Journal of Fluorine Chemistry 130 (2009) 1028–1034

Contents lists available at ScienceDirect

Journal of Fluorine Chemistry

journal homepage: www.elsevier.com/locate/fluor

Synthesis, characterization and antimicrobial activity of some new 1-(fluorobenzoyl)-3-(fluorophenyl)thioureas

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ARTICLE INFO

Article history:
Received 13 July 2009
Received in revised form 24 August 2009
Accepted 1 September 2009
Available online 18 September 2009

Keywords:
Fluorine substituted thioureas
Antibacterial activity
Antifungal activity
In vitro studies

ABSTRACT

Synthesis of a variety of new 1-(isomeric fluorobenzoyl)-3-(isomeric fluorophenyl)thioureas (**1a–t**) was accomplished in two steps. The synthetic route involves the reaction of equimolar quantities of isomeric fluorobenzoyl chlorides with potassium thiocyanate in anhydrous acetone to afford the corresponding isothiocyantes in situ, followed by treatment with equimolar quantities of isomeric fluoroanilines. All of the synthesized compounds (**1a–t**) were screened for their *in vitro* antibacterial activity using Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aureginosa*). The minimum inhibitory concentration (MIC) was also determined for the most active compounds. *In vitro* antifungal activity was also determined against the five fungal species (*Rhizopus oryzae*, *Aspergillus tereus*, *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus fumigatus*). In general, the antifungal activity of compounds was better than their antibacterial activity.

1. Introduction

Due to unique properties of fluorinated compounds, the substitution of hydrogen by fluorine has become a common strategy in drug development. The fluorinated molecules resulting from isosteric substitution of hydrogen by fluorine, generally also fit in the biological recognition site. The inclusion of fluorine into organic molecules may increase the lipophilicity and thus enhance the rate of cell penetration and transport of a drug to an active site. The higher polarizability due to the C–F bond may give new possibilities for binding to the receptor. The fluorinated compounds are more resistant to metabolic degradation due to the high bond energies and heats of formation of the H–O and C–O bonds relative to those of the F–O bond [1–3]. Fluorinated chemicals are of growing importance with applications in medicine, agrochemicals and organic electronics. Fluorine substitution at a certain position in a drug molecule can influence not only pharmacokinetic properties such as absorption, tissue distribution, secretion and the route and rate of biotransformation but also its pharmacodynamics, toxicology and improves the efficiency of medicine [4].

Fluorinated thioureas are convenient synthons for preparation of versatile fluorine-containing heterocycles: [1,3]-benzothiazin-4-ones [5,6], 1-aryl-2-ethylthio-quinazolin-4-one, thiazolidine and 1*H*-1,2,4-triazoles [7]. Fluorinated thioureas constitute a novel class of potent influenza virus neuraminidase inhibitors [8]. Thiourea derivatives are effective against HIV [9–10] and possess bactericidal action [11]. Thioureas containing both carbonyl and thiocarbonyl groups are versatile ambidentate donor ligands for transition metal ions [12]. N,N-dialkyl-N-aroyl thioureas are efficient ligands for the separation of platinum group metals [13]. 1,3-Dialkyl or diaryl thioureas exhibit significant antifungal activity against plant pathogens *Pyricularia oryzae* and *Drechslera oryzae* [14]. 1-Benzoyl-3-(4,6-disubstituted-pyrimidinyl)thioureas have shown excellent herbicidal activity [15]. Acyl thioureas are well known for their superior pesticidal, fungicidal, antiviral and plant growth regulating activity [16]; others are reported to show potent anti-inflammatory and analgesic activities [17]. Thioureas have extensively been used in enantioselective synthesis, such as nitro-Mannich reactions, Aza–Henry reaction and the Michael addition [18–20]. Symmetrical and asymmetrical phenethyl thioureas, 5-halo-substituted thiophene pyridyl thioureas and heterocyclic thioureas are non-nucleoside inhibitors of HIV-1 reverse transcriptase [21].

Condensation of thiourea derivatives with carbonyl compounds has been used in the synthesis of N-alkyl-1,3-thiazol-2-amines, 3-alkyl-1,3-thiazolimines [22], 1-aroyl-3-aryl-4-substituted imidazole-2-thiones [23] and 2-(aroylimino)-3-aryl-4-methyl/phenyl-1,3-thiazolines [24]. Cyclocondensation of unsymmetrical perfluoroalkyl substituted beta-diketones with urea, thiourea, and guanidine leads to various heterocycles [25]. Regioselective synthesis of 2*H*-[1,2,4]thiadiazolopyrimidine derivatives [26] and 2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-thiazole-4-carboxylic acids via N-Fmoc thioureas has recently been described [27].

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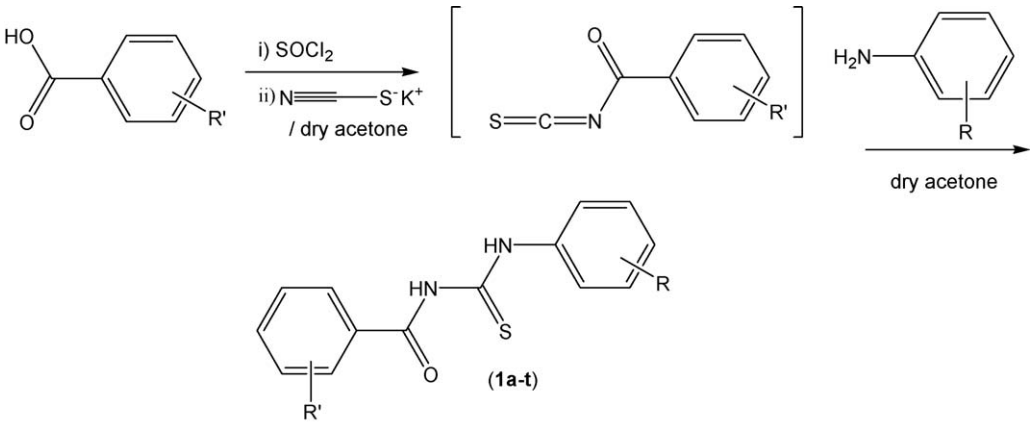
doi:10.1016/j.jfluchem.2009.09.003



a: R'= 2-Cl R=2-F, **b:** R'=3,4,5-tri-OCH_3 R=2-F
c: R'= 2-F R=2-F, **d:** R'=3-F R=2-F
e: R'= 4-F R=2-F,
f: R'=2-Cl R=3-F, **g:** R'=3,4,5-tri-OCH_3 R=3-F
h: R'=2-F R=3-F, **i:** R'=3-F R=3-F
j: R'=4-F R=3-F,

k: R'=2-Cl R=4-F, **l:** R'=3,4,5-tri-OCH_3 R=4-F,
m: R'=2-F R=4-F, **n:** R'=3-F-Ph R=4-F,
o: R'=4-F R=4-F,

p: R'=2-Cl R=3-Cl, 4-F, **q:** R'=3,4,5-tri-OCH_3 R=3-Cl, 4-F
r: R'=2-F R=3-Cl, 4-,F **s:** R'=4-F R=3-Cl, 4-F
t: R'Ph=t-butyl R=3-Cl, 4-F

Scheme 1. Synthesis of some new 1-(fluorobenzoyl)-3-(fluorophenyl)thioureas.

