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Synthesis, Structure-affinity Relationships and Radiolabeling of Selective High-affinity 5-HT₄ Receptor Ligands as Prospective Imaging Probes for PET

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Abstract

In a search for high-affinity receptor ligands that might serve for development as radioligands for the imaging of brain 5-HT₄ receptors in vivo with positron emission tomography (PET), structural modifications were made to the high-affinity 5-HT₄ antagonist, (1-butylpiperidin-4-yl)methyl 8-amino-7-iodo-2,3-dihydrobenzo[*b*][1,4]dioxine-5-carboxylate (1, SB 207710). These modifications were made mainly on the aryl side of the ester bond to permit possible rapid labeling of the carboxylic acid component with a positron-emitter, either carbon-11 ($t_{1/2} = 20.4$ min) or fluorine-18 ($t_{1/2} = 109.7$ min), and included, i) replacement of the iodine atom with a small substituent such as nitrile, methyl or fluoro, ii) methylation of the 8-amino group, iii) opening of the dioxan ring, and iv) alteration of the length of the *N*-alkyl group. High-affinity ligands were discovered for recombinant human 5-HT₄ receptors with amenability to labeling with a positron-emitter and potential for development as imaging probes. The ring-opened radioligand, (([methoxy-¹¹C]1-butylpiperidin-4-yl)methyl 4-amino-3-methoxybenzoate; [¹¹C]13), showed an especially favorable array of properties for future evaluation as a PET radioligand for brain 5-HT₄ receptors.

Keywords

PET; Imaging; 5-HT₄; Radioligand; Carbon-11; Fluorine-18

Introduction

Suitably effective radioligands, when applied with molecular imaging techniques, such as positron emission tomography (PET) or single photon emission computed tomography (SPECT), provide a unique means for measuring brain neurotransmitter receptor concentrations in living subjects, and therefore they constitute important clinical research tools.¹ Such radioligands may also be used with imaging techniques to assess the receptor binding of unlabeled ligands in vivo, whether exogenous (e.g., a developmental or therapeutic drug, or a substance of abuse) or endogenous (the neurotransmitter), and hence they are also useful for drug discovery and development.²

Serotonin (5-hydroxytryptamine; 5-HT) is an important neurotransmitter that is known to act on at least fourteen receptors in seven major sub-classes. Many of these receptors in brain

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Supporting Information Available: The chemical purities of compounds 1–21 and their HPLC methods of determination. This material is available free of charge via the Internet at <http://pubs.acs.org>.

have been implicated in neuropsychiatric disorders, and hence they have become targets for deep biomedical investigation and also for drug discovery and development programs. Although many effective radioligands exist for the *in vivo* imaging of some of the 5-HT receptors, especially the 5-HT_{1A}, 5-HT_{2A} and 5-HT_{1B} subtypes, radioligands for some other subtypes are not yet well explored (e.g., 5-HT₄).

The 5-HT₄ receptor is a well-characterized G-protein coupled receptor that exists abundantly in brain, especially in limbic and striatonigral regions.³ This receptor population is implicated in dopamine, serotonin and acetylcholine release, and possibly plays a significant role in normal cognition, learning, and memory. 5-HT₄ receptors have also been implicated in neuropsychiatric disorders, such as Alzheimer's disease, anxiety and depression.^{4,5,6}

A reason for the relatively slow development of 5-HT₄ PET radioligands is the wide array of properties that must ideally be found in any candidate. These include high target receptor affinity, high selectivity, generally moderate lipophilicity⁷, appropriate intrinsic activity, ability to cross the blood-brain barrier⁸, absence of troublesome radiometabolites⁸ and amenability to labeling with a suitable radioisotope.^{9,10} Suitable radioisotopes are generally short-lived carbon-11 ($t_{1/2} = 20.4$ min) or fluorine-18 ($t_{1/2} = 109.7$ min) for PET and iodine-123 ($t_{1/2} = 13.2$ h) for SPECT.

(1-Butylpiperidin-4-yl)methyl 8-amino-7-iodo-2,3-dihydrobenzo[*b*][1,4]dioxine-5-carboxylate (**1**, SB 207710; Chart 1) is an exceptionally high-affinity 5-HT₄ receptor antagonist.^{11,12} The use of [¹²³I]**1** with SPECT provided the first demonstration of 5-HT₄ receptor imaging in primate brain *in vivo*.¹³ Some analogs of **1**, labeled with either carbon-11 or fluorine-18 in the terminal *N*-alkyl group, have been prepared and one of these, [¹¹C]**2** ([¹¹C]SB 207145; Chart 1) has shown promise for PET imaging in animal¹⁴ and human subjects^{15,16}. This radioligand is an ester and rapidly metabolized *in vivo* by hydrolysis. Here, we aimed to develop alternative radioligands for 5-HT₄ receptor imaging with PET, again based on **1**, in which the radiolabel is located on the carbonyl side of the ester group to avoid potential issues from possibly brain-penetrant radiometabolites, and to permit control of lipophilicity through adjustment of *N*-alkyl chain length. The ester hydrolysis of such radioligands outside brain would produce carboxylic acids as radiometabolites; these radiometabolites would be expected to have poor entry into brain and therefore would not be expected to interfere with the measurement of brain receptor density with the parent radioligand.⁸ The 5-HT₄ radioligand [¹¹C]**2** has been shown to be amenable to quantification in human brain,¹⁶ implying that ester hydrolysis to generate benzoates as radiometabolites within brain should not be a major concern for ligands related to **1**. Control of lipophilicity can be important for adjusting properties that may impact on the potential success of a radioligand, including its plasma free fraction, ability to penetrate the blood-brain-barrier (permeability parameter)¹⁷ and susceptibility to metabolism.^{8–10} Our synthetic strategies included replacement of the aryl halo group, and/or alkylation of the aryl amino group, opening of the dioxan ring and manipulation of the *N*-alkyl chain length. As a result, several new high-affinity ligands were discovered and radiolabeled as prospective PET radioligands for 5-HT₄ receptors. The 3-methoxy compound, [¹¹C]**13**, was found to have an especially favorable array of properties for further evaluation as a new 5-HT₄ receptor PET radioligand.

Results

Chemistry

We considered several approaches for modifying the structure of **1** to allow carbon-11 to be introduced as a radiolabel into the structure on the carbonyl side of the ester bond, using readily accessible labeling agents such as [¹¹C]methyl iodide¹⁸, [¹¹C]methyl triflate¹⁹ or

[¹¹C]cyanide ion²⁰. These strategies included: i) replacing the iodo group in **1** with a group of similar or smaller size, namely nitrile, as in **5** and **11**, or methyl as in **7** (Scheme 1), ii) *N*-methylation, as in **6** and **8–11** (Scheme 1), and iii) replacement of the dioxan ring by a single *O*-methyl group, as in **12** and **13** (Scheme 2). Ligand **5** was obtained in low but useful yield (19%) by treating **1** with potassium cyanide. The *N*-methyl compound **6** was obtained in good yield (60%) by treating the primary arylamine **4** with paraformaldehyde and then sodium borohydride. Treatment of **6** with *N*-halosuccinimides (NXS, X = Cl, Br or I) gave the respective halo derivatives **8–10** in moderate yields (30–56%). Ligand **7** was obtained from **1** in moderate yield (44%) through Pd-catalyzed methylation with tetramethyltin. Ligand **11** was similarly obtained from ligand **10** but in much lower yield (7%). The ring *O*-methyl derivatives, **12** and **13**, were obtained in moderate (32%) and low (8%) overall yields, respectively, from the corresponding methyl esters **12a** and **13a** in two steps, namely *N*-protection and trans-esterification (Scheme 2). Analogs of **13**, in which either the ester group was replaced with an amido group, as in **14**, or in which the length of the *N*-alkyl group was altered to vary lipophilicity, as in **15** and **16**, were also prepared in two steps and moderate overall yields (30–69%) from 4-amino-3-methoxybenzoic acid (Scheme 3).

For the purpose of creating a radioligand that might be labeled with fluorine-18, we succeeded in preparing **17**, a 7-fluoro analog of **1**, in six steps from 5-fluoro-2-hydroxybenzoic acid (Scheme 4). All steps proceeded in > 75% yield, except the final two-stage esterification, involving Cbz-protection (34%) and trans-esterification (59%). Also, two fluoroalkoxy analogs of **13**, the fluoro-methoxy compound **18** and the 2-fluoroethoxy compound **19**, were prepared in two steps from methyl 4-amino-3-hydroxybenzoate in moderate overall yields of 37 and 20%, respectively (Scheme 5).

Potential precursors for radiolabeling were also synthesized, including the phenol precursor **20** by demethylation of **13** (42% yield) (Scheme 6), and the nitro compound **21** in three steps from 8-amino-7-nitro-2,3-dihydrobenzo[*b*][1,4]dioxine carboxylic acid methyl ester (**21a**) in 42% overall yield (Scheme 7).

Pharmacological Assays and Screen

Assay of compounds **1**, **5**, **8**, **13**, **15** and **17–19** for binding to 5-HT₄ receptors in guinea pig striatal membranes showed all these compounds to have sub-nanomolar *K*_i values (Table 1). However, assay of the same ligand set against human recombinant 5-HT₄ receptors (*h*5-HT₄), expressed in HEK293T cells, revealed a greater variation in *K*_i, with only some of the compounds (**1**, **8**, **13** and **17**) showing low nanomolar values. This assay was applied to the full range of new ligands and revealed interesting structure-activity information (Table 1).

Replacement of the iodine atom in **1** with a nitrile, methyl or nitro group resulted in approximately 15-, 3- and 3.5-fold decrease in binding affinity, respectively, whereas replacement with fluorine retained binding affinity and replacement with hydrogen slightly increased affinity. *N*-Methylation of **1** resulted in about a 4-fold reduction in binding affinity, while the *N*-methylation of the corresponding nitrile, **5**, resulted in only a marginal reduction in binding affinity. The *N*-methyl 8-chloro analog **8** showed a binding affinity comparable to that of **1**.

Replacement of the dioxan ring in **1** with a 2-methoxy group gave ligand **12** with much reduced affinity whereas replacement with a 3-methoxy group gave ligand **13** with affinity comparable to that of **1**. Shortening or lengthening of the *N*-alkyl chain length in **13** gave ligands of 5- and 7-fold lower affinity, respectively.

Replacement of the ester group in **13** with an amido group drastically reduced binding affinity.

Replacement of the methoxy group in **13** with fluoromethoxy or 2-fluoroethoxy led to 7- and 5-fold reduction in binding affinity, respectively.

A selection of the compounds was also assessed for intrinsic activity (Table 1). The majority of these ligands were found to be quite potent partial or full inverse agonists with pEC_{50} values in the range 7.70–8.23. The 2-methoxy ligand **12** was found to be a potent full agonist with a pEC_{50} value of 9.75. In stark contrast, the high-affinity regional isomer **13** was found to be an antagonist. The arylmethyl analog of **1** was also found to be an antagonist, as was the previously known ligand, **4**.

Ligand **13** showed greater than 2000-fold selectivity for $h5-HT_4$ receptors versus twelve other $h5-HT$ receptors and binding sites (Table 2). Ligands **8** and **17** were not quite as selective among $h5-HT$ receptors and binding sites. The lowest selectivity for **8** was 33-fold versus $h5-HT_{2B}$ receptors and for **17**, 80-fold versus the same receptors (Table 2). Ligand **13** also exhibited high selectivity for $h5-HT_4$ receptors versus a wide range of non-serotonergic receptors and binding sites (Table 3). For **13**, the lowest selectivities for $h5-HT_4$ receptors were versus σ_1 and σ_2 receptors, namely 88- and 62-fold, respectively. Ligands **8** and **17** were also generally selective for $h5-HT_4$ receptors versus other receptors and binding sites, except that they displayed much lower selectivities versus σ_1 and especially σ_2 receptors (Table 3). Ligand **8** also showed a quite low 22-fold selectivity versus D_4 receptors (Table 3).

Computation of $cLogD$

The $cLogD$ values of ligands ranged from 1.74 to 3.27 (Table 1).

Syntheses of Radioligands

Some ligands, namely ligands **5**, **8**, **13**, **17** and **18**, were selected to test their amenability for rapid labeling with a positron-emitter, either carbon-11 or fluorine-18. Ligand [^{11}C]**5**, a [^{11}C]nitrile, was produced in an average of 26% decay-corrected radiochemical yield (RCY) by Pd-mediated exchange of the iodine atom with no-carrier-added (NCA) [^{11}C]cyanide ion in the presence of potassium carbonate-K 2.2.2 in THF (Scheme 8). Use of potassium dihydrogen phosphate as base in THF or DMSO²¹ gave much lower RCY (Table 4). The *N*-[^{11}C]methyl ligand, [^{11}C]**8**, was obtained in 11% RCY by ^{11}C -methylation of **1** with NCA [^{11}C]methyl iodide in the presence of the solid base, Li_3N , under the influence of ultrasound (Scheme 9). Use of Li_2O as solid base resulted in a lower RCY (7%) of [^{11}C]**8**. The *O*-[^{11}C]methyl ligand [^{11}C]**13** was obtained from the phenol **20** by methylation with either NCA [^{11}C]methyl iodide or [^{11}C]methyl triflate in either 36 or 27% RCY, respectively (Scheme 9). An experiment based on $^{13}C/^{11}C$ co-labeling, followed by ^{13}C -NMR,²² confirmed the position of the radiolabel. This radioligand was obtained in moderately high specific radioactivity, namely 2,848 mCi/ μ mol from [^{11}C]methyl iodide and 2,517 mCi/ μ mol from [^{11}C]methyl triflate at the end of radiosynthesis.

All attempts to prepare [^{18}F]**17** by substitution of the nitro group in precursor **21** or the *N*-Boc-protected analog **21c** with cyclotron-produced [^{18}F]fluoride ion were unsuccessful. However, the [^{18}F]fluoro-*di*-deutero-methoxy ligand, NCA [^{18}F]**18** was obtained in 13% RCY from [^{18}F]fluoride ion by treating the phenol **21** with derived [^{18}F] d_2 -fluorobromomethane in acetonitrile with NaOH as base at 100 °C (Scheme 10). Other tested conditions gave inferior RCYs (Table 5).

