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RESEARCH ARTICLE

Reaction of pyridothiouracil with hydrazonoyl halides and the antimicrobial activity of the products, pyrido[2,3-d][1,2,4]triazolo-[4,3-*a*]pyrimidin-5(1H)-one derivatives

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New functionalized pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidin-5(1H)-one derivatives were synthesized *via* reaction of the hydrazonoyl halides with 7-(4-nitrophenyl)-5-phenyl-2,3-dihydro-2-thioxopyrido[2,3-*d*]pyrimidin-4(1H)-one or its 2-methylthio derivative. The biological activity of the products has been evaluated. The mechanism and the regioselectivity of the studied reactions have been discussed.

Keywords: Hydrazonoyl halides; 2-Thiouracil; Thiohydrazonates

1. Introduction

In continuation of our systematic studies of hydrazonoyl halides **1** devoted to the various aspects of their chemistry [1–6], it was thought interesting to investigate their reactions with both 7-(4-nitrophenyl)-5-phenyl-2,3-dihydro-2-thioxopyrido[2,3-d]pyrimidin-4(1H)-one **2** and its 2-methylthio derivative **3**. One of the objectives of the present study is to elucidate the regiochemistry of the reactions of **1** with **2** since such reactions can lead, theoretically, to compounds **4** and/or **5** (scheme 1). Furthermore, the interest in synthesis of the latter compounds is due to the fact that various derivatives of 1,2,4-triazolo[4,3-*a*]pyrimidin-5(1H)-one and pyrido[2,3-d]pyrimidin-5(1H)-one were reported to have various pharmacological activities. For example, some derivatives of 1,2,4-triazolo[4,3-*a*]pyrimidin-5(1H)-one were reported to be useful as calcium channel blocking vasodilators, some have antihypertensive [7], cardiovascular [8, 9] and anxiolytic activities [10]. Other derivatives are used as components in photographic materials [11]. In addition, some pyrido[2,3-d]pyrimidine derivatives have been reported to have medicinal applications such as inhibitors of adenosine kinase [12] or dihydrofolate reductase enzymes [13]. Thus, the other objective of the present study is to examine the antimicrobial activity of the products that will be isolated from the studied reactions.

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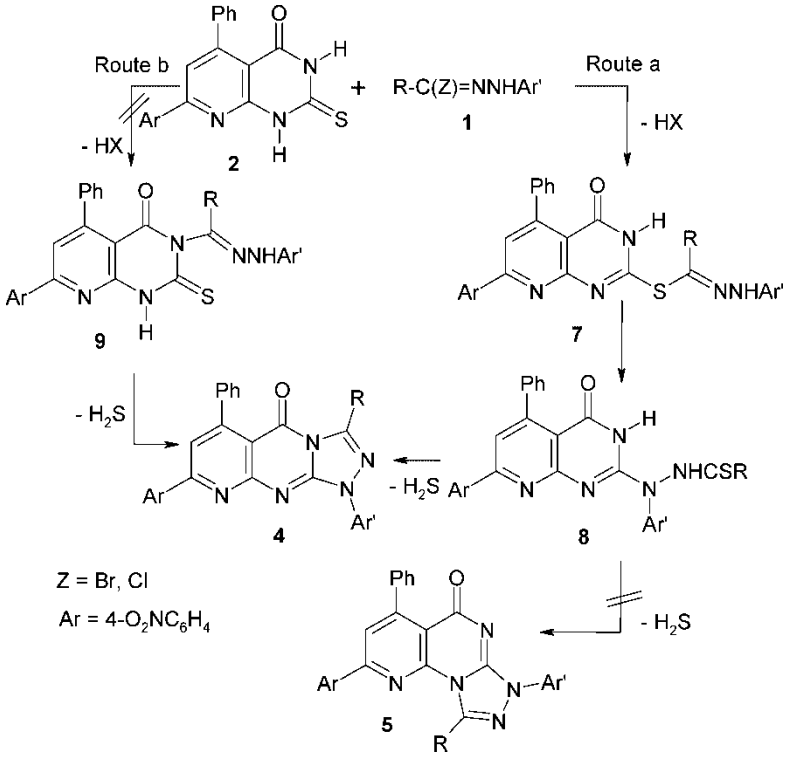
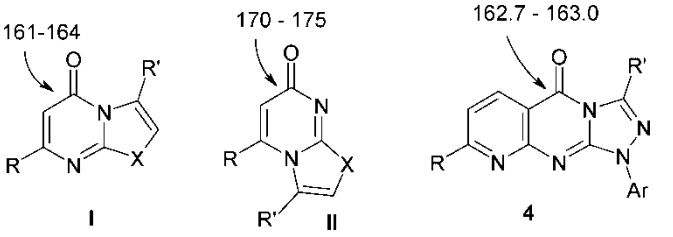


SCHEME 1

2. Results and discussion

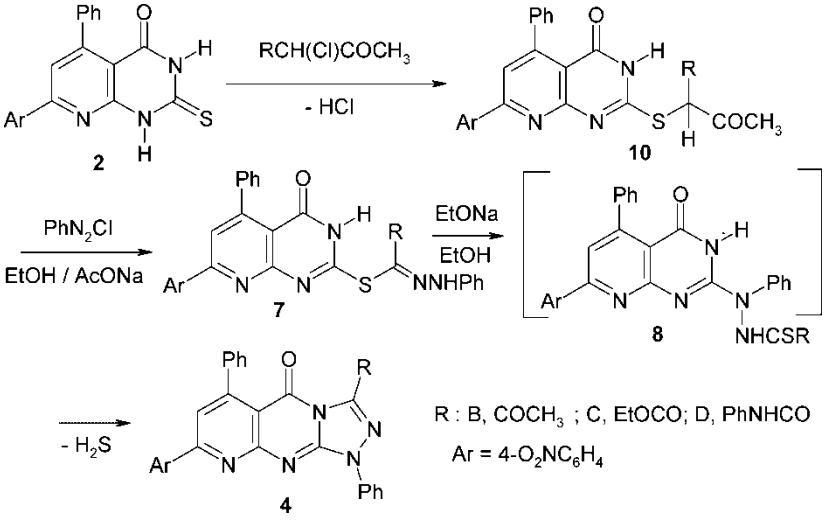
The starting 7-(4-nitrophenyl)-5-phenyl-2,3-dihydro-2-thioxopyrido[2,3-d]pyrimidin-4(1H)-one **2** [14] and the hydrazonoyl halides **1A–E** [15, 28, 29] were prepared by literature methods. 2-Methylthio-7-(4-nitrophenyl)-5-phenyl-pyrido[2,3-d]pyrimidin-4(1H)-one **3**, which has not been reported hitherto, was prepared by methylation of **2** with methyl iodide in dimethyl formamide in presence of potassium carbonate. Its structure was confirmed by spectral and elemental analysis data (see section 4, Experimental).

