

## Synthesis and Investigation of 2-Propylpentanoyl Amino Acid and Dipeptide Conjugates as Novel Anticonvulsants

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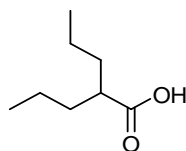
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THE CENTRAL nervous system (CNS) requires the anticipation of a number of neuro-active amino acids and peptides<sup>(1)</sup> for its normal function. Thus,  $\gamma$ -aminobutyric acid (GABA), glycine, histadine, tyrosine, arginine, taurine, aspartic and glutamic acids, are involved in the synthesis of neurotransmitters neuromodulators, or their neuro-agonists. In particular, glycine is an inhibitory neurotransmitter<sup>(1)</sup> that is found in mammalian proteins and tissues. Its role primarily appears to involve the inhibition of neuronal activity in the striatum, substantia nigra and cerebellum. Analogously,  $\gamma$ -aminobutyric acid (GABA)<sup>(2)</sup> is a constituent of mammalian tissues particularly the brain and spinal cord. It is synthesized in GABAergic neurons, and interacts with postsynaptic receptors to exert its inhibitory effects.

On the other hand, the collective terms “convulsive disorders”, “seizure disorders” and “cerebral seizures” are currently, synonymously considered, for epileptic convulsions. Such disorders are common neurological symptoms of a complex nature and, frequently, undetermined etiologies.

With the existing medications, however, approximately 25% of epileptic patients are not seizure free, regardless of their therapy optimization. In addition to the apparent health hazards, considerable socio-economic problems are intrinsically encountered with the prevalence of untreated epilepsy.

In that context, 2-propylpentanoic acid (*generic name: Valproic Acid* (trade names: *Depakene*®, “*Abbott Lab*”) is one of the essential antiepileptics, that are still predominant in the current clinical practice<sup>(3)</sup>.



Valproic Acid, 2-propylpentanoic acid (VPA), CAS: 99-66-1

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The use of valproic acid as an anticonvulsant, however, is frequently restricted by its two potentially life-threatening, side effects, namely, teratogenicity<sup>(4)</sup> and hepatotoxicity<sup>(5)</sup>.

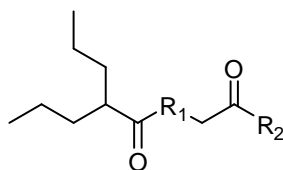
It is noteworthy to mention that neither GABA nor Glycine, as neuro-inhibitor amino acids, are antiepileptically effective upon oral or systemic administration since they can't cross blood brain barrier<sup>(8)</sup>. Furthermore, the dipeptides glycylglycine, glycylglycinamide and gabaloyl-glycinamide did not manifest a significant anticonvulsant activity following *i.p.* administration to mice. However, valproyl conjugates of amino acids, particularly their amides, appeared recently as attractive anticonvulsant candidates<sup>(6, 7)</sup>.

While glycinamide alone is active only when it is combined with a lipophilic anchor such as benzyl, acetyl, or benzyloxycarbonyl (Z-group), to be promoted to into an anticonvulsant active entity<sup>(9)</sup>. Contrarily, N<sup>α</sup>-valproyl glycine amide showed a promising anticonvulsant profile in the screening of animal antiepileptic models, due to its better pharmacokinetic profile<sup>(6, 10)</sup>. Moreover, it was interestingly reported that it acts as a novel drug, while doesn't serve as a chemical delivery system or a prodrug for either of its components: valproic acid or glycine<sup>(11)</sup>.

### Results and Discussion

Some rationalized novel amino acid and peptide conjugates of the antiepileptic drug "Valproic Acid" were, herein, chemically synthesized, characterized and pharmacologically investigated for their anticonvulsant potency.

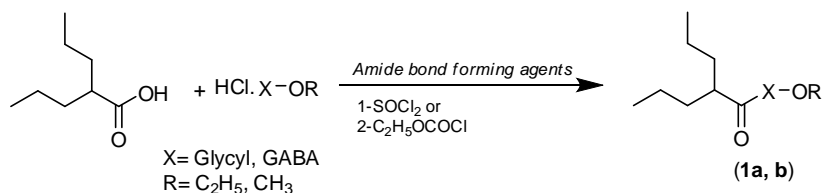
The hereinafter general structural formula represents the molecular edifice of the newly synthesized anticonvulsant candidates:



R<sub>1</sub>:- amino acid or dipeptide residue; R<sub>2</sub>:-OR (esters), -NH-NH<sub>2</sub> (Hydrazides; -NH-N=CHR<sub>3</sub> (Schiffs' bases; R<sub>3</sub>, aldehyde residue), -COOH(acids), -NH<sub>2</sub> (amides).

