Synthesis and Antioxidant Activity of Styrylsulfonylmethyl 1,3,4-Oxadiazoles, Pyrazolyl/Isoxazolyl-1,3,4-oxadiazoles

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Received August 18, 2013; accepted September 20, 2013

A new class of mono and bis heterocycles-styrylsulfonylmethyl 1,3,4-oxadiazoles, pyrazolyl/isoxazolyl-1,3,4-oxadiazoles were prepared and studied their antioxidant activity. The compound methyl substituted 2-(*p*-methylphenylamino-sulfonylmethyl)-5-[*Z*-(*p*-methylstyrylsulfonylmethyl)]-1,3,4-oxadiazole displayed slightly higher antioxidant activity than the standard ascorbic acid.

Key words pyrazolyl; isoxazolyl oxadiazole; nitrile imine; nitrile oxide; antioxidant activity

Nitrogen containing five-membered heterocyclic compounds have gained intensive research interest as they possess a wide range of applications in pharmaceutical and agrochemistry.[1–3] In fact, pyrazole, isoxazole and oxadiazole motifs make up the core structures of numerous natural products and biologically active synthetic compounds.[4–9] Substituted 1,3,4-oxadiazoles show antiallergic,[10] antiviral,[11] anti-inflammatory,[12] anticancer[13] and antimicrobial[14–19] activities. Additionally, pyrazole and their derivatives exhibit remarkable pharmacological activities such as antimicrobial, hypoglycemic, hyperlipidemia, and anti-inflammatory.[20–24] The isoxazole nucleus is well known for its commercial application in various realms of therapy, including cytotoxic agents.[25] The marketed drugs containing isoxazole moiety *viz*., acetylsulfisoxazole, cycloserine, drazoxol, sulfisoxazole and zonisamide have a great medicinal value. Recently, we have studied the antioxidant properties of arylsulfonylaminosulfonylmethyl oxadiazoles, pyrazolyl/isoxazolyl-oxadiazoles.[26] Fascinated by diverse bioactivity of these heterocycles and in continuation of our ongoing program[26–28] aiming at finding a new class of bis heterocycles with potential chemotherapeutic activities, we planned to synthesize and to study the antioxidant property of hitherto not reported styrylsulfonylmethyl 1,3,4-oxadiazoles and pyrazolyl/isoxazolyl-1,3,4-oxadiazoles.

Results and Discussion

Chemistry Initially, 2-(arylaminosulfonylmethyl)-5-[*Z*-(styrylsulfonylmethyl)]-1,3,4-oxadiazole (**3**) was prepared by the cyclocondensation of arylaminosulfonylacetic acid hydrazide (**1**) with *Z*-styrylsulfonylacetic acid (**2**) in the presence of $POCl_3$ (Chart 1). The ^{1}H-NMR spectrum of **3a** displayed two singlets at δ 5.11 and 4.86 ppm due to methylene protons attached to C-2 and C-5. A doublet was observed at 6.59 ppm due to the olefin proton H_B, while the other olefin proton H_A exhibited a doublet at much downfield region and merged with aromatic protons. The coupling constant value J_{AB}=9.5 Hz indicated that they possess *cis* geometry. Further, a broad singlet was appeared at 10.31 ppm due to NH which disappeared on deuteration. The 1,3-dipolar cycloaddition of dipolar reagents to dipolarophiles was one of the facile techniques to prepare five-membered heterocycles. Adopting this methodology, the olefin moiety present in compound **3** was utilized to develop pyrazoles and isoxazoles with appropriate dipolar reagents-nitrile imine and nitril oxide. In the literature it was reported that the cycloaddition of 1,3-dipolar reagents to α,β-unsaturated systems proceeds in such a way that the electron rich atom of 1,3-dipolar species attacks β-carbon of α,β-unsaturated systems followed by isomerization.[29] In fact the cycloaddition of nitrile imine generated from benzaldehyde phenylhydrazone in the presence of chloramine-T to compound **3** proceeded regioselectively with the formation of 2-((arylaminosulfonyl)-methyl)-5-((4′,5′-dihydro-1′,3′-diphenyl-5′-aryl-1′*H*-pyrazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazole (**4**). The ^{1}H-NMR spectrum of **4a** displayed two doublets at δ 5.10 ($C_{4'}$-H), 5.34 ($C_{5'}$-H) ppm, two singlets at 4.72 (CH_2-(C-5)), 5.13 (CH_2-(C-2)) ppm and a broad singlet at 10.47 ppm (NH). The signal due to NH disappeared when D_2O was added. In a much similar way, the cycloaddition of nitrile oxide generated from benzaldoxime in the presence of chloramine-T to compound **3** furnished 2-((arylaminosulfonyl)methyl)-5-((4′,5′-dihydro-3′-phenyl-5′-arylisoxazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazole (**5**). The addition of nitrile imines and nitrile oxides to **3** may lead to isomers. The other isomer if any formed during the course of the reaction was not isolated. However, only one pure regioisomer was isolated under the applied reaction conditions. This may be due to more electron withdrawing effect of sulfonyl group.[30] The ^{1}H-NMR spectrum of **5a** showed two doublets at δ 5.18 and 5.43 ppm due to $C_{4'}$-H and $C_{5'}$-H. Two singlets were observed at 5.20 and 4.89 ppm due to methylene protons attached to C-2 and C-5. The compound **5a** displayed a broad singlet at 10.52 ppm due to NH which disappeared when D_2O was added. The oxidation of pyrazoline and isoxazoline rings present in compounds **4** and **5** was performed with chloranil in xylene to obtain the aromatized compounds 2-((arylaminosulfonyl)methyl)-5-((1′,3′-diphenyl-5′-aryl-1′*H*-pyrazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazole (**6**) and 2-((arylaminosulfonyl)methyl)-5-((3′-phenyl-5′-arylisoxazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazole (**7**). In the ^{1}H-NMR spectra of **6** and **7** the absence of signals corresponding to pyrazoline and isoxazoline ring protons indicated their formation. The structures of **4**–**7** were further ascertained by IR and ^{13}C-NMR spectra.

