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SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME NEWER 3-*N*-ARYL AMINO ACETAMIDO THIENOPYRIMIDINONE DERIVATIVES

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ABSTRACT
Compounds containing thiophene and pyrimidine constitute major class of drugs with wide spectrum of biological activities. Many thienopyrimidinone analogs are also found to exhibit significant therapeutic value. Thus a new series of Thieno[2,3-d]-pyrimidin(3*H*)-4-ones have been synthesized and characterized. Starting from cycloheptanone and ethyl cyanoacetate the intermediate 2-Amino-3-carbethoxy-4, 5-penta methylene thiophene was prepared. This compound was acetylated and then condensed with hydrazine hydrate to yield the key intermediate 3-Amino -2-methyl-5, 6-pentamethylene thieno (2, 3-d) pyrimidin(3*H*)-4-one. This compound was chloro acetylated and then the active chlorine was replaced with aryl amino nucleophiles to get the title compounds. The title compounds have been purified and characterized by MP, TLC, IR, ^{1}H NMR, Mass spectral data and elemental analysis. The compounds were screened for *in vitro* antibacterial and antifungal activities. It was observed that the compounds containing electron withdrawing groups showed significant activity. These compounds can be further exploited to get the potent lead compounds.

KEY WORDS: Synthesis, Thieno [2,3-d]-pyrimidin(3*H*)-4-one, ,Characterization, Antibacterial, Antifungal.

INTRODUCTION
In recent years, heterocyclics containing sulphur and nitrogen play a major role in synthetic medicinal chemistry and pharmacological field owing to their extensive biological activities. These are extremely versatile building blocks for the manufacture of bioactive compounds in pharmaceutical drug design. This helped medicinal chemists to plan, organize and execute new approaches on discovery of novel drugs.

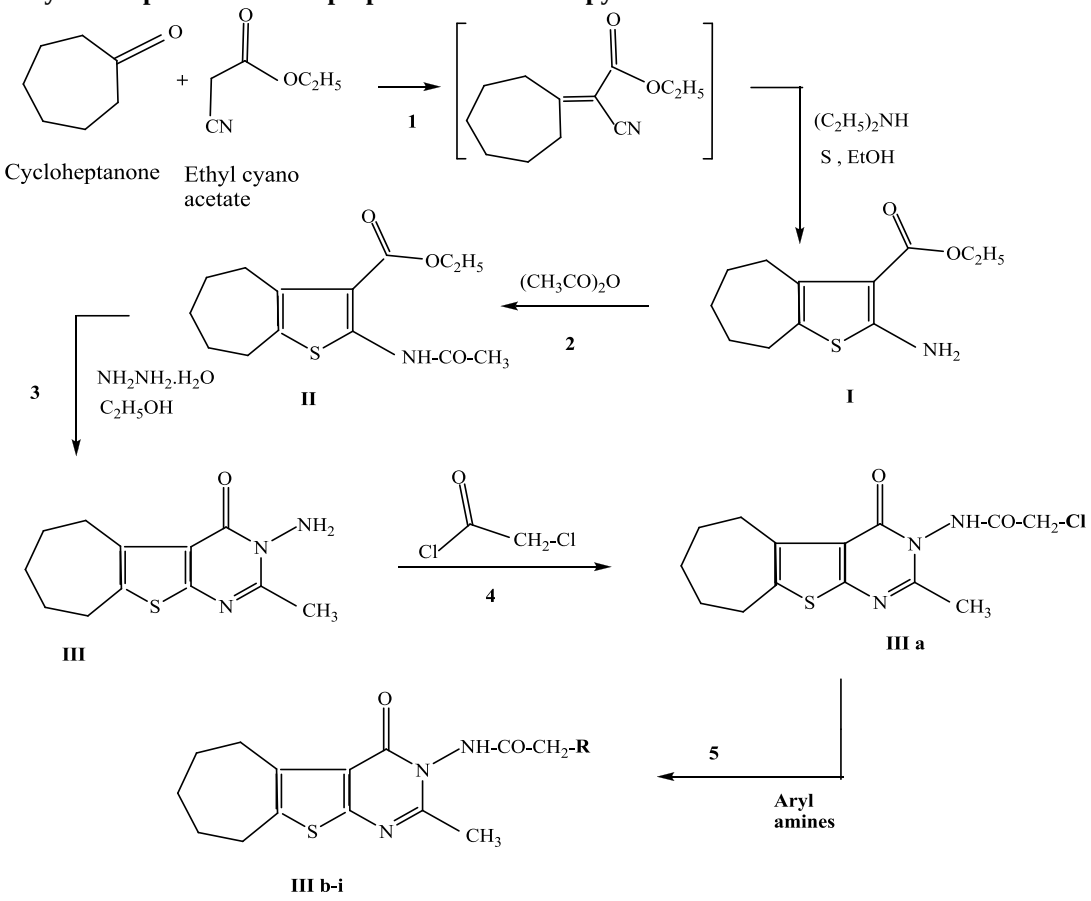
Compounds containing thiophene are distinctive molecules accounted to possess a wide spectrum of biological activities including antibacterial[1], antifungal[1], anti-inflammatory[2,3], CNS depressant[4], analgesic[5], antitumor activity[6], etc. Pyrimidines are more vital in many biological processes since they are present in several vitamines , co-enzymes, nucleic acids, etc. Many synthetic pyrimidine analogs are also known to exhibit a variety of therapeutic applications including antibacterial[7,8], antifungal[7,8], anti-inflammatory[8], analgesic[9], anti histaminic[10], antitumor activity[11], and so on. Thieno pyrimidinones are a class of bicyclic compounds involving the fusion of thiophene and pyrimidine rings. Many of their derivatives also show characteristic biological activities like antibacterial[12], antifungal[13], anti-tubercular[14], antitumor[15], protein kinase inhibiting[16,17], antiplatelet[18], anticonvulsant[19], and antiviral activities[20]. It was also noted that the reactions involving chloro acetylation followed by replacing the active chlorine by amino nucleophiles resulted in the introduction of many potent drug molecules[21,22].

