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Synthesis and Biological Evaluation of Some Mannich Bases of Benzothiazolyl Oxadiazoles

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A new series of 2-(5'-mercapto-4'-substituted methylamino-1',3',4'-oxadiazole-2'-yl)benzothiazoles (**4**) were synthesized and evaluated for their antimicrobial and pharmacological activity. 2-(5'-Mercapto-4'-substituted methylamino-1',3',4'-oxadiazole-2'-yl) benzothiazoles (**4**) were synthesized from 5'-(1,3-benzothiazole-2-yl)-1',3',4'-oxadiazole-2-(3*H*)-thione (**3**) upon treating with formaldehyde and substituted amines by fallowing Mannich reaction condition. 5'-(1,3-Benzothiazole-2-yl)-1',3',4'-oxadiazole-2'-(3*H*)-thione (**3**) was prepared in single step from 1,3-benzothiazole-2-carboxyhydrazide (**2**) upon reaction with carbon disulphide in the presence of potassium hydroxide in ethanolic medium. 2-(5'-Mercapto-4'-substituted methylamino-1',3',4'-oxadiazole-2'-yl)-benzothiazoles (**4a$_1$-a$_{10}$**) were screened for their antibacterial, antiinflammatory and analgesic activity. The synthesized compounds exhibited good analgesic activity compared to standard drug.

Key Words: Synthesis, Benzothiazoles, Antibacterial, Antiinflammatory and Analgesic activity.

INTRODUCTION

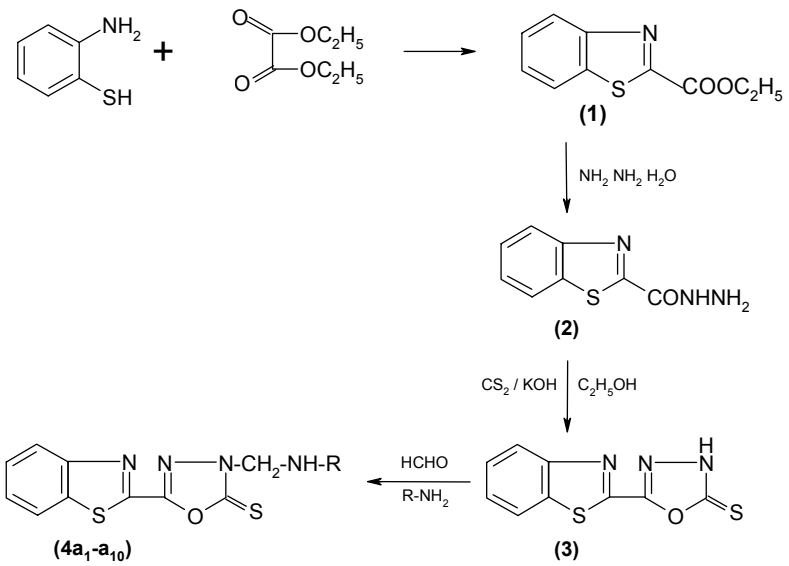
A number of Mannich bases have been associated with potent biological activities such as antitumor[1], antitubercular[2], antimicrobial[3-5], antimalarial[6], antifungal[7] *etc*., like wise 1,3,4-oxadiazoles are also known to possess antibacterial[8], antifungal[9], antiinflammatory and analgesic activity[10,11]. Similarly a number of benzothiazole derivatives are known for their varied biological activities like anti-bacterial[12-14], antiinflammatory, analgesic, antitumor activities[15-18], *etc.*, prompted by these observations it was targeted to synthesize a series of benzothiazole derivatives by incorporating benzothiazole nucleus with 1,3,4-oxadiazole and converting them to Mannich bases and screen the newly synthesized compounds for their antibacterial, antiinflammatory and analgesic activity.

EXPERIMENTAL

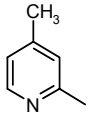
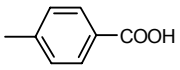
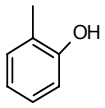
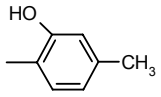
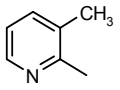
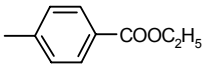
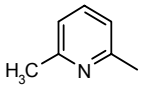
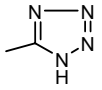
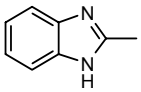
All the melting points were determined by Toshniwal apparatus in open capillaries and are uncorrected. The purity of the compounds was determined by using TLC on silica gel G plates using *n*-butanol:ethyl acetate (1:3) solvent system and UV lamp was used as a visualizing agent.

