Review Article

D$_4$/5-HT$_{2A}$ Receptor antagonists: LU-111995 and other potential new antipsychotics in development

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CONTENTS

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 D$_2$ receptor, was underlined by the excellent correlation between affinities of antipsychotic drugs for the D$_2$ 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 D$_2$ 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 neuroleptics, in negative symptoms, producing virtually no EPS (5). Despite its potential to induce agranulocytosis, clozapine is considered a mainstay of therapy of schizophrenics, especially for otherwise treatment-resistant patients.

Pharmacologically, clozapine differs in its receptor profile from typical antipsychotics by having high affinity for different neurotransmitter receptors such as histamine H$_1$, serotonin 5-HT$_2$, 5-HT$_6$, 5-HT$_7$, muscarinic acetylcholine, α-adrenoceptors, *etc.*, while binding with only moderate affinity to the dopamine D$_2$ receptor (6-10).

For two decades or more the search for new antipsychotics has focused on efforts to copy the receptor profile of clozapine. These efforts resulted in drugs combining D$_2$ and 5-HT$_2$ antagonism, such as risperidone.

The discovery of dopamine D$_3$ and D$_4$ receptors as members of the D$_2$ receptor family has opened new perspectives for the search for new antipsychotic agents (11-16). In particular, the D$_4$ receptor appears to be a promising target, since of all the dopamine receptors, clozapine exhibits highest affinity for this subtype. In addition, its ratio of D$_4$ affinity over the free plasma concentration at therapeutic doses fits well with that of other neuroleptics (17).

Investigations on D$_4$ receptor mRNA distribution in rats revealed a preferential location in cortical and midbrain regions such as the amygdala and hippocampus. In primate brains, D$_4$ receptor antibody labelling and binding was found on pyramidal cells as well as on interneurones of the cortex and the hippocampus (18). These areas have been linked to schizophrenic symptoms, especially negative symptoms and cognitive deficit. Only low density is detected in the corpus striatum (13-16, 19-21), believed to be the region in which D$_2$ receptor antagonism causes EPS. Furthermore, D$_4$ receptor levels were reported to be elevated in postmortem brains of schizophrenics (22, 23), although evidence for this finding is still under debate (24-27).

Difficulties arise in defining and quantifying typical and

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.*, D_2/5-HT$_{2/\alpha}$) might contribute to therapeutic efficacy.

Meltzer *et al.* concluded that, whereas D_2 affinity determines the potency of an antipsychotic drug, the 5-HT$_2$/D_2 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 D_3 and D_4 receptor subtypes within the D_2 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 D_4 affinity component but also bind to D_2 receptors (30-34). Among these, clozapine is the drug with the lowest D_2/D_4 affinity ratio.

Van Tol *et al.* proposed that the D_2/D_4 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 D_4 receptor, neither the D_4 affinity *per se* nor D_2/D_4 or 5-HT$_{2A}$/D_4 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 D_2, D_4 and 5-HT$_{2A}$ 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 D_4 receptor). According to these data, atypical neuroleptics appear to have either a low affinity for dopamine D_2 receptors or are selective for D_4 receptors.

In studies using positron emission tomography, therapeutic doses of typical antipsychotics like haloperidol were found to occupy 65-89% of brain dopamine D_2 receptors in patients, whereas therapeutic doses of the atypical antipsychotic, clozapine, typically result in a D_2 receptor occupancy of only 40-65% (36). Due to the lack of specific tracers the dopamine D_4 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 D_2 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 D_4 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 D_4 receptor (41-44). NGD94-1 from Neurogen appears to have been superseded in phase II by NGD94-4 due to pharmacokinetic reasons (45, 46), whereas 4-arylpiperazinyl-benzoxazine derivatives are selective dopamine D_4 ligands (47). PNU-101387 from Pharmacia & Upjohn is in phase II as an interesting highly selective D_4 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]pyridin-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 D_4 receptor affinity also exhibits a 10-fold lower D_3 affinity, was reported to have been discontinued in the preclinical phase (58). One compound from a series of arylpiperazinyl-benzamide derivatives showed high D_4 affinity and selectivity (59).

