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Biological Studies of Some 2,4-Dichloro-5-fluorophenyl Containing Triazolothiadiazoles

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Summary. A series of dichlorofluorophenyl containing triazolothiadiazoles were obtained by cyclocondensation of triazole with substituted benzoic, aryloxyacetic, and anilinoacetic acids using $POCl_3$ as cyclizing agent. The structures of the newly synthesized compounds were characterized and confirmed by IR, ^1H NMR, mass spectra, and elemental analysis. Selected compounds were screened for their antitubercular activity against *Mycobacterium tuberculosis*. The compound bearing 2,4-dichloro-5-fluorophenyl moiety at position 3, and 6 of the triazolothiadiazole showed excellent activity *in vitro* primary screening. Compounds with 3-chloro-4-fluorophenyl and 4-fluorophenoxymethyl moieties at position 6 of the triazolothiadiazole showed very good analgesic activity. Triazolothiadiazole with 4-chlorophenyl, 4-fluoro-3-phenoxyphenyl, and 2,4-dichloro-5-fluorophenyl moieties showed excellent antimicrobial activity against the tested strains at 6.25 $\mu g\,cm^{-3}$ concentrations.

Keywords. $POCl_3$ cyclization; Triazolothiadiazoles; Antitubercular; Analgesic; Antimicrobial.

Introduction

1,2,4-Triazoles represent an overwhelming and rapid developing field in modern heterocyclic chemistry. A degree of respectability has been bestowed for 1,2,4-triazole derivatives due to their antibacterial, antifungal [1], antitubercular [2], anticancer [3], antitumor [4], anticonvulsant [5], antiinflammatory, and analgesic properties [6]. Certain 1,2,4-triazoles also find applications in the preparation of photographic plates, polymers, and as analytical agents [7].

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The earliest use of thiadiazoles has been in the field of pharmaceuticals as antibacterials with similar properties to those of sulfonamide drugs [8]. 1,3,4-Thiadiazoles were found to possess antitumor [9], anti-inflammatory [6], antibacterial, antifungal, anticonvulsant [10], and antitubercular properties [11]. Incorporation of the toxophoric >N–C=S linkage in thiadiazoles has been found to be responsible for the broad spectrum of biological activities [12].

The triazolothiadiazole systems may be viewed as a cyclic analogue of two very important components, thiosemicarbazide and biguanide, which often display diverse pharmacological properties. The triazolothiadiazoles have been obtained by fusing the biolabile 1,2,4-triazole and 1,3,4-thiadiazole rings together [13]. Fused heterocycles such as triazolothiadiazoles have been reported to possess analgesic, antibacterial, antifungal, antiinflammatory, antitubercular, antiviral, and anthelmintic properties [13].

The importance of fluorine containing compounds in general, and heterocyclic in particular, has initiated active research on fluorine containing heterocycles. The replacement of hydrogen or hydroxyl by a fluorine atom can alter the pK_a, dipole moments, and even the chemical reactivity and stability of neighbouring functional groups [14].

From literature, it is predictable that 1,2,4-triazoles represent important pharmacophores, and have a wide range of therapeutic properties. They play vital role in medicinal agents due to different biolog-

ical activities and lot of work might be carried out on this moiety for obtaining better therapeutic molecules. At present several triazole bearing compounds, like Flutrox, Nefazodone, Trazodone, Triazoledione, *etc.*, are used in modern medicine. Fluorine incorporated heterocycles, triazoles and thiadiazoles displayed varied pharmacological properties. Since there have been few reports on dichlorofluorophenyl containing triazolothiadiazoles, it was contemplated to synthesize them and to pursue antitubercular, analgesic, and antimicrobial screenings. The corresponding results are presented in this paper.

