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Synthesis of *N*-(substituted phenyl) pyrrolidine-2-carboxamide as Novel Anticonvulsant Agents

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ABSTRACT

In present investigation a series of *N*-(substituted phenyl) pyrrolidine-2-carboxamide derivatives were synthesized and were evaluated for anticonvulsant activity by using maximal electroshock seizure (MES) test in mice. The structures of the synthesized compounds were confirmed on the basis of their spectral data and elemental analysis. Most of the compounds were found to be active in MES tests without any neurotoxicity. Compounds **3a** and **3d** were found the most active of the series without any neurotoxicity and less CNS depressant effect as compared to standard drug carbamazepine.

INTRODUCTION

Epilepsy is one of the most common disorders of brain, affecting about 50 million individuals worldwide and about 2.5 million in United States of America and characterized by recurrent seizure attack [1,2]. The conventional antiepileptic drugs (AEDs) like phenytoin, carbamazepine, benzodiazepine and ethosuximide are widely used but exhibit adverse side effects. In recent years several new drugs such as oxacarbazepine, lamotrigine, topiramate, gabapentine and vigabatrin have been added as therapeutic agents for the treatment of epilepsy. However there is a significant group of patients (up to 30%) who are resistant to the available antiepileptic drugs. The long established antiepileptic drugs control seizure in 50% of patients developing partial seizures and in 60-70% of those developing generalized seizures [3,4]. Hence there is an urgent need to develop new antiepileptic drugs which brought about substantial benefit to the patient population in the form of increase seizure control, increase tolerability, and better safety and pharmacokinetic properties. Many literatures reveal that the carboxamide nucleus has been found to posses' anticonvulsant, anti-inflammatory, analgesic, antitumor, anxiolytic and antimicrobial activity [5, 6, 7, 8, 9, 10, 11]. It was considered to be worth to work on the above mentioned novel analogues. In the present work fifteen new carboxamides were synthesized and the reaction sequence for the preparation is outlined in Scheme 1. Pyrrolidine-2-carbonyl chloride **2** was synthesized from pyrrolidine-2-carboxylic acid (L-proline) with PCl_5 in the presence of acetyl chloride. *N*-(Substituted phenyl) pyrrolidine-2-carboxamide derivatives (**3a-i**) were synthesized from pyrrolidine-2-carbonyl chloride and substituted aromatic amines in the presence of acetone. A few *N*-(substituted phenyl) pyrrolidine-2-carboxamide were further acetylated with acetic anhydride in the presence of triethylamine and dichloromethane to synthesized 1-acetyl-*N*-(substituted) pyrrolidine-2-carboxamides (**4a-f**). The purity of the compounds was checked by TLC and elemental analyses. Both analytical and spectral data (^{1}H NMR, IR) of all the synthesized compounds were in full agreement with the proposed structures.

MATERIALS AND METHODS

Chemistry

The melting points were determined in one end open capillary tubes on a Buchi 530 melting point apparatus and are uncorrected. Fourier Transforms Infra-red Spectroscopy (FT-IR) and proton nuclear magnetic resonance (^{1}H NMR) spectra were

recorded for the compounds on Win-IRrez (Bio-Rad) using the potassium bromide (KBr) disc technique and Brucker Avance (400 MHz) instrument respectively. Chemical shifts were reported in parts per million (ppm) using tetramethylsilane (TMS), as internal standard and Elemental analyses (C, H and N) were undertaken with Perkin-Elmer model 240C analyzer. Silica gel-G coated glass plate used for thin layer chromatography (TLC), and visualized by iodine-vapor as visualizing agents.

Synthesis of N-(substituted phenyl) pyrrolidine-2-carboxamide **(3a-i)**

A stirred suspension of the hydrochloride of pyrrolidine-2-carboxylic acid (17.4 mmol) in acetyl chloride (20 ml) was treated with phosphorous pentachloride (14.4 mmol) in dry condition. The reaction mixture was warmed to 35° C, and additional PCl_5 (9.6 mmol, 2.0 g) was added after 4 h. After stirring for an additional for an additional 4 h, the reaction mixture was cooled in an ice bath. The resultant product in one portion (1 mmol) was further suspended in acetone (40 ml) and with substituted aniline (1 mmol) and was heated to reflux for 8 h. After cooling to room temperature, the resultant product was treated with 1N NaOH, and extracted with ethyl acetate (75 ml). The organic layer was separated, dried (Na_2SO_4) and evaporated under reduced pressure to give a sticky product, which were purified with petroleum ether or diethyl ether by scratching the compounds with the wall of beaker and discarding the soluble impurities and further recrystallized with appropriate solvent. The process continued till pure product was obtained.