Antioxidant Activity The compounds **3**–**7** were tested for antioxidant property by 2,2,-diphenyl-1-picrylhydrazyl radi-

The authors declare no conflict of interest.

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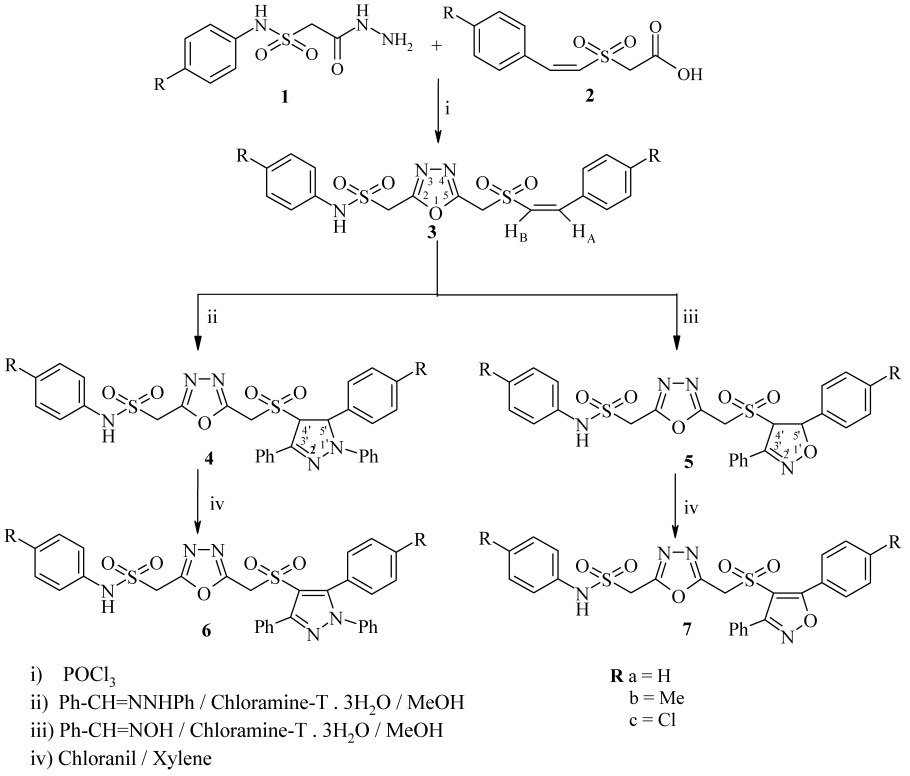

Chart 1

Table 1. The *in Vitro* Antioxidant Activity of **3–7** in DPPH Method

Compound	Concentration		
	50 μg/mL (%)	75 μg/mL (%)	100 μg/mL (%)
3a	74.61±0.21	77.22±0.06	79.19±0.58
3b	78.23±0.65	81.35±1.04	84.46±0.33
3c	57.46±0.01	61.54±0.50	63.82±0.29
4a	—	—	—
4b	45.11±0.98	48.29±1.24	51.31±1.04
4c	—	—	—
5a	—	—	—
5b	46.51±0.52	49.15±0.37	52.45±0.14
5c	—	—	—
6a	68.08±1.58	72.52±1.74	74.09±1.85
6b	73.14±0.41	76.06±0.57	78.78±0.82
6c	52.86±0.25	56.36±1.07	59.27±0.09
7a	70.41±0.71	73.61±0.39	75.50±1.34
7b	75.95±1.42	77.67±0.91	80.37±1.17
7c	55.16±1.05	58.23±0.58	62.52±1.26
Ascorbic acid	77.15±0.45	80.95±0.39	83.82±0.81
Blank	—	—	—

(—) Showed no scavenging activity. Values were the means of three replicate±S.D.

