European Journal of Medicinal Chemistry 45 (2010) 4246–4251

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and bioassay of aminosulfonyl-1,3,4-oxadiazoles and their interconversion to 1,3,4-thiadiazoles

V. Padmavathi*, S. Nagi Reddy, G. Dinneswara Reddy, A. Padmaja

Department of Chemistry, Sri Venkateswara University, Tirupati, Andhra Pradesh 517 502, India

ARTICLE INFO

Article history:
Received 17 February 2010
Received in revised form
10 June 2010
Accepted 15 June 2010
Available online 22 June 2010

Keywords:
1,3,4-oxadiazole
1,3,4-thiadiazole
Phosphorus oxychloride
Thiourea
Antimicrobial activity
Antioxidant activity

ABSTRACT

A new class of oxadiazoles is prepared by treating aminosulfonylacetic acids with different carboxylic acid hydrazides. Interconversion of oxadiazoles to thiadiazoles is carried out with thiourea. The compounds are screened for antimicrobial and antioxidant activities.

1. Introduction

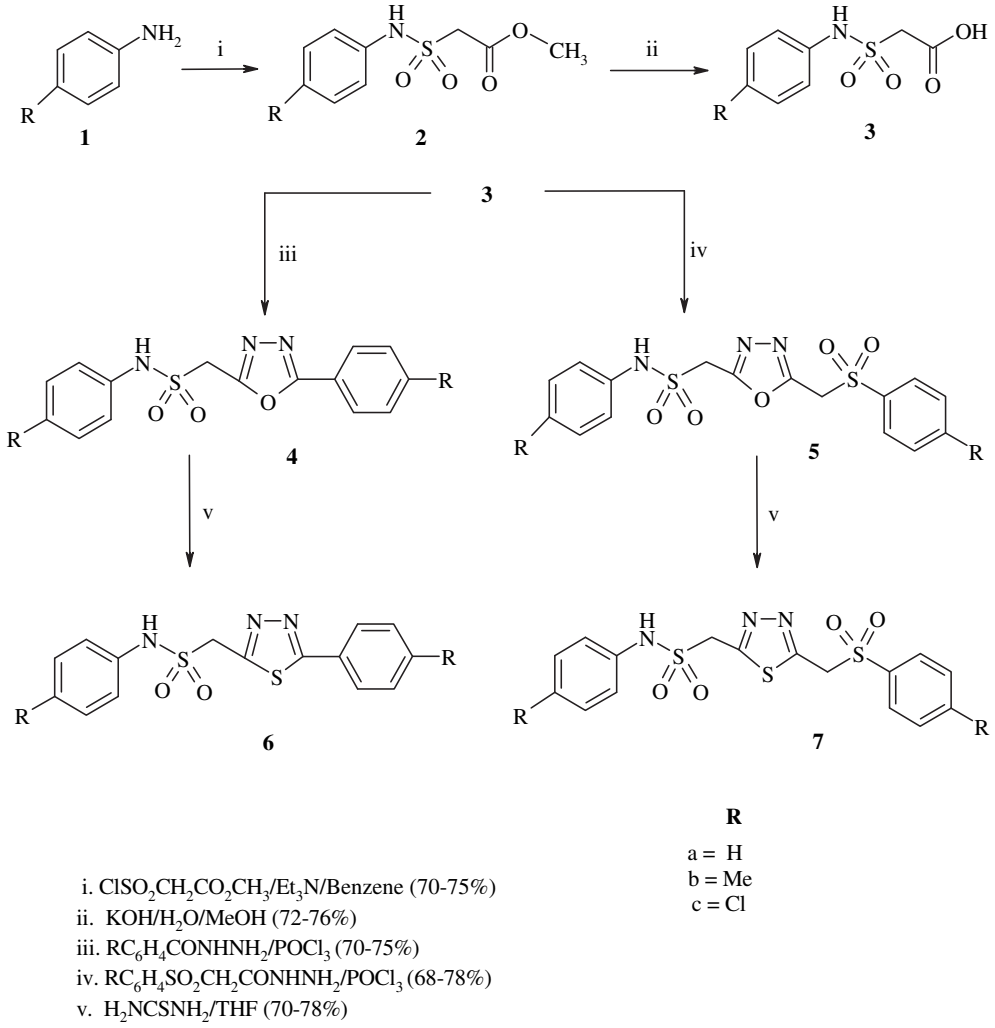
Heterocyclic compounds containing five-membered rings gained importance because of their versatile biological properties. In particular, compounds bearing 1,3,4-oxadiazole nucleus are known to have unique antioedema and anti-inflammatory activities [1]. Differently substituted oxadiazole molecules possess other interesting properties such as analgesic [1], antimicrobial [2], antitubercular [3], anticonvulsant [4] and antihepatitis B virus activities [5]. The advent of sulfur drugs and the later discovery of mesoionic compounds in fact accelerated the rate of interest in the field of sulfur containing heterocycles. Substituted oxadiazole and thiadiazole derivatives are potent cyclooxygenase/5-lipooxygenase inhibitors [6]. Indeed, 5-unsubstituted 1,3,4-thiadiazoles are used as intermediates in the synthesis of therapeutically potent antibiotic cefazolin [7]. Remarkable progress has been made by our group in the development of biologically potent heterocycles [8]. Replacement of -O- by -S- in some heterocycles was reported *viz.*, transformation of epoxide to episulfides by the action of thiocyanates or thiourea. However, there are few reports about the conversion of 1,3,4-oxadiazoles to 1,3,4-thiadiazoles [9]. The present communication deals with the synthesis and bioassay of arylaminosulfonylmethyl-5-aryl-1,3,4-oxadiazoles and their conversion to thiadiazoles.

