$CH_3C_6H_4$
5c, 8c	CH_3CO	4-ClC_6H_4	5g, 8g	CO_2Et	4-ClC_6H_4	5k, 8k	PhNHCO	4-ClC_6H_4
5d, 8d	CH_3CO	4-$NO_2C_6H_4$	5h, 8h	CO_2Et	4-$NO_2C_6H_4$	5l, 8l	PhNHCO	4-$NO_2C_6H_4$

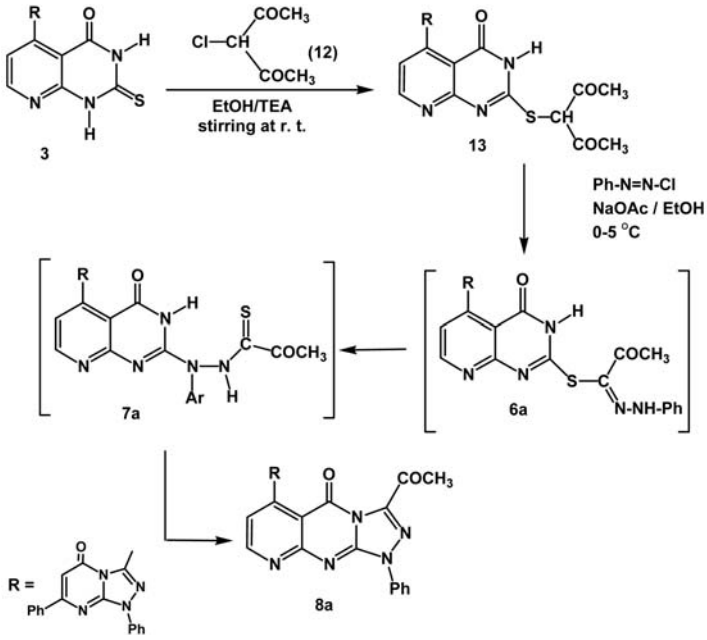
Scheme 2

Further evidence for the assigned structure of compounds **8** was obtained *via* alternative synthesis of these products. Thus, reaction of 7-amino-3-acetyl-1-phenyl [1,2,4]triazolo[4,3-*a*]pyrimidin-5(1*H*)-one (**10**)[20] with enaminone **1** in acetic acid under reflux gave a product identical in all respects (mp, mixed mp, IR) with the isolated product **8a** (Scheme 3).



Scheme 3

Final evidence for the assigned structure of the target products **8a-l** was based on alkylation of **3** with chloroactivemethylene compound **12** followed by coupling obtained with diazonium salt.[18,19,21] Thus, reaction of **3** with 3-chloropentane-2,4-dione[22-24] **12** in ethanol in the presence of triethylamine at room temperature yielded substitution product **13** (Scheme 4). The structure of the new product **13** was elaborated by its spectral data (IR, ^1H NMR, and MS) and elemental analysis. For example, the ^1H NMR spectrum showed two characteristic singlet signals near δ 2.4 and 4.9 ppm assigned to the protons of CH_3CO and SCH groups, in addition to the signals of the



Scheme 4

=NH and aromatic protons (see Experimental). Treatment of compound **13** with phenyldiazonium chloride in ethanol in the presence of sodium acetate at low temperature (0–5 °C) led to formation of a single substance as evidenced by TLC analysis of the crude product. The microanalyses and mass spectral data of the isolated product were consistent with structure **8a** (Scheme 4).

The formation of the product **8a** obtained by the reaction sequence outlined in Scheme 4, was reasonably formed by Japp-Klingemann[21] elimination of the acetyl group during the azo coupling reaction of compound **13**, to form the corresponding thiohydrazonate **6a**. The latter undergo Smiles rearrangement[18,19] as mentioned above to give the thiohydrazide **7a**, which in turn undergo *in situ* cyclization, to give **8a,** as the end product (Scheme 4).

Finally, we use microwave irradiation as an alternative method for the synthesis of products **8a**, **8e**, and **8i** using the same reaction sequence outlined in Scheme 1. It is noteworthy that the catalyst used in this method may be either triethylamine or chitosan, i.e., a copolymer containing both glucoseamine units and acetylglucoseamine units, which is a very efficient and environmentally benign heterogeneous basic catalyst. In Table 1 the reaction results (time, % yield) are compared with those of the traditional thermal procedure using triethylamine as basic catalyst.

