Asian Journal of Chemistry; Vol. 23, No. 3 (2011), 1305-1308

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Synthesis and Evaluation of Some 2-[(Substituted) ethanoyl]amino-5-aryl-1,3,4-thiadiazoles as Diuretic Agents

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(*Received*: 10 May 2010; *Accepted*: 10 November 2010) AJC-9292

Twelve 2-[(substituted)ethanoyl]amino-5-aryl-1,3,4-thiadiazoles were prepared by the reaction of 2-(chloro ethanoyl)amino-5-aryl-1,3,4-thiadiazoles and various secondary amines. These compounds were screened for diuretic activity. The compounds namely $4a_1$, $4b_2$ and $4c_2$ showed good diuretic activity comparable with the standard drug acetazolamide at a dose level of 100 mg/kg, orally in albino rats.

Key Words: Synthesis, Substituted thiadiazoles, Diuretic agents.

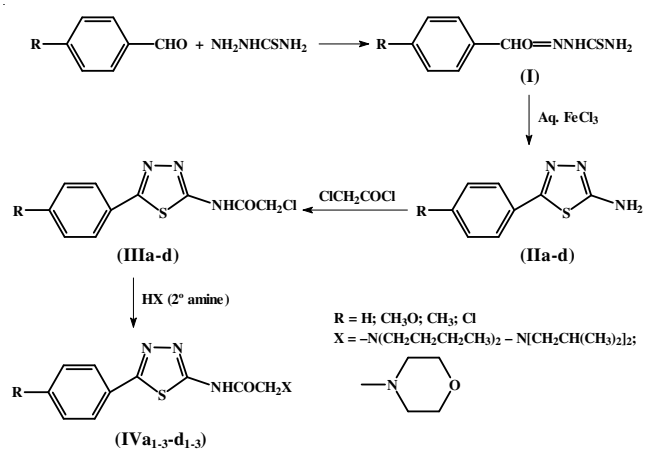
INTRODUCTION

1,3,4-Thiadiazole is a versatile pharmacophore, which exhibits a wide variety of biological activities. A few of them, are diuretic[1-5], CNS depressant[6,7], anticonvulsant[8,9], hypoglycaemic[10,11], antiinflammatory[12-14], antimicrobial activities[15-20] and anticancer[21,22]. In continuation of the earlier work on 1,3,4-thiadiazoles[23,24] of as diuretic agents, some 2-[(substituted) ethanoyl]amino-5-aryl-1,3,4-thiadiazoles were synthesized and evaluated for their diuretic activity.

2-[(Substituted)ethanoyl]amino-5-aryl-1,3,4-thiadiazoles synthesized according to **Scheme-I**. Aromatic aldehydes were reacted with thiosemicarbazide to give thiosemicarbazones (**I**a-d). Compounds **I** were oxidatively cyclized into 2-amino-5-aryl-1,3,4-thiadiazoles (**II**a-d) by aqueous ferric chloride. Compounds (**II**) were acetylated with chloroacetyl chloride to give compounds (**III**a-d). Finally reaction of 2-(chloro) ethanoyl amino-5-aryl-1,3,4-thiadiazoles (**III**) with various secondary amines gave 2-[(substituted) ethanoyl]amino-5-aryl-1,3,4-thiadiazoles (**IV**a_{1-3}, **IV**b_{1-3}, **IV**c_{1-3} and **IV**d_{1-3}). Physical data of the synthesized compounds are provided in Table-1. The compounds were characterized on the basis of elemental analysis and spectral studies. The IR, ^{13}C NMR and Mass spectral data of compounds are given in Table-2.

EXPERIMENTAL

The purity of the final compounds was ascertained by thin layer chromatography (TLC) using benzene-methanol-ammonia (75:25:0.25, v/v/v) as solvent system and iodine vapours as detecting agent. Melting points were determined by Toshniwal melting point determination apparatus and are uncorrected. For characterization of the synthesized compounds IR spectra were recorded on Shimadzu I R 470 double beam spectrophotometer in KBr phase. ^{13}C NMR spectra were recorded on 13C Avance Bruker 300 MHz spectrometer. Mass spectra were recorded on JEOL SX 102/DA-6000 mass spectrometer using fast atomic bombardment (FAB) technique. % Nitrogen analysis was also carried out.



