



## Research paper

## Synthesis and evaluation of novel serotonin 4 receptor radiotracers for single photon emission computed tomography



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## ABSTRACT

Despite its implication in several physiological and pathological processes the serotonin subtype-4 receptor (5-HT<sub>4</sub>R) has seen limited effort for the development of radiolabeling agent especially concerning single photon emission computed tomography (SPECT). Bearing an ester function, the available ligands are rapidly susceptible to hydrolysis which limits their use *in vivo*. In this study the synthesis of iodinated benzamide and ketone analogs were described. Their affinity for the 5-HT<sub>4</sub>R and their lipophilicity were evaluated and the most promising derivatives were evaluated *ex vivo* for their binding to the receptor and for their ability to displace the reference ligand [<sup>125</sup>I]-SB207710.

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## 1. Introduction

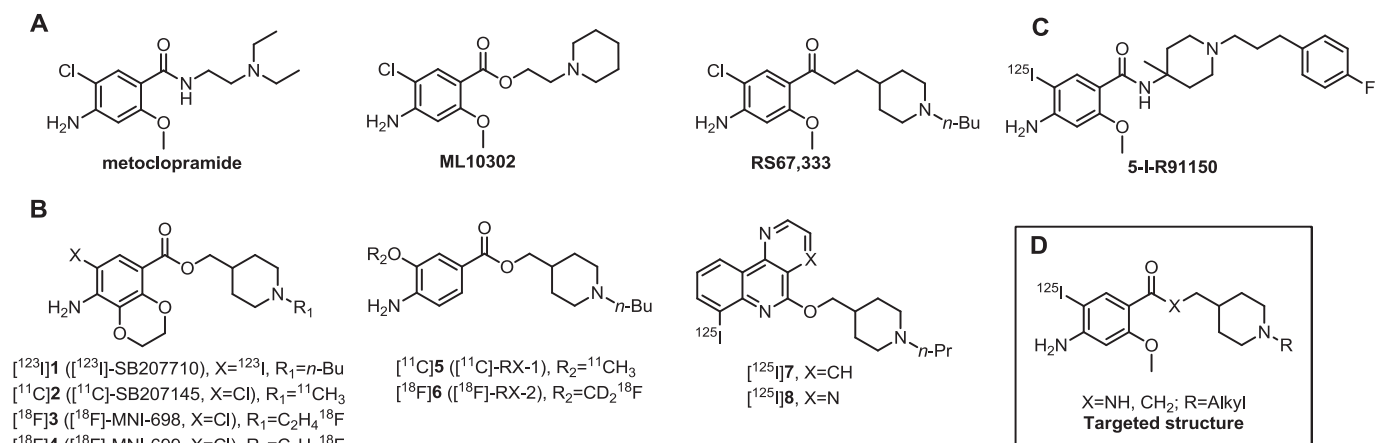
Serotonin (5-hydroxytryptamine, 5-HT) plays a central role in neurotransmission through its interaction with several receptor subtypes, and the deregulation of this system has been implicated in numerous pathologies [1]. In order to determine the implication of each receptor subtypes in these diseases, intense research has been conducted during the last few years to develop selective radioligands of interest for positron emission tomography (PET) and single photon emission computed tomography (SPECT) imaging [2,3]. Among those receptors, 5-HT<sub>4</sub>R are of particular interest. They have been described for the first time by Dumuis in 1988 and are positively linked to adenylate cyclase and could control the release of a wide variety of neurotransmitters [4]. These receptors are expressed in the central nervous system (CNS) and control brain physiological functions such as learning and memory, feeding and mood behavior but they are also located in the periphery and are implicated in gastro-intestinal transit [5]. Interactions with

peripheral 5-HT<sub>4</sub>R appear beneficial in gastrointestinal disorders [6], while interactions with the central 5-HT<sub>4</sub>R, mainly with agonists, result in cognitive improvement after chronic or acute administration [7]. For this reason it is now postulated that several major devastating illnesses could benefit from 5-HT<sub>4</sub>R-directed therapy including Alzheimer's disease (AD) [8] or depression [9].

Over the years, potent and selective ligands have been developed towards the 5-HT<sub>4</sub>R [10] which are mainly based on a typical 4-amino-5-chloro-2-methoxybenzoyl residue already described in the non-selective benzamide metoclopramide [11], as well as in the benzoate ML10302 and the benzophenone RS67,333 (Fig. 1A) [12]. In order to specify *in vivo* the 5-HT<sub>4</sub>R physiological or pathological role, selective radioligands have been developed for SPECT and PET such as benzodioxane [<sup>123</sup>I]SB-207710 ([<sup>123</sup>I]1) [13] or its chlorinated analogue [<sup>11</sup>C] SB-207145 ([<sup>11</sup>C]2) (Fig. 1B) [14]. The latter remains the only radiotracer that has been evaluated in human studies [15]. Two radiotracers containing fluorine-18 [<sup>18</sup>F]MNI-698 ([<sup>18</sup>F]3) and [<sup>18</sup>F]MNI-699 ([<sup>18</sup>F]4) were described in the same chemical series but possessing a radioactive atom as a substituent of the piperidine alkyl chain [16]. Among them [<sup>18</sup>F]3 gave promising results for imaging 5-HT<sub>4</sub>R in the brain in monkey [17,18]. More recently two novel tracers [<sup>11</sup>C]RX-1 ([<sup>11</sup>C]5) and [<sup>18</sup>F]RX-2

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**Fig. 1.** A: Structures of reference 5-HT<sub>4</sub>R ligands; B: 5-HT<sub>4</sub>R PET and SPECT radiotracers; C: 5-HT<sub>2A</sub>R SPECT radiotracer; D: targeted structures.

([<sup>18</sup>F]6) were developed and evaluated for PET imaging [19]. However each of these ligands containing an ester function are susceptible to ester hydrolysis *in vivo* which could limit their accuracy. Other chemical series were then explored with the recent development of azaphenanthrene derivatives [<sup>125</sup>I]7 and [<sup>125</sup>I]8 (Fig. 1) [20,21]. Considering our recent experience with the modulation of the metoclopramide and RS67,333 scaffold [22], we decided to investigate the influence of the replacement of the ester function by an amide or a ketone function both on 5-HT<sub>4</sub>R affinity and selectivity, lipophilicity but also on the amenability to introduce a iodine labeling atom on the aromatic ring. The amide modulation could be of particular interest since this function is present in R-91150 which was developed as brain 5-HT<sub>2A</sub>R SPECT radiotracers [23,24].

## 2. Results

### 2.1. Chemistry

Despite the traditionally reported strategy for obtaining iodinated single photon emission computed tomography radiotracers, based on a iododestannylation reaction of a stannylated precursor [25], and while our structures appear to be favorable for this reaction, we focused on the introduction of iodine atoms on non-functionalized precursors using electrophilic aromatic substitution reactions.

Initially, we planned to synthesize iodinated derivatives without radioactive source, by generating I<sub>2</sub> from NaI using an oxidant in acidic conditions [26]. Obtaining the iodinated derivatives from the non-functionalized compounds allowed us to confirm the selective halogenation at the expected position, to evaluate their potential affinity toward 5-HT<sub>4</sub>R and to validate the feasibility of this procedure for the radiolabeling of our non-functionalized derivatives. This strategy will also allow us to avoid the synthesis of stannylated precursor, typically used in this kind of radiolabeling strategies.

For the design of non-functionalized aryl ketone derivatives, acids **11a–b** were synthesized in a three-step reaction from 4-aminosalicylic acid: an esterification of carboxylic acid, an alkylation of phenol with an ethyl or a 2-fluoroethyl chain and a saponification reaction (Scheme 1). Compounds **14a–b** were finally prepared according to the general route as previously described: formation of  $\beta$ -keto ester, subsequent alkylation with a piperidine derivative, saponification-decarboxylation reaction, deprotection of *tert*-butoxycarbonyl group under acidic conditions and alkylation of the piperidine with (bromomethyl)cyclohexane (Scheme 1)

[27].

For the design of non-functionalized benzamide derivatives, aminopiperidine chains **16a–b** were synthesized in a two-step reaction, first by alkylation of the piperidine moiety with alkyl halides, then by reduction of amides using LiAlH<sub>4</sub>. Target compounds **17a–d** were finally obtained by a peptide coupling between carboxylic acids **11a–c** (previously synthesized, except commercially available 4-amino-2-methoxybenzoic acid **11c**) and substituted aminopiperidines **16a–b** (Scheme 2).

Iodinations from non-functionalized aryl ketone derivatives **14a–b** and benzamide derivatives **17a–d** were performed using NaI as the source of iodine in a mixture of acetic acid/hydrogen peroxide solution 30%. A total conversion was observed, after a purification by flash chromatography on silica gel column and compounds **18a–f** were obtained in 18–71% isolated yields (Scheme 3). Selective halogenation at the expected position validated the feasibility of our strategy.

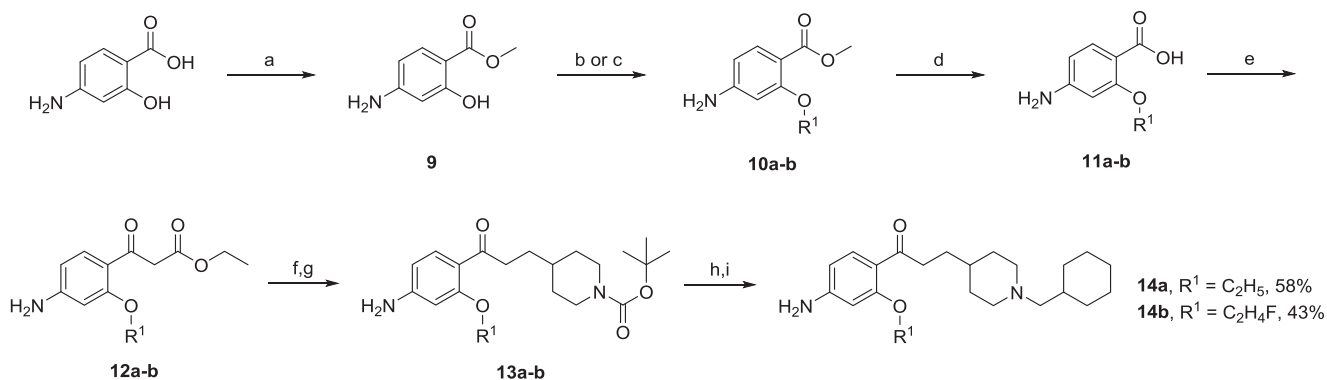
### 2.2. Synthesis of radioligand

The effectiveness of our synthetic route was based on a final electrophilic iodination step. Considering the *in vitro* activities of the iodinated compounds (Table 1), radioiodination of **17a–b** was performed (Scheme 3). [<sup>125</sup>I]**18c–d** were obtained using Na<sup>125</sup>I as the radioactive iodine source and peracetic acid (formed *in situ* by the reaction of H<sub>2</sub>O<sub>2</sub> and acetic acid) as the oxidant in order to *in situ* generate iodine and to conduct the aromatic electrophilic substitution (Scheme 3). Radiotracers were isolated by a linear gradient HPLC run.

### 2.3. *In vitro* assays

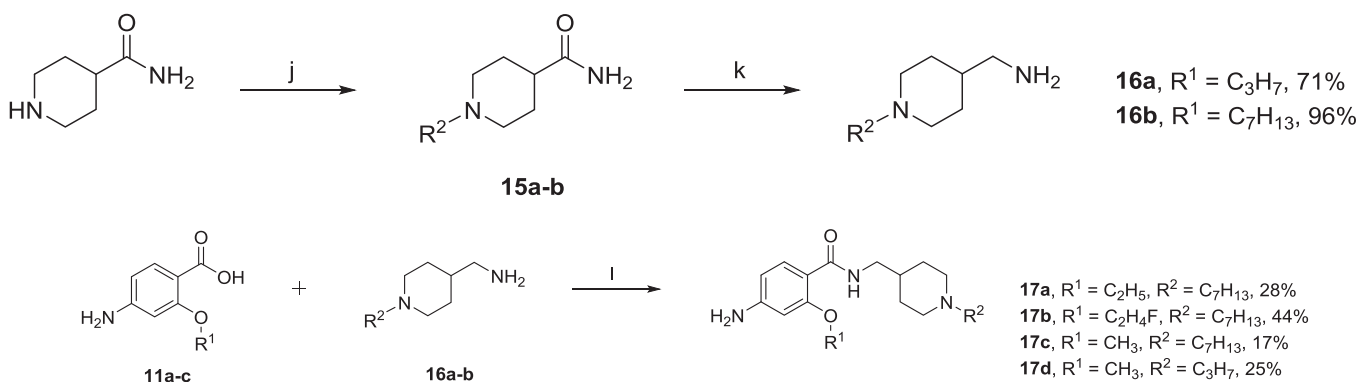
According to the work described by Grossman et al. [28], precursors **14a–b**, **17a–d** and final derivatives **18a–f**, were evaluated *in vitro* for their potential guinea-pig 5-HT<sub>4</sub>R affinity using a displacement assay of the tritiated ligand [<sup>3</sup>H]-GR113808, a specific and highly potent 5-HT<sub>4</sub>R antagonist used to label specific binding sites in human or guinea-pig brain (Table 1).

