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Synthesis, antimicrobial and cytotoxic activities of sulfone linked bis heterocycles

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ABSTRACT

A new class of sulfone linked bis heterocycles *viz*., pyrrolyl/pyrazolyl arylaminosulfonylmethyl 1,3,4-oxadiazoles, 1,3,4-thiadiazoles, and 1,2,4-triazoles were prepared and tested for antimicrobial activity and cytotoxicity. The chloro-substituted compounds **5c**, **8c** and **14c** showed comparable antibacterial activity to chloramphenicol against *Pseudomonasaeruginosa* and compound **5c** exhibited comparable antifungal activity to ketoconazole against *Penicilliumchrysogenum*. One of the compounds, vinylsulfonyl oxadiazole showed appreciably cytotoxic activity on A549 lung carcinoma cells with an IC_{50} at a concentration of 31.7 μM.

1. Introduction

In the past years considerable evidence has been accumulated to demonstrate the efficacy of five membered heterocycles *viz*., pyrrole, pyrazole, oxadiazole, thiadiazole, triazole and their derivatives. In fact tolmetin, ketorolac, the pyrrole derivatives were of pharmacological relevance due to their anti-inflammatory and analgesic properties [1]. Celecoxib, a pyrazole derivative is widely used in the market as an anti-inflammatory drug [1]. As a scaffold in medicinal chemistry, 1,3,4-oxadiazoles were established as a member of the privileged structures class [2]. In particular a few differently substituted 1,3,4-oxadiazoles have been found to exhibit anticancer activity [3–5]. 1,3,4-Thiadiazole nucleus constitutes the active part of several biologically active compounds including antibacterial [6–8], antimycotic [9,10] and anti-inflammatory agents [11–13]. The most frequently used triazoles are fluconazole and itraconazole that display a broad spectrum of antifungal activity and reduced toxicity when compared with imidazole antifungals [14–19]. Based on the above facts and our continued interest in the synthesis of biologically potent heterocyclic systems, the present work has been taken up to investigate the antimicrobial and cytotoxic activity of sulfone linked pyrrolyl-oxadiazoles/thiadiazoles/triazoles and pyrazolyl-oxadiazoles/thiadiazoles/triazoles.

2. Chemistry

The general synthetic pathway discussed hereafter is depicted in Scheme. The synthetic Scheme involves the cyclocondensation of arylaminosulfonylacetic acid hydrazide (**1**) and *E*-arylsulfonylethenesulfonylacetic acid (**2**) in the presence of $POCl_3$ to get 2-(arylaminosulfonylmethyl)-5-[*E*-(2-arylsulfonylvinylsulfonylmethyl)]-1,3,4-oxadiazole (**3**). The oxadiazole moiety was interconverted to thiadiazole by treating with thiourea in THF. Thus 2-(arylaminosulfonylmethyl)-5-[*E*-(2-arylsulfonylvinylsulfonylmethyl)]-1,3,4-thiadiazole (**4**) was prepared. However, compound 4-amino-3-(arylaminosulfonylmethyl)-5-[*E*-(2-arylsulfonylvinylsulfonylmethyl)]- 1,2,4-triazole (**5**) was obtained by the reaction of **3** with hydrazine hydrate in the presence of KOH in *n*-butanol (Scheme 1). The ^1H NMR spectra of **3a** and **4a** displayed two singlets at δ 5.50, 5.41 and 4.88, 5.12 due to methylene protons attached to C-2 and C-5 while **5a** at 5.30 and 5.15 ppm due to methylene protons attached to C-3 and C-5. The downfield signal was assigned to the one adjacent to sulfonamide moiety. In addition to this, one doublet was observed at 7.16, 6.83 and 6.77 ppm in these compounds due to olefin proton H_B. The olefin proton, H_A displayed a signal at downfield region, merged with

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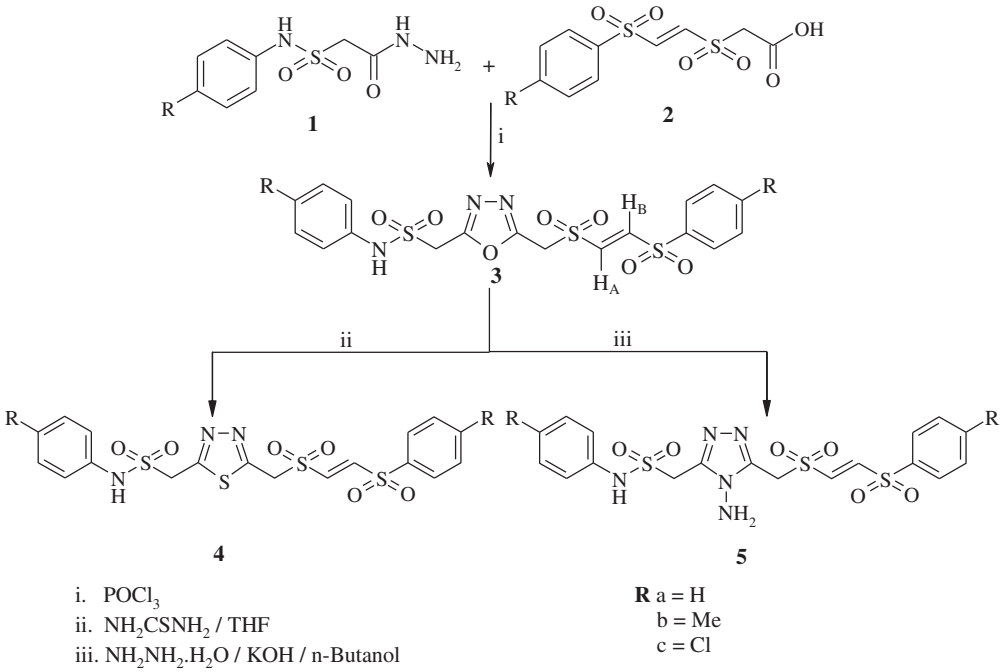


Scheme 1. Synthesis of 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazoles.

aromatic protons. The coupling constant value $J \approx 14.3$ Hz indicated that they possess *trans* geometry. The broad signals appeared at 10.24, 10.32 and 10.28 ppm in **3a**, **4a** and **5a** was assigned to NH which disappeared on deuteration. Compound **5a** also exhibited another broad signal at 5.68 ppm due to NH_2 which disappeared on deuteration.

The olefin moiety present in **3**, **4** and **5** was exploited to develop five membered heterocycles, pyrrole and pyrazole. The reaction of **3**, **4** and **5** with tosylmethyl isocyanide (TosMIC) in the presence of sodium hydride in a solvent mixture of DMSO and ether yielded 2-(arylamino-sulfonylmethyl)-5-(4′-arylsulfonyl- 1′H-pyrrol-3′-ylsulfonylmethyl)-1,3,4-oxadiazole (**6**), 2-(aryl-aminosulfonylmethyl)-5-(4′-arylsulfonyl-1′H-pyrrol-3′-ylsulfonylmethyl)-1,3,4-thiadiazole (**7**) and 4-amino-3-(arylaminosulfonylmethyl)-5-(4′-arylsulfonyl-1′H-pyrrol-3′-ylsulfonylmethyl)-1,2,4-triazole (**8**) (Scheme 2). The ^{1}H NMR spectra of **6a**, **7a** and **8a** displayed two singlets at δ 5.38, 5.29, 5.37 and 5.08, 5.18, 5.11 ppm due to two methylene protons attached to C-2 and C-5 of oxadiazole/thiadiazole and C-3 and C-5 of triazole units. Apart from these, one singlet was observed at 6.59, 6.65 and 6.73 due to $C_{5'}$—H of pyrrole ring whereas $C_{2'}$—H appeared at downfield region and merged with aromatic protons. Compounds **6a**, **7a** and **8a** also showed two broad singlets at 8.35, 8.39, 8.37 and at 10.40, 10.34, 10.35 ppm due to NH of pyrrole ring and NH—SO_2 which disappeared on deuteration. On the other hand, the 1,3-dipolar cycloaddition of diazomethane to **3**, **4** and **5** in the presence of Et_3N in ether at −20 °C for 40—48 h resulted in 2-(arylaminosulfonylmethyl)-5-(3′-arylsulfonyl-4′,5′-dihydro-1′ H-pyrazol-4′-ylsulfonylmethyl)-1,3,4-oxadiazole (**9**), 2-(arylaminosulfonylmethyl)-5-(3′-arylsulfonyl-4′,5′-dihydro-1′H-pyrazol-4′-ylsulfonylmethyl)-1,3,4-thiadiazole (**10**) and 4-amino-3-(arylaminosulfonylmethyl)-5-(3′-arylsulfonyl-4′,5′-dihydro-1′H-pyrazol-4′-ylsulfonylmethyl)-1,2,4-triazole (**11**). The pyrazoline ring protons displayed an AMX splitting pattern in the ^{1}H NMR spectra of **9a**, **10a** and **11a**. Thus three double doublets observed at δ 3.57, 4.24, 4.60 in **9a**, at 3.58, 4.29, 4.64 in **10a** and at 3.52, 4.21, 4.64 ppm in **11a** were assigned to H_X, H_M and H_A, respectively. The coupling constant values $J_{Ax} \approx 6.1$, $J_{Mx} \approx 10.2$ and $J_{AM} \approx 12.1$ Hz indicated that H_A, H_M are *cis*, H_A, H_x are *trans* while H_M, H_x are *geminal*. Oxidation of compound **9**, **10** and **11** was effected with chloranil in xylene to afford the aromatized compounds, 2-(arylaminos ulfonylmethyl)-5-(3′-arylsulfonyl-1′H-pyrazol-4′-ylsulfonylmethyl)-1,3,4-oxadiazole (**12**), 2-(arylaminosulfonylmethyl)-5-(3′-arylsulfonyl-1′H-pyrazol-4′-ylsulfonylmethyl)-1,3,4-thiadiazole (**13**) and 4-amino-3-(arylaminosulfonylmethyl)-5-(3′-arylsulfonyl-1′H-pyrazol-4′-ylsulfonylmethyl)-1,2,4-triazole (**14**) (Scheme 2). The absence of AMX splitting pattern due to pyrazoline ring protons in **12**, **13** and **14** indicated that aromatization took place. The structures of these compounds were further established by IR and ^{13}C NMR spectra.

