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Synthesis and Anticonvulsant Evaluation of *N*-Substituted-Isoindolinedione Derivatives

Atef Abdel-Monem Abdel-Hafez

Department of Pharmaceutical Medicinal Chemistry, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt

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A series of *N*-substituted-1,3-isoindolinedione derivatives (**2-16**) were synthesized for the purpose of defining the effect of N-substitution on the anticonvulsant activity of these derivatives. The target compounds (**2-16**) were obtained by condensation of phthalic anhydride with the corresponding amine derivative. The structures of the synthesized derivatives (**2-16**) were confirmed by means of IR, ^{1}H-NMR, ^{13}C-NMR, MS and elemental analyses. The anticonvulsant activity of all compounds (**2-16**) were evaluated by subcutaneous pentylenetetrazole seizure threshold test at doses of 0.2, 0.4 and 0.8 mmol/kg compared with sodium valproate as a positive control.Their neurotoxicity were determined by the rotorod test. Many of the present series of compounds showed good anticonvulsant activity at the tested doses, as compared to sodium valproate. Three of them (**4**, **6** and **11**) exhibited 100 % protection against convulsions, neurotoxicity and death at all tested doses. Out of the series, two compounds (**12** and **13**) were completely inactive with 100% mortality. 3-(*p*-chlorophenyl)-4-(1,3-dioxo-2,3-dihydro-1H-2-isoindolyl)butanoic acid derivative (**11**) has emerged as the most active compound which is 20 times more active than valproate with ED_{50} 8.7, 169 mg/kg; TD_{50} 413, 406 mg/kg and PI 47.5, 2.4. The results revealed the importance of the combination of baclofenic and phthalimide moieties (compound **11**) as a promising anticonvulsant candidate.

Key words: Isoindolinedione, Phthalimide, Baclofen, Lipophilicity, Anticonvulsant activity

INTRODUCTION

Isoindoline-1,3-dione appears to function as the pharmacophoric structure of many agents having diverse biological activities, which include sedative hypnotic (Hashimoto, 2002), hypoglycemic (Sou *et al.*, 2000; Takahashi *et al.*, 2000), antimalarial (Hashimoto, 2002), antiandrogenic (Miyachi *et al.*, 1997; Hashimoto, 1998), hypolipidimic (Chapman *et al.*, 1979; Chapman *et al.*, 1983; Hall *et al.*, 1983; Chapman *et al.*, 1984), antitumor (Al-Soud and Al-Masoudi, 2001), antiangiogenic (Shimazawa *et al.*, 1999), antiviral (Hashimoto, 2002) and anticonvulsant effects (Bailleux *et al.*, 1994a; Bailleux *et al.*, 1994b; Bailleux *et al.*, 1994c; Bailleux *et al.*, 1995; Usifoh *et al.*, 2001). The famous isoindoline-1,3-dione derivative, thalidomide **1** (Náphthalimidoglutarimide) was synthesized in 1953 by the Swiss pharmaceutical company Ciba and marketed in 1954 by the German company Chemie Grünenthal as anticonvulsant agent for the treatment of epilepsy (Hashimoto, 2002). Since the pioneering discovery of the anticonvulsant properties of **1**, the isoindoline-1,3-dione ring system became an important building block that led to the discovery of a number of *N*-substituted phthalimide derivatives with a marked antiepileptic activity. Worldwide, 1% of the population suffers from some kind of epilepsy and 25% of them have seizures that are resistant to the available medical therapies (Pandeya *et al.*, 1999). Furthermore, the anticonvulsant drugs that are presently used in clinical practice show a broad range of adverse effects. Consequently, a real need exists to develop new anticonvulsant compounds with broad antiseizure activity and minimal neurological

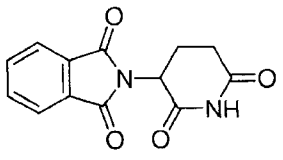

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Fig. 1. Structure of thaliclomide **1**

Correspondence to: Atef Abdel-Monem Abdel-Hafez, Department of Pharmaceutical Medicinal Chemistry, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt
Tel: 20-88-303006, Fax: 20-88-33-2776
E-mail: atef@acc.aun.edu.eg

toxicity and side effects. In the present study, we synthesized a number of *N*-substituted1,3-isoindolinedione derivatives (**2-16**) with alkyl, cycloalkyl, aryl, and heteroaryl substitution, in addition to condensation with baclofen, a well known anticonvulsant agent, in order to investigate the influence of substitution on their antiseizure activity.

