ISSN: 2230-7346

Srinivasa Murthy et al. / JGTPS/ 5(4)-(2014) 2199–2203

(Research Article)

Journal of Global Trends in Pharmaceutical Sciences

Journal home page: www.jgtps.com

SYNTHESIS, CHARACTERIZATION AND EVALUATION OF ANTICONVULSANT ACTIVITY OF SOME NEW 4-ARYL-8-ARYLIDENE-5,6-DIHYDRO-2-IMINO-6,6-DIMETHYL-4H,7H-[3,1] BENZOTHIAZINE DERIVATIVES

Bharath Rathna Kumar P[1], Srinivasa Murthy M[2*], Jayaveera K.N[3]

[1]Department of Pharmaceutical Chemistry, Oil Technological Research Institute-JNTUA, Ananthapuramu, A.P, India.

[2]Department of Pharmaceutical Chemistry, Vignan Institute of Pharmaceutical Sciences, Near Ramoji Film City, Deshmukhi, Nalgonda, India.

[3]Director, Research and Development, Adept Pharma and Bioscience Excellence Pvt., Ltd, Hyderabad, India.

ABSTRACT

The newly designed thiazine derivatives (IIIA-H) were synthesized by reacting 2,6-diarylidene-4,4-dimethylcyclohexanone (IIA-H) with thiourea in presence of potassium hydroxide. The compounds (IIA-H) were obtained by reaction of 4,4-dimethylcyclohexanone with different aromatic aldehydes. Their structures were confirmed by IR, ^{1}HNMR and mass spectra. The newly synthesized compounds were screened for *In-vivo* anticonvulsant activity. Anticonvulsant activity was investigated by maximal electroshock induced method. Among the test compounds, IIIA and IIIF has significantly decreased the duration of all phases of convulsions and exhibited potent anticonvulsant activity than other synthesized compounds.

Key Words: Synthesis, thiazines, anticonvulsant activity, phenytoin, electroshock.

INTRODUCTION

Benzothiazine derivatives are of significant interest due to their chemotherapeutic history. The title compounds 4-aryl-8-arylidene-5,6-dihydro-2-imino-6,6-dimethyl-4H,7H-[3,1]benzothiazines (Scheme 1) have been synthesized from 2,6-diarylidene-4-methyl cyclohexanone (IIA-H), characterized by IR, ^{1}HNMR and mass spectroscopy. The final compounds were investigated for their anticonvulsant activity. Thiazines are found to exhibit a variety of pharmacological activities such as anticonvulsant[1], anti-inflammatory[2], analgesic[3], antitumor[4], antimicrobial[5,6], antidiabetic[7], tuberculostatic and circulatory activities[8]. Epilepsy is a group of disorders of central nervous system characterized by excessive electrical discharge in brain, which causes seizures.

Address for correspondence

Dr. M. Srinivasa Murthy*

Department of Pharmaceutical Chemistry, Vignan Institute of Pharmaceutical Sciences, Near Ramoji Film City, Deshmukhi, -508284, Telangana, India,
Email ID:msmurthy1093@gmail.com
Ph: +91- 9866399799

It has been estimated that nearly 50 million people were suffering from epilepsy across the globe[9]. Epilepsy is usually controlled, but not cured, with medication, although surgery may be considered in difficult cases. However, over 30% of people with epilepsy do not have seizure control even with the best available drugs. Not all epilepsy syndromes are life long; some forms are confined to particular stages of childhood. Therefore, the need for more effective and less toxic antiepileptic drugs is highly imperative.

By considering all these facts it was planned to synthesize title compounds with an objective to get new potent anticonvulsant agents.

MATERIALS AND METHODS

Melting points of all synthesized compounds were determined in open capillary tubes on an electro thermal apparatus and are uncorrected. The reaction progress and purity of the compounds was checked by TLC on silica gel coated aluminium plates (Merck) as adsorbent. The spots were observed in iodine chamber and the distance travelled by spots was measured by using transparent ruler. IR spectra (ν_{max} cm^{-1}) were recorded on a BRUKER ATR-IR spectrophotometer in the range of 4000-400 cm^{-1} using attenuated total reflectance technique.^{1}HNMR spectra were recorded on a

INOVA (400 MHz) NMR spectrometer using $CDCl_3$ as solvent and TMS as an internal standard (chemical shifts in δ ppm). Mass spectra were recorded on a VG Autospec MS using ESI mode positive ion trap detector. The drugs and chemicals were purchased from Sigma-Aldrich, India. All other chemicals and solvents were obtained from local firms (India) and were of highest purity and analytical grade.

