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Synthesis, antiinflammatory and antimicrobial activities of some 2,4-dichloro-5-fluorophenyl substituted arylidenetriazolothiazolidinones

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Abstract A series of 2,4-dichloro-5-fluorophenyl substituted arylidenetriazolothiazolidinones were obtained by one-pot reaction of 3-(2,4-dichloro-5-fluorophenyl)-4*H*-1,2,4-triazole-5-thiol with substituted benzaldehydes and monochloroacetic acid in the presence of acetic anhydride, acetic acid, and sodium acetate. The structures of the newly synthesized compounds were characterized and confirmed by IR, ^{1}H NMR, mass spectra, and elemental analysis. Compounds bearing the 4-methylthiophenyl, 3,4-methylenedioxyphenyl, and 2,3,5-trichlorophenyl moiety showed excellent antiinflammatory activity. The antimicrobial screening studies revealed that compounds with 4-anisyl, 4-methylthiophenyl, 3,4-methylenedioxyphenyl, and 2,3,5-trichlorophenyl at position 5 of the arylidenetriazolothiazolidinone moiety showed excellent activity against all tested strains at 6.25 μg cm^{-3} concentrations.

Keywords 3-(2,4-Dichloro-5-fluorophenyl)-4*H*-1,2,4-triazole-5-thiol; One-pot reaction; Arylidenetriazolothiazolidinones; Antiinflammatory; Antimicrobial activity.

Introduction

Thiazolidinone derivatives constitute an important class of heterocyclic compounds. There has been considerable interest in the chemistry of thiazolidin-4-one ring systems, which is a core structure in various synthetic pharmaceuticals displaying a broad spectrum of biological activities [1]. The thiazolidin-4-one ring system also occurs in Nature as actithiazic acid, ((−)2-(5-carboxypentyl)thiazolidin-4-one), which is isolated from *Streptomyces* strains [2].

Thiazolidinone derivatives are known to exhibit diverse bioactivities such as anticonvulsant [3], antidiarrheal [4], antimicrobial [5], antidiabetic [6], cycloxygenase inhibitory [7], Ca^{2+} channel blocker [8], cardioprotective [9], anticancer [10], anti HIV [11], and tumor necrosis factor-α-antagonist activities [12].

1,2,4-Triazoles represent an overwhelming and rapid developing field in modern heterocyclic chemistry. From literature, it is predictable that 1,2,4-triazoles represent important pharmacophores, and have a wide range of therapeutic properties. They play a vital role as medicinal agents due to different biological 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. A degree of respectability has been bestowed for 1,2,4-triazole derivatives due to their antibacterial, antifungal [13], antitubercular [14], and anticancer [15] properties. Certain 1,2,4-triazoles also find applications in the preparation of photographic plates, polymers, and as analytical agents [16].

The importance of fluorine containing compounds in general, and heterocyclic in particular, has initiated active research on fluorine containing heterocycles.

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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 neighboring functional groups [17].

Fluorine containing heterocycles, triazoles, and thiazolidinones displayed varied pharmacological properties. Since there has been no report on dichlorofluorophenyl containing arylidenetriazolothiazolidinones, it was contemplated to synthesize them and to pursue their antibacterial and antifungal screenings. The corresponding results are presented in this paper.

