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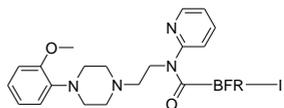
Summary and general discussion

Summary

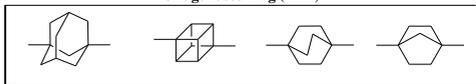
In human, the 5-HT_{1A} receptor is predominantly located in the brain stem raphe nuclei and in the limbic forebrain (hippocampus, entorhinal cortex and septum).¹ It is thought to be involved in different central nervous system (CNS) disorders such as major depression^{2,3}, anxiety⁴, schizophrenia^{5,6,7} and Alzheimer's disease^{8,9}. Indeed, studies using partial 5-HT_{1A} agonists such as the anxiolytic agent buspirone, suggested that the 5-HT_{1A} receptor is involved in the pathogenesis and treatment of depression.^{1,4} These data may implicate the importance of the 5-HT_{1A} 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_{1A} receptors. However, the clinical use of the well-known PET tracer, the 5-HT_{1A} receptor antagonist [*carbonyl*-¹¹C]WAY-100635, suffers from a drawback of a rapid hydrolysis of the amide bond *in vivo*.¹⁰ 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_{1A} 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_{1A} receptor in imaging studies in neuropsychiatric disorders. We explain why we consider molecular imaging (SPECT and PET) a useful tool to study 5-HT_{1A} 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.

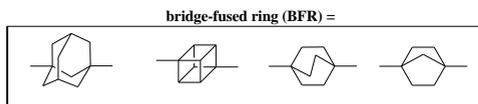
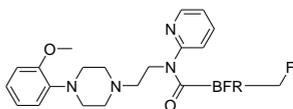


bridge-fused ring (BFR) =



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_{1A} 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_{1A} 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 [¹²³I]cubane analogue and the [¹²³I]cubane *O-desmethyl* analogue, respectively and a calculated specific activity of >1 TBq/μmol at the end of synthesis (EOS). The metabolic stability of the [¹²³I]cubane analogue was investigated in human hepatocytes and compared with that of the well-validated radiotracer for the 5-HT_{1A} receptor named [¹⁸F]*p*-MPPF. At 15 minutes of incubation, >90% of the parent compound of analogue of [¹²³I]cubane was still present compared to only 45% of the parent compound of [¹⁸F]*p*-MPPF, indicating a remarkable stabilizing effect of the cubyl moiety on the amide hydrolysis. In addition, there was no free iodide-123 detected up to 150 min of incubation, which indicates that the carbon-iodine bond is metabolically stable in human hepatocytes. This bond was metabolically stable in rats too. During the biodistribution studies, no uptake of a radioactive iodide was observed in the thyroid. Unfortunately, both radioligands showed a poor uptake in the rat brain at 45 minutes. In addition, low specific to non-specific binding ratios (ratios of uptake in the hippocampus over the cerebellum were 2.51 and 2.38, respectively) were observed in the brain for both analogues. The

above results indicate that the designed [^{123}I]ligands are not suitable for SPECT brain imaging.



Chapter 3 describes a new series of eight (WAY-100635 and *O-desmethyl* WAY-100635) analogues in which the same BFR's were used but in this case with a fluoromethyl group. This led to an improvement in the lipophilicity compared to the iodinated BFR analogues of WAY-100635. *In vitro*, all analogues showed a high (sub)nanomolar affinity and a good selectivity for the 5-HT_{1A} receptor. Three ligands (cubane, bicyclo[2.2.2]octane and bicyclo[2.2.1]heptane) were selected for further evaluation. The lipophilicity of these ligands ($\log D_{7.4}$ 2.94, 3.37 and 2.70, respectively) was in the same order of magnitude as that of WAY-100635 ($\log D_{7.4}$ 3.03). Investigation of the metabolic stability of the [^{18}F]fluoromethyl cubane analogue in human hepatocytes showed that >73% of the parent analogue was still present at 15 minutes of incubation. The *in vitro* results made these ligands worthwhile for further *in vivo* investigation as potential 5-HT_{1A} receptor binding ligands and promising ^{18}F -labeled PET tracers.

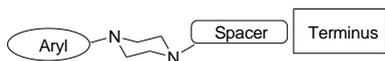
Chapter 4 presents the data for the three selected, and novel, radiofluorinated BFR analogues of WAY-100635. Routes were developed to synthesize the precursors required for a radiofluorination reaction. A nucleophilic substitution using fluorine-18 yielded the radioligands in moderate to good radiochemical yields (24-45%) and with specific activities of about 100 GBq/ μmol . The biodistribution studies in rats showed that the uptake of radioactivity of these radioligands is nearly similar to that of iodine-123 analogues, but the specific to non-specific binding ratios were remarkably higher (ratios of uptake in the hippocampus over the cerebellum were 5.55, 4.79 and 5.45, respectively). However, it was rather disappointing that the PET studies showed a high accumulation of $^{18}\text{F}^-$ in the bones. The rate of bone uptake and thus the order of defluorination was for [^{18}F]bicyclo[2.2.1]heptane \approx [^{18}F]cubane > [^{18}F]bicyclo[2.2.2]octane. This instability toward *in vivo* enzymatic defluorination in rats, suggest that these radioligands are not better than the already clinically used WAY-100635 analogues.

