Current Strategies for the Development of Novel Antipsychotic Drugs

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Abstract: While classical neuroleptics are characterized by dopamine D_2 antagonism, this is also considered to be the cause of their neurological side effects. In recent years, novel antipsychotic drugs with improved efficacy, devoid of extrapyramidal effects are being developed. The mechanisms of action of these new atypical antipsychotics can be classified into three general groups: a) binding to D_2 together with non-dopaminergic receptors, b) interaction with dopamine receptor subtypes other than D_2 and c) selective binding to non-dopaminergic systems, such as glutamatergic, sigma, neurotensin, and cannabinoid.

Keywords: schizophrenia, atypical antipsychotics, novel antipsychotics, extrapyramidal side effects, cognitive functions.

INTRODUCTION

Schizophrenia, the most common psychotic disorder occurring in about 1% of the population, includes an ensemble of severe and disabling mental diseases characterized by the deterioration of several basic mental functions, such as sensory perception, psychomotricity, affectivity, and thought [1]. For many years, the pharmacological treatment of schizophrenia and psychoses relied on conventional neuroleptics, belonging mainly to the structural families of the phenothiazines (chlorpromazine, fluphenazine) and butyrophenones (haloperidol, droperidol). These drugs are recognized as effective agents in alleviating the positive symptoms of schizophrenia, namely hallucinations and delusions. However, they show poor or no efficacy against the negative symptoms (apathy, social withdrawal) and cognitive deficits (attention and memory impairment) [2, 3], and their use is frequently limited by the appearance of serious side effects, such as extrapyramidal syndrome [4], tardive dyskinesia [5], and hyperprolactinemia [6]. For some time, these undesired effects were regarded as inherent to the own mechanism of action of the antipsychotic drugs. Thus, the term neuroleptic (that is a drug producing neurological side effects) was considered a synonym of antipsychotic drug. A common feature of classical neuroleptics is their antagonism at dopamine D_2 receptors, a property that has been correlated with the pharmacological potencies of the drugs [7]. As a matter of fact, this constitutes one of the supports of the hypothesis that postulates an increase in dopaminergic activity at the mesolimbic system of the brain as the biochemical basis of schizophrenia [8, 9]. On the other hand, many side effects of classical antipsychotics are also associated with dopamine antagonism in other areas of the brain [10]. In particular, extrapyramidal effects and hyperprolactinemia are related to the dopamine blockade at the nigrostriatal system and pituitary dopamine receptors, respectively. Moreover, a reduction in the activity of dopaminergic pathways innervating frontal and prefrontal cortex could contribute to the impaired affective and cognitive functions [11, 12].

Further efforts in the research for more effective antipsychotic medications gave a new impulse in the therapy of schizophrenia with the introduction of improved drugs challenging this initial concept. This new class of compounds, classified as "atypical antipsychotics", is characterized by its efficacy in both the positive and negative symptoms of schizophrenia along with minimal induction of extrapyramidal effects [13]. The first antipsychotic displaying this different pharmacological profile was the dibenzodiazepine clozapine (**1**) (**Fig. 1**) [14], considered to be the prototype of these new drugs. Unfortunately, clozapine is not devoid of other severe undesirable effects [15, 16], such as agranulocytosis, seizures and thromboembolism in a significant number of cases. For this reason, its clinical use has been limited, under strict hematological monitoring, to a small population of patients resistant to other drugs [17]. Attempts to rationalize the particular mechanism of action of clozapine are hampered by the complex binding profile of this drug. Indeed, clozapine displays high affinity to a variety of central nervous system receptors including dopaminergic, serotonergic, alpha adrenergic, histaminergic, and cholinergic [18]. At present, although several models have been proposed, there is not a unique theory that satisfactorily explains the atypical antipsychotic profile of clozapine. In this context, a number of putative atypical antipsychotics with different modes of action are currently being developed. The proposed profiles for the new atypical antipsychotics can be classified into three general groups: a) compounds that bind to dopamine D_2 along with other different receptors, b) compounds that bind to dopamine receptors other than D_2, and c) compounds that predominantly bind to non-dopaminergic receptors.

COMPOUNDS BINDING TO DOPAMINERGIC D_2 AND OTHER RECEPTORS

Schizophrenia is believed to be associated with a biochemical imbalance in the complex, interrelated neurotransmitter systems of the brain. Although there is

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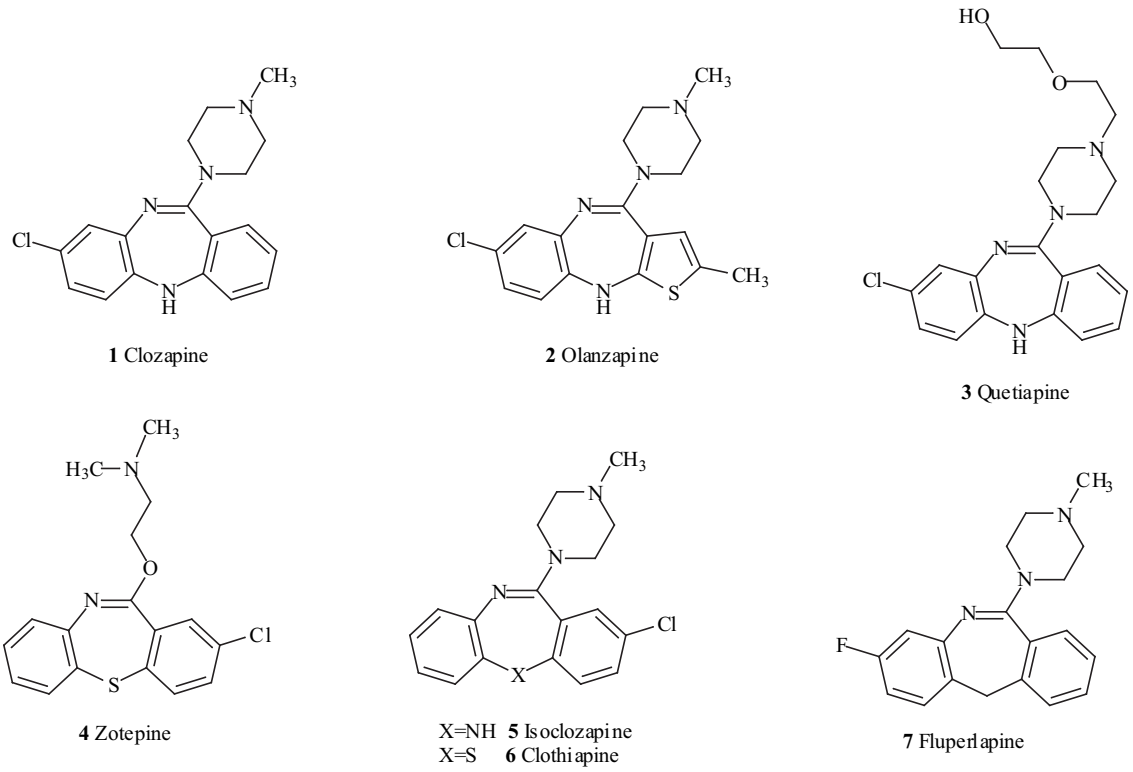


Fig. (1). Structure of clozapine and some tricyclic analogues.

clear evidence of altered dopaminergic transmission, the possibility that other chemical messengers are also involved in schizophrenia cannot be ruled out. Furthermore, the finding that clozapine binds to a variety of receptors led to some theories trying to explain the atypical profile by the interaction with more than one receptor system. Thus, the mesolimbic selectivity of the atypical antipsychotics has been suggested to be achieved by indirect modulation or compensation of their dopaminergic effects through action on other neurotransmitter systems [19].

Serotonergic Receptors

The involvement of serotonin in the pathophysiology of schizophrenia is widely recognized. Dopamine and serotonin neurotransmission share common anatomical locations and display complex functional interactions [20]. Moreover, serotonin dysfunction has been reported in schizophrenic patients [21]. These facts, along with the high affinity of most antipsychotics, including clozapine, for serotonin receptors [22, 23] underlie the hypothesis that serotonergic effects can contribute to the atypical antipsychotic profile. A suggested mechanism for this effect is that serotonin exerts a differential modulation of the dopaminergic activity in distinct brain systems. Indeed, serotonin antagonism facilitates dopamine output at the nigrostriatal and cortical regions, in which dopaminergic hypoactivity is associated with motor side effects and negative symptoms, respectively, while it has an inhibitory effect in the limbic areas, thus contributing to the behavioral control and reduction of the positive symptoms [24, 25].

Serotonin (5-hydroxytryptamine) is known to interact with a variety of specific receptors. At present, seven families and a number of subfamilies of 5-HT receptors have been identified [26]. One of the most acknowledged models for the development of new atypical antipsychotics has been a mixed profile of antagonism at dopamine D_2 and serotonin 5-HT_{2A} (formerly 5-HT_2) receptors. The observation that administration of the selective 5-HT_2 antagonist ritanserin in combination with conventional neuroleptics improved the efficacy against the negative symptoms and reduced the incidence of extrapyramidal effects in schizophrenic patients [27] encouraged the development of novel antipsychotic compounds with high affinity for the 5-HT_2 receptor [28]. Supporting these facts, selective 5-HT_2 antagonists have been shown to attenuate the effects of dopamine D_2 receptor blockade on the rat striatal neurotransmission by increasing dopamine synthesis and release, while having a negligible effect on D_2 blockade in regions innervated by mesolimbic dopaminergic neurons [29]. In a cluster analysis based on the affinity profiles of a number of typical and atypical antipsychotics for several neurotransmitter receptors, the atypical drugs were characterized by a greater affinity for 5-HT_2 than D_2 receptors [30, 31].

Small modifications of the dibenzo[*b,e*][1,4]diazepine heterocyclic structure of clozapine have been regarded as an effective approach to develop new antipsychotics with a safer profile. Some tricyclic derivatives (**Fig. 1**) such as olanzapine (**2**) [32], quetiapine (**3**) [33] and zotepine (**4**) [34] have been launched as atypical antipsychotics with greater affinity for 5-HT_{2A} than for D_2 receptors, and devoid of any significant tendency to cause agranulocytosis. Nevertheless, the subtle structural requirements defining the atypical properties and the absence of toxicological effects are poorly understood, and minor modifications of the structure of clozapine can lead to important variations in its

pharmacological profile. Thus, isoclozapine (**5**) and clothiapine (**6**), in which the halogen atom is situated in the opposite benzene ring as compared to (**1**), have a less favorable receptor binding profile and display pharmacological properties corresponding to typical neuroleptics [35], whereas the 5-deaza analogue fluperlapine (**7**), with an atypical profile similar to that of clozapine, has been associated with the induction of agranulocytosis in humans [36].