Generally, the followed synthetic procedures for preparing the candidates are those frequently manipulated in amino acid and synthetic peptide chemistry for mild amide and peptide bond formation, namely, acid chloride, carbodiimides and mixed anhydride procedures<sup>(12, 13)</sup>.

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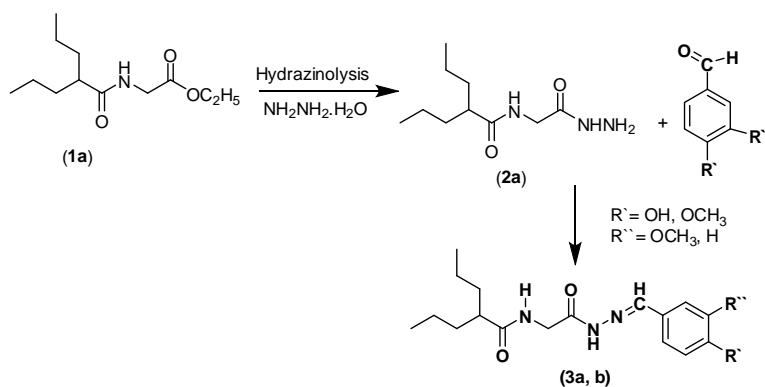
*Synthesis of N-valproyl amino acid esters*

The ester function is known as versatile synthetic identity, easily to be hydrolyzed, or hydrazinolysed to its corresponding acids or hydrazides, respectively. However, both valproyl amino acid esters and valproyl amino acids as revealed by the literature investigation, are of limited interest as anticonvulsant agents<sup>(6)</sup>.

The following synthetic procedure is based upon coupling valproyl chloride, at low temperature, with free Gly-OEt or GABA-OMe, in the presence of N-methyl morpholine, as a tertiary base.

Alternatively, the formation of valproyl mixed anhydride with ethylchloroformate at (~ -15 °C, in the presence of a tertiary base) followed by *in situ*, coupling of formed mixed anhydride with free *Gly-OEt* or *GABA-OMe*, was equally, manipulated

The acid chloride method, however, proved to be more advantageous than the mixed anhydride method in several aspects as the stability of the starting acid chloride and overall reaction yield.

*Synthesis of N<sup>α</sup>-Valproyl glycol hydrazones*



An alternative method involved the coupling of *N*<sup>γ</sup>-valproyl GABA (4b) with glycine OEt in the presence of the water soluble diisopropylcarbodiimide (DIC) affording (5b), in a 48% yield.

The mixed anhydride method, as used for the synthesis of the valproyl dipeptide ester (5b), proved, however, to be more advantageous than the DIC method in several aspects, exemplified by the associated higher yields, as well as minimizing the side products, in contrast to the difficulty encountered in the complete removal of diisopropylurea, as the main side product of DIC method.

The suggestion of synthesis of valproyl dipeptide amides was rationalized by the literature reports that two valproyl amino acid amides, namely that of glycine and taurine have a pronounced anticonvulsant activity. In addition, mechanistically, the reported candidates were not acting as prodrugs for valproic acid, contrarily, as novel integrated agents<sup>(6, 10)</sup>.

Ammonolysis of valproyl dipeptide esters (5a, b) with methanolic dry ammonia afforded the corresponding valproyl dipeptide amides (6a, b) in 89 and 85 % yields.

### Experimental

Purity of the synthesized compounds was evidenced by TLC and their elemental analysis which was, generally, found to be within  $\pm 0.4$  of the calculated theoretical values.

Melting points were determined in opened glass capillary tubes with an Electro thermal" digital melting point apparatus, (Model: IA9100- England) and were uncorrected. Infrared spectra (KBr) were recorded on "Nexus 670 FTIR Nicolet" Fourier Transform Infrared Spectrometer, USA, within the range 4000-400  $\text{cm}^{-1}$ . Proton nuclear magnetic resonance (<sup>1</sup>H-NMR) spectra ( $\text{CDCl}_3$  or DMSO) were run in a Jöel (Japan, 270 or 500 MHz ) spectrometer where TMS is considered as an internal standard. Mass spectra were run on "MAT Finnigan SSQ 7000 spectrometer" (Germany), using the electron impact (EI) technique. Elemental (micro-analysis) for the prepared compounds (Carbon, Hydrogen and Nitrogen, Microanalytical Unit, NRC, Cairo). Analytical thin layer chromatography (TLC) was performed on "Silica Gel" aluminium sheets, 60 F<sub>254</sub> (E. Merck).

