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Introduction

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CNS disorders and their influence on social life

Alzheimer's disease (AD) is the most common form of dementia. It is a progressive disease of the central nervous system (CNS), which restricts the patients' participation in social life. The population of patients with AD is currently estimated to be about 11 million people world-wide, while this is expected to be nearly doubled by the year 2025. According to a study done by the American Alzheimer's Association in 1998, AD costs the USA businesses about \$ 33 billion a year. \$ 26 billion of it is related to the absenteeism of caregiver's employees who are taking care of Alzheimer patients and \$ 7 billion is related to the businesses contributing toward the total cost care.¹ An even more common world-wide CNS disease is major depression. Depression is a mental disorder that presents with depressed mood, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, low energy, and poor concentration. These problems can become chronic or recurrent and lead to substantial impairments in an individual's ability to take care of his or her everyday responsibilities. At its worst, depression can lead to suicide, a tragic fatality associated with the loss of about 850.000 lives every year world-wide.² The lifetime prevalence for a depressive episode that last longer than 6 months is about 15%, a figure that is surprisingly constant across the world.

The lost days from work and economic burden from affective disorders are high and were estimated \$ 43.7 billion in 1990 in the USA.³ In Europe, at least 21 million residents may be affected by major depression, resulting in related total annual costs of € 120 billion in 2004.⁴ The World Health Organisation (WHO) estimates that depression will soon be the greatest health burden world-wide. It is one of the major reasons of disability, as measured by the years lived with disability (YLDs), and the fourth leading contributor to the global burden of disease, as measured by the disability adjusted life years (DALYs) in 2000. According to the WHO, major depression is now the second cause of DALYs for females and males in the age range of 15-44 years (121 million people world-wide), which is expected to happen in 2020.² The costs of treatment for depression and schizophrenia in The Netherlands alone was in 1999 about € 870 million, which is 2.6% of the total cost for health care.⁵ Schizophrenia is a mental disorder known by a disintegration of thoughts process and emotional responsiveness. The WHO has also reported that a study in 27 developing and developed countries there was no population

that was free of schizophrenia. It may therefore be stated that mental disorders are an enormous drain on world economy. These disorders, which additionally also heightens the risk for a heart attack or stroke and often reduces the quality of life, are an important public health issue and the study of their pathophysiology and treatment is a priority.⁶

5-HT and 5-HT_{1A} receptors related to CNS disorders

The 5-hydroxytryptamine-1A (5-HT_{1A}) receptor plays an important role in learning and memory⁷ and changed expression have been implicated in various disorders^{8,9} including major depression^{10,11}, anxiety disorders¹², AD^{13,14} and schizophrenia.^{15,16,17} These receptors may also play a role in sleep, food intake, mood and thermoregulation.^{18,19} For example, using the partial 5-HT_{1A} agonist buspirone, it has been shown, among others, that the 5-HT_{1A} receptor is involved in both pathogenesis and treatment of major depression^{12,20}, implicating the 5-HT_{1A} receptor as a potential therapeutic target and/or marker to study the pathophysiology of neuropsychiatric disorders. Though, according to Berlim²¹ and Rush²², a major problem in psychiatry is the lack of effective treatments to relieve the symptoms of depression in many patients. Fewer than 25% of those affected have access to effective treatments.

In AD, studies reported the correlation between the presence of intraneuronal neurofibrillary tangles and the degree of dementia.²³ In the earliest stage of the disease, there are a large number of pyramidal neurons in the cornus ammonis 1 and subicular regions of hippocampus as well as a part of the medial temporal lobe that are effected.²⁴ These pyramidal neurons receive inhibitory serotonergic input from the dorsal raphe nucleus via 5-HT_{1A} receptors located on their axonal hillock.²⁵ Furthermore, a significant decrease of 5-HT_{1A} receptor densities was reported in the hippocampal and raphe nuclei of AD patients, revealing the role of this receptor in the neuropathology of AD.^{13,14} On the other hand, the majority of post-mortem studies have related schizophrenia to an increase of 5-HT_{1A} receptor densities in the prefrontal cortex.^{15,16} All together, these findings may implicate the importance of 5-HT_{1A} receptor for drug target therapy in several CNS disorders.⁶ The 5-HT_{1A} receptor is one of the seven different types of 5-HT receptors (5-HT₁ to 5-HT₇) described, that mediate various functions of 5-HT, of which all but one (5-HT₃) are G- protein coupled receptors.^{18,26,27} In 1994, the International Union of