MATERIALS AND METHODS

Chemistry

Melting points were determined on electrothermal melting point apparatus and are uncorrected. IR spectra were recorded as KBr discs on a 470-Shimadzu infrared spectrophotometer. Thin layer chromatography was carried out on pre-coated silica gel 60 F254 plates (0.25 mm thickness, Merck, Darmstadt, Germany) and spots were detected under UV light. ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectra were measured with a JEOL-JNM-GX 400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) spectrometer, and all chemical shifts are given in δ ppm relative to tetramethylsilane (TMS). Electron impact (EI) mass spectra were measured with JEOL-JMS-AX 505 spectrometer at an ionization voltage of 70 eV. Elemental analyses were performed at the microanalytical centre of Toyama Medical and Pharmaceutical University, Toyama, Japan. All chemicals used were of analytical grade.

General method for synthesis of *N*-substituted phthalimide derivatives (2-16)

Phthalic anhydride (0.5 g), 1.1 eq. of the appropriate amine were added to a 50 mL round-bottomed flask equipped with a reflux condenser. The mixture was heated to gentle boiling at 150~250 °C for between 5~15 min. The reaction mixture was cooled and then benzene (30 mL) was added before solidification to form a slurry mixture. The precipitate was filtered, washed twice with water, and the crude material was crystallized from the appropriate solvent (Table I). The physical constants are listed in Table I.

Table I. Physical data of *N*-substituted-isoindolinedione Derivatives (**2-16**)[a]

Compound No.	M.P./(°C)	Crystallization Solvent[b]	Yield (%)	Molecular Formula[c]	MS (m/z) $[M]^+$
2	118-120[d]	A	91	$C_{12}H_{11}NO_4$	233
3	108-109[e]	A	90	$C_{14}H_{15}NO_4$	261
4	203-204[f]	B	92	$C_{13}H_{16}N_2O_2$	232
5	170-171[g]	C	97	$C_{14}H_{15}NO_2$	229
6	190-192	A	82	$C_{12}H_7NO_5$	245
7	155-157	D	96	$C_{15}H_{11}NO_3$	253
8	271-273	D	78	$C_{14}H_8N_2O_4$	268
9	213-214	D	85	$C_{14}H_8BrNO_2$	301 303 $[M^+{+}2]$
10	222-224	E	84	$C_{15}H_9NO_4$	267
11	140-142	E	84	$C_{18}H_{14}ClNO4$	343
12	201-203	D	84	$C_{11}H_6N_2O_2S$	230
13	233-234	D	82	$C_{11}H_6N_4O_2$	214
14	199-201	D	83	$C_{10}H_5N_3O_2S$	231
15	235-237	D	82	$C_{13}H_8N_2O_2$	301
16	258-260	E	88	$C_{15}H_9N_3O_2$	263

[a] The IR spectra were consistent with structural assignments.
[b] Solvent of crystallization: A) Ethanol
B) Light petrol ether 60-80°C
C) $CHCl_3$: MeOH
D) Benzene
E) Ethanol : water
[c] The results for C, H, N and S microanalysis were within 0.4% of the calculated theoretical values.
[d] Reported m.p. 116-117 °C (Chapman *et al.*, 1979)
[e] Reported m.p. 108 °C (Chapman *et al.*, 1979)
[f] Reported m.p. 204-205 °C (Moore and Rapala, 1946)
[g] Reported m.p. 168 °C (Shibata *et al.*, 1995)

4-(1,3-Dioxo2,3-dihydro-1*H*-2-isoindolyl)butanoic acid (2)

^{1}H-NMR: 1.80 (2H, p, *J* = 7.32 Hz, -$CH_2C\underline{H_2}CH_2$-); 2.25 (2H, t, *J* = 7.08 Hz,-$C\underline{H_2}$ COOH); 3.59 (2H, t, *J* = 6.84 Hz, N-$C\underline{H_2}$); 7.82 (4H, m, aromatic protons); 12.05 (1H, s, COOH). ^{13}C-NMR: 23.3, 31.0, 36.9 (aliphatic carbons); 122.9, 131.7, 134.3 (aromatic carbons), 168.0 (C=O), 173.8 (COOH).

6-(1,3-Dioxo2,3-dihydro-1*H*-2-isoindolyl)hexanoic acid (3)

^{1}H-NMR: 1.24 (2H, p, *J* = 7.08 Hz, *N*-$CH_2CH_2C\underline{H_2}CH_2CH_2$-COOH); 1.56 (4H, m, *N*-$CH_2C\underline{H_2}CH_2C\underline{H_2}CH_2COOH$); 2.17 (2H, t, *J* = 7.32 Hz, -$C\underline{H_2}$-COOH); 3.53 (2H, t, *J* = 7.32 Hz, N-$C\underline{H_2}$); 7.83 (4H, m, aromatic protons); 11.96 (1H, s, COOH). ^{13}C-NMR: 24.0, 25.7, 27.7, 33.4, 37.2 (aliphatic carbons); 132.0, 131.6, 134.3 (aromatic carbons), 167.9 (C=O), 173.3 (COOH).

