Synthesis and Pharmacological Properties of N-Substituted-*N*′-(4,6-dimethylpyrimidin-2-yl)-Thiourea Derivatives and Related Fused Heterocyclic Compounds

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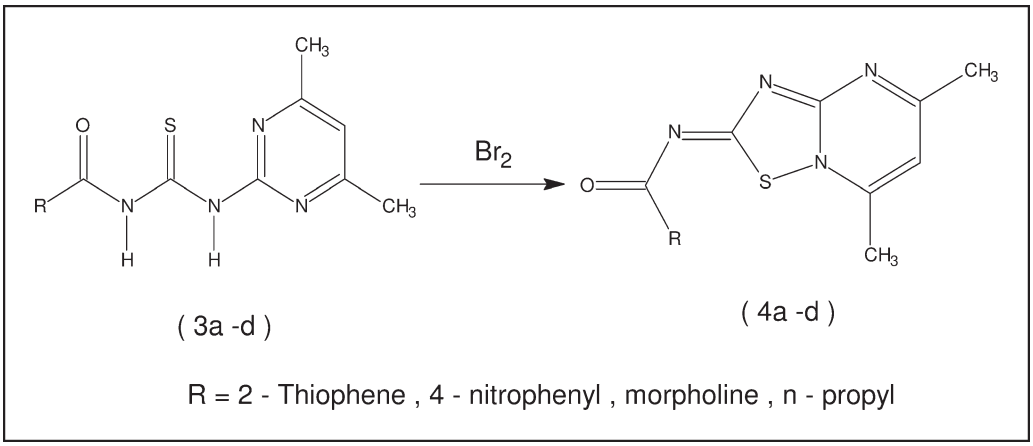

A series of new N-Substituted-*N*′-(4,6-dimethylpyrimidin-2-yl)-thiourea derivatives (**3a–d**) and related fused heterocyclic compounds (**4a–d**) were synthesized using tetrabutylammonium bromide as phase transfer catalyst (PTC). *N*-[(2*E*)-5,7-dimethyl-2*H*-[1,2,4] thiadiazolo [2,3-*a*] pyrimidin-2-ylidene] derivatives (**4a–d**) were prepared by oxidative cyclization of **3a–d**. The structures of these novel compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, mass spectrometry, and the elemental analysis. The crystal structures were determined from single crystal X-ray diffraction data. The results indicated that the compounds possessed a broad spectrum of activity against the tested microorganisms and showed higher activity against fungi than bacteria. Compounds **3d** and **3a** exhibited the greatest antimicrobial activity.

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INTRODUCTION

Recent years have seen a dramatic increase in fungal infections, mostly caused by *Candida albicans*; these infections are often spread through the use of broad-spectrum antibiotics, immunosuppressive agents, anticancer, and anti-AIDS drugs [1]. The main problem in the treatment of fungal infections is the increasing prevalence of drug resistance, especially in patient's chronically subjected to antimycotic therapy, such as persons infected with HIV [2]. For these reasons, serious attention has recently been directed toward the discovery and development of new antifungal drugs.

The biological and synthetic significance places this grouping in an important position in medicinal chemistry research. Substituted thioureas are an important class of compounds, precursors or intermediates towards the synthesis of a variety of heterocyclic systems such as imidazole-2-thiones [3], 2-imino-1,3-thiazolines [4], pyrimidine-2-thiones, and (benzothiazolyl)-4-quinazolinones[5], *N*-(substituted phenyl)-*N*-phenylthioureas have been developed as anion-binding site in a hydrogen-bonding receptor [6], calix [7], arenas-containing thioureas as neutral receptors towards α,α-dicarboxylate anions [8], and *N*-4-substituted-benzyl-*N*-ter-butylbenzyl thioureas as vanilloid receptors ligands and antagonists in rate DRG neurons [9]. Thioureas are also known to exhibit a wide range of biological activities including anticancer [10,11], antifungal [12], antiviral, antibacterial, anti-tubercular, anti-thyroidal, herbicidal and insecticidal activities[13] organocatalyst [14], and as agrochemicals [15,16].

Pyrimidine moiety is an important class of N-containing heterocycles widely used as key building blocks for pharmaceutical agents when attached with

thiourea functional group. It exhibits a wide spectrum of pharmacophore as it acts as bactericidal, fungicidal [17], analgesic [18], anti-hypertensive [19], and anti-tumor agents [20]. Among these, thiouracils are similarly used as anti-inflammatory and virucidal agents [21]. In addition, preclinical data from literature survey indicate continuing research in polysubstituted pyrimidine as potential anti-tumor agents [22]. The biological and synthetic significance places this scaffold at a prestigious position in medicinal chemistry research.

We became interested in the synthesis of thiourea derivatives containing the pyrimidine moiety and their fused heterocyclic compounds (**4a–d**) by using Br_2 as the oxidant [23]. Moreover, these compounds contain an inherently weak N—S bond, which can benefit plant absorption and metabolism. All the structures of these novel target compounds were characterized by spectroscopic techniques.