Table 2. The *in Vitro* Antioxidant Activity of **3–7** in NO Method

Compound	Concentration		
	50 μg/mL (%)	75 μg/mL (%)	100 μg/mL (%)
3a	73.10±0.03	73.43±0.50	75.25±0.21
3b	77.21±1.45	78.67±0.79	82.35±1.03
3c	56.92±0.38	60.76±0.42	63.23±0.61
4a	—	—	—
4b	41.57±0.15	44.40±0.65	46.24±0.41
4c	—	—	—
5a	—	—	—
5b	44.71±1.19	48.26±0.32	51.42±0.45
5c	—	—	—
6a	66.12±0.11	70.57±0.60	72.42±0.73
6b	71.75±1.44	74.13±1.29	76.26±1.51
6c	50.92±0.09	55.19±0.85	57.72±0.65
7a	68.81±1.92	71.21±1.68	74.97±1.45
7b	73.23±1.47	75.35±1.22	78.96±0.96
7c	54.20±1.55	56.37±1.45	60.11±1.86
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±S.D.

cal (DPPH),[31,32] nitric oxide (NO)[33,34] and hydrogen peroxide (H_2O_2)[35] methods. The results of the antioxidant activity of test compounds and control drug are presented in Tables 1–3 and Figs. 1–3. The aim of this study was to identify the potential pharmacophoric unit for antioxidant property. Amongst all the compounds the mono heterocyclic derivatives, styrylsulfonylmethyl 1,3,4-oxadiazoles **3** showed greater radical scavenging activity than the bis heterocyclic compounds. This may be due to the presence of more electron withdrawing styryl moiety. In fact, the methyl substituted styrylsulfonylmethyl oxadiazole **3b** displayed slightly higher radical scavenging activity when compared with the standard ascorbic acid. Further, it was observed that amongst bis heterocyclic compounds, the aromatized compounds **6** and **7** displayed greater activity than the respective dihydro derivatives **4** and **5**. In fact, isoxazolyl oxadiazoles showed comparatively higher radical scavenging activity than the pyrazolyl oxadiazoles. It was also noticed that unsubstituted and methyl substituted compounds **3a**, **3b**,

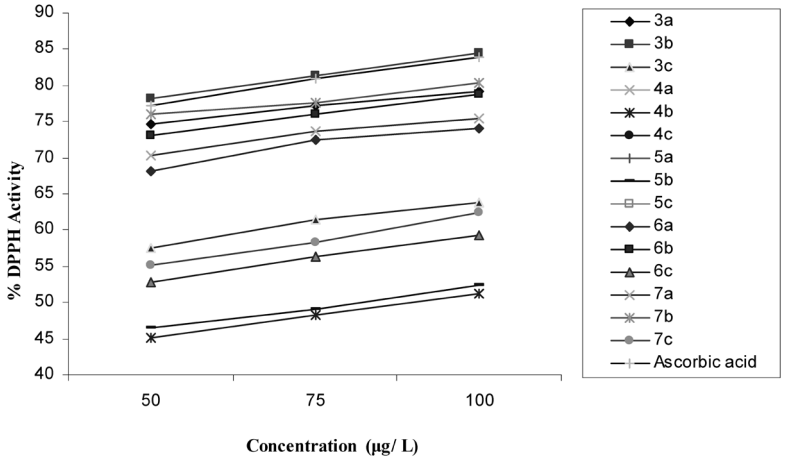

Fig. 1. The *in Vitro* Antioxidant Activity of Compounds **3**–**7** in DPPH Method

Table 3. The *in Vitro* Antioxidant Activity of **3**–**7** in H_2O_2 Method

Compound	Concentration		
	50 μg/mL (%)	75 μg/mL (%)	100 μg/mL (%)
3a	72.12±0.53	75.24±0.17	78.40±0.37
3b	76.51±0.15	78.79±0.07	81.44±0.45
3c	58.31±1.25	60.05±1.16	64.89±1.72
4a	—	—	—
4b	43.28±0.41	46.61±0.88	48.11±0.73
4c	—	—	—
5a	—	—	—
5b	45.82±1.41	47.57±1.12	50.27±1.33
5c	—	—	—
6a	66.19±1.04	70.39±0.92	74.65±0.69
6b	71.56±0.33	75.14±0.10	77.21±0.82
6c	53.74±1.36	55.65±0.94	58.62±1.29
7a	69.27±0.21	73.49±0.58	76.68±0.51
7b	72.54±1.69	76.43±1.45	79.77±1.81
7c	56.06±0.62	59.91±0.49	62.54 ±0.71
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±S.D.

6a, **6b**, **7a** and **7b** exhibited higher activity than the corresponding chloro substituted compounds **3c**, **6c** and **7c**. The compounds **4b** and **5b** exhibited low activity while **4a**, **4c**, **5a** and **5c** showed no activity. Besides, the perusal of Tables 1–3 indicated that radical scavenging activity increases with increase in concentration in all the three methods.

Conclusion

A variety of mono and bis heterocycles-styrylsulfonylmethyl 1,3,4-oxadiazoles, pyrazolyl/isoxazolyl-1,3,4-oxadiazoles were prepared from arylaminosulfonylacetic acid hydrazide and *Z*-styrylsulfonylacetic acid and studied their antioxidant activity. The styrylsulfonylmethyl 1,3,4-oxadiazoles, pyrazolyl/isoxazolyl-1,3,4-oxadiazoles were displayed greater antioxidant activity than the pyrazolinyl/isoxazolinyl-1,3,4-oxadiazoles. In fact, the methyl substituted compound **3b** showed slightly higher antioxidant activity than the standard ascorbic acid.

