EFFECTIVE MICROWAVE SYNTHESIS OF BIOACTIVE THIENO[2,3-*d*]PYRIMIDINES

TASLIMAHEMAD T. KHATRI[*,a, b], *VIRESH H. SHAH*[c]

[a]*Department of Chemistry, Lovely Professional University, Phagwara -144411, India*
[b]*Department of Chemistry, KSKV Kachchh University, Bhuj-370001, India*
[c]*Department of Chemistry. Saurashtra University, Rajkot-360009, India*

ABSTRACT

A series of novel 2-amino-3-cyanothiophenes (**2a-2j**) were synthesized using heterogeneous base (K_2CO_3) supported Gewald reaction. Cyclization of **2a-j** with formamide and urea in conventional heating as well as microwave irradiation gave thieno[2,3-*d*]pyrimidines (**3a-3j**) and thieno[2,3-*d*]pyrimidin-2(1*H*)-ones(**4a-4j**) respectively. The reaction rates were faster and yields were higher in the microwave conditions. The structures of the compounds were confirmed with elemental analysis, mass spectral analysis, FTIR, ^{1}H NMR and ^{13}C NMR techniques. All the synthesized compounds were subjected to antimicrobial activity (MIC) *in vitro* by broth dilution method and exhibited a moderate antimicrobial activity.

Key words: Gewald reaction; thieno[2,3-*d*]pyrimidines; thieno[2,3-*d*]pyrimidin-2(*1H*)-ones; antimicrobial activity

1. INTRODUCTION

The Gewald reaction is the most common and reliable reaction to synthesize 2-aminothiophenes since invented first by K. Gewald in 1961.[1-4] In the present work the reaction has been performed in the presence of heterogeneous catalyst at room temperature with constant stirring, avoiding drastic conditions and toxic solvents. Hence is an imperative to explore a true green chemistry approached one spot synthesis of novel 2-amino-3-cyanothiophenes; that can be constructive intermediate in the synthesis of novel thieno[2,3-*d*]pyrimidines and their derivatives.

The thienopyrimidines occupy a special position among fused pyrimidines, along with some other pyrimidines containing an annelated five membered hetero aromatic ring; they are structural analogues of biogenic purines and can be considered as potential nucleic acid antimetabolites. Many of thienopyrimidines are found to exhibit a variety of biological activities like antimicrobial,[5] analgesic and ulcerogenic,[6] anti-inflammatory,[6-8] antitubercular,[9] EGFR inhibitors,[10] inhibition of cancer cell proliferation,[11] antagonism of α1 adrenoceptors,[12] adenosine receptor antagonists[13] and other wide range of biological activities.[14-15]

Some recently reported thieno[2,3-*d*]pyrimidine derivatives have showed various biological activities (Fig. 1); compound **I** acts Potent SARS-CoV 3C-Like Protease Inhibitors,[16] compound **II** showed anticancer activity,[17] compound **III** antiproliferative,[18] compound **IV** acts as adenosine A_{2A} receptor agonist[19] and compound **V** is an anti-bacterial agent.[20]

