Med Chem Res (2013) 22:1747–1755
DOI 10.1007/s00044-012-0168-x

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, antimicrobial and cytotoxic activities of novel 4-trifluoromethyl-(1,2,3)-thiadiazolo-5-carboxylic acid hydrazide Schiff's bases

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Received: 15 November 2011 / Accepted: 28 June 2012 / Published online: 18 July 2012
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Abstract A series of novel 1,2,3-thiadiazolo-5-carboxylic acid hydrazide Schiff's bases **4a**–**4r** were prepared starting from ethyl-4,4,4-trifluoroacetoacetate and ethyl carbazate in four steps. All the compounds were screened for antibacterial activity against various bacterial strains at 150 μg/ml concentration and found no activity. Similarly, all the compounds were screened for antifungal activity against various fungal strains at 100 and 150 μg/ml concentrations. Compounds **4a**, **4m**, and **4q** found to show moderate activity against *Candida albicans*. Further, compounds were evaluated for cytotoxic activity against breast carcinoma cells MDA-MB 231 (aggressive), MCF-7 (non-aggressive) using doxorubicin as standard. Compound **4n** was found to show 25.39 % cell viability against MDA-MB 231 and 63.60 % cell viability against MCF-7 cells.

Keywords 1,2,3-Thiadiazole · Schiff's bases · Hurd–Mori synthesis · Antibacterial activity · Antifungal activity · Cytotoxic activity

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Introduction

The thiadiazole nucleus present in many biologically active compounds and more specifically 1,2,3-thiadiazole derivatives known to have antihelminthic (Sarett and Brown, 1962, 1967, 1969), insecticide (McConnell and Coover, 1963), antibacterial activities (Pain and Slack, 1965) and some of the derivatives was found as necroptosis inhibitors (Teng *et al.*, 2007). The 1,2,3-thiadiazole ring system is prepared mainly in five ways: (i) cyclization of hydrazones with thionyl chloride (Hurd–Mori synthesis) (Hurd and Mori, 1955), (ii) cycloaddition of diazoalkanes with C=S bond (Pechmann synthesis) (Pechmann and Nold, 1896), (iii) hetero-cyclization of α-diazo thiocarbonyl compounds (Wolff synthesis) (Wolff, 1904), (iv) ring transformation of other sulfur-containing heterocyclic compounds (Kurzer, 1973), and (v) elaboration of preformed 1,2,3-thiadiazole (Thomas, 1984; Bakulev and Mokrushin, 1986; L'abbé *et al.*, 1996; Morzherin *et al.*, 2003). Recent reports claim that the 1,2,3-thiadiazole derivatives are identified as novel class of potent HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTIs) (Peng *et al.*, 2008, 2009). In addition, it was found that the fluorine (Hertel *et al.*, 1988) or trifluoromethyl (Filler *et al.*, 1993; Chae *et al.*, 2004) group at a strategic position of an organic molecule dramatically alters the properties of molecule in terms of lipid solubility, oxidative thermal stability, permeability, oral bioavailability thereby enhancement of transport mechanism. Based on the importance of 1,2,3-thiadiazole nucleus and in continuation of our efforts (Manichandrika *et al.*, 2008, 2010; Sirisha *et al.*, 2010; Kurumurthy *et al.*, 2011) on synthesis of trifluoromethyl-substituted compounds, we have synthesized a series of novel 4-trifluoromethyl-1,2,3-thiadiazole-5-carboxylic acid hydrazide Schiff's bases in four steps starting from ethyl 4,4,4-trifluoroacetoacetate

and ethyl carbazate. All the final compounds screened for antibacterial, antifungal, and cytotoxic activities against breast carcinoma cells MDA-MB 231 (aggressive) and MCF-7 (non-aggressive) and reporting here for the first time.

Chemistry

A mixture of ethyl 4,4,4-trifluoroacetoacetate and ethyl carbazate in ethanol was heated at reflux for 6 h to obtain 3-(ethoxy carbonyl hydrazino)-4,4,4-trifluorobutyric acid ethylester **1**. The product **1** was further treated with thionyl chloride and heated at 60 °C for 8 h, by Hurd–Mori synthesis obtained 4-trifluoromethyl-(1,2,3)-thiadiazolo-5-carboxylic acid ethylester **2**. The compound **2** was refluxed in hydrazine hydrate to form 4-trifluoromethyl-(1,2,3)-thiadiazole-5-carboxylic acid hydrazide **3**. The hydrazide **3** was further condensed with different substituted benzaldehydes and ketones in ethanol and obtained 4-trifluoromethyl-(1,2,3)-thiadiazolo-5-carboxylic acid hydrazide Schiff's bases **4** in high yields. The details of reactions outlined in Scheme 1 and products are tabulated in Table 1.

