European Journal of Chemistry 1 (2) (2010) 102-109

European Journal of Chemistry

Journal homepage: www.eurjchem.com

Synthesis and preliminary biological screening of certain 5-aralkyl pyrrolidine-3-carboxylic acids as anticonvulsants

Mohamed Nabil Aboul-Enein[a,*], Aida Abd El-Sattar El-Azzouny[a], Ola Ahmed Saleh[a], Mahmoud Abd El- Moien Nawwar[b], Mohamed Abd El-Hamid Ismail[c], Mahmoud Gamal El-Din Elsedeek[a] and Yousreya Aly Maklad[a]

[a] *Medicinal and Pharmaceutical Chemistry Department, Pharmaceutical and Drug Industries Research Division, National Research Centre, Dokki-Giza, 12622, Egypt*
[b] *Phytochemistry and Chemosystematic Department, Pharmaceutical and Drug Industries Research Division, National Research Centre, Dokki-Giza, 12622, Egypt*
[c] *Pharmaceutical Chemistry Department, Faculty of Pharmacy, Ain Shams University, Cairo, 11566, Egypt*

**Corresponding author at: Medicinal and Pharmaceutical Chemistry Department, Pharmaceutical and Drug Industries Research Division, National Research Centre, Dokki-Giza, 12622, Egypt Tel.: +20122168624; fax: +20237601877. E-mail address: mnaboulenein@yahoo.com (M.N. Aboul-Enein).*

ARTICLE INFORMATION

Received: 23 March 2010
Received in revised form: 29 April 2010
Accepted: 30 April 2010
Online: 30 June 2010

KEYWORDS

Synthesis
5-aralkyl pyrrolidine-3-carboxylic acids
Epilepsy
Anticonvulsants

ABSTRACT

Synthesis of a series of 5-aralkyl pyrrolidine-3-carboxylic acid derivatives namely, 1-acetyl-4-hydroxy-5-benzyl or 5-(4-alkoxy-benzyl)-pyrrolidine-3-carboxylic acids (3a-e), 1-*H*-4-hydroxy-5-benzyl or 5-(4-alkoxy-benzyl)-pyrrolidine-3-carboxylic acids (4a-e), 1-acetyl-5-benzyl or 5-(4-alkoxy-benzyl)-pyrrolidine-3-carboxylic acids (8a-e), 1-*H*-5-benzyl or 5-(4-alkoxy-benzyl)-pyrrolidine-3-carboxylic acids (9a-e) have been accomplished. The structures of the new compounds were assigned from IR, ^{1}H NMR, ^{13}C NMR and elemental analyses. Compounds 3a-e, 4a-e, 8a-e and 9a-e were biologically screened for their anticonvulsant potential using the subcutaneous pentylenetetrazole seizures (scPTZ) assay and Gabapentin as reference standard. The 1-*H*-4-hydroxy-5-benzyl or 5-(4-alkoxy-benzyl)-pyrrolidine-3-carboxylic acids (4a-e) showed the highest anticonvulsant activity. Compound 4b was found to be the most potent one which exhibited 100% protection.

1. Introduction

Epilepsy is a common chronic neurological disorder characterized by recurrent unprovoked seizures [1,2]. These seizures are transient signs and/or symptoms of abnormal, excessive or synchronous neuronal activity in the brain [3]. About 50 million people worldwide suffer from epilepsy, with almost 90% of these people living in developing countries [4]. It is noteworthy that, many of the current antiepileptic drugs were developed empirically on the basis of their activity in animal models. The mechanism of action of these drugs at the cellular level is not fully understood. However, the ultimate goal of the antiepileptic is to prevent the paroxysmal discharge without affecting normal transmission [5].

A myriad of pyrrolidine derivatives cover a great section of the Medicinal Chemistry drug armamentarium. These compounds display a broad spectrum of pharmacodynamic and chemotherapeutic profiles [6]. Among these, are the compounds that display anticonvulsant activities [7-15].

Previously, we have reported the anticonvulsant properties of certain pyrrolidine surrogates (**I-III**) [16-18] that showed anticonvulsant activities at dose levels of 0.07, 0.04 and 0.01 mmol/kg b.wt. equivalent to 30, 15 and 3.97 mg/kg body weight (b.wt.), respectively.

Thus, in continuation to our work in this field it was of interest to design and synthesize four series of pyrrolidine derivatives related to structures of **I-III** namely: 1-acetyl-4-hydroxy-5-benzyl or 5-(4-alkoxy-benzyl)-pyrrolidine-3-carboxylic acids (**3a-e**, Scheme 1), 1-*H*-4-hydroxy-5-benzyl or 5-(4-alkoxy-benzyl)-pyrrolidine-3-carboxylic acids (**4a-e**, Scheme 1), 1-acetyl-5-benzyl or 5- (4-alkoxy-benzyl)-pyrrolidine-3-carboxylic acids (**8a-e**, Scheme 2), 1-*H*-5-benzyl or 5-(4-alkoxy-benzyl)-pyrrolidine-3-carboxylic acids (**9a-e**, Scheme 2) to be pharmacologically screened for their anticonvulsant activities. These compounds bear the pyrrolidine-3-carboxylic acid ring structure which may be considered as a rigid cyclized form of γ-aminobutyric acid (GABA), which acts as an important inhibitory neurotransmitter in the central nervous system. Similar ring system is also found in the anticonvulsants gamibetal [19], gabapentin [20] and baclofen [21] (Figure 1).

2. Experimental

2.1. Chemistry

All melting points are uncorrected and were determined with Electrothermal Capillary melting point apparatus. Infrared (IR) spectra were recorded as thin film (for oils) in NaCl discs or as KBr pellets (for solids) with JASCO FT/IR-6100 Spectrometer and values are represented in cm^{-1}. ^{1}H NMR and ^{13}C NMR spectra were recorded on Jeol ECA 500 MHz Spectrophotometer using TMS as internal standard and chemical). The δ shift values are reported in ppm (^{1}H NMR data were represented as follows: chemical shifts, multiplicity (s. singlet, d. doublet, t. triplet, q. quartet, m. multiplet, and br. broad) and number of protons. The ^{13}C NMR data were represented as chemical shifts and type of carbon, the chemical shifts bearing "*" could be interchanged. The mass spectra were recorded on Finnigan Mat SSQ-7000 Spectrophotometer and Jeol JMS-AX 500. Elemental analyses were carried out at the

ISSN 2153-2249 (Print) / ISSN 2153-2257 (Online)
DOI:10.5155/eurjchem.1.2.102-109.47

Microanalytical Unit, National Research Centre, Cairo, Egypt. Aluminium oxide 60G F_{254} neutral plates for TLC (Merck) were used for thin layer chromatography. Visualization was performed by illumination with UV light source (254 nm). Column chromatography was performed with alumina neutral III for gravity columns. The solvent system used in column elution was: Petroleum-ether (40-60 °C): ethyl acetate (9:1). The compounds throughout this work were named according to *AutoNom*® computer program version 2.1, MDL Information Systems, as component of *Chemdraw Ultra*® program package version 5. Synthesis of 1-acetyl-4-ethoxycarbonyl-2-benzyl or 2-(4-alkoxy-benzyl)-pyrrolidin-3-one (**1a-e**) [22,23] and 1-acetyl-4-hydroxy-5-benzyl or 5-(4-alkoxy-benzyl)-pyrrolidine-3-carboxylic acid ethyl esters (**2a-e**) [24] was performed according to the reported procedures.

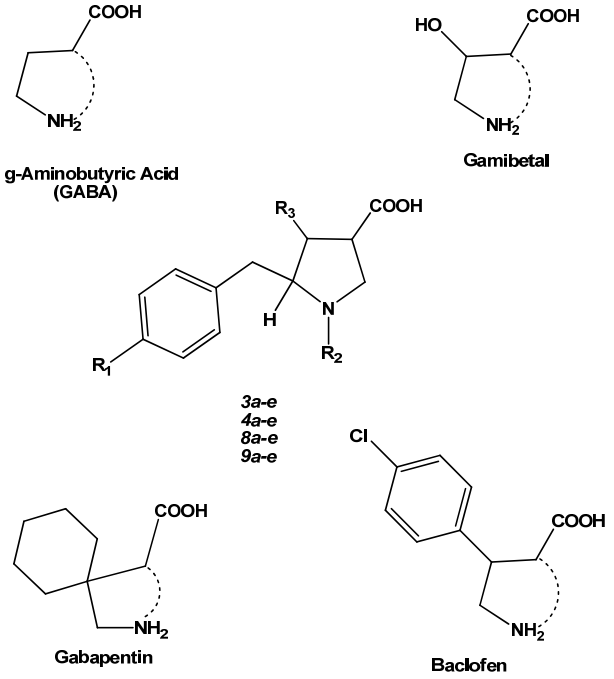


Figure 1. Structural relation between γ-aminobutyric acid (GABA), Gamibetal, Gabapentin, Baclofen and the target compounds, 3a-e, 4a-e, 8a-e and 9a-e.

