Chemistry Research Journal, 2022, 7(3):125-130
Available online www.chemrj.org

Research Article

ISSN: 2455-8990
CODEN(USA): CRJHA5

Synthesis and Anticonvulsant Activity of Novel Arylidene Hydrazide Derivative

Shalini Agarwal, Anju Goyal

Faculty of Pharmacy, B N University, Udaipur, Rajasthan 313001

Abstract
A novel Arylidene Hydrazides derivative was synthesised from o-chloro benzoic acid and aniline via Ulmann reaction. The series of chemical reaction was yielded novel Arylidien Hydrazide. The chemical structures of the synthesized molecules was confirmed by elemental and spectral (IR, ^{1}H NMR, and MS) analysis. The anticonvulsant activity of the compound was investigated using maximal electroshock seizure and subcutaneous pentylenetetrazole (scPTZ) model, showed significant activity. The results of the present study validated that the pharmacophore model with four binding sites is essential for anticonvulsant activity.

Keywords: Arylidene Hydrazides, Anti conversant, hydrazides derivative

Introduction

Epilepsy is a continual neurological sickness characterised by means of the periodic unexpected loss or impairment of consciousness, often observed through convulsions. approximately 50 million humans globally have epilepsy, with nearly 90% of those human beings in developing countries [1]. This disease can happen to any age group or any gender. Epilepsy additionally affects about 4% of people over their lifetime. despite the improvement of numerous new anticonvulsants, over 30% of human beings with epilepsy do no longer have seizure control and others do so best on the expense of sizeable dose-related toxicity and strange adverse consequences [2-5]. For that reason, there may be a large need for the synthesis of more powerful and safer antiepileptic drugs. The conformational research into the clinically active anticonvulsant drugs which includes phenytoin, carbamazepine, lamotrigine, rufinamide, and phenobarbitone has resulted a popular version for anticonvulsant activity Considering this poor efficacy and side effects within the latest years have brought about the development of numerous more recent and promising drugs like stiripentol, zonisamide, tiagabine, levetiracetam, topira mate, etc. with better seizure controlling efficacy. But, those newer capsules additionally convey serious and damaging side effects such as hepatotoxicity, anorexia, gastrointestinal disturbances, and hirsutism [6–10]. consequently, research is envisaged for improvement of anticonvulsant agents with higher advantage or risk ratio.

Material: Infrared (IR) spectra were recorded on an Agilent Cary 630 Fourier transform infrared (FTIR) spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AVANCE III HD 400 MHz spectrometer using TMS as an internal standard. DMSO was used as the solvents for dissolving the samples for NMR. All