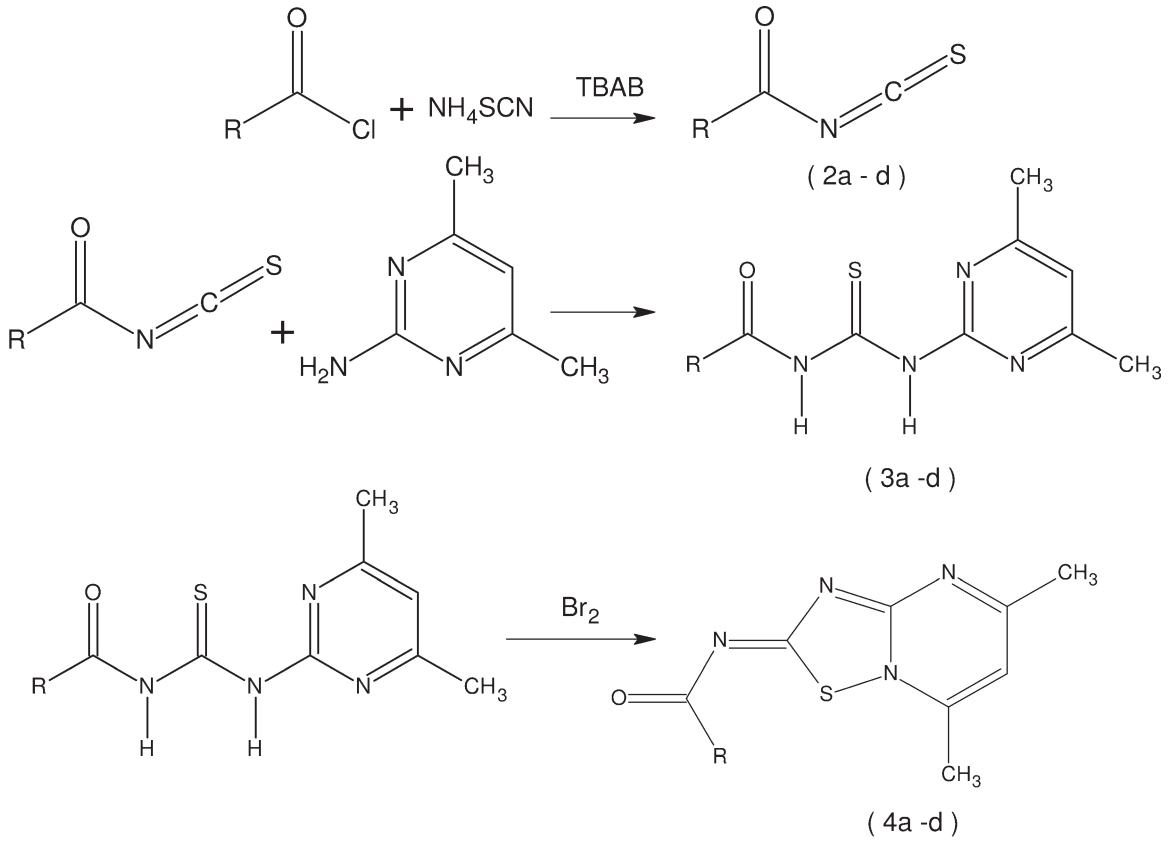
In this communication, results of synthesis, spectroscopic studies, crystal structure, and antimicrobial activity of pyrimidine-thiourea derivatives and related fused heterocyclic compounds are presented.

RESULTS AND DISCUSSION

Preparation and spectral studies. Synthesis of thiourea derivatives and related fused heterocyclic compounds was carried out by slight modification of our published procedures [24–26].The use of phase transfer catalyst (PTC) as a method of agitating a heterogeneous reaction system is gaining recognition [27,28]. In search of improving methods to prepare the target pyrimidine-thiourea derivatives by reacting isothiocyanates with nucleophiles, we have found the use of tetrabutylammonium bromide (TBAB) as PTC can afford aroyl, thiophenoyl and butanoyl isothiocyanates in good yield. In this article, we have conducted our reactions using TBAB as PTC to synthesize the intermediate pyrimidine-thioureas **(3a–d)**, then the compounds **3a–d** underwent a reaction with bromine at room temperature to give the corresponding compounds **(4a–d)**.

This provides a facile and convenient method for the synthesis of substituted *N*-[(2*E*)-5,7-dimethyl-2*H*-[1,2,4]thiadiazolo[2,3-*a*]pyrimidin-2-ylidene]derivatives, with the advantages of simple operation, short reaction times and good yield. Compounds **3a–d** and **4a–d** were prepared according to Scheme 1.

Scheme 1. Preparation of thiourea derivatives and related fused heterocyclic compounds.



All the structures of newly synthesized compounds were assigned on the basis of their elemental analysis and spectroscopic data, IR, and ^{1}H NMR. All the compounds were soluble in DMF, DMSO, ethanol, and ethyl acetate.

IR (KBr) spectrum of **3a–d** had strong N—H absorptions at about 3200–3350 cm^{-1} ,and displayed absorptions at about 1670–1680 cm^{-1}, 1440 cm^{-1} ,which were assigned to C=O, and C=S functions, respectively. The medium strong $\upsilon_{C=O}$ band in the IR spectra of all the compounds of series **3a–d** appeared at 1670–1680 cm^{-1}, which is lower than that of the ordinary carbonyl absorption (1730 cm^{-1}). The formation of H-bond leads an increase of their polarity, so the strength of their double bond decreased, and absorption moved to lower wave number. This possibility did not exist for compounds **4a–d**, hence, their C=O absorption is at comparatively higher wave number. Even though, there was a delocalized pi-bond in them, the wave number was not so high as that of the ordinary carbonyl, at about 1720 cm^{-1}.

The ^{1}H NMR spectrum exhibited the singlet and multiplet signals at about δ 6.20–6.50 and δ 7.10–8.50, which were assigned to pyrimidine-CH and phenyl-CH protons, respectively. Most compounds **3a–d** exhibited broad signals at 10.40–12.60 ppm, which were assigned to the N—H protons. The chemical shift of the proton of pyrimidine ring (Py-5-H) can change greatly, depending on the nature of the substituents. ^{13}C NMR showed peaks at δ 170.5–168.29, 180.02–179.02 for C=O (amide) and C=S (thioamide), respectively for compounds **3a–d**.The molecular structure of the compound **3a** is shown in Figure 1. The title compound (3a), $C_{12}H_{12}N_4OS_2$, crystallizes in thioamide forms with an intramolecular N5—H05···N3 hydrogen bond associated with the thiourea unit. Thiophene and pyrimidine units are planner. There is a strong intramolecular hydrogen bond N5—H05···N3, with H05···N3 = 1.937(14) Å, forming a six-membered ring. The C9—S1 and C10—O bonds show a typical double bond character with bond lengths of 1.660(11) and 1.214(13) Å, respectively. All of the C—N bonds, C2—N1 = 1.339(14) Å, C6—N1 = 1.338 (14) Å, C4—N3 = 1.356 (13) Å, C9—N4= 1.370 (14) Å, C9—N5 = 1.373 (13) Å, C10—N5 = 1.394(13) Å also indicate a partial double bond character. The C9—N5 bond because of its vicinity to the carbonyl group is slightly shorter than to the C2—N4 bond [29,30]. These bond distances are in good agreement with those observed in structures containing the *N*-benzoyl-N′-phenylthiourea moiety, as reported in the Cambridge Structural Database [31].The packing is three-dimensional, but a representative two-dimensional view along the short axis is given in Figure (Supporting Information). One classical H bond N4=H04···S1 and three

