Microwave-Assisted Synthesis of Tetrahydropyrimidines *via* Multicomponent Reactions and Evaluation of Biological Activities

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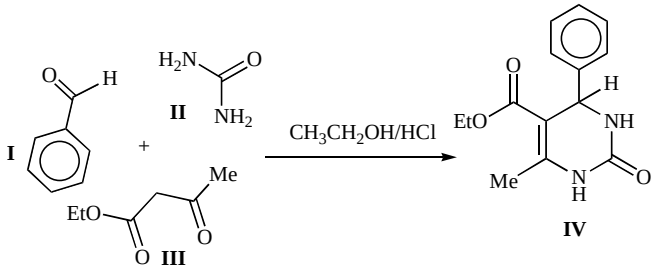
Abstract: Various pyrimidine derivatives (**1a-c**) were synthesized using the Biginelli three component cyclocondensation reactions of a β-diketone, arylaldehyde, and thiourea, under microwave irradiation and conventional conditions. The acetylation of compound **1a** gave 3-acetyl thioxopyrimidin **2**. Also, thiazolopyrimidine (**3**) and (**4**) derivatives were obtained by a simple one-pot condensation reaction of starting compounds **1a,** and **1b** with 2-bromopropionic acid. The prepared compounds were screened for their antimicrobial activities against Gram-positive, Gram-negative bacteria and fungi using micro dilution procedure. The results of the study showed that the compound **4** was effective and selective for antimicrobial activity against the tested bacteria and fungi. Thus, we suggested that the compound **4** might be a new potential antimicrobial substance for bacteria and fungi.

Keywords: Antimicrobial substances, microwave-assisted, multicomponent reactions.

INTRODUCTION

Recently, there has been focused interest in the multicomponent reactions of β-diketone, arylaldehyde, and (thio)urea under Brönsted acid catalysis that was firstly reported by Pietro Biginelli in 1893. This is very simple one-pot, acid catalyzed cyclocondensation reaction of benzaldehyde (**I**), urea (**II**) and ethyl acetoacetate (**III**). The reaction was carried out in ethanol with a few drops of concentrated hydrochloric acid and finalized 3,4-dihydropyrimidine-2(1*H*)-one (**IV**) (Scheme **1**) [1, 2].



Scheme 1.

The reaction products are dihydropyrimidine derivatives, which display various types of biological activities [3] such as anticancer [4], antifilarial [5], antihypertensive [6], anti-inflammatory [7] and as modulators for the transport of calcium channel blocking activity [8].

In the last few years, there has been an increasing interest in the use of microwave heating in organic synthesis [9-13] and it forms now the basis of a number of commercial systems. Some interesting features of this method are the rapid reaction rates, simplicity, solvent-free conditions, and the case of work-up after the reaction. Also, microwave irradiation generates rapid intense heating of polar substances, which result in the reduction of reaction time compared to conventional heating [14-17].

So far, our studies have been continuing on the synthesis of pyrimidine derivatives by using conventional heating and microwave irradiation. The purpose of the study is to extend the Biginelli reactions to synthesize some pyrimidine derivatives. Preparation of pyrimidine derivatives with microwave irradiation are simple and efficient route.

RESULTS AND DISCUSSION

In the present study, we achieved the synthesis of the pyrimidine derivatives *via* conventional heating as well as microwave irradiation experiments. The reactions **1a-c** were performed with 1,3-diphenyl-1,3-propanedione, thiourea and terephthalaldehyde (**1a**), 2-hydroxy-5-methylisophthalaldehyde (**1b**), 4-carboxybenzaldehyde (**1c**), respectively in glacial acetic acid containing a few drops of concentrated hydrochloric acid. The mixture was heated under reflux condition for 8 h. The reaction was finalized in good yields (60-81%) (Scheme **2**).

When 1,3-diketone, aryl aldehyde, thiourea and a few drops of concentrated HCl were reacted in glacial acetic acid under microwave irradiation for 15 min. the same final products (**1a-c**) were obtained.