#### *Synthesis of N-valproyl amino acid esters*

##### *Acid chloride method*

A cold solution of valproic acid (~20 mmol, ~ -5 °C) and thionyl chloride (3 ml; ~ 20 mmole), to which was added dimethylformamide (0.5 ml), was stirred for an hour. The reaction mixture was then refluxed for an additional hour, evaporated to dryness and triturated with benzene to afford the corresponding acid chloride in a 90% yield.

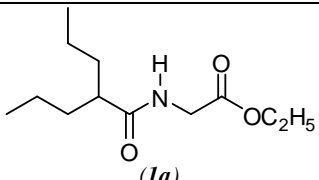
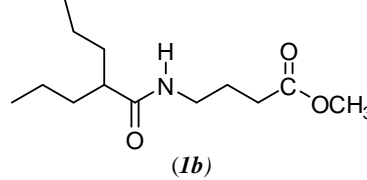
A THF solution of valproyl chloride (1 mmole) was dropwisely added to a cold (-15 °C) and stirred solution of the amino acid ester (1 mmole) after that (pH was adjusted up to ~ 7.5 using N-methyl morpholine). The reaction mixture was stirred for additional 3 hr at the same temperature, for 12 hr at room temperature, after which it was washed with water, 1N sodium bicarbonate, 0.5N HCl and finally with water, then it was dried over anhydrous sodium sulphate. The solvent was evaporated to dryness and the obtained residue was solidified by trituration with diethyl ether. The obtained solid (1a or 1b) was then filtered off, washed with pet.ether.

*Mixed anhydride method*

Triethylamine (0.13ml, 1 mmol) was added to a cold and stirred dichloromethane solution (25ml, -20°C) of valproic acid and ethyl chloroformate (0.1 ml, 1 mmole). Stirring was continued for 20 min after which the amino acid ester (1 mmole) was added. The reaction mixture was stirred at ~ -20°C, pH~ 7.5 for 3 hr, then for 12 hr at room temperature. The solution was then washed with water, 1N hydrochloric acid, 1N sodium bicarbonate and water followed by drying over anhydrous sodium sulphate. The solvent was evaporated under reduced pressure and the crude product was triturated with diethyl ether, filtered off, to afford the corresponding ester (1a or 1b).

1a: C<sub>12</sub>H<sub>23</sub>NO<sub>3</sub> (229.32), m.p., 80-81 °C and 1b: C<sub>13</sub>H<sub>25</sub>NO<sub>3</sub> (243.34), m.p., 71-72 °C.

**TABLE 1. Spectral data for N-valproyl amino acid esters (1a, b).**

Spectral Data	Structural Formula
<p><i>IR</i> (KBr, cm<sup>-1</sup>): 3297(NH st.), 2956 (CH aliphatic), 1731(C=O ester), 1640, 1550 (C=O stretching, amide I and N-H bending, amide II).</p> <p><i>MS</i> (m/z %): 229 (<b>M<sup>+</sup></b>, 1%), 187 (55%), 158 (100%), 57(92%).</p>	 <p style="text-align: center;"><b>(1a)</b></p>
<p><i>IR</i> (KBr, cm<sup>-1</sup>): 3396(NH st), 2955 (CH aliphatic), 1736(C=O ester), 1644, 1545 (C=O stretching, amide I and N-H bending, amide II).</p> <p><i>MS</i> (m/z %): 201 (<b>M<sup>+</sup></b>- 42, 17.48%), 172 (49%), 140 (25%), 127 (11.75%), 112(21%), 99(14%), 57 (100%).</p>	 <p style="text-align: center;"><b>(1b)</b></p>

*Synthesis of N<sup>α</sup>-valproyl glycyhydrazones*

Hydrazine hydrate (98%, 0.35ml, ~10 mmol) was added to a methanolic solution (20 ml) of compounds 1a (1 mmol). The reaction mixture was refluxed for 3 hr, after which the solvent was evaporated. The obtained residue was

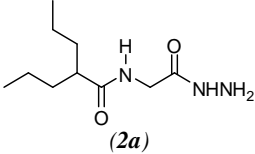
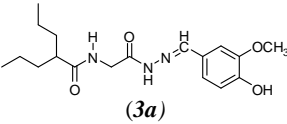
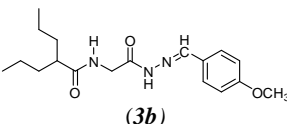
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trituated with ether, filtered off and crystallized from methanol/diethyl ether to give the corresponding hydrazide (2a).

