European Journal of Medicinal Chemistry 46 (2011) 5728–5735

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Design, synthesis and *in vitro* evaluation of bridgehead fluoromethyl analogs of *N*-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-*N*-(pyridin-2-yl) cyclohexanecarboxamide (WAY-100635) for the 5-HT_{1A} receptor

Rana Al Hussainy [a,*], Joost Verbeek [a], Dion van der Born [a], Jan Booij [b], J(Koos) D.M. Herscheid [a]

[a] Department of Nuclear Medicine & PET Research, VU University Medical Center, PO Box 7057, 1007MB Amsterdam, The Netherlands
[b] Department of Nuclear Medicine, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

ARTICLE INFO

Article history:
Received 18 March 2011
Received in revised form
10 June 2011
Accepted 16 June 2011
Available online 29 June 2011

Keywords:
5-HT_{1A} receptor
WAY-100635
Bridge-fused ring

ABSTRACT

Fluorinated analogs that are related to the 5-hydroxytryptamine (5-HT_{1A}) antagonist, *N*-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-*N*-(pyridin-2-yl)cyclohexanecarboxamide (WAY-100635), have been synthesized and their binding affinity for the 5-HT_{1A} receptor and other neurotransmitter receptors (adrenoceptors, sigma receptors, and dopamine receptors), and serotonin transporters was examined *in vitro*. These ligands were designed to provide a possible potential positron emission tomography (PET) ligand with high metabolic stability. To this end, the cyclohexyl moiety in WAY-100635 and in *O-desmethyl* WAY-100635 was replaced by a bridge-fused ring (BFR) such as adamantyl, cubyl, bicyclo[2.2.2] octyl and bicyclo[2.2.1]heptyl to reduce the metabolic rate of the amide bond hydrolysis, while a fluoromethyl group was introduced on the other bridgehead of the BFR to prevent defluorination by HF elimination. All synthesized analogs displayed high affinity in the (sub)nanomolar range for the 5-HT_{1A} receptor, comparable to WAY-100635. In addition, **6b**, **6c** and **6d** were reasonably selective to the 5-HT_{1A} receptor over the above mentioned receptors. In human hepatocytes, **6b** showed a suitable metabolic stability.

In conclusion, the obtained data provides a promising starting point for the synthesis of the corresponding ^{18}F-labeled PET analogs .

1. Introduction

Among many 5-HT_{1A}[1] receptor ligands, WAY-100635 was developed as the first potent, "silent" and selective 5-HT_{1A} receptor antagonist. This compound has a binding selectivity of at least >10-fold relative to other 5-HT receptor subtypes, major neurotransmitter receptors, re-uptake and ion channel sites, however it showed also high affinity for α_1-adrenoceptors and D_4 receptors [1]. Later, WAY-100635 was labeled with carbon-11 ([carbonyl-^{11}C] WAY-100635) and evaluated to study *in vivo* changes in the densities of 5-HT_{1A} receptors in several neuropsychiatric disorders, such as major depression and anxiety disorders [2,3], Alzheimer's disease [2,4,5] and schizophrenia [2,6] using PET. Studies of the metabolic pathway of [carbonyl-^{11}C] WAY-100635 in humans revealed rapid metabolism to WAY-100634 due to the hydrolysis of the amide bond, which interfere the PET measurements (Fig. 1) [7–9]. In addition, a disadvantage of using carbon-11 ($t_{1/2}$ = 20 min) radiotracers is that these tracers can only be used where both a cyclotron and a PET-camera are in close proximity due to it short half-life. Zhuang and co-workers have shown in a series of benzamido analogs of WAY-100635 a limited tolerance for variations at the amino pyridine position in the native receptor [10]. Therefore, research has been directed toward WAY-100635 analogs that were labeled with the longer lived fluorine-18 ($t_{1/2}$ = 110 min) isotope with the label outside the WAY-100634 moiety [8]. [^{18}F] fluorinated analogs of WAY-100635 that contain an aromatic

* Corresponding author. Tel.: +31 20 4449704; fax: +31 20 4449121.
E-mail address: rhussainy@rnc.vu.nl (R. Al Hussainy).

[1] *Abbreviations*: 5-HT, 5-hydroxytryptamine; BFR, bridge-fused ring; PET, positron emission tomography; D4, dopamine D4 receptor; [^{18}F]MPPF, 4-[^{18}F]fluoro-N-{2-[1-(2-methoxyphenyl)piperazine-1-yl]ethyl}-N-(pyridine-2-yl)benzamide; [^{18}F] MeFBWAY, 3-methyl-4-[^{18}F]fluoro-N-{2-[1-(2-methoxyphenyl)piperazine-1-yl] ethyl}-N-(pyridine-2-yl)benzamide; [^{18}F]FCWAY, 4-[^{18}F]fluoro-N-2-[4-(2-methoxyphenyl)piperazin-1-ylethyl]-N-(2-pyridyl) cyclohexanecarboxamide; [^{18}F] MeFWAY, N-{2-[4-(2-Methoxyphenyl)piperazinyl]ethyl}-N-(2-pyridyl)-N-(4-[^{18}F] fluoromethyl-cyclohexane)carboxamide; LG, leaving group; TBAF, tetrabutyl ammonium fluoride; IC_{50}, concentration producing 50% inhibition; K_i, apparent equilibrium inhibition constant; K_D, apparent equilibrium dissociation constant; t_R, retention time; Log $D_{7.4}$, log base ten of the partition coefficient at pH = 7.4; THF, tetrahydrofuran; DIPA, diisopropylamine.

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doi:10.1016/j.ejmech.2011.06.023

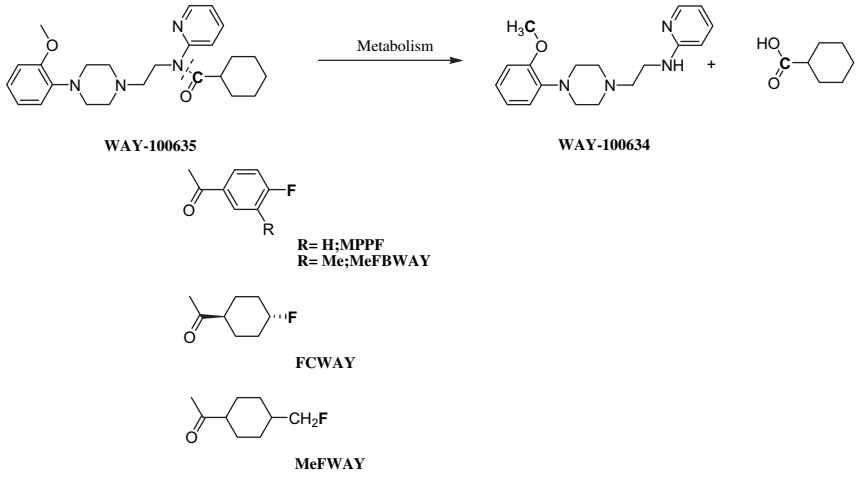
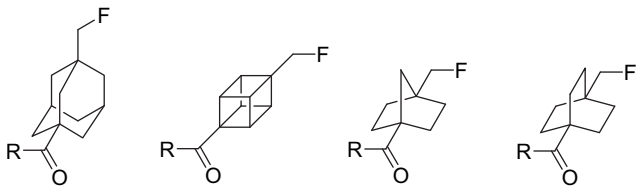


Fig. 1. WAY-100635, WAY-100634 and WAY-100635 analogs; MPPF, MeFBWAY, FCWAY and MeFWAY.

moiety in the carboxamide part instead of the cyclohexyl moiety such as 4-[^{18}F]fluoro-N-{2-[1-(2-methoxyphenyl)piperazine-1-yl] ethyl}-N-(pyridine-2-yl)benzamide ([^{18}F]*p*-MPPF) [11–13] and 3-methyl-4-[^{18}F]fluoro-N-{2-[1-(2-methoxyphenyl)piperazine-1-yl] ethyl}-N-(pyridine-2-yl)benzamide ([^{18}F]MeFBWAY) were prepared [11] (Fig. 1). [^{18}F]*p*-MPPF showed ca. 4–6 times lower *in vivo* binding potentials than [carbonyl-^{11}C] WAY-100635 [13]. [^{18}F]MeFBWAY showed ca. 7 times lower *in vitro* binding affinity than WAY-100635 [11]. Displacement experiments of [^{18}F]*p*-MPPF with WAY-100635 in the hippocampus of rats showed only 60% [11] to 77% [12] brain uptake reduction and 80% of [^{18}F]MeFBWAY [11]. [^{18}F]*p*-MPPF was rapidly metabolized in human subjects [13,14], while [^{18}F]MeFBWAY is less selective than [^{18}F]*p*-MPPF. [^{18}F] MeFBWAY has a relatively high affinity for α_1-adrenoceptors (K_i = 13.2 nM) compared to [^{18}F]*p*-MPPF (K_i = 151 nM) [11]. Alternatively, analogs with a saturated moiety such as [^{18}F]-4-fluoro-N-2-[4-(2-methoxyphenyl)piperazin-1-ylethyl]-N-(2-pyridyl) cyclohexanecarboxamide, ([^{18}F]FCWAY) were prepared (Fig. 1).

