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Phenytoin-based Bivalent Ligands: Design, Synthesis and Anticonvulsant Activity

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Synthesis, characterization and anticonvulsant properties of new bivalent ligands derived from phenytoin were described. Initial anticonvulsant screening was performed using maximal electroshock (MES) and pentylenetetrazole (PTZ) screens in mice. The neurotoxicity for compounds that showed significant anticonvulsant activity was determined applying the rotorod test. Most of the test compounds were found to be effective in at least one seizure model in a dose of 100 mg/kg. Compound **5e** exhibited marked anticonvulsant activity in both MES and PTZ screens. The computer-aided prediction of biological activity was carried out.

Key words: Phenytoin, Bivalent ligands, Anticonvulsant activity

INTRODUCTION

Epilepsy, one of the most common neurological disorders which results from a temporary electrical disturbance of the brain, affects about 1% of the world's population (Scheurer and Pedley, 1990). It is a syndrome of periodic and unpredictable seizures which is characterized by paroxysmal, excessive, and hyper synchronous discharges of large number of neurons (Löscher, 1998). Despite the development of new anticonvulsants, about one third of patients do not respond well to currently available treatment, even if multiple drugs with complementary activities are used (Upton, 1994; Löscher and Schmidt, 2006). Moreover, their usage is associated with undesirable numerous side effects such as headache, nausea, anorexia, ataxia, hepatotoxicity, drowsiness, gastrointestinal disturbance, gingival hyperplasia, and hirsutism (Greenwood, 2000; Cramer et al., 2010). Therefore, continued search for safer and more effective anticonvulsants is urgently necessary.

In the current context, hybrid molecules and bivalent ligands, which incorporate two pharmacophores connected by an appropriate spacer, have received much attention during recent years as a promising strategy in drug discovery (Wright and Usher, 2001). Such pharmacomolecules have the potential for bridging vicinal binding sites. It was postulated that these ligands would have greater potency as well as increased affinity and selectivity than the individual binding of two monovalent counterparts (Wright and Usher, 2001; George et al., 2002).

To date, only few reports on bivalent ligands designed as anticonvulsants appeared in the literature (Poupaert et al., 1988; Hudkins and DeHaven-Hudkins, 1994). With the aim to improve the pharmacological profile of potential anticonvulsants while minimizing common side effects, phenytoin **I**, a well established antiepileptic drug acting as a voltage-gated sodium channel blocker, was selected as a pharmacophore, to be engaged in the construction of homobivalent and heterobivalent ligands due to its chemical simplicity, selectivity, intrinsic rigidity and high potency against partial and generalized seizures (Lenkowski et al., 2007) (Fig. 1).

SAR studies on the phenytoin derivatives revealed that, beside the pharmacophoric elements essential for the interaction at the binding site (Dimmock et al., 1995), the attachment of second aryl ring, designated as the distal ring to the proximal aryl ring, result in increase the van der Waal's bonding at the binding site, and thus increase the potency of the ligand (Pandeya et al., 2000; Zha et al., 2004). This was substantiated by previous publications, which demonstrated that the aromatic

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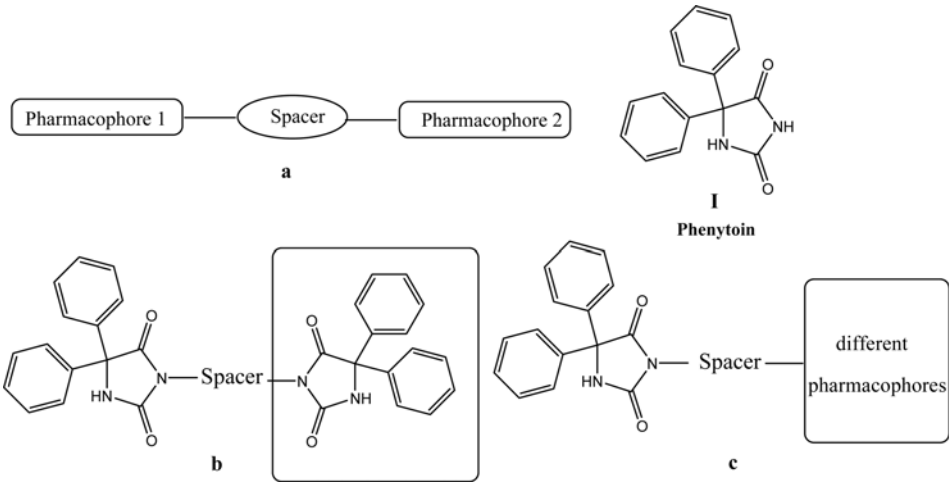


Fig. 1. (**a**) Bivalent ligands; (**b**) Phenytoin in the construction of homobivalent ligand; (**c**) Phenytoin in the construction of heterobivalent ligand.

ring of the tosyl group at *N*-3 in certain hydantoin derivatives, contributes to the sodium channel binding affinity (Obniska et al., 2009) (Fig. 2).

Herein, we report the synthesis of certain symmetric dimeric phenytoins linked through two or three oxyethylene units, compounds **3a**,**b**. The polyethylene glycol spacer has the advantage of being flexible linkage possessing good hydrophilic-hydrophobic properties, which should play an important role in the pharmacokinetic properties of the ligand (Huber et al., 2012).

Furthermore, considering the significant anticonvulsant activity observed by certain heterodimeric phenytoin derivatives (Obniska et al., 2009; Kaminski et al., 2011), we synthesized new phenytoin derivatives **4a**,**b** and **5a**-**j**, where two or three oxyethylene unit spacer connect the phenytoin pharmacophore at one end and another function at the other. These functions include the tosylate group, **4a**,**b**, amine function, arylpiperazine, phthalimide or aminoguanidine, **5a**-**j** (Scheme 1).

Besides, in the design of bivalent ligands, a crucial feature is the position and nature of the attachment point for linking the two pharmacophoric units. In

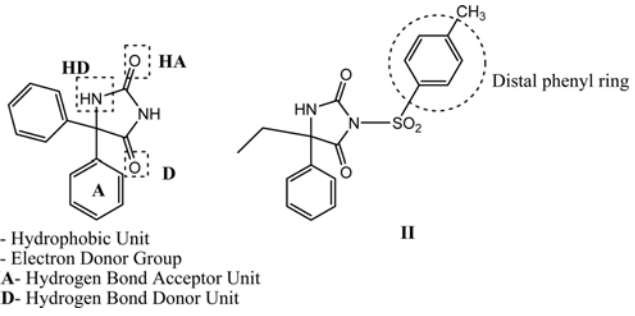


Fig. 2. The required pharmacophoric elements for anticonvulsant agents.

Scheme 2, we synthesized homodimeric phenytoin derivatives **7a**-**c** and **9**, connected through an amide linker and varying methylene unit spacer (n = 0-3).

