Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 1313–1316

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Pharmacological Evaluation of a Diarylmethylene-Piperidine Derivative: A New Potent Atypical Antipsychotic?

Patrice Talaga,* Alain Matagne and Henrik Klitgaard

UCB S.A., Pharma Sector, Chemin du Foriest, B-1420 Braine-l'Alleud, Belgium

Received 31 January 2001; revised 7 March 2001; accepted 16 March 2001

Abstract—A new diaryl-methylene piperidine derivative, **2**, displayed an atypical antipsychotic profile both in vitro and in vivo. The main pharmacological characteristics of this compound appears to reside in a more potent antagonism of the 5-HT_2 serotonergic receptor than of the D2 dopaminergic receptor. This confirms that molecules displaying a D2/5-HT_2 binding ratio <1 possess clozapine-like antipsychotic activity. © 2001 Elsevier Science Ltd. All rights reserved.

The notion of atypical antipsychotics is based on the observation that classical neuroleptics produce extrapyramidal side effects (EPS), whereas atypical neuroleptics display antipsychotic activity without inducing such extrapyramidal side effects. Among the neuroleptics, the prototype atypical antipsychotic is clozapine, **1**. Although clozapine appears to be efficacious in patients with negative symptoms and in therapy-resistant patients, its clinical use is hampered by the occurrence of fatal agranulocytosis and seizures.[1] Thus, there is an urgent need for more tolerable antipsychotic drugs with an atypical profile.[2]

A wide variety of neurochemical theories have been proposed as an etiological abnormality in schizophrenia.[3,4] Among the numerous neurotransmitters potentially involved in the pathogenesis of schizophrenia, dopamine and serotonin still seem to be of crucial importance.[5] Thus, the importance of dopamine antagonists in the treatment of schizophrenia has led the most extensive investigation being aimed at dopamine systems. Actually, most of the subtypes of dopamine receptors including D1–D5 are targets for antipsychotic drugs.[3,5,6] The unique antipsychotic profile of clozapine may relate to a relative selective blockade of subtypes of dopamine receptors. An elevated concentration of D4 receptors has been reported in brains from patients with schizophrenia.[7] Moreover, it has been reported that most typical neuroleptics primarily act at D2 receptors at their therapeutic concentrations,[8] whereas clozapine primarily acts at D4 receptors.[8] Besides blockade of dopamine receptors, it also appears that antagonism of serotonergic receptors is important, since coadministration of a 5-HT_2 antagonist with a typical antipsychotic has been suggested to reduce the appearance of EPS.[9,10] A correlation between an atypical profile and the ratio of D2/5-HT_2 receptor binding has been proposed.[11] Thus, it appears[12] that clozapine-like antipsychotics have a D2/5-HT_2 binding ratio <1 (pK_i values) whereas typical antipsychotics have a ratio >1.

In this communication, we describe the in vitro and in vivo pharmacological profile of a new diarylmethylene-piperidine derivative, **2**, which was identified via screening of UCB's internal compound library. The discovery approach was based upon the hypothesis that an atypical antipsychotic profile is related to a relatively higher affinity (antagonism) for the D4 than for the D2 receptor and to a relatively higher affinity (antagonism) for the 5-HT_2 than for the D2 receptor. We anticipated that such a binding profile would result in a relative selective inhibition of behaviors in mice mediated through the mesolimbic (A10) dopaminergic system (apomorphine-induced climbing/amphetamine-induced hyperactivity) in contrast to stereotyped behaviors mediated through the nigro-striatal (A9) dopaminergic system (apomorphine and d-amphetamine induced stereotypy).

Compound **2** may be regarded as belonging to the diarylbutylamine's group of antipsychotic drugs (e.g., Pimozide). Specifically, **2** is a diarylmethylene piperidine substituted on the nitrogen atom by a tetrahydrofuranoyl

*Corresponding author. Tel.: +32-2-386-2727; fax: +32-2-386-2704; e-mail: patrice.talaga@ucb-group.com

PII: S0960-894X(01)00200-1

amide group. This group may be considered as a rigidified bioisosteric analogue of the ethoxy-ethanol chain found in another recently claimed atypical antipsychotic, namely seroquel,[13] **3** (Fig. 1).

