Synthesis, Pharmacological Characterization and QSAR Modelling of 4-Phenylpiperidines and 4-Phenylpiperazines

Effects on the dopaminergic neurotransmission *in vivo*

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Synthesis, Pharmacological Characterization and QSAR Modelling of 4-Phenyl-piperidines and 4-Phenylpiperazines - Effects on the dopaminergic neurotransmission *in vivo*

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In loving memory of my mother Inga-Maj

Abstract

The endogenous neurotransmitter dopamine (DA) is involved in several functions that are controlled from the central nervous system (CNS), for example behaviour, memory, cognition and reward. A disturbed dopaminergic neurotransmission may lead to many severe conditions, such as schizophrenia, attention deficit hyperactivity disorder (ADHD) or Parkinson's disease (PD). The dopamine receptors belong to the G-protein coupled receptors (GPCRs) and are divided into five distinct subtypes (D_1-D_5). These subtypes can be either of the D_1- or D_2-types based on their effect on the production of cyclic adenosine monophosphate (cAMP). The most common dopaminergic receptor used as target for pharmaceuticals is by far the D_2 receptor and drugs acting as full agonists, partial agonists and antagonists at this receptor have been developed.

In the search for new dopaminergic ligands, a set of 4-phenylpiperidines and 4-phenylpiperazines have been synthesized and their effects have been tested in both *in vivo* and *in vitro* assays. Starting with the known partial agonist 3-(1-benzylpiperidin-4-yl)phenol, stepwise structural modifications of functional groups afforded mainly D_2 antagonists but with a conserved preference for binding to the agonist binding site and fast dissociation rates from the receptor. However, further modifications, including changes of the position of the aromatic substituent, indicated that other targets than the D_2 receptor was involved and binding affinity studies later concluded that some of these compounds had MAO A inhibiting properties. In order to fully elucidate what structural properties are related to the different pharmacological responses, QSAR models with physicochemical descriptors set against each respective response were acquired by means of partial least square (PLS) regression. Models with high predictivity (Q^2>0.53) were obtained and the interpretation of these models has provided an improved understanding of how structural modifications in this chemical class affect the response both *in vivo* and *in vitro*. The structural motifs that were investigated included the position and physicochemical properties of the aromatic substituent as well as the heterocycle being a piperazine or a piperidine. All these properties turned out to be significant for the different responses in some aspect. In addition, a strong correlation between the affinities to the D_2 receptor and to MAO A and the levels of the metabolite DOPAC in striatum has been established. This led us to the conclusion that it is primarily interactions with these two targets that lead to the *in vivo* response observed for this class of compounds.

Keywords: dopamine, D2, monoamine oxidase, DOPAC, *in vivo*, QSAR, dopaminergic stabilizer

Papers included in the thesis

This thesis is based on the following publications and manuscripts:

I. **Synthesis and evaluation of a set of 4-phenylpiperidines and 4-phenylpiperazines as D_2 receptor ligands and the discovery of the dopaminergic stabilizer 4-[3-(methylsulfonyl)phenyl]-1-propylpiperidine (Huntexil, Pridopidine, ACR16).**
Pettersson F, Pontén H, Waters N, Waters S, Sonesson C.
J Med Chem. **2010** Mar 25; 53(6):2510-20.

II. **Synthesis and Evaluation of a Set of *para*-Substituted 4-Phenylpiperidines and 4-Phenylpiperazines as MAO Inhibitors.**
Pettersson F, Svensson P, Waters N, Waters S, Sonesson C.
J Med Chem. **2012** Apr 12;55(7):3242-9

III. **Synthesis, Pharmacological Evaluation and QSAR Modeling of mono-Substituted 4-Phenylpiperidines and 4-Phenylpiperazines**
Pettersson F, Svensson P, Waters N, Waters S, Sonesson C.
Eur J Med Chem. **2012**, Submitted

IV. **New quantum mechanically derived electronic principal properties of aromatic substituents.**
Sunesson Y, Norrby P. O., Pettersson F, Sonesson C and Svensson P.
Manuscript

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Contributions to the Papers

I. Planned and synthesized most of the included compounds; Extracted the rawdata and calculated the correlations; interpreted results and wrote the manuscript

II. Planned and synthesized most of the included compounds; Tabulated data from the rawdata and calculated the correlations and QSARs; interpreted results and wrote the manuscript

III. Planned and synthesized most of the included compounds; Tabulated data from the rawdata and calculated the correlations and QSAR; interpreted results and wrote the manuscript

IV. Provided the data set necessary for comparison; wrote parts of the manuscript, assisted with calculations of QSAR models; provided feed-back and managed the format

Contents

Abbreviations

3-MT	3-Methoxytyramine
5-HT	5-Hydroxytryptamine (serotonin)
COMT	Catechol-*O*-methyltransferase
DA	Dopamine
DOPAC	3,4-Dihydroxyphenylacetic acid
EDG	Electron donating group
EWG	Electron withdrawing group
GPCR	G-protein-coupled seven-transmembrane receptor
HA	Hydrogen bond acceptor
HD	Hydrogen bond donor
HVA	Homovanillic acid
HPLC	High performance liquid chromatography
IA	Intrinsic activity
K*i*	Binding affinity constant
LMA	Locomotor activity
NE	Norepinephrine
OPLS	(Orthogonal) partial least square
PD	Parkinson's disease.
QSAR	(Quantitative) structure-activity relationship
Vol_R,	Calculated volume.
π	Calculated hydrophobicity
σ_m	Hammett´s sigma meta
σ_p	Hammett´s sigma para
μR	Group dipole moment

1. INTRODUCTION

1.1 Monoaminergic Neurotransmitters

Neurotransmitters are a group of endogenous chemicals that transmit an impulse from a neuron to a target cell across a synaptic cleft. Neurotransmitters can be broadly split into two groups – the small molecule neurotransmitters and the relatively larger neuropeptide neurotransmitters. Within the category of small molecule neurotransmitters are the monoaminergic neurotransmitters, consisting of one amino group attached to an aromatic moiety by a two carbon chain. They are synthesized in the body from different amino acids (a.a.) and belong to specific subclasses depending on which a.a. they are derived from. The major monoamine subclasses active in the brain are the catecholamines and the tryptamines. Dopamine (DA, **1**, Figure 1) and norepinephrine (NE, **2**, Figure 1) belong to the catecholamines and serotonin (5-HT, **3**, Figure 1) belongs to the tryptamine class.

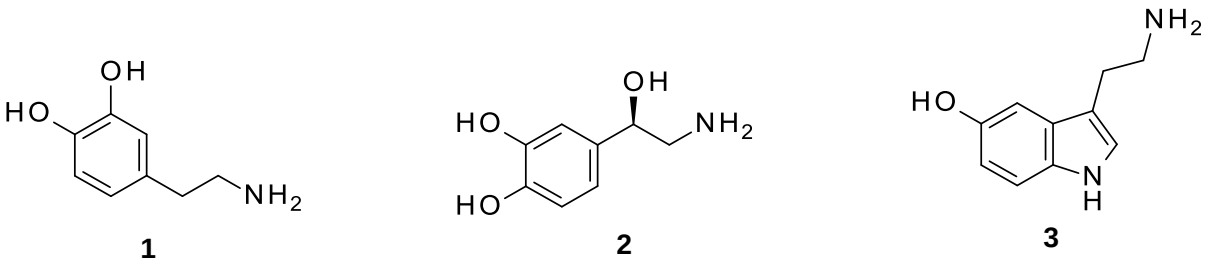


Figure 1. The monoamines: dopamine (**1**), norepinephrine (**2**) and serotonin/5-HT (**3**).

1.1.1 Catecholamine Synthesis and Catabolism. Since the catecholamines are unable to penetrate the blood-brain barrier (BBB), they have to be synthesised in the brain by specific enzymes (Figure 2). The precursor for catecholamine synthesis is tyrosine, an amino acid that is able to penetrate the BBB by a specific carrier. Tyrosine is oxidized to the catechol 3,4-dihydroxyphenylalanine (DOPA) by tyrosine hydroxylase and DOPA is then converted to dopamine (DA) by the enzyme aromatic L-amino acid decarboxylase. Hydroxylation of DA by dopamine β-hydroxylase produce Norepinephrine (NE) and *N*-methylation by phenylethanolamine *N*-methyltransferas leads to epinephrine (E). However, from here on the focus of this work will be limited to dopamine.

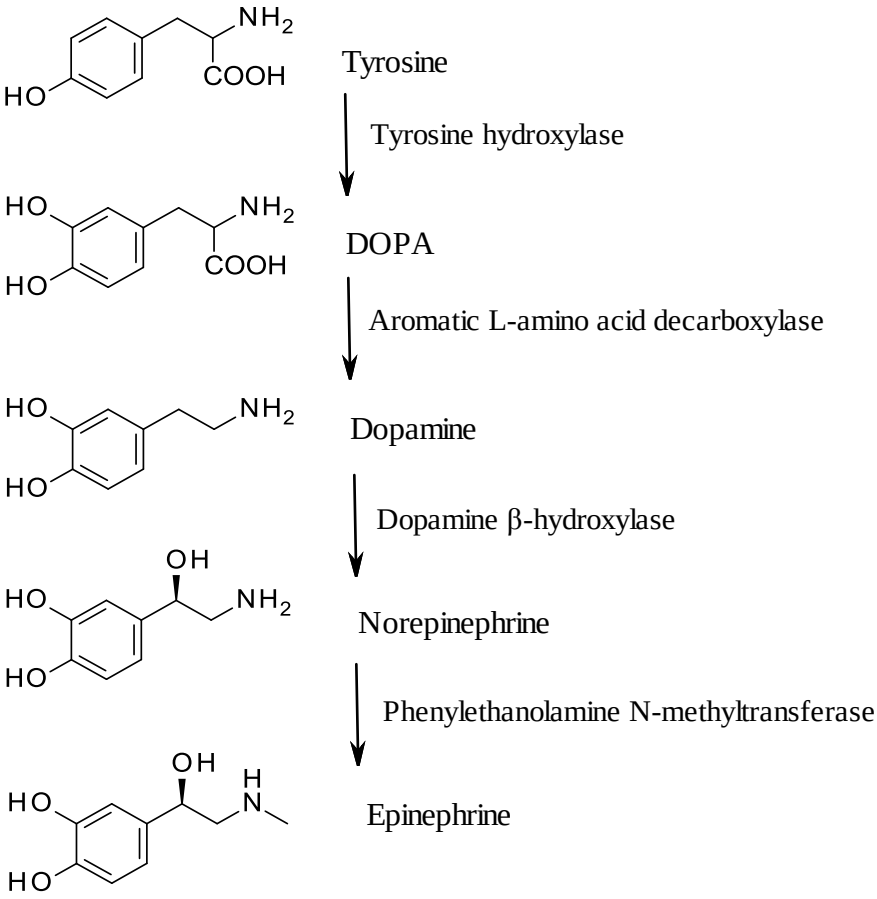


Figure 2. The synthesis of catecholamines

After being synthesized in the cytosol, dopamine is stored in presynaptic vesicles waiting for a signal. Neurotransmission occurs when an action potential causes the newly synthesised dopamine to be released into the synaptic cleft. There it activates post-synaptic receptors, which in turn propagate the signal further along the postsynaptic neuron. In addition, dopamine also affects pre-synaptic receptors, resulting in a feed-back control of the continued synthesis and release of neurotransmitters into the synaptic cleft (Figure 3).

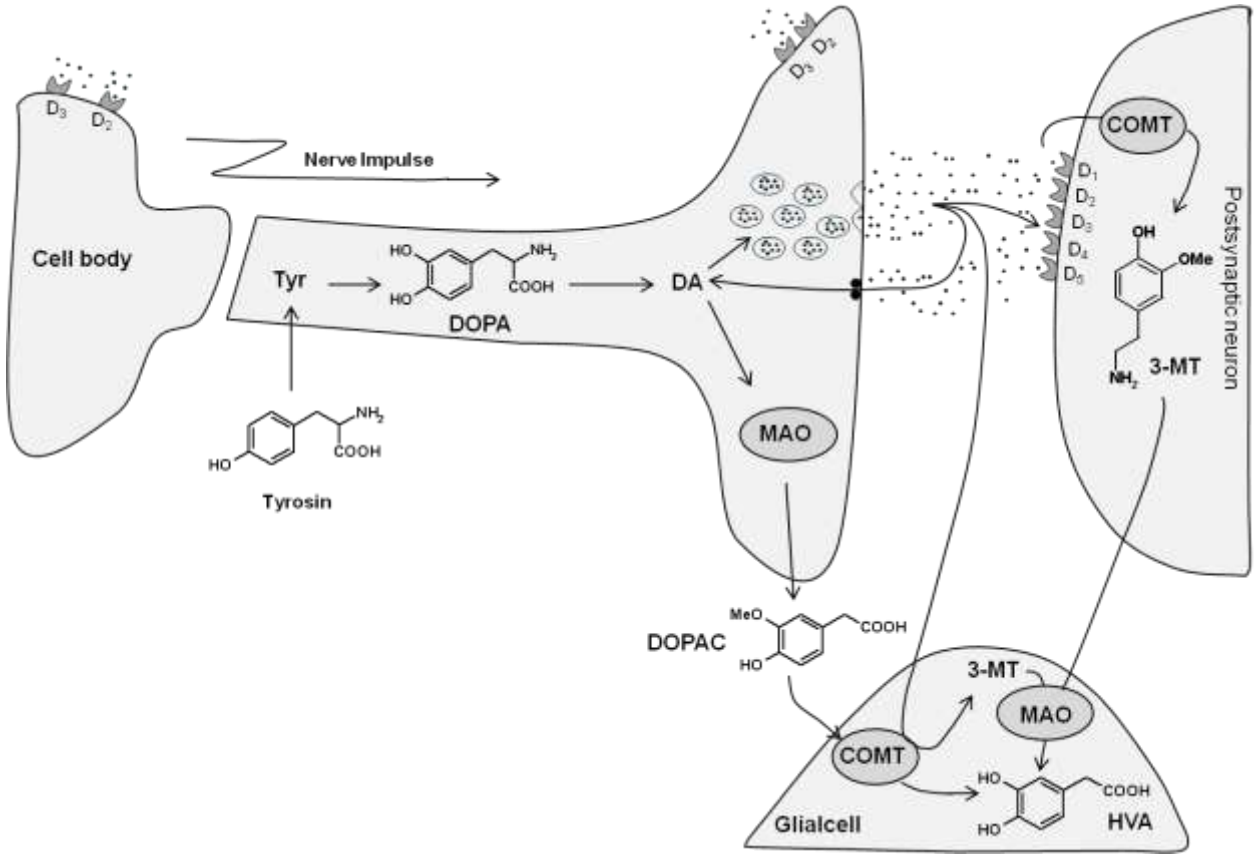


Figure 3. The dopamine neurone

After exerting its effects at the synapse, dopamine is cleared from the synaptic cleft by either reuptake or degradation; leading to a termination of the signalling. The degradation of dopamine in the brain is primarily mediated through two enzymes: monoamine oxidase (MAO) and catechol-*O*-methyl transferase (COMT). MAO metabolizes DA into 3,4-dihydroxyphenylacetaldehyde (DOPAL) which is further metabolized into 3,4-dihydroxyphenylacetic acid (DOPAC) by the enzyme aldehyde dehydrogenase (ALDH). COMT then methylates DOPAC to homovanillic acid (HVA), which is excreted via the urine. COMT is also able to directly metabolize dopamine, producing 3-methoxytyramine (3-MT) which in turn can be metabolized by MAO/ALDH into HVA (Figure 4).

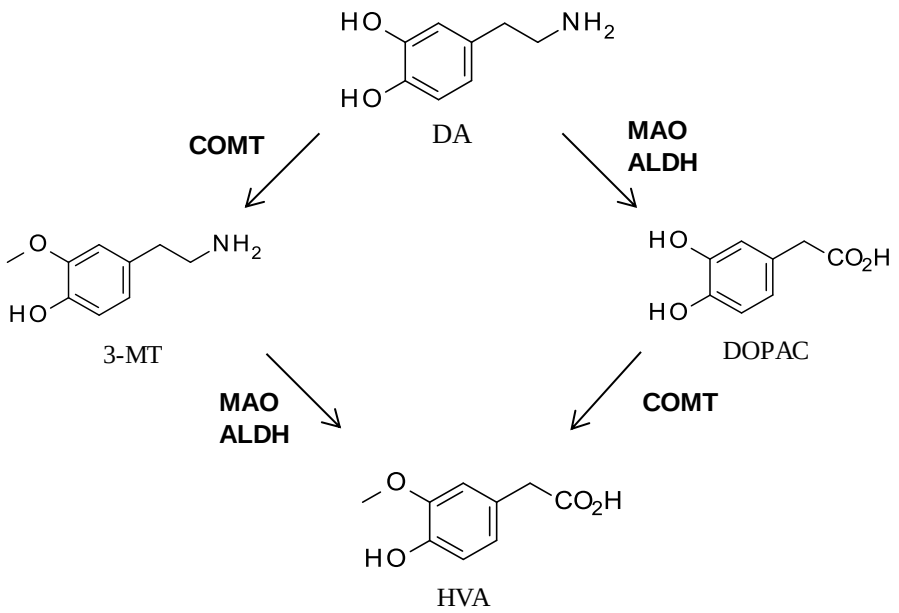


Figure 4. Metabolism of dopamine (DA) into 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxytyramine (3-MT) and homovanillic acid (HVA) by monoamine oxidase (MAO), aldehyde dehydrogenase (ALDH) and catechol-*O*-methyl transferase (COMT).

1.1.2. Monoamine Oxidases. There are two distinct types of MAOs, MAO A and MAO B, which share 70% amino acid sequence homology.[1-5] They are tightly bound to the outer membrane of the mitochondrion in the liver and in the brain.[6] Both MAO A and MAO B catalyze the oxidative deamination of 5-HT, DA and NA in the brain, albeit in rats this reaction is preferentially catalyzed by MAO A.[7, 8] Furthermore, MAO A is the isoform found primarily within dopaminergic nerve terminals[9] whereas MAO B is found mainly in striatal neurons and glial cells.[10] Thus, in rats it is mainly MAO A that affect the DA catabolism leading to production of the metabolite DOPAC and therefore MAO A inhibitors (e.g. clorgyline) reduces striatal DOPAC levels *in vivo*.[6, 11] In addition, when the MAO-mediated metabolism is blocked, more synaptic DA is metabolized by COMT to 3-MT and less 3-MT is metabolized to HVA by MAO (Figure 3 and 4), leading to a concomitant increase in 3-MT levels.

1.1.3. Dopamine Receptor Subtypes. In 1979, Kebabian *et al.* characterized two subtypes of the DA receptor as D_1 and D_2.[12] The location and function of these two receptors has since then been extensively investigated.[13-17] Even though there is some overlap in their distribution in the CNS, their pharmacological profiles are quite diverse. Both subtypes belong to the G-protein coupled

seven-transmembrane receptors (GPCRs), but where the D_1 receptor interacts with the G_s type protein, resulting in an activation of the adenylate cyclase enzyme and subsequent increased production of cyclic adenosine monophosphate (cAMP), the D_2 receptor instead interacts with the G_i complex, rendering an inhibition of the cAMP production. More recently, three additional subtypes of the DA receptor have been characterized, namely D_3, D_4 and D_5. Based on their amino acid sequences and structural similarities, D_5 has been identified as a D_1-like receptor,[18] while D_3 and D_4 have been classified as D_2-like.[13, 16, 17, 19, 20] Sequencing has shown 75% similarity between the transmembrane regions of D_2 and D_3 while a corresponding number for D_2/D_4 is 53%.[21] However, even though the homology is high, studies on their respective distribution and function have revealed some substantial diversity between the different subtypes.[21] This is also reflected in the respective *in vivo* responses of subtype specific compounds. For example, D_3 agonists induce hypoactivity in rats at doses where the synthesis and release of DA is unaffected, providing evidence that D_3 function mainly a postsynaptic receptor.[22-25] The role of the D_3 and D_4 receptors in neuropsychiatric and neurological conditions have been studied extensively, and while D_3 is claimed to be involved in several different brain disorders (e.g. schizophrenia, substance abuse etc.), the D_4 receptor holds less promise as a drug target in this area.[21, 26, 27]

1.1.4. The Dopamine D_2 Receptor

Dopamine type 2 receptors (D_2) are mainly located in the structure of the mammalian brain known as the basal ganglia, but are also present in other areas, for example the cortex. Dopamine in the brain exerts its action by means of synaptic as well as extrasynaptic release, affecting postsynaptic, presynaptic and dendritic D_2 receptor populations. DA acts as a high-affinity neurotransmitter at the D_2 receptor allowing for low concentration tonic signalling of the dopaminergic system. In addition, the system can respond to short surges of DA evoked by event-related firing of the dopaminergic neurons.[21] Two isoforms of the D_2 receptor are generated by differential splicing of the same gene and have been termed D_2S (D_2-short) and D_2L (D_2-long).[28, 29] These two alternatively spliced isoforms differ in the third intracellular loop (i.e. by the presence of 29 additional amino acids in D_2L), causing some diversity in their anatomical, physiological, signaling, and pharmacological properties. D_2S has been shown to be more densely expressed presynaptically and to be more involved in the autoreceptor functions, whereas D_2L seems to be the main isoform

postsynaptically.[30, 31] Presynaptic autoreceptors provide a negative feedback system that controls firing, synthesis and release of DA in response to extracellular neurotransmitter levels.[32-34] Besides the different splice isoforms, the D_2 receptor population can be distributed between two "activity-states"; either a resting, low-affinity state (D_2^{Low}) or a catalytically active, high-affinity state (D_2^{High}) in which DA binds with higher affinity.[20, 35]

1.2. Clinical Aspects of Dopaminergic Drugs

DA was first recognized in 1958 by Arvid Carlsson and Nils-Åke Hillarp at the Laboratory for Chemical Pharmacology of the National Heart Institute of Sweden.[36] Carlsson *et al*. demonstrated that reserpine depleted the levels of DA in the brain and that subsequent injection of L-DOPA restored these levels.[37] Furthermore, reserpine was discovered to induce catalepsy in both rabbit and cat, and administration of L-DOPA gave an acute reversal of the said symptoms. These findings subsequently led to the theory of DA's role in the control of motor functions and possible involvement in the pathophysiology of Parkinson's disease (PD)[38], a theory that was soon proven correct (Ehringer *et al*.[39]).

