

Synthesis, Anticonvulsant Activity and Cytotoxicity of Novel Valproic Acid Derivatives

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Abstract:

Objective: The aim of this work was to construct novel hydrazones and thiosemicarbazide derivatives of valproic acid. The new targets will be evaluated for their anticonvulsant activity and cytotoxicity effects.

Methods: Targets **7a-k**, **10**, **11** were synthesized starting from valproic acid using benzotriazole activation and hydrazide and thiosemicarbazide chemistry. The anticonvulsant activity was evaluated by pentylenetetrazole-induced seizures modes using sodium valproate as a standard for comparison of the activity. The compounds with high anticonvulsant activity were subsequently examined for cytotoxicity against HepG2 by MTT assay.

Results: The new targets were characterized using ¹HNMR and ¹³CNMR and their purity were authenticated by elemental analysis. Four compounds **7e**, **7j**, **10** and **11** exhibited the most potent anticonvulsant activity associated with low cytotoxicity.

Conclusion: Compounds **11** exhibited a moderate anticonvulsant activity and a significantly lower cytotoxicity than valproic acid and 5-fluorouracil suggesting that it could be used as a lead for the development of better anticonvulsant drug candidates.

Keywords: Hydrazones, 1,3,4-thiadiazole, 1,2,4-triazole, anticonvulsant and anticancer

I. Introduction

Epilepsy is a complex neurological disorder affecting about 50 million people of all ages, races, and social classes [1-3]. The life long consumption of antiepileptic drugs and their diverse side predisposes the risk of drug-drug interaction [4,5].

Valproic acid (VPA) is a leading antiepileptic drug for the treatment of various types of epileptic seizures especially generalized seizures. One of its main advantages over benzodiazepines is the lack of sedative effect. However, in spite of the broad spectrum antiepileptic activity, the clinical use of VPA, is restricted by serious side effects like teratogenicity and hepatotoxicity [6].

Valproic acid is used as lead compound for developing new improved agents with potential anticonvulsant activity such as propylisopropyl acetamide (PID), valroceamide (VGD) and valnoctamide (VCD) (Figure 1) [7,8].

Figure 1: Structure of valproic acid and its analogues.

Hydrazide-hydrazones derivatives enjoy various pharmaceutical activities e.g. antibacterial, antifungal, antimicrobial, anticancer and anticonvulsant activity [9,10]. Therefore, a set of valproic acid analogues containing heteroatomic system were synthesized and screened for their anticonvulsant activity using pentylenetetrazole (PTZ)-induced seizures model. The cytotoxicity of compounds **3**, **7e**, **7j**, **10** and **11** was assessed by measuring their effect on the viability HepG2 cell lines were evaluated. The effect of the compounds on the morphology of treated hepatocellular carcinoma cells was also investigated using the light microscope.

II. Materials And Methods

Sodium valproate, all reagents and solvents were purchased from commercial sources. A Fisher melting apparatus was used for determination of "uncorrected" melting points. ¹H NMR (400 MHz) and ¹³C NMR (100 MHz) spectra were recorded on Bruker a 400 MHz NMR spectrometer and using DMSO-*d*₆ as solvent, at Faculty of Science, Zagazig University. and Bruker 300 MHz NMR spectrometer, at Ministry of Defense, Chemical war Department, Cairo. The chemical shift (δ) are measured in ppm, and coupling constants (*J*) are given in Hz. Elemental analyses were performed on Carlo Erba-1106 instrument, at the regional center for mycology & biotechnology, Al-Azhar University.

Chemistry

Procedure for Synthesis of N-(valproyl)benzotriazole(3)

A mixture of Thionyl chloride (0.2 mL, 1 equiv) and 1*H*-benzotriazole (1.4 g, 4 equiv) was stirred for 30 min in methylene chloride at 25 °C. Valproic acid (0.5 g, 3 mmol, 1 equiv) was then added and stirring was continued for 2.5 h at room temperature. CH₂Cl₂ (50 mL) was added followed by water (100 mL). After transfer of the two layers to a separating funnel, the organic layer was washed with saturated Na₂CO₃ (20 mL, 3 x), H₂O (20 mL, 2 x), and brine (10 mL, 1 x) and dried over anhydrous Na₂SO₄. Evaporation of the methylene chloride under reduced pressure afforded compound **3**.

Pal yellow Oil; yield: 0.65 g, 88 %; ¹H NMR (300 MHz, DMSO-*d*₆): δ 0.78 (t, *J* = 7.4 Hz, 6H), 1.32–1.15 (m, 4H), 1.66–1.48 (m, 2H), 1.85–1.66 (m, 2H), 4.08–3.92 (m, 1H), 7.55 (t, *J* = 7.7 Hz, 1H), 7.72 (t, *J* = 7.7 Hz, 1H), 8.18 (d, *J* = 8.4 Hz, 1H), 8.22 (d, *J* = 8.4 Hz, 1H, Ar-H); ¹³C NMR (75 MHz, DMSO-*d*₆): δ : 13.5, 19.8, 33.8, 43.3, 113.9, 119.8, 126.2, 130.4, 145.6, 175.3; Anal. Calcd for C₁₄H₁₉N₃O: C, 68.54; H, 7.81; N, 17.13. Found: C, 68.78; H, 7.87; N, 17.22.

