Evaluation of Anticonvulsant Activities of Bis(3-aryl-3-oxo-propyl)ethylamine Hydrochlorides and 4-Aryl-3-arylcarbonyl-1-ethyl-4-piperidinol Hydrochlorides

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Abstract

Bis-Mannich bases, bis(3-aryl-3-oxo-propyl)ethylamine hydrochlorides 1-4, and their corresponding structural and non-classical isomers, 4-aryl-3-arylcarbonyl-1-ethyl-4-piperidinol hydrochlorides 5-8, were synthesized. The aryl part was phenyl in 1 and 5, p-methylphenyl in 2 and 6, p-chlorophenyl in 3 and 7, and 2-thienyl in 4 and 8. The chemical structures of the compounds were confirmed by 'H-NMR, 13C-NMR, UV, IR and elemental analyses. Anticonvulsant activities of the compounds were evaluated by the maximum electroshock (MES) and subcutaneous pentylenetetrazol (scMet) tests in the dose range of 30–300 mg/kg. Alterations in biological activity depending on modifications in chemical structure were also followed. Compounds 1–4, 6, and 8 were toxic and caused death of the animals 20 min after the injection. Compounds 2, 3, and 6 were also neurotoxic at the 100 mg/kg dose level. While only compound 7 was active in the scMet test at 300 mg/kg within 4 h, all the compounds showed activity in the MES test at different dose levels and time periods. In conclusion, compounds 5 and 7, which were not toxic and did not show neurotoxicity, seemed to be candidate compounds to develop new anticonvulsant compounds useful in the treatment of the grand mal (compounds 5, 7) and petit mal (compound 7) epilepsies.

1. Introduction

Epilepsy treatment is still one of the major problems in medicine because of the uncontrolled seizures in some types of epilepsy, toxicity of medication, several side effects of currently used drugs, which may limit their maximal usefulness, obligation of administration of some antiepileptics used symptomatically in the treatment of epilepsies for a long time even throughout one's life as they have to be used chronically, and also the risk of tolerance developing against the present drugs. Although there are a number of antiepileptic drugs available in the market, because of above mentioned reasons, development of new compounds having anticonvulsant activity is still necessary [1].

The maximal electroshock (MES) test is a predictor of compounds that are active against grand mal seizures. The subcutaneous pentylenetetrazol (scMet) test is used to detect compounds useful in treating petit mal seizures [2]. Most of the drugs used in the treatment of epilepsies today contain ureid structure such as...
barbiturates, glutamides, oxazolidinediones and succinimides [3–5]. Mannich bases are a group of compounds having several biological activities such as antimicrobial [6–10], cytotoxic [11–16], diuretic [17], antiinflammatory [18, 19], antimalarial [20–22] and also anticonvulsant [23–26] activities. Having the anticonvulsant activity of Mannich bases of conjugated aryldiene ketones [24], Mannich bases of 1-aryl-1-ethanones [27] and some bis-Mannich bases, namely, bis(3-aryl-3-oxo-propyl)methylamine hydrochlorides and their corresponding piperidinol derivatives [26] prompted us to design, synthesize, and investigate the anticonvulsant activity of bis(3-aryl-3-oxo-propyl)ethylamine hydrochlorides 1–4, and their corresponding structural and non-classical isomers, 4-aryl-3-arylcarbonyl-1-ethyl-4-piperidinol hydrochlorides 5–8 (Fig. 1).

2. Materials and methods

2.1. Chemistry

Melting points given are uncorrected. All chemicals used in this study were purchased from Aldrich Co. (Milwaukee, WI, USA). Microanalyses (C, H, N) were performed on a CHN rapid elemental analyser (Perkin Elmer Instruments, Norfolk, CT, USA). The results of the elemental analyses of the compounds were within ±0.4 %. Thin-layer chromatography plates were composed of silica gel with a fluorescent indicator. IR spectra were recorded from KBr pellets on Shimadzu IR470 (Kyoto, Japan), Mattson 1000 FTIR (Madison, WI, USA) and Perkin Elmer 298 IR spectrophotometer. H-NMR spectra were obtained with Bruker ARX 300 (300 MHz) spectrometer (Karlsruhe, Germany). Chemical shifts were reported as δ (ppm) values. Chemical ionization (CI) mass spectra were obtained on a Bruker ARX 300 instrument with NH₃ as the reagent, and the electron-impact (EI) mass spectrum was obtained on Varian-MAT 112S spectrometer (Palo Alto, CA, USA). UV spectra were recorded in MeOH with Shimadzu double-beam spectrometer UV-150-02.

2.1.1. Synthesis of the compounds 1–8 (Fig. 1)

Synthesis of bis(3-aryl-3-oxo-propyl)ethylamine hydrochlorides 1–4 and 4-aryl-3-arylcarbonyl-1-ethyl-4-piperidinol hydrochlorides 5–8 have been reported in our previous study (12). Chemical structures of the compounds were confirmed by H-NMR, ¹³C-NMR, UV, IR and elemental analyses (12).

