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Synthesis And Anticonvulsant Activity Of Novel 2,5-Disubstituted -1,3,4 - Thiadiazole Derivatives

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Abstract: A series of Carboxamide moiety with substituted 1,3,4-thiadiazole was designed and synthesized. These title compounds were prepared by condensation of benzoxazine with 2,5-disubstituted-1,3,4- thiadiazole . Structure elucidation of the synthesized compounds was done by spectral analysis. The anticonvulsant activity of the title compounds was evaluated by using PTZ model(60mg/kg) and carbamazepine taking as a reference standard (100 mg/kg). All synthesized compounds showed no sedation side effect as compared to reference standard (carbamazepine). The present study indicated that bromo substituted compounds MH-B_1T_2 (2-benzamido-5-bromo-*N*-(5-(2-chlorophenyl)-1,3,4-thiadiazol-2-yl)benzamide)showed significant protection against pentylenetetrazole induced convulsions which can be regarded as strong candidates for future investigations.
Keywords: 1,3,4- thiadiazole , Anticonvulsant , Carboxamide , Benzamide.

INTRODUCTION

Epilepsy is the third most frequent neurological disorder encountered in the elderly after cerebrovascular disease and dementia. Currently used anticonvulsant agents for treatment of epilepsy have certain disadvantages such as notable adverse effects and inefficient therapy in some seizure types, a clear need for safer and more effective antiepileptic drugs is well known. Therefore, the development of new antiepileptic drugs with approved therapeutic properties is an important challenge for medicinal chemists[1-5].

Extensive literature review revealed that two structures among the compounds studied for anticonvulsant activity are carboxamide moiety and 1,3,4- thiadiazole nucleus shows potent anticonvulsant activity. 4-pyridinecarboxamide[6], Ameltolide[7], Ralitoline[8], Soretolide[9] are the examples of carboxamide analogs and acetazolamide, methazolamide are the examples of 2,5-disubstituted-1,3,4- thiadiazole analogs[10,11]. These observations prompted us to prepare a series of (2- benzamido-5-substituted- *N* -(5- substituted phenyl)-1,3,4-thiadiazol -2- yl) benzamide derivatives to combine the Carboxamide moiety with substituted 1,3,4-thiadiazole moieties. In this paper, we report details of synthesis and evaluation of the anticonvulsant profiles of these compounds.

EXPERIMENTAL

CHEMISTRY

The synthesis of the title compounds was accomplished as shown in Fig. 2. Synthesis of monobromo anthranilic acid, 2-phenyl benzoxazin-4-one and 2,5-disubstituted 1,3,4-thiadiazoles were carried out according to the procedure mentioned in the literature.

I.Synthesis of monobromo anthranilic acid

Anthranilic acid (10g) was dissolved in glacial acetic acid (120cc) and cooled below 15^0C. Then bromine (4.7cc) in acetic acid has been run in, till the reddishbrown color of the bromine persisted. The product was filtered off washed with benzene. It was then boiled up with water (500cc) containing concentrated hydrochloric acid (25cc) and filtered while hot under suction. The insoluble residue was extracted twice with boiling water (250cc). The filtrate, upon cooling yielded an abundant precipitate of the monobromo anthranilic acid. The crude drug obtained was
recrystallized from appropriate solvents. The yields and the melting points of the compounds are 72% and 220-222 ^{0}C respectively. The solvent system for TLC :Ethyl acetate : n-Hexane(1:1).

II.Synthesis of 2-phenyl benzoxazin-4-one/6-bromo-2-phenyl benzoxazin-4-one

0.058 mole of anthranilic acid /6-bromo anthranilic acid dissolved in 50mL of pyridine and cooled to 0^0C. To this reaction mixture, 0.058 mole of benzoyl chloride was added and stirred for 30min at room temperature by using magnetic stirrer. The reaction mixture was treated with 50ml of 5% $NaHCO_3$ solution and solid mass was filtered, washed with water, dried and the crude product was recrystalised from hot ethanol. The yields and the melting points of the 2-phenyl-4*H*-3,1-benzoxazin-4-one : 59.90% and 120-121^{0}C respectively and 6-bromo-2-phenyl-4*H*-3,1-benzoxazin-4-one: 79.10 % and 168-171^{0}C respectively. The solvent system for TLC :Ethyl acetate : n-Hexane(1:1).

