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Brief Article

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Syntheses, Radiolabelings, and in Vitro Evaluations of Fluorinated PET Radioligands of 5-HT$_6$ Serotoninergic Receptors

Julie Colomb,[†,§] Guillaume Becker,[‡,§,∥] Sylvain Fieux,[‡,§] Luc Zimmer,[‡,§,∥] and Thierry Billard*[,†,§]

[†]Institute of Chemistry and Biochemistry (ICBMS-UMR CNRS 5246), University of Lyon, University Lyon 1, CNRS, 43 Boulevard du 11 Novembre 1918, 69622 Lyon, France

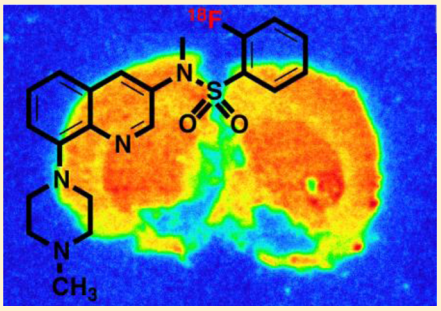
[‡]Lyon Neuroscience Research Center (UMR 5292, U 1028), University of Lyon, University Lyon 1, CNRS, INSERM, 59 Boulevard Pinel, 69003 Lyon, France

[§]CERMEP-In Vivo Imaging, Groupement Hospitalier Est, 59 Boulevard Pinel, 69003 Lyon, France

[∥]Hospices Civils de Lyon, Neurological Hospital, 59 Boulevard Pinel, 69003 Lyon, France

Supporting Information

ABSTRACT: The 5-HT$_6$ receptors are potent therapeutic targets for psychiatric and neurological diseases (schizophrenia, Alzheimer's disease, etc.). However, with lack of specific radiopharmaceuticals, their pharmacology is still incomplete and their exploration is limited to animal models. In this context, we have designed a fluorinated PET radiotracer, [^{18}F]2FNQ1P, that possesses a high affinity and selectivity for 5-HT$_6$. In vitro PET autoradiographies in rat brain sections with this radiotracer were in accordance with the 5-HT$_6$ distribution pattern.



INTRODUCTION

The 5-HT$_6$ receptor is one of the most recently discovered of the serotonin family of receptors. It was discovered by two independent groups using molecular cloning technologies and was first isolated from rat striatum.[1,2] Three years later, the human homologue was discovered by Kohen et al.[3] It is known that 5-HT$_6$ receptor density is particularly high in striatal areas in rats and humans. The first autoradiographic visualizations of 5-HT$_6$ receptor distribution used the 5-HT$_6$ receptor antagonists [^{3}H]4-amino-*N*-[2,6-bis(methylamino)pyridin-4-yl]benzenesulfonamide ([^{3}H]Ro-63-0563) and [^{125}I]**30**, in various species.[4,5] These studies consistently revealed heterogeneous binding throughout the brain, with the high levels in striatum, moderate levels in cerebral cortex, and low levels in cerebellum. Cross-species comparison showed similar distribution patterns between rats and non-human primates and humans.[6] At that stage, however, the role of the 5-HT$_6$ receptor in the brain was not yet clearly understood. Its specific brain localization, particularly in the basal ganglia and limbic regions, and the high affinity shown by several atypical antipsychotics suggested involvement in the serotonergic control of motor function, mood-dependent behavior, and related diseases. Recent studies identified 5-HT$_6$ receptors as promising targets for cognitive improvement in psychiatric or neurodegenerative diseases and for antiobesity drugs.[7,8]

In vitro imaging (e.g., autoradiography) allows post-mortem visualization and accurate delineation of receptors in animal models and humans,[9] but in vivo imaging (e.g., positron emission tomography (PET)) is essential to the development of the preclinical and clinical pharmacology[10] and of directed brain drug candidates[11] and to assess directly the involvement of serotonin 5-HT$_6$ receptors in neuropsychiatric diseases and possible therapies. This requires a PET radiotracer labeling 5-HT$_6$ receptors with high affinity, high selectivity, high signal-to-noise ratio, lipophilicity sufficient to penetrate the blood−brain barrier, relatively slow clearance, and a low level of labeled metabolites in the brain.[12] Although several selective 5-HT$_6$ antagonists exist,[7,13−17] few have been radiolabeled for successful use in PET imaging (Figure 1).

In 2005, a pharmaceutical company patented a new generation of 5-HT$_6$ receptor antagonists based on the 3-benzenesulfonyl-8-piperazine-1-ylquinoline scaffold.[18] Within this scaffold, *N*-methyl derivative **1** presented high binding affinity for 5-HT$_6$ (K_i = 0.16 nM). [^{11}C]**1** was then prepared by N-methylation of the corresponding desmethyl precursor with [^{11}C]MeOTf.[19] This radioligand readily entered the brain of anesthetized pigs, with a distribution consistent with reported 5-HT$_6$ receptor density patterns.[19,20] Selectivity, however, was by no means optimal, since [^{11}C]**1** also showed non-negligible affinity toward 5-HT$_{2A}$ receptors (K_i = 0.79 nM), a different serotoninergic receptor known to be colocalized with 5-HT$_6$ receptors, particularly in the striatum.[21] Nevertheless and because of the lack of other 5-HT$_6$ PET radiotracers, evaluation was pursued in pigs, non-human primates, and finally in humans.[22] More recently, another compound, **2**, was radio-

