ANTICONVULSANT ACTIVITIES OF NEW 1,4-DIHYDROPYRIDINE DERIVATIVES CONTAINING 4-NITROIMIDAZOLYL SUBSTITUENTS.

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ABSTRACT

Anticonvulsant activity of Alkyl, cycloalkyl and arylalkyl ester analogues of nifedipine in which the ortho-nitro phenyl group at position 4 is replaced by 1-methyl-4-nitro-5-imidazolyl substituent, were determined against pentylenetetrazole-induced seizures in mice.

The anticonvulsant effects of the compounds were evaluated by the measurement of seizure latency and duration. Significant differences were observed between treated animals with control group and nifedipine in seizure duration. Our results show that most of the compounds had similar activity to the reference drug nifedipine. In addition, compounds **6a**, **6b**, **6f**, **6g**, **6h**, **8e**, **8f**, **8g** , **8h** and **8i** were more active than the reference drug nifedipine.

Key Words: Calcium Channel blockers, Nitroimidazole, Dihydropyridines, Anticonvulsion

INTRODUCTION

Nifedipine and other dihydropyridine derivatives such as nimodipine, nitrendipine and nisoldipine are potent blockers of the calcium channels of smooth muscles and also bind with high affinity to the brain membranes (1). There are considerable evidences that calcium is an important factor for the induction of epilepcy. Specifically, different seizure-inducing agents or procedures cause a rapid intraneuronal influx of calcium ions, which is causally related to the subsequant epileptiform activity (2-8).

Conversely, calcium channel inhibitors are effective against the whole range of convulsive procedures including electro and pentylene-tetrazole convulsions (9), and sound and high pressure-induced seizures (10,11).

Previously, it was demonstrated that the dihydropyridine calcium channel blockers are effective anticonvulsant candidates in experimental seizures (12,13). In this article anticonvulsant properties of dialkyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-4-nitroimidazole-5-yl)-3,5-pyridine dicarboxylate (14) is reported.

MATERIAL AND METHODS

Chemistry

Compounds (6a-6i and 8a-8i) were prepared as shown in Scheme 1.

The preparation and the physical data of these compounds were reported previously (15).

Pharmacology

Male NMRI mice (20-25g; Razi Institute, Iran) were used throughout this study. The animals were maintained at constant room temperature (22.0±3.0°C) and submitted to 12h light/12h dark cycle with food and water available *ad libitum*. They were housed in standard poly-carbonate cages and acclimated at least 2 days before experiments. The experiments were carried out between 13:00 and 18:00 hours. Seven mice were used for each dose of each compound. Pentylenetetrazole (PTZ) was purchased from Sigma (poole, UK) and a 0.85% solution of this agent in 0.9% saline was prepared and kept at 37°C. For induction of convulsion a dose of 0.01 ml/kg (85 mg/kg body weight) was effective in 97% of mice which were used (16,17). Nifedipine as standard compound and test compounds were suspended in (1:8 v/v) ethanol-distilled water. The ethanol/water vehicle was also used as a control. Intraperitoneal injection of PTZ (85 mg/kg) induced clonic-tonic convulsions in mice. Doses of 5 and 10 mg/kg of the test compounds and nifedipine as reference drug were administered intraperitoneally 30 min before PTZ injection. After injection of PTZ mice were observed for 30 min to detect the seizure latency and duration and the number of seizure attacks and mortality. Seizure latency (SL) is the time that is required to observe the first tonic-clonic-seizure after PTZ injection and seizure duration is the duration (SD) of tonic-clonic seizure.