In order to evaluate the influence of these modulations on the physicochemical properties of the novel ligands **18a–f**, the evaluation of their capacity to cross the blood–brain barrier (BBB) was performed using a PAMPA assay and their lipophilicity was evaluated by mean of the determination of their Log P which were calculated using either MarvinSketch 5.2.6 or Molinspiration and estimated using chromatographic method adapted from the



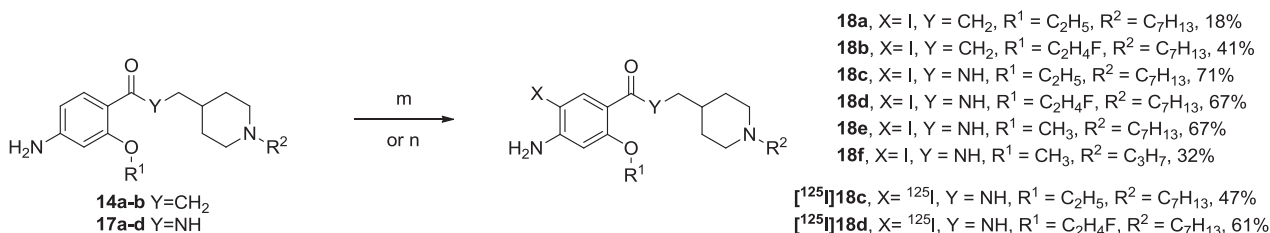
<sup>a</sup>Reagents, conditions and yields: (a)  $\text{H}_2\text{SO}_4$  cc., MeOH, 16h, reflux, 90%; (b) iodoethane,  $\text{K}_2\text{CO}_3$ , DMF,  $70^\circ\text{C}$ , overnight, 61%; (c) 2-fluoroethyl 4-methylbenzenesulfonate,  $\text{K}_2\text{CO}_3$ , DMF,  $110^\circ\text{C}$ , 2h, 62%; (d) 1N NaOH aq., EtOH, rt, overnight, 94-96%; (e) CDI, dry THF, rt, 15h, then potassium 3-ethoxy-3-oxopropanoate,  $\text{MgCl}_2$ ,  $40^\circ\text{C}$ , 24h, 23-36%; (f) *tert*-butyl 4-(iodomethyl)piperidine-1-carboxylate,  $\text{K}_2\text{CO}_3$ , DMF, rt, 48h; (g) KOH, EtOH/ $\text{H}_2\text{O}$  (5:1), reflux, 5h, 70-74% over two steps; (h) TFA, DCM, rt, 1h; (i) bromomethylcyclohexane,  $\text{K}_2\text{CO}_3$ ,  $110^\circ\text{C}$ , 5h, 43-58% over two steps.

**Scheme 1.** Synthetic route of aryl ketone derivatives 14a-b<sup>a</sup>.



<sup>b</sup>Reagents, conditions and yields: (j) alkyl-halogenated derivatives,  $\text{K}_2\text{CO}_3$ , EtOH, reflux, 15-24h, 92-93%; (k)  $\text{LiAlH}_4$ , dry THF, addition  $0^\circ\text{C}$  then rt, 1-2h, reflux, 3h, 71-96%; (l) EDCI, HOBT,  $\text{Et}_3\text{N}$ , DMF, rt, 18-72h, 17-44%.

**Scheme 2.** Synthetic route of benzamide derivatives 17a-d<sup>b</sup>.

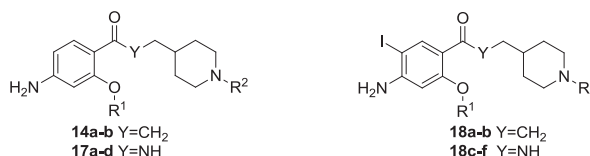


<sup>c</sup>Reagents, conditions and yields: (m) NaI, acetic acid/30%  $\text{H}_2\text{O}_2$  2:1, rt, 1-2h, 18-71%; (n)  $\text{Na}^{125}\text{I}$ , acetic acid/30%  $\text{H}_2\text{O}_2$  2:1, rt, 30 min, 47-61%.

**Scheme 3.** Synthesis of iodinated compounds 18a-f<sup>c</sup> and  $^{125}\text{I}$  radiolabeling of compounds 17a-b<sup>c</sup>.

**Table 1**

5-HT<sub>4</sub>R affinity for the compounds **14a–b**, **17a–d** and **18a–f**; calculated and measured lipophilicities and permeabilities in the PAMPA-BBB assay with their predictive penetration in the CNS for the compounds **18a–f**.



Compound	Y	R <sup>1</sup>	R <sup>2</sup>	K <sub>i</sub> 5-HT <sub>4</sub> R (nM) %Inhibition 10 <sup>−6</sup> M/10 <sup>−8</sup> M	Lipophilicity		Permeability	
					cLogP <sup>a</sup> –cLogP <sup>b</sup>	LogP exp.	Pe (10 <sup>−6</sup> cm s <sup>−1</sup> )	Prediction
<b>14a</b>	CH <sub>2</sub>	C <sub>2</sub> H <sub>5</sub>		24.7 ± 5.3 (n = 3) <sup>d</sup> 100%/38%	n.d.	n.d.	n.d.	n.d.
<b>18a</b>	CH <sub>2</sub>	C <sub>2</sub> H <sub>5</sub>		86 ± 19.4 (n = 3) <sup>d</sup> 100%/65%	5.06 – 6.13	6.4 ± 0.5	8.08	CNS ±
<b>14b</b>	CH <sub>2</sub>	C <sub>2</sub> H <sub>4</sub> F		39.9 ± 5.3 (n = 3) <sup>d</sup> 100%/27%	n.d.	n.d.	n.d.	n.d.
<b>18b</b>	CH <sub>2</sub>	C <sub>2</sub> H <sub>4</sub> F		521 ± 261 (n = 3) <sup>d</sup> 100%/22%	4.91 – 6.05	5.6 ± 0.4	10.36	CNS ±
<b>17a</b>	NH	C <sub>2</sub> H <sub>5</sub>		6.9 ± 0.6 (n = 3) <sup>d</sup> 100%/51%	n.d.	n.d.	n.d.	n.d.
<b>18c</b>	NH	C <sub>2</sub> H <sub>5</sub>		8.64 ± 0.37 (n = 3) <sup>d</sup> 100%/20%	3.95 – 5.63	4.9 ± 0.5	24.38	CNS +
<b>17b</b>	NH	C <sub>2</sub> H <sub>4</sub> F		8.1 ± 3.4 (n = 3) <sup>d</sup> 100%/38%	n.d.	n.d.	n.d.	n.d.
<b>18d</b>	NH	C <sub>2</sub> H <sub>4</sub> F		14.7 ± 2.9 (n = 3) <sup>d</sup> 100%/30%	3.79 – 5.55	5.2 ± 0.4	32.29	CNS +
<b>17c</b>	NH	CH <sub>3</sub>		n.d. 72%/40% <sup>g</sup>	n.d.	n.d.	n.d.	n.d.
<b>18e</b>	NH	CH <sub>3</sub>		13.9 ± 3.7 (n = 3) <sup>c</sup> 100%/29%	3.59 – 5.25	5.0 ± 0.4	31.60	CNS +
<b>17d</b>	NH	CH <sub>3</sub>		n.d. 79%/5% <sup>g</sup>	n.d.	n.d.	n.d.	n.d.
<b>18f</b>	NH	CH <sub>3</sub>		68.7 ± 11.4 (n = 3) <sup>c</sup> 96%/11%	2.36 – 3.84	3.5 ± 0.4	22.68	CNS +

n.d. not determined.

<sup>a</sup> Calculated with MarvinSketch.

<sup>b</sup> Calculated with Molinspiration.

<sup>c</sup> Guinea pig receptors.

<sup>d</sup> Human receptors.

#### 2.4. Imaging experiments

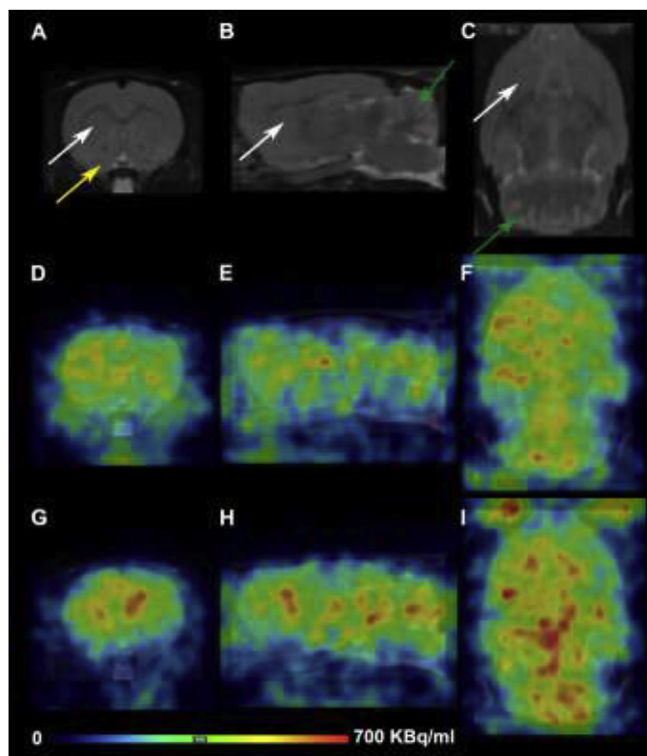
As detailed in the discussion section the most active *in vitro* compounds **18c–d** were selected for further preclinical evaluation as 5-HT<sub>4</sub>R SPECT radiotracers. Following their radioiodination [<sup>125</sup>I]**18c** and [<sup>125</sup>I]**18d** were injected in the tail vein of rats and SPECT images were obtained (Fig. 2). As illustrated by representative images [<sup>125</sup>I]**18c** and [<sup>125</sup>I]**18d** are able to enter the brain and are detected by the SPECT scanner. However, the brain distribution of both [<sup>125</sup>I]**18c** and [<sup>125</sup>I]**18d** did not show a specific accumulation in 5-HT<sub>4</sub>R rich region, such as olfactory tubercles, caudate-putamen, in comparison with the cerebellum, a region devoid of 5-HT<sub>4</sub>R according to *in vivo* SPECT imaging and *ex vivo* autoradiograms (Figs. 2–3).

Being able to penetrate the brain and to accumulate in different regions of the brain, we decided to evaluate the capacity of our ligands to target the 5-HT<sub>4</sub>R. A competition experiment was conducted between the specific 5-HT<sub>4</sub>R radiotracer [<sup>125</sup>I]**1** and our ligands **18c–d** and non radioactive **1** (Fig. 4). In this *in vitro* study rat brain slides were first incubated with [<sup>125</sup>I]**1** before being treated with increasing concentration of **18c** and **18d** (from 0.01 to 10 μM, Fig. 4F), B- or 1 μM of **1** (Fig. 4, G). **18c–d** can inhibit the specific

binding of [<sup>125</sup>I]**1** on 5-HT<sub>4</sub>R (Fig. 4).

### 3. Discussion

Our synthetic strategy led to the development of final derivatives **18a–f**, possessing an iodine atom on their structure which could be easily introduced through a late stage diversification process. Two of them were designed to possess a ketone function (**18a–b**) and four (**18c–f**) were obtained with an amide linker. Our initial objectives were to assess the influence of these pharmacomodulations on the biological activity but also on their lipophilicity and their ability to cross the BBB. All the synthesized derivatives showed moderate to good affinities for the 5-HT<sub>4</sub>R with low nanomolar K<sub>i</sub> demonstrating that the introduction of a large iodine atom on the aromatic ring is compatible with a conserved interaction with the receptor (Table 1). We could however noticed that the iodination is detrimental to 5-HT<sub>4</sub>R affinity for the two ketones **18a–b** (K<sub>i</sub> = 86 and 521 nM respectively) which were less potent ligands than their non-iodinated precursors **14a–b** (K<sub>i</sub> = 24.7 and 39.9 nM respectively). On the other hand introduction of iodine on the amides **18c–f** generally improves their affinities for the 5-HT<sub>4</sub>R and **18c** was found to be the most potent ligand with a K<sub>i</sub> of 8.64 nM



**Fig. 2.** *In vivo* SPECT imaging showing activity distribution in the brain after injection of [ $^{125}\text{I}$ ]18c or [ $^{125}\text{I}$ ]18d. Representative MRI images in coronal (A), sagittal (B) and axial (C) planes. Arrows indicate areas of interest: olfactory tubercles (yellow), caudate-putamen (white) and cerebellum (green). Images of SPECT scan between 10 and 60 min after injection of [ $^{125}\text{I}$ ]18c (D–F) or [ $^{125}\text{I}$ ]18d (G–I) coregistered with MRI template. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Table 1). Concerning the other pharmacomodulations, the different alkoxy chains developed did not influence the 5-HT<sub>4</sub>R binding and the methylenecyclohexyl linker was found superior than the propyl one (18e vs 18f).

Concerning the influence of these modulations on the lipophilicity, the amide linker appears again to be the most appropriate with calculated or measured LogP between 3.5 and 5.2 for 18f and 18d respectively. The amide derivatives were also identified to be able to cross the BBB in a predictive PAMPA assay since all compounds 18c–f could be considered as CNS + compounds. Based

on this positive preliminary studies compound 18c and 18d, the most potent compounds, were designated for the development of [ $^{125}\text{I}$ ]–ligand in order to evaluate their efficiency as SPECT radiotracers.

