Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

Synthesis and Characterization of a Novel Series of Agonist Compounds as Potential Radiopharmaceuticals for Imaging Dopamine $D_{2/3}$ Receptors in Their High-Affinity State

Jan-Peter van Wieringen,[†,#] Vladimir Shalgunov,[‡,#] Henk M. Janssen,[§] P. Michel Fransen,[§] Anton G. M. Janssen,[∥] Martin C. Michel,[⊥] Jan Booij,*[,†] and Philip H. Elsinga[‡]

[†]Department of Nuclear Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

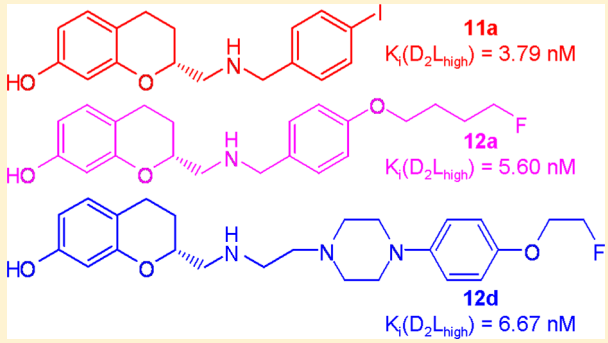
[‡]Department of Nuclear Medicine and Molecular Imaging, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

[§]SyMO-Chem BV, Eindhoven, The Netherlands

[∥]GE Healthcare, Eindhoven, The Netherlands

[⊥]Department of Pharmacology, Johannes Gutenberg University, Mainz, Germany

ABSTRACT: Imaging of dopamine $D_{2/3}$ receptors ($D_{2/3}R$) can shed light on the nature of several neuropsychiatric disorders in which dysregulation of $D_{2/3}R$ signaling is involved. Agonist $D_{2/3}$ tracers for PET/SPECT imaging are considered to be superior to antagonists because they are more sensitive to dopamine concentrations and may selectively label the high-affinity receptor state. Carbon-11-labeled $D_{2/3}R$ agonists have been developed, but these short-lived tracers can be used only in centers with a cyclotron. Here, we report the development of a series of novel D_2R agonist compounds based on the 2-aminomethylchromane (AMC) scaffold that provides ample opportunities for the introduction of longer-lived [^{18}F] or [^{123}I]. Binding experiments showed that several AMC compounds have a high affinity and selectivity for $D_{2/3}R$ and act as agonists. Two fluorine-containing compounds were [^{18}F]-labeled, and both displayed specific binding to striatal $D_{2/3}R$ in rat brain slices in vitro. These findings encourage further in vivo evaluations.



INTRODUCTION

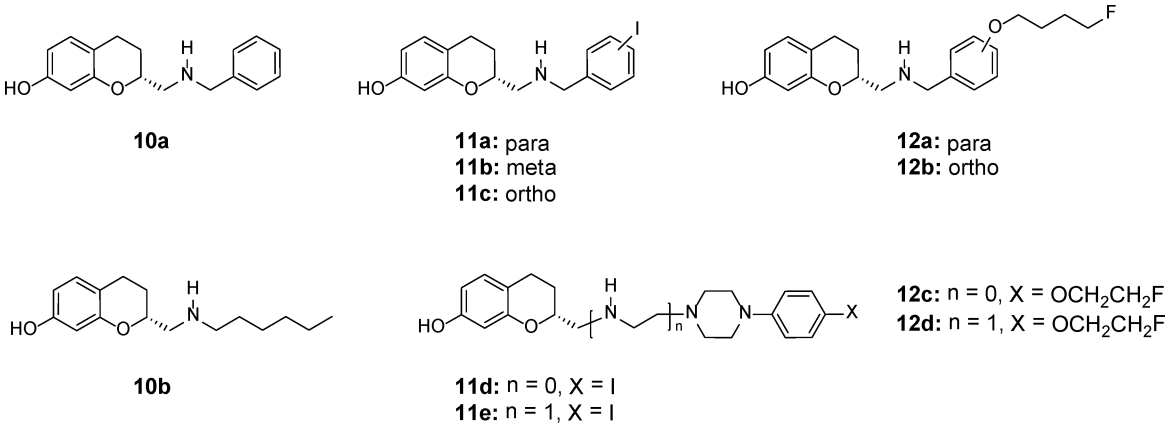
A disturbed dopamine system plays a central role in several neuropsychiatric disorders, including Parkinson's disease (PD),[1] dementia with Lewy bodies,[2] schizophrenia,[3] and drug addiction.[4] The prevalence of PD and schizophrenia is about 1% of the total population,[5,6] whereas the prevalence of (alcohol) addiction may be even higher,[7] creating a large disease burden. For dementia with Lewy body pathology, the prevalence among people above the age of 65 years is about 10%.[8]

Dopamine receptors belong to the superfamily of G-protein coupled receptors (GPCRs) and are divided into two subfamilies based on their action on adenylyl cyclase (AC). The dopamine D_1-like (D_1 and D_5) subfamily stimulates AC to produce the second messenger cyclic adenosine monophosphate (cAMP), whereas D_2-like receptors (D_2, D_3, and D_4) inhibit cAMP production by AC.[9,10] The D_2 and D_3 receptor subtypes are highly involved in the pathophysiology and treatment of PD and schizophrenia, and, like other GPCRs, they typically exhibit interconvertible high- and low-affinity states for agonists in vitro.[11−13] In the high-affinity state, the receptors are coupled to the G-protein, and this state is considered to be the active form of the receptor.[14] In the low-affinity state, receptors are uncoupled from the G-proteins and, as a result, are inactive. There is substantial evidence that alterations in the density of $D_{2/3}$ receptors in the high-affinity state may be more relevant to the pathophysiology of neuropsychiatric disorders than alterations in the total receptor density. This is supported mostly by data from Seeman and coworkers.[13] In several animal models of neuropsychiatric disorders, including psychosis, increases in striatal $D_{2/3}$ high-affinity receptors of up to 9-fold were registered as compared to control animals, but total receptor numbers did not increase.[15] Although agonists are selective for the high-affinity state, antagonists bind with equal affinity to the low- and high-affinity states of a receptor.[16] Therefore, antagonist radioligands cannot distinguish a shift in the state of receptors from low to high affinity.[17] Numerous compounds have been synthesized and characterized as agonist tracers for the imaging of $D_{2/3}$ in the high-affinity state by means of positron emission tomography (PET).[18] These compounds are of different chemical classes, including ergolines, aporphines, 2-aminotetralins, benzoquinolines, and naphthoxazines. Aporphines [^{11}C]NPA (*N*-propyl-

Received: September 9, 2013
Published: December 10, 2013

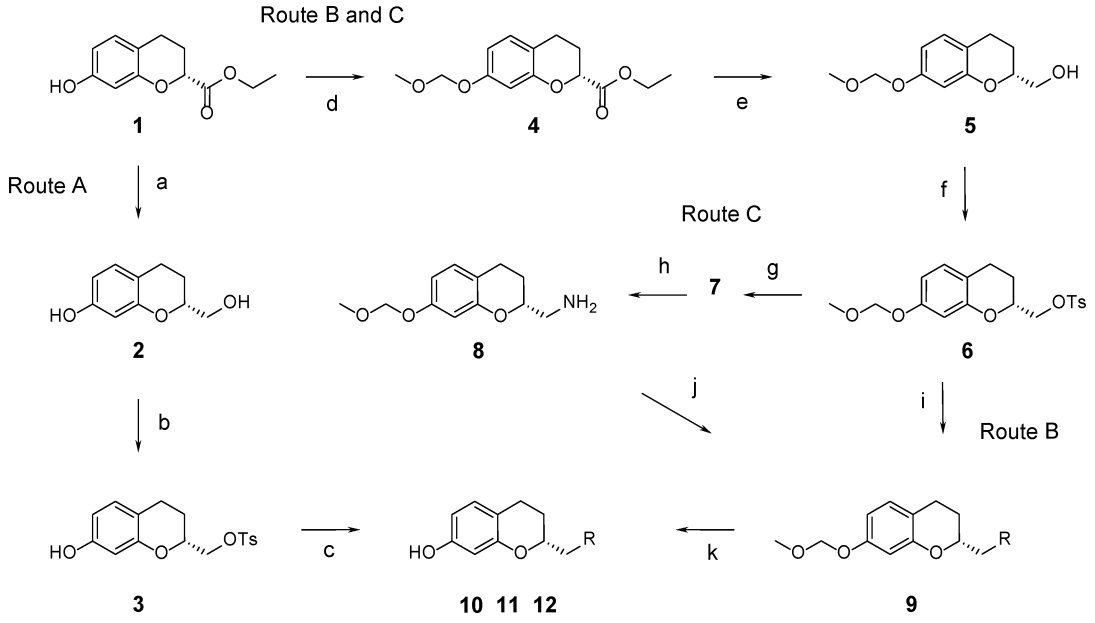
   dx.doi.org/10.1021/jm401384w | *J. Med. Chem.* 2014, 57, 391−410

Chart 1. (*R*)-2-Aminomethylchroman-7-ols (AMCs) Investigated in This Study[a]



[a]Reference compounds **10**, iodide series **11**, and fluoride series **12**. The oxalate salts of the shown amine structures have been used for all assays and binding studies.

Scheme 1. Synthesis of the Unlabelled AMC Compounds[a]

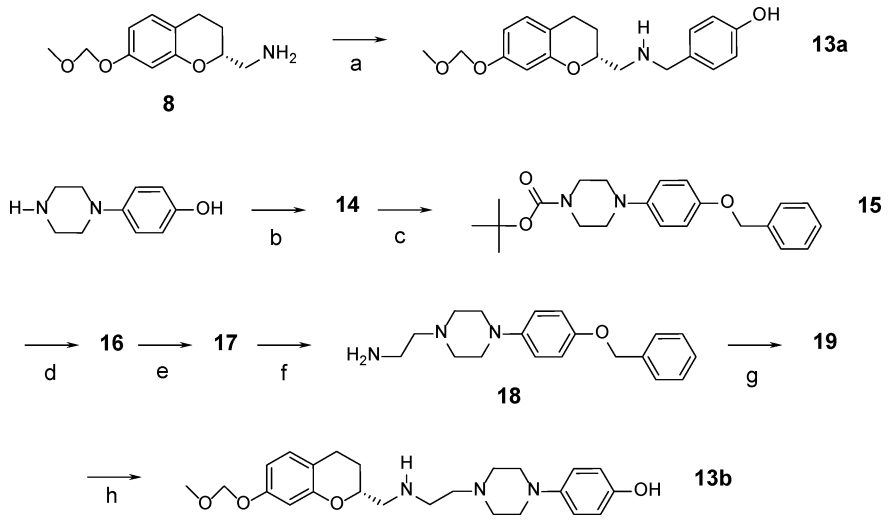


[a]Reagents and solvents: (a, e) $LiBH_4$, THF; (b, f) TsCl, pyridine; (c, i) amine building block, DIPEA, DMSO; (d) MOMCl, DIPEA, CH_2Cl_2; (g) potassium phthalimide, DMF; (h) H_2NNH_2 hydrate, EtOH; (j) (i) aldehyde building block, MeOH and (ii) $NaBH_4$, MeOH; (k) 4 M HCl, dioxane/isopropanol. The R group in the series of compounds **10**, **11**, and **12** denotes a secondary or tertiary amine residue; see Chart 1 for details on the specific R groups that were used.

norapomorphine) and [^{11}C]MNPA (2-methoxy-*N*-propylnorapomorphine) and naphthoxazine [^{11}C]PHNO (4-propyl-9-hydroxynaphthoxazine) turned out to be the most successful agonists and have also been evaluated as PET tracers in man.[19,20] In humans, agonists [^{11}C]PHNO and [^{11}C]NPA showed better sensitivity for the detection of endogenous dopamine release than antagonist [^{11}C]raclopride.[21−24] All three of these tracers also performed better than [^{11}C]-raclopride in dopamine-release measurement in rodents, pigs, cats, and nonhuman primates.[25] This corroborates the notion that agonist radiotracers are more prone to detect changes in neurotransmitter concentrations than antagonist tracers. However, all $D_{2/3}$ agonist PET ligands successfully evaluated in humans are [^{11}C]-labeled.[19,20] Because of the short half-life (20.4 min) of carbon-11, these radioligands can be used only when a cyclotron is present on-site. This expensive device is, however, available in only a minority of hospitals. To increase the worldwide availability of such agonist tracers, we are developing fluorine-18-labeled PET tracers (half-life 109.8 min) or iodine-123-labeled (half-life 13.2 h) agonist dopamine $D_{2/3}$ receptor tracers for single photon emission computed tomography (SPECT) imaging. Accordingly, we have prepared and evaluated a new series of agonist compounds based on the 2-aminomethylchroman-7-ol (AMC) scaffold. Structure−activity relationships for this class of compounds[26] and classes related to it,[27−31] including the preference for the high-affinity versus low-affinity state of D_2 receptor and selectivity for the D_2 receptor over other monoamine receptors such as the serotonin 1A (5-HT_{1A}) and α_1 adrenergic receptor, have already been examined in detail by Mewshaw and co-workers. Importantly, the D_2 affinity and agonism of AMCs shows considerable tolerance to structural modifications in the moiety attached to

dx.doi.org/10.1021/jm401384w | J. Med. Chem. 2014, 57, 391−410

Scheme 2. Synthesis of the Phenol Precursors to the [^{18}F]-Labelled AMCs[a]



[a]Reagents and solvents: (a) (i) 4-hydroxybenzaldehyde, AcOH, CH_2Cl_2 and (ii) $NaB(OAc)_3H$; (b) BOC-anhydride, DMF; (c) BnBr, DMF, K_2CO_3; (d) 4 M HCl, dioxane; (e) *tert*-butyl *N*-(2-bromoethyl)carbamate, DIPEA, DMF; (f) TFA, CH_2Cl_2; (g) tosylate **6**, DIPEA, DMSO/dioxane; (h) Pd/C, H_2, EtOH.

the nitrogen atom of the AMC core, which provides ample opportunities for the introduction of the [^{18}F] or [^{123}I] label into the structure without compromising the necessary pharmacological properties. We have used data from the Mewshaw studies to design our AMCs that either bear an iodide or a fluorine atom (Chart 1). We have included the nonhalogenated reference[26] compounds **10a** and **10b** that were already evaluated in vitro by Mewshaw, as this enables us to put receptor binding data on the new iodinated (**11**) and fluorinated (**12**) series of compounds into perspective. Introduction of the piperazine group in some of the AMC structures was inspired by studies on pramipexole[32] and 5-OH-DPAT,[33] piperazines that have shown potent and selective D_3-receptor binding, as well as by several Mewshaw studies.[27−31]

RESULTS

Chemical Synthesis. Scheme 1 outlines the synthetic routes to the prepared cold AMCs (i.e., the unlabeled regular ^{19}F and ^{127}I compounds). Details on specific transformations can be found in the Experimental Section. Even though racemates can be used for initial screening in receptor-binding studies, we have prepared enantiomerically pure (*R*)-AMC compounds, as it is already known that these enantiomers are the eutomers.[26] Accordingly, the test compounds **10a**, **10b**, **11a−e**, and **12a−d** reported in this work are all (*R*) enantiomers, and they have all been prepared from the same parent compound, **1** (i.e., (*R*)-7-hydroxychroman-2-carboxylic acid ethyl ester). The racemate of compound **1** was prepared from 2,4-dihydroxyacetophenone in two steps, according to Walenzyk[34] and Cohen,[35] and thereafter this racemic mixture was resolved to **1** (the *R* enantiomer) using the lipase kinetic resolution procedure as reported by Kalaritis.[36] GC analysis on a chiral capillary column showed two peaks of equal intensity for the racemate of **1**, whereas resolved **1** only showed one peak (99% ee).

Three routes to AMC target molecule series **10**, **11**, and **12** were employed. Route A is via unprotected tosylate **3** by reaction of **3** with the appropriate amine building blocks. This conveniently short route was used by Mewshaw[26] as well, and we have prepared molecules **10a**, **11b**, and **11d** via route A. However, we have found that yields remain low when using tosylate **3**, presumably because **3** can react with itself under basic conditions as opposed to reacting with the presented amine reactants. Furthermore, the synthesis of **3** in pyridine suffers from side-product formation, where the ditosylate side product has been observed in particular. As an alternative, we used route B that proceeds via tosylate **6**, which is MOM-protected at the 7 OH position of the chroman group. Route B is longer, as it requires extra (de)protection steps, but the syntheses have proved to be more reliable. Preparation of **10b**, **11a**, **11e**, **12a**, **12c**, and **12d** was achieved via route B. The MOM protective group was selected over the methoxy or benzyloxy group that was applied by Mewshaw because the methoxy group requires more harsh deprotection conditions and the benzyloxy group is or may be incompatibile with the (iodo)benzyl target structures. Route B still gave rise to side-product formation; for example, in the cases where primary amines were reacted with tosylate **6**, tertiary amine side products were formed next to the desired secondary amine main products. We have therefore also employed route C, which is the longest route and which route proceeds via primary amine **8**. In this case, a reductive amination coupling step with aldehyde building blocks can be performed so that side product formation is largely avoided. Compounds **11a**, **11c**, and **12b** were prepared according to route C. Provided that the aldehyde counterpart for preparing the desired AMC target molecule is easily accessible, route C is recommended. It may even be possible to use the unprotected amine (i.e., amine **8** without the MOM group) to prepare AMCs, but we have not tested this option. The synthesis of the amine or aldehyde building blocks that are required for coupling to tosylate **3** (route A), tosylate **6** (route B), or amine **8** (route C) are described in the Experimental Section unless these building blocks are commercially available. Test compounds **10**, **11**, and **12** were all converted to their oxalate salts, and these salt end products were used in all assays and studies described in this article.

dx.doi.org/10.1021/jm401384w | *J. Med. Chem.* 2014, 57, 391−410

Table 1. Affinity of Reference and Newly Synthesized Compounds for the Different Subtypes of Dopamine Receptors[a]

	D_1[b]	D_2L_{High}[b]	D_2L_{Low}[b]	D_2S_{Low}[c]	D_3[c]	D_4[b]	D_5[b]
dopamine	not determined	6.97 ± 0.66 (6)	5.25 ± 0.62 (6)	6.06 ± 0.18 (4)	7.90 ± 0.59 (5)	8.15 ± 0.98 (4)	5.99 ± 0.26 (3)
		231 nM	11.6 μM	920 nM	24.8 nM	33.8 nM	1.16 μM
PHNO	not determined	9.47 ± 0.45 (5)	7.68 ± 0.09 (4)	7.77 ± 0.24 (3)	9.00 ± 0.23 (3)	not determined	not determined
		0.45 nM	21.4 nM	18.7 nM	1.08 nM		
NPA	not determined	10.13 ± 0.08 (2)	8.79 ± 0.19 (5)	8.31 ± 0.5 (6)	8.74 ± 0.15 (3)	not determined	not determined
		0.075 nM	1.73 nM	10.6 nM	1.91 nM		
10a	5.21 ± 0.30 (3)	8.91 ± 0.62 (4)	7.40 ± 0.46 (5)	6.73 ± 0.91 (3)	8.50 ± 0.04 (4)	7.93 ± 0.08 (3)	5.43 ± 0.11 (3)
	7.25 μM	2.98 nM	70.2 nM	736 nM	3.18 nM	11.9 nM	3.83 μM
10b	4.98 ± 0.17 (3)	8.20 ± 0.40 (4)	6.82 ± 0.22 (5)	6.59 ± 0.11 (3)	7.72 ± 1.41 (3)	8.30 ± 0.26 (3)	5.56 ± 0.09 (3)
	10.9 μM	8.19 nM	168 nM	260 nM	547 nM	5.70 nM	27.7 μM
11a	5.09 ± 0.20 (3)	8.69 ± 0.64 (4)	7.45 ± 0.39 (6)	7.09 ± 0.55 (3)	8.25 ± 0.54 (4)	8.81 ± 0.14 (3)	5.45 ± 0.18 (3)
	8.67 μM	3.79 nM	51.8 nM	140 nM	13.9 nM	1.59 nM	3.79 μM
11b	5.41 ± 0.25 (3)	8.99 ± 0.60 (4)	7.47 ± 0.56 (6)	7.54 ± 0.19 (3)	8.54 ± 0.57 (4)	9.02 ± 0.07 (3)	5.34 ± 0.12 (3)
	4.29 μM	2.06 nM	72.9 nM	30.8 nM	7.87 nM	0.97 nM	4.64 μM
11c	6.38 ± 0.15 (3)	9.79 ± 0.83 (4)	7.53 ± 0.49 (4)	7.08 ± 0.67 (3)	8.78 ± 0.2 (4)	not determined	not determined
	436 nM	0.68 nM	51.2 nM	166 nM	1.79 nM		
11d	4.52 ± 0.48 (3)	no meaningful affinity	no meaningful affinity	no meaningful affinity	7.62 ± 0.05 (3)	7.84 ± 0.02 (3)	4.76 ± 0.11 (3)
	46.5 μM				24.2 nM	14.3 nM	17.8 μM
11e	5.41 ± 0.11 (3)	not detected	7.18 ± 0.32 (5)	7.02 ± 0.41 (3)	7.66 ± 0.20 (3)	8.02 ± 0.12 (3)	5.46 ± 0.09 (3)
	3.96 μM		80.5 nM	131 nM	23.0 nM	9.70 nM	3.48 μM
12a	5.18 ± 0.17 (3)	8.26 ± 0.13 (2)	7.21 ± 0.36 (5)	7.04 ± 0.1 (3)	8.04 ± 0.21 (3)	7.50 ± 0.09 (3)	5.61 ± 0.31 (3)
	6.91 μM	5.60 nM	80.5 nM	92.7 nM	12.1 nM	32.1 nM	2.89 μM
12b	5.94 ± 0.04 (3)	not detected	6,84 ± 0.21 (4)	6.45 ± 0.38 (3)	7.49 ± 0.1 (3)	not determined	not determined
	1.16 μM		156 nM	452 nM	33.0 nM		
12c	no meaningful affinity	no meaningful affinity	no meaningful affinity	no meaningful affinity	7.43 ± 0.17 (4)	7.32 ± 1.02 (3)	4.71 ± 0.05 (3)
					39.4 nM	131 nM	19.7 μM
12d	no meaningful affinity	8.30 ± 0.47 (3)	6.66 ± 0.18 (5)	6.63 ± 0.21 (3)	8.06 ± 0.27 (3)	7.64 ± 0.35 (3)	5.01 ± 0.01 (3)
		6.67 nM	235 nM	252 nM	10.0 nM	27.7 nM	9.70 μM

[a]Data are shown as the mean ± SD of −log K_i values, with the number of experiments given in parentheses; mean K_i values, in nanomolar or micromolar, are given in the bottom row for each compound. [b]Data from experiments using membranes made from HEK293 cells stably transfected with the concerning dopamine receptor subtypes. [c]Data from experiments using membranes purchased from PerkinElmer.