A different approach was used in the design of the atypical antipsychotic risperidone (**8**) (**Fig. 2**). This compound combines structural features associated with both dopamine and serotonin antagonism [37]. Yet an oversimplification, the 4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl moiety can be regarded as a bioisosteric replacement of the 4-fluoro-γ-aminobutyrophenone group present in some classes of typical neuroleptics and can be associated to dopamine D_2 antagonism. On the other side, the pyrido[1,2-*a*]pyrimidin-4-one heterocyclic system is closely related to the thiazolo[3,2-*a*]pyrimidin-5-one and the 2,4-quinazolinedione groups present in the 5-HT_2 antagonists ritanserin and ketanserin, respectively. Other putative atypical antipsychotics under clinical development

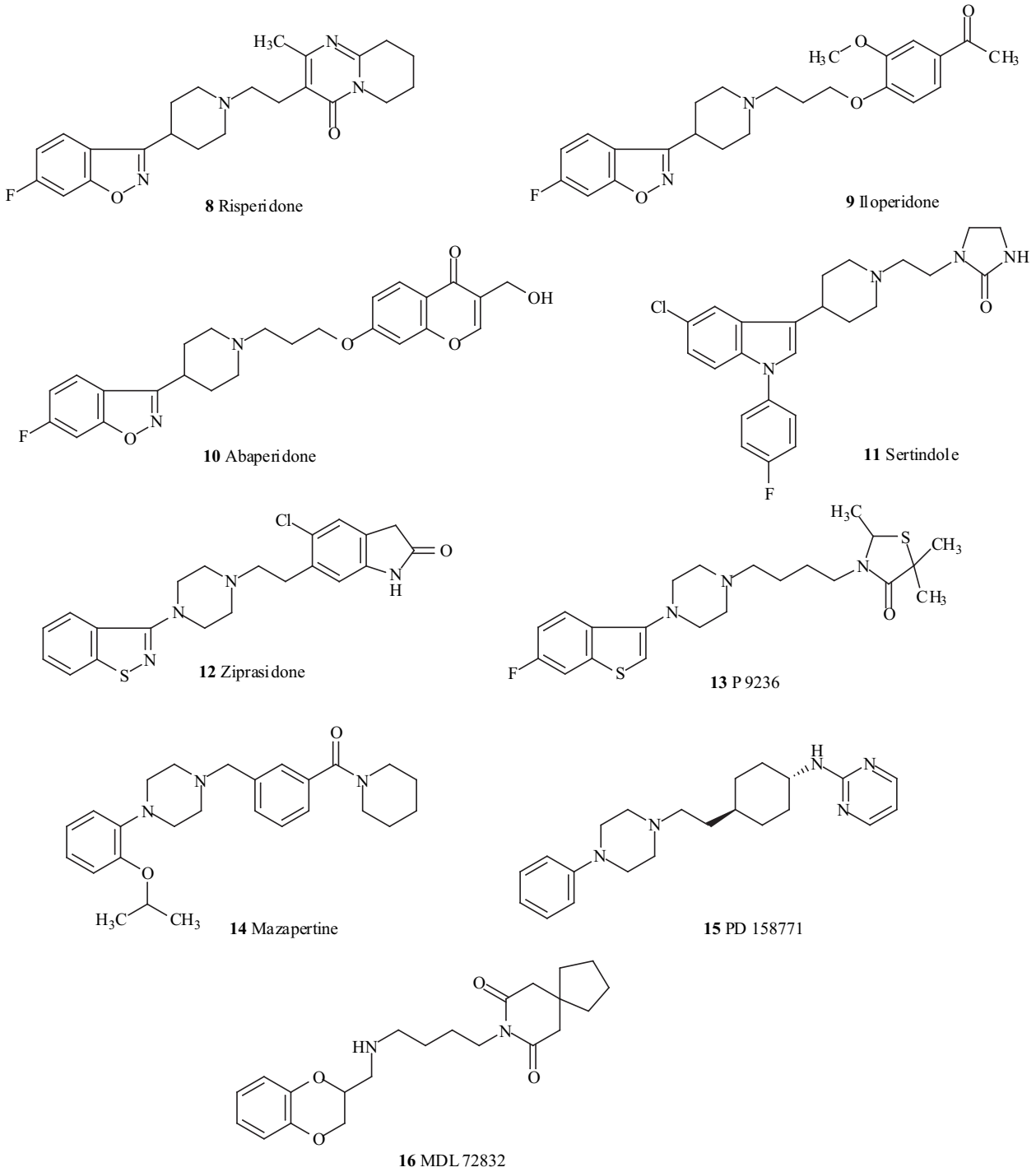


Fig. (2). Antipsychotics with affinity for dopamine and serotonin receptors.

incorporating the benzisoxazolylpiperidinyl group include iloperidone (**9**) [38] and abaperidone (**10**) [39]. The indolylpiperidine bioisosteric derivative sertindole (**11**) [40] showed an atypical profile with no tendency to induce extrapyramidal side effects in humans. Unfortunately, sertindole was withdrawn from the market due to its liability to prolong the QT interval, which could be associated to cardiotoxic effects. Some related compounds possess a benzisothiazolylpiperazine group, such as tiospirone [41] and ziprasidone (**12**) [42] or a benzothienylpiperazine group, such as P 9236 (**13**) [43].

The importance of serotonin 5-HT_{1A} receptors in the pharmacological profile of antipsychotic drugs has also been recognized. The synthesis and release of serotonin can be modulated through agonism at presynaptic 5-HT_{1A} receptors, which produces analogous effects to 5-HT_{2A} antagonism [44, 45]. Thus, the 5-HT_{1A} agonist 8-hydroxy-2-(dipropylamino)tetralin demonstrated antipsychotic-like properties and a selective reduction in limbic versus striatal dopamine synthesis [46, 47], and some 5-HT_{1A} agonists have been shown to potentiate the antipsychotic action and to reduce the occurrence of extrapyramidal side effects of D_2 antagonists [48, 49]. 5-HT_{1A} receptors are located both presynaptically in the raphe nuclei cells innervating the forebrain, and postsynaptically in limbic structures. An optimal profile would be activation of the highly sensitive presynaptic 5-HT_{1A} autoreceptors, while blocking the low-sensitivity postsynaptic receptors. This has been suggested to be achieved by partial agonism at 5-HT_{1A} receptors [50]. Additionally, 5-HT_{1A} partial agonism has been associated with anxiolytic and antidepressant properties, which may contribute to controlling the appearance of psychotic episodes in schizophrenic patients [51].

A number of atypical antipsychotics display partial agonism for 5-HT_{1A} receptors. Interestingly, few compounds have been found to act as pure 5-HT_{1A} antagonists [52]. Whereas the classical butyrophenone and phenothiazine neuroleptics have low affinities for these receptors, clozapine and the benzisoxazolylpiperidine derivatives have moderate 5-HT_{1A} affinities. A combination of higher 5-HT_{1A} than D_2 receptor affinity has been the rationale for designing several novel atypical antipsychotics with efficacy against negative and cognitive symptoms and reduced extrapyramidal effects [50]. Aryl piperazines constitute important pharmacophores leading to compounds with high affinity for 5-HT_{1A} receptors. Thus, the benzisothiazolylpiperazine derivative ziprasidone (**12**) displays nanomolar affinity for 5-HT_{1A} receptors, which has been suggested to play a significant role in its pharmacological actions [53]. Other potent dopamine and 5-HT_{1A} ligands in clinical or preclinical development as potential atypical antipsychotics incorporate a phenyl- or a (2-alkoxyphenyl)piperazine unit, such as mazapertine (**14**) [54] and PD 158771 (**15**) [55]. The (2,3-dihydro-1,4-benzodioxin-2-yl)methylamino group in some novel antipsychotics, such as MDL 72832 (**16**) [56], can also be regarded as a bioisosteric equivalent of the (2-alkoxyphenyl)piperazine framework (**Fig. 2**).

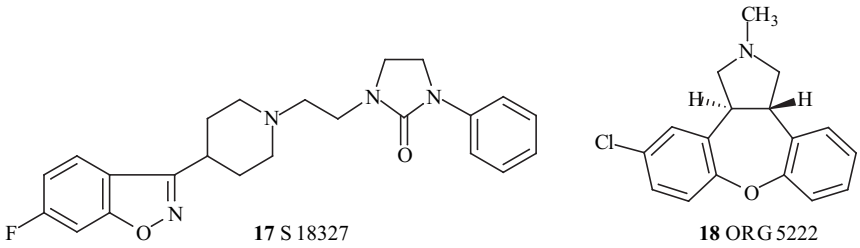
Other serotonin receptors have aroused interest as possible targets for novel antipsychotics with improved profiles. Clozapine and its major metabolite *N*-desmethyl-clozapine are potent 5-HT_{2C} antagonists [57]. Overactivity at 5-HT_{2C} receptors (formerly classified as 5-HT_{1C} receptors) that control the basal ganglia activity has been related to extrapyramidal effects and parkinsonism [58]. Olanzapine (**2**), sertindole (**11**) and ziprasidone (**12**) are examples of novel antipsychotics with potent 5-HT_{2C} antagonism. Clozapine and other antipsychotics also display high affinity for 5-HT_6 and 5-HT_7 receptors [59], which led to the suggestion of a possible role of these receptors in their pharmacological profile. Although preliminary data support a possible therapeutic relevance of these receptors (mainly the 5-HT_6 receptors) in the treatment of schizophrenia, this still remains to be proved due to the absence of specific ligands [60]. Sertindole (**11**) and olanzapine (**2**) display high affinities for 5-HT_6 receptors, whereas risperidone (**8**) and ziprasidone (**12**) are potent 5-HT_7 ligands.

Adrenergic Receptors

Current theories consider that binding to only dopaminergic and serotonergic receptors cannot completely account for the atypical properties of novel antipsychotics. Within the complex multireceptor binding profile of clozapine, alpha adrenergic effects have been suggested to play a significant role in their beneficial clinical profile [61, 62]. Thus, stimulation of α_1 adrenergic receptors in the prefrontal cortex has been related to an impairment of cognitive functions [63], and α_1 antagonism has been found to reverse deficits in sensorimotor gating mechanisms [64]. Moreover, an increase in cerebral noradrenergic activity has been correlated with both the severity of positive and negative symptoms of schizophrenia [65, 66]. Additionally, antagonism at central α_1-adrenoceptors has been shown to produce a selective inhibition of mesolimbic versus striatal dopamine release [67, 68], which could contribute to minimize the extrapyramidal side effects related to dopaminergic antagonism. Although α_1 antagonism has raised some controversy, mainly due to cardiovascular side effects (orthostatic hypotension, sedation, dizziness), slow titration and dosage optimization can greatly minimize these adverse effects of antipsychotic medications [69].

Most classical and atypical antipsychotics exhibit alpha adrenergic antagonism. However, some novel antipsychotics are characterized by a specially potent α_1 adrenergic antagonism. Iloperidone (**9**), quetiapine (**3**), S 18327 (**17**) [70, 71] and ORG 5222 (**18**) [72] (**Fig. 3**), are some examples of antipsychotic compounds in different stages of development that display higher affinity for α_1 than D_2 or 5-HT receptors.