Procedure for synthesis of 2-Propylpentanehydrazide (5) [11]

Hydrazine monohydrate (0.50 mL, 10 mmol) was added to a solution of **3** (0.49 g, 2 mmol) in diethylether and the mixture was stirred for 30 min at 25 °C. Diethylether was evaporated under reduced pressure and the residue was dissolved in CH₂Cl₂ (50 mL) then, washed with saturated Na₂CO₃ (20 mL, 3x), H₂O (20 mL, 2x), and brine (10 mL, 1x). Methylene chloride layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure to afford compound **5**.

White microcrystals; yield: 0.28 g, 87%; m.p: 122–123 °C [lit m.p 123–124 °C]; ¹H NMR (400 Hz, DMSO-*d*₆): δ : 0.82 (t, *J* = 8 Hz, 6H), 1.10–1.29 (m, 6H), 1.35 – 1.50 (m, 2H), 2.01–2.11 (m, 1H), 4.15 (s, 2H), 8.94 (s, 1H); ¹³C NMR (75 MHz, DMSO) δ : 13.9, 20.1, 34.7, 43.2, 174.2; Anal. Calcd. for C₈H₁₈N₂O: C, 60.72; H, 11.47; N, 17.70. Found: C, 60.97; H, 11.52; N, 17.83.

General procedures for synthesis of hydrazones (7a-k)

To a solution of the hydrazide **5** (0.5 gm, 3.2 mmol) in ethanol (20 mL), the appropriate aldehyde (3.2 mmol) and acetic acid (two drops) were added. The reaction was refluxed for 2 hour. Then it was cooled and quenched with ice. The separated solid was filtered, and recrystallized from ethanol.

N'-(4-Nitrobenzylidene)-2-propylpentanehydrazide (7a)

White microcrystals; yield: 0.3 g, 90%; m.p: 133–135 °C; ¹H NMR (400 Hz, DMSO-*d*₆): δ Isomer A (53.9 %): 0.82–0.91 (m, 6H), 1.19–1.29 (m, 4H), 1.32–1.43 (m, 2H), 1.49–1.63 (m, 2H), 3.43 – 3.49 (m, 1H), 8.25–8.30 (m, 4H), 8.32 (s, 1H), 11.65 (s, 1H). Isomer B (46.1 %): 0.82–0.91 (m, 6H), 1.19–1.29 (m, 4H), 1.32–1.43 (m, 2H), 1.49–1.63 (m, 2H), 2.27–2.32 (m, 1H), 7.88–7.96 (m, 4H), 8.09 (s, 1H), 11.56 (s, 1H); ¹³C NMR (75 MHz, DMSO-*d*₆): δ : 14.4, 20.6, 34.7, 35.1, 44.6, 124.4, 124.6, 127.9, 128.3, 141.2, 144.1, 172.5, 178.0; Anal. Calcd. for C₁₅H₂₁N₃O₃: C, 61.84; H, 7.27; N, 14.42. Found: C, 62.02; H, 7.36; N, 14.59.

N'-(4-Chlorobenzylidene)-2-propylpentanehydrazide (7b)[11]

White microcrystals; yield: 0.31 g, 87%; m.p: 153–155 °C [lit. m.p 155 °C]; ¹H NMR (400 Hz, DMSO-*d*₆): δ Isomer A (57.6 %) 0.82–0.87 (m, 6H), 1.20–1.28 (m, 4H), 1.29–1.43 (m, 2H), 1.44–1.63 (m, 2H), 2.20–2.29 (m, 1H), 7.45–7.53 (m, 2H), 7.62–7.73 (m, 2H), 7.98 (s, 1H), 11.31 (s, 1H). Isomer B (42.4 %) 0.82–0.87

(m, 6H), 1.20–1.28 (m, 4H), 1.29–1.43 (m, 2H), 1.44–1.63 (m, 2H), 3.39–3.48 (m, 1H), 7.45–7.53 (m, 2H), 7.62–7.73 (m, 2H), 8.21 (s, 1H), 11.40 (s, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆): δ: 13.9, 20.2, 34.3, 34.6, 44.1, 128.1, 128.6, 128.8, 128.9, 133, 141.0, 144.8, 171.7; Anal. Calcd. for C₁₅H₂₁ClN₂O: C, 64.16; H, 7.54; N, 9.98. Found: C, 64.37; H, 7.51; N, 10.13.

