Chapter 6:
Antipsychotic Activity
<table>
<thead>
<tr>
<th>6) Chapter 6:</th>
<th>Antipsychotic activity</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>6.1</td>
<td>Catalepsy in rodents</td>
<td>118</td>
</tr>
<tr>
<td>6.2</td>
<td>Pole climb avoidance in rats</td>
<td>118</td>
</tr>
<tr>
<td>6.3</td>
<td>Inhibition of Apomorphine climbing in rats</td>
<td>119</td>
</tr>
<tr>
<td>6.4</td>
<td>Spontaneous locomotor activity in rats</td>
<td>120</td>
</tr>
<tr>
<td>6.5</td>
<td>Result and Discussion</td>
<td>122</td>
</tr>
</tbody>
</table>
6 Antipsychotic activities (in vivo)\textsuperscript{239}

All the compounds, which have exhibited potent \textit{in vitro} \(D_2\) and 5-HT receptor antagonistic activity, were selected for their \textit{in vivo} antipsychotic activity to check its safety. Compound LMVSNMP02, LMVSNMP07, LMVSNMP13 and LMVSNMP19 were selected for \textit{in vivo} screening.

6.1 Catalepsy in rodents

Groups of 6 Wistar rats with a body weight between 120 and 250 g were used. They were dosed intraperitoneally with the test drug or the standard. Then, they were placed individually into translucent plastic boxes with a wooden dowel mounted horizontally 10 cm from the floor and 4 cm from one end of the box. The floor of the box was covered with approximately 2 cm of bedding material. White noise is presented during the test. The animals were allowed to adapt to the box for 2 min. Then, each animal was grasped gently around the shoulders and under the forepaws and placed carefully on the dowel. The amount of time spent with at least one forepaw on the bar is determined. When the animal removes its paws, the time is recorded and the rat is repositioned on the bar. Three trials are conducted for each animal at 30, 60, 120 and 360 min.

An animal is considered to be cataleptic if it remains on the bar for 60 seconds. Percentage of cataleptic animals is calculated. For dose-response curves, the test is repeated with various doses and more animals. ED\textsubscript{50} values can be calculated. A dose of 1 mg/kg i.p. of haloperidol/clozapine was found to be effective.

6.2 Pole climb avoidance in rats

Male rats of the Long-Evans strain with a starting body weight of 250 g were used. The training and testing of the rats is conducted in a 25 × 25 × 40 cm chamber that is enclosed in a dimly lit, sound-attenuating box. Scrambled shock was passed to the grid floor of the chamber. A 2.8-kHz speaker and a 28-V light are situated on top of the chamber. A smooth stainless-steel pole, 2.5 cm in diameter, was suspended by a counterbalance weight through a hole in the upper center of the chamber. A micro switch was activated when the pole was pulled down 3 mm by a weight greater than 200 g. A response was recorded when a rat jumps on the pole and activates the micro switch. The rat cannot hold the pole down while standing on the grid floor because of the counter balance tension and cannot remain on the pole any length of time because of its smooth surface. The activation of the light and the speaker together were used as the conditioning stimulus. The conditioning stimulus was
presented alone for 4 s and then was coincident with the unconditioned stimulus, a scrambled shock delivered to the grid floor, for 26 s. The shock current was maintained at 1.5 mA. A pole climb response during the conditioned stimulus period terminates the conditioned stimulus and the subsequent conditioned and unconditioned stimuli. This was considered an avoidance response. A response during the time when both the conditioned and unconditioned stimuli were present terminates both stimuli and was considered an escape response. Test sessions consist of 25 trials or 60 min, whichever comes first. There was a minimum intertrial interval of 90 s. Any time remaining in the 30 s allotted to make the pole climb was added to the 90 s intertrial interval. Responses during this time have no scheduled consequences; however, rats having greater than 10 inter trial interval responses should not be used in the experiment. Before testing experimental compounds, rats were required to make at least 80% avoidance responses without any escape failures.

Data were expressed in terms of the number of avoidance and escape failures relative to the respective vehicle control data. ED$_{50}$ values can be calculated using different doses.

6.3 Inhibition of Apomorphine climbing in rats

Groups of 6 male rats (150-200 g) were treated i.p. or orally with the test substance or the vehicle and placed individually in wire-mesh stick cages. Thirty min afterwards, they are injected s.c. with 3 mg/kg apomorphine. 10, 20 and 30 min after Apomorphine administration, they were observed for climbing behavior and scored as follows:

0 = four paws on the floor,
1 = forefeet holding the vertical bars,
2 = four feet holding the bars.

