Available online at www.sciencedirect.com
SCIENCE DIRECT®
IL FARMACO 59 (2004) 261–269
IL FARMACO
www.elsevier.com/locate/farmac

Lipophilic 4-imidazoly-1,4-dihydropyridines: synthesis, calcium channel antagonist activity and protection against pentylenetetrazole-induced seizure

Latifeh Navidpour [a], Hamed Shafaroodi [b], Ramin Miri [c], Ahmad Reza Dehpour [b], Abbas Shafiee [a,*]

[a] *Department of Medicinal Chemistry and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, P.O. Box 14155-6451, Tehran 14174, Iran*
[b] *Department of Pharmacology, Faculty of Medicine, Tehran University of Medical Sciences, P.O. Box 13145-784, Tehran 14174, Iran*
[c] *Medicinal and Natural Products Chemistry Research Center, Shiraz University of Medical Sciences, P.O. Box 71345-1718, 71345 Shiraz, Iran*

Received 31 July 2003; accepted 13 November 2003

Abstract

A group of alkyl, cycloalkyl and aryl ester analogs of nifedipine, in which the *o*-nitrophenyl group at position 4 is replaced by a 2-phenyl-4(5)-imidazolyl substituent, were synthesized and evaluated as calcium channel antagonist using the high K^+ contraction of guinea-pig ileal longitudinal smooth muscle, and the activity of **5a–d, 8b** and **8f** against pentylenetetrazole (PTZ)-induced seizure was assessed. The results for symmetrical esters showed that lengthening of the methylene chain in C_3 and C_5 ester substituents increased activity. When increasing of the length is accompanied by increasing the hindrance, the activity decreased. In contrast to symmetrical derivatives, comparison of the activities of asymmetrical esters showed that increasing the length of the methylene chain was accompanied by a decrease in their activity. The results demonstrate that **8a** was more active, and **5c** and **8f** were similar in effect to that of the reference drug nifedipine. The time-course of anticonvulsant effect on PTZ-induced seizure threshold of said compounds was assessed and showed that increasing the lipophilicity decreases the time needed for maximum effect. Mice treated with intraperitoneal injection of 25 mg/kg of these derivatives all exhibited increase seizure threshold as compared with control.

Keywords: 1,4-Dihydropyridine; Calcium channel antagonist; 2-Phenyl-imidazole; Pentylenetetrazole; Anticonvulsant

1. Introduction

Recent interest in calcium antagonists as possible antiepileptic drugs (AEDs) stems from various observations. Synaptosomal release of excitatory neurotransmitters is dependent on calcium influx [1], and many established AEDs, such as phenytoin and carbamazepine, inhibit this process by inhibiting calmodulin activation of calmodulin kinase II [2,3], although the relevance of this to their mode of action is still speculative. Initiation of seizure activity is associated with synchronization of intrinsic burst-firing which is synapse mediated [4]. The abnormal action potential (paroxysmal depolarizing shift, PDS), which occurs when epileptogenic activity begins [5] is also dependent on calcium cellular entry [6]. Both burst-firing [7] and the PDS [8] can be inhibited by calcium antagonists or exacerbated by a calcium agonist [9]. Depression of epileptic discharges by calcium antagonists has been shown in vivo in single neurons and neuronal populations [10].

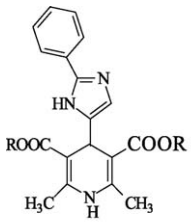
Animal models of epilepsy have helped to demonstrate anticonvulsant effects with flunarizine [11–13], nimodipine [4,14] and verapamil [15]. Another study [16] showed that many calcium antagonists were effective against audiogenic seizures in mice.

In epileptic patients, studies of flunarizine have disagreed as to whether it has useful anticonvulsant potential [17,18] or not [19,20]. Worrisome neurologic side effects with flunarizine [21] and the pharmacologic interactions of other AEDs with verapamil [22] and diltiazem [23] promote the dihydropyridines as candidates for investigation as AEDs in clinical practice. Their lack of sedative side effects adds to their benefits.

* Corresponding author.
E-mail address: ashafiee@ams.ac.ir (A. Shafiee).

doi:10.1016/j.farmac.2003.11.013

Table 1
Physical and calcium channel modulation data for compounds **5a–d**



Compound	R	Melting point (°C)	Yield (%)	Formula	Calcium channel antagonist activity (IC_{50} ± S.E.M.) [a]
5a	CH_3	247–249	80	$C_{20}H_{21}N_3O_4$	$1.36\ (\pm 0.88) \times 10^{-9}$
5b	CH_2CH_3	204–206	72	$C_{22}H_{25}N_3O_4$	$1.68\ (\pm 0.35) \times 10^{-9}$
5c	$CH_2CH_2CH_3$	224–226	53	$C_{24}H_{29}N_3O_4$	$3.11\ (\pm 0.59) \times 10^{-10}$
5d	$C(CH_3)_3$	243–245	54	$C_{26}H_{33}N_3O_4$	$2.40\ (\pm 0.31) \times 10^{-9}$
Nifedipine					$2.75\ (\pm 0.36) \times 10^{-10}$

[a] The molar concentration of antagonist test compound causing a 50% in the tonic contractile response (IC_{50} ± S.E.M.) in guinea-pig ileum smooth muscle by KCl (80 mmol/l) was determined graphically from dose–response curve. The number of experiments was six for all compounds.

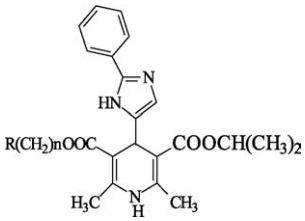
Studies suggest that substitution of C_4 *o*-nitrophenyl with heterocycles retains pharmacologic activity. We have found that bioisosteric replacement of the 4-aryl moiety with a imidazolyl group yields analogous 4-imidazolyl-1,4-dihydropyridines which exhibit potent calcium antagonist activity [24–28]. A major goal of our present research is to prepare lipophilic substituted 4-imidazolyl-1,4-dihydropyridine with improved ability to cross the blood–brain barrier.

The present study assessed the efficacy of some alkyl, cycloalkyl and aryl 1,4-dihydro-2,6-dimethyl-4-(2-phenyl-4(5)-imidazolyl)-3,5-pyridine dicarboxylate derivatives in terms of calcium channel blocking and anticonvulsive activity.

2. Chemistry

The synthesis of the 1,4-dihydropyridine derivatives **5a–d** (Table 1) and **8a–i** (Table 2) was achieved following the steps outlined in Fig. 1. Reaction of alcohol **2** with 2,2,6-trimethyl-4H-1,3-dioxin-4-one **3** afforded the corresponding acetoacetic esters **4** (76–92% yield) [29]. The symmetrical analogs **5a–d** (Table 1) were prepared by the classical Hantzsch condensation [30] in which 2-phenyl-1*H*-imidazole-4(5)-carboxaldehyde **1** was reacted with acetoacetic esters **4** and ammonium hydroxide. The asymmetrical analogs **8a–i** (Table 2) were synthesized by a modified Hantzsch reaction, using a procedure reported by Meyer et al. [31]. The physical properties of final compounds are summarized in Tables 1 and 2.