Discussion

In this study, variation of the structure of **1** led to several new high-affinity ligands for guinea pig and $h5-HT_4$ receptors, some of which proved amenable to labeling with a

positron-emitter to provide candidate radioligands for imaging brain 5-HT₄ receptors with PET. Interestingly, all seven new ligands (**5**, **8**, **13**, **15**, **17–19**) that were tested for binding to guinea pig 5-HT₄ receptors showed sub-nanomolar affinity within a narrow range ($K_i = 0.22–0.90$ nM), while the same set of ligands assayed against human recombinant receptors (*h*5-HT₄) showed somewhat lower binding affinity across a wider range ($K_i = 2.2–33$ nM) (Table 1). This species difference was unexpected, since 5-HT₄ receptor ligands from several other structural classes do not show such major differences in binding affinity between guinea pig 5-HT₄ and *h*5-HT₄ receptors.²³ Also, the affinities of **1**, the prototypic ligand for our series, were previously reported to be quite similar for human colon and guinea pig 5-HT₄ receptors.¹² Because any successfully developed PET radioligand would ultimately be used for imaging 5-HT₄ receptors in human brain, all new ligands from this study were assayed against *h*5-HT₄ receptors (Table 1).

Replacement of the iodine atom in **1** with a methyl or nitro substituent reduced *h*5-HT₄ receptor binding affinity by less than one order of magnitude, whereas replacement with a fluoro substituent caused negligible change, and replacement with hydrogen slightly improved affinity (Table 1). Replacement with a nitrile or nitro group caused a 15- or 4-fold reduction in affinity, respectively. Therefore, the *h*5-HT₄ receptor was quite tolerant of a small substituent ortho to the 8-amino group in **1**, irrespective of the electronic influence of the substituent.

Although a primary arylamino group is a frequent constituent of ligands reported for 5-HT₄ receptors,²⁴ the effect of *N*-alkylation of this group on binding affinity had not been examined previously. We found that the *N*-methylations of **1**, **4** and **5** caused only small reductions of binding affinity, of about 3-, 6.5- and 1.1-fold, respectively. The *N*-methylated **4** recovered its affinity to 2.0 nM after chlorination in the ortho position as seen in **8**. Another prepared *N*-methyl ligand, **9**, also exhibited low nanomolar affinity. Thus, the *h*5-HT₄ receptor readily tolerates the secondary 8-*N*-methylamino substituent in this structural class of ligand.

SB 204070 (**3**) is the 7-chloro analog of **1**. The non-dioxan 2-methoxy analog of **3** has nanomolar affinity for guinea pig 5-HT₄ receptors.²⁵ Here, opening of the dioxan ring in the proto analog of **1**, namely ligand **4**, by removal of either OCH₂ group gave the two *O*-methyl compounds, **12** and **13**. Binding affinity was reduced 36-fold in the 2-methoxy compound **12** but only 1.7-fold in the 3-methoxy compound **13**. Replacement of the 3-methoxy group in **13** with fluoromethoxy or 2-fluoroethoxy reduced affinity by 7- and 4.6-fold, respectively. Shortening or lengthening of the *N*-alkyl chain in **13**, similarly reduced binding affinity by 7- and 5-fold, respectively.

We noted that the conversion of the ester function in the benzodioxan **3** into an amido function has negligible effect on binding affinity ($1/IC_{50}$)²⁴ and in the ring-opened 2-methoxy analog causes only a 10-fold reduction in binding affinity.²⁵ We therefore considered replacing the ester function in the new radioligands with an amide function in order to confer greater resistance to hydrolysis *in vivo*. However, replacement of the ester group in the 3-methyl ether **13** with an amido group dramatically reduced affinity by several thousand-fold.

The maximal receptor-specific signal to be expected from the use of a radioligand with PET is related to the binding potential (BP), expressed as B_{max}/K_D , where B_{max} is the local concentration of receptors and K_D is the equilibrium dissociation constant of radioligand from the receptor.^{9,10} Therefore, high affinity ($1/K_D$ or as a surrogate measure, $1/K_i$) is a key parameter in determining whether a particular radioligand can be successful. Previously, it has been found that moderately sizeable receptor-specific signals can be obtained in mini-

Fig. 14, monkey¹³ and human¹⁶ subjects *in vivo* with 5-HT₄ receptor radioligands having K_D values in the low or sub-nanomolar range, such as [¹²³I]**1** and [¹¹C]**2**. Our new ligands **8**, **13** and **17** showed K_i values less than or comparable with that of **1** for *h5*-HT₄ receptors, and they therefore met the high-affinity criterion for development as PET radioligands. Since target selectivity, intrinsic activity²⁶ and lipophilicity also bear on the likely success of candidate PET radioligands,^{9,10} these three ligands were also assessed for these parameters. For comparison, lipophilicity was also computed for the other synthesized ligands, and intrinsic activity also assessed for **1**, **4** and **6–13** and **17**.

Ligands **1**, **8** and **17** appeared to be similarly potent inverse agonists in the GloSensor assay, whereas ligands **4** and **13** were antagonists (inactive) in the same assay (Table 1). Ligand **1** is widely considered to be an antagonist at 5-HT₄ receptors.^{11,12} However, it should be noted that ligands characterized as competitive antagonists often express inverse agonism in assays where constitutive receptor activity is present.²⁷ This fact likely explains why **1** appears to be an inverse agonist in the Glo-sensor assay. Ligand **7** was also inactive in the Glo-sensor assay, whereas ligands **6** and **9–11** were inverse agonists. Remarkably, ligand **12**, the close methoxy positional isomer of the antagonist **13**, was a potent agonist with quite high binding affinity.

Ligands **8**, **13** and **17** showed generally high selectivity for binding to *h5*-HT₄ receptors versus binding to other *h5*-HT receptors and binding sites (Table 2). The lowest selectivity was 33-fold for ligand **8** versus *h5*-HT_{2B} receptors. Although there is strong evidence that 5-HT_{2B} receptors exist in brain, their distribution and density in human brain remains unknown and is suspected to be low.^{28,29} Thus, the low-affinity binding of **8** to 5-HT_{2B} receptors in human brain *in vivo* would likely be insignificant compared to its high-affinity binding to relatively abundant 5-HT₄ receptors. Therefore, this off-target binding would probably not be a serious impediment to the development of **8** as a PET radioligand. Ligands **8**, **13** and **17** showed only low affinity for a wide range of other receptors and binding sites, except for σ_1 and σ_2 receptors in the cases of **8** and **17** and for D₄ receptors in the case of **8** (Table 3). Both sigma receptors are present in brain,³⁰ and indeed σ_1 receptors may be imaged with PET *in vivo* with moderately high-affinity radioligands, such as [¹¹C]SA4503 ($K_i = 17.4$ nM).³⁰ Thus, the affinities of ligands **8** ($K_i = 60$ nM) and **17** ($K_i = 55$ nM) for σ_1 receptors may be insufficiently low for successful 5-HT₄ receptor PET imaging. Imaging data suggest that the density of σ_2 receptors in human brain is much lower than that of σ_1 receptors.³⁰ Hence, it is uncertain whether the high-affinities of **8** ($K_i = 8$ nM) and **17** ($K_i = 13$ nM) for σ_2 receptors may be problematic for their development as PET 5-HT₄ receptor radioligands. The moderate affinity of **8** for D₄ receptors is unlikely to be problematic, since D₄ receptors only exist in very low density in human brain.^{31,32} As a candidate for development as a PET radioligand, **13** expressed excellent overall 5-HT₄ receptor selectivity, with greater than 60-fold selectivity against the full range of tested receptors and binding sites (Table 3).

Ligands **8**, **13** and **17** had computed lipophilicities (*cLogD* values) between 2.0 and 3.3, and these are within the range considered desirable for achieving adequate brain entry from blood without incurring excessive non-specific binding.^{7–10}

Given the array of favourable properties expressed by ligands **8**, **13** and **17**, it appeared especially attractive to attempt to label these ligands with a positron-emitter, either carbon-11 or fluorine-18, to give radioligands that might be tested and evaluated *in vivo* with PET.

Various conditions were attempted for the labeling of **8** with carbon-11 in its *N*-methyl group, all based on ¹¹C-methylation of the primary arylamine, **3**. However, **3**, as is quite

usual for primary arylamines, proved stubbornly reactive towards [^{11}C]methyl iodide or [^{11}C]methyl triflate under conventional conditions. Recently, we have developed methodology for labeling such precursors in the presence of a strong inorganic base under the influence of ultrasound agitation. This method, using DMF as solvent and Li_3N as solid base gave [^{11}C]**8** in low but still useful RCY (11%) from [^{11}C]methyl iodide after HPLC separation (Figure 9).

By contrast to **8**, the methoxy compound **13**, was readily labeled by reaction of the phenol **20** with [^{11}C]methyl iodide or [^{11}C]methyl triflate under basic conditions. No protection of the anilino nitrogen was necessary because of its low reactivity towards these labeling agents. The selectivity of the ^{11}C -methylation reaction for the phenol oxygen versus the anilino nitrogen was confirmed through a $^{11}\text{C}/^{13}\text{C}$ co-labelling experiment and subsequent ^{13}C -NMR spectroscopy.²² [^{11}C]**13** was readily separated by HPLC and was shown to be radiochemically pure by analytical HPLC.

The labeling of the fluoro compound **17** was first attempted through treatment of the *N*-Boc-protected **21** with NCA [^{18}F]fluoride ion because the presence of unprotected amino group plus the electron-rich nature of the aryl ring were expected to oppose facile aromatic nucleophilic substitution.³³ The *N*-Boc group was found to be unstable when temperature exceeded 120 °C. Nitro substitution occurred in deprotected **21** at high temperature (200 °C) but not without concomitant ester hydrolysis. The direct labeling of **21** also only gave [^{18}F]fluoride ion-substituted benzoic acid at similarly high temperature. Another attempt based on halogen exchange in methyl 7-iodo-8-nitro-2,3-dihydrobenzo[*b*][1,4]dioxine-5-carboxylate precursor resulted in the replacement of the nitro group instead of the iodo group by [^{18}F]fluoride ion. We considered other strategies for ^{18}F -labeling, including the production of a diaryliodonium salt precursor for radiofluorination.³⁴ However, we were unable to synthesize a suitable iodonium salt via a metalated-ring intermediate, since the aryl ring resisted clean stannylation, boronation or mercuration by conventional reagents and methods. Curiously, the *n*-tributyl stannyl analog of **1** is known¹³ but its synthesis, as far as we can ascertain, has never been published. Therefore, the radiosynthesis of [^{18}F]**17** remains a major challenge.

The radiolabeling of the relatively lower affinity ligands **5** and **18** with carbon-11 and fluorine-18, respectively, was shown to be feasible. Thus, [^{11}C]**5** was obtained by palladium-mediated ^{11}C -cyanation of **1** and [^{18}F]**18** by ^{18}F -fluoromethylation of the phenol **20** with [^{18}F] d_2 -fluoromethyl bromide. Deuterium was incorporated into [^{18}F]**18** to provide for greater resistance to defluorination *in vivo*.^{34–36}

Conclusions

In this study, manipulations of the structure of **1** led to several new ligands with high affinity towards guinea pig 5-HT₄ receptors and a few (**8**, **13** and **17**) with comparably high affinity towards *h*5-HT₄ receptors. Both **8** and **13** were amenable to labeling with carbon-11, whereas the labeling of **17** with NCA fluorine-18 remains a challenge. [^{11}C]**8** has affinity, selectivity, intrinsic activity and computed lipophilicity comparable to [^{123}I]**1** (Table 1), and should prove to be similarly effective for imaging 5-HT₄ receptors in monkey *in vivo*. [^{11}C]**13** is an easily-labeled, highly selective, high-affinity, and moderate lipophilicity antagonist for 5-HT₄ receptors and therefore merits evaluation as a PET radioligand for the study of 5-HT₄ receptors *in vivo* with PET. This radioligand is currently under evaluation in monkey, and findings will be published elsewhere.

Experimental Section

Materials

Methyl 3-methoxy-4-nitrobenzoate, 4-(aminomethyl)-1-(*n*-butyl)-piperidine, and *tetra*-*kis*(triphenylphosphine)palladium(0) were purchased from Alfa Aesar (Ward Hill, MA). Chlorofluoromethane and 1-chloro-2-fluoroethane were purchased from SynQuest (Alachua, FL). Other chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI) and used as received. Compounds **3** (SB 204070; 1-butylpiperidin-4-yl)methyl 8-amino-7-chloro-2,3-dihydrobenzo[*b*][1,4]dioxane-5-carboxylate and the des-chloro analog **4** were synthesized from 2,3-dihydro-benzodioxin by modified literature procedures.^{25, 37, 38}

General Methods

¹H- (400.13 MHz), ¹³C- (100.62 MHz) and ¹⁹F- (376.46 MHz) NMR spectra were recorded at rt on an Avance-400 spectrometer (Bruker, Billerica, MA). Chemical shifts are reported in δ units (ppm) downfield relative to the chemical shift for tetramethylsilane. Abbreviations s, d, t, dd, dt and bs denote singlet, doublet, triplet, doublet of doublet, doublet of triplet and broad singlet. Thin layer chromatography was performed with POLYGRAM[®] SIL G/UV₂₅₄ layers (0.2 mm silica gel with fluorescent indicator; Grace Davison Discovery Sciences; Deerfield, IL); compounds were visualized under UV light ($\lambda = 254$ nm).

High resolution mass spectra (HRMS) were acquired from the Mass Spectrometry Laboratory, University of Illinois at Urbana-Champaign (Urbana, IL) under electron ionization conditions using a double-focusing high-resolution mass spectrometer (Autospec, Micromass Inc., USA) with samples introduced through a direct insertion probe.

LC-MS analyses of synthesized compounds were performed on an LCQ Deca model instrument (Thermo Fisher Scientific Corp.; Waltham, MA). A gradient or isocratic LC analysis of sample was carried out with binary solvents (A: B; 150 μ L/min) composed of water-methanol-acetic acid (90: 10: 0.5 by vol.) (A) and methanol-acetic acid (100: 0.5, v/v) (B) on a Luna C18 column (3 μ m, 50 \times 2 mm; Phenomenex; Torrance, CA). Following electrospray ionization of the column effluent, ions *m/z* 150 through 750 were acquired.

Melting points were measured with a Mel-Temp manual apparatus (Electrothermal, Fisher Scientific) and were uncorrected.

γ Radioactivity from ¹¹C and ¹⁸F was measured using a calibrated dose calibrator (Atomlab 300; Biodex Medical Systems). Radioactivity measurements were corrected for physical decay. All radiochemistry was performed in lead-shielded hot-cells for personnel protection from radiation.