Scheme 2 shows the synthetic route to phenols **13a** and **13b**, which are the precursors to radiolabeled compounds [^{18}F]**12a** and [^{18}F]**12d**, respectively. MOM-protected amine **8** was coupled by reductive amination to 4-hydroxy benzaldehyde to prepare **13a.** Commercially available 1-(4-hydroxyphenyl)-piperazine was converted in five steps to primary amine reactant **18**, which can be coupled to tosylate **6** to provide **19**. Subsequent debenzylation of **19** affords **13b**. Precursors **13a** and **13b** both require protection at the 7-OH chroman position because a selective coupling of the respective [^{18}F]-alkyltosylates to the other phenol moiety is desired. Protection of the NH groups in the precursors is not needed because coupling under basic conditions with the [^{18}F]-alkyltosylates is favored at the phenol position over the NH position. For details on the preparation of [^{18}F]**12a** and [^{18}F]**12d**, see the Radiolabeling section in the Results.

Pharmacology. *Affinity of the Compounds to the Dopamine Receptors Determined by Radioligand Binding.* All novel AMC compounds (Chart 1) as well as the reference ligands dopamine, PHNO, and NPA were tested for their ability to compete with [^{3}H]spiperone or [^{3}H]SCH23390 for binding to various receptors of the dopamine receptor family. In Table 1, the results of these binding studies are compiled. For almost all compounds, the binding experiments on the D_2L (D_{2long}) receptor produced competition curves that were explained significantly better by biphasic models (using GraphPad Prism). This finding indicates agonism for these ligands.[37] See Figure 1 for a typical biphasic binding curve recorded for dopamine.

Among the AMC compounds tested, **11c** had the highest affinity for the D_2L_{high} receptor (pK_i 9.79 ± 0.83). Compounds **10a**, **10b**, **11a**, **11b**, **12a**, and **12d** showed high affinities, with recorded pK_i values above 8. The only tertiary amine

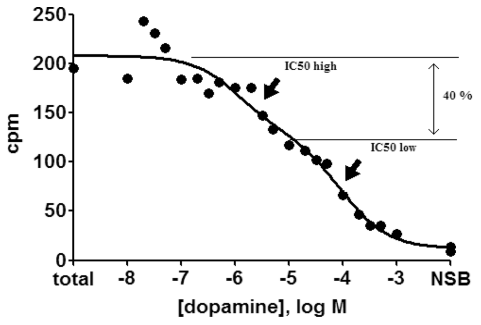


Figure 1. Representative biphasic competition binding curve for dopamine binding to the D_2L receptor. The data shown are from one experiment, where the bound fraction of [^{3}H]spiperone in counts per minute (cpm) is plotted against the log of the concentration (mol/L) of dopamine. According to the points of inflection (arrows), this example shows an IC_{50} for the D_2 high-affinity state at 10^{-6} M dopamine and an IC_{50} for the D_2 low-affinity state at 10^{-4} M dopamine (these IC_{50} values need to be transformed to K_i values using the Cheng−Prusoff equation).[38] The fraction of the receptors in the high-affinity state is 40 ± 7%.

dx.doi.org/10.1021/jm401384w | J. Med. Chem. 2014, 57, 391−410

compounds, AMCs **11d** and **12c**, did not show a meaningful affinity for the D_2L receptor. For **11e** and **12b**, no biphasic curves were observed and measured affinities were (well) below a pK_i of 8 (pK_i 7.18 ± 0.32 for **11e** and 6.84 ± 0.21 for **12b**), which were comparable to those recorded for D_2L, and all tested compounds showed affinities for the dopamine D_4 receptor in the same range as that measured for the D_2L receptor except for tertiary amines **11d** and **12c**, which had a high affinity for D_4 but no meaningful binding to D_2L. These two compounds also showed an evident affinity for D_3, with pK_i's of 7.62 for **11d** and 7.43 for **12c.** Finally, all of the tested compounds lacked reasonable affinity for the dopamine D_1-like receptors (D_1 and D_5) (Table 1).

Ligand-Induced Inhibition of Forskolin-Induced cAMP Accumulation. In HEK293 cells expressing the dopamine D_2L receptor, dopamine inhibits the forskolin-induced cAMP accumulation, where this inhibition becomes apparent at a certain dopamine concentration. In the cAMP assay (Table 2), the AMC test compounds were compared to dopamine with respect to their inhibition potency, and it was found that **11e** appeared to be less potent than the endogenous agonist dopamine; **11a** and **12a** were similarly potent, and all of the other test compounds were somewhat more potent than dopamine, with **11b** appearing to be the most potent of the tested halogenated compounds. All compounds had efficacies comparable to that of dopamine (Table 2).

Table 2. Potency and Efficacy Maximal Response (E_{max})[a]

ligand	pEC_{50}	E_{max} (% of dopamine)	N
dopamine	9.2 ± 0.4	100	4
10a	10.7 ± 0.3	78 ± 5	4
10b	10.3 ± 0.4	82 ± 6	4
11a	9.2 ± 0.4	86 ± 5	4
11b	10.5 ± 0.4	86 ± 6	4
11e	8.8 ± 0.4	86 ± 5	4
12a	9.4 ± 0.3	96 ± 5	3
12d	9.8 ± 0.2	94 ± 3	3

[a]Expressed as the percent of dopamine response for various ligands at the human D_2L receptor determined in a cAMP accumulation assay. All data are expressed as the mean ± SEM.

Lipophilicity. Data regarding lipophilicity are shown in Table 3. Compounds **10a**, **10b**, **11a**, **11b**, **11e**, **12a**, **12b**, and **12d** showed a cLogD ($cLogP_{7.4}$) in the range between 1 and 3.[39]

Radiolabeling. The two fluorinated compounds, **12a** and **12d**, were selected for [^{18}F]-labeling, and this was accomplished using a three-step two-pot procedure starting with the synthesis of a [^{18}F]fluoroalkyl synthon from [^{18}F]fluoride and the appropriate ditosylate precursor followed by [^{18}F]-fluoroalkylation of phenol precursors **13a** or **13b** (Scheme 2) and in situ removal of the MOM-protecting group under acidic conditions (Scheme 3). The [^{18}F]-labeled products, [^{18}F]**12a** and [^{18}F]**12d**, were purified by reversed-phase HPLC. A typical synthesis run lasted for 150 min and started from 5 GBq of [^{18}F]fluoride, from which 20−200 MBq of formulated [^{18}F]**12a** or [^{18}F]**12d** was obtained.

Decay-corrected yields, radiochemical purities, and specific activities of [^{18}F]**12a** and [^{18}F]**12d** are listed in Table 4.

In Vitro Autoradiography. Results of in vitro autoradiography of [^{18}F]**12a** and [^{18}F]**12d** are presented in Figure 2 and Table 5.

Table 3. Lipophilicity of AMC Compound Series 10, 11, and 12[a]

compound	cLogP	cLogD ($cLogP_{7.4}$)
10a	3.42	1.41
10b	3.90	1.46
11a	4.35	2.43
11b	4.35	2.46
11c	4.35	3.30
11d	4.74	4.15
11e	4.38	2.42
12a	4.04	2.12
12b	4.04	2.57
12c	3.86	3.27
12d	3.49	1.54

[a]Assessed with the BiologP (New cLogP) calculator program from the University of Massachusetts.

At nanomolar concentration, [^{18}F]**12a** showed rather homogeneous uptake in sagittal rat brain slices, with only slightly higher binding in the striatum. Striatum-to-cerebellum and striatum-to-cortex ratios were on average 1.19 and 1.16, respectively. In the presence of 10 μM of the dopamine $D_{2/3}$ antagonist raclopride, the striatum-to-cerebellum ratio was reduced by 14%, and the striatum-to-cortex ratio, by 11%. At subnanomolar concentration, however, [^{18}F]**12a** demonstrated moderate but clearly visible specific uptake in the striatum. At 0.19 nM, striatum-to-cerebellum ratios were, on average, 1.85 and were reduced by 40% in the presence of 10 μM raclopride and by 35% in the presence of 100 μM guanosinetriphosphate (GTP). Striatum-to-cortex ratios were, on average, 1.68, and coincubation with 10 μM raclopride or 100 μM GTP resulted in 39 and 33% decreases, respectively.

At nanomolar concentration, [^{18}F]**12d** demonstrated preferential binding in the striatum and hippocampus. In control animals, the mean striatum-to-cerebellum ratio was 2.64. In the presence of 10 μM raclopride or 100 μM GTP, striatum-to-cerebellum ratios were significantly reduced by 37%. Striatum-to-cortex ratios were, on average, 1.90 in control slices and were reduced by 37% by raclopride and by 22% by 100 μM GTP.

DISCUSSION

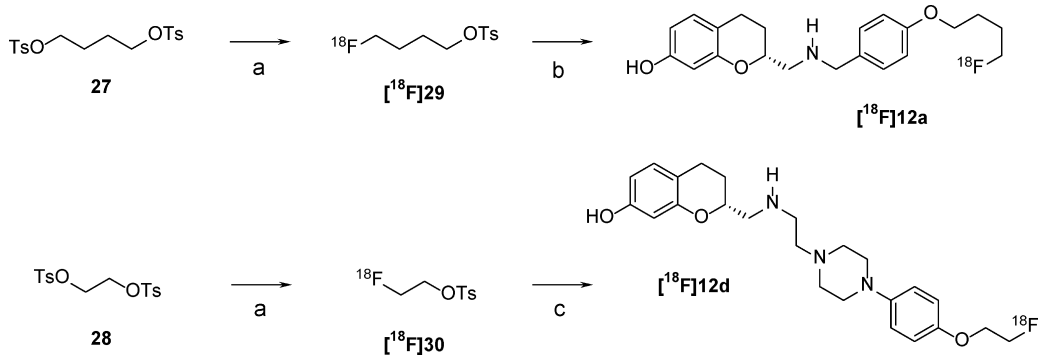
Our present effort is directed at developing new agonist radiotracers that can be used in centers without an on-site cyclotron with the aim of enabling visualization of dopamine $D_{2/3}$ receptors in the agonist high-affinity state. Accordingly, we synthesized a novel group of iodinated and fluorinated compounds based on the AMC scaffold, which is known for its agonistic properties on $D_{2/3}$ dopamine receptors and for its good selectivity of binding for these receptor subtypes.[26]

The primary binding site of the D_2 receptor is formed by transmembrane domains (TMs) 3, 5, and 6 of the receptor.[40] D_2 ligands contain an aromatic core that engages in interactions with the hydrophobic residues in TM3 and TM6. To this core are attached an amino group and a hydrogen-bond donating group to form an ionic bond with the conserved aspartate residue in TM3 and one or more hydrogen bonds with conserved serine residues in TM5, respectively (Figure 3).

The optimal distances between these groups and their optimal mutual orientations have been the subject of numerous studies that have produced more or less general pharmacophore models ranging from the McDermed criteria developed in the late 1970s[41] to more recent models.[42,43] Notably, it was

dx.doi.org/10.1021/jm401384w | J. Med. Chem. 2014, 57, 391−410

Scheme 3. Labelling Schemes of [^{18}F]12a and [^{18}F]12d[a]



[a]Reagents and solvents: (a) K^{18}F-Kryptofix-K_2CO_3, MeCN; (b) (i) **13a**, NaH, DMF and (ii) 0.4 M HCl; (c) (i) **13b**, NaH, DMF and (ii) 0.4 M HCl.

Table 4. Decay-Corrected Yields (RCY), Radiochemical Purities (RCP), and Specific Activities (SA) of [^{18}F]12a and [^{18}F]12d[a]

tracer	RCY, %	RCP, % (formulated tracer)	SA (GBq/umol), at end of synthesis
[^{18}F]**12a**	HPLC: 25 ± 11 (3–47)[b]	94 ± 4 (85–98)	92 ± 96 (1.4–312)
n = 10	formulated: 7 ± 8 (1–22)[c]		
[^{18}F]**12d**	HPLC: 14 ± 5 (6–20)	92 ± 5 (85–98)	29 ± 12 (8–40)
n = 6	formulated: 5 ± 3 (1.4–11)		

[a]Data are expressed as the mean ± SD, and the range is given in brackets. [b]HPLC fraction containing the tracer vs ^{18}F-fluoride taken for synthesis [c]Formulated tracer vs ^{18}F-fluoride taken for synthesis

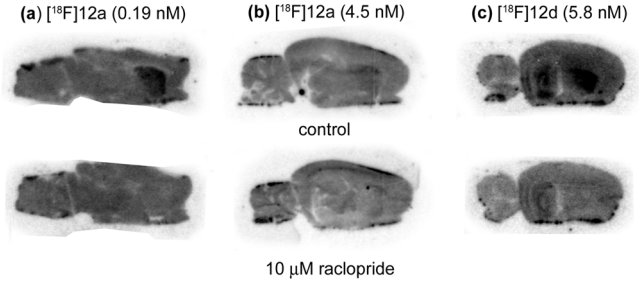


Figure 2. In vitro autoradiography with [^{18}F]**12a** at 0.19 nM (a), [^{18}F]**12a** at 4.5 nM (b), and [^{18}F]**12d** at 5.8 nM (c). Note the specific binding in the striatum for [^{18}F]**12a** (only at lower concentration) and for [^{18}F]**12d**.

found that the nitrogen atom can accommodate a bulky substituent extending into the ancillary binding pocket of the receptor formed by TM2 and TM7. The passage between the primary and ancillary binding pockets is more narrow in D_1-like receptors than in D_2-like receptors, so the presence of such bulky substituents imparts D_2-over-D_1 selectivity.[44] The second substituent at the nitrogen, however, cannot be greater than *n*-propyl.[45]

Mewshaw et al.[26] proposed AMCs as a new series of compounds that meet the criteria for agonists with a high D_2 affinity and an appropriate D_2-over-D_1 selectivity. The AMC scaffold offers convenient possibilities to accommodate the [^{123}I] or [^{18}F] label on the N substituent, and the same substituent can also be used to adjust the lipophilicity of the compound. On the basis of benzylated secondary amine compound **10a**, which was developed by Mewshaw and was found to have a high affinity (subnanomolar) for the D_2^{high} receptor, we designed three iodobenzyl and two fluoroalkyloxybenzyl candidate ligands. From the conformational and comparative molecular field analysis carried out for the AMC derivatives by the group of Mewshaw[46] and the modeling data for the D_2 receptor,[47,48] we concluded that the benzyl group of **10a** is likely to interact with extracellular loop 2 of the receptor and not with the ancillary pocket of D_2 formed by TM2 and TM7. To probe the spatial requirements of such alignment, we synthesized para- (**11a**), meta- (**11b**), and ortho-iodobenzyl (**11c**) as well as para- (**12a**) and ortho-(4-fluoro)-

Table 5. In Vitro Autoradiography Results[a]

tracer	Log D	conditions	striatum-to-cerebellum ratio	striatum-to-cortex ratio
[^{18}F]**12a**	2.45 ± 0.10	control (n = 3)	1.85 ± 0.23	1.68 ± 0.11
upper row: 0.19 nM			1.19 ± 0.18	1.16 ± 0.16
lower row: 4.5 ± 3.6 nM		raclopride (n = 1)	1.10 ± 0.19	1.03 ± 0.08
			1.04 ± 0.22	1.05 ± 0.11
		GTP (n = 1)	1.20 ± 0.16	1.13 ± 0.09
[^{18}F]**12d**	1.48 ± 0.01	control (n = 4)	2.64 ± 1.04	1.90 ± 0.47
5.8 ± 1.6 nM		raclopride (n = 4)	1.65 ± 0.37	1.20 ± 0.20
		GTP (n = 1)	1.67 ± 0.28	1.48 ± 0.17

[a]Values are the mean ± SD from the total number of slides assayed in n independent experiments. The number of slides per condition in each experiment varied from 4 to 12.

dx.doi.org/10.1021/jm401384w | J. Med. Chem. 2014, 57, 391–410

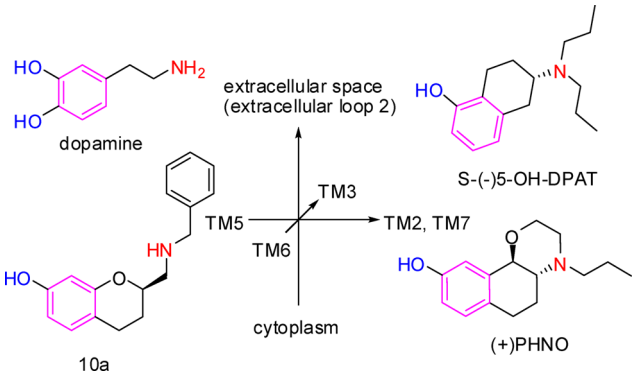


Figure 3. Comparison of the molecular structures of some D_2-agonist ligands, where their aryl cores are purple, basic nitrogens are red, and H-bond-donating hydroxyls are blue. The orientation of the functional groups was chosen to resemble roughly the putative receptor-bound conformations of the ligands. Note that for conformationally constrained (+)PHNO the lone electron pair of the nitrogen is directed roughly normally to the aryl ring and away from view, toward the conserved aspartate in TM3. In the 3D representation, the TM6–TM3 axis is directed away from view, that is, TM6 is closer to the viewer than TM5, whereas TM3 is farther.

butyloxybenzyl (**12b**) derivatives of **10a**. The butyl spacer in **12a** and **12b** was selected to bring the expected lipophilicity of the compounds within the Log D range of 2 to 3 (Table 3) to ensure passage of the blood–brain barrier.[49]

The arylpiperazine motif is known to act as a pharmacophoric core in a number of high-affinity D_2-agonists,[31] and apparently it can fit into the primary binding pocket of the D_2-like receptor.[48] Interestingly, Dutta and co-workers synthesized and evaluated a number of hybrid ligands based on an aminotetralin or a pramipexol scaffold to which an arylpiperazine moiety was attached via a spacer.[32,33] In these hybrid ligands, the arylpiperazine moiety is most probably bound in the ancillary binding pocket of the receptor and not in the primary binding site.[50] Inspired by these results, we designed four hybrid AMC derivatives with arylpiperazine side chains attached to the AMC core either directly (**11d** and **12c**) or through an ethylene spacer (**11e** and **12d**). Note that compounds **11d** and **12c** are the only tertiairy amines in the series of developed compounds, where all of the other compounds are secondary amine AMCs.

In the Mewshaw study that evaluated agonist ligands for the dopamine $D_{2/3}$ receptor, the affinity was measured in rat striatal membranes using the agonist [^{3}H]quinpirole, and it was claimed that this approach specifically assessed the binding to the D_2L_{high} receptor because [^{3}H]quinpirole should predominantly bind to D_2L_{high}. Measuring affinities for D_2L_{low} was also done in rat striatal membranes but in the presence of GTP to convert D_2L_{high} to D_2L_{low} and with antagonist [^{3}H]spiperone as the radioligand. In addition, ketanserin was administered to preclude binding of spiperone to serotonin receptors.[26] Finally, a selection of compounds were evaluated in binding assays using membranes from cells transfected with human D_2S, D_3, and D_4 receptors. We have also used membranes from cloned human dopamine receptors expressed in cell lines but included D_1, D_2L, and D_5 as well. We also used [^{3}H]spiperone as the radioligand, resulting in biphasic binding curves from which the affinities of the compounds for D_2L_{high} and D_2L_{low} were derived (Figure 1).

Several of the prepared compounds indeed showed high affinity and selectivity for the $D_{2/3}$ receptors and agonistic action on the D_2 receptor. Dopamine and most of the AMC compounds consistently showed biphasic competition curves for the D_2L receptor, which is typical for agonists. The inflection points in these curves reflect the agonist high- (D_2L_{high}) and low-affinity (D_2L_{low}) states (Figure 1). In line with this finding, the cAMP experiments also showed agonistic properties for the tested compounds.

Nevertheless, when we compare the affinity of reference compounds **10a** and **10b** to the values of Mewshaw et al., our findings show affinities that are 1 log scale lower (0.2 vs 3.0 nM for **10a** and 0.7 vs 8.2 nM for **10b**). This difference can be caused either by interspecies difference (Mewshaw used predominantly rat receptors, whereas we used only human receptors) or by differences in techniques. The latter is more likely in our opinion, as discrepancy in the techniques is a well-known cause of discrepancy in affinity measurements. For instance, for the D_2L_{high} receptor, we found a dopamine dissociation constant of 231 nM, whereas the literature reports values of 2 nM using [^{3}H]raclopride,[51] 4.7 nM using [^{3}H]spiperone,[52] and 56 nM using [^{125}I]iodosulpiride.[53]

Reported affinities of dopamine for the D_2S receptor range between 4.5 and 229 nM using [^{3}H]spiperone as radioligand,[54−56] whereas 22 nM was found using [^{125}I]-iodosulpiride.[57] Although we did not find a high-affinity state of dopamine for the D_2S receptor in our experiments, a comparison of the findings for the D_2S_{high} and the D_2L_{high} is still warranted because these receptors are almost equal structurally.

In our experiments, PHNO showed an affinity for D_2L_{high} of 0.45 nM, and this is in line with literature findings of 0.6[58] and 1.5 nM[59] for D_2L_{high}. NPA exhibited a K_i of 0.08 nM for D_2L_{high}, which is reasonably comparable to the literature data that report K_i's of 0.38[60] and 0.11 nM.[61]

All AMC derivatives designed by us had at least 100-fold lower affinities toward D_1-like receptors than D_2-like receptors. This is in agreement with the established notion that bulky substituents at the nitrogen impart D_2 selectivity.

Comparing the affinity toward D_2L_{high} of the para (**11a**), meta (**11b**) and ortho (**11c**) iodobenzyl compounds shows that **11a** and **11b** have about equal affinity (3.8 and 2.1 nM, respectively), with **11c** displaying a 5-fold higher affinity (0.68 nM). This finding is in accordance with our expectation that the large iodine atom at the para position of **11a** may be accommodated less easily within the binding pocket (possibly clashing somewhat with the receptor's extracellular loop) as compared to the placement of the meta- and ortho-substituted iodines in **11b** and **11c**. Nevertheless, **11a** still shows a high affinity. Compound **12a**, with the 4-fluorobutyloxy group at the para position of the benzyl ring, had a D_2L_{high} affinity of 5.6 nM, comparable to that of **11a**, whereas its ortho isomer, **12b**, surprisingly did not appear to recognize D_2L_{high} and had about 3-fold lower affinities for D_2L_{low} and D_3 than **12a**. Apart from steric interactions, intramolecular hydrogen-bonding interactions between the secondary NH and the O atom of the fluorobutoxy chain may also play a role here, where these interactions can only be conceived for **12b** (forming an ideal six-membered ring) and not for **12a**. Similarly, such interactions may exist with the I atom in **11c** but not with those in **11b** or **11a**. Note that these intramolecular hydrogen-bonding interactions are also reflected in the increased lipophilicities of compounds **11c** and **12b** relative to those of their para- or meta-substituted

dx.doi.org/10.1021/jm401384w | J. Med. Chem. 2014, 57, 391−410

counterparts (Table 3). However, it is difficult to be conclusive about the binding mode of **12a** and particularly **12b**.

