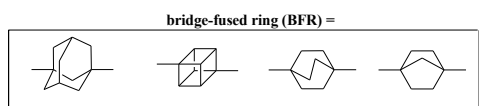
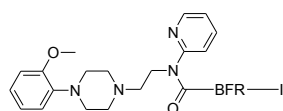


## Summary

In human, the 5-HT<sub>1A</sub> receptor is predominantly located in the brain stem raphe nuclei and in the limbic forebrain (hippocampus, entorhinal cortex and septum).<sup>1</sup> It is thought to be involved in different central nervous system (CNS) disorders such as major depression<sup>2,3</sup>, anxiety<sup>4</sup>, schizophrenia<sup>5,6,7</sup> and Alzheimer's disease<sup>8,9</sup>. Indeed, studies using partial 5-HT<sub>1A</sub> agonists such as the anxiolytic agent buspirone, suggested that the 5-HT<sub>1A</sub> receptor is involved in the pathogenesis and treatment of depression.<sup>1,4</sup> These data may implicate the importance of the 5-HT<sub>1A</sub> receptor as a target for drug therapy and/or as a marker to study the underlying pathophysiology of several major neuropsychiatric disorders. Molecular imaging techniques such as Positron Emission Tomography (PET) or Single Photon Emission Computerized Tomography (SPECT) may be valuable tools in the investigation of changes in the availability of 5-HT<sub>1A</sub> receptors. However, the clinical use of the well-known PET tracer, the 5-HT<sub>1A</sub> receptor antagonist [*carbonyl*-<sup>11</sup>C]WAY-100635, suffers from a drawback of a rapid hydrolysis of the amide bond *in vivo*.<sup>10</sup> In addition, due to the short half-life of the carbon-11 isotope ( $t_{1/2} = 20$  minutes) this tracer can only be used as both a cyclotron and a PET-camera are in close proximity.

This thesis describes the development of novel iodinated (iodine-123,  $t_{1/2} = 13.2$  hours) or fluorinated (fluorine-18,  $t_{1/2} = 109.8$  minutes) analogues of WAY-100635 as possible SPECT or PET radiotracers for the 5-HT<sub>1A</sub> receptor using the hypothesis that a bridge-fused ring (BFR) system might increase their metabolic stability.

Chapter 1 presents a general introduction to the background and context of this research, including an extensive survey of the potential role of the 5-HT<sub>1A</sub> receptor in imaging studies in neuropsychiatric disorders. We explain why we consider molecular imaging (SPECT and PET) a useful tool to study 5-HT<sub>1A</sub> receptor in CNS disorders. In addition, an overview of well-validated SPECT and PET radioligands, as well as their applications and drawbacks, are described followed by the aim of this thesis, which is defined at the end of this chapter.



Chapter 2 describes the synthesis, binding affinity and selectivity of iodinated BFR analogues of WAY-100635. To that end, the cyclohexyl moiety of the lead compounds (WAY-100635 and its *O-desmethylated* analogue) was replaced by a bridgehead iodinated BFR, like adamantane, cubane, bicyclo[2.2.2]octane and bicyclo[2.2.1]heptane. The pharmacological evaluation of these novel ligands resulted in a high (sub)nanomolar affinity and a good selectivity for the 5-HT<sub>1A</sub> receptor. Only the cubane analogues could easily be iodinated with iodine-123 and therefore were selected for further *in vivo* evaluation. Regarding the lipophilicity, replacement of the methoxy group by a hydroxy group had hardly any effect ( $\log D_{7.4}$  4.14 and 4.04, respectively). The affinity for the 5-HT<sub>1A</sub> receptor slightly increased ( $K_i$  1.11 and 0.64, respectively), but this did not lead to a better selectivity. The radiosynthesis was made straightforward by a non-isotopic exchange reaction on the corresponding bromo-compound resulting in a final radiochemical yield of 40% and 35% for the [<sup>123</sup>I]cubane analogue and the [<sup>123</sup>I]cubane *O-desmethyl* analogue, respectively and a calculated specific activity of >1 TBq/ $\mu$ mol at the end of

