Development of Novel Serotonin 5-HT$_6$ and Dopamine D$_2$ Receptor Ligands and MAO A Inhibitors

Synthesis, Structure-Activity Relationships and Pharmacological Characterization

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- Synthesis, Structure-Activity Relationships and Pharmacological Characterization

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In loving memory of my mother

Abstract

It is known since the 1950s that enhancement of the levels of the monoamines dopamine (DA), serotonin (5-hydroxytryptamine, 5-HT) and norepinephrine (NE) in the brain will relieve the symptoms of major depression, and current therapies are still based on this mechanism. However, all available antidepressants today are still suffering from slow onset of therapeutic action, as well as adverse effects and lack of efficacy. Therefore, development of compounds with new mechanisms of action for treatment of depression is needed.

One of the most important stages of the drug discovery process is the generation of lead compounds. Structure-activity relationships (SARs) are well integrated in modern drug discovery and have been used in the process of developing new leads. The tetrahydropyridine/piperidine indoles are known to affect multiple targets of the dopaminergic and serotonergic systems in the brain. This class of indoles can easily be modified and they possess the necessary properties for a lead, such as low molecular weight and high water solubility. This thesis is focused on further exploring the SAR around tetrahydropyridine/piperidine indoles by introduction of substituents and/or bioisosteric replacements of the indole core with the aim of developing novel compounds acting at the dopaminergic and serotonergic systems in the brain. By using *in vivo* and *in vitro* screening approaches, 5-HT type 6 receptor (5-HT$_6$) agonists, DA type 2 receptor (DA D$_2$) antagonists, 5-HT reuptake transporters (SERT) inhibitors, dual DA D$_2$ antagonists/SERT inhibitors and finally reversible monoamine oxidase A (MAO A) inhibitors were identified after modifications of the chemical lead. In addition, the SAR of 6-substituted 3-(pyrrolidin-1-ylmethyl)chromen-2-ones (coumarin derivatives) were also investigated and were identified as selective and reversible MAO A inhibitors.

Three compounds, i.e. the 5-HT$_6$ agonist **81**, the dual DA D$_2$ antagonist/SERT inhibitor **158** and the MAO A inhibitor **134** have been identified to be of potential interest as novel antidepressants.

Keywords: dopamine D$_2$ receptor, serotonin reuptake transporter, monoamine oxidase, 5-HT$_6$ receptor, DOPAC, 5-HIAA, 3-tetrahydropyridine indole, 3-piperidine indole, 3-(pyrrolidin-1-ylmethyl)chromen-2-one

Papers included in the thesis

This thesis is based on the following publications and manuscript, which will be referred to in the thesis by their Roman numerals.

I. **2-Alkyl-3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indoles as novel 5-HT$_6$ receptor agonists**
Mattsson C, Sonesson C, Sandahl A, Greiner HE, Gassen M, Plaschke J, Leibrock J, Boettcher H.
Bioorg Med Chem Lett. **2005**, *15*, 4230-4234

II. **Structure-activity relationship of 5-chloro-2-methyl-3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole analogues as 5-HT$_6$ receptor agonists**
Mattsson C, Svensson P, Boettcher H, Sonesson C.
Eur J Med Chem. **2013**, *63*, 578-588

III. **Systematic *in vivo* screening of a series of 1-propyl-4-aryl-piperidines against dopaminergic and serotonergic properties in rat brain: a scaffold-jumping approach**
Mattsson C, Andreasson T, Waters N, Sonesson C.
J Med Chem. **2012**, *55*, 9735-9750
Correction: *J Med Chem*. **2013**, *56*, 4130-4133

IV. **A novel series of 6-substituted 3-(pyrrolidin-1-ylmethyl)chromen-2-ones as selective monoamine oxidase (MAO) A inhibitors**
Mattsson C, Svensson P, Sonesson C.
Eur J Med Chem. **2013**, Submitted

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Contributions to the Papers

I.   Planned and synthesized most of the included compounds; interpreted results, and wrote the manuscript.

II.   Planned and synthesized most of the included compounds; interpreted results, and wrote the manuscript. Did not perform the conformation simulations.

III.   Planned and synthesized all of the included compounds; interpreted results, and wrote the manuscript. Did not perform the PLS correlations or *in vivo* studies.

IV.   Planned and synthesized all of the included compounds; interpreted results, and wrote the manuscript. Did not perform the docking study to the MAO A enzyme, conformation simulations or *in vivo* studies.

Contents

Abbreviations

3-MT	3-Methoxytyramine
5-HIAA	5-Hydroxyindoleacetic acid
5-HT	5-Hydroxytyramine (serotonin)
5-HTP	5-Hydroxytryptophan
AADC	Aromatic-L-amino acid decarboxylase
AC	Adenylyl cyclase
ALDH	Aldehyde dehydrogenase
α_2	Adrenergic α_2 receptor
aq.	Aqueous
BHK	Baby hamster kidney
Boc	*tert*-Butyloxycarbonyl
Bn	Benzyl
cAMP	3',5'-Cyclic adenosine monophosphate
CHO	Chinese hamster ovary
CNS	Central nervous system
COMT	Catecol-*O*-methyltransferase
Conc.	Concentrated
DA	Dopamine
DABCO	1,4-Diazabicyclo[2.2.2]octane
DA D_{2L}	Dopamine type 2 long receptor
DA D_{2S}	Dopamine type 2 short receptor
DA D_2^{High}	High-affinity dopamine type 2 receptor state
DA D_2^{Low}	Low-affinity dopamine type 2 receptor state
DAG	Diacyl glycerol
DAT	Dopamine reuptake transporter
DBH	Dopamine β-hydroxylase
DMF	*N,N*-Dimethylformamide
DOPAC	3,4-Dihydroxyphenylacetic acid
DOPAL	3,4-Dihydroxyphenylacetaldehyde
EPS	Extrapyramidal side effects
equiv.	Equivalent
Et	Ethyl
FAD	Flavin adenine dinucleotide
GABA	γ-Amino-butyric acid
$G_{i/o}$	Inhibitory G-protein
G_o	Inhibitory G-protein
Gln	Glutamine
GPCR	G-protein-coupled seven-transmembrane receptor
$G_{q/11}$	Stimulatory G-protein
G_s	Stimulatory G-protein
h	Hour
H_1	Histaminergic type 1 receptor
HEK	Human embryonic kidney
HVA	Homovanillic acid
IC_{50}	The concentration of an inhibitor required to inhibit an enzyme by 50%
Ile	Isoleucine
IP_3	Inositol triphosphate

*i*Pr	Isopropyl
K_i	Binding affinity constant
L-DOPA	L-3,4-Dihydroxyphenylalanine
Leu	Leucine
LMA	Locomotor activity
MAO	Monoamino oxidase
MAOI	Monoamino oxidase inhibitor
Me	Methyl
NDRI	Dopamine and norepinephrine reuptake inhibitor
NE	Norepinephrine
NET	Norepinephrine reuptake transporter
*n*Bu	*n*-Butyl
*n*Pr	*n*-Propyl
NRI	Selective norepinephrine reuptake inhibitors
Ph	Phenyl
Phe	Phenylalanine
PLS	Partial least square
RIMA	Reversible inhibitors of MAO A
rt	Room temperature
SAR	Structure-activity relationship
SAFIR	Structure-affinity relationship
SE	Standard error
SEM	Standard error of the mean
SERT	Serotonin reuptake transporter
SI	Selectivity index
SNRI	Dual serotonin and norepinephrine reuptake inhibitor
SSRI	Selective serotonin reuptake inhibitor
*t*Bu	*tert*-Butyl
TCA	Tricyclic antidepressant
TH	Tyrosine hydroxylase
THF	Tetrahydrofuran
THP	Tryptophan hydroxylase
TPH	2-Tetrahydropyranyl
Tyr	Tyrosine
Val	Valine

1. Introduction

1.1. Neurotransmission

Neurons within the human brain communicate through neurotransmission in a complex network between numerous different types of neurons ending in a physiological response such as movement, thinking, fear, stress etc. A neuron receives signals from other cells in the dendrite network (Figure 1), creating a depolarization wave that propagates from the synapse to the cell body of the neuron. In the axon, an action potential is generated and the electrical impulse is propagated to the axon terminal (presynaptic terminal), where it is transformed to a chemical signal through the release of neurotransmitters into the synapse. The neurotransmitters then diffuse over the synaptic cleft to the target cell (postsynaptic cell) where they interact with specific receptor proteins leading to an inhibitory or excitatory modulation of the signal in the postsynaptic cell (cellular response). Neurotransmitters are rapidly removed from the synaptic cleft by reuptake and/or degradation that leads to a termination of the signaling.[1]
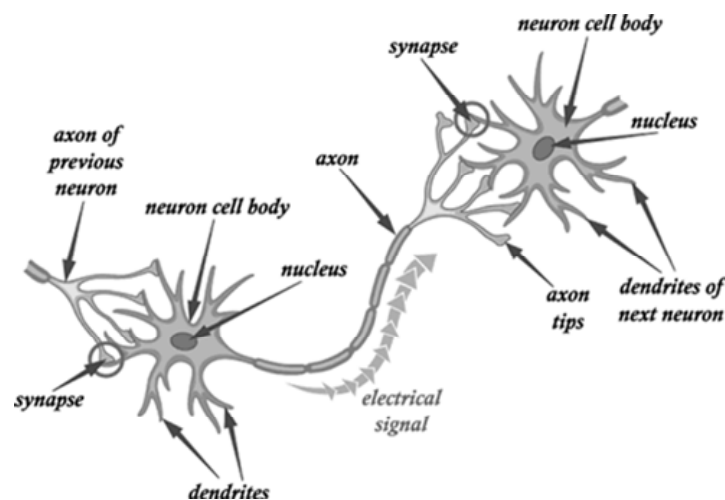
Figure 1. Neurons synapse in brain, modified from Totora and Derrickson.[2]

Numerous pharmaceuticals have their main target within the synaptic space (e.g. antipsychotics, antidepressants, pain killers and anti-migraine drugs). Compounds that stimulate the receptors in the same way as the endogenous ligands (neurotransmitters) are called *full agonists* (Figure 2); an agonist that can only activate the receptor to a limited extent is called a *partial agonist*. *Antagonists* are compounds that are able to interact and block the receptor for stimulation by neurotransmitters (having no biological effects of their own) whereas compounds that interact with the receptors and

activate a reversed physiological response compared to the endogenous ligands are called *inverse agonists*. Compounds that interact with and block the effect of enzymes and reuptake proteins within the synapse, without eliciting any cellular response are called *inhibitors*.[1]
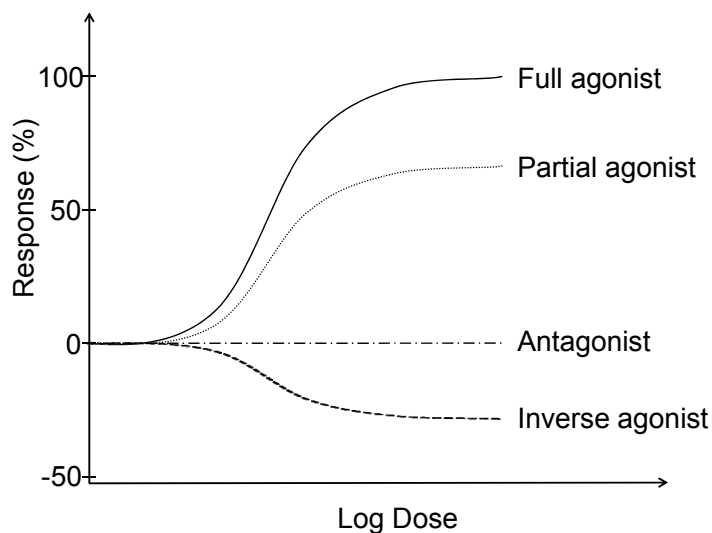
Figure 2. Dose-response curves illustrating the receptor response by an agonist, partial agonist, antagonist and, inverse agonist.

1.2. Monoaminergic neurotransmitters

Neurotransmitters are compounds that are responsible for the chemical transmission between neurons in the brain. One of the neurotransmitter systems in the human brain is the monoaminergic system, which is divided into three major parts: the dopaminergic, adrenergic and serotonergic systems, with their corresponding neurotransmitters, dopamine (DA), norepinephrine (NE) and serotonin (5-HT) (Figure 3) respectively.[1] 5-HT was the first compound in this system to be discovered. In the 1930s, Vittorio Erspamer isolated "enteramine" (5-HT) from enterochromaffin cells of the gut and the same substance was later found in blood serum by Irvine Page in 1948, who named it serotonin.[3] In 1946, the Swedish biologist Ulf von Euler discovered NE,[4] followed by Arvid Carlsson who discovered DA in 1958.[5-7] Both Ulf von Euler and Arvid Carlsson received the Nobel Prize (1970 and 2000, respectively) for their discoveries.[4] Since the discovery of these neurotransmitters it has been established that dysfunction in the monoaminergic system contributes to various disorders including Parkinson's disease, depression, schizophrenia and drug abuse.[8, 9]

Dopamine (DA) Norepinephrine (NE) Serotonin (5-HT)
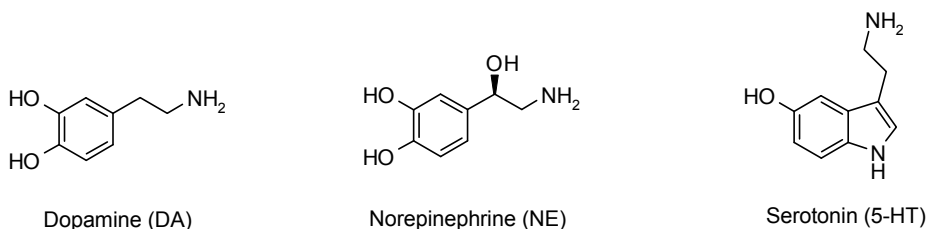
Figure 3. The monoamine neurotransmitters in the brain.

1.3. Monoamine synthesis and catabolism

The monoamines are not able to diffuse from the blood to the brain, since they are too hydrophilic to cross the blood-brain barrier.[10] Instead the monoamines are synthesized in the cell body of the neuron and transported to the axon terminal. The corresponding essential amino acids (L-tyrosine and L-tryptophan) are actively transported over the blood-brain barrier into the central nervous system (CNS). The neurotransmitters DA and NE are biosynthesized from the precursor L-tyrosine in a two or three step synthesis, respectively, as outlined in Figure 4.[11] The biosynthesis of 5-HT in two steps is starting from L-tryptophan (Figure 4).[12]
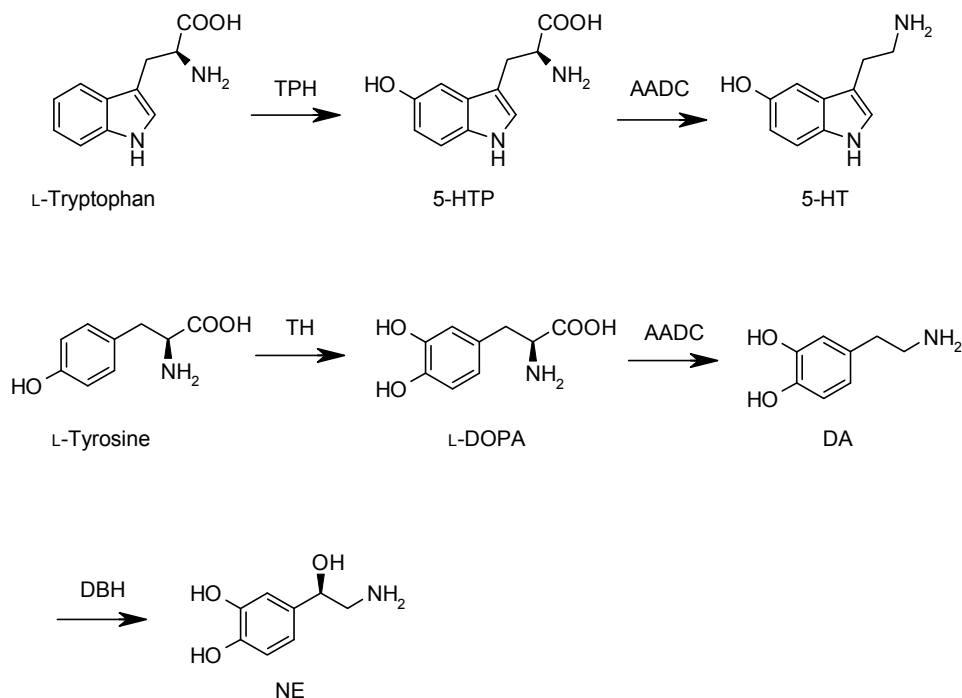
Figure 4. Biosynthetic route of the monoamines 5-HT, DA and NE. Abbreviations: TPH, L-tryptophan hydroxylase; 5-HTP, 5-hydroxy-L-tryptophan; AADC, aromatic L-amino acid decarboxylase; 5-HT, serotonin; TH, tyrosine hydroxylase; L-DOPA, L-3,4-dihydroxy phenylalanine; DA, dopamine; DBH, dopamine β-hydroxylase; NE, norepinephrine.

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Monoamines are degraded by two different enzymatic systems; monoamine oxidase (MAO) and catechol-*O*-methyl transferase (COMT) (Figure 5). MAOs are located intracellularly at the outer side of the mitochondrial membrane whereas COMT is located intracellularly within postsynaptic neurons and glial cells.[13] MAO metabolizes DA into 3,4-dihydroxyphenylacetaldehyde (DOPAL) which is immediately oxidized into 3,4-dihydroxyphenylacetic acid (DOPAC) by the enzyme aldehyde dehydrogenase (ALDH). DOPAC is then methylated to homovanillic acid (HVA) by COMT. However, COMT is also able to directly metabolize DA, producing 3-methoxytyramine (3-MT) which in turn can be metabolized by MAO/ALDH into HVA (Figure 5).[14] The other main neurotransmitter 5-HT is metabolized mainly by MAO generating 5-hydroxyindoleacetic acid (5-HIAA, Figure 5).[12]
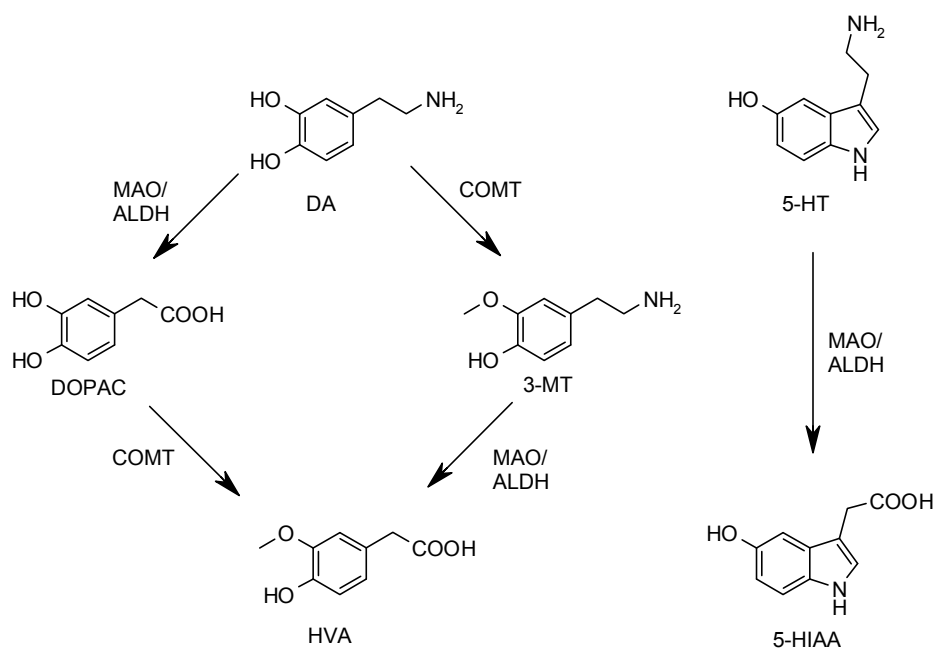
Figure 5. *In vivo* metabolism of the neurotransmitters DA and 5-HT. Abbreviations: MAO, monoamine oxidase; ALDH, aldehyde dehydrogenase; COMT, catechol-*O*-methyltransferase DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; 3-MT, 3-methoxytyramine; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; DA, dopamine.

1.4. The 5-HT neuron and receptor subtypes

The 5-HT receptor family is the largest family of the seven transmembrane G-protein-coupled receptors (GPCRs). Fourteen different receptor subtypes, grouped into seven families (5-HT$_3$ is a ligand gated ion channel), have now been described (Table 1).[15-17] The GPCRs act through intracellular signaling pathways [3',5'-cyclic adenosine monophosphate (cAMP), inositol triphosphate (IP$_3$) and diacyl glycerol (DAG)] to hyperpolarize (5-HT$_{1A-F}$) or depolarize (5-

HT$_{2/4/5/6/7}$) their target cells. All 5-HT receptors are localized postsynaptically on target cells. However, the 5-HT$_{1A}$ receptor is also located at the 5-HT dendrites and cell bodies (located in the brain stem, raphe nuclei) and 5-HT$_{1B/1D}$ subtypes at the 5-HT presynaptic axon terminals controlling synthesis, cell firing and release of neurotransmitters into the synaptic cleft (Figure 6).[18] [19] The main physiological role of serotonin reuptake transporters (SERT) is to remove the released 5-HT from the extracellular space, and thereby control the duration and magnitude of neurotransmission via 5-HT receptors (Figure 6).[20] The termination of the neurotransmission signaling is rapid with SERT. Back in the presynaptic terminal 5-HT is repacked in vesicles or degraded by MAO, yielding the oxidative degradation product 5-HIAA.
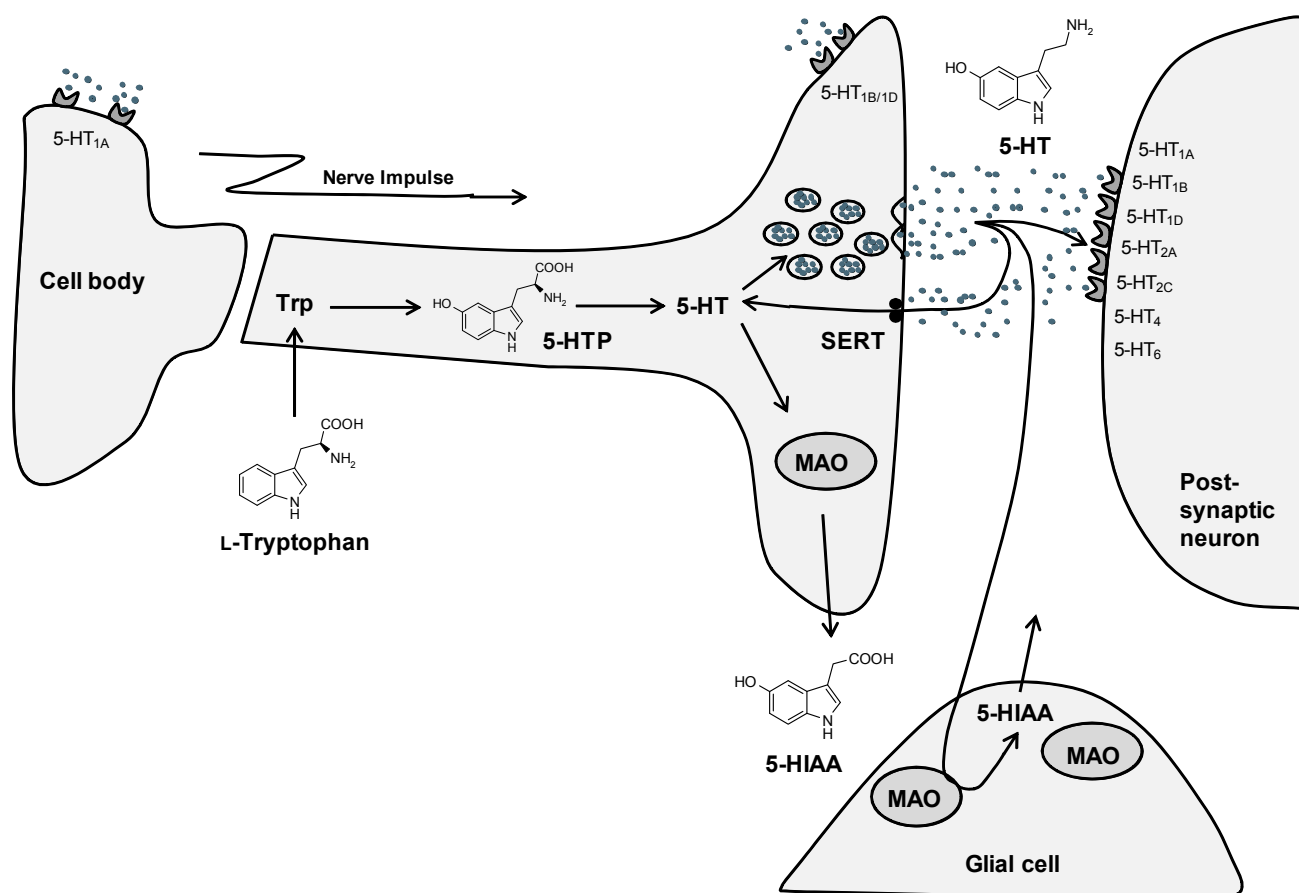
Figure 6. An overview of the serotonin (5-HT) neuron with a selection of the 5-HT receptors, the 5-HT biosynthetic pathway and degradation of 5-HT are outlined at/in various compartments, i.e. the cell body, presynaptic and postsynaptic neuron as well as in the glial cell. Abbreviations: MAO, monoamine oxidase; 5-HT; serotonin; Trp, L-tryptophan; 5-HTP, 5-hydroxy-L-tryptophan; 5-HIAA, 5-hydroxyindoleacetic acid; SERT, serotonin reuptake transporter.

Table 1. Serotonin (5-HT) receptor subtypes and their pharmacological and physiological functions in brain and their connections to possible diseases.

Subtype	Signaling pathway	Agonists/antagonists	Putative functions	Related clinical interests
5-HT$_{1A}$	G$_{i/o}$ ↓cAMP	8-OH-DPAT (ag) WAY100635 (ant)	thermoregulation, feeding, stress, pain, mood, emotion, cognition, learning, memory	anxiety/depression, schizophrenia neurodegenerative disorders
5-HT$_{1B}$	G$_{i/o}$ ↓cAMP	sumatriptan (ag) GR55562 (ant)	mood, feeding	anxiety/depression, migraine
5-HT$_{1D}$	G$_{i/o}$ ↓cAMP	sumatriptan (ag) BRL15572 (ant)	mood, feeding	anxiety/depression, migraine
5-HT$_{1E}$	G$_{i/o}$ ↓cAMP	-	-	-
5-HT$_{1F}$	G$_{i/o}$ ↓cAMP	LY334370 (ag)	mood, emotion	migraine
5-HT$_{2A}$	G$_{q/11}$ ↑IP$_3$/DG	DOI (ag) M100907 (ant)	mood, respiratory control, feeding, nociception	anxiety/depression, schizophrenia, drug abuse, pain, anorexia/bulimia Alzheimer's disease
5-HT$_{2B}$	G$_{q/11}$ ↑IP$_3$/DG	BW723C86 (ag) SB204741 (ant)	-	drug abuse
5-HT$_{2C}$	G$_{q/11}$ ↑IP$_3$/DG	Ro600175 (ag) mesulergine (ant)	mood, impulsivity, feeding, locomotor activity	anxiety/depression, schizophrenia, drug abuse, obesity
5-HT$_{3A-3E}$	Ion channel	2-Methyl-5-HT (ag) ondansetron (ant)	vomiting reflex, mood	nausea, anxiety/depression
5-HT$_{4A-4H}$	G$_s$ ↑cAMP	BIMU8 (ag) GR113808 (ant)	feeding, reward, cognition	anorexia, drug abuse, Alzheimer's disease
5-HT$_{5A}$	G$_{i/o}$ ↓cAMP	-	circadian rhythm, sleep, mood, cognition	-
5-HT$_{5B}$	-	-	-	-
5-HT$_6$	G$_s$ ↑cAMP	WAY181187 (ag) SB271046 (ant)	cognition, learning, memory, feeding	Alzheimer's disease, dementia, obesity
5-HT$_{7A-7D}$	G$_s$ ↑cAMP	8-OH-DPAT (ag) SB269970 (ant)	mood, sleep, cognition	anxiety/depression, schizophrenia

[a]The table is to a large extent based on the reviews of: Charnay and Leger,[20] Nichols and Nichols,[15] Alexander *et al.*,[17] Hannon and Hoyer,[16] and Filip and Bader.[21] Abbreviations: DAG, diacyl glycerol; IP$_3$, inositol triphosphate; cAMP, 3',5'-cyclic adenosine monophosphate; G$_{i/o}$, inhibitory G-protein; G$_s$ and G$_{q/11}$, stimulatory G-protein; ag, agonist; ant, antagonist.

1.4.1. The 5-HT$_6$ receptor

The 5-HT$_6$ receptor is one of the most recent additions to the large family of 5-HT receptors and was first identified in the early 1990s.[22] The exclusive localization of the 5-HT$_6$ receptors in the CNS, combined with the fact that a number of known antipsychotics and antidepressants display high affinity for this receptor, has resulted in a widespread interest in this field of research.[22, 23] The 5-HT$_6$ receptors are found in striatal, limbic and specific cortical areas expressed postsynaptically by non-serotonin containing neurons [i.e. acetylcholine, glutamate and γ-amino-butyric acid (GABA)] and their distribution is almost superimposable to that of DA receptors.[23-25] Altogether, this suggests that 5-HT$_6$ receptors may be involved in the control of motor function, mood, reward and motivation, making them an interesting drug target for CNS disorders such as schizophrenia, depression and epilepsy. They may also be of relevance to the understanding and treatment of obesity, impaired memory and cognitive function, and drug abuse.[26-30]

1.5. The dopamine neuron and receptor subtypes

The physiological actions of DA are mediated by five distinct (D$_1$-D$_5$) but closely related GPCRs that are divided into two major groups: the D$_1$-like and D$_2$-like receptors (Table 2, Figure 7).[31-33] This classification is based on their different transductions mechanisms, D$_1$-like receptors (D$_1$ and D$_5$) are positively linked to adenylyl cyclase (AC) through coupling with a stimulatory G-protein (G$_s$) resulting in an increase of cAMP, and subsequent stimulation of the postsynaptic cell. The D$_2$-like (D$_2$, D$_3$ and D$_4$) receptors are negatively linked to AC through coupling with an inhibitory G-protein (G$_i$ and G$_o$) resulting in a decrease in cAMP, and inhibition of the postsynaptic cell. The individual members of the subfamilies of the D$_1$ and D$_2$-like receptors share a high level of homology of their transmembrane domains and have distinct pharmacological properties; The D$_1$, D$_4$ and D$_5$ receptors are located postsynaptically, whereas D$_2$ and D$_3$ receptors are found both post- and presynaptically. Presynaptic autoreceptors provide a negative feedback system that controls firing, synthesis and release of DA in response to extracellular neurotransmitter levels. Termination of the neurotransmission signaling is rapid by clearing of DA via the DA reuptake transporter (DAT). Back in the presynaptic terminal DA is repacked in vesicles or degraded by MAO and COMT (postsynaptic neuron), yielding the oxidative degradation products DOPAC and HVA.[31-33]
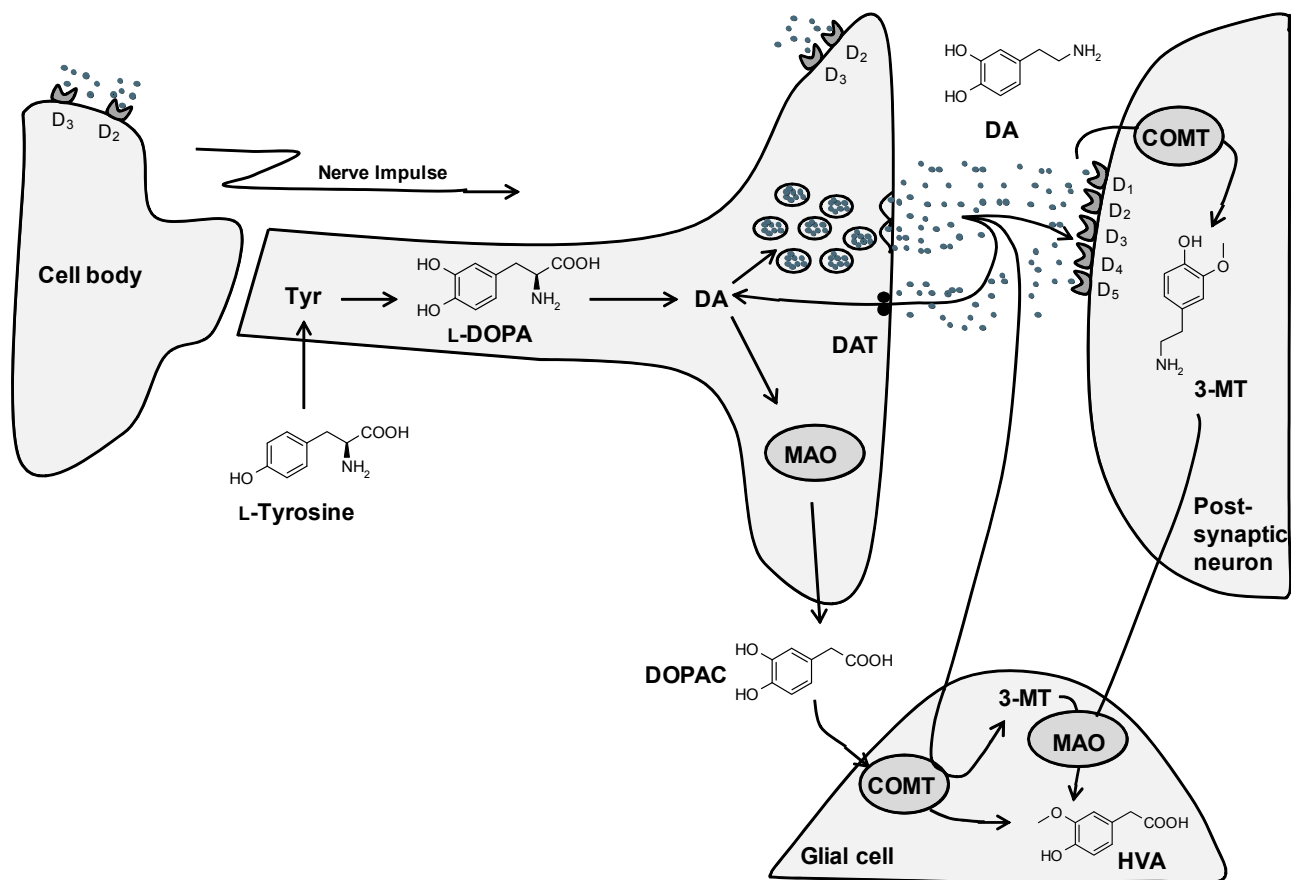
Figure 7. An overview of the dopamine (DA) neuron with D_1-D_5 receptors, the DA biosynthetic pathway and degradation of DA is outlined at/in various compartments, i.e. the cell body, presynaptic and postsynaptic neuron as well as in the glial cell. Abbreviations: MAO, monoamine oxidase; COMT, catechol-O-methyltransferase; DA, dopamine; Tyr; L-tyrosine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; 3-MT, 3-methoxytyramine; DAT, dopamine reuptake transporter.

1.5.1. The dopamine D_2 receptor

The DA D_2 receptor is the second most abundant DA receptor type in the mammalian forebrain and the highest levels of DA D_2 receptors are located in the striatum, the nucleus accumbens and the olfactory tubercle. DA D_2 receptors are also expressed at significant levels in the substantia nigra, ventral tegmental area, hypothalamus, cortical areas, septum, amygdala, and hippocampus. DA generally exerts its actions on neuronal circuitry, via a relatively slow modulation of the fast neurotransmission that is mediated by glutamate and GABA.[31] In addition, DA D_2 receptors have been found in two isoforms spliced from the same gene, termed DA D_2 short (D_{2S}) and DA D_2 long receptor (D_{2L}).[34] The DA D_{2S} receptor has been shown to be more densely expressed presynaptically and to be more involved in the autoreceptor functions, whereas DA D_{2L} seems to be the main

isoform postsynaptically. Therefore they differ in physiological, signaling and pharmacological properties.[35, 36] Besides the different splice isoforms, the DA D_2 receptor population can be distributed between two "activity states"; either a resting, low-affinity state (D_2^{Low}) or an active, high-affinity state (D_2^{High}) to which DA binds with higher affinity.[37] Additionally, the DA D_2 presynaptic receptors are reported to be more sensitive to low DA levels than the postsynaptic DA D_2 receptors.[38]

Table 2. Dopamine receptor subtypes and their pharmacological and physiological functions in brain and connections to possible diseases.

	Signaling pathway	Agonists/antagonists	Putative functions	Related clinical interests
D_1	G_s $\uparrow$cAMP	SKF38393 (ag) SCH23390 (ant)	locomotor activity, reinforcement and reward, working memory	schizophrenia, Parkinson's disease
D_2	$G_{i/o}$ $\downarrow$cAMP	ropinirole (ag) spiperone (ant)	locomotor activity, reinforcement and reward, working memory, cognition, emotion	schizophrenia, Parkinson's disease, movement disorders, drug abuse
D_3	$G_{i/o}$ $\downarrow$cAMP	7-OH-DPAT (ag) nafadotride (ant)	locomotor activity, reinforcement and reward	schizophrenia, drug abuse, Parkinson's disease
D_4	$G_{i/o}$ $\downarrow$cAMP	ABT670 (ag) FAUC213 (ant)	motor activity, initiation and inhibition of behavior, working memory	ADHD, schizophrenia
D_5	G_s $\uparrow$cAMP	-	-	-

[a]The table is to a large extent based on the reviews of: Beaulieu and Gainetdinov,[31] Zhang *et al.*,[39] and Boeckler and Gmeiner.[40] Abbreviations: DAG, diacyl glycerol; IP_3, inositol triphosphate; cAMP, 3',5'-cyclic adenosine monophosphate; $G_{i/o}$, inhibitory G-protein; G_s, stimulatory G-protein; ag, agonist; ant, antagonist; ADHD, attention deficit hyperactivity disorder.

1.6. Monoamine oxidase (MAO)

MAO is a flavoenzyme located intracellularly at the outer mitochondrial membrane responsible for the oxidative deamination of xenobiotic amines and monoamine neurotransmitters.[41-44] There are two distinct types of MAOs, MAO A and MAO B, which share 70% amino acid sequence homology. Both MAO A and MAO B catalyze the deamination of DA, tyramine and tryptamine, MAO A preferentially deaminates 5-HT and NE whereas MAO B preferentially deaminates benzylamines and β-phenethylamines. Within CNS, MAO B is reported to be the most dominating MAO isoenzyme, and is mainly present in serotonergic and histaminergic neurons and glial cells (ependyma, circumventricular organs, astrocytes). A major role for MAO B is to protect the brain

from a variety of trace amines (e.g. high densities in the blood-brain barrier). MAO A on the other hand is found in catecholaminergic neurons and is responsible for the metabolism of the major neurotransmitters 5-HT, NE and DA, offering a multi neurotransmitter strategy for the treatment of depression.[41-44] MAO inhibitors (MAOIs) can be classified on the basis on selectivity for either MAO A or MAO B, and whether the inhibitor is reversible or irreversible. The older MAOIs (e.g. iproniazid, **1**, Figure 8) were unselective and irreversible and had broad side effect profiles and dietary restrictions due to "the cheese reaction", a severe hypertensive crisis upon consumption of food containing large quantities of tyramine. Newer reversible inhibitors of MAO A (RIMA) are easily displaced by ingested tyramine in the gut and thus do not cause the "the cheese reaction" and no dietary restrictions are needed. The only RIMA approved today against depression is moclobemide (**2**, Figure 8).[45-47]

1 Iproniazid (A/B, I)   **2** Moclobemide (A, R)
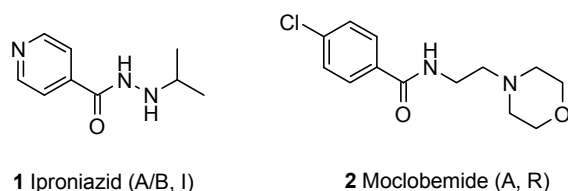
Figure 8. MAO inhibitors: Irreversible (I) or reversible (R) MAO A (A) and MAO B (B) inhibitors.

