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Original article

Synthesis, antimicrobial and cytotoxic activities of 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazoles

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ABSTRACT

A new class of 1,3,4-oxadiazoles were prepared from acid hydrazides on treatment with different carboxylic acids in the presence of phosphorus oxychloride. Interconversion of oxadiazoles to thiadiazoles and triazoles was carried out with appropriate reagents. The antimicrobial and cytotoxic activities of compounds **7a–d** to **12a–d** were tested. Compounds **10d** and **12d** showed pronounced antimicrobial activity. Further, compound **10d** exhibited maximum cytotoxicity.

1. Introduction

In the past years considerable evidence has been accumulated to demonstrate the efficacy of substituted 1,3,4-oxadiazoles in antibacterial, antifungal and HIV activities [1,2]. The advent of sulfur drugs and the later discovery of mesoionic compounds in fact accelerated the rate of progress in the field of sulfur containing heterocycles. Substituted oxadiazole and thiadiazole derivatives are potent cyclooxygenase/5-lipooxygenase inhibitors [3–7]. This novel dual inhibitory activity of the enzyme pathways hold promise as antiinflammatory agents with an improved efficacy. Symmetrical oxadiazoles were found to be effective insecticides towards houseflies, faceflies and hornflies [8]. The therapeutic effects of 1,2,4-triazole derivatives have been studied for a number of pathological conditions including inflammation, cancer, pain, tuberculosis and hypertension [9–17]. In fact, remarkable progress has been made by our group in the development of biologically potent heterocycles [18–22]. Replacement of –O– by –S– or –NH– in some heterocycles was reported *viz.*, Bordners [23] preparation of pyrroles from furan and the transformation of epoxides to episulfides by the action of thiocyanates or thiourea [24–26]. However, reports about the conversion of 1,3,4-oxadiazoles to 1,3,4-thiadiazoles and 1,2,4-triazoles are relatively less [27,28]. The present communication deals with the synthesis of 2,5-diaryloxadiazoles and their conversion to thiadiazoles and triazoles and also the biological evaluation for antimicrobial and cytotoxic activities.

2. Chemistry

The arylsulfonylacetic acid methyl esters **3a–d** and benzylsulfonylacetic acid methyl esters **4a–d** were prepared from arylsulfonylacetic acids **1a–d** and benzylsulfonylacetic acids **2a–d** by esterification. The corresponding acid hydrazides **5a–d** and **6a–d** were obtained by the reaction of 3/4 with hydrazine hydrate in the presence of pyridine. The acid hydrazides on treatment with different carboxylic acids in the presence of phosphorus oxychloride afforded 2-(arylsulfonylmethyl)-5-aryl-1,3,4-oxadiazoles **7a–d** and 2-(arylmethanesulfonylmethyl)-5-aryl-1,3,4-oxadiazoles **8a–d** (Scheme 1). Compounds **7a–d** and **8a–d** were treated with two-fold excess thiourea in tetrahydrofuran. The reaction mixture indicated two spots on TLC which were separated and identified as 2-(arylsulfonylmethyl)-5-aryl-1,3,4-thiadiazoles **9a–d** and 2-(arylmethanesulfonylmethyl)-5-aryl-1,3,4-thiadiazoles **10a–d** as major products apart from **7a–d** and **8a–d** as minor ones, respectively. On the other hand, the reaction of **7a–d** and **8a–d** with excess hydrazine produced 3-(arylsulfonylmethyl)-5-phenyl-4*H*-1,2,4-triazol-4-amines **11a–d** and 3-(arylmethanesulfonylmethyl)-5-phenyl-4*H*-1,2,4-triazol-4-amines **12a–d** (Scheme 1).

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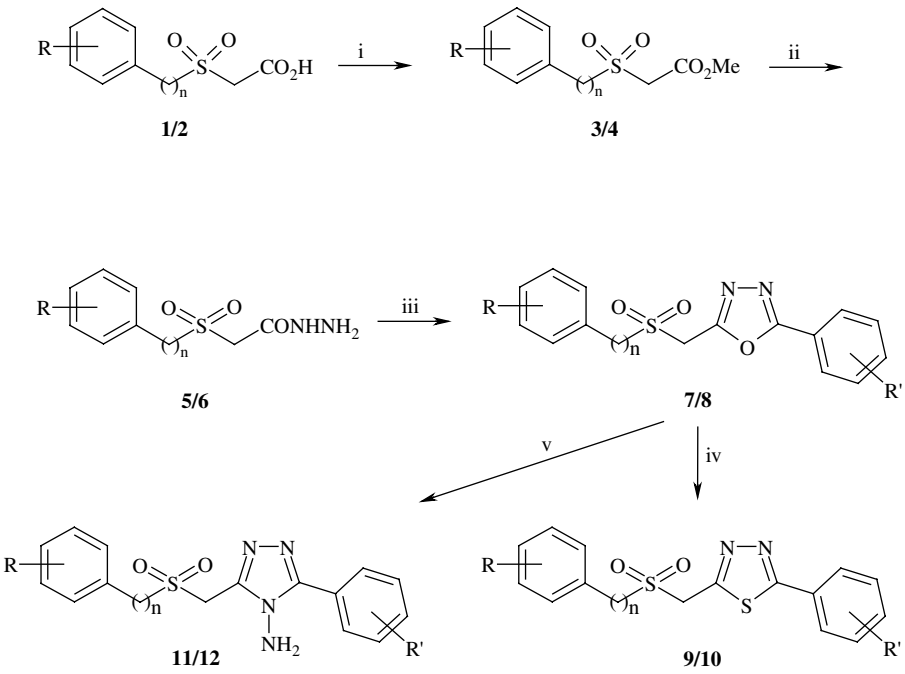

Scheme 1.

