PHARMACOLOGICAL EVALUATION OF ACUTE AND SUBACUTE TOXICITY AND ANTIDEPRESSANT EFFECT AFTER ACUTE ADMINISTRATION OF NOVEL N-SUBSTITUTED BENZAMIDES

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Abstract

The new synthesized N-substituted-benzamides are structurally related to tiapride, sulpiride or amisulpride, drugs mainly used in the treatment of psychosis and depressive states. This dual activity is based on the dose-dependent antagonist properties towards dopamine receptors. At low antidepressant doses, benzamides preferentially block presynaptic dopamine D3 autoreceptors, that control dopamine synthesis and its release into synaptic cleft, whereas at higher antipsychotic doses, the blocking is moved towards postsynaptic dopamine D2 receptors.

Our objective was to investigate the acute and subacute toxicity of the new synthesized compounds on mice, followed by the antidepressant activity after intraperitoneally (i.p.) acute administration in non-depressed mice, using the forced swimming test (FST).

LD50 after i.p. and per os (p.o.) administration were determined on mice, values being close to those of other benzamides therapeutically used. The histopathological examination showed, for one compound, renal and hepatic toxicity in one of five examined samples.

FST evidenced a significant antidepressant effect (p<0.05, „t” Student test), for two of three compounds, when compared with the control group.

Rezumat

Au fost sintetizați noi compuși benzamidici N-substituți, înruditi structural cu tiapridul, sulpiridul sau amisulpridul, medicamente folosite atât în tratamentul manifestărilor psihotice, cât și al stârtilor depresive. Această acțiune duală se datorează activității antagonistice, dependente de doză, asupra receptorilor dopaminergici. Astfel, la dozele mici antidepresive, benzamidele blochează receptorii presinapticii D3, care modulează sinteza dopaminii și eliberarea acesteia în fanta sinaptică, în timp ce la dozele mari antipsihotice, blocajul se mută asupra receptorilor dopaminergici D2 postsinaptic. 
Obiectivul lucrării de față a fost investigarea toxicității acute și subacute a noilor compuși, la șoarece, precum și cercetarea activității antidepresive, după administrare acută la șoarece non-depresiv, utilizând testul înotului forțat.

Au fost stabilite DL₅₀ după administrare intraperitoneală și orală, pentru cei trei compuși luați în lucru, valorile obținute fiind apropiați de cele ale benzamidelor folosite deja în terapeutică. Examenul histopatologic a evidențiat, pentru unul dintre compuși, toxicitate hepatică și renală, observată în una dintre cele cinci probe examine.

Testul înotului forțat a scos în evidență un efect antidepresiv, semnificativ statistic față de lotul martor (p<0.05, testul “t” Student), pentru doi dintre cei trei compuși investigați.

**Keywords:** benzamides; antidepressant; forced swimming test

**Introduction**

Many studies showed the dual dose-dependent activity, antipsychotic and antidepressant, of benzamidic drugs such tiapride [12], sulpiride [2] or amisulpride [7, 11]. At low doses, benzamides preferentially block presynaptic dopamine D₃ autoreceptors, that control dopamine synthesis and its release into synaptic cleft, whereas at higher doses, the blocking is moved towards postsynaptic dopamine D₂ receptors, the classical antipsychotic mechanism [10]. Thus, substituted benzamides represent an atypical class of antipsychotics, successfully used for both depressive states and schizophrenia. For therapeutics, this is an important step for the treatment of bipolar disorders [6].

The purpose of this paper was to investigate the acute and subacute toxicity of three new N-substituted benzamides, conventionally named I 5C, I 14C and II 5C [3, 4], structurally related to antipsychotics like tiapride or sulpiride (fig.1). Also, the antidepressant effect after acute administration was evaluated, using the forced swimming test.

![Chemical structures of tiapride and new N-substituted benzamides](image-url)
Materials and methods

Male NMRI mice (20-25 g) were supplied by the University of Medicine and Pharmacy “Carol Davila” biobase. Animals were housed in groups of 10, in plexiglass cages with sawdust litter, having free access to food and water. The temperature and relative humidity were continuously monitored using an electronic hygro-thermometer. The temperature was between 21ºC and 24ºC and the relative humidity was generally maintained at 45-60%. All procedures were carried out in accordance with the Directive 86/609/EEC of 24th November 1986, regarding the protection of animals used for experimental and other scientific purposes.