The promising bioactive diversity of thiophenes, pyrimidines and thienopyrimidinones prompted us to synthesize and biologically evaluate a new series of structural variants of thieno [2, 3-d] pyrimidinone derivatives. Thus the present communication describes the synthesis and utilization of 3-amino-2-methyl-5,6-pentamethylene thieno [2,3-d] pyrimidin (3*H*)-4-one[**III**] as key prototype structural unit, and treatment of the active 3-amino group with chloro acetyl chloride and then with various aryl amines to obtain a new series of compounds[**III a-i**] . The title compounds were subsequently subjected to antibacterial and antifungal investigations.

MATERIALS AND METHODS
All the solvents and chemicals were procured from either S.D.Fine chem. Ltd or Merck, Mumbai. They were analytical grades and directly used. The purity of the compounds was routinely ensured by TLC using silica

gel-G with solvents as a mixture of Benzene and Chloroform (1:1). UV absorption data for the intermediates and target compounds were collected by using UV-Visible Spectro- photometer SL-159, Elico India Ltd reported in λ max values. ^{1}H NMR spectra were recorded in DMSO at 400MHz on Bruker AMX using tetra methyl silane (TMS) as internal standard and the data was reported in ppm. IR spectra of the compounds were evidenced using KBr pellet on Shimadzu FTIR-8700 spectrophotometer and frequencies were recorded in wave numbers. Mass spectra were recorded in Shimadzu LC MS-2010A at Quest Research Training Institute Ltd, Bangalore. The chemical reactions involved in this work is given in scheme-1.

Scheme-1: Synthetic protocol for the preparation of Thieno pyrimidinones



R= - 2′-methyl anilino (**III b**) , - 4′-methyl anilino (**III c**), - 4′-hydroxy anilino (**III d**),
- 4′-nitro anilino (**III e**), -4′-fluoro anilino (**III f**) - 2′-chloro anilino (**III g**),
-4′-chloro anilino (**III h**), -3′-chloro-4′-fluoro anilino (**III i**)

Step-1: Synthesis of 2-Amino-3- carbethoxy-4, 5-pentamethylene thiophene[23,24]:[I]

To a mixture of the cycloheptanone (4.72 ml, 0.04 mol) ,ethyl cyano acetate (4.26 ml, 0.04 mol) and sulphur powder (1.28 g ; 0.04 mol) in ethanol (40 ml), diethyl amine (4.0 ml) was added drop wise with stirring. The mixture was stirred further for 1hour at 45-50^{O}C and chilled overnight. The solid obtained was filtered, washed and recrystallized from ethanol. White solid, Yield 50.87%, M.P 84^o C, R_f value 0.60

