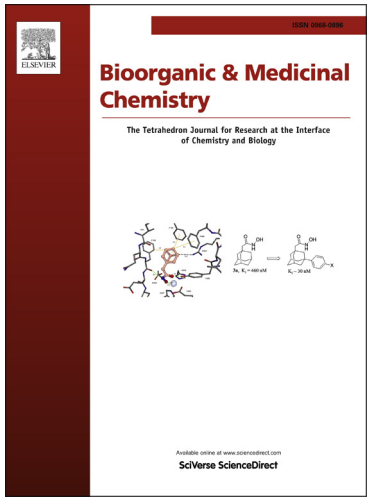


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## Graphical Abstract

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**Design of fluorinated 5-HT$_4$R antagonists: influence of the basicity and lipophilicity toward the 5-HT$_4$R binding affinities**

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# Design of fluorinated 5-HT$_4$R antagonists: influence of the basicity and lipophilicity toward the 5-HT$_4$R binding affinities

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ABSTRACT

Analogues of potent 5-HT$_4$R antagonists possessing a fluorinated *N*-alkyl chain have been synthesized in order to investigate the effect of the resulting change in basicity and lipophilicity on the affinity and selectivity profile. We demonstrate that for this series, the affinity is decreased with decreased basicity of the piperidine's nitrogen atom. In contrast, the resulting increase in lipophilicity has minimal impact on binding affinity and selectivity. 3,3,3-Trifluoropropyl and 4,4,4-trifluorobutyl derivatives **6d** and **6e** have shown to bind to the 5-HT$_4$R while maintaining their pharmacological profile and selectivity toward other 5-HT receptors.



## 1. Introduction

The serotonin 5-HT$_4$ receptors (5-HT$_4$R) constitute an important subtype of the seven serotonin (5-HT) receptors expressed in the central nervous system (CNS). Since its discovery in 1988,[1] intense efforts from both academia and pharmaceutical industry have led to the discovery of ligands exhibiting high affinity and selectivity for 5-HT$_4$Rs. These ligands, coupled with effective pharmacological evaluations, have led to a better knowledge of the anatomical distribution and functional roles of these receptors. Activation of the 5-HT$_4$R *in vivo* causes the production of cyclic adenosine mono phosphate (cAMP) *via* a coupling with adenylate cyclase,[2] thus promoting activation of $Ca^{2+}$ channels,[3] inhibition of $K^+$ channels[4] and activation of the rap1-rac pathway.[5] The 5-HT$_4$Rs can be found in the peripheral system where they have been shown to be implicated in gastrointestinal disorders[6] and heart failures.[7]

Brain 5-HT$_4$Rs are mainly expressed in striatum, globus pallidus, nucleus accumbens and substantia nigra.[8] Pharmacological studies of central 5-HT$_4$Rs using selective agonists and/or antagonists have shown that 5-HT$_4$Rs are implicated in cognition,[9] learning and memory processes,[10] and more recently in neuropsychiatric disorders such as Alzheimer's disease,[11,12] food intake[13] and depression.[14]

Our group has been involved in the design of 5-HT$_4$R antagonists as potential single photon emission computed tomography (SPECT) radiotracers.[15] Indeed, as the discovery of active 5-HT$_4$Rs agonists and antagonists remains of great interest in clinical research, molecular imaging techniques using positron emission tomography (PET) or SPECT have emerged as valuable tools in both clinical studies and drug discovery programs.[16,17,18] Despite these imaging techniques which have found applications in diagnosis, imaging of neurotransmitter receptors, *in vivo* binding studies of new ligands and establishing treatment strategies, research remains strongly hampered by the low availability of suitable radioligands. In CNS, one of the reasons for the difficulty to develop suitable radiotracers is the requirement to cross the blood brain barrier (BBB). BBB passage has been shown to be facilitated by low molecular weight (<500 Da), small cross-sectional area (<80Å$^2$), low hydrogen-bonding capacity, lack of formal charge and mainly a moderate lipophilicity ($2.0<\text{Log}D_{7.4}<3.5$).[19] Furthermore, CNS receptors (radio)ligands once they have passed through the BBB, must not be the substrate of brain efflux transporters such as the P-

