Synthesis, Antimicrobial and Antioxidant Activities of Sulfone Linked Bis Heterocycles—Pyrazolyl Oxadiazoles and Pyrazolyl Thiadiazole

Venkatapuram PADMAVATHI,* Sanapalli NAGI REDDY, and Konda MAHESH

Department of Chemistry, Sri Venkateswara University; Tirupati-517 502, India.
Received July 12, 2009; accepted September 14, 2009; published online September 24, 2009

A new class of bis heterocycles—sulfone linked pyrazolyl oxadiazoles and thiadiazoles were developed from *Z*-styrylsulfonylacetic acid. The pyrazolyl thiadiazoles exhibited excellent antimicrobial activity whereas pyrazolyl oxadiazoles displayed good antioxidant activity.

Key words *Z*-styrylsulfonylacetic acid; pyrazolyl oxadiazole; pyrazolyl thiadiazole; antimicrobial activity; antioxidant property

Five membered heterocycles natural as well as synthetic are important class of compounds due to their varied biological properties. Pyrazoles show a wide variety of pharmacological effects including anti-inflammatory,[1] antiobesity[2] alcohol dehydrogenase inhibitory[3] and phosphodiesterase inhibitory[4] activities. Some bis pyrazoline derivatives are also found with antimicrobial activity.[5] Huisgen's 1,3-dipolar cycloaddition is a versatile method to synthesize five membered nitrogen heterocycles.[6] Recent synthesis of pyrazoles *via* 1,3-dipolar cycloaddition includes reaction of nitrile imines and activated olefins[7,8] or enamines,[9,10] hydrazones and nitroolefins,[11,12] diazocompounds and activated olefins[13—17] and azomethine imines and alkynes.[18] 1,3,4-Thiadiazole nucleus constitutes the active part of several biologically active compounds including antibacterial,[19—21] antimycotic,[22,23] and anti-inflammatory agents.[24—26] There are a large number of methods for the synthesis of 2,5-disubstituted 1,3,4-thiadiazoles. The most universal is two-stage method consisting of acylation of thiosemicarbazide using carboxylic acid chlorides and subsequent cyclization of the intermediate acylthiosemicarbazides in the presence of various dehydrating agents *viz*., sulfuric acid, phosphorus oxychloride, benzoyl chloride and acetyl chloride.[27] In addition, several 1,3,4-oxadiazole derivatives exhibit significant antibacterial[28—32] and anti-inflammatory activities.[33—35] One of the popular methods for the synthesis of 1,3,4-oxadiazoles involves cyclization of diacylhydrazines prepared by the reaction of acyl chlorides and hydrazine. Several cyclodehydrating agents such as $Et_2O \cdot BF_3$,[36] 1,1,1,3,3,3-hexamethyldisilazane,[37] triflic anhydride,[38] phosphorus pentoxide,[39] polyphosphoric acid,[40] thionyl chloride,[41,42] phosphorus oxychloride[43] and sulfuric acid[44] have been used. Recently we have reported the synthesis, antimicrobial and cytotoxic activities of 1,3,4-oxadiazoles and 1,3,4-thiadiazoles.[45] In continuation of our interest on the chemical and pharmacological properties of sulfone linked bis heterocycles, we report herein the synthesis, antimicrobial and antioxidant activities of pyrazolyl oxadiazoles and thiadiazoles.

Chemistry

The starting material *Z*-styrylsulfonyl acetic acid **3** was obtained *via* the reaction of phenylacetylene with mercaptoacetic acid followed by oxidation (Chart 1).[46] The reaction of **3** with differently substituted benzoic acid hydrazides in the presence of phosphorus oxychloride afforded 2-aryl-5-((styrylsulfonyl)methyl)-1,3,4-oxadiazole (**4**). Compound **4** was treated with two fold excess thiourea in tetrahydrofuran. The reaction mixture indicated two spots on TLC which were separated and identified as 2-aryl-5-((styrylsulfonyl)methyl)-1,3,4-thiadiazole (**5**) as major product apart from **4** as minor one. The ^{1}H-NMR spectra of **4a** and **5a** displayed two doublets at 6.52, 6.48 (H_A) and 7.30, 7.34 ppm (H_B) for the olefin protons. A singlet at 4.55, 4.49 ppm was observed in both the compounds which was assigned to methylene protons in addition to signals due to aromatic protons. The coupling constant value J=11.7 Hz, indicated the *cis* geometry. The ^{13}C-NMR spectra of **4a** and **5a** exhibited signals at 50.8, 48.7 (SO_2CH_2), 130.2, 130.4 (C-H_A), 145.0, 142.3 (C-H_B), 156.7, 156.8 (C-5), and 165.3, 164.4 ppm (C-2), respectively. The olefin moiety present in **4** and **5** was used to develop pyrazoline ring by 1,3-dipolar cycloaddition of diazomethane. Treatment of **4** and **5** with diazomethane in the presence of catalytic amount of Et_3N at −20 to −15 °C for 40 h gave a solid which was identified as 2-((4′,5′-dihydro-4′-phenyl-1′-*H*-pyrazol-3′-ylsulfonyl)methyl)-5-aryl-1,3,4-oxadiazole (**6**) and 2-((4′,5′-dihydro-4′-phenyl-1′-*H*-pyrazol-3′-ylsulfonyl)methyl)-5-aryl-1,3,4-thiadiazole (**7**) (Chart 2). The ^{1}H-NMR spectra of **6a** and **7a** exhibited an AMX splitting pattern for pyrazoline ring protons at 4.49, 4.47 (H_A), 4.08, 4.17 (H_M) and 3.69, 3.61 ppm (H_X) respectively, in addition to the signals of methylene and aromatic protons. The observed coupling constant values J_{AM}=11.9, 12.6 Hz, J_{AX}=6.1, 6.6 Hz, J_{MX}=11.0, 11.5 Hz indicated that H_A, H_M are *cis*, H_A, H_X are *trans* and H_M, H_X are *geminal*. The compounds **6** and **7** on oxidation with chloranil in xylene gave the corresponding aromatized products, 2-((4′-phenyl-1′*H*-pyrazol-3′-ylsulfonyl)methyl)-5-aryl-1,3,4-oxadiazole (**8**) and 2-((4′-phenyl-1′*H*-pyrazol-3′-ylsulfonyl)methyl)-5-aryl-1,3,4-thiadiazole (**9**) (Chart 2). The disappearance of AMX splitting pattern and the appearance of a singlet in the downfield region, merged with aromatic protons confirmed their formation. The ^{13}C-NMR spectra of **8a** and **9a** exhibited signals at 52.3, 49.8 (SO_2CH_2), 134.9, 133.2 (C-4′), 139.2, 139.4

