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Research Article

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Design, Characterisation and Anticonvulsant Evaluation of Thienopyrimidinone Derivatives Synthesised by Green Chemistry

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ABSTRACT

The synthesis and biological evaluation of some novel thieno (2,3-d)pyrimidin-4(3H)one derivatives was aimed at creating a new scaffold. Considering the previous articles activity profile of thienopyrimidinone derivatives, ten new substituted thieno(2,3-d)pyrimidin-4(3H)one derivatives have been synthesized by reacting different 2-amino-4,5-substituted thiophene-3-carbonitrile with appropriate aliphatic carboxylic acid in presence of phosphoryl trihloride and alumina by conventional synthesis as well as microwave assisted route. Structural characterizations of all the synthesized compounds were performed by using spectral and elemental analysis. All the synthesized compounds evaluated for their antimicrobial and anticonvulsant activities by using disc diffusion method and maximal electroshock induced seizures (MES) method. It was found that the compounds having potent antimicrobial and anticonvulsant activities.

Keywords: Pyrimidinone; Anti-convulsant; Antimicrobial; Phosphoryl trichloride; Thiophene

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INTRODUCTION

Epilepsy is one of the world's oldest known neurological disorders affecting people of all ages. It is characterized by unpredictable and periodic seizures. It is estimated that it affects approximately 50 million of the global population and 2.4 million new cases are added to these figures every year.

Many of the heterocyclic compounds are being used for various therapeutic purposes and they are playing a vital role in biological life, so there is a need to synthesize and screen for newer heterocyclic compounds. The heterocyclic compounds pyrimidinone is the attractive nucleus of our present work. Many of the researchers had reported activities of pyrimidinone nucleus for analgesic [1], antibacterial [2], antimicrobial [3,4], antioxidant [5,6], antimalarial [7], antifungal [8,9], anti-inflammatory [10,11], anticancer [12-15], antiviral [16], anti-tubercular [17], antihypertensive [18,19], cardiovascular [20], CNS activity [21] antidiabetic [22] and anti-convulsant [23].

EXPERIMENTAL SECTION

Materials and Methods

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine and UV light. All compounds were purified by recrystallization with suitable organic solvents. IR spectra were recorded on Brooker-ALPHA FT-IR instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using direct inlet probe technique. ^{1}H NMR and ^{13}C NMR was determined in $CDCl_3$ solution on a Bruker Ac 400 MHz spectrometer. Purity of the synthesized compounds was checked by HPLC Agilent. The results are in agreements with the structures assigned. Elemental analysis of the all the synthesized compounds was carried out on Euro EA 3000 elemental analyser and the results are in agreements with the structures assigned.

Biological Evaluation

Antibacterial activity:

The synthesized compounds were tested for their antibacterial activity against namely *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96), *Pseudomonas aeruginosa* (MTCC 741), *Escherichia coli* (MTCC 443) by using agar well diffusion method using Mueller-Hinton agar media at concentration of 50 µg/ml. Nutrient agar medium was dissolved in water and pH was adjusted to 7.0. This was then disturbed in 20 ml quantity in boiling tubes; they were then plugged tightly with non-absorbent cotton and sterilized in an autoclave. The bacterial culture (50 µl) was then added aseptically to the agar medium maintained at 45°C, mixed well and poured in to petriplates. Test solutions of compound were prepared in DMSO. After hardening, cups of 6 mm diameter each were cut into agar and 50 µl test solution having 50 µg/ml were placed in these cups. The plates were incubated at 37°C for 24 hours and the diameter of inhibition zone was measured in mm. Solvent DMSO alone was kept as control, which did not have any inhibition zone. The activity was compared with standard antibiotic streptomycin (50 µg/ml). Each experiment was performed in triplicate and average zone of inhibition was taken. Inhibition zone of the compounds are presented in Table 1.

Antifungal activity:

Antifungal activity of the synthesized compounds was tested against *Candida albicans* (MTCC 227) and *Aspergilla niger* (MTCC 282) using agar well diffusion method. Potato dextrose agar was dissolved in water and pH was adjusted to 5.6. This was then distributed 20 ml each in boiling tubes which were plugged tightly with non-absorbent cotton and sterilized. To this 50 µl of fungal spore suspension was added and thoroughly mixed with 20 ml medium aseptically and poured in to petriplates. When agar solidified, cups of 6 mm diameter were made on each of the seeded plates. These cups were filled with 50 µl of test samples of concentration of 50 µg/ml the petriplates were incubated at 28°C for 2 days. The inhibition zones produced by test compounds were compared with inhibition zones produced by pure fluconazole (50 µg/ml) used as standard. Inhibition zone of the compounds are presented in Table 2.