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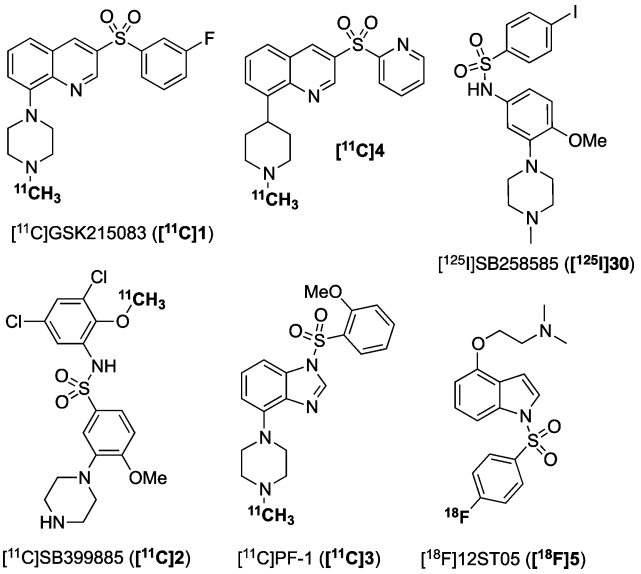

Figure 1. Published 5-HT$_6$ receptor radioligands.

labeled with carbon-11. Despite subnanomolar 5-HT$_6$ affinity (K_i = 0.78 nM) and straightforward radiolabeling, [^{11}C]**2** showed poor brain entry, limiting its usefulness for in vivo imaging of 5-HT$_6$ receptors.[23] In the same year, another group proposed various candidate 5-HT$_6$ PET ligands, of which [^{11}C]**3** appeared to be the most promising but was abandoned because of its poor radiopharmacological properties.[24] Very recently, a structural modification of [^{11}C]**1** was patented [^{11}C]**4**) as a potential PET radiotracer with subnanomolar affinity for 5-HT$_6$ receptor (K_i = 0.22 nM) and good selectivity toward 5-HT$_{2A}$ receptors (K_i = 123 nM).[25,26] Nevertheless and despite the interest of several of these radiotracers, the fact that they are radiolabeled using carbon-11 is a drawback for further development because of its short radioactive half-life ($T_{1/2}$ = 20 min). Fluorine-18 is the radionuclide of choice in PET because of its longer half-life ($T_{1/2}$ = 110 min), facilitating transfer between PET imaging centers if it can be successfully developed as a radiopharmaceutical. This last point justifies research for fluorinated candidates.

In 2007, we reported a fluorine-18 labeled ligand ([^{18}F]**5**) presenting in vitro affinity toward the 5-HT$_6$ receptor (4 nM); it showed a good brain penetration but no specific binding to 5-HT$_6$ receptors.[27] A new PET radiotracer, with greater specificity and selectivity for 5-HT$_6$ receptors and ideally with fluorine-18 radiolabeling, therefore remains an objective. This article presents the first developments in a new series of fluorinated 5-HT$_6$ tracer candidates and the emergence of a potent 5-HT$_6$ radiotracer.

RESULTS AND DISCUSSION

When this work started, the best radioligand so far described and fully validated was [^{11}C]**1**. Consequently, inspired by this structure, various structural modifications around the quinoline core were envisaged by taking care that the envisaged structural modifications always fit with the 5-HT$_6$ receptor pharmacophore.[13,28] The priority was for all these new structures to also be able to be radiolabeled with the fluoride anion fluorine-18, which is the only radioisotope source that can be introduced onto organic compounds by nucleophilic substitution. For our purposes of aromatic radiolabeling, the fluorine atom must be placed in the ortho or para position of an electron-withdrawing group to favor such a reaction.

The first envisaged modification concerned only the position of the fluorine atom in ortho or para position on the benzenesulfonyl core of **1** to determine a possible "fluorine position" effect (**6**, **7**, Scheme 1). These molecules have been previously described in literature; however, their 5-HT$_6$ activities have not been described.[29] Some replacements of the sulfonyl hydrogen acceptor part by bioisosteres, such as the sulfoxide (**8**) and sulfonamide (**9**, **10**) groups, were also considered.[30] Modifications to the piperazine substituent were also envisaged, in accordance with the pharmacophore. Consequently, the fluorobenzenesulfonyl group, which can be radiolabeled, could substitute for the methyl substituent (**11**, **12**). A combination of these modifications was also designed, modifying sulfonamide and the substituent (**13**, **14**). Finally, the moving of the fluorobenzenesulfonyl part from the quinoline position 3 to the position 5 was also envisaged (**15**).

From a retrosynthetic point of view, **6**−**14** can be obtained via two coupling reactions from 3-iodo-8-chloroquinoline **17**, easily obtained from the commercially available 8-chloroquinoline **16**, which then constitutes the common starting material (Scheme 1).

The 8-chloroquinoline **16** was regioselectively iodinated to furnish **17** with good yield.[18] Despite various attempts at direct sulfonylation of **17**,[31−34] no efficient methods proved effective, leading sometimes to desulfitative coupling.[35] Consequently, a two-step strategy was adopted: the corresponding fluorobenzene thiols were first introduced by chemoselective copper-catalyzed coupling reaction,[36] and the resulting sulfides (**18**) were then oxidized with MMPP (magnesium monoperoxyphthalate) into the expected sulfones (**19**). Buchwald previously demonstrated that the biphenylphosphine DavePhos facilitated the coupling reaction of chlorinated aromatic rings with various amino groups, such as piperazine.[37] In the case of **19**, satisfactory yields were obtained with *N*-methylpiperazine to afford the expected ligands **6** and **7**. A similar synthetic pathway was applied to obtain **8**. More moderate oxidation conditions (mCPBA) were used to stop at the sulfoxide **20**, which was then coupled to *N*-methylpiperazine. In this last coupling reaction, XPhos is preferable to DavePhos to obtain the expected product **8** in moderate yield (Scheme 1).[38]

The corresponding sulfonamides **9** and **10** were prepared by two successive palladium-catalyzed coupling reactions. Sulfonamides **21** were obtained by the chemoselective reaction of **17** with the corresponding fluorobenzenesulfonamide in good yields. A second coupling reaction with *N*-methylpiperazine, under conditions similar to those described above, led to the expected ligands **9** and **10** with satisfactory yields (Scheme 1).