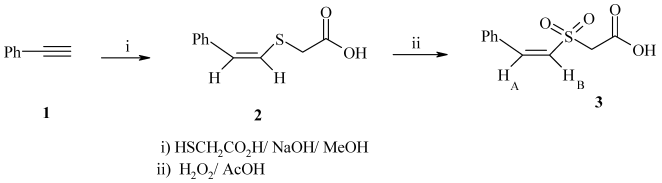

Chart 1

* To whom correspondence should be addressed. e-mail: vkpuram2001@yahoo.com

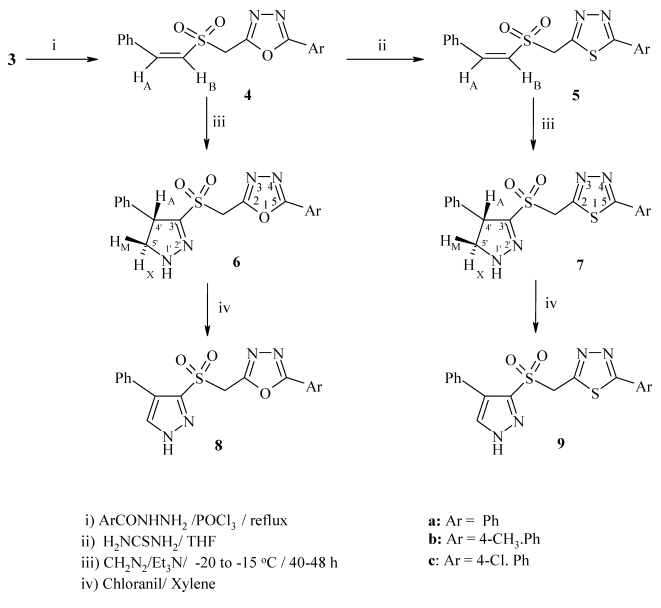

Chart 2

(C-5′), 149.1, 148.9 (C-3′), 158.7, 157.9 (C-5), 163.9, 164.1 ppm (C-2), besides signals due to aromatic carbons.

Antimicrobial Testing The compounds **6**—**9** were tested for antimicrobial activity at two different concentrations 100 and 200 μg/ml. The antibacterial activity was screened against *Staphylococcus aureus*, *Bacillus subtilis* (Gram-positive bacteria) and *Escherichia coli*, *Klebsiella pneumoniae* (Gram-negative bacteria) on nutrient agar plates at 37 °C for 24 h using chloramphenicol as reference drug. The compounds were also evaluated for their antifungal activity against *Fusarium solani*, *Curvularia lunata* and *Aspergillus niger* using ketoconazole as standard drug. Fungi cultures were grown on potato dextrose agar medium (PDA) at 25 °C for 3 d. The spore suspension was adjusted to 10^6 pores/ml at a mg/ml concentration by the Vincent and Vincent method.[47)]

The results of the compounds of preliminary antibacterial testing are shown in Table 1. The results revealed that the compounds 2-((4′,5′-dihydro-4′-phenyl-1′-*H*-pyrazol-3′-ylsulfonyl)methyl)-5-aryl-1,3,4-thiadiazole (**7**) and 2-((4′-phenyl-1′*H*-pyrazol-3′-ylsulfonyl)methyl)-5-aryl-1,3,4-thiadiazole (**9**) exhibited high activity (17—38 mm) on both Gram-positive and Gram-negative bacteria. In fact, compounds **7c** and **9c** showed pronounced activity (30—38 mm) towards Gram-positive bacteria. The compounds 2-((4′-phenyl-1′-*H*-pyrazol-3′-ylsulfonyl)methyl)-5-aryl-1,3,4-oxadiazole (**8**) displayed moderate activity towards Gram-positive bacteria (16—22 mm). On the other hand, 2-((4′,5′-dihydro-4′-phenyl-1′-*H*-pyrazol-3′-ylsulfonyl)methyl)-5-aryl-1,3,4-oxadiazole (**6**) exhibited least activity against both bacteria. All the test compounds inhibited the spore germination of tested fungi *A. niger*, *F. solani* and *C. lunata*. Results of the investigation presented in Table 2 revealed that all the compounds except **6** showed relatively high inhibitory effect on *F. solani* and *C. lunata* than on *A. niger*. The compounds **7c** and **9c** displayed high activity.

The minimum inhibitory concentration (MIC) values were determined as the lowest concentration that completely inhibited the visible growth of the microorganisms (Table 3).

Table 1. The *in Vitro* Antibacterial Activity of **6**—**9**

Compound	Concentration (μg/ml)	Zone of inhibition (mm)			
		Gram (+)ve		Gram (−)ve	
		S. aureus	*B. subtilis*	*E. coli*	*K. pneumoniae*
6a	100	10	12	7	—
	200	14	14	9	8
6b	100	8	10	—	—
	200	11	13	—	—
6c	100	9	12	8	—
	200	12	14	10	—
7a	100	24	23	21	23
	200	25	27	26	24
7b	100	19	19	17	18
	200	22	23	24	22
7c	100	32	30	25	23
	200	34	32	26	25
8a	100	17	16	16	14
	200	19	20	18	17
8b	100	15	16	14	12
	200	19	21	17	16
8c	100	18	19	16	15
	200	21	22	18	19
9a	100	26	23	23	23
	200	31	28	27	25
9b	100	23	25	22	22
	200	25	28	25	24
9c	100	35	36	30	29
	200	37	38	32	31
Chloramphenicol	100	35	38	40	42
	200	39	41	44	45

Table 2. The *in Vitro* Antifungal Activity of **6**—**9**

Compound	Concentration (μg/ml)	Zone of inhibition (mm)		
		F. solani	*C. lunata*	*A. niger*
6a	100	13	16	12
	200	15	19	14
6b	100	11	14	10
	200	13	17	12
6c	100	15	18	14
	200	17	21	16
7a	100	25	26	21
	200	28	28	24
7b	100	23	25	21
	200	25	26	22
7c	100	30	32	26
	200	32	34	29
8a	100	21	23	19
	200	24	26	22
8b	100	18	17	15
	200	21	22	19
8c	100	24	25	22
	200	26	28	24
9a	100	33	33	29
	200	36	35	32
9b	100	28	27	26
	200	32	33	30
9c	100	30	36	24
	200	32	39	26
Ketoconazole	100	38	41	36
	200	42	44	39

