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RESEARCH ARTICLE

Utility of a pyrimidine thione derivative in the synthesis of new fused pyrimido[4,5-*d*]pyrimidine, pyrido[2,3-*d*]pyrimidine and different types of thienopyrimidine derivatives

Hala M. Aly*[1, 2] . Nashwa M. Saleh[1]

1. Chemistry Department, Faculty of Science (Girl's), Al-Azhar University, P.O. box 11754, Nasr City, Cairo, Egypt.

2. Chemistry Department, Faculty of Arts and Sciences, Northern Border University, Rafha, Saudi Arabia

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Corresponding Author

Hala M. Aly

Abstract

Thioxodihydropyrimidine-4,6(1*H*,5*H*)dione **1** was exploited as a starting material for the syntheses of fused pyrimidopyrimidine, pyridopyrimidine and different types of thienopyrimidine derivatives such as aminothieno[3,4-*d*] and [3,2-*d*]pyrimidine. The characterization of synthesized compounds was done by analytical and spectral studies. The antimicrobial activity of the target synthesized compounds was tested against various microorganisms by the disc diffusion method. In general, the novel synthesized compounds showed a good antimicrobial activity against microorganisms.

Introduction

Recently, much attention has been paid to the development of new methods for the synthesis of heterocyclic compounds, due to their potential importance in the pharmaceutical and agricultural fields. Pyrimidines and their fused derivatives also constitute a very important class of compounds including natural products, Pharmaceuticals, and functional materials. They are accessible by variety of methods, including classical approaches and novel strategies (Hala *et al* 2011, Matthew *et al* 2008). These observations led us to interest, because they generally show diverse biological properties such as antitumor, analgesic, antibacterial, and fungicidal activities (Hala 2010, Alqasoumi *et al* 2009, Hala 2011, Keri et al 2010, Tirlapur et al 2010). This aroused considerable interest to design and synthesize pyrimidines compounds profound antibacterial and fungicidal activities. A large number of thienopyrimidines was reported in literature as virucides, bactericides, fungicides, acaricides, insecticides (Shishoo et al 2000, Aboulwafa et al 1992) and antimicrobial, anti-inflammatory, anticonvulsant activity (Ashalatha et al 2007). As a continuation of my research program to find out bioactive thienopyrimidines (Hala 2010, Mohamed et al 2006), we aimed at the synthesis and characterization of some new thienopyrimidine derivatives and evaluate these compounds for their various biological activities.

Experimental

General

All melting points 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 ^{13}C NMR (500 MHz) spectra were run in dimethylsulfoxide

(DMSO-d_6). Chemical shifts were related to that of the solvent. 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 Centre, Faculty of Science, Cairo University. Analytical results for C, H, N and S were within ±0.4 of the calculated values.

Chemistry

General procedure for the reaction of 2-thiobarbituric acid with aromatic aldehyde derivatives

A mixture of 2-thioxo-dihydropyrimidine-4,6(1*H*,5*H*)dione (2-thiobarbituric acid) **1** (1.44 g, 0.01 mol), 2-hydroxybenzaldehyde (1.22 g, 0.01 mol) and/or 3-phenylacryl-aldehyde (2.08 g, 0.01 mol), thiourea (0.76 g, 0.01 mol), absolute ethanol (70 ml) and 36% HCl (3 ml) as heated under reflux for an 4 h and the reaction mixture was allowed to cool. The product, which appeared as precipitate, was filtered off and washed with ethanol to give ***3a***, ***3b,*** respectively.

5-(2-Hydroxyphenyl)-2, 7-dithioxo-2, 3, 5, 6, 7, 8-hexahydropyrimido[4, 5-*d*]-pyrimidin-4(1*H*)one (3a)

Off white crystals: Yield, 86%; m.p. 244-246 °C; IR (KBr) ν cm^{-1}: 3423(OH), 3290, 3205 (br, 4NH), 3167 (CH arom.), 1699(C=O), 1570, 1560 (2C=S). ^{1}H NMR (DMSO-d_6) δ_H: 3.2 [d, 1H, H-5], 7.5-7.9[m, 4H, Ar-H], 9.3, 9.6 [2s, 2H, NH], 10.3[s, 1H, OH], 11.7[s, 1H, N_1-H], 12.4 ppm [s, 1H, N_3-H]. MS, m/z (%): 306 [M^+] (57.88), 177 (100). Anal. Calcd. For $C_{12}H_{10}N_4O_2S_2$ (306): C, 47.04%; H, 3.29%; N, 18.29%; S, 20.93%. Found: C, 47.34%; H, 3.49%; N, 18.09%; S, 20.83%.