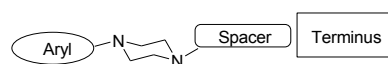


## General discussion

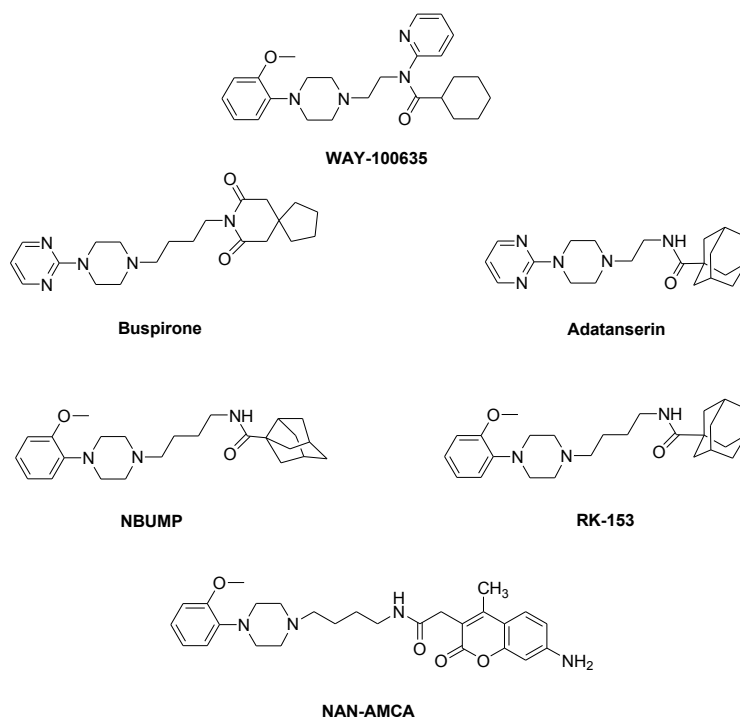
This thesis describes the development of possible SPECT and PET radiotracers for imaging the 5-HT<sub>1A</sub> receptor in the brain. Such a tracer should at least fulfill the following criteria:

- The binding affinity and selectivity for the 5-HT<sub>1A</sub> receptor should be high.
- It should have a low tendency to lead to racemic mixtures during the radiolabeling and during metabolism.
- The bond of the attached radiolabeled atom should be stable.
- The radiosynthesis should be amenable to high yield labeling with high specific activity.
- The lipophilicity should be within the range ( $\log D_{7.4} = 2-3.5$ ) to enable crossing of the blood brain barrier (BBB), and to prevent high non-specific binding.
- The rate of metabolism should be low, and labeled metabolites should not be able to cross the BBB.

All ligands that displayed a low nanomolar to subnanomolar affinity to the 5-HT<sub>1A</sub> receptor are sharing the same backbone chemical structure as shown in the figure below. The long-chain arylpiperazines (LCAPs) must include the two structural features necessary for recognition by sites at this receptor; an aromatic ring and a strongly basic nitrogen atom at a distance of 5.2-5.6 Å.<sup>11</sup> The selectivity of a large number of 5-HT<sub>1A</sub> receptor ligands depend on the nature of their N<sub>4</sub>-substituent (Terminus group and Spacer length). A modification of the Terminus moiety influences both selectivity and efficacy. By variation of the linker moiety of the LCAPs the



first 5-HT<sub>1A</sub> antagonist WAY-100635 was reported.<sup>12</sup> Compounds with slightly shorter or longer chain length were found to display affinity for 5-HT<sub>2</sub>, dopamine D<sub>2</sub> and or  $\alpha_1$ -adrenergic receptors, such as RK-153, which displayed enhanced affinity for D<sub>2</sub> receptors.<sup>13</sup> The Aryl group might be a phenyl, substituted phenyl, heteroaryl, or substituted heteroaryl group. If the aryl group is a pyrimidine an agonistic effect is observed such as for buspirone (5-HT<sub>1A</sub> agonist)<sup>1,4</sup> and adatsanerin (5-HT<sub>1A</sub> partial agonist/5-HT<sub>2</sub> antagonist)<sup>14</sup>. Modification of the Terminus might influence both selectivity and efficacy. NBUMP<sup>15</sup> e.g. behaves both as an antagonist and an agonist in an adenylate cyclase assay. In addition, *in vitro* screening studies of different of 5-HT<sub>1A</sub> ligands identified the LCAP Terminus region as one that could tolerate significant bulky groups without effecting its binding affinity as was found for NAN-AMCA ( $K_i = 0.4$  nM)<sup>16</sup>.



Based on the chemical structure of WAY-100635, a series of 16 bridgehead analogues of WAY-100635 and *O*-desmethyl WAY-100635 with an iodo-BFR or a fluoromethyl-BFR attached to the carboxamide were synthesized. As expected, **the binding affinity** for the 5-HT<sub>1A</sub> receptor of these compounds was comparable to that of WAY-100635 with  $K_i$  values in the (sub)nanomolar range. Five compounds were selected for further investigations; the iodo-cubane, *O*-desmethylated iodo-cubane, fluoromethyl-cubane, fluoromethyl-bicyclo[2.2.2]octane and fluoromethyl-bicyclo[2.2.1]heptane analogues. These compounds show a high **binding selectivity** for the 5-HT<sub>1A</sub> receptor over other relevant receptors.