The IR, ^{1}H and ^{13}C NMR spectra of the isolated compounds **1a-c** confirm the expected structures. The IR spectra of the thioxopyrimidine compounds (**1a-c**) displayed

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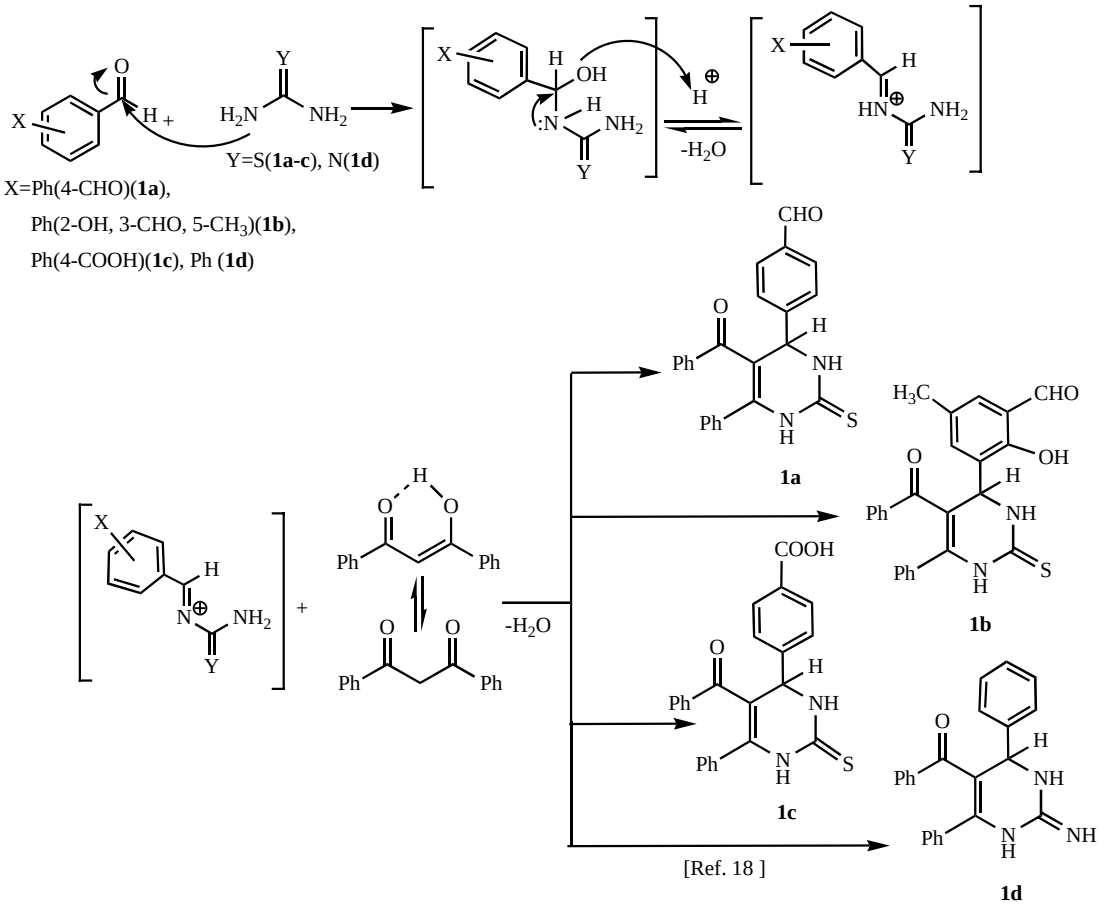


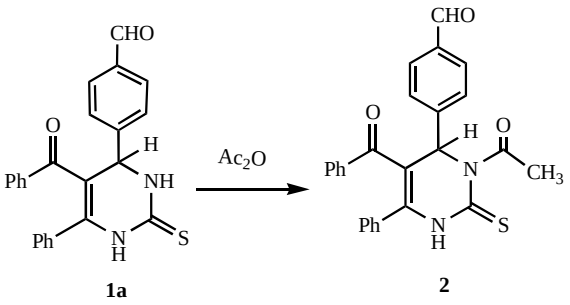
Scheme 2.

absorption bands characteristic for the NH (3200-3096 cm^{-1}), CHO (1707-1652 cm^{-1}), C=O (1692-1600 cm^{-1}) and C=S (1292-1273 cm^{-1}) functions.