EXPERIMENTAL

The reactant and product melting points were different from each other. It clearly indicates the formation of new chemical entities. All the synthesized compounds were observed as single spot on TLC plates and the R_f values of intermediate and final products were found different which confirms the purity of products and completion of reaction. The IR and ^{1}HNMR spectral values were in good agreement with the structure of the synthesized compounds and the mass spectral values also correspond to their calculated molecular weight.

Synthesis Procedures

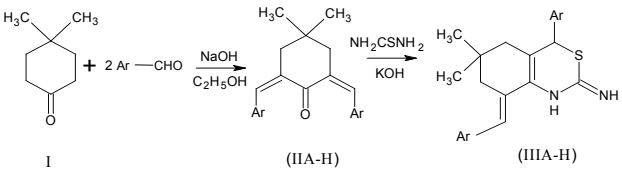
General procedure for the synthesis of 2,6-diarylidene-4,4-dimethylcyclohexanone (IIA-H)[10]:

A mixture of 10% sodium hydroxide, ethyl alcohol, 4,4-dimethylcyclohexanone (0.01mol) and aromatic aldehyde (0.02mol) was stirred at 20-25˚C for 2h. Later the reaction mixture was kept in an ice chest over night. The product was filtered, washed with ice cold water followed by ice cold ethanol, dried and recrystallized from DMF. The physical data of the compounds were given in the table 1.

General procedure for the synthesis of 4-aryl-8-arylidene-5,6-dihydro-2-imino-6,6-dimethyl-4H,7H-[3,1] benzothiazines (IIIA-H)[11]:

A mixture of 2,6-diarylidene cyclohexanone derivative (0.01 mol), thiourea (0.015 mol) and potassium hydroxide (0.01 mol) dissolved in 10ml of water was refluxed in isopropyl alcohol for 16h. Later the solvent was removed under reduced pressure and the residue obtained was treated with ice cold water, filtered dried and recrystallized from ethanol. The physical data of the compounds were given in the table 1.

Scheme 1: Schematic representation of synthesized compounds (IIIA-H)



I (IIA-H) (IIIA-H)

PHARMACOLOGICAL EVALUATION

The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) of Bharat Institute of Technology (Pharmacy), Hyderabad, Andhrapradesh with CPCSEA Registration No: 1015/c/06/CPCSEA.

Anticonvulsant activity

Animals

Albino Wistar rats of either sex weighing 180–220g, were placed in polypropylene cages with paddy husk as bedding in a controlled room temperature 24 ± 2°C, relative humidity 30–70% and provided with food and water *ad libitum* were used for acute oral toxicity study. The animals were starved for 24h prior to experimentation but fed with tap water throughout. All studies were carried out by using six rats in each group.

Acute oral toxicity studies

All the synthesized compounds viz. (IIIA-H) were tested for acute toxicity[12] by following OECD guidelines 420 after obtaining ethical clearance from animal ethics committee. Albino Wistar rats were (180-220g) fasted for 24h and divided into 3 groups of six animals each. The test compounds, suspended in sodium carboxy methyl cellulose (CMC) solution (0.5%w/v) were administered orally in doses of 5mg/kg, 50mg/kg, 300mg/kg and 2000mg/kg. None of the test compound had shown toxicity even at 2000 mg/kg, p.o. No signs of toxicity, weight variation, mortality were observed during initial 48h of observation. Thus the cut off LD_{50} was $1/10^{th}$ of 2000mg/kg i.e. 200mg/kg was selected for each test compound.

Maximal electroshock method[13]

The maximal electroshock (MES) was used to induce the convulsions. The stimulus was applied via ear clip electrodes and the current shock of 150mA for 0.25sec was applied and the convulsions of animals of different groups in different stages were compared. Male albino Wistar rats (180-220g) were treated with synthesized compounds (IIIA-H) (200mg/kg) orally using oral feeding needle. CMC treated animal served as control and phenytoin (20mg/kg, orally) was administered as standard drug to a group of six animals. The pharmacological effects were noted for myoclonic flexion, extension, clonus, stupor and recovery. Similarly for the standard drug, values were recorded.