Results and Discussion

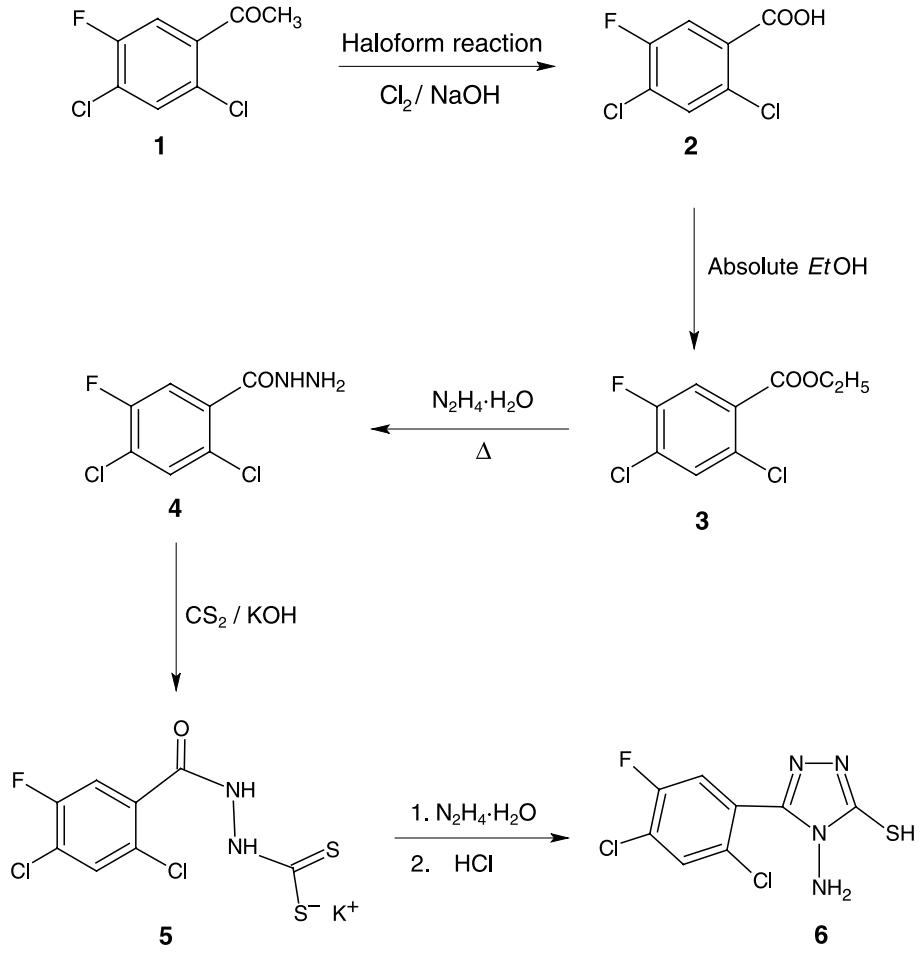
Chemistry

4-Amino-3-(2,4-dichloro-5-fluorophenyl)-5-mercapto-1,2,4-triazole (**6**) was obtained from 2,4-dichloro-5-fluorobenzoic acid according to Ref. [15]. Triazole **6** was treated with substituted benzoic, aryloxyacetic, and anilinoacetic acids using $POCl_3$ as cyclizing agent to yield 6-substituted triazolothiadiazoles **7**, **8**, and **9** in good yields. Aryloxyacetic and anilinoacetic acids used in the reaction were prepared according to Refs. [16, 17]. The reaction sequences are outlined in Schemes 1 and 2.