***N'*-(4-Fluorobenzylidene)-2-propylpentanehydrazide (7c)**

White microcrystals; yield: 0.3 g, 89%; m.p: 142–144 °C; ¹H NMR (400Hz, DMSO-*d*₆): δ Isomer A (55.5 %): 0.81–0.92(m, 6H), 1.19–1.29(m, 4H), 1.30–1.41 (m, 2H), 1.46–1.63(m, 2H), 3.41–3.47 (m, 1H), 7.27(t, *J* = 8 Hz, 2H), 7.65–7.77(m, 2H), 8.21(s, 1H), 11.34(s, 1H). Isomer B (45.5%): 0.81–0.92(m, 6H), 1.19–1.29(m, 4H), 1.30–1.41 (m, 2H), 1.46–1.63(m, 2H), 2.21–2.29(m, 1H), 7.27(t, *J* = 8 Hz, 2H), 7.65–7.77(m, 2H), 7.98(s, 1H), 11.25(s, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 14.4, 20.6, 20.7, 34.7, 35.1, 44.5, 116.1, 116.4, 129.1, 129.5, 129.6, 145.4, 172.0, 177.6; Anal. Calcd. for C₁₅H₂₁FN₂O: C, 68.16; H, 8.01; N, 10.60. Found: C, 68.42; H, 8.09; N, 10.87.

***N'*-(4-Methoxybenzylidene)-2-propylpentanehydrazide (7d)[11]**

White microcrystals; yield: 0.32 g, 91%; m.p: 147–149 °C, [lit. m.p 110–112°C]; ¹H NMR (400 Hz, DMSO-*d*₆): δ: Isomer A (55.8%): δ 0.83 – 0.89 (m, 6H), 1.21–1.29 (m, 4H), 1.31–1.42 (m, 2H), 1.47–1.62 (m, 2H) 2.20–2.26 (m, 1H), 3.80 (d, *J* = 4 Hz, 3H), 6.97–7.02 (m, 4H), 7.93 (s, 1H), 11.07 (m, 1H). Isomer B (44.2%): δ 0.83–0.89 (m, 6H), 1.21–1.29 (m, 4H), 1.31–1.42 (m, 2H), 1.47–1.62 (m, 2H), 3.40–3.47 (m, 1H), 3.80 (d, *J* = 4 Hz, 3H), 7.54–7.63 (m, 4H), 8.16 (s, 1H), 11.17 (m, 1H); ¹³C NMR (75 MHz, DMSO-*d*₆): δ 13.9, 20.0, 34.2, 34.7, 44.0, 55.2, 114.2, 114.3, 126.7, 128.0, 128.5, 145.9, 160.6, 171.2, 176.8; Anal. Calcd for C₁₆H₂₄N₂O₂: C, 69.53; H, 8.75; N, 10.14. Found: C, 69.76; H, 8.89; N, 10.36.

***N'*-(2,5-Dimethoxybenzylidene)-2-propylpentanehydrazide (7e)**

White microcrystals; yield: 0.35 g, 90%; m.p: 154–156 °C; ¹H NMR (400 Hz, DMSO-*d*₆): δ Isomer A (54.3%) 0.86 (t, *J*=8 Hz, 6H), 1.18–1.27(m, 4H), 1.30–1.39 (m, 2H), 1.47–1.61(m, 2H), 2.19–2.25(m, 1H), 3.71 (s, 3H), 3.74 (s, 3H), 6.95–6.99 (m, 1H), 7.01–7.06 (m, 1H), 7.26–7.30 (m, 1H), 8.27 (s, 1H) 11.22 (s, 1H); Isomer B (45.7%) 0.86 (t, *J*=8 Hz, 6H) 1.18–1.27(m, 4H), 1.30–1.39 (m, 2H), 1.47–1.61(m, 2H) 3.39–3.46(m, 1H), 3.78 (s, 3H), 3.80 (s, 3H), 6.95–6.99 (m, 1H), 7.01–7.06 (m, 1H), 7.26–7.30 (m, 1H), 8.53 (s, 1H), 11.36 (s, 1H); ¹³C NMR (75 MHz, DMSO-*d*₆): δ 14.1, 20.1, 34.1, 34.7, 44.1, 55.3, 56.1, 108.8, 113.3, 123.0, 141.4, 152.0, 153.1, 171.5, 177.1 (CO); Anal. Calcd for C₁₇H₂₆N₂O₃: C, 66.64; H, 8.55; N, 9.14. Found: C, 66.87; H, 8.61; N, 9.21.

2-Propyl-*N'*-(3,4,5-trimethoxybenzylidene)pentanehydrazide (7f)

White microcrystals; yield: 0.38 g, 89%; m.p: 162–164 °C; ¹H NMR (400 Hz, DMSO-*d*₆): δ Isomer A (60.4%) 0.84–0.89 (m, 6H), 1.18–1.30 (m, 4H), 1.31–1.40 (m, 2H), 1.47–1.64 (m, 2H), 2.22–2.23 (m, 1H), 3.69 (d, *J* = 4 Hz, 3H), 3.81(d, *J* = 8 Hz, 6H), 6.96 (d, *J* = 8 Hz, 2H), 7.90 (s, 1H), 11.27 (s, 1H); Isomer B (39.6%) 0.84–0.89 (m, 6H), 1.18–1.30 (m, 4H), 1.31–1.40 (m, 2H), 1.47–1.64 (m, 2H), 3.38–3.45 (m, 1H), 3.69 (d, *J* = 4 Hz, 3H), 3.81(d, *J* = 8 Hz, 6H), 6.96 (d, *J* = 8 Hz, 2H), 8.15 (s, 1H), 11.31 (s, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 13.9, 20.1, 34.1, 34.7, 44.1, 55.8, 55.9, 60.1, 103.7, 104.1, 129.9, 139.0, 146.2, 152.9, 171.5, 177.3; Anal. Calcd. for C₁₈H₂₈N₂O₄: C, 64.26; H, 8.39; N, 8.33. Found: C, 64.39; H, 8.44; N, 8.47.

