Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

Synthesis and Structure−Affinity Relationships of Selective High-Affinity 5-HT$_4$ Receptor Antagonists: Application to the Design of New Potential Single Photon Emission Computed Tomography Tracers

Emmanuelle Dubost,[†] Noé Dumas,[‡] Christine Fossey,[†] Rosa Magnelli,[†] Sabrina Butt-Gueulle,[†] Céline Ballandonne,[†] Daniel H. Caignard,[§] Fabienne Dulin,[†] Jana Sopkova de-Oliveira Santos,[†] Philippe Millet,[‡] Yves Charnay,[‡] Sylvain Rault,[†] Thomas Cailly,[†] and Frederic Fabis*[,†]

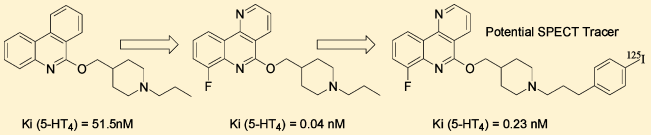
[†]UFR des Sciences Pharmaceutiques, Université de Caen Basse-Normandie, EA 4258 CERMN, FR CNRS 3038 INC3M, SF-4206 ICORE, Boulevard Becquerel, F-14032 Caen, France

[‡]Unité de Morphologie et Unité de Neuroimagerie, Service de Neuropsychiatrie, Hôpitaux Universitaires de Genève, Ch. du Petit-Bel-Air, 2, CH1225 Chêne-Bourg/Genève, Switzerland

[§]Institut de Recherches Servier, 125 Chemin de Ronde, F-78290 Croissy sur Seine, France

Supporting Information

ABSTRACT: The work described herein aims at finding new potential ligands for the brain imaging of 5-HT$_4$ receptors (5-HT$_4$Rs) using single-photon emission computed tomography (SPECT). Starting from the nonsubstituted phenanthridine compound **4a**, exhibiting a K_i value of 51 nM on the 5-HT$_4$R, we explored the structure−affinity in this series. We found that substitution in position 4 of the tricycle with a fluorine atom gave the best result. Introduction of an additional nitrogen atom inside the tricyclic framework led to an increase of both the affinity and selectivity for 5-HT$_4$R, suggesting the design of the antagonist **4v**, exhibiting a high affinity of 0.04 nM. Several iodinated analogues were then synthesized as potential SPECT tracers. The iodinated compound **11d** was able to displace the reference radioiodinated 5-HT$_4$R antagonist (1-butylpiperidin-4-yl)methyl-8-amino-7-iodo[^{123}I]-2,3-dihydrobenzo[*b*][1,4]dioxine-5-carboxylate {[^{123}I]**1**, [^{123}I]SB 207710} both in vitro and in vivo in brain. Compound **11d** was radiolabeled with [^{125}I]iodine, providing a potential SPECT candidate for brain imaging of 5-HT$_4$R.



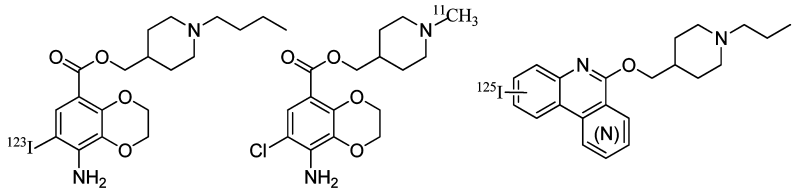
INTRODUCTION

Since its discovery more than 2 decades ago,[1,2] the serotonin 4 receptor subtype (5-HT$_4$R) has emerged as a promising target for drug discovery, development, and medical applications.[3,4] Pharmacological investigations coupled to the discovery of ligands exhibiting high affinity and selectivity for 5-HT$_4$Rs have led to knowing their anatomical distribution and functional roles. 5-HT$_4$Rs are found in the peripheral system, where they are implicated in gastrointestinal disorders[5] and heart failure.[6] Brain 5-HT$_4$Rs are mainly expressed in striatum, globus pallidus, nucleus accumbens, and substantia nigra.[7] Their distribution in the central nervous system and pharmacological studies using selective agonists and/or antagonists has shown that 5-HT$_4$Rs are implicated in cognition,[8] learning and memory processes,[9] and more recently in neuropsychiatric disorders such as Alzheimer's disease,[10,11] food intake,[12] and depression.[13] Although efforts have been made by both academics and pharmaceutical companies to develop 5-HT$_4$R ligands with potential medical applications, only peripheral agonists have yet reached the market for gastrointestinal disorders.[14] Brain 5-HT$_4$ receptor ligands have entered clinical trials for the treatment of Alzheimer disease but failed in phase IIb.[15,16] Discovery of active 5-HT$_4$Rs agonists and antagonists remains of great interest in clinical research. To this end, molecular imaging techniques using positron emission tomography (PET) or single photon emission computed tomography (SPECT) have emerged as valuable tools, both in clinical studies and drug discovery programs.[17,18] These noninvasive techniques have found broad applications, including diagnosis, imaging of neurotransmitter receptors, in vivo binding studies of new ligands, and establishing treatment strategies. The bottleneck of these techniques remains the limited availability of suitable radioligands.

(1-Butylpiperidin-4-yl)methyl-8-amino-7-iodo[^{123}I]-2,3-dihydrobenzo[*b*][1,4]dioxine-5-carboxylate {[^{123}I]**1**, [^{123}I]SB 207710}[19] and (1-methyl[^{11}C]piperidin-4-yl)methyl-8-amino-7-chloro-2,3-dihydrobenzo[*b*][1,4]dioxine-5-carboxylate {[^{11}C]**2**, [^{11}C]SB 207145}[20] have been described as potential radiotracers for respectively PET or SPECT imaging (Chart 1). [^{11}C]**2** has been successfully used in minipig for the determination of radioligand metabolism and binding ki-

Received: July 3, 2012
Published: October 26, 2012

Chart 1. Literature and Prospective 5-HT$_4$R Radiotracers



netics.[21] Further studies have shown that this radiotracer can be used for quantitative PET measurements of 5-HT$_4$R in the human brain.[22,23] Nevertheless, its short half-life (20.9 min) limits its use in facilities where both a cyclotron and a PET camera are in proximity. Other analogs of **1** have recently been shown to exhibit pharmacological properties that could make them new promising 5-HT$_4$R PET radiotracers.[24] Among them, fluorinated compounds could serve as PET radiotracers of longer half-life. [^{123}I]**1** is to date the only SPECT tracer reported for brain imaging of 5-HT$_4$R, but due to low brain penetration and rapid metabolism, no further investigations with this product have been reported. We report here work aimed at developing new 5-HT$_4$R radioligands for SPECT imaging. On the basis of previous works in the lab concerning the synthesis and evaluation of 5-HT$_4$R ligands,[25,26] we synthesized and evaluated new diversely substituted phenanthridine derivatives. As a result, we were able to design several selective and high-affinity 5-HT$_4$R antagonists among which some iodinated compounds were identified and successfully radiolabeled with ^{125}I, representing new potential radioligands for SPECT imaging studies of the 5-HT$_4$R (Chart 1).

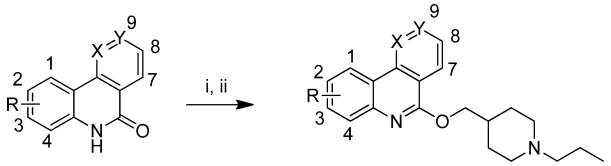
RESULTS

Chemistry. The starting (aza)phenanthridinones **3a–v** were prepared according to the general route as previously described.[27] Compounds **4a–v** were obtained using a two-step procedure involving the formation of an imidoyl chloride intermediate in phosphorus oxychloride at 80 °C and the subsequent nucleophilic aromatic substitution of the chlorine atom with (1-propylpiperidin-4-yl)methanol, using conditions based on previous results.[25,26] Compounds **4a–v** were obtained in 42–91% overall yields, except compound **4n**, which was prepared in 9% overall yield in a three-step procedure[28] (Table 1).

For the design of potential SPECT ligands, we were interested in the introduction of iodine atoms either on the tricyclic framework or on the lateral chain. Iodinated phenanthridinone **8a** and benzonaphthyridinone **8b** were obtained in good overall yields starting from 2-trimethylsilyl-fluorobenzene by a four-step procedure involving borylation, Suzuki cross-coupling reaction, iododesilylation, and anionic ring closure (Scheme 1). For the iodinated side chain, we chose to add a terminal iodoaryl group to the propyl chain.[25,26] Thus, **10** was prepared starting from 4-iododihydrocinnamic acid involving amidation with ethyl isonipecotate and reduction of both the carboxamide and ester function with diisobutylaluminium hydride (Scheme 2).

The iodinated compounds **11a–d** were obtained using the two-step chlorodehydroxylation/SNAr sequence starting from phenanthridinones **3a** and **8a**, benzonaphthyridinones **3v** and **8b**, and the appropriate piperidine derivative (Table 2).

Table 1. Synthesis of (Aza)Phenanthridines 4a–v[a]



3a-v 4a-v

starting material	R	X	Y	product	yield (%)[b]
3a	H	CH	CH	**4a**	76
3b	2-F	CH	CH	**4b**	57
3c	2-Cl	CH	CH	**4c**	70
3d	2-Me	CH	CH	**4d**	49
3e	2-MeO	CH	CH	**4e**	91
3f	3-Cl	CH	CH	**4f**	87
3g	3-MeO	CH	CH	**4g**	71
3h	3-Me	CH	CH	**4h**	59
3i	3-F	CH	CH	**4i**	65
3j	4-Me	CH	CH	**4j**	47
3k	4-MeO	CH	CH	**4k**	63
3l	4-Cl	CH	CH	**4l**	62
3m	4-F	CH	CH	**4m**	67
3n	7-F	CH	CH	**4n**	9[c]
3o	8-NO_2	CH	CH	**4o**	57
3p	8-MeO	CH	CH	**4p**	70
3q	8-F	CH	CH	**4q**	44
3r	9-Me	CH	CH	**4r**	71
3s	9-F	CH	CH	**4s**	62
3t	H	CH	N	**4t**	42
3u	H	N	CH	**4u**	54
3v	4-F	N	CH	**4v**	83

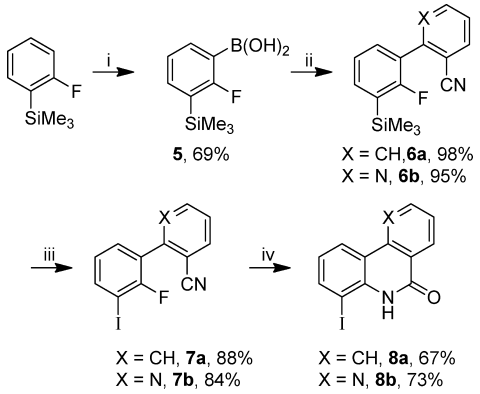
[a]Reagents and conditions: (i) $POCl_3$, 80 °C, overnight; (ii) (1-propylpiperidin-4-yl)methanol, NaH, DMF, 0 °C to rt, overnight. [b]Isolated yields. [c]Yield over three steps; see experimental data for details.

Synthesis of Radioligands. Stannylated precursors for radioiodination were prepared from iodinated compounds **11a,b,d** using palladium-catalyzed tin–iodine exchange in the presence of PPh_3 in toluene.[29] Radioiodination from the stannylated compounds **12a,b,d** was performed using Na^{125}I as the source of radioactive iodine H_2O_2 (30%) as the oxidant in acidic medium (Scheme 3). After HPLC purification, the radioiodinated compounds **13a,b,d** were obtained in 56–85% radiochemical yields. The products **13a,b,d** were found to be all carrier-free, exhibiting apparent specific radioactivities of respectively 240, 110, and 280 Ci/mmol (11%, 5% and 13% of the carrier free specific activity).

5-HT$_4$R Binding Affinity and Functional Assays. Twenty-six compounds (**4a–v**, **11a–d**) were initially screened for their affinity toward 5-HT$_4$R in guinea pig striatal

dx.doi.org/10.1021/jm300943r | J. Med. Chem. 2012, 55, 9693–9707

Scheme 1. Synthesis of 4-Iodo(aza)phenanthridin-6(5*H*)-ones 8a,b[a]



[a]Reagents and conditions: (i) *s*-BuLi 0.75 h, $B(OMe)_3$ 0.75 h, THF, −78 °C to rt; (ii) 2-BrPhCN or 2-Cl-3-CN-pyridine, Na_2CO_3, $Pd(OAc)_2$, PPh_3, DME/H_2O, 14 h, 90 °C; (iii) ICl, DCM, 4.5 h, rt; (iv) KOH, *t*-BuOH, sealed tube, 1 h, 150 °C.

membranes at 10^{-6} and 10^{-8} M. Twenty compounds were selected for K_i determination in 5-HT_4R guinea pig striatal membranes. These ligands show K_i values between 2.2 and 691 nM. Among them, 10 were chosen for human 5-HT_4R K_i determination and, except **4t**, all ligands showed better affinity for human 5-HT_4R compared to guinea pig 5-HT_4R, exhibiting K_i values between 0.04 and 33 nM (Table 3). All compounds were evaluated for their intrinsic activity and showed either an inverse agonist (**4k−m,u**) or a full antagonist profile (**4v**, **11a,b**, and **11d**), as shown in Table 4.

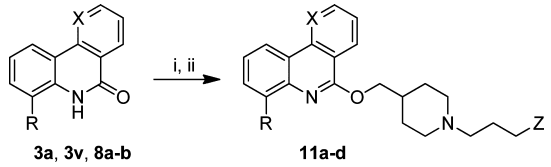
5-HTRs Binding Profile. Compounds **4k**, **4l**, **4m**, **4u**, **4v**, **11a**, **11b**, and **11d** with the highest affinities for 5-HT_4R were screened toward other 5-HTR subtypes. Results are shown in Table 5.

DISCUSSION

Unlike PET imaging using ^{18}F, for which both a suitable fluorinated ligand and a precursor for radiofluorination have to be designed, for SPECT imaging an iodinated ligand can serve as both the nonradioactive ligand for pharmacological studies and the precursor for the introduction of ^{123}I via successive stannylation−iododestannylation reactions. A major challenge with SPECT imaging is to find a suitable iodinated ligand exhibiting high affinity and receptor selectivity. Whereas a hydrogen atom often can be replaced by a fluorine atom without significant decrease in biological activities, introduction of an iodine atom can lead to a dramatic decrease in affinity due to its large atomic radius.

Starting from the unsubstituted phenanthridine **4a**, which exhibited good 5-HT_4R binding affinity (K_i = 51 nM), we explored the influence of substitution of the tricyclic ring system in this series to find the best positions for the introduction of substituents, including an iodine atom. The chemical routes we have developed allowed the introduction of substituents in position 2, 3, 4, 7, 8, and 9 (Table 3). The results show that substitution of the phenanthridine in position 2, 3, 8, or 9 was detrimental for affinity compared to unsubstituted compound **4a**; all compounds show K_i values above 100 nM, even with the small fluorine group. Better results were obtained when substituents were placed in positions 4 and 7. The 7-fluoro compound **4n** showed a slight increase in affinity (K_i = 22 nM), whereas substituents in position 4 led to significant improvements in affinity (compounds **4k−m**), except the 4-methyl-substituted compound **4j**. Interestingly, while compounds **4k−m** exhibited 35,

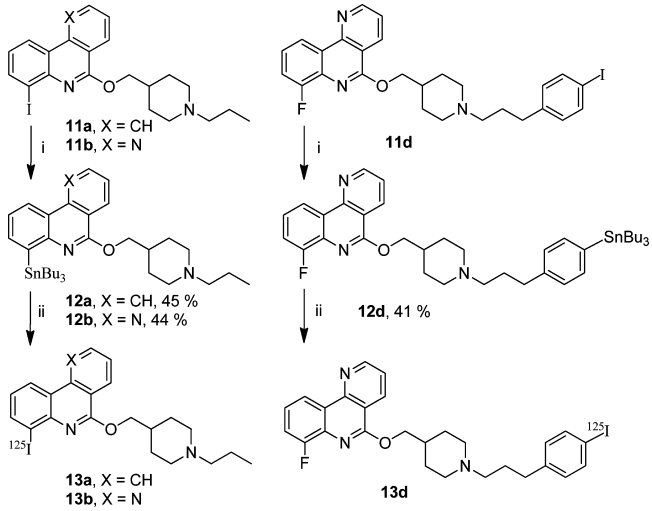
Table 2. Synthesis of Iodinated (Aza)Phenanthridines 11a−d[a]



starting material	R	X	Z	product	yield (%)[b]
8a	I	CH	H	**11a**	76
8b	I	N	H	**11b**	71
3a	H	CH	4-iodophenyl	**11c**	39
3v	F	N	4-iodophenyl	**11d**	41

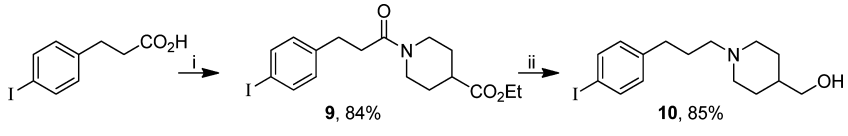
[a]Reagents and conditions: (i) $POCl_3$, 80 °C, overnight; (ii) 1-propylpiperidin-4-ylmethanol or **10**, NaH, DMF, 0 °C to rt, overnight. [b]Isolated yields.

