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Original Article

ASSESMENT OF ANTICONVULSANT AND SEDATIVE ACTIVITIES AFBIS PYRAZOLIDINE-3, 5-DIONE DERIVATIVES TETHERED WITH 1, 4- DIHYDROPYRIDINE MOIETY

ASMA SAMAUNNISA A[a*], RIAZUDDIN MOHAMMED[b], C.H.S. VENKATARAMANA[a],V MADHAVAN[c]

[a]Department of Pharmaceutical chemistry, M.S.Ramaiah College of Pharmacy, Bangalore, India, [b]Department of Medical biosciences, Linköping University, Linköping, Sweden.
Email: asmasamaunnisa@gmail.com

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ABSTRACT

Objective: In the present research work, 8 novel derivatives of 2,6-dimethyl-1,4-dihydropyridine-3,5-yl-bis[carbonyl-2- (phenyl)]pyrazolidine-3,5-diones] 3A-3D'were synthesized from cyclization of 2,6-dimethyl-N3,N5-diphenyl-1,4-dihydropyridine-3,5-dicarbohydrazide 2A- 2D', are subjected to anticonvulsant and sedative activities screening.

Methods: Anticonvulsant activity was tested using two models namely Maximal electroshock (MES) induced convulsion method and Pentylenetetrazole (PTZ) induced convulsion method. Sedative activity was also screened with two models vizlocomotor activity using actophotometer in mice and anxiolytic activity using the elevated plus maze in rats. Phenytoin, diazepam and Chlorpromazine, diazepam were used as standards for anticonvulsant and sedative activities respectively.

Results: At both the doses of 200mg/kg and 400 mg/kg, all of the tested derivatives 3A-3D' exhibited significant anticonvulsant activities. Conversely, none of the derivatives exhibited better sedative activity which can be compared with the standard.

Conclusion: In view of the results obtained, further studies are required to properly establish the anticonvulsant profile with minimal CNS side effects of the novel derivatives 3A-3D'.

Keywords: 1, 4-Dihydropyridine, pyrazolidine-3,5-dione,invivo Anticonvulsant, Sedative.

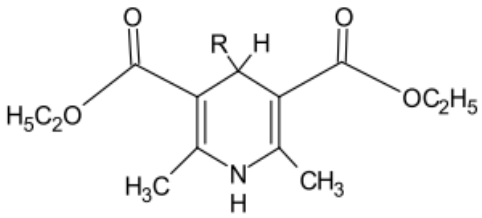
INTRODUCTION

Nitrogen containing heterocycles emerged as a class of compounds, endowed with expansive range of biological and pharmacological activities. Just by incorporating nitrogen as hetero atom in a number of homocyclicor heterocyclic rings, renders the moiety with profound qualities of encountering various health ailments. These moieties upon certain alteration or when blended with other ring systems exhibit superior activities or paves way for different pharmacological activity. Among such rings is a pyridine ring, from which diverse analogues can be obtained. One such analogue is 1,4-dihydropyridine, possessing varied activities like antibacterial, antifungal, anti- hypertensive, anticonvulsant, anti-inflammatory, Anticanceretc [1,2,3,4,5,6]. It belongs to the class of prominent calcium channel antagonists [7].

Pyrazolidinediones are a class of compounds containing two nitrogens as hetero atoms in a five membered completely saturated ring with two doubly bonded oxygen outside the ring. Even this ring is endowed with fine biological activities such as anti-microbial, anti-inflammatory, antihypertensive, antineoplastic and anticonvulsant activities[8,9,10,11].

Calcium ions acts as imperative factor for induction of epilepsy, which is a neurological disorder characterized by seizures. Diverse moieties used as anti-convulsant agents own a variety of neurological side effects. Consequently, search for new anticonvulsant agents with less possible neurologic adverse effects and effective therapeutic activity is going on. 1,4-dihydropyridines with their active calcium channel antagonistic property, has become a complementary class of compounds for the treatment of epilepsy [12].