Pharmacology Committee on Receptor Nomenclature and Drug Classification (NC-IUPHAR) has reclassified 5-HT receptors and sub-receptors into 5-HT₁ (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F}), 5-HT₂ (5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}), 5-HT₃, 5-HT₄, recombinant 5-HT_{5A/5B}, 5-HT₆, 5-HT₇ in addition to orphan receptors.²⁷ The table below shows the type, mechanism of action and effect of each receptor.²⁸ In human brain, high 5-HT_{1A} receptor densities are found in the brainstem raphe nuclei and in the limbic forebrain (hippocampus, entorhinal cortex and septum). Low densities are observed in the basal ganglia and in the adult cerebellum.²⁰ These receptors are expressed on the cell bodies of 5-HT neurons as somatodendritic autoreceptors and mediate the autoregulation of 5-HT neuronal function (inhibition of 5-HT cell firing and 5-HT release) in the raphe nuclei. They are also expressed as postsynaptic receptors in the 5-HT neuron terminal fields of the hippocampal pyramidal and granule cells and inhibit neuronal release.²⁹ Serotonin, “*sero* = serum and *tonin* = vasoconstriction” or 5-hydroxytryptamine (5-HT) was first isolated as an unknown vasoconstrictor substance from the blood and indicate its presence originally in the platelets.^{30,31} In human gut, 5-HT is a peripheral hormone produced by enterochromaffin cells and stored in platelets, which represents 80-90% of the production of 5-HT in the body.

Table 1. Serotonin receptors, type, mechanism of action and effect.

5-HT _r	Type	Mechanism of action	Effect
1	G _i /G _o -coupled receptor	Decreasing cellular levels of cAMP*	Inhibitory
2	G _q /G ₁₁ -coupled receptor	Increasing cellular levels of IP ₃ and DAG*	Excitatory
3	Ligand-gated Na ⁺ /K ⁺ channel	Depolarizing plasma membrane	Excitatory
4	G _s -coupled receptor	Increasing cellular levels of cAMP	Excitatory
5	G _i /G _o -coupled receptor	Decreasing cellular levels of cAMP	Inhibitory
6	G _s -coupled receptor	Increasing cellular levels of cAMP	Excitatory
7	G _s -coupled receptor	Increasing cellular levels of cAMP	Excitatory

*cAMP; cyclic adenosine monophosphate, IP₃; inositol triphosphate, DAG; diacylglycerol

5-HT is initially synthesized from tryptophan in a two-steps reaction (Figure 1).^{30,32} In the brain stem, the synthesis of 5-HT is generated by the rate-limiting enzyme “tryptophan hydroxylase 2 (TPH2)” using (6R)-L-erythro-5,6,7,8-tetrahydrobiopterin (BH₄) and dioxygen as cofactors to produce 5-hydroxytryptophan in neurons. In the

enterochromaffin cells in the intestine, pineal gland, spleen and thymus, tryptophan hydroxylase 1 (TPH1) is responsible for the synthesis.³⁴ Decarboxylation of the 5-hydroxytryptophan by amino acid decarboxylase will produce 5-HT.^{32,33}

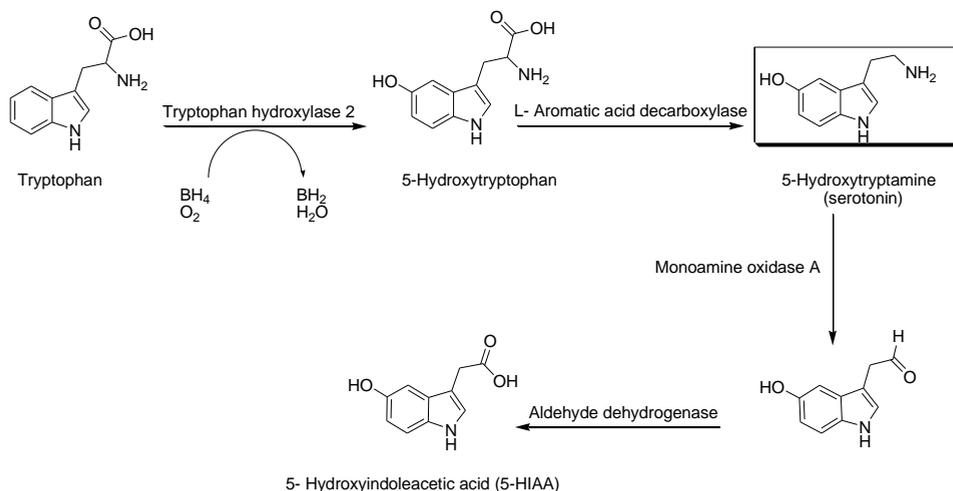


Figure 1. The biosynthesis of serotonin and its metabolism in the brain stem.

The newly synthesized 5-HT is transported from the cytoplasm into synaptic vesicles by the vesicular monoamine transporter 2 (VMAT2)³⁴ (Figure 2). 5-HT is stored as co-transmitter with diverse peptide hormones in neurons and chromaffin cells, such as somatostatin, substance P or vasoactive intestinal polypeptide (VIP).³⁰ After 5-HT has been released in the synaptic cleft, it will interact with 5-HT receptors. After this, the selective 5-HT reuptake from the synaptic cleft into the presynaptic neuron occurs via reuptake transporters (12-transmembrane-proteins, 5-HHT).³² Upon return to the cytoplasm, the actions of this neurotransmitter are terminated by three major mechanisms including diffusion, degradation by monoamine oxidase (MAO) type A³⁵ and uptake back to the synaptic vesicles through the actions of VMAT2. Degradation by MAO will be followed by dehydrogenation by aldehyde dehydrogenase leading to 5-hydroxyindoleacetic acid (5-HIAA), which can easily be excreted in the urine (Figure 1).^{30,32,36}