***N'*-(4-Hydroxybenzylidene)-2-propylpentanehydrazide (7g)[11]**

White microcrystals; yield: 0.3 g, 90%; m.p: 185– 187 °C [lit. m.p 190–192 °C]; ¹H NMR (400 Hz, DMSO-*d*₆): δ Isomer A (50.1%) 0.80–0.92(m, 6H), 1.18–1.28(m, 4H), 1.29–1.40(m, 2H), 1.45–1.61(m, 2H), 2.19–2.24(m, 1H), 6.78–6.83(m, 4H), 7.88 (s, 1, CH), 9.83 (s, 1H), 11.02(s, 1H); Isomer B (49.9 %) 0.80–0.92(m, 6H), 1.18–1.28(m, 4H), 1.29–1.40(m, 2H), 1.45–1.61(m, 2H), 3.38–3.46(m, 1H), 7.43–7.51(m, 4H), 7.88 (s, 1H), 9.87(s, 1H), 11.12(s, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 14.0, 20.1, 20.2, 34.3, 34.8, 44.1, 115.6, 115.7, 125.3, 128.2, 128.7, 146.4, 159.2, 171.2, 177.1; Anal. Calcd. for C₁₅H₂₂N₂O₂: C, 68.67; H, 8.45; N, 10.68. Found: C, 68.93; H, 8.62; N, 10.82.

***N'*-(4-Methylbenzylidene)-2-propylpentanehydrazide (7h)**

White microcrystals; yield: 0.29 g, 88%; m.p: 132–134 °C; ¹H NMR (400 Hz, DMSO-*d*₆): δ Isomer A (51.5%) 0.81–0.90 (m, 6H), 1.18–1.28 (m, 4H), 1.29–1.40 (m, 2H), 1.47–1.61 (m, 2H), 2.21–2.25 (m, 1H), 2.33 (d, *J* = 4 Hz, 3H), 7.21–7.26 (m, 2H), 7.45–7.58 (m, 2H), 7.95 (s, 1H), 11.17 (s, 1H). Isomer B (48.5%): 0.81–0.90 (m, 6H), 1.18–1.28 (m, 4H), 1.29–1.40 (m, 2H), 1.47–1.61 (m, 2H), 2.33 (d, *J* = 4 Hz, 3H), 3.40–3.48 (m, 1H), 7.21–7.26 (m, 2H), 7.45–7.58 (m, 2H), 8.17 (s, 1H), 11.27 (s, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 13.9, 20.1, 20.2, 21.0, 34.3, 34.7, 40.1, 126.5, 126.9, 129.4, 131.4, 139.5, 142.2, 146.1, 171.5, 177.0; Anal. Calcd for C₁₆H₂₄N₂O: C, 73.81; H, 9.29; N, 10.76. Found: C, 73.98; H, 9.32; N, 10.92.

***N'*-(2-(Benzyloxy)benzylidene)-2-propylpentanehydrazide (7i)**

White microcrystals; yield: 0.4 g, 89%; m.p: 180-182 °C; ¹H NMR (400 Hz, DMSO-*d*₆): δ Isomer A (56.7%): 0.81–0.92 (m, 6H), 1.20–1.29 (m, 4H), 1.29–1.43 (m, 2H), 1.46–1.64 (m, 2H), 2.18–2.28 (m, 1H), 5.15 (s, 2H), 7.04–7.11 (m, 2H), 7.31–7.36 (m, 1H), 7.37–7.43 (m, 2H), 7.44–7.46 (m, 2H), 7.55–7.63 (m, 2H), 7.93 (s, 1H), 11.08 (s, 1H); Isomer B (43.3%): 0.81–0.92 (m, 6H), 1.20–1.29 (m, 4H), 1.29–1.43 (m, 2H), 1.46–1.64 (m, 2H), 3.38–3.47 (m, 1H), 5.15 (s, 2H), 7.04–7.11 (m, 2H), 7.31–7.36 (m, 1H), 7.37–7.43 (m, 2H), 7.44–7.46 (m, 2H), 7.55–7.63 (m, 2H), 8.16 (s, 1H), 11.17 (s, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 13.9, 20.1, 20.2, 34.3, 34.7, 44.1, 69.0, 115.0, 115.2, 127.1, 127.3, 127.7, 127.9, 128.0, 128.4, 136.8, 142.1, 145.9, 159.7, 171.3, 176.9; Anal. Calcd for C₂₂H₂₈N₂O₂: C, 74.97; H, 8.01; N, 7.95. Found: C, 75.14; H, 8.13; N, 8.03.

