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Synthesis and Structure-Affinity Relationships of Selective High-Affinity 5-HT₄ Receptor Antagonists: Application to the Design of New Potential Single Photon Emission Computed Tomography (SPECT) Tracers

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Abstract

The work described herein aims at finding new potential ligands for the brain imaging of 5-HT₄ receptors using single-photon emission computed tomography (SPECT). Starting from the non-substituted phenanthridine compound **4a** exhibiting a K_i value of 51 nM on 5-HT₄R, we explored 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 increase both the affinity and the selectivity for 5-HT₄R 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-HT₄R antagonist (1-butylpiperidin-4-yl)methyl-8-amino-7-iodo[¹²³I]-2,3-dihydrobenzo[*b*][1,4]dioxine-5-carboxylate ([¹²³I]**1**, [¹²³I]SB 207710) both *in vitro* and *in vivo* in brain. Compound **11d** was radiolabeled with [¹²⁵I]iodine, providing a potential SPECT candidate for brain imaging of 5-HT₄R.

INTRODUCTION

Since its discovery more than two decades ago,^{1,2} the serotonin 4 receptor subtype (5-HT₄R) has emerged as a promising target for drug discovery, development and for medical applications.^{3,4} Pharmacological investigations coupled to the discovery of ligands exhibiting high affinity and selectivity for 5-HT₄R have led to know their anatomical distribution and functional roles. 5-HT₄R are found in the peripheral system, where they are implicated in gastrointestinal disorders⁵ and heart failures⁶. Brain 5-HT₄R 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

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X-ray crystallographic data of compounds **4c**, **4d**, **4g**, **4h**, **4j**, **4m**, **4p**, **4q**, **4r**, **4v** and **11d**. Amino-acid sequences alignment of 5-HT₄R and human β₂-adrenergic receptor. This material is available free of charge via the Internet at <http://pubs.acs.org>.

agonists and/or antagonists have shown that 5-HT₄Rs are implicated in cognition,⁸ learning and memory processes,⁹ and more recently in neuropsychiatric disorders such as Alzheimer's disease,^{10,11} food intake¹² and depression.¹³ Although efforts have been made by both academics and pharmaceutical companies to develop 5-HT₄R ligands with potential medical applications, only peripheral agonists have yet reached the market for gastrointestinal disorders.¹⁴ Brain 5-HT₄ receptor ligands have entered clinical trials for the treatment of Alzheimer Disease but failed in phase IIb.^{15,16} Discovery of active 5-HT₄Rs 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 non-invasive 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[¹²³I]-2,3-dihydrobenzo[*b*][1,4]dioxine-5-carboxylate ([¹²³I]**1**, [¹²³I]SB 207710)¹⁹ and (1-methyl[¹¹C]piperidin-4-yl)methyl-8-amino-7-chloro-2,3-dihydrobenzo[*b*][1,4]dioxine-5-carboxylate ([¹¹C]**2**, [¹¹C]SB 207145)²⁰ have been described as potential radiotracers for respectively PET or SPECT imaging (Chart 1). [¹¹C]**2** has been successfully used in minipig for the determination of radioligand metabolism and binding kinetics.²¹ Further studies have shown that this radiotracer can be used for quantitative PET measurements of 5-HT₄R 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 which could make them new promising 5-HT₄R PET radiotracers.²⁴ Among them, fluorinated compounds could serve as PET radiotracers of longer half-life. [¹²³I]**1** is to date the only SPECT tracer reported for brain imaging of 5-HT₄R, 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-HT₄R radioligands for SPECT imaging. Based on previous works in the lab concerning the synthesis and evaluation of 5-HT₄R 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-HT₄R antagonists among which some iodinated compounds were identified and successfully radiolabeled with ¹²⁵I, representing new potential radioligands for SPECT imaging studies of the 5-HT₄R (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 procedure²⁸ (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-trimethylsilylfluorobenzene 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).

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 PPh₃ in toluene.²⁹ Radioiodination from the stannylated compounds **12a,b,d** was performed using Na¹²⁵I as the source of radioactive iodine H₂O₂ (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-HT₄R binding affinity and functional assays

Twenty six compounds (**4a-v**, **11a-d**) were initially screened for their affinity toward 5-HT₄R in *Guinea Pig* striatal membranes at 10⁻⁶ and 10⁻⁸M. Twenty compounds were selected for K_i determination in 5-HT₄R *Guinea Pig* striatal membranes. These ligands show K_i values between 2.2 and 691 nM. Among them, ten were chosen for human 5-HT₄R K_i determination and except **4t**, all ligands showed better affinity for *h*-5-HT₄R compared to guinea pig 5-HT₄R, 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-HT₄R were screened towards other 5-HTR subtypes. Results are shown in Table 5.

Discussion

Unlike PET imaging using ¹⁸F 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 non radioactive ligand for pharmacological studies and the precursor for the introduction of ¹²³I *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 dramatic decrease in affinity due to its large atomic radius.

Starting from the unsubstituted phenanthridine **4a** which exhibited good 5-HT₄R 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, 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 position 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, 22 and 20 nM K_i values respectively for guinea pig 5-HT₄R, against human 5-HT₄R they showed significant 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-HT₄R and remarkably this compound exhibited a very high affinity on human 5-HT₄R 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-HT₄R 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-HT₄R binding affinity. Despite differences between fluorine and iodine in terms of size and electronic properties, **11a** and **11b** exhibited K_i values of 13 and 4.6 nM for guinea pig 5-HT₄R and respectively, 1.2 and 0.26 nM for human 5-HT₄R 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-HT₄R 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-HT₄R ($K_i = 2.5$ nM) and human 5-HT₄R ($K_i = 0.23$ nM) in 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-HT₄R ligands **4k-m**, **4u-v**, **11a-b** and **11d** were evaluated for both intrinsic activity and selectivity toward other 5-HTRs (Table 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 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 the 5-HT_{2B}R for which compounds **4k-m** exhibited almost the same potency as for 5-HT₄R. Interestingly, the additional nitrogen atom in position 10 not only led to increased affinities for 5-HT₄R 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-HT₄R 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-HT₄ receptor was constructed using the β_2 -adrenergic receptor crystal structure, one of the available GPCR structures that exhibit a sequence identity of about 40% with the 5-HT₄R.³³ The two crystallographic conformers of compound **4m** were docked into the 5-HT₄R model (see supporting information). In the selected pose (Figure 1A, mean Chem Score Fit =

