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Synthesis and characterization of novel 1,6-dihydropyrimidine derivatives for their pharmacological properties

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ABSTRACT

The present research work involves the use of commercially available thiophene-2-carbaldehyde as a starting material to construct novel pyrimidine compounds. Synthesis of pyrimidine derivatives has been done by the trimolecular Biginelli condensation reaction, which involves the use of thiophene-2-carbaldehyde with cyano ethylacetate and thiourea to yield 4-oxo-6-(thiophen-2-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (1). The intermediate 1 was methylated using methyl iodide and K_2CO_3 in dimethylformamide (DMF) which afforded dimethylated derivative 1-methyl-2-(methylthio)-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidine-5-carbonitrile (2). The intermediate compound 2 when refluxed with hydrazine hydrate in ethanol as a solvent led to the formation of the parent compound 2-hydrazineyl-1-methyl-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidine-5-carbonitrile (3), the parent compound 3 was used for the synthesis of carboxamides of *N′*-(5-cyano-1-methyl-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidin-2-yl)substituted benzohydrazide (4a–d) and Schiff bases of (*E*)-2-(2-substituted benzylidenehydrazineyl)-1-methyl-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidine-5-carbonitrile (5a–g). Selected title compounds are screened for antibacterial, analgesic, and antifungal activities.

INTRODUCTION

Heterocyclic compounds play an important roles in biological and pharmaceutical process. As several drug molecules contain heterocycles as a core structure, great efforts have been made to develop improved synthetic methods for this structure (Chandrashekharappa *et al*., 2018a; 2018b; Mallikarjuna *et al*., 2016; Nagesh *et al*., 2014; Sandeep *et al*., 2013a; 2013b; 2014; 2016a; 2016b; Siddesh *et al*., 2014a). Among all heterocycles, pyrimidine-based heterocycles are more interesting in biological applications (Bairagi *et al*., 2018; Dharma Rao *et al*., 2017; Devi *et al*., 2009; Siddesh *et al*., 2013; Siddesh *et al*., 2014a; Thriveni *et al*., 2014; Venugopala *et al*., 2014). Pyrimidines linked to thiopheno moiety has been reported in the literature for many years (Ram *et al*., 1987; Ramesh and Bhalgat, 2011). They have been found to possess a wide spectrum of biological activities (Ghith *et al*., 2017; Noravyan *et al*., 2012; Wu, 2012), and many of them have been used as drugs in the market (Shishoo *et al*., 2009), and some of the structures of active pharmaceutical ingredients are highlighted in Figure 1.

The above observations revealed the importance of thienopyrimidine derivatives for various pharmacological properties, such as anticancer (Bugge *et al*., 2016; Ghith *et al*., 2017; Ravez *et al*., 2015; Teo *et al*., 2015; Yong *et al*., 2015; Zhang *et al*., 2015), antiviral (Kankanala *et al*., 2017), antidiabetic

