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Chloropyrimidines as a New Class of Antimicrobial Agents†

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Abstract—In the course of our investigations of pyrimidines as antimycotic agents, we have identified a sub-class, with significant in vitro activity against mycobacteria. The salient feature of these pyrimidine derivatives (**3a–o** and **7a,b**) is their appended aryl, heteroaryl and alkylthio substituent at position 6 and also alkylthio substituent at position 2. The rational design, synthesis, and evaluation of the in vitro antibacterial activity against six pathogenic bacteria including virulent and non-virulent strains of *Mycobacterium tuberculosis* is described. Some of the synthesized compounds (**3c, 3h, 3i, 3o**) have displayed only potent in vitro antimycobacterial activity with MIC of 0.75 μg/mL except **3i** which also demonstrated activity against *Escherichia coli* at 12.5 μg/mL concentration. Only two compounds, **3a** and **3b,** demonstrated antibacterial activity against *Pseudomonas aeruginosa* and *E. coli* with MIC 12.5 μg/mL. All the synthesized compounds were also evaluated for their antimycotic activity against five pathogenic fungi but only some of them **3j–n** and **7a,b** were found most potent against *Aspergillus fumigatus* and *Trichophyton mentagrophytes*.

Introduction

The worldwide resurgence of tuberculosis followed by the recent emergence of multi-drug resistant strains of *Mycobacterium tuberculosis*[1] has refocused attention towards this disease with a view to develop new and more effective antibacterial agents. The clinical management of acquired immune deficiency syndrome (AIDS) has become very challenging because the opportunistic infections, particularly *M. tuberculosis*[2] and *Mycobacterium avium*[3] are more often responsible for the death of HIV-infected patients. Thus in order to control the rapid spread of tuberculosis, there is an urgent need to develop new antimycobacterial agents with unique modes of action to replace the current regimens. This preliminary communication demonstrates the in vitro antitubercular and antifungal activity of chloropyrimidine **3,** which has not been disclosed so far. The objective of our study is to generate new leads and to optimize the structure to display the potent efficacy.

Chemistry

The 4-chloropyrimidines were synthesized from a sequence of reactions as depicted in Schemes 1 and 2.

The precursor 6-aryl-5-cyano-2-thiouracil **(1)** was prepared as described earlier.[4,5] The regioselective alkylation of 2-thiouracil **(1)** was achieved[6] by slow addition of alkyl halide to a solution of **1** in DMF using K_2CO_3 as a base at 0–5 °C. The product isolated **(2)** was purified either by column chromatography or crystallization. The product 4-Aryl-2-methylsulfanyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile **(2)** was subjected for halogenation by refluxing with $POCl_3$ for 4–5 h to yield 2-alkylsulfanyl-6-aryl-4-chloropyrimidine-5-carbonitrile **(3)**. The crude chloro compound was purified either by crystallization from acetone or through silica gel column chromatography. The synthesis of **7a,b** was achieved in two steps by refluxing a mixture of ethyl 2-cyano-3,3-dimethylthioacrylate (**4**) with *S*-alkylthiourea (**5**) in ethanol followed by halogenation of the isolated pyrimidine derivative (**6**) with $POCl_3$.

Results and Discussion

All the synthesized chloropyrimidines (**3a–o** and **7a,b**) were screened for in vitro antimycobacterial activity by the microplate Alamar blue assay[7,8] (MABA). The antibacterial and antifungal activity was determined by double dilution method[9] as follows. The bacterial and fungal strains were maintained on nutrient agar (NA) and Sabouraud dextrose agar (SDA), respectively, at 37 and 28 °C. The inocula were prepared from the fresh

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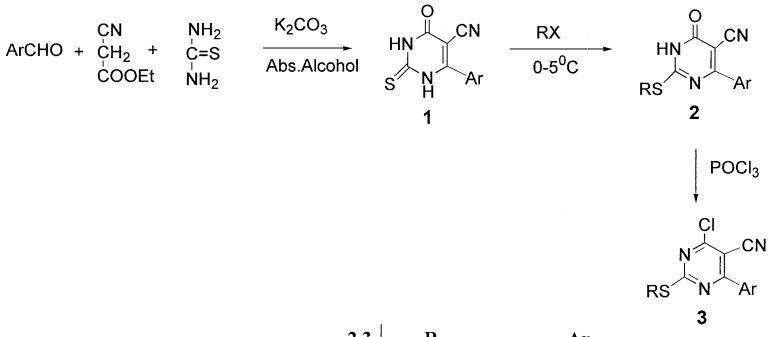
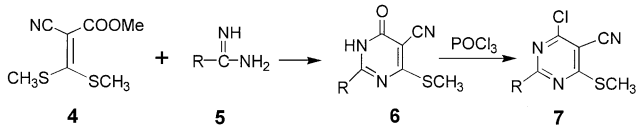


2,3	R	Ar
a	CH_3	2-Thienyl
b	C_2H_5	2-Thienyl
c	C_3H_7 (n-)	2-Thienyl
d	CH_2=CH-CH_2	2-Thienyl
e	C_4H_9 (n-)	2-Thienyl
f	C_4H_9 (iso)	2-Thienyl
g	$C_6H_5CH_2$	2-Thienyl
h	4-$ClC_6H_4CH_2$	2-Thienyl
i	C_3H_7	3-FC_6H_4
j	CH_3	2,4-$F_2C_6H_3$
k	C_2H_5	2,4-$F_2C_6H_3$
l	C_3H_7 (n-)	2,4-$F_2C_6H_3$
m	CH_3	2-Furyl
n	C_2H_5	2-Furyl
o	C_3H_7 (n-)	2-Furyl

Scheme 1.