Taking into consideration the aforementioned biological and synthetic significance of thioureas, synthesis of some new 1-(isomeric fluorobenzoyl)-3-(isomeric fluorophenyl)thiourea derivatives was undertaken as a continuation of our efforts towards synthesis, characterization and crystal structure of new thiourea derivatives as precursors towards novel heterocycles and for the systematic study of their bioactivity and complexation behavior.

2. Results and discussion

2.1. Synthesis

The synthetic sequence leading to the thioureas is outlined in Scheme 1. Isomeric fluoro benzoic acids were converted into corresponding acid chlorides by treatment with thionyl chloride

Table 1
Physicochemical elemental and mass spectral data of thioureas (**1a–t**).

Compound	R	R′	Yield (%)	R_f[a]	Mp (°C)	Molecular formula (MW)	EIMS $[M]^+$	Analysis (calcd./found)			
								C (%)	H (%)	N (%)	S (%)
1a	2-F	2-Cl	63	0.52	164	$C_{14}H_{10}N_2OSFCl$ (308.76)	302.0	54.46/54.16	3.26/3.36	9.07/9.02	10.39/10.32
1b	2-F	3,4,5-Tri-OCH_3	84	0.34	186	$C_{17}H_{17}N_2O_4SF$ (364.39)	364.0	56.03/56.05	4.70/4.72	7.69/7.65	8.80/8.82
1c	2-F	2-F	61	0.57	115	$C_{14}H_{10}N_2OSF_2$ (292.3)	292.0	57.53/57.46	3.45/3.43	9.58/9.61	10.97/10.98
1d	2-F	3-F	51	0.58	112	$C_{14}H_{10}N_2OSF_2$ (292.3)	292.0	57.53/57.42	3.45/3.41	9.58/9.52	10.97/11.08
1e	2-F	4-F	81	0.51	170	$C_{14}H_{10}N_2OSF_2$ (292.3)	292.0	57.53/57.59	3.45/3.42	9.58/9.62	10.97/11.01
1f	3-F	2-Cl	53	0.46	129	$C_{14}H_{10}N_2OSFCl$ (308.76)	302.0	54.46/54.22	3.26/3.21	9.07/9.03	10.39/10.41
1g	3-F	3,4,5-Tri-OCH_3	82	0.33	178	$C_{17}H_{17}N_2O_4SF$ (364.39)	364.0	56.03/56.13	4.70/4.80	7.69/7.62	8.80/8.78
1h	3-F	2-F	85	0.53	117	$C_{14}H_{10}N_2OSF_2$ (292.3)	292.0	57.53/57.49	3.45/3.41	9.58/9.53	10.97/10.95
1i	3-F	3-F	50	0.60	93	$C_{14}H_{10}N_2OSF_2$ (292.3)	292.0	57.53/57.46	3.45/3.35	9.58/9.47	10.97/10.91
1j	3-F	4-F	62	0.63	130	$C_{14}H_{10}N_2OSF_2$ (292.3)	292.0	57.53/57.47	3.45/3.40	9.58/9.62	10.97/10.93
1k	4-F	2-Cl	80	0.44	171	$C_{14}H_{10}N_2OSFCl$ (308.76)	302.0	54.46/54.40	3.26/3.21	9.07/9.03	10.39/10.36
1l	4-F	3,4,5-Tri-OCH_3	87	0.32	161	$C_{17}H_{17}N_2O_4SF$ (364.39)	364.0	56.03/56.09	4.70/4.72	7.69/7.71	8.80/8.79
1m	4-F	2-F	56	0.51	110	$C_{14}H_{10}N_2OSF_2$ (292.3)	292.0	57.53/57.57	3.45/3.42	9.58/9.54	10.97/10.93
1n	4-F	3-F	84	0.61	131	$C_{14}H_{10}N_2OSF_2$ (292.3)	292.0	57.53/57.50	3.45/3.47	9.58/9.59	10.97/11.01
1o	4-F	4-F	60	0.62	138	$C_{14}H_{10}N_2OSF_2$ (292.3)	292.0	57.53/57.59	3.45/3.39	9.58/9.48	10.97/10.90
1p	3-Cl-4-F	2-Cl	83	0.49	154	$C_{14}H_9N_2OSFCl_2$ (343.2)	342.0	48.99/50.02	2.64/2.61	8.16/8.19	9.34/9.37
1q	3-Cl-4-F	3,4,5-Tri-OCH_3	92	0.32	160	$C_{17}H_{16}N_2O_4SFCl$ (398.84)	398.0	51.19/51.11	4.04/4.07	7.02/7.08	8.04/8.00
1r	3-Cl-4-F	2-F	59	0.51	111	$C_{14}H_9N_2OSF_2Cl$ (326.75)	326.0	51.46/51.39	2.78/2.79	8.57/8.52	9.81/9.89
1s	3-Cl-4-F	4-F	69	0.64	158	$C_{14}H_9N_2OSF_2Cl$ (326.75)	326.0	51.46/51.36	2.78/2.73	8.57/8.59	9.81/9.75
1t	3-Cl-4-F	*t*-Butyl	89	0.63	114	$C_{12}H_{14}N_2OSFCl$ (288.77)	288.0	56.67/56.59	5.94/5.97	11.01/11.05	12.61/12.57

[a] Solvent system: hexane:ethyl acetate (8:2).
Recrystallization solvent: methanol.

Table 2
(IR, ^{1}H, ^{13}C NMR) spectral data of thioureas (**1a–t**).