III.Synthesis of 2,5-disubstituted 1,3,4-thiadiazoles

A reaction mixture of benzoic acid(0.048mol) and thiosemicarbazide(0.048mol) in phosphorous oxychloride (16 mL) was refluxed gently(at 75 ^{o}C) for 30 min . After cooling down to room temperature, water was added (90 mL) carefully. The reaction mixture was refluxed for 4 h. and filter during hot. Then filterate was basified to pH 8 by the dropwise addition of 50% aqueous potassium hydroxide solution under stirring. The precipitate was filtered and washed with water, dried and crystallized from ethanol. Solvent system for TLC : Ethyl acetate : n-Hexane(1:1).

IV.Synthesis of target compounds

A mixture of 2-phenyl-4*H*-3,1-benzoxazin-4-one (0.0035mol) / 6-bromo-2-phenyl-4*H*-3,1-benzoxazin-4-one (0.0035mol) and slight excess of 5-phenyl-1,3,4-thiadiazol-2-amine (0.0046mol) in 20 mL of dry pyridine was heated under reflux for 6-8 hrs, left to cool and then poured into cold water with constant stirring. The solid product that separated out was filtered off, thoroughly washed with water, dried and then recrystallized from methanol. The completion of the reaction was monitored on precoated TLCplates by using Ethyl acetate : n-Hexane (1:1) as the eluent and observed in UV light. The yield, melting point and other physical properties of synthesized compound are reported in **Table 1.** The structures of these novel synthesized compounds were elucidated by IR, ^{1}H NMR,^{13}C NMR and Mass spectroscopic tools. The summary of spectral analysis data is shown in **Table 2.**

Melting points were determined in open capillary tubes in VEEGO Corporation melting point apparatus and are uncorrected. The purity of the compounds was confirmed by thin layer chromatography using Precoated Silica Gel plates(Merck) and solvent system of Ethyl acetate : n-Hexane(1:1). The spots were developed in iodine chamber and visualized under ultraviolet lamp. The infrared spectra were recorded using KBr as the medium, using JASCO FTIR-6100 model within the institute. ^{13}C nuclear magnetic resonance (^{13}C NMR) and ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were recorded for the compounds on BRUKER AVANCE II 400 MHz spectrophotometer using DMSO(d_6) as a solvent and TMS as a internal standard at Panjab University , Chandigarh. The mass spectra were recorded on MASS-ESI instrument at Oxygen Healthcare , Ahmedabad.

PHARMACOLOGICAL TEST

Compounds were pharmacologically pre-evaluated using the testing procedures described elsewhere as part of the Antiepileptic Drug Development (ADD) Program (Epilepsy Branch, Neurological Disorders Program,NINCDS, Bethesda).

Animals(Male albino mice)were divided into three groups each consisting of six animals.

Group I served as control : received only the vehicle (Saline 10ml/kg)

Group II standard. : administered Carbamazepin (100mg/kg was suspended in 0.5% methylcellulose (in Saline) in a volume of 10ml/kg)

Group III test. : administered each test compounds (100mg/kg was suspended in 0.5% methylcellulose (in Saline) in a volume of 10ml/kg)

After 30 min drug treatment, PTZ (60 mg/kg subcutaneously) was given in the scruff of neck. The animals were placed in isolation cages to minimize stress and observed for onset of clonic convulsions, number of convulsions in 30 minutes, 1h and 24 h mortality[12-15].

STATISTICAL ANALYSIS

Unpaired student T-test was used for data expressed in mean ± S.E.M, using SPSS Statistic 17.0 software . Differences between means were considered to be significant at P<0.001, P<0.01, P<0.05 compared to control. Whereas, two proportions z-test(Pearson Chi – Square) was applied for the data expressed in percentage, using SPSS Statistic 17.0 software and in this case the 'P' value less than 0.05 was considered significant when compared to control.