Step-2: Synthesis of 2-Acetamido-3- carbethoxy-4, 5-pentamethylene thiophene[25,26] :[II]

A mixture of compound **I** (2.25g ;0.01mol), an excess of acetic anhydride(5.0ml; 0.05 mol) and zinc dust(0.30g; 0.01mol) was stirred and irradiated with microwave heating involving Kenstar microwave oven(2450 MHz, 900 W) for 15 seconds. After complete dissolution, the reaction mixture was cooled and the resulting white solid was crystallized from methanol. White solid, Yield 78.25%, M.P 114^{o}C, R_f value 0.53 , UV- λ max 234 nm

Step-3: Synthesis of 2-Methyl -3-*N*-amino-5, 6-pentamethylene thieno [2, 3-d] - pyrimidin (3*H*)–4-one[27]: [III]

To a mixture of compound **II** (2.67g; 0.01mol) and an excess of hydrazine hydrate (15 ml; 0.03mol), ethanol (20 ml) was added and the reaction mixture was irradiated for 20 seconds until the solid dissolved. Irradiation was continued until a solid separated out from the reaction mixture. Then the reaction mixture was cooled to room temperature, a white crystalline product

was obtained. This product was crystallized from aqueous acetone (1:2). White solid, Yield 60.42 %, M.P 180^{o}C, R_f value 0.59 ; UV- λ max 214 nm ; IR(KBr)cm^{-1}: 3361.65 (NH str., 1^{o}-NH_2), 2950.63 (aliph CH, str.), 1680.18(C=O, aryl), 1530.34 (-N– C= O cyclic str.), 1634.12 (NH bend), 1374 (CH_3 bend), 754.35(C-S); ^{1}H NMR (DMSO) δ in ppm : 4.74(s, 2H, NH_2), 2.55 (t, 4H, c.heptane), 1.62 (m, 2H, c.heptane) ,1.29 (m, 4H, c.heptane), 0.90 (s, -CH_3, 3H at 2) ; MS: m/z 249(M^+)

Step-4: Synthesis of 2-Methyl -3-*N*-(2′-chloro acetamido)-5, 6- pentamethylene thieno [2, 3-d]-pyrimidin (3*H*)–4-one[28] : [III a]

Compound **III** (2.49g, 0.01mol) was dissolved in an excess of glacial acetic acid (15.0 ml). Chloro acetyl chloride (1.13 ml, 0.01mol) was added drop wise until a solid mass was obtained. The reaction mixture was refluxed for 4 hours, cooled to room temperature and then poured into crushed ice. A cream coloured product was obtained, filtered and recrystallized from ethanol. White solid ; Yield 56.80%, MP 132 ^{O}C, R_f 0.42 ; UV- λ max 212 nm; IR(KBr)cm^{-1}: 3233.10(amide NH str.), 3050.52(Ar-CH str.), 2882.80 (aliph.CH str.), 1684.55 (C=O, aryl),1536.40 (-N–C=O cyclic str.), 1628.80 (NH bend), 1465.25 (CH_2 bend), 1378.00 (CH_3 bend), 812.80 (C-Cl, str), 756.70 (C-S) ; ^{1}H NMR (DMSO) δ in ppm : 8.05 (s, IH, NH of amide), 3.98 (d, 2H, -$COCH_2$-), 2.55 (t, 4H, c.heptane), 1.65 (m, 4H, c.heptane), 1.31 (m, 2H of c.heptane), 0.92 (s, -CH_3, 3H at 2); MS: m/z 325(M^+);

Step-5: Synthesis of 2-Methyl -3-*N*-[(substituted anilino) acetamido] -5, 6- penta methylene thieno [2, 3-d]- pyrimidin (3*H*)–4-ones [28][III b-i]

To a mixture of **IIIa** (0.01 mol) and the required aryl amine (0.01 mol), dioxane (30 ml) and potassium carbonate (2.0g) were added. The reaction mixture was refluxed for 7 hours, cooled to room temperature and poured into ice cold water (50 ml). The solid obtained as a mass was collected and dried over a watch glass and rinsing the solid with petroleum ether to remove the unreacted aryl amines. The product was recrystallized from ethanol.