D_4/5-HT$_2$ 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-HT$_{2A}$, 5-HT$_{2C}$, 5-HT$_3$, 5-HT$_6$, 5-HT$_7$) 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-HT$_2$ receptors in the brain, along with dopamine D_2 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-HT$_{2C}$ receptors does not appear to correlate with either its typical or atypical antipsychotic activity (63). Activity at the 5-HT$_{2A}$ receptor may be useful in improving negative symptoms of schizophrenia (64-66), whereas affinity for 5-HT$_{2C}$ receptors may be responsible for neuroleptic-induced weight gain (67).

The combination of 5-HT$_2$ and D_4 receptor antagonism could be an excellent basis for good antipsychotic activity without classical side effects. Dopamine D_4 receptors were found on GABAergic neurones in the primate cerebral cortex and GABAergic interneurones receive a major serotoninergic input. This led Mrzljak *et al.* (68) to hypothesize that synergistic effects mediated by
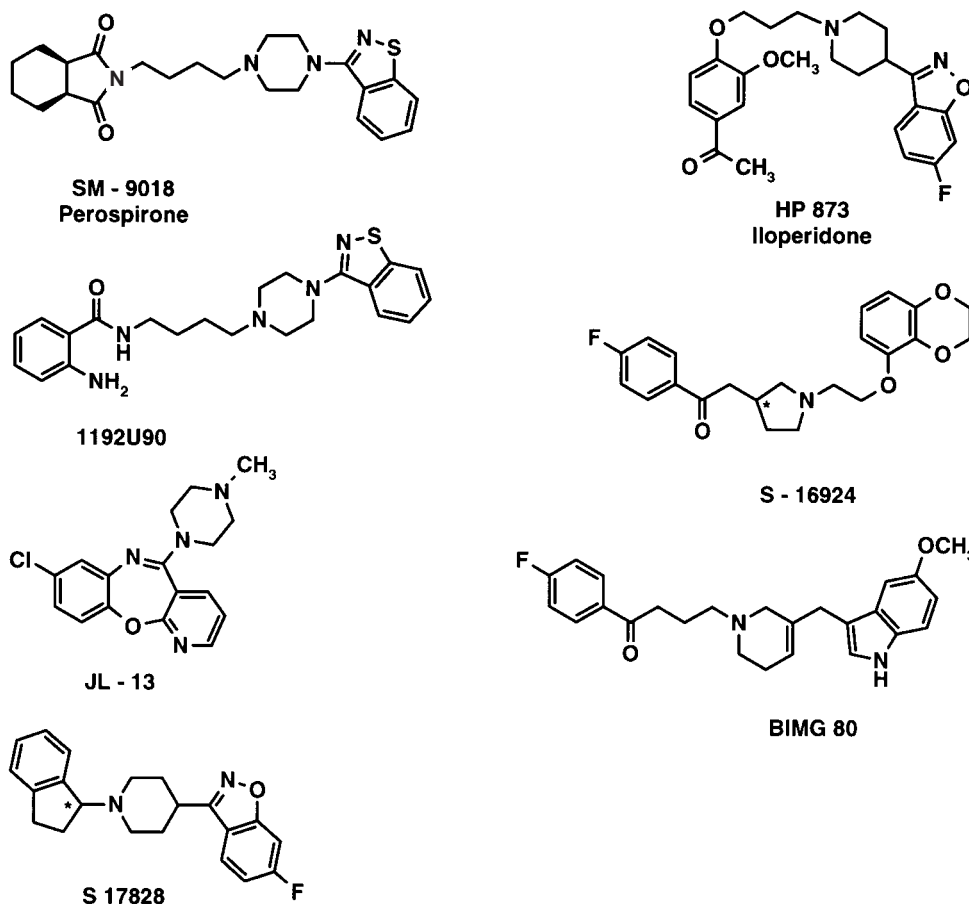
Fig. 1. Structure of nonselective D_4/5-HT_2 antagonists.

dopamine D_4 and by 5-HT_2 receptors may be one possible reason for the palliative effect of clozapine in schizophrenic patients.

Some of the newer atypical neuroleptics on the market, zotepine, risperidone, olanzapine and sertindole, combine high 5-HT_{2A} and high D_4 affinity but also bind to the D_2 receptor (30-34).