31.18), the basic piperidine nitrogen interacted with Asp₁₀₀ (consistent with the constraint used during the docking) and an additional polar interaction could form through this basic nitrogen and the Tyr₃₀₂ 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 beta 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 (Phe₁₈₆, Tyr₁₉₂, Phe₂₇₅, Phe₂₇₆), 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 Ser₁₉₇ hydroxyl group as seen in Figure 1B (mean ChemScore Fit = 33.10). While this additional interaction could explain the increased affinity for the 5-HT₄R 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 Ser₁₉₇ is present in both the 5-HT₄ 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: Tyr₁₉₂(5-HT₄R)/Phe₂₁₇(5-HT_{2B}R), Cys₁₉₆(5-HT₄R)/Gly₂₂₁(5-HT_{2B}R) and Ala₁₉₃(5-HT₄R)/Met₂₁₈(5-HT_{2B}R). We reasoned that the supplementary nitrogen atom in **4v** could interact with the hydroxyl group of the 5-HT₄R Tyr₁₉₂. 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 Tyr₁₉₂ 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 Tyr₁₉₂ as set during the docking and was located far from the Ser₁₉₇. Furthermore, in this docking pose, the fluorine, the N-6 nitrogen atom and the oxygen atom were placed near to the 5-HT₄R Trp₂₉₄ (TM7), so that electrostatic interactions can occur with this amino acid. Binding interactions with this Trp₂₉₄ has been previously shown to be essential for some 5-HT₄R 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 co-crystallized extended ligands such as the full agonist carmoterol in β_1 -adrenergic receptor³⁷ or for the antagonist JD_{Tic} 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-HT₄R specific binding capacity, *in vitro* competition experiments with the selective and specific antagonist radioligand [¹²⁵I]**1** were performed. Increasing concentrations of **11d** co-administered with [¹²⁵I]**1** show a decrease in the 5-HT₄R-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 experiment was performed *ex vivo*. The specific binding of [¹²⁵I]**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 co-injection 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 [¹²⁵I]**1** both *in vitro* and *in vivo* at very low concentration; 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-¹²⁵I 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 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-HT₄ receptors. Starting from a phenanthridine scaffold we designed new antagonists exhibiting high affinity and selectivity toward this receptor. The fluorinated compounds **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-HT₄R. 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-HT₄R 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 radiolabelled with [¹²⁵I] and owing to their favourable 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 discs using a PerkinElmer BX-FT-IR. ¹H and ¹³C NMR spectra were recorded on a Jeol Lambda 400 spectrometer with chemical shifts expressed in parts per million (in DMSO-*d*₆ or CDCl₃). 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 held for 3 min before return to initial conditions in 1 min. Initial conditions were then maintained for 5 min (A: H₂O, B: MeCN; each containing HCOOH: 0.1%; 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_α radiation (λ=0.71073 Å). The structures were solved using direct methods and refined by full-matrix least-squares analysis on *I*². Program(s) used to solve structures: SHELXS–97 (Sheldrick). Program(s) used to refine structures: SHELXL–97. Software used to prepare material for publication: SHELXL–97 (Sheldrick). 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 or <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)nicotinonitrile⁴⁰ (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. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.28 (m, 1H), 7.49 (m, 1H), 7.70 (dd, 1H, ³*J* = 7.8 Hz, ³*J* = 3.9 Hz), 8.43 (d, 1H, ³*J* = 7.8 Hz), 8.68 (dd, 1H, ³*J* = 7.8 Hz, ⁴*J* = 1.9 Hz), 9.07 (d, 1H, ³*J* = 3.9 Hz), 11.87 (brs, 1H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 116.5 (d, ²*J* = 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, ²*J* = 14 Hz), 135.9, 149.4 (d, ¹*J* = 243 Hz), 149.9 (d, *J* = 3 Hz), 154.3, 160.7. HRMS (EI) calcd. for C₁₂H₇FN₂O 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 POCl₃ (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 (3 times). The organic phase was dried with MgSO₄, 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 (3 times), dried over MgSO₄, filtered, evaporated and purified by silica gel chromatography.

6-(1-Propylpiperidin-4-yl)methoxyphenanthridine **4a**

Starting from **3a** (213 mg, 1.1 mmol) using general procedure A and cyclohexane/ethyl acetate 8/2 with 5% of NEt₃ 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. ¹H NMR (400 MHz, CDCl₃) δ 0.91 (t, 3H, ³*J* = 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, ³*J* = 6.0 Hz), 7.47 (ddd, 1H, ³*J* = 8.0 Hz, ³*J* = 6.8 Hz, ⁴*J* = 1.2 Hz), 7.59–7.64 (m, 2H), 7.80 (ddd, 1H, ³*J* = 8.4 Hz, ³*J* = 7.2 Hz, ⁴*J* = 1.6 Hz), 7.86 (dd, 1H, ³*J* = 8.4 Hz, ⁴*J* = 1.6 Hz), 8.38 (dd, 1H, ³*J* = 8.0 Hz, ⁴*J* = 1.2 Hz), 8.40–8.42 (m, 1H), 8.48–8.51 (m, 1H). ¹³C NMR (100 MHz, CDCl₃) 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 C₂₂H₂₆N₂O 334.2046, found 334.2046.