Since the initial discovery of DA's presence in the brain, a great deal of effort has been made to investigate how DA affects the CNS, in the normal state as well as in disrupted systems. For example, the role of DA in the reward system has been extensively studied in order to understand addiction and finding suitable drugs to treat such disorders.[40-43]

1.2.1. Schizophrenia. One of the fields where dopaminergic drugs have had the most profound impact is schizophrenia, where the DA hypothesis for a long time has been the leading pathophysiologic theory, and DA blocking drugs has been the standard treatment since the 1950's. Schizophrenia is a severe, world-wide disease affecting about 1% of the population. The symptoms are divided into positive (hallucinations, delusions etc.), negative (lack of motivation, anhedonia, etc.) and cognitive (memory- and attention-deficits).[44] The search for an ideal treatment of schizophrenia has moved from D_2-antagonists (e.g. haloperidol (**6**) and chlorpromazine) introduced in the 1950's,[45, 46] to atypical antipsychotics of various types and with a broad spectrum of mechanisms (e.g. clozapine, aripirazole etc.).[47] Although traditional D_2-antagonist antipsychotics are efficacious for the positive symptoms, they are also responsible for extrapyramidal side effects

(EPS) which occur as a result of excessive attenuation of brain DA neuronal activity due to the blockade of postsynaptic DA receptors.[48, 49]

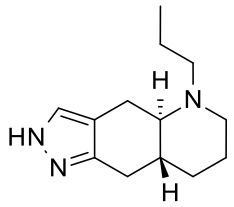
1.2.2. Neurological Diseases. As mentioned earlier, PD was the first disease where the involvement of DA in the brain was proven, and L-DOPA is still the main treatment for this condition. Since then, the importance of DA for both motor and cognitive functions in the patophysiology of many neurological diseases and disorders has been understood. Besides PD, dopaminergic drugs have also been found useful in the treatment of Huntington´s disease (HD), restless leg syndrome (RLS), Tourette's syndrome and attention deficit hyperactivity disorder (ADHD). The pharmacological profiles of the drugs used to treat these disorders are quite diverse, from DA antagonists in RLS and Tourette's syndrome to DA reuptake inhibitors in ADHD. In HD, the vesicular monoamine transporter (VMAT)-inhibitor tetrabenazine has shown to be effective in treatment of chorea.[50] However, there are many aspects of this disease and an effective treatment option for other symptoms is still being sought for. Recent clinical trials have shown promising results for the dopaminergic stabilizer pridopidine (ACR16, Huntexil®) (**16**, Figure 7) with beneficial effects on several manifestations of HD and a very favorable side effect profile.

1.3. Dopamine D_2 Ligands

Drugs that interact with the agonist binding site of D_2 receptors can be described as full agonists, partial agonists or antagonists/inverse agonists[51] and a number of such drugs have well-established applications in the treatment of various neurological and psychiatric disorders. The association and dissociation rate constants, k_{on} and k_{off}, besides defining the equilibrium state also describe how fast the ligand associate to and dissociate from the receptor system. Moreover, it has been proposed that the occurrence of side effects (e.g. extrapyramidal symptoms and sustained hyperprolactinaemia) of antipsychotic drugs is directly linked to the long D_2 dissociation rates.[52-54]

1.3.1. DA D_2 Agonists. *In vitro*, the D_2 agonists preferentially displaces agonist ligands over antagonist ligands in binding assays and induce a full catalytic reaction in functional assays (i.e. they have high intrinsic activity).[55-57] *In vivo*, the full D_2 agonists induce a decrease in DA release

through activation of presynaptic autoreceptors and affect locomotor activity in a biphasic manner (i.e. first decreased, then increased activity). The biphasic effect on behaviour is dose dependent and caused by differences in sensitivity between the autoreceptors and the postsynaptic receptors. In general, the autoreceptors are more sensitive and low doses of agonist only activate this population, leading to a decrease in DA release and a concomitant diminished locomotor activity. At higher doses, postsynaptic receptors are also affected with behavioural stimulation as a result. Examples of full D_2 agonists are DA (**1**), quinpirole (**4**) and ropinirole (**5**).



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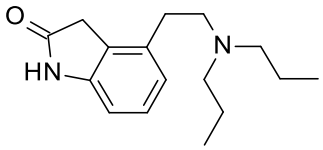

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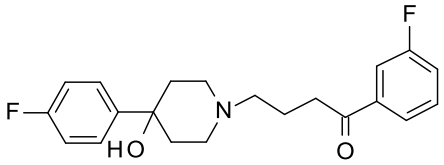
1.3.2. DA D_2 Antagonists. In contrast to the agonists, the D_2 antagonists in general show no preference in displacing agonist over antagonist ligands in binding assays and they induce no catalytic reaction in functional assays. *In vivo*, D_2 antagonists induce an increase in DA release through blockage of presynaptic autoreceptors and decreased locomotor activity through inhibition of postsynaptic receptors. D_2 antagonists are by far the most common type of dopaminergic ligands in medicine, for example haloperidol (**6**) and risperidone (**7**) used to treat schizophrenia (Figure 5).



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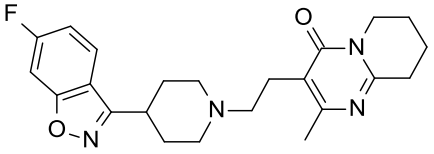

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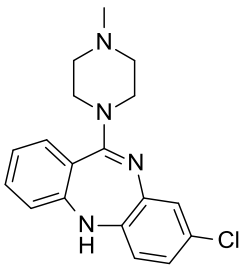

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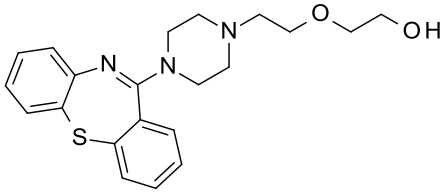

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Figure 5. Dopamine antagonists haloperidol (**6**), risperidone (**7**) clozapine (**8**) and quetiapine (**9**).

1.3.3. DA D_2 Partial Agonists. D_2 partial agonists, much like full agonists, in general preferentially displace agonist ligands over antagonist ligands in binding assays.[58] However, the partial agonists do not induce a full catalytic response in functional assays (i.e. they have lower intrinsic activity than the full agonist). *In vivo*, partial D_2 agonists affect DA release and locomotor activity differently depending on the level of intrinsic activity. If the level of intrinsic activity is very low, the *in vivo* effects are similar to those of an antagonist while higher intrinsic activity induces more agonist-like effects. The D_2 partial agonist aripiprazole (**10**, Figure 6) has very low intrinsic activity[59, 60] and is therefore thought to act as either a functional agonist or a functional antagonist, depending on the initial levels of DA. Aripiprazole has been approved for the treatment of schizophrenia, bipolar disorder and depression.

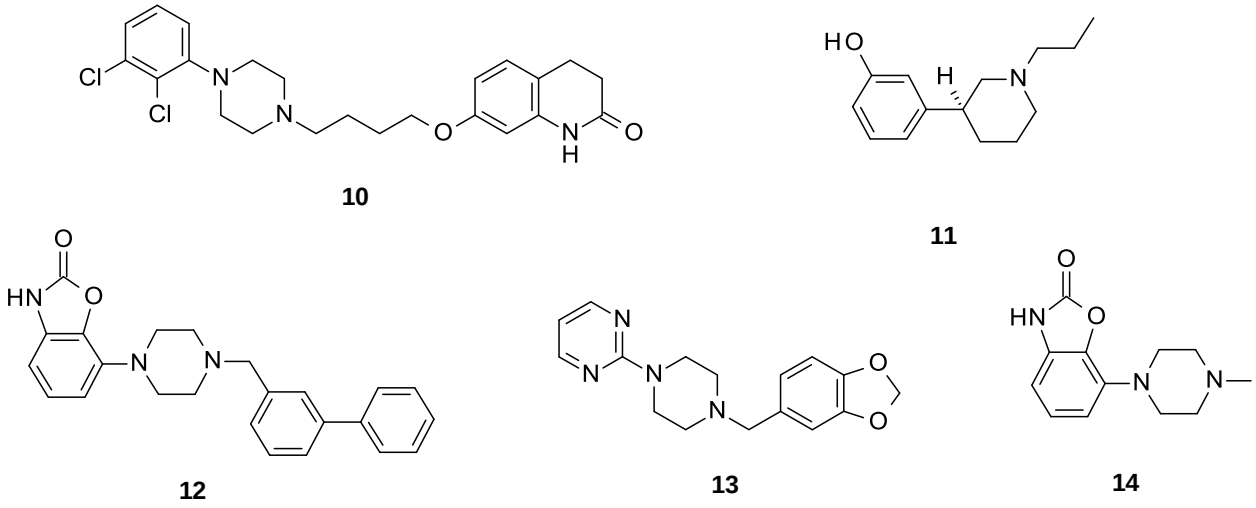


Figure 6. Dopamine partial agonists aripiprazole (**10**), (–)-3PPP (**11**), bifeprunox (**12**), piribedil (**13**) and pardoprunox (**14**).

1.3.4. Dopaminergic Stabilizers. For the last decades the bulk of medicinal chemistry optimization programs have generated high-affinity drugs with slow drug–receptor kinetics. In the meantime, limited attention has been set on optimizing D_2 ligands with low *in vitro* affinity and receptor kinetics comparable to those of natural DA signaling. Studies have shown that DA D_2 receptor kinetics differs among antipsychotic compounds and it has been proposed that fast-off kinetics (high k_{off}) is a requirement for atypicality.[54, 61] This is a new approach towards determining what properties are important in order to achieve an optimal antipsychotic profile with low propensity for side effects and the dopaminergic stabilizers have been characterized *in vitro* as low

affinity D_2 receptor ligands with fast-off receptor kinetics.[62, 63] It is however the *in vivo* effect that singles out the dopaminergic stabilizers from other D_2 ligands, having the ability to counteract states of both hyperactivity and hypoactivity, depending on the prevailing dopaminergic tone. To date, four dopaminergic stabilizers have been developed, namely (3*S*)-3-(3-methylsulfonylphenyl)-1-propylpiperidine ((-)-OSU-6162; **15**, Figure 7), 4-(3-methylsulfonylphenyl)-1-propylpiperidine (pridopidine; **16**, Figure 7), 1-ethyl-4-(2-fluoro-3-methylsulfonyl-phenyl)piperidine (ordopidine; **17**, Figure 7) and 1-ethyl-4-(3-fluoro-5-methylsulfonyl-phenyl)piperidine (seridopidine; **18**, Figure 7). Pridopidine has shown unique effects in clinical studies for symptomatic treatment of Huntington´s disease (HD) while **15** is being tested for treatment of alcohol dependence.[64] Other areas where dopaminergic stabilizers have shown promising results are PD, L-DOPA induced dyskinesia (LID), schizophrenia and stroke/traumatic brain injury.[65, 66]

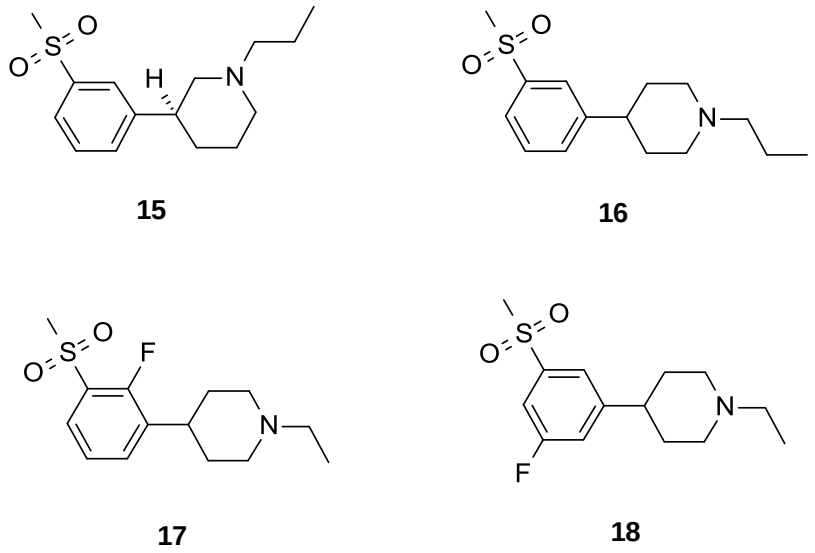
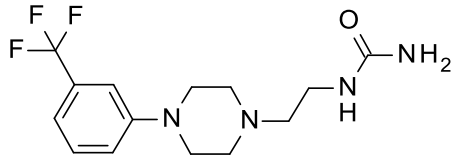


Figure 7. Dopaminergic stabilizers *S*-(-)-OSU6162 (**15**), pridopidine (**16**), ordopidine (**17**) and seridopidine (**18**).

1.4. Structure Activity Relationships

Structure activity relationships (SARs) describe the relationship between the structure of a molecule and its biological/pharmacological activity. There are different ways to describe a molecule and thus different ways to produce a SAR, for example using the 3D-structure or physicochemical properties of parts of, or the entire, molecule. The biological/pharmacological activity also includes a wide range of different parameters, like *in vitro* affinity to a certain receptor or the locomotor activity of a living animal. The SAR enables the medicinal chemist to understand how chemical modifications affect the biological response and this knowledge can be used to produce new compounds with a desired profile.

1.4.1 Phenylpiperidines and Phenylpiperazines. As structural backbones for pharmacologically active compounds, phenylpiperidines and phenylpiperazines have been extensively studied for several different targets. For example, many 5-HT ligands are based on these structures, like the 5-HT_{1A} agonist fluprazine (**19**), the SSRI paroxetine (**20**) and the 5-HT_{2A} antagonist nefazodone (**21**).



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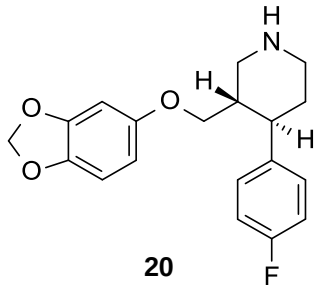

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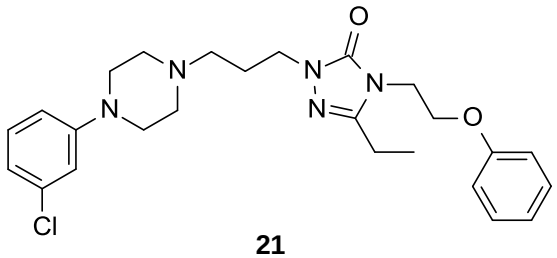

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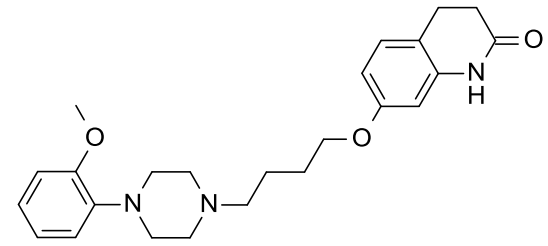
Other targets where phenylpiperidines and phenylpiperazines have been investigated as potential ligand scaffolds include GABA, NMDA and the adrenergic receptors. However, the main use of these structures in medicinal chemistry has been as dopaminergic ligands. Haloperidol (**6**), as

mentioned previously, is a D_2 antagonist and one of the first classical neuroleptics used as a treatment option in schizophrenia. Since then, many 4-phenylpiperidine analogues have been studied for their dopaminergic effects and potential use as antipsychotics. The partial D_2 agonists aripiprazole (**10**) and bifeprunox (**12**) instead have the phenylpiperazine backbone and a lot of attention has been devoted to find D_2/D_3 ligands in this structural class. Most series of phenylpiperidines and –piperazines acting on the D_2/D_3 receptors have an additional aromatic moiety attached at the basic amine and a linker of varying length in between. The linker has proved important for the D_2/D_3 selectivity and recent publications have concluded that the binding cavity in the extracellular loop region of D_2 is significantly shallower than the D_3 counterpart.[67, 68] The same group also reported compounds selective for both D_2 (SV-III-130s (**22**) and SV293 (**23**))[67] and D_3 (**24**)[69] receptors, but for most D_2-type ligands the affinities to these subtypes are similar. The D_4-ligand L-745870 (**25**)[70] is also of the phenylpiperazine class and the bulky *N*-substituent is proposed to be favorable for selectivity over D_2.



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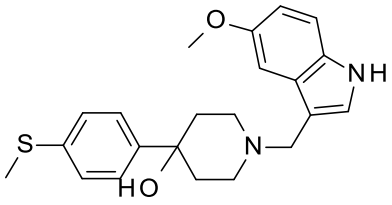

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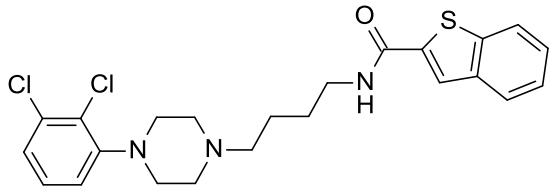

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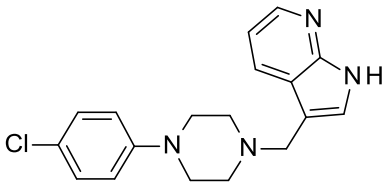

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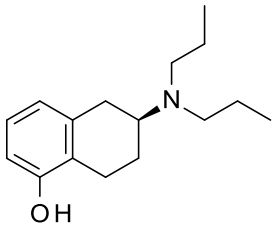
1.4.2. D_2 Ligands. Most compounds affecting the D_2 receptor has at least one aromatic moiety and one basic amine. In general, the agonists are relatively small, hydrophilic molecules whereas the antagonists are usually larger and more lipophilic.[71] Furthermore, the full agonists have certain pharmacophore elements that usually are required in order to achieve a full catalytic response at the D_2 receptor, for example a hydrogen bonding aromatic substituent (preferable in the *meta* position) and the basic amine in a position that resembles that of DA itself (e.g. 5-OH-DPAT (**26**) and

quinpirole (**4**)).[58] The D_2 receptor antagonists bind to the receptor but do not activate the G-protein and these compounds are usually of a bulky and hydrophobic character. D_2 receptor antagonists usually consist of two aromatic moieties with a basic amine in between (e.g. haloperidol (**6**) and risperidone (**7**)) and molecular modelling based on closely related receptor structures (i.e. D_3 and β_2) have confirmed that hydrophobic interactions of the aromatic parts stabilize the inactive conformation.[72, 73]

The SAR of partial D_2 agonists is more complex and both small and hydrophilic and bulky and hydrophobic structures with this profile have been developed. 3-[(3S)-1-Propyl-3-piperidyl]phenol ((–)-3PPP; **11**) is a partial agonist while the corresponding *R*-enantiomer (**27**) is a full agonist[74] and alignment of these two molecules with rigid full agonist analogues has revealed that the *R*-enantiomer fits perfectly while the aromatic ring and basic nitrogen of the *S*-enantiomer are unable to adapt the "right" conformation.[58, 74] 3-(4-Benzylpiperazin-1-yl)phenol (**29**) first published by Mewshaw *et al.*[75] lack the phenethylamine backbone of DA but still has intrinsic activity. The SAR of the phenylpiperazines indicated that a hydrogen-bonding group in the meta-position was preferred for the agonist properties and that the N-substituent could be either a small alkyl or a large aromatic group. The partial D_2 agonists, bifeprunox (**12**) and pardoprunox (**14**), are based on the phenylpiperazine backbone and have a benzoxazolone-group on the aromatic ring with the hydrogen-bonding functionality in the meta-position. Pardoprunox has a small methyl-group on the piperazine while bifeprunox has a bulky biphenyl-moiety, yet the intrinsic activity of the two analogues is similar.[76-78] The aripiprazole structure contains a 2,3-dichloro-substituted phenylpiperazine moiety that has been shown to stabilize the active conformation of the D_2 receptor through a hydrogen-bond between the 3-chloro group and a serine in the active site.[79] Recent studies have also shown that the chlorine-oxygen interaction can be relevant for binding affinities[80] albeit not as strong as the hydrogen bonding between a "full" hydrogen donor and acceptor. A weaker interaction with the receptor is a possible explanation to the fact that although aripiprazole act as a partial agonist, it has lower intrinsic activity than for example bifeprunox or (–)-3PPP. It should however be noted that even if a hydrogen-bonding substituent in the *meta*-position is positive for intrinsic activity, it is not essential. The π-π interactions are also likely to be important for the stabilization of the active conformation and the fact that (*S*)-2-dipropylaminotetralin (*S*-DPAT, **28**), which lack aromatic substituents, acts as a full DA D_2 agonist is a strong indication for this.[56, 58] Moreover, this could explain the intrinsic activity of piribedil (**13**)[81] as well as the fact that the 2-methoxy analogue of aripiprazole also act as a partial agonist.[82]



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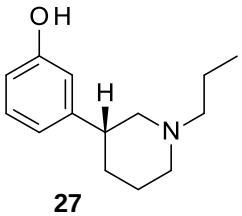

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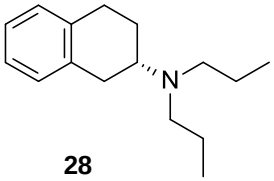

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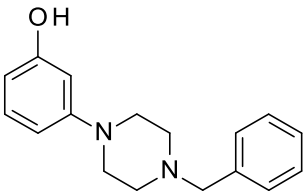

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1.4.3. MAO Inhibitors. Compounds that bind to and block the effect of MAO can be divided into reversible or irreversible inhibitors. Furthermore, the inhibitors can be selective for either MAO A or MAO B or non-selective (having equal effects on both isozymes). As an entity, the MAO inhibitors (MAOIs) are structurally quite diverse, but there is a distinct separation between reversible and irreversible inhibitors. While the irreversible inhibitors, for example ipronazid (**30**, Figure 8) and selegiline (**31**, Figure 8), have a functional group (e.g. propargylamine or hydrazine) that enables covalent binding to the enzyme, the reversible inhibitors lack such moiety. Structural separations between MAO A and MAO B is less evident, but most reversible MAO A inhibitors have an aromatic moiety with a basic amino group at 2-4 atoms distance from the ring (e.g. moclobemide (**32**, Figure 8) and pirlindole (**33**, Figure 8). Studies on *para*-substituted phenethylamines, benzylamines and amphetamines have shown that the physiochemical properties of the *para*-substituent are correlated to the affinity of the two isozymes. Size and electronic properties have been proposed to mainly impact affinity to MAO A, while the hydrophobicity of the substituent seems to influence MAO B affinity to a greater extent.

