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Design and Biological Evaluation of Novel Arylidene Hydrazides Derivatives for Anticonvulsant Activity

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Abstract

The aim of present study was to design and synthesized a novel series of compound which can be evaluate for anticonvulsant, anti-microbial activity. This novel derivatives of Arylidene Hydrazides (4a-4k) were synthesised from o-chloro benzoic acid and aniline via Ulmann reaction. The synthetic method used was simple, rapid and economical found to be accurate and reproducible. The series of chemical reaction was yielded as 11 novel Arylidiene Hydrazide according to the scheme. The chemical structures of the synthesized molecules were confirmed by different lab as well as instrumental analysis like TLC, IR, NMR, and MS. All the synthesised compounds were evaluated for anticonvulsant activity by using two different models on Wistar albino rats. The most common model for anticonvulsant activities are maximal electroshock seizure and subcutaneous pentylenetetrazole (scPTZ) which we were applied for the same. The title compounds 4a & 4b exhibited anticonvulsant potency against all the two screens methods with actuate toxicity. After completion of experiments the SAR of molecule suggest that it must be have site as hydrophobic aryl ring system, (HBD) hydrogen binding domain, (D) electron donor moiety, (C) distal aryl ring.

Keywords: Arylidene Hydrazides, Anticonvulsant activity, hydrazides derivatives

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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 being 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]. For that reason, there may be a large need for the improvement 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 inside the thought of a popular version for anticonvulsant activity[3-5]. considering this poor treatment, efforts within the latest years have brought about the development of numerous more recent and promising drugs like Stiripentol, Zonisamide, Tiagabine, Levetiracetam, Topiramate, (Figure 1) etc. with better seizure controlling efficacy. but, those newer capsules additionally convey serious and damaging side results 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.

As we had mention our aim to prepare antimicrobial and anticonvulsant compound by simple method. On this series We had prepared some novel compound for the same, the bio-screening data revealed that 4b, 4f, 4g, 4h, and 4j compounds exhibited good antibacterial activity against Gram-positive Bacillus subtilis (MTCC441), Bacillus cereus (MTCC-7190) and Gram-negative Escherichia coli and Aspergillus fumigates (ATTC 9197) where 4a and 4c showed good antifungal activity against Candida albicans. Among the compounds, 4a and 4c exhibited good antioxidant activity too.[11]

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Material

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 Thin-layer chromatography (T.L.C.) was performed on silica gel coated on aluminium 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. 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 reactions were carried out using dry glassware.

Methods

Experimental Chemistry: The novel compound were prepared according to scheme as mention in scheme (Figure 2)

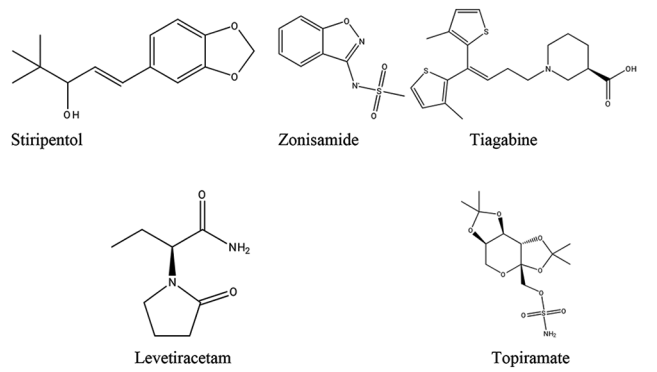

Figure 1: Newer drugs for anticonvulsant

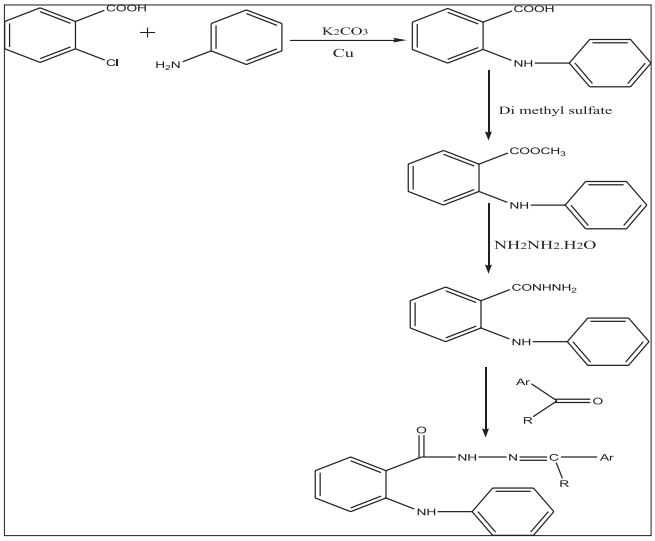

Figure 2: scheme for synthesised Novel Arylidene Hydrazides Derivatives, R: different aldehydes/ketones

