D4/5-HT2A Receptor antagonists: LU-111995 and other potential new antipsychotics in development

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Introduction

Schizophrenia affects about 1% of the population worldwide (1). The observation that amphetamines, which induce schizophrenic-like psychosis in humans, release dopamine and that the psychostimulant actions of amphetamines are antagonized by antipsychotic drugs, led to the dopamine hypothesis of schizophrenia (2-4). The key role played by dopamine, and particularly its D2 receptor, was underlined by the excellent correlation between affinities of antipsychotic drugs for the D2 receptor and their therapeutic doses. However, not only therapeutic activity but also extrapyramidal symptoms (EPS) such as acute dystonia, drug-induced parkinsonism, tardive dyskinesia, and hyperprolactinemia are intimately linked to dopamine D2 receptor affinity (1).

These close correlations were first challenged by clozapine which proved to be efficacious not only in positive symptoms but also, in contrast to classical neurolep-
atypical properties; many attempts have been made to correlate the typical or atypical properties of antipsychotic drugs with their affinities for special receptor subtypes. Most of the atypical neuroleptic drugs bind not only to dopamine receptors but also to other receptors such as serotonin, histamine, muscarinic and α-adrenergic receptors, thus displaying a broad and nonspecific receptor profile. Blockade of other receptors or combined blockade of different receptors (e.g., D2/5-HT2A) might contribute to therapeutic efficacy.

Meltzer et al. concluded that, whereas D2 affinity determines the potency of an antipsychotic drug, the 5-HT2A/D2 affinity ratio is responsible for the atypical character of the drug, i.e., efficacy without EPS (28, 29).

On the other hand, many of the typical and atypical antipsychotics with proven clinical efficacy are potent ligands of the interesting dopamine D3 and D4 receptor subtypes within the D2 family. Typical antipsychotics such as trifluoperazine, haloperidol, loxapine and thioridazine, and atypical neuroleptics like clozapine and zotepine, as well as the newest drugs on the market or in preregistration status, i.e., risperidone, olanzapine, sertindole and ziprasidone, show a moderate to high D3 affinity component but also bind to D2 receptors (30-34). Among these, clozapine is the drug with the lowest D3/D2 affinity ratio.

Van Tol et al. proposed that the D2/D3 ratios of antipsychotic drugs may constitute a criterion for differentiation of typical and atypical antipsychotic drugs (13-17). However, it was shown later that although several antipsychotic drugs had high affinities for the dopamine D3 receptor, neither the D3 affinity per se nor D2/D3 or 5-HT2A/D2 ratios reliably distinguish between typical and atypical antipsychotic drugs (30).

Seeman et al. (35) emphasized that the apparent dissociation constant of a drug at a given receptor is dependent on the radioligand used. He extrapolated the radioligand-independent dissociation constants of various antipsychotics at D2, D3 and 5-HT2A receptors. These values agree with the dissociation constants obtained directly with the radioactive antipsychotic drug itself (for example clozapine revealed a radioligand-independent value of 1.6 nM at the dopamine D2 receptor). According to these data, atypical neuroleptics appear to have either a low affinity for dopamine D2 receptors or are selective for D3 receptors.

In studies using positron emission tomography, therapeutic doses of typical antipsychotics like haloperidol were found to occupy 65-89% of brain dopamine D2 receptors in patients, whereas therapeutic doses of the atypical antipsychotic, clozapine, typically result in a D2 receptor occupancy of only 40-65% (36). Due to the lack of specific tracers the dopamine D4 receptor occupancy could not yet be determined. In this context it is especially intriguing that doses as low as 50 mg of clozapine, which should lead to an even lower D4 receptor occupancy, have been reported to be efficacious in psychotic symptoms elicited by dopaminergic treatments in parkinsonian patients (37).

A number of highly selective dopamine D4 antagonists without any relevant affinities for other dopamine and serotonin receptor subtypes have recently been reported. L-745,870 from Merck Sharp and Dohme, did not show any antipsychotic effect on 38 schizophrenic patients using 15 mg/day (38-40). The isoxazole derivative, L-741,742, was hampered by cardiovascular side effects in preclinical studies and some 4-heterocyclyl-piperidines and -tetrahydropyridines are selective ligands for the dopamine D4 receptor (41-44). NGD94-1 from Neuronor appears to have been superseded in phase II by NGD94-4 due to pharmacokinetic reasons (45, 46), whereas 4-arylpiiperazinyl-benzoxazine derivatives are selective dopamine D4 ligands (47). PNU-101387 from Pharmacia & Upjohn is in phase II as an interesting highly selective D4 antagonist with a very long half-life (48, 49), whereas U-99363 and U-101958 could not be developed as drugs due to their poor metabolic stability and low bioavailability (50). CP-293,019 from Pfizer (51), PD-165325, PD-168306 and some chromeno[3,4-c]pyrindin-5-ones from Parke-Davis (52-54), various 2-naphthoate esters and arylpiperazinealkylamides from SmithKline Beecham (55, 56) and YM-50001 from Yamanouchi (57) are all in preclinical development. YM-43611 from Yamanouchi, which in addition to high D4 receptor affinity also exhibits a 10-fold lower D3 affinity, was reported to have been discontinued in the preclinical phase (58). One compound from a series of arylpiperazinyl-benzamide derivatives showed high D4 affinity and selectivity (59).