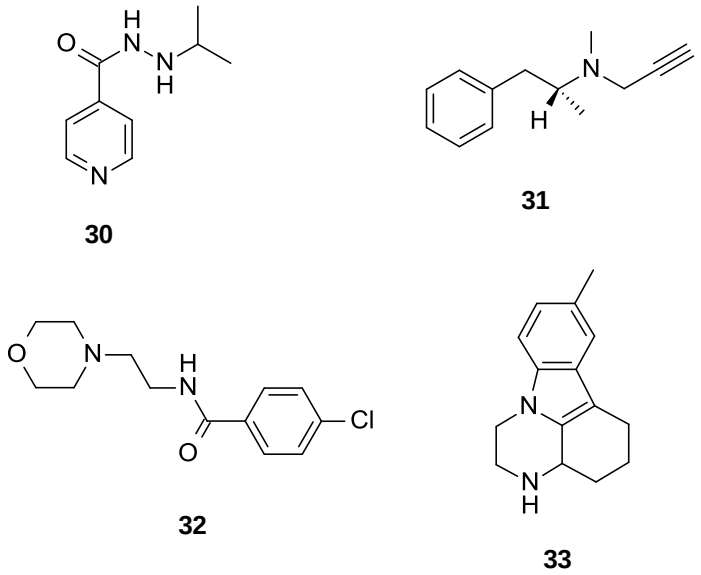


Figure 8. Irreversible MAOIs: iproniazid (**30**) and selegiline (**31**). Reversible MAO A inhibitors moclobemide (**32**) and pirlindole (**33**).

1.4.4. Quantitative structure activity relationships (QSARs). SAR is useful when comparing heterogeneous structural classes with diverse biological activities. There is however a shortcoming with this method; it assumes that similar molecules have similar activities. This is indeed not always the case, since many times small differences on the molecular level can have a major impact on the response. In order to find relationships between a homogenous group of compounds and their respective activity, quantitative structure activity relationships (QSARs) can instead be applied. QSAR models attempt to relate chemical structure to biological activity using quantitative regression by setting the chemical properties of a molecule, or parts thereof (e.g. Hammett constants of a substituent) against the response variable of a biological activity (e.g. affinity to a receptor). QSAR modeling generally involves three steps: (1) design of a training set of molecules; (2) decision on descriptors that are presumed relevant for the correlation between chemical structure and biological activity; and (3) application of statistical methods that correlate changes in structure with changes in biological activity. Since in QSAR, the physicochemical properties of chemical structures as well as biological response are expressed by numbers, a mathematical relationship can be established between the two. The model can then be used to predict the biological activity of new chemical structures and is therefore a powerful tool in medicinal chemistry.

Most QSAR models in the field are based on *in vitro* data as the biological response, or more specifically, binding affinities to one or many receptors. It has been a general resistance towards

using *in vivo* data in QSAR modeling, mostly derived from a skeptic view on the response obtained from a complex biological system such as a living animal. The data from an *in vivo* experiment is often linked to several different aspects of pharmacology, pharmacodynamics and pharmacokinetics, and therefore each specific contribution can be difficult to interpret. However, the sum of these aspects for the most part holds very valuable information and an *in vivo* response can even be superior to *in vitro* data in a QSAR model.

1.4.5. Drug Design. In drug design the knowledge of biological targets, usually proteins and enzymes in pathways that are related to a particular disease state, is used to find new drugs that affect these targets in a specific way. There are many different techniques that can be used to obtain this knowledge. The application of X-ray crystallography and NMR spectroscopic methods can resolve the structure of proteins to a very high resolution, making it possible to determine its 3D-structure. This information can in turn provide valuable insight into the optimization of the molecular interactions of a drug-target complex to achieve potency and selectivity of a drug candidate. However, in order to acquire the 3D-structure of a target protein, it has to be in a crystalline form and many biological targets are extremely difficult to crystallize. Especially the trans-membrane proteins have been problematic in this aspect, the main reason being the amphipathic nature of their surface. Instead computerized modeling, using the amino acid sequence of the target protein together with known 3D geometrical shape of homologue proteins, can be applied.

The 3D-structures of both MAO A and MAO B have been determined by X-ray crystallography with several different ligands[83-86] and these structures have been used in the development of novel classes of MAO inhibitors.[87-89] DA D_2, on the other hand, has not yet been successfully crystallized, but molecular modeling based on the 3D structure of the closely related DA D_3[72] and β_2 adrenergic[90, 91] receptors has provided a better understanding of the ligand-receptor interactions in this class.[79, 92, 93] These studies have revealed that Asp-114 on the third transmembrane helix (TM3) most likely forms of a salt bridge with the protonated nitrogen of DA and that serine residues in TM5 (Ser-193, Ser-194 and Ser-197) interact with the catechol function through hydrogen bonding (Figure 9).[94-99] More recent publications have also shown that His-393 on TM6 can form hydrogen bonds with the catechol or other hydrogen bonding groups of dopaminergic ligands.[92, 100] In addition, Phe-110, Met-117, Cys-118 (TM3), Phe-164 (TM4), Phe-189, Val-190 (TM5), Trp-386, Phe-389, Phe-390, and His-394 (TM6) contribute to the stabilization of the drug-receptor complex

via hydrophobic interactions.[92, 100] Ligand interactions with two amino acids, Ile-184 and Asn-186, in the second extracellular loop (EC2) have also shown to be important (Figure 9).[92, 101] It has been proposed that in the activation phase of GPCRs, TM6 undergoes a translational or rotational movement, and that the interaction with an agonist facilitates this movement.[102-105] In line with this proposal, Goddard *et al*. (2007) speculated that DA D_2 agonists interact with TM3 (Asp-114) and TM5 (Ser-193 and Ser-197) by pulling them closer together in the active state, allowing the flexible motion of TM6.[106] An antagonist (such as haloperidol) instead interacts strongly with TM3 and TM6 (having minimal contact with TM5), thus preventing such movement.[99, 106]

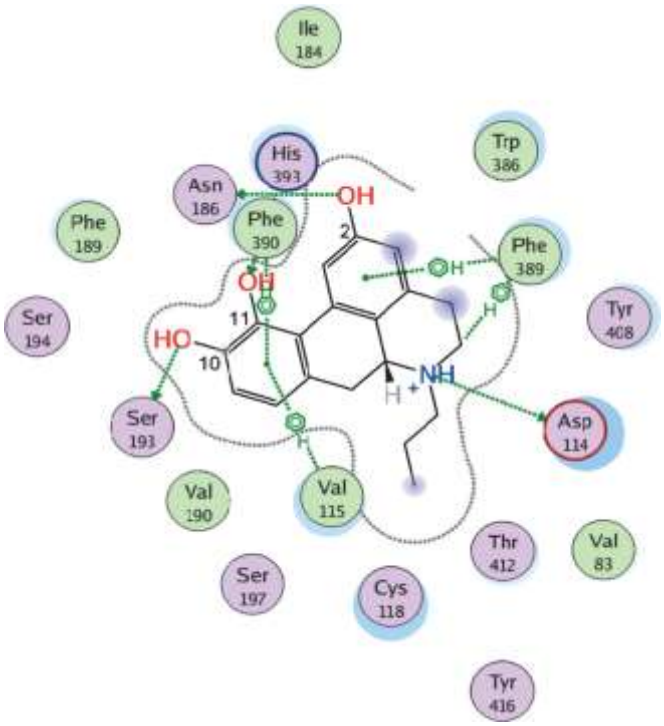


Figure 9. Schematic view of the interactions between the full agonist (*R*)-2-OH-NPA and the DA D_2 receptor in a homology model by Malo *et al*.[92] Amino acids in purple are polar, while green residues are hydrophobic. The blue shades indicate ligand–receptor solvent accessibility.

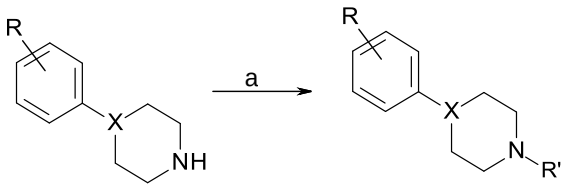
2. AIMS

This work is a part of a research project aimed at finding novel dopaminergic ligands with beneficial effects in several neurological and psychiatric disorders. The discovery and mechanism of action of the dopaminergic stabilizer pridopidine (ACR16, Huntexil®, **16**), currently being developed for Huntington's disease, are included. In addition, the QSARs of mono-substituted 4-phenylpiperidines/-piperazines have been investigated and correlations between the *in vivo* and *in vitro* profile of compounds in this structural class has been established.

3. CHEMISTRY (Papers I, II and III)

The compounds included in this work have been synthesized by various methods described in the literature. Alkylation of commercially available phenylpiperazines/-piperidines using standard conditions (Scheme 1) produced the bulk of target compounds. Other methods were applied when the desired starting material was unavailable, and these methods are shown in separate sections.

Scheme 1



X = N, CH
R' = n-Pr, Bn
R = H, OMe, SO_2Me, CN, Me, Cl, OH, CF_3, COMe, Ot-Bu

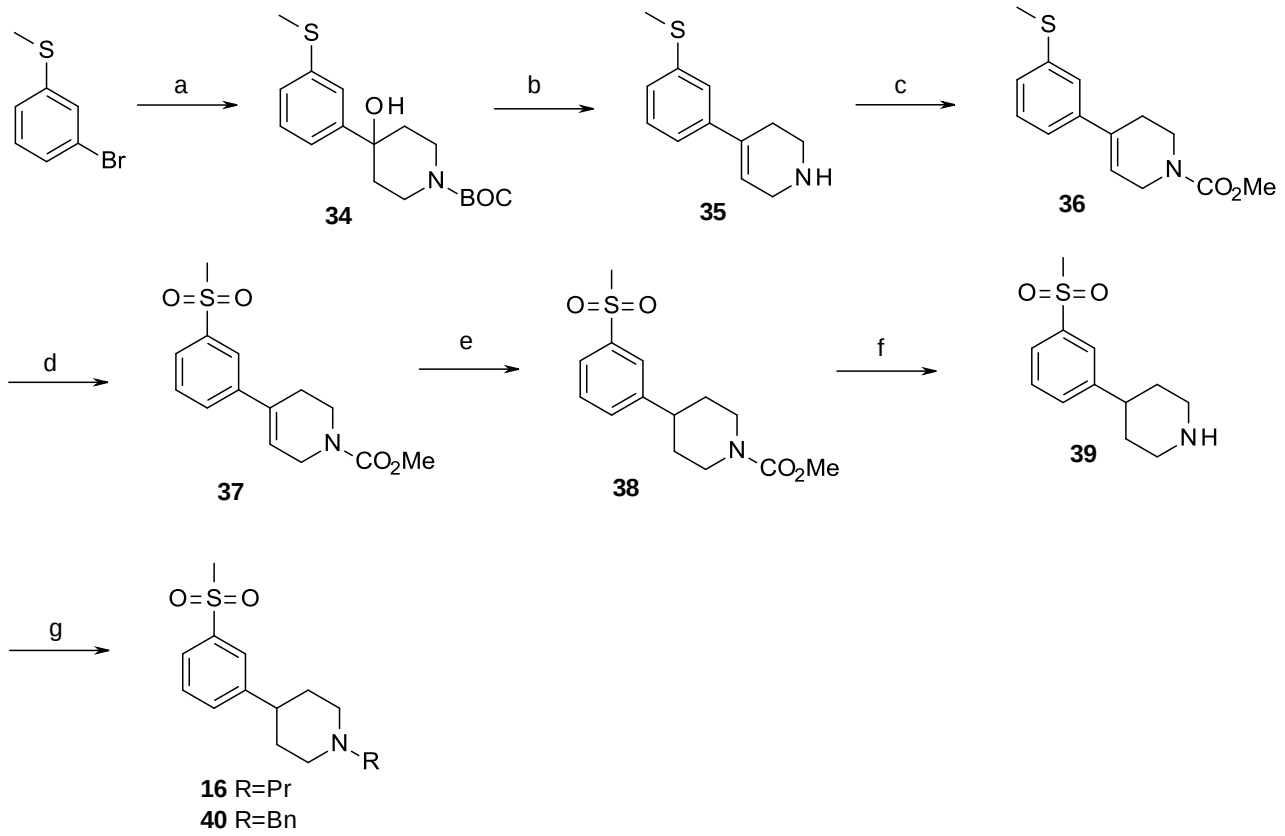
[a] Reagents and conditions: (a) PrI or BnBr, K_2CO_3, CH_3CN, Δ.

3.1. Original Synthetic Route to Pridopidine (Paper I)

Pridopidine (**16**), or ACR16 as the compound was first named, has recently been developed for large scale manufacturing and is currently being synthesized with an optimized synthetic route. However, the first synthesis of pridopidine/ACR16 was performed by a different route (Scheme 2, R=Pr). In the first step of seven in total, 1-bromo-3-methylthiobenzene was treated with n-butyllithium and quenched with 1-Boc-4-piperidone to yield **34**. Subsequent treatment with trifluoroacetic acid (TFA) in a solution of DCM led to both deprotection and dehydroxylation, producing **35** in excellent yield. It is well known in the trait that sulfides contaminate the palladium of the Pd/C-catalyst used in H_2-mediated reductions,[107] and therefore the sulfide had to be oxidized to the corresponding sulfone prior to the reduction step. Attempts to oxidize **35** directly with *m*-chloroperbenzoic acid (*m*-CPBA) did however lead to simultaneous oxidation of the tetrahydropyridine-ring along with the thiomethyl-group, producing the undesired 4-[3-(methylsulfonyl)phenyl]pyridine. In order to avoid this side-reaction, **35** was first protected by the addition of methylchloroformate to afford the carbamate **36**, after which quantitative oxidization by *m*-CPBA to the corresponding sulfone **37** was possible. **37** was then easily reduced with catalytic

hydrogenation (Pd/C), affording the piperidine-derivative **38** in good yield. After the deprotection of **38** with aqueous HCl (8 M), the secondary amine **39** was alkylated with 1-iodopropane, affording pridopidine/ACR16 (**16**) (Scheme 2). The corresponding benzyl-analogue (**40**) was obtained by alkylation of **39** with benzylbromide. In addition, the preparation of 4-(3-isopropylsulfonylphenyl)piperidine (**87**) followed the same synthetic route.

Scheme 2



[a] Reagents and conditions: (a) *n*-butyllithium, 1-Boc-4-piperidone, THF; (b) trifluoroacetic acid, CH_2Cl_2, Δ; (c) triethylamine, methylchloroformate, CH_2Cl_2; (d) *m*-CPBA, CH_2Cl_2; (e) Pd/C, H_2, MeOH, HCl; (f) HCl, EtOH, Δ; (g) PrI or BnBr, K_2CO_3, CH_3CN,

3.2. Suzuki Cross Coupling between Phenylbromides and 1-Pyridyl-4-boronic acid (Paper III)

The Suzuki cross coupling is a palladium catalyzed cross-coupling reaction between organic halides and organoboron compounds that leads to the formation of carbon-carbon bonds.[108-110] The mechanism of the Suzuki reaction has been studied extensively in order to fully optimize the reaction conditions (Figure 10).[111] The first step is an oxidative addition of palladium to the halide (**I**) which forms an organo-palladium complex (**II**). Further reaction with the required base (e.g. Na_2CO_3, K_3PO_4) gives an intermediate (**III**), which via transmetalation with the boronate complex (**V**) forms another organo-palladium species (**VII**). Reductive elimination yields the desired product (**VIII**) and restores the original palladium catalyst (**IX**) for further use.

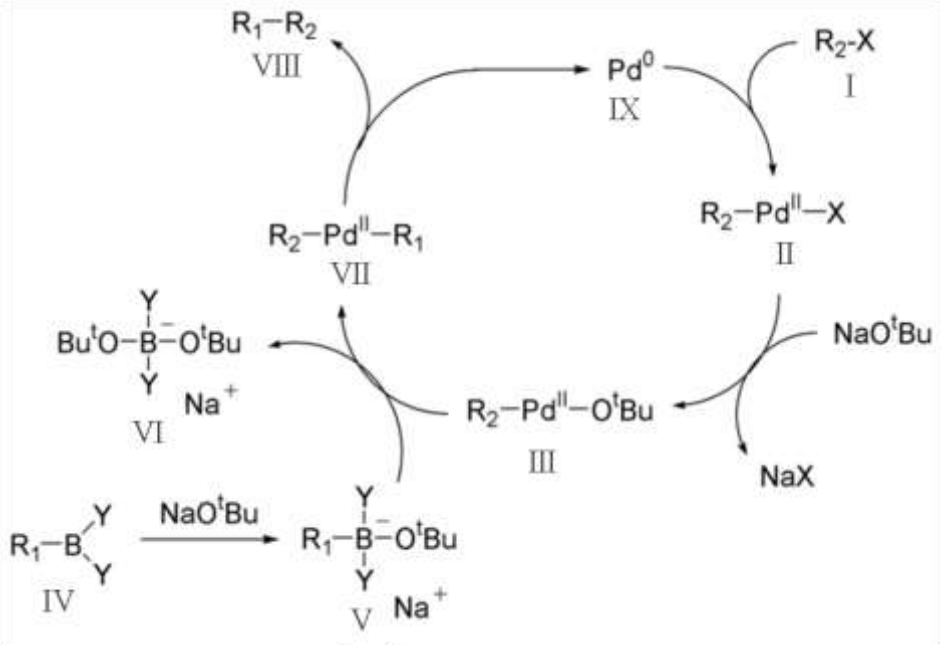


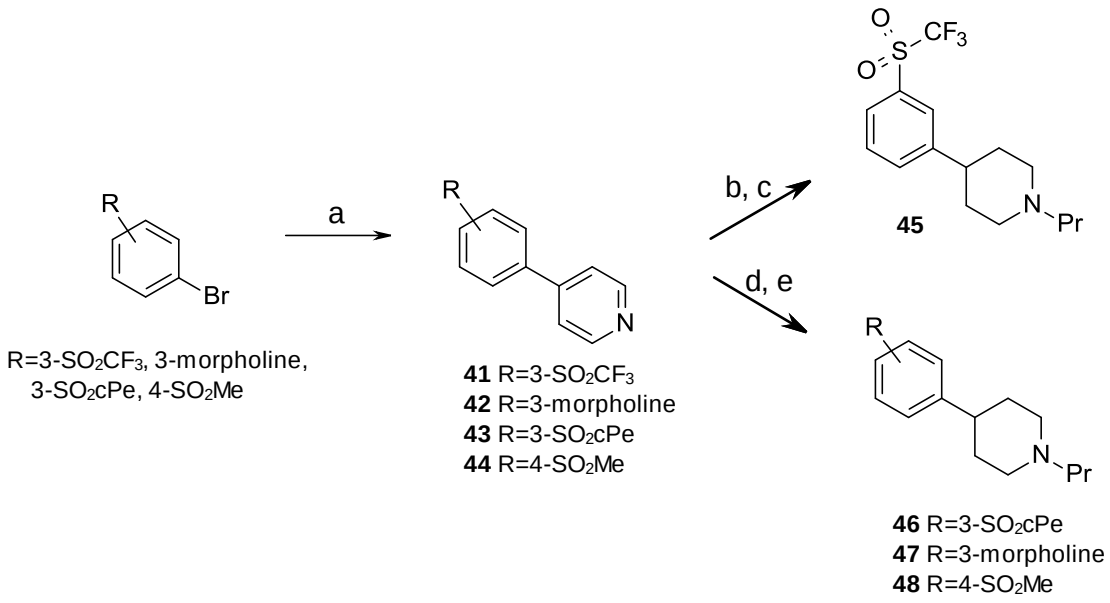
Figure 10. The proposed mechanism for the Suzuki cross coupling reaction.

In the cases were the desired phenylpiperidine starting material was commercially unavailable and lithiation or Grignard reaction of the phenylbromide was inapplicable (see Scheme 2), the desired phenylpiperidines were acquired through Suzuki cross-coupling of the substituted arylbromides and 4-pyridineboronic acid, followed by reduction of the pyridine ring (Scheme 2).

4-[3-(Trifluoromethylsulfonyl)phenyl]pyrididine (**41**), 4-[3-(4-pyridyl)phenyl]morpholine (**42**) , 4-(3-cyclopentylsulfonylphenyl)pyridine (**43**) and 4-(4-Methylsulfonylphenyl)pyridine (**44**) were all prepared through Suzuki-coupling, but only the pyridine ring of **41** could be reduced directly by platina-mediated catalytic hydrogenation.[112] For the other substrates this reaction was unsuccessful and instead quarterisation of the pyridine nitrogen by heating with 1-iodopropane preceded the

reduction.[113] Thus, the desired target compounds 4-(3-cyclopentylsulfonylphenyl)-1-propylpiperidine (**46**), 4-[3-(1-propyl-4-piperidyl)phenyl]morpholine (**47**) and 4-(4-methylsulfonylphenyl)-1-propylpiperidine (**48**) were obtained from the reduction step, while a subsequent *N*-propylation produced 1-propyl-4-[3-(trifluoromethylsulfonyl)phenyl]piperidine (**45**).

Scheme 3



[a] Reagents and conditions: (a) pyridyl-4-boronic acid, Na_2CO_3, $Pd(PPh_3)_4$, toluene/EtOH, Δ; (b) PtO_2, H_2, MeOH, konc HCl; (c) PrI, K_2CO_3, CH_3CN, Δ; (d) PrI, Δ; (e) PtO_2, H_2, MeOH, konc HCl.