5-Styryl-2,7-dithioxo-2,3,5,6,7,8-hexahydropyrimido[4,5-*d*]pyrimidin-4(1*H*) one (3b)

Yellow crystals: Yield, 88%; m.p. 260-262 °C; IR (KBr) ν cm^{-1}: 3300, 3297, 3288, 3280 (4NH), 2967(CH aliph.), 3155 (CH arom.), 1765(C=O), 1565, 1555 (2C=S). ^{1}H NMR (DMSO-d_6) δ_H: 3.4 [d, 1H, H-5], 6.3, 6.5[2s, 2H, CH=CH], 7.3-7.8[m, 5H, Ar-H], 9.4 [br, 2H, 2NH], 11.9 [s, 1H, N_1-H], 12.0 ppm [s, 1H, N_3-H]. MS, m/z (%): 316[M^+], 77(100). Anal. Calcd. For $C_{14}H_{12}N_4OS_2$ (316): C, 53.14%; H, 3.82%; N, 17.71%; S, 20.27%. Found: C, 53.34%; H, 3.92%; N, 17.91%; S, 20.47%.

6-Acetyl-5-(2-hydroxyphenyl)-2,7-dithioxo-2,3,5,6,7,8-hexahydropyrimido [4, 5-*d*] pyrimidin-4(1*H*)one (4)

A solution of **3b** (3.92 g, 0.01 mol) was refluxed in Ac_2O (30 ml) for 1 h. The separated product was filtered off and crystallized from C_2H_5OH to give black crystals **4**. Yield, 88%; m.p. 236-238 °C; IR (KBr) ν cm^{-1}: 3280, 3205 (br, 3NH), 3167 (CH arom.), 1735,1688(2C=O), 1570,1558 (2C=S). ^{1}H NMR (DMSO-d_6) δ_H: 2.08 [s, 3H, $COCH_3$], 3.1 [s, 1H, H-5], 6.4, 6.5[2s, 2H, CH=CH], 7.2-8.0[m, 5H, Ar-H], 8.3 [s, 1H, N_8-H], 11.8 [s, 1H, N_1-H], 12.3 ppm [s, 1H, N_3-H]. ^{13}C NMR (DMSO-d_6) δ_H: 174.4, 172.9, 171.7, 162.9, 161.3, 135, 130.1, 128.2, 127.5, 126.8, 123, 85.1, 51.3, 19.9. MS, m/z (%): 358 [M+ 1], 128(100). Anal. Calcd. For $C_{16}H_{14}N_4O_2S_2$ (358): C, 53.61%; H, 3.94%; N, 15.63%; S, 17.89%. Found: C, 53.31%; H, 3.74%; N, 15.43%; S, 17.49 %

6-(2,3-Dimethyl-5-oxo-1-phenyl-2,5-dihydro-1*H*-pyrazol-4-ylamino)-2-thioxo-2,3-dihydropyrimidin-4(1*H*)one (6)