As shown in Table 1, the use of microwave irradiation substantially reduced the reaction times from hours to minutes and appreciably increased the yield of the products. Also, chitosan can be used as an ecofriendly basic catalyst for preparation of the desired products **8a,e,i** in high yield under thermal heating or microwave irradiation.

3. Antimicrobial Activity

The compounds **8a-g,k** were tested for their antimicrobial activities using two bacteria species namely, Gram (+) bacteria as *Staphylococcus aureus* **Sa** and Gram (–) bacteria as *Esherichia coli* **Ec**. The organisms were tested against the activity of solutions of concentration 20 mg/mL of each compound and using an inhibition zone diameter (IZD) in (mm/mg sample) as criterion for the antimicrobial activity.[25,26] The bactericide **Tetracycline** was used as reference to evaluate the potency of the tested compounds under the same conditions. The results are depicted in Table 2. The tested products (**8a-g,k**) exhibited antimicrobial and antipseudomonal effect. Products **8a,c,e** have moderate activity against both of Gram-positive and Gram-negative bacteria.

Table 1. Formation of **8a**, **8e**, and **8i** using microwave and conventional heating techniques

Compound number	Microwave irradiation			Thermal heationg		
	Time (Minutes)	Yield (%) (Chitosan)	Yield (%) (TEA)	Time (Hours)	Yield (%) (Chitosan)	Yield (%) (TEA)
8a	4	93	92	2	88	82
8e	4	95	93	2	90	85
8i	4	82	82	2	81	80

Table 2. Antimicrobial activity of products **8***

Sample no.	Inhibition zone diameter (mm / mg sample)		Sample no.	Inhibition zone diameter (mm / mg sample)	
	Ec (G^-)	Sa (G^+)		Ec (G^-)	Sa (G^+)
8a	14	14	**8f**	11	11
8b	11	12	**8g**	11	11
8c	16	16	**8k**	10	10
8d	11	12	Tetracycline	30	30
8e	12	13			

* A solution of 20.0 mg/ml was tested *Ec*: Esherichia coli; (G^-): Gram negative bacteria
Sa: Staphylococcus aureus; (G^+): Gram positive bacteria Tetracycline used as standard antibacterial agent

4. Conclusions

Pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidin-5-ones **8,** with an incorporated [1,2,4]triazolo[4,3-*a*]pyrimidine moiety, have been prepared *via* one-pot reaction of pyridopyrimidine thione **3** or its methylthio derivative **4** with hydrazonoyl chloride **5** using triethylamine or chitosan as basic catalysts. Microwave irradiation and conventional thermal heating techniques were employed for carrying out these reactions. Some of the newly synthesized products **8** have a mild effect against *Sa* (G^+) and *Ec* (G^-) bacteria.

5. Experimental Section

All melting points were determined on an electrothermal Gallenkamp apparatus and they are uncorrected. Solvents were generally distilled and dried by standard literature procedures prior to use. The IR spectra were measured on a Pye-Unicam SP300 Infrared Spectrophotometers in potassium bromide discs. The ^{1}H NMR spectra were recorded on a Varian Mercury VXR-300 spectrometer (300 MHz) and the chemical shifts were related to that of the solvent DMSO-d_6. The mass spectra were recorded on a GCMS-Q1000-EX Shimadzu and GCMS 5988-A HP spectrometers, the ionizing voltage was 70 eV. Elemental analyses were carried out by the Microanalytical Center of Cairo University, Giza, Egypt. Microwave experiments were carried out using CEM Discover Labmate microwave apparatus (300 W with Chem. Driver Software).

The antimicrobial activity of products **8** was evaluated at the Microanalytical Center of Cairo University. Compounds **1**,[16] **2**,[17] **10,**[20], **12,**[22–24] and hydrazonoyl chlorides **5**[27–33] were prepared following literature methods.

Synthesis of 5-[(1,7-diphenyl-1,5-dihydro-5-oxo-[1,2,4] triazolo[4,3-*a*]pyrimidin-3-yl)]-2-thioxo-2,3-dihydro-1*H*-pyrido[2,3-*d*]pyrimidin-4-one (3)

A mixture of 3-[3-(dimethylamino)-2-propenoyl]-1,7-diphenyl-1,5-dihydro[1,2,4]triazolo[4,3-*a*]pyrimidin-5-one (**1**) (3.85 g, 10 mmol) and 6-amino-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one **(2)** (1.43 g, 10 mmol) in acetic acid (40 mL) was refluxed for 6 hours. The reaction mixture was cooled and diluted with methanol and the solid product was collected by filtration and recrystallized from dioxane to give **3**.