Step 1

Synthesis of N-phenyl Anthranilic acid ($C_{13}H_{11}NO_2$): A mixture of o-chloro benzoicacid (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.[12,13] 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.[14] 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 (8mole) 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%).[15]

Step 4

General synthesis of Arylidene hydrazide: The reaction between 2-(phenylamino) benzohydrazide and different 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 (IV a-k) were characterized by IR, 1H-NMR, mass, and melting point.[16]

Compound 4 a: 2-phenylamino benzoic acid (3-phenylallylidene) hydrazide: Molecular formula: $C_{22}H_{19}N_3O$, Yield (85%), bluish. M.P.–180°C. FTIR (KBr) V_{max}^{cm-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.

Compound 4 b: 2-Phenylamino benzoic acid (benzylidene) hydrazide: Molecular formula: $C_{20}H_{17}N_3O$, Yield (85%), brown. M.P.–160°C. FTIR (KBr) $V_{max}^{cm-1:}$ 3446.59 (N-H elongation), 3049.26 (Ar. C-H elongation), 1669.2 (C=O elongation), 1636.42 (Ar.C=C elongation), 1526.92 (C=N elongation), 1318.29 (C-N elongation), 1028.28 (Ar. C-C elongation). ^{1}H-NMR (500 MHz, Chloroform-d): δ (in ppm): 11.12 (1H, s) 8.74 (1H, s), 8.56 (2H, d) 8.28 (2H, d), 8.19 (1H, d), 7.71 (2H, d), 7.69 (2H, d), 7.60 (2H, d), 7.39 (2H, d), 7.32 (2H, d), 6.99 (1H, s), 6.70(1H, s), 6.0 (1H, s), 4.56 (1H, s). ESI MS of $C_{20}H_{17}N_3O$ found is 315.37.

Compound 4 C: 2-phenylaminobenzoic acid (2-hydroxybenzylidene hydrazide: Molecular formula: $C_{20}H_{17}N_3O_2$, yield (85%), brown. M.P.– 160°C. FTIR (KBr) V_{max}^{cm-1}:3455.46 (N-H elongation), 3290.42 (Ar O-H elongation), 3047.44 (Ar. C-H elongation), 1667.49 (C=O elongation), 1635.67 (Ar C=C elongation), 1525.29 (C=N elongation), 1316.48 (C-N elongation), 1027.36 (Ar.

Table 1: The Effect of MES Induced convulsions of different synthesized compounds

Drug	Dose mg/kg	Route of Administration	Time (Sec) in various phases of convulsions (Mean+SEM)				Recovery/Death
			Flexion	Extensor	Clonus	Stupor	
Control (Saline)		Oral	3.83±0.6	11.16±1.60	3.50±0.88	110.66±6.92	R
Std Phenytoin	25	I.P.	3.51±0.76	00±00	00±00	00±00	R
4a	25	Oral	3.83±0.71**	00±00	00±00	22.83±5.67***	R
4b	25	Oral	2.83±0.22***	00±00	00±00	28.33±1.17***	R
4c	25	Oral	3.23±0.55	6.22±0.23	3.33±0.12	30.35±2.41	R
4d	25	Oral	3.45±0.55	2.64±0.21	3.44±0.35	41.46±3.12	R
4e	25	Oral	3.11±0.58	5.59±0.34	3.61±0.11	52.85±1.44	R
4f	25	Oral	3.23±0.63	2.47±0.11	3.12±0.81	33.42±2.24	R
4g	25	Oral	3.56±0.41	4.35±0.59	3.32±0.25	31.15±3.11	R
4h	25	Oral	3.47±0.58	3.13±0.64	3.16±0.13	40.25±2.38	R
4i	25	Oral	3.59±0.41	9.63±0.51	3.22±0.22	34.11±2.12	R
4j	25	Oral	3.53±0.89	4.38±0.29	3.41±0.11	52.42±1.22	R
4k	25	Oral	3.44±0.34	2.99±0.27	3.86±0.34	31.32±2.41	R

