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Original article

Efficient one-pot preparation of novel fused chromeno[2,3-*d*]pyrimidine and pyrano[2,3-*d*]pyrimidine derivatives

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ABSTRACT

Some novel chromeno[2,3-*d*]pyrimidinone, pyrano[2,3-*d*]pyrimidine, dihydropyrimidine, pyridopyranopyrimidine and pyrimidopyranopyrimidine have been synthesized. The structures of target compounds were confirmed by elemental analyses and spectral data. The antimicrobial activity of all the target synthesized compounds were tested against various microorganismst such as *Pseudomonas aeruginosa*; *Staphylococcus aureus* (Bacteria), *Aspergillus flavus* (Fungus) and *Candida albicans* (Yeast fungus) by the disc diffusion method. In general, the novel synthesized compounds showed a good antimicrobial activity against the previously mentioned microorganisms.

1. Introduction

Pyrane and fused 4*H*-pyrane derivatives have attracted a great interest owing to their antimicrobial activity [1–3], inhibition of influenza, virus sialidases [4], mutagenic activity [5], antiviral [6] antiproliferaction agents [7], sex-pheromones [8] antitumor [9] and anti-inflammatory agents [10]. Moreover, pyrane derivatives are well known for their antihistaminic activity [11]. Also, pyrimidines and its fused derivatives play an essential role in several biological processes and chemical and pharmacological importance. In particular, pyrimidine nucleus can be found in a broad variety of antibacterial and antitumor agents as well as in agrochemical and veterinary products [12–15]. Based on the above information's and due to our interest in developed the synthetic strategies for the construction of novel fused pyrimidinones as a biologically active pharmacophore [16–18], we have reported a facile methodology for the synthesis and transformation of pyrimidindione to pyrimidinones fused at C-5 and C-6 position. Herein, a detailed account of our focused attention toward construction of the pyrimidinones was reported, having latent functionalities at C-5 position, which could form useful building blocks for the synthesis of various C-5, C-6 heterocyclic ring fused pyrimidinones via a *Knoevenagel* condensation and other reactions.

The significance of pyranopyrimidine derivatives is recognized as a result of their occurrence in the structure of various natural products, their biological activity, and their synthetic potential [19–21]. We thought it would be of interest to combine the above mentioned heterocyclic compounds in a molecular framework to investigate a possible additive effect of these rings regarding biological activity.

2. Results and discussion

2.1. Chemistry

A *Knoevenagel* condensation of 2-thioxo-dihydropyrimidine-4,6(1*H*,5*H*)-dione **1** with aromatic aldehyde in ethanol under reflux in the presence of piperidine yielded the chromeno[2,3-*d*]pyrimidine derivative **3**. Assignment of chromeno[2,3-*d*]pyrimidine derivative **3** was based on the basis of elemental and spectral data. Its infrared spectrum exhibited the absence of one carbonyl group absorption band while it showed the presence of broad band for OH group stretching at 3432 cm^{-1}. Its mass spectrum showed a molecular ion peak at *m/z* 246 (M^+) (10.98) together with a base peak at *m/z* 86 (100). The formation of compound **3** is assumed to proceed through the in situ intramolecular cyclization of the non-isolable intermediate **2** via nucleophilic addition of the hydroxyl group to the carbonyl group followed by elimination of water (Scheme 1).

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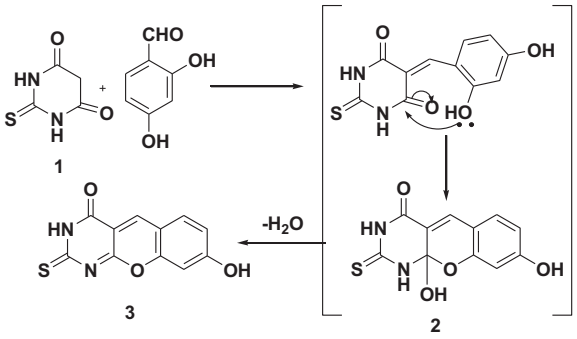
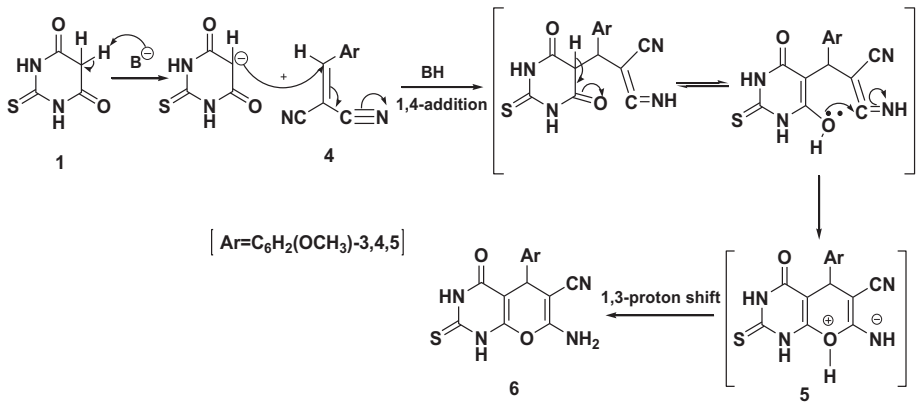


Scheme 1.