D4/5-HT2 antagonists in development

The possible importance of serotonin acting in conjunction with dopamine for the pathophysiology of schizophrenia and the mechanism of action of psychotropic drugs has been intensively studied (28, 29, 60, 61). Clozapine, for instance, has a relatively high affinity for at least five of the 5-HT receptor subtypes (5-HT2A, 5-HT2C, 5-HT3, 5-HT6, 5-HT7) and has been shown to be more efficacious than typical neuroleptic drugs in treatment-resistant patients (5-10). It has been suggested that agents which antagonize 5-HT2 receptors in the brain, along with dopamine D2 receptors, may have an improved ratio of therapeutic effect to extrapyramidal symptoms (6-8, 62). Additionally, the ability of an antipsychotic drug to bind to cloned 5-HT2C receptors does not appear to correlate with either its typical or atypical antipsychotic activity (63). Activity at the 5-HT3 receptor may be useful in improving negative symptoms of schizophrenia (64-66), whereas affinity for 5-HT2C receptors may be responsible for neuroleptic-induced weight gain (67).

The combination of 5-HT2 and D2 receptor antagonism could be an excellent basis for good antipsychotic activity without classical side effects. Dopamine D2 receptor antagonists were found on GABAergic neurones in the primate cerebral cortex and GABAergic interneurones receive a major serotonergic input. This led Mrzljak et al. (68) to hypothesize that synergistic effects mediated by
mine-induced hyperactivity (ED50 = 2.2 mg/kg p.o.), apomorphine-induced stereotypy (ED50 = 5.8 mg/kg p.o.) and climbing behavior (ED50 = 3.5 mg/kg p.o.). SM-9018 possesses only weak cataleptogenic activity in rats (ED50 = 150 mg/kg p.o.), despite its very potent D2 antagonistic activity. Moreover, SM-9018 induced weak central depressant effects such as inhibition of spontaneous locomotor activity in mice (ED50 = 15 mg/kg p.o.). These observations suggest that SM-9018 may improve both negative and positive symptoms in schizophrenic patients without strong extrapyramidal side effects.

The benzisoxazole derivative, HP-873 (iloperidone), developed by Hoechst Marion Roussel and licensed exclusively to Titan Pharmaceuticals, is expected to enter phase III clinical trials for the treatment of schizophrenia in the near future (72, 73). The drug shows potent affinity for human D2L (Ki = 6.3 nM, 3H-spiradoline), human D_4 (Ki = 7.1 nM, 3H-methylspiperone), human D_3 (Ki = 5.6 nM, 3H-LSD), and D_2 receptors (Ki = 22 nM, 3H-LSD) are lower. Therefore, iloperidone is classified primarily as a potent D_2, D_3, 5-HT_2 and α_1 ligand with an

Nonselective D_4/5-HT_2 antagonists (Fig. 1)

The piperazino-benzothiazole cis-isomeric derivative, SM-9018 (perospirone), developed by Sumitomo, is currently in phase III clinical trials in Japan (69-71). The drug possesses a broad receptor profile, showing high affinities for human D2L (Ki = 0.2 nM, 3H-spiradoline), human D_4 (Ki = 0.8 nM, 3H-spiradoline), 5-HT_2 (Ki = 0.6 nM, rat cortex, 3H-ketanserin) and 5-HT_1A receptors (Ki = 2.9 nM, rat hippocampus, 3H-8-OH-DPAT), and has moderate affinity for α_1-adrenoceptors (Ki = 17 nM, rat cortex, 3H-WB-4101) and D_3 receptors (Ki = 41 nM, rat striatum, 3H-SCH-23390). The drug also strongly inhibits 5-HT_2 receptor-mediated behavior in rats, such as tryptamine-induced clonic seizures (ED50 = 1.4 mg/kg p.o.) and D_2-like receptor-mediated behavior such as methamphetamine-induced hyperactivity (ED50 = 2.2 mg/kg p.o.), apomorphine-induced stereotypy (ED50 = 5.8 mg/kg p.o.) and climbing behavior (ED50 = 3.5 mg/kg p.o.). SM-9018 possesses only weak cataleptogenic activity in rats (ED50 = 150 mg/kg p.o.), despite its very potent D_2 antagonistic activity. Moreover, SM-9018 induced weak central depressant effects such as inhibition of spontaneous locomotor activity in mice (ED50 = 15 mg/kg p.o.). These observations suggest that SM-9018 may improve both negative and positive symptoms in schizophrenic patients without strong extrapyramidal side effects.
atypical multireceptor profile. In a mouse model of apomorphine-induced climbing, inhibition was observed with ED_{50} values of 0.16 and 0.25 mg/kg i.p. and p.o., respectively. Iloperidone was reported to be efficacious and well tolerated in phase II studies in schizophrenia.