Scheme 3. Preparation of Stannylated Precursors 12a,b,d and ^{125}I Labeling[a]



[a]Reagents and conditions: (i) $(Bu_3Sn)_2$, $Pd(OAc)_2$, PPh_3, PhMe/H_2O, 16 h, 90 °C. (ii) $Na^{125}I$, 30% H_2O_2, EtOH/AcOH, rt, 20 min.

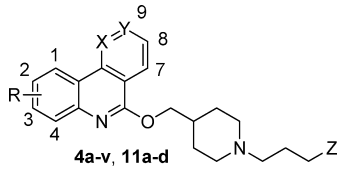
Scheme 2. Synthesis of {1-[3-(4-Iodophenyl)propyl]piperidin-4-yl}methanol 10[a]



[a]Reagents and conditions: (i) Ethyl isonipecotate, HOBt, EDCI, NEt_3, 24 h, rt; (ii) DIBALH, THF, 3 h, −10 °C to rt.

dx.doi.org/10.1021/jm300943r | *J. Med. Chem.* 2012, 55, 9693−9707

Table 3. Binding Affinities of New 5-HT$_4$R Ligands



4a-v, 11a-d

compd	R	X	Y	Z	% inhbn (10^{-6} M/10^{-8} M)[a]	5-HT$_4$ K_i (nM) guinea pig[b]	5-HT$_4$ K_i (nM) human[c]
4a	H	CH	CH	H	100/11	51.5	NM[d]
4b	2-F	CH	CH	H	47/0	NM	NM
4c	2-Cl	CH	CH	H	27/0	NM	NM
4d	2-Me	CH	CH	H	88/7	246	NM
4e	2-MeO	CH	CH	H	83/9	233	NM
4f	3-Cl	CH	CH	H	85/0	NM	NM
4g	3-MeO	CH	CH	H	NM	1900	NM
4h	3-Me	CH	CH	H	65/30	691	NM
4i	3-F	CH	CH	H	100/14	101	33.0[e]
4j	4-Me	CH	CH	H	100/0	100	NM
4k	4-MeO	CH	CH	H	100/22	35.0	17.0[e]
4l	4-Cl	CH	CH	H	100/24	21.9	5.0[e]
4m	4-F	CH	CH	H	100/63	20.1	3.1[e]
4n	7-F	CH	CH	H	100/0	21.6	NM
4o	8-NO_2	CH	CH	H	54/0	NM	NM
4p	8-MeO	CH	CH	H	85/0	NM	NM
4q	8-F	CH	CH	H	90/4	154	NM
4r	9-Me	CH	CH	H	28/0	NM	NM
4s	9-F	CH	CH	H	84/0	209	NM
4t	H	CH	N	H	100/50	22.8	403[e]
4u	H	N	CH	H	96/64	13.1	7.5[e]
4v	4-F	N	CH	H	100/98	2.2	0.04[f]
11a	4-I	CH	CH	H	100/0	13.5	1.20[f]
11b	4-I	N	CH	H	100/20	4.6	0.26[f]
11c	H	CH	CH	4-iodophenyl	100/3	115	NM
11d	4-F	N	CH	4-iodophenyl	100/94	2.5	0.23[f]

[a]Inhibition percentages were determined by using guinea pig striatal membrane 5-HT$_4$R. [b]Guinea pig striatal membrane 5-HT$_4$R (n = 3). [c]Human 5-HT$_4$R (n = 3). [d]NM = not measured. [e]K_i determinations were performed at NIMH PDSP. [f]K_i determinations were performed at CEREP. See Experimental Section for details.

Table 4. Intrinsic Activity and cLogD of 5-HT$_4$R Ligands 4k, 4l, 4m, 4u, 4v, 11a, 11b, and 11d

	4k	4l	4m	4u	4v	11a	11b	11d
human 5-HT$_4$ K_i(nM)	17	5.0	3.1	7.5	0.04	1.20	0.26	0.23
efficacy	inv ag[a]	inv ag[a]	inv ag[a]	inv ag[a]	antag[b]	antag[b]	antag[b]	antag[b]
EC_{50} (nM)	79.4	251	63.1	10	–	–	–	–
K_B (nM)	–	–	–	–	0.025	5.0	0.5	6.3
cLogD[c]	2.48	3.25	2.79	1.84	1.98	3.58	2.77	4.67

[a]Functional assays performed at NIMH PDSP; inv ag = inverse agonist. [b]Functional assays performed at CEREP; antag = antagonist. See Experimental Section for details. [c]Calculated log D (pH = 7.4) using MarvinSketch 5.2.6.

22, and 20 nM K_i values respectively for guinea pig 5-HT$_4$R, against human 5-HT$_4$R they showed significantly higher binding affinities with K_i values of respectively 17, 5, and 3 nM. Introduction of a nitrogen atom in position 9 or 10 led to benzonaphthyridines **4t** and **4u**, which exhibited increased activity compared to **4a** with K_i = 23 and 13 nM, respectively. When fluorine was introduced in position 4 to give benzonaphthyridine **4v**, the affinity was significantly increased to 2.2 nM on guinea pig 5-HT$_4$R, and remarkably, this compound exhibited a very high affinity on human 5-HT$_4$R with a K_i value of 0.04 nM (pK_i = 10.4, pK_B = 10.6). This compound represents one of the most active 5-HT$_4$R antagonist reported to date, being in the same range as the reference antagonists [1-(2-methanesulfonamidoethyl)-piperidin-4-yl]methyl 1-methylindole-3-carboxylate (GR 113808)[30] or **1**.[31]

With these structure–affinity results in hand, we investigated the design of iodinated compounds for potential development of SPECT radiotracers. As the position 4 seemed to give the best results in both the phenanthridine and benzonaphthyridine series, we chose to introduce an iodine atom in this position. Iodinated compounds **11a** and **11b** were synthesized and evaluated for their 5-HT$_4$R binding affinity. Despite differences between fluorine and iodine in terms of size and electronic

dx.doi.org/10.1021/jm300943r | J. Med. Chem. 2012, 55, 9693–9707

Table 5. Binding Affinities of Compounds 4k, 4l, 4m, 4u, 4v, 11a, 11b, and 11d toward 5-HT Receptors[a]

human 5-HTR	4k	4l	4m	4u	4v	11a	11b	11d
5-HT$_4$	17	5.0	3.1	7.5	0.04	1.20	0.26	0.23
5-HT$_{1A}$	>10^4	8836	>10^4	>10^4	4987	>10^4	5629	680
5-HT$_{1B}$	>10^4	>10^4	>10^4	>10^4	>10^4	>10^4	>10^4	>10^4
5-HT$_{1D}$	2569	2360	>10^4	>10^4	>10^4	>10^4	>10^4	>10^4
5-HT$_{1E}$	>10^4	>10^4	>10^4	>10^4	>10^4	>10^4	>10^4	NM
5-HT$_{2A}$	5962	1780	>10^4	>10^4	>10^4	1019	1825	810
5-HT$_{2B}$	29.3	98.0	40.4	627	136	162	>10^4	340
5-HT$_{2C}$	>10^4	729	1049	2793	492	772	341	400
5-HT$_3$	>10^4	2470	710	680	641	1314	>10^4	410
5-HT$_{5A}$	>10^4	>10^4	>10^4	>10^4	>10^4	5805	>10^4	1700
5-HT$_6$	>10^4	6256	>10^4	>10^4	>10^4	>10^4	>10^4	>10^4
5-HT$_7$	3473	1914	384	>10^4	1945	153	1018	150

[a]Selectivity was performed at NIMH PDSP, except for compound **11d**, for which the selectivity was performed at CEREP.

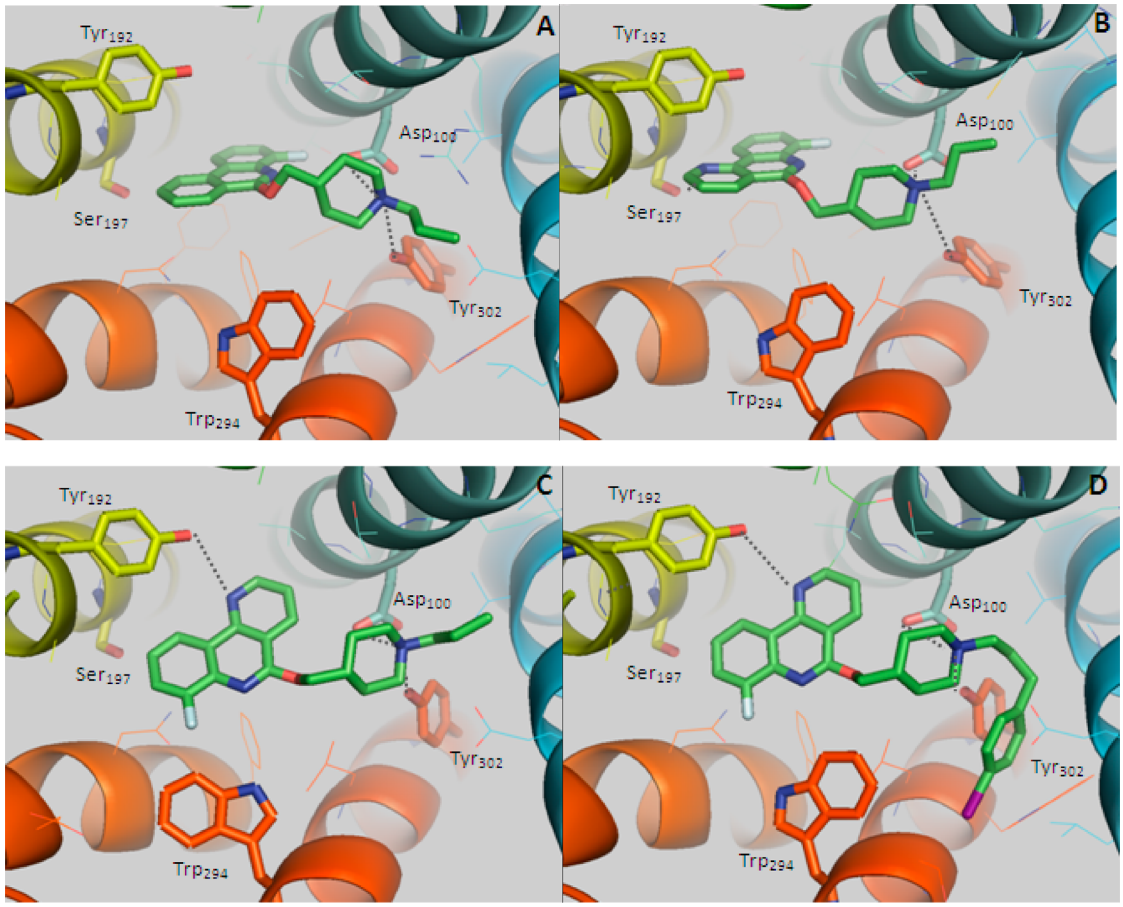


Figure 1. Docking poses of compounds **4m** (A), **4v** (B and C), and **11d** (D).

properties, **11a** and **11b** exhibited K_i values of 13 and 4.6 nM for guinea pig 5-HT$_4$R and respectively, 1.2 and 0.26 nM for human 5-HT$_4$R, in the same range as their fluorinated analogues **4m** and **4v**. Further, we investigated compounds iodinated on the *N*-propyl group. In order to avoid the expected elimination of the iodine atom if bonded to a saturated carbon, it was not directly attached to the *N*-propyl chain but to an aromatic group using the *N*-3-(4-iodophenyl)-propyl chain. Previous results have shown that some 5-HT$_4$R ligands bearing bulky aromatic group bonded to the lateral chain can exhibit high affinities.[3] Accordingly, compounds **11c** and **11d** were synthesized. Despite the introduction of the bulky iodoaryl group, the iodinated 4-fluorobenzonaphthyridine **11d** was found to exhibit high affinity for both guinea pig 5-HT$_4$R (K_i = 2.5 nM) and human 5-HT$_4$R (K_i = 0.23 nM) on the similar order of magnitude as compound **4v**. Along with high binding affinities, intrinsic activity and binding selectivity are other key parameters for the development of suitable ligands for imaging. The best 5-HT$_4$R ligands **4k−m**, **4u−v**, **11a,b**, and **11d** were evaluated for both intrinsic activity and selectivity toward other 5-HTRs (Tables 4 and 5). Compounds **4k−m** and **4u** appeared to be inverse agonists with pEC$_{50}$ ranging from 6.6 to 8.0, whereas compounds **4v**, **11a,b**, and **11d** were found to be full antagonists with pK_B values ranging from 8.2 to 10.6, in accordance with their K_i values. The difference observed in intrinsic activity could be due to high constitutive

dx.doi.org/10.1021/jm300943r | J. Med. Chem. 2012, 55, 9693−9707

activity of the cloned receptors used in the functional tests. Some ligands have been found to behave either as antagonists or as inverse agonists, depending on the functional models used.[32] These compounds were then evaluated for their selectivity toward other 5-HT receptors. All compounds showed high selectivity toward other 5-HT receptors except 5-HT_{2B}R, for which compounds **4k−m** exhibited almost the same potency as for 5-HT_4R. Interestingly, the additional nitrogen atom in position 10 not only led to increased affinities for 5-HT_4R but also contributes to decreased affinity for 5-HT_{2B}R, resulting in compounds with higher selectivity (>100). In order to try to rationalize this result we performed docking studies using a model of 5-HT_4R based on the crystal structures of compounds **4m**, **4v**, and **11d** assuming that the crystalline conformations are the most energetically stable (see Supporting Information). The homology model of the human 5-HT_4 receptor was constructed using the β_2-adrenergic receptor crystal structure, one of the available GPCR structures that exhibits a sequence identity of about 40% with the 5-HT_4R.[33] The two crystallographic conformers of compound **4m** were docked into the 5-HT_4R model (see Supporting Information). In the selected pose (Figure 1A, mean ChemScore fit = 31.18), the basic piperidine nitrogen interacted with Asp_{100} (consistent with the constraint used during the docking), and an additional polar interaction could form through this basic nitrogen and the Tyr_{302} hydroxyl group.[34] The tricyclic framework was oriented toward the transmembrane helix 5 (TM5, colored in yellow in Figure 1), in a position analogous to that observed for β-adrenergic receptor ligands in solved X-ray structures.[33,35] In the **4m** docking position, the tricycle is oriented parallel to the TM5 helix axis and is surrounded by several aromatic residues (Phe_{186}, Tyr_{192}, Phe_{275}, Phe_{276}), oriented approximately perpendicularly to the tricyclic framework, indicating possible π stacking interactions. The fluorine atom pointed inside the receptor. The addition of a supplementary nitrogen atom in derivative **4v** could lead to a new hydrogen bond with the Ser_{197} hydroxyl group as seen in Figure 1B (mean ChemScore fit = 33.10). While this additional interaction could explain the increased affinity for the 5-HT_4R of **4v** versus **4m**, it does not explain its lower affinity for the 5-HT_{2B}R. Indeed, a careful comparison of these receptors sequences showed that Ser_{197} is present in both the 5-HT_4 and 5-HT_{2B} receptors; therefore, it is not probable that the additional nitrogen interacts with this serine. Comparison of amino acid sequences in the TM5 part of the binding cavity shows three differences: Tyr_{192}(5-HT_4R)/Phe_{217}(5-HT_{2B}R), Cys_{196}(5-HT_4R)/Gly_{221}(5-HT_{2B}R), and Ala_{193}(5-HT_4R)/Met_{218}(5-HT_{2B}R). We reasoned that the supplementary nitrogen atom in **4v** could interact with the hydroxyl group of the 5-HT_4R Tyr_{192}. Therefore, the docking studies of **4v** were carried out by taking into account a supplementary hydrogen-bonding constraint between this additional nitrogen atom and the Tyr_{192} hydroxyl group. In the best scoring docking pose of this study (mean ChemScore fit = 41.55), the tricycle was oriented perpendicularly to the TM5 helix axis (Figure 1C), the N-1 nitrogen atom formed the H-bond with Tyr_{192} as set during the docking and was located far from the Ser_{197}. Furthermore, in this docking pose, the fluorine, the N-6 nitrogen atom, and the oxygen atom were placed near to the 5-HT_4R Trp_{294} (TM7), so that electrostatic interactions can occur with this amino acid. Binding interactions with this Trp_{294} have been previously shown to be essential for some 5-HT_4R ligands.[36] The docking of compound **11d** showed that it can be positioned in the same manner as **4v** (mean ChemScore fit = 33.54), the 4-iodophenyl group going up toward the extracellular entrance (Figure 1D), as it was observed for some cocrystallized extended ligands such as the full agonist carmoterol in β_1-adrenergic receptor[37] or for the antagonist JDTic in κ-opioid receptor.[38]

Among the iodinated compounds, **11a**, **11b**, and **11d** could be considered as promising candidates for the development of a SPECT tracer owing to their subnanomolar binding affinities, high selectivity over other 5-HTRs including 5-HT_{2B}R, and computed lipophilicities within a range adequate for brain penetration.[39] Compound **11d**, which exhibits the highest K_i value and selectivity, was chosen for further evaluation. In order to address its 5-HT_4R specific binding capacity, in vitro competition experiments with the selective and specific antagonist radioligand [^{125}I]**1** were performed. Increasing concentrations of **11d** coadministered with [^{125}I]**1** show a decrease in the 5-HT_4R-specific radioactivity at 10 pM of **11d**, while increasing the concentration to 1 nM led to the almost complete abolishment of the signal (Figure 2). The same

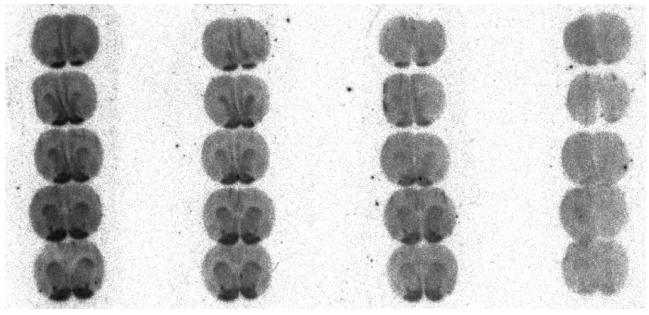


Figure 2. Autoradiograms obtained by incubation of sections with 100 pM of [^{125}I]**1** in the presence of growing concentrations of **11d** (1, 10, 100, and 1000 pM from left to right).

experiment was performed ex vivo. The specific binding of [^{125}I]**1** is slightly noticeable in the olfactory tubercles but is markedly reduced compared to a reference experiment (Figure 3). The activity between the hemispheres in the coinjection

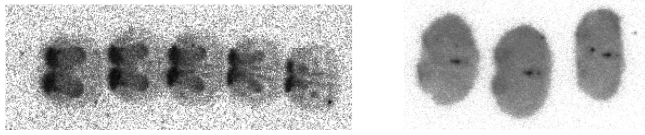


Figure 3. Ex vivo autoradiogram after intravenous injection of [^{125}I]**1** alone (left) and after coinjection with 50 μg/kg of **11d** to a mouse (right).

experiment is due to the presence of blood in the brain sections. Taken together, these two experiments show that (1) **11d** is able to compete with the specific radioligand [^{125}I]**1** both in vitro and in vivo at very low concentration and (2) **11d** is able to cross the blood−brain barrier, making **11d** a suitable candidate for use in SPECT imaging studies.

