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Synthesis and evaluation of some new substituted 1,4-dihydro pyridine derivatives and their anticonvulsant activity

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Abstract

A series of substituted 1, 4-Dihydropyridine derivatives were synthesized and the structures of these compounds were established on the basis of spectral and elemental analysis. All the compounds were evaluated for anti convulsant activity by Maximal Electroshock Induced convulsions in Rats, PTZ induced convulsions in Rats, and Strychnine induced Convulsions in Rats methods. Compounds A_1,A_2,A_3,A_4,B_1,B_2,B_3,B_4 have been found to exhibit anti-convulsant activity.

Key words: 1, 4-Dihydropyridine, Anti-convulsant, CHN analysis.

Introduction

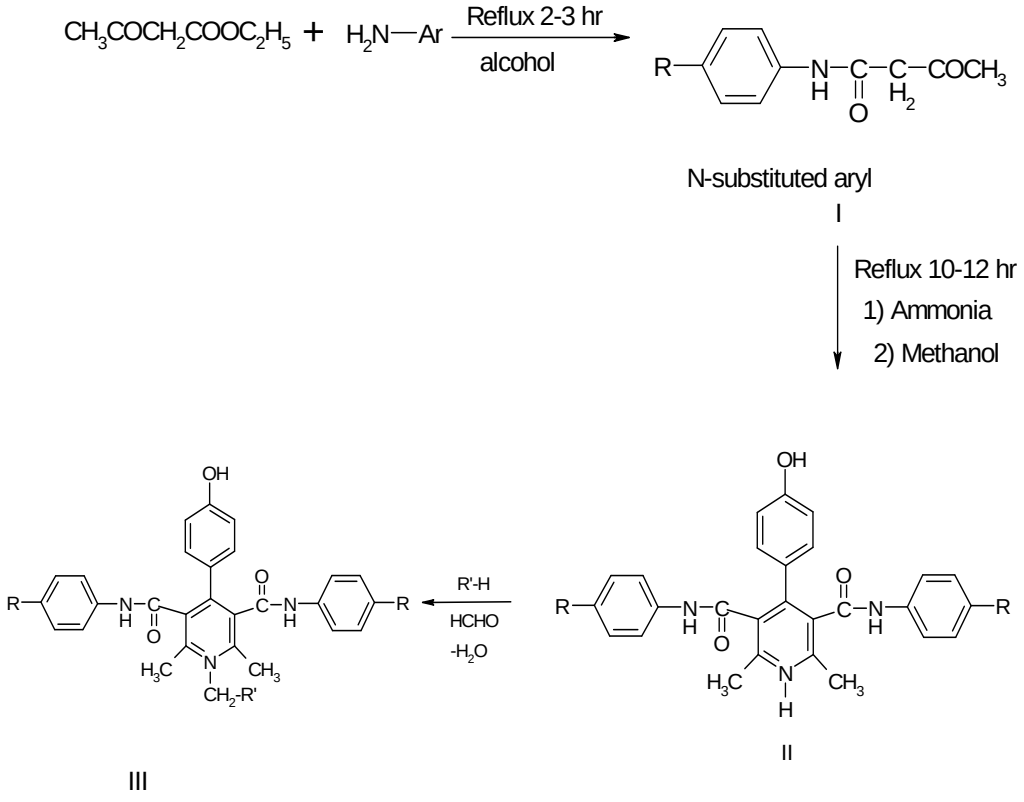
The word “Epilepsy” is derived from the Greek, meaning ‘to take hold of, seizer [1]. An epileptic seizure is a transient paroxysm of uncontrolled discharges of neurons causing an event that is discernible by the person experiencing the seizure and/or by the observer [2]. Epilepsy affects approximately 1% of the whole worldwide population and is the second most common Neurologic disorder after stroke. The incidence is highest in the first 10 years of life and declines thereafter through the age of 50 until the elderly years when the incidence increases again. Epilepsy begins before the age of 18 in over 75% of patients. [3] A recent meta-analysis of published and unpublished studies puts the overall prevalence rate of epilepsy in India at 5.59 per

1,000 populations, with no statistically different rates between men and women or urban or rural residence.1, 4-Dihydropyridine exhibit wide range of pharmacological activities like anti tubercular, anti-inflammatory, antibacterial, anti-convulsant and antifungal. These observations promoted us to synthesis the title compounds with presumption that incorporation of using diketones, substituted aldehydes and aromatic amines with pyridine nuclei would produce new compounds with significant anti-convulsant activity.