The average values of the drug-treated animals were compared with those of the controls, the decrease was expressed as percent. The ED$_{50}$-values and confidence limits are calculated by probit analysis. Three dose levels were used for each compound and the standard with a minimum of 10 animals per dose level.
6.4 Spontaneous locomotor Activity in Rats

The effect of tests on motor performance was examined in groups of 6 male rats. The animals were given either tests (0.125 mg/kg, 2 mg/kg, p.o.) or vehicle (2-hydroxypropyl-α-cyclodextrin, 10%) 60 min before the test. To assess the inhibition of spontaneous motor activity, rats were individually placed in Plexiglas activity cages (40*40 cm) with photocells on the walls. The photocells were connected through an interface to a computer. The consecutive interruption of photocell beams was taken as a locomotion count. Spontaneous activity was recorded for 30 min after administration of the test compound.
Table 10: *In vivo* Pharmacological Profile of the synthesized compounds (Inhibition of different responses after oral administration of the test and reference compounds)

![Chemical Structure](image)

<table>
<thead>
<tr>
<th>Com. Code</th>
<th>R₁</th>
<th>R₂</th>
<th>ED₅₀ ± SEM* of CAT (Catalepsy) mg/kg</th>
<th>ED₅₀ ± SEM* of CAR (Conditional Avoidance Response) mg/kg</th>
<th>ED₅₀ ± SEM* of Spontaneous Locomotor Activity mg/kg</th>
<th>ED₅₀ ± SEM* of Apomorphine induced Climbing mg/kg</th>
<th>CAT/CAR Ratio mg/kg ED₅₀ ± SEM*</th>
<th>pA₂ ± SEM* for D₂ receptor</th>
<th>pA₂ ± SEM* for 5-HT receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMVSNMP02</td>
<td>4-OCH₃</td>
<td>4-OCH₃</td>
<td>16.97 ± 0.01</td>
<td>1.65 ± 0.01</td>
<td>1.47 ± 0.01</td>
<td>1.77 ± 0.02</td>
<td>10.40 ± 0.07</td>
<td>6.87 ± 0.11</td>
<td>6.4 ± 0.02</td>
</tr>
<tr>
<td>LMVSNMP07</td>
<td>3-OCH₃</td>
<td>H</td>
<td>31.64 ± 0.01</td>
<td>2.27 ± 0.02</td>
<td>4.60 ± 0.01</td>
<td>2.50 ± 0.01</td>
<td>14.04 ± 0.19</td>
<td>6.8 ± 0.00</td>
<td>6.36 ± 0.02</td>
</tr>
<tr>
<td>LMVSNMP13</td>
<td>3-Cl</td>
<td>4-OCH₃</td>
<td>20.86 ± 0.03</td>
<td>2.01 ± 0.04</td>
<td>1.46 ± 0.01</td>
<td>3.58 ± 0.06</td>
<td>10.39 ± 0.23</td>
<td>6.71 ± 0.00</td>
<td>6.47 ± 0.02</td>
</tr>
<tr>
<td>LMVSNMP19</td>
<td>4-Cl</td>
<td>2,3-di Cl</td>
<td>29.54 ± 0.01</td>
<td>2.20 ± 0.11</td>
<td>1.53 ± 0.02</td>
<td>2.15 ± 0.01</td>
<td>14.75 ± 0.44</td>
<td>6.79 ± 0.00</td>
<td>6.2 ± 0.01</td>
</tr>
<tr>
<td>Clozapine</td>
<td>-</td>
<td>-</td>
<td>104.72 ± 0.01</td>
<td>5.24 ± 0.01</td>
<td>7.13 ± 0.01</td>
<td>3.60 ± 0.01</td>
<td>19.98 ± 0.04</td>
<td>6.23 ± 0.01</td>
<td>6.53 ± 0.04</td>
</tr>
</tbody>
</table>

*Under experimental conditions*
6.5 Result and Discussion:

Results

Catalepsy

As shown in Table 10 the ED$_{50}$ values for LMVSNMP02, LMVSNMP07, LMVSNMP13 and LMVSNMP19 were 16.97 mg/kg, 31.64 mg/kg, 20.86 mg/kg and 29.54 mg/kg respectively. Clozapine had ED$_{50}$ value 104.72 mg/kg. Clozapine was found more potent at all or median to high doses in the present experimental condition.

