Full Paper

Synthesis and Antioxidant Activity of a New Class of Mono- and Bis-Heterocycles

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A new class of mono- and bis-heterocycles, 2-arylsulfonylaminosulfonylmethyl-5-styrylsulfonylmethyl-oxadiazoles, pyrazolyl oxadiazoles, and isoxazolyl oxadiazoles, were synthesized and tested for their antioxidant activity. The styrylsulfonylmethyloxadiazole **5b** showed good antioxidant activity when compared with the standard ascorbic acid.

Keywords: 1,3-Dipolar cycloaddition / Antioxidant activity / Isoxazolyl oxadiazole / Pyrazolyl oxadiazole / Styrylsulfonylmethyloxadiazole

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Introduction

Heterocycles are ubiquitous in natural products, pharmaceuticals, organic materials, and numerous functional molecules. Therefore, the interest for developing new, versatile, and efficient methods for the synthesis of heterocycles has always been a thread in the synthetic community [1]. The 2,5-disubstituted 1,3,4-oxadiazole motif is of considerable importance in different areas of chemistry *viz*., medicinal, pesticide, polymer, and material science [2]. In drug discovery and development, the compounds containing oxadiazole moiety are in late stage clinical trials, including zibotentan as an anticancer agent [3] and ataluren used for the treatment of cystic fibrosis [4]. Raltegravir is an oxadiazole containing antiretroviral drug used for the treatment of HIV infection.

The 1,3,4-oxadiazoles are prepared by (a) oxidative cyclization of *N*-acylhydrazones with various oxidizing agents such as chloramine T [5], ceric ammonium nitrate [6], $FeCl_3$ [7], $KMnO_4$ under microwave irradiation [8], (b) cyclodehydration of 1,2-diacylhydrazines with thionyl chloride, PPA, phosphorus oxychloride, H_2SO_4 [9] and (c) direct reaction of carboxylic acids or acyl chlorides with acid hydrazides or hydrazines [10]. Additionally, a vast number of bioactive natural products and pharmaceutical drugs based on the pyrazole and isoxazole ring systems such as celecoxib, valdecoxib, leflunomide, and cloxacillin have become very important areas of research in natural product and pharmaceutical chemistry [11]. So far, extensive work has been generated for the synthesis of pyrazoles and isoxazoles based on [2+3] cycloaddition of 1,3-dipoles to alkynes [12] and olefins [13–15], condensation of hydrazine and hydroxylamine with β-diketone equivalents such as propargylic ketones, enones, and α,β-unsaturated nitriles [16]. As part of our continued interest to develop biologically active heterocycles, we report herein the synthesis and bioassay of a new class of mono- and bis-heterocycles.

Results and discussion

Chemistry

A new series of 2-arylsulfonylaminosulfonylmethyl-5-styrylsulfonylmethyloxadiazoles, pyrazolyl oxadiazoles, and isoxazolyl oxadiazoles were prepared from the simple, multifunctional starting compounds, methyl 2-((arylsulfonyl)aminosulfonyl)-acetate (**2**) and *Z*-styrylsulfonylacetic acid (**4**) (Scheme 1). Compound **2** was obtained by the reaction of arylsulfonamide (**1**) with chlorosulfonylmethylacetate in the presence of Et_3N. Treatment of compound **2** with hydrazine hydrate led to the formation of 2-((arylsulfonyl)aminosulfonyl)acetohydrazide (**3**). The condensation between compounds **3** and **4** in the presence of $POCl_3$ produced 2-(((arylsulfonyl-)aminosulfonyl)-methyl)-5-((styrylsulfonyl)methyl)-1,3,4-oxadiazoles (**5**). The IR

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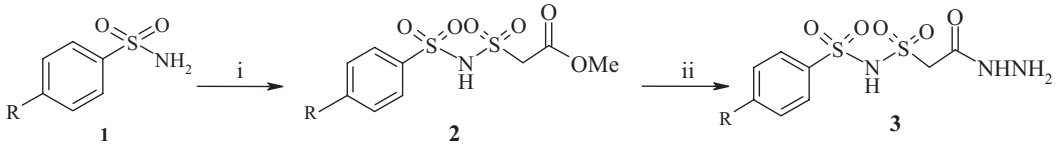
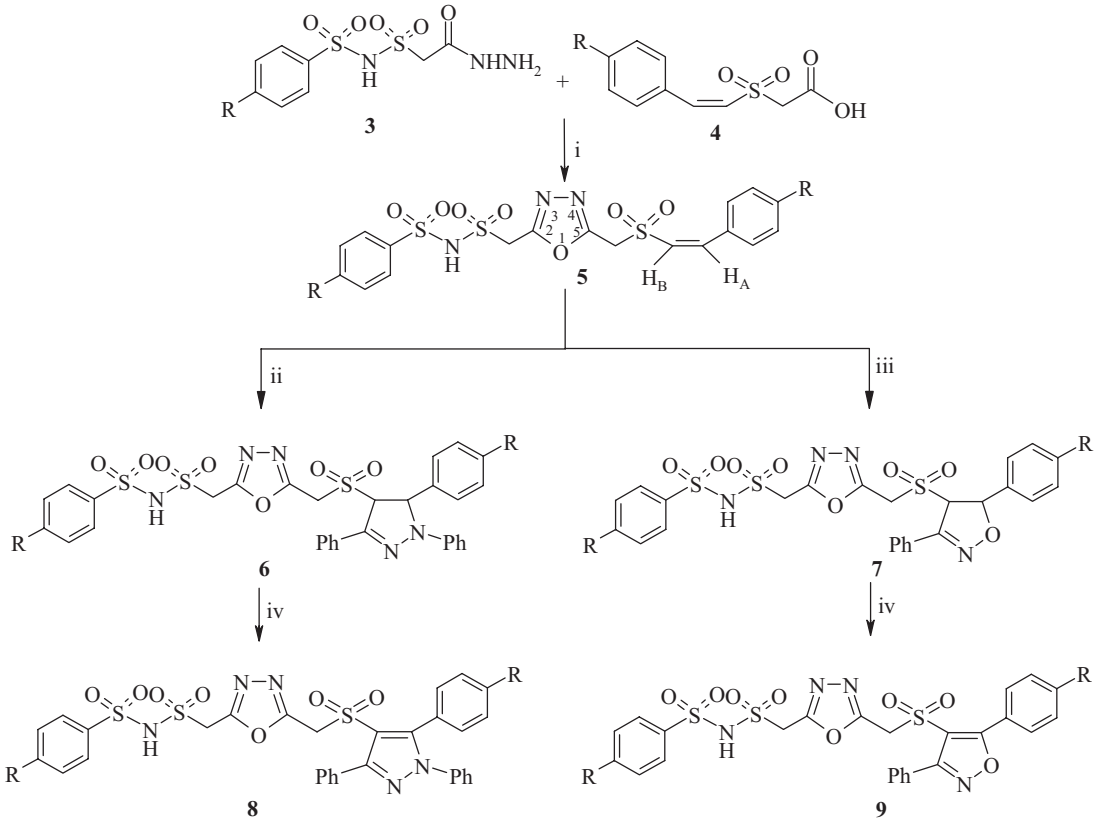