Materials and Methods

Melting points were determined in open capillary method and are uncorrected. IR spectra were recorded on Thermo Nicolet IR 200 spectrophotometer using KBr disc method. The ^{1} H-NMR spectra were recorded on sophisticated multinuclear FT-NMR Spectrometer model Advance – II (Bruker) using DMSO as a solvent and TMS as internal standard. The purity of the compounds was determined by TLC using suitable solvent system.

Scheme:



Synthesis of N – substituted aryl acetoacetamide **(I)** [5]

An equimolar amount of ethyl acetate and different aryl amine were taken in the round bottom flask and dissolved in alcohol and refluxed for about 3 hr.The reaction mixture was cooled. The solid that separated out was filtered, washed with cold water and dried. The crude solid product was purified by recrystallization twice from alcohol to give colourless crystals.

***Synthesis of 1, 4-Dihydropyridine* (II) [**5]
N- Substituted arylacetamide (0.01 moles) was dissolved in methanol and p-hydroxy benzaldehyde (0.05 moles) was added followed by addition of excess of ammonia (25%). The reaction mixture was mechanically stirred for 10 min. and then heated on water bath under reflux for 10-12 hr. Methanol was removed under reduced pressure and collected. The product was separated, filtered and wash with methanol. It was purified by recrystallization from alcohol to give yellowish crystalline compound.

Synthesis of 1, 4-dihydropyridine derivatives by mannich bases [6]
A mixture of 0.01 mol of 1, 4-dihydro-2, 6-dimethyl-4-[4-(hydroxylphenyl) {3, 6-(4, 4 disubstituted benzamide)} pyridine], 0.01 mole of different amines like 2-(2, 6-dichloro phenyl amino) benzohydrazide\2-hydroxybenzohydra-zide\pyrazine-2-carbohydrazide\5-amino tetra-zole and 0.02 mole of formaldehyde were taken in 15ml of alcohol and refluxed for 2hrs.then kept it overnight. The product thus separated was filtered and it was purified by recrystallization from alcohol.

Table no.1: Analytical data of synthesized compounds

Compd	Chemical formula	% Yield	M.P. ^{0}C	Mole wt.	Elemental Analyses Found (Calcd.)		
					C	H	N
A_1	$C_{44}H_{42}O_4N_6Cl_2$	72	112-14	799	70.47 (70.86)	5.71 (5.22)	11.11 (11.32)
A_2	$C_{36}H_{36}O_5N_6$	75	260-62	598	65.34	5.07	9.40
A_3	$C_{34}H_{34}O_4N_7$	65	129-31	604	71.20 (7.03)	5.75 (5.56)	14.60 (14.45)
A_4	$C_{30}H_{31}O_2N_8$	58	156-58	561	66.07	5.50	19.89
B_1	$C_{42}H_{37}O_4N_6Cl_4$	79	106-08	787	62.40 (62.92)	4.45 (4.87)	10.40 (10.17)
B_2	$C_{35}H_{32}O_5N_4Cl_2$	70	126-28	658	59.22	4.11	9.80
B_3	$C_{32}H_{27}O_3N_6Cl_2$	80	102-04	613	62.33	4.38	13.63
B_4	$C_{29}H_{27}O_3N_8Cl_2$	78	120-22	605	66.66 (66.58)	4.12 (4.32)	18.48 (18.22)

Spectral Data
A_1 : IR (KBr) (cm^{-1}): 3251 (O-H str.); 3035 (N-H str.);2919(Ar C-H str.); 1646 (C=O str.); 839 (Ar. C-H def.).^{1}H NMR (DMSO) δ in ppm: 10.56 (1H, s, OH); 8.69-10.40(5H, m, 5NH); 6.63-8.33 (20H, m, ArH); 4.45(2H s, CH_2); 2.13-2.45(12H, m, $4CH_3$).

A_2 : IR (KBr) (cm^{-1}): 3247 (O-H str.); 3079(N-H str.); 2927(Ar C-H str.); 1645 (C=O str.); 841(Ar. C-H def.).

A_3 : IR (KBr) (cm^{-1}): 3249 (O-H str.); 3024 (N-H str.); 2921(Ar C-H str.); 1646 (C=O str.); 893-C-H def; 844 C-F; 840 (Ar. C-H def.). ^{1}H NMR (DMSO) δ in ppm: 10.63 (1H,s,OH); 8.53-10.23(5H,m,5NH); 6.67-8.42 (16H, m, ArH); 4.43(2H s, CH_2); 2.17-2.28(12H, m, $4CH_3$)

A_4 : IR (KBr) (cm^{-1}): 3307(O-H str.); 3094(N-H str.); 2980(Ar C-H str.); 1703 (C=O str.); 838 (Ar. C-H def.); 808-C-H def; 787 C-F str.