2.1.1. General procedure for preparation of 1-acetyl-4-hydroxy-5-benzyl or 5-(4-alkoxy-benzyl)-pyrrolidine-3-carboxylic acid (3a-e)

To 0.0143 mol of **2a-e** in ethanol (15 mL), 14.3 mL of lithium hydroxide solution (1.7 N) was added. The reaction mixture was stirred for 24 h at room temperature and evaporated under reduced pressure. The residue was dissolved in water and washed with ethyl acetate. The aqueous layer was acidified with hydrochloric acid (1 N), extracted with ethyl acetate, dried and evaporated under reduced pressure to afford 3a-e as solids which were recrystallized from isopropanol.

2.1.1.1. 1-Acetyl-4-hydroxy-5-benzyl-pyrrolidine-3-carboxylic acid (3a)

Yield 85%. M.p.: 152-154 °C. IR (KBr, cm^{-1}): 3300-2700 (br, OH alcohol and acid), 1725 (C=O, acid) and 1594 (C=O, amide). ^{1}H NMR (DMSO-d_6 ppm: δ) 2.00 (s, 3H, C***H*₃**-CO-N); 2.70-3.10 (m, 3H, Ph-C***H*₂**, C***H***-COOH); 3.60-3.92 (m, 3H, C***H*₂**-N, N-C***H***); 4.06-4.08 (m, 1H, C***H***OH); 7.24-7.27 (m, 5H , Ar-***H***). EI/MS, m/z (%): 263, M^+; Microanalysis calcd. C, H, N for $C_{14}H_{17}NO_4$: 63.87, 6.51, 5.32; found, 63.95, 6.43, 5.50.

2.1.1.2. 1-Acetyl-4-hydroxy-5-(4-methoxy-benzyl)-pyrrolidine-3-carboxylic acid (3b)

Yield 80%. M.p.: 208-210 °C. IR (KBr, cm^{-1}) 3300-2700 (br, OH alcohol and acid), 1723 (C=O, acid) and 1608 (C=O, amide). ^{1}H NMR (DMSO-d_6 ppm: δ) 2.00 (s, 3H, C***H*₃**-CO-N); 2.67-2.95 (m, 3H, Ph-C***H*₂**, C***H***-COOH); 3.67 (s, 3H, C***H*₃**-O); 3.58-4.06 (m, 4H, C***H*₂**-N, N-C***H***, C***H***-OH); 6.83 (d, J= 8.6Hz, 2H, Ar-***H***); 7.15 (d, J= 8.6Hz, 2H, Ar-***H***). EI/MS, m/z (%):294, M^++1. Microanalysis calcd. C, H, N for $C_{15}H_{19}NO_5$: 61.42, 6.53, 4.78, found, 61.53, 6.67, 4.72.

2.1.1.3. 1-Acetyl-4- hydroxy-5-(4-ethoxy-benzyl)-pyrrolidine-3-carboxylic acid (3c)

Yield 83%. M.p.: 196-198 °C. IR (KBr, cm^{-1}) 3300-2700 (br, OH alcohol and acid), 1722 (C=O, acid) and 1607 (C=O, amide); ^{1}H NMR (DMSO-d_6 ppm: δ) 1.26 (t, J=6.9 Hz, 3H, C***H*₃**-$(CH_2)_2$-O); 1.94 (s, 3H, C***H*₃**-CO-N); 2.65-2.95 (m, 3H, Ph-C***H*₂**, CH-COOH); 3.58-4.07(m, 6H, C***H*₂**-N, N-C***H***, CH_3C***H*₂**O, C***H***-OH); 6.83 (d, J= 8.3Hz, 2H, Ar-***H***); 7.15 (d, J= 8.3Hz, 2H, Ar-***H***). ^{13}C NMR data (DMSO-d_6 ppm, δ) : 171.8 (1C, ***C***OOH); 170.1(1C, CH_3-***C***O-N); 156.9 (1C, Ar-***C***); 131.8 (1C, Ar-***C***); 130.6 (2C, Ar-***C***); 114.5 (2C, Ar-***C***); 70.4 (1C, CH_3***C***H_2O); 65.3 (1C, ***C***H-OH); 63.2 (1C, N-***C***H); 49.0* (1C, ***C***H-COOH); 48.0* (1C, C***H*₂**-N); 32.0 (1C, Ph-***C***H_2); 23.5(1C, ***C***H_3-CO-N); 15.0 (1C, ***C***H_3CH_2O). EI/MS, m/z (%):307, M^+. Microanalysis calcd. C, H, N for $C_{16}H_{21}NO_5$: 62.53, 6.89, 4.56; found, 62.59, 7.01, 4.50.

2.1.1.4. 1-Acetyl-4-hydroxy-5-(4-n-propoxy-benzyl)-pyrrolidine-3-carboxylic acid (3d)

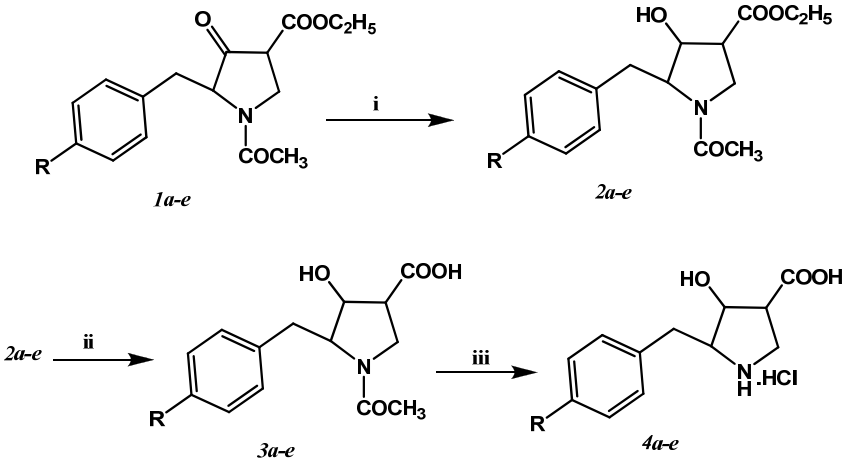
Yield 76%. M.p.: 168-170 °. IR (KBr, cm^{-1}) 3300-2700 (br, OH alcohol and acid), 1716 (C=O, acid) and 1572 (C=O, amide). ^{1}H NMR (DMSO-d_6 ppm: δ) 0.93 (t, J=7.2 Hz, 3H, C***H*₃** $(CH_2)_2$-O); 1.60-1.70 (m, 2H, CH_3-C***H*₂**-CH_2-O); 1.90 (s, 3H, C***H*₃**-CO-N); 2.60-3.00 (m, 3H,-Ph-C***H*₂** -, C***H***-COOH); 3.60-4.10 (m, 6H, C***H*₂**-N, N-C***H*,** O-C***H*₂**-CH_2-CH_3, C***H***OH); 6.77 (d, J= 7.6Hz, 2H, Ar-***H***); 7.15 (d, J= 7.6Hz, 2H, Ar-***H***). EI/MS, m/z (%): 321, M^+. Microanalysis calcd. C, H, N for $C_{17}H_{23}NO_5$: 63.54, 7.21, 4.36, found, 63.59, 7.28, 4.32.