Scheme 2.

slant growth (bacteria 24 h, yeasts 24–48 h and mycelial fungi 7 days) in normal saline and inoculated in nutrient broth (bacteria 2×10^5 cfu/mL) and Sabouraud dextrose broth (fungi $2\times10^{3-4}$ cfu/mL). The tests were performed in assay tubes (12×75 mm) in duplicate. The first tube was filled with 1.8 mL seeded broth (bacteria or fungus) and subsequent tubes contained 1 mL broth up to 12 tubes. The test compound (1 mg/mL), 0.2 mL was added to the first tube and 1 mL of this was transferred to the second tube and so on so forth. Suitable solvent control (DMSO), positive growth control and standard drug controls were also run simultaneously. The tubes were incubated at 37 °C for bacteria and 28 °C for fungi. The MICs were recorded by visual observation. 4-Chloropyrimidines (**3a**–**o** and **7a,b**) were submitted for preliminary evaluation of their in vitro activity against *M. tuberculosis* (*MT* H_{37} *Rv*), human isolates of *Klebsiella pneumoniae, Pseudomonas aeruginosa, S. faecalis, Staphylococcus aureus, E. coli* and antimycotic activity against *Candida albicans (SKF), Cryptococcus neoformans* (*CN-17) Sporothrix schenckii* (SS-1), *Aspergillus fumigatus* (*AF-27), T. mentagrophytes* (*A-280*) (Table 1). As evident from the antibacterial screening data, some of the compounds are in fact showing significant activity against *M. tuberculosis*. Among all the screened compounds **3c, 3h, 3i** and **3o** were found highly active with MIC 0.78 μg/mL followed by **3n** (1.5 μg/mL), **3b**, **3e, 3f, 3m** and **7b** (3.12 μg/mL). These active compounds were further screened against virulent strain (*MT* H_{37} *Rv*) but no change in their MIC profile was observed. It is obvious from the structure–activity profile of chloropyrimidines that substituents at positions 2 and 6 greatly influence the antitubercular activity. From the activity profile of compounds **3a**–**h**, it is evident that S–C_3H_7 (*n*-) substituent at position 2 is optimum chain length for expressing significant activity except **3l,** which was 16 times less active. An increase or decrease in alkyl chain length diminishes the activity. A normal or branched alkylthio group chain at position 2 does not affect the activity of compounds as evident from (**3e**) and (**3f**). A change in heteroaryl substituent at position 6 from 2-thienyl (**3c**) to 2-furyl (**3o**) with *n*-propylthio substituent at position 2, did not change the degree of activity. In exception, 6-aryl-4-chloro-2-(4-chlorobenzylthio)pyrimidine-5-carbonitrile (**3h**) was found equipotent as **3c** while 6-aryl-4-chloro-2-benzylthiopyrimidine-5-carbonitrile (**3g**) displayed 8-fold decrease in MIC. Similarly chloropyrimidines **7a,b** with 2-methylthio and 2-benzylthio substituents were 2- to 4-fold less active than the standard drugs PAS and EMB, respectively. The antibacterial screening data against pathogenic bacteria revealed that except **3a**, **3g** and **3i** none of the compounds were active against *P. aeruginosa, E. coli, S. faecalis* at 12.5 μg/mL concentration. However, **3i** was the only compound, which also demonstrated highly significant antitubercular activity apart from its activity against *S. faecalis*. All the remaining active compounds were specifically active against *M. tuberculosis*. So far we are not sure, about the mode of action of chloropyrimidines against bacteria and fungi.

All the compounds were further screened for their antimycotic activity against five pathogenic fungi *Ca, Cn, Ss, Af, Tm*. The antimycotic activity profile of synthesized compounds (Table 1), revealed that **7a** was more

Table 1. In vitro antimicrobial activities of 4-chloropyrimidines (**3a–o** and **7a,b**)

	MIC μg/mL											
No.	Mt	Kp*	Kp	Pa	Sf	Sa	Ec	Ca	Cn	Ss	Af	*T*m
3a	6.25	ND	>100	12.5	>100	>100	12.5	>100	>100	>100	12.5	25
3b	3.12	ND	>100	>100	>100	>100	>100	>100	>100	>100	25	50
3c	0.78	50	>100	>100	>100	>100	>100	>100	>100	>100	>100	50
3d	6.25	ND	>100	>100	>100	>100	>100	>100	>100	>100	0.78	6.25
3e	3.12	6.25	>100	>100	>100	>100	>100	>100	>100	>100	>100	25
3f	3.12	25	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
3g	6.25	ND	50	12.5	50	>100	12.5	100	>100	>100	>100	>100
3h	0.78	3.12	>100	>100	>100	>100	>100	100	>100	>100	>100	>100
3i	0.78	ND	>100	>100	12.5	>100	>100	>100	>100	>100	25	6.25
3j	>25	>100	>100	>100	>100	>100	>100	>100	3.12	>100	0.78	>100
3k	>25	>100	>100	>100	>100	>100	>100	50	6.25	>100	1.56	0.78
3l	12.5	>100	>100	>100	>100	>100	>100	>100	12.5	>100	3.12	3.12
3m	3.12	>100	>100	>100	>100	>100	>100	>100	3.12	25	0.09	3.12
3n	1.56	25	>100	>100	25	50	>100	>100	25	>100	0.39	1.56
3o	0.78	>100	>100	>100	50	>100	>100	>100	>100	50	3.12	3.12
7a	3.12	>100	>100	>50	>25	25	>100	>100	1.56	50	0.002	0.005
7b	3.12	12.5	>100	>100	>100	>100	>100	>50	6.25	50	0.78	0.19
EMB	1.56	—	—	—	—	—	—	—	—	—	—	—
PAS	0.78	—	—	—	—	—	—	—	—	—	—	—
Clo	—	—	—	—	—	—	—	0.39 0.19	0.09	3.12	0.19	
Cipr	—	0.19	3.1	0.78	1.5	0.78	1.5	—	—	—	—	—