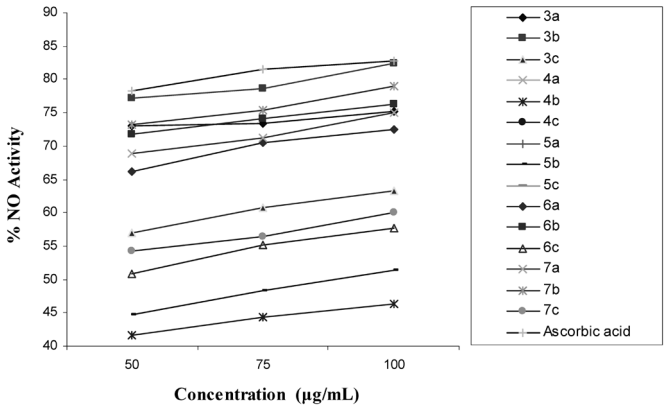

Fig. 2. The *in Vitro* Antioxidant Activity of Compounds **3**–**7** in NO Method

Experimental

General 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 recorded on a Thermo Nicolet IR 200 FT-IR spectrometer as KBr pellets and the wave numbers were given in cm^{-1}. The ^{1}H-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 tetramethylsilane (TMS) as an internal standard. The antioxidant property was carried out by using Shimadzu UV-2450 spectrophotometer. 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 elemental analyses were determined using Perkin-Elmer 240C elemental analyzer. The arylaminosulfonylacetic acid hydrazide (**1**) and *Z*-styrylsulfonylacetic acid (**2**) were prepared as per the literature procedure.[27,36,37]

General Procedure for the Synthesis of 2-(Arylaminosulfonylmethyl)-5-[*Z*-(styrylsulfonylmethyl)]-1,3,4-oxadiazole (3a–c) To an equimolar mixture (10 mmol) of arylaminosulfonylacetic acid hydrazide (**1**) and *Z*-styrylsulfonylacetic acid (**2**), $POCl_3$ (7 mL) was added and heated under reflux for 3–5 h. The excess $POCl_3$ was removed *in vacuo* and the residue was poured onto crushed ice. The resulting precipitate was filtered, washed with saturated sodium bicarbon-

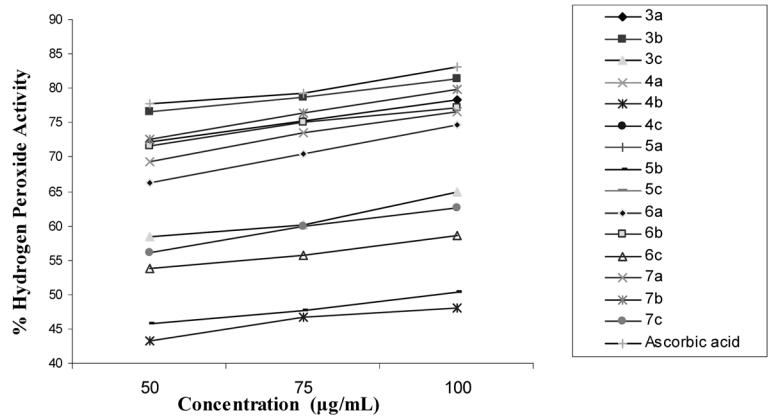

Fig. 3. The *in Vitro* Antioxidant Activity of Compounds **3**–**7** in H_2O_2 Method

ate solution followed by water. It was dried and recrystallized from ethanol.

2-(Phenylaminosulfonylmethyl)-5-[*Z*-(styrylsulfonylmethyl)]-1,3,4-oxadiazole (**3a**): White solid, yield 67%, mp 122–124°C. IR (KBr) cm^{-1}: 1160, 1315 (SO_2), 1572 (C=N), 1625 (C=C), 3227 (NH). ^{1}H-NMR (DMSO-d_6) δ: 4.86 (s, 2H, CH_2-(C-5)), 5.11 (s, 2H, CH_2-(C-2)), 6.59 (d, 1H, H_B, J=9.5 Hz), 7.11–7.69 (m, 11H, H_A, Ar-H), 10.31 (brs, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 44.3 (CH_2-(C-5)), 49.7 (CH_2-(C-2)), 121.6 (C-H_B), 140.2 (C-H_A), 157.3 (C-5), 158.2 (C-2), 124.7, 125.6, 126.4, 128.3, 128.9, 129.4, 130.2, 131.1 (aromatic carbons). MS *m/z*: 419.47 (M^+). *Anal.* Calcd for $C_{18}H_{17}N_3O_5S_2$: C, 51.54; H, 4.08; N, 10.02. Found: C, 51.60; H, 4.10; N, 10.12.

