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Research Article

SYNTHESIS CHARACTERIZATION AND PHARMACOLOGICAL ACTIVITY OF NOVEL THIADIAZOLE ANALOGUES

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ABSTRACT
A new series of Thiadiazole showed diversified pharmacological activities. In view of potential biological activities of Thiadiazole, it was considered worth will to synthesized2-Benzylidenylamino-5-(N—bezotriozolomethyl)-1,3,4-thiadiazoleand evaluate for their possible pharmacological activity.
KEYWORD: Thiadiazole, Analgesic activity, Anticonvulsant activity, Antibacterial activity and Anti tubercular activity.

INTRODUCTION

1,2,3-Benzotriazoles were reported to have potential antimicrobial[1] and antiparacitic activity[2]. Similarly1,3,4-Thiadiazole derivatives were also reported to possess fungicidal herbicidal, bactericidal[3], pestcidal, insecticidal, antihistamine and antiamoebic[4], CNS stimulant anticholinergic, hypoglycemic[5], cardiotonic, antihypertensive[7], anticonvulsant,hypnotic[8], antihelmintic, anti-inflammatory and antioxidant activity[9],potassium channel inhibitor, cardiovascular [10], local anesthetic, sedative, cholerectic, anti-tubercular[11], antiviral,anti-tumor, CNSdepressant[12], antihypertensive,hypoglycemic[13], antiallergic[14], hypertensive, antihelmenthic, antiinflamatory, antiparkinsonian[15], hypnotic[16], antithyroidal, fungicidal[17], cysticidal, antileukem-ic[18] and anti-oxidant activities of Thiadiazole. And synthesized (B1-B6) to probe how far these compounds could enhance the analgesic, anti-convulsant and anti-bacterial activity, anti-tubercular and anticancer activity.

MATERIAL AND METHODS

Melting points were determined by open capillary method and are uncorrected. IR (KBr) spectra were recorded on thermo Nicolet IR-200 spectrophotometer. The ^{1}H-NMR spectra were recorded on multi nuclear FT-NMR spectrophotometer model-II (Bruker), DMSO-d6 solvent. The instrument is equipped with a cryomagnet of field strength9.4 T. Its ^{1}H frequency is 400 MHZ. The purity of compounds was determined on pre-coated TLC using suitable solvent system 2-Benzylidenylamino-5-(N-Benztriazolomethyl)-1,3,4- Thiadiazoles were prepared as the described in the four steps as stated below.

STEP-I The synthesis of N-Benztriazolacetate

The (0.1M) Benztrizole, ethyl acetate (0.1M) and 0.3g of k_2co_3 in 60 Ml of acetone were stirred for 10 hrs. The solvent was removed under reduced pressure. A solid mass was produced and then needle shaped brown crystals were obtained after recrystallization from the mixture of chloroform and ether (8.2% v/v).The yield obtained was 60% and M.P was 40^{oC}.

STEP-II The synthesis of N-Benztrizole acetyl thiosemicarbazide

The crystals obtained from step-I (0.08M) and thiosemicarbazide (0.08M) were taken in 50 mL of ethanol, stirred for 6 hrs and then refluxed for 3 hrs. The yellow colored compound was obtained after recrystallization from the mixture of chloroform and hexane (9:1 v/v). The yield was 57% and M.P 102-103^{oC}.

STEP-III The synthesis of 2-amino 5-(N-Benztriazolomethyl)-1,3,4-Thiadiazole

Compound-ii (0.08M) was added in con.H_2SO_4 and kept overnight at room temperature, then neutralized with ammonia and extracted with ether. The other was distilled off and the product recrystallized from methanol, the yield was 52% and M.P 121^{oC}.