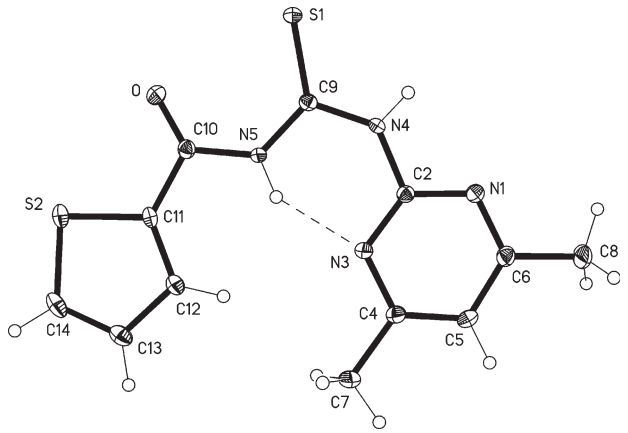


Figure 1. Perspective view of one of the independent molecules of **3a** with atom labeling scheme. Thermal ellipsoids are drawn at the 50% probability level. The dotted line shows the intramolecular Hbonding interaction.

''weak'' H bonds of the form C—H···X (X = O, S) are observed.

TESTING OF BIOLOGICAL ACTIVITIES

In vitro **antibacterial activity.** Six Gram-positive and Gram-negative bacterial strains (*Enterobacter cloacae*, *Escherichia coli*, *Proteus vulgaris*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, and *Staphylococcus aureus*) were included for the bioassay.

Compounds which showed minimal inhibitor concentration (MIC) of >100 μg/mL or above have not been included for the discussion. It was interesting to note that though several compounds show activity in different strains of bacteria only compounds **3a** and **3d** were found to be active against *E. cloacae* which is Gram negative strain of bacteria. Compounds **3a–d** displayed MICs of 25–100 μg/mL against other five strains of bacteria (Table 1)

Based on the MIC values against different stains of bacteria compound **3d** was found to be a potent antibacterial. The values of 15 μg/mL against *E. cloacae*, *E. coli* and 20 μg/mL against *S. aureus* and *S. epidermidis* were best amongst all the compounds screened. The compound **3a** showed MIC of 25 μg/mL against *E. cloacae*, 20 μg/mL against *E. coli*, 25 μg/mL against *S. aureus* and 20 μg/mL against *S. epidermidis.*

The reason for stronger antibacterial activity against Gram-negative and Gram-positive bacteria of **3d** and **3a** may be due to presence of thiophene ring and *n*-propyl group. This indicated that there are different structure requirements for binding of drug to bacterial or fungal targets, respectively. The antimicrobial activity values of the investigated compounds in this research were higher

Table 1

MIC values (μg/mL) of the synthesized pyrimidine-thiourea derivatives and related fused heterocyclic compounds against the tested gram-negative and gram-positive bacteria.

Compound	*E. cloacae* (ATCC 13047)	*E. coli* (ATCC 25922)	*P. vulgaris* (ATCC 13315)	*S. epidermidis* (ATCC 12228)	*E. faecalis* (ATCC 29212)	*S. aureus* (ATCC 25923)
3a	25	20	15	20	50	25
3b	25	25	50	25	50	50
3c	50	50	50	50	100	50
3d	15	15	15	20	25	20
4a	100	100	100	100	100	100
4b	100	100	100	100	100	100
4c	200	200	100	100	200	200
4d	100	100	100	100	100	100
Ref. Drugs	2‡	1‡	2‡	0.5†	4†	2†

‡ Gentamycin.
† Amikacin.

than that reported for other thiourea derivatives [32,33].The main difference in the thiourea derivatives reported in this article is the presence of pyrimidine moiety.

However, the thiophene and *n*-propyl based thiourea derivatives (**3d** and **3a**) containing the pyrimidine moiety showed good antimicrobial activity. Although the pyrimidine moiety is also present in all the fused heterocyclic compounds (**4a–d**) but their antimicrobial activity is comparatively lower than the thiourea derivatives (**3a–d**). Lipophilicity is another factor, which correlates well with the bioactivity of chemicals, is a very important molecular descriptor and different lipophilic behavior of compounds plays an important role in their biological activity mechanisms. The *n*-octanol/water partition coefficient (log P_{ow}) is widely used as a general measure of lipophilicity. The calculated value of partition coefficient (log P_{ow}) for compounds with 4-methylphenyl (**3c**) and 4-nitrophenyl (**3b**) have relatively higher log P_{ow} values of 2.31 ± 0.62 and 1.81 ± 0.63, respectively, and hence, show more lipophilic character [34].The antimicrobial activity for these compounds is comparatively lower than the compounds (**3d**) and (**3a**) because of its higher partition coefficient (log P_{ow}) value. The compounds with a thiophene ring (**3a**) and *n*-propyl group (**3d**), which have the log P_{ow} value of 1.45 ± 0.64 and 1.16 ± 0.62, respectively, show higher antibacterial activity than other investigated compounds due probability to their lower lipophilic character.