B_1 : IR (KBr) (cm^{-1}): 3266 (O-H str.); 3134 (N-H str.); 2977(Ar C-H str.); 1651(C=O str.); 824 (Ar. C-H def.).

B_2 : IR (KBr) (cm^{-1}): 3278 (O-H str.); 3178 (N-H str.); 2669(Ar C-H str.); 1650 (C=O str.); 827 (Ar. C-H def.). ^{1}H NMR (DMSO) δ in ppm: 10.59 (1H,s,OH); 8.60-10.34(4H,m,4NH); 6.68-8.38 (17H, m, ArH); 4.47(2H s, CH_2); 2.20-2.49(6H, m, $2CH_3$).

B_3 : IR (KBr) (cm^{-1}): 3260 (O-H str.); 3139(N-H str.); 2925(Ar C-H str.); 1650 (C=O str.); 823 (Ar. C-H def.).

B_4 : IR (KBr) (cm^{-1}): 3429(O-H str.); 3112 (N-H str.); 2975(Ar C-H str.); 2975(Ar C-H str.); 826 (Ar. C-H def.). ^{1}H NMR (DMSO) δ in ppm: 10.61 (1H, s, OH); 8.67-10.43(4H, m, 4NH); 6.72-8.39 (13H, m, ArH); 4.42(2H s, CH_2); 2.17-2.45(6H, m, $2CH_3$),

Anticonvulsant Activity

A) Maximal Electroshock Induced Convulsions in Rats [4]

The Anticonvulsant property of the drug in this model was assessed by its ability to protect against Maximal Electroshock Induced Convulsions. The animals were first weighed and were selected for the experiment depending on the weight. The rats were then divided into seven groups of six rats each. Group 1 was the control group; Group 2 received 20-mg/kg b.w of Phenobarbitone sodium; Groups 3, 4, 5, 6, 7, received 200-mg/kg b.w. of C_1, C_2, C_3, C_5, and C_8 respectively, which was prepared by dissolving in 1% Tween 80.Maximal electroshock (Inco Electroconvulsiometer model # 100-3) of 150mA current for 0.2 seconds was applied through ear electrodes to induce convulsions in the control and test compound treated animals. The drugs and chemicals were prepared fresh; the concentration, dose and the duration before induction of convulsions were as follows:

B) Pentylenetetrazole- Induced Convulsions in Rats [4]

The anticonvulsant property of the drug in this model was assessed by its ability to protect against PTZ induced convulsions. The method used was as described by Kulkarni S.K.The animals were first weighed and were selected for the experiment depending on the weight. Rats of either sex were used. The rats were then divided into seven groups of six rats each.Pentylenetetrazole (80 mg/kg, body weight) was administered intraperitoneally to induce convulsions in the control and drug treated animals. Group 1 was the control group; Group 2 received 4-mg/kg b.w of Diazepam; Groups 3, 4, 5, 6, 7, received 200-mg/kg b.w. of C_1, C_2, C_3, C_5, and C_8 respectively, which were prepared by dissolving in 1% Tween 80.The drugs and chemicals were prepared fresh; the concentration, dose and the duration before induction of convulsions were as follows: The onset time for action, jerky movements and convulsions were noted down for each group. The starting time for each phase was first noted and then converted to duration of each phase by negating the starting time of one phase from the starting time of the previous phase.

C) Strychnine Induced Convulsions in Rats [4]

The anticonvulsant property of the drug in this model was assessed by its ability to protect against Strychnine induced convulsions. The method used was as described by Kulkarni S.K. *et* al.The animals were first weighed and were selected for the experiment depending on the weight. Rats of either sex were used. The rats were then divided into seven groups of six rats each. Group 1 was the control group; Group 2 received 4-mg/kg b.w of Diazepam; Groups 3, 4, 5, 6, 7, received 200-mg/kg b.w. of C_1, C_2, C_3, C_5, and C_8 respectively, which was prepared by dissolving in 1% Tween 80. Strychnine (4mg/kg body weight) was administered to control and drug treated animals, which produced powerful opisthotonus tonic convulsions of the body and limbs. The drugs and chemicals were prepared fresh; the concentration, dose and the duration before induction of convulsions were as follows.

Table no.2A: Maximal electroshock induced convulsions of some prototype of compounds.