Chemical Synthesis

The diarylmethylene piperidine derivative **2** was prepared from the known ester **5** as shown in Scheme 1.[14] 5-Chloromethyl-furan-2-carboxylic acid methyl ester **5** was completely hydrolyzed with 5 N sodium hydroxide in dioxane to provide the corresponding 5-hydroxymethyl-furan-2-carboxylic acid. The acid was subsequently reesterified under acidic catalysis to provide **6** in 39% overall yield. 5-Chloromethyl-tetrahydrofuran-2-carboxylic acid ethyl ester **7** was obtained by hydrogenation of **6** in ethanol using Rh/C as catalyst followed by chlorination of the hydroxymethyl group with thionyl chloride in toluene. Compound **7** was not isolated and was directly coupled to the diarylmethylene piperidine derivative **8** in xylene at reflux to provide the 5-(4-[(4-fluoro-phenyl)-phenylmethylene]-piperidine-1-ylmethyl)-tetrahydrofuran-2-carboxylic ethyl ester derivative in 31% yield. The ester was then saponified under basic conditions and converted to the corresponding amide derivative **2** (overall yield 56%) by treatment with ethyl chloroformate in THF followed by the addition of gaseous ammonia. **2** has been obtained in a diastereomerically pure form (characterized by NMR and chiral HPLC) and is assumed to have a *cis* configuration.[15]

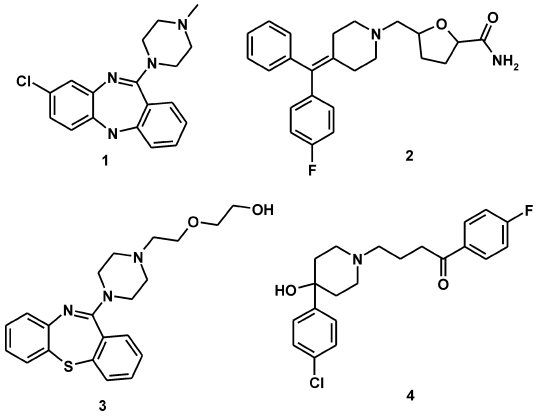
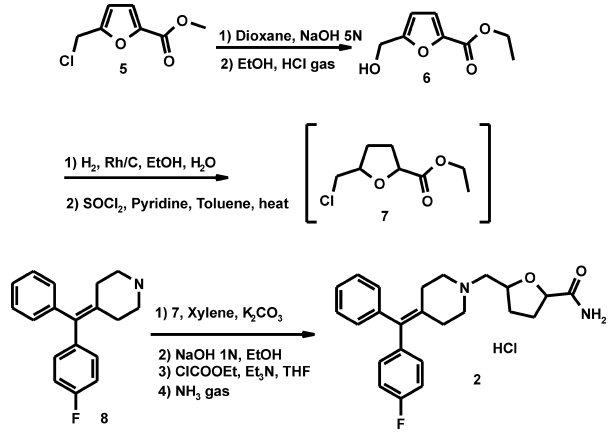


Figure 1. Structure of compound **2** and reference antipsychotics.



Scheme 1. Synthesis of compound **2**.

The antipsychotic activity of **2** was determined by using classical in vitro and in vivo assays, and was compared to clozapine **1**, seroquel **3**, and haloperidol **4**.

In Vitro Binding Studies

Affinity for the D2, D4, and 5-HT_2 receptors as well as the binding ratios D2/5-HT_2 and D2/D4 are listed in Table 1. The affinity of **2** for the 5-HT_2 receptor (pK_i 7.5) was relatively higher when compared to the similar affinities for the D2 or D4 receptors. This gives a D2/5-HT_2 ratio of 0.93 suggesting potential atypical antipsychotic activity in vivo.