3. Biology

3.1. Antimicrobial activity

Compounds **3**—**14** were tested for antimicrobial activity at two different concentrations 50 and 100 μg/ml.

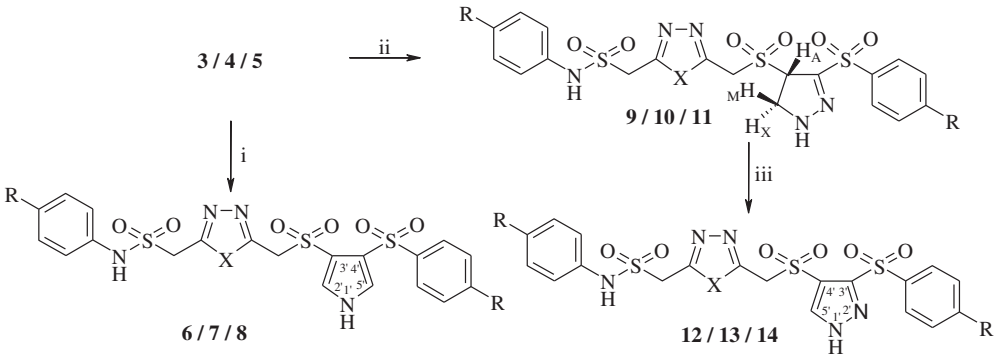
3.2. Cytotoxicity

Compounds **3**—**14** were subjected to MTT assay to determine cytotoxic capability.

4. Result and discussion

4.1. Antimicrobial activity

The results of antibacterial activity shown in Table 1 indicated that Gram-negative bacteria were more susceptible towards the tested compounds than Gram-positive ones. When compared to the standard drug Chloramphenicol it was seen that the chloro-substituted compounds **5c**, **8c** and **14c** were effective particularly against *Pseudomonas aeruginosa* at 100 μg/ml. Amongst bis heterocyclic compounds, the aromatized bis heterocycles **12**, **13** and **14** were effective than the non-aromatized compounds **9**, **10** and **11**. Amongst pyrrole and pyrazole containing bis heterocycles, the latter compounds **12**, **13** and **14** displayed greater activity. It was



Scheme 2. Synthesis of sulfonamido pyrrolyl/pyrazolyl 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazoles.

also observed that the compounds having thiadiazole **4**, **7**, **13** and triazole rings **5**, **8**, **14** were effective when compared with those having oxadiazole unit **3**, **6**, **12**.

All the tested compounds inhibited the spore germination against tested fungi except compound **9**. In general, most of the compounds showed slightly higher antifungal activity towards

Table 1
The *in vitro* antibacterial activity of compounds **3**–**14**.

Compound	Diameter of zone of inhibition (mm)							
	Gram-positive bacteria				Gram-negative bacteria			
	S. aureus		*B. subtilis*		*P. aeruginosa*		*K. pneumoniae*	
	50 μg/well	100 μg/well	50 μg/well	100 μg/well	50 μg/well	100 μg/well	50 μg/well	100 μg/well
3a	10	13	12	14	–	–	–	–
3b	–	–	–	–	–	–	–	–
3c	14	17	15	17	16	19	15	17
4a	19	21	22	24	16	17	24	26
4b	18	20	21	23	14	16	23	25
4c	29	32	27	29	25	27	33	35
5a	21	23	24	26	17	18	26	28
5b	20	22	23	25	15	17	25	27
5c	32	35	36	38	31	33	39	40
6a	12	14	13	15	–	–	–	–
6b	–	–	–	–	–	–	–	–
6c	18	20	17	21	18	20	23	25
7a	15	17	18	20	14	15	20	22
7b	14	16	17	19	13	14	19	21
7c	26	27	27	29	23	25	29	30
8a	17	19	20	22	15	16	22	24
8b	16	18	19	21	13	15	21	23
8c	30	33	29	33	25	28	34	36
9a	–	–	–	–	–	–	–	–
9b	–	–	–	–	–	–	–	–
9c	17	18	17	20	17	19	22	24
10a	12	14	14	16	–	–	–	–
10b	11	13	14	16	11	13	16	18
10c	24	27	25	26	22	23	25	28
11a	13	15	15	17	12	14	17	19
11b	13	15	16	18	12	15	18	20
11c	25	27	24	27	21	24	28	30
12a	10	12	13	15	10	12	15	17
12b	–	–	–	–	–	–	–	–
12c	20	23	21	22	19	21	23	24
13a	16	17	15	17	10	12	14	16
13b	12	14	10	13	8	11	13	15
13c	27	29	28	31	24	26	30	33
14a	19	21	19	23	15	17	24	26
14b	16	17	18	20	12	14	20	22
14c	31	34	33	35	30	32	37	39
Chloramphenicol	33	35	34	38	27	30	40	42
Control (DMSO)	–	–	–	–	–	–	–	–

(–) No activity.

Table 2
The *in vitro* antifungal activity of compounds **3**–**14**.

Compound	Diameter of zone of inhibition (mm)			
	A. niger		*P. chrysogenum*	
	50 μg/well	100 μg/well	50 μg/well	100 μg/well
3a	–	–	–	–
3b	–	–	–	–
3c	19	22	21	25
4a	19	22	21	24
4b	18	21	20	23
4c	31	33	32	35
5a	21	24	23	26
5b	19	23	22	25
5c	32	34	35	38
6a	–	–	–	–
6b	–	–	–	–
6c	21	24	22	26
7a	16	18	17	20
7b	14	17	16	19
7c	29	31	30	33
8a	18	20	19	22
8b	18	19	18	21
8c	32	34	33	36
9a	–	–	–	–
9b	–	–	–	–
9c	20	23	22	24
10a	–	–	–	–
10b	11	14	13	16
10c	28	29	28	31
11a	13	15	14	17
11b	13	16	15	18
11c	28	30	29	32
12a	10	13	12	15
12b	–	–	–	–
12c	20	23	21	24
13a	15	17	16	19
13b	10	13	11	13
13c	30	32	31	34
14a	19	23	20	23
14b	16	19	14	18
14c	30	34	33	37
Ketoconazole	33	36	36	38
Control (DMSO)	–	–	–	–

(–) No activity.

Penicillium chrysogenum than *Aspergillus niger*. Compounds **5c** and **14c** displayed comparable activity particularly against *P. chrysogenum* almost equivalent to the standard drug Ketoconazole (Table 2). This may be due to the presence of more electronegative atom *viz*., chlorine in the aromatic ring which may enhance the biological potency, bioavailability, metabolic stability and lipophilicity. Enhanced lipophilicity may lead to easier absorption and transportation of molecules within the biological systems.

The MIC, MBC and MFC values of the compounds tested are listed in Table 3. Compound **5c** exhibited low MIC values when compared with **8c** and **14c**. In addition MBC value is 2 × MIC in case of *Bacillussubtilis* and MFC value is 2 × MIC in case of

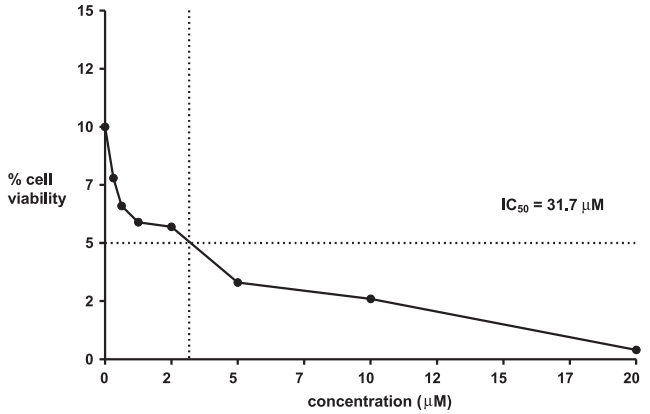


Fig. 1. The cytotoxic activity **3c** as measured by MTT assay on A549 lung carcinoma cells. *X*-axis shows the concentration of the compound and *Y*-axis, cell viability.

P. chrysogenum. However, the other compounds showed the bactericidal and fungicidal effects greater than 2 × MIC. The structure–antimicrobial activity relationship of the synthesized compounds revealed that the compounds having extended conjugation **3**, **4** and **5** exhibited greater activity than bis heterocycles **6**–**14**. Amongst bis heterocyclic compounds, the compounds having oxadiazole moiety exhibited least activity when compared with those having thiadiazole and triazole units. The compounds having chloro substituent on the aromatic ring showed greater activity.

4.2. Cytotoxicity

Compounds **3**–**14** were subjected to MTT assay to determine cytotoxic capability. Only compound **3c** showed appreciable cytotoxic activity on A549 cells ($IC_{50} = 31.7$ μM). However, all the other compounds did not show any cytotoxicity when used upto 0.2 mM concentration. Fig. 1 shows the results of cytotoxicity of **3c** using MTT assay. The cytotoxic activity observed with compound **3c** is concentration dependent. Compound **3c** at concentrations 100–200 μM showed lowest viability, while viability more than 75% was observed when this compound was used at concentrations below 12.50 μM. This suggests compound **3c** is a potential lead molecule for cytotoxic activity against tumour cells. Further modifications to **3c** may provide a more potent molecule that could be developed as therapeutic drug. Future efforts are in progress towards this goal.

5. Conclusion

In conclusion we have prepared a new class of sulfonamido mono and bis heterocycles and studied their antimicrobial and cytotoxic activities. The chloro-substituted compounds **5c**, **8c** and **14c** showed moderate and comparable antibacterial activity to chloramphenicol against *P. aeruginosa* and compound **5c** exhibited

Table 3
MIC, MBC and MFC of compounds **5c**, **8c** and **14c**.

Compound	Minimum inhibitory concentration					
	MIC (MBC/MFC) μg/ml					
	S. aureus	*B. subtilis*	*P. aeruginosa*	*K. pneumoniae*	*A. niger*	*P. chrysogenum*
5c	12.5(50)	12.5(25)	12.5(50)	25(100)	12.5(100)	25(50)
8c	50(200)	25(100)	50(>200)	100(>200)	25(100)	50(>200)
14c	50(200)	12.5(50)	25(100)	50(200)	12.5(50)	25(100)
Chloramphenicol	6.25	6.25	6.25	12.5	–	–
Ketoconazole	–	–	–	–	6.25	12.5

comparable antifungal activity to ketoconazole against *P. chrysogenum*. It was observed that compound **3c** displayed appreciable cytotoxic activity that could be used as a lead compound in the future studies.

