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Original article

Synthesis and antitumor activity studies of some new fused 1,2,4-triazole derivatives carrying 2,4-dichloro-5-fluorophenyl moiety

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ABSTRACT

A series of 3-(2,4-dichloro-5-fluorophenyl)-6-(substituted phenyl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines (**4**) (Fig. 1) have been synthesized by the cyclization of 3-(2,4-dichloro-5-fluorophenyl)-1,2,4-triazol-5-thiol (**3**) with substituted phenacyl bromides. All the newly synthesized compounds were confirmed by IR, ^{1}H NMR and mass spectral studies. Among the compounds tested for their antitumor activity three compounds exhibited *in vitro* antitumor activity with moderate to excellent growth inhibition against a panel of sixty cancer cell lines of leukemia, non-small cell lung cancer, melanoma, ovarian cancer, prostate and breast cancer. The compound **4d** showed promising antiproliferative activity with GI_{50} values in the range of 1.06–25.4 μM.

1. Introduction

The Chemistry of N-bridged heterocycles derived from 1,2,4-triazole has received considerable attention in recent years due to their usefulness in different areas of biological activities and as industrial intermediates. 1,2,4-triazole derivatives are known to exhibit antimicrobial [1–5], antitubercular [6], anticancer [7,8], anticonvulsant [9], anti-inflammatory and analgesic properties [10]. The arrangement of three basic nitrogen atoms in triazole ring induces the antiviral activities in the compounds containing triazole ring [11]. 1,2,4-triazole nucleus has been incorporated in to a wide variety of therapeutically interesting drug candidates including H1/H2 histamine receptor blockers, cholinesterase active agents, CNS stimulants, antianxiety and sedatives [12], antimycotic activity such as Fluconazole, Itraconazole and Voriconazole [13,14]. Also there are some known drugs containing 1,2,4-triazole moiety, eg: Triazolam[15], Alprazalam [16], Etizolam [17], Furacylin [18], Ribavirin [19], Hexaconazole [20], Triadimefon [21], Mycobutanil [22], Rizatriptan [23], Propiconazole [24], Fluotrimaole [25]. A series of 1,2,4-triazole derivatives have been extensively employed in agriculture as herbicides [26]. Certain 1,2,4-triazoles also find applications in the preparation of photographic plates, polymers and as analytical agents [27]. Triazolothiadiazines are reported to posses wide spectrum of biological activities [28–32].

Recently, we have reported the significant anticancer properties of 1,2,4-triazolo[3,4-*b*]thiadiazole and 1,3,4-oxadiazole derivatives having 4-fluoro-3-phenoxyphenyl and 2,4-dichloro-5-fluorophenyl moiety [33,34]. Prompted by the biological properties of 1,2,4-triazole derivatives and 1,3,4-thiadiazines and in continuation of our studies on N-bridged heterocycles derived from 1,2,4-triazoles [35], it was contemplated to synthesize some new 1,2,4-triazolo[3,4-*b*]-thiadiazine derivatives (Scheme 2) having 2,4-dichloro-5-fluorophenyl moiety at position 3 of the triazole ring and to screen them for their anticancer properties. Results of such studies are discussed in this paper.

2. Chemistry

Thiocarbohydrazide was prepared from hydrazine hydrate and carbon disulfide following the literature method [36]. This was heated with 2,4-dichloro-5-fluorobenzoic acid at its melting point to get 4-amino-3-(2,4-dichloro-5-fluorophenyl)-1,2,4-triazol-5-thiol (3) (Scheme 1). The reaction of 3-(2,4-dichloro-5-fluorophenyl)-1,2,4-triazol-5-thiol (3) with various phenacyl bromides