Analytical and Spectral data of title compounds [III b-i]

3-*N*-[2-(2′-methyl anilino) acetamido]-2-methyl-5,6-penta methylene thieno[2,3-d] pyrimidin (3*H*)-4-one : [III b]: White solid ; Yield 52.20%, MP 114 ^{O}C, R_f 0.56 ; UV- λ max 212 nm ; IR(KBr)cm^{-1} : 3277.00 (amide NH str.), 3066.44 (Ar-CH str.), 2886 (aliph. CH str), 1670.64 (C=O, str.), 1548.52(-N– C= O cyclic str.), 1615.46 (NH bend), 1460.20 (CH_2 bend), 1320.50 (arom C-N, str.), 1050.40 (aliph C-N str.), 1380.45(CH_3 bend), 754.52 (C-S); ^{1}H NMR (DMSO) δ in ppm : 8.06 (s, 1H, NH of amide) , 7.15-6.70 (m, 4H, Ar-H ′), 4.05 (t,1H, NH of amine), 3.90 (d, 2H, -$COCH_2$-), 3.25(s.3H, 2′-CH_3), 2.52 (t, 4H ,c.heptane), 1.67 (m, 4H, c.heptane), 1.30 (m, 2H of c.heptane), 0.90 (s, -CH_3, 3H at 2); MS: m/z 396 (M^+); Elemental analysis: Calculated for $C_{21}H_{24}O_2N_4S$: C-63.64, H-6.06, O- 8.08, N-14.14, S-8.08. Found: C-62.56, H-6.18, O- 8.20, N-14.20, S-8.02

3-*N*-[2-(4′-methyl anilino) acetamido]-2-methyl-5,6-penta methylene thieno[2,3-d] pyrimidin (3*H*)-4-one : [III c]: White solid ; Yield 63.50%, MP 116^{o} C, R_f 0.58 ; UV- λ max 214 nm ; IR(KBr) cm^{-1}: 3280.40 (amide NH str.), 3069.40 (Ar-CH str.), 2882.22 (aliph.CH str), 1680.30 (C=O, str.),1545.55 (-N– C= O cyclic str.), 1620.40 (NH bend), 1460.20 (CH_2 bend), 1322.70 (arom C-N, str.), 1053.48 (aliph C-N str.), 1381.48 (CH_3 bend), 756.50(C-S); ^{1}H NMR (DMSO) δ in ppm : 8.01(s, IH, NH of amide), 6.85 (d, 2H, Ar-H at 3′ and 5′) , 6.31(d, 2H, Ar-H at 2′ and 6′), 4.02 (t,1H, NH of amine), 3.95 (d,2H, -$COCH_2$-) 3.36 (s.3H, 4′-CH_3), 2.55 (t, 4H ,c.heptane), 1.62 (m, 4H, c.heptane), 1.29 (m, 2H of cyclo- heptane) , 0.90 (s, -CH_3, 3H at 2); MS: m/z 396(M^+) ; Elemental analysis: Calculated for $C_{21}H_{24}O_2N_4S$: C-63.64, H-6.06, O- 8.08, N-14.14, S-8.08. Found: C-64.32, H-6.45, O- 8.32, N-14.28, S-8.25.

3-*N*-[2-(4′-hydroxy anilino) acetamido]-2-methyl-5,6-penta methylene thieno [2,3-d] pyrimidin (3*H*)-4-one : [III d]: White solid ; Yield 58.45%, MP 120 ^{O}C, R_f 0.62 ; UV- λ max 213 nm ; IR(KBr) cm^{-1}: 3285.50(-OH), 3240.15 (amide NH str.), 3055.52 (Ar-CH str.), 2887.90 (aliph.CH str.), 1688.20 (C=O, aryl), 1535.44 (-N–C=O cyclic str.), 1622.80 (NH bend), 1335.55 (arom C-N str), 1052.45 (aliph C-N str.), 1467.20 (CH_2 bend), 1380.45 (CH_3 bend), 755.82 (C-S) ; ^{1}H NMR (DMSO) δ in ppm : 8.00 (s, IH, NH of amide), 6.85(d, 2H, Ar-H at 3′ and 5′) , 6.33(d.2H, Ar-H at 2′ and 6′), 5.10 (s, 1H, OH at 4′), 4.02 (t,1H, NH of amine), 3.90 (d,2H, -$COCH_2$-), 2.57 (t, 4H ,cycloheptane), 1.64 (m, 4H, c.heptane), 1.28 (m, 2H of c.heptane), 0.90 (s, -CH_3, 3H at 2); MS: m/z 398(M^+); Elemental analysis: Calculated for $C_{20}H_{22}O_3N_4S$: C-60.30, H-5.55, O-12.06, N-14.07, S-8.04. Found: C-60.70, H-5.74, O- 12.02, N-14.30, S-8.08