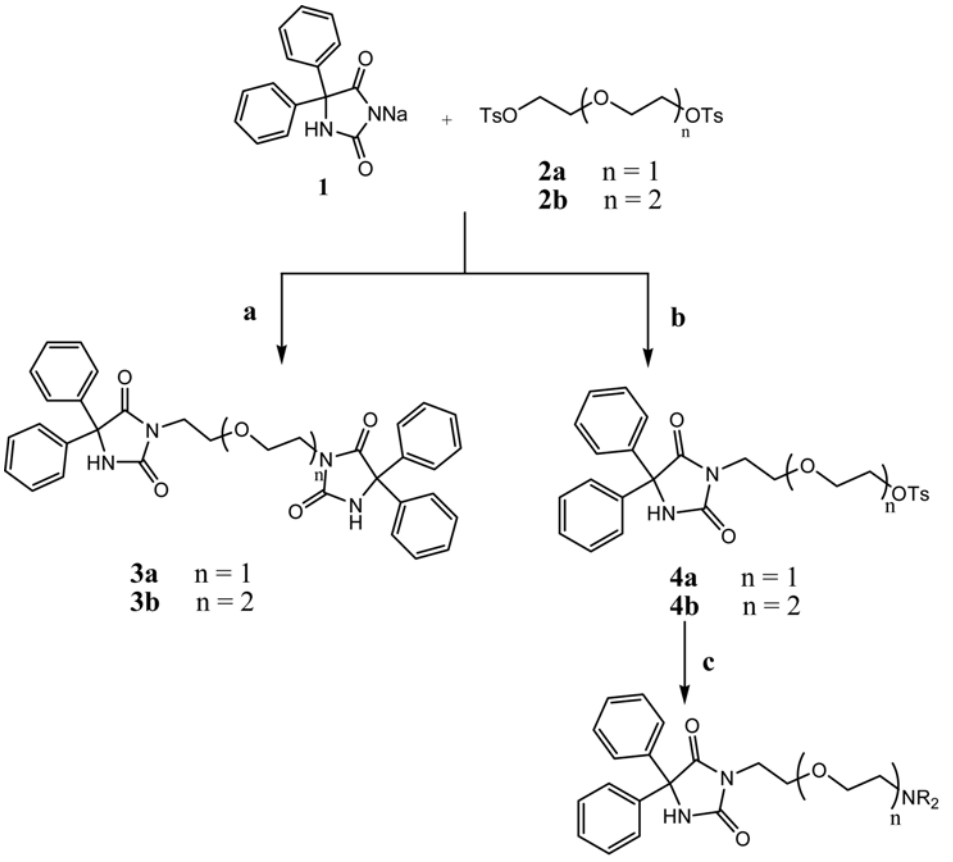
The chemical structures of the new compounds were characterized using IR, ^{1}H-NMR, ^{13}C-NMR, MS and elemental analysis techniques. The anticonvulsant activity was evaluated by using experimental epilepsy models, i.e., maximal electroshock (MES) and subcutaneous pentylenetetrazole (scPTZ) screens in mice. The rotorod assay was performed to evaluate the neurotoxicity of the most active compounds.

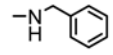
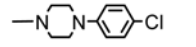
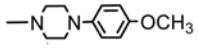
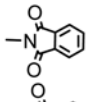
The computer-aided prediction of biological activity in relation to the chemical structure of a compound, including c Log *p* calculation and prediction of pharmacokinetic parameters, is now a commonly used technique in drug discovery (Lipinski et al., 2001).

MATERIALS AND METHODS

Chemistry

All chemicals and reagents were obtained from Aldrich (Sigma-Aldrich), and were used without further purification. Reactions were monitored by TLC, performed on silica gel glass plates containing 60 GF-254, and visualization on TLC was achieved by UV light or iodine indicator. IR spectra were determined on Shimadzu IR 435 spectrophotometer (KBr, cm^{-1}). ^{1}H-NMR spectra were carried out using a Mercury 300-BB 300 MHz using TMS as internal standard. Chemical shifts (δ) were recorded in ppm on δ scale, Micro analytical Center, Cairo University, Egypt. ^{13}C-NMR spectra were carried out using a Mercury 300-BB 300 MHz using TMS as internal standard. Chemical shifts (δ) were recorded in ppm on δ scale, Micro analytical Center, Cairo University, Egypt. Mass spectra were recorded on Shimadzu



5	NR_2	n	5	NR_2	n
a		1	f		2
b		1	g		1
c		1	h		1
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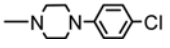
e		1	j	-NHNHC(NH)NH_2	1

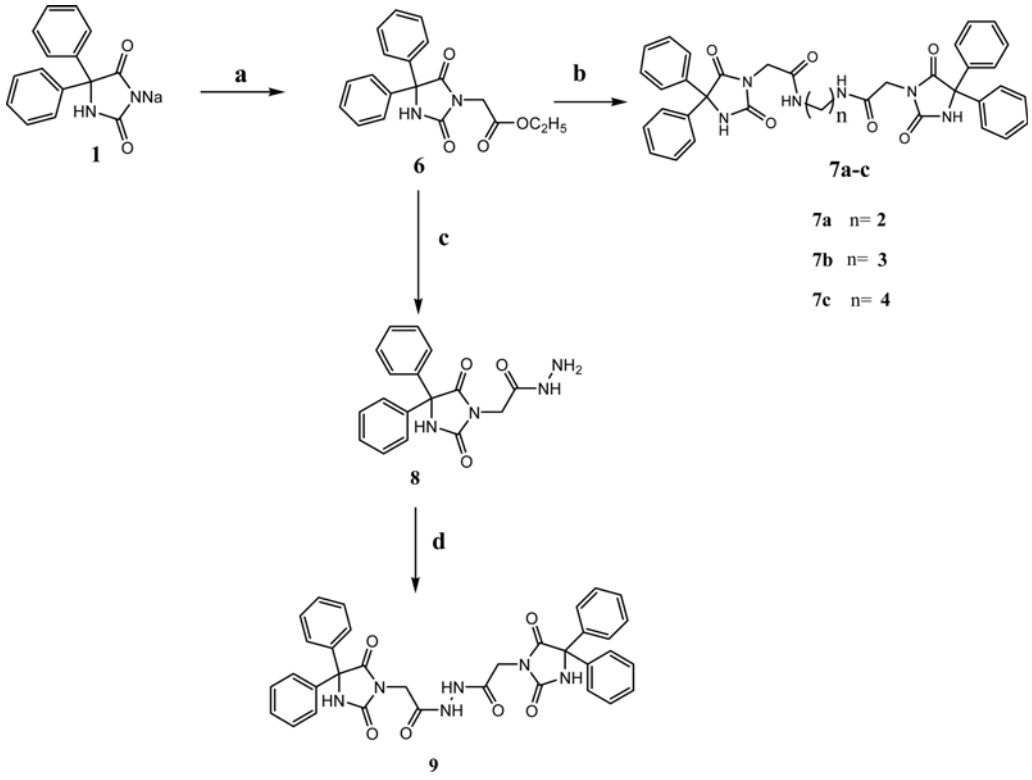
Scheme 1. Reagents and conditions: (**a**) 2:1 molar ratio, DMF, 5-7 h; (**b**) equimolar ratio, DMF, 4-5 h; (**c**) NHR_2, DMF, K_2CO_3, 12 h.