3. Biology

3.1. Antimicrobial activity

Compounds **7a–d** to **12a–d** were tested for *in vitro* antimicrobial activity against the Gram-positive bacteria *Staphylococcus aureus* (NCIM No. 5021), *Bacillus subtilis* (NCIM No. 2063), the Gram-negative bacteria *Klebsiella pneumoniae* (NCIM No. 2957), *Proteus vulgaris* (NCIM No. 2027) and fungi *Fusarium solani* (NCIM No. 1330), *Curvularia lunata* (NCIM No. 716) and *Aspergillus niger* (NCIM No. 596). The primary screen was carried out by agar disc-diffusion method [29] using nutrient agar medium. The minimum inhibitory concentration for the most active compounds **10d** and **12d** against the same microorganisms used in the preliminary screening was carried out using microdilution susceptibility method [30]. Chloramphenicol and ketoconazole were used as control drugs. The observed data on the antimicrobial activity of compounds and control drugs are given in Tables 1–3.

3.2. MTT assay for cell viability

Toxicity of compounds **8d**, **10d** and **12d** in different cell lines in the presence of 10% and 0.2% FBS, respectively, was determined using 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide reduction assay [31,32]. The compounds were dissolved in DMSO at 10 mM concentration and stored at −20 °C. The dilutions were made in culture medium before treatment.

Nearly 5000 cells/well were plated in 96 well plates. After 3–4 h, the compounds were added to the cells at different concentrations. After 48 h of incubation, 20 μl of MTT solution was added and the cells were incubated further for 4 h. Blue formazan crystals were seen in well when checked under microscope. Media was removed and 200 μl of DMSO was added per well. The absorbance was measured using microtiter plate reader. Control treatments were performed with DMSO. The % viability was then calculated as $[A_{590}(\text{treated cells}) - \text{background}]/[A_{590}(\text{untreated cells}) - \text{background}] \times 100$.

4. Results and discussion

We have synthesized a series of heterocycles, 2-(arylsulfonylmethyl)-5-aryl-1,3,4-oxadiazoles **7a–d**, 2-(arylmethanesulfonylmethyl)-5-aryl-1,3,4-oxadiazoles **8a–d**, 2-(arylsulfonylmethyl)-5-aryl-1,3,4-thiadiazoles **9a–d**, 2-(arylmethanesulfonylmethyl)-5-aryl-1,3,4-thiadiazoles **10a–d**, 3-(arylsulfonylmethyl)-5-aryl-4*H*-1,2,4-triazol-4-amines **11a–d** and 3-(arylmethanesulfonylmethyl)-5-aryl-4*H*-1,2,4-triazol-4-amines **12a–d** as illustrated in Scheme 1. Structures of all the compounds were established on the basis of elemental analyses, IR, ^{1}H NMR and ^{13}C NMR spectral data.

4.1. Biological results

The results of preliminary antibacterial testing of compounds **7a–d** to **12a–d** are shown in Table 1. The results revealed that 2-(arylmethanesulfonylmethyl)-5-aryl-1,3,4-thiadiazoles **10a–d**, 3-(arylmethanesulfonylmethyl)-5-aryl-4*H*-1,2,4-triazol-4-amines **12a–d** exhibited high activity (22–39 mm) on both Gram (+ve) and Gram (−ve) bacteria. In fact, compounds **10d** and **12d** showed pronounced activity (31–39 mm) towards Gram (+ve) bacteria. Compounds 2-(arylmethanesulfonylmethyl)-5-aryl-1,3,4-oxadiazoles **8a–d**, 2-(arylsulfonylmethyl)-5-aryl-1,3,4-thiadiazoles **9a–d**,

Table 1
Antibacterial activity of **7**–**12**.

Compound	Concentration (μg/disc)	Zone of inhibition (mm)			
		Gram-positive bacteria		Gram-negative bacteria	
		Staphylococcus aureus	*Bacillus subtilis*	*Klebsiella pneumoniae*	*Proteus vulgaris*
7a	100	12	11	16	14
	200	15	13	18	17
7b	100	14	16	12	14
	200	16	17	15	17
7c	100	15	15	14	12
	200	18	17	16	15
7d	100	15	16	14	16
	200	18	17	17	19
8a	100	16	14	15	12
	200	18	17	18	15
8b	100	17	15	14	15
	200	20	18	16	17
8c	100	18	18	16	15
	200	20	19	18	17
8d	100	20	19	17	15
	200	23	21	20	18
9a	100	19	15	16	18
	200	21	17	18	20
9b	100	20	15	17	16
	200	23	18	21	19
9c	100	20	18	15	16
	200	23	23	17	19
9d	100	21	19	16	19
	200	23	23	19	21
10a	100	24	22	17	16
	200	29	25	22	24
10b	100	26	25	19	18
	200	29	28	23	22
10c	100	28	24	19	17
	200	30	28	23	22
10d	100	29	27	20	20
	200	33	31	24	23
11a	100	16	14	15	16
	200	18	17	18	18
11b	100	17	19	17	16
	200	20	22	20	18
11c	100	19	17	17	18
	200	23	20	19	21
11d	100	19	16	18	19
	200	23	18	20	20
12a	100	22	23	18	17
	200	24	26	22	22
12b	100	29	30	24	26
	200	32	34	28	29
12c	100	27	28	27	26
	200	31	33	29	29
12d	100	32	37	33	31
	200	37	39	36	34
Chloramphenicol	100	35	38	37	42
	200	41	44	42	45

Table 2
Antifungal activity of **7**–**12**.