In comparison to the benzylamino AMCs, **10a**, **11a−c**, and **12a−b**, the hybrid AMCs, **11d−e** and **12c−d**, generally showed lower affinities toward all dopaminergic receptor subtypes, both D_1-like and D_2-like. This decrease in affinity was, however, not homogeneous, and the greatest decrease was observed for the D_2 subtype receptors, as tertiary amine hybrids **11d** and **12c** did not show a meaningful affinity toward these receptors at all, whereas ethylene-spaced secondary amine hybrid **11e** recognized only the low-affinity state of D_2L and D_2S. This observation is in agreement with the SAR studies on D_3-over-D_2 selectivity that suggest that the ancillary binding pocket of D_3 is more spacious than that of D_2, so compounds with bulkier groups attached to the nitrogen atom tend to prefer binding to D_3.[62,63] An interesting exception to this trend is ethylene-spaced secondary amine hybrid **12d**, which has a D_2L_{high} affinity comparable to that of fluorobutyloxybenzyl derivative **12a** and a D_2L_{high}-over-D_2L_{low} preference that is even higher than that of **12a**. It is impossible to determine how exactly the arylpiperazine moiety interacts with the receptor in these hybrid ligands, but, at least in the case of **12d**, the arylpiperazine must be bound in the ancillary pocket because arylpiperazines that interact with the primary binding pocket of the receptors have very low D_2 affinities when a bulky group, such as a fluoroalkoxy group, is placed at the para position of the aryl ring.[31,55]

We successfully labeled two of our compounds (**12a** and **12d**) with fluorine-18, where we made the selection for these two compounds based on the results of the earlier-described in vitro experiments: **12a** and **12d** show a high affinity for dopamine $D_{2/3}$ receptors, a low affinity for dopamine D_1-like receptors, agonism in the cAMP experiment, and have an appropriate lipophilicity. Scheme 3 shows our approach to prepare [^{18}F]**12a** and [^{18}F]**12d**, where we chose to use [^{18}F]fluoroalkylation onto a phenol precursor (either **13a** or **13b**) to arrive at the targeted tracers. The applied [^{18}F]-labeling approach is versatile, as we can quickly adapt the procedure for the preparation of homologous [^{18}F]fluoroalkyl derivatives of [^{18}F]**12a** or [^{18}F]**12d** in case it is necessary to adjust the hydrophobicity of the ligand. In contrast, an approach in which one would label compounds by direct [^{18}F]fluorination of tosyloxyalkyloxy or bromoalkyloxy precursors would require the preparation of every individual precursor (but it would benefit from fewer reaction steps involving [^{18}F] or [^{18}F] intermediates).

As can be seen from Table 4, only 25−30% of the HPLC-purified [^{18}F]**12a** or [^{18}F]**12d** ended up in the formulated tracer solution. The reason for this was that only 30−50% of the radioactivity could be eluted from the Oasis HLB SPE cartridge using the designated portions (1 to 2 mL) of pure ethanol, with the remainder of the [^{18}F]**12a** and [^{18}F]**12d** amines sticking to the cartridge. The elution efficiency rose to 80−90% when the ethanol was acidified with 1% v/v ortho-phosphoric or acetic acid, but it was decided to use ethanol as eluent.

Tracers [^{18}F]**12a** and [^{18}F]**12d** were obtained in reasonable yields and satisfactory specific activities. Radiochemical purities were also satisfactory; all radioactive impurities present in formulated [^{18}F]**12a** were much more polar than the tracer itself (not detectable on QC radio-HPLC, producing a spot with $R_f = 0$ on radio-TLC), and formulated [^{18}F]**12d** contained radioactive impurities both more and less hydrophobic than the tracer, but the former never amounted to more than 5% of the total radioactivity.

We evaluated labeled compounds [^{18}F]**12a** and [^{18}F]**12d** by in vitro autoradiography in rat brain slices. Both ligands exhibited preferential uptake in the striatum region, where for [^{18}F]**12a** this was visible only at subnanomolar free-ligand concentrations and for [^{18}F]**12d** it was found at low nanomolar levels (Figure 2). Given that the two compounds had comparable D_2L_{high} affinities, the lack of discernible $D_{2/3}$-specific binding of [^{18}F]**12a** at low nanomolar concentrations may be due to its higher lipophilicity (Table 3), which may have led to the $D_{2/3}$-specific signal being buried under the nonspecific signal resulting from [^{18}F]**12a** binding to membrane phospholipides.

For both compounds, binding in the striatum could be blocked with dopamine $D_{2/3}$ receptor antagonist raclopride and could be decreased to a similar extent in the presence of GTP, which suggests that the studied compounds indeed bind to the $D_{2/3}$ receptors in the striatum, showing a strong preference for the high-affinity state. This promising finding stimulates performing future in vivo PET-studies of [^{18}F]**12d** in rodents, especially because the lipophilicity of this compound is in the requested range for the development of accurate PET tracers.[64]

Most of our novel AMC compounds showed comparable affinities for both the dopamine D_2 and D_3 receptor. This is in line with presently available agonist dopamine receptor PET tracers such as [^{11}C]NPA and [^{11}C]MNPA. In contrast, [^{11}C]PHNO, which is D_3-preferring in vivo,[24] is currently being used to investigate the regional distribution of D_3 receptors in the brain,[65,66] and the latest clinical research data suggest that D_3-selective tracers might be advantageous over tracers with mixed $D_{2/3}$ affinity in some applications.[67,68]

Three of our four hybrid compounds (**11d−e** and **12c**) seem selective for the D_3 receptor over the D_2 subtype. Although their D_3 affinities are too low to consider them as candidate tracers, they confirm that high-affinity D_3-selective AMCs can be made if more studies into structure−activity relationship for this compound class are undertaken. In addition, several of our novel compounds display a non-negligible affinity for the D_4R (Table 1). For instance, **11a** and **11b**, which are attractive candidates for [^{123}I] labeling, have low nanomolar K_i values on D_4 receptors, on par with their K_i's at D_2L_{high}. However, in the human striatum, the D_4 receptor density is 16-fold lower than that of $D_{2/3}$ receptors.[69,70] In addition, in the rat striatum, the D_4 receptor density is 7- and 5-fold lower than that of the D_2 and D_3 receptors, respectively.[71] Consequently, we believe that striatal binding of our tracers will predominantly reflect binding to $D_{2/3}$ receptors.

Finally, our novel series of compounds may also offer the opportunity to develop [^{123}I]-labeled SPECT tracers; compound **11a** may be an attractive candidate to label with [^{123}I] because it has appropriate affinity, selectivity, and lipophilicity and because labeling seems feasible. Future studies will focus on this possibility.

CONCLUSIONS

We have identified a new series of agonist radiopharmaceuticals based on an AMC scaffold that bind with high affinity and selectivity to dopamine $D_{2/3}$ receptors in vitro. In cAMP assays, they all show nearly full agonism. In addition, two of these compounds were labeled successfully with [^{18}F] and were evaluated by in vitro autoradiography, where both of them showed specific binding in rat striatum: one at subnanomolar

dx.doi.org/10.1021/jm401384w | J. Med. Chem. 2014, 57, 391−410

([^{18}F]**12a**) and another at low nanomolar ([^{18}F]**12d**) concentrations. These findings will stimulate further in vivo evaluations, for example, animal PET and/or SPECT studies.

EXPERIMENTAL SECTION

Materials (Chemical Synthesis). Reagents, chemicals, materials, and solvents were obtained from commercial sources, and were used as received: Biosolve and Merck for solvents, Cambridge Isotope Laboratories for deuterated solvents, and Aldrich, Acros, ABCR, Merck, and Fluka for chemicals, materials, and reagents. All solvents were of AR quality. Moisture or oxygen-sensitive reactions were performed under an atmosphere of dry N_2 or argon.

Methods for Chemical Synthesis and Molecular Characterization. ^{1}H and ^{13}C NMR spectra were recorded on Varian Mercury (400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR) spectrometers at 298 K. Chemical shifts are reported in ppm downfield from tetramethylsilane (TMS) for ^{1}H NMR and applying deuterated chloroform ($CDCl_3$) or other deuterated solvents as internal reference for ^{13}C NMR. Abbreviations used for splitting patterns are s, singlet; t, triplet; q, quartet; m, multiplet; dd, double of doublets.

Melting points were measured on a Büchi melting point B-540 apparatus.

LC−PDA/MS analyses were performed on a Shimadzu LC-10 AD VP series LC coupled to a photo diode array (PDA) detector (Finnigan Surveyor PDA Plus detector, Thermo Electron corporation) and an ion-trap detector (LCQ Fleet, Thermo Scientific). Analyses were executed at 298 K using an Alltech Alltima HP C18 3μ column using an injection volume of 1−4 μL, a flow rate of 0.2 mL min^{-1}, and, typically, a MeCN in H_2O gradient (from 5 to 100% MeCN, where both MeCN and H_2O contain 0.1% formic acid).

Elemental and UPLC−HRMS analyses were performed on the aminomethylchroman-7-ol (AMC) test compound series **10**, **11**, and **12** in their isolated oxalate forms. Elemental analyses were done to assess the number of oxalate groups that were attached to the AMC amines and were performed on a PerkinElmer Series II CHNS/O Analyzer 2400. UPLC−HRMS was used to assess the purity and the exact molecular weight of the AMCs. Purities were typically ca. 95%, mostly higher, and are given for every AMC compound. Mass deviations from the theoretical mass were typically between 0 and 2 ppm. UPLC was performed on a Waters Acquity UPLC equipped with a sample manager (an autosampler) and a binary solvent manager. An Acquity UPLC BEH C18 1.7 μm column was used using H_2O and MeCN both with 0.1% formic acid as the two eluents and applying an 8 min gradient program where the amount of MeCN was increased from 5 to 60% in 5 min. A sample solution (10% MeCN in H_2O with 0.1% formic acid) with a concentration of ca. 0.1 mg/mL was prepared, and an injection volume of 0.1 μL was used. For HRMS detection, a Xevo G2 Qtof detector using Zspray lockspray ionization was applied.

GC−FID measurements to establish the ee of compound **1** were performed on a Shimadzu GC-2000 by applying the Varian WCOT fused silica column (25 m, i.d. 0.25 mm) that is coated with CP chirasil-dex cb df 0.25 μm particles. An isothermal program was used (200 °C), applying a carrier gas helium flow of 30 cm/sec.

GC−MS measurements were executed on a Shimadzu GC-17A gas chromatograph coupled to a Shimadzu GC−MS-QP5000 detector using a Phenomenex Zebron ZB-35 capillary column (30 m length, i.d. 0.25 mm, 0.25 μm film thickness) that was operated using helium as the carrier gas and typically applying a temperature gradient from 80 to 300 °C.

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) was performed on a PerSeptive Biosystems Voyager-DE PRO spectrometer using an α-cyano-4-hydroxycinnamic acid matrix.

Analytical thin-layer chromatography (TLC) was performed on Kieselgel F-254 precoated silica plates. Normal-phase column chromatography was carried out on flash silica gel (40−63 μm mesh) or regular silica gel (60−200 μm), both acquired from Screening Devices B.V., or on standardized aluminum oxide 90 from Merck. Reversed-phase (RP) chromatography was performed on the Biotage Isolera One applying a SNAP KP-C18-HS cartridge.

The applied centrifuge was a Thermo Electron corporation Heraeus Megafuge 1.0.

Synthesis of AMC-Derivative Series 10, 11, and 12. A series of nine potential agonist compounds for the dopamine $D_{2/3}$ receptor incorporating iodine or fluorine atoms in their structures was synthesized. Iodinated compounds were termed **11a**, **11b**, **11c**, **11d**, and **11e**. Fluoridated compounds were numbered **12a**, **12b**, **12c**, and **12d**. For internal standardization, two reference compounds without iodine or fluorine atoms in their structure were also synthesized (**10a** and **10b**). Structural formulas are shown in Chart 1. All of the test compound series **10**, **11**, and **12** reported in this work are (*R*) enantiomers, and they all originate from starting compound **1** (i.e., (*R*)-7-hydroxychroman-2-carboxylic acid ethyl ester).

(*R*)-7-Hydroxychroman-2-carboxylic Acid Ethyl Ester (1). The racemate of **1**[34,35] was resolved to **1** (the *R* enantiomer) using the lipase kinetic resolution procedure as reported by Kalaritis.[36] Accordingly, the racemate of **1** (10 g, 45.5 mmol) was dissolved in THF (34 mL), and demi-water (270 mL) and a 0.05 M phosphate buffer (68 mL) were added. Finally, amano lipase from *Pseudomonas fluorescens* (1.82 g) was added. The reaction mixture was treated with a 1 M NaOH solution (25 mL) by dropwise addition over 10 h. After NaOH addition, the reaction mixture was stirred for another 8 h. The mixture was evaporated to remove the THF, and the remaining aqueous mixture was extracted with ethyl acetate. Washing of the collected organic layers with saturated $NaHCO_3$, drying with Na_2SO_4, and concentrating gave an oil that slowly crystallized. Yield: typically ca. 45%.

Note that the reaction conversion (i.e., the hydrolysis of the *S* enantiomer to the acid) and the ee of the remaining desired *R*-enantiomer ester **1** was monitored using a chiral GC−FID method. Using this method, the racemate of **1** gives two peaks of equal intensity, whereas isolated **1** (i.e., the *R* enantiomer after resolution) gives one peak with no or very little sign of the *S* enantiomer (99% ee). The *S* enantiomer eluted first, and the desired *R* enantiomer eluted second. Analytical data of isolated **1** are according to those from the above-mentioned literature sources, particularly $[\alpha]_D^{rt} = -20.1$ ($CHCl_3$, *c* 1).

(*R*)-(7-Hydroxychroman-2-yl)methanol (2). Ethyl ester **1** (3.18 g, 14.4 mmol) was dissolved in THF (200 mL) and cooled to 0 °C. A 2 M $LiBH_4$ in THF solution (18.2 mL, 36.4 mmol, 2.5 equiv) was added. The reaction mixture was stirred at rt for 3 h, during which time the mixture became a milky suspension, and it was then poured into 1:1 water/brine (200 mL) that was brought to pH 2 using a 2N HCl solution. After stirring for 5 h, the mixture was extracted with diethyl ether and dried with Na_2SO_4. The fraction of starting compound (ca. 15%) that was still present was removed by silica column chromatography (1:1 hexane/ethyl acetate). Yield: 2.1 g (80%). Analytical data are according to those from literature.[26] ^{1}H NMR ($CDCl_3$): δ 6.9 (m, 1H), 6.35 (m, 2H), 5.35 (bs, 1H), 4.05 (m, 1H), 3.85 (m, 1H), 3.75 (m, 1H), 2.85−2.6 (multiple signals, 2H), 2.2 (bs, 1H), 1.95−1.75 (multiple signals, 2H). LC−MS: *m*/*z* 181.1 $[M + H]^+$ (calcd 180.20 for $C_{10}H_{12}O_3$).

Toluene-4-sulfonic Acid, (*R*)-(7-Hydroxychroman-2-yl)-methyl Ester (3). A solution of the alcohol **2** (0.5 g, 2.8 mmol) in pyridine (25 mL) was stirred at 0 °C. 4-Toluenesulfonyl chloride (0.56 g, 2.9 mmol, 1.05 equiv) was added in small portions, and the reaction mixture was stirred for 6 h at 4 °C. The mixture was then poured in ice water, and aqueous 2 N HCl was added to acidify to pH < 2. Extraction with CH_2Cl_2, drying of the collected organic layers with $MgSO_4$, and evaporation of the solvents gave a yellowish oil that was purified with silica column chromatography (2% MeOH/$CHCl_3$). Yield 0.56 g (60%). Analytical data are according to those from literature.[26] ^{1}H NMR ($CDCl_3$): δ 7.8 (m, 2H), 7.35 (m, 2H), 6.82 (m, 1H), 6.35 (m, 1H), 6.20 (m, 1H), 5.55 (bs, 1H), 4.15 (multiple signals, 3H), 2.75−2.6 (multiple signals, 2H), 2.45 (s, 3H), 1.95 (m, 1H), 1.75 (m, 1H). LC−MS: *m*/*z* 335.1 $[M + H]^+$ (calcd 334.39 for $C_{17}H_{18}O_5S$).

dx.doi.org/10.1021/jm401384w | J. Med. Chem. 2014, 57, 391−410

(*R*)-7-(Methoxymethoxy)chroman-2-carboxylic Acid Ethyl Ester (4). At rt, methoxylmethyl chloride (MOMCl; 0.188 mL, 2.07 mmol, 0.92 equiv) was added to a solution of ester **1** (0.50 g, 2.25 mmol) and DIPEA (1.2 mL, ca. 3 equiv) in CH_2Cl_2 (25 mL). Monitoring of the progress of the reaction using ^{1}H NMR and LC−MS showed that extra MOMCl was required to complete the reaction, so another portion of MOMCl (0.188 mL, 0.92 equiv) was added. Finally, the reaction mixture was washed with water and concentrated, and the remaining product was purified by silica column chromatography (2% MeOH/$CHCl_3$). Yield: 73%. ^{1}H NMR ($CDCl_3$): δ 6.92 (m, 1H), 6.66 (s, 1H), 6.56 (m, 1H), 5.11 (m, 2H), 4.65 (m, 1H), 4.25 (m, 2H), 3.45 (s, 3H), 2.85−2.6 (multiple signals, 2H), 2.3−2.2 (m, 1H), 2.2−2.1 (m, 1H), 1.29 (t, 3H). ^{13}C NMR ($CDCl_3$): δ 170.8, 156.6, 154.0, 129.8, 114.7, 109.4, 104.6, 94.5, 73.7, 61.3, 55.8, 24.7, 22.7, 14.1. LC−MS: m/z 267.3 $[M + H]^+$ and 235.4 $[M - CH_3O]^+$ (calcd 266.29 for $C_{14}H_{18}O_5$).

(*R*)-(7-(Methoxymethoxy)chroman-2-yl)methanol (5). Ethyl ester **4** (0.551 g, 2.07 mmol) was dissolved in THF (30 mL), after which $LiBH_4$ (2.39 mL of a 2 M solution in THF, 4.8 mmol) was added, and the solution was cooled using an ice bath. The reaction mixture was allowed to heat to rt over about 2 h. After verification that the reaction was completed by monitoring with LC−MS and ^{1}H NMR, the reaction mixture was poured in water and extracted multiple times with ethyl acetate. The organic layer was dried using Na_2SO_4 and concentrated to give a colorless oil. This crude product was purified using silica column chromatography (2% MeOH/$CHCl_3$). Yield: 90% (oil). ^{1}H NMR ($CDCl_3$): δ 6.92 (m, 1H), 6.54 (multiple signals, 2H), 5.08 (s, 2H), 4.05 (m, 1H), 3.85−3.75 (m, 1H), 3.75−3.65 (m, 1H), 3.43 (s, 3H), 2.85−2.6 (multiple signals, 2H), 2.4−2.3 (bs, 1H), 1.95−1.85 (m, 1H), 1.85−1.7 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 156.4, 155.0, 129.9, 115.4, 109.0, 104.3, 94.4, 76.4, 65.4, 55.8, 23.8, 23.7. LC−MS: m/z 225.0 $[M + H]^+$ and 193.1 $[M - CH_3O]^+$ (calcd 224.26 for $C_{12}H_{16}O_4$). GC−FID analysis gave a single peak when using the chiral capillary column (see GC−FID analysis of molecule **1**).

Toluene-4-sulfonic Acid, (*R*)-(7-(Methoxymethoxy)chroman-2-yl)-methyl Ester (6). Alcohol **5** (0.56 g, 2.50 mmol) was dissolved in pyridine (7.5 mL), and the solution was cooled to 0 °C. *p*-Toluenesulfonylchloride (1.05 g, 5.5 mmol, 2.2 equiv) in pyridine was added slowly, and the reaction mixture was stirred overnight at 4 °C. A small amount of ice was added to destroy the excess of *p*-toluenesulfonylchloride, the pH of the ice-cooled mixture was brought to 1 using a concentrated hydrochloric acid solution, and the product was extracted with CH_2Cl_2. The organic layer was washed with $NaHCO_3$, with a solution of $CuSO_4$ to remove traces of pyridine, and finally with NaCl(aq). Drying with Na_2SO_4 and concentration gave a slightly pinkish colored oil. Yield: 89%. ^{1}H NMR ($CDCl_3$): δ 7.83 (m, 2H), 7.38 (m, 2H), 6.95 (m, 1H), 6.55 (multiple signals, 2H), 6.41 (s, 1H), 5.08 (s, 2H), 4.20 (multiple signals, 3H), 3.45 (s, 3H), 2.85−2.6 (multiple signals, 2H), 2.45 (s, 3H), 2.05−1.95 (m, 1H), 1.85−1.7 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 156.5, 154.4, 144.9, 132.7, 129.9, 129.6, 128.0, 114.7, 110.0, 104.4, 94.4, 72.9, 71.1, 55.9, 23.8, 23.3, 21.6. LC−MS: m/z 401.3 $[M + Na]^+$, 347.4 $[M - CH_3O]^+$ (calcd 378.44 for $C_{19}H_{22}SO_6$).

(*R*)-*N*-(7-(Methoxymethoxy)chroman-2-yl)-methyl Phthalimide (7). Tosylate **6** (300 mg, 0.79 mmol) and potassium phthalimide (367 mg, 1.98 mmol, 2.5 equiv) were suspended in DMF (10 mL). The mixture was stirred for 16 h at 80 °C and was then allowed to cool to rt. Diethyl ether was added, and the mixture was washed twice with a saturated aqueous NH_4Cl solution, twice with water, and one more time with a saturated NaCl solution in water. The organic layer was dried with Na_2SO_4. After removal of the volatiles, the obtained white solid was purified with silica column chromatography (2% MeOH/$CHCl_3$). Yield: 78%. ^{1}H NMR ($CDCl_3$): δ 7.80 (m, 2H), 7.30 (m, 2H), 6.90 (d, 1H), 6.51 (m, 1H), 6.45 (s, 1H), 5.08 (m, 2H), 4.33 (m, 1H), 4.03 (dd, 1H), 3.84 (dd, 1H), 3.45 (s, 3H), 2.74 (m, 2H), 2.03 (m, 1H), 1.78 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 168.2, 156.4, 154.8, 134.0, 132.0, 129.8, 123.4, 115.0, 109.2, 104.5, 94.4, 73.0, 55.9, 41.9, 25.4, 23.5. LC−MS: m/z 322.2 $[M - CH_3O]^+$, 354.3 $[M + H]^+$, 376.4 $[M + Na]^+$, 643.3 $[2M - H\text{-}2CH_3O]^+$ (calcd 353.37 for $C_{20}H_{19}NO_5$).