Qp-2010 plus spectrometer, Micro analytical Center, Cairo University, Egypt. Elemental analyses were carried out at the Micro analytical Center, Cairo University, Egypt. Their results corresponded to the calculated values within experimental error. Melting points were determined with Stuart apparatus and are uncorrected.

The starting materials, phenytoin sodium (5,5-diphenylimidazolidine-2,4-dione sodium salt) (**1**) commercially available, diethylene glycol di(4-methylbenzenesulphonate) (**2a**), triethylene glycol di(4-methylbenzenesulphonate) (**2b**) (Ouchi et al., 1990), ethyl 2-(2,5-dioxo-4,4-diphenylimidazolidin-1-yl)acetate (**8**) (Paxton et al., 1976), and 2-(2,5-dioxo-4,4-diphenylimidazolidin-1-yl) acetohydrazide (**10**) (Schlögl et al., 1952), were prepared according to the reported procedures.

3-{2-[2-(4,4-Diphenyl-2,5-dioxoimidazolidin-1-yl) ethoxy]ethyl}-5,5-diphenylimidazolidine-2,4-dione (3a)

A mixture of phenytoin sodium (**1**) (0.55 g, 0.002 mol) and diethylene glycol ditosylate (**2a**) (0.41 g, 0.001 mol) in dry DMF (5 mL) was heated in a water bath (60-80°C) for 5 h. After cooling, the reaction mixture



Scheme 2. Reagents and conditions: (**a**) $ClCH_2COOC_2H_5$, DMF, 2 h; (**b**) $NH_2(CH_2)nNH_2$, 6 h; (**c**) $H_2N\text{-}NH_2$, abs.ethanol, 24 h; (**d**) compound **6**, 12 h.

was diluted with ice-cold water (25 mL) and the solid product so formed was collected by filtration, washed with water then with sodium hydroxide solution (5%, 20 mL), dried then crystallized from ethanol.

Yield 61%; mp 231-232°C; IR (KBr) (cm^{-1}): 3286 (N-H), 3070 (C-H aromatic), 2950, 2850 (C-H aliphatic), 1774, 1701 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.43 (t, 4H, $2CH_2$-N), 3.56 (t, 4H, $2CH_2$-O), 7.32-7.41 (m, 20H, Ar-H), 9.57 (s, 2H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6 ppm): δ 37.51 (N-CH_2), 66.04 (O-CH_2), 69.11 (C5 of imidazolidinedione), 126.68, 128.04, 128.42, 139.53 (aromatic C's), 155.06 (C=O), 173.18 (C=O); EIMS (% rel. abundance): 574 ($M^{\cdot+}$, 7.99), 180 (100); Anal. Calcd for $C_{34}H_{30}N_4O_5$ (574.63): C, 71.07; H, 5.26; N, 9.75; Found: C, 71.24; H, 5.18; N, 9.38.

3-{2-[2-(2-(4,4-Diphenyl-2,5-dioxoimidazolidin-1-yl) ethoxy)ethoxy]ethyl}-5,5-diphenylimidazolidine-2,4-dione (3b)

The title compound was prepared from **1** (0.55 g, 0.002 mol) and triethylene glycol ditosylate (**2b**) (0.45 g, 0.001 mol) for 7 h following the procedure described for the compound **3a**. Crystallized from dioxane/water mixture; yield 62%; mp 233-234°C; IR (KBr) (cm^{-1}): 3332 (N-H), 3080 (C-H aromatic), 2947, 2873 (C-H aliphatic), 1774, 1716 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.36 (t, 4H, 2 N-CH_2), 3.53 (t, 4H, 2 O-CH_2), 3.57 (s, 4H, 2 CH_2-O), 7.30-7.40 (m, 20H, Ar-H), 9.58 (s, 2H, NH, D_2O exchangeable); EIMS (% rel. abundance): 619 (M+H, 4.59), 618 ($M^{\cdot+}$, 11.25), 279 (100); Anal. Calcd for $C_{36}H_{34}N_4O_6$ (618.68): C, 69.89; H, 5.54; N, 9.06; Found: C, 69.66; H, 5.67; N, 8.86.

2-[2-(2,5-Dioxo-4,4-diphenylimidazolidin-1-yl)ethoxy]ethyl-4-methyl benzene sulphonate (4a)

A mixture of phenytoin sodium (**1**) (2.74 g, 0.01 mol) and diethylene glycol ditosylate (**2a**) (4.14 g, 0.01 mol) in dry DMF (5 mL) was heated in a water bath (60-80°C) for 4 h. After cooling, the reaction mixture was diluted with ice-cold water (25 mL), the deposited solid product was filtered, dried, washed with ether (10 mL) then with toluene (10 mL) and crystallized from aqueous ethanol.

Yield 71%; mp 123-124°C; IR (KBr) (cm^{-1}): 3448 (N-H), 3089 (C-H aromatic), 2943, 2873 (C-H aliphatic), 1770, 1705 (C=O), 1354, 1176 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.41 (s, 3H, CH_3), 3.42-3.60 (m, 6H, N-CH_2 and O-CH_2), 4.10 (t, 2H, CH_2-OSO_2), 7.30-7.38 (m, 10H, Ar-H), 7.44 (d, 2H, Ar-H), 7.74 (d, 2H, Ar-H), 9.56 (s, 1H, NH, D_2O exchangeable); EIMS (% rel. abundance): 495 (M+H, 7.57), 180 (100); Anal. Calcd for $C_{26}H_{26}N_2O_6S$ (494.56): C, 63.14; H, 5.30; N, 5.66; Found:

C, 63.28; H, 5.56; N, 5.71.

2-{2-[2-(2,5-Dioxo-4,4-diphenylimidazolidin-1-yl) ethoxy]ethoxy}ethyl-4-methylbenzenesulphonate (4b)

The title compound was prepared from **1** and triethylene glycol ditosylate (**2b**) (0.003 mol each) for 5 h following the procedure described for the compound **4a**. After cooling, the reaction mixture was diluted with ice-cold water (15 mL), extracted with ethyl acetate (3 × 15 mL) and dried (Na_2SO_4). The combined extract was evaporated under reduced pressure, the residue was triturated with benzene, and the precipitated solid product was crystallized from benzene/petroleum ether (60-80°C).

Yield 40%; mp 237-238°C; IR (KBr) (cm^{-1}): 3332 (N-H), 3066 (C-H aromatic), 2947, 2873 (C-H aliphatic), 1774, 1716 (C=O), 1338, 1176 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.41 (s, 3H, CH_3), 3.38 (t, 2H, N-CH_2), 3.70 (s, 4H, 2 O-CH_2), 4.07-4.14 (m, 4H, 2O-CH_2), 4.43 (t, 2H, CH_2-OSO_2), 7.12 (d, 2H, Ar-H), 7.22-7.42 (m, 10H, Ar-H), 7.78 (d, 2H, Ar-H), 9.27 (s, 1H, NH, D_2O exchangeable); EIMS (% rel. abundance): 539 (M+H, 0.39), 538 ($M^{+\cdot}$, 1.22), 91 (100). Anal. Calcd for $C_{28}H_{30}N_2O_7S$ (538.61): C, 62.44; H, 5.61; N, 5.20; Found: C, 62.63; H, 5.69; N, 5.44.

