Synthesis and Anticonvulsant Activity of Some Cinnamylpiperazine Derivatives

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Abstract: A series of cinnamylpiperazine derivatives was synthesized using different benzophenone as starting material. The structures of the compounds were proved by their IR, ^{1}H-NMR spectroscopic data and mass spectra data. The anticonvulsant activities of these compounds were evaluated with maximal electroshock (MES) test and rotarod test with intraperitoneal injection on KunMing mice. Among all the flunarizine analogues, no one exhibited better anticonvulsant activity than flunarizine. Flunarizine (**4i**) exhibited anticonvulsant activity with ED_{50} of 38.1 mg/kg, TD_{50} of 164.3 mg/kg and PI of 4.3 through administration intraperitoneal, and with ED_{50} of 56.8 mg/kg, TD_{50} of 456.3 mg/kg and PI of 8.0 through oral administration.

Keywords: Anticonvulsant, MES, Calcium channel, Flunarizine, Cinnarizine, Cinnamyl piperazine.

1. INTRODUCTION

An approximately 1% of the world population suffer from epilepsy. Ya-Juan Zhao *et al.* [1] proved that the seizures of epilepsy often caused excessive calcium influx by measuring intracellular calcium concentration of epilepsy patients. Calcium channel blockers are a class of drugs and natural substances which disrupt the conduction of calcium channels. Because they have effects on many excitable cells of the body, such as cardiac muscle, i.e. heart, smooth muscles of blood vessels, or neurons, so calcium channel blockers are mainly used to decrease blood pressure clinical and also used to other diseases, i.e. migraine. Desmedt [2] reported that flunarizine can protect rats and mice in controlling the seizure induced by MES test. The results of Overweg [3] of 77 cases treated for refractory epilepsy using additional flunarizine showed 71% reduction in the number of patients with seizures. According to the reports of double-blind placebo-controlled trial of flunarizine as add-on therapy in refractory childhood epilepsy [4], flunarizine is a difluorinated derivative of cinnarizine. It was reported that it showed anticonvulsant activity [5, 6]. Recently, the side effects of flunarizine were reported such as depression and parkinsonism [7]. To find better anticonvulsant compound and explain the structure-activity relationship, we designed and synthesized 14 flunarizine analogues and evaluated their anticonvulsant activity. The structures of the compounds were proved by means of IR, ^{1}H-NMR spectroscopic data and microanalyses. The anticonvulsant activity was evaluated by using the maximal electroshock (MES) test and neurotoxicity was evaluated using the rotarod test with intraperitoneal injection on KunMing mice.

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2. RESULTS AND DISCUSSION

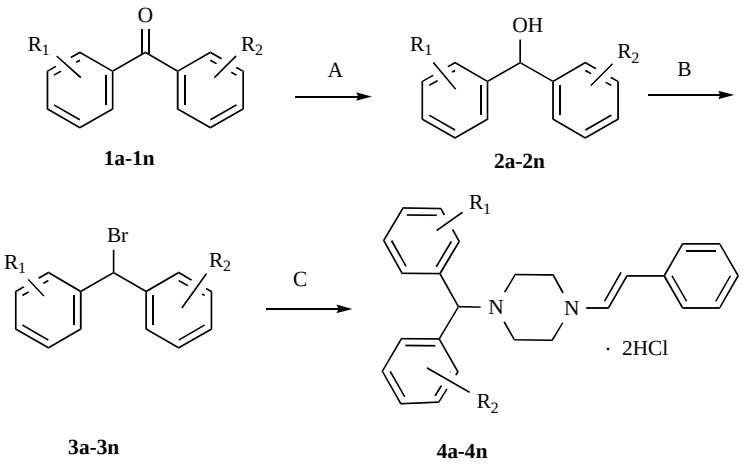
Target compounds **4a-4n** were synthesized according to Scheme **1**. Compounds **2a-2n** were achieved according Reduction Reaction using $NaBH_4$. Compounds **3a-3n** were accomplished by treatment of the HBr. Then compounds **4a-4n** were synthesized through the reaction of compounds **3a-3n** with cinnamylpiperazine in toluene.