The *trans*-isomer of [^{18}F]FCWAY showed higher binding affinity for the 5-HT$_{1A}$ receptor than [^{18}F]*p*-MPPF and [^{18}F]MeFBWAY, but now, in addition to the hydrolysis of the amide bond, defluorination was observed [11]. This led to a substantial skull uptake in human of [^{18}F] amounting to twice that of average uptake in the whole brain at 60 min post-injection, which hampers clinical applications [15–17]. Placing fluorine on a primary carbon as in N-{2-[4-(2-methoxyphenyl)piperazinyl]ethyl}-N-(2-pyridyl)-N-(4-[^{18}F]fluoromethyl-cyclohexane)carboxamide ([^{18}F]MeFWAY) greatly reduced this *in vivo* instability [18].

Recently, we reported the synthesis of bridgehead iodinated WAY-100635 analogs and the evaluation of their biological properties *in vitro* as potential SPECT tracers for imaging 5-HT$_{1A}$ receptor [19]. This study revealed that the bridge-fused rings (BFRs) are well tolerated with respect to affinity for the 5-HT$_{1A}$ receptor. Furthermore, it confirmed the finding of Wilson [20] that a more bulky group reduces the hydrolysis of the amide bond.



R = WAY-100634, *O-desmethyl* WAY-100634

Fig. 2. Novel analogs of WAY-100635 and *O-desmethyl* WAY-100635.

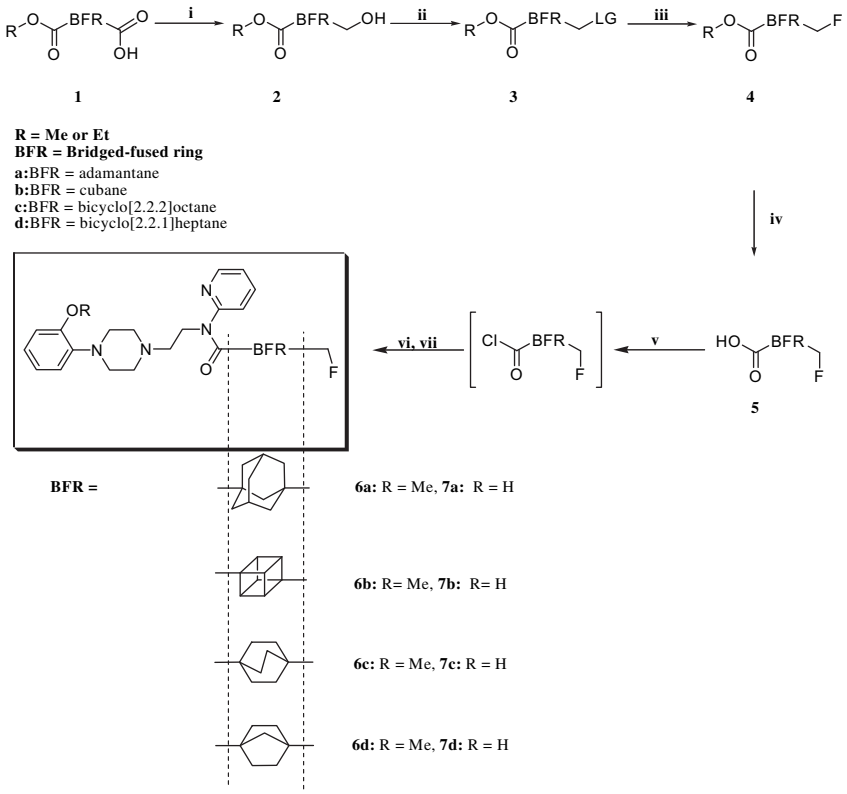
The aim of the present study was to synthesize analogs of WAY-100635 and *O-desmethyl* WAY-100635 with a BFR moiety attached to the carboxamide and with a fluoromethyl group on the other bridgehead of the BFR (Fig. 2). Based on their chemical structure, these compounds were expected to have a high affinity for the target 5-HT$_{1A}$ receptor, at least comparable to that of WAY-100635. The rapid *in vivo* hydrolysis of the amide bond is probably reduced due to steric hindrance. Defluorination might be prevented because fluorine is placed on a primary carbon and in such a position that makes HF elimination chemically impossible. In addition, it is also expected that these compounds will have a lipophilicity within the range (Log D$_{7.4}$ = 2–3.5) [21], which is considered optimal for brain penetration and low non-specific binding. An a-chiral structure is maintained which avoid the often difficult separation of enantiomers during the chemical synthesis. Moreover, the labeling with fluorine-18 can be performed in a last synthesis step when these compounds are being used as radiopharmaceuticals. Herein, their synthesis and *in vitro* binding affinity, selectivity and stability are described.

2. Chemistry

A general pathway for the synthesis of compounds **5a**–**d** is outlined in Scheme 1. Compounds **1a** and **1b** were synthesized by mono-saponification of the commercially available di-esters, according to literature procedures of Eaton [22] and Frazer [23]. 4-(Ethoxycarbonyl)bicyclo[2.2.2]octane-1-carboxylic acid (**1c**)[2] was obtained from diethyl 2,5-dioxocyclohexane-1,4-dicarboxylate in four steps as described by Boulerice and Frazer [23–26]. Compound **1d** was synthesized by mono-saponification of dimethyl bicyclo[2.2.1]heptane-1,4-dicarboxylate, which was synthesized according to a method of Della and Tsanaktsidis [27].

Compound **2** was synthesized starting from a reduction of the appropriate acid **1** with borane dimethyl sulfide complex [28]. In order to obtain the fluorinated compounds, both nucleophilic substitution reactions and electrophilic substitution reactions were

[2] 4-(Methoxycarbonyl)bicyclo[2.2.2]octane-1-carboxylic acid is nowadays commercially available, see Experimental section - Chemistry.



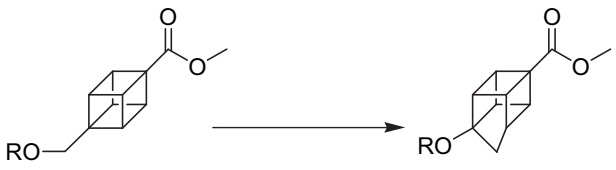
Scheme 1. General pathway for the synthesis of WAY-100635 and *O-desmethyl* WAY-100635 analogs : (i) $Me_2S.BH_3$, THF, 0 °C; (ii) conversion to a LG by several methods (see Discussion); (iii) nucleophilic fluorination (see Discussion); (iv) KOH, MeOH or EtOH, reflux. (v) thionyl chloride, MeCN, reflux; (vi) WAY-100634, NEt_3, MeCN, RT.; (vii, for *O-desmethyl* WAY-100634 analogs) MeOH:$NaHCO_3$:H_2O (2:1:1), 50 °C, 2 h.

studied carefully. Electrophilic fluorinations of compounds **2** using (dimethylamino)sulfur trifluoride (DAST) [29] or Yarovenko [30] reagent were unsuccessful. Maybe, due to the formation of an intermediate cation, a rearrangement has taken place leading to a ring enlargement. For a nucleophilic substitution reaction, the primary alcohols (**2a**–**d**) needed to be converted into a good leaving group (LG). Of all tested leaving groups (LG = tosylate, triflate, iodide) the triflate gave the best results in the subsequent fluorination step. The triflate compounds **3a**, **3c** and **3d** were obtained using triflic anhydride [31]. Fluorination with tetrabutyl ammonium bifluoride (TBABF) [32], TBAF (1 M in THF), TBAF·xH_2O [33], KF [34], KF in crown ether [35] or AgF did not give the desired products in a reasonable yield. The fluorination of **3a**, **3c** and **3d** (LG = Triflate) with TBAF·(t-BuOH)$_4$ complex gave **4a**, **4c** and **4d** in good yields [36,37]. Subsequent saponification gave the corresponding derivatives **5** [21,22].

