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ORIGINAL ARTICLE

$POCl_3$ catalyzed, one-step, solvent-free synthesis of some novel thieno[2,3-*d*]pyrimidin-4(3*H*)-one derivatives as antimicrobial agent

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KEYWORDS

$POCl_3$;
Thieno[2,3-*d*]pyrimidin-4(3*H*)-one;
Conventional heating;
MW assisted synthesis;
Antimicrobial activity

Abstract A $POCl_3$ catalyzed, efficient, one-step and solvent-free synthesis of novel thieno[2,3-*d*]pyrimidin-4(3*H*)-one derivatives from 2-amino-4,5-substitutedthiophene-3-carbonitrile has been developed under conventional heating and microwave irradiation. The formation of compounds was confirmed using elemental analysis and spectroscopic techniques like IR, NMR (^{1}H and ^{13}C) and mass spectroscopy. Furthermore, they were screened *in vitro* to study their antimicrobial activity, which shows weak to moderate activity against all tested microorganisms.

1. Introduction

In the current era, antibiotics and synthetic antimicrobial agents have changed the scenario of the medical field in the treatment of various bacterial and fungal infections. However, occurrence of various drug-resistant microbial strains posed a concrete contest to the medicinal chemists [1]. So, the medicinal chemists are working to develop new chemical entities to conquer drug resistant strains.

Thienopyrimidine, having structural resemblance to purine, is an important class of therapeutic drugs having a broad range of biological activities. Various diversified biological activities such as antibacterial [2–5], antimicrobial [6–8], antiviral [9], anti-HIV and anti-HSV [3], anti-avian influenza virus (H5N1) [10], anti-inflammatory [5,7], analgesic [5,11], antidepressant and sedative [11] have been reported for thienopyrimidine derivatives.

Various synthetic approaches have been utilized for the synthesis of thienopyrimidines [12]. Recently, Bakavoli et al. [4] used molecular iodine as an oxidizing agent for the synthesis of thienopyrimidine via an oxidative heterocyclisation reaction. However, the synthesis of thienopyrimidine from 2-amino-4,5-substitutedthiophene-3-carbonitrile requires two steps, we tried to develop a single step and solvent-free method to generate a series of thieno[2,3-*d*]pyrimidin-4(3*H*)-one derivatives.

In recent times, microwave assisted synthesis of medicinal compounds has gained appreciation among the synthetic chemists due to their improved selectivity, shorter reaction time, eco-friendliness and superior work-up procedures [13]. Litera-

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ture survey also revealed the importance of the microwave irradiation technique for the synthesis of thienopyrimidine from 2-amino-4,5-substituted-3-carbethoxythiophene [14,15].

So, herewith we are reporting the efficient synthesis of thieno[2,3-*d*] pyrimidin-4(3*H*)-one derivatives from 2-amino-4,5-substitutedthiophene-3-carbonitrile by conventional heating as well as by microwave assisted synthesis using $POCl_3$ as a chlorinating and oxidizing agent.

2. Experimental protocols

2.1. Materials and methods

All the chemicals and solvents used were of LR grade, obtained from SD fine chemicals and Merck (Mumbai, India). The progress of reaction was tested on precoated silica gel F_{254} plates obtained from Merck (Mumbai, India) using the mobile phase toluene and ethyl acetate in 7:3 ratio. Iodine chamber and UV lamp (λ = 254 nm) were used for visualization of the spots. Chemline CL726 melting point apparatus was used for measurement of melting points in an open capillary tube and are uncorrected. The IR spectra (ν_{max}, cm^{-1}) were recorded on Shimadzu FT-IR 157 spectrophotometer as KBr disk. ^1H NMR and ^{13}C NMR (δ, ppm) spectra were recorded on Bruker advance III 500 MHz NMR spectrophotometer operating at 500 MHz and 125 MHz for ^1H and ^{13}C respectively in $CDCl_3$/DMSO-d_6 using TMS as reference standard. Mass spectra were recorded on Shimadzu GC-MS-QP2010 mass spectrometer. Elementar Vario EL III CHN analyzer was used for elemental analysis and results were found within ±0.4% of the calculated value.

*2.2. General procedure for preparation of 2-amino-4,5-substitutedthiophene-3-carbonitrile (**1f–g**)*

4-Methylcyclohexanone or 1,3-cyclohexanedione (0.01 M), malanonitrile (0.01 M), sulfur (0.01 M) and ethanol (10 mL) were mixed in a conical flask. The reaction mixture was warmed up to 40-50 °C on a water bath and then diethylamine (1 mL) was added with constant stirring in such a way that the temperature does not exceed 50 °C. Stirring was continued for 1-2 h till solid crystals gets separated. The reaction mixture was then cooled and kept in a refrigerator. The fine crystals thus obtained were filtered, dried and recrystallized from a suitable solvent to give target compounds in good yields.