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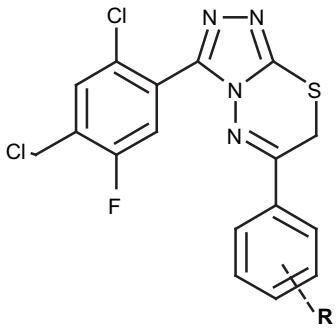
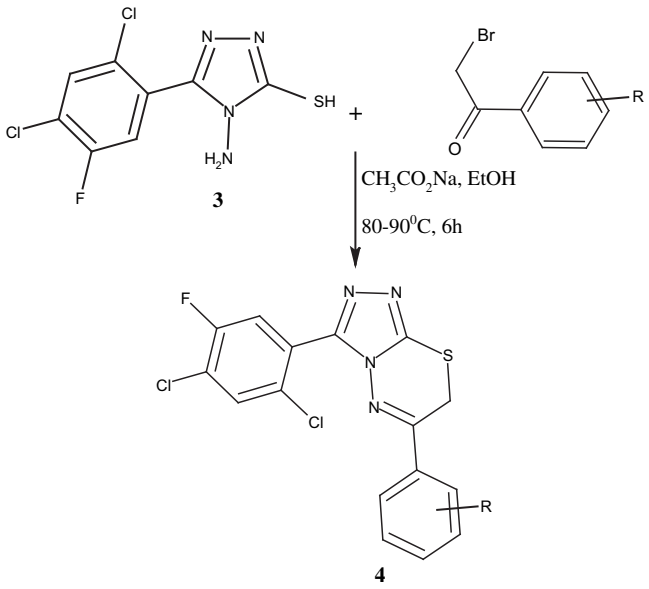


Fig. 1. General structure of 1,2,4-triazolo[3,4-*b*]thiadiazines (**4**).

in presence of anhydrous sodium acetate and absolute ethanol yielded 3-(2,4-dichloro-5-fluorophenyl)-6-aryl-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines (**4a**-**g**) (Scheme 2). The formation of these compounds (**4**) (Fig. 1) was confirmed by recording their IR, ^{1}H NMR and mass spectra.

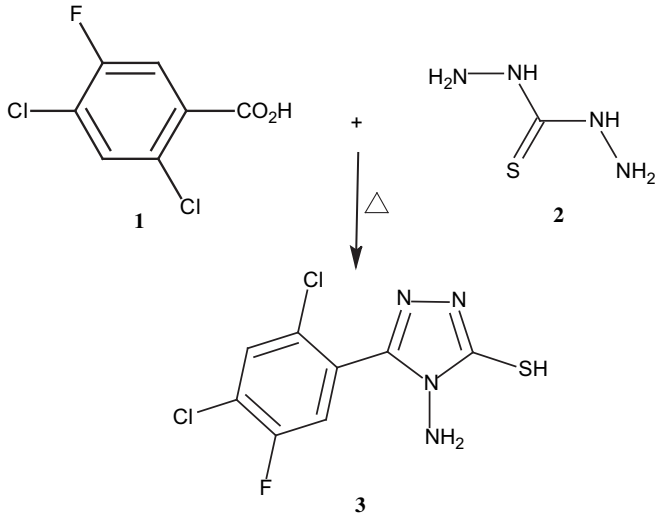


Scheme 2. Synthesis of triazolothiadiazine derivatives (**4**).

3. Pharmacology

3.1. Anticancer screening studies

Three of the newly synthesized compounds were screened for their anticancer activities under NCI screening programme [33,34]. The 3- cell line one dose assay has been done for the compounds **4d**, **4f** and **4g**. The 3- cell lines used in present investigation are NCI-H 460 (Lung), MCF7 (Breast) and SF 268 (CNS). In this current protocol, each cell line is preincubated on microtiter plate, the test agents are then added at a single concentration and the culture incubated for forty eight hours. End point determinations are made with Sulphorhodamine B, a protein binding dye. Results for each test agents are reported as the percent growth of the treated cells when compared to the untreated control cells. The compounds which reduce the growth of any one of the lines to 32% or less (negative numbers indicate the cell kill) are passed on for the evaluation in the full panel of 60 cell line over a five- log dose range. Compounds **4d**, **4f** and **4g** are found to be active against NCI-H 460 (Lung), MCF7 (Breast) and SF268 (CNS). Results of this study are given in Table 2. Further, 60 cell line anticancer assay of these compounds had been carried out. The concentration at which they cause 50% growth inhibition, GI_{50} values were determined and the results obtained are given in Table 3.



Scheme 1. Synthesis of 3-(2,4-dichloro-5-fluorophenyl)-1,2,4-triazol-5-thiol (**3**).