2. Chemistry

The general synthetic pathway for the synthesis of arylaminosulfonylmethyl oxadiazoles and thiadiazoles is depicted in Scheme 1. Experimental data and spectroscopic analysis of the compounds **4**–**7** are compiled in experimental section. The synthetic intermediate, arylaminosulfonylacetic acid methyl ester **2** is prepared by the condensation of substituted aniline with methyl chlorosulfonylacetate. The latter compound is obtained from methyl bromoacetate [10]. Hydrolysis of **2** in the presence of KOH in methanol gave the corresponding acid, arylaminosulfonylacetic acid **3** [10]. The cyclocondensation of **3** with different aryl acid hydrazides in the presence of phosphorus oxychloride afforded 2-arylaminosulfonylmethyl-5-aryl-1,3,4-oxadiazoles **4**. Similarly the reaction of **3** with arylsulfonylacetic acid hydrazide in the presence of phosphorus oxychloride produced 2-arylaminosulfonylmethyl-5-arylsulfonylmethyl-1,3,4-oxadiazole **5**. The ^{1}H NMR spectrum of **4a** displayed a singlet at δ 5.04 for methylene protons and another broad singlet at 10.45 ppm for NH. However **5a** exhibited two sharp singlets at δ 4.08 and 4.42 ppm for two methylene protons. The signal at downfield region is assigned to methylene protons adjacent to SO_2NH group. Apart from these, a broad singlet is observed at δ 10.54 ppm due to

* Corresponding author. Tel.: +91 877 2289303; fax: +91 877 2249532.
E-mail address: vkpuram2001@yahoo.com (V. Padmavathi).

0223-5234/$ – see front matter © 2010 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2010.06.022



Scheme 1. Synthesis of arylaminosulfonylmethyl oxadiazoles and thiadiazoles.

NH. Compounds **4** and **5** are treated with two-fold excess thiourea in tetrahydrofuran to give 2-arylaminosulfonylmethyl-5-aryl-1,3,4-thiadiazole **6** and 2-arylaminosulfonylmethyl-5-arylsulfonylmethyl-1,3,4-thiadiazole **7**, respectively. The ^{1}H NMR spectrum of **6a** showed two singlets at δ 5.05 and 10.42 ppm for methylene and NH protons while for **7a** three singlets at 4.03, 4.47 and 10.56 ppm for two methylene and NH protons. The structures of **4**–**7** were evidenced by ^{13}C NMR spectroscopy.

3. Biology

3.1. Antimicrobial activity

The compounds **4**–**7** 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), *Aspergillus niger* (NCIM No. 596). The primary screen was carried out by agar disc-diffusion method [11] using nutrient agar medium. The minimum inhibitory concentration for the most active compounds **6c, 7a, 7b** and **7c** against the same microorganisms used in the preliminary screening was carried out using microdilution susceptibility method [12]. Chloramphenicol and Ketoconazole were used as control drugs. The observed data on the antimicrobial activity of the compounds and control drugs are given in Tables 1–3.

3.2. Antioxidant testing

The compounds **4**–**7** are tested for antioxidant property by nitric oxide [13,14] and DPPH [15] methods. The observed data on the antioxidant activity are given in Table 4.