(i) $ClSO_2CH_2CO_2Me$ / Et_3N / Benzene
(ii) $NH_2NH_2.H_2O$ / Pyridine / MeOH

R a = H
b = Me
c = Cl



(i) $POCl_3$
(ii) Ph-CH=NNHPh / Chloramine-T . $3H_2O$ / MeOH
(iii) Ph-CH=NOH / Chloramine-T . $3H_2O$ / MeOH
(iv) Chloranil / Xylene

R a = H
b = Me
c = Cl

Scheme 1. Synthesis of mono- and bis-heterocycles.

spectra of compound **5** exhibited absorption bands in the regions 1149–1162 and 1302–1315 (SO_2), 1560–1577 (CN), 1610–1625 (C=C) and 3219–3230 cm^{-1} (NH). The ^1H NMR spectrum of compound **5a** showed two singlets at δ 5.16, 4.92 ppm which were assigned to methylene protons attached to C-2 and C-5, respectively. Apart from these, a doublet was observed at δ 6.72 ppm due to the olefin proton H_B while the other olefin proton H_A displayed a signal in a more downfield region and merged with aromatic protons. The coupling constant J = 9.6 Hz indicated that they are in *cis* geometry. The compound **5a** presented a broad singlet at δ 10.59 ppm due to NH and disappeared when D_2O was added. The olefin moiety in compounds **5** was exploited to develop pyrazole and isoxazole rings by 1,3-dipolar cycloaddition. Thus, the cycloaddition of

nitrile imines generated from araldehyde phenylhydrazone in the presence of chloramine-T to **5** resulted in tetrasubstituted pyrazolinyl derivative 2-(((arylsulfonyl)aminosulfonyl)methyl)-5-((4′,5′-dihydro-1′,3′-diphenyl-5′-aryl-1′*H*-pyrazol-4′-ylsulfonyl)-methyl)-1,3,4-oxadiazole (**6**). Likewise, the addition of nitrile oxides generated from araldoximes in the presence of chloramine-T to **5** gave 2-(((arylsulfonyl)aminosulfonyl)methyl)-5-((4′,5′-dihydro-3′-phenyl-5′-arylisoxazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazole (**7**). In the IR spectra of compounds **6** and **7**, the absorption bands observed at 1140–1151 and 1329–1347, 1565–1585 and 3241–3263 cm^{-1} were assigned to SO_2, C=N and NH, respectively. The ^1H NMR spectra of compounds **6a** and **7a** displayed two doublets at δ 5.13, 5.17 and 5.42, 5.50 ppm due to $C_{5'}$-H and $C_{4'}$-H, two singlets at δ 5.20, 5.22, and 4.83, 4.96 ppm due to methylene protons attached to C-2 and C-5, respectively. Moreover, a broad singlet was observed at δ 10.54 in **6a** and at 10.62 ppm in **7a** due to NH, which disappeared when D_2O was added. The oxidation of compounds **6** and **7** was carried out to afford the aromatized compounds. Thus, 2-(((arylsulfonyl)aminosulfonyl)methyl)-5-((1′,3′-diphenyl-5′-aryl-1′*H*-pyrazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazole (**8**) and 2-(((arylsulfonyl)-aminosulfonyl)methyl)-5-((3′-phenyl-5′-arylisoxazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazole (**9**) were prepared by the reaction of compounds **6** and **7** with chloranil in xylene. The absence of signals due to methine protons of pyrazoline and isoxazoline units confirmed their formation. The structures of the lead compounds were further established by ^{13}C NMR spectral data.

Biological results

Antioxidant activity

The compounds **5–9** were evaluated for antioxidant property by 2,2′,-diphenyl-1-picrylhydrazyl (DPPH) [17, 18], nitric oxide (NO) [19, 20], and hydrogen peroxide (H_2O_2) [21] methods.

The observed data on the antioxidant activity of the compounds and control drug are shown in Tables 1–3 and Figs. 1–3. The aim of this study is to identify the potential heterocyclic compound for antioxidant activity. The compounds **5b**, **8b**, and **9b** showed excellent radical scavenging activity in all the three methods due to the presence of electron donating group, Me, in benzene ring when compared with the standard drug ascorbic acid. The compounds **5a**, **8a**, and **9a** exhibited good activity whereas the compounds **5c**, **6b**, **7b**, **8c**, and **9c** displayed moderate activity. However, the other compounds showed no activity. Amongst bis-heterocyclic systems, the aromatized compounds **8** and **9** exhibited greater activity than the corresponding dihydro compounds **6** and **7**. It was also perceived that mono-heterocyclic compound **5b** displayed slightly higher activity than the corresponding bis-heterocyclic systems **8b** and **9b**. This may be due to extended conjugation in mono-heterocyclic systems. However, the compound isoxazole in combination with oxadiazole **9** exhibited greater activity which may be due to the presence of two oxygen atoms than the compounds having pyrazole and oxadiazole **8**. The IC_{50} values of the standard ascorbic acid in DPPH method was found to be 59.65 μg/mL at 100 μg/mL whereas the IC_{50} values of the

Table 1. The *in vitro* antioxidant activity of **5–9** in the DPPH method.

Compound	Concentration			
	50 μg/mL	75 μg/mL	100 μg/mL	IC_{50} μmol/mL
5a	68.19 ± 0.82	70.11 ± 0.42	72.91 ± 0.71	0.075 ± 0.04
5b	75.57 ± 1.06	78.24 ± 0.25	82.45 ± 1.01	0.064 ± 1.08
5c	50.42 ± 0.54	52.37 ± 0.16	57.40 ± 0.33	0.089 ± 0.69
6a	–	–	–	–
6b	55.15 ± 0.36	59.57 ± 1.17	61.85 ± 1.26	0.064 ± 1.32
6c	–	–	–	–
7a	–	–	–	–
7b	58.41 ± 1.87	62.28 ± 1.32	64.76 ± 1.41	0.067 ± 1.86
7c	–	–	–	–
8a	60.48 ± 0.15	63.18 ± 0.42	66.72 ± 0.96	0.061 ± 0.45
8b	70.23 ± 1.38	72.08 ± 0.49	75.61 ± 1.07	0.050 ± 1.66
8c	44.38 ± 0.91	46.70 ± 1.07	49.81 ± 0.09	0.075 ± 1.06
9a	65.38 ± 0.12	68.67 ± 0.61	71.50 ± 1.42	0.063 ± 0.07
9b	73.57 ± 1.03	76.24 ± 0.52	80.45 ± 1.54	0.054 ± 0.22
9c	48.33 ± 1.73	50.36 ± 0.78	52.17 ± 1.88	0.077 ± 1.13
Ascorbic acid	77.15 ± 0.45	80.95 ± 0.39	83.82 ± 0.81	0.183 ± 1.20
Blank	–	–	–	–

(–) Showed no scavenging activity.
Values were the means of three replicates ± SD.