Mt, *Mycobacterium tuberculosis*; Kp*, *Klebsiella pneumoniae* (ATCC 13883); Kp, *Klebsiella pneumoniae*; Pa, *Pseudomonas aeruginosa*; Sf, *Streptococcus faecalis*; Sf, *Staphylococcus aureus*; Ec, *Escherichia coli*; Ca, *Candida albicans*; Cn, *Cryptococcus neoformans*; Ss, *Sporothrix schenckii*; Af, *Aspergillus fumigatus*; Tm, *Trichophyton mentagrophytes*; EMB, ethambutol; PAS, *p*-aminosalicylic acid; Clo, clotrimazole; Cipro, ciprofloxacin; MIC, minimum inhibitory concentration. The tests were performed in duplicate and repeated twice. ND, not done.

active than **7b** against *Af* and *Tm*, displaying even better efficacy, than the standard drug, Clotrimazole. A mere change of substituent at position 2 in **7a** from methylthio to benzylthio (**7b**) reduced the activity several fold. Among other pyrimidine derivatives (**3a–o**), only **3m** exhibited significant activity followed by **3o**, **3k, 3n** and **3l** against *Af, Tm* and *Cn*. Compounds **3d** and **3i** were found active against *Af and Tm* while **3j** displayed activity against *Cn* and *Af*. Thus, an increase in alkyl chain length of 2-alkylthiosubstituted pyrimidines (**3a–o**), reduces the activity profile as evident from the screening data of **3a–f, 3j–l** and **3m–o**.

All those compounds (**3c, 3h, 3i** and **3o**) displaying significant antitubercular activity did not display antifungal activity except **3o** which demonstrated moderate activity with MIC 3.12 μg/mL against *A. fumigatus* and *T. mentagrophytes*.

Experimental

Chemistry

Melting points were determined on Buchi-530 capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on Brucker WM 200 MHz spectrometer in deuterated solvents with TMS as internal reference. IR spectra of all the compounds were recorded on Perkin–Elmer AC-1 spectrophotometer. Mass spectra of all compounds were measured with Jeol JMS-D 300 spectrometer (70eV). Microanalyses were determined on Carlo Erba EA-1108 element analyzer within ±0.5% of the theoretical values. Thin layer chromatography (TLC) was performed on 7×3 cm precoated silica gel plastic plates. For column chromatography, silica gel of 60–120 mesh from Acme Synthetic Chemicals, Bombay, India was used.

General procedure for the preparation of 4-aryl-2-methylsulfanyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (2a–o and 6a,b)

To a mixture of 6-aryl-5-cyano-2-thiouracil (**1**, 1 mmol) and K_2CO_3 (1.5 mmol) in DMF (10 mL), alkyl iodide (1.2 mmol) was added dropwise with stirring while maintaining the temperature of the reaction mixture at 0–5 °C. Stirring was continued for 3 h at this temperature and continued for additional 2 h at room temperature. Water was added to the mixture and filtered. The aqueous filtrate was neutralized with acetic acid and the precipitate was filtered and purified.

2-Methylsulfanyl-6-oxo-4-(2-thienyl)-1,6-dihydropyrimidine-5-carbonitrile (2a). Yield 58.4%; mp 239–241 °C; MS (EI) *m/z* 249 (M^+, 15.5), 183 (10.9), 175 (15.9), 147 (20.2); IR (KBr) 1666 (CO), 2220 (CN), 3476 cm^{-1} (NH); ^{1}H NMR (200 MHz, acetone) δ 2.71 (s, 3H, SCH_3), 7.20 (t, J=6.4 Hz, 1H, thienyl), 7.82 (d, J=8.0 Hz, 1H, thienyl), 8.25 (d, J=7.8 Hz, 1H, thienyl). Anal. calcd. for $C_{10}H_7N_3OS_2$: C, 48.17, H, 2.83, N, 16.85. Found: C, 48.08, H, 2.67, N, 16.73.

2-Ethylsulfanyl-6-oxo-4-(2-thienyl)-1,6-dihydropyrimidine-5-carbonitrile (2b). Yield 75.1%; mp >250 °C; MS (EI) *m/z* 263 (M^+, 67.4), 248 (17.5), 230 (44.4), 203 (11.4), 177 (37.2); IR (KBr) 1660 (CO), 2219 (CN), 3470 cm^{-1} (NH); ^{1}H NMR (200 MHz, acetone) δ 1.33 (t, J=6.6 Hz, 3H, CH_3), 3.22 (q, J=5.8 Hz, 2H, SCH_2), 7.21 (t, J=6.84 Hz, 1H, thienyl), 7.82 (d, J=8.0 Hz, 1H,

thienyl), 8.27 (d, $J=7.6$ Hz, 1H, thienyl). Anal. calcd for $C_{11}H_9N_3OS_2$: C, 50.17, H, 3.44, N, 15.96. Found: C, 50.33, H, 3.28, N, 15.76.

6-Oxo-2-(*n*)-propylsulfanyl-4-(2-thienyl)-1,6-dihydropyrimidine-5-carbonitrile (2c). Yield 72.2%; mp > 250 °C; MS (EI) *m/z* 277 (M^+, 22.9), 262 (37.3), 249 (54.0), 235 (100); IR (KBr) 1662 (CO), 2218 (CN), 3458 cm^{-1} (NH); ^1H NMR (200 MHz, acetone) δ 1.12 (t, $J=6.3$ Hz, 3H, CH_3), 1.78 (q, $J=5.3$ Hz, 2H, CH_2), 3.30 (t, $J=6.5$ Hz, 2H, SCH_2), 7.34 (t, $J=6.4$ Hz, 1H, thienyl), 7.97 (d, $J=7.8$ Hz, 1H, thienyl), 8.40 (d, $J=8.0$ Hz, 1H, thienyl). Anal. calcd for $C_{12}H_{11}N_3OS_2$: C, 51.96, H, 3.99, N, 15.15. Found: C, 51.76, H, 4.19, N, 15.23.