The structure–antimicrobial activity relationship of the synthesized compounds revealed that the compounds having pyrazoline in combination with oxadiazole (**6**) exhibited least

Table 3. Minimum Inhibitory Concentration of Compounds **7c** and **9c**

Compound	Minimum inhibitory concentration (MIC), μg/ml						
	S. aureus	*B. subtilis*	*E. coli*	*K. pneumoniae*	*F. solani*	*C. lunata*	*A. niger*
7c	100	50	200	200	100	100	100
9c	25	25	50	50	50	25	50
Chloramphenicol	6.25	6.25	6.25	12.5	—	—	—
Ketoconazole	—	—	—	—	12.5	6.25	6.25

Table 4. Antioxidant Activity of **6**—**9**

Compound	% Inhibition at 100 μM	
	Nitric oxide method	DPPH method
6a	69.5	70.3
6b	74.1	72.5
6c	78.5	79.3
7a	45.8	46.3
7b	47.3	46.8
7c	49.4	49.7
8a	74.8	75.3
8b	54.3	52.5
8c	89.6	88.4
9a	46.7	46.3
9b	45.5	46.9
9c	49.9	50.5

activity when compared with compounds having pyrazole with oxadiazole (**8**) and pyrazoline/pyrazole with thiadiazole (**7**/**9**) moieties. However, compounds **8** displayed moderate activity. On the other hand compounds **7** and **9** exhibited high activity. The presence of chloro substituent on the aromatic ring enhances the activity of the compounds. The maximum activity was observed with the compounds **7c** and **9c**.

Antioxidant Testing The compounds **6**—**9** were tested for antioxidant property by nitric oxide[48,49] and 1,1-diphenylpicrylhydrazyl (DPPH)[50] methods. The compounds **6a**, **6b**, **6c**, **8a** and **8c** exhibited high antioxidant activity in both nitric oxide and DPPH methods at 100 μM concentration (Table 4).

Conclusion

A new and novel bis heterocyclic systems pyrazolyl oxadiazoles and thiadiazoles were developed from *Z*-styrylsulfonylacetic acid by appropriate functionalization of acid and olefin groups. The compounds pyrazolyl thiadiazoles exhibited excellent antimicrobial activity, whereas pyrazolyl oxadiazoles showed good antioxidant property.

Experimental

Melting points were determined in open capillaries on a Mel-Temp apparatus and are uncorrected. The purity of the compounds was checked by TLC (silica gel H, BDH, ethyl acetate/hexane, 1 : 3). The IR spectra were recorded on a Thermo Nicolet IR 200 FT-IR spectrometer as KBr pellets and the wave numbers were given in cm^{-1}. The ^{1}H-NMR spectra were recorded in $CDCl_3$/DMSO-d_6 on a Bruker spectrospin operating at 400 MHz. The ^{13}C-NMR spectra were recorded in $CDCl_3$/DMSO-d_6 on Bruker spectrospin operating at 100 MHz. All chemical shifts are reported in δ (ppm) using TMS as an internal standard. The microanalyses were performed on a Perkin-Elmer 240C elemental analyzer. The antioxidant property was carried out by using Shimadzu UV-2450 spectrophotometer. The starting compound *Z*-styrylsulfonylacetic acid (**3**) was prepared by the literature procedure.[46]

General Procedure of Synthesis of 2-Aryl-5-((styrylsulfonyl)methyl)-1,3,4-oxadiazole (4a—c) To *Z*-styrylsulfonylacetic acid (5 mmol) and benzoic acid hydrazide (5 mmol), $POCl_3$ (4 ml) was added and heated under reflux for 5—6 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, dried and recrystallized from ethanol to get **4**.

2-Phenyl-5-((styrylsulfonyl)methyl)-1,3,4-oxadiazole (**4a**): White solid, yield 75%, mp 112—114 °C; IR (KBr) cm^{-1}: 1589 (C=N), 1542 (C=C), 1317, 1135 (SO_2); ^{1}H-NMR ($CDCl_3$) δ: 4.55 (s, 2H, SO_2–CH_2), 6.52 (d, 1H, H_A, J=11.7 Hz), 7.30 (d, 1H, H_B, J=11.7 Hz), 7.22—7.92 (m, 10H, Ar–H); ^{13}C-NMR ($CDCl_3$) δ: 50.8 (SO_2–CH_2), 130.2 (C–H_A), 145.0 (C–H_B), 156.7 (C-5), 165.3 (C-2), 121.8, 126.1, 127.2, 128.5, 130.1, 130.7, 131.5, 136.2 (aromatic carbons).

2-((Styrylsulfonyl)methyl)-5-*p*-tolyl-1,3,4-oxadiazole (**4b**): White solid, yield 74%, mp 105—107 °C; IR (KBr) cm^{-1}: 1593 (C=N), 1546 (C=C), 1318, 1138 (SO_2); ^{1}H-NMR ($CDCl_3$) δ: 2.26 (s, 3H, Ar–CH_3), 4.53 (s, 2H, SO_2–CH_2), 6.54 (d, 1H, H_A, J=11.8 Hz), 7.31 (d, 1H, H_B, J=11.8 Hz), 7.25—7.94 (m, 9H, Ar–H); ^{13}C-NMR ($CDCl_3$) δ: 21.8 (Ar–CH_3), 51.1 (SO_2–CH_2), 129.6 (C–H_A), 146.1 (C–H_B), 156.1 (C-5), 164.8 (C-2), 121.2, 125.2, 128.2, 128.9, 129.7, 130.9, 131.2, 136.4 (aromatic carbons).

2-(*p*-Chlorophenyl)-5-((styrylsulfonyl)methyl)-1,3,4-oxadiazole (**4c**): White solid, yield 74%, mp 127—129 °C; IR (KBr) cm^{-1}: 1608 (C=N), 1559 (C=C), 1320, 1143 (SO_2); ^{1}H-NMR ($CDCl_3$) δ: 4.58 (s, 2H, SO_2–CH_2), 6.56 (d, 1H, H_A, J=12.0 Hz), 7.33 (d, 1H , H_B, J=12.0 Hz), 7.27—7.96 (m, 9H, Ar–H); ^{13}C-NMR ($CDCl_3$) δ: 51.2 (SO_2–CH_2), 130.6 (C–H_A), 145.2 (C–H_B), 156.9 (C-5), 165.6 (C-2), 121.5, 126.3, 128.5, 129.5, 130.2, 130.6, 131.7, 138.6 (aromatic carbons).

