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Synthesis and in vivo biodistribution of F-18 labeled 3-*cis*-, 3-*trans*-, 4-*cis*-, and 4-*trans*-fluorocyclohexane derivatives of WAY 100635

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Abstract—Radioligands that are specific for the serotonin 5-HT_{1A} receptor will be useful in characterizing the physiological action of this receptor subtype. With radioligands of varying pharmacokinetic properties, investigators can measure not only receptor density, but also the effect of endogenous serotonin concentration. To this end, three additional fluorinated analogs of WAY 100635 were prepared and evaluated as 5-HT_{1A} receptor ligands of varyin|%1e[OK]g pharmacokinetic properties based on our previous studies. These four compounds are *cis*-4-fluoro-, *trans*-4-fluoro-, *cis*-3-fluoro-, and *trans*-3-fluoro-*N*-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-*N*-(pyridin-2-yl)cyclohexanecarboxamides (FCWAYs). All four compounds were characterized and radiolabeled with fluorine-18, a 109.7 min half-life radionuclide used in positron emission tomography. We then determined in vitro inhibition constants at the 5-HT_{1A} receptor; in vitro metabolic profile, using rat hepatocytes and liquid chromatography/mass spectroscopy (LC/MS); and the rate of defluorination and hippocampus to cerebellum ratio ex vivo. This led to the conclusion that high affinity 4-*trans*-F-18 FCWAY had the best properties for measuring receptor density given its high hippocampus to cerebellum ratio and 3-*cis*-F-18 FCWAY had the best properties for measuring dynamic change in receptors, with lower affinity and faster pharmacokinetics.

1. Introduction

The serotonin 5-HT_{1A} receptor was identified in 1981,[1] but silent antagonists (compounds possessing no intrinsic agonist activity) were not identified until 12 years later.[2] This delay prevented the clear definition of the action of this receptor due, in part, to the complexities of the 5-HT system, for which at least 15 separate receptor subtypes have been identified,[3] and in the difficulty of characterizing the agonist and antagonist properties of potential ligands using physiological models alone.[2] In general, radiolabeled silent antagonists with high affinity for a specific receptor subtype represent an 'ideal' ligand for studying that subtype in vivo by biomedical imaging techniques such as single photon emission tomography (SPET) and positron emission tomography (PET). The intricacies and problems associated with the 5-HT_{1A} receptor in particular have recently been elucidated,[2] and show the value, if not the necessity, of adhering to this ideal.

The first 5-HT_{1A} silent antagonist, 4-phenylethyl-1-(2-methoxyphenyl)piperazine, possessed high binding affinity for that subtype, but also bound to α_1 adrenoceptors. An added *N*-*t*-butyl carboxamide group produced (*S*)-*N*-*t*-butyl-3-[4-[(2-methoxyphenyl)piperazin-1-yl]-2-phenylpropanamide [(*S*)WAY-100135], a 5-HT_{1A} silent antagonist having the 'ideal' properties of both high specificity and high affinity.[4] Despite this decidedly advantageous combination of attributes, (*S*)WAY-100135, with an IC_{50} of 15 nM for displacement of [^{3}H]8-OH-DPAT [8-hydroxy-2-(di-*n*-propylamino)tetralin] binding to rat hippocampal 5-HT_{1A} receptors,[5] was still considered to be an order of magnitude too weak to be useful as an external imaging agent.

The introduction of WAY 100635 (**1**, Scheme 1),[6] with an IC_{50} of 1.35 nM against [^{3}H]8-OH-DPAT binding,[7] was a significant breakthrough in the development of external imaging agents. Mathis et al. radiolabeled this compound with carbon-11, a positron emitting radionuclide with a 20.4 min half-life, and studied its

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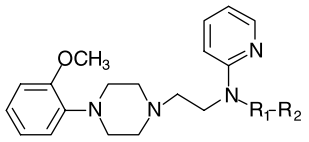


Number	Abbreviation	R_1 (N sub)	R_2
1	WAY 100635	Cyclohexanecarbonyl	None
2	WAY 100634	H	None
3	p-MPPI	Benzoyl	4-Iodo
4	p-MPPF (FBWAY)	Benzoyl	4-Fluoro
5	MeFBWAY	3-Methylbenzoyl	4-Fluoro
7	Trans 4-FCWAY	Cyclohexylcarbonyl	Trans-4-fluoro
8		Benzoyl	4-Fluoromethyl
9		3-Nitrobenzoyl	4-Fluoromethyl
10		Cyclohexylcarbonyl	Cis-4-methoxy
11		Cyclohexylcarbonyl	Trans-4-methoxy
12	Cis 4-FCWAY	Cyclohexylcarbonyl	Cis-4-fluoro
13	Trans 3-FCWAY	Cyclohexylcarbonyl	Trans-3-fluoro
14	Cis 3-FCWAY	Cyclohexylcarbonyl	Cis-3-fluoro
	CYCLOHEXANECARBOXYIC ACID (CH) ESTERS		
15	Cis pentamethylbenzylCH		4-Fluoro
16	Trans pentamethylbenzylCH		4-Fluoro
17	Trans pentamethylbenzylCH		3-Fluoro
18	Cis pentamethylbenzylCH		3-Fluoro
19	Cis pentamethylbenzylCH		4-Hydroxy
20	Trans pentylethylbenzyl CH		4-Hydroxy
21	Trans pentamethylbenzylCH		3-Hydroxy
22	Cis pentamethylbenzylCH		3-Hydroxy
23	Trans pentamethylbenzyl CH		4-Nosylate
24	Cis pentamethylbenzyl CH		4-Nosylate
25	Trans pentamethylbenzylCH		3 Mesylate
26	Cis pentamethylbenzylCH		3 Mesylate

Scheme 1. Structures based on the *N*-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-*N*-(pyridin-2-yl)amide platform.

biodistribution in monkeys.[8] A detailed analytical study of the major metabolic pathways (in vitro) for **1**, by liquid chromatography/mass spectroscopy (LC/MS), showed oxidation in the methoxyphenyl moiety as the primary mode in rat hepatocytes, while amide hydrolysis predominated in human hepatocytes.[9] Metabolism in monkeys (in vivo) paralleled the hydrolysis mode observed in human hepatocytes, when blood samples were analyzed by TLC.[10] When **1** was labeled with carbon-11 in the methoxyphenyl moiety, this predominant amide hydrolysis (in human and nonhuman primates) gave the radiolabeled metabolite corresponding to WAY-100634 (**2**), which easily crossed the BBB and bound weakly to both 5-HT_{1A} and a_1 receptors.[11] This problem was partially circumvented by labeling the carboxyl group with carbon-11, the acid liberated upon hydrolysis having markedly less BBB permeability.[12]

The next series of compounds developed were based on replacing the cyclohexanecarboxamide moiety of **1** with (*o*-, *m*-, and *p*-) substituted phenylcarboxamides.[13] Inhibition constants against the binding of [^{125}I]8-OH-PIPAT in rat hippocampal homogenates for all of the compounds tested (with the exception of the *o*-iodo-derivative) were comparable to **1**. The 4-iodophenylcarboxamide derivative, 4-iodo-*N*-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-*N*-(pyridin-2-yl)benzamide (p-MPPI, **3**), was selected for development and radiolabeled with iodine-123 and 125 for in vivo evaluation in rats.[14,13] Subsequently, the 4-fluorophenylcarboxamide derivative, p-MPPF (**4**),[13] was radiolabeled, first with tritium,[15] and followed shortly thereafter with fluorine-18 ($t_{1/2}$ = 109.7 min).[16,17]

At this point in the development of compounds that could be used specifically for PET imaging, studies of phenyl- and cyclohexanecarboxamides further diverged into two groups: those labeled with fluorine-18 and those labeled with carbon-11. The research group at NIH, concentrating on fluorine-18, prepared a series of derivatives of WAY 100635 from the following acids: 4-fluorobenzoic (**4**), 4-fluoro-3-methylbenzoic (**5**), trans-4-fluorocyclohexanecarboxylic (**7**), 4-fluoromethylbenzoic (**8**), and 4-fluoromethyl-3-nitrobenzoic (**9**).[18] The biological properties of the F-18 labeled compounds were compared to those of [carbonyl-^{11}C] WAY 100635 in rats. The two benzylic fluorides (**8** and **9**) were eliminated from further study due to rapid defluorination in vivo. Unlike other benzylic halides, fluorides are generally stable to nucleophilic attack even under fairly strenuous conditions. This anomalous behavior prompted further study, which confirmed that in vivo defluorination was not due to nucleophilic displacement,[19] however, the mechanism, presumed to be oxidation–elimination, is yet to be elucidated.