2.1.1.5. 1-Acetyl-4-hydroxy-5-(4-n-butoxy-benzyl)-pyrrolidine-3-carboxylic acid (3e)

Yield 73%. M.p.: 138-140 °C. IR (KBr, cm^{-1}) 3300-2700 (br, OH alcohol and acid), 1710 (C=O, acid) and 1585 (C=O, amide). ^{1}H NMR (DMSO-d_6 ppm δ)**:** 1.29 (t, J=6.9 Hz, 3H, C***H*₃**-$(CH_2)_3$-O); 1.80-2.41 (m, 7H, O-CH_2-CH_2-C***H*₂**-CH_3, O-CH_2 -C***H*₂**-CH_2-CH_3, C***H*₃**-CO-N); 2.76-3.08 (m, 3H,-Ph-C***H*₂**-, C***H***-COOH); 3.45-4.08 (m, 6H, C***H*₂**-N, N- C***H***, O-C***H*₂** -CH_2-CH_2-CH_3, C***H***-OH); 6.83 (d, J= 7.6Hz, 2H, Ar-***H***); 7.03 (d, J= 7.6Hz, 2H, Ar-***H***). EI/MS, m/z (%):335, M^+. Microanalysis calcd. C, H, N for $C_{18}H_{25}NO_5$: 64.46, 7.51, 4.18; found, 64.51, 7.65, 4.22.

2.1.2. *General procedure for preparation of 1-H-4-hydroxy-5-benzyl or 5- (4-alkoxy-benzyl)-pyrrolidine-3-carboxylic acid hydrochlorides (4a-e)*

To 0.0034 mol of the 1-acetyl-4-hydroxy-5-benzyl or -5-(4-alkoxy-benzyl)-pyrrolidine-3-carboxylic acid (***3a-e***) was added 7 mL of hydrochloric acid solution (5 N). The reaction mixture was refluxed for 5 h, then evaporated under reduced pressure and crystallized from ethyl acetate /alcohol (9:1) to afford the hydrochloride salts ***4a-e*** as solids (Scheme 1).



R = H, OCH_3, OC_2H_5, O-C_3H_7(*n*), O-C_4H_9(*n*)

i : H_2 / PtO_2 , 95% ethanol, room temp. and normal pressure, 48h.
ii : LiOH, H_2O, C_2H_5OH, room temp., 24h.
iii : HCl, H_2O, reflux, 6h.

Scheme 1

2.1.2.1. 1-H-4-Hydroxy-5-benzyl-pyrrolidine-3-carboxylic acid hydrochloride (4a)

Yield 80%. M.p.: 258-260 °C. IR (KBr, cm^{-1}) 3436-2850 (br, OH alcohol and acid) and 1724 (C=O, acid). ^{1}H NMR (DMSO-d_6 ppm δ)**:** 2.50-2.70 (m, 3H, Ph-C***H*₂**,C***H***-COOH); 2.91-3.42 (m, 4H, C***H*₂**-N,N-C***H***, C***H***-OH); 4.30 (s,1H,CH-O***H***); 6.82-7.15 (m, 5H, Ar-***H***); 9.4 (s ,1H,COO***H***). EI/MS, m/z (%): 221, M^+-HCl. Microanalysis calcd. C, H, N for $C_{12}H_{16}ClNO_3$: 56.15, 5.89, 5.46; found, 56.38, 5.92, 5.31.

2.1.2.2. 1-H-4-Hydroxy-5-(4-methoxy-benzyl)-pyrrolidine-3-carboxylic acid hydrochloride (4b)

Yield 85%. M.p.: 256-258 °C. IR (KBr, cm^{-1}) 3239-2925 (br, OH alcohol and acid) and 1716 (C=O, acid). ^{1}H NMR (DMSO-d_6 ppm δ)**:** 2.61-2.81 (m, 3H, Ph-C***H*₂**, C***H***-COOH); 2.90-3.40 (m, 4H, C***H*₂**-N, N-C***H***, C***H***-OH); 3.70 (s, 3H, C***H*₃**-O); 6.89 (d, J= 8.4Hz, 2H, Ar-***H***); 7.21(d, J= 8.4Hz, 2H, Ar-***H***); 9.40 (s, 1H, COO***H***). EI/MS, m/z (%):287.5, M^+. Microanalysis calcd. C, H, N for $C_{13}H_{18}ClNO_4$: 54.26, 6.31, 4.87; found, 54.35, 6.52, 4.96.

2.1.2.3. 1-H-4-Hydroxy-5-(4-ethoxy-benzyl)-pyrrolidine-3-carboxylic acid hydrochloride (4c)

Yield 78%. M.p.: 240-242 °C. IR (KBr, cm^{-1}) 3219-2770 (br, OH alcohol and acid) and 1715 (C=O, acid). ^{1}H NMR (DMSO-d_6 ppm δ): 1.29 (t, J=6.9 Hz, 3H, C***H*₃**-CH_2-O); 2.70-3.00 (m, 3H, Ph-C***H*₂**, C***H***-COOH); 3.10-3.60 (m, 4H, C***H*₂**-N, N-C***H***, C***H***-OH); 3.95 (q, J= 6.9Hz, 2H, CH_3-C***H*₂**-O); 6.89 (d, J= 8.4 Hz, 2H, Ar-***H***); 7.21 (d, J= 8.4Hz, 2H, Ar-***H***); 9.50 (s, 1H, COO***H***). EI/MS, m/z (%):265, M^+- HCl. Microanalysis calcd. C, H, N for $C_{14}H_{20}ClNO_4$: 55.72, 6.68, 4.64; found, 55.67, 6.62, 4.76.

2.1.2.4. 1-H-4-Hydroxy-5-(4-n-propoxy-benzyl)-pyrrolidine-3-carboxylic acid hydrochloride (4d)

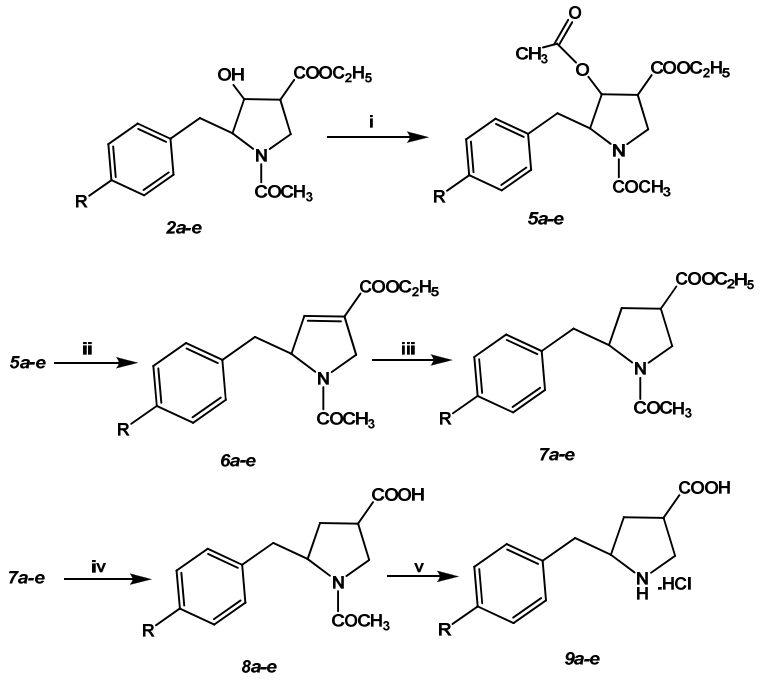
Yield 81% decomposition at 270°C. IR (KBr, cm^{-1}) 3228-2764 (br, OH alcohol and acid) and 1716 (C=O, acid). ^{1}H NMR (DMSO-d_6 ppm δ) **:** 0.93 (t, J= 6.9 Hz, 3H, C***H*₃**-$(CH_2)_2$-O); 1.66-1.68 (m, 2H, CH_3-C***H*₂**-CH_2-O); 2.75-2.93 (m, 3H, Ph-C***H*₂**, C***H***-COOH); 3.10-3.50 (m, 4H, C***H*₂**-N, N-C***H***, C***H***-OH); 3.87 (t, J=6.6 Hz, 2H, CH_3-CH_2-C***H*₂**-O); 6.80 (d, J= 8.4Hz, 2H, Ar-***H***); 7.20 (d, J= 8.4Hz, 2H, Ar-***H***); 9.50 (s, 1H, COO***H***). ^{13}C NMR data (DMSO-d_6 ppm δ):170.6 (1C, ***C***OOH); 158.1 (1C, Ar-***C***); 130.6 (1C, Ar-***C***); 129.1 (2C, Ar-***C***); 115.1 (2C, Ar-***C***); 71.2 (1C, CH_3 CH_2***C***H_2O); 69.5 (1C, ***C***H-OH); 66.2 (1C, N-***C***H); 49.0* (1C, C***H*₂**-N); 43.5* (1C, ***C***H-COOH); 32.0 (1C, Ph-***C***H_2); 23.5(1C, CH_3-***C***H_2-CH_2-O); 15.0 (1C, ***C***H_3CH_2 CH_2O). FAB/MS, m/z (%): 280 (M^++1)-HCl. Microanalysis calcd. C, H, N for $C_{15}H_{22}ClNO_4$: 57.05, 7.02, 4.44; found, 57.10, 7.09, 4.62.