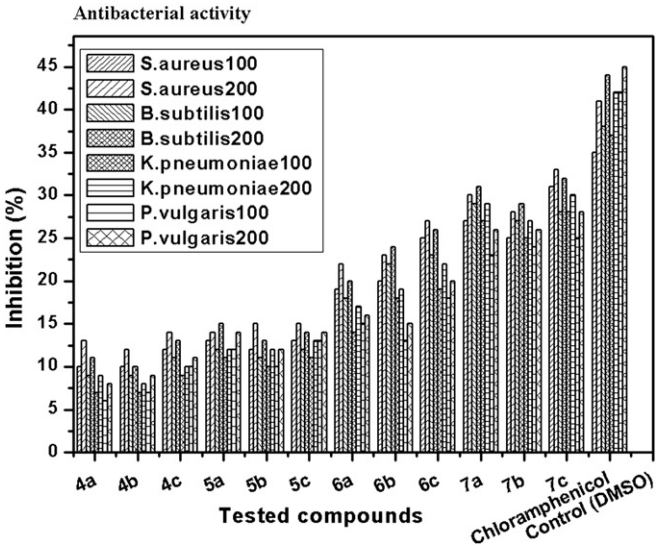
4. Result and discussion

We have synthesized 2-arylaminosulfonylmethyl-5-aryl-1,3,4-oxadiazole **(4)** and 2-arylaminosulfonylmethyl-5-arylsulfonylmethyl-1,3,4-oxadiazole **(5)** by the cyclocondensation of 2-arylaminosulfonylacetic acid with acid hydrazides and arylsulfonylacetic acid hydrazides in the presence of $POCl_3$. The interconversion of **4** and **5** to 2-arylaminosulfonylmethyl-5-aryl-1,3,4-thiadiazole (**6**) and 2-arylaminosulfonylmethyl-5-arylsulfonylmethyl-1,3,4-thiadiazole (**7**), respectively was effected with thiourea as presented in Scheme 1.

4.1. Biological results

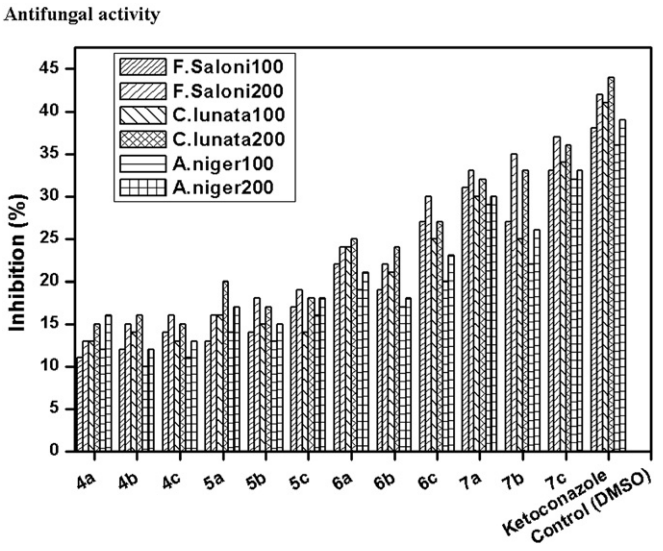
The results of preliminary antibacterial testing of compounds **4**–**7** are presented in Table 1. The results revealed that the

Table 1
Antibacterial activity of **4**–**7**.



Compound	Concentration (μg/disc)	Zone of inhibition (mm)			
		Gram-positive bacteria		Gram-negative bacteria	
		Staphylococcus aureus	*Bacillus subtilis*	*Klebsiella pneumoniae*	*Proteus vulgaris*
4a	100	10	09	07	06
	200	13	11	09	08
4b	100	10	09	07	07
	200	12	10	08	09
4c	100	12	11	09	10
	200	14	13	10	11
5a	100	13	12	11	12
	200	14	15	12	14
5b	100	12	11	10	10
	200	15	13	12	12
5c	100	13	12	11	13
	200	15	14	13	14
6a	100	19	18	14	15
	200	22	20	17	16
6b	100	20	22	18	13
	200	23	24	19	15
6c	100	25	23	19	18
	200	27	26	22	20
7a	100	27	29	27	23
	200	30	31	29	26
7b	100	25	27	25	24
	200	28	29	27	26
7c	100	31	28	28	25
	200	33	32	30	28
Chloramphenicol	100	35	38	37	42
	200	41	44	42	45
Control (DMSO)		–	–	–	–

Table 2
Antifungal activity of **4**–**7**.



