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Synthesis of various fused pyrimidine rings and their pharmacological and antimicrobial evaluation

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Abstract: Various fused pyrimidines, such as furo[2,3-*d*]pyrimidine, triazolo-[1,5-*a*]pyrimidine and tetrazolo[1,5-*a*]pyrimidine, were synthesized in the reactions of thioxopyrimidine-6(1*H*)-ones with ethyl chloroacetate (under different reaction conditions), thiourea and sodium nitrite. Pyrimidine thiones reacted with $POCl_3/PCl_5$ to give the chloro derivatives which reacted with sodium azide and thiourea to give tetrazolo[1,5-*c*]pyrimidines, and pyrimido pyrimidines. Thioxopyrimidine-6(1*H*)-ones reacted with benzylamine to give pyrrolo[2,3-*d*]pyrimidinethiones. Theoretical calculation using MIDO/3, Fukui indices and the heat of formation of some compounds were carried out. The pharmacological and antimicrobial activities of some of the synthesized products were also evaluated.

Keywords: fused pyrimidine; thiazoles; pyrimidopyrimidines; antitumor; antioxidants; antimicrobial.

INTRODUCTION

Heterocyclic compounds are of great importance in the synthesis of pharmacologically active compounds.[1–4] Pyrimidine nuclei are the active core of various bioactive molecules and are best known as the heterocyclic core of nucleic acid bases.

In general, heterocyclics encompassing a pyrimidine moiety have found applications in a wide spectrum of biological[5–7] and therapeutic areas.[8–13] Such a ring system is often incorporated into drugs designed as anticancer,[14,15] antiviral,[16] antihypertensive,[17] analgesic,[18,19] antipyretic,[20] anti-inflammatory,[21] antifungal,[22] antibacterial[22] and anti-psoriasis agents.[23] Some derivatives are active on the blood circulatory system,[24] stimulate skin preparative regeneration and increase the efficacy of antibiotic therapy of *Staphylococcus* and proteus-infected wounds.[25] As part of ongoing interest in the synthesis of heterocyclic com-

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pounds, convenient syntheses of 5-substituted 2-thioxo-4-aryl-1,2,3,6-tetrahydropyrimidin-6-ones and 4-aryl-2-(methylthio)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile were reported.[26] The study presented herein dealt with the efficient synthesis of a variety of fused pyrimidine derivatives, and an investigation of their biological activities.

RESULTS AND DISCUSSION

Chemistry

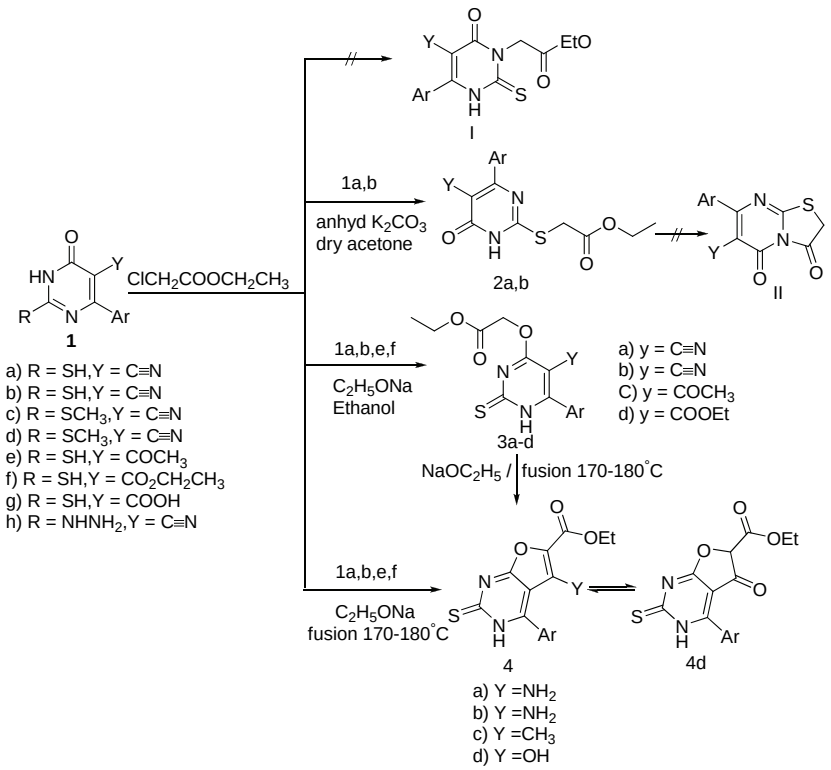
The hydrolysis of nitriles is generally considered one of the best methods for the preparation of carboxylic acids. The hydrolysis of nitriles proceeds in the distinct steps under acid or base treatment to achieve amides and carboxylic acids. The hydrolysis of the 5-cyano group in 2-thioxopyrimidine **1a** in basic medium was unsuccessful due to the low reactivity of the group. However, the hydrolysis was successfully achieved with 70 % sulfuric acid as the weakly nucleophilic nitrile was activated by protonation using H_2SO_4 to make it more electrophilic. This facilitates the reaction to give the corresponding 2-thioxopyrimidine-5-carboxylic acid (**1g**), Scheme 1. The structure of compound **1g** was confirmed by spectroscopic data, the IR spectrum revealed the presence of ν_{OH} at 3416 cm^{-1}, the ^{1}H-NMR spectrum showed a band at δ 11.0 ppm for the acidic OH and the ^{13}C-NMR spectrum showed the presence of a carboxylic C=O at δ 165.2 ppm and the absence of C≡N.