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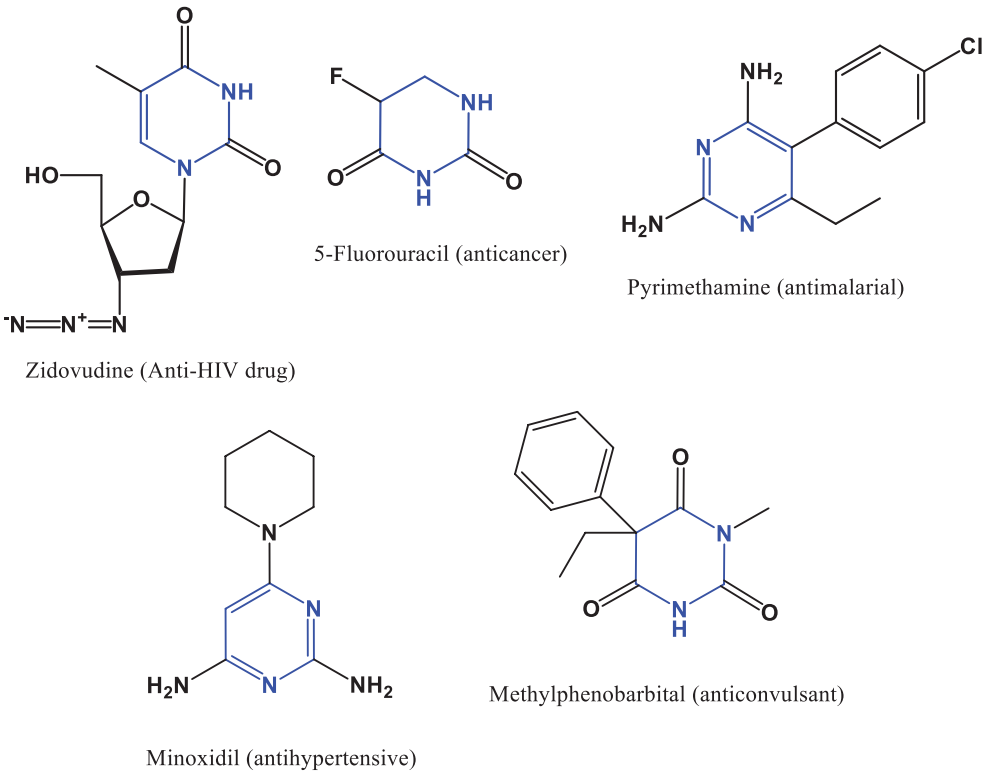


Figure 1. Chemical structure of active pharmaceutical ingredients having pyrimidine pharmacophore.

(Klossowski *et al*., 2017), antimicrobial (Aruna Kumari *et al*., 2017; Bari and Haswani, 2017; Joshi *et al*., 2011; Ortikov *et al*., 2017), antitubercular (Borate *et al*., 2016; Chandrashekharappa *et al*., 2019; Pisal *et al*., 2017; Rosado *et al*., 2017; Srnivasula *et al*., 2016; Venugopala *et al*., 2013; 2016), antiulcer (Amr *et al*., 2016), and immunosuppressive agents (Zhang *et al*., 2017). Synthesis of these molecular analogues is worth to get novel pharmacologically active leads. Encouraged by the research findings on pharmacological properties of thienopyrimidines, in this work, we have undertaken the synthesis of pyrimidine derivative linked to thiophene moieties (**4a–d**) and (**5a–g**). The sequence of reactions carried out to achieve the title compounds (**4a–d**) and (**5a–g**) is depicted in Scheme 1.