Data analysed using oneway ANOVA followed by dunnet's test, values are Mean±SEM, N=6***p<0.05, compared with standard group, R: Recovery, D: Death

Table 2: The Effect of PTZ Induced convulsions of different synthesized compounds

Drug	Dose mg/kg b.wg	Route of Administration	Onset of convulsions (Sec.±SEM)			Mortality%
			Onset (Time in Sec)	No. Of Animal Survived	No. of Animal Convulsed	
Control PTZ	80	I.P.	78.66±6.29	0/6	6/6	100.0%
Standard Diazepam + PTZ	4+80	I.P.	00±00	6/6	0/6	0%
4a	25	Oral	215.68±3.29***	6/6	2/6	0%
4b	25	Oral	209.36±3.12***	6/6	2/6	0%
4c	25	Oral	178.36±3.29	6/6	5/6	0%
4d	25	Oral	132.51±4.33	6/6	3/6	0%
4e	25	Oral	110.84±5.11	6/6	4/6	0%
4f	25	Oral	96.96±2.29	6/6	5/6	0%
4g	25	Oral	85.22±6.63	6/6	6/6	100%
4h	25	Oral	145.36±4.45	6/6	5/6	0%
4i	25	Oral	196.45±3.85	6/6	3/6	0%
4j	25	Oral	56.85±4.94	6/6	4/6	0%
4k	25	Oral	126.26±3.67	6/6	6/6	100%

Data analyse during oneway ANOVA followed by dunnet's test, values are Mean±SEM, N=6***p<0.05, compared with std group I.P.: Intraperitoneal

C-C elongation). ^{1}H-NMR (500 MHz, Chloroform-d): δ (in ppm):11.12 (1H, s), 8.74 (1H, s), 8.56 (2H, d), 8.28 (2H, d), 8.19 (1H, d), 7.71 (2H, d), 7.69 (2H, d), 7.60 (2H, d), 7.39 (2H, d), 7.32 (2H, d), 6.99 (1H, s), 6.70 (1H, s), 6.0 (1H, s), 4.56 (1H, s). ESI MS of $C_{20}H_{17}N_3O_2$ found is 315.37.

Compound 4 d: 2-phenylaminobenzoic acid (4-hydroxybenzylidene) hydrazide: Molecular formula: $C_20H_{17}N_3O_2$, yield (91%), dark yellow. M.P.-216°C. FTIR (KBr) $V_{max}^{cm-1:}$ 3454.39 (N-H elongation), 3289.52 (Ar O-H elongation), 3046.56 (Ar. C-H elongation), 1666.36 (C=O elongation), 1634.66 (Ar C=C elongation), 1524.24 (C=N elongation), 1315.66 (C-N elongation), 1026.36 (Ar. C-C elongation). ^{1}H-NMR (500 MHz, Chloroform-d): δ (in ppm):11.28 (1H, s), 9.82 (1H, s), 8.92 (1H, s) 8.28 (2H, d), 8.22 (2H, d), 8.19 (2H, d), 7.74 (2H, d), 7.60 (2H, d), 7.59 (2H, d), 6.50 (1H, s), 4.51 (1H, s). ESI MS of $C_20H_{17}N_3O_2$ found is 315.37.

Compound 4 e: 2-phenylaminobenzoic acid (4-chlorobenzylidene) hydrazide: Molecular formula: $C_{20}H_{16}$ ClN_3O, yield (86%), black. M.P.–180°C.FTIR (KBr) νmaxcm-1: 3453.78 (N-H elongation), 3044.74 (Ar. C-H elongation),1663.43 (C=O elongation), 1633.36 (Ar.C=C elongation), 1523.98 (C=N elongation), 1323.65 (C-N elongation), 1025.52 (Ar. C-C elongation), 712.59 (Ar. C-Cl elongation). ^{1}H-NMR (500 MHz, Chloroform-d): δ(in ppm): 11.22 (1H, s) 8.98 (1H, s), 8.32 (2H, d) 8.22 (2H, d), 8.19 (1H, d), 7.78 (2H, d), 7.59 (2H, d), 6.83 (2H, d), 6.83 (2H, d), 6.83 (2H, d), 6.38 (1H, s), 4.63 (1H, s). ESI MS of $C_{20}H_{16}$ ClN_3O found is 349.81.