General procedure for 5a-j.

An equimolar mixture of the appropriate **4a** or **4b**, the respective amino compound and anhydrous K_2CO_3 (0.001 mol each) in dry DMF (5 mL) was heated in a boiling water bath for 12 h. After cooling, the mixture was diluted with ice-cold water (25 mL), the precipitated solid was filtered, washed with water, dried then crystallized from absolute ethanol.

3-{2-[2-(Benzylamino)ethoxy]ethyl}-5,5-diphenylimidazolidine-2,4-dione (5a)

Yield 67%; mp 177-178°C; IR (KBr) (cm^{-1}): 3282 (N-H), 3062 (C-H aromatic), 2924, 2854 (C-H aliphatic), 1774, 1705 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.98-3.03 (m, 4H, 2 CH_2-N), 3.59-3.65 (m, 4H, 2 O-CH_2), 4.09 (s, 2H, benzylic CH_2), 7.08-7.45 (m, 15H, Ar-H), 8.89 (s, 1H, NH, D_2O exchangeable), 9.56 (s, 1H, NH, D_2O exchangeable); EIMS (% rel. abundance): 430 (M+H, 5.00), 429 ($M^{+\cdot}$, 3.20), 91 (100). Anal. Calcd for $C_{26}H_{27}N_3O_3$ (429.51): C, 72.71; H, 6.34; N, 9.78; Found: C, 72.92; H, 6.43; N, 9.95.

3-{2-[2-(Morpholin-4-yl)ethoxy]ethyl}-5,5-diphenylimidazolidine-2,4-dione (5b)

Yield 73%; mp 200-201°C; IR (KBr) (cm^{-1}): 3289 (NH), 3065 (C-H aromatic), 2920, 2845 (C-H aliphatic), 1774, 1701 (C=O); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.62-1.78 (m, 6H, 3CH_2-N), 3.62-3.68 (m, 6H, CH_2-N and 2CH_2-O), 3.71-3.80 (m, 4H, 2CH_2-O), 6.73 (s, 1H, NH, D_2O exchangeable), 7.27-7.39 (m, 10H, Ar-H); EIMS (% rel. abundance): 410 (M+H, 0.19), 409 ($M^{+\cdot}$, 0.22), 100 (100); Anal. Calcd for $C_{23}H_{27}N_3O_4$ (409.48): C, 67.46; H, 6.65; N, 10.26; Found: C, 67.73; H, 6.81; N, 10.00.

3-{2-[2-(Piperidin-1-yl)ethoxy]ethyl}-5,5-diphenylimidazolidine-2,4-dione (5c)

Yield 63%; mp 201-202°C; IR (KBr) (cm^{-1}): 3502 (NH), 3090 (C-H aromatic), 2935, 2804 (C-H aliphatic), 1770, 1705 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.30-1.42 (m, 6H, piperidine), 2.26-2.30 (m, 6H, 3CH_2-N), 3.36 (t, 2H, CH_2-N), 3.42-3.56 (m, 4H, 2O-CH_2), 7.32-7.42 (m, 10H, Ar-H), 9.60 (s, 1H, NH, D_2O exchangeable); EIMS (% rel. abundance): 407 ($M^{+\cdot}$, 3.51), 98 (100); Anal. Calcd for $C_{24}H_{29}N_3O_3$ (407.51): C, 70.74; H, 7.17; N, 10.31; Found: C, 70.79; H, 7.25; N, 10.56.

3-{2-[2-(4-Phenylpiperazin-1-yl)ethoxy]ethyl}-5,5-diphenylimidazolidine-2,4-dione (5d)

Yield 66%; mp 204-205°C; IR (KBr) (cm^{-1}): 3286 (NH), 3095 (C-H aromatic), 2960, 2800 (C-H aliphatic), 1774, 1701 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.40 (t, 2H, CH_2-N), 2.42-2.55 (m, 4H, piperazine), 3.00-3.05 (m, 4H, piperazine), 3.37 (t, 2H, CH_2-N), 3.53-3.65 (t, 4H, 2 O-CH_2), 6.82-7.50 (m, 15H, Ar-H); EIMS (% rel. abundance): 485 (M+H, 1.65), 484 ($M^{+\cdot}$, 20.15), 175 (100). Anal. Calcd for $C_{29}H_{32}N_4O_3$ (484.59): C, 71.88; H, 6.66; N, 11.56; Found: C, 71.91; H, 6.69; N, 11.78.

3-{2-[2-(4-(4-Chlorophenyl)piperazin-1-yl)ethoxy] ethyl}-5,5-diphenylimidazolidine-2,4-dione (5e)

Yield 64%; mp 216-217°C; IR (KBr) (cm^{-1}): 3286 (NH), 3100 (C-H aromatic), 2920, 2831 (C-H aliphatic), 1774, 1701 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.44 (t, 2H, CH_2-N), 2.50-2.55 (m, 4H, piperazine), 3.25-3.28 (m, 4H, piperazine), 3.39 (t, 2H, N-CH_2), 3.53-3.57 (m, 4H, O-CH_2), 6.85-7.42 (m, 14H, Ar-H), 9.60 (s, 1H, NH, D_2O exchangeable); EIMS (% rel. abundance): 521 (M+2, 4.10), 519 ($M^{+\cdot}$, 11.39), 209 (100); Anal. Calcd for $C_{29}H_{31}ClN_4O_3$ (519.03): C, 67.11; H, 6.02; N, 10.79; Found: C, 67.24; H, 5.66; N, 10.91.

3-{2-[2-(2-(4-(4-Chlorophenyl)piperazin-1-yl)ethoxy] ethoxy)ethyl}-5,5-diphenylimidazolidine-2,4-dione (5f)

Yield 60%; mp 200-201°C; IR (KBr) (cm^{-1}): 3329 (NH), 3089 (C-H aromatic), 2947, 2827 (C-H aliphatic), 1774, 1716 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.44 (t, 2H, CH_2-N), 2.55-2.61 (m, 4H, piperazine), 3.01-3.08 (m, 4H, piperazine), 3.37 (t, 2H, CH_2-N), 3.51-3.62 (m, 8H,

4 O-CH_2), 7.09 (d, 2H, Ar-H), 7.38-7.46 (m, 10H, Ar-H), 7.48 (d, 2H, Ar-H), 9.60 (s, 1H, NH, D_2O exghangeable); EIMS (% rel. abundance): 562 (M^+, 1.06), 279 (100); Anal. Calcd for $C_{31}H_{35}ClN_4O_4$ (563.09): C, 66.12; H, 6.27; N, 9.95; Found: C, 66.24; H, 6.31; N, 9.74.