3-*N*-[2-(4′-nitro anilino) acetamido]-2-methyl-5,6-penta methylene thieno[2,3-d] pyrimidin (3*H*)-4-one : [III e]: Pale yellow solid ; Yield 66.45%, MP 149 ^{O}C, R_f 0.42 ; UV- λ max 214 nm ; IR(KBr)cm^{-1}: 3235.10 (amide NH str.), 3052.52 (Ar-CH str.), 2888.70 (aliph.CH str.), 1685.20 (C=O, aryl), 1537.48 (-N–C=O cyclic str.), 1625.86 (NH bend), 1338.50 (arom C-N str), 1053.45 (aliph C-N str.), 1467.60 (CH_2 bend), 1377.45 (CH_3 bend),1332.20 (NO of NO_2) , 754.20 (C-S) ; ^{1}H NMR (DMSO) δ in ppm : 8.08 (s, IH, NH of amide), 6.82 (d, 2H, Ar-H at 3′ and 5′) , 6.33 (d.2H, Ar-H at 2′ and 6′), 4.05 (t,1H, NH of amine), 3.90 (d,2H, -$COCH_2$-), 2.55 (t, 4H ,c.heptane), 1.62 (m, 4H, c.heptane), 1.29 (m, 2H of c.heptane) , 0.90 (s, -CH_3, 3H at 2); MS: m/z 427(M^+) ; Elemental analysis: Calculated for $C_{20}H_{21}O_4N_5S$: C-56.20, H-4.91, O- 14.99, N-16.40, S-7.49. Found: C-59.54, H-4.66, O- 14.65, N-16.12, S-7.40

3-*N*-[2-(4′-fluoro anilino) acetamido]-2-methyl- 5,6-penta methylene thieno[2,3-d] pyrimidin (3*H*)-4-one : [III f]: White solid ; Yield 60.85%, MP 125 ^{O}C, R_f 0.45 ; UV- λ max 215 nm ; IR(KBr) cm^{-1}: 3237.10 (amide NH str.), 3053.50 (Ar-CH str.), 2887,10 (aliph.CH str.), 1685.00 (C=O, aryl), 1538.40(-N–C=O cyclic str.), 1625.86 (NH bend), 1334.58 (arom C-N str), 1052.40 (aliph C-N str.), 1467.90 (CH_2 bend), 1375.42 (CH_3 bend), 1130.70 (C-F str.) , 755.50 (C-S) ; ^{1}H NMR (DMSO) δ in ppm : 8.10(s, IH, NH of amide), 6.87(d, 2H, Ar-H at 3′ and 5′) ,6.34(d.2H, Ar-H at 2′ and 6′), 4.08 (t,1H, NH of amine), 3.92 (d,2H, -$COCH_2$-), 2.55 (t, 4H ,c.heptane), 1.64 (m, 4H, c.heptane), 1.27 (m, 2H of c.heptane), 0.90 (s, -CH_3, 3H at 2); MS: m/z 400(M^+) ; Elemental analysis: Calculated for $C_{20}H_{21}O_2N_4SF$: C-60.00, H-5.25, O- 8.00, N-14.00, S-8.00, F 4.75 . Found C-58.22, H-5.55, O- 8.40, N-14.20, S-8.10, F-4.55.