RESULTS AND DISCUSSION

The physical constants and characterization data of all the synthesized compounds reveals their successful synthesis. The structures of new compounds prepared during the present investigation have been authentically established by their IR, ^{1}HNMR and mass spectral studies. The result of anticonvulsant effect of newly

synthesized derivatives against MES induced convulsions are shown in table 2. The one way ANOVA analysis of the data observed indicated that the test compounds exhibited significant anticonvulsant effect against MES induced seizures. Control group animals exhibited hind limb tonic extension (HLTE) of 11.24±0.020sec. after the delivery of an electroshock. IIIE showed no effect on decreasing total duration of HLTE while IIIA, IIIB, IIIC, IIID, IIIF and IIIG abolished HLTE. Whereas the compounds IIIA and IIIF has significantly decreased the duration of all phases of convulsions such as flexion, extension, clonus, stupor and recovery exhibiting potent anticonvulsant activity. Statistically significant results were observed for compounds IIIA, IIIB, IIIC, IIID, IIIF and IIIG with $P<0.001$ using one way ANOVA, followed by student's t test where $P<0.001$, $P<0.01$ and $P<0.05$ was considered statistically significant.

Spectral data of 4-(3-nitrophenyl)-8-(3-nitrobenzylidene)-5,6-dihydro-2-imino-6,6-dimethyl-4H,7H-[3,1]-benzothiazine IIIA:

IR: 3395 (imine N-H stretching); 3101 (cyclic N-H stretching); 1081 (C-N stretching); 1525, 1447 (C=C stretching); 1616 (C=N stretching). ^{1}HNMR ($CDCl_3$): δ0.9 (s, $(CH_3)_2$, 6H), δ1.7 (d, CH_2, 2H), δ1.9 (d, CH_2, 2H), δ4.9 (s, -CH-S, 1H), δ8.7 (s, imine, 1H), δ7.4-8.4 (m, ArH, 8.0H), δ6.9 (s, cyclic NH, 1H), δ7.1 (s, bezylic H, 1H) MS-m/z 451 (M+).

Spectral data of 4-(4-fluorophenyl)-8-(4-fluorobenzylidene)-5,6-dihydro-2-imino-6,6-dimethyl-4H,7H-[3,1]-benzothiazine IIIB:

IR: 3371 (imine N-H stretching); 3160 (cyclic N-H stretching); 1015 (C-N stretching); 1546, 1494 (C=C stretching); 1597 (C=N stretching); 2948 (aliphatic C-H stretching). ^{1}HNMR ($CDCl_3$): δ0.9 (s, $(CH_3)_2$, 6H), δ1.7 (d, CH_2, 2H), δ1.9 (d, CH_2, 2H), δ4.9 (s, -CH-S, 1H), δ7.7 (s, imine, 1H), δ6.6 (s, cyclic NH, 1H), δ7.2-7.6 (m, ArH, 8H, benzylic H, 1H), MS-m/z 396(M+).

Spectral data of 4-(4-ethylphenyl)-8-(4-ethylbenzylidene)-5,6-dihydro-2-imino-6,6-dimethyl-4H,7H-[3,1]-benzothiazine IIIC:

IR: 3589 (imine N-H stretching); 3176 (cyclic N-H stretching); 1022 (C-N stretching); 1544, 1471 (C=C stretching); 1611 (C=N stretching); 2956 (aliphatic C-H stretching). ^{1}HNMR ($CDCl_3$): δ0.9 (s, $(CH_3)_2$, 6H), δ1.3 (t, $(CH_3)_2$, 6H), δ2.3 (d, CH_2, 2H), δ2.5 (d, CH_2, 2H), δ2.9 (q, $(CH_2)_2$, 4H), δ4.9 (s, -CH-S, 1H), δ7.6 (s, imine, 1H), δ6.6 (s, cyclic NH, 1H), δ7.2-7.5 (m, ArH, 8H), δ6.8 (s, bezylic H, 1H), MS-m/z 417(M+).

Spectral data of 4-(4-isopropylphenyl)-8-(4-isopropylbenzylidene)-5,6-dihydro-2-imino-6,6-dimethyl-4H,7H-[3,1]-benzothiazine IIID:

IR: 3445 (imine N-H stretching); 3189 (cyclic N-H stretching); 1017 (C-N stretching); 1544, 1465 (C=C stretching); 1606 (C=N stretching). ^{1}HNMR ($CDCl_3$): δ0.9 (s, $(CH_3)_2$, 6H), δ1.2 (d, $(CH_3)_4$, 12H), δ2.3-2.5 (m, $(CH)_2$, 2H), δ1.7 (d, CH_2, 2H), δ1.9 (d, CH_2, 2H), δ4.9 (s, -CH-S, 1H), δ7.7 (s, imine, 1H), δ6.6 (s, cyclic NH, 1H), δ7.2-7.5 (m, ArH, 8.0H), δ6.8 (s, bezylic H, 1H), MS-m/z 445(M+).