Table 2. The *in vitro* antioxidant activity of **5–9** in the nitric oxide method.

Compound	Concentration		
	50 μg/mL	75 μg/mL	100 μg/mL
5a	62.41 ± 1.15	64.32 ± 1.34	66.15 ± 1.01
5b	70.51 ± 0.20	74.14 ± 0.55	76.43 ± 1.50
5c	48.57 ± 0.97	50.01 ± 1.42	54.74 ± 1.11
6a	–	–	–
6b	52.10 ± 1.29	54.15 ± 1.02	58.67 ± 1.67
6c	–	–	–
7a	–	–	–
7b	55.24 ± 1.30	57.37 ± 1.72	60.14 ± 0.47
7c	–	–	–
8a	58.38 ± 0.59	60.65 ± 1.31	62.83 ± 1.17
8b	65.43 ± 1.66	68.21 ± 1.81	70.20 ± 1.41
8c	42.81 ± 1.69	45.26 ± 1.61	48.63 ± 0.31
9a	60.74 ± 0.16	62.33 ± 0.71	65.21 ± 0.37
9b	67.51 ± 1.25	69.40 ± 1.05	73.43 ± 0.89
9c	46.29 ± 1.51	48.10 ± 1.23	50.92 ± 0.77
Ascorbic acid	78.23 ± 0.17	81.46 ± 1.37	82.79 ± 0.80
Blank	–	–	–

(–) Showed no scavenging activity.
Values were the means of three replicates ± SD.

Table 3. The *in vitro* antioxidant activity of **5–9** in the H_2O_2 method.

Compound	Concentration		
	50 μg/mL	75 μg/mL	100 μg/mL
5a	63.59 ± 1.54	64.72 ± 1.76	69.21 ± 1.85
5b	71.42 ± 1.48	75.23 ± 1.25	77.67 ± 1.68
5c	49.12 ± 0.81	52.37 ± 1.63	56.01 ± 1.92
6a	–	–	–
6b	50.63 ± 1.04	53.47 ± 1.81	55.92 ± 1.96
6c	–	–	–
7a	–	–	–
7b	57.83 ± 0.45	59.08 ± 1.63	61.18 ± 1.45
7c	–	–	–
8a	59.76 ± 1.17	60.91 ± 0.73	62.15 ± 0.38
8b	66.70 ± 1.60	68.84 ± 1.74	70.62 ± 1.89
8c	43.16 ± 1.10	46.48 ± 0.60	50.43 ± 0.11
9a	61.30 ± 1.55	63.19 ± 1.86	65.77 ± 1.30
9b	69.27 ± 0.38	70.56 ± 0.19	72.68 ± 0.21
9c	46.46 ± 0.79	49.79 ± 1.15	51.14 ±1.01
Ascorbic acid	77.68 ± 0.51	79.27 ± 1.29	83.16 ± 0.44
Blank	–	–	–

(–) Showed no scavenging activity.
Values were the means of three replicates ± SD.

compounds **5b**, **8b**, and **9b** were found to be 33.08, 35.47, and 33.98 μg/mL. Besides, the perusal of Tables 1–3 indicated that radical scavenging activity in DPPH, nitric oxide, and hydrogen peroxide methods increases with increase in concentration.

Conclusion

In summary, we prepared a novel series of 2-arylsulfonylaminosulfonylmethyl-5-styrylsulfonylmethyloxadiazoles, pyrazolyl oxadiazoles, and isoxazolyl oxadiazoles from simple compounds **3** and **4** adopting versatile synthetic methodologies. Among the tested compounds, the styrylsulfonylmethyloxadiazole derivative (**5b**) is a potential antioxidant agent.

Experimental

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 wavenumbers are given in cm^{-1}. The ^1H NMR spectra were recorded in DMSO-d_6 on a Bruker-400 spectrometer (400 MHz). The ^{13}C NMR spectra were recorded in DMSO-d_6 on a Bruker spectrometer operating at 100 MHz. All chemical shifts are reported in δ (ppm) using TMS as an internal standard. The mass spectra were recorded on Jeol JMS-D 300 and Finnigan Mat 1210 B at 70 eV with an emission current of 100 μA. The microanalyses were performed on a Perkin-Elmer 240C elemental analyzer. The antioxidant property was determined by using a Shimadzu UV-2450 spectrophotometer. The *Z*-styrylsulfonylacetic acid (**4**) was prepared by the literature procedure [22, 23].

*General procedure for the synthesis of methyl 2-((arylsulfonyl)aminosulfonyl)acetate (**2a–c**)*

To a solution of chlorosulfonylmethylacetate (1.71 g, 10 mmol) in benzene (60 mL), arylsulfonamide (**1**) (10 mmol) and triethylamine (15 mL) in benzene (150 mL) were added dropwise while stirring. After addition was completed, the mixture was warmed gently for 5 min. Then, it was cooled and filtered. The filtrate was washed successively with water, dil. HCl, sat. $NaHCO_3$ solution, and brine solution. The organic layer was dried over an. Na_2SO_4 and the solvent was removed *in vacuo*. The resultant compound was purified by recrystallization from benzene/cyclohexane.

*Methyl 2-((phenylsulfonyl)aminosulfonyl)acetate **2a***

White solid in 66%; m.p.: 148–150°C; IR (KBr) υ_{max} (cm^{-1}): 1124, 1318 (SO_2), 1732 (CO_2Me), 3230 (NH); ^1H NMR (DMSO-d_6) δ (ppm): 3.60 (s, 3H, OCH_3), 4.42 (s, 2H, CH_2), 7.21–7.85 (m, 5H, Ar–H), 10.25 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 50.2 (OCH_3), 54.6 (CH_2), 162.5 (CO), 126.2, 127.3, 129.5, 131.5 (aromatic carbons); MS (*m*/*z*): 293.33 [M^+]. Anal. calcd. for $C_9H_{11}NO_6S_2$: C 36.85, H 3.78, N 4.77. Found: C 36.47, H 3.89, N 4.82%.

*Methyl 2-((4-methylphenylsulfonyl)aminosulfonyl)acetate **2b***

White solid in 78%; m.p.: 135–137°C; IR (KBr) υ_{max} (cm^{-1}): 1119, 1322 (SO_2), 1727 (CO_2Me), 3223 (NH); ^1H NMR (DMSO-d_6) δ (ppm): 2.32 (s, 3H, Ar–CH_3), 3.58 (s, 3H, OCH_3), 4.37 (s, 2H, CH_2), 7.19–7.68 (m, 4H, Ar–H), 10.21 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 24.5 (Ar–CH_3), 49.9 (OCH_3), 56.7 (CH_2), 163.2 (CO), 126.9, 128.4, 133.4, 134.1 (aromatic carbons); MS (*m*/*z*): 307.35 [M^+]. Anal. calcd.