In ^1H NMR spectra, the formation of the thioxopyrimidine ring in this reaction was clearly demonstrated by the fact that the C_4 methine proton of compounds **1a-c** appeared at 5.3-5.7 ppm as doublet. The signals of the N_3H and N_1H protons of compounds **1a-c** appeared on one proton singlets at 10.4, 9.8; 10.4, 9.4 and 10.7, 9.9 ppm, respectively. The signals of the other protons were appeared at the expected chemical shifts and integral values.

The 3-acetyl thioxopyrimidine derivative **2** was prepared *via* reaction of compound **1a** with acetic anhydride (Scheme **3**). ^1H NMR spectra of the compounds **2** revealed the absence of N_3H signals instead they exhibited three proton singlet at 2.9 ppm assigned for the N_3COCH_3 protons of the acetyl group.

On the other hand, the reactions of thioxopyrimidine derivatives having the NH and C=S group in the suitable position with various bromo compounds were convenient methods to build the thiazolopyrimidine and pyrimido[2,3-b]thiazine derivatives. Thus, the compound **1a,b** were cyclized with 2-bromopropionic acid to the thiazolopyrimidine (**3**) and (**4**) derivatives (Scheme **1**), in approximately 10% and 31% yields (Scheme **4**).

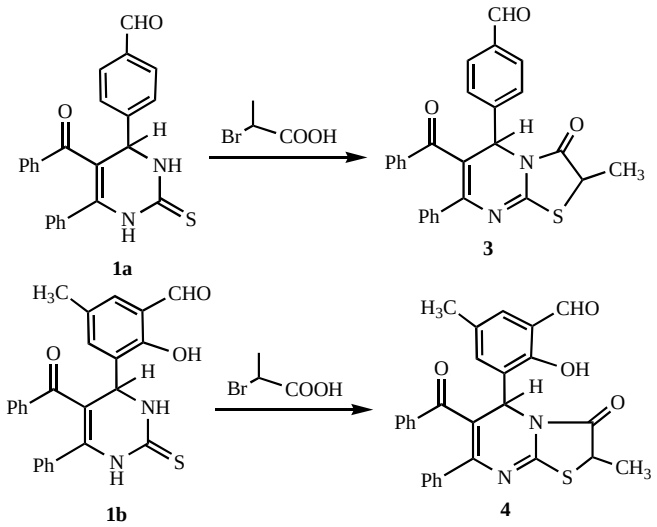


Scheme 3.

The absorptions at 3446-3200 cm^{-1} disappeared in the IR spectra of compounds **3** and **4**. The characteristic absorption of N_3H group of starting compounds is good evidence of the expected reactions. The ^1H NMR data of the compounds **3** and **4** are in accord with the expected reaction products (See experimental for details).

The compounds **1a-c, 2-4** were also prepared under microwave irradiation. In that way, we isolated the corresponding thioxopyrimidine derivatives in good yields within a few minutes. The reaction times are shortened from 480 min to 15 min (Table **1**).

All the prepared chemical compounds were screened *in vitro* for their antibacterial activity against 4 Gram-positive (*S. aureus* ATCC 6538, *S. aureus* ATCC 25923, *B. cereus*



Scheme 4.

ATCC 7064 and *M. luteus* ATCC 9345), one Gram-negative (*E. coli* ATCC 4230) bacteria, and 3 yeast (*C. albicans* ATCC 14053, *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019). Gram-positive and Gram-negative bacterial strains tested MIC values 20-40 μg mL^{-1}. However, the compound **4** was much less active against the tested organisms, compared with the standard drug (Table **2**).