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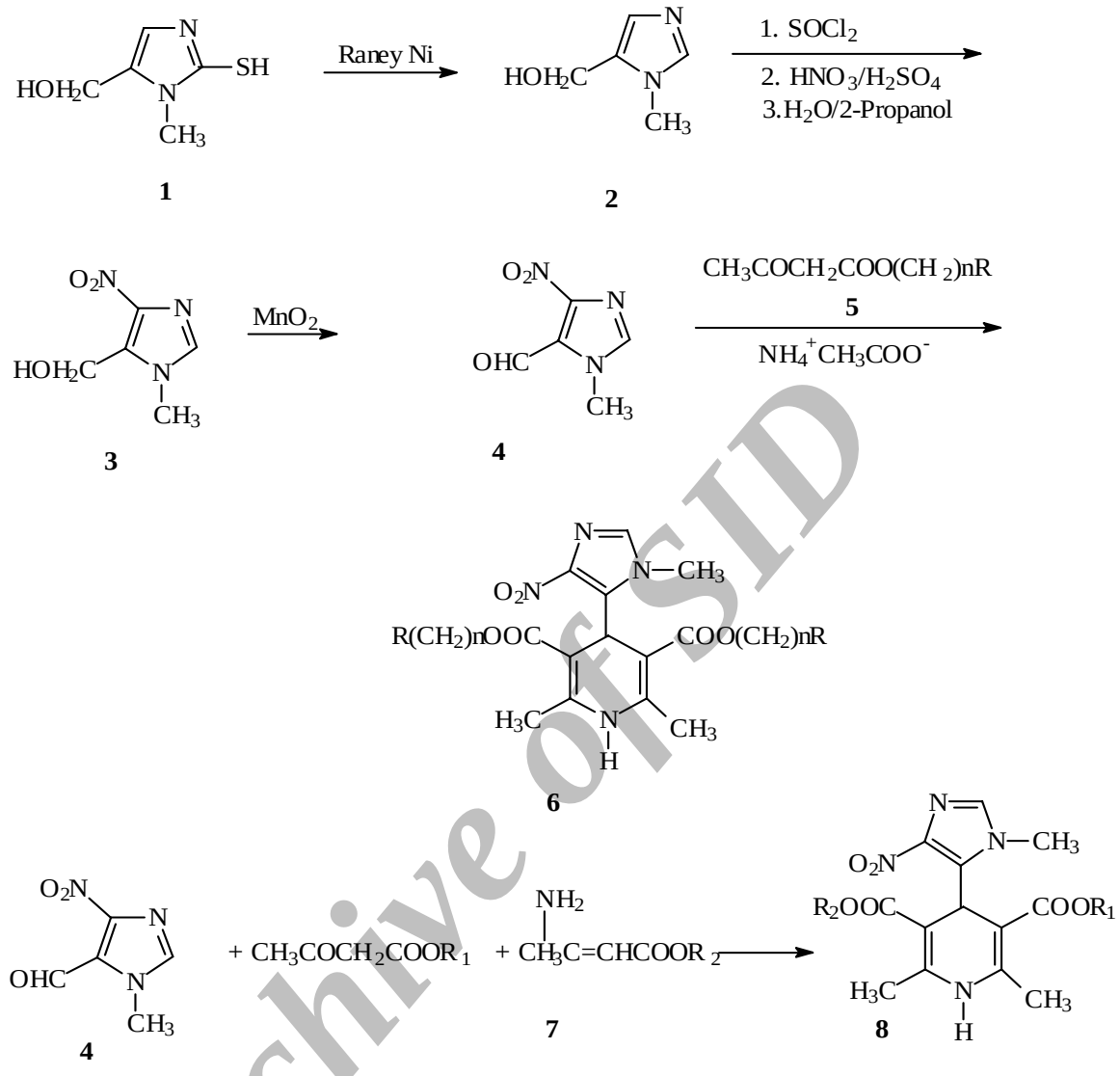


Synthesis of 1,4-Dihydropyridine Derivatives Containing 4-Nitroimidazolyl Substituents

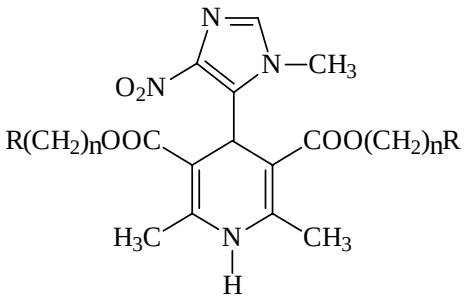
Statistics

Statistical significance of differences in seizure duration and seizure latency were estimated by analysis of variance (one way ANOVA) followed by Dunnets' test. Resutls were expressed as mean ± S.E. mortality rates and were analyzed by Chi-squared test and showed no significant differences.