Similar strategies were applied to the synthesis of **11**−**14** (Scheme 1). After a first coupling between **17** and benzenesulfinate (Cu-catalyzed) or benzenesulfonamide (Pd-catalyzed), **22** and **24** were respectively obtained with satisfactory yields. Then a second coupling reaction with *N*-Boc-piperazine afforded **23** and **25** with moderate to good yields. Finally, Boc deprotection, followed by sulfonylation with the corresponding fluorobenzenesulfonyl chloride, led to the expected ligands **11**−**14**.

The final targeting ligand **15** was synthesized by inverting the steps of quinoline iodination and coupling with piperazine. After introduction, mediated by palladium, of *N*-methylpiperazine in position 8, regioselective iodination gave rise to **27**, which was then coupled to fluorobenzenethiol with copper

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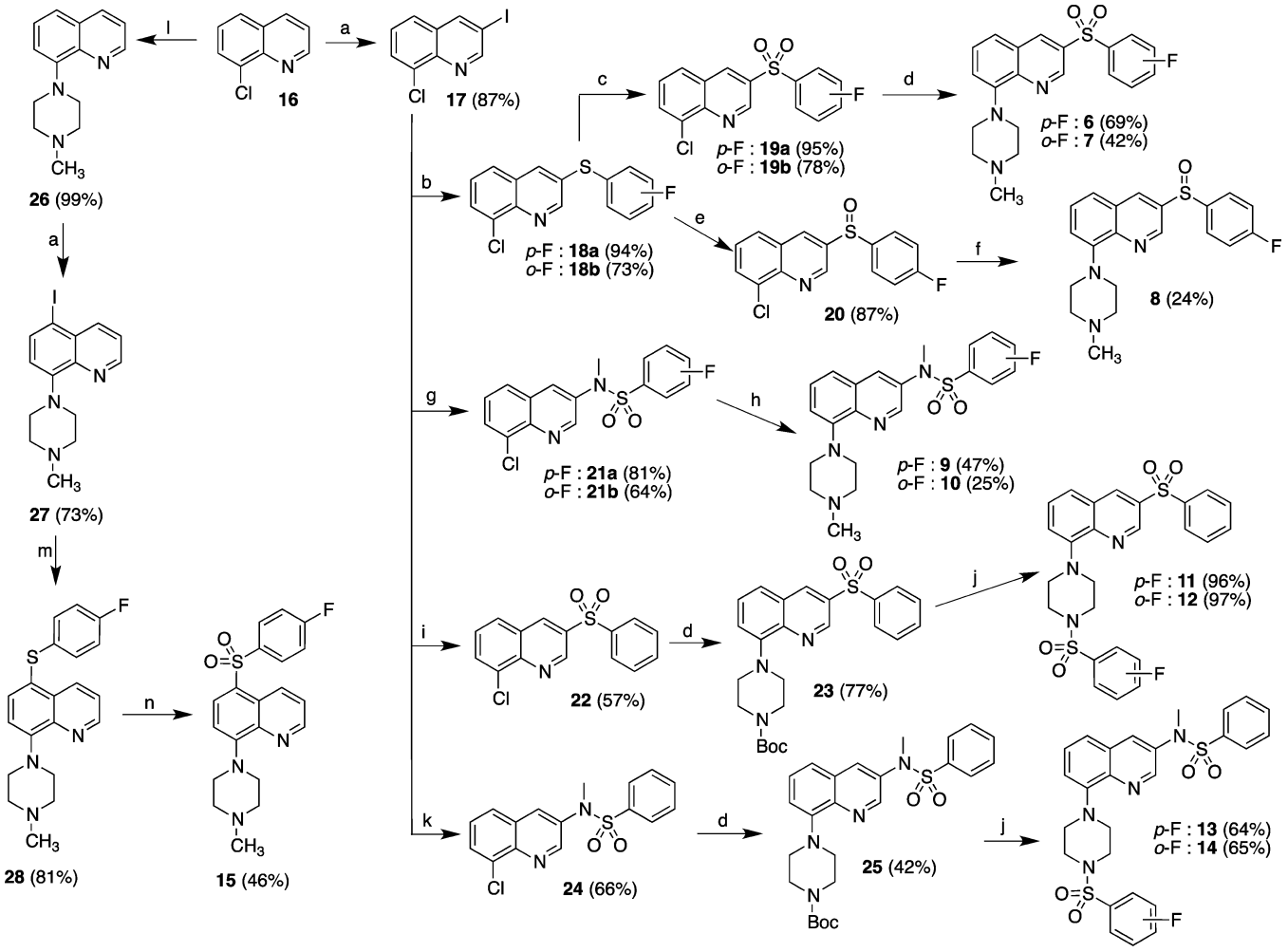
Scheme 1. Synthesis of Fluorinated Ligands 6−15[a]



[a]Reagents and conditions: (a) NIS, AcOH, 80 °C; (b) CuI (2.5%), Cs_2CO_3, thiol, DMF, 100 °C; (c) MMPP (2.25 equiv), CH_2Cl_2/MeOH; (d) Pd_2dba_3 (5%), DavePhos (10%), ^{t}BuONa (1.4 equiv), *N*-methylpiperazine, toluene, 110 °C; (e) mCPBA (1.1 equiv), CH_2Cl_2; (f) Pd_2dba_3 (5%), XPhos (10%), ^{t}BuONa (1.4 equiv), *N*-methylpiperazine, toluene, 110 °C; (g) Pd_2dba_3 (5%), XPhos (10%), Cs_2CO_3 (1.4 equiv), *N*-methylsulfonamide, toluene, 110 °C; (h) Pd_2dba_3 (5%), XPhos (10%), Cs_2CO_3 (1.4 equiv), *N*-methylpiperazine, toluene, 110 °C; (i) $PhSO_2Na$, CuI (10%), DMEDA (20%), DMSO, 110 °C; (j) (1) TFA, CH_2Cl_2; (2) $ArSO_2Cl$, Et_3N; (k) Pd_2dba_3 (5%), XPhos (10%), Cs_2CO_3 (1.4 equiv), $PhSO_2NHMe$, toluene, 110 °C; (l) Pd_2dba_3 (5%), DavePhos (10%), ^{t}BuONa (1.4 equiv), *N*-methylpiperazine, dioxane, 110 °C; (m) CuI (20%), Cs_2CO_3, thiol, DMF, 100 °C; (n) Oxone (2.05 equiv), MeOH/H_2O, 1/1.

catalysis, and the resultant sulfide **28** was oxidized into the expected sulfone **15** (Scheme 1).

