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Synthesis and biological activity of Schiff and Mannich bases bearing 2,4-dichloro-5-fluorophenyl moiety

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Abstract—A series of 2,4-dichloro-5-fluorophenyl bearing Mannich base (**4** and **5**) was prepared from triazole Schiff bases (**3**) by aminomethylation with formaldehyde and secondary/substituted primary amines. All newly synthesized compounds were screened for their antimicrobial activity. Compounds **3c**, **4c**, **4e** and **4f** exhibited promising antibacterial and compounds **3c**, **5c**, **5e** and **5f** showed good antifungal activity.

1. Introduction

Schiff bases derived from various heterocycles were reported to possess cytotoxic,[1] anticonvulsant,[2] antiproliferative[3], anticancer and antifungal activities.[4] Mannich reaction is an important tool for synthesis of novel compounds. Mannich bases are physiologically reactive because of the basic function rendering the molecule soluble in aqueous solvents when it is transformed into aminium salt. Mannich bases have been reported as potential biological agents. They find application as antitubercular,[5] antimalarial,[6] vasorelaxing,[7] anticancer[8] and analgesic drugs[9]. They are also used in the polymer industry as paints and surface active reagents[10]. Mannich bases of 1,2,4-triazole carrying *N*-methyl piperazine reported to possess protozocidal and antibacterial activity. The modern drugs such as Prazosin,[11] Lidoflazine[12] and Urapidil[13] which contains piperazine nucleus showed good cardiovascular activity. Some Mannich bases are reported to exhibit activity against Maurine P388 lymphocytic leukemia (Wi Dr Colon cancer) in vitro.[14,15]

Various 1,2,4-triazole derivatives have been reported to possess antibacterial, antifungal, anticancer,[16] antitubercular,[17] analgesic and anti-inflammatory properties.[18] 1,2,4-Triazole nucleus has been incorporated into wide variety of therapeutically interesting molecules to transform them into better drugs. Some of the modern-day drugs with triazole nucleus are Ribavirin (antiviral agent), Rizatriptan (antimigrane agent), Alprazolam (anxiolytic agent), Flucanozole and Itraconazole (antifungal agent).

Many compounds containing dichlorophenyl moiety are found to be biologically important and incorporation of fluorine atom in these molecules could also alter the pKa, dipole moments and even chemical reactivity and stability of neighbouring groups. Further, presence of fluorine atom in the molecule increases the lipophilicity of the molecule and hence affects the partitioning of a molecule into membranes and also facilitates hydrophobic interactions of the molecule with specific binding sites on either receptor or enzymes.[19,20]

Prompted by these observations, it was contemplated to synthesize some dichlorofluorophenyl containing congeners of triazole Schiff and Mannich bases with a view to explore their potency as better chemotherapeutic agents. All newly synthesized compounds were screened for the antibacterial and antifungal activity.

2. Result and discussion

4-Amino-3-(2,4-dichloro-5-fluorophenyl)-5-mercapto-1,2,4-triazole (**1**) was synthesized from 2,4-dichloro-5-

Keywords: 2,4-Dichloro-5-fluorophenyl triazole; Schiff base; Mannich base; Antibacterial; Antifungal activity.
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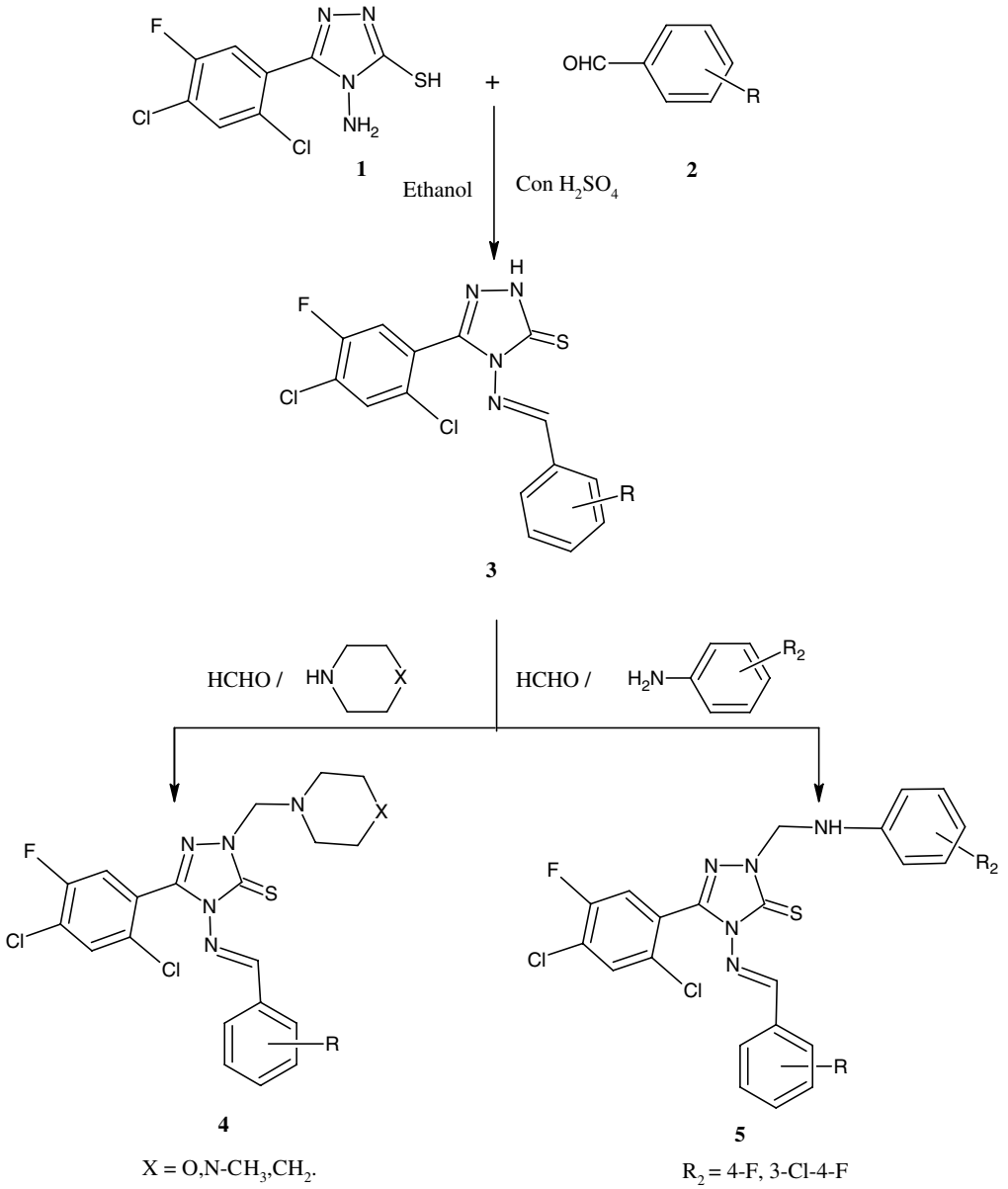
fluorobenzoic acid as per the literature.[21] Compound **1** was treated with substituted aromatic aldehydes (**2**) in presence of concentrated H_2SO_4 to yield Schiff base (**3**). Compound **3** when treated with secondary and substituted aromatic amines in presence of formaldehyde gave Mannich bases (**4** and **5**) in good yields. The reaction sequence is outlined in Scheme 1. The Characterization data of Schiff bases (**3**) and Mannich bases (**4** and **5**) are given in Tables 1 and 2.