Degradation also occur by catechol-*O*-methyltransferase (COMT) in the extracellular space, however this is less effective in the peripherally nerves system (PNS) than CNS.³²

In the brain, 5-HT can exert inhibitory or excitatory effects on individual neurons and it acts either presynaptically or postsynaptically.³⁷ In the nerve cells of the myenteric plexus it acts as excitatory neurotransmitter.³⁰

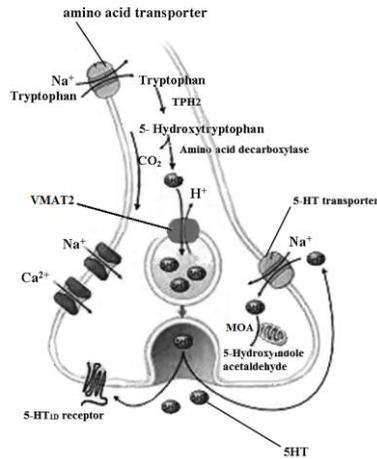


Figure 2. The synaptic regulation of 5-HT neurotransmitters.

The importance of molecular imaging of the brain is clear when we realize that, until now, most of the brain disorders cannot be treated effectively, which affects the quality of care, health outcomes, functioning of the patients and thus economic productivity. Molecular imaging techniques using Positron Emission Tomography (PET) or Single Photon Emission Computerized Tomography (SPECT) are valuable tools in the investigation of changes in 5-HT_{1A} receptor availability. These changes are believed to be related to common neuropsychiatric disorders such as major depression, anxiety, AD and schizophrenia. In the last decade, there is an increase of interest to provide integrated molecular medicine solutions using new radiolabeled ligands with dedicated and optimized imaging systems to investigate *in vivo* changes of 5-HT_{1A} receptors.^{13,14,15,17}

PET and SPECT techniques

In general, imaging can address two issues: structure and function. One can either view brain structure, image anatomy or examine physiological functions of the brain, the chemical processes and image biochemistry, either at rest or during activation. Structural imaging techniques are CT (Computerized Tomography) and MRI (Magnetic Resonance Imaging). Functional imaging techniques are MRI spectroscopy and functional MRI (fMRI), SPECT and PET. Although CT and MRI are most commonly used, they could not help to explain or examine the physiological function of the brain.³⁸ The benefit of SPECT and PET imaging is the ability to view the biochemical processes inside the brain. While CT and MRI can be compared with country maps, SPECT and PET show us the traffic on the routes.

Both SPECT and PET are emission techniques using the concept of a radiotracer (or radiopharmaceutical/radioligand) to image chemical processes in living subjects. A radiotracer is a ligand with a biological activity in which an atom is replaced by a radioactive atom or to which a radioactive atom is added. When a radiotracer with a high binding specificity is introduced into the body, its site-specific uptake can be traced by means of photons emitted by the labeled atom. The detection of the gamma ray (photons) in SPECT is done with solid-state detectors. When a photon enters such a crystal it will produce a visible light through interaction with the crystal, which is collected by photomultiplier tubes at the rear of the crystals and converted to a signal. Collimators are placed between the patient and detector to allow precise localization. These collimators are mostly made of thick perforated lead sheets. Almost only perpendicular incoming photons can pass the holes of the collimator and enter the detector, leading to a 2-dimensional image. Similar to CT, for SPECT imaging multiple acquisitions can be made from various angles and then reconstructed back to create a 3-dimensional map.³⁹

In brain SPECT, commonly the nuclides Tc-99m ($t_{1/2} = 6$ hours) and I-123 ($t_{1/2} = 13.2$ hours) are used for radiolabeling. Depending on the choice of the radiotracer, different brain functions can be demonstrated. SPECT can provide measures of cerebral function such as cerebral blood flow when using [^{99m}Tc]-hexamethylpropylene amine oxime ([^{99m}Tc]-HMPAO). This radiotracer is trapped in the brain predominantly within 2-5 minutes after intravenous injection. This technique gives a snapshot of cerebral blood

flow a few minutes after injection. Other examples of commonly used SPECT tracers are [^{123}I]iodobenzamide ([^{123}I]IBZM) to assess dopamine $D_{2/3}$ receptors in the basal ganglia, and the non-selective [^{123}I]2 β -carbomethoxy-3 β -(4-iodophenyl)-N-(3-fluoropropyl)nortropane ([^{123}I]FP-CIT) to assess dopamine transporters (Figure 3).⁴⁰

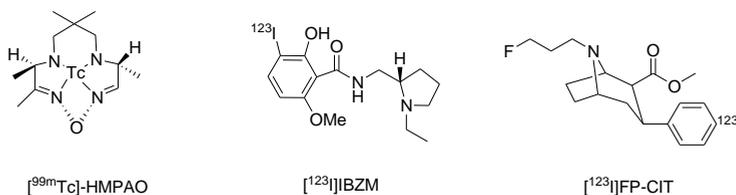


Figure 3. The chemical structure of some commonly used SPECT radiotracers.