2-Allylsulfanyl-6-oxo-4-(2-thienyl)-1,6-dihydropyrimidine-5-carbonitrile (2d). Yield 56.5%; mp > 250 °C; MS (EI) *m/z* 275 (M^+, 100), 260 (48.1), 242 (68.6), 177(14); IR (KBr) 1660 (CO), 2218 (CN), 3384 cm^{-1} (NH); ^1H NMR (200 MHz, $CDCl_3$ + DMSO) δ 3.93 (d, $J=7.8$ Hz, 2H, CH_2), 5.30 (dd, $J=4.7$ Hz, 2H, SCH_2), 5.99–6.03 (m, 1H, CH), 7.24 (t, $J=6.9$ Hz, 1H, thienyl), 7.75 (d, $J=8.0$ Hz, 1H, thienyl), 8.40 (d, $J=7.9$ Hz, 1H, thienyl). Anal. calcd for $C_{12}H_9N_3OS_2$: C, 52.34, H, 3.29, N, 15.26. Found: C, 52.14, H, 3.35, N, 15.12.

2-(*n*)-Butylsulfanyl-6-oxo-4-(2-thienyl)-1,6-dihydropyrimidine-5-carbonitrile (2e). Yield 87.64%; mp > 250 °C; MS (EI) *m/z* 291 (M^+, 69.9), 262 (28.6), 249 (57.7), 233 (36.8); IR (KBr) 1676 (CO), 2210 (CN), 3446 cm^{-1} (NH); ^1H NMR (200 MHz, $CDCl_3$) δ 1.09 (t, $J=6.2$ Hz, 3H, CH_3), 1.46–1.61 (m, 2H, CH_2), 1.70–1.82 (m, 2H, CH_2), 3.22 (t, $J=6.2$ Hz, 2H, SCH_2), 7.26 (t, $J=6.2$ Hz, 1H, thienyl), 7.82 (d, $J=7.8$ Hz, 1H, thienyl), 8.45 (d, $J=8.0$ Hz, 1H, thienyl). Anal. calcd. for $C_{13}H_{13}N_3OS_2$: C, 53.58, H, 4.49, N, 14.42. Found: C, 53.86, H, 4.32, N, 14.83.

2-(iso)-Butylsulfanyl-6-oxo-4-(2-thienyl)-1,6-dihydropyrimidine-5-carbonitrile (2f). Yield 76.7%; mp > 250 °C; MS (EI) *m/z* 291 (M^+, 58.9), 262 (38.6), 249 (77.7); IR (KBr) 1676 (CO), 2190 (CN), 3440 cm^{-1} (NH); ^1H NMR (200 MHz, $CDCl_3$) δ 1.09 (d, $J=7.8$ Hz, 6H, CH_3), 1.95–2.12 (m, 1H, CH), 3.00 (d, $J=7.2$ Hz, 2H, SCH_2), 7.23 (t, $J=6.1$ Hz, 1H, thienyl), 7.71 (d, $J=7.7$Hz, 1H, thienyl), 8.37 (d, $J=7.8$ Hz, 1H, thienyl). Anal. calcd for $C_{13}H_{13}N_3OS_2$: C, 53.58, H, 4.49, N, 14.42. Found: C, 53.95, H, 4.35, N, 14.86.

2-Benzylsulfanyl-6-oxo-4-(2-thienyl)-1,6-dihydropyrimidine-5-carbonitrile (2g). Yield 52.3%; mp > 250 °C; MS (EI) *m/z* 325 (M^+, 63.3), 292 (25.5), 247 (5.2); IR (KBr) 1656 (CO), 2224 (CN), 3348 cm^{-1} (NH); ^1H NMR (200 MHz, $CDCl_3$ + DMSO) δ 4.55 (s, 2H, SCH_2), 7.24–7.35 (m, 5H Ar–H), 7.42 (t, $J=6.0$ Hz, 1H, thienyl), 7.79 (d, $J=8.0$ Hz, 1H, thienyl), 8.39 (d, $J=7.8$ Hz, 1H, thienyl). Anal. calcd for $C_{16}H_{11}N_3OS_2$: C, 59.05, H, 3.41, N, 12.91. Found: C, 59.21, H, 3.35, N, 12.82.

2-(4-Chlorobenzylsulfanyl)-6-oxo-4-(2-thienyl)-1,6-dihydropyrimidine-5-carbonitrile (2h). Yield 72.4%, mp > 250 °C; MS (EI) *m/z* 359 (M^+, 21), 327 (3.6), 235 (7.1), 177 (9); IR (KBr) 1664 (CO), 2208 (CN), 3394 cm^{-1} (NH); ^1H NMR (200 MHz, acetone) δ 4.18 (s, 2H, SCH_2), 7.22 (d, $J=7.3$ Hz, 2H, Ar–H), 7.27 (t, $J=6.9$ Hz, 1H, thienyl), 7.32 (d, $J=7.8$ Hz, 2H, Ar–H), 7.52 (d, $J=7.6$ Hz, 1H, thienyl), 8.04 (d, $J=8.1$ Hz, 1H, thienyl). Anal. calcd for $C_{16}H_{10}ClN_3OS_2$: C, 53.40, H, 2.80, N, 11.68. Found: C, 53.30, H, 2.58, N, 11.39.

