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Research Article

SYNTHESIS AND ANTICONVULSANT SCREENING OF N-ARYL-2-(3-OXO-1,4-BENZOTHIAZIN-2YL) ACETAMIDES DERIVATIVE

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ABSTRACT

A series of N-aryl-2-(3-oxo-1, 4-benzothiazin-2-yl) acetamides have been synthesized by the condensation of different 3/4 substituted (3-nitro, 4-nitro, 3-methyl, 4-methyl, 3-chloro, 4-chloro) anilines with (2H)-oxo-3,3a-dihydrofuro [3,2-b][1,4-benzothiazine and screened for anticonvulsant activity. The condensation *o*-aminophenol with maleic anhydride yielded 2,3-dihydro-3-oxo (2H)-1,4-benzothiazin-2-yl-acetic acid which on reaction with thionyl chloride afforded (2H)-oxo-3,3a-dihydrofuro [3,2-b][1,4]-benzothiazine. The structures of the synthesized compounds have been established on the basis of elemental analysis and spectral data. All the synthesized compounds were subjected for anticonvulsant activity by using maximal electroshock seizure (MES) and subcutaneous pentylenetetrazole (scPTZ) methods. The synthesized compounds were administered intraperitoneally at the doses of 30, 100 and 300 mg/kg and were compared with the standard drugs i.e. phenytoin (MES group) and carbamazepine (scPTZ group). The abolition of hind limb tonic extensor spasm was recorded as a measure of anticonvulsant activity. All the compounds were found to possess anticonvulsant activity comparable to that of standard.

Keywords: Anticonvulsant activity, Benzothiazine, Pentylenetetrazole, Carbamazepine

INTRODUCTION

Epilepsy is a neurological disorder characterized by unprovoked seizures that affect millions of people worldwide. It is estimated that 25% of the epileptic population have seizures that are not responsive to presently available medical therapies. **[1].**

Conventional antiepileptic drugs (AEDs) phenobarbital, primidone, phenytoin, carbamazepine, ethosuximide and benzodiazepine, are widely used but exhibit an unfavorable side effect profile and failure to adequately control seizures **[2].** It is estimated that available medication controls the seizures in only 50% of patients or decrease incidence in only 75% of patients. These facts make the field of anticonvulsant drug discovery a high priority **[3].** The long-established AEDs control seizures in 50% of patients developing partial seizures and in 60-70% of those developing generalized seizures **[4].** Hence, there is an urgent need to develop new AEDs.

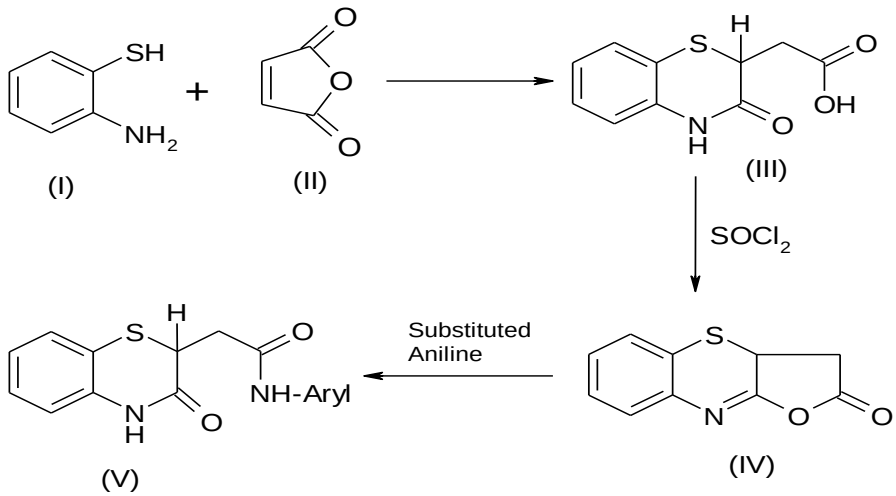
Symptoms of depression were significantly more likely to appear in patients taking vigabatrin. These results triggered the search for newer anticonvulsants **[5].**

Oxopyridobenzothiazines are also reported to have binding to the benzodiazepine receptor, anticonvulsant activity in the pentylenetetrazole-induced convulsion assay. The 7-chloro derivatives of it have shown receptor affinity comparable to chlorodiazepoxide with slightly improved *in vivo* activity **[6]**.

As benzothiazines are reported to have anticonvulsant activity, N-aryl-2-(3-oxo-1,4-benzothiazin-2-yl) acetamides derivatives were prepared and evaluated for anticonvulsant activity by maximal electroshock seizure (MES) and subcutaneous pentylenetetrazole (ScPTZ) methods.

