ORIGINAL ARTICLE

Anticonvulsant activity and neuroprotection assay of 3-substituted-N-aryl-6,7-dimethoxy-3a,4-dihydro-3H-indeno[1,2-c]pyrazole-2-carboxamide analogues

Mohamed Jawed Ahsan *

Department of Pharmaceutical Chemistry, Maharishi Arvind College of Pharmacy, Jaipur, Rajasthan 302 023, India

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1. Introduction

Despite the development of newer antiepileptic drugs (AEDs), epilepsy is still the 3rd most devastating neurological disorder and nearly 1–2% of the world’s population is afflicted with this disorder (Global Campaign Against Epilepsy, 2001; White, 2003). Treatment of epilepsy with the available AEDs is inadequate and associated with toxic and idiosyncratic effects (Duncan, 2002; Dimmock et al., 1995). Today anticonvulsant agents having neuroprotective actions have received considerable attention in the treatment of epilepsy since epileptic seizure and neurodegeneration share the common aspects of their underlying pathophysiology and some AEDs are equally effective neuroprotectives (Stanisaw et al., 2007). Phenytoin, topiramate, and zonisamide are examples of AEDs, which are also used as neuroprotective agents (Jain, 2011). It has been reported that neurodegeneration is the major neurobiological abnormality in epileptic brain (Naegle, 2007). Neuroprotection also aims to prevent or slow disease progression and secondary injuries by halting or at least slowing the loss of neuron (Seidl and Potashkin, 2011), and neuroprotective agents are used in an attempt to save ischaemic neurons in the brain for irreversible injury (Green, 2004). The development of a newer drug with increase seizure control, increased tolerability, better safety and pharmacokinetic properties, and with neuroprotective action might be a potential approach to reduce the number of epileptic cases. Earlier we have reported the anticonvulsant activity of pyrazoline analogues (Ahsan et al., 2012, 2013). Nowadays organotypic hippocampal brain slice cultures have been gaining importance as an

* Tel.: +91 9694087786; fax: +91 141 2335120. E-mail address: jawedpharma@gmail.com

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early stage drug screening tool in the neuroprotection arena (Norenberg et al., 2005). We can evaluate the ability of novel compounds to prevent excitotoxic cell death when these compounds are added either in conjunction with, or following, the treatment with agents that induce excitotoxic cell death. The aim of the present study was to investigate the anticonvulsant and neuroprotective activities of the synthesized pyrazoline compounds.

2. Experimental

2.1. Chemistry

The entire chemicals were supplied by E. Merck (Germany) and S.D. Fine Chemicals (India). Melting points were determined by open tube capillary method and are uncorrected. Purity of the compounds was checked on TLC plates (silica gel G) using eluants benzene–acetone (9:1), the spots were located under iodine vapours or UV light. IR spectra were obtained on a Schimadzu 8201 PC, FT-IR spectrometer (KBr pellets). 1H NMR spectra were recorded on a Bruker AC 300 MHz spectrometer using TMS as internal standard in DMSO. Mass spectra were recorded on a Bruker Esquire 3000 spectrometer using TMS as internal standard in N-(4-chlorophenyl)-6,7-dimethoxy-3a,4-dihydro-3H-indeno[1,2-c]pyrazole-2-carboxamide (4b) M.p. 116 °C; yield 72% (Ahsan et al., 2011).

2.2. General method for the synthesis of 2-substituted-5,6-dimethoxy-2,3-dihydro-1H-indene-1-one derivatives (3a–c)

5,6-Dimethoxy-2,3-dihydro-1H-inden-1-one (1) (0.001 mol) with appropriate aromatic aldehyde (2a–c) (0.001 mol) in diluted methanolic sodium hydroxide solution was stirred under room temperature for 4 h. The resulting solution was allowed to stand overnight and then the reaction mixture was poured into cold water and neutralized with dilute HCl. The solid was filtered, dried and recrystallized with ethanol furnished the 2-substituted-5,6-dimethoxy-2,3-dihydro-1H-indene-1-one (3a–c) (Ahsan et al., 2011).

2.3. General method for the synthesis of 3a,4-dihydro-3H-indeno[1,2-c]pyrazole-2-carboxamide analogues (4a–c)

2-substituted-5,6-dimethoxy-2,3-dihydro-1H-indene-1-one (3a–c) (0.01 mol) and substituted phenyl semicarbazide (0.01 mol) in 20 ml glacial acetic acid were refluxed for 12 h. The excess solvent was removed under reduced pressure and then the reaction mixture was poured into crushed ice. The solution mass was filtered, dried and recrystallized with ethanol furnished 3-substituted-N-aryl-6,7-dimethoxy-3a,4-dihydro-3H-indeno[1,2-c]pyrazole-2-carboxamide (Ahsan et al., 2011).