Results and discussion

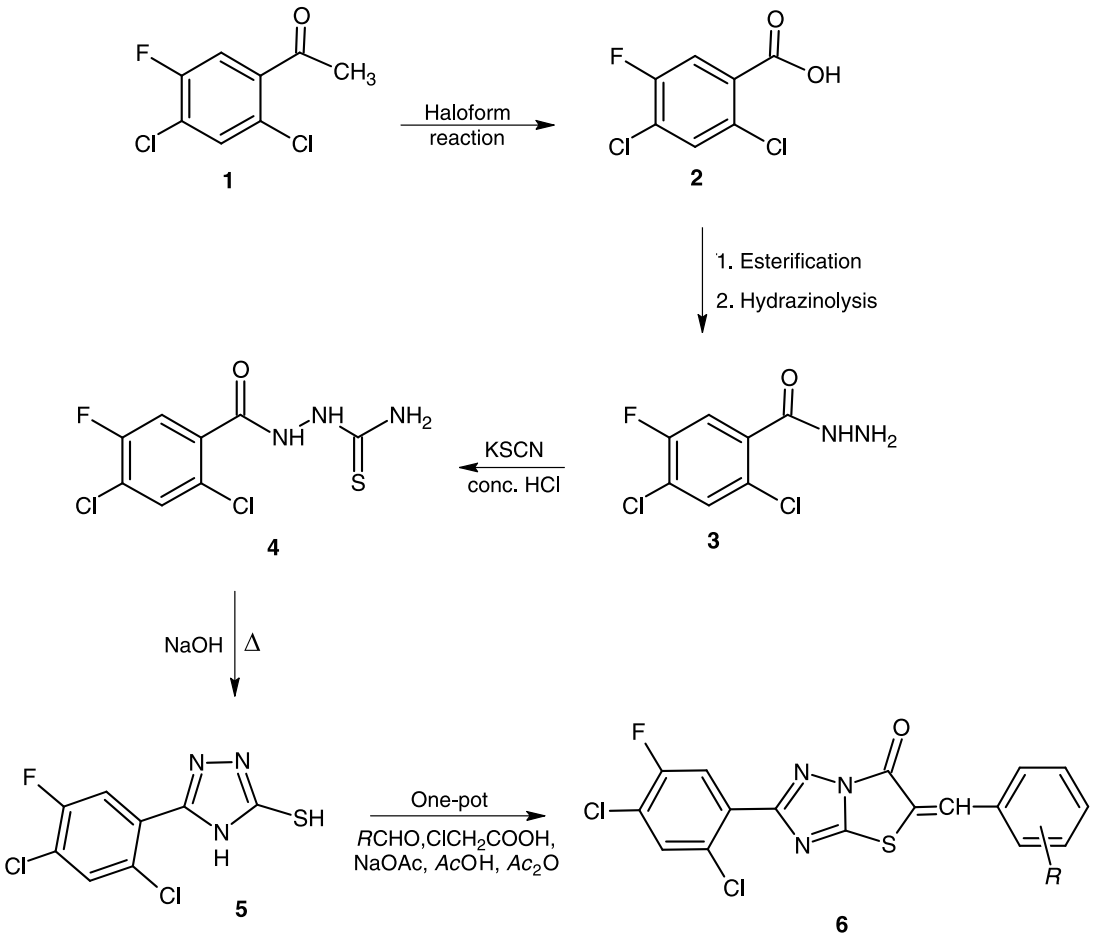
Chemistry

2,4-Dichloro-5-fluorobenzoic acid (**2**) was obtained (Scheme 1) from 2,4-dichloro-5-fluoroacetophenone (**1**) according to Ref. [18]. 2,4-Dichloro-5-fluorobenzoyl hydrazide (**3**) was prepared according to Ref. [19]. 2-(2,4-Dichloro-5-fluorobenzoyl)hydrazine carbothioamide (**4**) was obtained by the treatment of 2,4-dichloro-5-fluorobenzoyl hydrazide (**3**) with potassium thiocyanate in presence of concentrated hydrochloric acid, which on further cyclization in the presence of base yielded 3-(2,4-dichloro-5-fluorophenyl)-4*H*-1,2,4-triazole-5-thiol (**5**) in good yield. A series of 2,4-dichloro-5-fluorophenyl containing arylidenetriazolothiazolidinones (**6**) were obtained in good yields by an one-pot reaction of 3-(2,4-dichloro-5-fluorophenyl)-4*H*-1,2,4-triazole-5-thiol (**5**) with substituted benzaldehydes, and monochloroacetic acid in the presence of acetic anhydride, acetic acid, and sodium acetate.

Pharmacological studies

Antiinflammatory studies

Few selected compounds were screened for their antiinflammatory activity by *Carrageenan* induced rat



Scheme 1

Table 1 Antiinflammatory activity data of arylidenetriazolothiazolidinones

Compd. no.	Dose (mg/kg, p.o.)	Increase in paw volume/cm^3	Inhibition of paw oedema/%
6a	50	0.46 ± 0.0027	26.6
6b	50	0.34 ± 0.0037	48.4
6c	50	0.52 ± 0.0016	18.8
6d	50	0.58 ± 0.0017	9.4
6e	50	0.37 ± 0.0031	42.2
6f	50	0.69 ± 0.0017	0
6g	50	0.48 ± 0.0020	25
6h	50	0.65 ± 0.0011	0
6j	50	0.26 ± 0.0020	59.4
6l	50	0.16 ± 0.0026	75
control	10 cm^3/kg	0.65 ± 0.0096	–
standard*	2	0.24 ± 0.0014	62.9

* Indomethacin is used as the standard

paw oedema (Acute-inflammation model) as described by *Winter et al.* [20]. The results were expressed as % inhibition of oedema over the untreated control group. The anti-inflammatory results revealed that compounds **6b**, **6j**, and **6l** showed excellent antiinflammatory activity whereas compound **6e** showed moderate antiinflammatory activity compared to that of the standard drug, Indomethacin. The results of antiinflammatory studies are given in Table 1.