Fig.1. Designing strategy for (2-benzamido-5-substituted-*N*-(5- substitutedphenyl)-1,3,4-thiadiazol-2-yl)benzamide

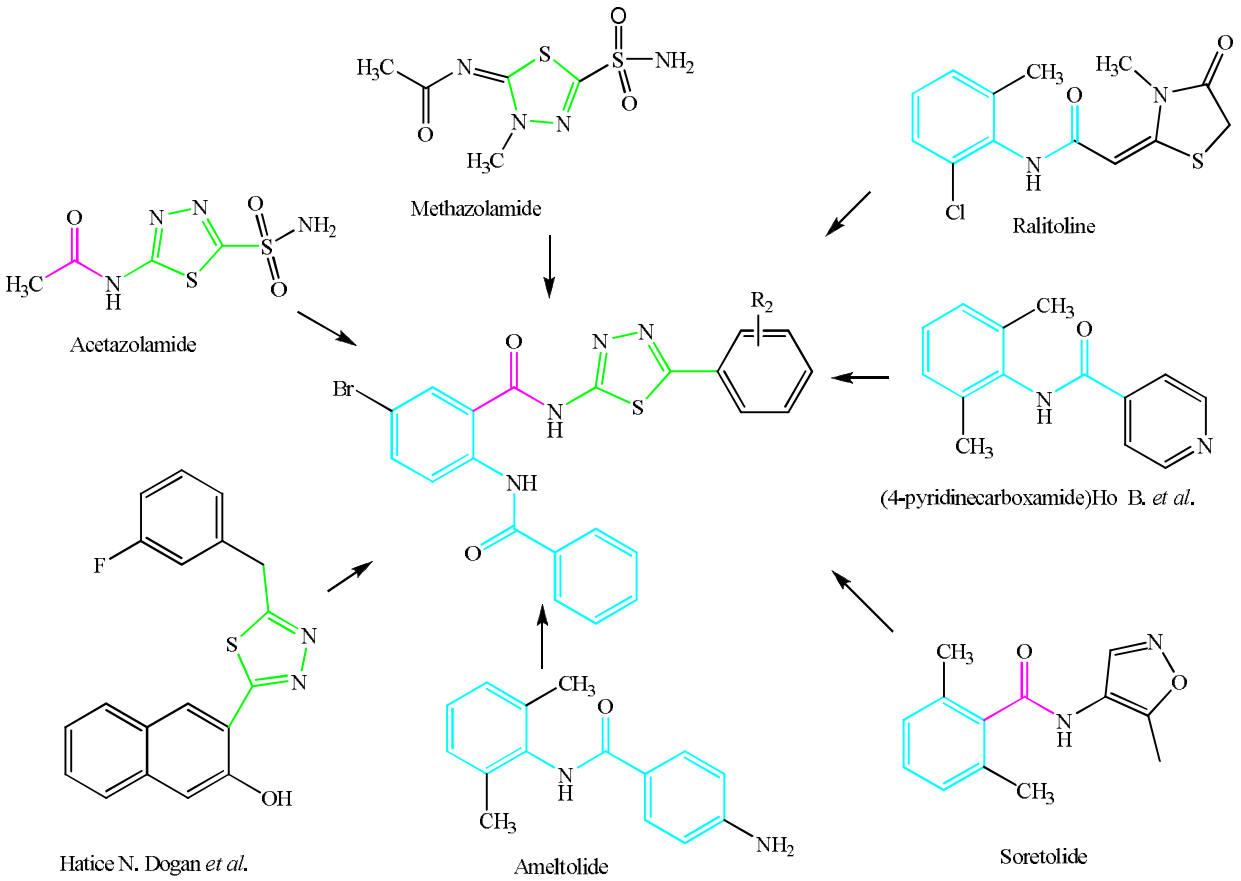


Fig. 2. Scheme for the synthesis of (2-benzamido-5-substituted-*N*-(5- substitutedphenyl)-1,3,4-thiadiazol-2-yl)benzamide