Anticonvulsant activity:

Anticonvulsant activity was performed by MES (maximal electroshock method). This method was approved by the Institutional Animal Ethical Committee at Chalapathi Institute of Pharmaceutical Sciences, Guntur (Ref No. IAEC/ CLPT/08/2015-16). In this MES method, adult male and female Albino rats (Wistar strain) weighing 100-200 gm were used. The animals are divided into three groups (control, standard and test) and each group comprising of three rats. The test compounds were suspended in 1% aqueous CMC suspension and were injected i.p in doses ranging from 15, 30 and 60 mg/kg body weight. Phenytoin sodium was used as a standard drug which was given in the dose 30 mg/kg by i.p which was observed to protect 100% against the induced convulsions. The control group received only 1% aqueous CMC suspension. The seizures were induced by electroconvulsiometer (SECOR INDIA, Scientific Engg., Corp; New Delhi). The aimals were subjected to electroshock by delivering the current of 150 mA through the corneal electrodes for a period of 2.0 seconds. The animals were observed for 30 min for convulsive responses. Different stages of convulsions i.e. the tonic flexion (towards upper extremities), tonic extensor phase (extension of lower extremities), clonic convulsions (intermediate jerking of limbs), stupor (unconsciousness) and recovery or death were observed for each animal. The anticonvulsant effect of newly synthesized compounds was assessed by absence or reduction of hind limb tonic extensor phase. Each value represents the mean SEM (standard error mean) of three rats significantly different from standard drug phenytoin ($t_{tab} < t_{cal}$, $P<0.05$) (students test).

RESULTS AND DISCUSSION

A series of thieno (2,3-d) pyrimidin-4(3H)-one derivatives (TP_1-TP_{15}) has been synthesized using the appropriate synthetic procedures as per the scheme by both conventional and microwave assisted method. In this method 4-Methylcyclohexanone or 1,3-cyclohexanedione, malononitrile, elemental sulphur and ethanol were taken in conical flask and warmed up to 40-50°C. Then diethylamine was added to get 2-amino-4,5-substituted thiophene-3-carbonitrile derivatives (T_1-T_7). Then these derivatives was dissolved in appropriate aliphatic acid and alumina. Then $POCl_3$ was added and mixture was refluxed. Then it was cooled and poured on ice cold water and washed with 10% $NaHCO_3$ solution, dried and recrystallized from suitable solvent to get pure crystals. All the newly synthesized compounds were characterized on the basis of M.P, R_f value, FTIR, 1H NMR, MASS spectra and elemental analysis.

Preparation of 2-Amino-4,5-Substituted Thiophene-3-Carbonitrile Derivatives (T_1-T_7)

4-Methylcyclohexanone or 1,3-cyclohexanedione (0.01 mol), malononitrile (0.01 mol), elemental sulphur (0.01 mol) and ethanol (10 ml) were taken in conical flask and warmed up to 40-50°C. Then diethylamine (1 ml) was added drop wise with constant stirring until the sulphur went into the solution. Stirring was continued for 1-2 hr till the solid separated out. It was then cooled to room temperature and kept in refrigerator overnight. The crystals obtained was filtered, dried and recrystallized from suitable solvent to give target compounds (Figure 1).

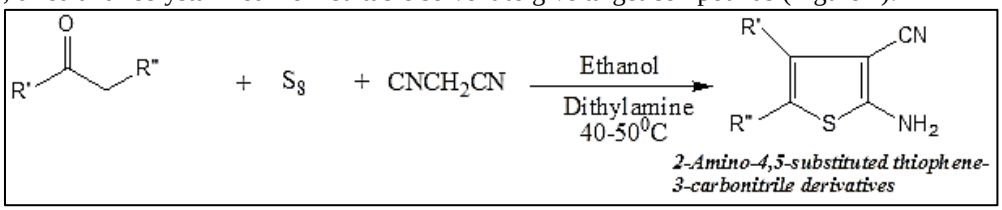

Figure 1: Synthesis of 2-amino-4,5-substituted thiophene-3-carbonitrile derivatives

2-Amino-5-ethyl-4-methylthiophene-3-carbonitrile (T_1):

Yield: 68% (Ethanol); M.P: 102-104°C; R_f : 0.76; IR (KBr, v_{max}, 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); MS: m/z 166 (M^+); Anal. Calcd. for $C_8H_{10}N_2S$: C, 57.80; H, 6.06; N, 16.85. Found: C, 57.83; H, 6.10; N, 16.81.