4-(3-Fluorophenyl)-6-oxo-2-(n)-propylsulfanyl-1,6-dihydropyrimidine-5-carbonitrile (2i). Yield 66%; mp 161–163 °C; MS (EI) *m/z* 289 (M^+, 42.4), 274 (31.0), 261 (42.7), 247 (100); IR (KBr) 1666 (CO), 2222 (CN), 3450 cm^{-1} (NH); ^1H NMR (200 MHz, DMSO) δ 1.02 (t, $J=6.0$ Hz, 3H, CH_3), 1.72 (q, $J=5.9$ Hz, 2H, CH_2), 3.71 (s, 2H, SCH_2), 7.43–7.81 (m, 4H, Ar–H). Anal. calcd for $C_{14}H_{12}FN_3OS$: C, 58.13, H, 4.14, N, 14.53. Found: C, 58.10, H, 4.15, N, 14.53.

4-(2,4-Difluorophenyl)-2-methylsulfanyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (2j). Yield 48.7%; mp > 250 °C; MS (EI) *m/z* 279 (M^+, 100), 276 (29.4), 267 (23.2), 247 (25.4); IR (KBr) 1682 (CO), 2220 (CN), 3560 cm^{-1} (NH); ^1H NMR (200 MHz, $CDCl_3$) δ 2.68 (s, 3H, SCH_3), 6.90–7.10 (m, 1H, Ar–H), 7.54–7.68 (m, 2H, Ar–H). Anal. calcd for $C_{12}H_7F_2N_3OS$: C, 51.60, H, 2.50, N, 15.05. Found: C, 51.33, H, 2.43, N, 15.19.

4-(2,4-Difluorophenyl)-2-ethylsulfanyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (2k). Yield 69.54%; mp > 250 °C; MS (EI) *m/z* 293 (M^+, 66.0), 278 (22.7), 260 (60.2), 207 (29.7); IR (KBr) 1662 (CO), 2222 (CN), 3440 cm^{-1} (NH); ^1H NMR (200 MHz, $CDCl_3$) δ 1.42 (t, $J=6.4$ Hz, 3H, CH_3), 3.22 (q, $J=5.8$ Hz, 2H, SCH_2), 6.95–7.08 (m, 1H, Ar–H), 7.59–7.71 (m, 2H, Ar–H). Anal. calcd for $C_{13}H_9F_2N_3OS$: C, 53.24, H, 3.07, N, 14.33. Found: C, 53.66, H, 3.13, N, 14.69.

4-(2,4-Difluorophenyl)-6-oxo-2-(*n*)-propylsulfanyl-1,6-dihydropyrimidine-5-carbonitrile (2l). Yield 71.5%; mp > 250 °C; MS (EI) *m/z* 307 (M^+, 34.5), 292 (32.7), 279 (55.4), 265 (69.4); IR (KBr) 1662 (CO), 2226 (CN), 3490 cm^{-1} (NH); ^1H NMR (200 MHz, $CDCl_3$) δ 1.22 (t, $J=6.6$ Hz, 3H, CH_3), 1.69–1.83 (m, 2H, CH_2), 3.25 (t, $J=6.5$ Hz, 2H, SCH_2), 7.14–7.24 (m, 1H, Ar–H), 7.64–7.81 (m, 2H, Ar–H). Anal. calcd for $C_{14}H_{11}F_2N_3OS$: C, 54.72, H, 3.61, N, 13.68. Found: C, 54.53, H, 3.44, N, 13.85.

4-(2-Furyl)-2-methylsulfanyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (2m). Yield 54.7%; mp > 250 °C; MS (EI) *m/z* 233 (M^+, 14.4), 219 (41.7), 161 (17.2); IR (KBr) 1669 (CO), 2228 (CN), 3384 cm^{-1} (NH); ^1H NMR (200 MHz, $CDCl_3$) δ 2.58 (s, 3H, SCH_3), 6.62–6.69 (m, 1H, furyl), 7.67 (d, $J=7.8$ Hz, 1H, furyl), 7.79 (d, $J=8.0$ Hz, 1H, furyl). Anal. calcd for $C_{10}H_7N_3O_2S$: C, 51.50, H, 3.03, N, 18.02. Found: C, 51.44, H, 2.98, N, 18.38.

2-Ethylsulfanyl-4-(2-furyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (2n). Yield 69.9%; mp > 250 °C; MS (EI) *m/z* 247 (M^+, 26.5), 193 (11.3), 150 (21.5); IR (KBr) 1652 (CO), 2224 (CN), 3489 cm^{-1} (NH); ^1H NMR (200 MHz, $CDCl_3$) δ 1.45 (t, $J=6.8$ Hz, 3H, CH_3), 3.29 (q, $J=5.6$ Hz, 2H, SCH_2), 6.60–6.64 (m, 1H, furyl), 7.65 (d, $J=7.9$ Hz, 1H, furyl), 7.77 (d, $J=8.2$ Hz, 1H, furyl). Anal. calcd for $C_{11}H_9N_3O_2S$: C, 53.44, H, 3.67, N, 17.00. Found: C, 53.68, H, 3.55, N, 16.88.

4-(2-Furyl)-6-oxo-2-(n)-propylsulfanyl-1,6-dihydropyrimidine-5-carbonitrile (2o). Yield 75.7%; mp > 250 °C; MS (EI) *m/z* 261 (M^+, 71.5), 246 (84.7), 233 (100), 219 (89.3); IR (KBr) 1660 (CO), 2222 (CN), 3438 cm^{-1} (NH); ^1H NMR (200 MHz, $CDCl_3$) δ 1.08 (t, $J = 6.6$ Hz, 3H, CH_3), 1.74–1.88 (m, 2H, CH_2), 3.15 (t, $J = 6.4$ Hz, 2H, SCH_2), 6.30 (d, $J = 7.8$ Hz, 1H, furyl), 7.26–7.50 (m, 1H, furyl), 7.76 (d, $J = 8$ Hz, 1H, furyl). Anal. calcd for $C_{12}H_{11}N_3O_2S$: C, 55.17 H, 4.24, N, 16.09. Found: C, 55.35, H, 4.11, N, 16.27.