***In vitro* antifungal activity.** In the study of antifungal activity of synthesized compounds, two methods, ''broth micro-dilution procedure'' and ''poisoned food technique'' were adopted. All the thiourea derivatives and related fused heterocyclic compounds inhibited the growth of anti-yeast activity with MIC values ranging between 10 and >200 μg/mL. MIC values for thiourea derivatives were ranged between 10–50 μg/mL (Table 2).

According to the antimicrobial studies, all the compounds showed such activity, albeit lower than their anti-yeast efficacy. This difference may be due to the differences between the cell structure of bacteria and yeast. Although the cell walls of fungi contain chitin, the cell wall of bacteria contains murein [35]. In addition; fungi contain ergosterol in their cell membranes instead of the cholesterol found in the cell membranes of animals [36].

In ''poisoned food technique'' the prepared thiourea derivatives were tested against seven isolated fungi, that is, *A. alternata, A. flavus, A. niger, Rhizopus spp., Curvularia lunata, D. tetramera, and Penicillium spp.* as presented in (Table 3). Efficacy of **3a–d** were determined at different concentrations (0.005, 0.01, and 0.015) against seven isolated fungi, that is, *A. alternata, A. flavus, A. niger, Rhizopus spp., Curvularia lunata, D. tetramera, and Penicillium spp.* as presented in (Table 3). In this significant decrease in colony diameter was observed by all fungicides (**3a–d**) in different concentrations, when compared with control having large colony

Table 2

MIC values (μg/mL) of the synthesized pyrimidine-thiourea derivatives and related fused heterocyclic compounds against the tested fungi.

Compound	*C. glabrata* (ATCC32554)	*C. albicans* (ATCC 90028)	C. *tropicalis* (ATCC 20336)
3a	15	10	20
3b	50	20	25
3c	25	50	50
3d	10	10	15
4a	100	100	100
4b	100	100	100
4c	200	100	100
4d	100	50	100
Gentamycin	2	1	2

Table 3

Effect of thiourea derivatives (**3a–d**) in the % inhibition growth of fungal colonies (cm) on agar medium at 25°C.

Compd.	Conc.	*Alternaria alternates*	*A. flavus*	*A. niger*	*Curvularia lunata*	*D. tetramera*	*Rhizopus spp.*	*Penicillium spp.*
3d	0	3.775A	4.425A	4.200A	3.425A	2.825A	2.775A	3.900A
	0.005	2.050E	2.225DE	2.025CD	2.125C-E	1.375E-G	1.900BC	1.425C
	0.01	0.8750I	1.775F	1.400EF	1.675EF	0.825G	0.525F-G	0.525D
	0.015	0.5500J	1.254G	1.150F	1.375FG	0.4500H	0.3750G	0.425D
3a	0	3.775A	4.425A	4.200A	3.425A	2.825A	2.775A	3.900A
	0.005	2.650C	2.650C	2.700B	2.725BC	2.400AB	1.950BC	2.000BC
	0.01	1.800FG	2.075D-F	1.550D-F	2.250C-E	1.900B-E	1.200C-F	1.800C
	0.015	1.650H	1.700F	1.250EF	1.875D-F	1.400E-G	0.6750E-G	1.525C
3b	0	3.775A	4.425A	4.200A	3.425A	2.825A	2.775A	3.900A
	0.005	3.050B	3.050B	2.550B	3.050B	2.300A-C	2.000B	3.375A
	0.01	2.800C	2.450CD	1.721C-E	2.475CD	2.075B-D	1.525B-D	2.525B
	0.015	2.000EF	1.950EF	1.400EF	2.050DE	1.650D-F	1.350B-E	1.950BC
3c	0	3.775A	4.425A	4.200A	3.425A		2.825A	2.775
	0.005	2.375D	1.950EF	2.200BC	2.250C-E	1.975B-E	1.750BC	1.575C
	0.01	1.800FG	1.275G	1.500EF	1.750EF	1.675C-F	0.8500D-G	1.350C
	0.015	1.150H	1.025G	1.050F	0.9500G	1.100FG	0.575FG	0.700D
LSD value		0.457	0.729	0.754	0.523	0.671	0.480	0.430

$P < 0.05$ values within the same column show the same letters are not significantly different from each other.

diameter. It was observed that the growth reduction and the concentration of material in the medium, both were directly proportional to each other. **3d** and **3a** showed remarkable antifungal property against all tested fungi and proved as a best substitute of hazardous fungicides. **3c** showed inhibition of *A. niger* 66.66 %, *A. flavus* 55.80 %, *Penicillium spp*. 56.63 at 0.015 (Fig. 2; 3a), and little reduction on other concentrations. Whereas **3b** showed inhibitory effect on *A. niger, Rhizopus spp*., and *Penicillium spp*. at 0.015 and at 0.005, 0.01 it showed moderate effect (Fig. 2; 3b), **3a** showed antifungal property on almost every concentration but at 0.015 it possess 60–82% inhibition of all tested fungi (Fig. 2; 3c). **3d** showed inhibition of *Penicillium spp*. 98.30%, *Rhizopus spp*. 97.50% at 0.015 concentration as shown in Fig. 2; 3d. **3a** and **3d** was found the best antifungal to control the fungal growth; this efficacy may be due to presence of heterocyclic rings of pyrimidine, and thiophene in its structure. It means **3a** and **3d** have an ability to minimize fungal population. In **3a** and **3d** treatment, active molecules might have entered the fungal mycelium, interacted with in a short time and paralyzed it. Chemical ingredients present in this fungicide may be inhibitory to germination of fungal propugules with respect to others. Fungicidal seed treatments are known to reduced the seed-borne *mycoflora* and there by improve the seed germination. These fungi isolated were found to reduce germination and viability of seed and vitality of sunflower seedlings. Two systemic fungicides, **3a** and **3d** were found to be significantly effective in the elimination of seed-borne fungi.