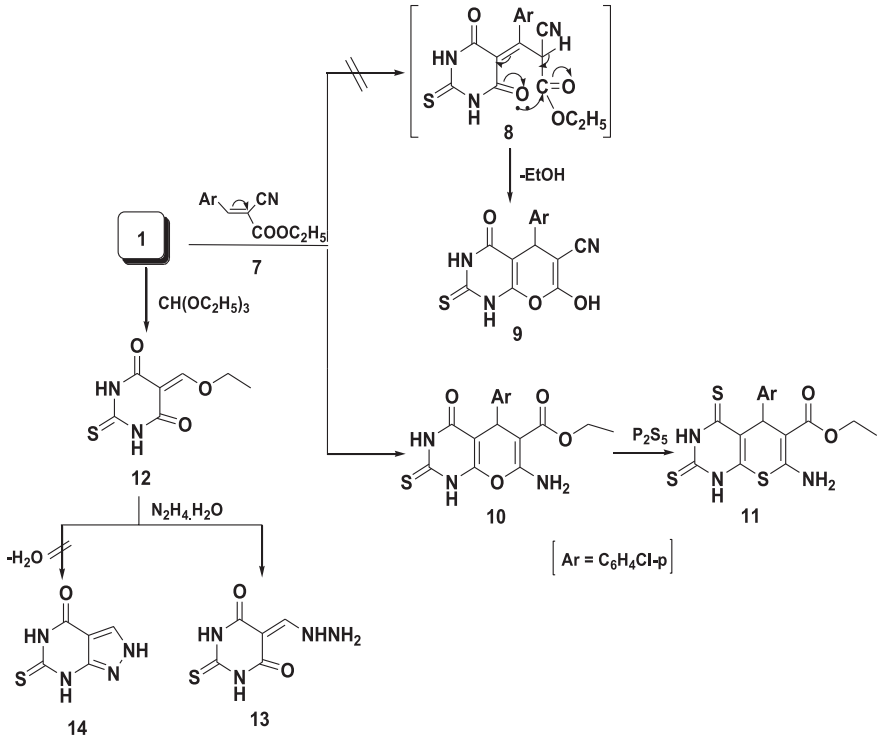
The active methylene group in pyrimidindione derivative **1** was exploited to synthesize novel pyranopyrimidine and hydrazinyl-methylene-2-thioxo-dihydropyrimidine derivatives through its reactions with some electrophiles. Thus, the 7-aminopyrano[2,3-*d*] pyrimidine-6-carbonitrile derivative **6** was synthesized in a good yield, as stable crystalline solids and easily recrystallized from dioxane, when treatment of compound **1** and 2-(3,4,5-trimethoxybenzylidene) malononitrile **4** under reflux in absolute ethanol, in the presence of catalytic amounts of piperidine. The structure of compound **6** was characterized by elemental and spectroscopic analysis. Thus, IR spectrum of compound **6** revealed the presence of characteristic bands for amino, cyano and one carbonyl functional groups. In addition, the ^{1}H NMR spectrum of compound **6** in DMSO-d_6 revealed the absence of methylene moiety. The structure of compound **6** was supported by its mass spectrum which revealed a molecular ion peak at *m/z* 388 (M^+) (2.86). Pyrano[2,3-*d*]pyrimidine derivative **6** was assumed to be formed via *Michael addition* of the active methylene group in pyrimidindione derivative **1** to the activated double bond in cyanoarylidine derivative **4** to form the non-isolable intermediate **5** followed by the intramolecular cyclization to furnish o-aminocarbonitrile of pyrano[2,3-*d*]pyrimidinone derivative **6** (Scheme 2).

In a similar manner, the reaction of starting material **1** with equimolar amount of ethyl 3-(4-chlorophenyl)-2-cyanoacrylate **7** under refluxing ethanol in the presence of piperidine afforded ethyl 7-amino-5-(4-chlorophenyl)-4-oxo-2-thioxo-2,3,4,5-tetrahydro-1*H*-pyrano[2,3-*d*]pyrimidine-6-carboxylate **10**. Both elemental and spectral data supported the proposed structure **10** and the formation of Michael adduct **8** followed by intramolecular cyclization through elimination of ethanol to afforded **9** was ruled out (Scheme 3).

