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ANTICONVULSANT AND NEUROTOXICITY EVALUATION OF NEW BROMOPHTHALIMIDOBUTYRYL AMIDE DERIVATIVES

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Abstract: A series of 4-(5-bromo-1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)-butyryl N-(substituted phenyl) amides **(3a-l)** were synthesized and evaluated for their anticonvulsant activity in MES test according to the protocols of Antiepileptic Drug Development (ADD) programme of National Institutes of Health (NIH, Bethesda, USA).The most active compounds of the series were **3a**, **3c**, **3d**, **3f**, **3g**, **3i** and **3k** at the dose of 30 mg/kg (*i.p.*) 0.5 h and 4 h after administration. In neurotoxic screen (NT), **3d**, **3i** and **3k** were less toxic when compared with the other compounds.

Keywords: anticonvulsants; MES test; neurotoxic; butyryl amides.

Epilepsy is not a disease, but a syndrome of different cerebral disorders of central nervous system (CNS), which is characterized by paroxysmal, excessive, and hyper synchronous discharges of large numbers of neurons (1). In India, studies have reported the prevalence rate of epilepsy varying from 1710 to 9780 cases per million population (2). Despite the optimal use of available antiepileptic drugs (AEDs), many patients with epilepsy fail to experience seizure control and others do so only at the expense of significant toxic effects (3). The limitations with the conventional AEDs highlighted the need for developing newer agents for epilepsy and the AED search has come a long way, particularly over the last two decades.

Many of the antiepileptic drugs exert their anticonvulsant actions by enhancing GABA-mediated inhibition in the CNS. This recognition has led to the rational development of several potentially useful new drugs that also enhance central inhibition through an interaction with the GABA system. One of them is progabide, a lipid soluble derivative of the amidated form of GABA, readily able to cross the blood brain barrier. Due to the fact that various substituted N-phenyl phthalimide derivatives have been reported as potential anticonvulsants (4, 5), an attempt has been made to synthesize phthalimidobutyryl amides by incorporating GABA into the phthalimide pharmacophore with an aim to reduce the toxicity and improve lipophilicity with promising anticonvulsant activity.

EXPERIMENTAL

Chemistry

The melting points were determined in open capillary tubes using Fisher 10-110 D Kjeldahl flask-300 mL containing liquid paraffin and are uncorrected. All the chemicals and solvents used were commercially procured from Central Drug House Pvt. Ltd. (C.D.H), E. Merk, S.D. Fine-Chem Ltd. and Qualigens. These solvents and reagents were of LR grade and purified before their use. The silica gel G (160-120 mesh) used for analytical chromatography (TLC) was obtained from E. Merck India Ltd. Solvent system used was chloroform: methanol (9:1, v/v). Ashless Whatman no.1 filter paper was used for vacuum filtration. The Proton Nuclear Magnetic Resonance Spectra (^{1}H-NMR) were recorded on Bruker model DRX-400 NMR spectrometer in $CDCl_3$/DMSO-d_6 using tetramethylsilane (TMS) as internal reference. Chemical shifts are reported in parts per million (δ, ppm). The Hitachi IR-spectrometer was used to record IR-spectra in KBr discs. Elemental analyses (C, H, and N) were undertaken with a Perkin-Elmer model analyzer and all analyses were consistent with theoretical values (within ± 0.4%) unless indicated otherwise.

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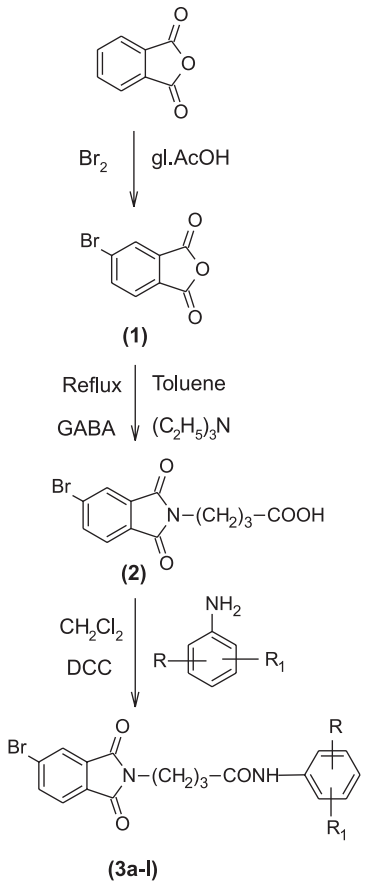
Table 1. Physical properties of 4-(5-bromo-1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)-butyryl N-(substituted phenyl)amides (**3a-l**).