CONCLUSIONS

An *in vitro* screen led to the identification of compounds **3a** and **3d** as potential antimicrobial candidates worthy of further structural modification and pharmacological evaluation. Moreover, the antimicrobial activity of this series suggests the pyrimidine-thiourea core offers a novel template for the development of a new class of antimicrobial agents. The *in vitro* antifungal study of the synthesized compounds, *N*-(4,6-dimethylpyrimidin-2-yl)-*N*′-(thiophene-5-carbonyl)-thiourea (**3a**) and *N*-butyryl-*N*′-(4,6-dimethylpyrimidin-2-yl)-thiourea (**3d**) shows good activity measured by broth micro-dilution method (BMD) and corroborated by poisoned food technique (PFT).

EXPERIMENTAL

4-Nitrobenzoyl chloride, thiophene-2-carbonyl chloride, 4-methylbenzoyl chloride, butanoyl chloride, ammonium thiocyanate, 2-amino-4,6-dimethylpyrimidine, TBAB and bromine of analytical grade from Fluka were used as received. Solvents; acetone, ethyl acetate, ethanol, methanol, dichloromethane, were obtained from RIEDEL and used as without further purification. The proton NMR and ^{13}C spectra were recorded in DMSO-d_6 solvent on Jeol ECS-400 and 300 MHz spectrophotometer using tetramethylsilane as an internal reference, respectively. The apparent resonance multiplicity is described as s (singlet), br s (broad singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), and m (multiplet). Infrared measurements were recorded in the range 400–4000 cm^{-1} on spectrum 2000 by Perkin Elmer. Elemental analysis was carried out using Perkin Elmer CHNS/O 2400. FAB MS

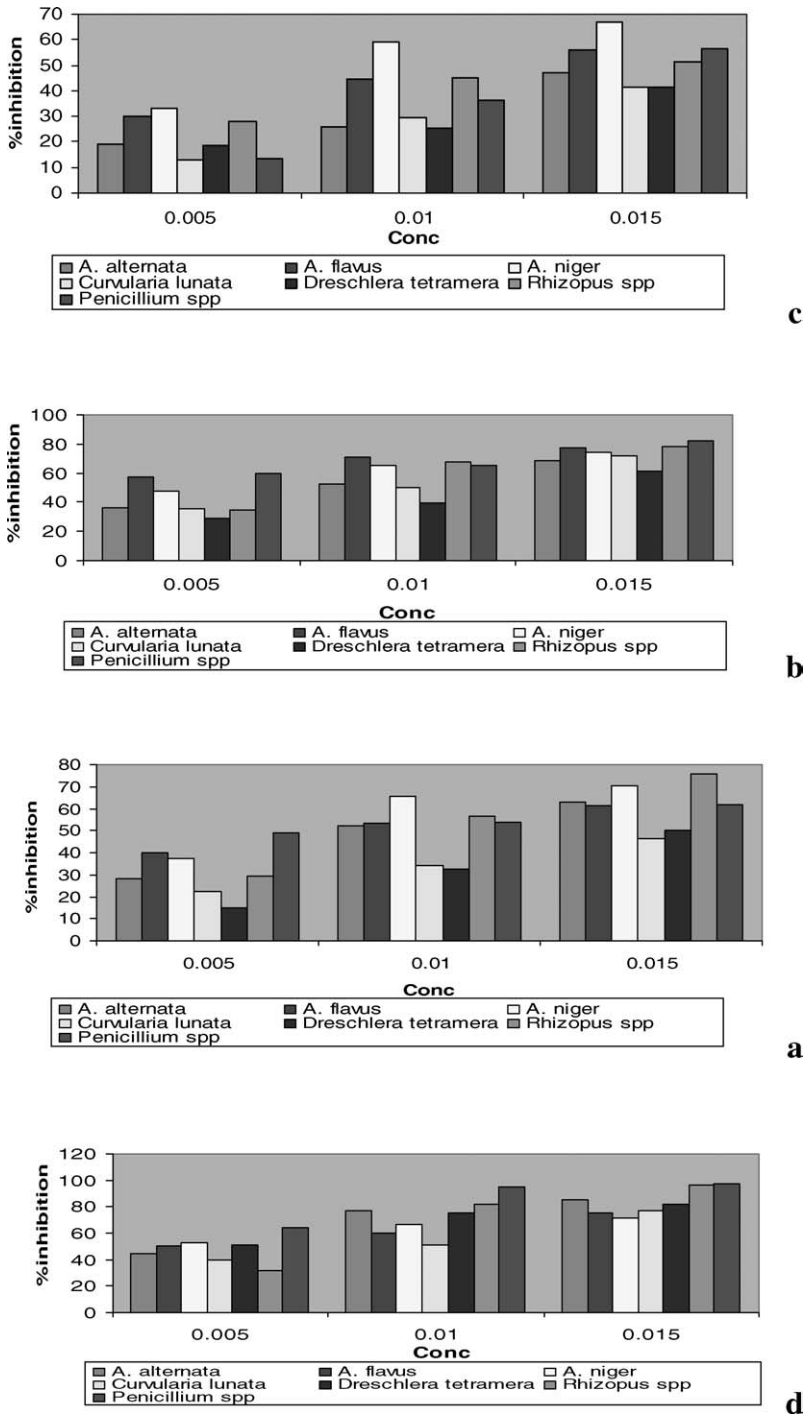


Figure 2. Effect of thiourea derivatives (**3a–d**) in the % inhibition growth of seven different fungi.

were obtained on a Finnigan MAG 90 mass spectrometer using glycerol as a matrix. Melting points were recorded on Electrothermal IA9000 series digital melting point apparatus. X-ray diffraction data were collected on Oxford Diffraction Xcalibur Nova diffractometer. Thin layer chromatography (TLC) analysis were carried out on 5 × 20 cm plate coated with silica gel GF_{254} type 60 (25–250 mesh) using an ethyl acetate-petroleum ether mixture (1:2) as solvent.