Compound 4 f: 2-phenylamino benzoic acid (4-hydroxy-3-methoxybenzylidene) hydrazide: Molecular formula: $C_{21}H_{19}N_3O_3$, yield (89%), red. M.P.– 160°C. FTIR (KBr) $V_{max}^{cm-1:}$ 3443.78 (N-H elongation), 3287.31 (Ar. O-H elongation), 3046.79 (Ar.C-H elongation), 2834.79 (C-O-CH3 elongation), 1661.36 (C=O elongation), 1630.92 (Ar.C=C elongation),1524.23 (C=N elongation), 1351.61 (C-N elongation), 1023.59 (Ar. C-C elongation). ^{1}H-NMR (500 MHz, Chloroform-d): δ (in ppm): 11.27 (1H, s) 9.93 (1H, s), 8.97 (1H, d) 8.56 (1H, s), 8.39 (1H, s), 8.22 (1H, s), 7.60 (2H, m), 7.52 (2H, m), 7.36 (2H, m), 6.89 (2H, m), 6.79 (2H, m), 6.50 (1H, s), 4.66 (1H, s), 4.60 (3H, S). ESI MS of $C_{21}H_{19}N_3O_3$ found is 361.39.

Compound 4 g: 2-phenylamino benzoic acid (2-bromo-3-hydroxy-4-methoxybenzylidene) hydrazide: Molecular formula: $C_{21}H_{18}BrN_3O_3$ Yield (86%), light yellow. M.P.– 235°C. FTIR (KBr) $V_{max}^{cm-1:}$ 3421.61 (N-H elongation), 3283.28 (Ar O-H elongation), 3041.36 (Ar. C-H elongation), 2830.38 (C-O-CH3 elongation), 1659.42 (C=O elongation),1629.59 (Ar.C=C elongation), 1528.38 (C=N elongation), 1362.44 (C-N elongation), 1020.42 (Ar. C-C elongation), 648.98 (Ar. C-Br elongation). ^{1}H-NMR (500 MHz, Chloroform-d): δ (in ppm): 11.368 (s, 1H, NH-N), 9.864 (s, 1H, Ar. OH), 8.989 (s, 1H, N=CH), 8.989 (s, 1H, N=CH), 8.021–8.366 (d, 2H, Ar. H), 7.428–7.862 (m, 4H, Ar. H), 6.261–6.787 (m, 5H, Ar. H), 4.686 (s, 1H, NH), δ 3.648 (s, 3H, OCH3), ESI MS of $C_{21}H_{18}BrN_3O_3$ found is 440.29.

Compound 4 h: 2-phenylamino benzoic acid (4-dimethylamino benzylidene) hydrazide: Molecular formula: $C_{22}H_{22}N_4O$, Yield (92%), yellow, M.P.–242°C. FTIR (KBr) $V_{max}^{cm-1:}$ 3438.51 (N-H elongation), 3046.62 (Ar. C-H elongation), 1661.12 (C=O elongation),1639.24(Ar C=C elongation),1523.94 (C= elongation),1317.92 (C-N elongation),1024.44 (Ar. C-C elongation),739.29 (C-N elongation

2 amine). ^{1}H-NMR (500 MHz, Chloroform-d): δ (in ppm): 11.369 (s, 1H, NH-N). 8.913 (s, 1H, N=CH), 8.101–8.468 (dd, J=9.15, 4H, Ar. H), 7.379–7.746 (m, 4H, Ar. H), 6.366–6.848 (m, 5H, Ar. H), 4.789 (s, 1H, NH), 3.016 (ds, 6H, N-(CH3)2), ESI MS of $C_{22}H_{22}N_4O$ found is 358.18.