(*R*)-(7-(Methoxymethoxy)chroman-2-yl)-methyl Amine (8). Phthalimide-protected amine **7** (200 mg, 0.566 mmol) was stirred in EtOH (3 mL) together with hydrazine monohydrate (0.029 mL, 0.58 mmol). The mixture was heated at an oil bath temperature of 80 °C for 16 h, during which time the reaction mixture changed from a suspension to a clear solution and back to a suspension. The mixture was allowed to cool to rt and was filtered; the filtrate was evaporated and redissolved in $CHCl_3$. The organic layer was washed once with a 0.1 M NaOH(aq) solution and once with water. The organic layer was dried with Na_2SO_4 and concentrated to give a colorless oil. Yield: 101 mg (80%). ^{1}H NMR ($CDCl_3$): δ 6.90 (m, 1H), 6.51 (multiple signals, 2H), 5.08 (s, 2H), 3.90 (m, 1H), 3.45 (s, 3H), 2.90 (d, 2H), 2.8−2.7 (m, 1H), 2.7−2.6 (m, 1H), 1.85 (m, 1H), 1.75 (m, 1H), 1.4 (bs, 2H). ^{13}C NMR ($CDCl_3$): δ 156.4, 155.3, 129.8, 115.4, 108.8, 104.3, 94.4, 77.6, 55.8, 46.6, 25.0, 24.0. LC−MS: m/z 224.1 $[M + H]^+$ (calcd 223.27 for $C_{12}H_{17}NO_3$).

Route A: Coupling of Tosylate 3 to the Appropriate Amines. (*R*)-2-[(Benzylamino)methyl]chroman-7-ol (10a). *Free Amine.* Reference compound **10a** was prepared using a similar method to that described by Mewshaw.[26] Benzylamine (0.266 g, 2.48 mmol, 3 equiv) was added to a solution of the tosylate **3** (0.285 g, 0.83 mmol) in DMSO (8 mL). The reaction mixture was stirred for 16 h at 100 °C, at which point it was poured in water (35 mL). The obtained suspension was extracted two times with ethylacetate, the collected organic layers were washed with water to remove DMSO, and the ethyl acetate solution was dried with Na_2SO_4. The crude free amine product was purified with silica column chromatography (5% MeOH/$CHCl_3$). Yield: 90 mg (40%). ^{1}H NMR ($CDCl_3$): δ 7.45−7.15 (multiple signals, 5H), 6.8 (m, 1H), 6.35 (multiple signals, 2H), 4.05 (m, 1H), 3.95 (d, 1H), 3.85 (d, 1H), 2.85 (m, 1H), 2.75 (m, 1H), 2.7−2.6 (multiple signals, 2H), 1.85 (m, 1H), 1.65 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 156.2, 154.5, 138.3, 130.0, 128.6, 128.6, 127.4, 112.6, 109.0, 103.3, 74.3, 54.0, 53.6, 26.2, 23.7. LC−MS: m/z 270.3 $[M + H]^+$ (calcd 269.34 for $C_{17}H_{19}NO_2$). MALDI-TOF-MS: m/z 270.18 $[M + H]^+$.

Oxalate Salt. To a solution of free amine **10a** (90 mg, 0.334 mmol) in THF (2 mL) was added a solution of oxalic acid dihydrate (84 mg, 0.67 mmol, 2 equiv) in THF (0.5 mL). Immediately after addition, a solid precipitated. The mixture was stirred for 3 h, a small amount of ether was added to improve the precipitation, the mixture was filtrated, and the white solid residue was washed with diethylether. Yield: 82 mg (68%). ^{1}H NMR (DMSO-d_6): δ 8.0−6.0 (broad signal acidic protons), 7.55 (m, 2H), 7.4 (m, 3H), 6.8 (m, 1H), 6.28 (m, 1H), 6.23 (s, 1H), 4.27 (m, 1H), 4.23 (s, 2H), 3.2−3.0 (multiple signals, 2H), 2.75−2.5 (multiple signals, 2H), 1.95 (m, 1H), 1.65 (m, 1H). ^{13}C NMR (DMSO-d_6): δ 164.4, 156.5, 153.9, 132.5, 130.0, 129.9, 128.8, 128.6, 111.9, 108.5, 102.9, 71.5, 50.6, 49.9, 24.7, 22.7. mp 212 °C (dec). LC−MS: m/z 270.2 $[M + H]^+$ (calcd 269.34 for $C_{17}H_{19}NO_2$). UPLC−HRMS $[M + H]^+$: calcd 270.1494; found, 270.1491 (1.1 ppm). UPLC: 99% purity. Anal. ($C_{17}H_{19}NO_2$ + 1 $C_2H_2O_4$ (fw 359.4)) C, H, N: Found 63.36, 5.27, 3.65; Calcd 63.51, 5.89, 3.90.

(*R*)-2-[(3-Iodobenzylamino)methyl]chroman-7-ol (11b). *Free Amine.* Tosylate **3** (0.38 g, 1.1 mmol) was mixed with commercially available 3-iodo-benzylamine (0.78 g, 3.3 mmol, 2.9 equiv) and DIPEA (0.6 mL, 3 equiv) in DMSO (10 mL), and this solution was stirred overnight at an oil bath temperature of 100 °C. According to LC−MS, no tosylate starting material was present, and the mixture was poured in water followed by extraction of the water layer with ethyl acetate (two times). The ethyl acetate layers were collected, dried with Na_2SO_4, and concentrated. Finally, the crude product was purified with silica column chromatography (2% MeOH/$CHCl_3$), giving 169 mg of an oily product (39%). ^{1}H NMR ($CDCl_3$): δ 7.7 (s, 1H), 7.6 (m, 1H), 7.35 (m, 1H), 7.05 (m, 1H), 6.8 (m, 1H), 6.35 (multiple signals, 2H), 4.05 (m, 1H), 3.85 (d, 1H), 3.80 (d, 1H), 2.85 (m, 1H), 2.75 (m, 1H), 2.7−2.55 (multiple signals, 2H), 1.85 (m, 1H), 1.65 (m, 1H).

Oxalate Salt. Free amine **11b** (169 mg, 0.43 mmol) was dissolved in THF (2 mL), and oxalic acid dihydrate (118 mg, 0.94 mmol, 2.2

dx.doi.org/10.1021/jm401384w | J. Med. Chem. 2014, 57, 391−410

equiv) was added. A precipitation formed, and after some stirring, the suspension was filtrated to give a white solid product. Yield: 152 mg (73%). ^{1}H NMR (DMSO-d_6): δ 8.0−6.0 (broad signal acidic protons), 7.9 (s, 1H), 7.75 (m, 1H), 7.55 (m, 1H), 7.25 (m, 1H), 6.85 (m, 1H), 6.28 (m, 1H), 6.25 (s, 1H), 4.25 (m, 1H), 4.15 (s, 2H), 3.2−3.0 (multiple signals, 2H), 2.75−2.5 (multiple signals, 2H), 1.95 (m, 1H), 1.65 (m, 1H). ^{13}C NMR (DMSO-d_6): δ 164.0, 156.5, 154.0, 138.4, 137.3, 135.5, 130.7, 129.9, 129.4, 111.9, 108.5, 102.9, 94.9, 71.7, 50.2, 49.9, 24.7, 22.8. mp 214 °C (dec). LC−MS: m/z 396.2 $[M + H]^+$ (calcd 395.24 for $C_{17}H_{18}NO_2I$). UPLC−HRMS $[M + H]^+$: calcd 396.0460; found, 396.0455 (1.3 ppm). UPLC: 94% purity. Anal. ($C_{17}H_{18}NO_2I$ + 1 $C_2H_2O_4$ (fw 485.3)) C, H, N: Found 47.23, 4.33, 2.38; Calcd 47.03, 4.15, 2.89.

(*R*)-1-(4-Iodophenyl)-4-((7-hydroxychroman-2-yl)methyl)-piperazine (11d). *Free Amine.* Tosylate **3** (289 mg, 0.865 mmol) was dissolved in DMSO (10 mL), and to the solution were added DIPEA (0.334 g, 2.59 mmol, 2.9 equiv) and 1-(4-iodophenyl)-piperazine (500 mg, 1.74 mmol, 2.0 equiv). 1-(4-Iodophenyl)-piperazine was synthesized from 1-phenyl-piperazine according to Hanson et al.[72] The reaction mixture was stirred overnight at 100 °C under an argon atmosphere. According to LC−MS, all of the tosylate reactant was converted. The reaction mixture was cooled to rt and poured into water. Extraction with ethyl acetate was followed by drying of the collected organic layers with Na_2SO_4. Evaporation of the solvent yielded crude product (844 mg) that was purified with silica column chromatography (2% MeOH/$CHCl_3$). A beige solid was obtained. Yield: 120 mg (31%). ^{1}H NMR ($CDCl_3$ with a drop of CD_3OD): δ 7.55 (m, 2H), 6.85 (m, 1H), 6.70 (m, 2H), 6.35 (m, 1H), 6.30 (s, 1H), 4.2 (m, 1H), 3.2 (m, 4H), 2.85−2.6 (multiple signals, 7H), 2.55 (m, 1H), 2.0 (m, 1H), 1.75 (m, 1H). LC−MS: m/z 451.3 $[M + H]^+$ (calcd 450.32 for $C_{20}H_{23}N_2O_2I$).

Oxalate Salt. To a solution of free amine **11d** (120 mg, 0.267 mmol) in THF (4 mL) was added a solution of oxalic acid dihydrate (74 mg, 0.59 mmol, 2.2 equiv) in THF (1 mL). Immediately, a white solid precipitated. After 3 h of stirring, the reaction mixture was centrifuged. The solvent was decanted, and the residue was washed with a mixture of THF and ether (1:1). After drying, a white solid residue was isolated (120 mg, 83%). ^{1}H NMR (DMSO-d_6): δ 7.5 (m, 2H), 6.8 (multiple signals, 3H), 6.25 (m, 1H), 6.20 (s, 1H), 4.3 (m, 1H), 3.25 (m, 4H), 3.1−2.9 (multiple signals, 6H), 2.8−2.6 (multiple signals, 2H), 2.0 (m, 1H), 1.65 (m, 1H). ^{13}C NMR (DMSO-d_6): δ 162.6, 156.5, 154.3, 150.0, 137.4, 129.9, 118.0, 112.1, 108.2, 102.9, 81.3, 71.8, 60.4, 52.3, 46.4, 25.5, 22.9. mp 177 °C (dec). LC−MS: m/z 451.2 $[M + H]^+$ (calcd 450.32 for $C_{20}H_{23}N_2O_2I$). UPLC−HRMS $[M + H]^+$: calcd 451.0882; found,, 451.0882 (0 ppm). UPLC: 96% purity. Anal. ($C_{20}H_{23}N_2O_2I$ + 1 $C_2H_2O_4$ (fw 540.4)) C, H, N: Found 48.88, 4.12, 4.81; Calcd 48.90, 4.66, 5.18.

Route B: General Procedure for Coupling of MOM-Protected Tosylate 6 to the Appropriate Amines. Tosylate **6** was added to a solution of the amine (typically in a molar excess relative to the tosylate) in DMSO. DIPEA was also present (in molar excess relative to the tosylate). The reaction mixture was heated to 80−100 °C under an inert atmosphere of argon, and the progress of the reaction was followed with LC−MS for 1 or 2 days. In cases where primary amine starting compounds were used, LC−MS analysis of the reaction mixture showed the excess of the primary amine starting compound and the secondary amine desired product as well as tertiary amine byproduct. After reaction, the mixture was poured into cold water, and the product was extracted using ethyl acetate. Evaporation yielded an oily product that requires column chromatographic purification. The obtained secondary amine products can severely stick to silica, so alumina was sometimes used as the stationary phase. The applied eluent was a mixture of $CHCl_3$ with a small percentage of MeOH (typically 1 to 2% v/v) or a gradient with gently increasing MeOH content starting from pure $CHCl_3$.

(*R*)-*N*-[7-(Methoxymethoxy)chroman-2-yl]methyl 1-Hexyl Amine (9a). *n*-Hexylamine (0.321 g, 3.18 mmol) was dissolved in DMSO (5 mL) with 5 equiv of DIPEA (0.9 mL). Tosylate **6** (397 mg, 1.05 mmol) was added, and the reaction mixture was heated overnight at 95 °C under an argon atmosphere. The mixture was cooled and poured into water followed by extraction of the aqueous layer ethyl acetate. Concentration of the organic layer gave a slightly colored oil (378 mg), which gave 193 mg of pure product after silica column chromatography (2% MeOH/$CHCl_3$). Yield 60%. ^{1}H NMR ($CDCl_3$): δ 6.92 (m, 1H), 6.53 (multiple signals, 2H), 5.10 (s, 2H), 4.11 (m, 1H), 3.44 (s, 3H), 2.90−2.55 (multiple signals, 5H), 2.0−1.9 (m, 1H), 1.8−1.6 (multiple signals, 3H), 1.50 (m, 2H), 1.4−1.2 (multiple signals, 6H), 0.88 (t, 3H). ^{13}C NMR ($CDCl_3$): δ 156.4, 155.2, 129.9, 115.5, 108.8, 104.3, 94.4, 75.2, 55.8, 54.3, 50.1, 31.7, 30.0, 27.0, 25.7, 24.0, 22.6, 14.0. LC−MS: m/z 308.3 $[M + H]^+$ (calcd 307.43 for $C_{18}H_{29}NO_3$).

(*R*)-*N*-[7-(Methoxymethoxy)chroman-2-yl]methyl 4-Iodobenzyl Amine (9b). Tosylate **6** (327 mg, 0.865 mmol) was added to a solution of commercially available *p*-iodobenzylamine (0.4 g; 1.83 mmol) in DMSO (5 mL) and DIPEA (0.8 mL). The mixture was heated at 85 °C, kept under argon, and stirred overnight. After cooling to rt, the reaction mixture was poured into water. Extraction with ethyl acetate and evaporation of the volatiles yielded a slightly colored oil. This crude material was purified with silica column chromatography (2% MeOH/$CHCl_3$), yielding 283 mg of the product (75%). ^{1}H NMR ($CDCl_3$): δ 7.64 (m, 2H), 7.12 (m, 2H), 6.95 (m, 1H), 6.53 (multiple signals, 2H), 5.11 (s, 2H), 4.15 (m, 1H), 3.80 (s, 2H), 3.45 (s, 3H), 2.9−2.6 (multiple signals, 4H), 2.0−1.9 (multiple signals, 2H), 1.75 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 156.4, 155.1, 139.9, 137.3, 130.0, 129.8, 115.4, 108.9, 104.3, 94.4, 92.1, 75.2, 55.8, 53.4, 53.1, 25.6, 23.9. LC−MS: m/z 440.2 $[M + H]^+$ (calcd 439.29 for $C_{19}H_{22}NO_3I$).

(*R*)-1-(4-Iodophenyl)-4-(4-(7-(methoxymethoxy)chroman-2-yl)-3-azabutyl)-piperazine (9d). Primary amine **24** (0.585 g, 1.77 mmol, 1.5 equiv) was dissolved in DMSO (5 mL) with 4 equiv of DIPEA (0.8 mL). Tosylate **6** (442 mg, 1.17 mmol) was added, and the reaction mixture was heated at 85 °C and kept overnight under a gentle flow of argon. The reaction mixture was then cooled and poured into water. Extraction with ethyl acetate and evaporation of the solvent yielded a slightly colored oil that was purified with alumina column chromatography (2% MeOH/$CHCl_3$). Yield: 250 mg (40%). ^{1}H NMR ($CDCl_3$): δ 7.50 (m, 2H), 6.95 (m, 1H), 6.67 (m, 2H), 6.53 (multiple signals, 2H), 5.08 (s, 2H), 4.15 (m, 1H), 3.44 (s, 3H), 3.17 (m, 4H), 2.95−2.65 (multiple signals, 6H), 2.65−2.55 (multiple signals, 6H), 2.0−1.9 (multiple signals, 2H), 1.75 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 156.4, 155.2, 150.9, 137.7, 129.9, 117.9, 115.5, 108.9, 104.3, 94.4, 81.2, 75.0, 57.8, 55.8, 54.2, 53.0, 48.7, 46.4, 25.7, 24.0. LC−MS: m/z 538.3 $[M + H]^+$ (calcd 537.44 for $C_{24}H_{32}N_3O_3I$).

(*R*)-*N*-[7-(Methoxymethoxy)chroman-2-yl]methyl 4-(4-Fluorobutoxy)benzyl Amine (9e). Amine **21** (0.198 g, 1.00 mmol, 1.2 equiv) was dissolved in DMSO (5 mL) with 5 equiv of DIPEA (0.75 mL). Tosylate **6** (318 mg, 0.84 mmol) was added, and the mixture was heated at 95 °C and left to react for 2 days under an argon atmosphere. The reaction was cooled and poured in water. Extraction with ethyl acetate yielded a slightly colored oil that was purified using silica column chromatography (2% MeOH/$CHCl_3$) and subsequent RP C18 chromatography using an aqueous eluent gradient. Yield: 124 mg (36%). ^{1}H NMR ($CDCl_3$): δ 7.25 (m, 2H), 6.90 (m, 1H), 6.85 (m, 2H), 6.53 (multiple signals, 2H), 5.05 (s, 2H), 4.6 (m, 1H), 4.45 (m, 1H), 4.15 (m, 1H), 3.95 (m, 2H), 3.8 (s, 2H), 3.4 (s, 3H), 2.9−2.65 (multiple signals, 4H), 2.0−1.7 (multiple signals, 7H). LC−MS: m/z 404.3 $[M + H]^+$ (calcd 403.49 for $C_{23}H_{30}NO_4F$).

(*R*)-1-(4-(2-Fluoroethoxy)phenyl)-4-((7-(methoxymethoxy)-chroman-2-yl)-methyl)-piperazine (9g). 1-(4-(2-Fluoroethoxy)-phenyl)-piperazine was prepared from commercially available 1-(4-hydroxyphenyl)-piperazine according to Tietze et al.[73] using the 1-fluoro-2-tosyloxy-ethane building block that was prepared[74] from commercially available 2-fluoroethanol. 1-(4-(2-Fluoroethoxy)-phenyl)-piperazine (0.305 g, 1.36 mmol, 1.2 equiv) was dissolved in DMSO (5 mL) with 3 equiv of DIPEA (0.6 mL). Tosylate **6** (428 mg, 1.13 mmol) was added, and the reaction mixture was heated at 95 °C. Overnight stirring under an inert argon atmosphere was followed by cooling the reaction mixture and pouring it in water. Extraction with ethyl acetate and concentration yielded a slightly colored crude oil that was purified using silica column chromatography (2% MeOH/$CHCl_3$). Yield: 320 mg (66%). ^{1}H NMR ($CDCl_3$): δ 7.0−6.8

dx.doi.org/10.1021/jm401384w | J. Med. Chem. 2014, 57, 391−410

(multiple signals, 5H), 6.56 (multiple signals, 2H), 5.12 (s, 2H), 4.79 (m, 1H), 4.67 (m, 1H), 4.20 (m, 2H), 4.15 (m, 1H), 3.46 (s, 3H), 3.13 (m, 4H), 2.9−2.55 (multiple signals, 8H), 2.1−2.0 (m, 1H), 1.85−1.7 (m, 1H). LC−MS: m/z 431.4 $[M + H]^+$ (calcd 430.52 for $C_{24}H_{31}N_2O_4F$).

(*R*)-1-(4-(2-Fluoroethoxy)phenyl)-4-(4-(7-(methoxymethoxy)chroman-2-yl)-3-azabutyl)-piperazine (9h). Primary amine **26** (0.364 g, 1.36 mmol, 1.2 equiv) was dissolved in DMSO (5 mL) with 3 equiv of DIPEA (0.6 mL). Tosylate **6** (428 mg, 1.13 mmol) was added, and the reaction mixture was heated overnight at 100 °C under a gentle flow of argon. The reaction mixture was cooled and poured into water. Extraction with ethyl acetate and concentration gave a slightly colored oil (0.36 g). Finally, purification with alumina column chromatography (2% MeOH/$CHCl_3$) gave a yield of 47% of pure product (250 mg). ^{1}H NMR ($CDCl_3$): δ 7.0−6.8 (multiple signals, 5H), 6.54 (multiple signals, 2H), 5.10 (s, 2H), 4.79 (m, 1H), 4.67 (m, 1H), 4.20 (m, 1H), 4.13 (m, 2H), 3.44 (s, 3H), 3.12 (m, 4H), 3.0−2.5 (multiple signals, 12H), 2.05−1.9 (multiple signals, 2H), 1.75 (m, 1H). LC−MS: m/z 474.4 $[M + H]^+$ (calcd 473.59 for $C_{26}H_{36}N_3O_4F$).

Route C: General Procedure for Coupling MOM-Protected Amine 8 to the Appropriate Aldehydes. MOM-protected primary amine **8** and the appropriate aldehyde (1 equiv) were mixed in MeOH and stirred overnight at rt. During this step, the imine was formed. The conversion was followed with ^{1}H NMR by inspecting the aldehyde signal (because of the reversibility of the reaction in the presence of water, monitoring by LC−MS was not possible). After completion of imine formation, 1.1 equiv of $NaBH_4$ was added, and the reaction mixture was stirred at rt for 1 to 3 h. ^{1}H NMR was used to monitor imine conversion. The reaction mixture was poured in water, and the aqueous solution was stirred for 1 h and then extracted with CH_2Cl_2 or, alternatively, with $CHCl_3$. Because of the presence of boron salts, it may be necessary to first adjust the pH of the water layer to become acidic (pH 1) and then back to slightly basic (pH 8) and then perform the extraction at this higher pH. Drying of combined organic layers with Na_2SO_4 was followed by removal of the solvents to yield the product.

(*R*)-*N*-[7-(Methoxymethoxy)chroman-2-yl]methyl 4-Iodobenzyl Amine (9b). 4-Iodobenzaldehyde was prepared from commercially available *p*-iodo benzoic acid using a two-step procedure[75] via the *p*-iodo benzyl alcohol. Amine **8** (418 mg, 1.87 mmol) and freshly prepared 4-iodobenzaldehyde (433 mg, 1.87 mmol) were stirred in MeOH (10 mL). $NaBH_4$ (77.7 mg, 2.06 mmol) was added followed by extraction with $CHCl_3$ and then CH_2Cl_2. An oily product was obtained (766 mg, 93%). The analytical data are identical to those reported for the same **9b** product prepared and isolated via route B (vide supra).