Alpha-2 receptors mediate most of the physiological actions of noradrenaline in the prefrontal cortex [73]. Three subtypes of human α_2 adrenoceptors have been cloned, and have been designated as α_{2A}, α_{2B} and α_{2C}. Affinity for the α_{2A} subtype has also been implicated in the atypical profile of clozapine [74, 75]. Alpha-2A receptors can be presynaptic autoreceptors or heteroreceptors, as well as postsynaptic receptors. Specific stimulation at postsynaptic prefrontal cortical α_2 receptors has been suggested to be responsible for the enhancement of cognitive functions elicited by the selective α_{2A} adrenergic agonist guanfancine [76], and



17 S 18327 **18** ORG 5222

Fig. (3). Antipsychotics with high affinity for alpha-adrenergic receptors.

selective potentiation of α_2 receptor activity has been proposed to have a beneficial effect in the treatment of the cognitive deficits associated with schizophrenia [77, 78]. Interestingly, some novel antipsychotics are characterized by α_2 antagonism. The effects of α_2 antagonism could be a combination of actions at pre- and postsynaptic receptors. In practice, antagonism at these receptors has been related to an enhancement of the cerebral noradrenergic transmission [70], which could be attributed to a preferential antagonism at the highly sensitive presynaptic receptors, and has been shown to improve attention and cognitive functions in humans [74, 79]. Since an increased level of noradrenergic activity at frontocortical α_1 receptors is predicted to exert the opposite effect in cognitive functions [80], concomitant α_1 antagonism could offer an improved profile. Antagonism at presynaptic α_2 heteroreceptors has also been related to a preferential facilitation of frontocortical versus subcortical dopaminergic transmission [71]. Additionally, α_2 antagonism has been implicated in the anticataleptic properties of clozapine and its lack of extrapyramidal side effects [81].

A number of antipsychotics show moderate affinities for α_2 receptors, but usually lower than affinities for α_1 or D_2 receptors. Risperidone (**8**) and ORG 5222 (**18**) are some of the most potent antagonists at these receptors, displaying the same order of affinities for α_2 as for α_1 receptors.

COMPOUNDS BINDING TO DOPAMINE RECEPTORS OTHER THAN D_2

Traditionally, D_2 receptors have been considered as a major target for neuroleptics due to the correlation between the effective doses of these drugs and their affinity for D_2 receptors. Nevertheless, most antipsychotics also interact with other pre- and postsynaptic dopamine receptors. Recent progress in molecular biology research has led to the identification of five dopamine receptor subtypes, which can be pharmacologically and structurally grouped into two major families: the D_1-like dopamine receptor family, comprising D_1 and D_5 subtypes, and the D_2-like family, including D_2, D_3 and D_4 subtypes [82]. The particular anatomical distribution of certain receptor subtypes in brain areas related to schizophrenia and the binding profile of some atypical drugs suggest that a preferential pharmacological action on dopaminergic receptors other than D_2 could improve the efficacy against psychotic symptoms without producing adverse motor and endocrine effects. During the last decade, anatomic, pharmacologic and genetic studies have gathered experimental evidence, either implicating or ruling out the role of dopaminergic receptors other than D_2 as pharmacological targets for psychotic disorders.

D_1 Receptors

The existence of functional interactions between D_1 and D_2 receptors raised the possibility that D_1 receptors could represent a target for novel antipsychotic drugs [83]. Although clinical trials with the selective D_1 antagonists SCH 39166 and NNC 01-0687 failed to demonstrate convincing efficacy against the positive symptoms, in one of these studies with SCH 39166 a modest improvement of negative symptoms was observed [84]. Further observations revealed a reduced D_1 receptor population in the prefrontal cortex of schizophrenic patients [85], a fact that has been related with the severity of cognitive deficits. Accordingly, it has been argued that antipsychotics with additional D_1 antagonism could display improved properties against the negative symptoms of schizophrenia.

D_3 Receptors

Several lines of evidence point to involvement of D_3 receptors in schizophrenia, especially their selective localization in mesolimbic dopaminergic projections. In the rat, mRNA for D_3 receptors has been detected in the ventral striatum, and particularly in the shell of nucleus accumbens. Interestingly, the anatomical location of D_3 receptors shows little overlap with D_2 receptors and contrasts with the broad distribution of that receptor subtype [86, 87]. Also in human forebrain, D_3 receptor expressing neurons have been found in several limbic structures, with small differences to the rat [88]. These findings are suggestive of a contribution of D_3 receptors to the dopaminergic transmission in the limbic areas, which are known to be directly involved in the emotional behavior and cognitive function deficits characteristic of schizophrenia.

Pharmacological data also support a main role for D_3 receptors in schizophrenia. In general, antipsychotics display poor selectivity between D_2 and D_3 receptor antagonism. Thus, at pharmacological doses, significant blockade of D_3 receptors should occur. Moreover, the observed tolerance to dopamine antagonists, exhibited as a progressive reduction in their extrapyramidal side effects, but not in their antipsychotic efficacy, suggests that different receptors could be involved in either effect. Furthermore, in experimental animals it was shown that, upon long term treatment with neuroleptics, an upregulation in the number of D_2 receptors occurred, whereas this was not observed with D_3 receptors [89]. Taken together, these data lead to the hypothesis that

D_2 receptors in basal ganglia are responsible for the extrapyramidal effects of antipsychotics, whereas D_3 receptors in mesolimbic regions account for the antipsychotic effects.

Evidence for alterations in D_3 receptors has been obtained in schizophrenic patients. A 45% increase in D_3 receptor population in ventral striatum was found in non-medicated patients, but not in those being treated by antipsychotic medications [90]. Recent studies [91, 92] found an elevation of D_3 receptor mRNA expression in lymphocytes from schizophrenic patients, a pattern which is probably reflected in the brain as it has been shown with other neurotransmitter receptors. More conflicting have been the data from genetic association studies searching for a possible link between D_3 receptors and schizophrenia. The most commonly analyzed polymorphism, *Bal*I, is a Ser^9 to Gly^9 substitution in the *N*-terminal extracellular domain. Since the initial report [93] of an association between homozygosity for either allele and schizophrenia, some reports have been in agreement with this initial finding [94], while the majority failed to confirm it [95, 96]. A significant but small relationship between D_3 polymorphism and schizophrenia was confirmed for some subpopulations in two published meta-analyses with gathered data from about 2,500 patients and controls [97, 98].

Within this framework, compounds acting on this receptor subtype have become an important focus of research efforts [99]. Most antipsychotics display high affinity for D_3 receptors, although this is usually slightly lower than for D_2 receptors. Some new atypical compounds with mixed

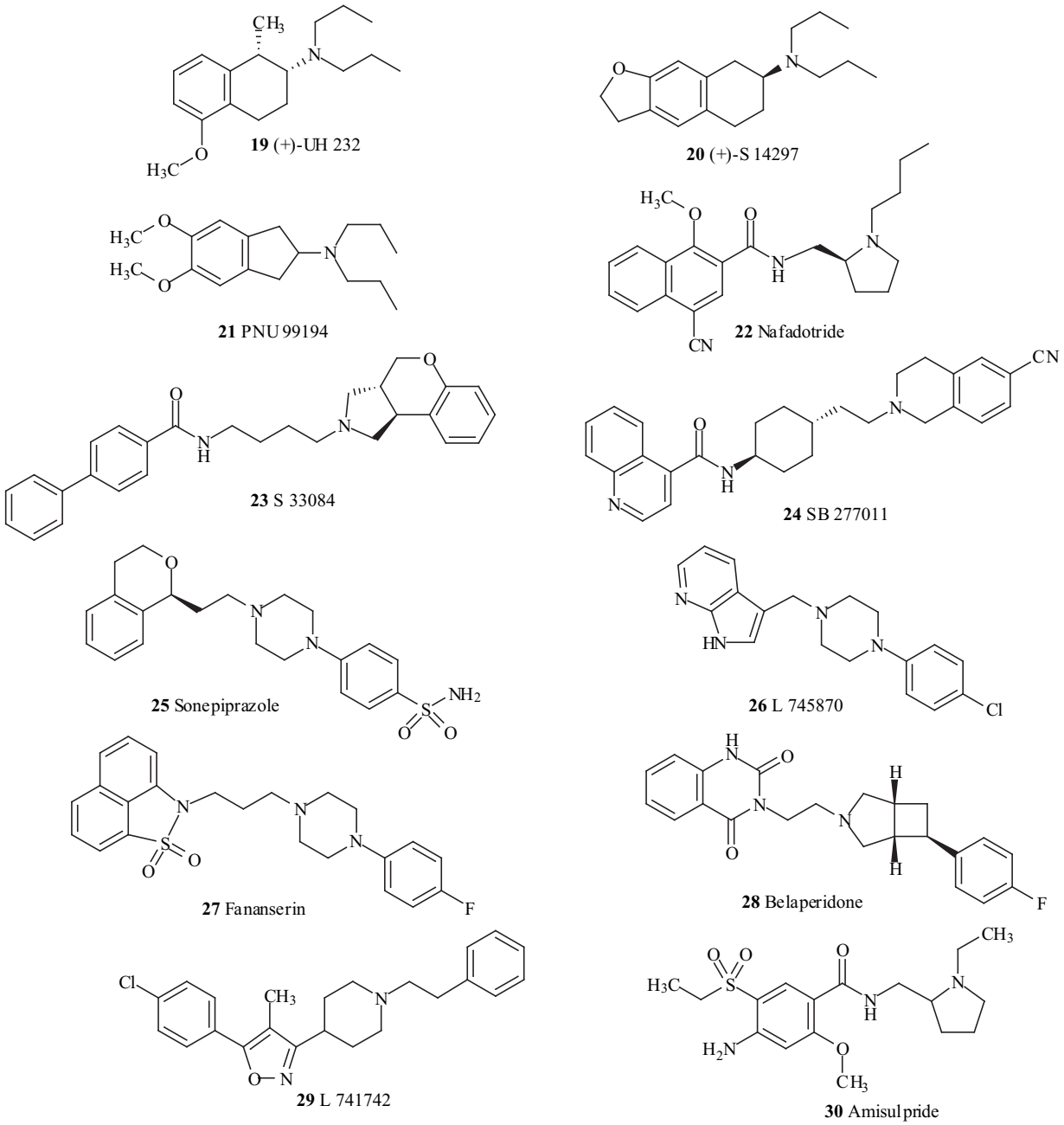


Fig. (4). Compounds binding to dopamine receptors other than D_2.

receptor binding profiles that display potent D_3 affinity include abaperidone (**10**) and ziprasidone (**12**). In recent years, a number of selective D_3 antagonists have been introduced as pharmacological tools, but most of them show inappropriate pharmacokinetic or pharmacodynamic properties. Nevertheless, some D_3 antagonists have been advanced to preclinical or clinical development.