Table 1 Physical properties of compounds **4a**–**4r**

S. no.	Compounds	R	R′	Yield (%)	mp (°C)
1	**4a**	H	C_6H_5	98	223–225
2	**4b**	H	4-F–C_6H_4	96	228–230
3	**4c**	H	4-Cl–C_6H_4	95	244–245
4	**4d**	H	4-Br–C_6H_4	92	223–225
5	**4e**	H	4-NO_2–C_6H_4	98	203–205
6	**4f**	H	4-$N(Me)_2$–C_6H_4	90	250–256
7	**4g**	H	4-OH–C_6H_4	98	178–180
8	**4h**	H	4-OMe–C_6H_4	94	260–263
9	**4i**	H	Furan-2-yl	96	219–221
10	**4j**	H	Thiophene-2-yl	95	246–248
11	**4k**	CH_3	C_6H_5	92	229–231
12	**4l**	CH_3	4-NO_2–C_6H_4	92	203–209
13	**4m**	CH_3	2,4-Br_2–C_6H_3	91	218–220
14	**4n**	CH_3	Pyridine-2-yl	88	208–211
15	**4o**	CH_3	CH_3	95	186–188
16	**4p**	(RR′=)–$CH_2(CH_2)_2CH_2$–		96	175–176
17	**4q**	(RR′=)–$CH_2(CH_2)_3CH_2$–		95	186–188
18	**4r**	(RR′=)–$CH_2(CH_2)_4CH_2$–		91	191–194

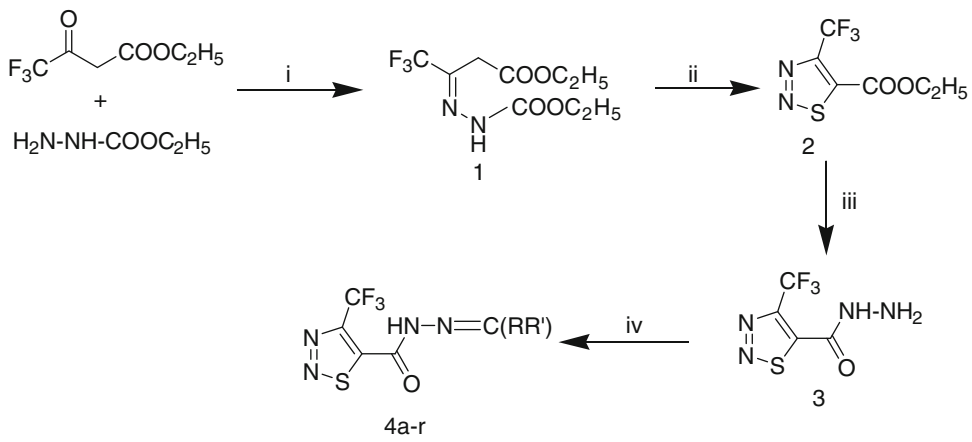
Antimicrobial activity

In vitro antibacterial assays

Compounds **4a**–**4r** were dissolved in acetone and screened for in vitro antibacterial activity against Gram-positive (*Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis*) and Gram-negative (*Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae*) bacteria. The MIC values of the compounds were compared with those obtained with penicillin and streptomycin. However, no compounds showed significant activity against all species of Gram-positive and Gram-negative bacteria. The details of compounds and their activity profile against various microorganisms are tabulated in Table 2.

In vitro antifungal activity assays

The in vitro antifungal activity of compounds **4a**–**4r** were screened against the fungal strains, viz., *Candida albicans* (MTCC 227), *Saccharomyces cerevisiae* (MTCC 36) and filamentous fungal cultures like *Aspergillus niger* (MTCC 1344), *Candida rugosa* (NCIM 3462) by agar cup diffusion method. The strains were obtained from the Institute of Microbial Technology, Chandigarh. Compounds **4a** and **4m** showed moderate activity against *C. albicans* at 100



Scheme 1 Preparation of compounds **4a**–**4r**. Reagents and conditions: (*i*) Ethanol, reflux, 6 h; (*ii*) $SOCl_2$, 60 °C, 8 h; (*iii*) hydrazine hydrate, reflux, 3 h; (*iv*) (RR′) C=O, ethanol, reflux, 1–2 h.

Table 2 Antibacterial activity of compounds **4a–4r**

Compounds	MIC (μg/ml)					
	B. subtilis	*S. aureus*	*S. epidermidis*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*
4a	150	150	150	150	150	150
4b	150	150	150	150	150	150
4c	150	150	150	150	150	150
4d	150	150	150	150	150	150
4e	150	150	150	150	150	150
4f	150	150	150	150	150	150
4g	150	150	150	150	150	150
4h	150	150	150	150	150	150
4i	150	150	150	150	150	150
4j	150	150	150	150	150	150
4k	150	150	150	150	150	150
4l	150	150	150	150	150	150
4m	150	150	150	150	150	150
4n	150	150	150	150	150	150
4o	150	150	150	150	150	150
4p	150	150	150	150	150	150
4q	150	150	150	150	150	150
4r	150	150	150	150	150	150
Penicillin	1.562	1.562	3.125	12.5	12.5	6.25
Streptomycin	6.25	6.25	3.125	6.25	1.562	3.125

and 150 μg/ml, whereas **4q** showed moderate activity against *C. albicans* at 150 μg/ml. The inhibitory zone diameters of the compounds are compared with those obtained with 50 μg/ml of standard amphotericin. The details of the results have been tabulated in Table 3.