2.1.2.5. 1-H-4-Hydroxy-5-(4-n-butoxy-benzyl)-pyrrolidine-3-carboxylic acid hydrochloride (4e)

Yield 76% decomposition at 270°C. IR (KBr, cm^{-1}) 3227-2929 (br, OH alcohol and acid) and 1716 (C=O, acid). ^{1}H NMR (DMSO-d_6 ppm δ)**:** 0.88 (t, J=7.4 Hz, 3H, C***H*₃**-$(CH_2)_3$-O); 1.36-1.41 (m, 2H, CH_3-C***H*₂**-$(CH_2)_2$-O); 1.60-1.67 (m, 2H, CH_3CH_2-C***H*₂**-CH_2-O); 2.79-3.00 (m, 3H, Ph-C***H*₂**, C***H***-COOH); 3.13-3.65 (m, 4H, C***H*₂**-N, N-C***H***, C***H***-OH); 3.89 (t, J= 6.6 Hz, 2H, CH_3-$(CH_2)_2$-C***H*₂**-O); 6.84 (d, J= 8.4 Hz, 2H, Ar-***H***); 7.17 (d, J= 8.4Hz, 2H, Ar-***H***); 9.50 (s, 1H, COO***H***). FAB/MS, m/z (%): 294 (M^++1)-HCl. Microanalysis calcd. C, H, N for $C_{16}H_{24}ClNO_4$: 58.27, 7.33, 4.25; found, 58.31, 7.28, 4.20.

2.1.3. General procedure for preparation of 1-acetyl-5-benzyl or 5-(4-alkoxy-benzyl)-pyrrolidine-3-carboxylic acid ethyl esters (7a-e)

A solution of 0.01 mol of 1-acetyl-4-hydroxy-5-benzyl or 5-(4-alkoxy-benzyl)-pyrrolidine-3-carboxylic acid ethyl ester (***2a-e***) in 9.4 mL (10.2 g, 0.1 mol) of acetic anhydride was refluxed for 18h. The reaction mixture was evaporated under vacuum, diluted with water and extracted with ethyl acetate (2x50 mL). The organic layers were shaken with 10% solution of sodium hydrogen carbonate (2x50 mL) then with water (1x50 mL). The organic layer was dried ($MgSO_4$) and evaporated under vacuum to afford 4-acetoxy-1-acetyl-5-benzyl or 5-(4-alkoxy- benzyl)-pyrrolidine-3-carboxylic acid ethyl esters (***5a-e***) as brown oils, which were used as such in the next step. The yields of ***5a-e*** were: 80%, 86%, 82%, 78% and 90%, respectively. The IR (liquid film, cm^1) spectra of ***5a-e*** showed bands at 1725-1747 (C=O, acetoxy and ester), 1644-1651 (C=O, amide) and absence of OH band at 3325-3335 cm^{-1}.



R : H, OCH_3, OC_2H_5, O-C_3H_7(*n*), O-C_4H_9(*n*)
i : $(CH_3CO)_2O$, reflux ,18h.
ii : DBU, THF, room temp., 2h.
iii : H_2 / PtO_2 , 95% ethanol, stirring at room temp. and normal pressure, 48h.
iv : LiOH, H_2O, C_2H_5OH, room temp., 24h.
v : HCl, H_2O (1:1), reflux, 6h.

Scheme 2

A solution of 0.01 mol of ***5a-e*** in 30 mL of freshly distilled tetrahydrofuran was stirred at room temperature for 2-3h in the presence of 1.49 mL (1.52 g, 0.01 mol) of 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU). The reaction mixture was evaporated under vacuum. The residue was diluted with water, acidified with hydrochloric acid (1 N) and extracted with diethyl ether (3x25 mL). The organic layers were separated, dried ($MgSO_4$) and evaporated under vacuum to afford 1-acetyl-5-benzyl or 5-(4-alkoxy-benzyl)-2,5-dihydro-1*H*-pyrrole-3-carboxylic acid ethyl ester (***6a-e***) as yellowish brown oils, which were pure enough to be used in the next step. The yields of ***6a-e*** were: 78%, 80%, 67%, 82% and 81%, respectively.

A solution of 0.01 mol of 1-acetyl-5-benzyl or 5-(4-alkoxy-benzyl)-2,5-dihydro-1*H*-pyrrole-3-carboxylic acid ethyl ester (***6a-e***) in 150 mL of ethanol 95% was hydrogenated at room temperature and normal pressure for 48 h, using 0.23 g (0.001 mol) of platinum IV oxide. Then, the catalyst was filtered off and ethanol was evaporated under vacuum to afford 1-acetyl-5-benzyl or 5-(4-alkoxy-benzyl)-pyrrolidine-3-carboxylic acid ethyl esters (***7a-e***) as yellow oils, which were purified by column chromatography using petroleum-ether (40-60 ^oC): ethyl acetate (9:1).

2.1.3.1. 1-Acetyl-5-benzyl-pyrrolidine-3-carboxylic acid ethyl ester (7a)

Yield 88%. IR (liquid film, cm^{-1}): 1729 (C=O, ester) and 1634 (C=O, amide). EI/MS, m/z (%): 275, M^+, $C_{16}H_{21}NO_3$.

2.1.3.2. 1-Acetyl-5-(4-methoxy-benzyl)- pyrrolidine-3-carboxylic acid ethyl ester (7b)

Yield 90%. IR (liquid film, cm^{-1}): 1731(C=O, ester) and 1637 (C=O, amide). EI/MS, m/z (%): 306, M^++1, $C_{17}H_{23}NO_4$.

2.1.3.3. 1-Acetyl-5-(4-ethoxy-benzyl)-pyrrolidine-3-carboxylic acid ethyl ester (7c)

Yield 87%.. IR (liquid film, cm^{-1}):1729 (C=O, ester) and 1646 (C=O, amide). EI/MS, m/z (%): 319, M^+, $C_{18}H_{25}NO_4$.

2.1.3.4. 1-Acetyl-5-(4-n-propoxy-benzyl)-pyrrolidine-3-carboxylic acid ethyl ester (7d)

Yield 86%. IR (liquid film, cm^{-1}): 1730 (C=O, ester) and 1642 (C=O, amide). EI/MS, m/z (%): 334, M^++1, $C_{19}H_{27}NO_4$.

2.1.3.5. 1-Acetyl-5-(4-n-butoxy-benzyl)- pyrrolidine-3-carboxylic acid ethyl ester (7e)

Yield 88%. IR (liquid film, cm^{-1}): 1730 (C=O, ester) and 1644 (C=O, amide). EI/MS, m/z (%): 348, M^++1, $C_{20}H_{29}NO_4$. ^1H NMR (DMSO-d_6) δ ppm of ***7e*** as a representative example of compounds ***7a-e***: ^1H NMR (DMSO-d_6) δ ppm of ***7e***: 0.93 (t, J= 7.1 Hz, 3H, C***H***$_3$-$(CH_2)_3O$); 1.25 (t, J=6.9 Hz, 3H, $COOCH_2C$***H***$_3$); 1.41-1.49 (m, 2H, CH_3-C***H***$_2$-$(CH_2)_2O$); 1.69-1.74 (m, 2H, CH_3-CH_2-C***H***$_2$-CH_2O); 2.02-2.59 (m, 5H, C***H***$_3$CON, C***H***$_2$-CHCOOH); 2.78-3.05 (m, 3H, Ph-C***H***$_2$, C***H***-COO- CH_2CH_3); 3.21-3.73 (m, 3H, C***H***$_2$-N, N-C***H***); 3.90 (t, J= 6.2Hz, 2H, CH_3-$(CH_2)_2$-C***H***$_2$O); 4.15 (q, J= 6.3 Hz, 2H, COOC***H***$_2CH_3$); 6.78(d, J= 7.2 Hz, 2H, Ar-***H***); 7.07 (d, J= 7.2 Hz, 2H, Ar-***H***).