Reaction of **1a** and **c** with hydrazine hydrate afforded the corresponding 2-hydrazino derivative **1h** by nucleophilic substitution of the SH or CH_3S group, respectively.

Masumoto *et al.* reported that treatment of 1-phenylpyrazolin-5-one with a base afforded a mixture of three adducts through carbon, nitrogen and oxygen anions.[27] On the other hand, some pyrimidinethiones in the presence of K_2CO_3/ /dry acetone yielded *S*-alkylated and/or *S*-and *N*-dialkylated products.[28–30] Thienopyridin-2-one bearing a cyano group at the α-position to oxygen, reacted with ethyl chloroacetate in the presence of sodium ethoxide to give the oxygen alkylated derivative as the sole product, which cyclized to afford the corresponding furothiopyridine.[31,32]

The reaction of **1a** and **b** with ethyl chloroacetate in the presence of a base depended on the reaction conditions. Furthermore, it was found that treatment of **1a** and **b** with ethyl chloroacetate in dry acetone, as a polar aprotic solvent, under reflux conditions in the presence of anhydrous K_2CO_3 afforded the *S*-alkylated products **2a** and **b** (Scheme 1). The *N*-alkylated product (**I**) and the corresponding cyclic product 7-aryl-2,3-dihydro-3,5-dioxo-5*H*-thiazolo[3,2-*a*]pyrimidine-6--carbonitrile (**II**) were not isolated.

To account for these results, theoretical calculations using MIDO/3 and Fukui indices were performed. As expected, the predicted electron density of the



Scheme 1. Synthesis of compounds **2a** and **b**; **4a** and **d**; Ar = C_6H_3-3,4-(O–CH_2–O) for **1a**, **c**, **g** and **h**, **2a**, **3a** and **4a**; Ar = C_6H_3-3,4-$(OCH_3)_2$ for **1b**, **d**, **e**, **f**, **2b**, **3b**–**d** and **4b**–**d**.

oxygen atom (–0.298 and –3.05 for **1a** and **b**, respectively) is larger than that of the sulfur atom (–0.172 and –0.188 for **1a** and **b**, respectively). However, since the size of the atom plays a significant role in the extent of its nucleophilicity, the S atom is more nucleophilic than the O atom. Consequently, the reaction proceeded *via S*-alkylation and afforded the thermodynamically more stable products **2a** ($\Delta H_f^{\ominus}$ = –82.03 kcal* mol^{-1}, $\Delta E = E_{LUMO}–E_{HOMO}$ = 8.048 eV) and **2b** (($\Delta H_f^{\ominus}$ = –83.667 kcal mol^{-1}, $\Delta E = E_{LUMO}–E_{HOMO}$ = 8.45 eV). The structure of **2a** was also confirmed by ^{13}C-NMR spectroscopy, which evidenced the presence of *S*-$CH_2COOC_2H_5$ by the appearance of a signal at δ 159.98 ppm, which is attributable to –C=S, and not at $\delta \approx$ 180 ppm.

On the other hand, refluxing **1a** and **b** with ethyl chloroacetate in ethanol and sodium ethoxide gave the corresponding *O*-alkylated products **3a** and **b**, respectively, Scheme 1. It should be noted that, although **3a** and **b** were predicted to be thermodynamically less stable, where the ($\Delta H_f^{\ominus}$ of **3a** is –55.95 kcal mol^{-1} and $\Delta E = E_{LUMO}–E_{HOMO}$= 7.018 eV, and that of **3b** is –56.595 kcal mol^{-1} and ΔE =

* 1 kcal = 4184 J

= E_{LUMO}–E_{HOMO} = 7.44 eV, and in addition, the keto (lactam) form is more stable than the enol (lactim) form by *ca*.13 kcal mol^{-1}, the formation of the *O*-alkylated products **3a** and **b** where the O atom with its higher electron density acted as the nucleophile were facilitated by the presence of the high boiling point, polar protic solvent and the strengthening of the employed base. In addition, analysis of the local reactivity of **1b** through an evaluation of the Fukui indices indicated that the Fukui indices for the S atom were f_k^- = 0.643, f_k^+ = 0.133 and q_N = –0.589, while for the O atom they were f_k^- = 0.026, f_k^+ = 0.046 and q_N = = 0.527. The presence of electron withdrawing group (C≡N) in the α-position to the carbonyl of the amide also acts as an effective factor in the reaction pathway leading to the formation of **3a** and **b**. In order to confirm this assumption, **1e** and **f** were prepared and allowed to react under reflux with ethyl chloroacetate in ethanol and sodium ethoxide, whereby the *O*-alkylated products **3c** and **d** were isolated in a good yield as the sole products.