2-[3-(Dimethylamino)propyl]-1,3-isoindolinedione (4)

^{1}H-NMR: 2.45 (6H, s, N-$(C\underline{H_2})_2$); 2.69 (2H, t, *J* = 7.08 Hz, N-$CH_2CH_2C\underline{H_2}N(CH_3)_2$); 3.18 (2H, p, *J* = 6.12 Hz, *N*-$CH_2C\underline{H_2}CH_2$-$N(CH_3)_2$); 3.59 (2H, t, *J* = 7.32 Hz, *N*-$C\underline{H_2}CH_2CH_2$-$N(CH_3)_2$); 7.56~7.86 (4H, m, aromatic protons). ^{13}C-NMR: 25.2, 37.0, 44.8, 56.4 (aliphatic carbons); 122.9, 127.4, 128.5, 128.7, 134.3, 135.8 (aromatic carbons), 167.8 (C=O).

2-Cyclohexyl-1,3-isoindolinedione (5)

^{1}H-NMR: 1.12~2.05 (10H, m, cyclohexyl moiety); 3.96 (1H, m, -N-C$\underline{H}$), 7.82 (4H. m, aromatic protons). ^{13}C-NMR: 24.9, 25.5, 29.4, 50.1 (cyclohexyl carbons); 122.9, 131.4, 134.3 (aromatic carbons), 167.8 (C=O).

2-(2,5-Dioxotetrahydro-3-furanyl)-1,3-isoindolinedione (6)

^{1}H-NMR: 3.03 (1H, m, -CH-); 2.92 (1H, dd, *J* =16.88, 7.08 Hz, -CH_2-), 3.14 (1H, dd, *J* = 16.84, 7.56 Hz, -CH_2-); 7.90

(4H, m, aromatic protons). ^{13}C-NMR: 33.8, (CH_2); 47.9 (CH); 123.4, 125.3, 131.1, 134.9, 136.2 (aromatic carbons); 167.0, 169.9, 171.4 (3 × C=O).

2-(4-Methoxyphenyl)-1,3-isoindolinedione (7)
^{1}H-NMR: 3.81 (3H, s, OCH_3); 7.07 (2H, d, *J* = 7.7 Hz, 2',6' aromatic protons); 7.35 (2H, d, *J* = 7.7 Hz, 3',5' aromatic protons); 7.36 (2H, s, 4,7 aromatic protons); 7.93 (2H, m, 5,6 aromatic protons): ^{13}C-NMR: 55.4 (OCH_3); 114.1, 123.3, 124.4, 128.3, 128.7, 131.5, 134.6, 158.8, (aromatic carbons); 167.3 (C=O).

2-(4-Nitrophenyl)-1,3-isoindolinedione (8)
^{1}H-NMR: 7.86 (2H, d, *J* = 9.28 Hz, 2',6' aromatic protons); 7.92 (2H, dd, *J* = 6.56, 3.64, Hz, 5,6 aromatic protons); 8.01 (2H, dd, *J* = 5.64, 2.96 Hz, 4,7 aromatic protons); 8.39 (2H, d, *J* = 9.28 Hz, 3',5' aromatic protons). ^{13}C-NMR: 123.6, 124.1, 127.7, 131.4, 135.0, 137.8, 146.1 (aromatic carbons); 166.4 (C=O).

2-(4-Bromophenyl)-1,3- isoindolinedione (9)
^{1}H-NMR: 7.43 (2H, d, *J* = 8.76 Hz, 2', 6' aromatic protons); 7.73 (2H, d, *J* = 9.04 Hz, 3',5' aromatic protons); 7.90 (2H, dd, *J* = 5.60, 2.92 Hz, 5,6 aromatic protons); 7.96 (2H, dd, *J* = 5.36, 2.68 Hz, 4,7 aromatic protons). ^{13}C-NMR: 121.0, 123.4, 129.4, 131.3, 131.5, 131.8, 134.7 (aromatic carbons), 166.7 (C= O).

2-(1,3-Dioxo-2,3-dihydro-1*H*-2- isoindolyl)benzoic acid (10)
^{1}H-NMR: 7.52~8.05 (8H, m, aromatic protons); 13.07 (1H, s, COOH). ^{13}C-NMR: 123.5, 129.2, 129.3, 130.7, 131.0, 131.4, 131.7, 133.0, 134.8 (aromatic carbons); 166.1, 167.0 (C = O, COOH). Elemental analysis was previously reported (Abd El-Wahed *et al.*, 1996).