MATERIAL AND METHODS

The condensation of *o*-aminothiophenol **(I)** with maleic anhydride **[7, 8, 9] (II)** yielded 2,3-dihydro-3-oxo (2H)- 1,4 benzothiazine acetic acid **(III)**, which on reaction with thionyl chloride **[10]** afforded 2(H)-Oxo-3,3a-dihydrofuro [3,2-b][1,4]-benzothiazine **(IV)**. Condensation of IV with substituted aniline give corresponding N-aryl-2-(3-oxo-1,4-benzothiazin-2-yl) acetamides **(V)**.The synthetic approach outlined in **Scheme I**.



Scheme I: Synthesis of N-aryl-2- (3-oxo-1, 4-benzothiazin-2-yl) acetamides.

The characterization of compounds **V a-f** has been done on the basis of their elemental analysis and spectral (IR, ^{1}HNMR) data **(Table 1).**

Table 1: Physical and Spectral data of compounds V a-f

Compound	Aryl	Yield (%)	M.P. (°C)	R_f	Mol. Formula	IR cm^{-1} and ^1H HMR	Found % Calculated, C	H	N
Va	-C_6H_4-4-NO_2	66	237-238	-	$C_{16}H_{13}N_3O_4S$	1620 (NHC=O), 1570 (C-N), 3030 (Ar C-H), 1662 (C=O). ^1H NMR: 2.8 (m, 2H, -CH_2CO), 4.3 (m, 1H, S-CH), 7-7.7 (m, 8H, ArH), 9.6 (s, 1H, NH), 10.3 (s, 1H, NH).	55.96 (55.97)	3.81 (3.82)	12.25 (12.24)
V b	-C_6H_4-3-NO_2	45	185-190	0.34	$C_{16}H_{13}N_3O_4S$	3450 (NH), 1570 (C-N), 3030 (Ar C-H), 1680 (C=O).	55.97 (55.95)	3.82 (3.80)	12.24 (12.26)
V c	-C_6H_4-3-CH_3	56	216-219	0.42	$C_{17}H_{16}N_2O_2S$	3360 (NH), 1675 (NHCO), 2560 (S-H), 3035 (Ar C-H), 1662 (C=O). 2.8 (m, 2H, -CH_2CO), 4.3 (m, 1H, S-CH), 7-7.7 (m, 8H, ArH), 9.6 (s, 1H, NH), 10.3 (s, 1H, NH).	65.36 (65.37)	5.16 (5.17)	8.97 (8.96)
V d	-C_6H_4-4-CH_3	72	212-215	0.07	$C_{17}H_{16}N_2O_2S$	3310 (NH), 1690 (NHCO), 2560 (S-H), 2980 (Ar C-H), 1650 (C=O).	65.36 (65.35)	5.16 (5.18)	8.97 (8.98)
V e	-C_6H_4-3-Cl	76	215-218	0.43	$C_{16}H_{13}N_2O_2SCl$	2560 (S-H), 3015 (Ar C-H), 1682 (C=O), 740 (C-Cl). 2.8 (m, 2H, -CH_2CO), 4.3 (m, 1H, S-CH), 7-7.7 (m, 8H, ArH), 9.6 (s, 1H, NH), 10.3 (s, 1H, NH).	57.74 (57.72)	3.94 (3.95)	8.42 (8.43)
V f	-C_6H_4-4-Cl	65	230-232	0.59	$C_{16}H_{13}N_2O_2SCl$	3335 (NH), 1680 (NHCO), 2560 (S-H), 3000 (Ar C-H), 740 (C-Cl).	57.74 (57.75)	3.94 (3.93)	8.42 (8.43)

The melting points were carried out in open capillary tube and were uncorrected. Thin layer chromatography was performed using silica gel coated on a glass plate and spots were visualized by exposure to iodine vapor. IR spectra in Nujol were recorded on a Shimadzu IR spectrophotometer. ^1H NMR spectra were recorded in $CDCl_3$+acetone-d_6 on a FX 90Q FTNMR spectrometer using TMS as an internal standard (chemical shifts in δ, ppm).

(1) Preparation of 2,3-dihydro-3-oxo (2H)- 1,4 benzothiazine acetic acid (III)

To a solution of pure maleic anhydride (4.9g, 0.05 mole) in ether (20 mL), a solution of *o*-aminothiophenol (6.25 mL, 0.05 mole) in ether (20 mL) was added with swirling at room temperature. The warm reaction mixture was cooled. When a colorless product separates out, it was filtered off, washed with ether and the crystalline product was purified by recrystallization from ethanol as colorless needles. Yield- 98%, m.p-201-02 °C, Calcd. For $C_{10}H_9NO_3S$: C, 53.80; H, 4.06; N, 6.27 %; found C, 54.2; H, 4.2; N, 6.13 %; IR (Nujol)(cm^{-1}) 3200 (NH), 1660 (NHCO), 3030 (Ar C-H), 1700 (COOH).