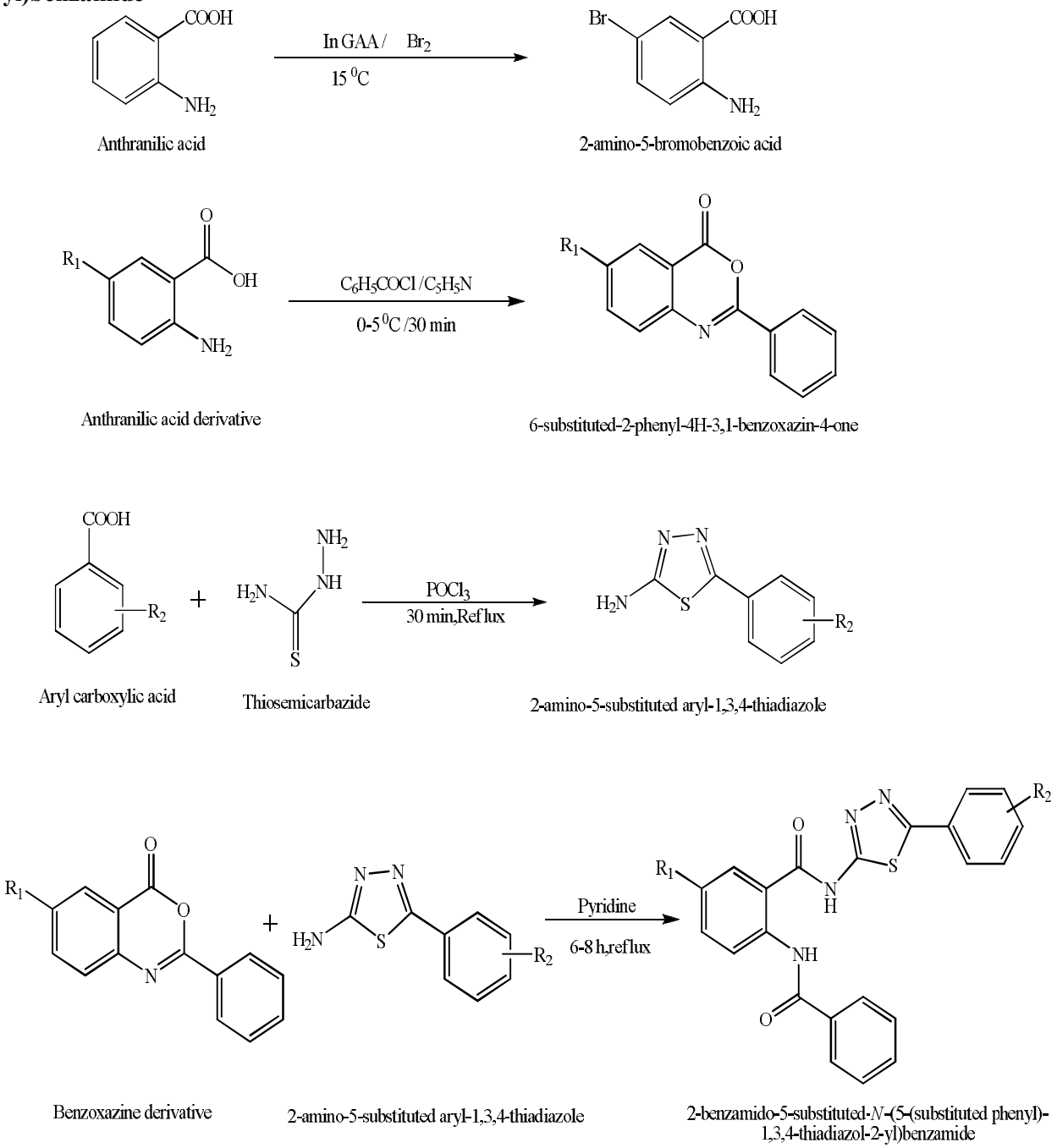
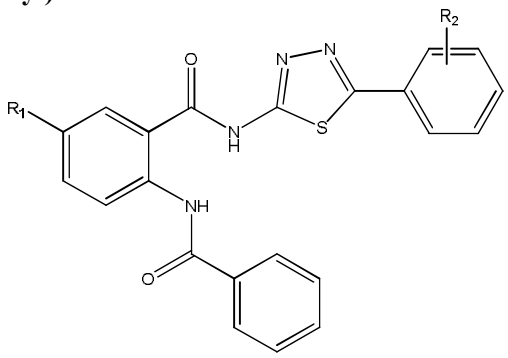


Table 1.Physical constants of the synthesized (2-benzamido-5-substituted-*N*-(5- substituted phenyl)-1,3,4-thiadiazol-2-yl)benzamide.