Of the remaining compounds, the two phenylcarboxamides p-MPPF, which was identified as FBWAY (**4**) in our early publication[18] and the 4-fluoro-3-methylphenyl derivative (**5**) showed (definitively in the first case and presumptively in the second) brain uptake and biphasic clearance in rats, the uptake phase peaking at approximately 5 min postinjection. The normalized uptake correlating with the beginning of the second phase for both compounds was considerably lower than for WAY 100635 [carbonyl-^{11}*C*], which was run concurrently as a control. The last compound, the *trans*-4-fluorocyclohexyl derivative (**7**), referred to as 4-*trans*-FCWAY, had a high affinity for the 5-HT_{1A} receptor and a high hippocampus to cerebellum [(H/Cb) target/nontarget] ratio.[18] By comparison, the [*O*-methyl-^{11}*C*]cis 4-methoxycyclohexanecarboxylic acid derivative (**10**) did not bind in vivo and the *trans*-4-methoxy (**11**) had a high affinity in vitro, but had a relatively low H/Cb ratio.[20] Two reviews, covering both historical and current trends in 5-HT_{1A} radioligand development pertinent to positron emission tomography, have recently been published.[2,21]

We have now synthesized additional F-18 labeled derivatives in an attempt to produce a range of pharmacokinetic properties with a longer half-life radionuclide that will facilitate the determination of the metabolic-corrected plasma input function.[22] Our hypothesis is that the use of subtype selective ligands with different receptor affinities will provide sensitivity to a range of serotonergic processes. Using a high affinity ligand should provide a more accurate measure of serotonin receptor density, suitable for comparison among patient groups. Lower affinity ligands may be useful for measuring

dynamic changes in neurotransmitter concentration. Recently, Rice et al.[23] showed that a high affinity ligand, such as WAY 100635, was unaffected by the 5-HT releasers, *p*-chloroamphetamine and fenfluramine. However, a recent publication showed that the binding of p-MPPF can be modulated by release of endogenous serotonin using fenfluramine.[24] The combination of these data supports our hypothesis that a lower affinity compound may be more susceptible to blocking by endogenous serotonin.

The first lower affinity compound, MeFBWAY (**5**), has a low H/Cb ratio in rats and, therefore, a low contrast between receptor-rich and receptor-poor regions, which limits the sensitivity of the radiopharmaceutical. We have previously reported on *trans*-4-FCWAY (**7**).[18] In this report, we compare this high affinity compound with *cis*-4-FCWAY (**12**), *trans*-3-FCWAY (**13**), and *cis*-3-FCWAY (**14**) in search of a less tightly bound radiotracer. The criteria are the ease of synthesis of the precursor, the radiochemical yield, the in vitro inhibition constant, metabolic profile, the rate of defluorination in vivo, and the target gray matter to cerebellum ratio.

2. Results and discussion

2.1. Synthesis of WAY 100635 analogs

Three new fluorinated WAY 100635 analogs (**12**, **13**, and **14**) were prepared using a similar procedure used for preparing (**7**) starting from corresponding Pentamethylbenzyl fluoroxycyclohexanecarboxylates (**15**, **17**, and **18**). The major difficulty in making these analogs is the synthesis of the fluorinated cyclohexanecarboxylates. Previously, when DAST was used as the fluorinating agent, the yield of fluorinated products was very low (~5%). The major side product was the unsaturated cyclohexenecarboxylate. The current procedure (Scheme 2) of using the mixture of DAST and HF/pyridine as the fluorinating agent gives a much higher yield (~70%). However, the products are the mixtures of four fluorinated isomers, which require extensive HPLC procedures to separate these isomers. The configuration of these fluorocyclohexanecarboxylates was established by proton NMR. A simple force field calculation of the possible 3- and 4-fluorocyclohexanecarboxylic methyl esters shows that in all cases, an axial carbomethoxy group is 7–8 kcal mol^{-1} higher in energy than an equatorial one, while there is no energetic preference for fluorine being either axial or equatorial (inhouse molecular mechanics calculation using software PC Model). Having established the basis for the carbomethoxy (or large) group's disposition toward remaining equatorial, the configuration at fluorine can be determined by a comparison of its geminal proton by ^{1}H NMR, an equatorial proton always appearing downfield of the corresponding axial proton in substituted cyclohexanes. This proton is easily discernible from the remaining ring protons, since it is strongly shifted downfield by its proximity to fluorine and additionally distinguishable by the large proton-fluorine coupling constant (doublet).

2.2. Synthesis of labeling precursors

Compounds **21** and **22** were prepared by hydrogenating 3-hydroxybenzoic acid using rhodium as the catalyst. The resulting mixture of 3-*cis* and 3-*trans* hydroxycyclohexanecarboxylic acid was converted to the pentamethylbenzyl ester and separated by silica gel flash chromatography (Scheme 3). Unlike the 4-hydroxy counterpart (**19 and 20**),[18] nosylate derivatives of **21** and **22** are unstable, and the mesylate derivatives were prepared instead. The reactivity of mesylate at the 3 position is similar to that of the nosylate at 4 position.

2.3. Synthesis of F-18 labeled WAY 100635 analogs

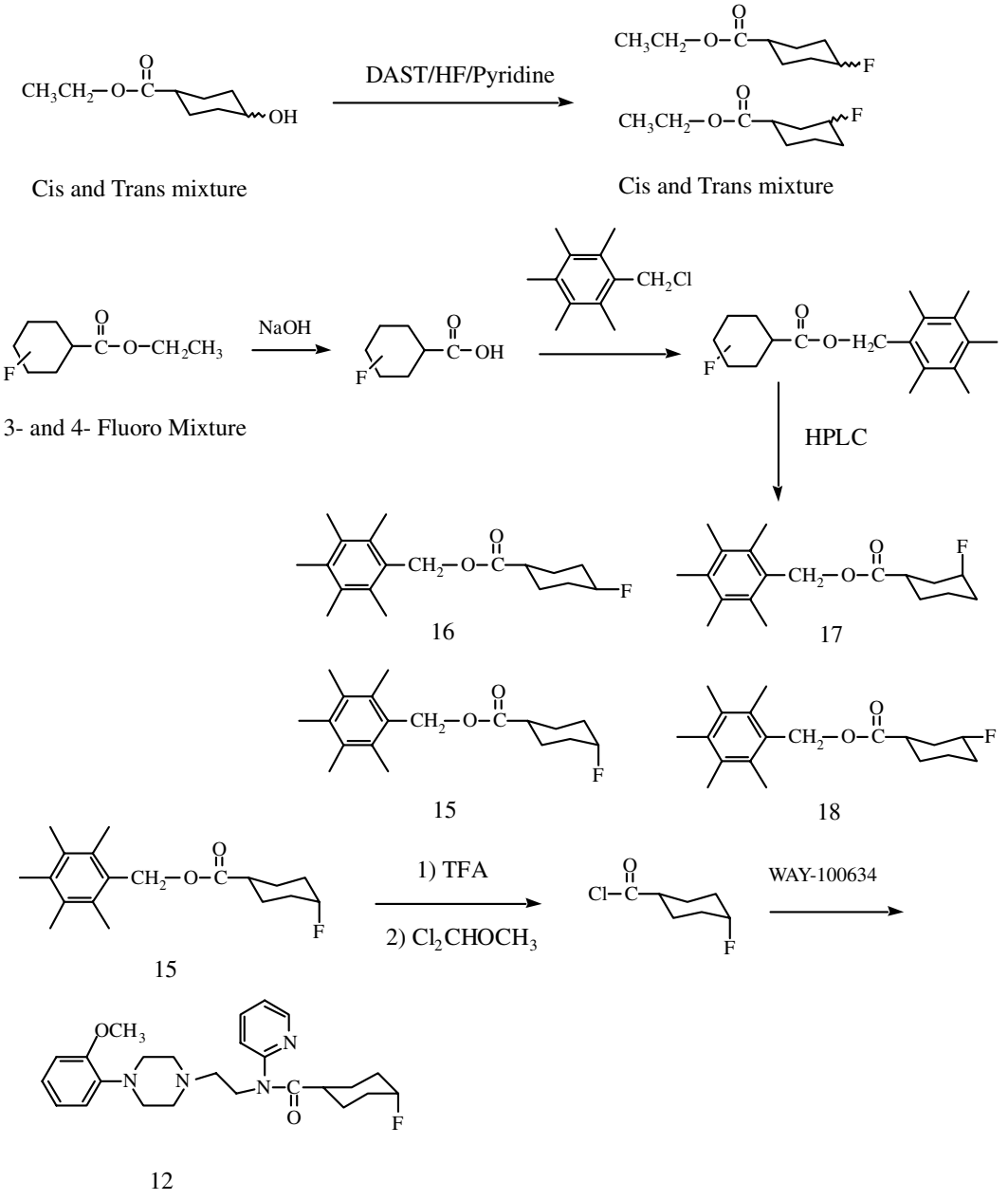
The four radiolabeled compounds were prepared by generating the radiolabeled acid first starting from either the 4-nosylate derivatives or 3-mesylate derivatives with F-18 and then combining this acid with the amine, WAY 100634, after conversion of the acid to the acid chloride. All substitution reactions undergo conversion of the configuration. The 4-*cis*-nosylate and the 4-*trans*-nosylate gave similar radiolabeling yields of 25–40% in acetonitrile and 35–50% in acetone. The 3-*trans*-mesylate gave 25–35% incorporation of F-18 and 3-*cis*-mesylate gave 15-25% incorporation of F-18 in acetonitrile. The reactions in acetone for both 3-*trans*- and 3-*cis*-mesylate gave only ~5% labeling yield. The 3-*trans*-mesylate had higher yields than the 3-*cis*-mesylate, but the radiochemical yield for substitution at the 3 position was lower than at the 4 position.