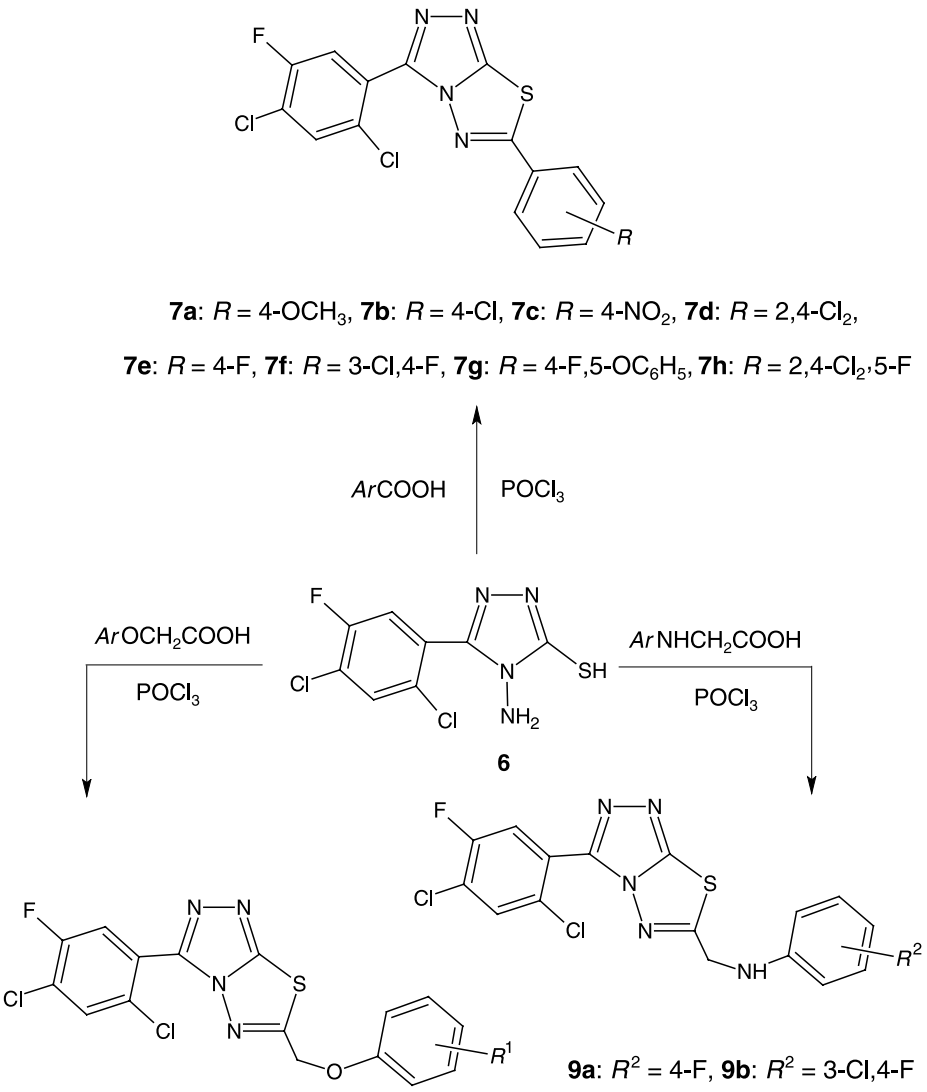
Pharmacological Studies

Antitubercular Studies

Tuberculosis Activity Antimicrobial Acquisition and Coordinating Facility (TAACF) of Southern Research evaluated some of the selected compounds for *in vitro* antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA) [18]. The antitubercular screening results are given



Scheme 1



Scheme 2

in Table 1. The antitubercular screening results revealed that, compound **7h** showed an excellent inhibition of 97%, and compound **7b** displayed a moderate inhibition of 72%.

Analgesic Studies

Selected compounds were screened for analgesic activity by hot plate test according to *Eddy* and *Leimbach* [19]. The analgesic screening results are given in Table 2. The analgesic screening results revealed that compounds **7f** and **8e** showed excellent analgesic activity whereas compounds **7b**, **7g**, **7h**, **8b**, and **8d** showed moderate to good analgesic activity compared with Pethidine.

Antibacterial Studies

The newly prepared compounds were screened for their antibacterial activity against *Escherichia coli* (ATCC-25922), *Staphylococcus aureus* (ATCC-25923), *Pseudomonas aeruginosa* (ATCC-27853), *Streptococcus pyogenes*, and *Klebsiella pneumoniae* (recultured) bacterial strains by the disc diffusion

Table 1. Antitubercular activity data of triazolothiadiazoles (Alamar assay)

Compd. no.	Sample ID	$MIC/\mu g\,cm^{-3}$	Inhibition/%
7a	300008	>6.25	49
7b	300009	>6.25	72
7c	300010	>6.25	29
7d	300011	>6.25	28
7e	300012	>6.25	33
7f	300013	>6.25	0
7g	300014	>6.25	29
7h	300015	<6.25	97
8a	300016	>6.25	27
8c	300017	>6.25	36
8d	300018	>6.25	43
8e	300019	>6.25	34

method [20, 21]. The diameter of the zone of inhibition and minimum inhibitory concentration values are given in Table 3. The antibacterial screening data revealed that the compounds **7b**, **7e**, **7g**, **7h**, and **8e** were active against *S. aureus* and *E. coli*. Especially compounds **7b**, **7g**, **7h**, and **8e** exhibited good antibacterial activity against all tested bacterial strains almost equivalent to that of the standard drug Ciprofloxacin.

Antifungal Studies

The newly prepared compounds were screened for their antifungal activity against *Aspergillus niger*, *Candida albicans*, *Aspergillus fumigatus*, *Penicillium marneffei*, and *Trichophyton mentagrophytes* (recultured) in *DMSO* by agar diffusion method [22, 23]. The diameter of zone of inhibition and minimum inhibitory concentration values are given in Table 4. The antifungal screening data showed that compounds **7b**, **7g**, **7h**, **8b**, and **8e** showed good activity against *C. albicans* and *A. fumigatus* at 6.25 $\mu g\,cm^{-3}$ concentrations. Compounds **7b**, **7g**, and **7h** exhibited

Table 2. Analgesic activity data of triazolothiadiazoles

Compd. no.	Dose/mg kg^{-1}	Time of reaction to pain stimulus at time/h [s] ± SEM		
		0	1	3
7a	50	8.1	8.2	8.4
7b	50	8.1	10.4	11.2
7e	50	7.9	8.1	8.2
7f	50	8.2	14.2	13.9
7g	50	7.9	10.2	10.8
7h	50	8.24	13.4	12.8
8a	50	8.4	8.3	8.2
8b	50	8.12	12.4	11.9
8d	50	8.2	13.2	12.9
8e	50	8.3	14.1	12.8
Control	10	8.25	8.2	8.8
Standard*	5	8.5	16.4	14.3