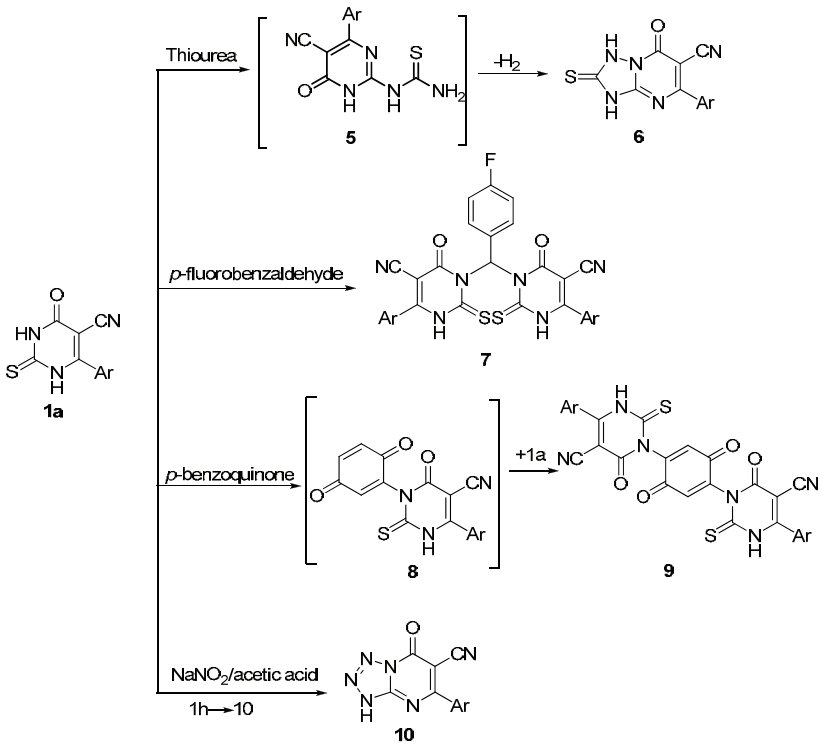
The ethyl 5-substituted-4-aryl-2-thioxo-1,2-dihydrofuro[2,3-*d*]pyrimidine-6--carboxylates **4a**–**d** were obtained *via* either fusion of **1a**, **b**, **e** and **f** with ethyl chloroacetate at 170–180 °C in the presence of sodium ethoxide or cyclization of the open chain products **3a**–**d** under similar reaction conditions (Scheme 1).

Treatment of **1a** with thiourea in *n*-butanol afforded the triazolo pyrimidine **6** without isolation of the intermediate **5** (Scheme 2). The ^{1}H-NMR spectrum of **6** showed the lack of an NH group adjacent to the C=O.

It was of interest to condense **1a** with 4-fluorobenzaldehyde[33] and also with *p*-benzoquinone,[34] to obtain the bis(pyrimidine-5-carbonitriles) **7** and **9** in 73 % and 68 % yield, respectively (Scheme 2).

It was found that the reaction of **1h** with aqueous sodium nitrite solution in the presence of acetic acid at 5 °C afforded the corresponding unisolated diazonium salt followed by cyclization to give the tetrazolo pyrimidine **10** in 52 % yield (Scheme 2). The IR spectra and the ^{1}H-NMR bands showed the absence of the NH_2 group and the appearance of a signal at δ 151.37 ppm in the ^{13}C-NMR spectrum indicated the formation of the tetrazolo ring.

Treatment of 2-thioxopyrimidines **1a**, **b** and **d** with a mixture of phosphorus oxychloride and phosphorus pentachloride afforded the corresponding 4-chloro-2-thioxopyrimidines **11a**–**c** in 62–74 % yield (Scheme 3). The structures of compounds **11b** and **c** were confirmed by their ^{13}C-NMR spectra, which showed signals for –C–Cl at δ 153.80 and 158.8 ppm, respectively, rather than at $\delta \approx$ 166 ppm, which is attributable to –C=O. Such compounds were utilized for the synthesis of several new fused heterocyclic systems bearing 2-thioxo-pyrimidin-4(1*H*)-one moieties as an active core of bioactive molecules with expected antimicrobial and pharmacological activities.[35–37]



Scheme 2. Synthesis of compounds **5**–**10**; Ar = C_6H_3-3,4-(O–CH_2–O) for **6**, **7**, **9** and **10**.

Treatment of **11a** with bi-functional nucleophilic substrates, *i.e.*, sodium azide and thiourea, afforded the tetrazolo pyrimidine **13**, and pyrimido pyrimidine **15**, respectively (Scheme 3).