Apomorphine-Induced Climbing-Stereotypy in Mice

Activities of **2** and the reference antipsychotics in the apomorphine-induced climbing-stereotypy model[18] are listed in Table 2. Among the reference molecules tested, only the atypical antipsychotic clozapine showed a significant separation between the doses that were active against climbing and stereotypy. In contrast, the typical neuroleptic haloperidol inhibited both apormophine induced hyperactivity and stereotypy at similar doses. Interestingly, a significant separation between the doses active against climbing and stereotypy was also observed for **2** (ED_{50} values for apomorphine induced climbing/stereotypy: 140/290 μmol/kg).

Table 1. Affinities (pK_i) for D2, D4, 5-HT_2 receptors and D2/5-HT_2, D2/D4 binding ratios for **2** and reference molecules

Compounds	D2	D4	5-HT_2	D2/5-HT_2	D2/D4
1	7.5	7.8	8.3[a]	0.8[b]	0.96
2	7.0	6.8	7.5	0.93	1.03
3	7.2	na[e]	220[c]	0.95[d]	na[e]
4	9.1	8.8	7.7[a]	1.17[b]	1.03

[a]Values taken from ref 26.
[b]Calculated from ref 16.
[c]K_i (nM) value taken from ref 4.
[d]Calculated from ref 17.
[e]na, not available.

Table 2. ED_{50} (μmol/kg) of **2** and reference molecules on apomorphine-induced climbing and stereotypy and DOI-induced head-twitches in mice

Compounds	Inhibition apomorphine-induced climbing[a] ip (po)	Inhibition of apomorphine-induced stereotypy[a] ip (po)	Inhibition of DOI-induced head-twitches[b] ip
1	28 (30)	67 (62)	1.5
2	46 (140)	69 (290)	16
3	51	60	9.0
4	0.32	0.31	0.61

[a]Climbing and stereotypy were induced by sc administration of apomorphine at a dose of 2.5 mg/kg.
[b]Head-twitches were induced by ip administration of (±)-DOI, at a dose of 1.6 mg/kg.

Amphetamine-Induced Hyperactivity and Stereotypy in Mice

The typical neuroleptic haloperidol inhibited both amphetamine-induced hyperactivity[19] and stereotypy[20] at similar doses. In contrast, **2** decreased d-amphetamine-induced hyperactivity from a dose of 10 μmol/kg (decrease of 44%; $p<0.01$), while a dose of 100 μmol/kg was required to slightly antagonize the stereotyped behavior induced by d-amphetamine (decrease of 13%; $p<0.05$). This was similar to clozapine which also antagonized amphetamine induced hyperactivity and stereotypy at doses of 10 and 100 μmol/kg, respectively.

DOI-Induced Head-Twitches in Mice

All reference antipsychotics were potent inhibitors of DOI-induced head-twitches,[21] a 5-HT_2 mediated behavior (Table 2).

Taken together, these results demonstrate that **2** reveals a profile similar to that of the atypical antipsychotic clozapine in mice. This was confirmed in rats using the catalepsy test[22] and the Sidman Avoidance paradigm.[23] Oral administration of **2** (40–160 mg/kg) dose-dependently decreased the number of avoidance responses in the Sidman Avoidance test (Fig. 2). It also clearly increased the number of shocks received and the number of escape failures. A similar activity was observed with clozapine from a dose of 20 mg/kg po in this model. No induction of catalepsy was observed with **2** (up to 160 mg/kg po) and clozapine (up to 40 mg/kg po). In contrast, the typical antipsychotic haloperidol both decreased the conditioned avoidance behavior (0.5 mg/kg po) and induced catalepsy (2 mg/kg po).

Adverse Effect Profile Evaluation in Mice and Rats

- Cardiovascular effects of **2** as well as of clozapine were evaluated in the awake rats (unpublished data). Oral administration of clozapine transiently increased heart rate (+49%) from the dose of 10.5 mg/kg, and decreased arterial blood pressure (−32%) at the dose of 32.7 mg/kg at 0.5–2.5 h post-administration. In contrast, **2** given orally (43.1–431 mg/kg) had no significant effect on heart rate, and blood pressure throughout the 24 h post-administration.
- Anticholinergic side effects were evaluated in mice using the oxotremorine-induced tremors/salivation test.[24] As for clozapine (ED_{50} values, μmol/kg ip salivation/tremor inhibition: clozapine 12/8; **2** 32/20), no important separation between tremors and salivation inhibitory activity was detected. In contrast to clozapine, **2** displayed no affinity for muscarinic receptors. Thus, an indirect effect obviously mediates the anticholinergic action induced by compound **2**.
- Testing in the IRWIN test[25] showed that **2**, compared to clozapine, induced effects like sedation (100/3.2), convulsions (320/180) and lethality (1000/320) at markedly higher doses (μmol/kg ip).