MATERIALS AND METHODS

Chemistry

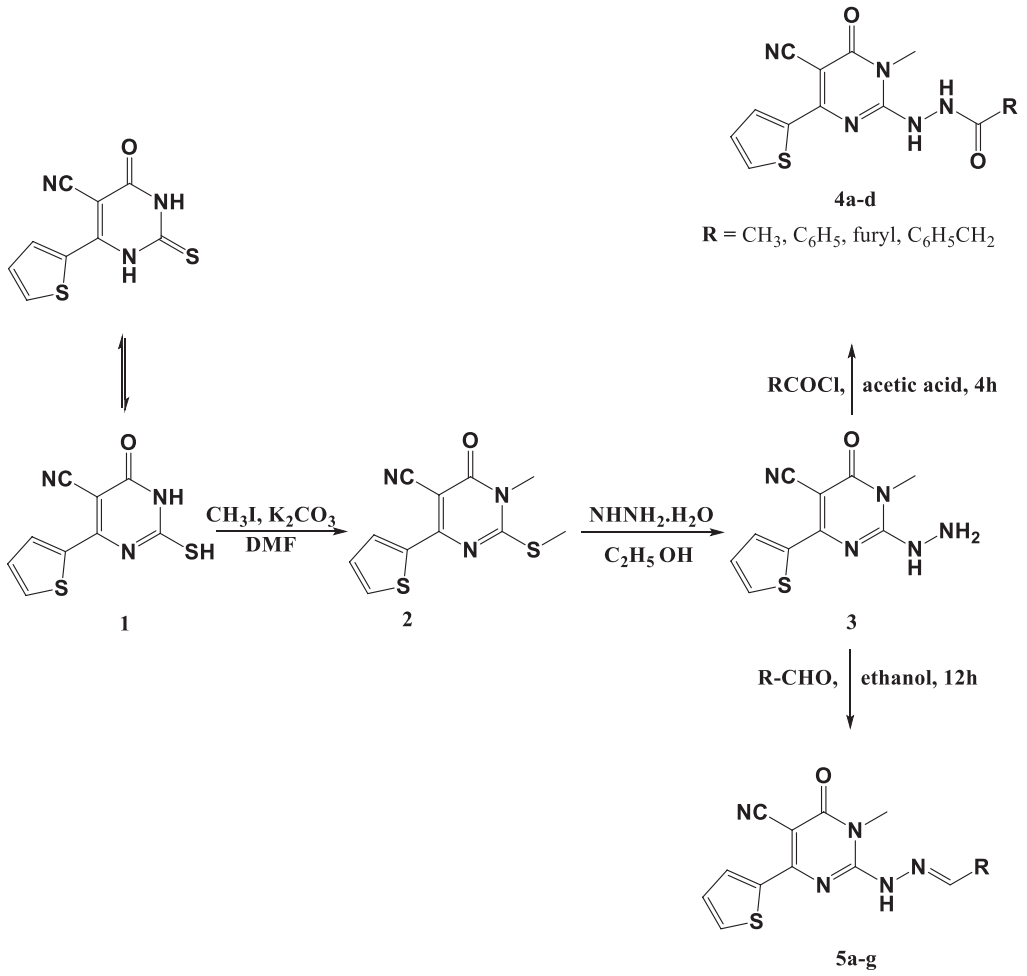
All the chemicals and solvents used were of AR grade and procured from Sigma-Aldrich, India. The Scheme 1 chemical reactions were carried out under a nitrogen atmosphere using a dry solvent. The progress of the reaction was monitored by thin layer chromatography (TLC). TLC was performed on Merck silica gel on TLC aluminum foil with ethyl acetate and hexane as the solvent system and visualization in a UV chamber. IR spectra were recorded on Thermo scientific Fourier-transform infrared spectroscopy (FT-IR) spectrophotometer, and ^{1}H Nuclear magnetic resonance (NMR) spectra were recorded at ambient temperature using dimethyl sulfoxide (DMSO) and $CDCl_3$ as a solvent using Bruker AV 800 spectrometer. The chemical shifts are expressed in δ ppm and were reference with tetramethyl silane. The peak multiplicities were specified as follows: s, singlet; d, doublet; t, triplet; q, quartet; and m, multiplet. Liquid chromatography–mass spectrometry (LC-MS) were performed on a Jeol JMS-D 300 mass spectrometer operating at 70 eV. Elemental analysis was performed on a Thermo Finnigan FLASH FA 1112 CHN analyzer.

General procedure for the synthesis of N′-(5-cyano-1-methyl-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidin-2-yl) benzohydrazide (4a)

A mixture of 2-hydrazineyl-1-methyl-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidine-5-carbonitrile (**3**) (2.49 g, 0.01 mol), acetyl chloride (1.40 ml, 0.01 mol) in acetic acid (20 ml) was refluxed for 4 hours. The completion of the reaction was monitored by TLC. After reaction completion, the resulting reaction medium was poured into the ice cold water, stirred well, the solid obtained was filtered and washed thoroughly with water. The crude compound was purified through recrystallization in ethyl alcohol afforded 2.13 g (60%) of *N*′-[5-cyano-1-methyl-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidin-2-yl]acetohydrazide (**4a**). Similarly, the remaining compounds **4b, 4c**, and **4d** were prepared by following a similar procedure. FT-IR (KBr) ν (cm^{-1}): 3,074 (NH), 2216 (CN), 1,679 (C=O); ^{1}H NMR δ = 8.25 (NH), 7.3–8.3 (m, 3H, ArH), 4.0 (s, 1H, NH), 2.3 (s, 1H, NH), 3.5 (s, 3H, −NCH_3), 2.6 (s, 3H, $COCH_3$); Elemental analysis calculated for $C_{12}H_{11}N_5O_2S$: C, 49.77; H, 3.80; N, 24.19; Found: C, 49.73; H, 3.76; N, 24.15; LC-MS: *m/z* = 290.3 (M+1).