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

The piperazino-benzisothiazole *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 D_{2L} (K_i = 0.2 nM, ^{3}H-spiperone), human $D_{4.4}$ (K_i = 0.8 nM, ^{3}H-spiperone), 5-HT_2 (K_i = 0.6 nM, rat cortex, ^{3}H-ketanserin) and 5-HT_{1A} receptors (K_i = 2.9 nM, rat hippocampus, ^{3}H-8-OH-DPAT), and has moderate affinity for α_1-adrenoceptors (K_i = 17 nM, rat cortex, ^{3}H-WB-4101) and D_1 receptors (K_i = 41 nM, rat striatum, ^{3}H-SCH-23390). The drug also strongly inhibits 5-HT_2 receptor-mediated behavior in rats, such as tryptamine-induced clonic seizures (ED_{50} = 1.4 mg/kg p.o.) and D_2-like receptor-mediated behavior such as methampheta-

mine-induced hyperactivity (ED_{50} = 2.2 mg/kg p.o.), apomorphine-induced stereotypy (ED_{50} = 5.8 mg/kg p.o.) and climbing behavior (ED_{50} 3.5 mg/kg p.o.). SM-9018 possesses only weak cataleptogenic activity in rats (ED_{50} = 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 (ED_{50} = 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 D_{2L} (K_i = 6.3 nM, ^{3}H-*N*-methylspiperone), human D_3 (K_i = 7.1 nM, ^{3}H-*N*-methylspiperone), human 5-HT_{2A} (K_i = 5.6 nM, ^{3}H-RP-62203) and α_1-adrenoceptors (IC_{50} = 0.09 nM), whereas the affinities for the human D_4 receptor (K_i = 25 nM, ^{3}H-spiperone), the rat 5-HT_6 (K_i = 43 nM, ^{3}H-LSD) and the rat 5-HT_7 receptor (K_i = 22 nM, ^{3}H-LSD) are lower. Therefore, iloperidone is classified primarily as a potent D_2, D_3, 5-HT_2 and α_1 ligand with an

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 $5\text{-}HT_2$ (K_i = 1.5 nM, rat cortex, ^{3}H-ketanserin), $5\text{-}HT_{1A}$ (K_i = 2.5 nM, rat hippocampus, ^{3}H-8-OH-DPAT) and α_1-adrenergic receptors (K_i = 0.3 nM, rat brain, ^{3}H-WB-4101) but showed comparatively lower affinity and no selectivity for $hD_{4.2}$ (K_i = 13 nM, ^{3}H-spiperone) and hD_{2S} receptors (K_i = 13 nM, ^{3}H-raclopride). Electrophysiological investigations revealed $5\text{-}HT_{1A}$ 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* $5\text{-}HT_2$ antagonistic effects, 1192U90 may relieve positive and negative symptoms of schizophrenia with an atypical antipsychotic profile.

JL-13, a bioisosteric 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 $5\text{-}HT_{2A}$ (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, $5\text{-}HT_{2C}$, $5\text{-}HT_6$ and $5\text{-}HT_7$ 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 ((–)-enantiomer), is currently in preclinical development at Servier as a D_4/$5\text{-}HT_2$ antagonist and $5\text{-}HT_{1A}$ ligand for the potential treatment of psychosis and schizophrenia (80). In binding studies the compound was 6-fold more potent than clozapine at hD_4 receptors (K_i = 6.2 nM, ^{3}H-*N*-methylspiperone) and 5-fold more potent at hD_3 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 $5\text{-}HT_{2A}$ affinity (pK_i = 8.6, rat cortex, ^{3}H-ketanserin), S-16924 binds strongly to $5\text{-}HT_{1A}$ (pK_i = 8.4, rat hippocampus, ^{3}H-8-OH-DPAT) and $5\text{-}HT_{2C}$ 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_4 (K_i = 2.0 nM, ^{3}H-*N*-methylspiperone) and hD_3 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 $5\text{-}HT_{2A}$ receptors (pK_i = 8.5), α_1 (pK_i = 8.2) and α_2-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 $5\text{-}HT_{2A}$ (K_i = 5.8 nM, rat cortex, ^{3}H-ketanserin), hD_{2L} (K_i = 5.7 nM, ^{3}H-pramipexole), hD_1 (K_i = 2.1 nM, ^{3}H-SCH-23390), hD_4 (K_i = 11 nM, ^{3}H-pramipexole) and α_1 affinities (K_i = 12 nM, rat cortex, ^{3}H-prazosin) and moderate $5\text{-}HT_6$ affinity (K_i = 51 nM, cloned rat receptor, ^{3}H-LSD). The functional test revealed agonist properties at the $5\text{-}HT_{1A}$ 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 $5\text{-}HT_2$ 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 apomorphine-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*-chloroamphetamine-induced hyperthermia in rats (ED_{50} = 3.0 - 5.6 mg/kg). Despite its potent D_2 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/$5\text{-}HT_2$ antagonists (Fig. 2)