Sr. No	Code	Substituent		Molecular Formula	Molecular Weight	% Yield	Melting Point	R_f*
		R_1	R_2					
1	MH-B_0T_1	-H	-H	$C_{22}H_{14}N_4OS$	382.43	62.04	195-198 °C	0.54
2	MH-B_0T_2	-H	2-Cl	$C_{22}H_{13}ClN_4OS$	416.88	43.62%	250-253 °C	0.61
3	MH-B_0T_3	-H	2,4-Cl	$C_{22}H_{12}Cl_2N_4OS$	451.32	59.57 %	187-190 °C	0.44
4	MH-B_0T_4	-H	4-Cl	$C_{22}H_{13}ClN_4OS$	416.88	67.78%	170-173 °C	0.40
5	MH-B_0T_5	-H	4-F	$C_{22}H_{13}FN_4OS$	400.42	71.2%	239-241 °C	0.56
6	MH-B_1T_1	-Br	-H	$C_{22}H_{13}BrN_4OS$	461.33	86.84%	243-246 °C	0.78
7	MH-B_1T_2	-Br	2-Cl	$C_{22}H_{12}BrClN_4OS$	495.77	84.75 %	226-228 °C	0.72
8	MH-B_1T_3	-Br	2,4-Cl	$C_{22}H_{11}BrCl_2N_4OS$	530.22	86.28%	242-245 °C	0.82
9	MH-B_1T_4	-Br	4-Cl	$C_{22}H_{12}BrClN_4OS$	495.77	73.78%	190-193 °C	0.74
10	MH-B_1T_5	-Br	4-F	$C_{22}H_{12}BrFN_4OS$	479.32	81.01%	230-232 °C	0.76

*Solvent: Ethyl acetate : n-Hexane(1:1)

Table 2.Spectroscopic data of the title compounds

Sr. No	Compounds (Code)	Spectral data(IR(KBr)n(cm^{-1}),^{1}H NMR(400MHz,δ),^{13}C NMR(400MHz,δ),ESIMS(m/z)						
		2^0amide Stretch	C=O stretch	C=N stretch	C-N stretch	C-S Stretch	O-C-N bending	C-X bending
1	MH-B_0T_1	3350	1683.7 1650	1539.09	1319.2	750.26	650	-
		7.94-7.96(4H,(2Ha,2Hb),Ar,m), 7.62-7.53(6H(6Hc),Ar,m), 7.26-7.23(1H(H'd),Ar,t, J-8Hz), 7.51-7.49(1H(Hd),Ar,t), 8.12(1H(Hj1),Ar,m),8.48(1H(Hj2),Ar,m), 13.18(1H,s(1),-CONH-,s), 11.53(1H,(2),-CONH-,s)						
		131.73(C-a1), 131.53(C-a2), 126.71(C-b1), 126.75(C-b2), 127.06(C-b3), 128.58 (C-b'1), 128.61(C-b'2), 129.02(C-b'3), 130.39(C-c1), 130.1(C-c2), 134.4(C-d1), 134.5(C-d2), 128.2(C-e), 127.3(C-m), 137.3(C-f), 126.5(C-g), 132.3(C-h), 133.07(C-i), 164.39(C-j1), 164.40(C-j2), 152(C-k), 174(C-l)						
		401(Molecular ion peak ,30%), 400.8(Base peak, 100 %), 223.8,177.8,135.8						
2	MH-B_0T_4	3300	1665 1625	1530.24	1320	764.63	600	1100(C-Cl)
3	MH-B_0T_5	3323.71	1763 1650	1520.6	1320	761.74	615	1250(C-F)
4	MH-B_1T_1	3300	1660 1690	1520	1320	775.24	670	1050(C-Br)
5	MH-B_1T_2	3320	1760 1671	1530	1327.2	750.26	650	1050(C-Cl) 1040(C-Br)
6	MH-B_1T_3	3300	1670 1630	1520	1327.7	775.24	610	1030(C-Cl) 1100(C-Cl) 1045(C-Br)
7	MH-B_1T_4	3531.02	1690 1620	1509.9	1332.5	686.53	600	1090(C-Cl) 1050(C-Br)