2-Fluoro-6-(1-propylpiperidin-4-yl)methoxyphenanthridine **4b**

Starting from **3b** (180 mg, 0.84 mmol), using general procedure A and cyclohexane/ethyl acetate 7/3 with 5% of NEt₃ 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. ¹H NMR (400 MHz, CDCl₃) 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, ³*J* = 6.1 Hz), 7.33 (ddd, 1H, ³*J* = 8.8 Hz, ³*J* = 8.0 Hz, ⁴*J* = 2.8 Hz), 7.64 (ddd, 1H, ³*J* = 8.0 Hz, ³*J* = 6.8 Hz, ⁴*J* = 1.2 Hz), 7.77 (ddd, 1H, ³*J* = 8.0 Hz, ³*J* = 7.2 Hz, ⁴*J* = 1.6 Hz), 7.81 (dd, 1H, ³*J* = 8.8 Hz, ³*J* = 5.2 Hz), 7.98 (dd, 1H, ³*J* = 10.0 Hz, ⁴*J* = 2.8 Hz), 8.33 (d, 1H, ³*J* = 8.4 Hz), 8.36 (dd, 1H, ³*J* = 8.0 Hz, ⁴*J* = 1.2 Hz). ¹³C NMR (100 MHz, CDCl₃) δ 12.1, 20.2, 29.3 (2C), 35.9, 53.6 (2C), 61.2, 70.5, 107.1 (d, ²*J* = 23 Hz), 116.9 (d, ²*J* = 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* =

4Hz), 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. 1H NMR (400 MHz, $CDCl_3$) δ 0.92 (t, 3H, $^3J = 6.8$ Hz), 1.50-1.61 (m, 4H), 1.91-2.02 (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. 1H 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. 1H 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. 1H 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. 1H 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. 1H 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. 1H 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. 1H 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,

CDCl₃) 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 C₂₃H₂₈N₂O 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 NEt₃ 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. ¹H NMR (400 MHz, CDCl₃) 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). ¹³C NMR (100 MHz, CDCl₃) 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 C₂₃H₂₈N₂O₂ 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 NEt₃ 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. ¹H NMR (400 MHz, CDCl₃) 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). ¹³C NMR (100 MHz, CDCl₃) 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 C₂₃H₂₆ClN₂O [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 NEt₃ 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. ¹H NMR (400 MHz, CDCl₃) 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). ¹³C NMR (100 MHz, CDCl₃) 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, ²J = 4 Hz), 120.3, 122.2, 123.8 (d, ²J = 8 Hz), 124.5 (d, ²J = 2 Hz), 125.2, 127.7, 131.2, 132.7 (d, ²J = 11 Hz), 134.2 (d, ²J = 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 C₂₂H₂₅FN₂O 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-carbonitrile⁴¹ (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. ¹H NMR (400 MHz, CDCl₃) 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). ¹³C NMR (100 MHz, CDCl₃) 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 C₂₂H₂₅FN₂O 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. ¹H NMR (400 MHz, CDCl₃) 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). ¹³C NMR (100 MHz, CDCl₃) 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 C₂₂H₂₅N₃O₃ 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. ¹H NMR (400 MHz, CDCl₃) 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). ¹³C NMR (100 MHz, CDCl₃) 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 C₂₃H₂₈N₂O₂ 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. ¹H NMR (400 MHz, CDCl₃) 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). ¹³C NMR (100 MHz, CDCl₃) 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 C₂₂H₂₅FN₂O 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. ¹H NMR (400 MHz, CDCl₃) 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). ¹³C NMR (100 MHz, CDCl₃) 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 C₂₃H₂₈N₂O 348.2201, found 348.2202.

9-Fluoro-6-(1-propylpiperidin-4-yl)methoxyphenanthridine 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 recrystallisation 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. ¹H NMR (400 MHz, CDCl₃) 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). ¹³C NMR (100 MHz, CDCl₃) 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 C₂₂H₂₅FN₂O 352.1950, found 352.1938.

5-(1-Propylpiperidin-4-yl)methoxybenzo[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. ¹H NMR (400 MHz, CDCl₃) 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, ³J = 11.7 Hz), 4.51 (d, 2H, ³J = 6.5 Hz), 7.55 (t, 1H, ³J = 7.6 Hz), 7.69 (t, 1H, ³J = 7.5 Hz), 7.90 (d, 1H, ³J = 8.3 Hz), 8.12 (d, 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). ¹³C NMR (100 MHz, CDCl₃) 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 C₂₁H₂₆N₃O [M+H]⁺ 336.2076, found 336.2067.

5-(1-Propylpiperidin-4-yl)methoxybenzo[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 NEt₃ 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. ¹H NMR (400 MHz, CDCl₃) 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, ³J = 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). ¹³C NMR (100 MHz, CDCl₃) 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 C₂₁H₂₅N₃O 335.1997, found 335.1998.

7-Fluoro-5-(1-propylpiperidin-4-yl)methoxybenzo[*h*]-1,6-naphthyridine 4v

Starting from **3v** (1 g, 4.6 mmol), following general procedure A and using ethyl acetate and ethyl acetate/NEt₃ 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. ¹H NMR (400 MHz, CDCl₃) δ 0.91 (t, 3H, ³*J* = 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, ³*J* = 6.8 Hz), 7.41-7.49 (m, 2H), 7.59 (dd, 1H, ³*J* = 8.3 Hz, ³*J* = 4.8 Hz), 8.64 (dd, 1H, ³*J* = 7.8 Hz, ⁴*J* = 1.9 Hz), 8.72 (dd, 1H, ³*J* = 8.3 Hz, ⁴*J* = 1.9 Hz), 9.11 (dd, 1H, ³*J* = 4.8 Hz, ⁴*J* = 1.9 Hz). ¹³C NMR (100 MHz, CDCl₃) δ 12.1, 20.2, 29.2 (2C), 35.8, 53.6 (2C), 61.2, 71.1, 115.6, 115.6 (d, ²*J* = 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, ²*J* = 11 Hz), 150.3 (d, *J* = 3 Hz), 153.3, 157.0 (d, ¹*J* = 250 Hz), 158.6. LC-MS (ESI): t_R = 6.00 min; [M+H]⁺: 354.37. HRMS/EI calcd. for C₂₁H₂₄FN₃O 353.1903, found 353.1915.