3-{2-[2-(4-(4-Methoxyphenyl)piperazinyl)ethoxy] ethyl}-5,5-diphenylimidazolidine-2,4-dione (5g)
Yield 62%; mp 213-214°C; IR (KBr) (cm^{-1}): 3286 (NH), 1774, 1701 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.45-2.50 (m, 4H, piperazine), 2.85-2.90 (m, 4H, piperazine), 3.25-3.30 (m, 4H, 2CH_2-N), 3.48-3.55 (m, 4H, 2CH_2-O), 3.67 (s, 3H, OCH_3), 6.81-6.82 (m, 4H, Ar-H), 7.32-7.41 (m, 10H, Ar-H), 9.61 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6 ppm): δ 37.50 (CH_2), 49.39 (2CH_2 piperazine), 53.13 (2CH_2 piperazine), 55.07 (CH_2), 57.07 (OCH_3), 66.02 (2 O-CH_2), 69.10 (C-5 imidazolidine), 114.08, 117.11, 126.60, 128.05, 128.43, 139.50, 145.30 (aromatic C's), 155.06 (C=O), 173.18 (C=O); EIMS (% rel. abundance): 515 (M+H, 14.34), 514 (M^+, 33.04), 205 (100); Anal. Calcd for $C_{30}H_{34}N_4O_4$ (514.62): C, 70.02; H, 6.66; N, 10.89; Found: C, 70.31; H, 6.82; N, 11.14.

***N*-{2-[2-(2,5-Dioxo-4,4-diphenylimidazolidin-1-yl) ethoxy]ethyl}phthalimide (5h)**
Yield 77%; mp 211-212°C; IR (KBr) (cm^{-1}): 3290 (NH), 3066 (C-H aromatic), 2935, 2877 (C-H aliphatic), 1774, 1701, 1658 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.24-3.30 (m, 4H, 2CH_2-N), 3.50-3.62 (m, 4H, 2O-CH_2), 7.31-7.42 (m, 14H, Ar-H), 9.57 (s, 1H, NH, D_2O exchangeable); EIMS (% rel. abundance): 470 (M+H, 3.41), 469 (M^+, 5.54), 174 (100); Anal. Calcd for $C_{27}H_{23}N_3O_5$ (469.49): C, 69.07; H, 4.94; N, 8.95; Found: C, 68.83; H, 4.74; N, 8.59.

***N*-{2-[2-(2-(2,5-Dioxo-4,4-diphenylimidazolidin-1-yl)ethoxy)ethoxy]ethyl} phthalimide (5i)**
Yield 75%; mp 216-217°C; IR (KBr) (cm^{-1}): 3329 (NH), 3062 (C-H aromatic), 2947, 2873 (C-H aliphatic), 1774, 1716, 1660 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.23-3.29 (m, 4H, 2 CH_2-N), 3.42 (s, 4H, 2 CH_2-O), 3.51-3.56 (m, 4H, 2 O-CH_2), 7.31-7.46 (m, 12H, Ar-H), 7.76-7.92 (m, 2H, Ar-H), 9.60 (s, 1H, NH, D_2O exchangeable); EIMS (% rel. abundance): 513 (M^+, 26.44); Anal. Calcd for $C_{29}H_{27}N_3O_6$ (513.54): C, 67.83; H, 5.30; N, 8.18; Found: C, 67.48; H, 5.39; N, 8.08.

***N*-{2-[2-(2,5-Dioxo-4,4-diphenylimidazolidin-1-yl) ethoxy]ethyl}aminoguanidine (5j)**
Yield 60%; mp 187-188°C; IR (KBr) (cm^{-1}): 3400-3286 (NH, NH_2), 3100 (C-H aromatic), 2920, 2880 (C-H aliphatic), 1774, 1701 (C=O); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.60-1.83 (br. s, 5H, NH, NH_2 aminoguanidine D_2O exchangeable), 2.89 (t, 2H, N-CH_2), 2.97 (t, 2H, CH_2-N), 3.57-3.79 (m, 4H, 2 CH_2-O), 6.30 (s, 1H, NH, D_2O exchangeable), 7.26-7.37 (m, 10H, Ar-H); EIMS (% rel. abundance): 295 (10.77), 279 (33.97), 251 (26.19), 180 (100); Anal. Calcd for $C_{20}H_{24}N_6O_3$ (396.44): C, 60.59; H, 6.10; N, 21.20; Found: C, 60.63; H, 6.18; N, 21.41.

General procedure for preparation of 7a-c

A mixture of **8** (0.67 g, 0.002 mol) and the appropriate diaminoalkane (0.001 mol) was heated in a boiling water bath with stirring for 6 h. After cooling, the reaction mixture was diluted with ice-cold water (15 mL), the resulting solid was filtered, washed with dilute HCl (5 mL) then with water (25 mL) and crystallized from aqueous ethanol.

***N,N*-(Ethane-1,2-diyl)bis[2-(2,5-dioxo-4,4-diphenylimidazolidin-1-yl)acetamide] (7a)**
Yield 44%; mp 223-224°C; IR (KBr) (cm^{-1}): 3425-3278 (NH), 3070 (C-H aromatic), 2951, 2854 (C-H aliphatic), 1774, 1724, 1650 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.41 (s, 4H, 2 CH_2-N), 4.00 (s, 4H, 2 CH_2-CO), 7.32-7.39 (m, 20H, Ar-H), 9.31 (s, 2H, 2 NH, D_2O exchangeable), 11.10 (s, 2H, 2 NH, D_2O exchangeable); EIMS (% rel. abundance): 644 (M^+, 51.33); Anal. Calcd for $C_{36}H_{32}N_6O_6$ (644.68): C, 67.07; H, 5.00; N, 13.04; Found: C, 66.80; H, 4.82; N, 13.28.

***N,N*-(Propane-1,3-diyl)bis[2-(2,5-dioxo-4,4-diphenylimidazolidin-1-yl)acetamide] (7b)**
Yield 48%; mp 228-229°C; IR (KBr) (cm^{-1}): 3275-3213 (NH), 3070 (C-H aromatic), 2951, 2854 (C-H aliphatic), 1774, 1720, 1674 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.30-1.35 (m, 2H, CH_2-$\underline{CH_2}$-CH_2), 3.42 (t, 4H, 2 CH_2-N), 4.05 (s, 4H, 2 CH_2-CO), 7.35-7.38 (m, 20H, Ar-H), 9.31 (s, 2H, 2 NH, D_2O exchangeable), 11.10 (s, 2H, 2 NH, D_2O exchangeable); EIMS (% rel. abundance): 658 (M^+, 9.85), 104 (100). Anal. Calcd for $C_{37}H_{34}N_6O_6$ (658.71): C, 67.47; H, 5.20; N, 12.76; Found: C, 67.56; H, 5.27; N, 12.98.