(*R*)-*N*-[7-(Methoxymethoxy)chroman-2-yl]methyl 2-Iodobenzyl Amine (9c). 2-Iodobenzaldehyde was prepared from its commercially available benzyl alcohol precursor.[76] Amine **8** (192 mg, 0.86 mmol) was stirred with 2-iodobenzaldehyde (200 mg, 0.86 mmol) in MeOH (5 mL). According to the ^{1}H NMR, ca. 4% of aldehyde was still present; nevertheless, $NaBH_4$ (42 mg, 1.11 mmol, 1.3 equiv) was added. After extraction, the crude yellow oil (331 mg) was purified by silica column chromatography (ethyl acetate/hexane 1:1) to yield 209 mg (56%) of product. ^{1}H NMR ($CDCl_3$): δ 7.80 (m, 1H), 7.40 (m, 1H), 7.28 (m, 1H), 6.95 (multiple signals, 2H), 6.53 (multiple signals, 2H), 5.08 (s, 2H), 4.15 (m, 1H), 3.87 (s, 2H), 3.45 (s, 3H), 2.9−2.6 (multiple signals, 4H), 2.05−1.85 (multiple signals, 2H), 1.85−1.75 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 156.4, 155.2, 142.0, 139.3, 129.8, 129.4, 128.7, 128.2, 115.5, 108.9, 104.4, 99.5, 94.4, 75.2, 58.0, 55.8, 53.2, 25.6, 23.9. LC−MS: m/z 440.2 $[M + H]^+$ (calcd 439.29 for $C_{19}H_{22}NO_3I$).

(*R*)-*N*-[7-(Methoxymethoxy)chroman-2-yl]methyl 2-(4-Fluorobutoxy)benzyl Amine (9f). Amine **8** (205 mg, 0.92 mmol) was stirred with 2-(4-fluorobutoxy) benzaldehyde **22** (179 mg, 0.92 mmol) in MeOH (5 mL). According to ^{1}H NMR, 10% of the aldehyde was still present; nevertheless, $NaBH_4$ (45 mg, 1.19 mmol, 1.3 equiv) was added. The crude yellowish oil (340 mg) was purified with silica column chromatography (ethyl acetate/hexane 1:1) to yield 221 mg of product (60%). ^{1}H NMR ($CDCl_3$): δ 7.26 (m, 1H), 7.23 (m, 1H), 6.85 (multiple signals, 2H), 6.80 (m, 1H), 6.53 (multiple signals, 2H), 5.10 (s, 2H), 4.56 (bs, 1H), 4.45 (t, 1H), 4.15 (m, 1H), 4.06 (t, 2H), 3.85 (dd, 2H), 3.44 (s, 3H), 2.90−2.6 (multiple signals, 4H), 2.05 (bs, 1H), 2.0−1.85 (multiple signals, 5H), 1.8−1.7 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 156.9, 156.4, 155.3, 129.9, 129.8, 128.3, 128.1, 120.4, 115.5, 111.0, 108.8, 104.3, 94.5, 84.5, 82.8, 75.6, 67.1, 55.8, 53.6, 49.4, 27.4, 27.2, 25.7, 25.4, 25.3, 24.0. LC−MS: m/z 404.3 $[M + H]^+$ (calcd 403.49 for $C_{23}H_{30}NO_4F$).

Routes B and C: General Procedure for Deprotection of the MOM Group. The appropriate MOM-protected compound **9** (100 to 300 mg) was dissolved in an 4 M HCl solution in dioxane (2−5 mL; HCl in high molar excess) and isopropanol (2−5 mL). The reaction mixture was stirred for about 3 h at rt, and a N_2 flow was maintained over the reaction mixture. From time to time, N_2 was bubbled through the solution to remove volatile reaction products from the mixture. The reaction mixture may turn hazy because of the precipitation of the ammonium salt. After completion of the reaction (the reaction was monitored by LC−MS), the mixture was added to water, and the pH was brought to slightly basic by addition of a NaOH solution. The product was isolated from the water layer by several extractions using $CHCl_3$ or CH_2Cl_2 with some added THF followed by drying of the collected organic layers with Na_2SO_4 and evaporation of the solvent.

General Procedure for Preparation of the Oxalate Salt. The isolated free amine compound was dissolved in THF, and a solution of oxalic acid dihydrate in THF was added. The reaction mixture turned hazy because of precipitation of the oxalate ammonium salt. After about 3−5 h of stirring, the reaction mixture was centrifuged, and the solvent was pipetted or decanted off. The residue was stirred in a fresh 1:1 mixture of diethyl ether and THF and was centrifuged again. The solvent was removed, and the precipitate was dried to yield a white, off-white, or slightly brownish powder product.

(*R*)-2-[(Hexylamino)methyl]chroman-7-ol (10b). *Free Amine.* Reference compounds **10b** was already reported by Mewshaw,[26] although the racemic mixture was prepared and not the (*R*) enantiomer. Furthermore, we prepared **10b** via its MOM-protected precursor, whereas Mewshaw prepared the racemate via its benzyl-protected precursor. Compound **9a** (121 mg, 0.39 mmol) was dissolved in a solution of 4 M HCl in dioxane (2 mL) and isopropanol (3 mL). Yield after work up: 100 mg (96%). ^{1}H NMR ($CDCl_3$): δ 6.78 (m, 1H), 6.45 (m, 1H), 6.32 (m, 1H), 4.07 (m, 1H), 2.90 (t, 1H), 2.8−2.5 (multiple signals, 5H), 1.85 (m, 1H), 1.75−1.5 (multiple signals, 3H), 1.4−1.2 (multiple signals, 6H), 0.88 (t, 3H). ^{13}C NMR ($CDCl_3$): δ 156.7, 154.5, 130.0, 112.2, 109.4, 103.0, 74.2, 54.2, 50.0, 31.6, 29.2, 26.9, 26.3, 23.7, 22.5, 14.0. LC−MS: m/z 264.3 $[M + H]^+$ (calcd 263.38 for $C_{16}H_{25}NO_2$).

Oxalate Salt. Free amine **10b** (120 mg, 0.456 mmol) was dissolved in THF (2 mL), and oxalic acid dihydrate (86 mg, 0.68 mmol, 1.5 equiv) in THF (1 mL) was added. A white solid precipitate was isolated (115 mg, 71%). ^{1}H NMR (DMSO-d_6): δ 9.0−6.0 (broad signal, acidic protons), 6.85 (m, 1H), 6.28 (m, 1H), 6.23 (s, 1H), 4.25 (m, 1H), 3.25−3.05 (multiple signals, 2H), 2.9 (m, 2H), 2.75−2.5 (multiple signals, 2H), 1.95 (m, 1H), 1.65 (multiple signals, 3H), 1.25 (m, 6H), 0.9 (t, 3H). ^{13}C NMR (DMSO-d_6): δ 164.6, 156.6, 153.9, 129.9, 111.9, 108.5, 102.9, 71.3, 50.1, 47.3, 30.7, 25.7, 25.2, 24.7, 22.7, 21.9, 13.9. mp 152 °C (dec). LC−MS: m/z 264.3 $[M + H]^+$ (calcd 263.38 for $C_{16}H_{25}NO_2$). UPLC−HRMS $[M + H]^+$: calcd 264.1964; found, 264.1966 (0.8 ppm). UPLC: 92% purity. Anal. ($C_{16}H_{25}NO_2$ + 1 $C_2H_2O_4$ (fw 353.4)) C, H, N: Found 60.59, 7.68, 3.55; Calcd 61.18, 7.70, 3.96.

(*R*)-2-[(4-Iodobenzylamino)methyl]chroman-7-ol (11a). *Free Amine.* Compound **9b** (171 mg, 0.389 mmol) was deprotected in 4 M HCl in dioxane (3.5 mL) and isopropanol (3.5 mL). After workup, an oil was obtained in 80% yield (123 mg). ^{1}H NMR (CD_3OD): δ 7.65 (m, 2H), 7.15 (m, 2H), 6.80 (m, 1H), 6.27 (m, 1H), 6.23 (m, 1H), 4.05 (m, 1H), 3.80 (bs, 2H), 2.9−2.5 (multiple signals, 4H), 1.93 (m, 1H), 1.65 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 156.0, 154.4, 137.8, 137.7, 130.6, 130.1, 112.7, 109.1, 103.2, 93.1, 74.2, 53.6, 53.5, 26.2, 23.6. LC−MS: m/z 396.2 $[M + H]^+$ (calcd 395.24 for $C_{17}H_{18}NO_2I$). MALDI-TOF-MS: m/z 396.0 $[M + H]^+$.

dx.doi.org/10.1021/jm401384w | J. Med. Chem. 2014, 57, 391−410

Oxalate Salt. Free amine **11a** (118 mg, 0.299 mmol) was dissolved in THF (4 mL), and oxalic acid dihydrate (49 mg, 0.389 mmol) in THF (1 mL) was added. A white solid was isolated (90 mg, 62%). ^{1}H NMR (DMSO-d_6): δ 8.0−6.0 (broad signal acidic protons), 7.75 (m, 2H), 7.30 (m, 2H), 6.80 (m, 1H), 6.28 (m, 1H), 6.25 (s, 1H), 4.25 (m, 1H), 4.15 (s, 2H), 3.2−3.0 (multiple signals, 2H), 2.75−2.5 (multiple signals, 2H), 1.95 (m, 1H), 1.65 (m, 1H). ^{13}C NMR (DMSO-d_6): δ 164.2, 156.5, 154.0, 137.4, 132.6, 132.2, 129.9, 111.9, 108.5, 102.9, 95.3, 71.7, 50.1, 50.0, 24.7, 22.8. mp 211 °C (dec). LC−MS: m/z 396.1 $[M + H]^+$ (calcd 395.24 for $C_{17}H_{18}NO_2I$). UPLC−HRMS $[M + H]^+$: calcd 396.0460; found, 396.0466 (1.5 ppm). UPLC: 95% purity. Anal. ($C_{17}H_{18}NO_2I$ + 1 $C_2H_2O_4$ (fw 485.3)) C, H, N: Found 48.39, 4.53, 2.32; Calcd 47.03, 4.15, 2.89.

(*R*)-2-[(2-Iodobenzylamino)methyl]chroman-7-ol (11c). *Free Amine.* Compound **9c** (209 mg, 0.48 mmol) was dissolved in a mixture of 4 M HCl dioxane (2.4 mL) and isopropanol (2.4 mL). After 1 h, extra amounts of 4 M HCl in dioxane (1 mL) and isopropanol (l mL) were added. After workup, a glassy product was obtained in quantitative yield (188 mg). ^{1}H NMR ($CDCl_3$): δ 7.82 (m, 1H), 7.40 (m, 1H), 7.35 (m, 1H), 6.95 (m, 1H), 6.76 (m, 1H), 6.35 (m, 1H), 6.30 (m, 1H), 4.10 (m, 1H), 4.05 (d, 1H), 3.90 (d, 1H), 2.9−2.75 (multiple signals, 2H), 2.75−2.5 (multiple signals, 2H), 1.8 (m, 1H), 1.65 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 155.9, 154.7, 140.6, 139.7, 130.2, 130.0, 129.3, 128.5, 112.8, 108.8, 103.4, 99.7, 74.4, 57.8, 53.0, 26.1, 23.7. LC−MS: m/z 396.2 $[M + H]^+$ (calcd 395.24 for $C_{17}H_{18}NO_2I$).

Oxalate Salt. Free amine **11c** (188 mg, 0.48 mmol) was dissolved in THF (2 mL), and oxalic acid dihydrate (132 mg, 1.05 mmol, 2.2 equiv) in THF (1 mL) was added. A white solid residue was isolated (219 mg, 94%). ^{1}H NMR (DMSO-d_6): δ 8.0−6.0 (broad signal acidic protons), 7.95 (m, 1H), 7.60 (m, 1H), 7.45 (m, 1H), 7.13 (m, 1H), 6.80 (m, 1H), 6.30−6.25 (multiple signals, 2H), 4.3−4.1 (multiple signals, 3H), 3.1 (m, 2H), 2.7−2.6 (multiple signals, 2H), 2.05 (m, 1H), 1.65 (m, 1H). ^{13}C NMR (DMSO-d_6): δ 163.6, 156.5, 154.1, 139.4, 136.8, 130.3, 130.2, 129.9, 128.6, 112.0, 108.4, 103.0, 100.9, 72.3, 55.5, 50.9, 24.9, 22.8. mp 209 °C (dec). LC−MS: m/z 396.2 $[M + H]^+$ (calcd 395.24 for $C_{17}H_{18}NO_2I$). UPLC−HRMS $[M + H]^+$: calcd 396.0460; found, 396.0466 (1.5 ppm). UPLC: 97% purity. Anal. ($C_{17}H_{18}NO_2I$ + 1 $C_2H_2O_4$ (fw 485.3)) C, H, N: Found 46.81, 4.03, 2.56; Calcd 47.03, 4.15, 2.89.

(*R*) 1-(4-Iodophenyl)-4-(4-(7-hydroxychroman-2-yl)-3-azabutyl)-piperazine (11e). *Free Amine.* Compound **9d** (115 mg, 0.214 mmol) was dissolved in 4 M HCl in dioxane (3.5 mL) and isopropanol (4 mL). The reaction was not complete after 3 h, so the reaction mixture was evaporated to yield an oily product. A fresh solution of 4 M HCl in dioxane was added, and after a further 90 min of stirring under N_2, the reaction was complete. A quantitative yield of an oil was obtained after workup. ^{1}H NMR ($CDCl_3$): δ 7.50 (m, 2H), 6.77 (m, 1H), 6.64 (m, 2H), 6.49 (s, 1H), 6.28 (m, 1H), 4.08 (m, 1H), 3.10 (m, 2H), 3.05 (m, 2H), 2.9−2.3 (multiple signals, 14H), 1.83 (m, 1H), 1.65 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 156.4, 154.5, 150.9, 137.7, 129.9, 117.7, 112.2, 109.2, 103.4, 81.0, 74.5, 56.4, 54.0, 52.6, 48.7, 46.0, 26.3, 23.7. LC−MS: m/z 494.2 $[M + H]^+$ (calcd 493.39 for $C_{22}H_{28}N_3O_2I$). MALDI-TOF-MS: m/z 494.0 $[M + H]^+$.

Oxalate Salt. Free amine **11e** (105 mg, 0.214 mmol) was dissolved in THF (4 mL), and oxalic acid dihydrate (63 mg, 0.50 mmol, 2.3 equiv) in THF (1 mL) was added. A white solid was isolated (93 mg, 57%). ^{1}H NMR (DMSO-d_6): δ 8.0−6.0 (broad signal acidic protons), 7.5 (m, 2H), 6.85 (m, 1H), 6.8 (m, 2H), 6.28 (m, 1H), 6.23 (s, 1H), 4.3 (m, 1H), 3.35−3.1 (multiple signals, 8H), 2.8−2.6 (multiple signals, 8H), 2.0 (m, 1H), 1.65 (m, 1H). ^{13}C NMR (DMSO-d_6): δ 162.9, 156.5, 153.8, 150.4, 137.3, 130.0, 117.8, 111.9, 108.6, 102.9, 80.7, 71.2, 52.9, 52.2, 50.2, 47.4, 43.7, 24.7, 22.7. mp 201 °C. LC−MS: m/z 494.2 $[M + H]^+$ (calcd 493.39 for $C_{22}H_{28}N_3O_2I$). UPLC−HRMS $[M + H]^+$: calcd 494.1304; found, 494.1294 (2.0 ppm). UPLC: 99% purity. Anal. ($C_{22}H_{28}N_3O_2I$ + 3 $C_2H_2O_4$ (fw 763.5)) C, H, N: Found 43.37, 4.77, 5.44; Calcd 44.05, 4.49, 5.50.

(*R*)-2-[(4-(4-Fluorobutoxy)benzylamino)methyl]chroman-7-ol (12a). *Free Amine.* Compound **9e** (124 mg, 0.308 mmol) was dissolved in a solution of 4 M HCl in dioxane (2 mL) and isopropanol (2 mL). No starting compound was present according to LC−MS analysis, but there were some impurities present. After aqueous workup, the crude product was purified by silica column chromatography (eluent: heptane/THF 1:1). Yield: 45 mg (41%) of a solid. ^{1}H NMR ($CDCl_3$): δ 7.35 (d, 2H), 6.90 (d, 2H), 6.78 (m, 1H), 6.38 (multiple signals, 2H), 4.60 (m, 1H), 4.45 (m, 1H), 4.08 (m, 1H), 4.00 (t, 2H), 3.90 (dd, 2H), 2.9−2.5 (multiple signals, 4H), 2.0−1.75 (multiple signals, 5H), 1.7 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 158.3, 156.3, 154.5, 130.2, 130.1, 130.0, 114.6, 112.4, 109.2, 103.3, 84.6, 82.9, 74.2, 67.2, 53.6, 53.5, 27.3, 27.1, 26.3, 25.2, 23.7. LC−MS: m/z 360.0 $[M + H]^+$ (calcd 359.44 for $C_{21}H_{26}NO_3F$). MALDI-TOF-MS: m/z 360.1 $[M + H]^+$.

Oxalate Salt. Free amine **12a** (87 mg, 0.242 mmol) was dissolved in THF (2 mL), and oxalic acid dihydrate (46 mg, 0.365 mmol, 1.5 equiv) in THF (1 mL) was added. The residue was a white solid (68 mg, 68%). ^{1}H NMR (DMSO-d_6): δ 7.5−5.0 (broad signal acidic protons), 7.45 (d, 2H), 6.95 (d, 2H), 6.8 (m, 1H), 6.3 (m, 1H), 6.25 (s, 1H), 4.55 (m, 1H), 4.45 (m, 1H), 4.25 (m, 1H), 4.1 (s, 2H), 4.0 (m, 2H), 3.2−3.0 (multiple signals, 2H), 2.75−2.6 (multiple signals, 2H), 1.95 (m, 1H), 1.85−1.7 (multiple signals, 4H), 1.65 (m, 1H). ^{13}C NMR (DMSO-d_6): δ 164.4, 158.9, 156.5, 153.9, 131.6, 129.9, 124.0, 114.5, 111.9, 108.5, 102.9, 84.4, 82.8, 71.5, 67.1, 50.0, 49.5, 26.7, 26.5, 24.7, 22.7. mp 177 °C. LC−MS: m/z 360.1 $[M + H]^+$ (calcd 359.44 for $C_{21}H_{26}NO_3F$). UPLC−HRMS $[M + H]^+$: calcd 360.1975; found, 360.1970 (1.4 ppm). UPLC: 95% purity. Anal. ($C_{21}H_{26}NO_3F$ + $C_2H_2O_4$ fw 449.5)) C, H, N: Found 60.40, 6.00, 2.76; Calcd 61.47, 6.28, 3.12.

(*R*)-2-[(2-(4-Fluorobutoxy)benzylamino)methyl]chroman-7-ol (12b). *Free Amine.* Compound **9f** (227 mg, 0.563 mmol) was dissolved in a mixture of 4 M HCl dioxane (2.4 mL) and isopropanol (2.4 mL). After 1 h, an extra amount of 4 M HCl in dioxane (1.5 mL) was added. After workup, an oily product was obtained (202 mg, 100%). ^{1}H NMR ($CDCl_3$): δ 7.25 (multiple signals, 2H), 6.95 (m, 1H), 6.90 (m, 1H), 6.75 (m, 1H), 6.40 (m, 1H), 6.27 (m, 1H), 4.45 (m, 1H), 4.36 (m, 1H), 4.1−4.0 (multiple signals, 4H), 3.80 (d, 1H), 2.85−2.5 (multiple signals, 4H), 2.0−1.75 (multiple signals, 5H), 1.65 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 157.0, 156.5, 154.6, 130.3, 129.9, 128.8, 126.5, 120.6, 112.4, 111.1, 109.1, 103.3, 84.5, 82.8, 74.4, 67.2, 53.2, 49.1, 27.4, 27.2, 26.3, 25.4, 25.3, 23.7. LC−MS: m/z 360.3 $[M + H]^+$ (calcd 359.44 for $C_{21}H_{26}NO_3F$).

Oxalate Salt. Free amine **12b** (202 mg, 0.563 mmol) was dissolved in THF (2 mL), and oxalic acid dihydrate (156 mg, 1.24 mmol, 2.2 equiv) in THF (1 mL) was added. In this case, no precipitate was formed after addition or after 3 h of stirring at rt, so diethylether was added to induce precipitation. Centrifugation, washing, and drying gave a white solid residue (159 mg, 63%). ^{1}H NMR (DMSO-d_6): δ 8.0−6.0 (broad signal acidic protons), 7.45 (m, 1H), 7.4 (m, 1H), 7.05 (m, 1H), 7.0 (m, 1H), 6.85 (m, 1H), 6.28 (m, 1H), 6.23 (s, 1H), 4.5 (m, 1H), 4.4 (m, 1H), 4.3 (m, 1H), 4.2 (q, 2H), 4.1 (m, 2H), 3.2−3.1 (multiple signals, 2H), 2.75−2.6 (multiple signals, 2H), 1.95 (m, 1H), 1.85−1.7 (multiple signals, 4H), 1.65 (m, 1H). ^{13}C NMR (DMSO-d_6): δ 164.2, 156.9, 156.5, 153.9, 131.3, 130.6, 129.9, 125.1, 120.4, 111.9, 111.8, 108.5, 102.9, 84.4, 82.8, 71.5, 67.4, 50.2, 45.6, 26.7, 26.5, 24.7, 22.7. mp 54 °C. LC−MS: m/z 360.3 $[M + H]^+$ (calcd 359.44 for $C_{21}H_{26}NO_3F$). UPLC−HRMS $[M + H]^+$: calcd 360.1975; found, 360.1978 (0.8 ppm). UPLC: 99% purity. Anal. ($C_{21}H_{26}NO_3F$ + $C_2H_2O_4$ (fw 449.5)) C, H, N: Found 61.27, 6.10, 2.76; Calcd 61.47, 6.28, 3.12.

