The medicinal chemistry of 2-aminotetralin-derived benzamides
Homan, Evert Jan

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
1998

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
1

INTRODUCTION

1.1 SCHIZOPHRENIA

Although the disease nowadays called schizophrenia has probably been present since early civilization, it was not until the beginning of the nineteenth century that the first detailed descriptions of the illness appeared in literature. In 1896, Emil Kraepelin named the disorder dementia praecox or early-onset dementia, distinguishing the patients from those with a late-onset dementia and from those suffering from manic-depressive illness. Recognizing that the common characteristic between the patients was a ‘thought disorder’ or the ‘splitting of the fabric of thought’, Eugen Bleuler in 1911 renamed the disease schizophrenia [schízein (Gk.) = to split, phrèn (Gk.) = mind].

Bleuler distinguished between ‘accessory’ and ‘fundamental’ symptoms, nowadays referred to as positive and negative symptoms, respectively. Positive symptoms of schizophrenia include hallucinations (sensory experiences without adequate external stimuli), delusions (belief held despite evidence to the contrary), positive formal thought disorder and bizarre or disorganized behaviour. Negative symptoms include alogia (poverty of speech), affective flattening (reduced emotional responsiveness), anhedonia (inability to feel pleasure), asociality (inability to initiate or maintain social contacts), avolition–apathy (lack of motivation, underachievement at work or school) and attentional impairment. Based on this differentiation in symptoms, a concept of two different syndromes in schizophrenia, referred to as Type I and Type II, has been postulated. In the Type I syndrome (or ‘florid state’) positive symptoms are most prominent, whereas in the Type II syndrome (or ‘deficit state’) negative symptoms dominate. Although the two syndromes seem to be relatively
independent, they are not mutually exclusive, since both positive and negative symptoms of schizophrenia can be prominent in a single patient.\textsuperscript{80}

Schizophrenia appears to have a worldwide incidence of about 1 percent. Throughout the world, the prevalence of schizophrenia seems to be fairly homogeneous, although there is evidence of geographical pockets with a relatively high prevalence. Incidence estimates, however, are highly dependent on the diagnostic criteria being used.\textsuperscript{375} Schizophrenia is highly variable across individuals and across time in the same individual. Whereas some individuals experience one or more episodes and return to normal or near-normal functioning, others have a gradual or intermittent course with increasing disability. Both men and women seem to be equally affected by schizophrenia, although men usually have a younger age of onset.\textsuperscript{410}

Despite its ancient history and the vast amount of research that has been devoted to the disease during the last century, little is understood about the etiology of schizophrenia. A genetic predisposition for schizophrenia has been generally accepted, based on family studies, twin studies and studies with adoptees.\textsuperscript{197} In addition to this genetic hypothesis, environmental factors such as (prenatal) exposure to viruses,\textsuperscript{198,249} perinatal complications,\textsuperscript{223} autoimmunity,\textsuperscript{200} season of birth,\textsuperscript{172} and social-cultural status\textsuperscript{323} have been put forward as possible causes underlying schizophrenia. In view of the complexity of the disease, it seems likely that a combination of both genetic and environmental factors is a prerequisite for developing schizophrenia. For a more extensive review on the history, etiology, pathophysiology and treatment of schizophrenia, the reader is referred to ref. 35.

### 1.2 Neurochemical Hypotheses of Schizophrenia

Whereas the true causes underlying schizophrenia are still subject of debate, evidence has been accumulating over the last three decades that the symptoms of the disease may be the result of neurochemical and/or anatomical abnormalities in the central nervous system (CNS). The implication of disturbed neurotransmission in schizophrenia was initiated by the findings of Carlsson and Lindqvist, who reported in 1963 that the antipsychotic drugs chlorpromazine and haloperidol enhanced the accumulation of the main metabolites of dopamine and noradrenaline in the rat brain, presumably by blockade of catecholamine receptors.\textsuperscript{56} These findings have laid the foundation for the dopamine hypothesis of schizophrenia, which has governed the development of antipsychotic drugs during the last thirty years.

#### 1.2.1 The Dopamine Hypothesis of Schizophrenia

Dopamine (3,4-dihydroxyphenylethylamine, \textbf{1}) is utilized as a neurotransmitter in specific neuronal pathways within the CNS. The localization of these pathways has been unraveled in the 1960's. Three major dopaminergic systems can be distinguished in the mammalian CNS (for reviews and references see refs. 34, 72, 121, and 266):
(1) the nigrostriatal dopamine system (A9), with cell bodies situated predominantly in the substantia nigra pars compacta and nerve terminals in the caudate nucleus/putamen (striatum), the globus pallidus and the subthalamic nucleus;

(2) the mesolimbocortical dopamine system (A10), with cell bodies predominantly in the ventral tegmental area and projections to limbic cortical areas, including the piriform cortex, the entorhinal cortex, the perirhinal cortex, the prefrontal cortex and the cingulate cortex (mesocortical projections), as well as to other limbic structures, including the nucleus accumbens, the septum, the hippocampus, the olfactory tubercle and the amygdala (mesolimbic projections);

(3) the tuberoinfundibular/tuberohypophyseal dopamine system (A12), with cell bodies lying in the arcuate and periventricular nuclei and nerve terminals in the median eminence and the pituitary, respectively.

Chart 1.1 Chemical structure of dopamine (1).

Basically, the dopamine hypothesis of schizophrenia posited that the symptoms of the disease are a manifestation of a hyperdopaminergic state of the CNS, in particular of the mesolimbic dopamine system, which traditionally has been implicated in the control of mood and emotional behaviours like aggression, anxiety and sexuality (for reviews and references, see refs. 54, 135, and 337). Both preclinical and clinical studies have provided consistent evidence for overactivity in central dopaminergic neurotransmission in the pathophysiology of schizophrenia. First, patients suffering from Parkinson’s disease who are treated with L-DOPA, a precursor in the biosynthesis of dopamine, sometimes experience psychotic episodes as side-effects. Furthermore, drugs that stimulate dopaminergic neurotransmission (e.g. d-amphetamine and L-DOPA) can exacerbate psychotic symptoms in schizophrenic patients. Chronic administration of high doses of such drugs to healthy individuals can induce a syndrome mimicking paranoid schizophrenia, which can be reversed by antipsychotic drugs. Second, a highly significant positive correlation between the affinity of antipsychotic drugs for brain dopamine D₂ receptors and their clinical potency has been demonstrated. Third, post-mortem analyses on the brains of schizophrenics have revealed significantly elevated numbers of dopamine D₂ receptors, which cannot be totally accounted for by the medication history of the patients. In vivo measurements of dopamine D₂ receptor densities in humans by positron emission tomography (PET) gave conflicting results in this regard, but these might be caused by differences in the radioligands and patients groups that were used.

Despite these consistencies in evidence pointing towards a hyperdopaminergic state of the CNS in schizophrenia, there is now general agreement that the dopamine hypothesis, as posited in its original form, is too simple for explaining the complete symptomatology of the disease. Several observations support its shortcomings. First, substantial evidence for elevated levels of dopamine in the schizophrenic brain is still lacking. In fact, many patients – particularly those in which negative
symptoms dominate – exhibit normal or subnormal levels of the dopamine metabolite homovanillic acid (HVA) in their cerebrospinal fluid (CSF). In addition, low doses of the indirect dopamine agonists \(d\)-amphetamine\(^{62,134}\) and L-DOPA\(^{52,287}\) have been reported to improve negative symptoms in a subgroup of patients. Taken together, these findings suggest that at least in a subgroup of patients, the CNS shows an overall hypo- rather than a hyperdopaminergic activity.\(^{395,409}\)

Several investigators conceived the possibility of the coexistence of areas with a hyper- and a hypodopaminergic activity in the brains of schizophrenics.\(^{85,137}\) Hypofrontality, a dysfunction of the prefrontal cortex, has been proposed as the cause of the hypodopaminergic state.\(^{404}\) In fact, animals and humans with frontal lobe damage show behaviour with striking similarities to negative symptoms of schizophrenia.\(^{70}\) These findings have led to a reconceptualization of the dopamine hypothesis of schizophrenia, which explains the existence of both positive and negative symptoms: as a consequence of hypofrontality a hypodopaminergic state of the prefrontal cortex arises, causing the more persistent negative symptoms of schizophrenia.\(^{58,85,137,385}\) This hypodopaminergic state of the prefrontal cortex can under certain circumstances (e.g. stress)\(^{162}\) lead to a dysinhibition of subcortical dopaminergic systems, in particular the mesolimbic dopamine system, and trigger an abnormally high release of dopamine in these brain areas.\(^{82,137}\) This latter phenomenon should in turn account for the occurrence of positive symptoms in schizophrenia.

A second shortcoming of the dopamine hypothesis is its failure to explain the delayed onset of action of antipsychotic drugs. Thus, while antipsychotic drugs produce complete occupancy of dopamine receptors within minutes after administration,\(^{335}\) it usually takes days or weeks before their clinical effects become apparent.\(^{297}\) These observations, however, may be explained in terms of initial compensatory mechanisms (e.g. receptor upregulation) in the dopaminergic neurotransmission developing after blockade of dopamine receptors, which upon long-term drug treatment slowly adapt towards a new homeostasis situation.

Finally, a significant population of schizophrenic patients persistently shows moderate to severe positive symptoms despite trials with several antipsychotic drugs of different classes. This subgroup of patients, referred to as ‘treatment-resistant’ or ‘treatment-refractory’, has been estimated to comprise 15 to 30% of all schizophrenics, depending on the diagnostic criteria.\(^{258}\) Taken together, these shortcomings of the dopamine hypothesis lead to the believe that, in addition to dopamine, other neurotransmitters may be involved in the pathophysiology of schizophrenia.\(^{55}\) In this respect, serotonin in particular has regained much attention during the last few years.

### 1.2.2 The Serotonin Hypothesis of Schizophrenia

Serotonin (5-hydroxytryptamine, 5-HT, 2), was recognized to function as a neurotransmitter in the CNS in the early 1950’s.\(^{382}\) Similar to dopamine, serotonergic neurotransmission is restricted to specific neuronal pathways in the brain (for reviews and references, see refs. 18, 177, and 377). The cell bodies of serotonergic neurons, designated as the clusters B1–B9, are located in the pons and upper brain stem of the CNS and can be divided into a superior and an inferior group. The superior group (B5–B9) consists of four main nuclei: the median raphe nucleus (MRN, B5 and B8), the dorsal raphe nucleus (DRN, B6 and B7), the caudal linear nucleus (CLN, B8), and the nucleus prosupralemniscus (NP, B9). The inferior group (B1–B4) consists of five main nuclei: the nucleus...
raphe obscurus (NRO, B2), the nucleus raphe pallidus (NRPa, B1 and B4), the nucleus raphe magnus (NRM, B3), the lateral paragigantocellular nucleus (LPGN), and the intermediate reticular nuclei (IRN, B1 and B3). The cells in the inferior group project to the ventral horn and central gray area of the spinal cord and therefore are probably irrelevant in the pathophysiology of schizophrenia. Cells in the superior group innervate the forebrain, their ascending afferents predominantly running via the medial forebrain bundle (MFB). Serotonergic fibers have been detected in virtually every area of the forebrain, but certain areas are innervated with relatively high densities. These include the cortex, the hippocampus, the suprachiasmatic nuclei of the hypothalamus, the substantia nigra zona compacta, the medial mammilary nucleus, the lateral septum, the periventricular nucleus of the thalamus, the ventrolateral geniculate, and the medial nucleus of the amygdala. In comparison with the dopaminergic system, the serotonergic projections are more diffuse: most cells in the superior group appear to innervate overlapping terminal fields. For example, the DRN and the CLN project to structures of the basal ganglia including the corpus striatum and the substantia nigra, while the MRN, but also the DRN project to limbic structures, such as the hippocampus and septum.

Four major lines of evidence exist which implicate serotonin in the pathophysiology of schizophrenia (for reviews see refs. 36, 47, and 288). First, the structural similarities between serotonin and lysergic acid diethylamide (d-LSD), a drug known to induce effects resembling certain aspects of schizophrenia, raised the idea in the early 1950’s that d-LSD might exert these effects by blocking serotonin receptors. A deficiency of serotonin in the brains of schizophrenic patients should thus account for the observed symptoms. Following this ‘serotonin deficiency hypothesis of schizophrenia’, other researchers have put forward the ‘transmethylation hypothesis of schizophrenia’, proposing that certain forms of schizophrenia may be caused by the formation of hallucinogenic N,N-dimethyltryptamines from naturally occurring biogenic amines. However, no increases in the concentrations of such compounds in urine, whole blood and plasma, or CSF of schizophrenics, as compared to normal controls, could be detected. Furthermore, hallucinogenic tryptamines and d-LSD predominantly induce visual hallucinations, symptoms which are actually quite rare in schizophrenia, but fail to induce other prominent features of the disease, such as formal thought disorders. These shortcomings, together with the increasing amount of evidence for a prominent role of dopamine in the pathophysiology of schizophrenia, which has been accumulated during the same period, have led to a gradual disbelief in these hypotheses. More recent pharmacological developments, however, have revived the interest in the possible role of serotonin in schizophrenia (see Section 1.6.4).