The benzisothiazole derivative, 1192U90, developed by Glaxo Wellcome, has completed phase I clinical trials (74-76). The drug bound potently to 5-HT_{2} (K_{i} = 1.5 nM, rat cortex, ^{3}H-ketanserin), 5-HT_{1A} (K_{i} = 2.5 nM, rat hippocampus, ^{3}H-8-OH-DPAT) and ^{3}H-adenrenergic receptors (K_{i} = 0.3 nM, rat brain, ^{3}H-WB-4101) but showed comparatively lower affinity and no selectivity for hD_{4} (K_{i} = 13 nM, ^{3}H-spiperone) and hD_{2S} receptors (K_{i} = 13 nM, ^{3}H-oraclpride). Electrophysiological investigations revealed 5-HT_{1A} agonism which could be an additional beneficial effect for antipsychotic efficacy. 1192U90 was also active in tests that predict antipsychotic activity; it antagonized apomorphine-induced climbing in mice (ED_{50} = 10 mg/kg p.o.) and amphetamine-induced hyperlocomotion in rats (ED_{50} = 6.6 mg/kg p.o.) and inhibited conditioned avoidance in rats (ED_{50} = 5.7 mg/kg p.o.). The drug only weakly antagonized apomorphine-induced stereotypy in rats (ED_{50} = 133 mg/kg p.o.) and induced catalepsy in mice only at high doses (ED_{50} = 192 mg/kg p.o.). In combination with its strong in vivo 5-HT_{2} antagonistic effects, 1192U90 may relieve positive and negative symptoms of schizophrenia with an atypical antipsychotic profile.

JL-13, a biosisosteric analog of clozapine, is in preclinical studies at Therabel as a clozapine successor with lower side effect potential (77-79). The drug exhibited potent affinity for 5-HT_{2A} (pK_{i} = 7.19) and D_{4} receptors (pK_{i} = 6.79) with a moderate selectivity for D_{4} versus D_{2} (pK_{i} = 5.92). On the other hand, the affinities for D_{1}, muscarinic, 5-HT_{2C}, 5-HT_{6} and 5-HT_{7} receptors are reduced compared with clozapine. In preclinical studies, JL-13 did not antagonize apomorphine-induced stereotypy and did not produce catalepsy, but antagonized apomorphine-induced climbing in rodents.

The benzodioxane derivative, S-16924 ((+-)-enantio), is currently in preclinical development at Servier as a D_{4}/5-HT_{2} antagonist and 5-HT_{1A} ligand for the potential treatment of psychosis and schizophrenia (80). In binding studies the compound was 6-fold more potent than clozapine at hD_{2} receptors (K_{i} = 6.2 nM, ^{3}H-N-methylspiiperone) and 5-fold more potent at hD_{4} receptors (K_{i} = 49 nM, ^{125}I-iodosulpride) but does not show selectivity versus hD_{2} receptors (K_{i} = 29 nM, ^{125}I-iodosulpride). In addition to a high 5-HT_{2A} affinity (pK_{i} = 8.6, rat cortex, ^{3}H-ketanserin), S-16924 binds strongly to 5-HT_{1A} (pK_{i} = 8.4, rat hippocampus, ^{3}H-8-OH-DPAT) and 5-HT_{2C} receptors (pK_{i} = 8.1, pig choroid plexus, ^{3}H-mesulergine). It inhibited amphetamine-induced locomotion in rodents (ID_{50} = 2.3 mg/kg s.c.), reduced conditioned avoidance responses (ID_{50} = 2.9 mg/kg s.c.) and failed to evoke catalepsy. This pharmacological profile indicates S-16924 as a potential atypical antipsychotic which may have advantages over clozapine.

Another atypical antipsychotic from Servier at the pre-clinical stage is the (+-)-enantiomeric benzisoxazole derivative, S-17828 (80-82). It shows high affinity for hD_{2} (K_{i} = 2.0 nM, ^{3}H-N-methylspiiperone) and hD_{4} receptors (K_{i} = 1.6 nM, ^{125}I-iodosulpride) but without any selectivity versus hD_{2} receptors (K_{i} = 3.2 nM, ^{125}I-iodosulpride). Furthermore, it also binds strongly to 5-HT_{2A} receptors (pK_{i} = 8.5), ^{3}H-8-OH-DPAT and ^{3}H-adrenoceptors (pK_{i} = 8.2). S-17828 elicited catalepsy in rats only at doses (ED_{50} = 1.7 mg/kg p.o.) higher than those inhibiting amphetamine-induced locomotion (ID_{50} = 0.04 mg/kg p.o.).