2-(*p*-Methylphenylaminosulfonylmethyl)-5-[*Z*-(*p*-methylstyrylsulfonylmethyl)]-1,3,4-oxadiazole (**3b**): White solid, yield 65%, mp 101–103°C. IR (KBr) cm^{-1}: 1155, 1312 (SO_2), 1565 (C=N), 1622 (C=C), 3224 (NH). ^{1}H-NMR (DMSO-d_6) δ: 2.21 and 2.27 (s, 6H, Ar-CH_3), 4.69 (s, 2H, CH_2-(C-5)), 5.06 (s, 2H, CH_2-(C-2)), 6.42 (d, 1H, H_B, J=9.3 Hz), 7.03–7.59 (m, 9H, H_A, Ar-H), 10.02 (brs, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 21.2 and 21.9 (Ar-CH_3), 42.8 (CH_2-(C-5)), 48.4 (CH_2-(C-2)), 120.8 (C-H_B), 137.1 (C-H_A), 154.5 (C-5), 155.1 (C-2), 122.5, 123.7, 124.4, 124.9, 127.1, 127.9, 129.5, 130.6 (aromatic carbons). MS *m/z*: 447.53 (M^+). *Anal.* Calcd for $C_{20}H_{21}N_3O_5S_2$: C, 53.68; H, 4.73; N, 9.39. Found: C, 53.63; H, 4.72; N, 9.47.

2-(*p*-Chlorophenylaminosulfonylmethyl)-5-[*Z*-(*p*-chlorostyrylsulfonylmethyl)]-1,3,4-oxadiazole (**3c**): White solid, yield 69%, mp 140–142°C. IR (KBr) cm^{-1}: 1169, 1318 (SO_2), 1580 (C=N), 1630 (C=C), 3235 (NH). ^{1}H-NMR (DMSO-d_6) δ: 5.02 (s, 2H, CH_2-(C-5)), 5.25 (s, 2H, CH_2-(C-2)), 6.87 (d, 1H, H_B, J=9.8 Hz), 7.17–7.71 (m, 9H, H_A, Ar-H), 10.45 (brs, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 47.6 (CH_2-(C-5)), 50.9 (CH_2-(C-2)), 122.5 (C-H_B), 143.1 (C-H_A), 158.1 (C-5), 159.7 (C-2), 127.7, 128.6, 129.0, 129.7, 130.5, 131.2, 132.6, 133.4 (aromatic carbons). MS *m/z*: 488.36 (M^+). *Anal.* Calcd for $C_{18}H_{15}Cl_2N_3O_5S_2$: C, 44.27; H, 3.10; N, 8.60. Found: C, 44.22; H, 3.07; N, 8.56.

General Procedure for the Synthesis 2-((Arylaminosulfonyl)methyl)-5-((4′,5′-sssdihydro-1′,3′-diphenyl-5′-aryl-1′*H*-pyrazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazole (4a–c) The compound **3** (1 mmol), benzaldehyde phenylhydrazone (1.2 mmol), and chloramine-T (1.2 mmol) and methanol (20 mL) were refluxed for 17–20 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-((Phenylaminosulfonyl)methyl)-5-((4′,5′-dihydro-1′,3′-diphenyl-5′-phenyl-1′*H*-pyrazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazole (**4a**): Pale yellow solid, yield 70%, mp 162–164°C. IR (KBr) cm^{-1}: 1132, 1327 (SO_2), 1575 (C=N), 3248 (NH). ^{1}H-NMR (DMSO-d_6) δ: 4.72 (s, 2H, CH_2-(C-5)), 5.10 (d, 1H, $C_{4'}$-H, J=7.1 Hz), 5.13 (s, 2H, CH_2-(C-2)), 5.34 (d, 1H, $C_{5'}$-H, J=7.1 Hz), 7.21–7.69 (m, 20H, Ar-H), 10.47 (brs, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 47.6 (CH_2-(C-5)), 51.5 (CH_2-(C-2)), 65.8 (C-5′), 84.5 (C-4′), 154.5 (C-3′), 157.7 (C-5), 158.5 (C-2), 124.6, 125.3, 126.7, 127.1, 128.1, 129.2, 130.6, 131.5, 132.7, 133.8, 134.2, 135.1 (aromatic carbons). MS *m/z*: 613.71 (M^+). *Anal.* Calcd for $C_{31}H_{27}N_5O_5S_2$: C, 60.67; H, 4.43; N, 11.41. Found: C, 60.82; H, 4.57; N, 11.65.

2-((*p*-Methylphenylaminosulfonyl)methyl)-5-((4′,5′-dihydro-1′,3′-diphenyl-5′-(*p*-methylphenyl)-1′*H*-pyrazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazole (**4b**): Pale yellow solid, yield 68%, mp 156–158°C. IR (KBr) cm^{-1}: 1128, 1321 (SO_2), 1566 (C=N), 3240 (NH). ^{1}H-NMR (DMSO-d_6) δ: 2.24 and 2.37 (s, 6H, Ar-CH_3), 4.52 (s, 2H, CH_2-(C-5)), 5.04 (d, 1H, $C_{4'}$-H, J=6.9 Hz), 5.11 (s, 2H, CH_2-(C-2)), 5.30 (d, 1H, $C_{5'}$-H, J=6.9 Hz), 7.15–7.52 (m, 18H, Ar-H), 10.35 (brs, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 21.7 and 22.5 (Ar-CH_3), 46.3 (CH_2-(C-5)), 52.7 (CH_2-(C-2)), 64.9 (C-5′), 83.1 (C-4′), 153.1 (C-3′), 156.5 (C-5), 157.2 (C-2), 123.3, 124.2, 125.5, 126.5, 127.1, 127.9, 129.7, 130.5, 131.4, 133.2, 133.9, 134.5 (aromatic carbons). MS *m/z*: 641.76 (M^+). *Anal.* Calcd for $C_{33}H_{31}N_5O_5S_2$: C, 61.76; H, 4.86; N, 10.91. Found: C, 61.83; H, 5.02; N, 10.99.