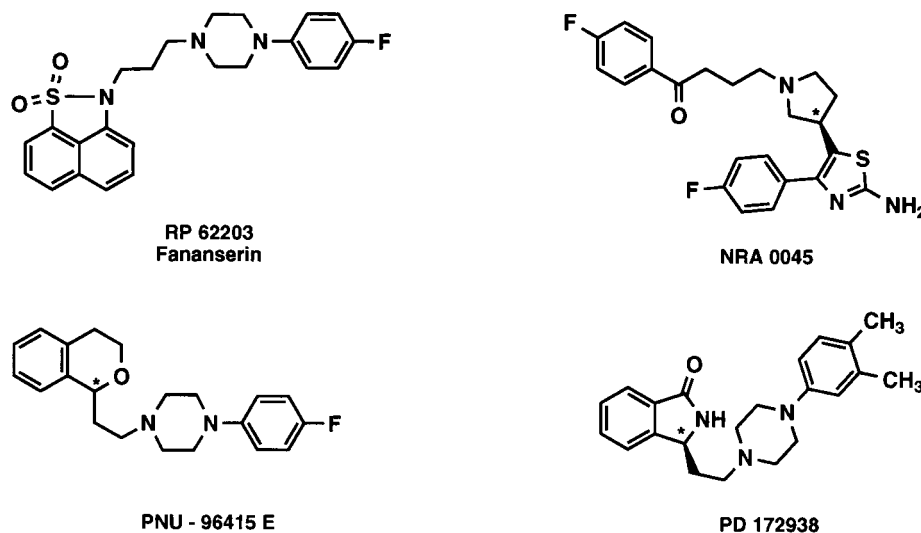The naphtosultam derivative, RP-62203 (fananserin), developed by Rhône Poulenc Rorer, was originally

RP 62203
Fananserin

NRA 0045

PNU - 96415 E

PD 172938

Fig. 2. Structure of selective D_4/5-HT_2 antagonists.

described as a potent 5-HT_2 ligand *in vitro* (K_i 5-HT_2 = 0.26 nM, rat brain, ^{3}H-ketanserin) with a more than 100-fold selectivity *versus* H_1-, α_1-, 5-HT_{1A} and D_2 receptors, indicating a profile superior to that of ritanserin (90). *In vivo* this compound is a potent orally effective and long-lasting 5-HT_2 antagonist in the mescaline-induced head twitch test in mice (ED_{50} 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 D_4 receptor (K_i 2.9 nM, ^{3}H-spiperone) and 250-fold selectivity *versus* the rat D_2 receptor was discovered (K_i D_2 = 726 nM, rat striatum, ^{3}H-spiperone) (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 D_4, 5-HT_{2A} and α_1 affinities (96-99). The drug shows a high dopamine D_4 selectivity ($K_{i(hD4.2)}$ = 2.5 nM, ^{3}H-spiperone, 90-fold selectivity against hD_{2L} receptors and 40-fold against hD_3 receptors), high 5-HT_{2A} affinity (K_i = 1.9 nM, ^{3}H-RP62203, rat cortex) and strong α_1-adrenoceptor affinity (K_i = 1.4 nM, ^{3}H-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 (ED_{50} = 0.4 mg/kg i.p. and 0.3 mg/kg p.o.), the disruption of prepulse inhibition in rats by apomorphine 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