Compound **3b** was totally unstable with all candidates of leaving groups, except the bromide. According to COSY and NOSY NMR spectra, this compound (**3b**) seems to rearrange to a homocubyl product (Scheme 2). Therefore, **4b** was synthesized by bromination of **2b** with tetrabromomethane and triphenylphoshine followed by fluorination with tetrabutyl ammonium fluoride (TBAF, 1 M in THF). This compound was purified by prep-HPLC instead of normal column chromatography to avoid rearrangement to the homocubyl derivatives. The collected fractions were directly converted into the acid **5b**.

Formation of the acid chlorides of **5a**–**d** was achieved with thionyl chloride in MeCN [7]. Acylation of WAY-100634 with the appropriate acid chloride gave the four analogs **6a**–**d** in moderate to high yields (59–97%). WAY-100634 (Fig. 1) was prepared according to procedures described by Pike [7] and Cliffe [38]. For the synthesis of *O-desmethyl* WAY-100634 the same route was used. However, it was also prepared by demethylation of WAY-100634 [39]. Upon reaction of the acid chlorides with *O-desmethyl* WAY-100634 a competition was always observed between N- and O-acylation. Therefore, this compound was first double acylated after which the O-acyl bond was broken with base (mixture of MeOH/saturated $NaHCO_3$/H_2O; 2:1:1) to afford the analogs **7a**–**d** also in moderate to high yields (32–87%), Scheme 1.

All synthesized analogs were stable in solvents like dimethylsulfoxide, ethanol and water for at least several days. Even after six months in cold (−20 °C) ethanol no decomposition was detected, both by HPLC and ^{1}H NMR analysis.

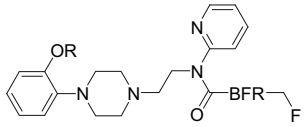


Scheme 2. Rearrangement of **3b** to a homocubyl compound. R = Tosylate, Nosylate, Triflate.

3. The lipophilicity of the investigated compounds

The lipophilicity (Log $D_{7.4}$ values) of all compounds was calculated by using MarvinSketch (http://www.chemaxon.com). Data are shown in Table 1. The Log $D_{7.4}$ of the three selected compounds **6b**, **6c**, and **6d** were determined in comparison to WAY-100635. The Log $D_{7.4}$ ± S.D. values for **6b**, **6c** and **6d** were 2.94 ± 0.01, 3.37 ± 0.05 and 2.70 ± 0.01, respectively and 3.03 ± 0.04 for WAY-100635.

Table 1
Calculated Log $D_{7.4}$ value and affinity for the human 5-HT_{1A} receptor of WAY-100635 analogs .



Compd	R	BFR	cLog $D_{7.4}$[a]	IC_{50} (nM)[b]	5-HT_{1A} K_i (nM)[c]
WAY-100635	CH_3	–	4.08	0.91 ± 0.43	0.39
6a	CH_3	Adamantyl	4.48	4.27 ± 0.15	1.82
6b	CH_3	Cubyl	2.04[d]	1.27 ± 0.10	0.54
6c	CH_3	Bicyclo[2.2.2]octyl	4.37	1.53 ± 0.01	0.65
6d	CH_3	Bicyclo[2.2.1]heptyl	3.93	2.02 ± 0.02	0.86
O-desmethyl WAY−100635	H	–	3.93	0.77 ± 0.36	0.33
7a	H	Adamantyl	4.33	2.59 ± 0.20	1.10
7b	H	Cubyl	1.89[d]	1.35 ± 0.04	0.58
7c	H	Bicyclo[2.2.2]octyl	4.22	1.76 ± 0.03	0.75
7d	H	Bicyclo[2.2.1]heptyl	3.78	1.74 ± 0.06	0.74

[a] Calculated Log $D_{7.4}$ using MarvinSketch (http://www.chemaxon.com).
[b] Results are presented as concentration producing 50% inhibition (IC_{50}) in nM and the mean of three experiments per drug ± SD, ($n = 3$).
[c] K_i values were calculated as $K_i = IC_{50}/(1 + C/K_D)$, with $C = 0.5$ nM, $K_D = 1.48$ nM [40].
[d] Probably, these values were not correctly calculated, due to the presence of the cubyl moiety.

4. Receptor binding *in vitro*

4.1. 5-HT_{1A} receptor binding

Radioligand binding experiments were performed using the 5-HT_{1A} agonist [^{3}H]8-OH-DPAT and a membrane suspension of cells expressing the cloned human 5-HT_{1A} receptor. Inhibition curves were measured for the eight compounds and the concentration producing 50% inhibition (IC_{50}) for each were derived from non-linear regression curve fitting and apparent equilibrium inhibition constant (K_i) values were calculated according to the Cheng–Prusoff equation [40]. Data are shown in Table 1. All compounds revealed affinity for the 5-HT_{1A} receptor in the low nanomolar range.

4.2. Receptor binding profile

The selectivity for the 5-HT_{1A} receptor over other relevant receptors of three promising WAY-100635 analogs for PET, **6b**–**d**, was determined by NIMH/Psychoactive Drug Screening Program (PDSP). The results are shown in Table 2. A typical inhibition curve is shown in Fig. 3.

5. Metabolic stability

The metabolic stability study was performed using radiolabeled [^{18}F]**6b** (see Supplementary data) and was compared with [^{18}F]*p*-MPPF [19]. The preliminary study showed that incubation of [^{18}F]**6b** with human hepatocytes demonstrated a better metabolic stability than [^{18}F]*p*-MPPF. Results are depicted in Table 3.

6. Discussion

Similar to central nervous system drug discovery, PET radiotracers should be developed to have high affinity and selectivity for the target protein. To reliably visualize the 5-HT_{1A} receptor, which exist in human brain at concentrations up to 100 fmol/mg protein (equivalent to 10 pmol/mL or 10 nM), a radioligand should have a K_D value in the low or (sub)nanomolar range [5]. Almost without exception all PET radiotracers for the 5-HT_{1A} such us [^{18}F]MPPF and [^{18}F]FCWAY were sharing the same WAY-100634 moiety to guarantee this high affinity as well as selectivity. For imaging purposes the letter can be expressed as the ratio of B_{max}/K_D for the target receptor over that of the other receptors. This ratio should be as high as possible. Translated to *in vitro* this means in practice that the K_i for the receptor of interest is preferably a factor of 100 lower than for the others with a comparable density. For a low-density non-target receptor this factor may of course be lower.

In this study, we were conscious to design and synthesize fluorinated compounds (Fig. 2) that have a similar or even higher affinity and a similar or even better selectivity than WAY-100635, but we mainly focussed our attention on the metabolic stability by using BFR groups. Defluorination is a major metabolic problem for some ^{18}F-labeled radiotracers. Unfortunately, there is no specific rule to enhance the metabolic stability and to prevent HF elimination. Species (rats, monkeys and human) differences in the ability to radiodefluorination exist for many radiotracers [21]. ^{18}F-labeling at an aromatic carbon atom within a phenyl or pyridinyl group showed less to no defluorination such as in [^{18}F]MPPF [12,14,21], while ^{18}F-labeling at an aliphatic carbon atom is often prone to defluorination such as in [^{18}F]FCWAY [17,21]. This is not unexpected since a fluorine-carbon bond tends to an axial position due to fluorine's electronegativity, which in turn facilitates elimination. For this reason, the corresponding *cis*-compound, in which fluorine is always axial, defluorinates even much faster. It is interesting to note that by placing fluorine on a primary carbon as in [^{18}F]MeFWAY, the defluorination rate was greatly reduced [18].