3. Biological activity

3.1. Antibacterial studies

The newly prepared compounds were screened for their antibacterial activity against *Escherichia coli* (ATCC-25922), *Staphylococcus aureus* (ATCC-25923), *Psuedomonas aeruginosa* (ATCC-27853) and *Klebsiella pneumoniae* (recultured) bacterial strains by disc diffusion method.[22,23] A standard inoculum ($1–2 \times 10^7$ c.f.u/ml 0.5 McFarland standards) was introduced onto the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculum. The discs measuring 6.25 mm in diameter were prepared from Whatman No.1 filter paper and sterilized by dry heat at 140 °C for an hour. The sterile discs previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. Solvent and growth controls were kept. The plates were inverted and incubated for 24 h at 37 °C. Ciprofloxacin was used as a standard drug. The inhibition zones were measured and compared with the controls. The bacterial zone of inhibition values are given in Table 3.



Scheme 1. Synthesis of Schiff bases and Mannich bases.

Table 1. Characterization data of Schiff bases **3a–g**

Compound	R	Mol. Formula	mp °C	Yield (%)	Analysis (%) Found (calculated)		
					C	H	N
3a	4-$N(CH_3)_2$	$C_{17}H_{14}Cl_2FN_5S$	224–26	73	49.55 (49.76)	3.39 (3.44)	16.71 (17.07)
3b	4-Cl	$C_{15}H_8Cl_3FN_4S$	225–27	87	44.52 (44.85)	1.96 (2.01)	13.64 (13.95)
3c	4-OCH_3	$C_{16}H_{11}Cl_2FN_4SO$	192–94	78	47.97 (48.30)	2.55 (2.79)	13.80 (14.10)
3d	4-NO_2	$C_{15}H_8Cl_2FN_5SO_2$	217–19	75	43.40 (43.70)	1.85 (1.96)	16.58 (16.99)
3e	4-F	$C_{15}H_8Cl_2F_2N_4S$	192–94	70	46.45 (46.77)	2.01 (2.09)	14.27 (14.54)
3f	3,4-(O–CH_2–O)	$C_{16}H_8Cl_3FN_4SO_2$	206–08	81	46.55 (46.75)	2.09 (2.15)	13.76 (14.07)
3g	2,4-Cl_2	$C_{15}H_7Cl_4FN_4S$	201–03	68	40.90 (41.31)	1.59 (1.62)	12.59 (12.85)

Table 2. Characterization data of Mannich bases **4a–i** and **5a–f**

Compound	X/R_2	R	Mol. Formula	mp °C	Yield (%)	Analysis (%) Found (calculated)		
						C	H	N
4a	O	4-$N(CH_3)_2$	$C_{22}H_{23}Cl_2FN_6OS$	150–52	81	51.72 (51.87)	4.51 (4.55)	16.33 (16.50)
4b	N–CH_3	4-$N(CH_3)_2$	$C_{23}H_{26}Cl_2FN_7S$	100–02	73	52.72 (52.87)	4.98 (5.02)	18.61 (18.77)
4c	CH_2	4-$N(CH_3)_2$	$C_{23}H_{25}Cl_2FN_6S$	112–14	78	54.21 (54.44)	4.93 (4.97)	16.47 (16.56)
4d	O	4-Chloro	$C_{20}H_{17}Cl_3FN_5OS$	185–87	83	47.75 (47.97)	3.38 (3.42)	13.81 (13.98)
4e	N–CH_3	4-Chloro	$C_{21}H_{20}Cl_3FN_6S$	143–45	69	48.85 (49.09)	3.89 (3.92)	16.22 (16.36)
4f	CH_2	4-Chloro	$C_{21}H_{19}Cl_3FN_5S$	167–69	80	50.36 (50.56)	3.81 (3.84)	13.85 (14.04)
4g	O	Piperonyl	$C_{21}H_{18}Cl_2FN_5O_3S$	154–56	86	49.26 (49.42)	3.45 (3.55)	13.60 (13.72)
4h	N–CH_3	Piperonyl	$C_{22}H_{21}Cl_2FN_6O_2S$	167–69	65	50.21 (50.48)	4.01 (4.04)	15.92 (16.06)
4i	CH_2	Piperonyl	$C_{22}H_{20}Cl_2FN_5O_2S$	114–16	73	51.76 (51.97)	3.89 (3.97)	13.61 (13.74)
5a	4-Fluoro	4-$N(CH_3)_2$	$C_{24}H_{20}Cl_2F_2N_6S$	128–30	66	53.85 (54.04)	3.72 (3.78)	15.65 (15.75)
5b	3-Chloro-4-fluoro	4-$N(CH_3)_2$	$C_{24}H_{19}Cl_3F_2N_6S$	177–79	70	50.57 (50.76)	3.29 (3.37)	14.66 (14.80)
5c	4-Fluoro	4-Chloro	$C_{22}H_{14}Cl_3F_2N_5S$	164–66	76	50.17 (50.35)	2.66 (2.69)	13.19 (13.34)
5d	3-Chloro-4-fluoro	4-Chloro	$C_{22}H_{13}Cl_4F_2N_5S$	169–71	69	47.18 (47.25)	2.30 (2.34)	12.37 (12.52)
5e	4-Fluoro	Piperonyl	$C_{23}H_{15}Cl_2F_2N_5O_2S$	173–75	73	51.55 (51.70)	2.80 (2.83)	12.85 (13.11)
5f	3-Chloro-4-fluoro	Piperonyl	$C_{23}H_{14}Cl_3F_2N_5O_2S$	172–74	77	48.35 (48.57)	2.41 (2.48)	12.12 (12.31)