Groups	Time (Sec) in various phases of convulsions (Mean±SEM)				
	Flexion	**Extension**	**Clonus**	**Stupor**	**Recovery**
Control	8.000±0.2582	12.33±0.6667	4.833±0.4014	23.83±0.7923	287.5±1.784
Standard	6.500±0.3416*	6.500±0.3416**	3.833±0.6009*	4.167±0.4773**	99.67±1.745**
A_1	4.167±0.4014**	8.333±0.3333**	3.500±0.4282*	6.167±1.0140**	118.2±4.269**
A_2	2.667±0.2108**	8.167±0.3073**	4.833±0.3073*	5.333±0.4944**	76.83±1.493**
A_3	2.833±0.4014**	8.667±0.4944**	5.167±0.3073*	2.667±0.3333**	105.7±1.358**
A_4	2.783±0.1952***	7.872±0.4374***	2.870±0.3982***	1.9833±0.3982***	132.3±1.356***
B_1	3.000±0.2582**	9.000±0.5774**	3.500±0.4282*	2.833±0.4014**	127.3±1.453**

Note: n= 6, *p< 0.05, **p< 0.01, ***p<0.001 compared with control (one-way ANOVA followed by Dunnett's test.)

Table no.2 B: Pentylenetetrazole induced convulsion

Groups	Onset time in Seconds (Mean±SEM)		
	Action	**Jerky Movements**	**Convulsions**
Control	211.5±48.87	68.17±1.447	75.83±1.973
Standard	0.0±0.0**	0.0±0.0**	0.0±0.0**
A_1	285.5 ± 2.473^{NS}	593.2±3.89**	719.0±4.81**
A_2	305.3±4.507*	602.7±4.485**	727.2±5.250**
A_3	297.5±4.448*	590.2±3.429**	701.3±12.07**
A_4	289.7±3.782***	523.7±1.223***	780.48±1.287***
B_1	291.3±4.432*	581.0±3.777**	679.2±20.83**
B_2	287.1±35.07	56.17±1.87	667.1±1.93
B_3	211±20.23	49.5±2.49	704±5.10
B_4	275.14±4.37	53.2±3.29	775.4±1.28

Note: n= 6, *p< 0.05, **p< 0.01, ***p<0.001. NS – Non-significant compared with control (one-way ANOVA followed by Dunnett's test)

Table no.2 C: Strychnine induced convulsion of prototype of compounds

Treatment Group	Onset of Convulsions (mins)	Death time (mins)	% Mortality
Control	1.698±0.01424	4.592±0.01537	100
Standard	0.0±0.0**	0.0±0.0**	0
A_1	25.17±0.1562**	28.01±0.3043**	100
A_2	25.36±0.2660**	29.11±0.1836**	100
A_3	23.74±0.1651**	27.12±0.05782**	100
A_4	24.37±0.1586***	26.92±0.1783***	100
B_1	26.09±0.06154**	29.92±0.05251**	100
B_2	24.97±0.255	26.01±0.311	100
B_3	27.45±0.33	28.01±0.412	100
B_4	26.5±0.43	27.01±0.366	100

Note: n= 6, *p< 0.05, **p< 0.01, ***p<0.001 compared with control (one-way ANOVA followed by Dunnett's test.)

Table No. 3: Anti-convulsant activity

A_1	273.15±24.07	566.24±2.97	113±1.19
A_2	267.09±29.09	524.13±1.43	709±1.13
A_3	279.09±20.07	567.14±1.13	711±1.29
A_4	249.03±19.04	577.09±1.09	698±1.11
B_1	253.01±17.09	523.02±1.20	701±1.47
B_2	277.04±11.08	497.03±1.19	747±1.13
B_3	288.01±14.09	445.04±1.17	766±1.04
A_1	293.01±11.02	551.05±1.56	697±1.12

Results and Discussion

The compounds were synthesized as per the scheme-I, where 1, 4-Dihydropyridine derivatives were synthesized by reacting N- Substituted aryl acetamide and p-hydroxy benzaldehyde followed by mannich reaction. The structures of synthesized compounds were confirmed by IR, NMR and CHN analysis. All these compounds were evaluated for anti-convulsant activity by known standard procedure (MES).it is very interesting to note that substituted 1, 4-Dihydropyridine derivatives have proved to be promising anticonvulsant agents. All the result obtained for anticonvulsant activity by one-way ANOVA followed by Dunnett's test. With the continuous efforts for the molecular modification of such substituted 1, 4-Dihydropyridine derivatives may prove for their promising anticonvulsant activities.

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