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-isooindol-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 (1H-NMR) were recorded on Bruker model DRX-400 NMR spectrometer in CDCl3/DMSO-d6 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).

<table>
<thead>
<tr>
<th>Compd.</th>
<th>R</th>
<th>R_1</th>
<th>Mol. formula</th>
<th>M. p. (°C)</th>
<th>Yield %</th>
<th>R/Rm value a (δ ppm)</th>
<th>1H-NMR (DMSO-d6) formula</th>
<th>δ ppm</th>
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</thead>
<tbody>
<tr>
<td>3a</td>
<td>H</td>
<td>4-NO2</td>
<td>C_19H_14BrN_3O_5</td>
<td>160-163</td>
<td>67</td>
<td>0.69/-0.28</td>
<td>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).</td>
<td></td>
</tr>
<tr>
<td>3b</td>
<td>3-Cl</td>
<td>4-Cl</td>
<td>C_18H_13BrClN_2O_3</td>
<td>154-157</td>
<td>55</td>
<td>0.72/-0.41</td>
<td>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).</td>
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</tr>
<tr>
<td>3c</td>
<td>H</td>
<td>4-OCH_3</td>
<td>C_19H_17BrN_2O_4</td>
<td>147-150</td>
<td>65</td>
<td>0.66/-0.28</td>
<td>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).</td>
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<tr>
<td>3d</td>
<td>H</td>
<td>2-C_2H_5</td>
<td>C_22H_23BrN_2O_3</td>
<td>170-173</td>
<td>58</td>
<td>0.71/-0.38</td>
<td>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).</td>
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</tr>
<tr>
<td>3e</td>
<td>H</td>
<td>2-OCH_3</td>
<td>C_19H_17BrN_2O_4</td>
<td>121-124</td>
<td>64</td>
<td>0.66/-0.28</td>
<td>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).</td>
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<tr>
<td>3f</td>
<td>H</td>
<td>2-C_3H_7</td>
<td>C_20H_19BrN_2O_3</td>
<td>160-163</td>
<td>62</td>
<td>0.74/-0.45</td>
<td>1.89 (m, 2H, CH_2), 2.35 (t, 2H, COCH_2, J = 6.4, 7.2), 2.48 (t, 2H, COCH_2, J = 6.4, 7.2), 3.62 (t, 2H, NCH_2, J = 5.6, 6.4), 7.00-7.83 (m, 7H, Ar-H), 9.21 (s, 1H, CONH).</td>
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<tr>
<td>3g</td>
<td>H</td>
<td>3-CH_3</td>
<td>C_19H_17BrN_2O_4</td>
<td>184-187</td>
<td>60</td>
<td>0.73/-0.43</td>
<td>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).</td>
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<tr>
<td>3h</td>
<td>H</td>
<td>H</td>
<td>C_19H_14BrN_3O_5</td>
<td>158-161</td>
<td>72</td>
<td>0.68/-0.32</td>
<td>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).</td>
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</tr>
<tr>
<td>3i</td>
<td>H</td>
<td>3-Cl</td>
<td>C_19H_16BrClN_2O_5</td>
<td>120-123</td>
<td>58</td>
<td>0.69/-0.34</td>
<td>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).</td>
<td></td>
</tr>
<tr>
<td>3j</td>
<td>2-C_2H_5</td>
<td>4-C_2H_5</td>
<td>C_22H_23BrN_2O_3</td>
<td>130-133</td>
<td>75</td>
<td>0.71/-0.38</td>
<td>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).</td>
<td></td>
</tr>
<tr>
<td>3k</td>
<td>H</td>
<td>4-F</td>
<td>C_19H_14BrF_3N_2O_3</td>
<td>203-206</td>
<td>63</td>
<td>0.66/-0.28</td>
<td>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).</td>
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<tr>
<td>3l</td>
<td>2-CH_3</td>
<td>6-CH_3</td>
<td>C_23H_18BrN_3O_5</td>
<td>140-143</td>
<td>70</td>
<td>0.74/-0.45</td>
<td>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).</td>
<td></td>
</tr>
</tbody>
</table>

aMelting points of the compounds at their decomposition. bSolvent 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°C. After complete addition of bromine the temperature was raised to 65-70°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).

<table>
<thead>
<tr>
<th>Compound</th>
<th>MES screen</th>
<th>Toxicity screen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5 h</td>
<td>4 h</td>
</tr>
<tr>
<td>3a</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>3b</td>
<td>-</td>
<td>300</td>
</tr>
<tr>
<td>3c</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>3d</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>3e</td>
<td>-</td>
<td>300</td>
</tr>
<tr>
<td>3f</td>
<td>30</td>
<td>100</td>
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<tr>
<td>3g</td>
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<td>3i</td>
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<td>3j</td>
<td>100</td>
<td>300</td>
</tr>
<tr>
<td>3k</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>3l</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>30</td>
<td>100</td>
</tr>
</tbody>
</table>

Notes:

- 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).
- Data from references (7, 8).
General procedure for the preparation of 4-(5-bromo-1,3-dioxo-1,3-dihydro-2H-isooindol-2-yl)butyryl N-(substituted phenyl)amides (3a-l)

Equimolar quantities of 4-(5-bromo-1,3-dioxo-1,3-dihydro-2H-isooindol-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°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=Ostr (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 1H-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 successfullly 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 alky 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.

Acknowledgments

The authors are thankful to Jamia Hamdard, New Delhi for 1H-NMR spectral data and Arbro Pharmaceuticals for IR data. Financial support to this work from UGC, New Delhi is gratefully acknowledged.

REFERENCES


Received: 15. 04. 2008