The MES test and rotarod test were carried out by the standard described in the Antiepileptic Drug Development Program (ADD) of the National Institutes of Health (USA). All compounds, which were dissolved in polyethylene Glycol-400, were evaluated for anticonvulsant activities with KunMing mice in the 18–22 g weight range. In the MES test, seizures were elicited with a 60-Hz alternating current of 50 mA intensity in mice. The current was applied *via* corneal electrodes for 0.2 s. Protection against the spread of MES-induced seizures was defined as the abolition of the hind leg and tonic maximal extension component of the seizure.

The activity profile for the tested compounds is summarized in Table **1** along with the literature data for standard drugs. All the compounds were active in the MES test, indicative of their ability to prevent seizure spread. All synthesized compounds **4a-4n** exhibited different anticonvulsant activity, among the 14 compounds **4a-4n: 4f, 4g, 4k, 4l** and **4n**, five compounds hardly exhibited anticonvulsant activity at the dose of 300mg/kg; **4b-4e**, **4h** and **4j**, six compounds exhibited weak anticonvulsant activity at the dose of 300mg/kg; (E)-1-benzhydryl-4-styrylpiperazine dihydrochloride (**4a**) and (E)-1-((2-methoxyphenyl)(4- methoxyphenyl)methyl)-4-styrylpiperazine dihydrochloride (**4m**), two compounds exhibited median anticonvulsant activity at the dose of 100mg/kg; and only one compound **4i** exhibited more stronger anticonvulsant activity at the dose of 30mg/kg, being flunarizine.

As a result of preliminary screening, compound **4i** was considered for phase II trials. This provides an evaluation of the median effective dose and median toxic dose. The slope

1570-1808/10 $55.00+.00



Scheme 1. Synthesis of compounds 4a-4n. Regents and conditions: **A**) $NaBH_4$; **B**) HBr, reflux; **C**) Cinnamylpiperazine, toluene, HCl

Table 1. Phase I nticonvulsant and Toxicity Data in Mice (ip)

Compound	R'	R	MES[a]		Toxicity	
			0.5h	4h	0.5h	4h
4a	-H	-H	100	300	300	—[b]
4b	-H	-3F	300	—	300	—
4c	-H	-4F	300	—	300	—
4d	-H	-4Br	300	—	300	—
4e	-H	$-4OCH_3$	300	—	300	—
4f	-H	-2Cl	>300	—	300	—
4g	-H	-4Cl	>300	—	300	—
4h	-4F	-2Cl	300	—	300	—
4i	-4F	-4F	30	100	300	—
4j	-4Cl	-2Cl	300	—	300	—
4k	-4Cl	-4Cl	>300	—	300	—
4l	$-4OCH_3$	$-4OCH_3$	>300	—	300	—
4m	$-4OCH_3$	$-2OCH_3$	100	—	100	—
4n	$-4CH_3$	$-4CH_3$	>300	—	300	—

[a]The unit is mg/kg.; [b]No activity.

of the regression line and the SE of the alope were then calculated. These data are shown in Table **2**. Compound **4i** was the most active compound with ED_{50} of 38.1 mg/kg, TD_{50} of 164.3 mg/kg and PI of 4.3 through intraperitoneal administration, and with ED_{50} of 56.8 mg/kg, TD_{50} of 456.3 mg/kg and PI of 8.0 through oral administration. In conclusion, 14 flunarizine analogs were synthesized, but no one exhibited better anticonvulsant activity than flunarizine.

The initiation of epileptogenic activity in the neuron is thought to be connected with the phenomenon known as "intrinsic burst firing", which is activated by an inward Ca^{2+} current. Ca^{2+} is described as the primary mediator of "excitotoxic" neuronal damage. Both necrotic and apoptotic cell death are associated with Ca^{2+} entry into the cells during status epilepticus. The Ca^{2+} channel blockers depressed epileptic depolarizations of neurons and inhibited the spread of epilepsy [8, 9]. In this study, the results of pharmacology test show that flunarizine and some of its analogs possess anticonvulsant effects thus further confirming the anticonvulsant activity of calcium channel blockers. Those compounds might exhibit the anticonvulsant activity via blocking the Ca^{2+} inward flow.

3. EXPERIMENTAL SECTION

3.1. Chemistry

Melting points were determined in open capillary tubes and were uncorrected. ^{1}H-NMR spectra were measured on a AV-300 (Bruker, Switzerland), and all chemical shifts were given in parts per million relative to tetramethysilane. Mass spectra were measured on an HP1100LC (Agilent Technologies, USA). Elemental analyses were performed on a 204Q CHN (PerkineElmer, USA). The major chemicals were pur-

Table 2. The Data of Flunarizine Dihydrochloride by Different Route of Administration

Administration	ED_{40} (mg/kg)	TD_{40} (mg/kg)	PI(TD_{40}/ED_{40})
Intraperitoneal	38.1	164.3	4.3
Oral	46.8	446.3	8.0

chased from Alderich Chemical Corporation. All other chemicals were of analytical grade.