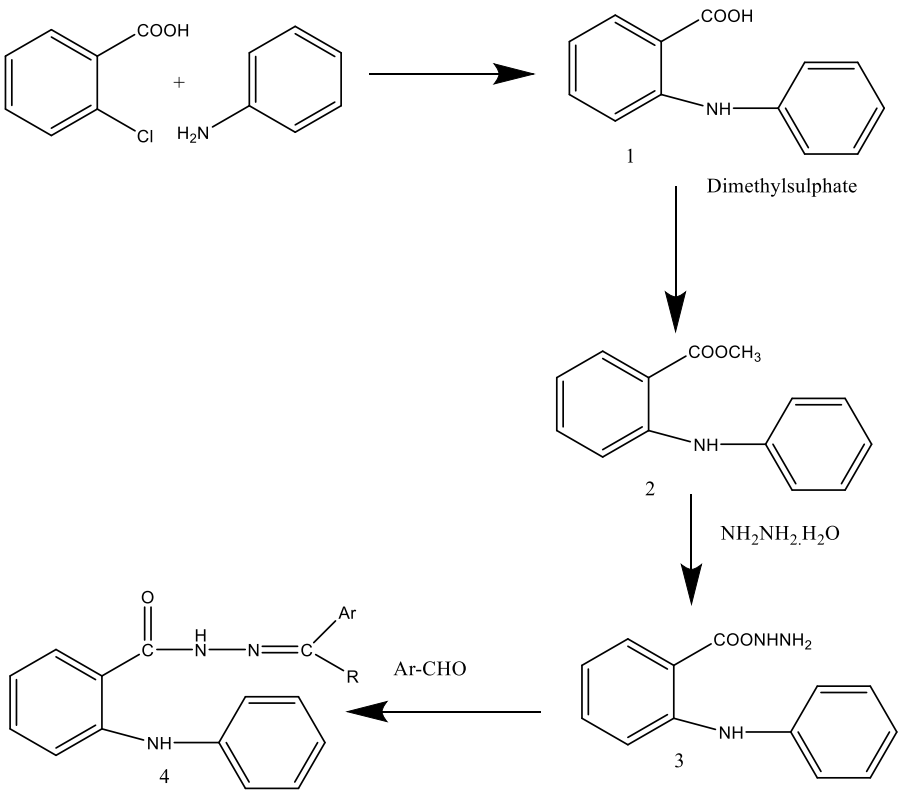

Figure 1: Scheme for Synthesis of N'-substituted arylidene-2-(phenylamino) benzohydrazide derivative

reactions were carried out using dry glassware. The chemicals used in this work were obtained from Sigma-Aldrich and Spectrochem Pvt. Ltd. and were used without further purification. Commercial grade solvents were used. Analytical hin-layer chromatography (T.L.C.) was performed on silica gel coated on aluminum sheets and was monitored using ultraviolet light of wavelength 254 nm. Column chromatography was performed on 60–120 mesh silica gel. Compounds were eluted by a mixture of hexane and ethyl acetate as required percentage.

Methods

Step 1: Synthesis of N-phenyl anthranilic acid ($C_{13}H_{11}NO^2$)

A mixture of o-chloro benzoic acid (1 mole), aniline (1 mole), copper power (0.2 g), and potassium carbonate (10 g) in 60 ml of iso-amyl alcohol were refluxed on a water bath for 8 h with occasional stirring. After the completion of the reaction, the mixture was allowed to cool at room temperature. The reaction mixture was filtered and acidified with conc. hydrochloric acid. The precipitate so obtained was again filtered and washed with hot water. The crude acid was dissolved in 0.1 N sodium hydroxide solution and reprecipitated by adding conc. hydrochloric acid. The crude acid was filtered and washed with water. The dried crude product (I) was recrystallized with alcohol. The completion of the reaction was monitored by T.L.C. with Mobile phase: n-hexane: ethyl acetate (6:4), melting point: 189°C, yield: 4.2 g (42%).

Step 2: Synthesis of methyl 2-(phenyl amino) benzoate ($C_{14}H_{13}NO_2$)

A solution of N-phenyl anthranilic acid (1 mole) in acetone was refluxed with dimethyl sulfate (2 mole) and anhydrous potassium carbonate (0.02 mole) on a water bath for 90 min. After the completion of the reaction, the reaction mixture was allowed to cool at room temperature and inorganic salt was filtered off. The filtrate was concentrated and after cooling to room temperature, poured into crushed ice. The precipitate so formed was filtered and washed with water, dried, and recrystallized (II) with methanol. The completion of the reaction was monitored by T.L.C. Melting point: 50°C, yield: 8.5 g (85%).

Step 3: Synthesis of 2-phenyl amino benzoic acid hydrazide ($C_{13}H_{13}N_3O$)

A solution of methyl 2-(phenyl amino) benzoate (3 mole) was dissolved in ethanol and refluxed with 99% hydrazine hydrate (8 mole) on a water bath for 5–6 h. After the completion of the reaction, the reaction mixture was allowed to cool at room temperature and poured into a beaker containing crushed ice. The reaction mixture was allowed to stand for 1 h. The precipitate so formed was filtered and washed with water. The crude product was dried and recrystallized with ether. The completion of the reaction was monitored by T.L.C. and melting point: 116°C, yield: 6.5 g (92.8%).