The infrared spectrum of compound **10** indicated the presence of characteristic absorption bands for NH_2 and ester functional groups. In addition, the molecular structure of compound **10** was established by ^{1}H NMR spectrum which exhibited lack of the characteristic signal of methylene group and the presence of triplet at δ 1.2 ppm, quartet at δ 4.4 ppm assigned for ethoxycarhonyl moiety in addition to amino protons at δ 5.8 ppm. The molecular ion peak of compound **10** was found in the mass spectrum at *m/z* 379 ($M^+-OC_2H_5$) (9.5) corresponding to the molecular formula $C_{16}H_{14}ClN_3O_4S$ with a base peak at *m/z* 186 (100). Thionation reaction of the o-aminoester of pyrano[2,3-*d*]pyrimidine derivative **10** was occurred by the interaction with phosphorus pentasulphide afforded the ethyl -7-amino-5-(4-chlorophenyl)-2,4-dithioxo-2,3,4,5-tetrahydro-1*H*-thiopyrano[2,3-*d*]pyrimidine-6-carboxylate **11**. The infrared spectrum of compound **11** indicated the presence of one carbonyl group absorption band and other characteristic absorption bands at 1567 and 1355 cm^{-1} for the two C=S functional group. The molecular ion peak of compound **11** was found in the mass spectrum at *m/z* 411(M^+-NH_2) (2.13) corresponding to the molecular formula $C_{16}H_{14}ClN_3O_2S_3$. 5-Ethoxymethylene derivative **12** was obtained in 87% yield by the alkylation reaction of starting material **1** with excess amount of triethyl orthoformate at 200 °C under solvent free condition (Scheme 3). The reactivity of compound **12** which contains chalcone system, toward hydrazine hydrate was investigated. Thus, hydrazinolysis of compound **12** with hydrazine hydrate furnished hydrazinyl methylene derivative **13** and attempts to cyclize the hydrazide derivative **13** by refluxing in ethanol containing piperidine and/or pyridine to afford 6-thioxo-6,7-dihydropyrazolo[3,4-*d*] pyrimidinone derivative **14** were unsuccessful on the basis of analytical and spectral data. Furthermore, *o*-aminoester of pyrano [2,3-*d*]pyrimidine derivative **6** gave characteristic reaction for enaminonitriles so it used as a key precursor for the synthesis of condensed heterocyclic compounds of expected biological activity. The interaction of pyrano[2,3-*d*]pyrimidine **6** with malononitrile in the presence of piperidine as basic condition afforded the corresponding pyridopyranopyrimidine **15**. 2-arylsulfonylamino pyrano [2,3-*d*]pyrimidine derivative **16** was obtained by refluxing of compound **6** with benzenesulfonyl chloride in dry benzene. The structure of compound **16** was demonstrated by spectral data. IR spectrum revealed band for (C≡N). Mass spectrum of **16** showed a molecular ion peak *m/z* at 528 [M^+] (2.1), with a base peak at 222 (100). Finally, the behavior of compound **6** toward acid derivative was also investigated. Thus, heating compound **6** with formic acid caused cyclization to give the corresponding pyrimidopyranopyrimidine derivative **17** with a new ring system. Compound **17** was formed via *Dimruth rearrangement* illustrated in Scheme 4. The



Scheme 2.

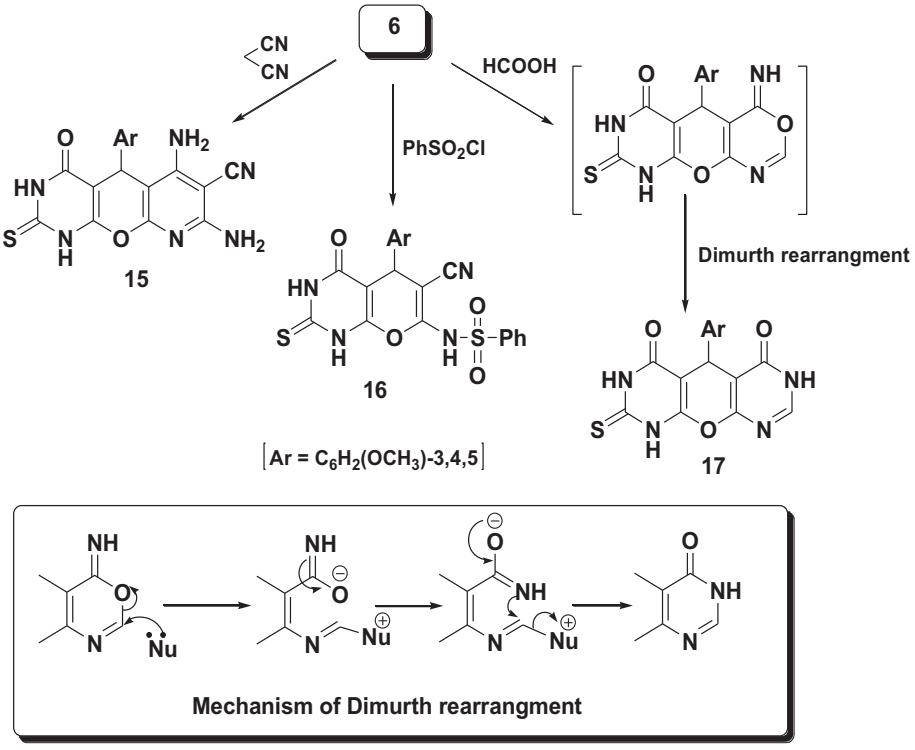


Scheme 3.