General Procedure of Synthesis of 2-Aryl-5-((styrylsulfonyl)methyl)-1,3,4-thiadiazole (5a—c) In a sealed test tube, a mixture of **4** (5 mmol), thiourea (20 mmol) dissolved in tertahydrofuran (5 ml) was taken. The contents were heated at 120—150 °C in an oil bath for 22—26 h. After the reaction was completed, it was extracted with dichloromethane. The organic layer was washed with water, brine solution and dried over anhydrous Na_2SO_4. The resultant solid was recrystallized from methanol to obtain **5**.

2-Phenyl-5-((styrylsulfonyl)methyl)-1,3,4-thiadiazole (**5a**): White solid, yield 64%, mp 133—135 °C; IR (KBr) cm^{-1}: 1580 (C=N), 1544 (C=C), 1318, 1140 (SO_2); ^{1}H-NMR ($CDCl_3$) δ: 4.49 (s, 2H, SO_2–CH_2), 6.48 (d, 1H, H_A, J=11.6 Hz), 7.34 (d, 1H, H_B, J=11.6 Hz), 7.17—7.92 (m, 10H, Ar–H); ^{13}C-NMR ($CDCl_3$) δ: 48.7 (SO_2–CH_2), 130.4 (C–H_A), 142.3 (C–H_B), 156.8 (C-5), 164.4 (C-2), 121.4, 125.2, 128.4, 128.9, 130.1, 130.3, 131.6, 134.5 (aromatic carbons).

2-((Styrylsulfonyl)methyl)-5-*p*-tolyl-1,3,4-thiadiazole (**5b**): White solid, yield 69%, mp 147—149 °C; IR (KBr) cm^{-1}: 1594 (C=N), 1548 (C=C), 1324, 1142 (SO_2); ^{1}H-NMR ($CDCl_3$) δ: 2.23 (s, 3H, Ar–CH_3) 4.46 (s, 2H, SO_2–CH_2), 6.51 (d, 1H, H_A, J=11.8 Hz), 7.32 (d, 1H, H_B, J=11.8 Hz), 7.19—7.83 (m, 9H, Ar–H); ^{13}C-NMR ($CDCl_3$) δ: 21.9 (Ar–CH_3), 49.3 (SO_2–CH_2), 129.7 (C–H_A), 145.3 (C–H_B), 155.6 (C-5), 165.7 (C-2), 121.6, 123.4, 125.6, 127.5 , 130.4, 130.5, 130.7, 133.7 (aromatic carbons).

2-(*p*-Chlorophenyl)-5-((styrylsulfonyl)methyl)-1,3,4-thiadiazole (**5c**): White solid, yield 72%, mp 155—157 °C; IR (KBr) cm^{-1}: 1561 (C=C), 1603 (C=N), 1322, 1144 (SO_2); ^{1}H-NMR ($CDCl_3$) δ: 4.48 (s, 2H, SO_2–CH_2), 6.53 (d, 1H, H_A, J=12.0 Hz), 7.36 (d, 1H , H_B, J=12.0 Hz), 7.23—7.99 (m, 9H, Ar–H); ^{13}C-NMR ($CDCl_3$) δ: 48.2 (SO_2–CH_2), 130.7 (C–H_A), 145.7 (C–H_B), 147.1 (C-5) 162.8 (C-2), 120.7, 126.4, 127.7, 128.6, 130.0, 130.8, 131.9, 134.8 (aromatic carbons).

General Procedure of Synthesis of 2-((4′,5′-Dihydro-4′-phenyl-1′*H*-pyrazol-3′-ylsulfonyl)methyl)-5-aryl-1,3,4-oxadiazole (6a—c)/2-((4′,5′-Dihydro-4′-phenyl-1′*H*-pyrazol-3′-ylsulfonyl)methyl)-5-aryl-1,3,4-thiadiazole (7a—c) To a cooled solution of **4**/**5** (2.5 mmol) in dichloromethane (10 ml), an ethereal solution of diazomethane (20 ml, 0.4 M) and triethylamine (0.06 g) were added. The reaction mixture was kept at −20 to −15 °C for 40—48 h. The solvent was removed under reduced pressure. The resultant solid was purified by column chromatography (silica gel (BDH)

60—120 mesh, hexane–ethyl acetate, 4 : 1).

2-((4′,5′-Dihydro-4′-phenyl-1′*H*-pyrazol-3′-ylsulfonyl)methyl)-5-phenyl-1,3,4-oxadiazole (**6a**): Yellow solid, yield 62%, mp 136—138 °C; IR (KBr) cm^{-1}: 3338 (NH), 1582 (C=N), 1331, 1130 (SO_2); ^{1}H-NMR (DMSO-d_6) δ: 3.69 (dd, 1H, H_X, J_{AX}=6.1 Hz, J_{MX}=11.0 Hz), 4.74 (d, 1H, SO_2–CH_2, J=14.6 Hz), 4.08 (dd, 1H, H_M, J_{AM}=11.9 Hz, J_{MX}=11.0 Hz), 4.49 (dd, 1H, H_A, J_{AM}=11.9 Hz, J_{AX}=6.1 Hz) 5.03 (d, 1H, SO_2–CH_2, J=14.6 Hz), 7.20—7.94 (m, 10H, Ar–H), 8.87 (br s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ: 48.6 (C-4′), 51.6 (SO_2–CH_2), 59.1 (C-5′), 149.4 (C-3′), 156.6 (C-5), 165.5 (C-2), 123.2, 125.6, 127.5, 129.1, 129.8, 137.2, 138.9 (aromatic carbons). *Anal.* Calcd for $C_{18}H_{16}N_4O_3S$: C, 58.68; H, 4.38; N, 15.21; Found: C, 58. 81; H, 4.43; N, 15.32.