2.3.1. 3-[(3,4-Dimethoxyphenyl)-N-(3-chloro-4-fluorophenyl)-6,7-dimethoxy-3a,4-dihydro-3H-indeno[1,2-c]pyrazole-2-carboxamide (4a)

Yield 78%, m.p. 202 °C, IR (KBr) cm⁻¹: 3331 (NH), 1680 (C=O), 1561 (C=N), 1157 (C–N), 789 (C–F), 765 (C–Cl); 1H NMR (300 MHz, DMSO-d₆); δ 3.18–3.22 (1H, m, CH), 3.31–3.33 (2H, J = 6.0 Hz, CH₂), 3.81 (6H, s, OCH₃), 3.83 (6H, s, OCH₃), 4.01 (1H, d, J = 6.1 Hz, CH), 7.29–7.89 (8H, m, Ar), 10.05 (1H, s, CONH); MS: m/z, M+ 525, M+ + 2 527.

2.3.2. 3-[(4-Fluorophenyl)-N-(4-chlorophenyl)-6,7-dimethoxy-3a,4-dihydro-3H-indeno[1,2-c]pyrazole-2-carboxamide (4b)

M.p. 116 °C; yield 72% (Ahsan et al., 2011).

2.3.3. 3-[(4-Chlorophenyl)-N-(4-chlorophenyl)-6,7-dimethoxy-3a,4-dihydro-3H-indeno[1,2-c]pyrazole-2-carboxamide (4c)

M.p. 128 °C; yield 70% (Ahsan et al., 2011).

2.4. Anticonvulsant screening

The anticonvulsant screening of the compound was done according to the Antiepileptic Drug Development Programme (ADD) protocol reported elsewhere (Swinyard et al., 1989; White et al., 1995a,b; Dunham and Miya, 1957; Barton et al., 2001; Toman et al., 1952).

2.5. In vitro hippocampal slice culture neuroprotection assay

The “Primary Screen Experiment” is a qualitative assessment of the ability of a compound to prevent excitotoxic cell death (Norenberg et al., 2005). Slice cultures were prepared as per the reported method (Stoppini et al., 1991; Norenberg, 2004). Sprague–Dawley rat pups (10–11 days) were anesthetized with pentobarbital, sacrificed, and their brains rapidly removed and placed on sterile filter paper. The brain was kept moist with sterile filtered dissection buffer. The brain was then bisected sagittally and the brainstem removed, revealing the cortex and underlying hippocampus. The tissue was then placed on a McElwain tissue chopper plate and then sliced into 400 μm thick sections. Slices were placed in a small petri dish and the hippocampi were then separated under microscopic control. Sections were then transferred into a six well culture plate containing a membrane insert (30 mm, Millipore). Each well contained four slices and 1 ml of media contained: 50% Opti MEM, 25% horse serum, 25% Hank’s balanced salt solution and d-glucose (25 mM). The cultures were then placed in an incubator (5% CO₂, 95% O₂) and maintained at 36 °C. One day prior to the experiment, the culture medium was replaced with 1.0 ml of serum-free media containing neurobasal

![Scheme 1](http://dx.doi.org/10.1016/j.arabjc.2013.10.023)
medium, 25 mM d-glucose, 1 mM l-glutamine and 2% B27 supplement. Organotypic hippocampal slice cultures were treated with N-methyl-D-aspartate (NMDA) or kainic acid (KA) to induce neuronal cell death. Propidium iodide, a membrane-impermeant compound, was included in all wells of the culture plate. Dying cells have compromised cell membranes, thus propidium iodide may diffuse into the cell, intercalate with DNA and fluoresce and the intensity of the propidium iodide fluorescence is proportional to the amount of cell death in the individual slices. Hippocampal slice cultures were treated with the excitotoxin alone or as indicated above, with the excitotoxin and either one or two investigational compounds at the concentrations indicated. If neuroprotection occurs as a consequence of the added compound, slice cultures will have a visibly reduced fluorescent intensity when compared to the slice cultures that have been treated with the excitotoxin alone.

### 3. Results and discussion

#### 3.1. Chemistry

The 3-substituted-N-aryl-6,7-dimethoxy-3a,4-dihydro-3H-indeno[1,2-c]pyrazole-2-carboxamide analogues (4a–e) described in this study was synthesized as per the reported method and is summarized in Scheme 1 (Ahsan et al., 2011) and their physical constants are described in Table 1. In the initial step equimolar mixtures of 5,6-dimethoxy-2,3-dihydro-1H-indene-1-one (1) and aromatic aldehydes (2a–c) in diluted methanolic sodium hydroxide solution were stirred at room temperature giving 2-(4-pyridyl)-5,6-dimethoxy-2,3-dihydro-1H-indene-1-one (3a–c). In the subsequent step 2-substituted-5,6-dimethoxy-2,3-dihydro-1H-indene-1-one was treated with appropriate substituted phenyl semicarbazides furnishing the title compound (4a–e). The substituted

<table>
<thead>
<tr>
<th>Compound</th>
<th>Excitotoxin</th>
<th>Insult duration (h)</th>
<th>Primary screen result</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>Kainic acid (KA)</td>
<td>4</td>
<td>No neuroprotection observed</td>
</tr>
<tr>
<td>4b</td>
<td>Kainic acid (KA)</td>
<td>4</td>
<td>Neuroprotection observed</td>
</tr>
<tr>
<td>4c</td>
<td>Kainic acid (KA)</td>
<td>4</td>
<td>No neuroprotection observed</td>
</tr>
</tbody>
</table>

Table 3 *In vitro* hippocampal slice culture neuroprotection assay of compound 4b.