A stirred solution of the hydrazide 2a (1mmol), in ethanol (20 ml) and vanillin or anisaldehyde (1mmol) was refluxed for 5 hr with stirring. The reaction mixture was evaporated under reduced pressure, the obtained residue was trituated with ether. The separated solid was collected by filtration, dried and crystallized from ethanol/ether to give the corresponding hydrazones (3a, b).

2a:  $C_{10}H_{21}N_3O_2$  (215.29) m.p., 130-133 °C; 3a:  $C_{18}H_{27}N_3O_4$  (349.42) m.p., 187-188 °C 3b:  $C_{18}H_{27}N_3O_3$  (333.43), m.p., 204-206 °C.

TABLE 2. Spectral data.

Spectral Data	Structural Formula
<p>IR (KBr, <math>cm^{-1}</math>): 3292 (NH st), 2941 (CH aliphatic), 1635, 1560 (C=O stretching, amide I and N-H bending, amide II).</p> <p>MS (m/z %): 173.11 (<math>M^+</math>, 42, 12.7%), 149.11(56%), 83 (51%), 57(100%).</p>	 <p>(2a)</p>
<p>IR (KBr, <math>cm^{-1}</math>): 3289(NH st), 3090(CH aromatic), 2957(CH aliphatic), 1680(C=N), 1636, 1548(C=O amide I stretching and N-H amide II, bending).</p> <p><math>^1H-NMR</math> (<math>\delta</math>, ppm, <math>CDCl_3</math>): 0.86(<i>t</i>, 6H, 2<math>CH_2CH_2CH_2</math>), 1.3(<i>m</i>, 4H, 2<math>CH_2CH_2CH_2</math>), 1.5(<i>m</i>, 4H, 2 <math>CH_3CH_2CH_2</math>), 2.20(<i>m</i>, 1H, <math>CHCO</math>), 3.90(<i>s</i>, 3H, Ph-O<math>CH_3</math>), 4.50(<i>s</i>, 2H, <math>NHCH_2</math>), 6.4(<i>s</i>, 1H, <math>NH-N=CH</math>), 6.90(<i>d</i>, 1H, C-6 H aromatic), 7.57(<i>d</i>, 1H, C-5 H-aromatic), 7.70(<i>s</i>, 1H, C-2 H aromatic), 7.78(<i>br</i>, H, <math>NHCO</math>), 8.59(<i>br</i>, 1H, <math>NH-N=CH</math>), 8.64(<i>s</i>, 1H, Ph-OH).</p> <p>MS (m/z %): 333 (<math>M^+</math>, 16, 14.68%), 206 (18%), 150 (100%), 166 (59%), 57(7%).</p>	 <p>(3a)</p>
<p>IR (KBr, <math>cm^{-1}</math>): 3429(OH-Ph st), 3292(NH st), 3093(CH aromatic), 2952(CH aliphatic), 1688(C=N), 1641, 1549(C=O amide I, stretching and N-H amide II, bending).</p> <p><math>^1H-NMR</math> (<math>\delta</math>, ppm, <math>CDCl_3</math>): 0.86(<i>t</i>, 6H, 2 <math>CH_2CH_2CH_2</math>), 1.25(<i>m</i>, 4H, 2 <math>CH_2CH_2CH_2</math>), 1.62(<i>m</i>, 4H, 2 <math>CH_3CH_2CH_2</math>), 2.20(<i>m</i>, 1H, <math>CHCO</math>), 3.85(<i>s</i>, 3H, Ph-O<math>CH_3</math>), 4.50(<i>s</i>, 2H, <math>NHCH_2</math>), 6.34(<i>s</i>, 1H, <math>NH-N=CH</math>), 7.29(<i>d</i>, 2H, C<sub>2</sub>, C<sub>6</sub>-H-aromatic), 7.65(<i>d</i>, 2H, C-3 C-5 H-aromatic), 7.93(<i>br</i>, H, <math>NHCO</math>), 8.91(<i>br</i>, 1H, <math>NH-N=CH</math>).</p> <p>MS (m/z %): 333.20 (<math>M^+</math>, 12%), 278.90 (63%), 264 (100%), 197.03 (39%), 166(63%).</p>	 <p>(3b)</p>

*Synthesis of N-valproyl dipeptide esters and amides*

Sodium hydroxide (0.5N, 20 ml) was dropwisely added to a cold and stirred methanolic solution (1 mmol, -5 °C,) of the corresponding ester (1a or 1b) .The cold reaction mixture (4 °C) was acidified with (1N) hydrochloric acid to pH~3. The obtained solid was filtered off, washed with water, dried and crystallized from ethanol/water to give the corresponding acids (4a, b).