N′-[5-Cyano-1-methyl-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidin-2-yl] benzohydrazide (4b)

FT-IR (KBr) ν (cm^{-1}): 3,318 (NH), 2,236 (CN), 1,690 (C=O); Elemental analysis calculated for $C_{17}H_{13}N_5O_2S$: C, 58.11;



Scheme 1. Synthetic scheme for the construction of N'-(5-cyano-1-methyl-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidin-2-yl)substituted benzohydrazide (**4a–d**) and (E)-2-(2-substituted benzylidenehydrazineyl)-1-methyl-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidine-5-carbonitrile (**5a–g**).

H, 3.73; N, 19.93; Found: C, 58.03; H, 3.64; N, 19.88; LC-MS: *m/z* = 352.3 (M+1).

N′-[5-Cyano-1-methyl-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidin-2-yl]furan-2-carbohydrazide (4c)

FT-IR (KBr) *v* (cm^{-1}): 3,350 (NH), 2,215 (CN), 1,680 (C=O); Elemental analysis calculated for $C_{15}H_{11}N_5O_3S$: C, 52.78; H, 3.25; N, 20.52; Found: C, 52.69; H, 3.18; N, 20.56; LC-MS: *m/z* = 342.3 (M+1).

N′-[5-Cyano-1-methyl-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidin-2-yl]-2-phenylacetohydrazide (4d)

FT-IR (KBr) *v* (cm^{-1}): 3,384 (NH), 2,233 (CN), 1,679 (C=O); ^{1}H NMR δ: 8.56 (NH), 7.89-7.69 (m, 5H, Ar-H), 6.25 (m, 3H-thiophene), 3.99 (2H, benzylic), 3.75 (s, 3H-NCH_3); Elemental analysis calculated for $C_{18}H_{15}N_5O_2S$: C, 59.17; H, 4.14; N, 19.17; Found: C, 59.07; H, 4.06; N, 19.11; LC-MS: *m/z* = 366.4 (M+1).

Synthetic procedure for the preparation of (E)-2-(2-benzylidenehydrazineyl)-1-methyl-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidine-5-carbonitrile (5a)

The compound 2-hydrazineyl-1-methyl-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidine-5-carbonitrile (**3**) (2.49 g, 0.01 mol) was refluxed with benzaldehyde (1.06 g, 0.01 mol) and a catalytic amount of acetic acid in absolute ethyl alcohol for 12 hours. The completion of the reaction was monitored through TLC, after the completion, the reaction mixture was poured into ice cold water with stirring; the solid obtained was filtered and washed thoroughly with water. The crude product was purified by several trials of recrystallization with rectified spirit afforded the pure 2-[(2*E*)-2-benzylidenehydrazinyl]-1-methyl-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidine-5-carbonitrile (**5a**) at 2.06 g (61%). Similarly, the other Schiff bases **5b–g** were prepared by following the similar procedure, and their physicochemical parameters are tabulated in Table 1. FT-IR (KBr) *v* (cm^{-1}): 3,113 (NH), 2,224 (CN), 1,671 (C=O), 1,551 (CH=N); ^{1}H NMR *δ* = 3.4(s, 1H, NH), 9.9 (s,

Table 1. Physicochemical constants of *N'*-(5-cyano-1-methyl-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidin-2-yl) substituted benzohydrazide (**4a–d**) and (*E*)-2-(2-substitued benzylidenehydrazineyl)-1-methyl-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidine-5-carbonitrile (**5a–g**).