2-((4′,5′-Dihydro-4′-phenyl-1′*H*-pyrazol-3′-ylsulfonyl)methyl)-5-*p*-tolyl-1,3,4-oxadiazole (**6b**): Yellow solid, yield 72%, mp 112—114 °C; IR (KBr) cm^{-1}: 3341 (NH), 1592 (C=N), 1334, 1131 (SO_2); ^{1}H-NMR (DMSO-d_6) δ: 2.32 (s, 3H, Ar–CH_3), 3.66 (dd, 1H, H_X, J_{AX}=6.2 Hz, J_{MX}=11.1 Hz), 4.06 (dd, 1H, H_M, J_{AM}=12.1 Hz, J_{MX}=11.1 Hz), 4.47 (dd, 1H, H_A, J_{AM}=12.1 Hz, J_{AX}=6.2 Hz), 4.72 (d, 1H, SO_2–CH_2, J=14.2 Hz), 5.04 (d, 1H, SO_2–CH_2, J=14.2 Hz), 7.21—7.85 (m, 9H, Ar–H), 8.95 (br s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ: 21.8 ($\underline{C}H_3$–Ar), 47.7 (C-4′), 51.8 (SO_2–CH_2), 58.2 (C-5′), 148.6 (C-3′), 157.8 (C-5), 164.6 (C-2), 122.1, 127.7, 128.3, 129.4, 130.1, 137.4, 140.2 (aromatic carbons). *Anal.* Calcd for $C_{19}H_{18}N_4O_3S$: C, 59.67; H, 4.74; N, 14.65; Found: C, 59.73; H, 4.68 ; N, 14.71.

2-((4′,5′-Dihydro-4′-phenyl-1′*H*-pyrazol-3′-ylsulfonyl)methyl)-5-(*p*-chlorophenyl)-1,3,4-oxadiazole (**6c**): Yellow solid, yield 74%, mp 148—150 °C; IR (KBr) cm^{-1}: 3343 (NH), 1606 (C=N), 1336, 1134 (SO_2); ^{1}H-NMR (DMSO-d_6) δ: 3.68 (dd, 1H, H_X, J_{AX}=6.3 Hz, J_{MX}=11.3 Hz), 4.13 (dd, 1H, H_M, J_{AM}=12.2 Hz, J_{MX}=11.3 Hz), 4.54 (dd, 1H, H_A, J_{AM}=12.2 Hz, J_{AX}=6.3 Hz), 4.77 (d, 1H, SO_2–CH_2, J=14.9 Hz), 5.06 (d, 1H, SO_2–CH_2, J=14.9 Hz), 7.23—7.98 (m, 9H, Ar–H), 8.97 (br s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ: 48.9 (C-4′), 51.9 (SO_2–CH_2), 59.3 (C-5′), 146.6 (C-3′), 158.0 (C-5), 164.8 (C-2), 122.2, 127.9, 128.8, 129.2, 130.2, 137.6, 140.4 (aromatic carbons). *Anal.* Calcd for $C_{18}H_{15}ClN_4O_3S$: C, 53.67; H, 3.75; N, 13.91; Found: C, 53.60; H, 3.82; N, 14.00.

2-((4′,5′-Dihydro-4′-phenyl-1′-*H*-pyrazol-3′-ylsulfonyl)methyl)-5-phenyl-1,3,4-thiadiazole (**7a**): Yellow solid, yield 70%, mp 145—147 °C; IR (KBr) cm^{-1}: 3339 (NH), 1583 (C=N), 1332, 1132 (SO_2); ^{1}H-NMR (DMSO-d_6) δ: 3.61 (dd, 1H, H_X, J_{AX}=6.6 Hz, J_{MX}=11.5 Hz), 4.17 (dd, 1H, H_M, J_{AM}=12.6 Hz, J_{MX}=11.5 Hz), 4.47 (dd, 1H, H_A, J_{AM}=12.6 Hz, J_{AX}=6.6 Hz), 4.61 (d, 1H, SO_2–CH_2, J=14.7 Hz), 5.15 (d, 1H, SO_2–CH_2, J=14.7 Hz), 7.25—7.84 (m, 10H, Ar–H), 8.91 (br s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ: 48.8 (C-4′), 49.3 (SO_2–CH_2), 59.3 (C-5′), 149.4 (C-3′), 157.8 (C-5), 164.7 (C-2), 122.8, 127.7, 128.5, 129.0 129.7, 134.6, 137.2 (aromatic carbons). *Anal.* Calcd for $C_{18}H_{16}N_4O_2S_2$: C, 56.23; H, 4.19; N, 14.57; Found: C, 56.17; H, 4.11 ; N, 14.63.

2-((4′,5′-Dihydro-4′-phenyl-1′*H*-pyrazol-3′-ylsulfonyl)methyl)-5-*p*-tolyl-1,3,4-thiadiazole (**7b**): Yellow solid, yield 66%, mp 156—158 °C; IR (KBr) cm^{-1}: 3342 (NH), 1592 (C=N), 1334, 1133 (SO_2); ^{1}H-NMR (DMSO-d_6) δ: 2.35 (s, 3H, Ar–CH_3), 4.64 (d, 1H, SO_2–CH_2, J=14.8 Hz), 4.97 (d, 1H, SO_2–CH_2, J=14.8 Hz), 3.67 (dd, 1H, H_X, J_{AX}=6.8 Hz, J_{MX}=11.2 Hz), 4.11 (dd, 1H, H_M, J_{AM}=12.1 Hz, J_{MX}=11.2 Hz), 4.50 (dd, 1H, H_A, J_{AM}=12.1 Hz, J_{AX}=6.8 Hz), 7.25—7.97 (m, 9H, Ar–H), 8.94 (br s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ: 22.1 ($\underline{C}H_3$–Ar), 50.2 (SO_2–CH_2), 49.1 (C-4′), 59.4 (C-5′), 147.9 (C-3′), 158.1 (C-5), 163.9 (C-2), 124.3, 127.3 128.7, 129.1, 130.2, 137.5, 139.4 (aromatic carbons). *Anal.* Calcd for $C_{19}H_{18}N_4O_2S_2$: C, 57.27; H, 4.55; N, 14.06; Found: C, 57.35; H, 4.49; N, 14.00.

