Research Article

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PHARMACOLOGICAL SCREENING OF N³, N⁵-DIPHENYL-1, 4-DIHYDROPYRIDINE-3, 5-DICARBOHYDRAZIDE DERIVATIVES FOR *IN VIVO* ANTICONVULSANT AND SEDATIVE ACTIVITY

Asma Samaunnisa A^{1*}, Riazuddin Mohammed², C.H.S. Venkataramana¹, V Madhavan³

¹Department of Pharmaceutical chemistry, M. S. Ramaiah College of Pharmacy, Bangalore, India

²Department of Medical biology, Linköping University, Linköping, Sweden

³Department of Pharmacognosy, M.S. Ramaiah College of Pharmacy, Bangalore, India

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*Corresponding author

Asma Samaunnisa A, Department of Pharmaceutical chemistry, M. S. Ramaiah College of Pharmacy, Bangalore, India

E-mail: asmasamaunnisa@gmail.com

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ABSTRACT

Diethyl-2, 6-dimethyl-1, 4-dihydropyridine-3, 5-dicarboxylate (1A) and Diethyl-4-(4-hydroxyphenyl)-2, 6-dimethyl-1, 4-dihydropyridine-3, 5-dicarboxylate (1A') were synthesized by the condensation of ethyl acetoacetate with formaldehyde and para hydroxy benzaldehyde for 1A and 1A' respectively. From these intermediates, novel derivatives of 2, 6-dimethyl-N³, N⁵-diphenyl-1,4-dihydropyridine-3,5-dicarbohydrazide (2A-2D') were prepared in the presence of methanol and respective phenyl hydrazine. In view of the biological activities possessed by 1,4-dihydropyridines, synthesized compounds were subjected to *in vivo* anticonvulsant and sedative activities. Anticonvulsant activity was evaluated using Maximal electroshock (MES) induced convulsion method and Pentylenetetrazole (PTZ) induced convulsion method. Sedative activity was assessed by two models namely, locomotor activity using actophotometer in mice and anxiolytic activity using the elevated plus maze in rats. Phenytoin and diazepam were taken as standards for anticonvulsant activity, Chlorpromazine and diazepam were used as standards for sedative activity. Dimethyl sulfoxide (DMSO) was used as control; activity of synthesized drugs was compared with that of standards. All the newly synthesized derivatives exhibited significant anticonvulsant activity and considerably low sedative activity.

Keywords: *In vivo* Anticonvulsant, Sedative, 1, 4-Dihydropyridine, Dicarbohydrazide.

INTRODUCTION

Epilepsy is a neurological disorder characterized by seizures. As a treatment of epilepsy, the available drugs have many undesirable side effects like irritability, toxicity, teratogenicity, dose related side effects etc., and therefore efforts are directed towards the search for more effective drugs with less possible side effects. Calcium ion is a key factor for the induction of epilepsy. Drugs blocking calcium channels may be chosen as complementary anticonvulsants. Calcium channel antagonists such as nifedipine, amlodipine or felodipine belonging to the class of dihydropyridines, can be employed as antiepileptic agents¹⁻⁴. Pyridine; which is a six membered heterocyclic ring; is biologically found in the clan of vitamin B; is a parent molecule for dihydropyridine⁵. It is nitrogen containing heterocyclic ring, well known for its diverse biological and pharmacological activity. 1, 4-dihydropyridines, very well acknowledged as calcium channel antagonists, exhibits effective anti-hypertensive activity^{6,7}. These drug candidates are being used to treat epileptic disorders⁸. The rationale behind this project was to screen the newly synthesized 2, 6-dimethyl-N³, N⁵-diphenyl-1, 4-dihydropyridine-3,5-dicarbohydrazide¹¹ (2A-2D') derivatives for anticonvulsant and sedative activities. The structures of the newly synthesized compounds were characterized using IR, ¹HNMR, Mass and elemental analysis.

Experimental

Chemicals and solvents used were of reagent grade and used without further purification, were procured from Spectro Chem, Hi-Media, Merck, Sigma Aldrich and Ranbaxy. The purity of the synthesized compounds was determined by melting point using open capillary method and is uncorrected. IR (infra-red) was performed using SHIMADZU FTIR- 8400S. The compounds 2A-2D' were identified by ¹HNMR (proton nuclear magnetic resonance) using amx-400 NMR, Mass using LC-MS 2010A and elemental analysis using Flash EA 1112 series Thermo finnigan. TLC was performed using Solvent system- Ethyl acetate: n-Hexane, Stationary phase- Silica Gel-G.