Table 2
Physical and calcium channel modulation data for compounds **8a–i**



Compound	R	*n*	Melting point (°C)	Yield (%)	Formula	Calcium channel antagonist activity (IC_{50} ± S.E.M.) [a]
8a	C_6H_{11}(cyclohexyl)	0	232–233	49	$C_{27}H_{33}N_3O_4$	$5.85\ (\pm 0.39) \times 10^{-11}$
8b	C_6H_{11}(cyclohexyl)	1	211–212	54	$C_{28}H_{35}N_3O_4$	$2.12\ (\pm 0.28) \times 10^{-9}$
8c	C_6H_{11}(cyclohexyl)	2	176–178	48	$C_{29}H_{37}N_3O_4$	$9.71\ (\pm 0.82) \times 10^{-9}$
8d	C_6H_{11}(cyclohexyl)	3	217–218	40	$C_{30}H_{39}N_3O_4$	$7.80\ (\pm 0.38) \times 10^{-9}$
8e	C_6H_{11}(cyclohexyl)	4	170–172	58	$C_{31}H_{41}N_3O_4$	$3.98\ (\pm 0.41) \times 10^{-8}$
8f	C_6H_5	1	194–195	48	$C_{28}H_{29}N_3O_4$	$2.77\ (\pm 0.46) \times 10^{-10}$
8g	C_6H_5	2	180–182	67	$C_{29}H_{31}N_3O_4$	$1.85\ (\pm 0.29) \times 10^{-9}$
8h	C_6H_5	3	175–178	58	$C_{30}H_{33}N_3O_4$	$3.52\ (\pm 0.60) \times 10^{-9}$
8i	C_6H_5	4	163–165	52	$C_{31}H_{35}N_3O_4$	$4.42\ (\pm 0.30) \times 10^{-9}$
Nifedipine						$2.75\ (\pm 0.36) \times 10^{-10}$

[a] Refer to footnote 'a' of Table 1.

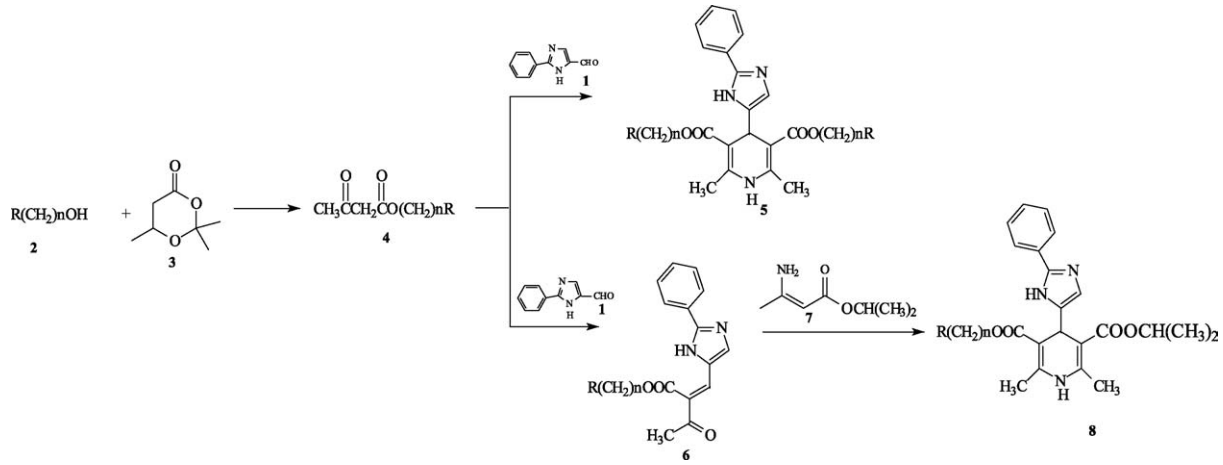


Fig. 1. Synthesis of 1,4-dihydropyridines having 2-phenyl-4(5)-imidazolyl substituents.

3. Experimental

3.1. Chemistry

Melting points were determined with a Reichert-Jung hot-stage microscope (A Cambridge Instrument Company, Vienna, Austria) and are uncorrected. ^{1}H NMR spectra were obtained with a Bruker 80 MHz spectrometer (Bruker Analytische Messetechnik, Rheinstetten, Germany) in d_1-chloroform or d_6-DMSO and tetramethylsilane (TMS) was used as an internal standard. Mass spectra were recorded with a Finnigan Mat TSQ-70 spectrometer (Finnigan Mat, Breman, Germany). Infrared spectra were acquired on a Nicolet Magna 550-FT spectrometer (Nicolet, Madison, USA). Elemental analyses were carried out with a Perkin-Elmer Model 240-C apparatus (Perkin-Elmer, Norwalk, CT, USA). The results of elemental analyses (C, H, N) were within ±0.4% of the calculated values. Silica gel HT-254 (E. Merck, Darmstadt, Germany) was used for thin layer chromatography.

Isopropyl 3-aminocrotonate **7** were purchased from Sigma-Aldrich Chemie GmbH (Deisenhofen, Germany).

3.1.1. General procedure for the synthesis of dialkyl 1,4-dihydro-2,6-dimethyl-4-(2-phenyl-4(5)-imidazolyl)-3,5-pyridine dicarboxylates (**5a–d**)

A solution of ammonium hydroxide (25%, 0.5 ml) was added to a stirring solution of compound **1** [32] (175 mg, 1 mmol) and corresponding alkyl 3-oxobutanoate (2 mmol) in methanol (10 ml). The mixture was protected from light and refluxed overnight. After cooling the precipitate was filtered and crystallized from methanol to give pure compounds **5a–d**.

*3.1.1.1. Dimethyl 1,4-dihydro-2,6-dimethyl-4-(2-phenyl-4(5)-imidazolyl)-3,5-pyridine dicarboxylate (**5a**).* Melting point: 247–249 °C; IR (KBr): ν (cm^{-1}) 3256 (NH), 1689 (CO); ^{1}H NMR ($CDCl_3$): δ 2.30 (s, 6H, C_2 and C_6-CH_3), 3.73 (s, 6H, $COOCH_3$), 5.05 (s, 1H, H_4), 6.62 (s, 1H, H_4-imidazole), 7.32 (m, 3H, aromatic), 7.77 (m, 2H, aromatic); MS, *m/z* (%): 367 (100), 351 (44), 335 (26), 307 (82), 275 (63), 248 (12), 223 (38), 165 (18), 145 (40), 104 (12), 77 (5).