(2) 2(H)-Oxo-3, 3a-dihydrofuro [3,2-b][1,4] benzothiazine (IV)

A mixture of 3-oxo-1,4-benzothiazin-2-yl-acetic acid (2.23 g, 0.01 mole) and sulphuryl chloride (5 mL) in 50 mL of benzene was refluxed for 4 hrs. The product was isolated and recrystallized from acetone to give (IV). Yield- 69%, m.p-209 °C, Calcd. For $C_{10}H_7NO_2S$:C, 58.5; H,3.4; N,6.8 %; found C, 58.7; H, 3.1; N, 6.5 %; IR (Nujol)(cm^{-1}) 1773 (lactone C=O).

(3) N-(4-nitrophenyl)-2-(3-oxo-1,4-benzothiazin-2-yl) acetamide (Va)

To a mixture of **IV** (2.07 gm, 0.01 mole) and 4-nitroaniline (2.76 g, 0.02 mole) in benzene (50ml), a few drops of glacial acetic acid were added and the reaction mixture was refluxed on a steam bath for 4 hrs. The solvent was distilled off and the residue filtered, and purified by column chromatography on silica gel using benzene-chloroform as eluent (10:90) to give pure Va. Yield- 66%, m.p-237-38 °C. The other compounds Vb-f were also prepared in a similar way from IV by using different substituted anilines. The physical and spectral data are given in table 1.

Anticonvulsant screening

The synthesized compounds were evaluated for their anticonvulsant activity using MES and sc PTZ methods. The animals, albino mice weighing 20-25 gm were kept under hygienic conditions and on standard laboratory diet and were randomly allocated in different groups of 3 animals each and were overnight fasted before the experiment with free access to water. All procedures were conducted as per guidelines of the committee for the purpose of control and supervision of experimental animals. The protocol for the use of animals for this study was approved by the Institutional Animal Ethics committee, Dr. Hari Singh Gour Central University, Sagar, Madhya Pradesh, India. The synthesized compounds (Va-Vf) were administered intraperitoneally at the doses of 30, 100 and 300 mg/kg and were compared with the standard drugs i.e. phenytoin (MES group) and carbamazepine (scPTZ group). Both the test compounds and standard drugs were administered as carboxymethyl cellulose suspension (0.5%). The anticonvulsant activity was assessed at the end of 0.5 and 4 h periods after the administration of test and standard drug. The abolition of hind limb extensor spasm was recorded as a measure of anticonvulsant activity. Most of the compounds were found as potent as phenytoin and carbamazepine.

Maximal electroshock seizure test (MES)

Supra-maximal electroshock of current intensity 50mA at a frequency of 60 Hz was given for duration of 0.2 sec via corneal electrodes. Two drop of 0.9% w/v sodium chloride instilled in each eye prior to application of electrodes assured adequate electrical contact. Test solutions of all the compounds were prepared in 0.5% v/v carboxymethyl cellulose in water and animals were dosed intraperitoneally 30 min prior to testing. Abolition of the hind limb extension component of the seizure was defined as anticonvulsant activity in the MES test **[11, 12, 13]**.

Subcutaneous pentylenetetrazole seizure test (scPTZ)

A dose of pentylenetetrazole 85 mg/kg administered subcutaneously to mice causes seizures in more than 97% of the animals. This is called the convulsive dose 97 (CD97). The test was carried out by giving the pentylenetetrazole injection approximately 10 minutes before the anticipated time of the peak anticonvulsant drug action. The animals were observed at 0.5 and 4 hours for the occurrence of seizures. A threshold convulsion is defined as one episode of clonic spasms which persists for at least 5 seconds. Absence of even a threshold convulsion during the period of observation is taken as the endpoint in this test **[11, 13]**.

RESULTS AND DISCUSSION

The synthesized compounds were screened for their anticonvulsant activity by the Maximal Electroshock (MES) induced seizures and scPTZ induced seizure method, wherein electroshocks were applied via ear-lip electrodes using phenytoin and carbamazepine as reference drugs.

