# Synthesis of novel group of multireceptoral ligands with antipsychotic and pro-cognitive properties

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## Introduction

First-generation antipsychotics, like e.g. haloperidol or chlorpromazine, allowed to effectively treat positive symptoms of schizophrenia and related psychotic disorders, but they also revealed high rate of side effects, e.g. extrapyramidal symptoms [1]. Discovery of second-generation antipsychotics (olanzapine, risperidone, etc.) significantly reduced the range of the observed adverse effects, but those drugs did not completely eliminate cognitive deficits in schizophrenia [1]. Therefore new therapeutic agents with dual effect i.e. suppression of psychotic symptoms and elimination of cognition impairment are still needed [2].

It is believed that antagonism at 5-$HT_6$ receptors is responsible for such pro-cognitive actions. This is supported by the exclusive central nervous system localization of the 5-$HT_6$ receptors, limited to the limbic and cortical brain areas, and relatively potent affinity and antagonistic activity of several atypical antipsychotics [3].

As a part of our ongoing efforts to discover effective antipsychotic agents that would also ameliorate the cognitive deficits, we designed and synthesized a new series of compounds **1**–**44** (Table 1).

## Results of affinity experiments

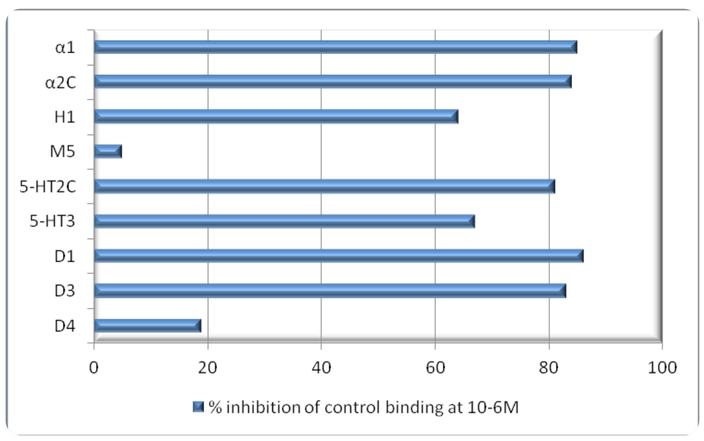
Membrane preparation and general assay procedures for 5-$HT_{1A}$ [4], 5-$HT_{2A}$ [4], 5-$HT_7$ [5,6], 5-$HT_6$ [7], and $D_2$ [8] receptors were performed exactly as previously described (Table 1).
For binding experiments 7–9 sample concentrations, each run in triplicate, were used to determine inhibition constant ($K_i$) on the base of Cheng-Prusoff'a equation: $K_i = IC_{50} / (1 + L/K_D)$. Values are means of three experiments run in triplicate, SEM ≤ 16%.

**Table 1.** The binding data of the library members **1**–**44** and reference compounds for 5-$HT_{1A}$, 5-$HT_6$, 5-$HT_7$ and $D_2$ receptors.