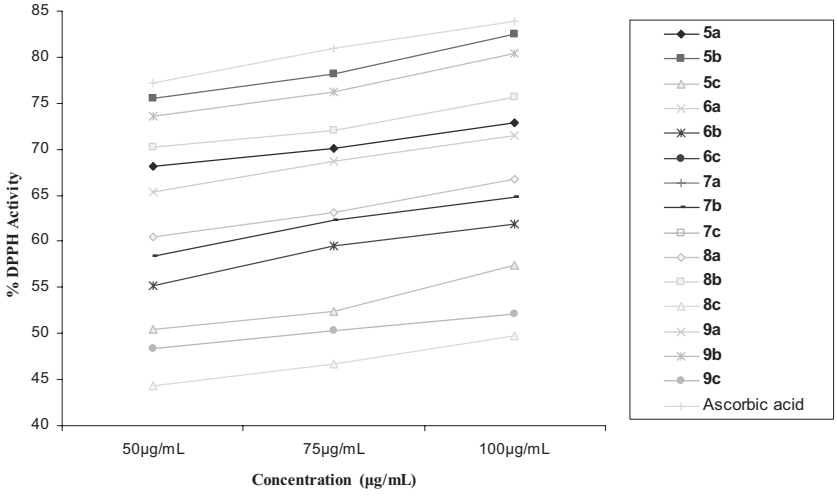


Figure 1. The *in vitro* antioxidant activity of **5**–**9** in the DPPH method.

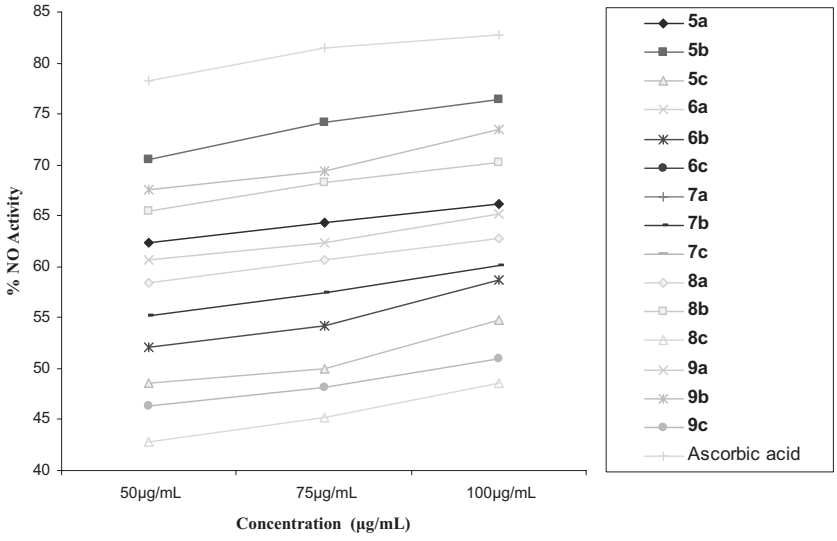


Figure 2. The *in vitro* antioxidant activity of **5**–**9** in the nitric oxide method.

for $C_{10}H_{13}NO_6S_2$: C 39.07, H 4.26, N 4.55. Found: C 39.40, H 4.29, N 4.77%.

Methyl 2-((4-chlorophenylsulfonyl)aminosulfonyl)acetate ***2c***

White solid in 80%; m.p.: 164–165°C; IR (KBr) υ_{max} (cm^{-1}): 1137, 1327 (SO_2), 1739 (CO_2Me), 3241 (NH); ^1H NMR (DMSO-d_6) δ (ppm): 3.64 (s, 3H, OCH_3), 4.45 (s, 2H, CH_2), 7.37–7.91 (m, 4H, Ar–H), 10.33 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 51.5 (OCH_3), 55.4 (CH_2), 162.7 (CO), 123.2, 125.7, 130.6, 134.1 (aromatic carbons); MS (*m/z*): 327.77 [M^+]. Anal. calcd. for $C_9H_{10}ClNO_6S_2$: C 32.98, H 3.07, N 4.27. Found: C 33.39, H 3.28, N, 4.63%.

*General procedure for the synthesis of 2-((arylsulfonyl)-aminosulfonyl)acetohydrazide (**3a–c**)*

A mixture of compound **2** (10 mmol), hydrazine hydrate (0.53 mL, 11 mmol), methanol (6 mL), and three drops of pyridine was refluxed for 5–7 h. The reaction mixture was cooled. The solid separated was collected by filtration, dried, and recrystallized from methanol.

2-((Phenylsulfonyl)aminosulfonyl)acetohydrazide ***3a***

White solid in 75%; m.p.: 166–168°C; IR (KBr) υ_{max} (cm^{-1}): 1150, 1321 (SO_2), 1647 (CONH), 3242 (NH), 3335, 3441 (NH_2); ^1H NMR (DMSO-d_6) δ (ppm): 3.91 (s, 2H, CH_2), 4.34 (bs, 2H, NH_2), 7.31–7.69 (m, 5H, Ar–H), 9.39 (bs, 1H, CO–NH), 10.45 (bs, 1H, NH–SO_2); ^{13}C NMR (DMSO-d_6) δ (ppm): 56.2 (CH_2), 159.3 (CO), 124.5, 125.9, 127.4, 132.9 (aromatic carbons); MS (*m/z*): 293.33 [M^+]. Anal. calcd. for $C_8H_{11}N_3O_5S_2$: C 32.75, H 3.78, N 14.32. Found: C 32.52, H 3.65, N, 14.72%.

2-((4-Methylphenylsulfonyl)aminosulfonyl)acetohydrazide ***3b***

White solid in 71%; m.p.: 152–154°C; IR (KBr) υ_{max} (cm^{-1}): 1144, 1316 (SO_2), 1643 (CONH), 3237 (NH), 3329, 3435 (NH_2); ^1H NMR

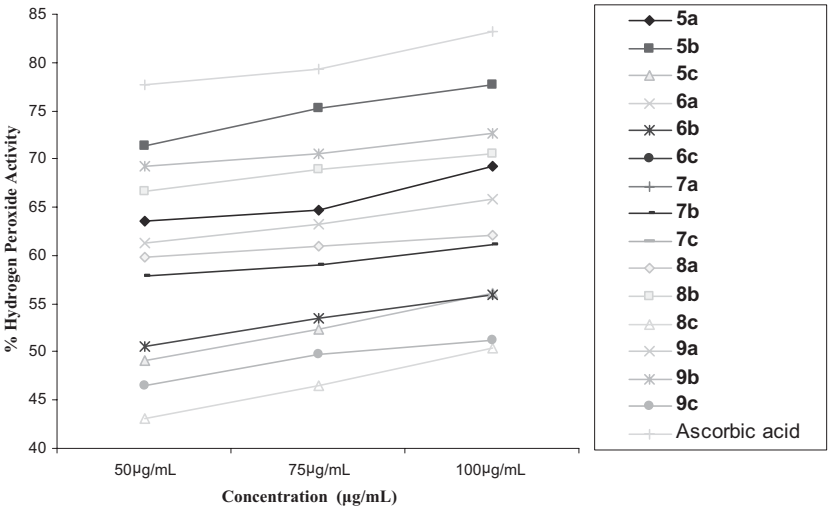


Figure 3. The *in vitro* antioxidant activity of **5–9** in the hydrogen peroxide method.