The Compounds Va (4-nitrophenyl-) group and Vb (3-nitrophenyl-) were found to be as potent as standards i.e.phenytoin for MES and carbamazepine for scPTZ. The compounds Vd (4-methylphenyl-) Vf (4-chlorophenyl) were also active against both the test model but less potent than Va for MES screen but has same potency as carbamazepine for scPTZ. The compound Vc containing (3-methylphenyl-) and Ve (3-

chlorophenyl-) were found to be least active anticonvulsant agent. The anticonvulsant activity can be attributed to the electron withdrawing nitro group.

Table 2: Anticonvulsant activity of synthesized compounds

S. No.	Compounds	Intraperitoneal injection in mice			
		MES Screen[a]		Sc PTZ screen[a]	
		0.5 h	4 h	0.5 h	4 h
1	Va	30	30	100	300
2	Vb	30	30	100	-
3	Vc	300	-	300	-
4	Vd	100	300	100	300
5	Ve	300	-	300	-
6	Vf	100	300	100	-
7	Phenytoin	30	30	-	-
8	Carbamazepine	-	-	100	300
9	Control	5%CMC	-	-	-

[a]Doses of 30, 100 and 300 mg/kg were administered. The figures in the table indicate the minimum dose whereby activity was shown in 3 or more of the mice from each group. The (-) indicates absence of activity at maximum dose i.e. 300 mg/kg.

CONCLUSION

The synthesized compounds were evaluated for their anticonvulsant activity using MES and sc PTZ methods. The abolition of hind limb extensor spasm was recorded as a measure of anticonvulsant activity. Most of the compounds were found as potent as phenytoin and carbamazepine. The anticonvulsant activity can be attributed to the electron withdrawing groups.

REFERENCE

1. Beraldo H, Gambino D. Wide pharmacological versatility of semicarbazones thiosemicarbazones and their metal complexes. Mini Rev Med Chem 2004; 4: 159-165.
2. Lopes LJM. The new drugs and strategies to manage epilepsy. Curr Pharmac Design 2000; 6: 873-878.
3. Porter RJ, Rogawski MA. New antiepileptic drugs: from serendipity to rational discovery. Epilepsia 1992: 33.
4. Berk M, Segal J, Janet L, Vorster M. Emerging options in the treatment of bipolar disorders. Drugs 2001; 61: 1407-1414.
5. Rall TW, Schleifer LS. Drugs effective in the treatment of epilepsies: Goodman and Gilman's, The Pharmacological Basis of Therapeutics. 6th edition, Macmillan Publishing Co, INC, New York, pp 448-474; 1980.
6. Chorvat RJ, Desai BN, Radak SE, Bloss J, Hirsch J, Tenen S. Synthesis, benzodiazepine receptor binding, and anticonvulsant activity of 2,3-dihydro-3-oxo-5H-pyrido[3,4-b][1,4]benzothiazine-4-carbonitriles. J Med Chem 1983; 26(6): 845-50.
7. Wagh SB, Shaikh AS, Balasubramaniyan V. Reactions of cyclic anhydrides: Part VIII-Formation of 2-Oxobenzothiazines from o-aminothiophenol & Maleic anhydride derivatives. Indian J Chem 1983; 22B: 868.
8. Balasubramaniyan P, Shaikh AS, Argade NP, Balasubramaniyan V. Regioselective reactions of o-Aminothiophenol with unsymmetrical maleic anhydrides. Indian J Chem 1989; 28B: 123-125.
9. Balasubramaniyan P, Patel MV, Wagh SB, Balasubramaniyan V. Reactions of cyclic anhydrides: Synthesis of 3-oxo-1,4-benzothiazines and 2-oxo-quinoxalines, A novel approach via maleanillic acids. Curr Sci 1982 51: 279.
10. Sastry CVR, Rao KS, Jain ML. Synthesis and biological activity of 2-oxo-1,2,3,3a-tetrahydropyrrolo [3,2-b]-[1,4] benzothiazines. Indian J Chem 1991; 30 B: 535.
11. Sharma PP, Pandeya SN, Roy RK, Verma AK, Gupta S. Synthesis and anticonvulsant activity of some novel isatin schiff's bases. Int J Chem Tech Res 2009; 1(3): 758-763.
12. Shaikh MF, Sancheti J, Sathaye S. Phytochemical and pharmacological investigations of *Eclipta alba* (linn.) hassak leaves for antiepileptic activity. Int J Pharm Pharm Sci 2012; 4(4): 319-323.
13. BhatJU, Nizami Q, Aslam M, Parray SA, Asiaf A, Khanam R, Mujeeb M. Anticonvulsant activity of methanolic extract of *Melissa parviflora* in experimentally inducedswiss albino mice. Int J Pharm Pharm Sci 2012; 4(2): 428-430.