*2.2.1. 2-Amino-5-ethyl-4-methylthiophene-3-carbonitrile (**1a**)*

Yield 68% (Ethanol); m.p. 102-3 °C; IR (KBr, cm^{-1}): 3332, 3185, 2968, 2912, 2215, 1622; ^1H NMR ($CDCl_3$, δ): 1.39 (t, 3H, -CH_3), 2.37 (s, 3H, -CH_3), 2.99 (q, 2H, -CH_2), 8.27 (s, 2H, -NH_2, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, δ): 9.68, 13.85, 20.73, 84.22, 116.4, 128.1, 135.07, 149.1; MS: *m*/*z* 124 (100%), 166 (M^+); Anal. Calcd. (Found) for $C_8H_{10}N_2S$: C, 57.80 (57.83); H, 6.06 (6.10); N, 16.85 (16.81).

*2.2.2. 2-Amino-6-methyl-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (**1f**)*

Yield 84% (Ethanol); m. p. 144-6 °C; IR (KBr, cm^{-1}): 3334, 3190, 2969, 2900, 2219, 1621; ^1H NMR ($CDCl_3$, δ): 1.24 (d, 3H, –CH_3), 2.05 (m, 1H, ═CH), 2.22 (d, 2H, -CH_2), 2.46 (t, 2H, -CH_2), 2.74 (t, 2H, -CH_2), 8.29 (s, 2H, -NH_2, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, δ): 20.33, 21.73, 28.3, 30.1, 31.55, 84.2, 116.4, 135.0, 138.1, 149.2; MS: *m*/*z* 150 (100%), 192 (M^+); Anal. Calcd. (Found) for $C_{10}H_{12}N_2S$: C, 62.46 (62.33); H, 6.29 (6.28); N, 14.57 (14.61).

*2.2.3. 2-Amino-7-oxo-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (**1g**)*

Yield 74% (Dioxane); m.p. 215-7 °C; IR (KBr, cm^{-1}): 3345, 3198, 2968, 2909, 2214, 1665, 1622; ^1H NMR (DMSO-d_6, δ): 2.25 (m, 2H, -CH_2), 2.85 (t, 2H, -CH_2), 3.01 (t, 2H, -CH_2), 8.30 (s, 2H, -NH_2, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, δ): 22.5, 22.7, 37.5, 84.25, 116.6, 139.5, 149.2, 163.7; MS: *m*/*z* 150 (100%), 192 (M^+); Anal. Calcd. (Found) for $C_9H_8N_2OS$: C, 56.23 (56.33); H, 4.19 (4.10); N, 14.57 (14.63).

*2.3. General procedure for preparation of thieno[2,3-d] pyrimidin-4(3H)-one derivatives (**2a–o**)*

2.3.1. Conventional synthesis

2-Amino-4,5-substitutedthiophene-3-carbonitrile (**1a–g**) (1 mM) was dissolved in appropriate aliphatic acid (2 mL). Then $POCl_3$ (0.2 mL) was added drop wise and the reaction mixture has been kept for reflux on a boiling water bath. The reaction progress was supervised using TLC. After completion of the reaction, the mixture was poured on ice-cold water (50 mL) and crude precipitates thus formed were filtered, washed with 10% sodium bicarbonate solution, dried and recrystallized from a suitable solvent.

2.3.2. Microwave assisted synthesis

A mixture of 2-amino-4,5-substitutedthiophene-3-carbonitrile (**1a–g**) (1 mM), appropriate aliphatic acid (2 mL) and alumina (0.5 g) were finely crushed and transferred to a glass vial and then phosphorus oxychloride (0.2 mL) was added to this mixture. The glass vial was then capped and microwaves were irradiated in a microwave oven (CEM, Discover microwave lab station, 2450 MHz with temperature control) at the power of 960 W for 2-4 min. After the completion of reaction (reaction monitoring by TLC), the mixture was poured on ice-cold water (50 mL). The precipitated product was filtered and washed with 10% sodium bicarbonate solution to give the desired compounds.