1.7. Depression

Finding the next generation of antidepressants with a new mechanism of action or a combination therapy with selective serotonin reuptake inhibitors (SSRI) has engaged many researchers in recent years.[48] It is known since the 1950s that enhancement of the monoamine levels of DA, 5-HT and NE will relieve the symptoms of major depression, and current therapies are still based on this hypothesis.[49] Approved antidepressant drugs (Figure 9) mediate their effect through different mechanisms; tricyclic antidepressant [TCA, combined reuptake inhibitor of 5-HT and NE, impramine (**3**)], selective serotonin reuptake inhibitors [SSRI, citalopram (**4**)], selective norepinephrine reuptake inhibitors [NRI, reboxetine (**5**)], dual serotonin and norepinephrine reuptake inhibitors [SNRI, venlafaxine (**6**)] and norepinephrine and dopamine reuptake inhibitors [NDRI, bupropion (**7**)] which all lead to an increase of monoamine availability by blocking reuptake of the monoamines. The "receptor blockers", exemplified with mirtazapine (**8**, Figure 9), bind to adrenergic α_2 receptors and postsynaptic 5-HT receptors such as 5-HT$_{2A}$ and 5-HT$_{2C}$ leading to an increase in 5-HT and NE levels.[49] The MAOI [selegiline (**9**), Figure 9] and RIMA [moclobemide (**2**), Figure 8] increase the monoamine availability by preventing the degradation of DA, NE and 5-HT (i.e. by

inhibition of MAO).[41, 50, 51] An increase of monoamines induces "neuronal changes" (i.e. receptor desensitization, alterations in intracellular transduction cascades and gene expression, induction of neurogenesis, and modification in synaptic architecture and signaling) that can relieve the symptoms of clinical depression.[52] The main drawbacks for all available antidepressants are a slow onset of therapeutic action (i.e. normally 2-6 weeks), intolerable side effects and lack of efficacy. Today 35-40% of all patients suffering from major depression are not sufficiently cured which leads to treatment resistant depression.[49, 53-55]
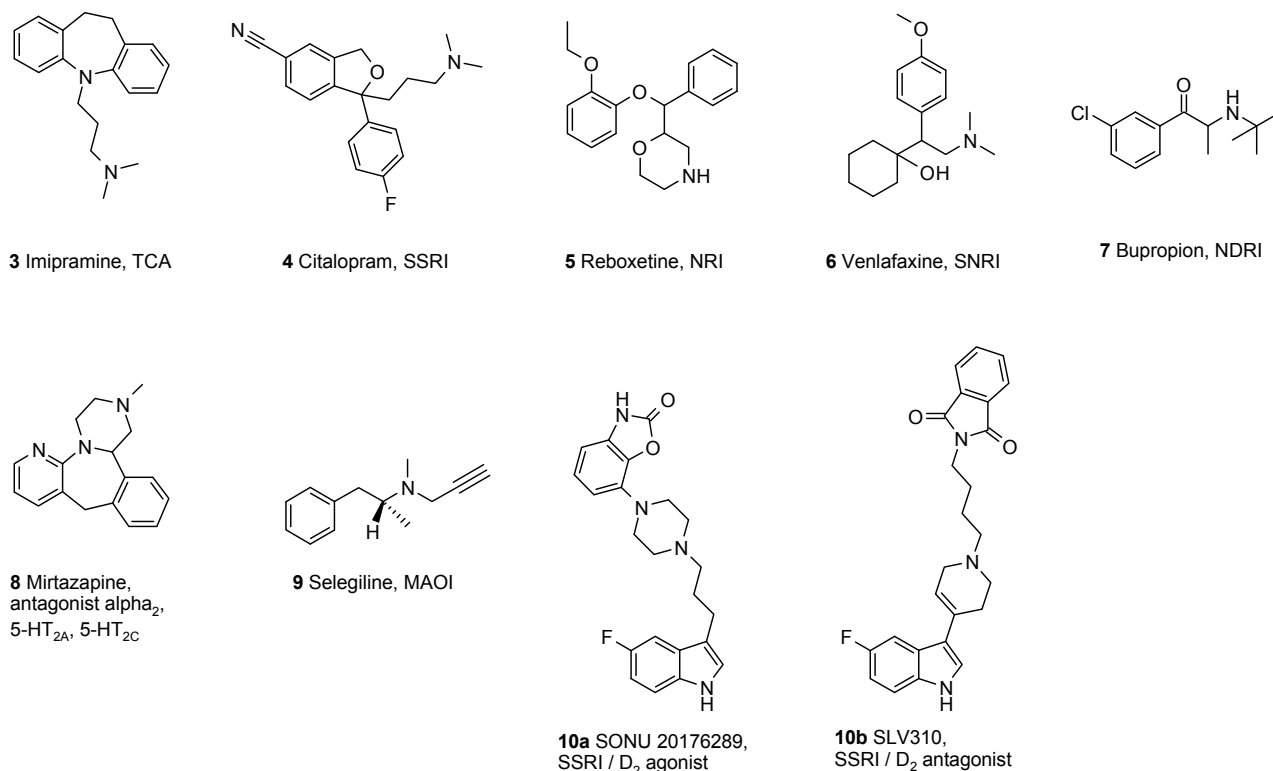
Figure 9. Antidepressants: tricyclic antidepressant (TCA), selective serotonin reuptake inhibitors (SSRI), selective norepinephrine reuptake inhibitors (NRI), dual serotonin and norepinephrine reuptake inhibitors (SNRI), norepinephrine and dopamine reuptake inhibitor (NDRI), unselective and irreversible monoamine oxidase inhibitor (MAOI).

For these reasons an improvement of the efficacy of existing antidepressants is needed. In recent years studies of antidepressant and electroconvulsive treatments have yielded insights on how to assign specific symptoms of depression to different monoaminergic neurotransmitters (Figure 10). NE may be related to alertness, energy, anxiety, attention, and interest in life; 5-HT to anxiety, obsessions, and compulsions; and DA to attention, motivation, pleasure, reward and interest in life. All three transmitters have an impact on mood but other symptoms may be related to a specific

monoamine.[56-58] The depressive symptoms can be divided into two groups; an increase in negative affect and a loss of positive affect. Negative affect means viewing the world as a hostile, unpleasant, disturbing and threatening place. Loss of positive affect means having the inability to enjoy rewards from normal activities such as family, work or hobbies that normally give one pleasure (Figure 11). The two groups can both contribute to the feeling of low mood and sadness. By using this type of model it is possible to better understand how to treat the symptoms of depression. Patients with symptoms associated with negative affect are best treated with 5-HT/NE acting drugs and patients experiencing loss of positive affect can be better treated with DA and/or NE acting drugs.[58] One of the main areas in the brain that is believed to be involved in the loss of positive affect is the prefrontal cortex.
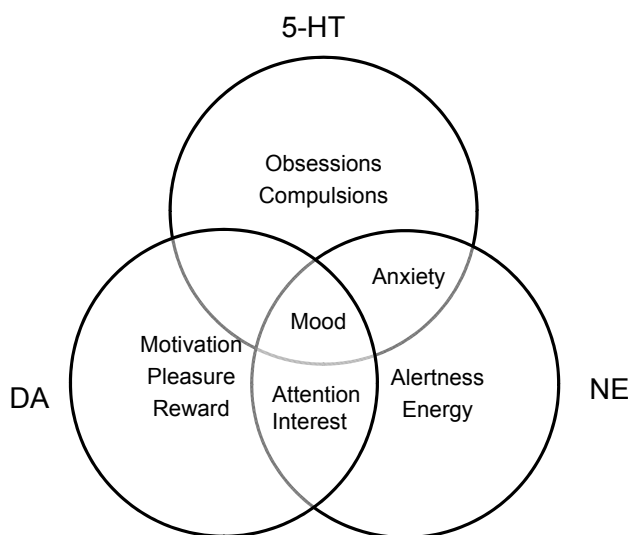
Figure 10. Monoamine neurotransmitter regulation of mood and behavior. Modified from Nutt.[58]

The new understanding on how different symptoms vary with the diverse monoamines has yielded an interest in introducing a dopaminergic component into antidepressant drugs.[59] Bupropion (**7**, Figure 9) is the only drug approved today with a direct dopaminergic mechanism, i.e. moderate DAT inhibition. Other drugs such as NRI, SNRI and "receptor blockers", increase DA in prefrontal cortex by indirect mechanisms, i.e. by blocking the NE reuptake transporter (NET) (in the frontal cortex the NET is mainly responsible for DA elimination) or through other receptor interactions.[60] In treatment resistant depression, combination treatments with SSRI and different atypical antipsychotics (DA D_2 antagonists) have been beneficial, and today aripiprazole, quetiapine and olanzapine are approved for adjunctive treatment in major depression (the combination of olanzapine and fluoxetine is registered

as Symbyax[®,61]).[62-65] In addition, data from clinical studies have shown that DA agonists such as pramipexole and ropinirole exhibit antidepressant properties.[59, 66, 67] Furthermore, compounds with dual effects such as DA D_2 agonism/SERT inhibition [e.g. SONU 20176289, (**10a**)][68, 69] and potent DA D_2 antagonism/SERT inhibition [e.g. SLV310, (**10b**), Figure 9][70-74] have been developed and investigated for their antidepressant properties. Another concept of elevating all three monoamines DA, NE and 5-HT, without any selectivity for different brain regions, is to use MAOIs. Selective MAO A inhibitors [RIMA, moclobemide (**2**, Figure 8)] and non-selective MAOIs [selegiline (**9**), Figure 9] are today used for treatment resistant depression.[41, 50, 75, 76]
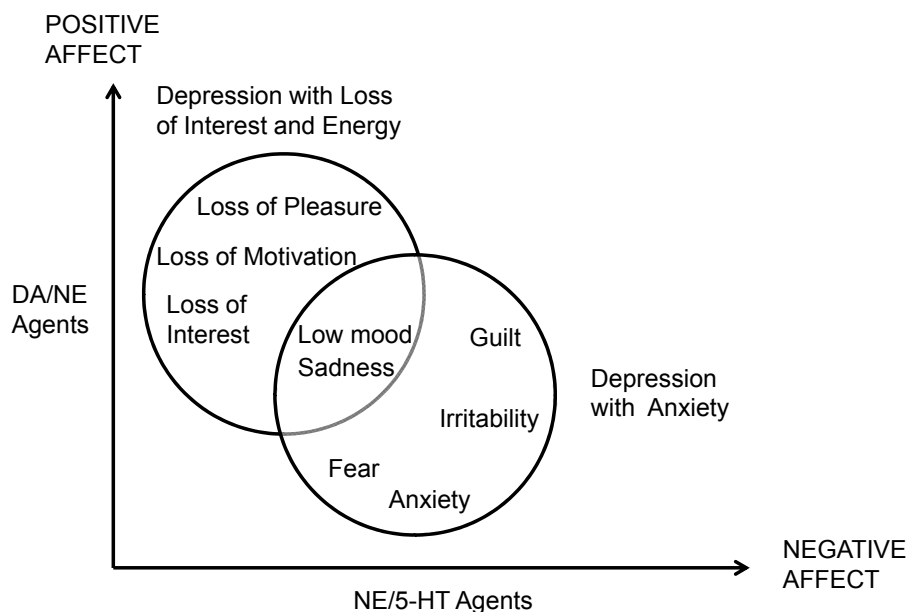
Figure 11. Hypothetical model showing differential actions of antidepressants agents on positive and negative affect. Modified from Nutt.[58]

In addition, a different hypothesis for finding new antidepressants is to explore the diverse postsynaptically located 5-HT receptor subtypes. The most used treatment of depressive symptoms is SSRIs, which yield an unspecific stimulation of all postsynaptic 5-HT subtypes by increasing extracellular 5-HT levels. Today it is not known which 5-HT subtype receptor or combination of subtype receptors that mediate the antidepressant effect of SSRIs. It is currently believed that 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{2C}$, 5-HT$_4$ and 5-HT$_6$ receptors may be involved in the antidepressive response.[29, 30, 77, 78]

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1.8. Structure-activity relationships

One of the most important stages of the drug discovery process is the generation of lead compounds. Structure-activity relationships (SARs) are well integrated in modern drug discovery and have been largely used in the process of finding new leads, optimization of their effects on receptors or enzymes, as well as optimization of pharmacokinetic and physicochemical properties.[79]

11 RU 24969

12 R = -H, -Cl

13

14

15

16 R^5 = -H   R = -Me
17 R^5 = -OMe R = -Me
18 R^5 = -OMe R = -H

19

20 Naratriptan
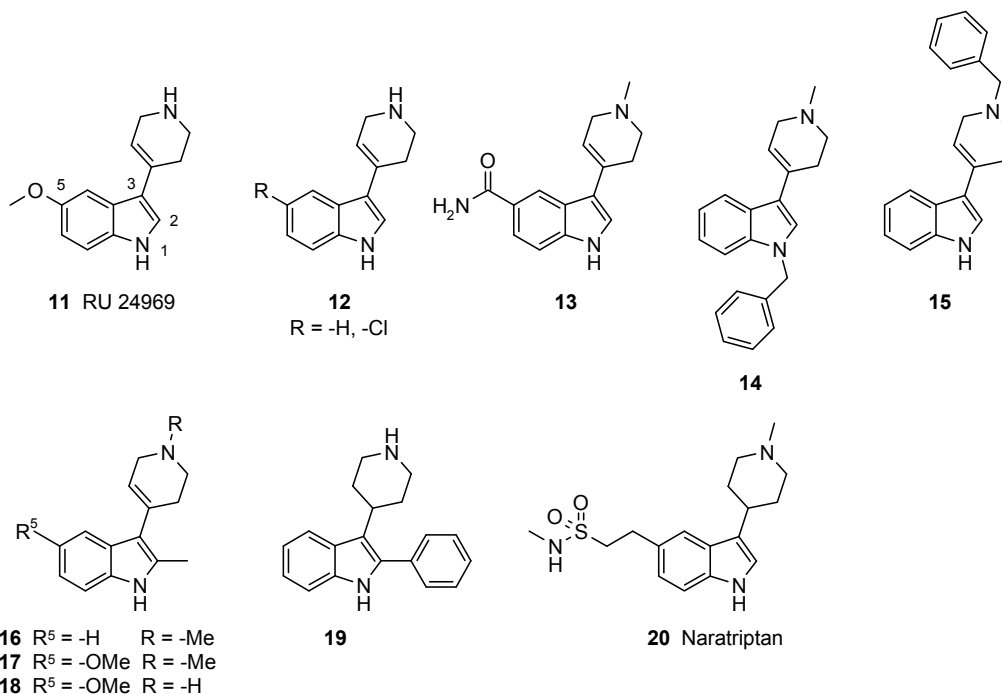
Figure 12. Tetrahydropyridine/piperidine-indoles with affinity/activity to the 5-HT receptors and/or SERT.

1.8.1. RU 24969 and analogs, SAR for 5-HT subtypes

As a structural class of pharmacologically active compounds, piperidine/tetrahydropyridine-indole derivatives (Figure 12) have been extensively studied for effects on different targets. The first ligand reported as a non-selective 5-HT receptor agonist within this class in 1980s was the tetrahydropyridine RU 24969 (**11**, Figure 12).[80] Currently, **11** is classified as a serotonin 5-HT$_{1A/1B}$ agonist and displays no activity on SERT, MAO or DA D$_2$ receptors.[80-85] However, the corresponding 5-H and 5-Cl analogs (**12**) of **11** have affinity for SERT (IC$_{50}$ = 160-300 nM) and weak affinity for MAO (IC$_{50}$ = 2.8-3.7 μM).[80, 81] Tetrahydropyridine-indoles substituted at the 5-position with methoxy, bromo, chloro, methyl ester or nitro groups have been found to display affinity to the 5-HT$_{1A}$ receptor. Most favored was however the carboxamido group (**13**, K$_i$ = 5 nM).[81, 86] Selectivity for 5-HT$_2$ over the 5-HT$_1$ receptor is possible to achieve by introducing large

hydrophobic groups, like benzyl, on the 1-position of the indole (**14**) or at the basic tetrahydropyridine nitrogen (**15**).[87] Several researchers have investigated the effects of introducing a methyl group in the 2-position (**16-18**) of **11** and found that generally the affinity for the 5-HT$_1$ and 5-HT$_2$ receptors decreases between 12-173 fold compared with the unsubstituted tetrahydropyridine-indoles.[81, 86-88] Larger groups such as 2-phenyl (**19**) is reported to enhance the 5-HT$_2$ affinity in the piperidine-indole series.[89] In addition, introduction of bulkier groups in the 5-position of piperidine indoles have been used to develop selective agonists for the 5-HT$_{1B/1D}$ receptors [i.e. naratriptan (**20**), a registered drug for migraine].[90]
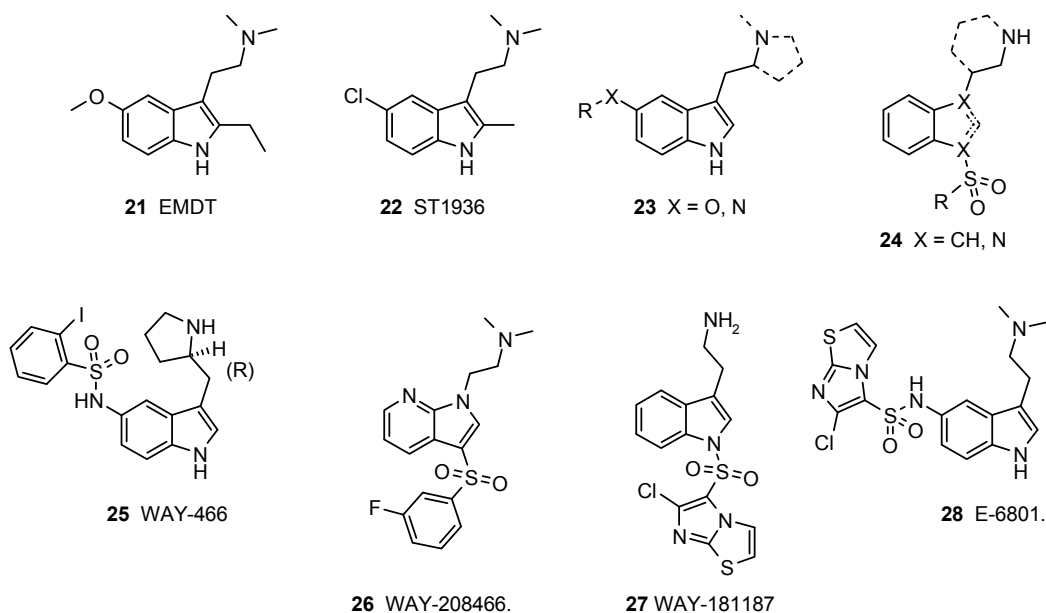
Figure 13. Known tryptamine based 5-HT$_6$ receptor agonists.

1.8.2. 5-HT$_6$ receptor agonists

All currently known 5-HT$_6$ receptor agonists are based on the 5-HT scaffold, and the first reported agonists had an alkyl group in the 2-position (**21** and **22**, Figure 13).[91, 92] More recently, a series of 5-HT$_6$ receptor agonists has been reported that are built on the two chemical motifs **23** and **24** (Figure 13), where the R-group is defined by a large aryl substituent.[93-100] From these two series, it is clear that the 5-HT$_6$ receptor can accommodate larger groups in both the N^1- and 5-positions when the basic amino group is positioned on an ethyl side chain in the 3-position of the indole. The amino group has also been incorporated in ring-closed motifs, such as the pyrrolidine and piperidine ring, with retained agonism. Furthermore, Holenz *et al.* have reported an elegant study on compounds based on the general structure **23**, from which potent 5-HT$_6$ receptor antagonists and agonists were

developed depending on the properties of the aryl-sulfonamide (R-group) used.[93] This means that the substitution in the 5-position is crucial for whether an agonist or antagonist will be formed, and this position may be used for fine tuning of agonist vs. antagonist properties. It has recently been shown that 5-HT$_6$ agonists such as EMDT (**21**),[91, 101] ST1936 (**22**),[102] LY-586713,[103] WAY-466 (**25**),[95] WAY-208466 (**26**),[77, 98, 104] WAY-181187 (**27**),[77, 104] and E-6801 (**28**)[105] (Figure 13) have antidepressant and/or cognition enhancing effects.[27-30, 106, 107]
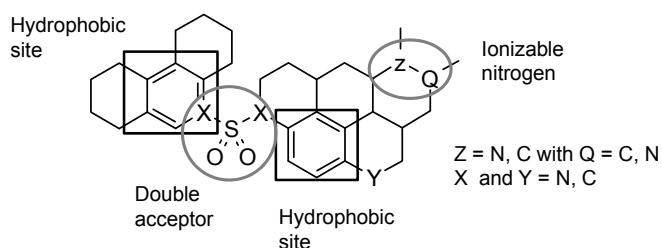
Figure 14. Hypothetical framework for 5-HT$_6$ antagonists with the common structural motifs outlined, modified from Holenz *et al*.[93, 94]

1.8.3. 5-HT$_6$ receptor antagonists

Selective 5-HT$_6$ receptor antagonists were discovered a few years after the discovery of the 5-HT$_6$ receptor through high-throughput screening and modification of the endogenous ligand 5-HT.[108] The common motifs for selective 5-HT$_6$ antagonists have four key elements (Figure 14), two hydrophobic areas (aromatics) connected via a hydrogen bond acceptor (sulfonamide or sulfonyl), and one ionizable often tertiary aliphatic amino function.[94, 100, 109] The early analogs lacked brain penetration properties and were stopped after clinical phase I studies (e.g. SB-271046, **29**, Figure 15). Today several 5-HT$_6$ antagonists [e.g. LY-483518 (**30**), PRX-07034 (**31**) and, SB-742457 (**32**), Figure 15] are in clinical development for the treatment of cognitive disorders (Alzheimer's disease) and obesity.[27-29, 110, 111] In addition, 5-HT$_6$ antagonists have shown antidepressant properties, which is controversial due to the fact that 5-HT$_6$ agonists also display antidepressant effects.[27-29]

| **29** SB-271046 | **30** SGS-518, LY-483518 | **31** PRX-07034 | **32** SB-742457 |
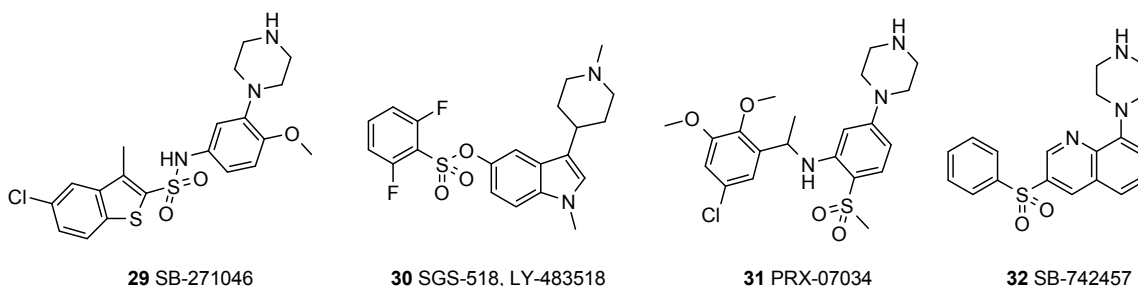
Figure 15. A selection of 5-HT$_6$ antagonists which have entered clinical development.

1.8.4. RU 24969 analogs and SAR for the 5-HT$_6$ receptor

Additional studies on the tetrahydropyridine/piperidine moiety have reported **33**[93] and **34**[112] to be potent 5-HT$_6$ antagonists (Figure 16). However, moving the nitrogen atom in the tetrahydropyridine ring one step yields a modest partial agonist 1-(benzenesulfonyl)-3-(1,2,3,6-tetrahydropyridin-5-yl)indole (**35**, K$_i$ = 4.6 nM, EC$_{50}$ = 159 nM, efficacy 41%, Figure 16),[113] while the saturated analog **36** (K$_i$ = 2 nM with EC$_{50}$ = 24 nM, Figure 16) is a full 5-HT$_6$ receptor agonist. Separation of the enantiomers yielded one enantiomer behaving as a full agonist whereas the other is a potent antagonist.[113]
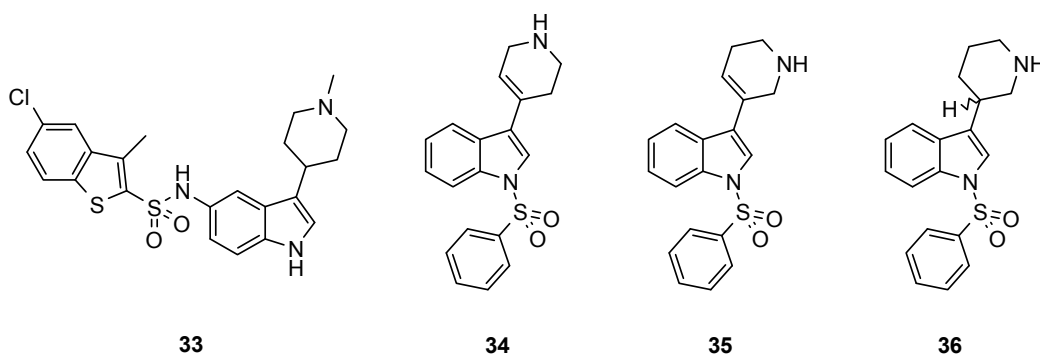
Figure 16. Tetrahydropyridine/piperidine-indole based 5-HT$_6$ receptor ligands.

1.8.5. Dopamine D$_2$ receptor antagonists

DA D$_2$ receptor antagonists were the first drugs used in the treatment of schizophrenia in the 1950s [e.g. haloperidol (**37**) and pimozide (**38**), Figure 17] and these drugs were classified as typical antipsychotics.[114] The symptoms for schizophrenia can be divided into two groups; positive symptoms (e.g. hallucinations and delusions) and negative symptoms (e.g. mood symptoms and cognitive deficits).[115] The first generation of antipsychotics (i.e. typical antipsychotics) in general has good effect on the positive symptoms, but the negative symptoms were left untreated, and patients usually suffered from a broad side effect profile, i.e. extrapyramidal side effects (EPS) such as parkinsonism and tardive dyskinesia.[116] This led to the development of the second generation antipsychotic drugs (i.e. atypical antipsychotics) represented by sertindole (**39**),[117] risperidone (**40**)[118], ziprasidone (**41**) and olanzapine (**42**) (Figure 17).[119, 120] The target mechanism for these ligands was a combination of DA D$_2$ and 5-HT$_{2A}$ receptor antagonism, but they also were found to have high affinity for a broad range of other receptors [5-HT$_{2C}$, 5-HT$_6$ and 5-HT$_7$, muscarinic, α-

adrenergic, histaminergic (H$_1$), and dopaminergic (D$_4$ and D$_1$)]. DA D$_2$ receptor antagonists are usually large lipophilic compounds that lack the essential pharmacophore elements for displaying agonist properties.[32] The aromatic moieties could be simple phenyl rings as in haloperidol (**37**, Figure 17) or in other cases built on bicyclic aromatic moieties as in pimozide (**38**), sertindole (**39**), risperidone (**40**), and ziprasidone (**41**). These large lipophilic aromatic moieties are believed to interact with hydrophobic residues that are not involved in agonist interactions in the receptor cavity, and thereby stabilizing the inactive state of the DA D$_2$ receptor.[121, 122]
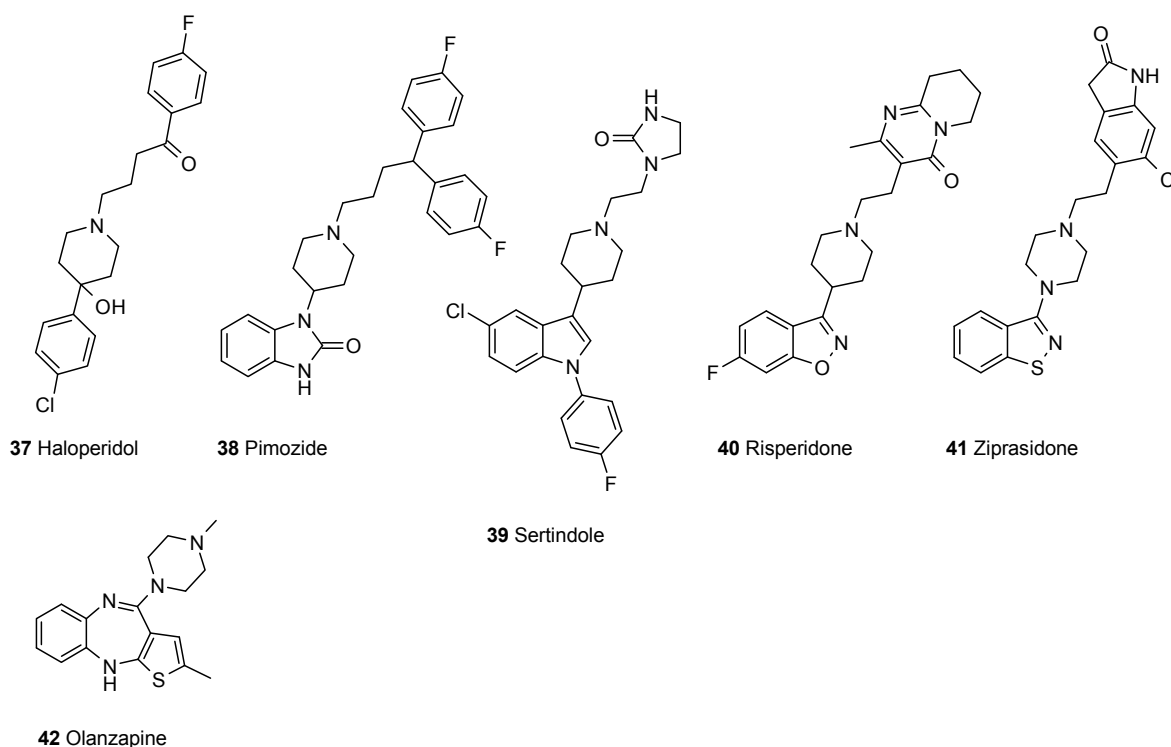
Figure 17. Dopamine D$_2$ receptor antagonists clinically developed as typical/atypical antipsychotics.

1.8.6. Dopamine D$_2$ receptor agonists

Dopamine D$_2$ receptor agonists are mainly hydrophilic compounds resembling the chemical structure of the endogenous ligand DA, e.g. ropinirole (**43**, Figure 18).[32] All DA D$_2$ agonists possess a basic nitrogen atom separated by a 5-7 Å chain or framework (ethyl amino side chain) from an aromatic ring with a hydrogen bond donating group in the *meta*-position. Substitution on the basic nitrogen with alkyl groups improves both DA D$_2$ receptor potency and efficacy. The *N*-propyl group has been found to be favored in several DA D$_2$ agonists, it binds in the propyl binding pocket in the DA D$_2$

receptor.[123, 124] DA D_2 agonists such as ropinirole (**43**) and pramipexole (**44**, Figure 18) are mainly used in the clinic for early treatment of Parkinson's disease.[125] On the other hand, partial DA D_2 agonists like (-)-3PPP (**46**, Figure 18)[126] and the more recently developed aripiprazole (**45**, Figure 18) have demonstrated efficacy in the treatment of schizophrenia.[120, 127] In addition, aripiprazole (**45**) has recently been found to counteract the induced weight gain by DA D_2 antagonists such as olanzapine (**42**, Figure 17). without interfering with the antipsychotic effects.[128]
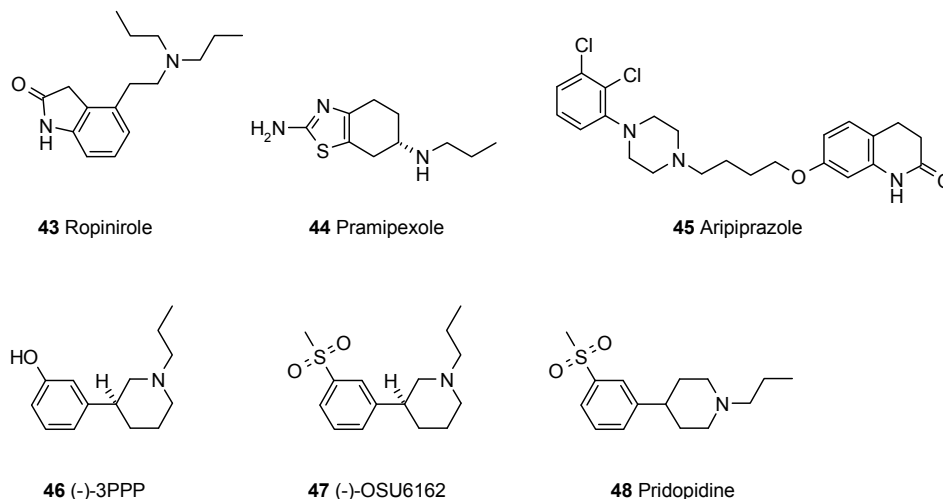
Figure 18. Dopamine D_2 receptor ligands: the full agonists ropinirole (**43**) and pramipexole (**44**), the partial agonists aripiprazole (**45**) and (-)-3PPP (**46**), the dopaminergic stabilizers (*S*)-(-)-OSU6162 (**47**) and pridopidine (**48**).

1.8.7. Dopamine D_2 receptor stabilizers

Recently, a new class of DA D_2 ligands was discovered, the so called dopaminergic stabilizers exemplified by (-)-OSU6162 (**47**)[129] and pridopidine (ACR16, **48**) (Figure 18).[130] Dopaminergic stabilizers have an *in vivo* profile that is distinct from DA D_2 antagonists, partial agonists and agonists. *In vivo* the dopaminergic stabilizers behave as DA D_2 antagonists but have the unique property to counteract states of both hyper- and hypoactivity (behavior), depending on the prevailing dopaminergic tone. From an *in vitro* perspective, dopaminergic stabilizers are DA D_2 receptor ligands with fast off kinetics that bind preferentially to the DA D_2^{High} affinity state without inducing any intrinsic activity. This is in sharp contrast to classical DA D_2 antagonists which binds with equal affinity to DA D_2^{High} and D_2^{Low}. The low affinity for DA D_2^{Low} and rapid dissociation is believed to allow for the DA D_2 receptors to regain responsiveness to DA relatively quickly, since the

dopaminergic stabilizers lose their occupancy much faster and thus allow for surges of DA to access the receptors.[130] In support of this, it was recently reported that the DA D_2 antagonists haloperidol (**37**) and sertindole (**39**) displayed insurmountable/noncompetitive-like DA D_2 receptor antagonistic properties while the dopaminergic stabilizers such as **47** and **48** were found to be surmountable/competitive in the presence of dopamine.[131] The dopaminergic stabilizer **48** is currently in Phase III development for the treatment of motor symptoms associated with Huntington's disease.[132, 133] The other dopaminergic stabilizer **47** has recently been found to be active in animal models for alcohol dependence,[134] improvement in stroke/traumatic brain injury in humans, and has a potential of treating L-DOPA induced dyskinesia in Parkinson's disease and schizophrenia.[135-137]
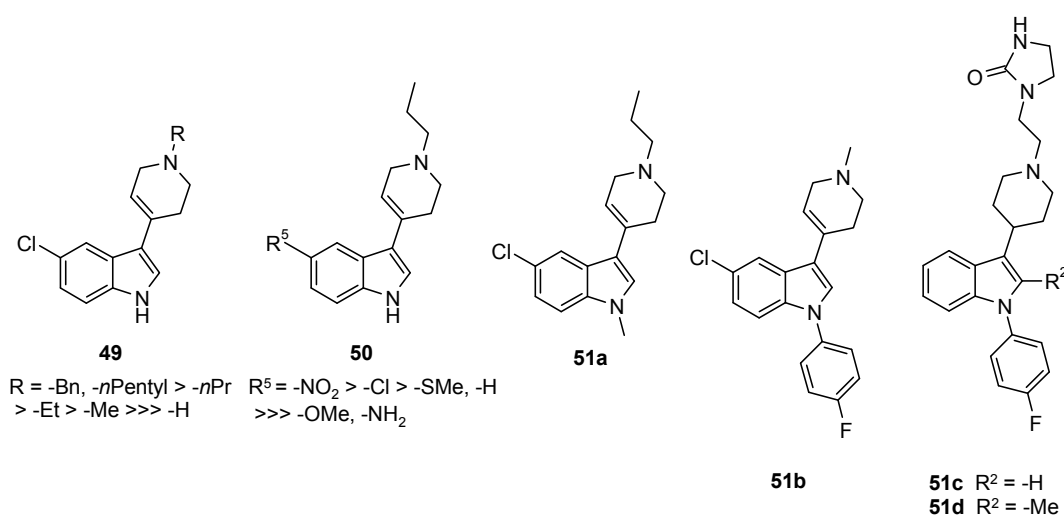
Figure 19. Tetrahydropyridine/piperidine-indole based dopamine D_2 ligands

1.8.8. RU 24969 analogs and SAR for dopamine D_2 receptors

Guillaume *et al.*[81] published a SAR study around the DA D_2 receptor for analogs of the tetrahydropyridine-indole derivative RU 24969 (**11**, Figure 12) and found that the secondary amines, regardless of different 5-substituents [methoxy (**11**), ethoxy, thiomethyl, nitro and, chloro (**12**)] lack activity at DA D_2 receptors. However, by substitution at the basic amine with alkyl groups, antagonistic dopaminergic effects were achieved (**49**, Figure 19). The most potent antagonists were the benzyl (IC_{50} = 40 nM) and *n*-pentyl (IC_{50} = 54 nM) derivatives followed by *n*-propyl (IC_{50} = 80 nM). Further investigations were made with different substituents in the 5-position together with an *n*-propyl substituent (**50**, Figure 19). The nitro (IC_{50} = 30 nM) and chloro (IC_{50} = 80 nM) derivatives turned out to be the most potent. In addition, adding a 1-methyl substituent (**51a**) reduced the DA D_2

20

receptor affinity 6-fold compared with the unsubstituted derivative.[81] However, a phenyl group attached to the 1-position yielded high affinity ligands for the DA D_2 receptor (**51b**, IC_{50} = 1.1 nM; **51c**, IC_{50} = 18 nM, Figure 19).[117, 138] In addition, Perregaard *et al.* reported that the substitution with a methyl group in the 2-position of the indole core (**51d**, Figure 19), decreased the affinity for DA D_2 receptors 21-fold compared to the unmethylated derivative (**51c**).[138]

1.8.9. RU 24969 analogs and SAR for MAO inhibition

A few examples of analogs of the 3-tetrahydropyridine-indole RU 24969 (**11**, Figure 12) such as the 5-H and 5-Cl derivatives (**12**, Figure 12) are reported to have moderate affinity for the MAO enzyme (IC_{50} = 2.8-3.7 µM, rat brain both subtypes).[80] However, moving the piperidine ring to the 2-position and exchanging the indole to benzofuran yields high affinity ligands as in the known RIMAs [i.e. brofaromine (**52**)[45] and sercloremine (**53**), Figure 20].[139] Both these derivatives also have moderate affinity for SERT. However, insertion of substituents in the 5- and 6-positions of benzofuran scaffold diminishes the MAO inhibitory activity and yields a potent SSRI (CGP 6085 A, **54**).[140]

52 Brofaromine   **53** Sercloremine   **54** CGP 6085A
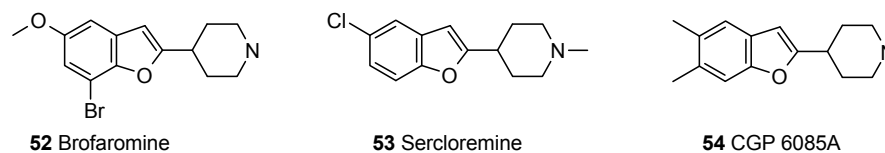
Figure 20. Monoamine oxidase inhibitors (MAO) **52** and **53** and the structurally related selective serotonin reuptake inhibitor (SSRI) **54**.

1.8.10. Coumarin analogs and SAR for MAO inhibition

Coumarins (2*H*-chromen-2-one) are naturally occurring in many plants and are well-known for displaying a variety of pharmacological properties depending on the substitution patterns.[141] Over the last decade, coumarin derivatives have been identified as inhibitors of therapeutically important enzymes such as aromatase and acetylcholinesterase.[142, 143] One of the most famous drugs that are based on the coumarin scaffold is the anticoagulant warfarin.[144] Derivatives containing the coumarin ring system have shown MAO inhibitory activity and in recent years the knowledge of how to develop selective MAO B ligands within this class has emerged.[145-147] However, only a few publications can be found describing MAO A selective coumarins. Esuprone (**55**) and LU 53439 (**56**, Figure 21) are two examples of MAO A and MAO B selective ligands, respectively, and the SAR

studies within this chemical class have revealed that the substitution pattern is crucial for both activity and selectivity.[146] Most of the attention has been focused on the C7 position where the type of substitution is extremely important for MAO A or MAO B selectivity. However, there is no clear chemical property of the substituent that correlates to either MAO A or MAO B selectivity. The C3 and/or C4 positions tolerate a large variety of groups such as alkyl, phenyl, carboxylic acid and ester, acyl, amides etc. and these compounds tends to be MAO B inhibitors (**56** and **57**).[145-155]
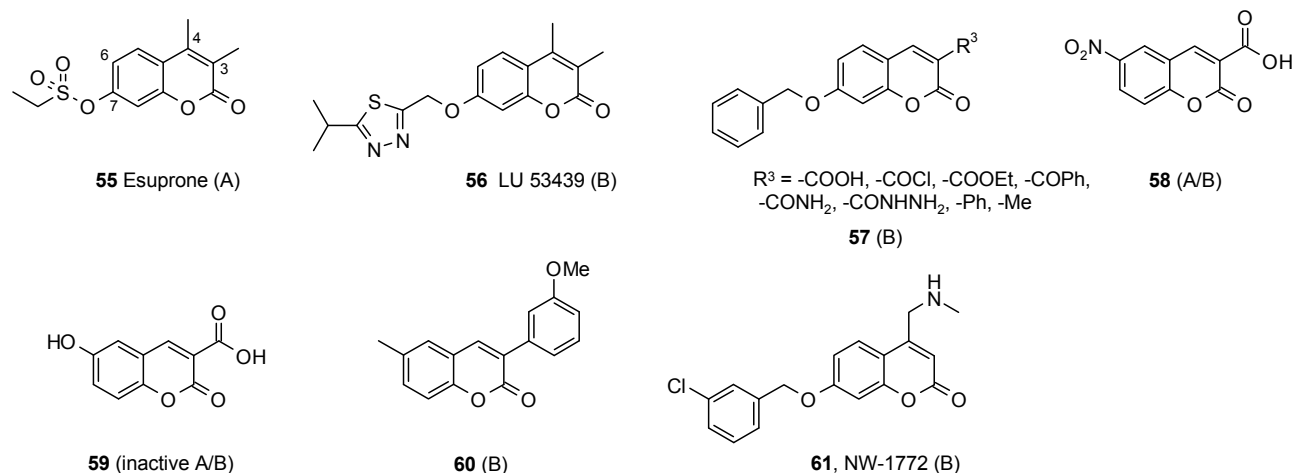
Figure 21. Reversible MAO A (A) and MAO B (B) coumarin based inhibitors. Abbreviations: MAO, monoamine oxidase.

Among the existing publications on coumarins functioning as MAO inhibitors, only a few have reported the effect of substitution at the C6 position. In general, such compounds have low activity at MAO A and MAO B (**58**, **59**, Figure 21),[152, 156] except for **60** which is a potent MAO B inhibitor (IC_{50} = 0.8 nM).[154] One of the major drawbacks with the coumarins developed so far are properties such as low aqueous solubility and weak metabolic stability, which hampers further development of clinical candidates.[157] Therefore a search for new coumarins with improved pharmacokinetic properties and better physicochemical properties is ongoing. Recently Pisani *et al.*[157] reported the discovery of a new selective MAO B inhibitor with improved pharmacokinetic and toxicity properties (NW-1772, **61**, Figure 21) by the introduction of a methylaminomethylene group in the 4-position of the coumarin core. This finding is encouraging for the development of more drug-like molecules within this class of compounds.[157, 158]

2. Aims

This thesis is part of an ongoing research project aimed at the development of novel drugs with effects in the serotonergic and dopaminergic systems useful for treatment of affective disorders. To maintain this goal, the specific objectives of this project were to:

- Investigate the SARs for 2-alkyl-3-(1,2,3,6-tetrahydropyridin-4-yl)-1H-indoles, for the development of 5-HT$_6$ receptor agonists.