2-((4′,5′-Dihydro-4′-phenyl-1′*H*-pyrazol-3′-ylsulfonyl)methyl)-5-(*p*-chlorophenyl)-1,3,4-thiadiazole (**7c**): Yellow solid, yield 73%, mp 172—174 °C; IR (KBr) cm^{-1}: 3344 (NH), 1602 (C=N), 1337, 1135 (SO_2); ^{1}H-NMR (DMSO-d_6) δ: 3.69 (dd, 1H, H_X, J_{AX}=6.6 Hz, J_{MX}=11.8 Hz), 4.19 (dd, 1H, H_M, J_{AM}=12.8 Hz, J_{MX}=11.8 Hz), 4.49 (dd, 1H, H_A, J_{AM}=12.8 Hz, J_{AX}=6.6 Hz), 4.69 (d, 1H, SO_2–CH_2, J=14.4 Hz), 5.01 (d, 1H, SO_2–CH_2, J=14.4 Hz), 7.26—8.01 (m, 9H, Ar–H), 8.96 (br s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ: 49.6 (C-4′), 50.7 (SO_2–CH_2), 59.7 (C-5′), 148.2 (C-3′), 158.3 (C-5), 164.9 (C-2), 122.4, 128.1, 128.9, 129.4, 130.4, 137.7, 140.6 (aromatic carbons). *Anal.* Calcd for $C_{18}H_{15}ClN_4O_2S_2$: C, 51.61; H, 3.61; N, 13.37; Found: C, 51.50; H, 3.65; N, 13.47.

General Procedure of Synthesis of 2-((4′-Phenyl-1′*H*-pyrazol-3′-ylsulfonyl)methyl)-5-aryl-1,3,4-oxadiazole (8a—c)/2-((4′-Phenyl-1′*H*-pyrazol-3′-ylsulfonyl)methyl)-5-aryl-1,3,4-thiadiazole (9a—c) A solution of **6**/**7** (1 mmol) and chloranil (1.4 mmol) in xylene (10 ml) was refluxed for 24—25 h. Then, the reaction mixture was treated with a 5% NaOH solution. The organic layer was separated and repeatedly washed with water. It was dried over anhydrous Na_2SO_4 and the solvent was removed on a rotary evaporator. The resultant solid was purified by recrystallization from 2-propanol.

2-((4′-Phenyl-1′*H*-pyrazol-3′-ylsulfonyl)methyl)-5-phenyl-1,3,4-oxadiazole (**8a**): Yellow solid, yield 64%, mp 157—159 °C; IR (KBr) cm^{-1}: 3341 (NH), 1584 (C=N), 1331, 1136 (SO_2); ^{1}H-NMR (DMSO-d_6) δ: 4.74 (d, 1H, SO_2–CH_2, J=14.6 Hz), 4.98 (d, 1H, SO_2–CH_2, J=14.6 Hz), 6.52 (br s, 1H, NH) 6.84—7.78 (m, 11H, $C_{5'}$–H, Ar–H); ^{13}C-NMR (DMSO-d_6) δ: 52.3 (SO_2–CH_2), 134.9 (C-4′), 139.2 (C-5′), 149.1 (C-3′), 158.7 (C-5), 163.9 (C-2), 122.1, 127.6, 128.4, 129.1, 129.8, 137.2, 140.1 (aromatic carbons). *Anal.* Calcd for $C_{18}H_{14}N_4O_3S$: C, 59.01; H, 3.85; N, 15.29; Found: C, 59.09; H, 3.90; N, 15.35.

2-((4′-Phenyl-1′*H*-pyrazol-3′-ylsulfonyl)methyl)-5-*p*-tolyl-1,3,4-oxadiazole (**8b**): Yellow solid, yield 69%, mp 163—165 °C; IR (KBr) cm^{-1}: 3342 (NH), 1596 (C=N), 1332, 1138 (SO_2); ^{1}H-NMR (DMSO-d_6) δ: 2.32 (s, 3H, Ar–CH_3), 4.71 (d, 1H, SO_2–CH_2, J=14.2 Hz), 5.04 (d, 1H, SO_2–CH_2, J=14.2 Hz), 6.56 (br s, 1H, NH), 6.81—7.75 (m, 10H, $C_{5'}$–H, Ar–H); ^{13}C-NMR (DMSO-d_6) δ: 22.5 (CH_3–Ar), 51.5 (SO_2–CH_2), 135.2 (C-4′), 141.4 (C-5′), 148.7 (C-3′), 157.8 (C-5), 164.2 (C-2), 122.3, 127.8, 128.6, 128.9, 130.2, 135.4, 138.8 (aromatic carbons). *Anal.* Calcd for $C_{19}H_{16}N_4O_3S$: C, 59.99; H, 4.24; N, 14.73; Found: C, 60.07; H, 4.30; N, 14.80.

2-((4′-Phenyl-1′*H*-pyrazol-3′-ylsulfonyl)methyl)-5-(*p*-chlorophenyl)-1,3,4-oxadiazole (**8c**): Yellow solid, yield 72%, mp 181—183 °C; IR (KBr) cm^{-1}: 3344 (NH), 1602 (C=N), 1334, 1142 (SO_2); ^{1}H-NMR (DMSO-d_6) δ: 4.80 (d, 1H, SO_2–CH_2, J=14.9 Hz), 5.06 (d, 1H, SO_2–CH_2, J=14.9 Hz) 6.53 (br s, 1H, NH), 6.87—7.78 (m, 10H, $C_{5'}$–H, Ar–H); ^{13}C-NMR (DMSO-d_6) δ: 52.6 (SO_2–CH_2), 135.4 (C-4′), 141.5 (C-5′), 149.7 (C-3′) 158.9 (C-5), 164.4 (C-2), 122.5, 127.4, 128.8, 129.5, 130.4, 137.6, 140.5 (aromatic carbons). *Anal.* Calcd for $C_{18}H_{13}ClN_4O_3S$: C, 53.94; H, 3.27; N, 13.98; Found: C, 54.02; H, 3.31; N, 14.07.

2-((4′-Phenyl-1′*H*-pyrazol-3′-ylsulfonyl)methyl)-5-phenyl-1,3,4-thiadiazole (**9a**): Yellow solid, yield 68%, mp 168—170 °C; IR (KBr) cm^{-1}: 3346 (NH), 1587 (C=N), 1336, 1139 (SO_2); ^{1}H-NMR (DMSO-d_6) δ: 4.62 (d, 1H, SO_2–CH_2, J=14.3 Hz), 4.89 (d, 1H, SO_2–CH_2, J=14.3 Hz), 6.55 (br s, 1H, NH), 6.76—7.71 (m, 11H, $C_{5'}$–H, Ar–H); ^{13}C-NMR (DMSO-d_6) δ: 49.8 (SO_2–CH_2), 133.2 (C-4′), 139.4 (C-5′), 148.9 (C-3′), 157.9 (C-5), 164.1 (C-2), 124.3, 125.7, 126.5, 129.3, 129.9, 137.4, 138.2 (aromatic carbons). *Anal.* Calcd for $C_{18}H_{14}N_4O_2S_2$: C, 56.53; H, 3.69; N, 14.65; Found: C, 56.45; H, 3.74; N, 14.55.