The radioiodinated compound **13d** was successfully prepared from **11d** by a two-step sequence involving stannylation and Sn−^{125}I exchange (Scheme 3). **13d** was obtained in a 85% radiochemical yield, and the specific radioactivity was measured at 280 Ci/mmol (13% of the carrier free specific activity). The two other iodinated compounds **11a** and **11b** were radioiodinated following the same strategy affording **13a** and **13b** in respectively 56 and 70% RCY and with a specific activity of

dx.doi.org/10.1021/jm300943r | J. Med. Chem. 2012, 55, 9693−9707

respectively 240 and 110 Ci/mmol (11 and 5% of the carrier free specific activity).

CONCLUSION

Our studies aimed at the development of new potential radiotracers for SPECT imaging of brain 5-HT_4 receptors. Starting from a phenanthridine scaffold we designed new antagonists exhibiting high affinity and selectivity toward this receptor. The fluorinated compound **4v** has shown the best profile with a low subnanomolar K_i value and a high selectivity toward other 5-HT receptor subtypes. Compared to the phenanthridine analog **4m**, the additional nitrogen atom in compound **4v** led to a significant improvement of both affinity and selectivity for the 5-HT_4R. Having established the structure−affinity relationships for this series, we have successfully introduced iodine atoms without negatively affecting either affinities or selectivities. Among these iodinated compounds, **11d** was chosen for further evaluation as a potential radiotracer. This compound was able to displace a specific 5-HT_4R ligand both in vitro and in vivo at subnanomolar concentrations, thereby demonstrating receptor specificity and capacity to cross the blood−brain barrier. Three iodinated compounds were successfully radiolabeled with [^{125}I] and owing to their favorable pharmacological properties represent novel candidates for further evaluation as SPECT radiotracers.

EXPERIMENTAL SECTION

All chemical reagents and solvents were purchased from commercial sources and used without further purification except THF, which was distilled from Na/benzophenone. Thin-layer chromatography (TLC) was performed on silica gel plates. Silica gel 0.06−0.2 mm, 60 Å was used for all column chromatography. Melting points were determined on a Kofler melting point apparatus. IR spectra were recorded as neat films on a Nicolet 380 FT-IR or on KBr disks using a PerkinElmer BX-FT-IR. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL Lambda 400 spectrometer with chemical shifts expressed in parts per million (in DMSO-d_6 or $CDCl_3$). High-resolution mass spectra (EI) were performed on a JEOL GC-Mate spectrometer. High-resolution mass spectra (ESI) were performed on a Bruker APEX III FT-ICR-MS system. Elemental analyses were performed at the "Institut de Recherche en Chimie Organique Fine" (Rouen, France). The purities of all tested compounds were analyzed by LC−MS, with the purity all being higher than 95%. Analyses were performed using a Waters alliance 2695 using the following gradient: A (95%)/B (5%) to A (5%)/B (95%) in 10 min. This ratio was held for 3 min before returning to initial conditions in 1 min. Initial conditions were then maintained for 5 min (A, H_2O; B, MeCN; each containing 0.1% HCOOH; column, C18 Xterra MSC118/2.1_50 mm). MS detection was performed with a Micromass ZMD 2000. Suitable crystals of solved structures were obtained by slow evaporation from MeCN solution. Data for crystal structures analysis were collected at 296 K with a Bruker−Nonius Kappa CCD area detector diffractometer with graphite−monochromatized Mo K_λ radiation (λ = 0.710 73 Å). The structures were solved using direct methods and refined by full-matrix least-squares analysis on F^2. SHELXS−97 (Sheldrick) was used to solve structures, to refine structures, and to prepare material for publication. Crystallographic data for compounds **4c**, **4d**, **4g**, **4h**, **4j**, **4m**, **4p**, **4q**, **4r**, **4v**, and **11d** have been deposited at the Cambridge Crystallographic Data Centre, CCDC No 889427, 889429−889437, and 889548. Copies of this information may be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (+44−1223−336408; E-mail, deposit@ccdc.cam.ac.uk; Web site, http://www.ccdc.cam.ac.uk).

7-Fluorobenzo[*h*]-1,6-naphthyridin-5(6*H*)-one (3v). In a sealed tube were introduced KOH (1.94 g, 34.6 mmol), 2-(2,3-difluorophenyl)nicotinonitrile[40] (1.5 g, 6.9 mmol), and *t*-BuOH (40 mL). The tube was heated to 150 °C for 0.5 h. Water (30 mL) was added and the obtained precipitate was filtered. The solid was then dried under vacuum to afford **3v** (1.3 g) as a white powder. Yield: 87%. Mp: >260 °C. IR (KBr): ν (cm^{-1}) 3022, 1675 (CO), 1587, 1419, 763. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.28 (m, 1H), 7.49 (m, 1H), 7.70 (dd, 1H, ^3J = 7.8 Hz, ^3J = 3.9 Hz), 8.43 (d, 1H, ^3J = 7.8 Hz), 8.68 (dd, 1H, ^3J = 7.8 Hz, ^4J = 1.9 Hz), 9.07 (d, 1H, ^3J = 3.9 Hz), 11.87 (brs, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 116.5 (d, ^2J = 17 Hz), 119.7 (d, J = 3 Hz), 121.0 (d, J = 2 Hz), 121.6, 122.2 (d, J = 7 Hz), 123.9, 126.5 (d, ^2J = 14 Hz), 135.9, 149.4 (d, ^1J = 243 Hz), 149.9 (d, J = 3 Hz), 154.3, 160.7. HRMS/EI: calcd for $C_{12}H_7FN_2O$ 214.0542, found 214.0548.

General Procedure A for the Synthesis of Compounds 4a−v and 11a−d. The chosen (aza)phenanthridin-6(5*H*)-one (**3a−v**, **8a−b**) and $POCl_3$ (5 mL mmol^{-1}) were heated to 90 °C overnight in a round-bottom flask. After cooling, the mixture was poured carefully on cold water and crushed ice. The pH was carefully adjusted to 12 using a 28% ammonia solution. The product was extracted using EtOAc (three times). The organic phase was dried with $MgSO_4$, filtered, and evaporated. The crude material was added at 0 °C to a solution of either (1-propylpiperidin-4-yl)methanol or {1-[3-(4-iodophenyl)-propyl]piperidin-4-yl}methanol **10** (1 equiv) and NaH (4 equiv) in anhydrous DMF (10 mL mmol^{-1}). The solution was allowed to reach room temperature, stirred overnight, hydrolyzed with water, and extracted with AcOEt (3 times). The combined organic phases were washed with water (three times), dried over $MgSO_4$, filtered, evaporated, and purified by silica gel chromatography.

6-(1-Propylpiperidin-4-yl)methyloxyphenanthridine (4a). Starting from **3a** (213 mg, 1.1 mmol) using general procedure A and cyclohexane/ethyl acetate 8/2 with 5% of NEt_3 as the eluent for the chromatography, **4a** was obtained as a white powder (277 mg). Yield: 76%. Mp: 74−76 °C. IR (KBr): ν (cm^{-1}) 2924, 1590, 1461, 1343, 1319. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91 (t, 3H, ^3J = 7.6 Hz), 1.50−1.62 (m, 4H), 1.91−2.03 (m, 5H), 2.30−2.34 (m, 2H), 3.00−3.03 (m,2H), 4.50 (d, 2H, ^3J = 6.0 Hz), 7.47 (ddd, 1H, ^3J = 8.0 Hz, ^3J = 6.8 Hz, ^4J = 1.2 Hz), 7.59−7.64 (m, 2H), 7.80 (ddd, 1H, ^3J = 8.4 Hz, ^3J = 7.2 Hz, ^4J = 1.6 Hz), 7.86 (dd, 1H, ^3J = 8.4 Hz, ^4J = 1.6 Hz), 8.38 (dd, 1H, ^3J = 8.0 Hz, ^4J = 1.2 Hz), 8.40−8.42 (m, 1H), 8.48−8.51 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.3, 20.4, 29.5 (2C), 36.1, 53.8 (2C), 61.4, 70.6, 120.4, 122.0, 122.2, 122.6, 124.4, 125.2, 127.3, 127.9, 128.9, 130.9, 134.9, 143.5, 159.1. LC−MS (ESI): t_R = 5.02 min; [M + H]$^+$ 335.58. HRMS/EI: calcd for $C_{22}H_{26}N_2O$ 334.2046, found 334.2046.

2-Fluoro-6-(1-propylpiperidin-4-yl)-methyloxyphenanthridine (4b). Starting from **3b** (180 mg, 0.84 mmol), using general procedure A and cyclohexane/ethyl acetate 7/3 with 5% of NEt_3 as the eluent for the chromatography, **4b** was obtained as a white powder (169 mg). Yield: 57%. Mp: 115−116 °C. IR (KBr): ν (cm^{-1}) 3076, 2927, 2764, 1618, 1591, 1495, 1453, 1436, 1348, 1315, 1243. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91 (t, 3H), 1.53−1.59 (m, 4H), 1.90−2.02 (m, 5H), 2.29−2.33 (m, 2H), 2.99−3.02 (m, 2H), 4.45 (d, 2H, ^3J = 6.1 Hz), 7.33 (ddd, 1H, ^3J = 8.8 Hz, ^3J = 8.0 Hz, ^4J = 2.8 Hz), 7.64 (ddd, 1H, ^3J = 8.0 Hz, ^3J = 6.8 Hz, ^4J = 1.2 Hz), 7.77 (ddd, 1H, ^3J = 8.0 Hz, ^3J = 7.2 Hz, ^4J = 1.6 Hz), 7.81 (dd, 1H, ^3J = 8.8 Hz, ^3J = 5.2 Hz), 7.98 (dd, 1H, ^3J = 10.0 Hz, ^4J = 2.8 Hz), 8.33 (d, 1H, ^3J = 8.4 Hz), 8.36 (dd, 1H, ^3J = 8.0 Hz, ^4J = 1.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.1, 20.2, 29.3 (2C), 35.9, 53.6 (2C), 61.2, 70.5, 107.1 (d, ^2J = 23 Hz), 116.9 (d, ^2J = 24 Hz), 120.2, 121.9, 123.3 (d, J = 9 Hz), 125.1, 127.7, 129.4 (d, J = 5 Hz), 130.8, 134.0 (d, J = 4 Hz), 139.8, 158.3 (d, J = 2 Hz), 159.7 (d, ^1J = 241 Hz). LC−MS (ESI): t_R = 5.23 min; [M + H]$^+$ 353.34. HRMS/EI: calcd for $C_{22}H_{25}FN_2O$ 352.1950, found 352.1938.

2-Chloro-6-(1-propylpiperidin-4-yl)-methyloxyphenanthridine (4c). Starting from **3c** (670 mg, 2.9 mmol), using general procedure A and cyclohexane/ethyl acetate 8/2 with 5% of NEt_3 as the eluent for the chromatography, **4c** was obtained as a white powder (753 mg). Yield: 70%. Mp: 110−111 °C. IR (KBr): ν (cm^{-1}) 2930, 1589, 1345, 1317, 1096, 820. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.92 (t, 3H, ^3J = 6.8 Hz), 1.50−1.61 (m, 4H), 1.91−

dx.doi.org/10.1021/jm300943r | J. Med. Chem. 2012, 55, 9693−9707

2.02 (m, 5H), 2.30−2.34 (m, 2H), 3.00−3.03 (m, 2H), 4.47 (d, 2H, ^3J = 5.9 Hz), 7.54 (dd, 1H, ^3J = 8.8 Hz, ^4J = 1.9 Hz), 7.65 (t, 1H, ^3J = 6.8 Hz), 7.76−7.81 (m, 2H), 8.33 (d, 1H, ^4J = 2.0 Hz), 8.35−8.39 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.1, 20.2, 29.3 (2C), 35.9, 53.6 (2C), 61.2, 70.6, 120.3, 121.8, 121.9, 123.5, 125.1, 127.8, 129.0, 129.1, 129.8, 131.0, 133.7, 141.8, 159.1. LC−MS (ESI): t_R = 5.58 min; $[M + H]^+$ 369.26, 371.26. HRMS/EI: calcd for $C_{22}H_{25}ClN_2O$ 368.1655, found 368.1659.

2-Methyl-6-(1-propylpiperidin-4-yl)-methyloxyphenanthridine (4d). Starting from **3d** (400 mg, 1.9 mmol), using general procedure A and cyclohexane/ethyl acetate 8/2 with 5% of NEt_3 as the eluent for the chromatography, **4d** was obtained as a white powder (326 mg). Yield: 49%. Mp: 87−89 °C. IR (KBr): ν (cm^{-1}) 2951, 2928, 2798, 2761, 1589, 1344, 1317, 1302, 1148, 1088, 820, 772. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91 (t, 3H, ^3J = 7.8 Hz), 1.52−1.60 (m, 4H), 1.92−2.02 (m, 5H), 2.30−2.34 (m, 2H), 2.56 (s, 3H), 3.00−3.03 (m, 2H), 4.48 (d, 2H, ^3J = 5.9 Hz), 7.43 (d, 1H, ^3J = 7.8 Hz), 7.61 (t, 1H, ^3J = 6.8 Hz), 7.75−7.80 (m, 2H), 8.20 (s, 1H), 8.36 (d, 1H, ^3J = 7.8 Hz), 8.48 (d, 1H, ^3J = 7.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.1, 20.2, 21.7, 29.3 (2C), 36.0, 53.7 (2C), 61.3, 70.4, 120.2, 121.8, 121.9, 122.2, 125.0, 127.0, 127.5, 130.3, 130.6, 133.8, 134.6, 141.4, 158.4. LC−MS (ESI): t_R = 5.53 min; $[M + H]^+$ 349.35. Anal. Calcd for $C_{22}H_{26}N_2O$: C, 79.01; H, 7.84; N, 8.38. Found: C, 79.27; H, 7.91; N, 8.01.

2-Methoxy-6-(1-propylpiperidin-4-yl)-methyloxyphenanthridine (4e). Starting from **3e** (300 mg, 1.33 mmol), using general procedure A and cyclohexane/ethyl acetate 9/1 with 5% of NEt_3 as the eluent for the chromatography, **4e** was obtained as a colorless oil (440 mg). Yield: 91%. IR (KBr): ν (cm^{-1}) 2935, 1620, 1591, 1498, 1345, 1316, 1243. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.84 (t, 3H, ^3J = 7.8 Hz), 1.38−1.45 (m, 4H), 1.81−1.91 (m, 5H), 2.19−2.22 (m, 2H), 2.86−2.89 (m, 2H), 3.94 (s, 3H), 4.36 (d, 2H, ^3J = 5.9 Hz), 7.27 (dd, 1H, ^3J = 8.8 Hz, ^4J = 2.9 Hz), 7.70−7.76 (m, 2H), 7.90 (t, 1H, ^3J = 6.8 Hz), 8.04 (d, 1H, ^4J = 2.9 Hz), 8.28 (d, 1H, ^3J = 7.8 Hz), 8.76 (d, 1H, ^3J = 8.8 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 11.9, 19.7, 28.8 (2C), 35.5, 53.1 (2C), 55.6, 60.3, 70.0, 104.3, 118.3, 119.2, 122.8, 122.9, 124.3, 127.9, 128.6, 131.1, 133.9, 137.1, 156.5, 156.7. LC−MS (ESI): t_R = 5.31 min; $[M + H]^+$ 365.38. HRMS/ESI: calcd for $C_{23}H_{29}N_2O_2$ $[M + H]^+$ 365.2229, found 365.2211.