(ED_{50} = 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 catelepsy in rats at the dose 30 mg/kg i.p. As a compound with combined dopamine D_4 and 5-HT_2 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 D_4/5-HT_2 antagonist (101). Compared with clozapine, the drug has a 10-fold higher affinity for the $hD_{4.2}$ receptor (K_i = 3.0 nM, ^{3}H-spiperone) and an equipotent affinity for the 5-HT_{2A} receptor (K_i = 5.8 nM, rat, ^{3}H-ketanserin), while its dopamine D_2 (K_i = 199 nM, cloned rat D_{2L} receptors, ^{3}H-raclopride) and α_1-adrenergic receptor affinities (K_i = 38 nM, rat cortex, ^{3}H-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 *d*-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 D_4/5-HT_{2A} receptor affinity, PNU-96415E may have potential antipsychotic efficacy with a lower side effect potential.
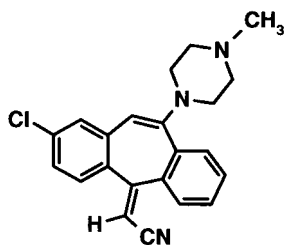
Rilapine
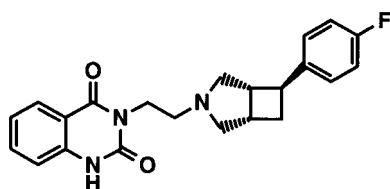
LU-111995

The benzisoindolone derivative ((*S*)-enantiomer), PD-172938 (102), has been identified by Parke Davis and is currently in preclinical development. It is a potent and selective dopamine D_4 receptor antagonist (K_i = 7.8 nM) with some 5-HT$_2$ affinity (K_i = 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 ED$_{50}$ 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 dopamine and serotonin receptor subtypes. Rilapine, a 5,11-dicarbo analog of clozapine with a pharmacophoric methylenenitrile function on carbon 11, was one of the interesting candidates (28, 29, 103, 104) with potent 5-HT$_{2A}$ (K_i = 0.3 nM, rat cortex, ^{3}H-ketanserin), 5-HT$_{1A}$ (K_i = 0.3 nM, rat hippocampus, ^{3}H-8-OH-DPAT), α_1 (K_i = 0.1 nM, rat brain, ^{3}H-prazosin), moderate D_1 (K_i = 12 nM, bovine nucleus caudatus, ^{3}H-SCH-23390) and weaker D_2 affinities (K_i = 70 nM, bovine nucleus caudatus, ^{3}H-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 [(+)-(1*S*,5*R*,6*S*)-*exo*-3-[2-[6-(4-fluorophenyl)-3-azabicyclo[3.2.0]heptane-3-yl]-ethyl]-1*H*,3*H*-quinazoline-2,4-dione] has been selected from a series of com-