Scheme-I

Synthesis of thiosemicarbazones[25,26] (I): Aromatic aldehydes (0.2 mol) in warm alcohol (300 mL) and a solution of thiosemicarbazide (0.2 mol, 18.2 g) in 300 mL of hot water, were mixed slowly with continuous stirring. The product,

which separated after cooling, was filtered off and crystallized from 50 % aqueous ethanol except I_d for which rectified spirit used. **I**a, R = H-, yield 92 %, m.p. 159-160 ºC (158.5-160 ºC lit.[25,26]), % N 23.66/23.46 (found/calculated); **I**b, R = CH_3O-, yield 95 %, m.p. 162-163 ºC (161-162 ºC lit.[25]), % N 21.58/21.74; **I**c, R = CH_3-, yield 94 %, m.p. 168-169 ºC (167-168.5 ºC lit.[25]), % N 20.35/20.09 and **I**d, R = Cl, yield 90 %, m.p. 207-209 ºC (208-209 ºC lit.[25]), % N 19.48/19.67.

Synthesis of 2-amino-5-aryl-1,3,4-thiadiazoles[23,25-27] (II): Thiosemicarbazone (**I**) (0.05 mol) was suspended in 300 mL distilled water in a 1 L beaker. Solution of ferric chloride (0.15 mol) in 300 mL distilled water was added to it. This was heated to 80-90 ºC and maintained for 45 min. Then the solution was filtered and cooled. A mixture of citric acid (0.11 mol) and sodium citrate (0.05 mol) dissolved in a minimum amount of distilled water was added to the filtered mixture and stirred. After cooling the whole solution was neutralized with aqueous ammonia (10 %). The precipitate so obtained was filtered and washed with distilled water and allowed to dry. The products (**II**a-d) were crystallized from 25 % aqueous ethanol except for **II**d (50 % ethanol). **II**a, R = H-, yield 65 %, m.p. 224-225 ºC (224 ºC lit.[26]), % N 23.88/23.72 (found/calculated); **II**b, R = CH_3O-, yield 62 %, m.p. 214-215 ºC (215-216 ºC lit.[25]), % N 22.15/21.98; **II**c, R = CH_3-, yield 57 %, m.p. 187-189 ºC (187-188 ºC lit.[27]), % N 20.50/20.28 and **II**d, R = Cl, yield 70 %, m.p. 227-229 ºC (229-230 ºC lit.[27]), % N 19.93/19.85.

Synthesis of 2-(chloro ethanoyl)amino-5-aryl-1,3,4-thiadiazoles (III): Compound **2** (0.05 mol) was taken in a two necked round bottom flask, containing 200 mL of dry benzene[28], fitted with a reflux condenser and a separating funnel. The separating funnel contained chloroacetyl chloride (0.05 mol) in dry benzene (50 mL). Chloroacetyl chloride was added dropwise with stirring. After the total addition, contents were refluxed for 3 h. The solvent was distilled off and the product dried under reduced pressure in a rotary vacuum evaporator. The dried product so obtained was taken out of the round bottom flask with the aid of distilled water and poured on crushed ice. The product was filtered off and washed several times with cold distilled water to free it from chloride. The crystallization was affected using aqueous dioxane (40-80 %). **III**a, R = H-, yield 95 %, m.p. 228-229 ºC, % N 24.15/23.73 (found/calculated); **III**b, R = CH_3O-, yield 94 %, m.p. 231-232 ºC, % N 20.60/20.29; **III**c, R = CH_3-, yield 92 %, m.p. 238-240 ºC, % N 21.56/21.99 and **III**d, R = Cl, yield 90 %, m.p. 243-244 ºC, % N 19.43/19.86.

Synthesis of 2-(substituted) ethanoyl amino-5-aryl-1,3,4-thiadiazoles (IV): Compound **III** (0.025 mol) and respective amine (0.03 mol) were taken in a round bottom flask containing dry benzene (100 mL) and dry pyridine[28] (0.025 mol). The contents were refluxed for 48 h on an electrically heated water bath. The solvent was distilled off and the product dried under reduced pressure in a rotary vacuum evaporator. The dried product so obtained was taken out of the flask with the aid of distilled water and poured on crushed ice. The product was filtered off and washed several times with cold distilled water to free it from chloride and crystallized using solvents given in Table-1.