MATERIALS AND METHODS

Step 1: Synthesis of Diethyl-2, 6-dimethyl- 1, 4-dihydropyridine-3, 5-dicarboxylate (1A) and diethyl-4-(4-hydroxyphenyl)-2, 6-dimethyl-1, 4-dihydropyridine-3, 5-dicarboxylate (1A')^{9,10}

Step 2: Synthesis of 2, 6-dimethyl-N3, N5- diphenyl-1, 4-dihydropyridine-3, 5- dicarbohydrazide (2A-2D')¹¹ **2A-2D'**

Substitutions for the derivatives are given in Table 1 -

Table 1: Substitutions of the synthesized derivatives

Compound	R ¹	R ²	R
1A	-	-	H
1A'	-	-	C ₆ H ₄ OH
2A	H	H	H
2B	NO ₂	NO ₂	H
2C	H	Cl	H
2D	H	NO ₂	H
2A'	H	H	C ₆ H ₄ OH
2B'	NO ₂	NO ₂	C ₆ H ₄ OH
2C'	H	Cl	C ₆ H ₄ OH
2D'	H	NO ₂	C ₆ H ₄ OH

Pharmacological Activity**Acute Toxicity studies**

Acute toxicity studies on wistar albino rats were carried out according to OECD guidelines. The derivatives were administered orally at a dose level of 100 mg/kg and 1000 mg/kg to separate groups of animals. The animals were under close observation after administration of doses during the first 24 h, especially during the first 2 h and subsequently for a total of 14 days¹².

Anticonvulsant Activity**Maximal electroshock (MES) induced Seizure method**

In this method, seizure was induced in animals by an electro-convulsive shock (150 mA, 0.2 sec) through an ear electrode using an electro convulse meter. Animals were divided into ten groups, each group comprising of six animals. The standard group-I was subjected to electro-convulsive shock, 30 minutes after intra peritoneal injection of phenytoin (25 mg/kg), group-II was treated with DMSO which was used as control. The newly synthesized derivatives 2A-2D' was orally administered to the respective groups (group-III to group-X) at doses of 200 and 400 mg/kg followed by electro convulsive shock after 1 h. The animals were then individually observed for various parameters such as tonic flexion, tonic extensor, clonic convulsions and stupor. The time taken for recovery or death after electro-convulsive shock was also noted^{13,16}.

Pentylenetetrazole (PTZ) induced convulsion method

In this method, seizure was induced in animals by administration of Pentylenetetrazole (PTZ). Animals were divided into ten groups of six animals each. The

synthesized derivatives 2A-2D' were administered to the respective groups at doses of 200 and 400 mg/kg followed by PTZ (70 mg/kg i.p.) after 1 h. The standard group was injected PTZ (70 mg/kg i.p.), 30 minutes after i.p. injection of diazepam (4 mg/kg). The animals were then individually placed in trays and parameters observed were Straub's tail, tonic-clonic convulsion, jerky movements of whole body, followed by recovery or death. The latency and duration of myoclonic jerks as well as incidence of seizures were recorded. Time taken for death/recovery was also noted^{13,14,16}.

Sedative Activity**Locomotor activity using Actophotometer**

Animals were divided into ten groups of six animals each. The control group received distilled water. The standard group received chlorpromazine (3 mg/kg body weight, i.p.) and the test groups received synthesized derivatives 2A-2D' at doses 200 and 400 mg/kg, p.o. The animals were placed individually in an actophotometer, half an hour after i.p. administration of chlorpromazine and 1 h after administration of derivatives, for 10 minutes. The number of counts corresponding to locomotor activity for each animal was noted^{13,14}.

Anxiolytic activity using Elevated Plus-Maze

The animals were individually placed at the center of the maze, head facing towards open arm, 1 hour after oral administration of derivatives 2A-2D', vehicle and 30 minutes after injection of diazepam. During 5 minutes test period the various parameters like first preference of rat to open or enclosed arms, number of entries into open and closed arms and average time spent by the animal in

each arms were observed. The control group received distilled water. The standard group received diazepam (4 mg/kg body weight, i.p.) and the test groups received derivatives 2A-2D' at doses 200 mg/kg and 400 mg/kg. Anxiolytic activity of drugs was indicated by number of entries and time spent in both the closed and opened arms. A statistical comparison was made between the control, standard and test groups^{13,15}.

Statistical Analysis

The experimental data were expressed as mean $\pm$ SEM. The data were analyzed using ANOVA and Tukey-Kramer multiple comparison test. The results were considered statistically significant if $P < 0.05$.

RESULTS AND DISCUSSION

Acute Toxicity Studies

As per the acute toxicity studies carried out, no mortality and toxicity symptoms were observed up to the dose range of 1000 mg/kg for 72 h after the oral administration of extract. Therefore a cut off value of greater than 1000 mg/kg was taken as LD₅₀. Two doses, 200 mg/kg and 400 mg/kg were selected to carry out further studies.