Compd.	R	R_1	Mol. formula	M. p.[a] (^oC)	Yield %	R_f/R_m value[b] (δ ppm)	^{1}H-NMR (DMSO-d_6)
3a	H	4-NO_2	$C_{18}H_{14}BrN_3O_5$	160-163	67	0.69/-0.28	1.89 (m, 2H, CH_2), 2.43 (t, 2H, $COCH_2$, *J* =5.4, 7.2), 3.72 (t, 2H, NCH_2, *J* = 4.4, 8.6), 7.60-7.84 (m, 7H, Ar-H), 10.2 (s, 1H, CONH).
3b	3-Cl	4-Cl	$C_{18}H_{13}BrCl_2N_2O_3$	154-157	55	0.72/-0.41	1.89 (m, 2H, CH_2), 2.35 (t, 2H, $COCH_2$, *J* = 4.2, 7.2), 3.62 (t, 2H, NCH_2, *J* = 6.4, 8.4), 7.32-7.83 (m, 6H, Ar-H), 10.13 (s, 1H, CONH).
3c	H	4-OCH_3	$C_{19}H_{17}BrN_2O_4$	147-150	65	0.66/-0.28	1.89 (m, 2H, CH_2), 2.27 (t, 2H, $COCH_2$, *J* = 4.2, 7.2), 3.59 (t, 2H, NCH_2, *J* = 4.5, 6.8), 3.75 (s, 3H, OCH_3), 6.78-7.84 (m, 7H, Ar-H), 9.68 (s, 1H, CONH).
3d	H	4-CH_3	$C_{19}H_{17}BrN_2O_3$	170-173	58	0.71/-0.38	1.70 (m, 2H, CH_2), 2.19 (s, 3H, CH_3), 2.48 (t, 2H, $COCH_2$, *J* = 6.4, 7.2), 3.61 (t, 2H, NCH_2, *J* = 5.6, 7.2), 7.00-7.82 (m, 7H, Ar-H), 9.73 (s, 1H, CONH).
3e	H	2-OCH_3	$C_{19}H_{17}BrN_2O_4$	121-124	64	0.66/-0.28	1.02 (m, 2H, CH_2), 2. 47 (t, 2H, $COCH_2$, *J* = 6.2, 8.4), 3.61 (t, 2H, NCH_2, *J* = 6.7, 6.9), 3.77 (s, 3H, OCH_3), 6.81-7.85 (m, 7H, Ar-H), 9.03 (s, 1H, CONH).
3f	H	2-CH_3	$C_{19}H_{17}BrN_2O_3$	160-163	62	0.74/-0.45	1.89 (m, 2H, CH_2), 2.35 (t, 2H, $COCH_2$, *J* = 6.4, 7.2), 2.48 (s, 3H, CH_3), 3.62 (t, 2H, NCH_2, *J* = 5.6, 6.4), 7.00-7.83 (m, 7H, Ar-H), 9.21 (s, 1H, CONH).
3g	H	3-CH_3	$C_{19}H_{17}BrN_2O_3$	184-187	60	0.73/-0.43	1.70 (m, 2H, CH_2), 2.19 (s, 3H, CH_3), 2.48 (t, 2H, $COCH_2$, *J* = 6.4, 7.2), 3.61 (t, 2H, NCH_2, *J* = 5.6, 7.2), 7.01-7.83 (m, 7H, Ar-H), 9.73 (s, 1H, CONH).
3h	H	H	$C_{18}H_{15}BrN_2O_3$	158-161	72	0.68/-0.32	1.56 (m, 2H, CH_2), 2.35 (t, 2H, $COCH_2$, *J* = 7.2, 7.5), 3.66 (t, 2H, NCH_2, *J* = 6.6, 8.8), 6.97-7.84 (m, 8H, Ar-H), 9.76 (s, 1H, CONH).
3i	H	3-Cl	$C_{18}H_{14}BrClN_2O_3$	120-123	58	0.69/-0.34	1.44 (m, 2H, CH_2), 2.34 (t, 2H, $COCH_2$, *J* = 6.9, 7.2), 3.65 (t, 2H, NCH_2, *J* = 6.3, 6.5), 7.03-7.83 (m, 7H, Ar-H), 10.01 (s, 1H, CONH).
3j	2-C_2H_5	4-C_2H_5	$C_{22}H_{23}BrN_2O_3$	130-133	75	0.71/-0.38	1.14 (q, 2H, 4-Ar-CH_2), 1.92 (t, 3H, 4-Ar-CH_3), 1.21 (q, 2H, 2-Ar-CH_2), 2.14 (t, 3H, 2-Ar-CH_3), 2.28 (m, 2H, CH_2), 2.35 (t, 2H, $COCH_2$, *J* = 6.2, 7.2), 3.62 (t, 2H, NCH_2, *J* = 6.6, 8.7), 6.89-7.86 (m, 6H, Ar-H), 9.14 (s, 1H, CONH).
3k	H	4-F	$C_{18}H_{14}BrFN_2O_3$	203-206	63	0.66/-0.28	1.88 (m, 2H, CH_2), 2.46 (t, 2H, $COCH_2$, *J* = 7.2, 8.3), 3.63 (t, 2H, NCH_2, *J* = 6.1, 7.4), 7.02-7.90 (m, 7H, Ar-H), 10.01 (s, 1H, CONH).
3l	2-CH_3	6-CH_3	$C_{20}H_{19}BrN_2O_3$	140-143	70	0.74/-0.45	1.89 (m, 2H, CH_2), 2.33 (s, 6H, 2 X Ar-CH_3), 2.35 (t, 2H, $COCH_2$, *J* = 6.2, 8.4), 3.62 (t, 2H, NCH_2, *J* = 6.4, 5.8), 6.82-7.90 (m, 6H, Ar-H), 9.22 (s, 1H, CONH).