2.4. In vitro assays

We have developed three classes of compounds: those based on cyclohexanecarboxylic acid, those based on benzoic acid, and those based on benzoic acid with replacement of the pyridine with other aromatic ring systems.[22] None of the compounds tested showed agonist properties in the GTP-γ-S binding (data not shown). Among the compounds in the first class of compounds, the *trans*-4-FCWAY has a higher affinity than the comparable cis compound (Table 1); the *trans*- and *cis*-3-FCWAY show the same trend (Table 1). *trans*-4-FCWAY has a higher affinity compared with the benzoic acid series and this was reflected in the pharmacokinetics of these two groups of compounds.

Of the compounds in the third class (i.e., those containing other types of aromatic rings in place of the parent's pyridine) showed a lower range of inhibition constants, which did not lead to a clear structure–activity relationship.[22]

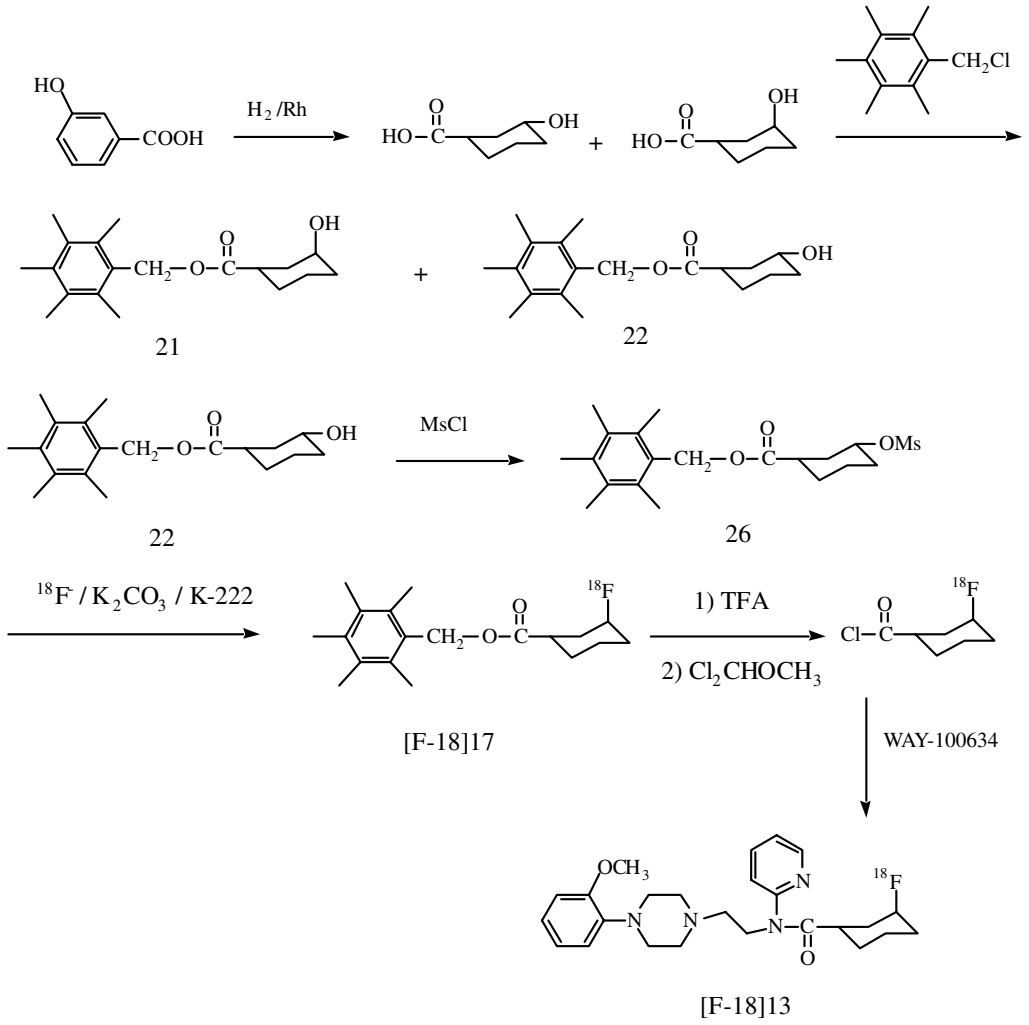
The study in rat hepatocytes showed that *trans*-4-FCWAY produced eight metabolites. The major metabolite was the oxidation product of metabolism of the aromatic ring. *cis*-4-FCWAY had a nearly identical metabolite profile.[25] The *cis*-3-FCWAY also had a similar profile, but the *trans*-3-FCWAY had a measurable amount of an oxidation product of the fluorocyclohexane ring (Figs. 1 and 2). *cis*-3-FCWAY had a slower rate of defluorination than that for the *trans*-3-FCWAY.



Scheme 2. Synthesis of FCWAYs.

2.5. In vivo studies in rats

The biodistribution of *trans*-4-FCWAY and *cis*-4-FCWAY showed the profound effects of the stereochemistry of the fluorine. The uptake of the 4-*cis* isomer at 30 min was roughly an order of magnitude lower than the 4-*trans* isomer in the rat hippocampus and cortex. The 4-*cis*-FCWAY had properties similar to those of the 4-fluorophenylcarboxamide derivatives (FBWAY and MeFBWAY) in that the expression for specific binding (H/Cb − 1) was 2.6, compared to values of 4.7 and 4.9 for the two 4-fluorophenylcarboxamides (Table 2). The blood clearance of the *cis*- and *trans*-4-fluoro compounds was similar. These compounds were blocked by co-injection of 50 nmol of WAY 100635 indicating saturable binding. As previously shown, 500 nmol of WAY 100635 was needed to reduce FBWAY hippocampal binding to that of the cerebellum.[18] The low net retention of *cis*-4-FCWAY at 30 min was due to rapid efflux, because the distribution of *cis*-4-FCWAY at 5 min was within a factor of 2 to that obtained with *trans*-4-FCWAY (Table 3). The uptake of *trans*-4-FCWAY did not seem to be related to α_1 receptor binding. Co-injection of 50 nmol of prazosin, an α_1 receptor antagonist, did not show a statistically significant change in the distribution of *trans*-4-FCWAY (Table 4). However, in the case of *cis*-4-FCWAY, binding in the cerebellum was blocked to some extent. This precluded a meaningful calculation of a target to non-target tissue ratio, where the cerebellum is (normally) used as the non-target tissue. A detectable decrease after prazosin co-injection occurred in both the thalamus and



Scheme 3. Synthesis F-18 labeled FCWAY.