The structure of (*R*)-(+)-7-hydroxy-2-(dipropylamino) tetralin, a dopaminergic agonist with more than 20-fold selectivity for D_3 over D_2 receptors, served as a starting point for the design of selective D_3 antagonists (**Fig. 4**). The aminotetralin derivative (+)-UH 232 (**19**) is a D_3 receptor ligand with little selectivity over D_2 receptors. However, this compound was found to induce catalepsy and increased prolactin secretion in rats [100], a fact that could be attributed to poor selectivity or to D_3 partial agonist activity [101]. Moreover, in a clinical trial in six schizophrenic patients, (+)-UH 232 did not produce an improvement but rather a worsening in the symptomatology [102]. The tricyclic derivative (+)-S 14297 (**20**), with higher D_3/D_2 selectivity, displayed a profile of functional D_3 antagonism without producing extrapyramidal or endocrine adverse effects [100]. The aminoindan PNU 99194A (**21**) [103] showed moderate D_3/D_2 selectivity and was found not to induce catalepsy in rats. This compound, however, induced weak increases in prolactin secretion.

The *ortho*-methoxybenzamide class of antipsychotics provided an alternative approach for the design of selective D_3 antagonists. For many years, sulpiride, a mixed D_2/D_3 antagonist with negligible affinity for non-dopaminergic receptors, has been recognized as an antipsychotic with atypical profile [104]. Nevertheless, sulpiride shows low *in vivo* efficacy, which has been attributed to poor bioavailability and brain penetration. Its more lipophilic analogue remoxipride showed higher *in vivo* efficacy, although it was withdrawn from the market due to some cases of induction of aplastic anemia. Increase in the selectivity for D_3 versus D_2 receptors has been achieved by variations in the benzamide aromatic ring. Nafadotride (**22**) [105], a naphthalenecarboxamide derivative, is a potent D_3 antagonist with moderate selectivity over D_2 receptors. This compound was found to induce catalepsy and to increase plasma prolactin levels, effects probably related to its D_2 antagonist properties. The structural similarity between the *N*-(2-aminoethyl)benzamide moiety in these compounds and the γ-aminobutyrophenone pharmacophore could account for this poor selectivity. In this direction, a greater separation between the amine and amide functions has led to some potent compounds with about 100-fold selectivity for D_3 receptors. The biphenylcarboxamide S 33084 (**23**) showed functional D_3 antagonism without inducing catalepsy, but it had little effect in preclinical models of potential antipsychotic activity [106]. The quinolinecarboxamide SB 277011 (**24**) was active in the isolation-induced prepulse inhibition deficit model, but failed to demonstrate significant activity in other pharmacological models characteristic of antipsychotic compounds [107].

D_4 Receptors

The role of D_4 receptors in schizophrenia remains controversial in spite of some encouraging early reports. The unique antipsychotic effects of clozapine have been suggested to be mediated by D_4 receptors. This concept was based on its higher affinity for D_4 compared to D_2 or D_3 receptors, and on the good correlation of the therapeutically effective plasma levels of clozapine with its affinity for D_4 but not for D_2 or D_3 receptors [108]. Moreover, the anatomical localization of these receptors, almost restricted to cortical and certain mesolimbic areas [109], could account for the improvement in negative symptoms seen with clozapine.

Histopathological data also support a role for these receptors. Increased levels of a D_4-like site in striatal *postmortem* brain tissues were reported for schizophrenic patients [110]. Although further neuropathological studies have questioned these findings [111], more recent reports seem to confirm them [112, 113].

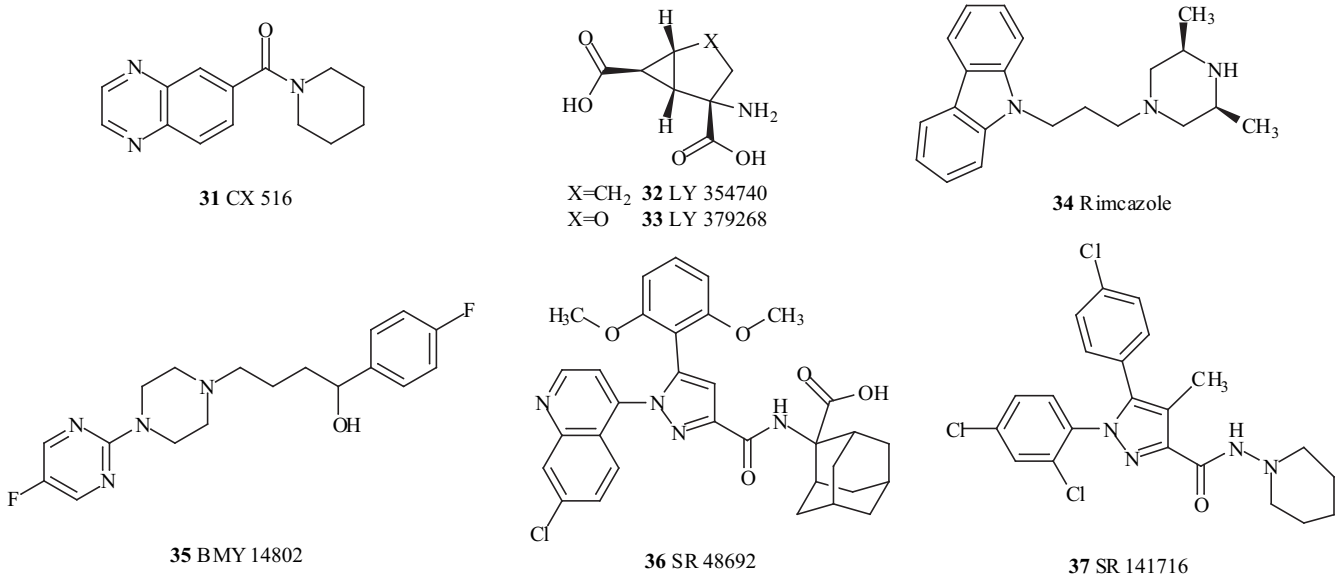


Fig. (5). Compounds binding to non-dopaminergic receptors.

Genetic studies have also provided contradictory results. The existence of a tandem of 48 base pairs in the third cytoplasmatic loop, which can be repeated from 2 to 10 times, creates a wide structural diversity ($D_{4.2}$ to $D_{4.10}$ receptors). Differences found in the affinity for some D_4 antagonists raised the possibility that this diversity could be linked to an increased susceptibility to schizophrenia or to variable responses to clozapine. However, several reported studies failed to sustain conclusive associations between the D_4 receptor gene and schizophrenia [114].

As for D_3 receptors, a number of typical and atypical antipsychotics (such as haloperidol, risperidone and olanzapine) display potent, non-selective dopamine D_4 antagonism. In the search for novel antipsychotics devoid of extrapyramidal side effects, the development of selective D_4 antagonists has recently attracted significant interest [115]. Some lines of research have also pointed to the development of D_4/5-HT_2 mixed antagonists [116]. Arylpiperazines are important pharmacophores for compounds with selectivity for D_4 over D_2 receptors, such as sonepiprazole (**25**) [117], CP 293019 [118], L 745870 (**26**) [119] and the mixed D_4/5-HT_2 antagonist fananserin (**27**) [120, 121] (**Fig. 4**). In preclinical tests, these compounds lacked extrapyramidal and neuroendocrine side effects, and displayed moderate activity in some animal models predictive of antipsychotic efficacy [122]. However, the reported results of the first clinical trials with L 745870 [123] and fananserin [124] failed to demonstrate antipsychotic efficacy. Other chemical structures are represented by the bicyclic amine belaperidone (**28**) [116] and the isoxazolylpiperidine L 741742 (**29**) [125]. Both compounds have also been discontinued. New selective D_4 antagonists, such as the arylpiperazine PD 172760 [126], with a higher potency in some animal models of schizophrenia, are awaiting clinical trials.

Although the first clinical reports obtained with D_3 and D_4 selective antagonists do not support a role for these receptors in schizophrenia, further trials are required to decide if either pure D_3/D_4 antagonism or in combination with other receptors could represent a useful strategy towards improved treatments for this disease.

Presynaptic Dopaminergic Receptors

Dopaminergic D_2 and D_3 receptors also exhibit a presynaptic location, controlling the synthesis and release of dopamine into the synaptic cleft and the neuronal firing of dopaminergic neurons in a feedback mechanism [127, 128]. Since stimulation of these autoreceptors is expected to reduce the dopamine output without complete dopaminergic blockade, compounds acting at presynaptic dopaminergic receptors have been developed as an alternative strategy for novel antipsychotic compounds free of the side effects characteristic of postsynaptic dopamine antagonists. The phenyltetrahydropyridine derivative roxindole [129] and the phenylpiperazine OPC 4392 [130] have been shown to produce moderate improvements in the negative symptoms in schizophrenic patients without causing extrapyramidal side effects. Unfortunately, the clinical development of both compounds has been discontinued. The benzamide derivative amisulpride (**30**) is a preferentially presynaptic D_2/D_3 receptor blocker with a reduced tendency to produce extrapyramidal side effects. At low doses, amisulpride has shown an improvement of negative symptoms, whereas at high doses it controls the positive symptoms due to postsynaptic dopaminergic blockade [131].

COMPOUNDS BINDING TO NON-DOPAMINERGIC RECEPTORS

Recent findings have provided evidence that atypical antipsychotic properties can be found in compounds devoid of direct dopaminergic activity. Action on non-dopaminergic receptors might lead to improved pharmacological properties, avoiding some of the side effects that appear with traditional dopamine antagonists. Two concepts support this pharmacological profile: a) certain neurotransmitter systems differentially modulate the dopaminergic transmission in distinct brain areas, a profile that could offer clear advantages over unselective direct blockade of D_2 receptors, and b) although dopamine is thought to play a prevailing role in the pathophysiology of schizophrenia, the involvement of other neurotransmitter systems could account for some facts not sufficiently explained by the dopaminergic model alone. The hypothesis of a dopaminergic dysfunction in the pathogenesis of schizophrenia has required in recent years some reconceptualization, and a role for other neurotransmitters has been recognized.

Glutamatergic Receptors

A dysfunction of glutamatergic neurotransmission has been implicated in the pathophysiology of schizophrenia. This concept was originally based on the effects of the dissociative anesthetic phencyclidine. This non-competitive glutamate antagonist was found to induce a psychotic state in humans which, in contrast to dopaminergic agents, mimics many features of schizophrenia, including the positive, negative and cognitive symptoms [132]. Moreover, *postmortem* studies in brain tissues of schizophrenics showed presynaptic abnormalities in glutamatergic transmission [133]. The glutamate hypothesis of schizophrenia is now regarded as a complementary model to the dopaminergic hypothesis rather than an alternative theory. Thus, it has been suggested that thalamic sensorimotor inflow to the cortex is attenuated by the cortico-striatal glutamatergic pathways, whereas it is enhanced by dopaminergic stimulation [134].

Glutamate is the main excitatory central neurotransmitter and is ubiquitously distributed in the central nervous system, being involved in a great variety of functions. The physiological effects of glutamate are mediated through two types of receptors: ionotropic, directly coupled to ligand-gated ion channels, and metabotropic, coupled to G proteins [135]. Ionotropic receptors have been classified into three groups according to their selective ligands: the *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and kainate receptors. Metabotropic receptors comprise eight receptor subtypes termed as $mGluR_1$ to $mGluR_8$, which have been categorized into three subgroups.