Compound	IR (υ, cm^{-1})	^{1}H NMR (δ, ppm, J (Hz))	^{13}C NMR (δ, ppm)
1a	3355 (NH), 1679 (C=O), 1538 (C=C), 1277 (C=S), 1129 (C–N)	12.48 (*br s*, 1H, NH), 9.34 (*br s*, 1H, NH), 8.44 (*dt*, J = 2.1, 7.5 Hz, 1H, Ar–H), 7.83 (*td*, J = 0.9, 7.2 Hz, 1H, Ar–H), 7.55–7.18 (*m*, 7H, Ar–H)	177.9 (C=S), 165.9 (C=O), 155.1 (*d*, ^{1}J = 247 Hz), 133.5, 131.8, 131.2, 131.0, 130.8, 127.8 (*d*, ^{3}J = 8.25 Hz), 127.6, 125.3, 124.1 (*d*, ^{4}J = 3.75 Hz), 125.94 (*d*, ^{3}J = 10.5 Hz), 115.97 (*d*, ^{2}J = 19.5 Hz)
1b	3315 (NH), 1662 (C=O), 1584 (C=C), 1239 (C=S), 1131 (C–N)	12.75 (*br s*, 1H, NH), 9.17 (*br s*, 1H, NH), 8.43 (*dt*, J = 2.1, 7.2 Hz, 1H, Ar–H), 7.31–7.17 (*m*, 3H, Ar–H), 7.12 (*s*, 2H, Ar–H), 3.96 (*s*, 3H, O–CH_3), 3.95 (*s*, 6H, 2[O–CH_3])	178.5 (C=S), 166.7 (C=O), 155.0 (*d*, ^{1}J = 247 Hz), 153.5 (2C), 142.9, 127.8 (*d*, ^{3}J = 8.25 Hz), 126.4, 125.9 (*d*, ^{3}J = 10.5 Hz), 125.3, 124.1 (*d*, ^{4}J = 3.75 Hz), 115.6 (*d*, ^{2}J = 19.5 Hz), 104.9 (2C), 61.0 (OCH_3), 56.5 (2C, 2[OCH_3])
1c	3300 (N–H), 1669 (C=O), 1550 (C=C), 1281 (C=S), 1138 (C–N)	12.57 (*br s*, 1H, NH), 9.70 (*br d*, J = 14.7 Hz, 1H, NH), 8.44 (*dt*, J = 2.1, 7.5 Hz, 1H, Ar–H), 8.11 (*dt*, J = 1.8, 7.8 Hz, 1H, Ar–H), 7.71–7.63 (*m*, 1H, Ar–H), 7.39 (*dt*, J = 1.2, 7.8 Hz, 1H, Ar–H), 7.30–7.23 (*m*, 4H, Ar–H)	177.5 (C=S), 166.2 (C=O), 160.5 (*d*, ^{1}J = 250 Hz), 155.2 (*d*, ^{1}J = 247 Hz), 135.9 (*d*, ^{3}J = 9.75 Hz), 132.2, 127.9 (*d*, ^{3}J = 8.25 Hz), 125.9 (*d*, ^{3}J = 10.5 Hz), 125.6 (*d*, ^{4}J = 3.75 Hz), 125.3, 124.1 (*d*, ^{4}J = 3.75 Hz), 118.8 (*d*, ^{3}J = 9.75 Hz), 116.7 (*d*, ^{2}J = 24 Hz), 115.7 (*d*, ^{2}J = 19.5 Hz)
1d	3240 (N–H), 1677 (C=O), 1557 (C=C), 1269 (C=S), 1152 (C–N)	12.63 (*br s*, 1H, NH), 9.28 (*br s*, 1H, NH), 8.39 (*dt*, J = 1.8, 7.5 Hz, 1H, Ar–H), 7.71–7.17 (*m*, 7H, Ar–H)	178.7 (C=S), 166.3 (C=O), 162.9 (*d*, ^{1}J = 251 Hz), 155.1 (*d*, ^{1}J = 247 Hz), 140.1 (*d*, ^{3}J = 8.75 Hz), 132.0 (*d*, ^{3}J = 10.75 Hz), 127.8 (*d*, ^{3}J = 8.25 Hz), 125.9 (*d*, ^{3}J = 10.5 Hz), 125.3, 124.1 (*d*, ^{4}J = 3.75 Hz), 122.3 (*d*, ^{4}J = 3 Hz), 116.2 (*d*, ^{2}J = 22 Hz), 115.6 (*d*, ^{2}J = 19.5 Hz), 114.3 (*d*, ^{2}J = 26.25 Hz)
1e	3350 (N–H), 1653 (C=O), 1581 (C=C), 1282 (C=S), 1146 (C–N)	12.43 (*br s*, 1H, NH), 9.65 (*br s*, 1H, NH), 8.45 (*dt*, J = 2.1, 7.5 Hz, 1H, Ar–H), 8.07–7.05 (*m*, 7H, Ar–H)	177.5 (C=S), 166.8 (C=O), 164.9 (*d*, ^{1}J = 250 Hz), 155.0 (*d*, ^{1}J = 247 Hz,), 133.0 (2C, *d*, ^{3}J = 8.25 Hz), 131.6, 127.8 (*d*, ^{3}J = 8.25 Hz), 125.9 (*d*, ^{3}J = 10.5 Hz), 125.2, 124.1 (*d*, ^{4}J = 3.75 Hz), 115.6 (*d*, ^{2}J = 19.5 Hz), 114.8 (2C, *d*, ^{2}J = 21.75 Hz)
1f	3200 (N–H), 1677 (C=O), 1545 (C=C), 1281 (C=S), 1156 (C–N)	12.49 (*br s*, 1H, NH), 9.33 (*br s*, 1H, NH), 7.79–6.97 (*m*, 8H, Ar–H)	177.6 (C=S), 166.2 (C=O), 165.6 (*d*, ^{1}J = 245 Hz), 138.9 (*d*, ^{3}J = 10.5 Hz), 133.5, 131.9, 131.2, 131.0, 130.5, 130.6 (*d*, ^{3}J = 9.75 Hz), 127.6, 119.4 (*d*, ^{4}J = 3 Hz), 113.7 (*d*, ^{2}J = 21.75 Hz), 111.2 (*d*, ^{2}J = 26.25 Hz)
1g	3290 (N–H), 1649 (C=O), 1634 (C=C), 1281 (C=S), 1142 (C–N)	12.75 (*br s*, 1H, NH), 9.17 (*br s*, 1H, NH), 7.75 (*dt*, J = 2.1, 10.2 Hz, 1H, Ar–H), 7.42–7.35 (*m*, 4H, Ar–H), 7.11 (*s*, 2H, Ar–H), 7.04–6.98 (*m*, 1H, Ar–H), 3.95 (*s*, 6H, O–CH_3), 3.95 (*s*, 3H, 2[O–CH_3])	178.5 (C=S), 166.4 (C=O), 165.5 (*d*, ^{1}J = 244 Hz, Ar-C), 153.6 (2C), 142.9, 139.6 (*d*, ^{3}J = 10.5 Hz), 130.6 (*d*, ^{3}J = 9.75 Hz), 125.4, 119.4 (*d*, ^{4}J = 3 Hz), 113.6 (*d*, ^{2}J = 21 Hz), 111.2 (*d*, ^{2}J = 26 Hz), 104.9 (2C), 61.2 (OCH_3), 56.6 (2C, 2[OCH_3])