Conditional avoidance response (CAR) in rats

All compounds effectively inhibited avoidance response in a dose-dependent manner. A significant effect of LMVSNMP02, LMVSNMP07, LMVSNMP13 and LMVSNMP19 was found on oral administration (p.o.). The calculated ED$_{50}$ values were 1.65 mg/kg, 2.27 mg/kg, 2.01 mg/kg and 2.20 mg/kg (p.o.). Clozapine showed a significant effect with ED$_{50}$ value 5.24 mg/kg (p.o.).

Spontaneous locomotor activity test in rats

Pretreatment time was fixed as 1 h for test compounds and clozapine. After the pretreatment time, each test compound dose-dependently suppressed spontaneous locomotor activity. A significant effect of LMVSNMP02, LMVSNMP07, LMVSNMP13 and LMVSNMP19 was found on oral administration (p.o.). The calculated ED$_{50}$ values were 1.47 mg/kg, 4.60 mg/kg, 1.46 mg/kg and 1.53 mg/kg (p.o.). Clozapine showed a significant effect with ED$_{50}$ value 7.13 mg/kg (p.o.) as shown in Table 10.

Effect on apomorphine-induced climbing behavior

As shown in Table 10, oral administration of test compounds significantly antagonized the apomorphine-induced cage climbing behavior in rats. The calculated ED$_{50}$ value for LMVSNMP02, LMVSNMP07, LMVSNMP13 and LMVSNMP19 were 1.77, 2.50, 3.58 and 2.15 mg/kg respectively. Clozapine, significantly blocked the apomorphine-induced climbing, showing 3.60 mg/kg (p.o.)
Discussion

All the compounds were tested for their affinity at dopamine D$_2$ and serotonin 5-HT receptors by *in vitro* models. Concurrently, potent compounds were screened for potential atypical antipsychotic profile by oral administration in some *in vivo* assays. All compounds had a greater affinity for 5-HT than D$_2$ receptors like clozapine. The results from binding affinities, therefore, indicate that LMVSNMP02, LMVSNMP07, LMVSNMP13 and LMVSNMP19 are more potent than currently available antipsychotic drugs. Apomorphine-induced climbing behavior is due to the stimulation of dopamine receptors and has been used as a convenient means to *in vivo* screen dopamine agonists or antagonists (neuroleptics) and to assess striatal dopamine activity. LMVSNMP02, LMVSNMP07, LMVSNMP13 and LMVSNMP19 blocked apomorphine-induced cage climbing behavior of rats when treated orally, without any hypoactivity. It is likely due to selective blockade of dopaminergic receptors, and the potency is similar to that of clozapine. The behavioral studies using rats, antagonism of dopamine agonist-induced hyper locomotion and conditioned avoidance response (CAR) paradigm have traditionally been used to predict the antipsychotic efficacy of novel agent. In the CAR, LMVSNMP02 showed an excellent effect with lower ED$_{50}$ value which is lower than that of clozapine. LMVSNMP02, however, induced catalepsy (CAT) only at the high dose group (150 mg/kg). The relative ratio (e.g. Therapeutic index: TI) of the ED$_{50}$ for CAT to the ED$_{50}$ for a sensitive pharmacological screen for APs, such as conditioned avoidance responding (CAR) in rats, has been used to predict the relative ability of a compound to induce extrapyramidal symptoms (Parkinson-like symptoms and tardive dyskinesia). Conditioned avoidance response (CAR) behavior and catalepsy (CAT) are the standard preclinical tests used to predict antipsychotic activity and motor side-effect liability, respectively. As an assessment of the proposed atypical antipsychotic profile, induction of catalepsy in rats was included as a measure of the potential for induction of extrapyramidal side effects. The test compounds shown in table 28 displayed an appreciable difference between doses required for inhibition of apomorphine induced climbing and those that induced catalepsy, thus showing meso limbic selectivity, which is consistent with the expected atypical profile. Comparison with standard drugs suggested that the most active compounds LMVSNMP02, LMVSNMP07, LMVSNMP13 and LMVSNMP19 would exhibit a reduced propensity to induce EPS, similar to the atypical antipsychotics clozapine. Notably, the broader the margin for the induction of catalepsy also corresponds to the most potent compounds in the climbing rat assay. In summary, LMVSNMP02, LMVSNMP07, LMVSNMP13 and
LMVSNMP19 shows general profiles of an atypical antipsychotic drug and have efficacies on negative and/or cognitive symptoms of schizophrenia patients. Moreover, it has better safety than currently available antipsychotic drugs.