When these three compounds are passed on for extensive evaluation in the full panel of 60 cell lines over a five- log dose range, they showed variable antitumor property against most of the tested subpanel tumor cell lines at GI_{50} level. The subpanel tumor cell lines median growth inhibitory concentration (the average sensitivity of each subpanel towards each of the test compounds) and the full panel mean graph midpoint (MG-MID: the average of all cell lines towards each of the test compounds) are reported in Table 4. Compounds **4d**, **4f** and **4g** showed good antiproliferative activity against most of the cell lines with GI_{50} values well below 100 μM concentrations. It is interesting to note that compound **4d** showed significant antitumor activity against the entire cancer cell lines screened (*cf.* Table 3). The cell lines showing activity with GI_{50} values at <10 μM are K-562, MOLT-4, NCI-H522, COLO 205, HCT-116, HCT 15, HT29, KM12, SW620, SNB-75, LOXIMVI, MALME-3M, M14, SK-MEL-28, UACC-62, IGROVI, OVCAR-4, 786-0, ACHN, CAKI-1, SN12C, TK-10, UO-31, DU-145, MCF7, NCI/ADR-RES, MDA-MB-435, BT-549 and T-47D. Compound **4g** showed significant activity against T-47D with GI_{50} value of 4.93 μM. However, compound **4f** is moderately active as GI_{50} values are in considerably higher range.

The ratio obtained by dividing the compounds full panel MG-MID (μM) by its individual subpanel MG-MID (μM) is considered as a measure of compound selectivity. Ratios between 3 and 6 refer to moderate selectivity, ratios >6 indicate high selectivity and compounds not meeting of either of these criteria are considered nonselective. Compound **4d** showed high selectivity against leukemia with selectivity index of 4.82. All the other active compounds in the present study prove to be nonselective against any of nine tumor sub panels tested (cf. Table 4).

Among the tested compounds, only **4d**, **4f** and **4g** have shown very good antiproliferative activity against most of the cell lines. These compounds contain chlorine atoms at various positions, whereas other compounds having other substituents are less active/inactive indicating that presence of chlorine atom is responsible for their activity. Highest activity was observed for **4d** having chlorine substituent at position 4 of phenyl ring.

Table 1
Characterization data of 3-(2,4-dichloro-5-fluorophenyl)-6-(substituted phenyl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadizine derivatives (**4a**-**g**).

Comp No.	R	M.P (°C)	Yield (%)	Mol. formula
4a	H	108–10	68	$C_{16}H_9Cl_2FN_4S$
4b	4-OMe	126–27	76	$C_{17}H_{11}Cl_2FN_4OS$
4c	4-CH_3	98–100	72	$C_{17}H_{11}Cl_2FN_4S$
4d	4-Cl	144–45	78	$C_{16}H_8Cl_3FN_4S$
4e	4-Br	166–68	84	$C_{16}H_8BrCl_2FN_4S$
4f	2,4-Cl_2	148–50	72	$C_{16}H_7Cl_4FN_4S$
4g	2,4-Cl_2-5-F	174–75	74	$C_{16}H_6Cl_4F_2N_4S$

All the compounds showed satisfactory C, H & N analysis. Solvent of crystallization: Ethanol + dioxan (4:1).

3.2. Toxicity studies

Among the compounds tested for their anticancer activity, **4d** showed very good anticancer activity in terms of growth inhibitory effect on cancer cell lines. Hence, it could be a potential drug candidate for cancer treatment. Hence this particular compound was analyzed for its acute toxicity (lethal dose) which is one of the basic requirements in fixing the therapeutic dose in drug development. Median lethal dose (LD_{50}) in male Swiss albino mice (*Mus musculus*) was determined by employing the standard methods [37,38]. From the experiment, oral LD_{50} of the test compound was found to be 227 mg/Kg body weight at 24 h duration.

4. Conclusions

This study demonstrates the significant antitumor property of compound **4d** against all the sixty cancer cell lines screened. Further, for the first time we have reported the new class of potential antitumor agents, with potential for structure activity studies and toxicological profiling. Therefore, it was concluded that 1,2,4-triazole derivatives with 2,4-dichloro-5-fluorophenyl moiety at position 3 is an excellent synthon for further study.