Second, post-mortem studies on the brains of schizophrenic patients have revealed some consistencies related to disturbances in serotonergic neurotransmission, although a number of studies
with conflicting data have been reported. Measured post-mortem parameters include brain levels of tryptophan, serotonin and 5-hydroxyindoleacetic acid (5-HIAA), the major metabolite of serotonin, and serotonin receptor densities. Inconsistencies in these parameters arise from differences in patient parameters such as age, cause of death, medication history, and brain region under investigation in the different studies. Thus, several researchers have reported increased levels of 5-HT and/or 5-HIAA in various brain areas, including the basal ganglia, while others found decreased or unaffected levels. Initial measurements of serotonin 5-HT₂ receptor densities, employing [³H]-LSD as the radioligand, gave conflicting results as well: some researchers found decreased numbers of [³H]-LSD binding sites in the frontal cortex of schizophrenics, while others reported unchanged or even increased cortical [³H]-LSD binding. The lack of receptor selectivity of LSD should probably in part account for these controversial observations, since studies performed with more specific radioligands gave more consistent results. Thus, more recently a group of Japanese researchers, employing the selective serotonin 5-HT₂ receptor antagonists ketanserin as a radioligand, found decreased densities of serotonin 5-HT₂ receptors in the prefrontal cortices of schizophrenic patients. Other scientists, also using [³H]-ketanserin or [³H]-spiperone, were able to reproduce these results. In contrast to cortical areas, increased serotonin 5-HT₂ receptor densities in the hippocampus, the nucleus accumbens and the ventral putamen have been observed. The increase in serotonin 5-HT₂ receptor density in the putamen was confirmed by Toru et al. Three post-mortem studies on the density of serotonin 5-HT₁A receptors in schizophrenics have been published. Joyce reported increased numbers of serotonin 5-HT₁A receptors in the prefrontal cortex and the hippocampus of schizophrenic patients. Hashimoto and coworkers also found increased prefrontal cortical serotonin 5-HT₁A receptor binding, but no differences in other brain areas, including the hippocampus. Increased serotonin 5-HT₁A receptor densities in the prefrontal cortex of schizophrenics were confirmed by Sumiyoshi et al. Interestingly, there seems to be general agreement in these reports that the observed changes in receptor densities of both serotonin 5-HT₂ and 5-HT₁A receptors are unrelated to the medication histories of the investigated patients.

A third line of evidence comes from measurements of cerebrospinal fluid (CSF) levels of 5-HIAA, and measurement of peripheral markers of serotonin function, including blood platelet serotonin and whole blood serotonin. CSF 5-HIAA levels can be taken as a crude indicator of central 5-HT metabolism. Initial data from a number of CSF 5-HIAA measurements were again inconsistent, although no increases were reported. Three independent studies later showed that cortical atrophy and ventricular enlargements, malformations of the brain which are frequently observed in schizophrenics with predominantly negative symptoms, were significantly correlated with a decrease in CSF 5-HIAA levels. In addition, there seems to be evidence for a relationship between low CSF 5-HIAA levels and suicidal behaviour in a subgroup of schizophrenic patients. Blood platelet serotonin content has been put forward as a measure of serotonin turnover in the CNS. The human blood platelets are neuroectodermal derivatives and therefore have many biochemical and morphological characteristics in common with CNS serotonergic synaptosomes. A number of studies on platelet and whole blood serotonin levels in schizophrenics has been reported and there seems to be general agreement on elevated levels in chronic schizophrenics. The clinical significance of these findings, however, is unclear.
Finally, attempts have been made to challenge the serotonergic neurotransmission in schizophrenic patients with selective serotonergic agents, in order to evoke alterations in the symptoms and hence shed more light on the role of serotonin in the disease. Thus, treatment studies with high doses of L-tryptophan and 5-hydroxytryptophan, two precursors in the biosynthesis of serotonin, but also tryptophan hydroxylase inhibitors such as para-chlorophenylalanine, non-selective serotonin receptor agonists like meta-chlorophenylpiperazine, and serotonin uptake inhibitors like fenfluramine have been undertaken, but the results were in general disappointing. 

In summary, the inconsistencies in the studies described above indicate that no solid conclusions can be drawn about the implications of serotonin in schizophrenia. These inconsistencies might be a reflection of the heterogeneity of the disease. Nevertheless, a few consistent observations, particularly the decreased and increased densities in prefrontal cortical serotonin 5-HT$_2$ and 5-HT$_1A$ receptors, respectively, and the elevations in blood and platelet serotonin levels at least suggest that serotonergic mechanisms are involved in the pathophysiology of schizophrenia.

1.2.3 Involvement of Other Neurotransmitters in Schizophrenia

Since the implication of disturbed neurotransmission in schizophrenia by Carlsson and Lindqvist, virtually every neurotransmitter of importance in the CNS has been suggested to play a role in the pathophysiology of the disease. Thus, a noradrenaline hypothesis, a glutamate hypothesis, an acetylcholine hypothesis, and a γ-aminobutyric acid (GABA) hypothesis of schizophrenia have been put forward to account for the symptoms observed in the disease. In addition, several neuropeptides, including neurotensin, cholecystokinin, and endogenous opioids, but also hormones, such as oestrogens and melatonin, have been implicated in the pathophysiology of schizophrenia. In view of the complexity of the disease, it cannot be excluded that, in addition to dopamine and serotonin, other neurotransmitters also play a role in the pathophysiology of the disease. A discussion on the possible role of these compounds, however, is beyond the scope of this thesis. For reviews and references, the reader is referred to the references quoted.

1.3 Dopamine Receptors

1.3.1 General Structural Features of G-Protein-Coupled Receptors

Upon release from dopaminergic neurons, dopamine exerts its action by interacting with specific dopamine receptors. At present, five subtypes of dopamine receptors have been identified and anatomically, and to a certain extent biochemically and pharmacologically characterized. All currently identified dopamine receptor subtypes belong to the superfamily of G-protein-coupled receptors (GPCRs).

Members of this family, to which among others the opsins, olfactory receptors and many neurotransmitter receptors belong, comprise large glycoproteins embedded in the cell membranes of the cells targeted by a specific endogenous ligand. GPCRs are, by definition, coupled to guanine-nucleotide-binding regulatory proteins (G-proteins), which link the receptors to intracellular effector mechanisms. The exact molecular structures of GPCRs are unknown, since attempts to crystallize
these proteins have failed thus far. Nevertheless, biophysical, biochemical and molecular biological studies on various GPCRs suggest that these receptors have many structural features in common. Thus, hydropathy analyses of the primary amino acid sequences and sequences alignments of the currently identified GPCRs revealed the presence of seven relatively hydrophobic regions of about 25 amino acids in length, which are interconnected by six relatively hydrophilic regions of variable lengths. The seven hydrophobic regions are believed to form seven transmembranal (TM) \( \alpha \)-helices, orientated in a more or less parallel manner. All GPCRs cloned thus far have been shown to possess a substantial degree of homology in their amino acid sequences, especially in the TM regions. The amino-terminal region of the protein, which contains one or more glycosylation sites, is located extracellularly, whereas the carboxyl-terminal region protrudes into the cytosol. The interconnecting loops are alternatively located intra- and extracellularly. The binding site for the endogenous ligand (the ‘active site’) is believed to be situated within the core formed by the seven TM domains, while the third cytoplasmatic loop is thought to be involved in the coupling of the receptor to the G-protein. Binding of the endogenous ligand to the active site presumably induces conformational changes in the receptor molecule, which trigger via the G-protein an intracellular response, e.g. the production of a second messenger molecule. In this way, the ‘information’ carried by the ligand is transduced over the plasma membrane into the cell (for reviews and references on GPCRs, see refs. 96, 97, and 363).

### 1.3.2 Dopamine Receptor Classification

The application of new molecular biology techniques in the field of receptor research has accounted for a revolution during the last decade. Until ten years ago, only two subtypes of dopamine receptors were discriminated, based on biochemical and pharmacological observations: dopamine D\(_1\) receptors, mediating the stimulation of intracellular cyclic adenosine monophosphate (cAMP) production by activating the enzyme adenylate cyclase, and dopamine D\(_2\) receptors, which mediate the inhibition of this second messenger system.\(^{196,360}\) The identification of the DNA sequence encoding the hamster \( \beta_2 \)-adrenergic receptor in 1986 proved to be a milestone in receptor research,\(^ {95}\) since it opened the possibility to locate the coding sequences of a number of other GPCRs, including those of the dopamine receptors. Thus, screening of genomic libraries has resulted in the identification, cloning and expression of at least five different subtypes of human dopamine receptors, termed D\(_1\), D\(_2\), D\(_3\), D\(_4\), and D\(_5\). Based on similarities and differences in gene organization, molecular structure, pharmacology and biochemistry these subtypes have been classified into two subfamilies: dopamine ‘D\(_1\)-like’ receptors, comprising the dopamine D\(_1\) and D\(_3\) receptor subtypes, and dopamine ‘D\(_2\)-like’ receptors, comprising the dopamine D\(_2\), D\(_3\), and D\(_4\) receptor subtypes, respectively. The properties of the two subfamilies closely resemble those of the dopamine D\(_1\) and D\(_2\) receptor subtypes as originally defined in the late 1970’s. The most important characteristics of the cloned human dopamine receptor subtypes are summarized in Table 1.1.
In addition to elucidating the molecular features of GPCRs, molecular cloning techniques have also allowed for the expression of GPCRs in cells that normally do not express such receptors. Thus,

<table>
<thead>
<tr>
<th>TABLE 1.1</th>
<th>Summary of the characteristics of cloned human dopamine receptor subtypes.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene</td>
<td>D₁-like</td>
</tr>
<tr>
<td>Reference</td>
<td>89, 366, 413</td>
</tr>
<tr>
<td>Chromosome localization</td>
<td>5q35.1</td>
</tr>
<tr>
<td>Introns</td>
<td>no</td>
</tr>
<tr>
<td>Expression</td>
<td>–</td>
</tr>
<tr>
<td>Protein</td>
<td></td>
</tr>
<tr>
<td>Amino acids</td>
<td>446</td>
</tr>
<tr>
<td>3rd Cytoplasmatic loop</td>
<td>short</td>
</tr>
<tr>
<td>C-terminus</td>
<td>long</td>
</tr>
<tr>
<td>Sequence homology</td>
<td>D₁</td>
</tr>
<tr>
<td>D₃</td>
<td>100</td>
</tr>
<tr>
<td>D₂</td>
<td>100</td>
</tr>
<tr>
<td>D₃</td>
<td>100</td>
</tr>
<tr>
<td>D₄</td>
<td></td>
</tr>
<tr>
<td>Localization</td>
<td>CPut, NAc, ICj, OT</td>
</tr>
<tr>
<td>Pharmacology</td>
<td></td>
</tr>
<tr>
<td>Dopamine affinity (nM)</td>
<td>2000</td>
</tr>
<tr>
<td>Specific agonist</td>
<td>SKF 38393 (3)</td>
</tr>
<tr>
<td>Specific antagonist</td>
<td>SCH 23390 (4)</td>
</tr>
<tr>
<td>Biochemistry</td>
<td></td>
</tr>
<tr>
<td>G-protein-coupled</td>
<td>yes</td>
</tr>
<tr>
<td>cAMP</td>
<td>↑</td>
</tr>
<tr>
<td>IP₃</td>
<td>↑</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>↑</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>?</td>
</tr>
<tr>
<td>Dopamine release</td>
<td>?</td>
</tr>
<tr>
<td>Mitogenesis</td>
<td>?</td>
</tr>
<tr>
<td>Acidification</td>
<td>?</td>
</tr>
</tbody>
</table>

ₐSequence homology in TM domains, expressed as percentages. ᵇBased on rat brain mRNA distribution data.
Abbreviations: CPut, caudate putamen; NAc, nucleus accumbens; ICj, islands of Calleja; OT, olfactory tubercle; Hipp, hippocampus; Thal, thalamus; Pit, pituitary; SN, substantia nigra; VTA, ventral tegmental area; Sept, septum; Cer, cerebellum; FC, frontal cortex; Amyg, amygdala; Mes, mesencephalon; MO, medulla oblongata (taken from ref. 176).
Values in the presence of a guanyl nucleotide (taken from ref. 354). ᵈFor chemical structures, see Chart 1.3.
Abbriviations: cAMP, cyclic adenosine monophosphate; IP₃, inositol triphosphate; ↑, increase; ↓, decrease; –, no effect; ?, unknown (taken from ref. 355).
mammalian cell lines can be transiently or permanently transfected with cDNAs encoding the different dopamine receptor subtypes. Because these cells usually express a single receptor subtype in high density, they are very suitable for determining receptor binding affinities of drug candidates. Since most newly synthesized target compounds presented in the subsequent chapters of this thesis have been evaluated for their ability to bind to cloned human dopamine D<sub>2</sub> and D<sub>3</sub> receptors, the characteristics of these two receptor subtypes will be described in more detail in the next sections. For reviews and references on the other dopamine receptor subtypes, see refs. 176, 180, 362, and 387.