2-((*p*-Chlorophenylaminosulfonyl)methyl)-5-((4′,5′-dihydro-1′,3′-diphenyl-5′-(*p*-chlorophenyl)-1′*H*-pyrazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazole (**4c**): Pale yellow solid, yield 74%, mp 177–179°C. IR (KBr) cm^{-1}: 1135, 1337 (SO_2), 1578 (C=N), 3252 (NH). ^{1}H-NMR (DMSO-d_6) δ: 4.84 (s, 2H, CH_2-(C-5)), 5.16 (d, 1H, $C_{4'}$-H, J=7.4 Hz), 5.15 (s, 2H, CH_2-(C-2)), 5.35 (d, 1H, $C_{5'}$-H, J=7.4 Hz), 7.29–7.74 (m, 18H, Ar-H), 10.54 (brs, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 48.7 (CH_2-(C-5)), 53.2 (CH_2-(C-2)), 67.9 (C-5′), 85.8 (C-4′), 155.9 (C-3′), 158.4 (C-5), 159.6 (C-2), 125.4, 126.2, 127.2, 128.2, 129.8, 130.6, 131.4, 132.7, 133.5, 134.2, 135.1, 136.3 (aromatic carbons). MS *m/z*: 682.61 (M^+). *Anal.* Calcd. for $C_{31}H_{25}Cl_2N_5O_5S_2$: C, 54.54; H, 3.69; N, 10.25. Found: C, 54.27; H, 3.82; N, 9.89.

General Procedure for the Synthesis of 2-((Arylaminosulfonyl)methyl)-5-((4′,5′-dihydro-3′-phenyl-5′-

arylisoxazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazole (5a–c) A mixture of compound **3** (1 mmol), benzaldoxime (1.2 mmol), chloramine-T (1.2 mmol) and methanol (20 mL) was refluxed for 14–17 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). Evaporation of the solvent under reduced pressure yielded a solid which was purified by column chromatography (silica gel, 60–120 mesh) using hexane–ethyl acetate (4 : 1) as eluent.

2-((Phenylaminosulfonyl)methyl)-5-((4′,5′-dihydro-3′-phenyl-5′-phenylisoxazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazoles (**5a**): White solid, yield 67%, mp 149–151°C. IR (KBr) cm^{-1}: 1140, 1330 (SO_2), 1560 (C=N), 3237 (NH). ^{1}H-NMR (DMSO-d_6) δ: 4.89 (s, 2H, CH_2-(C-5)), 5.18 (d, 1H, $C_{4'}$-H, J=7.6 Hz), 5.20 (s, 2H, CH_2-(C-2)), 5.43 (d, 1H, $C_{5'}$-H, J=7.6 Hz), 7.31–7.85 (m, 15H, Ar-H), 10.50 (brs, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 48.6 (CH_2-(C-5)), 53.4 (CH_2-(C-2)), 64.5 (C-4′), 85.3 (C-5′), 155.2 (C-3′), 158.1 (C-5), 158.9 (C-2), 125.7, 126.1, 127.3, 129.5, 130.6, 131.4, 132.1, 135.7 (aromatic carbons). MS m/z: 538.64 (M^+). *Anal.* Calcd for $C_{25}H_{22}N_4O_6S_2$: C, 55.74; H, 4.11; N, 10.40. Found: C, 56.03; H, 4.29; N, 10.47.

2-((*p*-Methylphenylaminosulfonyl)methyl)-5-((4′,5′-dihydro-3′-phenyl-5′-(*p*-methylphenyl)isoxazol-4′-ylsulfonyl)-methyl)-1,3,4-oxadiazoles (**5b**): White solid, yield 60%, mp 129–131°C. IR (KBr) cm^{-1}: 1134, 1324 (SO_2), 1555 (C=N), 3230 (NH). ^{1}H-NMR (DMSO-d_6) δ: 2.28 and 2.42 (s, 6H, Ar-CH_3), 4.94 (s, 2H, CH_2-(C-5)), 5.12 (d, 1H, $C_{4'}$-H, J=7.2 Hz), 5.14 (s, 2H, CH_2-(C-2)), 5.36 (d, 1H, $C_{5'}$-H, J=7.2 Hz), 7.21–7.72 (m, 13H, Ar-H), 10.49 (brs, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 22.6 and 24.2 (Ar-CH_3), 47.5 (CH_2-(C-5)), 52.1 (CH_2-(C-2)), 62.9 (C-4′), 84.2 (C-5′), 153.3 (C-3′), 157.4 (C-5), 158.1 (C-2), 124.5, 125.2, 127.6, 128.4 129.3, 130.6, 133.2, 134.8 (aromatic carbons). MS m/z: 566.67 (M^+). *Anal.* Calcd for $C_{27}H_{26}N_4O_6S_2$: C, 57.22; H, 4.62; N, 9.88. Found: C, 57.34; H, 4.90; N, 10.01.