N-(4-Chlorophenyl) pyrrolidine-2-carboxamide ***(3a)*** Recrystallized from petroleum ether (yield 72%). mp 46°C. H-NMR ($CDCl_3$) δ; 8.19 (s, 1H, CONH), 7.03-7.52 (m, 5H, 4 ArH & 1 ArNH), 5.19 (s, 1H, NH of pyrrolidine), 3.47 (d, 1H, C*H*CH_2), 1.64-2.17 (bm, 6H, pyrrolidine) - IR ν_{max} cm^{-1} (KBr): 3372 (NH), 1674(C=O) - Anal.: $C_{11}H_{13}ClN_2O$

N-(4-Fluorophenyl) pyrrolidine-2-carboxamide ***(3b)*** Recrystallized from petroleum ether (yield 68%). mp 84°C. H-NMR ($CDCl_3$) δ; 9.12 (s, 1H, CONH), 6.95-7.62 (m, 5H, 4 ArH & 1 NH), 5.12 (s, 1H, NH of pyrrolidine), 3.69 (d, 1H, C*H*CH_2), 1.59-2.75 (bm, 6H, pyrrolidine) - IR ν_{max} cm^{-1} (KBr): 3382 (NH), 1671 (C=O) - Anal.: $C_{11}H_{13}FN_2O$

N-(4-Bromophenyl) pyrrolidine-2-carboxamide ***(3c)*** Recrystallized from petroleum ether and n-Hexane (yield 52%). mp 92°C. H-NMR ($CDCl_3$) δ; 9.26 (s, 1H, CONH), 7.43-7.82 (m, 5H, 4 ArH & 1 NH), 5.18 (s, 1H, NH of pyrrolidine), 3.77 (d, 1H, C*H*CH_2), 1.12-2.87 (bm, 6H, pyrrolidine) - IR ν_{max} cm^{-1} (KBr): 3373 (NH), 1689 (C=O) - Anal.: $C_{11}H_{13}BrN_2O$

N-(4-Nitrophenyl) pyrrolidine-2-carboxamide ***(3d)*** Recrystallized from petroleum ether (yield 72%). mp 60°C. H-NMR($CDCl_3$) δ; 8.10 & 8.23 (d, 2H, 3,5-ArH), 7.9 (s, 1H, CONH), 6.35 & 6.23 (d, 2H, 2,6-ArH), 5.40 (s, 1H, NH of pyrrolidine), 3.36 (d, 1H, C*H*CH_2), 1.37-2.27 (s, 6H of pyrrolidine), - IR ν_{max} cm^{-1} (KBr): 3375 (NH), 1684 (C=O) - Anal.: $C_{11}H_{13}N_2O_3$

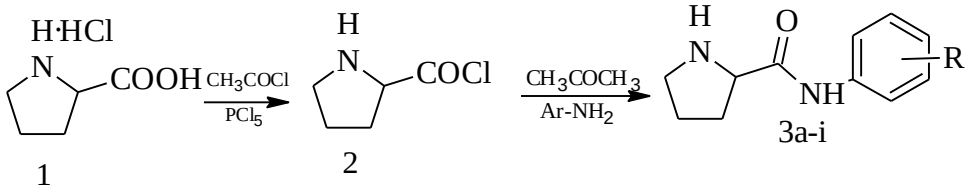
N-(4-Methylphenyl) pyrrolidine-2-carboxamide ***(3e)*** Recrystallized from petroleum ether and n-Hexane (yield 62%). mp 104°C. H-NMR ($CDCl_3$) δ; 9.21 (s, 1H, CONH), 7.04-7.52 (m, 5H, 4 ArH & 1 NH), 5.24 (s, 1H, NH of pyrrolidine), 3.47 (d, 1H, C*H*CH_2), 2.35 (s, 3H, CH_3), 1.12-2.17 (bm, 6H, pyrrolidine) - IR ν_{max} cm^{-1} (KBr): 3370 (NH), 1676 (C=O) - Anal.: $C_{12}H_{16}N_2O$

N-(2-Methylphenyl) pyrrolidine-2-carboxamide ***(3f)*** Recrystallized from petroleum ether and n-Hexane (yield 64%). mp 142°C. H-NMR ($CDCl_3$) δ; 9.29 (s, 1H, CONH), 7.03-7.83 (m, 5H, 4 ArH & 1 NH), 3.67 (s, 1H, C*H*CH_2), 1.98-2.77 (bm, 6H, pyrrolidine) - IR ν_{max} cm^{-1} (KBr): 3368, 1677 - Anal.: $C_{12}H_{16}N_2O$

N-(2,4-Dimethylphenyl)pyrrolidine-2-carboxamide ***(3g)*** Recrystallized from methanol and benzene (yield 58%). mp 56°C. H-NMR ($CDCl_3$) δ; 9.10 (s, 1H, CONH), 7.03-7.52 (m, 4H, 3 ArH & 1 NH), 3.37 (d, 1H, C*H*CH_2), 2.39 (s, 6H, CH_3), 1.08-2.19 (bm, 6H, pyrrolidine) - IR ν_{max} cm^{-1} (KBr): 3377 (NH), 1679 (C=O) - Anal.: $C_{13}H_{18}N_2O$