3-Chloro-6-(1-propylpiperidin-4-yl)-methyloxyphenanthridine (4f). Starting from **3f** (124 mg, 0.54 mmol), using general procedure A and cyclohexane/ethyl acetate 95/5 with 5% of NEt_3 as the eluent for the chromatography, **4f** was obtained as a white powder (173 mg). Yield: 87%. Mp: 84−86 °C. IR (KBr): ν (cm^{-1}) 3400, 2939, 1590, 1482, 1352, 1317, 1081, 765. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.90 (t, 3H, ^3J = 6.4 Hz), 1.70−1.80 (m, 4H), 2.02−2.04 (m, 5H), 2.31−2.34 (m, 2H), 2.93−3.00 (m, 2H), 4.48 (s, 2H), 7.57 (d, 1H, ^3J = 8.8 Hz), 7.76−7.81 (m, 2H), 7.96 (t, 1H, ^3J = 6.8 Hz), 8.37 (d, 1H, ^3J = 6.8 Hz), 8.67 (d, 1H, ^3J = 8.8 Hz), 8.75 (d, 1H, ^3J = 7.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.0, 20.5, 28.9 (2C), 36.2, 53.1 (2C), 61.2, 70.6, 119.7, 121.8, 122.2, 122.9, 125.1, 127.8, 128.7, 129.1, 129.8, 131.0, 133.7, 141.8, 159.1. LC−MS (ESI): t_R = 5.79 min; $[M + H]^+$ 369.32, 371.33. HRMS/EI: calcd for $C_{22}H_{25}ClN_2O$ 368.1655, found 368.1666.

3-Methoxy-6-(1-propylpiperidin-4-yl)-methyloxyphenanthridine (4g). Starting from **3g** (291 mg, 1.3 mmol), using general procedure A and cyclohexane/ethyl acetate 9/1 with 5% of NEt_3 as the eluent for the chromatography, **4g** was obtained as a white powder (334 mg). Yield: 71%. Mp: 76−77 °C. IR (KBr): ν (cm^{-1}) 2932, 1615, 1587, 1484, 1350, 1315, 1174, 1087, 983, 770. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91 (t, 3H, ^3J = 7.8 Hz), 1.50−1.62 (m, 4H), 1.92−2.03 (m, 5H), 2.30−2.34 (m, 2H), 3.01−3.03 (m, 2H), 3.96 (s, 3H), 4.48 (d, 2H, ^3J = 6.8 Hz), 7.09 (dd, 1H, ^3J = 8.8 Hz, ^4J = 2.0 Hz), 7.30 (d, 1H, ^4J = 2.0 Hz), 7.55 (t, 1H, ^3J = 6.8 Hz), 7.75 (t, 1H, ^3J = 8.8 Hz), 8.28 (d, 1H, ^3J = 8.8 Hz), 8.34 (d, 1H, ^3J = 7.8 Hz), 8.37 (d, 1H, ^3J = 7.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.1, 20.2, 29.3 (2C), 36.0, 53.7 (2C), 55.5, 61.3, 70.5, 108.5, 114.6, 116.2, 119.1, 121.3, 123.3, 125.1, 126.0, 130.9, 134.9, 145.0, 159.5, 160.3. LC−MS (ESI): t_R = 5.25 min; $[M + H]^+$ 365.38. HRMS/EI: calcd for $C_{23}H_{28}N_2O_2$ 364.2150, found 364.2140.

3-Methyl-6-(1-propylpiperidin-4-yl)-methyloxyphenanthridine (4h). Starting from **3h** (120 mg, 0.57 mmol), using general procedure A and cyclohexane/ethyl acetate 8/2 with 5% of NEt_3 as the eluent for the chromatography, **4h** was obtained as a white powder (118 mg). Yield: 59%. Mp: 107−108 °C. IR (KBr): ν (cm^{-1}) 3430, 2939, 1590, 1344, 1314, 1090, 774. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91 (t, 3H, ^3J = 6.8 Hz), 1.53−1.60 (m, 4H), 1.92−2.03 (m, 5H), 2.30−2.34 (m, 2H), 2.53 (s, 3H), 3.00−3.03 (m, 2H), 4.49 (d, 2H, ^3J = 5.8 Hz), 7.30 (d, 1H, ^3J = 8.8 Hz), 7.59 (t, 1H, ^3J = 7.8 Hz), 7.68 (s, 1H), 7.78 (t, 1H, ^3J = 7.8 Hz), 8.29 (d, 1H, ^3J = 7.8 Hz), 8.36 (d, 1H, ^3J = 8.8 Hz), 8.46 (d, 1H, ^3J = 7.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.1, 20.2, 21.5, 29.4 (2C), 36.0, 53.7 (2C), 61.3, 70.4, 119.9, 120.0, 121.6, 121.9, 125.0, 125.9, 126.7, 127.6, 130.7, 134.9, 138.9, 143.4, 159.0. LC−MS (ESI): t_R = 5.64 min; $[M + H]^+$ 349.33. HRMS/ESI: calcd for $C_{23}H_{29}N_2O$ $[M + H]^+$ 349.2280, found 349.2266.

3-Fluoro-6-(1-propylpiperidin-4-yl)-methyloxyphenanthridine (4i). Starting from **3i** (100 mg, 0.47 mmol), using general procedure A and cyclohexane/ethyl acetate 7/3 with 5% of NEt_3 as the eluent for the chromatography, **4i** was obtained as a colorless oil (107 mg). Yield: 65%. IR (KBr): ν (cm^{-1}) 2995, 2852, 1588, 1463, 1260, 1088, 1019, 799, 770. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91 (t, 3H, ^3J = 7.8 Hz), 1.54−1.64 (m, 9H), 1.90−2.02 (m, 5H), 2.30−2.34 (m, 2H), 3.01−3.04 (m, 2H), 4.48 (d, 2H, ^3J = 5.8 Hz), 7.19−7.28 (m, 1H), 7.52 (dd, 1H, ^3J = 10.8 Hz, ^3J = 2.9 Hz), 7.62 (t, 1H, ^3J = 7.8 Hz), 7.80 (t, 1H, ^3J = 6.8 Hz), 8.34−8.43 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.1, 20.2, 29.3 (2C), 35.9, 53.7 (2C), 61.3, 70.3, 112.7 (d, ^2J = 22 Hz), 112,9 (d, ^2J = 23 Hz), 119.0 (d, J = 1 Hz), 119.6, 121.7, 123.7 (d, J = 10 Hz), 125.2, 127.0, 131.1, 134.5, 144.8 (d, J = 12 Hz), 159.7, 163.0 (d, ^1J = 246 Hz). LC−MS (ESI): t_R = 5.47 min; $[M + H]^+$ 353.28. HRMS/EI: calcd for $C_{22}H_{25}FN_2O$ 352.1950, found 352.1941.

4-Methyl-6-(1-propylpiperidin-4-yl)-methyloxyphenanthridine (4j). Starting from **3j** (217 mg, 1.03 mmol), using general procedure A and cyclohexane/ethyl acetate 9/1 with 5% of NEt_3 as the eluent for the chromatography, **4j** was obtained as a white powder (170 mg). Yield: 47%. Mp: 93−95 °C. IR (KBr): ν (cm^{-1}) 2938, 2764, 1589, 1312, 760. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91 (t, 3H, ^3J = 7.5 Hz,), 1.51−1.62 (m, 4H), 1.92−2.04 (m, 5H), 2.30−2.33 (m, 2H), 2.73 (s, 3H), 3.01 (m, 2H), 4.52 (d, 2H, ^3J = 6.5 Hz), 7.37 (dd, 1H, ^3J = 8.0 Hz, ^3J = 7.0 Hz), 7.48−7.50 (m, 1H), 7.61 (ddd, 1H, ^3J = 8.0 Hz, ^3J = 7.5 Hz, ^4J = 1.5 Hz), 7.78 (ddd, 1H, ^3J = 8.0 Hz, ^3J = 7.0 Hz, ^4J = 1.5 Hz), 8.27−8.29 (m, 1H), 8.37 (dd, 1H, ^3J = 8.1 Hz, ^4J = 1.4 Hz), 8.49−8.51 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.1, 18.3, 20.2, 29.4 (2C), 35.9, 53.7 (2C), 61.2, 70.3, 119.8, 119.9, 122.0, 122.1, 123.7, 124.9, 126.9, 129.4, 130.6, 135.2, 135.7, 141.8, 157.6. LC−MS (ESI): t_R = 5.65 min; $[M + H]^+$ 349.57. HRMS/EI: calcd for $C_{23}H_{28}N_2O$ 348.2201, found 348.2215.

4-Methoxy-6-(1-propylpiperidin-4-yl)-methyloxyphenanthridine (4k). Starting from **3k** (150 mg, 0.66 mmol), using general procedure A and cyclohexane/ethyl acetate 9/1 with 5% of NEt_3 as the eluent for the chromatography, **4k** was obtained as a white powder (153 mg). Yield: 47%. Mp: 99−100 °C. IR (KBr): ν (cm^{-1}) 3433, 2951, 2768, 1590, 1321, 1256, 748. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.92 (t, 3H, ^3J = 7.8 Hz), 1.52−1.67 (m, 4H), 1.94−2.04 (m, 5H), 2.31−2.35 (m, 2H), 3.01−3.04 (m, 2H), 4.08 (s, 3H), 4.56 (d, 2H, ^3J = 5.9 Hz), 7.10 (d, 1H, ^3J = 7.8 Hz), 7.42 (t, 1H, ^3J = 8.8 Hz), 7.64 (t, 1H, ^3J = 7.8 Hz), 7.80 (t, 1H, ^3J = 8.8 Hz), 8.03 (d, 1H, ^3J = 7.8 Hz), 8.40 (d, 1H, ^3J = 7.8 Hz), 8.49 (d, 1H, ^3J = 7.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.1, 20.2, 29.3 (2C), 36.1, 53.7 (2C), 56.5, 61.3, 70.4, 109.4, 114.4, 120.2, 122.4, 123.6, 124.3, 125.1, 127.3, 130.8, 133.8, 134.9, 154.5, 158.4. LC−MS (ESI): t_R = 5.24 min; $[M + H]^+$ 365.38. HRMS/EI: calcd for $C_{23}H_{28}N_2O_2$ 364.2150, found 364.2139.

4-Chloro-6-(1-propylpiperidin-4-yl)-methyloxyphenanthridine (4l). Starting from **3l** (150 mg, 0.65 mmol), using general procedure A and cyclohexane/ethyl acetate 9/1 with 5% of NEt_3 as the eluent for the chromatography, **4l** was obtained

as a white powder (149 mg). Yield: 62%. Mp: 75−77 °C. IR (KBr): ν (cm^{-1}) 3434, 2919, 2852, 1591, 1458, 1399, 1342, 1097, 747. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91 (t, 3H, ^3J = 6.8 Hz), 1.52−1.62 (m, 4H), 1.92−2.03 (m, 5H), 2.30−2.34 (m, 2H), 3.00−3.03 (m, 2H), 4.60 (d, 2H, ^3J = 6.8 Hz), 7.38 (t, 1H, ^3J = 7.8 Hz), 7.67 (t, 1H, ^3J = 6.8 Hz), 7.73 (d, 1H, ^3J = 7.8 Hz), 7.83 (t, 1H, ^3J = 6.8 Hz), 8.33 (d, 1H, ^3J = 7.8 Hz), 8.40 (d, 1H, ^3J = 7.8 Hz), 8.49 (d, 1H, ^3J = 8.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.1, 20.2, 29.3 (2C), 35.9, 53.7 (2C), 61.2, 70.8, 120.1, 120.8, 122.2, 124.0, 124.1, 125.2, 127.8, 129.1, 131.2, 132.0, 134.7, 139.8, 159.2. LC−MS (ESI): t_R = 5.64 min; $[M + H]^+$ 369.35, 371.30. HRMS/ESI: calcd for $C_{23}H_{26}ClN_2O$ $[M + H]^+$ 369.1734, found 365.1729.

4-Fluoro-6-(1-propylpiperidin-4-yl)-methyloxyphenanthridine (4m). Starting from **3m** (260 mg, 1.22 mmol), using general procedure A and cyclohexane/ethyl acetate 8/2 with 5% of NEt_3 as the eluent for the chromatography, **4m** was obtained as a white powder (288 mg). Yield: 67%. Mp: 105−106 °C. IR (KBr): ν (cm^{-1}) 2925, 1591, 1348, 1317, 1234, 753. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91 (t, 3H, ^3J = 7.5 Hz), 1.51−1.64 (m, 4H), 1.93−2.03 (m, 5H), 2.31−2.34 (m, 2H), 3.02−3.04 (m, 2H), 4.55 (d, 2H, ^3J = 6.0 Hz), 7.33−7.41 (m, 2H), 7.67 (ddd, 1H, ^3J = 8.0 Hz, ^3J = 7.0 Hz, ^4J = 1.0 Hz), 7.82 (ddd, 1H, ^3J = 8.2 Hz, ^3J = 7.2 Hz, ^4J = 1.4 Hz), 8.17−8.18 (m, 1H), 8.39−8.41 (m, 1H), 8.46 (d, 1H, ^3J = 8.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.1, 20.2, 29.3 (2C), 36.0, 53.6 (2C), 61.2, 70.7, 113.9 (d, ^2J = 20 Hz), 117.5 (d, J = 4 Hz), 120.3, 122.2, 123.8 (d, J = 8 Hz), 124.5 (d, J = 2 Hz), 125.2, 127.7, 131.2, 132.7 (d, ^2J = 11 Hz), 134.2 (d, J = 3 Hz), 157.5 (d, ^1J = 250 Hz), 159.1. LC−MS (ESI): t_R = 5.32 min; $[M + H]^+$ 353.30. HRMS/EI: calcd for $C_{22}H_{25}FN_2O$ 352.1951, found 352.1943.

7-Fluoro-6-(1-propylpiperidin-4-yl)-methyloxyphenanthridine (4n). In sealed tube were introduced KOH (1.07 g, 19 mmol), 2′-fluoro-3-fluorobiphenyl-2-carbonitrile[41] (0.82 g, 3.8 mmol), and *t*-BuOH (30 mL). The tube was heated to 150 °C for 0.5 h. Water (20 mL) was added and the resulting precipitate was filtered. The solid was then dried under vacuum to afford a mixture of the expected 7-fluorophenanthridin-6(5*H*)-one along with side products. The mixture was then subjected to general procedure A using cyclohexane/ethyl acetate 9/1 as the eluent for the chromatography. **4n** was obtained as yellow oil (0.12 g). Yield: 9%. IR (KBr): ν (cm^{-1}) 3432, 2931, 2765, 1595, 1338, 758. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91 (t, 3H, ^3J = 6.8 Hz), 1.52−1.54 (m, 4H), 1.99−2.02 (m, 5H), 2.30−2.34 (m, 2H), 3.00−3.04 (m, 2H), 4.47 (d, 2H, ^3J = 6.8 Hz), 7.28−7.31 (m, 1H), 7.47 (t, 1H, ^3J = 6.8 Hz), 7.63 (t, 1H, ^3J = 6.8 Hz), 7.72 (m, 1H), 7.83 (d, 1H, ^3J = 9.8 Hz), 8.29 (d, 1H, ^3J = 8.7 Hz), 8.36 (d, 1H, ^3J = 6.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.1, 20.2, 29.3 (2C), 35.9, 53.7 (2C), 61.3, 70.3, 109.7 (d, J = 9 Hz), 111.7 (d, J = 5 Hz), 114.1 (d, ^2J = 23 Hz), 121.2, 122.5, 124.5, 127.6, 129.4, 131.4 (d, ^2J = 10 Hz), 137.7, 143.4, 157.6 (d, J = 7 Hz), 160.23 (d, ^1J = 262 Hz). LC−MS (ESI): t_R = 5.38 min; $[M + H]^+$ 353.47. HRMS/EI: calcd for $C_{22}H_{25}FN_2O$ 352.1950, found 352.1935.