Spectral data of 4-(3,4-dimethoxybenzylidene)-8-(3,4-dimethoxybenzylidene)-5,6-dihydro-2-imino-6,6-dimethyl-4H,7H-[3,1]-benzothiazine IIIE:

IR: 3239 (imine N-H stretching); 3140 (cyclic N-H stretching); 1021 (C-N stretching); 1513, 1440 (C=C stretching); 1602 (C=N stretching); 2910, 2813 (aliphatic C-H stretching); 1063 (C-O-C stretching). ^{1}HNMR ($CDCl_3$): δ0.9 (s, $(CH_3)_2$, 6H), δ1.8 (d, CH_2, 2H), δ1.9 (d, CH_2, 2H), δ4.8 (s,-CH-S, 1H), δ8.2 (s, imine, 1H), δ6.0 (s, cyclic NH, 1H), δ6.2 (s, bezylic H, 1H), δ6.4 (s, ArH, 1H), δ6.7 (d, ArH, 1H), δ6.8 (d, ArH, 1H), δ7.1 (d, ArH, 1H), δ7.2 (s, ArH, 1H), δ7.7 (d, ArH, 2H), 3.8 (s, 6H, 2×OCH_3), 4.1 (s, 6H, 2×OCH_3), MS-m/z 482(M+1).

Spectral data of 4-(4-hydroxyphenyl)-8-(4-hydroxybenzylidene)-5,6-dihydro-2-imino-6,6-dimethyl-4H,7H-[3,1]-benzothiazine IIIF:

IR: 3498 (O-H stretching); 3271 (imine N-H stretching); 3168 (cyclic N-H stretching); 1015 (C-N stretching); 1536, 1491 (C=C stretching); 1608 (C=N stretching); 2908, 2868 (aliphatic C-H stretching). ^{1}HNMR ($CDCl_3$): δ0.9 (s, $(CH_3)_2$, 6H), δ1.6 (d, CH_2, 2H), δ2.0 (d, CH_2, 2H), δ4.9 (s, -CH-S, 1H), δ8.6 (s, imine, 1H), δ7.4-8.2 (m, ArH, 8.0H), δ6.6 (s, cyclic NH, 1H), δ8.9 (s, $(4\text{-}OH)_2$, 2H), δ7.1 (s, bezylic H, 1H), MS-m/z 393(M+).

Spectral data of 4-(2,4-dihydroxyphenyl)-8-(2,4-dihydroxybenzylidene)-5,6-dihydro-2-imino-6,6-dimethyl-4H,7H-[3,1]-benzothiazine IIIG:

IR: 3462 (O-H stretching); 3385 (imine N-H stretching); 3105 (cyclic N-H stretching); 1025 (C-N stretching); 1563,1494 (C=C stretching); 1603 (C=N stretching); 2984, 2851 (aliphatic C-H stretching). ^{1}HNMR ($CDCl_3$): δ0.9 (s, $(CH_3)_2$, 6H), δ1.8 (d, CH_2, 2H), δ2.2 (d, CH_2, 2H), δ4.9 (s, -CH-S, 1H), δ8.2 (s, imine, 1H), δ7.0-8.0 (m, ArH, 6H), δ6.2 (s, cyclic NH, 1H), δ8.9 (s, $(4\text{-}OH)_2$, 2H), δ6.7 (s, bezylic H, 1H), δ9.2 (s, (2-OH), 2H), δ9.4 (s, (4-OH), 2H), MS-m/z 425(M+).

Spectral data of 4-(4-N,N-dimethylphenyl)-8-(4-N,N-dimethylbenzylidene)-5,6-dihydro-2-imino-6,6-dimethyl-4H,7H-[3,1]-benzothiazine IIIH:

IR: 3258 (imine N-H stretching); 3159 (cyclic N-H stretching); 1089 (C-N stretching); 1569, 1480 (C=C stretching); 1615 (C=N stretching); 2964, 2881 (aliphatic C-H stretching). ^{1}HNMR ($CDCl_3$): δ0.9 (s, $(CH_3)_2$, 6H), δ1.7 (d, CH_2, 2H), δ1.9 (d, CH_2, 2H), δ1.2 (s, $(CH_3)_4$, 12H), δ4.9 (s, -CH-S, 1H), δ6.5 (s, cyclic NH, 1H), δ7.2-7.9 (m, ArH, 8H and imine H, 1H), δ6.7 (s, bezylic H, 1H), MS-m/z 447(M+).