***N,N*-(Butane-1,4-diyl)bis[2-(2,5-dioxo-4,4-diphenylimidazolidin-1-yl)acetamide] (7c)**
Yield 52%; mp 271-272°C; IR (KBr) (cm^{-1}): 3390-3200 (NH), 3070 (C-H aromatic), 2924, 2854 (C-H aliphatic), 1774, 1716, 1685 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.41 (t, 4H, CH_2-$\underline{CH_2}$-$\underline{CH_2}$-CH_2), 3.09 (t, 4H, 2 CH_2-N), 4.30 (s, 4H, 2 CH_2-CO), 7.33-7.40 (m, 20H, Ar-H), 9.59 (s, 2H, 2 NH, D_2O exchangeable), 11.10 (s, 2H, 2 NH, D_2O exchangeable); EIMS (% rel. abundance): 673 (M+H, 10.04), 672 (M^+, 19.69); Anal. Calcd for $C_{38}H_{36}N_6O_6$ (672.73): C, 67.84; H, 5.39; N, 12.49; Found:

C, 67.70; H, 5.44; N, 12.63.

2-(2,5-Dioxo-4,4-diphenylimidazolidin-1-yl)-*N*-[2-(2,5-dioxo-4,4-diphenylimidazolidin-1-yl)acetyl] acetohydrazide (9)

An equimolar mixture of **8** and the hydrazide derivative **10** (0.001 mol each) in absolute ethanol (6 mL) was heated under reflux for 12 h. The grey solid precipitated on hot was filtered, dried and crystallized from DMF-water.

Yield: 56%; mp > 330ºC; IR (KBr) (cm^{-1}): 3414-3267 (NH), 3090 (C-H aromatic), 2935, 2850 (C-H aliphatic), 1774, 1712, 1650 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.78 (s, 4H, 2 CH_2), 7.30-7.60 (m, 20H, Ar-H), 9.42 (s, 2H, 2 NH, D_2O exchangeable); EIMS (% rel. abundance): 616 (M^+, 52.71); Anal. Calcd for $C_{34}H_{28}N_6O_6$ (616.62): C, 66.23; H, 4.58; N, 13.63; Found: C, 66.34; H, 4.56; N, 13.97.

Anticonvulsant screening

All synthesized target compounds were evaluated for anticonvulsant activity against PTZ and MES-induced seizures in mice.

Adult albino mice weighing 20-25 g of both sexes (obtained from the animal house colony in the National Research Center) were used throughout this study. Animals were housed in groups of 4-5 and were allowed free access to food pellets (vit mix 1%, mineral mix 4% corn oil 10%, sucrose 20%, cellulose 0.2%, casein (95% pure) 10.5%, starch 54.3%) and water except for the short time that animals were removed from their cages for testing. All behavioural experiments were conducted during the period between 10:00 and 13:00 with normal room light (12 h regular light/dark cycle) and temperature (22 ± 18ºC). Animals' housing and rearing had been followed optimum standard rules. Unnecessary disturbance of animals was avoided. Animals were treated gently; squeezing, pressure and tough manoeuvre is avoided. Instruments used for drug preparation and animal injection were previously sterilized. Anaesthesia had been used whenever applicable. The dose of each drug was calculated accurately. Animals' cadavers and parts of tissues were handled with care by following the principles of healthy hygiene; dead bodies were incinerated in the National Research Center incineration. All procedures were carried out in accordance with the (National Research Center, Cairo, Egypt) institutional guidelines for animal care and use as follows:

Pentylynetetrazole induced seizure test

The test compound or the reference drug (100 mg/kg) was given orally to groups of 6 mice. Another group of 6 mice have been taken as a control. Sixty min after oral administration, pentylenetetrazole (PTZ) (85 mg/kg) was injected subcutaneously. Each animal was placed into an individual plastic cage for observation over a period of 20 min. The incidence of tonic-clonic convulsions lasting for at least 5 sec was recorded (Fisher, 1989; Löscher et al., 1991).

Animals devoid of generalized convulsions were considered to be protected and the results were represented as percentage protection. Standard drug used was phenytoin sodium at a dose of 100 mg/kg (therapeutic dose) (Table II).

MES-maximal electroshock seizure pattern test

The procedure was carried out as described by Krall et al. (1998) and Kitano et al. (1996). Electroshock was applied via ear-lip electrodes and generated by a stimulator (deliver an alternating 60 HZ current by Ugo Basile ECT Unit (Pulse generator 57800-001), the stimulus duration was 2.5 sec the end point was tonic hind limb extension (Löscher et al., 1984). The maximum electro-shock was determined. The drugs were administered orally (100 mg/kg) 60 min before the test. The control animals were administered the reference antiepileptic drug + vehicle. The mean threshold current for electroshock-induced tonic hind limb extensor seizure was calculated for each drug (Table II).

The number of protected animals in the treated groups is calculated as percentage of affected animals in the control group and statistics was done using chi-squared test with the aid of Graphpad Prism software, version 5 (inc., San Diego).

Neurotoxicity screening

Minimal motor impairment was measured in mice or rats by using standardized rotorod test (Cuadrado and Armijo, 2005). Prior to the experiment, male albino mice were placed on 3 centimeter rod (*Ugo-Basile Accele. ROTA-ROD for mice, 7650*) rotating at 6 rpm, in two training sessions that last 10 and 15 min respectively. The candidates under investigation were injected *i.p.* (100 mg/kg) in four groups each of 6 mice. 1 h later, the animals were again tested on the rotorod to assess the locomotor coordination and neurological deficit (*e.g.*, ataxia, sedation, hyper-excitability), which are reflected by the inability of the animal to maintain equilibrium on the rod after the administration of a selected candidate. The end point for minimal neurotoxicity assessment was reflected by the inability of mice to maintain their equilibrium for at least 1 min in each of the four trials.

Prediction of ADME properties

A computational study of titled compounds was performed for prediction of ADME properties.. Polar surface area (TPSA), number of rotatable bonds, molecular volume, number of hydrogen donor and acceptor atoms and violations of Lipinski's rule of five (Lipinski et al., 2001) were calculated by Molinspiration and MolSoft (MolSoft, 2007). The computed c Log *p* values were calculated using Molinspiration (2008) software (Ahsan et al., 2011).

Absorption (% ABS) was calculated by:

ABS % = 109 (0.345 × TPSA) (Zhao et al., 2002).

RESULTS AND DISCUSSION

Scheme 1 describes the synthesis of phenytoin derivatives **3a**,**b**, **4a**,**b** and **5a-j**. Phenytoin dimers **3a**,**b** were obtained by heating two equivalents of the commercially available phenytoin sodium (**1**) with one equivalent of the appropriate ditosylate **2a**,**b** in DMF. The compounds **4a**,**b** were prepared in 71% and 40% yields respectively by using equimolar amounts of phenytoin sodium (**1**) and the appropriate **2a**,**b** adopting the same reaction conditions as for **3a**,**b**. Subsequent reaction of **4a**,**b** with various primary or secondary amino compounds in DMF and anhydrous K_2CO_3, afforded the target compounds **5a-j** in 60-77% yields.