A mixture of 2-thioxo-dihydropyrimidine-4,6(1*H*,5*H*)dione **1** (1.44 g, 0.01 mol) and 4-amino-1,5-dimethyl-2-phenyl-1,2-dihydro-pyrazol-3-one **5** (2.03 g, 0.01 mol) in ethanol (50 ml) was refluxed for 5 h. The reaction mixture was cooled and then poured onto cold water; the obtained solid was crystallized from ethanol to give white powder **6**. Yield, 90%; m.p. 222–224 °C; IR (KBr) ν cm^{-1}: 3254, 3233, 2210 (3NH), 3032 (CH arom.), 2940, 2870 (CH aliph.), 1690, 1667 (2C=O), 1588 (C=S). ^{1}H NMR (DMSO-d_6) δ_H: 2.3, 3.3 [2s, 6H, $2CH_3$], 5.3 [s, 1H, CH-5 pyrimidine], 7.0–7.6 [m, 5H, Ar-H], 9.0 [s, 1H, NH, D_2O-exchangable], 11.8 [s, 1H, N_1-H], 12.3 ppm [s, 1H, N_3-H]. MS, m/z (%): 329[M- 1], 175(100). Anal. Calcd. For $C_{15}H_{15}N_5O_2S$ (329): C, 54.70%; H, 4.59%; N, 21.26%; S, 9.74%. Found: C, 54.30%; H, 4.39%; N, 21.16%; S, 9.54%

7-Amino-5-(2-chlorophenyl)-8-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-4-oxo-2-thioxo-1,2,3,4,5,8-hexahydropyrido[2,3-*d*]pyrimidine-6-carbonitrile (9)

A mixture of enaminone **6** (3.29 g, 0.01 mol) and 2-(2-chloro-benzylidene) malononitrile **7** (1.88 g, 0.01 mol) in ethanol (50 ml) containing 3 drops of pipridine, was refluxed for 5 h. The reaction mixture was filtered while hot and the solid obtained was crystallized from dioxane to give yellow crystals **9**. Yield, 94%; m.p. 195-197 °C; IR (KBr) ν cm^{-1}:3344, 3337, 3210 (NH_2, NH), 3074 (CH arom.), 2959, 2887 (CH aliph.), 2171 (C≡N), 1724, 1699 (2C=O), 1595 (C=S), 705 (C–Cl). ^{1}H NMR (DMSO-d_6) δ_H: 2.4–3.1 [2s, 6H, $2CH_3$], 4.9 [s, 1H, CH-5 pyrimidine], 6.4 [s, 2H, NH_2, D_2O-exchangable], 7.2–8.5 [m, 9H, Ar-H], 11.9 [s, 1H, N_1-H], 12.2 ppm [s, 1H, N_3-H]. ^{13}C NMR (DMSO-d_6) δ_H: 171.6, 162.7, 160.4, 160.7, 154.1, 144.1, 136.9, 133.9, 131, 129.8, 129.1, 128.4, 126.8, 127.4, 120, 118.4, 113.8, 105.2, 81.5, 57.2, 38.3, 33.9, 14.3. MS, m/z (%): 517 [M-1] (55.7), 51 (100). Anal. Calcd. For

$C_{25}H_{20}ClN_7O_2S$ (517): C, 57.97%; H, 3.89%; N, 18.93%; S, 6.19% Found: C, 57.77%; H, 3.49%; N, 18.63%; S, 6.09%

Ethyl 4-oxo-5-(phenylamino)-2-thioxo-1,2,3,4-tetrahydrothieno[3,4-*d*]- pyrimidine-7-carboxylate (10)
A mixture of equivalent amount from 1,3-diketone **1** (1.44 g, 0.01 mol) in DMF (60 mL) and dried potassium carbonate (0.01mol) was added and the mixture was stirred for 1 h at room temperature. Phenyl isothiocyanate (1.35 g, 0.01 mol,1 equiv.) was then added dropwise and the mixture was stirred for 2 h at room temperature before adding ethyl bromoacetate (1.65 g, 0.01 mol) and dried potassium carbonate. The reaction was quenched with 100 mL of water after having stirred for 4 h at room temperature. The crude product precipitated and was purified by filtration followed by crystallization in ethanol to give pale yellow crystals **10**. Yield, 74%; m.p. 246-247 °C; IR (KBr) ν cm^{-1}: 3298, 3267 (br, 3NH), 3081 (CH arom.), 2929, 2891 (CH aliph.), 1768, 1684 (2C=O), 1579 (C=S). ^{1}H NMR (DMSO-d_6) δ_H: 1.14 (t, 3H, CH_3), 4.03 (q, 2H, CH_2), 7.5–8.4 [m, 6H, Ar-H+ NH], 11.8 [s, 1H, N_1-H], 12.3 ppm [s, 1H, N_3-H]. ^{13}C NMR (DMSO-d_6) δ_H: 171, 166.5, 161.7, 160.1, 144.1, 131.3, 128.9, 121.0, 118.3, 116.8, 104.1, 60.2, 14.7. MS, m/z (%):347 [M+2] (3.7), 148 (100). Anal. Calcd. For $C_{15}H_{13}N_3O_3S_2$ (347): C, 51.86%; H, 3.77%; N, 12.10%; S, 18.46%. Found: C, 51.96%; H, 3.87%; N, 12.50%; S, 18.06%