5. Experimental

Melting points were taken in open capillary tubes and are uncorrected. The purity of the compounds was confirmed by thin layer chromatography using Merk silica gel 60 F_{254} coated alumina plates. IR spectra were recorded on a SHIMADZU-FTIR Spectrometer in KBr (ν_{max} in cm^{-1}). ^1H NMR spectra were recorded in DMSO-d_6 on EM-390 (300 MHz) NMR spectrometer operating at 70 eV using TMS as internal standard. FAB MS spectra were recorded on a JEOL SX 102/DA-6000 Mass spectrometer using argon/Xenon (6kv, 10 mA) as the FAB gas.

5.1. 4-Amino-3-(2,4-dichloro-5-fluorophenyl)-5-mercapto-1,2,4-triazole (**3**)

A mixture of 2,4-dichloro-5-fluorobenzoic acid (**1**) (0.01 mol) and thiocarbohydrazide (0.015 mol) contained in a round-

Table 2
Anticancer activity data of 1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazine derivatives (**4d**, **4f** & **4g**).

Compd No.	Growth percentage			
	(Lung) NCI-H 460	(Breast) MCF7	(CNS) SF 268	Activity
4d	3	3	3	Active
4f	32	69	76	Active
4g	6	44	26	Active

Samples are tested at 1.00 x10^{-04} Molar concentrations. Growth percentages less than 32 are considered as active.

Table 3
GI_{50} values for compounds (**4d**, **4f** and **4g**).

Cell line	Compd		
	4d	**4f**	**4g**
Leukemia			
CCRF-CEM	–	27.6	22.0
HL-60(TB)	–	>100	80.4
K-562	1.69	47.5	17.8
MOLT-4	1.06	75.1	16.9
RPMI-8226	–	25.3	6.00
SR	–	>100	25.4
Non-small cell lung cancer			
A549/ATCC	18.3	58.0	23.7
EKVX	27.4	58.5	24.6
HOP-62	20.1	44.2	–
HOP-92	16.4	54.2	11.3
NCI-H226	18.1	36.5	14.9
NCI-H23	13.8	82.5	21.8
NCI-H322M	16.2	76.4	29.1
NCI-H460	19.1	37.3	17.6
NCI-H522	1.80	50.8	24.2
Colon cancer			
COLO 205	7.09	32.9	19.0
HCC-2998	13.6	35.0	31.6
HCT-116	2.36	25.4	–
HCT-15	1.86	38.3	20.2
HT29	4.6	40.5	20.1
KM12	8.08	41.9	28.5
SW-620	1.91	36.8	22.6
CNS cancer			
SF-268	13.2	36.6	18.5
SF-295	15.7	36.4	20.3
SF-539	–	39.2	18.9
SNB-19	19.5	25.4	22.7
SNB-75	3.37	>100	26.2
U251	10.6	38.2	20.4
Melanoma			
LOX IMVI	1.69	34.3	21.6
MALME-3M	2.37	26.6	28.4
M14	4.87	37.5	–
SK-MEL-28	6.41	40.2	21.5
UACC-62	3.63	24.9	13.3
UACC-257	15.7	86.0	30.2
Ovarian cancer			
IGROV1	3.39	50.4	29.8
OVCAR-3	15.1	22.8	11.5
OVCAR-4	5.35	>100	34.5
OVCAR-5	10.6	>100	56.8
OVCAR-8	14.8	57.3	26.0
SK-OV-3	25.4	48.0	29.2
Renal cancer			
786-0	2.08	47.1	–
A498	21.0	–	–
ACHN	4.82	66.6	26.6
CAKI-1	4.21	35.9	20.6
RXF 393	11.4	51.0	23.2
SN12C	3.60	56.4	18.4
TK-10	3.11	77.3	37.4
UO-31	1.89	60.2	31.3
Prostate cancer			
PC-3	18.7	39.2	16.9
DU-145	3.41	49.5	36.6
Breast cancer			
MCF7	6.24	31.3	16.8
NCI/ADR-RES	3.12	30.8	27.4
MDA-MB-231/ATCC	10.8	33.7	13.4
HS 578T	–	33.6	21.9
MDA-MB-435	4.55	24.4	27.8
BT-549	3.27	–	–
T-47D	3.90	17.7	4.93

Table 4
Sub panel Tumor cell lines, Median Growth Inhibitory Concentration (GI_{50}) and Full Panel Mean Graph Mid Point (MG-MID).