A variety of bridgehead WAY-100635 derivatives (**6a**–**d** and **7a**–**d**) with a fluoromethyl-BFR, such as -adamantyl, -cubyl, -bicyclo[2.2.2]octyl and -bicyclo[2.2.1]heptyl, attached to the carboxamide were synthesized in moderate to good yields. The attachment of a $-CH_2F$ moiety on the bridgehead carbon is expected to hardly influence the biological activity, but has the advantage that HF elimination is no longer possible. As also the

Table 2
Affinity (K_i value) of WAY-100635 analogs **6b**, **6c** and **6d** for selected receptors.[a,b]

Compd	5-HT_{1A}	5-HT_{2B}	5-HT_7	5-HT_{1D}	α_{1A}-A	α_{1B}-A	α_{1D}-A	D_4
WAY-100635	0.6	24[c]	>10000[c]	>10000[c]	20[c]	322[c]	5[c]	16[c]
6b	0.2	99	132	122	16	176	27	71
6c	1.1	79	77	361	74	110	73	84
6d	0.9	88	97	92	36	38	19	92

[a] The primary testing of the compounds on a number of receptors, (serotonin receptor subtypes: 5-HT_{1E}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_3, 5-HT_6; adrenoceptor subtypes: α_{2A}, α_{2B}; serotonin transporter sigma receptor: σ1R, σ2R) showed <50% inhibition at 10 μM. On other receptors (serotonin receptor subtypes: 5-HT_{1B}, 5-HT_{1D}, 5-HT_{5A}; adrenoceptor subtypes: α_{2C}, β_1, β_2, β_3 and dopamine receptor subtype D_2) where the compounds showed >50% inhibition at 10 μM the K_i values were at least a factor of 100 greater than those for the 5-HT_{1A} receptor.
[b] Results are presented as apparent equilibrium inhibition constant (K_i) in nM and are the mean of three experiments.
[c] Data taken from NIMH-PDSP [http://pdsp.med.unc.edu/pdsp.php].

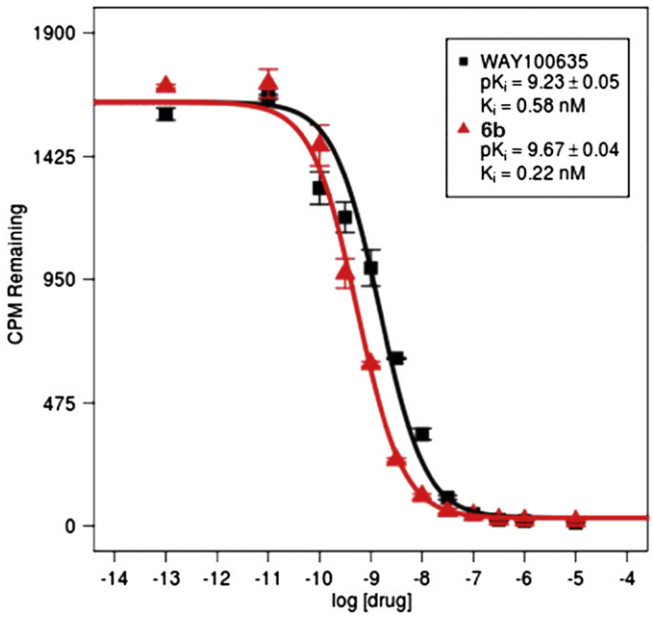


Fig. 3. The inhibition curves of **6b** and **WAY-100635** using the 5-HT_{1A} receptor agonist [^{3}H]8-OH-DPAT.

carboxamide function is at a bridgehead carbon, the proposed molecules have the advantage that no enantiomeric mixtures are formed and that the amide bond is more stable toward *in vivo* hydrolysis. Hence, the novel analogs are aliphatic, a-chiral and expected to be stable toward HF elimination.

The eight fluoromethyl WAY-100635 analogs have been tested in an *in vitro* radioligand binding assay to determine their affinity for 5-HT_{1A} receptors. All compounds showed a similar high affinity for the 5-HT_{1A} receptor as that of the parent compounds WAY-100635 and *O-desmethyl* WAY-100635. The presence of a hydroxyl group instead of the methoxy group in the aromatic moiety does not lead to a substantial better *in vitro* binding to the 5-HT_{1A} receptor or a real decrease in cLog $D_{7.4}$. Since on the other hand, this will complicate the radiolabeling with ^{18}F (the hydroxyl group must be protected before and deprotected after the fluorination step), we excluded compounds **7** for further extensive evaluation.

Of the other fluoromethyl-BFR analogs of WAY-100635, compound **6a** was found to have the lowest affinity for the 5-HT_{1A} receptor and the highest lipophilicity (cLog $D_{7.4}$ = 4.48), confirmed by a t_R on HPLC (see Experimental) which was more than twice that of the other compounds, which might be too high for *in vivo* application. Therefore, we further focused our attention on the BFR analogs of WAY-100635, **6b**–**d**. The Log $D_{7.4}$ of **6b**–**d** and of WAY-100635 for comparison was determined experimentally. The lipophilicity of all three compounds was comparable to that of WAY-100635. These measured Log $D_{7.4}$ values differ from the calculated values, but are now within the optimal range (2–3.5). Furthermore, it confirmed our assumption that the calculating software is not capable for handling a cubane structure.

The selectivity of **6b**–**d** for the 5-HT_{1A} receptor over other relevant receptors was determined at NIMH/Psychoactive Drug Screening Program (PDSP). The PDSP labs have used the same human (HEK-293 EBNA)-cells but other practical conditions, which explains the differences in K_i values between Table 1 and Table 2. For *in vivo* application, compound **6c** might show a better selectivity than WAY-100635 because of its lower binding affinities for α_{1A}-adrenoceptors receptor and the D_4 receptor. Compounds **6b** and **6d** might also show a better *in vivo* selectivity than WAY-100635 because of their lower binding affinities for the D_4 receptor. On the other hand, **6b**, **6c** and **6d** show higher affinity for the subreceptors 5-HT_7 and 5-HT_{1D} than WAY-100635 itself, albeit to be over 30–200 times lower than the affinity for the 5-HT_{1A} receptor.

Table 3
Stability of [^{18}F]**6b** and [^{18}F]MPPF in a medium with human hepatocytes.

	t = 0 min	t = 15 min	t = 60 min	t = 150 min
[^{18}F]**6b**	99.6%	73.2%	30.7%	17.4%
[^{18}F]MPPF	99.2%	45.2%	22.7%	8.4%

A metabolic stability study with human hepatocytes was performed using radiolabeled [^{18}F]**6b** and compared with that of [^{18}F] *p*-MPPF. This preliminary study showed that at 15 min of incubation >73% of the parent compound [^{18}F]**6b** was still present, whereas under the same conditions [^{18}F]MPPF was more rapidly metabolized as only 45% was left. This better metabolic stability of [^{18}F]**6b** compared to [^{18}F]MPPF must for a large part be due to the expected decrease in rate of the amide hydrolysis. *In vivo* studies with the radiolabeled compounds of **6** are planned to give a decisive answer.

7. Conclusions

Eight novel fluoromethyl-BFR analogs of WAY-100635 were synthesized successfully. They all bind to the 5-HT_{1A} receptor with high affinity (K_i values in the (sub)nanomolar range). The binding affinity and selectivity of the three selected analogs **6b**, **6c** and **6d** and preliminary stability of **6b** make them worthwhile for further *in vivo* investigation as potential 5-HT_{1A} receptor binding ligands and promising ^{18}F-labeled PET tracers.

8. Experimental section

8.1. Chemistry

The solvents were dried according to standard procedures. Reactions involving moisture sensitive compounds were performed under an anhydrous atmosphere of dry argon, unless indicated otherwise. Commercially available chemicals were used without further purification. Reactions were monitored by using thin-layer chromatography (TLC) on silica-coated plastic sheets (Merck silica gel 60 F_{254}) with the indicated eluent. The compounds were visualized by UV light (254 nm), I_2, Ceric Ammonium Molybdate (CAM), Bromocrysol or potassium permanganate staining, followed by charring at 130 °C. Flash chromatography refers to purification using the indicated eluent and Acros silica gel (0.030–0.075 mm). All other reagents were purchased from Fluka, Sigma–Aldrich, and Acros. Nuclear magnetic resonance spectra (^{1}H NMR and ^{13}C NMR) were determined in the indicated solvent using a Bruker AC 200 MHz (200.13 MHz and 50.32 MHz, respectively), a Bruker Avance 250 MHz (250.13 and 62.90 MHz, respectively) or a Bruker MSL 400 MHz (400.13 and 100.61 MHz, respectively). Chemical shifts (δ) are given in ppm downfield from tetramethylsilane (^{1}H, ^{13}C) and coupling constants *J* in Hz. Mass spectra were recorded on a Finnigan MAT-90 mass spectrometer. Products were detected at λ = 254 nm. All tested compounds have a purity of at least 95% according to NMR and HPLC analysis. Analytical HPLC was performed on a C18 – 10 μm column (kromasil, 4.6 mm × 250 mm), using MeOH/H_2O/DIPA (65/35/0.02) as eluent and a flow of 1 mL min^{-1}.