### 1.3.3 Dopamine D<sub>2</sub> Receptors

In 1988, Bunzow and coworkers identified and cloned the gene encoding the rat dopamine D<sub>2</sub> receptor, by applying a cloning strategy which was based on the presumed structural homology between different GPCRs.\(^{53}\) Thus, initially using the DNA sequence encoding the hamster β<sub>2</sub>-adrenergic receptor as a hybridization probe, in order to screen a rat genomic library for the coding sequences of other GPCRs, they ultimately were able to isolate the cDNA encoding a protein of 415 amino acids with the characteristics of a GPCR: the protein contained seven hydrophobic domains, a number of amino acids which are highly conserved among a large number of GPCRs, potential asparagine-linked glycosylation and phosphorylation sites in the N-terminal region, and a significant sequence homology with other GPCRs. Analysis of its mRNA distribution in the rat brain revealed high abundancies in brain areas classically associated with dopaminergic neurotransmission, including the striatum, nucleus accumbens, olfactory tubercle, pituitary, substantia nigra and ventral tegmental area. Expression of the receptor in different mammalian cell lines allowed for the establishment of the ligand specificity and the functional coupling of the receptor to effector systems. The receptor was shown to bind classical dopamine D<sub>2</sub> receptor antagonist like spiperone, (+)-butaclamol, haloperidol and (–)-sulpiride with high affinity and selectivity, and appeared to mediate the inhibition of adenylate cyclase activity, as well as prolactin secretion. Taken together, these findings strongly suggest that the cloned protein indeed corresponds to the classical dopamine D<sub>2</sub> (for review and references see ref. 138).

A human homologue of the rat dopamine D<sub>2</sub> receptor gene was cloned by the same research group in 1989 from human pituitary tissue.\(^{139}\) The protein encoded by the gene was 443 amino acids in length and showed an overall sequence similarity with the rat dopamine D<sub>2</sub> receptor of approximately 96%. The difference in length between the two species isoforms was shown to be the result of alternative splicing of pro-mRNA during the process of gene expression. Thus, several research groups have reported the existence of two splice variants of the dopamine D<sub>2</sub> receptor in rats\(^{66,84,129,264,286,307}\) and humans.\(^{84,139,343,361}\) In both species, the two isoforms differ by a stretch of 29 amino acid in the putative third cytoplasmatic loop. Both human isoforms are one amino acid shorter than their rat homologues. The long isoforms have been termed D<sub>2A</sub> [alternative nomenclature: D<sub>2L</sub>, D<sub>444</sub> (rat), or D<sub>443</sub> (human)], while the short isoforms have been termed D<sub>2B</sub> [alternative nomenclature: D<sub>2S</sub>, D<sub>415</sub> (rat), or D<sub>414</sub> (human)].

The biological significance of the existence of two isoforms of the dopamine D<sub>2</sub> receptor is unclear. All brain regions expressing dopamine D<sub>2</sub> receptors that have been investigated thus far
seem to express both splice variants, although their relative abundance in different brain regions is variable. In general, the short isoform is the least abundant of the two. Receptor binding studies have shown that the two isoforms cannot be pharmacologically distinguished, although some compounds seem to have some preference for the dopamine D$_{2B}$ receptor. Since the two isoforms differ only in the length of the putative third cytoplasmatic loop, differences in coupling to second messenger systems may be expected (for review and references, see ref. 106).

Virtually all compounds previously designated to be selective for the dopamine D$_2$ receptor also bind with high affinity to the dopamine D$_1$ receptor (see below). Therefore, in order to be able to further investigate the functional role of the dopamine D$_2$ receptor, there is currently a strong need for selective dopamine D$_2$ receptor agonists and antagonists.

Expression of cloned dopamine D$_2$ receptors in various cell lines has revealed that they utilize different signal transduction systems, presumably via coupling to different G-proteins (for reviews see refs. 176 and 362). In all cellular environments inhibition of adenylate cyclase has been detected,$^1,2,7,15,23,19,22,23,1,24,0,27,5,3,8$ but cell-specific signaling pathways may be present as well. Thus, dependent on the type of cells which express the receptors, stimulation of dopamine D$_2$ receptors, in addition to inhibition of intracellular cAMP production, may result in: (1) enhancement of phosphoinositide (PI) hydrolysis by activation of the enzyme phospholipase C$_c$,$^{23,1,3,8}$ (2) an increase,$^{15,3,2,3,1,3,8}$ or decrease$^{3,8}$ in the intracellular Ca$^{2+}$ concentration; (3) opening of K$^+$ channels,$^{3,8}$ and (4) extracellular release of arachidonic acid.$^{19,2,3,0,0}$

**Chart 1.3** Chemical structures of SKF 38393 (3), SCH 23390 (4), N-0923 (5), raclopride (6), PD 128907 (7), S 14297 (8), PD 168077 (9), and L-745,870 (10).
1.3.4 DOPAMINE D₃ RECEPTORS

In 1990, the gene encoding a novel dopamine receptor, termed D₃, was cloned by French researchers. The human dopamine D₃ receptor comprises a glycoprotein of 400 amino acid residues and shows 50% sequence homology with the human dopamine D₂ receptor, or 77% when only the presumed TM domains are considered. In addition, the two subtypes have more structural features in common, such as a long third intracellular loop, a short carboxylic acid terminal segment and several glycosylation sites (Table 1.1). Furthermore, in analogy with the dopamine D₂ receptor gene organization, the coding sequence of the dopamine D₃ receptor contains introns, which may give rise to the expression of splice variants encoded by the same gene. Various different splice variants indeed have been identified in mouse, rat, and human brain, but in most cases the structures of the encoded proteins made it unlikely that they comprise fully functional receptors.

The identification of 7-hydroxy-2-(N,N-di-n-propylamino)tetralin (7-OH-DPAT) as a selective dopamine D₃ receptor agonist allowed for the autoradiographic localization of the dopamine D₃ receptor in the brain. The overall abundance of the dopamine D₃ receptor turned out to be about two orders of magnitude lower, and more importantly, the regional distribution to be much more restricted than that of the dopamine D₂ receptor. Northern blot and in situ hybridization analyses confirmed high density expression of dopamine D₃ receptors predominantly in the olfactory tubercle/islands of Calleja complex, the anterior and shell parts of the nucleus accumbens, the bed nucleus of the stria terminalis, the mammillary nuclei of the hypothalamus, and the geniculate nuclei of the thalamus. The expression of the dopamine D₃ receptor being restricted to these phylogenetically old brain areas, referred to as the limbic system, suggests an important role of the dopamine D₃ receptor in the control of cognitive, emotional and reward processes, and hence as a major target for antipsychotic drug action. High densities of dopamine D₃ receptors were also identified in the cerebellum, but the physiological function of this cerebellar expression is unclear. The identification of low levels of dopamine D₃ receptor expression in the substantia nigra pars compacta and the ventral tegmental area suggest that this receptor subtype may function as an autoreceptor.

The pharmacological profile of the dopamine D₃ receptor is similar, but not identical to that of the dopamine D₂ receptor, and supports a possible role as an autoreceptor. Thus, all dopamine D₂ receptor agonists and antagonists bind with good affinities to dopamine D₃ receptors as well, but some compounds, previously designated as putative dopamine D₂ autoreceptor agonists (e.g. 7-OH-DPAT) or antagonists (e.g. (−)-AJ 76 and (−)-UH 232), show preference for the dopamine D₃ receptor. Remarkably, in comparison with the other dopamine receptor subtypes, the dopamine D₃ receptor displays an exceptional high affinity for dopamine itself (Table 1.1), the significance of which is unclear. In addition, various selective dopamine D₃ receptor agonists (e.g. PD 128907) and antagonists (see Section 1.6.1) have become available during the last few years, allowing to further study the functional role of the dopamine D₃ receptor.

Whereas the signal transduction pathways of the dopamine D₂ receptor have been unraveled to a large extent, the biochemistry of the dopamine D₃ receptor is much less clear. Initial studies failed to demonstrate any coupling to G-proteins. Binding of agonists to dopamine D₃ receptors, expressed in various cell types, was not or only weakly affected by guanide nucleotides, and no second messenger...
generation was observed. Later studies, however, revealed functional coupling of dopamine \( D_3 \) receptors to different transduction mechanisms in various cell lines. Thus, dopamine \( D_3 \) receptor-mediated inhibition of cAMP production,\(^{67}\) aggregation of melanophore pigment,\(^{303}\) acidification of the extracellular environment,\(^{315}\) and inhibition of \( Ca^{2+} \) currents\(^{331}\) have been reported. Nevertheless, the magnitudes of the effects seem to be consistently lower than the corresponding dopamine \( D_2 \) receptor-mediated effects. The relationships between these \textit{in vitro} observations and the functional role of the dopamine \( D_3 \) receptor \textit{in vivo} remain to be established (for reviews on the dopamine \( D_3 \) receptor, see refs. 330, 353, and 354).

1.4 SEROTONIN RECEPTORS

1.4.1 SEROTONIN RECEPTOR CLASSIFICATION

In analogy with dopamine and other neurotransmitter substances, serotonin interacts with its own specific receptors after its release from serotonergic neurons. Gaddum and Picarelli in 1957 were the first to distinguish between two types of serotonin receptors, which they termed D and M.\(^{125}\) Receptors of the D-type mediated the serotonin-induced contraction of smooth muscle, which could be blocked by dibenzyline. M-type receptors mediated the serotonin-induced release of acetylcholine from postganglionic nerve terminals, which could be blocked by morphine. A new classification, based on radioligand binding studies, was proposed by Peroutka and Snyder in the late 1970’s: receptors labeled by \([\text{^3H}]\)-5-HT were classified as 5-HT\(_1\), while those labeled by \([\text{^3H}]\)-spiperone were termed 5-HT\(_2\).\(^{290}\) It was soon realized that both classification schemes were incomplete, but also complemented each other. Therefore, in 1986 a group of scientists decided to combine the two classification schemes, taking into account rank order and affinities for agonists and antagonists, and to some extent the second messenger systems involved. Thus, the different serotonin receptor subtypes were grouped in three main classes, designated ‘5-HT\(_1\)-like’ (corresponding to some D receptors and 5-HT\(_1\) binding sites), 5-HT\(_2\) (corresponding to most D receptors and 5-HT\(_2\) binding sites), and 5-HT\(_3\) (equivalent to M receptors).\(^{46}\) This classification scheme has formed the basis for the classification scheme of serotonin receptors currently in use, and which is based on a combination of operational, transductional and structural considerations (for reviews, see refs. 165, 166, and 325).

Application of modern molecular biology techniques have revealed the existence of at least seven classes of serotonin receptors (5-HT\(_{1-7}\)) to date, and some of these consist of several subtypes, adding up to a total of fourteen serotonin receptor subtypes. Some of these have been identified as gene products only, and therefore are denoted in small characters. The serotonin 5-HT\(_1\) class comprises the subtypes 5-HT\(_{1A}\), 5-HT\(_{1B}\), 5-HT\(_{1D}\), 5-HT\(_{1E}\), and 5-HT\(_{1F}\). The non-rodent form of the serotonin 5-HT\(_{1B}\) receptor has previously been referred to as 5-HT\(_{1Db}\), while the serotonin 5-HT\(_{1D}\) receptor has been formerly termed 5-HT\(_{1Da}\). The serotonin 5-HT\(_2\) class consists of subtypes 5-HT\(_{2A}\), 5-HT\(_{2B}\) (previously named 5-HT\(_{2F}\)), and 5-HT\(_{2C}\). The serotonin 5-HT\(_{2C}\) receptor has been formerly referred to as the serotonin 5-HT\(_{1C}\) receptor, but based on clear operational and transductional similarities with the 5-HT\(_{2A}\) receptor, it was later assigned to the 5-HT\(_2\) class. The serotonin 5-HT\(_5\) class comprises the subtypes 5-HT\(_{5A}\) and 5-HT\(_{5B}\). The serotonin 5-HT\(_3\) receptor is a ligand-gated ion
channel, all other serotonin receptor subtypes belong to the superfamily of G-protein-coupled receptors. The most important features of the serotonin receptors have been summarized in Table 1.2. Since all of the compounds disclosed in this thesis have been evaluated for their affinities at the serotonin 5-HT\textsubscript{1A} receptor, the characteristics of this subtype will be addressed in more detail.