3.3. Buchwald-Hartwig Cross Coupling between Phenylbromides and Piperazines (Paper III)

All *ortho*- and *para*-substituted, and most *meta*-substituted, phenylpiperazines included in the data set could be obtained from commercially available starting materials via *N*-alkylation (Scheme 1). However, in order to obtain 1-(3-methylsulfonylphenyl)-4-propylpiperazine (**49**), 4-benzyl-1-(3-methylsulfonylphenyl)-piperazine (**50**) and 1-[3-(benzenesulfonyl)phenyl]piperazine (**51**), the corresponding phenylpiperazines had to be synthesized from the phenyl bromides and piperazine using the Buchwald-Hartwig cross coupling reaction[114, 115]. This is a C–N palladium-catalyzed cross-coupling reaction where the following general mechanism has been proposed:

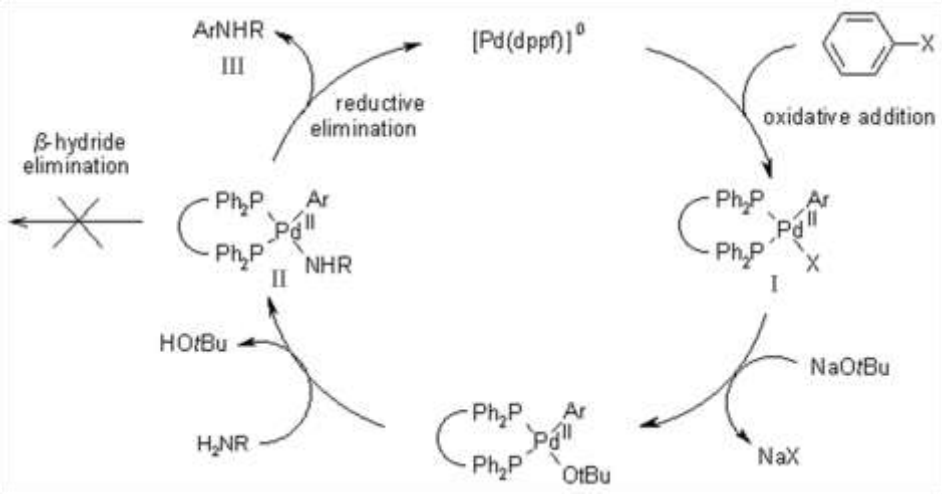
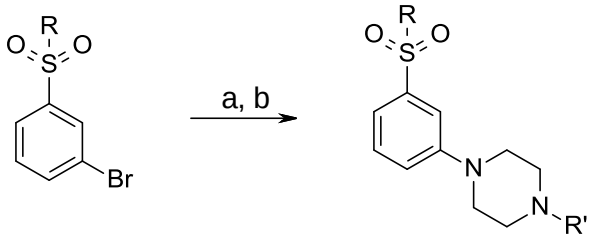


Figure 11. The proposed mechanism for the Buchwald-Hartwig cross coupling reaction.

Bidentate ligands are often used in these reactions to improve the yield, minimize the use of catalyst and shorten the reaction time.[114, 115] 1-Bromo-3-(methylsulfonyl)benzene and 1-(benzenesulfonyl)-3-bromo-benzene were coupled with piperazine using $Pd_2(dba)_3$ and rac-BINAP in refluxing toluene for 15h (Scheme 3). For chelating ligands, oxidative addition occurs directly from the ligand-palladium complex forming intermediate I (Figure 11). Deprotonation by base followed by amine ligation produces the palladium amide (II). This key intermediate reductively eliminates to produce the product (III) and regenerate the catalyst. β-Hydride elimination from intermediate II is avoided by the chelating phosphine, producing a 4-coordinate species which hinder the side reaction. The yields were 49% and 87%, respectively, without optimizations.

Scheme 4



R=Me, Ph

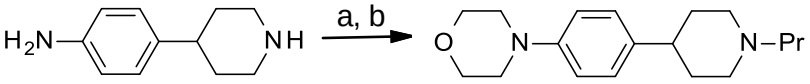
49 R=Me, R'=Pr
50 R=Me, R'=Bn
51 R=Ph, R'=Pr

[a]Reagents and conditions: (a) piperazine, NaOt-Bu, $Pd_2(dba)_3$, rac-BINAP, toluene, Δ; (b) PrI or BnBr, K_2CO_3, CH_3CN, Δ.

3.4 Conversion of Functional Groups

3.4.1 Aniline to Morpholine (Paper II). The commercially available 4-(4-piperidyl)aniline was used to prepare the desired *para*-morpholine compound. After *N*-alkylation with 1-iodopropane, a ring-closing reaction around the aniline nitrogen was achieved by a microwave assisted nucleophilic substitution using bis(2-chloroethyl)ether in DMF.[116] Thus, 4-[4-(1-propyl-4-piperidyl)phenyl]morpholine (**52**) was obtained through a 2-step synthesis in an overall yield of 63% (Scheme 5).

Scheme 5

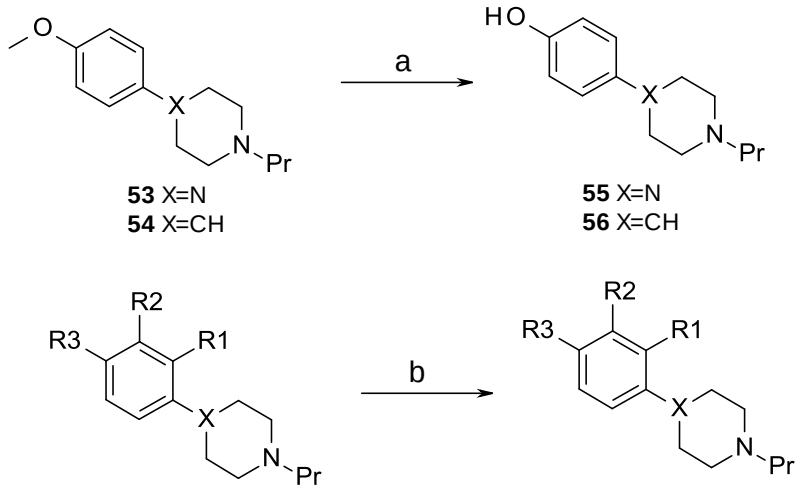


52

[a]Reagents and conditions: (a) PrI, K_2CO_3, CH_3CN, Δ; (b) bis(2-chloroethyl)ether, DMF, MW.

3.4.2 Phenols to Mesylates and Triflates (Paper III). The mesylate and triflate groups are often used as leaving groups in aromatic substitution reactions but they can also be used in biologically active compounds and were found by Sonesson *et al*. to have beneficial properties in both the 3-phenylpiperidine and aminotetraline series.[117] The transformation from the corresponding phenols was achieved by addition of triflic anhydride or mesylchloride, respectively, in the presence of triethylamine (Scheme 6).[118, 119]

Scheme 6



53 X=N
54 X=CH

55 X=N
56 X=CH

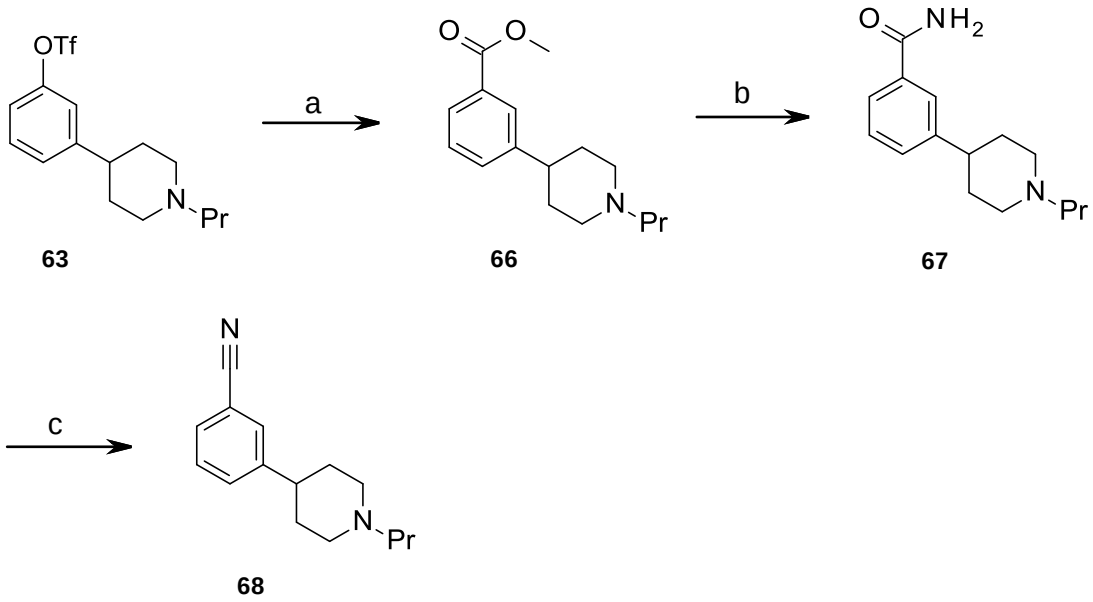
55 X=N, R1/R2=H, R3=OH
57 X=N, R1/R3=H, R2=OH
58 X=CH, R1/R3=H, R2=OH
59 X=CH, R1=OH, R2/R3=H
60 X=N, R1=OH, R2/R3=H

61 X=N, R1/R3=H, R2=OSO_2Me
62 X=N, R1/R3=H, R2=OSO_2CF_3
63 X=CH, R1/R3=H, R2=OSO_2CF_3
64 X=CH, R1=OSO_2CF_3, R2/R3=H
65 X=N, R1/R2=H, R3=OSO_2CF_3

[a] Reagents and conditions: (a) HBr (48%), Δ; (b) NEt_3, CH_3SO_2Cl or $(CF_3SO_2)_2O$, CH_2Cl_2.

3.4.3 Triflate to Nitrile (Paper III). In the 3-phenylpiperidine series, Sonesson *et al.*[117] provided a convenient route to the cyano-analogue from the partial agonist (-)-3-(3-hydroxyphenyl)-1-propylpiperidine ((-)-3-PPP). The same route was also used to obtain the *meta*-cyano compound in the 4-phenylpiperidine series. Starting from the triflate (**63**), palladium catalyzed carbonylation[120] using carbon monoxide and methanol furnished the methyl ester (**66**). The ester was converted to an amide (**67**) via a one-step reaction using formamide and sodium methoxide in DMF.[121] The target compound, 3-(4-propylpiperazin-1-yl)benzonitrile (**68**), was then obtained through a dehydration of the amide group by phosphorous oxychloride in DMF[122] (Scheme 7).

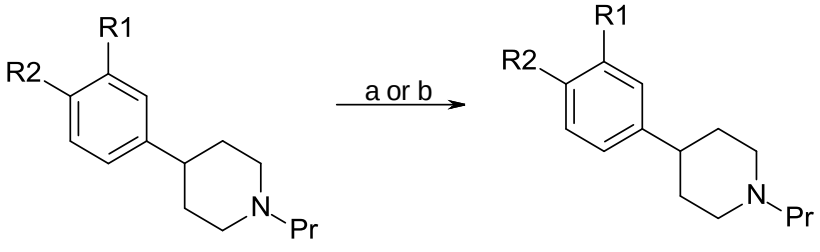
Scheme 7



[a] Reagents and conditions: (a) $Pd(OAc)_2$/dppp, CO(g), NEt_3, MeOH; (b) $HCONH_2$, NaOMe, DMF; (c) $POCl_3$, DMF.

3.4.4 Phenols to Alkoxy-groups (Paper II and III). Two different alkoxy compounds were synthesized from the corresponding phenols. The iso-propoxy derivate (**69**) was produced by reaction of the 3-hydroxyphenylpiperidine (**58**) with NaH in DMF and quenching with 2-iodopropane. Refluxing the *para*-isomer (**56**) in acetonitrile with a weak base (K_2CO_3) and *n*-butylbromide produced the *n*-butoxy analogue (**70**) (Scheme 8).

Scheme 8



56 R1=H, R2=OH
58 R1=OH, R2=H

69 R1=Oi-Pr, R2=H
70 R1=H, R2=On-Bu

[a] Reagents and conditions: (a) NaH, *i*-PrI, DMF; (b) *n*-BuBr, K_2CO_3, CHCN.

4. Pharmacology

4.1 Methods

The target compounds were tested both *in vivo* and *in vitro* in different pharmacological assays. The *in vivo* models were used to investigate both behaviour and neurochemical effects in freely moving rats while the *in vitro* models were used to measure the binding affinities to the DA D_2 receptor and MAO enzymes.

4.1.1. *In vitro* models. The DOPAC levels produced by a pharmaceutically active compound can be linked to a number of different targets and as previously mentioned two of these targets are the DA D_2 receptor and the MAO A enzyme. We therefore measured the affinity to these targets for a subset of compounds chosen to provide as much information about the *in vitro* SAR as possible. In addition to pure affinity, the level of intrinsic activity at the D_2 receptors is also a determinant for the DOPAC levels. Partial D_2 agonists in general produce less DOPAC than an antagonist at a dose where maximal effects for both are achieved (see Figure 12). The intrinsic activity was measured in a functional assay for a few compounds, but the effect in an *in vitro* model is not necessarily the same as in the living system. The efficacy data produced in the D_{2L}-$G\alpha_{qi5}$ HEK293 cells can therefore differ from the effects observed *in vivo*. Another indicator of the agonistic property of a compound is the ratio between the propensity to displace agonists rather than antagonists from the receptor. These two assays have been denoted D_2^{High} and D_2^{Low}, where high is the active state and low the inactive state of the receptor. The same compounds that were examined for intrinsic activity were also investigated in the D_2^{High} and D_2^{Low} binding affinity assays and the ratios ($K_i(D_2^{Low})/K_i(D_2^{High})$) from these studies clearly showed that the included compounds were more prone to displace an agonist than an antagonist. This ratio has also been used as a quantitative measurement and strong correlations to the results from both *in vitro* and *in vivo* assays of intrinsic activity have been shown.[123, 124]

In addition, the affinity to MAO B was investigated for the *para*-substituted compounds in order to clarify which physicochemical properties of the substituent that was important for interaction with the respective isozyme. In agreement with previous observations[6], we also found that affinity to MAO B alone was not a contributing factor to the DOPAC levels in the rat.

4.1.2. *In vivo* models. The level of DOPAC in different parts of the brain has been used as a measurement of the synthesis and turnover of DA. Striatum is the part of the brain that has the strongest correlation to behaviour and DA is the main neurotransmitter affecting locomotor activity. Therefore the level of DOPAC in striatum was the biomarker of choice for our *in vivo* models. Male Sprague-Dawley rats from B&K Scanbur (Sollentuna, Sweden) or Charles River (Köln, Germany) were used and five groups of animals, four animals per group, where dosed with either saline (control) or the test substance in escalating doses (usually up to a 100 μmol/kg). The behaviour was recorded using motility meters[125] and the distance travelled was used as a measurement of the rats activity. The rats were decapitated 1h after the injection and the effect of the target compounds on the levels of DOPAC was measured by high-performance liquid chromatography on the homogenates of the dissected brain. The rats treated with the test compounds were compared to the saline treated rats in the same experiment (effect expressed as "% of control"), both with regards to the biochemical markers and the locomotor activity (LMA). Several reference compounds have been tested in these models in order to compare if the response factors are in agreement with what is known from the literature. The effects on striatal DOPAC levels following administration of different D_2 ligands were as follows: antagonists (e.g. haloperidol (**6**)) gave sharp increases, full D_2 agonist (e.g. apomorphine) produced decreased levels and partial agonists (e.g. aripiprazole (**10**) (Figure 12), (-)-3PPP (**11**)) yielded varying levels (100 - ~150% of control) of DOPAC. This is in agreement with previously published results from *in vivo* studies on rat post mortem neurochemistry.[126-129] The dopaminergic stabilizer pridopidine (**16**) has effects on DOPAC similar to an antagonist, although compared to haloperidol, higher doses are required to achieve the maximal response (Figure 12).

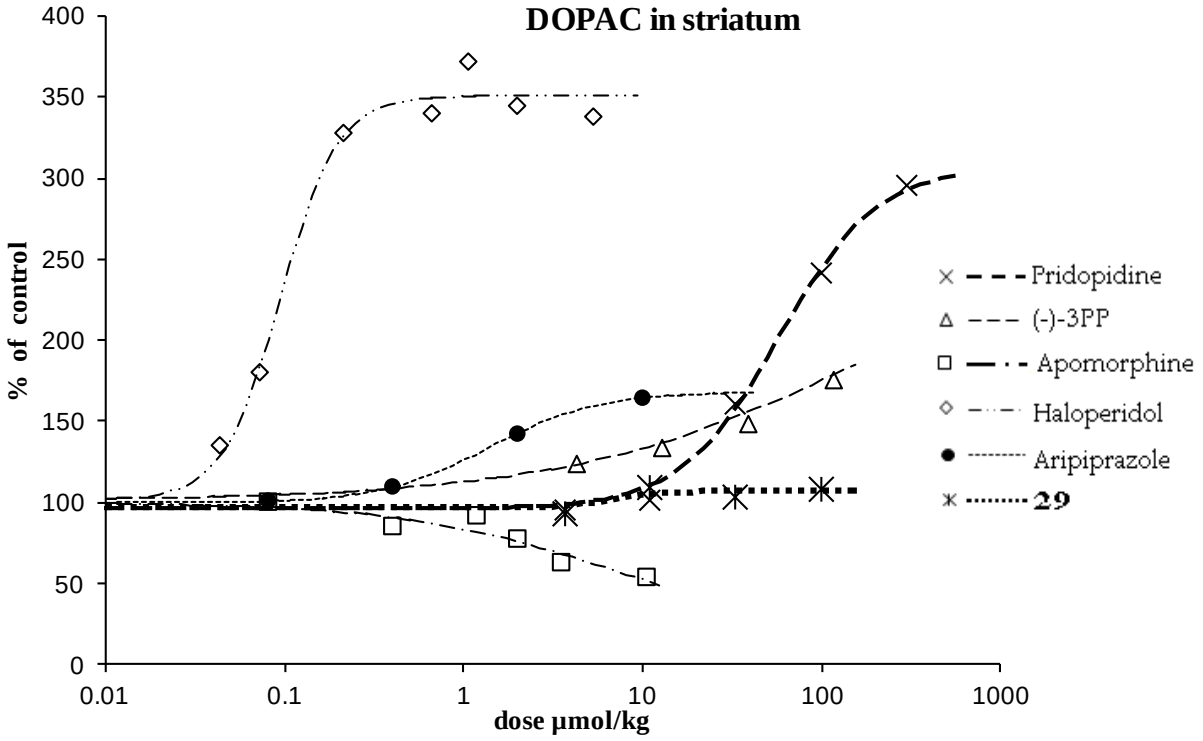


Figure 12. Dose-dependent effects of the DA D_2 agonist apomorphine, the partial agonists (-)-3PPP, aripiprazole and 3-(1-benzylpiperidin-4-yl)phenol (**29**), the antagonist haloperidol and the dopaminergic stabilizer pridopidine on the DOPAC levels in striatum.

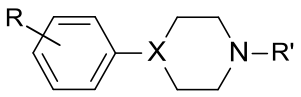
The effects on the locomotor activity by the different ligands were also in accordance with previous observations. Haloperidol and the partial agonists all produced strong inhibition of the normal behaviour while apomorphine gave a biphasic effect on locomotor activity (i.e. inhibition at low doses and stimulation at high doses). The dopaminergic stabilizer pridopidine (**16**), on the other hand, had no effect on normal exploratory behaviour and partly habituated rats were even mildly stimulated.[130] Pretreatment with amphetamine has often been used as a model of psychosis and the induced hyperactivity was blocked by most antipsychotics. However, as seen with the antagonists/partial agonists, this goes hand in hand with inhibition of the normal exploratory behaviour. Pridopidine (**16**) has the ability to counteract the amphetamine induced hyperactivity without impeding the normal state and this is a central trait of the dopaminergic stabilizers. Analysis of perfusates collected from microdialysis probes implanted in the striatum of freely moving rats was used to measure the DOPAC and 3-MT levels during a period of 180 min after administration of a MAO A inhibitor. The observed effects, with decreases in DOPAC and increased levels of 3-MT, were in agreement with previous investigations of known MAO A inhibitors.[11] All

experiments were carried out in accordance with Swedish animal protection legislation and with the approval of the local Animal Ethics Committee in Gothenburg

4.2 Results

The results from the different assays are included in Table 1. From the *in vivo* models, only the DOPAC levels (presented as % of control) are presented. Additional results can be found in Table 3, Paper I. The *in vitro* data from the binding assays are all included.

Table 1. *In vivo* levels of DOPAC and *in vitro* affinities to D_2 and MAO



Comp	R'	X	R	Pos	DOPAC, % of ctrl ± SEM[a]	pK*i* (D_2^{High})[b]	pK*i* (D_2^{Low})[b]	pK*i* (MAO A)[b]	pK*i* (MAO B)[b]
71	n-Pr	N	H	None	181 ± 6 [d]	6.3[e]		4.3[d]	
72	n-Pr	CH	H	None	131 ± 11 [d]	6.8 [e]		5.0[d]	
60	n-Pr	N	OH	Ortho	270 ± 23 [e]				
64	n-Pr	CH	OSO_2CF_3	Ortho	270 ± 16 [e]				
73	n-Pr	N	OMe	Ortho	370 ± 24 [e]	7.7 [e]	7.1	<3.2 [e f]	
74	n-Pr	CH	OMe	Ortho	277 ± 18[†] [†]	7.9 [e]		5.6 [e]	
75	n-Pr	N	SO_2Me	Ortho	283 ± 16 [e]	6.2 [e]		<3.2 [e f]	
76	n-Pr	N	CN	Ortho	313 ± 9[†] [†] [e]				
77	n-Pr	N	Me	Ortho	327 ± 16 [e]				
78	n-Pr	N	Cl	Ortho	368 ± 19 [e]				
79	n-Pr	CH	CF_3	Ortho	254 ± 20 [e]				
16	n-Pr	CH	SO_2Me	Meta	265 ± 10[c]	5.1[c]	4.5 [c]		
29	Bn	N	OH	Meta	108 ± 4 [c]	8.3[c]	7.0 [c]		
40	Bn	CH	SO_2Me	Meta	310 ± 16	6.4 [c]	6.1 [c]		
45	n-Pr	CH	SO_2CF_3	Meta	258 ± 15 [e]				
46	n-Pr	CH	SO_2c-Pe	Meta	170 ± 10 [e]	5.4 [e]		<3.2 [e f]	
47	n-Pr	CH	morph	Meta	105 ± 7 [e]				
49	n-Pr	N	SO_2Me	Meta	310 ± 16[†] [†] [c]	6.2[c]	5.9 [c]	3.9 [e]	
50	Bn	N	SO_2Me	Meta	248 ± 10 [c]	6.4 [c]	5.7 [c]		
51	n-Pr	N	SO_2Ph	Meta	152 ± 7 [e]				
57	n-Pr	N	OH	Meta	260 ± 12[†] [†] [c]	7.2[c]	6.1 [c]	<3.2 [e f]	
58	n-Pr	CH	OH	Meta	107 ± 4[†] [c]	6.5[c]	5.6 [c]		
61	n-Pr	N	OSO_2Me	Meta	254 ± 18[†] [e]				
62	n-Pr	N	OSO_2CF_3	Meta	285 ± 12 [e]				
63	n-Pr	CH	OSO_2CF_3	Meta	241 ± 6 [e]				

Cont.