Antibacterial studies

The newly prepared compounds were screened for their antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, and *Klebsiella pneumoniae* (recultured) bacterial strains by the disc diffusion method [21, 22]. The diameter of the zone of inhibition and minimum inhibitory concentration values are given in Table 2. The antibacterial screening data revealed that **6a**, **6b**, **6e**, **6g**, and **6l** were active against *S. aureus* and *E. coli*. Compounds **6a**, **6b**, **6e**, **6i**, and **6l** were active against *P. aeruginosa* and *S. pyogenes*. Especially compounds **6a**, **6b**, **6e**, **6j**, and **6l** 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) by the agar diffusion method [23, 24]. The diameter of zone of inhibition and minimum inhibitory concentration values are given in Table 3. The antifungal screening data showed that compounds **6a**, **6g**, **6j**, and **6l** showed good activity against *A. fumigatus* and *P. marneffei* at 6.25 $\mu g\,cm^{-3}$ concentrations. Compounds **6a**, **6j**, and **6l** exhibited good antifungal activity against all tested fungal strains almost equivalent to that of the standard drug, Amphotericin B.

Table 2 Antibacterial activity (diameter zones/mm) of inhibition ($MIC/\mu g\,cm^{-3}$) of arylidenetriazolothiazolidinones

Compd. no.	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Streptococcus pyogenes*
6a	21 (6.25)	25 (6.25)	29 (6.25)	19 (6.25)	24 (6.25)
6b	19 (6.25)	29 (6.25)	26 (6.25)	17 (6.25)	19 (6.25)
6c	14 (12.5)	24 (6.25)	8 (25)	–	–
6d	9 (25)	15 (12.5)	–	10 (25)	15 (12.5)
6e	23 (6.25)	29 (6.25)	32 (6.25)	20 (6.25)	18 (6.25)
6f	12 (12.5)	–	25 (6.25)	12 (12.5)	–
6g	18 (6.25)	27 (6.25)	15 (12.5)	–	19 (6.25)
6h	–	15 (12.5)	20 (12.5)	–	10 (25)
6i	12 (12.5)	19 (12.5)	28 (6.25)	15 (12.5)	20 (6.25)
6j	23 (6.25)	28 (6.25)	30 (6.25)	18 (6.25)	21 (6.25)
6k	10 (25)	8 (25)	–	15 (12.5)	–
6l	21 (6.25)	26 (6.25)	28 (6.25)	18 (6.25)	23 (6.25)
standard*	23 (6.25)	29 (6.25)	32 (6.25)	21 (6.25)	25 (6.25)

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

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

Table 3 Antifungal activity (diameter zones/mm) of inhibition ($MIC/\mu g\,cm^{-3}$) of arylidenetriazolothiazolidinones

Compd. no.	*Candida albicans*	*Aspergillus niger*	*Aspergillus fumigatus*	*Trichophyton mentagrophytes*	*Penicillium marneffei*
6a	19 (6.25)	23 (6.25)	18 (6.25)	16 (6.25)	20 (6.25)
6b	8 (25)	–	12 (12.5)	14 (12.5)	11 (12.5)
6c	12 (12.5)	15 (12.5)	10 (25)	–	14 (12.5)
6d	–	18 (6.25)	8 (25)	10 (25)	–
6e	10 (25)	–	–	13 (12.5)	9 (25)
6f	–	10 (25)	14 (12.5)	9 (25)	–
6g	14 (12.5)	14 (12.5)	16 (6.25)	–	18 (6.25)
6h	17 (6.25)	12 (12.5)	–	17 (6.25)	12 (12.5)
6i	9 (25)	7 (25)	11 (12.5)	13 (12.5)	–
6j	21 (6.25)	20 (6.25)	20 (6.25)	19 (6.25)	18 (6.25)
6k	15 (12.5)	15 (12.5)	9 (25)	12 (12.5)	–
6l	18 (6.25)	24 (6.25)	22 (6.25)	18 (6.25)	21 (6.25)
standard*	21 (6.25)	25 (6.25)	22 (6.25)	19 (6.25)	23 (6.25)