As both the above said rings have anticonvulsant profiles, derivatives blended with them might provide a synergistic action and can act as a lead molecules gifted with superior anticonvulsant activity. The current research work primarily aims to screen thesynthesized derivatives containing both 1, 4-dihydropyridine and pyrazolidine-3,5-diones for their anticonvulsant and sedative activities. The derivatives are2,6-dimethyl-1,4-dihydropyridine-3,5-yl-bis[carbonyl-2-(phenyl)]pyrazolidine-3,5-diones] 3A-3D'[13].Structures of these novel derivatives were characterized based on IR, ^{1}HNMR, Mass and elemental analysis.



1A-1A'

Step 1: Synthesis of Diethyl-2, 6-dimethyl- 1, 4-dihydropyridine-3, 5-dicarbohydrazide (1A) and diethyl-4-(4-hydroxyphenyl)-2, 6- dimethyl-1, 4-dihydropyridine-3, 5- dicarboxylate' (1A')[14,15]

MATERIALS AND METHODS

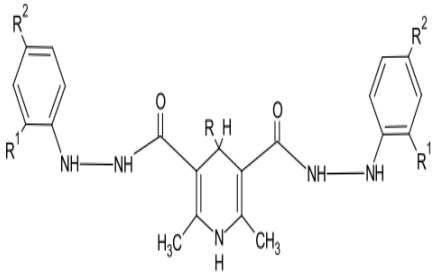
Experimental

Chemicals and solvents used were of reagent grade and used without further purification, were procured from SpectroChem, Hi-Media, Merck, Sigma Aldrich and Ranbaxy. The purity of the synthesized compounds was determined by melting point using open capillary method and are 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.

Animals

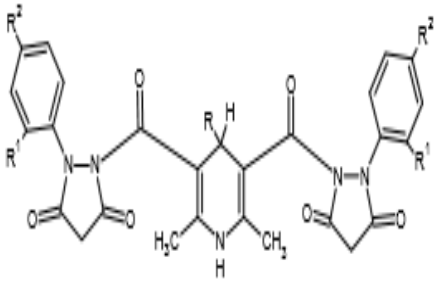
Albino Wistar rats of either sex with body weight 150-250g were used for the screening of antihypertensive activity. Animals were retained at standard conditions of temperature (24±2°C) and relative humidity (30-70%) and exposed to 12hr light and dark cycle. Animals were given standard diet and water *ad libitum*. All the procedures involving animals were carried out as per OECD guidelines and under the institutional animal ethical committee approval.

Synthesis of 1,4-dihydropyridines



2A-2D'

Step 2: Synthesis of 2, 6-dimethyl-N3, N5- diphenyl-1, 4-dihydropyridine-3, 5- dicarbohydrazide (2A-2D')[16]



3A-3D'

Substitutions for the derivatives 3A-3D' are given in table 1

Step 3:Synthesis of 2, 6-dimethyl-1, 4-dihydropyridine-3, 5-yl-bis [carbonyl-2-(phenyl)] pyrazolidine-3, 5- diones] (3A-3D')[13]

Biological Activity

1) Acute Toxicity studies[17]

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 2000 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 days.

Table 1: Substitutions of the derivatives 3A-3D'

Compound	R^1	R^2	R
3A	H	H	H
3B	NO_2	NO_2	H
3C	H	Cl	H
3D	H	NO_2	H
3A'	H	H	C_6H_4OH
3B'	NO^2	NO_2	C_6H_4OH
3C'	H	Cl	C_6H_4OH
3D'	H	NO_2	C_6H_4OH

2) Anticonvulsant Activity

Maximal electroshock (MES) induced Seizure method[18,19]

In this method, seizure was induced in animals by an electro-convulsive shock (150mA, 0.2 sec) through an ear electrode using an electro convulsometer. Animals were divided into ten groups, each group comprising of six animals. The standard group-I was subjected to electro-convulsive shock, 30 min after intraperitonial injection of phenytoin (25 mg/kg), group-II is treated with DMSO which is used as control. The newly synthesized derivatives 2A-2D' were 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 1h. 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.