Compd code	Mol formulae (Mol mass)	R	Yield (%)[a]	m.p (°C)	$_c$LogP[b]
4a	$C_{12}H_{11}N_5O_2S$ (289)	CH_3	60	192	−0.2347
4b	$C_{17}H_{13}N_5O_2S$ (351)	C_6H_5	40	175	0.9042
4c	$C_{15}H_{11}N_5O_3S$ (341)	Furyl	65	189	0.0802
4d	$C_{18}H_{15}N_5O_2S$ (365)	$C_6H_5CH_2$	38	203	1.3232
5a	$C_{17}H_{13}N_5OS$ (335)	C_6H_5	61	210	2.8042
5b	$C_{18}H_{15}N_5O_2S$ (365)	2-OCH_3,C_6H_4	42	221	2.5950
5c	$C_{15}H_{11}N_5OS_2$ (341)	Thienyl	35	193	2.5425
5d	$C_{17}H_{12}ClN_5OS$ (369)	4-Cl, C_6H_4	50	228	3.5208
5e	$C_{17}H_{13}N_5O_2S$ (351)	2-OH, C_6H_4	42	213	3.4032
5f	$C_{15}H_{11}N_5O_2S$ (325)	Furyl	30	197	1.9802
5g	$C_{17}H_{12}N_6O_3S$ (380)	4- NO_2, C_6H_4	60	217	2.5563

[a]Compounds purification by recrystallization method using ethanol as solvent.

[b]*c*Log*P* was calculated using ChemDraw Professional 16.0v.

1H, N=CH), 7.1–8.5 (m, 8H, ArH), 2.1 (s, 3H-NCH_3); Elemental analysis calculated for $C_{17}H_{13}N_5OS$: C, 60.88; H, 3.91; N, 20.88; Found C, 60.80; H, 3.83; N, 20.83; LC-MS: *m/z* = 336 (M+1).

2-[(2E)-2-(2-Methoxybenzylidene)hydrazinyl]-1-methyl-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidine-5-carbonitrile (5b)

FT-IR (KBr) *v* (cm^{-1}): 3,196 (NH), 2,223 (CN), 1,779 (C=O), 1662 (CH=N); Elemental analysis calculated for $C_{18}H_{15}N_5O_2S$: C, 59.17; H, 4.14; N, 19.17; Found C, 59.07; H, 4.06; N, 19.11; LC-MS: *m/z* = 366.4 (M+1).

1-Methyl-6-oxo-4-(thiophen-2-yl)-2-[(2E)-2-(thiophen-2-ylmethylidene) hydrazinyl]-1,6-dihydropyrimidine-5-carbonitrile (5c)

FT-IR (KBr) *v* (cm^{-1}): 3,249 (NH), 2,217 (CN), 1,654 (C=O), 1,549 (CH=N); ^{1}H NMR δ = 8.80 (NH), 8.58 (1H, N=CH), 7.04–6.29 (m, 6H-thiophenes), 3.82 (s, 3H-NCH_3 Elemental analysis calculated for $C_{15}H_{11}N_5OS_2$: C, 52.77; H, 3.25; N, 20.51; Found C, 52.68; H, 3.18; N, 20.46; LC-MS: *m/z* = 342.4 (M+1).

2-[(2E)-2-(4-Chlorobenzylidene)hydrazinyl]-1-methyl-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidine-5-carbonitrile (5d)

FT-IR (KBr) *v* (cm^{-1}): 3,097 (NH), 2,216 (CN), 1,657 (C=O), 1,633 (CH=N); ^{1}H NMR δ = 8.79 (NH), 8.65 (1H, N=CH), 8.01–7.86 (m, 4H, Ar-H), 6.94–6.60 (m, 3H-thiophene), 3.71 (s, 3H-NCH_3); Elemental analysis calculated for $C_{17}H_{12}ClN_5OS$: C, 55.21; H, 3.27; N, 18.94; Found C, 55.12; H, 3.20; N, 18.88; LC-MS: *m/z* = 369 (M^+).