General procedure for synthesis of 6-aminothieno[3,2-d]pyrimidine derivatives (12a,b)
A mixture of 1,3-diketone **1**(1.44 g, 0.01 mol), activated nitrile**11a**.**b** (0.66 g, 0.01mol), sulfur (0.011mol), ethanol (50 ml) and piperidine were stirred on a water bath for 3h at 40-50°C., the reaction temperature should not generally exceed 60°C. The separated solid was filtered, washed with ethanol, dried and crystallized from a suitable solvent (ethanol) to give **12a, b**

6-Amino-4-oxo-2-thioxo-1,2,3,4-tetrahydrothieno[3,2-*d*]pyrimidine-7-carbonitrile (12a)
Yield, 94%; m.p. 236-238 °C; IR (KBr) ν cm^{-1}: 3338, 3325, 3288 (NH, NH_2), 2198 (C≡N), 1701 (C=O), 1582 (C=S). MS, m/z (%): 223 [M] (9.4), 69(100). Anal. Calcd. For $C_7H_4N_4OS_2$ (223): C, 37.49 %; H, 1.80 %; N, 24.98 %; S, 28.60%. Found: C, 37.29%; H, 1.70%; N, 24.68%; S, 28.30%

Ethyl 6-amino-4-oxo-2-thioxo-1,2,3,4-tetrahydrothieno[3,2-*d*] pyrimidine-7-carboxylate (12b)
Yield, 65%; m.p.247-249 °C; IR (KBr) ν cm^{-1}: 3318, 3300, 3273 (NH, NH_2), 1777, 1695 (2C=O), 1576 (C=S). ^{1}H NMR (DMSO-d_6) δ_H: 1.6 (t, 3H, CH_3), 4.3 (q, 2H, CH_2), 7.8 [s, 1H, NH_2], 11.0 [s, 1H, N_1-H], 12.0 ppm [s, 1H, N_3-H]. MS, m/z (%): 271 [M] (13.0), 145 (100). Anal. Calcd. For $C_9H_9N_3O_3S_2$: C, 39.84 %; H, 3.34 %; N, 15.49 %; S, 23.64 %. Found: C, 39.44 %; H, 3.14 %; N, 15.09 %; S, 23.24 %

Microbiological studies

Antimicrobial assay

Materials and methods

Cultures of two fungal species, namely, *Aspergillus flavus* and one yeast fungus; *Candida albicans*, as well as two bacterial species, namely, gram negative bacteria; *Pseudomonas aeruginosa*, gram positive bacteria; *Staphylococcus aureus* which were used to investigate the antimicrobial activity of the newly synthesized compounds. The antimicrobial activity was biologically assayed using the diffusion plate technique. The used medium for growing bacteria was universal nutrient agar while Sabouraud and/or yeast malt extract agar were used for fungi (Srinivasan et al 2001). Briefly, the latter technique involved pouring a spore suspension of the fungal species (1 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. Solutions of the newly synthesized compounds **3a-12b** (1.0 mg/mL) dissolve in dimethyl formamide. They were 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 3h at 4°C prior to incubation at appropriate temperature for bacteria or fungi (Hala et al 2012). Growth of the tested organisms 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 *Tetracyclin* and the bactericide *Amphotericin B* 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.