Pentylenetetrazole (PTZ) induced convulsion method[18,19,20]

In this method, seizure was induced in animals by administration of Pentylenetetrazole (PTZ). Animals were divided into ten groups of six animals each. Standard group was treated with Pentylenetetrazoleintraperitonially (i. p.). 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 1h. The standard group was injected PTZ (70 mg/kg i.p.), 30 min 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.

3) Sedative Activity

Locomotor activity using Actophotometer[18,20]

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

Anxiolytic activity using Elevated Plus-Maze[18,20,21]

The animals were individually placed at the center of the maze, head facing towards open arm, 1hour after oral administration of derivatives 2A-2D', vehicle and 30min after injection of diazepam. During 5 min 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 (4mg/kg body weight, i.p.) and the test groups received derivatives 2A-2D' at doses 200mg/kg and 400mg/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.

Statistical Analysis

The experimental data were expressed as mean±SEM. The data were analyzed usin.g ANOVA and Tukey-Kramer multiple comparison test. The results were considered statistically significant if P<0.05.

RESULTS AND DISCUSSION

Acute Toxicity Studies

Up to the dosage range of 1000 mg/kg, no mortality and toxicity symptoms were observed as per the toxicity studies. These observations pertains to the 72h after oral administration of derivatives. As a result, a cut off value greater than 1000 mg/kg was taken as LD_{50}. To carry out further studies, two doses of 200 mg/kg and 400 mg/kg were chosen.

Anticonvulsant Activity

Maximal electroshock induced convulsions

In this method, an electro-convulsive shock of 150mA for 0.2 secis used to induce seizures in mice. Phenytoin was used as standard. Among the derivatives 3A-3D', tested for anticonvulsant activity, 3B, 3C, 3A', 3B', 3C' exhibited highly significant activity as of standard. Duration of extensor phase is almost very less and there was a rapid recovery indicating worthy anticonvulsant activity at both 200 and 400 mg/kg concentrations. Derivatives 3A, 3D, 3D' displayed significant activity but very slightly less compared to other candidates of the class at 200 along with 400 mg/kg. The slightly increased activity of 3A' compared to 3A might be pertaining to the presence of phenol ring at 4th position on the 1,4-dihydropridine ring and absence of any substitutions on the phenyl rings attached to pyrazolidine-3,5-dione moieties present at either side of 1,4-dihydropyridine moiety.Data represented in figures 1a and 1b.

Pentylenetetrazole induced convulsions

Pentylenetetrazole (PTZ) was employed to induce convulsions in mice. Diazepam was taken as standard drug. Derivatives 3C, 3D, 3A', 3C', 3D' displayed significant activity compared to standard. Although the onset of convulsion time is slightly higher than the standard, time taken for recovery was less which signifies that there was a rapid recovery from seizures. Other derivatives 3A, 3B, 3B' displayed marginally less activity compared to other derivatives but considerably good anticonvulsant activity. Data represented in figures 2a and 2b.

Sedative Activity

Locomotor activity

Actophotometer was used to evaluate the locomotor activity using Chlorpromazine as standard.All the tested derivatives at concentrations of 200 as well as 400 mg/kg displayed very less andalmost incomparable activity compared to standard. Data represented in figure 3.

Elevated Plus-Maze

Anxiolytic activity was assessed using elevated plus-maze with diazepam as standard drug. Complete homologous series of novel derivatives 3A-3D' exhibited interrelated activity. Number of entries in closed arms were exponentially high at both the concentrations of 200 mg/kg and 400 mg/kg there by suggesting weak and less anxiolytic activity. Similarly, the amount of time spent by the rats in closed arms was very high signifying almost very weak and unmatched anxiolytic activity with the standard. Data represented in figures 4a, 4b, 5a and 5b.