The mixed anhydride method, was followed as previously described for (1a, b) to give the corresponding valproyl dipeptide esters (5a, b).

Diisopropyl carbodiimide method: glycine ethyl ester, 1 mmol, was added to a solution N<sup>γ</sup>-valproyl GABA (4b) (1mmol) in dichloromethane (20 ml, -5°C). Diisopropylcarbodiimide (0.26 ml, 1mmol) was added. The solution was then stirred for 20 hr at room temperature, washed with 1N sodium bicarbonate, 0.5 N HCl and water, dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated under reduced pressure. The obtained oily residue was solidified by trituration with dry ether/n-hexane mixture, filtered off and crystallized from ethanol/diethyl ether to give the corresponding dipeptide ester (5b).

To a cold methanolic solution of 5a or 5b (1mmol, 20 ml, at -5 °C) a current stream of dry ammonia gas was passed till saturation. The reaction mixture was left over night at ~ -4 °C, evaporated under reduced pressure and the obtained residue was triturated with diethyl ether. The formed solid was filtered off, washed with ether, dried and crystallized from methanol/diethyl ether to give the corresponding amides (6a, b):

4a: C<sub>10</sub>H<sub>9</sub>NO<sub>3</sub> (201.26), *m.p.*, 122-123 °C;

4b: C<sub>12</sub>H<sub>23</sub>NO<sub>3</sub> (229.32) *m.p.*, 68.5-69 °C;

5a: C<sub>15</sub>H<sub>28</sub>N<sub>2</sub>O<sub>4</sub> (300.39) *m.p.*, 120-121 °C;

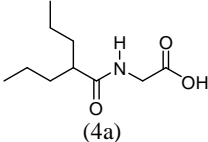
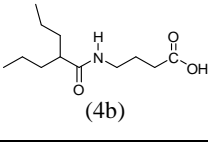
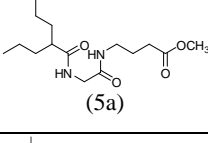
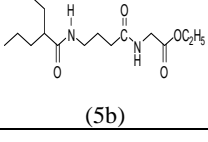
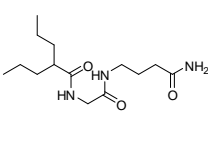
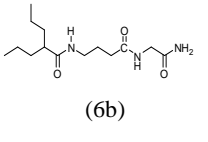
5b: C<sub>16</sub>H<sub>30</sub>N<sub>2</sub>O<sub>4</sub> (314.42) *m.p.*, 119-120°C;

6a: C<sub>14</sub>H<sub>27</sub>N<sub>3</sub>O<sub>3</sub> (285.38), *m.p.*, 126-127 °C;

6b: C<sub>14</sub>H<sub>27</sub>N<sub>3</sub>O<sub>3</sub> (285.21), *m.p.*, 103-105 °C.



TABLE 3. Spectral data.