Table 4 Cytotoxic activity of compounds **4a–4q** against MDA-MB 231 and MCF-7 cells treated for 48 h by MTT assay at 10 μM concentration

S. no.	Compounds	Percentage of cell viability	
		MDA-MB 231	MCF-7
1	Control (DMSO)	100.00	100.00
2	**4a**	101.55	101.95
3	**4b**	102.43	99.53
4	**4h**	87.27	101.30
5	**4i**	89.53	99.98
6	**4j**	91.93	102.15
7	**4k**	94.52	115.02
8	**4m**	94.73	122.72
9	**4n**	25.39	63.60
10	**4p**	84.20	97.94
11	**4q**	78.56	97.15
12	Doxorubicin (5 μM)	22.85	43.13

In vitro cytotoxic activity

The compounds **4a–4b**, **4h–4k**, **4m–4n**, and **4p–4q** were screened against breast carcinoma cells MDA-MB 231 (aggressive) and MCF-7 (non-aggressive) using

Table 3 Antifungal activity of compounds **4a–4r**

Compounds	Zone of inhibition							
	C. albicans		*C. rugosa*		*S. cerevisiae*		*A. niger*	
	100 μg	150 μg	100 μg	150 μg	100 μg	150 μg	100 μg	150 μg
4a	10	12	0	6	0	0	0	0
4b	0	0	0	0	0	0	0	0
4c	0	0	0	0	0	0	0	0
4d	0	0	0	0	0	0	0	0
4e	0	0	0	0	0	0	0	0
4f	0	0	0	0	0	0	0	0
4g	0	0	0	0	0	0	0	0
4h	0	0	0	0	0	0	0	0
4i	0	0	0	0	0	0	0	0
4j	0	0	0	0	0	0	0	0
4k	0	0	0	0	0	0	0	0
4l	0	0	0	0	0	0	0	0
4m	11	15	0	0	0	0	0	0
4n	0	0	0	0	0	0	0	0
4o	0	0	0	0	0	0	0	0
4p	0	0	0	0	0	0	0	0
4q	0	7	0	0	0	0	0	0
4r	0	0	0	0	0	0	0	0
Amphotericin B	23.5		21		22		25	

Table 5 % of cell viability of **4n** at different concentrations against MDA-MB 231 cells

Concentration (μM)	Percentage of cell viability
10	25.39
5	34.81
1	41.74

Table 6 IC_{50} (μg/ml) values of compound **4n** and doxorubicin

Compound	IC_{50}	
	MDA-MB 231	MCF-7
4n	2.74	6.01
Doxorubicin	1.81	4.12

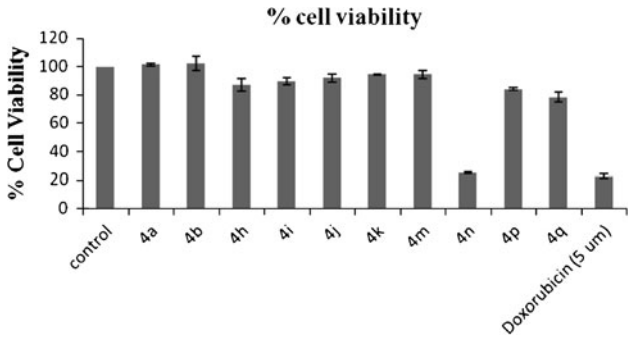


Fig. 1 Cytotoxic activity of compounds **4a**–**4q** against MDA-MB 231 breast carcinoma cells at 10 μM concentration

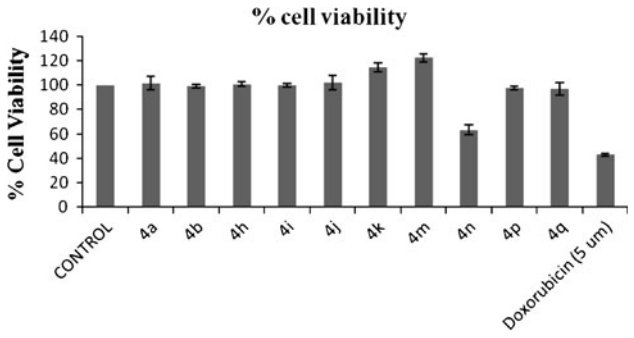


Fig. 2 Cytotoxic activity of compounds **4a**–**4q** against MCF-7 breast carcinoma at 10 μM concentration

doxorubicin as standard, in order to assess the growth inhibitory/cytotoxic effects. Compound **4n** was found to show promising cell viability against both the cell lines and is attributed to the presence of thiadiazole and pyridine ring in single molecule. Thus, compound **4n** was further evaluated at different concentrations and found to be increased

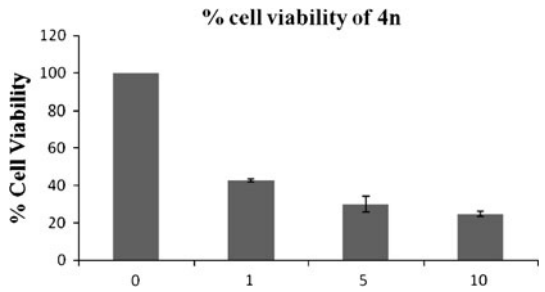


Fig. 3 Percentage of cell viability of **4n** at different concentrations against MDA-MB 231 cells

activity with increase in concentration. The data are outlined in Table 5 and IC_{50} values are calculated and compared with standard doxorubicin in Table 6. All other compounds showed moderate cell viability, however, compounds **4k** and **4m** showed proliferation of cells rather than viability against MCF-7 cell lines. Further work is in progress to identify the lead compound. The details are outlined in Table 4 and Figs. 1, 2.

Conclusion

A series of novel trifluoromethyl-substituted thiadiazole carboxylic acid hydrazide Schiff's bases were prepared in four steps and screened for antimicrobial and cytotoxic activities. The compounds **4a**, **4m**, and **4q** showed promising antifungal activity and compound **4n** showed promising percentage cell viability (Fig. 3).