Results and Discussion

Chemistry

Nitrogen or sulfur-containing heterocycles have received a great deal of interest in the medicinal, agricultural, and material sciences and this justifies continuing efforts in the development of new efficient and mild synthetic strategies (Renslo et al 1998). In this investigation, a series of new pyrimidine thione, *N*-acetyl pyrimidine, *N*-pyrazolodihydropyridine and thiophene derivatives attached to thioxopyrimidine moiety, **3a, b – 12a, b** were designed, synthesized (**Figure**s **1**–**5**) and biologically evaluated for their in vitro antimicrobial activity. Thus, pyrimido[4,5-*d*]pyrimidine derivatives **3a,b** were synthesized by acid catalyzed condensation of 2-thioxodihydro-pyrimidine-4,6(1*H*,5*H*)dione **1**, aromatic aldehydes and thiourea in ethanol, by a modification of the Biginelli reaction (Sarac et al 1997) **(**Figure 1).The structures of **3a,b** were confirmed by spectral data and elemental analysis. The IR spectrum of compound **3a** showed absorption bands at 3423 (OH), 3290, 3205 cm^{-1} (br, 4NH (in pyrimidine ring), 1699 (C=O), 1570, 1560 cm-1 (2C=S). The 1H NMR spectrum of **3a** displayed a doublet signals at δ 3.2 characteristics for CH-5 and five singlet signals at δ 12.4, 11.7, 9.6, 9.3 and 10.3 characteristic for 4NH pyrimidines and OH group, in addition to multiples at δ 7.5–7.9 due to four aromatic protons. The mass spectrum of 3a exhibited a molecular ion peak at m/z 306 (57.82%) together with a base peak at m/z 177.

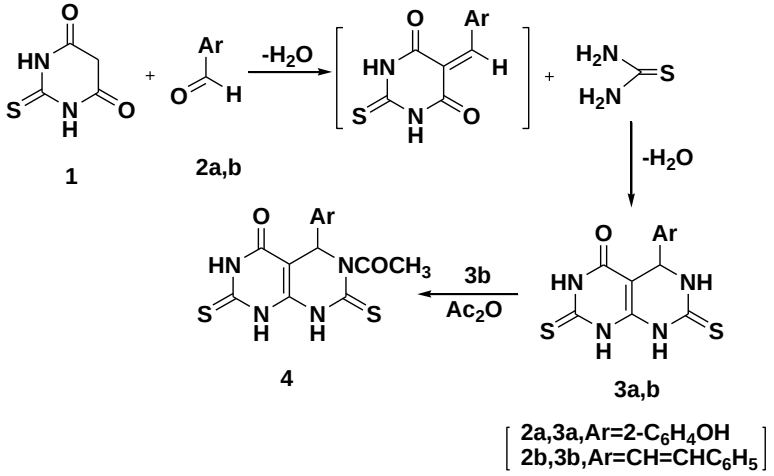

Fig.1. Synthetic pathways for compounds 3a,b and 4.

On the other hand, the ^{1}H NMR spectrum of compound (**3b**; DMSO-d_6) recorded the CH-5 proton in the ^{1}H NMR spectrum exhibited signal at δ 3.4 ppm region doublets. Also, two downfield one proton singlet at δ 12.0 and 11.9 ppm regions were assigned to the N3–H and N1–H protons and broad signal at δ 9.4 for 2NH of the pyrimido[4,5-*d*]pyrimidine derivative structures. Surprisingly, heating under refluxing compound **3b** with acetic anhydride leads to the formation of corresponding 3-acetyl derivative **4**. The site of acetylating in **4** was supported by ^{1}H NMR spectrum, it recorded a signal at δ 12.3, 11.8 and 8.4 ppm in deuterated dimethylsulfoxide which could be assigned to 3NH and the signal for CH-5 proton collapsed from a doublet in compound **3b** to a singlet in compound **4.**