Spectral Data	Structural Formula
<p><i>IR</i> (KBr, <math>\text{cm}^{-1}</math>): 3305-3110 (OH and NH st), 2945 (CH aliphatic), 1706 (C=O acid), 1612, 1493 (C=O, stretching, amide I and N-H, bending, amide II).</p> <p><i>MS</i> (<math>m/z</math> %): 201 (<math>M^+</math>, 19.1%), 184 (10.6%), 131 (38.3%), 99 (29.8%), 84 (23%), 57 (100%).</p>	 <p>(4a)</p>
<p><i>IR</i> (KBr, <math>\text{cm}^{-1}</math>): 3305-3199 (OH and NH st), 2960 (CH aliphatic), 1704 (C=O acid), 1635, 1550 (C=O stretching, amide I and N-H bending, amide II).</p> <p><i>MS</i> (<math>m/z</math> %): 230.14 (<math>M^+</math>+1, 100%), 187 (27%), 158 (17%), 140 (10%), 57 (19%).</p>	 <p>(4b)</p>
<p><i>IR</i> (KBr, <math>\text{cm}^{-1}</math>): 3303(NH st), 2956 (CH aliphatic), 1730(C=O Ester), 1639, 1550 (C=O stretching amide I and N-H bending, amide II).</p> <p><i>MS</i> (<math>m/z</math> %): 301 (<math>M^+</math>+1, 100%), 283(6%), 258 (23%), 229.2 (18%), 184 (16%), 157.2 (18%), 57 (37%).</p>	 <p>(5a)</p>
<p><i>IR</i> (KBr, <math>\text{cm}^{-1}</math>): 3289(NH st), 2958(CH aliphatic), 1748(C=O ester), 1642, 1553 (C=O stretching amide I and N-H bending, amide II).</p> <p><i>MS</i> (<math>m/z</math> %): 314 (<math>M^+</math>, 2.8%), 272 (9.1%), 243 (13.6%), 170 (19%), 158 (31%), 127 (11.8%), 86(46%), 57 (100%).</p>	 <p>(5b)</p>
<p><i>IR</i> (KBr, <math>\text{cm}^{-1}</math>): 3303 (NH st), 2958(CH aliphatic), 1653, 1549 (C=O stretching, amide I and N-H, bending, amide II).</p> <p><i><sup>1</sup>H-NMR</i> (<math>\delta</math>, ppm, <math>\text{CDCl}_3</math>): 0.88(<i>t</i>, 6H, 2 <math>\text{CH}_3\text{CH}_2\text{CH}_2</math>), 1.25(<i>m</i>, 4H, 2 <math>\text{CH}_3\text{CH}_2\text{CH}_2</math>), 1.60(<i>m</i>, 4H, 2 <math>\text{CH}_3\text{CH}_2\text{CH}_2</math>), 1.83(<i>m</i>, 2H, <math>\text{NHCH}_2\text{CH}_2\text{CH}_2</math>), 2.14(<i>m</i>, 1H, <math>\text{CHCO}</math>), 2.28 (<i>t</i>, 2H, <math>\text{NHCH}_2\text{CH}_2\text{CH}_2</math>), 3.33(<i>m</i>, 2H, <math>\text{NHCH}_2\text{CH}_2\text{CH}_2</math>), 3.89, 3.90(<i>d</i>, 2H, <math>\text{NHCH}_2\text{CO}</math>), 5.40(<i>s</i>, 2H, <math>\text{CONH}_2</math>, <math>\text{D}_2\text{O}</math> exchangeable), 6.26(<i>br</i>, 1H, <math>\text{CONHCH}_2\text{CH}_2</math>), 6.60(<i>br</i>, 1H, <math>\text{CONHCH}_2\text{CO}</math>).</p> <p><i>MS</i> (<math>m/z</math> %): 286 (<math>M^+</math>, 7%), 270(2%), 227.2 (8%), 184 (16%), 130 (27%), 86.10 (43%), 57 (100%).</p>	 <p>(6a)</p>
<p><i>IR</i> (KBr, <math>\text{cm}^{-1}</math>): 3392(NH st), 2956(CH aliphatic), 1637, 1551 (C=O stretching, amide I and N-H bending, amide II).</p> <p><i><sup>1</sup>H-NMR</i> (<math>\delta</math>, ppm, <math>\text{CDCl}_3</math>): 0.9(<i>t</i>, 6H, 2 <math>\text{CH}_3\text{CH}_2\text{CH}_2</math>), 1.25(<i>m</i>, 4H, 2 <math>\text{CH}_3\text{CH}_2\text{CH}_2</math>), 1.64(<i>m</i>, 4H, 2 <math>\text{CH}_3\text{CH}_2\text{CH}_2</math>), 1.82(<i>m</i>, 2H, <math>\text{NHCH}_2\text{CH}_2\text{CH}_2</math>), 2.02(<i>m</i>, 1H, <math>\text{CHCO}</math>), 2.28 (<i>t</i>, 2H, <math>\text{NHCH}_2\text{CH}_2\text{CH}_2</math>), 3.26(<i>m</i>, 2H, <math>\text{NHCH}_2\text{CH}_2\text{CH}_2</math>), 3.97(<i>d</i>, 2H, <math>\text{NHCH}_2\text{CO}</math>), 5.88 (<i>s</i>, 2H, <math>\text{CONH}_2</math>), 6.66(<i>br</i>, 1H, <math>\text{CONHCH}_2\text{CH}_2</math>), 7.12(<i>br</i>, 1H, <math>\text{CONHCH}_2\text{CO}</math>).</p> <p><i>MS</i> (<math>m/z</math> %): 286 (<math>M^+</math>, 99%), 243 (41%), 214(48%), 184 (4%), 168.1 (13%), 140 (48%), 98.97 (35%), 57(100%).</p>	 <p>(6b)</p>

### Anticonvulsant Screening

The assays were realized in the Pharmacology Unit, NRC, Cairo, Egypt.

#### *Animals*

The experimental work was based on male albino mice. Animals, weighing ~20gm. each, were obtained from the "Animal House" (National Research Centre Cairo, Egypt) and were kept under hygienic conditions, during which they were fed on standard pellet chow and water was provided *ad-lib*.