* Pethidine is used as the standard

Table 3. Antibacterial activity data of triazolothiadiazoles **7**, **8**, and **9**

Compd. no.	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Streptococcus pyogenes*
7a	–	10 (12.5)	–	–	15 (6.25)
7b	22 (6.25)	19 (6.25)	23 (6.25)	21 (6.25)	19 (6.25)
7c	–	15 (6.25)	–	9 (25)	12 (12.5)
7d	–	8 (25)	–	–	10 (12.5)
7e	19 (6.25)	18 (6.25)	15 (6.25)	10 (12.5)	16 (6.25)
7f	11 (12.5)	10 (12.5)	12 (12.5)	7 (25)	11 (12.5)
7g	20 (6.25)	20 (6.25)	22 (6.25)	23 (6.25)	19 (6.25)
7h	18 (6.25)	18 (6.25)	24 (6.25)	20 (6.25)	17 (6.25)
8a	15 (6.25)	12 (12.5)	–	17 (6.25)	12 (12.5)
8b	–	–	12 (12.5)	–	14 (12.5)
8c	9 (25)	–	–	–	10 (12.5)
8d	–	9 (25)	10 (12.5)	8 (25)	–
8e	21 (6.25)	20 (6.25)	23 (6.25)	19 (6.25)	18 (6.25)
9a	17 (6.25)	10 (12.5)	18 (6.25)	–	–
9b	–	12 (12.5)	–	12 (12.5)	9 (25)
Standard*	22 (6.25)	20 (6.25)	25 (6.25)	23 (6.25)	20 (6.25)

* Ciprofloxacin is used as the standard, – indicates bacteria is resistant to the compounds >100 $\mu g\,cm^{-3}$

Diameter zones of inhibitions in mm. *MIC* values are given in brackets

MIC ($\mu g\,cm^{-3}$) Minimum inhibitory concentration, *i.e.*, lowest concentration to completely inhibit bacterial growth

Table 4. Antifungal activity data of triazolothiadiazoles **7**, **8**, and **9**

Compd. no.	*Candida albicans*	*Aspergillus niger*	*Aspergillus fumigatus*	*Trichophyton mentagrophytes*	*Penicillium marneffei*
7a	–	11 (12.5)	–	–	12 (12.5)
7b	29 (6.25)	27 (6.25)	28 (6.25)	23 (6.25)	19 (6.25)
7c	–	–	20 (6.25)	–	–
7d	10 (25)	–	12 (25)	15 (6.25)	16 (6.25)
7e	–	16 (6.25)	–	12 (12.5)	–
7f	15 (12.5)	10 (12.5)	–	8 (25)	12 (12.5)
7g	30 (6.25)	28 (6.25)	26 (6.25)	22 (6.25)	19 (6.25)
7h	28 (6.25)	29 (6.25)	27 (6.25)	25 (6.25)	18 (6.25)
8a	17 (6.25)	12 (12.5)	8 (25)	12 (12.5)	15 (6.25)
8b	25 (6.25)	11 (12.5)	–	18 (6.25)	9 (25)
8c	–	–	22 (6.25)	9 (25)	15 (6.25)
8d	–	–	15 (6.25)	–	–
8e	21 (6.25)	–	28 (6.25)	11 (12.5)	12 (12.5)
9a	12 (12.5)	25 (6.25)	11 (12.5)	20 (6.25)	17 (6.25)
9b	27 (6.25)	15 (6.25)	17 (6.25)	10 (12.5)	18 (6.25)
Standard*	30 (6.25)	30 (6.25)	28 (6.25)	25 (6.25)	19 (6.25)

* Griseofulvin is used as the standard, – indicates fungus is resistant to the compounds $>100\,\mu g\,cm^{-3}$
Diameter zones of inhibitions in mm. *MIC* values are given in brackets
MIC ($\mu g\,cm^{-3}$) Minimum inhibitory concentration, *i.e.*, lowest concentration to completely inhibit fungal growth

good antifungal activity against all tested fungal strains almost equivalent to that of the standard drug Griseofulvin.

Conclusion

The antitubercular screening study revealed that compounds with 2,4-dichloro-5-fluorophenyl and 4-chlorophenyl at position 6 of the triazolothiadiazole moiety showed excellent and moderate antitubercular activity. The analgesic activity study revealed that compounds with 3-chloro-4-fluorophenyl and 4-fluorophenoxymethyl moieties at position 6 of the triazolothiadiazole showed excellent analgesic activity. The antimicrobial screening studies revealed that compounds with 4-chlorophenyl, 4-fluoro-3-phenoxyphenyl, and 2,4-dichloro-5-fluorophenyl moieties showed excellent activity. These inferences are based on preliminary tests, further studies in these classes of compounds are in progress.