6. Experimental

6.1. Chemistry

Melting points were determined in open capillaries on a Mel-Temp apparatus and are uncorrected. The purity of the compounds was checked by TLC (silica gel H, BDH, hexane/ethyl acetate, 3:1). The IR spectra were run on a Thermo Nicolet IR 200 FT-IR spectrometer as KBr pellets and the wave numbers were given in cm^{-1}. The ^1H NMR spectra were recorded in DMSO-d_6 on a Jeol JNM λ-400 MHz. The ^{13}C NMR spectra were recorded in DMSO-d_6 on a Jeol JNM spectrometer operating at 100 MHz. All chemical shifts are reported in δ (ppm) using TMS as an internal standard. The microanalyses were performed on a Perkin–Elmer 240C elemental analyser.

6.1.1. General procedure for the synthesis of 2-(arylaminosulfonyl methyl)-5-[E-(2-arylsulfonylvinylsulfonylmethyl)]-1,3,4-oxadiazole **3a–c**

A mixture of arylaminosulfonylacetic acid hydrazide (**1**) (10 mmol), *E*-arylsulfonylethenesulfonylacetic acid (**2**) (10 mmol) and $POCl_3$ (7 ml) was heated under reflux for 3–5 h. The excess $POCl_3$ was removed under reduced pressure and the residue was poured onto crushed ice. The resulting precipitate was filtered, washed with saturated sodium bicarbonate solution and then with water. It was dried and recrystallized from ethanol.

*6.1.1.1. 2-(Phenylaminosulfonylmethyl)-5-[E-(2-phenylsulfonylvinyl sulfonylmethyl)]-1,3,4-oxadiazole (**3a**).* White solid (3.48 g, 72%); m.p. 132–134 °C; IR (KBr): 1152, 1312 (SO_2), 1574 (C=N), 1635 (C=C), 3231 (NH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 4.88 (s, 2H, SO_2-CH_2), 5.50 (s, 2H, CH_2-SO_2-NH), 7.16 (d, 1H, H_B, $J = 14.3$ Hz), 7.23–8.00 (m, 11H, H_A & Ar–H), 10.24 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): δ 47.1 (SO_2-CH_2), 49.5 (CH_2-SO_2-NH), 120.7 (C–H_B), 142.9 (C–H_A), 157.8 (C-5), 159.4 (C-2), 125.1, 125.8, 126.3, 126.9, 128.0, 129.4, 130.1, 131.5 (aromatic carbons) ppm; Anal. Calcd. for $C_{18}H_{17}N_3O_7S_3$: C, 44.71; H, 3.54; N, 8.69; Found: C, 44.79; H, 3.52; N, 8.80%.

*6.1.1.2. 2-(p-Methylphenylaminosulfonylmethyl)-5-[E-(2-p-methylphenylsulfonylvinyl-sulfonylmethyl)]-1,3,4-oxadiazole (**3b**).* White solid (3.58 g, 70%); m.p. 114–116 °C; IR (KBr): 1148, 1308 (SO_2), 1562 (C=N), 1624 (C=C), 3217 (NH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.24 & 2.28 (s, 6H, Ar–CH_3), 4.84 (s, 2H, SO_2-CH_2), 5.39 (s, 2H, CH_2-SO_2-NH), 6.46 (d, 1H, H_B, $J = 14.3$ Hz), 7.14–7.92 (m, 9H, H_A & Ar–H), 10.25 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): δ 20.6 & 21.5 (Ar–CH_3), 46.8 (SO_2-CH_2), 45.5 (CH_2-SO_2-NH), 124.8 (C–H_B), 143.2 (C–H_A), 157.3 (C-5), 158.8 (C-2), 123.5, 124.1, 124.8, 125.2, 126.4, 127.3, 129.1, 132.4 (aromatic carbons) ppm; Anal. Calcd. for $C_{20}H_{21}N_3O_7S_3$: C, 46.95; H, 4.14; N, 8.21; Found: C, 46.90; H, 4.15; N, 8.28%.

*6.1.1.3. 2-(p-Chlorophenylaminosulfonylmethyl)-5-[E-(2-p-chlorophenylsulfonylvinyl-sulfonylmethyl)]-1,3,4-oxadiazole (**3c**).* White solid (4.14 g, 75%); m.p. 154–156 °C; IR (KBr): 1157, 1316 (SO_2), 1575 (C=N), 1631 (C=C), 3238 (NH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 4.92 (s, 2H, SO_2-CH_2), 5.45 (s, 2H, CH_2-SO_2-NH), 6.99 (d, 1H, H_B, $J = 14.6$ Hz), 7.24–8.00 (m, 9H, H_A & Ar–H), 10.39 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): δ 44.8 (SO_2-CH_2), 49.3 (CH_2-SO_2-NH), 126.1 (C–H_B), 145.5 (C–H_A), 158.5 (C-5), 159.2 (C-2), 125.6, 126.1, 128.4, 129.0, 129.8, 130.8, 132.4, 135.7 (aromatic carbons) ppm; Anal. Calcd. for $C_{18}H_{15}Cl_2N_3O_7S_3$: C, 39.17; H, 2.74; N, 7.61; Found: C, 39.17; H, 2.77; N, 7.58%.

6.1.2. General procedure for the synthesis of 2-(arylaminosulfonyl methyl)-5-[E-(2-arylsulfonylvinylsulfonylmethyl)]-1,3,4-thiadiazole **4a–c**

In a sealed test tube, compound **3** (5 mmol), thiourea (20 mmol) and tetrahydrofuran (5 ml) were taken and heated at 120–150 °C in an oil bath for 18–21 h. After the reaction was completed, it was extracted with dichloromethane. The organic layer was washed with water, brine solution and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the resultant solid was recrystallized from methanol.

*6.1.2.1. 2-(Phenylaminosulfonylmethyl)-5-[E-(2-phenylsulfonylvinyl sulfonylmethyl)]-1,3,4-thiadiazole (**4a**).* White solid (1.63 g, 68%); m.p. 165–167 °C; IR (KBr): 1138, 1321 (SO_2), 1568 (C=N), 1632 (C=C), 3248 (NH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 5.12 (s, 2H, SO_2-CH_2), 5.41 (s, 2H, CH_2-SO_2-NH), 6.83 (d, 1H, H_B, $J = 14.2$ Hz), 7.20–7.74 (m, 11H, H_A & Ar–H), 10.32 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): δ 44.3 (SO_2-CH_2), 52.2 (CH_2-SO_2-NH), 123.8 (C–H_B), 143.0 (C–H_A), 157.9 (C-5), 158.2 (C-2), 126.8, 127.3, 128.1, 128.8, 129.5, 131.2, 132.6, 134.6 (aromatic carbons) ppm; Anal. Calcd. for $C_{18}H_{17}N_3O_6S_4$: C, 43.27; H, 3.43; N, 8.41; Found: C, 43.23; H, 3.45; N, 8.51%.

*6.1.2.2. 2-(p-Methylphenylaminosulfonylmethyl)-5-[E-(2-p-methylphenylsulfonylvinyl-sulfonylmethyl)]-1,3,4-thiadiazole (**4b**).* White solid (1.71 g, 65%); m.p. 180–182 °C; IR (KBr): 1131, 1319 (SO_2), 1561 (C=N), 1625 (C=C), 3237 (NH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.26 & 2.31 (s, 6H, Ar–CH_3), 5.10 (s, 2H, SO_2-CH_2), 5.40 (s, 2H, CH_2-SO_2-NH), 6.79 (d, 1H, H_B, $J = 14.6$ Hz), 7.16–7.71 (m, 9H, H_A & Ar–H), 10.30 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): δ 21.1 & 22.4 (Ar–CH_3), 43.6 (SO_2-CH_2), 50.8 (CH_2-SO_2-NH), 122.6 (C–H_B), 142.8 (C–H_A), 158.2 (C-5), 159.8 (C-2), 126.4, 127.1, 127.7, 128.4, 129.7, 130.2, 130.9, 132.8 (aromatic carbons) ppm; Anal. Calcd. for $C_{20}H_{21}N_3O_6S_4$: C, 45.52; H, 4.01; N, 7.96; Found: C, 45.58; H, 3.99; N, 8.02%.

*6.1.2.3. 2-(p-Chlorophenylaminosulfonylmethyl)-5-[E-(2-p-chlorophenylsulfonylvinyl-sulfonylmethyl)]-1,3,4-thiadiazole (**4c**).* White solid (1.90 g, 67%); m.p. 192–194 °C; IR (KBr): 1128, 1327 (SO_2), 1572 (C=N), 1628 (C=C), 3255 (NH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 5.19 (s, 2H, SO_2-CH_2), 5.46 (s, 2H, CH_2-SO_2-NH), 6.89 (d, 1H, H_B, $J = 14.8$ Hz), 7.19–7.65 (m, 9H, H_A & Ar–H), 10.35 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): δ 43.5 (SO_2-CH_2), 51.8 (CH_2-SO_2-NH), 124.2 (C–H_B), 143.6 (C–H_A), 157.5 (C-5), 158.4 (C-2), 126.5, 127.4, 128.1, 128.8, 129.3, 130.4, 132.1, 134.6 (aromatic carbons) ppm; Anal. Calcd. for $C_{18}H_{15}Cl_2N_3O_6S_4$: C, 38.03; H, 2.66; N, 7.39; Found: C, 38.07; H, 2.70; N, 7.32%.

6.1.3. General procedure for the synthesis of 4-amino-3-(arylamino sulfonylmethyl)-5-[E-(2-arylsulfonylvinylsulfonylmethyl)]-1,2,4-triazole **5a–c**

To a solution of **3** (5 mmol) in *n*-butanol (25 ml), hydrazine hydrate (15 mmol) was added and refluxed for 7–9 h. Then KOH (0.01 mol) was added to the reaction media and the precipitate formed was filtered. The solid obtained was acidified with conc. HCl to pH = 3 and washed with water. It was dried and recrystallized from ethanol.