#### *Statistical analysis of results*

*Valproic acid* and dimethylsulfoxide (DMSO) were considered as the reference drug and negative control, respectively.

The obtained data were statistically analyzed *vis-à-vis* the control then valproic acid, as therein indicated. The "Least Significant Difference Test (LSD, SPSS statistics, 17.0" software, IBM®) was statistically manipulated.

Maximal electroshock seizure test (MES).

### Material and Methods

The test was, principally, realized according to Krall, *et al.*<sup>(17)</sup> and Kitona *et al.*<sup>(18)</sup>. A candidate under investigation was *p.o.* given to a group of six mice (10 mg/kg). One hour later, an increased electric current (starting from 1 mA) was applied *via* an ear electrode, for 0.2 seconds and at 50 Hz (Ugo Basil, ECT Unit, 57800).

Tonic extension of the hind limbs is taken as the end point<sup>(19)</sup>. The mean convulsion threshold was considered as the mean of the maximal electric current which could be tolerated by the animal at the end point.

#### *Results*

Results of the MES test were expressed in *mili-Ampere* of the mean convulsion threshold for each candidate. The obtained values represent a mean of that of six mice ( $\pm$  standard error).

#### *Statistical analysis of the results*

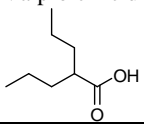
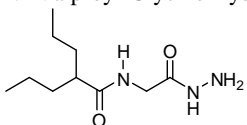
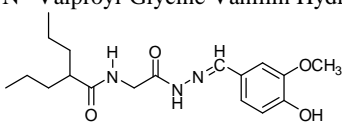
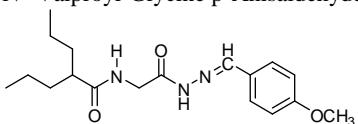
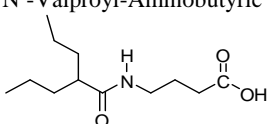
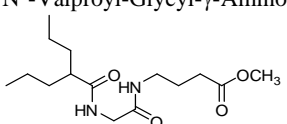
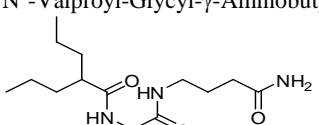
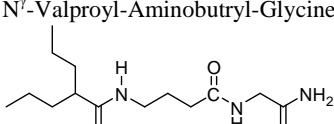
##### *1- LSD test (vis-à-vis control)*

LSD test was applied to determine the significant activity, considering  $P < 0.05$ , while compared to the control. All tested compounds were found statistically significant at (10 mg/kg), except *N*'-valproyl-aminobutryl-glycine amide (6b).

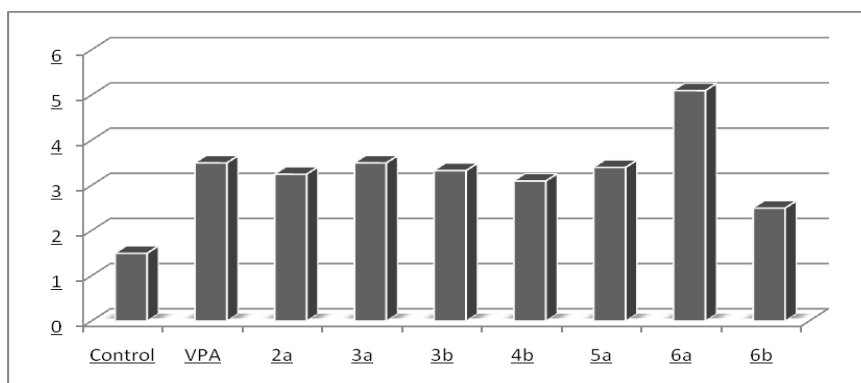
##### *2- LSD test (vis-à-vis valproic acid)*

Compound (6a) was found statistically significant, compared to valproic acid ( $P \leq 0.05$ ).

TABLE 4. Anticonvulsant potency of the tested candidates, (MES test).