*2.3.3. 6-Ethyl-5-methylthieno[2,3-d]pyrimidin-4(3H)-one (**2a**)*

IR (KBr, cm^{-1}): 3164, 3071, 2969, 2910, 1667, 1578; ^1H NMR (DMSO-d_6, δ): 1.32 (t, 3H, –CH_3), 2.38 (s, 3H, –CH_3), 2.90 (q, 2H, –CH_2), 8.10 (s, 1H, ═CH), 11.90 (s, 1H, –NH of pyrimidine, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, δ): 9.68, 13.85, 20.53, 118.4, 132.1, 134.07, 145.7, 156.2, 161.0; MS: *m*/*z* 194 (100%, M^+).

*2.3.4. 6-Ethyl-2,5-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (**2b**)*

IR (KBr, cm^{-1}): 3156, 3067, 2976, 2918, 1665, 1574; ^1H NMR ($CDCl_3$, δ): 1.32 (t, 3H, –CH_3), 2.37 (s, 3H, –CH_3), 2.49 (s, 3H, –CH_3), 2.91 (q, 2H, –CH_2), 11.87 (s, 1H, –NH of pyrimidine, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, δ): 9.7, 13.9, 20.5, 21.4, 118.3, 131.9, 133.87, 153.7, 156.2, 161.0; MS: *m*/*z* 208 (100%, M^+).

*2.3.5. 2,6-Diethyl-5-methylthieno[2,3-d]pyrimidin-4(3H)-one (**2c**)*

IR (KBr, cm^{-1}): 3159, 3070, 2973, 2908, 1668, 1583; ^1H NMR ($CDCl_3$, δ): 1.29 (t, 3H, $-CH_3$), 1.36 (t, 3H, $-CH_3$), 2.39 (s, 3H, $-CH_3$), 2.74 (q, 2H, $-CH_2$), 2.93 (q, 2H, $-CH_2$), 11.90 (s, 1H, –NH of pyrimidine, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, δ): 9.8, 10.4, 13.9, 20.39, 26.4, 118.25, 131.5, 133.3, 154.9, 156.2, 161.1; MS: *m/z* 222 (100%, M^+).

*2.3.6. 6-Ethyl-2-methylthieno[2,3-d]pyrimidin-4(3H)-one (**2d**)*

IR (KBr, cm^{-1}): 3168, 3070, 2968, 2909, 1664, 1580; ^1H NMR ($CDCl_3$, δ): 1.35 (t, 3H, $-CH_3$), 2.45 (s, 3H, $-CH_3$), 2.95 (q, 2H, $-CH_2$), 7.01 (s, 1H, =CH), 11.92 (s, 1H, –NH of pyrimidine, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, δ): 15.9, 21.4, 22.6, 124.45, 133.1, 139.4, 154.1, 156.3, 161.0; MS: *m/z* 194 (100%, M^+).

*2.3.7. 2,6-Diethylthieno[2,3-d]pyrimidin-4(3H)-one (**2e**)*

IR (KBr, cm^{-1}): 3164, 3073, 2970, 2905, 1661, 1587; ^1H NMR ($CDCl_3$, δ): 1.29 (t, 3H, $-CH_3$), 1.36 (t, 3H, $-CH_3$), 2.74 (q, 2H, $-CH_2$), 2.93 (q, 2H, $-CH_2$), 7.02 (s, 1H, =CH), 11.90 (s, 1H, –NH of pyrimidine, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, δ): 10.45, 16.0, 22.4, 26.6, 124.5, 133.1, 139.2, 154.1, 156.3, 161.0; MS: *m/z* 208 (100%, M^+).

*2.3.8. 5-Ethyl-2,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (**2f**)*

IR (KBr, cm^{-1}): 3154, 3063, 2977, 2918, 1666, 1575; ^1H NMR ($CDCl_3$, δ): 1.29 (t, 3H, $-CH_3$), 2.39 (s, 3H, $-CH_3$), 2.47 (s, 3H, $-CH_3$), 2.87 (q, 2H, $-CH_2$), 11.78 (s, 1H, –NH of pyrimidine, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, δ): 10.35, 15.4, 17.6, 21.45, 120.5, 127.0, 133.6, 154.2, 156.2, 161.0; MS: *m/z* 208 (100%, M^+).