1h	3295 (N–H), 1667 (C=O), 1574 (C=C), 1280 (C=S), 1138 (C–N)	12.67 (*br s*, 1H, NH), 9.68 (*br d*, J = 14.7 Hz, 1H, NH), 8.11 (*dt*, J = 1.8, 7.8 Hz, 1H, Ar–H), 7.77 (*td*, J = 2.1, 10.2 Hz, 1H, Ar–H), 7.70–6.96 (6H, *m*, Ar–H)	177.9 (C=S), 164.2 (C=O), 162.0 (*d*, ^{1}J = 247 Hz), 160.5 (*d*, ^{1}J = 250 Hz), 139.6 (*d*, ^{3}J = 10.5 Hz), 135.9 (*d*, ^{3}J = 9.75 Hz), 132.2, 130.0 (*d*, ^{4}J = 3 Hz), 125.5 (*d*, ^{4}J = 3.75 Hz), 119.4 (*d*, ^{4}J = 3 Hz), 118.8 (*d*, ^{3}J = 9.75 Hz), 116.8 (*d*, ^{2}J = 24 Hz), 113.6 (*d*, ^{2}J = 21 Hz), 111.2 (*d*, ^{2}J = 25.5 Hz)
1i	3275 (N–H), 1667 (C=O), 1574 (C=C), 1280 (C=S), 1138 (C–N)	12.62 (*br s*, 1H, NH), 9.20 (*br s*, 1H, NH), 7.51 (*td*, J = 1.5, 10.2 Hz, 1H, Ar–H), 7.69–6.98 (*m*, 7H, Ar–H)	178.6 (C=S), 166.4 (C=O), 162.9 (*d*, ^{1}J = 251 Hz), 162.6 (*d*, ^{1}J = 245 Hz), 140.2 (*d*, ^{3}J = 8.75 Hz), 139.7 (*d*, ^{3}J = 10.5 Hz), 132.0 (*d*, ^{3}J = 10.75 Hz), 130.1 (*d*, ^{3}J = 9.75 Hz), 112.3 (*d*, ^{4}J = 3 Hz), 119.4 (*d*, ^{4}J = 3.75 Hz), 116.2 (*d*, ^{2}J = 22 Hz), 114.6 (*d*, ^{2}J = 26.5 Hz), 113.7 (*d*, ^{2}J = 21.75 Hz), 111.2 (*d*, ^{2}J = 25 Hz)
1j	3325 (N–H), 1664 (C=O), 1579 (C=C), 1276 (C=S), 1159 (C–N)	12.39 (*br s*, 1H, NH), 9.69 (*br s*, 1H, NH), 8.08–8.00 (2H, *m*, Ar–H), 7.77 (*td*, J = 2.1, 10.2 Hz, 1H, Ar–H), 7.43–6.96 (*m*, 5H, Ar–H)	178.0 (C=S), 166.6 (C=O), 165.5 (*d*, ^{1}J = 244.5 Hz), 164.9 (*d*, ^{1}J = 250 Hz), 138.9 (*d*, ^{3}J = 10.5 Hz), 133.0 (2C, *d*, ^{3}J = 8.25 Hz), 131.6, 130.6 (*d*, ^{3}J = 9.75 Hz), 119.4 (*d*, ^{4}J = 3 Hz), 114.8 (2C, *d*, ^{2}J = 21.25 Hz), 113.8 (*d*, ^{2}J = 21.75 Hz), 114.8 (2C, *d*, ^{2}J = 21.75 Hz)
1k	3155 (N–H), 1675 (C=O), 1543 (C=C), 1279 (C=S), 1166 (C–N)	12.29 (*br s*, 1H, NH), 9.34 (*br s*, 1H, NH), 7.77 (*dd*, J = 1.2, 8.1 Hz, 1H, Ar–H), 7.70–7.10 (*m*, 7H, Ar–H)	178.3 (C=S), 166.2 (C=O), 161.0 (*d*, ^{1}J = 246 Hz), 133.5, 133.4, 132.0, 131.2, 131.0, 130.5, 127.6, 126.3 (2C, *d*, ^{3}J = 8.25 Hz), 115.8 (2C, *d*, ^{2}J = 22.5 Hz)
1l	3235 (N–H), 1608 (C=O), 1574 (C=C), 1240 (C=S), 1133 (C–N)	12.7 (*br s*, 1H, NH), 9.16 (*br s*, 1H, NH), 7.68–7.13 (*m*, 4H, Ar–H), 7.11 (*s*, 2H, Ar–H), 3.95 (*s*, 6H, 2[O–CH_3]), 3.85 (*s*, 3H, O–CH_3)	178.5 (C=S), 166.4 (C=O), 161.0 (*d*, ^{1}J = 246 Hz), 153.5 (2C), 142.9, 133.5 (*d*, ^{4}J = 3 Hz), 126.5, 126.3 (2C, *d*, ^{3}J = 8.75 Hz), 115.7 (2C, *d*, ^{2}J = 22.5 Hz), 104.9 (2C), 61.1 (OCH_3), 56.5 (2C, 2[OCH_3])
1m	3215 (N–H), 1660 (C=O), 1539 (C=C), 1279 (C=S), 1144 (C–N)	12.5 (*br s*, 1H, NH), 9.78 (*br d*, J = 15 Hz, 1H, NH), 8.12 (*dt*, J = 1.8, 7.8 Hz, 1H, Ar–H), 7.63–7.71 (*m*, 3H, Ar–H), 7.38 (*dt*, J = 1.2, 7.8 Hz, 1H, Ar–H), 7.30–7.10 (*m*, 3H, Ar–H)	178.7 (C=S), 163.1 (*d*, J = 9.75 Hz, C=O), 161.0 (*d*, ^{1}J = 246 Hz), 160.5 (*d*, ^{1}J = 250 Hz), 135.9 (*d*, ^{3}J = 9.75 Hz), 133.6 (*d*, ^{4}J = 3 Hz), 132.1, 126.3 (*d*, ^{3}J = 8.25 Hz), 125.5 (*d*, ^{4}J = 3 Hz), 118.9 (*d*, ^{3}J = 9.75 Hz), 116.8 (*d*, ^{2}J = 24 Hz), 115.8 (*d*, ^{2}J = 22.5 Hz)
1n	3210 (N–H), 1674 (C=O), 1523 (C=C), 1261 (C=S), 1156 (C–N)	12.5 (*br s*, 1H, NH), 9.14 (*br s*, 1H, NH), 7.69–7.52 (*m*, 5H, Ar–H), 7.38 (*ddt*, J = 0.9, 2.4, 8.1 Hz, 1H, Ar–H), 7.18–7.09 (*m*, 2H, Ar–H)	178.8 (C=S), 164.4 (C=O), 162.9 (*d*, ^{1}J = 251 Hz), 161.1 (*d*, ^{1}J = 247 Hz), 140.1 (*d*, ^{3}J = 8.75 Hz), 133.6 (*d*, ^{4}J = 3 Hz), 132.0 (*d*, ^{3}J = 10.75 Hz), 126.3 (2C, *d*, ^{3}J = 8.25 Hz), 122.3 (*d*, ^{4}J = 3.75 Hz), 116.2 (*d*, ^{2}J = 22 Hz), 115.8 (2C, *d*, ^{2}J = 22.5 Hz), 114.3 (*d*, ^{2}J = 26.25 Hz)