***2*-Propyl-*N'*-(pyridin-4-ylmethylene)pentanehydrazide (7j)**

White microcrystals; yield: 0.28 g, 89%; m.p: 174–176 °C; ¹H NMR (400 Hz, DMSO-*d*₆): δ Isomer A (50.9%): 0.83–0.88 (m, 6H), 1.21–1.28 (m, 4H), 1.31–1.41 (m, 2H), 1.49–1.62 (m, 2H), 3.41–3.51 (m, 1H), 7.57–7.62 (m, 2H), 8.22 (s, 1H), 8.60–8.64 (m, 2H), 11.62 (s, 1H); Isomer B (49.1%): δ 0.83–0.88 (m, 6H), 1.21–1.28 (m, 4H), 1.31–1.41 (m, 2H), 1.49–1.62 (m, 2H), 2.25–2.33 (m, 1H), 7.57–7.62 (m, 2H), 7.97 (s, 1H), 8.60–8.64 (m, 2H), 11.54 (s, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 13.9, 20.1, 20.2, 34.2, 34.6, 44.1, 120.5, 120.9, 139.9, 141.6, 143.8, 150.2, 172.0, 177.6; Anal. Calcd. for C₁₄H₂₁N₃O: C, 67.98; H, 8.56; N, 16.99. Found: C, 68.05; H, 8.64; N, 17.21.

***N'*-(Furan-2-ylmethylene)-2-propylpentanehydrazide (7k)**

White microcrystals; yield: 0.27 g, 90%; m.p: 152-154 °C; ¹H NMR (400 Hz, DMSO-*d*₆): δ Isomer A (58.1%): 0.82–0.89 (m, 6H), 1.18–1.28 (m, 4H), 1.29–1.42 (m, 2H), 1.44–1.62 (m, 2H), 2.20–2.30 (m, 1H), 7.47–7.51 (m, 3H), 7.98 (s, 1H), 11.31 (m, 1H); Isomer B (41.9%): 0.82–0.89 (m, 6H), 1.18–1.28 (m, 4H), 1.29–1.42 (m, 2H), 1.44–1.62 (m, 2H), 3.39–3.48 (m, 1H), 7.63–7.72 (m, 3H), 8.21 (s, 1H), 11.40 (s, 1H); ¹³C NMR (75 MHz, DMSO-*d*₆): δ 13.9, 13.9, 20.0, 20.1, 34.1, 34.6, 44.1, 112.0, 112.8, 136.1, 144.8, 149.6, 171.5, 177.1. Anal. Calcd for C₁₃H₂₀N₂O₂: C, 66.07; H, 8.53; N, 11.85. Found: C, 66.21; H, 8.61; N, 12.01.

Procedure for preparation of N-phenyl-2-(2-propylpentanoyl)hydrazine-1-carbothioamide (9)

A mixture of **5** (0.5 gm, 3.2 mmol) in ethanol (10 mL) and phenyl isothiocyanate (3.2 mmol) was refluxed for 15 hours. The precipitate was filtered, washed with ethanol and crystallized from ethanol/DMF (4:1). White microcrystals; yield: 0.35 g, 94%; m.p: 153–155 °C; ¹H NMR (300 Hz, DMSO-*d*₆): δ 0.85 (t, *J* = 6 Hz, 6H), 1.26–1.33 (m, 6H), 1.46–1.55 (m, 2H), 2.22–2.30 (m, 1H), 7.14 (t, *J* = 6 Hz, 1H), 7.33 (t, *J* = 6 Hz, 2H), 7.48 (d, *J* = 6 Hz, 3H), 9.58 (s, 1H), 9.89 (s, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 14.1, 20.0, 34.2, 42.9, 124.9, 128.3, 139.1, 174.4, 181.4; Anal. Calcd for C₁₅H₂₃N₃OS: C, 61.40; H, 7.90; N, 14.32. Found: C, 61.58; H, 7.96; N, 14.50.

Procedure for preparation of 3-mercapto-4-phenyl-5-[1-(1-propyl)-1-butyl]-4H-1,2,4-triazole (10)

A mixture of **9** (0.5 gm, 1.7 mmol) in ethanol (10 mL) and sodium hydroxide (1.7 mmol) was refluxed for 6 hours. The solution is neutralized using glacial acetic acid and the precipitate was filtered, washed with water and crystallized from Chloroform/Petroleum ether (4:1).

White microcrystals; yield: 0.31 g, 81%; m.p: 163-165 °C; ¹H NMR (300 Hz, DMSO-*d*₆): δ 0.71 (t, *J* = 6 Hz, 6H), 1.08–1.20 (m, 4H), 1.33–1.44 (m, 2H), 1.46–1.60 (m, 2H), 2.37–2.46 (m, 1H), 7.30–7.35 (m, 2H), 7.52–7.63 (m, 3H), 13.74 (s, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 13.7, 19.6, 34.8, 35.3, 128.7, 129.6, 129.6, 133.7, 155.2, 167.5; Anal. Calcd for C₁₅H₂₁N₃S: C, 65.41; H, 7.69; N, 15.26; Found: C, 65.57; H, 7.59; N, 14.98.