Yellow crystals [3.72 g, 80%], mp > 300 °C; IR (KBr) υ 3261, 3245 (2 NH), 1677, 1662 (2 CO), cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.70 (s, 1H, pyrimidine-H), 7.49–8.20 (m, 10H, Ar-H), 8.29 (d, 1H, *J* = 4.5 *Hz,* pyridine-H), 8.48 (d, 1H, *J* = 4.5 *Hz,* pyridine-H), 12.72 (s, 1H, NH), 13.34 (s, 1H, NH) ppm; MS, *m/z* (relative intensity) 465 (M^+, 40), 287 (20), 178 (40), 77 (100). *Anal*. Calcd. for $C_{24}H_{15}N_7O_2S$ (465.10): C, 61.93; H, 3.25; N, 21.06; S, 6.89. Found: C, 61.82; H, 3.44; N, 21.12; S, 6.79%.

Synthesis of 2-methylsulfanyl-5-[(1,7-diphenyl-1,5-dihydro-5-oxo-[1,2,4]triazolo[4,3-*a*] pyrimidin-3-yl)]-3*H*-pyrido[2,3-*d*]pyrimidin-4-one (4)

To a stirred solution of 5-[(1,7-diphenyl-1,5-dihydro-5-oxo-[1,2,4]triazolo[4,3-*a*]pyrimidin-3-yl)]-2-thioxo-2,3-dihydro-1*H*-pyrido[2,3-*d*]pyrimidin-4-one (**3**) (0.47 g, 1 mmol) in dimethylformamide (10 mL) was added anhydrous potassium carbonate (0.21 g, 1.5 mmol), and methyl iodide (0.14 g, 1 mmol). The reaction mixture was stirred overnight at room temperature then poured into ice-water. The solid formed was filtered, washed with water, dried and recrystallized from dioxane to give compound **4** as yellow solid, mp > 300 °C; IR (KBr) υ 3325 (NH), 1690, 1666 (2 CO), cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.58 (s, 3H, CH_3), 6.68 (s, 1H, pyrimidine-H), 7.25–8.13 (m, 10H, Ar-H), 8.24 (d, *J* = 5 *Hz*, 1H, , pyridine-H), 8.31 (d, *J* = 5 *Hz,*1H, pyridine-H), 11.94 (s, 1H, NH) ppm. *Anal*. Calcd. for $C_{25}H_{17}N_7O_2S$ (479.52): C, 62.62; H, 3.57; N, 20.45; S, 6.69. Found: C, 62.37; H, 3.28; N, 20.32; S, 6.51%.

Synthesis of pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidin-5-one derivatives (8a-l)

Method A: To a mixture of equimolar amounts of **3** and the appropriate hydrazonoyl chloride **5a-l** (1 mmol of each) in dioxane (15 mL) was added triethylamine (0.14

mL, 1 mmol). The reaction mixture was refluxed till all of the starting materials had disappeared and hydrogen sulfide gas ceased to evolve (2 h, monitored by TLC). The solvent was evaporated and the residue was treated with methanol. The solid formed was filtered and recrystallized from dioxane to give compounds **8a-l**.

Method B: To a mixture of equimolar amounts of **4** and the appropriate hydrazonoyl chloride **5a,e,i** (1 mmol of each) in dioxane (15 mL) was added triethylamine (0.14 mL, 1 mmol). The reaction mixture was refluxed until methanethiol gas ceased to evolve (2 h, monitored by TLC). The solvent was evaporated and the residue treated with methanol. The solid that formed was filtered and recrystallized from dioxane to give products identical in all respects (mp, mixed mp and IR) with **8a,e,i**..

Method C: To a solution of **3** and the appropriate hydrazonoyl chloride **5a,e,i** (1 mmol of each) in dioxane (15 mL) was added triethylamine (0.14 mL, 1 mmol) at room temperature. The reaction mixture was irradiated in a pressurized microwave (17.2 Bar, 140 °C) for 4 min. at a power of 300 W. After cooling, dil. HCl was added till the pH became acidic, and the solid product was collected and recrystallized from dioxane to give products **8a,e,i**.