2.1.4. General procedure for preparation of 1-acetyl -5-benzyl or 5-(4-alkoxy-benzyl)-pyrrolidine-3-carboxylic acids (8a-e)

To 0.0143 mol of ***7a-e*** in ethanol (15 mL), 14.3 mL of lithium hydroxide solution (1.7 N) was added. The reaction mixture was stirred for 24 h at room temperature and evaporated under reduced pressure. The residue was dissolved

in water and washed with ethyl acetate. The aqueous layer was acidified with hydrochloric acid (1 N), extracted with ethyl acetate, dried and evaporated under reduced pressure to afford ***8a-e*** as solids which were recrystallized from isopropanol.

2.1.4.1. 1-Acetyl-5-benzyl-pyrrolidine-3-carboxylic acid (8a)

Yield 87%. M.p.: 138-140 °C. IR (KBr, cm^{-1}): 3300-2700 (br, OH carboxylic), 1718 (C=O, acid) and 1584 (C=O, amide). ^{1}H NMR (DMSO-d_6) δ ppm: 2.00-2.32 (s, 5H, C***H***$_3$-CO-N, C***H***$_2$-CH-COOH); 2.80-3.10 (m, 3H, Ph-C***H***$_2$, C***H***-COOH); 3.61-3.80 (m, 3H, C***H***$_2$-N, N-C***H***); 6.82-7.15 (m, 5H, Ar-***H***). EI/MS, m/z (%): 248, M^++1. Microanalysis calcd. C, H, N for, $C_{14}H_{17}NO_3$: 68.00, 6.93, 5.66; found, 67.95, 6.87, 5.70.

2.1.4.2. 1-Acetyl-5-(4-methoxy-benzyl)-pyrrolidine-3-carboxylic acid (8b)

Yield 85%. M.p.: 140-142°C. IR (KBr, cm^{-1}): 3300-2700 (br, OH carboxylic), 1719 (C=O, acid) and 1590 (C=O, amide). ^{1}H NMR (DMSO-d_6) δ ppm: 2.00-2.46 (m, 5H, C***H***$_3$-CO-N, C***H***$_2$-CH-COOH); 2.75-3.05 (m, 3H, Ph-C***H***$_2$, C***H***-COOH); 3.60-4.00 (m, 6H, C***H***$_2$-N, N-C***H***, C***H***$_3$-O); 6.82 (d, J= 6.9 Hz, 2H, Ar-***H***); 7.02 (d, J= 6.9 Hz, 2H, Ar-***H***). EI/MS, m/z (%):277, M^+. Microanalysis calcd. C, H, N for, $C_{15}H_{19}NO_4$: 64.97, 6.91, 5.05; found, 64.90, 6.88, 5.11.

2.1.4.3. 1-Acetyl-5-(4-ethoxy-benzyl)-pyrrolidine-3-carboxylic acid (8c)

Yield 77%. M.p.: 178-181°C. IR (KBr, cm^{-1}): 3300-2700 (br, OH carboxylic), 1711 (C=O, acid) and 1586 (C=O, amide). ^{1}H NMR (DMSO-d_6) δ ppm: 1.39 (t, J=6.7 Hz, 3H, C***H***$_3$-CH_2-O); 2.05-2.32 (m, 5H, C***H***$_3$-CO-N, C***H***$_2$-CH-COOH); 2.48-3.29 (m, 3H, Ph-C***H***$_2$, C***H***-COOH); 3.62-3.76 (m, 3H, C***H***$_2$-N, N-C***H***); 3.98 (q, J=10.3 Hz, 2H, CH_3-C***H***$_2$-O); 6.80 (d, J= 8.3 Hz, 2H, Ar-***H***); 7.15 (d, J= 8.3 Hz, 2H, Ar-***H***). EI/MS, m/z (%): 291, M^+. Microanalysis calcd. C, H, N for, $C_{16}H_{21}NO_4$: 65.96, 7.27, 4.81; found, 65.91, 7.32, 4.69.

2.1.4.4. 1-Acetyl-5-(4-n-propoxy-benzyl) -pyrrolidine-3-carboxylic acid (8d)

Yield 73%. M.p.:134-136°C. IR (KBr, cm^{-1}): 3300-2700 (br, OH carboxylic), 1711 (C=O, acid) and 1588 (C=O, amide). ^{1}H NMR (DMSO-d_6) δ ppm: 0.92 (t, J=7.5 Hz, 3H, C***H***$_3$-$(CH_2)_2$-O); 1.64-1.72 (m, 2H, CH_3-C***H***$_2$-CH_2-O); 2.02-2.40 (m, 5H, C***H***$_3$-CO-N, C***H***$_2$- CH-COOH); 2.74-3.10 (m, 3H, Ph-C***H***$_2$, C***H***-COOH); 3.60-3.88 (m, 5H, C***H***$_2$-N, N-C***H***, CH_3-CH_2-C***H***$_2$-O); 6.80 (d, J= 8.6 Hz, 2H, Ar-***H***); 7.05 (d, J= 8.6 Hz, 2H, Ar-***H***). EI/MS, m/z (%): 305, M^+. Microanalysis calcd. C, H, N for, $C_{17}H_{23}NO_4$: 66.86, 7.59, 4.59; found, 66.79, 7.61, 4.52.

2.1.4.5. 1-Acetyl-5-(4-n-butoxy-benzyl)-pyrrolidine-3-carboxylic acid (8e)

Yield 80%. M.p.: 155-156°C. IR (KBr, cm^{-1}): 3300-2700 (br, OH carboxylic), 1719 (C=O, acid) and 1585 (C=O, amide). ^{1}H NMR (DMSO-d_6) δ ppm: 0.88 (t, J=7.5 Hz, 3H, C***H***$_3$-$(CH_2)_3$-O); 1.35-1.43 (m, 2H, CH_3-C***H***$_2$-$(CH_2)_2$-O); 1.60-1.67 (m, 2H, CH_3-CH_2-C***H***$_2$-CH_2-O); 1.90-2.38 (m, 5H, C***H***$_3$-CO-N, C***H***$_2$- CH-COOH); 2.72-3.06 (m, 3H, Ph-C***H***$_2$, C***H***-COOH); 3.65-3.90 (m, 5H, C***H***$_2$-N, N-C***H***, CH_3-$(CH_2)_2$- C***H***$_2$-O); 6.80 (d, J= 8.3 Hz, 2H, Ar-***H***); 7.01 (d, J= 8.3 Hz, 2H, Ar-***H***). ^{13}C NMR data (DMSO-d_6) δ ppm: 175.8 (1C, ***C***OOH); 175.0 (1C, CH_3-***C***O-N); 158.1 (1C, Ar-***C***); 130.8 (1C, Ar-***C***); 130.6 (2C, Ar-***C***); 114.5 (2C, Ar-***C***); 67.5 (1C, CH_3-CH_2-CH_2-***C***H_2-O); 58.5 (1C, N-***C***H); 49.6 (1C, C***H***$_2$-N); 42.1(1C, Ph-***C***H_2); 37.7 (1C, ***C***H-COOH);33.1 (1C, N-CH-***C***H_2); 31.3 (1C, CH_3-CH_2-***C***H_2-CH_2-O); 23.4(1C, ***C***H_3-CO-N); 19.3 (1C, CH_3-***C***H_2-CH_2-CH_2-O);14.2 (1C, ***C***H_3-CH_2-CH_2-CH_2-O). EI/MS, m/z (%):320, M^++1. Microanalysis calcd. C, H, N for, $C_{18}H_{25}NO_4$: 67.69, 7.89, 4.39; found, 67.22, 8.04, 4.34.

2.1.5. General procedure for preparation of 1-H-5-benzyl or 5-(4-alkoxy-benzyl)-pyrrolidine-3-carboxylic acid hydrochlorides (9a-e)

To 0.0034 mol of N-acetylated-4-hydroxy-5-benzyl or 5-(4-alkoxy- benzyl)-pyrrolidine-3-carboxylic acid (***8a-e***) was added 7 mL solution of hydrochloric acid (5 N). The reaction mixture was refluxed for 5 h, evaporated under reduced pressure to afford the hydrochlorides ***9a-e*** as solids which were crystallized from ethyl acetate/ alcohol (9:1).