Procedure for preparation of 2-Phenylamino-5-[1-(1-propyl)-1-butyl]-1,3,4-thiadiazole (11)

Compound **9** (0.5 gm, 1.7 mmol) was added to an ice cold sulphuric acid (5 mL) portionwise while stirring. The mixture was stirred for 24 hours then, poured on ice and neutralized with 10 M sodium carbonate. The formed solid was filtered, washed with water and crystallized from Chloroform/Petroleum ether (4:1).

White microcrystals; yield: 0.35 g, 91%; m.p: 167–169 °C; ¹H NMR (300 Hz, DMSO-*d*₆): δ 0.86 (t, *J* = 6 Hz, 6H), 1.19–1.28 (m, 4H), 1.52–1.65 (m, 4H), 2.99–3.08 (m, 1H), 7.49–7.56 (m, 5H), 10.31 (s, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 13.8, 19.9, 37.3, 40.3, 116.5, 126.6, 140.9, 141.4, 163.7, 164.8; Anal. Calcd. for C₁₅H₂₁N₃S: C, 65.41; H, 7.69; N, 15.26; Found: C, 65.70; H, 7.40; N, 15.11.

Anticonvulsant activity

Adult male white Swiss albino mice weighing 20–25 g (10–12 weeks old) were obtained from Experimental Animal Care Centre, National Research Centre, Dokki, Giza, Egypt. The animals were maintained

under standard conditions of humidity, temperature (25 ± 2 °C) and light (12 h light/12 h dark). They were fed with a standard mice pellet diet and had free access to water. Each treatment group and vehicle control group consisted of six animals. The anticonvulsant activity of compounds **3**, **5**, **7a-k**, **10** and **11** was evaluated by pentylenetetrazole (PTZ) models (12). The test compounds were dissolved 10% DMSO Solution of compounds **3**, **5**, **7a-k**, **10** and **11** was injected intraperitoneally (i.p.) at dose of 0.5 mmol/kg 30 minutes before seizures induction. Sodium valproate (300 mg/kg) was used as reference drugs. The PTZ test was carried out as follows: (i) i.p. injection of PTZ (100 mg/kg), (ii) seizures and tonic-clonic convulsions, hypnosis and death were recorded. In order to determine their protective and therapeutic indexes, a range of i.p. doses of one tested compounds were given to each group of 6 mice until at least four points were established in the range of 10-90% seizure protection or minimal observed neurotoxicity. The respective ED₅₀ and TD₅₀ values were calculated. The dose of tested compounds that prevented 50% of the treated animals from PTZ-induced clonic convulsion was calculated (ED₅₀). The animals that showed no convulsion within one hour after PTZ administration were considered to be protected (13).

Cytotoxic activity

Cell Cultures

A human liver cancer cell line (HepG₂), was propagated in RPMI-1640 medium L-Glutamine (Lonza Verviers SPRL, Belgium, cat#12-604F) supplemented with 10% fetal bovine serum (FBS) (Seralab, UK, cat# EU-000-H). The cells were incubated in 5% CO₂ humidified at 37°C for growth.

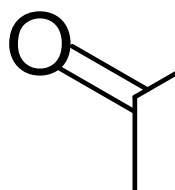
Evaluation of cell proliferation by MTT assay

The number of viable HepG₂ cells after treatment with different concentration of the compounds was evaluated by the MTT (3-[4,5-methylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay as reported previously with slight modification [14]. In brief, after evaluation of cell count and viability by trypan blue dye, HepG₂ cells (1×10^5 cells/well) were seeded in a 96-well plate in triplicate and were allowed to adhere and spread for 24 h. The tested compounds were dissolved in 500 µl Dimethyl sulfoxide (DMSO) to have stock solution of 100 mM, as the final concentration of DMSO in the culture medium never exceeded 0.2% (v/v) [14] and then various concentrations of tested compounds were prepared by further diluting in complete medium to have final concentration of 12.5, 50, 100, 200, and 400 µM. In the next day the medium was replaced with fresh medium with the indicated concentrations of tested compounds and cells were allowed to grow for 48 h. Four hours before completion of incubation, 10 µl of MTT (5 mg/mL in PBS w/o Ca, Mg, Lonza Verviers SPRL Belgium, cat#17-516F) was added in each well. After completing the incubation, 100 µl of Dimethyl sulfoxide (DMSO) was added to each well, then the 96 well plates were centrifuged for 5 minutes at 4000 rpm to precipitate the formazan crystals. Color developed after the reaction was measured at 490 nm using Bio-Tek microplate reader. The experiment was conducted in triplicate. Data were calculated as percent of cell viability by the following formula: % cell viability = (Mean absorbance in test wells / Mean absorbance in control wells) 100. The effect of tested compounds on the morphology of treated hepatocellular carcinoma cells was investigated by the light microscope and then photographed by SONY CYBER-SHORT [15].

III. Results And Discussion

The carboxylic group of valproic acid **1** was activated by treating with benzotriazole **2** in presence of thionylchloride in dichloromethane [16]. The activated valproic acid **3** was treated with hydrazine hydrate **4** in diethyl ether to yield the hydrazide of valproic acid **5**. Compound **5** was condensed with various aryl and heteroaryl aldehydes **6** in presence of acetic acid in ethanol under reflux condition to obtain the corresponding hydrazones **7a-k** as *anti-syn* isomers in good yield [11].