- Investigate 1-propyl-4-aryl-piperidines for their dopaminergic and serotonergic properties *in vivo* and *in vitro* (DA D$_2$, SERT, MAO), using a scaffold-jumping approach.

- Develop selective MAO A inhibitors based on 6-subsituted 3-(pyrrolidin-1-ylmethyl)chromen-2-ones (coumarins).

3. Chemistry

The compounds included in this work have been synthesized by various methods described in the literature. The 2-alkylindoles (Paper I and II) and the coumarins (Paper IV) were synthesized by ring closing reactions and by functional group transformation of available intermediates. The 4-aryl-piperidines (Paper III) were transformed to the target compounds by alkylation reactions. For reactions not discussed in detail, further information and specific conditions are given in the corresponding Papers I-IV as indicated below. In addition, a chemistry section and experimental part to Paper I has been added (Appendix 1).

3.1. Synthesis of 2-alkyl substituted 3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indoles (Paper I, II)

The target 2-alkyl substituted 3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole derivatives were prepared by an acid catalyzed condensation between 2-alkyl-1*H*-indoles and 4-piperidone/1-benzylpiperidin-4-one in 25-98% yield (Scheme 1).[159] The different 2-alkyl-1*H*-indoles were synthesized according to Scheme 2 using an improved Madelung ring synthesis (Paper I),[160, 161] or by modifications of the 5-substituted-2-methyl-1*H*-indoles (Scheme 3) (Paper II). A few of the 2-alkyl-1*H*-indoles were commercially available, i.e. 5-methoxy (**97**), 5-bromo (**102**), 5-amino (**105**), 5-chloro (**109**), 5-fluoro (**113**), 5-H (**114**) and 5-nitro-2-methyl-1*H*-indole (**115**). 5-Methylsulfonyl-1*H*-indole-2-carboxylic acid (**107**) was used as a precursor for 2-methyl-5-methylsulfanyl-1*H*-indole (**108**) (Paper I and II).[162]

Scheme 1. General synthesis of 2-alkyl-3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indoles.[a]

R = -H, -Bn
R^1 = -H, -Me, -Et, -*n*Pr
R^2 = -H, -Me, -Et, -*n*Pr, -*i*Pr
R^5 = -H, -F, -Cl, -Br, -SMe, -OMe, -O*i*Pr, -OSO$_2$CF$_3$, -OPh(2-NO$_2$), -NHSO$_2$Ph, -Ph, -(3-thienyl)
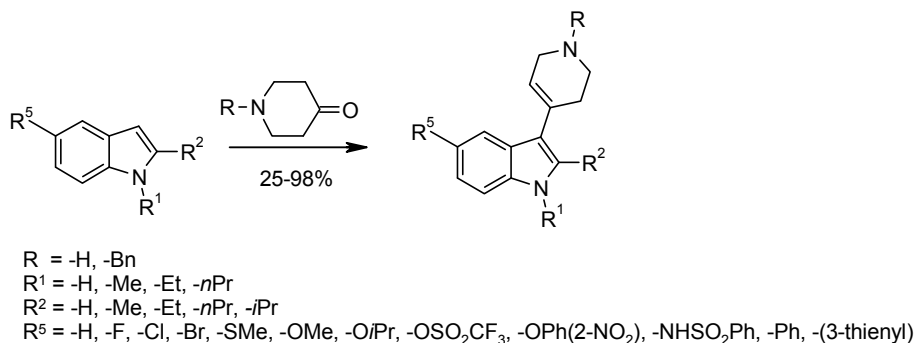
[a]*Reagents and conditions*: H$_3$PO$_4$, acetic acid, 80 °C.

25

3.1.1. Madelung synthesis of 2-alkyl-1H-indoles

The Madelung reaction is very useful for the preparation of 2-substituted indoles. However, in its original form it is run under harsh conditions using potassium *tert*-butoxide at elevated temperatures (250-350 ºC) in order to make the condensation between a non-activated aromatic methyl group and an *ortho*-acylamino substituent possible. Today, a modified version of the Madelung condensation has been developed, using alkyl lithium bases at low temperatures, allowing much milder reaction conditions and other starting materials. The reaction is outlined in Scheme 2. [160, 161, 163, 164]

Scheme 2. Madelung synthesis of 2-alkyl-1*H*-indoles and further reaction to 2-alkyl-3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indoles.[a]
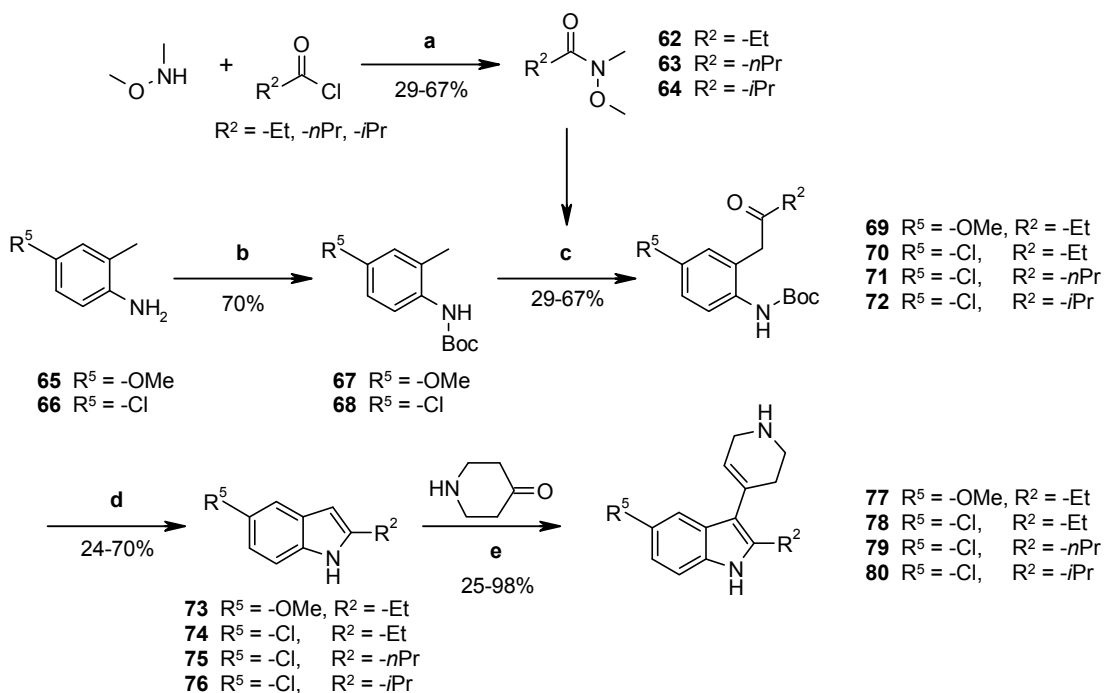
[a]*Reagents and conditions*: (a) 2 equiv. triethylamine, CH$_2$Cl$_2$, 0 °C to rt; (b) (*t*-BuO$_2$C)$_2$O, THF, Δ; (c) 2 equiv. *sec*-BuLi, R^{2}CON(OMe)Me (**62-64**), THF, -40 °C to rt; (d) trifluoroacetic acid, CH$_2$Cl$_2$; (e) H$_3$PO$_4$, acetic acid, 80 °C.

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The various 5-substituted 2-alkylindoles (**73-76**, Scheme 2) were synthesized starting from 2-methylanilines (**65**, **66**) protected with a *tert*-butyloxycarbonyl group (Boc) to give **67** and **68** in approx. 70% yield (Scheme 2). Treatment with 2 equiv. of strong base (i.e. *sec*-butyllithium) afforded a stabilized dianion which was acylated by different *N*-methyl-*N*-methoxyamides (**62-64**, Weinreb amides, Scheme 2)[165, 166] to give the ketones (**69-72**, Scheme 2) in moderate yields (29-67%). The methoxy moiety in the Weinreb amides facilitates the nucleophilic attack both inductively and through chelation. The ketones (**69-72**, Scheme 2) were subsequently treated with diluted trifluoroacetic acid to achieve cyclization and deprotection affording the 2-alkyl-1*H*-indoles in moderate yields (**73-76**, 24-70%, Scheme 2). In the last step the 2-alkyl-1*H*-indoles (**73-76**) were treated with 4-piperidone to give the 2-alkyl substituted 3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indoles in moderate to good yields (**77-80**, 25-98%).

3.1.2. Transformation of functional groups on the indole core structure (Paper II)

The 2-methyl-3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indoles **81-96** were prepared by transformation of functional groups on the indole core (Scheme 3). The different transformations used were: Mitsunobu coupling, palladium catalyzed cross coupling (Suzuki), nucleophilic aromatic substitution, sulfonylation of aniline, alkylation and dealkylation.[163] A few transformations were less successful such as the reduction of 5-methylsulfonyl-1*H*-indole-2-carboxylic acid (**107**) to the corresponding 2-methyl-5-methylsulfanyl-1*H*-indole (**108**). Using a large excess of LiAlH$_4$ (10 equiv.) gave simultaneous reduction of both functional groups (sulfone and acid) but in low yield (16%).[162] Also the nucleophilic substitution of 2-methyl-1*H*-indol-5-ol (**98**) with 1-fluoro-2-nitrobenzene (microwave heating) proceeded in only moderate yield (36%). This nucleophilic aromatic substitution needed a strong electron-withdrawing group to proceed (-NO$_2$). Attempts to remove the nitro group were unsuccessful, reduction to an aniline was possible, but during diazotization conditions the indole was decomposed. Therefore compound **90** was used for pharmacological studies without any further transformations. In addition, the nucleophilic substitutions on the indole N^1-position (**110-112**) also proceeded in moderate yields.

Scheme 3. Synthesis of various 2-methyl-3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indoles by functional group transformations.[a]

	R^1	R^5
81	-H	-Cl
82	-H	-Br
83	-H	-F
84	-H	-OMe
85	-H	-H
86	-H	-OSO$_2$CF$_3$
87	-H	-NHSO$_2$Ph
88	-H	-SMe
89	-H	-NO$_2$
90	-H	-OPh(2-NO$_2$)
91	-H	-O*i*Pr
92	-H	-(3-thienyl)
93	-H	-Ph
94	-Me	-Cl
95	-Et	-Cl
96	-*n*Pr	-Cl
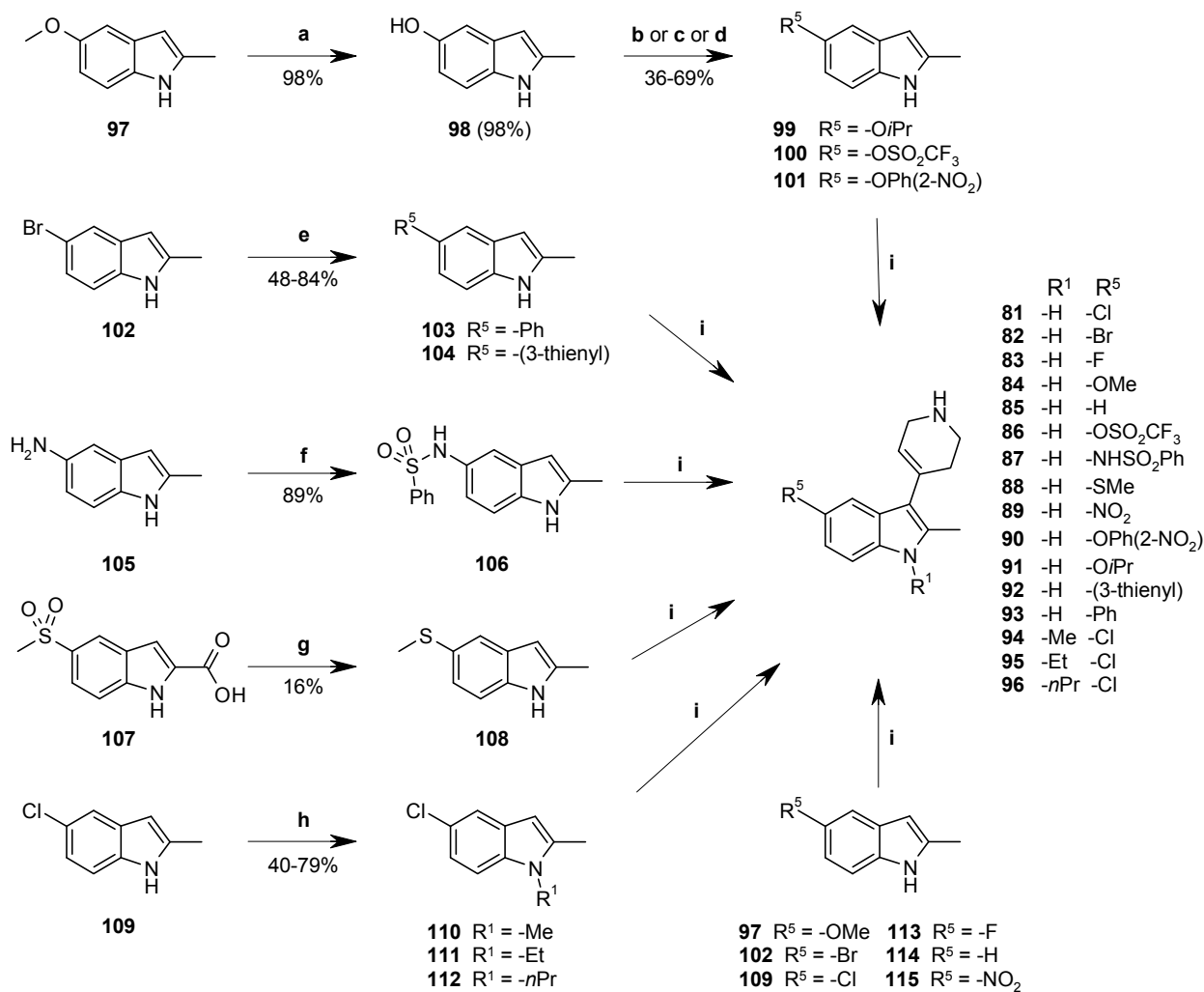
[a]*Reagents and conditions*: (a) BBr$_3$, CH$_2$Cl$_2$, rt; (b) 2-propanol, diethyl azodicarboxylate, Ph$_3$P, CH$_2$Cl$_2$; (c) PhN(SO$_2$CF$_3$)$_2$, triethylamine, CH$_2$Cl$_2$; (d) 1-fluoro-2-nitrobenzene, Cs$_2$CO$_3$, DMF, microwave heating 10 min, 140 °C; (e) phenylboronic acid/3-thiopheneboronic acid, Pd(PPh$_3$)$_4$, toluene, ethanol, aq. NaHCO$_3$, reflux; (f) PhSO$_2$Cl, pyridine, rt; (g) LiAlH$_4$, dioxane, 110 °C; (h) NaH, DMF, alkyl halide, Δ; (i) 4-piperidone hydrochloride, H$_3$PO$_4$, acetic acid, 80°C.

3.2. Synthesis of 1-propyl-4-aryl-piperidines (Paper III)

Most of the compounds in Paper III were synthesized by *N*-alkylation of commercially available 4-arylpiperidines under standard conditions (Scheme 4). However, the 2- and 3-benzothiophene and the 3-indazole derivatives were synthesized according to Schemes 5 and 6.

Scheme 4. General synthesis of 1-propyl-4-aryl-piperidine derivatives.[a]

Z = C, N
Y = CO, CH, N
X = CO, CH, NMe, NH, O, S
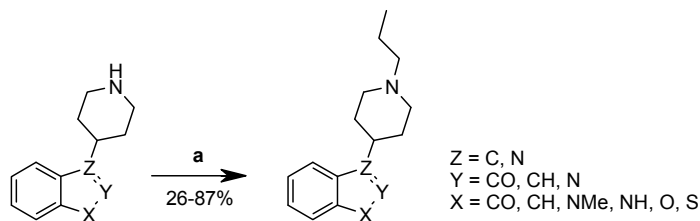
Cores: 3-Indole, 2-benzofuran, 3-benzothiophene, 3-benzisoxazole, 3-indazole, 3-benzimidazole, 3-benzimidazol-2-one, 3-isatin, *N*[1]-Indole, 1-naphthalene, 2-naphthalene, 2-benzothiophene

[a]*Reagents and conditions*: (a) 1-iodopropane, K_2CO_3, acetonitrile, Δ.

3.2.1. Synthesis of 3-(1-propyl-4-piperidyl)-1H-indazole (**119**)

The indazole ring system is a common bioisoster of indole and is frequently used in pharmaceutical compounds, although it has a rare occurrence in nature (Scheme 5).[167] The structural difference between the indole and indazole core is the replacement of C2 in indole by nitrogen. Therefore, the indazole C3 position is less nucleophilic for introduction of electrophiles compared to the corresponding indoles. This means that strong deprotonating agents are needed, which usually leads to ring opening and thus generating benzonitriles instead of the desired 3-substituted derivatives. Another issue with the indazole core is that regioisomers are formed during N^1-deprotonation. The deprotonated N^1-isomer is only slightly more stable than the N^2-isomer leading to mixtures of the regioisomers when indazoles are reacted with electrophiles under basic conditions.[168] Welch *et al.* developed a method where the stable dianion of 3-bromo-1*H*-indazole (**116**, Scheme 5) was generated by subsequent treatment with one equiv. *n*-butyllithium and two equiv. *tert*-butyllithium at −78 °C making C3-substitution with electrophiles possible.[169] The 3-substituted indazole **119**, was synthesized by the above mentioned method, where quenching with 1-propylpiperidin-4-one gave the 3-substituted indazole (**117**) in moderate yield (32%).[169] Subsequent treatment with

trifluoroacetic acid in CH$_2$Cl$_2$ gave the dehydrated compound **118** in excellent yield (98%). The tetrahydropyridine **118** was then reduced by catalytic hydrogenation (Pd/C), affording the piperidine-derivative **119** in moderate yield (46%, Scheme 5).

Scheme 5. Synthesis of 3-(1-propyl-4-piperidyl)-1*H*-indazole (**119**).[a]
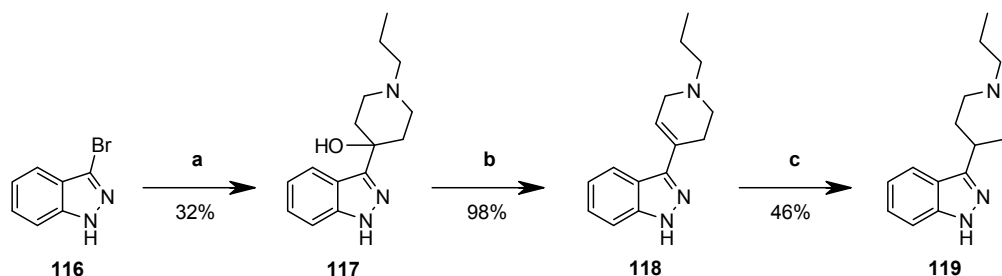
[a]*Reagents and conditions*: (a) *n*-BuLi (1 equiv.), *tert*-BuLi (2 equiv.), 1-propylpiperidin-4-one, THF; (b) trifluoroacetic acid, CH$_2$Cl$_2$, Δ; (c) Pd/C, H$_2$, ethanol.

3.2.2. Synthesis of 4-(benzothiophen-2 and 3-yl)-1-propyl-piperidine derivatives

Benzothiophenes can be selectively lithiated at the α-position to the heteroatom which gives a possibility to introduce electrophiles in the C2-position.[170] Lithiation at the C3-position can be achieved by halogen exchange at low temperatures (-78 ºC) in order to prevent isomerization to the more stable C2-lithiated intermediate.[163, 171] The two different regioisomers of benzothiophenes (**122** and **125**, Scheme 6) were synthesized by the above mentioned methodology. The 3-bromo-benzothiophene was lithiated with *n*-butyllithium at low temperature and quenched with 1-Boc-4-piperidone. Subsequent treatment with trifluoroacetic acid gave the dehydrated 3-substituted tetrahydropyridine **120** in moderate yield (35%). The corresponding 2-substituted benzothiophene derivative **123** was synthesized from benzothiophene by lithiation with *n*-butyllithium at room temperature and quenched with 1-Boc-4-piperidone. Subsequent treatment with trifluoroacetic acid yielded **123** in moderate yield (39%). Both tetrahydropyridine regioisomers (**120**, **123**) were alkylated with 1-iodopropane to afford **121** and **124** in excellent yield (98%). Reduction of the tetrahydropyridine ring with catalytic hydrogenation (Pd/C) gave the 2- and 3-substituted benzothiophene derivatives **125** and **122**, respectively (22-38%) (Scheme 6).

Scheme 6. Synthesis of 4-(benzothiophen-3-yl)-1-propylpiperidine (**122**) and 4-(benzothiophen-2-yl)-1-propylpiperidine (**125**).[a]
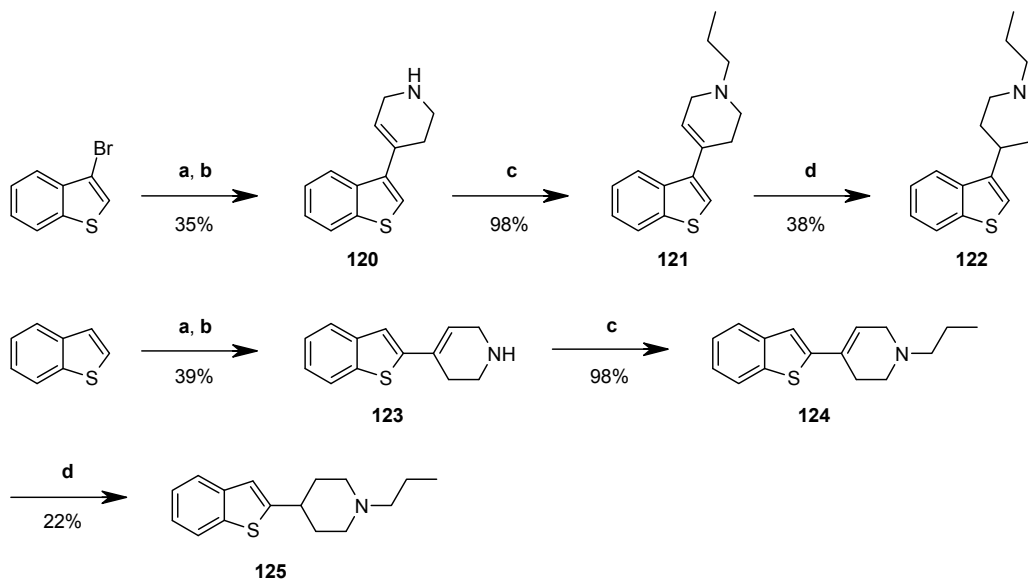
[a]*Reagents and conditions*: (a) *n*-BuLi, 1-Boc-4-piperidone, diethyl ether, THF; (b) trifluoroacetic acid, CH$_2$Cl$_2$, Δ; (c) 1-iodopropane, K$_2$CO$_3$, acetonitrile, Δ; (d) Pd/C, H$_2$, methanol, acetic acid, HCl.

3.3. Synthesis of 6-subsituted 3-(pyrrolidin-1-ylmethyl)chromen-2-ones (Paper IV)

The 6-subsituted 3-(pyrrolidin-1-ylmethyl)chromen-2-one derivatives described in Paper IV were synthesized by the use of the Baylis-Hillman reaction (Scheme 7) followed by ring closing reactions (Scheme 8 and 9) or by functional group transformation on the coumarin core (Scheme 3, Paper IV).

3.3.1. The Baylis-Hillman reaction

The Baylis-Hillman reaction (Scheme 7), is a versatile carbon-carbon bond forming reaction between the α-position of an activated alkene and an electrophile, often an aldehyde.[172] The reaction is catalyzed by tertiary amines such as 1,4-diazabicyclo[2.2.2]octane (DABCO) or other similar catalysts which typically gives multifunctional allylic alcohol products. The Baylis-Hillman product can serve as a precursor for several different ring systems (i.e. coumarin, chromene, indolizines and, quinolines) or to other biologically active compounds.[172-176]

Scheme 7. The general Baylis-Hillman reaction.[a]

R = -alkyl, -aryl
R^1 = -COMe, -CN, -CO$_2$Et etc.
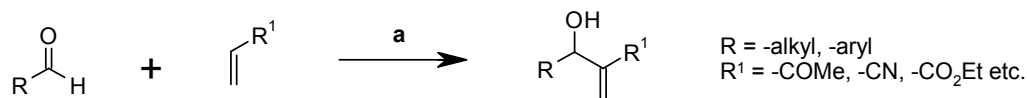
[a]*Reagents and conditions*: (a) tertiary amine (e.g. DABCO), neat or with solvent (e.g. CHCl$_3$, THF, DMF, 1,4-dioxane, MeOH), 0-70 °C, 1 h-weeks.

The majority of the 6-substituted coumarin derivatives in this series were prepared by the Baylis-Hillman methodology described by Kaye and Musa.[175] (Scheme 8 and 9). The different salicylaldehydes (**126a-e**, Scheme 8) were benzylated under standard conditions using potassium carbonate as base (48-97%, **127a-e**). Salicylaldehyde **126a** was synthesized from 4-butoxyphenol with a magnesium mediated *ortho*-formylation in excellent yield (98%).[177] The benzylated derivatives (**127a-e**) were mixed with methyl acrylate, DABCO and chloroform and stirred at room temperature for 1-7 weeks giving Baylis-Hillman products in good to excellent yields (73-97%, **128a-e**).[175] When the salicylaldehyde was substituted with electron withdrawing groups (**126b**, **126c**) the reaction rate increased (1-2 weeks), an observation that has been reported by others.[174, 178, 179] The

conjugate addition was performed with ethylamine, propylamine and pyrrolidine in methanol with excellent conversion (yield 80-98%, **129a-f**). Debenzylation by catalytic hydrogenation (Pd/C) achieved the ring opened hydroxyl derivatives **130a-f**, which after filtration were stirred over night at ambient temperature, thus inducing spontaneous cyclization to the coumarins **132-137** (21-62%, some cases required the addition of potassium carbonate). For the nitro substituted **129c**, a concomitant reduction of the nitro group to the corresponding aniline was observed (**130c**).

Scheme 8. Synthesis of 6-substituted coumarin derivatives **132-137**.[a]

compd	R^6	R^1, R^2
126a-131a, 132	-O*n*Bu	-H, -Et
126b-131b, 133	-OCF$_3$	-(CH$_2$)$_4$-
126c-129c	-NO$_2$	-(CH$_2$)$_4$-
130c-131c, 134	-NH$_2$	-(CH$_2$)$_4$-
126d-131d, 135	-OMe	-(CH$_2$)$_4$-
126e-131e, 136	-H	-H, -*n*Pr
129f-131f, 137	-H	-(CH$_2$)$_4$-
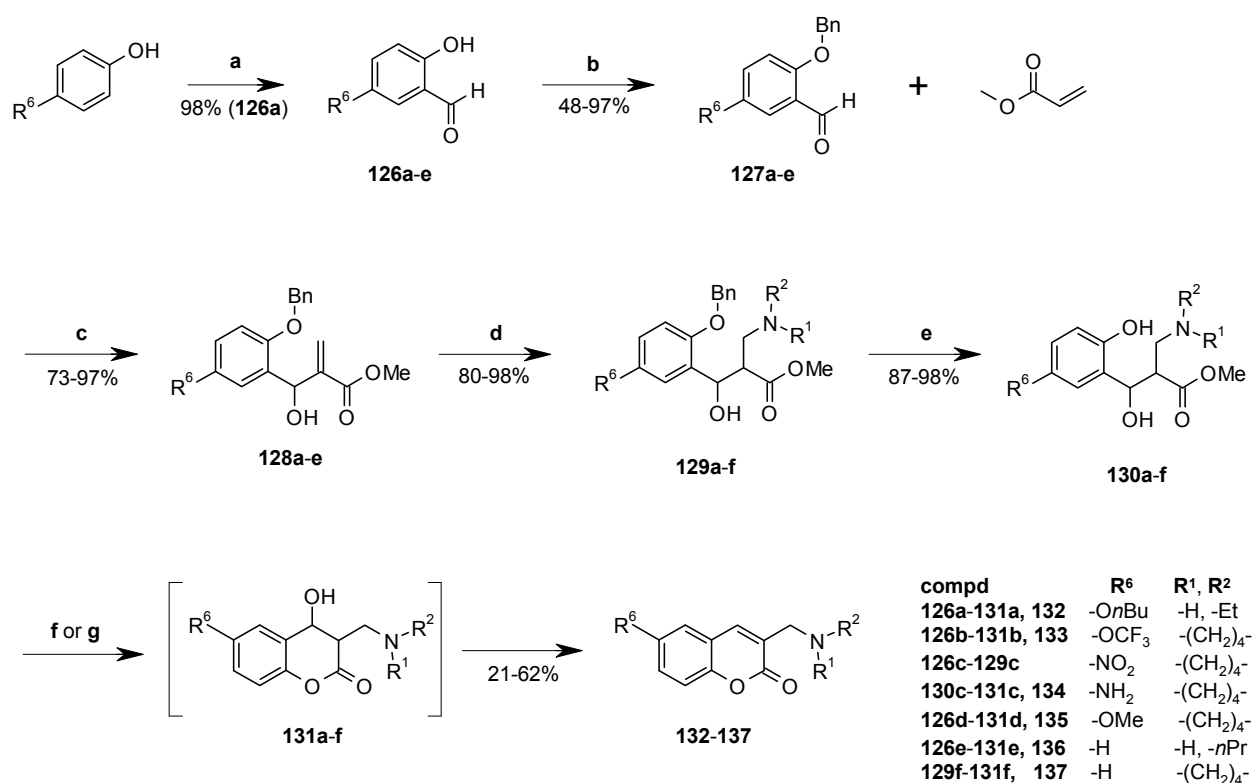
[a]*Reagents and conditions*: (a) 1. Mg(OMe)$_2$ 6-10% in methanol, 2. paraformaldehyde, toluene, 3. 10% HCl; (b) benzyl bromide, K$_2$CO$_3$, acetonitrile, 80 ºC; (c) DABCO, CDCl$_3$, rt, 1–7 weeks; (d) NR^{1}R^2: ethylamine, propylamine or pyrrolidine, methanol, rt; (e) H$_2$, Pd/C, methanol, rt; (f) methanol, rt; (g) K$_2$CO$_3$, methanol, rt.

Scheme 9. Synthesis of 3-(pyrrolidin-1-ylmethyl)-6-(trifluoromethyl)chromen-2-one **142**.[a]
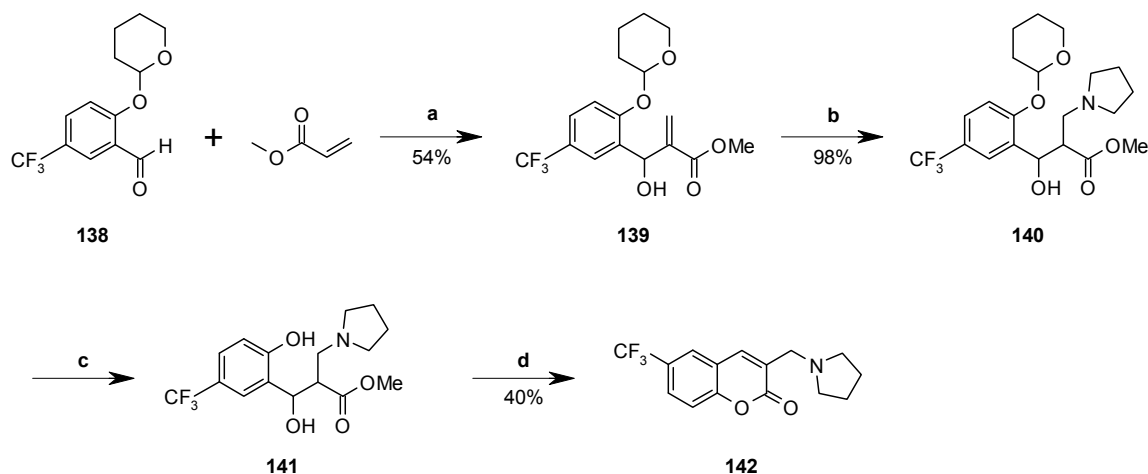
[a]*Reagents and conditions*: (a) DABCO, CDCl$_3$, rt, 5 days; (b) pyrrolidine, methanol, rt; (c) conc. HCl, methanol, rt; (d) triethylamine, methanol, microwave heating 100 °C. Compound **138** was synthesized according to Geneste and Schäfer.[180]

3.3.2. Baylis-Hillman reaction using 2-tetrahydropyranyl as a phenol protecting group

Compound **142** (Scheme 9), which is substituted with a 6-triflouromethyl group, was synthesized using a version of the Baylis-Hillman reaction. In this case, 2-tetrahydropyranyl (THP) was selected as protecting group for the phenol since benzylation of reactive *p*-trifluoromethyl phenols under basic conditions can give 1,6-elimination of hydrogen fluoride.[181, 182] The use of an acid labile protecting group such as THP solved this problem and 2-tetrahydropyran-2-yloxy-5-(trifluoromethyl)benzaldehyde (**138**, Scheme 9) was synthesized according to Geneste and Schäfer via directed *ortho*-lithiation of THP-protected 4-(trifluoromethyl)phenol in the presence of dimethylformamide.[180] The Baylis-Hillman product (**139**) was obtained from **138** and methyl acrylate with full conversion after five days (rate enhancement). Conjugate addition with pyrrolidine acting as both base and reactant gave **140** which was deprotected under acidic conditions to give the ring opened phenolic derivative **141**. Correction of pH to basic conditions (triethylamine) and concomitant heating (microwave) gave ring closure to afford **142** in 40% yield (Scheme 9).

4. Pharmacology

4.1. Methods

The target compounds were tested for their *in vivo* and *in vitro* effects in a range of pharmacological assays. The *in vivo* models were used to investigate both behavior and neurochemical effects in freely moving rats. The *in vitro* assays were used to measure the binding affinities/functional activity at the 5-HT$_6$, and DA D$_2$ receptors, and to SERT and MAO. For the most interesting ligands screening for other receptor/transporter off targets was also performed.

4.1.1. In vitro assays

In order to evaluate ligand affinity for various receptor systems, *in vitro* binding was performed by displacement of a high affinity radiolabeled ligand from the target receptor system, the radioactivity was determined with a scintillation counter. The 5-HT$_6$ binding was measured by displacement of [^{3}H]-LSD to cloned human 5-HT$_6$ receptors stably expressed in human embryonic kidney (HEK) 293 cells.[183] The intrinsic activity of the compounds at the 5-HT$_6$ receptors was determined by measuring their effect on cAMP production in baby hamster kidney (BHK) cells and compared to the effect elicited by 5-HT (Paper I and II).[108] In addition, the potency of the agonists was measured and presented as EC$_{50}$-values. The DOPAC levels produced in striatum by pharmacologically active compounds can be linked to a number of different targets and as previously mentioned (Sections 1.5., 1.6., 1.8.5.–1.8.7.) two of these targets are DA D$_2$ receptors and MAO A. We therefore in Paper III measured the affinity to these targets. The effects on 5-HIAA levels can be linked to activities on the 5-HT$_{1A}$ receptor, and to SERT and MAO A and therefore the affinity for SERT and 5-HT$_{1A}$ was included.[184] The target compounds were also evaluated for their affinity to human DA D$_{2S}$ receptors expressed in HEK cells. Two different ligands were used: the antagonist [^{3}H]methyl-spiperone, which labels the low affinity state DA D$_2$^{Low}, and the agonist [^{3}H]-7-OH DPAT (7-hydroxy-2-dipropylaminotetralin), which labels the high affinity state DA D$_2$^{High}.[185] The agonist affinity state of DA D$_2$ receptors (DA D$_2$^{High} or DA D$_2$^{Low}) is dependent on the degree of G-protein coupling, but the antagonists are believed to bind approximately equally well to both receptor states.[37, 122, 186-188] A DA D$_2$ receptor that is uncoupled from a G-protein is considered to be in its low affinity state, whereas coupling of the G-protein (a process promoted by agonists) gives a high affinity state. By using both

an agonist and an antagonist as the [^{3}H]-ligand, the affinity for DA D_2^{High} and DA D_2^{Low} can be determined, and the ratio between these two affinities (K_i^{Low}/K_i^{High}) correlates with the intrinsic activity of the compound (antagonists display ratios around 1 and agonists >50).[130, 188] The affinity to the human SERT was also performed by using [^{3}H]imipramine as the ligand in Chinese hamster ovary (CHO) cells[189] and affinity for MAO A from rat cerebral cortex, using [^{3}H]Ro 41-1049 as the ligand.[190] Inhibitory activity on the human MAO A and MAO B was measured with kynuramine as substrate for both subtypes. The determination of MAO catalytic rates in the presence of compounds was accomplished by measuring the concentration of 4-hydroxyquinoline, the MAO catalyzed oxidation product of kynuramine, using LC-MS/MS.[191] The corresponding IC_{50}-values and the MAO A selectivity [expressed as IC_{50} (MAO B)/IC_{50} (MAO A)] are reported in Paper IV. The antagonistic potencies for inhibition of the DA D_2 receptor in human HEK cells with DA D_2-G_{qi5} clone was also determined for a subset of compounds (Paper IV).[131]

4.1.2. In vivo models

The levels of DOPAC, 3-MT and 5-HIAA in striatum have been used as measurements of the synthesis and turnover of DA and 5-HT (Paper III and IV). Striatum is the part of the brain that has the strongest correlation to behavior and DA is the main neurotransmitter affecting locomotor activity (LMA, Paper III). Male Sprague-Dawley or Charles River rats were used and five groups of animals, four animals per group, were dosed with either saline (control) or the test substance in escalating doses (usually up to a 100 µmol/kg). The behavior was recorded using motility meters and the distance travelled was used as a measurement of the rats' activity.[192] The rats were decapitated 1 hour after the injection and the effect of the target compounds on the levels of DOPAC, 3-MT and 5-HIAA was measured by HPLC 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 regard to the biochemical markers and the 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 effect on LMA is reported at the dose when the compound reaches its maximal effect on DOPAC. In addition, the reported effect on LMA is during the last 45 min of the behavioral session, which is regarded as the hypoactive state of the animal. This is the time period during which dopaminergic stabilizers increase LMA compared with DA D_2 receptor antagonists, which decrease LMA (Paper III). The typical *in vivo* effects of DA D_2

receptor antagonists are dose dependent increases in the synthesis and release of DA in the striatum, measured as an increase of DOPAC levels (up to a maximum of 300–400% of control),[193] plus a concomitant potent reduction in spontaneous LMA in partly habituated rats, which is a hallmark for a potential risk for EPS side effect in patients [i.e. pimozide (**38**), sertindole (**39**),[117] risperidone (**40**),[118] and ziprasidone (**41**)[119] (Figure 17)].[194] Generally, they also bind with high affinity to DA D_2 receptors (K_i<12 nM, Table 5).[195] The different inhibitors against MAO have been extensively studied *in vivo* in rat striatum. MAO A is responsible for deamination of DA, NE and 5-HT, while MAO B has only minor effects on the neurotransmission in rat striatum. The selective irreversible MAO A inhibitor clorgyline inhibits deamination of DA and 5-HT and yields a concomitant decrease in DOPAC, 5-HIAA (5-HT metabolite) and an increase in the level of 3-MT (DA metabolized by COMT).[14, 196] In addition, inhibition of SERT by e.g. citalopram (**4**, SSRI, Figure 9) yields a significant decrease in 5-HIAA levels but leaves the dopaminergic system unchanged, i.e. DOPAC levels.[184] *In vivo* microdialysis was performed for the confirmation of the MAO A inhibitor effects of compound **134** (Paper IV) and for detection of the extracellular levels of 5-HT, NE and DA within striatum/prefrontal cortex (**134** and **158**, see Sections 6.2. and 6.3.). Analysis of perfusates collected from microdialysis probes implanted in the striatum/prefrontal cortex of freely moving rats was used to measure the DOPAC and 3-MT levels together with 5-HT, NE and DA during a period of 180 min after administration of the test compound.[196, 197] All experiments were carried out in accordance with Swedish animal protection legislation and after the approval of the local animal ethics committee in Gothenburg.

4.2. Affinity/activity studies of the 2-alkyl-3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indoles at the 5-HT$_6$ receptor (Paper I and II)

The synthesized 3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indoles were evaluated for their binding and intrinsic properties at the 5-HT$_6$ receptor. These results are reported in Table 3 and the corresponding selectivity profile against other 5-HT receptor subtypes and SERT are reported in Table 4.