pounds due to its high dopamine D_4 affinity and its D_4 *versus* D_2 receptor selectivity (106). Having additional high affinity for the 5-HT$_2$ 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-azabicylco[3.2.0]heptane building blocks with high (> 90%) diasteroselectivity *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 D_4/5-HT$_2$ 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 D_4 affinity (compare **12**). Introduction of fluorine into the 4-position significantly improved the D_4 affinity and selectivity *versus* D_{2A}, while the 5-HT$_{2A}$ affinity remained unchanged. The (+)-enantiomer of **2**, LU-111995, showed 30-fold higher D_4 affinity and selectivity *versus* D_{2A} than the corresponding (-)-enantiomer. The dopamine D_4 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 D_4 affinity and reversed selectivity in favor of the D_{2A} receptor. Replacement of the phenyl group by thiophen needs the introduction of 5-chlorine to achieve high D_4 affinity and selectivity. A basic nitrogen atom in the 4-membered ring substituent (*e.g.*, pyridine derivative **11**) abolishes D_4 and 5-HT$_{2A}$ 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.
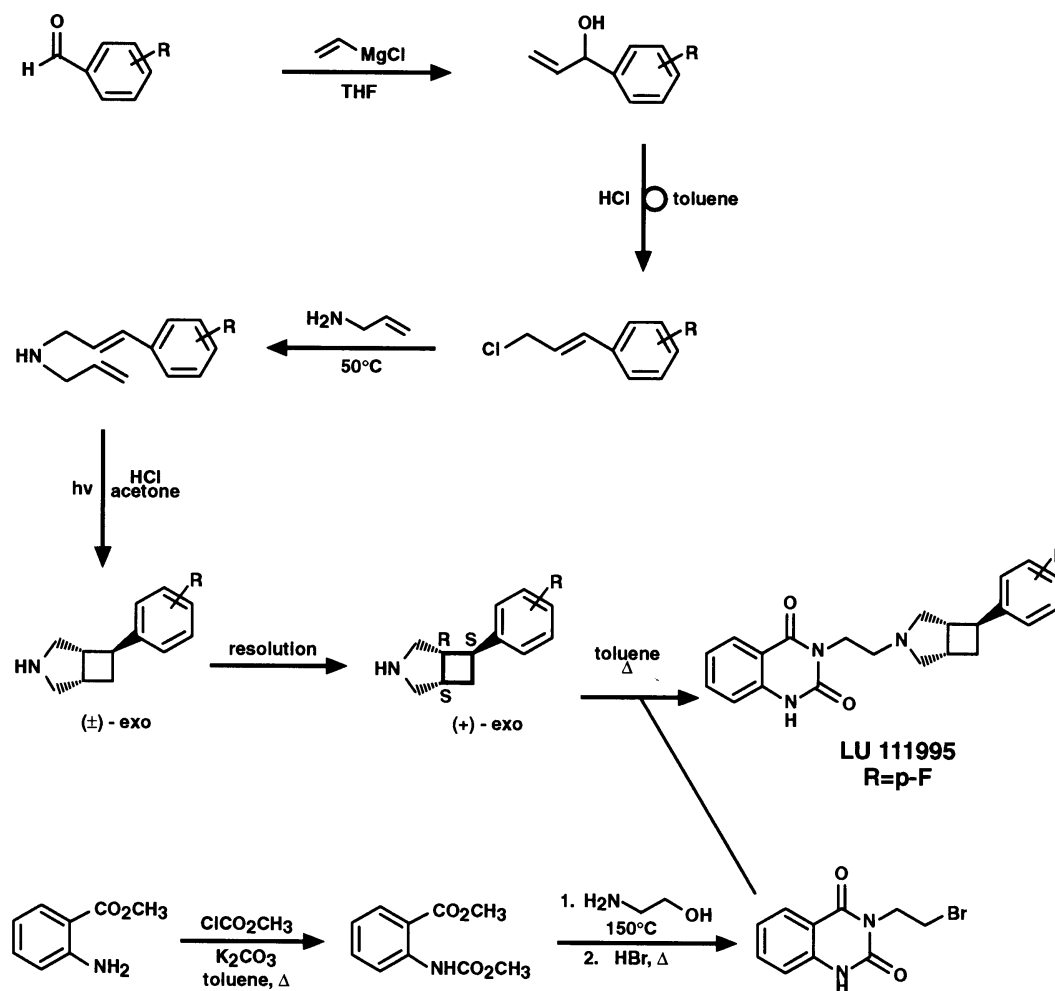
Fig. 3. Synthesis of LU-111995 and derivatives.

LU-111995 displayed highest affinities for D_4 ($K_i = 3.1$ nM) and 5-HT$_{2A}$ (3.3 nM) receptors. The D_{2A}/D_4 ratio was 34. Moderate affinity was found for histamine H_1 (16.5 nM) and α_1-adrenergic (19 nM) receptors. Lower affinity was seen for D_{2A} (105 nM), 5-HT$_{1D}$ (79 nM), α_2 (50 nM), 5-HT$_{2C}$ (51 nM) and σ (37 nM) receptors. K_i values of LU-111995 for all other receptors tested exceeded 200 nM.

The receptor profile of LU-111995 had remarkable similiarities to that of clozapine. In comparison to clozapine, however, the D_4 affinity of LU-111995 is 7-fold higher, and the D_4/D_{2A} selectivity is 5-fold more pronounced. Conversely, clozapine displays higher affinities for histamine H_1 (27-fold), α_1-, α_2-adrenergic and muscarinic receptors. Affinities of both compounds for the 5-HT$_{2A}$ receptor are almost equal.