The antifungal activities of the newly synthesized compounds against 3 yeast strains (*Candida albicans* ATCC 14053, *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019) were given in Table **3**. The MIC values calculated toward fungal strains revealed that the compounds **1a**, **1c** and **4** had effective and selective antifungal activity with MICs in the range of 40-80 μg mL^{-1}, while **1b**, **1d**, **2** and **3** compounds displayed poor activity. The compound **4** was the most effective compound against tested yeast strains (MIC values 40-80 μg mL^{-1}) (Table **3**).

A number of studies reported that the various chemical compounds containing pyrimidine ring and their derivatives had higher activity against microorganisms [19-22]. The data obtained from the study revealed that compound **4** displayed effective and selective antibacterial activity against the tested Gram-positive, Gram-negative bacteria and *Candida* strains. However, compound **4** showed high biological activity against the tested Gram-positive bacteria comparing to the Gram-negative bacterium (*E. coli* ATCC 4230) and the fungal strains. Here, we proposed that the reason for this low efficiency toward the Gram-negative bacteria and yeast strains might be related to rigid structure of cell wall of fungi. The composition of the presence of an extra outer membrane is acting as a barrier for foreign substances in the gram-negative cell wall, such as antibiotics and other antimicrobial drugs [23].

CONCLUSION

In conclusion, we have described easy and efficient yielding preparation for thioxopyrimidines by conventional

Table 1. Comparative Study of the Synthesis of 1a-c, 1d [Ref.18], 2- 4

	Yield (%)			Reaction time/min	
Compound	**MW**	**CONV.**	**Medium**	**MW**	**CONV.**
1a	65	62	Glacial acetic acid	15	480
1b	58	60	Glacial acetic acid	15	480
1c	76	81	Glacial acetic acid	15	480
1d	Ref. [18]				
2	46	44	Acetic anhydride/THF	15	60
3	15	10	1,4-Dioxane	5	180
4	34	31	1,4-Dioxane	15	180

Table 2. MICs of the Compounds 1a-d, 2- 4 Derivatives Against Gram-Negative and Gram-Positive Bacterial Strains

Compounds	***Bacillus cereus* ATCC 7064**	***Staphylococcus aureus* ATCC 6538**	***Staphylococcus aureus* ATCC 25923**	***Escherichia coli* ATCC 4230**	***Micrococcus luteus* ATCC 9345**
1a	80	80	80	80	40
1b	640	640	640	640	320
1c	80	40	40	80	40
1d	320	320	320	640	320
2	640	640	320	320	80
3	320	160	160	640	160
4	40	20	20	40	20
Ampicillin	5	5	10	20	10

*The MICs values were determined as μg ml^{-1} active compounds in medium.

Table 3. MICs of the Compounds 1a-d, 2- 4 Derivatives Against Yeast Strains

Compounds	*Candida albicans* ATCC 14053	*Candida parapsilosis* ATCC 22019	*Candida krusei* ATCC 6258
1a	80	80	40
1b	160	160	320
1c	80	40	40
1d	320	160	160
2	160	160	160
3	160	160	160
4	40	40	40
Fluconazole	5	5	10

*The MICs values were determined as μg ml^{-1} active compounds in medium.

heating and microwave irradiation *via* Biginelli three-component cyclocondensation reaction. Our investigations are continuing on this subject and the results will be published when our studies are completed. The new synthesized chemical compounds containing pyrimidine ring and their derivatives were screened invitro for the antibacterial and the antifungal activities against some bacteria and fungi. The results of the study showed that the compound **4** was effective and selective for antimicrobial activity against the tested bacteria and fungi. In recently, multi-drug resistant microorganisms pose a serious challenge to the medical community therefore there is an urgent need to develop new compounds. Thus, we think that the compound **4** could be a new potential antimicrobial substance toward bacteria and fungi.

GENERAL EXPERİMENTAL PROCEDURE

A mixture of 1,3-dione (1.6 mmol), arylaldehyde (1.1 mmol), urea derivative (1.1 mmol) and 20 ml of glacial acetic acid containing 0.2 ml concentrated hydrochloric acid was heated under reflux for 8 h.