2-Fluoro-3-(trimethylsilanyl)phenylboronic acid 5

In a three necks round-bottom flask under N₂ at -80 °C were introduced (2-fluorophenyl)trimethylsilane⁴² (7.4 g, 44 mmol), THF (90 mL) and *s*-BuLi 1.3 M 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 stirred again for 0.75 h. The mixture was allowed to reach room temperature, hydrolyzed with water (200 mL), washed with Et₂O (3 × 150 mL), acidified to pH = 1 using HCl 1 M and extracted with Et₂O (3 × 150 mL). The combined organic layers were dried over MgSO₄, 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. ¹H NMR (400 MHz, CDCl₃) δ 0.32 (s, 9H), 5.29 (s, 1H), 5.31 (s, 1H), 7.19 (td, 1H, ³*J* = 7.2 Hz, *J* = 1.5 Hz), 7.52 (m, 1H), 7.84 (td, 1H, ³*J* = 7.2 Hz, *J* = 1.6 Hz). ¹³C NMR (100 MHz, CDCl₃) δ -1.0, 124.3 (d, ⁴*J* = 2 Hz), 125.6 (d, ²*J* = 36 Hz), 138.2 (d, ³*J* = 7 Hz), 138.8 (d, ³*J* = 13 Hz), 172.7 (d, ¹*J* = 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 N₂, were introduced DME (15 mL) and water (15 mL). The solution was degazed by bubbling N₂ for 15 min and Pd(OAc)₂ (62 mg, 0.27 mmol) and PPh₃ (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 Na₂CO₃ (2.33 g, 22 mmol) were added. The solution was heated to 90 °C for 14h, cooled to room temperature, filtered over a pad of celite and extracted with EtOAc (3 × 50 mL). The combined organic layers were dried over MgSO₄, 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. ¹H NMR (400 MHz, CDCl₃) δ 0.35 (s, 9H), 7.25 (t, 1H, ³*J* = 7.8 Hz), 7.40-7.53 (m, 4H), 7.65 (t, 1H, ³*J* = 7.8 Hz), 7.77 (d, 1H, ³*J* = 7.8 Hz). ¹³C NMR (100 MHz, CDCl₃) δ -1.0, 112.9, 118.1, 124.1 (d, *J* = 3 Hz), 125.0 (d, ²*J* = 19 Hz), 127.5 (d, ³*J* = 36 Hz), 127.9, 130.9, 132.4, 132.4, 133.2, 136.1 (d, *J* = 13 Hz), 140.1, 163.5 (d, ¹*J* = 242 Hz). HRMS/EI calcd. for C₁₆H₁₆FNSi 269.1036, found 269.1034.

2-(2-Fluoro-3-trimethylsilanylphenyl)nicotinonitrile 6b

Same procedure as for **6a**, starting from 2-chloronicotinonitrile (1 g, 7.2 mmol), Pd(OAc)₂ (81 mg, 0.36 mmol), PPh₃ (189 mg, 0.72 mmol), **5** (2.29 g, 10.8 mmol) and Na₂CO₃ (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. ¹H NMR (400 MHz, CDCl₃) 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 $\text{C}_{15}\text{H}_{15}\text{FN}_2\text{Si}$ 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 $\text{Na}_2\text{S}_2\text{O}_3$ 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. ^1H 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 $\text{C}_{13}\text{H}_7\text{FIN}$ 322.9607, found 322.9601.

2-(2-Fluoro-3-iodophenyl)nicotinonitrile **7b**

Same procedure as for **7a**, 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. ^1H 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 $\text{C}_{12}\text{H}_6\text{FIN}_2$ 323.9568, found 323.9559.

4-Iodophenanthridin-6(5H)-one **8a**

Same procedure as for compound **3v**, using **7a** (1.11 g, 3.4 mmol), KOH (0.96 g, 14 mmol) and *t*-BuOH (30 mL), 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. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) 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, $\text{DMSO}-d_6$) 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 $\text{C}_{13}\text{H}_8\text{INO}$ 320.9651, found 320.9665.

7-Iodobenzo[*h*]-1,6-naphthyridin-5(6H)-one **8b**

Same procedure as for compound **3v**, using **7b** (0.7 g, 2.16 mmol), KOH (0.6 g, 10.8 mmol) and *t*-BuOH (35 mL), 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. ^1H 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 $\text{C}_{12}\text{H}_7\text{IN}_2\text{O}$ 321.9603, found 321.9592.

Ethyl 1-[3-(4-iodophenyl)propanoyl]piperidine-4-carboxylate 9

To a solution of 4-iododihydrocinamic acid⁴³ (5 g, 18.11 mmol) in DCM (50 mL) was added at room temperature HOBt (3.67 g, 27.17 mmol), EDCI (5.21 g, 27.17 mmol), NEt₃ (3.79 mL, 27.17 mmol) and ethyl isonipecotatate (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. ¹H NMR (400 MHz, CDCl₃) 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). ¹³C NMR (100 MHz, CDCl₃) 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 C₁₇H₂₂INO₃ 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 DIBALH 1M 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 MgSO₄, 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. ¹H NMR (400 MHz, CDCl₃) 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). ¹³C NMR (100 MHz, CDCl₃) 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 C₁₅H₂₂INO 359.0746, found 359.0759.

4-Iodo-6-(1-propylpiperidin-4-yl)methoxyphenanthridine 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. ¹H NMR (400 MHz, CDCl₃) 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, ³J = 7.8 Hz), 3.02 (d, 2H, ³J = 10.7 Hz), 4.63 (d, 2H, ³J = 6.8 Hz), 7.19 (t, 1H, ³J = 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). ¹³C NMR (100 MHz, CDCl₃) 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 C₂₂H₂₅IN₂O 460.1012, found 460.1008.

7-Iodo-5-(1-propylpiperidin-4-yl)methoxybenzo[*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 NEt₃ 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. ¹H NMR (400 MHz, CDCl₃) 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, ³J = 8.7 Hz), 4.63 (d, 2H, ³J = 5.8 Hz), 7.27 (t, 1H, ³J = 7.8 Hz), 7.59 (dd, 1H, ³J = 7.8 Hz, ⁴J = 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). ¹³C NMR (100 MHz, CDCl₃) 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 C₂₁H₂₄IN₃O 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. ¹H NMR (400 MHz, CDCl₃) 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, ³J = 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). ¹³C NMR (100 MHz, CDCl₃) 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 C₂₈H₃₀IN₂O 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. ¹H NMR (400 MHz, CDCl₃) 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, ³J = 7.8 Hz, ⁴J = 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). ¹³C NMR (100 MHz, CDCl₃) 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, ²J = 7 Hz), 125.4 (d, ²J = 1 Hz), 130.5 (2C), 133.1, 134.1 (d, ²J = 11 Hz), 137.2 (2C), 141.8, 150.2 (d, ²J = 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 C₂₇H₂₈FIN₃O 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 PPh₃ (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 Et₂O and Et₂O/NEt₃ (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. ¹H NMR (400 MHz, CDCl₃) δ 0.85 (t, 6H, ³J = 6.3 Hz), 0.92 (t, 6H, ³J = 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, ³J = 9.7 Hz), 2.06 (t, 1H, ³J = 11.2 Hz), 2.36 (t, 2H, ³J = 7.8 Hz), 3.05 (d, 2H, ³J = 10.7 Hz), 4.48 (d, 2H, ³J = 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, ³J = 7.8 Hz), 8.50 (d, 1H, ³J = 8.7 Hz). ¹³C NMR (100 MHz, CDCl₃) 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 C₃₄H₅₃N₂OSn 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. ^1H 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 $\text{C}_{33}\text{H}_{52}\text{N}_3\text{OSn}$ 626.3133, found 626.3136.