2-Amino-5-ethylthiophene-3-carbonitrile (T_2): Yield: 65% (Ethanol); M.P.: 62-64°C; R_f : 0.70.

2-Amino-4-ethyl-5-methylthiophene-3-carbonitrile (T_3): Yield: 53% (Ethanol); M.P: 104-106°C; R_f : 0.76.

2-Amino-5,6-dihydro-4H-cyclopenta(b)thiophene-3-carbonitrile (T_4): Yield; 51% (Ethanol); M.P: 152-154°C; R_f: 0.71.

2-Amino-5,6,7,8-tetrahydro-4H-cyclohepta(b)thiophene-3-carbonitrile (T_5): Yield: 48% (Ethanol); M.P: 126-128°C; R_f : 0.85.

2-Amino-6-methyl-4,5,6,7-tetrahydrobenzo(b)thiophene-3-carbonitrile (T_6): Yield: 84% (Ethanol); M.P: 144-146°C; R_f : 0.83; IR (KBr, v_{max}, 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); MS: m/z 192 (M^+); Anal. Calcd. for $C_{10}H_{12}N_2S$: C, 62.46; H, 6.29; N, 14.57. Found: C, 62.33; H, 6.28; N, 14.61.

2-amino-7-oxo-4,5,6,7-tetrahydrobenzo(b)thiophene-3-carbonitrile (T_7):

Yield: 74% (Dioxane); M.P: 214-216°C; R_f : 0.55; IR (KBr, v_{max}, 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); MS: m/z 192 (M^+); Anal. Calcd. for $C_9H_8N_2OS$: C, 56.23; H, 4.19; N, 14.57. Found: C, 56.33; H, 4.10; N, 14.63.

Preparation of Thieno(2,3-d)pyrimidin4(3H)-one Derivatives (TP_1-TP_{15})

Conventional method:

2-Amino-4,5-substituted thiophene-3-carbonitrile (T_1-T_7) (0.001 mol) was dissolved in appropriate aliphatic acid (2 ml). Then $POCl_3$ (0.2 ml) was added and mixture was refluxed. The progress of reaction was monitored by TLC. After the completion of reaction, mixture was cooled and poured on ice cold water (50 ml). The crude precipitate formed was filtered and washed with 10% $NaHCO_3$ solution, dried and recrystallized from suitable solvent to get pure crystals (Figure 2).

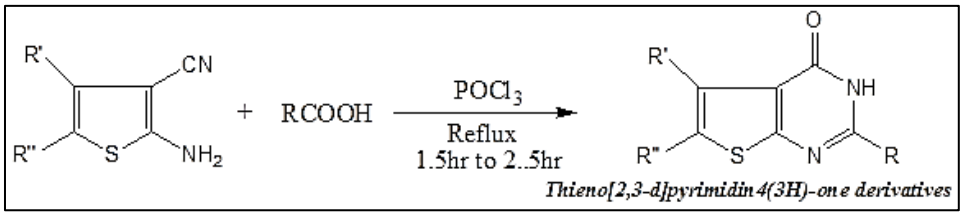

Figure 2: Synthesis of Thieno (2,3-d)pyrimidin4(3H)-one derivatives by conventional method

Microwave assisted method:

The mixture of 2-Amino-4,5-substituted thiophene-3-carbonitrile (T_1-T_7) (0.001 mol), appropriate aliphatic acid (2 ml) and alumina (0.5 gm) were finely ground with a mortar and pestle. Phosphorous oxychloride (0.2 ml) was added to this mixture in a glass vial and capped. Microwave irradiation (RAAGA) was applied for 2-4 min (one pulse each of 30 sec). After the completion of reaction (reaction monitoring by TLC), the mixture was poured into ice-cold water (50 ml). The precipitated product was filtered and washed with 10% $NaHCO_3$ solution to afford desired compound (Figure 3).