Compound **5** was also condensed with phenyl isothiocyanate in ethanol under reflux condition to afford *N*-phenyl-2-(2-propylpentanoyl)hydrazine-1-carbothioamide **9**, which was further treated with NaOH and H₂SO₄ to yield triazole derivative **10** and thiazazole derivative **11** respectively.



Scheme 1: Synthesis of valproic acid analogues with heteroatomic system **7a-k, 9-11**.

Anticonvulsant activity

Targets **7a-k, 10, 11** were subjected to standard subcutaneous pentetrazol (scPTZ) tests for anticonvulsant activity (12). The results of the scPTZ screening in mice after intraperitoneal administration of the compounds are summarized in Table 2. Compounds **7e, 7j, 10, 11** were proved to be active anticonvulsants at 0.5 mmol/kg. Compounds **7e, 7j, 10, 11** were subjected to further investigations at different doses for the quantification of their anticonvulsant activity (indicated by ED₅₀) in rats (Table 3). Compounds **7e, 7j, 10, 11** were showed anticonvulsant activity against PTZ-induced seizure with ED₅₀ values of 268, 115, 133 and 187 mg/kg, respectively. Sodium valproate was used as reference drug at ED₅₀ value 300 mg/kg. Interestingly, the ED₅₀ value of standard reference drug was found higher than the test compounds at molar doses.

Table 1. Preliminary anticonvulsant activity of the new synthesized compounds (200 mg/ kg) and Sodium valproate (300 mg/kg)

S. No.	Compound	PTZ (% of protection)
1	3	-
2	5	-
3	7a	-
4	7b	-
5	7c	-
6	7d	-
7	7e	50
8	7f	-
9	7g	-
10	7h	-
11	7i	-
12	7j	83
13	7k	-
14	10	83
15	11	67
16	PTZ	-
17	DMSO	-
18	Sodium valproate	100

Table 2. Comparison of the anticonvulsant activity (ED₅₀), median toxic dose (TD₅₀) and therapeutic index of the most promising anticonvulsant new synthesized compounds and sodium valproate in rats

S. NO.	Compound No.	ED ₅₀	TD ₅₀	Protective index
1	Sodium valproate	300	450	1.5
2	7e	268	457	1.71
3	7j	115	346	3.01
4	10	133	371	2.79
5	11	187	419	2.24

The LD₅₀ and protective index of the test compounds were also determined (Table 2). It is noteworthy, that the protective index of the test compounds was found higher as compared to the reference anticonvulsant drug (Sodium valproate) at molar doses (Table 2).

Cytotoxicity studies

The cytotoxicity of all the synthesized compounds was also studied against hepatoma cell line (HepG2) at 12.5, 50, 100, 200, and 400 μM concentrations using MTT assay colorimetric assay (Table 4).²⁴ Data illustrated in (Figure 2) shows the percentage of viability of HepG2 cells after 48 h from treatment with different concentrations of the compounds versus control. The results revealed that the inhibition of HepG2 cells proliferation was in a dose dependent manner, as increasing the concentration of the tested compound lead to increase in cell growth inhibition (Figure 3), which also was confirmed by morphology of the cells (Figure 3a & 3b). The cells underwent dramatic morphological changes, shrunken and the ratio of cytolysis increased after exposure to tested compounds at 200 μM and 400 μM ; where compound **7e** had the lowest IC_{50} value (44 μM), followed by compounds **7j** and **10**, with IC_{50} values equal 81.3, and 172 μM , respectively (Table 4). On the other hand, compounds **3** and **11** showed lower cytotoxicity, with high IC_{50} value > 400 μM compared to 5-Flurouracil (5-FU) (with IC_{50} =188 μM), which used as reference drug.

Table 3: Anticancer activity of valproic acid analogues on HepG₂ cell lines.

S. No	Compound	% Survival in HepG ₂ cells treated with 50 μM of compound	IC_{50} (μM)
1	3	72.60 \pm 0.257 ^a	>400
2	7e	46.86 \pm 0.042	44
3	7j	59.28 \pm 0.133	81.3
4	10	55.82 \pm 0.0322	172
5	11	79.81 \pm 0.0393	>400
6	5 FU	57.27 \pm 0.110	188
7	VPA	83.47 \pm 0.010	50