2-[(2E)-2-(2-Hydroxybenzylidene)hydrazinyl]-1-methyl-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidine-5-carbonitrile (5e)

FT-IR (KBr) *v* (cm^{-1}): 3,379 (NH), 2,254 (CN), 1,700 (C=O), 1,555 (CH=N); ^{1}H NMR δ = 8.78 (NH), 8.66 (1H, N=CH), 7.99–7.80 (m, 4H, ArH), 6.88–6.70 (m, 3H-thiophene), 3.80 (s, 3H-NCH_3); Elemental analysis calculated for $C_{17}H_{13}N_5O_2S$: C, 58.11; H, 3.73; N, 19.93; Found C, 57.86; H, 3.66; N, 19.88; LC-MS: *m/z* = 352.3 (M+1).

2-[(2E)-2-(Furan-2-ylmethylidene)hydrazinyl]-1-methyl-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidine-5-carbonitrile (5f)

FT-IR (KBr) *v* (cm^{-1}): 3,378 (NH), 2,266 (CN), 1,689 (C=O), 1,544 (CH=N); Elemental analysis calculated for $C_{15}H_{11}N_5O_2S$: C, 55.38; H, 3.41; N, 21.53; Found:- C, 55.33; H, 3.39; N, 21.51; LC-MS: *m/z* = 326.3 (M+1).

1-Methyl-2-[(2E)-2-(4-nitrobenzylidene)hydrazinyl]-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidine-5-carbonitrile (5g)

FT-IR (KBr) *v* (cm^{-1}): 3,368 (NH), 2,256 (CN), 1,670 (C=O), 1,545 (CH=N); Elemental analysis calculated for $C_{17}H_{12}N_6O_3S$: C, 53.68; H, 3.18; N, 22.09; Found C, 53.60; H, 3.11; N, 22.04; LC-MS: *m/z* = 381.3 (M+1).

Pharmacology

Antibacterial activity

The antibacterial activity of the synthesized compounds was performed using a cup plate method (Nagesh *et al*., 2015) employing Hi-Media agar medium against two Gram-positive bacteria *Bacillus subtilis* (ATCC 6633) and *Staphylococcus aureus* (ATCC 25923) and Gram-negative bacteria *Escherichia coli* (ATCC 35218) and *Pseudomonas aeruginosa* (ATCC 10145). The antibacterial results of the studied compounds are summarized in Table 2. The tested compounds exhibited slight to moderate antibacterial activity against all microorganisms when compared to the standard compounds.

Antifungal activity

The antifungal activity (Nagesh *et al*., 2015) of the test compounds was tested against two different fungal strains, namely, *Candida albicans* and *Aspergillus niger* by a filter paper disc technique at 50 and 100 µg/ml concentrations. After 48 hours incubation, the zone of inhibition was measured in

millimeter. Griseofulvin was used as the standard drug and dimethylformamide as a control. The antifungal results are tabulated in Table 3.

Analgesic activity

Analgesic activity of the test compounds was tested by an acetic-acid-induced writhing method using Albino mice of either sex (20–30 g) (Siddesh *et al*., 2014b). A 0.6% Acetic acid solution was used to induce writhing in mice. Eleven groups of animals were prepared with six animals in each. The analgesic response was assessed by counting the number of abdominal constrictions for 20 minutes starting 3 minutes after the injection of acetic acid solution. The test compounds were administered to group 1–10 at 100 mg/kg body weight and eleventh group received the standard drug at 100 mg/kg body weight. After 1 hour, the acetic acid solution was administered intraperitoneally, and a number of abdominal constrictions were documented for 20 minutes starting 3 minutes after the injection of acetic acid solution. The analgesic activity was calculated as the percentage of maximum possible effect, and the results are given in Table 4. Animal ethical clearance to conduct *in vivo* analgesic activity was obtained from the ethical committee from the institution.