Compound 4 i: 2-Phenylaminobenzoic acid (3hydroxy-4-methoxybenzylidene) hydrazide: Molecular formula: $C_{21}H_{19}N_3O_3$, yield (88%), brown. M.P.– 206°C. FTIR (KBr) $V_{max}^{cm-1:}$ 3441.87 (N-H elongation), 3281.39 (Ar O-H elongation), 3040.97(Ar C-H elongation), 2828.32 (C-O-CH3 elongation), 1660.92 (C=O elongation),1628.29 (Ar.C=C elongation), 1526.46 (C=N elongation), 136365 (C-N elongation), 1020.66 (Ar. C-C elongation). ^{1}H-NMR (500 MHz, Chloroform-d): δ (in ppm): 11.348 (s, 1H, NH-N), 9.896 (s,1H, Ar. OH), 8.918 (s, 1H, N=CH),8.696 (s, 1H, Ar. H), 8.131-8.261 (d, 2H, Ar. H), 7.481-7.868 (m, 4H, Ar. H) 6.332-6.816 (m, 5H, Ar. H), 4.743 (s, 1H, NH), 3.611 (s, 3H, OCH3). ESI MS of C21H19N3O3 found is 361.26.

Compound 4 j: 2-phenylamino benzoic acid (3,5 di-tertbutyl-2-hydroxy benzylidene) hydrazide: Molecular formula: $C_{28}H_{33}N_3O_2$ Yield (75%), light yellow, M.P.–196°C. FTIR (KBr) $V_{max}^{cm-1:}$ 3423.82 (N-H elongation), 3280.89 (Ar. O-H elongation), 3039.49 (Ar C-H elongation), 2876.49 (Aliphatic C-H elongation), 1657.17 (C=O elongation), 1627.95 (Ar C=C elongation), 1525.52 (C=N elongation), 1368.21 (C-N elongation), 1019.29 (Ar. C-C elongation). ^{1}H-NMR (500 MHz, Chloroform-d): δ (in ppm): 11.352 (s, 1H, NH-N), 9.818 (s, 1H, Ar. OH), 8.996 (s, 1H, N=CH), 8.789 (s, 1H, Ar. H), 8.585 (s, 1H, Ar. H), 7.332–7.722 (m, 4H, Ar. H), 6.341–6.818 (m, 5H, Ar. H), 4.717 (s, 1H, NH), 2.348 (ts, 9H, C-(CH3)3), 2.089 (ts, 9H, C-(CH3)3). ESI MS of $C_{28}H_{33}N_3O_2$ found is 329.15.

Compound 4 k: 2-phenylaminobenzoic acid (1-phenylethylidene) hydrazide: Molecular formula: $C_{21}H_{19}N_3O$ yield (75%), light yellow, M.P.–196°C. FTIR (KBr) $V_{max}^{cm-1:}$ 3420.87 (N-H elongation), 3038.46 (Ar. C-H elongation),2875.65 (Aliphatic C-H elongation), 1656.56 (C=O elongation), 1619.59 (Ar C=C elongation), 1528.18 (C=N elongation), 1371.78 (C-N elongation), 1017.96 (Ar. C-C elongation). ^{1}H-NMR (500 MHz, Chloroform-d): δ (in ppm): 11.391 (s, 1H, NH-N), 8.768 (s, 1H, N=CH), 8.022-8.551 (m, 5H, Ar. H), 7.369–7.791 (m, 4H, Ar. H), 6.331–6.818 (m,5H, Ar. H), 4.614 (s, 1H, NH), 2.161 (s, 3H, C-CH3). ESI MS of $C_{21}H_{19}N_3O$ found is 443.26.

Pharmacological Evaluation

The synthesized compounds were 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.

Acute Toxicity

The animals were administered with solution of synthesized compounds in distilled water and Tween 20 mixture. The aqueous solutions of 2% Tween 20 were administered to rats orally. The dose was administered by gavage using a stomach tube. Group 1 was kept as untreated control. Group 2-12 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. behavioral parameters, 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.[17,18,19]

PTZ-induced seizure[20,21] 30 min after the administration of the synthesized compounds at various doses, the animals were given a subcutaneous dose of pentylenetetrazole (85 mg/kg), a dose at which 100% of the naıve animals showed convulsive reactions. The dose which prevented 50% of the treated animals from tonic convulsions (ED50) was then calculated.