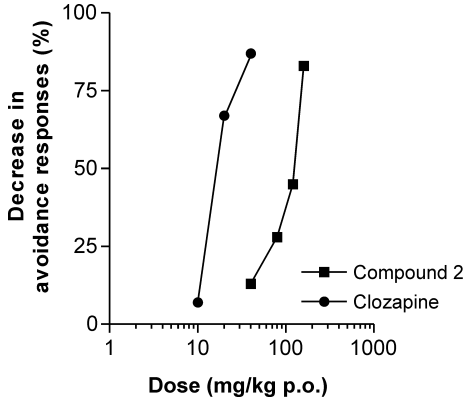


Figure 2. Effect of **2** and clozapine on conditioned avoidance responding in the rat.

In conclusion, the present study provides support that a more potent antagonism at the 5-HT_2 receptor as compared to the D2 receptor is important for an atypical profile of an antipsychotic. In contrast, a relatively selective antagonism of the D4 receptor appears not to be important. This finding seems in accordance with a study published by Roth et al.,[12] which reported that affinity for the D4 receptor is unable to distinguish between typical and atypical antipsychotics. Lead molecule **2** presented a favorable binding ratio between the 5-HT_2 and the D2 receptor in vitro and a relatively selective inhibition of mesolimbic versus nigro-striatal dopamine behaviors in vivo. The latter was comparable to the separation observed with clozapine. Thus, **2** should be considered for further preclinical investigations in order to delineate its clinical potential as an atypical antipsychotic.

Acknowledgements

We are grateful to Yves Lamberty, Michel Guyaux and Martine Vandevelde for performing the oxotremorine-induced tremors/salivation, Irwin, and cardiovascular tests, respectively. Furthermore, we wish to thank C. Chaussée and A. C. Michaux as well as B. Lallemand for their skillful technical assistance.

References and Notes

1. Keith, S. J. *N. Engl. J. Med.* **1997**, *12*, 851.
2. Armer, R.; Miller, D. J. *Idrugs* **2000**, *3*, 920.
3. Nakazato, A.; Okuyama, S. *Exp. Opin. Ther. Patents* **2000**, *10*, 75.
4. Kerwin, R.; Owen, M. *Br. J. Psychiatry* **1999**, *174* (Suppl. 38), 1.
5. Nakazato, A.; Okuyama, S. *Expert Opin. Ther. Patents* **2000**, *10*, 75.
6. McDonnel, M. E.; Reitz, A. *Expert Opin. Ther. Patents* **1994**, *4*, 1221.