The indole derivative, BIMG-80, is currently in preclinical development at Boehringer Ingelheim for the treatment of psychosis (83-85). This drug shows a broad antagonistic receptor profile with strong 5-HT_{2A} (K_{i} = 5.8 nM, rat cortex, ^{3}H-ketanserin), hD_{2A} (K_{i} = 5.7 nM, ^{3}H-ramiprilexole), hD_{1} (K_{i} = 2.1 nM, ^{3}H-SC-23390), hD_{2} (K_{i} = 11 nM, ^{3}H-ramiprilexole) and ^{3}H-affinities (K_{i} = 12 nM, rat cortex, ^{3}H-prazosin) and moderate 5-HT_{2} affinity (K_{i} = 51 nM, cloned rat receptor, ^{3}H-LSD). The functional test revealed agonist properties at the 5-HT_{1A} receptor (K_{i} = 52 nM). BIMG-80 antagonized the hyperlocomotion caused by d-amphetamine in rats (ED_{50} = 5.3 mg/kg i.p.) and did not reduce d-amphetamine-induced stereotypy up to 10 mg/kg i.p. There was no cataleptogenic potential up to 48 mg/kg i.p. BIMG-80 (0.3-3 mg/kg s.c.), like clozapine (2.5-10 mg/kg s.c.), produced greater percent increases in dopamine release in the medial prefrontal cortex than in the striatum and nucleus accumbens. Therefore, BIMG-80 appears to be a potential atypical antipsychotic with a low propensity to induce extrapyramidal side effects.

The atypical antipsychotic candidate, SM-13496, developed by Sumitomo, is reported to be in phase I clinical trials (86-89). The drug binds with high affinity for cloned dopamine D_{4} receptors (K_{i} = 1.4 nM) and shows high to moderate affinities for dopamine D_{2} (K_{i} = 14 nM) and 5-HT_{2} receptors (K_{i} = 27 nM). SM-13496 inhibited methamphetamine-induced hyperactivity in rats (ED_{50} = 0.9 mg/kg p.o.) which persisted for over 12 hours, blocked amphetamine-induced climbing behavior in mice (ED_{50} = 4.9 mg/kg p.o.) and selectively suppressed conditioned avoidance response in rats (ED_{50} = 4.7 mg/kg p.o.). It also inhibited serotonin receptor-mediated effects such as tryptamine-induced clonic seizure and p-chloro-amphetamine-induced hyperthermia in rats (ED_{50} = 3.0 - 5.6 mg/kg). Despite its potent D_{4} blocking activities, SM-13496 showed only negligible induction of extrapyramidal side effects, i.e., catalepsy (ED_{50} > 1.000 mg/kg p.o.). In conclusion, SM-13496 appears to be an atypical antipsychotic with very low propensity to induce extrapyramidal side effects.

Selective D_{4}/5-HT_{2} antagonists (Fig. 2)

The naphosultam derivative, RP-62203 (fanaserin), developed by Rhône Poulenc Rorer, was originally
described as a potent 5-HT₂ ligand in vitro (Kᵢ 5-HT₂ = 0.26 nM, rat brain, ³H-ketanserin) with a more than 100-fold selectivity versus H₁, α₁, 5-HT₁A and D₂ receptors, indicating a profile superior to that of ritanserin (90). In vivo this compound is a potent orally effective and long-lasting 5-HT₂ antagonist in the mescaline-induced head twitch test in mice (ED₅₀ 0.4 mg/kg p.o.) and rats (91). Fananserin was in phase I clinical trials in France and the U.S. Its development for dysthymic disorders, sleep disorders, depression and anxiety has been discontinued (92). Later, after the high affinity of fananserin for the human dopamine D₄ receptor (Kᵢ 2.9 nM, ³H-sperone) and 250-fold selectivity versus the rat D₂ receptor was discovered (Kᵢ D₂ = 726 nM, rat striatum, ³H-sperone) (93), the compound was developed as a third generation antipsychotic drug. It produced no extrapyramidal symptoms in phase I (94). However, it has recently been reported that the further development of fananserin has been discontinued due to lack of efficacy in a phase II trial (95).

The aminothiazole derivative, NRA-0045 ((R)- (+)-enantiomer), is in preclinical development at Taisho as an atypical antipsychotic with high D₄, 5-HT₂₅ and α₁ affinities (96-99). The drug shows a high dopamine D₄ selectivity (Kᵢ D₄ = 2.5 nM, ³H-sperone, 90-fold selectivity against hD₂ receptors and 40-fold against hD₃ receptors), high 5-HT₂₅ affinity (Kᵢ = 1.9 nM, ³H-RP62203, rat cortex) and strong α₁-adrenoceptor affinity (Kᵢ = 1.4 nM, ³H-prazosin, rat brain). The pharmacological profile suggests that NRA-0045 may have no propensity for causing motor side effects. Locomotor hyperactivity induced by methamphetamine in rats was dose-dependently antagonized (ED₅₀ = 0.4 mg/kg i.p. and 0.3 mg/kg p.o.), the disruption of prepulse inhibition in rats by amphetamine was significantly reversed (3 mg/kg i.p.), and the inhibitory effect of methamphetamine on the firing rate of A10 dopamine neurones was completely reversed (ED₅₀ = 0.1 mg/kg i.v.), whereas the inhibitory effect of methamphetamine on A9 dopamine neurones was not affected (in doses up to 1 mg/kg i.v.). NRA-0045 produced less than 50% induction of catalepsy in rats at the dose 30 mg/kg i.p. As a compound with combined dopamine D₄ and 5-HT₂ receptor antagonist activity, NRA-0045 may be an interesting new antipsychotic drug for the treatment of both positive and negative symptoms of schizophrenia. Some back-up candidates in this structural class have been reported very recently (100).