The synthesis of the desired targets **7a-c** and **9** was accomplished according to the reaction sequence illustrated in Scheme 2. The phenytoin dimers **7a-c** were prepared in 44-52% yields by heating two equivalents of the ester **6** with one equivalent of the appropriate diaminoalkane of 2, 3 and 4 carbon spacer. On the other hand, the compound **9** was obtained in 56% yield by heating the ester **6** with the hydrazide derivative **8** in ethanol for 12 h. Thin layer chromatography (TLC) was run throughout the reactions to optimize the reactions for purity and completion.

The structure of all new compounds was confirmed by both spectral data and elemental analysis. IR spectra of the dimeric phenytoins **3a**,**b** showed the appearance of C-H aliphatic bands in the range of 2947-2873 cm^{-1}. ^{1}H-NMR spectrum of **3b** revealed two sets of triplet signals at δ 3.36 ppm and δ 3.53 ppm assignable to the N-CH_2- and O-CH_2- protons respectively. A singlet signal corresponding to the four equivalent protons of -O-CH_2-CH_2-O- was displayed at δ 3.57 ppm. ^{13}C-NMR for the compound **3a** showed appearance of two signals at δ 37.51 ppm and 66.04 ppm corresponding to the N-CH_2- and of O-CH_2- carbons respectively in addition to the characteristic signals of phenytoin.

The formation of the intermediates **4a**,**b** was confirmed by its IR and ^{1}H-NMR spectral studies. IR spectra of the title compounds showed the appearance of two bands corresponding to the SO_2*str* in the range of 1354-1338 cm^{-1} and 1176 cm^{-1}. ^{1}H-NMR spectra for **4a**,**b** showed a singlet of CH_3 protons resonated at δ 2.41 ppm and a triplet assignable to -CH_2-OSO_2- protons in the range of δ 4.10-4.43 ppm.

The structural assignments to the new compounds **5a-j** were based on their elemental analyses and spectral data. IR spectra of compounds **5h** and **5i** showed the appearance of additional carbonyl group of phthalimide in the range 1660-1658 cm^{-1}. ^{1}H-NMR spectrum of **5a** showed a singlet at δ 4.09 ppm corresponding to benzylic CH_2 and a D_2O exchangeable signal at δ 8.89 ppm assignable to NH of benzylamine in addition to the phenytoin NH signal at δ 9.56 ppm. In the compound **5g**, a singlet assignable to OCH_3 protons appeared at δ 3.67 ppm.^{13}C-NMR of the compound **5g** confirmed the presence of the piperazine ring carbons at δ 49.39 and 53.13 ppm. In addition, a signal at δ 57.07 ppm was assignable to OCH_3. The mass spectra of the new compounds **3a**,**b**, **4a**,**b** and **5a-j** were in accordance with their structures.

Computational studies

Prediction of ADME properties

Oral bioavailability and potency of CNS-acting drugs is directly related to the good intestinal absorption, reduced molecular flexibility (measured by the number of rotatable bonds), low polar surface area or total hydrogen bond count (sum of donors and acceptors), as well as blood-brain barrier penetration, which is correlated to the molecular lipophilicity, and expressed by c Log *p* (*p* is the partition coefficient of the molecule in water/octanol system). On the basis of lipophilicity, c Log *p* values should be ≤ 5, with optimum lipophilicity at c Log *p* = 2-3, for any compound to be considered an oral drug (Veber et al*.,* 2002; Refsgaard et al*.,* 2005). In this context, we attempted to correlate the anticonvulsant activity of title compounds with their calculated Log *p* values. The computed c Log *p* values were calculated using Molinspiration (2008) software (Ahsan et al., 2011) (Table I).

A computational study for prediction of ADME properties of all molecules performed is presented in Table I.

Topological polar surface area (TPSA) is a sum of surfaces of polar atoms (usually oxygen, nitrogen and attached hydrogen) in a molecule. TPSA is a descriptor that was shown to correlate well with passive molecular transport through membranes and, therefore, allows prediction of transport properties of drugs in the intestines and blood-brain barrier crossing (Zhao

et al., 2002). Both TPSA and volume are inversely proportional to the percentage of absorption (% ABS), was calculated using TPSA. From all these parameters, it can be observed that all titled compounds exhibited % ABS ranging from 54.84 to 87.66%, among them seven compounds are supposed to have very high % ABS (83.35-87.66%) (Table I).

The number of rotatable bonds (NROTB), which is important for conformational changes of molecules under study and ultimately for the binding with receptors or channels, revealed that for passing oral bioavailability criteria, number of rotatable bond should be ≤ 10 (Veber et al., 2002). Furthermore, Lipinski's rule of five was calculated (Lipinski et al., 2001). The rule states that most molecules with good membrane permeability have c Log $p \leq 5$, molecular weight < 500, number of hydrogen bond acceptors ≤ 10, and number of hydrogen bond donors ≤ 5. This rule is widely used as a filter for drug-like properties. All the title compounds (**4**, **5a-e**, **6a-e**, **7a-e**) were subjected to molecular properties prediction by Molinspiration and MolSoft (MolSoft, 2007) software in order to filter the drugs for synthesis and biological screening and to reduce enormous wastage of expensive chemicals and precious time (Ahsan et al., 2011).

From the data in Table I, it is obvious that almost all compounds are supposed to have high lipophilicity and can be able to pass blood-brain barrier. In addition, the synthesized compounds in general possess 8-13 rotatable bonds and therefore, exhibit large conformational flexibility.

Most of the synthesized compounds have not revealed prominent violation in Lipinski's parameters, making them potentially promising agents for antiepileptic therapy (Table I).

Anticonvulsant screening

The pre-clinical discovery and development of new chemical agents for the treatment of epilepsy are based mainly on the use of predictable animal models, from which the PTZ and maximal electroshock seizure (MES) screens are recognized as the "gold standards" in the early stages of testing (Rogawski, 2006). All new synthesized compounds **3a**,**b**, **4a**,**b**, **5a-j** and **7a-c** and **9** were subjected to preliminary anticonvulsant screening by using PTZ and MES model of seizures. Being phenytoin derivatives, the test compounds were administered in a dose equivalent to the therapeutic dose of the reference drug, phenytoin (100 mg/kg). The MES test is associated with the electrical induction of the seizure, whereas PTZ method involves a chemical induction to generate the convulsion. The test compounds that showed significant anticonvulsant activity in either of screen models are tested for neurotoxicity using rotorod screen in mice (Table II). The initial anticonvulsant evaluation showed that ten of the tested compounds were effective in the MES and/or scPTZ screens. In the PTZ test, compounds **4b**, **5e** and **7c** demonstrated 100% protection against PTZ-induced seizures, which is more potent than the reference drug,

Table I. Pharmacokinetic parameters of the synthesized compounds important for good oral bioavailability

