A Facile Microwave-Assisted Synthesis of Novel Thienopyrimidines and Triazolothienopyrimidines and their Antimicrobial Activities

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***Abstract*—**Thienopyrimidines (2, 3, 4) and triazolo-thienopyrimidines (5a-c) were prepared from the precursor 2-amino-5, 5-dimethyl-7-oxo-4, 5, 6, 7-tetrahydro-1-benzothiophene-3-carbonitrile (1) by employing the microwave irradiation technique. The precursor 2-amino-3-cyanothiophene analogue (1) was synthesized by employing the well-known Gewald reaction. In the present work it has been found that the microwave supported syntheses is more efficient than the classical heating method. The structures of all the compounds were ascertained by spectral and analytical data. The compounds have showed promising antibacterial and antifungal activities.

***Keywords*—**Antibacterial activity, Antifungal activity, Microwave irradiation, Thienopyrimidine, Triazolothienopyrimidine.

I. Introduction

HETEROCYCLIC compounds with thienopyrimidine molecule have gained considerable interest due to their interesting pharmacological and biological activities [1 - 4]. These derivatives have also displayed analgesic [5, 6], antimicrobial [7], anti-inflammatory effects [8, 9], platelet aggregation inhibition activity [10], antogonism of α-adrenoceptors [11] and prevention of cartilage destruction in articular diseases [12]. In view of these fascinating and encouraging results and in continuation of our work on biologically active nitrogen and sulfur heterocycles [13], we have synthesized some thienopyrimidines and triazolothienopyrimidines by adopting a different methodology and evaluated them for their antimicrobial properties. Addition to the conventional method the microwave irradiation technique has also been employed for the synthesis of some thienopyrimidines **(2, 3, 4)** and triazolothienopyrimidines (**5a-c**) and the results have been compared. The well-known Gewald reaction was adopted for the synthesis of the precursor 2-amino-3-cyanothiophene **(1).**

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Some of the synthesized compounds were tested against two Gram (+) Bacteria (*staphylococcus aureus*, *Bacillus subtilis*), two Gram (-) bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*) and two yeast-like fungi *Candida albicans* and *Candida parapsilosis* using the broth microdilution method [14, 15]

II. Experimental

Experimental protocols

Melting points were determined in open capillaries and are uncorrected. The IR spectra were recorded on Shimadzu FT IR – 8400S spectrophotometer using KBr pellets. ^{1}H and ^{13}C NMR were recorded on Bruker 300 MHz FT NMR spectrometer in $CDCl_3$ and DMSO-d_6 with TMS as internal standard. Mass spectrum was recorded on Finnigan MAT (Model MAT8200) spectrometer and elemental analyses were carried out using Heraus CHN rapid analyzer.

Synthesis

*2-amino-5, 5-dimethyl-7-oxo-4, 5, 6, 7-tetrahydro-1-benzothio -phene-3-carbonitrile (**1**).*

To a well stirred mixture of dimedone (8.2 g, 37 mmole) and malononitrile (3.3 g, 50 mmole) in ethanol (45 mL) was added elemental sulfur (1.68 g, 50.25 mmole). To this cooled reaction mixture was added diethylamine (5 mL) with vigorous stirring during 1 min. Reaction mixture was stirred at 40-45^{o}C for about 1 hr. The yellow-orange solid separated was filtered, washed with hot ethanol and recrystallised from dioxane to yield analytically pure yellow needles. Yield 76%, m.p. 242-244^{o}C; IR (KBr) vcm^{-1}: 3328, 3180, 2973, 2909, 2219, 1659, 1616, 1530; ^{1}H NMR (300MHz, DMSO) δ: 1.02(s, 6H, gemdimethyl), 2.24(s, 2H, CH_2), 2.41(s, 2H, $COCH_2$), 8.17(s, 2H, NH_2, D_2O exchangeable). Anal. calcd. for $C_{11}H_{12}N_2OS$: C, 59.97; H, 5.49; N, 12.72. Found: 60.04; H, 5.55; N, 12.81% [13].

*6, 6-Dimethyl-6, 7-dihydro-3H,5H-benzo[4, 5]thieno[2, 3-d] pyrimidine-4, 8-dione (**2**).*

Method A: A mixture of **1 (**1 g**)** and formic acid (15 mL) was heated under microwave irradiation at 90 ^{0}C for 15 minutes.