Method D: To a solution of **3** (0.13 g, 1 mmol) and the appropriate hydrazonoyl chloride **5a,e,i** (1 mmol of each) in dioxane (15 mL) was added chitosan (0.1 g) at room temperature. The reaction mixture was irradiated under constant pressure (17.2 Bar, 140 °C) for 4 min. at a power of 300 W. The hot solution was filtered to remove chitosan. After cooling, dil. HCl was added till the pH became acidic, and the solid product was collected and recrystallized from dioxane to give products **8a,e,i**. The physical constants together with the spectral data of products **8 a-l** are listed below:

3-Acetyl-6-[(1,7-diphenyl-1,5-dihydro-5-oxo-[1,2,4]triazolo[4,3-*a*]pyrimidin-3-yl)]-1-phenyl-1,5-dihydropyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidin-5-one (8a)

Yellow crystals, mp 195–197 °C; IR (KBr) υ 1733, 1698, 1650 (3 CO), cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.81 (s, 3H, $COCH_3$), 6.69 (s, 1H, pyrimidine-H), 7.43–8.33 (m, 15H, Ar-H), 7.88 (d, *J* = 4.5 *Hz*, 1H, pyridine-H), 8.79 (d, *J* = 4.5 *Hz*, 1H, pyridine-H) ppm; ^{13}C NMR (300 MHz, DMSO-d_6) δ 30.3, 123.9, 124.2, 124.5, 125.9, 126.4, 126.5, 128.2, 128.4, 128.8, 129.1, 129.4, 129.8, 139.8, 142.2, 143.9, 147.4, 147.7, 148.8, 152.4, 153.3, 155.3, 159.1, 159.3, 167.7, 168.1, 176.4 ppm; MS, *m/z* (relative intensity) 591 (M^+, 25), 408 (20), 233 (40), 91 (100), 77 (60). *Anal*. Calcd. for $C_{33}H_{21}N_9O_3$ (591.18): C, 67.00; H, 3.58; N, 21.31. Found: C, 67.09; H, 3.48; N, 21.19%.

3-Acetyl-6-[(1,7-diphenyl-1,5-dihydro-5-oxo-[1,2,4]triazolo[4,3-*a*]pyrimidin-3-yl)]-1-(4-methylphenyl)-1,5-dihydropyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidin-5-one (8b)

Dark orange crystals, mp 285–287 °C; IR (KBr) υ 1726, 1691, 1654 (3 CO), cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.44 (s, 3H, Ar-CH_3), 2.79 (s, 3H, $COCH_3$), 6.67 (s, 1H, pyrimidine-H), 7.43–7.70 (m, 10H, Ar-H), 7.86 (d, *J* = 5 *Hz*, 1H, pyridine-H), 8.11 (d, *J* = 8 *Hz*, 2H, Ar-H), 8.30 (d, *J* = 8 *Hz*, 2H, Ar-H), 8.77 (d, *J* = 5 *Hz*,1H, pyridine-H) ppm; MS, *m/z* (relative intensity) 605 (M^+, 23), 233 (17), 132 (22), 91 (100), 77 (56). *Anal*. Calcd. for $C_{34}H_{23}N_9O_3$ (605.19): C, 67.43; H, 3.83; N, 20.82. Found: C, 67.29; H, 3.72; N, 21.03%.

3-Acetyl-6-[(1,7-diphenyl-1,5-dihydro-5-oxo-[1,2,4]triazolo[4,3-*a*]pyrimidin-3-yl)]-1-(4-chlorophenyl)-1,5-dihydropyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidin-5-one (8c)

Yellow crystals, mp 190–192 °C; IR (KBr) υ 1730, 1697, 1647 (3 CO), cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.81 (s, 3H, $COCH_3$), 6.68 (s, 1H, pyrimidine-H), 7.31–7.75 (m, 10H, Ar-H), 7.88 (d, *J* = 8 *Hz*, 2H, Ar-H), 8.19 (d, *J* = 8 *Hz*, 2H, Ar-H), 8.20 (d, *J* = 4.5*Hz*, 1H, pyridine-H), 8.79 (d, *J* = 4.5 *Hz,* 1H, pyridine-H) ppm; MS, *m/z* (relative intensity) 627 (M^++2, 14), 625 (M^+, 18), 129 (34), 103 (46), 91 (100), 77 (70). *Anal*. Calcd. for $C_{33}H_{20}ClN_9O_3$ (625.14): C, 63.31; H, 3.22; N, 20.14. Found: C, 63.19; H, 3.28; N, 20.25%.