8-Nitro-6-(1-propylpiperidin-4-yl)methyloxyphenanthridine (4o). Starting from **3o** (0.80 g, 3.3 mmol), following general procedure A and using cyclohexane/ethyl acetate (9/1) as eluent for the chromatography, **4o** was obtained as a white powder (0.72 g). Yield: 57%. Mp: 123−125 °C. IR (KBr): ν (cm^{-1}) 2940, 2765, 1604, 1344. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.92 (t, 3H, ^3J = 7.8 Hz), 1.53−1.58 (m, 4H), 1.95−2.05 (m, 5H), 2.31−2.35 (m, 2H), 3.02−3.05 (m, 2H), 4.52 (d, 2H, ^3J = 6.8 Hz), 7.54 (t, 1H, ^3J = 6.8 Hz), 7.73 (t, 1H, ^3J = 6.8 Hz), 7.90 (d, 1H, ^3J = 7.8 Hz), 8.42 (d, 1H, ^3J = 7.8 Hz), 8.55 (m, 2H), 9.19 (d, 1H, ^4J = 1.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.1, 20.2, 29.3 (2C), 35.9, 53.5 (2C), 61.3, 70.3, 119.8, 121.0, 121.4, 123.0, 123.5, 124.5, 125.1, 128.2, 130.9, 138.9, 144.7, 146.2, 158.4. LC−MS (ESI): t_R = 5.39 min; $[M + H]^+$ 380.36. HRMS/EI: calcd for $C_{22}H_{25}N_3O_3$ 379.1895, found 379.1913.

8-Methoxy-6-(1-propylpiperidin-4-yl)-methyloxyphenanthridine (4p). Starting from **3p** (0.80 g, 3.55 mmol), following general procedure A and using cyclohexane/ethyl acetate (9/1) as the eluent for the chromatography, **4p** was obtained as a white powder (0.90 g). Yield: 70%. Mp: 92−94 °C. IR (KBr): ν (cm^{-1}) 3430, 2765, 2940, 1590, 1462, 1219. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, 3H, ^3J = 6.8 Hz), 1.51−1.57 (m, 4H), 1.93−2.03 (m, 5H), 2.30−2.34 (m, 2H), 3.00−3.03 (m, 2H), 3.98 (s, 3H), 4.51 (d, 2H, ^3J = 6.8 Hz), 7.43 (m, 2H), 7.45 (t, 1H, ^3J = 6.8 Hz), 7.71 (d, 1H, ^3J = 2.9 Hz), 7.84 (d, 1H, ^3J = 8.8 Hz), 8.33 (d, 1H, ^3J = 7.8 Hz), 8.42 (d, 1H, ^3J = 8.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.1, 20.2, 29.3 (2C), 35.8, 53.7 (2C), 55.5, 61.3, 70.3, 105.3, 120.8, 121.3, 121.4, 122.4, 123.5, 124.3, 127.6, 127.7, 128.9, 142.2, 158.2, 158.7. LC−MS (ESI): t_R = 5.47 min; $[M + H]^+$ 365.33. HRMS/EI: calcd for $C_{23}H_{28}N_2O_2$ 364.2150, found 364.2135.

8-Fluoro-6-(1-propylpiperidin-4-yl)-methyloxyphenanthridine (4q). Starting from **3q** (0.26 g, 1.22 mmol), following general procedure A and using cyclohexane/ethyl acetate (9/1) as the eluent for the chromatography, **4q** was obtained as a white powder (0.19 g). Yield: 44%. Mp: 70−71 °C. IR (KBr): ν (cm^{-1}) 2939, 755. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.92 (t, 3H, ^3J = 7.0 Hz), 1.53−1.60 (m, 4H), 1.92−2.02 (m, 5H), 2.31−2.34 (m, 2H), 3.01−3.03 (m, 2H), 4.47 (d, 2H, ^3J = 6.8 Hz), 7.48 (t, 1H, ^3J = 7.5 Hz), 7.52 (dt, ^3J = 8.5 Hz, ^4J = 2.5 Hz, 1H), 7.61 (t, 1H, ^3J = 7.5 Hz), 7.86 (d, 1H, ^3J = 8.0 Hz), 7.97 (dd, 1H, ^3J = 9.0 Hz, ^4J = 2.5 Hz), 8.34 (d, 1H, ^3J = 8.0 Hz), 8.48 (dd, 1H, ^3J = 9.0 Hz, ^4J = 5.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.1, 20.2, 29.3 (2C), 35.9, 53.7 (2C), 61.3, 70.3, 110.1 (d, ^2J = 22 Hz), 119.8 (d, ^2J = 22 Hz), 121.6 (d, J = 7 Hz), 121.9 (d, J = 11 Hz), 122.0, 124.5 (d, J = 7 Hz), 124.7, 128.0, 128.7, 131.5 (d, J = 2 Hz), 143.0, 158.2 (d, J = 2 Hz), 161.7 (d, ^1J = 246 Hz). LC−MS (ESI): t_R = 5.41 min; $[M + H]^+$ 353.31. HRMS/EI: calcd for $C_{22}H_{25}FN_2O$ 352.1950, found 352.1958.

9-Methyl-6-(1-propylpiperidin-4-yl)-methyloxyphenanthridine (4r). Starting from **3r** (1.00 g, 4.77 mmol), following general procedure A and using cyclohexane/ethyl acetate (9/1) as the eluent for the chromatography **4r** was obtained as a white powder (1.18 g). Yield: 71%. Mp: 90−91 °C. IR (KBr): ν (cm^{-1}) 3411, 3064, 2933, 1596, 1336, 758. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90 (t, 3H, ^3J = 6.8 Hz), 1.50−1.61 (m, 4H), 1.92−2.01 (m, 5H), 2.30−2.32 (m, 2H), 2.60 (s, 3H), 2.99−3.02 (m, 2H), 4.48 (d, 2H, ^3J = 6.8 Hz), 7.42−7.46 (m, 2H), 7.59 (t, 1H, ^3J = 6.8 Hz), 7.83 (d, 1H, ^3J = 8.8 Hz), 8.23−8.26 (m, 2H), 8.37 (d, 1H, ^3J = 7.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.1, 20.2, 22.3, 29.3 (2C), 35.9, 53.7 (2C), 61.3, 70.3, 118.1, 121.6, 122.0, 122.3, 124.0, 124.9, 127.6, 128.6, 128.7, 134.8, 141.1, 143.6, 159.0. LC−MS (ESI): t_R = 5.61 min; $[M + H]^+$ 349.32. HRMS/EI: calcd for $C_{23}H_{28}N_2O$ 348.2201, found 348.2202.

9-Fluoro-6-(1-propylpiperidin-4-yl)-methyloxyphenanthridine (4s). Starting from **3s** (0.20 g, 0.94 mmol), following general procedure A and using cyclohexane/ethyl acetate (9/1) as the eluent for the chromatography, **4s** was obtained, after recrystalisation in MeCN, as a white powder (0.20 g). Yield: 62%. Mp: 79−80 °C. IR (KBr): ν (cm^{-1}) 3399, 2944, 2765, 1593, 1336, 762. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90 (t, 3H, ^3J = 7.2 Hz), 1.52−1.59 (m, 4H), 1.89−1.99 (m, 5H), 2.30−2.33 (m, 2H), 3.00 (m, 2H), 4.48 (d, 2H, ^3J = 6.8 Hz), 7.33 (ddd, 1H, ^3J = 8.9 Hz, ^3J = 8.2 Hz, ^4J = 2.5 Hz), 7.47 (ddd, 1H, ^3J = 8.1 Hz, ^3J = 7.1 Hz, ^4J = 1.3 Hz), 7.64 (ddd, 1H, ^3J = 8.1 Hz, ^3J = 7.1 Hz, ^4J = 1.4 Hz), 7.86 (dd, 1H, ^3J = 8.1 Hz, ^4J = 1.4 Hz), 8.04 (dd, 1H, ^3J = 10.7 Hz, ^4J = 2.9 Hz), 8.28 (dd, 1H, ^3J = 8.3 Hz, ^4J = 1.4 Hz), 8.39 (dd, 1H, ^3J = 8.8 Hz, ^3J = 5.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.1, 20.2, 29.3 (2C), 35.9, 53.7 (2C), 61.3, 70.3, 107.2 (d, ^2J = 22 Hz), 115.8 (d, ^2J = 23 Hz), 116.9 (d, ^4J = 1 Hz), 121.9 (d, ^4J = 3 Hz), 122.3, 124.3, 127.8, 127.9 (d, ^3J = 10 Hz), 129.3, 137.1 (d, ^3J = 11 Hz), 143.8, 158.5, 164.3 (d, ^1J = 249 Hz). LC−MS (ESI): t_R = 5.45 min; $[M + H]^+$ 353.28. HRMS/EI: calcd for $C_{22}H_{25}FN_2O$ 352.1950, found 352.1938.

5-(1-Propylpiperidin-4-yl)methyloxybenzo[*c*]-2,6-naphthyridine (4t). Starting from **3t** (0.1 g, 0.5 mmol), following general procedure A and using cyclohexane/ethyl acetate (4/1) and cyclohexane/ethyl acetate (1/1) as the eluents for the chromatography, **4t** was obtained as a white powder (72 mg). Yield: 42%. Mp: 113−115 °C. IR (KBr): ν (cm^{-1}) 2953, 2932, 1613, 1585, 1459, 1337, 1235, 1128, 1985, 844, 768, 673. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.92 (t, 3H, ^3J = 7.4 Hz), 1.50−1.61 (m, 4H), 1.91−2.03 (m, 5H), 2.32 (m, 2H), 3.02 (d, 2H, J = 11.7 Hz), 4.51 (d, 2H, ^3J = 6.5 Hz), 7.55 (t, 1H, J = 7.6 Hz), 7.69 (t, 1H, J = 7.5 Hz), 7.90 (d, 1H, ^3J = 8.3 Hz), 8.12 (d,

dx.doi.org/10.1021/jm300943r | J. Med. Chem. 2012, 55, 9693−9707

1H, ^3J = 5.3 Hz), 8.54 (d, 1H, ^3J = 8.3 Hz), 8.83 (d, 1H, ^3J = 5.3 Hz), 9.92 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.0, 20.1, 29.1 (2C), 35.7, 53.5 (2C), 61.1, 70.8, 117.1, 120.2, 121.4, 124.1, 125.2, 127.9, 128.6, 129.5, 143.5, 146.1, 143.4, 157.5. LC−MS (ESI): t_R = 4.59 min; $[M + H]^+$ 336.25. HRMS/ESI: calcd for $C_{21}H_{26}N_3O$ $[M + H]^+$ 336.2076, found 336.2067.

5-(1-Propylpiperidin-4-yl)methyloxybenzo[*h*]-1,6-naphthyridine (4u). Starting from **3u** (0.1 g, 0.5 mmol), following general procedure A and using cyclohexane/ethyl acetate (1/1) and ethyl acetate with 5% of NEt_3 as the eluents for the chromatography, **4u** was obtained as a white powder (92 mg). Yield: 54%. Mp: 114−115 °C. IR (KBr): ν (cm^{-1}) 2931, 1605, 1590, 1458, 1328, 767, 733. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.84 (t, 3H, ^3J = 7.3 Hz), 1.18 (brs, 1H), 1.43−1.56 (m, 3H), 1.18−1.96 (m, 5H), 2.23−2.27 (m, 2H), 2.95 (d, 2H, J = 11.2 Hz), 4.44 (d, 2H, ^3J = 6.3 Hz), 7.45−7.50 (m, 2H), 7.62−7.65 (m, 1H), 7.80 (d, 1H, ^3J = 7.5 Hz), 8.55 (dd, 1H, ^3J = 8.2 Hz, ^4J = 1.4 Hz), 8.88 (dd, 1H, ^3J = 8.1 Hz, ^4J = 1.4 Hz), 9.03 (dd, 1H, ^3J = 4.4 Hz, ^4J = 1.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.1, 20.2, 29.3 (2C), 35.9, 53.6 (2C), 61.2, 70.8, 115.4, 122.3, 123.5, 123.7, 124.9, 127.2, 130.4, 133.0, 144.9, 150.8, 152.9, 158.4. LC−MS (ESI): t_R = 4.84 min; $[M + H]^+$ 336.28. HRMS/EI: calcd for $C_{21}H_{25}N_3O$ 335.1997, found 335.1998.

7-Fluoro-5-(1-propylpiperidin-4-yl)methyloxybenzo[*h*]-1,6-naphthyridine (4v). Starting from **3v** (1 g, 4.6 mmol), following general procedure A and using ethyl acetate and ethyl acetate/NEt_3 98/2 as the eluents for the chromatography, **4v** was obtained as a white powder (1.37 g). Yield: 83%. Mp: 119−120 °C. IR (KBr): ν (cm^{-1}) 2952, 2932, 1605, 1589, 1329, 1235, 1152, 774. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91 (t, 3H, ^3J = 6.8 Hz), 1.50−1.64 (m, 4H), 1.77 (brs, 1H), 1.90−2.04 (m, 4H), 2.30−2.34 (m, 2H), 3.02 (d, 2H, J = 11.7 Hz), 4.56 (d, 2H, ^3J = 6.8 Hz), 7.41−7.49 (m, 2H), 7.59 (dd, 1H, ^3J = 8.3 Hz, ^3J = 4.8 Hz), 8.64 (dd, 1H, ^3J = 7.8 Hz, ^4J = 1.9 Hz), 8.72 (dd, 1H, ^3J = 8.3 Hz, ^4J = 1.9 Hz), 9.11 (dd, 1H, ^3J = 4.8 Hz, ^4J = 1.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.1, 20.2, 29.2 (2C), 35.8, 53.6 (2C), 61.2, 71.1, 115.6, 115.6 (d, ^2J = 19 Hz), 119.2 (d, J = 4 Hz), 122.8, 124.4 (d, J = 7 Hz), 125.5 (d, J = 2 Hz), 133.2, 134.1 (d, ^2J = 11 Hz), 150.3 (d, J = 3 Hz), 153.3, 157.0 (d, ^1J = 250 Hz), 158.6. LC−MS (ESI): t_R = 6.00 min; $[M + H]^+$ 354.37. HRMS/EI: calcd for $C_{21}H_{24}FN_3O$ 353.1903, found 353.1915.

2-Fluoro-3-(trimethylsilanyl)phenylboronic Acid (5). In a three necks round-bottom flask under N_2 at −80 °C were introduced (2-fluorophenyl)trimethylsilane[42] (7.4 g, 44 mmol), THF (90 mL), and 1.3 M *s*-BuLi in *n*-hexane/cyclohexane 98/2 (33.82 mL, 44 mmol). The yellow solution was stirred for 0.75 h, trimethyl borate (5.49 mL, 48.4 mmol) was added, and the solution was stirred again for 0.75 h. The mixture was allowed to reach room temperature, hydrolyzed with water (200 mL), washed with Et_2O (3 × 150 mL), acidified to pH = 1 using 1 M HCl and extracted with Et_2O (3 × 150 mL). The combined organic layers were dried over $MgSO_4$, filtered, and evaporated in vacuo to afford **5** (6.4 g) as white crystals. Yield: 69%. Mp: 93−94 °C. IR (KBr): ν (cm^{-1}) 3512, 3351, 2952, 1423, 1351, 844, 760, 605. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.32 (s, 9H), 5.29 (s, 1H), 5.31 (s, 1H), 7.19 (td, 1H, ^3J = 7.2 Hz, J = 1.5 Hz), 7.52 (m, 1H), 7.84 (td, 1H, ^3J = 7.2 Hz, J = 1.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ −1.0, 124.3 (d, ^4J = 2 Hz), 125.6 (d, ^2J = 36 Hz), 138.2 (d, ^3J = 7 Hz), 138.8 (d, ^3J = 13 Hz), 172.7 (d, ^1J = 235 Hz), signal of the carbon bonded to the boron is missing.

2′-Fluoro-3′-(trimethylsilanyl)biphenyl-2-carbonitrile (6a). In a round-bottom flask under N_2 were introduced DME (15 mL) and water (15 mL). The solution was degassed by bubbling N_2 for 15 min, and $Pd(OAc)_2$ (62 mg, 0.27 mmol) and PPh_3 (144 mg, 0.55 mmol) were added. The solution was heated to 50 °C for 10 min, and 2-bromobenzonitrile (1 g, 5.5 mmol), **5** (1.75 g, 8.25 mmol), and Na_2CO_3 (2.33 g, 22 mmol) were added. The solution was heated to 90 °C for 14 h, cooled to room temperature, filtered over a pad of Celite, and extracted with EtOAc (3 × 50 mL). The combined organic layers were dried over $MgSO_4$, filtered, evaporated in vacuo, and purified by flash chromatography using cyclohexane/AcOEt 9/1 as the eluent to afford **6a** (1.45 g) as a colorless oil. Yield: 98%. IR (KBr): ν (cm^{-1}) 2957, 2227 (CN), 1637, 1412, 585, 842, 761. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.35 (s, 9H), 7.25 (t, 1H, ^3J = 7.8 Hz), 7.40−7.53 (m, 4H), 7.65 (t, 1H, ^3J = 7.8 Hz), 7.77 (d, 1H, ^3J = 7.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ −1.0, 112.9, 118.1, 124.1 (d, J = 3 Hz), 125.0 (d, ^2J = 19 Hz), 127.5 (d, ^3J = 36 Hz), 127.9, 130.9, 132.4, 132.4, 133.2, 136.1 (d, J = 13 Hz), 140.1, 163.5 (d, ^1J = 242 Hz). HRMS/EI: calcd for $C_{16}H_{16}FNSi$ 269.1036, found 269.1034.