(*R*)-1-(4-(2-Fluoroethoxy)phenyl)-4-((7-hydroxychroman-2-yl)-methyl)-piperazine (12c). *Free Amine.* Compound **9g** (120 mg, 0.279 mmol) was dissolved in a solution of 4 M HCl in dioxane (2.5 mL) and isopropanol (4 mL). A slightly colored solid was obtained. ^{1}H NMR (THF-d_8): δ 7.75 (bs, 1H), 6.8−6.6 (multiple signals, 5H), 6.08 (m, 1H), 6.03 (m, 1H), 4.58 (m, 1H), 4.45 (m, 1H), 4.1−3.9 (multiple signals, 3H), 2.95 (bs, 4H), 2.7−2.4 (multiple signals, 8H), 1.98 (m, 1H), 1.6 (m, 1H). ^{13}C NMR (THF-d_8): δ 157.7, 156.5, 153.4, 147.2, 130.2, 118.3, 115.7, 113.2, 108.5, 103.8, 83.5, 81.9, 74.6, 68.5, 68.3, 63.1, 55.0, 51.1, 27.1, 24.5. LC−MS: m/z 387.3 $[M + H]^+$ (calcd 386.47 for $C_{22}H_{27}N_2O_3F$).

dx.doi.org/10.1021/jm401384w | *J. Med. Chem.* 2014, 57, 391−410

Oxalate Salt. Free amine **12c** (108 mg, 0.279 mmol) was dissolved in THF (4 mL), and oxalic acid dihydrate (155 mg, 1.23 mmol, ca. 4.5 equiv) in THF (1 mL) was added. A white solid residue was obtained (119 mg, 90%). ^{1}H NMR (DMSO-d_6): δ 8.0−6.0 (broad signal acidic protons), 6.95−6.8 (multiple signals, 5H), 6.28 (m, 1H), 6.2 (s, 1H), 4.75 (m, 1H), 4.65 (m, 1H), 4.35 (m, 1H), 4.15 (m, 1H), 4.1 (m, 1H), 3.25−3.0 (multiple signals, 10H), 2.75−2.6 (multiple signals, 2H), 1.98 (m, 1H), 1.6 (m, 1H). ^{13}C NMR (DMSO-d_6): δ 163.0, 156.5, 154.2, 152.1, 144.8, 129.9, 117.6, 115.1, 112.1, 108.3, 102.9, 83.1, 81.4, 71.5, 67.4, 67.2, 60.2, 52.5, 47.8, 25.4, 22.8. mp 105 °C. LC−MS: m/z 387.3 $[M + H]^+$ (calcd 386.47 for $C_{22}H_{27}N_2O_3F$). UPLC−HRMS $[M + H]^+$: calcd 387.2084; found, 387.2078 (1.5 ppm). UPLC: 95% purity. Anal. ($C_{22}H_{27}N_2O_3F + C_2H_2O_4$ (fw 476.5)) C, H, N: Found 60.33, 5.73, 5.54; Calcd 60.50, 6.14, 5.88.

(*R*)-1-(4-(2-Fluoroethoxy)phenyl)-4-(4-(7-hydroxychroman-2-yl)-3-azabutyl)-piperazine (12d). *Free Amine.* Compound **9h** (200 mg, 0.422 mmol) was dissolved in a 4 M HCl solution in dioxane (2 mL) and isopropanol (2 mL). The reaction was not complete after 3 h, so an extra portion of 4 M HCl solution in dioxane was added (2 mL), and the mixture was stirred overnight at rt under N_2. A slightly colored oil was obtained after workup. Yield: 160 mg (88%). ^{1}H NMR ($CDCl_3$): δ 6.85 (m, 4H), 6.8 (m, 1H), 6.5 (m, 1H), 6.3 (m, 1H), 4.8 (m, 1H), 4.7 (m, 1H), 4.2 (m, 1H), 4.15 (multiple signals, 2H), 3.1−2.4 (multiple signals, 16H), 1.8 (m, 1H), 1.65 (m, 1H). LC−MS: m/z 430.3 $[M + H]^+$ (calcd 429.54 for $C_{24}H_{32}N_3O_3F$).

Oxalate Salt. Free amine **12d** (160 mg, 0.373 mmol) was dissolved in THF (4 mL), and oxalic acid dihydrate (300 mg, 2.38 mmol, ca. 6 equiv) in THF (1 mL) was added. The isolated residue was a white solid (225 mg, 86%). ^{1}H NMR (DMSO-d_6): δ 8.0−6.0 (broad signal acidic protons), 6.9−6.8 (multiple signals, 5H), 6.28 (m, 1H), 6.24 (s, 1H), 4.75 (m, 1H), 4.64 (m, 1H), 4.30 (m, 1H), 4.20 (m, 1H), 4.12 (m, 1H), 3.3−3.1 (multiple signals, 4H), 3.1 (m, 4H), 2.8−2.5 (multiple signals, 8H), 1.95 (m, 1H), 1.65 (m, 1H). ^{13}C NMR (DMSO-d_6): δ 162.4, 156.5, 153.8, 151.8, 145.4, 130.0, 117.3, 115.1, 111.9, 108.6, 102.9, 83.1, 81.5, 71.2, 67.5, 67.3, 52.9, 52.4, 50.3, 49.0, 43.6, 24.7, 22.7. mp 198 °C (dec). LC−MS: m/z 430.3 $[M + H]^+$ (calcd 429.54 for $C_{24}H_{32}N_3O_3F$). UPLC−HRMS $[M + H]^+$: calcd 430.2506; found, 430.2499 (1.6 ppm). UPLC: 99% purity. Anal. ($C_{24}H_{32}N_3O_3F + 3\ C_2H_2O_4$ (fw 699.6)) C, H, N: Found 52.61, 5.40, 5.46; Calcd 51.51, 5.48, 6.01.

Synthesis of the Precursors to [^{18}F]12a and [^{18}F]12d: Phenols 13a and 13b. *Precursor to [^{18}F]**12a**: (R)-N-[7-(Methoxymethoxy)chroman-2-yl]methyl 4-Hydroxybenzyl Amine (**13a**).* Primary amine **8** (146 mg, 0.65 mmol), commercially available 4-hydroxybenzaldehyde (80 mg, 0.65 mmol), and acetic acid (43 mg, 0.72 mmol, 1.1 equiv) were stirred in CH_2Cl_2 (4 mL) for 2.5 h at rt. According to ^{1}H NMR, the reaction was complete, so sodium triacetoxyborohydride (305 mg, 1.44 mmol, 2.2 equiv) was added, and the reaction mixture was left to stir for 18 h at rt. Monitoring with LC−MS showed that conversion to the desired product was ca. 80%. The mixture was brought to a pH of 7 with a 0.1 M NaOH aqueous solution and was then extracted with CH_2Cl_2. Drying of the organic layer with Na_2SO_4, evaporation of the solvent, and purification with silica column chromatography (hexane/ethyl acetate 1:1) gave the product in a yield of 76% (164 mg). ^{1}H NMR ($CDCl_3$): δ 7.12 (m, 2H), 6.9 (m, 1H), 6.65 (m, 2H), 6.55 (multiple signals, 2H), 5.1 (s, 2H), 5.05 (bs, 2H), 4.2 (m, 1H), 3.75 (m, 2H), 3.4 (s, 3H), 2.9−2.6 (multiple signals, 4H), 1.9 (m, 1H), 1.7 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 156.4, 156.0, 155.0, 130.0, 129.9, 129.8, 115.8, 115.5, 109.0, 104.3, 94.5, 74.5, 55.9, 53.3, 53.2, 25.7, 23.9. LC−MS: m/z 330.1 $[M + H]^+$ (calcd 329.40 for $C_{19}H_{23}NO_4$).

*Precursor to [^{18}F]**12d**: Multistep Synthesis to Phenol **13b**.* Compounds **15** and **16** were prepared according to a patent procedure.[77]

4-(4-Benzyloxy-phenyl)-piperazine-1-carboxylic Acid *tert*-Butyl Ester (15). 4-(4-Hydroxyphenyl)-piperazin-1-carboxylic acid tert butyl ester (**14**) was prepared according to Tietze.[73] Compound **14** (2 g, 7.19 mmol), benzyl bromide (1.23 g, 7.19 mmol), and K_2CO_3 (0.99 g, 7.19 mmol) were mixed in DMF (2 mL). The reaction mixture was stirred at 50 °C under argon for 16 h and was then poured in water. The suspension was filtrated, and the residue was purified with silica column chromatography (2% MeOH/$CHCl_3$). Yield: 1.94 g (73%). Analytical data were in accordance with those reported in a patent.[77] ^{1}H NMR ($CDCl_3$): δ 7.5−7.3 (multiple signals, 5H), 6.9 (multiple signals, 4H), 5.02 (s, 2H), 3.56 (t, 4H), 3.0 (t, 4H), 1.48 (s, 9H). LC−MS: m/z 369.2 $[M + H]^+$ (calcd 368.48 for $C_{22}H_{28}N_2O_3$).

1-(4-Benzyloxyphenyl)piperazine (16). Compound **15** (1.75 g, 4.75 mmol) was mixed with a 4 N HCl dioxane solution (30 mL) and was stirred for 1 h at rt. The reaction mixture was filtrated and washed with ethyl acetate to afford the HCl salt, a white solid residue. Extraction using chloroform and a basic aqueous solution gave the amine product. Yield: 1.3 g (100%). Analytical data were in accordance with those reported in a patent.[77] ^{1}H NMR ($CDCl_3$): δ 7.5−7.3 (multiple signals, 5H), 6.9 (multiple signals, 4H), 5.0 (s, 2H), 3.0 (bs, 8H), 1.5 (bs, 1H). LC−MS: m/z 269.3 $[M + H]^+$ (calcd 268.36 for $C_{17}H_{20}N_2O$).

1-(4-Benzyloxyphenyl)-4-(2-*N*-(*tert*-butyl-carbamate)-ethyl)-piperazine (17). *tert*-Butyl *N*-(2-bromoethyl)carbamate was prepared according to a literature procedure.[78] Secondary amine **16** (0.4 g; 1.49 mmol) was mixed with DMF (5 mL), DIPEA (2.6 mL, 14.9 mmol, 10 equiv), and *tert*-butyl *N*-(2-bromoethyl)carbamate (0.33 g, 1.49 mmol), yielding a suspension. The mixture was stirred at 40 °C for 72 h and turned into a clear brown solution. Pouring into water gave a suspension that was extracted with diisopropyl ether. The organic layer was dried with Na_2SO_4 and evaporated to dryness; the crude product residue was purified using silica column chromatography (2:1 pentane/THF). Yield: 369 mg (60%). ^{1}H NMR ($CDCl_3$): δ 7.5−7.3 (multiple signals, 5H), 6.9 (multiple signals, 4H), 5.0 (s, 2H), 4.95 (bs, 1H), 3.25 (m, 2H), 3.05 (m, 4H), 2.6 (m, 4H), 2.5 (t, 2H), 1.45 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 152.9, 145.9, 137.3, 128.5, 127.8, 127.4, 125.5, 118.0, 115.5, 79.2, 70.5, 57.2, 53.0, 50.5, 37.1, 28.4. LC−MS: m/z 412.3 $[M + H]^+$ (calcd 411.55 for $C_{24}H_{33}N_3O_3$).

1-(4-Benzyloxyphenyl)-4-(2-aminoethyl)-piperazine (18). Boc-protected amine **17** (0.74 g; 1.79 mmol) was dissolved in CH_2Cl_2 (8 mL) and TFA (2 mL), and the solution was stirred for 1 h at rt. Evaporation of the solvents and redissolution into (8 mL) and TFA (2 mL) was followed by further stirring at rt until ^{1}H NMR and TLC analyses showed that the Boc group was deprotected. Dilution with $CHCl_3$ and washing of the organic layer with a 0.1 M NaOH (pH 10) to which NaCl was added to enhance the phase separation was followed by drying of the organic layer with Na_2SO_4 and evaporation of the volatiles to yield a beige solid (528 mg, 95%). ^{1}H NMR ($CDCl_3$): δ 7.5−7.3 (multiple signals, 5H), 6.9 (multiple signals, 4H), 5.0 (s, 2H), 3.15 (m, 4H), 2.8 (m, 2H), 2.6 (m, 4H), 2.5 (t, 2H), 1.65 (bs, 2H). LC−MS: m/z 312.3 $[M + H]^+$ (calcd 311.43 for $C_{19}H_{28}N_3O$).

(*R*)-1-(4-Benzyloxyphenyl)-4-(4-(7-(methoxymethoxy)-chroman-2-yl)-3-azabutyl)-piperazine (19). A solution of tosylate **6** (300 mg, 0.793 mmol) in 1:1 dioxane/DMSO (1 mL) was added to a mixture of DIPEA (1.4 mL, 7.93 mmol, 10 equiv) and amine **18** (306 mg, 0.952 mmol, 1.2 equiv) in 1:1 dioxane/DMF (1 mL). This mixture was stirred overnight at 90 °C. According to LC−MS, there was still some tosylate present, so DIPEA (0.5 mL) was added, and the reaction mixture was stirred at 90 °C for another 24 h. The mixture was then poured into water (pH became ca. 9 to 10), and the aqueous layer was extracted with $CHCl_3$. The organic layer was washed with NaCl(aq), dried with Na_2SO_4, and concentrated to give the crude product that was purified with alumina column chromatography (1:1 ethyl acetate/pentane). Yield: 156 mg (32%). ^{1}H NMR ($CDCl_3$): δ 7.45−7.3 (multiple signals, 5H), 6.9 (multiple signals, 5H), 6.55 (multiple signals, 2H), 5.1 (s, 2H), 5.0 (s, 2H), 4.15 (m, 1H), 3.4 (s, 3H), 3.1 (m, 4H), 2.9−2.7 (multiple signals, 6H), 2.6 (multiple signals, 6H), 1.95 (multiple signals, 2H), 1.75 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 156.5, 155.2, 152.9, 146.0, 137.4, 129.9, 128.5, 127.8, 127.4, 118.0, 115.5, 114.9, 108.9, 104.4, 94.5, 75.1, 70.5, 57.8, 55.9, 54.3, 53.4, 50.6, 46.5, 25.8, 24.0. LC−MS: m/z 540.4 $[M + Na]^+$, 518.5 $[M + H]^+$ and 259.8 $[M+2H]^{2+}$ (calcd 517.67 for $C_{31}H_{39}N_3O_4$).

(*R*)-1-(4-Hydroxyphenyl)-4-(4-(7-(methoxymethoxy)-chroman-2-yl)-3-azabutyl)-piperazine (13b). Benzylated molecule **19** (156 mg, 0.30 mmol) was dissolved in EtOH, and Pd/C

dx.doi.org/10.1021/jm401384w | J. Med. Chem. 2014, 57, 391−410

catalyst (Degussa type) was added. The mixture was shaken under hydrogen at 70 psi, and the conversion was monitored by ^{1}H NMR. Filtration over two paper filters and one using a syringe filter (45 μm regenerated cellulose) gave a yield of 121 mg product (94%). ^{1}H NMR ($CDCl_3$): δ 6.9 (m, 1H), 6.8 (m, 2H), 6.75 (m, 2H), 6.5 (multiple signals, 2H),5.1 (s, 2H), 4.2 (m, 1H), 3.4 (s, 3H), 3.1 (m, 4H), 2.9−2.7 (multiple signals, 6H), 2.6 (multiple signals, 6H), 1.95 (m, 1H), 1.75 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 156.5, 155.1, 150.3, 145.4, 130.0, 118.5, 116.0, 115.5, 109.0, 104.4, 94.5, 74.7, 57.4, 55.9, 54.1, 53.3, 50.8, 46.1, 25.8, 24.0. LC−MS: m/z 428.4 $[M + H]^+$ and 214.7 $[M + 2H]^{2+}$ (calcd 427.55 for $C_{24}H_{33}N_3O_4$).

Preparation of Amine and Aldehyde Building Blocks for Coupling to Tosylate 3 (Route A), Tosylate 6 (Route B), or Amine 8 (Route C). *tert*-Butyl (4-(4-Fluorobutoxy)-benzyl)-carbamate (20). *tert*-Butyl (4-hydroxybenzyl)-carbamate, as prepared[79] from the HBr salt of 4-hydroxy-benzylamine (3.17 g, 14.2 mmol), was mixed with commercially available 1-bromo-4-fluorobutane (2.62 g, 17 mmol, 1.2 equiv) and K_2CO_3 (2.36 g, 17 mmol, 1.2 equiv) in MeCN (75 mL). The reaction mixture was stirred overnight at 80 °C under an inert argon atmosphere, cooled to rt, and filtered to remove salts. The filtrate was concentrated, and the crude product was purified with silica column chromatography (2% MeOH/$CHCl_3$). Drying under vacuum removed traces of 1-bromo-4-fluorobutane. Yield: 2.9 g (68%). ^{1}H NMR ($CDCl_3$): δ 7.2 (m, 2H), 6.85 (m, 2H), 4.8 (bs, 1H), 4.55 (m, 1H), 4.45 (t, 1H), 4.2 (m, 2H), 3.95 (t, 2H), 1.95−1.8 (multiple signals, 4H), 1.45 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 158.2, 155.8, 131.0, 128.8, 114.5, 84.5, 82.9, 79.1, 67.2, 44.1, 28.3, 27.2, 27.0, 25.2, 25.1. LC−MS: m/z 242.0 $[M - C_4H_8]^+$ (calcd 297.37 for $C_{16}H_{24}NO_3F$).

4-(4-Fluorobutoxy)-benzyl Amine (21). Boc-protected amine **20** (0.56 g, 1.87 mmol) was stirred at rt for 10 min in 4 M HCl in dioxane (0.6 mL). The reaction mixture developed into a hazy suspension. The mixture was evaporated to dryness, and the residue was dissolved in water that was adjusted to a basic pH using a NaOH solution. The product was extracted using CH_2Cl_2. The organic layer was dried using Na_2SO_4 and evaporated to yield the desired amine product. Yield: 261 mg (71%). ^{1}H NMR ($CDCl_3$): δ 7.2 (m, 2H), 6.85 (m, 2H), 4.55 (m, 1H), 4.45 (t, 1H), 4.0 (t, 2H), 3.8 (s, 2H), 1.9 (multiple signals, 4H), 1.4 (bs, 2H). ^{13}C NMR ($CDCl_3$): δ 157.8, 135.6, 128.2, 114.5, 84.5, 82.9, 67.2, 45.9, 27.3, 27.1, 25.3, 25.2. LC−MS: m/z 198.1 $[M + H]^+$, 181.3 $[M - NH_2]^+$ (calcd 197.25 for $C_{11}H_{16}NOF$).

2-(4-Fluorobutoxy)-benzaldehyde (22). 2-Hydroxybenzalhyde (1 g, 8.18 mmol) and 1-bromo-4-fluorobutane (1.52 g, 9.8 mmol, 1.2 equiv), both commercially available, were mixed with K_2CO_3 (2.26 g, 16.3 mmol, 2 equiv) in DMF (40 mL). The suspension was stirred overnight under argon at an oil bath temperature of 50 °C. The next day, the reaction mixture was filtrated and diluted with water so that the product could be extracted from the water layer using diethyl ether (twice). The ether layer was washed twice with water and once with a saturated NaCl solution and was then dried with Na_2SO_4. Starting bromide was still present in the product, but it could be removed by coevaporation with diisopropylether. Yield: 1.43 g (89%). ^{1}H NMR ($CDCl_3$): δ 10.5 (s, 1H), 7.8, (m, 1H), 7.55 (m, 1H), 7.0 (m, 2H), 4.60 (m, 1H), 4.46 (m, 1H), 4.13 (t, 2H), 2.05−1.8 (multiple signals, 4H). ^{13}C NMR ($CDCl_3$): δ 189.6, 161.2, 135.9, 128.3, 124.8, 120.6, 112.4, 84.3, 82.7, 67.8, 27.2, 27.0, 25.2, 25.1. GC-MS: m/z 121 $[M - C_4H_8F]^+$ (calcd 196.22 for $C_{11}H_{13}O_2F$).

1-(2-*N*-(*tert*-Butylcarbamate)ethyl)-4-(4-iodophenyl)-piperazine (23). 1-(4-Iodophenyl)-piperazine[72] (4.6 g, 15.1 mmol), *tert*-butyl *N*-(2-bromoethyl)carbamate[78] 3.7 g, 16.6 mmol, 1.1 equiv), and DIPEA (5.3 mL, 30.4 mmol, 2 equiv) were dissolved in MeCN (30 mL). The mixture was stirred overnight at 50 °C and kept under argon. The reaction mixture was evaporated to dryness and was redissolved in $CHCl_3$. The solution was washed two times with water and once with a NaCl solution and was then dried over Na_2SO_4. After concentration, the crude product was purified with silica column chromatography (1% MeOH/$CHCl_3$). Yield 80%. ^{1}H NMR ($CDCl_3$): δ 7.50 (m, 2H), 6.65 (m, 2H), 4.95 (bs, 1H), 3.25 (m, 2H), 3.15 (m, 4H), 2.6 (m, 4H), 2.5 (t, 2H), 1.45 (s, 9H). LC−MS: m/z 432.1 $[M + H]^+$ (calcd 431.32 for $C_{17}H_{26}N_3O_2I$).

1-(2-Aminoethyl)-4-(4-iodophenyl)-piperazine (24). Boc-protected amine **23** (1.58 g, 3.65 mmol) was added slowly to a concentrated 10 M HCl solution in water (2 mL). The reaction was stirred for 15 min at rt, diluted with water (50 mL), and adjusted to a pH of 9 to 10 using a NaOH solution in water. The product was isolated by extraction of the water layer with ethyl acetate (twice), drying of the organic layer over Na_2SO_4, and evaporation of the volatiles. Yield: 0.78 g (65%). ^{1}H NMR ($CDCl_3$): δ 7.45 (m, 2H), 6.75 (m, 2H), 3.1 (m, 4H), 3.0 (bs, 2H), 2.65 (t, 2H), 2.5 (m, 4H), 2.35 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 150.9, 137.7, 118.0, 81.2, 61.1, 53.0, 48.7, 38.8. LC−MS: m/z 332.1 $[M + H]^+$ (calcd 331.20 for $C_{12}H_{18}N_3I$).

1-(2-*N*-(*tert*-Butylcarbamate)ethyl)-4-(4-(4-fluoroethoxy)-phenyl)-piperazine (25). 1-(4-(2-Fluoroethoxy)-phenyl)-piperazine[73] (1.88 g, 8.4 mmol), *tert*-butyl *N*-(2-bromoethyl)carbamate[78] (2.07 g, 9.24 mmol, 1.1 equiv), and DIPEA (mL) were mixed in MeCN (20 mL). The mixture was stirred overnight under argon at an oil bath temperature of 50 °C. The reaction mixture was concentrated and redissolved in CH_2Cl_2 to enable washing with water (twice) and with a saturated NaCl solution (once). The organic layer was dried with Na_2SO_4 and concentrated to afford crude product that was purified using silica column chromatography (elution gradient starting with $CHCl_3$ and finally using 3% MeOH/$CHCl_3$). After evaporation of the solvent, a yellowish solid was obtained. Yield: 2.42 g (78%). ^{1}H NMR ($CDCl_3$): δ 6.85 (multiple signals, 4H), 4.95 (bs, 1H), 4.75 (m, 1H), 4.65 (m, 1H), 4.2 (m, 1H), 4.15 (m, 1H), 3.25 (m, 2H), 3.05 (m, 4H), 2.6 (m, 4H), 2.5 (t, 2H), 1.45 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 155.9, 152.5, 146.2, 118.0, 115.5, 82.9, 81.2, 79.0, 67.8, 67.6, 57.2, 53.0, 50.4, 37.1, 28.4.