Reaction of **2** with each of the hydrazonoyl halides **1A–E** was carried out in chloroform in the presence of triethylamine while stirring the reaction mixture at room temperature. In all cases, hydrogen sulfide evolved during the course of the reaction and so stirring of the reaction mixture was continued till hydrogen sulfide ceased to evolve. Work up of the reaction mixture afforded, in each case, one isolable product as evidenced by tlc analysis of the crude product. On the basis of elemental analyses and IR, ^{1}H and ^{13}C NMR spectra which showed all the expected signals (see section 4, Experimental), the isolated products were assigned the structure of pyrido[2,3-d]-[1,2,4]triazolo[4,3-*a*]pyrimidin-5(1H)-one **4** rather than the isomeric structure of pyrido[2,3-d]-[1,2,4]triazolo[3,4-*a*]pyrimidin-5(1H)-one **5** (scheme 1). For example, the δ value (162.7–163.0) for the carbonyl carbon signal in the ^{13}C nmr spectrum of **4Aa**, taken as an example of the series prepared is similar to that of **I** (δ161–164) and different from its isomeric structure **II** (δ170–175) [15] (chart 1). This finding ruled out the acylimino type structure **5** for the isolated products.



The formation of compounds **4** from the thione **2** and hydrazonoyl halides **1** could be accounted for by one of the two pathways indicated in scheme 1. As depicted in this scheme, it is suggested that the studied reactions started with the hydrazonoylation of **2** to give the respective thiohydrazonate esters **7**. This is followed by Smiles type rearrangement [17, 18] of **7** to form the respective thiohydrazides **8**, which in turn underwent cyclization to give **4** as end products (route a, scheme 1). It seems that both intermediates **7** and **8** are consumed, under the employed reaction conditions as soon as they are formed since all attempts to isolate them failed. Alternatively, reaction of the thione **2** with hydrazonoyl halides **1** starts with the formation of the amidrazone intermediates **9** which cyclize to give **4** (route b, scheme 1). This alternative pathway has been ruled out, however, on the basis that alkylation and acylation of 2-thiouracil derivatives have been known to give S-alkyl and S-acyl derivatives, respectively [19–23].

Furthermore the suggested route a and the involvement of **7** and **8** as intermediates in the formation of **4** by this route were evidenced by alternate synthesis of **4Ba, 4Da** and **4Ea** (scheme 2). Thus, treatment of **2** with each of 3-chloro-2,4-pentanedione, ethyl α-chloroacetoacetate and α-chloroacetoacetanilide in chloroform in the presence of triethylamine afforded the respective substituted products **10Ba, 10Da** and **10Ea**.



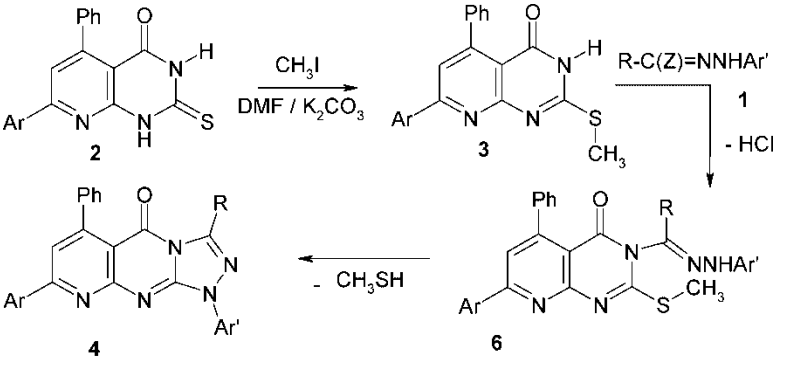
SCHEME 2

Coupling of each of these compounds with benzenediazonium chloride in ethanol in the presence of sodium acetate yielded the thiohydrazonates **7Ba, 7Da** and **7Ea**, respectively (scheme 2) *via* Japp-Klingemann cleavage of the acetyl group [24]. Treatment of the products **7** with sodium ethoxide in ethanol afforded the respective compounds **4Ba, 4Da** and **4Ea** identical in all respects with those obtained from reactions of **2** with each of **1Ba, 1Da** and

1Ea, respectively. This finding indicates that **7** and **8** are intermediates in the studied reactions of **1** with **3** and they are consumed as soon as they are formed under the employed reaction conditions.

Finally, the suggestion that the site of cyclization of the thiohydrazide intermediates **8** involves N-3 rather than N-1 to give **4** is consistent with literature reports. For example, it has been reported that cyclization of 2-substituted-uracil derivatives having no substituent on N-3 proceeds regioselectively to give the respective 1,2,4-triazolo[4,3-*a*]pyrimidin-5(1H)-ones [25–27].

The assignment of structure **4** was further evidenced by its alternate synthesis. In our hands, treatment of 2-methylthio derivative **3** with each of the hydrazonoyl halides **1A–E** in chloroform in the presence of triethylamine at room temperature resulted in the evolution of methanethiol and the formation of products that proved identical in all respects (IR, MS, mp and mixed mp) with **4** (scheme 3). In this case, reaction of **1** with **3** seems to proceed undoubtedly *via* the initial formation of the amidrazone intermediate **6** which in turn cyclizes to give **4** as end products (scheme 3). This conclusion is consistent with literature reports which indicate that alkylation and acylation of 2-methylthiopyrimidin-4(3H)-ones yields the respective 3-substituted-2-methylthiopyrimidinone derivatives [16].