Radioactive products were separated by HPLC on a Gemini or Gemini-NX C18 column (5 μ m, 10 \times 250 mm; Phenomenex) eluted with 10 mM-NH₄OH-MeCN or 100 mM-HCOONH₄-MeCN at the stated composition and flow rate. Eluates were monitored for radioactivity (pin diode detector; Bioscan) and absorbance at 294 nm (System Gold 166 detector; Beckman).

The purity of each new non-radioactive compound was assessed by reverse phase HPLC under the conditions tabulated in Supporting Information. Each compound was shown to have a chemical purity of > 98%. Radioactive compounds were analyzed with HPLC on a Gemini C18 column (5 μ m, 4.6 \times 150 mm) or Gemini-NX C18 column (5 μ m, 4.6 \times 250 mm; Phenomenex) eluted with 10 mM-NH₄OH-MeCN or 100 mM-HCOONH₄-MeCN at the later stated composition and flow rate. Eluates were monitored for radioactivity (pin diode detector; Bioscan). Samples were injected alone, and then co-injected with the

reference non-radioactive compound to check for co-elution. RCYs were calculated for labeled products isolated with HPLC.

Computation of *cLogP* and *cLogD*

cLogP and *cLogD* (at pH 7.4) values for ligands were computed with ACD software.

(1-Butylpiperidin-4-yl)methyl 8-amino-7-iodo-2,3-dihydrobenzo[*b*][1,4]dioxine-5-carboxylate (**1**)

N-Iodosuccinimide (498 mg, 2.21 mmol) was added portion-wise to a solution of **4** (770 mg, 2.21 mmol) in acetic acid (5 mL) at 0 °C and stirred at rt for 2 h. The acetic acid was evaporated off, and the residue was basified with NaHCO₃ solution and extracted twice with CH₂Cl₂. The combined organic layers were evaporated to dryness. Silica gel chromatography (MeOH-CH₂Cl₂, 1: 20 v/v) of the residue gave **1** as a light yellow oil (410 mg, 40%). ¹H-NMR (CDCl₃): δ 0.92 (3H, t, *J* = 7.2 Hz), 1.25–1.53 (6H, m), 1.74–1.79 (3H, m), 1.94 (2H, t, *J* = 11.2 Hz), 2.31–2.34 (2H, m), 2.96 (2H, d, *J* = 11.2 Hz), 4.10 (2H, d, *J* = 6.4 Hz), 4.30–4.38 (4H, m), 4.54 (2H, bs), 7.82 (1H, s). ¹³C-NMR (CDCl₃): δ 14.09, 20.92, 29.02, 29.17, 35.53, 53.44, 58.89, 63.97, 64.44, 68.86, 71.21, 110.80, 129.77, 133.29, 141.06, 144.75, 164.14.

(1-Butylpiperidin-4-yl)methyl 8-amino-7-cyano-2,3-dihydrobenzo[*b*][1,4]dioxine-5-carboxylate (**5**)³⁹

A mixture of **1** (200 mg, 0.42 mmol), KCN (42 mg, 0.64 mmol), CuI (17 mg, 0.084 mmol) and 1,10-phenanthroline (30 mg, 0.17 mmol) in DMF (500 μL) was stirred at 110 °C in an oven-dried sealed tube under Ar for 42 h. Then the mixture was cooled to rt and filtered through celite. The celite pad was rinsed twice with CH₂Cl₂. The combined filtrates were evaporated to dryness and dissolved in MeOH. Separation by HPLC on an XTerra RP18 column (10 μm, 19 × 250 mm; Waters) eluted with MeOH-*aq.* NH₄OH (0.025%) (9: 1 v/v) at 10 mL/min gave **5** as a pale yellow solid (*t*_R = 14 min; 30 mg, 19%). Mp: 108–110 °C. ¹H-NMR (CDCl₃): δ 0.92 (3H, t, *J* = 7.2 Hz), 1.28–1.50 (6H, m), 1.81–1.84 (3H, m), 2.15 (2H, t, *J* = 11.4 Hz), 2.51 (2H, m), 3.16 (2H, d, *J* = 11.6 Hz), 4.12 (2H, d, *J* = 6.0 Hz), 4.34–4.44 (4H, m), 4.87 (2H, s), 7.67 (1H, s). ¹³C-NMR (CDCl₃): δ 14.09, 20.92, 29.06, 29.23, 35.53, 53.42, 58.89, 63.73, 64.85, 69.13, 88.00, 110.11, 116.81, 128.80, 129.90, 143.32, 147.47, 163.64. LC-MS *m/z*: [M + H]⁺, 374.2. HRMS: calc'd for C₂₀H₂₇N₃O₄ (M⁺ + H), 374.2080; found, 374.2070.

(1-Butylpiperidin-4-yl)methyl 8-(methylamino)-2,3-dihydrobenzo[*b*][1,4]dioxine-5-carboxylate (**6**)⁴⁰

A mixture of **4** (2.24 g, 6.44 mmol) and paraformaldehyde (580 mg) in ethanol (58 mL) was heated at 60 °C overnight and then NaBH₄ (245 mg, 6.44 mmol) was added. The mixture was heated at 70 °C for 2 h and then evaporated to dryness. The residue was diluted with water and extracted thrice with CH₂Cl₂. The combined organic layers were dried on MgSO₄ and evaporated to dryness. Silica gel chromatography of the residue (MeOH-CH₂Cl₂, 1: 20 v/v) gave **6** as a colorless oil (1.4 g, 60%). ¹H-NMR (CDCl₃): δ 0.92 (3H, t, *J* = 7.2 Hz), 1.29–1.35 (2H, m), 1.52–1.53 (4H, m), 1.78–1.82 (3H, m), 1.99 (2H, t, *J* = 11.2 Hz), 2.39–2.35 (2H, m), 2.90 (2H, d, *J* = 5.2 Hz), 3.02 (2H, d, *J* = 11.2 Hz), 4.10 (2H, d, *J* = 6.0 Hz), 4.28–4.30 (2H, m), 4.34–4.36 (2H, m), 4.46–4.52 (1H, m), 6.18 (1H, d, *J* = 8.8 Hz), 7.51 (1H, d, *J* = 8.8 Hz). ¹³C-NMR (CDCl₃): δ 14.02, 20.85, 28.78, 28.88, 29.85, 35.47, 53.38, 58.75, 63.83, 64.50, 68.18, 100.99, 107.36, 125.60, 129.78, 143.15, 144.11, 165.40. LC-MS *m/z*: [M + H]⁺, 363.3. HRMS: calc'd for C₂₀H₃₀N₂O₄ (M⁺ + H), 363.2284; found, 363.2281.

(1-Butylpiperidin-4-yl)methyl 8-amino-7-methyl-2,3-dihydrobenzo[*b*][1,4]dioxine-5-carboxylate (7)⁴¹

1 (210 mg, 0.44 mmol), *N*-methyl pyrrolidone (560 μ L), Pd₂(dba)₃ (14 mg, 0.015 mmol) and PPh₃ (28 mg, 0.11 mmol) were added to an oven-dried sealed tube. The mixture was heated at 50 °C for 10 min and then CuI (6.0 mg, 0.031 mmol) was added. The mixture was stirred for another 10 min and then Me₄Sn (91 μ L, 0.65 mmol) was added. The mixture was heated at 70 °C for 48 h. The solvent was evaporated off and, the residue was diluted with water and extracted thrice with CH₂Cl₂. The combined organic layers were dried over MgSO₄ and evaporated to dryness. Silica gel chromatography (MeOH-CH₂Cl₂, 1: 20 v/v) of the residue gave **7** as a pale yellow oil (70 mg, 44%). ¹H-NMR (CDCl₃): δ 0.92 (3H, t, *J* = 7.2 Hz), 1.25–1.58 (7H, m), 1.72–1.81 (3H, m), 1.78–1.82 (3H, m), 1.95 (2H, t, *J* = 11.2 Hz), 2.11 (3H, s), 2.31–2.35 (2H, m), 2.98 (2H, d, *J* = 11.6 Hz), 4.08 (2H, s), 4.10 (2H, d, *J* = 6.4 Hz), 4.30–4.36 (4H, m), 7.28 (1H, s). ¹³C-NMR (CDCl₃): δ 14.02, 16.48, 20.84, 28.69, 35.39, 53.34, 58.72, 63.87, 64.45, 68.27, 107.79, 113.41, 125.34, 130.17, 138.86, 143.34, 165.46. LC-MS: *m/z* [M + H]⁺, 363.1. HRMS: calc'd for C₂₀H₃₀N₂O₄ (M⁺ + H), 363.2284; found, 363.2279.

(1-Butylpiperidin-4-yl)methyl 7-chloro-8-(methylamino)-2,3-dihydrobenzo[*b*][1,4]dioxine-5-carboxylate (8)

N-Chlorosuccinimide (70 mg, 0.52 mmol) was added in portions to a stirred solution of **6** (190 mg, 0.52 mmol) in acetic acid (5 mL) at rt and left for 1 h. The acetic acid was evaporated off and the residue was basified with NaHCO₃ solution and extracted thrice with CH₂Cl₂. The combined organic layers were evaporated to dryness. Silica gel chromatography (MeOH-CH₂Cl₂, 1: 20 v/v) of the residue gave **8** as a pale yellow oil (80 mg, 38%). ¹H-NMR (CDCl₃): δ 0.92 (3H, t, *J* = 7.2 Hz), 1.29–1.35 (2H, m), 1.44–1.53 (4H, m), 1.75–1.80 (3H, m), 1.98 (2H, t, *J* = 11.2 Hz), 2.34–2.38 (2H, m), 3.00 (2H, d, *J* = 11.2 Hz), 3.12 (3H, d, *J* = 4.8 Hz), 4.10 (2H, d, *J* = 6.4 Hz), 4.28–4.36 (5H, m), 7.45 (1H, s). ¹³C-NMR (CDCl₃): δ 14.03, 20.84, 28.83, 28.97, 33.82, 35.42, 53.35, 58.76, 63.63, 64.26, 68.72, 109.54, 112.30, 124.79, 133.42, 140.14, 144.06, 164.27. LC-MS: *m/z* [M + H]⁺, 397.6. HRMS: calc'd for C₂₀H₂₉ClN₂O₄ (M⁺ + H), 397.1894; found, 397.1886.

(1-Butylpiperidin-4-yl)methyl 7-bromo-8-(methylamino)-2,3-dihydrobenzo[*b*][1,4]dioxine-5-carboxylate (9)

The procedure for the synthesis of **8** was used with a solution of **6** (140 mg, 0.39 mmol) in acetic acid (4 mL) and with *N*-bromosuccinimide (70 mg, 0.39 mmol) replacing *N*-chlorosuccinimide, and gave **9** as a pale yellow oil (50 mg, 30%). ¹H-NMR (CDCl₃): δ 0.92 (3H, t, *J* = 7.2 Hz), 1.29–1.52 (6H, m), 1.73–1.78 (3H, m), 1.92 (2H, dt, *J* = 2.4, 11.6 Hz), 2.29–2.33 (2H, m), 2.96 (2H, d, *J* = 11.2 Hz), 3.10 (3H, d, *J* = 5.2 Hz), 4.10 (2H, d, *J* = 6.4 Hz), 4.27–4.37 (5H, m), 7.62 (1H, s). ¹³C-NMR (CDCl₃): δ 14.09, 20.93, 29.11, 29.27, 34.11, 35.58, 53.48, 58.93, 63.60, 64.31, 68.96, 102.04, 110.64, 127.61, 133.67, 141.18, 144.76, 164.21. LC-MS: *m/z* [M + H]⁺ 441.1. HRMS: calc'd for C₂₀H₂₉⁸⁰BrN₂O₄ (M⁺ + H), 441.1389; found, 441.1368.

(1-Butylpiperidin-4-yl)methyl 7-iodo-8-(methylamino)-2,3-dihydrobenzo[*b*][1,4]dioxine-5-carboxylate (10)

The procedure for the synthesis of **8** was used with a solution of **6** (195 mg, 0.54 mmol) in acetic acid (4 mL) and with *N*-iodosuccinimide (122 mg, 0.54 mmol) replacing *N*-chlorosuccinimide to give **10** as a colorless oil (147 mg, 56%). ¹H-NMR (CDCl₃): δ 0.92 (3H, t, *J* = 7.2 Hz), 1.19–1.54 (6H, m), 1.72–1.80 (3H, m), 1.96 (2H, t, *J* = 11.2 Hz), 2.33–2.37 (2H, m), 3.00 (2H, d, *J* = 11.2 Hz), 3.07 (3H, d, *J* = 3.2 Hz), 4.05 (1H, bs), 4.09–4.11 (3H, m), 4.28–4.38 (4H, m), 7.84 (1H, s). ¹³C-NMR (CDCl₃): δ 14.06, 20.89, 28.91, 29.06,

34.50, 35.47, 53.39, 58.83, 63.57, 64.35, 68.88, 112.25, 133.12, 133.80, 143.57, 145.67, 164.02. LC-MS: m/z $[M + H]^+$, 489.2. HRMS: calc'd for $C_{20}H_{29}IN_2O_4$ ($M^+ + H$), 489.1250; found, 489.1258.

(1-Butylpiperidin-4-yl)methyl 7-cyano-8-(methylamino)-2,3-dihydrobenzo[*b*][1,4]dioxine-5-carboxylate (**11**)³⁹

A mixture of **10** (140 mg, 0.29 mmol), KCN (21 mg, 0.32 mmol), CuI (6.0 mg, 0.029 mmol), 1,10-phenanthroline (11 mg, 0.057 mmol) in DMF (300 μ L) was stirred at 110 °C in an oven-dried sealed tube under Ar for 48 h. The mixture was cooled to rt and then filtered through celite. The celite pad was rinsed twice with CH_2Cl_2 . The combined filtrates were evaporated to dryness and then dissolved in MeOH. Separation by HPLC on an XTerra RP18 column (10 μ m, 19 \times 250 mm; Waters) eluted with MeOH-*aq.* NH_4OH (0.025%) (9: 1 v/v) at 15 mL/min gave **11** (t_R = 10.31 min; 8 mg, 7%). ¹H-NMR ($CDCl_3$): δ 0.92 (3H, t, J = 7.2 Hz), 1.25–1.52 (7H, m), 1.73–1.79 (3H, m), 1.90–1.96 (2H, t, J = 11.2 Hz), 2.30–2.34 (2H, m), 2.96 (2H, d, J = 11.2 Hz), 3.33 (3H, d, J = 5.2 Hz), 4.09 (2H, d, J = 6.0 Hz), 4.30–4.40 (4H, m), 5.04–5.06 (1H, m), 7.71 (1H, s). ¹³C-NMR ($CDCl_3$): δ 14.09, 20.92, 29.10, 29.26, 31.63, 35.53, 53.44, 58.91, 63.79, 64.61, 69.07, 85.21, 109.42, 119.52, 130.19, 131.81, 144.86, 145.98, 163.61. LC-MS: m/z $[M + H]^+$, 388.2. HRMS: calc'd for $C_{21}H_{29}N_3O_4$ ($M^+ + H$), 388.2236; found, 388.2219.