Compound	Concentration (μg/ml)	Zone of inhibition (mm)		
		Fusarium solani	*Curvularia lunata*	*Aspergillus niger*
7a	100	15	15	14
	200	18	19	17
7b	100	16	18	15
	200	20	22	17
7c	100	16	15	16
	200	20	17	19
7d	100	16	17	15
	200	19	19	16
8a	100	17	18	16
	200	20	22	17
8b	100	17	18	18
	200	19	23	21
8c	100	16	19	17
	200	19	22	21
8d	100	18	16	17
	200	22	19	21
9a	100	18	16	15
	200	22	19	17
9b	100	19	18	15
	200	22	21	18
9c	100	18	19	16
	200	21	21	19
9d	100	20	19	17
	200	24	22	21
10a	100	18	21	16
	200	20	23	19
10b	100	20	19	16
	200	23	21	19
10c	100	24	23	17
	200	27	25	20
10d	100	28	25	20
	200	31	27	23
11a	100	22	20	19
	200	25	24	22
11b	100	23	22	18
	200	25	25	21
11c	100	21	22	19
	200	24	25	22
11d	100	24	23	20
	200	27	25	24
12a	100	19	22	17
	200	21	24	21
12b	100	21	18	17
	200	23	20	21
12c	100	24	24	18
	200	26	27	20
12d	100	29	27	22
	200	32	31	24
Ketoconazole	100	38	41	36
	200	42	44	39

and 3-(arylsulfonylmethyl)-5-aryl-4*H*-1,2,4-triazol-4-amines **11a**–**d** displayed moderate to high activity towards Gram (+ve) bacteria (17–23 mm) and moderate activity (15–21 mm) towards Gram (−ve) bacteria. On the other hand, 2-(arylsulfonylmethyl)-5-aryl-1,3,4-oxadiazoles **7a**–**d** exhibited least activity against both bacteria.

All the test compounds inhibited the spore germination of tested fungi *A. niger, F. solani* and *C. lunata*. Results of the investigation presented in Table 2 revealed that all the compounds except **7a**–**d** possess relatively high inhibitory effect on *F. solani* and *C. lunata* than on *A. niger*. Further, compounds 2-(4-chlorobenzylsulfonylmethyl)-5-(2-chlorophenyl)-1,3,4-thiadiazole **10d** and 3-(4-chlorobenzylsulfonylmethyl)-5-(2-chlorophenyl)-4*H*-1,2,4-triazol-4-amine **12d** displayed greater activity.

The MIC values were determined as the lowest concentration that completely inhibited visible growth of the microorganisms (Table 3). The structure–antimicrobial activity relationship of the synthesized compounds revealed that the compounds having oxadiazole exhibited least activity when compared with compounds having triazole and thiadiazole moieties. Besides, the compounds with benzylsulfonyl group were the most active. The presence of chloro substituent enhances the activity of the compounds. The maximum activity was observed with compounds **10d** and **12d** (Table 3).

Compounds **8d**, **10d** and **12d**, were tested for their cytotoxic potential using A549 (lung adenocarcinoma) cells in the presence of fetal bovine serum. As shown in Fig. 1 **10d** showed maximum cytotoxicity at a concentration of 250 μM, with an EC_{50} of 150 μM approximately. The other compounds **12d** and **8d** showed appreciable cytotoxicity of about 50% of the vehicle control at a concentration of 250 μM. The effect of these compounds was similar in the presence of 0.2% serum (data not shown).

Table 3
Minimum inhibitory concentration (MIC), μg/ml of **7**–**12**.

Compound	Minimum inhibitory concentration (MIC), μg/ml						
	S. aureus	*B. subtilis*	*K. pneumoniae*	*P. vulgaris*	*F. solani*	*C. lunata*	*A. niger*
7a	–	–	–	–	–	–	–
7b	–	–	–	–	–	–	–
7c	–	–	–	–	400	–	–
7d	400	400	–	–	400	400	–
8a	400	–	–	–	400	–	–
8b	400	–	–	–	400	400	–
8c	400	–	–	–	400	400	–
8d	200	400	–	–	200	200	200
9a	400	400	–	–	400	400	–
9b	400	400	–	–	400	400	400
9c	400	400	–	–	400	400	400
9d	200	200	400	400	200	200	200
10a	200	200	200	400	200	200	200
10b	100	200	200	200	200	200	200
10c	100	200	200	200	200	200	200
10d	50	100	100	200	100	100	100
11a	400	400	–	–	400	400	–
11b	400	200	–	–	400	200	–
11c	400	400	–	400	200	200	200
11d	400	400	400	400	200	200	400
12a	400	400	200	200	200	200	400
12b	100	200	200	200	100	100	100
12c	100	100	200	200	100	200	200
12d	25	25	50	100	100	50	50
Chloramphenicol	6.25	6.25	6.25	12.5	–	–	–
Ketoconazole	–	–	–	–	12.5	6.25	6.25

5. Conclusion

A new class of heterocycles, 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazoles were developed adopting simple, elegant and well-versed methodologies. We have also evaluated preliminary antimicrobial activity and cytotoxic activity of the compounds. The maximum antimicrobial activity was observed with **10d** and **12d**. Compound **10d** showed maximum cytotoxic activity.

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, ethyl acetate/hexane, 1:3). The IR spectra were recorded 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 $CDCl_3$/DMSO-d_6 on a Varian EM-360 spectrometer (300 MHz). The ^{13}C NMR spectra were recorded in $CDCl_3$/DMSO-d_6 on a Varian VXR spectrometer operating at 75.5 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 analyzer. The starting compounds arylsulfonylacetic acid (**1**) and arylmethanesulfonylacetic acid (**2**) were prepared by the literature procedure [33–35].