1-(2-Aminoethyl)-4-(4-(4-fluoroethoxy)phenyl)-piperazine (26). Boc-protected amine **25** (2.42 g, 6.58 mmol) was stirred in concentrated 10 M HCl solution in water (20 mL). A thick white foam formed, and after 20 min, water (75 mL) was added as well as solid NaOH to adjust the pH to about 10. The product was extracted from the water layer using CH_2Cl_2 (twice). Drying of the collected organic layers (Na_2SO_4) and evaporation of the solvent gave a white solid. Yield: 1.47 g (84%). ^{1}H NMR ($CDCl_3$): δ 6.85 (multiple signals, 4H), 4.75 (m, 1H), 4.65 (m, 1H), 4.2 (m, 1H), 4.15 (m, 1H), 3.05 (m, 4H), 2.8 (t, 2H), 2.6 (m, 4H), 2.45 (t, 2H), 1.4 (bs, 2H). ^{13}C NMR ($CDCl_3$): δ 152.4, 146.2, 117.9, 115.4, 82.8, 81.1, 67.7, 67.5, 61.1, 53.3, 50.4, 38.7. LC−MS: m/z 268.3 $[M + H]^+$ (calcd 267.35 for $C_{14}H_{22}N_3OF$).

Preparation of Radiochemistry [^{18}F]Fluoroalkylation Precursors and References Compounds: Tosylates 27−30. 1,4-Butanediol Ditosylate (27). *p*-Toluenesulfonyl chloride (3.17 g, 16.6 mmol) was dissolved in 10 mL of dry CH_2Cl_2. To this solution was added 3.0 mL (2.29 g, 22.7 mmol) of dry triethylamine. The mixture was cooled to 0 °C in an ice bath, and 610 μL (616 mg, 7.6 mmol) of dry 1,4-butanediol was added. After 30 min of stirring, another 60 μL of 1,4-butanediol was added. The mixture was left to stir overnight, with the reaction mixture temperature coming to rt. On the next day, the mixture was diluted with an extra portion of CH_2Cl_2 (15 mL), cooled to 0 °C, and acidified by slowly adding 50 mL of 0.8 M HCl. The organic layer was separated and washed with 0.3 M HCl (3 × 50 mL). The CH_2Cl_2 solution of the crude product was then concentrated in vacuo, and the product was purified on a silica column eluted with hexane/CH_2Cl_2/ethyl acetate (6:6:1) to yield 1.93 g of final product as white crystals. Yield: 58%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.76 (d, J = 8.4 Hz, 4H), 7.35 (d, J = 8.4 Hz, 4H), 3.95−4.03 (m, 4H), 2.45 (s, 6H), 1.65−1.74 (m, 4H).

1,2-Ethanediol Ditosylate (28). This compound was prepared and isolated in as similar fashion as its butane-spaced counterpart, **27**. Analytical data were according to expectations.

4-Fluorobutyl Tosylate (29). Tetrabutylammonium fluoride trihydrate (66 mg, 0.25 mmol) was dried by coevaporation in vacuo with three 3 mL portions of acetonitrile (the temperature in the flask was not allowed to rise above 30 °C to prevent decomposition of the tetrabutyl ammonium fluoride). The dried tetrabutyl ammonium

dx.doi.org/10.1021/jm401384w | J. Med. Chem. 2014, 57, 391−410

fluoride was redissolved in a small quantity of dry acetonitrile, and this solution was added to a solution of 1,4-butanediol ditosylate (100 mg, 0.25 mmol). The reaction was conducted in refluxing acetonitrile for 24 h, and the conversion was monitored by TLC on silica plates (hexane/ethyl acetate 2:1, R_f = 0.75). After the reaction ended, acetonitrile was evaporated in vacuo, the residue was redissolved in hexane/ethyl acetate (2:1 v/v), and the product was purified on a silica column eluted with hexane/ethyl acetate (2:1 v/v) to yield 30 mg of final product as brownish crystals. Yield: 48%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.79 (d, J = 8.1 Hz, 2H), 7.35 (d, J = 8.1 Hz, 2H), 4.44 (dt, J_1 = 47.0 Hz, J_2 = 5.6 Hz, 2H), 4.08 (t, J = 4.08 Hz, 2H), 2.46 (s, 3H), 1.67−1.84 (m, 4H).

2-Fluoroethyl Tosylate (30). 2-Fluoroethyl tosylate was synthesized according to the same protocol as 1,4-butanediol ditosylate. Instead of 1,4-butanediol, 2-fluoroethanol (240 μL, 268 mg, 4.2 mmol) was reacted with *p*-toluenesulfonyl chloride (755 mg, 4.0 mmol). Yield: 760 mg of final product was obtained (86%) as a colorless slightly yellowish oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.82 (d, J = 7.6 Hz, 2H), 7.37 (d, J = 6.8 Hz, 2H), 4.58 (dt, J_1 = 47.0 Hz, J_2 = 4.2 Hz, 2H), 4.27 (dt, J_1 = 27.6 Hz, J_2 = 4.0 Hz, 2H), 2.48 (s, 3H).

Cell Culture and Preparation of Membranes. Human embryonic kidney 293 cells (HEK293) stably expressing the human dopamine receptor subtypes were cultured in RPMI 1640 medium with 2 mM L-glutamine in the presence of 100 U/mL of penicillin and 100 U/mL of streptomycin. To maintain selection pressure, 50 μg/mL of neomycin and 5 μg/mL of puromycin were also added to the medium of cells expressing dopamine D_2L, D_2S, D_3, and D_4 receptors and D_1 and D_5 receptors, respectively. Cells were harvested by scraping the culture dishes, washed by centrifugation at 200*g*, and lysed in ice-cold buffer containing 5 mM Tris and 5 mM $MgCl_2$ at pH 7.4. The suspension was homogenized with 8 strokes in a glass/Teflon homogenizer and centrifuged twice for 1 h at 30 000*g* at 4 °C. Pellets were resuspended in buffer (50 mM Tris, pH 7.4) and rehomogenized with 4 strokes in the glass/Teflon homogenizer, and aliquots were frozen in liquid nitrogen and stored at −80 °C. Protein content was measured using the method of Bradford.[80] For experiments on dopamine D_2S and D_3 receptors, membrane preparations were obtained from PerkinElmer (Waltham, MA, USA).

Receptor Binding Assays. For the radioligand experiments, the samples were thawed and rehomogenized in Mg-containing buffer (50 mM Tris, 5 mM KCl, 2 mM $CaCl_2$, and 2 mM $MgCl_2$ pH 7.4) to allow formation of the agonist high-affinity state. Rehomogenization was done with a short burst (10 s) of an ultra turrax at full speed. Binding experiments were performed with 40−60 μg of protein per assay, an assay volume of 1000 μL, and a radioligand concentration of 0.15 nM for the [^{3}H]spiperone (D_2L, D_2S, D_3, and D_4 receptors) assays and 1.5 nM for [^{3}H]SCH23390 (D_1 and D_5 receptor) assays, with an incubation time of 2 h at 25 °C and a preincubation of 15 min at 25 °C. Nonspecific binding was defined using 1 μM (+)-butaclamol (antagonist for dopamine receptors). To avoid oxidation of the compounds, 0.05% ascorbic acid was added to all solutions. All experiments were performed in duplicate in a 96-well platform, and reactions were terminated by rapid vacuum filtration over Whatman GF/C filter plate with ice-cold buffer (50 mM Tris). For the D_3 receptor binding experiments, plates were precoated with 0.1% PEI. Filter radioactivity was measured in a PerkinElmer Topcount with Microscint-O cocktail as scintillant. The competition experiments were analyzed using the Prism program (version 5.01, GraphPad Software, San Diego, CA, USA) by fitting sigmoidal curves to the data. Resulting IC_{50} values were converted to K_i values on the basis of the Cheng−Prusoff equation.[38]

cAMP Accumulation. To determine the concentrations of cAMP formed in the assay, the LANCE cAMP 384 kit from PerkinElmer was used according to the manufacturer's protocol. Transfected HEK293 cells stably expressing the dopamine D_2L were cultured as mentioned earlier. They were detached from the surface of the culture dish with 2 mL of dissociation solution, washed with HBSS, and resuspended in a standard buffer (SB) containing HBSS, 0.05% BSA, and 5 mM HEPES. The stimulation solutions contained SB with 1.0 mM 3-isobutyl-1-methylxanthine (IBMX), 63.2 μM forskolin, and different concentrations of the dopamine agonists. After the stimulation solutions were added to the well plate, the cell suspensions (diluted to 6000 cells/well) were added in a 1:1 ratio so that the final concentrations of IBMX and forskolin in the assay were 0.5 mM and 31.6 μM, respectively. The plate was incubated for 30 min at room temperature for the stimulation, and subsequently different detection reagents and antibody solutions were added to a total volume of 20 μL/well. Measurements were carried out on a Victor 2 plate reader (Wallac, PerkinElmer, Zaventum, Belgium) 3 h after adding the detection mixtures.

Lipophilicity. Lipophilicity (cLogP and cLogD) of the unlabeled compounds was calculated using the BiologP (New cLogP) calculator program from the University of Massachusetts. LogD of the [^{18}F]-labeled compounds was determined using radioactivity remaining on the HLB cartridge after its elution with ethanol during the formulation procedure (vide infra). The cartridge was dried for 5 min in strong N_2 flow and eluted with at least 1.5 mL of *n*-octanol. Then, 500 μL of octanol eluate was mixed with 500 μL of 1 M phosphate buffer, pH 7.4, and vigorously vortexed for 5 min at rt. After that, the mixture was centrifuged at 17 000*g* for 10 min and three 100 μL aliquots of organic and aqueous phase were loaded into a gamma-counter for radioactivity measurement. To correct for the possible presence of radioactive impurities in the tracer, aliquots of organic and aqueous phases were later analyzed by radio-TLC, and the counts obtained from the gamma-counter were corrected according to the percentage of impurities found in aqueous and organic phases. LogD was calculated with the formula

$$\mathrm{LogD} = \lg\left(\frac{\mathrm{CPM}_{org} X_{org}^{tracer}}{\mathrm{CPM}_{aq} X_{aq}^{tracer}}\right)$$

where CPM_{org} is the mean cpm in the organic aliquots, X_{org}^{tracer} is the mean percent activity accounting for intact tracer in organic aliquots (as determined from radio-TLC), and CPM_{org} and X_{org}^{tracer} are the corresponding values for the aqueous phase.

For each tracer batch used for LogD determination, the experiment was conducted in duplicate. LogD values are the mean calculated from at least two independent experiments.

Materials (in Vitro Experiments). The transfected dopamine receptor cell lines were a generous gift of Prof. Gross (Abbott). Human D_2S and D_3 membranes were obtained from PerkinElmer (Waltham, MA, USA). [^{3}H]Spiperone (specific activity 16.2 Ci/mmol) and [^{3}H]SCH23390 (specific activity 73.1 Ci/mmol) were obtained from PerkinElmer. (+)-Butaclamol, (*R*)-(−)-propylnorapomorphine HCl, and dopamine HCl were obtained from Sigma-Aldrich (Zwijndrecht, The Netherlands). (+)-PHNO HCl was obtained from Axon Medchem (Groningen, The Netherlands).

Radiochemistry. *Preparation of [^{18}F]**12a**.* ^{18}F-fluoride in enriched $H_2{}^{18}O$ water was adsorbed on an anion-exchange column (QMA) and later eluted into a reaction vial with 1−1.5 mL of 1.5 mg/mL of K_2CO_3 and 5 mg/mL of Kryptofix solution in 9:1 v/v mixture of acetonitrile and water. After the elution of QMA, acetonitrile and water were evaporated at 130 °C under nitrogen flow. For further water removal, 1 mL of anhydrous acetonitrile was added into the reaction vial and evaporated. Then, 5 mg of 1,4-butanediol ditosylate (**27**) in 1 mL of anhydrous acetonitrile was added into the vial, the vial was sealed, and the reaction was carried out for 4 min at 130 °C followed by cooling for 5 min . One milliliter of water was added to the cooled reaction mixture, and the resulting mixture was loaded into the HPLC loop. 4-[^{18}F]fluorobutyltosylate was purified by reverse-phase HPLC using Alltima C18 5u 250 × 10 mm column eluted with acetonitrile/10 mM H_3PO_4 (65:35 v/v) at 5 mL/min (t_R = 7.0 min, k' = 3.9, as confirmed using the reference compound 4-fluorobutyltosylate **29**). The collected radioactive fraction was diluted 2- to 3-fold with water, and 4-[^{18}F]fluorobutyltosylate was adsorbed on a tC18 light Seppak cartridge. The cartridge was rinsed with 5 mL of pure water and dried for 5 min in strong nitrogen flow. Afterward, 4-[^{18}F]fluorobutyltosylate was eluted from the cartridge with 0.7 mL of dry DMF into a v-vial containing 0.6 mg of precursor **13a**, 30 μL of dry DMF, and 2 to 3 mg of NaH. The reaction vial was sealed, and the

dx.doi.org/10.1021/jm401384w | J. Med. Chem. 2014, 57, 391−410

alkylation reaction was conducted for 10 min at 120 °C; then, 1 mL of 0.4 M HCl was added into the vial with a syringe. The MOM group was then deprotected, which lasted 10 min at 120 °C. [^{18}F]**12a** was purified by reverse-phase HPLC using Alltima C18 5u 250 × 10 mm column eluted with acetonitrile/10 mM H_3PO_4 (40:60 v/v) at 5 mL/min (t_R = 9.8 min, k' = 5.4; Scheme 3).

The collected HPLC fraction was diluted with 2 to 3 volumes of water, and [^{18}F]**12a** was adsorbed on a Oasis HLB SPE cartridge. The cartridge was rinsed with 5 mL of pure water, and [^{18}F]**12a** was then eluted with 1 to 2 mL of absolute ethanol (typically 20−40% recovery). After the partial or complete evaporation of ethanol in nitrogen flow (with or without application of vacuum), the tracer was formulated in physiological saline (ethanol content in the formulated tracer was kept below 10%). The identity of [^{18}F]**12a** was confirmed by coelution with the unlabeled **12a** on reverse-phase HPLC and radio-TLC. From the same data, radiochemical purity was assessed. Reverse-phase HPLC was carried out on Platinum EPS 5u 250 × 4.6 column eluted with acetonitrile/physiological saline/formic acid (70:30:0.1 v/v) at 2 mL/min (t_R = 6.4 min, k' = 4.5). For quality control, radio-TLC was carried out on silica plates eluted with dichloromethane/methanol/ammonia (100:5:1, R_f = 0.25) and ethyl acetate/methanol/triethylamine (100:5:1, R_f = 0.5).

*Preparation of [^{18}F]**12d**.* The procedure described above for [^{18}F]**12a** was also used for the preparation of [^{18}F]**12d**, with the following modifications: ethylene glycol ditosylate (**28**) and precursor **13b** were used instead of 1,4-butanediol ditosylate (**27**) and precursor **13a**, respectively. 2-[^{18}F]fluoroethyl tosylate purification conditions were as follows: Alltima C18 5u 250 × 10 mm column eluted with acetonitrile/10 mM sodium acetate buffer pH 4.0 (60:40 v/v) at 5 mL/min (t_R = 6.8 min, k'= 3.8, as confirmed using the reference tosylate **30**). At the end of the deprotection step, the reaction mixture was diluted with 10 mL of water, titrated with 1 M NaOH to pH 4−7, and then put through the Oasis HLB SPE cartridge. Adsorbed activity was then eluted from the cartridge with 1 mL of an acetonitrile/DMF mixture (1:1 v/v). The eluate was further diluted with water to a total volume of 1.5−2 mL and injected onto the Alltima C18 5u 250 × 10 mm HPLC column for purification of [^{18}F]**12d** (Scheme 3).

[^{18}F]**12d** purification conditions were as follows: Alltima C18 5u 250 × 10 mm column eluted with a gradient of acetonitrile in 10 mM sodium acetate buffer: isocratic 45% acetonitrile during the first 2 min, then a linear decrease from 45% at 2 min to 30% acetonitrile at 15 min (t_R [^{18}F]**12d** = 10.6 min, k' = 5.9).

HPLC conditions for the identity confirmation and RCP assessment were as follows: platinum EPS 5u 250 × 4.6 column eluted with acetonitrile/10 mM phosphate buffer pH 7 (65:35 v/v) at 2 mL/min (t_R = 11.0 min, k' = 7.3).

Quality control TLC conditions were as follows: dichloromethane/methanol/ammonia 100:5:1 (R_f = 0.25) and ethyl acetate/methanol/ammonia/triethylamine 100:5:5:1 (R_f = 0.3). Before putting radioactive samples onto the TLC plates, drops of **12d** oxalate standard solution containing 2 μg of compound were put onto the start positions of each lane to exclude on-plate decomposition of no-carrier-added [^{18}F] compound.

Autoradiography. Frozen brains of young (10−12 weeks of age; 300−350 g body weight) male Sprague−Dawley rats (Harlan, The Netherlands) were cut into halves along the sagittal symmetry plane. Each half was mounted, lateral side up, on a paper slide pretreated by Tissue-Tek fixing cocktail (Sakura, The Netherlands) and fixed by placing it onto a petri dish floating on liquid nitrogen. After fixing, brains were cut at −12 °C into sagittal slices 20 μm thick using a Leica microtome, and the slices were thaw-mounted on Superfrost (70 × 22 mm, Fischer) adhesive slides. Only slices containing both striatal and cerebellar regions (representing nonspecific binding) were used. Slides were organized in pairs, and in each pair, new slices were mounted alternately on the first or the second slide of the pair so that slices in corresponding positions on the two slides forming a pair would represent adjacent tissue layers. The slices were allowed to dry and were then put into storage boxes with desiccator (silicagel) bags and kept at −80 °C until they were used (no longer than 1 week).

On the day of the experiment, the slides with mounted slices were taken out of storage and allowed to come to room temperature for 5−10 min. After that, incubation buffer (50 mM Tris-HCl, 5 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, and 120 mM NaCl, pH 7.4, 25 °C) was applied to the slides using an automatic pipet (1−1.2 mL/slide), and the slides were preincubated for 15 min at room temperature. The preincubation buffer was then removed, and the slides were placed into staining jars containing incubation buffer and radioligand ([^{18}F]**12a** or [^{18}F]**12d**) at a concentration of about 5 nM (4.5 ± 3.6 nM for [^{18}F]**12a** and 5.8 ± 1.6 nM for [^{18}F]**12d**, n = 4). Four conditions were tested: radioligand only, radioligand in NaCl-free buffer, radioligand and 10 μM raclopride (nonspecific binding only), and radioligand and 100 μM GTP (low-affinity state only). The slides were incubated for 35 min at 37 °C, washed once with ice-cold incubated buffer (3.5 min), and dipped for 30 s into ice-cold sterile water to remove buffer salts.

After drying the slides in a stream of room-temperature air, they were exposed on phosphor storage screens for 6−10 h. Afterward, the storage screens were read by a Cyclone Storage Phosphor System (Packard Instruments Co). Quantification of plate readings was done with Optiquant software (version 3.00, Packard Instruments Co) by drawing regions of interest on the striatum and cerebellum manually.

The animal experiments were performed by licensed investigators in compliance with the Law on Animal Experiments of The Netherlands. The protocol was approved by the Committee on Animal Ethics of the University of Groningen. The rats were maintained on a 12 h light/12 h dark regime and were fed standard laboratory chow ad libitum.

AUTHOR INFORMATION

Corresponding Author

*E-mail: j.booij@amc.uva.nl. Tel: +31 20 5662397.

Author Contributions

#These authors contributed equally to this work.

Notes

The authors declare the following competing financial interest(s): A.G.M.J. is an employee of GE Healthcare, a company marketing the DA $D_{2/3}$ receptor antagonist radiopharmaceutical [^{123}I]IBZM. M.C.M. is an employee of Boehringer Ingelheim, a company marketing the DA D_2 agonist pramipexole. J.B. is consultant at GE Healthcare. The other authors declare that they have no competing interest.

ACKNOWLEDGMENTS

J.A.J.M. (Jef) Vekemans, Eindhoven University of Technology, (TU/e), is thanked for his advice on synthetic design; J.L.J. (Joost) van Dongen and R.A.A. (Ralf) Bovee (both TU/e) are thanked for their expertise on and execution of the HRMS measurements and elemental analyses, respectively. Prof. E.W. (Bert) Meijer (TU/e and ICMS Eindhoven) is thanked for enabling the execution of the synthetic part of the presented research. Jan Keijser (Center for Behavior and Neurosciences, University of Groningen) is thanked for his advice on the design and execution of in vitro autoradiography experiments. Jan Koerts (Laboratory of Pathology and Medical Biology, UMCG) is thanked for providing access to the equipment needed for the preparation of brain slices. This work was supported by a grant from the Dutch Technology Foundation STW (grant 10127).

ABBREVIATIONS USED

AMC, 2-aminomethylchromane; DIPEA, diisopropylethylamine; UPLC, ultra-performance liquid chromatography

REFERENCES

(1) Booij, J.; Tissingh, G.; Winogrodzka, A.; van Royen, E. A. Imaging of the dopaminergic neurotransmission system using single-photon emission tomography and positron emission tomography in patients with parkinsonism. *Eur. J. Nucl. Med.* **1999**, *26*, 171−182.

(2) McKeith, I.; O'Brien, J.; Walker, Z.; Tatsch, K.; Booij, J.; Darcourt, J.; Padovani, A.; Giubbini, R.; Bonuccelli, U.; Volterrani, D.; Holmes, C.; Kemp, P.; Tabet, N.; Meyer, I.; Reininger, C. Sensitivity and specificity of dopamine transporter imaging with ^{123}I-FP-CIT SPECT in dementia with Lewy bodies: A phase III, multicentre study. *Lancet Neurol.* **2007**, *6*, 305−313.

(3) Kapur, S.; Mamo, D. Half a century of antipsychotics and still a central role for dopamine D_2 receptors. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **2003**, *27*, 1081−1090.

(4) Volkow, N. D.; Fowler, J. S.; Wang, G. J.; Swanson, J. M. Dopamine in drug abuse and addiction: Results from imaging studies and treatment implications. *Mol. Psychiatry* **2004**, *9*, 557−569.

(5) von Campenhausen, S.; Bornschein, B.; Wick, R.; Bötzel, K.; Sampaio, C.; Poewe, W.; Oertel, W.; Siebert, U.; Berger, K.; Dodel, R. Prevalence and incidence of Parkinson's disease in Europe. *Eur. Neuropsychopharmacol.* **2005**, *15*, 473−490.

(6) Kirkbride, J. B.; Errazuriz, A.; Croudace, T. J.; Morgan, C.; Jackson, D.; Boydell, J.; Murray, R. M.; Jones, P. B. Incidence of schizophrenia and other psychoses in England, 1950−2009: A systematic review and meta analyses. *PLoS One* **2012**, *7*, e31660-1−e31660-21.

(7) Goldstein, R. B.; Dawson, D. A.; Chou, P.; Grant, B. F. Sex differences in prevalence and comorbidity of alcohol and drug use disorders: Results from wave 2 of the national epidemiologic survey on alcohol and related conditions. *J. Stud. Alcohol Drugs* **2012**, *73*, 938−950.

(8) James, B. D.; Bennett, D. A.; Boyle, P. A.; Leurgans, S.; Schneider, J. A. Dementia from alzheimer disease and mixed pathologies in the oldest old. *JAMA* **2012**, *307*, 1798−1800.