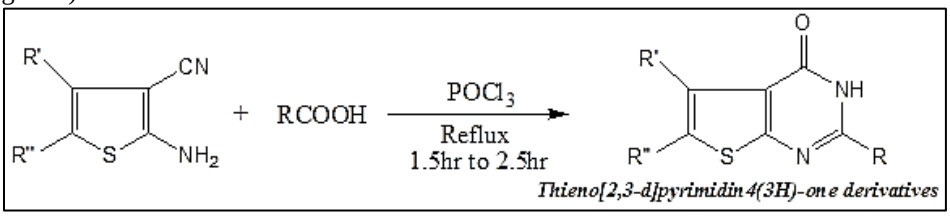

Figure 3: Synthesis of Thieno(2,3-d)pyrimidin4(3H)-one derivatives by microwave assisted method

6-ethyl-5-methylthieno(2,3-d)pyrimidin-4(3H)-one (TP_1):

Yield: 90.6% (Methanol); M.P: 202-204°C; R_f : 0.35; IR (KBr): 3164, 3071, 2969, 2910, 1667, 1578 cm^{-1}; ^1H NMR ($CDCl_3$, δ, ppm); DMSO-d_6: 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, D_2O exchangeable); MS: m/z 194 (M^+); Anal. Calcd. for $C_9H_{10}N_2OS$; C 55.53 (55.65); H 5.20 (5.19); N 14.53 (14.42).

6-ethyl-2,5-dimethylthieno(2,3-d)pyrimidin-4(3H)-one (TP_2):

Yield: 86.1% (Ethanol); M.P: 208-210°C; R_f : 0.37; IR (KBr): 3156, 3067, 2976, 2918, 1665, 1574 cm^{-1}; ^1H NMR ($CDCl_3$, δ, ppm); 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, D_2O exchangeable); MS: m/z 208 (M^+); Anal. Calcd. for $C_{10}H_{12}N_2OS$; C 57.63 (57.67); H 5.75 (5.81); N 13.48 (13.45).

2,6-diethyl-5-methylthieno(2,3-d)pyrimidin-4(3H)-one (TP_3):

Yield: 88.5% (Ethanol); M.P: 200-202°C; R_f : 0.42; IR (KBr): 3159, 3070, 2973, 2908, 1668, 1583 cm^{-1}; ^1H NMR ($CDCl_3$, δ, ppm); 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, D_2O exchangeable); MS: m/z 222 (M^+); Anal. Calcd. for $C_{11}H_{14}N_2OS$; C 59.56 (59.43); H 6.40 (6.35); N 12.49 (12.60).

6-ethyl-2-methylthieno(2,3-d)pyrimidin-4(3H)-one (TP_4):

Yield: 89.3% (Ethanol); M.P: 206-208°C; R_f : 0.32; IR (KBr): 3168, 3070, 2968, 2909, 1664, 1580 cm^{-1}; ^1H NMR ($CDCl_3$, δ, ppm); 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, D_2O exchangeable); MS: m/z 194 (M^+); Anal. Calcd. for $C_9H_{10}N_2OS$; C 55.53 (55.65); H 5.20 (5.19); N 14.53 (14.42).

2,6-diethylthieno(2,3-d)pyrimidin-4(3H)-one (TP_5): Yield: 85.4% (Ethanol); M.P: 192-194°C; R_f : 0.39; IR (KBr): 3164, 3073, 2970, 2905, 1661, 1587 cm^{-1}; ^1H NMR ($CDCl_3$, δ, ppm); 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, D_2O exchangeable); MS: m/z; 208 (M^+); Anal. Calcd. for $C_{10}H_{12}N_2OS$; C 57.60 (57.67); H 5.80 (5.81); N 13.48 (13.45).

5-ethyl-2,6-dimethylthieno(2,3-d)pyrimidin-4(3H)-one (TP_6): Yield: 84.2% (Ethanol); M.P: 210-212°C; R_f : 0.37; IR (KBr): 3154, 3063, 2977, 2918, 1666, 1575 cm^{-1}; ^1H NMR ($CDCl_3$, δ, ppm); 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, D_2O exchangeable); MS: m/z 208 (M^+); Anal. Calcd. for $C_{10}H_{12}N_2OS$; C 57.61 (57.67); H 5.75 (5.81); N 13.43 (13.45).