3-Acetyl-6-[(1,7-diphenyl-1,5-dihydro-5-oxo-[1,2,4]triazolo[4,3-*a*]pyrimidin-3-yl)]-1-(4-nitrophenyl)-1,5-dihydropyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidin-5-one (8d)

Yellow crystals, mp > 300 °C; IR (KBr) υ 1738, 1698, 1655 (3 CO), cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.82 (s, 3H, $COCH_3$), 6.69 (s, 1H, pyrimidine-H), 7.46–7.79 (m, 10H, Ar-H), 8.47 (d, *J* = 8 *Hz*, 2H, Ar-H), 8.52 (d, *J* = 8 *Hz*, 2H, Ar-H), 7.79 (d, *J* = 4.5 *Hz*, 1H, pyridine-H), 8.87 (d, *J* = 4.5 *Hz*, 1H, pyridine-H) ppm; MS, *m/z* (relative intensity) 636 (M^+, 25), 465 (60), 129 (40), 103 (35), 91 (100), 77 (50). *Anal*. Calcd. for $C_{33}H_{20}N_{10}O_5$ (636.16): C, 62.26; H, 3.17; N, 22.00. Found: C, 62.19; H, 3.24; N, 22.15%.

Ethyl 5-oxo-6-[(1,7-diphenyl-1,5-dihydro-5 -oxo-[1,2,4]triazolo[4,3-*a*]pyrimidin-3-yl)]-1-phenyl-1,5-dihydropyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidine-3-carboxylate (8e)

Yellow crystals, mp 215–217 °C; IR (KBr) υ 1750, 1707, 1645 (3 CO), cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.44 (t, *J* = 7 *Hz*, 3H, CH_3), 4.57 (q, *J* = 7 *Hz*, 2H, CH_2), 6.69 (s, 1H, pyrimidine-H), 7.44–8.30 (m, 15H, Ar-H), 7.87 (d, *J* = 5 *Hz*, 1H, pyridine-H), 8.77 (d, *J* = 5 *Hz,*1H, pyridine-H) ppm; ^{13}C NMR (300 MHz, DMSO-d_6) δ 30.6, 35.6, 122.9, 124.1, 124.8, 125.7, 126.6, 126.9, 127.9, 128.4, 128.9, 129.3, 129.8, 129.9, 139.5, 142.7, 143.9, 147.7, 147.9, 148.8, 152.9, 153.8, 155.4, 159.6,

159.9, 167.8, 168.6, 177.8 ppm; MS, *m/z* (relative intensity) 621 (M^+, 25), 212 (75), 157 (20), 91 (100), 77 (80). *Anal*. Calcd. for $C_{34}H_{23}N_9O_4$ (621.19): C, 65.70; H, 3.73; N, 20.28. Found: C, 65.59; H, 3.68; N, 20.39%.

Ethyl 5-oxo-6-[(1,7-diphenyl-1,5-dihydro-5-oxo-[1,2,4]triazolo[4,3-*a*]pyrimidin-3-yl)]-1-(4-methylphenyl)-1,5-dihydropyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidine-3-carboxylate (8f)

Yellow crystals, mp 256–258 °C; IR (KBr) υ 1753, 1709, 1687 (3 CO), cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.42 (t, *J* = 7 *Hz*, 3H, CH_3), 2.39 (s, 3H, Ar-CH_3), 4.56 (q, *J* = 7 *Hz*, 2H, CH_2), 6.69 (s, 1H, pyrimidine-H), 7.55–7.77 (m, 10H, Ar-H), 7.85 (d, *J* = 5 *Hz*, 1H, pyridine-H,), 8.11 (d, *J* = 8 *Hz*, 2H, Ar-H), 8.31 (d, *J* = 8 *Hz,* 2H, Ar-H), 8.78 (d, *J* = 5 *Hz,* 1H, pyridine-H) ppm; MS, *m/z* (relative intensity) 635 (M^+, 25), 591 (25), 261 (40), 171 (20), 91 (100), 77 (60). *Anal*. Calcd. for $C_{35}H_{25}N_9O_4$ (635.20): C, 66.14; H, 3.96; N, 19.83. Found: C, 65.99; H, 3.88; N, 20.03%.