*6.1.3.1. 4-Amino-3-(phenylaminosulfonylmethyl)-5-[E-(2-phenylsulfonylvinylsulfonyl-methyl)]-1,2,4-triazole (**5a**).* White solid (1.71 g, 69%); m.p. 170–172 °C; IR (KBr): 1132, 1324 (SO_2), 1559 (C=N), 1624 (C=C), 3251 (NH), 3358, 3474 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): δ 5.15 (s, 2H, SO_2-CH_2), 5.30 (s, 2H, CH_2-SO_2-NH), 5.68 (bs, 2H, NH_2), 6.77 (d, 1H, H_B, $J = 14.4$ Hz), 7.11–7.78 (m, 11H, H_A & Ar–H),

10.28 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): δ 45.8 (SO_2-CH_2), 51.3 (CH_2-SO_2-NH), 121.8 (C–H_B), 143.2 (C–H_A), 158.1 (C-5), 159.3 (C-3), 125.4, 126.1, 126.7, 128.4, 129.0, 130.2, 132.1, 133.4 (aromatic carbons) ppm; Anal. Calcd. for $C_{18}H_{19}N_5O_6S_3$: C, 43.45; H, 3.85; N, 14.08; Found: C, 43.41; H, 3.82; N, 14.17%.

6.1.3.2. 4-Amino-3-(p-methylphenylaminosulfonylmethyl)-5-[E-(2-p-methylphenyl-sulfonylvinylsulfonylmethyl)]-1,2,4-triazole (***5b***). White solid (1.73 g, 66%); m.p. 195–197 °C; IR (KBr): 1126, 1321 (SO_2), 1561 (C=N), 1622 (C=C), 3254 (NH), 3356, 3470 (NH_2) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.22 & 2.26 (s, 6H, Ar–CH_3), 5.14 (s, 2H, SO_2-CH_2), 5.35 (s, 2H, CH_2–SO_2–NH), 5.59 (bs, 2H, NH_2), 6.74 (d, 1H, H_B, J = 14.2 Hz), 7.18–7.90 (m, 9H, H_A & Ar–H), 10.26 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): δ 20.8 & 21.4 (Ar–CH_3), 45.1 (SO_2-CH_2), 51.0 (CH_2-SO_2-NH), 122.7 (C–H_B), 140.6 (C–H_A), 158.5 (C-5), 159.7 (C-3), 126.3, 127.3, 127.9, 128.6, 130.1, 130.8, 131.4, 133.2 (aromatic carbons) ppm; Anal. Calcd. for $C_{20}H_{23}N_5O_6S_3$: C, 45.70; H, 4.41; N, 13.32; Found: C, 45.73; H, 4.45; N, 13.38%.

6.1.3.3. 4-Amino-3-(p-chlorophenylaminosulfonylmethyl)-5-[E-(2-p-chlorophenylsulfonyl-vinylsulfonylmethyl)]-1,2,4-triazole (***5c***). White solid (1.98 g, 70%); m.p. 206–208 °C; IR (KBr): 1137, 1330 (SO_2), 1567 (C=N), 1630 (C=C), 3267 (NH), 3369, 3479 (NH_2) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.14 (s, 2H, SO_2-CH_2), 5.32 (s, 2H, CH_2–SO_2–NH), 5.72 (bs, 2H, NH_2), 6.82 (d, 1H, H_B, J = 14.7 Hz), 7.14–7.67 (m, 9H, H_A & Ar–H), 10.31 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): δ 46.1 (SO_2-CH_2), 51.5 (CH_2-SO_2-NH), 123.0 (C–H_B), 142.3 (C–H_A), 158.2 (C-5), 158.8 (C-3), 127.3, 127.9, 128.4, 129.0, 129.6, 130.2, 131.4, 133.7 (aromatic carbons) ppm; Anal. Calcd. for $C_{18}H_{17}Cl_2N_5O_6S_3$: C, 38.17; H, 3.02; N, 12.36; Found: C, 38.22; H, 3.00; N, 12.44%.

6.1.4. General procedure for the synthesis of 2-(arylaminosulfonyl methyl)-5-(4′-arylsulfonyl-1′H-pyrrol-3′-ylsulfonylmethyl)-1,3,4-oxadiazole ***6a–c****/2-(arylaminosulfonylmethyl)-5-(4′-arylsulfonyl-1′H-pyrrol-3′-ylsulfonylmethyl)-1,3,4-thiadiazole* ***7a–c****/4-amino-3-(aryl aminosulfonylmethyl)-5-(4′-arylsulfonyl-1′H-pyrrol-3′-ylsulfonyl methyl)-1,2,4-triazole* ***8a–c***

Compound **3**/**4**/**5** (1 mmol) and TosMIC (1 mmol) in Et_2O–DMSO (2:1) were added dropwise under stirring to a suspension of NaH (50 mg) in Et_2O (20 ml) at room temperature and stirring was continued for 6–8 h. Then, water was added and the reaction mass was extracted with Et_2O. The ethereal layer was dried over anhydrous Na_2SO_4 and filtered. Evaporation of the solvent *in vacuo* gave a crude product which was purified by column chromatography using silica gel (hexane–ethyl acetate; 4:1).

6.1.4.12-(Phenylaminosulfonylmethyl)-5-(4′-phenylsulfonyl-1′H-pyrrol-3′-ylsulfonyl-methyl)-1,3,4-oxadiazole (***6a***). White solid (0.35 g, 67%); m.p. 140–142 °C; IR (KBr): 1141, 1335 (SO_2), 1581 (C=N), 1631 (C=C), 3250 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.08 (s, 2H, SO_2-CH_2), 5.38 (s, 2H, CH_2–SO_2–NH), 6.59 (s, 1H, $C_{5'}$–H), 7.22–7.81 (m, 11H, $C_{2'}$–H & Ar–H), 8.35 (bs, 1H, NH), 10.40 (bs, 1H, NH–SO_2) ppm; ^{13}C NMR (DMSO-d_6): δ 45.6 (SO_2-CH_2), 50.8 (CH_2-SO_2-NH), 106.2 (C-4′), 111.8 (C-3′), 117.2 (C-5′), 122.6 (C-2′), 155.2 (C-5), 158.7 (C-2), 126.4, 127.1, 127.6, 128.2, 129.2, 130.4, 132.8, 135.2 (aromatic carbons) ppm; Anal. Calcd. for $C_{20}H_{18}N_4O_7S_3$: C, 45.97; H, 3.47; N, 10.72; Found: C, 46.00; H, 3.50; N, 10.67%.

6.1.4.22-(p-Methylphenylaminosulfonylmethyl)-5-(4′-p-methylphenylsulfonyl-1′H-pyrrol-3′-ylsulfonylmethyl)-1,3,4-oxadiazole (***6b***). White solid (0.35 g, 65%); m.p. 153–155 °C; IR (KBr): 1136, 1327 (SO_2), 1577 (C=N), 1628 (C=C), 3252 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.23 & 2.28 (s, 6H, Ar–CH_3), 5.16 (s, 2H, SO_2-CH_2), 5.37 (s, 2H, CH_2–SO_2–NH), 6.53 (s, 1H, $C_{5'}$–H), 7.19–7.74 (m, 9H, $C_{2'}$–H & Ar–H), 8.38 (bs, 1H, NH), 10.38 (bs, 1H, NH–SO_2) ppm; ^{13}C NMR (DMSO-d_6): δ 21.5 & 22.9 (Ar–CH_3), 44.8 (SO_2-CH_2), 51.2 (CH_2-SO_2-NH), 105.6 (C-4′), 110.2 (C-3′), 116.5 (C-5′), 121.2 (C-2′), 155.8 (C-5), 158.1 (C-2), 126.3, 127.4, 128.0, 128.7, 129.3, 130.4, 132.6, 134.5 (aromatic carbons) ppm; Anal. Calcd. for $C_{22}H_{22}N_4O_7S_3$: C, 47.99; H, 4.03; N, 10.18; Found: C, 47.93; H, 4.00; N, 10.28%.

6.1.4.32-(p-Chlorophenylaminosulfonylmethyl)-5-(4′-p-chlorophenyl sulfonyl-1′H-pyrrol-3′-ylsulfonylmethyl)-1,3,4-oxadiazole (***6c***). White solid (0.41 g, 71%); m.p. 162–164 °C; IR (KBr): 1144, 1336 (SO_2), 1585 (C=N), 1634 (C=C), 3265 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.13 (s, 2H, SO_2-CH_2), 5.35 (s, 2H, CH_2–SO_2–NH), 6.62 (s, 1H, $C_{5'}$–H), 7.25–7.94 (m, 9H, $C_{2'}$–H & Ar–H), 8.40 (bs, 1H, NH), 10.45 (bs, 1H, NH–SO_2) ppm; ^{13}C NMR (DMSO-d_6): δ 46.4 (SO_2-CH_2), 51.7 (CH_2-SO_2-NH), 106.9 (C-4′), 112.3 (C-3′), 117.8 (C-5′), 123.5 (C-2′), 156.3 (C-5), 159.4 (C-2), 127.3, 128.1, 129.5, 130.8, 132.7, 133.2, 134.8, 136.7 (aromatic carbons) ppm; Anal. Calcd. for $C_{20}H_{16}Cl_2N_4O_7S_3$: C, 40.61; H, 2.73; N, 9.47; Found: C, 40.65; H, 2.76; N, 9.52%.