STEP-IV Synthesis of 2-Benzylidenylamino-5-(N-Benztriazolomethyl)-1,3,4- Thiadiazole

(0.2M) compound iii,(0.2M) carbonyl compound (R_1R_2 C=O) and (2mL) glacial acetic acid were refluxed in 50mL methanol for 8 hrs. Solvent was distilled off and product recrystallized from mixture of benzene and chloroform (1:6 v/v), M.P 129^{oC} and yield was 50%.The purity of the synthesized compounds was established by TLC using 2% silica gel-G, n-butanol: glacial acetic acid: water (4:1:5) as eluents. Iodine chamber and U.V light used for detecting the compounds. M.P of compounds (B1 to B6) was 138^{oC}, 130^{oC},163^{oC}, 156^{oC}, 122^{oC} and 126^{oC}respectively. IR and NMR spectra, the physical data is given in Table no.1

Acute toxicity studies[20]

The acute toxicity of synthesized compound was determined by using albino mice of either sex (20-30gm) maintained under standard husbandry conditions. The animals were fasted overnight prior to the experiment and fixed dose (OECD) guideline no.425 method of (CPCSEA) was adopted for toxicity studies. Effective dose (ED_{50} –therapeutic dose) is taken as 1/10th of lethal dose.

Analgesic activity[21]

The analgesic activity of all the synthesized compounds were carried out by Eddy's hot plate method using albino mice's of either sex(20-30gm),animals were deprived of food for 18 hours. Prior to experiment [the first group was control group and received standard pentazocine drug (10mg/kg IP) and the remaining groups reward the compounds under investigation at 200 mg/kg P.O]. All the synthesized compounds (200mg/kg P.O) concentration were subjected to the determination of analgesic activity and compared the basal reaction time in seconds at different time intervals against the drug pentazocine (10mg/kg) and gum acacia 2% was used as control. The results are shown in table no.3.

Anti-convulsant activity[22]

The anti-convulsant activity of all the synthesized compounds were carried out by MES induced convulsions method using albino mice's of either sex (20-30gm) animals were deprived of food for 18hours. Prior to experiment [the first group was control group and received standard phenytoin drug (10mg/kg IP) and the remaining groups reward the compounds under investigation at 200mg/kg P.O]

A 60 mA current was delivered transauricularly for 0.2seconds in mice via small alligator clips attached to cornea for recording various parameters. Mice were placed in a rectangular plastic cage with an open top, permitting full view of animal's motor response to seizure in the pilot study of various phases of convulsions. The following parameters were recorded during 30min test session. Tonic flexion, tonic extension, clonus convulsions, percent protection. The values were expressed as mean ± SEM from 6 animals.The results were subjected to statistical analysis by using ANOVA followed by Tukell-kramer test to calculate the significance difference if any among the groups $P<0.05$ was considered as significant. The results are shown in table No.4.

Anti-bacterial activity[23]

Anti-bacterial activity of all synthesized compound wasdetermined by the disc diffusion method against the gram +ve organism.

Bacillus subtills and Bacillus pumilis and gram –ve organisms E-coli, pseudomonas aeruginosa at 100mg/mL concentration. The bacteria's were sub cultured in nutrient agar medium. The petri dishes were incubated at 37^0C for 24hours. Standard antibacterial drug ampicillin at 100mg/ml concentration was also increased under similar conditions. The results are shown in table No.5

Antitubercular Activity[24]

The Antitubercular screening was medium (L.J medium) as described by Barry A.L against H_{37} Rv strain. L.J medium was inoculated with mycobacterium tuberculosis of H_{37} Rv strain. The inoculated medium was incubated for 37^{Oc} for 6 weeks. At the end of 6 weeks, the growth of mycobacterium tuberculosis was read. The results are shown in table No.6