Methyl 4-(benzyloxycarbonylamino)-2-methoxybenzoate (**12b**)⁴²

Saturated $NaHCO_3$ solution (50 mL) and then $CbzCl$ (1.70 mL, 12.1 mmol) were added to a solution of methyl 4-amino-2-methoxybenzoate (**12a**, 2.0 g, 11 mmol) in THF (50 mL). The mixture was stirred at rt for 4 h and then filtered through celite. The filtrate was acidified to pH < 1 and extracted with EtOAc. After concentration of this solution, the residue was purified by recrystallization from EtOAc-hexane to give **12b** as a white solid (3.09 g, 89%). Mp: 132–134 °C. ¹H-NMR ($CDCl_3$): δ 3.84 (3H, s), 3.87 (3H, s), 5.19 (2H, s), 6.77 (1H, dd, J = 1.6, 8.4 Hz), 6.66, 7.11 (1H, s), 7.35–7.39 (5H, m), 7.79 (1H, d, J = 8.4 Hz). ¹³C-NMR ($CDCl_3$): δ 51.84, 55.97, 67.30, 101.72, 109.43, 114.05, 128.32, 128.52, 128.68, 133.06, 135.67, 143.19, 152.96, 160.76, 166.07.

(1-Butylpiperidin-4-yl)methyl 4-amino-2-methoxybenzoate (**12**)⁴³

A THF solution of *n*-BuLi (1.6M; 612 μ L, 0.98 mmol) was added drop-wise to a solution of (4-butylpiperidin-1-yl)methanol (190 mg, 1.11 mmol) in THF (3 mL) in an oven-dried flask under Ar at 0 °C. After stirring the mixture for 10 min, a solution of **12b** (350 mg, 1.11 mmol) in THF (3 mL) was added drop-wise. The mixture was stirred for 2 h, poured into water and extracted with CH_2Cl_2 . The combined organic layers were dried on $MgSO_4$ and evaporated to dryness. Silica gel chromatography of the residue (MeOH- CH_2Cl_2 , 1: 15 v/v) gave a white solid (200 mg). MeOH (15 mL) was added to this solid (170 mg) plus Pd/C (10%; 25 mg). The mixture was degassed with H_2 for 30 min, stirred at rt overnight, filtered and evaporated to dryness. Silica gel chromatography (MeOH- CH_2Cl_2 , 1: 20 v/v) of the residue gave **12** as a colorless oil (108 mg, 36%). ¹H-NMR ($CDCl_3$): δ 0.91 (3H, t, J = 7.6 Hz), 1.28–1.53 (6H, m), 1.71–1.80 (3H, m), 1.95 (2H, t, J = 11.2 Hz), 2.31–2.35 (2H, m), 2.97 (2H, d, J = 11.6 Hz), 3.81 (3H, s), 4.09 (2H, d, J = 6.0 Hz), 4.22 (2H, br), 6.18–6.22 (2H, m), 7.72 (1H, d, J = 8.4 Hz). ¹³C-NMR ($CDCl_3$): δ 14.06, 20.88, 28.94, 29.07, 35.53, 53.45, 55.66, 58.84, 68.33, 97.58, 106.23, 108.78, 134.06, 152.29, 161.87, 165.63. LC-MS: m/z $[M + H]^+$, 321.1. HRMS: calc'd for $C_{18}H_{28}N_2O_3$ ($M^+ + H$), 321.2178; found, 321.2181.

Methyl 4-amino-3-methoxybenzoate (**13a**)

Pd/C (10%; 1.8 g) and HCOOK (7.4 g, 88 mmol) were added to a solution of methyl 3-methoxy-4-nitrobenzoate (2.0 g, 9.5 mmol) in MeOH (50 mL). The mixture was refluxed at

80 °C for 1 h. The suspension was cooled and filtered through celite. The filtrate was evaporated to dryness. Silica gel chromatography (30% EtOAc in hexane) of the residue gave **13a** as a white solid (1.72 g, 100%). Mp: 127–129 °C. Lit. mp 127–128 °C.⁴⁴ ¹H-NMR (CDCl₃): δ 3.86 (3H, s), 3.90 (3H, s), 4.22 (2H, br), 4.45 (1H, s), 6.66 (1H, d, *J* = 8.4 Hz), 7.55 (1H, d, *J* = 8.4 Hz). ¹³C-NMR (CDCl₃): δ 51.69, 55.58, 111.15, 113.09, 119.41, 124.09, 141.21, 146.10, 167.35.

Methyl 4-(benzyloxycarbonylamino)-3-methoxybenzoate (**13b**)

As described for **12b** from **12a**, **13b** was obtained from **13a** as a white solid (58%). Mp: 95–96 °C. ¹H-NMR (CDCl₃): δ 3.89 (6H, 2s), 5.22 (2H, s), 7.34–7.52 (6H, m), 7.69 (1H, dd, *J* = 1.6, 8.4 Hz), 8.20 (1H, d, *J* = 8.4 Hz). ¹³C-NMR (CDCl₃): δ 52.05, 55.90, 67.27, 110.73, 116.92, 123.48, 124.25, 128.43, 128.49, 128.68, 132.06, 135.84, 146.96, 152.93, 166.81.

(1-Butylpiperidin-4-yl)methyl 4-amino-3-methoxybenzoate (**13**)

As described for **12** from **12b**, compound **13** was obtained from **13b** as a white solid in 13% yield. Mp: 162–164 °C. ¹H-NMR (CDCl₃): δ 0.94 (3H, t, *J* = 7.2 Hz), 1.31–1.39 (2H, m), 1.72–1.80 (2H, m), 1.95–1.99 (5H, br), 2.55 (2H, br), 2.75–2.79 (2H, m), 3.37 (2H, d, *J* = 11.6 Hz), 3.90 (3H, s), 4.18 (2H, d, *J* = 4.0 Hz), 4.38 (2H, br), 6.69 (1H, d, *J* = 8.0 Hz), 7.43 (1H, d, *J* = 2 Hz), 7.52–7.53 (1H, dd, *J* = 1.6, 8 Hz); ¹³C-NMR: δ (CDCl₃) 14.09, 20.94, 29.10, 29.25, 35.71, 53.50, 55.63, 58.94, 68.84, 111.21, 113.05, 119.75, 124.02, 141.09, 146.16, 166.86. LC-MS: *m/z* [M + H]⁺, 321.3. HRMS: calc'd for C₁₈H₂₈N₂O₃ (M⁺ + H), 321.2178; found, 321.2190.

4-Amino-N-((1-butylpiperidin-4-yl)methyl)-3-methoxybenzamide (**14**)³⁷

CDI (485 mg, 2.99 mmol) was added in portions to a suspension of 4-amino-3-methoxybenzoic acid (500 mg, 2.99 mmol) in MeCN (30 mL) in an oven-dried flask and then stirred for 2 h at rt. (1-Butylpiperidin-4-yl)methanamine (510 mg, 2.99 mmol) in MeCN (10 mL) was added drop-wise. The above solution was stirred overnight and evaporated to dryness. Silica gel chromatography (from 9% to 33% MeOH in CH₂Cl₂) of the residue gave an oil which was then dissolved in MeCN and filtered through an Iso-Disc™ Filter (PTFE 25-4, 25 mm × 0.45 μm). The filtrate was dried to give **14** as a light brown foam-like oil (660 mg, 69%). ¹H-NMR (CDCl₃): δ 0.91 (3H, t, *J* = 7.2 Hz), 1.26–1.38 (4H, m), 1.42–1.48 (2H, m), 1.57–1.64 (1H, m), 1.88 (2H, dt, *J* = 2.0, 11.6 Hz), 2.27–2.31 (2H, m), 2.92 (2H, d, *J* = 11.6 Hz), 3.30 (2H, t, *J* = 6.4 Hz), 3.86 (3H, s), 4.14 (2H, bs), 6.34 (1H, t, *J* = 5.6 Hz), 6.63 (1H, d, *J* = 8.0 Hz), 7.14 (1H, dd, *J* = 2.0, 8.4 Hz), 7.37 (1H, d, *J* = 1.6 Hz). ¹³C-NMR (CDCl₃): δ 12.58, 19.41, 27.72, 28.58, 34.79, 43.98, 52.06, 54.08, 57.36, 108.34, 111.67, 118.05, 122.76, 138.15, 145.21, 166.04. LC-MS: *m/z* [M + H]⁺, 320.2. HRMS: calc'd for C₁₈H₃₀N₃O₂ (M⁺ + H), 320.2338; found, 320.2328.

(1-Propylpiperidin-4-yl)methyl 4-amino-3-methoxybenzoate (**15**)

CDI (485 mg, 2.99 mmol) was added in portions to a suspension of 4-amino-3-methoxybenzoic acid (500 mg, 2.99 mmol) in MeCN (15 mL) in an oven-dried flask and stirred for 30 min at rt. Solvent was then evaporated off and the residue dissolved in anhydrous THF (7 mL). In another oven-dried flask, (1-propylpiperidin-4-yl)methanol (319 mg, 2.03 mmol) was dissolved in anhydrous THF (7 mL) and cooled (ice-bath). A THF solution of *n*-BuLi (1.6M, 1.27 mL, 2.03 mmol) was added drop-wise to this solution and stirred for 10 min. The solution of CDI-activated acid was then added, stirred overnight and evaporated to dryness. The residue was diluted with water and extracted thrice with CH₂Cl₂. The combined organic layers were dried on MgSO₄ and evaporated to dryness. Silica gel chromatography (MeOH-CH₂Cl₂, 1: 30 v/v) of the residue gave **15** as a yellow oil (312 mg, 50%). ¹H-NMR (CDCl₃): δ 0.89 (3H, t, *J* = 7.2 Hz), 1.37–1.56 (4H, m), 1.72–1.78 (2H, m),

1.94 (2H, dt, $J = 2.0, 12.0$ Hz), 2.25–2.29 (2H, m), 2.94 (2H, d, $J = 11.6$ Hz), 3.86 (3H, s), 4.13 (2H, d, $J = 6.0$ Hz), 4.36 (2H, bs), 6.63 (1H, d, $J = 8.4$ Hz), 7.44 (1H, d, $J = 2.0$ Hz), 7.54 (1H, dd, $J = 1.6, 8.0$ Hz). $^{13}\text{C-NMR}$ (CDCl_3): δ 12.05, 20.15, 29.06, 35.67, 53.40, 55.50, 61.09, 68.76, 111.08, 112.90, 119.35, 124.03, 141.31, 146.03, 166.83. LC-MS: m/z $[\text{M} + \text{H}]^+$, 307.2. HRMS: calc'd for $\text{C}_{17}\text{H}_{26}\text{N}_2\text{O}_3$ ($\text{M}^+ + \text{H}$), 307.2022; found, 307.2022.

(1-Pentylpiperidin-4-yl)methyl 4-amino-3-methoxybenzoate (16)

As described for **15**, compound **16** was obtained from 4-amino-3-methoxybenzoic acid and 1-pentylpiperidin-4-ylmethanol as a pale yellow oil (200 mg, 30%). $^1\text{H-NMR}$ (CDCl_3): δ 0.89 (3H, t, $J = 7.2$ Hz), 1.24–1.52 (8H, m), 1.77–1.79 (3H, m), 1.89–1.95 (2H, dt, $J = 2.4, 12$ Hz), 1.95–2.32 (2H, m), 2.96 (2H, d, $J = 11.6$ Hz), 3.89 (3H, s), 4.13 (2H, d, $J = 6.4$ Hz), 4.26 (2H, bs), 6.65 (1H, d, $J = 8$ Hz), 7.45 (1H, d, $J = 1.6$ Hz), 7.55 (1H, dd, $J = 2, 8.4$ Hz). $^{13}\text{C-NMR}$ (CDCl_3): δ 14.06, 22.64, 26.78, 29.10, 29.94, 35.71, 53.49, 55.59, 59.23, 68.82, 111.16, 113.00, 119.61, 124.03, 141.17, 146.12, 166.86. LC-MS: m/z $[\text{M} + \text{H}]^+$ 335.2. HRMS: calc'd for $\text{C}_{19}\text{H}_{30}\text{N}_2\text{O}_3$ ($\text{M}^+ + \text{H}$), 335.2335; found, 335.2325.

3-Bromo-5-fluoro-2-hydroxybenzoic acid (17a)

5-Fluoro-2-hydroxybenzoic acid (20 g, 0.13 mol) and *N*-bromosuccinimide (23 g, 0.13 mol) were added to acetic acid (200 mL). The mixture was heated at 80 °C for 24 h. After evaporation of all acetic acid, the residue was recrystallized from EtOAc and hexane to give **17a** (27 g, 90%). Mp 233–235 °C. Lit. mp 233 °C.⁴⁵ $^1\text{H-NMR}$ (MeOD): δ 7.48–7.50 (2H, br). $^{13}\text{C-NMR}$ (MeOD): δ 111.91 (d, $J = 10.0$ Hz), 114.92 (d, $J = 7.0$ Hz), 116.22 (d, $J = 24.0$ Hz), 127.13 (d, $J = 26.0$ Hz), 155.70 (d, $J = 239$ Hz), 156.56, 172.24.

5-Fluoro-2,3-dihydroxybenzoic acid (17b)⁴⁶

NaOH solution (2.5M; 300 mL) was stirred under an aspirator for 2 h. Then CuSO_4 (250 mg, 1.57 mmol) was added and the solution further stirred for 1 h. To the filtrate of this solution was added **17a** (22 g, 0.094 mol) and the mixture was refluxed overnight. The reaction mixture was cooled, acidified with 37% HCl to pH < 2 and then evaporated to dryness. The residue was dissolved in MeOH and filtered through celite. The combined filtrates were evaporated to dryness. Silica gel chromatography (30% EtOAc in hexane with 1% HOAc) of the residue gave **17b** as a white solid (12 g, 75%). Mp 184–186 °C. $^1\text{H-NMR}$ (MeOD): δ 6.58 (1H, dd, $J = 3.2, 9.6$ Hz), 7.01 (1H, dd, $J = 3.0, 9.0$ Hz). $^{13}\text{C-NMR}$ (MeOD): δ 105.80 (d, $J = 24.0$ Hz), 109.44 (d, $J = 26.0$ Hz), 113.48 (d, $J = 9.0$ Hz), 148.36, 148.50 (d, $J = 9.0$ Hz), 156.24 (d, $J = 234$ Hz), 173.03. $^{19}\text{F-NMR}$ (CDCl_3): δ -125.39. HRMS: calc'd for $\text{C}_7\text{H}_5\text{FO}_4$ ($\text{M}^+ + \text{H}$), 172.01719; found, 172.01717.