Functional $D_{4.2}$ receptor assay

In vitro studies to determine agonist/antagonist properties of LU-111995 were carried out on HEK293 cells stably expressing $D_{4.2}$ receptors. A DNA construct encod-

ing the luciferase gene driven by a cAMP-dependent promotor 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-HT$_2$ receptor found *in vitro*, effects in the antidopaminergic tests (apomorphine or methamphetamine antagonism) and the conditioned avoidance response seem to reflect

Table I: In vitro *binding*[a] *of 3-substituted quinazolinediones.*

Compound		R¹	R²	R³	hD_4 K_i [nM]	hD_{2A} K_i [nM]	$5\text{-}HT_{2A}$ K_i [nM]	D_{2A}/D_4
(±)	1	H	H	phenyl	30	127	11	4.2
(±)	2	H	H	phenyl–F	4.8	120	11	25
LU-111995		H	H	phenyl–F	3.1	105	3.3	34
(-)	2	H	H	phenyl–F	87	115	5.4	1.3
(±)	3	H	CH₃	phenyl–F	8.6	77	6.2	8.9
(±)	4	6-F	H	phenyl–F	3.6	55	16	15
(±)	5	H	H	phenyl–Cl	15	44	10	2.9
(±)	6	H	H	phenyl–NO₂	17	470	~75	28
(±)	7	H	H	phenyl–OCH₃	365	210	~51	0.6
(±)	8	H	H	phenyl–NH₂	5400	600	~135	0.1
(±)	9	H	H	thiophene	150	109	~54	0.7
(±)	10	H	H	thiophene–Cl	3.1	98	18	32
(±)	11	H	H	pyridine	5150	~700	>3000	
(±)	12	H	H	H	>10 000	>5000	>3000	

[a]Conditions for receptor binding assays see Table II.

dopamine D_2-mediated effects rather than the drug's high D_4 affinity.

No cataleptogenic effects were found even at high doses, predicting a low propensity to induce extrapyramidal symptoms.

Low ED_{50} values in the tests for serotonin antagonism prove the good oral bioavailability of the compound in animal experiments.

In addition, LU-111995 inhibited the orientational motility in mice ($ED_{50} = 13.4$ mg/kg p.o.); it was 2-5 times less effective than clozapine or chlorpromazine, respectively. In a further sign of a central sedative action, LU-111995 (doses 21.5 and 46.4 mg/kg p.o.) potentiated the effect of threshold doses of hexobarbital. By comparison, chlorpromazine was about 5 times as potent.

Preliminary results of studies still in progress such as dialysate levels of dopamine in the prefrontal cortex, drug-induced pattern of *c-fos* expression and prepulse inhibition, appear to confirm the atypical character of the drug.

Pharmacokinetics and metabolism

LU-111995 is rapidly absorbed in rats and dogs. Its absolute bioavailability is high: 70% in the dog (capsules) and 40% in the rat (solution).

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-

Table II: In vitro *receptor binding of LU-111995 in comprison with clozapine.*[a]

Receptor	Receptor source	Labelled ligand	Nonspecific binding	LU-111995 K_i [nM]	Clozapine K_i [nM]
Dopamine D_1	Bovine caudate	^{3}H-SCH 23390	Butaclamol (10 μM)	580	172
Dopamine D_2	Human cloned	^{125}I-Iodospipreone	Haloperidol (1 μM)	105	139
Dopamine D_3	Human cloned	^{125}-I-Iodosulpride	Spiperone (1 μM)	2234	305
Dopamine D_4	Human cloned	^{125}I-Iodospiperone	Haloperidol (1 μM)	3.1	22
Serotonin 5-HT$_{1A}$	Rat hippocampus	^{3}H-8-OH-DPAT	5-HT (1 μM)	1030	81
Serotonin 5-HT$_{1D}$	Human cloned	^{3}H-5-CT	5-HT (1 μM)	79	59
Serotonin 5-HT$_{2A}$	Rat cortex	^{3}H-Ketanserin	Cyproheptadine (0.1 μM)	3.3	2.5
Serotonin 5-HT$_{2C}$	Pig chorioid plexus	^{3}HMesulergine	5-HT (1 μM)	51	13
Serotonin 5-HT$_3$	N1E-115 neuroblastoma	^{3}H-GR 65630	Metoclopramid (0.1 μM)	4000	79
Histamine H_1	Guinea pig cerebellum	^{3}H-Pyrilamine	Pyrilamine (1 μM)	17	0.6
Histamine H_2	Guinea pig cortex	^{3}H-Tiotidine	Histamine (5 μM)	241	775
Histamine H_3	Rat cortex	^{3}H-Methylhistamine	Methylhistamine (1 μM)	1700	400
Adrenoceptor α_1	Rat brain	^{3}H-Prazosin	Prazosin (0.5 μM)	19	3.9
Adrenoceptor α_2	Rat brain	^{3}H-RX-821002	(-)Epinephrine (100 μM)	57	11
Sigma	Guinea pig cortex	^{3}H-(+)-3PPP	Haloperidol (10 μM)	37	n.d.[b]
Muscarinic	Rat cortex	^{3}H-QNB	Atropine (1 μM)	>1000	7