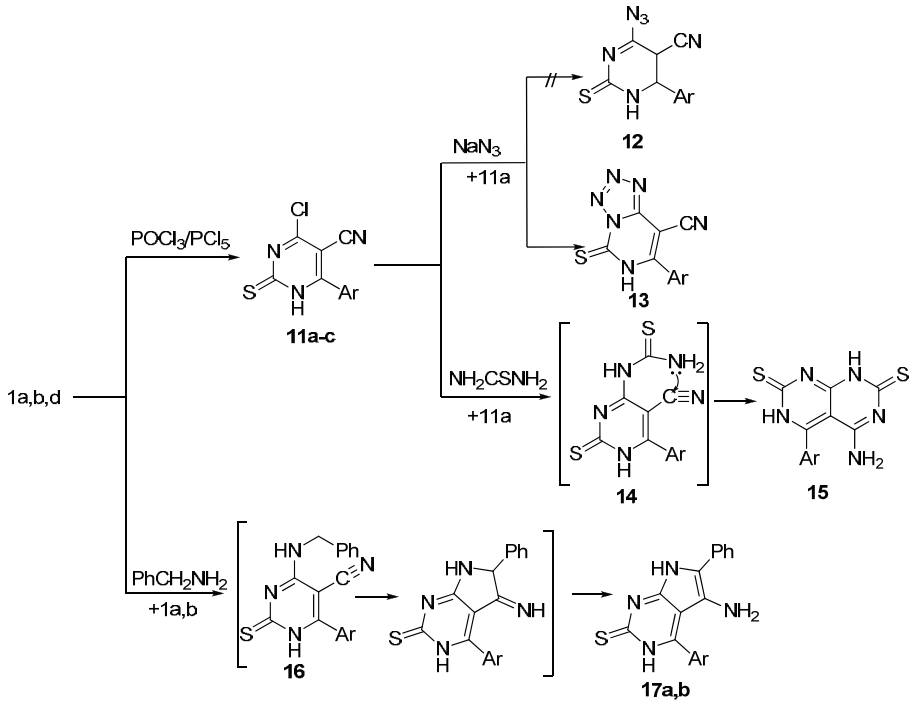
Reaction of 2-thioxopyrimidines **1a** and **b** with benzylamine in *n*-butanol afforded the pyrrolo pyrimidine-2-thiones **17a** and **b**, respectively, *via* the formation of the intermediate **16**.

Biological activities

Antimicrobial, anticancer and antioxidant activities of some compounds were investigated using standard methods and the results were compared with those of standard drugs.

Antimicrobial activity

It was observed that some of the thirteen tested compounds showed good activities against Gram-positive and Gram-negative bacteria, and the fungi *Candida albicans* and *Aspergillus niger*. Compounds **3b**, **3d**, **4a** and **11a** showed inhibition towards all the tested organisms (Table I).



Scheme 3. Synthesis of compounds **11a**–**c**, **13**, **15** and **17a** and **b**; Ar = C_6H_3-3,4-(O–CH_2–O) for **11a** and **c**, **13**, **15** and **17a**; Ar = C_6H_3-3,4-$(OCH_3)_2$ for **11b** and **17b**.

The following points were noticed. On comparison between the compounds **1a, 1c** and **1g**, it was noticed that compound **1a** did not inhibit any of the tested organisms, while conversion of the C=S group to $CSCH_3$ or CN group into COOH groups in compounds **1c** and **1g**, respectively, resulted in activity of **1c** against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* and of **1g** against *S. aureus*, *P. aeruginosa* and *C. albicans*.

Comparing the esters **2a** and **2b**, it was noticed that compound **2a** showed activity against *S. aureus*, *E. coli* and *P. aeruginosa* only, while compound **2b** exhibited activity against all the tested organisms, indicating that the presence of the 3,4-dimethoxy phenyl group in **2a** was more effective than the 1,3-benzo-dioxole group in **2b**.

The (aryloxy)acetate compounds **3b** and **3d** showed activity against all the tested organisms, while the (aryloxy)acetate **3a** showed activity against *S. aureus*, *E. coli* and *P. aeruginosa* only; this indicated that the presence of the 3,4--dimethoxyphenyl group in **3b** and **3d** is more effective than the 1,3-benzodioxole group in **3a**.

The furocarboxylate compound **4a** showed activity against all the tested organisms while **4c** showed activity against *S. aureus*, *E. coli*, *P. aeruginosa* and

C. albicans. **4d** exhibited activity against *Bacillus subtilis*, *S. aureus*, *C. albicans* and *A. niger*; this indicated that the NH_2 group in **4a** is more effective than the CH_3 and OH groups in **4c** and **4d**, respectively.

TABLE I. Antimicrobial activity for some of the products; the well diameter was 1 cm (100 μL of each one was tested); St = standard, that is chloramphenicol at 1 mg mL^{-1} for Gram-positive bacteria, cefalexin for Gram-negative bacteria at 1 mg mL^{-1}, fluconazole for *A. niger* at 1 mg mL^{-1} and flucoral for *C. albicans* at 1 mg mL^{-1}

Compound	Inhibition zone diameter, mm					
	B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
1a	–	–	–	–	–	–
1c	–	24	21	14	–	–
1g	–	23	–	18	23	–
2a	–	19	18	21	–	–
2b	24	21	26	23	–	–
3a	–	26	18	24	–	–
3b	22	26	24	23	19	20
3d	24	28	26	26	21	22
4a	20	21	22	24	22	20
4c	–	22	20	23	22	–
4d	20	26	–	–	24	26
7	–	–	–	24	–	26
11a	20	26	22	18	22	20
St	32.5	32.5	32.5	32.5	28	23

Compound **7** exhibited activity against *P. aeruginosa* and *A. niger*. Conversion of C=O group in **1a** to a chlorine atom in **11a** resulted in inhibition of all the tested organisms.

Pharmacological activity

Antitumor activity using the in vitro *Ehrlich ascites assay.* Cancer still continues to be a major health problem worldwide. The development of new anticancer therapeutic agents is one of the fundamental goals in medicinal chemistry. Compound **4d** proved to have the highest cytotoxic activity, 100 % mortality with 5-flurouracil as a standard, followed by **4b**, **11b**, **1b** and **6**, having mortalities of 97.5, 93.5, 91 and 84.1 %, respectively, at 100 μg mL^{-1}. Compounds **1e**, **1a** and **11a** exhibited medium activity, while compounds **17a**, **1f**, **10** and **2b** showed low activity. The variations of inhibition of the Ehrlich antitumor activity with concentration of the test compounds are listed in Table II.