Affinities of these ligands for 5-HT_6 receptors were determined by binding assays on human recombinant receptors expressed in CHO cells. 5-HT_6 receptor affinity results varied according to the compound (Table 1). It thus appeared that replacing the sulfonyl group by the sulfonamide moiety was not deleterious for the affinity, confirming the bioisostery between SO_2 and $NHMeSO_2$ (**6**, **7** vs **9**, **10**) for 5-HT_6 receptor. 5-HT_6 receptor affinities decreased with increasing of steric hindrance onto piperazine (**11**, **12**). However, excessive hindrance is highly deleterious for the affinity with K_i > 1 μM (**13**, **14**). The drop in the basicity of the piperazine nitrogen substituted by the sulfonyl group can also contribute to the loss in binding for 5-HT_6 receptor. Finally, modifying geometry by changing the substituent positions also reduces affinity (**15**).

The second receptor target that held our attention was the 5-HT_{2A} receptor, since it is known that this family is the closest to 5-HT_6 receptors, ranging between 33% and 40% in sequence homology.[39] Only molecules with K_i(5-HT_6) < 5 nM were selected. i.e., **6**−**8**, **10** (Table 1).

As expected, only modifying the position of the fluorine atom onto the benzenesulfonyl part (**6**, **7**) did not bring significantly improved affinity or selectivity in comparison to the parent **1**. A simple change in the oxidation state of the sulfur atom (**8**) decreased 5-HT_6 receptor affinity and did not improve 5-HT_{2A} receptor selectivity. Finally, bioisosteric replacement of sulfone by sulfonamide **10** led to subnanomolar 5-HT_6 receptor affinity and very low affinity for 5-HT_{2A} receptor. Because lipophilicity and molecular polar surface (PSA) could be predictive of blood−brain barrier penetration,[40−42] the lipophilicity (log *D*) and PSA of **6**−**8**, **10** were also calculated with the ACD/Labs and Marvin software applications (Table 1). PSA values were lower than 70 Å^2, predicting good brain penetration.[42] The log *D* values at physiological pH were also compatible with good brain permeation.

Consequently, radiolabeling of **10** was envisaged to obtain the corresponding ^{18}F analogues. To obtain the nitro precursor

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Table 1. Lipophilicity, PSA, 5-HT$_7$. and 5-HT$_{2A}$ Affinities (K_i) of 6−15

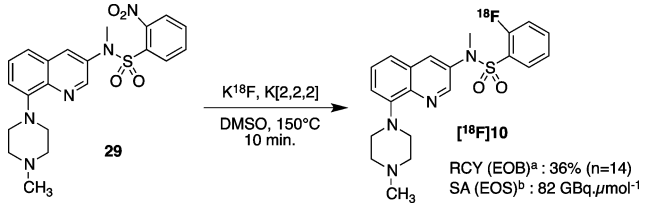
	K_i (nM)[a]				
ligand	5-HT$_6$	5-HT$_{2A}$	5-HT$_{2A}$/5-HT$_6$	log D (pH 7.4)[b]	PSA (pH 7.4) (Å^2)[c]
6	0.26	1.7	6.5	3.01	61.89
7	0.10	14	140	2.33	61.89
8	4.4	54	12.3	2.00	56.85
9	27				
10	0.9	>1 μM	>1000	1.86	66.33
11	7.6				
12	30				
13	>1 μM				
14	>1 μM				
15	53				

[a]Determined on CHO cells. [b]Calculated with ACD/Labs, version 7.09, Advanced Chemistry Development, Inc., Toronto, Ontario, Canada, www.acdlabs.com, 2014. [c]Calculator plugins were used for structure−property prediction and calculation, Marvin 6.0.3, 2013, ChemAxon (http://www.chemaxon.com).

(**29**), strategy similar to that described above (Scheme 1) was applied, with some adjustments probably required because of the presence of the highly electron-withdrawing nitro substituent, which would tend to modify substrate reactivity. In particular, triflate of quinoline had to be used to enable the final coupling step with *N*-methylpiperazine (see Supporting Information).

The nitro precursor **29** has been obtained with an overall yield of 13% and has been labeled by nucleophilic aromatic substitution of the nitro group, in classical conditions at 150 °C, in dimethyl sulfoxide (DMSO), and in presence of Kryptofix (Scheme 2).[43−45]

Scheme 2. Radiolabeling of 29[a]



[a]RCY: radiochemical yield, based on the fluorine-18 activity recovered from the resin. EOB: end of bombardment. Mean of radiochemical yields of several radiolabelings (in brackets, number of radiolabeling experiments). [b]SA: specific activity. EOS: end of synthesis.

The radiolabeled compound [^{18}F]**10** was produced with good radiochemical yield, chemical and radiochemical purity of >90%, and good specific activity of 82 GBq·μmol^{-1}.