*3.1.1.2. Diethyl 1,4-dihydro-2,6-dimethyl-4-(2-phenyl-4(5)-imidazolyl)-3,5-pyridine dicarboxylate (**5b**).* Melting point: 204–206 °C; IR (KBr): ν (cm^{-1}) 3295 (NH), 1696 (CO); ^{1}H NMR ($CDCl_3$): δ 1.26 (t, 6H, CH_3-ethyl), 2.28 (s, 6H, C_2 and C_6-CH_3), 4.20 (q, 4H, $COOCH_2$), 5.02 (s, 1H, H_4), 6.56 (s, 1H, H_4-imidazole), 7.35 (m, 3H, aromatic), 7.69 (m, 2H, aromatic).

*3.1.1.3. Dipropyl 1,4-dihydro-2,6-dimethyl-4-(2-phenyl-4(5)-imidazolyl)-3,5-pyridine dicarboxylate (**5c**).* Melting point: 224–226 °C; IR (KBr): ν (cm^{-1}) 3303 (NH), 1683 (CO); ^{1}H NMR ($CDCl_3$): δ 0.90 (t, 6H, CH_3-propyl), 1.65 (m, 4H, CH_2-propyl), 2.29 (s, 6H, C_2 and C_6-CH_3), 4.10 (t, 4H, $COOCH_2$), 5.08 (s, 1H, H_4), 6.54 (bs, 1H, NH-imidazole), 6.64 (s, 1H, H_4-imidazole), 7.36 (m, 3H, aromatic), 7.71 (m, 2H, aromatic).

*3.1.1.4. Ditertiarybutyl 1,4-dihydro-2,6-dimethyl-4-(2-phenyl-4(5)-imidazolyl)-3,5-pyridine dicarboxylate (**5d**).* Melting point: 243–245 °C; IR (KBr): ν (cm^{-1}) 3302 (NH), 1700 (CO); ^{1}H NMR ($CDCl_3$): δ 1.40 (s, 18H, $C(CH_3)_3$), 2.19 (s, 6H, C_2 and C_6-CH_3), 4.90 (s, 1H, H_4), 6.55 (s, 1H, H_4-imidazole), 7.34 (m, 3H, aromatic), 7.81 (m, 2H, aromatic).

3.1.2. General procedure for the synthesis of alkyl(aryl) 2-[(2-phenyl-4(5)-imidazolyl)-methylene]-3-oxobutanoate derivatives (**6a–i**)

A solution of compound **1** (345 mg, 2 mmol), corresponding alkyl(aryl) acetoacetate (2 mmol), glacial acetic acid (0.2 ml), piperidine (0.08 ml) and dry benzene (20 ml) was refluxed for 2 h, during which the resultant water was removed via a Dean-Stark trap. After cooling the benzene was removed and the residue was purified by chromatography on silica gel with chloroform/methanol (20:1 v/v), to give pure compounds **6a–i** as semisolid.

*3.1.3. General procedure for the synthesis of alkyl(aryl) isopropyl 1,4-dihydro-2,6-dimethyl-4-(2-phenyl-4(5)imidazolyl)-3,5-pyridine dicarboxylates (**8a–i**)*

A solution of compounds **6a–i** (1 mmol) and isopropyl 3-aminocrotonate **7** (140 mg, 1 mmol) in methanol was protected from light and refluxed overnight. After cooling, the solution was concentrated under reduced pressure and purified by chromatography on silica gel with chloroform/methanol (20:1 v/v). The product was crystallized from ethyl acetate/acetone to give pure compounds **8a–i**.

*3.1.3.1. Cyclohexyl isopropyl 1,4-dihydro-2,6-dimethyl-4-(2-phenyl-4(5)imidazolyl)-3,5-pyridine dicarboxylate (**8a**).* Melting point: 232–233 °C; IR (KBr): ν (cm^{-1}) 3276 (NH), 1696 (CO); ^1H NMR ($CDCl_3$): δ 1.24 (2d, 6H, CH_3-isopropyl), 1.70 (m, 10H, cyclohexyl), 2.28 (s, 6H, C_2 and C_6-CH_3), 4.95 (m, 2H, CO_2CH), 5.02 (s, 1H, H_4), 6.08 (bs, 1H, NH-imidazole), 6.59 (s, 1H, H_4-imidazole), 7.35 (m, 3H, aromatic), 7.71 (m, 2H, aromatic); MS, *m/z* (%): 463 (20), 420 (22), 380 (82), 338 (100), 294 (44), 278 (25), 249 (48), 236 (35), 196 (43), 149 (15), 123 (7).

*3.1.3.2. Cyclohexylmethyl isopropyl 1,4-dihydro-2,6-dimethyl-4-(2-phenyl-4(5)imidazolyl)-3,5-pyridine dicarboxylate (**8b**).* Melting point: 211–212 °C; IR (KBr): ν (cm^{-1}) 3276 (NH), 1696 (CO); ^1H NMR ($CDCl_3$): δ 1.26 (2d, 6H, CH_3-isopropyl), 1.30 (m, 11H, cyclohexyl), 2.30 (s, 6H, C_2 and C_6-CH_3), 3.72 (m, 3H, CO_2CH_2 and CO_2CH), 5.05 (s, 1H, H_4), 5.90 (bs, 1H, NH-imidazole), 6.65 (s, 1H, H_4-imidazole), 7.45 (m, 3H, aromatic), 7.79 (m, 2H, aromatic).

*3.1.3.3. Cyclohexylethyl isopropyl 1,4-dihydro-2,6-dimethyl-4-(2-phenyl-4(5)imidazolyl)-3,5-pyridine dicarboxylate (**8c**).* Melting point: 176–178 °C; IR (KBr): ν (cm^{-1}) 3276 (NH), 1703 (CO); ^1H NMR ($CDCl_3$): δ 1.24 (2d, 6H, CH_3-isopropyl), 1.56 (m, 13H, CH_2-cyclohexyl), 2.30 (s, 6H, C_2 and C_6-CH_3), 4.15 (t, 2H, CO_2CH_2), 5.01 (m, 1H, CO_2CH), 5.04 (s, 1H, H_4), 6.46 (s, 1H, H_4-imidazole), 6.88 (bs, 1H, NH-imidazole), 7.34 (m, 3H, aromatic), 7.70 (m, 2H, aromatic).

*3.1.3.4. Cyclohexylpropyl isopropyl 1,4-dihydro-2,6-dimethyl-4-(2-phenyl-4(5)imidazolyl)-3,5-pyridine dicarboxylate (**8d**).* Melting point: 217–218 °C; IR (KBr): ν (cm^{-1}) 3269 (NH), 1683 (CO); ^1H NMR ($CDCl_3$): δ 1.25 (2d, 6H, CH_3-isopropyl), 1.60 (m, 15H, CH_2CH_2-cyclohexyl), 2.29 (s, 6H, C_2 and C_6-CH_3), 4.12 (t, 2H, CO_2CH_2), 5.02 (m, 1H, CO_2CH), 5.10 (s, 1H, H_4), 5.62 (bs, 1H, NH-imidazole), 6.65 (s, 1H, H_4-imidazole), 7.42 (m, 3H, aromatic), 7.78 (m, 2H, aromatic).