2-((*p*-Chlorophenylaminosulfonyl)methyl)-5-((4′,5′-dihydro-3′-phenyl-5′-(*p*-chlorophenyl)isoxazol-4′-ylsulfonyl)-methyl)-1,3,4-oxadiazoles (**5c**): White solid, yield 73%, mp 171–173°C. IR (KBr) cm^{-1}: 1146, 1333 (SO_2), 1564 (C=N), 3245 (NH). ^{1}H-NMR (DMSO-d_6) δ: 4.99 (s, 2H, CH_2-(C-5)), 5.23 (d, 1H, $C_{4'}$-H, J=7.7 Hz), 5.27 (s, 2H, CH_2-(C-2)), 5.51 (d, 1H, $C_{5'}$-H, J=7.7 Hz), 7.38–7.93 (m, 13H, Ar-H), 10.59 (brs, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 49.5 (CH_2-(C-5)), 53.8 (CH_2-(C-2)), 61.4 (C-4′), 83.7 (C-5′), 152.8 (C-3′), 158.8 (C-5), 159.2 (C-2), 123.7, 125.8, 126.9, 127.5, 128.8, 130.1, 131.9, 136.6 (aromatic carbons). MS m/z: 607.51 (M^+). *Anal.* Calcd for $C_{25}H_{20}Cl_2N_4O_6S_2$: C, 49.42; H, 3.31; N, 9.22. Found: C, 49.15; H, 3.37; N, 9.08.

General Procedure for the Synthesis of 2-((Arylaminosulfonyl)methyl)-5-((1′,3′-diphenyl-5′-aryl-1′*H*-pyrazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazole (6a–c)/2-((Arylaminosulfonyl)methyl)-5-((3′-phenyl-5′-arylisoxazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazoles (7a–c) The compound **4**/**5** (1 mmol), chloranil (1.2 mmol) in xylene (10 mL) was refluxed for 23–26 h. Then, it was treated with 5% sodium hydroxide solution. The organic extract was separated, washed with water and dried (an. Na_2SO_4). The solvent was removed under reduced pressure. The resultant solid was recrystallized from 2-propanol.

2-((Phenylaminosulfonyl)methyl)-5-((1′,3′-diphenyl-5′-phenyl-1′*H*-pyrazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazole (**6a**): White solid, yield 62%, mp 180–182°C. IR (KBr) cm^{-1}: 1125, 1329 (SO_2), 1562 (C=N), 1627 (C=C), 3260 (NH). ^{1}H-NMR (DMSO-d_6) δ: 5.09 (s, 2H, CH_2-(C-5)), 5.30 (s, 2H, CH_2-(C-2)), 7.06–7.71 (m, 20H, Ar-H), 10.55 (brs, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 45.6 (CH_2-(C-5)), 50.2 (CH_2-(C-2)), 136.9 (C-4′), 146.2 (C-3′), 149.7 (C-5′), 157.5 (C-5), 158.3 (C-2), 123.3, 123.6, 124.1, 124.9, 126.6, 127.4, 128.2, 129.5, 130.1, 131.7, 132.4, 133.5 (aromatic carbons). MS m/z: 611.70 (M^+). *Anal.* Calcd for $C_{31}H_{25}N_5O_5S_2$: C, 60.86; H, 4.12; N, 11.45. Found: C, 61.25; H, 4.31; N, 11.69.

2-((*p*-Methylphenylaminosulfonyl)methyl)-5-((1′,3′-diphenyl-5′-(*p*-methylphenyl)-1′*H*-pyrazol-4′-ylsulfonyl)-methyl)-1,3,4-oxadiazole (**6b**): White solid, yield 66%, mp 168–170°C. IR (KBr) cm^{-1}: 1130, 1335 (SO_2), 1570 (C=N), 1624 (C=C), 3265 (NH). ^{1}H-NMR (DMSO-d_6) δ: 2.26 and 2.39 (s, 6H, Ar-CH_3), 5.03 (s, 2H, CH_2-(C-5)), 5.28 (s, 2H, CH_2-(C-2)), 6.94–7.52 (m, 18H, Ar-H), 10.50 (brs, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 21.5 and 22.4 (Ar-CH_3), 45.8 (CH_2-(C-5)), 49.9 (CH_2-(C-2)), 136.3 (C-4′), 145.9 (C-3′), 148.7 (C-5′), 156.6 (C-5), 157.6 (C-2), 122.7, 123.1, 123.9, 124.8, 124.9, 126.8, 127.5, 128.6, 129.7, 130.3, 131.6, 134.1 (aromatic carbons). MS m/z: 639.76 (M^+). *Anal.* Calcd for $C_{33}H_{29}N_5O_5S_2$: C, 61.95; H, 4.56; N, 10.94. Found: C, 62.28; H, 4.93; N, 11.13.