Enaminones are versatile reagents for the synthesis of quinoline and pyrimidine derivatives (Mohamed et al 2006, Goerdeler et al 1963 and Pulakiyoti et al 1990). As a part of program directed towards the synthesis of new suitably functionalized pyrimidines with higher potential biological activity. We reported here the possible utility of 6-(2,3-dimethyl-5-oxo-1-phenyl-2,5-dihydro-1*H*-pyrazol-4-ylamino)-2-thioxo-2,3-dihydropyrimidin-4(1*H*)one (enaminone) **6** with its bulky *N*-substituted pyrazolone moiety in the synthesis of *N*-pyrazolopyridopyrimidine systems. Enaminone **6** was obtained from condensation of 2-thiobarbituric acid **1** with 4-aminoantipyrine **5** (Soad et al 2005). The derivatives of pyridopyrimidines have been the focus of great interest over many years. This is due to the wide range of biological activities associated with this heterocyclic scaffold (Qingyun et al 2007, Behalo 2009). Therefore, treatment of enaminone **6** with cinnamonitriles **7**, in ethanol containing a catalytic amount of piperidine, resulted in cycloaddition affording the pyrido[2,3-*d*]pyrimidine carbonitrile derivative **9** presumably via Michael type product **8** (Soad et al 2005). The structure of the enaminone **6** was established by elemental analyses and spectral data. IR spectrum of compound **6** showed the presence of the characteristic bands for (3NH), and (2C=O). Also, the ^{1}H NMR spectrum indicated the presence of a singlet at δ 9.0 ppm which could be assigned to NH of

enaminone **6**. IR spectrum of compound **9** exhibited bands for (NH_2), (2C=O), (C=S) in addition to the carbonitrile band. The mass spectrum of **9** revealed a molecular ion peak m/z at 517 [M-1] (55.7), with a base peak at 51 (100). (**Figure 2**)

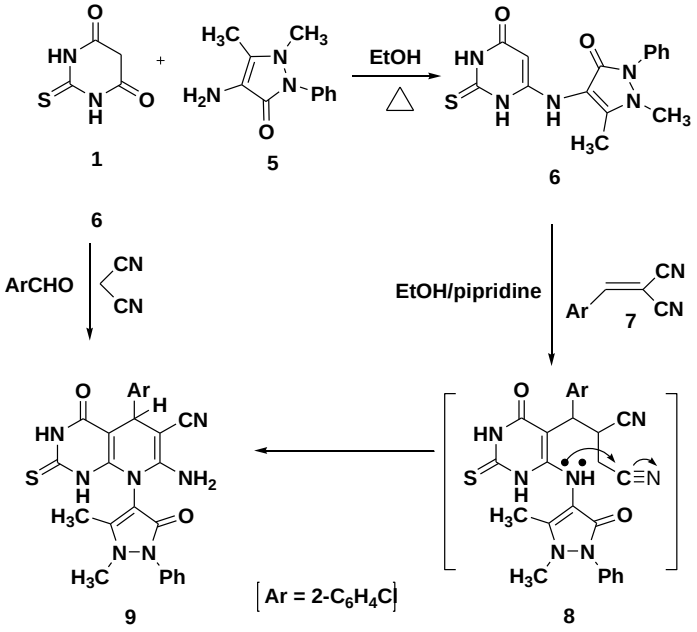

Fig.2. Synthetic pathways for compounds 6 and 9.

Sulfur-containing derivatives are important in the pyrimidine chemistry because their different reactions make them convenient intermediates. Among these reactions, the alkylation of mercaptopyrimidines is a very useful synthetic procedure for the pyrimidine ring functionalization (Aly et al 2007). Generally, the described synthetic access required appropriate thiophene that obtained after multi-step synthesis. Due to the interesting biological properties of thienopyrimidines, we decided to develop new preparation methods based on the use of methylene active compound and isothiocyanate. The first step is the condensation of activated methylene compound with alkyl or aryl isothiocyanates in a basic medium, which is well documented in the literature (Sommen et al 2003). Potassium carbonate was used as the base in order to obtain the intermediate ketene aminothioacetal. The addition of equivalents of alkyl bromoacetate leads only to the thiophenes **10** in moderate to good yields (**Figure 3**). Condensation of the intermediate salt ketene-*N*,*S*-acetal with the halide currently leads to the corresponding aminothioacetal which smoothly undergoes a Dieckmann or Thorpe–Ziegler cyclisation in basic medium at room temperature. Beside the correct values in elemental analyses, the IR, ^{1}H NMR, and mass spectra of **10** are in agreement with the assigned structure, (See Experimental Section).