3-*N*-[2-(2′-chloro anilino) acetamido]-2-methyl-5,6-penta methylene thieno[2,3-d] pyrimidin (3*H*)-4-one : [III g]: White solid ; Yield 55.55%, MP 117 ^{O}C, R_f 0.48 ; UV- λ max 216 nm ; IR(KBr) cm^{-1}: 3230.12 (amide NH str.), 3050.52 (Ar-CH str.), 2886.00 (aliph. CH str.),1685.28 (C=O, aryl),1536.44 (-N–C=O cyclic str.), 1625.86 (NH bend), 1335.55 (arom C-N str), 1050.40 (aliph C-N str.), 1465.20 (CH_2 bend), 1378.45 (CH_3 bend), 812.22 (C-Cl, str), 754.52(C-S) ; ^{1}H NMR (DMSO) δ in ppm: 8.01 (s, 1H, NH of amide,) , 7.10-6.60 (m, 4H, Ar-H ′), 4.02(t,1H, NH of amine),3.95 (d,2H, -$COCH_2$-), 2.55 (t, 4H, c.heptane), 1.65 (m, 4H, c.heptane), 1.29 (m, 2H of c.heptane), 0.92 (s, -CH_3,3H at 2); MS: m/z 416(M^+) ; Elemental analysis: Calculated for $C_{20}H_{21}O_2N_4SCl$: C-57.69, H-5.04, O-7.69, N-13.46, S-7.67, Cl- 8.53 . Found: C-55.20, H-5.35, O- 7.43, N-13.26, S-7.60, Cl- 8.58

3-*N*-[2-(4′-chloro anilino) acetamido]-2-methyl-5,6-penta methylene thieno[2,3-d] pyrimidin (3*H*)-4-one : [III g]: White solid ; Yield 59.40%, MP 127 ^{o} C, R_f 0.52 ; UV- λ max 217 nm ; IR(KBr)cm^{-1}: 3238.52 (amide NH str.), 3060.66(Ar-CH str.), 2890.12 (aliph. CH str.), 1685.80 (C=O, aryl),1540.50 (-N–C=O cyclic str.), 1622.55 (NH bend),.1337.25 (arom C-N str), 1053.20 (aliph C-N str.), 1469.20 (CH_2 bend), 1375.15 (CH_3 bend), 814.20 (C-Cl, str), 754.50 (C-S) ; ^{1}H NMR (DMSO) δ in ppm: 8.06 (s, IH, NH of amide), 6.88 (d, 2H, Ar-H at 3′ and 5′) , 6.35(d.2H, Ar-H at 2′ and 6′), 4.08 (t,1H, NH of amine), 3.90 (d,2H, -$COCH_2$-), 2.50 (t, 4H ,c.heptane), 1.64 (m, 4H, c.heptane), 1.28 (m, 2H of c.heptane) , 0.92 (s, -CH_3, 3H at 2); MS: m/z 416(M^+) ; Elemental analysis: Calculated for $C_{20}H_{21}O_2N_4SCl$: C-57.69, H-5.04, O- 7.69, N-13.46, S-7.67, Cl- 8.53 . Found : C-56.33, H-5.35, O- 7.85, N-13.76, S-7.17, Cl-8.76.

3-*N*-[2-(3′-chloro-4′-fluoro anilino) acetamido]-2-methyl-5,6- penta methylene thieno [2,3-d] pyrimidin (3*H*)-4-one : [III i]: White solid ; Yield 70.20%, MP 110 ^{O}C, R_f 0.60 ; UV- λ max 215 nm ; IR(KBr)cm^{-1}: 3233.15 (amide NH str.), 3055.50 (Ar-CH str.), 2884.40 (aliph.CH str.), 1680.48 (C=O, aryl),1536.80(-N–C=O cyclic str.), 1627.46 (NH bend), 1335.70 (arom C-N str), 1050.70 (aliph C-N str.), 1466.00 (CH_2 bend), 1376.22 (CH_3 bend), 1132.50 (C-F str.), 812.22 (C-Cl, str), 754.52(C-S) ; ^{1}H NMR (DMSO) δ in ppm: 8.04 (s, IH, NH of amide), 7.10-6.85 (m, 3H, Ar-H), 4.02 (t,1H, NH of amine), 3.92 (d,2H, -$COCH_2$-), 3.34 (s, 3H,4′-CH_3), 2.55 (t, 4H ,c.heptane), 1.64 (m, 4H, c.heptane), 1.29 (m, 2H of c.heptane), 0.92 (s, -CH_3, 3H at 2); MS: m/z 416(M^+) ; Elemental analysis: Calculated for $C_{20}H_{20}O_2N_4SClF$: C-55.30, H-4.83, O- 7.37, N-12.90, S-7.37, Cl- 8.06, F-4.37 . Found: C-53.38, H-4.44, O- 7.70, N-12.50, S-7.24, Cl- 8.50, F-4.42.