structure of compound **17** was supported by its analytical and spectral data. The infrared spectrum of compound **17** showed the absence of (C≡N) band and the presence bands at 3293,3224 cm^{-1} (br, NH), 1690, 1676 cm^{-1} (2C═O), 1620 cm^{-1} (C═N). The mass spectrum of compound **17** revealed a molecular ion peak *m/z* at 416 [M^+] (39.71), with a base peak at 180 (100).

2.2. Antimicrobial activity

All of the target synthesized compounds were screened against cultures of two fungal species, namely *Aspergillus flavus* and *Candida albicans* as well as two bacteria species, namely *Pseudomonas aeruginosa* and *Staphylococcus aureus* to detect their



Scheme 4.

Table 1
Antimicrobial activity of chemical substances tested.

Compound No.	Inhibition zone diameter (mm)			
	Antibacterial		Antifungal	
	Pseudomonas aeruginosa	*Staphylococcus aureus*	*Aspergillus flavus*	*Candida albicans*
3	14	15	0	0
6	13	13	0	10
10	19	16	0	13
11	18	16	14	14
12	13	13	0	12
13	18	18	36	0
15	12	13	0	10
16	13	14	0	12
17	13	15	0	11
Tetracyclin	28	26	–	–
Amphotericin B	–	–	16	19

Tetracyclin used as a standard (antibacterial agent).
Amphotericin B used as a standard (antifungal agent).

antimicrobial activities. The antimicrobial activity was biologically assayed using the diffusion technique. The investigation of antibacterial and antifungal screening data revealed that all the tested compounds **3, 6, 10, 11, 12, 13, 16** and **17** showed comparatively good activity against all the bacterial strains. The organisms were tested against the activity of solutions with concentration; 1.0 mg/mL of each compound; and using inhibition zone diameter (IZD) in centimeter as the criterion for antimicrobial activity. *Amphotericin B* as an antifungal agent and *Tetracyclin* as an antibacterial agent were used as references to evaluate the potency of the tested compounds under the same conditions. The minimum inhibitory concentration (MIC) of the biologically active compounds was measured by a two-fold serial dilution method. The results are depicted in Table 1. The good activity is attributed to the presence of pharmacologically active 3-hydoxy, $-OCH_3$, 4-chloro groups attached to phenyl ring on the pyrane ring and $-OC_2H_5$,$-NHNH_2$ groups attached to pyrimidine moiety and 2,4-diamino-3-cyanopyridyl, benzenesulfonamide and pyrano[2,3-*d*] pyrimidine moiety attached to the pyrano[2,3-*d*]pyrimidine. In conclusion, we reported herein a simple and convenient route for the synthesis of some new heterocyclic compounds based on 2-thiobarbituric acid for antimicrobial evaluation. Most of the compounds were effective against *C. albicans*. In case of *Amphotericin B*; compounds **6, 10, 11,12,15,16 and 17** are mostly effective, while **3, 13** have no activity. The pyrano[2,3-*d*]pyrimidine-6-carboxylate **11** nearly as active as the standard fungicide *Amphotericin B*. Among these nine active compounds, the most and the only more effective compound against *A. flavus* than the standard fungicide *Amphotericin B* was **13**, which includes hydrazide subsistent on pyrimidine moiety while other effective compounds does not have. However, it was also observed that the substations on the pyrane derivatives had no determining influence on the antifungal activity (MIC values <50 μg/mL). All of the other compounds exhibited no activity against the tested species (Table 1).

3. Conclusions

The research study reports the successful synthesis and antimicrobial activity of new chromene, pyrane and pyranopyridine derivatives bearing 2-thiobarbituric acid moiety. The antimicrobial activity study revealed that all the tested compounds showed moderate to good antibacterial and antifungal activities against pathogenic strains. Structure and biological activity relationship of title compounds showed that the presence of thiopyrane moiety and biologically active groups like 3-hydoxy, $-OCH_3$, 4-chloro groups attached to phenyl ring of the pyrane ring and $-OC_2H_5$,$-NHNH_2$ groups attached to pyrimidine moiety and 2,4-diamino-3-cyano-pyridyl, benzenesulfonamide and pyrano[2,3-*d*] pyrimidine moiety attached to the pyrano[2,3-*d*]pyrimidine of the title compounds are responsible for the good antimicrobial activity. From the obtained antifungal and antibacterial results, we can conclude that the entire tested compounds are more active toward bacteria than fungi.