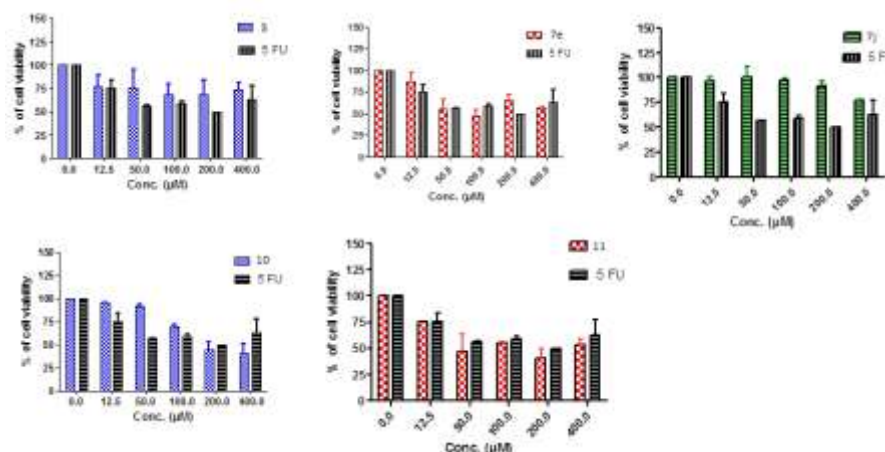


Figure2. Cytotoxic effect of the tested compounds on HepG₂ cells: different concentration of compounds starting from 12,5 to 400 μM were prepared. 1×10^4 cell/well of HepG₂ cells were treated with different concentration of tested compound for 48 h and cytotoxic effect was detected by MTT assay. The figure shows the % of cell growth viability compared to control which were untreated cell.

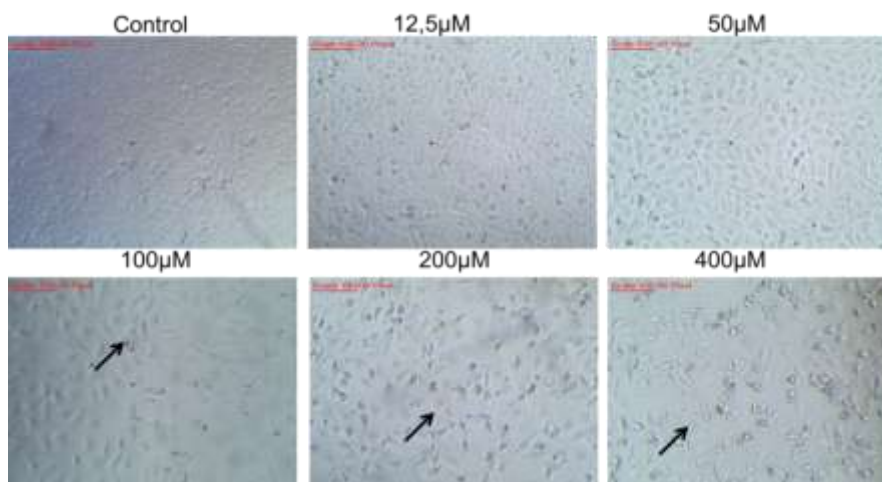


Figure 3a: Effect of 5-FU on HepG₂ cell line growth.

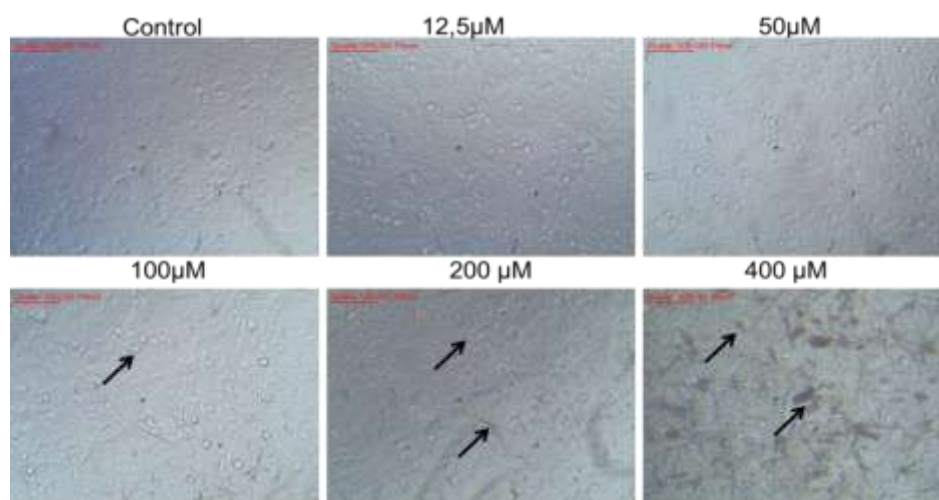


Figure 3b: Effect of 7e on HepG₂ cell line growth.

IV. Conclusion

In conclusion, novel derivatives of valproic acid were constructed and evaluated for their anticonvulsant activity in mice. Four compounds **7e**, **7j**, **10** and **11** showed good anticonvulsant activity. Compound **11** showed anticonvulsant activity comparable to that of valproic acid and exhibited significantly lower cytotoxicity than valproic acid. Thus it could be used as a lead compound for further investigations.

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Author Contributions

pharmacist TaghreedAbdelstarSheha, Dr.Tarek Salah Ibrahimaand Dr. Nader E. Abo-Dyaplanned and executed the chemistry experimental work in addition to reporting data.Dr. Mohamed Tantawy and Dr. Mostafa El-Nagar performed the anticonvulsant screening and MTT assay. Prof.ZakariaKamel Abdel-Samii supervised the research and wrote the paper together with Dr. Nader E. Abo-Dya

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