Thus, the radioactive labeling of compounds 17a–b was performed and the resulting [ $^{125}\text{I}$ ]18c and [ $^{125}\text{I}$ ]18d were evaluated as radiotracers *in vivo*, *ex vivo* and *in vitro*. After injection of each radioligands in rat, *in vivo* SPECT imaging showed activity distribution in the brain and validated their ability to cross the BBB. Mdr1a KO rats were used for the *in vivo* experiments in order to evaluate only the ability of the [ $^{125}\text{I}$ ]18c and [ $^{125}\text{I}$ ]18d tracers to specifically bind to 5-HT<sub>4</sub>R regardless of the Mdr1a expression. Autoradiograms obtained for *ex vivo* imaging showed an inability to obtain specific labeling in regions known to contain high densities of 5-HT<sub>4</sub>R. In order to address their 5-HT<sub>4</sub>R specific binding capacity, *in vitro* competition experiments with [ $^{125}\text{I}$ ]1 were performed. The selective and specific antagonist radioligand was co-administered with 18c and 18d. Increasing concentrations of both iodinated derivatives showed a decrease in the 5-HT<sub>4</sub>R-specific radioactivity, while increasing the concentration to 10  $\mu\text{M}$  led to the almost complete abolishment of the signal.

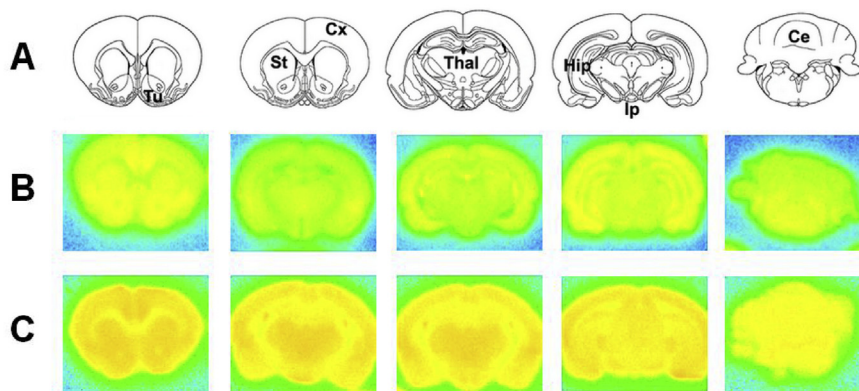
#### 4. Conclusion

In conclusion we have demonstrated that the modulation of the ester function of the reference 5-HT<sub>4</sub>R ligands and their replacement by an amide or a ketone function do not affect the ability of the ligand to cross the blood–brain barrier. Among the synthesized derivatives, the most promising results were obtained with the amide analog 18c ( $K_i = 8.64$  nM) which was able to bind to the 5-HT<sub>4</sub>R *in vitro* as demonstrated by the displacement of [ $^{125}\text{I}$ ]1. However the non-specific interactions of [ $^{125}\text{I}$ ]18c–d with other brain regions might be explained by a fast metabolism or by their high lipophilicity which would be optimized in a future study.

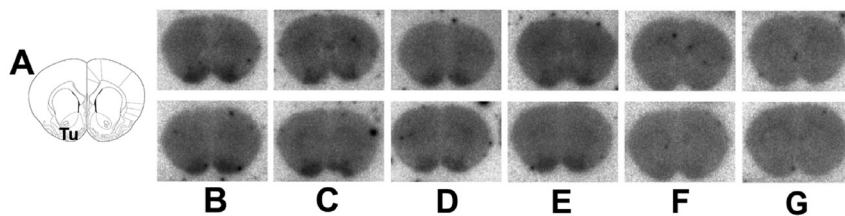
#### 5. Experimental section

##### 5.1. Chemistry

All commercially available compounds were used without further purification. Melting points were determined on a Kofler apparatus. Analytical thin-layer chromatography (TLC) was performed on silica gel 60 F<sub>254</sub> on aluminium plates (Merck) and visualized with UV light (254 nm). Flash chromatography was conducted on a VWR SPOT II Essential instrument with silica gel 60



**Fig. 3.** *Ex vivo* autoradiograms in the brain after injection of [ $^{125}\text{I}$ ]18c or [ $^{125}\text{I}$ ]18d. Anatomic atlas templates adapted from the Paxinos and Watson atlas (A), autoradiograms obtained 60 min after injection of [ $^{125}\text{I}$ ]18c (B) or [ $^{125}\text{I}$ ]18d (C). Cx: cortex, Ce: cerebellum, Hip: hippocampus, Ip: interpeduncular nucleus, Tu: olfactory tubercles, St: striatum, Thal: thalamus.



**Fig. 4.** *In vitro* competition between [ $^{125}\text{I}$ ]**1** and **18c**, **18d** or **1**. Localisation of olfactory tubercles (Tu) is shown in a coronal slice, adapted from the Paxinos and Watson atlas (A). Representative autoradiograms obtained by incubation of [ $^{125}\text{I}$ ]**1** in the presence of growing concentrations (0, 0.01, 0.1, 1, 10  $\mu\text{M}$ , from B to F, respectively) of **18c** (topline) or **18d** (line below) or in the presence of 1  $\mu\text{M}$  of **1** (G).

(40–63  $\mu\text{m}$ ). Column's size and flow rate were used according to manufacturer's recommendation. NMR spectra were recorded at 400 or 500 MHz (Bruker Avance III 400/500 MHz) for  $^1\text{H}$  NMR, at 100 or 125 MHz for  $^{13}\text{C}$  NMR and at 376.1 MHz for  $^{19}\text{F}$  in chloroform- $d$ , methanol- $d_4$  or DMSO- $d_6$  with chemical shift ( $\delta$ ) given in parts per million (ppm) relative to TMS as  $^1\text{H}$  and  $^{13}\text{C}$  NMR internal standard and  $\text{CFCl}_3$  as  $^{19}\text{F}$  NMR reference standard, and recorded at 295 K. The following abbreviations are used to describe peak splitting patterns when appropriate: br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, dt = doublet of triplet. Coupling constants  $J$  are reported in hertz units (Hz). Infrared spectra (IR) were obtained on a PERKIN-ELMER FT-IR spectrometer and are reported in terms of frequency of absorption ( $\text{cm}^{-1}$ ) using KBr discs. High-resolution mass spectra (HRMS) were obtained by electronic impact (HRMS/EI), or by electrospray (HRMS/ESI) on a Bruker maXis mass spectrometer. LC-MS (ESI) analyses were realized with Waters Alliance 2695 as separating module using the following gradients: A (95%)/B (5%) to A (5%)/B (95%) in 4.00 min. This ratio was hold during 1.50 min before return to initial conditions in 0.50 min. Initial conditions were then maintained for 2.00 min (A =  $\text{H}_2\text{O}$ , B =  $\text{CH}_3\text{CN}$ ; each containing  $\text{HCOOH}$ : 0.1%; column XBridge C18 2.5  $\mu\text{m}$ /4.6  $\times$  50 mm; flow rate 0.8 mL/min). MS were obtained on a SQ detector by positive ESI. Mass spectrum data are reported as  $m/z$ .

**Methyl 4-amino-2-hydroxybenzoate (9).** To a stirred solution of 4-amino-2-hydroxybenzoic acid (2.0 g, 13.1 mmol, 1.0 eq.) in MeOH (40 mL) was added dropwise concentrated aqueous solution of  $\text{H}_2\text{SO}_4$  (2.8 mL) and the resulting mixture was refluxed for 16 h. After cooling to room temperature, it was neutralized with saturated aqueous  $\text{NaHCO}_3$  solution until no further gas evolution was observed and the mixture was concentrated *in vacuo*. The residue was dissolved in water and extracted several times with EtOAc. The combined organic extracts were dried over  $\text{MgSO}_4$  and concentrated under reduced pressure to afford (**9**), as a brown solid (1.97 g, 90% yield); mp 113  $^\circ\text{C}$  (litt.: 115  $^\circ\text{C}$  [30]);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  10.95 (br s, 1H, OH), 7.60 (d,  $^3J = 8.9$  Hz, 1H), 6.14 (m, 2H), 4.15 (br s, 2H,  $\text{NH}_2$ ), 3.86 (s, 3H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  170.6, 163.6, 153.5, 131.7, 106.9, 103.0, 100.7, 51.8; IR (neat,  $\text{cm}^{-1}$ )  $\nu$  3475, 3381, 3249, 3025, 2952, 2851, 1642, 1437, 1356, 1283, 780; MS  $m/z$  [ $\text{M}+\text{H}$ ] $^+$  168.17.

**Methyl 4-amino-2-ethoxybenzoate (10a).** To a stirred solution of Methyl 4-amino-2-hydroxybenzoate (**9**) (500 mg, 3.0 mmol, 1.0 eq.) in DMF (25 mL) were added  $\text{K}_2\text{CO}_3$  (829 mg, 6.0 mmol, 2.0 eq.) and Iodoethane (288  $\mu\text{L}$ , 3.6 mmol, 1.2 eq.), then the resulting mixture was stirred at 70  $^\circ\text{C}$  overnight. After cooling to room temperature, the mixture was concentrated *in vacuo*. The crude was dissolved with EtOAc, then the organic layer was washed with brine, dried over  $\text{MgSO}_4$  and concentrated *in vacuo*. The crude was purified by chromatography on silica gel column (DCM/EtOAc, gradient 100:0 to 90:10) and concentrated under reduced pressure to afford (**10a**), as a white solid (360 mg, 61% yield); mp 104  $^\circ\text{C}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.70 (d,  $^3J = 8.6$  Hz, 1H), 6.18 (dd,

$^3J = 8.6$  Hz,  $^4J = 2.2$  Hz, 1H), 6.15 (d,  $^4J = 2.2$  Hz, 1H), 4.13 (br s, 2H,  $\text{NH}_2$ ), 3.99 (q,  $^3J = 7.1$  Hz, 2H), 3.79 (s, 3H), 1.42 (t,  $^3J = 7.1$  Hz, 3H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  166.4, 161.2, 152.2, 134.2, 109.0, 106.5, 98.8, 64.4, 51.4, 14.8; IR (neat,  $\text{cm}^{-1}$ )  $\nu$  3500, 3356, 3223, 2978, 2947, 1694, 1608, 1254, 1038, 811; HRMS (ESI)  $m/z$  calcd. for  $\text{C}_{10}\text{H}_{14}\text{NO}_3$  [ $\text{M}+\text{H}$ ] $^+$  196.0968, found 196.0965.

**Methyl 4-amino-2-(2-fluoroethoxy)benzoate (10b).** To a stirred solution of Methyl 4-amino-2-hydroxybenzoate (**9**) (500 mg, 3.0 mmol, 1.0 eq.) in DMF (25 mL) were added  $\text{K}_2\text{CO}_3$  (829 mg, 6.0 mmol, 2.0 eq.) and 2-fluoroethyl 4-methylbenzenesulfonate (720 mg, 3.3 mmol, 1.1 eq.) and the resulting mixture was stirred at 110  $^\circ\text{C}$  for 2 h. After cooling to room temperature, the mixture was concentrated *in vacuo*. The crude was dissolved with EtOAc, then the organic layer was washed with brine, dried over  $\text{MgSO}_4$  and concentrated *in vacuo*. The crude was purified by chromatography on silica gel column (DCM/EtOAc, gradient 100:0 to 90:10) and concentrated under reduced pressure to afford (**10b**), as a white solid (397 mg, 62% yield); mp 105  $^\circ\text{C}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.74 (d,  $^3J = 8.5$  Hz, 1H), 6.28 (dd,  $^3J = 8.5$  Hz,  $^4J = 2.2$  Hz, 1H), 6.20 (d,  $^4J = 2.2$  Hz, 1H), 4.78 (dt,  $^2J = 47.4$  Hz,  $^3J = 4.2$  Hz, 2H), 4.23 (dt,  $^3J = 27.0$  Hz,  $^3J = 4.3$  Hz, 2H), 4.07 (br s, 2H,  $\text{NH}_2$ ), 3.82 (s, 3H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  166.2, 160.8, 152.0, 134.4, 110.1, 107.6, 100.3, 82.1 (d,  $^1J = 171.0$  Hz), 68.8 (d,  $^2J = 20.7$  Hz), 51.6;  $^{19}\text{F}$  NMR ( $\text{CDCl}_3$ , 376 MHz)  $\delta$  -223.6 (tt,  $^2J = 47.4$  Hz,  $^3J = 27.0$  Hz); IR (neat,  $\text{cm}^{-1}$ )  $\nu$  3497, 3370, 3236, 2965, 2947, 1690, 1607, 1254, 1087, 776; HRMS (ESI)  $m/z$  calcd. for  $\text{C}_{10}\text{H}_{13}\text{FNO}_3$  [ $\text{M}+\text{H}$ ] $^+$  214.0874, found 214.0872.

**Representative procedure (d) for the synthesis of 11a-b.** To a stirred solution of methyl ester derivatives (1.0 eq.) in EtOH (10 mL/mmol) was added an aqueous 1N NaOH solution (10.0 eq.) under nitrogen atmosphere at room temperature. The resulting mixture was stirred overnight at room temperature, and then concentrated *in vacuo* to remove EtOH. The residue was diluted with water. The aqueous layer was acidified by addition of hydrochloric acid until acidic pH, and extracted several times with ethyl acetate. The combined organic extract was washed with brine, dried over  $\text{MgSO}_4$  and concentrated under reduced pressure to give the title compounds **11a-b**.