Although most antipsychotics are devoid of significant affinities for glutamate receptors, glutamatergic neurotransmission has been related to their mechanism of action. Thus, the behavioral effects of NMDA receptor antagonists can be reversed by antipsychotic compounds, especially those with atypical profiles. Mediation of dopaminergic, serotonergic and adrenergic mechanisms is suggested to underlie these effects of the antipsychotic drugs [136].

Other compounds with direct effect on different glutamate receptor subtypes are being developed as potential antipsychotics. NMDA receptors have attracted special interest since phencyclidine and other psychotomimetics were shown to act as non-competitive antagonists at these receptors. The NMDA receptor has a complex structure formed by an ion channel coupled to recognition sites for glutamate along with a number of accessory modulatory sites, including those for the co-agonist glycine, polyamine modulators, channel blockers such as phencyclidine, and several metal cations [137]. Exogenous administration of glutamate or other nonselective agonists appears not to be a useful approach since these compounds are associated to neurotoxic and convulsive effects. Instead, enhancement of glutamatergic transmission by allosteric modulation of the NMDA receptors at the $glycine_B$ site could have positive effects. Thus, administration of glycine [138] or its partial agonist D-cycloserine [139] has been reported to improve the negative symptoms in schizophrenic patients. Nevertheless, high doses of these compounds were required due to poor brain penetrability, and D-cycloserine was associated to some cases of worsening of positive symptoms. Moreover, experience with the glycine prodrug milacemide provided inconsistent results [140]. In recent years, compounds with antagonistic effects at the glycine site, such as L 701324, have been reported to display some features characteristic of atypical antipsychotics [141].

Other glutamate receptor subtypes have been suggested to modulate brain dopaminergic neurons, thus being potential targets for novel antipsychotics. Allosteric modulation of the AMPA receptor is another approach to enhance the glutamatergic transmission. The AMPA positive modulator CX 516 (**31**) was shown to act synergistically with other established antipsychotics [142].

Some subtypes of metabotropic receptors have a basal ganglia localization and exert modulatory effects on dopaminergic neurotransmission [143]. Thus, agonists at group II metabotropic glutamate receptors ($mGlu_{2/3}$) have been found to display potential antipsychotic effects in some animal models. The bicyclic analogues of glutamic acid LY 354740 (**32**) and LY 379268 (**33**) [144] are agonists at group II mGluR with good brain penetration, although these compounds have demonstrated inconclusive antipsychotic effects.

Sigma Receptors

The question of the precise nature of σ receptors has been surrounded by great controversy. Although this receptor was originally related to the opiate receptor class [145], later studies demonstrated that sigma receptors did not involve opioid activity [146]. In addition, despite some initial confusion, this receptor was definitely distinguished from the phencyclidine binding site of the NMDA receptor [147, 148]. Moreover, some speculation aroused about a possible non-receptor nature of the σ binding site [149]. Notwithstanding this, certain facts contributed to put forward the theory that σ receptors could mediate the effects of antipsychotic drugs. The σ receptor agonist *N*-allylnormetazocine (SKF 10047) caused psychotomimetic effects in dogs and in man induced hallucinations and delusions similar to those of schizophrenia. In the brain, σ receptors are localized in high densities in limbic structures as well as in motor-related areas [150] and have been shown to indirectly modulate the dopaminergic transmission [151]. Furthermore, some typical (haloperidol, perphenazine) and atypical antipsychotics (remoxipride, tiospirone) were found to display potent binding affinity for σ receptors [152]. It has been suggested that the antipsychotic activity of haloperidol could be mediated by σ effects, whereas the extrapyramidal side effects could be associated to its D_2 antagonism. Selective ligands for σ receptors are thus regarded as potential candidates for novel antipsychotics. From the two sigma receptor subtypes (σ_1 and σ_2) identified in the brain [153], the σ_1 subtype is usually implicated in schizophrenia due to the selectivity of SKF 10047 for this receptor. In apparent opposition to this evidence, a number of compounds belonging to different pharmacological classes (such as the antitussive dextromethorphan, the local anesthetic cocaine and the steroid hormone progesterone) display moderate to high affinity for σ receptors, but are devoid of antipsychotic activity [154, 155].

The structural features defining σ receptor affinity comprise a great variety of chemical structures [155]. The carbazole derivative rimcazole (**34**) [156], a moderately potent but selective σ ligand, was discontinued from clinical studies due to insufficient efficacy and to the appearance of side effects at higher doses. The pyrimidinylpiperazine BMY 14802 (**35**), with affinity for σ and $5\text{-}HT_{1A}$ receptors, did not produce extrapyramidal side effects but failed to demonstrate antipsychotic efficacy in clinical trials [157]. The arylpiperidine derivative panamesine showed antipsychotic activity but also motor and endocrine side effects, which were attributed to an active metabolite that, unlike the parent compound, displayed D_2 antagonism [158, 159]. Other σ receptor ligands under development as potential antipsychotics include the phenethylamine derivative NE 100 [160] and the aryltetrahydropyridine E 5842 [161]. In recent years, attention has been focused in other potential clinical applications of σ ligands, such as neuroprotection, anxiety, and motor disorders.

Neurotensin Receptors

Several peptide molecules have been found to act as central neurotransmitters modulating other transmitter systems. Neurotensin is a tridecapeptide widely distributed in the central nervous system as well as in peripheral tissues. In the central nervous system, neurotensin is co-localized with dopaminergic neurons, acting mainly as a modulator of dopaminergic transmission [161] through interaction with specific NT receptors. Two neurotensin receptor subtypes

have been cloned, NT_1 and NT_2. Some concerns exist, however, about the role of the NT_2 subtype, since neurotensin seems to act as an antagonist at these receptors [162]. Injection of neurotensin into the brain was found to produce effects similar to those of the atypical antipsychotics [163], a fact that led to the suggestion that neurotensin could be implicated in the pathophysiology of schizophrenia. In addition, whereas chronic administration of classical neuroleptics was found to increase neurotensin release in both limbic and striatal regions, atypical antipsychotics selectively increased the neurotensin levels in the limbic system [164]. Moreover, reduced levels of neurotensin have been found in the cerebrospinal fluid from psychotic patients [165]. Non-peptide, metabolically stable, neurotensin agonists have thus been proposed as potential antipsychotics free of neurologic side effects [166]. However, neurotensin exhibits complex pharmacological effects and, interestingly, there has also appeared some evidence indicating that neurotensin antagonists display antipsychotic properties [167]. The non-peptidic NT_1 antagonist SR 48692 (**36**) [168], which has affinity for NT_2 receptors, is currently in clinical trials as a potential antipsychotic.

Cannabinoid Receptors

Most pharmacological effects of cannabis (marijuana), especially the induction of schizophrenic-like symptoms, are attributed to its principal active component, Δ^9-tetrahydrocannabinol. Its effects were found to be mediated by specific cannabinoid receptors (CB_1 and CB_2) for which endogenous ligands (anandamide and palmitoyl ethanolamine) were further identified. A dysfunction of the endogenous cannabinoid system has been related to schizophrenia. Thus, agonists at CB_1 receptors have been found to induce an activation of dopaminergic mesolimbic neurons [169] and elevations of endogenous cannabinoid levels have been determined in the cerebrospinal fluid of schizophrenic patients [170]. The cognitive impairments during psychotic episodes have been related to a disregulation of the endogenous cannabinoid system [171]. A selective CB_1 antagonist, SR 141716 (**36**), showed a pharmacological profile consistent with antipsychotic activity [172]. This compound is currently undergoing clinical trials for schizophrenia as well as for obesity.

Other Non-dopaminergic Receptors

A number of other receptor systems have been implicated in schizophrenia or in the effects of antipsychotics. Most of them are co-localized with dopamine D_2 receptors and have been shown to modulate the dopaminergic transmission in selected areas of the brain. Some proposed pharmacological targets for novel antipsychotic drugs include serotonin 5-HT_3 antagonists [173], adenosine A_2 agonists [174], cholecystokinin CCK-B antagonists [175] and tachykinins NK_{1-3} antagonists [176]. More selective ligands are required to assess the actual role of these receptors in the pathology of schizophrenia and the potential of these compounds as novel treatments for schizophrenia.

CONCLUSIONS

Although blockade of the hyperactive dopaminergic neurotransmission in the mesolimbic system of the brain is still regarded as the key mechanism for controlling psychotic symptoms, the extrapyramidal and neuroendocrine adverse effects associated to D_2 antagonism constitute a main drawback of classical neuroleptics. However, the appearance of clozapine provides evidence that antipsychotic and side effects are not necessarily associated. New mechanisms are now emerging as potential targets for atypical antipsychotics with improved side effect profiles. Action on more selective dopaminergic receptor subtypes and indirect dopamine modulation through a variety of non-dopaminergic receptors are among the multiple hypotheses to explain the mechanisms of action of the novel compounds. Since antipsychotics with improved profiles can display different mechanisms of action, it could be likely that these hypotheses each only partially account for the atypical profile of clozapine. It remains possible that an optimal combination of these mechanisms could offer new strategies to improved treatments for positive, negative and cognitive symptoms of schizophrenia and be devoid of unpleasant side effects. Recent insights into the biochemical basis of schizophrenia are expected to furnish interesting opportunities for an efficient control of this devastating disease.

REFERENCES

[1] Andreasen, N.C.; Arndt, S.; Alliger, R.; Miller, D.; Flaum, M. *Arch. Gen. Psychiatry*, **1995**, *52*, 341-351.
[2] Lewine, R.R.J.; Fogg, L.; Meltzer, H.Y. *Schizophr. Bull.*, **1983**, *9*, 368-376.
[3] Lader, M. *J. Int. Med. Res.*, **1989**, *17*, 1-16.
[4] Marder, S.R.; Wirshing, W.C.; Van Putten, T. *Schizophr. Res.*, **1991**, *4*, 81-90.
[5] Baldessarini, R.J.; Tarsy, D. *Annu. Rev. Neurosci.*, **1980**, *3*, 23-41.
[6] Boyd, A.E.; Reichlin, S. *Psychoneuroendocrinology*, **1978**, *3*, 113-130.
[7] Seeman, P.; Lee, T.; Chau-Wong, M.; Wong, K. *Nature*, **1976**, *261*, 717-719.
[8] Seeman, P. *Synapse*, **1987**, *1*, 133-152.
[9] Davis, K.L.; Kahn, R.S.; Ko, G.; Davidson, M. *Am. J. Psychiatry*, **1991**, *148*, 1474-1486.
[10] Costall, B.; Naylor, R.J. *Neuropharmacology*, **1974**, *13*, 353-364.
[11] Knable, M.B.; Weinberger, D.R. *J. Psychopharmacology*, **1997**, *11*, 123-131.
[12] Parellada, E.; Catafau, A.M.; Bernardo, M.; Lomeña, F.; Catarineu, S.; González-Monclús, E. *Biol. Psychiatry*, **1998**, *44*, 787-790.
[13] Lowe, J.A., III; Seeger, T.F.; Vinick, F.J. *Med. Res. Rev.*, **1988**, *8*, 475-497.
[14] Kane, J.; Honigfield, G.; Singer, J.; Meltzer, H. *Arch. Gen. Psychiatry*, **1988**, *45*, 789-796.
[15] Povlsen, U.J.; Noring, U.; Fog, R.; Gerlach, J. *Acta Psychiatr. Scand.*, **1985**, *71*, 176-185.
[16] Lieberman, J.A.; Hohn, C.A.; Mikane, J.; Rai, K.; Pisciotta, A.V.; Salz, B.L.; Howard, A. *J. Clin. Psychiatry*, **1988**, *49*, 271-277.
[17] Fitton, A.; Heel, R.C. *Drugs*, **1990**, *40*, 722-747.
[18] Meltzer, H.Y. *J. Clin. Psychiatry*, **1994**, *55* (Suppl. B), 47-52.