Table 3. 5-HT$_6$ receptor binding affinity and functional cAMP data for different 3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indoles and derivatives: variation in N^1-, 2-, 5- and the tetrahydropyridine *N*-positions.
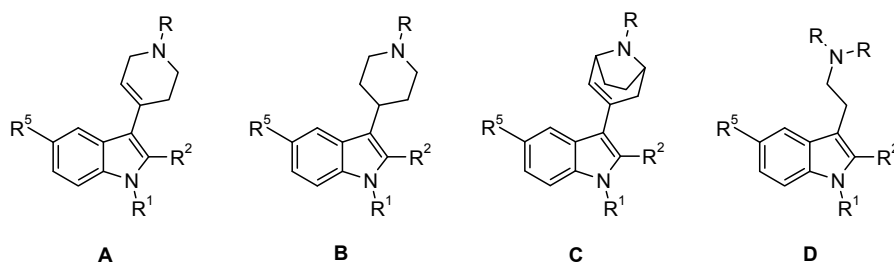
Compound	R^5	R^2	R^1	R	Substructure	IC$_{50}$[a] (nM)	EC$_{50}$[b] (nM)	IA (%)	Functional activity
77	-OMe	-Et	-H	-H	A	90	7.9	100	AGO
78	-Cl	-Et	-H	-H	A	30	ND	ND	-
79	-Cl	-*n*Pr	-H	-H	A	40	ND	ND	-
80	-Cl	-*i*Pr	-H	-H	A	100	ND	ND	-
81	-Cl	-Me	-H	-H	A	7.4[c]	1.0[d]	92	AGO
82	-Br	-Me	-H	-H	A	10	2.7	100	AGO
83	-F	-Me	-H	-H	A	30	5.3	100	AGO
84	-OMe	-Me	-H	-H	A	80	5.8	100	AGO
85	-H	-Me	-H	-H	A	200	1100	78	pAGO
86	-OSO$_2$CF$_3$	-Me	-H	-H	A	50	32	65	pAGO
87	-NHSO$_2$Ph	-Me	-H	-H	A	29[e]	21	59.6	pAGO
88	-SMe	-Me	-H	-H	A	20	6.6	58	pAGO
89	-NO$_2$	-Me	-H	-H	A	10	32	28	pAGO
90	-OPh(2-NO$_2$)	-Me	-H	-H	A	9	ND	ND	-
91	-O*i*Pr	-Me	-H	-H	A	100	ND	ND	-
92	-(3-thienyl)	-Me	-H	-H	A	700	ND	ND	-

Compound	R[5]	R[2]	R[1]	R	Substructure	IC$_{50}$[a] (nM)	EC$_{50}$[b] (nM)	IA (%)	Functional activity
93	-Ph	-Me	-H	-H	A	1000	ND	ND	-
94	-Cl	-Me	-Me	-H	A	40	ND	ND	-
95	-Cl	-Me	-Et	-H	A	100	ND	ND	-
96	-Cl	-Me	-*n*Pr	-H	A	80	ND	ND	-
143	-Cl	-Me	-H	-Me	A	10	2.9	152	AGO
144	-Cl	-Me	-H	-Et	A	30	ND	ND	-
145	-Cl	-Me	-H	-*n*Pr	A	60	ND	ND	-
146	-Cl	-Me	-H	-*n*Bu	A	50	ND	ND	-
147	-Cl	-Me	-H	-Bn	A	600	ND	ND	-
148	-Cl	-Me	-SO$_2$Ph	-H	A	1.7[e]	0	0	ANT[f]
149	-Cl	-Et	-H	-Et	A	70	ND	ND	-
150	-H	-Ph	-H	-H	A	2	0	0	ANT[f]
151	-CN	-H	-H	-H	A	20	130	44	pAGO
152	-OMe	-Et	-H	-H	B	300	ND	ND	-
153	-Cl	-Me	-H	-Me	C	40	10000	75	pAGO
RU24969 (**11**)	-OMe	-H	-H	-H	A	79	200	55	pAGO
EMDT (**21**)	-OMe	-Et	-H	-Me	D	85[g]	710	113	AGO
ST1936 (**22**)	-Cl	-Me	-H	-Me	D	40	16	53	pAGO

[a]Displacement of [^{3}H]LSD binding to cloned human 5-HT$_6$ receptors stably expressed in HEK cells,[183] single determination. [b]Stimulation of cAMP production to cloned human 5-HT$_6$ receptors stably expressed in BHK cells,[108] single determination. [c]Mean of four determinations (SEM ± 1.6). [d]Mean of three determinations (SEM ± 0.40). [e]Displacement of [^{3}H]LSD binding to cloned human 5-HT$_6$ receptors stably expressed in CHO cells,[22] mean of two determinations. [f]Confirmed to be antagonists by inhibition of cAMP production of 5-HT induced stimulation of cAMP accumulation in HeLa cells stably expressing the human 5-HT$_6$ receptors.[108] [g]Mean of two determinations (SEM ± 15). Abbreviations: AGO, full agonist; ANT, antagonist; IA, intrinsic activity; ND, not determined; pAGO, partial agonist; SEM, standard error of the mean.

Table 4. 3-(1,2,3,6-Tetrahydropyridin-4-yl)-1*H*-indoles, selectivity data for other 5-HT receptors and serotonin transporter protein (SERT).[a]
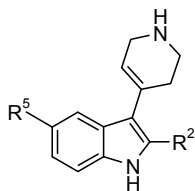
Compd	R^5/R^2	5-HT IC_{50} (nM)[a]								SERT IC_{50} (nM)[a]
		1A	1B	1D	2A	2C	3	4	7	
77	-OMe/-Et	>1000	79	30	1800	7400	9300	>1000	>1000	>1000
78	-Cl/-Et	420	58	22	150	690	ND	ND	>1000	>1000
81	-Cl/-Me	660[b]	180[b]	110[c]	240[c]	450[c]	34[b]	620[c]	3000	4500
82	-Br/-Me	660	75	130	400	420	ND	ND	480	4800
83	-F/-Me	>1000	200	160	950	700	ND	ND	3100	1900
84[d]	-OMe/-Me	430	310	>1000	>1000	>1000	>1000	9800	9800	>1000
85	-H/-Me	5700	890	1200	1500	2900	2300	1000	>1000	>1000
86	-OSO$_2$CF$_3$/-Me	>1000	29	35	>1000	990	ND	ND	1800	>1000
89	-NO$_2$/-Me	890	570	310	1000	>1000	ND	ND	>1000	>1000
92	-(3-thienyl)/-Me	>1000	12	33	>1000	>1000	ND	ND	690	>1000
93	-Ph/-Me	>1000	140	390	>1000	>1000	ND	ND	3700	>1000
150[e]	-H/-Ph	>1000	>1000	>1000	50	100	270	ND	ND	ND
RU24969, (11)[f]	-OMe/-H	4	6	20	>1000	300	>1000	ND	ND	ND

[a]Binding methods according to Bartoszyk *et al.*[198] and Hinrich *et al.*[199] 5-HT$_{1A}$ ([^{3}H]-8-OH-DPAT, rat), 5-HT$_{1B}$ ([^{125}I]-iodocyanopindolol, rat), 5-HT$_{1D}$ ([^{3}H]-5-HT, calf), 5-HT$_{2A}$ ([^{3}H]-ketanserin, human), 5-HT$_{2C}$ ([^{3}H]-mesulergine, human), 5-HT$_3$ ([^{3}H]-GR65630, NG 108 cells), 5-HT$_4$ ([^{3}H]-GR113808, guinea pig), 5-HT$_7$ ([^{3}H]-LSD, human) and SERT ([^{3}H]-5-HT, rat), single determination. [b]Mean of two determinations (± SEM). [c]Mean of four determinations (± SEM). [d]5-HT$_{1A}$-data reported before by Guillaume *et al.*[81] [e]5-HT$_{2A/2C}$-data reported before by Crawforth *et al.*[89] [f]Selectivity profile reported before by e.g. Macor *et al.*[84] and Zifa and Fillion.[82] Abbreviations: ND, not determined.

In a high-throughput screening for novel lead structures at Merck KGaA, 2-phenyl-3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole (**150**) was found to bind with high affinity to the 5-HT$_6$ receptor (IC_{50} = 2 nM) and turned out to be an antagonist measuring the cAMP response of 5-HT on the human 5-HT$_6$ receptor. Within the tryptamine series it is known that the size of the 2-alkyl/aryl group influences the agonist/antagonist property at the 5-HT$_6$ receptors, smaller alkyl groups in the 2-position such as 2-methyl and 2-ethyl are reported to provide agonists, e.g. EMDT (**21**) and ST1936 (**22**), while exchanging to a larger 2-phenyl group yields a 5-HT$_6$ antagonist.[91, 92, 200] Therefore, we speculated that a similar approach could switch compound **150** into a 5-HT$_6$ agonist by replacing the 2-phenyl group with smaller alkyl groups.
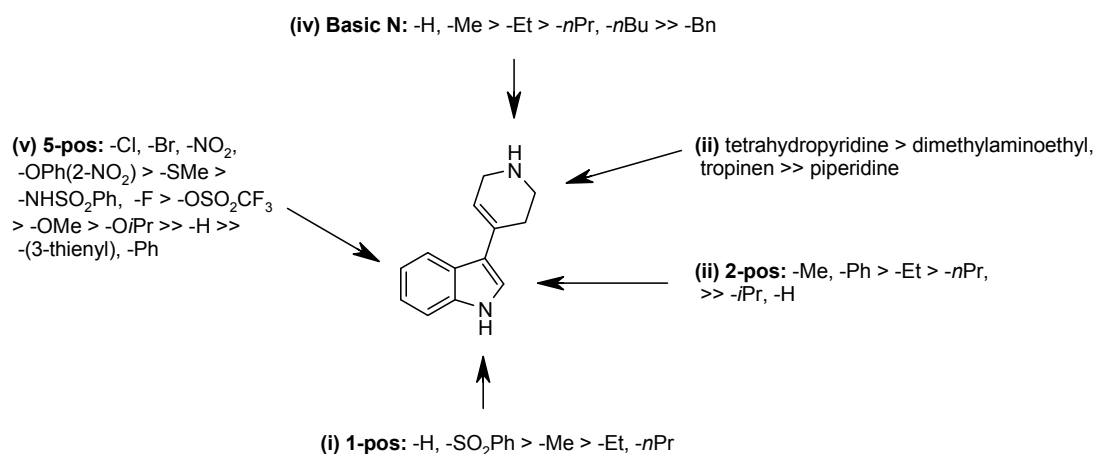
(iv) Basic N: -H, -Me > -Et > -nPr, -nBu >> -Bn

(v) 5-pos: -Cl, -Br, -NO$_2$,
 -OPh(2-NO$_2$) > -SMe >
 -NHSO$_2$Ph, -F > -OSO$_2$CF$_3$
 > -OMe > -OiPr >> -H >>
 -(3-thienyl), -Ph

(ii) tetrahydropyridine > dimethylaminoethyl,
 tropinen >> piperidine

(ii) 2-pos: -Me, -Ph > -Et > -nPr,
 >> -iPr, -H

(i) 1-pos: -H, -SO$_2$Ph > -Me > -Et, -nPr

Figure 22. Summary of SAFIR for the 2-alkyl-3-(1,2,3,6-tetrahydropyridin-4-yl)-1H-indoles at the 5-HT$_6$ receptor.

4.2.1. Affinity to the 5-HT$_6$ receptor

A summary of the structure-affinity relationships (SAFIRs) for 2-alkyl-3-(1,2,3,6-tetrahydropyridin-4-yl)-1H-indoles at the 5-HT$_6$ receptor is outlined in Figure 22: (**i**) Small alkyl substituents (i.e. methyl, ethyl, n-propyl) at the N^1-position decrease affinity, whereas an un-substituted indole nitrogen and -SO$_2$Ph substitution enhance affinity. (**ii**) A methyl or ethyl group is optimal at the 2-position, while further homologation decreases the affinity. However, a phenyl group yields a potent ligand. (**iii**) The tetrahydropyridine ring was the most potent moiety at the 5-HT$_6$ receptor compared with the other investigated linkers such as the saturated analog piperidine (**152**), flexible 2-dimethylaminoethyl side chain (**21**, **22**) or the more rigid tropinen ring (**153**). (**iv**) N-Methylated and an un-substituted basic nitrogen yield potent ligands. Further homologation decreases affinity and N-benzyl is not tolerated. (**v**) Substitution at the 5-position of the indole ring with chloro (**81**), bromo (**82**), nitro (**89**) or phenoxy (**90**) substituents yields high affinity ligands. The bulky phenoxy (**90**) and phenylsulfone amide (**87**) groups yield a positive interaction with the 5-HT$_6$ receptor (bent geometrical shape) compared with the almost inactive 3-thienyl (**92**) and phenyl (**93**) compounds (negative steric interactions). These results are in agreement with previous findings by others, a flexible aromatic group enhances binding at the indole 5-position.[93, 95, 200] A more lipophilic substituent as the methylsulfanyl group (**88**) enhances 5-HT$_6$ affinity compared with a methoxy group (**84**). This further supports that hydrogen bond formation of a methoxy or hydroxy group is not important for binding at the 5-HT$_6$ receptor, which has also been shown in the tryptamine series.[200]
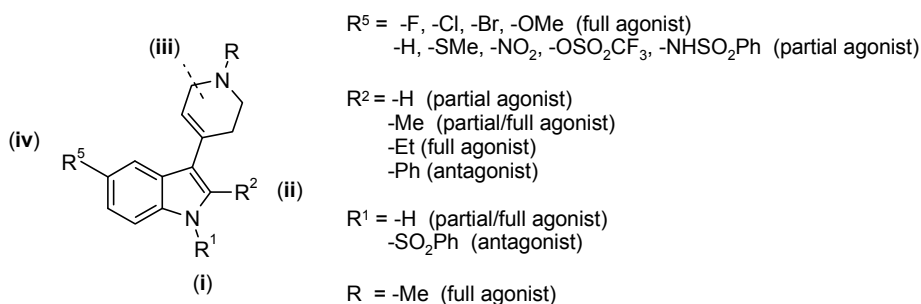
R⁵ = -F, -Cl, -Br, -OMe (full agonist)
      -H, -SMe, -NO₂, -OSO₂CF₃, -NHSO₂Ph (partial agonist)

R² = -H (partial agonist)
    -Me (partial/full agonist)
    -Et (full agonist)
    -Ph (antagonist)

R¹ = -H (partial/full agonist)
    -SO₂Ph (antagonist)

R = -Me (full agonist)

Figure 23. Summary of the functional activity for the 2-substituted 3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indoles at the 5-HT$_6$ receptor.

4.2.2. Functional activity at the 5-HT$_6$ receptor

An overview of the functional activity for the 2-substituted 3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indoles at the 5-HT$_6$ receptor is outlined in Figure 23. (**i**) For a compound to demonstrate potent 5-HT$_6$ receptor agonist properties, the indole N^1 should be unsubstituted. Substitution with an arylsulfonyl group, switched the full agonist **81** to a 5-HT$_6$ receptor antagonist **148**. This in line with what has been reported previously for this type of substitution (e.g. for **34**, Figure 16).[112] (**ii**) An alkyl group such as 2-methyl/ethyl is needed for full intrinsic activities. Compounds lacking a methyl/ethyl group in the 2-position (**11**, **151**) are partial agonists at 5-HT$_6$ receptors, whereas substitution with a 2-phenyl group (**150**) yields antagonistic effect. Therefore, the presence of a small alkyl group in the 2-position is significant for high intrinsic activity, indicating that there must be a specific interaction with the receptor site or an influence on the conformation of the tetrahydropyridine ring that is important for intrinsic activity (see Section 4.2.4. conformational analysis). (**iii**) Replacing the tetrahydropyridine ring in **81** with a tropinen ring (**153**) or a 2-dimethylaminoethyl side chain (**22**) reduced the potency and intrinsic activity at 5-HT$_6$ receptors. For instance, exchanging the ethyl amino chain in EMDT (**21**, EC$_{50}$ = 710 nM) to the more rigid tetrahydropyridine with retained 5-methoxy and 2-ethyl groups yielded a full agonist (**77**, EC$_{50}$ = 7.9 nM) with 90-fold improved potency at the 5-HT$_6$ receptor.[91] (**iv**) According to Table 3 and Figure 23, intrinsic activity varies with different substituents in the 5-position. Interestingly, the chloro (**81**), bromo (**82**), fluoro (**83**) and methoxy (**84**) derivatives display full agonist activity whereas the triflate (**86**), methylsulfanyl (**88**), nitro (**89**) and the bulky phenylsulfonamido (**87**) analogs were found to be partial agonists.

4.2.3. Selectivity for off targets

Introduction of a 2-methyl/ethyl group has been found to improve the selectivity profile to other 5-HT receptor subtypes and SERT (Table 4). For instance, comparing the 2-unsubstituted partial 5-HT$_6$ agonist **11** (RU 24969, 5-HT$_{1A/1B}$ agonist)[84] with the corresponding 2-methyl analog **84** reveals that the selectivity towards 5-HT$_{1A}$, 5-HT$_{1B}$ and, 5-HT$_{1D}$ receptors has increased >50-fold, yielding a selective partial 5-HT$_6$ agonist (**84**, Table 3 and 4). However, the 5-HT$_{1B}$ and 5-HT$_{1D}$ subtypes seem to accommodate a 2-methyl substituent to some extent since the 5-bromo (**82**), 5-triflate (**86**) and 5-(3-thienyl) (**92**) all display affinities below 130 nM, as well as a 2-ethyl group, as in the 5-methoxy (**77**) and 5-chloro (**78**) derivatives. Furthermore, compound **81** was also tested for affinity for the dopaminergic receptors (i.e. D$_2$, D$_3$ and D$_4$) and found to display no affinity (Table 4, Paper I).

4.2.4. Conformational analysis

(+)-Lysergic acid diethylamide (LSD) is a high-affinity partial agonist at 5-HT$_6$ receptors, with a K$_i$ of 2 nM (Figure 24). It is proposed that the ergoline moiety of LSD should represent the optimal conformation by which tryptamine based ligands bind to 5-HT$_6$ receptors. This is because the ergoline skeleton locks the aminoethyl side chain in the tryptamine substructure, in a fully extended fashion.[200-203] Rigid body alignment of LSD and three 3-(1,2,5,6-tetrahydropyridin-4-yl)-1*H*-indoles with -H, -methyl (**85**) or –phenyl (**150**) in the indole 2-position is shown in Figure 24. Low-energy conformations from stochastic searches were used for LSD and the 3-(1,2,5,6-tetrahydropyridin-4-yl)-1*H*-indole analogs in the alignment. In the alignment there are matches between N^1 in the indole rings and the basic nitrogen atoms in the alkyl rings. This may explain the high affinity observed in this series of 5-HT$_6$ ligands. The 2-methyl (**85**) moiety overlaps better with the slightly extended hydrophobic volume that LSD occupies in this region than does the 2-H analog. The 2-phenyl (**150**) moiety extends even further in this direction and could, therefore, participate in other interactions that may explain its antagonistic profile.
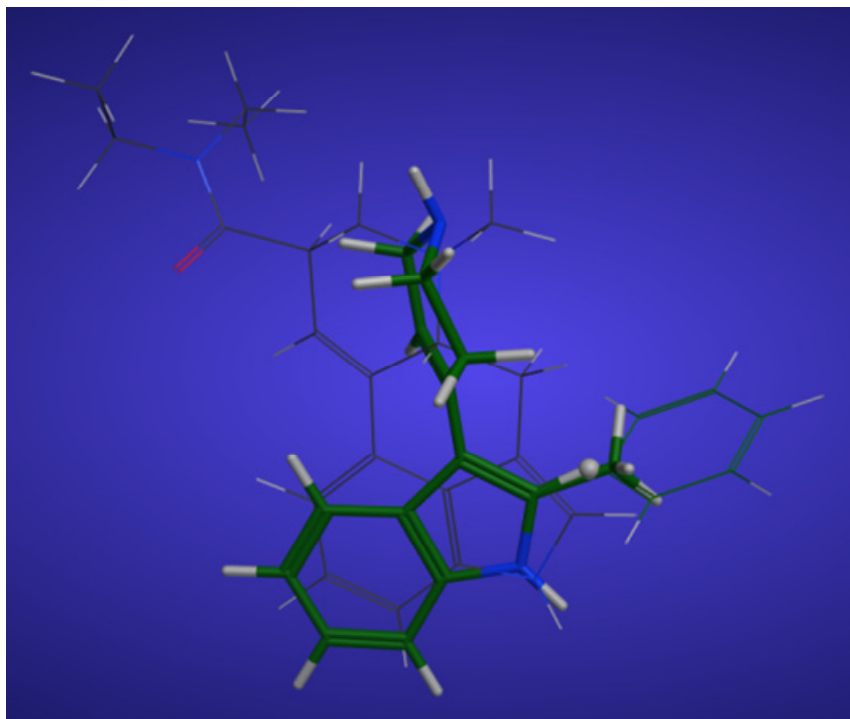
43

Figure 24. Rigid body alignment of 3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indoles **85** (thick bonds, green), its 2-H analog (white small sphere on the 2-H) and **150** (thin bonds, green) against LSD (thin bonds, grey).

4.2.5. Concluding remarks

By systematic substitution of the tetrahydropyridine-indoles we have gained good insight in how to achieve *in vitro* affinity to, as well as agonistic activity at the 5-HT$_6$ receptors. In order for a compound to demonstrate potent 5-HT$_6$ receptor agonist properties, the N^1-position of the indole should be unsubstituted, an alkyl group such as 2-methyl is needed and finally halogen (fluoro, chloro or bromo) or methoxy substituents in the indole 5-position were essential requirements. The most potent full agonist at the 5-HT$_6$ receptor within this series was the 5-chloro-2-methyl-3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole (**81**, IC$_{50}$ = 7.4 nM, EC$_{50}$ = 1 nM) with good selectivity versus all other 5-HT receptors (>20-fold), except for the 5-HT$_3$ receptor (only a 6-fold difference). In addition, the 5-HT$_6$ receptor agonist **81** has been used for investigation of the 5-HT$_6$ receptor functions *in vivo* and *in vitro* and **81** was found to have antidepressant as well as cognition enhancing effects.[105, 204-206]

4.3. 1-Propyl-4-aryl-piperidines as dopamine D$_2$ receptor ligands and serotonin reuptake (SERT) and monoamine oxidase (MAO) inhibitors (Paper III)

In Paper III we have used an *in vivo* screening approach for the identification of novel 1-propyl-4-arylpiperidines (Figure 25). The effects on locomotor activity (LMA) and brain neurochemistry such as DOPAC and 5-HIAA levels in rat were determined and correlated to reference compounds (Table 6). In addition an *in vitro* screening was included for relevant targets of DA D$_2$ (D$_{2S}^{High}$ and D$_{2S}^{Low}$) receptors, MAO A and SERT (Table 5) and for other off-target receptors (**125**, **154**, **157**, **160**, **164**, **165**, Table 3 and 4, Paper III).
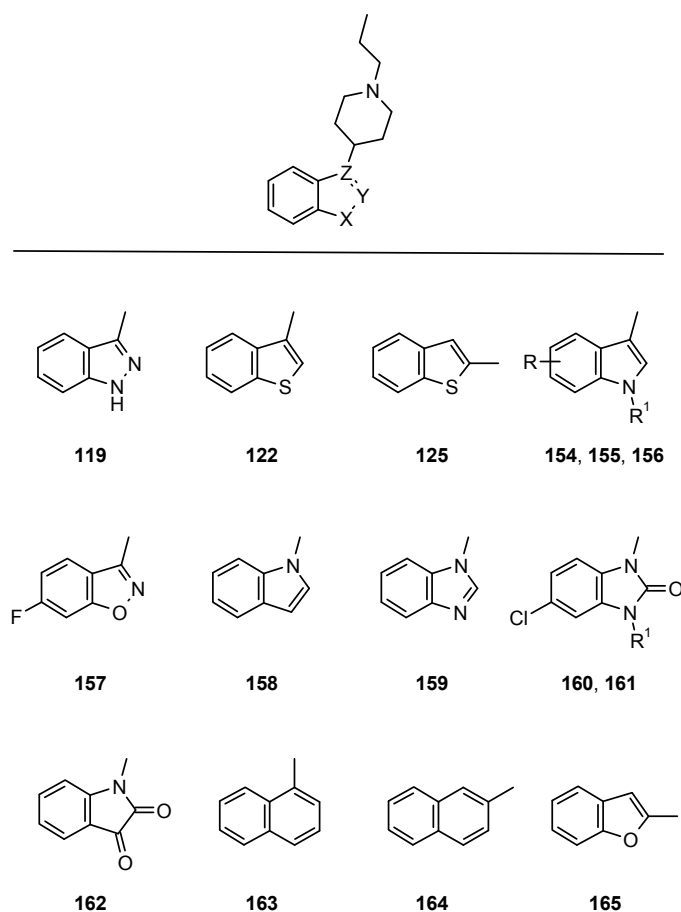

Figure 25. Overview of 1-propyl-4-aryl-piperidines and their bicyclic aryl core building blocks. Compounds: **154**, 6-F, R^1 = H; **155**, 6-F, R^1 = methyl; **156**, 5-F, R^1 = methyl; **160**, R^1 = H; **161** R^1 = methyl.

Table 5. *In vitro* data for compounds **119**, **122**, **125**, **154–165** and reference compounds.[a]

Compound	D_{2S}^{High} (nM)[b]	D_{2S}^{Low} (nM)[b]	K_i^{Low}/K_i^{High}	MAO A (nM)[b]	SERT (nM)[b]
119	187	1066	5.7	7442	5638
122	26	121	4.6	13990	6.5
125	574	2325	4.0	18	130
154	43	27	0.6	15780	45
155	157	275	1.7	4053	45
156	19	32	1.7	6863	58
157	34	194	5.7	2232	29830
158	109	1136	10	74900	54
159	10820	>580000[c]	n.c.[d]	>580000[c]	122
160	456	371	0.8	>580000[c]	3512
161	5182	2002	0.4	>580000[c]	2241
162	12830	12160	0.9	>580000[c]	23830
163	41	112	2.7	5417	52
164	1535	2870	1.9	63	127
165	803	2717	0.3	92	827
Pimozide (**38**)	ND	11.70[e]	n.c.[d]	ND	ND
Risperidone (**40**)	ND	2.7[e]	n.c.[d]	ND	ND
Ziprasidone (**41**)	ND	8.50[e]	n.c.[d]	ND	ND
(-)-OSU6162 (**47**)	755	3884	5.1	ND	ND
Pridopidine (**48**)[h]	7521	17550	2.3	ND	ND
Moclobemide (**2**)	ND	ND	n.c.[d]	11500[g]	ND

[a]Confidence intervals are reported in supporting information (Paper III). [b]Binding affinities (apparent K_i) using [³H]7-OH-DPAT as agonist ligand at the human dopamine D_{2S}^{High} receptor, [³H]methyl-spiperone as antagonist ligand for the human D_{2S}^{Low} receptor, [³H]Ro 41-1049 as ligand for MAO A (rat) and [³H]imipramine as ligand for human SERT. [c]IC_{50}-value less than 50% displacement at the highest concentration tested (1.0E-04 M). [d]Not calculated due to missing binding values. [e]From Kongsamut *et al.* using CHO cells for dopamine D_{2L} receptor antagonist binding with [³H]methyl-spiperone.[195] [f]Data from Pettersson *et al.*[130]. [g]Measured in bovine brain, Di Santo *et al.*[207]. Abbreviations: [³H]7-OH-DPAT, [³H]7-hydroxy-2-dipropylaminotetralin;Ro41-1049, *N*-(2-aminoethyl)-5-(*m*-fluorophenyl)-4-thiazole carboxamide HCl; ND, not determined; n.c., not calculated.

Table 6. *In vivo* data for compounds **119**, **122**, **125**, **154–165** and reference compounds in rats.

Compound	clogP[a]	ED$_{50}$ DOPAC (µmol/kg)[b]	Dose (µmol/kg)	DOPAC % of control ± SEM[c]	5-HIAA % of control ± SEM[d]	LMA % of control ± SEM[e]
119	2.5	55	33	227 ± 7.7*	105 ± 4.9	7 ± 4.7*
122	4.4	54	100	290 ± 25*	79 ± 4.8*	48 ± 24
125	4.7	n.c.[f]	100	28 ± 3.7*	76 ± 9*	37 ± 7
154	3.5	4.1	33	299 ± 9*	93 ± 2.6	2.8 ± 2.2*
155	4.3	33	100	264 ± 18*	95 ± 4.8	27 ± 5
156	4.2	3.0	33	275 ± 15*	87 ± 6.2	9 ± 2.1*
157	3.2	4.0	33	350 ± 17*	111 ± 0.7	8.3 ± 2.9*
158	4.4	126	100	227 ± 17*	87 ± 8.8	92 ± 43
159	3.0	i.a.	100	111 ± 8.6	98 ± 3.4	20 ± 8*
160	3.0	7.0	33	334 ± 18*	101 ± 1	54 ± 13
161	3.8	47	100	253 ± 4.3*	113 ± 7.5	27 ± 9
162	2.4	i.a.	100	101 ± 2.9	95 ± 2.7	118 ± 48
163	4.5	103	100	218 ± 11*	76 ± 6.3*	62 ± 16
164	4.6	n.c.[f]	100	32 ± 1.4*	70 ± 2.5*	22 ± 8*
165	4.3	n.c.[f]	100	35 ± 3.4*	84 ± 3.3	75 ± 34
Pimozide (**38**)	4.4	1.6	5.8	416 ± 18*	101 ± 5	7.3*
Risperidone (**40**)	2.7	0.5	2.4	347 ± 8*	115 ± 4.8*	11 ± 4.8*
Ziprasidone (**41**)	4.2	1.2	6.4	311 ± 10*	98 ± 0.5	13 ± 4.9*
(-)-OSU6162 (**47**)	2.36	63	100	260 ± 15*[g]	100 ± 1.8*	215 ± 62[g]
Pridopidine (**48**)[h]	2.21	81	300	298*	106	200
Moclobemide (**2**)	n.c.	n.c.[f]	37	18 ± 0.4*	81 ± 1.7*	164 ± 48
Citalopram (**4**)	n.c.	i.a.	25	101 ± 2.3	69 ± 2*	50 ± 20

[a]Calculated logarithm of the compound's partition coefficient between *n*-octanol and water with Advanced Chemistry Development (ACD) version 12 (Toronto, Canada). [b]Calculated using methodology as described in Ponten *et al.*[197] [c]Post mortem neurochemistry (subcutaneous injection) analysis of striatal DOPAC levels compared with saline control (n = 4). [d]Post mortem neurochemistry (subcutaneous injection) analysis striatal 5-HIAA levels compared with saline control (n = 4). [e]LMA 15–60 min after subcutaneous injection, measured at 25 Hz, compared with saline control. To compare the LMA of different compounds, the lowest dose required to produce a maximal DOPAC response was selected. [f]not calculated due to decreasing values in dose response. [g]Data from Pettersson *et al.*[130]. [h]Data from Ponten *et al.*[197]. *p-values < 0.05 using student's t-test. Abbreviations: n.c., not calculated; i.a., inactive.

In the search for new chemical scaffolds to serve as starting points for development of dopaminergic stabilizers, we examined whether it would be possible to start from a DA D_2 receptor antagonist, (rather than agonist) motif. As a starting point we focused on the typical/atypical antipsychotics (i.e. DA D_2 receptor antagonists), such as pimozide (**38**), sertindole (**39**),[117] risperidone (**40**),[118] and ziprasidone (**41**)[119] (Figure 17). They all share a common motif with different bicyclic cores attached to a piperidine ring. However, by removing the cyclic "alkyl/aryl" ring(s) in the side chain attached to the basic amine, the propyl group known to be "optimal" for dopaminergic stabilizer properties would be retained (**166**, Figure 26).[129, 130] In order to fully explore the SAR for 1-propyl-4-aryl-piperidines, a wide spectrum of core building blocks were included in the data set (Figure 25). Many of these building blocks are often included in compounds with known effects on the dopaminergic and the serotonergic systems in the brain.[170, 208-212] However, they have been imbedded in larger compounds and it is, therefore, harder to judge the contribution that each core building block makes with regards to SAR on the dopaminergic system (i.e. DA D_2 receptors).
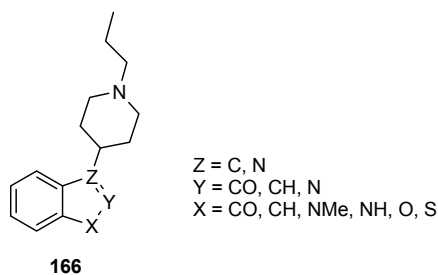

Figure 26. Generic structure of 1-propyl-4-aryl piperidines.

4.3.1. In vivo and in vitro effects of screening 1-propyl-4-aryl-piperidines

The results from the *in vivo/in vitro* screening of 1-propyl-4-aryl-piperidines were quite surprising. The effects of different bicyclic ring structures of **166** (Figure 25 and 26) were found to have a marked impact on the dopaminergic and serotonergic system (i.e. effects on DOPAC and 5-HIAA levels), effects on LMA, binding to DA D_2 receptors, SERT and affinity for MAO A (Figure 27, Table 5 and 6). The change in levels of the DA metabolite DOPAC is an *in vivo* indicator of effects on DA D_2 receptors controlling synthesis, release and turnover of DA in brain regions such as striatum. In the same way the corresponding metabolite 5-HIAA is an indicator of effects on synthesis, release and turnover of 5-HT. Figure 27 summarizes the effects on *in vivo* DOPAC levels (% of control) in rat striatum of a selection of compounds, i.e. **119**, **122**, **125**, **154**, **157-160**, **163-165**,

moclobemide (**2**), pimozide (**38**), risperidone (**40**), and ziprasidone (**41**). As can be seen the effects span from a large increase in DOPAC levels (300-350% of control) to a decrease in DOPAC levels (28-35% of control) within the 1-propyl-4-aryl-piperidine series. Compounds to the left in Figure 27 producing large increases in DOPAC (**154**, **157**, ED_{50} 4.1 and 4 μmol/kg, respectively) are comparable in efficacy and potency with the DA D_2 antagonists pimozide (**38**), risperidone (**40**), and ziprasidone (**41**) (yielding maximal DOPAC levels 350-400% of control, ED_{50} 0.5-1.6 μmol/kg). They also share the DA D_2 antagonist common features such as strong reduction in LMA (<10% control) and potent affinity to DA D_2 receptors. In Paper III a correlation between the above mentioned reference compounds [pimozide (**38**), risperidone (**40**), and ziprasidone (**41**)] and compounds **154**, **156** and, **157** were found to correlate well for DA D_2^{High} and the corresponding *in vivo* potencies (ED_{50}) observed for DOPAC (Figure 1S, Paper III), and therefore it can be concluded that compounds **154**, **156** and, **157** act as DA D_2 antagonists *in vivo*. The surprising dose dependent decrease in DOPAC levels for compounds on the right side of Figure 27 (i.e. **125**, **164** and **165**), was at a first glance believed to be an effect of direct DA D_2 agonistic response from these compounds, which is known to yield a decrease in DOPAC levels.[130] However, when we investigated further the effects *in vivo* (e.g. effects on 3-methoxytyramine; 3-MT), the profile of these three compounds was shown to be similar to that of moclobemide (**2**, Table 6, Figure 8), a known selective and reversible inhibitor of MAO A (metabolizes DA to DOPAC, Figure 5).[46] This was further supported by subsequent affinity screening, where these three compounds displayed high affinity for MAO A (18, 63 and 92 nM, for **125**, **164** and, **165**, respectively, Table 5), but lacked essential affinity for DA D_2 receptors (Table 5). This is also in agreement with the literature where the 2-piperidinyl-benzofuran analogs brofaromine (**52**, Figure 20)[45] and sercloremine (**53**, Figure 20)[139] are reported to be reversible MAO A inhibitors. We have not measured whether the new compounds (**125**, **164** and **165**) are reversible or irreversible MAO A inhibitors. However, due to the fact that these compounds share the same chemical motif as **52** and **53** and lack reactive functional groups, it is most likely that they are reversible MAO A inhibitors. From a SAFIR perspective the MAO A activity seems to relate to geometrical aspects and substitution in the "*para*" position of the aromatic ring is not tolerated by DA D_2 receptors but seems to be positive for MAO A activity. This is further supported by the MAO A properties for *para*-substituted 4-phenylpiperidines. [45, 46, 213]
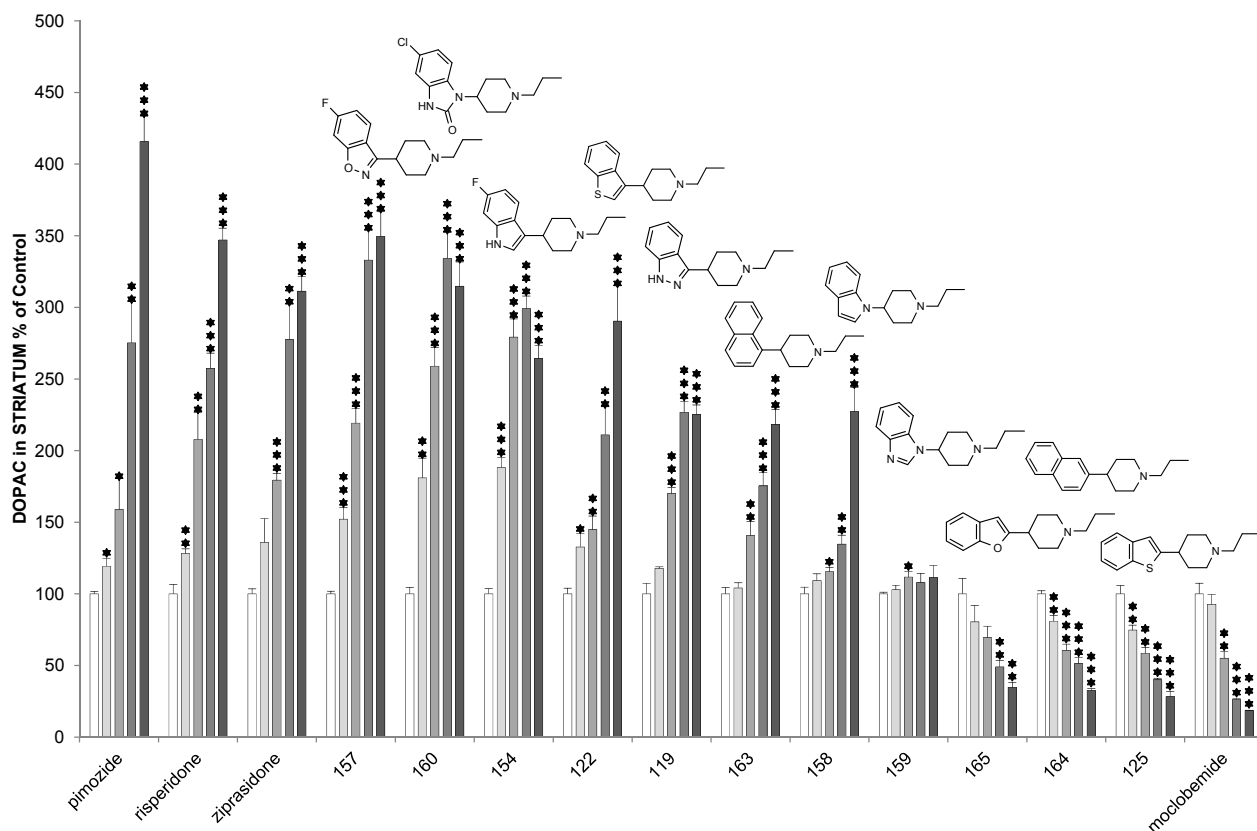
Figure 27. A dose response study of effects on *in vivo* DOPAC levels (% of control) in rat striatum (subcutaneous injection) of compounds **119**, **122**, **125**, **154**, **157-160**, **163-165**, moclobemide (**2**), pimozide (**38**), risperidone (**40**) and ziprasidone (**41**). Controls are indicated with a white bar. **119**, **122**, **125**, **154**, **158-160**, **163-165** are used in doses of 3.7, 11, 33 and 100 µmol/kg. **157**: 1.2, 3.7, 11 and 33 µmol/kg, ziprasidone: 0.2, 0.7, 2.1 and 6.4 µmol/kg, pimozide: 0.2, 0.6, 1.9 and 5.8 µmol/kg, risperidone: 0.07, 0.2, 0.7 and 2.4 µmol/kg, and moclobemide: 1.4, 4, 12 and 37 µmol/kg. Statistical significance was assessed using t-test (2 tailed) versus controls. $*p<0.05$, $**p<0.01$, $***p<0.001$ in this respect. Error bars shows Standard Error of the Mean (SEM) ($n = 4$).

4.3.2. Correlation between in vivo DOPAC and in vitro dopamine D_2 receptors and MAO A

The major contributor to DOPAC levels in striatum is believed to be dopamine D_2 antagonism/agonism and MAO A inhibition, and as such they may counteract or interact with each other yielding a net outcome of DOPAC in striatum. However, other targets such as DAT, NET and COMT also contribute to the net effects on DOPAC levels, but are in our series believed to be less important.[214, 215] For a few compounds the *in vivo* and *in vitro* potencies at DA D_2 receptors did not correlate, e.g. indazole **119** and 1-naphtalene **163** displayed lower DOPAC levels (227% respective 218% of control, Table 6) than excepted compared with their *in vitro* potency at DA D_2 receptors (Table 5). In order to more thoroughly investigate what mechanisms are connected to the *in vivo* response, the effect on DOPAC levels was modeled against the binding affinity for DA D_2 receptors,

50

MAO A and SERT, using partial least squares (PLS) regression (Figure 28 and supporting information Paper III).[216, 217] A strong correlation could be observed between *in vitro* binding to MAO A and DA D_2 receptors and the *in vivo* DOPAC levels. However, the affinity for SERT had a minor contribution to the model and is, therefore, difficult to interpret. Based on this result, we conclude that the overall effect of any particular compound on DOPAC levels results from a combination of its ability to antagonize DA D_2 receptors and inhibit MAO A, which both counteract each other. Compounds **119** and **163** fit well with this model and support the hypothesis that lower efficacy on DOPAC levels are due to inhibition of MAO A. It is also worth mentioning that the MAO A inhibitor moclobemide (**2**) displays low affinity for MAO A ($K_i = 11.5$ µM, Table 5), but is still able to inhibit the enzyme efficiently. As such, the low affinity observed for some of the new compounds may at first glance appear insufficient to explain the effects on DOPAC levels; however, the supporting evidence indicates that low affinity is an important contributor to the net effect on DOPAC.
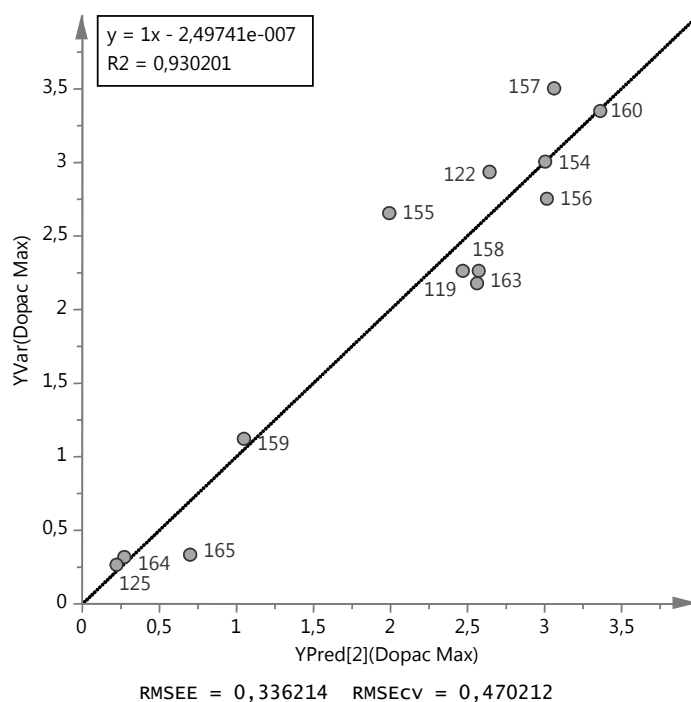

Figure 28. Partial least squares regression on DOPAC was modeled against the binding affinity for DA D_2 receptors, MAO A and SERT.