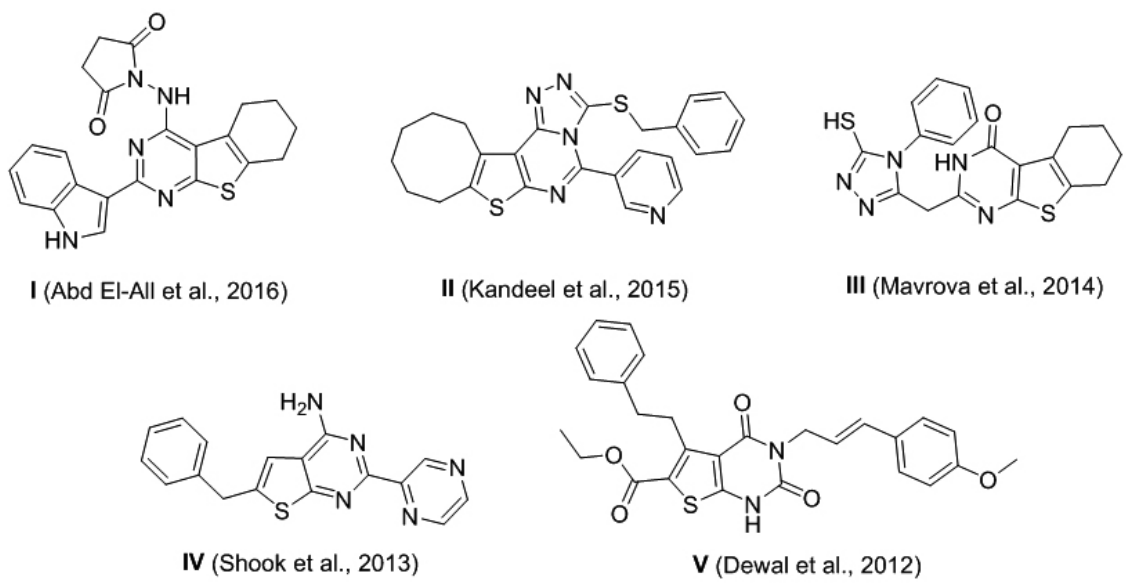


Figure 1. Structures of some bioactive thieno[2,3-*d*]pyrimidines and thieno[2,3-*d*]pyrimidones.

Thienopyrimidines can be prepared *via* cyclization of diamides intermediates, generated from amino carbamoyl thiophenes by reaction with acylating agents such as orthoesters[21] acid anhydrides or acid chlorides.[22] Alternatively, the synthesis of thienopyrimidines can be achieved from amino alkoxycarbonyl thiophenes, amidine intermediates, by the reaction of thiophenes with amides;[23] nitrites under acidic conditions,[24] and by intermolecular cyclization of orthoesters and amines.[25] Herein we report the synthesis of 2-amino thiophenes by Gewald synthesis and their cyclization with formamide and urea in microwave as well as conventional heating. Since our group is looking for improved options for antimicrobial agents[26,27] we have also tested the synthesized compounds for their antimicrobial activities.

2. EXPERIMENTAL

2.1 General

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC (Kieselgel 60, F_{254}) of 0.5 mm thickness and spots were located by iodine and UV. The microwave-assisted reactions were realized in a QPro-M microwave synthesizer. IR spectra were recorded on Shimadzu FT-IR-8400 instrument

e-mail : khatritaslim@gmail.com

using KBr pellet method. Mass spectra were recorded on Shimadzu GCMS-QP-2010 model using Direct Injection Probe technique. ^{1}H-NMR and ^{13}C NMR were determined in DMSO-d_6 solution on a Bruker Ac 400 MHz and some on Bruker Ac 500 MHz FT NMR spectrometer with TMS as internal standard. Elemental analysis of the all the synthesized compounds was carried out on Elemental Vario EL III Carlo Erba 1108 model and the results are in agreements with the structures assigned.

2.2 General procedure for the synthesis of 5-amino-4-cyano-N-(aryl)-3-methylthiophene-2-carboxamides (2a-2j).

An appropriate *N*-(aryl)-3-oxobutanamides (**1a-1j,** 10.0 mmoles) was dissolved in ethanol (10.0 mL), malononitrile (10.0 mmoles) and powdered sulphur (10.0 mmoles) were added to the same solution. Potassium carbonate (1.0 g) was added to the resulting solution as inorganic basic support.[28] The heterogeneous mixture was stirred at room temperature for 14-16 h at 600 rpm (Scheme 1). After the completion of the reaction as monitored by TLC, the reaction mixture was filtered off to separate K_2CO_3 as residue and the filtrate was poured onto of ice-cold water (50.0 mL). The product got precipitated out, which was filtered and recrystallized from methanol and was extracted in Chloroform thrice (Table 1).

***2.2.1 5-amino-4-cyano-N-(4-chlorophenyl)-3-methylthiophene-2-carboxamide* (2d).** Colourless amorphous; IR (ν_{max}, cm^{-1}): 3367 and 3351 (NH_2), 3242 (N-H, amide), 3049 (C-H_{arom}), 2877 (CH_3), 2231 (C≡N), 1711 (C=O, amide), 1562, 1489 and 1453 (C=C_{arom}), 700 (C-S-C). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 2.41 (3H, s, CH_3), 7.27 (2H, s, NH_2), 7.40-7.44 (2H_{arom}, d, $^3J_{HH}$ 8.4 Hz, 2CH), 7.78-7.83 (2H, d, $^3J_{HH}$ 8.4Hz, 2CH), 9.30 (1H, s, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ_c: 9.1 (-CH_3), 85.7 (=C-CN), 117.3 (-CN), 122.2 (2C_{arom}), 130.4 (2C_{arom}), 133.3 (1C_{arom}), 136.7 (1C_{arom}), 142.2 (C-CONH), 147.0 (C-Me), 156.4 (C-NH_2), 167.0 (-CONH); MS, *m/z* 291, 264, 256, 193, 180, 165, 154, 111; Anal. Calcd. for $C_{13}H_{10}ClN_3OS$: C, 53.52; H, 3.45; N, 14.40%. Found: C, 53.46; H, 3.29; N, 14.30%.