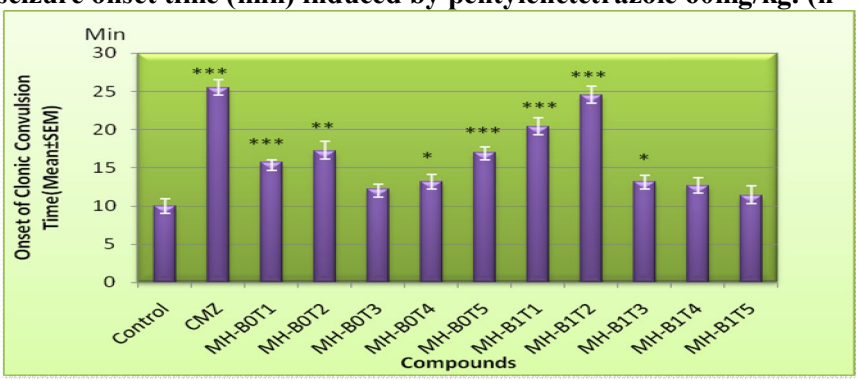
8	MH-B_1T_5	3116.75	1664 1630	1521.7	1319.2	754.12	610	1260(C-F) 1030(C-Br)
		7.98-7.95(4H(2Ha,2Hb),Ar,m), 7.60-7.51(3H(3He),m), 7.29-7.24(2H(2H'e),Ar,t ,J-8Hz), 8.32(1H(Hj),Ar,s), 8.47-8.44(1H(Hd),Ar,d,J-12Hz), 8.32(1H(Hc),Ar,dd, J-varify), 13.73(1H,(1),-CONH-,s), 11.77(1H,(2),-CONH-,s)						
		132.31(C-a1), 131.84(C-a2), 116.25(C-b1), 116.03(C-b2), 122.99(C-c1), 122.76(C-c2), 128.98(C-d1), 128.89(C-d2), 128.60(C-d3), 134.21(C-e), 138.71(C-f), 114.80(C-g), 135.44(C-h), 125.4(C-i), 164.72(C-j1), 164.68(C-j2), 160(C-k), 174(C-l), 126.49(C-m), 162.19(C-n), 127.10(C-O), 127(C-p)						
		497(Molecular ion peak,30%),400.8(Base peak,100%),458.9,423.0,223.8,195,177.8,153.8						

Table 3. Effect of synthesized compounds on PTZ induce seizure in mice.

Sr. No.	Drug (Code) Dose-100mg/kg Volume-10ml/kg	Clonic convulsions (Mean±S.E.M)		% of protection in 1 hrs mortality of animals N/F	% of protection in 24 hrs mortality of animals N/F	Intensity of sedation
		Onset of clonic convulsion	No. of clonic convulsions (in 30 min)			
1	Vehicle	10.00±0.89	3.00±0.25	2/6=0.33%	1/6=16.66%	-
2	Carbamazepine	25.50±1.05	1.16±0.16	6/6=100%	6/6=100%	++
3	MH-B_0T_1	15.66±0.33	1.66±0.33	6/6=100%	6/6=100%	+
4	MH-B_0T_2	17.16±1.30	1.16±0.33	6/6=100%	6/6=100%	+
5	MH-B_0T_3	12.16±0.70	2.00±0.36	4/6=66.66%	3/6=50%	+
6	MH-B_0T_4	13.16±0.98	2.83±0.30	4/6=66.66%	3/6=50%	+
7	MH-B_0T_5	17.00±0.73	2.00±0.25	6/6=100%	5/6=83.33%	+
8	MH-B_1T_1	20.33±1.20	1.33±0.21	6/6=100%	6/6=100%	+
9	MH-B_1T_2	24.50±1.17	1.16±0.16	6/6=100%	6/6=100%	+
10	MH-B_1T_3	13.16±0.87	2.83±0.47	3/6=50%	3/6=50%	+
11	MH-B_1T_4	12.66±1.05	1.66±0.33	5/6=83.33%	4/6=66.66%	+
12	MH-B_1T_5	11.33±1.33	2.16±0.47	5/6=83.33%	3/6=50%	+
13	MH-A_0T_1-P	21.66±1.45	1.66±0.33	6/6=100%	6/6=100%	+
14	MH-A_0T_2-P	19.83±0.94	1.33±0.21	6/6=100%	6/6=100%	+
15	MH-A_0T_3-P	16.83±1.16	1.50±0.22	6/6=100%	6/6=100%	+
16	MH-A_0T_4-P	16.66±0.66	1.50±0.22	6/6=100%	6/6=100%	+
17	MH-A_0T_5-P	18.66±2.04	1.66±0.33	6/6=100%	4/6=66.66%	+
18	MH-A_1T_1-P	24.33±1.60	1.16±0.16	6/6=100%	6/6=100%	+
19	MH-A_1T_2-P	21.83±1.47	1.16±0.16	6/6=100%	6/6=100%	+
20	MH-A_1T_3-P	15.00±1.50	1.33±0.21	6/6=100%	5/6=83.33%	+
21	MH-A_1T_4-P	17.00±0.34	1.50±0.34	5/6=83.33%	4/6=66.66%	+
22	MH-A_1T_5-P	20.50±1.23	1.33±0.21	6/6=100%	6/6=100%	+

N/F=Protected animal against mortality in 1hr or 24hrs over treated animal, Intensity of sedation: + animals stopped moving but responded to physical stimuli, ++ animals had ataxia on moving, +++ animals did not respond to physical stimuli but responded to painful stimuli, ++++ absence of wrighting reflex as well as no response to painful stimuli.