1,4,6	A	B	C	D	E
R	Ph	CH_3CO	PhCO	EtOCO	PhNHCO
1,4,6	**a**	**b**	**c**	**d**	**e**
X	H	4-MeO	4-Me	4-Cl	$4\text{-}NO_2$

SCHEME 3

In conclusion, the studied reactions of hydrazonoyl halides **1** with the thione **2** and its 2-methylthioanalog **3** are both site and regioselective and lead to the title ring system.

3. Antimicrobial activity

The compounds **4A–D** were tested for their antimicrobial activities against four fungal species namely *Aspergillus fumigatus* **AF**, *Penicillium italicum* **PI**, *Syncephalastrum racemosum* **SR** and *Candida albicans* **CA** as well as four bacteria species namely *Staphylococcus aureus* **SA**,

Table 1. Antimicrobial activity of the products 4A–E.

	Micro-organism/IZD(cm)[f]							
Compd. No.	AF	PI	SR	CA	SA	PA	BS	EC
4Aa	+	+	0	+	++	0	+	0
4Ba	+	0	+	0	+	0	+	0
4Bb	0	0	0	0	+	+	+	0
4Bc	0	0	0	0	0	+	0	0
4Bd	+	0	0	0	+	+	++	+
4Be	+	0	0	0	0	0	0	0
4Ca	+	0	0	0	0	0	0	0
4Da	++	0	+	+	0	0	+	0
4Dc	+	0	0	+	0	0	+	0
4Dd	++	0	+	0	+	+	+	0
CA[a]						++	++	++
TE[b]	++	++	++	++	++			

[f] 50 ml of solution in DMF whose concentration 1.0 μg/ml was tested.
[a]Chloramphenicol as standard antibacterial agent, [b]Terbinfin as standard antifungal agent. ++, inhibition value 0.6–1.0 cm; +, inhibition value 0.1–0.5 cm beyond control; 0, no inhibition detected.

Pseudomonas aeruginosa **PA**, *Bacillus subtilis* **BS** and *Escherichia coli* **EC**. The organisms were tested against the activity of solutions of concentration of 1.0 μg/ml of each compound and using inhibition zone diameter in cm (IZD) as criterion for the antimicrobial activity. Terbinafin as an antifungal agent and Chloramphenicol as an antibacterial agent were used as references to evaluate the potency of the tested compounds under the same conditions. The results are depicted in table 1. The results reveal that compounds **4Aa** and **4Bd** exhibited the highest degree of inhibition against the tested organisms **SA** and **BS**, respectively, whereas compounds **4Da** and **4Dd** exhibited maximum inhibition against **AF**. Their activity is similar to that of the standard antifungal and antibacterial agents used. All other compounds either exhibit no activity or being less active against the tested species.

4. Experimental

Melting points were determined on a Gallenkamp apparatus and are uncorrected. IR spectra were recorded in potassium bromide using Pye-Unicam SP300 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in deuterated DMSO-d_6 using a Varian Gemini 300 NMR spectrometer. 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 at the Microanalytical Laboratory of National Research Center, Giza, Egypt. 6-Amino-1,2-dihydro-2-thioxo-4(3H)-pyrimidinone [30, 31], benzylidene-4-nitroacetophenone [32], 1,2-dihydro-7-(4-nitrophenyl)-5-phenyl-2-thioxo-pyrido[2,3-d]pyrimidin-4(3H)-one **2** [14] and the hydrazonoyl halides **1A–E** were prepared by literature methods as previously described [15, 28, 29].

4.1 *Synthesis of 2-methylthio-7-(4-nitrophenyl)-5-phenyl-pyrido[2,3-d]pyrimidin-4(1H)-one (3)*

To a stirred solution of 2-thioxopyrido[2,3-d]pyrimidin-4(1H)-one (**2**) (3.76 g, 0.01 mole) in dimethylformamide (40 mL) in the presence of anhydrous K_2CO_3 (2.07 g, 0.015 mole), methyl iodide (1.42 g, 0.01 mol) was added. The reaction mixture was stirred overnight and the solvent

was evaporated. The residue was poured into ice-water mixture and the solid product was collected, washed with water and crystallized from dimethylformamide to give pure compound **3**. Yellow solid, (Yield 3.1 g, 70%), m.p. 340–342 °C; IR ν 3168 (NH), 1705 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.74 (s, 3H), 7.41–8.01 (m, 6H), 8.36 (d, $J = 8$ Hz, 2H), 8.50 (d, $J = 8$ Hz, 2H), 12.25 (s, 1H); MS, m/z (%) 390 (M^+, 40); 389 (48), 388 (67), 135 (10), 120 (100), 93 (79), 77 (34); Anal. Calcd for $C_{20}H_{14}N_4O_3S$ (390.42): C, 61.53; H, 3.61; N, 14.35, S, 8.21; Found: C, 61.31; H, 3.56; N, 14.11; S, 8.33.

4.2 *Reactions of 2 with active chloromethylene compounds*

To a solution of **2** (3.76 g, 0.01 mol) in chloroform was added triethylamine (0.7 mL, 0.01 mol) and the mixture was stirred for 10 min at room temperature. To the resulting clear solution was added active chloromethylene derivative (0.01 mol) dropwise while stirring the reaction mixture. After complete addition, the reaction mixture was stirred for further 24 h at room temperature. The solid that precipitated was filtered off, washed with water, dried and finally crystallized from dimethylformamide to give pure **10**. The compounds **10** prepared are listed below together with their physical constants.