Experimental Protocols

Melting points were determined by open capillary method. The IR spectra (in KBr pellets) were recorded on a Shimadzu FT-IR 157 spectrophotometer. ^{1}H NMR spectra were recorded (in $CDCl_3$/*DMSO*-d_6) on a Bruker 400 MHz NMR spectrometer using *TMS* as an internal standard. The mass spectra were recorded on a MASPEC/FAB mass spectrometer operating at 70 eV. The purity of the compounds was checked by thin layer chromatography (TLC) on silica gel plates using a mixture of petroleum ether and ethyl acetate. Iodine was used as visualizing agent. Elemental analyses were found to agree favorably with calculated values.

4-Amino-3-(2,4-dichloro-5-fluorophenyl)-5-mercapto-1,2,4-triazole (**6**) was synthesized by literature method [15]. Aryloxyacetic acids were prepared according to Ref. [16]. Anilinoacetic acids were prepared according to Ref. [17].

*General Procedure for the Synthesis of 6-Substituted-3-(2,4-dichloro-5-fluorophenyl)-1,2,4-triazolo[3,4-b]-thiadiazoles **7**, **8**, and **9***

A mixture of 0.01 mol **6**, 0.01 mol substituted aromatic, aryloxy-, or anilinoacetic acid, and 10 cm^3 $POCl_3$ was refluxed on a water bath for about 9 h. Excess of $POCl_3$ was removed under reduced pressure. The reaction mixture was cooled, poured onto crushed ice, and neutralized with aqueous ammonia. The resulting solid product was filtered off, washed with water, dried, and recrystallized from a mixture of ethanol and dimethylformamide.

3-(2,4-Dichloro-5-fluorophenyl)-6-(4-methoxyphenyl)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole (**7a**, $C_{16}H_9Cl_2FN_4OS$)
Yield 82%, mp 144–146°C (*Et*OH:*DMF* = 2:1); IR (KBr): $\bar{\nu} = 3089$ (Ar–H), 2922 (C–H), 1604 (C=N), 1105 (C–F), 831, 729 (C–Cl) cm^{-1}; ^{1}H NMR (400 MHz, *DMSO*-d_6): $\delta = 3.86$ (s, CH_3), 6.94 (d, 2H-*p*-anisyl, $J = 8.4$ Hz), 7.42 (d, 1H-dichlorofluorophenyl, $J_{\text{H-Fortho}} = 8.8$ Hz), 7.60 (d, 1H-dichlorofluorophenyl, $J_{\text{H-Fmeta}} = 6.4$ Hz), 7.68 (d, 2H-*p*-anisyl, $J = 8.4$ Hz) ppm; FABMS: m/z (%) = 394 (M^+, 90), 396 (M^+, 100).

3-(2,4-Dichloro-5-fluorophenyl)-6-(4-chlorophenyl)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole (**7b**, $C_{15}H_6Cl_3FN_4S$)
Yield 70%, mp 208–300°C (*Et*OH:*DMF* = 2:1); IR (KBr): $\bar{\nu} = 3090$ (Ar–H), 1600 (C=N), 1089 (C–F), 854, 740 (C–Cl) cm^{-1}; ^1H NMR (400 MHz, *DMSO*-d_6): $\delta = 7.55$ (d, 2H-*p*-chlorophenyl, $J = 8.6$ Hz), 7.71 (d, 1H-dichlorofluorophenyl, $J_{H\text{-}Fmeta} = 6.5$ Hz), 7.86 (d, 1H-dichlorofluorophenyl, $J_{H\text{-}Fortho} = 8.6$ Hz), 8.10 (d, 2H-*p*-chlorophenyl, $J = 8.6$ Hz) ppm; FABMS: m/z (%) = 398 (M^+, 10), 400 ($M^+ + 2$, 7).

3-(2,4-Dichloro-5-fluorophenyl)-6-(4-nitrophenyl)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole (**7c**, $C_{15}H_6Cl_3FN_5O_2S$)
Yield 87%, mp 228–230°C (*Et*OH:*DMF* = 2:1); IR (KBr): $\bar{\nu} = 3097$ (Ar–H), 2950 (C–H), 1602 (C=N), 1529 (NO_2 asymmetric), 1348 (NO_2 symmetric), 1105 (C–F), 854, 740 (C–Cl) cm^{-1}; ^1H NMR (400 MHz, *DMSO*-d_6): $\delta =$ 8.12 (d, 1H-dichlorofluorophenyl, $J_{H\text{-}Fortho} = 9.4$ Hz), 8.24 (d, 1H-dichlorofluorophenyl, $J_{H\text{-}Fmeta} = 6.7$ Hz), 8.33 (d, 2H-*p*-nitrophenyl, $J = 8.8$ Hz), 8.45 (d, 2H-*p*-nitrophenyl, $J = 8.8$ Hz) ppm; FABMS: m/z (%) = 409 (M^+, 5), 410 ($M^+ + 1$, 100).