Distributions of [^{18}F]**10** were assessed by semiquantitative autoradiography in rat brain (Figure 2). Autoradiograms obtained after incubation with a constant in vitro radiotracer concentration demonstrated the presence of structures able to concentrate radioactivity (i.e., cortex and striatum). These binding areas corresponded to those described as rich in 5-HT$_6$ receptors.[5] [^{18}F]**10** radioactivity levels were markedly reduced after addition of their respective cold molecules (1 μM). Furthermore, the binding level of [^{18}F]**10** decreased significantly after addition of 1 μM **30**, a 5-HT$_6$ antagonist.[46] These

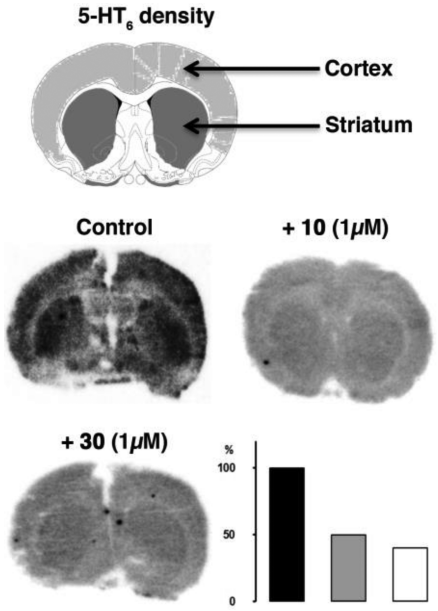


Figure 2. In vitro autoradiograms of rat brain sections incubated with [^{18}F]**10** and after competition with cold **10** or 5-HT$_6$ antagonist **30** (both at 1 μM). The corresponding anatomic slice shows 5-HT$_6$ receptor locations and density (proportional to gray level) in cortex and striatum. Histogram: decrease of [^{18}F]**10** binding after cold compound or 5-HT$_6$ antagonist addition (gray and white bars versus the control black bar).

preliminary biological results demonstrated the favorable properties of [^{18}F]**10** as a 5-HT$_6$ radiotracer.

To confirm the potential of [^{18}F]**10** as a PET radiotracer, affinities toward other receptors were explored in vitro: serotoninergic 5-HT$_{1B}$, 5-HT$_{2A}$, 5-HT$_{2B}$, 5-HT$_{2C}$, and 5-HT$_4$, adrenergic α_{1B}, and dopaminergic D$_2$ and D$_3$ receptors were selected because of their shared location in the target brain area, the striatum.[47] Compound **10** presented very low affinity toward the main receptors located in the striatal region and toward other 5-HT$_2$ family receptors, confirming its excellent selectivity for the 5-HT$_6$ receptor (Table 2).

Table 2. In Vitro Selectivity of Compound 10

receptor	K_i[a]	receptor	K_i[a]
5-HT$_6$	0.9 nM	5-HT$_4$	>10^{-6} M
5-HT$_{1B}$	>10^{-6} M	α_{1B}	>10^{-6} M
5-HT$_{2A}$	>10^{-6} M	D$_2$	>10^{-6} M
5-HT$_{2B}$	260 nM	D$_3$	>10^{-6} M
5-HT$_{2C}$	>10^{-6} M		

[a]Determined on CHO cells.

CONCLUSION

To our knowledge, this is the first report of a promising fluorinated PET radioligand for in vivo imaging of the serotonin 5-HT$_6$ receptor. We synthesized 10 compounds according to a quinoline-based scaffold. Four of these were selected for their 5-HT$_6$ receptor affinity and selectivity toward 5-HT$_{2A}$ receptor. These nonradioactive fluorinated ligands and their radiolabeling precursors were obtained from a bis-functionalized quinoline core followed by coupling reactions in three steps. Radiolabeling used ^{18}F-nucleophilic aromatic substitution. Three presented high in vitro binding in the striatum (an area rich in

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5-HT_6 receptors), but only **10** specifically presented favorable in vitro binding for 5-HT_6 receptors versus striatal 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_4, α_{1B}, D_2, and D_3 receptors. In vitro PET autoradiographies in rat brain sections confirmed the 5-HT_6 distribution pattern of [^{18}F]**10**. In conclusion, this study enabled selection of a fluorinated radiotracer candidate with suitable characteristics for PET imaging of 5-HT_6 receptors, justifying further radiopharmacological evaluation in animal models before considering human brain imaging. For the following studies, this promising molecule will be named 2FNQ1P.

EXPERIMENTAL SECTION

Chemistry. The purity of the tested compounds and their nitro precursors was assessed by RP-HPLC and elemental analysis. All compounds reported are at least 95% pure.

2-Fluoro-*N*-methyl-*N*-[8-(4-methylpiperazin-1-yl)quinolin-3-yl]benzenesulfonamide (10). **10** was obtained in 25% yield. Brown oil. ^{1}H NMR: δ 8.76 (d, J = 2.6 Hz, 1H), 7.91 (d, J = 2.6 Hz, 1H), 7.69 (ddd, J = 7.6 Hz, J = 7.6 Hz, J = 1.7 Hz, 1H), 7.57 (m, 1H), 7.43 (m, 1H), 7.35 (dd, J = 8.2 Hz, J = 1.2 Hz, 1H), 7.23−7.12 (m, 3H), 3.49−3.45 (m, 7H), 2.86 (m, 4H), 2.47 (s, 3H). ^{13}C NMR: δ 159.28 (d, J = 256.6 Hz), 149.4, 146.9, 141.2, 135.9 (d, J = 8.3 Hz), 134.7, 132.9, 131.9, 129.6, 128.1, 126.1 (d, J = 14.6 Hz), 124.9 (d, J = 3.9 Hz), 122.2, 117.7 (d, J = 21.6 Hz), 117.0, 55.4, 51.9, 46.2, 38.9. ^{19}F NMR: δ −107.64 (m). Anal. Calcd for $C_{21}H_{23}FN_4O_2S$: C, 60.85; H, 5.59; N, 13.52. Found: C, 61.03; H, 5.67; N, 13.69.