Step 4: Synthesis of 2-phenylamino benzoic acid (3-phenylallylidene) hydrazide (Compound 4)

The reaction between 2-(phenylamino) benzohydrazide and an aldehydes (unimolar quantity) was done for 1 h at room temperature. The progress of the reaction was checked by performing T.L.C. in the solvent mixture of petroleum ether and ethyl acetate in the ration of 7:3. The target compounds were purified by column chromatography at different solvent mixture of petroleum ether and ethyl acetate in the ration of 7:3. The resulted compounds was characterized by IR, ^{1}H-NMR, mass, and melting point.

Molecular formula: $C_{22}H_{19}N_3O$, Yield (85%), bluish. M.P.–180°C. FTIR (KBr) νmaxcm-1: 3457.29 (N-H elongation), 3051.32 (Ar. C-H elongation), 1670.33 (C=O elongation), 1638.41 (Ar.C=C elongation), 1528.25 (C=N elongation), 1317.71 (C-N elongation), 1030.59 (Ar. C-C elongation). ^{1}H-NMR (500 MHz, Chloroform-d): δ(in ppm): 11.32 (1H, s) 8.64 (1H, s), 8.48 (2H, d) 8.37 (1H, d), 7.99 (2H, d), 7.70 (2H, d), 7.60, 2H), 7.39 (2H, d), 7.36 (2H, d), 6.98 (1H, s), 6.88 (1H, s), 6.10 (1H, s), 5.60 (1H, s), 4.56 (1H, s). ESI MS of $C_{22}H_{19}N_3O$ found is 341.41

Pharmacological evaluation:

The synthesized compound was evaluated for anticonvulsant activity following the standard procedures proposed by OECD guidelines 423 on Wistar albino rats. Wistar albino rats of either sex weighing 185-210 g were divided into different groups as per the requirements, each containing five animals. The rats were fasted for 18 hours, with water and libitum.

The animals were administered with solution of synthesized compound in distilled water and Tween 20 mixture. The aqueous solutions of 2% Tween 20 were administered to rats orally. Group 01 was kept as untreated control. Group 02-05 were administered orally with a dose of 25, 50, 100 and 200 mg/kg body weight respectively. After administration of different compounds, animals were observed for 24 hrs for the different parameters i.e. behavioural paramerts, hypersensitivity reactions, tremor, anxiety etc. After screening, Anticonvulsant activity will be screened against MES and PTZ induced convulsions on group of six albino rats either sex. The activity will be compared with standard Phenytoin.

MES test Seizures were elicited with a 150-mA for 0.2sec in mice. The current was applied via corneal electrodes for 0.2 s. Protection against the spread of MES-induced seizures was defined as the abolition of the hind leg and tonic maximal extension component of the seizure. 30 min after the administration of the compounds, the activities were evaluated in MES test [11,12,13].

PTZ-induced seizure [14,15] 30 min after the administration of the synthesized compound at various doses, the animals were given a subcutaneous dose of pentylenetetrazole (85 mg/kg), a dose at which 100% of the animals showed convulsive reactions. The dose which prevented 50% of the treated animals from tonic convulsions (ED_{50}) was then calculated.

Resuls

Acute Toxicity Studies

After conduction of acute toxicity studies of synthesized compound, It was found that the animals treated by 25 mg/kg do not produce any toxic effects in rats while on increasing the dose i.e. 50, 100 and 200 mg/kg, they showed a significant hypersensitivity reactions and some behavioral abnormalities. So for the entire study, It was decided to select 25 mg/kg dose for further in vivo evaluation. Analysis of results when compared with control

(3.83±0.6) indicates there is a significant reduction in flexion at dose 25 mg/kg(3.83±0.71, 2.83±0.22). Phenytoin which is used as standard has shown (3.51±0.76). The test compound has shown significant statistical protection (P<0.001). Similarly in case of Extension Clonus and Stupor, the synthesized compound produced a significant change or significant statistical protection (P<0.001).