[d] These authors contributed equally to this work.

glycoprotein 1 (P-gp) in order to reach efficiently their target *in vivo*. Affinity for P-gp is known to be promoted by high lipophilicity, positive charge at pH 7.4 and multiple aromatic groups.[19a]

With the above in mind, our investigation related to 5-$HT_4$R brain (radio)ligands was extended to investigate the influence of modification of basicity on affinity, selectivity and pharmacological profile of 5-$HT_4$R antagonists **1**–**3** we have previously described (Figure 1), *via* fluorination of their *n*-propyl groups.[15] Fluorination of alkyl chains nearby amine functional groups is a well-known approach to decrease its basicity,[20,21] and is also expected to increase the ligand lipophilicity.[21] This is confirmed by the calculated p$K_{a(H)}$ and log*D* values (see below). Moreover, there is significant precedence that shows how fluorination of related 5-HTR ligands, all containing basic nitrogen, improves pharmacokinetic parameters while maintaining activity.[22]

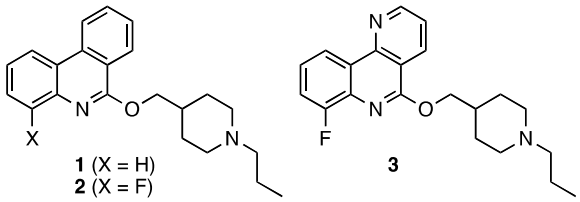
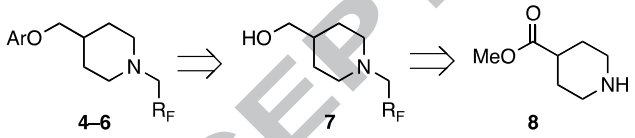



**Figure 1.** Previously described 5-$HT_4$R antagonists. $K_i$ 5-$HT_4$R (guinea pig): **1**, 51.5 nM ; **2**, 20.1 nM and **3**, 2.2 nM.

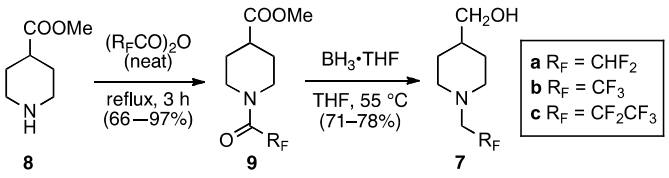
Hence, the evaluation of fluorinated analogues **4**–**6** was proposed (Scheme 1), incorporating the three aromatic groups shown above, each with an *N*-alkyl lateral chain ($R_F$) containing a varying degree/relative position of the fluorination. The synthesis of **4**–**6** was envisioned by conventional nucleophilic aromatic substitution of **7** to the appropriate aromatic electrophile, with **7** to be obtained from methyl isonipecotate **8**. The introduction of the fluorinated substituent was investigated by either an acylation or alkylation strategy.




**Scheme 1.** Fluorinated target molecules **4**–**6**, with retrosynthetic analysis leading to methyl isonipecotate **8**.

## 2. Chemistry

Acylation of methyl isonipecotate **8** to give the corresponding amide **9**, followed by reduction, was deemed the most convenient way to introduce the fluorinated side chain. The reaction of **8** with various commercially available anhydrides gave **9** in good to excellent yield. It was found that prior purification of **8** facilitated the handling of the compound: reaction of **8** with carbon dioxide leads to a gummy solid. In addition, overnight reflux was required to achieve complete conversion. The lower yield of **9b** (66%) was surprising, but trifluoromethylation of the isonipecotic acid with trifluoroacetic anhydride yielded the corresponding amide in 88% yield (not shown).[23] In contrast, reaction of **8** with ethyl pentafluoroacetate as reagent, with DMAP as catalyst, only led to **9c** in 42% yield.[24]