7-Fluoro-5-{1-[3-(4-tributylstannylphenyl)propyl]piperidin-4-yl}methoxybenzo[*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. ^1H NMR (400 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 $\text{C}_{39}\text{H}_{55}\text{FN}_3\text{OSn}$ 720.3353, found 720.3356.

Pharmacological Assay and Screen

Binding of all compounds to native 5-HT₄R 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 sec), and ultracentrifugation (23,000 $\times g$, 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 Lowry⁴⁵ using bovine serum albumin as standard. For radioligand binding studies, 600 μg of membrane were incubated in duplicate at 37 °C for 30 min with [^3H]GR 113808 (Perkin Elmer), 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-pres soaked 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: 6 concentrations of [^3H]GR 113808 were used to give final concentrations of 0.02–0.8 nM, non-specific binding of [^3H]GR 113808 was defined in the presence of 30 μM serotonin to determine the K_d and the B_{max} . For competition studies, [^3H]GR 113808 was used to give a final concentration of 0.1 nM. Percentages of inhibition of the binding of [^3H]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 5-point inhibition curves using the EBDA-Ligand software, and expressed as $K_i \pm \text{SD}$.

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-HT₄ receptors and to other serotonin receptors (5-HT_{1A-E}, 5-HT_{2A-C}, 5-HT₃, 5-HT_{5A}, 5-HT₆, 5-HT₇). For *h*5-HT₄R K_i determinations, [^3H]GR 113808 was used

as hot ligand and GR 113808 as reference. 5-HT₄R membrane was made with HEK T cells transiently transfected with human 5-HT₄ DNA. Binding protocol is the same as 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-HT₄R 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-HT₄ and other serotonin receptors (5-HT_{1A-E}, 5-HT_{2A-C}, 5-HT₃, 5-HT_{5A}, 5-HT₆, 5-HT₇) 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

0.05 M NaOH solution containing 165 µCi of carrier free Na¹²⁵I (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 pH5 - 350 µL) was added and **13a** was isolated by an isocratic HPLC using a Bondclone C18 10 µm 300X7.8 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

0.05 M NaOH solution containing 128 µCi of carrier free Na¹²⁵I (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 pH5 - 350 µL) was added and **13b** was isolated by an isocratic HPLC using a Bondclone C18 10 µm 300X7.8 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

0.05 M NaOH solution containing 210 µCi of carrier free Na¹²⁵I (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 pH5 - 350 µL) was added and **13d** was isolated by an isocratic HPLC using a Bondclone C18 10 µm 300X7.8 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

0.05 M NaOH (1 μ L) solution containing 210 μ Ci of carrier free Na 125 I (PerkinElmer) was added to a mixture made of **1** trimethylstannyl precursor (ERAS Labo, France 100 μ g) in a mixture containing EtOH (10 μ L), glacial acetic acid (5 μ L) and 30% hydrogen peroxide solution (5 μ L). After an incubation at room temperature for 20 min, HPLC phase (MeCN/water 30/70, 10 mM H₃PO₄ 350 μ L) was added and [125 I]1 was isolated by an isocratic HPLC using a Bondclone C18 10 μ m 300X7.8 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 minutes in ice-cold buffer, dipped in ice-cold deionised water, and rapidly dried under a cold air stream. The labelled sections were exposed on phosphorimaging plates overnight before detection with 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 μ g/kg of **11d** and 30 μ Ci [125 I]1 (146 Ci/mmol specific activity, 6.6% of the carrier free). The animal was then sacrificed with CO₂ and 20 μ m thin cryosections were collected in 5HT₄R-rich regions (striatum, accumbens nucleus, olfactory tubercles), and finally exposed overnight on phosphorimaging plates before detection with Fujifilm BAS scanner.

Molecular Modeling study

Receptor Model—First, the sequence of the human 5-HT₄R was retrieved from the [UniProt Knowledgebase \(UniProtKB\)](#)⁴⁷ (ID : Q712M9_HUMAN). Using screening methods like FUGUE,⁴⁸ SP3,⁴⁹ PSIBLAST,^{50,51} HHSEARCH⁵² and the @tome-2 server⁵³, the β 2 adrenergic receptor has been identified as the best 3D experimental template for the homology modeling of the 5-HT₄R (Sequence identity = 40%). The high-resolution (2.4 Å) crystal structure of the human β 2 adrenergic receptor (β 2AR)-T4 lysozyme fusion protein bound to the carazolol (PDB: 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 info for final sequence alignment). 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 Modeller software.⁵⁴ The resulting model was then evaluated by methods like verify3D⁵⁵ and Eval23D.⁵⁶

Docking studies—The docking of the compounds into the 5-HT₄R was carried out with the GOLD program (v5.0) 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-HT₄R model was defined as a 10 Å sphere centred on the aspartic acid residue Asp₁₀₀. Because the mutagenesis studies have shown that the interaction between the positively ionisable amine of ligands and Asp₁₀₀ of 5-HT₄R is crucial for ligand binding, a hydrogen bond constraint between positively ionisable amine ligand and OD atom of Asp₁₀₀ 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: Arg₉₆, Asp₁₀₀, Thr₁₀₄, Tyr₁₉₂, Ser₁₉₇ and Trp₂₉₄. For **4v** docking a second hydrogen bond constraint between the tricycle nitrogen and the hydroxyle group of Tyr₁₉₂ was added. For the two crystal conformers available for each compound, a dozen separate docking procedures were carried out and analyzed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