Table 1. Inhibition constants (K_i, nM) obtained by in vitro assay

Compound	K_i versus [^{3}H]8-OH-DPAT human clone
MeFBWAY	0.79 ± 0.11
FBWAY	1.1
trans-4-FCWAY	0.25 ± 0.08
cis-4-FCWAY	1.45
trans-3-FCWAY	<1
cis-3-FCWAY	1.2
FPWAY	<1

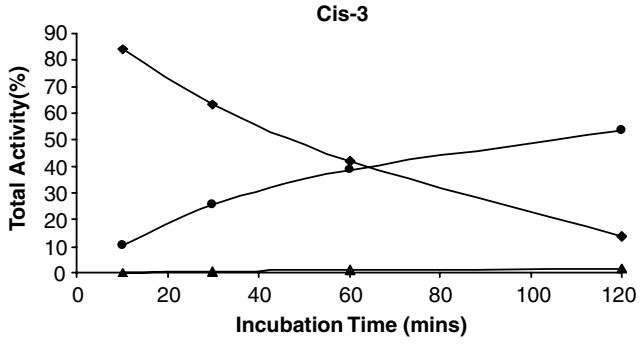


Figure 1. F-18 labeled *cis*-3-FCWAY and metabolites present after incubation with rat hepatocytes. *cis*-3-FCWAY (diamond), fluoride (triangle), and aromatic oxidation (circle).

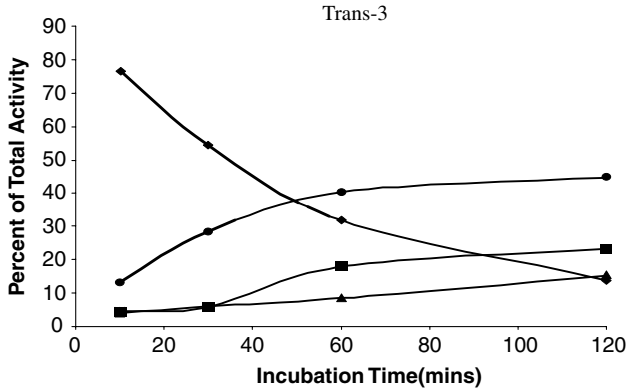


Figure 2. F-18 labeled *trans*-3-FCWAY and metabolites present after incubation with rat hepatocytes. *trans*-3-FCWAY (diamond), fluoride (triangle), aromatic oxidation (circle), and fluorocyclohexane oxidation (square).

cortex, but none in the hippocampus, most likely because of the relatively high concentration of 5 HT_{1A} receptors in that tissue. From these data, the *cis*-4-FCWAY appears to be inferior to the FBWAY and MeFBWAY as the target to nontarget ratio is lower and binding to the α_1 receptor is evident.

Table 2. Biodistribution (DUR) of radiolabeled WAY derivatives at 30 min in rats with and without 50 nmol of co-injected WAY 100635 ($n = 4$)

Compound	Cortex (Ctx)	Hippocampus (H)	Hypothalamus	Cerebellum	(Ctx − Cb)/Cb	(H − Cb)/Cb
FBWAY	0.17 ± 0.02	0.38 ± 0.11	0.13 ± 0.02	0.066 ± 0.008	1.5	4.7
+50 nmol	0.09 ± 0.01	0.14 ± 0.01	0.03 ± 0.007	0.05 ± 0.01	1.0	2.1
MeFBWAY	0.13 ± 0.02	0.32 ± 0.05	ND	0.05 ± 0.01	1.4	4.9
+50 nmol	0.05 ± 0.02	0.04 ± 0.01	ND	0.04 ± 0.006	0.1	0.0
4-*trans*-FCWAY	2.01 ± 0.36	3.05 ± 0.42	1.35 ± 0.29	0.16 ± 0.03	11.6	18.1
+50 nmol	0.56 ± 0.03	1.48 ± 0.14	0.37 ± 0.04	0.10 ± 0.006	4.6	13.8
4-*cis*-FCWAY	0.14 ± 0.02	0.26 ± 0.05	0.19 ± 0.04	0.07 ± 0.008	1.0	2.6
+50 nmol	0.07 ± 0.009	0.07 ± 0.007	0.11 ± 0.02	0.07 ± 0.007	0.0	0.0

Table 3. Biodistribution (DUR) of radiolabeled WAY derivatives at 5 min and 30 min after injection of *cis*-4-FCWAY and *trans*-4-FCWAY in rats ($n \geqslant 4$)

FCWAY	Blood	Caudate	Thalamus	Hippocampus	Brain stem	Cerebellum	Cortex
5′-*cis*	0.85 ± 0.12	0.44 ± 0.05	0.71 ± 0.13	1.30 ± 0.32	0.74 ± 0.13	0.39 ± 0.07	0.95 ± 0.21
30′-*cis*	0.52 ± 0.06	0.08 ± 0.02	0.11 ± 0.03	0.23 ± 0.03	0.14 ± 0.04	0.07 ± 0.02	0.13 ± 0.03
5′-*trans*	1.05 ± 0.09	0.89 ± 0.18	1.68 ± 0.24	4.31 ± 0.20	1.87 ± 0.04	0.59 ± 0.02	3.82 ± 0.11
30′-*trans*	0.64 ± 0.04	0.28 ± 0.05	0.88 ± 0.12	3.06 ± 0.20	ND	0.18 ± 0.04	1.97 ± 0.16

(DUR) differential uptake ratio: [(% ID/g) × body weight (g)/100].

Table 4. Percent reduction of *cis*-4-FCWAY and *trans*-4-FCWAY in rats 30 min after co-injection of 50 nmol 4-FCWAY, 200 nmol WAY 100635, or 50 nmol of prazosin

	% reduction[a]					
	50 nmol of 4-FCWAY		200 nmol of WAY 100635		50 nmol of prazosin	
	4-*trans*	4-*cis*	4-*trans*	4-*cis*	4-*trans*	4-*cis*
Blood	−2.42	−3.66	−0.23	17.77	−8.47	−2.70
Caudate	26.52	**16.81**[b]	27.23	22.67	9.08	**20.13**[b]
Thalamus	**76.80**[b]	**26.16**[b]	**77.38**[b]	27.99	17.14	**26.83**[b]
Hippocampus	**88.77**[b]	**66.96**[b]	**92.93**[b]	**70.82**[b]	15.67	2.66
Cerebellum	13.74	10.33	18.51	11.33	−6.41	**26.76**[b]
Cortex	**88.95**[b]	**48.28**[b]	**91.58**[b]	**46.69**[b]	11.43	**30.62**[b]

[a] % reduction = [(tissue uptake − tissue uptake with inhibitor)/tissue uptake] × 100.

[b] Values in bold represent reduction which is significantly different from the control tissue ($p < 0.05$; $n \geqslant 4$).

The second group of radiopharmaceuticals studied included the two 3-FCWAY analogs, *cis*-3-FCWAY and *trans*-3-FCWAY. The cis analog has an H/Cb ratio between 9 and 10, and a cortex/cerebellum (Ctx/Cb) ratio between 3 and 4 at 30 min (Table 5). Data obtained on two separate days show that reproducibility is high in these experiments. These ratios are higher than those obtained for MeBWAY (Table 2) and thus meet our criterion for a compound with an effective specific binding between that of MeBWAY and *trans*-4-FCWAY. The 3-trans derivative had H/Cb and Ctx/Cb ratios as high as those for *trans*-4-FCWAY but gave lower radiochemical yields (Table 5). To determine if α_1 adrenoceptor binding was involved in the 3-trans compound's biodistribution, 200 nmol of prasozin was co-injected, and blocking was observed (Table 6). As a result of these findings, this compound was not pursued.

Table 6. Biodistribution (DUR) of F-18 3-*trans* FCWAY in rats after 30 min with and without coinjection of 200 nmol of prazosin ($n \geqslant 4$)

	NCA	+200 nmol prazosin	% reduction
Blood	0.44 ± 0.03	0.54 ± 0.07	−22.3
Caudate	0.36 ± 0.05	0.25 ± 0.04	**31.8**[a]
Thalamus	1.56 ± 0.25	1.12 ± 0.34	28.3
Hippocampus	3.66 ± 0.60	3.11 ± 0.41	15.0
Brain stem	1.35 ± 0.15	1.12 ± 0.14	17.3
Cortex	2.81 ± 0.28	2.48 ± 0.31	11.8
Cerebellum	0.18 ± 0.03	0.14 ± 0.04	22.9

[a] Values in bold represent reduction which is significantly different from the control tissue ($p < 0.05$; $n \geqslant 4$).