(DMSO-d_6) δ (ppm): 2.30 (s, 3H, Ar–CH_3), 3.85 (s, 2H, CH_2), 4.22 (bs, 2H, NH_2), 7.23–7.37 (m, 4H, Ar–H), 9.34 (bs, 1H, CO–NH), 10.38 (bs, 1H, NH–SO_2); ^{13}C NMR (DMSO-d_6) δ (ppm): 23.2 (Ar–CH_3), 54.3 (CH_2), 157.6 (CO), 121.9, 123.2, 126.6, 129.5, 132.4 (aromatic carbons); MS (*m/z*): 307.35 [M^+]. Anal. calcd. for $C_9H_{13}N_3O_5S_2$: C 35.17, H 4.26, N 13.67; Found: C 35.59, H 4.73, N 13.89%.

2-((4-Chlorophenylsulfonyl)aminosulfonyl)acetohydrazide ***3c***

White solid in 79%; m.p.: 163–165°C; IR (KBr) υ_{max} (cm^{-1}): 1158, 1324 (SO_2), 1651 (CONH), 3248 (NH), 3341, 3450 (NH_2); ^{1}H NMR (DMSO-d_6) δ (ppm): 3.98 (s, 2H, CH_2), 4.42 (bs, 2H, NH_2), 7.39–7.47 (m, 4H, Ar–H), 9.47 (bs, 1H, CO–NH), 10.52 (bs, 1H, NH–SO_2); ^{13}C NMR (DMSO-d_6) δ (ppm): 58.8 (CH_2), 162.3 (CO), 124.1, 125.7, 128.4, 135.2 (aromatic carbons); MS (*m/z*): 327.77 [M^+]. Anal. calcd. for $C_8H_{10}ClN_3O_5S_2$: C 29.31, H 3.07, N 12.81. Found: C 29.39, H 3.52, N 12.89%.

*General procedure for the synthesis of 2-(((arylsulfonyl)-aminosulfonyl)methyl)-5-((styrylsulfonyl)methyl)-1,3,4-oxadiazole (**5a–c**)*

To an equimolar mixture of compounds **3** (10 mmol) and **4** (10 mmol), $POCl_3$ (7 mL) was added and heated under reflux for 6–8 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.

2-(((Phenylsulfonyl)aminosulfonyl)methyl)-5-((styrylsulfonyl)methyl)-1,3,4-oxadiazole ***5a***

White solid in 63%; m.p.: 128–130°C; IR (KBr) υ_{max} (cm^{-1}): 1154, 1302 (SO_2), 1565 (C=N), 1614 (C=C), 3224 (NH); ^{1}H NMR (DMSO-d_6) δ (ppm): 4.92 (s, 2H, CH_2-(C-5)), 5.16 (s, 2H, CH_2-(C-2)), 6.72 (d, 1H, H_B, J = 9.6 Hz), 7.35–7.86 (m, 11H, H_A and Ar–H), 10.59 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 45.2 (CH_2-(C-5)), 49.2 (CH_2-(C-2)), 122.3 (C-H_B), 141.3 (C-H_A), 158.2 (C-5), 159.7 (C-2), 125.2, 126.6, 127.3, 128.9, 130.2, 131.4, 132.7, 133.1 (aromatic carbons); MS (*m/z*): 483.55 [M^+]. Anal. calcd. for $C_{18}H_{17}N_3O_7S_3$: C 44.71, H 3.54, N 8.69. Found: C 44.85, H 3.59, N 8.99%.

2-(((4-Methylphenylsulfonyl)aminosulfonyl)methyl)-5-((4-methylstyrylsulfonyl)methyl)-1,3,4-oxadiazole ***5b***

White solid in 61%; m.p.: 115–117°C; IR (KBr) υ_{max} (cm^{-1}): 1149, 1309 (SO_2), 1560 (C=N), 1610 (C=C), 3219 (NH); ^{1}H NMR (DMSO-d_6) δ (ppm): 2.37 and 2.41 (s, 6H, Ar–CH_3), 4.85 (s, 2H, CH_2-(C-5)), 5.12 (s, 2H, CH_2-(C-2)), 6.69 (d, 1H, H_B, J = 9.2 Hz), 7.15–7.62 (m, 9H, H_A and Ar–H), 10.58 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 21.7 and 22.5 (Ar–CH_3), 42.9 (CH_2-(C-5)), 48.7 (CH_2-(C-2)), 121.2 (C-H_B), 138.5 (C-H_A), 156.9 (C-5), 157.8 (C-2), 123.4, 124.6, 125.2, 127.4, 128.1, 129.3, 130.5, 131.7 (aromatic carbons); MS (*m/z*): 511.61 [M^+]. Anal. calcd. for $C_{20}H_{21}N_3O_7S_3$: C 46.95, H 4.11, N 8.21; Found: C 46.57, H 4.45, N 8.33%.

2-(((4-Chlorophenylsulfonyl)aminosulfonyl)methyl)-5-((4-chlorostyrylsulfonyl)methyl)-1,3,4-oxadiazole ***5c***

White solid in 65%; m.p.: 139–141°C; IR (KBr) υ_{max} (cm^{-1}): 1162, 1315 (SO_2), 1577 (C=N), 1625 (C=C), 3230 (NH); ^{1}H NMR (DMSO-d_6) δ (ppm): 4.98 (s, 2H, CH_2-(C-5)), 5.23 (s, 2H, CH_2-(C-2)), 6.75 (d, 1H, H_B, J = 9.8 Hz), 7.36–7.89 (m, 9H, H_A and Ar–H), 10.60 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 48.3 (CH_2-(C-5)), 51.5 (CH_2-(C-2)), 123.5 (C-H_B), 141.1 (C-H_A), 157.5 (C-5), 159.2 (C-2), 127.9, 128.3, 129.1, 129.8, 130.4, 131.6, 133.8, 137.2 (aromatic carbons); MS (*m/z*): 552.45 [M^+]. Anal. calcd. for $C_{18}H_{15}Cl_2N_3O_7S_3$: C 39.13, H 2.73, N 7.60. Found: C 38.82, H 2.98, N 7.41%.

*General procedure for the synthesis of 2-(((arylsulfonyl)-aminosulfonyl)methyl)-5-((4′,5′-dihydro-1′,3′-diphenyl-5′-aryl-1′H-pyrazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazole (**6a–c**)*

The compound **5** (1 mmol), araldehyde phenylhydrazone (1.2 mmol) and chloramine-T (0.33 g, 1.2 mmol) in methanol (20 mL) were refluxed for 20–22 h. The precipitated inorganic salts were filtered off. The filtrate was concentrated and the residue was extracted with dichloromethane. The organic layer was washed with water, brine and dried (an. Na_2SO_4). The solvent

was removed under vacuum. The resultant residue was purified by column chromatography (silica gel, 60–120 mesh) using hexane/ethyl acetate (4:1) as eluent.