6.1.1. General procedure for the synthesis of arylsulfonylacetic acid methyl esters ***3a–d***/arylmethanesulfonylacetic acid methyl esters ***4a–d***

A solution of **1**/**2** (10 mmol) in methanol (10 ml) and conc. H_2SO_4 (1 ml) was refluxed on steam bath for 8–10 h. The contents of the flask were cooled and poured onto crushed ice. The solid separated was collected by filtration, washed with cold water and dried. The crude product was recrystallized from methanol.

6.1.2. General procedure for the synthesis of arylsulfonylacetic acid hydrazides ***5a–d***/arylmethanesulfonylacetic acid hydrazides ***6a–d***

To a solution of **3**/**4** (10 mmol) in methanol (6 ml), hydrazine hydrate (11 mmol) and 3 drops of pyridine were added and refluxed for 6–7 h. The reaction mixture was cooled and the solid separated was collected by filtration, dried and recrystallized from methanol.

6.1.3. General procedure for the synthesis of 2-(arylsulfonylmethyl)-5-aryl-1,3,4-oxadiazoles ***7a–d***/2-(arylmethanesulfonylmethyl)-5-aryl-1,3,4-oxadiazoles ***8a–d***

A mixture of **5**/**6** (10 mmol), substituted aromatic carboxylic acid (10 mmol) and $POCl_3$ (7 ml) was heated under reflux for 5–6 h. The

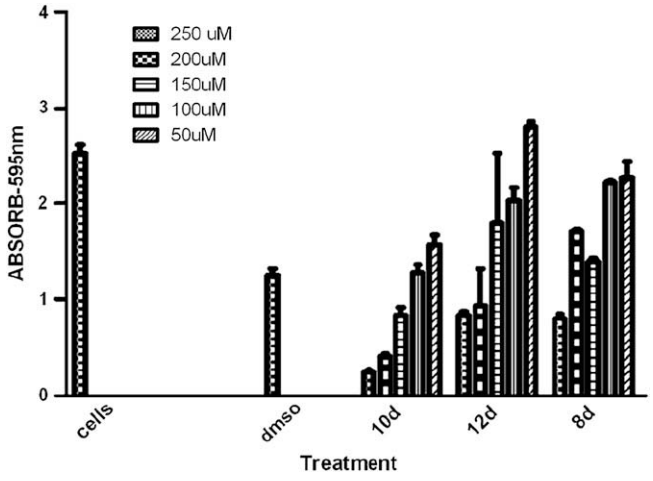

Fig. 1. Cytotoxic activity of compounds **8d**, **10d** and **12d** tested in A549 cells by MTT assay. A549 cells are highly invasive lung carcinoma cells. Cells were cultured in a medium containing DMEM, 10% fetal bovine serum and penicillin–streptomycin. Cells were plated in a 96 well tissue culture plate at a density of 3×10^4 per well. After cells attached (2–4 h), the compounds in indicated concentrations were added to the wells, in DMSO. The bars reflect the viable cells in each treatment. Cells, cells alone without any treatment, DMSO denotes the vehicle control. The experiment was done in duplicate with triplicate readings of each experiment.

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, dried and recrystallized from ethanol.

6.1.3.1. 2-(Phenylsulfonylmethyl)-5-phenyl-1,3,4-oxadiazole ***7a***. White solid (3.00 g, 74%); m.p. 75–77 °C; IR (KBr): 1138, 1335 (SO_2), 1632 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.29 (s, 2H, CH_2), 7.18–7.49 (m, 10H, Ar-H) ppm; ^{13}C NMR ($CDCl_3$) δ 55.1 (CH_2SO_2), 157.1 (C_2), 164.3 (C_5), 126.8, 127.6, 128.1, 128.7, 129.2, 129.6, 134.5, 136.8 ppm (aromatic carbons). Anal. Calcd. for $C_{15}H_{12}N_2O_3S$: C, 59.99; H, 4.03; N, 9.33; Found: C, 60.10; H, 4.07; N, 9.42%.

6.1.3.2. 2-(Phenylsulfonylmethyl)-5-(2-chlorophenyl)-1,3,4-oxadiazole ***7b***. White solid (3.34 g, 77%); m.p. 68–70 °C; IR (KBr): 1125, 1332 (SO_2), 1625 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.24 (s, 2H, CH_2), 7.24–7.52 (m, 9H, Ar-H) ppm; ^{13}C NMR ($CDCl_3$) δ 54.4 (CH_2SO_2), 157.8 (C_2), 164.7 (C_5), 124.5, 125.2, 126.7, 127.8, 128.6, 129.2, 130.5, 133.9 ppm (aromatic carbons). Anal. Calcd. for $C_{15}H_{11}ClN_2O_3S$: C, 53.82; H, 3.31; N, 8.37; Found: C, 53.89; H, 3.33; N, 8.41%.

6.1.3.3. 2-(4-Chlorophenylsulfonylmethyl)-5-phenyl-1,3,4-oxadiazole ***7c***. White solid (3.34 g, 79%); m.p. 79–81 °C; IR (KBr): 1128, 1334 (SO_2), 1627 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.32 (s, 2H, CH_2), 7.16–7.57 (m, 9H, Ar-H) ppm; ^{13}C NMR ($CDCl_3$) δ 55.0 (CH_2SO_2), 157.5 (C_2), 165.8 (C_5), 126.8, 127.2, 128.3, 128.6, 129.2, 130.4, 132.2, 136.6 ppm (aromatic carbons). Anal. Calcd. for $C_{15}H_{11}ClN_2O_3S$: C, 53.82; H, 3.31; N, 8.37; Found: C, 53.91; H, 3.35; N, 8.32%.