The racemic isochroman derivative, PNU-96415E, from Pharmacia & Upjohn, is currently in preclinical development as a D₄/5-HT₂ antagonist (101). Compared with clozapine, the drug has a 10-fold higher affinity for the hD₄ receptor (Kᵢ = 3.0 nM, ³H-sperone) and an equipotent affinity for the 5-HT₂₅ receptor (Kᵢ = 5.8 nM, rat, ³H-ketanserin), while its dopamine D₂ (Kᵢ = 199 nM, cloned rat D₂ receptors, ³H-raclopride) and α₁-adrenergic receptor affinities (Kᵢ = 38 nM, rat cortex, ³H-prazosin) are considerably lower. The results from in vivo studies are consistent with a clozapine-like profile: PNU-96415E inhibited exploratory locomotor activity in mice and rats with approximately one-third lower potency than clozapine, it antagonized apomorphine-induced climbing with approximately 10-fold lower potency than clozapine and blocked head and body twitch produced by 5-HTP in mice with about one-third the potency of clozapine. The drug did not antagonize stereotypic behaviors produced by a high dose of ¾-amphetamine or methylphenidate in rats and mice at doses up to 30 mg/kg. It blocked conditioned avoidance response in rats with a lower potency than clozapine and produced only very weak catalepsy at doses that reduced locomotion or impaired conditioned avoidance in rats. Having a clozapine-like profile with respect to D₄/5-HT₂ receptor affinity, PNU-96415E may have potential antipsychotic efficacy with a lower side effect potential.
The benzisoldolone derivative ((S)-enantioner), PD-172938 (102), has been identified by Parke Davis and is currently in preclinical development. It is a potent and selective dopamine D₄ receptor antagonist (Kᵦ = 7.8 nM) with some 5-HT₂ affinity (Kᵦ = 15 nM). In rats, the compound at 20 mg/kg p.o. showed a 37% increase in dopamine synthesis in the hippocampus and inhibited apomorphine-induced locomotion with an ED₅₀ value of 1.8 mg/kg p.o. PD-172938 has strong structural similarities with the Upjohn compound, PNU-101387.

**LU-111995**

For several years in our laboratory we have been synthesizing potential atypical antipsychotic compounds having combined affinities for dopaminergic and serotonergic receptor subtypes. Rilapine, a 5,11-dicarbo analog of clozapine with a pharmacophore nitenitrile function on carbon 11, was one of the interesting candidates (28, 29, 103, 104) with potent 5-HT₂A (Kᵦ = 0.3 nM, rat cortex, [³H]-ketanserin), 5-HT₁A (Kᵦ = 0.3 nM, rat hippocampus, [³H]-8-OH-DPAT), α₁ (Kᵦ = 0.1 nM, rat brain, [³H]-prazosin), moderate D₁ (Kᵦ = 12 nM, bovine nucleus caudatus, [³H]-SCH-23390) and weaker D₂ affinities (Kᵦ = 70 nM, bovine nucleus caudatus, [³H]-spiperone).

As part of our synthetic program it was our goal to replace the conformationally flexible amino side chains of atypical neuroleptics with new, more rigid 3-azabicyclo[3.2.0]heptane building blocks accessible by intramolecular [2+2] cycloaddition reactions (105). A second aim was to synthesize a structure completely different from clozapine.

LU-111995 ([(+)-(1S,5R,6S)-exo-3-[2-[6-4-fluorophenyl]-3-azabicyclo[3.2.0]heptane-3-yl]-ethyl]-1H,3H-quinazoline-2,4-dione] has been selected from a series of compounds due to its high dopamine D₄ affinity and its D₄ versus D₂ receptor selectivity (106). Having additional high affinity for the 5-HT₂ receptor, it appears to be particularly promising for development as an antipsychotic with an improved efficacy/side effect profile.

**Chemistry**

Figure 3 shows the chemistry of LU-111995 and derivatives. The benzaldehyde derivative reacts with vinylmagnesium chloride to give the aromatic allylic alcohol, which is then rearranged by addition of hydrogen chloride to produce the 3-chlorine substituted aromatic allylic chloride in > 90% yield. Nucleophilic substitution with excess allylamine at 50 °C leads to the monosubstituted diallyl amine in > 80% yield. The N-cinnamyl-N-allylamines react upon irradiation with UV light via a photochemical [2+2] cycloaddition in excellent yield (> 85%) to give the new exo-6-aryl-3-azabicyclo[3.2.0]heptane building blocks with high (> 90%) diastereoselectivity versus the endo diastereomer (107). Separation of the (+)-enantiomers proceeds effectively by classical resolution with (-)-2,3-di-p-toluoyl-L-tartaric acid in > 30% yield. The absolute configurations of the azabicyclo[3.2.0]heptane intermediates were determined by X-ray crystallographic analysis of the HCl salts (107). Coupling with the heterocyclic alkylbromide leads to the selective D₄/5-HT₂ receptor antagonists in > 70% yield.