4.2.1 3-[7-(4-Nitrophenyl)-5-phenyl-4(1H)-oxo-pyrido[2,3-d]pyrimidin-2-yl]thio-2,4-pentanedione (10Ba). Yellow solid (2.1 g, 60%), m.p. 220 °C (DMF-EtOH); IR (KBr) ν 3410 (NH), 1712, 1702, 1651 (3CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.7 (s, 3H), 2.8 (s, 3H), 4.7 (s, 1H), 7.20–7.80 (m, 6H, ArH), 7.90 (d, $J = 8$ Hz, 2H), 8.10 (d, $J = 8$ Hz, 2H), 12.70 (s, 1H); MS m/z (%) 475 ($M^+ + 1$, 17), 474 (M^+, 3), 432 (100), 389 (44), 243 (16); Anal. Found (Calcd) for $C_{24}H_{18}N_4O_5S$ (474.50): C, 60.54 (60.75); H, 3.75 (3.82); N, 11.63 (11.81); S, 6.43 (6.78)%.

4.2.2 Ethyl 2-[7-(4-nitrophenyl)-5-phenyl-4(1H)-oxo-pyrido[2,3-d]pyrimidin-2-yl]thio-3-oxobutanoate (10Da). Yellow solid (3.7 g, 75%), m.p. 230 °C (DMF-EtOH); IR (KBr) ν 3429 (NH), 1705, 1686, 1668 (3CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.31 (t, $J = 7$ Hz, 3H), 2.58 (s, 3H), 4.30 (q, $J = 7$ Hz, 2H), 4.89 (s, 1H), 7.43–7.95 (m, 6H, ArH), 8.34 (d, $J = 8$ Hz, 2H), 8.50 (d, $J = 8$ Hz, 2H), 9.31 (s, 1H); MS m/z (%) 504 (M^+, 2), 462 (20), 416 (20), 388 (100), 270 (10), 214 (5), 77 (5); Anal. Found (Calcd) for $C_{25}H_{20}N_4O_6S$ (504.53): C, 59.32 (59.52); H, 3.86 (4.00); N, 11.02 (11.11); S, 6.11 (6.36)%.

4.2.3 N-Phenyl 2-[7-(4-nitrophenyl)-5-phenyl-4(1H)-oxo-pyrido[2,3-d]pyrimidin-2-yl] thio-3-oxobutanamide (10Ea). Yellow solid (4.4 g, 80%), m.p. 225 °C (DMF-EtOH); IR (KBr) ν 3390, 3263 (2NH), 1725, 1672, 1652 (3CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.53 (s, 3H), 4.52 (s, 1H), 7.27–7.50 (m, 11H, ArH), 7.75 (d, $J = 8$ Hz, 2H), 7.9 (d, $J = 8$ Hz, 2H), 12.04 (s, 1H), 13.01 (s, 1H); MS m/z (%) 553 ($M^+ + 2$, 70), 552 ($M^+ + 1$, 2), 551 (M^+, 2), 434 (100), 388 (65), 272 (20), 187 (42), 162 (52), 100 (58); Anal. Found (Calcd) for $C_{29}H_{21}N_5O_5S$ (551.59): C, 62.98 (63.15); H, 3.66 (3.84); N, 12.59 (12.70); S, 5.67 (5.81)%.

4.3 *Synthesis of the thiohydrazonates (7)*

To a solution of each of **10Ba, 10Da** and **10Ea** (10 mmol) in ethanol (40 mL) was added sodium acetate trihydrate (1.38 g, 10 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 (10 mmol) dissolved in hydrochloric acid (6 M, 6 mL) with a solution of sodium nitrite (0.7 g, 10 mmol) in water (10 mL). After complete addition of the diazonium salt, the reaction mixture was stirred for further 12 h at room temperature. The solid precipitated was filtered off, washed with water, dried and crystallized from DMF to give the respective pure **7Ba, 7Da** and **7Ea**.

4.3.1 [7-(4-Nitrophenyl)-5-phenyl-4(1H)-oxo-pyrido[2,3-d]pyrimidin-2-yl] N-phenyl-2-oxopropanethiohydrazonate (7Ba). Yellow solid (4.01 g, 75%), m.p. 300 °C (EtOH); IR (KBr) ν 3320, 3241 (2NH), 1706, 1667 (2CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.51 (s, 3H), 7.18–8.34 (m, 11H, ArH), 8.37 (d, $J = 8$ Hz, 2H), 8.60 (d, $J = 8$ Hz, 2H), 10.34 (s, 1H), 11.04 (s, 1H); MS m/z (%) 536 (M^+, 3), 535 (4), 502 (76), 432 (15), 413 (10), 297 (19), 214 (18), 91 (34), 77 (100); Anal. Found (Calcd) for $C_{28}H_{20}N_6O_4S$ (536.57): C, 62.54 (62.68); H, 3.54 (3.76); N, 15.63 (15.66); S, 6.73 (6.98)%.

4.3.2 [7-(4-Nitrophenyl)-5-phenyl-4(1H)-oxo-pyrido[2,3-d]pyrimidin-2-yl] N-phenyl-2-ethoxy-3-oxo-ethanethiohydrazonate (7Da). Yellow solid (4.5 g, 80%), m.p. > 300 °C (DMF); IR (KBr) ν 3390, 3251 (2NH), 1700, 1689 (2CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.33 (t, $J = 7$ Hz, 3H), 4.49 (q, $J = 7$ Hz, 2H), 7.45–8.11 (m, 11H, ArH), 8.33 (d, $J = 8$ Hz, 2H), 8.59 (d, $J = 8$ Hz, 2H), 10.31 (s, 1H), 11.31 (s, 1H); MS m/z (%) 566 (M^+, 7), 521 (100), 493 (4), 422 (12), 343 (21), 214 (43), 125 (37), 111 (35), 77 (9); Anal. Found (Calcd) for $C_{29}H_{22}N_6O_5S$ (566.60): C, 61.32 (61.48); H, 3.79 (3.91); N, 14.76 (14.83); S, 5.48 (5.66)%.