3-(2,4-Dichloro-5-fluorophenyl)-6-(4-fluorophenyl)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole (**7e**, $C_{15}H_6Cl_2F_2N_4S$)
Yield 82%, mp 253–255°C (*Et*OH:*DMF* = 2:1); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 6.77$ (m, 2H-*p*-fluorophenyl), 6.94 (d, 1H-dichlorofluorophenyl, $J_{H\text{-}Fmeta} = 6.8$ Hz), 7.28 (d, 1H-dichlorofluorophenyl, $J_{H\text{-}Fortho} = 8.6$ Hz), 7.65 (m, 2H-*p*-fluorophenyl) ppm.

6-(3-Chloro-4-fluorophenyl)-3-(2,4-dichloro-5-fluorophenyl)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole (**7f**, $C_{15}H_5Cl_3F_2N_4S$)
Yield 72%, mp 224–226°C (*Et*OH:*DMF* = 2:1); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.33$ (m, 1H-chlorofluorophenyl), 7.67 (m, 2H-dichlorofluorophenyl), 7.78 (m, 1H-chlorofluorophenyl), 7.99 (m, 1H-chlorofluorophenyl) ppm.

3-(2,4-Dichloro-5-fluorophenyl)-6-(4-fluoro-3-phenoxyphenyl)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole (**7g**, $C_{21}H_{10}Cl_2F_2N_4OS$)
Yield 68%, mp 197–199°C (*Et*OH:*DMF* = 2:1); IR (KBr): $\bar{\nu} = 3083$ (Ar–H), 1591 (C=N), 1107 (C–F), 813, 740 (C–Cl) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.03$ (d, 2H-phenoxy, $J = 8$ Hz), 7.18 (m, 1H-fluorophenoxyphenyl), 7.36 (m, 3H-fluorophenoxyphenyl, and phenoxy), 7.61 (m, 4H-dichlorofluorophenyl, and fluorophenoxyphenyl) ppm.

3,6-Bis(2,4-dichloro-5-fluorophenyl)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole (**7h**, $C_{15}H_5Cl_4F_2N_4S$)
Yield 70%, mp 184–186°C (*Et*OH:*DMF* = 2:1); IR (KBr): $\bar{\nu} = 3078$ (Ar–H), 1600 (C=N), 1101 (C–F), 883, 736 (C–Cl) cm^{-1}; ^1H NMR (400 MHz, *DMSO*-d_6): $\delta = 8.05$ (d, 1H-dichlorofluorophenyl, $J_{H\text{-}Fortho} = 9.6$ Hz), 8.10 (d, 1H-dichlorofluorophenyl, $J_{H\text{-}Fortho} = 9.6$ Hz), 8.16 (d, 1H-dichlorofluorophenyl, $J_{H\text{-}Fmeta} = 6.4$ Hz), 8.19 (d, 1H-dichlorofluorophenyl, $J_{H\text{-}Fmeta} = 6.8$ Hz); FABMS: m/z (%) = 451 (M^+, 85), 453 ($M^+ + 1$, 100), 191 (dichlorofluorobenzonitrile, 45).

6-[(4-Chlorophenoxy)methyl]-3-(2,4-dichloro-5-fluorophenyl)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole (**8a**, $C_{16}H_8Cl_3FN_4OS$)
Yield 75%, mp 142–144°C (*Et*OH:*DMF* = 2:1); IR (KBr): $\bar{\nu} = 3091$ (Ar–H), 2922 (C–H), 1595 (C=N), 1097 (C–F), 823, 732 (C–Cl) cm^{-1}; ^1H NMR (400 MHz, *DMSO*-d_6): $\delta = 5.31$ (s, OCH_2), 7.73 (d, 2H-*p*-chlorophenyl, $J = 7.3$ Hz), 8.03 (m, 2H-dichlorofluorophenyl), 8.17 (d, 2H-*p*-chlorophenyl, $J = 7.3$ Hz) ppm; FABMS: m/z (%) = 301 (M^+, 127), 189 (dichlorofluorobenzonitrile cation, 42), 167 (*p*-chlorophenoxymethyl nitrile cation 12), 127 (*p*-chlorophenoxy cation, 33).