[19] Carlsson, A.; Waters, N.; Carlsson, M.L. *Biol. Psychiatry*, **1999**, *46*, 1388-1395.

[20] Abi-Dargham, A.; Laruelle, M.; Charney, D.; Krystal, J. *Drugs Today*, **1996**, *32*, 171-185.

[21] Bleich, A.; Brown, S-L.; Kahn, R.; van Praag, M. *Schizophr. Bull.*, **1988**, *14*, 297-315.

[22] Busatto, G.F.; Kerwin, R.W. *J. Psychopharmacol*., **1997**, *11*, 3-12.

[23] Lieberman, J.A.; Mailman, R.B.; Duncan, G.; Sikich, L.; Chakos, M.; Nichols, D.E.; Kraus, J.E. *Biol. Psychiatry*, **1998**, *44*, 1099-1117.

[24] Meltzer, H.Y. *Psychopharmacol. Ser.*, **1993**, *10*, 70-81.

[25] Kapur, S.; Remington, G. *Am. J. Psychiatry*, **1996**, *153*, 466-476.

[26] Hoyer, D.; Martin, G. *Neuropharmacology*, **1997**, *36*, 419-428.

[27] Gelders, Y.G. *Br. J. Psychiatry*, **1989**, *155*, 33-36.

[28] De Simoni, M.G.; Dal Toso, G.; Sokola, A.; Algeri, S. *Brain Res.*, **1987**, *411*, 81-88.

[29] Saller, C.F.; Czupryna, M.J.; Salama, A.I. *J. Pharmacol. Exp. Ther.*, **1990**, *253*, 1162-1170.

[30] Meltzer, H.Y.; Matsubara, S.; Lee, J.-C. *J. Pharmacol. Exp. Ther.*, **1989**, *251*, 238-246.

[31] Meltzer, H.Y.; Matsubara, S.; Lee, J.-C. *Psychopharmacol. Bull.*, **1989**, *25*, 390-392.

[32] Tamminga, C.A.; Kane, J.M. *Exp. Opin. Invest. Drugs*, **1997**, *6*, 1743-1752.

[33] Gunasekara, N.S.; Spencer, C.M. *CNS Drugs*, **1997**, *8*, 153-159.

[34] Uchida, S.; Honda, F.; Otsuka, M.; Satoh, Y.; Mori, J.; Ono, T.; Hitomi, M. *Arzneim.-Forsch. Drug Res.*, **1979**, *29*, 1588-1594.

[35] Schmutz, J. *Arzneim.-Forsch. Drug Res.*, **1975**, *25*, 712-720.

[36] Lai, W.G.; Gardner, I.; Zahid, N.; Uetrecht, J.P. *Drug Metab. Dispos.*, **2000**, *28*, 255-263.

[37] Rabasseda, X.; Mealy, N.; Peuskens, J. *Drugs Today*, **1993**, *29*, 535-553.

[38] Strupczewski, J.T.; Bordeau, K.J.; Chiang, Y.; Glamkowski, E.J.; Conway, P.G.; Corbett, R.; Hartman, H.B.; Szewczak, M.R.; Wilmot, C.A.; Helsey, G.C. *J. Med. Chem.*, **1995**, *38*, 1119-1131.

[39] Bolós, J.; Anglada, L.; Gubert, S.; Planas, J.M.; Agut, J.; Príncep, M.; De la Fuente, À.; Sacristán, A.; Ortiz, J.A. *J. Med. Chem.*, **1998**, *41*, 5402-5409.

[40] Sánchez, C.; Arnt, J.; Dragsted, N.; Hyttel, J.; Lembol, H.; Meier, E.; Perregaard, J.; Skarsfeldt, T. *Drug Dev. Res.*, **1991**, *22*, 239-250.

[41] Yevich, J.P.; New, J.S.; Smith, D.W.; Lobeck, W.G.; Catt, J.D.; Minielli, J.L.; Eison, M.S.; Taylor, D.P.; Riblet, L.A.; Temple, D.L.Jr. *J. Med. Chem.*, **1986,** *29*, 359-369.

[42] Seeger, T.F.; Seymour, P.A.; Schmidt, A.W.; Zorn, S.H.; Schulz, D.W.; Lebel, L.A.; McLean, S.; Guanowsky, V.; Howard, H.R.; Lowe, J.A.; Heym, J. *J. Pharmacol. Exp. Ther.*, **1995**, *275*, 101-113.

[43] Hrib, N.J.; Jurcak, J.G., Bregna, D.E.; Dunn, R.W.; Geyer, H.M., Hartman, H.B.; Roehr, J.E.; Rogers, K.L., Rush, D.K., Szczepanik, A.M.; Szewczak, M.R.; Wilmot, C.A.; Conway, P.G. *J. Med. Chem.*, **1992**, *35*, 2712-2715.

[44] DeVry, J. *Psychopharmacology*, **1995**, *121*, 1-26.

[45] Meltzer, H.Y. *Neuropsychopharmacology*, **1999**, *21* (2 Suppl), 106S-115S.

[46] Ahlenius, S. *Pharmacol. Toxicol.*, **1989**, *64*, 3-5.

[47] Andersen, H.L.; Kilpatrick, I.C. *Br. J. Pharmacol.*, **1996**, *118*, 421-427.

[48] Wadenberg, M-L.; Cortizo, L.; Ahlenius, S. *Pharmacol. Biochem. Behav.*, **1986**, *24*, 1409-1415.

[49] Prinssen, E.P.; Kleven M.S.; Koek W. *Psychopharmacology*, **1999**, *144*, 20-29.

[50] Millan, M.J. *J. Pharmacol. Exp. Ther.*, **2000**, *295*, 853-861.

[51] Schreiber, R.; De Vry, J. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry*, **1993**, *17*, 87-104.

[52] Griebel, G. *Drug News Perspect.*, **1999**, *12*, 484-490.

[53] Sprouse, J.S.; Reynolds, L.S.; Braselton, J.P.; Rollema, H.; Zorn, S.H. *Neuropsychopharmacology*, **1999**, *21*, 622-631.

[54] Reitz, A.B.; Bennett, D.J.; Blum, P.S.; Codd, E.E.; Maryanoff, C.A.; Ortegon, M.E.; Renzi, M.J.; Scott, M.K.; Shank, R.P.; Vaught, J.L. *J. Med. Chem.*, **1994,** *37*, 1060-1062.

[55] Akunne, H.C.; Zoski, K.T.; Davis, M.D.; Cooke, L.W.; Meltzer, L.T.; Whetzel, S.Z.; Shih, Y.H.; Wustrow, D.J.; Wise, L.D.; MacKenzie, R.G.; Georgic, L.M.; Heffner, T.G.; Pugsley, T.A. *Neuropharmacology*, **2000**, *39*, 1197-1210.

[56] Hibert, M.F.; Gittos, M.W.; Middlemiss, D.N.; Mir, A.K.; Fozard, J.R. *J. Med. Chem.*, **1988**, *31*, 1087-1093.

[57] Canton, H.; Verrièle, L.; Colpaert, F.C. *Eur. J. Pharmacol.*, **1990**, *191*, 93-96.

[58] Fox, S.H.; Brotchie, J.M. *Drug News Perspect.*, **1999**, *12*, 477-483.

[59] Roth, B.L.; Craigo, S.C.; Choudhary, M.S., Uluer, A.; Monsma, F.J.; Shen, Y.; Meltzer, H.Y., Sibley, D.R. *J. Pharmacol. Exp. Ther.*, **1994**, *268*, 1403-1410.

[60] Sleight, A.J.; Boess, F.G.; Bourson, A.; Sibley, D.R.; Monsma, F.J. *Drug News Perspect.*, **1997**, *10*, 214-224.

[61] Baldessarini, R.J.; Huston-Lyons, D.; Campbell, A.; Marsh, E.; Cohen, B.M. *Br. J. Psychiatry*, **1992**, *160* (Suppl. 17), 12-16.

[62] Prinssen, E.P.; Ellenbroek, B.A.; Cools, A.R. *Eur. J. Pharmacol.*, **1994**, *262*, 167-170.

[63] Arnsten, A.F.T.; Mathew, R.; Ubriani, R.; Taylor, J.R.; Li, B-M. *Biol. Psychiatry*, **1999**, *45*, 26-31.

[64] Bakshi, V.P.; Geyer, M.A. *J. Pharmacol. Exp. Ther.*, **1997**, *283*, 665-674.

[65] van Kammen, D.P.; Peters, J.; Yao, J.; van Kammen, W.B.; Neylan, T.; Shaw, D.; Linnoila, M. *Arch. Gen. Psychiatry*, **1990**, *47*, 161-168.

[66] Maas, J.W.; Contreras, S.A.; Miller, A.L.; Berman, N.; Bowden, C.L.; Javors, M.A.; Seleshi, E.; Weintraub, S. *Neuropsychopharmacology*, **1993**, *8*, 97-109.

[67] Lane, R.F.; Blaha, C.D.; Rivet J-M. *Brain Res.*, **1990**, *460*, 398-401.

[68] Svensson, T.H.; Mathé, J.M.; Andersson, J.L.; Nomikos, G.G.; Hildebrand, B.E.; Marcus, M. *J. Clin. Psychopharmacol.*, **1995**, *15* (Suppl. 1), 11S-18S.

[69] Keks, N.A. A*cta Psychiatr. Scand. Suppl.*, **1996**, *389*, 18-24.

[70] Millan, M.J.; Gobert, A.; Newman-Tancredi, A.; Lejeune, F.; Cussac, D.; Rivet, J-M.; Audinot, V.; Adhumeau, A.; Brocco, M.; Nicolas, J-P.; Boutin, J.A.; Despaux, N.; Peglion J-L. *J. Pharmacol. Exp. Ther.*, **2000**, *292*, 38-53.

[71] Millan, M.J.; Brocco, M.; Rivet, J-M.; Audinot, V.; Newman-Tancredi, A.; Maiofiss, L.; Queriaux, S.; Despaux, N.; Peglion J-L.; Dekeyne, A. *J. Pharmacol. Exp. Ther.*, **2000**, *292*, 54-66.

[72] De Boer, T.; Berendsen, H.; Broekkamp, C.L.E.; Vrijmoedde Vries, M.C.; Vos, R.M.E.; Tonnaer, J.A.D.M., Van Delft, A.M.L. *Drugs Future*, **1993**, *18*, 1117-1123.

[73] Aantaa, R.; Marjamaki, A.; Scheinin, M. *Ann. Med.*, **1995**, *27*, 439-449.