- 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. [PubMed: 3350057]
- 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. [PubMed: 2849052]
- Bureau R, Boulouard M, Dauphin F, Lezoualc'h F, Rault S. Review of 5-HT₄R ligands: state of arts and clinical applications. *Curr. Top. Med. Chem.* 2010; 10:527–553. [PubMed: 20166943]
- Lucas G. Serotonin receptors type 4: a new hope ? *Curr. Drug Targets.* 2009; 10:1085–1095. [PubMed: 19702554]
- Beattie DT, Smith JAM. Serotonin pharmacology in the gastrointestinal tract: a review. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 2008; 377:181–203.
- Qvigstad E, Brattelid T, Sjaastad I, Andressen KW, Krobert KA, Birkeland JA, Sejersted OM, Kaumann AJ, Skomedal T, Osnes JB, Levy FO. Appearance of a ventricular 5-HT₄ receptor-mediated inotropic response to serotonin in heart failure. *Cardiovasc. Res.* 2005; 65:869–878. [PubMed: 15721867]
- Eglen RM, Wong EHF, Dumuis A, Bockaert J. Central 5-HT₄ receptors. *TIPS.* 1995; 16:391–398. [PubMed: 8578609]
- 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. [PubMed: 11181892]
- Meneses A, Hong E. Effects of 5-HT₄ receptor agonists and antagonists in learning. *Pharmacol., Biochem. Behav.* 1997; 56:347–351. [PubMed: 9077568]
- Lezoualc'h F. 5-HT₄ receptor and Alzheimer's disease: the amyloid connection. *Exp. Neurol.* 2007; 205:325–329. [PubMed: 17346704]
- Cho S, Hu Y. Activation of 5-HT₄ receptors inhibits secretion of beta-amyloid peptides and increases neuronal survival. *Exp. Neurol.* 2007; 203:274–278. [PubMed: 16978609]

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-HT₄ receptors is mediated by increases in CART in the nucleus accumbens. *Proc. Natl. Acad. Sci. USA*. 2007; 104:16335–16340. [PubMed: 17913892]
13. Lucas G, Rymar W, Du J, Mnie-Filali O, Bisgaard C, Manta S, Lambas-Senas L, Wiborg O, Haddjeri N, Pineyro G, Sadikot AF, Debonnel G. Serotonin(4) (5-HT₄) receptor agonists putative antidepressant with a rapid onset of action. *Neuron*. 2007; 55:712–725. [PubMed: 17785179]
14. Manabe N, Wong BS, Camilleri M. New generation 5-HT₄ receptor agonists: potential for treatment of gastrointestinal motility disorders. *Expert Opin. Investig. Drugs*. 2010; 19:765–775.
15. Moser PC, 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. [PubMed: 12130738]
16. Mohler EG, Shacham S, Noiman S, Lezoualc'h F, Robert S, Gastineau M, Rutkowski J, Marantz Y, Dumuis A, Bockaert J, Gold PE, Ragozzino ME. VRX-03011, a novel 5-HT₄ agonist, enhances memory and hippocampal acetylcholine efflux. *Neuropharmacology*. 2007; 53:563–573. [PubMed: 17692343]
17. Wong DF, Gründer G, Brasi JR. Brain imaging research: does the science serve clinical practice? *Int. Rev. Psychiatry*. 2007; 19:541–558. [PubMed: 17896234]
18. Gibson RE, Burns HD, Hamill TG, Eng WS, Francis BE, Ryan C. Non-invasive radiotracer imaging as a tool for drug development. *Curr. Radiopharm. Des*. 2000; 6:973–989.
19. Pike VW, Halldin C, Nobuhara K, Hiltunen J, Mulligan RS, Swahn C-G, Karlsson P, Olsson H, Hume SP, Hirani E, Whalley J, Pilowsky LS, Larson S, Schnell P-O, Ell PJ, 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. [PubMed: 14579092]
20. Gee AD, Martarello L, Passchier J, Wishart M, Parker C, Matthews J, Comley R, Hopper R, Gunn R. Synthesis and evaluation of [¹¹C]SB207145 as the first in vivo serotonin 5-HT₄ receptor radioligand for PET imaging in man. *Curr. Radiopharm*. 2008; 1:110–114.
21. Kornum BR, Lind NM, Gillings N, Marner L, Andersen F, Knudsen GM. *J. Cereb. Blood Flow Metab*. 2009; 29:186–196. [PubMed: 18797470]
22. Madsen K, Haahr MT, Marner L, Keller SH, Baaré WF, Svarer C, Hasselbalch SG, Knudsen GM. Age and sex effects on 5-HT₄ receptors in the human brain: a [(11)C]SB207145 PET study. *J. Cereb. Blood Flow Metab*. 2011; 31:1475–1481. [PubMed: 21364600]
23. Marner L, Gillings N, Madsen K, Erritzoe D, Baaré WF, Svarer C, Hasselbalch SG, Knudsen GM. Brain imaging of serotonin 4 receptors in humans with [11C]SB207145-PET. *Neuroimage*. 2010; 50:855–861. [PubMed: 20096787]
24. Xu R, Hong J, Morse CL, Pike VW. Synthesis, Structure-Affinity Relationships, and Radiolabeling of Selective High-Affinity 5-HT₄ Receptor Ligands as Prospective Imaging Probes for Positron Emission Tomography. *J. Med. Chem*. 2010; 53:7035–7047. [PubMed: 20812727]
25. Hirschberger 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*]quinoline Derivatives as selective Antagonists of 5-HT₄ Receptors: Binding Profile and Pharmacological Characterization. *J. Med. Chem*. 2003; 46:138–147. [PubMed: 12502367]
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. [PubMed: 19261477]
27. Dubost E, Magnelli R, Cailly T, Legay R, Fabis F, Rault S. General method for the synthesis of substituted phenanthridin-6(*5H*)-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 SR, Varnäs K, Jia Z, Gulyás B, Pike VW, 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. [PubMed: 19767206]
30. Kaumann AJ. Blockade of human atrial 5-HT₄ receptors by GR 113808. *Br. J. Pharmacol.* 1993; 110:1172–1174. [PubMed: 8298805]
31. Kaumann AJ, Gaster LM, King FD, Brown AM. Blockade of human atrial 5-HT₄ receptors by SB 207710, a selective and high affinity 5-HT₄ receptor antagonist. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 1994; 349:546–548. [PubMed: 7915008]
32. Kenakin T. Efficacy as a vector: the relative prevalence and paucity of inverse agonism. *Mol. Pharmacol.* 2004; 66:2–11. [PubMed: 14722230]
33. Cherezov V, Rosenbaum DM, Hanson MA, Rasmussen SG, Thian FS, Kobilka TS, Choi HJ, Kuhn P, Weis WI, Kobilka BK, Stevens RC. High-resolution crystal structure of an engineered human beta₂-adrenergic G protein-coupled receptor. *Science.* 2007; 318:1258–1265. [PubMed: 17962520]
34. Rosenbaum DM, Cherezov V, Hanson MA, Rasmussen SG, Thian FS, Kobilka TS, Choi HJ, Yao XJ, Weis WI, Stevens RC, Kobilka BK. GPCR Engineering Yields High-Resolution Structural Insights into β_2 Adrenergic Receptor Function. *Science.* 2007; 318:1266–1273. [PubMed: 17962519]
35. Moukhametzianov R, Warne T, Edwards PC, Serrano-Vega MJ, Leslie AG, Tate CG, Schertler GF. Two distinct conformations of helix 6 observed in antagonist-bound structures of a beta₁-adrenergic receptor. *Proc. Natl. Acad. Sci. USA.* 2011; 108:8228–8232. [PubMed: 21540331]
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. [PubMed: 15351779]
37. Warne A, Moukhametzianov R, Baker JG, Nehme R, Edwards PC, Leslie AGW, Schertler GFX, Tate CG. The structural basis for agonist and partial agonist action on a β_1 -adrenergic receptor. *Nature.* 2011; 469:241–244. [PubMed: 21228877]
38. Wu H, Wacker D, Katritch V, Mileni M, Han GW, Vardy E, Liu W, Thompson AA, Huang XP, Carroll FI, Mascarella SW, Westkaemper RB, Mosier PD, Roth BL, Cherezov V, Stevens RC. Structure of the human κ -opioid receptor in complex with JDTic. *Nature.* 2012; 485:327–332. [PubMed: 22437504]
39. Waterhouse RN. 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. [PubMed: 14667492]
40. Bedjeguel 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 JL, Vedsø V, Begtrup M. Synthesis of Pentacyclic 13-Azadibenzo[*a,de*]anthracenes via Anionic Cascade Ring Closure. *J. Org. Chem.* 2003; 68:4091–4092. [PubMed: 12737598]
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. Kawasaki 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 CJ, Kilpatrick GJ, Bunc KT. Development of a radioligand binding assay for 5-HT₄ receptors in guinea pig and rat brain. *Br. J. Pharmacol.* 1993; 109:618–624. [PubMed: 8358562]
45. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 1951; 193:265–275. [PubMed: 14907713]
46. Allen JA, Yost JM, Setola V, Chen X, Sassano MF, Chzn M, Peterson S, Yadav PN, Huang XP, Feng B, Jensen NH, Che X, Bai X, Frye SV, Wetsel WC, Caron MG, Javitch JA, Roth BL, Jin J. Discovery of β -arrestin-biased dopamine D₂ ligands for probing signal transduction pathways essential for antipsychotic efficacy. *Proc. Natl. Acad. Sci. USA.* 2011; 108:18488–18493. [PubMed: 22025698]
47. Jain E, Bairoch A, Duvaud S, Phan I, Redaschi N, Suzek BE, Martin MJ, McGarvey P, Gasteiger E. Infrastructure for the life sciences: design and implementation of the UniProt website. *BMC Bioinformatics.* 2009; 10:136–155. [PubMed: 19426475]