**4-amino-2-ethoxybenzoic acid (11a).** The compound was prepared from Methyl 4-amino-2-ethoxybenzoate (**10a**) (870 mg, 4.5 mmol) according to procedure (d) and was obtained as a pale yellow solid (756 mg, 94% yield); mp 146  $^\circ\text{C}$  (litt.: 152–154  $^\circ\text{C}$  [31]);  $^1\text{H}$  NMR ( $\text{MeOD}-d_4$ , 400 MHz)  $\delta$  7.70 (d,  $^3J = 8.6$  Hz, 1H), 6.33 (d,  $^4J = 2.0$  Hz, 1H), 6.29 (dd,  $^3J = 8.6$  Hz,  $^4J = 2.0$  Hz, 1H), 4.21 (q,  $^3J = 7.0$  Hz, 2H), 1.47 (t,  $^3J = 7.0$  Hz, 3H);  $^{13}\text{C}$  NMR ( $\text{MeOD}-d_4$ , 100 MHz)  $\delta$  169.4, 161.8, 156.9, 135.5, 108.3, 106.1, 98.2, 66.1, 14.8; IR (neat,  $\text{cm}^{-1}$ )  $\nu$  3435, 3353, 3242, 2982, 2930, 2851, 1690, 1604, 1404, 1273, 1197, 1029; MS  $m/z$  [ $\text{M}+\text{H}$ ] $^+$  182.42.

**4-amino-2-(2-fluoroethoxy)benzoic acid (11b).** The compound was prepared from Methyl 4-amino-2-(2-fluoroethoxy)benzoate (**10b**) (940 mg, 4.4 mmol) according to procedure (d) and was obtained as a pale yellow solid (840 mg, 96% yield); mp 124  $^\circ\text{C}$ ;

$^1\text{H}$  NMR (MeOD- $d_4$ , 400 MHz)  $\delta$  7.70 (d,  $^3J$  = 8.4 Hz, 1H), 6.34–6.30 (m, 2H), 4.79 (dt,  $^2J$  = 47.7 Hz,  $^3J$  = 4.1 Hz, 2H), 4.34 (dt,  $^3J$  = 27.9 Hz,  $^3J$  = 4.1 Hz, 2H);  $^{13}\text{C}$  NMR (MeOD- $d_4$ , 100 MHz)  $\delta$  169.3, 161.7, 156.8, 135.6, 108.6, 106.8, 98.8, 82.7 (d,  $^1J$  = 167.7 Hz), 69.8 (d,  $^2J$  = 19.8 Hz);  $^{19}\text{F}$  NMR (MeOD- $d_4$ , 376 MHz)  $\delta$  -226.22 (tt,  $^2J$  = 47.7 Hz,  $^3J$  = 27.9 Hz); IR (neat,  $\text{cm}^{-1}$ )  $\nu$  3490, 3365, 3282, 3240, 2984, 2949, 1697, 1609, 1388, 1274, 1196, 1039, 884; HRMS (ESI)  $m/z$  calcd. for  $\text{C}_9\text{H}_{11}\text{FNO}_3$   $[\text{M}+\text{H}]^+$  200.0717, found 200.0714.

**Representative procedure (e) for the synthesis of 12a-b.** To a solution of benzoic acid derivatives (1.0 eq.) in dry THF (10 mL/mmol) was added CDI (1.1 eq.) and the resulting mixture was stirred at room temperature for 15 h. Then potassium 3-ethoxy-3-oxopropanoate (1.2 eq.) and  $\text{MgCl}_2$  (1.2 eq.) were added portionwise. The reaction mixture was stirred at 40 °C for 24 h. After removal of the solvent, the residue was dissolved with EtOAc, and washed with a saturated aqueous  $\text{NaHCO}_3$  solution then brine. The organic layer was dried ( $\text{MgSO}_4$ ) and concentrated *in vacuo*. Chromatographic separation gave the title compounds.

**Ethyl 3-(4-amino-2-ethoxyphenyl)-3-oxopropanoate (12a).** The compound was prepared from 4-amino-2-ethoxybenzoic acid (**11a**) (750 mg, 4.1 mmol) according to procedure (e). After a purification by chromatography on silica gel column (cyclohexane/EtOAc, gradient 100:0 to 70:30), the compound (**12a**) was obtained as a white solid (233 mg, 23% isolated yield); mp 119 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.82 (d,  $^3J$  = 8.6 Hz, 1H), 6.25 (dd,  $^3J$  = 8.6 Hz,  $^4J$  = 2.1 Hz, 1H), 6.10 (d,  $^4J$  = 2.0 Hz, 1H), 4.18 (q,  $^3J$  = 7.1 Hz, 2H), 4.05 (q,  $^3J$  = 7.0 Hz, 2H), 3.94 (s, 2H), 1.45 (t,  $^3J$  = 7.0 Hz, 3H), 1.24 (t,  $^3J$  = 7.1 Hz, 3H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  190.9, 169.2, 161.3, 153.2, 133.7, 117.1, 107.4, 97.1, 64.1, 60.9, 50.7, 14.7, 14.3; IR (neat,  $\text{cm}^{-1}$ )  $\nu$  3477, 3381, 3244, 2977, 2938, 2889, 1720, 1594, 1457, 1338, 1204, 1017, 832; HRMS (ESI)  $m/z$  calcd. for  $\text{C}_{13}\text{H}_{18}\text{NO}_4$   $[\text{M}+\text{H}]^+$  252.1230, found 252.1227.

**Ethyl 3-[4-amino-2-(2-fluoroethoxy)phenyl]-3-oxopropanoate (12b).** The compound was prepared from 4-amino-2-(2-fluoroethoxy)benzoic acid (**11b**) (840 mg, 4.21 mmol) according to procedure (e). After a purification by chromatography on silica gel column (cyclohexane/EtOAc, gradient 100:0 to 50:50), the compound (**12b**) was obtained as a white solid (406 mg, 36% isolated yield); mp 117 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.85 (d,  $^3J$  = 8.6 Hz, 1H), 6.30 (dd,  $^3J$  = 8.6 Hz,  $^4J$  = 2.1 Hz, 1H), 6.09 (d,  $^4J$  = 2.0 Hz, 1H), 4.79 (dt,  $^2J$  = 47.3 Hz,  $^3J$  = 4.1 Hz, 2H), 4.24 (dt,  $^3J$  = 27.6 Hz,  $^3J$  = 4.2 Hz, 2H), 4.18 (q,  $^3J$  = 7.1 Hz, 2H), 3.96 (s, 2H), 1.24 (t,  $^3J$  = 7.1 Hz, 3H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  190.8, 169.1, 160.5, 153.2, 133.8, 117.2, 108.1, 97.3, 81.5 (d,  $^1J$  = 170.8 Hz), 67.6 (d,  $^2J$  = 20.3 Hz), 61.0, 50.6, 14.2;  $^{19}\text{F}$  NMR ( $\text{CDCl}_3$ , 376 MHz)  $\delta$  -223.19 (tt,  $^2J$  = 47.4 Hz,  $^3J$  = 27.4 Hz); IR (neat,  $\text{cm}^{-1}$ )  $\nu$  3471, 3375, 3244, 2990, 2962, 2927, 2854, 1710, 1612, 1446, 1341, 1208, 1015, 822; HRMS (ESI)  $m/z$  calcd. for  $\text{C}_{13}\text{H}_{17}\text{FNO}_4$   $[\text{M}+\text{H}]^+$  270.1136, found 270.1132.

**Representatives procedures (f) and (g) for the synthesis of 13a-b.** To a solution of  $\beta$ -keto ester derivatives (1.0 eq.) in DMF (10 mL/mmol) were added  $\text{K}_2\text{CO}_3$  (2.0 eq.) and *Tert*-butyl 4-(iodomethyl)piperidine-1-carboxylate (1.2 eq.). The resulting mixture was stirred at room temperature for 48 h then concentrated *in vacuo*. The residue was dissolved with EtOAc and washed with brine. The organic layer was dried ( $\text{MgSO}_4$ ) and concentrated *in vacuo*. To a stirred solution of residue (1.0 eq.), used without any purification, in a mixture of EtOH/ $\text{H}_2\text{O}$  5:1 (24 mL/mmol) was added KOH (4.5 eq.) and the resulting mixture was refluxed for 5 h. After removal of the solvent, EtOAc was added. The organic layer was washed with brine, dried over  $\text{MgSO}_4$  and concentrated *in vacuo*. The residue was purified by chromatography on silica gel to give the title compounds **13a-b**.

***Tert*-butyl 4-[3-(4-amino-2-ethoxyphenyl)-3-oxopropyl]piperidine-1-carboxylate (13a).** The compound was prepared

from Ethyl 3-(4-amino-2-ethoxyphenyl)-3-oxopropanoate (**12a**) (249 mg, 1.0 mmol) according to procedures (f) and (g). After a purification by chromatography on silica gel column (cyclohexane/EtOAc, gradient 100:0 to 60:40), the compound (**13a**) was obtained as a yellow oil (279 mg, 74% isolated yield over 2 steps);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.69 (d,  $^3J$  = 8.5 Hz, 1H), 6.23 (dd,  $^3J$  = 8.5 Hz,  $^4J$  = 2.1 Hz, 1H), 6.12 (d,  $^4J$  = 2.0 Hz, 1H), 4.05 (m, 4H), 2.96 (t,  $^3J$  = 7.3 Hz, 2H), 2.66 (m, 2H), 1.67–1.58 (m, 4H), 1.45 (t,  $^3J$  = 7.0 Hz, 3H), 1.44 (s, 9H), 1.41 (m, 1H), 1.10 (m, 2H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  200.1, 160.7, 155.0, 152.2, 133.2, 118.6, 107.2, 97.6, 79.3, 63.9, 44.1 (2C), 40.9, 36.0, 32.2 (2C), 31.3, 28.6 (3C), 15.0; IR (neat,  $\text{cm}^{-1}$ )  $\nu$  3445, 3356, 3239, 2979, 2931, 2858, 1675, 1646, 1594, 1277, 1037, 818; HRMS (ESI)  $m/z$  calcd. for  $\text{C}_{21}\text{H}_{33}\text{N}_2\text{O}_4$   $[\text{M}+\text{H}]^+$  377.2435, found 377.2435.

***Tert*-butyl 4-[3-[4-amino-2-(2-fluoroethoxy)phenyl]-3-oxopropyl]piperidine-1-carboxylate (13b).** The compound was prepared from Ethyl 3-[4-amino-2-(2-fluoroethoxy)phenyl]-3-oxopropanoate (**12b**) (400 mg, 1.49 mmol) according to procedures (f) and (g). After a purification by chromatography on silica gel column (cyclohexane/EtOAc, gradient 100:0 to 60:40), the compound (**13b**) was obtained as a yellow oil (414 mg, 70% isolated yield over 2 steps); mp 119 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.66 (d,  $^3J$  = 8.5 Hz, 1H), 6.23 (dd,  $^3J$  = 8.5 Hz,  $^4J$  = 1.9 Hz, 1H), 6.08 (d,  $^4J$  = 1.9 Hz, 1H), 4.72 (dt,  $^2J$  = 47.5 Hz,  $^3J$  = 5.3 Hz, 2H), 4.32 (br s, 2H,  $\text{NH}_2$ ), 4.16 (dt,  $^3J$  = 5.3 Hz,  $^3J$  = 28.0 Hz, 2H), 4.02 (m, 2H), 2.95 (t,  $^3J$  = 7.4 Hz, 2H), 2.63 (m, 2H), 1.65–1.55 (m, 4H), 1.41 (s, 9H), 1.37 (m, 1H), 1.05 (m, 2H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  199.9, 160.0, 154.9, 152.7, 133.1, 118.0, 107.6, 97.4, 81.6 (d,  $^1J$  = 170.9 Hz), 79.4, 67.4 (d,  $^2J$  = 19.7 Hz), 43.7 (2C), 40.8, 35.8, 32.0 (2C), 31.1, 28.4 (3C);  $^{19}\text{F}$  NMR ( $\text{CDCl}_3$ , 376 MHz)  $\delta$  -223.28 (tt,  $^2J$  = 47.5 Hz,  $^3J$  = 27.8 Hz); IR (neat,  $\text{cm}^{-1}$ )  $\nu$  3450, 3355, 3239, 2926, 2858, 1677, 1641, 1595, 1277, 1062; HRMS (ESI)  $m/z$  calcd. for  $\text{C}_{21}\text{H}_{32}\text{FN}_2\text{O}_4$   $[\text{M}+\text{H}]^+$  395.2341, found 395.2341.

**Representatives procedures (h) and (i) for the synthesis of 14a-b.** To a stirred solution of *Tert*-butyl piperidine-1-carboxylate derivatives (1.0 eq.) in DCM (20 mL/mmol) was added TFA (2 mL/mmol). The resulting mixture was stirred at room temperature for 1 h. Removal of the solvent under vacuum afforded the crude product, which was directly engaged in the next step. The residue obtained (1.0 eq.) was dissolved in DMF (10 mL/mmol) and Bromomethylcyclohexane (1.1 eq.) and  $\text{K}_2\text{CO}_3$  (10.0 eq.) were added. The resulting mixture was stirred at 110 °C for 5 h, and then concentrated *in vacuo*. Ethyl acetate was added, the organic layer was washed several times with brine, dried over  $\text{MgSO}_4$  and concentrated *in vacuo*. The crude was purified by chromatography on silica gel column and concentrated under reduced pressure to afford the corresponding alkylated compounds **14a-b**.