[a]Melting points of the compounds at their decomposition, [b]Solvent system-$CHCl_3$-CH_3OH (9:1, v/v), Elemental analysis for C, H, N was within ± 0.4% of the theoretical values.



Scheme 1. Synthetic route of compounds **(3a-l)**

Iodine chambers and UV lamps were used for visualization of TLC spots.

Preparation of 5-bromo-2-benzofuran-1,3-dione (**1**)

Phthalic anhydride (9 g, 0.06 mol) was dissolved in glacial acetic acid (40 mL) in 250 mL conical flask. Bromine (3.2 mL, 0.06 mol) dissolved in glacial acetic acid (45.6 mL) was added dropwise at 25-30^{o}C. After complete addition of bromine the temperature was raised to 65-70^{o}C until all the bromine has disappeared and the evolution of hydrogen bromide has almost ceased. The reaction mixture was poured into 300 mL of water, just sufficient sodium bisulphite solution was added to remove the orange color. Then the product was filtered, washed with cold water and dried. The compound (**1**) was recrystallized from ethanol.

Preparation of 4-(5-bromo-1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)butanoic acid (**2**)

5-Bromophthalic anhydride (22.7 g, 0.1 mol) and GABA (10.3 g, 0.1 mol) were taken in toluene and catalytic amount of triethylamine was added. The reaction mixture was strongly heated under reflux for 24 h. The resulting mixture was concentrated to half of its volume and poured into a beaker containing crushed ice. The crude product was filtered, washed with water and dried. The compound (**2**) was recrystallized from ethanol.

Table 2. Anticonvulsant activity and minimal motor impairment of compounds **(3a-l)**.

Compound No.	MES screen[a]		Toxicity screen[a]	
	0.5 h	4 h	0.5 h	4 h
3a	30	100	100	300
3b	-	300	-	300
3c	30	100	100	300
3d	30	100	300	300
3e	-	300	100	300
3f	30	100	100	300
3g	30	100	100	300
3h	100	300	300	300
3i	30	100	300	-
3j	100	300	-	-
3k	30	100	300	300
3l	30	30	-	300
Phenytoin[b]	30	30	100	100
Carbamazepine[b]	30	100	300	300

[a]Doses of 30, 100 and 300 mg/kg were administered *i.p.* The figures in the Table indicate the minimum dose whereby bioactivity was demonstrated in half or more of the mice. The animals were examined 0.5 and 4 h after injections were made. The dash (-) indicates an absence of activity at maximum dose administered (300 mg/kg).
[b]Data from references (7, 8).