Compound	cLog *p*	TPSA (A^2)	% ABS	NROTB	MW	Molecular volume	HBD	HBA	Lipiniski's violation
Rule	≤ 5	-	-	≤ 10	< 500	-	≤ 5	≤ 10	≤ 1
3a	2.92	108.048	71.73	10	574.6	512.0	2	9	1
3b	2.79	117.282	68.54	13	618.6	554.6	2	10	1
4a	2.94	102.017	73.81	10	494.5	428.8	1	8	0
4b	2.81	111.251	70.62	13	538.6	471.4	1	9	1
5a	3.09	70.668	84.62	10	429.5	401.0	2	6	0
5b	1.76	71.113	84.47	8	409.4	378.5	1	7	0
5c	2.98	61.879	87.66	8	407.5	386.4	1	6	0
5d	3.12	65.117	86.54	9	484.6	453.8	1	7	0
5e	3.87	65.117	86.54	9	519.0	467.3	1	7	1
5f	3.74	74.351	83.35	12	563.0	509.9	1	8	1
5g	3.01	74.351	83.35	10	479.3	514.6	1	8	1
5h	2.84	97.717	75.29	8	469.5	411.7	1	8	0
5i	2.71	106.951	72.11	10	499.5	437.5	1	9	0
5j	-0.014	132.57	63.27	10	396.0	358.8	6	9	1
7a	0.61	157.01	54.84	11	644.6	565.8	4	12	2
7b	0.81	157.01	54.84	12	658.7	658.7	4	12	2
7c	0.26	157.01	54.84	13	672.7	672.7	4	12	2
9	0.38	157.01	54.84	9	616.6	532.2	4	12	2

phenytoin. Besides, compounds **5c**, **5i** and **7b** were found to be equipotent to phenytoin (80% protection). Compounds **3a**, **3b**, **4a**, **5a**, **5d**, **5g**, **5h**, **7a** and **9** showed moderate protective effect. The only compound that showed weak activity was the compound **5b** (20% protection). In the MES test, excluding compound **5d**, which showed remarkable anti-seizure effect more potent than phenytoin, all other test compounds showed from good to moderate protection against electrically induced seizures. Compounds **4a**, **5a** and **5e** showed protection comparable to reference drug, phenytoin.

This can be explained on the basis of drug lipophilicity and their permeability to the blood-brain barrier which can be expressed as the Log *p*. The calculated octanol/water partition coefficient c Log *p* being a measure of hydrophobicity/lipophilicity demonstrated that the compounds which showed high protection against either scPTZ or MES induced seizures except **7b**,**c** and **9** possess optimum c Log *p* values in the range of 2.71-3.87.

Analyzing the activities of synthesized compounds **3a**,**b**, **4a**,**b**, **5a-j** and **7a-c** and **9** the following SAR was gained. Generally, the anticonvulsant activity may be increased remarkably after the introduction of a halogen atom. Thus, within the aryl piperazine derivatives **5d**, **5e**, **5g**, the 4-chlorosubstituted derivative **5e** was the strongest and showed higher protection than the unsubstituted and 4-methoxysubstituted analogues against PTZ-induced convulsions. However, the unsubstituted derivative, **5d** showed higher protection against electrically induced seizures.

To demonstrate the effect of the spacer length on the anticonvulsant activity, within the different pairs of targets and intermediates with either two or three oxyethylene unit spacer, the following results were observed. Within the dimeric phenytoin derivatives **3a** (n = 1) and **3b** (n = 2), there is no difference in the seizure-protecting activity of the two compounds in both MES and scPTZ models. On the other hand, the benzene sulphonate intermediates **4a**, in which phenytoin pharmacophore is connected to the tosylate group through two oxyethylene unit spacer, displayed higher protection against MES-induced seizures, which is almost equal to the reference drug, phenytoin, compared to its analogue containing three oxyethylene unit spacer **4b**. The opposite effect was observed in the scPTZ screen, in which the compound **4b** displayed 100% protection against seizures compared to only 40% produced by **4a**. Within the two 4-chlorophenylpiperazine derivatives **5e** (n = 1) and **5f** (n = 2), it was observed that the shorter spacer analogue **5e**, displayed higher protection against seizures in both scPTZ and MES screens compared to **5h** containing longer chain spacer. In contrast, compound **5i** with terminal phthalimide substituent, and three oxyethylene unit spacer showed 80% protection against scPTZ-induced convulsions, which is more potent than its shorter spacer analogue **5h** (60% protection). However, both compounds **5h** and **5i** showed almost equal anticonvulsant activity in the MES screen.

Within the compounds **7a-c** and **9** in which two phenytoin pharmacophores are linked through amide linker and varying methylene chain spacer (n = 2-4), the length of the spacer appeared to have a direct effect on the anticonvulsant activity in the scPTZ screen (Table II). The protection against scPTZ-induced convulsions increases with the increase of the spacer length. The optimum spacer length was found to be 4-carbon spacer, as shown by the compound **7c** (100% protection). On the other hand, spacer length seems to have no effect in the MES assay, except for the compound **9** (n = 0), which exhibited significant protection against electrically-induced seizures.

In summary, compounds **4a**, **4b**, **5a**, **5c**, **5d**, **5e**, **5i**, **7b**, **7c** and **9** showed significant anticonvulsant activity in either of the tested models. Three compounds

Table II. Anticonvulsant activity and neurotoxicity (NT) of compounds **3a**,**b**, **4a**,**b**, **5a-j**, **7a-c** and **9**

Compound	PTZ	MES	NT[a]
	%protection		
3a	60	4.40 ± 0.8124	-
3b	60	4.33 ± 1.054	-
4a	40	7.00 ± 0.6325*	16.67
4b	100*	5.00 ± 0.3162	80*
5a	40	7.20 ± 0.7348*	16.67
5b	20	5.00 ± 0.4082	-
5c	80*	6.60 ± 0.9274*	33.33
5d	40	8.40 ± 0.7483*	50
5e	100*	7.00 ± 0.325*	33.33
5f	60	5.60 ± 0.2662	-
5g	40	5.60 ± 0.2449	-
5h	60	5.60 ± 0.8124	-
5i	80*	5.50 ± 0.8660	25
5j	40	4.80 ± 0.3742	-
7a	60	5.00 ± 0.4472	-
7b	80*	4.25 ± 0.4787	50
7c	100*	4.40 ± 0.7483	75*
9	40	6.20 ± 0.3742*	40
Control	-	2.80 ± 0.3742	0
Phenytoin	80*	7.25 ± 0.2500*	-

Values represent the mean ± S.E. of 5-6 animals for each group. Values with * are statistically significant ($p < 0.05$) from the control group by using one way ANOVA (followed by Tukey's as *p*ost tests). [a]Percentage of animals that fail to maintain equilibrium on the accelerating rod for 300 sec.

(**4b**, **5e**, **7c**) exhibited 100% protection against scPTZ-induced seizures. On the other hand, compound **5d** displayed more potent anticonvulsant activity than the standard drug in the MES screen. In addition, most of the compounds showed low neurotoxic properties in the neurotoxicity screen. The compound **5e** exhibited potent anticonvulsant properties in both tested models with low neurotoxicity, therefore, can be regarded as a strong candidate for future investigations. In addition, the majority of the pharmacologically active test compounds did not violated Lipinski parameters, and thus may possess good intestinal absorption and can penetrate the blood brain barrier.

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