Table 5. Biodistribution (DUR) of F-18 3 *cis*-FCWAY in rats after 30 min with and without co-injection of 200 nmol of WAY 100635 ($n \geqslant 6$) and biodistribution (DUR) of F-18 3-*trans*-FCWAY in rats after 30 min with and without coinjection of 200 nmol of WAY 100635 ($n \geqslant 4$)

	F-18 3 cis-FCWAY			F-18 3-*trans* FCWAY		
	NCA	+200 nmol WAY	% reduction	NCA	+200 nmol WAY	% reduction
Blood	0.31 ± 0.05	0.33 ± 0.05	−3.88	0.52 ± 0.03	0.54 ± 0.07	−5.14
Caudate	0.17 ± 0.04	0.11 ± 0.03	**33.30**[a]	0.44 ± 0.06	0.24 ± 0.03	45.39
Thalamus	0.24 ± 0.06	0.13 ± 0.04	**48.44**[a]	1.07 ± 0.10	0.23 ± 0.02	**78.82**[a]
Hippocampus	1.09 ± 0.06	0.12 ± 0.02	**89.12**[a]	3.73 ± 0.27	0.31 ± 0.04	**91.62**[a]
Brain stem	0.26 ± 0.02	0.11 ± 0.03	**56.20**[a]	1.23 ± 0.07	0.18 ± 0.02	**85.64**[a]
Cerebellum	0.12 ± 0.01	0.09 ± 0.02	18.39	0.16 ± 0.01	0.16 ± 0.01	1.59
Cortex	0.41 ± 0.06	0.12 ± 0.03	**71.88**[a]	2.91 ± 0.36	0.24 ± 0.01	**91.68**[a]
Femur	0.32 ± 0.04	0.34 ± 0.03	−5.54	0.83 ± 0.05	1.11 ± 0.03	−34.56

[a] Values in bold represent reduction which is significantly different from the control tissue ($p < 0.05$; $n \geqslant 6$). Average of two different days.

The final criterion is the rate of defluorination in vivo. It is clear from these data at 30 min in rats that the uptake in bone is low compared to fluoride itself, which gave a DUR of 5.1 whereas FBWAY gave a DUR at 0.25. The 3-*cis* is clearly the best of the four FCWAY compounds in this regard with a femur uptake at 30 min in rat of 0.3 DUR compared to 1.4 for 4-*trans*-FCWAY, 1.3 for 4-*cis*-FCWAY, 0.8 for 3-*trans* and 5.1 for fluoride. Rat metabolite study showed that all these radioligands were rapidly metabolized in the blood with ~70% parent activity at 5 min decreasing to 30% at 30 min. However, the metabolites did not get into the brain, after 30 min the brain was >97% parent for all the F-18 FCWAYs.

The position and configuration of the fluorine substitution can affect both affinity and stability of F-18 labeled WAY 100635 derivatives. The 4-*cis* isomer has the weakest affinity of the four isomers and the lowest hippocampus to cerebellum ratio. Although the 3-*trans* had a useable radiochemical yield, the H/Cb ratio was similar to that obtained with *trans*-4-FCWAY and we observed α_1 adrenoceptor binding. The 3-cis isomer has the lowest defluorination, nanomolar affinity, and an intermediate H/Cb ratio. The closest competitor is the previously reported compound with the 1,3-pyrimidine substituted for the pyridine in the FBWAY compound (FPWAY).[22] It has slightly poorer properties when compared to *cis*-3-FCWAY with an H/Cb ratio of 6.5 compared to an H/Cb ratio of 10 for *cis*-3-FCWAY. Neither of these compounds has yet to be shown to be sensitive to changes in serotonin concentration in vivo and, until that has been shown, *trans*-4-FCWAY is the compound of choice. However, two compounds, *cis*-3-FCWAY and FPWAY, are now available to test whether serotonin ligands can be used to measure endogenous serotonin.

In conclusion, *cis*-3-FCWAY has a factor of 2 higher H/Cb ratio compared to FPWAY and MeBWAY. *cis* 3-FCWAY has a low rate of defluorination in this series of four alkyl fluoride containing compounds, but not as low as that obtained for FPWAY. A series of radiofluorinated compounds that bind to the 5-HT_{1A} receptor are now available to study a range of serotonergic processes. High affinity 4-*trans*-FCWAY had the best properties for measuring receptor density given its high hippocampus to cerebellum ratio and 3-*cis*-FCWAY had the best properties of the analogs with lower affinity and faster pharmacokinetics to be responsive to endogenous serotonin concentrations.

3. Experimental

3.1. General chemistry

WAY 100634 (**2**) was obtained from Med-Life Systems, Inc. Upper Darby, PA. All other chemicals were purchased from Aldrich Chemical Company, Milwaukee, WI. The ^1H and ^{13}C NMR spectra were obtained on a Varian Gemini-2000 at 200 and 50 MHz, respectively. Chemical shifts are reported in ppm (δ) downfield of tetramethylsilane and coupling constants are given in Hertz. Mass spectra were recorded on a Hewlett Packard 5989B Mass Spectrometer linked to a Hewlett Packard 5890 Series II Plus Gas Chromatograph. Elemental analyses were performed by Galbraith Laboratories (Knoxville, TN). Thin-layer chromatography of the radioactive products was performed on Whatman LK6DF silica gel glass-backed plates (5×20 cm, 250 μm). TLC radio chromatograms were obtained using Fuji Bio-imaging Analysis System 1500. Semi-prep HPLC was carried out using a Perkin-Elmer series 200 LC pump and UV absorbance was monitored with a Waters 486 UV detector and radioactivity was monitored using a Beckman Model 170 radioisotope detector. The purification of radiolabeled compounds was performed using a reversed-phase semi-prep Rainin Microsorb, 5 μm (10×250 mm) C-18 column.

3.2. Mixture of *trans*-, *cis*-ethyl 4-fluorocyclohexanecarboxylates and *trans*-, *cis*-ethyl 3-fluorocyclohexanecarboxylates

To an ice cold solution of 100 g of 70% hydrogen fluoride in pyridine containing 2.52 g of sodium fluoride (60.0 mmol) was added 6.89 g of commercially available ethyl 4-hydroxycyclohexanecarboxylate (40.0 mmol, mixture of cis and trans isomers) and 5.28 ml (diethylamino)sulfur trifluoride (40.0 mmol). The mixture was stirred at room temperature for 4 h and then treated with 300 ml of 25% sodium carbonate solution and more solid sodium carbonate was added slowly until the solution was neutral. The aqueous solution was extracted with 2× 200 ml of dichloromethane and treated with 2 ml of bromine to add across the double bond of the ethyl cyclohex-3-enecarboxylate formed during the reaction. The solution was dried over anhydrous sodium sulfate and solvent evaporated at reduced pressure. The residue was distilled at 100 °C under the vacuum (0.1 mm) to give a mixture of 4.81 g of the four cis and trans fluorinated products in 70% yield.

3.3. Mixture of *trans*-, *cis*-4-fluorocyclohexanecarboxylic acids and *trans*-, *cis*-3-fluorocyclohexanecarboxylic acids

To an aqueous solution of 10% sodium hydroxide (40 ml) was added 4.01 g of above fluorinated mixture (23.0 mmol). The solution was stirred at room temperature overnight and then acidified with 12 N HCl dropwise until the solution was tested acidic by pH paper. Upon standing at room temperature, the hydrolyzed products precipitated out of the solution as the mixture of four fluorinated cyclohexanecarboxylic acids (2.61 g, yield 78%).

3.4. Mixture of *trans*-, *cis*-pentamethylbenzyl 4-fluorocyclohexanecarboxylates and *trans*-, *cis*-pentamethylbenzyl 3-fluorocyclohexanecarboxylates

To a solution of the above mixture of the fluorinated cyclohexanecarboxylic acids (2.00 g, 13.7 mmol) in 20 ml *N*,*N*-dimethylformamide were added pentamethylbenzyl chloride (2.69 g, 13.7 mmol) and triethylamine (1.39 g, 13.7 mmol). The mixture was stirred at room temperature overnight. The reaction mixture was

poured into a 200 ml 10% sodium bicarbonate solution to give a white precipitate. The solid products were collected by filtration and dried under vacuum to give 3.62 g of mixture of four pentamethylbenzyl esters of fluorocyclohexanecarboxylic acid (86%).