4. Experimental

4.1. Chemistry

All melting points are uncorrected and were determined on a *Stuart* melting point apparatus. IR spectra were recorded on a *Shimadzu*-440 IR spectrophotometer using the KBr technique (Shimadzu, Japan). ^{1}H NMR spectra were measured on a Varian Mercury VX-300 NMR spectrometer in DMSO-d_6 as a solvent and were run at 300 MHz, using tetramethylsilane (TMS) as an internal standard. The mass spectra were recorded on Shimadzu GCMS-QP-1000EX mass spectrometers at 70 eV. The purity of the synthesized compounds was monitored by TLC. Elemental analyses were carried out by the Microanalytical Research Center, Faculty of Science, Cairo University. Analytical results for C, H, N and S were within ±0.4 of the calculated values.

4.1.1. 8-Hydroxy-2-thioxo-2,3-dihydrochromeno[2,3-d]pyrimidin-4-one (*3*)

A mixture of compound **1** (0.72 g, 0.005 mol), 2,4-dihydroxy benzaldehyde (0.69 g, 0.005 mol), and piperidine (0.005 mol) in ethanol (60 mL) was heated under reflux for 3 h, The solid product was filtered on hot, dried, and crystallized from dioxane to give orange powders **3**: Yield, 80%; m.p. 185–186 °C; IR, cm^{-1}: 3432 (br,OH), 3325 (NH), 1694 (C=O), 1563 (C=S). ^{1}H NMR (DMSO-d_6) δ: 6.9–7.4 (m, 4H, Ar–H), 10.0 (s, H, OH), 11.6 (s, H, NH pyrimidine). MS, *m*/*z* (%): 246 (M) (10.98), 86 (100). Anal. Calcd. For $C_{11}H_6N_2O_3S$: C, 53.65; H, 2.46; N, 11.38; S, 13.02. Found: C, 53.55; H, 2.06; N, 11.18; S, 13.00.

4.1.2. General procedure for the reaction of barbituric acid with arylidine derivatives

A mixture of **1** (2.88 g, 0.02 mol), and either 2-(3,4,5-trimethoxy- benzylidene)malononitrile **4** (4.64 g, 0.02 mol) or ethyl 3-(4-chlorophenyl)-2-cyanoacrylate **7** and triethylamine (0.02 mol) in ethanol (10 mL) was refluxed for 3 h. After cooling, the resulting solid product was collected by filtration, washed with water, and the crude product recrystallized from ethanol to give **6** and **10**, respectively.

*4.1.2.1. 7-Amino-4-oxo-2-thioxo-5-(3,4,5-trimethoxyphenyl)-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidine-6-carbonitrile (**6**).* Yellow crystals: Yield, 85%; m.p. 221–223 °C. IR, cm^{-1}: 3369, 3330, 3251(NH_2,NH), 3189 (CH arom.), 2986 (CH aliph.), 2203(C≡N), 1689(C=O), 1567(C=S), ^{1}H NMR (DMSO-d_6) δ: 3.7(s,9H, 3OCH_3), 4.16 (s, 1H, H-5), 7.19 (s, 2H, NH_2), 7.20–7.35 (m,2H, Ar–H), 11.5, 12.01 ppm (2s, 2H, 2NH pyrimidine). MS, *m*/*z* (%): 388 (M^+) (2.86), 244 (100). Anal. Calcd. For $C_{17}H_{16}N_4O_5S$: C, 52.57; H, 4.15; N, 14.43; S, 8.26. Found: C, 52.87; H, 4.35; N, 14.53; S, 8.46.

*4.1.2.2. Ethyl-7-amino-5-(4-chlorophenyl)-4-oxo-2-thioxo-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidine-6-carboxylate (**10**).* Off white crystals: yield, 88%; m.p. 210–212 °C; IR, cm^{-1}: 3372, 3334, 3245 (NH_2, NH), 3132 (CH arom.), 3091 (CH aliph.), 1756, 1694 (2C=O), 1562 (C=S). ^{1}H NMR (DMSO-d_6) δ: 1.2 (t, 3H, CH_3), 3.4 (s, 1H, H-

5), 4.4 (q, 2H, CH_2), 5.8 (s, 2H, NH_2), 7.4–7.6 (m,4H, Ar–H), 11.6, 12.1 ppm (2s, 2H, 2NH pyrimidine).MS, *m/z* (%): 379 ($M^+-OC_2H_5$) (9.5), 186 (100). Anal. Calcd. For $C_{16}H_{14}ClN_3O_4S$: C, 50.60; H, 3.72; N, 11.06; S, 8.44. Found: C, 50.40; H, 3.42; N, 11.04; S, 8.04.