*3.1.3.5. Cyclohexylbutyl isopropyl 1,4-dihydro-2,6-dimethyl-4-(2-phenyl-4(5)imidazolyl)-3,5-pyridine dicarboxylate (**8e**).* Melting point: 170–172 °C; IR (KBr): ν (cm^{-1}) 3335 (NH), 1670 (CO); ^1H NMR ($CDCl_3$): δ 1.20 (2d, 6H, CH_3-isopropyl), 1.57 (m, 17H, $CH_2CH_2CH_2$-cyclohexyl), 2.23 (s, 6H, C_2 and C_6-CH_3), 4.10 (t, 2H, CO_2CH_2), 5.02 (m, 2H, H_4 and CO_2CH), 6.08 (bs, 1H, NH-imidazole), 6.59 (s, 1H, H_4-imidazole), 7.29 (m, 3H, aromatic), 7.67 (m, 2H, aromatic).

*3.1.3.6. Benzyl isopropyl 1,4-dihydro-2,6-dimethyl-4-(2-phenyl-4(5)imidazolyl)-3,5-pyridine dicarboxylate (**8f**).* Melting point: 194–195 °C; IR (KBr): ν (cm^{-1}) 3276 (NH), 1696 (CO); ^1H NMR ($CDCl_3$): δ 1.27 (2d, 6H, CH_3-isopropyl), 2.30 (s, 6H, C_2 and C_6-CH_3), 5.02 (m, 4H, H_4 and CO_2CH and CO_2CH_2), 5.95 (bs, 1H, NH-imidazole), 6.62 (s, 1H, H_4-imidazole), 7.28 (s, 5H, phenyl), 7.35 (m, 3H, aromatic), 7.70 (m, 2H, aromatic).

*3.1.3.7. Isopropyl phenylethyl 1,4-dihydro-2,6-dimethyl-4-(2-phenyl-4(5)imidazolyl)-3,5-pyridine dicarboxylate (**8g**).* Melting point: 180–182 °C; IR (KBr): ν (cm^{-1}) 3262 (NH), 1696 (CO); ^1H NMR ($CDCl_3$): δ 1.25 (2d, 6H, CH_3-isopropyl), 2.25 (s, 6H, C_2 and C_6-CH_3), 2.95 (t, 2H, CH_2-Ph), 4.38 (t, 2H, CO_2CH_2), 5.03 (s, 1H, H_4), 5.13 (m, 1H, CO_2CH), 6.32 (bs, 1H, H-imidazole), 6.62 (s, 1H, H_4-imidazole), 7.01 (s, 5H, phenyl), 7.35 (m, 3H, aromatic), 7.69 (m, 2H, aromatic).

*3.1.3.8. Isopropyl phenylpropyl 1,4-dihydro-2,6-dimethyl-4-(2-phenyl-4(5)imidazolyl)-3,5-pyridine dicarboxylate (**8h**).* Melting point: 175–178 °C; IR (KBr): ν (cm^{-1}) 3302 (NH), 1689 (CO); ^1H NMR ($CDCl_3$): δ 1.26 (2d, 6H, CH_3-isopropyl), 1.92 (t, 2H, CH_2-Ph), 2.30 (s, 6H, C_2 and C_6-CH_3), 2.62 (t, 2H, CH_2), 4.20 (t, 2H, CO_2CH_2), 5.08 (m, 2H, H_4 and CO_2CH), 6.12 (bs, 1H, NH-imidazole), 6.65 (s, 1H, H_4-imidazole), 7.28 (s, 5H, phenyl), 7.35 (m, 3H, aromatic), 7.70 (m, 2H, aromatic).

*3.1.3.9. Isopropyl phenylbutyl 1,4-dihydro-2,6-dimethyl-4-(2-phenyl-4(5)imidazolyl)-3,5-pyridine dicarboxylate (**8i**).* Melting point: 163–165 °C; IR (KBr): ν (cm^{-1}) 3276 (NH), 1703 (CO); ^1H NMR ($CDCl_3$): δ 1.23 (2d, 6H, CH_3-isopropyl), 1.63 (m, 4H, CH_2CH_2-Ph), 2.28 (s, 6H, C_2 and C_6-CH_3), 2.57 (m, 2H, CH_2), 4.14 (m, 2H, CO_2CH_2), 5.04 (m, 2H, H_4 and CO_2CH), 6.20 (bs, 1H, NH-imidazole), 6.62 (s, 1H, H_4-imidazole), 7.13 (s, 5H, phenyl), 7.32 (m, 3H, aromatic), 7.69 (m, 2H, aromatic).

3.2. Pharmacology

3.2.1. Determination of calcium channel antagonist activity

Male albino guinea pigs (300–450 g) were purchased from Pasteur Institute (Karaj, Iran). They had free access to standard rodent chow (Dam-Pars Co., Tehran, Iran) and tap water at all times. The animals were housed in a room maintained at 23 ± 2 °C temperature, 55 ± 10% humidity and on a 12 h light/dark cycle. The feeding was disrupted 1 d before starting in vitro tests. The animals were sacrificed by a

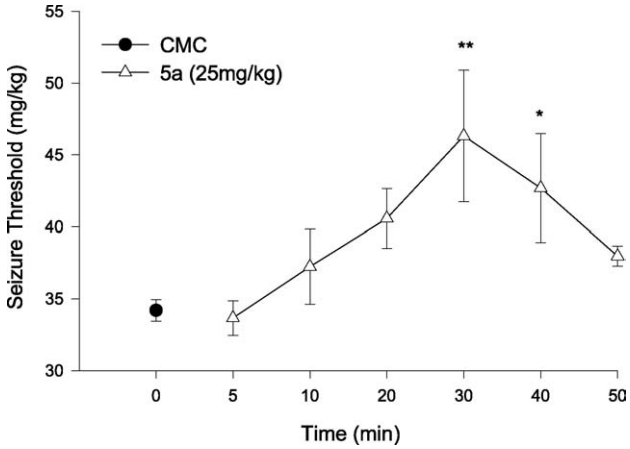


Fig. 2. The time-course of anticonvulsant effect of **5a** (25 mg/kg, i.p.) on PTZ-induced seizure threshold: **5a** was administered 5, 10, 20, 30, 40 and 50 min before PTZ and compared with CMC-treated mice. Data are expressed as mean ± S.E.M. of eight mice. * $P < 0.05$, ** $P < 0.01$ compared with CMC group.