<table>
<thead>
<tr>
<th>Table 1.2</th>
<th>Summary of the characteristics of the currently identified serotonin receptor subtypes.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptor</td>
<td>Subtype</td>
</tr>
<tr>
<td>5-HT(_1)</td>
<td>5-HT(_1)A</td>
</tr>
<tr>
<td>5-HT(_1)B</td>
<td>SN, GP</td>
</tr>
<tr>
<td>5-HT(_1)D</td>
<td>Hipp, OT</td>
</tr>
<tr>
<td>5-ht(_1)E</td>
<td>CPut</td>
</tr>
<tr>
<td>5-ht(_1)F</td>
<td>Hipp, FC</td>
</tr>
<tr>
<td>5-HT(_2)</td>
<td>5-HT(_2)A</td>
</tr>
<tr>
<td>5-HT(_2)B</td>
<td>not in CNS</td>
</tr>
<tr>
<td>5-HT(_2)C</td>
<td>SN</td>
</tr>
<tr>
<td>5-HT(_3)</td>
<td>–</td>
</tr>
<tr>
<td>5-HT(_4)</td>
<td>–</td>
</tr>
<tr>
<td>5-ht(_5)</td>
<td>5-ht(_5)A</td>
</tr>
<tr>
<td>5-ht(_5)B</td>
<td>Hipp</td>
</tr>
<tr>
<td>5-ht(_6)</td>
<td>–</td>
</tr>
<tr>
<td>5-HT(_7)</td>
<td>–</td>
</tr>
</tbody>
</table>

\(^a\)Abbreviations: Hipp, hippocampus; RNu, raphe nuclei; SN, substantia nigra; GP, globus pallidus; OT, olfactory tubercle; Hyp, hypothalamus; Thal, thalamus. \(^b\)For chemical structures, see Chart 1.4. \(^c\)Abbreviations: cAMP, cyclic adenosine monophosphate; IP\(_3\), inositol triphosphate; DAG, diacyl glycerol; ↓, decrease; ↑, increase; i, intracellular. \(^d\)No selective ligand available.
Chemical structures of 8-OH-DPAT (11), WAY 100,635 (12), CP 93129 (13), SB 224289 (14), L-694,247 (15), GR 127935 (16), LY 334370 (17), DOI (18), ketanserin (19), BW 723C86 (20), and SB 204741 (21).
Chapter 1

**Chart 1.4** (continued) Chemical structures of Ro 60-0175 (22), SB 242084 (23), m-CPBG (24), MDL 72222 (25), cisapride (26), GR 113808 (27), 5-CT (28), and SB 258719 (29).

1.4.2 Serotonin 5-HT$_{1A}$ Receptors

The heterogeneousity of the 5-HT$_1$ class of serotonin receptors, as defined by Bradley and co-workers, was demonstrated by Pedigo et al., who showed that a subclass of the serotonin 5-HT$_1$ receptors had relatively high affinity for spiperone. These sites were termed 5-HT$_{1A}$, while the sites with lower affinity for spiperone were named 5-HT$_{1B}$. The simultaneous identification of 8-hydroxy-2-(N,N-di-n-propylamino)tetralin (8-OH-DPAT, 3) as a highly potent and selective serotonin 5-HT$_{1A}$ receptor agonist turned out to be a major breakthrough in the characterization of the serotonin 5-HT$_{1A}$ receptor. Autoradiography studies with [³H]-8-OH-DPAT in the rat and human brain revealed high densities of serotonin 5-HT$_{1A}$ receptors in the dentate gyrus of the hippocampus (CA1 region), the lateral septum, the entorhinal and frontal cortex, and the amygdala, brain areas which are associated with mood control. Stimulation of these postsynaptic...
receptors in rats by selective serotonin 5-HT_{1A} receptor agonists induces several aspects of a behavioural pattern called the ‘5-HT syndrome’, characterized by hyperlocomotion, lower lip retraction, flat body posture, Straub tail, hindlimb abduction, reciprocal forepaw treading or ‘piano playing’, wet dog shakes, and head weaving.\textsuperscript{32,379,380} In addition to these postsynaptic receptors, serotonin 5-HT_{1A} receptors have also been found on the cell bodies of the serotonergic neurons in the median and dorsal raphe nuclei. Stimulation of these somatodendritic autoreceptors inhibits neuronal cell firing and release of serotonin onto the postsynaptic receptors.\textsuperscript{98}

Serotonin 5-HT_{1A} receptors are generally considered to be important targets for the treatment of mood disorders like anxiety and depression. An overactivity of the serotonergic system is thought to be responsible for developing anxiety. Therefore, stimulation of somatodendritic serotonin 5-HT_{1A} autoreceptors may prove to be a suitable therapy against this disease.\textsuperscript{148} Depressions on the other hand seem to be related to a reduced serotonergic activity,\textsuperscript{38} and may be treated by stimulating postsynaptic serotonin 5-HT_{1A} receptors with selective agonists.\textsuperscript{276}

In 1987 Kobilka and co-workers cloned a gene encoding a protein belonging to the family of GPCRs.\textsuperscript{201} The identity of the receptor encoded by the gene was resolved a year later and shown to correspond to the human 5-HT_{1A} receptor.\textsuperscript{110} The protein consisted of 421 amino acids and seemed to have many structural features in common with other GPCRs. In 1993, Chanda \textit{et al.} reported that the Kobilka sequence lacked one amino acid.\textsuperscript{64} Two different amino acid sequences of the rat serotonin 5-HT_{1A} receptor have also been reported. Both are constituted of 422 amino acids, but the sequences differ at one position.\textsuperscript{4,120} Thus, the human and rat serotonin 5-HT_{1A} receptor are equal in length and have an overall sequence identity of 89\%, or 99\% when only the putative TMs are considered.

Similar to the dopamine D_{2} receptor, the serotonin 5-HT_{1A} receptor contains several asparagine-linked glycosylation sites in the extracellular N-terminus region, as well as potential sites for phosphorylation and palmitoylation have been identified in the intracellular loops. There is general agreement that stimulation of serotonin 5-HT_{1A} receptors is predominantly associated with a decrease in intracellular cAMP production both \textit{in vitro} and \textit{in vivo}, mediated through an inhibitory effect on adenylate cyclase.\textsuperscript{87,88,111,149}

Due to the early recognition of 8-OH-DPAT as a selective serotonin 5-HT_{1A} receptor agonist, this serotonin receptor subtype is the best studied to date. Since its discovery, a number of potent and selective agonists (\textit{e.g.} flesinoxan) and antagonists (\textit{e.g.} WAY 100,635), belonging to various chemical classes, have become available.\textsuperscript{131,144,273,308} However, the recent notification that 8-OH-DPAT also has considerable affinity for the serotonin 5-HT_{7} receptor suggests that some of its activities, which have previously been attributed to its effects on serotonin 5-HT_{1A} receptors, may be mediated via stimulation of serotonin 5-HT_{7} receptors.\textsuperscript{102} Since it is quite well possible that new serotonin receptor subtypes will be identified in the future,\textsuperscript{295} it is therefore essential that the development of new selective serotonin 5-HT receptor ligands is continued.
1.5 CLASSICAL VERSUS ATYPICAL ANTIpsychotic AGENTS: BENEFICIAL VERSUS SIDE-EFFECTS

In 1950, Charpentier at Rhône-Poulenc synthesized the phenothiazine derivative encoded 4560RP, later to be known as chlorpromazine. Searching for adjuvants in surgical anesthesia, the surgeon Laborit tested chlorpromazine and noted that the compound induced ataraxia (indifference). Soon it was realized that chlorpromazine might be used for treatment of psychiatric disorders. The first clinical tests were performed on manic patients, and moderate responses were observed. In 1952, Deniker and co-workers reported the results of a trial with 38 schizophrenic patients who had been resistant to all existing therapies. All patients showed considerable improvement of schizophrenic symptoms. The serendipitous discovery of chlorpromazine as an antipsychotic agent proved to be a milestone in the pharmacotherapeutic treatment of schizophrenia, and has laid the foundation for the dopamine hypothesis of schizophrenia. Since then, numerous compounds with antipsychotic efficacy have been developed and have become available for the treatment of schizophrenia and related disorders.

1.5.1 CHEMICAL CLASSIFICATION OF Antipsychotic AGENTS

Several classification schemes for the currently clinically available antipsychotic agents have been proposed. Compounds may be classified based on chemical structure, pharmacological profiles, potency and nonneurologic side-effect profile, or clinical efficacy and neurologic side-effect liability. A chemical classification is chosen here. However, this section is not intended to provide a detailed review on the structure-activity relationships of the currently available antipsychotic agents, but rather to give an impression of the chemical diversity of the available compounds. Most antipsychotic agents which are currently in use in the Netherlands belong to one of five main chemical classes: (1) phenothiazines; (2) thioxanthenes; (3) butyrophenones; (4) diphenylbutylpiperidines, and (5) benzamides (for reviews and references, see refs. 163, 168, 218, 274, and 296).

Phenothiazines were the first antipsychotic drugs to become available, and are exemplified by the prototypical compound of this class, chlorpromazine (30). Large series of analogues of 30 have been made since its discovery, and these fall into three subclasses of phenothiazine derivatives, based on

![Chemical structures of chlorpromazine (30), fluphenazine (31), thioridazine (32) and chlorprotixene (33).](chart1.5)

**Chart 1.5** Chemical structures of chlorpromazine (30), fluphenazine (31), thioridazine (32) and chlorprotixene (33).
the different alkyl side chains: compounds with an aliphatic side chain, as in 30; compounds with a piperazine side chain, exemplified by fluphenazine (31); compounds with a piperidine side chain, as present in thioridazine (32). The antipsychotic activity of phenothiazine derivatives is dependent on the nature of the side chain, the basic amine, and the aromatic substituents. Derivatives with a piperazine side chain are generally the most potent. Regardless of the type of side chain, a three-carbon chain between the phenothiazine nitrogen atom and the side chain nitrogen atom is crucial for optimal activity. The basic nitrogen atom requires three substituents for optimal potency. The presence of an electron-withdrawing substituent at the 2-position of the phenothiazine nucleus is also essential for high potency. Phenothiazine derivatives are characterized by high affinities for dopamine D1, D2, and D3, serotonin 5-HT2, α1-adrenergic, histamine H1, and muscarinic receptors, the ratio of the affinities being dependent on the type of side chain.

Thioxanthenes (e.g. chlorprothixene, 33) are structurally related to phenothiazines, and their SARs with respect to requirements for side chains, basic nitrogen, and aromatic substituents are similar, but thioxanthenes are generally less potent than their phenothiazine analogues. The presence of the double bond in the side chain infers the existence of geometric isomers, referred to as cis and trans. In the cis isomers, which are more potent than the trans isomers, the side chain is directed towards the aromatic 2-substituent.

In the early 1950’s a synthesis program was set up by Paul Janssen and co-workers at the laboratories of Janssen Pharmaceutica, aimed at the development of analgesics more potent than morphine. A number of compounds emerging from this program showed chlorpromazine-like effects in addition to analgetic activity. Structural optimization of the antipsychotic-like activity resulted in the synthesis of haloperidol (34, Chart 1.6) in 1958. Since its discovery, over 5,000 structurally related analogues of 34 have been synthesized and pharmacologically evaluated. Nevertheless, 34 proved to be one of the best compounds in terms of activity and toxicity, and still is the most prescribed antipsychotic agent to date. Virtually all structural modifications to the para-fluorobutyrophenone side chain lead to a decrease in antipsychotic activity. Thus, the para-fluor substituent, the carbonyl moiety, and an unbranched propylene chain connecting the aryl group and

![Chemical structures of haloperidol (34), spiperone (35), and pimozide (36).](chart1.6.png)
the basic nitrogen are essential for high potency. The structural requirements for the basic amine seem to be less stringent, although maximum activity is usually obtained when the basic nitrogen atom is incorporated in a ring system. In case of a piperidine ring, like in 34, the presence of a substituent at the piperidine 4-position is beneficial for the antipsychotic activity. This substituent does not necessarily have to be aromatic in nature, as demonstrated by the spirocarbocyclic analogue spiperone (35), a derivative with an extremely high affinity for dopamine D₂ receptors. Radiolabeled spiperone is frequently used as a radioligand in dopamine D₂ receptor binding studies. In general, butyrophenones like haloperidol are relatively selective for dopamine D₂ receptors, but most of them also display high affinities for serotonin 5-HT₂ and α₁-adrenergic receptors.