Pharmacological activity

Determination of lethal dose (LD_{50}): In the present study the Horn's procedure[28] was followed. For the LD_{50} determination, albino mice (20-40 g) of either sex were used. The animals were divided into different groups of four animals each. To these groups, compounds were administered at 100, 316, 1000 and 3160 mg/kg of body weight orally and observations made for mortality upto 7 days. The lethal dose was then taken from the Horn's table. LD50 is reported in Table-3.

Diuretic activity[29,30]: For diuretic activity only male albino rats (100-200 g) were used (dose 100 mg/kg, orally). Diuretic activity was measured by collecting total excreted urine of rats kept in metabolic cage. The cages together with the funnel and the measuring cylinder used in the studies were coated with liquid paraffin before each experiment to facilitate the collection of urine. The primary test was conducted in conscious male albino rats. The rats were deprived of food for 18 h but tap water was allowed (*ad libitum*). The animals were grouped separately. Each group had four animals. Depending on the average body weight the animals of the groups received normal saline (5 mL/100 g) orally. Except the control group the animals of the other groups (T) received the compounds. The standard drug acetazolamide (45 mg/kg, orally) was given to a group. The animals were kept in the metabolic cage. The urine was collected quantitatively in a measuring cylinder (attached with metabolic cage) for a total period of 4 h (urine collected initially of 20 min was discarded). The effect of the compounds was compared with standard and the control group. The ratio (T/C, T/S) of urine volume of treated and control group was determined. The results are reported in Table-3.

RESULTS AND DISCUSSION

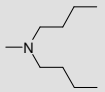
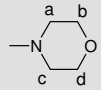
Chloroacetylation of 2-amino-5-aryl-1,3,4-thiadiazoles (**II**) followed by the reaction with various secondary amines gave 2-(substituted acetamido)-5-aryl-1,3,4-thiadiazoles (**IV**) (Table-1). The structural assignments of the products were done on the basis of their spectral data and the % nitrogen analysis (Tables-2).

For the LD_{50} determination, albino mice (20-40 g) of either sex were used. For diuretic activity only male albino rats (100-200 g) were used.

Compound **IV**c_1 was found to have LD_{50} 681 mg/kg body weight. Compounds **IV**a_1, **IV**b_1 and **IV**d_1 were found to have LD_{50} of 1000 mg/kg body weight. Rest of the compounds has LD_{50} > 3160 mg/kg body weight. Results indicate that the compounds showed diuretic activity. Compounds **IV**a_1 (85.2 %), **IV**b_2 (85.9 %) and **IV**c_2 (83.9 %) showed appreciable diuretic activity in comparison to the standard drug acetazolamide. Compounds **IV**a_2, **IV**a_3, **IV**b_1 and **IV**c_1 also showed moderate activity (Table-3).

The infrared spectra of the title compounds (**IV**) showed characteristic absorption band between 1700-1680 cm^{-1} due to C=O str. (amide I); between 1560-1540 cm^{-1} due to N-H def. (amide II); 1640-1620 cm^{-1} due to C=N str.; 760 and 680 cm^{-1} due to the presence of monosubstituted benzene ring (in compound **IV**a_{1-3}) and between 840-810 cm^{-1} due to *para* substituted benzene ring (in **IV**b_{1-3}, **IV**c_{1-3} and **IV**d_{1-3}). In ^{13}C NMR spectra of the synthesized compounds, C-2 and C-5 of 1,3,4-thiadiazole ring, were observed between 155-161 and 162-172 (δ, ppm), respectively. Carbonyl carbon and methylene carbon of -$NHCOCH_2X$ were observed between 163-171 and 56-61 ppm, respectively. Methoxy and methyl carbons were observed between 53-56 ppm and between 20-22 ppm for compounds **IV**b_{1-3} and **IV**c_{1-3}, respectively. In addition peaks

TABLE-1
CHARACTERIZATION DATA OF THE SYNTHESIZED COMPOUNDS (**IV**a_{1-3}-d_{1-3})