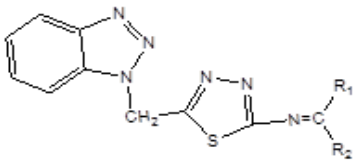
RESULTS AND DISCUSSION

Analgesic activity

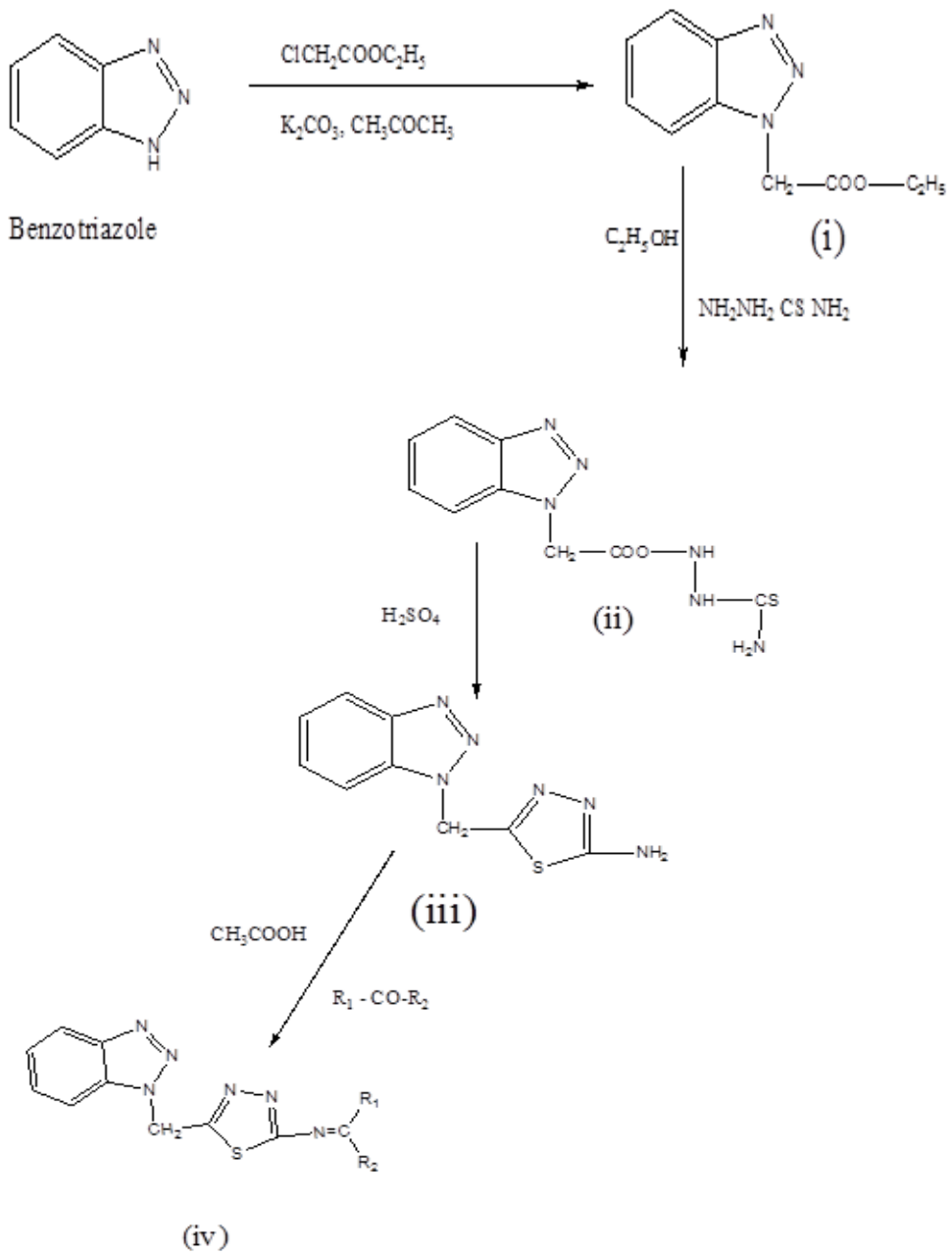
The synthesized thiadiazole derivatives B1, B2, B3 and B4, were selected for the screening of analgesic activity at the dose of 2mg/kg using Eddy's hot plate method in albino mice. The results are shown in table no. III. The basal reaction time of compounds B1, B2, B3 and B4are 14.15,13.39,13.16 and 11.72 seconds respectively at 60 minutes, where the standard drug pentazocine (10mg/kg) used was 13.65seconds at 60mins.