| Compound | $K_i$ [nM] | | | | |
|---|---|---|---|---|---|
| | 5-$HT_{1A}$ | 5-$HT_{2A}$ | 5-$HT_6$ | 5-$HT_7$ | $D_2$ |
| **1** | - | - | +++ | + | ++ |
| **2** | - | - | +++ | + | +++ |
| **3** | + | - | +++ | + | +++ |
| **4** | - | +++ | +++ | + | ++ |
| **5** | - | +++ | +++ | +++ | ++ |
| **6** | - | +++ | ++ | + | ++ |
| **7** | - | +++ | +++ | + | ++ |
| **8** | ++ | - | ++ | + | ++ |
| **9** | +++ | - | +++ | ++ | ++ |
| **10** | - | +++ | ++++ | ++ | - |
| **11** | + | +++ | +++ | + | +++ |
| **12** | ++ | ++++ | ++ | ++ | ++ |
| **13** | + | +++ | ++ | + | ++ |
| **14** | +++ | ++++ | +++ | ++ | ++ |
| **15** | + | ++++ | +++ | + | +++ |
| **16** | + | +++ | +++ | ++ | ++ |
| **17** | - | +++ | +++ | +++ | + |
| **18** | ++ | +++ | +++ | ++ | + |
| **19** | + | - | +++ | + | ++ |
| **20** | - | +++ | +++ | ++ | ++ |
| **21** | - | +++ | +++ | ++ | ++ |
| **22** | ++ | +++ | ++ | ++ | ++ |
| **23** | + | +++ | + | + | + |
| **24** | ++ | - | + | + | ++ |
| **25** | ++ | ++ | + | + | ++ |
| **26** | | + | + | | + |
| **27** | + | - | ++ | + | ++ |
| **28** | ++ | - | ++ | + | + |
| **29** | - | ++++ | ++ | ++ | + |
| **30** | + | - | ++ | + | - |
| **31** | ++ | ++++ | +++ | ++ | ++ |
| **32** | - | +++ | ++ | + | + |
| **33** | - | ++ | ++ | + | + |
| **34** | + | - | +++ | + | - |
| **35** | - | ++++ | ++++ | ++ | ++++ |
| **36** | - | +++ | +++ | ++ | +++ |
| **37** | ++ | +++ | +++ | ++ | +++ |
| **38** | ++ | - | ++ | ++ | +++ |
| **39** | +++ | +++ | +++ | ++ | ++ |
| **40** | +++ | +++ | ++ | ++ | ++ |
| **41** | +++ | +++ | ++ | ++ | ++ |
| **42** | + | - | ++ | | + |
| **43** | +++ | +++ | ++ | ++ | + |
| **44** | +++ | ++ | ++ | +++ | +++ |
| Haloperidol | 1703 | 166* | 1083 | 408 | 2 |
| Sertindole | 635 | < 1 | 0.2 | 14 | 12 |
| Clozapine | 143 | 13 | 4 | 30 | 72 |
| Olanzapine | 3442 | 10* | 7 | 185 | 7 |

(–): not determined, (++++): $K_i$<10, (+++): 10<$K_i$<100, (++): 100<$K_i$<1000, (+): 1000<$K_i$<10000
* data from PDSP $K_i$ database (http://pdsp.med.unc.edu), ** % inhibition of control binding at $10^{-6}$M.

**Figure 1.** The binding data of the lead compound **39** for $\alpha_1$, $\alpha_{2C}$, $H_1$, $M_5$, 5-$HT_{2C}$, 5-$HT_3$, $D_1$, $D_3$ and $D_4$ receptors. Assays were performed by CEREP (www.cerep.com).




## Behavioral studies

### 1. Sedation (side-effect)

Antipsychotics are associated with a range of side effects. It is well-recognized that many people stop taking them (around two-thirds even in controlled drug trials) due in part to adverse effects [9]. Sedation is common with antipsychotic medications and is dose related. It can be a cause of poor compliance and, if persistent, can interfere with social and vocational functioning [10].

Selected compounds **12** and **39** of the presented series induced sedation in the animal model at a dose (18 mg/kg) (Table 2).

### 2. Hyperactivity

Phencyclidine PCP is a drug of abuse that has a wide range of psychotomimetic effects in humans. Many researchers have drawn parallels between these effects of PCP in humans and some symptoms of schizophrenia. PCP is a noncompetitive antagonist at NMDA receptors which also acts as an indirect releaser of dopamine and serotonin and as a monoamine reuptake inhibitor. PCP produces a variety of unusual behaviors in rodents, dominated by increases in locomotor activity and stereotyped behaviors [3].

Compounds **12** and **39** were shown to inhibit PCP-induced hyperactivity in a dose 3 and 9 mg/kg respectively. (Table 2).

### 3. NOR

Patients with schizophrenia also show impairment of both face and visual object recognition tasks. Hence, the NOR test in rodents has been increasingly used as an ethologically relevant paradigm for studying visual episodic memory. This task is based on spontaneous exploration of novel and familiar objects. Successful object recognition is displayed by a longer time spent interacting with the novel object in the retention trial [3].

The NOR deficit was reduced by both compounds **12** and **39** at a comparable dose (3 mg/kg) that was determined for quetiapine (Table 2).