2-(2-Fluoro-3-trimethylsilanylphenyl)nicotinonitrile (6b). The same procedure as for **6a**, starting from 2-chloronicotinonitrile (1 g, 7.2 mmol), $Pd(OAc)_2$ (81 mg, 0.36 mmol), PPh_3 (189 mg, 0.72 mmol), **5** (2.29 g, 10.8 mmol) and Na_2CO_3 (3.06 g, 28.8 mmol). Cyclohexane/AcOEt 8/2 was used as the eluent for chromatography to afford **6b** (1.85 g) as a colorless oil. Yield: 95%. IR (KBr): ν (cm^{-1}) 2957, 2231 (CN), 1604, 1428, 1414, 1251, 862, 842, 762. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.36 (s, 9H), 7.29 (m, 1H), 7.43 (dd, 1H, ^3J = 8.3 Hz, ^3J = 4.9 Hz), 7.53−7.60 (m, 2H), 8.08 (d, 1H, ^3J = 7.8 Hz), 8.90 (dd, 1H, ^3J = 4.9 Hz, ^4J = 1.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ −1.0, 110.6, 116.5, 122.0, 124.2 (d, J = 3 Hz), 124.7 (d, ^2J = 19 Hz), 127.5 (d, ^2J = 31 Hz), 132.3 (d, J = 2 Hz), 137.2 (d, J = 11 Hz), 140.6, 152.5, 157.9, 163.8 (d, ^1J = 243 Hz). HRMS/EI: calcd for $C_{15}H_{15}FN_2Si$ 270.0988, found 270.0979.

2′-Fluoro-3′-iodobiphenyl-2-carbonitrile 7a. In a round-bottom flask were introduced **6a** (1.05 g, 3.7 mmol), DCM (30 mL) and ICl (279 μL, 5.6 mmol). The solution was allowed to stir for 4.5 h and a saturated $Na_2S_2O_3$ solution in water (40 mL) was added. The aqueous layer was then extracted with DCM (3 × 30 mL) and the combined organic layers were dried $MgSO_4$, filtered and evaporated in *vacuo* to afford **7a** (1.11 g) as a white powder. Yield: 88%. Mp: 123−125 °C. IR (KBr): ν (cm^{-1}) 2222 (CN), 1443, 1425, 788, 759. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.03 (t, 1H, ^3J = 7.8 Hz), 7.40 (m, 1H), 7.49−7.53 (m, 2H), 7.67 (td, 1H, J = 7.8 Hz, J = 1.9 Hz), 7.67 (d, 1H, ^3J = 7.8 Hz), 7.84 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 82.2 (d, ^2J = 25 Hz), 112.6, 117.7, 125.8 (d, J = 5 Hz), 126.3 (d, ^2J = 17 Hz), 128.6, 130.8 (d, J = 2 Hz), 131.4 (d, J = 2 Hz), 132.6, 133.3, 138.6, 140.2 (d, J = 2 Hz), 158.2 (d, ^1J = 246 Hz). HRMS/EI: calcd for $C_{13}H_7FIN$ 322.9607, found 322.9601.

2-(2-Fluoro-3-iodophenyl)nicotinonitrile (7b). The same procedure as for **7a** was used, starting from **6b** (0.82 g, 3 mmol) and ICl (228 μL, 4.5 mmol). The crude was purified by flash chromatography using cyclohexane/AcOEt 8/2 as the eluent to afford **7b** (0.82 g) as a white powder. Yield: 84%. Mp: 100−101 °C. IR (KBr): ν (cm^{-1}) 2228 (CN), 1424, 1227, 805, 761, 725. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.08 (t, 1H, ^3J = 7.8 Hz), 7.48 (dd, 1H, ^3J = 7.8 Hz, ^3J = 4.9 Hz), 7.56 (m, 1H), 7.92 (m, 1H), 8.11 (dd, 1H, ^3J = 7.8 Hz, ^4J = 1.9 Hz), 8.92 (dd, 1H, ^3J = 4.9 Hz, ^4J = 1.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 82.3 (d, ^2J = 26 Hz), 110.4, 116.1, 122.6, 126.0 (d, J = 5 Hz), 126.1 (d, ^2J = 16 Hz), 131.4 (d, J = 2 Hz), 140.8, 141.4 (d, J = 2 Hz), 152.6, 156.5, 158.5 (d, ^1J = 247 Hz). HRMS/EI: calcd for $C_{12}H_6FIN_2$ 323.9568, found 323.9559.

4-Iodophenanthridin-6(5*H*)-one (8a). The same procedure as for compound **3v** was used, with **7a** (1.11 g, 3.4 mmol), KOH (0.96 g, 14 mmol), and *t*-BuOH (30 mL) and heating at 150 °C for 1 h. **8a** (0.73 g) was obtained as a white powder. Yield: 67%. Mp: 240−242 °C. IR (KBr): ν (cm^{-1}) 3274, 1654 (CO), 1603, 1351, 752, 709, 625. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.04 (t, 1H, ^3J = 7.8 Hz), 7.66 (t, 1H, ^3J = 7.8 Hz), 7.86 (t, 1H, ^3J = 7.8 Hz), 8.00 (d, 1H, ^3J = 6.8 Hz), 8.33 (d, 1H, ^3J = 6.8 Hz), 8.42 (d, 1H, ^3J = 7.8 Hz), 8.50 (d, 1H, ^3J = 8.8 Hz), 9.29 (brs, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 105.1, 119.0, 122.6, 123.6, 125.9, 127.5, 128.2, 132.5, 134.0, 137.3, 137.7, 139.3, 172.9. HRMS/EI: calcd for $C_{13}H_8INO$ 320.9651, found 320.9665.

7-Iodobenzo[*h*]-1,6-naphthyridin-5(6*H*)-one (8b). The same procedure as for compound **3v** was used, with **7b** (0.7 g, 2.16 mmol), KOH (0.6 g, 10.8 mmol), and *t*-BuOH (35 mL) and heating at 150 °C for 1 h. **8b** (0.51 g) was obtained as a white powder. Yield: 73%. Mp: 230−231 °C. IR (KBr): ν (cm^{-1}) 3325, 1660 (CO), 1594, 1424, 765, 621. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.11 (t, 1H, ^3J = 7.8 Hz), 7.56 (dd, 1H, ^3J = 7.8 Hz, ^3J = 4.8 Hz), 8.01 (d, 1H, ^3J = 7.8 Hz), 8.74 (dd, 1H, ^3J = 7.8 Hz, ^4J = 1.9 Hz), 8.80 (d, 1H, ^3J = 6.8 Hz), 8.90 (brs, 1H), 9.04 (dd, 1H, ^3J = 4.8 Hz, ^4J = 1.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 84.4, 121.1, 121.3, 123.5, 124.6, 125.6, 136.4, 136.8, 141.0, 150.8,

154.4, 161.4. HRMS/EI: calcd for $C_{12}H_7IN_2O$ 321.9603, found 321.9592.

Ethyl 1-[3-(4-Iodophenyl)propanoyl]piperidine-4-carboxylate (9). To a solution of 4-iododihydrocinamic acid[43] (5 g, 18.11 mmol) in DCM (50 mL) were added at room temperatue HOBt (3.67 g, 27.17 mmol), EDCI (5.21 g, 27.17 mmol), NEt_3 (3.79 mL, 27.17 mmol), and ethyl isonipecotate (3.07 mL, 19.92 mmol). The solution was allowed to stir at room temperature for 24 h, evaporated to dryness, and purified by silica gel chromatography using cyclohexane/EtOAc 9/1 and 7/3 as the eluents to afford **9** (6.31 g) as white powder. Yield: 84%. Mp: 73−74 °C. IR (KBr): ν (cm^{-1}) 2953, 2930, 1729 (CO), 1644 (CO), 1447, 1178, 1040, 1006, 811. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.26 (t, 3H, ^3J = 6.8 Hz), 1.55−1.65 (m, 2H), 1.90 (m, 2H), 2.49 (m, 1H), 2.58 (d, 2H, ^3J = 7.8 Hz), 2.79 (m, 1H), 2.91 (d, 2H, ^3J = 7.8 Hz), 3.03 (m, 1H), 3.74 (m, 1H), 4.14 (t, 2H, ^3J = 6.8 Hz), 4.42 (m, 1H), 6.97 (d, 2H, ^3J = 8.3 Hz), 7.60 (d, 2H, ^3J = 8.3 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.2, 27.8, 28.3, 30.8, 34.7, 40.9, 41.0, 44.7, 60.6, 91.2, 130.5 (2C), 137.5 (2C), 140.9, 170.1, 174.1. HRMS/EI: calcd for $C_{17}H_{22}INO_3$ 415.0644, found 415.0634.

{1-[3-(4-Iodophenyl)propyl]piperidin-4-yl}methanol (10). To a solution of **9** (1.31 g, 3.15 mmol) in anhydrous THF (20 mL) at −5 °C was added 1 M DIBALH solution in *n*-hexane (12.62 mL, 12.62 mmol). The mixture was allowed to stir at room temperature for 15 h, carefully hydrolyzed with water (30 mL), extracted with EtOAc (3 × 50 mL), dried with $MgSO_4$, filtered, and evaporated. The crude product was then purified by silica gel chromatography using cyclohexane/EtOAc 9/1 and 7/3 as the eluents to afford **10** (0.96g) as a colorless oil. Yield: 85%. IR (KBr): ν (cm^{-1}) 3400 (OH), 2922, 1728, 1483, 1042, 1006, 797. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.26 (d, 1H, ^3J = 5.9 Hz), 1.26 (m, 2H), 1.70−1.80 (m, 5H), 1.92 (m, 2H), 2.32 (t, 2H, ^3J = 7.8 Hz), 2.56 (t, 2H, ^3J = 7.8 Hz), 2.92 (m, 2H), 3.49 (d, 2H, ^3J = 6.8 Hz), 6.93 (d, 2H, ^3J = 8.3 Hz), 7.58 (d, 2H, ^3J = 8.3 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.6, 28.8 (2C), 33.3, 38.6, 53.5 (2C), 58.2, 67.9, 92.0, 130.5 (2C), 137.3 (2C), 141.9. HRMS/EI: calcd for $C_{15}H_{22}INO$ 359.0746, found 359.0759.

4-Iodo-6-(1-propylpiperidin-4-yl)methyloxyphenanthridine (11a). Starting from **8a** (0.47 g, 1.46 mmol), using general procedure A and cyclohexane/ethyl acetate 8/2 as the eluent for the chromatography, **11a** was obtained as a yellow powder (0.51 g). Yield: 76%. Mp: 87−89 °C. IR (KBr): ν (cm^{-1}) 2926, 1591, 1396, 1339, 1136, 751. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91 (t, 3H, ^3J = 7.3 Hz), 1.51−1.64 (m, 4H), 1.93−2.09 (m, 5H), 2.31 (t, 2H, J = 7.8 Hz), 3.02 (d, 2H, J = 10.7 Hz), 4.63 (d, 2H, ^3J = 6.8 Hz), 7.19 (t, 1H, J = 6.8 Hz), 7.67 (t, 1H, ^3J = 7.8 Hz), 7.82 (t, 1H, ^3J = 7.8 Hz), 8.20 (d, 1H, ^3J = 7.8 Hz), (t, 2H, ^3J = 7.3 Hz), 8.49 (d, 1H, ^3J = 8.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.1, 20.2, 29.2 (2C), 35.6, 53.5 (2C), 61.1, 71.2, 101.8, 120.2, 122.0, 122.6, 123.0, 125.2, 125.5, 127.8, 131.1, 132.9, 134.9, 138.9, 159.5. LC−MS (ESI): t_R = 5.84 min; $[M + H]^+$ 461.28. HRMS/EI: calcd for $C_{22}H_{25}IN_2O$ 460.1012, found 460.1008.

7-Iodo-5-(1-propylpiperidin-4-yl)methyloxybenzo[*h*]-1,6-naphthyridine (11b). Starting from **8b** (0.16 g, 0.5 mmol), using general procedure A and cyclohexane/ethyl acetate 8/2 with 5% of NEt_3 as the eluent for the chromatography, **11b** was obtained as a yellow powder (0.16 g). Yield: 71%. Mp: 120−122 °C. IR (KBr): ν (cm^{-1}) 2917, 1603, 1588, 1341, 1093, 762. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91 (t, 3H, ^3J = 7.3 Hz), 1.51−1.59 (m, 4H), 1.91−2.08 (m, 5H), 2.32 (m, 2H), 3.02 (d, 2H, J = 8.7 Hz), 4.63 (d, 2H, ^3J = 5.8 Hz), 7.27 (t, 1H, J = 7.8 Hz), 7.59 (dd, 1H, ^3J = 7.8 Hz, ^3J = 4.9 Hz), 8.28 (d, 1H, ^3J = 8.7 Hz), 8.64 (dd, 1H, ^3J = 7.8 Hz, ^4J = 1.9 Hz), 8.96 (m, 1H), 9.11 (dd, 1H, ^3J = 4.9 Hz, ^4J = 1.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.0, 20.0, 29.1 (2C), 35.5, 53.5 (2C), 61.0, 71.5, 100.6, 115.2, 122.7, 124.1, 124.3, 125.9, 133.1, 140.4, 144.1, 150.7, 153.2, 158.8. LC−MS (ESI): t_R = 5.22 min; $[M + H]^+$ 462.22. HRMS/EI: calcd for $C_{21}H_{24}IN_3O$ 461.0964, found 461.0970.

6-{1-[3-(4-Iodophenyl)propyl]piperidin-4-yl}-methyloxyphenanthridine (11c). Starting from **3a** (0.5 g, 2.56 mmol), using general procedure A and cyclohexane/ethyl acetate 8/2 as the eluent for the chromatography, **11c** was obtained as a colorless oil (0.54 g). Yield: 39%. IR (KBr): ν (cm^{-1}) 2925, 1637, 1620, 1486, 1317, 759, 727. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.58 (m, 2H), 1.79−2.05 (m, 7H), 2.37 (t, 2H, ^3J = 7.8 Hz), 2.59 (t, 2H, ^3J = 6.7 Hz), 3.00 (d, 2H, ^3J = 10.7 Hz), 4.50 (d, 2H, ^3J = 5.8 Hz), 6.95 (d, 2H, ^3J = 8.8 Hz), 7.48 (d, 1H, ^3J = 6.8 Hz), 7.59 (d, 2H, ^3J = 8.8 Hz), 7.63 (m, 2H), 7.82 (d, 1H, ^3J = 6.8 Hz), 7.86 (d, 1H, J = 8.8 Hz), 8.38 (d, 1H, ^3J = 6.8 Hz), 8.42 (d, 1H, ^3J = 7.8 Hz), 8.51(d, 1H, ^3J = 7.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.6, 29.3 (2C), 33.3, 35.8, 53.6 (2C), 58.3, 70.4, 90.7, 120.1, 121.8, 122.1, 122.3, 124.3, 125.0, 127.1, 127.7, 128.7, 130.5 (2C), 130.8, 134.7, 137.3 (2C), 141.8, 143.3, 158.9. LC−MS (ESI): t_R = 6.33 min; $[M + H]^+$ 537.25. HRMS/ESI: calcd for $C_{28}H_{30}IN_2O$ 537.1397, found 537.1401.

7-Fluoro-5-{1-[3-(4-iodophenyl)propyl]piperidin-4-yl}-methyloxybenzo[*h*]-1,6-naphthyridine (11d). Starting from **3v** (0.5 g, 2.33 mmol), using general procedure A and cyclohexane/ethyl acetate 8/2 as the eluent for the chromatography, **11d** was obtained as a white powder (0.53 g). Yield: 41%. Mp: 142−144 °C. IR (KBr): ν (cm^{-1}) 2925, 1603, 1589, 1354, 1330, 1315, 1106, 794, 770. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.59 (m, 2H), 1.78−2.05 (m, 7H), 2.37 (t, 2H, ^3J = 7.8 Hz), 2.59 (t, 2H, ^3J = 6.7 Hz), 2.99 (d, 2H, ^3J = 10.7 Hz), 4.56 (d, 2H, ^3J = 5.8 Hz), 6.95 (d, 2H, ^3J = 8.8 Hz), 7.41−7.49 (m, 2H), 7.56−7.62 (m, 3H), 8.64 (dd, 1H, J = 7.8 Hz, J = 1.9 Hz), 8.73 (dd, 1H, ^3J = 8.3 Hz, ^4J = 1.9 Hz), 9.11 (dd, 1H, ^3J = 4.9 Hz, ^4J = 1.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.5, 29.2 (2C), 33.3, 35.8, 53.5 (2C), 58.2, 71.0, 90.7, 115.6, 115.7 (d, ^2J = 19 Hz), 119.2, 122.7, 124.4 (d, J = 7 Hz), 125.4 (d, J = 1 Hz), 130.5 (2C), 133.1, 134.1 (d, ^2J = 11 Hz), 137.2 (2C), 141.8, 150.2 (d, J = 3 Hz), 153.2, 157.0 (d, ^1J = 250 Hz), 158.5. LC−MS (ESI): t_R = 6.04 min; $[M + H]^+$ 556.38. HRMS/ESI: calcd for $C_{27}H_{28}FIN_3O$ 556.1256, found 556.1260.