3-(*p*-Chlorophenyl)-4-(1,3dioxo2,3-dihydro-1*H*-2-isoindolyl)butanoic acid (11)
^{1}H-NMR: 2.60 (1H, dd, *J* = 16.32, 9.0 Hz, -C<u>H</u>$_2$-COOH); 2.76 (1H, d, *J* = 16.36, 5.88 Hz, -C<u>H</u>$_2$-COOH); 3.52 (1H, p, *J* = 8.8 Hz ,CH); 3.75 (1H, d, *J* = 1.24 Hz, N-CH_2); 3.77 (1H, d, *J* = 2.2 Hz, N-CH_2-); 7.27 (4H, m, aromatic protons); 7.79 (4H, m, aromatic protons); 12.09 (1H, s, COOH). ^{13}C-NMR: 37.6, 42.6 (aliphatic carbons); 123.0, 128.2, 129.6, 131.3, 134.4, 140.1 (aromatic carbons), 167.6 (C=O); 172.5 (COOH).

2-(1,3-Thiazol-2-yl)-1,3-isoindolinedione (12)
^{1}H-NMR: 7.78 (1H, d, *J* = 3.68 Hz, 4' aromatic proton); 7.81 (1H, d, *J* = 3.4 Hz, 5' aromatic proton); 7.93 (2H, dd, *J* = 5.6, 2.92 Hz, 5,6 aromatic protons): 8.01(2H, dd, *J* = 5.12, 3.2 Hz, 4,7 aromatic protons). ^{13}C-NMR: 120.0, 123.9, 131.1, 135.2, 139.8, 151.7 (aromatic carbons), 164.9 (C=O).

2-(1*H*-1,2,4-Triazol-2-yl)-1,3- isoindolinedione (13)
^{1}H-NMR: 7.90~8.03 (5H, m, aromatic protons); 8.73 (1H, brs, NH). ^{13}C-NMR: 124.0, 131.0, 135.3, 150.4 (aromatic carbons); 166.1 (C=O).

2-(1,3,4-Thiadiazol-2-yl)-1,3-isoindolinedione (14)
^{1}H-NMR: 7.96 (2H, dd, *J* = 6.84, 3.9 Hz, 4,7 aromatic protons); 8.05 (2H, dd, *J* = 5.4, 2.9 Hz, 5,6 aromatic protons); 9.61(1H, s, thiadiazolyl proton). ^{13}C-NMR: 124.2, 131.1, 135.5, 153.2, 153.9 (aromatic carbons), 164.2 (C=O).

2-(4-Pyridyl)-1,3-isoindolinedione (15)
^{1}H-NMR: 7.57 (2H, dd, *J* = 6.84 Hz, 3',5' aromatic protons); 7.92 (2H, dd, *J* = 8.8, 3.68 Hz, 5,6 aromatic protons); 8.01 (2H, dd, *J* = 8.68, 3.68 Hz, 4,7 aromatic protons); 8.72 (2H, d, *J* = 5.36, 6.48 Hz, 2',6' aromatic protons). ^{13}C-NMR: 120.6, 123.7, 131.4, 135.0, 139.1, 150.4 (aromatic carbons); 166.2 (C=O).

2-(1*H*-Benzo[d]imidazol-2-yl)-1,3-isoindolinedione (16)
^{1}H-NMR: 6.99~8.11 (8H, m, aromatic protons). ^{13}C-NMR: 111.4, 120.7, 121.3, 122.5, 124.1, 130.2, 131.1, 132.3, 134.9, 135.5, 138.6, 153.2 (aromatic carbons); 165.7 (C=O).

Evaluation of the anticonvulsant activity (subcutaneous pentylenetetrazole (PTZ) seizure threshold test) (Swinyard, 1972)

Male albino mice weighing 22-30 g and maintained under a controlled temperature (25±2 °C) with 45% humidity at the Assiut University animal care centre were used as experimental animals. The animals were allowed free access to food and water except when removed from their cages for the experimental procedures. For preliminary screening, each compound was injected intraperitoneally (i.p.) in groups of 4 animals at doses of 0.2, 0.4 and 0.8 mmol/kg (suspended in 0.5% methylcellulose/water mixture). Thirty min later, PTZ was injected subcutaneously at a dose of 100 mg/kg dissolved in 0.9% sodium chloride solution. The animals were then observed for 1 h for convulsions or for death. For the experimental compounds, protection against convulsions was defined as the failure to observe an episode of clonic spasm of at least 5 seconds duration during this time period. The median anticonvulsant potency ED_{50} was determined by the graphic presentation method.