2.2. Anticonvulsant activity

Stimulator (Grass S88, Astro-Med., Grass Instrument Division, W. Warwick, RI, USA), constant current unit (Grass CCU1A, Grass Medical Instrument, Quincy, MA, USA), and a corneal electrode were used for the evaluation of anticonvulsant activity. PEG 400 and pentylenetetrazole (metrazol) were purchased from Aldrich Co. All synthesized compounds were administered i. p. as 30 % aqueous PEG 400 suspensions.

Twelve Swiss albino male mice (20 ± 2 g) obtained from the Hacettepe University Animal Farm and used according to the Hacettepe University, "Test Animals Ethic Committee" October 13, 2005 date 2005/52-10 number decision) were used for each compound. Control animals received 30 % aqueous PEG 400.

The compounds were tested for their anticonvulsant activities against MES and scMet induced seizures. The rotorod toxicity test was performed for neurological toxicity according to the phase I tests of the ADD (Antiepileptic Drug Development) programme (2–5) which has also been used for evaluation in various previous studies [25, 26, 28, 29]. Pentylenetetrazole was administered s. c. from the back of the neck. The rotorod toxicity test was performed on a 1-inch diameter knurled wooden rod, rotating at 6 rpm (the rotorod used in Screening I test was made by the Hacettepe University Technical Department).

2.2.1. Maximal electroshock seizure test

Maximal electroshock seizures were elicited with a 60-cycle alternating current of 50 mA intensity (5–7 times the intensity required to elicit minimal electroshock seizures) delivered for 0.2 s via corneal electrodes. A drop of 0.5 % saline was instilled into the eye prior to application of the electrodes in order to prevent the death of the animal. Abolition of the hind limb tonic extension component of the seizure was defined as protection.

2.2.2. Subcutaneous pentetrazole test

85 mg/kg of pentylenetetrazole (produces seizures in greater than 95 % of mice) was administered as a 0.5 % solution subcutaneously into the posterior midline. The animal was observed for 30 min. The absence of even a threshold seizure (a single episode of clinical spasms of at least 5 s duration) was defined as protection.

2.2.3. Neurotoxicity

The rotorod test was used to evaluate the neurotoxicity. The animal was placed on a 1-inch diameter knurled wooden rod rotating at 6 rpm. Normal mice remain on a rod rotating at this speed indefinitely. Neurologic toxicity was defined as failure of the animal to remain on the rod for 1 min.
3. Results

Anticonvulsant activity results of the compounds are shown in Table 1. According to the results of the anticonvulsant activity studies presented in Table 1, all compounds tested (compounds 1–6) were found to be protective in the MES test at 30 mg/kg and/or above, while compound 7 was found to be protective in the scMet test at 300 mg/kg within 4 h. Compounds 2, 3, and 6 demonstrated neurotoxicity at the 100 mg/kg dose level within 0.5 h. When compounds 2, 3, 4, 6, and 8 were administered to the animals at the dose level of 300 mg/kg and compound 1 at 100 mg/kg, no measurements were taken as the mice died within 20 min.

4. Discussion

Compounds 1–4 were reported in our previous study for the first time [12]. We had previously reported the anticonvulsant activities of the compounds corresponding to the compounds 1–5 and 7 in the series in which methyl on nitrogen was substituted, in other words, methylamine hydrochloride was used in the reactions as amine component [26]. The substituent on nitrogen was ethyl in this study. The compounds were prepared by using ethylamine hydrochloride.

All compounds (1–8) in the ethyl series in this study were effective in the MES test at and/or above 30 mg/kg doses. Of the compounds in the methyl series, only 2, 3, and 5 were effective in the MES test at 30 mg/kg doses and above [26]. The compounds in the series with methyl, which correspond to compounds 1, 2, and 3 in the ethyl series, were found to be effective in the scMet test at 100 and/or 300 mg/kg doses, however, only compound 7 in the series with ethyl was effective at the 300 mg/kg dose level. It seems that the compounds in the series with ethyl are more suitable for the MES test, while derivatives with methyl are suitable for the scMet test [26]. In the series with ethyl, compounds 2, 3, and 6 were found to be neurotoxic, while in the series with methyl, derivatives, which correspond to 4 and 5 in the ethyl series, were found to be neurotoxic; only compound 2 was toxic in the series with methyl. Compounds 3, 4, 6, and 8, in addition to compound 2, were found to be toxic at 300 mg/kg, and compound 1 was toxic at 100 mg/kg in the series with ethyl. The increase in toxicity in the series with ethyl compared with the series with methyl can be attributed to the increase in lipophilicity. Lipophilicity is important for the compounds to pass the blood-brain barrier and thus for their anticonvulsant activity. On the other hand, when we compared the results of this study with the results of our previous study [26], replacement of methyl amine with ethylamine in this study increased the protective effect at 0.5 and 4 h in MES induced seizures.

In conclusion, compounds 5 and 7, which were not toxic and did not show neurotoxicity, seemed to be candidate compounds to develop new anticonvulsant compounds useful in the treatment of grand mal (compounds 5, 7) and petit mal (compound 7) epilepsies.

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References

CNS-active Drugs. Hypnotics. Psychotropics. Sedatives