Antioxidant activity using 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) inhibition. Twelve compounds were tested for antioxidant activity as reflected in their ability to inhibit oxidation in rat brain and kidney homogenates, Table II. Compounds **4d**, **11b**, **6**, **4b** and **1b** showed a similar antioxidant

activity to ascorbic acid, used as standard. Compounds **1e**, **1a**, **10** and **11a** exhibited moderate antioxidant activity, while **1f**, **17a** and **2b** showed lower activity.

TABLE II. Antitumor (dead, %) and antioxidant activities (ABTS method) for some of the products

Compound	Antitumor activity c / μ mL^{-1}			Antioxidant activity		Bleomycin dependent-DNA damage
	100	50	25	Absorbance	Inhibition, %	
1a	68.9	37	20	0.213	59.4	0.118
1b	91	43	18	0.038	89.1	0.079
1e	79.8	38.6	21.1	0.201	61.7	0.124
1f	45.7	28.6	14	0.447	14.8	0.140
2b	33.1	28.6	14	0.485	7.6	0.173
4b	97.5	63	36.7	0.056	89.3	0.090
4d	100	100	98.2	0.033	93.7	0.086
6	84.1	11.4	25.6	0.049	91.4	0.089
10	36.8	19.1	11.6	0.22	56.4	0.129
11a	56	30	17.2	0.300	42.8	0.138
11b	93.5	48	21.4	0.045	92.8	0.084
17a	48.2	27.3	13.8	0.453	13.7	0.159
St[a]	98	62.7	37	–	–	–
ABTS	–	–	–	0.525	–	–
Ascorbic acid[b]	–	–	–	0.029	94.5	0.093

[a]5-Flurouracil was used as standard for the antitumor activity; [b]standard for the antioxidant activity

Bleomycin-dependent DNA damage. The bleomycins are a family of glycopeptide antibiotics[38] that are routinely used as antitumor agents. The bleomycin assay has been adopted for assessing the pro-oxidant activity of food antioxidants. The antitumor antibiotic bleomycin binds iron ions and DNA. If the samples to be tested are able to reduce bleomycin–Fe^{3+} to bleomycin–Fe^{2+}, DNA degradation in the system will be stimulated, resulting in a positive test for pro-oxidant activity. DNA degradation is accompanied by the formation of a product similar to malondialdehyde. L-ascorbic acid was used as the reducing agent to reduce Fe^{3+} to Fe^{2+}. Twelve compounds were selected for bleomycin-dependent DNA-damage testing (Table II).

Results in Table II showed that compounds **1b**, **4d**, **4b**, **6** and **11b** have the ability to protect DNA from the damage induced by bleomycin. On the other hand, the rest of the compounds exhibited weak activities. By comparing the obtained results of the investigated compounds to their structures the following structure–activity relationships were postulated; *i*) compounds **1b**, **4b**, **4d**, **6** and **11b** were more potent than ascorbic acid, which may be attributed to the presence of the thioxopyrimidine moiety in **1b**, **4b**, **4d**, and **11b**, and the thioxo triazolo moiety in 6; *ii*) compounds **4b** and **11b** were less potent than **1b**, which may be due to the replacement of the C=O and C≡N moieties into ethyl aminofuran-

carboxylate in compound **4b** and C=O into chlorine atom in compound **11b**; *iii*) compound **6** was more potent than compound **1a**, which may be attributable to the presence of the thioxotriazolo moiety.

EXPERIMENTAL

Chemistry

Chemicals were obtained from Alfa Aesar and used as provided. Melting points were measured on a Gallenkanp or a Griffin melting point apparatus. The infrared absorption spectra were measured on a Pye Unicam SP 2000 infrared spectrophotometer using the KBr wafer technique. The EI-MS spectra were determined using an AE1 MS 902 mass spectrometer. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker AC-300 instrument at 300 and 75 MHz, respectively, using DMSO-d_6 as the solvent. Chemical shifts are expressed as δ / ppm and TMS was used as an internal standard. All the spectral measurements were performed at the Micro analytical Center of Cairo University, Egypt, the Micro analytical Center of Ain Shams University or the Main Defense Chemical Laboratory. Elemental analysis were realized at the Faculty of Science, Ain Shams University, using a Perkin–Elmer 2400 C, H and N elemental analyzer and satisfactory analytical data (±0.3 %) were obtained for all compounds. The antimicrobial activities were determined at Al-Azhar University, Faculty of Science, Fermentation Biotechnology and Applied Microbiology (Ferm-BAM) Center, Egypt. The pharmacological activities were performed at the Pharmacology Department, Faculty of Pharmacy, Mansoura University, Egypt. The completion of chemical reactions was monitored by TLC. Compounds **1a**–**f** were prepared according to the literature.[26]

The physical, analytic and spectral data for the prepared compounds are given in Supplementary Material to this paper.