2,5-diethyl-6-methylthieno(2,3-d)pyrimidin-4(3H)-one (TP_7): Yield: 81.7% (Ethanol); M.P: 202-204°C; R_f : 0.41; IR (KBr): 3157, 3074, 2970, 2912, 1663, 1578 cm^{-1}; ^1H NMR ($CDCl_3$, δ, ppm); 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, D_2O exchangeable); MS: m/z 222 (M^+); Anal. Calcd. for $C_{11}H_{14}N_2OS$; C 59.56 (59.43); H 6.40 (6.35); N 12.49 (12.60).

2-ethyl-5,6,7-trihydrocyclopenta(b)thieno(2,3-d)pyrimidin-4(3H)-one (TP_8): Yield: 79.6% (Ethyl acetate); M.P: 198-200°C; R_f : 0.39; IR (KBr): 3221, 3074, 2970, 2856, 1674, 1578 cm^{-1}; ^1H NMR ($CDCl_3$, δ, ppm); 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, D_2O exchangeable); MS: m/z 220 (M^+); Anal. Calcd. for $C_{11}H_{12}N_2OS$; C 59.90 (59.97); H 5.44 (5.49); N 12.68 (12.72).

2-ethyl-5,6,7,8,9-pentahydrocyclohepta(b)thieno(2,3-d)pyrimidin-4(3H)one (TP_9): Yield: 78.9% (Ethyl acetate); M.P: 210-212°C; R_f : 0.46; IR (KBr): 3210, 3064, 2975, 2857, 1679, 1568 cm^{-1}; ^1H NMR ($CDCl_3$, δ, ppm); 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, D_2O exchangeable); MS: m/z 248 (M^+); Anal. Calcd. for $C_{13}H_{16}N_2OS$; C 62.90 (62.87); H 6.44 (6.49) ; N 11.23 (11.28).

7-methyl-5,6,7,8-tetrahydrobenzo(b)thieno(2,3-d)pyrimidin-4(3H)-one (TP_{10}): Yield: 82.1% (Ethanol); M.P: 154-156°C; R_f : 0.39; IR (KBr): 3178, 3076, 2972, 2856, 1665, 1576 cm^{-1}; ^1H NMR ($CDCl_3$, δ, ppm); 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, D_2O exchangeable); MS: m/z 220 (M^+); Anal. Calcd. for $C_{11}H_{12}N_2OS$; C 59.89 (59.97); H 5.69 (5.49) ; N 12.43 (12.72).

2,7-dimethyl-5,6,7,8-tetrahydrobenzo(b)thieno(2,3-d)pyrimidin-4(3H)one (TP_{11}): Yield: 84.5% (Ethanol); M.P: 208-210°C; R_f : 0.40; IR (KBr): 3175, 3074, 2972, 2856, 1666, 1577 cm^{-1}; ^1H NMR ($CDCl_3$, δ, ppm); 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, D_2O exchangeable); MS: m/z 234 (M^+); Anal. Calcd. for $C_{12}H_{14}N_2OS$; C 61.38 (61.51); H 5.96 (6.02) ; N 12.04 (11.96).

2-ethyl-7-methyl-5,6,7,8-tetrahydrobenzo(b)thieno(2,3-d)pyrimidin4(3H)-one (TP_{12}): Yield: 81.4% (Ethyl acetate); M.P: 160-162°C; R_f : 0.46; IR (KBr): 3176, 3076, 2978, 2850, 1665, 1572 cm^{-1}; ^1H NMR ($CDCl_3$, δ, ppm); 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, D_2O exchangeable); MS: m/z 248 (M^+); Anal. Calcd. for $C_{13}H_{16}N_2OS$; C 62.79 (62.87); H 6.41 (6.49) ; N 11.31 (11.28).

6,7-dihydrobenzo(b)thieno(2,3-d)pyrimidin-4,8(3H,5H)-dione (TP_{13}): Yield: 83.1% (Ethanol); M.P: 254-256°C; R_f : 0.22; IR (KBr): 3225, 3086, 2965, 2857, 1672, 1565 cm^{-1}; ^1H NMR ($CDCl_3$, δ, ppm); 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, D_2O exchangeable); MS: m/z 220 (M^+); Anal. Calcd. for $C_{10}H_8N_2O_2S$; C 54.41 (54.53); H 3.59 (3.66) ; N 12.80 (12.72).