General Procedure B for the Synthesis of Stannylated Compounds 12a,b,d. In a Schlenk flask under nitrogen were introduced toluene (4 mL), water (0.4 mL), $Pd(OAc)_2$ (0.025 mmol), and PPh_3 (0.05 mmol). The mixture was heated at 50 °C for 0.3 h, and the iodinated derivative (**11a**, **11b**, or **11d**; 0.5 mmol in 7 mL of toluene) and hexa-*n*-butylditin (0.75 mmol) were added. The mixture was heated at 90 °C for 16 h, filtered on Celite, and evaporated in vacuo. The obtained crude oil was then purified by chromatography on silica gel using Et_2O and Et_2O/NEt_3 (99/1) as the eluents.

6-(1-Propylpiperidin-4-yl)methyloxy-4-(tributylstannyl)-phenanthridine (12a). Starting from **11a** and following general procedure B, **12a** was obtained as a colorless oil (140 mg). Yield: 45%. IR (KBr): ν (cm^{-1}) 2955, 2922, 2870, 2851, 1589, 1459, 1339, 1311, 761. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85 (t, 6H, J = 6.3 Hz), 0.92 (t, 6H, J = 6.8 Hz), 1.16−1.20 (m, 4H), 1.27−1.43 (m, 10H), 1.50−1.62 (m, 10H), 1.97 (d, 2H, J = 9.7 Hz), 2.06 (t, 1H, J = 11.2 Hz), 2.36 (t, 2H, J = 7.8 Hz), 3.05 (d, 2H, J = 10.7 Hz), 4.48 (d, 2H, J = 5.8 Hz), 7.46 (t, 1H, ^3J = 7.8 Hz), 7.61 (t, 1H, ^3J = 7.8 Hz), 7.78 (m, 2H), 8.38 (t, 2H, J = 7.8 Hz), 8.50 (d, 1H, ^3J = 8.7 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 10.2 (3C), 12.0, 13.7 (3C), 20.0, 27.4 (3C), 29.1 (2C), 29.4 (3C), 35.7, 53.5 (2C), 61.1, 70.6, 119.9, 121.4, 121.9, 122.3, 124.1, 124.9, 126.9, 130.6, 135.4, 136.9, 143.6, 148.0, 157.7. HRMS/ESI: calcd for $C_{34}H_{53}N_2OSn$ 625.3181, found 625.3186.

7-(1-Propylpiperidin-4-yl)methyloxy-5-(tributylstannyl)-benzo[*h*]-1,6-naphthyridine (12b). Starting from **11b** and following general procedure B, **12b** was obtained as a colorless oil (139 mg). Yield: 44%. IR (KBr): ν (cm^{-1}) 2956, 2924, 2871, 2852, 1603, 1460, 1328, 1153, 773. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85 (t, 6H, J = 7.3 Hz), 0.92 (t, 6H, J = 6.8 Hz), 1.16−1.21 (m, 4H), 1.25−1.43 (m, 10H), 1.51−1.63 (m, 10H), 1.95 (d, 2H, J = 10.7 Hz), 2.06 (t, 1H, J = 11.2 Hz), 2.36 (t, 2H, J = 7.8 Hz), 3.06 (d, 2H, J = 10.7 Hz), 4.48 (d, 2H, J = 5.8 Hz), 7.51−7.56 (m, 2H), 7.86 (dd, 1H, ^3J = 6.8 Hz, ^4J = 1.9 Hz), 8.62 (dd, 1H, ^3J = 7.8 Hz, ^4J = 1.9 Hz), 8.92 (d, 1H, ^3J = 7.8 Hz), 9.09 (dd, 1H, ^3J = 4.9 Hz, ^4J = 1.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 10.2 (3C), 12.0, 13.5 (3C), 20.0, 27.4 (3C), 29.1 (3C), 29.3 (2C), 35.6, 53.4 (2C), 61.1, 70.9, 115.1, 122.0, 122.7, 123.9, 124.7, 132.9, 138.6, 142.9, 149.7, 151.3, 152.8, 157.3. HRMS/ESI: calcd for $C_{33}H_{52}N_3OSn$ 626.3133, found 626.3136.

7-Fluoro-5-{1-[3-(4-tributylstannylphenyl)propyl]piperidin-4-yl}methyloxybenzo[*h*]-1,6-naphthyridine (12d). Starting from **11d** and following general procedure B, **12d** was obtained as a colorless oil (147 mg). Yield: 41%. IR (KBr): ν (cm^{-1}) 2954, 2925, 1608, 1591, 1462, 1331, 1314, 1240, 1151, 1083, 769. ^{1}H NMR (400

dx.doi.org/10.1021/jm300943r | J. Med. Chem. 2012, 55, 9693−9707

MHz, $CDCl_3$): δ 0.85 (t, 9H, J = 7.3 Hz), 0.92 (t, 6H, J = 8.1 Hz), 1.18−1.29 (m, 8H), 1.42−1.52 (m, 6H), 1.79−1.96 (m, 7H), 2.34 (t, 2H, J = 7.8 Hz), 2.06 (t, 2H, J = 7.8 Hz), 2.94 (d, 2H, J = 11.2 Hz), 4.48 (d, 2H, J = 6.1 Hz), 7.10 (d, 2H, ^3J = 7.8 Hz), 7.30 (d, 2H, ^3J = 7.8 Hz), 7.34−7.41 (m, 2H), 7.52 (dd, 1H, ^3J = 8.2 Hz, ^3J = 4.58 Hz), 8.57 (dd, 1H, ^3J = 8.2 Hz, ^4J = 1.8 Hz), 8.64 (m, 1H), 9.04 (dd, 1H, ^3J = 4.5 Hz, ^4J = 1.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 9.5 (3C), 13.6 (3C), 27.3 (3C), 28.7, 29.1 (3C), 29.2 (2C), 33.8, 35.8, 53.5 (2C), 58.6, 71.0, 115.6, 115.6 (d, ^2J = 20 Hz), 119.2 (d, J = 4 Hz), 122.8, 124.4 (d, J = 7 Hz), 125.5 (d, J = 2 Hz), 128.1 (2C), 133.2, 134.1 (d, ^2J = 11 Hz), 136.4 (2C), 138.5, 141.8, 150.3 (d, J = 4 Hz), 153.3, 157.0 (d, ^1J = 250 Hz), 158.6. HRMS/ESI: calcd for $C_{39}H_{55}FN_3OSn$ 720.3353, found 720.3356.

Pharmacological Assay and Screen. Binding of all compounds to native 5-HT$_4$R from guinea pig was determined using the method of Grossman.[44] For membrane preparations, male guinea pigs (300−350 g, Charles River) were subjected to euthanasia by cervical dislocation and decapitated. Brains were rapidly removed at 4 °C and striatal regions carefully dissected and pooled. The tissues were then suspended in 10 volumes of HEPES buffer (50 mM, pH 7.4) at 4 °C. After homogenization at 4 °C (Ultra-Turrax, maximal speed, 15 s), and ultracentrifugation (23 000g, 60 min, 4 °C), the pellet was resuspended in 10 volumes of HEPES buffer (50 mM, pH 7.4) at 4 °C in order to obtain a tissue concentration of about 100 mg protein/mL. The protein concentration was determined by the method of Lowry[45] using bovine serum albumin as standard. For radioligand binding studies, 600 μg of membrane was incubated in duplicate at 37 °C for 30 min with [^{3}H]GR 113808 (Perkin-Elmer), a fixed concentration of compound, and HEPES buffer (50 mM, pH 7.4) at 37 °C. Incubation was terminated by rapid vacuum filtration through 0.5% polyethylenimine-presoaked Whatman GF/B filters (Alpha Biotech) using a Brandel cell harvester. Filters were subsequently washed three times with 4 mL of HEPES buffer (50 mM, pH 7.4) at 4 °C. The method was validated from saturation studies: six concentrations of [^{3}H]GR 113808 were used to give final concentrations of 0.02−0.8 nM, and nonspecific binding of [^{3}H]GR 113808 was defined in the presence of 30 μM serotonin to determine the K_d and the B_{max}. For competition studies, [^{3}H]GR 113808 was used to give a final concentration of 0.1 nM. Percentages of inhibition of the binding of [^{3}H]GR 113808 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 $K_i \pm$ SD.

Ligands **4i**, **4k**, **4l**, **4m**, **4t**, and **4u** were submitted to the National Institute of Mental Health Psychoactive Drug Screening Program (NIMH PDSP) for assessment of binding affinity to human recombinant 5-HT$_4$ receptors and to other serotonin receptors (5-HT$_{1A-E}$, 5-HT$_{2A-C}$, 5-HT$_3$, 5-HT$_{5A}$, 5-HT$_6$, 5-HT$_7$). For human 5-HT$_4$R K_i determinations, [^{3}H]GR 113808 was used as hot ligand and GR 113808 as reference. 5-HT$_4$R membrane was made with HEK T cells transiently transfected with human 5-HT$_4$ DNA. The binding protocol is the same as for other 5-HT subtypes in the PDSP assay protocol book. Selected ligands **4k**, **4l**, **4m**, and **4u** were also assessed for agonist/partial agonist activity and for antagonist activity. For 5-HT$_4$R-mediated Gs activation, cAMP was measured using GloSensor tech from Promega, with serotonin as a reference agonist according to a literature procedure.[46] For other experimental details, please refer to the PDSP Web site http://pdsp.med.unc.edu/ and click on "Binding Assay".

Other selected ligands **4v**, **11a**, **11b**, and **11d** were evaluated for binding to human 5-HT$_4$ and other serotonin receptors (5-HT$_{1A-E}$, 5-HT$_{2A-C}$, 5-HT$_3$, 5-HT$_{5A}$, 5-HT$_6$, 5-HT$_7$) as well as for intrinsic activity at CEREP. Detailed assay protocols are available at the CEREP Web site (http://www.cerep.com).

Radiosynthesis of 13a. A 0.05 M NaOH solution containing 165 μCi of carrier-free Na^{125}I (PerkinElmer, 1 μL) was added to a mixture made of **12a** (3 μg) in EtOH (1.5 μL), glacial acetic acid (5 μL), and 30% hydrogen peroxide solution (5 μL). After incubation at room temperature for 20 min, HPLC phase (MeCN/water 80/20, 10 mM AcOH buffer, pH 5, 350 μL) was added, and **13a** was isolated by an isocratic HPLC using a Bondclone C18 (10 μm, 300 × 7.8 mm, Phenomenex) column at 3 mL min^{-1}. The apparent specific activity was determined thanks to a calibration curve measured using growing amounts of the cold reference compound and to UV monitoring during the HPLC purification step. The overall radiochemical yield was 56%. The apparent specific radioactivity was 240 Ci/mmol (11% of the carrier-free specific activity).

Radiosynthesis of 13b. A 0.05 M NaOH solution containing 128 μCi of carrier-free Na^{125}I (PerkinElmer, 1 μL) was added to a mixture made of **12b** (3 μg) in EtOH (1.5 μL), glacial acetic acid (5 μL), and 30% hydrogen peroxide solution (5 μL). After incubation at room temperature for 20 min, HPLC phase (MeCN/water 80/20, 10 mM AcOH buffer, pH 5, 350 μL) was added and **13b** was isolated by an isocratic HPLC using a Bondclone C18 (10 μm, 300 × 7.8 mm, Phenomenex) column at 3 mL min^{-1}. The apparent specific activity was determined thanks to a calibration curve measured using growing amounts of the cold reference compound and to UV monitoring during the HPLC purification step. The overall radiochemical yield was 70%. The apparent specific radioactivity was 110 Ci/mmol (5% of the carrier-free specific activity).

Radiosynthesis of 13d. A 0.05 M NaOH solution containing 210 μCi of carrier-free Na^{125}I (PerkinElmer, 1 μL) was added to a mixture made of **12b** (10 μg) in EtOH (1 μL), glacial acetic acid (5 μL), and 30% hydrogen peroxide solution (5 μL). After incubation at room temperature for 20 min, HPLC phase (MeCN/water 80/20, 10 mM AcOH buffer, pH 5, 350 μL) was added and **13d** was isolated by an isocratic HPLC using a Bondclone C18 (10 μm, 300 × 7.8 mm, Phenomenex) column at 3 mL min^{-1}. The apparent specific activity was determined thanks to a calibration curve measured using growing amounts of the cold reference compound and to UV monitoring during the HPLC purification step. The overall radiochemical yield was 85%. The apparent specific radioactivity was 280 Ci/mmol (13% of the carrier-free specific activity).

Radiosynthesis of [^{125}I]1. A 0.05 M NaOH (1 μL) solution containing 210 μCi of carrier -free Na^{125}I (PerkinElmer) was added to a mixture made of **1** trimethylstannyl precursor (ERAS Labo, 100 μg) in a mixture containing EtOH (10 μL), glacial acetic acid (5 μL), and 30% hydrogen peroxide solution (5 μL). After an incubation at room temperature for 20 min, HPLC phase (MeCN/water 30/70, 10 mM H_3PO_4 350 μL) was added and [^{125}I]**1** was isolated by an isocratic HPLC using a Bondclone C18 (10 μm, 300 × 7.8 mm, Phenomenex) column at 3 mL min^{-1}. The apparent specific activity was determined thanks to a calibration curve measured using growing amounts of the cold reference compound and to UV monitoring during the HPLC purification step. After collection, pooling, and evaporation of the corresponding fractions, the overall radiochemical yield of iodination with ^{125}I was 70%. The apparent specific radioactivity was 146 Ci/mmol (6.6% of the carrier-free specific activity).

In Vitro Competition Experiments. For each in vitro autoradiographic experiment, frozen rat brain coronal sections at the level of the striatum and accumbens nucleus were thawed and dried, preincubated in 50 mM Tris-HCl pH = 7.4 for 5 min at room temperature, and then incubated in the same buffer with given concentrations of the radioligand for 30 min at room temperature. During this step, competition experiments were done by incubating the sections with 100 pM of [^{125}I]**1** and various concentrations of **11d**. After incubation, sections were washed three times for 5 min in ice-cold buffer, dipped in ice-cold deionized water, and rapidly dried under a cold air stream. The labeled sections were exposed on phosphorimaging plates overnight before detection with a Fujifilm BAS scanner.

In Vivo Competition Experiments. For the in vivo competition experiment, a mouse was injected intravenously with a saline solution containing a dose of 50 μg/kg of **11d** and 30 μCi [^{125}I]**1** (146 Ci/mmol specific activity, 6.6% of the carrier free). The animal was then sacrificed with CO_2, and 20-μm-thin cryosections were collected in 5HT$_4$R-rich regions (striatum, accumbens nucleus, olfactive tubercles), and finally exposed overnight on phosphorimaging plates before detection with a Fujifilm BAS scanner.

Molecular Modeling Study. *Receptor Model.* First, the sequence of the human 5-HT$_4$R was retrieved from the UniProt Knowledgebase

(UniProtKB)[47] (ID: Q712M9_HUMAN). Using screening methods like FUGUE,[48] SP3,[49] PSIBLAST,[50,51] HHSEARCH,[52] and the @ tome-2 server,[53] the β2 adrenergic receptor has been identified as the best 3D experimental template for the homology modeling of the 5-HT_4R (sequence identity = 40%). The high-resolution (2.4 Å) crystal structure of the human β2 adrenergic receptor (β2AR)-T4 lysozyme fusion protein bound to the carazolol (PDB ID: 2RH1)[33] was used as the 3D template. The alignment between the two sequences was manually optimized to avoid insertions and deletions in secondary structure elements (see Supporting Information for final sequence alignment). The disulfide bond C93−C184 between the transmembrane helix 3 (TM3) and the extracellular loop (ECL2) was conserved. This alignment was used as the basis for the homology modeling with the Modeler software.[54] The resulting model was then evaluated by methods like verify3D[55] and Eval23D.[56]

Docking Studies. The docking of the compounds into the 5-HT_4R was carried out with the GOLD program (v 5.0) using the default parameters.[57,58] This program applies a genetic algorithm to explore conformational spaces and ligand binding modes. To evaluate the proposed ligand positions, the ChemScore fitness function was applied in these docking studies. The binding site in the 5-HT_4R model was defined as a 10 Å sphere centered on the aspartic acid residue Asp_{100}. Because the mutagenesis studies have shown that the interaction between the positively ionizable amine of ligands and Asp_{100} of 5-HT_4R is crucial for ligand binding, a hydrogen bond constraint between positively ionizable amine ligand and OD atom of Asp_{100} was used during the docking.[36] Furthermore, special attention was paid during the docking procedure to the following amino acids in the binding site, which were kept flexible: Arg_{96}, Asp_{100}, Thr_{104}, Tyr_{192}, Ser_{197}, and Trp_{294}. For **4v** docking, a second hydrogen bond constraint between the tricycle nitrogen and the hydroxyl group of Tyr_{192} was added. For the two crystal conformers available for each compound, a dozen separate docking procedures were carried out and analyzed.