**1-(4-amino-2-ethoxyphenyl)-3-[1-(cyclohexylmethyl)-4-piperidyl]propan-1-one (14a).** The compound was prepared from *Tert*-butyl 4-[3-(4-amino-2-ethoxyphenyl)-3-oxopropyl]piperidine-1-carboxylate (**13a**) (280 mg, 0.74 mmol) according to procedures (h) and (i). After a purification by chromatography on silica gel column (cyclohexane/EtOAc, gradient 100:0 to 20:80), the compound (**14a**) was obtained as a yellow solid (159 mg, 58% isolated yield over 2 steps); mp 84 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.68 (d,  $^3J$  = 8.5 Hz, 1H), 6.23 (dd,  $^3J$  = 8.5 Hz,  $^4J$  = 2.1 Hz, 1H), 6.12 (d,  $^4J$  = 2.0 Hz, 1H), 4.09 (br s, 2H,  $\text{NH}_2$ ), 4.05 (q,  $^3J$  = 7.0 Hz, 2H), 3.02 (m, 2H), 2.95 (t,  $^3J$  = 7.4 Hz, 2H), 2.28 (m, 2H), 2.07 (m, 2H), 1.79–1.59 (m, 9H), 1.56 (m, 1H), 1.45 (t,  $^3J$  = 7.0 Hz, 3H), 1.38 (m, 1H), 1.27–1.08 (m, 5H), 0.90 (m, 2H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  200.1, 160.8, 152.2, 133.2, 118.5, 107.1, 97.6, 65.4, 63.9, 54.2 (2C), 41.0, 35.2, 34.7, 32.1 (2C), 31.2 (2C), 31.0, 26.6, 26.1 (2C), 15.0; IR (neat,  $\text{cm}^{-1}$ )  $\nu$  3445, 3349, 3242, 2919, 2846, 1637, 1587, 1454, 1273, 1036, 980, 827; HRMS (ESI)  $m/z$  calcd. for  $\text{C}_{23}\text{H}_{37}\text{N}_2\text{O}_2$   $[\text{M}+\text{H}]^+$  373.2850, found 373.2850.

**1-[4-amino-2-(2-fluoroethoxy)phenyl]-3-[1-(cyclohexylmethyl)-4-piperidyl]propan-1-one (14b).** The compound was prepared from *Tert*-butyl 4-[3-[4-amino-2-(2-fluoroethoxy)phenyl]-3-oxopropyl]piperidine-1-carboxylate (**13b**) (240 mg, 0.61 mmol) according to procedures (h) and (i). After a purification by chromatography on silica gel column (cyclohexane/EtOAc, gradient 100:0 to 20:80), the compound (**14b**) was obtained as a yellow solid (102 mg, 43% isolated yield over 2 steps); mp 72 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.70 (d, <sup>3</sup>J = 8.5 Hz, 1H), 6.27 (dd, <sup>3</sup>J = 8.5 Hz, <sup>4</sup>J = 2.0 Hz, 1H), 6.10 (d, <sup>4</sup>J = 2.0 Hz, 1H), 4.77 (dt, <sup>2</sup>J = 47.4 Hz, <sup>3</sup>J = 4.1 Hz, 2H), 4.22 (dt, <sup>3</sup>J = 4.1 Hz, <sup>3</sup>J = 27.6 Hz, 2H), 4.10 (br s, 2H, NH<sub>2</sub>), 2.96 (t, <sup>3</sup>J = 7.6 Hz, 2H), 2.83 (m, 2H), 2.06 (d, <sup>3</sup>J = 7.0 Hz, 2H), 1.82–1.57 (m, 11H), 1.46 (m, 1H), 1.27–1.08 (m, 6H), 0.84 (m, 2H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 200.3, 160.0, 152.1, 133.3, 118.9, 107.8, 97.8, 81.7 (d, <sup>1</sup>J = 171.2 Hz), 67.5 (d, <sup>2</sup>J = 20.2 Hz), 66.4, 54.7 (2C), 41.3, 36.0, 35.4, 32.5 (2C), 32.3 (2C), 31.4, 26.9, 26.4 (2C); <sup>19</sup>F NMR (CDCl<sub>3</sub>, 376 MHz) δ -223.3 (tt, <sup>2</sup>J = 47.1 Hz, <sup>3</sup>J = 27.8 Hz); IR (neat, cm<sup>-1</sup>) ν 3429, 3347, 3240, 2921, 2849–2768, 1637, 1591, 1443, 1274; HRMS (ESI) *m/z* calcd. for C<sub>23</sub>H<sub>36</sub>FN<sub>2</sub>O<sub>2</sub> [M+H]<sup>+</sup> 391.2755, found 391.2755.

**Representative procedure (j) for the synthesis of 15a–b.** To a stirred solution of piperidine-4-carboxamide (1.0 eq.) in EtOH (2 mL/mmol) were added K<sub>2</sub>CO<sub>3</sub> (2.0 eq.) and alkyl-halogenated compound (1.1–1.25 eq.) and the resulting mixture was refluxed for 15–24 h. After cooling to room temperature, the mixture was concentrated *in vacuo*. The crude was dissolved with CHCl<sub>3</sub>, then the organic layer was washed with brine, dried over MgSO<sub>4</sub> and concentrated *in vacuo*. The residue obtained was precipitated with petroleum ether and filtered to afford alkylated compound (92–93% yields).

**1-propylpiperidine-4-carboxamide (15a).** The compound was prepared from Piperidine-4-carboxamide (6.0 g, 46.8 mmol) and 1-iodopropane (9.94 g, 58.5 mmol) according to procedure (j), with a reflux for 15 h, and was obtained as a white solid (7.4 g, 92% yield); mp 162 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 5.49 (br s, 2H, NH<sub>2</sub>), 2.97 (m, 2H), 2.29–2.25 (m, 2H), 2.19–2.11 (m, 1H), 1.96–1.88 (m, 4H), 1.80–1.68 (m, 2H), 1.54–1.45 (m, 2H), 0.89 (t, <sup>3</sup>J = 7.4 Hz, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 177.4, 61.0, 53.4 (2C), 43.1, 29.2 (2C), 20.3, 12.1; IR (neat, cm<sup>-1</sup>) ν 3382, 3194, 2953, 2931, 2872, 2809–2680, 1653, 1425, 1143, 675; HRMS (ESI) *m/z* calcd. for C<sub>9</sub>H<sub>19</sub>N<sub>2</sub>O [M+H]<sup>+</sup> 171.1492, found 171.1489.

**1-(cyclohexylmethyl)piperidine-4-carboxamide (15b).** The compound was prepared from piperidine-4-carboxamide (2.92 g, 22.8 mmol) and bromomethylcyclohexane (4.44 g, 25.1 mmol) according to procedure (j), with a reflux for 24 h, and was obtained as white crystals (4.77 g, 93% yield); mp 178 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ 2.89 (m, 2H), 2.09 (m, 1H), 2.08 (d, <sup>3</sup>J = 7.1 Hz, 2H), 1.91–1.83 (m, 4H), 1.76–1.64 (m, 7H), 1.46 (m, 1H), 1.24–1.10 (m, 3H), 0.84 (m, 2H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 126 MHz) δ 118.0, 66.0, 53.8 (2C), 43.2, 35.3, 32.1 (2C), 29.1 (2C), 26.9, 26.3 (2C); IR (neat, cm<sup>-1</sup>) ν 3387, 3184, 2939, 2922, 2850, 1648, 1455, 1448, 1126, 635; HRMS (ESI) *m/z* calcd. for C<sub>13</sub>H<sub>25</sub>N<sub>2</sub>O [M+H]<sup>+</sup> 225.1961, found 225.1959.

**Representative procedure (k) for the synthesis of 16a–b.** To a stirred solution of alkylated piperidine-4-carboxamide (1.0 eq.) in anhydrous THF (5 mL/mmol) at 0 °C under nitrogen atmosphere was carefully added LiAlH<sub>4</sub> (3.0 eq.). The resulting mixture was stirred at room temperature for 1–2 h and then refluxed for 3 h. The reaction was then slowly quenched at 0 °C with *n* mL of water (*n* mL for *n* grams of LiAlH<sub>4</sub> used), *n* mL of an aqueous 15% NaOH solution followed by 3*n* mL of water to afford a granular inorganic precipitate. The solution was filtered over a pad of celite, the solid was rinsed with diethyl ether and EtOAc, then the filtrate was concentrated under reduced pressure to give reduced compound (71–96% yields).

**(1-propyl-4-piperidyl)methanamine (16a).** The compound

was prepared from 1-propyl piperidine-4-carboxamide (**15a**) (0.2 g, 1.17 mmol) according to procedure (k), with a stirring at room temperature for 1 h and a reflux for 3 h, and was obtained as a yellow oil (0.13 g, 71% yield); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 2.93 (m, 2H), 2.55 (d, <sup>3</sup>J = 5.9 Hz, 2H), 2.25 (m, 2H), 1.90–1.83 (m, 2H), 1.70–1.44 (m, 5H), 1.23–1.17 (m, 2H), 0.86 (t, <sup>3</sup>J = 7.3 Hz, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 126 MHz) δ 61.2, 53.8 (2C), 48.2, 39.5, 30.0 (2C), 20.3, 12.9; IR (neat, cm<sup>-1</sup>) ν 3378, 2956, 2930, 2874–2735, 1539, 1482, 1309, 1141.

**[1-(cyclohexylmethyl)-4-piperidyl]methanamine (16b).** The compound was prepared from 1-(cyclohexylmethyl)piperidine-4-carboxamide (**15b**) (3.0 g, 13.4 mmol) according to procedure (k), with a stirring at room temperature for 2 h and a reflux for 3 h, and was obtained as a white solid (2.70 g, 96% yield); mp 83 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ 2.91 (br s, 2H, NH<sub>2</sub>), 2.85 (m, 2H), 2.56 (d, <sup>3</sup>J = 6.4 Hz, 2H), 2.07 (d, <sup>3</sup>J = 7.0 Hz, 2H), 1.81 (m, 2H), 1.72 (m, 2H), 1.69–1.61 (m, 5H), 1.45 (m, 1H), 1.30–1.26 (m, 1H), 1.25–1.08 (m, 5H), 0.83 (m, 2H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 126 MHz) δ 66.3, 54.3 (2C), 47.7, 38.9, 35.3, 32.2 (2C), 29.9 (2C), 26.9, 26.3 (2C); IR (neat, cm<sup>-1</sup>) ν 3400, 3272, 2920, 2850, 1535, 1481, 1300, 1130; HRMS (ESI) *m/z* calcd. for C<sub>13</sub>H<sub>27</sub>N<sub>2</sub> [M+H]<sup>+</sup> 211.2169, found 211.2169.

**Representative procedure (l) for the synthesis of 17a–d.** To a stirred solution of acid derivative (1.0 eq.) in DMF (5 mL/mmol) were added Et<sub>3</sub>N (1.0 eq.), EDC (1.0 eq.), HOBt (1.0 eq.) and amine derivatives (1.0 eq.) under nitrogen atmosphere and the resulting mixture was stirred at room temperature for 18–72 h. After evaporation *in vacuo* to remove DMF, the residue was purified by chromatography on silica gel column and concentrated under reduced pressure to afford expected amide derivative (25–44% isolated yields).

**4-amino-N-[[1-(cyclohexylmethyl)-4-piperidyl]methyl]-2-ethoxybenzamide (17a).** The compound was prepared from [1-(cyclohexylmethyl)-4-piperidyl]methanamine (**16b**) (221 mg, 1.05 mmol) and 4-amino-2-ethoxybenzoic acid (**11a**) (190 mg, 1.05 mmol) according to procedure (l) with a stirring at room temperature for 72 h. After a purification by flash chromatography on silica gel column (DCM/EtOAc, gradient 100:0 to 90:10), the compound (**17a**) was obtained as a yellow oil (110 mg, 28% isolated yield); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ 8.01 (d, <sup>3</sup>J = 8.5 Hz, 1H), 7.95 (br s, 1H, NH), 6.31 (dd, <sup>3</sup>J = 8.5 Hz, <sup>4</sup>J = 1.5 Hz, 1H), 6.16 (d, <sup>4</sup>J = 1.6 Hz, 1H), 4.09 (q, <sup>3</sup>J = 7.0 Hz, 2H), 3.99 (br s, 2H, NH<sub>2</sub>), 3.31 (t, <sup>3</sup>J = 6.2 Hz, 2H), 2.85 (m, 2H), 2.07 (d, <sup>3</sup>J = 7.0 Hz, 2H), 1.83 (m, 2H), 1.75–1.63 (m, 7H), 1.55 (m, 1H), 1.48 (t, <sup>3</sup>J = 6.9 Hz, 3H), 1.45 (m, 1H), 1.34–1.29 (m, 2H), 1.24–1.10 (m, 3H), 0.84 (m, 2H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 126 MHz) δ 165.7, 158.6, 150.9, 134.0, 111.9, 107.7, 98.1, 66.2, 64.4, 54.2 (2C), 45.3, 36.4, 35.4, 32.2 (2C), 30.3 (2C), 26.9, 26.3 (2C), 15.1; IR (neat, cm<sup>-1</sup>) ν 3402, 3340, 3229, 2921, 2849, 1633, 1601, 1541, 1270, 1198, 1111, 1036; HRMS (ESI) *m/z* calcd. for C<sub>22</sub>H<sub>36</sub>N<sub>3</sub>O<sub>2</sub> [M+H]<sup>+</sup> 374.2802, found 374.2802.