1o	3285 (N–H), 1665 (C=O), 1538 (C=C), 1262 (C=S), 1143 (C–N)	12.7 (*br s*, 1H, NH), 9.16 (*br s*, 1H, NH), 8.07–6.95 (*m*, 8H, Ar–H)	178.2 (C=S), 166.4 (C=O), 164.9 (*d*, ^1J = 250 Hz), 161.0 (*d*, ^1J = 246 Hz), 133.6 (*d*, ^4J = 3 Hz), 133.0 (2C, *d*, ^3J = 8.25 Hz), 131.7, 126.3 (2C, *d*, ^3J = 8.25 Hz), 115.8 (2C, *d*, ^2J = 22.5 Hz), 114.9 (2C, *d*, ^2J = 21.7 Hz)
1p	3170 (N–H), 1691 (C=O), 1541 (C=C), 1260 (C=S), 1163 (C–N)	12.3 (*br s*, 1H, NH), 9.35 (*br s*, 1H, NH), 7.87 (*dd*, *J* = 2.7, 6.7 Hz, 1H, Ar–H), 7.81 (*td*, *J* = 0.9, 7.2 Hz, 1H, Ar–H), 7.60–7.43 (*m*, 4H, Ar–H), 7.21 (*t*, *J* = 8.7 Hz, 1H, Ar–H)	178.2 (C=S), 166.5 (C=O), 156.4 (*d*, ^1J = 248 Hz), 134.1 (*d*, ^4J = 3.75 Hz), 133.5, 133.4, 131.9, 131.1, 130.6, 127.6, 124.3 (*d*, ^3J = 7.5 Hz), 121.2 (*d*, ^2J = 18.75 Hz), 116.9 (*d*, ^2J = 15 Hz), 116.6 (*d*, ^3J = 12 Hz)
1q	3255 (N–H), 1662 (C=O), 1570 (C=C), 1251 (C=S), 1163 (C–N)	12.8 (*br s*, 1H, NH), 9.18 (*br s*, 1H, NH), 7.93 (*dd*, *J* = 2.7, 6.6 Hz, 1H, Ar–H), 7.59–7.54 (*m*, 1H, Ar–H), 7.20 (*t*, *J* = 8.7, 1H, Ar–H), 7.12 (*s*, 1H, Ar–H), 3.97 (*s*, 6H, 2[O–CH_3]), 3.96 (*s*, 3H, O–CH_3)	178.4 (C=S), 166.8 (C=O), 156.4 (*d*, ^1J = 248 Hz), 153.5 (2C), 143.0, 134.1 (*d*, ^4J = 3.75 Hz), 126.4, 124.3 (*d*, ^3J = 7.5 Hz), 121.2 (*d*, ^2J = 18.75 Hz), 116.9 (*d*, ^2J = 15 Hz), 116.6 (*d*, ^3J = 11.75 Hz), 105.0 (2C), 61.4 (OCH_3), 55.9 (2C, 2[OCH_3])
1r	3285 (N–H), 1666 (C=O), 1555 (C=C), 1277 (C=S), 1132 (C–N)	12.5 (*br s*, 1H, NH), 9.71 (*br d*, *J* = 14.7 Hz, 1H, NH), 8.11 (*dt*, *J* = 1.8, 7.8 Hz, 1H, Ar–H), 7.89 (*dd*, *J* = 2.7, 6.6 Hz, 1H, Ar–H) 7.71–7.54 (*m*, 2H, Ar–H), 7.39 (*dt*, *J* = 0.9, 7.8 Hz, 1H, Ar–H), 7.28 (*dd*, *J* = 0.9, 12.3 Hz, 1H, Ar–H), 7.20 (*t*, *J* = 8.7 Hz, 1H, Ar–H)	178.7 (C=S), 163.3 (*d*, *J* = 3 Hz, C=O), 160.6 (*d*, ^1J = 250 Hz), 156.4 (*d*, ^1J = 248 Hz), 136.1 (*d*, ^3J = 9.75 Hz), 134.1 (*d*, ^4J = 3.75 Hz), 132.2–126.6 (2C), 125.6 (*d*, ^4J = 3 Hz), 124.3 (*d*, ^3J = 7.5 Hz), 121.2 (*d*, ^2J = 18.75 Hz), 118.7 (*d*, ^3J = 9.75 Hz), 116.9 (*d*, ^2J = 15 Hz), 116.6 (*d*, ^3J = 12.75 Hz)
1s	3305 (N–H), 1675 (C=O), 1531 (C=C), 1273 (C=S), 1151 (C–N)	12.6 (*br s*, 1H, NH), 9.73 (*br s*, 1H, NH), 8.08–8.04 (*m*, 2H, Ar–H), 7.88 (*dd*, *J* = 2.7, 6.6 Hz, 1H, Ar–H). 7.58–7.54 (*m*, 1H, Ar–H), 7.21 (*t*, *J* = 8.7. 1H, Ar–H), 7.02–6.93 (*m*, 2H, Ar–H)	178,3 (C=S), 166.0 (C=O), 164.7 (*d*, ^1J = 250 Hz), 156.4 (*d*, ^1J = 248 Hz), 134.2 (*d*, ^4J = 3.75 Hz), 133.2 (2C, *d*, ^3J = 8 Hz). 131.5, 124.3 (*d*, ^3J = 7.5 Hz), 121.3 (*d*, ^2J = 18.75 Hz), 116.9 (*d*, ^2J = 15 Hz), 116.7 (*d*, ^2J = 12.75 Hz), 114.9 (2C, *d*, ^2J = 21.75 Hz), 116.6 (*d*, ^3J = 12.75 Hz)
1t	3305(N–H), 1683 (C=O), 1534 (C=C), 1271 (C=S), 1162 (C–N)	12.5 (*br s*, 1H, NH), 8.59 (*br s*, 1H, NH), 7.84 (*dd*, *J* = 2.4, 6.6 Hz, 1H, Ar–H), 7.52–7.46 (*m*, 1H, Ar–H), 7.22 (*t*, *J* = 8.7 Hz, 1H, Ar–H), 1.33 (*s*, 9H, 3[CH_3])	179.7 (C=S), 179.0 (C=O), 156.4 (*d*, ^1J = 247.5 Hz), 134.1 (*d*, ^4J = 3 Hz), 126.5, 124.2 (*d*, ^3J = 6.75 Hz), 121.2 (*d*, ^2J = 18.75 Hz), 116.6 (*d*, ^2J = 22.5 Hz), 40.1, 26.9 (3C, 3[CH_3])