2.1.5.1. 1-H-5-Benzyl-pyrrolidine-3-carboxylic acid hydrochloride (9a)

Yield 70 %, decomposition at 270°C. IR (KBr, cm^{-1}): 3410 (br, OH acid), 1727 (C=O, acid). ^{1}H NMR (DMSO-d_6) δ ppm: 1.71-2.10 (m, 2H, C***H***$_2$-CHCOOH); 2.60-3.42 (m, 6H, Ph-C***H***$_2$, C***H***-COOH ,C***H***$_2$-N,N-C***H***); 6.84-7.15(m, 5H, Ar-***H***); 9.55 (s, 1H, COO***H***). FAB/MS, m/z (%): 242, M^++0.5. Microanalysis calcd. C, H, N for, $C_{12}H_{16}ClNO_2$: 59.63, 6.67, 5.79; found, 59.68, 6.72, 5.81.

2.1.5.2. 1-H-5-(4-Methoxy-benzyl)-pyrrolidine-3-carboxylic acid hydrochloride (9b)

Yield 67 %. M.p.: 178-180°C. IR (KBr, cm^{-1}): 3432 (br, OH acid), 1727 (C=O, acid). ^{1}H NMR (DMSO-d_6) δ ppm: 1.72-2.20 (m, 2H, C***H***$_2$-CHCOOH); 2.62-3.41 (m, 6H, Ph-C***H***$_2$, C***H***-COOH ,C***H***$_2$-N, N-C***H***); 3.70 (s, 3H, C***H***$_3$-O); 6.84 (d, J= 8.4Hz, 2H, Ar ***H***); 7.12 (d, J= 8.4Hz, 2H, Ar-***H***); 9.52 (s, 1H, COO***H***). EI/MS, m/z (%): 235, M^+-HCl. Microanalysis calcd. C, H, N for, $C_{13}H_{18}ClNO_3$: 57.46, 6.68, 5.15; found, 57.50, 6.70, 5.11.

2.1.5.3. 1-H-5-(4-Ethoxy-benzyl)-pyrrolidine-3-carboxylic acid hydrochloride (9c)

Yield 75 %. M.p.:146-149°C. IR (KBr, cm^{-1}): 3400 (br, OH acid), 1733 (C=O, acid). ^{1}H NMR (DMSO-d_6) δ ppm: 1.29 (t, J=7.3 Hz, 3H, C***H***$_3$-CH_2-O); 1.73-2.25 (m, 2H, C***H***$_2$-CHCOOH); 2.81-3.60 (m, 6H, Ph-C***H***$_2$, C***H***-COOH , C***H***$_2$-N, N-C***H***); 3.97 (q, J=7.1Hz, 2H, CH_3-C***H***$_2$-O); 6.87 (d, J= 8.4 Hz, 2H, Ar-***H***); 7.18 (d, J= 8.4 Hz, 2H, Ar-***H***); 9.52(s, 1H, COO***H***). FAB/MS, m/z (%):286, M^++ 0.5. Microanalysis calcd. C, H, N for, $C_{14}H_{20}ClNO_3$: 58.84, 7.05, 4.90; found, 58.79, 7.00, 4.99.

2.1.5.4. 1-H-5-(4-n-Propoxy-benzyl)-pyrrolidine-3-carboxylic acid hydrochloride (9d)

Yield 80 %. M.p.:131-134°C. IR (KBr, cm^{-1}): 3449 (br, OH acid), 1733 (C=O, acid); ^{1}H NMR (DMSO-d_6) δ ppm: 0.99 (t, J=7.3 Hz, 3H, C***H***$_3$-$(CH_2)_2$-O); 1.80-2.10(m, 4H, CH_3-C***H***$_2$-O, C***H***$_2$-CH-COOH); 2.50-3.70 (m, 6H, Ph-C***H***$_2$, C***H***$_2$-N, N-C***H***, C***H***-COOH); 3.93 (t, J=6.6 Hz, 2H, CH_3-CH_2-C***H***$_2$-O); 6.87 (d, J= 8.4 Hz, 2H, Ar-***H***); 7.18 (d, J= 8.4 Hz, 2H, Ar-***H***). ^{13}C NMR data (DMSO-d_6) δ ppm: 170.7(1C, ***C***OOH); 158.1 (1C, Ar-***C***); 130.6 (1C, Ar-***C***); 128.0 (2C, Ar-***C***); 115.0 (2C, Ar-***C***); 68.1 (1C, CH_3-CH_2-***C***H_2-O); 62.2 (1C, N-***C***H); 47.0 (1C, C***H***$_2$-N); 37.0(1C, Ph-***C***H_2); 35.7 (1C, ***C***H-COOH);35.1 (1C, N-CH-***C***H_2); 32.1 (1C, CH_3 -***C***H_2-CH_2-O); 19.3 (1C, ***C***H_3- CH_2-CH_2-O). FAB/MS, m/z (%):299, M^+-0.5. Microanalysis calcd. C, H, N for, $C_{15}H_{22}ClNO_3$: 60.09, 7.40, 4.67; found, 60.12, 7.38, 4.70.

2.1.5.5. 1-H-5-(4-n-Butoxy-benzyl)-pyrrolidine-3-carboxylic acid hydrochloride (9e)

Yield 65 %. M.p.:140-142°C. IR (KBr, cm^{-1}): 3405 (br, OH acid), 1728 (C=O, acid). ^{1}H NMR (DMSO-d_6) δ ppm: 0.92 (t, J=7.3 Hz, 3H, C***H***$_3$-$(CH_2)_3$-O); 1.36-2.20 (m, 6H, CH_3-C***H***$_2$-$(CH_2)_2$-O, CH_3-CH_2-C***H***$_2$-CH_2-O, C***H***$_2$- CH-COOH); 2.84-3.70 (m, 6H, Ph- C***H***$_2$, C***H***-COOH, C***H***$_2$-N, N-C***H*);** 3.92 (t, J=6.5 Hz, 2H, CH_3-$(CH_2)_2$-C***H***$_2$-O); 6.88 (d, J= 8.7 Hz, 2H, Ar-***H***); 7.16 (d, J= 8.7Hz, 2H, Ar-***H***);

9.20 (s, 1H, COO***H***). FAB/MS, m/z (%):312, M^+-1.5. Microanalysis calcd. C, H, N for, $C_{16}H_{24}ClNO_3$: 61.24, 7.71, 4.46; found, 61.19, 7.68, 4.51.

2.2. Pharmacology

2.2.1. Materials

2.2.1.1. Animals

The anticonvulsant activity of the target compounds ***3a-e***, ***4a-e***, ***8a-e*** and ***9a-e*** was tested on adult male Swiss albino mice weighing 18-35 g. Animals were obtained from Animals House Colony of the National Research Centre, Cairo, Egypt. All animals were allowed free access to water and kept at constant diet.

2.2.1.2. Chemicals and drugs

Tween 80 (Sigma, USA), Pentylenetetrazole (Sigma,USA) and Gabapentin (Neurontin®; Parke Davis, USA) were used in this study. All the tested compounds were dissolved in 7% tween-80 saline solutions.

2.2.2. Determination of the anticonvulsant activity using Subcutaneous pentyleneteterazole seizures (scPTZ) test

Experiments [25,26] were carried out with groups of (6-10) mice each. The first group was divided into 3 subgroups and received intraperitoneally (ip) gabapentin (0.084, 0.14 and 0.28 mmol/kg b.wt equivalent to 15, 25, 50 mg/kg b.wt., respectively) [27] as reference drug. The second group was divided into 20 subgroups, each of which received (ip) one of the tested compounds 3a-e, 4a-e, 8a-e and 9a-e at dose levels of 0.0087, 0.0174, 0.0261, 0.05, 0.059 and 0.065 mmol / kg b.wt. One hour later [26] pentylenetetrazole (85 mg/kg) was administered (sc). The minimum dose of compounds and reference standard that induced maximum % protection was evaluated. The absence of seizures was chosen as an index for protective effect against pentylenetetrazole induced seizures as shown in Tables 1, 2, 3 and 4.