Methyl 7-fluoro-2,3-dihydrobenzo[b][1,4]dioxine-5-carboxylate (17c)⁴⁷

TMSCHN_2 (2.0M, 45 mL, 90 mmol) was added in portions to a solution of **17b** (6.0 g, 35 mmol) in MeOH (150 mL) and Et_2O (150 mL). After 20 min the solvent was evaporated off. Silica gel chromatography (15% EtOAc in hexane) of the residue gave the methyl ester (5.6 g, 93%). A mixture of this ester (3.9 g, 20 mmol) and Cs_2CO_3 (16.2 g, 49.8 mmol) in DMF (40 mL) was stirred at rt for 0.5 h. Then 1,2-dibromoethane (5.07 g, 27.0 mmol) was added and the mixture stirred at 80 °C for 16 h. The mixture was cooled to rt and filtered through celite, which was then rinsed twice with DMF. The combined DMF rinses were evaporated to dryness under high vacuum to give a dark red residue, which after silica gel chromatography (20% EtOAc in hexane with 1% HOAc) gave **17c** as a white solid (3.37 g, 77%). Mp 108–110 °C. $^1\text{H-NMR}$ (MeOD): δ 3.89 (3H, s), 4.28–4.34 (4H, m), 6.77 (1H, dd, $J = 3.2, 8.8$ Hz), 7.11 (1H, dd, $J = 3.2, 8.8$ Hz). $^{13}\text{C-NMR}$ (MeOD): δ 52.27, 64.10, 64.30, 108.71 (d, $J = 26.0$ Hz), 109.73 (d, $J = 24.0$ Hz), 120.07 (d, $J = 9.0$ Hz), 140.65, 144.69 (d, J

= 12.0 Hz), 155.69 (d, $J = 238$ Hz), 164.99. ^{19}F -NMR (CDCl_3): $\delta -121.14$. HRMS: calc'd for $\text{C}_{10}\text{H}_9\text{FO}_4$ ($\text{M}^+ + \text{H}$), 213.0563; found, 213.0561.

Methyl 7-fluoro-8-nitro-2,3-dihydrobenzo[b][1,4]dioxine-5-carboxylate (17d)

17c (3.4 g, 15.9 mol) was added in portions to a flask cooled between -50 and -60 °C and containing HNO_3 (90%; 30 mL). After 16 min, the reaction mixture was warmed gradually and water was added. The precipitate was filtered off and washed with water to give **17d** as a yellow solid (3.58 g, 88%). **17d** was used in the next step without further purification. Mp 133 – 135 °C. ^1H -NMR (CDCl_3): δ 3.92 (3H, s), 4.33–4.40 (4H, m), 6.83 (1H, d, $J = 11.2$ Hz). ^{13}C -NMR (CDCl_3): δ 53.59, 64.22, 64.80, 107.12 (d, $J = 25.0$ Hz), 119.75 (d, $J = 1.0$ Hz), 137.57 (d, $J = 3.0$ Hz), 148.50 (d, $J = 12.0$ Hz), 150.10 (d, $J = 257$ Hz), 162.83 (d, $J = 3.0$ Hz). ^{19}F -NMR (CDCl_3): $\delta -124.76$. HRMS: calc'd for $\text{C}_{10}\text{H}_8\text{FNO}_6$ ($\text{M}^+ + \text{Na}$), 280.0233; found, 280.0225.

8-Amino-7-fluoro-2,3-dihydrobenzo[b][1,4]dioxine-5-carboxylic acid (17e)

Pd/C (10%, 340 mg) and potassium formate (1.4 g, 17 mmol) were added to a solution of **17d** (500 mg, 1.94 mmol) in MeOH (10 mL). The mixture was refluxed at 80 °C for 2 h, and then cooled and filtered through celite. 37% HCl was added to the filtrate until no CO_2 was released. The white solid was filtered off and the filtrate evaporated to dryness. Silica gel chromatography (MeOH- CH_2Cl_2 , 1: 30 v/v) of the residue gave **17e** as light brown solid (350 mg, 85%). Mp 174 – 176 °C. ^1H -NMR (MeOD): δ 4.08–4.10 (2H, m), 4.18–4.21 (2H, m), 6.67 (1H, d, $J = 11.6$ Hz). ^{13}C -NMR (MeOD): δ 65.06, 66.29, 106.06 (d, $J = 4.0$ Hz), 109.21 (d, $J = 23.0$ Hz), 134.30 (d, $J = 11.0$ Hz), 134.44, 140.54 (d, $J = 3.0$ Hz), 147.01 (d, $J = 232$ Hz), 169.25. ^{19}F -NMR (MeOD): $\delta -143.14$. HRMS: calc'd for $\text{C}_9\text{H}_8\text{FNO}_4$ ($\text{M}^+ + \text{H}$), 214.0516; found, 214.0510.

(1-Butylpiperidin-4-yl)methyl 8-amino-7-fluoro-2,3-dihydrobenzo[b][1,4]dioxine-5-carboxylate (17)

Saturated NaHCO_3 solution (5 mL) and THF (20 mL), followed by CbzCl (662 mg, 3.87 mmol) were added to a flask containing **17e** (750 mg, 3.52 mmol). After stirring the mixture overnight, the THF was evaporated off. The solution was diluted with water, acidified with 37% HCl to pH < 1 and extracted thrice with EtOAc. The combined organic layers were dried on MgSO_4 and evaporated to dryness. Silica gel chromatography (30% EtOAc in hexane) of the residue gave Cbz-protected **17e** as a white solid (420 mg, 34%).

Cbz-protected **17e** (420 mg, 1.21 mmol), CDI (196 mg, 1.21 mmol) and MeCN (30 mL) were added to an oven-dried flask under Ar. The mixture was stirred for 2 h and evaporated to dryness. The residue was dissolved in THF (10 mL). In another oven-dried flask, a solution of (4-butylpiperidin-1-yl)methanol (207 mg, 1.21 mmol) in THF (10 mL) was added drop-wise to a THF solution of *n*-BuLi (1.6M; 760 μL , 1.21 mmol) under Ar at 0 °C and stirred at this temperature for 10 min. Then the activated acid in THF was added drop-wise to the prepared lithium alkoxide solution. The mixture was warmed to rt and stirred overnight. The reaction mixture was evaporated to remove THF, diluted with water and extracted with CH_2Cl_2 . The residue was purified by silica gel chromatography (MeOH- CH_2Cl_2 , 1: 15 v/v) to give an oil. Pd/C (10%; 25 mg) was then added to a solution of the oil in MeOH (15 mL). The suspension was degassed for 30 min with H_2 and then stirred at rt overnight under H_2 . The mixture was filtered through celite and evaporated to dryness. Silica gel chromatography (MeOH- CH_2Cl_2 , 1: 15 v/v) of the residue gave **17** as a yellow solid (260 mg, 59%). Mp 160 – 162 °C. ^1H -NMR (CDCl_3): δ 0.95 (3H, t, $J = 7.4$ Hz), 1.33–1.39 (2H, m), 1.70–1.95 (7H, m), 2.39–2.42 (2H, m), 2.67–2.71 (2H, m), 3.31 (2H, d, $J = 11.6$ Hz), 4.19–4.27 (6H, m), 4.88 (2H, s), 6.71 (1H, d, $J = 11.6$ Hz). ^{13}C -NMR (CDCl_3): δ 13.74, 20.46, 26.97, 34.35, 52.56, 57.84, 63.84, 64.56, 68.14, 105.60 (d, $J = 4.0$ Hz), 108.19

(d, $J = 23.0$ Hz), 132.20 (d, $J = 15.0$ Hz), 133.43 (d, $J = 11.0$ Hz), 139.81 (d, $J = 3.0$ Hz), 145.24 (d, $J = 232$ Hz), 166.72 (d, $J = 4$ Hz). ^{19}F -NMR (CDCl_3): $\delta -141.99$. LC-MS: m/z $[\text{M} + \text{H}]^+$, 367.1. HRMS: calc'd for $\text{C}_{19}\text{H}_{27}\text{FN}_2\text{O}_4$ ($\text{M}^+ + \text{H}$), 367.2033; found, 367.2033.

Methyl 4-amino-3-(fluoromethoxy)benzoate (**18a**) ⁴⁸

Chlorofluoromethane was bubbled into a tube (30-mL) containing anhydrous DMF (10 mL), methyl 4-amino-3-hydroxybenzoate (500 mg, 2.99 mmol) and Cs_2CO_3 (1.95 g, 5.98 mmol) for 14 min at -70 °C. The tube was then sealed and slowly warmed to rt. The mixture was stirred for 5 d then filtered through celite which was then rinsed thrice with EtOAc. The combined rinses were evaporated to dryness. Silica gel chromatography (20% EtOAc in hexane) of the residue gave **18a** as a pale yellow solid (368 mg, 62%). Mp: 69–70 °C. ^1H -NMR (CDCl_3): δ 3.84 (3H, s), 4.46 (2H, br), 5.71 (2H, d, $J = 54.4$ Hz), 6.69 (1H, d, $J = 8$ Hz), 7.63 (1H, dd, $J = 8.4, 1.6$ Hz), 7.68 (1H, s). ^{13}C -NMR (CDCl_3): δ 51.73, 100.22, 102.40, 114.25, 116.72, 119.23, 126.79, 142.44, 142.92, 142.95, 166.90. ^{19}F -NMR (CDCl_3): $\delta -147.56$. LC-MS: m/z $[\text{M} + \text{H}]^+$ 200.1. HRMS: calc'd for $\text{C}_9\text{H}_{11}\text{FNO}_3$ ($\text{M}^+ + \text{H}$), 200.0723; found, 200.0723.

(1-Butylpiperidin-4-yl)methyl 4-amino-3-(fluoromethoxy)benzoate (**18**)

n-BuLi (1.6M; 1.6 mmol; 1 mL) was added drop-wise to a solution of (4-butylpiperidin-1-yl)methanol (280 mg, 1.64 mmol) in THF (2 mL) contained in an oven-dried flask under Ar at 0 °C. The solution was stirred for 10 min and **18a** (160 mg, 0.80 mmol) in THF (2 mL) was added drop-wise. The mixture was stirred overnight, poured into water and extracted with CH_2Cl_2 . The combined organic layers were dried on MgSO_4 and evaporated to dryness. Silica gel chromatography (MeOH- CH_2Cl_2 , 1: 20 v/v) of the residue gave **18** as a pale yellow oil (160 mg, 59 %). ^1H -NMR (CDCl_3): δ 0.92 (3H, t, $J = 7.6$ Hz), 1.43–1.53 (6H, m), 1.75–1.80 (3H, m), 1.91–1.98 (2H, t, $J = 12.0$ Hz), 2.33 (2H, t, $J = 8$ Hz), 2.98 (2H, d, $J = 11.6$ Hz), 4.14 (2H, d, $J = 6.0$ Hz), 4.28 (2H, br), 5.76 (2H, d, $J = 54.4$ Hz), 6.71 (1H, d, $J = 8.0$ Hz), 7.67 (1H, dd, $J = 2.0, 8.4$ Hz), 7.70 (1H, s). ^{13}C -NMR (CDCl_3): δ 12.81, 19.65, 27.73, 27.89, 34.37, 52.18, 57.63, 67.66, 100.17 (d, $J = 219$ Hz), 113.04, 115.81, 118.78, 125.58, 140.86, 141.85 (d, $J = 2.0$ Hz), 165.00. ^{19}F -NMR (CDCl_3): $\delta -147.50$. LC-MS m/z $[\text{M} + \text{H}]^+$, 214.1. HRMS: calc'd for $\text{C}_{18}\text{H}_{28}\text{FN}_2\text{O}_3$ ($\text{M}^+ + \text{H}$), 339.2084; found, 339.2088.

Methyl 4-amino-3-(2-fluoroethoxy)benzoate (**19a**)

1-Chloro-2-fluoroethane (760 mg, 8.97 mmol) was added to a tube (15-mL) containing anhydrous DMF (5 mL), methyl 4-amino-3-hydroxybenzoate (500 mg, 2.99 mmol) and Cs_2CO_3 (1.95 g, 5.98 mmol). The tube was sealed and stirred for 88 h. The mixture was then filtered through celite which was rinsed thrice with EtOAc. The combined filtrate and rinses were evaporated to dryness. Silica gel chromatography (20% EtOAc in hexane) of the residue gave **19a** as a white solid (270 mg, 42%). Mp. 86–87 °C. ^1H -NMR (CDCl_3): δ 3.84 (3H, s), 4.18–4.27 (2H, dt, $J = 28.4, 4$ Hz), 4.39 (2H, br), 4.65–4.79 (2H, dt, $J = 47.2, 3.7$ Hz), 6.66 (1H, d, $J = 8$ Hz), 7.43 (1H, d, $J = 1.6$ Hz), 7.55 (1H, dd, $J = 8.4, 1.6$ Hz). ^{19}F -NMR (CDCl_3): $\delta -147.56$. LC-MS: m/z $[\text{M} + \text{H}]^+$, 214.1. HRMS: calc'd for $\text{C}_{10}\text{H}_{13}\text{FNO}_3$ ($\text{M}^+ + \text{H}$), 214.0879; found, 214.0876.