8.1.1. General procedure for the synthesis of compounds *6a*, *6b*, *6c* and *6d*

In a flame dried flask under argon atmosphere, compound **5** was dissolved in MeCN and thionyl chloride (1 equivalent) was added.

The reaction mixture was refluxed at 70 °C in MeCN for 45 min to 1 h and the excess of thionyl chloride was totally evaporated at room temperature (because of volatility of the acid chlorides) under reduced pressure, then co-evaporated with MeCN. Afterward, WAY-100634 (1 equivalent) in MeCN and NEt_3 (2 equivalents) were added to the corresponding acid chloride and stirred at room temperature for 1 h. The solvent was evaporated and the residue was dissolved in water and extracted with CH_2Cl_2. The organic phase was collected, dried over anhydrous Na_2SO_4 and evaporated to dryness.

*8.1.1.1. 4-(Fluoromethyl)-N-(2-(4-(2-methoxyphenyl)piperazin-1-yl) ethyl)-N-(pyridin-2-yl)adamantane-1-carboxamide (**6a**).* Starting with **5a** (50 mg, 0.24 mmol) and WAY-100634 (73.8 mg, 0.24 mmol) gave 116 mg (95%) of **6a** as colorless glass after column chromatography (EtOAc/NEt_3, 1/0.01). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.28–1.44 (m, 4H, 2× CH_2), 1.48–1.55 (m, 4H, 2× CH_2), 1.67 (q, J = 12 Hz, 4H, 2× CH_2), 1.95 (s, 2H, 2× CH), 2.64 (m, 6H, $N(CH_2)_3$), 2.90–3.10 (m, 4H, $N(CH_2)_2$), 3.80–3.86 (m, 5H, CH_3, NCH_2), 3.84 (d, J = 49 Hz, 2H, CH_2F), 6.81–7.01 (m, 4H, 4× CH), 7.23–7.37 (m, 2H, 2× CH), 7.75 (dt, J = 7.6 Hz, 1H, CH), 8.50 (d, J = 4.9 Hz, 1H, CH). ^{13}C NMR (100.61 MHz, $CDCl_3$): δ 28.07, 34.62, 34.79, 35.74, 37.02, 37.06, 39.34, 40.56, 40.61, 44.27, 49.06, 50.61, 53.51, 55.33, 55.76, 91.33, 93.05, 111.25, 118.08, 120.94, 122.79, 123.13, 126.11, 138.23, 141.35, 148.85, 152.24, 156.88, 178.13. HRMS (EI) m/z calcd for $C_{30}H_{39}FN_4O_2$; 506.3057; found: 507.3127 (M + H), t_R: 48.8 min.

*8.1.1.2. 4-(Fluoromethyl)-N-(2-(4-(2-methoxyphenyl)piperazin-1-yl) ethyl)-N-(pyridin-2-yl)cubane-1-carboxamide (**6b**).* Starting with **5b** (300 mg, 1.09 mmol) and WAY-100634 (346 mg, 1.11 mmol) gave 441 mg (84%) of **6b** as colorless glass after column chromatography (EtOAc/NEt_3, 1/0.01). ^{1}H NMR (200.13 MHz, $CDCl_3$): δ 2.46–3.20 (m, 10H, $N(CH_2)_3$ and $N(CH_2)_2$), 3.51–3.85 (m, 9H, 6× CH and CH_3), 3.90–4.12 (m, 2H, NCH_2), 4.40 (d, J = 48 Hz, 2H, CH_2F), 6.70–7.05 (m, 4H, 4× CH), 7.15–7.30 (m, 2H, 2× CH), 7.60 (dt, J = 7.6 Hz, 1H, CH), 8.50 (dd, J = 4.9 Hz, 1H, CH). ^{13}C NMR (50.32 MHz, $CDCl_3$): δ 43.63, 43.83, 44.98, 47.34, 50.42, 53.18, 54.55, 54.96, 55.14, 56.01, 59.34, 81.13, 84.41, 110.96, 117.87, 119.93, 120.72, 121.61, 122.65, 137.96, 141.09, 148.79, 152.01, 155.23, 171.49. HRMS (EI) m/z calcd for $C_{28}H_{31}FN_4O_2$; 474.2431; found: 475.2505 (M + H), t_R: 15.8 min.

*8.1.1.3. 4-(Fluoromethyl)-N-(2-(4-(2-methoxyphenyl)piperazin-1-yl) ethyl)-N-(pyridin-2-yl)bicyclo[2.2.2]octane-1-carboxamide (**6c**).* Starting with **5c** (25 mg, 0.13 mmol) and WAY-100634 (42 mg, 0.13 mmol) gave 38 mg (61%) of **6c** after column chromatography (EtOAc/MeOH, 1/0.07). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.23–1.40 (m, 6H, 3× CH_2), 1.58–1.73 (m, 6H, 3× CH_3), 2.60–2.70 (m, 6H, $N(CH_2)_3$), 3.03 (bs, 4H, $N(CH_2)_2$), 3.82–3.91 (m, 5H, CH_3 and NCH_2), 3.93 (d, J = 49 Hz, 2H, CH_2F), 6.83–7.02 (m, 4H, 4× CH), 7.24–7.32 (m, 2H, 2× CH), 7.77 (dt, J = 7.6 Hz, 1H, CH), 8.52 (d, J = 4.9 Hz, 1H, CH). ^{13}C NMR (100.61 MHz, $CDCl_3$): δ 26.95, 27.00, 28.60, 32.28, 32.46, 42.44, 48.88, 50.58 53.48, 55.35, 55.71, 89.85, 91.54, 111.27, 118.11, 120.96, 122.66, 122.82, 123.44, 138.20, 141.36, 149.00, 152.27, 156.88, 178.05. HRMS (EI) m/z calcd for $C_{28}H_{37}FN_4O_2$; 480.2901; found: 481.2967 (M + H), t_R: 23.6 min.

*8.1.1.4. 4-(Fluoromethyl)-N-(2-(4-(2-methoxyphenyl)piperazin-1-yl) ethyl)-N-(pyridin-2-yl)bicyclo[2.2.1]heptane-1-carboxamide (**6d**).* Starting with **5d** (50 mg, 0.29 mmol) and WAY-100634 (90.48 mg, 0.29 mmol) gave 88 mg (65%) of **6c** after column chromatography (EtOAc/MeOH, 1/0.07). ^{1}H NMR (250.13 MHz, $CDCl_3$): δ 1.10–1.40 (m, 4H, 2× CH_2), 1.40–1.70 (m, 4H, 2× CH_2), 1.78–1.98 (m, 2H, CH_2), 2.50–2.75 (m, 6H, $N(CH_2)_3$), 2.80–3.10 (m, 4H, $N(CH_2)_2$), 3.88 (s, 3H, CH_3), 3.90–3.40 (m, 2H, NCH_2), 4.33 (d, J = 48 Hz, 2H, CH_2F), 6.78–7.08 (m, 4H, 4× CH), 7.20–7.40 (m, 2H, 2× CH), 7.75 (dt, J = 7.4 Hz, 1H, CH), 8.52 (dd, J = 4.9 Hz, 1H, CH). ^{13}C NMR (50.32 MHz, $CDCl_3$): δ 31.42, 31.50, 34.15, 44.77, 44.85, 46.98, 47.29, 50.36, 53.24, 55.16, 55.48, 55.69, 84.92, 88,53, 110.98, 117.93, 120.75, 122.72, 122.89, 123.56, 137.97, 141.05, 148.98, 152.03, 155.53, 175.42. HRMS (EI) m/z calcd for $C_{27}H_{35}FN_4O_2$; 466.2744; found: 467.2811 (M + H), t_R: 16.6 min.