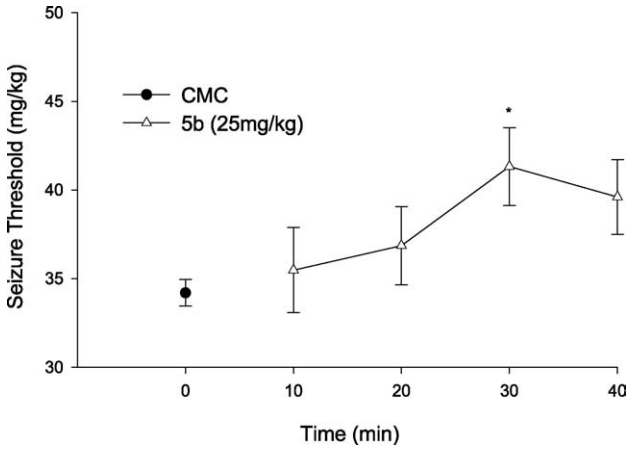


Fig. 3. The time-course of anticonvulsant effect of **5b** (25 mg/kg, i.p.) on PTZ-induced seizure threshold: **5b** was administered 10, 20, 30 and 40 min before PTZ and compared with CMC-treated mice. Data are expressed as mean ± S.E.M. of eight mice. * $P < 0.05$ compared with CMC group.

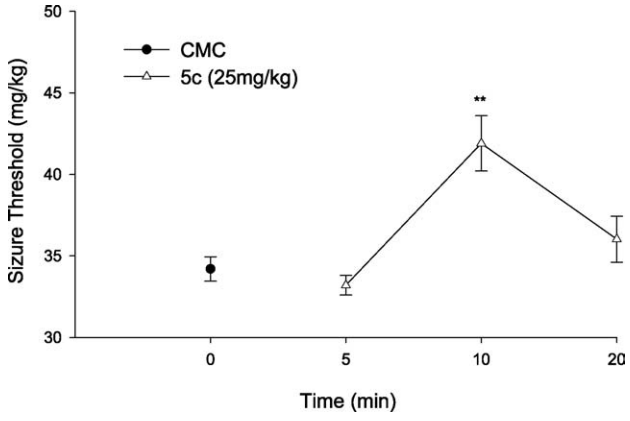


Fig. 4. The time-course of anticonvulsant effect of **5c** (25 mg/kg, i.p.) on PTZ-induced seizure threshold: **5c** was administered 5, 10 and 20 min before PTZ and compared with CMC-treated mice. Data are expressed as mean ± S.E.M. of eight mice. ** $P < 0.01$ compared with CMC group.

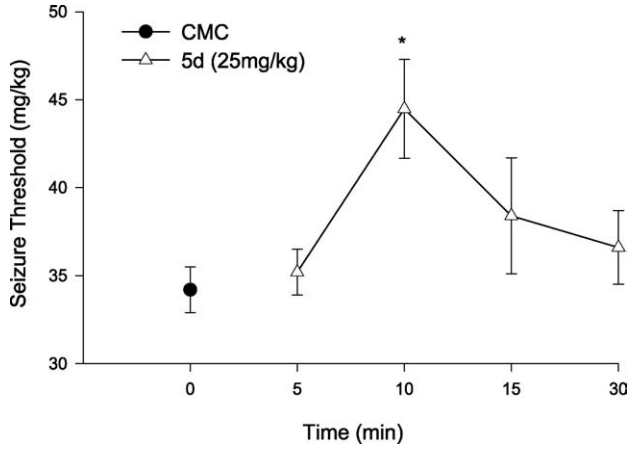


Fig. 5. The time-course of anticonvulsant effect of **5d** (25 mg/kg, i.p.) on PTZ-induced seizure threshold: **5d** was administered 5, 10, 15 and 30 min before PTZ and compared with CMC-treated mice. Data are expressed as mean ± S.E.M. of eight mice. * $P < 0.05$ compared with CMC group.

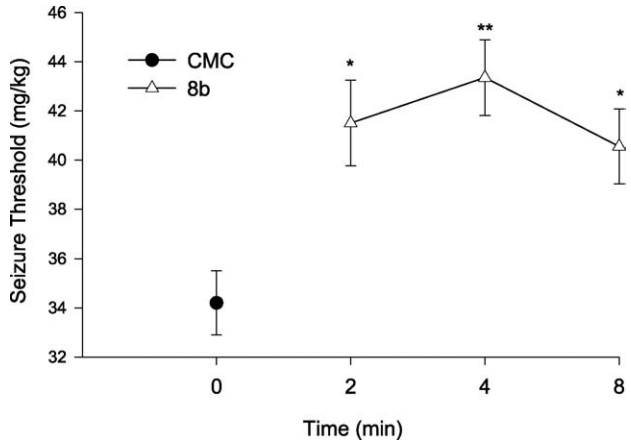


Fig. 6. The time-course of anticonvulsant effect of **8b** (25 mg/kg, i.p.) on PTZ-induced seizure threshold: **8b** was administered 2, 4 and 8 min before PTZ and compared with CMC-treated mice. Data are expressed as mean ± S.E.M. of eight mice. * $P < 0.05$, ** $P < 0.01$ compared with CMC group.

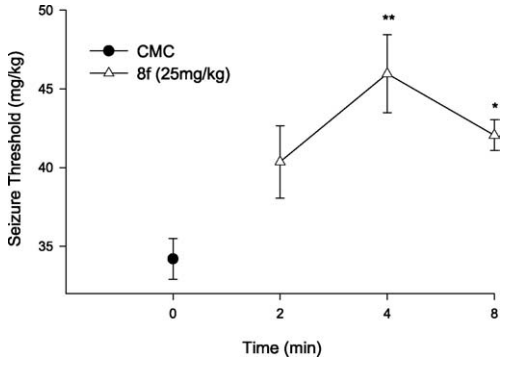


Fig. 7. The time-course of anticonvulsant effect of **8f** (25 mg/kg, i.p.) on PTZ-induced seizure threshold: **8f** was administered 2, 4 and 8 min before PTZ and compared with CMC-treated mice. Data are expressed as mean ± S.E.M.

blow to the head. The intestine was removed above the ileocecal junction and a longitudinal smooth muscle segments of 2 cm length were mounted under a resting tension of 0.5 g. The segments were maintained at 37 °C in a 20 ml jacketed organ bath containing oxygenated physiological saline solution of the following (mmol) composition: NaCl, 137; $CaCl_2$, 1.8; KCl, 2.7; $MgSO_4$, 1.1; NaH_2PO_4, 0.4; $NaHCO_3$, 12 and glucose, 5. The muscles were equilibrated for 1 h with a solution change every 15 min. The contractions were recorded with a force displacement transducer (F-50) on a Narco Physiograph (Narco Biosystems, Houston, TX, USA). Test agents were prepared as 10^{-2} mol/l stock solutions in DMSO and stored protected from light. Dilutions were made with DMSO. The contractile response was taken as the 100% value for the tonic (slow) component of the response. The contraction was elicited with 80 mmol/l KCl. Test compounds were cumulatively added and compound-induced relaxation of contracted muscle was expressed as percent of control. The IC_{50} values (concentration needed to produce 50% relaxation on contracted ileal smooth muscle) were graphically determined from the concentration–response curves [33,34].

The research protocol and experimental animals have been approved by the ethics committee of Tehran University of Medical Sciences.