*2.3.9. 2,5-Diethyl-6-methylthieno[2,3-d]pyrimidin-4(3H)-one (**2g**)*

IR (KBr, cm^{-1}): 3157, 3074, 2970, 2912, 1663, 1578; ^1H NMR ($CDCl_3$, δ): 1.29 (t, 3H, $-CH_3$), 1.34 (t, 3H, $-CH_3$), 2.36 (s, 3H, $-CH_3$), 2.74 (q, 2H, $-CH_2$), 2.93 (q, 2H, $-CH_2$), 11.90 (s, 1H, –NH of pyrimidine, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, δ): 10.32, 10.45, 15.42, 17.7, 26.45, 118.5, 127.0, 133.6, 154.2, 156.3, 161.0; MS: *m/z* 222 (100%, M^+).

*2.3.10. 2-Ethyl-6,7-dihydro-3H-cyclopenta[4,5]thieno[2,3-d]pyrimidin-4(5H)-one (**2h**)*

IR (KBr, cm^{-1}): 3221, 3074, 2970, 2856, 1674, 1578; ^1H NMR ($CDCl_3$, δ): 1.31 (t, 3H, -CH_3), 2.47 (pentet, 2H, $-CH_2$), 2.73 (q, 2H, $-CH_2$), 2.94 (t, 2H, $-CH_2$), 3.03 (q, 2H, $-CH_2$), 11.97 (s, 1H, –NH of pyrimidine, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, δ): 10.42, 24.8, 25.5, 26.45, 32.0, 118.3, 125.6, 139.5, 154.2, 156.3, 161.0; MS: *m/z* 220 (100%, M^+).

*2.3.11. 2-Ethyl-6,7,8,9-tetrahydro-3H-cyclohepta[4,5]thieno[2,3-d]pyrimidin-4(5H)-one (**2i**)*

IR (KBr, cm^{-1}): 3210, 3064, 2975, 2857, 1679, 1568; ^1H NMR ($CDCl_3$, δ): 1.33 (t, 3H, $-CH_3$), 1.69 (m, 4H, -CH_2), 1.90 (pentet, 2H, $-CH_2$), 2.47 (pentet, 2H, $-CH_2$), 2.82 (t, 2H, $-CH_2$), 3.33 (t, 2H, $-CH_2$), 11.85 (s, 1H, –NH of pyrimidine, D_2O exchangeable): ^{13}C NMR ($CDCl_3$, δ): 10.4, 20.1, 26.2, 26.55, 27.8, 28.8, 29.3, 118.3, 125.6, 139.5, 154.2, 156.3, 161.1; MS: *m/z* 248 (100%, M^+).

*2.3.12. 7-Methyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4(3H)-one (**2j**)*

IR (KBr, cm^{-1}): 3178, 3076, 2972, 2856, 1665, 1576; ^1H NMR ($CDCl_3$, δ): 1.31 (d, 3H, $-CH_3$), 2.06 (m, 1H, =CH), 2.18 (d, 2H, $-CH_2$), 2.43 (t, 2H, $-CH_2$), 2.74 (t, 2H, $-CH_2$), 7.84 (s, 1H, =CH), 11.82 (s, 1H, –NH of pyrimidine, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, δ): 20.4, 21.8, 28.7, 30.1, 31.6, 118.2, 125.5, 139.4, 145.7, 155.2, 161.0; MS: *m/z* 220 (100%, M^+).

*2.3.13. 2,7-Dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4(3H)-one (**2k**)*

IR (KBr, cm^{-1}): 3175, 3074, 2972, 2856, 1666, 1577; ^1H NMR ($CDCl_3$, δ): 1.30 (d, 3H, $-CH_3$), 2.06 (m, 1H, =CH), 2.18 (d, 2H, $-CH_2$), 2.37 (s, 3H, $-CH_3$), 2.43 (t, 2H, $-CH_2$), 2.74 (t, 2H, $-CH_2$), 11.81 (s, 1H, –NH of pyrimidine, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, δ): 20.4, 21.3, 21.8, 28.7, 30.1, 31.6, 118.2, 125.5, 139.4, 154.2, 155.25, 161.0; MS: *m/z* 234 (100%, M^+).

*2.3.14. 2-Ethyl-7-methyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4(3H)-one (**2l**)*

IR (KBr, cm^{-1}): 3176, 3076, 2978, 2850, 1665, 1572; ^1H NMR ($CDCl_3$, δ): 1.26 (t, 3H, $-CH_3$), 1.31 (d, 3H, $-CH_3$), 2.05 (m, 1H, =CH), 2.18 (d, 2H, $-CH_2$), 2.43 (t, 2H, $-CH_2$), 2.74 (t, 2H, $-CH_2$), 2.87 (q, 2H, $-CH_2$), 11.80 (s, 1H, –NH of pyrimidine, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, δ): 10.5, 20.45, 21.8, 26.4, 28.7, 30.15, 31.6, 118.2, 125.45, 139.42, 155.2, 156.4, 161.0; MS: *m/z* 248 (100%, M^+).