3.5. *cis*-Pentamethylbenzyl 4-fluorocyclohexanecarboxylate (15)

Part of the mixture (1.98 g) was dissolved in 10% ethyl acetate/hexane and about 60 mg of mixture in 0.5 ml solution was injected each time onto a normal phase prep-HPLC column running with 5% ethyl acetate/hexane at 10 ml/min. The pure *cis*-pentamethylbenzyl 4-fluoroxycyclohexanecarboxylate came out at about 11 min and remaining mixture came out about 13 min. After 30 injections, the fractions containing the product were combined to yield 250 mg of *cis*-pentamethylbenzyl 4-fluorocyclohexanecarboxylate as white crystals, mp 132–133 °C. ^{1}H NMR ($CDCl_3$) δ 1.28–1.62 (m, 2H), 1.72–2.12 (m, 6H), 2.17–2.48 (m, 16H), 4.76 (dm, J_{HF} = 47.9 Hz, 1H), 5.24 (s, 2H). ^{13}C NMR ($CDCl_3$) δ 16.4, 16.8, 17.2, 23.4 (d, J_{CF} = 2.7 Hz), 30.0 (d, J_{CF} = 18.7 Hz), 41.9, 62.4, 88.4 (d, J_{CF} = 168.9 Hz), 129.5, 133.1, 134.1, 136.2, 175.6. MS (EI) 306 (M^+), 160, 145,131, 105.

3.6. *trans*-Pentamethylbenzyl 4-fluoroxycyclohexanecarboxylate, *trans*- and *cis*-pentamethylbenzyl 3-fluoroxycyclohexanecarboxylate (16, 17, and 18)

The remaining of the above mixture was further separated with reversed-phase semi-prep HPLC with 60% acetonitrile running at 6 ml/min to give 410 mg of *trans*-pentamethylbenzyl 4-fluorocyclohexanecarboxylate (**16**), 320 mg of trans pentamethylbenzyl 3-fluorocyclohexanecarboxylate (**17**), and 210 mg of *cis*-pentamethylbenzyl 3-fluorocyclohexanecarboxylate (**18**).

Compound **16**: mp 104–105 °C. ^{1}H NMR ($CDCl_3$) δ 1.40–1.67 (m, 4H), 1.92–2.18 (m, 4H), 2.20–2.40 (m, 16H), 4.50 (dm, J_{HF} = 48.4 Hz, 1H), 5.25 (s, 2H). ^{13}C NMR ($CDCl_3$) δ 16.4, 16.8, 17.2 26.1 (d, J_{CF} = 10.8 Hz), 31.8 (d, J_{CF} = 19.0 Hz), 41.7 (d, J_{CF} = 1.5 Hz), 62.5, 91.2 (d, J_{CF} = 172.4 Hz), 129.4, 133.2, 134.1, 136.3, 175.6 (d, J_{CF} = 2.4 Hz). MS (EI) 306 (M^+), 160, 145, 131, 105.

Compound **17**: mp 105–106 °C. ^{1}H NMR ($CDCl_3$) δ 1.22–1.78 (m, 5H), 1.82–2.02 (m, 3H), 2.10–2.38 (m, 15H), 2.75 (tt, 1H), 4.90 (dm, J_H = 48.4 Hz, 1H), 5.24 (s, 2H). ^{13}C NMR ($CDCl_3$) δ 16.4, 16.8, 17.2, 19.7 (d, J_{CF} = 2.0 Hz), 28.3, 30.3, (d, J_{CF} = 21.0 Hz), 33.2 (d, J_{CF} = 21.0 Hz), 38.0, (d, J_{CF} = 1.5 Hz), 62.3, 88.6 (d, J_{CF} = 168.9 Hz), 129.4, 133.1, 134.0, 136.2, 176.0. MS (EI) 306 (M^+), 160, 145, 131, 105.

Compound **18**: mp 99–100 °C. ^{1}H NMR ($CDCl_3$) δ 1.22–1.57 (m, 3H), 1.59–1.69 (m, 2H), 1.80–2.00 (m, 2H), 2.00–2.14 (m, 1H), 2.18–2.37 (m, 16H), 4.46 (dm, J_{HF} = 48.2 Hz, 1H) 5.25 (s, 2H). ^{13}C NMR ($CDCl_3$) δ 16.4, 16.8, 17.2, 22.6 (d, J_{CF} = 11.2 Hz), 27.9 (d, J_{CF} = 2.0 Hz), 32.2 (d, J_{CF} = 18.6 Hz), 35.0 (d, J_{CF} = 20.5 Hz), 41.4 (d, J_{CF} = 11.2 Hz), 62.6, 91.4 (d, J_{CF} = 173.3 Hz), 129.4, 133.2, 134.1, 136.3, 174.9 (d, J_{CF} = 2.4 Hz). MS (EI) 306 (M^+), 160, 145, 131, 105.

3.7. *cis*- and *trans*-Pentamethylbenzyl 4-hydroxycyclohexanecarboxylates (19, 20)

Preparation of pure *cis*-pentamethylbenzyl 4-hydroxycyclohexanecarboxylate (**19**) has been described previously starting from the mixture of *cis*- and *trans*-ethyl 4-hydroxycyclohexanecarboxylates.[18] The pure cis isomer was obtained by fractional recrystallization from the mixture of cis and trans isomers of pentamethylbenzyl 4-hydroxycyclohexanecarboxylate. After recrystallization and collection of cis isomer, the mother liquor was evaporated to obtain a mixture of isomers, enriched in trans (**20**). This could be carried through to obtain pure *trans*-nosylate (**23**) in the next step.

3.8. *trans*- and *cis*-Pentamethylbenzyl 3-hydroxycyclohexanecarboxylates (21, 22)

To a solution of 2.00 g of 3-hydroxybenzoic acid in 100 ml ethyl acetate in a 300 ml pressure bottle were added 300 mg of rhodium (5 wt. % on alumina) and 1.0 ml of acetic acid. The mixture was hydrogenated at 60 psi overnight. Analysis by GC/MS indicated a mixture of unreduced 3-hydroxybenzoic acid, fully reduced cyclohexanecarboxylic acid, and 3-*cis*-and 3-*trans*-hydroxycyclohexanecarboxylic acid. The catalyst was then filtered and the solvent evaporated to give 1.95 g of solid mixture. The mixture was redissolved in 20 ml of *N*,*N*-dimethylformamide and 2.85 g of pentamethylbenzyl chloride, 2.0 ml of triethylamine were added to the solution. After stirring at room temperature overnight, the solution was poured into a 200 ml 10% sodium bicarbonate solution to give a white precipitate.

The solid was collected by filtration and air-dried to give 3.40 g of a mixture of *cis*- and *trans*-pentamethylbenzyl 3-hydroxycyclohexanecarboxylate. This mixture was dissolved in minimal amount of dichloromethane, loaded onto a 5.5 × 20 cm flash chromatography silica gel column, and eluted with 10% ethyl acetate/hexanes (v/v). The fractions containing the product were combined and solvent evaporated to give 0.74 g of *trans*-pentamethylbenzyl 3-hydroxycyclohexanecarboxylate (**21**) (17%) and 1.38 g of *cis*-pentamethylbenzyl 3-hydroxycyclohexanecarboxylate (**22**) (32%).

Compound **21**: mp 139.5–141.0 °C. ^{1}H NMR ($CDCl_3$) δ 1.4–1.7 (m, 6H), 1.7–1.9 (m, 2H), 2.2–2.4 (m, 15H), 2.8 (m, 1H), 4.0 (m, 1H), 5.2 (s, 2H). ^{13}C NMR ($CDCl_3$) δ 16.5, 16.9, 17.3, 20.0, 28.4, 33.0, 35.7, 38.2, 62.4, 66.3, 129.5, 133.0, 134.0, 136.1, 176.3. MS (EI) 304 (M^+), 160, 145, 131 105.

Compound **22**: mp 134–135 °C. ^{1}H NMR ($CDCl_3$) δ 1.2–1.6 (m, 4H), 1.8–2.1 (m, 4H), 2.2–2.4 (m, 16H), 3.7 (m, 1H), 5.3 (s, 2H). ^{13}C NMR ($CDCl_3$) d 16.5, 16.9, 17.3, 23.3, 28.2, 35.1 37.8, 42.1, 62.6, 69.9, 129.3, 133.1, 134.0, 136.2, 175.5. MS (EI) 304 (M^+), 160, 145, 131 105.