2-(((Phenylsulfonyl)aminosulfonyl)methyl)-5-((4′,5′-dihydro-1′,3′-diphenyl-5′-phenyl-1′H-pyrazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazole ***6a***

Pale yellow solid in 64%; m.p.: 173–175°C; IR (KBr) υ_{max} (cm^{-1}): 1145, 1332 (SO_2), 1580 (C=N), 3252 (NH); ^{1}H NMR (DMSO-d_6) δ (ppm): 4.83 (s, 2H, CH_2-(C-5)), 5.13 (d, 1H, C5′-H, J = 7.3 Hz), 5.20 (s, 2H, CH_2-(C-2)), 5.42 (d, 1H, C4′-H, J = 7.3 Hz), 7.28–7.82 (m, 20H, Ar–H), 10.54 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 49.5 (CH_2-(C-5)), 53.7 (CH_2-(C-2)), 66.4 (C-5′), 86.2 (C-4′), 158.2 (C-3′), 159.1 (C-5), 159.4 (C-2), 126.5, 127.6, 128.3, 128.9, 129.7, 130.8, 132.3, 133.5, 134.2, 135.7, 136.1, 136.9 (aromatic carbons): MS (*m/z*): 677.78 [M^+]. Anal. calcd. for $C_{31}H_{27}N_5O_7S_3$: C 54.93, H 4.01, N 10.33; Found: C 55.42, H 4.17, N 10.65%.

2-(((4-Methylphenylsulfonyl)aminosulfonyl)methyl)-5-((4′,5′-dihydro-1′,3′-diphenyl-5′-(4-methylphenyl)-1′H-pyrazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazole ***6b***

Pale yellow solid in 60%; m.p.: 166–168°C; IR (KBr) υ_{max} (cm^{-1}): 1142, 1329 (SO_2), 1576 (C=N), 3249 (NH); ^{1}H NMR (DMSO-d_6) δ (ppm): 2.32 and 2.41 (s, 6H, Ar–CH_3), 4.79 (s, 2H, CH_2-(C-5)), 5.09 (d, 1H, C5′-H, J = 7.1 Hz), 5.18 (s, 2H, CH_2-(C-2)), 5.40 (d, 1H, C4′-H, J = 7.1 Hz), 7.21–7.65 (m, 18H, Ar–H), 10.49 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 22.5 and 23.2 (Ar–CH_3), 48.2 (CH_2-(C-5)), 53.2 (CH_2-(C-2)), 65.7 (C-5′), 85.8 (C-4′), 156.8 (C-3′), 157.9 (C-2), 158.7 (C-5), 125.8, 126.1, 127.3, 127.9, 128.4, 130.1, 131.6, 132.5, 133.8, 134.2, 135.1, 135.9 (aromatic carbons); MS (*m/z*): 705.83 [M^+]. Anal. calcd. for $C_{33}H_{31}N_5O_7S_3$: C 56.15, H 4.42, N 9.92. Found: C 56.37, H 4.59, N 10.03%.

2-(((4-Chlorophenylsulfonyl)aminosulfonyl)methyl)-5-((4′,5′-dihydro-1′,3′-diphenyl-5′-(4-chlorophenyl)-1′H-pyrazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazole ***6c***

Pale yellow solid in 65%; m.p.: 185–186°C; IR (KBr) υ_{max} (cm^{-1}): 1151, 1337 (SO_2), 1585 (C=N), 3263 (NH); ^{1}H NMR (DMSO-d_6) δ (ppm): 4.85 (s, 2H, CH_2-(C-5)), 5.18 (d, 1H, C5′-H, J = 7.6 Hz), 5.24 (s, 2H, CH_2-(C-2)), 5. 45 (d, 1H, C4′-H, J = 7.6 Hz), 7.32–7.91 (m, 18H, Ar–H), 10.58 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 51.2 (CH_2-(C-5)), 54.5 (CH_2-(C-2)), 68.1 (C-5′), 86.9 (C-4′), 158.7 (C-3′), 159.9 (C-5), 160.5 (C-2), 126.5, 127.6, 128.3, 129.6, 130.7, 131.2, 132.7, 134.3, 135.9, 136.7, 137.2, 137.7 (aromatic carbons); MS (*m/z*): 746.68 [M^+]. Anal. calcd. for $C_{31}H_{25}Cl_2N_5O_7S_3$: C 49.86, H 3.37, N 9.37. Found: C 49.96, H 3.82, N 9.71%.

*General procedure for the synthesis of 2-(((arylsulfonyl)-aminosulfonyl)methyl)-5-((4′,5′-dihydro-3′-phenyl-5′-arylisoxazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazole (****7a–c****)*

A mixture of compound **5** (1 mmol), araldoxime (1.2 mmol), chloramine-T (0.33 g, 1.2 mmol) and methanol (20 mL) was refluxed for 16–18 h. The precipitated inorganic salts were filtered off. The filtrate was concentrated and the residue was extracted with dichloromethane. The organic layer was washed with water, brine and dried (an. Na_2SO_4). The solvent was removed under reduced pressure. The resultant residue was purified by column chromatography (silica gel, 60–120 mesh) using hexane/ethyl acetate (4:1) as eluent.

2-(((Phenylsulfonyl)aminosulfonyl)methyl)-5-((4′,5′-dihydro-3′-phenyl-5′-phenylisoxazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazole ***7a***

White solid in 70%; m.p.: 161–163°C; IR (KBr) υ_{max} (cm^{-1}): 1148, 1336 (SO_2), 1570 (C=N), 3245 (NH); ^{1}H NMR (DMSO-d_6) δ (ppm): 4.96 (s, 2H, CH_2-(C-5)), 5.17 (d, 1H, C5′-H, J = 7.2 Hz), 5.22 (s, 2H, CH_2-(C-2)), 5.50 (d, 1H, C4′-H, J = 7.2 Hz), 7.34–7.81 (m, 15H, Ar–H), 10.62 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 49.7 (CH_2-(C-5)), 55.2 (CH_2-(C-2)), 63.2 (C-5′), 87.2 (C-4′), 157.6 (C-3′), 159.5 (C-2), 160.2 (C-5), 127.9, 129.5, 130.3, 131.1, 131.9, 132.3, 133.7, 134.4 (aromatic carbons); MS (*m/z*): 602.68 [M^+]. Anal. calcd. for $C_{25}H_{22}N_4O_8S_3$: C 49.82, H 3.67, N 9.29. Found: C 49.66, H 3.83, N 9.50%.