While MRI is helpful in the diagnosis of AD if temporal lobe atrophy can be detected, SPECT imaging is useful when structural imaging is normal or equivocal or when MRI is not available. In 60-90% of the cases perfusion SPECT helps to distinguish those with AD from normal controls and those with other causes of cognitive impairment, particularly frontotemporal dementia.³⁸ In addition, dopamine transporters imaging may be helpful to differentiate AD from dementia with Lewy bodies.³⁹

Generally speaking, SPECT is cheaper than PET and therefore more widely available.^{38,40} However, SPECT suffers from several drawbacks, with sensitivity being a main issue, since many photons are lost by absorption by the collimator. Moreover, in clinical studies, the spatial resolution is poorer (10-15 mm) than for PET.^{38,40} Another well-known limitation of SPECT applicability is the fact that SPECT isotopes are relatively large atoms and cannot be used to label some compounds due to issues of steric hindrance.⁴⁰ On the other hand, due to the long half-life of the used isotopes, scans can be acquired later after injection at a time point after the washout of non-specific binding has occurred.

PET is a technique with higher sensitivity than SPECT. In PET the photons are naturally collimating through a physical process without using absorbing collimators. PET uses radiotracers that are labeled with positron emitting isotopes, such as F-18 ($t_{1/2} = 109.8$

minutes), C-11 ($t_{1/2} = 20$ minutes), and O-15 ($t_{1/2} = 122.24$ seconds). PET tracers have a relatively short half-life which reduces the substantially increasing long-term health risks associated with the ionizing radiation and therefore allows repeated PET imaging procedures. As the radioisotope decays in the body, the emitted positron annihilates with an electron. Such an event produces two 511 keV photons at almost 180 degrees to each other, a requirement of linear momentum conservation and the fact that the positron-electron system (positronium) right before annihilation form essentially a zero-momentum frame.⁴¹ The PET detector is set up in such a way that only events are accepted in which both annihilation photons are detected in coincidence. Depending on the used system, two photons are identified as coming from a single event if they arrive at detectors within about 6-12 nanoseconds of one another. No collimators are required, since the two crystals of the photon detectors are along the line, termed line-of-response (LOR), where the annihilation occurred.⁴¹ Absence of physical collimators increases the sensitivity of PET over SPECT by about 10-50 times, as in SPECT imaging many photons are lost by absorption by the SPECT collimators. Attenuation correction (AC) is an essential step in PET and SPECT imaging, since photons emitted from deep-seated tissues have more chance to be absorbed or scattered than more superficially emitted photons. AC can be performed by using an external (transmission) source or (co-registered) CT images.⁴² [¹⁸F]Fluorodeoxyglucose ([¹⁸F]FDG) is a good example of a PET radiotracer for defining brain metabolic activity in AD and its pharmacological modulation⁴³, while N-Methyl-[¹¹C]-2-(4'-methylamino phenyl)-6- hydroxybenzothiazole ([¹¹C]PIB) gives specific information concerning the deposition of amyloid, which is thought to be related directly to mechanisms of neurodegeneration⁴⁴ (Figure 4).

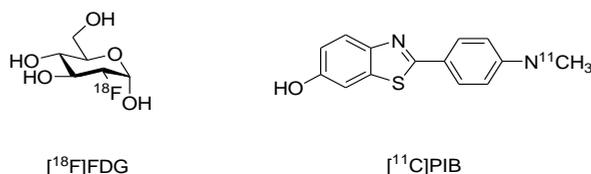


Figure 4. The chemical structure of several PET radiotracers.

SPECT and PET ligands for the 5-HT_{1A} receptor

The 5-HT_{1A} receptor antagonist *N*-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl} *N*-(pyridin-2-yl)cyclohexanecarboxamide (WAY-100635) was labeled with C-11 in the carbonyl position or the methyl position of the anisole. Since the main metabolic pathway of WAY-100635 is the hydrolysis of the amide bond, a label in the cyclohexanecarbonyl moiety was favored.^{45,46} Otherwise, the lipophilic metabolite WAY-100634 carries the label and since it also enters the brain, and binds to α_1 adrenoreceptors, it interferes with the preferred scintigraphic measurements.^{45,46,47,48,49} Using this radiotracer it has been shown that 5-HT_{1A} receptor binding was reduced by 42% in the brainstem raphe nuclei and by 27% in mesiotemporal cortex (hippocampus-amygdala) in patients suffering from major depressive disorder compared to controls.⁴⁵