*Synthesis of 6-(1,3-benzodioxol-5-yl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid (**1g**).* A mixture of **1a** (2.73 g, 10.0 mmol) and sulfuric acid (15 mL, 70 %) was heated under reflux for 3 h. The reaction mixture was left to cool and then poured onto cold water. The obtained solid was filtered off, dried and recrystallized from benzene. Pale brown crystals, Yield: 53 %

*Synthesis of 4-(1,3-benzodioxol-5-yl)-2-hydrazino-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**1h**).* To a solution of **1a** or **1c** (0.01 mol) in *n*-butanol (40 mL), hydrazine hydrate (0.75 mL, 0.015 mol, 98 %) was added and the reaction mixture was heated under reflux for 6 h. The solvent was evaporated and the obtained solid was collected by filtration, washed with water, dried and then recrystallized from methanol. Brown crystals, yield: 65 % from **1a** and 72 % from **1c**.

*General procedure for the synthesis of (dihydropyrimidin-2-ylthio)acetates **2a** and **b**.* A mixture of **1a** or **1b** (0.01 mol), ethyl chloroacetate (1.3 mL, 0.012 mol) and anhydrous K_2CO_3 (5.0 g, 40 mmol) in dry acetone (60 mL) was refluxed for 18 h and then filtered while hot, concentrated to half its volume and poured onto cold water. The obtained solid was filtered off, dried and recrystallized from a suitable solvent to afford **2a** and **2b**, respectively. **2a**: brown crystals (acetic acid), yield: 71 %, and **2b**: orange crystals (toluene/few drops of light petroleum 60–80 °C), yield: 72 %.

*General procedure for the synthesis of (dihydropyrimidin-4-yloxy)acetates **3a**–**d**.* A mixture of **1a**, **1b**, **1e** or **1f** (0.01 mol) and ethyl chloroacetate (1.3 mL, 0.012 mol) in sodium ethoxide solution (0.46 g of Na in 20 mL of absolute ethanol) was heated under reflux for 2 h.

Most of the solvent was evaporated and then the remaining acidified with cold diluted HCl (20 mL, 2 M). The oily residue obtained was extracted with ethyl acetate (3×50 mL) and dried over anhydrous $MgSO_4$. The solid obtained after evaporation of the solvent was filtered off, dried and recrystallized from a suitable solvent to give **3a**–**d**. **3a**: brown crystals (acetic acid), yield: 57 %; **3b**: orange crystals (toluene/light petroleum, 60–80 °C, 1:1 *V*/*V*), yield: 61 %; **3c**: yellow crystals (acetic acid), yield: 70 %; **3d**: pale brown crystals (acetic acid), yield: 64 %.

*General procedure for the synthesis of furo[2,3-*d*]pyrimidine-6-carboxylates **4a**–**d***. A mixture of **1a**, **1b**, **1e** or **1f** (0.01 mol) and ethyl chloroacetate (1.30 mL, 0.012 mol, 99 %) in sodium ethoxide solution (0.92 g of Na in 40 mL of absolute ethanol) was fused at 170–180 °C for 3 h. The reaction mixture was left to cool, poured onto ice water and acidified with HCl (15 mL, 2 M). The product was extracted with ethyl acetate (3×40 mL), dried over anhydrous $MgSO_4$ and most of the solvent was evaporated. The obtained solid was filtered off, dried and recrystallized from an appropriate solvent to give **4a**–**d**. **4a**: brown crystals (methanol), yield: 63 %; **4b**: brown crystals (ethanol), yield 61 %; **4c**: brown crystals (ethanol), yield 66 %; **4d**: brown crystals (toluene), yield 55 %.

*Synthesis of authentic samples of **4a** and **c***. A mixture of **3a** or **3c** (0.01 mol) and sodium ethoxide (0.46 g of Na metal in 20 mL of absolute ethanol) was heated at 170–180 °C for 1 h. The reaction mixture was left to cool, poured onto ice water and then acidified with HCl (15 mL, 2 M). The solid obtained was filtered off, dried and recrystallized from a proper solvent to yield **4a** or **4c**.

*Synthesis of 5-(1,3-benzodioxol-5-yl)-7-oxo-2-thioxo-1,2,3,7-tetrahydro[1,2,4]triazolo-[1,5-*a*]pyrimidine-6-carbonitrile (**6**)*. A mixture of **1a** (2.73 g, 10.0 mmol) and thiourea (0.94 g, 0.012 mol, 97 %) in *n*-butanol (40 mL) was heated under reflux for 4 h. Most of the solvent was evaporated and the obtained solid was filtered off, washed with water, dried and recrystallized from ethanol. Brown crystals, yield: 76 %.

*Synthesis of 3,3′-[(4-fluorophenyl)methylene]bis[6-(1,3-benzodioxol-5-yl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile] (**7**)*. A mixture of **1a** (5.46 g, 20.0 mmol) and 4-fluorobenzaldehyde (1.27 g, 10.0 mol) in acetic anhydride (15 mL) in the presence of a catalytic amount of anhydrous K_2CO_3 was heated at 70–80 °C for 3.5 h. The solid obtained after cooling was filtered off, dried and then recrystallized from ethanol. White crystals, yield: 73 %.

*Synthesis of 3,3′-(3,6-dioxocyclohexa-1,4-diene-1,4-diyl)bis[6-(1,3-benzodioxol-5-yl)-4--oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile] (**9**)*. A mixture of **1a** (5.46 g, 0.02 mol) and *p*-benzoquinone (1.30 g, 0.012 mol) in aqueous acetone (80 mL, 50 %) was stirred for 6 h at room temperature. The reaction mixture was kept overnight and then poured onto ice/water. The obtained solid was filtered off, washed with hot water, dried and recrystallized from ethanol/toluene (2:1 by volume). Pale brown crystals, yield: 68 %.