Infrared spectra were recorded using KBr pellets on a Shimadzu 8000 series spectrophotometer. The ^{1}H NMR spectra were recorded on a Varian EM-200, avance 300 MHz spectrophotometer using $CDCl_3$/DMSO-d_6 as solvent and TMS as internal standard (chemical shifts are expressed in δ ppm). Mass Spectra were recorded on LCMS Spectrophotometer.

All the compounds were synthesized according to **Scheme-I**. The physical data of all synthesized compounds **4a$_1$-4a$_{10}$** were represented in Table-1.

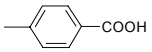
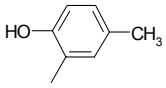
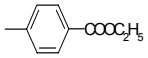
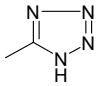
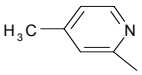
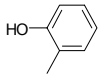
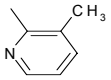
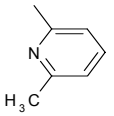
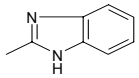


R =

4a$_1$		**4a$_6$**	
4a$_2$		**4a$_7$**	
4a$_3$		**4a$_8$**	
4a$_4$		**4a$_9$**	
4a$_5$		**4a$_{10}$**	

Scheme-I

TABLE-1
PHYSICAL DATA OF COMPOUNDS (**4a$_1$-a$_{10}$**)

Product code	R	m.f.	m.p. (ºC)	m.w.	Yield (%)
4a$_1$		$C_{15}H_{11}N_5OS_2$	216-220	341	47
4a$_2$		$C_{17}H_{12}N_4O_3S_2$	228-232	384	51
4a$_3$		$C_{17}H_{14}N_4O_2S_2$	188-190	370	75
4a$_4$		$C_{19}H_{16}N_4O_3S_2$	168-170	412	46
4a$_5$		$C_{11}H_8N_8OS_2$	226-230	332	45
4a$_6$		$C_{16}H_{13}N_5OS_2$	174-178	355	48
4a$_7$		$C_{16}H_{12}N_4O_2S_2$	186-190	356	76
4a$_8$		$C_{16}H_{13}N_5OS_2$	134-138	355	45
4a$_9$		$C_{16}H_{13}N_5OS_2$	180-184	355	51
4a$_{10}$		$C_{17}H_{12}N_6OS_2$	232-236	380	59

Preparation of ethyl-2-benzothiazole carboxylate (1): Ethyl-2-benzothiazole carboxylate was synthesized by treating a mixture of *o*-aminothiophenol (0.1 mol) and diethyl oxalate (0.2 mol) according to the reported procedure[19]. ^{1}H NMR (DMSO-d_6, δ ppm): 1.4 to 1.6 (3H, t, CH_3 of -$COOCH_2CH_3$), 4.5 to 4.7 (2H, q, CH_2 of -$COOCH_2CH_3$), 7.5 to 8.3 (4H, m, Ar-H).

Preparation of 1,3-benzothiazole-2-carboxyhydrazide (2): In a clean dry 100 mL round bottomed flask the ethyl-1,3-benzothiazole-2-carboxylate (**1**) (0.01 mol) (2.1 g) was dissolved in ethanol (60 mL). The hydrazine hydrate (0.02 mol) (1.1 g) (99 %) was added drop by drop with constant stirring and refluxed for 8 h and cooled to room temperature. The solid separated was filtered and washed with water and dried. The crude product was melted at 175-177 ºC and was recrystallized from ethanol. ^{1}H NMR (DMSO-d_6, δ ppm): 4.6 to 4.8 (2H, s, NH_2 of -$CONH.NH_2$), 7.5 to 8.3 (4H, m, Ar-H), 10.4 to10.6 (1H, s, NH of -$CONH.NH_2$).

Preparation of 5'-(1,3-benzothiazol-2-yl)-1',3',4'-oxadiazole-2(3*H*)-thione (3): In to a clean dry 250 mL round bottomed flask introduced 1,3-benzothiazole-2-carboxyhydrazide (**2**) (0.05 mol) (9.65 g) and dissolved in ethanol (100 mL) with gentle heating there after KOH solution (10 %, 10 mL) was added followed by carbon disulphide (10 mL) and the mixture was subjected to reflux till hydrogen sulphide gas was ceased (16-18 h). The reaction mixture was cooled to room temperature, poured on to a mixture of ice and water (500 mL) and acidified with chilled dil HCl. The separated solid was collected by filtration and dried. The crude product was recrystallized from ethanol and melting point was 244-248 ºC. ^{1}H NMR (DMSO-d_6, δ ppm): 3.0 to 3.8 (1H, bs, -NH of oxadiazole), 7.5 to 8.4 (4H, m, Ar-H).