Compd. No.	R	X	m.f.	Yield (%)	Found (%)	m.p. (ºC)	Solvent*
IV$_{a1}$	H	 (Di-*n*-butyl amino)	$C_{18}H_{26}N_4OS$	63	16.79	134-5	A
IV$_{a2}$	H	 (Di-iso-butyl amino)	$C_{18}H_{26}N_4OS$	68	15.86	175-7	A
IV$_{a3}$	H	 (Morpholino)	$C_{14}H_{16}N_4O_2S$	62	17.20	179-181	B
IV$_{b1}$	CH_3O	Di-*n*-butyl amino	$C_{19}H_{28}N_4O_2S$	61	14.72	131-2	A
IV$_{b2}$	CH_3O	Di-iso-butyl amino	$C_{19}H_{28}N_4O_2S$	52	14.13	170-2	A
IV$_{b3}$	CH_3O	Morpholino	$C_{15}H_{18}N_4O_2S$	46	16.10	200-01	C
IV$_{c1}$	CH_3	Di-*n*-butyl amino	$C_{19}H_{28}N_4OS$	62	14.94	123-4	A
IV$_{c2}$	CH_3	Di-iso-butyl amino	$C_{19}H_{28}N_4OS$	56	14.74	144-6	A
IV$_{c3}$	CH_3	Morpholino	$C_{15}H_{18}N_4O_2S$	58	17.27	206-8	C
IV$_{d1}$	Cl	Di-*n*-butyl amino	$C_{18}H_{25}N_4OSCl$	58	14.81	112-3	A
IV$_{d2}$	Cl	Di-iso-butyl amino	$C_{18}H_{25}N_4OSCl$	53	14.72	152-4	A
IV$_{d3}$	Cl	Morpholino	$C_{14}H_{15}N_4O_2SCl$	56	15.28	205-6	D

*Solvent for crystallization: A = Chloroform-Petroleum ether; B = 95 % ethanol; C, absolute ethanol; D, absolute ethanol-ether.