Table 3. Zone of Inhibition of Schiff and Mannich bases

Compound	*Staphylococcus aureus* (ATCC-25923)	*Escherichia coli* (ATCC-25922)	*Pseudomonas aeruginosa* (ATCC-27853)	*Klebsiella pneumoniae* (recultured)
3a	12	9	14	—
3b	10	12	10	—
3c	23	28	21	20
3d	—	—	10	—
3e	18	23	—	—
3f	6	10	8	11
3g	12	10	14	
4a	6	15	11	—
4b	13	—	—	—
4c	24	27	24	19
4d	21	29	26	—
4e	23	28	22	17
4f	26	29	25	20
4g	—	10	—	—
4h	14	15	12	6
4i	—	—	9	11
5a	7	6	—	—
5b	12	11	14	12
5c	17	—	12	7
5d	17	—		—
5e	14	9	13	—
5f	—	—	—	—
Standard	25	30	25	20

— Indicates bacteria are resistant to the compounds >100 μg/ml. Zone of Inhibition in mm.

Minimum inhibitory concentration (MIC) was determined by broth dilution technique. The Nutrient Broth, which contained logarithmic serially two fold diluted amount of test compound and controls, was inoculated with approximately 5×10^5 c.f.u of actively dividing bacteria cells. The cultures were incubated for 24 h at 37 °C and the growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentrations (MIC). To obtain the minimum bacterial concentration (MBC), 0.1 ml volume was taken from each tube and spread on agar plates. The number of c.f.u was counted after 18–24 h of incubation at 35 °C. MBC was defined as the lowest drug concentration at which 99.9% of the inoculum was killed. The minimum inhibitory concentration and minimum bactericidal concentration are given in Table 4.

The investigation of antibacterial screening data revealed that all the tested compounds showed moderate to good bacterial inhibition. The compounds **3c**, **4c**, **4e**, and **4f** showed good inhibition towards all tested species. Compound **3e** showed good inhibition against *S. aureus* and *E. coli* species at 6.25 μg/ml concentrations. The compounds **3c**, **4c**, **4e** and **4f** exhibited good antibacterial activity almost equivalent to that of standard. The MBC of few compounds were found to be the same as MIC but in most of the compounds it was two- or three- or fourfold higher than the corresponding MIC result.

3.2. Antifungal studies

The newly prepared compounds were screened for their antifungal activity against *Aspergillus flavus* (NICM No. 524), *Aspergillus fumigatus* (NICM No. 902), *Penicillium marneffei* and *Trichophyton mentagrophytes* (recultured) in DMSO by serial plate dilution method.[24,25] Sabourand's agar media were prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 ml) and adjusting pH to 5.7. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 ml saline to get a suspension of corresponding species. Twenty millilitres of agar media was poured into each petric dish. Excess suspension was decanted and the plates were dried by placing in an incubator at 37 °C for 1 h. Using an agar punch wells were made into each well labelled. A control was also prepared in triplicate and maintained at 37 °C for 3–4 days. The fungal activity of each compound was compared with that of Flucanozole as standard drug. The inhibition zones were measured and compared with the controls. The fungal zone of inhibition values are given in Table 5. The Nutrient Broth, which contained logarithmic serially twofold diluted amount of test compound and controls, was inoculated with approximately 1.6×10^4–6×10^4 c.f.u/ml was used. The cultures were incubated for 48 h at 35 °C and the growth was monitored. The lowest concentration (highest dilution) required to arrest the growth of fungus was regarded

Table 4. MIC and MBC results of Schiff and Mannich bases

Compound	*Staphylococcus aureus* (ATCC-25923)		*Escherichia coli* (ATCC-25922)		*Pseudomonas aeruginosa* (ATCC-27853)		*Klebsiella pneumoniae* (recultured)	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
3a	12.5	50	25	100	12.5	25	—	—
3b	12.5	100	12.5	50	12.5	100	—	—
3c	6.25	12.5	6.25	12.5	6.25	12.5	12.5	100
3d	—	—	—	—	12.5	100	—	—
3e	6.25	25	6.25	50	—	—	—	—
3f	25	100	25	100	25	100	12.5	50
3g	12.5	50	12.5	100	12.5	100	—	—
4a	25	100	12.5	100	12.5	50	—	—
4b	12.5	100	—	—	—	—	—	—
4c	6.25	25	6.25	12.5	6.25	6.25	12.5	100
4d	6.25	12.5	6.25	6.25	6.25	25	—	—
4e	6.25	12.5	6.25	25	6.25	12.5	12.5	50
4f	6.25	6.25	6.25	6.25	6.25	25	12.5	50
4g	—	—	25	100	—	—	—	—
4h	12.5	50	12.5	50	12.5	100	25	100
4i	—	—	—	—	25	100	25	50
5a	25	100	25	100	—	—	—	—
5b	12.5	25	12.5	100	12.5	100	12.5	100
5c	6.25	50	—	—	12.5	25	25	100
5d	6.25	12.5	—	—	—	—	—	—
5e	12.5	100	25	100	12.5	50	—	—
5f	—	—	—	—	—	—	—	—
Standard	6.25	12.5	12.5	25	6.25	12.5	6.25	12.5

— Indicates bacteria are resistant to the compounds >100 μg/ml.
MIC (μg/ml) = minimum inhibitory concentration, that is lowest concentration to completely inhibit bacterial growth.
MBC (μg/ml) = minimum bactericidal concentration, that is lowest concentration to completely kill bacteria.