4.3.3. In vivo and in vitro effects of compound 160

A notable finding in the 1-propyl-4-aryl-piperidine series was the results for the benzimidazol-2-one **160** which was well explained in our PLS model regarding effects on DOPAC, but was found to be much more potent *in vivo* ($ED_{50} = 7$, Table 6) compared to what should be expected from the *in vitro* affinity at DA D_2 receptors (DA D_2^{Low} $K_i = 371$ nM and DA D_2^{High} $K_i = 456$ nM, Table 5). At the same time, compound **160** differed from the potent DA D_2 antagonists with respect to the effects on LMA, only a partial reduction in LMA was observed for **160** (54% of control, Table 6). One underlying explanation for the observed behavior effects might be the relatively low affinity for DA D_2 receptors. We have recently demonstrated that there is a correlation between affinity for DA D_2^{Low} and effects on spontaneous LMA.[130] A compound such as the dopaminergic stabilizer pridopidine (**48**) has a very low affinity for DA D_2^{Low} ($K_i = 17550$ nM, Table 5), but induces an increase in DOPAC levels to the same extent as the most potent and efficacious DA D_2 receptor antagonists. However, in sharp contrast to these compounds, **48** induces an increase in spontaneous LMA (Table 6). Its unique mechanism of action (surmountable, low affinity and fast-off receptor kinetics) may account for the increase in LMA, since it is believed to allow DA receptors to rapidly regain responsiveness to the released DA.[130] Tighter binding to DA D_2 receptors means, therefore, that the responsiveness to DA is reduced, which consequently leads to reduced spontaneous LMA. In agreement with this, compound **160** binds with moderate affinity to DA D_2^{Low} ($K_i = 371$ nM, Table 5) and demonstrates only a partial reduction in LMA. In addition, among the compounds tested **160** was the most interesting, demonstrating efficacy in several animal models of psychosis with only a partial reduction of spontaneous LMA, indicating that it may have a low propensity to induce EPS in patients (see Paper III).

4.3.4. Affinity for SERT and effects on 5-HIAA levels in vivo

An interesting finding of several compounds in this series was that they, in addition to effects on DA D_2 receptors, also have affinity for SERT (i.e. a SSRI effect). *In vivo*, it is known that 5-HIAA levels in different brain regions (striatum, limbic and cortex), can be decreased by direct stimulation of serotonin 5-HT$_{1A}$ receptors [e.g. by agonists such as (+)-8-OH-DPAT][218] or indirectly by increased synaptic levels of 5-HT induced by SSRIs [e.g. citalopram (**4**)][184] or RIMAs [e.g. moclobemide (**2**), Table 6].[219] Therefore, the observed decrease in 5-HIAA levels for compounds **122**, **156**, **158** and **163** correlates well with the high affinity for SERT and is also comparable to the effects seen for

citalopram (**4**, Table 6). Two compounds, **154** and **155** display high affinity to SERT but did not show any significant effect on 5-HIAA levels *in vivo* post mortem (Table 6). However, by using microdialysis compound **155** was found to increase 5-HT release in striatum and cortex (250% of control for each region) with a concomitant decrease in 5-HIAA levels. The reason for lack of effects on 5-HIAA in post mortem neurochemistry compared to the observed effects in microdialysis is not clear but the dialysis data correlate well with the affinity for SERT. The dual effects on DA D_2 receptor and SERT found for these compounds and especially for compound **158** is interesting in relation to the concept of Symbyax[®,61] (combination of olanzapine and fluoxetine) for the treatment in major depression.[62-65] This will be highlighted and discussed in more detail in Section 6.2. However, three compounds (**125**, **164** and **165**, Table 5 and Table 6) also displayed potent inhibition of MAO A in combination with affinity for SERT. They also induced a clear decrease in 5-HIAA levels *in vivo*, whether the effect is correlated to SERT and/or MAO A is not possible to elucidate. In addition, one compound among all in this series turned out to be a selective SERT ligand *in vitro*, i.e. the benzimidazole **159**. Furthermore, the low *in vitro* binding affinities of 1,2-benzisoxazole **157**, 1*H*-indazole **119**, benzimidazolones **160** and **161**, and isatin **162** for SERT indicate that these structural motifs are not tolerated in the interaction with the SERT protein (Table 5). From a SAR perspective, it is interesting to note that these five compounds have a heteroatom in the 2-position of the 5-membered ring while remaining compounds which display affinity for SERT has a methine carbon in this position.
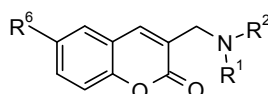
4.3.5. Concluding remarks

The screening of various five- and six-membered bicyclic aryl ring derivatives in the 1-propyl-4-piperidine series led to the discovery that the position and properties of the bicyclic aryl ring had a marked impact on the effects of compounds on the dopaminergic and serotonergic system in rat brain. Potent and selective DA D_2 receptor antagonists were achieved using 3-indoles, 3-benzoisoxazoles, 3-benzimidazol-2-one, and 3-benzothiophenes, whereas 3-isatin and 3-benzimidazole derivatives were devoid of dopaminergic activity. In addition, several of these bicyclic aryl derivatives displayed potent affinity for SERT (i.e. 3-indoles, 3-benzothiophenes, and 3-benzimidazole). In contrast, the 2-benzofuran and 2-benzothiophene analogs were potent and selective MAO A inhibitors. Furthermore, it was also discovered that the effect on DOPAC levels in striatum is correlated to a dual effect of blocking DA D_2 receptors and inhibition of MAO A.

4.4. 6-Substituted 3-(pyrrolidin-1-ylmethyl)chromen-2-ones as monoamine oxidase inhibitors (Paper IV)

In Paper IV we have used an *in vivo* screening approach for the identification of novel 6-subsituted 3-(pyrrolidin-1-ylmethyl)chromen-2-ones (Figure 29, Table 7). The effects on brain neurochemistry such as DOPAC, 3-MT and 5-HIAA levels in rat were determined and compared with reference compounds (Table 7). Additionally, *in vitro* screening of functional inhibition of MAO A and MAO B (Table 7) as well as in off-target assays have been performed (**134**, **142**, Table 4, Paper IV).

Table 7. Monoamine oxidase inhibitory activity and *in vivo* data of target compounds.

Compound	R^1, R^2	R^6	MAO B[a] IC$_{50}$ (µM)	MAO A[a] IC$_{50}$ (µM)	SI[b]	Dose (µmol/ kg)	DOPAC[c] % of control	3-MT[c] % of control	5-HIAA[c] % of control
132	-H, -Et	-OnBu	7.34	1.95	3.7	-	-	-	-
133	-(CH$_2$)$_4$-	-OCF$_3$	>100	4.24	>23.5	-	-	-	-
134	-(CH$_2$)$_4$-	-NH$_2$	>100	3.77	>26.5	86	45*	170*	72*
135	-(CH$_2$)$_4$-	-OMe	>100	4.48	>22.3	100	65*	129	90
136	-H, -nPr	-H	62.3	17.1	3.6	100	70*	89	116
137	-(CH$_2$)$_4$-	-H	>100	6.32	>15.8	100	58	164*	106
142	-(CH$_2$)$_4$-	-CF$_3$	>100	8.46	>11.8	100	77	88	104
166	-(CH$_2$)$_4$-	-NO$_2$	>100	>100	-	100	105	75	118
167	-(CH$_2$)$_4$-	-OH	>100	1.46	>68	80	47*	126	86*
168	-(CH$_2$)$_4$-	-OBn	>100	>100	-	-	-	-	-
169	-(CH$_2$)$_4$-	-OnPr	22.3	2.16	10.3	-	-	-	-
Tranylcypromine	-	-	0.253	0.247	1	0.14	20*	257*	87
Moclobemide (**2**)	-	-	-	-	-	37	18*	398*	81*
Selegiline (**9**)	-	-	-	-	-	53	36*	146*	96
Risperidone (**40**)	-	-	-	-	-	2.7	347*	72	115*

[a]Inhibitory activity of human MAO A and MAO B using kynuramine as substrate,[191] all values are expressed as the mean ± SE of duplicate determinations (Table 2, Paper IV). [b]The selectivity index is the selectivity for the MAO A isoform and is given as the ratio of IC$_{50}$ (MAO B)/IC$_{50}$ (MAO A). [c]Post mortem neurochemistry (subcutaneous injection) analysis of striatal DOPAC, 3-MT, and 5-HIAA levels compared with saline control (n = 4), ± SEM are reported in supporting information (Paper IV, Table 1S). *p-values <0.05 using student's t-test. Abbreviations: Kynuramine, 3-(2-aminophenyl)-3-oxopropanamine; SI, selectivity index; SE, standard error; SEM, standard error of the mean.

In our search for novel compounds active on the dopaminergic system in the brain, we applied scaffold jumping from the known partial DA D_2 agonist, 3-[(benzylamino)methyl]-2,3-dihydro-1,4-benzodioxin-6-ol (**170**, Figure 29).[220] The aim was to investigate whether the dopaminergic properties (i.e. DA D_2) of the benzodioxane core were transferable to the coumarin core (scaffold jumping, **171**, Figure 29).
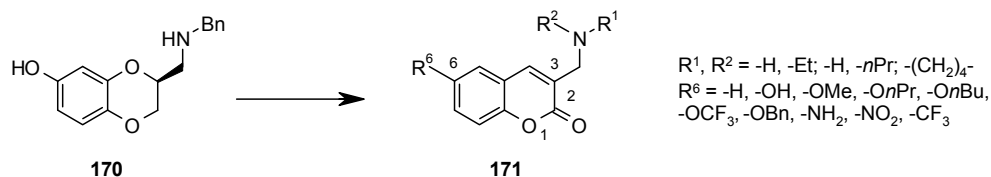

Figure 29. Design strategy "scaffold jumping" from the dopamine agonist 1,4-benzodioxan core (**170**) to a coumarin core (**171**, generic structure).

We maintained the known DA D_2 pharmacophore groups, a basic amino function in the 3-position with different electron withdrawing and donating groups in the 6-position (i.e. the *meta* position) on the coumarin core. The new compounds were screened *in vivo* for effects on the dopaminergic and serotonergic system in the rat brain (i.e. striatum) and the aim was to identify compounds with DA D_2 antagonistic properties. However, the 3-(aminomethyl)chromen-2-one substituted ligands showed a different profile *in vivo* than expected. Instead of large increases in DOPAC levels [350-400% of control, e.g. risperidone (**40**), Table 7] we found a decrease in DOPAC levels combined with a concomitant increase in 3-MT levels (**134**, **135**, **137**, **167**, Table 7).[196, 221] These data are in line with the previously reported *in vivo* profile for the MAO A inhibitor moclobemide (**2**).[219] The large increase of 3-MT levels is a hallmark for MAO A inhibitors since the metabolic pathway of DA to DOPAC is blocked and therefore the available DA is instead metabolized by COMT to 3-MT (Figure 5). The *in vivo* finding was further confirmed by *in vitro* functional DA D_2 receptor (**133-136**, **142**, and **166**, Table 1S, Paper IV), MAO A and MAO B assays (Table 7). The new compounds were devoid of any DA D_2 antagonistic properties and from Table 7, which shows the MAO inhibition data for all new compounds (**132-137**, **142**, **166-169**), it can be seen that most of the compounds display weak to moderate inhibitory activity at MAO A (range IC_{50} 1.46-17.1 µM, Table 7), but with a clear selectivity against MAO B (range SI 3.6 - >68).

R⁶: -OH, -NH₂ > -O*n*Pr > -OCF₃, -OMe >> -H, -CF₃ >>> -OBn
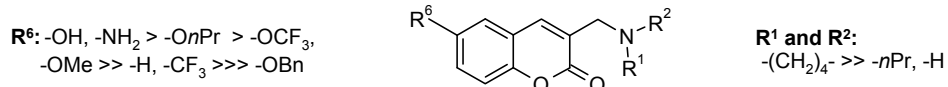
R¹ and R²: -(CH₂)₄- >> -*n*Pr, -H

Figure 30. Summary SAR for 6-subsituted 3-(pyrrolidin-1-ylmethyl)chromen-2-ones series against MAO A.

The SAR for the new 6-subsituted 3-(pyrrolidin-1-ylmethyl)chromen-2-ones series are outlined in Figure 30 with corresponding data in Table 7. As can be seen from Table 7 the tertiary pyrrolidine ring seems to abolish all MAO B activity in comparison with the secondary amines [ethylamine (**132**) and *n*-propylamine (**136**)]. Another important SAR finding was that the inhibitory activity at MAO A is favored by small hydrogen bond donating/accepting groups such as amino (**134**) and hydroxyl (**167**) in the C6 position. Adding small alkyl groups to the C6-hydroxy group slightly reduced the activity at MAO A [i.e. trifluoromethoxy (**133**) and methoxy (**135**)], while the introduction of a bulky C6-benzyloxy group (**168**) abolished all activity at both MAO A and B (see Section 4.4.2. molecular modeling). However, intermediate size alkoxy groups such as *n*-propoxy (**169**) and *n*-butoxy group (**132**) were well tolerated at MAO A, but the activity at MAO B was also enhanced and the selectivity towards MAO B was therefore reduced (SI = 3.7 for **132** and 10.3 for **169**). In addition, the introduction of electron withdrawing groups at the C6-position was found to be unfavorable [i.e. trifluoromethyl (**142**) and nitro (**166**)]. In a test panel for off target affinity for various receptors and protein transporters which may have an impact on the *in vivo* profile, compound **134** did not show any affinity of interest (Table 4, Paper IV) and therefore we can conclude that the *in vivo* effects seen for **134** is most likely attributed to the inhibition of MAO A. We have not measured whether the new compounds (i.e. **134** and **167**) are reversible or irreversible MAO A inhibitors. However, due to the fact that these compounds share the chemical motif known for coumarins which are classified as reversible MAO inhibitors (plus that they lack reactive functional groups), it is most likely that these new coumarins also are reversible MAO A inhibitors.[156, 157, 222]

4.4.1. The dopamine D$_2$ receptor interactions

The complete lack of effects on DA D$_2$ receptors for this new series of compounds was to a certain extent a surprise as they in comparison with the benzodioxane series have similar pK$_a$-values for the basic amines [8.21 (coumarin) and 7.38 (benzodioxane), ACDlabs]. The amino group has been demonstrated to be important for interactions with DA D$_2$ receptors.[220, 223, 224] In addition, conformational analysis of the coumarin vs. benzodioxane scaffold revealed that the compounds could adopt a similar conformation (Figure 31).[220] The alternate conformation for the coumarin ring with an intramolecular hydrogen bonding interaction with the carbonyl oxygen was not possible [Figure 31, all calculations were made with Chemical Computing Group's (www.chemcomp.com) 2011.10 Molecular Operating Environment (MOE), MMFF94s force field and Born solvation]. Therefore, the only likely explanation for the lack of effects on DA D$_2$ receptors is the presence of the carbonyl group in the 2-position of the coumarin ring which may have a negative impact on the interaction with DA D$_2$ receptors.
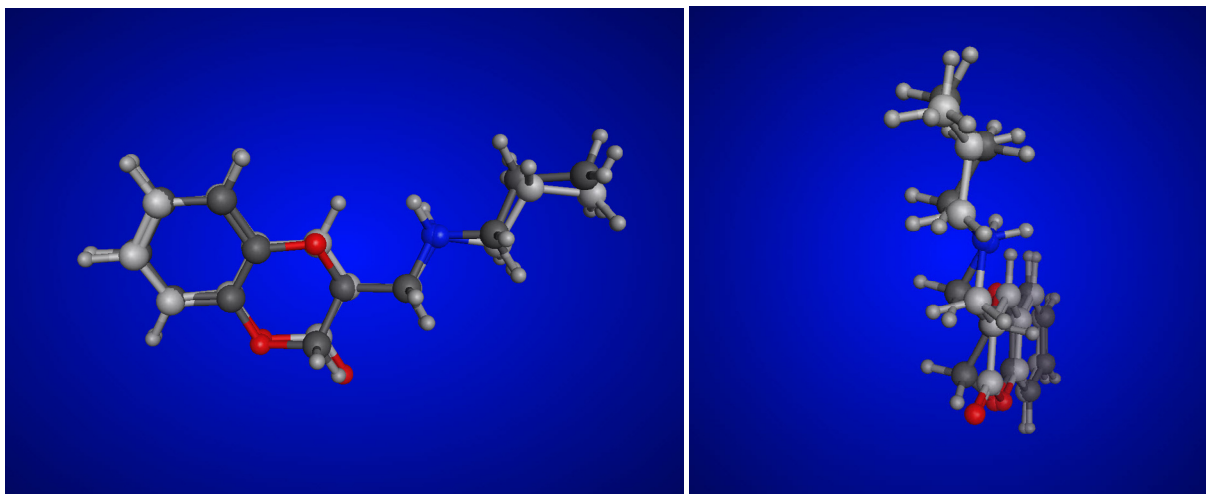

Figure 31. An overlay of global minimum energy conformations of benzodioxane (*N*-(2,3-dihydro-1,4-benzodioxin-3-ylmethyl)propan-1-amine) and the corresponding coumarin (**136**). All calculations were performed in the 2011.10 release of Chemical Computing Group's (www.chemcomp.com) Molecular Operating Environment (MOE) software using low mode following conformational analysis with the MMFF94s force field and Born solvation.
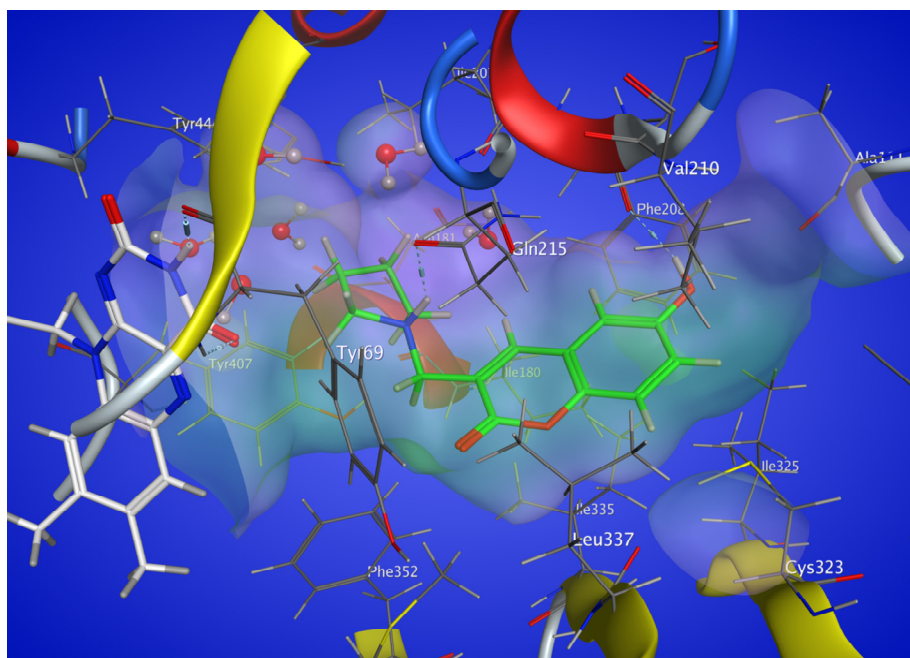
Figure 32. Binding pose of compound **167** (thick bonds with green carbon atoms) in the active site of human MAO A (PDB: 2Z5Y) viewed alongside the active site. Part of FAD is shown in thick bonds with white carbon atoms. The molecular surface of the active site is shown as a transparent shape. Abbreviations: FAD, Flavin adenine dinucleotide.
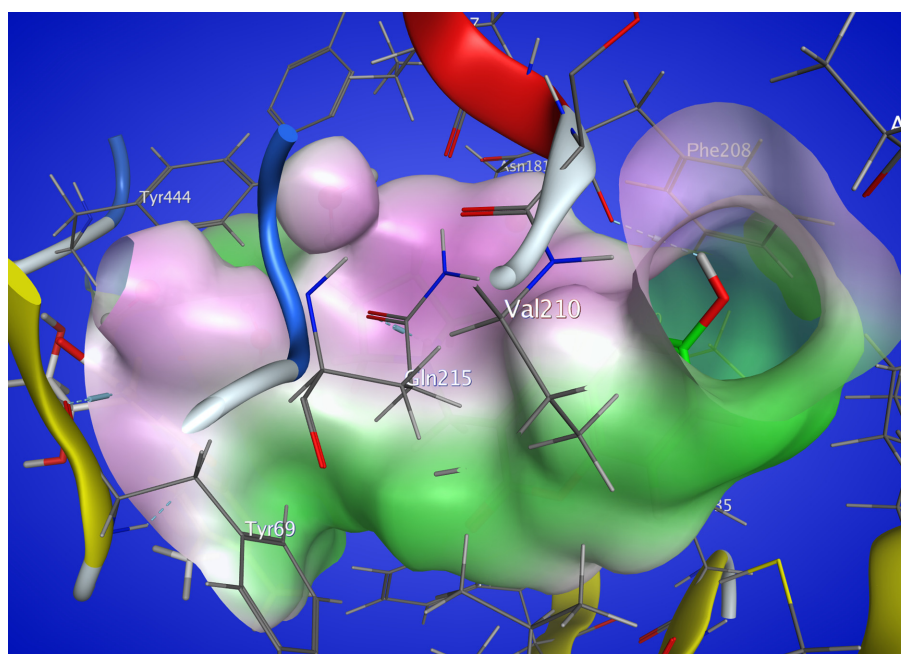

Figure 33. Binding pose of compound **167** in the active site of human MAO A (PDB: 2Z5Y) viewed from the active site entrance. The molecular surface of the binding cavity is shown as an opaque shape, color coded by lipophilicity (green: lipophilic, purple: polar). Only the 6-hydroxy part of **167** is visible from the entrance.

4.4.2. Molecular modeling

To further support the findings of the SAR in this new series of MAO A ligands, the new compounds were subjected to molecular modeling, based on the high resolution X-ray crystal complex of MAO A and the reversible inhibitor harmine (PDB entry 2Z5Y).[225] Docking was made with the most potent and selective ligands 6-amino-3-(pyrrolidin-1-ylmethyl)chromen-2-one (**134**, Figure 2S, Paper IV) and 6-hydroxy-3-(pyrrolidin-1-ylmethyl)chromen-2-one (**167**, Figure 32 and 33), with flexible automatic docking using GLIDE.[226, 227] Molecular docking studies of **134** and **167** revealed that these two compounds show a similar binding mode to MAO A, where the protonated amino group (C3) makes a hydrogen bond interaction to the amide group (oxygen) of the Gln-215 side chain. Moreover, a hydrogen bond between the 6-hydroxy group of **167** and the carbonyl oxygen of Phe-208 backbone is also observed. The Phe-208 residue is one of two unique amino acids (i.e. Phe-208, Ile-335) that determine the substrate/inhibitor cavity for MAO A. In the MAO B cavity they are replaced with Ile-199 and Tyr-326 and these amino acid pairs appear to be the major determinants in directing the different substrate/inhibitor specificities of these two enzymes.[225, 228, 229] The 6-benzyloxy derivative (**168**) was found to be inactive at MAO A and GLIDE failed to produce a binding mode for **168** neither when applying standard nor extra precision mode docking. In addition, by examination of the MAO A cavity docked with phenol **167** (Figure 32 and 33) shows that there is not enough space for such a large group in the C6-position. The binding mode would not permit the phenyl ring to point towards the entrance of the cavity but would instead clash into the protein. The opening would in any case be too narrow to accommodate a benzyl group. However, smaller and more flexible alkyls like *n*-propyl (**169**) are more likely to fit within the opening and possibly enhance binding through hydrophobic interactions with Phe-208, Leu-97 and Val-210. It is interesting to note that for other reversible MAO A inhibitors (i.e. other structural series) with a basic amino group a similar binding mode has been reported with a hydrogen bond between the protonated amine and the amide group (oxygen) of the Gln-215 side chain (Pettersson *et al.*,[213] Gallardo-Godoy *et al.*[230] and La Regina *et al.*[231]).

4.4.3. Chemical properties

Major drawbacks with the coumarins developed so far are the chemical properties, such as low aqueous solubility (high lipophilicity) and weak metabolic stability, which hamper further development of clinical candidates. Therefore a search for new coumarins with improved pharmacokinetic properties and better aqueous solubility is ongoing. Introduction of the polar amino group seems to be in favor of previously developed MAO coumarin inhibitors in terms of these properties (**61**, Figure 21).[157] The new series of 6-substituted 3-(pyrrolidin-1-ylmethyl)chromen-2-ones were all of low molecular weight (approx. 244 g/mol) with high aqueous solubility (>5 mg/mL in saline solution, see Paper IV). The metabolic stability was found to be sensitive to the substitution at the 6-position, i.e. compound **133** (6-OCF$_3$), **136** (6-H) and **137** (6-H) are not metabolically stable while compound **134** (6-amino) is stable (80% remaining compound after 60 min in the presence of rat liver microsomes, Table 3S, Paper IV). This indicates a potential for further development of new selective MAO A inhibitors within the coumarin series, combined with better metabolic stability and aqueous solubility. However, the relatively modest potency for these new reversible MAO A inhibitors may warrant further exploration of the SAR in other positions (i.e. C5, C7 and C8) to be able to identify more potent MAO A ligands.

4.4.4. Concluding remarks

The new series of 6-substituted 3-(pyrrolidin-1-ylmethyl)chromen-2-ones were found to be reversible MAO A inhibitors with moderate potency and a clear selectivity against MAO B. The most potent compounds were substituted with a hydroxy or amino group in the C6 position. Molecular modeling studies of these two compounds on MAO A implicated possible interactions between the protonated basic amino group of the ligands and the Gln-215 side chain. The 6-hydroxyl group was also proposed to make a hydrogen bonding interaction with the backbone carbonyl of Phe-208. The inactive 6-benzyloxy group was found to not fit within the MAO A cavity which supports the SAR for this series. These new compounds, despite their modest potency, have favorable properties such as low molecular weight, high aqueous solubility and compound **134** (6-amino) was also found to be metabolically stable in rat microsomes. This warrants further SAR investigations in other positions than the investigated C3 and C6-positions to identify more potent MAO A inhibitors.

5. SAR from a RU 24969 perspective

One of the key elements in the drug discovery process is to identify a chemical lead with potential to be developed for the target of interest and also has acceptable physicochemical properties. Nowadays the standard method in industry for chemical lead identification is the use of high-throughput screening of chemical libraries with large diversity in terms of chemical properties, but also *in silico* screening is becoming more and more important.[232-234] From this perspective, it is fascinating to see how diverse the pharmacological space can be based on one single chemical lead such as RU 24969 (**11**, Figure 12). RU 24969 is a suitable template on which many modifications can be done easily and also a chemical structure with the necessary physicochemical properties, i.e. low molecular weight and high water solubility. RU 24969 (**11**) is classified as a 5-HT$_{1A/1B}$ agonist with no activity at other 5-HT subtypes (Table 4),[82, 84, 85] SERT,[80] MAO[80] and DA D$_2$ receptors.[81] However, by R-group decoration and/or bioisosteric replacements of the indole core, 5-HT$_6$ agonists (**A**), dual DA D$_2$ antagonist/SERT inhibitors, (**B**), DA D$_2$ antagonists (**C**), SERT inhibitors (**D**) and reversible MAO A inhibitors (**F**) can be achieved (Figure 34). In many cases only a small change in the molecular structure leads to a complete switch in pharmacological profile. One such example is the introduction of a methyl group in the 2-position of RU 24969 which leads to a selective 5-HT$_6$ agonist instead of being a 5-HT$_{1A/1B}$ agonist (**A**, Figure 34). Another modification is the introduction of an *n*-propyl group on the basic nitrogen and reduction of the tetrahydropyridine ring which turn the compounds into active DA D$_2$ receptor ligands (**B** and **C**, Figure 34). On the other hand, by fine tuning the 5-membered heterocyclic ring a selective SERT inhibitor (**D**, Figure 34) was discovered. A more "drastic" modification, at least considering geometrical aspects, was the attachment of the piperidine ring in the 2-position instead of the 3-position which leads to MAO A inhibitors (**F**, Figure 34). It is also important to stress that inactive compounds are also found within the chemical space, i.e. **E** (Figure 34). However, even though many modifications of the RU 24969 chemical lead was made, numerous ones are yet to be explored, e.g. by introducing substituents in different positions of the aromatic ring and/or the piperidine ring, which can lead to new discoveries in terms of targets/profiles. This is an example of a commonly encountered phenomenon, that the pharmacological properties of molecules are highly sensitive to small changes in the structure. Therefore, fine tuning of the chemical structure seems to be enough in many cases for identification of new pharmacologically active compounds with different profiles. Hence, hunting for new

chemical spaces by large scale diversity approaches might often be a somewhat overelaborated way to achieving the same thing, except when finding better patent positions and/or avoiding class effects (side effects) etc. are needed.
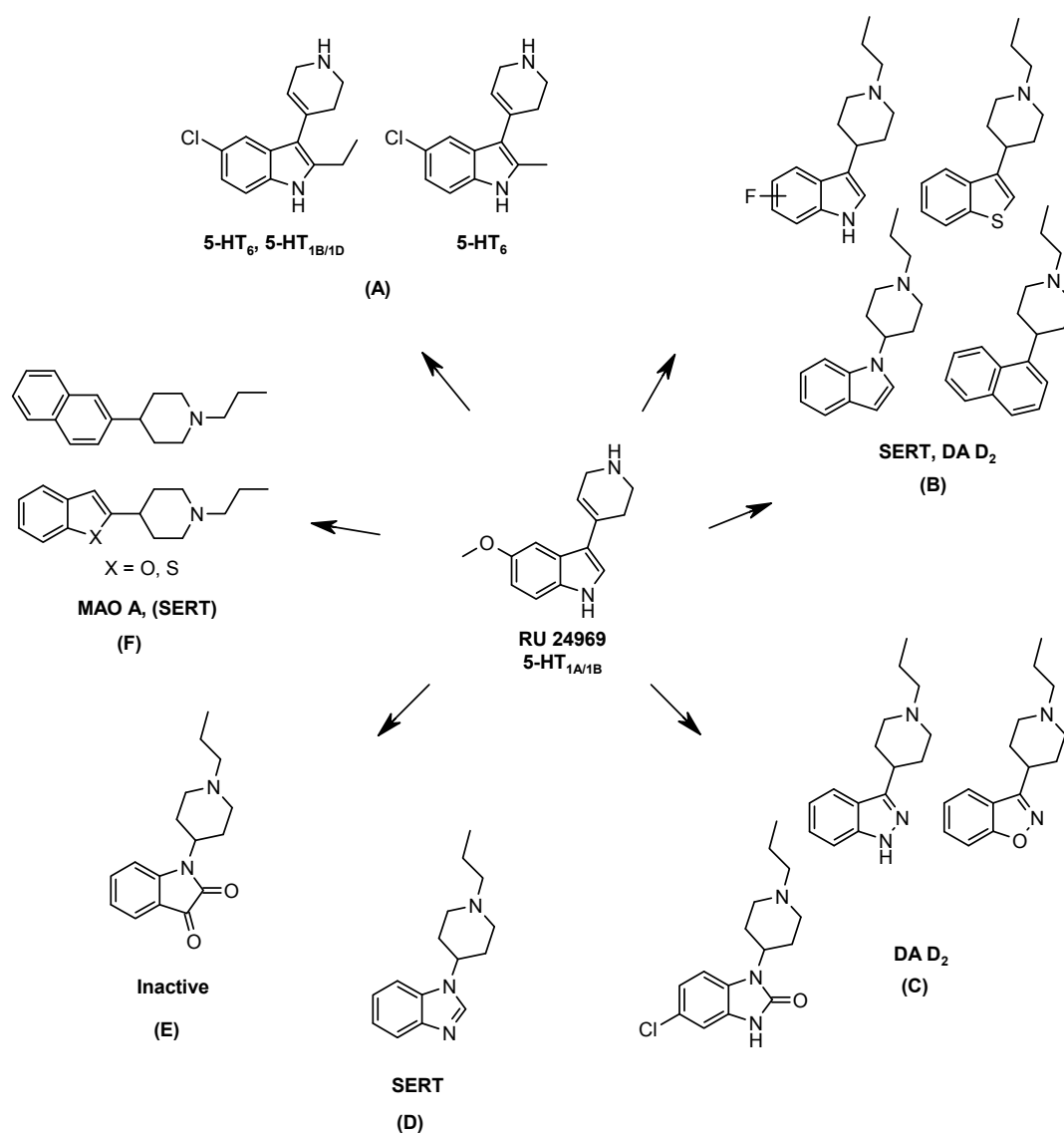

Figure 34. Overview of investigated structural variations of aryl-piperidine/tetrahydropyridines related to the 5-HT$_{1A/1B}$ agonist RU 24969.

6. Depression – and different targets

In this thesis three possible strategies for obtaining antidepressant effects have been explored:

- $5\text{-}HT_6$ agonism
- SERT inhibition combined with DA D_2 modulation
- Selective MAO A inhibition

6.1. $5\text{-}HT_6$ agonists and depression

The $5\text{-}HT_6$ agonist **81** (EMD386088, Paper I and II) has since 2005 been used as a tool compound for the investigation of the $5\text{-}HT_6$ receptor function *in vivo*/*in vitro*.[102, 105, 204-206, 235-241] However, the $5\text{-}HT_6$ receptor is one of the latest discovered receptors and therefore its physiological response is not fully elucidated. The $5\text{-}HT_6$ receptor is located postsynaptically and as such it could possibly be one of the receptors mediating an antidepressive response via a nonselective stimulation of SSRIs on different 5-HT subtypes.[29] The rat forced swim test measures behavioral patterns of the response to stress which are correlated with treatment for depression. The test involves placing the rat in a cylindrical container of water from which it is unable to escape. The immobility (passive behavior) and swimming and climbing (active response) time are then measured. This is a general test for antidepressant activity, and all classes of antidepressants today, decrease the time spent immobile by increasing their active behavior (swimming or climbing).[78] The $5\text{-}HT_6$ agonist **81** was found to reduce the immobility time and increase the swimming in a dose-dependent manner.[204] (Figure 35) This response was fully blocked by the selective $5\text{-}HT_6$ receptor antagonist SB-399885 (administrated at an inactive dose), indicating that this is a $5\text{-}HT_6$ receptor mediated effect. In addition, the $5\text{-}HT_6$ agonist **81** has been found to be active in cognition models like the ketamine-induced cognitive impairment in the novel object recognition (NOR) task,[205] as well as other cognition models,[27, 29, 105, 206] and therefore **81** may have potential for treating cognitive deficits in depression, but also in other conditions, such as schizophrenia and Alzheimer's disease.[27] However, as a paradox both $5\text{-}HT_6$ agonists and antagonists are effective as antidepressants, cognition enhancers and as anti-obesity agents, although the reason for these conflicting results is currently

unclear.[205] In summary, these results together with other *in vivo/in vitro* reports for 5-HT$_6$ agonists (see Section 1.8.2., 5-HT$_6$ agonists), may imply that 5-HT$_6$ agonists have a therapeutic potential as antidepressant alone or in combination with for instance SSRI.[29, 30, 77, 101-104]
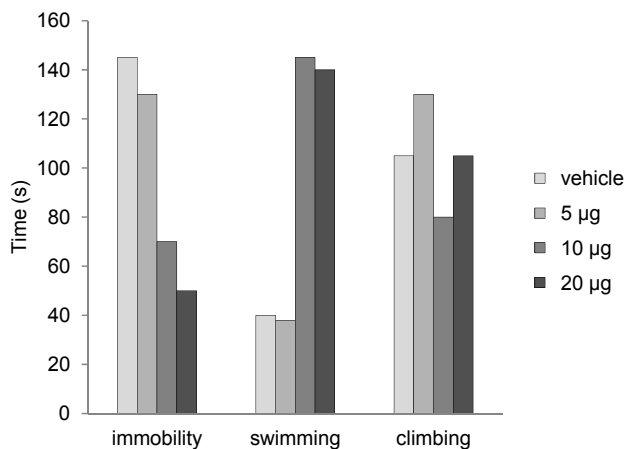

Figure 35. Effects of **81** (EMD386088) in the forced swim test in rats. **81** and its vehicle were administrated intrahippocampally for 10 min before the test. Data represent the mean of the total duration of immobility, swimming, and climbing during the 5-min test session. The number of animals in experimental groups was 8-10. Modified from Nikiforuk *et al.*[204]

6.2. SERT inhibition combined with dopamine D$_2$ modulation and depression

The clinical pharmacology literature is broadly consistent regarding the hypothesis that increases in DA function elevate mood and attenuates symptoms related to impaired DA function in depression.[56-59, 242-244] Of the two major forebrain DA projections, the larger nigrostriatal pathway is involved primarily in extrapyramidal motor control, whereas the smaller mesocorticolimbic system, which innervates limbic structures such as nucleus accumbens, amygdala, ventral hippocampus and prefrontal cortex, supports a variety of behavioral functions related to pleasure, motivation, reward and affect.[243] A dysfunction (e.g. hypofunction) in this projection may underlie the symptoms of loss of motivation, loss of interest and the inability to experience pleasure observed in major depressive disorder.[56] Therefore, compounds that enhance DA release in the mesocorticolimbic regions may improve symptoms of loss of pleasure, interests and lack of motivation. The first-line agents such as the SSRIs are known to increase the release of 5-HT via blockade of SERT. However, the SSRIs have also been found to decrease both NE and DA neurotransmission, probably via stimulation of 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors.[55, 62] The combination of SSRIs, such as fluoxetine, with certain atypical

antipsychotic drugs (olanzapine, quetiapine and risperidone) has been found to overcome some of the afore-mentioned limitations and give synergistic antidepressant effects.[62, 65] The broad binding profile and the effects of these atypical antipsychotic drugs have been suggested to be the underlying cause to counteract the SSRI-induced suppression of DA and NE activity.[61, 62, 195, 245] For instance, in the presence of 5-HT reuptake inhibition, addition of olanzapine has been shown to increase the release of DA and NE in prefrontal cortex and nucleus accumbens by blockade of DA D_2, 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors and by stimulation of 5-HT$_{1A}$ receptors, leading to synergistic antidepressant effects.[55, 246] However, a drawback of using the atypical antipsychotic drugs in the treatment of depression is the severe side effects observed in man. Olanzapine and quietapine are known to have EPS liability and induce weigh gain and thereby type II diabetes. A second problem with the adjunctive treatment regime is the problem of dosing due to different pharmacokinetic properties of the drugs used.[53] Therefore, it would be beneficial to develop a compound with dual activity such as increase in 5-HT (SERT inhibitor) and DA/NE release in brain regions suggested to be involved in depression (prefrontal cortex and nucleus accumbens).

Table 8. *In vivo* data on 5-HIAA and DOPAC levels (% of control) in striatum, limbic region and prefrontal cortex in rat brain.[a]

Compound	Dose (μmol/kg)	5-HIAA % of control			DOPAC % of control		
		striatum	limbic region	prefrontal cortex	striatum	limbic region	prefrontal cortex
158	11	82*	84*	78*	115*	113	105
158	33	83	85	82*	134*	140*	117*
158	100	87	91	85	227*	209*	190*
134	86	72*	66*	70*	45*	52*	43*
Citalopram (**4**)	25	69*	67*	71*	101	102	92
Moclobemide (**2**)	37	81*	71*	75*	18*	24*	28*

[a]Post mortem neurochemistry (subcutaneous injection) analysis of 5-HIAA and DOPAC levels (striatum, limbic region and prefrontal cortex) compared with saline control (n = 4) *p-values < 0.05 using student's t-test.

In the 1-propyl-4-arylpiperidine series (Paper III) we have identified compounds with dual DA D_2 antagonist/SERT inhibitor activity (Figure 25, Table 5 and 6), the indole **158**, is one such compound. *In vitro* **158** displays moderate affinity to DA D_2^{High} (K_i = 109 nM) and low affinity to DA D_2^{Low} (K_i = 1136 nM) combined with SERT inhibitor properties (K_i = 54 nM). *In vivo*, **158** induced a dose dependent increase in DOPAC levels in striatum, limbic system and prefrontal cortex (Table 8) which is a hallmark of DA D_2 receptors antagonism. A concomitant decrease in 5-HIAA levels in all three regions (Table 8) was also observed. This decrease was similar to the effects seen for the SSRI citalopram (**4**, Table 8) and indicates an increase in 5-HT release via blockade of SERT.[184] The postulated SSRI effect of **158** was further supported by *in vivo* microdialysis studies in freely moving rats. Compound **158** was found to elevate the extracellular 5-HT levels to about 250 and 300% of control in striatum and prefrontal cortex, respectively (Figure 36 and 37). The effect is similar to what is known for SSRIs in general.[184, 247] We have not measured the 5-HT release in the limbic region but since we see a decrease in 5-HIAA levels (Table 8) similar to what we see in striatum and prefrontal cortex, we can assume that there is an increase in 5-HT release in the limbic region as well.[247] In addition, compound **158** was also found to increase the levels of DA and NE in striatum and prefrontal cortex (Figure 36 and 37). Again, we have not measured the release in the limbic region but we can assume that there is an increase in DA levels based on the fact that **158** also increases DOPAC levels in the limbic region to same extent as in striatum (Table 8).[197] The underlying mechanism for these effects is most likely the DA D_2 receptor antagonism (blockade of inhibitory DA D_2 autoreceptors). However, the increase in DA and NE release in prefrontal cortex may be related to DA D_2 receptor antagonism but we cannot rule out that other targets are involved, i.e. α_2, 5-HT_{1A}, 5-HT_{2A} and, 5-HT_{2C}.[48, 49, 59, 246, 248] Moreover, compound **158** was also found to completely lack effects on LMA in a wide dose range suggesting low probability to induce EPS in humans (Table 6), this is in sharp contrast to typical and atypical DA D_2 antagonists.[194] This indicates also a weak (if any) antagonism of postsynaptic DA D_2 receptors while the increase in DA release is mostly attributed to the blockade of DA D_2 autoreceptors, i.e. compound **158** may be classified as a preferential DA D_2 autoreceptor antagonist.[242, 249, 250] In addition, SLV310 (**10b**, Figure 9), also a compound with dual activity (DA D_2 antagonism/SERT inhibition; DA D_{2S} K_i = 5 nM, SERT K_i = 2.5 nM), has been reported to lack effects in the forced swim test and the authors speculated that this could be linked to the strong reduction in LMA induced by SLV310.[73, 74] The lack of effects on LMA for **158** may therefore increase the likelihood of a positive effect in the forced swim test. In conclusion, the reversed indole **158** has shown to strengthen the 5-HT, NE and

DA neurotransmission in brain regions which are believed to be important for motivation and affect. These effects are suggested to correlate to blockade of DA D_2 receptors and inhibition of SERT. Therefore, compound **158** offers a "new" mechanism of action (MOA) for the treatment of major depression disorder, in addition to the known SNRIs, NDRIs and combination of SSRIs and atypical antipsychotic drugs. A drawback with compound **158** is the poor metabolic stability (6% remaining compound after 15 min in presence of rat liver microsomes) which hampers further development but as a concept molecule it warrants further investigation in more specific animal models of depression and anxiety.
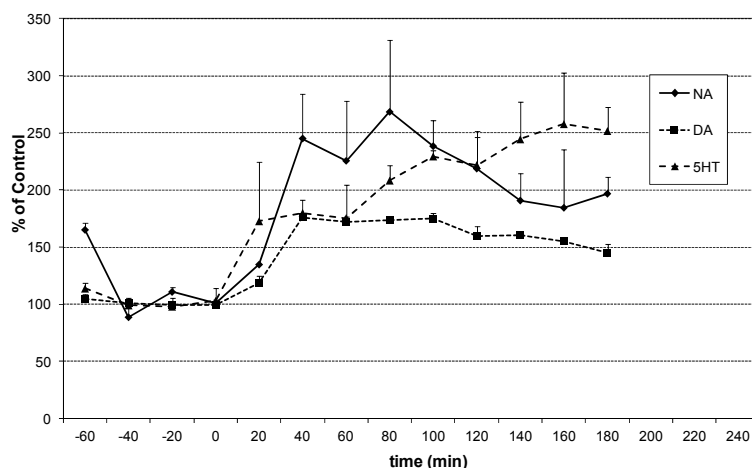

Figure 36. Striatum levels of DA, NE and 5-HT, measured by microdialysis in freely moving rats (n = 1-2) and expressed as percentage of saline control, after administration of **158** (50 µmol/kg, subcutaneous injection) at 0 min.