The proposed molecules were designed to have the labeled atom and the WAY-100634 moiety attached to a bridgehead by which these compounds remain achiral and have the advantage that **no racemic mixtures** can be formed. Hydrolysis of the **carbon-iodine bond** ( $S_N1$  substitution) seems unlikely since the formation of an intermediate cation is expected to be very slow. Shielding by the ring structure will prevent a backside  $S_N2$  attack, while elimination will lead to a highly strained ring system. Attachment of the **-CH<sub>2</sub>F moiety on the bridgehead carbon** has the advantage that HF elimination is no longer possible. As expected, all novel analogues were stable in solvents like dimethyl sulfoxide, ethanol and water for at least several days. **The radiosynthesis** of [<sup>123</sup>I]cubane analogues was a straightforward step with good final radiochemical yields and calculated specific activity at EOS. The [<sup>18</sup>F]fluorinated analogues were also easily synthesized from appropriate precursors in good radiochemical yields and with high effective specific activities. Different reaction conditions were needed for each radiosynthesis, depending on the used leaving group of the precursor.

A useful tracer, for imaging central 5-HT<sub>1A</sub> receptors accurately, has to penetrate to the brain to reach the target receptor and should have a low level of non-specific binding. Molecules could only cross cerebral endothelium by means of active transport or by diffusion through endothelial membranes. For the latter process, it is important that the molecules are lipophilic and have a molecular weight of 400-600 Da.<sup>17</sup> High **lipophilicity** leads to high affinity for plasma proteins resulting in a small free fraction in plasma. Only this small free

fraction is able to cross the BBB.<sup>18</sup> Since the introduction of an iodine atom is known to increase the lipophilicity, which might result in compounds that will not cross the BBB or have a high degree of nonspecific binding, the corresponding *O-desmethyl* analogues were also prepared in order to compensate for this possible and unfavorable effect. Unfortunately, this modification did not lead to a better lipophilicity or selectivity. In Wistar rats, the biodistribution studies showed a poor brain uptake of radioactivity. This indicates that the designed [<sup>123</sup>I]ligands are not suitable for brain SPECT imaging. Although the lipophilicity of the [<sup>18</sup>F]fluoromethyl ligands was within the required range, the brain uptake of radioactivity in rats was almost similar to that of the [<sup>123</sup>I]ligands at 45 minutes. It has been demonstrated that high density of P-glycoprotein (Pgp) is present in the brain endothelium. Pgp is a transmembrane protein that functions as an ATP-dependent efflux pump.<sup>19,20,21,22,23</sup> Many structurally diverse compounds have been identified as a substrate for Pgp such as the fluorinated analogue of WAY-100635, [<sup>18</sup>F]*p*-MPPF.<sup>1</sup> It is believed that tracers with aromatic rings and cationic centers are possible Pgp substrates.<sup>24</sup> It is possible that the poor brain uptake of the radioactivity of both series is because the cage structure makes them an effective Pgp substrate.

*In vitro* there was no significant difference in the binding affinity and selectivity for both series. However, *in vivo* the specific to non-specific binding ratios of the [<sup>18</sup>F]fluoromethyl ligands were much better than that of the [<sup>123</sup>I]ligands. This might be caused by the bulkiness of the BFR-I Terminus or by the higher lipophilicity leading to more specific binding.

The brain uptake over time and the specific binding of the fluorinated radioligands in rats were determined using PET. Unfortunately, there was a high accumulation of radioactive fluoride in bones at the end of the study. Although it is not clear which step is occurring first (the hydrolysis of the amide bond or the defluorination), all three radioligands defluorinated *in vivo* despite the fact that HF elimination is chemically impossible. Possible mechanisms for this **metabolic** instability might be  $\alpha$ -hydroxylation by e.g. cytochrome P450 isozyme 2E1 or a nucleophilic substitution by glutathione S-transferase.

The **metabolic** stability in human hepatocytes of the [<sup>123</sup>I] and [<sup>18</sup>F] radiolabeled analogues of the cubane was studied and compared with a well-known 5-HT<sub>1A</sub> receptor radiotracer [<sup>18</sup>F]*p*-MPPF. Both radiotracers showed a lower rate of hydrolysis than [<sup>18</sup>F]*p*-MPPF. This higher metabolic stability can be largely ascribed to a decrease in rate of the amide hydrolysis due to steric hindrance by the cage structure.

In conclusion, novel BFR analogues of WAY-100635 were designed with the aim to obtain SPECT or PET ligands with high metabolic stability. Although the ultimate goal was not reached, these synthesized ligands have provided us more insight in the structural requirements that are important for the lipophilicity, affinity and selectivity for the 5-HT<sub>1A</sub> receptor, as well as for the metabolic stability regarding the amide bond hydrolysis and the deiodination or defluorination. Ultimately, this knowledge might help to develop radioligands with an improved stability profile such as derivatives with a CF<sub>3</sub> group instead of the CH<sub>2</sub>F or which have the fluoro atom directly attached to the bridgehead.