The excess of formic acid was removed under reduced pressure. The resulting residue was crystallized from ethanol to yield pale yellow granules. Yield 80%, m.p. 138-140^{o}C; IR (KBr) νcm^{-1}: 3324, 3110, 1678, 1664, 1535, 1356, 975; ^{1}H NMR (300MHz, $CDCl_3$) δ: 0.99(s, 6H, gemdimethyl), 2.27(s, 2H, CH_2), 2.50(s, 2H, $COCH_2$), 8.05(s, C2-H, pyrimidine), 11.80(br s, 1H, NH, D_2O exchangeable). Anal. calcd. for $C_{12}H_{12}N_2O_2S$: C, 58.05; H, 4.87; N, 11.28. Found: C, 58.12; H, 5.01; N, 11.32% [13].

Method B: A mixture of **1 (**1 g**)** and formic acid (15 mL) was heated under reflux for 5 h. The excess of formic acid was removed under reduced pressure. The resulting residue was crystallized from ethanol to yield pale yellow granules. Yield 78%

*4-Chloro-6, 6-dimethyl-5, 6, 7, 8-tetrahydro[1]-benzothieno [2, 3-d]pyrimidin-8-one (***3***).*

Method A: Mixture of compound **2** (0.55 g, 2.5 mmol) with phosphorus oxychloride (1.75 mL) was heated under microwave irradiation at 95^{0} C for 10 minutes The reaction mixture was allowed to cool to room temperature and poured into ice-water (200 g), the solid that separated was filtered off and crystallized from petroleum ether. Yield 76%, m.p. 103-105^{o}C; IR (KBr) νcm^{-1}: 3110, 1678, 1535, 1343, 975; ^{1}H NMR (300MHz, $CDCl_3$) δ: 1.09(s, 6H, gemdimethyl), 2.35(s, 2H, CH_2), 2.56(s, 2H, $COCH_2$), 8.46(s, C2-H, pyrimidine). Anal. calcd. for $C_{12}H_{11}ClN_2OS$: C, 54.03; H, 4.16; N, 10.50. Found: C, 54.08; H, 4.22; N, 11.01%.

Method B: A solution of **2** (0.55 g, 2.5 mmol) in dry dioxane (7.5 mL) was treated with phosphorus oxychloride (1.75 mL) and the mixture was stirred under reflux for 3 h. The reaction mixture was allowed to cool to room temperature and poured into ice-water (200 g), the solid that separated was filtered off and crystallized from petroleum ether. Yield 74%.

*4-Hydrazino-6, 6-dimethyl-6, 7-dihydro[1]benzothieno[2, 3-d]pyrimidin-8-one (***4***).*

Method A: A mixture of compound **3** (0.6 g, 2.5 mmol) and hydrazine hydrate 80 % (1.8 mL) in ethanol (13 mL) was heated under microwave irradiation at 50^{o}C for 20 minutes. The reaction mixture was allowed to cool to room temperature. The deposited so precipitate was filtered off and crystallized from dioxane. Yield 83%, m.p. 170-172^{o}C; IR (KBr) νcm^{-1}: 3360, 3313, 3113, 1645, 1571; ^{1}H NMR (300MHz, $CDCl_3$) δ: 1.02(s, 6H, gemdimethyl), 2.27(s, 2H, CH_2), 2.45(s, 2H, $COCH_2$), 4.61(br s, 2H, NH_2, D_2O exchangeable), 5.42 (br s, 1H, NH, D_2O exchangeable), 8.57(s, C2-H, pyrimidine). Anal. calcd. for $C_{12}H_{14}N_4OS$: C, 54.94; H, 5.38; N, 21.36. Found: C, 54.99; H, 5.42; N, 21.41%.

Method B: A mixture of compound **3** (0.6 g, 2.5 mmol) and hydrazine hydrate 80 % (1.8 mL) in ethanol (13 mL) was heated at 50^{o}C for 3 h. The reaction mixture was allowed to cool to room temperature. The deposited so precipitate was filtered off and crystallized from dioxane. Yield 76%.