2-methyl-6,7-dihydrobenzo(b)thieno(2,3-d)pyrimidin-4,8(3H,5H)-dione (TP_{14}): Yield: 82.5% (Ethanol); M.P: 286-288°C; R_f : 0.25; IR (KBr): 3215, 3096, 2961, 2854, 1671, 1565 cm^{-1}; ^1H NMR ($CDCl_3$, δ, ppm); 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, D_2O exchangeable); MS: m/z 234 (M^+); Anal. Calcd. for $C_{11}H_{10}N_2O_2S$; C 56.41 (56.39); H 4.41 (4.30) ; N 11.86 (11.76).

2-ethyl-6,7-dihydrobenzo(b)thieno(2,3-d)pyrimidin-4,8(3H,5H)-dione (TP_{15}): Yield: 85.6% (Ethanol); M.P: 260-262°C; R_f : 0.30; IR (KBr): 3221, 3090, 2967, 2855, 1670, 1563 cm^{-1}; ^1H NMR ($CDCl_3$, δ, ppm); 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, D_2O exchangeable); MS: m/z 248 (M^+); Anal. Calcd. for $C_{12}H_{12}N_2O_2S$; C 58.10 (58.05); H 4.90 (4.87); N 11.18 (11.28).

Antimicrobial Activity

All the synthesized compounds were screened for antimicrobial activity against various microorganisms and anticonvulsant activity by MES method. From the results of antimicrobial screening of compounds TP_1-TP_{15} are shown in Table 3 and anticonvulsant data shown in Table 4. The result showed that the entire series of synthesized compounds exhibit weak to good activities compared to standard drugs against all tested microorganisms. From antimicrobial data it can be concluded that methyl cyclohexane and cyclohexanone group on thiophene ring, exhibited better activity against all tested microorganisms compared to other alkyl and cycloalkyl substituted thieno(2,3-d)pyrimidines. The compounds bearing methylcyclohexyl group (TP_{10}, TP_{11} and TP_{12}) has shown the highest sensitivity against *S. aureus*, *E. coli*, *P. aeruginosa*, *A. niger* and *C. albicans*. Cyclohexanone substituents containing compounds ($TP_{13,}$ TP_{14} and TP_{15}) found to possess good activity against *B. subtilis* and *P. aeruginosa*. Furthermore, it is also concluded that -H, $-CH_3$ and $-C_2H_5$ substitution at 6^{th} position has less effect and slightly modifies the antimicrobial activity over wide range of tested microorganisms.

Table 1: Physical data of thieno (2,3-d)pyrimidin4(3H)-one derivatives

Compounds	R'	R''	R	M.F	M.W	R_f	M.P (°C)
TP_1	CH_3	C_2H_5	H	$C_9H_{10}N_2OS$	194.26	0.36	202-204
TP2	CH_3	C_2H_5	CH_3	$C_{10}H_{12}N_2OS$	208.28	0.38	208-210
TP_3	CH_3	C_2H_5	C_2H_5	$C_{11}H_{14}N_2OS$	222.31	0.42	200-202
TP_4	H	C_2H_5	CH_3	$C_9H_{10}N_2OS$	194.26	0.33	206-208
TP_5	H	C_2H_5	C_2H_5	$C_{10}H_{12}N_2OS$	208.28	0.38	192-194
TP_6	C_2H_5	CH_3	CH_3	$C_{10}H_{12}N_2OS$	208.28	0.38	210-212
TP_7	C_2H_5	CH_3	C_2H_5	$C_{11}H_{14}N_2OS$	222.31	0.42	202-204
TP_8	$-(CH_2)_3-$		C_2H_5	$C_{11}H_{12}N_2OS$	220.29	0.4	198-200
TP_9	$-(CH_2)_5-$		C_2H_5	$C_{13}H_{16}N_2OS$	234.32	0.45	210-212
TP_{10}	$-CH_2CH_2CH(CH_3)CH_2-$		H	$C_{11}H_{12}N_2OS$	220.29	0.38	154-156
TP_{11}	$-CH_2CH_2CH(CH_3)CH_2-$		CH_3	$C_{12}H_{14}N_2OS$	234.32	0.41	208-210
TP_{12}	$-CH_2CH_2CH(CH_3)CH_2-$		C_2H_5	$C_{13}H_{16}N_2OS$	248.35	0.45	160-162
TP_{13}	$-(CH_2)_3CO-$		H	$C_{10}H_8N_2O_2S$	220.25	0.23	254-256
TP_{14}	$-(CH_2)_3CO-$		CH_3	$C_{11}H_{10}N_2O_2S$	234.28	0.24	286-288
TP_{15}	$-(CH_2)_3CO-$		C_2H_5	$C_{12}H_{12}N_2O_2S$	248.3	0.31	260-262