Neurological toxicity test

According to the procedure of Dunham and Miya (Dunham and Miya, 1957), rolling roller performance (RRP) test was used for the evaluation of any neurological deficit (e.g. ataxia, sedation or hyperexcitability). The mouse is placed on a 1-inch diameter rod rotating at 6 ppm. Neurological toxicity is indicated by the inability of

the animal to maintain equilibrium on the rod for at least 1 min, in each of three trials. The median neurotoxic dose (TD_{50}) of each compound was calculated by the up and down method (Brownlee *et al.*, 1953; Bruce, 1985).

Table II. Lipophilicity value (Clog P), preliminary anticonvulsant activity and neurological toxicity after intraperitoneal administration of *N*-substituted-1,3-isoiondolinedione derivatives (2-16) to mice

Compound No.	Clog P[a]	Dose (mmol/kg)	Anticonvulsant Activity (sc PTZ)[b]	Toxicity[c]	Mortality[d] (%)
2	1.12	0.2	1/4	1/4	0
		0.4	2/4	2/4	25
		0.8	3/4	3/4	50
3	2.17	0.2	2/4	2/4	0
		0.4	3/4	3/4	0
		0.8	4/4	4/4	25
4	1.24	0.2	4/4	4/4	0
		0.4	4/4	4/4	0
		0.8	4/4	4/4	0
5	3.46	0.2	1/4	1/4	25
		0.4	2/4	2/4	50
		0.8	3/4	3/4	75
6	0.62	0.2	4/4	4/4	0
		0.4	4/4	4/4	0
		0.8	4/4	4/4	0
7	2.48	0.2	0/4	0/4	50
		0.4	1/4	1/4	75
		0.8	1/4	1/4	100
8	2.74	0.2	3/4	3/4	0
		0.4	4/4	4/4	0
		0.8	4/4	4/4	0
9	3.54	0.2	3/4	3/4	0
		0.4	4/4	4/4	0
		0.8	4/4	4/4	0
10	2.46	0.2	0/4	0/4	100
		0.4	0/4	0/4	100
		0.8	1/4	1/4	75
11	3.12	0.2	4/4	4/4	0
		0.4	4/4	4/4	0
		0.8	4/4	4/4	0
12	1.69	0.2	0/4	0/4	100
		0.4	0/4	0/4	100
		0.8	0/4	0/4	100
13	0.62	0.2	0/4	0/4	100
		0.4	0/4	0/4	100
		0.8	0/4	0/4	100
14	1.06	0.2	0/4	0/4	100
		0.4	0/4	0/4	75
		0.8	1/4	1/4	50
15	1.79	0.2	2/4	2/4	25
		0.4	3/4	3/4	0
		0.8	4/4	4/4	0
16	2.72	0.2	0/4	0/4	100
		0.4	1/4	1/4	75
		0.8	2/4	2/4	0
Valproic acid	2.72	0.2	0/4	0/4	100
		0.4	0/4	0/4	100
		0.8	1/4	1/4	75

[a] Calculated partition coefficient (Leo, 1993).
[b] No. of animals protected / total No. of animals.
[c] No. of animals showed no toxicity/ total No. of animals.
[d] No. of animals died/ total No. of animals × 100.

Log P calculations

The log P values of the synthesized derivatives (**2-16**) were computed with a routine method called calculated log P (Clog P) contained in a PC-software package (Mac logP 2.0, BioByte Corp., CA, USA). A representation of the molecular structure where hydrogens are omitted, or suppressed (SMILES notation), is entered into the program, which computes the log P based on the fragment method developed by Leo (Leo, 1993). Results are given in Table II.