Pimozide (36) is the best-known representative of the class of diphenylbutylpiperidines. These compounds may be considered as structural analogues of the butyrophenones, in which the carbonyl oxygen atom has been replaced by a phenyl ring, and as such, the SARs of the two classes of compounds are fairly similar. Diphenylbutylpiperidines are characterized by a relatively high affinity for dopamine D₂ receptors, a strong antipsychotic potency and a long duration of action.

The interesting pharmacological and clinical profile of the benzamide derivative sulphiride (37) prompted researchers at Astra Läkemedel (presently Astra Arcus) to search for analogues of 37 with higher oral bioavailability and brain penetration capabilities, two important characteristics of a CNS drug which 37 lacks due to its relatively low lipophilicity. In order to achieve this goal they introduced halogen atoms and a second flanking alkoxy group at the benzamide nucleus. Remoxipride (38) was one of the most promising compounds that emerged from this project. It has been clinically available during a short period, and showed a superior antipsychotic profile compared to standard therapies. Substituted benzedrines possess a unique pharmacological profile: they bind to dopamine D₂ and D₃ receptors only. Radiolabeled raclopride (6, Chart 1.3) is frequently employed as a radioligand in dopamine D₂ and D₃ receptor binding and PET studies. The antidopaminergic properties of this class of benzedrines have been shown to reside predominantly in the (S)-enantiomers. Benzamides with different types of side chain have been reported, and they have different structural requirements for high activity. Thus, benzamides with 4-piperidinyl side chains,

![Chemical structures of sulpiride (37), remoxipride (38), clebopride (39), and nemonapride (40).](image-url)
such as clebopride (39), and with 3-pyrrolidinyl side chains, such as nemonapride (40), are also potent dopamine D2 receptor antagonists, provided that they bear a large lipophilic N-substituent.

A few compounds are available which do not belong to any of the aforementioned chemical classes, the most important representative being the dibenzodiazepine derivative clozapine (41). In addition, several compounds of different chemical classes, including risperidone (42), olanzapine (43), and sertindole (44), have recently been launched on the market. The pharmacological and clinical profiles of these compounds will be addressed in more detail in Sections 1.5.3. and 1.6.

![Chemical structures of clozapine (41), risperidone (42), olanzapine (43), and sertindole (44).](chart1.8)

**1.5.2 CLASSICAL ANTIPLATFORMIC AGENTS: THE NEUROLEPTICS**

The observation by Carlsson and Lindqvist in 1963 that chlorpromazine and haloperidol affected the dopaminergic and noradrenergic neurotransmission in the CNS not only laid the basis for the dopamine hypothesis of schizophrenia\(^{56}\) (see Section 1.2.1), but also initiated the search for a common site of action of antipsychotic agents. The development of the radioligand binding technique in the early 1970’s allowed for the measurement of the affinities of drugs for specific binding sites. In 1976, Creese et al.\(^{78}\) and Seeman et al.\(^{342}\) independently reported that a highly significant correlation existed between the affinity of antipsychotic agents for central dopamine receptors in vitro and their clinical potency. After the identification of two distinct subtypes of dopamine receptors,\(^{396}\) it was soon generally accepted that the dopamine D2 receptor was the principal target for antipsychotic activity.\(^{337}\) As a consequence, development of new antipsychotic drugs during the last 30 years has been primarily focused on compounds which potently block dopamine D2 receptors.

Blockade of dopamine D2 receptors in the mesolimbic and mesocortical dopaminergic (A10) system of the CNS is generally believed to be the mechanism of action of the classical antipsychotic
agents. Chronic treatment with antipsychotic agents presumably leads to a so-called ‘depolarization block’ of dopaminergic neurons projecting to the limbic and cortical areas, which prevents firing of these cells (for review and references, see ref. 285). Classical antipsychotic agents are frequently referred to as ‘neuroleptics’, a term which reflects their capabilities to ‘take control of the neurons’. However, whereas blockade of dopamine D_2 receptors in the A10 system probably is responsible for the antipsychotic effects, simultaneous blockade of dopamine D_2 receptors in other brain areas may give rise to the induction of serious side-effects (for reviews and references, see refs. 24 and 293).

Antagonism of postsynaptic dopamine D_2 receptors in the nigrostriatal dopaminergic system (A9) leads to a functional deficiency of dopamine in the basal ganglia, which results in the occurrence of typical motor side-effects, referred to as extrapyramidal side-effects (EPS). Acute EPS include parkinsonism, dystonia, akathisia, and dyskinesia. Neuroleptic-induced parkinsonism (secondary parkinsonism) is characterized by hypo- or bradykinesia (reduction in amplitude and velocity of voluntary movements), muscular rigidity and tremor. These symptoms closely resemble those of Parkinson’s disease (idiopathic parkinsonism), a neurodegenerative disorder caused by a deficiency of dopamine in the striatum as a result of the degeneration of the nigrostriatal dopaminergic projections. Acute dystonia is characterized by sustained muscle contractions which may result in repetitive movements or abnormal postures. Acute akathisia consists of a feeling of restlessness and compulsion to move the limbs, and this side-effect is the least tolerable by the patients. Acute dyskinesia comprises the involuntary movements of the limbs and orofacial musculature, the latter being characterized by protrusion or twisting of the tongue, smacking, chewing, pursing and sucking movements of the lips, puffing of the cheeks, and lateral jaw movements. These acute EPS, which have been estimated to occur in 10–50% of all patients, are usually observed within days after initiation of the treatment, and disappear soon after termination of the treatment. Upon chronic antipsychotic drug treatment, however, more persistent, so-called ‘tardive’ forms of EPS may become apparent. The tardive EPS tend to develop in a later phase or even after treatment, and, as opposed to acute EPS, dose reduction or discontinuation of drug treatment does not provide immediate relief but usually tends to worsen the symptoms. Of the tardive EPS, tardive dystonia (~2% incidence) and tardive dyskinesia (TD, ~15% incidence) seem to be the most severe, since they are frequently irreversible. Whereas the acute occurrence of acute EPS seems to be accounted for by the acute blockade of dopamine D_2 receptors in the nigrostriatal system, the mechanisms causing the tardive EPS are less well understood. The most plausible theory at the moment seems to be the ‘dopamine D_1/D_2 imbalance hypothesis’ proposed by Gerlach et al. According to this hypothesis, blockade of postsynaptic dopamine D_2 receptors in the nigrostriatal system at treatment onset leads to acute EPS. Simultaneous blockade of presynaptic dopamine D_2 receptors causes an increase in synthesis and release of dopamine, which stimulates the unoccupied postsynaptic dopamine D_1 receptors. A resulting supersensitivity of the dopamine D_1 receptors, due to relative overstimulation, in combination with dysfunctional dopamine D_2 receptors, should then account for the occurrence of tardive EPS.

Secretion of the hormone prolactin from the pituitary is inhibited by dopamine. Treatment with antipsychotic agents therefore frequently results in hyperprolactinaemia, as blockade of dopamine D_2 receptors by the antipsychotic agents prevents dopamine to properly regulate the prolactin secretion.
Hyperprolactinaemia can lead to gynaecomastia (breast growth in man), galactorrhea (increased milk production), amenorrhea (no menstruation), and loss of sexual drive.

In addition to these dopamine D$_2$ receptor-mediated side-effects, most antipsychotic agents produce a number of other side-effects, the extent of which is related to their receptor binding profiles. Thus, blockade of muscarinic cholinergic receptors leads to autonomic side-effects, including dry mouth, blurred vision, constipation, urinary retention, orthostatic hypotension, and tachycardia. Blockade of muscarinic receptors, however, also has antiparkinsonian effects. Therefore, antipsychotic agents with high affinity for muscarinic receptors, such as chlorpromazine and thioridazine, usually show a lower incidence of EPS. Antipsychotic agents with high affinity for histamine H$_1$ receptors have strong sedative effects, experienced by the patients as feelings of slowness, lethargy and weakness. In the majority of patients these effects are undesirable, but in agitated, excited patients they may be beneficial. Blockade of $\alpha_1$-adrenergic receptors also contributes to sedation, and in addition, may cause orthostatic hypotension. Taken together, phenothiazine derivatives, by virtue of their strong muscarinic, $\alpha_1$-adrenergic and histamine H$_1$ receptor blocking properties, are very sedative, often induce autonomic side-effects, but have a relatively low EPS liability. Thioxanthenes derivatives generally have somewhat lower affinities at histamine H$_1$ and muscarinic Therefore, they are less sedative than phenothiazine derivatives, but have a higher risk of inducing EPS. Butyrophenones and diphenylbutylpiperidines lack significant affinity for muscarinic and histamine H$_1$ receptors, and therefore hardly induce autonomic side-effects, but due to their high affinity for dopamine D$_2$ receptors, they have a strong potential to induce EPS.

Besides these receptor binding-related side-effects, classical antipsychotic agents can induce various nonspecific side-effects which seem not to be related to their receptor binding properties. Of these side-effects, the neuroleptic malignant syndrome (NMS) and agranulocytosis are the most serious, since they are potentially lethal. NMS occurs usually within a few days after initiation of neuroleptic treatment, and is characterized by muscular rigidity, hyperpyrexia (high fever), autonomic dysregulation, and delirium. Incidence estimates range from 0.02% to 2.4%. The mortality rate has been estimated to be 20–30%. Agranulocytosis is characterized by a suppression of the production of white blood cells, which makes a patient very susceptible for infections. Phenothiazines have a reputation for inducing agranulocytosis, with an estimated incidence risk of 0.05%. The mechanisms which cause NMS and agranulocytosis are poorly understood. Other aspecific side-effects, which are frequently observed, but less threatening, are weight gain, dermatological reactions, ophthalmological reactions, and sexual dysfunction.

In addition to these side-effects, treatment of schizophrenia with classical antipsychotic agents has two other major drawbacks. First, virtually all neuroleptics only improve the positive symptoms of the disease in as much as 60–70% of the patients, while the negative symptoms are only marginally or not at all affected. Second, the large number of treatment-resistant patients not only undermines the dopamine hypothesis of schizophrenia (see Section 1.2.1), but also emphasizes the need for antipsychotics with an improved clinical efficacy.

1.5.3 Atypical Antipsychotic Agents
A few compounds seem to have an antipsychotic profile which is different from those of the classical antipsychotic agents. These so-called ‘atypical antipsychotics agents’ are characterized by a significantly lower incidence of EPS, sometimes combined with an improved antipsychotic efficacy. Clozapine (42) is generally considered to be the prototypical atypical antipsychotic agents, since its superior clinical efficacy in combination with its low EPS liability has not been matched by any other compound thus far. The definition of the term ‘atypical antipsychotic’ has been the subject of debate. From a preclinical perspective, an atypical antipsychotic agents should show a large dose separation in animal models with predictive value for antipsychotic activity (e.g. conditioned avoidance responding, inhibition of locomotor activity, prepulse inhibition) and models predicting EPS liability (e.g. catalepsy), whereas from a clinical perspective, an atypical antipsychotic agent should combine a superior antipsychotic activity and a low propensity to induce EPS. Ideally, an atypical antipsychotic agent should fulfill the following clinical requirements: (1) improvement of both positive and negative symptoms; (2) low acute and tardive EPS liability; (3) efficacy in therapy-resistant patients, and (4) no increase in plasma prolactin levels.

The history of clozapine is peculiar (for reviews see refs. 158, 250, and 358). The compound was synthesized in 1958 at Sandoz-Wander Ltd., as part of a research program devoted to the discovery of new tricyclic antidepressants. In animal studies it was soon noted that clozapine behaved more like an antipsychotic agent, but failed to induce catalepsy, an effect which at that time was generally accepted to be a prerequisite for any compound to show antipsychotic activity in man. Clinical trials started in the early 1960’s, and the results were encouraging. The compound was launched in a number of countries, and evidence for its superior profile started to accumulate. In 1975, however, shortly after its launch in Finland, 16 patients developed agranulocytosis as a results of treatment with clozapine, 8 of which were fatal. This resulted in the withdrawal of clozapine in several countries, while its use was restricted in many others. Nevertheless, a certain myth started to develop around clozapine during this period of restricted use, as it was consistently shown to possess a superior antipsychotic efficacy in comparison with classical compounds, to be effective in therapy-resistant patients, and to have a low propensity to induce acute and tardive EPS. These findings have been confirmed by several clinical trials performed in the 1980’s. Predominantly as a result of the multi-centre study by Kane and co-workers in 1988, the use of clozapine for treatment of neuroleptic-responsive and treatment-resistant patients has been re-approved in the USA and a number of European countries.