4.1.3. Ethyl-7-amino-5-(4-chlorophenyl)-2,4-dithioxo-2,3,4,5-tetrahydro-1H-thiopyrano[2,3-d]pyrimidine-6-carboxylate (**11**)

A mixture of **10** (1.8 g, 0.005 mol,) and phosphorus pentasulphide (1.1 g, 0.005 mol) in dry xylene (50 mL) was heated under reflux for 1 h then the solution was concentrated. The product was obtained by filtration and recrystallized from xylene to give yellow crystals **11**: Yield, 75%; m.p. 220–222 °C; IR, cm^{-1}: 3361, 3339, 3219 (NH_2, NH), 3176 (CH arom.), 3101 (CH aliph.), 1769 (C=O), 1567, 1355 (2C=S). ^1H NMR (DMSO-d_6) δ: 1.4 (t, 3H, CH_3), 4.2 (q, 2H, CH_2), 6.2 (s, 2H, NH_2), 7.3–7.8 (m, 4H, Ar–H), 11.8, 12.0 (2s, 2H, NH). MS, *m/z* (%): 411 (M^+-NH_2) (2.13), 144 (100). Anal. Calcd. For $C_{16}H_{14}ClN_3O_2S_3$: C, 46.65; H, 3.43; N, 10.20; S, 23.35. Found: C, 47.05; H, 3.63; N, 10.03; S, 23.65.

4.1.4. 5-(Ethoxymethylene)-2-thioxo-dihydropyrimidine-4,6(1H,5H) dione (**12**)

A mixture of compound **1** (0.7 g, 0.005 mol) and triethyl orthoformate (8 mL) was heated under reflux for 2 h. The obtained solid was crystallized from ethanol to give yellow crystals **12**: Yield, 87%; m.p. 230–232 °C; IR, cm^{-1}: 3322 (NH), 3091, 3008 (CH aliph.), 1690, 1678 (2C=O), 1569 (C=S). ^1H NMR (DMSO-d_6) δ: 1.3 (t, 3H, CH_3), 4.0 (q, 2H, CH_2), 8.0 (s, 1H, C=CH–OC_2H_5), 11.4, 12.3 ppm (2s, 2H, 2NH pyrimidine). Anal. Calcd. For $C_7H_8N_2O_3S$: C, 41.99; H, 4.03; N, 13.99; S, 16.02. Found: C, 41.79; H, 3.83; N, 13.69; S, 15.72.

4.1.5. 5-(Hydrazinylmethylene)-2-thioxo-dihydropyrimidine-4,6(1H,5H)dione (**13**)

A mixture of **12** (1 g, 0.005 mol, 5 mmol) and hydrazine hydrate (0.25 g, 0.005 mol) in EtOH (60 mL) was heated under reflux for 3 h. The solid product was filtered on hot, dried, and crystallized from proper solvent to give yellow crystals **13**: Yield, 78%; m.p. >260 °C; IR, cm^{-1}: 3372, 3380,3205 (NH_2,NH), 3077 (CH aliph.), 1731, 1688 (2C=O), 1575 (C=S). ^1H NMR (DMSO-d_6) δ: 6.5 (s, 2H, NH_2 exchangeable), 7.9 (s, 1H, CH-$NHNH_2$), 8.9 (s, 1H, NH exchangeable), 11.4, 12.3 ppm (2s, 2H, 2NH pyrimidine). MS, *m/z* (%): 186 (M^+) (100). Anal. Calcd. For $C_5H_6N_4O_2S$: C, 32.25; H, 3.25; N, 30.09; S, 17.22. Found: C, 32.05; H, 3.55; N, 30.39; S, 17.02.

4.1.6. 6,8-Diamino-4-oxo-2-thioxo-5-(3,4,5-trimethoxyphenyl)-2,3,4,5-tetrahydro-1H-pyrido [3′,2′:4,5]pyrano[2,3-d]pyrimidine-7-carbonitrile (**15**)

A suspension of **6** (1.94 g, 0.005 mol) in C_2H_5OH (50 mL) containing a catalytic amount of piperidine was treated with malononitrile (0.35 g, 0.005 mol). The reaction mixture was refluxed for 3 h. The separated solid was filtered off on hot and crystallized from dioxane to give off white crystals **15**: Yield, 70%; m.p. 188–190 °C; IR, cm^{-1}: 3378, 3299 (br, NH_2+NH), 3166 (CH arom.), 2906 (CH aliph.), 2198(CN), 1674 (C=O), 1561 (C=S). ^1H NMR (DMSO-d_6) δ: 3.7(s, 9H, $3OCH_3$), 4.2(s, 1H, CH-5 pyrane), 5.8, 6.4 (2s, 4H, NH_2), 6.9–7.8 (m, 2H, Ar–H), 11.3, 12.3 ppm (2s, 2H, 2NH pyrimidine). MS, *m/z* (%): 456 (M) (7.25), 144 (100). Anal. Calcd. For $C_{20}H_{18}N_6O_5S$: C, 52.86; H, 3.99; N, 18.49; S, 7.06. Found: C, 52.46; H, 3.79; N, 18.09; S, 7.36.