*8.1.2. General procedure for synthesis of compounds **7a**, **7b**, **7c** and **7d***

The acid chlorides of **5a**–**d** were synthesized as mentioned above. Then *O-desmethyl* WAY-100634 (0.5 equivalent) and NEt_3 (2 equivalents) were dissolved in MeCN and added to the corresponding acid chloride. This new reaction mixture was stirred at room temperature for 30 min. Then the solvent was evaporated, the residue was dissolved in a mixture of MeOH/saturated $NaHCO_3$/H_2O (2/1/1) and heated at 50 °C for 2 h, unless stated otherwise. The solution was evaporated and the residue was dissolved in water, extracted with EtOAc, unless noted otherwise, dried over Na_2SO_4, filtered and evaporated to dryness.

*8.1.2.1. 4-(Fluoromethyl)-N-(2-(4-(2-hydroxyphenyl)piperazin-1-yl) ethyl)-N-(pyridin-2-yl)adamantane-1-carboxamide (**7a**).* Starting with **5a** (50 mg, 0.24 mmol) and *O-desmethyl* WAY-100634 (35 mg, 0.12 mmol) in MeCN (4 mL) gave after column chromatography (EtOAc/Hexane, 5/2) 47 mg (80%) of **7a** as colorless glass. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.30–1.80 (m, 12H, 6× CH_2), 2.09 (s, 2H, 2× CH), 2.60–2.72 (m, 6H, $N(CH_2)_3$), 2.79–2.89 (m, 4H, $N(CH_2)_2$), 3.83–3.90 (m, 2H, $N(CH_2)$), 3.86 (d, J = 50 Hz, 2H, CH_2F), 6.72–6.89 (m, 2H, 2× CH), 6.94–7.08 (m, 2H, 2× C), 7.17–7.25 (m, 2H, 2× CH), 7.70 (dt, J = 7.6 Hz, 1H, CH), 8.44 (d, J = 4.9 Hz, 1H, CH). ^{13}C NMR (62.90 MHz, $CDCl_3$): δ 28.10, 34.61, 34.89, 35.91, 37.21, 37.28, 39.39, 40.62, 40.70, 44.33, 49.10, 52.54, 53.98, 55.73, 90.86, 93.59, 114.04, 119.99, 121.34, 122.85, 123.02, 126.39, 138.20, 139.02, 148.95, 151.52, 156.90, 178.27. HRMS (EI) m/z calcd for $C_{29}H_{37}FN_4O_2$; 492.2901; found: 493.297 (M + H), t_R: 24.9 min.

*8.1.2.2. 4-(Fluoromethyl)-N-(2-(4-(2-hydroxyphenyl)piperazin-1-yl) ethyl)-N-(pyridin-2-yl)cubane-1-carboxamide (**7b**).* Starting with **5b** (50 mg, 0.27 mmol) and *O-desmethyl* WAY-100634 (41 mg, 0.14 mmol) in MeCN (4 mL) gave after column chromatography (EtOAc/MeOH, 1/0.1 to only EtOAc) 38 mg (59%) of **7b** as white solid. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 2.50–2.90 (m, 10H, $N(CH_2)_3$ and $N(CH_2)_2$), 3.60–3.89 (m, 6H, 6× CH), 4.05–4.12 (m, 2H, NCH_2), 4.46 (d, J = 4.9 Hz, 2H, CH_2F), 6.80–7.30 (m, 6H, 6× CH), 7.81 (dt, J = 7.6 Hz, 1H, CH), 8.54 (d, J = 4.9 Hz, 1H, CH). ^{13}C NMR (100.61 MHz, $CDCl_3$): δ 43.91, 44.01, 45.17, 47.58, 52.53, 53.81, 54.91, 55.11, 56.16, 59.58, 82.15, 83.79, 114.04, 119.96, 121.28, 121.75, 126.37, 138.11, 138.98, 140.46, 149.07, 151.49, 155.48, 171.75. HRMS (EI) m/z calcd for $C_{27}H_{29}FN_4O_2$; 460.2275; found: 461.2344 (M + H), t_R: 11.6 min.

*8.1.2.3. 4-(Fluoromethyl)-N-(2-(4-(2-hydroxyphenyl)piperazin-1-yl) ethyl)-N-(pyridin-2-yl)bicyclo[2.2.2]octane-1-carboxamide (**7c**).* Starting with **5c** (50 mg, 0.27 mmol) and *O-desmethyl* WAY-100634 (35 mg, 0.13 mmol) in MeCN (4 mL) gave after column chromatography (EtOAc) 20 mg (33%) of **7c** as colorless glass. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.20–1.38 (m, 6H, 3× CH_2), 1.59–1.74 (m, 6H, 3× CH_2), 2.49–2.72 (m, 6H, $N(CH_2)_3$), 2.74–2.90 (m, 4H, $N(CH_2)_2$), 3.83–3.90 (m, 2H, CH_2), 4.00 (d, J = 49 Hz, 2H, CH_2F), 6.79–7.17 (m, 4H, 4× CH), 7.23–7.31 (m, 2H, 2× CH), 7.77 (dt, J = 7.6 Hz, 1H, CH), 8.50 (d, J = 4.9 Hz, 1H, CH). ^{13}C NMR (100.61 MHz, $CDCl_3$): δ 26.95, 26.99, 28.62, 32.29, 32.47, 42.47, 48.94, 52.32, 53.95, 55.65, 89.83, 91.53, 114.02, 119.97, 121.34, 122.71, 123.31, 126.42, 138.26, 138.96, 149.08, 151.51, 156.86, 178.06.

HRMS (EI) *m/z* calcd for $C_{27}H_{35}FN_4O_2$; 466.2744; found: 467.2817 (M + H), t_R: 16.5 min.

*8.1.2.4. 4-(Fluoromethyl)-N-(2-(4-(2-hydroxyphenyl)piperazin-1-yl) ethyl)-N-(pyridin-2-yl)bicyclo[2.2.1]heptane-1-carboxamide (**7d**).* Starting with **5d** (50 mg, 0.19 mmol) and *O-desmethyl* WAY-100634 (28 mg, 0.09 mmol) in MeCN (5 mL) and subsequent hydrolysis in a mixture of MeOH/saturated $NaHCO_3/H_2O$ (4/1/1), the solvent was evaporated and the residue was extracted with CH_2Cl_2 (3 × 1 mL). Column chromatography (CH_2Cl_2/MeOH, 1/0.025) afforded **7d** (20 mg, 49%). ^{1}H NMR (250.13 MHz, $CDCl_3$): δ 1.08–1.35 (m, 4H, 2× CH_2), 1.40–1.66 (m, 4H, 2× CH_2), 1.75–2.00 (m, 2H, CH_2), 2.48–2.90 (m, 10H, $N(CH_2)_3$ and $N(CH_2)_2$), 3.83–4.00 (m, 2H, NCH_2), 4.34 (d, J = 48 Hz, 2H, CH_2F), 6.77–7.33 (m, 6H, 6× CH), 7.79 (dt, J = 7.6 Hz, 1H, CH), 8.54 (d, J = 4.9 Hz, 1H, CH). ^{13}C NMR (100.61 MHz, $CDCl_3$): δ 31.56, 31.59, 34.32, 44.92, 44.95, 47.25, 47.40, 53.73, 55.38, 55.80, 59.05, 86.00, 87.32, 114.12, 120.07, 121.40, 123.19, 123.51, 126.57, 138.31, 141.09, 149.21, 151.36, 155.53, 175.48. HRMS (EI) *m/z* calcd for $C_{26}H_{33}FN_4O_2$; 452.2588; found: 453.2658 (M + H), t_R: 11.7 min.

8.2. In vitro receptor binding

Materials: [^{3}H]8-OH-DPAT (154.2 Ci/mmol, 5.71 TBq/mmol), membrane preparations of HEK-293 EBNA-cells expressing the cloned human serotonin 5-HT_{1A} receptor (HEK-293 EBNA-cells, 1 unit = 2.5 μL), the 96-well microplates and an UniFilter-96 GF/C filter plate were obtained from Perkin Elmer Life Sciences (Wellseley, MA). Tris-(hydroxymethyl)-aminomethane was purchased from AppliChem (Darmstadt, Germany). HCl and $MgSO_4$ were purchased from Sigma–Aldrich (Zwijndrecht, The Netherlands). The filtration was carried out with a MicroBeta FilterMate-96 Harvester (PerkinElmer) and the radioactivity was quantified using a Wallac MicroBeta TriLux counter (PerkinElmer).