48. Shi J, Blundell TL, Mizuguchi K. FUGUE: sequence-structure homology recognition using environment-specific substitution tables and structure-dependent gap penalties. *J. Mol. Biol.* 2001; 310:243–257. [PubMed: 11419950]
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. [PubMed: 15523666]
50. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucl. Acids Res.* 1997; 25:3389–3402. [PubMed: 9254694]
51. Schäffer AA, Aravind L, Madden TL, Shavirin S, Spouge JL, Wolf YI, Koonin EV, Altschul SF. Improving the accuracy of PSI-BLAST protein database searches with composition-based statistics and other refinements. *Nucl. Acids Res.* 2001; 29:2994–3005. [PubMed: 11452024]
52. Soding J. Protein homology detection by HMM-HMM comparison. *Bioinformatics.* 2005; 21:951–960. [PubMed: 15531603]
53. Pons JL, Labesse G. @TOME-2: a new pipeline for comparative modeling of protein–ligand complexes. *Nucleic Acids Research.* 2009; 37(Web Server issue):W485–W491. [PubMed: 19443448]
54. Eswar N, Eramian D, Webb B, Shen M, Sali A. Protein structure modeling with MODELLER. *Methods Mol. Biol.* 2008; 426:145–159. [PubMed: 18542861]
55. Eisenberg D, Lüthy R, Bowie JU. VERIFY3D: assessment of protein models with three-dimensional profiles. *Methods Enzymol.* 1997; 277:396–404. [PubMed: 9379925]
56. Gracy J, Chiche L, Sallantin J. Improved alignment of weakly homologous protein sequences using structural information. *Protein Eng.* 1993; 6:821–829. [PubMed: 8309929]
57. Jones G, Willett P, Glen RC. Molecular recognition of receptor sites using a genetic algorithm with a description of desolvation. *J. Mol. Biol.* 1995; 245:43–53. [PubMed: 7823319]
58. Jones G, Willett P, Glen RC, Leach AR, Taylor R. Development and validation of a genetic algorithm for flexible docking. *J. Mol. Biol.* 1997; 267:727–748. [PubMed: 9126849]

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

Figure 2.

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

Figure 3.

Ex vivo autoradiogram after intravenous injection of [^{125}I]**1** alone (left) and after co-injection with 50 $\mu\text{g/kg}$ of **11d** to a mouse (right).

Scheme 1.

Synthesis of 4-iodo(aza)phenanthridin-6(5*H*)-ones **8a,b**.^a

^aReagents 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, Na₂CO₃, Pd(OAc)₂, PPh₃, DME/H₂O, 14 h, 90 °C; (iii) ICl, DCM, 4.5 h, rt; (iv) KOH, *t*-BuOH, sealed tube, 1 h, 150 °C.