Figure 37. Prefrontal cortex levels of DA, NE and 5-HT, measured by microdialysis in freely moving rats (n = 1-2) and expressed as percentage of saline control, after administration of **158** (50 µmol/kg, subcutaneous injection) at 0 min.
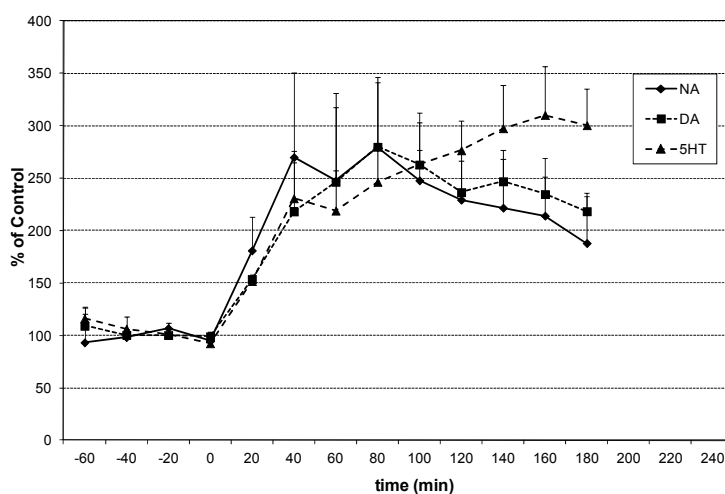
6.3. Selective MAO A inhibition and depression

In recent years a renewed interest in RIMAs has emerged, due to their efficacy in treatment resistant depression, where patients no longer respond to the first line treatment, i.e. SSRIs.[51, 251] RIMAs are also a concept for introducing a dopaminergic component into antidepressants, which has been postulated to be beneficial in treating "positive affect symptoms".[58] The use of old MAOIs is limited due to the risk of serious and potentially lethal adverse events such as hypertensive crises and serotonin syndrome, and the requirement for strict dietary restrictions. However, the newly developed RIMAs are found to be much safer with less adverse side effects, due to their reversibility which means they are easily displaced by endogenous amines. As such, there is no food restrictions needed for RIMAs.[219, 252] Furthermore, in a double-blind trial, treatment of the reversible and selective MAO A inhibitor moclobemide resulted in earlier improvement in anhedonia and blunted affect in patients with major depression compared with the predominantly serotonergic TCA clomipramine.[56, 253] In the search for novel compounds within the coumarin series we discovered and developed a new reversible MAO A inhibitor (**134**) with improved physicochemical properties, such as high water solubility and good metabolic stability. Compound **134** displays moderate inhibitory potency for MAO A (IC_{50} = 3.77 µM, Table 7), but with a clear selectivity against MAO B. In the dopaminergic/serotonergic *in vivo* screening in rat brain, **134** was shown to produce a typical MAO A inhibition profile *in vivo* with decreased DOPAC and 5-HIAA levels in striatum, limbic region and prefrontal cortex (Table 8) together with a concomitant increase in 3-MT levels (Table 7). The *in vivo* MAO A inhibitory effect was confirmed with moclobemide (**2**, Table 7 and 8).[219] However, the envisaged increase in release of 5-HT, NE and DA after inhibition of MAO A was not observed in microdialysis studies in striatum and prefrontal cortex.[219] In Figure 38 and 39 it is shown that there is no effect at all on DA and 5-HT in striatum after treatment with **134** and only minor effects on the DA levels in prefrontal cortex. The reason for the lack of effects on the extracellular levels of monoamines is not fully understood but since we have a clear *in vivo* effect on DOPAC and 5-HIAA levels in the post-mortem neurochemistry (Table 8) plus a clear increase in 3-MT levels and a concomitant decrease in DOPAC in striatum in the microdialysis study (Paper IV, Figure 5) the "negative" outcome was a bit surprising. The low potency of compound **134** might be an explanation but since moclobemide (**2**) is also known to be a low potency inhibitor of MAO A (IC_{50} = 6.1 µM in rat brain)[219] but with a clear effect on DA elevation in microdialysis studies (increase to 200% of control, intraperitoneal injection 74 µmol/kg) this explanation seems to be doubtful.[219, 254] However,

the dose used for **134** (43 µmol/kg) in the microdialysis study might be too low for a significant increase in DA levels plus the fact that only few animals were used in the study may further counteract a clear statistical effect. In conclusion, the new series of coumarins with improved chemical properties was found to be reversible inhibitors of MAO A with moderate potency but good selectivity against MAO B. However, the lack of effects for compound **134** on elevation of the extracellular levels of monoamines (5-HT, NE and DA) in different brain regions such as striatum and prefrontal cortex means that the project did not meet the screening goals. However, the basic amine-substituted coumarin scaffold is a promising template that warrants further SAR investigations in other positions than the investigated C3 and C6.
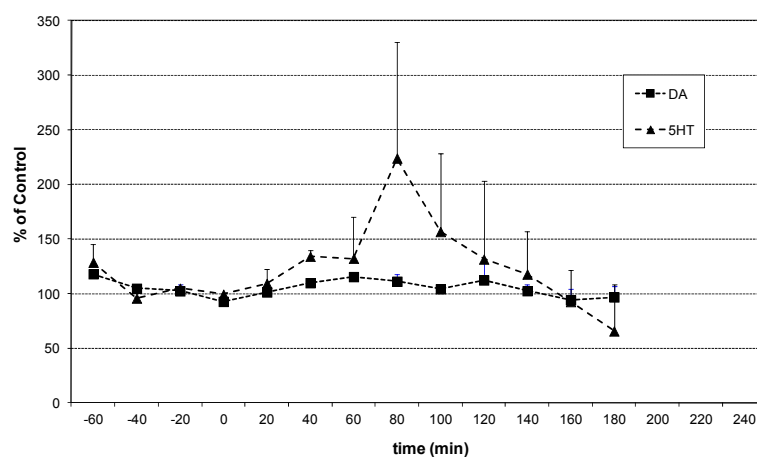

Figure 38. Striatum levels of DA and 5-HT, measured by microdialysis in freely moving rats (n = 1-2) and expressed as percentage of saline control, after administration of **134** (43 µmol/kg, subcutaneous injection) at 0 min.
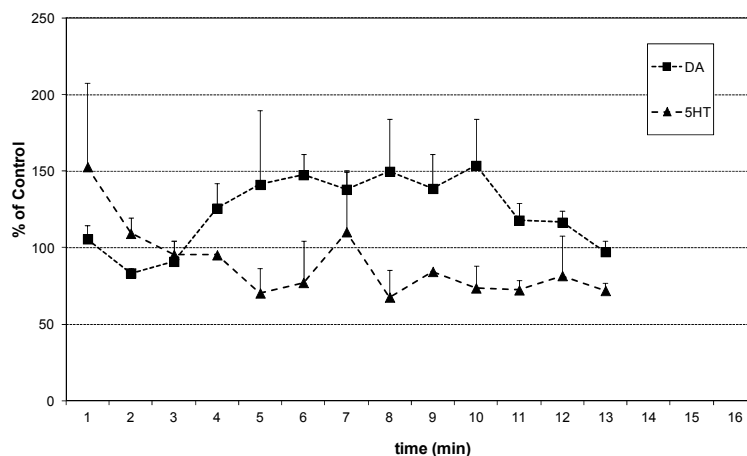

Figure 39. Prefrontal cortex levels of DA and 5-HT, measured by microdialysis in freely moving rats (n = 1-2) and expressed as percentage of saline control, after administration of **134** (43 µmol/kg, subcutaneous injection) at 0 min.

7. Concluding remarks

Major depression is one of the most common human CNS diseases today and the underlying mechanism is still unknown. All medications used today for the treatments of major depression (and depression) are built on the concept of increasing the monoaminergic neurotransmission in the brain. However, antidepressants today suffer from slow onset of therapeutic action, adverse effects and lack of efficacy. Therefore, development of new compounds based on new mechanism of actions is needed. In this thesis we have developed and evaluated three different pharmacological profiles with potential action as antidepressants:

- A potent selective 5-HT$_6$ agonist [i.e. 5-chloro-2-methyl-3-(1,2,3,6-tetrahydropyridin-4-yl)-1H-indole (**81**)].
- A dual DA D$_2$ antagonist/SERT inhibitor [i.e. 1-(1-propyl-4-piperidyl)indole (**158**)].
- A selective and reversible MAO A inhibitor [i.e. 6-amino-3-(pyrrolidin-1-ylmethyl)chromen-2-one (**134**)].

The 5-HT$_6$ receptor has been postulated to be one of the postsynaptic 5-HT receptors mediating the nonselective antidepressive response of SSRIs. The 5-HT$_6$ receptor agonist **81** was evaluated in the rat forced swim test, a model for antidepressant effects, and was found to reduce immobility time and increase swimming in a dose-dependent manner, indicating an antidepressive response. These results together with other *in vivo*/*in vitro* reports for 5-HT$_6$ agonists may imply that such compounds have a therapeutic potential as antidepressant alone or in combination with for instance SSRI.

In recent years, the notion that DA plays a major role in depression has emerged. Increased DA levels are believed to counteract some of the negative effects of SSRIs on DA and NE neurotransmission. DA is also believed to be linked to the positive affect symptoms (i.e. loss of motivation and/or interest and the inability to experience pleasure) which are less well treated today. By combination of an SSRI effect and DA D$_2$ antagonism in one compound (**158**) we were able to induce release of all monoamines (DA, NE and 5-HT) in regions of interest for depression, thereby increasing the monoamine neurotransmission in these regions. Compound **158** was also found to completely lack effects on LMA which may indicate low EPS liability in humans. This is in sharp contrast to typical and atypical DA D$_2$ antagonists and therefore, compound **158** offers a "new"

mechanism of action (MOA) for the treatment of major depression disorder and warrants further investigation in more specific animal models of depression and anxiety.

Another concept for increasing the neurotransmission in the noradrenergic, dopaminergic and serotonergic system in the brain is the use of reversible MAO A inhibitors (RIMAs), which have been found to be safer and better tolerated compared to the old irreversible MAO A inhibitors. However, the new selective and reversible MAO A inhibitor **134** failed to demonstrate any clear elevation of NA, DA or 5-HT release in a microdialysis study. The reason for this lack of effect is not fully understood but may be related to the modest potency of **134** as MAO A inhibitor (IC_{50} = 3.77 µM). However, **134** displays good physicochemical properties, such as high aqueous solubility and metabolic stability, and therefore warrants further SAR studies to find and develop more potent MAO A inhibitors.

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9. References

1. Brunton, L. L.; Chabner, B. A.; Knollmann , B. C. *Goodman & Gilman's: The Pharmacological Basis of Therapeutics*. 12th ed.; Macmillan Publishing Co: New York, 2012; p 169-649.
2. Tortora, G. J.; Derrickson, B. *Introduction to the Human Body: The Essentials of Anatomy and Physiology* 8th ed.; John Wiley & Sons, Inc. : Hoboken, NJ, 2010.
3. Whitaker-Azmitia, P. M. The discovery of serotonin and its role in neuroscience. *Neuropsychopharmacol.* **1999,** 21, 2S-8S.
4. Rubin, R. P. A Brief History of Great Discoveries in Pharmacology: In Celebration of the Centennial Anniversary of the Founding of the American Society of Pharmacology and Experimental Therapeutics. *Pharmacol. Rev.* **2007,** 59, 289-359.
5. Carlsson, A.; Lindqvist, M.; Magnusson, T.; Waldeck, B. On the presence of 3-hydroxytyramine in brain. *Science* **1958,** 127, 471.
6. Carlsson, A. Detection and assay of dopamine. *Pharmacol. Rev.* **1959,** 11, 300-304.
7. Abbott, A. Neuroscience: The molecular wake-up call. *Nature* **2007,** 447, 368-370.
8. Alex, K. D.; Pehek, E. A. Pharmacologic mechanisms of serotonergic regulation of dopamine neurotransmission. *Pharmacol. Ther.* **2007,** 113, 296-320.
9. Green, A. R. Gaddum and LSD: the birth and growth of experimental and clinical neuropharmacology research on 5-HT in the UK. *Br. J. Pharmacol.* **2008,** 154, 1583-1599.
10. Ronaldson, P. T.; Davis, T. P. Targeting blood-brain barrier changes during inflammatory pain: an opportunity for optimizing CNS drug delivery. *Ther. Deliv.* **2011,** 2, 1015-1041.
11. Elsworth, J. D.; Roth, R. H. Dopamine synthesis, uptake, metabolism, and receptors: relevance to gene therapy of Parkinson's disease. *Exp. Neurol.* **1997,** 144, 4-9.
12. Kema, I. P.; de Vries, E. G.; Muskiet, F. A. Clinical chemistry of serotonin and metabolites. *J. Chromatogr. B Biomed. Sci. Appl.* **2000,** 747, 33-48.
13. Kaenmaki, M.; Tammimaki, A.; Myohanen, T.; Pakarinen, K.; Amberg, C.; Karayiorgou, M.; Gogos, J. A.; Mannisto, P. T. Quantitative role of COMT in dopamine clearance in the prefrontal cortex of freely moving mice. *J. Neurochem.* **2010,** 114, 1745-1755.
14. Gesi, M.; Santinami, A.; Ruffoli, R.; Conti, G.; Fornai, F. Novel aspects of dopamine oxidative metabolism (confounding outcomes take place of certainties). *Pharmacol. Toxicol.* **2001,** 89, 217-224.
15. Nichols, D. E.; Nichols, C. D. Serotonin Receptors. *Chem. Rev.* **2008,** 108, 1614-1641.
16. Hannon, J.; Hoyer, D. Molecular biology of 5-HT receptors. *Behav. Brain. Res.* **2008,** 195, 198-213.
17. Alexander, S. P.; Mathie, A.; Peters, J. A. Guide to receptors and channels, 1st edition. *Br. J. Pharmacol.* **2004,** 141 S1-126.
18. McDevitt, R. A.; Hiroi, R.; Mackenzie, S. M.; Robin, N. C.; Cohn, A.; Kim, J. J.; Neumaier, J. F. Serotonin 1B autoreceptors originating in the caudal dorsal raphe nucleus reduce expression of fear and depression-like behavior. *Biol. Psychiatry* **2011,** 69, 780-787.
19. Sari, Y. Serotonin1B receptors: from protein to physiological function and behavior. *Neurosci. Biobehav. Rev.* **2004,** 28, 565-582.
20. Charnay, Y.; Leger, L. Brain serotonergic circuitries. *Dial. Clin. Neurosci.* **2010,** 12, 471-487.
21. Filip, M.; Bader, M. Overview on 5-HT receptors and their role in physiology and pathology of the central nervous system. *Pharmacol. Rep.* **2009,** 61, 761-777.
22. Monsma, F. J., Jr.; Shen, Y.; Ward, R. P.; Hamblin, M. W.; Sibley, D. R. Cloning and expression of a novel serotonin receptor with high affinity for tricyclic psychotropic drugs. *Mol. Pharmacol.* **1993,** 43, 320-327.
23. Woolley, M. L.; Marsden, C. A.; Fone, K. C. 5-HT6 receptors. *Curr. Drug Targ. CNS Neuro. Disord.* **2004,** 3, 59-79.
24. Gerard, C.; Martres, M.-P.; Lefevre, K.; Miquel, M.-C.; Verge, D.; Lanfumey, L.; Doucet, E.; Hamon, M.; El Mestikawy, S. Immuno-localization of serotonin 5-HT6 receptor-like material in the rat central nervous system. *Brain. Res.* **1997,** 746, 207-219.
25. Ward, R. P.; Dorsa, D. M. Molecular and behavioral effects mediated by Gs-coupled adenosine A2a, but not serotonin 5-Ht4 or 5-Ht6 receptors following antipsychotic administration. *Neurosci.* **1999,** 89, 927-938.
26. Arnt, J.; Olsen, C. K. 5-HT6 receptor ligands and their antipsychotic potential. *Int. Rev. Neurobiol.* **2011,** 96, 141-161.

27. Fone, K. C. F. An update on the role of the 5-hydroxytryptamine6 receptor in cognitive function. *Neuropharmacol.* **2008,** 55, 1015-1022.

28. Heal, D. J.; Smith, S. L.; Fisas, A.; Codony, X.; Buschmann, H. Selective 5-HT6 receptor ligands: progress in the development of a novel pharmacological approach to the treatment of obesity and related metabolic disorders. *Pharmacol. Ther.* **2008,** 117, 207-231.

29. Yun, H. M.; Rhim, H. The serotonin-6 receptor as a novel therapeutic target. *Exp. Neurobiol.* **2011,** 20, 159-168.

30. Wesolowska, A. Potential role of the 5-HT6 receptor in depression and anxiety: an overview of preclinical data. *Pharmacol. Rep.* **2010,** 62, 564-577.

31. Beaulieu, J. M.; Gainetdinov, R. R. The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacol. Rev.* **2011,** 63, 182-217.

32. Seeman, P. Brain dopamine receptors. *Pharmacol. Rev.* **1980,** 32, 229-313.

33. 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.

34. 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, 923-926.

35. 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 D2 receptors. *Nature* **2000,** 408, 199-203.

36. De Mei, C.; Ramos, M.; Iitaka, C.; Borrelli, E. Getting specialized: presynaptic and postsynaptic dopamine D2 receptors. *Curr. Opin. Pharmacol.* **2009,** 9, 53-58.

37. 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. *J. Biol. Chem.* **1982,** 257, 6351-6361.

38. Widzowski, D. V.; Cory-Slechta, D. A. Apparent mediation of the stimulus properties of a low dose of quinpirole by dopaminergic autoreceptors. *J. Pharmacol. Exp. Ther.* **1993,** 266, 526-534.

39. Zhang, A.; Neumeyer, J. L.; Baldessarini, R. J. Recent progress in development of dopamine receptor subtype-selective agents: potential therapeutics for neurological and psychiatric disorders. *Chem. Rev.* **2007,** 107, 274-302.

40. Boeckler, F.; Gmeiner, P. Dopamine D3 receptor ligands Recent advances in the control of subtype selectivity and intrinsic activity. *Biochim. Biophys. Acta. Biomembr.* **2007,** 1768, 871-887.

41. Youdim, M. B.; Bakhle, Y. S. Monoamine oxidase: isoforms and inhibitors in Parkinson's disease and depressive illness. *Br. J. Pharmacol.* **2006,** 147 Suppl 1, S287-S296.

42. Lopez-Munoz, F.; Alamo, C.; Juckel, G.; Assion, H. J. Half a century of antidepressant drugs: on the clinical introduction of monoamine oxidase inhibitors, tricyclics, and tetracyclics. Part I: monoamine oxidase inhibitors. *J. Clin. Psychopharmacol.* **2007,** 27, 555-559.

43. Saura, J.; Kettler, R.; Da Prada, M.; Richards, J. G. Quantitative enzyme radioautography with 3H-Ro 41-1049 and 3H-Ro 19-6327 in vitro: localization and abundance of MAO-A and MAO-B in rat CNS, peripheral organs, and human brain. *J. Neurosci.* **1992,** 12, 1977-1999.

44. Billett, E. E. Monoamine Oxidase (MAO) in Human Peripheral Tissues. *Neuro.Toxicol.* **2004,** 25, 139-148.

45. Lotufo-Neto, F.; Trivedi, M.; Thase, M. E. Meta-analysis of the reversible inhibitors of monoamine oxidase type A moclobemide and brofaromine for the treatment of depression. *Neuropsychopharmacol.* **1999,** 20, 226-247.

46. Nair, N. P.; Ahmed, S. K.; Kin, N. M. Biochemistry and pharmacology of reversible inhibitors of MAO-A agents: focus on moclobemide. *J. Psych. Neurosci.* **1993,** 18, 214-225.

47. Thase, M. E. The role of monoamine oxidase inhibitors in depression treatment guidelines. *J. Clin. Psychiatry* **2012,** 73 Suppl 1, 10-16.

48. Millan, M. J. Dual- and triple-acting agents for treating core and co-morbid symptoms of major depression: novel concepts, new drugs. *Neurotherapeut.* **2009,** 6, 53-77.

49. Millan, M. J. Multi-target strategies for the improved treatment of depressive states: Conceptual foundations and neuronal substrates, drug discovery and therapeutic application. *Pharmacol. Ther.* **2006,** 110, 135-370.

50. Goldberg, J. F.; Thase, M. E. Monoamine Oxidase Inhibitors Revisited: What You Should Know. *J. Clin. Psychiatry* **2013,** 74, 189-191.

51. Bortolato, M.; Chen, K.; Shih, J. C. Monoamine oxidase inactivation: from pathophysiology to therapeutics. *Adv. Drug. Deliv. Rev.* **2008,** 60, 1527-1533.

52. Delgado, P. L.; Moreno, F. A. Role of norepinephrine in depression. *J. Clin. Psychiatry* **2000,** 61 Suppl 1, 5-12.

53. Preston, T. C.; Shelton, R. C. Treatment resistant depression: strategies for primary care. *Curr. Psychiatry Rep.* **2013,** 15, 370.

54. Racagni, G.; Popoli, M. The pharmacological properties of antidepressants. *Int. Clin. Psychopharmacol.* **2010,** 25, 117-131.

55. Blier, P.; Szabo, S. T. Potential mechanisms of action of atypical antipsychotic medications in treatment-resistant depression and anxiety. *J. Clin. Psychiatry* **2005,** 66 Suppl 8, 30-40.

56. Nutt, D.; Demyttenaere, K.; Janka, Z.; Aarre, T.; Bourin, M.; Canonico, P. L.; Carrasco, J. L.; Stahl, S. The other face of depression, reduced positive affect: the role of catecholamines in causation and cure. *J. Psychopharmacol.* **2007,** 21, 461-471.

57. Nutt, D. J. The role of dopamine and norepinephrine in depression and antidepressant treatment. *J. Clin. Psychiatry* **2006,** 67 Suppl 6, 3-8.

58. Nutt, D. J. Relationship of neurotransmitters to the symptoms of major depressive disorder. *J. Clin. Psychiatry* **2008,** 69 Suppl E1, 4-7.

59. El Mansari, M.; Guiard, B. P.; Chernoloz, O.; Ghanbari, R.; Katz, N.; Blier, P. Relevance of norepinephrine-dopamine interactions in the treatment of major depressive disorder. *CNS Neurosci. Ther.* **2011,** 16, e1-17.

60. Tanda, G.; Carboni, E.; Frau, R.; Di Chiara, G. Increase of extracellular dopamine in the prefrontal cortex: a trait of drugs with antidepressant potential? *Psychopharmacol. (Berlin)* **1994,** 115, 285-288.

61. Bobo, W. V.; Shelton, R. C. Efficacy, safety and tolerability of Symbyax for acute-phase management of treatment-resistant depression. *Expert. Rev. Neurother.* **2010,** 10, 651-670.

62. Bobo, W. V.; Shelton, R. C. Fluoxetine and olanzapine combination therapy in treatment-resistant major depression: review of efficacy and safety data. *Expert Opin. Pharmacother.* **2009,** 10, 2145-2159.

63. Gabriel, A. Risperidone, quetiapine, and olanzapine adjunctive treatments in major depression with psychotic features: a comparative study. *Neuropsychiatric Dis. Treat.* **2013,** 9, 485-492.

64. Spielmans, G. I.; Berman, M. I.; Linardatos, E.; Rosenlicht, N. Z.; Perry, A.; Tsai, A. C. Adjunctive atypical antipsychotic treatment for major depressive disorder: a meta-analysis of depression, quality of life, and safety outcomes. *PLoS Med.* **2013,** 10, e1001403.

65. Möller, H. Antipsychotic and antidepressive effects of second generation antipsychotics. *Eur. Arch. Psychiatry Clin. Neurosci.* **2005,** 255, 190-201.

66. Corrigan, M. H.; Denahan, A. Q.; Wright, C. E.; Ragual, R. J.; Evans, D. L. Comparison of pramipexole, fluoxetine, and placebo in patients with major depression. *Depress. Anxiety* **2000,** 11, 58-65.

67. Cassano, P.; Lattanzi, L.; Fava, M.; Navari, S.; Battistini, G.; Abelli, M.; Cassano, G. B. Ropinirole in treatment-resistant depression: a 16-week pilot study. *Can. J. Psychiatry* **2005,** 50, 357-360.

68. Brennan, J. A.; Graf, R.; Grauer, S. M.; Navarra, R. L.; Pulicicchio, C. M.; Hughes, Z. A.; Lin, Q.; Wantuch, C.; Rosenzweig-Lipson, S.; Pruthi, F.; Lai, M.; Smith, D.; Goutier, W.; van de Neut, M.; Robichaud, A. J.; Rotella, D.; Feenstra, R. W.; Kruse, C.; Broqua, P.; Beyer, C. E.; McCreary, A. C.; Pausch, M. H.; Marquis, K. L. WS-50030 [7-{4-[3-(1H-inden-3-yl)propyl]piperazin-1-yl}-1,3-benzoxazol-2(3H)-one]: A Novel Dopamine D2 Receptor Partial Agonist/Serotonin Reuptake Inhibitor with Preclinical Antipsychotic-Like and Antidepressant-Like Activity. *J. Pharmacol. Exp. Ther.* **2010,** 332, 190-201.

69. Michael-Titus, A. T.; Albert, M.; Michael, G. J.; Michaelis, T.; Watanabe, T.; Frahm, J.; Pudovkina, O.; van der Hart, M. G.; Hesselink, M. B.; Fuchs, E.; Czeh, B. SONU20176289, a compound combining partial dopamine D(2) receptor agonism with specific serotonin reuptake inhibitor activity, affects neuroplasticity in an animal model for depression. *Eur. J. Pharmacol.* **2008,** 598, 43-50.

70. van Hes, R.; Smid, P.; Stroomer, C. N. J.; Tipker, K.; Tulp, M. T. M.; van der Heyden, J. A. M.; McCreary, A. C.; Hesselink, M. B.; Kruse, C. G. SLV310, a novel, potential antipsychotic, combining potent dopamine D2 receptor antagonism with serotonin reuptake inhibition. *Bioorg. Med. Chem. Lett.* **2003,** 13, 405-408.

71. Lange, J. H. M.; Reinders, J.-H.; Tolboom, J. T. B. M.; Glennon, J. C.; Coolen, H. K. A. C.; Kruse, C. G. Principal Component Analysis Differentiates the Receptor Binding Profiles of Three Antipsychotic Drug Candidates from Current Antipsychotic Drugs. *J. Med. Chem.* **2007,** 50, 5103-5108.

72. Smid, P.; Coolen, H. K. A. C.; Keizer, H. G.; van Hes, R.; de Moes, J.-P.; den Hartog, A. P.; Stork, B.; Plekkenpol, R. H.; Niemann, L. C.; Stroomer, C. N. J.; Tulp, M. T. M.; van Stuivenberg, H. H.; McCreary, A. C.; Hesselink, M. B.; Herremans, A. H. J.; Kruse, C. G. Synthesis, Structure-Activity Relationships, and Biological Properties of 1-Heteroaryl-4-[w-(1H-indol-3-yl)alkyl]piperazines, Novel Potential Antipsychotics Combining Potent Dopamine D2 Receptor Antagonism with Potent Serotonin Reuptake Inhibition. *J. Med. Chem.* **2005,** 48, 6855-6869.

73. Hesselink, M. B.; Van Vliet, B.; Kruse, C. G.; Long, S. K. SLV310, a molecule combining potent dopamine D2 receptor antagonism with serotonin reuptake inhibition: In vitro and in vivo neurochemistry. *Eur. Neuropsychopharmacol.* **2001,** 11, S252.

74. Tuinstra, T.; Herremans, A. H. J.; Van der Heyden, J. A. M.; McCreary, A. C.; Hesselink, M. B.; Kruse, C. G.; Long, S. K. SLV310, a molecule combining potent dopamine D2 receptor antagonism with serotonin reuptake

inhibition, active in preclinical models for antipsychotic as well as anxiolytic activity. *Eur. Neuropsychopharmacol.* **2001,** 11, S251.

75.   Song, M. S.; Matveychuk, D.; Mackenzie, E. M.; Duchcherer, M.; Mousseau, D. D.; Baker, G. B. An update on amine oxidase inhibitors: Multifaceted drugs. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2013,** 44C, 118-124.

76.   Duncan, J.; Johnson, S.; Ou, X. M. Monoamine oxidases in major depressive disorder and alcoholism. *Drug. Discov. Ther.* **2012,** 6, 112-122.

77.   Schechter, L. E.; Lin, Q.; Smith, D. L.; Zhang, G.; Shan, Q.; Platt, B.; Brandt, M. R.; Dawson, L. A.; Cole, D.; Bernotas, R.; Robichaud, A.; Rosenzweig-Lipson, S.; Beyer, C. E. Neuropharmacological profile of novel and selective 5-HT6 receptor agonists: WAY-181187 and WAY-208466. *Neuropsychopharmacol.* **2008,** 33, 1323-1335.

78.   Carr, G. V.; Lucki, I. The role of serotonin receptor subtypes in treating depression: a review of animal studies. *Psychopharmacol. (Berl)* **2011,** 213, 265-287.

79.   Beale, J. M.; Block , J. H. *Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry* 11th ed.; Lippincott Williams & Wilkins: Philadelphia, 2004; p 1-194.

80.   Hunt, P.; Oberlander, C. The interaction of indole derivatives with the serotonin receptor and non-dopaminergic circling behaviour. *Adv. Exp. Med. Biol.* **1981,** 133, 547-562.

81.   Guillaume, J.; Dumont, C.; Laurent, J.; Nedelec, L. (Tetrahydro-1,2,3,6 pyridinyl-4)-3 1 H-indoles: synthese, proprietes serotoninergique et anti-dopaminergiques. *Eur. J. Med. Chem.* **1987,** 22, 33-43.

82.   Zifa, E.; Fillion, G. 5-Hydroxytryptamine receptors. *Pharmacol. Rev.* **1992,** 44, 401-458.

83.   Taylor, E. W.; Nikam, S.; Weck, B.; Martin, A.; Nelson, D. Relative selectivity of some conformationally constrained tryptamine analogs at 5-HT1, 5-HT1A and 5-HT2 recognition sites. *Life Sci.* **1987,** 41, 1961-1969.

84.   Macor, J. E.; Burkhart, C. A.; Heym, J. H.; Ives, J. L.; Lebel, L. A.; Newman, M. E.; Nielsen, J. A.; Ryan, K.; Schulz, D. W.; Torgersen, L. K.; et al. 3-(1,2,5,6-Tetrahydropyrid-4-yl)pyrrolo[3,2-b]pyrid-5-one: a potent and selective serotonin (5-HT1B) agonist and rotationally restricted phenolic analogue of 5-methoxy-3-(1,2,5,6-tetrahydropyrid-4-yl)indole. *J. Med. Chem.* **1990,** 33, 2087-2093.

85.   Bruss, M.; Kiel, S.; Bonisch, H.; Kostanian, A.; Gothert, M. Decreased agonist, but not antagonist, binding to the naturally occurring Thr92Lys variant of the h5-HT7(a) receptor. *Neurochem. Int.* **2005,** 47, 196-203.

86.   Nelson, D. L. Structure-activity relationships at 5-HT1A receptors: binding profiles and intrinsic activity. *Pharmacol. Biochem. Behav.* **1991,** 40, 1041-1051.

87.   Taylor, E. W.; Nikam, S. S.; Lambert, G.; Martin, A. R.; Nelson, D. L. Molecular determinants for recognition of RU 24969 analogs at central 5-hydroxytryptamine recognition sites: use of a bilinear function and substituent volumes to describe steric fit. *Mol. Pharmacol.* **1988,** 34, 42-53.

88.   Agarwal, A.; Pearson, P. P.; Taylor, E. W.; Li, H. B.; Dahlgren, T.; Herslof, M.; Yang, Y.; Lambert, G.; Nelson, D. L.; Regan, J. W.; et al. Three-dimensional quantitative structure-activity relationships of 5-HT receptor binding data for tetrahydropyridinylindole derivatives: a comparison of the Hansch and CoMFA methods. *J. Med. Chem.* **1993,** 36, 4006-4014.

89.   Crawforth, J.; Goodacre, S.; Maxey, R.; Bourrain, S.; Patel, S.; Marwood, R.; O'Connor, D.; Herbert, R.; Hutson, P.; Rowley, M. 3-(4-Piperidinyl)- and 3-(8-aza-bicyclo[3.2.1]oct-3-yl)-2-phenyl-1H-indoles as bioavailable h5-HT2A antagonists. *Bioorg. Med. Chem. Lett.* **2000,** 10, 2701-2703.

90.   Lambert, G. A. Preclinical neuropharmacology of naratriptan. *CNS Drug. Rev.* **2005,** 11, 289-316.

91.   Glennon, R. A.; Lee, M.; Rangisetty, J. B.; Dukat, M.; Roth, B. L.; Savage, J. E.; McBride, A.; Rauser, L.; Hufeisen, S.; Lee, D. K. 2-Substituted tryptamines: agents with selectivity for 5-HT(6) serotonin receptors. *J. Med. Chem.* **2000,** 43, 1011-1018.

92.   Riccioni, T.; Bordi, F.; Minetti, P.; Spadoni, G.; Yun, H. M.; Im, B. H.; Tarzia, G.; Rhim, H.; Borsini, F. ST1936 stimulates cAMP, Ca2+, ERK1/2 and Fyn kinase through a full activation of cloned human 5-HT6 receptors. *Eur. J. Pharmacol.* **2011,** 661, 8-14.

93.   Holenz, J.; Merce, R.; Diaz, J. L.; Guitart, X.; Codony, X.; Dordal, A.; Romero, G.; Torrens, A.; Mas, J.; Andaluz, B.; Hernandez, S.; Monroy, X.; Sanchez, E.; Hernandez, E.; Perez, R.; Cubi, R.; Sanfeliu, O.; Buschmann, H. Medicinal chemistry driven approaches toward novel and selective serotonin 5-HT6 receptor ligands. *J. Med. Chem.* **2005,** 48, 1781-1795.

94.   Holenz, J.; Pauwels, P. J.; Diaz, J. L.; Merce, R.; Codony, X.; Buschmann, H. Medicinal chemistry strategies to 5-HT(6) receptor ligands as potential cognitive enhancers and antiobesity agents. *Drug Discov. Today* **2006,** 11, 283-299.

95.   Cole, D. C.; Lennox, W. J.; Lombardi, S.; Ellingboe, J. W.; Bernotas, R. C.; Tawa, G. J.; Mazandarani, H.; Smith, D. L.; Zhang, G.; Coupet, J.; Schechter, L. E. Discovery of 5-arylsulfonamido-3-(pyrrolidin-2-

ylmethyl)-1H-indole derivatives as potent, selective 5-HT6 receptor agonists and antagonists. *J. Med. Chem.* **2005,** 48, 353-356.

96. Cole, D. C.; Stock, J. R.; Lennox, W. J.; Bernotas, R. C.; Ellingboe, J. W.; Boikess, S.; Coupet, J.; Smith, D. L.; Leung, L.; Zhang, G. M.; Feng, X.; Kelly, M. F.; Galante, R.; Huang, P.; Dawson, L. A.; Marquis, K.; Rosenzweig-Lipson, S.; Beyer, C. E.; Schechter, L. E. Discovery of N1-(6-chloroimidazo[2,1-b][1,3]thiazole-5-sulfonyl)tryptamine as a potent, selective, and orally active 5-HT(6) receptor agonist. *J. Med. Chem.* **2007,** 50, 5535-5538.

97. Alcalde, E.; Mesquida, N.; Lopez-Perez, S.; Frigola, J.; Merce, R. Indene-based scaffolds. 2. An indole-indene switch: discovery of novel indenylsulfonamides as 5-HT6 serotonin receptor agonists. *J. Med. Chem.* **2009,** 52, 675-687.

98. Bernotas, R. C.; Lenicek, S.; Antane, S.; Cole, D. C.; Harrison, B. L.; Robichaud, A. J.; Zhang, G. M.; Smith, D.; Platt, B.; Lin, Q.; Li, P.; Coupet, J.; Rosenzweig-Lipson, S.; Beyer, C. E.; Schechter, L. E. Novel 1-aminoethyl-3-arylsulfonyl-1H-pyrrolo[2,3-b]pyridines are potent 5-HT(6) agonists. *Bioorg. Med. Chem.* **2009,** 17, 5153-5163.

99. Bernotas, R. C.; Antane, S.; Shenoy, R.; Le, V. D.; Chen, P.; Harrison, B. L.; Robichaud, A. J.; Zhang, G. M.; Smith, D.; Schechter, L. E. 3-(Arylsulfonyl)-1-(azacyclyl)-1H-indoles are 5-HT(6) receptor modulators. *Bioorg. Med. Chem. Lett.* **2010,** 20, 1657-1660.

100. Glennon, R. A.; Siripurapu, U.; Roth, B. L.; Kolanos, R.; Bondarev, M. L.; Sikazwe, D.; Lee, M.; Dukat, M. The medicinal chemistry of 5-HT6 receptor ligands with a focus on arylsulfonyltryptamine analogs. *Curr. Top. Med. Chem.* **2010,** 10, 579-595.

101. Svenningsson, P.; Tzavara, E. T.; Qi, H.; Carruthers, R.; Witkin, J. M.; Nomikos, G. G.; Greengard, P. Biochemical and behavioral evidence for antidepressant-like effects of 5-HT6 receptor stimulation. *J. Neurosci.* **2007,** 27, 4201-4209.

102. Scheggi, S.; Marchese, G.; Borsini, F.; Bordi, F.; De Montis, M. G. Effects of the 5-HT(6) receptor agonist ST 1936 on depression- and anhedonia-like experimental models. *Behav. Brain. Res.* **2011,** 224, 35-43.

103. de Foubert, G.; O'Neill, M. J.; Zetterstrom, T. S. Acute onset by 5-HT(6)-receptor activation on rat brain brain-derived neurotrophic factor and activity-regulated cytoskeletal-associated protein mRNA expression. *Neurosci.* **2007,** 147, 778-785.

104. Carr, G. V.; Schechter, L. E.; Lucki, I. Antidepressant and anxiolytic effects of selective 5-HT6 receptor agonists in rats. *Psychopharmacol. (Berl)* **2011,** 213, 499-507.

105. Kendall, I.; Slotten, H. A.; Codony, X.; Burgueno, J.; Pauwels, P. J.; Vela, J. M.; Fone, K. C. E-6801, a 5-HT6 receptor agonist, improves recognition memory by combined modulation of cholinergic and glutamatergic neurotransmission in the rat. *Psychopharmacol. (Berlin)* **2011,** 213, 413-430.

106. Terry Jr, A. V.; Buccafusco, J. J.; Wilson, C. Cognitive dysfunction in neuropsychiatric disorders: Selected serotonin receptor subtypes as therapeutic targets. *Behav. Brain. Res.* **2008,** 195, 30-38.

107. Mitchell, E. S.; Neumaier, J. F. 5-HT6 receptors: a novel target for cognitive enhancement. *Pharmacol. Ther.* **2005,** 108, 320-333.

108. Sleight, A. J.; Boess, F. G.; Bos, M.; Levet-Trafit, B.; Riemer, C.; Bourson, A. Characterization of Ro 04-6790 and Ro 63-0563: potent and selective antagonists at human and rat 5-HT6 receptors. *Br. J. Pharmacol.* **1998,** 124, 556-562.

109. Liu, K. G.; Robichaud, A. J. 5-HT6 medicinal chemistry. *Int. Rev. Neurobiol.* **2010,** 94, 1-34.

110. Ruiz, N. V.; Oranias Olsina, G. Patents. *Int. Rev. Neurobiol.* **2010,** 94, 35-66.

111. Upton, N.; Chuang, T. T.; Hunter, A. J.; Virley, D. J. 5-HT6 receptor antagonists as novel cognitive enhancing agents for Alzheimer's disease. *Neurotherapeut.* **2008,** 5, 458-469.