Experimental

Melting points were recorded on Cassia-Siamia (VMP-AM) melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer FT-IR 240-C spectrophotometer using KBr optics. ^{1}H NMR spectra were recorded on Bruker AV 300 MHz in $CDCl_3$ and DMSO-d_6 using TMS as internal standard. ^{13}C NMR spectra were recorded on Bruker AV 75 MHz in $CDCl_3$ and DMSO-d_6. Electron impact (EI) and chemical ionization mass spectra were recorded on a VG 7070 H instrument at 70 eV. All the reactions were monitored by thin layer chromatography (TLC) on precoated silica gel 60 F_{254} (mesh); spots were visualized with UV light. Merck silica gel (60–120 mesh) was used for column chromatography. CHN analysis was recorded on a Vario EL analyser. Six bacterial test organisms and four fungal strains were selected for screening and obtained from the Institute of Microbial Technology, Chandigarh. The breast carcinoma cells

MDA-MB 231 (aggressive) and MCF-7 (non-aggressive) were used for cytotoxic effects.

Preparation of ethyl 2-(4-ethoxy-1,1,1-trifluoro-4-oxobutan-2-ylidene) hydrazinecarboxylate (**1**)

Procedure

A mixture of ethyl-4,4,4-trifluoroacetoacetate (1.0 g, 5.4 mmol) and ethyl carbazate (0.56 g, 5.4 mmol) in 10 ml ethanol was heated at reflux for 6 h. After completion of reaction, the solvent was removed under vacuum and the crude residue was purified by passing through a column packed with silica gel using petroleum ether/EtOAc (6:4) as eluents. White solid, Yield: 92 %, mp 111–113 °C; IR (KBr, cm^{-1}): 3227 (–NH), 1747 (–C=O), 1619 (C=N), 1363 (–C–F); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.20 (t, $J = 6.6$ Hz, 3H, CH_3), 1.40 (t, $J = 6.7$ Hz, 3H, CH_3), 3.61 (s, 2H, –CH_2–), 4.22 (q, $J = 6.6$ Hz, 2H, OCH_2), 4.29 (q, $J = 6.7$ Hz, 2H, OCH_2), 10.71 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): 171.2, 159.1, 155.1, 118.6, 59.2, 58.2, 56.6, 13.6, 13.3; MS (EI, 70 eV): *m/z* (%) : 270(M^+, 100), 201 (M^+-CF_3, 28); Anal. Calcd. for $C_9H_{13}F_3N_2O_4$: C, 40.01; H, 4.85; N, 10.37 %. Found: C, 40.03; H, 4.84; N, 10.35 %.

Preparation of ethyl 4-(trifluoromethyl)-1,2,3-thiadiazole-5-carboxylate (**2**)

Procedure

The 3-(ethoxycarbonyl hydrazino)-4,4,4-trifluorobutyric acid ethyl ester **1** (1.0 g, 4 mmol) was cooled at 0 °C, and $SOCl_2$ (2.0 ml) was added dropwise with stirring during 10 min, and the temperature of reaction mixture was raised to 60 °C and stirring was continued for 8 h. The reaction mixture was cooled to room temperature and excess $SOCl_2$ was removed under vacuum. The residue was treated with cold water and neutralized with aqueous $NaHCO_3$. The product was extracted thrice with ethyl acetate, and combined extracts were washed with water and dried over anhydrous sodium sulfate. The solvent was removed under vacuum and the crude product was purified by passing through a column packed with silica gel using petroleum ether/EtOAc (6:4) as eluents. Yellow oil, Yield: 48 %; IR (KBr, cm^{-1}): 1725 (–C=O), 1519 (C=C), 1361 (–C–F); ^{1}H NMR ($CDCl_3$, 300 MHz): 1.41 (t, $J = 6.6$ Hz, 3H, CH_3), 4.33(q, $J = 6.6$ Hz, 2H, OCH_2); ^{13}C NMR (DMSO-d_6, 75 MHz): 167.2, 148.4, 146.9, 118.3, 59.4, 15.6; MS (EI, 70 eV): *m/z* (%): 226 (M^+, 100), 181 ($M^+-OC_2H_5$, 49), 157 (M^+-CF_3, 31); Anal. Calcd. for $C_6H_5F_3N_2O_2S$: C, 31.86; H, 2.23; N, 12.39 %. Found: C, 31.88; H, 2.21; N, 12.37 %.

Preparation of 4-(trifluoromethyl)-1,2,3-thiadiazole-5-carbohydrazide (**3**)

Procedure

The 4-trifluoromethyl-(1,2,3)-thiadiazol-5-carboxylic acid ethylester **2** (1.0 g, 4.5 mmol) and hydrazine hydrate (0.5 ml, 10 mmol) in ethanol (10 mL) was refluxed for 3 h. On cooling, yellow solid was separated, filtered, dried, and recrystallised to obtain pure compound **3**. Yellow solid, Yield: 72 %; mp 180 °C; IR (KBr, cm^{-1}): 3311, 3369 (–$NHNH_2$), 1705 (–C=O), 1515 (C=C), 1359 (–C–F); ^{1}H NMR (DMSO-d_6, 300 MHz): 10.60 (br., 1H, NH), 4.32(br., 2H, NH_2); ^{13}C NMR (DMSO-d_6, 75 MHz): 167.8, 148.1, 146.8, 118.6; MS (EI, 70 eV): *m/z* (%) : 212 (M^+, 100), 181 (M^+-NHNH_2, 65), 143 (M^+-CF_3, 32); Anal. Calcd. for $C_4H_3F_3N_4OS$: C, 22.65; H, 1.43; N, 26.41 %. Found: C, 22.66; H, 1.44; N, 26.39 %.