TABLE-2
SPECTRAL DATA OF THE SYNTHESIZED COMPOUNDS*

Compd.†	λ_{max} in nm (DMF)	^{13}C NMR (ppm)	Mass (m/z)
IV$_{a1}$	307.0	13.6 ($\underline{C}H_3CH_2CH_2CH_2N<$), 19.9 ($CH_3\underline{C}H_2CH_2CH_2N<$), 29.3 ($CH_3\ CH_2CH_2\underline{C}H_2N<$), 54.4 ($CH_3CH_2CH_2\underline{C}H_2N<$), 164.5 (C-2), 157.7 (C-5), 127.2 (C-1"), 128.7 (C-2",6"), 126.2 (C-3",5"), 129 (C-4"), 166.3 (C-1'), 59.8 (C-2')	347 [quassi-molecular ion peak, $(M+H)^+$], 142 $(-CH_2X)^+$
IV$_{a2}$	310.5	21.38 [$(\underline{C}H_3)_2CHCH_2N<$), 26.58 [$(CH_3)_2\underline{C}HCH_2N<$), 64.51 [$(CH_3)_2CH\underline{C}H_2N<$), 163.4 (C-2), 157.7 (C-5), 127.4 (C-1"), 129.5 (C-2",6"), 126.9 (C-3",5"), 131 (C-4"), 167.6 (C-1'), 59.35 (C-2')	347 $(M+H)^+$], 142 $(-CH_2X)^+$
IV$_{a3}$	297.0	64.38 (C-a,d of A*), 51.51 (C-b,c of A*), 163.66 (C-2), 156.4 (C-5), 127.5 (C-1"), 128.1 (C-2",6"), 125.3 (C-3",5"),128.8 (C-4"), 166.34 (C-1'), 58.59 (C-2')	305 $(M+H)^+$, 100 $(-CH_2X)^+$
IV$_{b1}$	311.5	13.7 ($\underline{C}H_3CH_2CH_2CH_2N<$), 20.4 ($CH_3\underline{C}H_2CH_2CH_2N<$), 29.1 ($CH_3\ CH_2CH_2\underline{C}H_2N<$), 54.9 ($CH_3CH_2CH_2\underline{C}H_2N<$), 164.2 (C-2), 160.8 (C-5), 121.4 (C-1"), 128.1 (C-2",6"), 114.2 (C-3",5"), 158.3 (C-4"), 167.6 (C-1'), 60.05 (C-2')	377 $(M+H)^+$, 142 $(-CH_2X)^+$
IV$_{b2}$	318.5	21.7 [$(\underline{C}H_3)_2CHCH_2N<$), 30.8 [$(CH_3)_2\underline{C}HCH_2N<$), 65.8 [$(CH_3)_2CH\underline{C}H_2N<$), 163.5 (C-2), 155.9 (C-5), 122.3 (C-1"), 128.5 (C-2",6"), 112.9 (C-3",5"), 159.5 (C-4"), 168.2 (C-1'), 57.5 (C-2')	377 $(M+H)^+$, 142 $(-CH_2X)^+$
IV$_{b3}$	308.5	51.7 (C-a,d of A*), 64.7 (C-b,c of A*), 161.0 (C-2), 155.8 (C-5), 121.2 (C-1"), 126.8 (C-2",6"), 112.8 (C-3",5"), 159.6 (C-4"), 166.6 (C-1'), 59.1(C-2')	335 $(M+H)^+$, 100 $(-CH_2X)^+$
IV$_{c1}$	302.5	13.7 ($\underline{C}H_3CH_2CH_2CH_2N<$), 20.1 ($CH_3\underline{C}H_2CH_2CH_2N<$), 28.1 ($CH_3\ CH_2CH_2\underline{C}H_2N<$), 54.8 ($CH_3CH_2CH_2\underline{C}H_2N<$), 163.1 (C-2), 157.4 (C-5), 127.6 (C-1"), 129.3 (C-2",6"), 126.2 (C-3",5"), 139.3 (C-4"), 167.3 (C-1'), 59.8 (C-2')	361 $(M+H)^+$, 142 $(-CH_2X)^+$
IV$_{c2}$	305.5	21.0 [$(\underline{C}H_3)_2CHCH_2N<$), 29.7 [$(CH_3)_2\underline{C}HCH_2N<$), 64.2 [$(CH_3)_2CH\underline{C}H_2N<$), 164 (C-2), 156.9 (C-5), 127.3 (C-1"), 129.8 (C-2",6"), 126.4 (C-3",5"), 141 (C-4"), 169.8 (C-1'), 59.2 (C-2')	361 $(M+H)^+$, 142 $(-CH_2X)^+$
IV$_{c3}$	297.5	53.4 (C-a,d of A*), 66.4 (C-b,c of A*), 163.5 (C-2), 157.4 (C-5), 127.3 (C-1"), 129.5 (C-2",6"), 126.8 (C-3",5"), 140.7 (C-4"), 168.1 (C-1'), 61.0 (C-2')	320 $(M+H)^+$, 100 $(-CH_2X)^+$
IV$_{d1}$	303.5	13.0 ($\underline{C}H_3CH_2CH_2CH_2N<$), 19.6 ($CH_3\underline{C}H_2CH_2CH_2N<$), 28.3 ($CH_3\ CH_2CH_2\underline{C}H_2N<$), 54.5 ($CH_3CH_2CH_2\underline{C}H_2N<$), 161.6 (C-2), 156.7 (C-5), 127.5 (C-1"), 128.4 (C-2",6"), 128.0 (C-3",5"), 135.7 (C-4"), 169.5 (C-1'), 56.9 (C-2')	381 $(M+H)^+$, 142 $(-CH_2X)^+$
IV$_{d2}$	306.0	21.0 [$(\underline{C}H_3)_2CHCH_2N<$), 29.3 [$(CH_3)_2\underline{C}HCH_2N<$), 64.3 [$(CH_3)_2CH\underline{C}H_2N<$), 164.8 (C-2), 157.4 (C-5), 127.8 (C-1"), 129.4 (C-2",6"), 128.5 (C-3",5"), 136.6 (C-4"), 170.0 (C-1'), 59.2 (C-2')	381 $(M+H)^+$, 142 $(-CH_2X)^+$
IV$_{d3}$	303.5	53.8 (C-a,d of A*), 66.6 (C-b,c of A*), 162.6 (C-2), 157.9 (C-5), 128.4 (C-1"), 129.4 (C-2",6"), 128.7 (C-3",5"), 136.7 (C-4"), 168.3 (C-1'), 61.3 (C-2')	339 $(M+H)^+$, 100 $(-CH_2X)^+$

†IR: 1700-1680 ν(C=O) cm^{-1}; 1560-1540 ν(N-H); 1640-1620 ν(C=N); 760 & 680 due to mono-substituted benzene ring (**IV**$_{a1-4}$) and 840-810 due to disubstituted benzene ring (**IV**$_{b1-4}$, **IV**$_{c1-4}$ and **IV**$_{d1-4}$).