2-(((4-Methylphenylsulfonyl)aminosulfonyl)methyl)-5-((4′,5′-dihydro-3′-phenyl-5′-(4-methylphenyl)isoxazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazole ***7b***

White solid in 68%; m.p.: 150–152°C; IR (KBr) υ_{max} (cm^{-1}): 1140, 1343 (SO_2), 1565 (C=N), 3241 (NH); ^{1}H NMR (DMSO-d_6) δ (ppm): 2.34 and 2.47 (s, 6H, Ar–CH_3), 5.07 (s, 2H, CH_2-(C-5)), 5.14 (d, 1H, C5′-H, J = 6.9 Hz), 5.26 (s, 2H, CH_2-(C-2)), 5.48 (d, 1H, C4′-H, J = 6.9 Hz), 7.28–7.88 (m, 13H, Ar–H), 10.57 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 23.4 and 25.1 (Ar–CH_3), 48.6 (CH_2-(C-5)), 54.8 (CH_2-(C-2)), 62.7 (C-5′), 85.6 (C-4′), 157.9 (C-3′), 158.2 (C-2), 160.8 (C-5), 126.2, 127.4, 139.7, 130.3, 131.5, 132.2, 132.9, 133.5 (aromatic carbons); MS (*m/z*): 630.74 [M^+]. Anal. calcd. for $C_{27}H_{26}N_4O_8S_3$: C 51.41, H 4.15, N 8.88. Found: C 51.59, H 4.38, N 9.07%.

2-(((4-Chlorophenylsulfonyl)aminosulfonyl)methyl)-5-((4′,5′-dihydro-3′-phenyl-5′-(4-chlorophenyl)isoxazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazole ***7c***

White solid in 76%; m.p.: 179–181°C; IR (KBr) υ_{max} (cm^{-1}): 1144, 1347 (SO_2), 1572 (C=N), 3250 (NH); ^{1}H NMR (DMSO-d_6) δ (ppm): 5.21 (d, 1H, C5′-H, J = 7.4 Hz), 5.26 (s, 2H, CH_2-(C-5)), 5.34 (s, 2H, CH_2-(C-2)), 5.60 (d, 1H, C4′-H, J = 7.4 Hz), 7.38–7.93 (m, 13H, Ar–H), 10.65 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 50.4 (CH_2-(C-5)), 55.9 (CH_2-(C-2)), 64.1 (C-4′), 89.5 (C-5′), 158.2 (C-3′), 159.8 (C-2), 161.6 (C-5), 128.7, 129.9, 132.5, 133.7, 134.6, 136.5, 137.1, 137.8 (aromatic carbons); MS (*m/z*): 671.58 [M^+]. Anal. calcd. for $C_{25}H_{20}Cl_2N_4O_8S_3$: C 44.71, H 3.00, N 8.34. Found: C 45.01, H 2.85, N 8.42%.

*General procedure for the synthesis of 2-(((arylsulfonyl)-aminosulfonyl)methyl)-5-((1′,3′-diphenyl-5′-aryl-1′H-pyrazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazole (****8a–c****)/2-(((arylsulfonyl)aminosulfonyl)methyl)-5-((3′-phenyl-5′-arylisoxazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazole (****9a–c****)*

A solution of **6**/**7** (1 mmol) in xylene (10 mL) and chloranil (0.29 g, 1.2 mmol) was refluxed for 25–30 h. Then, it was treated with a 5% sodium hydroxide solution. The organic extract was separated, repeatedly washed with water and dried (an. Na_2SO_4). The solvent was removed *in vacuo*. The resultant solid was recrystallized from 2-propanol.

2-(((Phenylsulfonyl)aminosulfonyl)methyl)-5-((1′,3′-diphenyl-5′-phenyl-1′H-pyrazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazole ***8a***

White solid in 67%; m.p.: 186–187°C; IR (KBr) υ_{max} (cm^{-1}): 1135, 1335 (SO_2), 1570 (C=N), 1633 (C=C), 3277 (NH); ^{1}H NMR (DMSO-d_6)

δ (ppm): 5.25 (s, 2H, CH_2-(C-5)), 5.36 (s, 2H, CH_2-(C-2)), 7.13–7.77 (m, 20H, Ar–H), 10.59 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 46.2 (CH_2-(C-5)), 53.2 (CH_2-(C-2)), 138.5 (C-4′), 149.4 (C-3′), 150.5 (C-5′), 158.2 (C-5), 158.7 (C-2), 124.5, 125.0, 125.8, 126.2, 127.4, 128.1, 129.3, 130.7, 131.1, 132.8, 133.7, 134.3 (aromatic carbons); MS (*m/z*): 675.77 [M^+]. Anal. calcd. for $C_{31}H_{25}N_5O_7S_3$: C 55.09, H 3.73, N 10.36. Found: C 55.54, H 4.02, N 10.49%.

2-(((4-Methylphenylsulfonyl)aminosulfonyl)methyl)-5-((1′,3′-diphenyl-5′-(4-methylphenyl)-1′H-pyrazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazole ***8b***

White solid in 64%; m.p.: 173–175°C; IR (KBr) υ_{max} (cm^{-1}): 1131, 1333 (SO_2), 1573 (C=N), 1629 (C=C), 3272 (NH); ^{1}H NMR (DMSO-d_6) δ (ppm): 2.43 and 2.51 (s, 6H, Ar–CH_3), 5.15 (s, 2H, CH_2-(C-5)), 5.29 (s, 2H, CH_2-(C-2)), 6.97–7.65 (m, 18H, Ar–H), 10.57 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 22.5 and 22.9 (Ar–CH_3), 45.8 (CH_2-(C-5)), 53.8 (CH_2-(C-2)), 138.1 (C-4′), 149.2 (C-3′), 150.8 (C-5′), 157.3 (C-5), 158.4 (C-2), 123.9, 124.5, 125.2, 126.5, 127.2, 127.9, 128.4, 128.9, 129.3, 131.7, 132.5, 133.6 (aromatic carbons); MS (*m/z*): 703.82 [M^+]. Anal. calcd. for $C_{33}H_{29}N_5O_7S_3$: C 56.31, H 4.15, N 9.94; Found: C 55.82, H 4.57, N 10.41%.

2-(((4-Chlorophenylsulfonyl)aminosulfonyl)methyl)-5-((1′,3′-diphenyl-5′-(4-chlorophenyl)-1′H-pyrazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazole ***8c***

White solid in 71%; m.p.: 191–192°C; IR (KBr) υ_{max} (cm^{-1}): 1147, 1340 (SO_2), 1582 (C=N), 1635 (C=C), 3286 (NH); ^{1}H NMR (DMSO-d_6) δ (ppm): 5.31 (s, 2H, CH_2-(C-5)), 5.40 (s, 2H, CH_2-(C-2)), 7.26–7.96 (m, 18H, Ar–H), 10.64 (bs, 1 NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 47.2 (CH_2-(C-5)), 53.9 (CH_2-(C-2)), 139.2 (C-4′), 150.1 (C-3′), 151.5 (C-5′), 159.24 (C-5), 159.8 (C-2), 125.4, 126.1, 126.7, 127.5, 128.3, 130.3, 131.6, 132.2, 133.9, 134.8, 135.4, 136.5 (aromatic carbons); MS (*m/z*): 744.66 [M^+]. Anal. calcd. for $C_{31}H_{23}Cl_2N_5O_7S_3$: C 50.00, H 3.11, N 9.40. Found: C 50.17, H 3.34, N 9.67%.