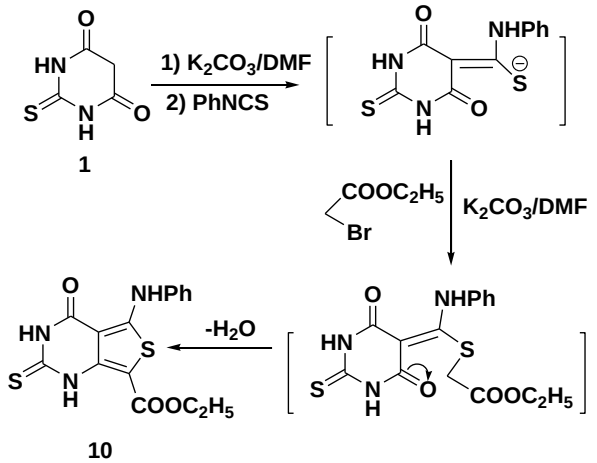

Fig.3. Synthetic pathway for compound 10.

The reactivity of compound **1** towards some nucleophilic reagents was also studied. Gewald *et al*. devised the most facile and promising set of synthetic routes leading to 2-aminothiophene (Gewald et al 1965). Gewald's synthesis of 6-aminothieno[3,2-*d*] pyrimidine derivative **12a,b** reinvestigated by treatment of 2-thiobarbituric acid **1** with an active nitrile **11a,b** bearing an electron withdrawing groups such as ethyl cyanoacetate and/or malononitrile in ethanol in the presence of a basic catalyst such as piperidine at 50 °C (**Figure 4**). The formation of **12a,b** is assumed to proceed via Gewald reaction that an activated nitrile first condenses with a ketone yielding a Knoevenagel-Cope condensation product which is then thiolated at the methylene function group with elemental sulfur followed by ring closure (**Figure 5**)

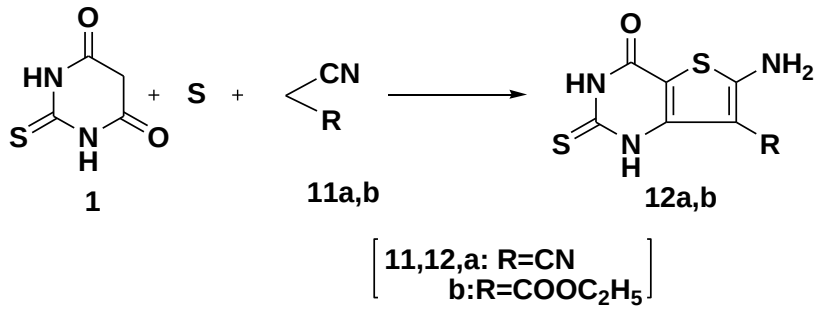

Fig 4. Synthetic pathways for compounds 12a, b.