8.2.1. 5-HT_{1A} receptor binding

Binding experiments were performed using the 5-HT_{1A} agonist [^{3}H]8-OH-DPAT and a cell membrane suspension containing the human 5-HT_{1A} receptor. A quality control of the radioligand and the membrane preparation was done by performing radioligand saturation binding experiments. The membrane suspension was diluted (factor 1:40) in binding buffer (50 mM Tris–HCl, pH 7.4, 5 mM $MgSO_4$) and incubated in the dark on 96-well microplates for 1 h at 37 °C with the following radioligand concentrations: 0.25, 0.5, 0.75, 1.00, 2.00, 2.50, 3.00, 4.00, 5.00, 6.00, 8.00, and 10.00 nM in a volume of 200 μL. Non-specific binding was measured at each radioligand concentration in the presence of 10 μM WAY-100635. Incubation was rapidly terminated by filtration over an UniFilter-96 GF/C filter plate presoaked in 0.3% polyethyleneimine (PEI), followed by three rapid washes with ice-cold washing buffer (50 mM Tris–HCl, pH 7.4, 5 mM $MgSO_4$) using a MicroBeta FilterMate-96 Harvester. Filter plates were dried for 2 h in an oven at 54 °C. Radioactivity was quantified with 10 μL of liquid scintillation fluid using a Wallac MicroBeta TriLux counter. The measured K_D value for the 5-HT_{1A} receptor in this membrane preparation was comparable to the one provided by the manufacturer (1.48 nM and 1.86 nM, respectively).

For the competitive binding experiments, an assay volume of 200 μL per well was used containing 100 μL of the diluted cell membrane suspension, 50 μL of 2 nM of [^{3}H]8-OH-DPAT radioligand dissolved in binding buffer, 10 μL of each novel analog in increasing concentrations (range 10^{-11} to 10^{-6} M) and 40 μL of the binding buffer. Incubation in the 96-well microplates was carried out in the dark for 2 h at 37 °C. Incubation was rapidly terminated (*vide supra*). Filter plates were dried overnight in an oven at 54 °C. Radioactivity was quantified (*vide supra*). IC_{50} values were derived from non-linear regression curve fitting using computer program Graphpad Prism® (version 4.00) and K_i values were calculated according to Cheng–Prusoff equation using the measured K_D of [^{3}H]8-OH-DPAT radioligand.

8.2.2. Receptor binding profile

The receptor binding profile of compound **6b**, **6c** and **6d** was investigated by radioligand binding by NIMH/psychoactive Drug Screening Program (PDSP). For assay conditions and assay protocol see http://pdsp.med.unc.edu/pdspw/binding.php.

8.3. Lipophilicity of the investigated compounds

The lipophilicity of the investigated compounds was determined by measuring the partition of the compounds between 1-octanol and water (buffer), and is expressed as the log $D_{7.4}$ value. The investigated compounds were dissolved in 1-octanol (3 mL) in a concentration of 1 mg/mL. The phosphate buffer (25 mM, pH = 7.4) was made of 1.160 g of NaH_2PO_4 and 2.175 g of $NaHPO_4$ in 1 L water. The HPLC eluent of a mixture of methanol: phosphate buffer (80:20) was used for further dilutions.

The HPLC samples were made as follows: the same amounts of the buffer and the 1-octanol stock (250 μL) were added to each other in a mixing vial and the mixture was shaken for 1 min. After resting for 30 min, a sample was taken from the 1-octanol layer (50 μL) and diluted with 450 μL of the HPLC eluent in a new vial. Another 50 μL was taken from this new mixture and diluted again with 450 μL of the HPLC eluent. This mixture was again diluted in the same previous manner and 50 μL of this sample was injected into the HPLC. A 50 μL sample of the buffer layer was also injected into the HPLC. This whole procedure was repeated five times.

As a reference, the partition of WAY-100635 between 1-octanol and water (buffer) was measured in a similar way. The log $D_{7.4}$ was calculated according to the formula: The log $D_{7.4}$ = ^{10}Log(A_{oct}/A_{buffer}), with A_{oct} = average concentration of the five octanol samples and A_{buffer} = average concentration of the five buffer samples.

8.4. Stability toward human hepatocytes

Approximately 4 million of cryopreserved human liver cells (In Vitro Technologies, Leipzig, Germany) were thawed rapidly and suspended in RPMI 1640 supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Cells were maintained at 5% CO_2 humidified atmosphere and at 37 °C, and washed with phosphate buffered saline (PBS). Viability was determined by trypan blue exclusion. After centrifugation, cells were washed and dissolved in the same medium as above. The viable cell concentration was adjusted to 1.0 million per mL. To 1 mL of this cell suspension 75 μL of [^{18}F]**6b** (50 MBq) or [^{18}F]MPPF (50 MBq) was added. After incubation for 15, 60 and 150 min at 37 °C, a 200 μL sample was taken, added to 200 μL of MeOH, sonicated and centrifuged. The composition of the supernatant was determined using HPLC combined with online radioactivity detection.

Acknowledgment

The authors would like to acknowledge the Dutch Technology Foundation STW for the financial support of the project VBC.7048, BV Cyclotron (VU University) for the supply of fluorine-18, Covidien Mallinckrodt Int. and Philips for financial support and Dr. Bryan Roth of the NIMH-PDSP program for performing the receptor profiling of compounds **6b**, **6c** and **6d**. The authors also would like

to acknowledge Prof. Josée E. Leysen for advice regarding the *in vitro* 5-HT_{1A} receptor binding affinity measurements.

Appendix. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2011.06.023.