Subpanel tumor cell line	4d	4f	4g
Leukemia	1.37	>100	28.08
Lung cancer	16.8	55.37	20.90
Colon cancer	5.64	35.82	23.66
CNS cancer	12.47	>100	21.16
Melanoma	5.77	43.25	23.0
Ovarian cancer	12.44	>100	31.3
Renal cancer	6.51	56.35	26.25
Prostate cancer	11.05	44.35	26.75
Breast cancer	5.31	28.58	18.70
MG-MID	6.60	44.66	22.38

bottomed flask was heated in a mantle until the contents were melted. The mixture was maintained at this temperature for 15–20 min (Scheme 1). The product obtained on cooling was treated with sodium bicarbonate solution to dissolve the unreacted carboxylic acid if any. The solid mass was then washed with water and collected by filtration. The product was recrystallized from a mixture of dioxan and ethanol to afford the compound **3**. Yield 60%, m.p. 186–88 °C.

5.2. Procedure for the preparation of 3-(2,4-dichloro-5-fluorophenyl)-6-(substituted phenyl)-1,2,4-triazolo[3,4-b]-1,3,4-thiadizines ***4a-g***

A mixture of 4-amino-3-(2,4-dichloro-5-fluorophenyl)-5-mercapto-1,2,4-triazole **3** (10 mmol) and substituted phenacyl bromide (10 mmol) in ethanol (25 ml) was kept under reflux on a water bath for about 6 h. The reaction mixture was cooled; precipitated solid was filtered, washed with water, dried and recrystallized from suitable solvents. Their characterization data are given in Table 1.

5.2.1. ***3***. *IR (KBr, cm^{-1})*

3236 and 3153 (NH_2 assymmetric and symmetric stretching), 3054(Ar-H), 1625(C═N), 1561(C═C), 1188(C–F), 892(C–Cl); ^{1}H NMR (DMSO-d_6, δ): 4.74(s, 2H, NH_2), 7.33(s, 1H, $J_{H\text{-}F\ ortho} = 8.3$ HZ, 2,4-dichloro-5-fluorophenyl) 7.62(d, 1H,, $J_{H\text{-}F\ meta} = 1.8$ HZ, 2,4-dichloro-5-fluorophenyl), 10.46(s, 1H, NH/SH); FAB MS (*m*/*z*, %): 282(M + 4, 57) 280(M + 2, 54) 279(M^+ + 1, 100), 278(M^+, 64), 137(53), 189(33).

5.2.2. ***4a***: *IR (KBr, cm^{-1})*

3070(Ar-H), 2930 & 2876(–CH_2–), 1646(C═N), 1576(C═C), 1101(C–F), 865(C–Cl); ^{1}H NMR (DMSO-d_6, δ): 3.90(s, 2H, SCH_2), 7.0–7.8(m, 7H, aromatic protons); FAB MS (*m*/*z*, %):382(M + 4, 58), 380(M + 2, 47) 379(M^+ + 1, 100), 278(M^+, 71), 137(53), 189(33).

5.2.3. ***4b***:*IR (KBr, cm^{-1})*

3028(Ar-H), 2963 & 2895(-CH_2-), 1667(C═N), 1589(C═C), 1156(C–F), 878(C–Cl); ^{1}H NMR (DMSO-d_6, δ): 3.84(s, 2H, SCH_2), 6.93(d, 2H, $J = 8.8$ Hz, 4-Methoxyphenyl), 7.66(d, 2H, $J = 8.8$ Hz, 4-Methoxyphenyl), 7.28(s, 1H, $J_{H\text{-}F\ ortho} = 8.3$ Hz, 2,4-dichloro-5-fluorophenyl), 7.57(d, 1H, $J_{H\text{-}F\ meta} = 2.2$ Hz, 2,4-dichloro-5-fluorophenyl); FAB MS (*m*/*z*, %): 412(M + 4, 59),410 (M + 2, 55), 409 (M^+ + 1, 100), 143(63), 189(51).