Preparation of *N*′-(arylmethylidene)-4-(trifluoromethyl)-1,2,3-thiadiazole-5-carbohydrazide (**4a**–**4r**)

General procedure

A mixture of 4-trifluoromethyl-(1,2,3)-thiadiazol-5-carboxylic acid hydrazide **3** (0.2 g, 1 mmol) and aryl aldehyde (1 mmol) in ethanol (5 ml) was refluxed on a water bath for 2 h. The solvent was removed and the residue was purified by column chromatography using petroleum ether/EtOAc (8:2) as eluents.

N′*-(phenylmethylidene)-4-(trifluoromethyl)-1,2,3-thiadiazole-5-carbohydrazide (**4a**)*

Yellow solid, Yield: 98 %, mp 223–225 °C; IR (KBr, cm^{-1}): 3429 (–NH), 1689 (–C=O), 1621 (C=N), 1369 (–C–F); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.41 (m, 3H, Ar–H), 7.59 (t, $J = 8.3$ Hz, 2H, Ar–H), 8.21 (s, 1H, –CH=N), 12.70 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): 170.8, 154.8, 147.6, 146.8, 131.2, 130.6, 129.6, 128.6, 128.4, 128.3, 119.6; MS (EI, 70 eV): *m/z* (%): 300 (M^+, 100), 231(M^+-CF_3, 31); Anal. Calcd. for $C_{11}H_7F_3N_4OS$: C, 44.00; H, 2.35; N, 18.66 %. Found: C, 44.02; H, 2.34; N, 18.68 %.

N′*-((4-fluorophenyl)methylidene)-4-(trifluoromethyl)-1,2,3-thiadiazole-5-carbohydrazide (**4b**)*

Pale yellow solid, Yield: 96 %, mp 228–230 °C; IR (KBr, cm^{-1}): 3420 (–NH), 1689 (–C=O), 1624 (C=N), 1371 (–C–F);

^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.30 (d, J = 8.4 Hz, 2H, Ar–H), 7.61 (d, J = 8.4 Hz, 2H, Ar–H), 8.39 (s, 1H, –CH=N–), 12.79 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): 169.8, 158.8, 148.6, 146.9, 146.8, 132.2, 131.6, 122.6, 124.6, 127.4, 119.6; MS (EI, 70 eV): *m/z* (%): 318 (M^+, 100), 249 (M^+-CF_3, 35); Anal. Calcd. for $C_{11}H_6F_4N_4OS$: C, 41.51; H, 1.90; N, 17.60 %. Found: C, 41.49; H, 1.89; N, 17.62 %.

*N′-((4-chlorophenyl)methylidene)-4-(trifluoromethyl)-1,2,3-thiadiazole-5-carbohydrazide (**4c**)*

Pale yellow solid, Yield: 95 %, mp 244–245 °C; IR (KBr, cm^{-1}): 3418 (–NH), 1686 (–C=O), 1625 (C=N), 1368 (–C–F); ^{1}H NMR (DMSO–d_6, 300 MHz): δ 7.31 (d, J = 8.3 Hz, 2H, Ar–H), 7.62 (d, J = 8.3 Hz, 2H, Ar–H), 8.18 (s, 1H, –CH=N–), 12.71(s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): 170.2, 154.8, 148.6, 146.9, 136.8, 132.2, 131.6, 129.6, 129.2, 127.4, 119.6; MS (EI, 70 eV): *m/z* (%): 334(M^+, 100), 265(M^+-CF_3, 38); Anal. Calcd. for $C_{11}H_6ClF_3N_4OS$: C, 39.47; H, 1.81; N, 16.74 %. Found: C, 39.49; H, 1.82; N, 16.71 %.

*N′-((4-bromophenyl)methylidene)-4-(trifluoromethyl)-1,2,3-thiadiazole-5-carbohydrazide (**4d**)*

Pale yellow solid, Yield: 92 %, mp 223–225 °C; IR (KBr, cm^{-1}): 3420 (–NH), 1680 (–C=O), 1629 (C=N), 1365 (–C–F); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.29 (d, J = 8.3 Hz, 2H, Ar–H), 7.60 (d, J = 8.3 Hz, 2H, Ar–H), 8.31 (s, 1H, –CH=N–), 12.77 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): 170.1, 154.7, 148.6, 146.9, 132.8, 132.2, 131.6, 129.6, 129.2, 125.4, 119.2; MS (EI, 70 eV): *m/z* (%): 379(M^+, 100), 310(M^+-CF_3, 28); Anal. Calcd. for $C_{11}H_6BrF_3N_4OS$: C, 34.85; H, 1.60; N, 14.78 %. Found: C, 34.87; H, 1.61; N, 14.77 %.

*N′-((4-nitrophenyl)methylidene)-4-(trifluoromethyl)-1,2,3-thiadiazole-5-carbohydrazide (**4e**)*

Brown solid, Yield: 98 %, mp 203–205 °C; IR (KBr, cm^{-1}): 3429 (–NH), 1689 (–C=O), 1621 (C=N), 1525 (–NO_2), 1367 (–C–F); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.41 (d, J = 8.5 Hz, 2H, Ar–H), 7.59 (d, J = 8.5 Hz, 2H, Ar–H), 8.21 (s, 1H, –CH=N–), 12.70 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): 170.4, 154.8, 150.6, 146.9, 146.8, 137.2, 131.6, 129.6, 123.2, 123.1, 118.4; MS (EI, 70 eV): *m/z* (%): 345(M^+, 100), 276(M^+-CF_3, 32); Anal. Calcd. for $C_{11}H_6F_3N_5O_3S$: C, 38.27; H, 1.75; N, 20.28 %. Found: C, 38.29; H, 1.74; N, 20.29 %.

