Synthesis and Anticonvulsant Activity of Some New Proline-Benzofuran-Acetamide/Propanamide/Butanamide Hybrids

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ABSTRACT Six new proline-benzofuran-acetamide/propanamide/butanamide hybrids (6a-b, 7a-b, 8a-b) were synthesized. The structures of the synthesized compounds were confirmed on the basis of their elemental analyses and spectral characterization. The anticonvulsant screening was determined using the two classical animal models, namely, maximal electroshock seizure (MES) and subcutaneous metrazol (scMET) models followed by motor impairment study by rotarod test in mice. Compounds 6b, 7b, 8a, and 8b showed potent anticonvulsant activity in MES model with median effective dose (ED50) of 65.3 mg/kg, 36.5 mg/kg, 56.7 mg/kg, and 31.6 mg/kg, respectively. Compounds 7b (100 mg/kg, 0.5 h; 300 mg/kg, 4 h) and 8b (100 mg/kg, 0.5 h and 4 h) also showed significant anticonvulsant activity against scMET test.

KEYWORDS Proline, Benzofuran, Acetamide/propanamide/butanamide, Anticonvulsant activity.

INTRODUCTION

Epilepsy is one of the most common chronic central nervous system diseases which is characterized by the appearance of recurrent spontaneous convulsions, in which there is a disturbance of movement, behavior, sensation, perception, and/or consciousness. According to the epidemiological survey report, almost 1% of the worldwide population suffers from epilepsy.[1] Epilepsy is an illness that can happen in both sexes and at all age groups.[2] Many of the

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currently available antiepileptic drugs are associated with severe adverse effects that limit their usage in short- or long-term treatment. These comprise drowsiness, ataxia, gingival hyperplasia, megaloblastic anemia, nausea, hepatic dysfunction, vomiting, osteomalacia, rashes, Stevens–Johnson syndrome, etc. Furthermore, about one-third of epileptic patients do not indicate satisfactory response to the available drugs in the market.[13-5] Therefore, in antiepileptic drug development research, there is an intense requirement and demand for the development of more effective anticonvulsant drugs with lesser side effects and distinctive mechanisms of action.

Amides are a class of compounds offering a broad range of biological activities. Many acacetamide derivatives are reported to have good anticonvulsant activity.[11-13] Propanamides and butanamides are also reported to have significant anticonvulsant activity.[14-19] Benzofuran derivatives have miscellaneous pharmacological activities[20] such as anti-inflammatory,[21] anticonvulsant,[22,23,22] analgesic,[23] antimicrobial,[24,25] antioxidant,[26] neuroprotective,[26,28] anticancer,[26] inhibition of NF-kB, protein kinase, and 24-hydroxylase.[27,29,30] Benzofuran, a multipurpose heterocyclic moiety holding a preliminary anticonvulsant activity, has been carefully chosen. These findings encouraged us to hypothesize that molecule comprising both benzofuran-acetamide/propanamide/butanamide and an amino acid proline would have a beneficial effect in fighting this disease. The previous research from our laboratory has validated diversified anticonvulsant activity of benzofuran acetamides, propanamides, butanamides, leucine, and phenylalanine-benzofuran-acetamide/propanamide/butanamide hybrids.[11-14,16,19,30]

In this study, the target compounds, 1-(2-(2-benzoyl/4-chlorobenzoyl)benzofuran-3-ylamino)-4-oxobutyl)propyridine-2-carboxylic acid (6a-b), 1-(2-(2-benzoyl/4-chlorobenzoyl)benzofuran-3-ylamino)-3-oxopropyl)pyridine-2-carboxylic acid (7a-b), and 1-(4-(2-benzoyl/4-chlorobenzoyl)benzofuran-3-ylamino)-4-oxobutyl)pyridine-2-carboxylic acid (8a-b) were synthesized by stirring 2-chloro-N-(2-benzoyl)4-chlorobenzoyl benzofuran-3-ylacetamide (3a-b), 3-chloro-N-(2-benzoyl)4-chlorobenzoyl benzofuran-3-ylacetamide (3a-b), 3-chloro-N-(2-benzoyl)4-chlorobenzoyl benzofuran-3-yl)propanamide (4a-b), and 4-chloro-N-(2-benzoyl)4-chlorobenzoyl benzofuran-3-yl)-butanamide (5a-b) with proline (pyrrolidine-2-carboxylic acid) in 1-butanol containing anhydrous K₂CO₃ in the presence of KI to obtain the title compounds 6a-b, 7a-b, and 8a-b, respectively. All the compounds were confirmed by 1H-nuclear magnetic resonance (NMR), mass spectroscopy, infrared (IR), and elemental analyses.