2-((4′-Phenyl-1′*H*-pyrazol-3′-ylsulfonyl)methyl)-5-*p*-tolyl-1,3,4-thiadiazole (**9b**): Yellow solid, yield 73%, mp 181—183 °C; IR (KBr) cm^{-1}: 3341 (NH), 1595 (C=N), 1334, 1138 (SO_2); ^{1}H-NMR (DMSO-d_6) δ: 2.23 (s, 3H, Ar–CH_3), 4.67 (d, 1H, SO_2–CH_2, J=14.5 Hz), 4.90 (d, 1H, SO_2–CH_2, J=14.5 Hz), 6.52 (br s, 1H, NH), 6.84—7.76 (m, 10H, C_5'–H, Ar–H); ^{13}C-NMR (DMSO-d_6) δ: 22.4 ($\underline{C}H_3$–Ar), 48.1 (SO_2–CH_2), 134.3 (C-4′), 141.5 (C-5′), 147.9 (C-3′), 159.7 (C-5), 164.3 (C-2), 122.4, 127.9, 128.7, 129.5 130.3, 137.5, 138.5 (aromatic carbons). *Anal.* Calcd for $C_{19}H_{16}N_4O_2S_2$: C, 57.56; H, 4.07; N, 14.13; Found: C, 57.63; H, 4.02; N, 14.18.

2-((4′-Phenyl-1′*H*-pyrazol-3′-ylsulfonyl)methyl)-5-(*p*-chlorophenyl)-1,3,4-thiadiazole (**9c**): Yellow solid, yield 66%, mp 194—196 °C; IR (KBr) cm^{-1}: 3345 (NH), 1604 (C=N), 1336, 1141 (SO_2); ^{1}H-NMR (DMSO-d_6) δ: 4.72 (d, 1H, SO_2–CH_2, J=14.9 Hz), 5.03 (d, 1H, SO_2–CH_2, J=14.9 Hz), 6.58 (br s, 1H, NH), 6.89—7.78 (m, 10H, $C_{5'}$–H, Ar–H); ^{13}C-NMR (DMSO-d_6) δ: 48.7 (SO_2–CH_2), 132.6 (C-4′), 139.7 (C-5′), 148.7 (C-3′) 159.2 (C-5), 165.6 (C-5), 125.4 127.2, 128.9, 129.5, 129.7, 134.8, 139.7 (aromatic carbons). *Anal.* Calcd for $C_{18}H_{13}ClN_4O_2S_2$: C, 51.86; H, 3.13; N, 13.44; Found: C, 51.78; H, 3.16; N, 13.52.

Antimicrobial Testing The compounds **6**—**9** were dissolved in DMSO at different concentrations of 100, 200 and 800 μg/ml.

Antibacterial and Antifungal Assays Preliminary antimicrobial activity of compounds **6**—**9** was tested by agar disc-diffusion method. Sterile filter paper discs (6 mm diameter) moistened with the test compound solution in DMSO of specific concentration 100 μg and 200 μg/disc were carefully placed on the agar culture plates that had been previously inoculated separately with the microorganisms. The plates were incubated at 37 °C and the diameter of the growth inhibition zones were measured after 24 h in case of bacteria and after 48 h in case of fungi.

The MIC's of the compound assays were determined using micro dilution susceptibility method. Chloramphenicol was used as reference antibacterial agent. Ketoconazole was used as reference antifungal agent. The test compounds, chloramphenicol and ketoconazole were dissolved in DMSO at concentration of 800 μg/ml. The two-fold dilution of the solution was prepared (400, 200, 100, …, 6.25 μg/ml). The microorganism suspensions were incubated at 36 °C for 24 and 48 h for bacteria and fungi, respectively. The minimum inhibitory concentrations of the compounds were recorded as the lowest concentration of each chemical compound in the tubes with no turbidity

(*i.e.*, no growth) of inoculated bacteria/fungi.

Antioxidant Testing The compounds **6**—**9** are tested for antioxidant property by nitric oxide and DPPH methods.

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.

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 spectrophotometric studies. A radical scavenging antioxidant reacts with DPPH stable free radical and converts 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/methanol. The tubes were kept at an ambient temperature for 20 min and the absorbance was measured at λ 517 nm. The difference between the test and the control experiments was taken and expressed as the per cent scavenging of the DPPH radical.