*2.3.15. 6,7-Dihydrobenzo[4,5]thieno[2,3-d]pyrimidin-4,8(3H,5H)-dione (**2m**)*

IR (KBr, cm^{-1}): 3225, 3086, 2965, 2857, 1672, 1565; ^1H NMR ($CDCl_3$, δ): 2.43 (t, 2H, $-CH_2$), 2.74 (pentet, 2H, $-CH_2$), 2.92 (t, 2H, $-CH_2$), 7.53 (s, 1H, =CH), 12.03 (s, 1H, –NH of pyrimidine, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, δ): 22.6, 22.8, 38.1, 119.8, 139.4, 145.65, 161.1, 162.2, 166.9, 191.2; MS: *m/z* 220 (100%, M^+).

*2.3.16. 2-Methyl-6,7-dihydrobenzo[4,5]thieno[2,3-d]pyrimidine-4,8(3H,5H)-dione (**2n**)*

IR (KBr, cm^{-1}): 3215, 3096, 2961, 2854, 1671, 1565; ^{1}HNMR ($CDCl_3$, δ): 2.37 (s, 3H, $-CH_3$), 2.43 (t, 2H, $-CH_2$), 2.73 (pentet, 2H, $-CH_2$), 2.92 (t, 2H, $-CH_2$), 12.05 (s, 1H, –NH of pyrimidine, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, δ): 21.3, 22.6, 22.8, 38.1, 119.8, 139.4, 154.4, 161.1, 162.2, 166.9, 191.2; MS: *m/z* 234 (100%, M^+).

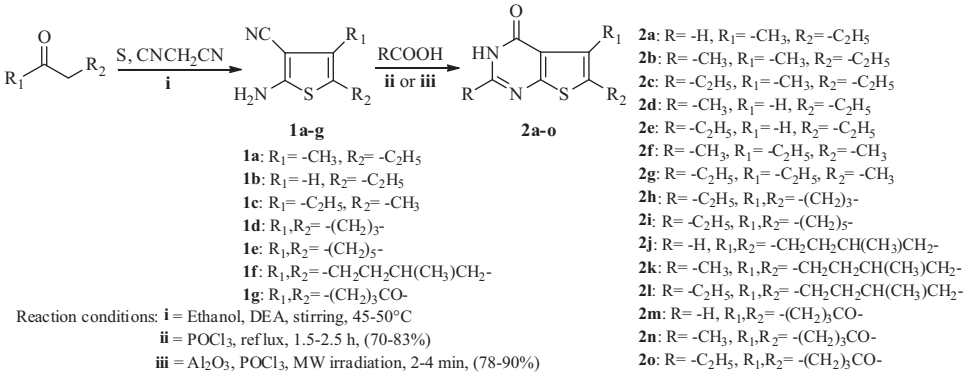
*2.3.17. 2-Ethyl-6,7-dihydrobenzo[4,5]thieno[2,3-d]pyrimidine-4,8(3H,5H)-dione (**2o**)*

IR (KBr, cm^{-1}): 3221, 3090, 2967, 2855, 1670, 1563; ^1H NMR ($CDCl_3$, δ): 1.29 (t, 3H, $-CH_3$), 2.42 (t, 2H, $-CH_2$), 2.73 (pentet, 2H, $-CH_2$), 2.87 (q, 2H, $-CH_2$), 2.93 (t, 2H, $-CH_2$), 12.07 (s, 1H, –NH of pyrimidine, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, δ): 10.4, 22.6, 22.8, 26.5, 38.1, 119.8, 139.4, 155.4, 161.0, 162.2, 166.9, 191.3; MS: *m/z* 248 (100%, M^+).

2.4. Biological screening

2.4.1. Antibacterial activity

Both Gram (+)ve (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram (-)ve (*Pseudomonas aerusinosa* and *Escherichia coli*)



Scheme 1 Synthetic route of compounds **2a**–**o**.

Table 1 Physicochemical and analytical data of thieno[2,3-*d*]pyrimidin-4(3*H*)-one derivatives (**2a**–**o**).