2.3 General Procedure for the Synthesis of 4-amino-5-methyl-N-phenylthieno[2,3-d]pyrimidine-6-carboxamides (3a-3j)

A mixture of an appropriate **2a-2j** (10 mmol) and formamide (10 mL) was irradiated in the microwave condition (180 MW), at 600 rpm as shown in scheme 2, the same reaction was carried out under conventional heating (Table 2) on oil bath (under TLC analysis). The reaction mixture was allowed to cool to room temperature. The solid thus formed was collected by filtration, washed with methanol (20 mL), dried and crystallized from dimethylformamide to afford the desired products (**3a-3j**).

***2.3.1 4-amino-N-(4-fluorophenyl)-5-methylthieno[2,3-d]pyrimidine-6-carboxamide* (3j).** Colourless crystals; IR (ν_{max}, cm^{-1}): 3443 (NH_2), 3231 (NH, amide), 3072 (C-H_{arom}), 2912 (CH_3), 1712 (C=O, amide), 1576 (C=N, pyrimidine ring), 1581 and 1542 (C=C_{arom}), 1217 (C-N, pyrimidine ring), 711 (C-S-C). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 2.60 (3H, s, CH_3), 7.21-7.25 (2H_{arom}, d, $^3J_{HH}$ 8.4 Hz, 2CH), 7.57-7.60 (2H_{arom}, d, $^3J_{HH}$ 7.6 Hz, 2CH), 7.83 (2H, s, NH_2), 8.52 (1H, s, -N=CH-$N_{Pyrimidine}$), 10.24 (1H, s, NH, amide); (100 MHz, DMSO-d_6) δ_c: 9.2 (-CH_3), 117.8 (-C=C-NH_2), 118.5 (2C_{arom}), 128.2 (2C_{arom}), 134.1 (1C_{arom}), 136.4 (=C-CONH-), 147.3 (=C-Me), 156.7 (N=C-N), 159.5 (C-NH_2), 160.1 (N=C-S), 162.7 (1C_{arom}), 168.7 (-CONH-); MP: 310-312°C; MS, *m/z* 302, 283, 270, 207, 192, 164, 138, 110, 95; Anal. Calcd. for $C_{14}H_{11}FN_4OS$: C, 55.62; H, 3.67; N, 18.53%. Found: C, 55.60; H, 3.63; N, 18.51%

2.4 General Procedure for the Synthesis of 4-amino-5-methyl-2-oxo-N-phenyl-1,2-dihydrothieno[2,3-d]pyrimidine-6-carboxamides (4a-4j).

A mixture of an appropriate **2a-j** (10.0 mmol) and urea (3.0 g) was irradiated in the microwave condition (180 MW), at 600 rpm as shown in scheme 2, the same reaction was carried out under conventional heating on (Table 2) oil bath (under TLC analysis). The reaction mixture was allowed to cool to room temperature. The solid thus formed was washed with water (20.0mL) followed by methanol (20.0 mL), dried and crystallized from dimethylformamide to afford the desired products (**4a-4j**).

***2.4.1 4-amino-N-(2-methoxyphenyl)-5-methyl-2-oxo-1,2-dihydrothieno[2,3-d]pyrimidine-6-carboxamide* (4h).** Colour less crystals; IR (ν_{max}, cm^{-1}): 3358 (NH_2), 3223 (NH, amide), 2974 (C-H_{arom}), 2881 (CH_3), 1701 (C=O, amide), 1668 (C=O, amide of pyrimidine ring), 1587 (C=C_{arom}) 1536 (C=N, pyrimidine ring), 1245 (C-N, pyrimidine ring), 664 (C-S-C). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 2.46 (3H, s, CH_3), 3.79 (3H, s, OCH_3), 7.21-7.26 (3H_{arom}, m, 3CH), 7.36-7.40 (1H_{arom}, d, $^3J_{HH}$ 8.0 Hz, 1CH), 7.65 (2H, s, NH_2), 9.57 (1H, s, NH, amide), 10.98(1H, s, $NH_{amide\ pyrimidine\ ring}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ_c: 9.05 (-CH_3), 56.9 (-OCH_3), 109.1 (-C=C-NH_2), 112.4 (1C_{arom}), 115.7 (1C_{arom}), 121.5 (1C_{arom}), 122.4 (1C_{arom}), 127.7 (=C-CONH-), 128.9 (1C_{arom}), 145.1 (=C-Me), 148.5 (-NH-CO-N-), 150.5 (1C_{arom}), 155.3 (C-NH2), 164.2 (-CONH-), 182.6 (NH=C-S); MS, *m/z* 330, 314, 299, 223, 208, 180, 122, 107; Anal. Calcd for $C_{15}H_{14}N_4O_3S$: C, 54.53; H, 4.27; N, 16.96%. Found C, 54.50; H, 4.22; N, 16.93%