* Amphotericin B is used as the standard, – indicates fungus is resistant to the compounds $>100\,\mu g\,cm^{-3}$
MIC ($\mu g\,cm^{-3}$) = minimum inhibitory concentration, *i.e.*, the lowest concentration to completely inhibit fungal growth

Conclusion

The antiinflammatory study revealed that compounds with 4-methylthiophenyl, 3,4-methylenedioxyphenyl, and 2,3,5-trichlorophenyl residues at position 5 of the arylidenetriazolothiazolidinone moiety showed excellent antiinflammatory activity. The antimicrobial screening studies revealed that compounds with 4-anisyl, 4-methylthiophenyl, 3,4-methylenedioxyphenyl, and 2,3,5-trichlorophenyl at position 5 of the arylidenetriazolothiazolidinones moiety showed excellent activity.

Experimental

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 300/400 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.

2,4-Dichloro-5-fluorobenzoic acid (**2**) was obtained from 2,4-dichloro-5-fluoroacetophenone (**1**) according to Ref. [18]. 2,4-Dichloro-5-fluorobenzoyl hydrazide (**3**) was prepared according to Ref. [19].

2-(2,4-Dichloro-5-fluorobenzoyl)hydrazine carbothioamide (**4**, $C_8H_6Cl_2FN_3OS$)

A mixture of 0.01 mol 2,4-dichloro-5-fluorobenzoyl hydrazide (**3**), 0.01 mol, and then 0.02 mol of potassium thiocyanate was added to 10 cm^3 of water containing 2 cm^3 of conc. HCl. The mixture was warmed on a water bath for 2 h. The reaction mixture was cooled, and poured onto crushed ice. The resulting solid was filtered, dried, and recrystallized from ethanol. Yield 90%; mp 203–205°C; FABMS: m/z (%) = 282 (M + 1, 100), 191 (85).

5-(2,4-Dichloro-5-fluorophenyl)-4H-1,2,4-triazole-3-thiol (**5**, $C_8H_4Cl_2FN_3S$)

To a solution of 100 cm^3 of 5% NaOH, 0.01 mol of 2-(2,4-dichloro-5-fluorobenzoyl)hydrazine carbothioamide (**4**) was added and refluxed for 3 h. The reaction mixture was cooled, poured onto crushed ice, and neutralized with conc. HCl. The resulting solid was filtered, dried, and recrystallized from a mixture of ethanol and dimethylformamide. Yield 82%; mp 256–258°C; ^{1}H NMR (300 MHz, *DMSO*-d_6): $\delta = 7.86$ (d, 1H-dichlorofluorophenyl, $J_{H-F_{ortho}} = 9.6$ Hz), 8.05 (d, 1H-dichlorofluorophenyl, $J_{H-F_{meta}} = 7.2$ Hz), 13.84 (s, 1H–NH/SH) 13.92 (s, 1H–NH/SH) ppm.

5-Arylidene-2-(2,4-dichloro-5-fluorophenyl)thiazolo[3,2-b]-1,2,4-triazol-6(5H)-one (***6***)

To 0.01 mol of 5-(2,4-dichloro-5-fluorophenyl)-4*H*-1,2,4-triazole-3-thiol (**5**), 0.015 mol of monochloroacetic acid, 0.01 mol of substituted benzaldehyde, 5 cm^3 of acetic anhydride, 0.01 mol of anhydrous sodium acetate, and 10 cm^3 of glacial acetic acid was added and refluxed for 3 h. The reaction mixture was cooled, and poured onto crushed ice. The resulting solid was filtered, dried, and recrystallized from a mixture of ethanol and dimethylformamide.

2-(2,4-Dichloro-5-fluorophenyl)-5-(4-methoxybenzylidene)-1,3-thiazolo[3,2-b]-1,2,4-triazol-6(5H)-one (**6a**, $C_{18}H_{10}Cl_2FN_3O_2S$)

Yield 79%; mp 226–228°C (*Et*OH:*DMF* = 2:1); IR (KBr): $\bar{\nu} = 3090$ (*Ar*–H), 2952 (C–H), 1706 (C=O), 1109 (C–F), 836 (C–Cl) cm^{-1}; ^{1}H NMR (400 MHz, *DMSO*-d_6): $\delta = 3.87$ (s, OCH_3), 7.19 (d, 2H-*p*-anisyl, $J = 8.8$ Hz), 7.78 (d, 2H-*p*-anisyl, $J = 8.8$ Hz), 8.04–8.08 (m, 2H-dichlorofluorophenyl), 8.29 (s, =CH) ppm; FABMS: m/z (%) = 422 (M + 1, 25).