Whereas the superiority of clozapine in treatment-resistant patients is beyond doubt, its efficacy against negative symptoms is less evident. Results from several clinical trials suggest that clozapine indeed is superior to classical antipsychotic agents in the treatment of negative symptoms, but these effects may in fact be the result of the reduction in EPS and depression caused by the compound.

Despite almost 40 years of research, the mechanism of action responsible for the unique profile of clozapine is poorly understood. The compound has high affinities for a number of receptor subtypes (see Table 1.3), the most prominent being dopamine D₄, serotonin 5-HT₂, 5-HT⁶, and 5-HT⁷, cholinergic muscarinic, α₁- and α₂-adrenergic, and histamine H₁ receptors. In contrast to most classical antipsychotic agents, clozapine displays a relatively low affinity for dopamine D₂ receptors. Furthermore, PET studies have shown that clozapine occupies a significantly lower number of
central dopamine D2 receptors at therapeutic doses than classical antipsychotic agents.\textsuperscript{107-109} Moreover, whereas it has been generally accepted that antipsychotic agents exert their antipsychotic effect by blocking dopamine D2 receptors, observations from \textit{in vitro} and \textit{in vivo} studies suggest that clozapine may behave as a dopamine D2 agonist.\textsuperscript{175,241} In addition, clozapine has also been shown to possess intrinsic efficacy at muscarinic m4,\textsuperscript{411,414} and serotonin 5-HT1A receptors.\textsuperscript{314}

Although clozapine seems to be the ideal antipsychotic agent, therapy with this drug has one major drawback: in approximately 1–2% of all patients it causes agranulocytosis.\textsuperscript{207} Consequently, the blood of patients treated with clozapine has to be monitored for leukocytes on a regular basis. The formation of free radical metabolites has been proposed as the cause of this potential lethal blood discrasia.\textsuperscript{115} In addition, clozapine is strongly sedative, and may induce seizures, weight gain, orthostatic hypotension, hypersalivation and constipation.\textsuperscript{60}

Of the older antipsychotic agents, thioridazine (32), sulpiride (37) and remoxipride (38) have been categorized as atypical, since therapy with these compounds seems to be associated with a reduced incidence of EPS. The reduced EPS liability of thioridazine may be the result of its high affinity for muscarinic receptors. The low propensity of sulpiride and remoxipride is remarkable in view of their high selectivity for dopamine D2 receptors (Table 1.3), and re-emphasizes the hypothesis that dopamine D2 receptor blockade is the primary mode of antipsychotic drug action. Remoxipride was

<table>
<thead>
<tr>
<th>Table 1.3</th>
<th>Receptor binding affinities (K\textsubscript{i}, nM) of haloperidol and putative atypical antipsychotic agents.\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptor</td>
<td>Hal</td>
</tr>
<tr>
<td>D\textsubscript{1}</td>
<td>15</td>
</tr>
<tr>
<td>D\textsubscript{2L}</td>
<td>2.2</td>
</tr>
<tr>
<td>D\textsubscript{3}</td>
<td>7.8</td>
</tr>
<tr>
<td>D\textsubscript{4,2}</td>
<td>11</td>
</tr>
<tr>
<td>5-HT\textsubscript{1A}</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>5-HT\textsubscript{2A}</td>
<td>25</td>
</tr>
<tr>
<td>5-HT\textsubscript{3C}</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>5-HT\textsubscript{6}</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>5-HT\textsubscript{7}</td>
<td>380</td>
</tr>
<tr>
<td>AchM</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>(\alpha)\textsubscript{1}</td>
<td>19</td>
</tr>
<tr>
<td>(\alpha)\textsubscript{2}</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>(\beta)\textsubscript{1}</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>(\beta)\textsubscript{2}</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>H\textsubscript{1}</td>
<td>790</td>
</tr>
<tr>
<td>sigma\textsubscript{1}</td>
<td>1.1</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Data adapted from refs. 12, 204, 316, 317 and 329. Abbreviations: Hal, haloperidol; Cloz, clozapine; Remox, remoxipride; Risp, risperidone; Olanz, olanzapine; Sert, sertindole; Zipr, ziprasidone; Quet, quetiapine. \textsuperscript{b}No data available.
launched in 1990 but had to be withdrawn in 1994, due to the induction of aplastic anemia in 8 out of 45,000 patients. During the short period the remoxipride has been clinically available, the compound has been shown to improve both positive and negative symptoms, being at least as effective as haloperidol in both first-episode and severely psychotic patients. In addition, it can prevent relapse in chronic schizophrenics and was shown to be effective in one third of therapy-resistant patients. Moreover, remoxipride showed a minimal tendency to induce EPS and does not affect plasma prolactin levels. Remoxipride may have proven to be the most significant advance in antipsychotic therapy since the reappraisal of clozapine, as it meets virtually all the requirements of the ideal atypical antipsychotic agent, and it is therefore truly unfortunate that this compound is no longer clinically available. Possible mechanisms underlying the atypical properties of remoxipride, which may also hold true for sulpiride, have been suggested to be either a selective effect on a subpopulation of dopamine D$_2$ receptors, a preferential effect on subsets of functionally coupled dopamine D$_2$ receptors, or differential effects on subsystems in the corpus striatum. Like haloperidol, remoxipride also has considerable affinity for sigma binding sites in addition to dopamine D$_2$ and D$_3$ receptors. However, since clinical trials with selective sigma site antagonists have failed to shown that such compounds possess antipsychotic activity, this action of remoxipride is unlikely to contribute to its clinical profile.

1.6 Pharmacological Approaches for the Development of Potential Atypical Antipsychotic Agents

The considerations above make it clear that there is still need for the development of antipsychotic agents with an improved clinical profile, i.e. antipsychotic agents which combine the superior antipsychotic activity and low neurological side-effect liability of clozapine, but also lack its potential to induce agranulocytosis. Furthermore, such compounds may help to better understand the exact mode of action of antipsychotic agents and to unravel the etiology of schizophrenia and related disorders. Since it has been generally accepted that a dopamine D$_2$ antagonistic component is required for antipsychotic activity, most pharmacological approaches which are currently under investigation rely on the development of compounds which interfere to some extent with dopamine D$_2$-like receptors. An atypical antipsychotic profile may possibly be achieved with compounds which selectively block a subtype of dopamine D$_2$-like receptors (i.e. D$_3$ or D$_4$), compounds which possess some intrinsic activity at dopamine D$_2$ receptors (i.e. dopamine D$_2$ autoreceptor agonists or partial dopamine D$_2$ receptor agonists), or by compounds which, in addition to dopamine D$_2$ receptors, also bind to specific serotonin receptor subtypes (i.e. serotonin 5-HT$_2$ or 5-HT$_{1A}$). These different pharmacological approaches will be addressed in more detail in the subsequent sections.

1.6.1 Selective Dopamine D$_3$ Receptor Antagonists

The rationale for the development of selective dopamine D$_3$ receptor antagonists as potential atypical antipsychotic agents is predominantly based on two observations. First, the specific regional distribution of the dopamine D$_3$ receptor in the CNS suggests that this receptor subtype may be an interesting target for antipsychotic drug action (for reviews, see refs. 130, 330, 350, 352, and 354). After the identification of the dopamine D$_3$ receptor as a new dopamine receptor subtype,
localization studies have shown that dopamine D₃ receptors are predominantly expressed in limbic structures, including the olfactory tubercle/Islands of Calleja complex, the mammilary nuclei of the hypothalamus, the amygdala, the bed nucleus of the stria terminalis, the nucleus accumbens, the septum and the hippocampus. In contrast, the expression of the dopamine D₂ receptor in the midbrain is more restricted to areas which are associated with motor functions. This raised the idea that selective dopamine D₃ receptor antagonists should alleviate psychotic symptoms by blocking dopamine D₃ receptors in the limbic areas, while at the same time leaving the dopamine D₂ receptors in the extrapyramidal areas unaffected, and hence be devoid of EPS. Second, molecular genetic studies have suggested that an association may exist between the occurrence of schizophrenia and the gene encoding the dopamine D₃ receptor (for reviews, see refs. 199, 267, and 268).

Encouraged by the identification of the dopamine D₃ receptor as a potential target for atypical antipsychotic agents, much effort has been devoted to the development of selective dopamine D₃ receptor antagonists during the last years, and various selective compounds have emerged (for review see ref. 374). (+)-AJ 76 (45, Chart 1.9) and (+)-UH 232 (46), two compound previously designated as selective dopamine D₂ autoreceptor antagonists, were the first compounds shown to have some preference (Kᵢ ratio D₂/D₃ of 3.0 and 4.4, respectively) for the dopamine D₃ receptor. More selective agents are exemplified by S 14297 (8, Chart 1.3, 23-fold selective), which is structurally closely related to the selective dopamine D₃ receptor agonist (R)-(+)–7-OH–DPAT, the 6-substituted 2-aminotetralin derivative GR 218231 (47, 400-fold selective), and the benzamide-related compounds nafadotride (48, 10-fold selective) and GR 103691 (49, 125-fold selective). It should be noted however, that the claimed selectivity ratios are dependent on the radioligand employed in the dopamine D₂ binding assays. Despite the availability of various compounds, no

**Chart 1.9**  Chemical structures of the selective dopamine D₃ receptor antagonists (+)-AJ 76 (45), (+)-UH 232 (46), GR 218231 (47), nafadotride (48), and GR 103691 (49).
selective dopamine D₃ receptor antagonist has been evaluated against schizophrenia in clinical trials thus far, presumably due to the lack of knowledge concerning the functional role of the dopamine D₃ receptor *in vivo*.

### 1.6.2 Selective Dopamine D₄ Receptor Antagonists

The hypothesis that selective dopamine D₄ receptor antagonists might have potential as atypical antipsychotic agents has predominately been founded on the observation that clozapine possesses a relatively high affinity for the dopamine D₄ receptor.³⁸⁸ Subsequently, based in this presumed selectivity, Seeman and co-workers have proposed a concept of dopamine D₄/D₂ receptor occupancy of atypical antipsychotic drug action. Their hypothesis was based on analyses of the so-called ‘radioligand-independent’ affinities of a number of classical and atypical antipsychotic agents for dopamine D₂ receptors, and estimations of free plasma concentrations of these compounds at therapeutic doses. Clozapine, at therapeutic plasma concentrations of 10–20 nM, would primarily occupy dopamine D₄ receptors, while other antipsychotic agents would primarily occupy dopamine D₂ receptors.³³⁸,³⁴⁰ The hypothesis was based on clozapine having an affinity of 10 nM for the dopamine D₄ receptor. However, significantly lower affinities, and hence selectivity ratios for clozapine have been reported by others.⁶³,¹⁰¹,²¹₃,²¹⁷ In addition, whereas the affinity of clozapine for dopamine D₂ receptors is generally considered to be relatively low (~150 nM), Malmberg *et al.* reported an affinity for the cloned dopamine D₂B receptor of 35 nM, again suggesting that clozapine may not be as selective for dopamine D₄ receptors as originally claimed.²⁴¹ Moreover, the affinities of clozapine for serotonin 5-HT₆, 5-HT₇, muscarinic, α₁- and α₂-adrenergic, and histamine H₁ receptors are all in the order of magnitude of the estimated therapeutic plasma levels (Table 1.3), making it equally likely that clozapine exerts its unique profile by interacting with one or more of these receptor subtypes.