RESULTS AND DISCUSSION

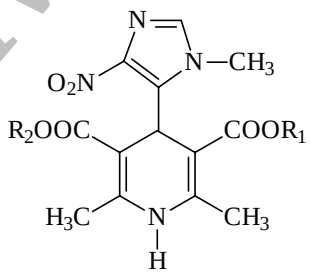
A number of novel antiepileptic compounds which are believed to act largely by inhibition of the voltage-dependent calcium channels are currently undergoing clinical trials or have been approved for clinical uses (18). Several articles have demonstrated that DHP substituted at C-4 with heterocycles were potent antagonists of calcium channels (19,20). In the previous paper we reported that C-4 imidazole substituents gave very active compounds as calcium channel antagonist (14,15). In this article anticonvulsant activity of these compounds (6a-i and 8a-i) are described. Anticonvulsant effects of the compounds were evaluated by the measurement of seizure latency and duration and mortality. These agents decreased seizure latency in treated animals with no significant differences. The same result also was obtained with nifedipine as a standard.

Table 1. Substituent pattern of symmetrical esters **6a-i**.



No.	R	n
6a	CH_3	0
6b	CH_3	1
6c	CH_3	2
6d	$CH(CH_3)_2$	0
6e	$C(CH_3)_3$	0
6f	C_6H_5	1
6g	C_6H_5	2
6h	C_6H_5	3
6i	C_6H_{11}(Cyclohexyl)	1

Table 2. Substituent pattern of asymmetrical esters **8a-i.**



No.	R_1	R_2
8a	$CH_2C_6H_5$	CH_3
8b	$CH_2C_6H_5$	CH_2CH_3
8c	$CH_2CH_2C_6H_5$	CH_3
8d	$CH_2CH_2C_6H_5$	CH_2CH_3
8e	$CH_2CH_2CH_2C_6H_5$	CH_3
8f	$CH_2CH_2CH_2C_6H_5$	CH_2CH_3
8g	$CH_2C_6H_{11}$(Cyclohexyl)	CH_3
8h	$CH_2CH_2C_6H_{11}$(Cyclohexyl)	CH_3
8i	$CH_2CH_2CH_2C_6H_{11}$(Cyclohexyl)	CH_3

Table 3. Effects of different doses of compound (**6a-6i**) on PTZ-induced seizures in mice.

Compound	Dose	Seizure latency	Seizure duration	Mortality %
6a	5	14.85±4.52	14.28±3.26	57.15
6a	10	12.57±2.64	13.28±4.70	28.57
6b	5	6.00±1.21	13.71±2.95	42.86
6b	10	8.28±2.65	12.71±2.12	42.86
6c	5	7.57±1.39	30.28±4.09	57.15
6c	10	1.71±0.42	26.28±3.07	28.57
6d	5	4.57±1.73	32.42±3.54	71.43
6d	10	4.00±1.17	26.30±3.09	57.15
6e	5	4.57±1.81	38.71±6.64	71.43
6e	10	4.00±1.81	33.14±5.81	57.15
6f	5	7.00±1.74	18.14±4.54	57.15
6f	10	3.14±1.28	15.71±4.62	28.58
6g	5	4.88±1.80	19.85±4.46	28.58
6g	10	4.14±1.83	16.28±4.51	42.86
6h	5	5.14±1.50	22.71±5.37	57.15
6h	10	6.28±2.40	16.42±5.4	42.86
6i	5	5.00±1.96	36.42±9.98	42.86
6i	10	6.00±1.30	29.14±6.44	42.86
Nifedipine	5	2.71±0.52	25.00±3.70	57.15
Nifedipine	10	3.27±0.42	24.28±3.19	42.86
Control	-	9.00±2.60	49.57±9.82	71.43

Intraperitoneal injecation of the compounds 30 minutes before PTZ decreased seizure duration significantly but did not show any significant effects on seizure latency and mortrality. Each point show mean ± S. E M 7 animals. * $p<0.05$ showed significant difference statistically.

Table 4. Effect of different doses of compounds (**8a-i**) on PTZ-induced seizures in mice.