Preparation of 5'-(1,3-benzothiazol-2-yl)-3'-substituted methylamino-1',3',4'-oxadiazole-2(3*H*)-thione ($4a_1$-a_{10}): A mixture of oxadiazole (**3**) (0.01 mol), formaldehyde (40 %, 1.5 mL), substituted amine (0.01 mol) and ethanol (20 mL) was taken in a 100 mL round bottomed flask and stirred for 10-15 h at room temperature and left over night. The separated solid was collected by filtration, washed with ethanol, dried and crystallized from suitable solvent. The physical characteristic data of synthesized compounds are presented in Table-1.

Compound $4a_1$: IR (KBr, cm^{-1}): 3244, -NH stretching, 3001 and 3053, Ar-CH and -CH_2 Stretching. ^{1}H NMR (DMSO-d_6, δ ppm): 5.8 (2H, s, 2H of CH_2), 6.1 (1H, s, H of NH), 7.3 to 8.3 (8H, m, Ar-H). MS (LCMS) m/e: 341.

Compound $4a_6$: ^{1}H NMR (DMSO-d_6, δ ppm): 2.2 (3H, s, 3H of CH_3), 5.6-5.7 (2H, t, 2H of CH_2), 7.0-8.3 (7H, m, Ar-H), 8.8-8.9 (1H, s, 1H of NH).

Compound $4a_7$: IR (KBr, cm^{-1}): 3379 (NH, stretching), 3052, 2990, 2917 (Ar-CH and CH_2 stretching), 849 and 770 (disubstituted benzene). ^{1}H NMR (DMSO-d_6, δ ppm): 6.1-6.2 (2H, t, 2H of CH_2), 6.9-8.3 (8H, m, Ar-H), 10.0 (1H, s, 1H of OH), 12.3 (1H, s, 1H of NH).

Antibacterial activity: All the synthesized compounds (**$4a_1$-a_{10}**) were evaluated for *in vitro* antibacterial activity against Gram+ve and Gram-ve bacterial strains such as *Bacillus subtilis*, *Bacillus pumilus*, *Escherichia coli* and *Pseudomonas aureginosa* at concentration 100 µg/mL by disc diffusion method 20 by using DMSO as solvent control and nutrient agar was employed as culture media[20]. After 24 h of incubation at 37 ºC the zone of inhibition were measured in mm. The activity was compared with known antibiotic ciprofloxacin and the data is represented in the Table-2.

Antiinflammatory activity: The synthesized compounds (**$4a_1$-a_{10}**) were assessed for antiinflammatory activity at a concentration of 200 mg/kg oral dose by acute carrageenan-induced oedema in rat hind paw by following the reported technique[21,22]. The activity were performed on albino rats of either sex weighing 150-200 g were selected in group of 6 each. The oedema volume of injected paws were measured plethysmographically by mercury displacement method. The activity was compared with standard diclofenac sodium and the data is represented in the Table-3.

TABLE-2
ANTIBACTERIAL ACTIVITY OF THE SYNTHESIZED COMPOUNDS (**4a$_1$-a$_{10}$**)

Sample code	*Inhibition of zone diameter (mm)			
	B. subtilis	*B. pumillis*	*E. coli*	*P. aeruginosa*
	100 µg	100 µg	100 µg	100 µg
4a$_1$	14	18	14	19
4a$_2$	16	13	16	15
4a$_3$	14	14	15	15
4a$_4$	15	18	15	20
4a$_5$	16	16	15	16
4a$_6$	15	15	16	16
4a$_7$	17	16	16	21
4a$_8$	14	15	16	17
4a$_9$	14	17	16	17
4a$_{10}$	15	16	16	15
Ciprofloxacin	22	24	24	24
DMSO	-	-	-	-

*Each value is an average of three independent determination ± Standard deviation;
Note: '–' denotes no activity, 8-12 mm poor activity, 13-17 mm moderate activity,
18-20 and above good activity.

TABLE-3
ANTIINFLAMMATORY ACTIVITY OF THE SYNTHESIZED COMPOUNDS (**4a$_1$-a$_{10}$**)

Treatment	Dose (mg/kg)	Paw oedema volume							
		After 1st h		After 2nd h		After 3rd h		After 4th h	
		Mean	%ROV	Mean	%ROV	Mean	%ROV	Mean	%ROV
Control	1.5 ml	1.21	-	2.49	-	3.10	-	4.39	-
Standard	50	0.168	57.25	0.166	67.75	0.186	73.93	0.050	90.90
4a$_1$	200	0.61	49.58	1.08	56.62	1.08	65.16	1.1	74.94
4a$_2$	200	0.71	41.32	1.1	55.82	1.05	66.12	1.03	76.53
4a$_3$	200	0.75	38.01	1.06	57.42	0.96	69.03	0.93	78.81
4a$_4$	200	0.66	45.45	0.95	61.84	0.85	72.58	0.80	81.77
4a$_5$	200	0.65	46.28	1.05	57.83	1.01	67.41	0.93	78.81
4a$_6$	200	0.70	42.14	1.03	58.63	0.98	68.38	0.95	78.35
4a$_7$	200	0.76	37.19	0.98	60.64	1.14	63.22	1.01	76.99
4a$_8$	200	0.66	45.45	1.05	57.83	0.88	71.61	0.81	81.54
4a$_9$	200	0.76	37.19	1.16	53.41	1.06	65.80	1.05	76.08
4a$_{10}$	200	0.90	25.61	0.98	60.64	0.98	68.38	0.93	78.82