*Synthesis of 5-(1,3-benzodioxol-5-yl)-7-oxo-1,7-dihydrotetrazolo[1,5-*a*]pyrimidine-6-carbonitrile (**10**)*. An aqueous solution of sodium nitrite (1.07 g, 15.0 mol) in water (10 mL) was added to a stirred cold (0 °C) solution of **1 h** (2.7 g, 10 mmol) in acetic acid (30 mL). The reaction mixture was stirred at 0 °C for 3 h, poured onto ice/saturated $NaHCO_3$ (40 mL) and the product was extracted with ethyl acetate (60 mL). The organic layer was dried over anhydrous $MgSO_4$ and half of the solvent was removed. The obtained solid was filtered off, and recrystallized from dioxane. Brown crystals, yield: 52 %.

*General procedure for the synthesis of dihydropyrimidine-5-carbonitriles **11a**–**c***. A mixture of **1a**, **1b** or **1d** (0.01 mol), phosphorus oxychloride (20 mL) and phosphorus pentachloride (1.5 g, 7.0 mmol) was heated on a water bath for 4 h. The reaction mixture was

poured onto cold water. The obtained solid was filtered off, washed several times with light petroleum 60–80 °C, dried and recrystallized from a suitable solvent. **11a**: brown crystals (ethanol), yield: 74 %; **11b**: brown crystals (ethanol), yield: 62 %; **11c**: brown crystals (ethanol), Yield: 64 %.

*Synthesis of 7-(1,3-benzodioxol-5-yl)-5-thioxo-5,6-dihydrotetrazolo[1,5-c]pyrimidine-8--carbonitrile (**13**)*. To a stirred solution of **11a** (2.9 g, 10 mmol) in DMF (30 mL), sodium azide (0.76 g, 12 mmol) was added in portions and the mixture was stirred at room temperature for 4 h. The mixture was poured onto ice water and the obtained solid was filtered off, dried and then recrystallized from methanol. Yellow crystals, yield: 54 %.

*Synthesis of 4-amino-5-(1,3-benzodioxol-5-yl)pyrimido[4,5-d]pyrimidine-2,7(1*H,3H*)--dithione (**15**)*. A mixture of **11a** (2.8 g, 0.010 mol), thiourea (0.69 g, 0.012 mol) and few drops of piperidine in *n*-butanol (40 mL) was heated at 130–140 °C for 4 h. The mixture was left to cool to room temperature and poured onto cold HCl (40 mL, 2 M). The obtained solid was filtered off, washed with water (3×30 mL), dried and recrystallized from *n*-butanol. Yellow crystals, yield: 62 %.

*Synthesis of 5-amino-4-aryl-6-phenyl-1,3-dihydro-2*H*-pyrrolo[2,3-*d*]pyrimidine-2-thiones **17a** and **b***. A mixture of chloropyrimidine **1a** or **b** (0.01 mol) and benzylamine (1.35 mL, 12.0 mmol) in *n*-butanol (40 mL) was heated under reflux for 4 h. The reaction mixture was concentrated to half its volume and poured onto cold water. The obtained solid was filtered off, dried and recrystallized from the proper solvent. **17a**: brown crystals (DMF), yield: 72 %; **17b**: brown crystals (*n*-butanol), yield: 67 %.

Biological and pharmaceutical activities

Antimicrobial activity. Some of the products were screened for their antimicrobial activity including Gram-positive bacteria (*B. subtilis* and *S. aureus*), Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and antifungal activity (*C. albicans* and *A. niger*).

Agar well diffusion method. The agar well diffusion method[39] was used for the determination of the inhibition zone. DMSO was used as the solvent and the blank with appropriate drugs as standards. The results are given in Table I. With a sterile loop, pure colonies of the bacterial cultures were picked up. The colonies were suspended in 5 mL of sterile physiological saline. Using sterile forceps, the wells containing the biomaterial were placed onto the agar surface and gently pressed down to ensure contact. The plates were per-incubated for 1 h in a refrigerator followed by incubation at 37 °C for 24 h.

Cell line and cell culture. Different concentrations of the tested compounds were prepared (ED_{100}, ED_{50} and ED_{25} as μg/ml DMSO). The amount of DMSO was adjusted to give a final concentration of 0.1%. Ascites fluid was obtained from the peritoneal cavity of the donor animal from (National cancer Institute, Cairo, Egypt) contain Ehrlich cell was as aseptically aspirated. The cells were grown partially floating and attach in a suspension culture (RPMI 1660 medium, Sigma Chemical Co. St. Louis, USA), supplemented with 10% fetal bovine serum 9GIBCO, UK). They were maintained at 37 °C in humidified atmosphere with 5% CO_2 for 2 h. The viability in control experiments (DMSO only without drug) exceeded 95% as determined by microscopical examination using a hemocytometer and trypan blue stain (stains only the dead cells).[40]

MTT assay. Briefly, cells were seeded in a 96-well plate at a density of 1×10^4 cells well^{-1} as previously described.[41-43] Drugs at different concentrations were added to each well and cultured for 48 h, followed by incubation with 5 mg L^{-1} MTT (3-(4,5-dimethylthiazol-2-yl)--2,5-diphenyltetrazolium bromide) for 4 h, after which the supernatant was removed after centrifugation. Finally, 100 μL of DMSO was added and the absorbance, *A*, at a wavelength

of 490 nm was measured by using an Elisa Reader EXL 800. The relative cell proliferation inhibition rate (*IR*) is given by:

$$IR = \left(1 - \frac{A_{490,\text{exp}}}{A_{490,\text{control}}}\right) \times 100$$

where $A_{490,\text{exp}}$ and $A_{490,\text{control}}$ are the absorbances at 490 nm for the experimental and control, respectively.