2,4-Dimethylsulfanyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (6a). Yield 89.9%; mp > 250 °C; MS (EI) *m/z* 213 (M^+, 33.5), 195 (30.7), 182 (15.9), 168 (35.3); IR (KBr) 1662 (CO), 2218 (CN), 3468 cm^{-1} (NH); ^1H NMR (200 MHz, $CDCl_3$) δ 2.50 (s, 3H, SCH_3), 2.56 (s, 3H, SCH_3). Anal. calcd for $C_7H_7N_3OS_2$: C, 39.41, H, 3.44, N, 19.98. Found: C, 39.35, H, 3.11, N, 19.27.

4-Benzylsulfanyl-2-methylsulfanyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (6b). Yield 87.86%; mp > 250 °C; MS (EI) *m/z* 289 (M^+, 25.0), 213 (16.8), 140 (21.7); IR (KBr) 1662 (CO), 2218 (CN), 3468 cm^{-1} (NH); ^1H NMR (200 MHz, $CDCl_3$) δ 2.56 (s, 3H, SCH_3), 3.66 (s, 2H, SCH_2), 7.14–7.28 (m, 2H, Ar–H), 7.34–7.42 (m, 3H, Ar–H). Anal. calcd for $C_{13}H_{11}N_3OS_2$: C, 53.97, H, 3.83, N, 14.53. Found: C, 53.71, H, 3.60, N,14.48.

General procedure for the preparation of 2-alkylsulfanyl-6-aryl-4-chloropyrimidine-5-carbonitrile (3a–o and 7a,b). 4-Aryl-2-methylsulfanyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**2**, 1 mmol) in $POCl_3$ (10 mL) was refluxed at 140 °C for 5 h. After completion of the reaction mixture was poured on crushed ice slowly with constant stirring. Crude product obtained was filtered, washed with water, dried and purified by using $CHCl_3$/hexane (1:1) as eluent and crystallized from acetone.

4-Chloro-2-methylsulfanyl-6-(2-thienyl)pyrimidine-5-carbonitrile (3a). Yield 41.24%; mp 208–210 °C; MS (EI) *m/z* 267.5 (M^+, 66.2), 220 (12.3), 186 (13.4), 159 (41.7); IR (KBr) 2228 cm^{-1} (CN); ^1H NMR (200 MHz, acetone) δ 2.71 (s, 3H, SCH_3), 7.44 (t, $J = 6.8$ Hz, 1H, thienyl), 8.06 (d, $J = 7.8$ Hz, 1H, thienyl), 8.50 (d, $J = 7.6$ Hz, 1H, thienyl). Anal. calcd for $C_{10}H_6ClN_3S_2$: C, 44.85, H, 2.26, N, 15.69. Found: C, 44.63, H, 2.15, N, 15.39.

4-Chloro-2-ethylsulfanyl-6-(2-thienyl)pyrimidine-5-carbonitrile (3b). Yield 41%; mp 129–130 °C; MS (EI) *m/z* 281.5 (M^+, 100), 266 (26.4), 248 (65.2), 221 (18.4); IR (KBr) 2222 cm^{-1} (CN); ^1H NMR (200 MHz, acetone) δ 1.44 (t, $J = 4.4$ Hz, 3H, CH_3), 3.31 (q, $J = 6.2$ Hz, 2H, SCH_2), 7.41 (t, $J = 6.7$ Hz, 1H, thienyl), 8.06 (d, $J = 8.0$ Hz, 1H, thienyl), 8.57 (d, $J = 8.2$ Hz, 1H, thienyl). Anal. calcd for $C_{11}H_8ClN_3S_2$: C, 46.89, H, 2.86, N, 14.92. Found: C, 46.75, H, 2.77, N, 14.83.

4-Chloro-2-(*n*)-propylsulfanyl-6-(2-thienyl)pyrimidine-5-carbonitrile (3c). Yield 45.9%; mp 112–114 °C; MS (EI) *m/z* 295.5 (M^+, 50.6), 280 (32.2), 267 (33), 253 (44.1); IR (KBr) 2222 cm^{-1} (CN); ^1H NMR (200 MHz, acetone) δ 1.15 (t, $J = 6.5$ Hz, 3H, CH_3), 1.77–1.95 (m, 2H, CH_2), 3.27 (t, $J = 6.6$ Hz, 2H, SCH_2), 7.41 (t, $J = 6.86$ Hz, 1H, thienyl), 8.07 (d, $J = 7.8$ Hz, 1H, thienyl), 8.49 (d, $J = 7.8$ Hz, 1H, thienyl). Anal. calcd for $C_{12}H_{10}ClN_3S_2$: C, 48.73, H, 3.41, N, 14.2. Found: C, 48.68, H, 3.34, N, 14.33.

2-Allylsulfanyl-4-chloro-6-(2-thienyl)pyrimidine-5-carbonitrile (3d). Yield 14.8%; mp 108–110 °C; MS (EI) *m/z* 293.5 (M^+, 47.6), 280 (34.5), 278 (90.7), 260 (67.8) ; IR (KBr) 2222 cm^{-1} (CN); ^1H NMR (200 MHz, acetone) δ 3.95 (d, $J = 8.0$ Hz, 2H, CH_2), 5.32 (dd, $J = 4.6$ Hz, 2H, SCH_2), 6.05–6.07 (m, 1H, CH), 7.39 (t, $J = 6.4$ Hz, 1H, thienyl), 8.05 (d, $J = 7.6$ Hz, 1H, thienyl), 8.47 (d, $J = 8.0$ Hz, 1H, thienyl). Anal. calcd for $C_{12}H_8ClN_3S_2$: C, 49.14, H, 2.73, N, 14.33. Found: C, 49.09, H, 2.73, N, 14.31.