### Experimental Section

**Chemistry**

All the solvents and reagents used were of laboratory grade and obtained from the commercial sources. The progress of the reactions was observed by thin-layer chromatography (TLC) on silica gel G pre-coated plates using cyclohexane:ethyl acetate (8:2) as solvent system. Iodine chamber was used for the visualization of the TLC spots. The melting points (°C) of the synthesized compounds are shown in Table 1. In the first step, the reaction of 2-hydroxybenzonitrile with phenacetyl bromide/p-chlorophenacyl bromide in dimethylformamide containing anhydrous K₂CO₃ produced intermediate

### RESULTS AND DISCUSSION

**Chemistry**

Target compounds proline-benzofuran-acetamide/propanamide/butanamide hybrids (6a-b, 7a-b, and 8a-b) were synthesized according to Scheme 1 and their physicochemical properties are shown in Table 1. In the first step, the reaction of 2-hydroxybenzonitrile with phenacetyl bromide/p-chlorophenacyl bromide in dimethylformamide containing anhydrous K₂CO₃ produced intermediate

### Table 1: Physicochemical properties of proline-benzofuran-acetamide/propanamide/butanamide hybrids (6a-b, 7a-b, and 8a-b)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mol. formula</th>
<th>°M.P. (°C)</th>
<th>Yield (%)</th>
<th>Rf value</th>
</tr>
</thead>
<tbody>
<tr>
<td>6a</td>
<td>C₂₂H₂₀N₂O₅</td>
<td>392.40</td>
<td>276-278</td>
<td>73</td>
</tr>
<tr>
<td>6b</td>
<td>C₂₂H₂₀N₂O₅</td>
<td>426.85</td>
<td>298-300</td>
<td>76</td>
</tr>
<tr>
<td>7a</td>
<td>C₂₂H₂₀N₂O₅</td>
<td>406.43</td>
<td>286-288</td>
<td>76</td>
</tr>
<tr>
<td>7b</td>
<td>C₂₂H₂₀N₂O₅</td>
<td>440.88</td>
<td>308-310</td>
<td>77</td>
</tr>
<tr>
<td>8a</td>
<td>C₂₂H₂₀N₂O₅</td>
<td>420.46</td>
<td>318-320</td>
<td>80</td>
</tr>
<tr>
<td>8b</td>
<td>C₂₂H₂₀N₂O₅</td>
<td>454.90</td>
<td>330-332</td>
<td>78</td>
</tr>
</tbody>
</table>

*Melting point of the compounds at their decomposition. *Solvent system – cyclohexane: ethyl acetate (8:2)*
Scheme 1: Synthesis of target compounds. Reagents and conditions: (a) Dimethylformamide, potassium carbonate ($\text{K}_2\text{CO}_3$), stir, 3 h; (b) MeOH, MeONa, stir, 1 h; (c) ClCH$_2$COCl, reflux, 30 min; (d) ClCH$_2$CH$_2$COCl, reflux, 30 min; (e) ClCH$_2$CH$_2$CH$_2$COCl, reflux, 30 min; and (f) Proline, 1-butanol, K$_2$CO$_3$, KI, reflux, 10 h

Table 2: Anticonvulsant activity of proline-benzofuran-acetamide/propanamide/butanamide hybrids (6a-b, 7a-b, and 8a-b).

<table>
<thead>
<tr>
<th>Compound</th>
<th>MES</th>
<th>*ED$_{50}$ (mg/kg)</th>
<th>Subcutaneous metrazol</th>
<th>Neurotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5 h</td>
<td>4 h</td>
<td>0.5 h</td>
<td>4 h</td>
</tr>
<tr>
<td>6a</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6b</td>
<td>100</td>
<td>300</td>
<td>65.3</td>
<td>-</td>
</tr>
<tr>
<td>7a</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7b</td>
<td>30</td>
<td>100</td>
<td>36.5</td>
<td>100</td>
</tr>
<tr>
<td>8a</td>
<td>100</td>
<td>300</td>
<td>56.7</td>
<td>-</td>
</tr>
<tr>
<td>8b</td>
<td>30</td>
<td>30</td>
<td>31.6</td>
<td>100</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>30</td>
<td>30</td>
<td>10.3</td>
<td>-</td>
</tr>
<tr>
<td>Carbamazepine$^*$</td>
<td>30</td>
<td>100</td>
<td>-</td>
<td>100</td>
</tr>
</tbody>
</table>

*ED$_{50}$ was calculated using XLSOFT (Evaluation Ver. 2012.2.01) on the basis of maximum effect observed at 30 min. *Activity reported$^{[39]}$
compounds were determined in open glass capillary on a Veego Melting Point Apparatus (India) and are uncorrected. 