7. Seeman, P.; Guan, H. C.; Van Tol, H. H. M. *Nature* **1993**, *365*, 441.
8. Seeman, P. *Neuropsychopharmacology* **1992**, *7*, 261.
9. Bersani, G.; Grispini, A.; Marimi, S.; Pasini, A.; Valducci, M.; Ciani, N. *Curr. Ther. Res.* **1986**, *40*, 492.
10. Ceulemans, D. L. S.; Geldens, Y. G.; Hoppenbrouwers, M. L.; Reyntjens, A. J. M.; Janssen, P. A. J. *Psychopharmacology* **1985**, *85*, 329.
11. Mewshaw, R. E.; Abreu, M. E.; Silverman, L. S.; Mathew, R. M.; Tiffany, C. W.; Baily, M. A.; Karbon, E. W.; Ferkany, J. W.; Kaiser, C. J. *J. Med. Chem.* **1993**, *36*, 3073.
12. Stockmeier, C. A.; Dicarlo, J. J.; Zhang, Y.; Thompson, P.; Melter, H. Y. *J. Pharmacol. Exp. Ther.* **1993**, *266*, 1374.
13. Saller, C. F.; Salama, A. I. *Psychopharmacology* **1993**, *112*, 285.
14. Compound **2** was characterized by NMR, mass spectrometry, and HPLC. For experimental details see: Moldenhauer, O.; Trautman, G.; Pfluger, R.; Döser, H. *Justus Liebigs Ann. Chem.* **1953**, *580*, 176. Watanabe, Y.; Usui, H.; Kobayashi, S.; Yoshiwara, H.; Shibano, T.; Tanaka, T.; Morishima, Y.; Yasuoka, M.; Kanao, M. *J. Med. Chem.* **1992**, *35*, 189.
15. Hydrogenation of 2,5-disubstituted furan derivatives usually gives the corresponding *cis*-derivative as described, for example, in: Eberbach W. In Houben-Weyl, *Band E6a, Hetarene* I, Teil 1; Georg Thieme Verlag: Stuttgart, New York, pp 16–166.
16. Leysen, J. E.; Janssen, P. M. F.; Schotte, A.; Luyten, W. H. M. L.; Megens, A. H. P. *Psychopharmacology* **1993**, *112*, S40.
17. Roth, B. L.; Tandra, S.; Burgess, L. H.; Sibley, D. R.; Meltzer, H. Y. *Psychopharmacology* **1995**, *120*, 365.
18. Climbing and stereotypy were induced by sc administration of apomorphine hydrochloride, at a dose of 2.5 mg/kg. Compounds were administered ip/po 30 min prior to apomorphine (group of eight mice). The ED_{50} values for inhibition of climbing and stereotypy were calculated by non-linear regression analysis.
19. Hyperactivity was induced by sc administration of d-amphetamine sulfate at a dose of 4 mg/kg, 30 min before testing. Compounds were administered ip 30 min prior to d-amphetamine, using groups of eight mice. The minimal active dose, defined as the lowest dose which significantly ($p<0.05$) inhibited d-amphetamine induced hyperactivity, was calculated using the Mann–Whitney U-test (two-tailed).
20. A stereotyped behavior was induced by sc administration of d-amphetamine sulfate, at a dose of 12 mg/kg. Compounds were administered ip 30 min prior to d-amphetamine, using groups of eight mice. The minimal active dose, defined as the lowest dose which significantly ($p<0.05$) inhibited d-amphetamine induced stereotypy, was calculated using the Kolmogorov–Smirnov two sample test.
21. A head twitch behavior was induced by ip administration of (±)-DOI hydrochloride (2,5-dimethoxy-4-iodo-amphetamine-HCl), at a dose of 1.6 mg/kg. The compounds were administered ip 30 min prior to DOI, using groups of eight mice. The number of head-twitches was counted 5 min after DOI administration for a duration of 5 min. The ED_{50} values were calculated by non-linear regression analysis.
22. Induction of catalepsy was performed at Porsolt & Partners Pharmacology, France, according to standard procedures. The compounds were administered po in groups of six rats and the induction of catalepsy was examined at 30 min intervals up to 360 min.
23. Conditioned avoidance response inhibition was performed at Porsolt & Partners Pharmacology, France, using the Sidman avoidance test. Compounds were administered po in groups of eight trained rats for which stable baseline performance has been verified over 2 consecutive weeks. The number of avoidance responses, the number of shocks received and the number of escape failures were measured during a session of 20 min starting 60 min after dosing.
24. Tremors and salivation were induced by ip administration of oxotremorine at a dose of 0.25 mg/kg. The compounds were administered ip 30 min prior to oxotremorine, using groups of six mice. Salivation and tremors were scored each 5 min for a duration of 30 min. The ED_{50} values were calculated by non-linear regression analysis.
25. An Irwin test was performed according to a standard protocol after ip administration. Activities like onset of sedation, incidence of convulsion and lethality were selected to assess the neurotoxic profile of the compounds.
26. Meltzer, H. Y.; Matsubara, S.; Lee, J. C. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 238.