*N′-((4-(dimethylamino)phenyl)methylidene)-4-(trifluoromethyl)-1,2,3-thiadiazole-5-carbohydrazide (**4f**)*

Red solid, Yield: 90 %, mp 250–256 °C; IR (KBr, cm^{-1}): 3389 (–NH), 1669 (–C=O), 1619 (C=N), 1369 (–C–F); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.02 (s, 6H, CH_3), 7.11 (d, J = 8.6 Hz, 2H, Ar–H), 7.29 (d, J = 8.6 Hz, 2H, Ar–H), 8.11 (s, 1H, –CH=N–), 12.66 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): 170.4, 154.7, 150.6, 146.9, 146.8, 137.2, 131.6, 131.4, 116.2, 116.1, 118.9, 41.6, 41.5; MS (EI, 70 eV): *m/z* (%): 343(M^+, 100), 274(M^+-CF_3, 29); Anal. Calcd. for $C_{13}H_{12}F_3N_5OS$: C, 45.48; H, 3.52; N, 20.40 %. Found: C, 45.51; H, 3.53; N, 20.38 %.

*N′-((4-hydroxyphenyl)methylidene)-4-(trifluoromethyl)-1,2,3-thiadiazole-5-carbohydrazide (**4g**)*

Yellow solid, Yield: 98 %, mp 178–180 °C; IR (KBr, cm^{-1}): 3420 (–OH), 3386 (–NH), 1683 (–C=O), 1632 (C=N), 1358 (–C–F); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 6.91 (d, J = 8.1 Hz, 2H, Ar–H), 7.52 (d, J = 8.1 Hz, 2H, Ar–H), 8.32 (s, 1H, –CH=N), 9.81 (s, 1H, –OH), 12.15 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): 170.1, 159.8, 154.6, 146.3, 146.2, 130.2, 130.1, 128.6, 118.8, 128.7, 118.6; MS (EI, 70 eV): *m/z* (%): 316(M^+, 100), 247(M^+-CF_3, 41); Anal. Calcd. for $C_{11}H_7F_3N_4O_2S$: C, 41.78; H, 2.23; N, 17.72 %. Found: C, 40.77; H, 2.22; N, 17.74 %.

*N′-((4-methoxyphenyl)methylidene)-4-(trifluoromethyl)-1,2,3-thiadiazole-5-carbohydrazide (**4h**)*

Yellow solid, Yield: 94 %, mp 260–263 °C; IR (KBr, cm^{-1}): 3389 (–NH), 1686 (–C=O), 1629 (C=N), 1354 (–C–F); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.90 (s, 3H, CH_3), 6.91 (d, J = 8.1 Hz, 2H, Ar–H), 7.62 (d, J = 8.1 Hz, 2H, Ar–H), 8.10 (s, 1H, –CH=N), 12.71 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): 170.8, 158.8, 155.6, 147.1, 146.8, 131.2, 131.1, 128.8, 128.7, 118.7, 118.6, 56.8; MS (EI, 70 eV): *m/z* (%): 330(M^+, 100), 261(M^+-CF_3, 38); Anal. Calcd. for $C_{12}H_9F_3N_4O_2S$: C, 43.64; H, 2.75; N, 16.96 %. Found: C, 43.66; H, 2.76; N, 16.94 %.

*N′-(furan-2-ylmethylidene)-4-(trifluoromethyl)-1,2,3-thiadiazole-5-carbohydrazide (**4i**)*

Yellow solid, Yield: 96 %, mp 219–221 °C; IR (KBr, cm^{-1}): 3360 (–NH), 1690 (–C=O), 1615 (C=N), 1349 (–C–F); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 6.51 (d, J = 8.3 Hz, 1H, Ar–H), 6.80 (m, 1H, Ar–H), 7.10 (d, J = 8.2 Hz, 1H, Ar–H), 8.1 (s, 1H, –CH=N–), 12.59 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): 170.1, 154.8, 146.6, 146.2, 143.6, 143.5, 114.9, 110.7, 110.6; MS (EI, 70 eV): *m/z* (%): 290(M^+, 100), 221(M^+-CF_3, 42); Anal. Calcd. for

$C_9H_5F_3N_4O_2S$: C, 37.25; H, 1.74; N, 19.30 %. Found: C, 37.26; H, 1.73; N, 19.32 %.

*N′-(thiophene-2-ylmethylidene)-4-(trifluoromethyl)-1,2,3-thiadiazole-5-carbohydrazide (**4j**)*

Yellow solid, Yield: 95 %, mp 246–248 °C; IR (KBr, cm^{-1}): 3396 (–NH), 1696 (–C=O), 1621 (C=N), 1364 (–C–F); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.11 (d, $J = 7.9$ Hz, 1H, Ar–H), 7.40 (m, 1H, Ar–H), 7.51 (d, $J = 8.1$ Hz, 1H, Ar–H), 8.30 (s, 1H, –CH=N–), 12.61 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): 169.6, 154.1, 145.6, 145.3, 125.5, 125.4, 123.5, 123.4, 115.6; MS (EI, 70 eV): *m/z* (%): 306 (M^+, 100), 237 (M^+-CF_3, 46); Anal. Calcd. for $C_9H_5F_3N_4OS_2$: C, 35.29; H, 1.65; N, 18.29 %. Found: C, 35.31; H, 1.66; N, 18.27 %.