Table 5. Zone of Inhibition of Schiff and Mannich bases

Compound	*Aspergillus fumigatus* (NICM No. 902)	*Aspergillus flavus* (NICM No. 524)	*Trichophyton mentagrophytes* (recultured)	*Penicillium marneffei* (recultured)
3a	12	11	17	15
3b	12	10	7	14
3c	19	21	18	20
3d	15	16	14	16
3e	18	14	19	21
3f	11	15	10	12
3g	12	—	15	14
4a	—	12	16	14
4b	12	15	10	8
4c	10	—	5	—
4d	11	16	—	14
4e	12	15	—	10
4f	—	—	—	—
4g	9	7	—	10
4h	5	10	—	13
4i	7	—	10	11
5a	12	8	14	10
5b	—	—	—	—
5c	18	19	17	18
5d	—	—	—	—
5e	17	21	19	21
5f	19	19	18	19
Standard	18	22	19	21

— Indicates fungus is resistant to the compounds >100 μg/ml. Zone of inhibition in mm.

as minimum inhibitory concentrations (MIC). To obtain the minimum fungicidal concentration (MFC), 0.1 ml volume was taken from each tube and spread on agar plates. The number of c.f.u was counted after 48 h of incubation at 35 °C. MFC was defined as the lowest drug concentration at which 99.9% of the inoculum was killed. The minimum inhibitory concentration and minimum fungicidal concentration are given in Table 6.

The antifungal screening data showed only moderate activity. Among the screened compounds, compounds **3c**, **5c**, **5e** and **5f** emerged as active against all the fungal strains. Compound **3e** showed good inhibition against *A. fumigatus*, *T. mentagrophytes* and *P. marneffei* at 6.25 μg/ml concentrations. The compounds **3c**, **5c**, **5e** and **5f** showed good antifungal activity almost equivalent to that of standard. The MBC of few compounds were found to be the same as MIC but in most of the compounds it was two- or three- or fourfold higher than the corresponding MIC result.

4. Conclusion

We have synthesized series of dichlorofluorophenyl bearing Schiff and Mannich bases. Among the synthesized compounds, Schiff bases with *p*-methoxyphenyl substituents showed good antibacterial and antifungal activity. In case of Mannich Bases, compounds containing *N*-methyl piperazinyl and piperidinyl moiety showed good antibacterial activity against all strains comparable to the standard. Mannich bases with 4-fluorophenyl and 3-chloro-4-fluorophenyl substituents showed good antifungal activity. Hence, it is concluded that, there is ample scope for further study.

5. Experimental

Melting points were determined by an open capillary method and are uncorrected. The IR spectra (in KBr pellets) were recorded on a Shimadzu FT-IR 157 spectrophotometer. ^{1}H NMR spectra were recorded using DMSO-d_6 as solvent and TMS as an internal standard either on a Bruker or 300 MHz or 400 MHz NMR spectrometer. Chemical shift values are given in δ scale. The mass spectra were recorded on a MASPEC low resolution mass spectrometer operating at 70 eV. The purity of the compounds was checked by thin-layer chromatography (TLC) on silica gel plate using petroleum ether and ethyl acetate.

5.1. Procedure for the preparation of 4-amino-3-(2,4-dichloro-5-fluorophenyl)-5-mercapto-1,2,4-triazole (1)

4-Amino-3-(2,4-dichloro-5-fluorophenyl)-5-mercapto-1,2,4-triazole (**1**) was synthesized by literature method.[21] The solid product obtained was recrystallized from ethanol. Yield 92%; mp 214–218 °C; IR (KBr) nu/cm^{-1}: 3242 and 3153 (NH_2 asymmetric and symmetric), 3097 (Ar–H), 1631 (C=N), 1456 (C=C), 1095 (C–F), 881/729 (C–Cl).

^{1}H NMR (δ, $CDCl_3$): 4.74 (s, 1H, NH_2), 7.33 (d, 1H, C_6H, $J_{H-F\ ortho}$ = 8.3 Hz), 7.92 (d, 1H, C_3H, $J_{H-F\ meta}$ = 6.5 Hz), 10.47 (s, 1H, NH/SH); Mass (%): M^+ 278 (52), M+2 (36), M+4 (12).

5.2. General procedure for the preparation of Schiff base (3a–g)

To a suspension of substituted benzaldehyde (**2**) (0.01 mol) in ethanol (15 ml), an equimolar amount of

Table 6. MIC and MFC results of Schiff and Mannich bases

Compound	*Aspergillus fumigatus* (NICM No. 902)		*Aspergillus flavus* (NICM No. 524)		*Trichophyton mentagrophytes* (recultured)		*Penicillium marneffei* (recultured)	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
3a	12.5	100	25	100	12.5	50	12.5	50
3b	12.5	25	25	50	25	100	12.5	100
3c	6.25	12.5	6.25	6.25	6.25	12.5	6.25	12.5
3d	6.25	25	12.5	100	12.5	50	6.25	25
3e	6.25	50	12.5	100	6.25	25	6.25	12.5
3f	25	50	12.5	25	25	50	12.5	50
3g	6.25	25	—	—	6.25	25	12.5	50
4a	—	—	12.5	100	12.5	50	12.5	100
4b	12.5	100	12.5	50	25	50	25	50
4c	12.5	100	—	—	25	100	—	—
4d	25	50	12.5	100	—	—	12.5	100
4e	12.5	100	12.5	50	—	—	25	100
4f	—	—	—	—	—	—	—	—
4g	25	100	25	50	—	—	12.5	50
4h	12.5	25	12.5	25	—	—	12.5	100
4i	25	100	—	—	25	50	12.5	100
5a	12.5	100	25	50	12.5	25	25	50
5b	—	—	—	—	—	—	—	—
5c	6.25	6.25	6.25	12.5	6.25	12.5	6.25	12.5
5d	—	—	—	—	—	—	—	—
5e	6.25	50	6.25	12.5	6.25	25	6.25	50
5f	6.25	6.25	6.25	12.5	6.25	12.5	6.25	6.25
Standard	6.25	12.5	6.25	12.5	6.25	12.5	6.25	12.5

— Indicates bacteria are resistant to the compounds >100 μg/ml.
MIC (μg/ml) = minimum inhibitory concentration, that is lowest concentration to completely inhibit fungal growth.
MFC (μg/ml) = minimum fungicidal concentration, that is lowest concentration to completely kill fungus.

triazole (**1**) was added. The suspension was heated until clear solution was obtained. Then few drops of concentrated sulfuric acid were added as catalyst. The solution was refluxed for 3–4 h on a water bath and the precipitated solid was filtered off, washed with water and recrystallized from ethanol–DMF.