Compound	Concentration (μg/disc)	Zone of inhibition (mm)		
		Fusarium solani	*Curvularia lunata*	*Aspergillus niger*
4a	100	11	13	12
	200	13	15	16
4b	100	12	14	10
	200	15	16	12
4c	100	14	13	11
	200	16	15	13
5a	100	13	16	14
	200	16	20	17
5b	100	14	15	13
	200	18	17	15
5c	100	17	14	16
	200	19	18	18
6a	100	22	24	19
	200	24	25	21
6b	100	19	21	17
	200	22	24	18
6c	100	27	25	20
	200	30	27	23
7a	100	31	30	29
	200	33	32	30
7b	100	27	25	22
	200	35	33	26
7c	100	33	34	32
	200	37	36	33
Ketoconazole	100	38	41	36
	200	42	44	39
Control (DMSO)		–	–	–

compounds **6c, 7a, 7b** and **7c** displayed excellent activity against Gram-positive bacteria (inhibitory zone >26 mm) and good activity against Gram-negative bacteria (inhibitory zone >20 mm). The compound **6a** displayed moderate to high activity towards gram (+ve) bacteria and moderate activity towards gram (−ve) bacteria. The compounds **4** and **5** are less active against both bacteria. All the tested compounds except **4** and **5** showed moderate to high inhibitory effect towards tested fungi (Table 2).

The MIC values represent 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 thiadiazole moiety exhibited higher antimicrobial activity than the compounds with oxadiazole unit. Further the compound having arylsulfonylmethane moiety **7** showed excellent antimicrobial activity. The presence of chloro substituent in the aromatic ring enhanced the activity.

4.2. Antioxidant testing

The compounds **4**–**7** are tested for antioxidant property by nitric oxide and DPPH methods. The results revealed that the compounds having oxadiazole moiety exhibited good antioxidant

Table 3
Minimum inhibitory concentration (MIC), μg/ml of **6c, 7a, 7c**.

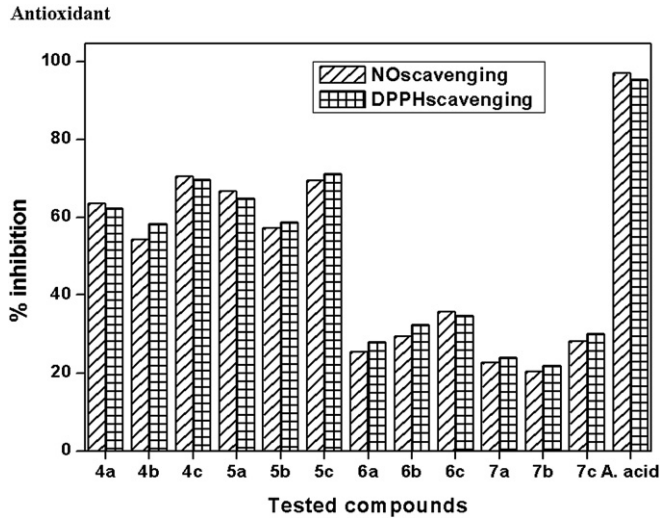
Compound	*Minimum inhibitory concentration* MIC, μg/ml						
	S. aureus	*B. subtilis*	*K. pneumoniae*	*P. vulgaris*	*F. solani*	*C. lunata*	*A. niger*
6c	100	100	200	200	200	100	200
7a	50	25	50	100	100	100	50
7b	50	50	100	100	50	100	100
7c	25	25	50	50	50	50	50
Chloramphenicol	6.25	6.25	12.5	12.5	–	–	–
Ketoconazole	–	–	–	–	12.5	6.25	6.25

property when compared with those having thiadiazole unit. In fact, the compounds **4a**, **4c**, **5a** and **5c** exhibited high antioxidant property in both nitric oxide and DPPH methods at a 100 μM concentration (Table 4).

5. Conclusion

In conclusion, a new class of heterocycles, 1,3,4-oxadiazoles and 1,3,4-thiadiazoles are developed adopting simple, elegant and well-versed methodologies. The preliminary antimicrobial activity studies revealed that the compounds having thiadiazole moiety exhibited a higher antimicrobial activity in comparison to compounds having oxadiazole unit. On the other hand, compounds with oxadiazole unit showed a good antioxidant property.

Table 4
Antioxidant property of **4**–**7**.



Compound	NO scavenging (%)	DPPH scavenging (%)
4a	63.52	62.27
4b	54.33	58.10
4c	70.54	69.61
5a	66.78	64.71
5b	57.19	58.64
5c	69.39	70.98
6a	25.45	27.80
6b	29.39	32.28
6c	35.66	34.58
7a	22.64	23.82
7b	20.26	21.74
7c	28.19	29.93
Ascorbic acid	96.90	95.37

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, 1:3 ethyl acetate–hexanes). The IR spectra were recorded on a Thermo Nicolet IR200 FT-IR spectrometer as KBr pellets and the wave numbers were given in cm^{-1}. The NMR spectra were recorded in DMSO-d_6 on a Bruker spectrospin operating at 400 MHz. All chemical shifts are reported in δ (ppm) using TMS as an internal standard. The microanalyses were performed on a Perkin-Elmer 240 C elemental analyzer. The starting compound arylaminosulfonylacetic acid **3** was prepared by the literature procedure [10].