6.1.4.42-(Phenylaminosulfonylmethyl)-5-(4′-phenylsulfonyl-1′H-pyrrol-3′-ylsulfonyl-methyl)-1,3,4-thiadiazole (***7a***). White solid (0.38 g, 72%); m.p. 167–169 °C; IR (KBr): 1141, 1324 (SO_2), 1591 (C=N), 1627 (C=C), 3241 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.18 (s, 2H, SO_2-CH_2), 5.29 (s, 2H, CH_2–SO_2–NH), 6.65 (s, 1H, $C_{5'}$–H), 7.10–7.81 (m, 11H, $C_{2'}$–H & Ar–H), 8.39 (bs, 1H, NH), 10.34 (bs, 1H, NH–SO_2) ppm; ^{13}C NMR (DMSO-d_6): δ 45.9 (SO_2-CH_2), 51.3 (CH_2-SO_2-NH), 104.3 (C-4′), 112.2 (C-3′), 115.3 (C-5′), 121.8 (C-2′), 154.8 (C-5), 158.4 (C-2), 126.8, 127.5, 128.1, 129.4, 130.3, 130.9, 132.5, 136.4 (aromatic carbons) ppm; Anal. Calcd. for $C_{20}H_{18}N_4O_6S_4$: C, 44.60; H, 3.37; N, 10.40; Found: C, 44.57; H, 3.38; N, 10.47%.

6.1.4.52-(p-Methylphenylaminosulfonylmethyl)-5-(4′-p-methylphenylsulfonyl-1′H-pyrrol-3′-ylsulfonylmethyl)-1,3,4-thiadiazole (***7b***). White solid (0.36 g, 65%); m.p. 175–177 °C; IR (KBr): 1133, 1322 (SO_2), 1583 (C=N), 1622 (C=C), 3237 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.28 & 2.32 (s, 6H, Ar–CH_3), 5.12 (s, 2H, SO_2-CH_2), 5.26 (s, 2H, CH_2–SO_2–NH), 6.68 (s, 1H, $C_{5'}$–H), 7.08–7.86 (m, 9H, $C_{2'}$–H & Ar–H), 8.32 (bs, 1H, NH), 10.29 (bs, 1H, NH–SO_2) ppm; ^{13}C NMR (DMSO-d_6): δ 20.6 & 21.8 (Ar–CH_3), 44.6 (SO_2-CH_2), 50.9 (CH_2-SO_2-NH), 103.8 (C-4′), 111.5 (C-3′), 112.4 (C-5′), 120.5 (C-2′), 154.6 (C-5), 158.3 (C-2), 126.5, 127.4, 128.1, 128.9, 131.2, 132.4, 133.5, 136.7 (aromatic carbons) ppm; Anal. Calcd. for $C_{22}H_{22}N_4O_6S_4$: C, 46.63; H, 3.91; N, 9.89; Found: C, 46.68; H, 3.90; N, 9.84%.

6.1.4.62-(p-Chlorophenylaminosulfonylmethyl)-5-(4-p-chlorophenylsulfonyl-1′H-pyrrol-3′-ylsulfonylmethyl)-1,3,4-thiadiazole (***7c***). White solid (0.44 g, 73%); m.p. 192–194 °C; IR (KBr): 1143, 1331 (SO_2), 1594 (C=N), 1631 (C=C), 3245 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.16 (s, 2H, SO_2-CH_2), 5.33 (s, 2H, CH_2–SO_2–NH), 6.71 (s, 1H, $C_{5'}$–H), 7.13–7.78 (m, 9H, $C_{2'}$–H & Ar–H), 8.36 (bs, 1H, NH), 10.33 (bs, 1H, NH–SO_2) ppm; ^{13}C NMR (DMSO-d_6): δ 46.8 (SO_2-CH_2), 52.1 (CH_2-SO_2-NH), 105.2 (C-4′), 112.9 (C-3′), 116.7 (C-5′), 122.7 (C-2′), 155.0 (C-5), 159.8 (C-2), 127.1, 128.4, 129.6, 130.2, 130.8, 132.2, 133.8, 136.2 (aromatic carbons) ppm; Anal. Calcd. for $C_{20}H_{16}Cl_2N_4O_6S_4$: C, 39.54; H, 2.65; N, 9.22; Found: C, 39.50; H, 2.62; N, 9.26%.

6.1.4.74-Amino-3-(phenylaminosulfonylmethyl)-5-(4′-phenylsulfonyl-1′H-pyrrol-3′-ylsulfonylmethyl)-1,2,4-triazole (***8a***). White solid (0.39 g, 74%); m.p. 179–181 °C; IR (KBr): 1135, 1334 (SO_2), 1596 (C=N), 1630 (C=C), 3266 (NH), 3318, 3445 (NH_2) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.11 (s, 2H, SO_2-CH_2), 5.37 (s, 2H, CH_2–SO_2–NH), 5.50 (bs, 2H, NH_2), 6.73 (s, 1H, $C_{5'}$–H), 7.15–7.80 (m, 11H, $C_{2'}$–H & Ar–H), 8.37 (bs, 1H, NH), 10.35 (bs, 1H, NH–SO_2) ppm; ^{13}C NMR (DMSO-d_6): δ 47.0 (SO_2-CH_2), 52.8 (CH_2-SO_2-NH), 105.7 (C-4′), 108.3 (C-3′), 114.3 (C-5′), 123.4 (C-2′), 155.6 (C-5), 158.7 (C-3), 127.1, 128.4,

128.9, 129.5, 131.7, 132.4, 133.3, 137.5 (aromatic carbons) ppm; Anal. Calcd. for $C_{20}H_{20}N_6O_6S_3$: C, 44.77; H, 3.76; N, 15.66; Found: C, 44.72; H, 3.77; N, 15.75%.

*6.1.4.84-Amino-3-(p-methylphenylaminosulfonylmethyl)-5-(4′-p-methylphenylsulfonyl-1′H-pyrrol-3′-ylsulfonylmethyl)-1,2,4-triazole (**8b**).* White solid (0.42 g, 76%); m.p. 187–189 °C; IR (KBr): 1132, 1326 (SO_2), 1583 (C=N), 1627 (C=C), 3262 (NH), 3312, 3437 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.25 & 2.30 (s, 6H, Ar–CH_3), 5.16 (s, 2H, SO_2–CH_2), 5.33 (s, 2H, CH_2–SO_2–NH), 5.52 (bs, 2H, NH_2), 6.68 (s, 1H, $C_{5'}$–H), 7.11–7.76 (m, 9H, $C_{2'}$–H & Ar–H), 8.29 (bs, 1H, NH), 10.38 (bs, 1H, NH–SO_2) ppm; ^{13}C NMR (DMSO-d_6): δ 21.4 & 22.7 (Ar–CH_3), 46.6 (SO_2–CH_2), 52.2 (CH_2–SO_2–NH), 104.3 (C-4′), 107.5 (C-3′), 113.8 (C-5′), 121.1 (C-2′), 154.2 (C-5), 158.5 (C-3), 127.4, 128.1, 128.6, 129.2, 130.0, 131.2, 133.4, 135.8 (aromatic carbons) ppm; Anal. Calcd. for $C_{22}H_{24}N_6O_6S_3$: C, 46.80; H, 4.28; N, 14.88; Found: C, 46.86; H, 4.30; N, 14.94%.

*6.1.4.9. 4-Amino-3-(p-chlorophenylaminosulfonylmethyl)-5-(4′-p-chlorophenylsulfonyl-1′H-pyrrol-3′-ylsulfonylmethyl)-1,2,4-triazole (**8c**).* White solid (0.43 g, 72%); m.p. 206–208 °C; IR (KBr): 1139, 1338 (SO_2), 1598 (C=N), 1634 (C=C), 3272 (NH), 3327, 3448 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): δ 5.14 (s, 2H, SO_2–CH_2), 5.39 (s, 2H, CH_2–SO_2–NH), 5.54 (bs, 2H, NH_2), 6.79 (s, 1H, $C_{5'}$–H), 7.17–7.84 (m, 9H, $C_{2'}$–H & Ar–H), 8.33 (bs, 1H, NH), 10.42 (bs, 1H, NH–SO_2) ppm; ^{13}C NMR (DMSO-d_6): δ 48.2 (SO_2–CH_2), 53.0 (CH_2–SO_2–NH), 106.2 (C-4′), 109.2 (C-3′), 116.2 (C-5′), 124.0 (C-2′), 155.9 (C-5), 159.1 (C-3), 127.3, 127.9, 128.5, 129.4, 130.7, 131.8, 132.5, 136.6 (aromatic carbons) ppm; Anal. Calcd. for $C_{20}H_{18}Cl_2N_6O_6S_3$: C, 39.67; H, 3.00; N, 13.88; Found: C, 39.72; H, 3.01; N, 13.84%.

*6.1.5. General procedure for the synthesis of 2-(arylaminosulfonylmethyl)-5-(3′-arylsulfonyl-4′,5′-dihydro-1′H-pyrazol-4′-ylsulfonylmethyl)-1,3,4-oxadiazole **9a**–**c**/2-(arylaminosulfonyl-methyl)-5-(3′-arylsulfonyl-4′,5′-dihydro-1′H-pyrazol-4′-ylsulfonylmethyl)-1,3,4-thiadiazole **10a**–**c**/4-amino-3-(arylaminosulfonylmethyl)-5-(3′-arylsulfonyl-4′,5′-dihydro-1′H-pyrazol-4′-ylsulfonylmethyl)-1,2,4-triazole **11a**–**c***

To a cooled solution of compound **3**/**4**/**5** (5 mmol) in dichloromethane (20 ml), an ethereal solution of diazomethane (40 ml, 0.4 M) and triethylamine (0.12 g) were added. The reaction mixture was kept at −20 to −15 °C for 48 h. The solvent was removed under reduced pressure and the resultant residue was passed through a column of silica gel using hexane and ethyl acetate (3:1) as eluent.

*6.1.5.1. 2-(Phenylaminosulfonylmethyl)-5-(3′-phenylsulfonyl-4′,5′-dihydro-1′H-pyrazol-4′-ylsulfonylmethyl)-1,3,4-oxadiazole (**9a**).* White solid (1.83 g, 70%); m.p. 152–154 °C; IR (KBr): 1141, 1335 (SO_2), 1586 (C=N), 3253 (NH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.57 (dd, 1H, H_x, J_{Ax} = 6.3 Hz, J_{Mx} = 10.8 Hz), 4.24 (dd, 1H, H_M, J_{AM} = 12.1 Hz), 4.60 (dd, 1H, H_A), 4.99 (s, 2H, SO_2–CH_2), 5.31 (s, 2H, CH_2–SO_2–NH), 7.17–7.79 (m, 10H, Ar–H), 10.03 (bs, 1H, NH), 10.35 (bs, 1H, NH–SO_2) ppm; ^{13}C NMR (DMSO-d_6): δ 47.1 (SO_2–CH_2), 51.6 (CH_2–SO_2–NH), 53.2 (C-5′), 67.1 (C-4′), 153.2 (C-3′), 157.6 (C-5), 159.2 (C-2), 127.4, 128.8, 129.6, 130.9, 131.7, 132.5, 133.4, 134.9 (aromatic carbons) ppm; Anal. Calcd. for $C_{19}H_{19}N_5O_7S_3$: C, 43.42; H, 3.64; N, 13.33; Found: C, 43.48; H, 3.66; N, 13.39%.