Scheme 2.

Synthesis of {1-[3-(4-iodophenyl)propyl]piperidin-4-yl}methanol **10**.^a

^aReagents and conditions: (i) Ethyl isonipecotat, HOBt, EDCI, NEt₃, 24 h, rt; (ii) DIBALH, THF, 3 h, -10 °C to rt.

Scheme 3. Preparation of stannylated precursors **12a,b,d** and ^{125}I labeling.^a^aReagents and conditions: (i) $(\text{Bu}_3\text{Sn})_2$, $\text{Pd}(\text{OAc})_2$, PPh_3 , $\text{PhMe}/\text{H}_2\text{O}$, 16 h, 90 °C. (ii) Na^{125}I , 30% H_2O_2 , EtOH/AcOH , rt, 20 min.

Previous Work

This Work

Chart 1.
Literature and prospective 5-HT₄R radiotracers.

Table 1

Synthesis of (aza)phenanthridines **4a-v**.^a

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

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^aReagents and conditions: (i) POCl₃, 80 °C, overnight; (ii) (1-propylpiperidin-4-yl)methanol, NaH, DMF, 0 °C to rt, overnight.

^bIsolated yields.

^cYield over 3 steps see experimental data for details.

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) POCl₃, 80 °C, overnight; (ii) 1-propylpiperidin-4-ylmethanol or **10**, NaH, DMF, 0 °C to rt, overnight.

^b Isolated yields.

Table 3

Binding affinities of new 5-HT₄R ligands.

compd	R	X	Y	Z	% inh (10 ⁻⁶ M/10 ⁻⁸ M) ^a	5-HT ₄ K _i (nM) ^b	h5-HT ₄ K _i (nM) ^c
4a	H	CH	CH	H	100/11	51.5	n.m. ^d
4b	2-F	CH	CH	H	47/0	n.m.	n.m.
4c	2-Cl	CH	CH	H	27/0	n.m.	n.m.
4d	2-Me	CH	CH	H	88/7	246	n.m.
4e	2-MeO	CH	CH	H	83/9	233	n.m.
4f	3-Cl	CH	CH	H	85/0	n.m.	n.m.
4g	3-MeO	CH	CH	H	n.m.	1900	n.m.
4h	3-Me	CH	CH	H	65/30	691	n.m.
4i	3-F	CH	CH	H	100/14	101	33.0 ^e
4j	4-Me	CH	CH	H	100/0	100	n.m.
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	n.m.
4o	8-NO ₂	CH	CH	H	54/0	n.m.	n.m.
4p	8-MeO	CH	CH	H	85/0	n.m.	n.m.
4q	8-F	CH	CH	H	90/4	154	n.m.
4r	9-Me	CH	CH	H	28/0	n.m.	n.m.
4s	9-F	CH	CH	H	84/0	209	n.m.

compd	R	X	Y	Z	% inh (10 ⁻⁶ M/10 ⁻⁸ M) ^a	5-HT ₄ K _i (nM) ^b	h5-HT ₄ K _i (nM) ^c
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	n.m.
11d	4-F	N	CH	4-iodophenyl	100/94	2.5	0.23 ^f

^a Inhibition percentages were determined using guinea pig striatal membrane 5-HT₄R.

^b Guinea pig striatal membrane 5-HT₄R (n=3).

^c Human 5-HT₄R (n=3).

^d n.m. = not measured.

^e K_i determinations performed at NIMH-PDSP – USA.

^f K_i determinations performed at CEREP- France. See experimental section for details.

Table 4
Intrinsic activity and cLogD of 5-HT₄R ligands **4k**, **4l**, **4m**, **4u**, **4v**, **11a**, **11b** and **11d**.

	4k	4l	4m	4u	4v	11a	11b	11d
h5-HT ₄	17	5.0	3.1	7.5	0.04	1.20	0.26	0.23
Ki(nM)								
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

^aFunctional assays performed at NIMH-PDSP – USA. Inv Ag = inverse agonist.

^bFunctional assays performed at CEREP – France. Antag = Antagonist. See experimental section for details.

^cCalculated LogD (pH = 7.4) using MarvinSketch 5.2.6.

Table 5
Binding affinities of compounds **4k**, **4l**, **4m**, **4u**, **4v**, **11a**, **11b** and **11d** toward 5-HT receptors

5-HT _R	4k	4l	4m	4u	4v	11a	11b	11d
h5-HT ₄	17	5.0	3.1	7.5	0.04	1.20	0.26	0.23
h5-HT _{1A}	> 10 ⁴	8836	> 10 ⁴	> 10 ⁴	4987	> 10 ⁴	5629	680
h5-HT _{1B}	> 10 ⁴	> 10 ⁴	> 10 ⁴	> 10 ⁴	> 10 ⁴	> 10 ⁴	> 10 ⁴	> 10 ⁴
h5-HT _{1D}	2569	2360	> 10 ⁴	> 10 ⁴	> 10 ⁴	> 10 ⁴	> 10 ⁴	> 10 ⁴
h5-HT _{1E}	> 10 ⁴	> 10 ⁴	> 10 ⁴	> 10 ⁴	> 10 ⁴	> 10 ⁴	> 10 ⁴	n.m.
h5-HT _{2A}	5962	1780	> 10 ⁴	> 10 ⁴	> 10 ⁴	1019	1825	810
h5-HT _{2B}	29.3	98.0	40.4	627	136	162	> 10 ⁴	340
h5-HT _{2C}	> 10 ⁴	729	1049	2793	492	772	341	400
h5-HT ₃	> 10 ⁴	2470	710	680	641	1314	> 10 ⁴	410
h5-HT _{5A}	> 10 ⁴	> 10 ⁴	> 10 ⁴	> 10 ⁴	> 10 ⁴	5805	> 10 ⁴	1700
h5-HT ₆	> 10 ⁴	6256	> 10 ⁴	> 10 ⁴	> 10 ⁴	> 10 ⁴	> 10 ⁴	> 10 ⁴
h5-HT ₇	3473	1914	384	> 10 ⁴	1945	153	1018	150

Selectivity was performed at NIMH-PDSP – USA except for compound **11d** for which the selectivity was performed at CEREP – France.