112. Cole, D. C.; Ellingboe, J. W.; Lennox, W. J.; Mazandarani, H.; Smith, D. L.; Stock, J. R.; Zhang, G.; Zhou, P.; Schechter, L. E. N1-arylsulfonyl-3-(1,2,3,6-tetrahydropyridin-4-yl)-1H-indole derivatives are potent and selective 5-HT6 receptor antagonists. *Bioorg. Med. Chem. Lett.* **2005,** 15, 379-383.

113. Cole, D. C.; Lennox, W. J.; Stock, J. R.; Ellingboe, J. W.; Mazandarani, H.; Smith, D. L.; Zhang, G.; Tawa, G. J.; Schechter, L. E. Conformationally constrained N1-arylsulfonyltryptamine derivatives as 5-HT6 receptor antagonists. *Bioorg. Med. Chem. Lett.* **2005,** 15, 4780-4785.

114. Kapur, S.; Mamo, D. Half a century of antipsychotics and still a central role for dopamine D2 receptors. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **2003,** 27, 1081-1090.

115. Tandon, R.; Nasrallah, H. A.; Keshavan, M. S. Schizophrenia, "just the facts" 4. Clinical features and conceptualization. *Schizophr. Res.* **2009,** 110, 1-23.

116. Casey, D. E. Neuroleptic drug-induced extrapyramidal syndromes and tardive dyskinesia. *Schizophr. Res.* **1991,** 4, 109-120.

117. Perregaard, J.; Arnt, J.; Bogeso, K. P.; Hyttel, J.; Sanchez, C. Noncataleptogenic, centrally acting dopamine D-2 and serotonin 5-HT2 antagonists within a series of 3-substituted 1-(4-fluorophenyl)-1H-indoles. *J. Med. Chem.* **1992,** 35, 1092-1101.

118. Strupczewski, J. T.; Allen, R. C.; Gardner, B. A.; Schmid, B. L.; Stache, U.; Glamkowski, E. J.; Jones, M. C.; Ellis, D. B.; Huger, F. P.; Dunn, R. W. Synthesis and neuroleptic activity of 3-(1-substituted-4-piperidinyl)-1,2-benzisoxazoles. *J. Med. Chem.* **1985,** 28, 761-769.

119. Howard, H. R.; Lowe, J. A., 3rd; Seeger, T. F.; Seymour, P. A.; Zorn, S. H.; Maloney, P. R.; Ewing, F. E.; Newman, M. E.; Schmidt, A. W.; Furman, J. S.; Robinson, G. L.; Jackson, E.; Johnson, C.; Morrone, J. 3-Benzisothiazolylpiperazine derivatives as potential atypical antipsychotic agents. *J. Med. Chem.* **1996,** 39, 143-148.

120. Mailman, R. B.; Murthy, V. Third generation antipsychotic drugs: partial agonism or receptor functional selectivity? *Curr. Pharm. Des.* **2011,** 16, 488-501.

121. Kalani, M. Y. S.; Vaidehi, N.; Hall, S. E.; Trabanino, R. J.; Freddolino, P. L.; Kalani, M. A.; Floriano, W. B.; Kam, V. W. T.; Goddard, W. A. The predicted 3D structure of the human D2 dopamine receptor and the binding site and binding affinities for agonists and antagonists. *Proc. Natl. Acad. Sci.* **2004,** 101, 3815-3820.

122. Goddard, W. A., 3rd; Abrol, R. 3-Dimensional structures of G protein-coupled receptors and binding sites of agonists and antagonists. *J. Nutr.* **2007,** 137, 1528S-1538S; discussion 1548S.

123. Liljefors, T.; Bogeso, K. P.; Hyttel, J.; Wikstrom, H.; Svensson, K.; Carlsson, A. Pre- and postsynaptic dopaminergic activities of indolizidine and quinolizidine derivatives of 3-(3-hydroxyphenyl)-N-(n-propyl)piperidine (3-PPP). Further developments of a dopamine receptor model. *J. Med. Chem.* **1990,** 33, 1015-1022.

124. Malo, M.; Brive, L.; Luthman, K.; Svensson, P. Selective pharmacophore models of dopamine D(1) and D(2) full agonists based on extended pharmacophore features. *Chem. Med. Chem.* **2010,** 5, 232-246.

125. Singer, C. Managing the patient with newly diagnosed Parkinson disease. *Cleve. Clin. J. Med.* **2012,** 79, S3-S7.

126. Hjorth, S.; Carlsson, A.; Wikstrom, H.; Lindberg, P.; Sanchez, D.; Hacksell, U.; Arvidsson, L. E.; Svensson, U.; Nilsson, J. L. 3-PPP, a new centrally acting DA-receptor agonist with selectivity for autoreceptors. *Life Sci.* **1981,** 28, 1225-1238.

127. Tamminga, C. A.; Cascella, N. G.; Lahti, R. A.; Lindberg, M.; Carlsson, A. Pharmacologic properties of (-)-3PPP (preclamol) in man. *J. Neural.Transm. Gen. Sect.* **1992,** 88, 165-175.

128. Wang, L. J.; Ree, S. C.; Huang, Y. S.; Hsiao, C. C.; Chen, C. K. Adjunctive effects of aripiprazole on metabolic profiles: comparison of patients treated with olanzapine to patients treated with other atypical antipsychotic drugs. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2013,** 40, 260-266.

129. Sonesson, C.; Lin, C. H.; Hansson, L.; Waters, N.; Svensson, K.; Carlsson, A.; Smith, M. W.; Wikstrom, H. Substituted (S)-phenylpiperidines and rigid congeners as preferential dopamine autoreceptor antagonists: synthesis and structure-activity relationships. *J. Med. Chem.* **1994,** 37, 2735-2753.

130. Pettersson, F.; Ponten, H.; Waters, N.; Waters, S.; Sonesson, C. Synthesis and evaluation of a set of 4-phenylpiperidines and 4-phenylpiperazines as D2 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.

131. Dyhring, T.; Nielsen, E. O.; Sonesson, C.; Pettersson, F.; Karlsson, J.; Svensson, P.; Christophersen, P.; Waters, N. The dopaminergic stabilizers pridopidine (ACR16) and (-)-OSU6162 display dopamine D(2) receptor antagonism and fast receptor dissociation properties. *Eur. J. Pharmacol.* **2010,** 628, 19-26.

132. de Yebenes, J. G.; Landwehrmeyer, B.; Squitieri, F.; Reilmann, R.; Rosser, A.; Barker, R. A.; Saft, C.; Magnet, M. K.; Sword, A.; Rembratt, A.; Tedroff, J. Pridopidine for the treatment of motor function in patients with Huntington's disease (MermaiHD): a phase 3, randomised, double-blind, placebo-controlled trial. *Lancet Neurol.* **2011,** 10, 1049-1057.

133. Lundin, A.; Dietrichs, E.; Haghighi, S.; Goller, M. L.; Heiberg, A.; Loutfi, G.; Widner, H.; Wiktorin, K.; Wiklund, L.; Svenningsson, A.; Sonesson, C.; Waters, N.; Waters, S.; Tedroff, J. Efficacy and safety of the dopaminergic stabilizer Pridopidine (ACR16) in patients with Huntington's disease. *Clin. Neuropharmacol.* **2010,** 33, 260-264.

134. Steensland, P.; Fredriksson, I.; Holst, S.; Feltmann, K.; Franck, J.; Schilström, 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.

135. Carlsson, M. L.; Carlsson, A.; Nilsson, M. Schizophrenia: from dopamine to glutamate and back. *Curr. Med. Chem.* **2004,** 11, 267-277.

136. 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.

137. 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 Neuropsychiatry* **2012,** 24, 266-274.

138. Perregaard, J.; Andersen, K.; Hyttel, J.; Sanchez, C. Selective, centrally acting serotonin 5-HT2 antagonists. 1. 2- and 6-substituted 1-phenyl-3-(4-piperidinyl)-1H-indoles. *J. Med. Chem.* **1992,** 35, 4813-4822.

139. Waldmeier, P. C.; Tipton, K. F.; Bernasconi, R.; Felner, A. E.; Baumann, P. A.; Maitre, L. CGP 4718 A, a new potential antidepressant with a dual mode of action. *Eur. J. Pharmacol.* **1985,** 107, 79-89.

140. Waldmeier, P. C.; Baumann, P. A.; Maitre, L. CGP 6085 A, a new, specific, inhibitor of serotonin uptake: neurochemical characterization and comparison with other serotonin uptake blockers. *J. Pharmacol. Exp. Ther.* **1979,** 211, 42-49.

141. Hoult, J. R.; Paya, M. Pharmacological and biochemical actions of simple coumarins: natural products with therapeutic potential. *Gen. Pharmacol.* **1996,** 27, 713-722.

142. Chen, S.; Cho, M.; Karlsberg, K.; Zhou, D.; Yuan, Y. C. Biochemical and biological characterization of a novel anti-aromatase coumarin derivative. *J. Biol. Chem.* **2004,** 279, 48071-48078.

143. Bruhlmann, C.; Ooms, F.; Carrupt, P. A.; Testa, B.; Catto, M.; Leonetti, F.; Altomare, C.; Carotti, A. Coumarins derivatives as dual inhibitors of acetylcholinesterase and monoamine oxidase. *J. Med. Chem.* **2001,** 44, 3195-3198.

144. Link, K. P. The Discovery of Dicumarol and Its Sequels. *Circulation* **1959,** 19, 97-107.

145. Gnerre, C.; Catto, M.; Leonetti, F.; Weber, P.; Carrupt, P. A.; Altomare, C.; Carotti, A.; Testa, B. Inhibition of monoamine oxidases by functionalized coumarin derivatives: biological activities, QSARs, and 3D-QSARs. *J. Med. Chem.* **2000,** 43, 4747-4758.

146. Rendenbach-Muller, B.; Schlecker, R.; Traut, M.; Weifenbach, H. Synthesis of coumarins as subtype-selective inhibitors of monoamine oxidase. *Bioorg. Med. Chem. Lett.* **1994,** 4, 1195-1198.

147. Helguera, A. M.; Perez-Machado, G.; Cordeiro, M. N.; Borges, F. Discovery of MAO-B Inhibitors - Present Status and Future Directions Part I: Oxygen Heterocycles and Analogs. *Mini Rev. Med. Chem.* **2012,** 12, 907-919.

148. Novaroli, L.; Daina, A.; Favre, E.; Bravo, J.; Carotti, A.; Leonetti, F.; Catto, M.; Carrupt, P. A.; Reist, M. Impact of species-dependent differences on screening, design, and development of MAO B inhibitors. *J. Med. Chem.* **2006,** 49, 6264-6272.

149. Carotti, A.; Carrieri, A.; Chimichi, S.; Boccalini, M.; Cosimelli, B.; Gnerre, C.; Carotti, A.; Carrupt, P.-A.; Testa, B. Natural and synthetic geiparvarins are strong and selective MAO-B inhibitors. synthesis and SAR studies. *Bioorg. Med. Chem. Lett.* **2002,** 12, 3551-3555.

150. Chimenti, F.; Secci, D.; Bolasco, A.; Chimenti, P.; Bizzarri, B.; Granese, A.; Carradori, S.; Yanez, M.; Orallo, F.; Ortuso, F.; Alcaro, S. Synthesis, molecular modeling, and selective inhibitory activity against human monoamine oxidases of 3-carboxamido-7-substituted coumarins. *J. Med. Chem.* **2009,** 52, 1935-1942.

151. Matos, M. J.; Vina, D.; Quezada, E.; Picciau, C.; Delogu, G.; Orallo, F.; Santana, L.; Uriarte, E. A new series of 3-phenylcoumarins as potent and selective MAO-B inhibitors. *Bioorg. Med. Chem. Lett.* **2009,** 19, 3268-3270.

152. Secci, D.; Carradori, S.; Bolasco, A.; Chimenti, P.; Yanez, M.; Ortuso, F.; Alcaro, S. Synthesis and selective human monoamine oxidase inhibition of 3-carbonyl, 3-acyl, and 3-carboxyhydrazido coumarin derivatives. *Eur. J. Med. Chem.* **2011,** 46, 4846-4852.

153. Matos, M. J.; Vazquez-Rodriguez, S.; Uriarte, E.; Santana, L.; Vina, D. MAO inhibitory activity modulation: 3-Phenylcoumarins versus 3-benzoylcoumarins. *Bioorg. Med. Chem. Lett.* **2011,** 21, 4224-4227.

154. Matos, M. J.; Teran, C.; Perez-Castillo, Y.; Uriarte, E.; Santana, L.; Vina, D. Synthesis and study of a series of 3-arylcoumarins as potent and selective monoamine oxidase B inhibitors. *J. Med. Chem.* **2011,** 54, 7127-7137.

155. Pisani, L.; Barletta, M.; Soto-Otero, R.; Nicolotti, O.; Mendez-Alvarez, E.; Catto, M.; Introcaso, A.; Stefanachi, A.; Cellamare, S.; Altomare, C.; Carotti, A. Discovery, Biological Evaluation, and Structure-Activity and -Selectivity Relationships of 6'-Substituted (E)-2-(Benzofuran-3(2H)-ylidene)-N-methylacetamides, a Novel Class of Potent and Selective Monoamine Oxidase Inhibitors. *J. Med. Chem.* **2013,** 56, 2651-2664.

156. Chimenti, F.; Secci, D.; Bolasco, A.; Chimenti, P.; Granese, A.; Befani, O.; Turini, P.; Alcaro, S.; Ortuso, F. Inhibition of monoamine oxidases by coumarin-3-acyl derivatives: biological activity and computational study. *Bioorg. Med. Chem. Lett.* **2004,** 14, 3697-3703.

157. Pisani, L.; Muncipinto, G.; Miscioscia, T. F.; Nicolotti, O.; Leonetti, F.; Catto, M.; Caccia, C.; Salvati, P.; Soto-Otero, R.; Mendez-Alvarez, E.; Passeleu, C.; Carotti, A. Discovery of a novel class of potent coumarin monoamine oxidase B inhibitors: development and biopharmacological profiling of 7-[(3-chlorobenzyl)oxy]-4-[(methylamino)methyl]-2H-chromen-2-one methanesulfonate (NW-1772) as a highly potent, selective, reversible, and orally active monoamine oxidase B inhibitor. *J. Med. Chem.* **2009,** 52, 6685-6706.

158. Binda, C.; Wang, J.; Pisani, L.; Caccia, C.; Carotti, A.; Salvati, P.; Edmondson, D. E.; Mattevi, A. Structures of human monoamine oxidase B complexes with selective noncovalent inhibitors: safinamide and coumarin analogs. *J. Med. Chem.* **2007,** 50, 5848-5852.

159. Freter, K. 3-cycloalkenylindoles. *J. Org. Chem.* **1975,** 40, 2525–2529.

160. Dillard, R. D.; Bach, N. J.; Draheim, S. E.; Berry, D. R.; Carlson, D. G.; Chirgadze, N. Y.; Clawson, D. K.; Hartley, L. W.; Johnson, L. M.; Jones, N. D.; McKinney, E. R.; Mihelich, E. D.; Olkowski, J. L.; Schevitz, R. W.; Smith, A. C.; Snyder, D. W.; Sommers, C. D.; Wery, J. P. Indole inhibitors of human nonpancreatic secretory phospholipase A2. 2. Indole-3-acetamides with additional functionality. *J. Med. Chem.* **1996,** 39, 5119-5136.

161. Clark, D. R.; Muchowski, J. M.; Fisher, E. L.; Flippin, L. A.; Repke, B. D.; Souchet, M. Preparation of indoles and oxindoles from N-(tert-butoxycarbonyl)-2-alkylanilines. *Synthesis* **1991**, 871-877.

162. Black, D. S. C.; Kumar, N.; Wong, L. C. H. Synthesis of 4,6-Dimethoxyindoles. *Australian J. Chem.* **1986,** 39, 15-20.

163. Joule, J. A.; Mills, K. *Heterocyclic Chemistry*. 4th ed.; Blackwell Science Ltd: 2000; p 324-379.

164. Houlihan, W. J.; Parrino, V. A.; Uike, Y. Lithiation of N-(2-alkylphenyl)alkanamides and related compounds. A modified Madelung indole synthesis. *J. Org. Chem.* **1981,** 46, 4511-4515.

165. Qu, B.; Collum, D. B. Mechanism of Acylation of Lithium Phenylacetylide with a Weinreb Amide. *J. Org. Chem.* **2006,** 71, 7117-7119.

166. Nahm, S.; Weinreb, S. M. N-methoxy-n-methylamides as effective acylating agents. *Tetrah. Lett.* **1981,** 22, 3815-3818.

167. Thangadurai, A.; Minu, M.; Wakode, S.; Agrawal, S.; Narasimhan, B. Indazole: a medicinally important heterocyclic moiety. *Med. Chem. Res.* **2012,** 21, 1509-1523.

168. Schmidt, A.; Beutler, A.; Snovydovych, B. Recent Advances in the Chemistry of Indazoles. *Eur. J. Org. Chem.* **2008,** 2008, 4073-4095.

169. Welch, W. M.; Hanau, C. E.; Whalen, W. M. A novel synthesis of 3-substituted indazole derivatives. *Synthesis* **1992**, 937-939.

170. Takeuchi, K.; Kohn, T. J.; Honigschmidt, N. A.; Rocco, V. P.; Spinazze, P. G.; Koch, D. J.; Nelson, D. L.; Wainscott, D. B.; Ahmad, L. J.; Shaw, J.; Threlkeld, P. G.; Wong, D. T. Advances toward new antidepressants beyond SSRIs: 1-aryloxy-3-piperidinylpropan-2-ols with dual 5-HT1A receptor antagonism/SSRI activities. Part 1. *Bioorg. Med. Chem. Lett.* **2003,** 13, 1903-1905.

171. Rocco, V. P.; Spinazze, P. G.; Kohn, T. J.; Honigschmidt, N. A.; Nelson, D. L.; Bradley Wainscott, D.; Ahmad, L. J.; Shaw, J.; Threlkeld, P. G.; Wong, D. T.; Takeuchi, K. Advances toward new antidepressants beyond SSRIs: 1-aryloxy-3-piperidinylpropan-2-ols with dual 5-HT1A receptor antagonism/SSRI activities. Part 4. *Bioorg. Med. Chem. Lett.* **2004,** 14, 2653-2656.

172. Basavaiah, D.; Rao, A. J.; Satyanarayana, T. Recent Advances in the Baylis-Hillman Reaction and Applications. *Chem. Rev.* **2003,** 103, 811-892.

173. Narender, P.; Srinivas, U.; Ravinder, M.; Ananda Rao, B.; Ramesh, C.; Harakishore, K.; Gangadasu, B.; Murthy, U. S. N.; Jayathirtha Rao, V. Synthesis of multisubstituted quinolines from Baylis-Hillman adducts obtained from substituted 2-chloronicotinaldehydes and their antimicrobial activity. *Bioorg. Med. Chem.* **2006,** 14, 4600-4609.

174. Kaye, P. T. The Baylis-Hillman entrée to heterocyclic systems—the Rhodes contribution. *South African J. Sci.* **2004,** 100, 545-548.

175. Kaye, P. T.; Musa, M. A. Application of Baylis-Hillman methodology in the synthesis of coumarin derivatives. *Synth. Commun.* **2003,** 33, 1755-1770.

176. Basavaiah, D.; Veeraraghavaiah, G. The Baylis–Hillman reaction: a novel concept for creativity in chemistry. *Chem. Soc. Rev.* **2012,** 41, 68-78.

177. Aldred, R.; Johnston, R.; Levin, D.; Neilan, J. Magnesium-mediated ortho-specific formylation and formaldoximation of phenols. *J. Chem. Soc. Perkin Trans 1: Org. and Bio-Org. Chem. (1972-1999)* **1994,** 13, 1823-1831.

178. Ahn, S. H.; Lim, H. N.; Lee, K. J. Application of the acetate of Baylis-Hillman adducts of salicylaldehydes in the synthesis of methyl 2-oxo-2,3-dihydrobenzo[b]oxepine-4-carboxylates. *J. Heterocyclic. Chem.* **2008,** 45, 1701-1706.

179. Aggarwal, V. K.; Emme, I.; Fulford, S. Y. Correlation between pKa and Reactivity of Quinuclidine-Based Catalysts in the Baylis-Hillman Reaction: Discovery of Quinuclidine as Optimum Catalyst Leading to Substantial Enhancement of Scope. *J. Org. Chem.* **2003,** 68, 692-700.

180. Geneste, H.; Schäfer, B. 2-Substituted 4-(trifluoromethyl)phenols by directed ortho-lithiation. *Synthesis* **2001,** 15, 2259-2262.

181. Dyer, R. G.; Turnbull, K. D. Hydrolytic Stabilization of Protected p-Hydroxybenzyl Halides Designed as Latent Quinone Methide Precursors. *J. Org. Chem.* **1999,** 64, 7988-7995.

182. Sakai, T. T.; Santi, D. V. Hydrolysis of hydroxybenzotrifluorides and fluorinated uracil derivatives. General mechanism for carbon-fluorine bond labilization. *J. Med. Chem.* **1973,** 16, 1079-1084.

183. Kohen, R.; Metcalf, M. A.; Khan, N.; Druck, T.; Huebner, K.; Lachowicz, J. E.; Meltzer, H. Y.; Sibley, D. R.; Roth, B. L.; Hamblin, M. W. Cloning, characterization, and chromosomal localization of a human 5-HT6 serotonin receptor. *J. Neurochem.* **1996,** 66, 47-56.

184. Stenfors, C.; Ross, S. B. Changes in extracellular 5-HIAA concentrations as measured by in vivo microdialysis technique in relation to changes in 5-HT release. *Psychopharmacol. (Berl)* **2004,** 172, 119-128.

185. Grandy, D. K.; Marchionni, M. A.; Makam, H.; Stofko, R. E.; Alfano, M.; Frothingham, L.; Fischer, J. B.; Burke-Howie, K. J.; Bunzow, J. R.; Server, A. C. Cloning of the cDNA and gene for a human D2 dopamine receptor. *Proceedings of the National Academy of Sciences* **1989,** 86, 9762-9766.

186. Wreggett, K. A.; De Lean, A. The ternary complex model. Its properties and application to ligand interactions with the D2-dopamine receptor of the anterior pituitary gland. *Mol. Pharmacol.* **1984,** 26, 214-227.

187. Hamblin, M. W.; Leff, S. E.; Creese, I. Interactions of agonists with D-2 dopamine receptors: evidence for a single receptor population existing in multiple agonist affinity-states in rat striatal membranes. *Biochem. Pharmacol.* **1984,** 33, 877-887.

188. 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. *Mol. Pharmacol.* **1992,** 42, 432-439.

189. Tatsumi, M.; Jansen, K.; Blakely, R. D.; Richelson, E. Pharmacological profile of neuroleptics at human monoamine transporters. *Eur. J. Pharmacol.* **1999,** 368, 277-283.

190. Cesura, A. M.; Bos, M.; Galva, M. D.; Imhof, R.; Da Prada, M. Characterization of the binding of [3H]Ro 41-1049 to the active site of human monoamine oxidase-A. *Mol. Pharmacol.* **1990,** 37, 358-366.

191. Yan, Z.; Caldwell, G. W.; Zhao, B.; Reitz, A. B. A high-throughput monoamine oxidase inhibition assay using liquid chromatography with tandem mass spectrometry. *Rapid Commun. Mass. Spectr.* **2004,** 18, 834-840.

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

193. Burki, H. R.; Ruch, W.; Asper, H. Effects of clozapine, thioridazine, perlapine and haloperidol on the metabolism of the biogenic amines in the brain of the rat. *Psychopharmacol.* **1975,** 41, 27-33.

194. Joyce, J. N. Multiple dopamine receptors and behavior. *Neurosci. Biobehav. Rev.* **1983,** 7, 227-256.

195. Kongsamut, S.; Kang, J.; Chen, X. L.; Roehr, J.; Rampe, D. A comparison of the receptor binding and HERG channel affinities for a series of antipsychotic drugs. *Eur. J. Pharmacol.* **2002,** 450, 37-41.

196. 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.

197. 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.

198. Bartoszyk, G. D.; Van Amsterdam, C.; Greiner, H. E.; Rautenberg, W.; Russ, H.; Seyfried, C. A. Sarizotan, a serotonin 5-HT1A receptor agonist and dopamine receptor ligand. 1. Neurochemical profile. *J. Neural. Transm.* **2004,** 111, 113-126.

199. Heinrich, T.; Bottcher, H.; Gericke, R.; Bartoszyk, G. D.; Anzali, S.; Seyfried, C. A.; Greiner, H. E.; Van Amsterdam, C. Synthesis and structure--activity relationship in a class of indolebutylpiperazines as dual 5-HT(1A) receptor agonists and serotonin reuptake inhibitors. *J. Med. Chem.* **2004,** 47, 4684-4692.

200. Glennon, R. A.; Bondarev, M.; Lee, M. 5-HT6 serotonin receptor binding of indolealkylamines: A preliminary structure-affinity investigation. *Med. Chem. Res.* **1999,** 9, 108-117.

201. Unsworth, C. D.; Molinoff, P. B. Characterization of a 5-hydroxytryptamine receptor in mouse neuroblastoma N18TG2 cells. *J. Pharmacol. Exp. Ther.* **1994,** 269, 246-255.

202. Russell, M. G.; Dias, R. Memories are made of this (perhaps): a review of serotonin 5-HT(6) receptor ligands and their biological functions. *Curr. Top. Med. Chem.* **2002,** 2, 643-654.

203. Roth, B. L.; Craigo, S. C.; Choudhary, M. S.; Uluer, A.; Monsma, F. J., Jr.; Shen, Y.; Meltzer, H. Y.; Sibley, D. R. Binding of typical and atypical antipsychotic agents to 5-hydroxytryptamine-6 and 5-hydroxytryptamine-7 receptors. *J. Pharmacol. Exp. Ther.* **1994,** 268, 1403-1410.

204. Nikiforuk, A.; Kos, T.; Wesolowska, A. The 5-HT6 receptor agonist EMD 386088 produces antidepressant and anxiolytic effects in rats after intrahippocampal administration. *Psychopharmacol. (Berlin)* **2011,** 217, 411-418.

205. Nikiforuk, A.; Fijal, K.; Potasiewicz, A.; Popik, P.; Kos, T. The 5-hydroxytryptamine (serotonin) receptor 6 agonist EMD 386088 ameliorates ketamine-induced deficits in attentional set shifting and novel object recognition, but not in the prepulse inhibition in rats. *J. Psychopharmacol.* **2013,** 27, 469-476.

206. Woods, S.; Clarke, N. N.; Layfield, R.; Fone, K. C. 5-HT(6) receptor agonists and antagonists enhance learning and memory in a conditioned emotion response paradigm by modulation of cholinergic and glutamatergic mechanisms. *Br. J. Pharmacol.* **2012,** 167, 436-449.

207. Di Santo, R.; Costi, R.; Roux, A.; Artico, M.; Befani, O.; Meninno, T.; Agostinelli, E.; Palmegiani, P.; Turini, P.; Cirilli, R.; Ferretti, R.; Gallinella, B.; La Torre, F. Design, synthesis, and biological activities of pyrrolylethanoneamine derivatives, a novel class of monoamine oxidases inhibitors. *J. Med. Chem.* **2005,** 48, 4220-4223.

208. Timms, G. H.; Boot, J. R.; Broadmore, R. J.; Carney, S. L.; Cooper, J.; Findlay, J. D.; Gilmore, J.; Mitchell, S.; Moore, N. A.; Pullar, I.; Sanger, G. J.; Tomlinson, R.; Tree, B. B.; Wedley, S. SAR development of a selective 5-HT1D antagonist/serotonin reuptake inhibitor lead using rapid parallel synthesis. *Bioorg. Med. Chem. Lett.* **2004,** 14, 2469-2472.

209. Forbes, I. T.; Cooper, D. G.; Dodds, E. K.; Douglas, S. E.; Gribble, A. D.; Ife, R. J.; Lightfoot, A. P.; Meeson, M.; Campbell, L. P.; Coleman, T.; Riley, G. J.; Thomas, D. R. Identification of a novel series of selective 5-HT7 receptor antagonists. *Bioorg. Med. Chem. Lett.* **2003,** 13, 1055-1058.

210. Hrib, N. J.; Jurcak, J. G.; Burgher, K. L.; Conway, P. G.; Hartman, H. B.; Kerman, L. L.; Roehr, J. E.; Woods, A. T. Benzisoxazole- and benzisothiazole-3-carboxamides as potential atypical antipsychotic agents. *J. Med. Chem.* **1994,** 37, 2308-2314.

211. Zhang, D.; Kohlman, D.; Krushinski, J.; Liang, S.; Ying, B. P.; Reilly, J. E.; Dinn, S. R.; Wainscott, D. B.; Nutter, S.; Gough, W.; Nelson, D. L.; Schaus, J. M.; Xu, Y. C. Design, synthesis and evaluation of bicyclic benzamides as novel 5-HT1F receptor agonists. *Bioorg. Med. Chem. Lett.* **2004,** 14, 6011-6016.

212. Andersen, K.; Perregaard, J.; Arnt, J.; Nielsen, J. B.; Begtrup, M. Selective, centrally acting serotonin 5-HT2 antagonists. 2. Substituted 3-(4-fluorophenyl)-1H-indoles. *J. Med. Chem.* **1992,** 35, 4823-4831.

213. Pettersson, F.; Svensson, P.; Waters, S.; Waters, N.; Sonesson, C. Synthesis and Evaluation of a Set of Para-Substituted 4-Phenylpiperidines and 4-Phenylpiperazines as Monoamine Oxidase (MAO) Inhibitors. *J. Med. Chem.* **2012,** 55, 3242-3249.

214. Huotari, M.; Passlin, M.; Nordberg, H. L.; Forsberg, M.; Kotisaari, S.; Tuomisto, L.; Shintani, F.; Tanaka, K. F.; Reenila, I.; Laitinen, K.; Mannisto, P. T. Effect of intracerebral 6-nitronoradrenaline, an endogenous catechol-O-methyltransferase (COMT) inhibitor, on striatal dopamine metabolism in anaesthetised rats. *J. Neurosci. Methods* **2001,** 109, 47-52.

215. Zetterstrom, T.; Sharp, T.; Collin, A. K.; Ungerstedt, U. In vivo measurement of extracellular dopamine and DOPAC in rat striatum after various dopamine-releasing drugs; implications for the origin of extracellular DOPAC. *Eur. J. Pharmacol.* **1988,** 148, 327-334.

216. Wold, S.; Sjöström, M.; Eriksson, L. PLS-regression: a basic tool of chemometrics. *Chemometr. Intell. Lab. Syst.* **2001,** 58, 109-130.

217. Wold, S.; Trygg, J.; Berglund, A.; Antti, H. Some recent developments in PLS modeling. *Chemometr. Intell. Lab. Syst.* **2001,** 58, 131-150.

218. Teismann, P.; Ferger, B. In vivo effects of the putative cognitive enhancer KA-672.HCl in comparison with 8-hydroxy-2-(DI-N-propylamino) tetralin and haloperidol on dopamine, 3,4-dihydroxypheny-lacetic acid, serotonin and 5-hydroxyindoleacetic acid levels in striatal and cortical brain regions. *Prog. Neuro-Psychopharmacol. Biol. Psych.* **2000,** 24, 337-348.

219. Haefely, W.; Burkard, W. P.; Cesura, A. M.; Kettler, R.; Lorez, H. P.; Martin, J. R.; Richards, J. G.; Scherschlicht, R.; Da Prada, M. Biochemistry and pharmacology of moclobemide, a prototype RIMA. *Psychopharmacol. (Berlin)* **1992,** 106 Suppl., S6-14.

220. 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. *J. Med. Chem.* **1997,** 40, 4235-4256.

84

221. Waldmeier, P. C.; Delini-Stula, A.; Maitre, L. Preferential deamination of dopamine by an A type monoamine oxidase in rat brain. *Naunyn Schmiedeberg's Arch. Pharmacol.* **1976,** 292, 9-14.

222. Thull, U.; Testa, B. Screening of unsubstituted cyclic compounds as inhibitors of monoamine oxidases. *Biochem. Pharmacol.* **1994,** 47, 2307-2310.

223. Ross, P. C.; Burkman, A. M. Inhibition of prolactin release from anterior pituitary lactotrophs in culture by sulfur-containing analogs of dopamine. *Proc. Soc. Exp. Biol. Med.* **1988,** 188, 87-91.

224. Miller, D. D.; Harrold, M.; Wallace, R. A.; Wallace, L. J.; Uretsky, N. J. Dopaminergic drugs in the cationic form interact with D2 dopamine receptors. *Trends Pharmacol. Sci.* **1988,** 9, 282-284.

225. 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. USA* **2008,** 105, 5739-5744.

226. Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Mainz, D. T.; Repasky, M. P.; Knoll, E. H.; Shelley, M.; Perry, J. K.; Shaw, D. E.; Francis, P.; Shenkin, P. S. Glide: A New Approach for Rapid, Accurate Docking and Scoring. 1. Method and Assessment of Docking Accuracy. *J. Med. Chem.* **2004,** 47, 1739-1749.

227. Friesner, R. A.; Murphy, R. B.; Repasky, M. P.; Frye, L. L.; Greenwood, J. R.; Halgren, T. A.; Sanschagrin, P. C.; Mainz, D. T. Extra Precision Glide: Docking and Scoring Incorporating a Model of Hydrophobic Enclosure for Protein-Ligand Complexes. *J. Med. Chem.* **2006,** 49, 6177-6196.

228. Edmondson, D. E.; Binda, C.; Wang, J.; Upadhyay, A. K.; Mattevi, A. Molecular and mechanistic properties of the membrane-bound mitochondrial monoamine oxidases. *Biochem.* **2009,** 48, 4220-4230.

229. 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. USA* **2005,** 102, 12684-12689.

230. 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.

231. La Regina, G.; Silvestri, R.; Artico, M.; Lavecchia, A.; Novellino, E.; Befani, O.; Turini, P.; Agostinelli, E. New Pyrrole Inhibitors of Monoamine Oxidase: Synthesis, Biological Evaluation, and Structural Determinants of MAO-A and MAO-B Selectivity. *J. Med. Chem.* **2007,** 50, 922-931.

232. McInnes, C. Virtual screening strategies in drug discovery. *Curr. Opin. Chem. Biol.* **2007,** 11, 494-502.

233. Duffy, B. C.; Zhu, L.; Decornez, H.; Kitchen, D. B. Early phase drug discovery: cheminformatics and computational techniques in identifying lead series. *Bioorg. Med. Chem.* **2012,** 20, 5324-5342.

234. Lopez-Vallejo, F.; Giulianotti, M. A.; Houghten, R. A.; Medina-Franco, J. L. Expanding the medicinally relevant chemical space with compound libraries. *Drug Discov. Today* **2012,** 17, 718-726.

235. Ly, S.; Pishdari, B.; Lok, L. L.; Hajos, M.; Kocsis, B. Activation of 5-HT6 receptors modulates sleep-wake activity and hippocampal theta oscillation. *ACS Chem. Neurosci.* **2013,** 4, 191-199.

236. Godinez-Chaparro, B.; Lopez-Santillan, F. J.; Orduna, P.; Granados-Soto, V. Secondary mechanical allodynia and hyperalgesia depend on descending facilitation mediated by spinal 5-HT(4), 5-HT(6) and 5-HT(7) receptors. *Neurosci.* **2012,** 222, 379-391.

237. Pratt, W. E.; Schall, M. A.; Choi, E. Selective serotonin receptor stimulation of the medial nucleus accumbens differentially affects appetitive motivation for food on a progressive ratio schedule of reinforcement. *Neurosci. Lett.* **2012,** 511, 84-88.

238. Yun, H. M.; Rhim, H. 5-HT6 receptor ligands, EMD386088 and SB258585, differentially regulate 5-HT6 receptor-independent events. *Toxicol. In Vitro* **2011,** 25, 2035-2040.

239. Zhang, X.; Andren, P. E.; Glennon, R. A.; Svenningsson, P. Distribution, level, pharmacology, regulation, and signaling of 5-HT6 receptors in rats and marmosets with special reference to an experimental model of parkinsonism. *J. Comp. Neurol.* **2011,** 519, 1816-1827.

240. Castaneda-Corral, G.; Rocha-Gonzalez, H. I.; Araiza-Saldana, C. I.; Ambriz-Tututi, M.; Vidal-Cantu, G. C.; Granados-Soto, V. Role of peripheral and spinal 5-HT6 receptors according to the rat formalin test. *Neurosci.* **2009,** 162, 444-452.

241. Meneses, A.; Perez-Garcia, G.; Liy-Salmeron, G.; Flores-Galvez, D.; Castillo, C.; Castillo, E. The effects of the 5-HT(6) receptor agonist EMD and the 5-HT(7) receptor agonist AS19 on memory formation. *Behav. Brain. Res.* **2008,** 195, 112-119.

242. Millan, M. J. The role of monoamines in the actions of established and "novel" antidepressant agents: a critical review. *Eur. J. Pharmacol.* **2004,** 500, 371-384.

243. Dunlop, B. W.; Nemeroff, C. B. The role of dopamine in the pathophysiology of depression. *Arch. Gen. Psychiatry* **2007,** 64, 327-337.

244. Porcelli, S.; Drago, A.; Fabbri, C.; Serretti, A. Mechanisms of antidepressant action: an integrated dopaminergic perspective. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2011,** 35, 1532-1543.

245. Di Giovanni, G.; Esposito, E.; Di Matteo, V. Role of serotonin in central dopamine dysfunction. *CNS Neurosci. Ther.* **2010,** 16, 179-194.

246. Lladó-Pelfort, L.; Assié, M. B.; Newman-Tancredi, A.; Artigas, F.; Celada, P. Preferential in vivo action of F15599, a novel 5-HT1A receptor agonist, at postsynaptic 5-HT1A receptors. *Br. J. Pharmacol.* **2010,** 160, 1929-1940.

247. Millan, M. J.; Gobert, A.; Girardon, S.; Dekeyne, A. Citalopram elicits a discriminative stimulus in rats at a dose selectively increasing extracellular levels of serotonin vs. dopamine and noradrenaline. *Eur. J. Pharmacol.* **1999,** 364, 147-150.

248. Gobert, A.; Rivet, J. M.; Cistarelli, L.; Melon, C.; Millan, M. J. Buspirone modulates basal and fluoxetine-stimulated dialysate levels of dopamine, noradrenaline and serotonin in the frontal cortex of freely moving rats: activation of serotonin1A receptors and blockade of alpha2-adrenergic receptors underlie its actions. *Neurosci.* **1999,** 93, 1251-1262.

249. Sonesson, C.; Waters, N.; Svensson, K.; Carlsson, A.; Smith, M. W.; Piercey, M. F.; Meier, E.; Wikstrom, H. Substituted 3-phenylpiperidines: new centrally acting dopamine autoreceptor antagonists. *J. Med. Chem.* **1993,** 36, 3188-3196.

250. Svensson, K.; Johansson, A. M.; Magnusson, T.; Carlsson, A. (+)-AJ 76 and (+)-UH 232: central stimulants acting as preferential dopamine autoreceptor antagonists. *Naunyn Schmiedebergs Arch. Pharmacol.* **1986,** 334, 234-245.

251. Culpepper, L. The Use of Monoamine Oxidase Inhibitors in Primary Care. *J. Clin. Psychiatry* **2012,** 73, 37-41.

252. Fowler, J. S.; Logan, J.; Azzaro, A. J.; Fielding, R. M.; Zhu, W.; Poshusta, A. K.; Burch, D.; Brand, B.; Free, J.; Asgharnejad, M.; Wang, G. J.; Telang, F.; Hubbard, B.; Jayne, M.; King, P.; Carter, P.; Carter, S.; Xu, Y.; Shea, C.; Muench, L.; Alexoff, D.; Shumay, E.; Schueller, M.; Warner, D.; Apelskog-Torres, K. Reversible inhibitors of monoamine oxidase-A (RIMAs): robust, reversible inhibition of human brain MAO-A by CX157. *Neuropsychopharmacol.* **2010,** 35, 623-631.

253. Jouvent, R.; Le Houezec, J.; Payan, C.; Mikkelsen, H.; Fermanian, J.; Millet, V.; Dufour, H. Dimensional assessment of onset of action of antidepressants: a comparative study of moclobemide vs. clomipramine in depressed patients with blunted affect and psychomotor retardation. *Psychiatry Res.* **1998,** 79, 267-275.

254. Colzi, A.; d'Agostini, F.; Cesura, A. M.; Da Prada, M. Brain microdialysis in rats: a technique to reveal competition in vivo between endogenous dopamine and moclobemide, a RIMA antidepressant. *Psychopharmacol. (Berlin)* **1992,** 106 Suppl., S17-20.

255. Blair, J. B.; Kurrasch-Orbaugh, D.; Marona-Lewicka, D.; Cumbay, M. G.; Watts, V. J.; Barker, E. L.; Nichols, D. E. Effect of Ring Fluorination on the Pharmacology of Hallucinogenic Tryptamines. *J. Med. Chem.* **2000,** 43, 4701-4710.