Compound **3a**
IR (KBr) ν/cm^{-1}: 3310 (NH), 3099 (Ar–H), 2922 (C–H), 1670 (C=N), 1571 (C=N), 1471 (C=C), 1080 (C–F), 798 and 758 (C–Cl). ^{1}H NMR (δ, $CDCl_3$): 3.5 (s, 6H, *N*,*N*-dimethylaminophenyl protons, J = 8.7 Hz), 7.40 (d, 1H, dichlorofluorophenyl proton, $J_{H-F\ ortho}$ = 8.5 Hz), 7.55 (d, 1H, dichlorofluorophenyl proton, $J_{H-F\ meta}$ = 6.7 Hz), 7.60 (d, 2H, *N*,*N*-dimethylaminophenyl protons, J = 8.7 Hz), 9.72 (s, H, N=CH), 10.50 (s, 1H, NH/SH).
Compound **3b**
IR (KBr) ν/cm^{-1}: 3345 (NH), 3085 (Ar–H), 1608 (C=N), 1576 (C=N), 1490 (C=C), 1101 (C–F), 819 and 735 (C–Cl). ^{1} H NMR (δ, $CDCl_3$): 7.59 (d, 2H, *p*-chlorophenyl protons, J = 8.5 Hz), 7.78 (d, 2H, *p*-chlorophenyl protons, J = 8.5 Hz), 7.93 (d, 1H, dichlorofluorophenyl proton, $J_{H-F\ ortho}$ = 9.2 Hz), 8.09 (d, 1H, dichlorofluorophenyl proton, $J_{H-F\ meta}$ = 6.7 Hz), 10.03 (s, H, N=CH), 14.49 (s, 1H, NH/SH).
Compound **3f**
IR (KBr) ν/cm^{-1}: 3225 (NH), 3095 (Ar–H), 2972 (C–H), 1610 (C=N), 1495 (C=C), 1105 (C–F), 827 and 745 (C–Cl). ^{1}H NMR (δ, $CDCl_3$): 6.03 (s, 2H, O–CH_2–O protons), 6.86 (d, 1H, piperonyl proton, J = 8 Hz), 7.18–7.24 (m, 2H, piperonyl protons), 7.37 (d, 1H, dichlorofluorophenyl proton, $J_{H-F\ ortho}$ = 8.4 Hz), 7.58 (d, 1H, dichlorofluorophenyl proton, $J_{H-F\ meta}$ = 6.5 Hz), 10.03 (s, 1H, N=CH), 10.60 (s, 1H, NH/SH).
Compound **3g**
IR (KBr) ν/cm^{-1}: 3245 (NH), 3095 (Ar–H), 1635 (C=N), 1486 (C=C), 1087 (C–F), 835 and 727 (C–Cl). ^{1}H NMR (δ, $CDCl_3$): 7.31 (d, 1H, dichlorophenyl proton, J = 8.4 Hz), 7.46 (d, 1H, dichlorophenyl proton, J = 8.4 Hz), 7.48 (s, 1H, dichlorophenyl proton), 7.65 (d, 1H, dichlorofluorophenyl proton, $J_{H-F\ meta}$ = 6.3 Hz), 7.79 (d, 1H, dichlorofluorophenyl proton, $J_{H-F\ ortho}$ = 8.4 Hz), 10.98 (s, 1H, N=CH), 14.21 (s, 1H, NH/SH).

5.3. General procedure for the synthesis of Mannich bases (4 and 5)

The Schiff base (**3**) (0.01 mol) was dissolved in a mixture of ethanol and DMF. Then formaldehyde (40%, 1.5 ml) and primary/secondary amine (0.01 mol) were introduced to this solution. The mixture was stirred for 2–3 h and kept overnight at room temperature. The resulting solid was collected by filtration, washed with cold ethanol and recrystallized from ethanol and DMF to yield the title compound.

Compound **4a**
IR (KBr) ν/cm^{-1}: 3099 (Ar–H), 2975 (C–H), 1640 (C=N), 1471 (C=C), 1295 (C=S), 1085 (C–F), 835 and 727 (C–Cl). ^{1}H NMR (δ, $CDCl_3$): 2.88 (t, 4H,

N–CH_2), 3.72 (t, 4H, O–CH_2), 3.05 (s, 6H, $N(CH_3)_2$, 5.23 (s, 2H, N–CH_2-N), 6.66 (d, 2H, *N*,*N*-dimethylaminophenyl protons, J = 8.8 Hz), 7.40 (d, 1H, dichlorofluorophenyl proton, $J_{H–F\ ortho}$ = 8.6 Hz), 7.54 (d, 1H, dichlorofluorophenyl proton, $J_{H–F\ meta}$ = 6.5 Hz), 7.61 (d, 2H, *N*,*N*-dimethylaminophenyl protons, J = 8.8 Hz), 9.67 (s, 1H, N=CH).