Acute toxicity

Research was performed using two routes of drug administration, i.p. and p.o.. Lethal doses were selected after we determined the maximum tolerated dose, the dose which produced no lethal effect. For every compound and route of administration we used three doses in arithmetical progression. Mortalities and all signs of toxicity were recorded. The animals remained alive were observed for 14 days.

LD50 were calculated using the regression equation.

Subacute toxicity

The test substances were administered once a day, seven days a week, during three weeks. The route of administration was i.p.. Doses were selected regarding LD50 obtained in acute toxicity studies, approximately 1/20 LD50, i.p.:

Group 1 (I 5C) - 10 mg/kg body weight
Group 2 (I 14C) - 11 mg/kg body weight
Group 3 (II 5C) - 8 mg/kg body weight
Group 4: Control – distilled water, equal volume as treated groups, i.p.

During three weeks, the mice were observed at least twice daily with the purpose of recording any symptoms of illness-health or behavioral changes. The observations included, but were not limited, to changes in skin and fur, in the eyes and mucous membranes, in the respiratory, circulatory and central nervous systems, somatomotor activity and behavior. The bodyweight of each mouse was recorded before the start of treatment and every four days during three weeks. The mean weight was calculated for each group. After 21 days, five mice of each group were sacrificed for the histological examination of brain, liver and kidney. The samples were fixed in 10% formaldehyde solution, stained with hematoxylin and eosin, and examined with light microscope.
Antidepressant effect after acute administration

The antidepressant activity was evaluated according to a modified forced swimming test (FST), originally described by Porsolt [5, 8, 9]. The mice were individually placed into a glass cylinder (25 cm height, 30 cm diameter) containing 20 cm of water maintained at 21–23°C. The total duration of the test was 6 minutes, but the immobility time was recorded only for 4 minutes, the first 2 minutes allowing the accommodation. Each mouse was considered immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water.

The tested compounds were intraperitoneally administered, as it follows:
Group 1 (control): distilled water, same volume with the other groups
Group 2 (reference): sulpiride 5 mg/kg body weight
Group 3 (reference): sulpiride 10 mg/kg body weight
Group 4: I 5C 10 mg/kg body weight
Group 5: I 14C 11 mg/kg body weight
Group 6: II 5C 8 mg/kg body weight.

FST was performed 30 minutes after the i.p. administration. A decrease in the duration of immobility during the FST was taken as a measure of antidepressant activity.

Statistical analyses
Immobility time in FST was analysed using “t” Student test and one-way ANOVA.

Results and discussion

Acute toxicity
At experimental doses used to establish LD50, mice developed tonic-clonic seizures, followed by death from a tetanic spasm. At lower doses, we observed acoustic and tactile hyperreflectivity, muscle hypertonia and hypokinesia. Minor or major catatonia was present, depending on the compound and dose.

Values for LD50 after i.p. and p.o. administration are given in table I:

<table>
<thead>
<tr>
<th>Substance (hydrochloride)</th>
<th>LD50 male mouse (mg/kg bw)</th>
<th>p.o.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I 5C</td>
<td>196.34</td>
<td>837.53</td>
</tr>
<tr>
<td>I 14C</td>
<td>214.04</td>
<td>595.66</td>
</tr>
<tr>
<td>II 5C</td>
<td>156.50</td>
<td>687.07</td>
</tr>
</tbody>
</table>
According to the Hodge and Sterner scale, the compounds are slightly toxic after oral administration, with a “4” toxicity rating [1]. LD50 values are similar to those of therapeutically used antipsychotic/antidepressant benzamides, which are also slightly toxic (table II).

<table>
<thead>
<tr>
<th>Benzamide</th>
<th>LD50 male mouse (mg/kg bw) i.p.</th>
<th>LD50 male mouse (mg/kg bw) p.o.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amisulpride</td>
<td>175</td>
<td>1024</td>
</tr>
<tr>
<td>Sulpride</td>
<td>170</td>
<td>1700</td>
</tr>
<tr>
<td>Sultopride</td>
<td>300</td>
<td>665</td>
</tr>
<tr>
<td>Tiapride</td>
<td>336</td>
<td>1340</td>
</tr>
</tbody>
</table>

**Subacute toxicity**

No mortality was recorded during the three weeks of compounds administration in doses approximately 1/20 LD50, i.p. The body weight of the animals did not show any significant change when compared to control group, although all compounds had the tendency to increase the body weight during the treatment (table III).