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

References

1) Penning T. D., Talley J. J., Bertenshaw S. R., Carter J. S., Collins P. W., Docter S., Graneto M. J., Lee L. F., Malecha J. W., Miyashiro J. M., Rogers R. S., Rogier D. J., Yu S. S., Anderson G. D., Burton E. G., Cogburn J. N., Gregory S. A., Koboldt C. M., Perkins W. E., Seibert K., Veenhuizen A. W., Zhang Y. Y., Isakson P. C., *J. Med. Chem.*, **40**, 1347—1365 (1997).
2) Rinaldi-Carmona M., Barth F., Héaulme M., Shire D., Calandra B., Congy C., Martinez S., Maruani J., Néliat G., Caput D., Ferrara P., Soubrieé P., Breliére J. C., Le Fur G., *FEBS Lett.*, **350**, 240—244 (1994).
3) Baud F. J., Bismuth C., Garnier R., Galliot M., Astier A., Maistre G., Soffer M., *Clin. Toxicol.*, **24**, 463—483 (1986).
4) Terrett N. K., Bell A. S., Brown D., Ellis P., *Bioorg. Med. Chem. Lett.*, **6**, 1819—1824 (1996).
5) Bhat Abdul R., Athar F., Azam A., *Eur. J. Med. Chem.*, **44**, 426— 431 (2009).
6) Huisgen R., *J. Org. Chem.*, **41**, 403—419 (1976).
7) Conti P., Pinto A., Tamborini L., Rizzo V., De Micheli C., *Tetrahedron*, **63**, 5554—5560 (2007).
8) Molteni G., *ARKIVOC*, **ii**, 224—246 (2007).
9) Donohue S. R., Halldin C., Pike V. W., *Tetrahedron Lett.*, **49** 2789—2791 (2008).
10) Oh L. M., *Tetrahedron Lett.*, **47**, 7943—7946 (2006).
11) Deng X., Mani N. S., *Org. Lett.*, **10**, 1307—1310 (2008).
12) Deng X., Mani N. S., *Org. Lett.*, **8**, 3505—3508 (2006).
13) Padmavathi V., Jagan Mohan Reddy B., Chandra Obula Reddy B., Padmaja A., *Tetrahedron*, **61**, 2407—2411 (2005).
14) Padmavathi V., Jagan Mohan Reddy B., Venkata Subbaiah D. R. C., *New J. Chem.*, **28**, 1479—1483 (2004).
15) Hari Y., Tsuchida S., Sone R., Aoyama T., *Synthesis*, **2007**, 371—375 (2007).
16) Jiang N., Li C. J., *Chem. Commun.*, **2004**, 394—395 (2004).
17) Aggarwal V. K., de Vicente J., Bonnert R. V., *J. Org. Chem.*, **68**, 5381—5383 (2003).
18) Komatsu M., Minakata S., Oderaotoshi Y., *ARKIVOC*, **VII**, 370—389 (2006).
19) Foroumadi A., Mansouri S., Kiani Z., Rahmani A., *Eur. J. Med. Chem.*, **38**, 851—854 (2003).
20) Thomasco L. M., Gadwood R. C., E. Weaver A., Ochoada J. M., Ford C. W., Zurenko G. E., Hamel J. C., Stapert D., Moerman J. K., Schaadt R. D., Agi B. H., *Bioorg. Med. Chem. Lett.*, **13**, 4193—4196 (2003).
21) Karaku S., Rollas S., *Il Farmaco*, **57**, 577—581 (2002).
22) Dogan H. N., Duran A., S. Rollas., Sener G., Uysal M. K., Gülen D., *Bioorg. Med. Chem.*, **10**, 2893—2898 (2002).
23) Mamolo M. G., Vio L., Banfi E., *Il Farmaco*, **51**, 71—74 (1996).
24) Schenone S., Brullo C., Bruno O., Bondavalli F., Ranise A., Filippelli W., Rinaldi B., Capuano A., Falcone G., *Bioorg. Med. Chem.*, **14**, 1698—1705 (2006).
25) Santagati M., Modica M., Santagati A., Russo F., Amico-Roxas M., *Pharmazie*, **49**, 880—884 (1994).
26) Palaska E., Sahin G., Kelecin P., Durlu N. T., Altinok G., *Il Farmaco*, **57**, 101—107 (2002).
27) Matysiak J., Niewiadomy A., *Synth. Commun.*, **31**, 2537—2547 (2001).
28) Sxahin G., Palaska E., Ekizŏglu M., Zalp M. O., *Il Farmaco*, **57**, 539—542 (2002).
29) Sx. G. Kuc. u kgu zel., E. E. Oruc., Rollas S., Sxahin. F., Ozbek. A., *Eur. J. Med. Chem.*, **37**, 197—206 (2002).
30) Macaev F., Rusu G., Bogrebnoi S., Gudima A., Stingaci E., Vlad L., Shvets N., Kandemirli F., Dimoglo A., Reynolds R., *Bioorg. Med. Chem.*, **13**, 4842—4850 (2005).
31) Zitouni G. T., Kaplancikli Z. A., Yildiz. M. T., Chevallet P., Kaya D., *Eur. J. Med. Chem.*, **40**, 607—613 (2005).
32) Mamolo M. G., Zampieri D., Vio L., Fermeglia M., Ferrone M., Pricl S., Scialino G., Banfi E., *Bioorg. Med. Chem.*, **13**, 3797—3809 (2005).
33) Jakubkiene V., Burbuliene M. M., Mekuskiene G., Udrenaite E., Gaidelis G., Vainilavicius P., *Il Farmaco*, **58**, 323—328 (2003).
34) Narayana B., Vijaya Raj K. K., Ashalatha B. V., Kumari N. S., *Arch. Pharm.* (Weinheim), **338**, 373—377 (2005).
35) Amir M., Shikla K., *Il Farmaco*, **39**, 535—545 (2004).
36) Tandon V. K., Chhor R. B., *Synth. Commun.*, **31**, 1727—1732 (2001).
37) Diana G. D., Volkots D. L., Nitz T. J., Biailly T. R., Long M. A., Vesico N., Aldous A., Pevear D. C., Dukto F. J., *J. Med. Chem.*, **37**, 2421—2436 (1994).
38) Liras S., Allen M. P., Segelstein B. E., *Synth. Commun.*, **30**, 437—444 (2000).
39) Carlsen H. J., Jorgensen K. B., *J. Heterocycl. Chem.*, **31**, 805—807 (1994).
40) Tully W. R., Cardner C. R., Gillespie R. J., Westwood R., *J. Med. Chem.*, **34**, 2060—2067 (1991).
41) Al-Talib M., Tashtoush H., Odeh N., *Synth. Commun.*, **20**, 1811—1817 (1990).
42) Kerr N. V., Ott D. G., Hayes F. N., *J. Am. Chem. Soc.*, **82**, 186—189 (1960).
43) Theocharis A. B., Alexandrou N. E., *J. Heterocycl. Chem.*, **27**, 1685—1688 (1990).
44) Short F. W., Long L. M., *J. Heterocycl. Chem.*, **6**, 707—712 (1969).
45) Padmavathi V., Sudhakar Reddy G., Padmaja A., Kondaiah P., Ali-Shazia., *Eur. J. Med. Chem.*, **44**, 2106—2112 (2009).
46) Bhaskar Reddy D., Muralidhar Reddy M., Subbaraju G. V., *Indian J. Chem.*, **34B**, 816—822 (1995).
47) Vincent J. C., Vincent H. W., *Proc. Soc. Exp. Biol. Merd.*, **55**, 162—164 (1944).
48) Shirwaiker A., Rajendran K., Dinesh Kumar C., *Indian J. Exp. Biol.*, **42**, 803—807 (2004).
49) Babu B. H., Shailesh B. S., Paddikala J., *Fitotherapia*, **72**, 272—279 (2001).
50) Kato K., Terao S., Shimamoto N., Hirata M., *J. Med. Chem.*, **31**, 793—798 (1988).