*N′-(1-phenylethylidene)-4-(trifluoromethyl)-1,2,3-thiadiazole-5-carbohydrazide (**4k**)*

Pale yellow solid, Yield: 92 %, mp 229–231 °C; IR (KBr, cm^{-1}): 3390(–NH), 1696 (–C=O), 1626 (C=N), 1361 (–C–F); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.41 (s, 3H, CH_3), 7.42 (m, 3H, Ar–H), 7.71 (d, $J = 8.4$ Hz, 2H, Ar–H), 12.60 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): 170.2, 155.8, 146.4, 146.2, 131.8, 131.1, 129.8, 129.7, 128.8, 128.6, 114.7, 12.8; MS (EI, 70 eV): *m/z* (%): 314(M^+, 100), 245(M^+-CF_3, 45); Anal. Calcd. for $C_{12}H_9F_3N_4OS$: C, 45.86; H, 2.89; N, 17.83 %. Found: C, 46.84; H, 2.90; N, 17.84 %.

*N′-(1-(4-nitrophenyl)ethylidene)-4-(trifluoromethyl)-1,2,3-thiadiazole-5-carbohydrazide (**4l**)*

Yellow solid, Yield: 92 %, mp 203–209 °C; IR (KBr, cm^{-1}): 3410(–NH), 1686 (–C=O), 1624 (C=N), 1513 (–NO_2), 1361 (–C–F); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.51 (s, 3H, CH_3), 7.42 (d, $J = 8.6$ Hz, 2H, Ar–H), 7.61 (d, $J = 8.6$ Hz, 2H, Ar–H), 12.79 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): 170.6, 154.2, 150.7, 146.6, 146.2, 131.1, 129.8, 129.7, 128.8, 128.6, 115.1, 12.1; MS (EI, 70 eV): *m/z* (%): 359 (M^+, 100), 290 (M^+-CF_3, 38); Anal. Calcd. for $C_{12}H_8F_3N_5O_3S$: C, 40.12; H, 2.24; N, 19.49 %. Found: C, 40.14; H, 2.23; N, 19.47 %.

*N′-(1-(2,4-dibromophenyl)ethylidene)-4-(trifluoromethyl)-1,2,3-thiadiazole-5-carbohydrazide (**4m**)*

Yellow solid, Yield: 91 %, mp 218–220 °C; IR (KBr, cm^{-1}): 3410(–NH), 1696 (–C=O), 1619 (C=N), 1358 (–C–F); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.55 (s, 3H, CH_3), 7.52 (d, $J = 8.1$ Hz, 1H, Ar–H), 7.59 (d, $J = 8.1$ Hz, 1H, Ar–H), 7.66 (s, 1H, Ar–H), 12.68 (s,1H, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): 170.8, 155.8, 146.4, 146.2, 136.6, 136.2, 134.5, 134.1, 122.8, 122.6, 115.9, 12.6; MS (EI, 70 eV): *m/z* (%): 472(M^+, 100), 403(M^+-CF_3, 42); Anal. Calcd. for $C_{12}H_7Br_2F_3N_4OS$: C, 30.53; H, 1.49; N, 11.87 %. Found: C, 30.56; H, 1.48; N, 11.88 %.

*N′-(1-(pyridine-2-yl)ethylidene)-4-(trifluoromethyl)-1,2,3-thiadiazole-5-carbohydrazide (**4n**)*

White solid, Yield: 88 %, mp 208–211 °C; IR (KBr, cm^{-1}): 3930(–NH), 1659 (–C=O), 1632 (C=N), 1359 (–C–F); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.59 (s, 3H, CH_3), 7.40 (d, $J = 7.9$ Hz, 1H, Ar–H), 7.80 (m, 2H, Ar–H), 8.61 (d, $J = 8.1$ Hz, 1H, Ar–H), 12.71 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): 170.3, 155.8, 152.3, 148.9, 146,7, 146.5, 136.8, 128.9, 125.1, 116.1, 11.9; MS (EI, 70 eV): *m/z* (%): 315(M^+, 100), 246(M^+-CF_3, 29); Anal. Calcd. for $C_{11}H_8F_3N_5OS$: C, 41.91; H, 2.56; N, 22.21 %. Found: C, 41.92; H, 2.55; N, 22.23 %.

*N′-(1-methylethylidene)-4-(trifluoromethyl)-1,2,3-thiadiazole-5-carbohydrazide (**4o**)*

White solid, Yield: 95 %, mp 186–188 °C; IR (KBr, cm^{-1}): 3410 (–NH), 1696 (–C=O), 1626 (C=N), 1369 (–C–F); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.21 (s, 6H, CH_3), 11.21 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): 170.2, 155.9, 146.8, 146.6, 115.6, 16.8, 12.4; MS (EI, 70 eV): *m/z* (%): 252 (M^+, 100), 183(M^+-CF_3, 34); Anal. Calcd. for $C_7H_7F_3N_4OS$: C, 33.33; H, 2.80; N, 22.21 %. Found: C, 33.35; H, 2.79; N, 22.23 %.