Although this tracer produces nice images of the 5-HT_{1A} receptor in human brain, it has a major drawback as it is labeled with the isotope C-11 which has a short half-life time, and thus can only be used as both a cyclotron and a PET-camera are in close proximity. Relatively longer lived isotopes, like I-123, can be used for SPECT cameras where studies can be easily scheduled since these cameras are available in almost all hospitals. Therefore, it was highly desirable to develop I-123 analogues of WAY-100635 like 4-iodo-*N*-{2-[1-(2-methoxyphenyl)piperazin-1-yl]ethyl}-*N*-(pyridin-2-yl)benzamide (*p*-MPPI) and 3-iodo-*N*-{2-[1-(2-methoxyphenyl)-piperazin-1-yl]ethyl}-*N*-(pyridin-2-yl)benzamide (*m*-MPPI) ($K_i = 2.6$ and 1.7 nM, respectively). To enhance the *in vivo* stability of these compounds, the iodine was attached to an aromatic moiety instead of the cyclohexyl ring. However, as found for all analogues of WAY-100635 with an aromatic substituent, both [¹²³I]*p*-MPPI and [¹²³I]*m*-MPPI showed low binding potentials and rapid metabolism in human subjects.^{20,50,51,52} Until now, there is no successful SPECT tracer available to image 5-HT_{1A} receptors. Instead, PET tracers labeled with a longer half-life time isotope than C-11 were developed such as [¹⁸F]*p*-MPPF. This radioligand was rapidly metabolized in human subjects^{53,54,55} and seems to be a substrate for *p*-glycoprotein (*p*-gp)⁵⁶, probably due to the extra aromatic group.⁵⁸ Alternatively, analogues with a saturated moiety such as in *trans*-[¹⁸F]-4-fluoro-*N*-2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl-*N*-(2-pyridyl)cyclohexanecarboxamide, ([¹⁸F]FCWAY) were prepared. The latter showed higher affinity for 5-HT_{1A} receptor than [¹⁸F]*p*-MPPF, but it was unstable *in*

in vivo as elimination of ^{18}F (in the form H^{18}F) of the cyclohexane ring was observed in addition to hydrolysis of the amide bond.^{56,57}

Lately, N-{2-[4-(2-methoxyphenyl)piperazinyl]ethyl} -N-(2-pyridyl)-N-(4-[^{18}F] fluoromethylcyclohexane)carboxamide ([^{18}F]MefWAY) was synthesized and studied *in vivo*. Although it showed promising results, it is still used as an enantiomeric mixture.⁵⁸ In conclusion, all the currently clinical used radiopharmaceuticals for imaging the 5-HT_{1A} receptor have some limitations. Figure 5 shows the chemical structure of some well-validated SPECT and PET radioligands.

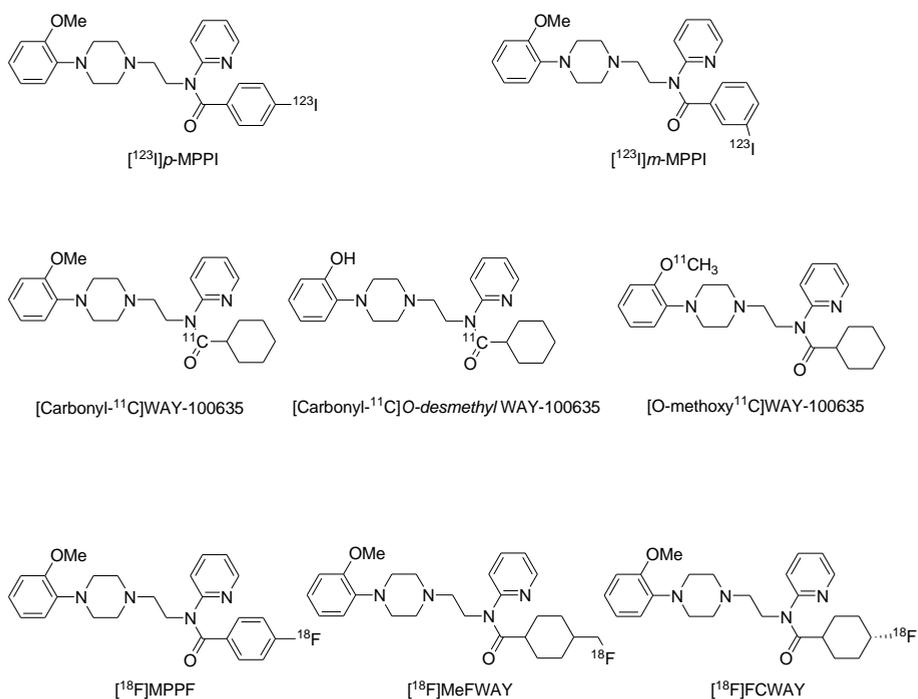


Figure 5. The chemical structure of SPECT and PET radiotracers for 5-HT_{1A} receptor.

Aim of this thesis

The aim of this thesis is to develop novel metabolically stable SPECT and PET radiopharmaceuticals to elucidate the function of the 5-HT_{1A} receptor in neuropsychiatric

disorders. The scope of the thesis includes the design, synthesis and pharmacological evaluation of bridge-fused ring (BFR) analogues of WAY-100635 with a BFR system attached to the carboxamide such as adamantane, cubane, bicyclo[2.2.2]octane and bicyclo[2.2.1]heptane. Such ligands may serve as important tools to investigate the role of the 5-HT_{1A} receptor in CNS disorders.