Ethyl 5-oxo-6-[(1,7-diphenyl-1,5-dihydro-5-oxo-[1,2,4]triazolo[4,3-*a*]pyrimidin-3-yl)]-1-(4-chlorophenyl)-1,5-dihydropyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidine-3-carboxylate (8g)

Yellow crystals, mp 175–177 °C; IR (KBr) υ 1749, 1698, 1662 (3 CO), cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.43 (t, *J* = 7 *Hz,* 3H, CH_3), 4.56 (q, *J* = 7 *Hz*, 2H, CH_2), 6.68 (s, 1H, pyrimidine-H), 7.55–8.30 (m, 10H, Ar-H), 8.31 (d, *J* = 8 *Hz*, 2H, Ar-H), 8.35 (d, *J* = 8 *Hz*, 2H, Ar-H), 8.38 (d, *J* = 5 *Hz,* 1H, pyridine-H), 8.78 (d, *J* = 5 *Hz*, 1H, pyridine-H) ppm; MS, *m/z* (relative intensity) 657 (M^++2, 10), 655 (M^+, 25), 281 (20), 194 (10), 91 (100), 77 (60). *Anal*. Calcd. for $C_{34}H_{22}ClN_9O_4$ (655.15): C, 62.25; H, 3.38; N, 19.22. Found: C, 62.19; H, 3.30; N, 19.13%.

Ethyl 5-oxo-6-[(1,7-diphenyl-1,5-dihydro-5-oxo-[1,2,4]triazolo[4,3-*a*]pyrimidin-3-yl)]-1-(4-nitrophenyl)-1,5-dihydropyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidine-3-carboxylate (8h)

Yellow crystals, mp > 300 °C; IR (KBr) υ 1751, 1706, 1669 (3 CO), cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.44 (t, *J* = 7 *Hz*, 3H, CH_3), 4.56 (q, *J* = 7 *Hz*, 2H, CH_2), 6.69 (s, 1H, pyrimidine-H), 7.56–8.29 (m, 10H, Ar-H), 8.38 (d, *J* = 8 *Hz,* 2H, Ar-H), 8.41 (d, *J* = 8 *Hz*, 2H, Ar-H), 7.84 (d, *J* = 4.5 *Hz,* 1H, pyridine-H), 8.82 (d, *J* = 4.5 *Hz,* 1H, pyridine-H) ppm; MS, *m/z* (relative intensity) 666 (M^+, 25), 281 (20), 194 (10), 91 (100), 77 (60). *Anal*. Calcd. for $C_{34}H_{22}N_{10}O_6$ (666.17): C, 61.26; H, 3.33; N, 21.01. Found: C, 61.19; H, 3.38; N, 21.11%.

***N*3,1-Diphenyl-5-oxo-6-[(1,7-diphenyl-1,5-dihydro-5-oxo-[1,2,4]triazolo[4,3-*a*]pyrimidin-3-yl)]-1,5-dihydropyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidine-3-carboxamide (8i)**

Yellow crystals, mp 170–172 °C; IR (KBr) υ 3388 (NH), 1697, 1688, 1651 (3 CO), cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 6.65 (s, 1H, pyrimidine-H), 7.21–8.35 (m, 20H, Ar-H), 7.76 (d, *J* = 5.5 *Hz,* 1H, pyridine-H), 8.80 (d, *J* = 5.5 *Hz,* 1H, pyridine-H), 11.12 (s, 1H, NH) ppm; ^{13}C NMR (300 MHz, DMSO-d_6) δ 108.4, 120.3, 120.9, 121.5, 122.7, 124.1, 124.7, 125.7, 126.5, 126.9, 127.6, 128.4, 128.9, 129.1, 129.5, 129.9, 139.3, 142.7, 143.8, 145.9, 147.5, 147.8, 148.6, 152.7, 153.8, 155.4, 159.6, 163.9, 167.8, 168.6 ppm; MS, *m/z* (relative intensity) 668 (M^+, 25), 548 (20), 120 (100), 91 (80), 77 (75). *Anal*. Calcd. for $C_{38}H_{24}N_{10}O_3$ (668.20): C, 68.26; H, 3.62; N, 20.95. Found: C, 68.09; H, 3.58; N, 21.09%.