**Scheme 2.** Synthesis of precursors **7** by acylation and reduction.

NMR spectroscopic studies on **9a**-**c** showed that all hydrogen and carbon atoms on the piperidine ring were inequivalent. Indeed, IR spectroscopic analysis indicated that the delocalisation of the nitrogen lone pair (leading to a resonance structure such as shown in Figure 2) is more extensive for the fluorinated amides compared to a non-fluorinated amide, as shown by the higher wavenumbers for the fluorinated amides **9a**–**c** (**9a,** $\nu$ = 1665 $cm^{-1}$; **9b**, $\nu$ = 1687 $cm^{-1}$; **9c**, $\nu$ = 1677 $cm^{-1}$) compared to a nonfluorinated propionamide derivative of isonipecotic ester[15] ($\nu$ = 1644 $cm^{-1}$). Interestingly, one of the piperidine carbon atoms adjacent to nitrogen appeared as a multiplet (Figure 2), as a result of the long-range $^4J_{C\text{-}F}$ fluorine-carbon coupling. Furthermore, a $^1$H-$^{19}$F HOESY experiment demonstrated that the fluorinated groups were close to only one of the two equatorial hydrogen atoms on the carbons adjacent to nitrogen atom (Figure 2). Correspondingly, $^1$H {$^{19}$F} NMR showed a simplified peak for this hydrogen atom, indicating the long-range fluorine-hydrogen coupling. Similar couplings and assignments were reported for dimethyl monofluoro- and trifluoroacetamides.[25]

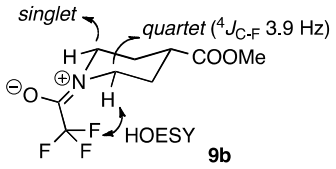
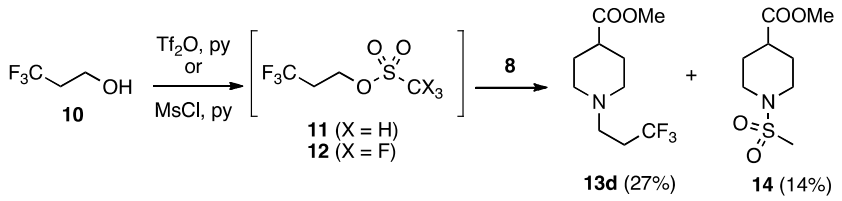


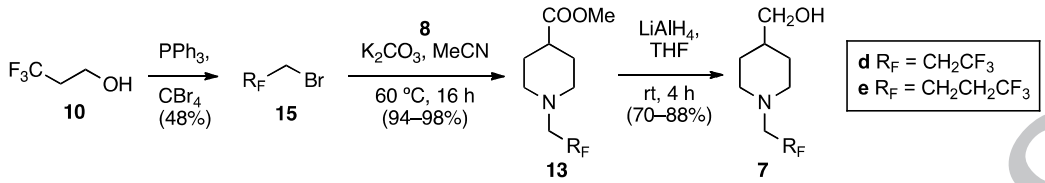

**Figure 2.** Mesomeric form of **9b** and HOESY coupling

The reduction of **9a**-**c** with $BH_3$•THF led, to our surprise, directly to **7a**-**c**, with both the amide and the ester groups converted to the amine and alcohol respectively (Scheme 2). Given ester groups are generally unreactive towards $BH_3$, it was assumed that after amide reduction a reactive boron oxide intermediate was formed, which enabled reduction of the methyl ester. Alternatively, the borane reactivity may have been increased *via* coordination with the piperidine nitrogen, facilitating intramolecular hydride delivery. A similar double reduction was also reported by Jiang *et al*.[26]

The required anhydrides for the synthesis of **7d**,**e** were not commercially available, and the synthesis of **7d** was first tried with the corresponding acid chloride, DMAP and triethylamine.[27] However, the reaction led to a complex mixture. A number of alkylation reactions were then investigated. Reaction of methyl isonipecotate **8** with triflate **12**, synthesized *in situ* from 3,3,3-trifluoropropan-1-ol and trifluoromethanesulfonic anhydride with pyridine,[28] also led to a complex mixture. However, the reaction of two equivalents of freshly purified **8** with the mesylate **11** for nearly three days did give the product, but in low yield (27%). Interestingly, a sulfonamide by-product **14** was isolated that originated from nucleophilic attack of the amine at the sulfur atom, illustrating the deactivating effect of a trifluoromethyl group towards $S_N2$ reactions.[29]