4.3.3 [7-(4-Nitrophenyl)-5-phenyl-4(1H)-oxo-pyrido[2,3-d]pyrimidin-2-yl] N-phenyl-2-(phenylamino)-2-oxo-ethanethiohydrazonate (7Ea). Yellow solid (4.3 g, 70%), m.p. 298 °C (EtOH); IR (KBr) ν 3303, 3184 (2NH), 1679, 1640, (2CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.19–8.11 (m, 16H, ArH), 8.60 (d, $J = 8$ Hz, 2H), 8.65 (d, $J = 8$ Hz, 2H), 10.43 (s, 1H), 11.04 (s, 1H), 11.32 (s, 1H); MS m/z (%) 613 (M^+, 2), 534 (3), 118 (6), 77 (100); Anal. Found (Calcd) for $C_{33}H_{23}N_7O_4S$ (613.66): C, 64.33 (64.59); H, 3.66 (3.78); N, 15.77 (15.98); S, 5.11 (5.23)%.

4.4 *Synthesis of pyrido[2,3-d][1,2,4]triazolo[4,3-a]pyrimidines (4A–E)*

4.4.1 Method A. To a mixture of 2-thioxopyrido[2,3-d]pyrimidin-4(1H)-one (**2**) (3.76 g, 0.01 mol) and appropriate hydrazonoyl halide **1** (0.01 mol) in chloroform (40 mL), trimethylamine (1.4 mL, 0.01 mol) was added. The reaction mixture was stirred at room temperature till hydrogen sulfide ceased to evolve (4–6 h). The solvent was evaporated and the residue was treated with ice/HCl mixture. The solid product was collected, washed with water and crystallized from proper solvent to give the respective **4** in 60–80% yield.

4.4.2 Method B. To a solution of 2-methylthio pyrido[2,3-d]pyrimidine (**3**) (3.9 g, 0.01 mol) in chloroform (30 mL) containing triethylamine (1.4 mL, 0.01 mol), the appropriate hydrazonoyl halide (**1**) was added (0.01 mol) and the resulting solution was stirred at room temperature overnight. The product was collected washed with water and crystallized from proper solvent to give the respective products **4** (65–80% yield). The products isolated were identical in all respects (m.p., mixed m.p. and IR) to compounds **4** prepared from method A.

4.4.3 Method C. To a stirred ethanolic sodium ethoxide solution, prepared from sodium metal (0.23 g, 10 mg atom) and absolute ethanol (20 mL), was added each of the compound **7Ba, 7Da** and **7Ea** (10 mmol) and the reaction mixture was stirred at room temperature for 12 h, during which the starting reactants **7** dissolved and the crude product preciptated. The latter was filtered, washed with water, dried and finally crystallized from the proper solvent to give a product identified as **4Ba**, **4Da** and **4Ea,** respectively. The latter products proved to be identical in all respects (mp, mixed mp, IR) with that obtained from **2** and the respective hydrazonoyl halides **1**. Their yields were 70–80%.

4.4.4 1,3,6-Triphenyl-8-(4-nitrophenyl)-pyrido[2,3-d]-[1,2,4]triazolo[4,3-a]pyrimidin-5(1H)-one (4Aa). Yellow solid (70%), m.p. $> 350\,^{\circ}$C (DMF); IR (KBr) ν 1705 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.40–7.80 (m, 16H, ArH), 8.33 (d, $J = 8$ Hz, 2H), 8.58 (d, $J = 8$ Hz, 2H); ^{13}C NMR (DMSO-d_6/CF_3COOH) δ 162.7, 162.1, 161.6, 161.0, 156.7, 149.3, 139.7, 138.3, 134.4, 133.2, 133.1, 132.4, 132.1, 131.3, 131.0, 130.8, 127.8, 127.1, 125.7, 123.6, 119.8, 116.1, 112.3; MS m/z (%) 538 ($M^+ +2$, 4), 537 ($M^+ +1$, 15), 536 (M^+, 35), 432 (62), 360 (4), 313 (9), 110 (18), 91 (100), 77 (28); Anal. Found (Calcd) for $C_{32}H_{20}N_6O_3$ (536.55): C, 72.06 (71.63); H, 3.96 (3.76); N, 15.33 (15.66)%.

4.4.5 3-Acetyl-1,6-diphenyl-8-(4-nitrophenyl)-pyrido[2,3-d]-[1,2,4]triazolo[4,3-a] pyrimidin-5(1H)-one (4Ba). Pale yellow solid (70%), m.p. 325 °C (DMF); IR (KBr) ν 1703, 1662 (2CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.68 (s, 3H), 7.50–8.20 (m, 11H, ArH), 8.33 (d, $J = 8$ Hz, 2H), 8.59 (d, $J = 8$ Hz, 2H); ^{13}C NMR (DMSO-d_6/CF_3COOH) δ 176, 168, 159.3, 155.3, 152.1, 148.0, 147.7, 147.4, 143.9, 142.2, 139.8, 129.8, 129.0, 128.8, 128.4, 128.2, 124.5, 121.7, 119.9, 30.13; MS m/z (%) 503 ($M^+ +1$, 30), 502 (M^+, 100), 443 (18), 432 (20), 287 (17), 227 (10), 214 (11), 140 (10), 91 (6), 77 (28); Anal. Found (Calcd) for $C_{28}H_{18}N_6O_4$ (502.49): C, 66.83 (66.93); H, 3.91 (3.61); N, 16.73 (16.61)%.

4.4.6 3-Acetyl-1-(4-methoxyphenyl)-8-(4-nitrophenyl)-6-phenyl-pyrido[2,3-d]-[1,2,4] triazolo[4,3-a]pyrimidin-5(1H)-one (4Bb). Pale yellow solid (78%), m.p. 320 °C (DMF); IR (KBr) ν 1703, 1655 (2CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.57 (s, 3H), 2.68 (s, 3H), 7.50–8.20 (m, 10H, ArH), 8.33 (d, $J = 8$ Hz, 2H), 8.59 (d, $J = 8$ Hz, 2H); MS m/z (%) 533 ($M^+ +1$, 3), 532 (M^+, 6), 457 (9), 359 (100), 313 (21), 214 (7), 77 (6); Anal. Found (Calcd) for $C_{29}H_{20}N_6O_5$ (532.52): C, 65.34 (65.41); H, 3.60 (3.75); N, 15.57 (15.78)%.