General procedure for synthesis of thiourea derivatives. A solution of substituted carbonyl chloride (0.01 mol) in anhydrous acetone (80 mL) and 3% TBAB in acetone was added drop wise to a suspension of ammonium

thiocyanate (0.01 mol) in acetone (50 mL) and the reaction mixture was refluxed for 30 min. After cooling at room temperature, a solution of 2-amino-4,6-dimethylpyrimidine (0.01 mol) in acetone (25 mL) was added and the resulting mixture refluxed for 1.5 h. The reaction mixture was poured into five times its volume of cold water when the thiourea precipitated as a solid. The product was recrystallized from dichloromethane: ethanol (1:2) mixture.

N-(4,6-dimethylpyrimidin-2-yl)-N′-(thiophene-5-carbonyl) thiourea (3a). Elemental analysis for $C_{12}H_{12}N_4OS_2$ (MW = 292.38) in wt % calc. C = 62.1, H = 3.28, N = 8.36, S = 9.55 and found to be C = 62.12, H = 3.34, N = 8.18, S = 9.43. m.p. 115°C, yield 93 %. IR (KBr pellet) in cm^{-1}: 3362 (free NH), 3215 (assoc. NH),1670 (C=O), 1529 (benzene ring), 1140 (C=S); ^{1}H NMR (400 MHz, DMSO-d_6) in δ (ppm) and *J* (Hz): 12.80 (1H, br s, NH), 11.63 (1H,br s, NH), 7.62 (1H, d, J = 7.2 Hz, Thiophene CH), 7.15 (1H, dd, J_1 = 7.5 Hz, J_2 = 8.2 Hz, Thiophene CH),7.03 (1H, d, J = 6.7 Hz, Thiophene CH), 6.50 (1H, s, pyrimidine-5-H), 1.23 (6H,s, pyrimidine-CH_3); ^{13}C NMR (300 MHz, DMSO-d_6) in δ (ppm): 179.2 (C=S), 168.9 (C=O), 159.5, 153.0, 138.6, 137.3, 135.1, 128.4, 28.0 (2C); FAB MS, *m/z* (%): 292.

N-(4, 6-dimethylpyrimidin-2-yl)-N′-(4-nitrobenzoyl) thiourea (3b). Elemental analysis for $C_{14}H_{13}N_5O_3S$ (MW = 331.35) in wt % calc. C = 50.75, H = 3.92, N = 21.14, S = 9.66 and found to be C = 50.62, H = 3.95, N = 21.12, S = 9.65, m.p 168–169°C, yield 91%. IR (KBr pellet) in cm^{-1}: 3332 (free NH), 3205(assoc. NH), 1685 (C=O), 1529 (benzene ring), 1140 (C=S), 1404 (C—N stretching); ^{1}H NMR (400 MHz, DMSO-d_6) in δ (ppm) and *J* (Hz): 12.80 (1H, br s, NH), 11.63 (1H,br s, NH), 7.75 (2H, d, J = 8.2 Hz), 7.65 (2H, d, J = 6.9 Hz), 6.40 (1H, s, pyrimidine-5-H), 1.23 (6H, s, pyrimidine-CH_3); ^{13}C NMR (300 MHz, DMSO-d_6) in δ (ppm): 180.3 (C=S), 167.0 (C=O), 159.4,154.3,151.0, 128.0, 121.4, 28.9(2C) ; FAB MS, *m/z* (%): 331.

N-(4,6-dimethylpyrimidin-2-yl)-N′-(4-methylbenzoyl) thiourea (3c). Elemental analysis for $C_{15}H_{16}N_4OS$ (MW = 300.37) in wt % calc. C = 59.98, H = 5.37, N = 18.65, S = 10.67 and found to be C = 59.97, H = 5.39, N = 18.66, S = 10.67, m.p 136°C, yield 91%. IR (KBr pellet) in cm^{-1}: 3338 (free NH), 3218 (assoc. NH), 1677 (C=O), 1527 (benzene ring), 1143 (C=S), 1404 (C—N stretching); ^{1}H NMR (400 MHz, DMSO-d_6) in δ (ppm) and *J* (Hz): 12.80 (1H, br s, NH), 11.63 (1H, br s, NH), 7.75 (2H, d, J = 8.2 Hz), 7.65 (2H, d, J = 6.9 Hz), 6.40 (1H, s, pyrimidine-5-H), 1.24 (6H, s, pyrimidine-CH_3), 0.93(3H,s, CH_3Ph); ^{13}C NMR (300 MHz, DMSO-d_6) in δ (ppm): 180.3 (C=S), 167.0 (C=O), 159.4, 154.3, 151.0, 128.0, 121.4, 28.2 (2C), 23.5 ; FAB MS, *m/z* (%): 300.

N-butyryl-N′-(4, 6-dimethylpyrimidin-2-yl) thiourea (3d). Elemental analysis for $C_{11}H_{16}N_4OS$ (MW = 252.33) in wt % calc. C = 52.36, H = 6.39, N = 22.20, S = 12.71 and found to be C = 52.36, H = 6.41, N = 22.18, S = 12.73, m.p 125–126°C, yield 91%. IR (KBr pellet) in cm^{-1}: 3331 (free NH), 3216(assoc. NH), 1681 (C=O), 1529 (benzene ring), 1140 (C=S), 1404 (C—N stretching); ^{1}H NMR (400 MHz, DMSO-d_6) in δ (ppm) and *J* (Hz): 12.80 (1H, br s, NH), 11.63 (1H, br s, NH), 6.45 (1H, s, pyrimidine-5-H), 1.25 (6H, s, pyrimidine-CH_3), 2.48 (2H, t, —CH_2, J = 7.3 Hz), 1.95 (2H, m, —CH_2), 0.93 (3H, t, —CH_3 , J = 7.1 Hz); ^{13}C NMR (300 MHz, DMSO-d_6) in δ (ppm): 180.3 (C=S), 167.0 (C=O), 159.4, 154.3, 151.0, 128.0, 121.4, 60.3, 45.5,28.1 (2C),23.2 ; FAB MS, *m/z* (%): 252.