2.5 Antimicrobial Activity

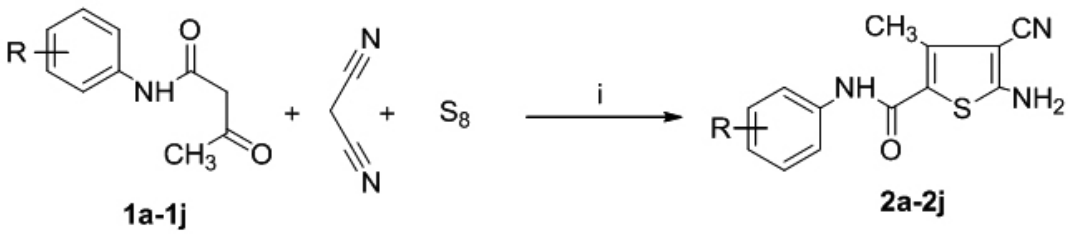
All the synthesized compounds were tested for their antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method[29-31] with two Gram-positive bacteria *Staphylococcus aureus* (S.a.) MTCC-96, *Streptococcus pyogenes* (S.p.) MTCC 443, two Gram-negative bacteria *Escherichia coli* (E.c.) MTCC 442, *Pseudomonas aeruginosa* (P.a.) MTCC 441 and three fungal strains *Candida albicans* (C.a.) MTCC 227, *Aspergillus niger* (A.n.) MTCC 282, *Aspergillus clavatus* (A.c.) MTCC 1323 taking ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, nystatin, and griseofulvin as standard drugs.

Serial dilutions of the test compounds and reference drugs were prepared in Muellere-Hinton agar. Drugs (10 mg) were dissolved in dimethylsulfoxide (DMSO, 1 mL). Further progressive dilutions with melted Muellere-Hinton agar were performed to obtain the required concentrations of 1.56, 3.12, 6.25, 10.0, 12.5, 25.0, 50.0, 62.5, 100.0, 125.0, 250.0, 500.0 and 1000.0 µg mLP^{-1}P. The tubes were inoculated with 10^8 cfu mL^{-1} (colony forming unit mL^{-1}) and incubated at 37 ºC for 24 h. The MIC was the lowest concentration of the tested compound that yields no visible growth (turbidity) on the plate. To ensure that the solvent had no effect on the bacterial growth, a control was performed with the test medium supplemented with DMSO at the same dilutions as used in the experiments and it was observed that DMSO had no effect on the microorganisms in the concentrations studied.

3. RESULTS AND DISCUSSION

3.1 Novel Synthetic Approach

The study began with synthesis of *N*-phenyl-3-oxobutanamide (**1a**). The fusion of ethyl acetoacetate with various substituted aromatic amines under solvent free condition yielded **1a.**[18] To carry out the reaction in a green approach under milder conditions, the Gewald reaction of **1a** in heterogeneous conditions was carried out with malanonitrile, K_2CO_3 and sulphur powder. The mixture was stirred at room temperature with constant stirring for 17hr in absolute ethanol, resulted in 5-amino-4-cyano-*N*-(phenyl)-3-methylthiophene-2-carboxamide (**2a**) in 67% yield (Scheme 1). Based on the remarkable results obtained with the stated reaction conditions, and in order to show the generality and scope of this protocol, we used various *N*-(aryl)-3-oxobutanamides (**1a-j**). Different substituents on the phenyl ring didn't distress the reaction as all the components provided moderate to good yield of products (Table 1).