2-(*n*)-Butylsulfanyl-4-chloro-6-(2-thienyl)pyrimidine-5-carbonitrile (3e). Yield 58.6%; mp 115 °C; MS (EI) *m/z* 309 (M^+, 25.5), 266(42.1), 253 (82.0); IR (KBr) 2220 cm^{-1} (CN); ^1H NMR (200 MHz, $CDCl_3$) δ 1.00 (t, $J = 6.5$ Hz, 3H, CH_3), 1.46–1.61 (m, 2H, CH_2), 1.70–1.84 (m, 2H, CH_2), 3.25 (t, $J = 6.5$ Hz, 2H, SCH_2), 7.24 (t, $J = 6.3$ Hz, 1H, thienyl), 7.82 (d, $J = 7.7$ Hz, 1H, thienyl), 8.48 (d, $J = 8.0$ Hz, 1H, thienyl). Anal. calcd for $C_{13}H_{12}ClN_3S_2$: C, 50.40, H, 3.90, N, 13.56. Found: C, 50.72, H, 3.72, N, 13.26.

2-(iso)-Butylsulfanyl-4-chloro-6-(2-thienyl)pyrimidine-5-carbonitrile (3f). Yield 63.48%; mp 110 °C; MS (EI) *m/z* 309 (M^+, 25.5), 294 (17.7), 266 (42.1), 253 (82.0); IR (KBr) 2220 (CN)); ^1H NMR (200 MHz, $CDCl_3$) δ 1.08 (d, $J = 8.1$ Hz, 6H, CH_3), 1.95–2.15 (m, 1H, CH), 3.10 (d, $J = 7.6$ Hz, 2H, SCH_2), 7.22 (t, $J = 6.5$ Hz, 1H, thienyl), 7.75 (d, $J = 7.8$ Hz, 1H, thienyl), 8.46 (d, $J = 7.7$ Hz, 1H, thienyl). Anal. calcd for $C_{13}H_{12}ClN_3S_2$: C, 50.40, H, 3.90, N, 13.56. Found: C, 50.22, H, 3.65, N, 13.76.

2-Benzylsulfanyl-4-chloro-6-(2-thienyl)pyrimidine-5-carbonitrile (3g). Yield 90.18%; mp 141–143 °C; MS (EI) *m/z* 343.5 (M^+, 64.7), 310 (43.5), 265 (9.1), 159 (16.4); IR (KBr) 2224 cm^{-1} (CN); ^1H NMR (200 MHz, acetone) δ 4.58 (s, 2H, SCH_2), 7.33–7.55 (m, 5H Ar–H), 7.56 (t, $J = 6.8$ Hz, 1H, thienyl), 8.09 (d, $J = 8.0$ Hz, 1H, thienyl), 8.50 (d, $J = 7.8$ Hz, 1H, thienyl). Anal. calcd for $C_{16}H_{10}ClN_3S_2$: C, 55.97, H, 2.93, N, 12.22. Found: C, 55.89, H, 2.81, N, 12.14.

2-(4-Chlorobenzylsulfanyl)-4-chloro-6-(2-thienyl)pyrimidine-5-carbonitrile (3h). Yield 17.78%; mp 120–122 °C; MS (EI) *m/z* 378 (M^+, 36.5), 346 (13.4), 309 (12.8), 159 (16.3); IR (KBr) 2230 cm^{-1} (CN); ^1H NMR (200 MHz, acetone) δ 4.73 (s, 2H, SCH_2), 7.39–7.42 (m, 3H, thienyl, Ar–H), 7.71 (d, $J = 7.2$ Hz, 2H, Ar–H), 8.15 (d, $J = 7.8$ Hz, 1H, thienyl), 8.63 (d, $J = 7.6$ Hz, 1H, thienyl). Anal. calcd for $C_{16}H_9Cl_2N_3S_2$: C, 50.79, H, 2.40, N, 11.12. Found: C, 50.90, H, 2.23, N, 11.22.

4-Chloro-6-(3-fluorophenyl)-2-(*n*)propylsulfanylpyrimidine-5-carbonitrile (3i). Yield 50.6%; mp 67–69 °C; MS (EI) *m/z* 307 (M^+, 100), 291(44.7), 278 (52.4), 273 (42.8); IR (KBr) 2230 cm^{-1} (CN); ^1H NMR (200 MHz, acetone) δ 1.29 (t, $J = 6.3$ Hz, 3H, CH_3), 1.96–2.19 (m, 2H, CH_2), 3.00 (t, 2H, $J = 6.3$ Hz, SCH_2), 7.66–8.18 (m, 4H, Ar–H). Anal. calcd for $C_{14}H_{11}ClFN_3S$: C, 54.63, H, 3.60, N, 13.65. Found: C, 54.53, H, 3.47, N, 13.55.

4-Chloro-6-(2,4-difluorophenyl)-2-methylsulfanylpyrimidine-5-carbonitrile (3j). Yield 40.56%; mp 150 °C; MS (EI) *m/z* 297 (M^+, 40.1), 294 (100), 279 (66.2), 265 (37.4); IR (KBr) 2218 cm^{-1} (CN); ^1H NMR (200 MHz, $CDCl_3$) δ 2.63 (s, 3H, SCH_3), 6.92–7.16 (m, 1H, Ar–H), 7.67–7.78 (m, 2H, Ar–H). Anal. calcd for $C_{12}H_6ClF_2N_3S$: C, 48.41, H, 2.03, N, 14.11. Found: C, 48.64, H, 2.14, N, 14.30.