3.2.2. Determination of anticonvulsant activity

3.2.2.1. Chemicals. Pentylenetetrazole (PTZ) was purchased from Sigma (UK). It was dissolved in physiological saline solution and all selected derivatives were dispersed in carboxy methyl cellulose (CMC, 2%) to such concentrations that requisite doses were administered in a volume of 10 ml/kg. In all experiments PTZ was administered intravenously (i.v.) and all other drugs were administered intraperitoneally (i.p.).

3.2.2.2. Subjects. Male NMRI mice (24–30 g, Pasteur Institute of Iran) were used throughout this study. The animals were housed in temperature-controlled room (24 ± 1 °C) on a 12 h light/dark cycle with free access to food and water. All procedures were carried out in accordance with institutional guidelines for animal care and use. Each mouse was used only once and each treatment group consisted of at least eight animals.

3.2.2.3. Determination of seizure threshold. Threshold of PTZ-induced seizures was determined by inserting a 30-gauge butterfly needle into the tail vein of mice and the infusion of PTZ (0.5%) at a constant rate of 0.5 ml/min to unrestrained animals. Infusion was halted when forelimb clonus followed by full clonus of the body was observed. Minimal dose of PTZ (mg/kg of mice weight) needed to induce clonic seizure was measured as an index of seizure threshold.

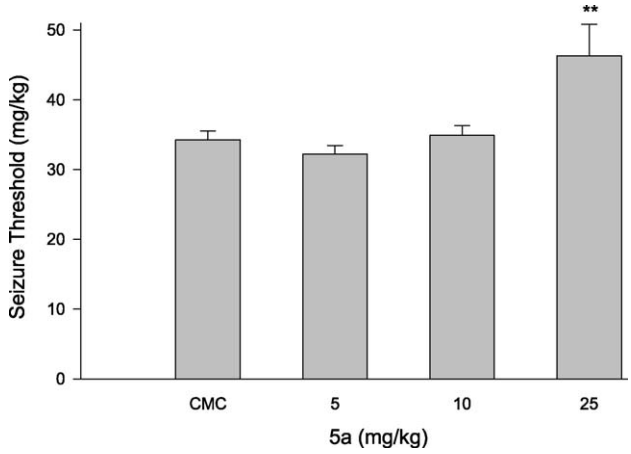


Fig. 8. Effect of different dosage of **5a** (5, 10 and 25 mg/kg) on PTZ-induced seizure threshold in mice: **5a** was administered 30 min before PTZ. Data are expressed as mean ± S.E.M. of eight mice. ** $P < 0.01$ compared with CMC group.

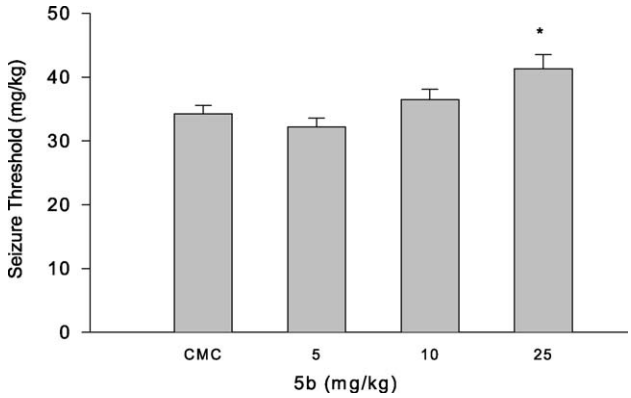


Fig. 9. Effect of different dosage of **5b** (5, 10 and 25 mg/kg) on PTZ-induced seizure threshold in mice: **5b** was administered 30 min before PTZ. Data are expressed as mean ± S.E.M. of eight mice. * $P < 0.05$ compared with CMC group.

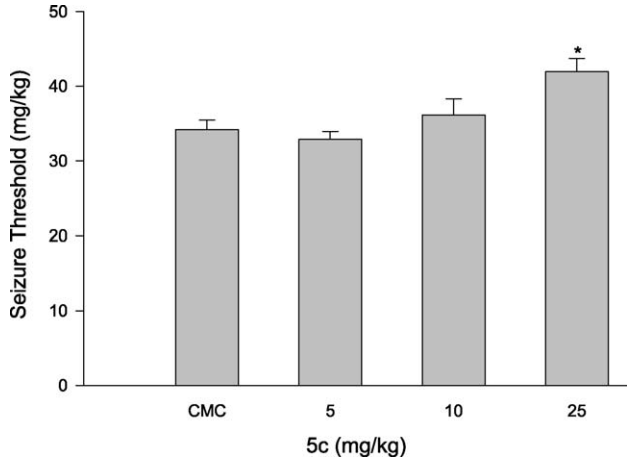


Fig. 10. Effect of different dosage of **5c** (5, 10 and 25 mg/kg) on PTZ-induced seizure threshold in mice: **5c** was administered 30 min before PTZ. Data are expressed as mean ± S.E.M. of eight mice. * $P < 0.05$ compared with CMC group.

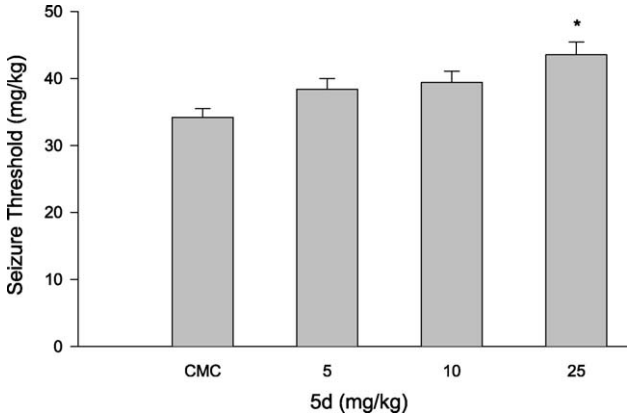


Fig. 11. Effect of different dosage of **5d** (5, 10 and 25 mg/kg) on PTZ-induced seizure threshold in mice: **5d** was administered 30 min before PTZ. Data are expressed as mean ± S.E.M. of eight mice. * $P < 0.05$ compared with CMC group.

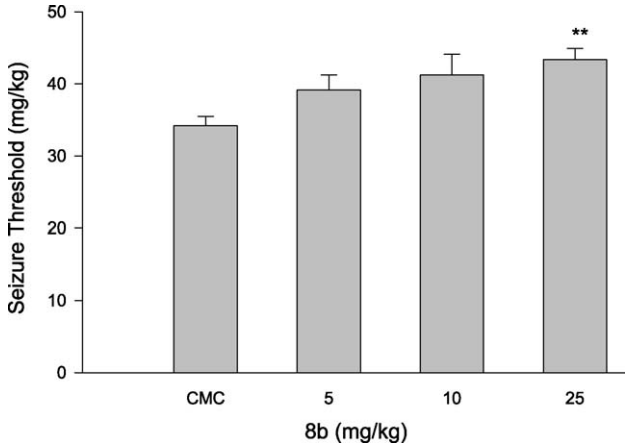


Fig. 12. Effect of different dosage of **8b** (5, 10 and 25 mg/kg) on PTZ-induced seizure threshold in mice: **8b** was administered 30 min before PTZ. Data are expressed as mean ± S.E.M. of eight mice. ** $P < 0.01$ compared with CMC group.