General procedure for synthesis of fused heterocyclic compounds. To a solution of compounds (**3a–d**) in 20 mL of acetone, an equimolar quantity of bromine was added dropwise with cooling in a water bath. After addition, the flask with the reaction mixture was stirred at room temperature for about 4 h. At the end of the reaction, the resulting precipitate was collected by filtration; the crude product was purified by column chromatography using petroleum ether/ethyl acetate to yield corresponding compound **4a–d**.

N-[(2E)-5,7-dimethyl-2H-[1,2,4] thiadiazolo [2,3-a] pyrimidin-2-ylidene] thiophene-2-carboxamide (4a). Elemental analysis for $C_{12}H_{10}N_4OS_2$ (MW = 290.36) in wt % calc. C = 49.65, H = 3.49, N = 19.30, S = 22.06 and found to be C = 49.67, H = 3.46, N = 19.28, S = 22.09. m.p 185°C, yield 86 %. IR (KBr pellet) in cm^{-1}: 1683 (C=O), 1587 (aromatic C=C),1403 (C—N stretching); ^{1}H NMR (400 MHz, DMSO-d_6) in δ (ppm) and *J* (Hz): 7.62 (1H, d, J = 7.2 Hz, Thiophene CH), 7.15 (1H, dd, J_1 = 7.5 Hz, J_2 = 8.2 Hz, Thiophene CH), 7.03 (1H, d, J = 6.7 Hz, Thiophene CH),6.21(1H, s, pyrimidine-5-H), 1.28 (6H,s, pyrimidine-CH_3); ^{13}C NMR (300 MHz, DMSO-d_6) in δ (ppm): 168.2 (C=O), 165.0 (C=N), 161.6, 154.2,151.3,145.3,136.0,128.6, 26.8 (2C) ; FAB MS, *m/z* (%): 290.

N-[(2E)-5,7-dimethyl-2H-[1,2,4]thiadiazolo[2,3-a]pyrimidin-2-ylidene]-4-nitrobenzamide (4b). Elemental analysis for $C_{14}H_{11}N_5O_3S$ (MW = 329.33) in wt % calc. C = 51.04, H = 3.34, N = 21.27, S = 9.72 and found to be C = 51.24, H = 3.34, N = 22.01, S = 9.75. m.p 210–212°C, yield 79%. IR (KBr pellet) in cm^{-1}: 1686 (C=O), 1615 (C=N stretching), 1592 (aromatic C=C), 1510 (NO_2); ^{1}H NMR (400 MHz, DMSO-d_6) in δ (ppm) and *J* (Hz): 7.74 (2H, d, J = 8.1 Hz), 7.66 (2H, d, J = 6.7 Hz), 6.20 (1H, s, pyrimidine-5-H) , 1.28 (6H,s, pyrimidine-CH_3); ^{13}C NMR (300 MHz, DMSO-d_6) in δ (ppm):169.1(C=O),166.4(C=N),162.4 ,153.5,141.2,130.0, 121.4 , 26.1 (2C) ; FAB MS, *m/z* (%): 329.

N-[(2E)-5,7-dimethyl-2H-[1,2,4]thiadiazolo[2,3-a]pyrimidin-2-ylidene]-4-methylbenzamide (4c). Elemental analysis for $C_{15}H_{14}N_4OS$ (MW = 298.36) in wt % calc. C = 60.38, H = 4.73, N = 18.78, S = 10.75 and found to be C = 60.36, H = 4.76, N = 18.79, S = 10.74. m.p 192–193°C, yield 89%. IR (KBr pellet) in cm^{-1}: 1684 (C=O), 1625 (C=N stretching), 1575 (aromatic C=C); ^{1}H NMR (400 MHz, DMSO-d_6) in δ (ppm) and *J* (Hz): 7.69 (2H, d, J = 7.5 Hz), 7.52 (2H, d, J = 7.1 Hz), 6.25 (1H, s, pyrimidine-5-H), 1.27 (6H, s, pyrimidine-CH_3), 0.94 (3H,s, CH_3Ph); ^{13}C NMR (300 MHz, DMSO-d_6) in δ (ppm): 168.2 (C=O),165.0 (C=N),162.4,153.5,141.2, 130.0,121.4,26.6(2C),21.9 ; FAB MS, *m/z* (%): 298.

N-[(2Z)-5,7-dimethyl-2H-[1,2,4]thiadiazolo[2,3-a]pyrimidin-2-ylidene]butanamide (4d). Elemental analysis for $C_{11}H_{14}N_4OS$ (MW = 250.32) in wt % calc. C = 52.78, H = 5.64, N = 22.38, S = 12.81 and found to be C = 52.75, H = 5.66, N = 22.38, S = 12.84. m.p 178°C, yield 85%. IR (KBr pellet) in cm^{-1}: 1678 (C=O), 1605 (C=N stretching), 1587 (aromatic C=C); ^{1}H NMR (400 MHz, DMSO-d_6) in δ (ppm) and *J* (Hz): 7.74 (2H, d, J = 8.1 Hz), 7.66 (2H, d, J = 6.7 Hz), 6.26 (1H, s, pyrimidine-5-H) , 1.29 (6H, s, pyrimidine-CH_3), 2.48 (2H, t, —CH_2, J = 7.3 Hz), 1.95 (2H, m, —CH_2), 0.93 (3H, t, —CH_3 , J = 7.1 Hz); ^{13}C NMR (300 MHz, DMSO-d_6) in δ (ppm):167.8(C=O),165.5(C=N),162.4 ,153.5, 141.2, 130.0, 121.4, 61.5, 45.1, 27.9, 22.1, 20.0 ; FAB MS, *m/z* (%): 250.