4-Chloro-6-(2,4-difluorophenyl)-2-ethylsulfanylpyrimidine-5-carbonitrile (3k). Yield 38.6%; mp 157 °C; MS (EI) *m/z* 311 (M^+, 100), 296 (25.5), 280 (23.9), 278 (54.2), 264 (10.6); IR (KBr) 2232 cm^{-1}(CN); ^1H NMR (200 MHz, $CDCl_3$) δ 1.42 (t, $J=6.4$ Hz, 3H, CH_3), 3.22 (q, $J=5.6$ Hz, 2H, SCH_2), 7.02–7.19 (m, 1H, Ar–H), 7.66–7.78 (m, 2H, Ar–H). Anal. calcd for $C_{13}H_8ClF_2N_3S$: C, 50.58, H, 2.58, N, 13.48. Found: C, 50.36, H, 2.48, N, 13.33.

4-Chloro-6-(2,4-difluorophenyl)-2-(*n*)-propylsulfanylpyrimidine-5-carbonitrile (3l). Yield 50.9%; mp 135 °C; MS (EI) *m/z* 325 (M^+, 62.6), 309 (52.4), 296 (58), 282 (43.3), 278 (50.2); IR (KBr) 2226 cm^{-1} (CN); ^1H NMR (200 MHz, $CDCl_3$) δ 1.06 (t, $J=6.8$ Hz, 3H, CH_3), 1.77–1.89 (m, 2H, CH_2), 3.19 (t, $J=6.5$ Hz, 2H, SCH_2), 7.02–7.11 (m, 1H, Ar–H), 7.63–7.76 (m, 2H, Ar–H). Anal. calcd for $C_{14}H_{10}ClF_2N_3S$: C, 51.62, H, 3.09, N, 12.90. Found: C, 51.46, H, 3.15, N, 12.67.

4-Chloro-6-(2-furyl)-2-methylsulfanylpyrimidine-5-carbonitrile (3m). Yield 67.86%; mp 160 °C; MS (EI) *m/z* 251 (M^+, 100), 218 (18.3), 208 (34.9), 179 (20.5); IR (KBr) 2226 cm^{-1} (CN); ^1H NMR (200 MHz, $CDCl_3$) δ 2.56 (s, 3H, SCH_3), 6.72–6.77 (m, 1H, furyl), 7.67 (d, $J=7.8$ Hz, 1H, furyl), 7.85 (d, $J=8$ Hz, 1H, furyl). Anal. calcd for $C_{10}H_6ClN_3OS$: C, 47.72, H, 2.40, N, 16.73. Found: C, 48.19, H, 2.69, N, 16.81.

4-Chloro-2-ethylsulfanyl-6-(2-furyl)pyrimidine-5-carbonitrile (3n). Yield 75.2%; mp 160 °C; MS (EI) *m/z* 265 (M^+, 100), 250 (37.9), 232 (73.5), 205 (25.1); IR (KBr) 2226 cm^{-1} (CN); ^1H NMR (200 MHz, $CDCl_3$) δ 1.43 (t, $J=6.7$ Hz, 3H, CH_3), 3.28 (q, $J=5.8$ Hz, 2H, SCH_2), 6.60–6.68 (m, 1H, furyl), 7.67 (d, $J=7.8$ Hz, 1H, furyl), 7.83 (d, $J=8$ Hz, 1H, furyl). Anal. calcd for $C_{11}H_8ClN_3OS$: C, 49.72, H, 3.03, N, 15.82. Found: C, 49.99, H, 3.18, N, 15.46.

4-Chloro-6-(2-furyl)-2-(n)-propylsulfanylpyrimidine-5-carbonitrile (3o). Yield 73.2%; mp 158 °C; MS (EI) *m/z* 279 (M^+, 34.8), 264 (26.6), 249 (100), 237 (25.7); IR (KBr) 2226 cm^{-1} (CN); ^1H NMR (200 MHz, $CDCl_3$) δ 1.07 (t, $J=6.1$ Hz, 3H, CH_3), 1.68–1.82 (m, 2H, CH_2), 3.19 (t, $J=6.4$ Hz, 2H, SCH_2), 6.67 (d, $J=7.8$ Hz, 1H, furyl), 7.66–7.77 (m, 1H, furyl), 7.89 (d, $J=7.7$ Hz, 1H, furyl). Anal. calcd for $C_{12}H_{10}ClN_3OS$: C, 51.52, H, 3.60, N, 15.02. Found: C, 51.41, H, 3.86, N, 15.19.

4-Chloro-2,6-dimethylsulfanylpyrimidine-5-carbonitrile (7a). Yield 64%; mp 150 °C; MS (EI) *m/z* 231 (M^+), 216 (32.9), 197 (34.5), 170 (26.7); IR (KBr) 2216 cm^{-1} (CN); ^1H NMR (200 MHz, $CDCl_3$) δ 2.50 (s, 3H, SCH_3), 2.60 (s, 3H, SCH_3). Anal. calcd for $C_7H_6ClN_3S_2$: C, 36.28, H, 2.60, N, 18.13. Found: C, 36.22, H, 2.87, N, 18.03.

2-Benzylsulfanyl-4-chloro-6-methylsulfanylpyrimidine-5-carbonitrile (7b). Yield 64.2%; mp 150 °C; MS (EI) *m/z* 307 (M^+, 41.6), 273 (20), 232 (13), 230 (24.3); IR (KBr) 2216 cm^{-1} (CN); ^1H NMR (200 MHz, $CDCl_3$) δ 2.60 (s, 3H, SCH_3), 3.92 (s, 2H, CH_2), 7.20–7.33 (m, 2H, Ar–H) 7.45–7.67 (m, 3H, Ar–H). Anal. calcd for $C_{13}H_{10}ClN_3S_2$: C, 50.72, H, 3.27, N, 13.65. Found: C, 50.52, H, 3.57, N, 13.43.

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