Radiosynthesis. Fluorine-18 was produced via the ^{18}O(p,n)^{18}F nuclear reaction (IBA Cyclone 18/9 cyclotron). Nitro/fluoro exchange was performed on a standard Neptis synthesizer (Ora): after initial fluoride preparation (collection, drying, and Kryptofix activation), 1.7−2.5 mg of nitro precursor **29** was introduced in 3 mL of DMSO, and the mixture was heated at 150 °C for 10 min. After dilution with 15 mL of water, the mixture was passed through an activated C18 cartridge for prepurification, and the crude product was eluted from the cartridge with 1.5 mL of methanol. Pure [^{18}F]**10** was obtained after separation on a preparative high-performance liquid chromatography (HPLC) (C18 Symmetry Prep Waters, 7 μm, 7.8 mm × 300 mm), eluting with H_2O/CH_3CN/TFA, 78/22/0.1%, at 3 mL·min^{-1} (λ = 254 nm). For biological use, the radiotracer was formulated via SPE techniques.[48] The product was diluted in 20 mL of sterile water and loaded on a SEP-Pak Light C18 cartridge (Waters, Milford, MA, U.S.). The loaded cartridge was rinsed with water and eluted with 1 mL of ethanol, and the final product was diluted with isotonic saline and sterilized by filtration (sterile filter Millex-GS, 0.22 mm). The radiochemical purity and specific activity of [^{18}F]**10** were assayed by analytical HPLC (MachereyeNagel EC 250/4.6 Nucleodur 100-5-C18ec C18 column; mobile phase H_3PO_4 20 mM/THF, 77/23; flow rate, 0.9 mL·min^{-1}). The identity of [^{18}F]**10** was confirmed by co-injection with an authentic nonradioactive sample.

In Vitro Affinities. Respective affinities toward 5-HT_6 receptors (K_i) were determined by CEREP (http://www.cerep.fr).

In Vitro Rat Autoradiographies. All animal experiments were performed in accordance with European Guidelines for Care of Laboratory Animals (86/609EEC) and were approved by the Animal Use Ethics Committee of the Université Claude Bernard Lyon 1. After euthanasia by inhaled isoflurane overdose, rat brains were carefully removed and immediately frozen in 2-methylbutane cooled with dry ice (−29 °C). Briefly, coronal sections (30 μm thick) across the hippocampus and cerebellum were cut using a −20 °C cryostat (Leica SM1850), thaw-mounted on glass slides, and allowed to air-dry before storage at −80 °C until use. On the day of radiotracer synthesis, the slides were allowed to reach room temperature and then incubated for 20 min in Tris phosphate buffered saline buffer (138 mM NaCl, 2.7 mM KCl, pH adjusted to 7.6) containing 37 kBq/mL (1 μCi/mL) [^{18}F]**10**. For competition experiments, the slides were placed in the same buffer supplemented with their respective cold compounds (**10** at 1 μM) or **30**, a selective 5-HT_6 antagonist (1 μM). After incubation, slides were dipped in cold buffer (4 °C) for 90 s, in distilled cold water (4 °C) for 90 s, and then dried and juxtaposed to a phosphor imaging plate for 60 min (BAS-1800 II, Fujifilm).

ASSOCIATED CONTENT

Supporting Information

Synthesis procedures and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: Thierry.billard@univ-lyon1.fr. Phone: (+33) 472 44 81 29.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

5-HT_6, serotoninergic receptor 6; 5-HT_{1B}, serotoninergic receptor 1B; 5-HT_{2A}, serotoninergic receptor 2A; 5-HT_{2B}, serotoninergic receptor 2B; 5-HT_{2C}, serotoninergic receptor 2C; 5-HT_4, serotoninergic receptor 4; α_{1B}, adrenergic receptor 1B; Boc, *tert*-butyloxycarbonyl; CHO, Chinese hamster ovary; D_2, dopaminergic receptor 2; D_3, dopaminergic receptor 3; DavePhos, 2-dicyclohexylphosphino-2′-(*N*,*N*-dimethylamino)-biphenyl; EOB, end of bombardment; EOS, end of synthesis; HPLC, high-performance liquid chromatography; mCPBA, *m*-chloroperbenzoic acid; MMPP, magnesium monoperoxyphthalate; NIS, *N*-iodosuccinimide; PET, positron emission tomography; PSA, polar surface area; RCY, radiochemical yield; RP-HPLC, reverse phase high-performance liquid chromatography; SA, specific activity; S_NAr, nucleophilic aromatic substitution; SPE, solid-phase extraction; XPhos, 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl

REFERENCES

(1) Monsma, F. J., Jr.; Shen, Y.; Ward, R. P.; Hamblin, M. W.; Sibley, D. R. Cloning and expression of a novel serotonin receptor with high affinity for tricyclic psychotropic drugs. *Mol. Pharmacol.* **1993**, *43*, 320−327.

(2) Ruat, M.; Traiffort, E.; Arrang, J. M.; Tardivel-Lacombe, J.; Diaz, J.; Leurs, R.; Schwartz, J. C. A novel rat serotonin (5-HT6) receptor: molecular cloning, localization and stimulation of cAMP accumulation. *Biochem. Biophys. Res. Commun.* **1993**, *193*, 268−276.

(3) Kohen, R.; Metcalf, M. A.; Khan, N.; Druck, T.; Huebner, K.; Lachowicz, J. E.; Meltzer, H. Y.; Sibley, D. R.; Roth, B. L.; Hamblin, M. W. Cloning, characterization, and chromosomal localization of a human 5-HT6 serotonin receptor. *J. Neurochem.* **1996**, *66*, 47−56.

(4) Boess, F. G.; Riemer, C.; Bos, M.; Bentley, J.; Bourson, A.; Sleight, A. J. The 5-hydroxytryptamine6 receptor-selective radioligand [3H]Ro 63-0563 labels 5-hydroxytryptamine receptor binding sites in rat and porcine striatum. *Mol. Pharmacol.* **1998**, *54*, 577−583.