References

[1] B.R. Chemel, B.L. Roth, B. Armbruster, V.J. Watts, D.E. Nichols, WAY-100635 is a potent dopamine D_4 receptor agonist, Psychopharmacology 188 (2006) 244–251.
[2] J. Passchier, A. Van Waarde, Visualisation of serotonin-1A (5-HT_{1A}) receptors in the central nervous system. Eur, J. Nucl. Med. 28 (2001) 113–129.
[3] S. Matsubara, R.C. Arora, H.Y. Meltzer, Serotonergic measures in suicide brain – 5-HT_{1A} binding-sites in frontal cortex of suicide victims, J. Neural Transm. 85 (1991) 181–194.
[4] V. Kepe, J.R. Barrio, S. Cheng Huang, L. Ercoli, P. Siddarth, K. Shoghi Jadid, G.M. Cole, N. Satyamurthy, J.L. Cummings, G.W. Small, M.E. Phelps, . Serotonin 1A receptors in the living brain of Alzheimer's disease patients, PNAS 103 (2006) 702–707.
[5] V.W. Pike, C. Halldin, H.V. Wikström, Radioligands for the study of the brain 5-HT_{1A} receptors *in vivo*, Prog. Med. Chem. 38 (2001) 189–247.
[6] F. Yasuno, T. Suhara, T. Ichimiya, A. Takano, T. Ando, Y. Okubo, Decreased 5-HT_{1A} receptor binding in amygdala of schizophrenia, Biol. Psychiatry 55 (2004) 439–444.
[7] V.W. Pike, J.A. McCarron, S.P. Hume, S. Ashworth, J. Opacka-Juffry, S. Osman, A.A. Lammertsma, K.G. Poole, A. Fletcher, A.C. White, I.A. Cliffe, Pre-clinical development of a radioligand for studies of central 5-HT-$_{1A}$ receptors in vivo [^{11}C]-WAY 100635, Med. Chem. Res. 5 (1994) 208–227.
[8] I.A. Cliffe, A retrospect on the discovery of WAY 100635 and the prospect for improved 5-HT_{1A} receptor PET radioligands, Nucl. Med. Biol. 27 (2000) 441–447.
[9] V.W. Pike, C. Halldin, H. Wikström, S. Marchais, J.A. McCarron, J. Sandell, B. Nowicki, C.-G. Swahn, S. Osman, S.P. Hume, M. Constantinou, B. Andree, L. Farde, Radioligands for the study of brain 5-HT1A receptors in vivo–development of some new analogues of WAY, Nucl. Med. Biol. 27 (2000) 449–455.
[10] Z. Zhuang, M. Kung, H.F. Kung, 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 (1994) 1406–1407.
[11] L. Lang, E. Jagoda, B. Schmall, B.K. Vuong, H. Richard Adams, D.L. Nelson, R.E. Carson, W.C. Eckelman, Development of fluorine-18-labeled 5-HT_{1A} antagonist, J. Med. Chem. 42 (1999) 1576–1582.
[12] A. Plenevaux, D. Weissmann, J. Aerts, C. Lemaire, C. Brihaye, C. Degueldre, D. Le Bars, D. Comar, J. Pujo, A. Luxen, Tissue distribution, autoradiography and metabolism of MPPF a new 5-HT_{1A} antagonist for PET: an in vivo study in rat, J. Neurochem. 75 (2000) 803–811.
[13] J. Passchier, P.H. Elsinga, Quantitative imaging of 5-HT1A receptor binding in healthy volunteers with [^{18}F]MPPF, Nucl. Med. Biol. 27 (2000) 473–476.
[14] L. Zimmer, N. Aznavour, [^{18}F]MPPF as a tool for the in vivo imaging of 5-HT_{1A} receptors in animal and human brain, Neuropharmacology 52 (2007) 695–707.
[15] L. Lang, E. Jagoda, Y. Ma, M.B. Sassaman, W.C. Eckelman, Synthesis and in vivo biodistribution of [^{18}F] labeled 3-cis-3-trans,4-cis-, and 4-trans-fluorocyclohexane derivatives of WAY 100635, Bioorg. Med. Chem. 14 (2006) 3737–3748.
[16] R.E. Carson, Y. Wu, L. Lang, Y. Ma, M.G. Der, P. Herscovitch, W.C. Eckelman, Brain uptake of the acid metabolites of F-18-labeled WAY 100635 analogs, J. Cereb. Blood Flow Metab. 23 (2003) 249–260.
[17] Y. Hoon Ryu, J. Liow, S. Zoghbi, M. Fujita, J. Collins, D. Tipre, J. Sangare, J. Hong, V.W. Pike, R.B. Innis, Disulfiram inhibits defluorination of ^{18}F- FCWAY, reduces bone radioactivity, and enhances visualization of radioligand binding to serotonin 5-HT_{1A} receptors in human brain, J. Nucl. Med. 48 (2007) 1154–1161.
[18] N. Saigal, R. Pichik, B. Easwaramoorthy, D. Collins, B.T. Christian, B. Shi, T.K. Narayanan, S.G. Potkin, J. Mukherjee, Synthesis and biological evaluation of novel serotonin 5-HT_{1A} receptor radioligand, ^{18}F-labeled mefway, in rodents and imaging by PET in nonhuman primate, J. Nucl. Med. 47 (2006) 1697–1706.
[19] R. Al Hussainy, J. Verbeek, D. van der Born, A.H. Braker, J.E. Leysen, R.J. Knol, J. Booij, J.D.M. Herscheid, Design, synthesis, radiolabeling, and in vitro and in vivo evaluation of bridgehead iodinated analogues of N-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-N-(pyridin-2-yl)cyclohexanecarboxamide (WAY-100635) as potential SPECT ligands for the 5-HT_{1A} receptor, J. Med. Chem. 54 (2011) 3480–3491.
[20] A.A. Wilson, T. Inaba, N. Fischer, L.M. Dixon, J. Nobrega, J.N. DaSilva, S. Houle, Analogues of WAY 100635 as potential imaging agents for 5-HT_{1A} receptors: syntheses, radiosyntheses, and *in vitro* and *in vivo* evaluation, Nucl. Med. Biol. 25 (1998) 769–776.
[21] V.W. Pike, PET radiotracers: crossing the blood–brain barrier and surviving metabolism, Trends Pharmacol. Sci. 30 (2009) 431–440.
[22] P.E. Eaton, N. Nordari, J. Tsanaktsidis, S.P. Upadhyaya, Barton decarboxylation of cubane-1,4-dicarboxylic acid: optimized procedures for cubanecarboxylic acid and cubane, Synthesis (1995) 501–502.
[23] J.D. Roberts, W.T. Moreland, W. Frazer, Synthesis of some 4-substituted bicyclo[2.2.2]octane-1-carboxylic acids, J. Am. Chem. Soc. 75 (1953) 637–640.
[24] L.G. Humber, G. Myers, L. Hawkins, C. Schmidt, M. Boulerice, Agents affecting lipid metabolism XIII. The synthesis of 1,4-disubstituted bicyclo[2.2.2]octane derivatives, Can. J. Chem. 42 (1964) 2852–2860.
[25] R. Mozingo, H. Adkins, L. Richards, Catalyst, Raney Nickel, W-2, Org. Syn. Coll. 3 (1955) 181 21 (1941) 15.
[26] C.N. Sukenik, S.S. Wang, K. Kumar, Synthesis, characterization, and chemistry of bridgehead-functionalized bicyclo[2.2.2]octanes: reactions at neopentyl sites, J. Org. Chem. 49 (1984) 665–670.
[27] E.W. Della, J. Tsanaktsidis, Synthesis of bridgehead-bridgehead substituted bicycloalkanes, Aust. J. Chem. 38 (1985) 1705–1718.
[28] R. Priefer, P.G. Farrell, D.N. Harpp, Effective synthetic routes to cubylcarbinol derivatives, Synthesis (2002) 2671–2673.
[29] W.J. Middleton, New fluorinating reagents. Dialkylaminosulfur fluorides, J. Org. Chem. 40 (1975) 574–578.
[30] M. Aleksandrov, G.I. Danilenko, L.M. Yagupol'skii, Fluorine derivatives of adamantane, J. Org. Chem. USSR 9 (1973) 976–977.
[31] J. Christopher Mc Williams, F.J. Fleitz, N. Zheng, D. Armstrong, Preparation of n-butyl-4-chlorophenyl sulfide, Org. Syn. Coll. 10 (2004) 147 79 (2002) 43.
[32] P. Bosch, F. Camps, E. Chamorro, Trtrabutylammonuim bifluoride: a versatile and efficient fluorinating agent, Tetrahedron Lett. 28 (1987) 4733–4736.
[33] C.R. Davis, D.C. Swenson, D.J. Burton, Tetramethyl 1,1,4,4-cyclohexanetetacarboxylate: preparation and conversion to key precursors of fluorinated, stereochemically defined cyclohexanes, J. Org. Chem. 58 (1993) 6843–6850.
[34] P.A. Grieco, T.R. Vedananda, C(12)-substituted prostaglandins. An enantiospecific total synthesis of (+)-12-(Fluoromethyl)prostaglandin $F_{2\alpha}$ methyl ester, J. Org. Chem. 48 (1983) 3497–3502.
[35] A. Ricci, A. Degl'innocenti, M. Fiorenza, M. Taddei, M.A. Spartera, D.R.M. Walton, Fluoride ion induced reactions of organosilanes with electrophiles, Tetrahedron Lett. 23 (1982) 577–578.
[36] D.W. Kim, H.J. Jeong, S.T. Lim, M.H. Sohn, Tetrabutylammonium tetra-butanol coordinated fluoride as a facile fluoride source, Angew. Chem. Int. Ed. 47 (2008) 8404–8406.
[37] D.W. Kim, H.J. Jeong, S.T. Lim, M.H. Sohn, J.A. Katzenellenbogen, D.Y. Chi, Facile nucleophilic fluorination reactions using *tert*-alcohol as reaction medium: significantly enhanced reactivity of alkali metal fluorides and improved selectivity, J. Org. Chem. 73 (2008) 957–962.
[38] I.A. Cliffe, H.L. Mansell. European Patent nr. 0 512 755 A2, 1993.
[39] D. Lednicer, M.F. Grostic, Selective demethylation of deoxyanision. Mass spectra of the products, J. Org. Chem. 32 (1967) 3251–3253.
[40] Y.C. Cheng, W.H. Prusoff, Relationship between the inhibition constant (K_i) and the concentration of inhibition which causes 50 percent inhibition (IC_{50}) of an enzymatic reaction, Biochem. Pharmacol. 22 (1973) 3099–3108.