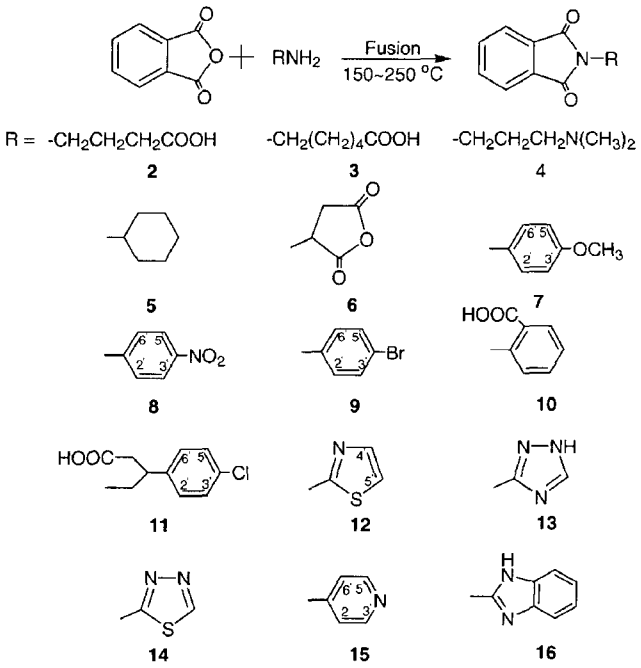
RESULTS AND DISCUSSION

Chemistry

N-Substituted-1,3-isoindolinedione derivatives (**2-16**) were synthesized according to the preferred synthetic route through one-pot synthesis outlined in Scheme 1. These derivatives (**2-16**) were prepared *via* direct fusion, at 150~250 °C for 5~15 min, from phthalic anhydride with the amine derivative in a convenient procedure with a yield varying from 78% to 97%. The physical properties of the target compounds (**2-16**) are reported in Table I. The purity of these compounds was determined by TLC and elemental analyses, and their structures were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR as well as MS.

Anticonvulsant screening

The preliminary screening (qualitative assay) of the anti-



Scheme 1. Pathway for the synthesis of the target compounds (**2-16**)

convulsant activity of the synthesized compounds (**2-16**) was evaluated in mice using PTZ-induced generalized tonic-clonic seizure as described earlier (Swinyard, 1972). Sodium valproate as a positive control and test compounds (**2-16**) were administered intraperitoneally at a dose of 0.2, 0.4 and 0.8 mmol/kg, half an hour after administration of PTZ (100 mg/kg, subcutaneously). The neurotoxicity of the tested compounds was determined using the rotorod toxicity test (Dunham and Miya, 1957). The results of the initial evaluation of the target compounds (**2-16**) are presented in Table II. From the results obtained, 2-[3-(dimethlamino)propyl]-1,3-isoindolinedioine (**4**), 2-(2,5-dioxotetrahydro-3-furanyl)-1,3-isoindolinedione (**6**) and 3-(*p*-chlorophenyl-4-(1,3dioxo2,3dihydro1H-2-isoindolyl) butanoic acid (**11**) showed 100% protection against convulsions without neurotoxicity and mortality. Complete protection against seizures with no toxicity or mortality was achieved by compounds **8** and **9** at doses of 0.4 and 0.8 mmol/kg. While at a dose of 0.8 mmol/kg, compounds **3** and **15** exhibited full protection against convulsions and toxicity. Inhibition of seizure activity and neurotoxicity occurred in a dose-dependent manner for compounds **2**, **3**, **5**, **15**, and **16**. At the dose range tested, weak activity was observed for compounds **7**, **10**, **14**, and sodium valproate (positive control). Two compounds (**12** and **13**) out of the series showed no anticonvulsant activity with 100% neurotoxicity and mortality at all doses used. The lipophilicity (ClogP calculated lipophilicity) of the target compounds (Table II) was estimated by calculation using the ClogP program based on the fragment method developed by Leo (Leo 1993). The ClogP values for the synthesized compounds (**2-16**) are within the acceptable range for CNS active drugs (Clog P range from 0.62 to 3.54 values). From the results of the initial screening of 1,3-isoindolinedoine derivatives (**2-16**), compounds **4**, **6**, and **11** were selected for quantification of the anticonvulsant activities and neurotoxicity effects. The given data in Table III represent the results of these evaluations. The ED_{50} results were determined against subcutaneous PTZ-induced convulsions, and TD_{50} values were measured by the rotorod procedure for testing neurologic deficit. 3-(*p*-Chlorophenyl)-4-(1,3-dioxo-2,3-dihydro-1H-2-isoindolyl) butanoic acid (**11**) had the highest protective index (PI) at 47.5, with ED_{50} and TD_{50} values of 8.7 and 413 mg/kg, respectively. The ED_{50}, TD_{50} and PI values for compound **4** were determined to be 22.4, 362 mg/kg and 13.5, respectively, while for compound **6** were 13.4, 356 mg/kg and 26.6. The data for compounds **4**, **6**, and **11** are compared to that for the anticonvulsant drug sodium valproate (Table III) which produced an ED_{50} of 169 mg/kg, and TD_{50} value of 406 mg/kg with PI 2.4.

In the present study, the PTZ induced seizure threshold method was used to evaluate the anticonvulsant activity and the relative potencies of the target compounds in comparison with sodium valproate. The qualitative experiment was designed to identify compounds with anticonvulsant activity with relatively fewer side effects than the positive control. Most of the tested compounds (**2-16**) displayed anticonvulsant properties, with the exception of two compounds, **12** and **13**. Compounds **4**, **6**, and **11** appear to possess the highest anticonvulsant potential of the present *N*-substituted-1,3-isoindolinedione series. Since this report was designed to clarify the effect of *N*-substitution on the anticonvulsant activity of 1,3-isoindolinedoine derivatives, the order of decreasing activity in relation to the *N*-substitution was: dimethylaminopropyl = 2,5-dioxotetrahydro-3-furanyl = 3-substituted GABA (baclofen) > 4-nitrophenyl = 4-bromophenyl > 5-carboxyphenyl = 4-pyridyl > 3-carboxypropyl = cyclohexyl > 1*H*-benzo[d] imidazol-2-yl > 4-methoxyphenyl > 2-carboxyphenyl = 1,3,4-thiadiazol-2-yl > 1,3-thiazol-2-yl = 1H-1,2,4-triazol-3-yl.