Antibacterial and antifungal activity Screening[29,30]

The antimicrobial activity of the new compounds was determined *in vitro* by cup-plate agar diffusion method using nutrient agar medium and DMSO as solvent. Antibacterial activity was determined against gram-positive bacteria *Staphylococcus aureus* (ATCC 11632) and *Bacillus subtilis* (ATCC 60711) and gram-negative bacteria *Escherichia coli*(ATCC 10536) and *Klebsiella pneumoniae*(ATCC 13883), while antifungal activity against *Aspergillus niger* (ATCC 1781), *Candida albicans* (ATCC 2501) and *Cryptococcus neoformans* (ATCC 32042) at 50 µg/0.1 ml concentration. After 24 hours of incubation at 37±1^{o}C, the antibacterial activity was determined by measuring zones of inhibitions in mm with Ampicillin as a standard used under similar conditions for comparision. Similarly antifungal activity was measured at 28±1^{o}C for 48 hours with Miconazole nitrate as standard. Control test with solvents were performed for every assay but showed no inhibition of microbial growth. The responses of organisms to the synthesized compounds were measured as mean of three values and compared with standards. Standard deviation was also calculated. (**Table -1 & 2)**.

Table-1: Antibacterial activity of new compounds.

Code	R	Zone of inhibition in mm.			
		S.aureus	*B.subtilis*	*E.coli*	*K.pneumoniae*
III b	2′-methyl anilino	10.66 ±1.52	09.33±0.57	NA	NA
III c	4′-methyl anilino	09.66±1.52	08.00±1.00	NA	NA
III d	4′-hydroxy anilino	09.33±0.57	08. 66±1.15	NA	NA
III e	4′-nitro anilino	14.33±0.57	13.66±0.57	13.66±1.52	12.33±1.52
III f	4′-fluoro anilino	17.33±0.57	15.66±1.52	15.00±1.00	15.33±1.15
III g	2′-chloro anilino	15.33±1.52	15.00±1.00	12. 33±0.57	12.66 ±1.52
III h	4′-chloro anilino	18.66±1.52	15.33±1.15	14.66±1.52	14.00±1.00

III i	3′-chloro-4′-fluoro anilino	20.66±0.57	17.33±0.57	18.66±1.52	15.00±1.00
Ampicillin	.....	22.66±0.57	18.00±1.00	23.33±0.57	18.66±1.15

NA-no activity

Table-2: Antifungal activity of new compounds

Code	**-R**	**Zone of inhibition(mm)**		
		A. niger	*C.albicans*	*C.neoformans*
III b	2′-methyl anilino	NA	NA	NA
III c	4′-methyl anilino	NA	NA	NA
III d	4′-hydroxy anilino	NA	NA	NA
III e	4′-nitro anilino	08.66±1.15	08.33±1.52	NA
III f	4′-fluoro anilino	12. 33±1.52	10.33±1.15	11.00±0.57
III g	2′-chloro anilino	09.66±1.52	09.00±1.00	08.66±1.15
III h	4′-chloro anilino	12.66±1.15	11.33±0.57	09.33±1.52
III i	3′-chloro-4′-fluoro anilino	13.66±1.52	12. 33±1.52	10.66±1.15
Miconazole nitrate	….	27.33±1.52	25.33±1.15	23.33±1.52

NA-no activity.

RESULTS AND DISCUSSION

The present work highlights the synthesis of a new series of Thienopyrimidinones invoving five steps. In the first step, cycloheptanone and ethyl cyano acetate were reacted in presence of sulphur and diethylamine involving the well-known Gewald reaction to get the inermediate 2-amino-3- carbethoxy-4,5-pentamethylene thiophene [**I**]. This intermediate was subjected to *N*-acetylation with zinc as a catalyst to obtain the corresponding 2-acetamido derivative [**II**]. The compound **II** when treated with hydrazine hydrate in ethanol under microwave irradiation gave the tricyclic intermediate 3-*N*-amino -2-methyl- 5, 6- pentamethylene thieno [2, 3-d]- pyrimidin (3*H*)–4-one [**III**].The primary amino group in the key intermediate **III** was chloro acetylated to get the product **III a**. The active chlorine in this compound was replaced by reacting with various aromatic amines as nucleophiles to obtain the title compounds **III b-i**, chemically 3-*N*-[(substituted amino) acetamido] -2-methyl- 5, 6-penta methylene thieno [2, 3-d]- pyrimidin (3*H*)–4-ones. The structures of prepared compounds have been characterized by spectral evidences.