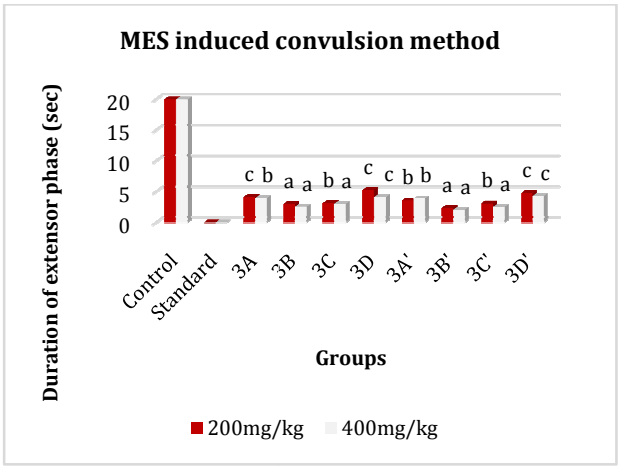


Fig. 1a: Anticonvulsant activity using MES induced Convulsion method (Duration of extensor phase).The results are expressed as Mean ± SEM. Control-DMSO 20% in distilled water, Standard-phenytoin 25mg/kg, n=6, [a]P< 0.001,[b]P< 0.01, [c]P< 0.05, in comparison with the control group.

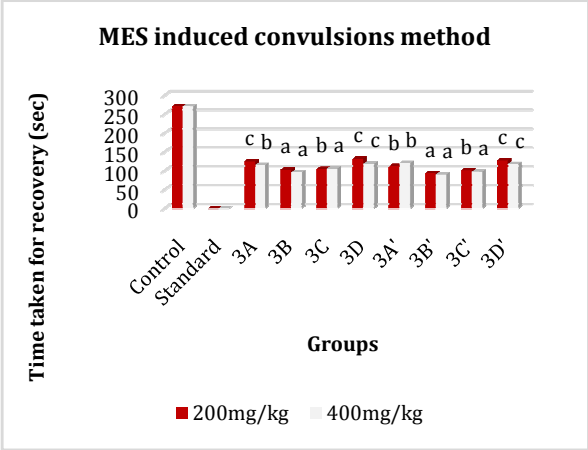


Fig. 1b: Anticonvulsant activity using MES induced Convulsion method (Time taken for recovery).The results are expressed as Mean ± SEM. Control-DMSO 20% in distilled water, Standard-phenytoin 25mg/kg, n=6, [a]P< 0.001, [b]P< 0.01, [c]P< 0.05, in comparison with the control group.

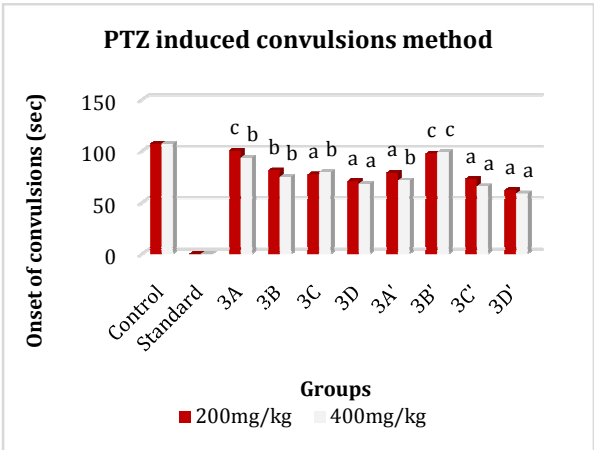


Fig. 2a: Anticonvulsant activity using PTZ induced Convulsion method (onset of convulsions). The results are expressed as Mean ± SEM. Control-DMSO 20% in distilled water, Standard-diazepam 4mg/kg, n=6, [a]P< 0.001, [b]P< 0.01, [c]P< 0.05, in comparison with the control group.