**Structure-activity relationship**

Table I shows the variation of substituents on the 4-membered ring. The rigid 3-azabicyclo[3.2.0]heptane side chain must be substituted on the 4-membered ring with an exo-configured aromatic ring to achieve high dopamine D₄ affinity (compare 12). Introduction of fluorne into the 4-position significantly improved the D₄ affinity and selectivity versus D₂A, while the 5-HT₂A affinity remained unchanged. The (+)-enantiomer of 2, LU-111995, showed 30-fold higher D₄ affinity and selectivity versus D₂A than the corresponding (-)-enantiomer. The dopamine D₄ affinity and selectivity was not improved by m-chlorine or p-nitro substitution. Electron donating substituents such as m-methoxy or p-amino eliminated the dopamine receptor D₄ affinity and reversed selectivity in favor of the D₂A receptor. Replacement of the phenyl group by thiophen needs the introduction of 5-chlorine to achieve high D₄ affinity and selectivity. A basic nitrogen atom in the 4-membered ring substituent (e.g., pyridine derivative 11) abolishes D₄ and 5-HT₂A affinity.

**Receptor binding profile**

Binding of LU-111995 to a variety of monoaminergic, peptidergic and other receptors (total 38) has been investigated by radioligand binding following standard procedures. The most relevant data are summarized in Table II.
ing the luciferase gene driven by a cAMP-dependent promoter was added as a reporter. Forskolin was used to increase the intracellular cAMP concentration. Addition of the agonist, quinpirole, dose-dependently reduced the luciferase bioluminescent signal, indicating a decrease of cAMP. Antagonists shifted the quinpirole dose-response curve to the right.

LU-111995 as well as the reference compounds, clozapine and haloperidol, were found to be pure competitive antagonists causing parallel shifts of quinpirole dose-response curves to the right.

In vivo pharmacology

LU-111995 displays highest affinities for D₄ (Kᵢ = 3.1 nM) and 5-HT₂A (3.3 nM) receptors. The D₂A/D₄ ratio was 34. Moderate affinity was found for histamine H₁ (16.5 nM) and α₁-adrenergic (19 nM) receptors. Lower affinity was seen for D₂A (105 nM), 5-HT₁D (79 nM), α₂ (50 nM), 5-HT₁C (61 nM) and σ (37 nM) receptors. Kᵢ values of LU-111995 for all other receptors tested exceeded 200 nM.

The receptor profile of LU-111995 had remarkable similarities to that of clozapine. In comparison to clozapine, however, the D₄ affinity of LU-111995 is 7-fold higher, and the D₂/D₄ selectivity is 5-fold more pronounced. Conversely, clozapine displays higher affinities for histamine H₁ (27-fold), α₁⁻, α₂-adrenergic and muscarinic receptors. Affinities of both compounds for the 5-HT₂A receptor are almost equal.

Functional D₄₂ receptor assay

In vitro studies to determine agonist/antagonist properties of LU-111995 were carried out on HEK293 cells stably expressing D₄₂ receptors. A DNA construct encoding the luciferase gene driven by a cAMP-dependent promoter was added as a reporter. Forskolin was used to increase the intracellular cAMP concentration. Addition of the agonist, quinpirole, dose-dependently reduced the luciferase bioluminescent signal, indicating a decrease of cAMP. Antagonists shifted the quinpirole dose-response curve to the right.

LU-111995 as well as the reference compounds, clozapine and haloperidol, were found to be pure competitive antagonists causing parallel shifts of quinpirole dose-response curves to the right.

In vivo pharmacology

LU-111995 shows clear dose-dependent antidopaminergic as well as antiserotonergic effects in vivo (Table III). Whereas the serotonin antagonistic potency corresponds quite well to the high affinity for the 5-HT₂ receptor found in vitro, effects in the antidopaminergic tests (apomorphine or methamphetamine antagonism) and the conditioned avoidance response seem to reflect
The compound showed good tissue penetration ($V_{ss} = 6$ l/kg b.w. in the dog, 5 l/kg b.w. in the rat). Brain tissue concentrations were 5- to 6-fold higher than plasma concentrations.

LU-111995 is highly bound to plasma proteins in man (98.6%), dog, rabbit and rat (97.5-96.5%). Plasma clearance was 1.3 l/h/kg b.w. in the dog and 3.8 l/h/kg b.w. in the rat. The elimination of LU-111995 from plasma was rapid, with half-lives of 45 min in dogs and 15 min in rats. The terminal half-lives are much longer with values of 6 h in the dog and 1.6 h in the rat. LU-111995 is extensively metabolized by oxidation and subsequent conjugation in dogs and rats.

In man, LU-111995 is well absorbed after oral administration and almost completely excreted in the form of metabolites. Excretion occurs via urine and feces in roughly equal amounts. In plasma three main metabolites were found in addition to the unchanged drug: the 5-membered lactam derivative of LU-111995 and two derivatives, hydroxylated in position-6 and -8 of the quinazoline ring system.