A six-fold increase in the density of dopamine D₄ receptors in post-mortem brain tissue of schizophrenic patients was also claimed by Seeman and co-workers. Dopamine D₄ receptor densities were measured by determining the differences in *B*ₘₐₓ values for the binding of [³H]-nemonapride, which has high affinities for dopamine D₂, D₃, and D₄ receptors, and [³H]-raclopride, which has high affinities for dopamine D₂ and D₃ receptors only. Although similar results were reported by others using the same methodology,²⁶⁹ they should be taken with caution. For example, Assié *et al.* have reported an affinity of 4.5 nM of nemonapride for the serotonin 5-HT₁A receptor.¹⁶ Simultaneous labeling of this receptor subtype therefore may also have accounted for the observed differences in *B*ₘₐₓ values. Furthermore, the fact that substituted benzamides such as raclopride and nemonapride consistently label almost twice as much binding sites as the prototypical dopamine D₂ receptor ligand spiperone may also have contributed to the reported observations.³⁴¹ For more reliable assessments of dopamine D₄ receptor densities, a highly selective dopamine D₄ receptor antagonist (see below) should be employed as a radioligand in such studies.
In view of the larger difference in sequence homology between dopamine D₄ receptors on the one hand and dopamine D₂ and D₃ receptors on the other hand (Table 1.1), it is not surprising that a considerable number of highly selective dopamine D₄ receptor antagonists have become available recently (for reviews see refs. 228 and 374). The piperaziny lazaindole L-745,870 (50, Chart 1.10) was reported to have 2200-fold and >5,000-fold selectivity for dopamine D₄ over dopamine D₂ and D₃ receptors, respectively. The same researchers recently reported the structurally related morpholine derivative 51, with >1,000-fold and >2,000-fold selectivity over dopamine D₂ and D₃ receptors. The isoxazole 52, which was also prepared in the same lab, is an isomer of the highly selective L-741,742 (10, Chart 1.3), but is slightly less selective. The optical pure isochromane derivative U-101387 (53) was shown to possess high affinity for the dopamine D₄ receptors, while lacking significant affinity for dopamine D₁, D₂ and D₃, serotonin 5-HT₁A and 5-HT₂, and α₁- and α₂- adrenergic receptors. This compound is currently undergoing clinical trials. YM-43611 (54) is a derivative of nemonapride (40, Chart 1.7) with 110-fold D₄ selectivity and 10-fold D₃ preference over dopamine D₂ receptors. In addition, this benzamide was shown to have negligible affinities for a number of representative neurotransmitter receptors. Compound 55 was the most dopamine D₄-selective representative of a series of naphthoate esters, having a 1,260-fold selectivity over dopamine D₂ receptors. Furthermore, 55 lacked significant affinities for dopamine D₃ and serotonin 5-HT₁A, 5-HT₂A, and 5-HT₂C receptors.

![Chemical structures of the selective dopamine D₄ receptor antagonists L-741,870 (50), 51, 52, U-101387 (53), YM-43611 (54), and 55.](chart.jpg)
L-745,870 (50) has been evaluated for antipsychotic efficacy in schizophrenic patients. At a dose of 15 mg/day this compound was ineffective,49 suggesting that blockade of dopamine D₄ receptors alone is not sufficient for exerting antipsychotic effects. Nevertheless, such selective dopamine D₄ antagonists may be useful in unraveling the functional role of the dopamine D₄ receptor and the relationship it bears to the pathophysiology of schizophrenia.

### 1.6.3 Dopamine D₂ Autoreceptor Agonists and Partial Dopamine D₂ Receptor Agonists

Several lines of evidence indicate that compounds which possess intrinsic efficacy at dopamine D₂ receptors may have potential as atypical antipsychotic agents (for reviews see refs. 29 and 30). The indirect dopamine receptor agonists d-amphetamine62,134 and L-DOPA52,287 have been shown to improve negative symptoms in some schizophrenic patients when co-administered with traditional antipsychotic agents. Furthermore, the dopamine D₁/D₂ receptor agonist alone at low doses has been reported to improve certain aspects of schizophrenia.370 In preclinical neurochemical and behavioural models, apomorphine at low doses simultaneously inhibits dopamine synthesis and locomotor activity in rats. These effects have been ascribed to activation of dopamine D₂ autoreceptors, located presynaptically at the nerve terminals of the dopaminergic neurons. Stimulation of these receptors leads to a decrease in synthesis and release of dopamine, and hence provides a feedback mechanism for regulation of the amount of dopamine available for stimulation of postsynaptic receptors in the synaptic cleft. Therefore, selective stimulation of terminal dopamine D₂ autoreceptors by selective agents has been proposed as a mechanism for treatment of schizophrenia.278 In brain areas with dopaminergic hyperactivity, stimulation of terminal dopamine D₂ autoreceptors would normalize the dopaminergic neurotransmission and hence reduce psychotic symptoms. Beneficial effects against negative symptoms of schizophrenia may stem from the compound’s ability to stimulate postsynaptic dopamine D₂ receptors in areas with dopaminergic hypoactivity. Furthermore, some degree of intrinsic efficacy would also reduce the propensity to cause EPS by mild stimulation of postsynaptic dopamine D₂ receptors in the nigrostriatal system. This hypothesis of dopamine D₂ autoreceptor selectivity also implies that terminal dopamine D₂ autoreceptors and postsynaptic dopamine D₂ receptors represent two distinct pharmacological entities. However, Drukarch and Stoof showed that the two types of receptors display similar pharmacological characteristics, suggesting that they are identical.99 Nevertheless, several compounds with putative preferential action on dopamine D₂ autoreceptors have been developed during the last decade. Examples of such compounds are (S)-(−)-3-PPP (preclamol, 56),403 B-HT 920 (talipexole, 57),8 SND 919 (pramipexole, 58),328 EMD 49980 (roxindole, 59),44 and OPC-4392 (60).21

Detailed analyses of the pharmacological profiles of these compounds, however, has revealed that they merely act as partial dopamine D₂ receptor agonists with varying degrees of intrinsic efficacy at both presynaptic and postsynaptic dopamine D₂ receptors. Moreover, most of the compounds previously designated as putative dopamine D₂ autoreceptor agonists have been shown to bind preferentially to dopamine D₃ receptors, and a correlation between their dopamine D₃ receptor binding properties and their potency to regulate dopamine autoreceptor-mediated cell activity has been reported.206 Furthermore, the inhibitory effects of presumed selective dopamine D₂
autoreceptor agonists on locomotor activity in rodents are probably mediated by stimulation of postsynaptic dopamine D<sub>3</sub> receptors. Taken together, the effects of these compounds, which have been previously ascribed to their alleged preferential action on terminal dopamine D<sub>2</sub> autoreceptors, are probably the result of simultaneous stimulation of both pre- and postsynaptic dopamine D<sub>2</sub> and D<sub>3</sub> receptors. The combination of the degree of intrinsic efficacy and preference for the dopamine D<sub>3</sub> receptor presumably determines the overall profile of these compounds.

Preclamol, talipexole, pramipexole, and roxindole have been evaluated in small clinical trials for efficacy in schizophrenia. In a single-blind trial with four patients, preclamol showed antipsychotic activity in two subjects and was well tolerated. Talipexole was evaluated in twelve schizophrenics in an open trial: in only four subjects significant amelioration of positive symptoms was observed. Pramipexole has recently been shown to significantly improve both positive and negative symptoms when used in combination with haloperidol. In addition, the compound has proven to be of value in the treatment of Parkinson’s disease, and has recently become available for this indication. Roxindole has been reported to be ineffective in patients in which positive symptoms dominate, but in a subgroup of patients with predominantly negative symptoms, moderate to significant improvements were observed. These effects of roxindole on negative symptoms may be attributed to antidepressant properties of the compound: roxindole binds with high affinity to serotonin 5-HT<sub>1A</sub> receptors and inhibits the reuptake of serotonin. This antidepressant action was confirmed by an open clinical trial with patients suffering from major depression, in which roxindole proved to be very effective. In general, from these preliminary clinical data it can be concluded that the usefulness of putative selective dopamine D<sub>2</sub> autoreceptor agonists as atypical antipsychotic agents is limited.

### 1.6.4 Mixed Dopamine D<sub>2</sub>/Serotonin 5-HT<sub>2</sub> Receptor Antagonists

The implication of serotonin in the pathophysiology of schizophrenia has been addressed in Section 1.2.2. A renewed interest in serotonergic agents as potential antipsychotic agents started

![Chemical structures of the putative selective dopamine D<sub>2</sub> autoreceptor agonists](#)

**Chart 1.11** Chemical structures of the putative selective dopamine D<sub>2</sub> autoreceptor agonists (S)-(−)-3-PPP (56), B-HT 920 (57), SND 919 (58), EMD 49980 (59), and OPC-4392 (60).
when in 1984 clozapine was shown to act as a potent antagonists at serotonin receptors. Altar and co-workers in 1986 suggested that the serotonin 5-HT<sub>2</sub>/dopamine D<sub>2</sub> receptor affinity ratio might be the key to the unique profile of clozapine. Based on cluster analyses, performed on the receptor binding profiles of a number of classical and supposedly atypical antipsychotic agents, Meltzer and coworkers in 1989 hypothesized that compounds which combine 5-HT<sub>2</sub> receptor antagonism and dopamine D<sub>2</sub> receptor antagonism in an appropriate ratio (5-HT<sub>2</sub>/D<sub>2</sub> pK<sub>i</sub> ratio ≥ 1.12) should possess atypical antipsychotic properties. This hypothesis should thus account for the superior clinical profile of clozapine. Measurement of receptor occupancy in rat brain revealed that a number of putative atypical antipsychotic agents preferably occupy serotonin 5-HT<sub>2</sub> receptors in vivo. Furthermore, PET studies in schizophrenics have shown that clozapine displays a very high serotonin 5-HT<sub>2</sub> receptor occupancy, while occupation of dopamine D<sub>2</sub> receptors by clozapine is consistently lower than by classical antipsychotic agents.

Support for a role of serotonin 5-HT<sub>2</sub> receptors in the pathophysiology of schizophrenia and in the mode of action of atypical antipsychotic agents also comes from clinical studies with the partial serotonin 5-HT<sub>2</sub> agonist m-chlorophenylpiperazine (MCPP). In unmedicated schizophrenic patients MCPP has been shown to exacerbate psychotic symptoms, but in healthy volunteers it does not induce symptoms resembling psychosis. In addition, the increase in plasma levels of prolactin, growth hormone and cortisol induced by MCPP in schizophrenic patients can be blocked by clozapine. Remarkably, patients who benefited most from treatment with clozapine were also those in which MCPP induced the strongest endocrine response when drug-free. The observation that fluphenazine, which predominantly blocks dopamine D<sub>2</sub> receptors, was unable to counteract the MCPP responses, supports the hypothesis that the effects of clozapine in these studies are mediated via blockade of serotonin 5-HT<sub>2</sub> receptors.

Substantial evidence also comes from clinical studies with the selective serotonin 5-HT<sub>2</sub> receptor antagonist ritanserin. When added to the regular antipsychotic medication of chronic schizophrenics with severe EPS, ritanserin markedly reduced EPS in these subjects. Reyntjens and co-workers also reported significant improvement of EPS and negative symptoms by ritanserin in an add-on trial in chronic schizophrenics. In another add-on study ritanserin proved to be particularly effective against negative symptoms. Finally, Wiesel et al. reported that, in an open trial, ritanserin alone proved to be effective against both positive and negative symptoms, without exacerbating EPS. The latter findings suggest that blockade of serotonin 5-HT<sub>2</sub> receptors alone might be sufficient for exerting an atypical antipsychotic profile. However, in order to validate this hypothesis, double-blind clinical trials are required.

Particularly the findings with ritanserin have stimulated the development of new potential atypical antipsychotic agents based on the concept of mixed dopamine D<sub>2</sub>/serotonin 5-HT<sub>2</sub> receptor antagonism during the last decade (for reviews see refs. 92, 171, 234, 254, and 327). Risperidone was the first compound developed on the basis of this concept, and was launched on the market in 1994. Risperidone has higher affinity for serotonin 5-HT<sub>2</sub> receptors than for dopamine D<sub>2</sub> receptors (Table 1.3). In addition, it also possesses high affinities for dopamine D<sub>3</sub>, serotonin 5-HT<sub>7</sub>, and α<sub>1</sub>- and α<sub>2</sub>-adrenergic receptors. In contrast to clozapine, risperidone lacks high affinities for muscarinic and histamine receptors. In several open and double-blind clinical trials, risperidone has been shown to be effective against both positive and negative symptoms of schizophrenia, being
Introduction

superior to haloperidol and at least as effective as clozapine. Risperidone produces maximal efficacy and less EPS than classical antipsychotic agents at doses between 4–8 mg/day. At larger doses, however, the compound may induce marked EPS. As risperidone treatment does not seem to be associated with agranulocytosis, it may prove to be a valuable alternative for clozapine. However, the efficacy of risperidone in treatment-refractory patients remains to be established (for reviews and references, see refs. 39, 41, 143, and 258). Side-effects associated with risperidone treatment are mild sedation, orthostatic hypotension, prolactin increase and weight gain.  