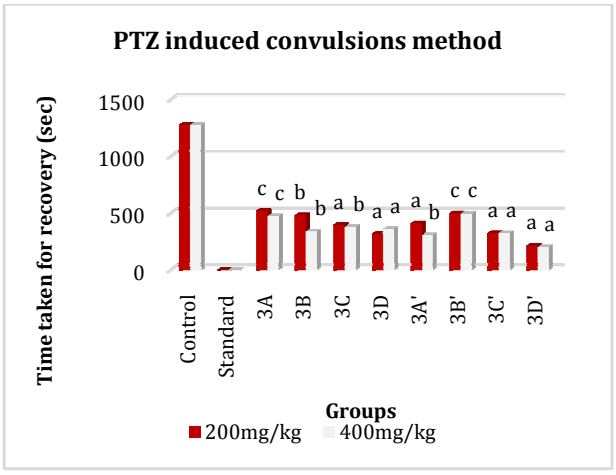


Fig. 2b: Anticonvulsant activity using PTZ induced Convulsion method (time taken for recovery). The results are expressed as Mean ± SEM. Control-DMSO 20% in distilled water, Standard- diazepam 4mg/kg, n=6, [a]P< 0.001, [b]P< 0.01, [c]P< 0.05, in comparison with the control group.

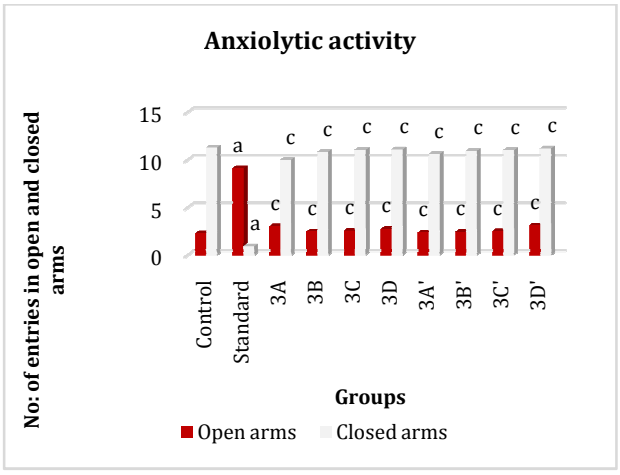


Fig. 4b: Effects of derivatives 3A-3D' (400mg/kg) on number of entries in open and closed arms (Elevated plus-maze). The results are expressed as Mean ± SEM. Control-DMSO 20% in distilled water, Standard- diazepam 4mg/kg, n=6, [a]P< 0.001, [b]P< 0.01, [c]P< 0.05, in comparison with the control group.

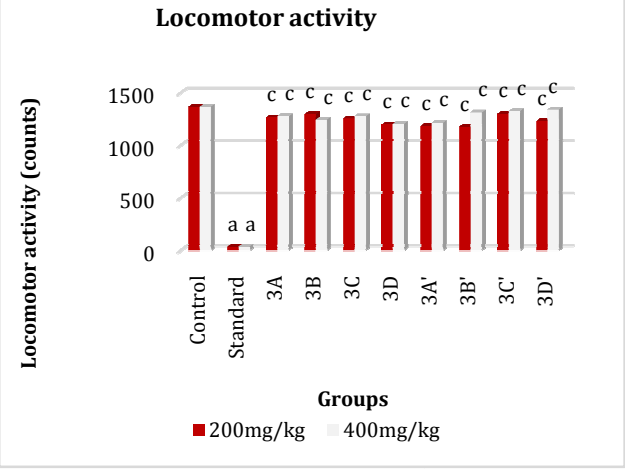


Fig. 3: Locomotor activity using Actophotometer. The results are expressed as Mean ± SEM. Control-DMSO 20% in distilled water, Standard- chlorpromazine 3mg/kg, n=6, [a]P< 0.001, [b]P< 0.01, [c]P< 0.05, in comparison with the control group.