(E)-1-benzhydryl-4-styrylpiperazine dihydrochloride (4a)

Yield = 75%, mp 180-182 ℃. ^{1}H-NMR ($CDCl_3$ ppm): 13.63 (s, 1H, H-Cl) 13.30 (s, 1H, H-Cl) 7.92-7.27 (m, 15H, Ar-H) 6.79 (d, 1H, *J* = 15.6 Hz, CH) 6.42-6.32 (m, 1H, CH) 5.13 (s, 1H, CH) 4.36 (d, 2H, *J* = 7.4 Hz, CH_2) 3.90-3.55 (m, 8H, CH_2-CH_2). IR (KBr) cm^{-1}: 3421, 1638, 920, 700. MS m/z 355 (M+1).

(E)-1-((3-fluorophenyl)(phenyl)methyl)-4-styrylpiperazine dihydrochloride (4b)

Yield = 56%, mp 178-188 ℃. ^{1}H-NMR ($CDCl_3$ ppm): 13.38 (s, 1H, H-Cl) 12.87 (s, 1H, H-Cl) 7.45-7.36 (m, 14H, Ar-H) 6.82 (d, 1H, *J* = 15.7 Hz, CH) 6.42-6.37 (m, 1H, CH) 5.35 (s, 1H, CH) 4.43 (d, 2H, *J* = 7.5 Hz, CH_2) 4.01-3.9 (m, 4H, CH_2-CH_2) 3.58-3.38 (m, 4H, CH_2). IR (KBr) cm^{-1}: 3391, 1618, 911, 690. MS m/z 373 (M+1).

(E)-1-((4-fluorophenyl)(phenyl)methyl)-4-styrylpiperazine dihydrochloride (4c)

Yield = 65%, mp 208-216 ℃. ^{1}H-NMR ($CDCl_3$ ppm): 13.38 (s, 1H, H-Cl) 12.87 (s, 1H, H-Cl) 7.39-7.28 (m, 14H, Ar-H) 6.82 (d, 1 H, *J* = 15.6 Hz, CH) 6.42-6.37 (m, 1H, CH) 5.36 (s, 1 H, CH) 4.43 (d, 2 H, *J* = 7.5 Hz, CH_2) 4.01-3.58 (m, 8 H, CH_2-CH_2). IR (KBr) cm^{-1}: 3361, 1663, 920, 693. MS m/z 373 (M+1).

(E)-1-((4-bromophenyl)(phenyl)methyl)-4-styrylpiperazine dihydrochloride (4d)

Yield = 59%, mp 220-224 ℃. ^{1}H-NMR ($CDCl_3$ ppm): 13.24 (s, 1H, H-Cl) 12.59 (s, 1H, H-Cl) 7.92-7.28 (m, 14H, Ar-H) 6.87 (d, 1 H, *J* = 15.7 Hz, CH) 6.45 (m, 1H, CH) 5.89 (s, 1H, CH) 4.51 (d, 2 H, *J* = 7.5 Hz, CH_2) 3.97-3.56 (m, 8H, CH_2-CH_2). IR (KBr) cm^{-1}: 3360, 1663, 916, 696. MS m/z 433 (M+1).

(E)-1-((4-methoxyphenyl)(phenyl)methyl)-4-styrylpiperazine dihydrochloride (4e)

Yield = 46%, mp 128-136 ℃. ^{1}H-NMR ($CDCl_3$ ppm): 13.57 (s, 1H, H-Cl) 13.24 (s, 1H, H-Cl) 7.90-7.27 (m, 14H, Ar-H) 6.80 (d, 1 H, *J* = 15.7 Hz, CH) 6.40-6.35 (m, 1H, CH) 4.95 (s, 1H, CH) 4.34 (d, 2 H, *J* = 7.5 Hz, CH_2) 3.85-3.80 (m, 4H, CH_2-CH_2) 3.78 (s, 3H, OCH_3) 3.55-3.51 (m, 4H, CH_2-CH_2). IR (KBr) cm^{-1}: 3321, 1629, 917, 701. MS m/z 385 (M+1).