<table>
<thead>
<tr>
<th>Compound concentration (μM)</th>
<th>No. of slices</th>
<th>Total propidium iodide uptake (%) ± (S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>16</td>
<td>51.4 ± 1.6</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>36.6 ± 2.8*</td>
</tr>
<tr>
<td>30</td>
<td>8</td>
<td>43.4 ± 2.7*</td>
</tr>
<tr>
<td>100</td>
<td>8</td>
<td>26.2 ± 1.9*</td>
</tr>
<tr>
<td>300</td>
<td>8</td>
<td>26.4 ± 1.5*</td>
</tr>
</tbody>
</table>

* Data is significantly different from excitotoxin treatment alone, p < 0.05.
phenyl semicarbazides was synthesized as per the reported method (Amir et al., 2010). The yields of the compounds ranged from 70% to 78% after recrystallization with absolute ethanol. The reactions were monitored by TLC using benzene–acetone (9:1) as mobile phase and the purity of the compounds was checked by elemental analysis and mass spectroscopy.

3.2. Anticonvulsant screening

The compound 4b showed protection against maximal electroshock induced seizure (MES) and subcutaneous metrazole (scMET) induced seizure at 300 mg/kg dose at 0.5 and 4 h, and 100% (4/4, 0.25 h), 75% (3/4, 1.0 h) and 50% (2/4, 0.5 h) protection in 6 Hz psychomotor seizure test devoid of any neurotoxicity or toxicity. The compound 4b also showed 50% (2/4, 1.0 h) and 25% (1/4, 2.0 h) protection in MES screen after oral administration in rat at dose 30 mg/kg without any toxicity (Ahsan et al., 2013). The compound 4a showed 50% (2/4, 0.5–1.0 h) and 25% (1/4, 2.0 h), while compound 4c showed 100% (4/4, 0.25–0.5 h), 75% (3/4, 1.0 h) and 25% (1/4, 4.0 h) protection in 6 Hz psychomotor seizure test (Ahsan et al., 2012).

3.3. In vitro hippocampal slice culture neuroprotection assay

The compounds 4a and 4c have not shown any neuroprotection activity excitotoxin against kainic acid (Table 2). In vitro hippocampal slice culture neuroprotection assay of compound 4b showed that the percent of total propidium iodide uptake was 36.6 ± 2.8, 43.4 ± 2.7, 26.2 ± 1.9 and 26.4 ± 1.5 when compound 4b was added with KA at concentrations 10, 30, 100 and 300 μM respectively. The data are given in Table 3 which show significant neuroprotection of compound 4b when coapplied with KA. The concentration–response curve was determined using Probit analysis to determine the IC50. The IC50 of compound 4b was found to be 159.20 ± 1.21 μM. The compound 4b attenuated KA-mediated cell death in organotypica hippocampal slice cultures is shown in Fig. 1. The intensity of the propidium iodide fluorescence reduced as the concentration of the compound 4b increased.

Table 1: Physical constant of 3-substituted-N-aryl-6,7-dimethoxy-3a,4-dihydro-3H-indeno[1,2-c]pyrazole-2-carboxamide analogues (4a–c)

<table>
<thead>
<tr>
<th>Compound</th>
<th>ADD No.</th>
<th>Ar1</th>
<th>Ar2</th>
<th>Yield (%)</th>
<th>M.p. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>439066</td>
<td>3,4-Dimethoxyphenyl-</td>
<td>3-Chloro-4-fluorophenyl-</td>
<td>78</td>
<td>202</td>
</tr>
<tr>
<td>4b</td>
<td>439068</td>
<td>4-Pyrinyl-</td>
<td>4-Chlorophenyl-</td>
<td>72</td>
<td>116</td>
</tr>
<tr>
<td>4c</td>
<td>439070</td>
<td>4-Fluorophenyl-</td>
<td>4-Chlorophenyl-</td>
<td>70</td>
<td>128</td>
</tr>
</tbody>
</table>

4. Conclusions

In summary compound 4b was found to be active and showed protection against seizure in MES, scMET and 6 Hz psychomotor seizure test. The compound 4b also showed neuroprotection against in vitro hippocampal slice culture when coapplied with KA. The pyrazoline derivative reported in this study may provide valuable therapeutic intervention for the treatment of epilepsy.

Conflict of interest

The author declares no conflict of interest.

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References