6.1.3.4. 2-(4-Chlorophenylsulfonylmethyl)-5-(2-chlorophenyl)-1,3,4-oxadiazole ***7d***. Colourless crystals (3.69 g, 82%); m.p. 88–90 °C; IR (KBr): 1126, 1330 (SO_2), 1629 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.25 (s, 2H, CH_2), 7.18–7.90 (m, 8H, Ar-H) ppm; ^{13}C NMR ($CDCl_3$) δ 54.8 (CH_2SO_2), 157.7 (C_2), 164.8 (C_5), 126.3, 127.1, 127.6, 128.5, 129.2, 133.2, 135.4, 136.2, 137.4, 138.5 ppm (aromatic carbons). Anal. Calcd. for $C_{15}H_{10}Cl_2N_2O_3S$: C, 48.79; H, 2.73; N, 7.59; Found: C, 48.73; H, 2.75; N, 7.65%.

6.1.3.5. 2-(Benzylsulfonylmethyl)-5-phenyl-1,3,4-oxadiazole ***8a***. White solid (3.14 g, 79%); m.p. 85–87 °C; IR (KBr): 1122, 1334 (SO_2), 1620 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.45 (s, 2H, CH_2), 4.48 (s, 2H, Ar-CH_2), 7.28–7.62 (m, 10H, Ar-H) ppm; ^{13}C NMR ($CDCl_3$) δ 46.5 (CH_2SO_2), 57.4 ($ArCH_2$), 157.3 (C_2), 164.1 (C_5), 127.4, 128.1, 128.9, 129.5, 131.2, 131.3, 134.8, 135.7 ppm (aromatic carbons). Anal. Calcd. for $C_{16}H_{14}N_2O_3S$: C, 61.13; H, 4.49; N, 8.91; Found: C, 61.05; H, 4.43; N, 8.99%.

6.1.3.6. 2-(Benzylsulfonylmethyl)-5-(2-chlorophenyl)-1,3,4-oxadiazole ***8b***. White solid (3.48 g, 74%); m.p. 99–101 °C; IR (KBr): 1134, 1328 (SO_2), 1632 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.44 (s, 2H, CH_2), 4.47 (s, 2H, Ar-CH_2), 7.32–7.88 (m, 9H, Ar-H) ppm; ^{13}C NMR ($CDCl_3$) δ 47.8 (CH_2SO_2), 57.6 ($ArCH_2$), 157.9 (C_2), 164.5 (C_5), 125.4, 126.4, 128.1, 129.5, 130.7, 132.1, 133.7, 139.2 ppm (aromatic carbons). Anal. Calcd. for $C_{16}H_{13}ClN_2O_3S$: C, 55.09; H, 3.76; N, 8.03; Found: C, 55.15; H, 3.80; N, 8.11%.

6.1.3.7. 2-(4-Chlorobenzylsulfonylmethyl)-5-phenyl-1,3,4-oxadiazole ***8c***. White solid (3.48 g, 72%); m.p. 112–114 °C; IR (KBr) 1130, 1336 (SO_2), 1625 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.46 (s, 2H, CH_2), 4.50 (s, 2H, Ar-CH_2), 7.39–7.90 (m, 9H, Ar-H) ppm; ^{13}C NMR ($CDCl_3$) δ 47.3 (CH_2SO_2), 56.5 ($ArCH_2$), 158.1 (C_2), 164.2 (C_5), 125.3, 125.9, 126.7, 127.4, 129.1, 131.3, 137.8, 138.3 ppm (aromatic carbons). Anal. Calcd. for $C_{16}H_{13}ClN_2O_3S$: C, 55.09; H, 3.76; N, 8.03; Found: C, 55.03; H, 3.81; N, 8.09%.

6.1.3.8. 2-(4-Chlorobenzylsulfonylmethyl)-5-(2-chlorophenyl)-1,3,4-oxadiazole ***8d***. White crystals (3.83 g, 79%); m.p. 118–120 °C; IR (KBr): 1126, 1320 (SO_2), 1630 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.47 (s, 2H, CH_2), 4.49 (s, 2H, Ar-CH_2), 7.41–8.13 (m, 8H, Ar-H) ppm; ^{13}C NMR ($CDCl_3$) δ 46.8 (CH_2SO_2), 57.5 ($ArCH_2$), 157.5 (C_2), 164.7 (C_5), 122.1, 125.5, 127.1, 127.2, 129.5, 131.3, 131.4, 132.4, 133.0, 133.3, 135.8 ppm (aromatic carbons). Anal. Calcd. for $C_{16}H_{12}Cl_2N_2O_3S$: C, 50.14; H, 3.16; N, 7.31; Found: C, 50.21; H, 3.14; N, 7.35%.

6.1.4. General procedure for the synthesis of 2-(arylsulfonylmethyl)-5-aryl-1,3,4-thiadiazoles ***9a–d****/2-(arylmethanesulfonylmethyl)-5-aryl-1,3,4-thiadiazoles* ***10a–d***

In a sealed test tube, a mixture of **7**/**8** (5 mmol), thiourea (20 mmol) and tertahydrofuran (5 ml) was taken and heated at 120–150 °C in an oil bath for 24–30 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 resultant solid was recrystallized from methanol.