Compound **4b**

IR (KBr) ν/cm^{-1}: 3076 (Ar–H), 2998 (C–H), 1645 (C=O), 1515 (C=C), 1285 (C=S), 1110 (C–F), 813 and 731 (C–Cl). ^{1}H NMR (δ, $CDCl_3$): 2.17 (s, 3H, N–CH_3), 2.46 (t, 4H, CH_2), 2.93 (t, 4H, CH_2) 3.05 (s, 6H, $N(CH_3)_2$), 5.27 (s, 2H, N–CH_2–N), 6.66 (d, 2H, *N*,*N*-dimethylaminophenyl protons, J = 8.4 Hz), 7.39 (d, 1H, dichlorofluorophenyl proton, $J_{H–F\ ortho}$ = 8.8 Hz), 7.53 (d, 1H, dichlorofluorophenyl proton, $J_{H–F\ meta}$ = 6.4 Hz), 7.60 (d, 2H, *N*,*N*-dimethylaminophenyl protons, J = 8.4 Hz), 9.65 (s, 1H, N=CH). Mass (%): 522 (M^+), 410 (M^+-piperazinyl moiety), 147 (M^+-*N*,*N*-dimethylaminobenzonitrile cation).

Compound **4c**

IR (KBr) ν/cm^{-1}: 3102 (Ar–H), 2975 (C–H), 1657 (C=N), 1521 (C=N), 1453 (C=C), 1262 (C=S), 1097 (C–F), 815 and 721 (C–Cl), ^{1}H NMR (δ, $CDCl_3$): 1.51–1.63 (m, 6H, CH_2), 2.76 (t, 4H, N–CH_2), 3.05 (s, 6H, $N(CH_3)_2$), 5.25 (s, 2H, N–CH_2–N), 6.64 (d, 2H, *N*,*N*-dimethylaminophenyl protons, J = 8.5 Hz), 7.55 (d, 2H, *N*,*N*-dimethylaminophenyl protons, J = 8.5 Hz), 7.42 (d, 1H, dichlorofluorophenyl proton, $J_{H–F\ ortho}$ = 8.8 Hz), 7.58 (d, 1H, dichlorofluorophenyl proton, $J_{H–F\ meta}$ = 6.8 Hz), 9.69 (s, 1H, N=CH).

Compound **4d**

IR (KBr) ν/cm^{-1}: 3079 (Ar–H), 2925 (C–H), 1635 (C=N), 1558 (C=N), 1475 (C=C), 1288 (C=S), 1089 (C–F), 812 and 745 (C–Cl). ^{1}H NMR (δ, $CDCl_3$): 2.88 (t, 4H, N–CH_2), 3.72 (t, 4H, O–CH_2), 5.23 (s, 2H, N–CH_2–N), 7.36–7.42 (m, 3H, *p*-chlorophenyl and dichlorofluorophenyl protons), 7.59 (d, 1H, dichlorofluorophenyl proton, $J_{H–F\ meta}$ = 6.5 Hz), 7.65 (d, 2H, *p*-chlorophenyl protons, J = 8.4 Hz), 10.44 (s, 1H, N=CH). Mass (%): M^+ 498, 400 (25), 361 (15), 262 (26), 137 (10), 98 (100).

Compound **4e**

IR (KBr) ν/cm^{-1}: 3106 (Ar–H), 2972 (C–H), 1625 (C=N), 1578 (C=N), 1464 (C=C), 1269 (C=S), 1112 (C–F), 825 and 732 (C–Cl); ^{1}H NMR (δ, $CDCl_3$): 2.29 (s, 3H, N–CH_3), 2.45 (t, 4H, CH_2), 2.95 (t, 4H, CH_2), 5.27 (s, 2H, N–CH_2–N), 7.41 (d, 2H, *p*-chloro-phenyl protons, J = 8.4 Hz), 7.48 (d, 1H, dichlorofluoro-phenyl proton $J_{H–F\ ortho}$ = 8.8 Hz), 7.57 (d, 1H, dichlorofluorophenyl proton, $J_{H–F\ meta}$ = 6.5 Hz), 7.65 (d, 2H, *p*-chlorophenyl protons, J = 8.4 Hz), 10.48 (s, 1H, N=CH).

Compound **4f**

IR (KBr) ν/cm^{-1}: 3085 (Ar–H), 2985 (C–H), 1658 (C=N), 1568 (C=N), 1435 (C=C), 1272 (C=S), 1092 (C–F), 813 and 735 (C–Cl). ^{1}H NMR (δ, $CDCl_3$): 1.55-1.61 (m, 6H, CH_2), 2.82 (t, 4H, N–CH_2), 5.23 (s, 2H, N–CH_2–N), 7.36–7.42 (m, 3H, *p*-chlorophenyl and dichlorofluorophenyl protons), 7.58 (d, 1H, dichlorofluorophenyl proton, $J_{H–F\ meta}$ = 6.5 Hz), 7.64 (d, 2H, *p*-chlorophenyl protons, J = 8.5 Hz), 10.48 (s, 1H, N=CH).

Compound **4g**

IR (KBr) ν/cm^{-1}: 3106 (Ar–H), 2925 (C–H), 1656 (C=N), 1571 (C=N), 1448 (C=C), 1097 (C–F), 812 and 745 (C–Cl), ^{1}H NMR (δ, $CDCl_3$): 2.87 (t, 4H, N–CH_2), 3.72 (t, 4H, O–CH_2), 5.23 (s, 2H, N–CH_2–N), 6.06 (s, 2H, O–CH_2–O), 6.86 (d, 1H, piperonyl proton, J = 7.9 Hz), 7.19 (d, 1H, piperonyl proton J = 7.9 Hz), 7.24 (s, 1H, piperonyl proton), 7.38 (d, 1H, dichlorofluorophenyl proton, $J_{H–F\ ortho}$ = 8.4 Hz), 7.56 (d, 1H, dichlorofluorophenyl proton, $J_{H–F\ meta}$ = 6.4 Hz), 10.04 (s, 1H, N=CH).