Table III. Anticonvulsant potency and neurotoxicity of compounds **4**, **6**, and **11**

Compound No.	ED_{50} mg/kg[a]	TD_{50} mg/kg[b]	PI[c]
4	22.4	302	13.5
6	13.4	356	26.6
11	8.7	413	47.5
Valproic acid	169	406	2.4

[a] The dose of compound required to produce 100% protection in 50% of the animals calculated by graphical presentation method.

[b] *Minimal neurological toxicity in 50% of the animals calculated by up* and down method.

[c] PI, protective index calculated as TD_{50} / ED_{50}.

Partition coefficient, log P value, is an important parameter for determining the biological properties of compounds affecting the central nervous system (CNS). Since the crossing of the blood-brain barrier by CNS agents appears to be optimal at a log P value of 2.0 (Maryanoff *et al.*, 1998), we correlated the anticonvulsant activity of the tested compounds with their log P values calculated by computer program. We found that all log P values of the synthesized compounds (**2-16**) are with in 2.0 ± 2, which allow these compounds to cross the blood-brain barrier to the CNS by passive mechanisms. On the other hand, it is difficult to correlate the log P values with the anticonvulsant potency of the target derivatives, which may reflect that the *N*-substitution on 1,3-isoindolinedione moiety affects the intrinsic activity and not the bioavailability.

Compounds **4**, **6**, and **11** showed higher protective indices (TD_{50}/ED_{50}) than sodium valproate (Table III), indicating their maximum anticonvulsant protection is achieved in non-neurotoxic doses. Combination of baclofenic and phthalimide moieties in compound **11**, reflects the greatest anticonvulsant potential of the present series with ED_{50}

value 8.7 mg/kg.

It is interesting to note that 2-(2,5-dioxotetrahydro-3-furanyl)-1,3-isoindolinedione **6** which is an analogue to thalidomide **1**, possesses higher anticonvulsant potency without neurotoxicity indicated by its ED_{50}, TD_{50} and PI values, in comparison to the non-specified anticonvulsant activity, detectable side effects and neurotoxicity of **1** (Hashimito, 2002).

Although initial evidence obtained from the present experiment examined where N-substituted analogues of 1,3-isoindolinedione with potent anticonvulsant activities were synthesized, further experiments are necessary to clarify the mechanisms of activity of these compounds.

REFERENCES

Abd El-Wahed, M. G., El-Manakhly, K., Hammad, H., and Barakat, A., Electrical behaviour of some phthalimide derivatives and their complexes with transition metals. *Bull. Korean Chem. Soc.*, 17, 285-288 (1996).

Al-Soud, Y. A. and Al-Masoudi, N. A., Synthesis and antitumor activity of some new phthalimide analogues. *Pharmazie*, 56, 372-375 (2001).

Bailleux, V., Vallée, L., Nuyts, J. P., Hamoir, G., Poupaert, J. H, Stables, J. P., and Vamecq, J., Compared the anticonvulsant activity and neurotoxicity of 4-amino-*N*-(2,6 dimethylphenyl) phthalimide and prototype antiepileptic drugs in mice and rats. *Epilepsia*, 36, 559-565 (1994a).

Bailleux, V., Vallée, L., Nuyts, J. P., and Vamecq, J., Synthesis and anticonvulsant of some *N*-phenylphthalimides. *Chem. Pharm. Bull.*, 42, 1817-1821 (1994b).

Bailleux, V., Vallée, L., Nuyts, J. P., and Vamecq, J., Original anticonvulsant properties of two *N*-phenylphthalimide derivatives. *Biomed. Pharmacother.*, 47, 463-464 (1994c).

Bailleux, V., Vallée, L., Nuyts, J. P., and Vamecq, J., Anti-convulsant activity of some 4-amino-*N*-phenylphthalimides and *N*-(3-amino-2-methylphenyl)phthalimides. *Biomed. Pharmacother.*, 48, 95-101 (1995).

Brownlee, K. A., Hodges, J. L., and Rosenblatt, M., The up and down method with small samples. *American Statistical Association Journal*, 262-277 (1953).

Bruce, R. D., An up and down procedure for acute toxicity testing. *Fundamental and Applied Toxicology*, 5, 151-157 (1985).