### 4. PPI

Schizophrenic patients also suffer from disturbances in information processing, reflected as a deficient sensorimotor gating, which may contribute to the cognitive deficits that characterize this disorder. Prepulse inhibition (PPI) of the startle reflex, an operational measure of the sensorimotor gating, is the reduction of a startle response to an intense acoustic stimulus (pulse), when this stimulus is immediately preceded by a stimulus of lower intensity (prepulse). In rodents, the PPI test has been extensively used to study and screen putative antipsychotics .

Dizocilpine-evoked cognitive deficit was ameliorated by compounds **39** at almost two fold higher dose (9 mg/kg) than for clozapine was assessed (Table 3).

### 5. ASST

It is widely accepted that cognitive deficits, including cognitive inflexibility are a core feature of schizophrenia. Cognitive flexibility may be assessed in rodents using the attentional set-shifting task (ASST). In this paradigm, rats must select a bowl containing a food reward based on the ability to discriminate the odors and the media covering the bait. The ASST requires rats to initially learn a rule and form an attentional 'set' within the same stimulus dimensions. At the extra-dimensional (ED) shift stage, the essential phase of the task, animals must switch their attention to a new, previously irrelevant stimulus dimension and, for example, discriminate between the odors and no longer between the media covering the bait. The animals' performance at the ED stage is regarded as an index of cognitive flexibility [3].

Administration of **39**, in over two times lower than for sertindole dose (1 mg/kg), ameliorated the ketamine-induced deficit and promoted cognitive flexibility in control rats (Table 3).

**Table 2.** The results of compounds **12** and **39** in tests: PCP-induced hyperactivity, PCP-induced novel object recognition in rats and sedation studies.

| Compound | Sedation | Protection from PCP-induced hyperactivity | Protection from PCP-induced Novel Object Recognition deficit (NOR) |
|---|---|---|---|
| | side-effect | anti-psychotic effect | procognitive effect in psychotic-like conditions |
| **12** | 18.0 | 3.0 | 3.0 |
| **39** | 18.0 | 9.0 | 3.0 |
| Sertindole | 9.0 | 1.0 | 1.5 |
| Clozapine | 3.0 | 1.0 | 1.0 |
| Olanzapine | 1.2 | 1.9 | 1.2 |
| Quetiapine | 9.0 | 9.0 | 3.0 |

Data given as MED (mg/kg)

**Table 3.** The results of lead compound **39** in tests: prepulse inhibition (PPI) and attentinal set shifting (ASST).

| Compound | Prepulse inhibition in dizocilpine-disturbed conditions (PPI) | Attentional set shifting in ketamine-disturbed condition (ASST) |
|---|---|---|
| | antipsychotic drug action | cognitive functions dependent on prefrontal cortex |
| **39** | 9.0 | 1.0 |
| Haloperidol | NA (0.1-0.2) | NA (0.01-0.1) |
| Sertindole* | NT | 2.5 |
| Clozapine | 5.0 | NA (0.1-5.0) |
| Olanzapine | NT | NA (1.5-3.0) |

Data given as MED (mg/kg), NT – not tested, NA – not active. * PO, other IP

## Conclusions

- The synthesized series of compounds **1**–**44** revealed broad spectrum of affinities for 5-$HT_{1A}$, 5-$HT_{2A}$, 5-$HT_6$ , 5-$HT_7$ and $D_2$ receptors, and some of them showed multireceptoral profile similar to second generation antipsychotics. Expanded receptor profile ($\alpha_1$, $\alpha_{2C}$, $H_1$, $M_5$, 5-$HT_{2C}$, 5-$HT_3$, $D_1$, $D_3$ and $D_4$ receptors) for lead compound **39** confirmed its multi-target activity (Figure 1).
- Functional in vitro assays revealed that compounds **35** and **39**, analogously as second generation antipsychotics, behaved as 5-$HT_{2A}$/5-$HT_6$/$D_2$ antagonists.
- The behavioral studies demonstrated the effectiveness of the lead compound **39** in ameliorating ketamine and dizocilpine-induced cognitive disruptions (PPI and ASST) and recognition memory impairment (NOR). Like other antipsychotic agents, compounds **12** and **39** reversed PCP-induced hyperactivity. .
- The lead compound **39** may be further develop as potential medication in the treatment of disorders characterized by cognitive impairments, such as schizophrenia and Alzheimer's disease.

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