*10, 10-dimethyl-8, 9, 10, 11-Tetrahydro[1]benzothieno[3, 2-e][1, 3, 4]triazolo[1, 5-c] pyrimidin-8-one (***5a***).*

Method A: A mixture of compound **4** (0.4 g, 2 mmol), formic acid (2 mL), and a catalytic amount of concentrated hydrochloric acid was heated under microwave irradiation at 90^{0} C for 30 minutes. The reaction mixture was allowed to cool to room temperature and poured into water (200 mL). The precipitate that was formed was collected by filteration, washed with ethanol (50 mL), dried and crystallized from dioxane:ethanol mixture(2:1). Yield 90%, m.p. 158-160^{o}C; IR (KBr) νcm^{-1}: 3045, 2949, 1658, 1617, 1522, 1485, 1432; ^{1}H NMR (300MHz, $CDCl_3$) δ: 1.15 (s, 6H, gemdimethyl), 2.14 (s, 2H, CH_2), 2.22 (s, 2H, $COCH_2$), 8.35 (s, C2-H, pyrimidine), 9.12 (s, 1H, triazole). Anal. calcd. for $C_{13}H_{12}N_4OS$: C, 57.34; H, 4.44; N, 20.57. Found: C, 57.39; H, 4.50; N, 20.65%.

Method B: A mixture of compound 4a (0.4 g, 2 mmol), formic acid (2 mL), and a catalytic amount of concentrated hydrochloric acid was heated under reflux for 7 hours. The reaction mixture was allowed to cool to room temperature and poured into water (200 mL). The precipitate that was formed was collected by filteration, washed with ethanol (50 mL), dried and crystallized from dioxane:ethanol mixture(2:1). Yield 85 %.

*3-Methyl-10, 10-dimethyl-8, 9, 10, 11-Tetrahydro[1]benzo- thieno[3, 2-e][1, 3, 4]- triazolo[1, 5-c]pyrimidin-8-one (***5b***).*

Method A: A mixture of compound **4** (0.4 g, 2 mmol), glacial acetic acid (6 mL) was heated under microwave irradiation at 85^{0} C for 25 minutes. The reaction mixture was allowed to cool to room temperature and poured into water (200 mL). The precipitate that was formed was collected by filteration, dried and crystallized from acetic acid. Yield 62%, m.p. 186-188^{o}C; IR (KBr) νcm^{-1}: 3022, 2934, 1659, 1604, 1518, 1430; ^{1}H NMR (300MHz, $CDCl_3$) δ: 1.23 (s, 6H, gemdimethyl), 3.00 (s, 3H, CH_3), 2.31(s, 2H, CH_2), 2.48 (s, 2H, $COCH_2$), 8.55 (s, C2-H, pyrimidine). Anal. calcd. for $C_{14}H_{14}N_4OS$: C, 58.72; H, 4.93; N, 19.57. Found: C, 58.79; H, 4.98; N, 19.65%.

Method B: A mixture of compound **4** (0.4 g, 2 mmol), glacial acetic acid (6 mL) was heated under reflux for 15 hours. The reaction mixture was allowed to cool to room temperature and poured into water (200 mL). The precipitate that was formed was collected by filteration, dried and crystallized from acetic acid. Yield 54%.

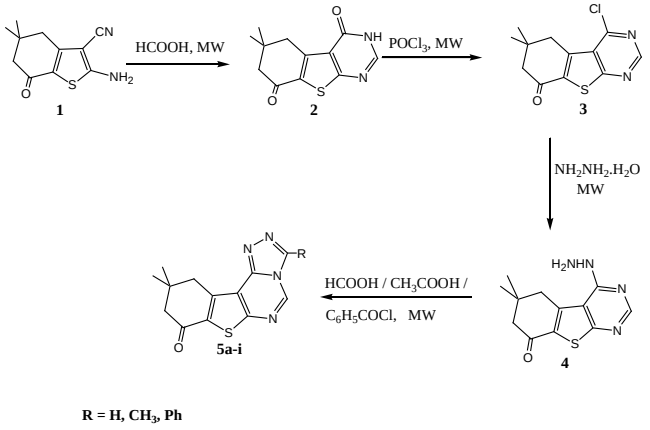
*3-Phenyl-10, 10-dimethyl-8, 9, 10, 11-tetrahydro[1]benzo-thieno[3, 2-e] [1, 3, 4]triazolo [1, 5-c]pyrimidin-8-one (***5c***).*