6.1.1. General procedure for the synthesis of 2-arylaminosulfonylmethyl-5-aryl-1,3,4-oxadiazole ***4a–c***/2-arylaminosulfonylmethyl-5-arylsulfonylmethyl-1,3,4-oxadiazole ***5a–c***

To an equimolar solution (5 mmol) of arylaminosulfonylacetic acid and benzoic acid hydrazide/arylsulfonylacetic acid hydrazide, $POCl_3$ (4 ml) is added and refluxed for 5–8 h. The excess $POCl_3$ is removed under vacuum and the residue is poured onto crushed ice. The resulting precipitate is filtered, washed with saturated sodium bicarbonate solution and then with water, dried and recrystallized from ethanol to get **4a–c/5a–c.**

*6.1.1.1. 2-Phenylaminosulfonylmethyl-5-phenyl-1,3,4-oxadiazole (**4a**).* White solid (1.18 g, 75%); m.p. 147–149 °C; IR (KBr): 1329, 1135 (SO_2), 1589 (C=N), 3334 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 5.04 (s, 2H, SO_2–CH_2), 7.25–7.88 (m, 10H, Ar–H), 10.45 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 47.2, 121.7, 123.1, 128.4, 129.2, 130.2, 130.5, 137.1, 137.8, 157.8, 164.3 ppm; Anal. Calcd. For $C_{15}H_{13}N_3O_3S$: C, 57.13; H, 4.16; N, 13.33; Found: C, 57.10; H, 4.22; N, 13.43%.

*6.1.1.2. 2-(p-methylphenylaminosulfonylmethyl)-5-(p-methylphenyl)-1,3,4-oxadiazole (**4b**).* White solid (1.25 g, 72%); m.p. 161–163 °C; IR (KBr): 1318, 1138 (SO_2), 1593 (C=N), 3341 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.28 (s, 3H, Ar–CH_3), 2.31 (s, 3H, Ar′–CH_3), 4.98 (s, 2H, SO_2–CH_2), 7.20–7.94 (m, 8H, Ar–H), 10.51 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 22.5, 22.9, 47.6, 121.2, 123.4, 127.9, 129.9, 130.7, 131.2, 137.2, 138.3 159.2, 165.1 ppm; Anal. Cacld. For $C_{17}H_{17}N_3O_3S$: Anal. Calcd. For C, 59.46; H, 4.99; N, 12.24; Found: C, 59.57; H, 5.04; N, 12.32%.

*6.1.1.3. 2-(p-chlorophenylaminosulfonylmethyl)-5-(p-chlorophenyl)-1,3,4-oxadiazole (**4c**).* White solid (1.34 g, 70%); m.p. 182–184 °C; IR (KBr): 1320, 1143 (SO_2), 1608 (C=N), 3343 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.09 (s, 2H, SO_2–CH_2), 7.23–7.91 (m, 8H, Ar–H), 10.47 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 47.8, 121.9, 122.2, 128.8, 128.9, 129.7, 130.2, 136.8, 137.5, 158.5, 164.8 ppm; Anal. Calcd. For $C_{15}H_{11}Cl_2N_3O_3S$: C, 46.89; H, 2.89; N, 10.94; Found: C, 46.82; H, 2.93; N, 10.90%

*6.1.1.4. 2-phenylaminosulfonylmethyl-5-phenylsulfonylmethyl-1,3,4-oxadiazole (**5a**).* White solid (1.34 g, 68%); m.p. 172–174 °C; IR (KBr): 1331, 1130 (SO_2), 1582 (C=N), 3347 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.08 (s, 2H, SO_2-CH_2), 4.42 (s, 2H, CH_2-SO_2), 7.18–7.72 (m, 10H, Ar–H), 10.54 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 54.3, 59.0, 122.8, 129.2, 130.3, 129.8, 130.6, 137.9, 138.7, 139.5, 158.5, 160.2 ppm; Anal. Cald. For $C_{16}H_{15}N_3O_5S_2$; C, 48.84; H, 3.84; N, 10.68; Found: C, 48.91; H, 3.80; N, 10.53%.

*6.1.1.5. 2-(p-methylarylaminosulfonylmethyl-5-(p-methylphenylsulfonylmethyl)-1,3,4-oxadiazole (**5b**).* White solid (1.64 g, 78%); m.p. 196–198 °C; IR (KBr): 1334, 1132 (SO_2), 1592 (C=N), 3342 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.35 (s, 3H, Ar–CH_3), 2.38 (s, 3H, Ar′–CH_3), 3.98 (s, 2H, SO_2-CH_2), 4.48 (s, 2H, CH_2-SO_2), 7.12–7.82 (m, 8H, Ar–H), 10.48 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 23.1, 23.4, 54.1, 59.2, 121.8, 127.8, 129.7, 130.5, 131.8, 136.5, 137.9, 138.7, 157.8, 158.7 ppm; Anal. Calcd. For $C_{18}H_{19}N_3O_5S_2$: C, 51.29; H, 4.54; N, 9.97; Found: C, 51.38; H, 4.49; N, 9.94%.