**4-amino-N-[[1-(cyclohexylmethyl)piperidin-4-yl]methyl]-2-(2-fluoroethoxy)benzamide (17b).** The compound was prepared from [1-(cyclohexylmethyl)-4-piperidyl]methanamine (**16b**) (242 mg, 1.15 mmol) and 4-amino-2-(2-fluoroethoxy)benzoic acid (**11b**) (230 mg, 1.15 mmol) according to procedure (l) with a stirring at room temperature for 48 h. After a purification by flash chromatography on silica gel column (DCM/EtOAc, gradient 100:0 to 90:10), the compound (**17b**) was obtained as a yellow oil (200 mg, 44% isolated yield); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ 8.03 (d, <sup>3</sup>J = 8.5 Hz, 1H), 7.79 (br s, 1H, NH), 6.37 (dd, <sup>3</sup>J = 8.5 Hz, <sup>4</sup>J = 2.1 Hz, 1H), 6.15 (d, <sup>4</sup>J = 2.1 Hz, 1H), 4.80 (dt, <sup>2</sup>J = 47.4 Hz, <sup>3</sup>J = 4.0 Hz, 2H), 4.28 (dt, <sup>3</sup>J = 27.4 Hz, <sup>3</sup>J = 4.1 Hz, 2H), 3.96 (br s, 2H, NH<sub>2</sub>), 3.32 (t, <sup>3</sup>J = 6.5 Hz, 2H), 2.86 (m, 2H), 2.08 (d, <sup>3</sup>J = 7.1 Hz, 2H), 1.84 (m, 2H), 1.75–1.63 (m, 7H), 1.56 (m, 1H), 1.47 (m, 1H), 1.33 (m, 2H), 1.25–1.12 (m, 3H), 0.84 (m, 2H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 126 MHz) δ 165.4, 157.8, 150.7, 134.3, 112.7, 108.5, 98.3, 81.5 (d, <sup>1</sup>J = 172.1 Hz), 67.8 (d, <sup>2</sup>J = 19.3 Hz), 66.3,

54.3 (2C), 45.5, 36.3, 35.4, 32.2 (2C), 30.3 (2C), 27.0, 26.4 (2C);  $^{19}\text{F}$  NMR ( $\text{CDCl}_3$ , 376 MHz)  $\delta$  -224.4 (tt,  $^2J = 47.3$  Hz,  $^3J = 27.3$  Hz); IR (neat,  $\text{cm}^{-1}$ )  $\nu$  3411, 3340, 3222, 2921, 2850, 1635, 1602, 1541, 1283, 1120, 1065, 889; HRMS (ESI)  $m/z$  calcd. for  $\text{C}_{22}\text{H}_{35}\text{FN}_3\text{O}_2$   $[\text{M}+\text{H}]^+$  392.2708, found 392.2708.

**4-amino-N-[[1-(cyclohexylmethyl)piperidin-4-yl]methyl]-2-methoxybenzamide (17c).** The compound was prepared from [1-(cyclohexylmethyl)-4-piperidyl]methanamine (**16b**) (975 mg, 4.63 mmol) and commercial 4-amino-2-methoxybenzoic acid (**11c**) (774 mg, 4.63 mmol) according to *procedure (l)* with a stirring at room temperature for 48 h. After a purification by flash chromatography on silica gel column (gradient: DCM to DCM/EtOAc 90:10), the compound (**17c**) was obtained as a white powder (280 mg, 17% isolated yield);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  8.02 (d,  $^3J = 8.5$  Hz, 1H), 7.79 (br s, 1H, NH), 6.34 (dd,  $^3J = 8.5$  Hz,  $^4J = 2.1$  Hz, 1H), 6.20 (d,  $^4J = 2.1$  Hz, 1H), 3.98 (br s, 2H,  $\text{NH}_2$ ), 3.90 (s, 3H), 3.31 (t,  $^3J = 6.3$  Hz, 2H), 2.87 (m, 2H), 2.10 (d,  $^3J = 7.0$  Hz, 2H), 1.86 (m, 2H), 1.76–1.64 (m, 7H), 1.58 (m, 1H), 1.47 (m, 1H), 1.34 (m, 2H), 1.25–1.10 (m, 3H), 0.85 (m, 2H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 126 MHz)  $\delta$  165.6, 159.2, 150.9, 134.1, 112.1, 107.8, 97.4, 66.2, 55.9, 54.2 (2C), 45.3, 36.4, 35.4, 32.2 (2C), 30.2 (2C), 26.9, 26.3 (2C); IR (neat,  $\text{cm}^{-1}$ )  $\nu$  3408, 3342, 3224, 2921, 2849, 1638, 1601, 1538, 1505, 1281, 1208; HRMS (ESI)  $m/z$  calcd. for  $\text{C}_{21}\text{H}_{34}\text{N}_3\text{O}_2$   $[\text{M}+\text{H}]^+$  360.2646, found 360.2644.

**4-amino-2-methoxy-N-[(1-propyl-4-piperidyl)methyl]benzamide (17d).** The compound was prepared from (1-propyl-4-piperidyl)methanamine (**16a**) (562 mg, 3.60 mmol) and commercial 4-amino-2-methoxybenzoic acid (**11c**) (600 mg, 3.60 mmol) according to *procedure (l)* with a stirring at room temperature for 18 h. After a first purification by chromatography on silica gel column (DCM/EtOAc, gradient 100:0 to 90:10), the crude was finally purified by C18 reversed phase column ( $\text{H}_2\text{O}/\text{ACN}$ , gradient 100:0 to 90:10) to separate expected product and side product of peptide coupling (similar polarity on silica gel), and concentrated under reduced pressure to afford the compound (**17d**) as a white solid (275 mg, 25% isolated yield); mp 134 °C;  $^1\text{H}$  NMR ( $\text{MeOD}-d_4$ , 500 MHz)  $\delta$  7.71 (d,  $^3J = 8.5$  Hz, 1H), 6.35 (d,  $^4J = 2.0$  Hz, 1H), 6.30 (dd,  $^3J = 8.5$  Hz,  $^4J = 2.0$  Hz, 1H), 3.91 (s, 3H), 3.55 (m, 2H), 3.36 (m, 2H), 3.00 (m, 2H), 2.96 (m, 2H), 1.99 (m, 2H), 1.93 (m, 1H), 1.75 (m, 2H), 1.55 (m, 2H), 1.01 (t,  $^3J = 7.4$  Hz, 3H);  $^{13}\text{C}$  NMR ( $\text{MeOD}-d_4$ , 126 MHz)  $\delta$  168.9, 161.1, 155.2, 133.9, 110.1, 107.9, 97.6, 59.6, 56.2, 53.5 (2C), 44.7, 35.5, 28.3 (2C), 18.7, 11.2; IR (neat,  $\text{cm}^{-1}$ )  $\nu$  3394, 3210, 2937, 2655, 2534, 1624, 1600, 1543, 1505, 1282, 1210; HRMS (ESI)  $m/z$  calcd. for  $\text{C}_{17}\text{H}_{28}\text{N}_3\text{O}_2$   $[\text{M}+\text{H}]^+$  306.2176, found 306.2173.

**Representative procedure (m) for iodination with standard conditions of radiolabeling.** To a stirred solution of amide derivative (1.0 eq.) in peracetic acid (acetic acid/30%  $\text{H}_2\text{O}_2$  2:1, 50 mL/mmol) was added NaI (1.1 eq.) (typical brown iodine color was observed) and the resulting mixture was stirred at room temperature for 1–2 h. The reaction was cooled at 0 °C in an ice-water bath, and were added respectively water and a saturated aqueous  $\text{Na}_2\text{S}_2\text{O}_3$  solution. The mixture was then neutralized by addition of an aqueous 2N NaOH solution until basic pH (~10), and extracted several times with DCM. The combined organic extract was dried over  $\text{MgSO}_4$  and was concentrated *in vacuo*. The residue was purified by chromatography on silica gel column and concentrated under reduced pressure to afford iodinated compound (18–71% isolated yields).

**1-(4-amino-2-ethoxy-5-iodophenyl)-3-[1-(cyclohexylmethyl)-4-piperidyl]propan-1-one (18a).** The compound was prepared from 1-(4-amino-2-ethoxyphenyl)-3-[1-(cyclohexylmethyl)-4-piperidyl]propan-1-one (**14a**) (44 mg, 0.12 mmol) according to *procedure (m)* with a stirring at room temperature for 1 h. After a purification by flash chromatography on silica gel column (DCM/MeOH, gradient 100:0 to 95:5), the compound (**18a**) was obtained as a yellow solid (11 mg, 18% isolated yield); mp 98 °C;

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  8.11 (s, 1H), 6.23 (s, 1H), 4.53 (br s, 2H,  $\text{NH}_2$ ), 4.05 (q,  $^3J = 6.9$  Hz, 2H), 3.29 (m, 2H), 2.95 (t,  $^3J = 7.2$  Hz, 2H), 2.57 (m, 2H), 2.40 (m, 2H), 1.88–1.64 (m, 10H), 1.51 (m, 1H), 1.46 (t,  $^3J = 7.0$  Hz, 3H), 1.30–1.09 (m, 5H), 1.01 (m, 2H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  198.3, 160.7, 151.7, 142.0, 120.3, 97.2, 73.3, 64.7, 64.3, 54.0 (2C), 40.4, 33.9 (2C), 32.0 (2C), 29.8 (3C), 26.1, 25.9 (2C), 14.9; IR (neat,  $\text{cm}^{-1}$ )  $\nu$  3429, 3187, 2924, 2851, 1625, 1575, 1436, 1260, 1211, 1189, 1039; HRMS (ESI)  $m/z$  calcd. for  $\text{C}_{23}\text{H}_{36}\text{IN}_2\text{O}_2$   $[\text{M}+\text{H}]^+$  499.1816, found 499.1817.

**1-[4-amino-2-(2-fluoroethoxy)-5-iodophenyl]-3-[1-(cyclohexylmethyl)-4-piperidyl]propan-1-one (18b).** The compound was prepared from 1-[4-amino-2-(2-fluoroethoxy)phenyl]-3-[1-(cyclohexylmethyl)-4-piperidyl]propan-1-one (**14b**) (77 mg, 0.20 mmol) according to *procedure (m)* with a stirring at room temperature for 1 h. After a purification by flash chromatography on silica gel column (cyclohexane/EtOAc, gradient 100:0 to 20:80), the compound (**18b**) was obtained as a pale yellow solid (42 mg, 41% isolated yield); mp 129 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  8.13 (s, 1H), 6.21 (s, 1H), 4.78 (dt,  $^2J = 47.4$  Hz,  $^3J = 4.0$  Hz, 2H), 4.49 (br s, 2H,  $\text{NH}_2$ ), 4.22 (dt,  $^3J = 4.1$  Hz,  $^3J = 27.5$  Hz, 2H), 2.95 (t,  $^3J = 7.6$  Hz, 2H), 2.83 (m, 2H), 2.07 (d,  $^3J = 7.0$  Hz, 2H), 1.83–1.56 (m, 11H), 1.47 (m, 1H), 1.25–1.09 (m, 6H), 0.85 (m, 2H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  199.0, 159.8, 151.3, 142.2, 120.9, 97.4, 81.5 (d,  $^1J = 171.6$  Hz), 74.1, 67.8 (d,  $^2J = 20.2$  Hz), 66.4, 54.7 (2C), 41.2, 36.0, 35.4, 32.4 (2C), 32.3 (2C), 31.3, 27.0, 26.4 (2C);  $^{19}\text{F}$  NMR ( $\text{CDCl}_3$ , 376 MHz)  $\delta$  -223.3 (tt,  $^2J = 47.4$  Hz,  $^3J = 27.4$  Hz); IR (neat,  $\text{cm}^{-1}$ )  $\nu$  3459, 3320, 3203, 2920, 2845, 1637, 1569, 1430, 1214, 1071, 899; HRMS (ESI)  $m/z$  calcd. for  $\text{C}_{23}\text{H}_{35}\text{FIN}_2\text{O}_2$   $[\text{M}+\text{H}]^+$  517.1722, found 517.1722.

**4-amino-N-[[1-(cyclohexylmethyl)-4-piperidyl]methyl]-2-ethoxy-5-iodobenzamide (18c).** The compound was prepared from 4-amino-N-[[1-(cyclohexylmethyl)-4-piperidyl]methyl]-2-ethoxybenzamide (**17a**) (50 mg, 0.13 mmol) according to *procedure (m)* with a stirring at room temperature for 1 h. After a purification by flash chromatography on silica gel column (DCM/MeOH, gradient 100:0 to 90:10), the compound (**18c**) was obtained as a pale yellow solid (46 mg, 71% isolated yield); mp 153 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  8.44 (s, 1H), 7.86 (br s, 1H, NH), 6.25 (s, 1H), 4.41 (br s, 2H,  $\text{NH}_2$ ), 4.08 (q,  $^3J = 7.0$  Hz, 2H), 3.31 (t,  $^3J = 6.2$  Hz, 2H), 2.89 (m, 2H), 2.10 (d,  $^3J = 6.9$  Hz, 2H), 1.87 (m, 2H), 1.76–1.63 (m, 7H), 1.56 (m, 1H), 1.48 (t,  $^3J = 7.0$  Hz, 3H), 1.46 (m, 1H), 1.39–1.33 (m, 2H), 1.25–1.10 (m, 3H), 0.84 (m, 2H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 126 MHz)  $\delta$  164.4, 158.6, 150.4, 142.8, 114.0, 97.5, 73.7, 66.1, 64.8, 54.1 (2C), 45.3, 36.3, 35.3, 32.2 (2C), 30.1 (2C), 26.9, 26.3 (2C), 15.0; IR (neat,  $\text{cm}^{-1}$ )  $\nu$  3463, 3386, 3322, 3206, 2916, 2847, 2797, 2759, 1651, 1626, 1581, 1254, 1123, 815, 577; HRMS (ESI)  $m/z$  calcd. for  $\text{C}_{22}\text{H}_{35}\text{IN}_3\text{O}_2$   $[\text{M}+\text{H}]^+$  500.1769, found 500.1768.