Method A: A mixture of compound **4** (0.4 g, 2 mmol), Benzoyl chloride (6 mL) was heated under microwave irradiation at 80^{0} C for 30 minutes. The reaction mixture was allowed to cool to room temperature and poured into water

(200 mL). The precipitate that was formed was collected by filteration, washed with ethanol (50 mL), dried and crystallized from dioxane:ethanol mixture (2:1). Yield 68%, m.p.170-172^{o}C; IR (KBr) νcm^{-1}: 2998, 2948, 1657, 1609, 1517, 1475, 1411; ^{1}H NMR (300MHz, $CDCl_3$) δ: 1.14 (s, 6H, gemdimethyl), 2.18 (s, 2H, CH_2), 2.28 (s, 2H, $COCH_2$), 7.34-7.61 (m, 5H, phenyl protons), 8.45 (s, C2-H, pyrimidine). Anal. calcd. for $C_{19}H_{16}N_4OS$: C, 65.50; H, 4.63; N, 16.08. Found: C, 65.56; H, 4.71; N, 16.12%.

Method B: A mixture of compound **4** (0.4 g, 2 mmol), Benzoyl chloride (6 mL) was stirred under reflux for 8 hours. The reaction mixture was allowed to cool to room temperature and poured into water (200 mL). The precipitate that was formed was collected by filteration, washed with ethanol (50 mL), dried and crystallized from dioxane:ethanol mixture (2:1). Yield 60%.

SCHEME I



III. Antimicrobial Activity

Minimum inhibitory concentration (MIC) values for the synthesized compounds were determined by using the broth microdilution method [14, 15]. Two Gram-positive (*S. aureus* ATCC 25923 and *B. subtilis* ATCC 6633) and two Gram-negative (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853) bacteria were used as quality control strains. For determining anti-yeast activities of the compounds, the following reference strains were tested: *Candida albicans* ATCC 10231 and *Candida parapsilosis* ATCC 90018. Ampicillin trihydrate and fluconazole were used as standard antibacterial and antifungal agents, respectively. Fluconazole was dissolved in sterile distilled water, ampicillin trihydrate in phosphate buffer (pH 8) and the stock solution of the synthesized compounds was dissolved in dimethyl sulfoxide (DMSO) and distilled water (50%) at a concentration of 2048 µg/mL. Twofold dilutions of the synthesized compounds were prepared (1024, 512… …2 µg/mL), and twofold dilutions of the reference compounds were prepared at 64 – 0.125 µg/mL. All bacteria were cultivated in Mueller-Hinton Agar (Merck). The bacteria inoculums was prepared in Mueller-Hinton Broth (Merck) which had been kept at 36^{0}C overnight and was diluted with broth to give a final concentration of 5 x10^{5} cfu/mL. All fungi were cultivated in sabouraud Dextrose Agar (Merck). The fungi inculums were prepared in sabouraud liquid medium (oxoid), which had been kept at 36^{0}C overnight and was diluted with RPMI-1640 medium with L-glutamine buffered with 3-[N-morpholino]-propane sulfonic acid (MOPS) at pH 7 to give a final concentration of 2.5 x 10^{3} cfu/mL. The microplates were incubated at 36^{0}C and read visually after 24 h, except for candida species when it was at 48 h. The incubation chamber was kept humid. At the end of the incubation period, MIC values were recorded as the lowest concentrations of the substances that gave no visible turbidity. The DMSO diluents at a maximum final concentration of 12.5% had no effect on the microorganism's growth. The minimum inhibitory concentrations (MIC) were recorded in Table I.

TABLE I
ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF THE COMPOUNDS AS MIC VALUES (µG/ML).

Compounds	*Staphylococcus aureus* ATCC 25923	*Bacillus subtilis* ATCC 6633	*Escherichia coli* ATCC 25922	*Pseudomonas aeruginosa* ATCC 27853	*Candida albicans* ATCC 10231	*Candida parapsilosis* ATCC 90018
2	512	13	256	256	64	128
3	512	13	256	256	64	64
4	256	64	128	256	16	64
5a	512	13	256	256	32	128
5b	512	32	256	256	16	256
5c	512	13	256	256	16	64
Ampicillin	4	8	4	-	-	-
Fluconazole	-	-	-	-	8	0.25