Comp	R'	X	R	Pos	DOPAC, % of ctrl ± SEM[a]	pK*i* (D_2^{High})[b]	pK*i* (D_2^{Low})[b]	pK*i* (MAO A)[b]	pK*i* (MAO B)[b]
68	n-Pr	CH	CN	Meta	275 ± 10 [e]				
69	n-Pr	CH	Oi-Pr	Meta	112 ± 6 [e]				
80	n-Pr	N	OMe	Meta	255 ± 16 [e]	6.5 [e]		4.8[e]	
81	n-Pr	CH	OMe	Meta	140 ± 8 [e]	5.8 [e]		4.7 [e]	
82	n-Pr	N	CN	Meta	314 ± 17 [e]	6.6 [e]		<3.2 [e f]	
83	n-Pr	N	CF_3	Meta	315 ± 15† [e]				
84	n-Pr	CH	CF_3	Meta	260 ± 9 [e]	6.7 [e]		3.9 [e]	
85	n-Pr	N	Cl	Meta	250 ± 9 [e]				
86	n-Pr	N	COMe	Meta	221 ± 9 [e]				
87	n-Pr	CH	Me	Meta	115 ± 7 [e]				
88	n-Pr	CH	SO_2i-Pr	Meta	205 ± 9 [e]				
89	n-Pr	CH	Ot-Bu	Meta	196 ± 9 [e]				
90	Bn	CH	OH	Meta	317 ± 13††	7.4 [c]	6.5 [c]		
48	n-Pr	CH	SO_2Me	Para	94 ± 5† [d]			<3.2[d f]	3.23 [d]
52	n-Pr	CH	morph	Para	38 ± 2 [d]			5.9[d]	4.89 [d]
53	n-Pr	N	OMe	Para	72 ± 3 [d]	5.2 [e]		5.9[d]	3.23[d]
54	n-Pr	CH	OMe	Para	22 ± 1 [d]	4.6 [e]		6.6[d]	3.66 [d]
65	n-Pr	N	OSO_2CF_3	Para	122 ± 3 [d]			4.8[d]	7.48 [d]
70	n-Pr	CH	On-Bu	Para	41 ± 2 [d]			6.4[d]	5.8 [d]
91	n-Pr	CH	CN	Para	94 ± 2 [d]			4.0[d]	3.23 [d]
92	n-Pr	CH	Cl	Para	68 ± 4 [d]	6.0 [e]		5.8[d]	4.42 [d]
93	n-Pr	CH	CF_3	Para	86 ± 5 [d]			5.2[d]	4.89 [d]

[a]Post-mortem biochemistry of levels of DOPAC in the striatum compared to saline control (n = 4) at 1h after administration of 100, †50 or ††33 μmol/kg of test compound (the dose where maximum DOPAC response is produced). [b]Negative logarithm of binding affinities (apparent K_i) to human recombinant HEK-293 cells with [^{3}H]7-OH-DPAT as ligand for D_2^{High} and [^{3}H]spiperone as ligand for D_2^{Low} and to rat cerebral cortex cells with [^{3}H]Ro 41-1049 as ligand for MAO A and Ro 16-6491 for MAO B. [c]Data from paper I. [d]Data from paper II. [e]Data from paper III. [f]C_{50} higher than 1 mM = K_i higher than 0.58 mM.

4.2.1. *In Vitro* Binding: D_2^{High}, D_2^{Low}, MAO A and MAO B and Intrinsic Activity at D_2 Receptors (Paper I-III). The starting point for the development of the new dopaminergic ligands in the 4-phenylpiperidine/-piperazine series was the partial agonist 3-(1-benzylpiperidin-4-yl)phenol (**29**) previously reported by Mewshaw *et al.*[75, 131] This compound has a high preference for displacing agonist over antagonist ligands at the D_2 receptor (i.e. high K*i*(D_2^{Low})/K*i*(D_2^{High}) ratio) and has therefore been classified as a potential partial agonist. In analogy with the development of *S*-(-)-OSU6162 (**15**) from the partial agonist (-)-3-PPP (**11**), we wanted to modify the key elements of **29** that are responsible for its intrinsic activity in order to produce compounds with little or no

intrinsic activity but with a sustained agonist-like interaction with the receptor. The portions of the molecule that we speculated as being most likely to contribute to stabilization of the active conformation were the phenol group, the piperazine and the large, aromatic *N*-alkyl group. Binding affinities of **29** to D_2^{Low} and D_2^{High} (Table 1) in our hands confirmed Mewshaw's results and in the functional assay **29** also showed a relatively high intrinsic activity (Table 2 in paper I), verifying that this compound indeed is a partial D_2 agonist. Any modification of the key elements in the structure led to a loss of efficacy in the functional assay while the $Ki(D_2^{Low})/Ki(D_2^{High})$ ratio, albeit significantly lower than for **29**, stayed ≥ 2, regardless of which portions were exchanged. However, *in vitro* assays measuring intrinsic activity can show varying results depending on which model that is used and it can therefore be difficult to determine the intrinsic activity observed *in vivo* with such assays.[67, 132]

Another interesting aspect of the interaction between the receptor and the ligand is the receptor kinetics. Many recent publications have presented fast dissociation from the D_2 receptor as a possible link to atypicality for the DA mediated antipsychotics used in the clinic.[54, 61, 133, 134] In a model using multiple washes of ligand-pretreated D_2 cells in order to detect how long it takes for the cell to regain responsiveness to DA, all tested compounds in this structural class displayed fast dissociation from the receptor (shown in Figure 2 of Paper I and as reported by Dyhring *et al.*[62]). This competitive interaction means that the effect of normal DA surges is less affected, which according to the theory would lead to an improved side effect profile. In sharp contrast, haloperidol had a slow dissociation, indicating a non-competitive antagonism where the inactive conformation of the DA receptor is stabilized and the responsiveness is diminished for a long time.

These intriguing results led us to a further exploration of how substitution of the phenyl ring affects the biological response. In order to investigate the effects of aromatic substitution and type of heterocycle, the *N*-alkyl moiety was set to 1-propyl. The substituent in mono-substituted phenylpiperidines/-piperazines can be located in three different positions; *ortho*, *meta* or *para* in relation to the heterocycle. All positions were investigated and the effect on the affinity, primarily to the D_2 receptor, was determined for a selected subgroup. Three compounds with substituents in the *ortho*-position were tested for their affinity to D_2 and the electron-donating methoxy group yielded a higher affinity than the corresponding electron-withdrawing methylsulfone. The meta-substituted subclass showed no such differences and the only compound having somewhat higher affinity to the D_2 receptor was the phenol-piperazine (**57**, Table 1). For compounds with substituents in the *para*-position, the D_2 receptor affinity was lower than for the corresponding

ortho- and *meta*-analogues and it seems that this position is not preferred for the interaction with this receptor.

However, affinity and intrinsic activity at D_2 was not sufficient to explain the observed neurochemical effects for all compounds and especially the *para*-substituted subclass differed greatly in their *in vivo* response compared to the other two positions. Their effects on the DOPAC levels led us to suspect that they were actually MAO inhibitors rather than D_2 ligands, and therefore the affinity to the two MAO isozymes was also measured. The affinity, primarily to MAO A, for the *para*-substituted compounds confirmed our hypothesis and moreover a strong correlation between the electronic properties of the substituent and the affinity to MAO A was observed. Affinity to MAO B was also apparent for a few of these compounds, but the correlation to the *in vivo* effects were absent in this class. Furthermore, in addition to the *para*-substituted class, MAO A affinity could be detected for the *ortho*- and *meta*-substituted compounds as well, albeit not as high and only secondary to the D_2 affinity. MAO A affinity was only measured for a few *ortho*- and *meta*-substituted compounds but the connection between electronic properties of the substituent and affinity to MAO A seems to be highly relevant in these positions as well. In order to explore which structural motifs that influence the *in vitro* effects, quantitative models with physicochemical descriptors were produced and these are addressed separately.

The D_2 receptor and MAO A are the targets with the most abundant impact on the DOPAC response in the brain. However, other targets are involved in the process of synthesis, storage, release, reuptake and metabolism of DA. COMT is involved in the metabolism of DA but in complete contrast to MAO A, inhibition of this enzyme leads to increased levels of DOPAC and decreased levels of 3-MT. However, all of the known COMT-inhibitors (e.g. tolcapone, entacapone) are structurally dissimilar to our compounds and it is therefore highly unlikely that this mechanism is involved in the observed *in vivo* effects. Dopamine and norepinephrine transporter (DAT and NAT) inhibitors also affect the DOPAC levels in striatum, albeit in general to a much lesser degree than compounds acting on the D_2 receptor or MAO A. A few of the compounds in the data set have been investigated in both DAT and NAT assays where the % displacement at 1 μM was determined (Appendix A). The only compounds displaying any relevant affinity are the *para*-susbtituted compounds **91** and **92**, which have 22% displacement for NET and 36% displacement for DAT, respectively. The limited impact on DOPAC and low affinity of our compounds makes it unlikely that these targets are of any major importance for the models. As mentioned in the introduction, the sequence homology between the TM regions of DA D_2 and DA D_3 receptors are

~75%, making subtype-selective ligands difficult to obtain. Even though we have no data to support it, the compounds presented here are most likely affecting the D_3 receptor very much the same way they affect D_2. However, since D_3-ligands have no relevant effect on the DOPAC levels, this interaction is insignificant for our correlations.[24]

4.2.2. *In Vivo* Effects: Neurochemistry and Locomotor Activity. In agreement with the *in vitro* effects, **29** displayed an *in vivo* profile that can be directly related to partial agonism. The DOPAC levels were unchanged within the whole dose range, and this can be attributed to a perfect balance of intrinsic activity at the DA releasing presynaptic receptors. As a full agonist produces a significant decrease in DOPAC levels compared to the untreated animals, partial agonists yield different DOPAC responses dependent on the level of intrinsic activity. In the case of **29** it is therefore likely that the activation of the presynaptic receptors is just enough to keep the release of DA at a rate comparable to the unaffected system. At the same time, this compound induces a strong inhibition of the locomotor activity, which indicates that the normal postsynaptic effect of DA is blocked and the agonist property of **29** is too low to stimulate the behaviour.

When modifying **29** the levels of DOPAC tended to increase dramatically, and as the binding affinities simultaneously were diminished (**29** has the highest affinity to D_2 of all tested 4-phenylpiperidines/-piperazines), decreased intrinsic activity is the likely explanation. However, in contrary to the intrinsic activity data from the *in vitro* efficacy model, a few of the compounds in this subgroup could be expected to have weak intrinsic activity *in vivo*. For example, **57** has a maximum DOPAC response of 260% of control and this level is reached at 33 μmol/kg (higher dose does not increase the DOPAC response). This indicates that the presynaptic effects are not fully antagonistic, despite the lack of intrinsic activity in the *in vitro* assay. Similar to **29**, **57** also induce an inhibition of the normal behaviour and this is most likely linked to the relatively high affinity to D_2 receptors. In Figure 5 of Paper I a strong correlation between affinity to D_2 receptors and locomotor activity can be observed. The set of compounds included in this correlation are both D_2 receptors antagonists and partial agonists and this indicates that the intrinsic activity, unless very high, does not affect the behaviour to any relevant degree.

Even if specific compounds are likely to be partial agonists and the *in vivo* model can reveal this, it is not a feasible method to use DOPAC levels as an indicator of intrinsic activity on the whole data set. Firstly, the potency of each compound differs and while some reaches the full effect at doses below 100 μmol/kg (the highest dose in the standard interval) others are likely to require

higher doses in order to reach the maximum possible response. This issue can be exemplified with **16**, for which the standard dose interval is not sufficient to achieve maximum DOPAC response and at a 100 μmol/kg the neurochemical profile could be mistaken for being a product of partial agonism. When increasing the dose to 300 μmol/kg, higher DOPAC levels, that instead indicate antagonist effects *in vivo*, are obtained. For **16** it is likely that 100 μmol/kg is not a sufficient dose to reach the maximum effect due to the low affinity (Table 1). However, even compounds with high affinity *in vitro* may require higher doses to achieve the maximum effect *in vivo*, since low bioavailability, poor penetration of the blood-brain barrier etc. can lead to low concentrations of the compound at the site of action. So unless the maximum DOPAC levels are high enough to rule out any intrinsic activity at the highest dose tested (e.g. **78**) or the maximum effect is produced at a dose below 100 μmol/kg (e.g. **57**), the DOPAC response can not by itself be used as an indicator for intrinsic activity. Moreover, the affinity and efficacy at D_2 receptors are not the only determining factors for the DOPAC response and other mechanisms must be considered. In the *para*-substituted class sharp decreases in DOPAC levels was observed and this was concluded to be connected to their inhibitory effects on MAO A rather than the effect on D_2 receptors. As it has become clear that substitution in the *ortho*- and *meta*-position also can lead to inhibition of MAO A, and thus a depressing effect on the DOPAC levels, it is most likely that we have two separate mechanisms producing the *in vivo* response. In order to more thoroughly investigate what mechanisms are connected to the *in vivo* response, binding data for both D_2 receptors and MAO A was acquired for a subset of molecules. Using Partial Least Square (PLS) regression[135-137], the p*Ki* values for D_2 and MAO A could be set against the DOPAC levels in a multivariate model and to our surprise a very strong correlation could be observed between the *in vivo* and *in vitro* effects (Figure 13).

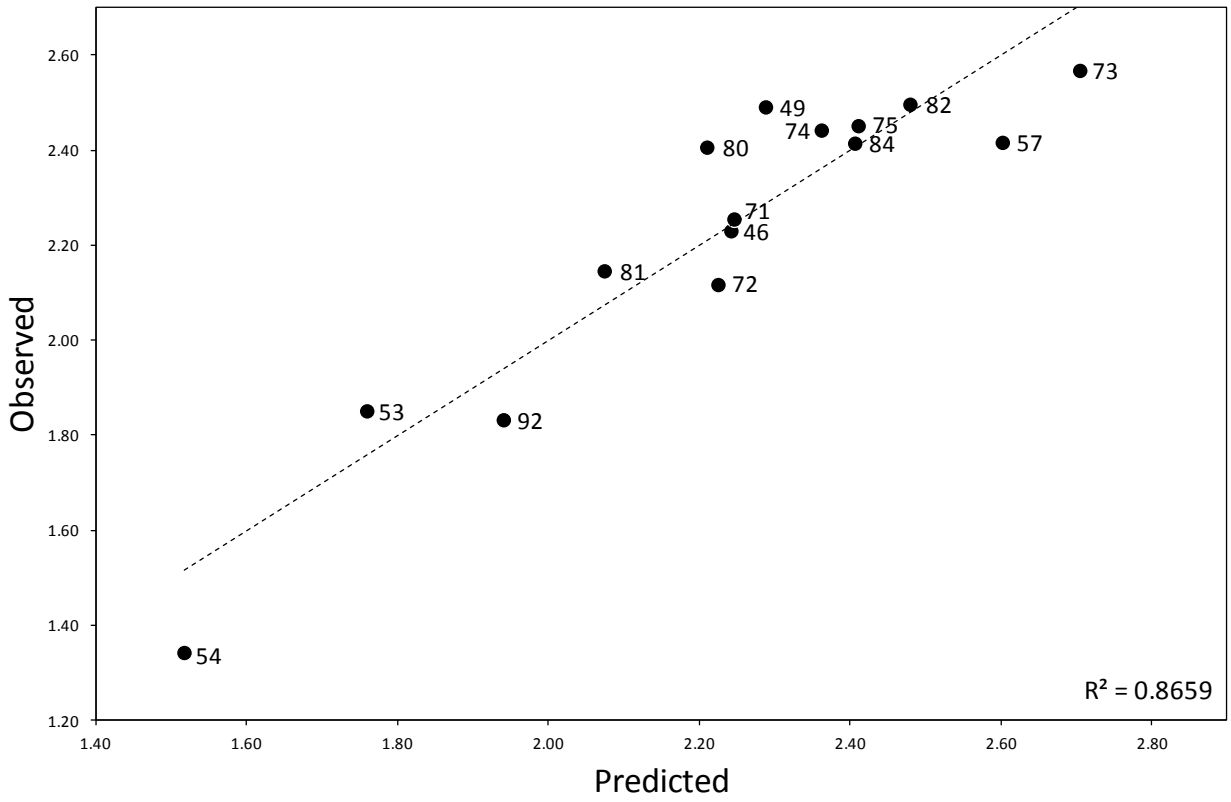


Figure 13. Relationship between observed versus predicted response in the PLS model of log(DOPAC) versus pK_i(MAO A) and $pK_i(D_2)$ for compounds in Table 1 that have binding data from both the D_2^{High} and MAO A assays.

This correlation led us to suspect that D_2 antagonism and MAO A inhibition are the dominating mechanisms behind the *in vivo* data for this structural class and that intrinsic activity at D_2 is secondary in affecting the DOPAC response.

Moreover, the position and physicochemical character of the substituent as well as choice of heterocycle is clearly of high relevance to both the *in vivo* and *in vitro* response. Although some SARs can be manifested from the data by qualitative methods, the relatively large data set makes the task at hand quite demanding. Thus, in order to further explore the effect of different aromatic substituents in different positions as well as the impact the choice of heterocycle has on the response, quantitative methods were employed.

5. Quantitative Structure Activity Relationships (QSARs)

The connection between structural motif and biological response in the mono-substituted phneylpiperidines/-piperazines is not easily interpreted and the use of qualitative descriptors combined with multivariate calculations is a way to illustrate which properties are related to each response in an efficient manner. Additionally, these models can be used to predict the response of new compounds with a desired pharmacological profile within this structural class.

5.1 QSAR models of *in vivo* and *in vitro* responses (Paper III)

The position of the aromatic substituent is clearly of great importance for both the *in vitro* and *in vivo* responses and the dataset was built on these premises. In addition, the physicochemical property of the substituent and the choice of heterocycle also influence the pharmacological effect. We therefore chose to include the following descriptors:

- A qualitative variable (QV) describing position relative to the heterocycle (i.e. none, *ortho*, *meta* and *para*)
- Descriptors for the whole molecule (i.e. clogP and LClogD)
- Seven descriptors that together describe the substituent's physicochemical character (i.e. hydrogen bond donating/-accepting properties (HD and HA), Hammet's electronic constants (σ_m and σ_p), group dipole moment (μR), volume (volR) and lipophilicity (π))
- Number of nitrogen atoms in the heterocycle (RingN) which in effect separates piperidines from piperazines.

QVs are discrete variables that for this model are split into four dummy variables (Rpos = none, *ortho*, *meta* or *para*) containing group-belongings (coded as 1 for yes and 0 for no). ClogP is a calculated descriptor that describes the lipophilicity of the whole compound. LClogD is an experimental descriptor that stems from HPLC retention times at pH 7.4 and therefore combines lipophilicity and ionization at physiological conditions. The substituent-descriptors are obtained from the literature (σ_m, σ_p and μR), calculated (volR and π) or coded as 1, 0.5 or 0 depending on their ability to participate in hydrogen bond (HD and HA). Recently published results[80] indicate that chlorine has some hydrogen accepting capacity and it is therefore set to 0.5.

The chosen descriptors were modelled against the maximum level of DOPAC and the affinity to MAO A and D_2 receptor respectively, using PLS regression.[135-137] The DOPAC model have the

largest number of observations (43) since *in vivo* data is present for all compounds in the data set. This yields a two component model with a R^2Y of 0.85 and a Q^2 of 0.78 which indicate good quality and high predictivity, especially for a model based on *in vivo* data.

The QSAR models with the *in vitro* binding affinities to MAO A and D_2 receptors, respectively, as response have fewer observations than the *in vivo* model (21 for MAO A and 17 for D_2). However, the number of observations still supersedes the number of descriptors which is usually desirable in order to avoid over-interpretation. For MAO A, a one component PLS regression model with $R^2Y = 0.68$ and $Q^2 = 0.53$ was obtained while the D_2 model had two components and $R^2Y = 0.82$ and $Q^2 = 0.54$. Comparing the values of Q^2, it becomes obvious that the *in vitro* models are less predictive than the DOPAC model. This may be related to the previous assumption, that the *in vivo* responses of these compounds are primarily a result of the combined *in vitro* binding affinities to MAO A and D_2 receptors. The DOPAC model can therefore be said to describe both the *in vitro* effects simultaneously, which in combination with the larger set of *in vivo* observations would help produce a better model.

The coefficient plots of the predictive components are a convenient way to interpret the QSAR models and get an overview of which structural elements that are important for each response. The plots show which descriptors have the highest influence on the response as well as if the influence is positively or negatively correlated to the effect. A general rule of thumb is that a descriptor that is positive for D_2 is also positive for DOPAC while a descriptor that is positive for MAO A is negative for DOPAC and vice versa. Thus, the sum of the impact a descriptor has on D_2 and MAO A is generally reflected in the impact the same descriptor has on DOPAC (Figure 14).

The position of the substituent is of great importance in all models. The *ortho*- and *meta*-position correlates positively with the DOPAC levels while a negative correlation is observed for the *para*-position (Figure 14). The opposite is true for MAO A affinity, where substitution in the *para*-position is essential for high affinity while *ortho* and *meta* have a negative impact. The position-effects in the D_2 model are similar to those of the DOPAC model, but the *meta*-position has only minimal influence on this response. Taken together, this explains how the location of the aromatic substituent is connected to the *in vivo* and *in vitro* effects and how these effects are related to each other. However, the response is also influenced by the physicochemical character of the substituent and the coefficient plots can help us understand these correlations.

The electronic components (σ_m, σ_p and μR) show that electron-withdrawing properties are positively correlated to the DOPAC response, negatively correlated to the MAO A affinity and of

minor importance to the affinity to D_2 receptors (Figure 14). This indicates that compounds with electron withdrawing groups have lower MAO A affinities, and with that higher DOPAC levels, compared to the compounds substituted with electron donating groups.

The size of the substituent (volR) affects the DOPAC response negatively and according to the coefficient plots from the *in vitro* models, this is related to a diminished binding to D_2. Larger groups are not well accepted according to the DOPAC response and a poor fit in the active site of the D_2 receptor is a possible explanation. The phenylsulfone (**51**) moiety is predicted to produce high DOPAC levels, probably linked to the fact that it is a piperazine-analogue with electron-withdrawing properties of the meta-substituent (both are positive for DOPAC increase). However, the observed response is much weaker than predicted (Figure 3, Paper III) and it is likely that this effect is connected to the size of the substituent.