(5) Roberts, J. C.; Reavill, C.; East, S. Z.; Harrison, P. J.; Patel, S.; Routledge, C.; Leslie, R. A. The distribution of 5-HT(6) receptors in rat brain: an autoradiographic binding study using the radiolabelled 5-

HT(6) receptor antagonist [(125)I]SB-258585. *Brain Res.* **2002**, *934*, 49−57.
(6) East, S. Z.; Burnet, P. W.; Leslie, R. A.; Roberts, J. C.; Harrison, P. J. 5-HT6 receptor binding sites in schizophrenia and following antipsychotic drug administration: autoradiographic studies with [^{125}I]SB-258585. *Synapse* **2002**, *45*, 191−199.
(7) Holenz, J.; Pauwels, P. J.; Díaz, J. L.; Mercè, R.; Codony, X.; Buschmann, H. Medicinal chemistry strategies to 5-HT6 receptor ligands as potential cognitive enhancers and antiobesity agents. *Drug Discovery Today* **2006**, *11*, 283−299.
(8) Marazziti, D.; Baroni, S.; Borsini, F.; Picchetti, M.; Vatteroni, E.; Falaschi, V.; Catena-Dell'Osso, M. Serotonin receptors of type 6 (5-HT6): from neuroscience to clinical pharmacology. *Curr. Med. Chem.* **2013**, *20*, 371−377.
(9) Heckl, S.; Pipkorn, R.; Nagele, T.; Vogel, U.; Kuker, W.; Voight, K. Molecular imaging: bridging the gap between neuroradiology and neurohistology. *Histol. Histopathol.* **2004**, *19*, 651−668.
(10) Lancelot, S.; Zimmer, L. Small-animal positron emission tomography as a tool for neuropharmacology. *Trends Pharmacol. Sci.* **2010**, *31*, 411−417.
(11) Zimmer, L.; Luxen, A. PET radiotracers for molecular imaging in the brain: past, present and future. *Neuroimage* **2012**, *61*, 363−370.
(12) Lee, C. M.; Farde, L. Using positron emission tomography to facilitate CNS drug development. *Trends Pharmacol. Sci.* **2006**, *27*, 310−316.
(13) Kim, H. J.; Doddareddy, M. R.; Choo, H.; Cho, Y. S.; No, K. T.; Park, W. K.; Pae, A. N. New serotonin 5-HT6 ligands from common feature pharmacophore hypotheses. *J. Chem. Inf. Model.* **2008**, *48*, 197−206.
(14) Shireman, B. T.; Bonaventure, P.; Carruthers, N. I. Recent advances on the 5-HT5A, 5-HT6 and 5-HT7 receptors. *Annu. Rep. Med. Chem.* **2008**, *43*, 25−42.
(15) Glennon, R. A. Higher-end serotonin receptors: 5-HT5, 5-HT6, and 5-HT7. *J. Med. Chem.* **2003**, *46*, 2795−2812.
(16) Ivachtchenko, A. V.; Ivanenkov, Y. A. Small molecule 5-HT6R ligands: a comprehensive insight into their selectivity and activity. *Curr. Bioact. Compd.* **2013**, *9*, 64−100.
(17) Vázquez-Villa, H.; González-Vera, J. A.; Benhamú, B.; Viso, A.; Fernández de la Pradilla, R.; Junquera, E.; Aicart, E.; López-Rodríguez, M. L.; Ortega-Gutiérrez, S. Development of molecular probes for the human 5-HT6 receptor. *J. Med. Chem.* **2010**, *53*, 7095−7106.
(18) Johnson, C. N.; Moss, S. F.; Witty, D. R. Preparation of piperazinyl-quinoline derivatives useful for the treatment of CNS disorders. WO2005030724A1, 2005.
(19) Gee, A. D.; Martarello, L.; Johnson, C. N.; Witty, D. R. Preparation of isotopomeric piperazine-containing ligands labeling and diagnostic imaging of 5-HT6 receptors. WO2006053785A1, 2006.
(20) Martarello, L.; Ahmed, M.; Chuang, A. T.; Cunningham, V. J.; Jakobsen, S.; Johnson, C. N.; Matthews, J. C.; Medhurst, A.; Moss, S. F.; Rabiner, E. A.; Ray, A.; Rivers, D.; Stemp, G.; Gee, A. D. Radiolabelling and in vivo evaluation of [^{11}C]GSK215083 as a potential 5-HT6 pet radioligand in the porcine brain. *J. Labelled Compd. Radiopharm.* **2005**, *48*, S7.
(21) Ward, R. P.; Dorsa, D. M. Colocalization of serotonin receptor subtypes 5-HT2A, 5-HT2C, and 5-HT6 with neuropeptides in rat striatum. *J. Comp. Neurol.* **1996**, *370*, 405−414.
(22) Parker, C. A.; Gunn, R. N.; Rabiner, E. A.; Slifstein, M.; Comley, R.; Salinas, C.; Johnson, C. N.; Jakobsen, S.; Houle, S.; Laruelle, M.; Cunningham, V. J.; Martarello, L. Radiosynthesis and characterization of ^{11}C-GSK215083 as a PET radioligand for the 5-HT6 receptor. *J. Nucl. Med.* **2012**, *53*, 295−303.
(23) Liu, F.; Majo, V. J.; Prabhakaran, J.; Milak, M. S.; John Mann, J.; Parsey, R. V.; Kumar, J. S. Synthesis and in vivo evaluation of [*O*-methyl-^{11}C] *N*-[3,5-dichloro-2-(methoxy)phenyl]-4-(methoxy)-3-(1-piperazinyl)benzenesulfonamide as an imaging probe for 5-HT6 receptors. *Bioorg. Med. Chem.* **2011**, *19*, 5255−5259.
(24) Zhang, L.; Villalobos, A.; Anderson, D.; Beck, E.; Blumberg, L.; Bocan, T.; Bronk, B.; Chen, L.; Brown-Proctor, C.; Grimwood, S.; Heck, S.; Skaddan, M.; McCarthy, T.; Zasadny, K. Development of design and selection parameters to accelerate the discovery process of novel CNS PET ligands and their application in the identification of a potential 5HT6 PET ligand [^{11}C]PF-1. *J. Labelled Compd. Radiopharm.* **2011**, *54*, S292.
(25) Rosse, G. Quinoline derivatives as 5-HT6 receptor PET ligands. *ACS Med. Chem. Lett.* **2014**, *5*, 275−276.
(26) Black, L. A. Radiolabeled 5-HT6 ligands. US20130343993A1, 2013.
(27) Tang, S.; Verdurand, M.; Joseph, B.; Lemoine, L.; Daoust, A.; Billard, T.; Fournet, G.; Le Bars, D.; Zimmer, L. Synthesis and biological evaluation in rat and cat of [^{18}F]12ST05 as a potential 5-HT6 PET radioligand. *Nucl. Med. Biol.* **2007**, *34*, 995−1002.
(28) Bojarski, A. J. Pharmacophore models for metabotropic 5-HT receptor ligands. *Curr. Top. Med. Chem.* **2006**, *6*, 2005−2026.
(29) Ahmed, M.; Johnson, C. N.; Jones, M. C.; MacDonald, G. J.; Moss, S. F.; Thompson, M.; Wade, C. E.; Witty, D. Preparation of arylsulfonyl(diazacycloalkyl)quinolines for treatment of CNS disorders. WO2003080580A2, 2003.
(30) Barillari, C.; Brown, N. Classical Bioisosteres. In *Bioisosteres in Medicinal Chemistry*; Brown, N., Ed.; Wiley: Weinheim, Germany, 2012; pp 15−29.
(31) Suzuki, H.; Abe, H. Copper-assisted displacement reaction of nonactivated Iodoarenes with arenesulfinates. Convenient alternative synthesis of unsymmetrical diaryl sulfones. *Tetrahedron Lett.* **1995**, *36*, 6239−6242.
(32) Baskin, J. M.; Wang, Z. An efficient copper catalyst for the formation of sulfones from sulfinic acid salts and aryl iodides. *Org. Lett.* **2002**, *4*, 4423−4425.
(33) Zhu, W.; Ma, D. Synthesis of aryl sulfones via l-proline-promoted CuI-catalyzed coupling reaction of aryl halides with sulfinic acid salts. *J. Org. Chem.* **2005**, *70*, 2696−2700.
(34) Cacchi, S.; Fabrizi, G.; Goggiamani, A.; Parisi, L. M. An efficient palladium-catalyzed synthesis of unsymmetrical diaryl sulfones from aryl bromides/triflates and arenesulfinates. *Synlett* **2003**, *2003*, 361−364.
(35) Colomb, J.; Billard, T. Palladium-catalyzed desulfitative arylation of 3-haloquinolines with arylsulfinates. *Tetrahedron Lett.* **2013**, *54*, 1471−1474.
(36) Sperotto, E.; van Klink, G. P. M.; de Vries, J. G.; van Koten, G. Ligand-free copper-catalyzed C−S coupling of aryl iodides and thiols. *J. Org. Chem.* **2008**, *73*, 5625−5628.
(37) Old, D. W.; Wolfe, J. P.; Buchwald, S. L. A highly active catalyst for palladium-catalyzed cross-coupling reactions: room-temperature Suzuki couplings and amination of unactivated aryl chlorides. *J. Am. Chem. Soc.* **1998**, *120*, 9722−9723.
(38) Shen, Q.; Ogata, T.; Hartwig, J. F. Highly reactive, general and long-lived catalysts for palladium-catalyzed amination of heteroaryl and aryl chlorides, bromides, and iodides: scope and structure−activity relationships. *J. Am. Chem. Soc.* **2008**, *130*, 6586−6596.
(39) Mitchell, E. S.; Neumaier, J. F. 5-HT6 receptors: a novel target for cognitive enhancement. *Pharmacol. Ther.* **2005**, *108*, 320−333.
(40) Clark, D. E. In silico prediction of blood−brain barrier permeation. *Drug Discovery Today* **2003**, *8*, 927−933.
(41) Norinder, U.; Haeberlein, M. Computational approaches to the prediction of the blood-brain distribution. *Adv. Drug Delivery Rev.* **2002**, *54*, 291−313.
(42) Kelder, J.; Grootenhuis, P. D. J.; Bayada, D. M.; Delbressine, L. P. C.; Ploemen, J.-P. Polar molecular surface as a dominating determinant for oral absorption and brain penetration of drugs. *Pharm. Res.* **1999**, *16*, 1514−1519.
(43) Andries, J.; Lemoine, L.; Le Bars, D.; Zimmer, L.; Billard, T. Synthesis and biological evaluation of potential 5-HT7 receptor PET radiotracers. *Eur. J. Med. Chem.* **2011**, *46*, 3455−3461.
(44) Le Bars, D. Fluorine-18 and medical imaging: radiopharmaceuticals for positron emission tomography. *J. Fluorine Chem.* **2006**, *127*, 1488−1493.
(45) Le Bars, D.; Lemaire, C.; Ginovart, N.; Plenevaux, A.; Aerts, J.; Brihaye, C.; Hassoun, W.; Leviel, V.; Mekhsian, P.; Weissmann, D.; Pujol, J. F.; Luxen, A.; Comar, D. High-yield radiosynthesis and