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 1</th>
<th>Day 5</th>
<th>Day 9</th>
<th>Day 13</th>
<th>Day 17</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23.4 ± 1.14</td>
<td>23.1 ± 1.55</td>
<td>23.1 ± 1.59</td>
<td>22.9 ± 1.85</td>
<td>22.4 ± 2.11</td>
<td>22.7 ± 1.95</td>
</tr>
<tr>
<td>I 5C</td>
<td>23.2 ± 1.73</td>
<td>24.3 ± 1.75</td>
<td>24.4 ± 1.87</td>
<td>24.7 ± 1.84</td>
<td>24.5 ± 1.82</td>
<td>25.1 ± 2.05</td>
</tr>
<tr>
<td>I 14C</td>
<td>23.1 ± 1.27</td>
<td>24.1 ± 1.55</td>
<td>24.6 ± 1.79</td>
<td>24.7 ± 1.87</td>
<td>24.7 ± 1.98</td>
<td>26.2 ± 2.24</td>
</tr>
<tr>
<td>II 5C</td>
<td>23.3 ± 1.75</td>
<td>24.2 ± 1.83</td>
<td>25.0 ± 1.72</td>
<td>25.2 ± 1.68</td>
<td>26.0 ± 2.26</td>
<td>26.4 ± 2.19</td>
</tr>
</tbody>
</table>

The macroscopic analysis of the organs removed for histological examination showed no significant changes in color or texture when compared with the control group.

At the histopathological examination, no differences were found between the brains from treated groups and control. Hepatotoxicity was present in one of five liver samples examined for the compound II 5C, the hepatocytes being degenerated, some with pyknotic nuclei. Granulovacuolar distrophy and medium, potentially reversible hepatosis were also found (fig. 2). However, the other four samples examined were similar to those of the control group.
The histopathological examination of the kidney samples showed one of five tissues with serious degenerative disorders, such as granulovacuolar tubulonephrosis, for the compound II 5C (fig. 3). The other four samples examined were similar to those of the control group.

**Antidepressant effect**

Two of the three tested compounds significantly decreased the immobility time in the FST (table IV). The antidepressant effect compared with the control group was 46.94% for compound I 14C ($p < 0.05$, “t” Student test) and 45.33% for compound II 5C ($p < 0.02$). The effect of the
two new benzamides was similar to the effect of sulpiride 10 mg/kg body weight: 46.47% (p < 0.02).

### Table IV

<table>
<thead>
<tr>
<th></th>
<th>Control group (s)</th>
<th>Sulpiride 5 mg/kg bw (s)</th>
<th>Sulpiride 10 mg/kg bw (s)</th>
<th>15C 10 mg/kg bw (s)</th>
<th>114C 11 mg/kg bw (s)</th>
<th>115C 8 mg/kg bw (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>72.44</td>
<td>59.10</td>
<td>45.90</td>
<td>38.44</td>
<td>39.60</td>
<td>39.60</td>
</tr>
<tr>
<td>SD</td>
<td>23.943</td>
<td>34.420</td>
<td>25.543</td>
<td>44.135</td>
<td>34.825</td>
<td>29.083</td>
</tr>
<tr>
<td>Normal distribu -tion</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Unpaired „t“ Student test</td>
<td>NS*</td>
<td>&lt;0.02</td>
<td>NS*</td>
<td>&lt;0.05</td>
<td>&lt;0.02</td>
<td></td>
</tr>
<tr>
<td>One way ANOVA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NS (&gt;0.05)</td>
<td></td>
</tr>
</tbody>
</table>

Note: NS* = non significant

*Figure 4*

Antidepressant effect of the new benzamides compared with the control group

* p<0.02; ** p<0.05

### Conclusions

The new synthesized N-substituted benzamides were assessed for acute and subacute toxicity. LD50 values are similar to those of the therapeutically used antipsychotic/antidepressant benzamides (tiapride, sulpiride or amisulpride), which are slightly toxic. Subacute toxicity research evidenced renal and hepatic toxicity for the compound II 5C, in one of the five samples.
All the compounds had antidepressant effect after acute administration in forced swimming test. Two of the results were statistically significant compared with the control group.

References

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