Hydrogen donating/accepting properties are not a major determinant for the most part, but in the D_2 model the hydrogen donating groups yield high affinity. This can be directly related to the 3-hydroxyl group, which in general makes good substrates of dopamine-like compounds. However, the influence of HD on DOPAC is not in proportion to the influence on D_2 and this deviation is a further indication that the phenol contributes to intrinsic activity.

Lipophilicity (π and clogP) has a substantial positive impact on the MAO A affinity and no relevant influence on D_2 affinity. Following the general relationship, this would lead to lipophilicity having a negative correlation to DOPAC and yet the DOPAC response is not affected by this property. It is a common phenomenon that highly lipophilic compounds have better affinity to different targets *in vitro* than more polar analogues. However, this is not always relevant to the effect these compounds have in a living system. Plasma protein binding and the propensity to be metabolized by liver enzymes are only two examples of *in vivo* related mechanisms that may counteract the overall effect of a lipophilic compound, even if the interaction with the target itself is optimal. However, what abolishes the effect of lipophilicity on the DOPAC response in this case remains to be investigated.

The number of nitrogen atoms in the ring shows a positive correlation to DOPAC and these effects are related to the impact the choice of heterocycle has on both MAO A and D_2 affinity. Piperazines have lower affinity to MAO A than the piperidines which are manifested by the negative bar in the coefficient plot from the MAO A model (Figure 14). The opposite is true for D_2 were piperazines tend to have higher affinity than the piperidines. Thus the negative correlation between MAO A and DOPAC together with the positive correlation between D_2 and DOPAC make

the *in vitro* effects of this property additive towards the *in vivo* response. The experimental descriptor LClogD is also highly affected by the choice of heterocycle. The retention time from HPLC which this descriptor is based on is to a large extent affected by the level of ionization of the tested compound. Piperazines and piperidines have different pK_a values and the ionization at physiological pH therefore differs, subsequently leading to variations in the retention time. Piperazines have lower pKa than the corresponding piperidines and are therefore less ionized at pH 7.4. This in turn leads to longer retention-times which are expressed as higher LClogD-values (method for calculating LClogD is described in the method-section of Papers II and III). Albeit highly influential, the level of ionization is only in part determining the retention-time in the HPLC. Lipophilicity of the compound is also a major contributor and since the column used in the method is llipophilic (reversed phase), more lipophilic compounds yields higher values. LClogD can therefore be said to be the sum of the lipohilicity of a compound and its propensity to be ionized at physiological pH and these two properties are basically described by clogP and Ring N. This also become obvious when observing the coefficient plots, where the direction and significance of the LClogD-descriptor is more or less the sum of clogP and Ring N.

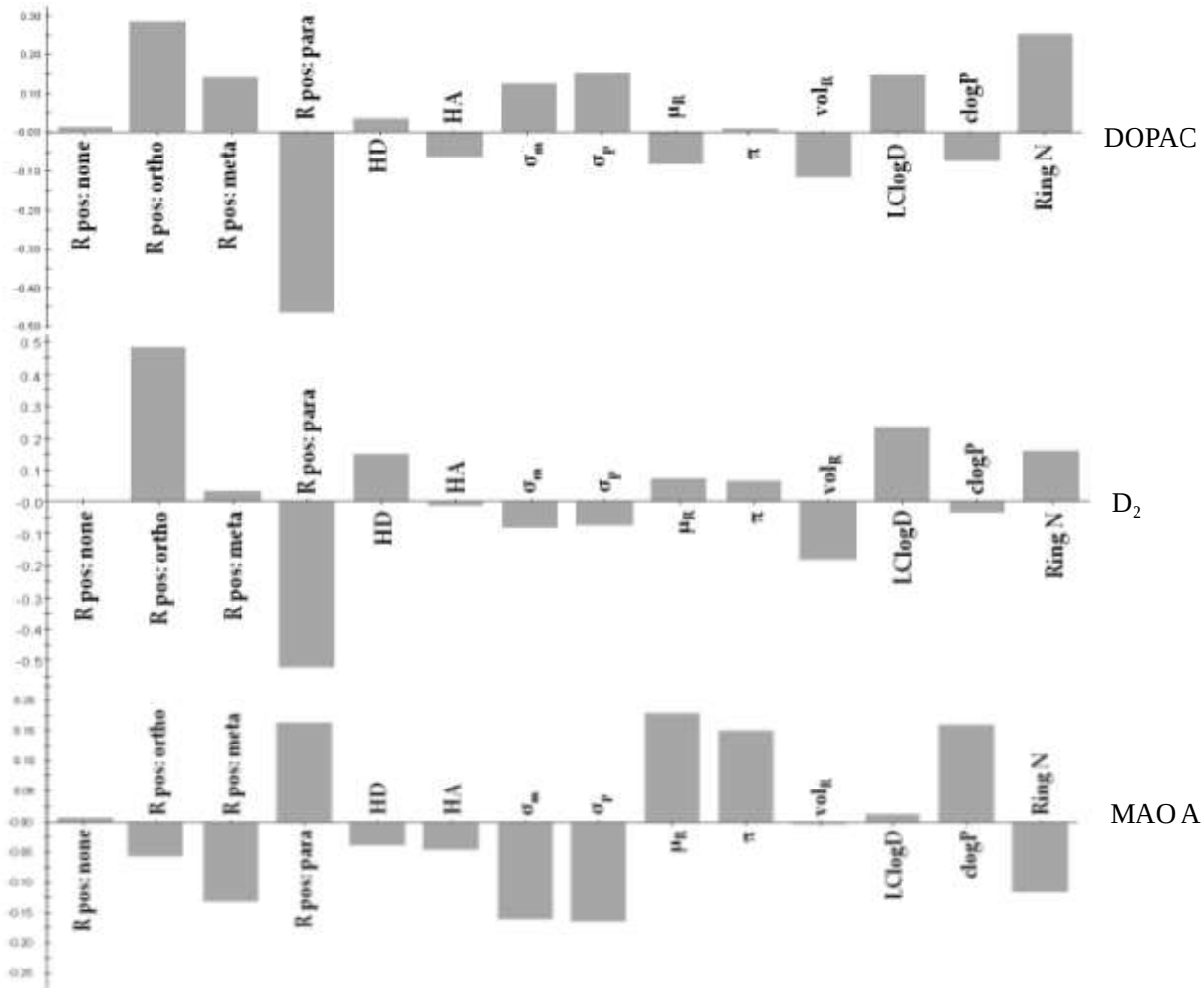


Figure 14. PLS coefficient plots of the predictive components from each of the QSAR models with DOPAC, D_2 and MAO A, respectively, as response.

5.2 Development of new electronic descriptors (Paper IV)

The relationship between electronic properties of the substituent and the DOPAC response made us interested in further investigating these effects. The most common descriptors in QSAR modelling are the Hammet's constants σ_m and σ_p. Although useful in describing basic electron-withdrawing/donating properties, these descriptors are not developed for explaining the physicochemical character of substituted aromatics in a biological system but rather the reactivity of an aromatic carboxylic acid with the substituent located in the *meta*- (σ_m) or *para*- (σ_p) position. In addition, the Hammett constants are derived experimentally and therefore values for substituents that are not tabulated in the literature will be difficult to obtain. On these premises we sought to find an alternative to the classical constants using quantum mechanical calculations to get descriptors

that were interpretable, calculable and significant for use in QSAR models. The method used to calculate these new descriptors is described in the methods section of Paper IV.

The calculated descriptors were assessed in order to reproduce σ_m, σ_p and the absolute value of the group dipole moment (μ) in a PLS regression model. This analysis clearly showed that the two sets of descriptors are highly correlated (see Figure 4 in Paper IV) and we therefore decided to exchange the common electronic descriptors in each of the QSAR models for DOPAC, D_2 and MAO A with these new descriptors, while leaving all the other parameters unaffected. The new models are very similar to the original models in terms of R^2Y and Q^2 and the combined plots of observed versus predicted DOPAC models, with the classical or the new electronic descriptors, shown in Figure 15 is a good illustration of the existing correlations. In addition, the impact of the electronic properties in each of the *in vitro* models was investigated by removing the electronic descriptors and regenerating the model without them. In the D_2 model, the predictivity (Q^2) actually increased when the electronic descriptors were removed, while for the MAO A model a sharp decrease in Q^2 followed the exclusion (see Table 2, Paper IV). These results gave further support to the previously established theory, that electronic properties are important mainly for the affinity to MAO A and that this is the main mechanism behind the influence of electronic properties on the DOPAC response.

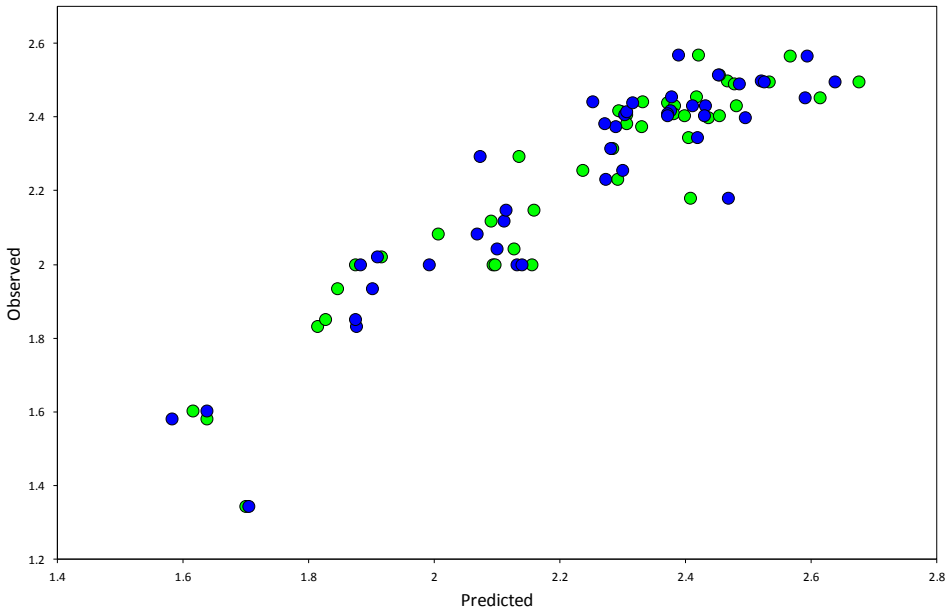


Figure 15. The observed versus predicted levels of DOPAC in striatum as log(percent of control). Green: QSAR based on QM descriptors. Blue: The Pettersson *et.al* QSAR based on empirical parameters.

6. Ligand-Target interactions at MAO A and D_2 receptors

The interaction between the *para*-substituted phenylpiperidines/-piperazines and the MAO A enzyme was exemplified by compound **54**, which was fitted in the active site by means of molecular modelling based on an X-ray crystal structure of MAO A (Figure 4, Paper II). This model revealed a hydrophobic pocket in which the aromatic substituent fitted while an interaction between the methoxy-oxygen and Cys323 further stabilized the complex. The binding pose of **54** is very similar to the 4-substituted phenethylamines presented by Gallardo-Godoy *et al.*[138] and it is interesting to compare how substitution in other positions of the aromatic ring affects the MAO A affinity in the two structural classes. The 2- and 3-position of the phenethylamines are unfavourable compared to the 4-position, very much like substitution in *ortho*- or *meta*-position in the phenylpiperidines/-piperazines are unfavourable compared to the *para*-substituted analogues. Gallardo-Godoy *et al.* proposed that sterically disfavoured areas, especially where the 3-substituents are located, led to the diminished affinity. The substituents in the phenethylamine series are primarily alkoxy-groups and a comparison of positional effects with the methoxy-substituted phenylpiperidines (**74**, **81** and **54**) reveal that affinity to MAO A follow the same trend in both structural classes (i.e. *para*>*ortho*>*meta*). The same relationship is however not seen with the phenylpiperazines, where a methoxy-group in the *ortho*-position (**73**) rendered a compound inactive in the MAO A assay while the corresponding *meta*-analogue (**80**) had the same affinity as the piperidine. Gallardo-Godoys group proposed a π-π stacking interaction between the aromatic ring of the phenethylamines and Phe208 in the active site of MAO A and thus a possible explanation to the deviating effects of the phenylpiperazines is a different character of the π-system. Since a lone pair on the anilinic nitrogen is partly delocalized, the π-π stacking interaction between the aromatic ring and the MAO A receptor may be disturbed, leading to a generally weaker interaction. Another possibility is that the angle between the phenyl and heterocyclic rings are important for the interaction with MAO A and since these differ in the low-energy conformations of phenylpiperidine and phenylpiperazine (see below), this could affect the affinity. Regardless of the reason, it is likely that the phenylpiperidines and phenethylamines interact with the active site of MAO A in a similar fashion while in the phenylpiperazine class some of these interactions are different.

The interaction between the ligand and the D_2 receptor is decisive for both the affinity and intrinsic activity and it is therefore of interest to elucidate what structural motifs in the phenylpiperidines/-piperazines that are important for both these properties. We have concluded that 3-(1-benzylpiperidin-4-yl)phenol (**29**) has high affinity to D_2 receptors and intrinsic activity both *in*

vitro and *in vivo*. The structural properties that we expected to be responsible for these effects are the phenylic 3-hydroxyl group, the piperazine and the *N*-benzyl. The 3-hydroxyl group is a mimic of the catechol in dopamine and therefore an important motif in the interaction with the D_2 receptor. Exchanging this group with a methylsulfon (**50**) leads to lower affinity to both D_2^{Low} and D_2^{High}, a major drop in the Ki^{Low}/Ki^{High} ratio and a complete loss of intrinsic activity *in vitro*. It is therefore safe to say that the phenol in **29** is highly involved in the stabilization of the active conformation of the D_2 receptor. The distance between the aromatic ring and the nitrogen atom of the basic amino group differ between DA and phenylpiperidines/-piperazines and thus the compounds in our data set are less "dopamine-like" than compounds based on the phenethylamine backbone (e.g. the 3-phenylpiperidines described by Sonesson *et al.*[139]). Studies have shown that for the 4-phenylpiperidines, the most stable conformation is when the piperidine ring and the aromatic ring are perpendicular to each other, while for phenylpiperazines co-planarity between the piperazine and aromatic ring is the most stable conformation (due to the sp^2 hybridization of the anilinic nitrogen).[131, 140] Rotation from these conformations in order to get a better fit in the receptor costs energy and such "energy-penalties" are associated with lower affinities and less intrinsic activity.[92] It is therefore likely that the angle between the phenyl and piperidine in **90** is less optimal for the ligand-receptor interaction than it is for the corresponding piperazine (**29**) and that this is the reason for the lower affinity and less intrinsic activity of **90**. We have also made MMF94S energy minimization studies[141, 142] on the 3-hydroxypiperazine (**57**) and 3-methylsulfonpiperidine (**16**) in order to compare the angle between the aromatic ring and the heterocycle for the low energy conformations. These studies show that, in accordance with previous studies, the phenyl and piperazine rings are co-planar while the phenyl and piperidine are perpendicular (Figure 16).

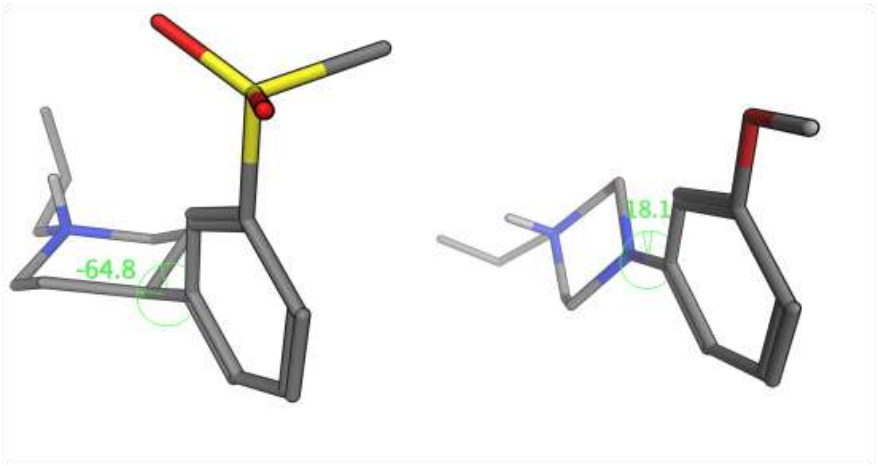


Figure 16. The 3D structures of **16** (left) and **57** (right) in their respective low energy conformation.

The piperazines in general have higher affinity to D_2 receptors than the corresponding piperidines and the *in vivo* effects also reflect this. Therefore the angle between the aromatic ring and the heterocycle is likely to be important and may suggest that the stable conformation of the piperazine is more optimal for the interaction with the DA D_2 receptors compared to the piperidines. There are however some deviations from this rule. The unsubstituted and the *ortho*-methoxy substituted phenylpiperidines (i.e. **72** and **74**, respectively) have higher affinity to D_2 receptors than the corresponding phenylpiperazines (**71** and **73**, respectively). Lacking substituents in the aromatic ring (i.e. no substituent in the *meta*-position that needs to recognize an interaction-point in the receptor) leaves the hydrophobic interactions as the most important factor for stabilization of the ligand receptor complex. These interactions are likely to be less sensitive to an optimal conformation of the piperidine/piperazine which would eliminate the energy-penalty, and thus the lower affinity, for the phenylpiperidine. In addition, other properties, such as lipophilicity, would become more important for the affinity and that may be the reason for the more lipophilic piperidine to bind harder than the corresponding piperazine. In a study by Dijkstra *et al.*[140], the conformation of arylpiperidines/-piperazines with substitution in different positions has been studied and it is obvious that the orientation between the aryl and heterocyclic rings is highly influenced by the location of the substituent. *Ortho*-substitution inevitably forces the heterocycle towards a perpendicular orientation in relation to the aryl, regardless of whether the heterocycle is a piperidine or piperazine. This means that the *ortho*-substituted phenylpiperidine/-piperazine all have the same phenyl-heterocycle conformation and therefore no major difference in affinity to D_2 are observed.

The *N*-alkyl group is also of some importance as exchanging the benzyl in **29** to a propyl-group (**57**) led both to a diminished affinity and a loss of intrinsic activity *in vitro*. The same replacement for the corresponding piperidines (**90** and **58**) also led to an attenuated affinity, indicating that the extra aromatic ring is beneficial for the ligand-receptor interaction in the phenol series. However, for the methylsulfones, the drop in affinity was not observed for the piperazines (**50** and **49**) whereas for the piperidines (**40** and **16**), the *N*-alkyl effect was similar to the phenols. This is further implications that interactions between specific parts of the ligands and the receptor are more or less important depending on which other interactions are present.

Even if the *in vitro* model shows no intrinsic activity for the tested *N*-propyl analogues, the *in vivo* data indicate that some of the meta-substituted compounds are likely to act as partial agonists. Due to the structural resemblance to the ligands modelled in the D_3 receptor by Newman *et al.*[79], we decided it to be relevant to use this work as a reference for the proposed interactions of the

compounds in our data set. However, the interaction between the *meta*-chloro of 2,3-dichlorophenylpiperazine and Ser194 (5.43) on TM5 that Newman *et al*. has claimed to lead to a stabilization of the active conformation of D_3, is depending on the absence of *N*-alkyl on the piperazine. With an N-alkyl group present the compounds in this model got twisted in such a way that the bond between the chlorine and oxygen could not be formed. Yet even with an *N*-alkyl group as long as butyl, some intrinsic activity could be detected in the Go BRET assay, indicating that the receptor was not completely stabilized in the inactive conformation. In the same model, the 2-methoxyphenylpiperazine could not interact with the serine in a way that led to a stabilization of the active conformation and subsequently the intrinsic activity was lower than for the corresponding dichloro-analogue. The homology between D_2 and D_3 is high with similar 3D conformations, but some differences have been observed, especially in the extracellular loop regions.[68] Since some of the meta-substituted compounds in our series are thought to act as partial agonists at D_2 while the *ortho*-substituted compounds all have antagonist profiles, we expect that the ligand-receptor interaction of our compounds is similar to the Newman model.

Besides the intrinsic activity, the *in vivo* response is influenced by both the affinity to MAO A and the potency at D_2 receptors and it is therefore hard to decide if a compound acts as a partial agonist based on the DOPAC levels alone. However, the *meta*-hydroxyl compounds **57** and **58** are likely to have intrinsic activity based on their affinity to MAO A/D_2 and DOPAC levels (although **58** have only been tested *in vivo* up to 50 μmol/kg) and **57** also has lower DOPAC than predicted in the *in vivo*/*in vitro* correlation (Figure 13). The hydroxyl-group is both a good hydrogen bond donor and acceptor and a hydrogen bond to a serine or a histidine residue in the active site, leading to a stabilization of the active conformation of D_2, is therefore likely. And if this is possible for the *meta*-hydroxy compounds, other analogues with hydrogen-bonding *meta*-substituents could also interact in this manner.

The π-π interactions are an important class of noncovalent ligand−receptor interactions and have been proposed to be involved in the stabilization of the active conformation of D_2.[73, 92, 143-145] A good example of this is (*S*)-dipropyl-2-aminotetralin (*S*-DPAT), which lack aromatic substituents but is still a full DA D_2 agonist.[56, 58] Furthermore, the electron withdrawing/donating properties of aromatic substituents are related to the electron density of the aromatic ring, which in turn has an impact on the proneness of the ring to participate in a π-π interaction.[146, 147] In the phenylpiperidine/-piperazine class, it is possible that the electronic properties of the substituents affect the ability of the aromatic ring to π-interact with the receptor and thus influence the intrinsic

activity. Although the impact of the electronic properties on the DOPAC response is connected primarily to the MAO A affinity, intrinsic activity could be an additional link in the relationship between structure, *in vivo* response and *in vitro* response.

Compound **16** has been fitted in the active site of the D_2 receptor using the docking method published by Malo *et al.*[92] (Figure 17) and according to this model the oxygen atoms in the methylsulfone-group show possible hydrogen bond interactions with both Ser193 and His393 while the protonated nitrogen interacts with Asp114. In addition, a hydrophobic interaction is feasible between the phenyl ring of the ligand and Phe389 in the binding site. The "agonist-fit" of **16** together with the low affinity is the likely reasons for the unique profile of this compound. Since **16** prefer the high-affinity state of the D_2 receptor it is more prone to bind when DA is present and it is also able to displace the endogenous ligand. However, once DA is displaced, the low affinity of **16** enables it to leave the receptor quickly and thus make way for DA to bind again. The impact on normal DA surges is therefore minimal while the hyperdopaminergic states can be efficiently inhibited.