1H-NMR spectra were recorded on Bruker model DRX-300-NMR spectrometer in CDCl₃ using tetramethylsilane as an internal standard. Elemental analyses (C, H, and N) were conducted with a PerkinElmer model 2400 analyzer and all analyses were found to be consistent with theoretical values unless indicated (within 0.4%). IR spectra were recorded in PerkinElmer model spectrometer by preparing KBr pellets for the synthesized compounds.

**Syntheses**

2-chloro-N-(2-(benzoyl)/(4-chlorobenzoyl)benzofuran-3-yl)-acetamide (3a-b), 3-chloro-N-(2-(benzoyl)/4-chlorobenzoyl)benzofuran-3-yl)-propanamide (4a-b), and 4-chloro-N-(2-(benzoyl)/(4-chlorobenzoyl) benzofuran-3-yl)-butanamide (5a-b) were prepared following the reported methods. [11,14-16]

**General synthetic method for** 1-(2-(benzoyl)/(4-chlorobenzoyl)benzofuran-3-ylaminom)-3-oxopropyl)pyrrolidine-2-carboxylic acid (6a/b), 1-(3-(2-(benzoyl)/(4-chlorobenzoyl)benzofuran-3-ylaminom)-3-oxopropyl)pyrrolidine-2-carboxylic acid (7a/b) and 1-(4-(2-(benzoyl)/(4-chlorobenzoyl)benzofuran-3-ylaminom)-4-oxobutyl)pyrrolidine-2-carboxylic acid (8a/b) were prepared according to the reported methods. [32-34]

**Antiepileptic Drug Development Program protocol in mice** 6a-b, 7a-b, and 8a-b was performed according to the Antiepileptic Drug Development Program protocol in mice. [32-37]

**Maximal electroshock seizure (MES)**

A 60 Hz alternating current of 50 mA was delivered for 0.2 s by pinnal electrodes in mice. The mice were administered with 30, 100, and 300 mg/kg of the synthesized compounds by i.p. route after 0.5 and 4 h intervals in 0.05 ml volume. An animal is considered protected from MES-induced seizure on termination of the hindlimb tonic extensor component of the convulsion. [32-34]
scMET-induced seizure

Metrazol s.c. injection produces clonic seizures in mice. scMET test model identifies the ability of a test compound to rise the seizure threshold of an animal and thus protects it from showing clonic seizure. The mice were administered with 30, 100, and 300 mg/kg of the synthesized compounds by i.p. route after 0.5 and 4 h intervals with standard metrazol, 50 mg/kg, s.c. An animal is considered protected from scMET-induced seizure on nonappearance of incidence of clonic spasms of the fore- and/or hindlimbs, jaws, or vibrissae, for approximately 3–5 s.[35]

Neurotoxicity study

The possible neurotoxic effects of the drug in mice were measured using a rotarod test. The synthesized compounds were administered at the dose of 30, 100, and 300 mg/kg, i.p. The mice were trained to stay on the knurled rod of diameter 3.2 cm that rotated with 10 rpm. Control mice can maintain their equilibrium on a rotating rod for longer period of time. Neurotoxicity was manifested in the form of violations of coordination of movement on the rotating rod for at least 1 min (1 min observation period) in each of three trials.[38]

Doses of 30, 100, and 300 mg/kg were prepared in PEG400 and were administered i.p. The animals were examined 0.5 and 4 h after injections were administered.

CONCLUSION

All the proline-benzofuran-acetamide/propanamide/butanamide hybrids (6a-b, 7a-b, and 8a-b) were synthesized in good yield, characterized, and evaluated for anticonvulsant activity and neurotoxicity. Compounds 6b, 7b, 8a, and 8b showed potent anticonvulsant activity in MES model with median effective dose (ED₅₀) of 65.3 mg/kg, 36.5 mg/kg, 56.7 mg/kg, and 31.6 mg/kg, respectively. Compounds 7b (100 mg/kg, 0.5 h; 300 mg/kg, 4 h) and 8b (100 mg/kg, 0.5 h and 4 h) also proved significant anticonvulsant activity against scMET test. All the compounds showed no sign of neurotoxicity at the dose of 300 mg/kg except 7b and 8b. The compounds 7b and 8b showed maximum activity and would be considered as a lead for further optimization as anticonvulsant agent.

ACKNOWLEDGMENTS

This publication was supported by the Deanship of Scientific Research at Prince Sattam bin Abdulaziz University, Al-Kharj, Kingdom of Saudi Arabia.

REFERENCES