Compound	Mean convulsion Threshold (mA $\pm$ SE)
Control	1.5 $\pm$ 0.34
Valproic Acid (Reference drug) 	3.50 $\pm$ 0.15
N <sup>α</sup> -Valproyl-Glycine Hydrazide (2a) 	3.25 $\pm$ 0.16
N <sup>α</sup> -Valproyl-Glycine Vanillin Hydrazone (3a) 	3.50 $\pm$ 0.22
N <sup>α</sup> -Valproyl-Glycine p-Anisaldehyde Hydrazone (3b) 	3.33 $\pm$ 0.21
N <sup>γ</sup> -Valproyl-Aminobutyric Acid (4b) 	3.1 $\pm$ 0.47
N <sup>α</sup> -Valproyl-Glycyl-γ-Aminobutyric Methyl Ester (5a) 	3.4 $\pm$ 0.22
N <sup>α</sup> -Valproyl-Glycyl-γ-Aminobutyric Amide (6a) 	5.1 $\pm$ 0.38
N <sup>γ</sup> -Valproyl-Aminobutryl-Glycine Amide (6b) 	2.5 $\pm$ 0.22

Principally, the maximal electroshock seizure test (MES), as a mean of electro-shock inducing convulsions, reflecting the generalized tonic-clonic (grand mal) type of epileptic seizures, was adopted<sup>(20)</sup>. The method was reported as, probably, the best validated for evaluating of antiepileptic drugs<sup>(18,19)</sup>.



**Fig. 1. Anticonvulsant potency of the synthesized compounds, valproic acid and control.**

From the molecular structural point of view, the least active dipeptide candidate  $N^{\alpha}$ -valproyl-aminobutryl-glycine amide (6b), however, the alteration of the amino acid positions in its peptide sequence, to be  $N^{\alpha}$ -valproyl-glycyl- $\gamma$ -aminobutyric amide (6a), was associated with an enhanced activity.

### Conclusions and Perspectives

The starting conceptual hypothesis of the probable amelioration of the biological activity, namely, anticonvulsant potency upon conjugation with rationalized amino acids or peptides derivatives, seemed herein, explicitly validated.

The novel amino acid dipeptide conjugate of the neuroinhibitor amino acids glycine and  $\gamma$ -aminobutyric acid (6a) proved as statistically significant anticonvulsants and more potent than the parent drug valproic acid, in the protection of the generalized tonic-clonic seizures (grand mal) animal model (MES), of epileptic convulsions.

The realized results could be, promisingly, extrapolated to encounter additional rationalized amino acid and peptide structural variations, as well as more profound pharmacological, namely, toxicological and pharmacokinetic investigations.

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## تشبيد ودراسة مشتقات الأحماض الأمينية وثنائي البيبتيدات لحامض 2- بروبييل بنتانويك (فالبرويك) كمضادات حديثة للتشنجات

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يهدف البحث إلى دراسة فرض إمكانية تحسين فاعلية المواد النشطة بيولوجيا بصفة عامة والعقاقير بصفة خاصة عن طريق تصميم وتشبيد مشتقات جديدة ومختارة لها مع الأحماض الأمينية والبيبتيدات ، وتطبيقا على حمض الفالبرويك ، كعقار متناول مضاد للتشنجات الصرعية .

ويعد حامض "الفالبرويك" (VPA) أحد الأدوية الأساسية في هذا المجال ، على الرغم ان استخدام هذا العقار يرتبط ببعض الآثار الجانبية التي لا يمكن إغفالها ، ومنها زيادة احتمالات حدوث السمية الكبدية والتسبب في إحداث التشوهات الجنينية ، مما مازال يحفز البحث عن بدائل جديدة أكثر فاعلية وأقل سمية ، مع اتساع مجال فاعليتها لتشمل الأنماط المتعددة للتشنجات الصرعية .

وانطلاقا من ذلك فقد بدأ مجديا، وكهدف لذلك البحث اقتراح التصميم البنائي ثم التشبيد الكيميائي لبعض مشتقات الأحماض الأمينية والبيبتيدات المدروسة لحامض الفالبرويك، وذلك لتقييم فاعليتها المضادة للتشنجات مقارنة بحامض " الفالبرويك" ذاته.

وتحديدا لما أجمل فيما سلف ، فقد تم التشبيد الكيميائي لمركبات جديدة وهي : مشتقات لحامض الفالبرويك مع الحمض الأميني جليسين والحمض الأميني "جبا". وبالإخص قد وجد عمليا ان مشتق حامض الفالبرويك مع اميد ثنائي البيبتيدات جليسين جابا(βα) له فاعلية مضادة للتشنجات وقد أظهر وقاية مؤكده مضادة للتشنجات المحدثة كهربائيا مع الجرعة المستخدمة .

وأخير يتضح أن الافتراض المبني بإمكان تحسين الفاعلية البيولوجية المضادة للتشنجات نتيجة ارتباطها المدروس ببعض الأحماض الأمينية والبيبتيدات قد تم إثباته عمليا من خلال هذا البحث وتطبيقا على حمض الفالبرويك .