3.9. *trans*- and *cis*-Pentamethylbenzyl 4-(4-nitrobenzenesulfonyl)-oxycyclohexanecarboxylates (23, 24)

To a solution of 0.61 g (2.0 mmol) mixture of **19** and **20** obtained from the mother liquor as described earlier in 10 ml methylene chloride were added 4-nitrobenzenesulfonyl chloride (0.44 g, 2.0 mmol) and triethylamine (0.28 ml, 2.0 mmol). The mixture was stirred at room temperature overnight. At the end of the reaction, the solid was filtered and the solution was loaded on a 5.5×20 cm silica gel and eluted with 20% ethyl acetate/hexane to give 0.41 g **23** (42%) and 0.15 g of **24** (16%) as a white solid.

Compound **23**: mp 131 °C (d). ^{1}H NMR ($CDCl_3$) δ 1.4–1.6 (m, 4H), 1.9–2.1 (m, 4H), 2.2–2.4 (m, 16H), 4.6 (m, 1H), 5.22 (s, 2H), 8.1 (d, 2H), 8.4 (d, 2H). ^{13}C NMR ($CDCl_3$) δ 16.4, 16.8, 17.2, 26.5, 31.3, 41.3, 62.6, 82.4, 124.7, 129.2, 129.2, 133.2, 134.0, 136.4, 143.5, 150.9, 175.0. MS (EI) 446, 412, 363, 322, 308, 286, 271, 225, 203, 160, 147, 131, 108, 81.

Compound **24**: mp 137 °C (d). ^{1}H NMR ($CDCl_3$) δ 1.4–2.0 (m, 8H), 2.2–2.3 (m, 15H), 2.4 (m, 1H) 4.9 (m, 1H), 5.22 (s, 2H), 8.1 (d, 2H), 8.4 (d, 2H). ^{13}C NMR ($CDCl_3$) δ 16.6, 16.9, 17.3, 23.5, 30.2, 41.1, 62.6, 80.6, 124.6, 129.1, 129.2, 133.1, 134.0, 136.3, 143.5, 150.8, 174.9. MS (EI) 488 (M^+), 446, 442, 362, 354, 308, 286, 243, 225, 203, 160, 147, 131, 81.

3.10. *trans*-Pentamethylbenzyl 3-(methanesulfonyl)oxycyclohexanecarboxylate (25)

To a solution of **21** (81 mg, 0.27 mmol) in 2 ml methylene chloride were added methanesulfonyl chloride (22 μl, 0.28 mmol) and diisopropylethylamine (51 μl, 0.29 mmol). The mixture was stirred at room temperature overnight. At the end of the reaction, the solution was loaded on to a 3.8×20 cm flash chromatography silica gel column and eluted with 3% ethyl acetate in methylene chloride. The fractions containing the product were combined and the solvent evaporated to give 55 mg (54%) of white solid. Mp 120–121 °C. ^{1}H NMR ($CDCl_3$) δ 1.4–1.8 (m, 4H), 1.8–2.0 (m, 4H), 2.1–2.4 (m, 15H), 2.8 (m, 1H), 3.0 (s, 3H), 5.0 (m, 1H), 5.25 (s, 2H). ^{13}C NMR ($CDCl_3$) δ 16.6, 16.9, 17.3, 20.0, 28.1, 31.0, 33.4, 38.3, 38.7, 62.7, 78.3, 129.2, 133.1, 134.0, 136.3, 175.2. MS (EI) 382 (M^+), 338, 308, 286, 256, 205, 175, 160, 145, 131,108, 81.

3.11. *cis*-Pentamethylbenzyl 3-(methanesulfonyl)oxycyclohexanecarboxylate (26)

To a solution of **22** (200 mg, 0.66 mmol) in 4 ml methylene chloride were added methanesulfonyl chloride (54 μl, 0.68 mmol) and diisopropylethylamine (126 μl, 0.71 mmol). The mixture was stirred at room temperature overnight. At the end of the reaction, the product was purified by flash chromatography on silica gel as described above to give 146 mg white solid (58%). mp 120.0–121.5 °C. ^{1}H NMR ($CDCl_3$) δ 1.2–2.0 (m, 6H), 2.1–2.5 (m, 17H), 3.0 (s, 3H), 4.6 (m, 1H), 5.25 (s, 2H). ^{13}C NMR ($CDCl_3$) δ 16.5, 16.9,17.3, 23.4, 27.8, 32.6, 35.1, 39.1, 42.0, 62.8, 80.1, 129.2, 133.1, 134.0, 136.3, 174.2. MS (EI) 382 (M^+), 338, 308, 286, 256, 205, 175, 160, 145, 131, 108, 81.

3.12. *cis*-4-Fluoro-*N*-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-*N*-(2- pyridyl)cyclohexanecarboxamide (12)

To a 5 ml V-vial were added 150 mg of **15**, 0.3 ml anisole, and 2 ml of trifluoroacetic acid. The mixture was stirred at room temperature for 5 min. Trifluoroacetic acid was evaporated and 0.20 ml of dichloromethyl methylether was added and heated at 90 C for 5 min. The vial was cooled and evaporated with argon flow. To the above residue, 0.20 g of WAY-100634 (**2**) and 85 μL of TEA in 3.0 ml of methylene chloride were added and stirred at room temperature for 2 h. At the end of the reaction, the solution was loaded onto a flash chromatography silica gel column and eluted with ethyl acetate containing 0.1% TEA to give 140 mg of product (65%). ^{1}H NMR ($CDCl_3$) δ 1.16–1.44 (m, 2H), 1.58–1.63 (m, 2H), 1.86–2.06 (m, 4H), 2.27–2.33 (m, 1H), 2.59–2.66 (m, 6H) 2.99 (s, broad, 4H), 3.84 (s, 3H), 3.99 (t, 2H), 4.71 (dm, J_{HF} = 48.8 Hz, 1H), 6.82–7.03 (m, 4H), 7.21–7.34 (m, 2H), 7.76 (td, 1H), 8.51 (dd, 1H). ^{13}C NMR ($CDCl_3$) δ 23.6, 30.0 (d, J_{CF} = 21.5 Hz), 41.2, 45.4, 50.7, 53.5, 55.4, 56.3, 87.8 (d, J_{CF} = 168.4 Hz), 111.4, 118.3, 121.1, 122.4, 122.5, 123.0, 138.5, 141.6, 149.5, 152.5, 156.2, 176.6. MS (EI) 440 (M^+), 425, 311, 278, 249, 218, 205, 190, 162, 149. Anal. Calcd ($C_{25}H_{33}FN_4O_2F$): C, 68.16; H, 7.55; N, 12.72. Found, C, 67.85; H, 7.80, N, 12.50. Compounds **13** and **14** are prepared using a similar procedure.

3.13. *trans*-3-Fluoro-*N*-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-*N*-(2-pyridyl)cyclohexanecarboxamide (13)

^{1}H NMR ($CDCl_3$) δ 1.22–2.22 (m, 9H), 2.58–2.81 (m, 6H), 2.99 (s, broad, 4H), 3.84 (s, 3H), 4.00 (t, 2H), 4.89 (dm, J_{HF} = 48.2 Hz, 1H), 6.83–7.04 (m, 4H), 7.22–7.33 (m, 2H), 7.77 (dt, 1H), 8.53 (dd, 1H). ^{13}C NMR ($CDCl_3$) δ 19.6, 28.7, 30.1 (d, J_{CF} = 21.5 Hz), 33.7 (d, J_{CF} = 20.0 Hz), 36.6, 45.4, 50.8, 53.6, 55.5, 56.4, 89.1 (d, J_{CF} = 167.0 Hz), 111.5, 118.4, 121.2, 122.5, 122.7, 123.1, 138.5, 141.7, 149.6, 152.6, 155.9, 175.9. MS (EI) 440 (M^+), 425,311, 218, 205, 190, 162, 149. Purity: 95% by HPLC.

3.14. *cis*-3-Fluoro-*N*-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-*N*-(2- pyridyl)cyclohexanecarboxamide (14)

^{1}H NMR ($CDCl_3$) δ 1.01–1.14 (m, 1H), 1.26–1.56 (m, 2H), 1.76–1.85 (m, 3H), 1.99–2.38 (m, 3H), 2.59–2.66 (m, 6H), 2.99 (s, broad, 4H), 3.84 (s, 3H), 3.98 (t, 2H), 4.24 (dm, J_{HF} = 48.8 Hz, 1H), 6.82–7.02 (m, 4H), 7.22–7.34 (m, 4H), 7.78 (td, 1H), 8.53 (dd, 1H). ^{13}C NMR ($CDCl_3$) δ 23.0 (d, J_{CF} = 12.2 Hz), 28.4, 32.3 (d, J_{CF} = 18.1 Hz), 35.6 (d, J_{CF} = 20.0 Hz), 40.4 (d, J_{CF} = 11.22 Hz), 45.7, 50.8, 53.6, 55.5, 56.3, 91.7 (d, J_{CF} = 173.3 Hz), 111.5, 118.4, 121.2, 122.3, 122.7, 123.1, 138.6, 141.6, 149.7, 152.6, 156.0, 174.6. MS (EI) 440 (M^+), 425,311, 218, 205, 190, 162, 149. Purity: 96% by HPLC.