N-(2,6-Dimethylphenyl)pyrrolidine-2-carboxamide ***(3h)*** Recrystallized from petroleum ether (yield 66%). mp 68°C. H-NMR ($CDCl_3$) δ; 8.21 (s, 1H, CONH), 6.98-7.47 (m, 5H, 4 ArH & 1 NH), 3.41 (s, 1H, C*H*CH_2), 2.47 (s, 6H, CH_3), 1.98-2.37 (bm, 6H, pyrrolidine) - IR ν_{max} cm^{-1} (KBr): 3375 (NH), 1674 (C=O) - Anal.: $C_{13}H_{18}N_2O$

N-(3-Chloro-4-fluorophenyl) pyrrolidine-2-carboxamide ***(3i)*** Recrystallized from petroleum ether (yield 62%). mp 76°C. H-NMR ($CDCl_3$) δ; 9.09 (s, 1H, CONH), 6.97-7.56 (m, 5H, 4 ArH & 1 NH), 3.49 (s, 1H, C*H*CH_2), 1.18-2.07 (bm, 6H, pyrrolidine) - IR ν_{max} cm^{-1} (KBr): 3373 (NH), 1678 (C=O) - Anal.: $C_{11}H_{12}ClFN_2O$



Scheme 1: Protocol for the synthesis o carboxamide analogues.

PHARMACOLOGY

Animals

Male Swiss albino mice (20–25 g) were used for all experiments. The animals were kept in colony cages (six mice each), maintained on standard pellet diet, water and left for 2 days for acclimatization before the experimental session. The food was withdrawn on the day before the experiment, but free access of water was allowed. All experiments were carried out according to the suggested ethical guidelines for the care of laboratory animals.

Anticonvulsant Activity and Neurotoxicity

The new derivatives obtained by the above mentioned procedure were undertaken for the initial anticonvulsant by reported methods [12,13]. Anticonvulsant activity and neurotoxicity screening data are summarized in Table 1. Each compound was administered as an intraperitoneally (i.p.) injection at three doses levels (30, 100 and 300 mg/kg) and the anticonvulsant activity was assessed after 30 min and 4 hr intervals of administration. The anticonvulsant activity was evaluated by maximal electroshock (MES). Neurotoxicity (NT) in mice was measured by Rotorod test.

Anticonvulsant Screening

Initially all the compounds were administered i.p. at doses of 30, 100 and 300 mg/kg to a group of six mice. Activity was established using the MES and s.c PTZ and these data are presented in Table 1.

Neurotoxicity (NT) Screening

Minimal motor impairment was measured in mice by the rotorod test. The mice were trained to stay on an accelerating rotorod that rotate at 10 revolutions per minute. The rod diameter was 3.2 cm. trained animals were given i.p. injection of the test compound in doses of 30, 100 and 300 mg/kg. Neurotoxicity was indicated by the inability of the mice to maintain equilibrium on the rod for at least 1 min in each of the three trails (Table 1).

Table 1: Anticonvulsant and Neurotoxicity Screening of Titled Compounds.

Compound	Intraperitoneal injection in mice[a]			
	Maximal Electroshock Screen		Neurotoxicity Screen	
	0.5 h	4 h	0.5 h	4 h
3a	100	100	–	–
3b	×	×	×	×
3c	300	–	×	×
3d	30	100	–	–
3e	300	–	×	×
3f	–	–	×	×
3g	300	–	×	×
3h	300	300	–	–
3i	–	–	×	×
Phenytoin	30	30	100	100

The dash (–) indicates absence of activity. The cross (×) indicated activity not done.

[a]Dose of 30, 100 and 300 mg/kg were administered. The figures in the table indicate the minimum dose whereby bioactivity was demonstrated in half or more of the mice. The animals were examined 0.5 and 4 h after administration All solutions prepared in polyethylene glycol 400.

RESULT AND DISCUSSION

The carboxamide N-(substituted phenyl) pyrrolidine-2-carboxamide **(3a-i)** and 1-acetyl-N-(4-chlorophenyl) pyrrolidine-2-carboxamide **(4a-f)** were prepared according to the previously reported procedures [5]. Pyrrolidine-2-carbonyl chloride **2** was synthesized from pyrrolidine-2-carboxylic acid (L-proline), which was then treated with appropriate substituted aromatic amines in dry acetone to obtain the resulting carboxamide derivatives (**3a-i**). The synthetic route for the resulting carboxamides derivatives (**3a-i**) is outlined in Scheme 1.

Anticonvulsant activity was determined by MES. As seen in Table 1, most of the compounds were active in MES at 0.5 h period. The compound **3d** was the most active compounds of the series showed protection against MES seizure at 30 mg/kg at 0.5 h comparable to standard drug phenytoin without any neurotoxicity. Especially, *N*-(substituted phenyl) pyrrolidine-2-carboxamide derivatives with 4-nitrophenyl and 4-chlorophenyl substituent (**3a** and **3d**) exhibited protection against MES induced seizure. The compounds **3a**, **3h**, **3c**, **3e** and **3g** showed good to moderate protection against MES induced seizure. *N*-(4-sustituted phenyl) pyrrolidine-2-carboxamide having 4-chlorophenyl and 4-nitrophenyl substituent (**3a** and **3d**) have maximum activity.

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