Table-1: Physical data of the compounds (IIA-H) and (IIIA-H)

Compound. no.	Ar	M.F	M.W	M.P (˚C)	R_f value	Yield (%)
IIA	*m*-Nitrophenyl	$C_{22}H_{20}N_2O_5$	392.4	140-142	0.80	77
IIB	*p*-Fluorophenyl	$C_{22}H_{20}F_2O$	338.3	115-117	0.89	95
IIC	*p*-Ethylphenyl	$C_{26}H_{30}O$	358.5	175-177	0.87	79
IID	*p*-Isopropylphenyl	$C_{28}H_{34}O$	386.5	193-195	0.85	90
IIE	*m,p*-Dimethoxyphenyl	$C_{26}H_{30}O_5$	422.5	122-124	0.78	72
IIF	*p*-Hydroxyphenyl	$C_{22}H_{22}O_3$	334.4	182-184	0.70	86
IIG	*o,p*-Dihydroxyphenyl	$C_{22}H_{22}O_5$	366.4	150-152	0.73	76
IIH	*p* -Dimethylaminophenyl	$C_{26}H_{32}N_2O$	388.5	120-122	0.69	78
IIIA	*m*-Nitrophenyl	$C_{23}H_{22}N_4O_4S$	450.5	210-212	0.46	78
IIIB	*p*-Fluorophenyl	$C_{23}H_{22}F_2N_2S$	396.4	280-282	0.59	90
IIIC	*p*-Ethylphenyl	$C_{27}H_{32}N_2S$	416.6	270-272	0.45	86
IIID	*p*-Isopropylphenyl	$C_{29}H_{36}N_2S$	444.6	260-262	0.50	83
IIIE	*m,p* -Dimethoxyphenyl	$C_{27}H_{32}N_2O_4S$	480.6	188-190	0.52	84
IIIF	*p*-Hydroxyphenyl	$C_{23}H_{24}N_2O_2S$	392.5	212-214	0.58	82
IIIG	*o,p* -Dihydroxyphenyl	$C_{23}H_{24}N_2O_4S$	424.5	223-225	0.47	65
IIIH	*p*-Dimethylaminophenyl	$C_{27}H_{34}N_4S$	446.6	140-142	0.62	65

Table-2: Anticonvulsant activity of synthesized compounds (IIIA-H)

Groups	Time (sec.) in various phases of convulsions (mean±SEM)				
	Flexion	**Extension**	**Clonus**	**Stupor**	**Recovery**
Control	3.05±0.016	11.24±0.020	15.56±0.765	95.34±0.663	124.16±0.149
Standard	1.30±0.261***	0.0***	8.77±0.524**	51.56±0.238**	82.25±0.892***
IIIA	2.00±0.034**	0.00***	10.26±0.903	56.75±0.663**	80.65±0.402**
IIIB	9.00±0.205	0.00***	30.00±0.936	40.00±0.134***	60.96±0.218***
IIIC	9.66±0.657	0.00***	17.50±0.752	56.66±0.264**	101.24±0.229
IIID	12.16±0.021	0.00***	15.83±0.876	60.50±0.700*	202.33±0.453
IIIE	3.47±0.308	11.44±0.276	13.65±0.376	90.78±0.478	140.80±0.739
IIIF	8.90±0.043	0.00***	8.94±0.163**	52.89±0.648**	71.67±0.532**
IIIG	7.89±0.185	0.00***	10.23±0.198	62.00±0.724	110.44±0.413
IIIH	3.44±0.204	5.19±0.875*	12.55±0.635	71.65±0.238	109.94±0.876

N=6, Values are Mean±SEM; values are compared with control group. P<0.001*, P<0.01** and P<0.05* was considered statistically significant.**

CONCLUSION

New thiazine derivatives were synthesized and evaluated for the anticonvulsant activity. Some of these derivatives were shown to be fairly effective. Presence of nitro, hydroxyl and fluoro substituent has shown better anticonvulsant activity than other compounds.

ACKNOWLEDGEMENT

The authors thank Prof. Dr. N. Devanna, Director, OTRI, JNTUA, Ananthapuramu for the encouragement and facilities provided to carry out this research work. We also thank the IICT, Hyderabad for providing spectral data.

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How to cite this article:
Bharath Rathna Kumar P, Srinivasa Murthy M*, Jayaveera K.N: synthesis, characterization and evaluation of anticonvulsant activity of some new 4-aryl-8-arylidene-5,6-dihydro-2-imino-6,6-dimethyl-4H,7H-[3,1] benzothiazine derivatives : 5(4): 2199 -2203. (2014)