*6.1.1.6. 2-(p-chlorophenylaminosulfonylmethyl-5-(p-chlorophenylsulfonylmethyl)-1,3,4- oxadiazole (**5c**).* White solid (1.73 g, 75%); m.p. 221–223 °C; IR (KBr): 1336, 1134 (SO_2), 1606 (C=N), 3344 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.05 (s, 2H, SO_2-CH_2), 4.45 (s, 2H, CH_2-SO_2), 7.27–7.89 (m, 8H, Ar–H), 10.59 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 54.7, 58.5, 122.5, 128.5, 129.5, 129.7, 130.6, 137.3, 138.5, 139.2, 158.9, 159.4 ppm; Anal. Cacld. For $C_{16}H_{13}Cl_2N_3O_5S_2$: C, 41.57; H, 2.83; N, 9.07; Found: C, 41.66; H, 2.87; N, 9.15%.

*6.1.2. General procedure for the synthesis of arylaminosulfonylmethyl-5-aryl-1,3,4-thiadiazole **6a–c**/arylaminosulfonylmethyl-5-arylsulfonylmethyl-1,3,4-thiadiazole **7a–c***

In a sealed test tube, a mixture of **4**/**5** (5 mmol), thiourea (20 mmol) dissolved in tetrahydrofuran (5 ml) is taken and refluxed at 120–150 °C in an oil bath for 22–26 h. After the reaction is completed, it is extracted with dichloromethane. The organic layer is washed with water, brine solution and dried over anhydrous Na_2SO_4. The resultant solid is recrystallized from methanol to get **6a–c/7a–c.**

*6.1.2.1. 2-phenylaminosulfonylmethyl-5-phenyl-1,3,4-thiadiazole (**6a**).* White solid (1.16 g, 70%); m.p. 162–164 °C; IR (KBr): 1318, 1140 (SO_2), 1580 (C=N), 3339 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.05 (s, 2H, SO_2-CH_2), 7.21–7.87 (m, 10H, Ar–H), 10.42 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 47.4, 121.7, 122.2, 128.5, 129.7, 130.9, 131.5, 137.3, 138.1, 58.3, 164.9 ppm; Anal. Calcd. For $C_{15}H_{13}N_3O_2S_2$: C, 54.36; H, 3.95; N, 12.68; Found: C, 54.29; H, 4.00; N, 12.78%.

*6.1.2.2. 2-(p-methylphenylaminosulfonylmethyl-5-(p-methylphenyl)-1,3,4-thiadiazole (**6b**).* White solid (1.29 g, 72%); m.p. 187–189 °C; IR (KBr): 1324, 1142 (SO_2), 1594 (C=N), 3342 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.32 (s, 3H, Ar–CH_3), 2.36 (s, 3H, Ar′–CH_3), 5.08 (s, 2H, SO_2-CH_2), 7.25–7.92 (m, 8H, Ar–H), 10.51 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 22.8, 23.2, 47.9, 121.8, 123.5, 127.7, 128.8, 130.1, 130.2, 136.5, 136.9, 158.1, 164.5 ppm; Anal. Calcd. For $C_{17}H_{17}N_3O_2S_2$: C, 56.80; H, 4.77; N, 11.69; Found: C, 56.75; H, 4.81; N, 11.76%.

*6.1.2.3. 2-(p-chlorophenylaminosulfonylmethyl-5-(p-chlorophenyl)-1,3,4-thiadiazole (**6c**).* White solid (1.50 g, 75%); m.p. 161–163 °C; IR (KBr): 1322, 1144 (SO_2), 1603 (C=N), 3344 (NH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 5.12 (s, 2H, SO_2-CH_2), 7.21–7.89 (m, 8H, Ar–H), 10.52 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): δ 48.3, 121.3, 122.3, 128.9, 129.3, 130.4, 130.7, 136.9, 137.7, 158.7, 165.3 ppm; Anal. Calcd. For $C_{15}H_{11}Cl_2N_3O_2S_2$: C, 45.01; H, 2.77; N, 10.50; Found: C, 45.09; H, 2.74; N, 10.44%.