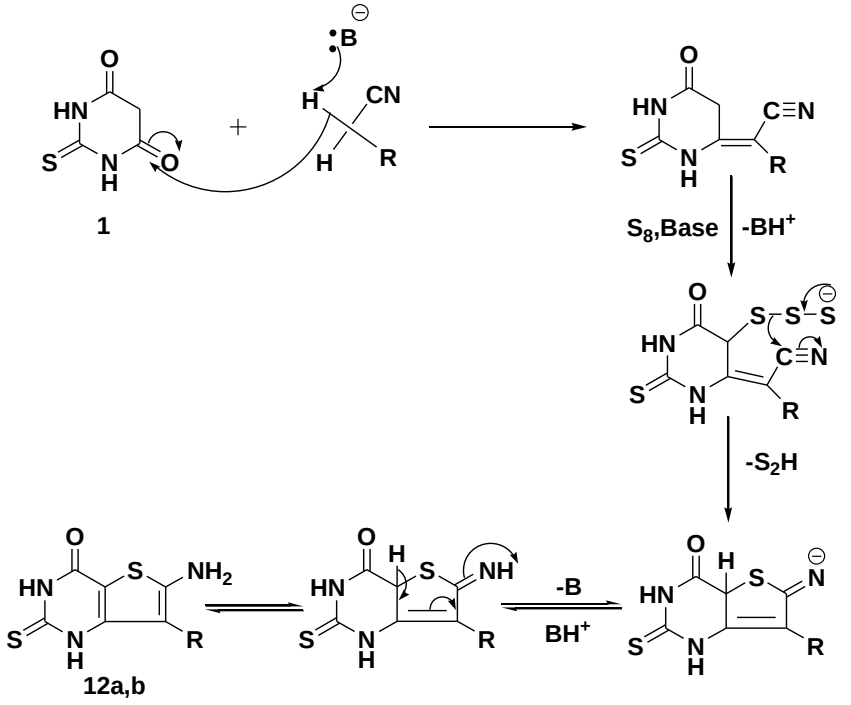

Fig 5. Postulated mechanism for the formation of compounds 12a, b.

Biological screening

Antimicrobial activity

The most important part of the results that were obtained from antimicrobial activities of synthesized compounds that screened against two fungal species, namely *Aspergillus flavus* and *Candida albicans* as well as two bacteria species, namely *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The antimicrobial activity was biologically

assayed using the diffusion plate technique. The investigation of antibacterial and antifungal screening data revealed that all the tested compounds **3a, 3b, 4, 6, 9, 10, 12a** and **12b** showed comparatively good activity against all the bacterial strains. The organisms were tested against the activity of solutions with concentration of 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 results were depicted in Table **1**. The good activity is attributed to the presence of pharmacologically active 2-hydoxy, 2-chloro groups attached to phenyl ring of the pyrimidine ring and $-NCOCH_3$ groups attached to pyrimidine moity. It is worth mentioning that incorporation of antipyrine to the pyridopyrimidine nucleus at position 8 via an aryilidine produced a high antimicrobial activity. Conversion of 2-thiobarbituric acid **1** to thieno[3,4-*d*]pyrimidine derivative **10** enhanced the antimicrobial activity. On other hand, incorporation of the thiophene nucleus to pyrimidine at position 4 and 5 in **12a, b** unfortunately produced strong antimicrobial activity. In conclusion, we reported herein a simple and convenient route for the synthesis of some new heterocycles based on thiobarbituric for antimicrobial evaluation. Most of the compounds were effective against *Candida albicans*. In comparison the tested synthesized compounds **3a, 3b, 4, 9, 10,12a** and **12b** with *Amphotericin B*; compound **4** showed high activity while **6** has no activity. Among these eight compounds, the most effective compounds against *Aspergillus flavus* were **3b, 4, 12a** and **12b**, which include pyrimidine and thiophen moieties while the other effective compounds do not have. However, it was also observed that the substations on the pyrane derivatives had no determining influence on the antifungal activity. All the other compounds exhibited no activity against the tested species (Table 1).

Table 1. Antimicrobial activity of chemical substances tested

Compound No.	Inhibition zone diameter (mm/gm sample)			
	Antibacterial		Antifungal	
	Pseudomonas aeruginosa	***Staphylococcus aureus***	***Aspergillus flavus***	***Candida albicans***
3a	17	19	0	13
3b	17	17	15	17
4	18	18	15	18
6	13	14	0	0
9	12	12	0	10
10	13	12	0	10
12a	14	14	13	14
12b	13	14	14	13
Tetracyclin	28	26	-	-
Amphotericin B	-	-	16	19

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

Conclusion

The research study reported the successful synthesis and antimicrobial activity of new pyrimidine thione, pyridine and thiophene derivatives bearing 2-thiobarbituric acid moiety. The antimicrobial activity study revealed that all the tested compounds showed moderate to good antibacterial activity whereas some of them had antifungal activities against pathogenic strains. Structure and biological activity relationship of new compounds was studied. The title compounds showed that the presence of pyrimidine moiety and biologically active groups like 2-hydoxy and 2-chloro groups that attached to phenyl ring of the pyrimidine thione, pyridine ring respectively and thiophene moiety attached to 2-thiobarbituric acid moiety are responsible for the highly antimicrobial activity. It is conclude that, the entire tested compounds are more active towards bacteria than some fungi.

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