[74] Nutt, D.J. *J. Psychopharmacol.*, **1994**, *8*, 193-195.

[75] Elman, I.; Goldstein, D.S.; Eisenhofer, G.; Folio, J.; Malhotra, A.K.; Adler, C.M.; Pickar, D.; Breier, A. *Neuropsychopharmacology*, **1999**, *20*, 29-34.

[76] Arnsten, A.F.T.; Cai, J.X.; Goldman-Rakic, P.S. *J. Neurosci.*, **1988**, *8*, 4287-4297.

[77] Friedman, J.I.; Temporini, H.; Davis, K.L. *Biol. Psychiatry*, **1999**, *45*, 1-16.
[78] Friedman, J.I.; Adler, D.N.; Davis, K.L. *Biol. Psychiatry*, **1999**, *46*, 1243-1252.
[79] Coull, J.T.; Sahakian, B.J.; Hodges, J.R. *Psychopharmacology*, **1996**, *123*, 239-249.
[80] Arnsten, A.F.T.; Steere, J.C.; Jentsch, D.J.; Li, B.M. *Adv. Pharmacol.*, **1998**, *42*, 764-767.
[81] Kalkman, H.O.; Neumann, V.; Hoyer, D.; Tricklebank, M.D. *Br. J. Pharmacol.*, **1998**, *124*, 1550-1556.
[82] Missale, C.; Nash, S.R.; Robinson, S.W.; Jaber, M.; Caron, M.G. *Physiol. Rev.*, **1998**, *78*, 189-225.
[83] Lynch, M.R. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry*, **1992**, *16*, 797-832.
[84] Barnes, T.R.E.; Gerlach, J. *Psychopharmacology*, **1995**, *121*, 287-288.
[85] Okubo, Y.; Suhara, T.; Suzuki, K.; Kobayashi, K.; Inoue, O.; Terasaki, O.; Someya, Y.; Sassa, T.; Sudo, Y.; Matsushima, E.; Iyo, M.; Tateno, Y.; Toru, M. *Nature*, **1997**, *385*, 634-636.
[86] Sokoloff, P.; Giros, B.; Martres, M.P.; Bouthenet, M.L.; Schwartz, J.C. *Nature*, **1990**, *347*, 146-151.
[87] Diaz, J.; Levesque, D.; Griffon, N.; Lammers, C.H.; Martres, M.P.; Sokoloff, P.; Schwartz, J.C. *Eur. J. Neurosci*., **1994**, *6*, 1384-7.
[88] Gurevich, E.V.; Joyce, J.N. *Neuropsychopharmacology*, **1999**, *20*, 60-80.
[89] Schwartz, J.C.; Diaz, J., Bordet, R.; Griffon, N.; Perachon, S.; Pilon, C.; Ridray, S.; Sokoloff, P. *Brain Res. Rev*., **1998**, *26*, 236-42.
[90] Joyce, J.N.; Gurevich, E.V. *Ann. N. Y. Acad. Sci*., **1999**, *877*, 595-613.
[91] Ilani, T.; Ben-Shachar, D.; Strous, R.D.; Mazor, M.; Sheinkman, A.; Kotler, M.; Fuchs, S. *Proc. Natl. Acad. Sci. U. S. A.*, **2001**, *98*, 625-628.
[92] Kwak, Y.T.; Koo, M.-S.; Choi, C.-H.; Sunwoo, I. *BMC Med. Genet.*, **2001**, *2*, 3.
[93] Crocq, M.A.; Mant, R.; Asherson P.; Williams, J.; Hode, Y.; Mayerova, A.; Collier, D.; Lannfelt, L.; Sokoloff, P.; Schwartz, J.C.; Gill, M.; Macher, J.P.; McGuffin, P.; Owen, M.J. *J. Med. Genet.*, **1992**, *29*, 858-860.
[94] Ebstein, R.P.; Macciardi, F.; Heresco-Levi, U.; Serretti, A.; Blaine, D.; Verga, M.; Nebamov, L.; Gur, E.; Belmaker, R.H.; Avnon, M.; Lerer, B. *Hum. Hered.*, **1997**, *47*, 6-16.
[95] Jonsson, E.; Lannfelt, L.; Sokoloff, P.; Schwartz J.C.; Sedvall, G. *Acta Psychiatr. Scand.*, **1993**, *87*, 345-349.
[96] Yang, L.; Li, T.; Wiese, C.; Lannfelt, L.; Sokoloff, P.; Xu, C.T.; Zeng, Z.; Schwartz, J-C.; Liu, X.; Moises, H.W. *Am. J. Med. Genet.*, **1993**, *48*, 83-86.
[97] Williams, J.; Spurlock, G.; Holmans, P.; Mant, R.; Murphy, K.; Jones, L.; Cardno, A.; Asherson, P.; Blackwood, D.; Muir, W.; Meszaros, K.; Aschauer, H.; Mallet, J.; Laurent, C.; Pekkarinen, P.; Seppala, J.; Stefanis, C.N.; Papadimitriou, G.N.; Macciardi, F.; Verga, M.; Pato, C.; Azevedo, H.; Crocq, M.A.; Gurling, H.; Kalsi, G.; McGuffin, P.; Owen, M.J. *Mol. Psychiatry*, **1998**, *3*, 141-149.
[98] Dubertret, C.; Gorwood, P.; Ades, J.; Feingold, J.; Schwartz, J.C.; Sokoloff, P. *Am. J. Med. Genet.*, **1998**, *81*, 318-322.
[99] Crider, M.A.; Scheideler, M.A. *Mini Rev. Med. Chem.*, **2001**, *1*, 89-99.
[100] Audinot, V.; Newman-Tancredi, A.; Gobert, A.; Rivet, J-M.; Brocco, M.; Lejeune, F.; Gluck, L.; Desposte, I.; Bervoets, K.; Dekeyne, A.; Millan, M. *J. Pharmacol. Exp. Ther.*, **1998**, *287*, 187-197.
[101] Griffon, N.; Pilon, C.; Schwartz, J-C.; Sokoloff, P. *Eur. J. Pharmacol.*, **1995**, *282*, R3-R4.
[102] Lahti, A.C.; Weiler, M.; Carlsson, A.; Tamminga, C.A. *J. Neural Transm.*, **1998**, *105*, 719-734.
[103] Waters, N.; Svensson, K.; Haadsma-Svensson, S.R.; Smith, M.W.; Carlsson, A. *J. Neural Transm.*, **1993**, *94*, 11-19.
[104] Caley, C.F.; Weber, S.S. *Ann. Pharmacother.*, **1995**, *29*, 152-160.
[105] Sautel, F.; Griffon, N.; Sokoloff, P.; Schwartz, J-P.; Launey, C.; Simon, P.; Costentin, J.; Schoenfelder, A.; Garrido, F.; Mann, A.; Wermuth, C.G. *J. Pharmacol. Exp. Ther.*, **1995**, *275*, 1239-1246.
[106] Millan, M.J.; Dekeyne, A.; Rivet, J.M.; Dubuffet, T.; Lavielle, G.; Brocco, M. *J. Pharmacol. Exp. Ther.*, **2000**, *293*, 1063-1073.
[107] Reavill, C.; Taylor, S.G.; Wood, M.D.; Ashmeade, T.; Austin, N.E.; Avenell, K.Y.; Boyfield, I.; Branch, C.L.; Cilia, J.; Coldwell, M.C.; Hadley, M.S.; Hunter, A.J.; Jeffrey, P.; Jewitt, F.; Johnson, C.N.; Jones, D.N.; Medhurst, A.D.; Middlemiss, D.N.; Nash, D.J.; Riley, G.J.; Routledge, C.; Stemp, G.; Thewlis, K.M.; Trail, B.; Vong, A.K.; Hagan, J.J. *J. Pharmacol. Exp. Ther.*, **2000**, *294*, 1154-1165.
[108] Seeman, P. *Neuropsychopharmacology*, **1992**, 7, 261-284.
[109] Wedzony, K.; Chocyk, A.; Mackowiak, M.; Fijal, K.; Czyrak, A. *J. Physiol. Pharmacol.*, **2000**, *51*, 205-221.
[110] Seeman, P.; Guan H.C.; Van Tol H.H. *Nature*, **1993**, *365*, 441-445.
[111] Reynolds, G.P.; Mason, S.L. *J. Neurochem*., **1994**, *63*, 1576-1577.
[112] Lahti, R.A.; Roberts R.C.; Cochrane, E.V.; Primus, R.J.; Gallager, D.W.; Conley, R.R.; Tamminga, C.A. *Mol. Psychiatry*, **1998**, *3*, 528-533.
[113] Stefanis, N.C.; Bresnick, J.N.; Kerwin, R.W.; Schofield, W.N.; McAllister, G. *Brain Res. Mol. Brain Res.*, **1998**, *53*, 112-119.
[114] Wong, A.H.C.; Buckle, C.E.; Van Tol, H.H.M. *Eur. J. Pharmacol.*, **2000**, *410*, 183-203.
[115] Sanner, M.A. *Exp. Opin. Ther. Patents*, **1998**, *8*, 383-393.
[116] Steiner, G.; Bach, A.; Bialojan, S.; Greger, G.; Hege, H.G.; Höger, T.; Jochims, K.; Munschauer, R.; Neumann, B.; Teschendorf, H.J.; Traut, M.; Unger, L.; Gross, G. *Drugs Fut.*, **1998**, *23*, 191-204.
[117] Merchant, K.M.; Gill, G.S.; Harris, D.W.; Huff, R.M.; Eaton, M.J.; Lookingland, K.; Lutzke, B.S.; McCall, R.B.; Piercey, M.F.; Schreur, P.J.K.D.; Sethy, V.H.; Smith, M.W.; Svensson, K.A.; Tang, A.H.; Vonvoigtlander, P.F.; Tenbrink, R.E. *J. Pharmacol. Exp. Ther.*, **1996**, *279*, 1392-1403.
[118] Sanner, M.A.; Chappie, T.A.; Dunaiskis, A.R.; Fliri, A.F.; Desai, K.A.; Zorn, S.H.; Jackson, E.R.; Johnson, C.G.; Morrone, J.M.; Seymour, P.A.; Majchrzak, M.J.; Faraci, W.S.; Collins, J.L.; Duignan, D.B.; Di Prete, C.C.; Lee, J.S.; Trozzi, A. *Bioorg. Med. Chem. Lett.*, **1998**, *8*, 725-730.
[119] Kulagowski, J.; Broughton, H.B.; Curtis, N.R.; Mawer, I.M.; Ridgill, M.P.; Baker, R.; Emms, F.; Marwood, R.; Patel, S.; Patel, S.; Ragan, C.I.; Leeson, P.A. *J. Med. Chem.*, **1996**, *39*, 1941-1942.
[120] Malleron, J.-L.; Comte, M.-T.; Gueremy, C.; Peyronel, J.F.; Truchon, A.; Blanchard, J.C.; Doble, A.; Piot, O.; Zundel, J.-L.; Huon, C.; Martin, B.; Mouton, P.; Viroulaud, A.; Allam, D.; Betschart, J. *J. Med. Chem.*, **1991**, *43*, 2477-2483.
[121] Heuillet, E.; Petitet, F.; Mignani, S.; Malleron, J.-L.; Lavayre, J.; Neliat, G.; Doble, A.; Blanchard, J.-C. *Eur. J. Pharmacol.*, **1996**, *314*, 229-233.
[122] Rowley, M.; Bristow, L.J.; Hutson, P.H. *J. Med. Chem.*, **2001**, *44*, 477-501.