RESULTS AND DISCUSSION

Chemistry

In the present work, synthesis of proposed compounds *N*′-(5-cyano-1-methyl-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidin-2-yl)substituted benzohydrazide (**4a–d**) and (*E*)-2-(2-substituted benzylidenehydrazineyl)-1-methyl-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidine-5-carbonitrile (**5a–g**) were achieved by exploiting commercially available thiophene-2-carbaldehyde as a starting compound. Synthesis of parent compound 2-hydrazineyl-1-methyl-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidine-5-carbonitrile (**3**) is achieved based on the reported procedure (Ram *et al*., 1987; Ramesh and Bhalgat, 2011). Novel compounds (**4b–d**) are prepared by following the procedure involved in the synthesis of title compound *N*′-[5-cyano-1-methyl-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidin-2-yl] acetohydrazide (**4a**) (Ram *et al*., 1987). Schiff bases of novel title compounds (**5a–g**) are prepared by refluxing parent compound 2-hydrazineyl-1-methyl-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidine-5-carbonitrile (**3**) with substituted, phenyl, thienyl, and furyl aldehydes in absolute ethanol medium.

Synthesis of 4-oxo-6-(thiophen-2-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**1**) involves the trimolecular

Table 2. Antibacterial activity of test compounds **3a**, **4a**, **4b**, **5a**, **5b**, **5c,** and **5f** against Gram-positive and Gram-negative organisms in comparison with controls.

Comp code	R	Zone of Inhibition (mm)							
		S. aureus		*B. subtilis*		*E. coli*		*P. aeruginosa*	
		50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml
3a	-	14	16	13	15	10	13	11	14
4a	CH_3	10	12	15	20	13	19	17	23
4b	C_6H_5	11	16	14	17	10	14	10	13
5a	C_6H_5	14	20	18	23	10	14	14	20
5b	2-OCH_3-C_6H_4	15	21	16	21	15	18	20	25
5c	C_4H_3S	10	14	14	20	12	17	19	23
5f	Furyl	10	12	12	18	15	18	16	19
DMF	-	-	-	-	-	-	-	-	-
Penicillin	-	15	20	16	22	-	-	-	-
Streptomycin	-					21	26	20	25

Table 3. Antifungal activity of test compounds **3a**, **4a**, **4b**, **5a**, **5b**, **5c,** and **5f** in comparison with controls.

Comp code	R	Zone of inhibition (mm)			
		C. albicans		*A. niger*	
		50 µg/mll	100 µg/ml	50 µg/ml	100 µg/ml
3a	-	15	19	13	17
4a	CH_3	21	25	20	25
4b	C_6H_5	17	21	17	21
5a	C_6H_5	14	20	18	22
5b	2-OCH_3, C_6H_4	18	21	14	17
5c	C_4H_3S	19	21	15	19
5f	Furyl	11	18	11	16
DMF	-	-	-	-	-
Griseofulvin	-	20	24	22	25

Table 4. Analgesic activity of test compounds **3a**, **4a**, **4b**, **5a**, **5b**, **5c,** and **5f** in comparison with controls.

Comp code	R	Mean no. of Writhings ± SEM	Percentage protection
3a	-	20.83 ± 2.43	54.54
4a	CH_3	18.42 ± 1.84	59.80
4b	C_6H_5	20.84 ± 2.16	54.52
5a	C_6H_5	17.57 ± 1.69	61.16
5b	2-OCH_3, C_6H_4	16.84 ± 1.54	63.32
5c	C_4H_3S	18.09 ± 1.64	60.05
5f	Furyl	23.51 ± 2.94	48.70
Tween 80	-	45.83 ± 3.66	-
Aspirin	-	11.99 ± 1.27	73.83

Biginelli condensation of thiophene-2-carbaldehyde with cyanoethylacetate and thiourea. The compound **1** was methylated using methyl iodide and K_2CO_3 in dimethylformamide (DMF) which afforded dimethylated derivative 1-methyl-2-(methylthio)-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidine-5-carbonitrile (**2**). The formation of the intermediate compounds **1** and **2** have been confirmed by our research group. The compound **2** when refluxed with hydrazine hydrate in ethanol medium led to the formation of parent compound 2-hydrazineyl-1-methyl-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidine-5-carbonitrile (**3**) which was the key intermediate for the synthesis of target compounds **4a–d** and **5a–g**.