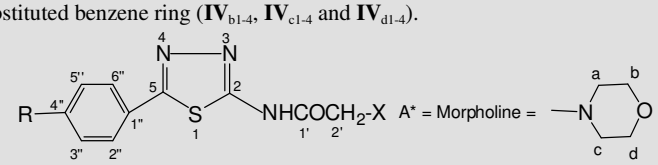
* =  A* = Morpholine =

TABLE-3
BIOLOGICAL DATA OF THE SYNTHESIZED COMPOUNDS

Compd.	Lethal dose*		Diuretic activity			
	Estimated LD_{50} (mg/kg)	Confidence limit (mg/kg)	% Output (%)	T/C	T/S	Diuretic activity**
$\mathbf{IV}_{a1}$	1000	246-4060	53.40	1.470	0.85	85.20
$\mathbf{IV}_{a2}$	>3160	–	49.80	1.360	0.80	79.40
$\mathbf{IV}_{a3}$	>3160	–	47.55	1.310	0.76	75.80
$\mathbf{IV}_{b1}$	1000	338-2960	50.48	1.390	0.80	80.46
$\mathbf{IV}_{b2}$	>3160	–	53.89	1.480	0.86	85.90
$\mathbf{IV}_{b3}$	>3160	–	44.21	1.220	0.70	70.47
$\mathbf{IV}_{c1}$	681	205-2260	47.62	1.310	0.76	75.90
$\mathbf{IV}_{c2}$	>3160	–	52.64	1.450	0.84	83.90
$\mathbf{IV}_{c3}$	>3160	–	42.16	1.160	0.67	67.20
$\mathbf{IV}_{d1}$	1000	246-4060	28.70	0.790	0.46	45.70
$\mathbf{IV}_{d2}$	>3160	–	35.10	0.960	0.56	56.00
$\mathbf{IV}_{d3}$	>3160	–	26.70	0.730	0.43	42.60
Control (C)	–	–	36.36	1.000	–	–
ACZ# (S)	–	–	62.74	1.725	1.00	100.00

*Total number of animals in each group = 4; **(T/S) × 100; #Acetazolamide (standard drug).

at δ 77.0 ppm for $CDCl_3$ (solvent) and at δ 39.0 ppm for DMSO-d_6 (solvent) were also observed in respective cases. In the mass spectra (FAB) two prominent peaks were observed. One due to quasi-molecular or adduct ion peak $(M+H)^+$ and second due to -CH_2X (Fig. 1) fragment ion (A). Peaks at m/z 136, 137, 154, 289 and 307 appeared due to the matrix used in this technique, *i.e.*, *m*-nitrobenzyl alcohol (NBA). All the spectral data and the % nitrogen analysis were in agreement with the structures of the compounds synthesized (Table-2).

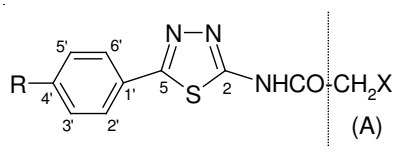


Fig. 1. General structure of 1,3,4-thiadiazoles

Results indicates that compounds with R = H, CH_3O-, CH_3- and X = di-*n*-butyl/di-iso-butyl amino substituents showed good diuretic activity, comparable with the standard drug acetazolamide. Although the activity was slightly lower than the standard drug. As compared to di-*n*-butyl amino derivatives, di-iso-butyl amino derivatives are more active except $\mathbf{IV}a_1$. Compounds with X = morpholine substituent showed lesser activity. Compounds with R = Cl^- ($\mathbf{IV}d_{1\text{-}3}$) showed reduced diuretic activity as compared to the other compounds in which R = H, CH_3O- and CH_3- ($\mathbf{IV}a_{1\text{-}3}$, $\mathbf{IV}b_{1\text{-}3}$ and $\mathbf{IV}c_{1\text{-}3}$).

ACKNOWLEDGEMENTS

The authors are thankful to Head, Department of Pharmaceutical Sciences, Dr. H.S. Gour Vishwavidyalaya, Sagar for providing necessary facilities. Thanks are also due to Head, RSIC, Lucknow for mass spectral analysis and to the Director, NIPER, Mohali (Punjab) for elemental and NMR spectral studies. One of the authors, SKJ is grateful to UGC, New Delhi (India) for award of research fellowship during Ph.D.

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