Standard: Diclofenac sodium; ROV = Reduction in paw oedema volume.

Analgesic activity: The analgesic activity of the synthesized compounds (**4a$_1$-a$_{10}$**) was carried out by Eddy's Hot Plate method[23] on albino mice of either sex each group comprising of 6 animals, weighing between 20-36 g. Pentazocin was used as standard drug to compare the analgesic activity and the data is represented in Table-4.

TABLE-4
ANALGESIC ACTIVITY OF THE SYNTHESIZED COMPOUNDS (**4a$_1$-a$_{10}$**)

Treatment	No. of animals	Average wt. of animals (g)	Average dose (mg/mL)	Basal reaction time (min) after				
				0 min	15 min	30 min	60 min	90 min
Control gum acacia	6	34.00	-	5.00±0.70	4.00±0.81	5.75±0.96	5.25±0.47	7.0±0.40
Pentazocin	6	28.00	30	7.50±0.94	10.50±0.64	11.75±0.47	12.2±0.25	12.50±0.28
4a$_1$	6	26.00	25	6.50±1.16	7.95±0.67	9.16±0.37	10.21±1.10	11.54±1.00
4a$_2$	6	36.00	20	5.36±1.05	7.57±0.89	8.49±0.35	9.14±0.27	10.29±1.39
4a$_3$	6	30.67	20	5.49±0.78	7.84±0.59	9.86±0.27	10.85±0.35	11.69±0.52
4a$_4$	6	26.67	15	4.66±0.93	6.46±1.02	9.46±1.08	9.98±1.31	11.18±0.85
4a$_5$	6	29.33	15	4.90±0.17	7.10±0.76	7.78±0.98	8.14±0.83	9.42±0.75
4a$_6$	6	26.67	15	6.92±0.73	7.68±0.89	8.06±0.76	10.64±1.03	11.25±0.84
4a$_7$	6	25.38	20	5.09±1.01	7.08±0.75	9.02±0.41	10.05±0.30	11.20±1.37
4a$_8$	6	29.00	15	4.56±0.91	7.10±1.00	9.59±1.07	10.01±1.40	11.01±0.79
4a$_9$	6	28.05	20	4.81±0.19	6.91±0.78	7.89±0.99	9.01±0.84	10.25±0.82
4a$_{10}$	6	30.15	25	6.39±1.11	8.01±0.72	9.26±0.38	10.41±1.21	12.01±1.20

RESULTS AND DISCUSSION

All the synthesized compounds were screened for antibacterial activity. The data in the Table-2 indicate that **4a$_1$**, **4a$_4$** and **4a$_7$** compounds were found to posses a broad-spectrum activity. While compounds **4a$_2$**, **4a$_3$**, **4a$_5$**, **4a$_6$**, **4a$_8$**, **4a$_9$** and **4a$_{10}$** were found to exhibit moderate activities. Among the synthesized compounds, the compounds **4a$_1$**, **4a$_4$** and **4a$_7$** showed good activity.

All the synthesized compounds were screened for antiinflammatory activity. Compounds **4a$_1$**, **4a$_2$**, **4a$_3$**, **4a$_4$**, **4a$_5$**, **4a$_6$**, **4a$_7$**, **4a$_8$**, **4a$_9$** and **4a$_{10}$** exhibited maximum inhibition of 74.94, 76.53, 78.81, 81.77, 78.81, 78.35, 76.99, 81.54, 76.08 and 78.82 %, respectively where as standard diclofenac sodium showed reduction in oedema volume by 90.90 % in Carrageenan induced rat hind paw oedema model. Among the tested compounds, the compounds **4a$_4$** and **4a$_8$** showed good antiinflammatory activity.

The synthesized compounds have shown a significant analgesic activity. The compound **4a$_1$**, **4a$_2$**, **4a$_3$**, **4a$_4$**, **4a$_5$**, **4a$_6$**, **4a$_7$**, **4a$_8$**, **4a$_9$** and **4a$_{10}$** have shown equipotent analgesic activity when compared to standard (pentazocin).

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