Data for the Compounds

Compound 1a

mp 205-206ºC (benzyl alcohol). IR (KBr): 3200 cm^{-1} (NH), 1707 cm^{-1} (CHO), 1604 (C=O) and 1288 cm^{-1} (C=S). ^{1}H-NMR (400MHz, DMSO-d_6): δ 11.8 (s, 1H, CHO), 10.4 (s, 1H, NH), 9.8 (s, 1H, NH), 7.4-6.9 (m, 14H, Harom.), 5.3 (d, *J*= 3.6 *Hz*, 1H, C4-CH). ^{13}C NMR (DMSO-d_6): δ 195.4 (C=O, benzoyl), 175.8 (C=S), 172.6 (C=O, CHO), 145.9, 143.0, 142.9, 139.6, 133.0, 131.6, 130.6, 130.5, 129.1, 128.3, 128.1, 127.5, 127.4, 110.5, 55.8 ppm. MS (100eV): *m/e*: 255.0, 265.0, 279.0, 284.1, 297.0, 305.2, 311.1, 314.0, 319.0, 325.1, 329.0, 339.1, 347.0, 349.2, 353.0, 357.1, 363.2, 369.1, 374.1, 383.1, 389.1, 399.1(M+1). Anal. Calcd. for $C_{24}H_{18}N_2O_2S$ (398): C, 72.34; H, 4.55; N, 7.03. Found: C, 72.54; H, 4.52; N, 7.01.

Compound 1b

mp 245-246ºC (butanol). IR (KBr) 3443 (OH), 3208 (NH), 1652 (CHO), 1600 (C=O), 1273 cm^{-1}(C=S). ^{1}H-NMR (400MHz, DMSO-d_6) δ 11.1 (s,1H, OH), 10.4 (s, 1H, NH), 9.9 (s, 1H, CHO), 9.4 (s, 1H, NH), 7.5-7.0(m, 12H, Harom.), 5.7(d, *J*= 3.2*Hz*, 1H, C4-CH), 2.3 ppm (s, 3H, CH_3). ^{13}C-NMR (DMSO-d_6) δ 197.3(C=O, benzoyl), 195.2 (C=O, CHO), 175.7(C=S), 156.6, 144.3, 139.2, 136.6, 133.2, 131.9, 130.9, 130.3, 130.0, 129.5, 129.2, 129.1, 128.4, 128.2, 122.0, 109.7, 51.7, 20.7 ppm. Anal. Calc. for Anal. Calc. for $C_{25}H_{20}N_2O_3S$(428): C, 70.07; H, 4.70; N, 6.54. Found: C, 71.00; H, 4.72; N, 6.56.

Compound 1c

mp 270-271ºC (acetic acide), IR (KBr) 3283 (OH), 3182-3096 (NH), 1692(C=O, acid), 1649 (C=O, benzoyl), 1292 cm^{-1} (C=S). ^{1}H-NMR (400MHz, DMSO-d_6) δ 12.3 (s, 1H, OH), 10.7 (s, 1H, N_3H), 9.9 (s, 1H, N_2H), 7.9-6.9 (m, 14H, Harom.), 5.4 (d, *J*= 3.6*Hz*, 1H, C4-CH). ^{13}C-NMR (DMSO-d_6) δ 195.4(C=O, benzoyl), 175.9 (C=S), 167.7 (C=O, acid), 148.1, 146.1, 145.4, 139.5, 132.9, 131.7, 130.9, 130.8, 130.4, 129.2, 128.4, 128.2, 127.4, 110.2, 55.8 ppm. MS (100eV): *m/e*: 267.1, 321.1, 350.1, 377.1, 415.1(M+1). Anal. Calc. for $C_{24}H_{18}N_2O_3S$ (414). C, 69.55; H, 4.38; N, 6.76. Found: C, 69.55; H, 4.38; N, 6.76.