(1-Butylpiperidin-4-yl)methyl 4-amino-3-(2-fluoroethoxy)benzoate (**19**)

As described for **18** from **18a**, compound **19** was obtained from **19a** as a white solid in 48% yield. Mp 72–74 °C. ^1H -NMR (CDCl_3): δ 0.92 (3H, t, $J = 7.6$ Hz), 1.27–1.53 (6H, m), 1.74–1.79 (3H, m), 1.94 (2H, t, $J = 12.0$ Hz), 2.33 (2H, t, $J = 7.6$ Hz), 2.97 (2H, d, $J = 11.2$ Hz), 4.13 (2H, d, $J = 6$ Hz), 4.24–4.33 (2H, dt, $J = 28.4, 4.0$ Hz), 4.36 (2H, br), 4.69–4.83 (2H, dt, $J = 47.2, 4.0$ Hz), 6.67 (1H, d, $J = 8.0$ Hz), 7.45 (1H, d, $J = 2.0$ Hz), 7.57 (1H, dd, $J =$

1.6, 8.4 Hz). ^{13}C -NMR (CDCl_3): δ 14.07, 20.89, 29.03, 29.17, 35.65, 53.45, 58.88, 67.92 (d, $J = 20.0$ Hz), 68.82, 81.77 (d, $J = 170$ Hz), 112.94, 113.39, 119.45, 124.79, 141.70, 144.70, 166.67. ^{19}F -NMR (CDCl_3): δ -147.49. LC-MS m/z [$\text{M} + \text{H}$] $^+$, 353.2. HRMS: calc'd for $\text{C}_{19}\text{H}_{30}\text{FN}_2\text{O}_3$ ($\text{M}^+ + \text{H}$), 353.2240; found, 353.2238.

(1-Butylpiperidin-4-yl)methyl 4-amino-3-hydroxybenzoate (**20**)^{49,50}

AlCl_3 (311 mg, 2.34 mmol) and NaI (351 mg, 2.34 mmol) were added to a solution of **13** (500 mg, 1.56 mmol) in MeCN (10 mL). The mixture was refluxed overnight and extracted thrice with EtOAc. The combined organic layers were dried on MgSO_4 and evaporated to dryness. Silica gel chromatography (MeOH/ CH_2Cl_2 , 1:20 v/v) of the residue gave **20** as an orange-brown solid (200 mg, 42%). Mp: 210–212 °C. ^1H -NMR (CDCl_3): δ 0.98 (3H, t, $J = 7.6$ Hz), 1.31–1.43 (2H, m), 1.51–1.63 (4H, m), 1.87–1.93 (3H, m), 2.28 (2H, t, $J = 11.2$ Hz), 2.54–2.58 (2H, m), 3.17 (2H, d, $J = 12.0$ Hz), 4.14 (2H, d, $J = 5.6$ Hz), 6.68 (1H, d, $J = 8.0$ Hz), 7.34 (1H, d, $J = 2.0$ Hz), 7.38–7.41 (1H, dd, $J = 1.6, 8.0$ Hz); ^{13}C -NMR (CDCl_3): δ 13.96, 21.05, 27.46, 34.81, 53.45, 58.09, 68.05, 114.47, 116.05, 119.06, 124.34, 143.61, 144.84, 168.55. LC-MS: m/z [$\text{M} + \text{H}$] $^+$, 307.2. HRMS: calc'd for $\text{C}_{17}\text{H}_{26}\text{N}_2\text{O}_3$ ($\text{M}^+ + \text{H}$), 307.2022; found, 307.2025.

(1-Butylpiperidin-4-yl)methyl 8-amino-7-nitro-2,3-dihydrobenzo[*b*][1,4]dioxine-5-carboxylate (**21**)

A mixture of **21a**⁵¹ (200 mg, 0.79 mmol), $(\text{Boc})_2\text{O}$ (515 mg, 2.36 mmol) and DMAP (44 mg, 0.039 mmol) in CH_2Cl_2 (15 mL) was refluxed at 50 °C for 1 h, then quenched with water and extracted thrice with CH_2Cl_2 . The combined organic layers were dried on MgSO_4 and then condensed to a crude product, which after silica gel chromatography (30% EtOAc in hexane) gave *di*-Boc-protected **21a** as a yellow solid (**21b**; 343 mg, 95%). ^1H -NMR (CDCl_3): δ 1.41 (18H, s), 3.94 (3H, s), 4.37–4.49 (4H, m), 8.30 (1H, s). ^{13}C -NMR (CDCl_3): δ 27.78, 52.63, 63.77, 64.80, 83.66, 118.16, 120.06, 126.55, 138.19, 140.71, 148.53, 149.49, 163.61.

A THF solution of *n*-BuLi (1.6M; 0.95 mL, 1.52 mmol) was added drop-wise to a solution of (4-butylpiperidin-1-yl)methanol (260 mg, 1.52 mmol) in THF (2 mL) in an oven-dried flask, under Ar at 0 °C. After stirring this solution for 10 min, a solution of **21b** (343 mg, 0.76 mmol) in THF (4 mL) was added dropwise. The mixture was stirred overnight, poured into water and extracted with CH_2Cl_2 . The combined organic layers were dried on MgSO_4 and evaporated to dryness. Silica gel chromatography (MeOH/ CH_2Cl_2 , 1: 15 v/v) of the residue gave *mono*-Boc-protected **21** as a white solid (**21c**; 186 mg, 50%). ^1H -NMR (CDCl_3): δ 0.92 (3H, t, $J = 7.2$ Hz), 1.29–1.49 (15H, m), 1.78 (3H, t, $J = 12.4$ Hz), 1.93 (2H, t, $J = 10.0$ Hz), 2.31 (2H, t, $J = 7.6$ Hz), 2.96 (2H, d, $J = 11.6$ Hz), 4.16 (2H, d, $J = 6.4$ Hz), 4.39–4.48 (4H, m), 7.20 (1H, br), 8.15 (1H, s). ^{13}C -NMR (CDCl_3): δ 13.06, 19.88, 27.05, 28.01, 28.24, 34.43, 52.34, 57.85, 62.79, 63.70, 68.82, 81.43, 119.75, 124.57, 134.69, 135.44, 146.92, 150.51, 162.21.

21c (100 mg, 0.20 mmol) was stirred overnight in a dioxane solution of HCl (4M). The mixture was evaporated to dryness, diluted with water and then neutralized to pH 7 with *aq.* NH_4OH (1M). The solution was extracted thrice with CH_2Cl_2 . The organic layers were combined and dried on MgSO_4 . Evaporation of solvent gave **21** as a yellow solid (71 mg, 89%). Mp 108–110 °C ($n = 2$). ^1H -NMR (CDCl_3): δ 0.92 (3H, t, $J = 7.2$ Hz), 1.29–1.51 (7H, m), 1.79 (3H, d, $J = 8.4$ Hz), 1.92 (2H, t, $J = 11.6$ Hz), 2.33 (2H, t, $J = 7.6$ Hz), 2.98 (2H, d, $J = 11.6$ Hz), 4.14 (2H, d, $J = 6.4$ Hz), 4.38–4.46 (4H, m), 8.45 (1H, s). ^{13}C -NMR (CDCl_3): δ 14.06, 20.89, 28.99, 29.16, 35.47, 53.38, 58.85, 63.82, 64.84, 69.28, 108.62, 123.12, 125.47, 130.17, 139.42, 147.16, 163.70. LC-MS: m/z [$\text{M} + \text{H}$] $^+$, 394.2. HRMS: calc'd for $\text{C}_{19}\text{H}_{28}\text{N}_3\text{O}_6$ ($\text{M}^+ + \text{H}$), 394.1978; found, 394.1972.

Pharmacological Assay and Screen

Some ligands (**1**, **5**, **8**, **13**, **15**, **17–19**) were evaluated at Caliper Life Sciences (Hanover, MD) for binding to 5-HT₄ receptors in guinea pig striatal membranes at 37 °C with [³H]GR 113808 as reference radioligand.⁵²

All new ligands (**2**, **3** and **5–21**) plus the already known ligands **1** and **4** were submitted to the National Institute of Mental Health Psychoactive Drug Screening Program (NIMH-PDSP) for assessment of binding affinity to human recombinant 5-HT₄ receptors (reference radioligand, [³H]GR 113808) and a wide range of other receptors and binding sites (5-HT_{1A-1E}, 2A-2C, 3, 5A, 6, 7, α_{1A}, 1B, 1D, 2A-2C, BZP (rat brain site), β₁₋₃, σ_{1,2}, D1-5, DAT, DOR, GABAA, H1-4, KOR, M1-5, MOR, NET and SERT). Selected ligands (**1**, **4**, **6–13** and **17**) were also assessed for agonist/partial agonist activity in a GloSensor L9 assay for cAMP response and for antagonist activity. Detailed assay protocols are available at the NIMH-PDSP web site (<http://pdsp.cwru.edu>).

Radiochemistry

Production of NCA [¹¹C]carbon dioxide

No-carrier-added (NCA) [¹¹C]carbon dioxide was produced with a PETrace cyclotron (GE; Milwaukee, WI) according to the ¹⁴N(p,α)¹¹C reaction²⁰ by irradiation of nitrogen gas (300 psi) containing 1% oxygen with a proton beam (16.5 MeV, 45 DA) for either 20 or 40 min. A 40-min irradiation produced about 2.0 Ci of [¹¹C]carbon dioxide.

Production of NCA [¹¹C]hydrogen cyanide²⁰

A PETrace Radiotracer Production System was used to produce this labeling agent, as follows. Cyclotron-produced NCA [¹¹C]carbon dioxide was trapped on molecular sieve (13 Å, 80–100 mesh; 0.55g) at 40 °C, while residual [¹³N]nitrogen was directed to waste. The [¹¹C]carbon dioxide was then released with a stream of nitrogen (250 mL/min), mixed with a stream of hydrogen (30 mL/min) and passed through a heated (400 °C) glass tube (10 mm × 200 mm) containing nickel catalyst (Ni-3266 Engelhardt). The effluent containing the generated [¹¹C]methane was passed through an OXY-TRAP® (PNr.-4001R; Alltech), mixed with anhydrous ammonia (research grade, 20 ml/min) and then passed over a wad of platinum wire (d 0.127 mm; 2.6 g) at 920 °C. The generated [¹¹C]hydrogen cyanide was delivered to a hot-cell for subsequent radiochemistry in a mixture of hydrogen, ammonia, and nitrogen carrier gas at 300 mL/min.

Production of NCA [¹¹C]methyl iodide

NCA [¹¹C]methyl iodide was produced from NCA [¹¹C]carbon dioxide (~ 2.0 Ci) via reduction to [¹¹C]methane and then vapour phase iodination¹⁸, either in a TRACERlab FX C Pro module (GE; Milwaukee, WI) for the synthesis of [¹¹C]**8** or a MeI MicroLab apparatus (GE; Milwaukee, WI) for the synthesis of [¹¹C]**13**.

Production of NCA [¹¹C]methyl triflate

A quartz column (i.d. 2.6 mm, length 26 cm) was packed around its center with a 6 cm length of AgOTf/Graphac (50: 50 w/w) held in place at each end with glass wool. NCA [¹¹C]methyl triflate (~ 350 mCi) was produced by passing [¹¹C]methyl iodide in helium gas (17 mL/min) into the heated (180 °C) column.¹⁹

Production of NCA [¹⁸F]d₂-fluoromethyl bromide⁵³

Cyclotron-produced [¹⁸F]fluoride ion (~ 150 mCi) in [¹⁸O]water was delivered into a glass vial containing K 2.2.2 (5.0 mg, 13.3 μmol) and potassium carbonate (0.50 mg, 3.6 μmol) in

MeCN-H₂O (0.1 mL, 9: 1 v/v). This solution was transferred to a modified version of a TRACERlab FX_{F-N} module and diluted with MeCN (1 mL). The mixture was evaporated to dryness at 90 °C under reduced pressure with a nitrogen flow. MeCN (2 mL) was again added and then evaporated to dryness. The vessel was sealed and then CD₂Br₂ (100 μL) in MeCN (1.0 mL) was added to the dry [¹⁸F]fluoride ion-K 2.2.2-K⁺ complex which was then heated at 95 °C for 15 min. The reaction vessel was then cooled to 35 °C. Nitrogen gas was used to transfer the volatile [¹⁸F]*d*₂-fluoromethyl bromide through a series of four silica gel cartridges (SepPak Plus) and then into a V-vial (1-mL) having a crimp-sealed silicon-Teflon septum cap. The RCY of [¹⁸F]*d*₂-fluoromethyl bromide was typically about 28%.⁵³

Radiosynthesis of [¹¹C]5

NCA [¹¹C]hydrogen cyanide (~ 200 mCi) was trapped in a V-vial (5-mL) containing THF (500 μL), precursor (**1**, ~ 1.0 mg), Pd(PPh₃)₄ (1.5–2.0 mg), base [K₂CO₃ (~ 2.0 mg) plus K 2.2.2 (~ 5.0 mg), or usually KH₂PO₄ (~ 2.0 mg) only]. The reaction mixture was heated at 80 °C for 5 min. HPLC mobile phase (3 mL) was added to the V-vial and [¹¹C]**5** isolated with radio-HPLC on a Gemini C18 column (5 μm, 10 × 250 mm; MeCN-*aq.* NH₄OH (10 mM; 3: 2 v/v; 6 mL/min; *t*_R = 7.2 min). The identity of [¹¹C]**5** was confirmed by analytical radio-HPLC on a Gemini C18 column (5 μm, 4.6 × 150 mm) eluted at 1 mL/min with MeCN-10 mM NH₄OH (16: 9 v/v; *t*_R = 5.8 min) and also by LC-MS of associated carrier. The radiochemical purity of [¹¹C]**5** was > 99%. The decay-corrected radiochemical yield (RCY) from [¹¹C]hydrogen cyanide was 26% (*n* = 3).

Radiosynthesis of [¹¹C]8

[¹¹C]Methyl iodide (~ 15 mCi) was trapped in a capped fluoro-polymer custom-made reaction vial (1.5-mL) containing anhydrous DMF (300 μL), precursor (**3**, ~ 1.0 mg) and base (Li₃N or Li₂O; ~ 5.0 mg). The mixture was sonicated in an ultrasound apparatus (UIS250L, Hielscher Ultrasonics, Germany) for 10 min and then filtered through an Iso-Disc™ filter (PFTE13-4, 13 mm × 0.45 μm) which was then rinsed twice with DMF. The combined filtrates were diluted with water and then the [¹¹C]**8** was isolated with radio-HPLC (MeCN-HCOONH₄; 2: 3 v/v; 3 mL/min; *t*_R = 13.1 min). The identity of [¹¹C]**8** was confirmed by analytical radio-HPLC on a Gemini-NX C18 column (5 μm, 4.6 × 250 mm) eluted with MeCN-100 mM HCOONH₄ (16: 9 v/v) at 1 mL/min (*t*_R = 7.0 min) and also by LC-MS of associated carrier. The RCYs from [¹¹C]methyl iodide were 7% (Li₂O) and 11% (Li₃N).