Table 1: The Effect of MES Induced convulsions of synthesized compound

Compound	Dose mg/kg b.w	Route of Administration	Time (Sec) in various phases of convulsions (Mean+ SEM)				Recovery/ Death
			Flexion	Extensor	Clonus	Stupor	
Control (Salaine)		Oral	3.83±0.6	11.16±1.60	3.50±0.88	110.66±6.92	Recovery
Standard Phenytoin	25	Oral	3.51±0.76	00±00	00±00	00±00	Recovery
Compound 4	25	Oral	3.83±0.71**	00±00	00±00	22.83±5.67***	Recovery

Data analysed using oneway ANOVA followed by dunnet's test, values are Mean±SEM, N=6, ***p<0.05, compared with std group

Pentylenetetrazol (PTZ) Induced Convulsions

Analysis of results when compared to control (78.66±6.29) indicate there is increase in

Table 2: The Effect of PTZ Induced convulsions of synthesized compound

Drug	Dose mg/kg b.w	Route of Administration	Onset of convulsions (Sec. ± SEM)			Mortality %
			Onset (Time in Sec)	No. of Animal Survived	No. of Animal Convulsed	
Control PTZ	80	Intraperitoneal	78.66±6.29	0/6	6/6	100.0%
Standard Diazepam+PTZ	4+80	Intraperitoneal	00±00	6/6	0/6	0%
Compound 4	25	Oral	215.68±3.29***	6/6	2/6	0%

Data analysed using oneway ANOVA followed by dunnet's test, values are Mean±SEM, N=6, ***p<0.05, compared with std group

latency of seizures at dose 25 mg/kg (215.68±3.29, 209.36±3.12).But the onset of Clonic convulsions is abolished by standard drug diazepam.

Discussion

Epilepsy is characterized by recurrent episodes of seizures. A seizure is due to abnormal discharge of some neurons in the brain. Antiepileptic drugs may have a stabilizing influence on neuronal membrane; prevent detonation of normal brain cells by the focal discharge, these drugs act only on those neurons which are firing repeatedly. Some drugs reduce low threshold Ca^{++} current and abolish absence seizures whereas some drugs increase GABA activity in the synapse causing neuronal inhibit ion hence antiseizure effect [16, 17]. The ability of compound to prevent MES is believed to correlate with its ability to prevent spread of seizure discharge through neural tissue. Whereas the ability of compound to prevent threshold seizures (PTZ), has been correlated with the ability to raise the threshold for excitation of neural tissue. Inhibition of the MES test predicts activity against generalized tonic-clonic and cortical focal seizures, lack of activity against MES induced seizures suggests that the drugs are effective in suppressing seizures [18].

It has often been stated that antiepileptic drugs that block MES-induced tonic extension act by blocking voltage dependent Na+ channels. In the present study compound showed significant results in MES model. PTZ seizure threshold is well acknowledged animal model used for screening anticonvulsant effects of various chemical entities, represent the petitmal type of seizures and this has been primarily utilized as animal model to evaluate the

anti epileptic drugs and is known to block the post synaptic GABA receptor mediated Cl- conductance and thus produce seizures. GABA is an important endogenous inhibitory neurotransmitter widely distributed throughout the CNS. As far as the GABA is concerned, the following facts support its involvement. After the acute toxicity study, and screened for the anti-convulsant activity by using MES and PTZ-induced convulsion in albino rats, It lead to the conclusion that the synthesized compound was showed significant anticonvulsant activity when compared to standard phenytoin and diazepam, results shown in table respectively [19,20] .

Conclusion

In this paper, it is described the multicomponent, synthesis of a novel Arylidene hydrazides derivative in short reaction times and from easily affordable starting material and anti-convulsant activity. The result of anti-convulsant study indicated mild to moderate Anti-convulsant activity.