3-(2,4-Dichloro-5-fluorophenyl)-6-[(4-methylphenoxy)methyl]-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole (**8b**, $C_{17}H_{11}Cl_2FN_4OS$)
Yield 80%, mp 187–188°C (*Et*OH:*DMF* = 2:1); IR (KBr): $\bar{\nu} = 3035$ (Ar–H), 2923 (CH), 1548 (C=N), 1101 (C–F), 813, 729 (C–Cl) cm^{-1}; ^1H NMR (400 MHz, *DMSO*-d_6): $\delta = 2.31$ (s, CH_3), 5.31 (s, OCH_2), 6.89 (d, 2H-*p*-cresyloxy, $J = 8.5$ Hz), 7.13 (d, 2H-*p*-cresyloxy, $J = 8.5$ Hz), 7.64 (m, 2H-dichlorofluorophenyl) ppm.

6-[(4-Chloro-3-methylphenoxy)methyl]-3-(2,4-dichloro-5-fluorophenyl)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole
(**8c**, $C_{17}H_{10}Cl_3FN_4OS$)
Yield 85%, mp 150–152°C (*Et*OH:*DMF* = 2:1); IR (KBr): $\bar{\nu} = 3091$ (Ar–H), 2925 (C–H), 1577 (C=N), 1097 (C–F), 812, 732 (C–Cl) cm^{-1}; ^1H NMR (400 MHz, *DMSO*-d_6): $\delta = 2.36$ (s, CH_3), 5.33 (s, OCH_2), 6.78 (m, 1H-chlorocresyloxy), 6.89 (d, 1H-chlorocresyloxy, $J = 2.8$ Hz), 7.28 (m, 1H-chlorocresyloxy), 7.65 (m, 2H-dichlorofluorophenyl) ppm.

6-[(4-Chloro-3-methylphenoxy)methyl]-3-(2,4-dichloro-5-fluorophenyl)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole
(**8d**, $C_{16}H_7Cl_4FN_4OS$)
Yield 78%, mp 220–222°C (*Et*OH:*DMF* = 2:1); IR (KBr): $\bar{\nu} = 3093$ (Ar–H), 2925 (C–H), 1554 (C=N), 1107 (C–F), 813, 736 (C–Cl) cm^{-1}; ^1H NMR (400 MHz, *DMSO*-d_6): $\delta = 5.38$ (s, OCH_2), 6.95 (d, 1H-dichlorophenyl, $J = 8.8$ Hz), 7.45 (d, 1H-dichlorophenyl, $J = 2.4$ Hz), 7.23 (dd, 1H-dichlorophenyl, $J = 2.4$ Hz), 7.65 (m, 2H-dichlorofluorophenyl) ppm, FABMS: m/z (%) = 463 ($M^+ + 1$, 55).

3-(2,4-Dichloro-5-fluorophenyl)-6-[(4-fluorophenoxy)methyl]-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole (**8e**, $C_{16}H_8Cl_2F_2N_4OS$)
Yield 81%, mp 176–178°C (*Et*OH:*DMF* = 2:1); IR (KBr): $\bar{\nu} = 3082$ (Ar–H), 2920 (C–H), 1546 (C=N), 1099 (C–F), 829, 732 (C–Cl) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta =$ 5.33 (s, OCH_2), 7.00 (m, 4H-*p*-fluorophenoxy), 7.65 (m, 2H-dichlorofluorophenyl) ppm.

Antitubercular Assay
A primary screening was conducted at 6.25 $\mu g\,cm^{-3}$ (or molar equivalent of highest molecular weight compound in a series of congeners) against *Mycobacterium tuberculosis* H37Rv (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA) [18]. Compounds exhibiting fluorescence were tested in the

BACTEC 460 radiometric system. Compounds effecting <90% inhibition in the primary screen ($MIC > 6.25\ \mu g\,cm^{-3}$) were not generally evaluated further. Compounds demonstrating at least 90% inhibition in the primary screen were retested at lower concentrations against *M. tuberculosis* H37Rv to determine the actual minimum inhibitory concentration (*MIC*) using MABA. The *MIC* was defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls.

Analgesic Assay

Male Albino mice of either sex with weight between 20 and 25 g were used for analgesic study. The animals were divided into 12 experimental groups each consisted of 6 animals. Gum Acacia (2%) was administered to group 1. Group 2 received Pethidine at a dose $5\,mg\,kg^{-1}$ by intraperitoneal injection. Other groups were given the test compounds (**7a**, **7b**, **7e–7h**, **8a**, **8d**, and **8e**) at a dose $50\,mg\,kg^{-1}$ orally. The animals were housed and fed in laboratory kept at constant temperature of 22°C under standard conditions (12:12 h light-dark cycle, standard pellet diet, tap water). In this test, reaction of mice to painful stimulus was measured. Mice were placed on the metal plate heated to 55 ± 0.4°C and covered with a glass cylinder (25 cm high, 15 cm in diameter). The time(s) elapsing to the first pain response (licking or jumping) was determined by a stop watch and then recorded as response latency, prior to 60 and 180 min following the *po* administration of the investigated compounds. Institutional ethics Committee approved all the experiments.