Radiosynthesis of [¹¹C]13 from [¹¹C]methyl triflate

[¹¹C]Methyl triflate (30–20 mCi) was trapped in a tapered bottom vial (0.9-mL) containing **20** (0.15–0.8 mg), 0.5M NaOH (3 eq.) and MeCN (300 μL). The mixture was heated at 80 °C for 5 min, diluted with HPLC mobile phase (500 μL), and then the [¹¹C]**13** (*t*_R = 8.7 min) isolated with radio-HPLC (MeCN-HCOONH₄; 2: 3 v/v; 4 mL/min). The identity of [¹¹C]**13** was confirmed by analytical radio-HPLC on a Gemini-NX C18 column (5 μm, 4.6 × 250 mm) eluted with MeCN-100 mM HCOONH₄ (2: 3 v/v) at 1 mL/min (*t*_R = 4.4 min) and also by LC-MS of associated carrier. The RCY was 27% (*n* = 12) from [¹¹C]methyl triflate and the radiochemical purity > 99%. The absorbance detector response of the analytical HPLC system was calibrated for mass of carrier ligand **13**. This allowed the mass of **13** in measured samples of [¹¹C]**13** to be determined, and the specific radioactivity to be calculated. The specific radioactivity was 2,517 mCi/μmol at end of synthesis (EOS).

Radiosynthesis of [¹¹C]13 from [¹¹C]methyl iodide

[¹¹C]Methyl iodide (30–550 mCi) was trapped in a tapered bottom vial (0.9-mL) containing **20** (~ 1.0 mg, 3.2 μmol), 1.0M (*n*-Bu)₄NOH (3 eq., 10 μL, 3.2 μmol) and DMF (300 μL).

The mixture was heated at 80 °C for 5 min and then diluted with water (500 μ L). [^{11}C]**13** was separated and analyzed as described above. The RCY of [^{11}C]**13** from [^{11}C]iodomethane was 36% ($n = 6$) and the radiochemical purity > 99%. The specific radioactivity was 2,848 mCi/ μ mol at EOS.

The following experiment²² was performed to confirm the position of radiolabel in [^{11}C]**13**. [^{11}C]Methyl iodide (~ 47 mCi) was trapped in a solution of **20** (~ 1.0 mg) in DMF (300 μ L). Then [^{13}C]methyl iodide (10 μ L from 47.7 mM stock solution in DMF) was added. [^{11}C]/[^{13}C]**13** was isolated by HPLC, as described above. A sample of the collected radioactive fraction was then analyzed by HPLC and LC-MS. The radioactive fraction was then evaporated to dryness, dissolved in CDCl_3 and, analyzed by ^{13}C -NMR{DEPT 135}.

Radiosynthesis of [^{18}F]**18**

Phenol **20** (~ 0.15 mg, 0.49 μ mol), MeCN (300 μ L) and 0.5M NaOH (1.5 μ mol, 3 μ L) were added to a V-vial (1-mL). [^{18}F]FCD₂Br (~ 20 mCi) was transferred to the solution under computer control from a TRACERlab FX_{F-N} module. Radioactivity transfer was monitored by two external radioactivity detectors (Bioscan) and was stopped when radioactivity maximized. The mixture was heated at 100 °C for 15 min and then diluted with water (700 μ L). [^{18}F]**18** was isolated with HPLC (MeCN-HCOONH₄; 2: 3 v/v; 3 mL/min; $t_R = 10.1$ min). The identity of [^{18}F]**18** was confirmed by analytical HPLC on a Gemini-NX C18 column (5 μ m, 4.6 \times 250 mm) eluted with MeCN-100 mM HCOONH₄ (1: 1 v/v) at 1 mL/min ($t_R = 4.2$ min) and also by LC-MS. The RCY of [^{18}F]**18** was 13% ($n = 1$) from [^{18}F]fluoride ion and the radiochemical purity > 99%.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

| | |
|-------------|------------------------------------|
| BZP | peripheral benzodiazepine receptor |
| Cbz | benzyloxycarbonyl |
| CDI | <i>N,N'</i> -carbonyldiimidazole |
| D | dopamine |
| DAT | dopamine transporter |
| DOR | δ opiate receptor |
| DMAP | 4-(dimethylamino)pyridine |
| DMF | dimethylformamide |
| EOS | end of synthesis |
| H | histamine |

| | |
|----------------|---|
| K 2.2.2 | 4,7,13,18-tetraoxo-1,10-diazabicyclo[8,8,8]hexacosane |
| M | muscarinic |
| MOR | μ opiate receptor |
| NBS | <i>N</i> -bromo-succinimide |
| NCA | no-carrier-added |
| NET | noradrenalin transporter |
| NMP | <i>N</i> -methyl-2-pyrrolidinone |
| NXS | <i>N</i> -halo-succinimide (X = halo atom) |
| PET | positron emission tomography |
| rt | room temperature |
| RCY | decay-corrected radiochemical yield |
| SERT | serotonin transporter |
| SPECT | single photon emission computed tomography |
| THF | tetrahydrofuran |
| TMS | trimethylsilyl |
| TPP | triphenylphosphine |
| 5-HT | serotonin |

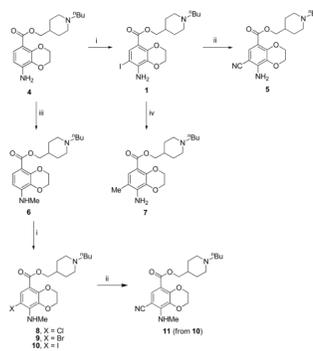
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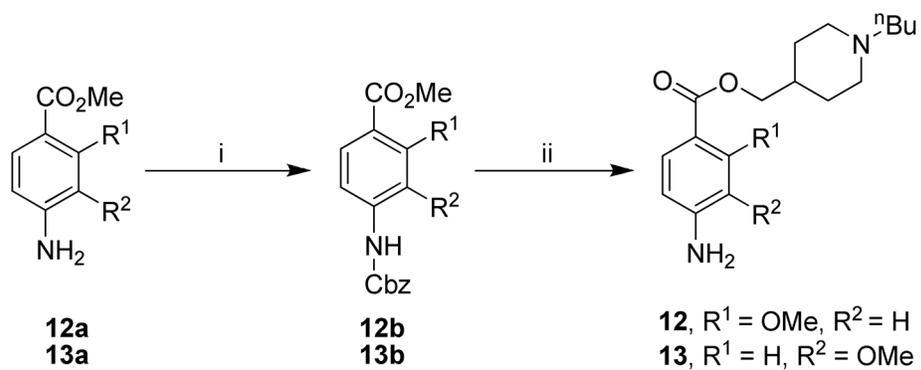
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**Scheme 1.**

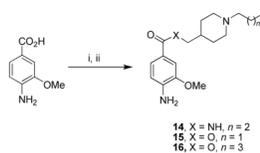
Synthesis of ring methyl, halo and nitrile ligands, and *N*-methyl ligands **5–11**.^a

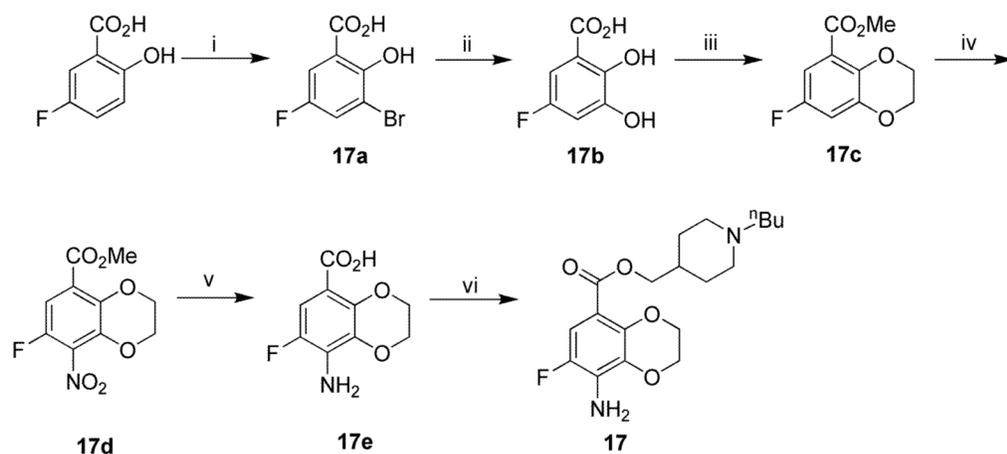
^aReagents and conditions: (i) NXS (X = Cl, Br or I), AcOH; (ii) KCN, CuI, 1,10-phenanthroline, DMF, 42 h, 110 °C; (iii) 1) paraformaldehyde, EtOH, 20 h, 60 °C; 2) NaBH₄; (iv) Me₄Sn, CuI, Pd₂(dba)₃, TPP, NMP, 70 °C, 48 h.

**Scheme 2.**

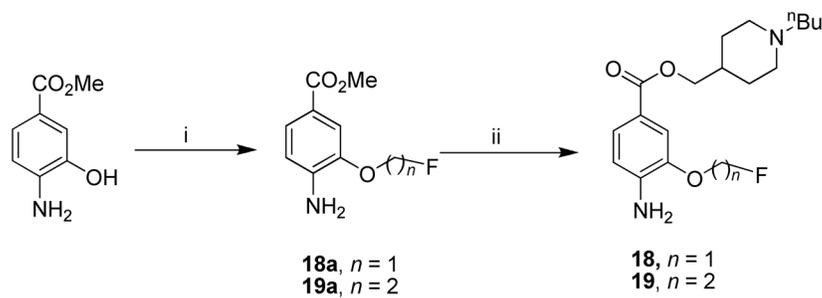
Synthesis of ring methoxy ligands, **12** and **13**.^a

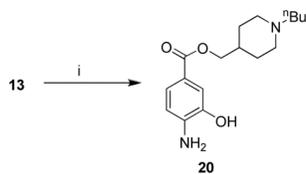
^aReagents and conditions: (i) CbzCl, NaHCO₃, overnight; (ii) 1) lithium (1-butylpiperidin-4-yl)methanolate, THF, 0 °C, then rt overnight; 2) H₂, Pd/C.

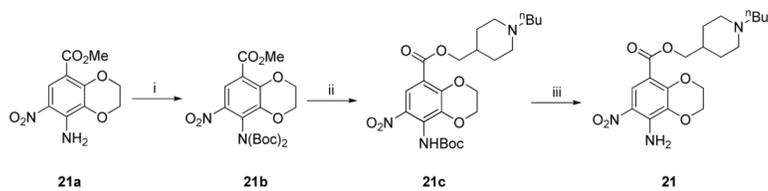
**Scheme 3.**Synthesis of amide (**14**) and *N*-alkyl analogs (**15** and **16**) of **13**.^a^aReagents and conditions: i) CDT; ii) (1-butylpiperidin-4-yl)methanamine or lithium (1-propylpiperidin-4-yl)methanolate or lithium (1-pentylpiperidin-4-yl)methanolate.

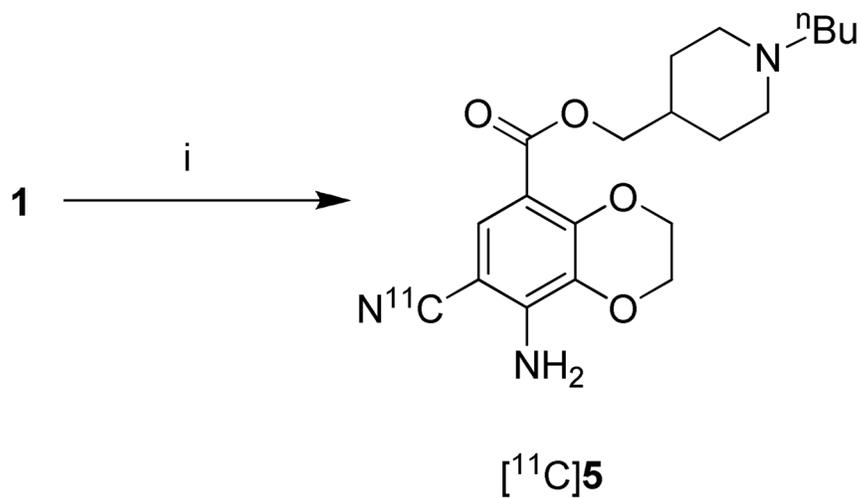
**Scheme 4.**Synthesis of ring fluoro ligand, **17^a**.

^aReagents and conditions: (i) NBS, AcOH; (ii) NaOH, CuSO₄; (iii) 1) TMSCHN₂; 2) Cs₂CO₃, 1,2-dibromoethane, DMF, 80 °C, 16 h; (iv) HNO₃ - 50 °C, 16 min; (v) 10% Pd/C, HCOOK, MeOH, 80 °C, 2 h; (vi) 1) CbzCl, aq. NaHCO₃; 2) CDI, MeCN, rt, 2 h; 3) lithium (1-butylpiperidin-4-yl)methanolate, THF, 0 °C, then rt overnight; 4) H₂, Pd/C.

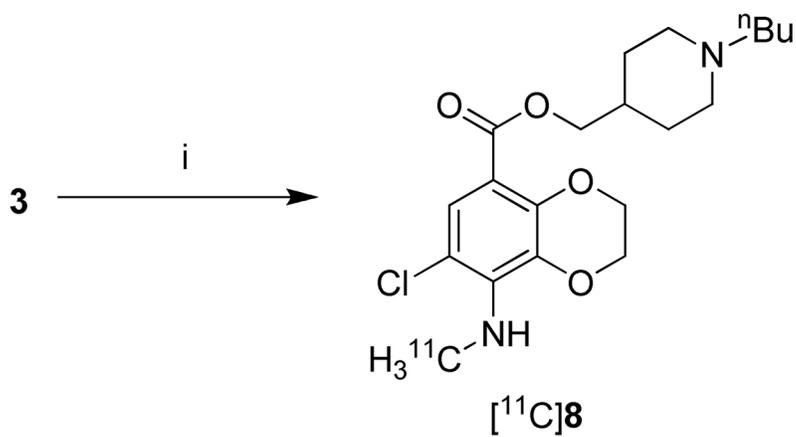
**Scheme 5.**Synthesis of fluoroalkoxy ligands, **18** and **19**.^a^aReagents and conditions: i) $F(CH_2)_nCl$, Cs_2CO_3 , DMF, rt; (ii) lithium (1-butylpiperidin-4-yl)methanolate, THF, 0 °C, then rt overnight.