4.4.7 3-Acetyl-1-(4-methylphenyl)-8-(4-nitrophenyl)-6-phenyl-pyrido[2,3-d]-[1,2,4] triazolo[4,3-a]pyrimidin-5(1H)-one (4Bc). Pale yellow solid (78%), m.p. 330 °C (DMF); IR (KBr) ν 1710, 1672 (2CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.52 (s, 3H), 2.68 (s, 3H), 7.45–8.30 (m, 10H, ArH), 8.35 (d, $J = 8$ Hz, 2H), 8.59 (d, $J = 8$ Hz, 2H); MS m/z (%) 517 (M^+, 9), 505 (100), 477 (21), 413 (17), 241 (40), 201 (9), 164 (10), 99 (5), 77 (14); Anal. Found (Calcd) for $C_{29}H_{20}N_6O_4$ (517.52): C, 68.04 (67.44); H, 4.09 (3.90); N, 16.15 (16.27)%.

4.4.8 3-Acetyl-1-(4-chlorophenyl)-8-(4-nitrophenyl)-6-phenyl-pyrido[2,3-d]-[1,2,4] triazolo[4,3-a]pyrimidin-5(1H)-one (4Bd). Yellow solid (70%), m.p. 315–317 °C (DMF); IR (KBr) ν 1649, 1712 (2CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.53 (s, 3H), 7.51–8.20 (m, 10H, ArH), 8.33 (d, $J = 8$ Hz, 2H), 8.59 (d, $J = 8$ Hz, 2H); MS m/z (%) 538 ($M^+ +2$, 30), 537 ($M^+ +1$, 16), 536 (M^+, 100), 468 (49), 343 (21), 297 (26), 214 (34), 125 (59), 111 (55), 91

(28), 77 (35); Anal. Found (Calcd) for $C_{28}H_{17}ClN_7O_4$ (536.94): C, 62.68 (62.64); H, 3.17 (3.19); N, 15.67 (15.65)%.

4.4.9 3-Acetyl-1,8-di(4-nitrophenyl)-6-phenyl-pyrido[2,3-d]-[1,2,4]triazolo[4,3-a] pyrimidin-5(1H)-one (4Be). Yellow solid (70%), m.p. 320 °C (DMF); IR (KBr) ν 1712, 1649 (2CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.51 (s, 3H), 7.50–7.91 (m, 6H, ArH), 8.51 (d, $J = 8$ Hz, 2H), 8.61 (d, $J = 8$ Hz, 2H); MS m/z (%) 547 (M^+, 10), 505 (100), 477 (27), 413 (10), 348 (10), 297 (22), 287 (40), 201 (20), 164 (10), 91 (57), 77 (42); Anal. Found (Calcd) for $C_{28}H_{17}N_7O_6$ (547.49): C, 61.51 (61.43); H, 3.50 (3.13); N, 17.72 (17.91)%.

4.4.10 1,6-Diphenyl-8-(4-nitrophenyl)-3-benzoyl-pyrido[2,3-d]-[1,2,4]triazolo[4,3-a] pyrimidin-5(1H)-one (4Ca). Dark yellow solid (75%), m.p. 313 °C (DMF); IR (KBr) ν 1712, 1666 (2CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.40–8.32 (m, 16H, ArH), 8.37 (d, $J = 8$ Hz, 2H), 8.65 (d, $J = 8$ Hz, 2H); ^{13}C NMR (DMSO-d_6/CF_3COOH) δ 176, 162.1, 148, 130.3, 130.0, 129.3, 129.2, 129.1, 128.9, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 127.4, 127.3, 127.2, 126.5, 123.7, 123.5, 120.7, 120.5, 119.9; MS m/z (%) 565 ($M^+ + 1$, 12), 564 (M^+, 20), 460 (27), 432 (12), 287 (17), 140 (2), 105 (100), 91 (15), 77 (48); Anal. Found (Calcd) for $C_{33}H_{20}N_6O_4$ (564.57): C, 69.81 (70.21); H, 3.81 (3.57); N, 14.72 (14.89)%.

4.4.11 1,6-Diphenyl-3-ethoxycarbonyl-8-(4-nitrophenyl)-pyrido[2,3-d]-[1,2,4]triazolo [4,3-a]pyrimidin-5(1H)-one (4Da). Pale yellow solid (80%), m.p. 298 °C (DMF); IR (KBr) ν 1725, 1672 (2CO) cm^1; ^1H NMR (DMSO-d_6) δ 1.3 (t, $J = 7$ Hz, 3H), 4.4 (q, $J = 7$ Hz, 2H), 7.45–8.30 (m, 11H, ArH), 8.35 (d, $J = 8$ Hz, 2H), 8.57 (d, $J = 8$ Hz, 2H); ^{13}C NMR (DMSO-d_6/CF_3COOH) δ 175.4, 162.1, 156.5, 154.1, 148.5, 139.7, 137.9, 135.6, 135.1, 134.5, 130.3, 130.1, 129.3, 129.2, 129.1, 128.9, 128.6, 128.5, 128.4, 128.1, 120.5, 35.6, 30.6; MS m/z (%) 533 ($M^+ + 1$, 33) 532 (M^+, 100), 460 (50), 413 (17), 297 (12), 227 (14), 91 (40), 77 (32); Anal. Found (Calcd) for $C_{29}H_{20}N_6O_5$ (532.52): C, 66.01 (65.41); H, 3.98 (3.79); N, 15.36 (15.78)%.

4.4.12 1-(4-Methylphenyl)-6-phenyl-3-ethoxycarbonyl-8-(4-nitrophenyl)-pyrido[2,3-d]-[1,2,4]triazolo[4,3-a]pyrimidin-5(1H)-one (4Dc). Pale yellow solid (78%), m.p. 322 °C (DMF/H_2O); IR (KBr) ν 1749, 1707 (2CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.3 (t, $J = 7$ Hz, 3H), 2.4 (s, 3H), 4.4 (q, $J = 7$ Hz, 2H), 7.45–8.30 (m, 10H, ArH), 8.49 (d, $J = 8$ Hz, 2H), 8.52 (d, $J = 8$ Hz, 2H); MS m/z (%) 547 ($M^+ + 1$, 30), 546 (M^+, 100), 433 (17), 400 (9), 297 (10), 200 (14), 91 (27), 77 (11); Anal. Found (Calcd) for $C_{30}H_{22}N_6O_5$ (546.93): C, 66.31 (65.93); H, 4.21 (4.06); N, 15.12 (15.38)%.