The IR spectrum of compound **3** exhibited stretching bands at 3,350, 2,220, and 1,670 cm^{-1} due to NH, CN, and C=O, respectively. ^1H NMR spectrum of parent compound **3** taken in DMSO exhibited multiplet at δ 8.2–7.2 and singlet at δ 3.2 toward three aromatic protons and *N*-methyl protons, respectively. Two characteristic signals for NH and NH_2 at δ 3.4 and 2.4, respectively. The mass spectrum of the compound exhibited its molecular ion peak at *m/z* 248 corresponding to its molecular weight.

Intermediate compound **3** on acetylation with acetyl chloride in an acetic acid solvent for 8 hours refluxation led to the formation of *N*′-[5-cyano-1-methyl-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidin-2-yl] acetohydrazide (**4a**). The IR spectrum of compound **4a** exhibited peaks at 3,074, 2,216, 1,679 cm^{-1} due to NH, CN, and C=O groups, respectively. ^1H NMR spectrum of the same compound was recorded in DMSO, the multiplet observed at 7.3–8.3 corresponding to three aromatic protons, two NH protons appeared at 2.4 and 4.0, $COCH_3$ proton appeared at 2.6, and a singlet at 3.5 belongs to three protons of *N*-methyl. The structure of **4a** was further confirmed by the appearance of a molecular ion peak at *m/z* 290 (M+1) in its mass spectrum. Similarly, the compounds **4b–d** were prepared by using the corresponding acid chlorides.

The compound **3** was also refluxed with the benzaldehyde in ethanol with a catalytic amount of acetic acid for 12 hours to yield 2-[(2*E*)-2-benzylidenehydrazinyl]-1-methyl-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidine-5-carbonitrile (**5a**). The IR spectrum of compound **5a** exhibited peaks at 3,113, 2,224, 1,671, and 1,551 cm^{-1} due to NH, CN, C=O, and CH=N groups, respectively. ^1H NMR spectrum of the same compound recorded in DMSO, exhibited a peak at δ 3.4 corresponding to one proton of amine, at δ 9.9 for CH=N proton, multiplet between δ 7.1 and 8.5 corresponds to eight aromatic protons, singlet at 2.1 belongs to three protons of N-CH_3. The structure of **5a** was further confirmed by the appearance of a molecular ion peak at *m/z* 336 (M+1) in its mass spectrum. Similarly, compounds **5b–g** were prepared by using the corresponding aldehydes.

The detailed experimental procedure, analysis data for the compounds mentioned above have been incorporated in the experimental section. The structures of all the synthesized compounds have been elucidated by IR, ^1H NMR, LC-MS, and elemental analysis data. Some of the selected compounds have been tested for antibacterial, antifungal, and analgesic activities, and the results have been discussed.

Pharmacology

The tested compounds exhibited significant to moderate antibacterial activity (Table 2) compared to the standard drugs against all microorganisms. Compounds **4a** and **5b** showed significant antibacterial activity against all the bacterial strains. Compound **4a** exhibited equipotent antifungal activity as that of a standard compound (Table 3). Compounds **5a** and **5b** showed considerable analgesic activity when compared to a standard substance (Table 4).

CONCLUSION

Reactions performed to achieve Schiff bases, and carboxamides of parent compound 2-hydrazineyl-1-methyl-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidine-5-carbonitrile (**3**) were eco-friendly and yields obtained were satisfactory. Purification of the compounds was achieved by recrystallization method, and the purity was over 99% which was ascertained by High-performance liquid chromatography (HPLC). Characterization of the compounds was completed by spectral analysis. Title compounds were screened for antibacterial, antifungal, and analgesic properties. Title compounds **4a** and **5b** showed significant antibacterial activity against all the bacterial strains. For antifungal activity, test compound **4a** exhibited equipotent as that of standard compound. Title compounds **5a** and **5b** exhibited considerable analgesic activity when compared to a standard substance.

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interests.

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