Antibacterial Assay

The newly prepared compounds were screened for their antibacterial activity against five bacterial strains by disc diffusion method. A standard inoculum ($1–2 \times 10^7$ c.f.u. cm^{-3} 0.5 *McFarland* standards) was introduced on to 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 1 h. The sterile disc 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. The inhibition zones were measured and compared with the controls. 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 were inoculated with approximately 5×10^5 c.f.u. cm^{-3} 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 concentration (*MIC*). Ciprofloxacin was used as a standard drug.

Antifungal Assay

The newly prepared compounds were screened for their antifungal activity against five fungal strains by the agar diffusion method. Sabourauds agar media was prepared by dissolving 1 g peptone, 4 g D-glucose, and 2 g agar in $100\,cm^3$ distilled water, and adjusting *pH* to 5.7 using buffer. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to $3\,cm^3$ saline to get a suspension of corresponding species. $20\,cm^3$ of agar media were poured into each Petri dish. Excess of 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 and each well was labeled. A control was also prepared in triplicate and maintained at 37°C for 3–4 d. The inhibition zones in diameter were measured and compared with the controls. The Nutrient Broth, which contained logarithmic serially two fold diluted amounts of test compound and controls were inoculated with approximately 1.6×10^4–6×10^4 c.f.u. cm^{-3}. 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 as minimum inhibitory concentration (*MIC*). Griseofulvin was used as the standard drug.

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References

[1] Zitouni GT, Kaplancıklı ZA, Yıldız MT, Chevallet P, Kaya D (2005) Eur J Med Chem **40**: 607
[2] Walczak K, Gondela A, Suwiński J (2004) Eur J Med Chem **39**: 849
[3] a) Holla BS, Poojary KN, Rao BS, Shivananda MK (2002) Eur J Med Chem **37**: 511; b) Holla BS, Veerendra B, Shivananda MK, Boja P (2003) Eur J Med Chem **38**: 759
[4] Al-Soud YA, Al-Masoudi NA, Ferwanah ARS (2003) Bioorg Med Chem **11**: 1707
[5] Almasirad A, Tabatabai SA, Faizi M, Kebriaeezadeh A, Mehrabi N, Dalvandi A, Shafiee A (2004) Bioorg Med Chem Lett **14**: 6057
[6] Amir M, Shikha K (2004) Eur J Med Chem **39**: 535
[7] Kawashima Y, Ishikawa H, Kida S, Tanaka T, Masuda T (1986) Japan Kokai **61**: 260085; (1987) Chem Abstr **106**: 138475x
[8] Holla BS, Gonsalves R, Shenoy S (1998) Il Farmaco **53**: 574
[9] Rostom SAF, Shalaby MA, El-Demellauy MA (2003) Eur J Med Chem **38**: 959
[10] Dogan HN, Duran A, Rollas S, Sener G, Uysal MK, Gulen D (2002) Bioorg Med Chem **10**: 2893
[11] Gadad AK, Noolvi MN, Karpoormath RV (2004) Bioorg Med Chem **12**: 5651
[12] a) John Stanley W (1977) Ger Offen 2704288, (1977) Chem Abstr **87**: 201546; b) Suzuki F, Kawakami J,

Yamamoto S, Kosai Y (1977) Japan Kokai 7776432; (1978) Chem Abstr **88**: 100351
[13] Zhang ZY, Sun XW (1998) Heterocycles **48**: 561
[14] Kevin Park B, Kitteringham NR, O'Neill PM (2001) Ann Rev Pharmacol Toxicol **41**: 443
[15] Karthikeyan MS, Prasad DJ, Boja Poojary, Bhat KS, Holla BS, Kumari NS (2006) Bioorg Med Chem **14**: 7482
[16] (1996) Vogel's Text Book of Practical Organic Chemistry, 5th edn, Longman, England, p 1160
[17] Walls LS (1969) African Pat 6,604,864, Chem Abstr **70**: 87320w
[18] Collins L, Franzblau SG (1997) Antimicrob Agents Chemother **41**: 1004
[19] Eddy NB, Leimbach D (1953) J Pharmacol Exp Ther **7**: 385
[20] Cruickshank R, Duguid JP, Marmion BP, Swain RHA (1975) Medicinal Microbiology, 12th edn, Vol. II. Churchill Livingstone, London, p 196
[21] Collins AH (1976) Microbiological Methods, 2nd edn, Butterworth, London
[22] Khan ZK (1997) *In vitro* and *in vivo* screening techniques for bioactivity screening and evaluation. Proc Int Workshop UNIDO-CDRI, p 210
[23] Varma RS (ed) (1998) Antifungal Agents: Past, Present and Future Prospects, National Academy of Chemistry and Biology, India, Lucknow

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