**Scheme 3. Synthesis of 13d *via* alkylation with 3,3,3-trifluoropropyl sulfonates**

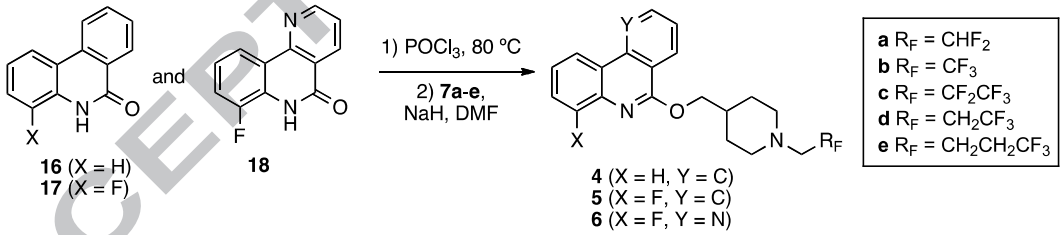



**Scheme 4.** Synthesis of **7d,e** *via* an alkylation strategy

Hence, the alkylation was attempted using the corresponding bromides (Scheme 4). 1-Bromo-3,3,3-trifluoropropane **15d** was prepared *via* an Appel reaction from **10**, while 1-bromo-4,4,4-trifluorobutane **15e** was commercially available. Reaction of either bromide with **8** proceeded in excellent yield, and ester reduction with $LiAlH_4$[30] then gave the remaining precursors **7d**,**e**.

Finally, reaction of the fluoroalkylpiperidines **7a-e** with (aza)phenanthridinones **16**–**18** according to a known procedure,[15,31,32] afforded phenanthridines **4** and **5**, and benzonaphthyridines **6** (Table 1). Initially, a side reaction was observed during the $S_NAr$ reaction in which the strong base initiated HF elimination in the side chain, as evidenced by the appearance of characteristic peaks of an alkene function in both $^1H$ and $^{19}F$ NMR spectra. However, this process could be eliminated by reducing the concentration of the reaction.

**Table 1.** Synthesis of substituted 6-(1-fluoroalkylpiperidin-4-yl)methyloxy(aza)phenanthridines **4-6**



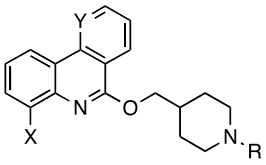

| Starting material | Product | Yield (%)[a] | Starting material | Product | Yield (%)[a] |
|---|---|---|---|---|---|
| **16** | **4a** | 80 | **17** | **5d** | 54 |
| **16** | **4b** | 68 | **17** | **5e** | 41 |
| **16** | **4c** | 50 | **18** | **6a** | 41 |
| **17** | **5a** | 79 | **18** | **6d** | 44 |
| **17** | **5b** | 69 | **18** | **6e** | 67 |
| **17** | **5c** | 71 | | | |

[a]Isolated yield.

### 3. Basicity and lipophilicity

For all the synthesized compounds **4**–**6**, as well as for the corresponding non-fluorinated compounds **1**–**3**, the $pK_{a(H)}$ and log *D* values were calculated. The data are given in Table 2, and represented according to relative basicity. The reduction in $pK_{a(H)}$ with the position of the fluorination (and number of fluorine atoms) is as expected, except for the values of **4b** and **5b**, where the calculated values were lower than expected: measured literature values for closely related compounds show a similar influence of a trifluoromethyl and pentafluoroethyl group on amine basicity ($pK_{a(H)}$ of $Me_2NCH_2CF_3$ and $Me_2NCH_2CF_2CF_3$ are 4.75 and 5.0 respectively).[33]