Fig.3.Effect of intraperitoneal injection of synthesized compounds and standard(Carbamazepine) on clonic seizure onset time (min) induced by pentylenetetrazole 60mg/kg. (n = 6) * p < 0.05 ** p < 0.01 *** p < 0.001.



RESULT AND DISCUSSIONS

The N–H and C=O stretching bands observed in the region of 3486–3000 and 1658–1700 cm^{-1} respectively, indicate the presence of an amide moiety. Title compounds showed other band like C-S stretching in the region of 700-850 cm^{-1}, C=N stretching of thiadiazole in the 1500-1600 cm^{-1} region, C-N stretching at 1350-1300 cm^{-1} and aromatic C=C stretching band near1600cm^{-1} and 1450cm^{-1} with overtone in the region of 1700-2000cm^{-1}. A weak band was observed in the region of 3100cm^{-1}. It is attributed to a Fermi resonance overtone of N-H bending and C-N stretching. Two signals were observed at downfield due to high deshielding effect of carbonyl groups. In case of MH-B_1T_5, 12 aromatic protons gave signal in the range of 7.24-8.47 ppm whereas in case of MH-B0T1, 14 aromatic protons gave signal in the range of 7.23-8.48 ppm. The molecular weight of the title compounds was further verified by ESIMS(Electrospray ionization mass spectrometry) where the *m/z* values of molecular ion peaks were in complete agreement with the calculated molecular weight for both compounds (MH-B_0T_1, MH-B_1T_5). The compounds having bromo and fluoro substituent (MH-B_1T_5) showed relatively small molecular ion peaks whereas the base peak obtained in MH-B_1T_5 was due to the loss of chlorine and fluorine ions.

The anticonvulsant activity of the title compounds were evaluated using scPTZ model(60mg/kg) and carbamazepine taken as a reference standard at a dose similar to that of the test compound i.e. 100 mg/kg body weight.

MH-B_0T_1 and MH-B_0T_2 showed 100% protection against 1 h and 24 h mortality and MH-B_0T_5 showed 100% and 83. 33 % protection against 1 h and 24 h mortality respectively. which were statistically significant ($P<0.05$) in comparison to control. It also decreased number of clonic convulsions($P<0.05$) and increased the onset time for clonic convulsion($P<0.01$) which was statistically significant in comparison to control. MH-B_0T_3 and MH-B_0T_4 showed 66.66% protection against 1 h mortality and 50% protection against 24 h mortality as well as decrease no of clonic convulsion and increase onset for clonic convulsion which however was not-significant in comparison to control as shown in **fig.3.**

MH-B_1T_1 and MH-B_1T_2 showed 100% protection against 1 h($P<0.05$) and 24 h($P<0.05$) mortality as well as decreased no of clonic convulsions($P<0.05$) and increased time for onset for clonic convulsion($P<0.001$) which were statistically significant in comparison to control. MH-B_1T_4 and MH-B_1T_5 showed 83. 33 % protection against 1 hr mortality and showed respectively 66.66% and 50% protection against 24 hrs mortality whereas MH-B_1T_3 showed 50% protection against both 1 h and 24 h mortality which however not-statistically significant in comparison to control as shown in fig.3. MH-B_1T_3 , MH-B_1T_4 and MH-B_1T_5 increase onset of clonic convulsion ($P<0.05$), as well as decrease no of clonic convulsion which however was not-significant in comparison to control.

CONCLUSION

All the synthesized compounds showed little sedation, a prominent side effect observed with the reference standard (carbamazepine). While considering all the newly synthesized compounds of this series together, use may conclude that bromo substituted (MH-B_1T_2) gave anticonvulsant activity equivalent to the standard carbamazepine. They also showed 100% protection against 1h and 24h mortality. These can be regarded as strong candidates for future investigations.

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