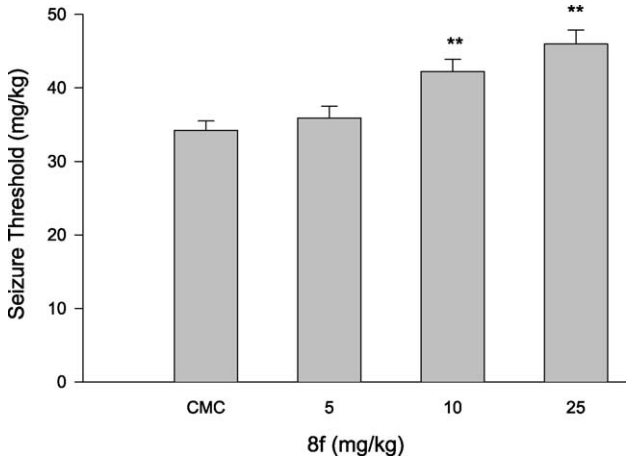


Fig. 13. Effect of different dosage of **8f** (5, 10 and 25 mg/kg) on PTZ-induced seizure threshold in mice: **8f** was administered 30 min before PTZ. Data are expressed as mean ± S.E.M. of eight mice. **$P < 0.01$ compared with CMC group.

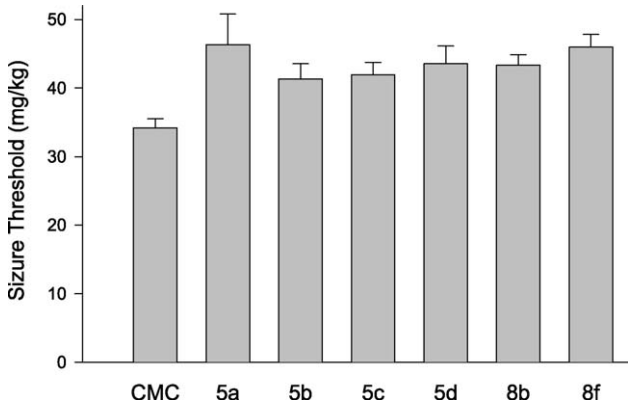


Fig. 14. Comparison of the highest dosage (25 mg/kg) of derivatives on PTZ-induced seizure threshold.

Animals in experiment 1 received the highest doses of selected derivatives (25 mg/kg), 2.5, 5, 10, 20, 30, 40 and 50 min prior to PTZ in distinct groups of mice in order to determine the time of maximal effect.

In experiment 2, different doses of the said derivatives (5, 10 and 25 mg/kg) or vehicle were administered in appropriate time thus obtained before determination of seizure threshold [35].

3.2.2.4. Statistical analysis. Data are expressed as mean ± S.E.M. The one-way analysis of variance (ANOVA) followed by Turkey–Kramer multiple comparisons was used to analyze the data. $P < 0.05$ was considered as the significance level between the groups.

4. Results and discussion

The calcium channel antagonist activity of **5a–d** and **8a–i** determined as the concentration needed to produce 50% inhibition of the guinea-pig ileal longitudinal smooth muscle (GPILSM) contractility, are summarized in Table 1 (symmetrical compounds) and Table 2 (asymmetrical compounds).

Comparison of the activities of symmetrical esters in alkyl ester series (Table 1, **5a–d**) indicates that lengthening of the methylene chain in C_3 and C_5 ester substituents increases activity (**5a–c**). When increasing of the length is accompanied by increasing the hindrance (**5d**), the activity decreases. Finally the results show that compound **5c** is similar in effect to the reference drug nifedipine.

In contrast to symmetrical derivatives, comparison of the activities of asymmetrical esters shows that increasing the length of the methylene chain is accompanied by a decrease in their activity. The activity of compounds **8f** is similar in effect and **8a** is more active than the reference drug nifedipine.

The structure–activity data indicate that the 4-(2-phenyl-4(5)-imidazolyl) moiety is a bioisoester of *o*-nitrophenyl group.

As lipophilic aromatic substituents attached to the C-2 position of imidazole ring are supposed to improve penetration into brain, the activity of **5a–d, 8b** and **8f** against PTZ-induced seizure was assessed. Following i.p. injection and due to different substituents, each derivative achieves its peak blood–brain concentration at a different time. Thus it seems that a rough estimate of the time needed by each derivative to reach its highest effect is essential at this point, and time-course of anticonvulsant on PTZ-induced seizure threshold of each compound was assessed and are shown in Figs. 2–7.

As could be predicted, increase in lipophilicity, decreases the time needed for maximum effect.

Mice treated with i.p. injection of 25 mg/kg of these derivatives all exhibited increase in seizure threshold as compared with controls. However, doses of 5 and 10 mg/kg did not affect seizure threshold significantly, compared with control, shown in Figs. 8–13. It is interesting to note that, as it is seen in Fig. 14, there were no significant differences between groups treated with different doses of each derivative.

Acknowledgements

This research was supported by a grant from the research council of Tehran University of Medical Sciences.