5-Oxo-*N*3-phenyl-6-[(1,7-diphenyl-1,5-dihydro-5-oxo-[1,2,4]triazolo[4,3-*a*]pyrimidin-3-yl)]-1-(4-methylphenyl)-1,5-dihydropyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidine-3-carboxamide (8j)

Yellow crystals, mp 202–204 °C; IR (KBr) υ 3386 (NH), 1695, 1682, 1650 (3 CO), cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 2.22 (s, 3H, Ar-CH_3), 6.58 (s, 1H, pyrimidine-H), 7.13–8.25 (m, 19H, Ar-H), 7.85 (d, *J* = 5 *Hz*, 1H, pyridine-H), 8.74 (d, *J* = 5 *Hz,*1H, pyridine-H), 11.24 (s, 1H, NH) ppm; MS, *m/z* (relative intensity) 682 (M^+, 25), 562 (50), 120 (100), 91 (80), 77 (60). *Anal*. Calcd. for $C_{39}H_{26}N_{10}O_3$ (682.22): C, 68.61; H, 3.84; N, 20.52. Found: C, 68.49; H, 3.68; N, 20.49%.

5-Oxo-*N*3-phenyl-6-[(1,7-diphenyl-1,5-dihydro-5-oxo-[1,2,4]triazolo[4,3-*a*]pyrimidin-3-yl)]-1-(4-chlorophenyl)-1,5-dihydropyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidine-3-carboxamide (8k)

Yellow crystals, mp 166–168 °C; IR (KBr) υ 3382 (NH), 1693, 1684, 1652 (3 CO), cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 6.69 (s, 1H, pyrimidine-H), 7.36–8.35 (m, 15H, Ar-H), 7.90 (d, *J* = 8 *Hz,* 2H, Ar-H), 8.45 (d, *J* = 8 *Hz*, 2H, Ar-H), 8.52 (d, *J* = 5 *Hz,* 1H, pyridine-H), 8.65 (d, *J* = 5 *Hz*, 1H, pyridine-H), 11.15 (s, 1H, NH) ppm; MS, *m/z* (relative intensity) 704 (M^+ +2, 10), 702 (M^+, 25), 582 (20), 120 (40), 111 (40), 91 (60), 77 (100). *Anal*. Calcd. for $C_{38}H_{23}ClN_{10}O_3$ (702.16): C, 64.91; H, 3.30; N, 19.92. Found: C, 64.79; H, 3.38; N, 20.09%.

5-Oxo-*N*3-phenyl-6-[(1,7-diphenyl-1,5-dihydro-5-oxo-[1,2,4]triazolo[4,3-*a*]pyrimidin-3-yl)]-1-(4-nitrophenyl)-1,5-dihydropyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidine-3-carboxamide (8l)

Yellow crystals, mp >300 °C; IR (KBr) υ 3381 (NH), 1696, 1689, 1657 (3 CO), cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 6.69 (s, 1H, pyrimidine-H), 7.41–8.45 (m, 15H, Ar-H), 8.43 (d, *J* = 8 *Hz*, 2H, Ar-H), 8.49 (d, *J* = 8 *Hz*, 2H, Ar-H), 8.58 (d, *J* = 5 *Hz*, 1H, pyridine-H), 8.69 (d, *J* = 5 *Hz*, 1H, pyridine-H), 11.19 (s, 1H, NH) ppm; MS, *m/z* (relative intensity) 713 (M^+, 25), 593 (20), 122 (40), 91 (60), 77 (100). *Anal*. Calcd. for $C_{38}H_{23}N_{11}O_5$ (713.19): C, 63.95; H, 3.25; N, 21.59. Found: C, 63.78; H, 3.18; N, 21.49%.

Alternative synthesis of 3-acetyl-6-[(1,7-diphenyl-1,5-dihydro-5-oxo-[1,2,4]triazolo[4,3-*a*]pyrimidin-3-yl)]-1-phenyl-1,5-dihydropyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidin-5-one (8a)

A mixture of 7-amino-1,3-diphenyl[1,2,4]triazolo[4,3-*a*]pyrimidin-5(1*H*)-one (**10**)[20] (0.30 g, 1 mmol) and enaminone (**1**) (0.22 g, 1 mmol) in acetic acid was refluxed for 6 hours. After cooling, the mixture was poured into ice and the solid product was filtered off and recrystallized from dioxane to give product identical with **8a** (mp, mixed mp, IR).