IV. Results and Discussion

The reaction sequences employed for the synthesis of title compounds is shown in SCHEME I. α-Aminocarbonitriles [16, 17] were used as general precursors for the synthesis of broad range of biologically active thienopyrimidines and triazolothienopyrimidines. The precursor 2-amino-5,5-dimethyl-7-oxo-4,5,6,7-tetrahydro-1-benzothiophene-3-carbo-nitrile **(1)** was prepared by the reaction of 1,3-dimedone respectively under conditions reported by K. Gewald [13, 18,

19]. Formation of thiophene having α-aminonitrile was characterized by the presence of band at 2210 cm^{-1} due to cyano group and N-H stretching bands at 3339 and 3190 cm^{-1}. Further it is also supported by the presence of D_2O exchangeable broad singlet at δ 7.48 in ^1H NMR spectrum due to NH_2 group.

Thienopyrimidin-4-one **2** was prepared by the microwave irradiation of 2-amino-3-cyanothiophene **1** in presence of formic acid. The structure of **2** was ascertained by the absence of 2210 cm^{-1} due to cyano group and the presence of $\nu_{c=o}$ in IR and a signal at δ 8.21 due to N=CH. And also a D_2O exchangeable broad singlet at δ 11.8 for NH in the ^1H NMR spectrum, along with the other expected signals.

Thienopyrimidin-4-one **2** on treatment with dry dioxane and phosphorous oxychloride afforded the 4-chloro-thienopyrimidine **3**. Formation of these products was confirmed by the absence of ν_{NH} and $\nu_{C=O}$ bands in IR. Thus obtained 4-chlorothienopyrimidine **3** on treatment with hydrazine hydrate afforded the hydrazino derivative **4.** Formation of the products was confirmed by the presence of bands at 3420, 3349 and 3246 cm^{-1} in IR spectrum, due to amino functional groups. ^1H NMR spectrum shows D_2O exchangeable singlets at δ 4.72 and 4.35 due to amino groups and the C_2-H of pyrimidine resonated at δ 8.24 as a singlet along with other expected signals.

TABLE II
COMPARATIVE DATA OF CONVENTIONAL AND MICROWAVE-ASSISTED SYNTHESIS OF COMPOUNDS

Compounds	Microwave-assisted (method-A)		Conventional (method-B)	
	Time (min) at 560 W	% Yield	Time (h)	% Yield
2	15	80	5	78
3	10	76	3	74
4	20	83	3	76
5a	30	90	7	85
5b	25	62	15	54
5c	30	68	8	60

The compound **4** was further converted into triazolothienopyrimidine derivatives **(5a-c)** by treatment with aliphatic acids such as formic or acetic acid or acid chlorides such as benzoyl chloride. The formation of triazole ring involving both amino groups was evident by the absence of absorption bands due to either of these groups in the IR spectrum of **4**. Further ^1H NMR spectrum also exhibited the presence of two characteristic protons each as singlet at δ 8.56 and δ 9.12 due to pyrimidine and triazole proton respectively.

Under conventional heating, these reactions have require long reaction time, high-energy consumption and the need of large amounts of solvents for work up and purification. But, the microwave assistance resulted in a remarkable acceleration of the reactions, with the reaction times decreasing significantly from hours to minutes and also the increase in the yield which as shown in TABLE II.

V. CONCLUSION

In the present investigation we have synthesized thienopyrimidines and tetracyclic triazole fused thienopyrimidines by an efficient and facile microwave irradiation method from the precursor 2-amino-5, 5-dimethyl-7-oxo-4, 5, 6, 7-tetrahydro-1-benzothiophene-3-carbonitrile **1**, which in turn was prepared by employing the well-known Gewald reaction. We report a comparative study of these syntheses under microwave irradiation and by classical heating. A fast, environment-friendly, and facile method under microwave irradiation is presented. The microwave irradiation provided a remarkable rate of acceleration for the reaction, and the reaction time decreased significantly.

Biological screening revealed that these compounds have showed promising antimicrobial activities. The antibacterial activity of compounds **2, 3, 5a** and **5c** was 62 % of that of ampicillin against *B. subtilis*. Therefore it can be suggested that these compounds show promising antibacterial activity. The antifungal activity of compound **4**, **5b** and **5c** was 50% of that of fluconazole against *C. albicans.* None of the title compounds had activity against *S. aureus*, *P. aeruginosa* and *E. Coli.*

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