(E)-1-((2-chlorophenyl)(phenyl)methyl)-4-styrylpiperazine dihydrochloride (4f)

Yield = 58%, mp 178-186 ℃. ^{1}H-NMR ($CDCl_3$ ppm): 13.44 (s, 1H, H-Cl) 13.01 (s, 1H, H-Cl) 7.95-7.31 (m, 14H, Ar-H) 6.80 (d, 1 H, *J* = 15.8 Hz, CH) 6.24-6.10 (m, 1H, CH) 5.78 (s, 1H, CH) 4,38 (d, 2 H, *J* = 7.4 Hz, CH_2) 3.93-3.64 (m, 8H, CH_2-CH_2). IR (KBr) cm^{-1}: 3351, 1638, 912, 691. MS m/z 389 (M+1).

(E)-1-((4-chlorophenyl)(phenyl)methyl)-4-styrylpiperazine dihydrochloride (4g)

Yield = 64%, mp 178-182 ℃. ^{1}H-NMR ($CDCl_3$ ppm): 13.51 (s, 1H, H-Cl) 13.13 (s, 1H, H-Cl) 7.42-7.27 (m, 14H, Ar-H) 6.80 (d, 1 H, *J* = 15.6 Hz, CH) 6.39-6.25 (m, 1H, CH) 5.21 (s, 1H, CH) 4.60 (d, 2 H, *J* = 7.5 Hz, CH_2) 3.88-3.51 (m, 8H, CH_2-CH_2). IR (KBr) cm^{-1}: 3421, 1625, 920, 692. MS m/z 389 (M+1).

(E)-1-((2-chlorophenyl)(4-fluorophenyl)methyl)-4-styrylpiperazine dihydrochloride (4h)

Yield = 49%, mp198-208 ℃. ^{1}H-NMR ($CDCl_3$ ppm): 13.24 (s, 1H, H-Cl) 12.86 (s, 1H, H-Cl) 7.87-7.28 (m, 13H, Ar-H) 6.79 (d, 1 H, *J* = 15.8 Hz, CH) 6.41-6.22 (m, 1H, CH) 5.74 (s, 1H, CH) 4.21 (d, 2 H, *J* = 7.6 Hz, CH_2) 3.90-3.57 (m, 8H, CH_2-CH_2). IR (KBr) cm^{-1}: 3381, 1600, 951, 750. MS m/z 407 (M+1).

(E)-1-(bis(4-fluorophenyl)methyl)-4-styrylpiperazine dihydrochloride (4i)

Yield = 63%, mp201-208 ℃. ^{1}H-NMR ($CDCl_3$ ppm): 13.85 (s, 1H, H-Cl) 13.15 (s, 1H, H-Cl) 7.12-7.39 (m, 13H, Ar-H) 6.82 (d, 1 H, *J* = 15.8 Hz, CH) 6.34-6.39 (m, 1H, CH) 5.35 (s, 1H, CH) 4.28 (d, 2 H, *J* = 7.5 Hz, CH_2) 3.95-3.48 (m, 8H, CH_2-CH_2). IR (KBr) cm^{-1}: 3401, 1608, 920, 690. MS m/z 391 (M+1).

(E)-1-((2-chlorophenyl)(4-chlorophenyl)methyl)-4-styrylpiperazine dihydrochloride (4j)

Yield = 52%, mp188-185 ℃. ^{1}H-NMR ($CDCl_3$ ppm): 13.44 (s, 1H, H-Cl) 13.01 (s, 1H, H-Cl) 8.71-8.69 (d, 1H, *J* = 7.83, H-Cl) 7.85-7.28 (m, 13H, Ar-H) 6.80 (d, 1 H, *J* = 15.8 Hz, CH) 6.44-6.36 (m, 1H, CH) 5.76 (s, 1H, CH) 4.18 (d, 2 H, *J* = 7.5 Hz, CH_2) 3.49-3.97 (m, 8H, CH_2-CH_2). IR (KBr) cm^{-1}: 3381, 1661, 916, 696. MS m/z 423 (M+1).