Chapman, J. M., Jr., Cocolas, G. H., and Hall, I. H., Hypolipidemic activity of phthalimide derivatives. 1. *N*-substituted phthalimide derivatives. *J. Med. Chem.*, 22, 1399-1402 (1979).

Chapman, J. M., Jr., Cocolas, G. H., and Hall, I. H., Hypolipidemic activity of phthalimide derivatives. IV: Further chemical modification and investigation of the hypolipidemic activity of *N*-substituted imides. *Journal of Pharmaceutical Sciences*, 72, 1344-1347 (1983).

Chapman, J. M, Jr., Wyrick S. D., Voorstad P. J., Maguire, G. H., Cocolas, G. H., and Hall, I. H., Hypolipidemic activity of phthalimide derivatives. V: Reduced and hydrolytic products of simple cyclic amides. *Journal of Pharmaceutical Sciences*, 73, 1482-1484 (1984).

Dunham, N. W. and Miya, T. S., A note on a simple apparatus for detecting neurological deficit in rats and mice. *Journal of the American Pharmaceutical Association*, XLVI, 208-209 (1957).

Hall, I. H., Voorstad P. J., Chapman, J. M, and Cocolas, G. H., Antihyperlipidemic activity of phthalimide analogues in rodents. *Journal of Pharmaceutical Sciences*, 72, 845-851 (1983).

Hashimoto, Y., Novel biological response modifiers derived from thalidomide. *Curr. Med. Chem.*, 5, 163-178 (1998).

Hashimoto, Y., Structural development of biological response modifiers based on thalidomide. *Bioorg. Med. Chem.*, 10, 461-479 (2002).

Leo, A. J., Calculating log P_{oct} from structures. *Chem. Rev.*, 63,1281-1306 (1993).

Maryanoff, B. E., Costanzo, M. J., Nortey, S. O., Greco, M. N., Shank, R. P., Schupsky, J. J., Ortegon, M. P., and Vaught, J. L., Structure-activity studies on anticonvulsant sugar sulfamates related to topiramate. Enhanced potency with cyclic sulfate derivatives. *J. Med. Chem.*, 41, 1315-1343 (1998).

Miyachi, H., Azuma, A., Kitamoto, T., Hayashi, K., Kato, S., Koga, M., Sato, B., and Hashimoto, Y., Potent novel nonsteroidal androgen antagonists with a phthalimide skeleton. *Bioorg. Med. Chem. Lett.*, 7, 1483-1488 (1997).

Moore, M. B. and Rapala, R. T., N-alkamine substituted phthalimides. *J. Amer. Chem. Soc.*, 68, 1657-1658 (1946).

Pandeya, S. N., Aggarwal, N., and Jain, J. S., Evaluation of semicarbazones for anticonvulsant and sedative-hypnotic properties. *Pharmazie*, 54, 300-302 (1999).

Shibata, Y., Shichita, M., Sasaki, K., Nishimura, K., Hashimoto, Y., and Iwasaki, S., *N*-alkylphthalimides: Structural requirements of thalidomidal action on 12-*O*- tetradecanoylphorbol-13-acetate induced tumor necrosis factor α production by human leukemia HL-60 cells. *Chem. Pharm. Bull.*, 43, 177-179 (1995).

Shimazawa, R., Miyachi, H., Takayama, H., Kuroda, K., Kato, F., Kato, M., Hashimoto, Y., Antiangiogenic activity of tumor necrosis factor-α-production regulators derived from thalidomide. *Biol. Pharm. Bull.*, 22, 224-226 (1999).

Sou, S., Mayumi, S., Takahashi, H., Yamasaki, R., Kadoya, S., Sodeoka M., and Hashimoto, Y., Novel α-glucosidase inhibitors with a tetrachlorophthalimide skeleton. *Bioorg. Med. Chem. Lett.*, 10, 1081-1084 (2000).

Swinyard, E. A., "Assay of antiepileptic drug activity in experimental animals" Standard tests in "Anticonvulsant drugs, International encyclopedia of Pharmacology and therapeutics" section 19, volume 1, Ed. By J. Mercier,

Pergamon press, Oxford/ New York. pp 47-65 (1972).

Takahashi, H., Sou, S., Yamasaki, R., and Sodeoka, M., Hashimoto Y. α-Glucosidase inhibitors with a phthalimide skeleton: Structure-activity relationship study. *Chem. Pharm. Bull.*, 48, 1494-1499 (2000).

Usifoh, C. O., Lambert, D. M., Wouters, J., and Scriba, G. K., Synthesis and anticonvulsant activity of *N,N*-phthaloyl derivatives of central nervous system inhibitory amino acids. *Arch. Pharm.*, 334, 323-331 (2001).