Cytotoxic activity. Ehrlich cells[44-46] (Ehrlich ascites carcinoma, EAC) were derived from the ascitic fluid from diseased mouse (purchased from the National Cancer institute, Cairo, Egypt). The cells were grown in suspension culture, partly floating and partly attached, in RPMI 1640 medium, supplemented with 10 % fetal bovine serum. They were maintained at 37 °C in a humidified atmosphere with 5 % CO_2. The viability of the cells used in the control experiments (DMSO only without drug) exceeded 95 %, as determined with trypan blue. The test compounds were prepared initially at a concentration of 1 mg mL^{-1} DMSO.

Antioxidant activity screening assay (ABTS method). For each of the investigated compounds,[47,48] 2 mL of ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) solution (60 μM) was added to 3 mL MnO_2 suspension (25 mg mL^{-1}), all prepared in 5 mL aqueous phosphate buffer solution (pH 7, 0.1 M). The mixture was shaken, centrifuged, filtered and the absorbance of the resulting green blue solution (ABTS radical solution) at 734 nm was adjusted to *ca*. 0.5. Then, 50 μl of a 2 mM solution of a test compound in spectroscopic grade MeOH/phosphate buffer (1:1) was added. The absorbance was measured and the reduction in the color intensity was expressed as inhibition percentage. L-Ascorbic acid was used as the standard antioxidant (positive control). A blank sample was run without ABTS and using MeOH/phosphate buffer (1:1) instead of a test compound. A negative control was run with ABTS and MeOH/phosphate buffer (1:1) only.

Bleomycin-dependent DNA damage assay. To the reaction mixtures in a final volume of 1.0 mL, the following reagents at the final concentrations were added: DNA (0.5 mg mL^{-1}), bleomycin sulfate (0.05 mg mL^{-1}), $FeCl_3$ (0.025 mM), magnesium chloride (5 mM), KH_2PO_4–KOH buffer pH 7.0 (30 mM), and ascorbic acid (0.24 mM) or a test fraction diluted with MeOH to give a concentration of 0.1 mg mL^{-1}. The reaction mixtures were incubated in a water bath at 37 °C for 1 h. At the end of the incubation period, 0.1 mL ethylenediaminetetraacetic acid (EDTA) (0.1 M) was added to stop the reaction (the iron–EDTA complex is unreactive in the bleomycin assay).[49] DNA damage was assessed by adding 1 mL 1 % (*w*/*V*) thiobarbituric acid (TBA) and 1 mL of 25 % (*V*/*V*) hydrochloric acid followed by heating in a water-bath maintained at 80 °C for 15 min. The chromogen formed was extracted into 1-butanol, and the absorbance was measured at 532 nm.

CONCLUSIONS

The type of the products from reactions of thioxopyrimidin-6(1*H*)-ones with ethyl chloroacetate were found in dependence on the reaction conditions to afford *S*-alkylated or *O*-alkylated or furo[2,3-*d*]pyrimidine products. Reactions of 2-thioxo-4-chloropyrimidine with bi-functional nucleophiles provided a convenient route for the synthesis of the corresponding tetrazolo[1,5-*c*]pyrimidine, pyrimido[4,5-*d*]pyrimidine and pyrrolo[2,3-*d*]pyrimidine. Some of the products exhibited promising antimicrobial, antitumor and antioxidant activities.

SUPPLEMENTARY MATERIAL

The physical, analytic and spectral data for the prepared compounds are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

ИЗВОД

СИНТЕЗА РАЗЛИЧИТИХ КОНДЕНЗОВАНИХ ПИРИМИДИНА И ИСПИТИВАЊЕ ФАРМАКОЛОШКЕ И АНТИМИКРОБНЕ АКТИВНОСТИ

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Синтетисана је група деривата пиримидина који садрже кондензоване прстенове, као што су фуро[2,3-*d*]пиримидин, триазоло[1,5-*a*]пиримидин и тетразоло[1,5-*a*]пиримидин, реакцијом тиоксопиримидин-6(1*H*)-она са етил-хлорацетатом, под различитим реакционим условима. Пиримидин-тиони у реакцији са $POCl_3/PCl_5$ дају хлор-деривате који реакцијом са натријум-азидом и тиоуреом дају као производе тетразоло[1,5-*c*]пиримидин и пиримидо-пиримидине. Тиоксопиримидин-6(1*H*)-он реагује са бензил-амином и као производ даје пироло[2,3-*d*]пиримидинтионе. Извршена су израчунавања топлота стварања једињења применом Фукуи индекаса. Испитана је фармаколошка и антимикробна активност неким од синтетисаних деривата.

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