3. Results and Discussion

3.1. Chemistry

The starting synthon for the preparation of 1-acetyl 4-hydroxy-5-benzyl or 5-(4-alkoxy-benzyl)-pyrrolidine-3-carbo xylic acid ethyl esters (***2a-e***, Scheme 1) is the β-ketoesters 1-acetyl-4-ethoxycarbonyl-2-benzyl or-2-(4-alkoxy-benzyl) pyrro lidin-3-ones (***1a-e***) [22,23]. The latter underwent hydro genation at room temperature and normal pressure using platinum IV oxide as a catalyst in ethanol (95%) to afford ***2a-e*** [24].

Subsequent hydrolysis of pyrrolidine esters ***2a-e*** using lithium hydroxide solution (1.7 N) gave the corresponding acids ***3a-e*** in 73-85% yields (c.f. Experimental). Their structures were confirmed through infrared, NMR, mass spectroscopy and microanalytical data. Deacetylation of ***3a-e*** was achieved by refluxing with hydrochloric acid (5 N) for 5h to afford the hydrochloride salts ***4a-e*** in 76-85% yields (c.f. Experimental). The Synthetic pathway for preparation of 1-acetyl-5-benzyl or 5-(4-alkoxy-benzyl)-pyrrolidine-3-carboxylic acids (***8a-e***) was performed according to the previously reported procedure of Aboul-Enein *et al.* [18] (Scheme 2) in 80- 90% yields. Subsequent deacetylation by stirring solutions of ***5a-e*** in tetrahydrofuran in the presence of 1, 8-diazabicyclo [5.4.0] undec-7-ene (DBU) resulted in the double bond formation between C-3 and C-4 in the pyrrolidine ring to afford ***6a-e*** in good yields. Catalytic hydrogenation of the pyrroline double bond of compounds ***6a-e*** by platinum IV oxide in ethanol (95%) under room temperature and normal pressure for 48h gave 1-acetyl-5- or 5-(4-alkoxy-benzyl)-pyrrolidine-3-carboxylic acid ethyl esters (***7a-e***) in quantitative yields (c.f. Experimental). Subsequent hydrolysis of the esters ***7a-e*** was performed using lithium hydroxide solution (1.7 N) to give the corresponding 1-acetyl-5-benzyl or 5-(4-alkoxy-benzyl)-pyrrolidine-3-carboxylic acids (***8a-e***) as solids in good yields. Their structures were confirmed through infrared, NMR, mass spectroscopy and microanalytical data (c.f. Experimental). The target compounds ***9a-e*** were achieved through refluxing ***8a-e*** with hydrochloric acid (5N) to afford the hydrochlorides ***9a-e*** as solids in good yields (c.f. Experimental).

3.*2. Pharmacology*

Compounds ***3a-e***, ***4a-e***, ***8a-e*** and ***9a-e*** were evaluated for their anticonvulsant potential using subcutaneous pentylen etetrazole seizures (scPTZ) test and gabapentin as a reference drug. The anticonvulsant activity of 1-acetyl-4-hydroxy-5-benzyl or 5-(4-alkoxy-benzyl)-pyrrolidine-3-carboxylic acids series (***3a-e***, Table 1) was screened. The data presented in Table 1 illustrated that compounds ***3a***, ***3c*** and ***3d*** are the most active of the series and exhibited equipotent anticonvulsant potential of 84% protection against pentylenetetrazole seizures at dose level of 0.065mmol/kg b.wt. The anticonvulsant potential of the different congeners of this series is arranged in the following decreasing order: ***3a= 3c =3d >3e > 3b***.

Table 1. Anticonvulsant activity of 1-acetyl-4-hydroxy-5-benzyl or 5-(4-alkoxy-benzyl)-pyrrolidine-3-carboxylic acids (***3a-e***) using sc PTZ-induced seizures in adult male albino mice.

Compound No	Dose*, mmol/ kg b.wt. (mg/kg b.wt.)	% Protection**
Control	-	0
Gabapentin	0.14 (25.00)	100
I	0.07 (30.00)	100
II	0.04 (15.00)	100
III	0.01 (3.97)	100
3a	0.065 (17.10)	84
3b	0.065 (19.11)	50
3c	0.065 (19.96)	84
3d	0.065 (20.87)	84
3e	0.059 (19.77)	50

* Minimum doses induced maximum % protection in the observed period.
** % protection against seizures induced by pentylenetetrazole.

Also, the anticonvulsant potential of 1-*H*-4-hydroxy-5-benzyl or 5-(4-alkoxy-benzyl)-pyrrolidine-3-carboxylic acid hydro chlorides (***4a-e***, Table 2) showed that compound 1-*H*-4-hydroxy-5-(4-methoxy- benzyl)-pyrrolidine-3-carboxylic acid hydrochloride (***4b***) possessed 100% protection against pentylenetetrazole seizures at dose level of 0.0087 mmol/kg b.wt., meanwhile compounds 1-*H*-4-hydroxy-5-(4-ethoxy-benzyl)-pyrrolidine-3-carboxylic acid hydrochloride (***4c***) and 1-*H*-4-hydroxy-5-(4-propoxy-benzyl)-pyrrolidine-3-carboxylic acid hydrochloride (***4d***) showed equipotent protection activity of 84% at dose level of 0.0261 mmol/kg b.wt. On the other hand, compound ***4a*** (0.0261 mmol/kg b.wt.) displayed 72% protection level compared with gabapentin (0.14 mmol/kg b.wt.). The different congeners of this series showed a decrease in the anticonvulsant potential in the following order: ***4b> 4c= 4d > 4a>4e*** (c.f. Table 2).

Table 2. Anticonvulsant activity of compounds 1-*H*-4-hydroxy-5-benzyl or 5- (4-alkoxy-benzyl-pyrrolidine-3-carboxylic acid hydrochlorides (***4a-e***) using sc PTZ-induced seizures in adult male albino mice.

Compound No.	Dose*, mmol/ kg b.wt. (mg / kg b.wt.)	% Protection**
Control	-	0
Gabapentin	0.14 (25.00)	100
I	0.07 (30.00)	100
II	0.04 (15.00)	100
III	0.01 (3.97)	100
4a	0.0261 (6.72)	72
4b	0.0087 (2.50)	100
4c	0.0261 (7.87)	84
4d	0.0261 (8.23)	84
4e	0.0174 (5.73)	67

*Minimum doses induced maximum % protection in the observed period.
** % protection against seizures induced by pentylenetetrazole.

The anticonvulsant profile of 1-acetyl-5-benzyl or 5-(4-alkoxy-benzyl)-pyrrolidine-3-carboxylic acids series (***8a-e***, Table 3) showed that compound ***8d*** displayed 100% protection at dose level of 0.05mmol/kg b. wt., nearly 3-fold more potent than the reference drug gabapentin (0.14 mmol/kg b. wt.), while ***8b*** and ***8e*** exhibited 84% protection potential at dose level of 0.05 and 0.065 mmol/kg b.wt., respectively. Meanwhile ***8c*** showed 67% protection at dose level of 0.065 mmol/kg b.wt. On the other hand, 1-acetyl-5- benzyl-pyrrolidine-3-carboxylic acid (***8a***) gave 33% protection at dose level of 0.05 mmol/kg b.wt. compared with gabapentin (0.14 mmol/kg b.wt.). The anticonvulsant potential for the different congeners showed the following decreasing order: ***8d*** >***8b*** >***8e*** >***8c*** >***8a***.

Table 3. Anticonvulsant activity of 1-acetyl-5-benzyl or 5-(4-alkoxy-benzyl)–pyrrolidine-3-carboxylic acids (***8a-e***) using sc PTZ-induced seizures in adult male albino mice.

Compound No.	Dose*, mmol/ kg b.wt. (mg/ kg b.wt.)	% Protection**
Control	-	0
Gabapentin	0.14 (25.00)	100
I	0.07 (30.00)	100
II	0.04 (15.00)	100
III	0.01 (3.97)	100
8a	0.05 (12.35)	33
8b	0.05 (13.85)	84
8c	0.065 (18.92)	67
8d	0.05 (15.25)	100
8e	0.065 (20.80)	84

*Minimum doses induced maximum % protection in the observed period.
** % protection against seizures induced by pentylenetetrazole.