*6.1.2.4. 2-phenylaminosulfonylmethyl-5-phenylsulfonylmethyl-1,3,4-thiadiazole (**7a**).* White solid (1.49 g, 73%); m.p. 185–187 °C; IR (KBr): 1331, 1133 (SO_2), 1583 (C=N), 3346 (NH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 4.03 (s, 2H, SO_2-CH_2), 4.47 (s, 2H, CH_2-SO_2), 7.22–7.91 (m, 10H, Ar–H), 10.56 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): δ 53.1, 59.3, 122.3, 127.8, 128.8, 128.1, 130.4, 136.7, 137.6, 138.9, 157.9, 152.9 ppm; Anal. Calcd. For $C_{16}H_{15}N_3O_4S_3$: C, 46.93; H, 3.69; N, 10.26: Found: C, 47.03; H, 3.74; N, 10.20%.

*6.1.2.5. 2-(p-methylphenylaminosulfonylmethyl-5-(p-methylphenylsulfonylmethyl)-1,3,4-oxadiazole (**7b**).* White solid (1.71 g, 78%); m.p. 216–218 °C; IR (KBr): 1336, 1135 (SO_2), 1592 (C=N), 3341 (NH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.27 (s, 3H, Ar–CH_3), 2.30 (s, 3H, Ar′–CH_3), 4.10 (s, 2H, SO_2-CH_2), 4.52 (s, 2H, CH_2-SO_2), 7.25–7.95 (m, 8H, Ar–H), 10.60 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): δ 23.5, 23.7, 55.3, 60.4, 123.3, 127.9, 129.3, 130.5, 128.8, 137.5, 139.3, 140.2, 158.9, 160.4 ppm; Anal. Calcd. For $C_{18}H_{19}N_3O_4S_3$: C, 49.41; H, 4.38; N, 9.60; Found: C, 49.49, H, 4.34, N, 9.68%.

*6.1.2.6. 2-(p-chlorophenylaminosulfonylmethyl-5-(4-chlorophenylsulfonylmethyl)-1,3,4-thiadiazole (**7c**).* White solid (1.82 g, 76%); m.p. 238–240 °C; IR (KBr): 1338, 1136 (SO_2), 1602 (C=N), 3345 (NH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 4.06 (s, 2H, SO_2-CH_2), 4.44 (s, 2H, CH_2-SO_2), 7.19–7.88 (m, 8H, Ar–H), 10.58 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): δ 55.2, 59.2, 123.2, 129.4, 130.1, 130.5, 131.3, 136.8, 138.1, 140.5, 160.3, 161.2 ppm; Anal. Calcd. For $C_{16}H_{13}Cl_2N_3O_4S_3$: C, 40.17; H, 2.74; N, 8.78; Found: C, 40.10; H, 2.78; N, 8.86%.

6.2. Biological assays

6.2.1. Compounds

The compounds **4–7** are 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. pneumoniae* 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 **4–7** are 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. All determinations were made in triplicate for each compound. Average of three independent readings for each organism was recorded.

The MIC values 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 a concentration of 800 μg/ml and two-fold dilution of the solution was prepared (400, 200, 100,…, 6.25 μg/ml). The microorganism suspensions were inoculated to the corresponding wells. The plates were incubated at 36 °C for 24 and 48 h for bacteria and fungi, respectively. The minimum inhibitory concentrations of the compounds represent as the lowest

concentration of each chemical compounds in the tubes with no turbidity (i.e. no growth) of inoculated bacteria/fungi.

6.2.4. Antioxidant testing

The compounds **4**–**7** were tested for antioxidant property by nitric oxide and DPPH methods.

6.2.5. Assay for nitric oxide (NO) scavenging activity

Sodium nitroprusside (5 μM) in phosphate buffer pH 7.4 was incubated with 100 μM concentration of test compounds dissolved in a suitable solvent (dioxane/methanol) and tubes were incubated at 25 °C for 120 min. Control experiment was conducted with equal amount of solvent in an identical manner. At intervals, 0.5 ml of incubation solution was taken and diluted with 0.5 ml of Griess reagent (1% Sulfanilamide, 0.1% *N*-naphthylethylenediamine dihydrochloride and 2% *o*-phosphoric acid dissolved in distilled water). The absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and subsequent *N*-naphthylethylenediamine dihydrochloride was read at λ 546 nm. The experiment was repeated in triplicate.

6.2.6. Reduction of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (DPPH method)

The nitrogen centered stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) has often been used to characterize antioxidants. It is reversibly reduced and the odd electron in the DPPH free radical gives a strong absorption maximum at λ 517 nm, which is purple in color. This property makes it suitable for spectrophotometer studies. A radical scavenging antioxidant reacts with DPPH stable free radical and converts it into 1,1-diphenyl-2-picrylhydrazine. The resulting decolorization is stoichiometric with respect to the number of electrons captured. The change in the absorbance produced in this reaction has been used to measure antioxidant properties.