Outline of the thesis

Chapter 2 encompasses the synthesis of a variety of bridgehead iodinated WAY-100635 analogues and their binding affinity and selectivity for the 5-HT_{1A} receptor. It is expected that these analogues will be more stable *in vivo*, in contrast to normal aliphatic iodine compounds, which deiodinate quickly. A S_N2-reaction is impossible and elimination of the iodine is unlikely since this would lead to a highly strained ring system. Furthermore, it is expected that the bulky structures would reduce the rate of hydrolysis of the amide bond. Beneficially, having WAY-100635 also attached to a bridgehead will keep all analogues a-chiral. Further in this chapter, a no carrier added (nca) synthesis route of the most promising iodinated BFR analogues ([¹²³I]cubane analogue of WAY-100635 and [¹²³I]cubane analogue of *O-desmethylate* WAY-100635) is described, as well as the biodistribution of these two compounds in rats.

Chapter 3 shows the synthesis of fluoromethyl BFR analogues of WAY-100635 and their binding affinity and selectivity for 5-HT_{1A} receptor. Analogues were designed in order to keep high affinity for the 5HT_{1A} receptor, at least comparable to that of WAY-100635, to avoid a-chiral structures, to prevent rapid hydrolysis of the amide bond and ready HF elimination and to enable labeling with F-18 in a last synthesis step. Also, to get a lipophilicity within the range considered optimal for brain penetration and low nonspecific binding.

Chapter 4 describes the radiosynthesis and biodistribution of the most promising fluoromethyl WAY-100635 analogues with BFR = cubane, bicyclo[2.2.2]octane or bicyclo[2.2.1]heptane. The results of the biodistribution and PET studies in rats are described and discussed.

References

1. http://www.who.int/world-health-day/previous/2001/files/whd2001_dare_to_care_en.pdf
2. http://www.who.int/mental_health/management/depression/definition/en/.
3. Paul E, Greenberg MS, Kessler RC, Birnbaum HG, Leong SA, Lowe SW, Berglund PA, Corey-Lisle PK. The economic burden of depression in the United States: How did it change between 1990 and 2000? *J Clin Psychiatry* 64 1465-1475; 2003.
4. Sobocki P, Jönsson B, Angst J, Rehnberg C. Cost of depression in Europe. *J Ment Health Policy Econ* 9 87-98; 2006.
5. Takken J, Polder JJ, Meerding WJ, Kommer GJ, Stokx LJ. *Kosten van ziekten in Nederland*; 2002 (www.kostenvanziekten.nl).
6. *De toekomst van ons zelf*. Rapport van de Gezondheidsraad, p51, Den Haag; 2002.
7. King MV, Marsden CA, Fone KCF. A role for the 5-HT_{1A}, 5-HT₂ and 5-HT₆ receptors in learning and memory. *Trends Pharmacol Sci* 29 482-492; 2008.
8. Saxena PR. Serotonin receptors: subtypes, functional responses and therapeutic relevance. *Pharmacol Ther* 66 339-368; 1995.
9. PJ Cowen. Psychopharmacology of 5-HT_{1A} receptors. *Nucl Med Biol* 27 437-439; 2000.
10. Mastubara S, Arora RC, Meltzer HY. Serotonergic measures in suicide brain -5-HT_{1A} binding-sites in frontal cortex of suicide victims. *J Neural Transm* 85 181-194; 1991.
11. Drevets WC, Frank E, Price CJ, Kupfer DJ, Holt D, Greer PJ, Huang Y, Gautier C, Mathis C. PET Imaging of serotonin 1A receptor binding in depression. *Biol Psychiatry* 46 1375-1387; 1999.
12. Handley SL, McBlane JW. 5-HT drugs in animal models of anxiety. *Psychopharmacology* 112 13-20; 1993.
13. Kepe V, Barrio JR, Cheng Huang S, Ercoli L, Siddarth P, Shoghi Jadid K, Cole GM, Satyamurthy N, Cummings JL, Small GW, Phelps ME. Serotonin 1A receptors in the living brain of Alzheimer's disease patients. *PNAS* 103 702-707; 2006.
14. Pike VW, Halldin C, Wikström HV. Radioligands for the study of the brain 5-HT_{1A} receptors *in vivo*. *Prog Med Chem* 38 189-247; 2001.
15. Bantick RA, Deakin JFW, Grasby PM. The 5-HT_{1A} receptor in schizophrenia: a promising target for novel atypical neuroleptics? *Psychopharmacology* 15 37-46; 2001.
16. Millan MJ. Improving the treatment of schizophrenia: Focus on serotonin (5-HT)_{1A} receptors. *J Pharmacol Exp Ther* 295 853-861; 2000.
17. Yasuno F, Suhara T, Ichimiya T, Takano A, Ando T, Okubo Y. Decreased 5-HT_{1A} receptor binding in amygdala of schizophrenia. *Biol Psychiatry* 55 439-444; 2004.
18. Frazer A, Maayani S, Wolfe BB. Subtypes of receptors for serotonin. *Annu Rev Pharmacol Toxicol* 30 307-348; 1990.
19. Schoeffter P, Bobirnac I, Boddekke E, Hoyer D. Inhibition of cAMP accumulation via recombinant human serotonin 5-HT_{1A} receptors: considerations on receptor effector coupling across systems. *Neuropharmacology* 36 429-437; 1997.