■ ASSOCIATED CONTENT

Supporting Information

X-ray crystallographic data of compounds **4c**, **4d**, **4g**, **4h**, **4j**, **4m**, **4p**, **4q**, **4r**, **4v**, and **11d** and amino acid sequences alignment of 5-HT_4R and human β2-adrenergic receptor. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +33(0)231566808. E-mail: frederic.fabis@unicaen.fr.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the National Institute of Mental Health's Psychoactive Drug Screening Program, Contract #HHSN-271-2008-00025-C (NIMH PDSP) which generously provided K_i determinations and functional assays. The NIMH PDSP is Directed by Bryan L. Roth MD, Ph.D. at the University of North Carolina at Chapel Hill and Project Officer Jamie Driscol at NIMH, Bethesda, MD. We thank Aurélien Lesnard for helpful management of biological results. This work was in part supported by the Swiss National Science Foundation (Grant No. 310030_120369).

■ REFERENCES

(1) Dumuis, A.; Bouhelal, R.; Sebben, M.; Bockaert, J. A 5-HT receptor in the central nervous system, positively coupled with adenylate cyclase, is antagonized by ICS 205930. *Eur. J. Pharmacol.* **1988**, *146*, 187−188.

(2) Dumuis, A.; Bouhelal, R.; Sebben, M.; Cory, R.; Bockaert, J. A non-classical 5-hydroxytryptamine receptor positively coupled with adenylate cyclase in the central nervous system. *Mol. Pharmacol.* **1988**, *34*, 880−887.

(3) Bureau, R.; Boulouard, M.; Dauphin, F.; Lezoualc'h, F.; Rault, S. Review of 5-HT_4R ligands: State of arts and clinical applications. *Curr. Top. Med. Chem.* **2010**, *10*, 527−553.

(4) Lucas, G. Serotonin receptors type 4: A new hope? *Curr. Drug Targets* **2009**, *10*, 1085−1095.

(5) Beattie, D. T.; Smith, J. A. M. Serotonin pharmacology in the gastrointestinal tract: A review. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2008**, *377*, 181−203.

(6) Qvigstad, E.; Brattelid, T.; Sjaastad, I.; Andressen, K. W.; Krobert, K. A.; Birkeland, J. A.; Sejersted, O. M.; Kaumann, A. J.; Skomedal, T.; Osnes, J. B.; Levy, F. O. Appearance of a ventricular 5-HT4 receptor-mediated inotropic response to serotonin in heart failure. *Cardiovasc. Res.* **2005**, *65*, 869−878.

(7) Eglen, R. M.; Wong, E. H. F.; Dumuis, A.; Bockaert, J. Central 5-HT_4 receptors. *TIPS* **1995**, *16*, 391−398.

(8) Matsumoto, M.; Togashi, H.; Mori, K.; Hueno, K.; Ohashi, S.; Kogima, T.; Yoshioka, M. Evidence for involvement of central 5-HT4 receptors in cholinergic function associated with cognitive processes: Behavioural, electrophysiological, and neurochemical studies. *J. Pharmacol. Exp. Ther.* **2001**, *296*, 676−682.

(9) Meneses, A.; Hong, E. Effects of 5-HT_4 receptor agonists and antagonists in learning. *Pharmacol., Biochem. Behav.* **1997**, *56*, 347−351.

(10) Lezoualc'h, F. 5-HT_4 receptor and Alzheimer's disease: The amyloid connection. *Exp. Neurol.* **2007**, *205*, 325−329.

(11) Cho, S.; Hu, Y. Activation of 5-HT_4 receptors inhibits secretion of beta-amyloid peptides and increases neuronal survival. *Exp. Neurol.* **2007**, *203*, 274−278.

(12) Jean, A.; Conductier, G.; Manrique, C.; Bouras, C.; Berta, P.; Hen, R.; Charnay, Y.; Bockaert, J.; Compan, V. Anorexia induced by activation of serotonin 5-HT_4 receptors is mediated by increases in CART in the nucleus accumbens. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 16335−16340.

(13) Lucas, G.; Rymar, W.; Du, J.; Mnie-Filali, O.; Bisgaard, C.; Manta, S.; Lambas-Senas, L.; Wiborg, O.; Haddjeri, N.; Pineyro, G.; Sadikot, A. F.; Debonnel, G. Serotonin(4) (5-HT(4)) receptor agonists putative antidepressant with a rapid onset of action. *Neuron* **2007**, *55*, 712−725.

(14) Manabe, N.; Wong, B. S.; Camilleri, M. New generation 5-HT4 receptor agonists: Potential for for treatment of gastrointestinal motility disorders. *Expert Opin. Invest. Drugs* **2010**, *19*, 765−775.

(15) Moser, P. C.;, E.; Bergis, O.; Jegham, S.; Lochead, A.; Duconseille, E.; Terranova, J.-P.; Caille, D.; Berque-Bestel, I.; Lezoualc'h, F.; Fischmeister, R.; Dumuis, A.; Bockaert, J.; George, P.; Soubrie, P.; Scatton, B. SL65.0155, a novel 5-hydroxytryptamine4 receptor partial agonist with potent cognition-enhancing properties. *J. Pharmacol. Exp. Ther.* **2002**, *302*, 731−742.

(16) Mohler, E. G.; Shacham, S.; Noiman, S.; Lezoualc'h, F.; Robert, S.; Gastineau, M.; Rutkowski, J.; Marantz, Y.; Dumuis, A.; Bockaert, J.; Gold, P. E.; Ragozzino, M. E. VRX-03011, a novel 5-HT4 agonist, enhances memory and hippocampal acetylcholine efflux. *Neuropharmacology* **2007**, *53*, 563−573.

(17) Wong, D. F.; Gründer, G.; Brasić, J. R. Brain imaging research: Does the science serve clinical practice? *Int. Rev. Psychiatry* **2007**, *19*, 541−558.

(18) Gibson, R. E.; Burns, H. D.; Hamill, T. G.; Eng, W. S.; Francis, B. E.; Ryan, C. Non-invasive radiotracer imaging as a tool for drug development. *Curr. Radiopharm. Des.* **2000**, *6*, 973−989.

(19) Pike, V. W.; Halldin, C.; Nobuhara, K.; Hiltunen, J.; Mulligan, R. S.; Swahn, C.-G.; Karlsson, P.; Olsson, H.; Hume, S. P.; Hirani, E.; Whalley, J.; Pilowsky, L. S.; Larson, S.; Schnell, P.-O.; Ell, P. J.; Farde, L. Radioiodinated SB 207710 as a radioligand in vivo: Imaging of brain 5-HT4 receptors with SPET. *Eur. J. Nucl. Med. Mol. Imaging* **2003**, *30*, 1520−1528.

(20) Gee, A. D.; Martarello, L.; Passchier, J.; Wishart, M.; Parker, C.; Matthews, J.; Comley, R.; Hopper, R.; Gunn, R. Synthesis and evaluation of [^{11}C]SB207145 as the first in vivo serotonin 5-HT$_4$ receptor radioligand for PET imaging in man. *Curr. Radiopharm.* **2008**, *1*, 110−114.
(21) Kornum, B. R.; Lind, N. M.; Gillings, N.; Marner, L.; Andersen, F.; Knudsen, G. M. *J. Cereb. Blood Flow Metab.* **2009**, *29*, 186−196.
(22) Madsen, K.; Haahr, M. T.; Marner, L.; Keller, S. H.; Baaré, W. F.; Svarer, C.; Hasselbalch, S. G.; Knudsen, G. M. Age and sex effects on 5-HT(4) receptors in the human brain: A [(11)C]SB207145 PET study. *J. Cereb. Blood Flow Metab.* **2011**, *31*, 1475−1481.
(23) Marner, L.; Gillings, N.; Madsen, K.; Erritzoe, D.; Baaré, W. F.; Svarer, C.; Hasselbalch, S. G.; Knudsen, G. M. Brain imaging of serotonin 4 receptors in humans with [11C]SB207145-PET. *Neuroimage* **2010**, *50*, 855−861.
(24) Xu, R.; Hong, J.; Morse, C. L.; Pike, V. W. Synthesis, structure−affinity relationships, and radiolabeling of selective high-affinity 5-HT$_4$ receptor ligands as prospective imaging probes for positron emission tomography. *J. Med. Chem.* **2010**, *53*, 7035−7047.
(25) Hinschberger, A.; Butt, S.; Lelong, V.; Boulouard, M.; Dumuis, A.; Fauphin, F.; Bureau, R.; Pfeiffer, B.; Renard, P.; Rault, S. New benzo[*h*]-1,6-naphthyridine and azepino[3,2-*c*]quinoleine derivatives as selective antagonists of 5-HT$_4$ receptors: Binding profile and pharmacological characterization. *J. Med. Chem.* **2003**, *46*, 138−147.
(26) Lemaitre, S.; Lepailleur, A.; Bureau, R.; Butt-Gueulle, S.; Lelong-Boulouard, V.; Duchatelle, P.; Boulouard, M.; Dumuis, A.; Daveu, C.; Lezoualc'h, F.; Pfeiffer, B.; Dauphin, F.; Rault, S. Novel antagonists of serotonin-4 receptors: Synthesis and biological evaluation of pyrrolothienopyrazines. *Bioorg. Med. Chem.* **2009**, *17*, 2607−2622.
(27) Dubost, E.; Magnelli, R.; Cailly, T.; Legay, R.; Fabis, F.; Rault, S. General method for the synthesis of substituted phenanthridin-6(5*H*)-ones using a KOH-mediated anionic ring closure as the key step. *Tetrahedron* **2010**, *66*, 5008−5016.
(28) See experimental data for details.
(29) Donohue, S. R.; Varnäs, K.; Jia, Z.; Gulyás, B.; Pike, V. W.; Halldin, C. Synthesis and in vitro autoradiographic evaluation of a novel high affinity radioiodinated ligand for imaging brain cannabinoid subtype-1 receptors. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6209−6212.
(30) Kaumann, A. J. Blockade of human atrial 5-HT$_4$ receptors by GR 113808. *Br. J. Pharmacol.* **1993**, *110*, 1172−1174.
(31) Kaumann, A. J.; Gaster, L. M.; King, F. D.; Brown, A. M. Blockade of human atrial 5-HT$_4$ receptors by SB 207710, a selective and high affinity 5-HT4 receptor antagonist. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1994**, *349*, 546−548.
(32) Kenakin, T. Efficacy as a vector: The relative prevalence and paucity of inverse agonism. *Mol. Pharmacol.* **2004**, *66*, 2−11.
(33) Cherezov, V.; Rosenbaum, D. M.; Hanson, M. A.; Rasmussen, S. G.; Thian, F. S.; Kobilka, T. S.; Choi, H. J.; Kuhn, P.; Weis, W. I.; Kobilka, B. K.; Stevens, R. C. High-resolution crystal structure of an engineered human beta2-adrenergic G protein-coupled receptor. *Science* **2007**, *318*, 1258−1265.
(34) Rosenbaum, D. M.; Cherezov, V.; Hanson, M. A.; Rasmussen, S. G.; Thian, F. S.; Kobilka, T. S.; Choi, H. J.; Yao, X. J.; Weis, W. I.; Stevens, R. C.; Kobilka, B. K. GPCR Engineering yields high-resolution structural insights into β2 adrenergic receptor function. *Science* **2007**, *318*, 1266−1273.
(35) Moukhametzianov, R.; Warne, T.; Edwards, P. C.; Serrano-Vega, M. J.; Leslie, A. G.; Tate, C. G.; Schertler, G. F. Two distinct conformations of helix 6 observed in antagonist-bound structures of a beta1-adrenergic receptor. *Proc. Natl, Acad. Sci. U. S. A.* **2011**, *108*, 8228−8232.
(36) Rivail, L.; Giner, M.; Gastineau, M.; Berthouze, M.; Soulier, J.-L.; Fischmeister, R.; Lezoualc'h, F.; Maigret, B.; Sicsic, S.; Berque-Bestel, I. New insights into the human 5-HT4 receptor binding site: Exploration of a hydrophobic pocket. *Br. J. Pharmacol.* **2004**, *143*, 361−370.
(37) Warne, A.; Moukhametzianov, R.; Baker, J. G.; Nehme, R.; Edwards, P. C.; Leslie, A. G. W.; Schertler, G. F. X.; Tate, C. G. The structural basis for agonist and partial agonist action on a β_1-adrenergic receptor. *Nature* **2011**, *469*, 241−244.
(38) Wu, H.; Wacker, D.; Katritch, V.; Mileni, M.; Han, G. W.; Vardy, E.; Liu, W.; Thompson, A. A.; Huang, X. P.; Carroll, F. I.; Mascarella, S. W.; Westkaemper, R. B.; Mosier, P. D.; Roth, B. L.; Cherezov, V.; Stevens, R. C. Structure of the human κ-opioid receptor in complex with JDTic. *Nature* **2012**, *485*, 327−332.
(39) Waterhouse, R. N. Determination of lipophilicity and its use as a predictor of blood brain barrier penetration of molecular imaging agents. *Mol. Imaging Biol.* **2005**, *5*, 376−389.
(40) Bedjeguelal, K.; Rabot, R.; Kaloun El B.; Mayer P.; Marchand A.; Rahier N.; Schambel P.; Bienayme H. Pyrazolopyridine derivatives as anticancer agent. PCT Int. Appl. WO 2011045344 (A1), 2011.
(41) Kristensen, J. L.; Vedsø, V.; Begtrup, M. Synthesis of pentacyclic 13-azadibenzo[*a*,*de*]anthracenes via anionic cascade ring closure. *J. Org. Chem.* **2003**, *68*, 4091−4092.
(42) Heiss, C.; Marzi, E.; Mongin, F.; Schlosser, M. Remote trimethylsilyl groups interfering with the *ortho* deprotonation of fluoroarenes and chloroarenes. *Eur. J. Org. Chem.* **2007**, 669−675.
(43) Kawaksaki, M.; Goto, M.; Kawabata, S.; Kometani, T. The effect of vinyl esters on the enantioselectivity of the lipase-catalysed transesterification of alcohols. *Tetrahedron: Asymmetry* **2001**, *12*, 585−596.
(44) Grossman, C. J.; Kilpatrick, G. J.; Bunc, K. T. Development of a radioligand binding assay for 5-HT$_4$ receptors in guinea pig and rat brain. *Br. J. Pharmacol.* **1993**, *109*, 618−624.
(45) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265−275.
(46) Allen, J. A.; Yost, J. M.; Setola, V.; Chen, X.; Sassano, M. F.; Chzn, M.; Peterson, S.; Yadav, P. N.; Huang, X. P.; Feng, B.; Jensen, N. H.; Che, X.; Bai, X.; Frye, S. V.; Wetsel, W. C.; Caron, M. G.; Javitch, J. A.; Roth, B. L.; Jin, J. Discovery of β-arrestin-biased dopamine D$_2$ ligandsfor probing signal transduction pathways essential for antipsychotic efficacy. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 18488−18493.
(47) Jain, E.; Bairoch, A.; Duvaud, S.; Phan, I.; Redaschi, N.; Suzek, B. E.; Martin, M. J.; McGarvey, P.; Gasteiger, E. Infrastructure for the life sciences: Design and implementation of the UniProt website. *BMC Bioinf.* **2009**, *10*, 136−155.
(48) Shi, J.; Blundell, T. L.; Mizuguchi, K. FUGUE: Sequence-structure homology recognition using environment-specific substitution tables and structure-dependent gap penalties. *J. Mol. Biol.* **2001**, *310*, 243−257.
(49) Zhou, H.; Zhou, Y. Fold recognition by combining sequence profiles derived from evolution and from depth-dependent structural alignment of fragments. *Proteins* **2005**, *58*, 321−328.
(50) Altschul, S. F.; Madden, T. L.; Schäffer, A. A.; Zhang, J.; Zhang, Z.; Miller, W.; Lipman, D. J. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res.* **1997**, *25*, 3389−3402.
(51) Schäffer, A. A.; Aravind, L.; Madden, T. L.; Shavirin, S.; Spouge, J. L.; Wolf, Y. I.; Koonin, E. V.; Altschul, S. F. Improving the accuracy of PSI-BLAST protein database searches with composition-based statistics and other refinements. *Nucleic Acids Res.* **2001**, *29*, 2994−3005.
(52) Soding, J. Protein homology detection by HMM−HMM comparison. *Bioinformatics* **2005**, *21*, 951−960.
(53) Pons, J. L.; Labesse, G. @TOME-2: A new pipeline for comparative modeling of protein−ligand complexes. *Nucleic Acids Res.*, **2009**, *37*, Web Server issue W485−W491.
(54) Eswar, N.; Eramian, D.; Webb, B.; Shen, M.; Sali, A. Protein structure modeling with MODELLER. *Methods Mol. Biol.* **2008**, *426*, 145−159.
(55) Eisenberg, D.; Lüthy, R.; Bowie, J. U. VERIFY3D: Assessment of protein models with three-dimensional profiles. *Methods Enzymol.* **1997**, *277*, 396−404.

(56) Gracy, J.; Chiche, L.; Sallantin, J. Improved alignment of weakly homologous protein sequences using structural information. *Protein Eng.* **1993**, *6*, 821−829.
(57) Jones, G.; Willett, P.; Glen, R. C. Molecular recognition of receptor sites using a genetic algorithm with a description of desolvation. *J. Mol. Biol.* **1995**, *245*, 43−53.
(58) Jones, G.; Willett, P.; Glen, R. C.; Leach, A. R.; Taylor, R. Development and validation of a genetic algorithm for flexible docking. *J. Mol. Biol.* **1997**, *267*, 727−748.