The solutions of test compounds (100 μM) were added to DPPH (100 μM) in dioxane/ethanol. The tubes were kept at an ambient temperature for 20 min and the absorbance was measured at λ 517 nm. The inhibition percentage was calculated by using the formula $\%\,\text{inhibition} = [(A_{\text{Control}} - A_{\text{Sample}})/A_{\text{Control}}] \times 100$.Where A_{Control} is the absorbance of the L-ascorbic acid and A_{Sample} is the absorbance of different compounds.

Acknowledgements

The authors are thankful to CSIR, New Delhi, India for the financial assistance under major research project.

References

[1] [1a] F.A. Omar, N.M. Mahfouz, M.A. Rahman, Eur. J. Med. Chem. 31 (1996) 819–825;
(b) B. Narayana, Pharm. (Weinheim) 338 (2005) 373–377;
(c) M. Amir, S. Kumar, Acta Pharm. 57 (2007) 31–45.
[2] S.L. Gaonkar, K.M. Rai, B. Prabhuswamy, Eur. J. Med. Chem. 41 (2006) 841–846.
[3] M.A. Ali, M.S. Yar, Bioorg. Med. Chem. Lett. 17 (2007) 3314–3316.
[4] A. Zarghi, S.A. Tabatabai, M. Faizi, A. Ahadian, P. Navabi, V. Zanganeh, A. Shafiee, Bioorg. Med. Chem. Lett. 15 (2005) 1863.
[5] T.M. Tan, Y. Chen, K.H. Kong, J. Bai, Y. Li, S.G. Lim, T.H. Ang, Y. Lam, Antiviral Res. 71 (2006) 7.
[6] [6a] D.H. Boschelli, D.T. Connor, D.A. Bornemeier, R.D. Dyer, J.A. Kennedy, P. J. Kuipers, G.C. Okonkwo, D.J. Schrier, C.D. Wright, J. Med. Chem. 36 (1993) 1802–1810;
(b) P.C. Unangst, G.P. Shrum, D.T. Conner, R.D. Dyer, D.J. Schrier, J. Med. Chem. 35 (1992) 3691;
(c) M.D. Mullican, M.W. Wilson, D.T. Conner, C.R. Kostlan, D.J. Shrier, R.D. Dyer, J. Med. Chem. 36 (1993) 1090–1099;
(d) I.P. Singh, A.K. Saxena, K. Shankar, Eur. J. Med. Chem. Chim. Ther. 21 (1986) 267–269;
(e) L.V.G. Nargund, G.R.N. Reddy, V.J. Hariprasad, Pharm. Sci. 83 (1994) 246–248.
[7] K. Kariyone, H. Harada, M. Kuritha, T. Takaano, J. Antibiot. 23 (1970) 131.
[8] [8a] V. Padmavathi, P. Triveni, G. Sudhakar Reddy, D. Deepti, Eur. J. Med. Chem. 43 (2008) 917–924;
(b) V. Padmavathi, A.V. Nagendra Mohan, K. Mahesh, Chem. Pharm. Bull. 56 (2008) 815–820;
(c) V. Padmavathi, G. Sudhakar Reddy, A. Padmaja, P. Kondaiah, A. Shazia, Eur. J. Med. Chem. 44 (2009) 2106–2112;
(d) V. Padmavathi, A.V. Nagendra Mohan, P. Triveni, A. Shazia, Eur. J. Med. Chem. 44 (2009) 2313–2321.
[9] [9a] N. Linganna, K.M. Lokanatha Rai, Synth. Commun. 28 (1998) 4611;
(b) F. Kiyoshi, T. Senji, Chem. Pharm. Bull. 8 (1960) 908.
[10] J.E. Oliver, A.B. Demilo, Synthesis (1975) 321–323.
[11] A. Nagai, Y. Matsushita, N. Ono, Y. Takechi, Jpn. Kokai Tokkyo JP 04173780 (1992) Chem. Abstr. 117 (1992) 212485.
[12] National Committee for Clinical Laboratory Standards (NCCLS) Approved Standard Document M-7A. Villanova, PA, 1985.
[13] P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, R.H. Yolken, in: G.L. Wood, J. A Washington (Eds.), Manual of Clinical Microbiology, Am. Soc. Microbiol., Washington DC, 1995.
[14] A. Shirwaiker, K. Rajendran, C. Dinesh Kumar, Indian J. Expl. Biol. 42 (2004) 803–807.
[15] B.H. Babu, B.S. Shailesh, J. Paddikala, Fitotherapia 72 (2001) 272–279.