Compd.	M.P. (°C)	Recrystalisation solvent	Molecular formula (M.W.)	Elemental analysis					
				Calculated %			Found %		
				C	H	N	C	H	N
2a	203-4	Methanol	$C_9H_{10}N_2OS$ (194.25)	55.65	5.19	14.42	55.53	5.20	14.53
2b	208-9	Ethanol	$C_{10}H_{12}N_2OS$ (208.28)	57.67	5.81	13.45	57.63	5.75	13.48
2c	200-1	Ethanol	$C_{11}H_{14}N_2OS$ (222.31)	59.43	6.35	12.60	59.56	6.40	12.49
2d	206-7	Ethanol	$C_9H_{10}N_2OS$ (194.25)	55.65	5.19	14.42	55.53	5.20	14.53
2e	192-4	Ethanol	$C_{10}H_{12}N_2OS$ (208.28)	57.67	5.81	13.45	57.60	5.80	13.48
2f	211-2	Ethanol	$C_{10}H_{12}N_2OS$ (208.28)	57.67	5.81	13.45	57.61	5.75	13.43
2g	202-3	Ethanol	$C_{11}H_{14}N_2OS$ (222.31)	59.43	6.35	12.60	59.56	6.40	12.49
2h	198-9	Ethyl acetate	$C_{11}H_{12}N_2OS$ (220.29)	59.97	5.49	12.72	59.90	5.44	12.68
2i	210-1	Ethyl acetate	$C_{13}H_{16}N_2OS$ (248.34)	62.87	6.49	11.28	62.90	6.44	11.23
2j	154-6	Ethanol	$C_{11}H_{12}N_2OS$ (220.29)	59.97	5.49	12.72	59.89	5.69	12.43
2k	208-9	Ethanol	$C_{12}H_{14}N_2OS$ (234.32)	61.51	6.02	11.96	61.38	5.96	12.04
2l	160-2	Ethyl acetate	$C_{13}H_{16}N_2OS$ (248.34)	62.87	6.49	11.28	62.79	6.41	11.31
2m	254-6	Ethanol	$C_{10}H_8N_2O_2S$ (220.25)	54.53	3.66	12.72	54.41	3.59	12.80
2n	286-7	Ethanol	$C_{11}H_{10}N_2O_2S$ (234.27)	56.39	4.30	11.96	56.41	4.41	11.86
2o	260-2	Ethanol	$C_{12}H_{12}N_2O_2S$ (248.30)	58.05	4.87	11.28	58.10	4.90	11.18

bacteria were used for *in vitro* antibacterial study by the agar well diffusion method [16]. Various solutions (50 μg/mL) of all test compounds and streptomycin were prepared in DMSO. Mueller-Hinton agar media was sterilized for 15 min. at 121 °C and transferred to sterile petri plates to form a uniform layer and then allowed to solidify. The different strains of bacteria were then inoculated uniformly on solidified agar media using a sterile cotton swab. The bores were made in agar plates using a 6 mm sterile borer and filled with 0.1 mL of all test solutions and streptomycin solution. The plates were then incubated at 37 °C for a period of 24 h and the zone of inhibition (mm) was measured. DMSO solution was used as control.

2.4.2. Antifungal activity

In vitro antifungal activity of all the compounds was screened against *Candida albicans* and *Aspergillus niger* by agar well diffusion method [17]. Various solutions (50 μg/mL) of all test compounds and amphotericin B were prepared in DMSO. Sterilized molten mass of potato dextrose agar was poured into previously sterilized petri plates and kept at room temperature to solidify. The fungi were then inoculated on the solidified media using a sterile cotton swab. The bores were made in agar plates with the help of a 6 mm sterile borer and 0.1 mL of all test solutions, DMSO and amphotericin B solution was added. All the plates were then incubated at 37 °C for a period of 24-48 h and the zone of inhibition was measured in mm.

3. Results and discussion

The starting material 2-amino-4,5-substitutedthiophene-3-car bonitrile (**1a**–**g**) was attained in good yield by condensation reaction of appropriate ketone/aldehyde, elemental sulfur and malanonitrile as reported earlier [18]. Although, solvent free microwave assisted synthetic method has been recently reported [19,20], Gewald's conventional method is more practical and economical as far as the synthesis of 2-aminothiophene is concerned. Therefore, compounds (**1a**–**g**) were prepared in sufficient quantities by Gewald reaction.

The formation of compounds was confirmed from analysis of their spectral and elemental data. The IR spectrum of compound **1a** showed symmetric and asymmetric stretching of the primary amino group (3185 and 3332 cm^{-1}) and the presence of a cyano group (2215 cm^{-1}). The ^1H NMR of **1a** showed a triplet and quartet at δ 1.39 (3H) and 2.99 (2H) respectively, indicating the presence of an ethyl at 5th position of the thiophene ring and a singlet at δ 2.37 indicating the presence of

Table 2 Comparison of conventional and microwave assisted synthesis.