From UV absorption results it was observed that the intermediate 2-amino-3- carbethoxy-4,5-pentamethylene thiophene [**II**] exhibited λ max at 234 nm, but the main intermediate 3-*N*-amino -2-methyl- 5, 6- pentamethylene thieno [2, 3-d]- pyrimidin (3*H*)–4-one [**III**] and its derivatized title compounds [**III a-i**] exhibited λ max at 212-217 nm. This might be due to hypsochromic shifts and hence cyclic hetero aromatic cyclization in the products. The compound **III** which was obtained by the cyclization of **II** showed no IR absorption band at 1735-1750 cm^{-1} due to the absence of the CO group of the ester. It was also observed that compound **III** and its derivatives **III a-i** exhibited characteristic strong bands at 1670-1688 cm^{-1} arising from C = O cyclic stretching vibrations due to the presence of pyrimidinone ring. There is one more strong band at 1530-1548 cm^{-1} in these compounds confirming the -N– C = O cyclic stretching vibrations.

In compound **III** there is the appearance of a specific IR absorption band at 3361.65 cm^{-1} due to the presence of primary amino group. This band is absent in the derivatives **III a-i**, but there is appearance of band at 3230-3280 cm^{-1} for N-H stretching vibrations due to the formation of amide linkage from amino group. The replacement of chlorine in **III a** by aryl amino groups to form **III b-i** has been confirmed by ′aromatic C-N stretching′ vibrations at 1320-1337 cm^{-1} in the title compounds.There is also ′′aliphatic C-N stretching′ vibrations in these compounds, indicated by a band at 1050-1057 cm^{-1}. The mass spectrum of compounds **III a-i** showed (M^+) peak in agreement with their molecular formula. The nature and number of protons have been supported by ^{1}H NMR data.

Among all the compounds screened for antibacterial activity, only the compounds bearing electron withdrawing groups like 4′-nitro (**IIIe**), 4′-fluoro (**III f**), 2′-chloro (**III g**), 4′-chloro (**III h**) and 3′-chloro-4′fluoro (**III i**) on the aryl ring showed significant activity against both gram-positive and gram-negative bacteria .They exhibited zone of inhibition of 14.33-20.66, 13.66-17.33, 12.33-18.66 and 12.33-15.33 mm against the bacterial strains *S.aureus*, *B.subtilis*, *E.coli* and *K..pneumoniae* respectively while comparing with that of standard drug Ampicillin, having values of 22.66, 18.00, 23.33 and 18.66 mm. The compounds 2′-methyl (**III b**), 4′-methyl (**III c**) and 4′-hydroxy (**III d**) displayed only mild activity against gram- positive bacteria (9.33-10.66 and 8.00-9.33mm) but inactive against gram-negative bacteria.

Fungicidal screening data also revealed similar pattern of activity, but the results were not appreciable. Only a few compounds like 4′-fluoro (**III f**), 2′-chloro (**III g**), 4′-chloro (**III h**) and 3′-cholro-4′-flouoro (**III i**) analogues imparted considerable antifungal activity against *A. niger* , *C.albicans* , and *C.neoformans* with zone of inhibition of 9.66-13.66, 09.00-12.33 and 09.33-11.00 mm respectively, while comparing with the standard drug Miconazole nitrate (27.33, 25.33 and 23.33). 4-nitro analog (**III e**) showed weak activity against *A.niger* and *C.albicans* (8.66 and 8.33 mm) but no activity against *C.neoformans.* The remaining compounds (**III b-d**) were found to be inactive against all the fungal organism.

CONCLUSION

Owing to the pronounced biological importance associated with the drugs containing thiophene and pyrimidine rings, we have hereby studied and reported the synthesis and structural characterization of some new thieno pyrimidinone derivatives. All the derivatives have been evaluated for their antibacterial and antifungal activities. The results indicated that some of the target compounds bearing electron withdrawing groups on the amino aryl ring like 4′-nitro (**III e**), 4′-fluoro (**III f**), 2′-chloro (**III g**), 4′-chloro (**III h**) and 3′-cholro-4′fluoro (**III i**) exhibited better antibacterial activity compared to the other compounds. Only a few compounds coded **III f, III g, III h** and **III i** imparted comparable fungicidal activity. Suitable molecular modifications of thienopyrimidinones may generate potent antimicrobial agents and further bio-evaluations in future.

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