3.15. Synthesis of [^{18}F] labeled WAY 100635 analogs

To a 1 ml V-vial containing 3 μmol of potassium carbonate in 15 μl of water and 6 μmol of Kryptofix-2.2.2. in 30 μl of acetonitrile, 300–500 μl of aqueous F-18 fluoride activity (30–50 mCi) was added. The water was removed with argon flow three times using anhydrous acetonitrile on a 105 °C heating block. To the above vial containing the anhydrous [^{18}F]fluoride ion, 3 mg of substrate (**23**, **24**, **25**, or **26**,) in 0.1 ml of acetonitrile or acetone was added. The vial was sealed and heated on the heating block for 10 min. The reaction mixture was cooled to room temperature and diluted with 1 ml ether. The ether solution was passed through a small silica gel column (0.5 ml) to a 5 ml V-vial and rinsed with another 1 ml ether. The solvent was evaporated with argon flow and the pentamethylbenzyl ester was hydrolyzed with 0.1 ml trifluoroacetic acid in 2 min at room temperature. The trifluoroacetic acid was removed with argon flow and 40 μL of α,α-dichloromethyl methylether was added to the reaction vial. The vial was sealed using a cap with Teflon seal, heated at 90 °C for 5 min, and cooled in ice water for 2 min. The vial cap was opened and the remaining α,α-dichloromethyl methylether was evaporated with argon flow. The vial containing the acid chloride was added 5 mg of substrate (**2**) in 0.2 ml acetonitrile containing 5 μL triethylamine. The vial was sealed and heated at 90 °C for 5 min. The vial was cooled in ice water and the reaction mixture diluted with 0.3 ml HPLC solvent (40–50% acetonitrile in 5 mM phosphate buffer containing triethylamine at a flow rate of 5 ml/min) and injected onto the reversed-phase HPLC column. The fraction containing the product was collected, diluted with 2 volumes of water, and passed through a 1 ml C-18 Bond Elute column. The column was washed with 10 ml of water and the product trapped on the column was eluted with 0.5 ml ethanol. The over radiochemical yields are 10–15% for 4-*cis* and 4-*trans* compounds and 5–10% for 3-*cis* and 3-*trans* compounds. The radiochemical purities were >98% for all four compounds. The specific activity for these compounds was between 2000 and 8000 Ci/mmol EOB.

3.16. In vitro assays (Table 1)

Receptor binding studies were carried out using 1.5 nM H-3 8-OH-DPAT with human recombinant CHO-K1 Cells containing serotonin 5-HT$_{1A}$ receptors. Five concentrations of each compound were used to determine an IC$_{50}$, which was converted to a K_i. Binding to adrenergic alpha$_1$ and dopamine D$_2$ receptors was also determined. All compounds showed inhibition of binding to the adrenergic α_1 and dopamine D$_2$ receptors at a concentration of 10 μM. The inhibition at the adrenergic α_1 receptor was greater than 95% for all compounds at this concentration, whereas the inhibition at the dopamine D$_2$ receptor was much lower and always less than 50% inhibition. Testing was performed at MDS Panlabs, Seattle, WA, on two separate occasions.

In addition, a complete analysis ($K_i \pm$ SEM) was carried out for MeFBWAY (0.791 ± 0.113) and *trans*-4-FCWAY (0.247 ± 0.085 nM). The biogenic amine profile for *trans*-4-FCWAY gave the following K_i (nM) values: adrenergic α_1 40.9 ± 4.3, adrenergic α_2 3600 ± 400, dopamine D$_2$ 152 ± 15.2, dopamine D$_3$ 79 ± 23, and serotonin 5-HT$_{2A}$ 514 ± 143. The biogenic amine profile for MeFBWAY gave the following K_i (nM) values: adrenergic α_1 12.5 ± 2, adrenergic α_2 342 ± 14, dopamine D$_2$ 119 ± 7, dopamine D$_3$ 26 ± 10, serotonin 5-HT$_{2A}$ 133 ± 32, σ_1 527 ± 162, and σ_2 5300 ± 1000. Testing was performed by MDS Panlabs, Seattle, WA.

3.17. Rat biodistribution studies

In the biodistribution studies, the rats were injected intravenously with 50 μCi of ^{18}F radioligand or coinjected with 50 μCi of ^{18}F radioligand and 50 nmol of nonradioactive WAY 100635. The rats were sacrificed at 30 min and the brain was immediately placed in 0.3 M sucrose on ice. The blood and other tissues were excised from each animal and weighed. The brain was dissected on ice and the various brain regions weighed. The radioactive content of the blood and various tissues was assessed by gamma counting.

3.18. Rat metabolite studies

The rats were injected intravenously with 100 μCi of the ^{18}F WAY 100635 analogs and sacrificed at 5, 10, 15, and 30 min. Blood and brain were removed, weighed, and counted to determine the total activity. After the blood was centrifuged, the serum was removed, mixed with an equal volume of acetonitrile, and centrifuged. The brain was placed in an equal volume of acetonitrile and homogenized for 15–30 s bursts. Following centrifugation, the radioactive content of the supernatant and pellets was determined. The supernatants were applied to TLC plates, developed, and placed on a phosphorimaging plate overnight. The plates were scanned for the WAY analog and its metabolites the next day using a Fuji Bio-imaging Analysis System 1500.

3.19. Incubation of cold and F-18 labeled FCWAYs with hepatocytes

The cryopreserved hepatocytes from male Sprague–Dawley rats (In Vitro Technologies, Inc., Baltimore, MD) were used for in vitro metabolism studies of FCWAYs. The cells, which were stored in liquid nitrogen, were thawed rapidly at 37 °C in a water bath and gradually diluted with cell culture medium (RPMI Medium 164O media, Life Technologies, Rockville, MD). After washing the cells with the medium, the viable cell concentration was adjusted to 1.0 million per ml and the resulting cell suspension was incubated at 37 °C for 15 min prior to the introduction of test compound. From stock solution of FCWAY (2.0 mg/ml in 10% EtOH in water), 10 μl was added into 1 ml of cell suspension, to give a final concentration of 20 μg/ml. The suspension was maintained at 37 °C, 100 μl of cell

suspension was removed and added to 100 μl acetonitrile at 10, 30, 60, and 120 min. Each suspension was centrifuged at 5000 rpm for 5 min. The metabolites in 20 μl supernatant were analyzed by LC/MS.

For the analysis of radiolabeled compounds, 0.5 mCi of [^{18}F]FCWAY in 20 μl ethanol was added to 1.0 ml suspension of cells and corresponding unlabeled compound, prepared as described in the preceding paragraph. Metabolites at 10, 30, 60, and 120 min were chosen for analysis, which was performed in the same manner as before. Supernatant (20 μl) was injected into the HPLC with a radioactivity detector and analyzed by LC/MS.

3.20. LC/MS

All experiments were performed with a Finnigan LCQ MS (Finnigan Corporation, San Jose, CA, USA) coupled with HP series 1100 HPLC system (Agilent Technologies Company, Palo Alto, CA, USA). HPLC utilized a YMC-pack ProC-18 reversed-phase column (150 × 4.6 mm ID, YMC, Inc c/o Waters, Milford, MA, USA), eluting with 50 mM ammonium acetate and acetonitrile at 0.5 ml/min flow rate and a gradient of 0–65% acetonitrile over 10 min followed by isocratic elution at 65% for an additional 10 min. The entire column eluent was introduced into the ESI MS source with a standard high flow tune method. Ion detection was achieved with the Finnigan ESI using a spray voltage of +4200 V, capillary heater temperature of 200 °C, sheath gas flow of 80 ml/min (N_2), and an auxiliary gas flow of 20 ml/min (N_2). By using the entire column eluent, FCWAYs could be determined with the detection limit of 1 pmol on-column.

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