4.4.13 1-(4-Chlorophenyl)-6-phenyl-3-ethoxycarbonyl-8-(4-nitrophenyl)-pyrido[2,3-d]-[1,2,4]triazolo[4,3-a]pyrimidin-5(1H)-one (4Dd). Pale yellow solid (75%), m.p. 312 °C (DMF/H_2O); IR (KBr) ν 1749, 1706 (2CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.3 (t, $J = 7$ Hz, 3H), 4.4 (q, $J = 7$ Hz, 2H), 7.45–7.89 (m, 6H, ArH), 7.74 (d, $J = 8$ Hz, 2H), 8.26 (d, $J = 8$ Hz, 2H), 8.35 (d, $J = 8$ Hz, 2H), 8.58 (d, $J = 8$ Hz, 2H); MS m/z (%) 568 ($M^+ + 2$, 25), 567 ($M^+ + 1$, 28), 566 (M^+, 70), 524 (21), 521 (100), 499 (80), 422 (12), 400 (9), 343 (30), 297 (35), 214 (13), 125 (30), 111 (35), 91 (10), 77 (9); Anal. Found (Calcd) for $C_{29}H_{19}ClN_6O_5$ (566.96): C, 60.92 (61.44); H, 3.42 (3.38); N, 14.61 (14.82)%.

4.4.14 1,6-Diphenyl-3-phenylaminocarbonyl-8-(4-nitrophenyl)-pyrido[2,3-d]-[1,2,4] triazolo[4,3-a]pyrimidin-5(1H)-one (4Ea). Yellow solid (75%), m.p. > 350 °C (AcOH); IR (KBr) ν 3255 (NH), 1706, 1666 (2CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.10–8.20 (m, 16H, ArH), 8.41 (d, $J = 8$ Hz, 2H), 8.64 (d, $J = 8$ Hz, 2H), 11.03 (s, 1H); ^{13}C NMR (DMSO-d_6/CF_3COOH) δ 163, 159, 155.8, 155.7, 154.2, 149, 146, 143, 139.8, 139.2, 138.3, 137, 130, 129.8, 129.7, 129.1, 128.8, 128.3, 128.1, 125.5, 124.6, 121, 120.5, 120, 108; MS m/z (%) 579 (M^+, 7), 551 (14), 459 (67), 434 (20), 287 (16), 201 (18), 119 (8), 91 (31), 77 (30); Anal. Found (Calcd) for $C_{33}H_{21}N_7O_4$ (579.58): C, 68.31 (68.39); H, 3.42 (3.65); N, 16.72 (16.92)%.

4.4.15 1-(4-Methylphenyl)-6-phenyl-3-phenylaminocarbonyl-8-(4-nitrophenyl)-pyrido [2,3-d]-[1,2,4]triazolo[4,3-a]pyrimidin-5(1H)-one (4Ec). Yellow solid (77%), m.p. 330–332 °C (DMF); IR (KBr) ν 3247 (NH), 1705, 1662 (2CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.45 (s, 3H), 7.20–7.91 (m, 11H, ArH), 8.01 (d, $J = 8$ Hz, 2H), 8.18 (d, $J = 8$ Hz, 2H), 8.36 (d, $J = 8$ Hz, 2H), 8.63 (d, $J = 8$ Hz, 2H), 11.05 (s, 1H); MS m/z (%) 594 ($M^+ + 1$, 4), 593 (M^+, 6), 565 (7), 474 (18), 448 (9), 343 (8), 287 (10), 217 (11), 119 (100), 91 (83), 77 (13); Anal. Found (Calcd) for $C_{34}H_{23}N_7O_4$ (593.61): C, 68.51 (68.80); H, 4.10 (3.91); N, 16.41 (16.52)%.

4.4.16 1-(4-Chlorophenyl)-6-phenyl-3-phenylaminocarbonyl-8-(4-nitrophenyl)-pyrido [2,3-d]-[1,2,4]triazolo[4,3-a]pyrimidin-5(1H)-one (4Ed). Pale yellow solid (65%), m.p. 345 °C (DMF); IR (KBr) ν 3255 (NH), 1712, 1666 (2CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.20–8.20 (m, 15H, ArH), 8.39 (d, $J = 8$ Hz, 2H), 8.60 (d, $J = 8$ Hz, 2H), 11.05 (s, 1H); MS m/z (%) 616 ($M^+ + 2$, 6), 615 ($M^+ + 1$, 8), 614 (M^+, 19), 586 (8), 546 (16), 540 (10), 483 (12), 404 (12), 368 (14), 313 (10), 239 (11), 119 (100), 91 (19), 77 (22); Anal. Found (Calcd) for $C_{33}H_{20}ClN_7O_4$ (614.02): C, 64.88 (64.55); H, 3.81 (3.58); N, 15.81 (15.97)%.

4.4.17 1,8-Di(4-Nitrophenyl)-6-phenyl-3-phenylaminocarbonyl-pyrido[2,3-d]-[1,2,4] triazolo[4,3-a]pyrimidin-5(1H)-one (4Ee). Pale yellow solid (65%), m.p. 348 °C (DMF); IR (KBr) ν 3188 (NH), 1683, 1662 (2CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.16–7.93 (m, 11H, ArH), 8.34 (d, $J = 8$ Hz, 2H), 8.37 (d, $J = 8$ Hz, 2H), 8.42 (d, $J = 8$ Hz, 2H), 8.64 (d, $J = 8$ Hz, 2H), 11.05 (s, 1H); MS m/z (%) 624 (M^+, 52), 428 (62), 245 (60), 214 (71), 128 (76), 111 (80), 69 (100); Anal. Found (Calcd) for $C_{33}H_{20}N_8O_6$ (624.58): C, 63.55 (63.46); H, 3.38 (3.23); N, 17.97 (17.94)%.