## References

1. Passchier J, Van Waarde A. Visualisation of serotonin-1A (5-HT<sub>1A</sub>) receptors in the central nervous system. *Eur J Nucl Med* 28 113-129; 2001.
2. Matsubara S, Arora R C, Meltzer HY. Serotonergic measures in suicide brain -5-HT<sub>1A</sub> binding-sites in frontal cortex of suicide victims. *J Neural Transmissions* 85 181-194; 1991.
3. 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.
4. Handley SL, McBlane JW. 5-HT drugs in animal models of anxiety. *Psychopharmacology* 112 13-20; 1993.
5. Bantick RA, Deakin JFW, Grasby PM. The 5-HT<sub>1A</sub> receptor in schizophrenia: a promising target for novel atypical neuroleptics? *Psychopharmacology* 15 37-46; 2001.
6. Millan MJ. Improving the treatment of schizophrenia: Focus on serotonin (5-HT)<sub>1A</sub> receptors. *J Pharmacol Exp Ther* 295 853-861; 2000.
7. Yasuno F, Suhara T, Ichimiya T, Takano A, Ando T, Okubo Y. Decreased 5-HT<sub>1A</sub> receptor binding in amygdala of schizophrenia. *Biol Psychiatry* 55 439-444; 2004.
8. 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.
9. Pike VW, Halldin C, Wikström HV. Radioligands for the study of the brain 5-HT<sub>1A</sub> receptors *in vivo*. *Prog Med Chem.* 38 189-247; 2001.
10. Cliffe IA. A retrospect on the discovery of WAY 100635 and the prospect for improved 5-HT<sub>1A</sub> receptor PET radioligands. *Nucl Med Biol* 27 441-447; 2000.
11. Orjales A, Alonso L, Labeaga L, Corcóstegui R. New (2-Methoxyphenyl)piperazine derivatives as 5-HT<sub>1A</sub> receptor ligands with reduced  $\alpha_1$ -adrenergic activity. Synthesis and structure- affinity relationships. *J Med Chem* 38 1273-1277; 1995.
12. Glennon RA, Dukat M. 5-HT<sub>1</sub> receptor ligands: update 1997. *Invest Drugs Res Alert* 2 351-372; 1997.
13. Raghupathi RK, Rydelek-Fitzgerald L, Teitler M, Glennon RA. Analogues of the 5-HT<sub>1A</sub> serotonin antagonist 1-(2-methoxyphenyl)-4-[4-(2-phthalimidio)-butyl]-piperazine with reduced  $\alpha_1$ -adrenergic affinity. *J Med Chem* 34 2633-2638; 1991.
14. Abou-Gharbia MA, Childers WE jr., Fletcher H, McGaughey G, Patel U, Webb MB, Tardley J, Andree T, Boast C, Kucharik RJ, Marquis K, Morris H, Scemi R, Moyer JA. Synthesis and SAR of adatsensin: novel adamantly aryl- and heteroaryl piperazine with dual serotonin 5-HT<sub>1A</sub> and 5-HT<sub>2</sub> activity as potential anxiolytic and antidepressant agents. *J Med Chem* 42 5077-5094; 1999.
15. Glennon RA. Concepts for the design of 5-HT<sub>1A</sub> serotonin agonist and antagonists. *Drug Dev Res* 26 251-274; 1992.
16. Roth BL. *The receptors: the serotonin receptors: from molecular pharmacology to human therapeutics*. Totowa, New Jersey, Humana 115; 2006.
17. Pardridge WM. Transport of small molecules through the blood-brain barrier: biology and methodology. *Adv Drug Deliv Rev* 15 5-36; 1995.
18. Waterhouse RN. Determination of lipophilicity and its use as a predictor of blood-brain barrier penetration of molecular imaging agents. *Mol Imaging Biol* 5 376-389; 2003.
19. Abbott NJ, Romero IA. Transporting therapeutics across the blood-brain barrier. *Mol Med Today* 2 106-113; 1996.
20. Bradbury MW. The blood-brain barrier. *Exp physiol* 78 453-472; 1993.
21. Begley DJ. The blood-brain barrier: principles for targeting peptides and drugs to central nervous system. *J Pharm Pharmacol* 48 136-146; 1996
22. Kartner N, Riordan JR, Ling V. Cell surface P-glycoprotein associated with multidrug resistance in mammalian cell lines. *Science* 221 1285-1288; 1983.
23. Schinkel AH. The physiological function of drug-transporting P-glycoproteins. *Semin Cancer Biol* 8 161-170; 1997.
24. Paschier J, van Waard A, Doze P, Elsinga PH, Vaalburg W. Influence of P-glycoprotein on brain uptake of [<sup>18</sup>F]MPPF in rats. *Eur J Pharmacol* 407 273-280; 2000.