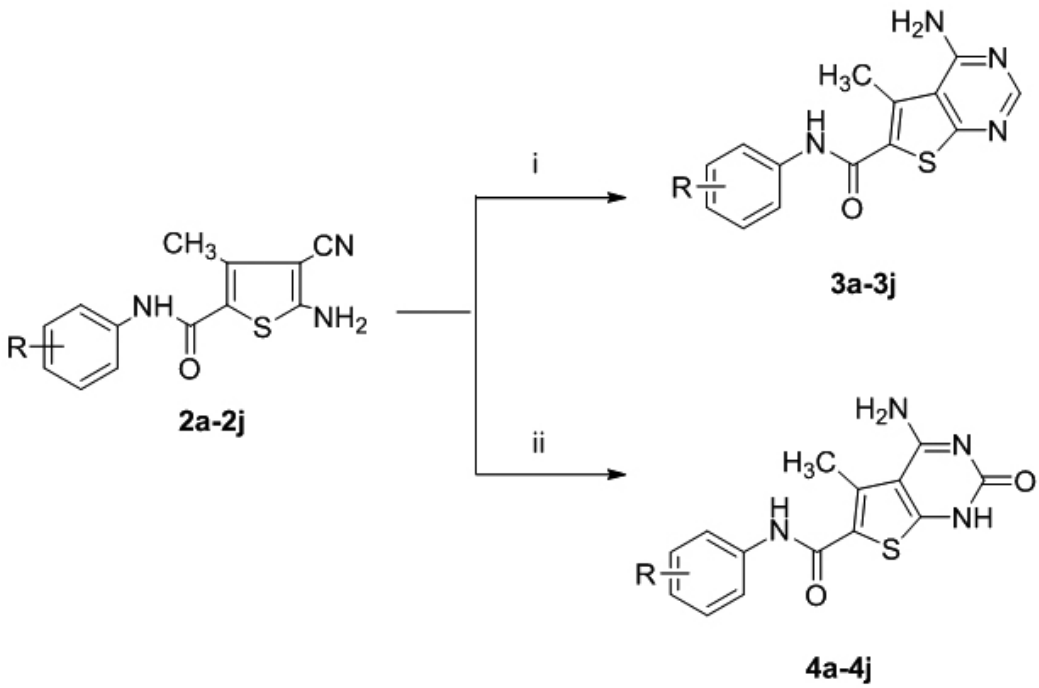
Scheme 1. *Reagents and conditions:* i: K_2CO_3, C_2H_5OH, stirring, 14-17 hours.

Table 1 : Preparation of 5-amino-4-cyano-*N*-(phenyl)-3-methylthiophene-2-carboxamides.

Code	R	M.F.	M.W. (g/ mole)	M.P. (°C)	Yield[1] (%)
2a	H	$C_{13}H_{11}N_3OS$	257	156-158	67
2b	4-CH_3	$C_{14}H_{13}N_3OS$	271	154-158	68
2c	2-Cl	$C_{13}H_{10}ClN_3OS$	291	181-183	65
2d	4-Cl	$C_{13}H_{10}ClN_3OS$	291	199-201	73
2e	3-NO_2	$C_{13}H_{10}N_4O_3S$	302	201-204	70
2f	4-NO_2	$C_{13}H_{10}N_4O_3S$	302	179-181	72
2g	4-Br	$C_{13}H_{10}BN_3OS$	334	222-226	69
2h	2-OCH_3	$C_{14}H_{13}N_3O_2S$	287	221-223	63
2i	4-OCH_3	$C_{14}H_{13}N_3O_2S$	287	203-205	69
2j	4-F	$C_{13}H_{10}FN_3OS$	275	254-256	51