Table 2: Reaction method, time and % yield of thieno (2,3-d)pyrimidin4(3H)-one derivatives

Compds	Conventional method		Microwave assisted method	
	Reaction time (hrs)	% Yield	Reaction time (min)	% Yield
TP_1	1.5	82	2	90
TP2	2	79	2.5	86
TP_3	2.5	81	3	88
TP_4	2	83	2.5	89
TP_5	2.5	79	3.5	85
TP_6	2	78	2.5	84
TP_7	2.5	75	3.5	81
TP_8	2.5	71	3.5	79
TP_9	2.5	70	4	78
TP_{10}	2	76	2	82
TP_{11}	2	77	2.5	84
TP_{12}	2.5	75	3	81
TP_{13}	2	78	2.5	83
TP_{14}	2	73	2.5	82
TP_{15}	2.5	72	3.5	85

Table 3: Antimicrobial activity of thieno (2,3-d)pyrimidin4(3H)-one derivatives

Compds	*Zone inhibition (in mm) ± S.D					
	Gram +ve bacteria		Gram -ve bacteria		Fungi	
	B.subtilis	*S.aureus*	*E.coli*	*P.aeruginosa*	*C.albicans*	*A.niger*
TP_1	8.33 ± 0.58	7.86 ± 1.15	11.15 ± 1.15	9.76 ± 1.15	10.00 ± 1.00	10.33 ± 0.58
TP2	8.65 ± 0.58	8.45 ± 0.58	11.67 ± 0.58	10.44 ± 0.58	10.44 ± 0.58	9.76 ± 1.15
TP_3	8.43 ± 0.58	7.86 ± 0.58	11.76 ± 0.58	10.00 ± 1.00	9.76 ± 1.15	9.33 ± 0.58
TP_4	9.45 ± 1.12	9.67 ± 0.58	10.33 ± 0.58	10.76 ± 0.58	10.33 ± 0.58	10.00 ± 1.00
TP_5	8.95 ± 0.56	7.85 ± 0.58	10.00 ± 1.00	11.15 ± 1.00	1015 ± 1.00	10.44 ± 0.58
TP_6	8.86 ± 0.58	8.68 ± 1.25	11.67 ± 1.15	11.44 ± 0.58	10.76 ± 0.58	10.76 ± 0.58
TP_7	9.05 ± 1.00	9.67 ± 0.58	11.67 ± 0.58	11.00 ± 1.00	10.35 ± 1.15	10.44 ± 0.58
TP_8	9.76 ± 0.58	8.00 ± 1.00	11.45 ± 0.58	11.76 ± 0.58	11.15 ± 1.00	11.50 ± 1.15
TP_9	11.32 ± 0.58	9.00 ± 1.00	12.67 ± 0.58	11.44 ± 0.58	10.76 ± 0.58	10.67 ± 1.00
TP_{10}	10.46 ± 0.58	9.55 ± 0.58	13.00 ± 1.15	11.66 ± 1.15	12.76 ± 0.58	11.76 ± 0.58
TP_{11}	10.67 ± 0.58	9.87 ± 0.58	15.44 ± 0.58	12.00 ± 1.00	12.44 ± 1.53	11.67 ± 0.58
TP_{12}	11.44 ± 0.58	11.00 ± 1.00	12.00 ± 1.00	11.76 ± 1.15	12.44 ± 1.15	11.00 ± 1.00
TP_{13}	12.76 ± 0.58	10.67 ± 1.15	13.00 ± 1.00	11.76 ± 0.58	11.76 ± 0.58	11.33 ± 1.15
TP_{14}	12.76 ± 1.15	10.67 ± 0.58	12.76 ± 1.15	11.44 ± 0.58	11.53 ± 0.58	10.76 ± 0.58
TP_{15}	12.44 ± 0.58	9.00 ± 1.00	11.76 ± 0.58	12.00 ± 1.00	11.00 ± 1.00	10.44 ± 1.15
Streptomycin	17.00 ± 1.00	18.67 ± 1.15	20 ± 0.58	19.44 ± 0.58	---	---
Fluconazole	---	---	---	---	16.44 ± 0.58	17.44 ± 0.58
Control	---	---	---	---	---	---