according to standard procedure. The latter were treated with an equimolar quantity of potassium thiocyanate in acetone to afford the corresponding isothiocyanate intermediates which were not separated. Condensation of the isothiocyanates with isomeric fluoroanilines furnished the 1-(substituted fluorobenzoyl)-3-(substituted fluoroaryl)thiourea derivatives (**1a–t**) [28].

Typically, thioureas are characterized by IR absorptions at 3350, 3200 for free and associated NH, at 1600–1695 for carbonyl and at 1230–1285 cm^{-1} for thiocarbonyl groups respectively. The characteristic broad singlets at *ca.* δ 9.0 and 12.0 ppm for HN(1) and HN(3) and peaks at *ca.* 167, 178 for carbonyl and thiocarbonyl were also observed in the ^1H and ^{13}C NMR spectra respectively. The physicochemical properties and the spectroscopic data of thioureas **1a–t** are given in Tables 1 and 2 respectively. Mass spectra of all of the compounds showed the molecular ion peaks. The major fragment corresponds to the N-McLafferty rearrangement and the base peaks are derived from the aroyl cation.

2.2. Biological activities

2.2.1. Antibacterial activity

In vitro evaluation of antibacterial activity was carried out by disk diffusion method (Kirby–Bauer method) against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aureginosa* [29]. The tests were repeated three times and the results are reported as means of at least three determinations and the results are summarized in Table 3. The figures represent the zone of inhibition in millimeters. As is evident from the table all compounds **1a–t** exhibited good to significant inhibitory activity against the two strains, viz. *B. subtilis* and *P. aureginosa*, compared to standard drug at the tested concentration. Some of them were active against *S. aureus* but most of them are inactive against *E. coli*. Good to significant activity against *B. subtilis* is observed for all of the compounds. Four compounds which show excellent activity are **1e** (73%), **1f** (78%), **1i** (66%) and **1s** (66%). Overall good results are obtained for compounds **1f–j** (R = 3-F) against *B. subtilis*. Compounds **1b–d**, **1g**, **1m**, **1o**, and **1t** have been found to exhibit moderate activity against *S. aureus*. All compounds show weak to

Table 3
Antibacterial bioassay screening of thioureas (**1a–t**).

Compound	Antibacterial activity[a]			
	Gram-positive		Gram-negative	
	Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aureginosa*
1a	–	07	–	05
1b	–	08	–	05
1c	–	09	–	06
1d	06	09	–	05
1e	–	13	–	14
1f	–	14	–	06
1g	–	10	–	–
1h	05	07	–	05
1i	10	12	–	04
1j	–	06	–	03
1k	–	06	–	03
1l	–	06	–	01
1m	07	09	–	06
1n	07	07	–	05
1o	–	08	–	08
1p	–	07	01	04
1q	07	10	–	05
1r	07	07	–	07
1s	–	12	–	11
1t	06	08	–	05
Imipenem	21	18	16	18

Concentration used: 2 mg/1 ml; –: no activity.
[a] Zone of inhibition (radius, mm).

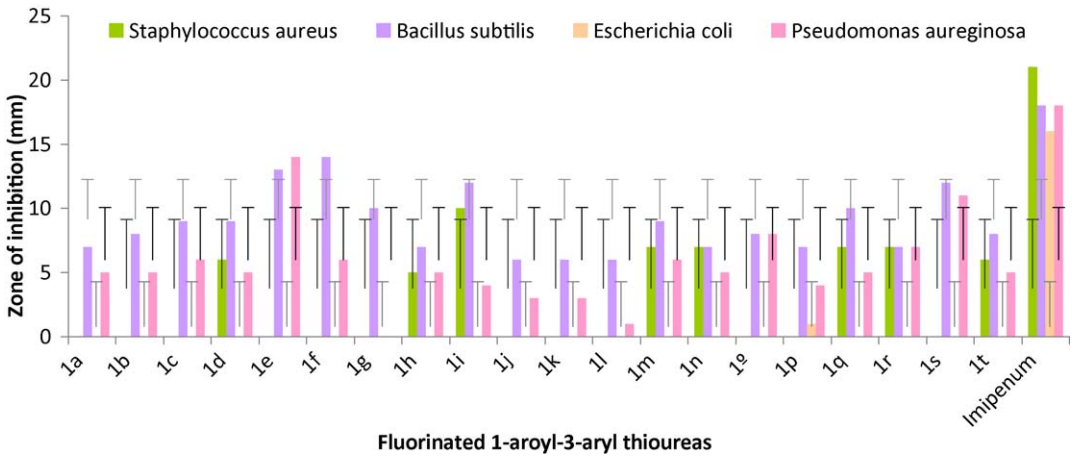


Fig. 1. Antibacterial bioassay screening of thioureas (**1a–t**).

Table 4
Minimum inhibitory concentration (MIC) of selected compounds.

Compound	Minimum inhibitory concentration (MIC) (μg/ml)			
	S. aureus	*B. subtilis*	*E. coli*	*P. aureginosa*
1c	–	250	–	500
1d	500	250	–	500
1e	–	125	–	125
1g	–	250	–	–
1i	250	125	–	1000
1m	500	250	–	500
1q	250	250	–	500
1s	–	125	–	125

moderate activity against bacterial strain *P. aureginosa* with the exception of compounds **1e** (78%) and **1s** (62%) that possess noteworthy activity (Fig. 1). Imipenem (5*R*,6*S*)-6-[(1*R*)-1-hydroxy ethyl]-3-({2-[(iminomethyl)amino] ethyl}thio)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (molecular mass 299.347) was used as the standard drug. It is an intravenous β-lactam antibiotic (subgroup carbapenems) having an extremely broad spectrum of activity. Imipenem is principally active against *Pseudomonas aeruginosa* and the *Enterococcus species* but is not active against *S. aureus*. Imipenem exhibits its antimicrobial action through inhibiting cell wall synthesis of bacteria and is very stable in the presence of β-lactamase produced by some bacteria. Since it is rapidly degraded by the renal enzyme dehydropeptidase 1 when administered alone, therefore it is always co-administered with cilastatin to prevent this inactivation [30]. Compounds **1e** (4-F on aroyl 2-F on aryl ring; zone of inhibition 14 mm against *B. subtilis*) and **1f** (2-Cl on aroyl, 3-F on aryl, zone of inhibition 14 mm against *P. aureginosa*) possess antibacterial action close to that of Imipenem having zone of inhibition 18 mm for each of these strains.

The minimum inhibitory concentrations (MICs) of the compounds were recorded as the lowest concentration of each of the compounds in the petri plates with no turbidity (i.e. no growth) of

Table 5
Antifungal bioassay screening of thioureas (**1a–t**).

Compound	Antifungal activity									
	Rhizopus oryzae		*Aspergillus tereus*		*Fusarium oxysporum*		*Aspergillus niger*		*Aspergillus fumigatus*	
	ZI	PI	ZI	PI	ZI	PI	ZI	PI	ZI[a]	PI
1a	7.5	16.7	5.0	37.5	6.0	25.0	5.0	44.4	6.0	33.3
1b	8.5	5.60	5.0	37.5	6.0	25.0	9.0	–	8.5	5.50
1c	1.5	83.4	5.0	37.5	3.2	60.0	4.0	55.5	4.0	55.5
1d	9.0	–	4.5	43.8	3.0	62.5	5.5	38.8	6.0	33.3
1e	7.5	16.7	4.0	50.0	4.5	43.8	5.5	38.8	7.0	22.2
1f	9.0	–	7.5	6.30	6.5	18.8	9.0	–	8.5	5.50
1g	1.0	88.9	5.5	31.3	3.2	60.0	9.0	–	9.0	–
1h	7.0	22.3	3.0	62.5	4.5	43.8	5.0	44.4	7.5	16.6
1i	7.5	16.7	3.5	56.3	4.5	43.8	9.0	–	7.5	16.6
1j	3.0	66.7	3.5	56.3	2.0	75.0	9.0	–	9.0	–
1k	8.0	11.2	5.5	31.3	6.5	18.8	4.0	55.5	4.5	50.0
1l	8.5	5.60	4.5	43.8	3.5	56.3	9.0	–	9.0	–
1m	2.0	77.8	5.5	31.3	4.5	43.8	8.0	11.1	8.5	5.50
1n	8.5	5.60	4.0	50.0	5.4	32.5	6.0	33.3	5.5	38.8
1o	7.0	22.3	3.0	62.5	5.0	37.5	9.0	–	9.0	–
1p	9.0	–	5.0	37.5	6.0	25.0	9.0	–	7.0	22.2
1q	9.0	–	4.0	50.0	3.0	62.5	5.0	44.4	5.0	44.4
1r	8.5	5.6	3.5	56.3	6.0	25.0	4.0	55.5	5.0	44.4
1s	9.0	–	5.0	37.5	4.0	50.0	5.0	44.4	4.0	55.5
1t	8.0	11.2	6.0	25.0	5.0	37.5	5.0	44.4	6.0	33.3
Control	9.0	–	8.0	–	8.0	–	9.0	–	9.0	–
Nystatin	0	100	0	100	0	100	0	100	0	100

ZI: zone of inhibition; PI : percent inhibition = 100 − fungal growth in sample(cm)/fungal growth in control(cm) × 100; –: no activity.
[a] Radius (cm).