Compd.	Conventional method		Microwave assisted method	
	Reaction time (h)	% Yield*	Reaction time (min)	% Yield*
2a	1.5	82	2	90
2b	2	79	2.5	86
2c	2.5	81	3	88
2d	2	83	2.5	89
2e	2.5	79	3.5	85
2f	2	78	2.5	84
2g	2.5	75	3.5	81
2h	2.5	71	3.5	79
2i	2.5	70	4	78
2j	2	76	2	82
2k	2	77	2.5	84
2l	2.5	75	3	81
2m	2	78	2.5	83
2n	2	73	2.5	82
2o	2.5	72	3.5	85

* Yield refers to pure isolated products.

a methyl at 4th position of the thiophene ring. The two protons of the NH_2 at 2nd position of thiophene ring appeared at 8.27 which is D_2O exchangeable, whereas its ^{13}C NMR spectra showed the characteristic peaks at δ 9.68 (CH_3 at C-4), 13.85 and 20.73 (CH_3 and CH_2 at C-5), 84.22 (C-3), 116.4 (C═N), 128.1 (C-4), 135.07 (C-5), 149.1 (C-2) ppm respectively. In mass spectrum, the molecular ion peak (M^+) appeared at *m*/*z* 166, which was identical with its molecular formula.

A number of syntheses of thieno[2,3-*d*] pyrimidin-4(3*H*)-one have been reported in the literature [21–23]. However, these methods suffer from some disadvantages like the use of hydrogen chloride gas, low yield (20–40%) etc. Literature survey revealed that attempts were made to convert *ortho* amino ester of thiophene to thienopyrimidines via thienooxazinones which truncated the usage of HCl gas. However, in this route the yield was reduced upto 40–50% [24]. This gave us an impetus to develop a new route to synthesize the target molecule that may restrict the use of HCl gas, without affecting % yield.

Nanda and Trotter [25] reported the use of $POCl_3$ in the synthesis of 2-trifluoroethylbenzimidazoles by intramolecular cyclization reaction. Therefore compounds (**1a**–**g**) were reacted with aliphatic acids in the presence of $POCl_3$ to give thieno[2,3-*d*]pyrimidin-4(3*H*)-one derivatives. A microwave assisted, solvent free method is also developed which gives better yield and requires a shorter reaction time compared to conventional synthesis. These reactions are summarized in Scheme 1. The physicochemical and micro-analytical data of synthesized compounds are presented in Table 1. Both synthetic methods are compared in terms of % yield and reaction time. The results are presented in Table 2.

The spectral and micro analytical data confirmed the structures of newly synthesized compounds. For example, IR spectrum of **2a** showed a peak at 3164 cm^{-1} for the secondary amino group, disappearance of cyano group at 2215 cm^{-1} and appearance of a carbonyl group at 1667 cm^{-1} indicated cyclization of 3-cyano-2-aminothiophene. The ^1H NMR spectrum in $CDCl_3$ showed a triplet of methyl and quartet of methylene proton of ethyl at δ 1.32 and 2.90 ppm respectively as well as a singlet of methyl at δ 2.38 ppm. Spectrum also showed a singlet broad peak at δ 11.90 ppm of D_2O exchangeable secondary amino group of pyrimidine. The characteristic signal for the aromatic proton was observed at δ 8.10 ppm. Similarly, its ^{13}C NMR spectrum showed the characteristic peaks at δ 9.68 (CH_3 at C-5), 13.85 and 20.53 (CH_3 and CH_2 at C-6), 118.4 (C-4a), 132.1 (C-5), 134.07 (C-6), 145.7 (C-2), 156.2 (C-1a), 161.0 (C═O) ppm respectively, which confirmed replacement of the cyano group by a carbonyl group i.e. formation of thieno[2,3-*d*]pyrimidin-4(3*H*)-one. The molecular

Table 3 Antimicrobial activity of newly synthesized thieno[2,3-*d*]pyrimidin-4(3*H*)-one derivatives (**2a-o**).