2-(2,4-Dichloro-5-fluorophenyl)-5-[4-(methylthio)-benzylidene]-1,3-thiazolo[3,2-b]-1,2,4-triazol-6(5H)-one
(**6b**, $C_{18}H_{10}Cl_2FN_3OS_2$)
Yield 88%; mp 228–230°C (*Et*OH:*DMF* = 2:1); IR (KBr): $\bar{\nu}$ = 3092 (*Ar*–H), 1702 (C=O), 1089 (C–F), 852, 736 (C–Cl) cm^{-1}; ^1H NMR (300 MHz, *DMSO*-d_6): δ = 3.50 (s, SCH_3), 7.48 (d, 2H-*p*-methylthio, J = 8.8 Hz), 7.73 (d, 2H-*p*-methylthio, J = 8.8 Hz), 8.05–8.09 (m, 2H-dichlorofluorophenyl), 8.28 (s, =CH) ppm.

2-(2,4-Dichloro-5-fluorophenyl)-5-[4-(dimethylamino)-benzylidene]-1,3-thiazolo[3,2-b]-1,2,4-triazol-6(5H)-one
(**6c**, $C_{19}H_{13}Cl_2FN_4OS$)
Yield 63%; mp 271–273°C (*Et*OH:*DMF* = 2:1); IR (KBr): $\bar{\nu}$ = 3093 (*Ar*–H), 2958 (C–H), 1702 (C=O), 1089 (C–F), 854 (C–Cl) cm^{-1}; ^1H NMR (300 MHz, *DMSO*-d_6): δ = 3.09 (s, $N(CH_3)_2$), 6.90 (d, 2H-*p*-dimethylamino, J = 9 Hz), 7.73 (d, 2H-*p*-dimethylamino, J = 9 Hz), 8.02–8.09 (m, 2H-dichlorofluorophenyl), 8.18 (s, =CH) ppm.

5-(4-Chlorobenzylidene)-2-(2,4-dichloro-5-fluorophenyl)-[1,3]thiazolo[3,2-b]-1,2,4-triazol-6(5H)-one
(**6e**, $C_{17}H_7Cl_2FN_3OS$)
Yield 87%; mp 232–234°C (*Et*OH:*DMF* = 2:1); ^1H NMR (400 MHz, $CDCl_3$): δ = 7.54–7.63 (m, 3H-*p*-chloro, dichlorofluorophenyl) 7.92 (d, 1H-dichlorofluorophenyl, $J_{H-F_{ortho}}$ = 9.4 Hz), 8.22 (s, =CH) ppm; FABMS: m/z (%) = 426 (M + 1, 28).

5-(2-Chloro-5-nitrobenzylidene)-2-(2,4-dichloro-5-fluorophenyl)-1,3-thiazolo[3,2b]-1,2,4-triazol-6(5H)-one
(**6g**, $C_{17}H_6Cl_3FN_4O_3S$)
Yield 78%; mp 216–218°C (*Et*OH:*DMF* = 2:1); ^1H NMR (400 MHz, $CDCl_3$): δ = 7.62 (d, 1H-dichlorofluorophenyl, $J_{H-F_{meta}}$ = 6.4 Hz), 7.77 (d, 1H-chloronitro, J = 8.7 Hz), 7.92 (d, 1H-dichlorofluorophenyl, $J_{H-F_{ortho}}$ = 9.2 Hz), 8.32 (d, 1H-chloronitro, J = 7.2 Hz), 8.52 (s, 1H-chloronitro), 8.56 (s, =CH) ppm; FABMS: m/z (%) = 471 (M + 1, 50).

5-(2,4-Dichlorobenzylidene)-2-(2,4-dichloro-5-fluorophenyl)-1,3-thiazolo[3,2-b]-1,2,4-triazol-6(5H)-one
(**6h**, $C_{17}H_6Cl_4FN_3OS$)
Yield 65%; mp 222–224°C (*Et*OH:*DMF* = 2:1); IR (KBr): $\bar{\nu}$ = 3090 (*Ar*–H), 1712 (C=O), 1105 (C–F), 882, 726 (C–Cl) cm^{-1}; ^1H NMR (300 MHz, *DMSO*-d_6): δ = 7.95 (d, 1H-dichloro, J = 2 Hz), 7.66–7.75 (m, 2H-dichloro), 8.05–8.09 (m, 2H-dichlorofluorophenyl), 8.28 (s, =CH) ppm.