Compound	Dose	Seizure latency	Seizure duration	Mortality %
8a	5	7.42±1.96	28.57±2.91	71.43
8a	10	6.14±1.54	27.57±4.99	57.15
8b	5	1.85±0.50	28.57±6.36	57.15
8b	10	2.28±0.60	33.57±6.88	71.43
8c	5	4.85±1.60	25.57±5.27	57.15
8c	10	4.71±1.18	19.14±3.95	57.15
8d	5	4.00±1.63	25.00±5.50	71.43
8d	10	3.4±1.42	19.28±5.95	28.58
8e	5	6.71±0.07	17.57±4.91	57.15
8e	10	4.71±1.18	14.57±5.30	57.15
8f	5	4.28±1.78	19.85±5.19	57.15
8f	10	6.00±2.81	14.5±5.30	28.58
8g	5	5.00±1.69	17.57±3.68	42.86
8g	10	8.00±2.50	16.85±3.66	28.58
8h	5	5.14±1.40	19.77±4.11	57.15
8h	10	4.71±1.37	18.00±3.43	71.43
8i	5	5.00±1.48	17.57±4.91	42.86
8i	10	6.00±2.81	17.50±3.68	28.58
Nifedipine	5	2.71±0.52	25.00±3.70	57.15
Nifedipine	10	3.27±0.42	24.28±3.19	42.86
Control	-	9.0±2.60	49.57±9.82	71.43

Intraperitoneal injecation of the compounds 30 minutes before PTZ decreased seizure duration significantly but did not show any significant effects on seizure latency and mortrality. Each point show mean ± S. E M 7 animals. * $p<0.05$ showed significant difference statistically.

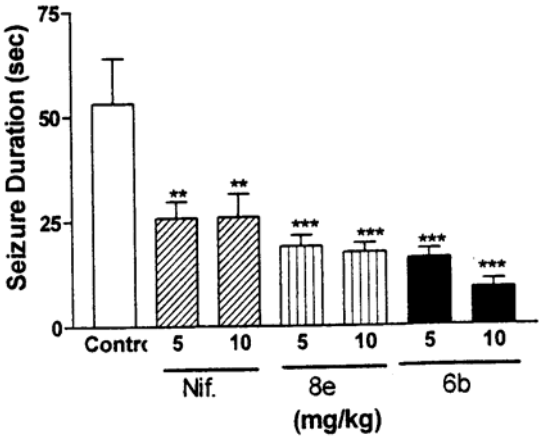


Figure 1. Effects of compound 6a, 8e and nifedipine on PTZ-induced seizure duration. Intraperitoneal injections of 6b (5, 10 mg/kg), 8e (5, 10 mg/kg) and nifedipine (5,10 mg/kg), 30 min before PTZ (85 mg/kg), decrease seizure duration significantly. Each point shows mean ± S.E. of seven animals. **P<0.01 and ***P<0.001 show statistically differences between control group and treated animals.

These contraversial effects may be related to various pharmacological actions of the agents on calcium channels or neurotransmitters in different regions of the brain. Significant differences were observed between treated animals with control group and nifedipine only in seizure duration [F(30, 186)= 2.934]. Comparison of the activities of symmetrical esters in alkyl ester series (tables 1 and 3, **6a**-**6e**) indicate that increasing the length of methylene chain of the C3 and C5 ester substituents to more than one methylene units (n>0), and increase in the steric hindrances of the substituent decrease activity. Therefore t-butylester (**6e**) is the weakest compound in this series.

In phenylalkyl ester series (tables 1 and 3, **6f**-**6h**) increasing the length of methylene chain did not change the efficacy significantly and all compounds had similar activity and were more active than nifedipine.

A comparison of the activity of compounds **6f** relative to **6i** show that phenyl derivative is more active than cyclohexyl derivative. Finally, the results show that most compounds had activity comparable to nifedipine and compounds **6a**, **6b**, **6f** and **6h** were more active than this reference drug.

In asymmetrical series of phenylalkyl esters (tables 2 and 4, **8a**-**8f**) when R_2 is methyl or ethyl, increasing the length of methylene chain increased activity. However, in cyclohexyl esters (**8g**-**8i**) when R_2 is a small substituent like methyl (R_2=Me), increasing the length of methylene chain did not change the efficacy significantly and all compounds had similar activity. A comparison of the activity of phenylalkyl series (**8a, 8c**) with cyclohexyl series (**8g, 8h**) shows that cyclohexyl derivatives were more active than phenyl alkyl derivatives. In this study the effect of doses of 1 and 25 mg/kg were also investigated. No significant alteration with dose of 1 mg/kg on SL and SD (data not shown) was observed. High volume of the solvent required for making the solution for the dose of 25 mg/kg reduced SL and SD responses, Data for these doses are not included. Finally, the results show that most compounds of the asymmetrical phenalkyl esters had activity comparable to nifedipine and some compounds of this series (**8e**-**8i**) were more active than the reference drug.

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