4.1.7. N-(6-Cyano-4-oxo-2-thioxo-5-(3,4,5-trimethoxyphenyl)-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidin-7-yl) benzenesulfonamide (**16**)

A mixture of compound **6** (1.94 g, 0.005 mol) and benzene sulfonylchloride (0.8 g, 0.005 mol) in dry benzene (30 mL) was refluxed for 2 h, the reaction mixture was cooled and poured on to ice water, then acidified with dilute HCl, the obtained solid was crystallized from dioxane to give pale yellow powder **16**: Yield, 69%; m.p. 228–230 °C; IR, cm^{-1}: 3270, 3251 (3NH), 3093 (CH arom.), 2940, 2886 (CH aliph.), 2185 (CN), 1677(C=O), 1578 (C=S). ^1H NMR (DMSO-d_6) δ: 3.8 (s, 9H, $3OCH_3$), 4.5 (s, 1H, CH-5 pyrane), 7.0–7.5 (m, 7H, Ar–H), 11.5, 12.2 ppm (2s, 2H, 2NH pyrimidine). MS, *m/z* (%): 528 (M) (2.1), 222 (100). Anal. Calcd. For $C_{23}H_{20}N_4O_7S_2$: C, 52.26; H, 3.81; N, 10.60; S, 12.13. Found: C, 52.36; H, 3.91; N, 10.90; S, 12.23.

4.1.8. 4,6-Dioxo-2-thioxo-5-(3,4,5-trimethoxyphenyl)-2,3,4,5,6,7-sexahydro-1H-pyrimido [2′,3′:4,5]pyrano[2,3-d]pyrimidine (**17**)

A solution of compound **6** (1.94 g, 0.005 mol) in formic acid (10 mL) was heated under reflux for 12 h. The reaction mixture was concentrated in vacuum and the obtained solid was crystallized from ethanol to give yellow powder **17**: Yield, 73%; m.p. 110–112 °C; IR, cm^{-1}: 3293, 3224 (br, NH), 3098 (CH aliph.), 3098 (CH arom.), 1690, 1676 (2C=O), 1620 (C=N), 1562 (C=S). ^1H NMR (DMSO-d_6) δ: 3.6 (s, 9H, $3OCH_3$), 4.1 (s, 1H, CH benzylic), 6.9–7.3 (d, 2H, Ar–H), 7.5 (s, 1H, CH=N), 8.0 (s, 1H, NH exchangeable), 11.8, 12.3 ppm (2s, 2H, 2NH pyrimidine). MS, *m/z* (%): 416(M) (39.71), 180 (100). Anal. Calcd. For $C_{18}H_{16}N_4O_6S$: C, 51.92; H, 3.87; N, 13.45; S, 7.70. Found: C, 51.72; H, 3.67; N, 13.65; S, 7.90.

4.3. Microbiological studies

4.3.1. Antibacterial

Antibacterial activities were investigated using agar well diffusion method. The activity of tested samples was studied against the *S. aureus* (as gram positive bacteria) while *P. aeruginosa* (as gram negative bacteria). Centrifuged pelletes of bacteria from a 24 h old culture containing approximately 10^4–10^6 CFU (colony forming unit) per ml were spread on the surface of Nutrient agar (typetone 1%, yeast extract 0.5%, NaCl 0.5%, agar 1%, 1000 mL of distilled water, PH 7.0) which was autoclaved under 121 °C for at least 20 min. Wells were created in medium with the help of a sterile metallic bores and then cooled down to 45 °C. The activity was determined by measuring the diameter of the inhibition zone (in mm). 100 μl of the tested samples (10 mg/mL) were loaded into the wells of the plates. All compounds was prepared in Dimethyl Sulfoxide (DMSO), DMSO was loaded as control. The plates were kept for incubation at 37 °C for 24 h and then the plates were examined for the formation of zone of inhibition. Each inhibition zone was measured three times by caliper to get an average value. The test was performed three times for each bacterium culture. *Tetracyclin* was used as antibacterial standard drugs [22]. Zones of inhibition were determined for **3, 6, 10, 11, 12, 13, 15**, **16** and **17** and the results are summarized in Table 1.

4.3.2. Antifungal

Newly prepared compounds were screened separately *in vitro* for their antifungal activity against cultures of two fungal species, namely, Fungus, *A. flavus* and one yeast fungus *C. albicans* on *Sabouraud dextrose agar plates*. The culture of fungi was purified by single spore isolation technique. The antifungal activity was detected by agar well diffusion method [23] by the following procedure *Sabouraud dextrose agar plates:* A homogeneous mixture of glucose–peptone–agar (40:10:15) was sterilized by autoclaving at 121 °C for 20 min. The sterilized solution (25 mL) was poured in each sterilized petridish in laminar flow and left for 20 min to form the solidified sabouraud dextrose agar plate. These plates were inverted and kept at 30 °C in incubator to remove the moisture and to check for any contamination.