6.1.4.1. 2-(Phenylsulfonylmethyl)-5-phenyl-1,3,4-thiadiazole ***9a***. White solid (1.58 g, 64%); m.p. 174–176 °C; IR (KBr): 1133, 1327 (SO_2), 1632 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.45 (s, 2H, CH_2), 7.14–7.42 (m, 10H, Ar-H) ppm; ^{13}C NMR (DMSO-d_6) δ 54.1 (CH_2SO_2), 162.4 (C_2), 167.5 (C_5), 125.4, 126.1, 127.3, 128.7, 129.8, 131.7, 134.2, 138.1 ppm (aromatic carbons). Anal. Calcd. for $C_{15}H_{12}N_2O_2S_2$: C, 56.94; H, 3.82; N, 8.85; Found: C, 56.84; H, 3.81; N, 8.93%.

6.1.4.2. 2-(Phenylsulfonylmethyl)-5-(2-chlorophenyl)-1,3,4-thiadiazole ***9b***. White solid (1.75 g, 65%); m.p. 185–187 °C; IR (KBr): 1135, 1333 (SO_2), 1635 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.50 (s, 2H, CH_2), 7.26–7.83 (m, 9H, Ar-H) ppm; ^{13}C NMR (DMSO-d_6) δ 54.5 (CH_2SO_2), 163.3 (C_2), 169.4 (C_5), 125.5, 126.4, 126.8, 127.8, 129.5, 131.3, 134.5, 136.2 ppm (aromatic carbons). Anal. Calcd. for $C_{15}H_{11}ClN_2O_2S_2$: C, 51.35; H, 3.16; N, 7.98; Found: C, 51.43; H, 3.18; N, 8.04%.

6.1.4.3. 2-(4-Chlorophenylsulfonylmethyl)-5-phenyl-1,3,4-thiadiazole ***9c***. White crystals (1.75 g, 68%); m.p. 169–171 °C; IR (KBr): 1130, 1328 (SO_2), 1628 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.48 (s, 2H, CH_2), 7.21–7.77 (m, 9H, Ar-H) ppm; ^{13}C NMR (DMSO-d_6) δ 54.8 (CH_2SO_2), 163.5 (C_2), 167.5 (C_5), 126.5, 128.9, 129.2, 129.6, 130.5, 130.7, 131.3, 133.5 ppm (aromatic carbons). Anal. Calcd. for $C_{15}H_{11}ClN_2O_2S_2$: C, 51.35; H, 3.16; N, 7.98; Found: C, 51.40; H, 3.19; N, 8.02%.

6.1.4.4. 2-(4-Chlorophenylsulfonylmethyl)-5-(2-chlorophenyl)-1,3,4-thiadiazole ***9d***. White solid (1.92 g, 66%); m.p. 194–196 °C; IR (KBr): 1146, 1328 (SO_2), 1639 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.46 (s, 2H, CH_2), 7.37–8.21 (m, 8H, Ar-H) ppm; ^{13}C NMR (DMSO-d_6) δ 55.1 (CH_2SO_2), 162.8 (C_2), 168.0 (C_5), 124.9, 128.8, 129.3, 130.8, 131.1, 132.3, 133.4, 134.6, 135.2 ppm (aromatic carbons). Anal. Calcd. for $C_{15}H_{10}Cl_2N_2O_2S_2$: C, 46.76; H, 2.62; N, 7.27; Found: C, 46.83; H, 2.58; N, 7.32%.

6.1.4.5. 2-(Benzylsulfonylmethyl)-5-phenyl-1,3,4-thiadiazole ***10a***. White solid (1.65 g, 65%); m.p. 188–190 °C; IR (KBr): 1137, 1326 (SO_2), 1625 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.57 (s, 2H, CH_2), 4.65 (s, 2H, Ar-CH_2), 7.25–7.62 (m, 10H, Ar-H) ppm; ^{13}C NMR (DMSO-d_6) δ 52.8 (CH_2SO_2), 58.5 ($ArCH_2$), 164.5 (C_2), 167.9 (C_5), 127.2, 128.5, 129.3, 129.9, 130.6, 131.5, 131.8, 134.3 ppm (aromatic carbons). Anal. Calcd. for $C_{16}H_{14}N_2O_2S_2$: C, 58.16; H, 4.27; N, 8.48; Found: C, 58.13; H, 4.31; N, 8.40%.

6.1.4.6. 2-(Benzylsulfonylmethyl)-5-(2-chlorophenyl)-1,3,4-thiadiazole ***10b***. White solid (1.82 g, 68%); m.p. 181–183 °C; IR (KBr): 1128, 1313 (SO_2), 1634 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.52 (s, 2H, CH_2), 4.60 (s, 2H, Ar-CH_2), 7.29–7.68 (m, 9H, Ar-H) ppm; ^{13}C NMR (DMSO-d_6) δ 51.0 (CH_2SO_2), 57.8 ($ArCH_2$), 163.7 (C_2), 167.5 (C_5), 128.6, 129.2,

129.8, 130.2, 132.4, 133.1, 134.6, 134.9 ppm (aromatic carbons). Anal. Calcd. for $C_{16}H_{13}ClN_2O_2S_2$: C, 52.67; H, 3.59; N, 7.68; Found: C, 52.60; H, 3.55; N, 7.73%.

6.1.4.7. 2-(4-Chlorobenzylsulfonylmethyl)-5-phenyl-1,3,4-thiadiazole ***10c***. White solid (1.82 g, 67%); m.p. 195–197 °C; IR (KBr): 1131, 1321 (SO_2), 1630 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.48 (s, 2H, CH_2), 4.57 (s, 2H, Ar-CH_2), 7.30–7.75 (m, 9H, Ar-H) ppm; ^{13}C NMR (DMSO-d_6) δ 51.5 (CH_2SO_2), 58.8 ($ArCH_2$), 164.1 (C_2), 168.8 (C_5), 127.2, 128.8, 129.2, 130.4, 130.8, 131.3, 132.2, 134.8 ppm (aromatic carbons). Anal. Calcd. for $C_{16}H_{13}ClN_2O_2S_2$: C, 52.67; H, 3.59; N, 7.68; Found: C, 52.60; H, 3.55; N, 7.64%.