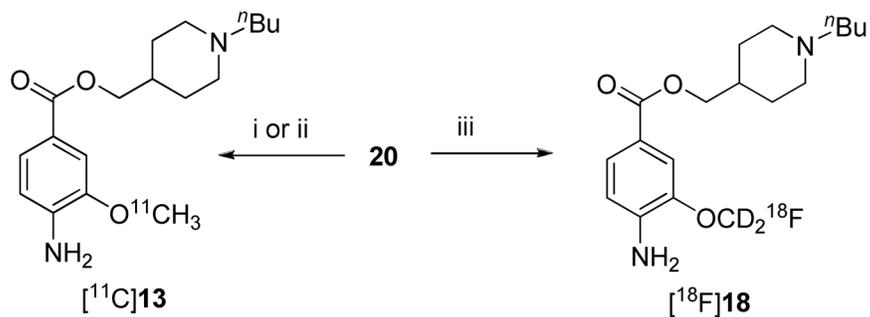
**Scheme 6.**Synthesis of phenol precursor, **20**.^a^aReagents and conditions: (i) AlCl₃, NaI, MeCN, reflux, overnight.

**Scheme 7.**Synthesis of nitro analog, **21**.^a^aReagents and conditions: (i) (Boc)₂O, DMAP, 50 °C, 1 h; (ii) lithium (1-butylpiperidin-4-yl)methanolate, THF, 0 °C, then rt overnight; (iii) 4M HCl in dioxane, overnight.

**Scheme 8.**Radiosynthesis of [^{11}C]5.^a

^a Reagents, conditions and yield: (i) [^{11}C]HCN, $\text{Pd}(\text{PPh}_3)_4$, K_2CO_3 , THF, K 2.2.2, 80 °C, 5 min, RCY = 26%.

**Scheme 9.**Radiosynthesis of [¹¹C]8.^a^a Reagents, conditions and yield: (i) [¹¹C]MeI, Li₃N, DMF, rt, ultrasound, 10 min. RCY= 11%.

**Scheme 10.**Radiosyntheses of [¹¹C]**13** and [¹⁸F]**18**.^a

^aReagents, conditions and yields: (i) [¹¹C]MeI, DMF, 1.0 M (*n*-Bu)₄NOH in MeOH, 80 °C, 5 min, RCY = 36%; (ii) [¹¹C]MeOTf, MeCN, 0.5 M NaOH, heat, 5 min, RCY = 27%; (iii) [¹⁸F]FCD₂Br, MeCN, 0.5 M NaOH, 100 °C, 15 min. RCY = 13% from [¹⁸F]fluoride ion.

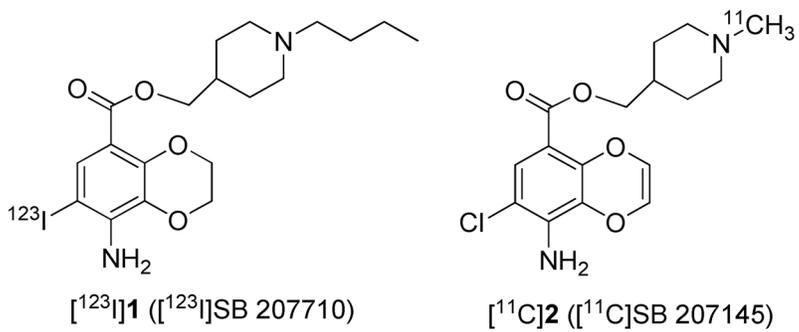
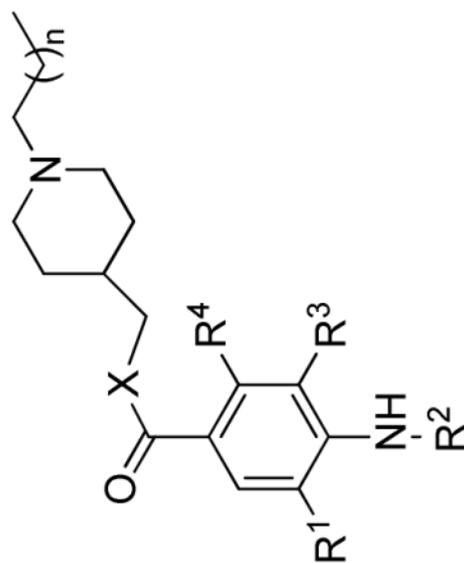


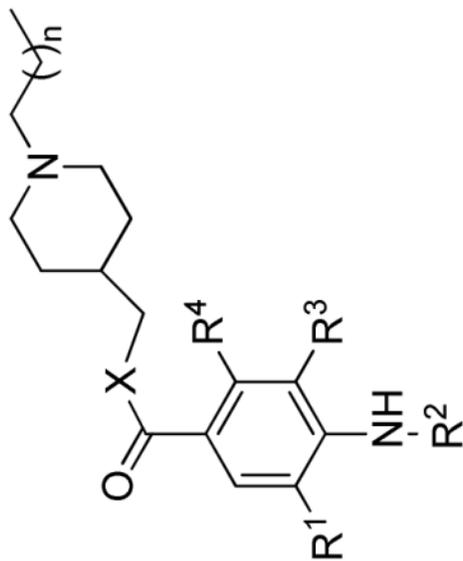
Chart 1.
Current analogs of **1** for 5-HT₄ receptor imaging in vivo.

Table 1

Binding affinities ($1/K_i$),^a efficacies and $cLogD$ values of new 5-HT₄ ligands.



| Compound | R ¹ | R ² | R ³ | R ⁴ | X | n | 5-HT ₄ K _i (nM) ^a | 1/5-HT ₄ K _i (nM) ^b | Efficacy ^c | pEC ₅₀ | cLogD ^d |
|----------|----------------|----------------|------------------|------------------|---|---|--|--|-----------------------|-------------------|--------------------|
| 1 | H | H | OCH ₂ | OCH ₂ | O | 2 | 0.20 | 2.2 ± 0.3 | Inv. Ag. | 7.9 | 3.20 |
| 4 | H | H | OCH ₂ | OCH ₂ | O | 2 | 0.20 | 1.4 ± 0.2 | Antagonist | - | 1.74 |
| 5 | CN | H | OCH ₂ | OCH ₂ | O | 2 | 0.45 | 33 ± 5 | n.m. | n.m. | 2.59 |
| 6 | H | Me | OCH ₂ | OCH ₂ | O | 2 | 0.45 | 9.1 ± 0.7 | Inv. Ag. | 8.05 | 2.37 |
| 7 | Me | H | OCH ₂ | OCH ₂ | O | 2 | 0.577 | 6.3 ± 1 | Antagonist | - | 2.20 |
| 8 | Cl | Me | OCH ₂ | OCH ₂ | O | 2 | 0.577 | 2.0 ± 0.2 | Inv. Ag. | 7.85 | 3.27 |
| 9 | Br | Me | OCH ₂ | OCH ₂ | O | 2 | 0.577 | 4.5 ± 0.4 | Inv. Ag. | 8.0 | 3.36 |
| 10 | I | Me | OCH ₂ | OCH ₂ | O | 2 | 0.577 | 7.4 ± 0.5 | Inv. Ag. | 7.7 | 3.41 |
| 11 | CN | Me | OCH ₂ | OCH ₂ | O | 2 | 0.738 | 37 ± 5 | Inv. Ag. | 7.8 | 2.52 |
| 12 | H | H | H | OMe | O | 2 | 0.738 | 50 ± 7 | Ag. | 9.75 | 1.77 |
| 13 | H | H | OMe | H | O | 2 | 0.738 | 2.4 ± 0.3 | Antagonist | - | 2.07 |
| 14 | H | H | OMe | H | N | 2 | 0.901 | 8,664 ± 857 | n.m. | n.m. | 0.16 |
| 15 | H | H | OMe | H | O | 1 | 0.901 | 17 ± 1 | n.m. | n.m. | 1.54 |
| 16 | H | H | OMe | H | O | 3 | 0.901 | 12 ± 1 | n.m. | n.m. | 2.60 |



| Compound | R ¹ | R ² | R ³ | R ⁴ | X | n | 5-HT ₄ K _i (nM) ^a | h5-HT ₄ K _i (nM) ^b | Efficacy ^c | pEC ₅₀ | cLogD ^d |
|----------|-----------------|----------------|------------------------------------|------------------|---|---|--|---|-----------------------|-------------------|--------------------|
| 17 | F | H | OCH ₂ | OCH ₂ | O | 2 | 0.334 | 2.1 ± 0.3 | Inv. Ag. | 8.23 | 2.42 |
| 18 | H | H | OCH ₂ F | H | O | 2 | 0.334 | 17 ± 1 | n.m. | n.m. | 1.97 |
| 19 | H | H | O(CH ₂) ₂ F | H | O | 2 | 0.215 | 11 ± 1 | n.m. | n.m. | 2.30 |
| 21 | NO ₂ | H | OCH ₂ | OCH ₂ | O | 2 | | 7.8 ± 0.9 | n.m. | n.m. | 3.04 |

^aFor guinea pig striatal membrane 5-HT₄ receptors. Values are averages of triplicate measurements.

^bFor h5-HT₄ receptors. Binding assay results are averages of triplicate measurements.

^cIn agonist/inverse agonist assay. Ag. = agonist; Inv. Ag. = Inverse agonist; n.m. = not measured.

^dcLogD was calculated with ACD software; estimated errors, are approximately ± 1.0.

Table 2Binding affinities of 5-HT₄ ligands **8**, **13** and **17** for other 5-HT receptors/binding sites.

| Binding site or receptor | Binding affinities (K_i) (nM) | | |
|-----------------------------|-----------------------------------|-----------|-----------|
| | Ligand 8 | Ligand 13 | Ligand 17 |
| <i>h</i> 5-HT ₄ | 2.0 | 2.4 | 2.1 |
| <i>h</i> 5-HT _{1A} | 300 | >10,000 | >10,000 |
| <i>h</i> 5-HT _{1B} | 820 | >10,000 | >10,000 |
| <i>h</i> 5-HT _{1D} | 1,304 | 5,686 | 2,856 |
| <i>h</i> 5-HT _{1E} | >10,000 | >10,000 | >10,000 |
| <i>h</i> 5-HT _{2A} | 1,776 | >10,000 | >10,000 |
| <i>h</i> 5-HT _{2B} | 66 | 2,861 | 168 |
| <i>h</i> 5-HT _{2C} | 685 | >10,000 | 8,363 |
| <i>h</i> 5-HT ₃ | 1,072 | 3,511 | >10,000 |
| <i>h</i> 5-HT _{5A} | 8,589 | >10,000 | >10,000 |
| <i>h</i> 5-HT ₆ | 489 | >10,000 | >10,000 |
| <i>h</i> 5-HT ₇ | 5,702 | >10,000 | >10,000 |
| <i>h</i> SERT | 9,006 | >10,000 | >10,000 |

Table 3Binding affinities of 5-HT₄ ligands **8**, **13** and **17** for non-serotonergic receptors and binding sites.

| Binding site or receptor | Binding affinities (K _i) (nM) | | |
|-----------------------------|---|-----------|-----------|
| | Ligand 8 | Ligand 13 | Ligand 17 |
| <i>h</i> α _{1A} | 522 | >10,000 | 87.6 |
| <i>h</i> α _{1B} | >10,000 | 4,228 | 9,076 |
| <i>h</i> α _{1D} | 2,276 | >10,000 | >10,000 |
| <i>h</i> α _{2A} | 2,239 | >10,000 | >10,000 |
| <i>h</i> α _{2B} | 671.2 | >10,000 | 773 |
| <i>h</i> α _{2C} | 556 | 989 | 593 |
| β ₁ (rat) | >10,000 | 4,407 | >10,000 |
| β ₂ (rat) | >10,000 | >10,000 | >10,000 |
| β ₃ (rat) | >10,000 | >10,000 | >10,000 |
| σ ₁ (rat) | 60 | 211 | 55 |
| σ ₂ (rat) | 8 | 148 | 12.9 |
| BZP (rat brain site) | >10,000 | >10,000 | >10,000 |
| <i>h</i> DAT | 2,020 | >10,000 | >10,000 |
| <i>h</i> DOR | >10,000 | >10,000 | >10,000 |
| D ₁ (rat) | 2,291 | >10,000 | >10,000 |
| D ₂ (rat) | 220 | >10,000 | 615 |
| D ₃ (rat) | 336 | 6,272 | 1,011 |
| D ₄ (rat) | 43 | 1,272 | 316 |
| <i>h</i> D ₅ | 972 | >10,000 | >10,000 |
| <i>h</i> GABA _A | >10,000 | >10,000 | >10,000 |
| H ₁ (guinea pig) | 2,727 | >10,000 | >10,000 |
| H ₂ (guinea pig) | >10,000 | >10,000 | >10,000 |
| H ₃ (guinea pig) | >10,000 | 1,649 | 5,038 |
| H ₄ (guinea pig) | >10,000 | >10,000 | >10,000 |
| <i>h</i> KOR | >10,000 | >10,000 | >10,000 |
| <i>h</i> MOR | >10,000 | >10,000 | >10,000 |
| <i>h</i> M ₁ | >10,000 | >10,000 | >10,000 |
| <i>h</i> M ₂ | 433 | >10,000 | >10,000 |
| <i>h</i> M ₃ | 823 | 1,095 | >10,000 |
| <i>h</i> M ₄ | 760 | 2,579 | >10,000 |
| <i>h</i> M ₅ | 972 | 4,058 | >10,000 |
| <i>h</i> NET | 3,226 | >10,000 | >10,000 |

Table 4RCYs for [^{11}C]5 under various conditions.

| Solvent | Base | RCY ^a (%) | <i>n</i> |
|---------|--|----------------------|----------|
| THF | KH ₂ PO ₄ | 2 | 1 |
| DMSO | KH ₂ PO ₄ | 10 | 2 |
| THF | K ₂ CO ₃ , K 2.2.2 | 26 | 3 |

^aFrom [^{11}C]HCN.

Table 5RCYs of [^{18}F]**18** under various conditions.

| Solvent | Base | T (°C) | Time (min) | RCY ^a (%) |
|---------|---|--------|------------|----------------------|
| MeCN | 0.5M NaOH | 80 | 5 | 1 |
| DMF | K ₂ CO ₃ , 18-crown-6 | 110 | 10 | 7 |
| MeCN | 0.5M NaOH | 100 | 15 | 13 |

^a Overall from starting [^{11}C]fluoride ion.