Antifungal assay: Fungal strain was grown in 5 mL sabouraud dextrose broth (glucose: peptone; 40:10) for 3–4 days to achieve 10^5 CFU/mL cells. The fungal culture (0.1 mL) was spread out

uniformly on the Sabouraud dextrose agar plates by sterilized triangular folded glass rod. Plates were left for 5–10 min so that culture is properly adsorbed on the surface of sabouraud dextrose agar plates. Now small wells of size (4 mm × 2 mm) were cut into the plates with the help of well cutter and bottom of the wells were sealed with 0.8% soft agar to prevent the flow of test sample at the bottom of the well. 100 μl of the tested samples (10 mg/mL) were loaded into the wells of the plates. All compounds was prepared in dimethyl sulfoxide (DMSO), DMSO was loaded as control. The plates were kept for incubation at 30 °C for 3–4 days and then the plates were examined for the formation of zone of inhibition. Each inhibition zone was measured three times by caliper to get an average value. The test was performed three times for each fungus. Amphotericin B was used as references to evaluate the potency of the tested compounds under the same conditions. Zones of inhibition were determined for 3, 6, 10, 11, 12, 13, 15, 16 and 17 and the results are summarized in Table 1.

References

[1] A.M. El-Agrody, M.S. Abd El-Latif, N.A. El-Hady, A.H. Fakery, A.H. Bedair, Molecules 6 (2001) 519–527.
[2] A.H. Bedair, Nagwa A. El-Haddy, M.S. Abd El-Latif, A.H. Fakery, A.M. El-Agrody, lL Farmaco 55 (2000) 708–714.
[3] A.M. El-Agrody, M.H. El-Hakim, M.S. Abd El-Latif, A.H. Fakery, E.M. El-Sayed, K.A. El-Ghareab, Acta Pharm. 50 (2000) 111–120.
[4] R.N. Taylor, A. Cleasby, O. Singh, T. Skarzynski, J. Med. Chem. 41 (1998) 798–807.
[5] K. Hirmoto, A. Nasuhara, K. Michiloshi, T. Kato, K. Kikugawa, Mutat. Res. 395 (1997) 47–56.
[6] A.G. Martinez, L.J. Marco, Bioorg. Med. Chem. Lett. 7 (1997) 3165–3170.
[7] C.P. Dell, C.W. Smith, Eur. Pat. 537, 94, 9, 21 Apr 1993; ref. Chem. Abstr. 119 (1993) 139102d.
[8] G. Bianchi, A. Tava, Agric. Biol. Chem. 51 (1987) 2001–2002.
[9] F. Eiden, F. Denk, Arch. Pharm. Weinheim Ger. 324 (1991) 353–354.
[10] C.J. Shishoo, M.B. Devani, G.V. Ullas, S. Ananthan, V.S. Bahadit, J. Heterocyclic Chem. 18 (1981) 43–46.
[11] K. Noda, A. Nakagawa, Y. Nakajima, H. Ide, Japan. Kokai 7785, 194, 15 Jul 1977;ref. Chem. Abstr. 88 (1978) P50908q.
[12] Z.H. Ismail, M.M. Ghorab, E.M.A. Mohamed, H.M. Aly, M.S.A. El-Gaby, Phosphorus, Sulfur and Silicon 183 (2008) 2541.
[13] M.S.A. El-Gaby, S.M. Abdel-Gawad, M.M. Ghorab, H.I. Heiba, H.M. Aly, Phosphorus, Sulfur and Silicon 181 (2006) 279.
[14] L.P. Prikazchikova, B.M. Khutova, I.F. Vladimirtsev, I.V. Boldyrev, N.I. Zhuravskaya, Fiziol. Akt. Veshchestva 7 (1975) 84 ref. Chem. Abstr. 83:127346m.
[15] D. Brown, in: A.R. Katritzky, C.W. Rees (Eds.), J. Comprehensive Heterocyclic Chemistry, 3, Pergamon Press, Oxford, 1984, p. 443.
[16] H.M. Aly, Phosphorus, Sulfur and Silicon 185 (2010) 211–221.
[17] H.M. Aly, Monatsch. Chem. 142 (2011) 935–941.
[18] H.M. Aly, N.M. Saleh, H.A. Elhady, Eur. J. Med. Chem. 46 (2011) 4566–4572.
[19] J. Hren, F. Požgan, A. Bunič, V.I. Parvulescu, S. Polanc, M. Kočevar, Tetrahedron 65 (2009) 8216–8221.
[20] H.M.F. Madkour, M.R. Mahmoud, M.H. Nassar, M.M. Habashy, Molecules 5 (2000) 746–755.
[21] Y. Jing, W. Hanqing, Synth. Commun. 35 (2005) 3133–3140.
[22] A. Rahman, M.I. Choudhary, W.J. Thomsen, Bioassay Techniques for Drug Development. Harwood Academic Publishers, the Netherlands, 2001, pp. 16–26.
[23] H.S. Rathore, S. Mittal, S. Kumar, Pestic. Res. J. 12 (2000) 103.