4.5 *Antimicrobial assay*

Cultures of four fungal species *namely Aspergillus fumigatus* **AF**, *Penicillium italicum* **PI**, *Syncephalastrum racemosum* **SR** and *Candida albicans*. **CA** as well as four bacterial species namely *Staphylococcus aureus* **SA**, *Pseudomonas aeruginosa* **PA**, *Bacillus subtilis* **BS** and *Escherichia coli* **EC** were used to investigate the antimicrobial activity of the compounds **5a-j**. The antimicrobial activity was assayed biologically using the diffusion plate technique. The latter technique was carried out by pouring a spore suspension of the fungal species (one ml of sterile water contains approximately 10^8 conidia) or spreading bacterial suspension over a solidified malt agar medium. The layer is allowed to set for 30 min. A solution of the test compound **4A–E** (1.0 μg/ml) in dimethylformamide was placed onto sterile 5 mm filter paper discs and allowed to dry, then the discs were placed on the centre of the malt agar plate and incubated at optimum incubation temperature 28 ± 2°C. Test organism growth may be affected by the inhibitory action of the test compound, so a clear zone around the disc

appears as an indication of the inhibition of test organism growth. The size of the clearing zone is proportional to the inhibitory action of the compound. The fungicide Terbinafin and the bactericide Chloramphenicol were used as standards under the same conditions. Measurements were considered after 72 h for fungi and 24 h for bacteria. The results are summarized in table 1.

References

[1] A.S. Shawali, M.A.N. Mosselhi. *J. Heterocyclic. Chem*, **40**, 725 (2003).
[2] A.S. Shawali, S.M. Elsheikh. *J. Heterocyclic Chem*., **38**, 541 (2001).
[3] A.S. Shawali, M.A. Abdallah. *Adv. Heterocyclic Chem*, **63**, 277 (1995).
[4] A.S. Shawali. *Chem. Rev*., **93**, 2731 (1993).
[5] A.S. Shawali. *Heterocycles*, **20**, 2239 (1983).
[6] A.S. Shawali, C. Parkanyi. *J. Heterocyclic Chem*., **17**, 833 (1980).
[7] K. Awal. Ger. Pat., 3839711 (1989); *Chem. Abstr*., **112**, 55902o (1990).
[8] N. Bru-Magniez, T. Guengor, J.M. Teulon. U.S. Pat. 5387747 (1995); *Chem. Abstr.*, **123**, 228204p (1995).
[9] G. Barthelemy, A. Hallot, J.N. Vallat. Fr. Pat. 2549834 (1985), *Chem. Abstr*., **103**, 71335u (1985).
[10] J.D. Albright; J.P. Dusza; R.A. Hardy Jr. U.S. 4209621 (1980), *Chem. Abstr*., **93**, 168298x (1980).
[11] H. Nakamura, Y. Hosoi. J. Fukawa. *Jpn. Kokai Pat.* 0313934 (1991), *Chem. Abstr.*, **115**, 60769k (1991).
[12] J. Audox, N. Ple', A. Turk, G. Que. *Tetrahedron*, **60**, 4107 (2004).
[13] J. Audox, N. Ple', A. Turk, G. Que. *Tetrahedron*, **60**, 6353 (2004).
[14] J. Quiroga, B. Insuasty, A. Sanchez, M. Nogueras, H. Meie. *J. Heterocyclic Chem*., **29**, 1045 (1992).
[15] A.S. Shawali, A.H. Elghandour, A.R. Sayed. *Synthetic Commun*., **31**, 731 (2001).
[16] M.A.E. Shaban, M.A. Taha, A.Z. Nasr, A.E.A. Morgan. *Pharmazie*, **50**, 784 (1995).
[17] K. Ishii, M. Hatanaka, I. Ueda. *Chem. Pharm. Bull*., **39**, 3331 (1991).
[18] J.F. Bunnett. *Quart. Rev*., **12**, 12 (1958).
[19] D.T. Hurst, C. Beaumont, D.T.E. Jones, D.A. Kingsley, J.D. Partridge, T.J. Rutherford. *Aust. J. Chem*., **41**, 1209 (1988).
[20] V. Sodereviciute, P. Vainilavicius. *Chemija*, **2**, 70 (1993).
[21] K.M. Ghoneim, F.A. El-Telbany, M. El-Enany, K. Youssef. *Egypt. J. Pharm. Sci.*, **28**, 127 (1987).
[22] A.A. Geies, A.M. Kamal-Eldeen, A.A. Abdelhafez, A.M. Gaber, *Phosphorus, Sulfur & Silicon*, **56**, 87 (1991).
[23] Z.H. Khalil, A.A. Abdelhafez, A.A. Ahmed. *Phosphorus, Sulfur & Silicon*, **54**, 81 (1987).
[24] R.R. Phillip. In *Organic Reactions*, R. Adams (Ed.), vol. 10, chap. 2, p. 143, John Wiley Sons, New York (1959).
[25] S.A. Abdel-Aziz, H.A. Allimony, H.M. ElShaar, F.A. Usama, R.M. Abdel-Rahman, *Phosphorus, Sulfur & Silicon*, **113**, 67 (1996).
[26] A.M. Abdelfattah, A.M. Negm, A.E. Gaafar. *Phosphorus, Sulfur & Silicon*, **72**, 145 (1992).
[27] N. Rashed, A. Mousaad, A. Saleh. *J. Chin. Chem. Soc*., **40**, 393 (1993).
[28] M.A.N. Mosselhi, N.M. Tawfik, A.S. Shawali. *Monatsch. Chem.*, **134**, 565 (2003).
[29] A.S. Shawali, M.A.M. Mosselhi, N.M. Tawfik. *J. Org. Chem.*, **66**, 4055 (2001).
[30] E.C. Taylor, C.C. Cheng. *J. Org. Chem*., **25**, 1481 (1960).
[31] W. Traube, L. Winter. Liebigs Ann. Chim., **439**, 331 (1904).
[32] B.S. Furness, A.J. Hannaford, P.W.G. Smith, A.R. Tatchell. In *Vogel's Textbook of Practical Organic Chemistry*, p. 796, Longman, Essex (1980).