2-((*p*-Chlorophenylaminosulfonyl)methyl)-5-((1′,3′-diphenyl-5′-(*p*-chlorophenyl)-1′*H*-pyrazol-4'-ylsulfonyl)-methyl)-1,3,4-oxadiazole (**6c**): White solid, yield 69%, mp 188–190°C. IR (KBr) cm^{-1}: 1137, 1338 (SO_2), 1575 (C=N), 1632 (C=C), 3272 (NH). ^{1}H-NMR (DMSO-d_6) δ: 5.16 (s, 2H, CH_2-(C-5)), 5.39 (s, 2H, CH_2-(C-2)), 7.14–7.91 (m, 18H, Ar-H), 10.64 (brs, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 46.8 (CH_2-(C-5)), 52.2 (CH_2-(C-2)), 137.5 (C-4′), 147.3 (C-3′), 150.2 (C-5′), 157.5 (C-5), 158.9 (C-2), 124.3, 124.6, 125.9, 126.3, 127.6, 128.5, 129.8, 130.5, 131.6, 132.3, 133.6, 134.7 (aromatic carbons). MS m/z: 680.59 (M^+). *Anal.* Calcd for $C_{31}H_{23}Cl_2N_5O_5S_2$: C, 54.70; H, 3.40; N, 10.28. Found: C, 54.79; H, 3.65; N, 10.34.

2-((Phenylaminosulfonyl)methyl)-5-((3′-phenyl-5′-phenylisoxazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazole (**7a**): White solid, yield 71%, mp 153–155°C. IR (KBr) cm^{-1}: 1120, 1323 (SO_2), 1580 (C=N), 1620 (C=C), 3268 (NH). ^{1}H-NMR (DMSO-d_6) δ: 5.26 (s, 2H, CH_2-(C-5)), 5.41 (s, 2H, CH_2-(C-2)), 7.19–7.84 (m, 15H, Ar-H), 10.57 (brs, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 47.1 (CH_2-(C-5)), 51.5 (CH_2-(C-2)), 138.2 (C-4′), 147.3 (C-3′), 151.4 (C-5′), 157.9 (C-5), 158.5 (C-2), 125.1, 126.6, 127.1, 128.4, 129.2, 130.4, 131.8, 133.5 (aromatic carbons). MS m/z: 536.60 (M^+). *Anal.* Calcd for $C_{25}H_{20}N_4O_6S_2$: C, 55.95; H, 3.75; N, 10.44. Found: C, 56.07; H, 3.84; N, 10.71.

2-((*p*-Methylphenylaminosulfonyl)methyl)-5-((3′-phenyl-5′-(*p*-methylphenyl)isoxazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazole (**7b**): White solid, yield 68%, mp 141–143°C. IR (KBr) cm^{-1}: 1115, 1325 (SO_2), 1583 (C=N), 1618 (C=C), 3264 (NH). ^{1}H-NMR (DMSO-d_6) δ: 2.30 and 2.45 (s, 6H, Ar-CH_3), 5.21 (s, 2H, CH_2-(C-5)), 5.32 (s, 2H, CH_2-(C-2)), 7.24–7.88 (m, 13H, Ar-H), 10.60 (brs, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 22.4 and 23.1 (Ar-CH_3), 46.4 (CH_2-(C-5)), 51.1 (CH_2-(C-2)), 137.5 (C-4′), 146.5 (C-3′), 150.4 (C-5′), 157.2 (C-5), 158.1 (C-2), 124.5, 125.7, 126.4, 127.8, 128.7, 129.7, 130.6, 132.9 (aromatic carbons). MS m/z: 564.65 (M^+). *Anal.* Calcd for $C_{27}H_{24}N_4O_6S_2$: C, 57.43; H, 4.28; N, 9.92. Found: C, 57.38; H, 4.61; N, 9.74.

2-((*p*-Chlorophenylaminosulfonyl)methyl)-5-((3′-phenyl-5′-(*p*-chlorophenyl)isoxazol-4′-ylsulfonyl)methyl)-1,3,4-oxadiazole (**7c**): White solid, yield 76%, mp 170–172°C. IR (KBr) cm^{-1}: 1141, 1332 (SO_2), 1585 (C=N), 1627 (C=C), 3279 (NH). ^{1}H-NMR (DMSO-d_6) δ: 5.37 (s, 2H, CH_2-(C-5)), 5.57 (s, 2H, CH_2-(C-2)), 7.30–7.89 (m, 13H, Ar-H), 10.68 (brs, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 48.6 (CH_2-(C-5)), 52.5 (CH_2-(C-2)), 138.8 (C-4′), 147.9 (C-3′), 153.3 (C-5′), 158.4 (C-), 159.4 (C-2), 126.7, 127.6, 128.2, 129.7, 130.5, 130.9, 132.2, 135.1 (aromatic carbons). MS *m/z*: 605.49 (M^+). *Anal.* Calcd for $C_{25}H_{18}Cl_2N_4O_6S_2$: C, 49.59; H, 2.99; N, 9.25. Found: C, 49.81; H, 3.14; N, 9.39.

Antioxidant Activity The compounds **3**–**7** were tested for antioxidant property by DPPH, NO, 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. 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, 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

$$I\% = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100$$

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.

NO Scavenging Activity NO scavenging activity was measured by slightly modified methods of Green *et al.*[33] and Marcocci *et al.*[34] NO radicals 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, 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 the standard. Nitric oxide scavenging activity was calculated by the following equation

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

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.

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.*[35] A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). Fifty, 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. Ascorbic acid was used as the standard. The percent of scavenging of H_2O_2 was calculated by the following equation

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

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.

Acknowledgment The authors are grateful to Council of Scientific and Industrial Research (CSIR), New Delhi, India for financial assistance under major research project.

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