*6.1.5.22-(p-Methylphenylaminosulfonylmethyl)-5-(3′-p-methylphenylsulfonyl-4′,5′-dihydro-1′H-pyrazol-4′-ylsulfonylmethyl)-1,3,4-oxadiazole (**9b**).* White solid (1.82 g, 66%); m.p. 159–161 °C; IR (KBr): 1136, 1331 (SO_2), 1588 (C=N), 3256 (NH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.27 & 2.32 (s, 6H, Ar–CH_3), 3.55 (dd, 1H, H_x, J_{Ax} = 6.2 Hz, J_{Mx} = 10.3 Hz), 4.15 (dd, 1H, H_M, J_{AM} = 12.0 Hz), 4.53 (dd, 1H, H_A), 4.92 (s, 2H, SO_2–CH_2), 5.26 (s, 2H, CH_2–SO_2–NH), 7.10–7.72 (m, 8H, Ar–H), 9.98 (bs, 1H, NH), 10.33 (bs, 1H, NH–SO_2) ppm; ^{13}C NMR (DMSO-d_6): δ 20.6 & 21.7 (Ar–CH_3), 46.5 (SO_2–CH_2), 52.4 (CH_2–SO_2–NH), 52.8 (C-5′), 68.5 (C-4′), 152.7 (C-3′), 156.5 (C-5), 158.7 (C-2), 126.7, 127.4, 128.1, 128.7, 129.3, 130.4, 132.6, 134.8 (aromatic carbons) ppm; Anal. Calcd. for $C_{21}H_{23}N_5O_7S_3$: C, 45.56; H, 4.19; N, 12.65; Found: C, 45.52; H, 4.18; N, 12.72%.

*6.1.5.32-(p-Chlorophenylaminosulfonylmethyl)-5-(3′-p-chlorophenylsulfonyl-4′,5′-dihydro-1′H-pyrazol-4′-ylsulfonylmethyl)-1,3,4-oxadiazole (**9c**).* White solid (2.11 g, 71%); m.p. 166–168 °C; IR (KBr): 1148, 1342 (SO_2), 1597 (C=N), 3264 (NH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.69 (dd, 1H, H_x, J_{Ax} = 6.5 Hz, J_{Mx} = 10.7 Hz), 4.27 (dd, 1H, H_M, J_{AM} = 12.5 Hz), 4.62 (dd, 1H, H_A), 5.00 (s, 2H, SO_2–CH_2), 5.27 (s, 2H, CH_2–SO_2–NH), 7.18–7.81 (m, 8H, Ar–H), 10.08 (bs, 1H, NH), 10.45 (bs, 1H, NH–SO_2) ppm; ^{13}C NMR (DMSO-d_6): δ 47.7 (SO_2–CH_2), 52.1 (CH_2–SO_2–NH), 53.5 (C-5′), 67.4 (C-4′), 153.6 (C-3′), 158.2 (C-5), 159.5 (C-2), 127.1, 127.7, 128.3, 129.4, 130.8, 131.6, 132.4, 135.6 (aromatic carbons) ppm; Anal. Calcd. for $C_{19}H_{17}Cl_2N_5O_7S_3$: C, 38.39; H, 2.88; N, 11.78; Found: C, 38.43; H, 3.00; N, 11.75%.

*6.1.5.42-(Phenylaminosulfonylmethyl)-5-(3′-phenylsulfonyl-4′,5′-dihydro-1′H-pyrazol-4′-ylsulfonylmethyl)-1,3,4-thiadiazole (**10a**).* White solid (1.86 g, 69%); m.p. 182–184 °C; IR (KBr): 1128, 1327 (SO_2), 1602 (C=N), 3268 (NH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.58 (dd, 1H, H_x, J_{Ax} = 5.8 Hz, J_{Mx} = 9.9 Hz), 4.29 (dd, 1H, H_M, J_{AM} = 11.7 Hz), 4.64 (dd, 1H, H_A), 4.95 (s, 2H, SO_2–CH_2), 5.24 (s, 2H, CH_2–SO_2–NH), 7.24–7.71 (m, 10H, Ar–H), 9.95 (bs, 1H, NH), 10.42 (bs, 1H, NH–SO_2) ppm; ^{13}C NMR (DMSO-d_6): δ 48.3 (SO_2–CH_2), 51.6 (CH_2–SO_2–NH), 52.7 (C-5′), 65.2 (C-4′), 152.8 (C-3′), 156.4 (C-5), 158.4 (C-2), 126.1, 127.2, 127.8, 128.7, 129.9, 132.1, 134.7, 137.3 (aromatic carbons) ppm; Anal. Calcd. for $C_{19}H_{19}N_5O_6S_4$: C, 42.13; H, 3.54; N, 12.93; Found: C, 42.18; H, 3.58; N, 13.00%.

*6.1.5.52-(p-Methylphenylaminosulfonylmethyl)-5-(3′-p-methylphenylsulfonyl-4′,5′-dihydro-1′H-pyrazol-4′-ylsulfonylmethyl)-1,3,4-thiadiazole (**10b**).* White solid (1.90 g, 67%); m.p. 187–189 °C; IR (KBr): 1124, 1322 (SO_2), 1589 (C=N), 3262 (NH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.28 & 2.33 (s, 6H, Ar–CH_3), 3.50 (dd, 1H, H_x, J_{Ax} = 5.9 Hz, J_{Mx} = 9.8 Hz), 4.19 (dd, 1H, H_M, J_{AM} = 11.5 Hz), 4.58 (dd, 1H, H_A), 4.93 (s, 2H, SO_2–CH_2), 5.36 (s, 2H, CH_2–SO_2–NH), 7.18–7.68 (m, 8H, Ar–H), 9.90 (bs, 1H, NH), 10.39 (bs, 1H, NH–SO_2) ppm; ^{13}C NMR (DMSO-d_6): δ 21.1 & 21.8 (Ar–CH_3), 47.6 (SO_2–CH_2), 50.5 (CH_2–SO_2–NH), 52.3 (C-5′), 64.3 (C-4′), 152.4 (C-3′), 156.2 (C-5), 157.9 (C-2), 126.3, 128.1, 128.9, 129.5, 131.4, 133.2, 134.3, 136.4 (aromatic carbons) ppm; Anal. Calcd. for $C_{21}H_{23}N_5O_6S_4$: C, 44.27; H, 4.07; N, 12.29; Found: C, 44.34; H, 4.04; N, 12.35%.

*6.1.5.62-(p-Chlorophenylaminosulfonylmethyl)-5-(3′-p-chlorophenylsulfonyl-4′,5′-dihydro-1′H-pyrazol-4′-ylsulfonylmethyl)-1,3,4-thiadiazole (**10c**).* White solid (2.22 g, 73%); m.p. 198–200 °C; IR (KBr): 1131, 1334 (SO_2), 1606 (C=N), 3276 (NH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.55 (dd, 1H, H_x, J_{Ax} = 6.0 Hz, J_{Mx} = 10.2 Hz), 4.24 (dd, 1H, H_M, J_{AM} = 11.8 Hz), 4.67 (dd, 1H, H_A), 4.98 (s, 2H, SO_2–CH_2), 5.28 (s, 2H, CH_2–SO_2–NH), 7.25–7.80 (m, 8H, Ar–H), 9.98 (bs, 1H, NH), 10.44 (bs, 1H, NH–SO_2) ppm; ^{13}C NMR (DMSO-d_6): δ 49.6 (SO_2–CH_2), 52.8 (CH_2–SO_2–NH), 53.9 (C-5′), 66.7 (C-4′), 154.1 (C-3′), 157.9 (C-5), 160.5 (C-2), 127.8, 128.3, 130.2, 131.6, 132.4, 133.4, 136.2, 137.7 (aromatic carbons) ppm; Anal. Calcd. for $C_{19}H_{17}Cl_2N_5O_6S_4$: C, 37.38; H, 2.81; N, 11.47; Found: C, 37.42; H, 2.82; N, 11.42%.

*6.1.5.7. 4-Amino-3-(phenylaminosulfonylmethyl)-5-(3′-phenylsulfonyl-4′,5′-dihydro-1′H-pyrazol-4′-ylsulfonylmethyl)-1,2,4-triazole (**11a**).* White solid (1.94 g, 72%); m.p. 184–186 °C; IR (KBr): 1135, 1314 (SO_2), 1610 (C=N), 3251 (NH), 3372, 3485 (NH_2) cm^{-1}; ^1H NMR

(DMSO-d_6): δ 3.52 (dd, 1H, H_x, J_{Ax} = 6.2 Hz, J_{Mx} = 10.4 Hz), 4.21 (dd, 1H, H_M, J_{AM} = 12.3 Hz), 4.64 (dd, 1H, H_A), 5.07 (s, 2H, SO_2–CH_2), 5.29 (s, 2H, CH_2–SO_2–NH), 5.72 (bs, 2H, NH_2), 7.20–7.83 (m, 10H, Ar–H), 9.87 (bs, 1H, NH), 10.26 (bs, 1H, NH–SO_2) ppm; ^{13}C NMR (DMSO-d_6): δ 46.9 (SO_2–CH_2), 51.7 (CH_2–SO_2–NH), 53.1 (C-5′), 66.8 (C-4′), 153.3 (C-3′), 158.6 (C-5), 159.5 (C-3), 127.1, 128.2, 129.3, 130.7, 131.9, 133.5, 135.3, 137.2 (aromatic carbons) ppm; Anal. Calcd. for $C_{19}H_{21}N_7O_6S_3$: C, 42.29; H, 3.92; N, 18.17; Found: C, 42.25; H, 3.90; N, 18.37%.