*Average of triplicate reading (conc. 50 µg/ml); S.D – Standard Deviation

Anticonvulsant Activity

All the synthesized compounds were screened for anticonvulsant activity by maximal electroshock-induced seizure (MES) method in albino rats (Wistar strain) of either sex. This method claimed to detect compounds possessing activity against generalized tonic clonic (grandmal) seizures. The MES test is a measure of an anticonvulsant drug to abolish or reduce the time of tonic extensor component of the hind limb in the maximal seizure pattern induced by 150 mA of current delivered for 0.2 seconds. The results of anticonvulsant activity data given in Table 4. In the primary MES screening compounds TP_3, TP_5, TP_7, TP_{10}, TP_{12} and TP_{14} afforded protection against seizures confirming their potential utility as prototypic molecules. The anticonvulsant activity data revealed that all the compounds showed remarkable reduction of hind limb tonic extensor phase when given in the dose of 30 mg/kg i.p and compounds TP_3, TP_{10}, TP_{12} and TP_{14} were found to be more potent compounds in this series. Moreover the anticonvulsant activity of the other tested compounds was found to be less effective than phenytoin used as standard anticonvulsant drug.

Table 4: Anticonvulsant activity of thieno(2,3-d)pyrimidin4(3H)-one derivatives

Compds	Time taken for onset of action				
	Flexion (min) (mean ± SEM)	Extensor (min) (mean ± SEM)	Clonus (min) (mean ± SEM)	Stupor (min) (mean ± SEM)	Recovery / Death
TP_1	4.5 ± 0.5	11.6 ± 0.5	13.5 ± 0.4	112.1 ± 0.4	R
TP2	5.0 ± 0.4	7.2 ± 0.2	11.8 ± 0.5	110.5 ± 0.4	R
TP_3	4.0 ± 0.4	7.5 ± 0.8	10.5 ± 0.7	110.5 ± 0.6	R
TP_4	4.8 ± 0.2	8.2 ± 0.5	12.5 ± 0.3	114.1 ± 0.7	R
TP_5	4.1 ± 0.6	6.5 ± 0.4	7.5 ± 0.4	107.3 ± 0.2	R
TP_6	5.0 ± 0.8	8.8 ± 0.4	12.5 ± 0.3	110.8 ± 0.1	R
TP_7	3.6 ± 0.4	8.2 ± 0.2	11.2 ± 0.6	118.6 ± 0.7	R
TP_8	5.2 ± 0.5	9.2 ± 0.3	10.5 ± 0.4	118.5 ± 0.5	R
TP_9	5.6 ± 0.4	6.9 ± 0.5	13.5 ± 0.7	118.2 ± 0.8	R
TP_{10}	3.7 ± 0.3	6.3 ± 0.6	7.5 ± 0.2	106.1 ± 0.9	R
TP_{11}	5.2± 0.5	9.2 ± 0.3	10.5 ± 0.4	118.5 ± 0.5	R
TP_{12}	4.0 ± 0.4	7.5 ± 0.8	10.5 ± 0.7	110.5 ± 0.6	R
TP_{13}	5.0 ± 0.6	8.6 ± 0.3	11.5 ± 0.3	120.8 ± 0.1	R
TP_{14}	3.5 ± 0.4	8.0 ± 0.2	10.2 ± 0.6	108.6 ± 0.7	R
TP_{15}	5.0 ± 0.3	7.5 ± 0.2	12.8 ± 0.5	112.5 ± 0.4	R
Control	4.1 ± 0.7	9.5 ± 0.2	11.5 ± 0.4	115.2 ± 0.6	R
Phenytoin sodium	Absent	5.8 ± 0.4	3.5 ± 0.5	102 ± 0.2	R

*Phenytoin sodium (30 mg/kg, i.p). The compounds tested for a dose of 30 mg/kg.

CONCLUSION

In this work thieno (2,3-d)pyrimidin4(3H)-one derivatives were synthesized by an efficient, solvent free, conventional and microwave assisted methods form 2-Amino-4,5-substituted thiophene-3-carbonitrile derivatives with $POCl_3$. All the newly synthesized thieno (2,3-d)pyrimidin4(3H)-one derivatives (TP_1-TP_{15}) exhibited weak to moderate antimicrobial activity. Compounds TP_3, TP_{10}, TP_{12} and TP_{14} exhibited more potent anticonvulsant activity.

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Conflict of Interest: NO

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