5-(1,3-Benzodioxol-5-ylmethylene)-2-(2,4-dichloro-5-fluorophenyl)-1,3-thiazolo[3,2-b]-1,2,4-triazol-6(5H)-one
(**6j**, $C_{18}H_8Cl_2FN_3O_3S$)
Yield 82%; mp 238–240°C (*Et*OH:*DMF* = 2:1); IR (KBr): $\bar{\nu}$ = 3098 (*Ar*–H), 2927 (C–H), 1700 (C=O), 1098 (C–F), 825, 736 (C–Cl) cm^{-1}; ^1H NMR (300 MHz, *DMSO*-d_6): δ = 6.19 (s, OCH_2O), 7.19 (d, 1H-methylenedioxy, J = 8.1 Hz), 7.34–7.42 (m, 2H-methylenedioxy), 8.04–8.1 (m, 2H-dichlorofluorophenyl), 8.25 (s, =CH) ppm; FABMS: m/z (%) = 436 (M + 1, 27).

2-(2,4-Dichloro-5-fluorophenyl)-5-(2,3,5-trichloro-benzylidene)-1,3-thiazolo[3,2-b]-1,2,4-triazol-6(5H)-one
(**6l**, $C_{17}H_5Cl_5FN_3OS$)
Yield 63%; mp 235–237°C (*Et*OH:*DMF* = 2:1); IR (KBr): $\bar{\nu}$ = 3090 (*Ar*–H), 2922 (C–H), 1702 (C=O), 1105 (C–F), 812, 731 (C–Cl) cm^{-1}; ^1H NMR (300 MHz, *DMSO*-d_6): δ = 8.04 (s, 1H-trichloro), 8.09 (d, 1H-dichlorofluorophenyl, $J_{H-F_{ortho}}$ = 7.8 Hz), 8.12–8.13 (m, 2H-trichloro and dichlorofluorophenyl), 8.25 (s, =CH) ppm.

Antiinflammatory assay
Wister albino rats of either sex weighing 180–250 g were used for the experiment. They were housed in clean polypropylene cages and kept under room temperature (25°C), relative humidity (60–70%) in 112 h light-dark cycle. The animals were given standard laboratory diet and water *ad libitum*. Food was withdrawn 12 h before and during experimental hours. Experiments were approved by the Institutional ethics committee.

The animals were divided into 12 groups each group contained six animals. A mark was made on the hind paw (left) just beyond the tibio-tarsal junction, so that every time the paw was dipped in the mercury column up to fixed mark constant paw volume was ensured. The initial paw volume of each rat was noted by plethysmometrically (Ugo Basile, Italy). First group received normal saline and the second group received Indomethacin orally at a dose of 1.5 mg/kg. The 3rd to 12th groups were administered the test compounds (at a dose of 50 mg/kg suspended in 10 cm^3/kg of 2% gum acacia) orally. After 30 min of treatment of test compounds, 0.1 cm^3 of 1% (w/v) carrageenan was injected in the subplantar region of the left hind paw. The right paw served as a reference to non-inflammed paw for comparison. The initial paw volume was measured with in 30 sec of the injection. The relative increase in paw volume was measured in control, standard and test compounds at 1, 2, and 3 h after the carrageenan injection. The difference between the two readings was taken as the volume of oedema and the percentage inhibition by the drugs was calculated using the formula,

$$\text{Percentage of oedema inhibition} = 100 - (V_{test}/V_{control}) \times 100,$$

where, $V_{control}$ = volume of paw oedema in control group; V_{test} = volume of paw oedema in the test compounds treated group.

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. 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*). 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. *Sabouraud* 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 was poured in to each Petri dish. Excess of suspension was decanted and the plates were dried by placing in a incubator at 37°C for 1 h. Using an agar punch, wells were made and each wells are labeled. A control was also prepared in triplicate and maintained at 37°C for 3–4d. The inhibition zones in diameter were measured and compared with the controls. The Nutrient Broth, which contained logarithmic serially two fold diluted amount of test compound and controls were inoculated with approximately 1.6×10^4–6×10^4 c.f.u. cm^{-3} 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 as minimum inhibitory concentrations (*MIC*). Amphotericin B was used as the standard drug.

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