Pharmacological Result: Acute Toxicity Studies

After conduction of acute toxicity studies of all synthesized compounds, we 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 behavioural abnormalities.

So for the entire study, we have decided or selected 25 mg/kg dose for the further in vivo evaluation. Analysis of results when compared with control (3.83±0.6) indicates 4a & 4bshowed 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 4a and 4b produced a significant change or significant statistical protection ($P<0.001$) (Table 1).

Pentylenetetrazol (PTZ) Induced Convulsions

Analysis of results when compared to control (78.66±6.29) indicate there is increase in 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 (Table 2).

Discussion

Chemistry

Arlidene hydrazides have received the attention of chemists due to their wide range of biological activities, which anticonvulsant, antifungal and antibacterial activities. In the present study it was envisaged that a drug molecule possessing the above mentioned pharmacophore could be of advantage since it might possess anticonvulsant, antifungal and antibacterial activities.

The synthesis of the title compounds was effected as outlined in the scheme. O-chloro benzoic acid was refluxed with aniline in presence of potassium carbonate and copper via Ulmann Reaction to give N-phenyl anthranilic acid. The reaction of N phenyl anthranilic acid with dimethyl sulphate in the presence of potassium carbonate yielded methyl 2- (phenyl amino) benzoate. This esterified product of N-phenyl anthranilic acid on reaction with hydrazine hydrate gave 2-phenyl amino benzoic acid hydrazide. Now this hydrazide derivative on reaction with substituted aromatic aldehyde/ketone yielded Arylidene hydrazides as title compounds (4a-4k).

All the title compounds have been characterized by their analytical and spectral data and all data agreed with proposed structure of synthesized compounds (4a-4k). All the compound showed 1700 cm^{-1} range for starching of C=O in IR band which indicated the link between aryl ring to amide chain. The appearance of IR band around 3200 cm^{-1} showed the presence of NH linkage of amide bond of hydrazide derivatives. The presence of peaks slightly above or below 2900 cm^{-1} showed the presence of an aromatic portion in the synthesized compounds.

The appearance of singlet ranging from δ 8.41- 9.325 indicated the presence of NH of hydrazide derivatives. The presence of aromatic protons was confirmed by the multiplte signal in the range of δ 6.50-7.90 ppm.

According to different studies a molecule for good anticonvulsant activity needs four essential binding sites: (A) hydrophobic aryl ring system, (HBD) hydrogen binding domain, (D) electron donor moiety, (C) distal aryl ring. Figure 3

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 where as some drugs increase GABA activity in the synapse causing neuronal inhibit ion hence anti-seizure effect.[22, 23] The ability of compound to prevent MES is believed to correlate with it stability 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 or 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.[24]

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 formulation 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, the various extracts were screened individual for the anti-convulsant activity by using MES and PTZ-induced convulsion in albino rats, lead to the conclusion that the synthesized compound i.e. 4a & 4b were showed significant anticonvulsant activity when compared to standard phenytoin and diazepam, results shown in table respectively.[25,26] Hence, 4a & 4b were selected for designing the possible modern formulations to exploit their anticonvulsant activity.

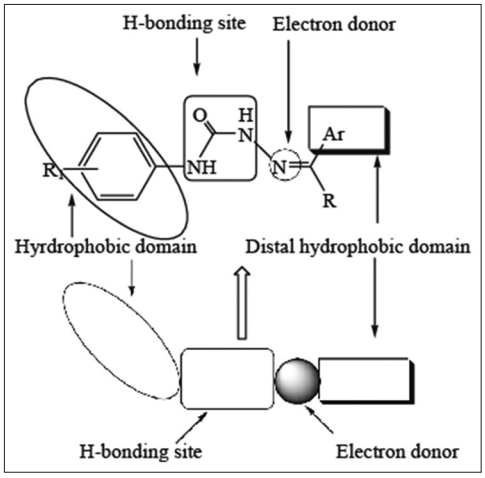

Figure 3: Pharmacophore model for anticonvulsant activity

Conclusion

A number of strategic derivatives of novel arylidene hydrazides derivatives were synthesized and evaluated for anticonvulsant activity using MES and scPTZ screens. Two of them synthesised compounds showed good in vivo seizure control.

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