From the above data it is cleared that former 6 compounds are having equi potent analgesic activity and later two compounds are having moderate to less analgesic activity when compared to that of standard drug pentazocine. From the analgesic activity evaluation data it is cleared that compounds B1,B2andB3 were shown very good analgesic activity whereas the compound B4 was shown less analgesic activity when compared to that of standard as well as other Thiadiazole derivatives.

Table 1 Physical characterization data of compounds (B1-B6)



S.no	Compounds	R1	R2	M.P	Mol.Formula	Mol.Wieght
1	B1	H	- C_6H_5	138^{oC}	$C_{17}H_{12}N_6S$	322
2	B2	C_6H_5	-4-Br-C_6H_4	130^{oC}	$C_{23}H_{15}N_6SBr$	466
3	B3	H	-4-Cl-C_6H_4	163^{oC}	$C_{15}H_{11}N_6SCl$	342
4	B4	CH_3	- C_6H_5	156^{oC}	$C_{18}H_{14}N_6S$	346
5	B5	CH_3	- C_2H_5	122^{oC}	$C_{14}H_{14}N_6S$	298
6	B6	C_6H_5	- C_6H_5	126^{oC}	$C_{23}H_{16}N_6S$	408



SCHEME

Anti-Convulsant activity

The Thiadiazole derivatives were selected and screened for the anticonvulsant activity at the dose of 200 mg/kg using MES induced model in albino mice. The anticonvulsant activity data is given in the table No4. The anticonvulsant activity result of the synthesized Thiadiazolewas studied by considering two main parameters,

1) Lesser duration of tonic extensor phase
2) Maximum protection of animals after 24 hrs.

The compounds B1, B2 and B3 were exhibited significant anticonvulsant activity against electrically induced convulsions. The protected animal's upto 60.66% and the tonic extensor phase of these agents is 5,93,9.19 and 9.65 seconds respectively. Whereas rest of the compounds thought they showed good decrease in the tonic extensor phase phenyl to in exerts its anticonvulsant action against generalized tonic seizures by preventing seizure spread.From anticonvulsant evaluation it was found that significant anticonvulsant activity of B1, B2 and B3may be due to the presence of electron withdrawing groups at R position.

Antibacterial Activity

The synthesized 6 compounds were screened for the Antibacterial activity studies at 50µg/mL and 100µg/mL using DMSO as a control against staphylococcus aureus, Bacillus pumilis, Bacillus subtilis, Escherichisa coli and Pseudomonas aeruginosa by disk-diffusion method on nutrient agar media, Ampicillin was used as standard drug for the comparison at the concentration 50µg/mL and 100µg/mL against Gram-positive and Gram-negative bacteria used for the study.

Data in the Table.No-5 clearly indicates that none of the compound exhibits antibacterial activity. The zone of inhibition of the entire synthesized compounds was between 7-10 mm at 50µg/mL concentration and 11-13 mm at 100µg/mL concentration.

Whereas the zone of inhibition of standard drug Ampicillin was 21-24 mm at 50µg/mL concentration and 32-35 mm 100µg/mL concentration, many studies have revealed that Thiadiazoles are having good antibacterial activity, but in the present study none of the synthesized compound exhibits such anti-bacterial activity. Which may be leads to the rigidity of the compounds and this may hinders the cleavage of molecules in physiological pH, which is a basic requirement for the activity of Thiadiazole derivatives.

Antitubercular Activity

The synthesized compounds were screened for Antitubercular activity against L.J media method using streptomycin as standard drug. Compound B2, B3 and B6 have shown excellent Antitubercular activity Table no.6.