References

[1] R.J. De Lorenzo, S.D. Freedman, Possible role of calcium dependent protein phosphorylation in mediating neurotransmitter release and anticonvulsant action, Epilepsia 18 (1977) 357–365.
[2] R.S. Sohn, J.A. Ferrendelli, Anticonvulsant drug mechanisms, Arch. Neurol. 33 (1976) 626–629.
[3] J.M. Crowder, H.F. Bradford, Common anticonvulsants inhibit Ca^{2+} uptake and amino acid neurotransmitter release in vitro, Epilepsia 28 (1987) 378–382.
[4] F.B. Meyer, Calcium, neuronal hyperexcitability and ischemic injury, Brain Res. Rev. 14 (1989) 227–243.
[5] E.S. Goldensohn, D.P. Purpura, Intracellular potentials of corticalneurons during focal epileptogenic discharges, Science 139 (1963) 840–842.
[6] R.K.S. Wong, D.A. Prince, Dentric mechanisms underlying penicillin-induced epileptiform activity, Science 204 (1979) 1229–1231.
[7] D. Bingmann, E.J. Speckmann, Specific suppression of pentylenetetrazol-induced epileptiform discharges in CA3 neurons (hippocampal slice, guinea pig) by the organic calcium antagonists flunarizine and verapamil, Exp. Brain Res. 74 (1989) 239–248.
[8] O.W. Witte, E.J. Speckmann, J. Walden, Motor cortical epileptic foci in vivo: actions of a calcium channel blocker on paroxysmal neuronal depolarizations, Electroencephalogr. Clin. Neurophysiol. 66 (1987) 43–55.
[9] J. Walden, H. Pockberger, E.J. Speckmann, H. Petache, Paroxysmal neuronal depolarizations in the rat motocortex in vivo: intracellular injection of the calcium agonist BAY K 8644, Exp. Brain Res. 64 (1986) 607–609.
[10] E.J. Speckmann, O.W. Witte, J. Walden, Involvement of calcium ions in focal epileptic activity of the neocortex, in: U. Heinemann, M. Klee, E. Neher, W. Singer (Eds.), Calcium Electrogenesis and Neuronal Functioning, Springer, Berlin, 1986, pp. 111–126.
[11] L.K.C. Desmedt, G.J.E. Niemergeers, P.A.J. Janssen, Anticonvulsive properties of cinnarizine and flunarizine in rats and mice, Arzneimittel-Forschung 25 (1975) 1408–1413.
[12] D. Ashton, A. Wauquier, Effects of some antiepileptic, neuroleptic and gabaminergic drugs on convulsions induced by D,L-allylglycine, Pharmacol. Biochem. Behav. 11 (1979) 221–226.
[13] G.B. De Sarro, G. Nistico, B.S. Meldrum, Anticonvulsant properties of flunarizine on reflex and generalized models of epilepsy, Neuropharmacology 25 (1986) 695–701.
[14] F.B. Meyer, R.E. Anderson, T.M. Sundt, T.L. Yaksi, F.W. Sharbrough, Suppression of pentylenetetrazole seizures by oral administration of a dihydropyridine Ca^{2+} antagonist, Epilepsia 28 (1987) 409–414.
[15] J. Walden, E.J. Speckmann, Suppression of recurrent generalized tonic–clonic seizure discharges by intraventricular perfusion of a calcium antagonist, Electroencephalogr. Clin. Neurophysiol. 69 (1988) 353–362.
[16] G.B. De Sarro, B.S. Meldrum, Anticonvulsant properties of some calcium entry blockers in DBA/2 mice, Br. J. Pharmacol. 93 (1988) 147–156.
[17] J. Overweg, D. Ashton, A. De Beukellar, C.D. Binnie, A. Wauquier, Add-on therapy in epilepsy with calcium entry blockers, Eur. Neurol. 25 (Suppl. 1) (1986) 93–101.
[18] E. Starreveld, F. De Beukellar, A.F. Wilson, D.R. Mclean, H.P. Findlay, Double-blind cross-over placebo controlled study of flunarizine in patients with therapy resistant epilepsy, Can. J. Neurol. Sci. 16 (1989) 187–190.
[19] J. Alving, O. Kristensen, I. Tsiropulos, K. Mondrup, Double-blind placebo-controlled evaluation of flunarizine as adjunct therapy in epilepsy with complex partial seizures, Acta Neurol. Scand. 79 (1989) 128–132.
[20] D. Keene, S. Whiting, P. Humphreys, P. Jacob, Flunarizine as a supplementary medication in refractory epilepsy: a double blind crossover study, Can. J. Neurol. Sci. 16 (1989) 191–193.
[21] C. Chouza, J.L. Caamano, R. Aljanati, A. Scaramelli, O. De Medina, S. Romero, Parkinsonism, tardive dyskinesia, akathisia and depression induced by flunarizine, Lancet 1 (1986) 655–661.
[22] G.J.A. Macphee, G.G. Thomson, G.T. McInnes, M.J. Bordie, Verapamil potentiates carbamazepine neurotoxicity: a clinically important inhibitory interaction, Lancet 1 (1986) 700–703.
[23] M.J. Bordie, G.J.A. Macphee, Carbamazepine toxicity precipitated by diltiazem, Br. Med. J. 292 (1986) 1170–1171.
[24] A. Shafiee, R. Miri, A.R. Dehpour, F. Soleymani, Synthesis and calcium channel antagonist-activity of nifedipine analogues containing nitroimidazolyl substituents, Pharm. Sci. 2 (1996) 541–543.
[25] F. Pourmorad, F. Hadizadeh, A. Shafiee, Synthesis and calcium-channel antagonist activity of 4-imidazolyl-1,4-dihydropyridines, Pharm. Sci. 3 (1997) 165–168.
[26] A. Shafiee, A.R. Dehpour, F. Hadizadeh, M. Azimi, Synthesis and calcium channel antagonist activity of nifedipine analogues with ethylsulfonylimidazolyl substituent, Pharm. Acta Helv. 73 (1998) 75–79.
[27] M. Amini, A.A. Golabchifar, A.R. Dehpour, H.M. Pirali, A. Shafiee, Synthesis and calcium channel antagonist activity of new 1,4-dihydropyridine derivatives containing dichloroimidazolyl substituents, Arzneimittel-Forsch./Drug Res. 52 (2002) 21–26.
[28] A. Shafiee, N. Rastkary, M. Jorjani, B. Shafaghi, Synthesis and antihypertensive activities of new 1,4-dihydropyridine containing nitroimidazolyl substituent with a nitrooxy group at the 3-ester position, Arch. Pharm. Pharm. Med. Chem. 2 (2002) 69–76.
[29] R.J. Clemens, H.A. Hyatt, Acetoacetylation with 2,2,6-trimethyl-4H-1,3-dioxine-4-one: a convenient alternative to diketen, J. Org. Chem. 50 (1985) 2431–2435.

[30] A. Hantzsch, Über die synthese pyridinartiger Verbindungen aus Acetessigather und Aldehydammoniak, Justus Liebigs Ann. Chem. 215 (1882) 1–82.

[31] H. Meyer, F. Bossert, E. Wehinger, K. Stoepel, W. Vater, Synthese und vergleichende pharmakologische Untersuchungen von 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-carbosäureestern mit nicht-identischen Esterfunktionen, Arzneimittel-Forsch./Drug Res. 31 (I) (1981) 407–409.

[32] I. Sircar, G. Bobowski, J.A. Bristol, R.E. Weishaar, D.B. Evans, Cardiotonic agents. Synthesis and biological activity of novel 6-(substituted 1H-imidazole-4(5)-yl)-3(2)H-pyridazinone, J. Med. Chem. 29 (1986) 261–267.

[33] G.T. Bolger, P. Gengo, R. Klochowski, R.H. Siegel, R.A. Janis, A.M. Triggle, et al., Characterization of binding of the Ca^{2+} channel antagonist, [^{3}H] nitrendipine to guinea pig ileal smooth muscle, J. Pharm. Exp. Ther. 225 (1983) 291–309.

[34] G.C. Rovnyak, K.S. Atwal, A. Hedberg, S.D. Kimball, S. Moreland, J.Z. Gougoutas, et al., Dihydropyridine calcium channel blockers: 3-substituted-4-aryl-1,4-dihydropyridine-5-carboxylic acid esters, potent antihypertensive agent, J. Med. Chem. 35 (1992) 3236–3263.

[35] H. Homayoun, S. Khavandgar, Kh. Namiranian, S.A. Gaskari, A.R. Dehpour, The role of nitric oxide in anticonvulsant and proconvulsant effects of morphine in mice, Epilepsy Res. 48 (2002) 33–41.