(9) Stoof, J. C.; Kebabian, J. W. Opposing roles for D-1 and D-2 dopamine receptors in efflux of cyclic AMP from rat neostriatum. *Nature* **1981**, *294*, 366−368.

(10) Strange, P. G. New insights into dopamine receptors in the central nervous system. *Neurochem. Int.* **1993**, *22*, 223−236.

(11) Sibley, D. R.; De Lean, A.; Creese, I. Anterior pituitary dopamine receptors. Demonstration of interconvertible high and low affinity states of the D-2 dopamine receptor. *J. Biol. Chem.* **1982**, *257*, 6351−6361.

(12) Chio, C. L.; Lajiness, M. E.; Huff, R. M. Activation of heterologously expressed D3 dopamine receptors: Comparison with D2 dopamine-receptors. *Mol. Pharmacol.* **1994**, *45*, 51−60.

(13) Seeman, P.; Weinshenker, D.; Quirion, R.; Srivastava, I. A. K.; Bhardwaj, S. K.; Grandy, D. K.; Premont, R. T.; Sotnikova, T. D.; Boksa, P.; El-Ghundi, M.; O'Dowd, B. F.; George, S. R.; Perreault, M. L.; Mannisto, P. T.; Robinson, S.; Palmiter, R. D.; Tallerico, T. Dopamine supersensitivity correlates with $D2^{High}$ states, implying many paths to psychosis. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 3513−3518.

(14) Zahniser, N. R.; Molinoff, P. B. Effect of guanine nucleotides on striatal dopamine receptors. *Nature* **1978**, *275*, 453−455.

(15) van Wieringen, J.-P.; Booij, J.; Shalgunov, V.; Elsinga, P.; Michel, M. Agonist high- and low-affinity states of dopamine D_2 receptors: Methods of detection and clinical implications. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2013**, *386*, 135−154.

(16) George, S. R.; Watanabe, M.; Seeman, P. Dopamine D2-receptors in the anterior-pituitary − A single population without reciprocal antagonist agonist states. *J. Neurochem.* **1985**, *44*, 1168−1177.

(17) Seeman, P.; Schwarz, J.; Chen, J. F.; Szechtman, H.; Perreault, M.; McKnight, G. S.; Roder, J. C. Psychosis pathways converge via $D2^{high}$ dopamine receptors. *Synapse* **2006**, *60*, 319−346.

(18) Finnema, S. J.; Bang-Andersen, B.; Wikstrom, H. V.; Halldin, C. Current state of agonist radioligands for imaging of brain dopamine D2/D3 receptors in vivo with positron emission tomography. *Curr. Top. Med. Chem.* **2010**, *10*, 1477−1498.

(19) Narendran, R.; Frankle, W. G.; Mason, N. S.; Laymon, C. M.; Lopresti, B. J.; Price, J. C.; Kendro, S.; Vora, S.; Litschge, M.; Mountz, J. M.; Mathis, C. A. Positron emission tomography imaging of $D_{2/3}$ agonist binding in healthy human subjects with the radiotracer [^{11}C]-N-propyl-norapomorphine: Preliminary evaluation and reproducibility studies. *Synapse* **2009**, *63*, 574−584.

(20) Ginovart, N.; Willeit, M.; Rusjan, P.; Graff, A.; Bloomfield, P. M.; Houle, S.; Kapur, S.; Wilson, A. A. Positron emission tomography quantification of [^{11}C]-(+)-PHNO binding in the human brain. *J. Cereb. Blood Flow Metab.* **2007**, *27*, 857−871.

(21) Ginovart, N.; Galineau, L.; Willeit, M.; Mizrahi, R.; Bloomfield, P. M.; Seeman, P.; Houle, S.; Kapur, S.; Wilson, A. A. Binding characteristics and sensitivity to endogenous dopamine of [^{11}C]-(+)-PHNO, a new agonist radiotracer for imaging the high-affinity state of D_2 receptors in vivo using positron emission tomography. *J. Neurochem.* **2006**, *97*, 1089−1103.

(22) Narendran, R.; Mason, N. S.; Laymon, C. M.; Lopresti, B. J.; Velasquez, N. D.; May, M. A.; Kendro, S.; Martinez, D.; Mathis, C. A.; Frankle, W. G. A comparative evaluation of the dopamine $D_{2/3}$ agonist radiotracer [^{11}C](−)-N-propyl-norapomorphine and antagonist [^{11}C]-raclopride to measure amphetamine-induced dopamine release in the human striatum. *J. Pharmacol. Exp. Ther.* **2010**, *333*, 533−539.

(23) Narendran, R.; Hwang, D. R.; Slifstein, M.; Talbot, P. S.; Erritzoe, D.; Huang, Y.; Cooper, T. B.; Martinez, D.; Kegeles, L. S.; Abi-Dargham, A.; Laruelle, M. In vivo vulnerability to competition by endogenous dopamine: Comparison of the D_2 receptor agonist radiotracer (−)-N-[^{11}C]propyl-norapomorphine ([^{11}C]NPA) with the D_2 receptor antagonist radiotracer [^{11}C]-raclopride. *Synapse* **2004**, *52*, 188−208.

(24) Shotbolt, P.; Tziortzi, A. C.; Searle, G. E.; Colasanti, A.; van der Aart, J.; Abanades, S.; Plisson, C.; Miller, S. R.; Huiban, M.; Beaver, J. D.; Gunn, R. N.; Laruelle, M.; Rabiner, E. A. Within-subject comparison of [^{11}C]-(+)-PHNO and [^{11}C]raclopride sensitivity to acute amphetamine challenge in healthy humans. *J. Cereb. Blood Flow Metab.* **2012**, *32*, 127−136.

(25) Skinbjerg, M.; Sibley, D. R.; Javitch, J. A.; Abi-Dargham, A. Imaging the high-affinity state of the dopamine D2 receptor in vivo: Fact or fiction? *Biochem. Pharmacol.* **2012**, *83*, 193−198.

(26) Mewshaw, R. E.; Kavanagh, J.; Stack, G.; Marquis, K. L.; Shi, X. J.; Kagan, M. Z.; Webb, M. B.; Katz, A. H.; Park, A.; Kang, Y. H.; Abou-Gharbia, M.; Scerni, R.; Wasik, T.; Cortes-Burgos, L.; Spangler, T.; Brennan, J. A.; Piesla, M.; Mazandarani, H.; Cockett, M. I.; Ochalski, R.; Coupet, J.; Andree, T. H. New generation dopaminergic agents.1. Discovery of a novel scaffold which embraces the D_2 agonist pharmacophore. Structure-activity relationships of a series of 2-(aminomethyl)chromans. *J. Med. Chem.* **1997**, *40*, 4235−4256.

(27) Mewshaw, R. E.; Shi, X. J.; Mazandarani, H.; Coupet, J.; McGaughey, G. B.; Andree, T. H. New generation dopaminergic agents. 3. Accessing the D-2 pharmacophore via the 7-hydroxy-2-aminomethyl benzoxazine moiety. *Med. Chem. Res.* **1997**, *7*, 429−435.

(28) Mewshaw, R. E.; Verwijs, A.; Shi, X. J.; McGaughey, G. B.; Nelson, J. A.; Mazandarani, H.; Brennan, J. A.; Marquis, K. L.; Coupet, J.; Andree, T. H. New generation dopaminergic agents. 5. Heterocyclic bioisosteres that exploit the 3-OH-N-1-phenylpiperazine dopaminergic template. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2675−2680.

(29) Mewshaw, R. E.; Marquis, K. L.; Shi, X. J.; McGaughey, G.; Stack, G.; Webb, M. B.; Abou-Gharbia, M.; Wasik, T.; Scerni, R.; Spangler, T.; Brennan, J. A.; Mazandarani, H.; Coupet, J.; Andree, T. H. New generation dopaminergic agents. 4. Exploiting the 2-methyl chroman scaffold. Synthesis and evaluation of two novel series of 2-(aminomethyl)-3,4,7,9-tetrahydro-2H-pyrano[2,3-e]indole and indol-8-one derivatives. *Tetrahedron* **1998**, *54*, 7081−7108.

(30) Mewshaw, R. E.; Webb, M. B.; Marquis, K. L.; McGaughey, G. B.; Shi, X. J.; Wasik, T.; Scerni, R.; Brennan, J. A.; Andree, T. H. New generation dopaminergic agents. 6. Structure-activity relationship studies of a series of 4-(aminoethoxy)indole and 4-(aminoethoxy)-

indolone derivatives based on the newly discovered 3-hydroxyphenoxyethylamine D_2 template. *J. Med. Chem.* **1999**, *42*, 2007−2020.
(31) Mewshaw, R. E.; Husbands, M.; Gildersleeve, E. S.; Webb, M. B.; Xiaojie, S.; Mazandarani, H.; Cockett, M. I.; Ochalski, R.; Brennan, J. A.; Abou-Gharbia, M.; Marquis, K.; McGaughey, G. B.; Coupet, J.; Andree, T. H. New generation dopaminergic agents. 2. Discovery of 3-OH-phenoxyethylamine and 3-OH-N1-phenylpiperazine dopaminergic templates. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 295−300.
(32) Biswas, S.; Hazeldine, S.; Ghosh, B.; Parrington, I.; Kuzhikandathil, E.; Reith, M. E. A.; Dutta, A. K. Bioisosteric heterocyclic versions of 7-{[2-(4-phenyl-piperazin-1-yl)ethyl]-propylamino}-5,6,7,8-tetrahydronaph thalen-2-ol: Identification of highly potent and selective agonists for dopamine D_3 receptor with potent in vivo activity. *J. Med. Chem.* **2008**, *51*, 3005−3019.
(33) Biswas, S.; Zhang, S. H.; Fernandez, F.; Ghosh, B.; Zhen, J.; Kuzhikandathil, E.; Reith, M. E. A.; Dutta, A. K. Further structure-activity relationships study of hybrid 7-{[2-(4-phenylpiperazin-1-yl)ethyl]propylamino}-5,6,7,8-tetrahydronapht halen-2-ol analogues: Identification of a high-affinity D_3 preferring agonist with potent in vivo activity with long duration of action. *J. Med. Chem.* **2008**, *51*, 101−117.
(34) Walenzyk, T.; Carola, C.; Buchholz, H.; Konig, B. Chromone derivatives which bind to human hair. *Tetrahedron* **2005**, *61*, 7366−7377.
(35) Cohen, N.; Weber, G.; Banner, B. L.; Lopresti, R. J.; Schaer, B.; Focella, A.; Zenchoff, G. B.; Chiu, A. M.; Todaro, L.; Odonnell, M.; Welton, A. F.; Brown, D.; Garippa, R.; Crowley, H.; Morgan, D. W. 3,4-Dihydro-2H-1-benzopyran-2-carboxylic acids and related-compounds as leukotriene antagonists. *J. Med. Chem.* **1989**, *32*, 1842−1860.
(36) Kalaritis, P.; Regenye, R. W.; Partridge, J. J.; Coffen, D. L. Kinetic resolution of 2-substituted esters catalyzed by a lipase ex. Pseudomonas-fluorescens. *J. Org. Chem.* **1990**, *55*, 812−815.
(37) Hamblin, M. W.; Leff, S. E.; Creese, I. Interactions of agonists with D_2 dopamine receptors: Evidence for a single receptor population existing in multiple agonist affinity-states in rat striatal membranes. *Biochem. Pharmacol.* **1984**, *33*, 877−887.
(38) Cheng, Y.-C.; Prusoff, W. H. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.
(39) Wong, D. F.; Pomper, M. G. Predicting the success of a radiopharmaceutical for in vivo imaging of central nervous system neuroreceptor systems. *Mol. Imaging Biol.* **2003**, *5*, 350−362.
(40) Floresca, C. Z.; Schetz, J. A. Dopamine receptor microdomains involved in molecular recognition and the regulation of drug affinity and function. *J. Recept. Signal Transduction Res.* **2004**, *24*, 207−239.
(41) Mcdermed, J. D.; Mckenzie, G. M.; Phillips, A. P. Synthesis and pharmacology of some 2-aminotetralins. Dopamine receptor agonists. *J. Med. Chem.* **1975**, *18*, 362−367.
(42) Malo, M.; Brive, L.; Luthman, K.; Svensson, P. Selective pharmacophore models of dopamine D1 and D2 full agonists based on extended pharmacophore features. *ChemMedChem* **2010**, *5*, 232−246.
(43) Malo, M.; Brive, L.; Luthman, K.; Svensson, P. Investigation of D_2 receptor−agonist interactions using a combination of pharmacophore and receptor homology modeling. *ChemMedChem* **2012**, *7*, 471−482.
(44) Lan, H.; DuRand, C. J.; Teeter, M. M.; Neve, K. A. Structural determinants of pharmacological specificity between D_1 and D_2 dopamine receptors. *Mol. Pharmacol.* **2006**, *69*, 185−194.
(45) Seiler, M. P.; Stoll, A. P.; Closse, A.; Frick, W.; Jaton, A.; Vigouret, J. M. Structure-activity relationships of dopaminergic 5-hydroxy-2-aminotetralin derivatives with functionalized N-alkyl substituents. *J. Med. Chem.* **1986**, *29*, 912−917.
(46) McGaughey, G. B.; Mewshaw, R. E. Application of comparative molecular field analysis to dopamine D_2 partial agonists. *Bioorg. Med. Chem.* **1999**, *7*, 2453−2456.
(47) McRobb, F. M.; Capuano, B.; Crosby, I. T.; Chalmers, D. K.; Yuriev, E. Homology modeling and docking evaluation of aminergic G protein-coupled receptors. *J. Chem. Inf. Model.* **2010**, *50*, 626−637.
(48) Ehrlich, K.; Gotz, A.; Bollinger, S.; Tschammer, N.; Bettinetti, L.; Harterich, S.; Hubner, H.; Lanig, H.; Gmeiner, P. Dopamine D_2, D_3, and D_4 selective phenylpiperazines as molecular probes to explore the origins of subtype specific receptor binding. *J. Med. Chem.* **2009**, *52*, 4923−4935.
(49) Waterhouse, R. N. Determination of lipophilicity and its use as a predictor of blood−brain barrier penetration of molecular imaging agents. *Mol. Imaging Biol.* **2003**, *5*, 376−389.
(50) Kortagere, S.; Cheng, S. Y.; Antonio, T.; Zhen, J.; Reith, M. E. A.; Dutta, A. K. Interaction of novel hybrid compounds with the D_3 dopamine receptor: Site-directed mutagenesis and homology modeling studies. *Biochem. Pharmacol.* **2011**, *81*, 157−163.
(51) Malmberg, A.; Mikaels, A.; Mohell, N. Agonist and inverse agonist activity at the dopamine D_3 receptor measured by guanosine 5′-[γ-thio]triphosphate-[^{35}S] binding. *J. Pharmacol. Exp. Ther.* **1998**, *285*, 119−126.
(52) Chio, C. L.; Lajiness, M. E.; Huff, R. M. Activation of heterologously expressed D3 dopamine receptors: Comparison with D2 dopamine receptors. *Mol. Pharmacol.* **1994**, *45*, 51−60.
(53) Perachon, S.; Schwartz, J. C.; Sokoloff, P. Functional potencies of new antiparkinsonian drugs at recombinant human dopamine D_1, D_2 and D_3 receptors. *Eur. J. Pharmacol.* **1999**, *366*, 293−300.
(54) Wiens, B. L.; Nelson, C. S.; Neve, K. A. Contribution of serine residues to constitutive and agonist induced signaling via the D_{2S} dopamine receptor: Evidence for multiple, agonist-specific active conformations. *Mol. Pharmacol.* **1998**, *54*, 435−444.
(55) Payne, S. L.; Johansson, A. M.; Strange, P. G. Mechanisms of ligand binding and efficacy at the human $D_{2(short)}$ dopamine receptor. *J. Neurochem.* **2002**, *82*, 1106−1117.
(56) Grunewald, S.; Reilander, H.; Michel, H. In vivo reconstitution of dopamine D_{2S} receptor-mediated G protein activation in baculovirus-infected insect cells: Preferred coupling to G_{i1} versus G_{i2}. *Biochemistry* **1996**, *35*, 15162−15173.
(57) Sokoloff, P.; Andrieux, M.; Besancon, R.; Pilon, C.; Martres, M. P.; Giros, B.; Schwartz, J. C. Pharmacology of human dopamine D_3 receptor expressed in a mammalian-cell line: Comparison with D_2 receptor. *Eur. J. Pharmacol.* **1992**, *225*, 331−337.
(58) Seeman, P. Dopamine agonist radioligand binds to both D2High and D2Low receptors, explaining why alterations in D2High are not detected in human brain scans. *Synapse* **2012**, *66*, 88−93.
(59) Seeman, P.; Van Tol, H. H. M. Dopamine receptor pharmacology. *Trends Pharmacol. Sci.* **1994**, *15*, 264−270.
(60) Gardner, B.; Strange, P. G. Agonist action at D2(long) dopamine receptors: Ligand binding and functional assays. *Br. J. Pharmacol.* **1998**, *124*, 978−984.
(61) Kara, E.; Lin, H.; Strange, P. G. Co-operativity in agonist binding at the D2 dopamine receptor: evidence from agonist dissociation kinetics. *J. Neurochem.* **2010**, *112*, 1442−1453.
(62) Salama, I.; Hocke, C.; Utz, W.; Prante, O.; Boeckler, F.; Hübner, H.; Kuwert, T.; Gmeiner, P. Structure−selectivity investigations of D_2-like receptor ligands by CoMFA and CoMSIA guiding the discovery of D_3 selective PET radioligands. *J. Med. Chem.* **2007**, *50*, 489−500.
(63) Wang, Q.; Mach, R. H.; Luedtke, R. R.; Reichert, D. E. Subtype selectivity of dopamine receptor ligands: Insights from structure and ligand-based methods. *J. Chem. Inf. Model.* **2010**, *50*, 1970−1985.
(64) Laruelle, M.; Slifstein, M.; Huang, Y. Relationships between radiotracer properties and image quality in molecular imaging of the brain with positron emission tomography. *Mol. Imaging Biol.* **2003**, *5*, 363−375.
(65) Rabiner, E. A.; Laruelle, M. Imaging the D_3 receptor in humans in vivo using [^{11}C](+)-PHNO positron emission tomography (PET). *Int. J. Neuropsychopharmacol.* **2010**, *13*, 289−290.
(66) Tziortzi, A. C.; Searle, G. E.; Tzimopoulou, S.; Salinas, C.; Beaver, J. D.; Jenkinson, M.; Laruelle, M.; Rabiner, E. A.; Gunn, R. N. Imaging dopamine receptors in humans with [^{11}C]-(+)-PHNO: Dissection of D_3 signal and anatomy. *NeuroImage* **2011**, *54*, 264−277.

(67) Boileau, I.; Payer, D.; Houle, S.; Behzadi, A.; Rusjan, P. M.; Tong, J. C.; Wilkins, D.; Selby, P.; George, T. P.; Zack, M.; Furukawa, Y.; McCluskey, T.; Wilson, A. A.; Kish, S. J. Higher binding of the dopamine D_3 receptor-preferring ligand [^{11}C] -(+)-propyl-hexahydro-naphtho-oxazin in methamphetamine polydrug users: A positron emission tomography study. *J. Neurosci.* **2012**, *32*, 1353−1359.
(68) Caravaggio, F.; Raitsin, S.; Gerretsen, P.; Nakajima, S.; Wilson, A.; Graff-Guerrero, A.Ventral striatum binding of a dopamine $D_{2/3}$ receptor agonist but not antagonist predicts normal body mass index*Biol. Psychiatry* [Online early access] DOI: 10.1016/j.biopsych.2013.02.017. Published Online: March 27, **2013**.
(69) Cumming, P. Absolute abundances and affinity states of dopamine receptors in mammalian brain: A review. *Synapse* **2011**, *65*, 892−909.
(70) Lahti, R. A.; Roberts, R. C.; Cochrane, E. V.; Primus, R. J.; Gallager, D. W.; Conley, R. R.; Tamminga, C. A. Direct determination of dopamine D_4 receptors in normal and schizophrenic postmortem brain tissue: A [^{3}H]NGD-94-1 study. *Mol. Psychiatry* **1998**, *3*, 528−533.
(71) Primus, R. J.; Thurkauf, A.; Xu, J.; Yevich, E.; McInerney, S.; Shaw, K.; Tallman, J. F.; Gallager, D. W. Localization and characterization of dopamine D_4 binding sites in rat and human brain by use of the novel, D_4 receptor-selective ligand [^{3}H]NGD 94-1. *J. Pharmacol. Exp. Ther.* **1997**, *282*, 1020−1027.
(72) Hanson, R. N.; Hariharan, S.; Astik, R. Synthesis of 4-(4-iodophenyl)piperazine and the 1-carboxamidino derivative. *J. Heterocycl. Chem.* **1985**, *22*, 47−48.
(73) Tietze, R.; Hocke, C.; Lober, S.; Hubner, H.; Kuwert, T.; Gmeiner, P.; Prante, O. Syntheses and radiofluorination of two derivatives of 5-cyano-indole as selective ligands for the dopamine subtype-4 receptor. *J. Labelled Compd. Radiopharm.* **2006**, *49*, 55−70.
(74) Parenty, A. D. C.; Smith, L. V.; Pickering, A. L.; Long, D. L.; Cronin, L. General one-pot, three-step methodology leading to an extended class of N-heterocyclic cations: Spontaneous nucleophilic addition, cyclization, and hydride loss. *J. Org. Chem.* **2004**, *69*, 5934−5946.
(75) Fesser, P.; Iacovita, C.; Wackerlin, C.; Vijayaraghavan, S.; Ballav, N.; Howes, K.; Gisselbrecht, J. P.; Crobu, M.; Boudon, C.; Stohr, M.; Jung, T. A.; Diederich, F. Visualizing the product of a formal cycloaddition of 7,7,8,8-tetracyano-p-quinodimethane (TCNQ) to an acetylene-appended porphyrin by scanning tunneling microscopy on Au(111). *Chem.—Eur. J.* **2011**, *17*, 5246−5250.
(76) Tummatorn, J.; Dudley, G. B. Generation of medium-ring cycloalkynes by ring expansion of vinylogous acyl triflates. *Org. Lett.* **2011**, *13*, 1572−1575.
(77) Sandanayaka, V. Preparation of phenylpiperazine derivatives as LTA4H (leukotriene A4 hydrolase) inhibitors for treating inflammation. U.S. Patent 20070149544, June 28, 2007.
(78) Shoji, A.; Kuwahara, M.; Ozaki, H.; Sawai, H. Modified DNA aptamer that binds the (R)-isomer of a thalidomide derivative with high enantioselectivity. *J. Am. Chem. Soc.* **2007**, *129*, 1456−1464.
(79) Chichak, K. S.; Peters, A. J.; Cantrill, S. J.; Stoddart, J. F. Nanoscale borromeates. *J. Org. Chem.* **2005**, *70*, 7956−7962.
(80) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248−254.