[1]Isolated yield

The 2-amino-3-cyano thiophenes has been used by Abdelaziz et al.,[32] and Shook et al.,[19] to synthesize bioactive thieno pyrimidines, inspired by this virtuous results and to demonstrate synthetic utility of synthesized 2-amino-3-cyano thiophenes, the reaction of **(2a-2j)** was carried out with formamide and urea under solvent free condition as shown in scheme 2, which yielded the novel thieno[2,3-d]pyrimidines (**3a-3j**) and thieno[2,3-*d*]pyrimidin-2(*1H*)-ones (**4a-4j**). Cyclization of different 2-amino-3-cyano thiophenes have been previously reported with formamide[33] and urea[34] under conventional heating for 4hr, which resulted in 68% and 70% yield respectively. In our protocol we have also carried out the reactions in Microwave condition, which shortened the reaction time dramatically, for this microwave magnetron power was varied for the all the reactions performed, but maximum yield was obtained at lower irradiation (180 MW). There were substantial differences regarding the nature of substrate and conventional versus microwave-assisted reactions. 5-amino-4-cyano-*N*-(pyridyl)-3-methylthiophene-2-carboxamide have been cyclized in reflux for 7hrs with formamide[35], while the highest time taken in our protocol for similar synthesis is 16minutes (for **3a** and **3i**, Table 2). There was a clear improvement in using microwave heating over conventional heating in all of our studied substrates. The reaction time for microwave-assisted reactions was up to twenty times shorter than for comparable reactions under conventional heating. When the reaction time was shortened, thermal decomposition was also minimized, resulting in higher isolated yields and more simplified product purification (Table 2).



Scheme 2. *Reagents and conditions:* i: formamide, microwave irradiation 180 Watts for or reflux on oil bath. ii: urea, microwave irradiation 180Watts for or heating on oil bath.

While cyclization of **2a-j** with formamide and urea we have observed that 3-NO_2 yields slightly more than that of 4-NO_2, may be because of electron withdrawing effect of NO_2 group at para position. There is a little effect on the yield due to electron donating and withdrawing groups, hence this protocol can widely be used for synthesis of different derivatives. To demonstrate the practicality of the developed microwave protocol, large-scale experiments (30 mmol of **2e** and **2g**) were carried out in the synthesis of **3e**, **3g**, **4e** and **4g** using a 250 mL Erlenmeyer flask as the reaction vessel. High yields of **3e** (79%), **3g** (75%), **4e** (72%) and **4g** (79%) were afforded under microwave irradiation at 180 MW with mentioned exposure times in Table 2.

3.2 Antimicrobial Activity

Although many antimicrobial agents have been introduced into therapy; however, the field still needs extensive efforts for the development of new antimicrobial agents to overcome the highly resistant strains of microorganisms. Therefore all of the synthesized compounds were tested for their antibacterial and antifungal activity (MIC) *in vitro* using two Gram-positive bacteria *Staphylococcus aureus* (S.a.), *Streptococcus pyogenes* (S.p.), two Gram-negative bacteria *Escherichia coli* (E.c.), *Pseudomonas aeruginosa* (P.a.) and three fungal strains *Candida albicans* (C.a.), *Aspergillus niger* (A.n.), *Aspergillus clavatus* (A.c), only promising results are shown here (Table 3). The other compounds' data can be found in the supplementary material.

Table 2: Comparison of microwave and conventional methods for the synthesis of thieno[2,3-*d*]pyrimidines (**3a-3j**) and thieno[2,3-*d*]pyrimidin-2(*1H*)-ones (**4a-4j**).

Code	R	M. F.	M.W. (g/mole)	M.P. (°C)	Conventional		Microwave	
					Time (min)	Yield[1] (%)	Time[2](min)	Yield[1](%)
3a	H	$C_{14}H_{12}N_4OS$	284	270-274	265	50	16	74
3b	4-CH_3	$C_{15}H_{14}N_4OS$	298	292-296	250	41	15	71
3c	2-Cl	$C_{14}H_{11}ClN_4OS$	318	310-312	220	56	12	69
3d	4-Cl	$C_{14}H_{11}ClN_4OS$	318	316-318	225	60	13	76
3e	3-NO_2	$C_{14}H_{11}N_5O_3S$	329	280-284	210	65	15	78
3f	4-NO_2	$C_{14}H_{11}N_5O_3S$	329	278-280	215	53	14	72
3g	4-Br	$C_{14}H_{11}BN_4OS$	363	320-322	240	48	13	68
3h	2-OCH_3	$C_{15}H_{14}N_4O_2S$	314	286-290	255	55	14	74
3i	4-OCH_3	$C_{15}H_{14}N_4O_2S$	314	298-300	240	51	16	70
3j	4-F	$C_{14}H_{11}FN_4OS$	302	280-284	195	53	11	73
4a	H	$C_{14}H_{12}N_4O_2S$	300	240-244	360	55	20	73
4b	4-CH_3	$C_{15}H_{14}N_4O_2S$	314	272-274	360	60	18	74
4c	2-Cl	$C_{14}H_{11}ClN_4O_2S$	334	284-286	315	62	21	76
4d	4-Cl	$C_{14}H_{11}ClN_4O_2S$	334	280-282	315	65	18	74
4e	3-NO_2	$C_{14}H_{11}N_5O_4S$	345	256-258	290	57	19	78
4f	4-NO_2	$C_{14}H_{11}N_5O_4S$	345	262-268	285	58	18	73
4g	4-Br	$C_{14}H_{11}BN_4O_2S$	379	296-300	330	57	16	76
4h	2-OCH_3	$C_{15}H_{14}N_4O_3S$	330	268-274	385	56	18	72
4i	4-OCH_3	$C_{15}H_{14}N_4O_3S$	330	280-284	395	49	21	73
4j	4-F	$C_{14}H_{11}FN_4O_2S$	318	270-274	350	52	22	69