preliminary in vivo evaluation of p-[^{18}F]MPPF, a fluoro analog of WAY-100635. *Nucl. Med. Biol.* **1998**, *25*, 343−350.
(46) Hirst, W. D.; Minton, J. A. L.; Bromidge, S. M.; Moss, S. F.; Latter, A. J.; Riley, G.; Routledge, C.; Middlemiss, D. N.; Price, G. W. Characterization of [^{125}I]-SB-258585 binding to human recombinant and native 5-HT6 receptors in rat, pig and human brain tissue. *Br. J. Pharmacol.* **2000**, *130*, 1597−1605.
(47) Sharman, J. L.; Benson, H. E.; Pawson, A. J.; Lukito, V.; Mpamhanga, C. P.; Bombail, V.; Davenport, A. P.; Peters, J. A.; Spedding, M.; Harmar, A. J. IUPHAR-DB: updated database content and new features. *Nucleic Acids Res.* **2013**, *41*, D1083−D1088.
(48) Lemaire, C.; Plenevaux, A.; Aerts, J.; Del Fiore, G.; Brihaye, C.; Le Bars, D.; Comar, D.; Luxen, A. Solid phase extraction—an alternative to the use of rotary evaporators for solvent removal in the rapid formulation of PET radiopharmaceuticals. *J. Labelled Compd. Radiopharm.* **1999**, *42*, 63−75.