2-(((Phenylsulfonyl)aminosulfonyl)methyl)-5-((3′-phenyl-5′-phenylisoxazol-4′-ylsulfonyl)-methyl)-1,3,4-oxadiazole ***9a***

White solid in 74%; m.p.: 169–171°C; IR (KBr) υ_{max} (cm^{-1}): 1129, 1330 (SO_2), 1584 (C=N), 1624 (C=C), 3279 (NH); ^{1}H NMR (DMSO-d_6) δ (ppm): 5.34 (s, 2H, CH_2-(C-5)), 5.52 (s, 2H, CH_2-(C-2)), 7.31–7.93 (m, 15H, Ar–H), 10.72 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 48.5 (CH_2-(C-5)), 54.1 (CH_2-(C-2)), 142.5 (C-4′), 147.2 (C-3′), 153.7 (C-5′), 159.1 (C-2), 159.7 (C-5), 126.4, 128.8, 129.2, 130.7, 131.5, 131.9, 132.3, 133.5 (aromatic carbons); MS (*m/z*): 600.67 [M^+]. Anal. calcd. for $C_{25}H_{20}N_4O_8S_3$: C 49.99, H 3.35, N 9.32. Found: C 49.57, H 3.39, N 9.65%.

2-(((4-Methylphenylsulfonyl)aminosulfonyl)methyl)-5-((3′-phenyl-5′-(4-methylphenyl)isoxazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazole ***9b***

White solid in 72%; m.p.: 158–160°C; IR (KBr) υ_{max} (cm^{-1}): 1124, 1327 (SO_2), 1579 (C=N), 1619 (C=C), 3271 (NH); ^{1}H NMR (DMSO-d_6) δ (ppm): 2.39 and 2.46 (s, 6H, Ar–CH_3), 5.28 (s, 2H, CH_2-(C-5)), 5.48 (s, 2H, CH_2-(C-2)), 7.18–7.82 (m, 13H, Ar–H), 10.68 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 23.9 and 24.9 (Ar–CH_3), 48.1 (CH_2-(C-5)), 53.7 (CH_2-(C-2)), 142.1 (C-4′), 146.8 (C-3′), 153.4 (C-5′), 158.4 (C-2), 159.1 (C-5), 125.7 127.2, 128.4, 129.5, 130.6, 131.4, 131.8, 132.2 (aromatic carbons); MS (*m/z*): 628.72 [M^+]. Anal. calcd. for $C_{27}H_{24}N_4O_8S_3$: C 51.58, H 3.84, N 8.91. Found: C 51.93, H 4.15, N 9.23%.

2-(((4-Chlorophenylsulfonyl)aminosulfonyl)methyl)-5-((3′-phenyl-5′-(4-chlorophenyl)isoxazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazole ***9c***

White solid in 78%; m.p.: 182–184°C; IR (KBr) υ_{max} (cm^{-1}): 1132, 1339 (SO_2), 1590 (C=N), 1628 (C=C), 3283 (NH); ^{1}H NMR (DMSO-d_6) δ (ppm): 5.42 (s, 2H, CH_2-(C-5)), 5.56 (s, 2H, CH_2-(C-2)), 7.36–7.97 (m, 13H, Ar–H), 10.76 (bs, 1 NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 49.3 (CH_2-(C-5)), 54.9 (CH_2-(C-2)), 143.8 (C-4′), 148.4 (C-3′), 154.3 (C-5′), 160.3 (C-5), 161.1 (C-2), 127.9, 129.2, 130.3, 131.4, 133.2, 133.9, 134.5, 135.2 (aromatic carbons); MS (*m/z*): 669.56 [M^+]. Anal. calcd. for $C_{25}H_{18}Cl_2N_4O_8S_3$: C 44.84, H 2.70, N 8.36. Found: C 45.05, H 3.14, N 8.23%.

Biological assays

Antioxidant activity

The compounds **5–9** were tested for antioxidant property by DPPH, nitric oxide, and H_2O_2 methods.

DPPH radical scavenging activity

The hydrogen atom or electron donation ability of the compounds was measured from the bleaching of the purple colored methanol solution of DPPH radical. The spectrophotometric assay uses the stable radical DPPH as a reagent. To 4 mL of 0.004% w/v methanol solution of DPPH, 1 mL of various concentrations of the test compounds (50, 75, and 100 μg/mL) in methanol were added. After 30 min incubation period at room temperature, the absorbance was read against blank at 517 nm. Ascorbic acid was used as the standard. The percent of inhibition (*I*%) of free radical production from DPPH was calculated by the following equation

$$I\,\% = \left[\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{blank}}}\right] \times 100$$

where A_{control} is the absorbance of the control reaction (containing methanolic DPPH and ascorbic acid), A_{sample} is the absorbance of the test compound (containing methanolic DPPH and test compound) and A_{blank} is the absorbance of the blank (containing only methanolic DPPH). Tests were carried out in triplicate.

Nitric oxide (NO) scavenging activity

Nitric oxide scavenging activity was measured by slightly modified methods of Green *et al.* [19] and Marcocci *et al.* [20]. Nitric oxide radicals (NO) were generated from sodium nitroprusside. One milliliter of sodium nitroprusside (10 mM) and 1.5 mL of phosphate buffer saline (0.2 M, pH 7.4) were added to different concentrations (50, 75, and 100 μg/mL) of the test compounds and incubated at 25°C for 150 min. After incubation, 1 mL of the reaction mixture was treated with 1 mL of Griess reagent (1% sulfanilamide, 2% H_3PO_4, and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromatophore was measured at 546 nm. Ascorbic acid was used as standard. Nitric oxide scavenging activity was calculated by the following equation

$$\%\ \text{of scavenging} = \left[\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{blank}}}\right] \times 100$$

where $A_{control}$ is the absorbance of the control reaction (containing all reagents and Ascorbic acid), A_{sample} is the absorbance of the test compound (containing all reagents and test compound) and A_{blank} is the absorbance of the blank (containing only reagents). Tests were carried out in triplicate.

Hydrogen peroxide (H_2O_2) scavenging activity

The H_2O_2 scavenging ability of the test compound was determined according to the method of Ruch *et al.* [21]. A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). 10, 25, 50, 75, and 100 μg/mL concentrations of the test compounds in 3.4 mL phosphate buffer were added to H_2O_2 solution (0.6 mL, 40 mM). The absorbance value of the reaction mixture was recorded at 230 nm. The percent of scavenging of H_2O_2 was calculated by the following equation

$$\% \text{ of scavenging} = \left[\frac{(A_{control} - A_{sample})}{A_{blank}}\right] \times 100$$

where $A_{control}$ is the absorbance of the control reaction (containing all reagents and ascorbic acid), A_{sample} is the absorbance of the test compound (containing all reagents and test compound) and A_{blank} is the absorbance of the blank (containing only reagents). Tests were carried out in triplicate.

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