Reaction of 5-[(1,7-diphenyl-1,5-dihydro-5-oxo-[1,2,4] triazolo[4,3-*a*]pyrimidin-3-yl)]-2-thioxo-2,3-dihydro-1*H*-pyrido[2,3-*d*]pyrimidin-4-one (3) with 3-chloro-2,4-pentanedione (12)

To a stirred solution of **3** (0.47 g, 1 mmol) in absolute ethanol (20 mL) was added triethylamine (0.2 mL), and the mixture was warmed for 10 min at 60 °C and cooled. To the resulting clear solution was added 3-chloro-2,4-pentanedione (**12**) (1 mmol) dropwise while stirring the reaction mixture. After complete addition, the reaction mixture was stirred overnight at room temperature. The solid that precipitated was filtered off, washed with water, dried and finally crystallized from ethanol to give 3-[4-oxo-5-(1,7-diphenyl-1,5-dihydro-5-oxo-[1,2,4]triazolo [4,3-*a*]pyrimidin-3-yl)-3,4-dihydropyrido[2,3-*d*]pyrimidin-2-yl]sulfanyl-2,4-pentanedione (**13**) as yellow crystals, mp >300 °C; IR (KBr) υ 3225 (NH), 1710, 1700, 1692, 1685 (4 CO), cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.39 (s, 3H, $COCH_3$), 2.45 (s, 3H, $COCH_3$), 4.95 (s, 1H, CH), 6.35 (s, 1H, pyrimidine-H), 7.12–8.06 (m, 10H, Ar-H), 8.58 (d, 1H, J = 4.5 *Hz*, pyridine-H), 8.69 (d, 1H, J = 4.5 *Hz,* pyridine-H), 11.57 (s, 1H, NH) ppm; MS, *m/z* (relative intensity) 563 (M^+, 40), 520 (70), 464 (60), 432 (50), 131 (40), 99 (60), 77 (100). *Anal*. Calcd. for $C_{29}H_{21}N_7O_4S$ (563.13): C, 61.80; H, 3.76; N, 17.40; S, 5.69. Found: C, 61.71; H, 3.58; N, 17.29; S, 5.52%.

Alternative synthesis of 8a *via* coupling of 13 with benzenediazonium chloride

To a solution of **13** (1 mmol) in ethanol (20 mL) was added sodium acetate trihydrate (0.14 g, 1 mmol), and the mixture was cooled to 0–5 °C in an ice bath. To the resulting cold solution was added portionwise a cold solution of benzenediazonium chloride [prepared by diazotizing aniline (1 mmol) dissolved in hydrochloric acid (6 M, 1 mL) with a solution of sodium nitrite (0.07 g, 1 mmol) in water (2 mL)]. After complete addition of the diazonium salt, the reaction mixture was stirred for a further 30 min in an ice bath. The solid precipitated was filtered off, washed with water, dried and crystallized from dioxane to give the respective product identical in all respects (mp, mixed mp, IR) with **8a**.

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Povzetek

V prispevku je opisana priprava vrste pirido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pirimidin-5-onov (**8**) z reakcijo hidrazonil kloridov **5** z 5-substituiranimi-2-tiokso-2,3-dihidro-1*H*-pirido[2,3-*d*]pirimidin-4-oni (**3**) oziroma njihovimi metiltio derivati **4**. Produkti **8** so bili pripravljeni tudi po dveh alternativnih poteh; a) z reakcijo enaminona **1** in 7-amino-1,3-disubstituiranega[1,2,4]triazolo[4,3-*a*]pirimidin-5-ona (**10**), oziroma b) z Japp-Klingemannovo reakcijo spojine **13**. Za sintezo ciljnih spojin **8** sta bili uporabljeni tako konvencionalna tehnika kot tudi obsevanje z mikrovalovi. Narejena je primerjalna študija obeh tehnik z uporabo trietilamina oziroma citozana kot bazičnega katalizatorja. Avtorji v študiji razpravljajo o možnih mehazmih študiranih reakcij. Na novih produktih je bila preizkušena tudi antimikrobna aktivnost.