**4-amino-N-[[1-(cyclohexylmethyl)-4-piperidyl]methyl]-2-(2-fluoroethoxy)-5-iodobenzamide (18d).** The compound was prepared from 4-amino-N-[[1-(cyclohexylmethyl)-4-piperidyl]methyl]-2-(2-fluoroethoxy)benzamide (**17b**) (75 mg, 0.19 mmol) according to *procedure (m)* with a stirring at room temperature for 1 h. After a purification by flash chromatography on silica gel column (DCM/MeOH, gradient 100:0 to 90:10), the compound (**18d**) was obtained as a white solid (66 mg, 67% isolated yield); mp 162 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  8.46 (s, 1H), 7.70 (br s, 1H, NH), 6.23 (s, 1H), 4.79 (dt,  $^2J = 47.3$  Hz,  $^3J = 4.1$  Hz, 2H), 4.44 (br s, 2H,  $\text{NH}_2$ ), 4.25 (dt,  $^3J = 27.2$  Hz,  $^3J = 4.1$  Hz, 2H), 3.31 (t,  $^3J = 6.3$  Hz, 2H), 2.85 (m, 2H), 2.07 (d,  $^3J = 7.1$  Hz, 2H), 1.83 (m, 2H), 1.75–1.63 (m, 7H), 1.55 (m, 1H), 1.46 (m, 1H), 1.32 (m, 2H), 1.25–1.10 (m, 3H), 0.85 (m, 2H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 126 MHz)  $\delta$  164.1, 157.8, 150.4, 143.1, 114.5, 97.7, 81.3 (d,  $^1J = 172.4$  Hz), 74.6, 68.0 (d,  $^2J = 19.3$  Hz), 66.3, 54.2 (2C), 45.5, 36.3, 35.4, 32.2 (2C), 30.2 (2C), 27.0, 26.3 (2C);  $^{19}\text{F}$  NMR ( $\text{CDCl}_3$ , 476 MHz)  $\delta$  -224.44 (tt,  $^2J = 48.1$  Hz,  $^3J = 27.7$  Hz); IR (neat,  $\text{cm}^{-1}$ )  $\nu$  3441, 3410, 3295, 3181, 2921, 2851, 1628, 1259, 1069, 891, 596; HRMS (ESI)  $m/z$  calcd. for  $\text{C}_{22}\text{H}_{34}\text{FIN}_3\text{O}_2$   $[\text{M}+\text{H}]^+$  518.1674,

found 518.1675.

**4-amino-N-[[1-(cyclohexylmethyl)-4-piperidyl]methyl]-5-iodo-2-methoxybenzamide (18e).** The compound was prepared from 4-amino-N-[[1-(cyclohexylmethyl)-4-piperidyl]methyl]-2-methoxybenzamide (**17c**) (200 mg, 0.56 mmol) according to *procedure (m)* with a stirring at room temperature for 2 h. After a purification by flash chromatography on silica gel column (DCM/MeOH, gradient 100:0 to 90:10), the compound (**18e**) was obtained as a white solid (182 mg, 67% isolated yield); mp 174 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  8.44 (s, 1H), 7.69 (t,  $^3J = 5.5$  Hz, 1H, NH), 6.27 (s, 1H), 4.44 (br s, 2H,  $\text{NH}_2$ ), 3.88 (s, 3H), 3.30 (t,  $^3J = 6.2$  Hz, 2H), 2.87 (m, 2H), 2.09 (d,  $^3J = 7.0$  Hz, 2H), 1.85 (m, 2H), 1.76–1.62 (m, 7H), 1.57 (m, 1H), 1.47 (m, 1H), 1.34 (m, 2H), 1.27 (m, 3H), 0.85 (m, 2H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  164.3, 159.2, 150.5, 142.9, 114.1, 96.8, 73.8, 66.1, 56.1, 54.2 (2C), 45.3, 36.4, 35.4, 32.2 (2C), 30.1 (2C), 26.9, 26.3 (2C); IR (neat,  $\text{cm}^{-1}$ )  $\nu$  3452, 3314, 3196, 2917, 2845, 1636, 1583, 1551, 1409, 1267, 1211; HRMS (EI)  $m/z$  calcd. for  $\text{C}_{21}\text{H}_{32}\text{IN}_3\text{O}_2$   $[\text{M}]^+$  485.1540, found 485.1537.

**4-amino-5-iodo-2-methoxy-N-[(1-propyl-4-piperidyl)methyl]benzamide (18f).** The compound was prepared from 4-amino-2-methoxy-N-[(1-propyl-4-piperidyl)methyl]benzamide (**17d**) (50 mg, 0.16 mmol) according to *procedure (m)*. After a purification by chromatography on silica gel column (DCM/MeOH 90:10 to DCM/MeOH/Et<sub>3</sub>N 90:10:2), the compound (**18f**) was obtained as a white solid (22 mg, 32% isolated yield); mp 114 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  8.41 (s, 1H), 7.79 (br s, 1H, NH), 6.29 (s, 1H), 4.47 (br s, 2H,  $\text{NH}_2$ ), 3.90 (s, 3H), 3.33 (m, 4H), 2.68 (m, 2H), 2.43 (m, 2H), 1.96–1.76 (m, 7H), 0.95 (t,  $^3J = 7.4$  Hz, 3H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 126 MHz)  $\delta$  164.7, 159.3, 150.8, 142.8, 113.5, 96.8, 73.7, 59.7, 56.2, 52.8 (2C), 44.5, 34.3, 27.8 (2C), 18.3, 11.6; IR (neat,  $\text{cm}^{-1}$ )  $\nu$  3395, 3197, 2959, 2933, 2649, 2531, 1623, 1587, 1538, 1257, 1213; HRMS (ESI)  $m/z$  calcd. for  $\text{C}_{17}\text{H}_{27}\text{IN}_3\text{O}_2$   $[\text{M}+\text{H}]^+$  432.1150, found 432.1142.

## 5.2. Synthesis of radiotracers

Iodine-125 radionuclide (specific activity ~629GBq/mg) was obtained from Perkinelmer.

$[\text{I}^{125}]\textbf{18c}$  and  $[\text{I}^{125}]\textbf{18d}$  were obtained by incubation, for 30 min at room temperature, of a mixture containing 5  $\mu\text{l}$  of precursor (10  $\mu\text{g}/\mu\text{l}$  in ethanol), 3  $\mu\text{l}$  of glacial acetic acid, 3  $\mu\text{l}$  of 30%  $\text{H}_2\text{O}_2$  and 10 mCi of carrier-free  $^{125}\text{I}$  sodium iodide in 0.1 M aqueous NaOH. Radiotracers were isolated by a linear gradient HPLC run (from 5% acetonitrile, ACN, to 95% ACN, 10 mM aqueous  $\text{H}_3\text{PO}_4$ , in 10 min).

HPLC was equipped with a reverse-phase column (Phenomenex Bonclone C18, Phenomenex, Schlieren, Switzerland) and radiotracers were eluted at a flow of 3 mL/min. Fractions containing radiotracers were diluted in water and loaded on a Sep-Pak cartridge (Sep-Pak C18, Waters, Switzerland). Radiotracers were eluted with 0.5 mL of 95% ACN, 10 mM  $\text{H}_3\text{PO}_4$  and concentrated using a rotary evaporator, and the final products were diluted in saline prior to animal administration.

During the HPLC run, ultraviolet absorbance and radioactivity were monitored, allowing for the measurement of specific activity thanks to calibration curves established with the cold reference compound. Radiochemical yields were 47% and 61% for  $[\text{I}^{125}]\textbf{18c}$  and  $[\text{I}^{125}]\textbf{18d}$ , respectively. Specific activity of radiotracers was superior to 200 GBq/ $\mu\text{mol}$ .

## 5.3. In vivo and Ex vivo imaging experiments

Three Sprague Dawley Mdr1a KO (SD-Abcb1<sup>tm1sage</sup>, Sigma Advance Genetic Engineering Labs, Boyertown, PA) rats (390  $\pm$  22 g) were used to determine the efficacy of  $[\text{I}^{125}]\textbf{18c}$  ( $n = 1$ ) and  $[\text{I}^{125}]\textbf{18d}$  ( $n = 2$ ) to image 5HT<sub>4</sub>R *in vivo*. Anesthetized animals (4%

isoflurane for induction, 2.5% for maintenance) were placed in the USPECT with their head positioned in the center of the field of view. Body temperature was maintained at  $37 \pm 1$  °C using a thermostatically controlled heating blanket. A 60 min acquisition was initiated upon a tail vein injection of tracer ( $26 \pm 2.4$  MBq). Immediately after the end of the acquisition, rats were euthanized by decapitation. The brains were quickly removed and frozen in pre-cold isopentane. Transverse sections (20  $\mu\text{m}$ ) were cut on a cryostat and exposed to phosphor imaging plates overnight (Fuji Photo Film Co., Tokyo, Japan). All experimental procedures were performed in accordance with the Swiss Federal Law on animal care under a protocol approved by the Ethical Committee on Animal Experimentation of the Canton of Geneva, Switzerland.

## 5.4. In vitro competition

Transverse sections (20  $\mu\text{m}$ ), from two Sprague Dawley Mdr1a KO rats, at the level of olfactory tubercles were cut on a cryostat. Slides were air-dried and stored at -20 °C. Sections were pre-incubated in 50 mM Tris-HCl buffer (pH = 7.4) for 15 min. Sections were then treated with  $[\text{I}^{125}]\textbf{SB207710}$  (0.24 MBq/ml) in 50 mM Tris-HCl buffer (pH = 7.4) for 90 min in the presence of increased **18c** or **18d** concentrations (from 0.01 to 10  $\mu\text{M}$ ) or in the presence of 1  $\mu\text{M}$  of SB207710. Sections were washed 3 times in ice cold buffer, dipped in ice-cold deionized water, and air-dried. Finally, sections were exposed to phosphor imaging plates overnight (Fuji Photo Film Co., Tokyo, Japan).

## 5.5. Biological methods

### 5.5.1. 5-HT<sub>4</sub>R binding experiment

For radioligand binding studies, 2.5  $\mu\text{g}$  of proteins (5-HT<sub>4</sub>R membrane preparations, HTS110M, Millipore) were incubated in duplicate at 25 °C for 60 min in the absence or the presence of  $10^{-6}$  or  $10^{-8}$  M of each drug and 1 nM  $[\text{I}^{125}]\textbf{GR113808}$  (VT 240, ViTrax) in 25 mM Tris buffer (pH 7.4). At the end of the incubation, homogenates were filtered through Whatman GF/C filters (Alpha Biotech) presoaked with 0.5% polyethylenimine using a Brandel cell harvester. Filters were subsequently washed three times with 4 mL of ice-cold 25 mM Tris buffer (pH 7.4). Non specific binding was evaluated in parallel in the presence of 30  $\mu\text{M}$  serotonin.

The method was validated from saturation studies: six concentrations of  $[\text{I}^{125}]\textbf{GR113808}$  were used to give final concentrations of 0.0625–2 nM, and nonspecific binding of  $[\text{I}^{125}]\textbf{GR113808}$  was defined in the presence of 30  $\mu\text{M}$  serotonin to determine the K<sub>d</sub> and the B<sub>max</sub>. For competition studies,  $[\text{I}^{125}]\textbf{GR113808}$  was used to give a final concentration of 0.2 nM.

Percentages of inhibition of the binding of  $[\text{I}^{125}]\textbf{GR113808}$  were obtained for concentrations of  $10^{-6}$  and  $10^{-8}$  M of the ligands tested. For some of these compounds, affinity constants were calculated from five-point inhibition curves using the EBDA-Ligand software and expressed as K<sub>i</sub>  $\pm$  SD.

### 5.5.2. Lipophilicity evaluation

Log P estimation was carried out using an isocratic liquid chromatography method for basic compounds derived from method described by Henchoz [29].

All experiments were performed on a UHPLC Agilent 1290 Infinity system (Agilent Technologies, Santa Clara, California, USA) equipped with a PDA detector 1260 operating at 220, 240, 254, 290 and 350 nm for all compounds. The chromatographic system was controlled by Open LAB CDS LC Chemstation™ software (revision C01.05). Retention time measurement was performed at 27 °C, with a flow rate 0.6 mL min<sup>-1</sup>, and by using an Acquity BEH Shield RP18 column (1.7  $\mu\text{m}$ , 2.1  $\times$  50 mm) from Waters (Milford, MA, USA).

Mobile phase was composed by various percentage of methanol as organic modifier (35–85%) and an aqueous basic buffer, a triethylammonium acetate solution at pH 11.5 adjusted by sodium hydroxide addition, in order to keep calibration and newly synthesized basic compounds in their neutral form.

Briefly, Log P was estimated by plotting the known log P values of 13 basic standard compounds with their log  $k_{49\%}$ , which is the retention factor at 49% methanol. Calculation of log  $k_{49\%}$  values was performed from retention time ( $t_R$ ) measurement at three different mobile phase compositions. Each compound was injected (1  $\mu$ L) once with each mobile phase composition and  $t_R$  determined from the apex of the peak. Finally, log  $k_{49\%}$  values were obtained by extrapolation to 49% organic modifier using linear relationships between log  $k$  values and methanol percentage ( $r^2 > 0.99$  for all compounds).

Calibration curve was built with Log Poct values of standards obtained from literature versus their calculated Log  $K_{49\%}$  which is the logarithm of retention factor  $K$  at 49% methanol in mobile phase. Linear relationships between Log Poct and calculated log  $K_{49\%}$  was expressed by the following equation:

$\text{Log } K_{49\%} = 0.5226 \times \text{Log Poct} - 0.7058$  with  $r^2 = 0.978$  and  $F = 444$ .