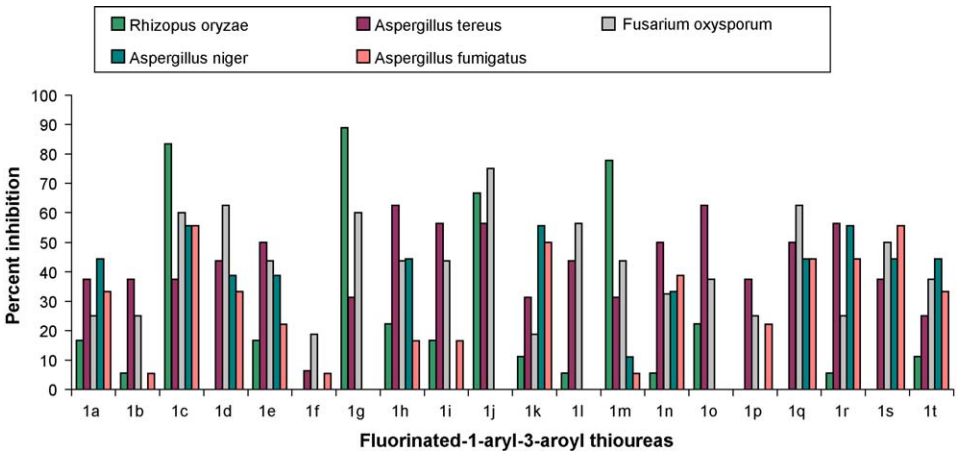


Fig. 2. Antifungal bioassay screening of thioureas (**1a**–**t**).

inoculated bacteria (Table 4). Compounds **1e** having a fluorine substituent at 4-position of aroyl ring and 2-position of aryl ring and **1s** having fluorine at 4-position of both aroyl ring and aryl ring along with a chloro substituent at 3-position of aryl ring show good antibacterial activity at very low concentration (125 μg/ml) against *B. subtilis* and *P. aureginosa*. Moreover, compound **1i** exhibited significant activity against *B. subtilis* at low concentration.

2.2.2. Antifungal activity

Antifungal activity is also affected by the nature and position of substituents on aroyl and aryl ring. Significant activity is observed when fluorine is present at ortho position of aroyl ring and at meta position of aryl ring whereas for absence of fluorine on aroyl ring lowers the activity (Table 5). Fig. 2 graphically presents the percent inhibition of isomeric 1-fluoroaroyl-3-fluoroaryl thioureas against different fungal strains. In case of *R. oryzae* the compounds showing significant to excellent activity are **1c** (83.4%), **1g** (88.9%), **1j** (66.7%) and **1m** (77.8%). In the case *A. tereus*, all of the compounds possess low to good inhibition with reference to control. Good to significant results are obtained for compounds **1h**–**j** (R = 3-F; R′ = F). Some of the compounds possessing moderate to good activity are **1h** (62.5%), **1i** (56.3%), **1j** (56.3%), **1o** (62.5%) and **1r** (56.3%). For fungal strain *F. oxysporum* good to significant activity was observed. Thus compounds **1c** (60%), **1d** (62.5%), **1g** (60%), **1j** (75%), **1l** (56.3%) and **1q** (62.5%) exhibited significant inhibition. In the case *A. niger* some of the compounds are inactive while the compounds **1c** (55.5%), **1k** (55.5%) and **1r** (55.5%) showed good inhibitions. Low activity is observed for compounds **1f–1e** against *A. fumigates* for R = 3-F whilst good results are observed for R = 2-F and R = 3-Cl-4-F and significant results have been observed for compounds **1c** (55.5%), **1k** (50%) and **1s** (55.5%).

Nystatin (mol. mass 926.09) isolated from *Streptomyces noursei* is a polyene antifungal drug effective against many molds and fungi including *Candida* and *Cryptococcus* spp. It is considered a relatively safe drug for treating oral or gastrointestinal fungal infections. Nystatin like amphotericin B and natamycin, binds to ergosterol, a major component of the fungal cell membrane. When present in sufficient concentrations, it forms pores in the membrane that lead to K^+ leakage and death of the fungus [31]. Nystatin was used as negative control (as standard drug) with no growth (100% inhibition) of all fungal strains at the same concentration.

3. Conclusions

A variety of new fluorinated thioureas (**1a**–**t**) were synthesized characterized and screened for *in vitro* antibacterial and antifungal activity. In general fluorinated thioureas show better antifungal activity than the antibacterial activity. The position of fluorine substituent on aroyl and aryl rings has significant effect on the antimicrobial activity.

4. Experimental

Melting points were recorded using a digital Gallenkamp (SANYO) model MPD.BM 3.5 apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were determined in $CDCl_3$ at 300 MHz and 75 MHz respectively using a Bruker spectrophotometer. IR spectra were recorded on an IR Shimadzu 460 spectrophotometer as KBr pellets. Mass Spectra (EI, 70 eV) on a GC–MS instrument Agilent technologies and elemental analyses were conducted using a LECO-183 CHNS analyzer. Bioactivities were carried out at the Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan. Thin layer chromatography (TLC) was conducted on 0.25 mm silica gel plates (60 F254, Merck). Visualization was made with ultraviolet light. Reagents were obtained commercially and used as received.

4.1. General procedure for the preparation of 1-(fluorobenzoyl)-3-(fluorophenyl)thioureas ***(1a–t)***

A freshly distilled solution of substituted fluorobenzoyl chloride (10 mmol) in anhydrous acetone (50 ml) was added dropwise to a suspension of potassium thiocyanate (10 mmol) in acetone (30 ml) at room temperature. The reaction mixture was refluxed for 30 min. and cooled to room temperature. A solution of the fluorinated aniline (10 mmol) in anhydrous acetone (20 ml) was added dropwise and the reaction mixture was refluxed further for 2–3 h. On completion of reaction (t.l.c.) the mixture was poured into cold water and the precipitated thioureas were filtered and recrystallized from methanol.

The physicochemical elemental and mass spectral data of compounds is given in Table 1 whereas IR and NMR data is presented in Table 2.