*N′,N″-pentane-1,5-diylidenebis(4-(trifluoromethyl)-1,2,3-thiadiazole-5-carbohydrazide) (**4p**)*

White solid, Yield: 96 %, mp 175–176 °C; IR (KBr, cm^{-1}): 3390(–NH), 1696 (–C=O), 1619 (C=N), 1361 (–C–F); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.91 (m, 4H, CH_2–), 2.40 (m, 4H, –CH_2–), 11.71 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): 170.8, 155.3, 146.9, 146.7, 115.6, 35.4, 31.2, 31.1, 30.8; MS (EI, 70 eV): *m/z* (%): 278(M^+, 100), 209(M^+-CF_3, 38); Anal. Calcd. for $C_9H_9F_3N_4OS$: C, 38.85; H, 3.26; N, 20.14 %. Found: C, 38.84; H, 3.27; N, 20.16 %.

*N′,N″-hexane-1,6-diylidenebis(4-(trifluoromethyl)-1,2,3-thiadiazole-5-carbohydrazide) (**4q**)*

White solid, Yield: 95 %, mp 186–188 °C; IR (KBr, cm^{-1}): 3390 (–NH), 1696 (–C=O), 1617 (C=N), 1356 (–C–F); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.70 (m, 2H, CH_2–), 1.95 (m, 4H, –CH_2–), 2.34 (m, 4H, –CH_2–), 11.81 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): 170.3, 155.6, 146.2,

146.1, 115.7, 33.4, 32.3, 26.9, 26.2, 26.1; MS (EI, 70 eV): *m/z* (%): 292(M^+, 100), 223(M^+-CF_3, 31); Anal. Calcd. for $C_{10}H_{11}F_3N_4OS$: C, 41.09; H, 3.79; N, 19.17 %. Found: C, 41.08; H, 3.78; N, 19.19 %.

*N′,N″-heptane-1,7-diylidenebis(4-(trifluoromethyl)-1,2,3-thiadiazole-5-carbohydrazide) (**4r**)*

White solid, Yield: 91 %, mp 191–194 °C; IR (KBr, cm^{-1}): 3380 (–NH), 1689 (–C=O), 1611 (C=N), 1352 (–C–F); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.74 (m, 4H, CH_2–), 1.92 (m, 4H, –CH_2–), 2.44 (m, 4H, –CH_2–), 11.83 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): 170.4, 155.9, 146.3, 146.1, 115.9, 29.9, 29.6, 29.5, 25.4, 24.7, 24.6; MS (EI, 70 eV): *m/z* (%): 306(M^+, 100), 237(M^+-CF_3, 28); Anal. Calcd. for $C_{10}H_{10}F_3N_4OS$: C, 43.13; H, 4.28; N, 18.29 %. Found: C, 43.15; H, 4.27; N, 18.31 %.

Antibacterial activity

Procedure

Five bacterial test organisms such as *B. subtilis* (MTCC 441), *S. aureus* (MTCC 96), *S. epidermidis* (MTCC 435), *E. coli* (MTCC 443) *P. aeruginosa* (MTCC 741), and *K. pneumoniae* were selected and obtained from the Institute of Microbial Technology, Chandigarh. Cultures of test organisms were maintained on nutrient agar slants and were subcultured in Petri dishes prior to testing. The media used were nutrient agar, nutrient broth procured from Himedia Laboratories, Mumbai. The minimum inhibitory concentration was determined by broth dilution method.

Antifungal activity

Procedure

Antifungal activity was studied by agar cup diffusion method. The readymade potato dextrose agar (PDA) medium (Himedia, 39 g) was suspended in distilled water (1000 ml) and heated until it gets dissolved completely. The medium and Petri dishes were autoclaved at pressure of 15 lb/inch for 20 min. Agar cup bioassay was employed for testing antifungal activity. The medium was poured into sterile Petri dishes under aseptic conditions in a laminar flow chamber. When the medium in the plates solidified, 0.5 ml of (week-old) culture of test organism was inoculated and uniformly spread over the agar surface with a sterile L-shaped rod. Solutions were prepared by dissolving the compound in acetone and different concentrations (30, 100 μg/ml) were made. After inoculation, cups were scooped out with 6-mm sterile cork borer and the lids of the dishes were replaced. To each cup, different concentrations (30, 100 μg/ml) of test solutions were added. Controls were maintained with acetone and amphotericin B (50 μg/ml). The treated and the controls were kept at 28 °C for 48 h. Inhibition zones were measured and the diameter was calculated in millimeter.

Cytotoxic activity

Procedure

The breast carcinoma cells MDA-MB 231 (aggressive) and MCF-7 (non-aggressive) from ATCC were grown in Dulbecco's modified Eagle's medium (DMEM) with 10 % FBS supplemented with antibiotics and treated with test compounds at 10 μM concentration and standard doxorubicin at 5 μM concentration for a period of 48 h and cytotoxicity was measured by standard MTT assay. At the end of the treatments, medium was removed and cells were washed with Dulbecco's phosphate buffered saline (DPBS) and 10 μl of 5 mg/ml MTT solution in 200 μl of culture medium was added and incubated for 1 h at 37 °C. Cells were solubilized with 200 μl of DMSO and absorbance was measured at 562 nm in a spectrophotometer. The absorbance of the wells containing treated cells was compared with that of the wells in which the drug is omitted (control).

Acknowledgments Authors are thankful to Dr. J.S.Yadav, Director, IICT for his constant encouragement and authors (P. Sambasiva Rao, C. Kurumurthy, B. Veeraswamy, and G. Santhosh kumar) are thankful to CSIR for providing financial assistance in the form of Senior Research Fellowship.

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