General discussion

This thesis describes the development of possible SPECT and PET radiotracers for imaging the 5-HT_{1A} receptor in the brain. Such a tracer should at least fulfill the following criteria:

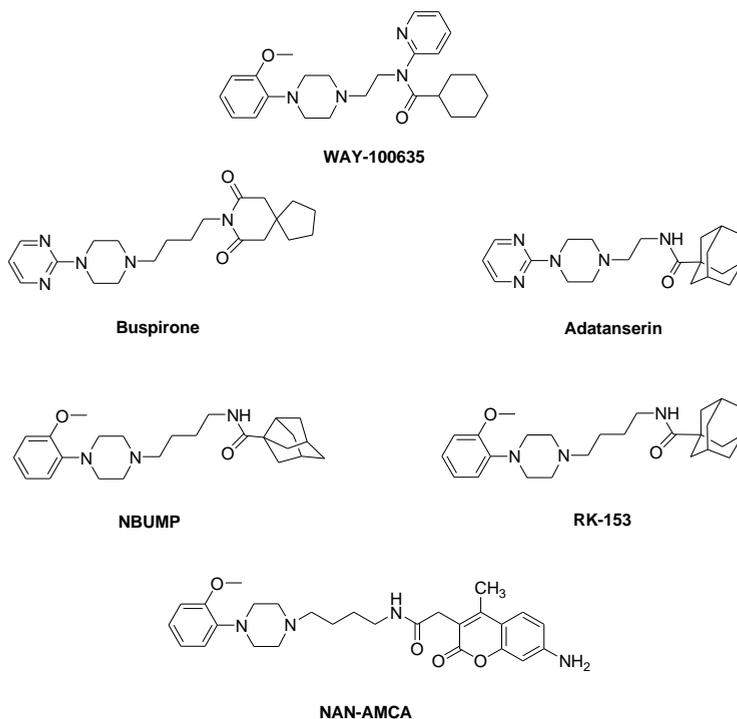
- The binding affinity and selectivity for the 5-HT_{1A} receptor should be high.
- It should have a low tendency to lead to racemic mixtures during the radiolabeling and during metabolism.
- The bond of the attached radiolabeled atom should be stable.
- The radiosynthesis should be amenable to high 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_{1A} 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.¹¹ The selectivity of a large number of 5-HT_{1A} receptor ligands depend on the nature of their N₄-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_{1A} antagonist WAY-100635 was reported.¹² Compounds with slightly shorter or longer chain length were found to display affinity for 5-HT₂, dopamine D₂ and or α_1 -adrenergic receptors, such as RK-153, which displayed enhanced affinity for D₂ receptors.¹³ 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_{1A} agonist)^{1,4} and adatsersin (5-HT_{1A} partial

agonist/5-HT₂ antagonist)¹⁴. Modification of the Terminus might influence both selectivity and efficacy. NBUMP¹⁵ 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_{1A} 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)¹⁶.



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_{1A} 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 analo-

gues. These compounds show a high **binding selectivity** for the 5-HT_{1A} 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 **-CH₂F 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 [¹²³I]cubane analogues was a straightforward step with good final radiochemical yields and calculated specific activity at EOS. The [¹⁸F]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_{1A} 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.¹⁷ 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.¹⁸ 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 [¹²³I]ligands are not suitable for brain SPECT imaging. Although the lipophilicity of the [¹⁸F]fluoromethyl ligands was within the required range, the brain uptake of radioactivity in rats was almost similar to that of the [¹²³I]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.^{19,20,21,22,23} Many structurally diverse compounds have been identified as a substrate for Pgp such as the fluorinated analogue of WAY-100635, [¹⁸F]*p*-MPPF.¹ It is believed that tracers with aromatic rings and cationic centers are possible Pgp substrates.²⁴ 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 [¹⁸F]fluoromethyl ligands were much better than that of the [¹²³I]ligands. This might be caused by the bulkiness of the BFR-I Terminus or by the higher lipophilicity leading to more aspecific 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 α -hydroxylation by e.g. cytochrome P450 isozyme 2E1 or a nucleophilic substitution by glutathione S-transferase.

The **metabolic** stability in human hepatocytes of the [¹²³I] and [¹⁸F] radiolabeled analogues of the cubane was studied and compared with a well-known 5-HT_{1A} receptor radiotracer [¹⁸F]*p*-MPPF. Both radiotracers showed a lower rate of hydrolysis than [¹⁸F]*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_{1A} 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_3 group instead of the CH_2F or which have the fluoro atom directly attached to the bridgehead.

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