Appendices

Appendix 1: Chemistry section and experimental part to Paper I

Chemistry. The different 2-alkyl-3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole derivatives (**15-21**, **22**, **28**, Scheme A1) (compound numbering refers to that in Paper I) were synthesized by acid-catalyzed condensation between a 2-alkyl-1*H*-indoles and 4-piperidone/tropinone/1-benzylpiperidin-4-one in 25-98% yield.[159] The 2-alkyl-1*H*-indoles substituted with an ethyl, *n*-propyl or *iso*-propyl group in the 2-position, were synthesized according to Scheme A1 using an improved Madelung synthesis. The corresponding 2-methyl analogs 5-chloro-2-methyl-1*H*-indole (**41**) and 5-methoxy-2-methyl-1*H*-indole (**42**) were commercially available.[160, 161] The synthesis starts with Boc protection of commercially available 2-methylanilines (4-chloro and 4-methoxy) to give their corresponding Boc protected amines **31** and **32** in approx. 70% yield. Condensation of **31** and **32** with different *N*-methoxy-*N*-methyl-alkylamides (**43-45**) gave ketones **33-36** in moderate yields (29-67%). Treatment with trifluoroacetic acid afforded cyclization and deprotection to afford 2-alkyl-1*H*-indoles **37-40** in moderate yields (24-70%, Scheme A1). Alkylation of the tetrahydropyridine nitrogen was performed by reductive amination with glacial acetic acid, formaldehyde and sodium triacetoxyborohydride to obtain the *N*-methyl derivative **24** in good yield (84%). The homologous ethyl, *n*-propyl and *n*-butyl derivatives were synthesized in good yields with K_2CO_3 and the appropriate alkyl halide in acetonitrile (**25-27** and **29**, 57-98%, Scheme A1). In addition, 5-methoxy-2-ethyl-3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole (**15**) was reduced to the corresponding piperidine derivative **23** with ammonium formate and Pd/C in 43% yield. Acylation of 5-methoxy-2-ethyl-1*H*-indole (**37**, Scheme A2) and 5-chloro-2-methyl-1*H*-indole (**41**) by treatment of oxalyl chloride gave the corresponding acid chloride intermediates,[255] which were directly reacted with an excess of *N,N,-*dimethylamine to give **46** (93% yield) and **47** (40% yield). The target 2-alkyltryptamines (**4**, **30**) were synthesized by subsequent reduction with 2 equiv. $LiAlH_4$ in moderate to good yields (44-95%). The synthesis of compound **4** has been published earlier using an alkylation method starting from 5-methoxy-*N,N*-dimethyltryptamine.[91]

Scheme A1.[a]
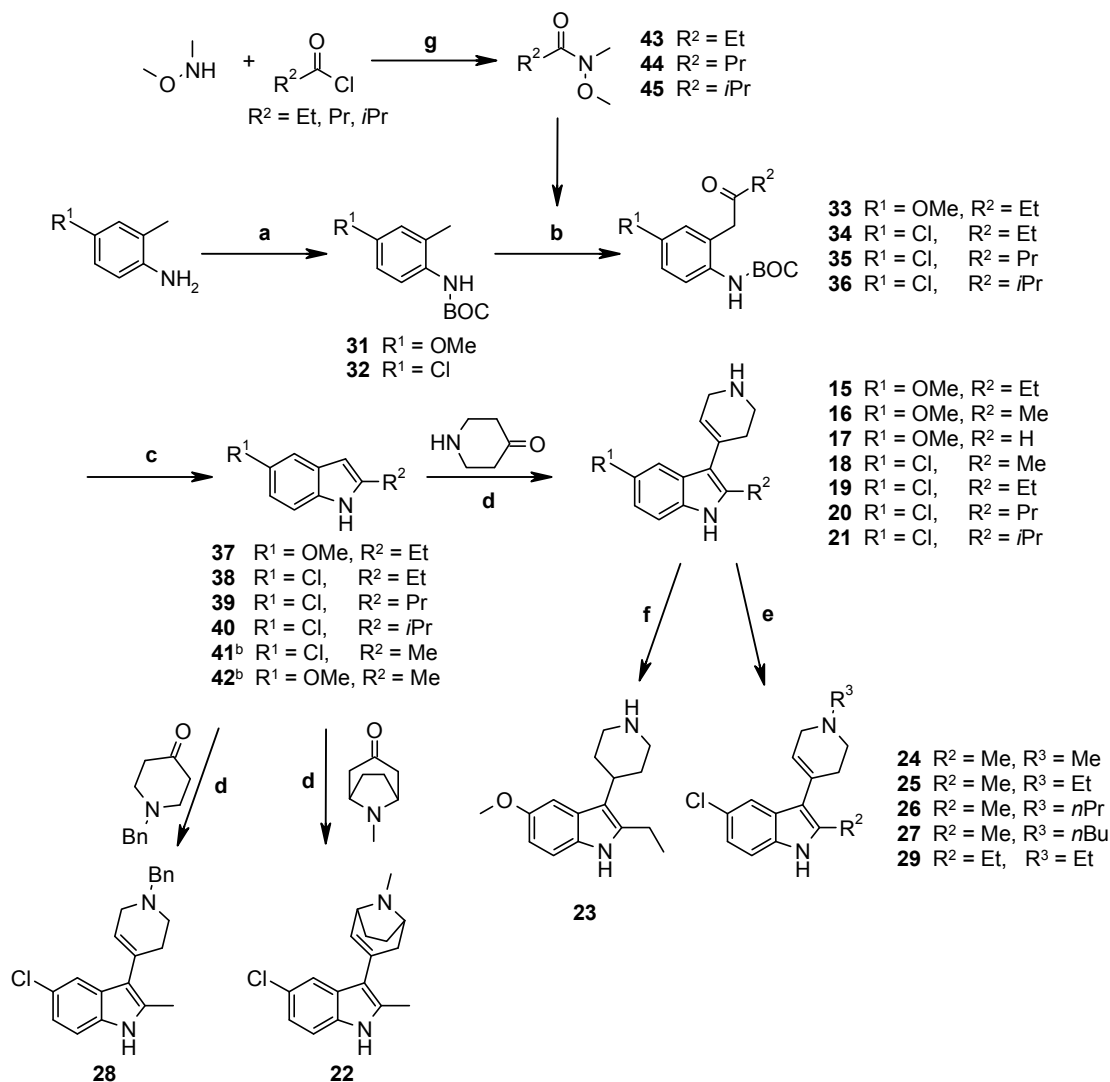
[a]*Reagents and conditions*: (a) (*t*-BuO₂C)₂O, THF, heat; (b) 2 equiv. *sec*-BuLi, R₂CON(OMe)Me (**43-45**), THF, -40 °C to rt; (c) trifluoroacetic acid, CH₂Cl₂; (d) H₃PO₄, acetic acid, 80 °C; (e) NaB(OAc)₃H, formaldehyde, acetic acid, THF or R³-X, K₂CO₃, acetonitrile; (f) Pd/C, NH₄HCO₂, EtOH; (g) 2 equiv. triethylamine, CH₂Cl₂, 0 °C to rt. [b]Commercially available.

Scheme A2.[a]
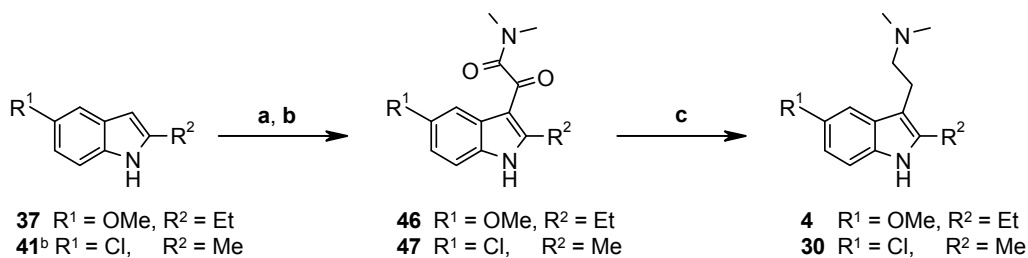
[a]*Reagents and conditions*: (a) oxalyl chloride, diethyl ether 0 °C to rt; (b) *N,N*-dimethylamine, THF, rt (c) LiAlH₄, THF, 60 °C. [b]Commercially available.

Materials and methods. All ^{1}H NMR and ^{13}C NMR experiments were performed on a Varian 300 MHz spectrometer (Varian, Darmstadt, Germany). Chemical shifts are reported as δ (ppm) relative to tetramethylsilane (TMS) as internal standard. The following abbreviations are used: singlet (s), doublet (d), doublet of doublet (dd), triplet (t), quadruplet (q), multiplet (m), broad singlet (br s). Electrospray ionization mass spectra (ESIMS) were recorded on Agilent 1200 Series Liquid Chromatography/Mass Selective Detector (Agilent Technologies, Stockholm, Sweden). Low resolution mass spectra (EI, 70 eV) were recorded on HP5700 mass detector interfaced with a HP 5970 A gas chromatograph (Agilent Technologies, Stockholm, Sweden) equipped with a fused silica column HP-1. Melting points were determined using a Büchi 545 instrument and are uncorrected (Kebo lab, Göteborg, Sweden). Elemental analyses (C, H, N) were performed by Merck KGaA (Darmstadt, Germany). All purifications were performed using a Flash Master II automated flash chromatography system (Biotage, Stockholm, Sweden), equipped with 20 g columns packed with E. Merck silica gel 60 (0.040–0.063 mm) using gradient solvent system isooctane/ethyl acetate/methanol. The starting materials 5-chloro-2-methyl-1*H*-indole (**41**) and 5-methoxy-2-methyl-1*H*-indole (**42**) were purchased from commercial suppliers and were used without purification. The purity of all target compounds was assessed to be greater than 95% by elemental analysis (C, H, N) or HPLC.

General procedure for the synthesis of the 2-Methyl-3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole analogs (15, 16, 18-22, 28). The 2-methyl-1*H*-indole derivative (0.5–6 mmol, 1 equiv.) was stirred at 80 °C in acetic acid (2 mL/1 mmol), then 4-piperidone hydrochloride hydrate/tropinone/1-benzylpiperidin-4-one (1.5–18 mmol, 3 equiv.) and 1 M H_3PO_4 (1 mL/1 mmol) were added. After 1–2 h, the mixture was poured into ice/ammonia, and extracted with ethyl acetate (3×25 mL). The combined organic layers were washed with water and brine, dried, and concentrated *in vacuo* to give the title compounds. The crude products were purified by silica gel column chromatography (ethyl acetate-methanol, gradient) and most of them were converted to the corresponding salts by dissolving the free base in methanol or ethanol and adding one equiv. of oxalic acid or ethanolic HCl solution. The solvent was removed and azeotroped with absolute ethanol *in vacuo* followed by recrystallization from appropriate solvents.

2-Ethyl-5-methoxy-3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole (15). The product was obtained in 74% yield from **37**. ESIMS: *m/z* 257.0 (M + H)$^+$. ^{1}H NMR (CD$_3$OD) δ 1.26 (t, *J* = 7.5 Hz, 3H), 2.41-2.43 (m, 2H), 2.75 (q, *J* = 7.5 Hz, 2H), 3.00 (t, *J* = 5.7 Hz, 2H), 3.42-3.43 (m, 2H), 3.77 (s, 3H), 5.64 (s, 1H), 6.69 (dd, *J* = 8.7, 2.7 Hz, 1H), 6.97 (d, *J* = 2.4 Hz, 1H), 7.14-7.17 (m, 1H). ^{13}C NMR (CD$_3$OD) δ 15.05, 20.98, 30.97, 43.93, 45.56, 56.41, 102.49, 111.14, 112.05, 114.96, 124.64, 129.18, 132.24, 132.78, 139.34, 154.92. The amine was converted to the oxalate salt and recrystallized in methanol/diethyl ether, mp 175-176 °C. Anal. (C$_{16}$H$_{20}$N$_2$O·C$_2$H$_2$O$_4$·1/3H$_2$O) C, H, N.

5-Methoxy-2-methyl-3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole (16). The product was obtained in 98% yield from **42**. ESIMS: *m/z* 243.1 (M + H)$^+$. ^{1}H NMR (CD$_3$OD) δ 2.36 (s, 3H), 2.44 (s, 2H), 3.02 (t, *J* = 5.7 Hz, 2H), 3.45 (s, 2H), 3.77 (s, 3H), 5.65 (s, 1H), 6.67 (dd, *J* = 8.7, 2.4 Hz,

1H), 6.97 (s, 1H), 7.10 (d, J = 9 Hz, 1H). NMR (CD$_3$OD) δ 12.67, 30.75, 43.96, 45.59, 56.47, 102.62, 111.04, 111.91, 115.58, 124.44, 129.28, 132.23, 132.80, 133.36, 155.01. Conversion to the HCl salt and recrystallization in methanol/diethyl ether gave **16** as a brown powder, mp 226–228 °C. Anal: (C$_{15}$H$_{18}$N$_2$O·HCl·1/3H$_2$O) C, H, N.

5-Chloro-2-methyl-3-(1,2,3,6-tetrahydropyridin-4-yl)-1H-indole (**18**). The product was obtained in 81% yield from **41**. ESIMS: m/z 247.1 (M + H)$^+$. ^{1}H NMR (CD$_3$OD) δ = 2.37-2.42 (m, 5H), 3.02 (t, J = 5.1 Hz, 2H), 3.46 (s, 2H), 5.66 (s, 1H), 6.96 (dd, J = 8.4, 1.8 Hz, 1H), 7.19 (d, J = 8.7 Hz, 1H), 7.43 (d, J = 2.1, 1H). ^{13}C NMR (CD$_3$OD) δ 12.58, 30.72, 43.87, 45.53, 112.46, 115.48, 118.91, 121.41, 125.18, 125.45, 130.01, 132.14, 134.35, 135.28. Conversion to the oxalate salt and recrystallization in methanol/diethyl ether gave **18** as a yellow powder, mp 196–197 °C (dec). Anal. (C$_{14}$H$_{15}$ClN$_2$·C$_2$H$_2$O$_4$) C, H, N.

5-Chloro-2-ethyl-3-(1,2,3,6-tetrahydropyridin-4-yl)-1H-indole (**19**). The product was obtained in 98% yield from **38**. ESIMS: m/z 261.0 (M + H)$^+$. ^{1}H NMR (CD$_3$OD) δ 1.27 (t, J = 7.5 Hz, 3H), 2.39 (s, 2H), 2.77 (q, J = 7.5 Hz, 2H), 3.01 (t, J = 5.7 Hz, 2H), 3.43 (s, 2H), 5.65 (s, 1H), 6.98 (dd, J = 8.7, 2.0 Hz, 1H), 7.21 (d, J = 8.7 Hz, 1H), 7.43 (s, 1H). ^{13}C NMR (CD$_3$OD) δ 14.88, 20.92, 31.01, 43.87, 45.53, 112.59, 114.88, 118.97, 121.49, 125.37, 125.41, 129.97, 132.12, 135.34, 140.26. The amine was converted to the oxalate salt and recrystallized in ethanol/diethyl ether, Anal. (C$_{15}$H$_{17}$ClN$_2$·C$_2$H$_2$O$_4$·0.5C$_2$H$_6$O·0.5H$_2$O) C, H, N.

5-Chloro-2-propyl-3-(1,2,3,6-tetrahydropyridin-4-yl)-1H-indole (**20**). The product was obtained in 42% yield from **39**. MS m/z (relative intensity, 70 eV) 274 (M$^+$, 45), 231 (46), 203 (45), 167 (23), 56 (bp). ESIMS: m/z 275.0 (M + H)$^+$. ^{1}H NMR (CD$_3$OD) δ 0.95 (t, J = 7.35 Hz, 3H), 1.65-1.73 (m, 2H), 2.39 (br s, 2H), 2.72 (t, J = 7.6 Hz, 2H), 3.03 (t, J = 5.7 Hz, 2H), 3.45 (s, 2H), 5.65 (s, 1H), 6.95 (dd, J = 8.4, 2.1 Hz, 1H), 7.19 (d, J = 8.7 Hz, 1H), 7.38 (d, J = 2.1, 1H). ^{13}C NMR (CD$_3$OD) δ 14.23, 24.21, 29.64, 31.15, 43.93, 45.60, 112.54, 115.62, 118.91, 121.49, 125.35, 125.66, 130.03, 132.21, 135.37, 138.73. Anal. (C$_{16}$H$_{19}$ClN$_2$·0.5H$_2$O) C, H, N.

5-Chloro-2-isopropyl-3-(1,2,3,6-tetrahydropyridin-4-yl)-1H-indole (**21**). The product was obtained in 41% yield from **40**. MS m/z (relative intensity, 70 eV) 274 (M$^+$, bp), 231 (63), 218 (62), 205 (62), 167 (31), 56 (88). ESIMS: m/z 275.0 (M + H)$^+$. ^{1}H NMR (CD$_3$OD) δ 1.30 (d, J = 6.9 Hz, 6H), 2.39 (br s, 2H), 3.06 (t, J = 5.7 Hz, 2H), 3.24-3.33 (m, 1H), 3.45 (br s, 2H), 5.65 (s, 1H), 6.95 (dd, J = 8.4, 2.1 Hz, 1H), 7.21 (d, J = 8.7 Hz, 1H), 7.36 (d, J = 2.1, 1H). ^{13}C NMR (CD$_3$OD) δ 23.15, 27.34, 31.39, 43.95, 45.62, 45.60, 112.66, 114.20, 118.89, 121.50, 125.31, 126.03, 129.95, 132.32, 135.47, 144.22. Anal. (C$_{16}$H$_{19}$ClN$_2$O·2/3H$_2$O) C, H, N.

5-Chloro-2-methyl-3-(8-methyl-8-azabicyclo[3.2.1]oct-2-en-3-yl)-1H-indole (**22**). The product was obtained in 25% yield from **41** and tropinone. MS m/z (relative intensity, 70 eV) 286 (M$^+$, 13), 259 (35), 258 (28), 257 (bp), 128 (9). ESIMS: m/z 287.0 (M + H)$^+$. ^{1}H NMR (CD$_3$OD) δ 1.7-2.7 (m,

6H), 2.37 (s, 3H), 2.46 (s, 3H), 2.75-2.92 (m, 1H), 3.35-3.45 (m, 1H), 5.74 (s, 1H), 6.95 (dd, J = 8.4, 1.8 Hz, 1H), 7.17 (d, J = 9.3 Hz, 1H), 7.38 (s, 1H). The amine was converted to the oxalate salt and recrystallized in EtOH, mp 193-198 °C. Anal. ($C_{17}H_{19}ClN_2 \cdot C_2H_2O_4 \cdot 0.5H_2O$) C, H, N.

3-(1-Benzyl-3,6-dihydro-2*H*-pyridin-4-yl)-5-chloro-2-methyl-1*H*-indole (**28**). The product was obtained in 40% yield from **41** and 1-benzylpiperidin-4-one. MS *m/z* (relative intensity, 70 eV) 336 (M^+, 41), 245 (20), 178 (18), 167 (17), 91 (bp). ESIMS: *m/z* 337 $(M + H)^+$. ^{1}H NMR (CD$_3$OD) δ 2.35 (s, 3H), 2.46 (br s, 2H), 2.70 (t, J = 6 Hz, 2H), 3.12 (br s, 2H), 3.61 (s, 2H), 5.59 (br s, 1H), 6.93 (dd, J = 6.9, 2.1, 1H), 7.16 (d, J = 8.4 Hz, 1H), 7.20-7.40 (m, 6H). ^{13}C NMR (CD$_3$OD) δ 12.64, 31.16, 51.04, 53.90, 63.68, 112.46, 114.87, 118.97, 121.43, 123.84, 125.47, 128.48, 129.34, 130.01, 130.87, 131.85, 134.43, 135.27, 138.24. The amine was converted to the oxalate salt and recrystallized in methanol/diethyl ether, mp 211-213 °C. Anal. ($C_{21}H_{21}ClN_2 \cdot C_2H_2O_4 \cdot 1/3C_2H_6O$) C, H, N.

2-Ethyl-5-methoxy-3-(4-piperidyl)-1*H*-indole (**23**). To a solution of **15** (0.24 g, 0.9 mmol) in methanol (10 mL), ammonium formate (0.4 g, 6.3 mmol) and Pd/C (10%, 0.04 g) were added under N$_2$ and the reaction mixture was refluxed for 24 h. Filtration and evaporation of the filtrate afforded crude product **23**. Aqueous work up with Na$_2$CO$_3$ (10%, 50 mL) and ethyl acetate (2 × 50 mL) was performed, and the combined organic phases were dried (MgSO$_4$) and concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate/methanol gradient) to give the title compound **23** (0.10 g, 43%). MS *m/z* (relative intensity, 70 eV) 258 (M^+, 49), 202 (44), 177 (40), 176 (63), 57 (bp). ESIMS: *m/z* 259.0 $(M + H)^+$. ^{1}H NMR (CD$_3$OD) δ 1.24 (t, J = 7.5 Hz, 3H), 1.63 (d, 2H), 2.11-2.25 (m, 2H), 2.65-2.87 (m, 5H), 3.11 (d, J = 11.7 Hz, 2H), 3.81 (s, 3H), 6.65 (dd, J = 8.7, 2.1 Hz, 1H), 7.12 (d, J = 8.7 Hz, 1H), 7.20 (d, J = 2.1 Hz, 1H). ^{13}C NMR (CD$_3$OD) δ 15.37, 20.65, 33.53, 35.90, 48.03, 56.58, 103.09, 110.84, 112.06, 115.02, 128.78, 132.68, 138.61, 154.39. The amine was converted to the oxalate salt and recrystallized in methanol, mp 193-195 °C. Anal. ($C_{16}H_{22}N_2O \cdot C_2H_2O_4 \cdot 0.5H_2O$) C, H, N.

5-Chloro-2-methyl-3-(1-methyl-3,6-dihydro-2*H*-pyridin-4-yl)-1*H*-indole (**24**). Compound **18** (240 mg, 0.97 mmol), glacial acetic acid (190 μL, 0.97 mmol) and formaldehyde (37%, 0.9 mL, 1.07 mmol) were mixed in THF (17 mL). Sodium triacetoxyborohydride (0.4 g, 1.45 mmol) was added to the solution, and the reaction mixture was stirred at room temperature under a nitrogen atmosphere for 1 h. The reaction was quenched with saturated aq. NaHCO$_3$, and the product was extracted with ethyl acetate. The combined organic phases were dried (MgSO$_4$), filtered and the solvent was evaporated to afford **24** as a residue. The residue was purified by flash chromatography on silica gel (ethyl acetate/methanol, gradient) to give the title compound **24** (0.21 g, 84%). MS *m/z* (relative intensity, 70 eV) 260 (M^+, 69), 217 (35), 167 (54), 94 (65), 70 (bp). ESIMS: *m/z* 261.0 $(M + H)^+$. ^{1}H NMR (CD$_3$OD) δ 2.35 (d, J = 4.5 Hz, 6H), 2.51 (br s, 2H), 2.63 (t, J = 6 Hz, 2H), 3.06 (br s, 2H), 5.58 (s, 1H), 6.96 (dd, J = 8.7, 1.9 Hz, 1H), 7.18 (d, J = 8.1 Hz, 1H), 7.43 (d, J = 2.1 Hz, 1H). ^{13}C NMR (CD$_3$OD) δ 12.64, 31.09, 45.63, 53.13, 55.41, 112.50, 114.65, 118.91, 121.45, 123.55, 125.47, 129.95, 131.48, 134.46, 135.23. The amine was converted to the oxalate salt and recrystallized in methanol, mp 192-193 °C. Anal. ($C_{15}H_{17}ClN_2 \cdot C_2H_2O_4$) C, H, N.

General procedure for the alkylation of 5-chloro-2-methyl-3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indoles 18 and 19. 5-Chloro-2-alkyl-3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole (**18** or **19**, 0.5–0.8 mmol, 1 equiv.) was dissolved in acetonitrile (10 mL) and potassium carbonate (1.5-2.4 mmol, 3 equiv.) and the appropriate alkyl halide (0.6-1.0 mmol, 1.2 equiv., iodoethane, 1-iodopropane or *n*-bromobutane) was added and the mixture was allowed to stir at ambient temperature overnight. The solid base was filtered off, washed with acetonitrile (3×10 mL) and the filtrate was concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (ethyl acetate/methanol, gradient) to give the title compound.

5-Chloro-3-(1-ethyl-3,6-dihydro-2*H*-pyridin-4-yl)-2-methyl-1*H*-indole (**25**). The product was obtained in 98% yield from iodoethane and **18**. MS *m/z* (relative intensity, 70 eV) 274 (M$^+$, bp), 259 (55), 167 (60), 108 (55), 84 (65). ESIMS: *m/z* 275 (M + H)$^+$. ^{1}H NMR (DMSO-*d$_6$*) δ 1.27 (t, *J* = 7.2 Hz, 3H), 2.44 (s, 3H), 2.70 (br s, 2H), 3.05 (q, *J* = 7.2 Hz, 2H), 3.15-3.35 (m, 4H), 5.71 (s, 1H), 7.06 (dd, *J* = 9.0, 1.8 Hz, 1H), 7.33 (d, *J* = 8.7 Hz, 1H), 7.53 (s, 1H), 11.29 (1H). ^{13}C NMR (DMSO-*d$_6$*) δ 10.21, 12.67, 27.70, 48.70, 50.38, 50.73, 112.06, 112.16, 117.47, 119.18, 120.23, 123.52, 127.97, 129.94, 133.49, 134.18. The amine was converted to the oxalate salt and recrystallized in methanol/diethyl ether, mp 208-210 °C. Anal. (C$_{16}$H$_{19}$ClN$_2$·C$_2$H$_2$O$_4$·2/3H$_2$O) C, H, N.

5-Chloro-2-methyl-3-(1-propyl-3,6-dihydro-2*H*-pyridin-4-yl)-1*H*-indole (**26**). The product was obtained in 88% yield from 1-iodopropane and **18**. MS *m/z* (relative intensity, 70 eV) 288 (M$^+$, bp), 259 (95), 202 (44), 167 (39), 122 (28). ESIMS: *m/z* 289 (M + H)$^+$. ^{1}H NMR (CD$_3$OD) δ 0.94 (t, *J* = 7.4 Hz, 3H), 1.58 (q, *J* = 7.8 Hz, 2H), 2.37-2.42 (m, 5H), 2.52 (s, 2H), 2.66 (t, *J* = 5.7 Hz, 2H), 3.11 (s, 2H), 5.60 (s, 1H), 6.95 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.18 (d, *J* = 8.7 Hz, 1H), 7.42 (d, *J* = 1.5 Hz, 1H). ^{13}C NMR (CD$_3$OD) δ 12.30, 12.66, 20.73, 31.13, 51.47, 53.86, 61.58, 112.49, 114.81, 118.96, 121.44, 123.78, 125.49, 130.02, 131.83, 134.45, 135.28. The amine was converted to the oxalate salt and recrystallized in methanol/diethyl ether, mp 195-196 °C. Anal. (C$_{17}$H$_{21}$ClN$_2$·C$_2$H$_2$O$_4$·2/3H$_2$O) C, H, N.

3-(1-Butyl-3,6-dihydro-2*H*-pyridin-4-yl)-5-chloro-2-methyl-1*H*-indole (**27**). The product was obtained in 66% yield from *n*-bromobutane and **18**. MS *m/z* (relative intensity, 70 eV) 302 (M$^+$, 82), 259 (bp), 178 (37), 167 (35), 129 (25). ESIMS: *m/z* 303 (M + H)$^+$. ^{1}H NMR (CD$_3$OD) δ 0.96 (t, *J* = 7.2 Hz, 3H), 1.34 (q, *J* = 7.5 Hz, 2H), 1.51-1.54 (m, 2H), 2.36-2.44 (m, 5H), 2.51 (s, 2H), 2.66 (t, *J* = 5.7 Hz, 2H), 3.10 (s, 2H), 5.59 (s, 1H), 6.96 (dd, *J* = 8.7, 2.0 Hz, 1H), 7.18 (d, *J* = 8.4 Hz, 1H), 7.42 (d, *J* = 1.5 Hz, 1H). ^{13}C NMR (CD$_3$OD) δ 12.67, 14.38, 21.89, 29.79, 31.15, 51.49, 53.91, 59.39, 112.48, 114.81, 118.97, 121.44, 123.81, 125.48, 130.03, 131.82, 134.43, 135.27. The amine was converted to the oxalate salt and recrystallized in methanol/diethyl ether, mp 197-200 °C. Anal. (C$_{18}$H$_{23}$ClN$_2$·2/3C$_2$H$_2$O$_4$·1/3H$_2$O) C, H, N.

5-Chloro-2-ethyl-3-(1-ethyl-3,6-dihydro-2*H*-pyridin-4-yl)-1*H*-indole (**29**). The product was obtained in 57% yield from iodoethane and **19**. MS *m/z* (relative intensity, 70 eV) 288 (M$^+$, 70), 287

(38), 181 (29), 97 (36), 84 (bp): ESIMS: *m/z* 289 (M + H)$^+$. ^{1}H NMR (CD$_3$OD) δ 0.91 (t, *J* = 7.3 Hz, 3H), 1.54 (q, *J* =7.5 Hz, 2H), 1.76-1.83 (m, 3H), 1.96-2.05 (m, 2H), 2.32 (t, *J* = 7.9 Hz, 3H), 2.40-2.52 (m, 1H), 3.04 (d, *J* = 11.7 Hz, 2H), 5.78 (s, 1H), 6.99-7.04 (m, 2H), 7.16-7.25 (m, 1H). ^{13}C NMR (CD$_3$OD) δ 11.91, 14.91, 20.94, 31.41, 50.99, 53.00, 53.34, 112.62, 114.17, 118.99, 121.53, 123.94, 125.41, 129.99, 131.90, 135.37, 140.39. Anal. (C$_{17}$H$_{21}$ClN$_2$·2/3H$_2$O) C, H, N.

2-(2-Ethyl-5-methoxy-1*H*-indol-3-yl)-*N*,*N*-dimethylethylamine (**4**). A solution of the compound **46** (0.60 g, 2.19 mmol) in dry THF (20 mL) was added dropwise to a slurry of LiAlH$_4$ (1 M, 2.4 mL, 4.3 mmol) at room temperature. The mixture was heated to 60 ºC for 2 h until LC-MS indicated that the reaction was complete. The mixture was then cooled, quenched with water, filtered through Celite, and the filtrate was concentrated under reduced pressure. The residue was taken up in ethyl acetate, washed with aqueous 1 M NaOH and brine, dried over MgSO$_4$ and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (ethyl acetate/methanol, gradient) to give the title compound **4** (0.59 g, 95%). MS *m/z* (relative intensity, 70 eV) 246 (M$^+$, 5), 188 (9), 173 (3), 158 (5), 58 (bp). ESIMS: *m/z* 247.0 (M + H)$^+$. ^{1}H NMR (CDCl$_3$) δ 1.26 (t, *J* = 7.5 Hz, 3H), 2.36 (s, 6H), 2.49-2.55 (m, 2H), 2.72 (q, *J* = 7.5 Hz, 2H), 2.83-2.89 (m, 2H), 3.84 (s, 3H), 6.76 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.98 (d, *J* = 2.4 Hz, 1H), 7.14 (d, *J* = 8.7 Hz, 1H), 7.90-8.28 (br s, 1H). ^{13}C NMR (CDCl$_3$) δ 14.32, 19.46, 22.75, 45.36, 56.06, 60.50, 100.79, 108.54, 110.45, 110.94, 129.07, 130.47, 138.02, 153.87. The amine was converted to the oxalate salt and recrystallized in ethanol/diethyl ether, mp 181-182 °C. Anal. (C$_{15}$H$_{22}$N$_2$O·C$_2$H$_2$O$_4$) C, H, N.

2-(5-Chloro-2-methyl-1*H*-indol-3-yl)-*N*,*N*-dimethylethylamine (**30**). A solution of the compound **47** (0.4 g, 1.5 mmol) in dry THF (20 mL) was added dropwise to a slurry of LiAlH$_4$ (1 M, 1.7 mL, 3 mmol) at room temperature. The mixture was heated to 60 ºC for 4 h until LC-MS indicated that the reaction was complete. The mixture was then cooled, quenched with water, filtered through Celite, and the filtrate was concentrated under reduced pressure. The residue was taken up into ethyl acetate, washed with aqueous 1 M NaOH and brine, dried over MgSO$_4$ and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (ethyl acetate/methanol, gradient) to give the title compound **30** (0.15 g, 44%). MS *m/z* (relative intensity, 70 eV) 236 (M$^+$, 2), 178 (4), 143 (2), 115 (2), 58 (bp). ESIMS: *m/z* 237.0 (M + H)$^+$. ^{1}H NMR (CDCl$_3$) δ 2.33 (d, *J* = 10.5 Hz, 9H), 2.48 (t, *J* = 8.2 Hz, 2H), 2.81 (t, *J* = 8.2 Hz, 2H), 7.01 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.09 (d, *J* = 8.4 Hz, 1H), 7.41 (s, 1H), 8.20-8.32 (br s, 1H). ^{13}C NMR (CDCl$_3$) δ 11.57, 22.62, 45.35, 60.14, 109.49, 111.12, 117.33, 120.91, 124.70, 129.79, 132.79, 133.65. The amine was converted to the HCl salt and recrystallized in acetonitrile/diethyl ether, mp 196-198 °C. Anal. (C$_{13}$H$_{17}$ClN$_2$·HCl) C, H, N.

General method for Boc protection of the aniline (**31**, **32**).[160] A solution of 3-substituted 2-methylaniline (36 mmol, 1 equiv.) was dissolved in THF (100 mL), di-*tert*-butyl dicarbonate (41.9 mmol, 1.1 equiv.) was added and the mixture was refluxed for 2 h. After cooling the reaction mixture was evaporated *in vacuo* and the residue was dissolved in ethyl acetate and washed with 1 M citric acid solution, dried over MgSO$_4$ and concentrated *in vacuo* to give the title compound.

1-*tert*-Butoxycarbonylamino-4-methoxy-2-methylbenzene (**31**). The product was obtained in 68% yield from 4-methoxy-2-methylaniline. MS *m/z* (relative intensity, 70 eV) 237 (M$^+$, 8), 181 (77), 137 (29), 122 (70), 57 (bp). ESIMS: *m/z* 260.0 (M + Na)$^+$.

1-*tert*-Butoxycarbonylamino-4-chloro-2-methylbenzene (**32**). The product was obtained in 71% yield from 4-chloro-2-methylaniline. MS *m/z* (relative intensity, 70 eV) 241 (M$^+$, 3), 185 (21), 141 (20), 77 (14), 57 (bp). ESIMS: *m/z* 264.0 (M + Na)$^+$.

General procedure for synthesis of 4-substituted 1-*tert*-butoxycarbonylamino-2-(2-oxoalkyl)benzene derivatives 33-36. A solution of 1.3 M *sec*-butyllithium/cyclohexane (20.6 mmol, 2 equiv.) was added slowly to (10.3 mmol, 1 equiv.) **31** or **32** dissolved in THF (30 mL) while the temperature was below -70 ºC. After the addition the reaction mixture were stirred for 20 min at -70 ºC. Then *N*-methoxy-*N*-methyl-alkylamide (**43-45**) was dissolved in THF (5 mL) and added to the reaction mixture at -70 ºC and the temperature was maintained for another 30 min. The cooling bath was removed and the reaction was stirred at ambient temperature for 1 h. Ethyl acetate was added and the mixture was poured into 1 M citric acid. The phases were separated, and the organic portion was washed with 10% aq. Na$_2$CO$_3$, dried with MgSO$_4$ and concentrated *in vacuo*. The crude products were purified by silica gel column chromatography (ethyl acetate/isooctane, gradient) to give the title compounds.

1-(2-*tert*-Butoxycarbonylamino-4-methoxyphenyl)-2-butanone (**33**). The product was obtained in 40% yield from **31** and **43**. MS *m/z* (relative intensity, 70 eV) 293 (M$^+$, 1), 175 (58), 160 (bp), 117 (37), 57 (77).

1-(2-*tert*-Butoxycarbonylamino-4-chlorophenyl)-2-butanone (**34**). The product was obtained in 67% yield from **32** and **43**. MS *m/z* (relative intensity, 70 eV) 297 (M$^+$, 4), 197 (8), 168 (13), 140 (17), 57 (bp). ESIMS: *m/z* 320.0 (M + Na)$^+$.

1-(2-*tert*-Butoxycarbonylamino-4-chlorophenyl)-2-pentanone (**35**). The product was obtained in 29% yield from **32** and **44**. MS *m/z* (relative intensity, 70 eV) 311 (M$^+$, 3), 193 (18), 164 (50), 71 (49), 57 (bp).

1-(2-*tert*-Butoxycarbonylamino-4-chlorophenyl)-3-methyl-2-butanone (**36**). The product was obtained in 66% yield from **32** and **45**. MS *m/z* (relative intensity, 70 eV) 311 (M$^+$, 3), 193 (28), 178 (55), 71 (43), 57 (bp).

General procedure for the ring closure of compounds 33-36 to afford 2-alkyl-1*H*-indoles 37-40. Compound **33-36** (3 mmol, 1 equiv.) was dissolved in CH$_2$Cl$_2$ and trifluoroacetic acid (2.5 mL) was added at 0 ºC. After addition the cooling bath was removed and the reaction mixture was stirred at ambient temperature for 24 h, washed with H$_2$O, aq. 10% Na$_2$CO$_3$, dried (MgSO$_4$) and concentrated

in vacuo. The residue was purified by silica gel column chromatography (ethyl acetate/isooctane, gradient) to give the title compound.

2-Ethyl-5-methoxy-1*H*-indole (37). The product was obtained in 70% yield from **33**. MS *m/z* (relative intensity, 70 eV) 175 (M$^+$, 61), 160 (bp), 145 (13), 132 (28), 117 (45). ESIMS: *m/z* 176.0 (M + H)$^+$.

5-Chloro-2-ethyl-1*H*-indole (38). The product was obtained in 50% yield from **34**. MS *m/z* (relative intensity, 70 eV) 179 (M$^+$, 45), 166 (32), 164 (bp), 143 (9), 128 (9). ^{1}H NMR (CDCl$_3$) δ 1.31 (t, *J* = 7.55 Hz, 3H), 2.73 (q, *J* = 7.39 Hz, 2H), 6.17 (s, 1H), 6.99-7.08 (m, 1H), 7.09-7.20 (m, 1H), 7.47 (s, 1H), 7.81 (br s, 1H). ^{13}C NMR (CDCl$_3$) δ 13.04, 21.38, 98.55, 111.16, 119.16, 121.11, 125.16, 129.99, 134.21, 142.91.

5-Chloro-2-propyl-1*H*-indole (39). The product was obtained in 24% yield from **35**. MS *m/z* (relative intensity, 70 eV) 193 (M$^+$, 14), 165 (35), 164 (bp), 128 (33), 102 (36). ^{1}H NMR (CDCl$_3$) δ 0.98 (t, *J* = 7.39 Hz, 3H), 1.61-1.92 (m, 2H), 2.68 (t, *J* = 7.55 Hz, 2H), 6.16 (s, 1H), 7.04 (dd, *J* = 8.4, 1.5 Hz, 1H), 7.14 (d, *J* = 8.4 Hz, 1H), 7.47 (s, 1H), 7.81 (br s, 1H). ^{13}C NMR (CDCl$_3$) δ 13.81, 22.31, 30.24, 99.34, 111.17, 119.12, 121.05, 125.13, 129.99, 134.15, 141.39.

5-Chloro-2-*iso*propyl-1*H*-indole (40). The product was obtained in 32% yield from **36**. MS *m/z* (relative intensity, 70 eV) 193 (M$^+$, 38), 180 (35), 178 (bp), 143 (55), 115 (13). ^{1}H NMR (CDCl$_3$) δ 1.32 (t, *J* = 6.88 Hz, 6H), 3.01 (quin, *J* = 6.92 Hz, 1H), 6.17 (s, 1H), 6.98-7.09 (m, 1H), 7.16 (d, *J* = 8.4 Hz, 1H), 7.47 (s, 1H), 7.86 (br s, 1H). ^{13}C NMR (CDCl$_3$) δ 22.27, 28.12, 97.28, 111.24, 119.27, 121.13, 125.11, 129.77, 134.07, 147.45.

General method for preparation of *N*-methoxy-*N*-methyl-alkylamides (43-45).[161] A mixture of *N,O*-dimethylhydroxylamine hydrochloride (30.7 mmol, 1 equiv.) and triethylamine (61.4 mmol, 2 equiv.) in CH$_2$Cl$_2$ (200 mL) was cooled in a icebath. Acid chloride (33.8 mmol, 1.1 equiv., propionyl chloride, butyryl chloride, and *iso*-butyryl chloride) in CH$_2$Cl$_2$ (25 mL) was added dropwise and the reaction was stirred at room temperature for 12 h and then washed with H$_2$O, dilute HCl, 10% aq. Na$_2$CO$_3$ and brine. The dried CH$_2$Cl$_2$ (Na$_2$SO$_4$) solution was concentrated and the residue was distilled using a Kugelrohr apparatus to afford the compound as a colorless liquid.

N-Methoxy-N-methyl-propanamide (43). The product was obtained in 53% yield from propionyl chloride. MS *m/z* (relative intensity, 70 eV) 117 (M$^+$, 6), 87 (6), 61 (86), 60 (11), 57 (bp). ESIMS: *m/z* 117.0 (M + H)$^+$.

N-Methoxy-N-methyl-butanamide (44). The product was obtained in 25% yield from butyryl chloride. ESIMS: *m/z* 132.0 (M + H)$^+$.

N-Methoxy-N,2-dimethyl-propanamide (**45**). The product was obtained in 58% yield from *iso-*butyryl chloride. ESIMS: *m/z* 132.0 (M + H)$^+$.

2-(2-Ethyl-5-methoxy-1*H*-indol-3-yl)-N,N-dimethyl-2-oxo-acetamide (**46**). A solution of oxalyl chloride (0.45 g, 3.56 mmol) in anhydrous diethyl ether (5 mL) was added dropwise over 15 min to a 0 °C solution of 2-ethyl-5-methoxy-1*H*-indole (**37**, 0.48 g, 2.74 mmol) in anhydrous diethyl ether (20 mL). The reaction mixture was stirred at room temperature for 3 h, then cooled to 0 °C and a solution of dimethylamine in THF (2 M, 10 mL) was added dropwise over 15 min. The solid formed was filtered off and washed with water to provide crude **46** (0.7 g, 93%). MS *m/z* (relative intensity, 70 eV) 274 (M$^+$, 6), 202 (bp), 187 (11), 131 (16), 72 (52). ESIMS: *m/z* 297.0 (M + Na)$^+$.

2-(5-Chloro-2-methyl-1*H*-indol-3-yl)-N,N-dimethyl-2-oxo-acetamide (**47**). A solution of oxalyl chloride (0.69 g, 5.48 mmol) in anhydrous diethyl ether (5 mL) was added dropwise over 15 min to a 0 °C solution of 5-chloro-2-methyl-1*H*-indole (**41**, 0.7 g, 4.22 mmol) in anhydrous diethyl ether (20 mL). The reaction mixture was stirred at room temperature for 5 h, then cooled to 0 °C and a solution of dimethylamine in THF (2 M, 15 mL) was added dropwise over 15 min. The solid formed was filtered off and washed with water to provide crude **47** (0.45 g, 40%). MS *m/z* (relative intensity, 70 eV) 264 (M$^+$, 7), 194 (32), 192 (bp), 164 (7), 128 (7). ESIMS: *m/z* 287.0 (M + Na)$^+$. ^{13}C NMR (DMSO-*d$_6$*) δ 3.12, 33.13, 36.25, 109.09, 113.16, 119.18, 122.64, 126.84, 127.70, 133.66, 148.35, 167.95, 186.53.