Crystal structure determination of *N*-(4, 6-dimethylpyrimidin-2-yl)-*N*′-(thiophene-5-carbonyl) thiourea (3a). Crystal data: $C_{12}H_{12}N_4OS_2$, monoclinic, space group $P2_1/n$, a = 13.8228(3), b = 5.9272(2), c = 16.6425(3) Å, β = 95.207(3)°, V = 1324.81 Å^3, T = 100K, Z = 4, F (000) = 608, D_x = 1.466 g cm^{-3}, μ = 4.0 mm^{-1}. Single crystals suitable for X-ray diffraction studies were obtained by evaporation dichloromethane from ethanol. A colorless plate 0.35 × 0.20 × 0.08 mm^3 was mounted on a glass fiber in inert oil. Measurements were performed at 100 K on an Oxford Diffraction Xcalibur Nova diffractometer with mirror-focused Cu-Kα radiation to $2\theta_{max}$ 152° (99.1% complete to 30.03°). The data were corrected for absorption using the multiscan method. Of 40077 intensities, 3842 were independent (R_{int} 0.0268). The structure was refined anisotropically using SHELXL-97 [37]. NH hydrogens were refined freely, other H atoms using a riding model. The final *wR*2 was 0.0806, with a conventional *R*1 of 0.0268, for 189 parameters; S = 1.100; max. Δρ 0.449 e Å^{-3}. CCDC 724710 contains the supplementary crystallographic data for this article. Copies of this information may be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge, CBZ IEZ, UK. Facsimile (44) 01223 336 033, E-mail: deposit@ccdc.cam.ac.uk or http//www.ccdc.com.ac.uk/deposit.

MICROBIOLOGY

Antibacterial screening. For determining antibacterial activity, the synthesized compounds and the control drug was dissolved in absolute dimethylsulfoxide (DMSO).Further dilutions were prepared at the required quantities of 1024, 512, 256, 128, 64, 32, 16, 8, 4, and 2 μg mL^{-1} on the microorganisms at the concentrations studied. The stock solutions were prepared in DMSO and DMSO had no effect on the microorganisms in the concentrations studied. Antibacterial activities of the compounds were determined using the broth dilution method proposed by the National Committee for Clinical Laboratory Standards (CLSI). MIC, which is the lowest concentration of a compound that completely inhibits microbial growth, was determined by a standard broth dilution technique adapted from the CLSI [38,39]. Minimal inhibitory concentrations for each compound were investigated against standard bacterial strains; *S. aureus* (ATCC 25923), *E. faecalis* (ATCC 29212), *E. coli* (ATCC 25922), *S. epidermidis* (ATCC 12228), *E. cloacae* (ATCC 13047), *P. vulgaris* (ATCC 13315).

Gentamycin and amikacin were used as reference drugs for Gram-negative antibacterial activity and Gram-positive antibacterial activity, respectively. All the inoculated plates were incubated at 37°C and results were evaluated after 24 h for bacteria. Every experiment in the antibacterial assays was replicated twice to define the MIC values. The lowest concentration of the compounds that prevented visible growth was considered MICs.

ANTIFUNGAL SCREENING

Broth microdilution procedure. Antifungal activity was determined by the *broth* micro-dilution procedures and principles of the Clinical and Laboratory Standards Institute (CLSI). Minimal inhibitory concentrations for each compound were investigated against standard yeast-like fungi, *C. albicans* (ATCC90028), *C. labrata* (ATCC 32554), *C. tropicalis*(ATCC 20336).Fungal colonies of the test organisms were suspended directly into a small volume of 0.9% saline and further diluted until turbidity matched the Mc Farland Standard no: 0.5 Petri dishes Sabouraud and Dextrose agar for fungi were impregnated with these microbial suspensions. The stock solutions of the synthesized compounds were prepared in dimethyl sulfoxide (DMSO), which had no effect on the organisms in the concentrations studied. The initial concentration was 200 mg/ml. All of the dilutions were done with distillated water. The concentrations of tested compounds were 100, 50, 25, 12.5, 6.25, 3.125 μg/mL. DMSO was used as negative control. Nystatin was used as reference drug for antifungal activity. All the inoculated plates were incubated at 37°C and results were evaluated after 48 h for fungi. The lowest concentration of the compounds that prevented visible growth was considered MICs.

Poisoned food technique. In the blotter paper method, surface-sterilized seeds (with 2.5% bleach-NaOCl for one minute) and untreated seeds were placed on moistened blotter paper. In this method, five seeds, sterilized and unsterilized, were used and replicated four times. The Petri plates were incubated at 25°C ± 2 under 12h alternating cycles of fluorescent light and darkness for a week. Fungi were identified on the basis of their typical structure and basic characters as suggested by Barnett [40] and Melone [41].

The pure cultures of the fungi isolated were maintained on PDA in tubes, which were stored in the refrigerator at 4°C and used frequently. These were multiplied on 2% PDA for 2–3 weeks. The inoculum was prepared by taking 1mg culture in 20 mL distilled water. Twenty seeds of sunflower cultivar, both sterilized and untreated lots were planted in each pot, replicated five times. All the thiourea derivatives **3a–d** were tested for the fungicidal activity according with the following procedure. Three seed dressing fungicides (**3a–c**) were tested as inhibitor of growth of the fungi isolated through ''Poisoned Food Technique'' [42].One mg of each thiourea derivative was dissolved in 20 mL of Potato Dextrose Agar (PDA) in Petri plates, and one set of agar plates without addition of fungicide was kept as control. The plates were incubated at 25°C for 7 days, colony of each fungus was measured in cm and percentage inhibition was calculated as:

$$\%\text{Inhibition} = \frac{\text{Diameter of colony of control} - \text{diameter of colony of fungi} \times 100}{\text{Diameter of colony of control}}$$

Statistical analysis. Data were analyzed statistically by applying ANOVA and comparing means by using Least Significant Difference test [43].

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