Compound **4h**

IR (KBr) ν/cm^{-1}: 3078 (Ar–H), 2976 (C–H), 1638 (C=N), 1548 (C=N), 1452 (C=C), 1242 (C=S), 1078 (C–F), 824 and 738 (C–Cl). ^{1}H NMR (δ, $CDCl_3$): 2.28 (s, 3H, N–CH_3), 2.45 (t, 4H, NCH_2), 2.95 (t, 4H, N–CH_2), 5.26 (s, 2H, N–CH_2–N), 6.03 (s, 2H, O–CH_2–O), 6.85 (d, 1H, piperonyl protons, J = 8 Hz), 7.19 (dd, 1H, piperonyl proton), 7.23 (d, 1H, piperonyl proton, J = 1.4 Hz), 7.36 (d, 1H, dichlorofluorophenyl proton, $J_{H–F\ ortho}$ = 8.5 Hz), 7.56 (d, 1H, dichlorofluorophenyl proton, $J_{H–F\ meta}$ = 6.5 Hz), 10.09 (s, 1H, N=CH).

Compound **4i**

IR (KBr) ν/cm^{-1}: 3075 (Ar–H), 2988 (C–H), 1635 (C=N), 1543 (C=N), 1448 (C=C),1272 (C=S), 1109 (C–F), 812 and 727 (C–Cl). ^{1}H NMR (δ, $CDCl_3$): 1.57–1.63 (m, 6H, CH_2), 2.78–2.84 (m, 4H, N–CH_2), 5.23 (s, 2H, N–CH_2–N), 6.03 (s, 2H, O–CH_2–O), 6.85 (d, 1H, piperonyl proton, J = 8 Hz), 7.19 (dd, 1H, piperonyl proton), 7.24 (d, 1H, piperonyl proton, J = 1.5 Hz), 7.37 (d, 1H, dichlorofluorophenyl proton, $J_{H–F\ ortho}$ = 8.5 Hz), 7.57 (d, 1H, dichlorofluorophenyl proton, $J_{H–F\ meta}$ = 6.5 Hz), 10.16 (s, 1H, N=CH).

Compound **5a**

IR (KBr) ν/cm^{-1}: 3325 (NH), 3078 (Ar–H), 2985 (C–H), 1623 (C=N), 1468 (C=C), 1288 (C=S), 1108 (C–F), 798 and 727 (C–Cl). ^{1}H NMR (δ, $CDCl_3$): 3.04 (s, 6H, NCH_3), 5.25 (t, 1H, NH), 5.63 (d, 2H, N–CH_2–N), 6.65 (d, 2H, *N*,*N*-dimethylaminophenyl protons, J = 8.8 Hz), 6.87–7.01 (m, 4H, *p*-fluorophenyl protons), 7.30 (d, 1H, dichlorofluorophenyl proton, $J_{H–F\ ortho}$ = 8.64 Hz), 7.52 (d, 1H, dichlorofluorophenyl proton, $J_{H–F\ meta}$ = 6.5 Hz), 7.58 (d, 2H, *N*,*N*-dimethylaminophenyl protons, = 8.9 Hz), 9.63 (s, 1H, N=CH).

Compound **5b**

IR (KBr) ν/cm^{-1}: 3313 (NH), 3079 (Ar–H), 1670 (C=N), 1545 (C=N), 1445 (C=C), 1266 (C=S), 1095 (C–F), 827 and 748 (C–Cl). ^{1}H NMR (δ, $CDCl_3$): 3.04 (s, 6H, $N(CH_3)_2$), 5.37 (t, 1H, NH), 5.61 (d, 2H, N–CH_2–N), 6.65 (d, 2H, *N*,*N*-dimethylaminophenyl protons, J = 8.8 Hz), 6.76–6.82 (m, 1H, 3-chloro-4-fluorophenyl proton), 6.96–7.04 (m, 1H, 3-chloro-4-fluorophenyl proton), 7.34 (d, 1H, dichlorofluorophenyl proton, $J_{H–F\ ortho}$ = 8.5 Hz), 7.53 (d, 1H, dichlorofluorophenyl proton, $J_{H–F\ meta}$ = 6.6 Hz), 7.58 (d, 2H, *N*,*N*-dimethylaminophenyl protons, J = 8.8 Hz), 10.4 (s, 1H, N=CH).

Compound **5c**
IR (KBr) ν/cm^{-1}: 3325 (NH), 3078 (Ar–H), 1656 (C=N), 1438 (C=C), 1102 (C–F), 831 and 728 (C–Cl). ^1H NMR (δ, $CDCl_3$): 5.59 (t, 1H, NH), 6.21 (d, 2H, N–CH_2–N), 6.83–6.95 (m, 1H, *p*-fluorophenyl proton), 6.96–7.05 (m, 2H, *p*-fluorophenyl protons), 7.38–7.42 (m, 3H, *p*-chlorophenyl and dichlorofluorophenyl protons), 7.63–7.66 (m, 3H, *p*-chlorophenyl and dichlorofluorophenyl proton), 10.35 (s, 1H, N=CH).
Compound **5d**
IR (KBr) ν/cm^{-1}: 3289 (NH), 3083 (Ar–H), 1625 (C=N), 1476 (C=C), 1097 (C–F), 825 and 756 (C–Cl). ^1H NMR (δ, $CDCl_3$): 5.31 (t, 1H, NH), 5.61 (d, 2H, N–CH_2–N), 6.76–6.81 (m, 2H, *p*-fluorophenyl protons), 6.96–7.02 (m, 2H, *p*-fluorophenyl protons), 7.31 (d, 2H, *p*-chlorophenyl protons, $J = 8.5$ Hz), 7.40 (d, 1H, dichlorofluorophenyl proton, $J_{H-F\ ortho} = 8.5$ Hz), 7.58 (d, 1H, dichlorofluorophenyl proton, $J_{H-F\ meta} = 6.5$ Hz), 7.63 (d, 2H, *p*-chlorophenyl protons, $J = 8.5$ Hz), 10.4 (s, 1H, N=CH). Mass (%): M^+ 557, 401 (75), 190 (12), 158 (38), 137 (47).
Compound **5e**
IR (KBr) ν/cm^{-1}: 3275 (NH), 3078 (Ar–H), 1635 (C=N), 1458 (C=C), 1108 (C–F), 827 and 748 (C–Cl). ^1H NMR (δ, $CDCl_3$): 5.28 (t, 1H, NH), 5.64 (d, 2H, N–CH_2–N), 6.02 (s, 2H, O–CH_2–O), 6.91–7.06 (m, 4H, *p*-fluorophenyl protons), 7.16–7.21 (m, 3H, piperonyl protons), 7.27 (d, 1H, dichlorofluorophenyl proton, $J_{H-F\ ortho} = 8.5$ Hz), 7.55 (d, 1H, dichlorofluoro phenyl proton, $J_{H-F\ ortho} = 6.5$ Hz), 10.06 (s, 1H, N=CH). Mass (%): 489 (50), 411 (M-124, base peak), 147 (piperonyl nitrile cation, 55), 124 (*p*-fluoro anilinomethyl cation, 40), 190 (dichlorofluorophenyl nitrile cation, 10)
Compound **5f**
IR (KBr) ν/cm^{-1}: 3246 (NH), 3077 (Ar–H), 1666 (C=N), 1458 (C=C), 1089 (C–F), 812 and 731 (C–Cl). ^1H NMR (δ, $CDCl_3$): 5.61 (t, 1H, NH), 6.03 (d, 2H, N–CH_2–N), 6.18 (s, 2H, O–CH_2–O), 6.76–6.81 (m, 2H, piperonyl protons), 6.84–6.88 (t, 1H, chlorofluorophenyl proton), 6.96–7.02 (m, 1H, chlorofluorophenyl proton), 7.16–7.21 (dd, 1H, piperonyl proton), 7.29–7.33 (dd, 1H, chlorofluorophenyl proton), 7.55 (d, 1H, dichlorofluorophenyl proton, $J_{H-F\ ortho} = 8.3$ Hz), 7.62 (d, 1H, dichlorofluorophenyl proton, $J_{H-F\ meta} = 6.2$ Hz), 10.06 (s, 1H, N=CH).