Calculated logP were obtained using MarvinSketch 5.2.6 (<http://www.chemaxon.com/products/marvin/marvinsketch/http://www.chemaxon.com/products/marvin/marvinsketch/>) or Molinspiration (<http://www.molinspiration.com/cgi-bin/properties>).

### 5.5.3. Parallel Artificial Membrane Permeability Assay (PAMPA)

The PAMPA-BBB experiment were conducted using the Pampa Explorer Kit (Pion Inc) according to manufacturer's protocol. Each stock compound solution (20 mM in DMSO) were diluted in Prisma HT buffer pH 7.4 (pION) to 100  $\mu$ M. 200  $\mu$ L of this solution ( $n = 6$ ) was added to donor plate (P/N 110243). 5  $\mu$ L of the BBB-1 Lipid (P/N 110672) was used to coat the membrane filter of the acceptor plate (P/N 110243). 200  $\mu$ L of the Brain Sink Buffer (P/N 110674) was added to each well of the acceptor plate. The sandwiches were incubated at room temperature for 4 h, without stirring. After incubation, the UV–visible spectra were measured with the microplate reader (Tecan infinite M200) and the  $-\log P_e$  were calculated for each compound by using the PAMPA Explorer software v. 3.7 (pION). Quality control standards with known  $-\log P_e$  values were used as references: the highly permeable corticosterone ( $-\log P_e = 4.6$ ) and the low permeable theophylline ( $-\log P_e < 6.0$ ) for the PAMPA-BBB experiments, and the low/moderately permeable ketoprofen and antipyrine ( $-\log P_e = 5.8$  at pH 7.4) for the PAMPA-GIT assays.

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### Abbreviations

AD	Alzheimer's Disease
BBB	blood brain barrier
5-HT	5-Hydroxytryptamine, serotonin
Ce	cerebellum
CNS	central nervous system

Cx	cortex
Hip	hippocampus
Ip	interpeduncular nucleus
PET	positron emission tomography
SPECT	single photon emission computed tomography
St	striatum
Thal	thalamus
Tu	olfactory tubercles

### Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ejmech.2016.03.059>.

### References

- [1] J.D. McCorvy, B.L. Roth, Structure and function of serotonin G protein-coupled receptors, *Pharmacol. Ther.* 150 (2015) 129–142.
- [2] J.S.D. Kumar, M.J. Mann, PET tracers for serotonin receptors and their applications, *Cent. Nerv. Syst. Agents Med. Chem.* 14 (2014) 92–112.
- [3] L.M. Paterson, B.R. Kornum, D.J. Nutt, V.W. Pike, G.M. Knudsen, 5-HT radioligands for human brain imaging with PET and SPECT, *Med. Res. Rev.* 33 (2013) 54–111.
- [4] A. Dumuis, R. Bouhelal, M. Sebben, R. Cory, J. Bockaert, A nonclassical 5-hydroxytryptamine receptor positively coupled with adenylate cyclase in the central nervous system, *Mol. Pharmacol.* 34 (1988) 880–887.
- [5] J. Bockaert, S. Claeysen, V. Compan, A. Dumuis, 5-HT<sub>4</sub> receptors, a place in the sun: act two, *Curr. Opin. Pharmacol.* 11 (2011) 87–93.
- [6] J. Tack, M. Camilleri, L. Chang, W.D. Chey, J.J. Galligan, B.E. Lacy, S. Müller, E.M. Quigley, J. Schuurkes, J.H. De Maeyer, V. Stanghellini, Systematic review: cardiovascular safety profile of 5-HT<sub>4</sub> agonists developed for gastrointestinal disorders, *Aliment. Pharmacol. Ther.* 35 (2012) 745–767.
- [7] P. Giannoni, F. Gaven, D. de Bundel, K. Baranger, E. Marchetti-Gauthier, F.S. Roman, E. Valjent, P. Marin, J. Bockaert, S. Rivera, S. Claeysen, Early administration of RS 67333, a specific 5-HT<sub>4</sub> receptor agonist, prevents amyloidogenesis and behavioral deficits in the 5XFAD mouse model of Alzheimer's disease, *Front. Aging Neurosci.* 5 (2013) 96.
- [8] S. Claeysen, J. Bockaert, P. Giannoni, Serotonin: a new hope in Alzheimer's disease?, *ACS Chem. Neurosci.* 6 (2015) 940–943.
- [9] R. Vidal, E. Castro, F. Pilar-Cuellar, J. Pascual-Brazo, A. Diaz, M.L. Rojo, R. Linge, A. Martin, E.M. Valdizan, A. Pazos, Serotonin 5-HT<sub>4</sub> Receptors: a new strategy for developing fast acting antidepressants? *Curr. Pharm. Des.* 20 (2014) 3751–3762.
- [10] R. Bureau, M. Boulouard, F. Dauphin, F. Lezoualc'h, S. Rault, Review of 5-HT<sub>4</sub>R ligands: state of art and clinical applications, *Curr. Top. Med. Chem.* 10 (2010) 527–553.
- [11] R.M. Pinder, R.N. Brogden, P.R. Sawyer, T.M. Speight, G.S. Avery, Metoclopramide: a review of its pharmacological properties and clinical use, *Drugs* 12 (1976) 81–131.
- [12] R.M. Eglen, D.W. Bonhaus, L.G. Johnson, E. Leung, R.D. Clark, Pharmacological characterization of two novel and potent 5-HT<sub>4</sub> receptor agonists, RS 67333 and RS 67506, in vitro and in vivo, *Br. J. Pharmacol.* 115 (1995) 1387–1392.
- [13] V.W. Pike, C. Hallidin, K. Nobuhara, J. Hiltunen, R.S. Mulligan, C.-G. Swahn, P. Karlsson, H. Olsson, S.P. Hume, E. Hirani, J. Whalley, L.S. Pilowsky, S. Larsson, P.O. Schnell, P.J. Ell, L. Farde, Radioiodinated SB 207710 as a radioligand in vivo: imaging of brain 5-HT<sub>4</sub> receptors with SPET, *Eur. J. Nucl. Med. Mol. Imaging* 30 (2003) 1520–1528.
- [14] B.R. Kornum, N.M. Lind, N. Gillings, L. Marner, F. Andersen, G.M. Knudsen, Evaluation of the novel 5-HT<sub>4</sub> receptor PET ligand [<sup>11</sup>C]SB207145 in the Göttingen minipig, *J. Cereb. Blood Flow. Metab.* 29 (2009) 186–196.
- [15] M.E. Haahr, P.M. Fisher, C.G. Jensen, V.G. Frokjaer, B.M. Mahon, K. Madsen, W.F. Baare, S. Lehel, A. Norremolle, E.A. Rabiner, G.M. Knudsen, Central 5-HT<sub>4</sub> receptor binding as biomarker of serotonergic tone in humans: a [<sup>11</sup>C] SB207145 PET study, *Mol. Psychiatry* 19 (2014) 427–432.
- [16] F. Caillé, T.J. Morley, A.A.S. Tavares, C. Papin, N.M. Twardy, D. Alagille, H.S. Lee, R.M. Baldwin, J.P. Seibyl, O. Barret, G.D. Tamagnan, Synthesis and biological evaluation of positron emission tomography radiotracers targeting serotonin 4 receptors in brain: [<sup>18</sup>F]MNI-698 and [<sup>18</sup>F]MNI-699, *Bioorg. Med. Chem. Lett.* 23 (2013) 6243–6247.
- [17] A.A.S. Tavares, F. Caillé, O. Barret, C. Papin, H. Lee, T.J. Morley, K. Fowles, D. Holden, J.P. Seibyl, D. Alagille, G.D. Tamagnan, In vivo evaluation of [<sup>18</sup>F]MNI-698: an [<sup>18</sup>F]-labeled radiotracer for imaging of serotonin 4 receptors in brain, *J. Nucl. Med.* 55 (2014) 858–864.
- [18] A.A.S. Tavares, F. Caillé, O. Barret, C. Papin, H. Lee, T.J. Morley, K. Fowles, D. Holden, J.P. Seibyl, D. Alagille, G.D. Tamagnan, Whole-body biodistribution and dosimetry estimates of a novel radiotracer for imaging of serotonin 4 receptors in brain: [<sup>18</sup>F]MNI-698, *Nucl. Med. Biol.* 41 (2014) 432–439.
- [19] T.G. Lohith, R. Xu, T. Tsujikawa, C.L. Morse, K.B. Anderson, R.L. Gladding, S.S. Zoghbi, M. Fujita, R.B. Innis, V.W. Pike, Evaluation in monkey of two candidate PET radioligands, [(11)C]RX-1 and [(18)F]RX-2, for imaging brain 5-

- HT4 receptors, *Synapse* 68 (2014) 613–623.
- [20] E. Dubost, N. Dumas, C. Fossey, R. Magnelli, S. Butt-Gueulle, C. Ballandonne, D.H. Caignard, F. Dulin, J. Sopkova de-Oliveira Santos, P. Millet, Y. Charnay, S. Rault, T. Cailly, F. Fabis, Synthesis and structure-affinity relationships of selective high-affinity 5-HT<sub>4</sub> receptor antagonists: application to the design of new potential single photon emission computed tomography tracers, *J. Med. Chem.* 55 (2012) 9693–9707.
- [21] N. Fresneau, N. Dumas, B.B. Tournier, C. Fossey, C. Ballandonne, A. Lesnard, P. Millet, Y. Charnay, T. Cailly, J.P. Bouillon, F. Fabis, Design of a serotonin 4 receptor radiotracer with decreased lipophilicity for single photon emission computed tomography, *Eur. J. Med. Chem.* 94 (2015) 386–396.
- [22] C. Lecoutey, D. Hedou, T. Freret, P. Giannoni, F. Gaven, M. Since, V. Bouet, C. Ballandonne, S. Corvaisier, A. Malzert-Freon, S. Mignani, T. Cresteil, M. Boulouard, S. Claeysen, C. Rochais, P. Dallemagne, Design of donecopride, a dual serotonin subtype 4 receptor agonist/acetylcholinesterase inhibitor with potential interest for Alzheimer's disease treatment, *Proc. Natl. Acad. Sci. U. S. A.* 111 (2014) E3825–E3830.
- [23] A.M. Catafau, M. Danus, S. Bullich, G. Nucci, J. Llop, S. Abanades, V.J. Cunningham, J.L. Eersels, J. Pavia, M. Farre, Characterization of the SPECT 5-HT<sub>2A</sub> receptor ligand 123 I-R91150 in healthy volunteers: Part 2 — Ketanserin displacement, *J. Nucl. Med.* 47 (2006) 929–937.
- [24] N. Dumas, M. Moulin-Sallanon, N. Ginovart, B.B. Tournier, P. Suzanne, T. Cailly, F. Fabis, S. Rault, Y. Charnay, P. Millet, Small-animal single-photon emission computed tomographic imaging of the brain serotonergic systems in wild-type and Mdr1a knockout rats, *Mol. Imaging* 13 (2014) 1–12.
- [25] S.R. Donohue, K. Varnäs, Z. Jia, B. Gulyás, V.W. Pike, C. Halldin, Synthesis and in vitro autoradiographic evaluation of a novel high-affinity radioiodinated ligand for imaging brain cannabinoid subtype-1 receptors, *Bioorg. Med. Chem. Lett.* 19 (2009) 6209–6212.
- [26] J. Mertens, D. Terriere, V. Sipido, W. Gommeren, P.M.F. Janssen, J.E. Leysen, Radiosynthesis of a new radioiodinated ligand for serotonin-5HT<sub>2</sub>-receptors, a promising tracer for  $\gamma$ -emission tomography, *J. Label. Compd. Radiopharm.* 34 (1994) 795–806.
- [27] C. Rochais, C. Lecoutey, F. Gaven, P. Giannoni, K. Hamidouche, D. Hedou, E. Dubost, D. Genest, S. Yahiaoui, T. Freret, V. Bouet, F. Dauphin, J. Sopkova de-Oliveira Santos, C. Ballandonne, S. Corvaisier, A. Malzert-Freon, R. Legay, M. Boulouard, S. Claeysen, P. Dallemagne, Novel Multi-Target Directed Ligands (MTDLs) with acetylcholinesterase (AChE) inhibitory and serotonergic subtype 4 receptor (5-HT<sub>4</sub>R) agonist activities as potential agents against Alzheimer's disease: the design of donecopride, *J. Med. Chem.* 58 (2015) 3172–3187.
- [28] C.J. Grossman, G.J. Kilpatrick, K.T. Bunce, Development of a radioligand binding assay for 5-HT<sub>4</sub> receptors in guinea-pig and rat brain, *Br. J. Pharmacol.* 109 (1993) 618–624.
- [29] Y. Henchoz, D. Guillarme, S. Martel, S. Rudaz, J.-L. Veuthey, P.-A. Carrupt, Fast log P determination by ultra-high-pressure liquid chromatography coupled with UV and mass spectrometry detections, *Anal. Bioanal. Chem.* 394 (2009) 1919–1930.
- [30] J.-Y. Park, S.-W. Kim, H.-J. Park, W.B. Im, J.-K. Lee, S.-H. Yoon, Synthesis and antioxidant effect of caffeic acid analogues bearing a carboxy and hydroxymethyl group, *Bull. Korean Chem. Soc.* 31 (2010) 3860–3863.
- [31] B. Biasotti, S. Dallavalle, L. Merlini, C. Farina, S. Gagliardi, C. Parini, P. Belfiore, Synthesis of photoactivable inhibitors of osteoclast vacuolar ATPase, *Bioorg. Med. Chem.* 11 (2003) 2247–2254.