Up to 15 metabolites were excreted via the renal and fecal routes. In the urine most of the metabolites were conjugated with glucuronic and sulfuric acid. In vitro investigation with human liver microsomes showed meta-
bolic degradation mainly by cytochrome P450 3A4 and, to a lesser extent, by cytochrome P450 2D6. Because of the lower lipophilicity of the metabolites and their weaker or negligible affinity for dopamine D4 receptors, a contribution of metabolites to the effects of LU-111995 in vivo is unlikely.

### Toxicology

LU-111995 has been studied in laboratory animals to identify and delineate its toxic properties in doses well above the proposed clinical dose of 2-4 mg/kg b.w.

Data from acute toxicity studies in rats and mice indicated that LU-111995 was readily and rapidly absorbed by the oral route. In rats and mice, acute toxicity after oral

### Table II: In vitro receptor binding of LU-111995 in comparison with clozapine.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Receptor source</th>
<th>Labelled ligand</th>
<th>Nonspecific binding</th>
<th>K_i [nM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine D1</td>
<td>Bovine caudate</td>
<td>^3H-SCH 23390</td>
<td>Butaclamol (10 µM)</td>
<td>580</td>
</tr>
<tr>
<td>Dopamine D2</td>
<td>Human cloned</td>
<td>^125I-Iodosuprime</td>
<td>Haloperidol (1 µM)</td>
<td>105</td>
</tr>
<tr>
<td>Dopamine D3</td>
<td>Human cloned</td>
<td>^125I-5-HT</td>
<td>Spiperone (1 µM)</td>
<td>2234</td>
</tr>
<tr>
<td>Dopamine D4</td>
<td>Human cloned</td>
<td>^125I-5-CT</td>
<td>Haloperidol (1 µM)</td>
<td>3.1</td>
</tr>
<tr>
<td>Serotonin 5-HT1A</td>
<td>Rat hippocampus</td>
<td>^3H-8-OH-DPAT</td>
<td>5-HT (1 µM)</td>
<td>1030</td>
</tr>
<tr>
<td>Serotonin 5-HT1D</td>
<td>Human cloned</td>
<td>^3H-5-CT</td>
<td>5-HT (1 µM)</td>
<td>79</td>
</tr>
<tr>
<td>Serotonin 5-HT2A</td>
<td>Rat cortex</td>
<td>^3H-Ketanserin</td>
<td>Cyprioheptadine (0.1 µM)</td>
<td>3.3</td>
</tr>
<tr>
<td>Serotonin 5-HT2C</td>
<td>Pig choroid plexus</td>
<td>^3H-Mesulergine</td>
<td>5-HT (1 µM)</td>
<td>51</td>
</tr>
<tr>
<td>Histamine H1</td>
<td>Guinea pig cerebellum</td>
<td>^3H-Pyrimidin</td>
<td>Pyrimidine (1 µM)</td>
<td>17</td>
</tr>
<tr>
<td>Histamine H2</td>
<td>Guinea pig cortex</td>
<td>^3H-Triotide</td>
<td>Histamine (5 µM)</td>
<td>241</td>
</tr>
<tr>
<td>Histamine H3</td>
<td>Rat cortex</td>
<td>^3H-Methylhistamine</td>
<td>Methylhistamine (1 µM)</td>
<td>1700</td>
</tr>
<tr>
<td>Adrenoceptor α1</td>
<td>Rat brain</td>
<td>^3H-Prazosin</td>
<td>Prazosin (0.5 µM)</td>
<td>19</td>
</tr>
<tr>
<td>Adrenoceptor α2</td>
<td>Rat brain</td>
<td>^3H-RX-621002</td>
<td>(-)Epinephrine (100 µM)</td>
<td>57</td>
</tr>
<tr>
<td>Sigma</td>
<td>Guinea pig cortex</td>
<td>^3H-(+)-3PPP</td>
<td>Haloperidol (10 µM)</td>
<td>37</td>
</tr>
<tr>
<td>Muscarinic</td>
<td>Rat cortex</td>
<td>^3H-QNB</td>
<td>Atropine (1 µM)</td>
<td>&gt;1000</td>
</tr>
</tbody>
</table>

### Table III: Comparison of LU-111995 with clozapine and haloperidol in pharmacological in vivo tests.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Apomorphine antagonism^a Mouse ED50 [mg/kg]</th>
<th>Methamphetamine antagonism^a Mouse ED50 [mg/kg]</th>
<th>Serotonin antagonism^a Mouse ED50 [mg/kg]</th>
<th>Conditioned avoidance response Rat ED50 [mg/kg]</th>
<th>Cataleptogenic effect Rat ED50 [mg/kg]</th>
</tr>
</thead>
<tbody>
<tr>
<td>LU 111995</td>
<td>18.1 (12.9/27.4)</td>
<td>4.1 (1.9/7.86)</td>
<td>1.0 (0.52/1.56)</td>
<td>6.1 (3.94/8.91)</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>215 (0/16)</td>
</tr>
<tr>
<td>Clozapine</td>
<td>5.2 (3.85/7.19)</td>
<td>2.45 (1.2/5.7)</td>
<td>0.32 (0.2/0.5)</td>
<td>6.01 (4.98/7.19)</td>
<td>10.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100 (0/8)</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>0.05 (0.03/0.09)</td>
<td>0.07 (0.06/0.09)</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.15 (9/12)</td>
</tr>
</tbody>
</table>

^aLU-111995 and clozapine were administered p.o. 60 min, haloperidol 120 min before test. ^bmg/kg p.o. (95% confidence limits).
LU-111995 was well tolerated in healthy volunteers after single oral doses up to 350 mg and after repeated dosing (up to 200 mg/day p.o.).