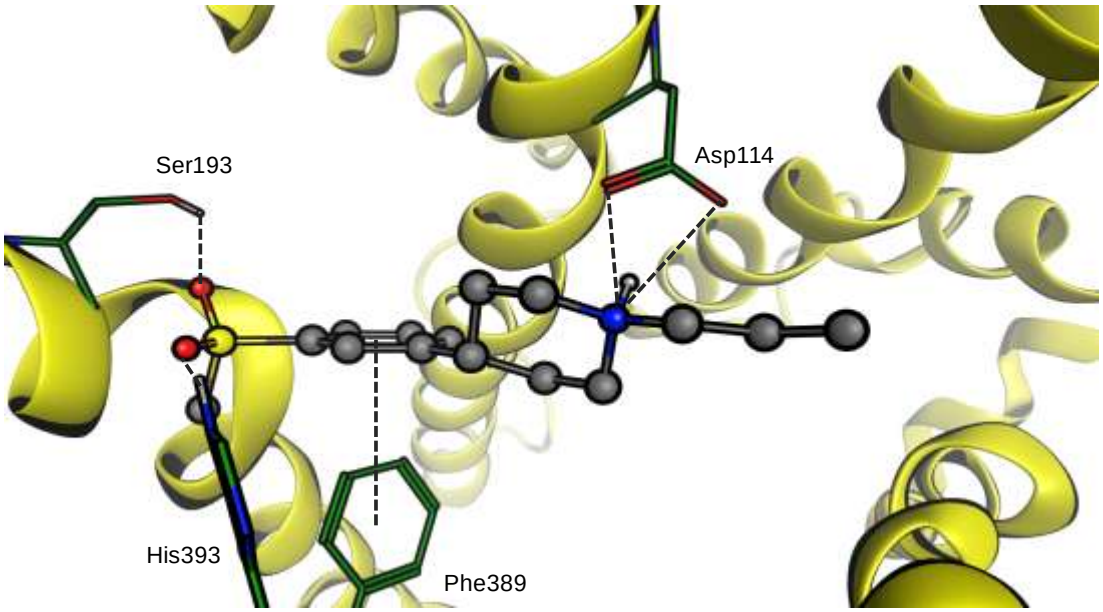


Figure 17. Pridopidine (**16**) in the active site of D_2 with possible interactions to aromatic acids marked (dotted lines).

7. Concluding remarks

A set of mono-substituted 4-phenylpiperidines and 4-phenylpiperazines have been synthesized and evaluated *in vivo* and *in vitro* for their effect on the dopaminergic system. The levels of DOPAC in striatum were measured for all compounds and the behavioural effects were reported for a chosen subset. Binding affinities to D_2 and MAO A as well as intrinsic activity and receptor kinetics was determined for some of the compounds in order to investigate the mechanisms behind the *in vivo* effects. Based on these data, the structural requirements for intrinsic activity at D_2 in this class of compounds have been elucidated and a method for obtaining D_2 antagonists which preferentially displaces an agonist over an antagonist and have fast dissociation rates from the receptor are described. This has also led to the discovery of the dopaminergic stabilizer pridopidine which has been shown to display low affinity and surmountable D_2 antagonism with a preference for binding to the active conformation of the receptor. These properties, together with a fast dissociation rate once bound to the receptor, would allow an attenuated physiological neurotransmission to persist in the normal state while hyperdopaminergic conditions are effectively inhibited.

In addition, QSAR models with physicochemical descriptors set against the different pharmacological responses (i.e. DOPAC, K*i*(D_2) and K*i*(MAO A)) have led to an improved understanding of how the observed effects are related. A strong correlation between the affinities to D_2 and MAO A and the levels of DOPAC in striatum has been established and the structural properties that are linked to each response have been annotated. The location of the aromatic substituent have proven utterly important for the pharmacological effects and distinct properties, such as D_2 antagonism, D_2 partial agonism and MAO A inhibition, can be produced by substituting different positions with functional groups of altering physicochemical properties. An electron-donating substituent in the *para*-position produces a MAO A inhibitor, having only minor interactions with the D_2 receptor. Any substituent in the *ortho*-position yields a D_2 antagonist while the *meta*-substituted compounds are more diverse, acting either as D_2 antagonists or partial agonists. Although the *ortho*- and *meta*-substituted compounds mainly affect the D_2 receptor, they can also have some MAO affinity depending on the physicochemical properties of the substituent.

The QSAR models and assimilated understanding of the mechanisms underlying the *in vivo* effects can be used to discover novel dopaminergic ligands with a desired pharmacological profile and future use as CNS active drugs for a wide variety of diseases and symptoms.

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My family
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My wife Jeevani, for your love and devotion that got me through this ordeal. I love you very much.
Sofia, min älskade dotter. Förlåt att jag varit borta så mycket, nu kan vi äntligen åka och bada.

9. Appendices

Appendix A

Table A1. Singlepoint displacement data of selected compounds on DAT and NET

Compound	DAT (h) (1µM)	NET (r) (1µM)	NET (h) (1µM)
16		9	
47		10	
48	6		-1
54	-9		11
68		-12	
73	-5		-4
76	7		-3
82		-13	
83	-12		-4
84	-2	-9	0
91	-2		22
92	36		11
93	-3		5

The results are expressed as a percent of control specific binding obtained in the presence of the test compounds from the assays listed below. Data are obtained from Cerep (Poitiers, France).

Assay Reference Compound	IC_{50} (M)	K_i (M)	n_H
NE transporter (h)			
protriptyline	5.5E-09	4.1E-09	*1.4*
DA transporter (h)			
BTCP	1.1E-08	5.6E-09	*1.2*

10. References

1. Bach, A. W.; Lan, N. C.; Johnson, D. L.; Abell, C. W.; Bembenek, M. E.; Kwan, S. W.; Seeburg, P. H.; Shih, J. C. cDNA cloning of human liver monoamine oxidase A and B: molecular basis of differences in enzymatic properties. *Proc Natl Acad Sci U S A* **1988,** 85, 4934-8.
2. Ito, A.; Kuwahara, T.; Inadome, S.; Sagara, Y. Molecular cloning of a cDNA for rat liver monoamine oxidase B. *Biochem Biophys Res Commun* **1988,** 157, 970-6.
3. Johnston, J. P. Some observations upon a new inhibitor of monoamine oxidase in brain tissue. *Biochem Pharmacol* **1968,** 17, 1285-97.
4. Kuwahara, T.; Takamoto, S.; Ito, A. Primary structure of rat monoamine oxidase A deduced from cDNA and its expression in rat tissues. *Agric Biol Chem* **1990,** 54, 253-7.
5. Kwan, S. W.; Abell, C. W. cDNA cloning and sequencing of rat monoamine oxidase A: comparison with the human and bovine enzymes. *Comp Biochem Physiol B* **1992,** 102, 143-7.
6. Waldmeier, P. C.; Delini-Stula, A.; Maitre, L. Preferential deamination of dopamine by an A type monoamine oxidase in rat brain. *Naunyn Schmiedebergs Arch Pharmacol* **1976,** 292, 9-14.
7. Green, A. R.; Mitchell, B. D.; Tordoff, A. F.; Youdim, M. B. Evidence for dopamine deamination by both type A and type B monoamine oxidase in rat brain in vivo and for the degree of inhibition of enzyme necessary for increased functional activity of dopamine and 5-hydroxytryptamine. *Br J Pharmacol* **1977,** 60, 343-9.
8. Neff, N. H.; Yang, H. Y.; Fuentes, J. A. Proceedings: The use of selective MAO inhibitor drugs to modify amine metabolism in brain. *Psychopharmacol Bull* **1974,** 10, 9-10.
9. Demarest, K. T.; Smith, D. J.; Azzaro, A. J. The presence of the type A form of monoamine oxidase within nigrostriatal dopamine-containing neurons. *J Pharm Exp Ther* **1980,** 215, 461-8.
10. Francis, A.; Pearce, L. B.; Roth, J. A. Cellular localization of MAO A and B in brain: evidence from kainic acid lesions in striatum. *Brain Res* **1985,** 334, 59-64.
11. Kato, T.; Dong, B.; Ishii, K.; Kinemuchi, H. Brain dialysis: in vivo metabolism of dopamine and serotonin by monoamine oxidase A but not B in the striatum of unrestrained rats. *J Neurochem* **1986,** 46, 1277-1282.
12. Kebabian, J. W.; Calne, D. B. Multiple receptors for dopamine. *Nature* **1979,** 277, 93-96.
13. Andersen, P. H.; Gingrich, J. A.; Bates, M. D.; Dearry, A.; Falardeau, P.; Senogles, S. E.; Caron, M. G. Dopamine receptor subtypes: beyond the D1/D2 classification. *Trends Pharmacol Sci* **1990,** 11, 231-236.
14. Civelli, O.; Bunzow, J. R.; Grandy, D. K. Molecular diversity of the dopamine receptors. *Annu Rev Pharmacol Toxicol* **1993,** 33, 281-307.

15. Sibley, D. R.; Monsma, F. J., Jr. Molecular biology of dopamine receptors. *Trends Pharmacol Sci* **1992,** 13, 61-69.
16. Sokoloff, P.; Martres, M. P.; Giros, B.; Bouthenet, M. L.; Schwartz, J. C. Heterogeneity and regulation of central dopamine receptor subtypes studied by cDNA cloning methodology. *Neurochem Int* **1992,** 20 Suppl, 27S-32S.
17. Vallone, D.; Picetti, R.; Borrelli, E. Structure and function of dopamine receptors. *Neurosci Biobehav Rev* **2000,** 24, 125-132.
18. Tiberi, M.; Jarvie, K. R.; Silvia, C.; Falardeau, P.; Gingrich, J. A.; Godinot, N.; Bertrand, L.; Yang-Feng, T. L.; Fremeau, R. T., Jr.; Caron, M. G. Cloning, molecular characterization, and chromosomal assignment of a gene encoding a second D1 dopamine receptor subtype: differential expression pattern in rat brain compared with the D1A receptor. *Proc Natl Acad Sci U S A* **1991,** 88, 7491-7495.
19. Niznik, H. B.; Van Tol, H. H. Dopamine receptor genes: new tools for molecular psychiatry. *J Psychiatry Neurosci* **1992,** 17, 158-180.
20. Sibley, D. R.; Lean, A. D.; Creese, I. Anterior pituitary dopamine receptors. Demonstration of interconvertible high and low affinity states of the D-2 dopamine receptor. *The Journal of biological chemistry* **1982,** 257, 6351-6361.
21. Beaulieu, J. M.; Gainetdinov, R. R. The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacol Rev* **2011,** 63, 182-217.
22. Svensson, K.; Carlsson, A.; Huff, R. M.; Kling-Petersen, T.; Waters, N. Behavioral and neurochemical data suggest functional differences between dopamine D2 and D3 receptors. *Eur J Pharmacol* **1994,** 263, 235-243.
23. Waters, N.; Lagerkvist, S.; Lövfberg, L.; Piercey, M.; Carlsson, A. The dopamine D3 receptor and autoreceptor preferring antagonists (+)-AJ76 and (+)-UH232; a microdialysis study. *European Journal of Pharmacology* **1993,** 242, 151-163.
24. Waters, N.; Lofberg, L.; Haadsma-Svensson, S.; Svensson, K.; Sonesson, C.; Carlsson, A. Differential effects of dopamine D2 and D3 receptor antagonists in regard to dopamine release, in vivo receptor displacement and behaviour. *J Neural Transm Gen Sect* **1994,** 98, 39-55.
25. Waters, N.; Svensson, K.; Haadsma-Svensson, S. R.; Smith, M. W.; Carlsson, A. The dopamine D3-receptor: a postsynaptic receptor inhibitory on rat locomotor activity. *J Neural Transm Gen Sect* **1993,** 94, 11-19.
26. Joyce, J. N. Dopamine D3 receptor as a therapeutic target for antipsychotic and antiparkinsonian drugs. *Pharmacol Ther* **2001,** 90, 231-259.

27. Sokoloff, P.; Giros, B.; Martres, M. P.; Bouthenet, M. L.; Schwartz, J. C. Molecular cloning and characterization of a novel dopamine receptor (D_3) as a target for neuroleptics. *Nature* **1990,** 347, 146-151.
28. Giros, B.; Sokoloff, P.; Martres, M. P.; Riou, J. F.; Emorine, L. J.; Schwartz, J. C. Alternative splicing directs the expression of two D2 dopamine receptor isoforms. *Nature* **1989,** 342, 923-936.
29. Monsma, F. J., Jr.; Mahan, L. C.; McVittie, L. D.; Gerfen, C. R.; Sibley, D. R. Molecular cloning and expression of a D_1 dopamine receptor linked to adenylyl cyclase activation. *Proc Natl Acad Sci U S A* **1990,** 87, 6723-6727.
30. De Mei, C.; Ramos, M.; Iitaka, C.; Borrelli, E. Getting specialized: presynaptic and postsynaptic dopamine D2 receptors. *Curr Opin Pharmacol* **2009,** 9, 53-58.
31. Usiello, A.; Baik, J. H.; Rouge-Pont, F.; Picetti, R.; Dierich, A.; LeMeur, M.; Piazza, P. V.; Borrelli, E. Distinct functions of the two isoforms of dopamine D_2 receptors. *Nature* **2000,** 408, 199-203.
32. Missale, C.; Nash, S. R.; Robinson, S. W.; Jaber, M.; Caron, M. G. Dopamine receptors: from structure to function. *Physiol Rev* **1998,** 78, 189-225.
33. Sibley, D. R. New insights into dopaminergic receptor function using antisense and genetically altered animals. *Annu Rev Pharmacol Toxicol* **1999,** 39, 313-341.
34. Wolf, M. E.; Roth, R. H. Autoreceptor regulation of dopamine synthesis. *Ann N Y Acad Sci* **1990,** 604, 323-343.
35. George, S. R.; Watanabe, M.; Paolo, T. D.; Falardeau, P.; Labrie, F.; Seeman, P. The functional state of the dopamine receptor in the anterior pituitary is in the high affinity form *Endocrinology* **1985,** 117, 690-697.
36. Benes, F. M. Carlsson and the discovery of dopamine. *Trends in Pharmacological Sciences* **2001,** 22, 46-47.
37. Carlsson, A.; Lindqvist, M.; Magnusson, T. 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. *Nature* **1957,** 180, 1200.
38. Carlsson, A. The occurrence, distribution and physiological role of catecholamines in the nervous system. *Pharmacol Rev* **1959,** 11, 490-493.
39. Ehringer, H.; Hornykiewicz, O. [Distribution of noradrenaline and dopamine (3-hydroxytyramine) in the human brain and their behavior in diseases of the extrapyramidal system]. *Klin Wochenschr* **1960,** 38, 1236-1239.
40. Di Chiara, G.; Bassareo, V. Reward system and addiction: what dopamine does and doesn't do. *Curr Opin Pharmacol* **2007,** 7, 69-76.
41. Di Chiara, G.; Bassareo, V.; Fenu, S.; De Luca, M. A.; Spina, L.; Cadoni, C.; Acquas, E.; Carboni, E.; Valentini, V.; Lecca, D. Dopamine and drug addiction: the nucleus accumbens shell connection. *Neuropharmacology* **2004,** 47 Suppl 1, 227-241.

42. Franken, I. H.; Booij, J.; van den Brink, W. The role of dopamine in human addiction: from reward to motivated attention. *Eur J Pharmacol* **2005,** 526, 199-206.

43. Melis, M.; Spiga, S.; Diana, M. The dopamine hypothesis of drug addiction: hypodopaminergic state. *Int Rev Neurobiol* **2005,** 63, 101-154.

44. Andreasen, N. C. Negative v positive schizophrenia. Definition and validation. *Arch Gen Psychiatry.* **1982,** 39, 789-794.

45. López-Muñoz, F.; *et al.* History of the discovery and clinical introduction of chlorpromazine. *Ann Clin Psychiatry.* **2005,** 17, 113-135.

46. Shen, W. W.; *et al.* A history of antipsychotic drug development. *Compr Psychiatry.* **1999,** 40, 407-414.

47. Kane, J. M.; *et al.* A review of their pharmacology and therapeutic potential. *Drugs* **1993,** 5, 74-93.

48. Casey, D. E. Neuroleptic drug-induced extrapyramidal syndromes and tardive dyskinesia. *Schizophrenia Research* **1991,** 4, 109-120.

49. Casey, D. E.; Keepers, G. A. Neuroleptic side effects: acute extrapyramidal syndromes and tardive dyskinesia. *Psychopharmacology Series* **1988,** 5, 74-93.

50. Zheng, G.; Dwoskin, L.; Crooks, P. Vesicular monoamine transporter 2: Role as a novel target for drug development. *The AAPS Journal* **2006,** 8, E682-E692.

51. Strange, P. G. Antipsychotic drug action: antagonism, inverse agonism or partial agonism. *Trends in Pharmacological Sciences* **2008,** 29, 314-321.

52. Fitzgerald, P.; Dinan, T. G. Prolactin and dopamine: what is the connection? A review article. *J Psychopharmacol* **2008,** 22, 12-19.

53. Kapur, S.; Roy, P.; Daskalakis, J.; Remington, G.; Zipursky, R. Increased dopamine D_2 receptor occupancy and elevated prolactin level associated with addition of haloperidol to clozapine. *Am J Psychiatry* **2001,** 158, 311-314.

54. Seeman, P. Atypical antipsychotics: mechanism of action. *Canadian Journal of Psychiatry* **2002,** 47, 27-38.

55. Kenakin, T. Collateral efficacy in drug discovery: taking advantage of the good (allosteric) nature of 7TM receptors. *Trends Pharmacol Sci* **2007,** 28, 407-415.

56. Payne, S. L.; Johansson, A. M.; Strange, P. G. Mechanisms of ligand binding and efficacy at the human D2(short) dopamine receptor. *Journal of Neurochemistry* **2002,** 82, 1106-1117.

57. Seeman, P.; Tallerico, T.; Ko, F. Dopamine displaces [^{3}H]Domperidone from high-affinity sites of the dopamine D2 receptor, but not [^{3}H]Raclopride or [^{3}H]Spiperone in isotonic medium: Implications for human positron emission tomography. *Synapse* **2003,** 49, 209-215.

58. Malmberg, Å.; Mohella, N.; Höök, B. B.; Johansson, A. M.; Hacksell, U.; Nordvall, G. Interactions of ligands with active and inactive conformations of the dopamine D2 receptor. *European Journal of Pharmacology* **1998,** 346, 299-307.

59. Burris, K. D.; Molski, T. F.; Xu, C.; Ryan, E.; Tottori, K.; Kikuchi, T.; Yocca, F. D.; Molinoff, P. B. Aripiprazole, a novel antipsychotic, is a high-affinity partial agonist at human dopamine D2 receptors. *The Journal of Pharmacology and Experimental Therapeutics* **2002,** 302, 381-389.

60. Tadori, Y.; Miwa, T.; Tottori, K.; Burris, K. D.; Stark, A.; Mori, T.; Kikuchi, T. Aripiprazole's low intrinsic activities at human dopamine D2L and D2S receptors render it a unique antipsychotic. *European Journal of Pharmacology* **2005,** 515, 10-19.

61. Kapur, S.; Seeman, P. Does fast dissociation from the dopamine D_2 receptor explain the action of atypical antipsychotics?: A new hypothesis. *Am J Psychiatry* **2001,** 158, 360-369.

62. Dyhring, T.; Nielsen, E. Ø.; Sonesson, C.; Pettersson, F.; Karlsson, J.; Svensson, P.; Christophersen, P.; Waters, N. The dopaminergic stabilizers pridopidine (ACR16) and (–)-OSU6162 display dopamine D2 receptor antagonism and fast receptor dissociation properties. *European Journal of Pharmacology* **2010,** 628, 19-26.

63. Pettersson, F.; Ponten, H.; Waters, N.; Waters, S.; Sonesson, C. Synthesis and evaluation of a set of 4-phenylpiperidines and 4-phenylpiperazines as D_2 receptor ligands and the discovery of the dopaminergic stabilizer 4-[3-(methylsulfonyl)phenyl]-1-propylpiperidine (huntexil, pridopidine, ACR16). *J Med Chem* **2010,** 53, 2510-2520.

64. Steensland, P.; Fredriksson, I.; Holst, S.; Feltmann, K.; Franck, J.; Schilstrom, B.; Carlsson, A. The Monoamine Stabilizer (-)-OSU6162 Attenuates Voluntary Ethanol Intake and Ethanol-Induced Dopamine Output in Nucleus Accumbens. *Biol Psychiatry* **2012,** 72, 823-831.

65. Gefvert, O.; Lindström, L. H.; Dahlbäck, O.; Sonesson, C.; Waters, N.; Carlsson, A.; Tedroff, J. (-)-OSU6162 induces a rapid onset of antipsychotic affect after singledose. A double-blind placebo-controlled pilot study. *Nord. J. Psychiatry* **2000,** 54, 93-94.

66. Johansson, B.; Carlsson, A.; Carlsson, M. L.; Karlsson, M.; Nilsson, M. K. L.; Nordquist-Brandt, E.; Rönnbäck, L. Placebo-controlled cross-over study of the monoaminergic stabiliser (−)-OSU6162 in mental fatigue following stroke or traumatic brain injury. *Acta Neuropsychiatrica* **2012,** 24, 266-274.

67. Luedtke, R. R.; Mishra, Y.; Wang, Q.; Griffin, S. A.; Bell-Horner, C.; Taylor, M.; Vangveravong, S.; Dillon, G. H.; Huang, R.-Q.; Reichert, D. E.; Mach, R. H. Comparison of the Binding and Functional Properties of Two Structurally Different D2 Dopamine Receptor Subtype Selective Compounds. *ACS Chemical Neuroscience* **2012**.

68. Wang, Q.; Mach, R. H.; Luedtke, R. R.; Reichert, D. E. Subtype Selectivity of Dopamine Receptor Ligands: Insights from Structure and Ligand-Based Methods. *Journal of Chemical Information and Modeling* **2010,** 50, 1970-1985.

69. Chu, W.; Tu, Z.; McElveen, E.; Xu, J.; Taylor, M.; Luedtke, R. R.; Mach, R. H. Synthesis and in vitro binding of N-phenyl piperazine analogs as potential dopamine D_3 receptor ligands. *Bioorganic & Medicinal Chemistry* **2005,** 13, 77-87.