[123] Kramer, M.S.; Last, B.; Getson, A.; Reines, S.A. *Arch. Gen. Psychiatry*, **1997**, *54*, 567-572.
[124] Truffinet, P.; Tamminga, C.A.; Fabre, L.F.; Meltzer, H.Y.; Riviere, M.E., Papillon-Downey, C. *Am. J. Psychiatry*, **1999**, *156*, 419-425.
[125] Rowley, M.; Broughton, H.B.; Collins, I.; Baker, R.; Emms, F.; Marwood, R.; Patel, S.; Patel, S.; Ragan, I.; Freedman, S.B.; Leeson, P.D. *J. Med. Chem.*, **1996**, *39*, 1943-1945.
[126] Belliotti, T.R.; Wustrow, D.J.; Brink, W.A.; Zoski, K.T.; Shih, Y.-H.; Whetzel, S.Z.; Georgic, L.M.; Corbin, A.E.; Akunne, H.C.; Heffner, T.G.; Pugsley, T.A.; Wise, L.D. *J. Med. Chem.*, **1999**, *42*, 5181-5187.
[127] Langer, S.Z.; Lehman, J. In *Catecholamines I*. Trendelenburg U., Weiner, N., Eds.; Springer-Verlag: Berlin, pp. 419-507.
[128] Meltzer, H.Y. *Pharmacol. Biochem. Behav.*, **1982**, *17* Suppl. 1, 1-10.
[129] Klimke, A.; Klieser, E. *Pharmacopsychiat.*, **1991**, *24*, 107-112.
[130] Yasuda, Y.; Kikuchi, T.; Suzuki, S.; Tsushi, M.; Yamada, K.; Hiyama, T. *Life Sci.*, **1988**, *42*, 1941-1954.
[131] Coukell, A.J.; Spencer, C.M.; Benfield, P. *CNS Drugs*, **1996**, *6*, 237-256.
[132] Javitt, D.C.; Zukin, S.R. *Am. J. Psychiatry*, **1991**, *148*, 1301-1308.
[133] Sherman, A.D.; Davidson, A.T.; Baruah, S.; Hegwood, T.S.; Waziri, R. *Neurosci. Lett.*, **1991**, *121*, 77-80.
[134] Carlsson, A. In *Schizophrenia*; Hirsch, S.R.; Weinberger, D.R., Eds.; Blackwell Science: Oxford, **1995**, pp. 379-400.
[135] Nakanishi, S.; Nakajima, Y.; Masu, M.; Ueda, Y.; Nakahara, K.; Watanabe, D.; Yamaguchi, S.; Kawabata, S.; Okada, M. *Brain Res. Rev.*, **1998**, *26*, 230-235.
[136] Löscher, W.; Hönack, D. *Eur. J. Pharmacol.*, **1992**, *215*, 199-208.
[137] McBain, C.J.; Mayer, M.L. *Physiol. Rev.*, **1994**, *74*, 723-760.
[138] Javitt, D.C.; Zylberman, I.; Zukin, S.; Heresco-Levy, U.; Lindemayer, J. *Am. J. Psychiatry*, **1994**, *151*, 1234-1236.
[139] Goff, D.C., Tsai, G., Manoach, D., Coyle, J.T. *Am. J. Psychiatry*, **1995**, *152*, 1213-1215.
[140] Rosse, R.B.; Schwartz, B.L.; Leighton, M.P.; Davis, R.E.; Deutsch, S.I. *Clin. Neuropharmacol.*, **1990**, *13*, 348-354.
[141] Bristow, L.J.; Flatman, K.L.; Hutson, P.H.; Kulagowski, J.J.; Leeson, P.D.; Young, L.; Tricklebank, M.D. *J. Pharmacol. Exp. Ther.*, **1996**, *277*, 578-585.
[142] Johnson, S.A.; Luu, N.T.; Herbst, T.A.; Knapp, R.; Lutz, D.; Arai, A.; Rogers, G.A.; Lynch, G. *J. Pharmacol. Exp. Ther.*, **1999**, *289*, 392-397.
[143] Gang, H.; Duffy, P.; Swanson, C.; Ghasemzadeh, M.B.; Kalivas, P.W. *J. Pharmacol. Exp. Ther.*, **1999**, *289*, 412-416.
[144] Monn, J.A.; Valli, M.J.; Massey, S.M.; Hansen, M.M.; Kress, T.J.; Wepsiec, J.P.; Harkness, A.R.; Grutsch, J.L.; Wright, R.A.; Johnson, B.G.; Andis, S.L.; Kingston, A.A.; Tomlinson, R.; Lewis, R.; Griffey, K.R.; Tizzano, J.P.; Schoepp, D.D. *J. Med. Chem.*, **1999**, *42*, 1027-1040.
[145] Martin, W.R.; Eades, C.G.; Thompson, J.A.; Huppler, R.E.; Gilgert, P.E. *J. Pharmacol. Exp. Ther.*, **1976**, *197*, 517-532.
[146] Vaupel, D.B. *Eur. J. Pharmacol.*, **1983**, *92*, 269-274.
[147] Gundlach, A.L.; Largent, B.L.; Snyder, S.H. *Eur. J. Pharmacol.*, **1985**, *113*, 465-466.
[148] Largent, B.L.; Gundlach, A.L.; Snyder, S.H. *J. Pharmacol. Exp. Ther.*, **1986**, *238*, 739-745.
[149] Lehmann, J. *Drug News Perspect.*, **1991**, *4*, 208-210.
[150] McLean, S.; Weber, E. *Neuroscience*, **1988**, *25*, 259-269.
[151] Iyengar, S.; Dilworth, V.M.; Mick, S.J.; Contreras, P.C.; Monahan, J.B.; Rao, T.S.; Wood, P.L. *Brain Res.*, **1990**, *524*, 322-326.
[152] Snyder, S.H.; Largent, B.L. *J. Neuropsychiatry*, **1989**, *1*, 7-15.
[153] Walker, J.M.; Bowen, W.D.; Walker, F.O.; Matsumoto, R.R., De Costa, B.R.; Rice, K.C. *Pharmacol. Rev.*, **1990**, *42*, 355-402.
[154] Largent, B.L.; Wikstrom, H.; Gundlach, A.L.; Snyder, S.H. *Mol. Pharmacol.*, **1987**, *32*, 772-784.
[155] Ferris, R.M.; Tang, F.L.M.; Chang, K.J.; Russell, A. *Life Sci.*, **1986**, *38*, 2329-2337.
[156] Gewirtz, G.R.; Gorman, J.M.; Volavka, J.; Macaluso, J.; Gribkoff, G.; Taylor, D.P.; Borison, R. *Neuropsychopharmacology*, **1994**, *10*, 37-40.
[157] Frieboes, R.M.; Murck, H.; Wiedemann, K.; Holsboer, F.; Steiger, A. *Psychopharmacology*, **1997**, *132*, 82-88.
[158] Huber, M.T.; Gotthardt, U.; Schreiber, W.; Krieg, J.C. *Pharmacopsychiatry*, **1999**, *32*, 68-72.
[159] Nakazato, A.; Ohta, K.; Sekiguchi, Y.; Okuyama, S.; Chaki, S.; Kawashima, Y.; Hatayama, K. *J. Med. Chem.*, **1999**, *42*, 1076-1087.
[160] Guitart, X.; Ballarín, M.; Codony, X.; Dordal, A.; Farré, A.J.; Frigola, J.; Mercè, R. *Drugs Fut.*, **1999**, *24*, 386-392.
[161] Kasckow, J.; Nemeroff, C.B. *Regul. Pept.*, **1991**, *36*, 153-164.
[162] Vita, N.; Oury-Donat, F.; Chalon, P.; Guilemot, M.; Kaghad, M.; Bachy, A.; Thurneyssen, O.; García, S.; Poinet-Chazel, C.; Casellas, P.; Keane, P.; Le Fur, G.; Maffrand, J.P.; Soubrie, P.; Caput, D.; Ferrara, P. *Eur. J. Pharmacol.*, **1998**, *360*, 265-272.
[163] Jolicoeur, F.B.; Gagne, M.A.; Rivest, R.; Drumheller, A.; St.-Pierre, S. *Brain. Res. Bull.*, **1993**, *32*, 487-491.
[164] Radke, J.M.; Owens, M.J.; Ritchie, J.C.; Nemeroff, C.B. *Proc. Natl. Acad. Sci. USA*, **1998**, *95*, 11462-11464.
[165] Sharma, R.P.; Janicack, P.G.; Bissette, G.; Nemeroff, C.B. *Am. J. Psychiatry*, **1997**, *154*, 1019-1021.
[166] Kinkead, B.; Binder, E.B.; Nemeroff, C.B. *Biol. Psychiatry*, **1999**, *49*, 340-351.
[167] Feifel, D.; Reza, T.L.; Robeck, S.L. *Peptides*, **1997**, *18*, 1457-1460.
[168] Azzi, M.; Betancur, C.; Sillaber, I.; Spangel, R.; Rostene, W.; Berod, A. *J. Neurochem.*, **1998**, *71*, 1158-1167.
[169] Gessa, G.; Melis, M.; Muntoni, A.; Diana, M. *Eur. J. Pharmacol.*, **1998**, *341*, 39-43.
[170] Leweke, F.M.; Giuffrida, A.; Wurster, U.; Emrich, H.M.; Piomelli, D. *Neuroreport*, **1999**, *10*, 1665-1669.
[171] Emrich, H.M.; Leweke, F.M.; Schneider, U. *Pharmacol. Biochem. Behav.*, **1997**, *56*, 803-807.
[172] Rinaldi-Carmona, M.; Congy, C.; Santucci, V.; Simlarid, J.; Gautret, B.; Neliat, G.; Labeeuw, B.; Le Fur, G.; Soubrie, P.; Breliere, J.-C. *J. Pharmacol. Exp. Ther.*, **1992**, *262*, 759-767.
[173] Kilpatrick, G.J.; Bunce, K.T.; Tyers, M.B. *Med. Res. Rev.*, **1990**, *10*, 441-475.
[174] Dixon, D.A.; Fenix, L.A.; Kim, D.M.; Raffa, R.B. *Ann. Pharmacother.*, **1999**, *33*, 480-488.
[175] Payeur, R.; Nixon, M.J.; Bourin, M.; Bradwejn, J.; Legrand, J.M. *Eur. J. Psychiatry*, **1993**, *57* (Suppl. 11), 4-11.
[176] Longmore, J.; Swain, C.J.; Hill, R.G. *Drug News Perspect.*, **1995**, *8*, 5-23.