Compound	*Zone of inhibition (in mm) ± S.D.					
	Gram (+)ve bacteria		Gram (-)ve bacteria		Fungi	
	B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
2a	7.33 ± 0.58	7.67 ± 1.15	10.00 ± 1.00	8.67 ± 1.15	9.00 ± 1.00	9.33 ± 0.58
2b	7.67 ± 0.58	8.33 ± 0.58	10.67 ± 0.58	9.33 ± 0.58	9.33 ± 0.58	8.67 ± 1.15
2c	7.33 ± 0.58	7.67 ± 0.58	9.33 ± 0.58	9.00 ± 1.00	8.67 ± 1.15	8.33 ± 0.58
2d	8.33 ± 0.58	8.67 ± 0.58	10.00 ± 1.00	9.67 ± 0.58	9.33 ± 0.58	9.00 ± 1.00
2e	7.67 ± 1.15	7.67 ± 0.58	9.67 ± 0.58	10.00 ± 1.00	9.00 ± 1.00	9.33 ± 0.58
2f	8.33 ± 0.58	8.67 ± 1.15	10.67 ± 1.15	10.33 ± 0.58	9.67 ± 0.58	9.67 ± 0.58
2g	8.00 ± 1.00	9.67 ± 0.58	10.67 ± 0.58	10.00 ± 1.00	9.33 ± 1.15	9.33 ± 0.58
2h	7.67 ± 0.58	8.00 ± 1.00	11.33 ± 0.58	10.67 ± 0.58	10.00 ± 1.00	10.00 ± 1.00
2i	8.67 ± 0.58	9.00 ± 1.00	11.67 ± 0.58	10.33 ± 0.58	9.67 ± 0.58	9.67 ± 1.15
2j	10.33 ± 0.58	9.33 ± 0.58	12.00 ± 1.00	10.67 ± 1.15	11.67 ± 0.58	10.67 ± 0.58
2k	10.67 ± 0.58	9.67 ± 1.15	13.33 ± 0.58	11.00 ± 1.00	11.33 ± 1.53	10.67 ± 0.58
2l	10.33 ± 0.58	10.00 ± 1.00	11.00 ± 1.00	10.67 ± 1.15	11.33 ± 1.15	10.00 ± 1.00
2m	11.67 ± 0.58	9.67 ± 1.15	12.00 ± 1.00	10.67 ± 0.58	10.67 ± 0.58	10.33 ± 1.15
2n	11.67 ± 1.15	9.67 ± 0.58	11.67 ± 1.15	10.33 ± 0.58	10.33 ± 0.58	9.67 ± 0.58
2o	11.33 ± 0.58	9.00 ± 1.00	10.67 ± 0.58	11.00 ± 1.00	10.00 ± 1.00	9.33 ± 1.15
Streptomycin	17.00 ± 1.00	19.67 ± 1.53	20.67 ± 0.58	19.33 ± 0.58	–	–
Amphotericin B	–	–	–	–	16.33 ± 0.58	17.33 ± 0.58
Control	–	–	–	–	–	–

* Average of triplicate reading at conc. 50 μg/mL; S.D. = Standard Deviation.

ion peak of compound **2a** was observed at m/z 194 (M^+), which is in full agreement with its molecular formula.

All the newly synthesized compounds were screened *in vitro* for their preliminary antimicrobial activity against various microorganisms. The results of preliminary *in vitro* antimicrobial testing of compounds **2a**–**o** are shown in Table 3.

The result showed that the entire series of synthesized compounds exhibit weak to good activities as compared to standard drugs against all tested microorganisms. From antimicrobial data it can be concluded that methyl cyclohexane and cyclohexanone group on the thiophene ring, exhibited better activity against all tested microorganisms compared to other alkyl and cycloalkyl substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-one. The compounds bearing methylcyclohexyl group (**2j**, **2k** and **2l**) has shown the highest sensitivity against *S. aureus*, *E. coli*, *P. aerusinosa*, *A. niger* and *C. albicans*. Cyclohexanone substituent containing compounds (**2m**, **2n** and **2o**) were found to possess good activity against *B. subtilis* and *P. aeruginosa*. Furthermore, it is also concluded that –H, $–CH_3$ and $–C_2H_5$ substitutions at 6^{th} position have less effect and slightly modify the antimicrobial activity over a wide range of tested microorganisms.

4. Conclusion

To conclude, we have successfully developed $POCl_3$ catalyzed, efficient, one-step, solvent-free synthesis of thieno[2,3-*d*]pyrimidin-4(3*H*)-one derivatives by conventional and microwave irradiation techniques. All the tested compounds showed weak to moderate antimicrobial activity against all bacteria and fungi used, it indicates that thieno[2,3-*d*]pyrimidin-4(3*H*)-one can be a potential lead for the development of antimicrobial agents.

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