*6.1.5.84-Amino-3-(p-methylphenylaminosulfonylmethyl)-5-(3′-p-methylphenylsulfonyl-4′,5′-dihydro-1′H-pyrazol-4′-ylsulfonylmethyl)-1,2,4-triazole (**11b**).* White solid (1.90 g, 67%); m.p. 197–199 °C; IR (KBr): 1131, 1308 (SO_2), 1603 (C=N), 3252 (NH), 3364, 3481 (NH_2) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.21 & 2.26 (s, 6H, Ar–CH_3), 3.57 (dd, 1H, H_x, J_{Ax} = 6.3 Hz, J_{Mx} = 10.3 Hz), 4.20 (dd, 1H, H_M, J_{AM} = 12.1 Hz), 4.62 (dd, 1H, H_A), 4.99 (s, 2H, SO_2–CH_2), 5.24 (s, 2H, CH_2–SO_2–NH), 5.64 (bs, 2H, NH_2), 7.16–7.79 (m, 8H, Ar–H), 9.89 (bs, 1H, NH), 10.24 (bs, 1H, NH–SO_2) ppm; ^{13}C NMR (DMSO-d_6): δ 21.1 & 22.5 (Ar–CH_3), 46.1 (SO_2–CH_2), 51.5 (CH_2–SO_2–NH), 52.8 (C-5′), 66.4 (C-4′), 152.9 (C-3′), 158.1 (C-5), 159.7 (C-3), 128.1, 128.9, 129.8, 131.2, 132.7, 133.4, 135.2, 137.9 (aromatic carbons) ppm; Anal. Calcd. for $C_{21}H_{25}N_7O_6S_3$: C, 44.43; H, 4.44; N, 17.27; Found: C, 44.49; H, 4.48; N, 17.34%.

*6.1.5.94-Amino-3-(p-chlorophenylaminosulfonylmethyl)-5-(3′-p-chlorophenylsulfonyl-4′,5′-dihydro-1′H-pyrazol-4′-ylsulfonylmethyl)-1,2,4-triazole (**11c**).* White solid (2.28 g, 75%); m.p. 218–220 °C; IR (KBr): 1136, 1321 (SO_2), 1608 (C=N), 3272 (NH), 3376, 3492 (NH_2) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 3.53 (dd, 1H, H_x, J_{Ax} = 6.1 Hz, J_{Mx} = 10.5 Hz), 4.29 (dd, 1H, H_M, J_{AM} = 12.3 Hz), 4.67 (dd, 1H, H_A), 5.18 (s, 2H, SO_2–CH_2), 5.32 (s, 2H, CH_2–SO_2–NH), 5.70 (bs, 2H, NH_2), 7.22–7.69 (m, 8H, Ar–H), 9.84 (bs, 1H, NH), 10.28 (bs, 1H, NH–SO_2) ppm; ^{13}C NMR (DMSO-d_6): δ 47.0 (SO_2–CH_2), 51.9 (CH_2–SO_2–NH), 53.6 (C-5′), 67.2 (C-4′), 154.1 (C-3′), 158.5 (C-5), 159.3 (C-3), 127.8, 129.1, 129.9, 130.7, 131.5, 133.4, 135.2, 138.1 (aromatic carbons) ppm; Anal. Calcd. for $C_{19}H_{19}Cl_2N_7O_6S_3$: C, 37.50; H, 3.15; N, 16.11; Found: C, 37.55; H, 3.18; N, 16.20%.

*6.1.6. General procedure for the synthesis of 2-(arylaminosulfonylmethyl)-5-(3′-arylsulfonyl-1′H-pyrazol-4′-ylsulfonylmethyl)-1,3,4-oxadiazole **12a**–**c**/2-(arylaminosulfonylmethyl)-5-(3′-arylsulfonyl-1′H-pyrazol-4′-ylsulfonylmethyl)-1,3,4-thiadiazole **13a**–**c**/4-amino-3-(arylaminosulfonylmethyl)-5-(3′-arylsulfonyl-1′H-pyrazol-4′-ylsulfonylmethyl)-1,2,4-triazole **14a**–**c***

A mixture of **9**/**10**/**11** (1 mmol) and chloranil (1.4 mmol) in xylene (10 ml) was refluxed for 20–23 h. Then, the reaction mixture was treated with a 5% NaOH solution. The organic layer was separated and repeatedly washed with water. It was dried over anhydrous Na_2SO_4 and the solvent was removed on a rotary evaporator. The resultant solid was recrystallized from methanol.

*6.1.6.12-(Phenylaminosulfonylmethyl)-5-(3′-phenylsulfonyl-1′H-pyrazol-4′-ylsulfonyl-methyl)-1,3,4-oxadiazole (**12a**).* White solid (0.34 g, 66%); m.p. 200–202 °C; IR (KBr): 1141, 1327 (SO_2), 1596 (C=N), 1638 (C=C), 3281 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.09 (s, 2H, SO_2–CH_2), 5.26 (s, 2H, CH_2–SO_2–NH), 6.47 (bs, 1H, NH), 6.92–7.57 (m, 11H, $C_{5'}$–H & Ar–H), 10.35 (bs, 1H, NH–SO_2) ppm; ^{13}C NMR (DMSO-d_6): δ 50.2 (SO_2–CH_2), 54.5 (CH_2–SO_2–NH), 134.6 (C-4′), 140.2 (C-5′), 149.4 (C-3′), 159.3 (C-5), 162.3 (C-2), 126.5, 127.4, 128.3, 129.7, 131.6, 132.8, 135.9, 138.5 (aromatic carbons) ppm; Anal. Calcd. for $C_{19}H_{17}N_5O_7S_3$: C, 43.59; H, 3.27; N, 13.38; Found: C, 43.63; H, 3.25; N, 13.44%.

*6.1.6.22-(p-Methylphenylaminosulfonylmethyl)-5-(3′-p-methylphenylsulfonyl-1′H-pyrazol-4′-ylsulfonylmethyl)-1,3,4-oxadiazole (**12b**).* White solid (0.37 g, 68%); m.p. 211–213 °C; IR (KBr): 1134, 1319 (SO_2), 1587 (C=N), 1636 (C=C), 3275 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.23 & 2.28 (s, 6H, Ar–CH_3), 5.07 (s, 2H, SO_2–CH_2), 5.32 (s, 2H, CH_2–SO_2–NH), 6.42 (bs, 1H, NH), 6.84–7.51 (m, 9H, $C_{5'}$–H & Ar–H), 10.32 (bs, 1H, NH–SO_2) ppm; ^{13}C NMR (DMSO-d_6): δ 21.6 & 22.4 (Ar–CH_3), 49.8 (SO_2–CH_2), 53.9 (CH_2–SO_2–NH), 133.8 (C-4′), 139.6 (C-5′), 148.6 (C-3′), 158.7 (C-5), 161.4 (C-2), 127.8, 128.4, 129.8, 131.6, 132.7, 133.5, 135.8, 136.9 (aromatic carbons) ppm; Anal. Calcd. for $C_{21}H_{21}N_5O_7S_3$: C, 45.72; H, 3.84; N, 12.70; Found: C, 45.70; H, 3.85; N, 12.64%.

*6.1.6.32-(p-Chlorophenylaminosulfonylmethyl)-5-(3′-p-chlorophenylsulfonyl-1′H-pyrazol-4′-ylsulfonylmethyl)-1,3,4-oxadiazole (**12c**).* White solid (0.43 g, 73%); m.p. 219–221 °C; IR (KBr): 1135, 1334 (SO_2), 1593 (C=N), 1642 (C=C), 3278 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.10 (s, 2H, SO_2–CH_2), 5.38 (s, 2H, CH_2–SO_2–NH), 6.46 (bs, 1H, NH), 7.01–7.66 (m, 9H, $C_{5'}$–H & Ar–H), 10.37 (bs, 1H, NH–SO_2) ppm; ^{13}C NMR (DMSO-d_6): δ 50.7 (SO_2–CH_2), 54.8 (CH_2–SO_2–NH), 135.1 (C-4′), 141.2 (C-5′), 150.4 (C-3′), 160.3 (C-5), 162.9 (C-2), 127.8, 128.2, 129.3, 130.6, 131.8, 132.4, 134.6, 137.8 (aromatic carbons) ppm; Anal. Calcd. for $C_{19}H_{15}Cl_2N_5O_7S_3$: C, 38.52; H, 2.55; N, 11.82; Found: C, 38.58; H, 2.53; N, 11.88%.

*6.1.6.42-(Phenylaminosulfonylmethyl)-5-(3′-phenylsulfonyl-1′H-pyrazol-4′-ylsulfonyl-methyl)-1,3,4-thiadiazole (**13a**).* White solid (0.37 g, 69%); m.p. 232–234 °C; IR (KBr): 1129, 1342 (SO_2), 1584 (C=N), 1641 (C=C), 3254 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.11 (s, 2H, SO_2–CH_2), 5.33 (s, 2H, CH_2–SO_2–NH), 6.53 (bs, 1H, NH), 6.99–7.62 (m, 11H, $C_{5'}$–H & Ar–H), 10.42 (bs, 1H, NH–SO_2) ppm; ^{13}C NMR (DMSO-d_6): δ 49.6 (SO_2–CH_2), 53.5 (CH_2–SO_2–NH), 133.5 (C-4′), 138.7 (C-5′), 149.4 (C-3′), 158.5 (C-5), 161.3 (C-2), 126.4, 127.5, 128.8, 129.5, 131.6, 132.8, 135.1, 138.2 (aromatic carbons) ppm; Anal. Calcd. for $C_{19}H_{17}N_5O_6S_4$: C, 42.29; H, 3.18; N, 12.98; Found: C, 42.36; H, 3.20; N, 12.94%.