6.1.4.8. 2-(4-Chlorobenzylsulfonylmethyl)-5-(2-chlorophenyl)-1,3,4-thiadiazole ***10d***. White crystals (1.99 g, 68%); m.p. 206–208 °C; IR (KBr): 1138, 1330 (SO_2), 1629 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.50 (s, 2H, CH_2), 4.59 (s, 2H, Ar-CH_2), 7.41–8.17 (m, 8H, Ar-H) ppm; ^{13}C NMR (DMSO-d_6) δ 51.7 (CH_2SO_2), 57.7 ($ArCH_2$), 164.6 (C_2), 167.6 (C_5), 127.9, 128.7, 129.7, 130.4, 131.2, 131.8, 132.6, 133.1, 134.3, 135.6 ppm (aromatic carbons). Anal. Calcd. for $C_{16}H_{12}Cl_2N_2O_2S_2$: C, 48.13; H, 3.03; N, 7.02; Found: C, 48.19; H, 3.00; N, 6.93%.

6.1.5. General procedure for the synthesis of 3-(arylsulfonylmethyl)-5-aryl-4H-1,2,4-triazol-4-amines ***11a–d****/3-(arylmethanesulfonylmethyl)-5-aryl-4H-1,2,4-triazol-4-amines* ***12a–d***

To a solution of **7**/**8** (5 mmol) in *n*-butanol (25 ml), hydrazine hydrate (15 mmol) was added and refluxed for 4 h. Then, KOH (10 mmol) 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. The resultant solid was recrystallized from ethanol.

6.1.5.1. 3-(Phenylsulfonylmethyl)-5-phenyl-4H-1,2,4-triazol-4-amine ***11a***. White solid (1.57 g, 65%); m.p. 147–149 °C; IR (KBr): 1141, 1337 (SO_2), 1630 (C=N), 3243, 3269 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.52 (s, 2H, CH_2), 5.61 (bs, 2H, NH_2), 7.17–7.48 (m, 10H, Ar-H) ppm; ^{13}C NMR (DMSO-d_6) δ 55.2 (CH_2SO_2), 162.9 (C_2), 168.3 (C_5), 126.5, 127.1, 127.9, 129.2, 130.5, 132.5, 135.8, 136.3 ppm (aromatic carbons). Anal. Calcd. for $C_{15}H_{14}N_4O_2S$: C, 57.31; H, 4.49; N, 17.82; Found: C, 57.37; H, 4.54; N, 17.94%.

6.1.5.2. 3-(Phenylsulfonylmethyl)-5-(2-chlorophenyl)-4H-1,2,4-triazol-4-amine ***11b***. White solid (1.74 g, 68%); m.p. 133–135 °C; IR (KBr): 1128, 1331 (SO_2), 1626 (C=N), 3247, 3259 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.56 (s, 2H, CH_2), 5.57 (bs, 2H, NH_2), 7.19–7.85 (m, 9H, Ar-H) ppm; ^{13}C NMR (DMSO-d_6) δ 54.9 (CH_2SO_2), 163.4 (C_2), 167.9 (C_5), 125.1, 125.7, 126.6, 127.4, 128.3, 131.7, 133.8, 139.9 ppm (aromatic carbons). Anal. Calcd. for $C_{15}H_{13}ClN_4O_2S$: C, 51.65; H, 3.76; N, 16.06; Found: C, 51.73; H, 3.74; N, 16.17%.

6.1.5.3. 3-(4-Chlorophenylsulfonylmethyl)-5-phenyl-4H-1,2,4-triazol-4-amine ***11c***. White solid (1.74 g, 69%); m.p. 142–144 °C; IR (KBr): 1145, 1338 (SO_2), 1625 (C=N), 3240, 3252 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.53 (s, 2H, CH_2), 5.63 (bs, 2H, NH_2), 7.22–7.79 (m, 9H, Ar-H) ppm; ^{13}C NMR (DMSO-d_6) δ 54.7 (CH_2SO_2), 162.7 (C_2), 168.8 (C_5), 126.4, 126.9, 127.2, 129.3, 130.4, 131.3, 133.6, 138.7 ppm (aromatic carbons). Anal. Calcd. for $C_{15}H_{13}ClN_4O_2S$: C, 51.65; H, 3.76; N, 16.06; Found: C, 51.60; H, 3.73; N, 16.00%.

6.1.5.4. 3-(4-Chlorophenylsulfonylmethyl)-5-(2-chlorophenyl)-4H-1,2,4-triazol-4-amine ***11d***. White crystals (1.91 g, 66%); m.p. 179–181 °C; IR (KBr): 1142, 1334 (SO_2), 1637 (C=N), 3244, 3253 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.49 (s, 2H, CH_2), 5.58 (bs, 2H, NH_2), 7.38–8.14 (m, 8H, Ar-H) ppm; ^{13}C NMR (DMSO-d_6) δ 54.5 (CH_2SO_2), 163.2 (C_2), 168.2 (C_5), 125.3, 126.6, 127.1, 128.6, 129.5, 131.7, 133.9, 134.6, 135.2, 137.5 ppm (aromatic carbons). Anal. Calcd. for $C_{15}H_{12}Cl_2N_4O_2S$: C, 47.01; H, 3.16; N, 14.62; Found: C, 47.04; H, 3.14; N, 14.71%.