[1]Isolated yield, [2]Continuous irradiation,

Table 3. Antimicrobial activity of the synthesized compounds[1]

Code	Minimum inhibition concentration (μg mL^{-1})						
	Gram-positive		Gram-negative		Fungal species		
	S.a.	***S. p.***	***E.c.***	***P.a.***	***C. a.***	***A. n.***	***A.c.***
2d	25	100	200	100	1000	500	500
2e	50	125	62.5	100	500	500	500
2g	50	100	25	100	>1000	500	1000
3d	25	100	200	100	1000	500	500
3g	50	100	25	100	>1000	500	1000
3j	25	50	50	50	500	500	1000
4d	50	125	100	200	>1000	500	>1000
4h	500	500	500	1000	>1000	500	125
4j	50	125	250	100	100	1000	500
Amp	250	100	100	100	-	-	-
Chl	50	50	50	50	-	-	-
Cip	50	50	25	25	-	-	-
Nor	10	10	10	10	-	-	-
Nys	-	-	-	-	100	100	100
Gri	-	-	-	-	500	100	100

[1]Microorganisms selected are as follows: *S.a., Staphylococcus aureus; S.p.,Streptococcus pyogenes; E.c. Escherichia coli;P.a., Pseudomonas aeruginosa; C.a., Candida albicans; A.n., Aspergillus niger; A.c. Aspergillus clavatus.* Standards: Amp,Ampicillin; Chl, Chloramphenicol; Cip, Ciprofloxacin; Nor, Norfloxacin; Nys, Nystatin; and Gri, Griseofulvin. Values are expressed as mean ± standard deviation of the three replicates.

It is worth noting here that compounds **2d** and **3d** exhibited significant activity against *Staphylococcus aureus,* while compound **3g** was found active against *Escherichia coli.* The compound **2j** and **2h** didn't show notable activity against all the tested microorganism but after cyclization resulted in compound **3j** and **4h r**espectively, the **3j** exhibited significant activity against all the bacterial species while **4h** against *Aspergillus clavatus* fungal species, may be due to presence of 2-aminopyrimidine precursor in the later ones as all the **2a-j** compounds showed low activity against tested microorganism except **2d**. On the other hand all the other synthesized compounds showed moderate-to-low activity against bacterial species and very low activity against fungal species.

4. CONCLUSION

In conclusion, we developed a general approach for the preparation of some biologically active thieno[2,3-*d*]pyrimidines and thieno[2,3-*d*]pyrimidin-2(*1H*)-ones via 2-aminothiophene intermediate using Gewald synthesis followed by cyclization reactions in conventional as well as microwave conditions. Compared to conventional heating, the microwave technique provides a rapid, simple, and effective method to synthesize such compounds that may have the potential application in the field of drug discovery. Moreover the reaction is simple, one pot, solvent free and also gives excellent yields without catalyst at larger scales. The synthesized compounds were characterized by spectral data (Mass Spectrum, IR, ^{1}H and ^{13}C NMR) and elemental analysis. The compounds were subjected to *in vitro* antimicrobial activity assays. The results showed that the synthesized compounds (**2a** to **4j**) possessed weak to good antimicrobial activities against the tested microorganism, with compounds **2d**, **3d**, **3g**, **3j** displaying good activity against bacterial species while all the synthesized compounds were poor at displaying activities against the fungal species, however compound **4h** showed moderate activity against *Aspergillus clavatus*. Further studies are currently underway to establish a definite structure activity relationship.

5. DECLARATION OF INTEREST

The authors report no conflicts of interest.

6. SUPPLEMENTARY MATERIAL

The spectroscopic data and biological activities of the remaining compounds are provided in the supplementary material.

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