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References and notes

1. Tarafder, M. T.; Kasbollah, A.; Saravan, N.; Crouse, K. A.; Ali, A. M.; Tin, O. K. *J. Biochem., Mol. Biol. Biophys.* **2002**, *6*, 85.
2. Kucukguzel, I.; Kucukguzel, S. G.; Rollas, S.; Sanis, G. O.; Ozdemir, O.; Bayrak, I.; Altug, T.; Stables, J. *Il Farmaco.* **2004**, *59*, 839.
3. Vicini, P.; Geronikaki, A.; Incerti, M.; Busonera, B.; Poni, G.; Kabras, C. A.; Colla, P. L. *Bioorg. Med. Chem.* **2003**, *11*, 4785.
4. Pignatello, R.; Panico, A.; Mazzane, P.; Pinizzotto, M. R.; Garozzo, A.; Fumeri, P. M. *Eur. J. Med. Chem.* **1974**, *29*, 781.
5. Joshi, S.; Khosla, N.; Tiwari, P. *Bioorg. Med. Chem.* **2004**, *12*, 571.
6. Lopes, F.; Capela, R.; Goncaves, J. O.; Horton, P. N.; Hursthouse, M. B.; Iley, J.; Casimiro, C. M.; Bom, J.; Moreira, R. *Tetrahedron Lett.* **2004**, *45*, 7663.
7. Ferlin, M. G.; Chiarelotto, G.; Antonucci, F.; Caparrotta, L.; Froldi, G. *Eur. J. Med. Chem.* **2002**, *37*, 427.
8. Holla, B. S.; Veerandra, B.; Shivanada, M. K.; Boja Poojary *Eur. J. Med. Chem.* **2003**, *38*, 759.
9. Malinka, W.; Swiatek, P.; Filipek, B.; Sapa, J.; Jezierska, A.; Koll, A. *Il Farmaco* **2005**, *60*, 961.
10. Tramontini; Agioline, L.; Ghedeni, N. *Polymer* **1988**, *29*, 771.
11. Seriabine et al. *Experientia* **1968**, *24*, 1150; Cohen *J. Clin. Pharmacol., J. New. Drugs* **1970**, *10*, 408.
12. Schaper et al. *J. Pharmacol. Exp. Ther.* **1966**, *152*, 265.
13. Schoentensack, V. W.; Bischler, P.; Dittmann, E.; Steinijans, Ch. *Arzneium Forsch* **1977**, *27*, 1908.
14. Dimmock, J. R.; Chamankkah, M.; Allen, T. M.; Halleran, S. *Pharmazie* **1995**, *50*, 221; Dimmock, J. R.; Kumar, P. *Curr. Med. Chem.* **1997**, *4*, 1.
15. Filler, R.; Kobayashi, Y. In *Biomedical Aspects of Fluorine Chemistry*; Elsevier: Amsterdam, 1982.
16. Holla, B. S.; Sooryanarayana Rao, B.; Sarojini, B. K.; Akberali, P. M.; Suchetha Kumari, N. *Eur. J. Med. Chem.* **2006**, *41*, 657.
17. Walczak, K.; Gondela, A.; Suwiński, J. *Eur. J. Med. Chem.* **2004**, *39*, 849.
18. Amir, M.; Shikha, K. *Eur. J. Med. Chem.* **2004**, *39*, 535.
19. Strunecka, A.; Patocka, J.; Connett, P. *J. Appl. Biomed.* **2004**, *2*, 141.
20. Kevin Park, B.; Kitteringham, N. R.; O'Neill, P. M. *Ann. Rev. Pharmacol. Toxicol.* **2001**, *1*, 443.
21. Reid, K. R.; Heindel, N. D. *J. Heterocycl. Chem.* **1976**, *13*, 925; Subrahmanya Bhat, K., Ph.D. thesis, unpublished work.
22. Cruickshank, R.; Duguid, J. P.; Marion, B. P.; Swain, R. H. A. *Medicinal Microlbiology*, 12th ed.; Churchil Livingstone: London, 1975; Vol. 2, pp. 196–202.
23. Collins, A. H. *Microbiological Methods*, 2nd ed.; Butterworth: London, 1976.
24. Khan, Z. K.; In Vitro and Vivo Screening techniques for bioactivity screening and evaluation. Proceeding Int. workshop UNIDO-CDRI (1997), 210–211.
25. Varma, R. S.; (Eds.). Antifungal Agents: Past, Present & Future prospects, (National Academy of Chemistry & Biology, India, Lucknow-1998).