Also, the results of the anticonvulsant activity of 1-*H*-5-benzyl or 5-(4-alkoxy-benzyl)-pyrrolidine-3-carboxylic acid hydrochlorides (***9a-e,*** Table 4) showed that compound 1-*H*-5-(4-butoxy-benzyl)-pyrrolidine-3-carboxylic acid hydrochloride (***9e***) exhibited 100% protection at dose level of 0.0261 mmol/kg b.wt., while compounds ***9a-d*** exerted 72, 67, 67 and 63% protection, respectively at the same dose (0.0261 mmol/kg b.wt.) as compared with gabapentin (0.14 mmol/kg b.wt.). The different congeners of this series showed anticonvulsant potential in the following decreasing order: ***9e*** >***9a***> ***9b*** =***9c*** >***9d***.

Table 4. Anticonvulsant activity of 1-*H*-5-benzyl or 5-(4- alkoxy-benzyl)-pyrrolidine-3-carboxylic acid hydrochlorides (***9a-e***) using sc PTZ-induced seizures in adult male albino mice.

Compound No.	Dose*, mmol/kg b.wt. (mg/kg b.wt.)	% Protection**
Control	-	0
Gabapentin	0.14 (25.00)	100
I	0.07 (30.00)	100
II	0.04 (15.00)	100
III	0.01 (3.97)	100
9a	0.0261 (6.30)	72
9b	0.0261 (7.086)	67
9c	0.0261 (7.45)	67
9d	0.0261 (7.81)	63
9e	0.0261 (8.18)	100

*Minimum doses induced maximum % protection in the observed period.
** % protection against seizures induced by pentylenetetrazole.

4. Structure-activity relationship

Regarding the structure-activity relationship of the target compounds, the results revealed that the 1-*H*-4-hydroxy-5-benzyl or 5-(4-alkoxy-benzyl)-pyrrolidine-3-carboxylic acid hydrochlorides (***4a-e***) series showed higher anticonvulsant activity than the other series ***3a-e, 8a-e*** and ***9a-e*** as well as the reference standard. The most potent of series ***4a-e*** is compound ***4b*** which exhibited 100% protection at dose level of 0.0087mmol/kg b.wt. which is about 16-fold that of gabapentin. Moreover, compound ***4b*** exhibited higher potency than our previously reported compounds **I-III** [16-18]. Two compounds ***4c, 4d*** showed 84% protection, one compound ***4a*** exhibited 72% and the compound ***4e*** exhibited 67% protection. In series ***4a-e***, it could be deduced that the presence of the carboxylic, hydroxyl and the free pyrrolidine amino groups all together, in one and the same compound, is a requirement to achieve potent anticonvulsant potential. Mostly, the substitution of the phenyl ring at the 4-position with an alkoxy group resulted in a better activity than the unsubstituted one. Meanwhile, the presence of acetyl group in 1-acetyl-4-hydroxy-5-benzyl or 5-(4-alkoxy-benzyl)-pyrrolidine-3-carboxylic acid series ***3a-e***, resulted in a decrease in the anticonvulsant potential than series ***4a-e***. The presence of the acetyl group in series ***8a-e*** led to a decrease in the anticonvulsant activity relative to series ***9a-e***. Within the same series ***8a-e***, the substitution of the phenyl ring resulted in increasing the anticonvulsant activity. Meanwhile, the absence of hydroxyl group in series ***9a-e*** resulted in a decrease in the anticonvulsant activity relative to series ***4a-e***.

References

[1]. Lindsay, J. M. *Epilepsia* **1971**, *12*, 47-54.
[2]. Blume, W.; Lüders, H.; Mizrahi, E.; Tassinari, C.; Boas, W. V. E.; Engle, J. *Epilepsia* **2001**, *42*, 1212-1218.
[3]. Fisher, R.; Boas, W. V. E.; Blume, W.; Elger, C.; Genton, P.; Lee, P.; Engel, J. *Epilepsia* **2005**, *46*, 470-472.
[4]. Engel, Jr. J.; Padley, T. A.; "Epilepsy a comprehensive text book" Lippincott-Raven, Philadelphia, 1998.
[5]. Baker, G. A.; Hargis, E.; Hsih, M. M.-S.; Mounfield, H.; Arzimanoglou, A.; Glauser, T.; Pellock, J.; Lund, S. *Epilepsy and Behavior* **2008**, *12*, 395-401.
[6]. Ismail, M. A. H.; Aboul-Enein, M. N.; Abouzid, K. A. M.; Abou El Ella, D. A.; Ismail, N. S. M. *Bioorg. Med. Chem*. **2009**, *17*, 3739-3746.
[7]. Sircar, S. S. G. *J. Chem. Soc.* **1927**, 1252-1256.
[8]. Long, M. *J. Amer. Chem. Soc.* **1951**, *73*, 4895-4898.
[9]. Marshall, P. G.; Vallance, D. K. *J. Pharm. Pharmacol.* **1954**, *6*, 740-746.
[10]. Waser, V. P. G.; Ganz, A. J.; Pfirmann, R. W. *Arzneim.-Forsch.* **1977**, *27*, 1942-1953.

[11]. Noyer, M.; Gillard, M.; Matagne, A. *Eur. J. Pharmacol*. **1995**, *286*, 137-146.
[12]. Goodman; Y.; Mattson, M. P. *J. Neurochem.* **1996**, *66*, 869-72; *Chem. Abst.* **1996**, *124*, 165104d.
[13]. Labouta, I. M.; Jacobsen, P.; Thorbek, P.; Krogsgaard- Larsen, P.; Hjeds, H. *Acta Chemica Scandinavica* **1982**, *B 36*, 669-74.
[14]. Rogawski, M. *Br. J. Pharmacol.* **2008**, *154*, 1555-1557.
[15]. Matagne, A.; Margineanu, D-G.; Potschka, H.; Loscher, W.; Michel, P.; Kenda, B.; Klitgaard, H. *Eur. J. Pharmacol.* **2009**, *614*, 30-37.
[16]. Aboul-Enein, M. N.; El-Azzouny, A. A.; Abdallah, N. A.; Maklad, Y. A.; Attia, M. I.; Ebeid, M. Y. *Sci. pharm.* **1997**, *65*, 225-245.
[17]. Aboul-Enein, M. N.; Ismail, M. A. H.; Maklad, Y. A.; Mohamed, N. S. *Egypt. Pharm. J.* **2003**, *2*, 94-121.
[18]. Aboul-Enein, M. N.; El-Azzouny, A. A.; Safwat, H. M.; Saleh, O. A.; Maklad, Y. A. *Egypt. Pharm. J.* **2005**, *4*, 549-578.
[19]. Balenovic, K.; Bregent, N. *J. Org. Chem.***1964**, *19*, 1589-1592**.**
[20]. Satzinger, G.; Hartenstien, J.; Herrmann, M.; Heldt, W. *U. S. Patent.* **1977**, *4,024, 175*.
[21]. Keberel, H.; Faigle, J. W.; Wilhelm, M. *U. S. Pat.* **1969**, *3,471, 548.*
[22]. Aboul-Enein, M.N.; Khalifa, M.; El-Difrawy, S.M. *Pharm. Acta Helv*. **1973**, *48*, 405-411.
[23]. Aboul-Enein, M. N.; El-Difrawy, S. M.; Gadalla, K. Z.; Macklouf, A. A.; El Sayed, M. E. *Egypt. J. Pharm. Sci.* **1990**, *31*, 29-40.
[24]. Aboul-Enein, M. N.; El-Difrawy, S. M.; Nofal, Z. M.; El-Azzouny, A. A. *Acta Pharm. Suec.* **1982**, *19*, 309-316.
[25]. Pandeya, S. N.; Yogeeswari, P; Stables, J. P. *Eur. J. Med.Chem.* **2000**, *35*, 879-886. c) Parmar, S. S.; Gupta, A. K.; Gupta, T. K.; Stenberg, V. I. *J. Pharm. Sci.* **1975**, *64*, 154-157.
[26]. Hassert, G. L.; Poutsiaka, J.W.; Papandrianos, D.; Burke, J.C.; Carver, B.V. *Toxicol. Appl. Pharmacol.* **1961**, *3*, 726-741.
[27]. Lado, F. A.; Sperber, E. F.; Moshe, S. L. *Epilepsia* **2001**, *42,* 458-463.