20. J. Passchier, A. van Waarde. Visualisation of serotonin-1A (5-HT_{1A}) receptors in the central nervous system. *Eur J Nucl Med* 28 113-129; 2001.
21. Berlim MT, Fleck MP, Turecki G. Current trends in the assessment and somatic treatment of resistant/refractory major depression: an overview. *Ann Med* 40 149-159; 2008.
22. Rush AJ, Thase ME, Dube S. Research issues in the study of difficult-to-treat depression. *Biol Psychiatry* 53 743-753; 2003.
23. Gómez-Isla T, Hollister R, West H, Mui S, JH Growdon JH, Petersen RC, Parisi JE, Hyman BT. Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. *Ann Neurol* 41 17-24; 1997.
24. Morrison J H, Hof PR. Life and Death of Neurons in the Aging Brain. *Science* 278 412-419; 1997.
25. DeFelipe J, Arellano JI, Gomez A, Azmitia EC, Munoz A. Pyramidal cell axons show a local specialization for GABA and 5-HT inputs in monkey and human cerebral cortex. *J Comp Neurol* 433 148-155; 2001.
26. Kroeze WK, Kristiansen K, Roth LB. Molecular biology of serotonin receptors- structure and function at the molecular level. *Curr Top Med Chem* 2 507-528; 2002.
27. Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR, Humphrey PP. International union of pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). *Pharmacol Rev* 46 157-203; 1994.
28. Roth BL. *The receptors: the serotonin receptors: from molecular pharmacology to human therapeutics*. Totowa, New Jersey, Humana 148; 2006.
29. Chalmers DT, Watson SJ. Comparative anatomical distribution of 5-HT_{1A} receptor messenger-RNA and 5-HT_{1A} binding in rat-brain- a combined *in situ* hybridization *in vitro* receptor autoradiography study. *Brain Res* 561 51-60; 1991.
30. Rang HP, Dale MM, Ritter JM, Moore PK. *Pharmacology*. London, Elsevier Science Limited 184-193; 2003.
31. Rapport MM, Green AA, Page IH. Serum vasoconstrictor (serotonin) IV. Isolation and characterization. *J Biol Chem* 176 1243-1251; 1948.
32. Golan DE, Tashjian AH, Galanter JM, Armstrong AW, Arnaout RA, Rose HS. *Principles of pharmacology: The pathophysiologic basis of drug therapy*. Baltimore, MD, Lippincott Williams & Wilkins 209; 2005.
33. Walther DJ, Bader M. A unique central tryptophan hydroxylase isoform. *Biochem Pharmacol* 66 1673-1680; 2003.
34. Eiden LE, Schäfer M K, Weihe E, Schütz B. The vesicular amine transporter family (SLC18): amine/proton antiporters required for vesicular accumulation and regulated exocytotic secretion of monoamines and acetylcholine. *Pflugers Arch* 447 636-640; 2004.
35. Rang HP, Dale MM, Ritter JM, Moore PK. *Pharmacology*. London, Elsevier Science Limited 545; 2003.
36. Zigmond MJ, Bloom FE, Landis SC, Roberts JL, Squire LR. *Fundamental Neuroscience* San Diego, Academic Press 203-210; 1999.