5.2.4. ***4c*** *IR (KBr, cm^{-1})*

3033 (Ar–H), 2981, 2962 2879(CH_3 & –CH_2–), 1633(C═N0), 1542(C═C), 1176(C-F), 914(C–Cl),^{1}H NMR (DMSO-d_6, δ): 2.3(s, 3H, CH_3), 3.87(s, 2H, SCH_2), 7.17(d, 2H, $J = 8$ Hz, 4-Metthylphenyl),7.43(d, 2H, $J = 8$.Hz, 4-Methylphenyl), 7.39(s, 1H, $J_{H\text{-}F\ ortho} = 5.8$ Hz, 2,4-dichloro-5-fluorophenyl) 7.66(d, 1H, $J_{H\text{-}F\ meta} = 1.9$ Hz, 2,4-dichloro-5-fluorophenyl); FAB MS (*m*/*z*, %): 396(M + 4, 78), 392(M + 2, 74), (M^+ + 1, 100), 390((M^+, 49), 155(100), 189(37)).

5.2.5. ***4d*** *IR (KBr, cm^{-1})*

3048(Ar-H), 2988, 2872(–CH_2–), 1578(C═N), 1491(C═C),1155(C–F), 963(C–Cl); ^{1}H NMR (DMSO-d_6, δ): 2.3(s, 3H, CH_3), 3.89(s, 2H, SCH_2), 7.31(d, 2H, $J = 8.4$ Hz, 4-chlorophenyl),7.55(d, 2H, $J = 8.4$ Hz, 4-chlorophenyl),7.42(s, 1H, $J_{H\text{-}F\ ortho} = 6.2$ Hz, 2,4-dichloro-5-fluorophenyl) 7.78(d, 1H, $J_{H\text{-}F\ meta} = 2.3$ Hz, 2,4-dichloro-5-fluorophenyl); FAB MS (*m*/*z*, %): 416(M + 4, 78), 414(M + 2, 74), 413(M^+ + 1, 100), 412((M^+, 49), 137(100), 189(37)).

5.2.6. ***4e*** *IR (KBr, cm^{-1})*

3067(Ar-H), 2962, 2889(–CH_2–), 1666(C═N), 1551(C═C),1181(C–F), 963(C–Cl), 742 & 721(C–Br); ^{1}H NMR (DMSO-d_6, δ): 3.97(s, 2H, SCH_2), 7.35(d, 2H, $J = 8.2$ Hz, 4-chlorophenyl),7.62(d, 2H, $J = 8.2$ Hz, 4-chlorophenyl),7.49(s, 1H, $J_{H\text{-}F\ ortho} = 6.6$ Hz, 2,4-dichloro-5-fluorophenyl) 7.84(d, 1H, $J_{H\text{-}F\ meta} = 2.1$ Hz, 2,4-dichloro-5-fluorophenyl); FAB MS (*m*/*z*, %): 461(M + 4, 78), 459(M + 2, 74), 458(M^+ + 1, 100), 457((M^+, 49), 155(51), 189(44)).

5.2.7. ***4f*** *IR (KBr, cm^{-1})*

3078(Ar-H), 2940(-CH_2-), 1661(C═N), 1559(C═C),1197(C–F), 873 & 833(C–Cl); ^{1}H NMR (DMSO-d_6, δ): 3.84(s, 2H, SCH_2), 7.26(d, 1H, $J = 7.8$ Hz, 2,4-dichlorophenyl),7.44(d, 1H, $J = 7.8$ Hz, 2,4-dichlorophenyl), 7.61(s, 1H, 2,4-dichlorophenyl) 7.57(s, 1H, $J_{H\text{-}F\ ortho} = 5.2$ Hz, 2,4-dichloro-5-fluorophenyl) 7.73(d, 1H, $J_{H\text{-}F\ meta} = 1.9$ Hz, 2,4-dichloro-5-fluorophenyl); FAB MS (*m*/*z*, %): 450(M + 4, 83), 448(M + 2, 74), 447(M^+ + 1, 91), 446((M^+, 59), 155(33), 189(26)).

5.2.8. ***4g*** *IR (KBr, cm^{-1})*

3086(Ar–H), 2976 & 2866(–CH_2–), 1664(C═N), 1593(C═C),1126(C–F), 896 & 833(C–Cl); ^{1}H NMR (DMSO-d_6, δ): 3.89(s, 2H, SCH_2), 7.26–7.81(m, 4H, aromatic protons), FAB MS (*m*/*z*, %): 468(M + 4, 71), 466(M + 2, 67), 465(M^+ + 1, 100), 464((M^+, 76), 155(47), 189(38)).

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