70. Kulagowski, J. J.; Broughton, H. B.; Curtis, N. R.; Mawer, I. M.; Ridgill, M. P.; Baker, R.; Emms, F.; Freedman, S. B.; Marwood, R.; Patel, S.; Patel, S.; Ragan, C. I.; Leeson, P. D. 3-((4-(4-Chlorophenyl)piperazin-1-yl)-methyl)-1*H*-pyrrolo-2,3-b-pyridine: an antagonist with high affinity and selectivity for the human dopamine D_4 receptor. *J Med Chem* **1996,** 39, 1941-1942.

71. Smith, J. H. *Introduction to the Principles of Drug Design and Action, 3rd Edition*. Harwood academic publishers: London, 1998; p 387 - 433.

72. Chien, E. Y.; Liu, W.; Zhao, Q.; Katritch, V.; Han, G. W.; Hanson, M. A.; Shi, L.; Newman, A. H.; Javitch, J. A.; Cherezov, V.; Stevens, R. C. Structure of the human dopamine D_3 receptor in complex with a D_2/D_3 selective antagonist. *Science* **2010,** 330, 1091-1095.

73. Tschammer, N.; Dorfler, M.; Hubner, H.; Gmeiner, P. Engineering a GPCR-Ligand Pair That Simulates the Activation of D_2L by Dopamine. *ACS Chemical Neuroscience* **2010,** 1, 25-35.

74. Liljefors, T.; Wikstrom, H. A molecular mechanics approach to the understanding of presynaptic selectivity for centrally acting dopamine receptor agonists of the phenylpiperidine series. *J Med Chem* **1986,** 29, 1896-1904.

75. Mewshaw, R. E.; Husbands, M.; Gildersleeve, E. S.; Webb, M. B.; Shi, X.; Mazandarani, H.; Cocke, M. I.; Ochalski, R.; Brennan, J. A.; Abou-Gharbia, M.; Marquis, K.; McGaughey, G. B.; Coupet, J.; Andree, T. H. New generation dopaminergic agents. 2. Discovery of 3-OH-phenoxyethylamine and 3-OH-NT-phenylpiperazine. *Bioorganic & Medicinal Chemistry Letters* **1998,** 8, 295-300.

76. Albert, J. S. *Targets and Emerging Therapies for Schizophrenia*. John Wiley & Sons, Inc: Hoboken, NJ, 2012.

77. Glennon, J. C.; Van Scharrenburg, G.; Ronken, E.; Hesselink, M. B.; Reinders, J. H.; Van Der Neut, M.; Long, S. K.; Feenstra, R. W.; McCreary, A. C. In vitro characterization of SLV308 (7-[4-methyl-1-piperazinyl]-2(3H)-benzoxazolone, monohydrochloride): a novel partial dopamine D_2 and D3 receptor agonist and serotonin 5-HT_{1A} receptor agonist. *Synapse* **2006,** 60, 599-608.

78. Tadori, Y.; Forbes, R. A.; McQuade, R. D.; Kikuchi, T. In vitro pharmacology of aripiprazole, its metabolite and experimental dopamine partial agonists at human dopamine D_2 and D_3 receptors. *European Journal of Pharmacology* **2011,** 668, 355-365.

79. Newman, A. H.; Beuming, T.; Banala, A. K.; Donthamsetti, P.; Pongetti, K.; LaBounty, A.; Levy, B.; Cao, J.; Michino, M.; Luedtke, R. R.; Javitch, J. A.; Shi, L. Molecular Determinants of

Selectivity and Efficacy at the Dopamine D3 Receptor. *Journal of Medicinal Chemistry* **2012,** 55, 6689-6699.

80. Jorgensen, W. L.; Schyman, P. Treatment of Halogen Bonding in the OPLS-AA Force Field: Application to Potent Anti-HIV Agents. *Journal of Chemical Theory and Computation* **2012,** 8, 3895-3901.
81. Millan, M. J.; Cussac, D.; Milligan, G.; Carr, C.; Audinot, V.; Gobert, A.; Lejeune, F.; Rivet, J. M.; Brocco, M.; Duqueyroix, D.; Nicolas, J. P.; Boutin, J. A.; Newman-Tancredi, A. Antiparkinsonian agent piribedil displays antagonist properties at native, rat, and cloned, human alpha(2)-adrenoceptors: cellular and functional characterization. *J Pharmacol Exp Ther* **2001,** 297, 876-887.
82. Vangveravong, S.; Zhang, Z.; Taylor, M.; Bearden, M.; Xu, J.; Cui, J.; Wang, W.; Luedtke, R. R.; Mach, R. H. Synthesis and characterization of selective dopamine D2 receptor ligands using aripiprazole as the lead compound. *Bioorganic & Medicinal Chemistry* **2011,** 19, 3502-3511.
83. Binda, C.; Li, M.; Hubalek, F.; Restelli, N.; Edmondson, D. E.; Mattevi, A. Insights into the mode of inhibition of human mitochondrial monoamine oxidase B from high-resolution crystal structures. *Proc Natl Acad Sci U S A* **2003,** 100, 9750-9755.
84. De Colibus, L.; Li, M.; Binda, C.; Lustig, A.; Edmondson, D. E.; Mattevi, A. Three-dimensional structure of human monoamine oxidase A (MAO A): relation to the structures of rat MAO A and human MAO B. *Proc Natl Acad Sci U S A* **2005,** 102, 12684-9.
85. Ma, J.; Yoshimura, M.; Yamashita, E.; Nakagawa, A.; Ito, A.; Tsukihara, T. Structure of rat monoamine oxidase A and its specific recognitions for substrates and inhibitors. *J Mol Biol* **2004,** 338, 103-14.
86. Son, S. Y.; Ma, J.; Kondou, Y.; Yoshimura, M.; Yamashita, E.; Tsukihara, T. Structure of human monoamine oxidase A at 2.2-A resolution: the control of opening the entry for substrates/inhibitors. *Proc Natl Acad Sci U S A* **2008,** 105, 5739-5744.
87. Coelho Cerqueira, E.; Netz, P. A.; Diniz, C.; Petry do Canto, V.; Follmer, C. Molecular insights into human monoamine oxidase (MAO) inhibition by 1,4-naphthoquinone: evidences for menadione (vitamin K3) acting as a competitive and reversible inhibitor of MAO. *Bioorg Med Chem* **2011,** 19, 7416-7424.
88. Geldenhuys, W. J.; Darvesh, A. S.; Funk, M. O.; Van der Schyf, C. J.; Carroll, R. T. Identification of novel monoamine oxidase B inhibitors by structure-based virtual screening. *Bioorg Med Chem Lett* **2010,** 20, 5295-5298.
89. Mu, L. H.; Wang, B.; Ren, H. Y.; Liu, P.; Guo, D. H.; Wang, F. M.; Bai, L.; Guo, Y. S. Synthesis and inhibitory effect of piperine derivates on monoamine oxidase. *Bioorg Med Chem Lett* **2012,** 22, 3343-3348.

90. Rosenbaum, D. M.; Zhang, C.; Lyons, J. A.; Holl, R.; Aragao, D.; Arlow, D. H.; Rasmussen, S. G.; Choi, H. J.; Devree, B. T.; Sunahara, R. K.; Chae, P. S.; Gellman, S. H.; Dror, R. O.; Shaw, D. E.; Weis, W. I.; Caffrey, M.; Gmeiner, P.; Kobilka, B. K. Structure and function of an irreversible agonist-beta(2) adrenoceptor complex. *Nature* **2011,** 469, 236-240.

91. Xu, F.; Wu, H.; Katritch, V.; Han, G. W.; Jacobson, K. A.; Gao, Z. G.; Cherezov, V.; Stevens, R. C. Structure of an agonist-bound human A_{2A} adenosine receptor. *Science* **2011,** 332, 322-327.

92. Malo, M.; Brive, L.; Luthman, K.; Svensson, P. Investigation of D2 receptor-agonist interactions using a combination of pharmacophore and receptor homology modeling. *ChemMedChem* **2012,** 7, 471-482.

93. Yap, B. K.; Buckle, M. J.; Doughty, S. W. Homology modeling of the human 5-HT(1A), 5-HT (2A), D_1, and D_2 receptors: model refinement with molecular dynamics simulations and docking evaluation. *J Mol Model* **2012,** 18, 3639-3655.

94. Cho, W.; Taylor, L. P.; Mansour, A.; Akil, H. Hydrophobic Residues of the D2 Dopamine Receptor Are Important for Binding and Signal Transduction. *Journal of Neurochemistry* **1995,** 65, 2105-2215.

95. Cox, B. A.; Henningsen, R. A.; Spanoyannis, A.; Neve, R. L.; Neve, K. A. Contributions of Conserved Serine Residues to the Interactions of Ligands with Dopamine D2 Receptors. *Journal of Neurochemistry* **1992,** 59, 627-635.

96. Mansour, A.; Meng, F.; Meador-Woodruff, J. H.; Taylor, L. P.; Civelli, O.; Akil, H. Site-directed mutagenesis of the human dopamine D2 receptor. *European Journal of Pharmacology* **1992,** 227, 205-214.

97. Wiens, B. L.; Nelson, C. S.; Neve, K. A. Contribution of Serine Residues to Constitutive and Agonist-Induced Signaling via the D2S Dopamine Receptor: Evidence for Multiple, Agonist-Specific Active Conformations *Molecular Pharmacology* **1998,** 54, 435-444.

98. Wilcox, R. E.; Huang, W.-H.; Brusniak, M.-Y. K.; Wilcox, D. M.; Pearlman, R. S.; Teeter, M. M.; DuRand, C. J.; Wiens, B. L.; Neve, K. A. CoMFA-Based Prediction of Agonist Affinities at Recombinant Wild Type versus Serine to Alanine Point Mutated D2 Dopamine Receptors. *Journal of Medicinal Chemistry* **2000,** 43, 3005-3019.

99. Yashar, M.; Kalani, S.; Vaidehi, N.; Hall, S. E.; Trabanino, R. J.; Freddolino, P. L.; Kalani, M. A.; Floriano, W. B.; Kam, V. W. T.; GoddardIII, W. A. The predicted 3D structure of the human D_2 dopamine receptor and the binding site and binding affinities for agonists and antagonists. *Proceedings of the National Academy of Sciences* **2004,** 101, 3815-3820.

100. Tschammer, N.; Bollinger, S.; Kenakin, T.; Gmeiner, P. Histidine 6.55 is a major determinant of ligand-biased signaling in dopamine D2L receptor. *Mol Pharmacol* **2011,** 79, 575-85.

101. Shi, L.; Javitch, J. A. The second extracellular loop of the dopamine D_2 receptor lines the binding-site crevice. *Proc Natl Acad Sci U S A* **2004,** 101, 440-5.

102. Ballesteros, J. A.; Jensen, A. D.; Liapakis, G.; Rasmussen, S.; Shi, L.; Gether, U.; Javitch, J. A. Activation of the 2-Adrenergic Receptor Involves Disruption of an Ionic Lock between the Cytoplasmic Ends of Transmembrane Segments 3 and 6 *Journal of Biological Chemistry* **2001,** 276, 29171-29177.

103. Hubbell, W. L.; Altenbach, C.; Hubbell, C. M.; Khorana, H. G. Rhodopsin structure, dynamics, and activation: A perspective from crystallography, site-directed spin labeling, sulfhydryl reactivity, and disulfide cross-linking. *Advances in Protein Chemistry* **2003,** 63, 243-290.

104. Vilardaga, J.-P. Switching modes for G protein-coupled receptor activation. *Nature Chémical Biology* **2006,** 2, 395-396.

105. Yao, X.; Parnot, C.; Deupi, X.; Ratnala, V. R. P.; Swaminath, G.; Farrens, D.; Kobilka, B. Coupling ligand structure to specific conformational switches in the beta(2)-adrenoceptor. *Nature Chemical Biology* **2006,** 2, 417-422.

106. Goddard(III), W. A.; Abrol, R. 3-Dimensional Structure of G Protein-Coupled Receptors and Binding Sites of Agonists and Antagonists. *The Journal of Nutrition* **2007,** 137, 1528-1538.

107. Bartholomew, C. H.; Agrawal, P. K.; Katzer, J. R. Sulphur poisoning of metals. *Advances in Catalysis* **1982,** 31, 135-242.

108. Miyaura, N.; Suzuki, A. Stereoselective synthesis of arylated (E)-alkenes by the reaction of alk-1-enylboranes with aryl halides in the presence of palladium catalyst. *Journal of the Chemical Society, Chemical Communications* **1979**, 866-867.

109. Miyaura, N.; Suzuki, A. Palladium-Catalyzed Cross-Coupling Reactions of Organoboron Compounds. *Chemical Reviews* **1995,** 95, 2457-2483.

110. Miyaura, N.; Yamada, K.; Suzuki, A. A new stereospecific cross-coupling by the palladium-catalyzed reaction of 1-alkenylboranes with 1-alkenyl or 1-alkynyl halides. *Tetrahedron Letters* **1979,** 20, 3437-3440.

111. Matos, K.; Soderquist, J. A. Alkylboranes in the Suzukiâ Miyaura Coupling Stereochemical and Mechanistic Studies. *The Journal of Organic Chemistry* **1998,** 63, 461-470.

112. Hamilton, T. S.; Adams, R. Reduction of pyridine hydrochloride and pyridonium salts by means of hydrogen and platinum-oxide platinum black. XVIII1. *Journal of the American Chemical Society* **1928,** 50, 2260-2263.

113. Bull, J. A.; Mousseau, J. J.; Pelletier, G.; Charette, A. B. Synthesis of Pyridine and Dihydropyridine Derivatives by Regio- and Stereoselective Addition to N-Activated Pyridines. *Chemical Reviews* 112, 2642-2713.

114. Driver, M.; Hartwig, J.; Broadley, K. A second-generation catalyst for aryl halide amination: Mixed secondary amines from aryl halides and primary amines catalyzed by (DPPF)$PdCl_2$. *Journal of the American Chemical Society* **1996,** 118, 7217-7218.

115. Wolfe, J.; Wagaw, S.; Buchwald, S. An Improved Catalyst System for Aromatic Carbon-Nitrogen Bond Formation: The Possible Involvement of Bis(phosphine) Palladium Complexes as Key Intermediates *Journal of the American Chemical Society* **1996,** 118, 7215-7216.

116. Cretcher, L. H.; Koch, J. A.; Pittenger, W. H. Further synthesis with I²,I²-dichloro diethyl-ether. *Journal of the American Chemical Society* **2012,** 47, 1173-1177.

117. Sonesson, C.; Lin, C.-H.; Hansson, L.; Waters, N.; Svensson, K.; Carlsson, A.; Smith, M. W.; Wikström, H. Substituted (S)-Phenylpiperidines and Rigid Congeners as Preferential Dopamine Autoreceptor Antagonists: Synthesis and Structure-Activity Relationships. *Journal of Medicinal Chemistry* **1994,** 37, 2735-2753.

118. Baraznenok, I. L.; Nenajdenko, V. G.; Balenkova, E. S. Chemical Transformations Induced by Trific Anhydride. *Tetrahedron* **2000,** 56, 3077-3119.

119. Truce, W. E.; Campbell, R. W.; Norell, J. R. Sulfene, an Intermediate in the Alcoholysis of Methanesulfonyl Chloride. *Journal of the American Chemical Society* **1964,** 86, 288-288.

120. Dolle, R. E.; Schmidt, S. J.; Kruse, L. I. Palladium catalysed alkoxycarbonylation of phenols to benzoate esters. *Journal of the Chemical Society, Chemical Communications* **1987**, 904-905.

121. Jagdmann, G. E.; Munson, H. R.; Gero, T. W. A Mild Efficient Procedure for the Conversion of Carboxylic Acid Esters to Primary Amides Using Formamide/Methanolic Sodium Methoxide. *Synth Commun* **1990,** 20, 1203-1208.

122. Surrey, A. R. Malononitrile. In *Org. Synth. coll.*, 1955; Vol. III, pp 535-536.

123. Lahti, R. A.; Figur, L. M.; Piercey, M. F.; Ruppel, P. L.; Evans, D. L. Intrinsic activity determinations at the dopamine D2 guanine nucleotide-binding protein-coupled receptor: utilization of receptor state binding affinities. *Molecular Pharmacology* **1992,** 42, 432-439.

124. Mewshaw, R. E.; Kavanagh, J.; Stack, G.; Marquis, K. L.; Shi, X.; Kagan, M. Z.; Webb, M. B.; Katz, A. H.; Park, A.; Kang, Y. H.; Abou-Gharbia, M.; Scerni, R.; Wasik, T.; Cortes-Burgos, L.; Spangler, T.; Brennan, J. A.; Piesla, M.; Mazandarani, H.; Cockett, M. I.; Ochalski, R.; Coupet, J.; Andree, T. H. New generation dopaminergic agents. 1. Discovery of a novel scaffold which embraces the D2 agonist pharmacophore. Structure-activity relationships of a series of 2-(aminomethyl)chromans. *Journal of Medicinal Chemistry* **1997,** 40, 4235-4256.

125. Ponten, H.; Sönniksen, K.; Abrahamsson, T.; Waters, N.; Gustafsson, B.; Hanse, E.; Groc, L. Behavioral and neurochemical repercussions of hippocampal network activity blockade during the neonatal period. *Developmental Brain Research* **2005,** 155, 81-86.

126. Hallman, H.; Jonsson, G. Neurochemical studies on central dopamine neurons--regional characterization of dopamine turnover. *Med Biol* **1984,** 62, 198-209.

127. Jordan, S.; Koprivica, V.; Dunn, R.; Tottori, K.; Kikuchi, T.; Altar, C. A. In vivo effects of aripiprazole on cortical and striatal dopaminergic and serotonergic function. *Eur J Pharmacol* **2004,** 483, 45-53.

128. Nissbrandt, H.; Carlsson, A. Turnover of dopamine and dopamine metabolites in rat brain: comparison between striatum and substantia nigra. *J Neurochem* **1987,** 49, 959-967.

129. Nissbrandt, H.; Sundstrom, E.; Jonsson, G.; Hjorth, S.; Carlsson, A. Synthesis and release of dopamine in rat brain: comparison between substantia nigra pars compacts, pars reticulata, and striatum. *J Neurochem* **1989,** 52, 1170-1182.

130. Ponten, H.; Kullingsjo, J.; Lagerkvist, S.; Martin, P.; Pettersson, F.; Sonesson, C.; Waters, S.; Waters, N. In vivo pharmacology of the dopaminergic stabilizer pridopidine. *Eur J Pharmacol* **2010,** 644, 88-95.

131. McGaughey, G. B.; Mewshaw, R. E. Molecular Modeling and the Design of Dopamine D_2 Partial Agonists. http://www.netsci.org/Science/Compchem/feature20.html

132. Jordan, S.; Johnson, J. L.; Regardie, K.; Chen, R.; Koprivica, V.; Tadori, Y.; Kambayashi, J.; Kitagawa, H.; Kikuchi, T. Dopamine D2 receptor partial agonists display differential or contrasting characteristics in membrane and cell-based assays of dopamine D_2 receptor signaling. *Prog Neuropsychopharmacol Biol Psychiatry* **2007,** 31, 348-356.

133. Seeman, P. An Update of Fast-Off Dopamine D2 Atypical Antipsychotics. *American Journal of Psychiatry* **2005,** 162, 1984-1985.

134. Vauquelin, G.; Bostoen, S.; Vanderheyden, P.; Seeman, P. Clozapine, atypical antipsychotics, and the benefits of fast-off D_2 dopamine receptor antagonism. *Naunyn Schmiedebergs Arch Pharmacol* **2012,** 385, 337-372.

135. Wold, S. Crossvalidatory estimation of the number of components in factor and principal components models. *Technometrics* **1978,** 20, 397-405.

136. Wold, S.; Sjöström, M.; Eriksson, L. PLS-regression: a basic tool of chemometrics. . *Chemometrics and Intelligent Laboratory Systems* **2001,** 58, 109-130.

137. Wold, S.; Trygg, J.; Berglund, A.; Antti, H. Some recent developments in PLS modeling. *Chemometrics and Intelligent Laboratory Systems* **2001,** 58, 131-150.

138. Gallardo-Godoy, A.; Fierro, A.; McLean, T. H.; Castillo, M.; Cassels, B. K.; Reyes-Parada, M.; Nichols, D. E. Sulfur-substituted alpha-alkyl phenethylamines as selective and reversible MAO-A inhibitors: biological activities, CoMFA analysis, and active site modeling. *J Med Chem* **2005,** 48, 2407-2419.

139. Sonesson, C.; Waters, N.; Svensson, K.; Carlsson, A.; Smith, M. W.; Piercey, M. F.; Meier, E.; Wikström, H. Substituted 3-phenylpiperidines: new centrally acting dopamine autoreceptor antagonists. *Journal of Medicinal Chemistry* **1993,** 36, 3188-3196.

140. Dijkstra, G. D. H. Conformational analysis of 1-arylpiperazines and 4-arylpiperidines. *Recueil des Travaux Chimiques des Pays-Bas* **1993,** 112, 151-160.

141. Halgren, T. A. MMFF VII. Characterization of MMFF94, MMFF94s, and Other Widely Available Force Fields for Conformational Energies and for Intermolecular-Interaction Energies and Geometries. *Journal of Computational Chemistry* **1999,** 20, 730-748.

142. Halgren, T. A. MMFF VI. MMFF94s Option for Energy Minimization Studies. *Journal of Computational Chemistry* **1999,** 20, 720-729.

143. Burley, S. K.; Petsko, G. A. Aromatic-aromatic interaction: a mechanism of protein structure stabilization. *Science* **1985,** 229, 23-28.

144. Meyer, E. A.; Castellano, R. K.; Diederich, F. Interactions with aromatic rings in chemical and biological recognition. *Angew Chem Int Ed Engl* **2003,** 42, 1210-1250.

145. Salonen, L. M.; Ellermann, M.; Diederich, F. Aromatic rings in chemical and biological recognition: energetics and structures. *Angew Chem Int Ed Engl* **2011,** 50, 4808-4842.

146. Cockroft, S. L.; Hunter, C. A.; Lawson, K. R.; Perkins, J.; Urch, C. J. Electrostatic control of aromatic stacking interactions. *J Am Chem Soc* **2005,** 127, 8594-8595.

147. Cockroft, S. L.; Perkins, J.; Zonta, C.; Adams, H.; Spey, S. E.; Low, C. M.; Vinter, J. G.; Lawson, K. R.; Urch, C. J.; Hunter, C. A. Substituent effects on aromatic stacking interactions. *Org Biomol Chem* **2007,** 5, 1062-1080.