General procedure for the preparation of 4-(5-bromo-1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)butyryl N-(substituted phenyl)amides (**3a-l**)

Equimolar quantities of 4-(5-bromo-1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)butanoic acid (1.49 g, 0.005 mol) and substituted anilines (0.005 mol) were condensed in the presence of N,N'-dicyclohexylcarbodiimide (DCC) (1.03 g, 0.005 mol) in dichloromethane, by stirring at ice-cold conditions (0-3^{o}C) for 6-8 h. The content was poured onto crushed ice and the obtained crude product was filtered, washed and dried. The compounds (**3a-l**) were recrystallized from ethanol.

In the IR spectra of compounds (**3a-l**) absorption bands for C-Br_{str} rotations appeared in the region 715.23-636.11 cm^{-1}, absorption bands of characteristic amide bonds C=O_{str} and NH_{str} and C=O_{str} (cyclic) vibration were observed in the range of 1703.91-1623.23 cm^{-1}, 3374.82-3318.83 cm^{-1} and 1762.63-1701.87 cm^{-1}, respectively. The physical properties and ^{1}H-NMR data are shown in Table 1.

RESULTS AND DISCUSSION

In order to reveal potential anticonvulsant profiles of the synthesized compounds, the MES model was employed according to the protocols of Antiepileptic Drug Development (ADD) programme of National Institutes of Health (NIH) (6). The anticonvulsant activity of (**3a-l**) were examined at 30,100 and 300 mg/kg *i.p.* in mice using MES test with data of standard drugs (7, 8). The MES test was applied 0.5 and 4 h after drug *i.p.* injection and protection was noted when compound had prevented tonic-clonic seizures.

Compound **3l** showed potent anticonvulsant activity in MES test (30 mg/kg *i.p.*, 0.5 h and 4 h). Compounds effective at a dose of 30 mg/kg *i.p.* after 0.5 h were **3a, 3c, 3d, 3f, 3g, 3i, 3k** and **3l**, whereas at a dose of 100 mg/kg *i.p.* after 0.5 h were **3h** and **3j**. Compound **3b** and **3e** did not show any protection at 0.5 h but showed protection at 4 h at a dose of 300 mg/kg. All the compounds were screened for the neurotoxicity successfully and passed the rotorod test without any sign of neurological deficit at 30 mg/kg *i.p.* after 0.5 h while the compounds **3a, 3c, 3e, 3f** and **3g** exhibited neurological deficit at the dose of 100 mg/kg *i.p.* after 0.5 h and the compounds **3d, 3h, 3i** and **3k** exhibited neurological deficit at the dose of 300 mg/kg *i.p.* after 0.5 h. Compounds **3j** was devoid of neurotoxicity after 0.5 h and 4 h whereas compound **3b** and **3l** were neurotoxic after 4 h (Table 2).

In conclusion, the studies revealed that the alkyl substitution at the aromatic ring was essential for activity being lipophilic in nature. Further modifications in the structure of these molecules might lead to the discovery of more potent anticonvulsant agents with lower neurotoxic effect.

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REFERENCES

1. Chen C., Chen Y.: Tetrahedron Lett. 45, 113 (2004).
2. Tale H.R.: Organic Lett. 4, 1641 (2002).
3. Mathis C.A., Wang Y., Holt D.P., Huang G.F., Debnath M.L.: J. Med. Chem. 46, 2740 (2003).
4. Vamecq J., Bac P., Herrenknecht C., Maurois P., Delcourt P., Stables P.J.: J. Med. Chem. 43, 1311 (2000).
5. Poupaert J., Hamoir G., Barbeaux P., Lambert D., Henichart P.J.: J. Pharm. Pharmacol. 47, 89 (1995).
6. Stables J.P., H.J. Kupferberg H.J. : The NIH anticonvulsant drug development (ADD) program: preclinical anticonvulsant screening project. In: Molecular and Cellular Targets for Anti-Epileptic Drugs. Avanzini G., Tanganelli P., Avoli M. Eds., pp. 191–198, John Libbey and Co. Ltd., London 1997.
7. White H.S., Woodhead J.H., Franklin M.R., Levy R.H., Mattson R.H.: Antiepileptic drugs 4, 99 (1995).
8. Dimmock J.R., Pandeya S.N., Quail J.W., Pugazhenthi U., Allen T.M., Kao G.Y.: Eur. J. Med. Chem. 30, 303 (1995).

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