4.2. Biological assay

4.2.1. Antibacterial activity

The antibacterial activity of all synthesized fluorinated 1-aroyl-3-aryl thioureas has been investigated against four strains of bacteria (*E. coli* ATCC 25922, *P. aureginosa* ATCC 10197, *B. subtilis* DSM 3256, *S. aureus* ATCC 25923) by the Agar well diffusion assay. Imipenem was used as a standard antibiotic, 2 mg of compounds were dissolved in 1 ml of DMSO. In nutrient broth medium 24 h

fresh bacterial culture was prepared. To compare the turbidity of bacterial culture McFarland 0.5% barium sulphate solution was used (as turbidity standard).

To perform antibacterial assay, nutrient agar petri plates were prepared with sterile cotton swabs, respective bacterial colony lawns were prepared with sterile cork borer (4 mm). Using a micropipette, 30 μl of test solution were poured into respective wells and these petri plates were incubated at 37 °C for 24 h. After 24 h of incubation, the radius of the clear zones showing no bacterial growth was measured around each well. The zones of inhibition (mm) was calculated and compared with the standard drug imipenem. The minimum inhibitory concentration for the most active compounds **1c**, **1d**, **1e**, **1g**, **1i**, **1m** and **1s** against the same microorganisms used in the preliminary screening was carried out using microdilution susceptibility method [32].

4.2.2. Antifungal activity

The antifungal activity of the all synthesized fluorinated 1-aroyl-3-aryl thioureas has been investigated against five different fungal strains, i.e., *Aspergillus terreus*, *Rhizopus oryzae*, *Fusarium oxysporium*, *Aspergillus niger* and *Aspergillus fumigatus*. 2 mg of compounds were dissolved in 1 ml of DMSO. All fungal strains were grown on saboraud dextrose agar SDA (pH 5.7) at 28 °C.

To perform antifungal assay SDA medium petri plates were prepared. 50 μl of test samples were added to respective petri plates with the help of a micropipette and spreaded through spreader and were allowed to absorb for some time. Fungal strains then inocubated at 30 °C in their respective petri plates. For every fungal strain, a positive control containing no test sample was also prepared. After 4–6 days of incubation, diameter of the fungal strains was measured in each petri plate and percentage of fungal inhibition was calculated using the formula:

$$\%\ \text{age of fungal inhibition} = 100 - \frac{\text{fungal growth in test sample(cm)}}{\text{fungal growth in control(cm)}} \times 100$$

Nystatin was used as negative control (as standard drug) with no growth (100% inhibition) of all fungal strains at the same concentration.

References

[1] B. Wakefield, Innov. Pharm. Technol. (2000) 74–78.
[2] J.M. Antelo, J. Crugeiras, J.R. Leis, A. Rios, J. Chem. Soc. Perkin Trans. 2 (2000) 2071–2076.
[3] I. Alkorta, I. Rozas, J. Elguero, J. Fluor. Chem. 101 (2000) 233–238.
[4] C. Wakselman, Ann. Pharm. Fr. 57 (1999) 108–115.
[5] E.V. Nosova, G.N. Lipunova, A.A. Laeva, L.P. Sidorova, V.N. Charushin, Zh. Org. Khim. 43 (2007) 68–76.
[6] E.V. Nosova, G.N. Lipunova, A.A. Laeva, V.N. Charushin, Zh. Org. Khim. 42 (2006) 1544–1550.
[7] G.N. Lipunova, E.V. Nosova, A.A. Laeva, T.V. Trashakhova, P.A. Slepukhin, V.N. Charushin, Russ. J. Org. Chem. 44 (2008) 741–749.
[8] C. Sun, H. Huang, M. Feng, X. Shi, X. Zhang, P. Zhoub, Bioorg. Med. Chem. Lett. 16 (2006) 162–166.
[9] T.K. Venkatachalam, C. Mao, F.M. Uckun, Bioorg. Med. Chem. 12 (2004) 4275–4284.
[10] H. Zhang, L. Vrang, K. Bäckbro, P. Lind, C. Sahlberg, T. Unge, B. Öberg, Antivir. Res. 28 (1995) 331–342.
[11] G.Y. Sarkis, E.D. Faisal, J. Heterocycl. Chem. 22 (1985) 137–140.
[12] M.F. Emen, H. Arslan, N. Kulcu, U. Florke, N. Duran, Pol. J. Chem. 79 (2005) 1615–1626.
[13] K.R. Koch, Coord. Chem. Rev. (2001) 473–488.
[14] R. Krishnamurthy, S. Govindaraghavan, J. Narayanasamy, Pestic. Sci. 52 (1999) 145–151.
[15] X. Sijia, D. Liping, K. Shaoyong, J. Liangbin, Chem. J. Internet 5 (2003) 67–70; S.Y. Ke, S.-J. Xue, ARKIVOC x (2006) 63–68.
[16] J.S. Upadlgaya, P.K. Srivastava, J. Ind. Chem. Soc. 59 (1982) 767–769.
[17] V. Alagarsamy, S. Meena, K.V. Ramseshu, V.R. Solomon, K. Thirumurugan, K. Dhanabal, M. Muruga, Eur. J. Med. Chem. 41 (2006) 1293–1300.
[18] T.P. Yoon, E.N. Jacobsen, Angew. Chem. Int. Ed. Engl. 44 (2005) 466–468.
[19] T. Okino, S. Nakamura, T. Furukawa, Y. Takemoto, Org. Lett. 6 (2004) 625–629.
[20] H.B. Huang, E.N. Jacobsen, J. Am. Chem. Soc. 128 (2006) 7170–7171.
[21] T.K. Venkatachalam, F.M. Uckun, Synth. Commun. 35 (2005) 2039–2056.
[22] C. Boga, L. Forlani, C. Silvestroni, A.B. Corradi, P. Sgarabotto, J. Chem. Soc. Perkin Trans. 1 (1999) 1363–1368.
[23] R.S. Zeng, J.P. Zou, S.J. Zhi, J. Chen, Q. Shen, Org. Lett. 5 (2003) 1657–1659; X.-C. Wang, F. Wang, Z.-J. Quan, M.-G. Wang, Z. Li, J. Chem. Res. 61 (2005) 689–690.
[24] A. Saeed, S. Zaman, M. Bolte, Synth. Commun. 38 (2008) 2185–2199.
[25] L.M. Popova, A.I. Ginak, Russ. J. Org. Chem. 44 (2008) 489–494.
[26] M. Kidwai, A. Mishra, J. Serb. Chem. Soc. 69 (2004) 247–254.
[27] L. Kang, A. Robert, Goodnow, J. Synth. Commun. 34 (2004) 1891–1895.
[28] M.L. Xiao, F.H. Chen, Z.J. Chen, B.S. Guo, X.H. Lv, W.M. Tang, Chin. Chem. Lett. 18 (2007) 258–260.
[29] J.G. Cappuccino, N. Sherman, Microbiology: A Laboratory Manual, The Benjamin/Cummings Publishing Co., California, USA, 1996, pp. 247–251.
[30] S.P. Clissold, P.A. Todd, R.D.M. Campoli, Drugs 33 (1987) 183–241.
[31] N. Akaike, N. Harata, Jpn. J. Physiol. 44 (1994) 433–473.
[32] P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, R.H. Yolken, in: G.L. Wood, J.A. Washington (Eds.), Manual of Clinical Microbiology, Am. Soc. Microbiol., Washington, DC, 1995.