Following risperidone, several new putatively atypical antipsychotic agents based on the concept of mixed dopamine D₂/serotonin 5-HT₂ antagonism have been developed. Two of these, olanzapine (43) and sertindole (44), have recently become clinically available. Olanzapine has strong structural resemblance to clozapine, and their receptor binding profiles are also similar (Table 1.3), but olanzapine has generally somewhat higher receptor affinities than clozapine. Therefore, olanzapine should be effective at lower doses, which should reduce the risk of aspecific side-effects such as agranulocytosis. Clinical trials have revealed that olanzapine is equally effective as haloperidol against positive and significantly more effective against negative symptoms, has a reduced incidence risk of EPS, and does not elevate plasma prolactin levels. 28,39 Olanzapine also seems to be effective in treatment-resistant patients. 245 Results of a recent double-blind multi-center comparison study between risperidone and olanzapine suggested that the latter has a superior clinical profile. 378 The superiority of olanzapine to clozapine remains to be established since these compounds have not been compared in clinical trials thus far. Olanzapine is mildly sedative, may cause orthostatic hypotension, and tends to induce weight gain. 60 

Sertindole is structurally unrelated to any of the currently available antipsychotic agents. It has particularly high affinities for dopamine D₂, serotonin 5-HT₂ and α₁-adrenergic receptors. 261 In several clinical trials, sertindole was shown to be equally effective as haloperidol against positive and significantly more effective against negative symptoms. 39,369 Sertindole’s efficacy in treatment-resistant patients, as well as its relative efficacy compared to clozapine remain to be established. Prolongation of the QT interval, decreased ejaculatory volume, nasal congestion and weight gain have been frequently reported as side-effects of sertindole treatment. 60 

Ziprasidone (CP-88,059, 61) and quetiapine (seroquel, ICI 204,636, 62) are putative atypical antipsychotic agents, which have been developed based on the concept of mixed dopamine D₂/serotonin 5-HT₂ receptor antagonism, and are on the verge of becoming clinically available. Ziprasidone has some structural features in common with risperidone, and their receptor binding

![Chemical structures of the mixed dopamine D₂/serotonin 5-HT₂ receptor antagonists ziprasidone (61) and quetiapine (62).]
profiles are also similar (Table 1.3). Unlike risperidone, however, ziprasidone also has high affinity for serotonin 5-HT$_{1A}$ receptors, which may be beneficial for its EPS liability (see Section 1.6.5). In clinical trials ziprasidone was shown to be effective against both positive and negative symptoms of schizophrenia, and to have antidepressant and anxiolytic properties. Furthermore, it seems to have a low tendency to induce EPS and other side-effects. Its efficacy in treatment-resistant patients remains to be established. Quetiapine has structural resemblance with clozapine and like clozapine it has a broad receptor binding profile, but the affinities are generally lower (Table 1.3). Quetiapine displays relatively high affinities for histamine H$_1$, $\alpha_1$-adrenergic, and serotonin 5-HT$_2$ receptors. The outcome of several clinical trials with quetiapine in schizophrenic patients has revealed that it has an antipsychotic efficacy comparable to haloperidol, but with a more favorable side-effect profile.

In summary, the clinical results obtained thus far with risperidone, olanzapine, sertindole, ziprasidone and quetiapine strongly suggest that the concept of mixed dopamine D$_2$/serotonin 5-HT$_2$ receptor antagonism, with a predominant occupancy of serotonin 5-HT$_2$ receptors, has proven to be successful for the development of antipsychotic agents with an improved efficacy and side-effect profile. Nevertheless, none of the compounds has proven to be superior to clozapine thus far.

### 1.6.5 Mixed Dopamine D$_2$ Receptor Antagonists/Serotonin 5-HT$_{1A}$ Receptor Agonists

The outcome of both preclinical and clinical investigations suggest that the serotonin 5-HT$_{1A}$ receptor may prove to be an interesting target for improving antipsychotic therapy. In preclinical behavioural and neurochemical models selective serotonin 5-HT$_{1A}$ receptor agonists have been shown to interact with antipsychotic agents. For example, several selective serotonin 5-HT$_{1A}$ receptor agonists, including 8-OH-DPAT, flesinoxan, buspirone, ipsapirone and gepirone, have consistently been shown to reverse catalepsy induced by dopamine D$_2$ receptor antagonists, such as haloperidol and raclopride, in rats and monkeys. Furthermore, 8-OH-DPAT has been shown to possess antipsychotic-like properties and to enhance the antipsychotic properties of raclopride in animals models with predictive value for antipsychotic activity. Catalepsy in animals has been generally accepted as a model for EPS in man. Thus, antipsychotic agents which potently induce catalepsy in animals show a high propensity to cause EPS in humans. Clozapine has only weak cataleptogenic properties at high doses and is even capable of reversing catalepsy induced by other antipsychotic agents, such as loxapine and olanzapine. In addition, whereas clozapine acts as an antagonist at virtually all receptor subtypes it binds to, it has been shown to act as a partial serotonin 5-HT$_{1A}$ receptor agonist. For example, Rollema et al. recently reported that the preferential increase in prefrontal cortical extracellular dopamine induced by clozapine could be partially blocked by the selective serotonin 5-HT$_{1A}$ receptor antagonist WAY 100,635, suggesting that clozapine exerts this action by stimulation of serotonin 5-HT$_{1A}$ receptors. Nevertheless, Bartoszyk et al. demonstrated that the anticataleptic properties of clozapine are not mediated by its action on serotonin 5-HT$_{1A}$ receptors.

Several clinical observations suggest a role for the serotonin 5-HT$_{1A}$ receptor in schizophrenia. First, post-mortem studies on the brains of schizophrenic patients have revealed increased densities of serotonin 5-HT$_{1A}$ receptors in the frontal cortex, which were unrelated to the medication history.
of the patients (see Section 1.2.2). Second, clinical studies with buspirone, a compound with mixed dopamine D₂ receptor antagonistic and partial serotonin 5-HT₁A receptor agonistic properties, suggest that it may have an atypical antipsychotic profile. Buspirone (63, Chart 1.13) has originally been developed and marketed as an anxiolytic agent. Early observations suggested that buspirone may also possess antipsychotic properties. When added to haloperidol in an open trial with schizophrenic patients, buspirone improved negative symptoms and EPS. Buspirone was also reported to reduce TD and EPS when given alone in an open trial. Furthermore, the compound has repetitively been reported to suppress akathisia induced by several neuroleptics, although worsening of movement disorders due to treatment with buspirone in a few patients has also been reported. Nevertheless, these results with buspirone suggest that it would be worthwhile to evaluate compounds with similar pharmacological profiles in preclinical models for antipsychotic activity and side-effect liability. Finally, it has been suggested that a serotonin 5-HT₁A receptor agonistic component may be beneficial in relieving the anxiety that can trigger psychotic episodes in schizophrenics.

In summary, these findings suggest that compounds which combine dopamine D₂ receptor antagonism with serotonin 5-HT₁A receptor agonism may have enhanced antipsychotic activity and a reduced EPS liability. However, the dopamine D₂/serotonin 5-HT₁A receptor affinity ratio, as well as the degrees of intrinsic efficacy at these receptor subtypes required for an optimal clinical profile need to be established. Nevertheless, several compounds with the indicated pharmacological profile have recently been disclosed (Chart 1.13). Mazapertine (RWJ-37796, 64) has high affinity for dopamine D₂, D₃, and serotonin 5-HT₁A receptors (Kᵢ values of 2.2, 1.8 and 1.7 nM, respectively) but also for α₁-adrenergic receptors (Kᵢ = 1.3 nM). At serotonin 5-HT₁A receptors, it behaves as a partial agonist. The chromane derivative EMD 128130 (65) has high affinities for dopamine D₂-like (D₂: Kᵢ = 20 nM) and serotonin 5-HT₁A (Kᵢ = 1 nM) receptors only, the activity residing in the (R)-enantiomer. EMD 128130 behaves in vivo as a dopamine D₂ receptor antagonist and a serotonin 5-HT₁A receptor agonist. PD 158771 (66) is the lead compound of a series of aminopyrimidine derivatives with high affinities for both dopamine D₂, D₃, and serotonin 5-HT₁A receptors (Kᵢ values of 5.2, 13.7 and 3.5 nM respectively). At both dopamine D₂ and serotonin 5-HT₁A receptors, 66 behaved as a partial agonist. Recently several compounds have been disclosed, which in addition to dopamine D₂ and serotonin 5-HT₁A receptors, also possess high affinity for serotonin 5-HT₃ receptors. The anthranilamide 1192U90 (67) is the lead compound of a series of substituted benzamides with such receptor binding profiles. Like ziprasidone it bears a 4-[(1,2-benzisothiazol-3-yl)-1-piperazine moiety, which presumably is responsible for the high serotonin 5-HT₂ receptor affinity. A similar receptor binding profile was reported for the structurally closely related thiazolidinone derivative P-9236 (HP-236, 68). Compounds 64–68 were all active in animal models with predictive value for antipsychotic activity (e.g. conditioned avoidance responding, inhibition of apomorphine-induced mouse climbing). Furthermore, evaluation in animal models with predictive value for EPS liability in man (e.g. catalepsy, inhibition of apomorphine-induced stereotyped behaviour) showed that they all are likely to have a low propensity to cause EPS. These results are promising and suggest that compounds with mixed dopamine D₂ receptor antagonistic and serotonin 5-HT₁A receptor agonistic properties may have potential as atypical antipsychotic agents. Mazapertine and 1192U90 are currently undergoing clinical trials.
The Department of Medicinal Chemistry at the University of Groningen has a history of more than 25 years in the design, synthesis, and pharmacological evaluation of drugs acting at the CNS. Research within the Department has been focused in particular on 2-aminotetralin-derived and structurally closely related compounds, such as octahydrobenzo[f]quinolines, hexahydronaphthoxazines and tetrahydrobenzopyranoxazines, with activity at dopaminergic, serotonergic or melatonergic receptors during the last 15 years (e.g. see refs. 23, 74, 93, 179 and 277). Examples of compounds that have been developed within the Department are the selective dopamine D$_3$ receptor agonists (R)-7-OH-DPAT$^{113}$ and PD 128907, the melatonin receptor agonist 8-methoxy-2-acetamidotetralin, and the dopamine D$_2$ receptor agonist N-0923, which is currently undergoing clinical trials for the treatment of Parkinson’s disease.

Encouraged by the promising concept of mixed dopamine D$_2$ receptor antagonism and serotonin 5-HT$_{1A}$ receptor agonism for the development of potential atypical antipsychotic agents, the idea was raised to design compounds with such a pharmacological profile using the 2-aminotetralin system as a structural base. This thesis is the result of that idea. In the subsequent chapters, the design,
synthesis, pharmacological evaluation, and molecular modeling aspects of a series of 2-aminotetralin-derived benzamides and structurally closely related compounds are described. It is the hope that the medicinal chemistry of the compounds disclosed in this thesis will contribute to a better understanding of the pathophysiology of schizophrenia and the mechanism of action of potential atypical antipsychotic agents.

1.8 References


Chapter 1


42

Chapter 1


Introduction


149 Harrington MA, Oksenberg D and Peroutka SJ (1988) 5-Hydroxytryptamine₁A receptors are linked to a Gₛ-adenylate cyclase complex in rat hippocampus. Eur J Pharmacol 154, 95–98.


Introduction


238 Lübbert H, Snutch TP, Dascal N, Lester HA and Davidson N (1987) Rat brain 5-HT\textsubscript{1C} receptors are encoded by a 5-6 kbase mRNA size class and are functionally expressed in injected Xenopus oocytes. J Neurosci \textbf{7}, 1159–1165.


Introduction


Introduction


Introduction

364 Sumiyoshi T, Stockmeier CA, Overholser JC, Dilley GE and Meltzer HY (1996) Serotonin_1A receptors are increased in postmortem prefrontal cortex in schizophrenia. Brain Res 708, 209–214.


Voigt MM, Laurie DJ, Seeburg PH and Bach A (1991) Molecular cloning and characterization of a rat brain cDNA encoding a 5-hydroxytryptamine\textsubscript{6} receptor. EMBO J 10, 4017–4023.


Weinshank RL, Zgombick JL, Branshek TA and Hartig PR (1992) Human serotonin\textsubscript{1D} receptor is encoded by a subfamily of two distinct genes: 5-HT\textsubscript{1Da} and 5-HT\textsubscript{1Db}. Proc Natl Acad Sci USA 89, 3630–3634.


Whitaker PM, Crow TJ and Ferrier IN (1981) Tritiated LSD binding in frontal cortex in schizophrenia. Arch Gen Psychiatry 38, 278–280.