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37. Range HP, Dale MM, Ritter JM, Moore PK. *Pharmacology*. London, Elsevier Science Limited 480-482; 2003.
 38. O'Brien J, Barben B. Neuroimaging in dementia and depression. *Adv Psychiatr Treat* 6 109-119; 2000.
 39. McKeith I, Volterrani D, Holmes C, Kemp P, Tabet N, Meyer I, Reiningger C, O'Brien J, Walker Z, Tatsch K, Booij J, Darcourt J, Padovani A, Giubbini R, Bonuccelli U. Sensitivity and specificity of dopamine transporter imaging with 123I-FP-CIT SPECT in dementia with Lewy bodies: a phase III, multicentre study. *Lancet Neurology* 6 305-315; 2007.
 40. <http://www.pet.ubc.ca/spect>.
 41. <http://www.pet.ubc.ca/pet>.
 42. <http://www.med-ed.virginia.edu/courses/rad/petct/Attenuation.html>.
 43. Tzimopoulou S, Cunningham VJ, Nichols TE, Searle G, Brid NP, Mistry P, Dixon IJ, Hallett WA, Whitcher B, Brown AP, Zvartau-Hind M, Lotay N, Lai RY, Castiglia M, Jeter B, Matthews Chen K, Bandy D, Reiman EM, Gold M, Rabiner EA, Matthews PM. A multi-center randomized proof-of-concept clinical trial applying [¹⁸F]FDG-PET for evaluation of metabolic therapy with rosiglitazone XR in mild to moderate Alzheimer's disease. *J Alzheimers Dis* 22 1241-1256; 2010.
 44. Rinne JO, Brooks DJ, Rossor MN, Fox NC, Bullock R, Klunk WE, Mathis CA, Blennow K, Barakos J, Okello AA, Rodriguez Martinez de Liano S, Liu E, Koller M, Gregg KM, Schenk D, Black R, Grundman M. 11C-PiB PET assessment of change in fibrillar amyloid-beta load in patients with Alzheimer's disease treated with bapineuzumab: a phase 2, double-blind, placebo-controlled, ascending-dose study. *Lancet Neurol* 9 363-372; 2010.
 45. Cliffe A. A retrospect on the discovery of WAY 100635 and the prospect for improved 5-HT_{1A} receptor PET radioligands. *Nucl Med Biol* 27 441-447; 2000.
 46. Pike VW, Halldin C, Wikström H, Marchais S, McCarron JA, Sandell J, Nowicki B, Swahn C-G, Osman S, Hume SP, Constantinou M, Andree B, Farde L. Radioligands for the study of brain 5-HT_{1A} receptors *in vivo*—Development of some new analogues of WAY. *Nucl Med Biol* 27 449-455; 2000.
 47. Plenevaux A, Weissmann D, Aerts J, Lemaire C, Brihaye C, Degueldre C. Tissue distribution, autoradiography and metabolism of MPPF a new 5-HT_{1A} antagonist for PET: an *in vivo* study in rat. *J Neurochem* 2 803-811, 2000.
 48. Passchier J, Elsinger PH. Quantitative imaging of 5-HT_{1A} receptor binding in healthy volunteers with [¹⁸F]MPPF. *Nucl Med Biol* 27 473-476; 2000.
 49. Pike VW, McCarron JA, Hume SP, Ashworth S, Opacka-Juffry J, Osman S, Lammertsma AA, Poole KG, Fletcher A, White AC, Cliffe IA. Pre-clinical development of a Radioligand for studies of central 5-HT_{1A} receptors *in vivo* [¹¹C]- WAY 100635. *Med Chem Res* 5 208-227; 1994.
 50. Kung HF, Fredrick D, Kim HJ, McElgin W, Kung MP, MU MU, Mozley PD, Vessotskie JM, Stevenson DA, Kushner SA, Zhuang ZP. *In vivo* SPECT imaging of 5-HT_{1A} receptors with [¹²³I]p-MPPI in nonhuman primates. *Synapse* 24 273-281; 1996.
-

51. Zhuang Z, Kung M, Kung HF. Synthesis and evaluation of 4-(2'-Methoxyphenyl)-1-[2'-[N-(2"-pyridinyl)- p-iodobenzamido]ethyl]piperazine (p-MPPI): A new iodinated 5-HT_{1A} ligand. *J Med Chem* 37 1406-1407; 1994.
52. Fletcher A, Bill DJ, Corn IA, Forster EA, Jones D, Reilly Y. A pharmacological profile of WAY-100635, a potent and selective 5-HT_{1A} receptor antagonist. *Br J Pharmacol* 112: 91P; 1994.
53. Zimmer L, Aznavour N. [¹⁸F]MPPF as a tool for the *in vivo* imaging of 5-HT_{1A} receptors in animal and human brain. *Neuropharmacol* 52 695-707; 2007.
54. Lang L, Jagoda E, Schmall B, Vuong BK, Richard Adams H, Nelson DL, Carson RE, Eckelman WC. Development of fluorine-18- labeled 5-HT_{1A} antagonist. *J Med Chem* 42 1576-1582; 1999.
55. Lang L, Jagoda E, Ma Y, Sassaman MB, Eckelman WC. Synthesis and *in vivo* biodistribution of [¹⁸F] labeled 3-cis-3-trans,4-cis-, and 4-trans-fluorocyclohexane derivatives of WAY 100635. *Bioorg Med Chem* 14 3737-3748; 2006.
56. Carson RE, Wu Y, Lang L, Ma Y, Der MG, Herscovitch P, Eckelman WC. Brain uptake of the acid metabolites of F-18- labeled WAY 100635 analogs. *J Cereb Blood Flow Metab.* 23, 249-260; 2003.
57. Hoon Ryu Y, Liow J, Zoghbi S, Fujita M, Collins J, Tipre D, Sangare J, Hong J, Pike VW, Innis RB. Disulfiram inhibits defluorination of ¹⁸F- FCWAY, reduces bone radioactivity, and enhances visualization of radioligand binding to serotonin 5-HT_{1A} receptors in human brain. *J Nucl Med* 48 1154- 1161; 2007.
58. Saigal N, Pichik R, Easwaramoorthy B, Collins D, Christian BT, Shi B, Narayanan TK, Potkin SG, Mukherjee J. Synthesis and biological evaluation of novel serotonin 5-HT_{1A} receptor radioligand, ¹⁸F-labeled Mefway, in rodents and imaging by PET in nonhuman primate. *J Nucl Med* 47 1697-1706; 2006.