Table 2 Spectral data of2-Benzylidenylamino-5-(N-Benztriazolomethyl)-1,3,4- Thiadiazole IR Spectra

Type	Vibration	Frequency
Thiadiazole ring	N-N str C=N str C-S str	1634.7 1656 659
Benzotriazoles ring	C-N str C=C str Ar-H str N=N str	1262.40 1542.20 3022.20 1372

^{1}H NMR Spectrum

Signal position	Relative no of proton	Inferences
7.56-7.37 8.01 3.85-3.75	9H 1H 2H	Ar-H -CH of Thiadiazole S-C-CH_2 ofThiadiazole

Table 3 Analgesic activity data of Thiadiazolederivatives

Treatment	No.of Animals	Avg.wt of animals (g)	Dose (mg/kg)	Basal reaction time(sec) after							
				0 sec	20sec	40sec	60sec	80sec	100sec	120sec	180sec
Control (gum acacia)	6	34	---	3.90 ±0.367	4.12 ±0.473	4.12 ±0.553	4.27 ±0.324	4.27 ±0.324	3.88 ±0.372	5.20 ±0,372	---
Standard (pentazocine)	6	28	0.28	6.53 ±0.889	12.87 ±1.332	12.91 ±1.320	13.65 ±0.851	13.65 ±0.851	11.83 ±1.042	12.33 ±0.918	13.14
B1	6	30.66	6.13	3.97 ±0.058	10.73 ±1.597	12.45 ±1.613	14.15 ±0.449	14.15 ±0.449	14.54 ±0.290	11.91 ±1.052	14.03
B2	6	29.33	5.86	5.49 ±o.416	11.66 ±1.889	14.66 ±0.210	13.39 ±0.605	13.39 ±0.605	14.09 ±0.573	11.79 ±1.028	10.49
B3	6	32	6.4	5.62 ±o.158	12.13 ±1.813	13.40 ±1.008	13.16 ±1.164	13.16 ±1.164	13.16 ±1.164	12.97 ±0.028	12.19
B4	6	28	5.6	5.46 ±0.717	9.92 ±1.346	12.51 ±1.576	11.72 ±1.084	11.72 ±1.084	11.72 ±1.084	13.36 ±0.519	---

Table 4 Anticonvulsant activity data of Thiadiazole derivatives

Group No	Treatment	Avg.wt	Avg.Dose(mL)	Latency(onset of clonus) (sec/30 min)mean±SEM	Duration of tonic extensor (sec/30 min)mean±SEM	% Production (24 hrs.)
I	Control(2%gum acacia)	26.75	0.26	3.52±0.036	13.66±0.018	12.5
II	Standard(phenytoin 25 mg/kg P.O)	22.25	0.22	10.25±0.036	No	100
III	Compound B1 (200g/kg P.O)	28.00	0.33	11.73±0.450	9.29±0.26**	66.66
IV	Compound B2 (200mg/kg P.O)	27.33	0.32	12.76±1.061	9.55±0.191**	66.66
V	Compound (200mg/kg P.O)	28.00	0.33	7.85±0.413	5.83±0.810**	66.66
VI	Compound One way ANOVA	26.00	0.30 F Df	7.77±0.602 12.912 59	5.02±2.058** 31.428 59	

n=6 Significance at P<0.05*,<0.05*,<0.001*** and ns-not significant.

Table 5 Antibacterial activity of newly synthesized Thiadiazole derivatives

Sample code	Inhibition zone diameter in nm							
	B.subtills		B.pumills		E.coli		P.aureaginosa	
	50µg	100 µg	50µg	100 µg	50µg	100 µg	50µg	100 µg
B1	9	13	9	12	9	13	8	13
B2	8	12	8	13	8	11	8	12
B3	9	12	9	12	9	12	9	12
B4	8	13	8	11	8	13	9	11
B5	6	11	7	8	7	10	8	11
B6	6	10	7	10	7	9	7	9
Ampicillin	22	34	21	32	22	35	24	34
DMSO	-	-	-	-	-	-	-	-

*Average of triplicate ± standard deviation

Note _ denotes no activity, 7-9 mm better activity,10-13 mm good activity.

Table 6-Antitubercular activities of the synthesized compounds

Compound	Anti-tubercular activity	
	50 mcg/mL	100 mcg/mL
B1	M	S
B2	S	S
B3	S	S
B4	*M*	S
B5	*M*	S
B6	*S*	S
Streptomycin	*S*	S

M-denotes with less than 20 colonies, S-denotes no growth.

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