**SH-I-048A, a novel positive modulator of GABAA receptor: in vitro and behavioral profile**


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**Introduction**

Numerous studies showed that anxiolytic effects induced by benzodiazepines (BZs) are mediated via α2/α3-GABAA receptors while its sedative effects are attributed to α1-GABAA receptor population. Nevertheless, our research of behavioral effects mediated by different GABAA receptor subtypes was encouraged mainly because the correlation between in vitro and in vivo results obtained with BZ site ligands is not yet well established.

**Materials and methods**

The BZ site ligand (S,E)-7-bromo-5-(2-fluorophenyl)-3-methyl-1Hbenzo[e][1,4]diazepin-2(3H)-one (SH-I-048A) was synthesized at the University of Wisconsin-Milwaukee. Its affinity was examined by competition binding assay using [3H] flunitrazepam as the radiolabel while efficacy profile was obtained by two-electrode voltage clamp experiments in Xenopus oocytes expressing recombinant GABAA receptor subtypes. The behavioral activity of SH-I-048A (2, 5 and 10 mg/kg) was examined using a battery of behavioral tests (spontaneous locomotor activity, elevated plus maze and water maze). All in vivo experiments were performed on male Wistar rats, 20 min after intraperitoneal administration of treatment.

**Results**

**In vitro studies**

In vitro affinity experiment revealed very high (subnanomolar) and comparable affinity of SH-I-048A for α1, α2, α3 and α5-GABAA receptors (Table 1). Regarding its efficacy in general, SH-I-048A acted as a positive modulator of the GABA-triggered current at all four GABAA receptor subtypes (Figure 1).

**In vivo studies**

Influence of treatment on total distance travelled in the spontaneous locomotor activity test (SLA) did not reach statistical significance, as assessed by one-way ANOVA (Figure 2). However, it showed significance for the other relevant parameter – total time immobile (F(3,28) = 3.3367, p = 0.033) (Figure 3). In the elevated plus maze (EPM), one-way ANOVA revealed a significant effect of treatment on the percentage of time spent on open arms (Figure 4), as a parameter related to anxiety (F(3,21) = 4.3496, p = 0.016), but not on the parameters related to locomotor activity. In addition, there were significantly fewer stretch-attend postures in rats treated with 5 and 10 mg/kg of SH-I-048A (Figure 5). The newly developed ligand had no significant effect on the water maze behavior.

**Conclusion**

- The in vitro profile of SH-I-048A could not be regarded as a genuine breakthrough in the development of novel, affinity/efficacy selective, BZ site ligands.
- However, the results from in vivo studies suggest that this ligand possesses a distinct anxiolytic potential, assessed by both conventional and ethological parameters in the elevated plus maze, without apparent effects on learning and memory and with somewhat diminished sedative properties.

**Table 1. Kᵢ values (nM) obtained from competition binding assay for α₁β₂γ₃,GABAA receptors**

<table>
<thead>
<tr>
<th>Ligand</th>
<th>α₁</th>
<th>α₂</th>
<th>α₃</th>
<th>α₅</th>
<th>β₂</th>
<th>γ₃</th>
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<tr>
<td>SH-I-048A</td>
<td>0.774</td>
<td>0.172</td>
<td>0.383</td>
<td>ND</td>
<td>0.110</td>
<td>ND</td>
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</tbody>
</table>

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**Figures**

1. Concentration-effect curves for modulation of GABA elicited currents by SH-I-048A on Xenopus oocytes expressing GABA₄ receptor subtypes α₁β₂γ₃, α₂β₂γ₃, α₃β₂γ₃ or α₅β₂γ₃. Concentrations of GABA eliciting 3% of the maximum GABA-triggered current of the respective cells were applied alone and in the presence of various concentrations of SH-I-048A.


3. Newly synthesized ligand influenced the rats’ behavior in the SLA test. *P<0.05 vs SOL.

4. Anxiety-like effects of SH-I-048A in the EPM. *P<0.05 compared to SOL.

5. Novel ligand altered one of the ethological parameters observed in the EPM. *P<0.05 compared to SOL.