*6.1.6.52-(p-Methylphenylaminosulfonylmethyl)-5-(3′-p-methylphenylsulfonyl-1′H-pyrazol-4′-ylsulfonylmethyl)-1,3,4-thiadiazole (**13b**).* White solid (0.40 g, 71%); m.p. 240–242 °C; IR (KBr): 1126, 1335 (SO_2), 1589 (C=N), 1636 (C=C), 3251 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.23 & 2.27 (s, 6H, Ar–CH_3), 5.13 (s, 2H, SO_2–CH_2), 5.36 (s, 2H, CH_2–SO_2–NH), 6.59 (bs, 1H, NH), 6.97–7.59 (m, 9H, $C_{5'}$–H & Ar–H), 10.38 (bs, 1H, NH–SO_2) ppm; ^{13}C NMR (DMSO-d_6): δ 21.0 & 21.9 (Ar–CH_3), 48.5 (SO_2–CH_2), 52.8 (CH_2–SO_2–NH), 132.8 (C-4′), 137.9 (C-5′), 148.6 (C-3′), 157.6 (C-5), 159.8 (C-2), 126.6, 127.2, 128.1, 128.7, 130.1, 131.5, 133.2, 137.4 (aromatic carbons) ppm; Anal. Calcd. for $C_{21}H_{21}N_5O_6S_4$: C, 44.43; H, 3.73; N, 12.34; Found: C, 44.48; H, 3.76; N, 12.40%.

*6.1.6.62-(p-Chlorophenylaminosulfonylmethyl)-5-(3′-p-chlorophenylsulfonyl-1′H-pyrazol-4′-ylsulfonylmethyl)-1,3,4-thiadiazole (**13c**).* White solid (0.44 g, 73%); m.p. 247–249 °C; IR (KBr): 1134, 1344 (SO_2), 1596 (C=N), 1644 (C=C), 3267 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.17 (s, 2H, SO_2–CH_2), 5.40 (s, 2H, CH_2–SO_2–NH), 6.55 (bs, 1H, NH), 7.04–7.62 (m, 9H, $C_{5'}$–H & Ar–H), 10.45 (bs, 1H, NH–SO_2) ppm; ^{13}C NMR (DMSO-d_6): δ 49.7 (SO_2–CH_2), 54.1 (CH_2–SO_2–NH), 133.9 (C-4′), 139.2 (C-5′), 149.2 (C-3′), 158.8 (C-5), 161.7 (C-2), 128.1, 128.9, 131.5, 132.6, 133.3, 134.1, 137.3, 137.9 (aromatic carbons) ppm; Anal. Calcd. for $C_{19}H_{15}Cl_2N_5O_6S_4$: C, 37.50; H, 2.48; N, 11.51; Found: C, 37.44; H, 2.46; N, 11.48%.

*6.1.6.74-Amino-3-(phenylaminosulfonylmethyl)-5-(3′-phenylsulfonyl-1′H-pyrazol-4′-ylsulfonylmethyl)-1,2,4-triazole (**14a**).* White solid (0.39 g, 74%); m.p. 234–236 °C; IR (KBr): 1140, 1330 (SO_2), 1611 (C=N), 1638 (C=C), 3265 (NH), 3335, 3458 (NH_2) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.08 (s, 2H, SO_2–CH_2), 5.34 (s, 2H, CH_2–SO_2–NH), 5.68 (bs, 2H, NH_2), 6.53 (bs, 1H, NH), 7.09–7.76 (m, 11H, $C_{5'}$–H & Ar–H), 10.31 (bs, 1H, NH–SO_2) ppm; ^{13}C NMR (DMSO-

d_6): δ 48.8 (SO_2–CH_2), 51.6 (CH_2–SO_2–NH), 133.6 (C-4′), 139.5 (C-5′), 148.5 (C-3′), 158.7 (C-5), 160.1 (C-3), 127.1, 128.3, 129.0, 129.8, 131.4, 132.5, 133.6, 136.4 (aromatic carbons) ppm; Anal. Calcd. for $C_{19}H_{19}N_7O_6S_3$: C, 42.45; H, 3.56; N, 18.24; Found: C, 42.50; H, 3.59; N, 18.32%.

*6.1.6.84-Amino-3-(p-methylphenylaminosulfonylmethyl)-5-(3′-p-methylphenylsulfonyl-1′H-pyrazol-4′-ylsulfonylmethyl)-1,2,4-triazole (**14b**).* White solid (0.39 g, 69%); m.p. 254–256 °C; IR (KBr): 1133, 1326 (SO_2), 1603 (C=N), 1629 (C=C), 3257 (NH), 3329, 3463 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.25 & 2.30 (s, 6H, Ar–CH_3), 5.10 (s, 2H, SO_2–CH_2), 5.32 (s, 2H, CH_2–SO_2–NH), 5.65 (bs, 2H, NH_2), 6.52 (bs, 1H, NH), 7.11–7.81 (m, 9H, $C_{5'}$–H & Ar–H), 10.34 (bs, 1H, NH–SO_2) ppm; ^{13}C NMR (DMSO-d_6): δ 20.4 & 21.6 (Ar–CH_3), 47.9 (SO_2–CH_2), 51.8 (CH_2–SO_2–NH), 133.0 (C-4′), 139.4 (C-5′), 147.6 (C-3′), 157.9 (C-5), 159.7 (C-3), 128.4, 129.1, 129.9, 131.2, 132.6, 133.2, 135.3, 136.7 (aromatic carbons) ppm; Anal. Calcd. for $C_{21}H_{23}N_7O_6S_3$: C, 44.59; H, 4.10; N, 17.33; Found: C, 44.55; H, 4.09; N, 17.39%.

*6.1.6.94-Amino-3-(p-chlorophenylaminosulfonylmethyl)-5-(3′-p-chlorophenylsulfonyl-1′H-pyrazol-4′-ylsulfonylmethyl)-1,2,4-triazole (**14c**).* White solid (0.47 g, 78%); m.p. 260–262 °C; IR (KBr): 1144, 1337 (SO_2), 1614 (C=N), 1636 (C=C), 3262 (NH), 3341, 3476 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): δ 5.15 (s, 2H, SO_2–CH_2), 5.39 (s, 2H, CH_2–SO_2–NH), 5.72 (bs, 2H, NH_2), 6.57 (bs, 1H, NH), 7.19–7.87 (m, 9H, $C_{5'}$–H & Ar–H), 10.43 (bs, 1H, NH–SO_2) ppm; ^{13}C NMR (DMSO-d_6): δ 48.7 (SO_2–CH_2), 52.5 (Ch_2–SO_2–NH), 134.3 (C-4′), 140.8(C-5′), 149.1 (C-3′), 159.2 (C-5), 160.5 (C-3), 127.1, 128.4, 129.5, 131.4, 132.6, 133.8, 135.2, 137.8 (aromatic carbons) ppm; Anal. Calcd. for $C_{19}H_{17}Cl_2N_7O_6S_3$: C, 37.63; H, 2.83; N, 16.17; Found: C, 37.70; H, 2.86; N, 16.14%.

6.2. Biological assays

6.2.1. Compounds

Compounds **3**–**14** were dissolved in DMSO at different concentrations of 50 & 100 μg/ml.

6.2.2. Cells

Bacterial strains *Staphylococcus aureus*, *B. subtilis*, *P. aeruginosa*, *Klebsiella pneumoniae* and fungi *A. niger & P. chrysogenum* were obtained from Department of Microbiology, S.V University, Tirupati, India.

6.2.3. Antibacterial and antifungal assays

The *in vitro* antimicrobial studies were carried out by agar well diffusion method against test organisms [20,21]. Nutrient broth (NB) plates were swabbed with 24 h old broth culture (100 μl) of test bacteria. Using the sterile cork borer, wells (6 mm) were made into each petriplate. Various concentrations of DMSO dissolved compounds (50, 100 μg/well) were added into the wells by using sterile pipettes. Simultaneously the standard antibiotics, Chloramphenicol for antibacterial activity and Ketoconazole for antifungal activity (as positive control) were tested against the pathogens. The samples were dissolved in DMSO which showed no zone of inhibition acts as negative control. The plates were incubated at 37 °C for 24 h for bacteria and at 28 °C for 48 h for fungi. After appropriate incubation, the diameter of zone of inhibition of each well was measured. Duplicates were maintained and the average values were calculated for eventual antibacterial activity.

Broth dilution test is used to determine Minimum Inhibitory Concentration (MIC) of the above mentioned samples [22,23]. Freshly prepared nutrient broth was used as diluents. The 24 h old culture of the test bacteria *S. aureus*, *B. subtilis*, *P. aeruginosa and K. pneumoniae* and the test fungi *A. niger & P. chrysogenum* were diluted 100 folds in nutrient broth (100 μl bacterial cultures in 10 ml NB). Increasing concentrations of the test samples (6.25, …200 μg) were added to the test tubes containing the bacterial and fungal cultures. All the tubes were incubated at 37 °C for 24 h for bacteria and at 28 °C for 48 h for fungi. The tubes were examined for visible turbidity and using NB as control. Control without test samples and with solvent was assayed simultaneously. The lowest concentration that inhibited visible growth of the tested organisms was recorded as MIC.

To determine the Minimum Bactericidal Concentration (MBC) [24] and Minimum Fungicidal Concentration (MFC) [25] for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes which did not show any growth and inoculated on sterile nutrient broth (for bacteria) and PDA (for fungi) by streaking. Plates inoculated with bacteria and fungi were incubated at 37 °C for 24 h and at 28 °C for 48 h, respectively. After incubation, that concentration was noted as MBC (for bacteria) or MFC (for fungi) at which no visible growth was observed.

6.2.4. MTT assay for cell viability

The cytotoxicity of the compounds was tested using A549 lung carcinoma cells. 5×10^4 cells were plated in each well of a 96-well tissue culture cluster (Nunc Inc Germany) and incubated at 37 °C in a medium containing DMEM, 10% foetal bovine serum and antibiotics (Invitrogen, USA), in 5% CO_2 atmosphere [26,27]. After attachment of the cells (usually 3–4 h), different concentrations of the compound were added and incubated for 72 h. MTT solution (20 μl of 5 mg/ml) was added to each well and the incubation continued for additional 3 h. The dark blue formazan crystals formed within the healthy cells were solubilized with DMSO and the absorbance was estimated in ELISA plate reader (7520 Microplate reader, Cambridge technologies, Inc) at 550 nm and the absorbance was correlated with the cell number. Experiments were performed in triplicates and the values are the average of three ($n = 3$) independent experiments. The inhibitory concentration (IC_{50}) of the compound was assessed by Graph Pad Prism software.

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