Compound 2

m.p. 230-231°C, IR(KBr): 3366 (NH), 3016 (CH), 1694 (C=O, CHO), 1687 (C=O, acetyl), 1614 (C=O, benzoyl), 1286 cm^{-1} (C=S). ^{1}H-NMR (400MHz, DMSOd_6): *δ* 11.8 (s, 1H, CHO), 8.6 (bs, 1H, NH), 7.4-7.0 (m,14H, Harom.), 6.4 (s, 1H, C4-CH), 2.9 ppm (s, 3H, CH_3). Anal. Calcd. for $C_{26}H_{20}N_2O_3S$ (440). C, 70.89; H, 4.58; N, 6.36. Found: C, 71.01; H, 4.62; N, 6.56.

Compound 3

mp 137-138ºC, IR(KBr): 3058, 2927, 2853 cm^{-1} (CH), 1766 (C=O, thiazolo), 1698 (C=O, CHO), 1649 cm^{-1} (C=O, benzoyl). ^{13}C-NMR (DMSO-d_6): δ 196.4 (C=O, benzoyl), 193.2 (C=O, thiazolo), 174.9 (C=O, CHO), 159.7, 147.9, 146.4, 138.1, 137.7, 136.7, 132.9, 130.9, 129.9, 129.8, 129.2, 128.6, 128.4, 116.1, 67.0, 57.7, 43.2, 19.4 (CH_3) ppm. Anal. Calc. for $C_{27}H_{20}N_2O_3S$ (452). C, 71.66; H, 4.45; N, 6.19. Found: C, 71.68; H, 4.40; N, 6.21.

Compound 4

mp 105-106ºC. IR (KBr) 3446 (OH), 1738 (C=O, thiazolo), 1698(C=O, CHO), (C=O, CHO), 1658 (C=O, benzoyl), 1597 cm^{-1} (C=N). ^{1}H-NMR (400MHz, DMSO-d_6)

δ 11.2 (bs, 1H, OH), 9.9 (s, 1H, CHO), 7.6-6.3 (m, 12H, Harom.), 6.3 (s, 1H, C5-CH), 4.1 (q, *J*= 6*Hz*, 1H, C2-CH), 1.2 (d, *J*= 6.4*Hz*, 3H, C2-CH_3). ^{13}C-NMR (DMSO-d_6) δ 197.5(C=O, benzoyl), 196.7 (C=O, thiazolo), 171.6 (C=O, CHO), 160.1, 157.3, 146.9, 138.8, 134.2, 132.9, 129.7, 129.6, 129.5, 129.3, 129.0, 128.8, 128.6, 128.4, 128.1, 126.9, 122.0, 114.9, 62.7, 55.3, 32.7 ppm.

Microwave Mediated Synthesis

Compounds 1a-c

A mixture of 1,3-diphenyl-1,3-propanedione (1.6 mmol), arylaldehydes (1.1 mmol), thiourea (1.1 mmol) and concentrated hydrochloric acid (0.2 ml) with glacial acetic acid (10 ml) were stirred at room temperature for 5 min with a magnetic stirrer, and then was inserted into the microwave oven. The mixture was subjected to microwave irradiation for 15 min. Then the reaction mixture was cooled and the crude products were filtered and became recrystallized suitable solvent.

Compound 2

A mixture of **1a** (1 mmol) in acetic anhydride (3 ml) was subjected to microwave irradiation, 15 min. then the reaction mixture was allowed to cool to room temperature, and poured over crushed ice and stirred for several minutes. The separated solid filtered off, washed with water and were recrystallized from dioxane.

Compounds 3, 4

A mixture of **1a** or **1b** (1mmol) and 2-bromopropionic acid (1.1 mmol) in dioxane (5 ml) were subjected to microwave irradiation for 15 min. The reaction mixture was cooled and the precipitate filtered off and then washed with water. The crude products were recrystallised from ethanol for compound **3**, and 2-propanol for compound **4**.

General Experimental Procedure for the Biological assay

Test compounds were dissolved in DMSO (12.5%) at an initial concentration of 1280 μg mL^{-1} and then they were serially diluted in culture medium.

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