[a]Receptor binding studies were performed *in vitro* using homogenates from animal brain or cloned human receptors stably expressed in mammalian cell lines. Data analysis were performed by non-linear regression analysis with a modified version of the program LIGAND.
[b]Not determined.

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 D_4 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

(up to 300 mg/kg b.w.) and intravenous (up to 20 mg/kg b.w.) administration was low.

Repeated-dose toxicity studies with daily oral doses for 4 weeks, or 13 and 26 weeks were performed in rats and dogs. The tablet as the final formulation is being used in the chronic dog study over 52 weeks. The evaluation is still ongoing.

These results justify the use of the intended clinical doses of 2-4 mg/kg b.w. per day in patients.

LU-111995 did not reveal any teratogenic potential in studies for effects on embryo and fetal development with Sprague Dawley rats (25, 45.5, 82.5 and 150 mg/kg b.w.) and Himalayan rabbits (10, 27.4 and 75 mg/kg b.w.).

In four mutagenicity tests (Ames test, V79 HPRT test, and chromosome aberration tests *in vitro* and *in vivo*), LU-111995 was negative for activity in both activated and nonactivated systems.

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

Compound	Apomorphine antagonism[a] Mouse ED$_{50}$[b]	Methamphe- tamine antagonism[a] Mouse ED$_{75}$[b]	Serotonin antagonism[a] Mouse ED$_{50}$[b]	Rat ED$_{50}$[b]	Conditioned avoidance response Rat ED$_{50}$	Cataleptogenic effect Rat Dose po [mg/kg]	Incidence [x/n]
LU 111995	18.1 (12.9/27.4)	4.1 (1.98/7.86)	1.0 (0.52/1.56)	6.1 (3.94/8.91)	74	215	0/16
Clozapine	5.2 (3.85/7.19)	2.45 (1.2/5.7)	0.32 (0.2/0.5)	6.01 (4.98/7.19)	10.1	100	0/8
Haloperidol	0.05 (0.03/0.09)	0.07 (0.06/0.09)	n.d.	n.d.	0.23	2.15	9/12

[a]LU-111995 and clozapine were administered p.o. 60 min, haloperidol 120 min before test. [b]mg/kg p.o. (95% confidence limits).

Clinical pharmacology

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 D_2 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. C_{max} 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 D_4 receptor antagonist, L-745,870, and the D_4/5-HT$_2$ 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 D_4 receptor antagonists or mixed-type dopamine/serotonin antagonists with a potent D_4 component. These drugs are aimed at a clozapine-like atypical antipsychotic activity, avoiding the serious side effects of clozapine.

The anatomical localization of D_4 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 D_4 receptors, however, is difficult to elucidate. Many of the classical behavioral paradigms which are still used to pre-

dict antipsychotic activity in patients have been validated and optimized by available antipsychotics with a preference for dopamine D_2 receptors.

The three highly selective dopamine D_4 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 D_4 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 D_4 receptors are a valuable target for antipsychotics, and that the efficacy of clozapine is at least partly attibutable to its relatively high D_4 affinity. L-745,870 was devoid of any clinical improvement in 38 acutely psychotic patients. Therefore, phase II results with the highly selective D_4 antagonist, PNU-101387, are eagerly awaited.

In contrast to pure D_4 antagonists, mixed D_4/5-HT$_{2A}$ antagonists may possess important therapeutic advantages, since their effect on 5-HT$_{2A}$ and/or further receptors could result in effects synergistic to D_4 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.

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