Single oral doses of 100-350 mg, as well as multiple oral doses of 50 mg, 100 mg and 200 mg once daily for 10 days resulted in slight to moderate sedation. No change in prolactin levels and in 6-hydroxycortisol/cortisol ratio was observed, indicating no relevant dopamine D2 receptor antagonism and no cytochrome P450 A4 enzyme induction, respectively. No seizures have been observed.

The plasma concentration-time curves of LU-111995 suggest a distinct two-compartment model. Cmax is reached after 1-3 h. Elimination t½ values are approximately 12 h (6-21 h). Dose linearity is given for the tested doses of 25-350 mg.

The results of a single-center, double-blind phase I/II study in 12 severely ill, chronic schizophrenic patients (8 patients on active drug, 4 on placebo, 5 weeks treatment) with a forced dose titration up to 350 mg/day demonstrated that LU-111995 is very well tolerated; only 1 patient showed akathisia at 350 mg, which disappeared after dose reduction.

Six out of 8 patients treated with LU-111995 showed reduction of schizophrenic symptoms, as did 2 of the 4 patients treated with placebo. Improvement in total PANSS score for active treatment in comparison to placebo was 32% (median values) in the endpoint analysis. In patients enrolled into a long-term extension of the trial because of their excellent response, no decrease in efficacy was observed after dose reduction. Taking into consideration the patient population and size, the tolerability and efficacy compared to placebo was remarkable and very encouraging.

These preliminary results obviously differ from the results obtained with the pure dopamine D4 receptor antagonist, L-745,870, and the D4/5-HT2A antagonist, fananserin. This discrepancy cannot be resolved currently, since the available information on pharmacological properties, pharmacokinetic parameters, dose ranging, receptor occupancy, etc. is limited.

Conclusions

Many drug companies are focusing on the identification and development of either pure dopamine D4 receptor antagonists or mixed-type dopamine/serotonin antagonists with a potent D4 component. These drugs are aimed at a clozapine-like atypical antipsychotic activity, avoiding the serious side effects of clozapine.

The anatomical localization of D4 receptors, especially their occurrence in the human cortex, make them an attractive target for antipsychotics with an improved effect on negative symptoms and cognitive deficits. The physiological and pharmacotherapeutic role of dopamine D4 receptors, however, is difficult to elucidate. Many of the classical behavioral paradigms which are still used to predict antipsychotic activity in patients have been validated and optimized by available antipsychotics with a preference for dopamine D2 receptors.

The three highly selective dopamine D4 receptor antagonists (L-745,870, PNU-101387 and NGD94-1) known to have entered clinical investigations so far, appeared to be without effect in standard animal models for antipsychotics. Therefore, clinical evaluation of the hypothesis that dopamine D4 receptor antagonism is responsible for, or at least contributes to the antipsychotic effect of clozapine is indispensable. In view of the limited information on L-745,870, the observation that this drug at a single dosage level did not improve psychotic symptoms may not be sufficient to refute the hypothesis that dopamine D4 receptors are a valuable target for antipsychotics, and that the efficacy of clozapine is at least partly attributable to its relatively high D4 affinity. L-745,870 was devoid of any clinical improvement in 38 acutely psychotic patients. Therefore, phase II results with the highly selective D4 antagonist, PNU-101387, are eagerly awaited.

In contrast to pure D4 antagonists, mixed D4/5-HT2A antagonists may possess important therapeutic advantages, since their effect on 5-HT2A and/or further receptors could result in effects synergistic to D4 antagonism and thus enhance the antipsychotic efficacy. The mixed-type compounds discussed in this review show effects in standard animal models of antipsychotic activity in varying degrees. Preliminary clinical data with LU-111995 on tolerability and efficacy in severely ill schizophrenic patients are very encouraging, and further phase II data with this drug are eagerly anticipated. Less sedation, a lack of increased salivation and a shorter titration phase may differentiate LU-111995 from clozapine.

Clinical results with dopamine/serotonin subtype-selective antagonists aiming at a certain set of monoaminergic receptors will show whether therapeutic progress in the treatment of one of the most disabling human diseases can be achieved by the envisaged mechanisms or if we must wait for a new drug generation beyond the dopamine/serotonin antagonists.

Acknowledgements

The contribution of Prof. Seeman and Dr. van Tol to the testing of dopamine D4 receptor binding is gratefully acknowledged.

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