(E)-1-(bis(4-chlorophenyl)methyl)-4-styrylpiperazine dihydrochloride (4k)

Yield = 55%, mp202-208 ℃. ^{1}H-NMR ($CDCl_3$ ppm): 13.86 (s, 1H, H-Cl) 13.35 (s, 1H, H-Cl) 7.83-7.28 (m, 13H, Ar-H) 6.78 (d, 1 H, *J* = 15.7 Hz, CH) 6.43-6.33 (m, 1H, CH) 5.10 (s, 1H, CH) 4.26 (d, 2 H, *J* = 7.5 Hz, CH_2) 3.87-3.57 (m, 8H, CH_2-CH_2). IR (KBr) cm^{-1}: 3389, 1645, 932, 692. MS m/z 423 (M+1).

(E)-1-(bis(4-methoxyphenyl)methyl)-4-styrylpiperazine dihydrochloride (4l)

Yield = 48%, mp120-125 ℃. ^{1}H-NMR ($CDCl_3$ ppm): 13.47 (s, 1H, H-Cl) 13.02 (s, 1H, H-Cl) 7.78-6.85 (m, 13H, Ar-H) 6.78 (d, 1 H, *J* = 15.7 Hz, CH) 6.40-6.34 (m, 1H, CH) 4.90 (s, 1H, CH) 4.30 (d, 2 H, *J* = 7.5 Hz, CH_2) 4.89-3.51

(m, 8H, CH_2-CH_2) 3.78 (s, 6H, OCH_3). IR (KBr) cm^{-1}: 3401, 1613, 934, 697. MS m/z 415 (M+1).

(E)-1-((2-methoxyphenyl)(4-methoxyphenyl)methyl)-4-styrylpiperazine dihydrochloride (4m)

Yield = 60%, mp150-160 ℃. ^{1}H-NMR ($CDCl_3$ ppm): 13.62 (s, 1H, H-Cl) 13.26 (s, 1H, H-Cl) 7.90-7.15 (m, 13H, Ar-H) 6.85 (d, 1 H, *J* = 15.8 Hz, CH) 6.41-6.38 (m, 1H, CH) 4.91 (s, 1H, CH) 4.34 (d, 2 H, *J* = 7.5 Hz, CH_2) 4.00-3.85 (m, 8H, CH_2-CH_2) 3.70 (s, 6H, OCH_3). IR (KBr) cm^{-1}: 3411, 1652, 933, 702. MS m/z 415 (M+1).

(E)-1-(dip-tolylmethyl)-4-styrylpiperazine dihydrochloride (4n)

Yield = 51%, mp194-198 ℃. ^{1}H-NMR ($CDCl_3$ ppm): 13.36 (s, 1H, H-Cl) 13.17 (s, 1H, H-Cl) 7.75-7.08 (m, 13H, Ar-H) 6.77 (d, 1 H, *J* = 15.6 Hz, CH) 6.39 (m, 1H, CH) 4.98 (s, 1H, CH) 4.32 (d, 2 H, *J* = 7.6 Hz, CH_2) 3.85-3.51 (m, 8H, CH_2-CH_2) 2.33 (s, 6H, CH_3). IR (KBr) cm^{-1}: 3391, 1599, 901, 699. MS m/z 383 (M+1).

3.2. Pharmacology

The MES test and rotarod test were carried out by the standard described in the Antiepileptic Drug Development Program (ADD) of the National Institutes of Health following previously described testing procedures (USA) [10, 11]. All compounds, which were dissolved in polyethyleneglycol-400, were evaluated for anticonvulsant activities with KunMing mice in the 18–25 g weight range. Groups of 10 mice were given a range of intraperitoneal doses of the test drug until at least three points were established in the range of 10–90% seizure protection or minimal observed neurotoxicity. From the plots of these data, the respective ED_{50} and TD_{50} values, 95% confidence intervals, slopes of the regression line and the standard error of the slopes were calculated by means of a computer program written by the National Institute of Neurological Disorders and Stroke.

3.2.1. MES Test

Seizures were elicited with a 60-Hz alternating current of 50 mA intensity in mice. The current was applied *via* corneal electrodes for 0.2 s. Protection against the spread of MES-induced seizures was defined as the abolition of the hind leg and tonic maximal extension component of the seizure. At 15 min after the administration of the compounds, the activities were evaluated in MES test.

3.2.2. Rotarod Test [12]

After the administration of the compounds with different doses for 15 min, the animals were tested on a 1-in. diameter; knurled plastic rod rotating at 6 rpm for 1 min. Neurotoxicity was indicated by the inability of an animal to maintain equilibrium in each of the three trials.

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