Acknowledgments: The authors are thankful to Faculty of Pharmacy, B N University, Udaipur for providing research facilities to pursue this work.

Reference

[1]. Fisher R., Boas W.V.E., Blume W., Elger C., Genton P., Lee P., Engel J. (2005) Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). Epilepsia;46:470–472.

[2]. Brown T.R., Holmes G.L. (2001) Primary care: epilepsy. N Engl J Med;344:1145–1151.

[3]. Pandeya S.N., Ponnilavarasan I., Pandey A., Lakhan R., Stables J.P. (1999) Evaluation of p-nitrophenyl substituted semicarbazones for anticonvulsant properties. Pharmazie;54:923–925.

[4]. Wong M.G., Dejina J.A., Andrews P.R. (1986) Conformational analysis of clinically active anticonvulsant drugs. J Med Chem;29:562–571.

[5]. Aggarwal N., Mishra P. (2004) Synthesis of 4-aryl substituted semicarbazones of some terpenes as novel anticonvulsants. J Pharm PharmaceutSci;7:260–264.

[6]. K. J. Meader, J. (2003) Clin. Psychiatry, 64, 30–34.

[7]. H. Stefan, T. J. Feuerstein(2007) Pharmacol. Ther., 113, 165–183.

[8]. J. R. Pollard, J. French (2006) Lancet Neurol., 5, 1064– 1067.

[9]. E. J. Donner, O. C. Snead (2006) NeuroRX , 3, 170–180.

[10]. M. Bialer, S. I. Johannessen, H. J. Kupferberg, R. H. Levy, E. Perucca, T. Tomson, (2004) Epilepsy Res., 61, 1–48.

[11]. Neeraj C, Bhupinder Singh S. (2011) An overview of advances in the standardization of herbal drugs. Journal of Pharmaceutical Education and Research. 2(2): 55-70.

[12]. Kalpana J, Preeti C, Dnyaneshwar W, Bhushan P. (2004) Molecular markers in herbal drug technology. Current Science. 87:159-165.

[13]. Pravin HN, Joseph K, Aruna J, Vilasrao K. (2012) Future Trends in Standardization of Herbal Drugs. Journal of Applied Pharmaceutical Science. 2(06):38-44.

[14]. Vishnukanta, Rana AC. Evaluation of anticonvulsant activity of *plumbagozeylanica*Linn leaf extracts. Asian Journal of Pharmaceutical and Clinical Research. 2010; 3(1):76-78.

[15]. Duraisami R, Srinivasan D, Ramaswamy S. (2009) Anticonvulsant activity of bioflavonoid gossypin. Journal of the Bangladesh Pharmacological Society. (4):51-54.

[16]. Tripathi KD. (2003) Anti-epileptic Drugs. Essential of Medical Pharmacology 5th edition, Jaypee Brothers, Medical Publishers, New Delhi. 369-373.

[17]. Raymond Adams, Maurice, Victor, Allan H. Rooper. (1997) Epilepsy and other seizure disorders, Chapter 16, 'Principles of neurology', 6th Edition, McGraw-Hill Health Profession Divisions, .

[18]. Karunakar H, Shalin PT, Arun BJ, Shastry CS, Chandrashekhar KS. (2009) Anticonvulsant Activity of *Carissa carandas*Linn. Root Extract in Experimental Mice. Tropical Journal of Pharmaceutical Research. 8 (2): 117-125.

[19]. Amole OO, Yemitan OK, Oshikoya KA. (2009) Activity of *Rauvolfia Vomitoria* (Afzel). African Journal of Pharmacy and Pharmacology 3(6):319-322.

[20]. Mohammad J, Khoshnood M, Mahmood Reza M, Narjes O**.** (2010) Anticonvulsant Activity of *Teucriumpolium*Against Seizure Induced by PTZ and MES in Mice**.** Iranian Journal of Pharmaceutical Research. 9 (4):395-401.