Anticonvulsant Activity

Maximal electroshock induced convulsions

In MES induced convulsions, 200 mg/kg and 400 mg/kg doses of 2A-2D' were administered. Among the derivatives tested, 2B and 2B' exhibited significant activity comparable to standard and between the two doses administered, 200 mg/kg showed fractionally more activity than 400 mg/kg. Derivatives 2A, 2C, 2A' and 2C' displayed moderate but low activity compared to the former mentioned derivatives of the series. 2D and 2D'

showed less activity compared to the other derivatives but exhibited considerable anticonvulsant activity. All the derivatives other than 2B and 2B' exhibited slightly higher anticonvulsant activity at a dose of 400 mg/kg rather than 200 mg/kg. In general, all the derivatives displayed significantly good anticonvulsant activity towards MES induced convulsions. Results given in Figures 1a and 1b

Pentylenetetrazole induced convulsions

For almost all the derivatives, 400 mg/kg dose showed good activity compared to 200 mg/kg counterparts. Amongst the derivatives tested, 2C, 2D, 2C' and 2D' showed good anticonvulsant activity. Whereas the other derivatives 2A, 2B, 2A' and 2B' presented moderate activity. Although, time taken for the onset of convulsion largely vary compared to the standard, all the derivatives exhibited fast recovery time. Results are presented in figure 2a and 2b

Sedative Activity

All the derivatives 2A-2D' was evaluated for their sedative activity using actophotometer for locomotor activity and elevated plus-maze for anxiolytic activity. The locomotor activity presented by all the derivatives was nowhere seen in the vicinity of standard drug. Results given in Figure 3; for anxiolytic activity, number of entries as well as the amount of time spent in closed arms is exponentially high compared to the open arms. All the derivatives presented noticeably increased tendency towards closed arms rather than open arms there by suggesting considerably very low anxiolytic activity. Results presented in Figures 4a, 4b, 5a and 5b.

Figure 1a: Anticonvulsant activity using MES induced Convulsion method (Duration of extensor phase)

The results are expressed as Mean $\pm$ SEM. Control-DMSO 20 % in distilled water, Standard- phenytoin 25 mg/kg, n=6, ***P < 0.001, **P < 0.01, *P < 0.05, in comparison with the control group

Figure 1b: Anticonvulsant activity using MES induced Convulsion method (Time taken for recovery)

The results are expressed as Mean $\pm$ SEM. Control-DMSO 20 % in distilled water, Standard- phenytoin 25 mg/kg, n=6, ***P < 0.001, **P < 0.01, *P < 0.05, in comparison with the control group.

Figure 2a: Anticonvulsant activity using PTZ induced Convulsion method (onset of convulsions)

The results are expressed as Mean $\pm$ SEM. Control-DMSO 20 % in distilled water, Standard- diazepam 4 mg/kg, n=6, ***P < 0.001, **P < 0.01, *P < 0.05, in comparison with the control group

Figure 2b: Anticonvulsant activity using PTZ induced Convulsion method (time taken for recovery)

The results are expressed as Mean $\pm$ SEM. Control-DMSO 20 % in distilled water, Standard- diazepam 4 mg/kg, n=6, ***P < 0.001, **P < 0.01, *P < 0.05, in comparison with the control group

Figure 3: Locomotor activity using Actophotometer

The results are expressed as Mean $\pm$ SEM. Control-DMSO 20 % in distilled water, Standard- chlorpromazine 3 mg/kg, n = 6, ****P < 0.001, **P < 0.01, *P < 0.05, in comparison with the control group

Figure 4a: Effects of derivatives 2A-2D' (200 mg/kg) on number of entries in open and closed arms (Elevated plus-maze)

The results are expressed as Mean $\pm$ SEM. Control-DMSO 20 % in distilled water, Standard- diazepam 4 mg/kg, n=6, ****P < 0.001, **P < 0.01, *P < 0.05, in comparison with the control group

Figure 4b: Effects of derivatives 2A-2D' (400 mg/kg) on number of entries in open and closed arms (Elevated plus-maze)
The results are expressed as Mean $\pm$ SEM. Control-DMSO 20 % in distilled water, Standard- diazepam 4 mg/kg, n=6, ***P < 0.001, **P < 0.01, *P < 0.05, in comparison with the control group

Figure 5a: Effects of derivatives 2A-2D' (200 mg/kg) on time spent in open and closed arms (Elevated plus-maze)
The results are expressed as Mean $\pm$ SEM. Control-DMSO 20 % in distilled water, Standard- diazepam 4 mg/kg, n = 6, ***P < 0.001, **P < 0.01, *P < 0.05, in comparison with the control group

Figure 5b: Effects of derivatives 2A-2D' (400 mg/kg) on time spent in open and closed arms (Elevated plus-maze)
The results are expressed as Mean $\pm$ SEM. Control-DMSO 20 % in distilled water, Standard- diazepam 4 mg/kg, n=6, ***P < 0.001, **P < 0.01, *P < 0.05, in comparison with the control group

CONCLUSION

In the present study, pharmacological screening of 2,6-dimethyl-N³,N⁵-diphenyl-1,4-dihydropyridine-3,5-dicarbohydrazide (2A-2D') derivatives for their anticonvulsant as well as sedative activity was reported. Derivatives 2A-2D' displayed very poor sedative activity. All the tested derivatives exhibited significantly good anticonvulsant activity and ascertained to be promising lead candidates. Further studies concerning anticonvulsant activity of these candidates might provide a better perception of their therapeutic ability.

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