6.1.5.5. 3-(Benzylsulfonylmethyl)-5-phenyl-4H-1,2,4-triazol-4-amine ***12a***. White solid (1.64 g, 63%); m.p. 189–191 °C; IR (KBr): 1125, 1324 (SO_2), 1639 (C=N), 3242, 3256 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.57 (s, 2H, CH_2), 4.63 (s, 2H, Ar-CH_2), 5.62 (bs, 2H, NH_2), 7.14–7.65 (m, 10H, Ar-H) ppm; ^{13}C NMR (DMSO-d_6) δ 53.0 (CH_2SO_2), 58.4 ($ArCH_2$), 163.0 (C_2), 168.5 (C_5), 124.4, 128.3, 129.0, 130.5, 131.3, 132.2, 133.4, 135.8 ppm (aromatic carbons). Anal. Calcd. for $C_{16}H_{16}N_4O_2S$: C, 58.52; H, 4.91; N, 17.06; Found: C, 58.63; H, 4.96; N, 17.16%.

6.1.5.6. 3-(Benzylsulfonylmethyl)-5-(2-chlorophenyl)-4H-1,2,4-triazol-4-amine ***12b***. White solid (1.81 g, 68%); m.p. 185–187 °C; IR (KBr): 1132, 1318 (SO_2), 1633 (C=N), 3239, 3246 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.54 (s, 2H, CH_2), 4.66 (s, 2H, Ar-CH_2), 5.65 (bs, 2H, NH_2), 7.23–7.71 (m, 9H, Ar-H) ppm; ^{13}C NMR (DMSO-d_6) δ 53.4 (CH_2SO_2), 58.9 ($ArCH_2$), 164.4 (C_2), 168.2 (C_5), 127.6, 128.3, 128.9, 129.6, 131.5, 132.6, 133.4, 134.8 ppm (aromatic carbons). Anal. Calcd. for $C_{16}H_{15}ClN_4O_2S$: C, 52.96; H, 4.17; N, 15.44; Found: C, 53.04; H, 4.14; N, 15.53%.

6.1.5.7. 3-(4-Chlorobenzylsulfonylmethyl)-5-phenyl-4H-1,2,4-triazol-4-amine ***12c***. White solid (1.81 g, 67%); m.p. 198–200 °C; IR (KBr): 1137, 1325 (SO_2), 1636 (C=N), 3245, 3251 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.58 (s, 2H, CH_2), 4.64 (s, 2H, Ar-CH_2), 5.60 (bs, 2H, NH_2), 7.16–7.74 (m, 9H, Ar-H) ppm; ^{13}C NMR (DMSO-d_6) δ 52.6 (CH_2SO_2), 59.1 ($ArCH_2$), 164.7 (C_2), 167.6 (C_5), 126.7, 127.1, 128.1, 128.6, 129.0, 129.4, 131.7, 134.4 ppm (aromatic carbons). Anal. Calcd. for $C_{16}H_{15}ClN_4O_2S$: C, 52.96; H, 4.17; N, 15.44; Found: C, 52.93; H, 4.13; N, 15.50%.

6.1.5.8. 3-(4-Chlorobenzylsulfonylmethyl)-5-(2-chlorophenyl)-4H-1,2,4-triazol-4-amine ***12d***. White solid (1.98 g, 65%); m.p. 202–204 °C; IR (KBr): 1134, 1337 (SO_2), 1638 (C=N), 3248, 3257 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.61 (s, 2H, CH_2), 4.69 (s, 2H, Ar-CH_2), 5.58 (bs, 2H, NH_2), 7.43–8.22 (m, 8H, Ar-H) ppm; ^{13}C NMR (DMSO-d_6) δ 52.8 (CH_2SO_2), 58.2 ($ArCH_2$), 163.5 (C_2), 167.3 (C_5), 127.0, 127.4, 129.7, 130.4, 130.0, 132.7, 133.8, 134.2, 135.6, 137.1 ppm (aromatic carbons). Anal. Calcd. for $C_{16}H_{14}Cl_2N_4O_2S$: C, 48.37; H, 3.55; N, 14.10; Found: C, 48.30; H, 3.53; N, 14.16%.

6.2. Biological assays

6.2.1. Compounds

Compounds **7a–d** to **12a–d** were dissolved in DMSO at different concentrations of 100, 200 and 800 μg/ml.

6.2.2. Cells

Bacterial strains *S. aureus*, *B. subtilis*, *E. coli*, *K. pneumonie* and fungi *F. solani*, *C. lunata* and *A. niger* were obtained from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune, India.

6.2.3. Antibacterial and antifungal assays

Preliminary antimicrobial activities of compounds **7a–d** to **12a–d** were tested by Agar disc-diffusion method. Sterile filter paper discs (6 mm diameter) moistened with the test compound solution in DMSO of specific concentration 100 μg and 200 μg/disc were carefully placed on the agar culture plates that had been previously inoculated separately with the microorganisms. The plates were incubated at 37 °C and the diameter of the growth inhibition zones were measured after 24 h in case of bacteria and after 48 h in case of fungi.

The MICs of the compound assays were carried out using microdilution susceptibility method. Chloramphenicol was used as reference antibacterial agent. Ketoconazole was used as reference

antifungal agent. The test compounds, chloramphenicol and ketoconazole were dissolved in DMSO at concentration of 800 μg/ml and two-fold serial dilution of the solution was prepared (400, 200, 100, ., 6.25 mg/ml). The microorganism suspensions were inoculated to the corresponding wells. The plates were incubated at 36 °C for 24 h and 48 h for bacteria and fungi, respectively. The minimum inhibitory concentrations of the compounds were recorded as the lowest concentration of each chemical compounds in the tubes with no turbidity (i.e. no growth) of inoculated bacteria/fungi.

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