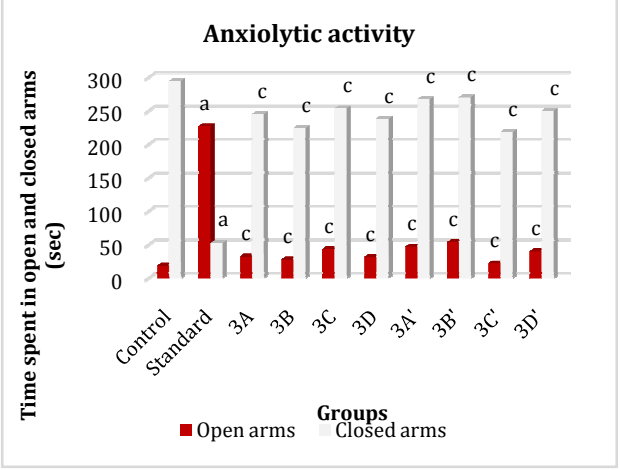


Fig. 5a: Effects of derivatives 3A-3D' (200mg/kg) on time spent in open and closed arms (Elevated plus-maze).The results are expressed as Mean ± SEM. Control-DMSO 20% in distilled water, Standard- diazepam 4mg/kg, n=6, [a]P< 0.001, [b]P< 0.01, [c]P< 0.05, in comparison with the control group.

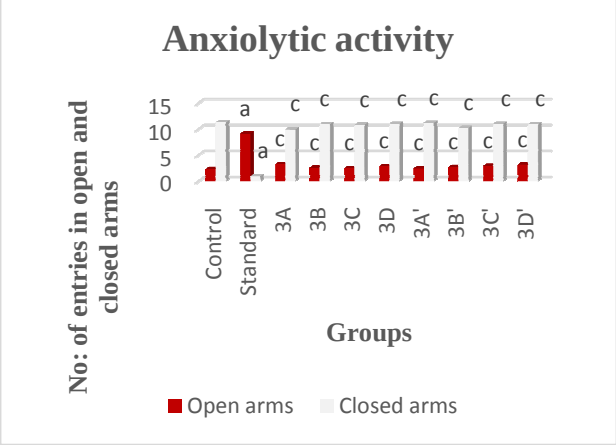


Fig. 4a: Effects of derivatives 3A-3D' (200mg/kg) on number of entries in open and closed arms (Elevated plus-maze).The results are expressed as Mean ± SEM. Control-DMSO 20% in distilled water, Standard- diazepam 4mg/kg, n=6, [a]P< 0.001, [b]P< 0.01, [c]P< 0.05, in comparison with the control group.

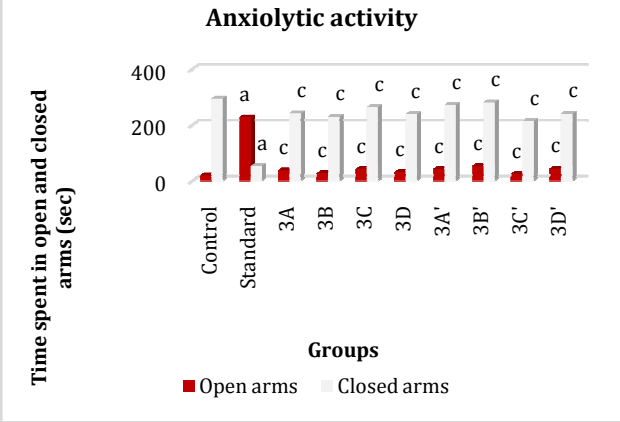


Fig. 5b: Effects of derivatives 3A-3D' (400mg/kg) on time spent in open and closed arms (Elevated plus-maze).The results are expressed as Mean ± SEM. Control-DMSO 20% in distilled water, Standard- diazepam 4mg/kg, n=6, [a]P< 0.001, [b]P< 0.01, [c]P< 0.05, in comparison with the control group.

CONCLUSION

Current study covers the pharmacological screening of 2, 6-dimethyl-1, 4-dihydropyridine-3, 5-yl-bis[carbonyl-2-(phenyl)]pyrazolidine-3,5-diones] 3A-3D' derivatives for their anticonvulsant as well as sedative activity. Mostly all the derivatives revealed highly significant anticonvulsant activity, although there was almost no sedative activity. The most major CNS side effects of presently available anticonvulsant drugs are sedation and hypnosis. As the derivatives 3A-3D' displayed very low sedative activity and strong anticonvulsant activity, these might prove to be the long awaited blockbuster drugs to treat epilepsy. Further studies are required to properly establish the anticonvulsant profile with minimal CNS side effects of the novel derivatives 3A-3D'.

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