**Table 2.** Acidity and lipophilicity data



**1**,**4** (X = H, Y = C)
**2**,**5** (X = F, Y = C)
**3**,**6** (X = F, Y = N)

| R | p$K_{a(H)}$[a] | | | clog$D$[b] | | | $R_f$[c] |
|---|---|---|---|---|---|---|---|
| $CH_2CH_2CH_3$ | **1** (9.70) | **2** (9.70) | **3** (9.68) | **1** (2.65) | **2** (2.79) | **3** (1.98) | |
| $CH_2CH_2CH_2CF_3$ | - | **5e** (8.74) | **6e** (8.72) | - | **5e** (4.34) | **6e** (3.53) | **5e** (0.13) |
| $CH_2CH_2CF_3$ | - | **5d** (8.31) | **6d** (8.29) | - | **5d** (4.22) | **6d** (3.38) | **5d** (0.31) |
| $CH_2CHF_2$ | **4a** (6.06) | **5a** (6.06) | **6a** (6.04) | **4a** (4.49) | **5a** (4.63) | **6a** (3.80) | **5a** (0.36) |
| $CH_2C_2F_5$ | **4c** (5.12) | **5c** (5.12) | - | **4c** (5.70) | **5c** (5.84) | | **5c** (0.63) |
| $CH_2CF_3$ | **4b** (3.34) | **5b** (3.22) | - | **4b** (5.00) | **5b** (5.14) | | **5b** (0.54) |

[a]Calculated p*K*a of the piperidine nitrogen using MarvinSketch 5.2.6. [b]Calculated Log*D* at pH = 7.4 using MarvinSketch 5.2.6. [c] $R_f$ values determined on a $SiO_2$ plate with an ethyl acetate/hexane mixture (15:85) as eluent.

The calculated log*D* values (pH 7.4) show that fluorination causes a large jump in lipophilicity, even for the 4,4,4-trifluorobutyl group. It is interesting to note that compounds with a 3,3,3-trifluoropropyl substituent are somewhat less lipophilic than these having a 4,4,4-trifluorobutyl group, despite their lower p$K_{a(H)}$ value (compare **5e** with **5d**, and **6e** with **6d**). As expected, the pentafluoroethyl substituent leads to a larger lipophilicity than the trifluoromethyl group (compare **4c** with **4b**, and **5c** with **5b**).

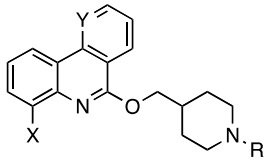
Interestingly, the decrease in polarity and basicity due to the fluorination clearly manifested itself in their retention time on normal phase silica gel. With an aprotic eluent (ethyl acetate/ hexane), a significant difference in retention time was observed, with the $R_f$ value reflecting the basicity of the compounds (reduced interaction of the less basic amine with silica gel), and a superposing influence of lipophilicity. With a protic eluent (isopropanol/hexane), the range in retention factors was much reduced (0.26–0.34), while the overall elution order was still maintained (see supporting information).

### 4. 5-$HT_4$R binding affinity, functional assays and 5-HTRs binding profile

The compounds **4-6** were first screened for their relative inhibition toward 5-$HT_4$R in *Guinea Pig* striatal membranes at $10^{-6}$ and $10^{-8}$ M (Table 3, col A), and compared to their parent nonfluorinated compounds **1**–**3** (entries 1, 5, 11). Compared to **1** (entry 1), compounds **4a**–**c** (entries 2-4) show a significantly reduced affinity with **4a**, which has the least electron withdrawing side chain, being the least affected. Evidently, this shows that the protonated form is crucial for binding, as fluorination close to the amine will result in a decrease of the p*K*a of the conjugate acid of the amine, and hence a decrease of the percentage of the protonated form at physiological pH.

The correlation of declining affinity with electron withdrawing character of the side chain effect was also seen for the other compounds. Compared to **2** (entry 5), **5a**–**c**, with fluorination at the β-position, showed no binding at $10^{-8}$ M, but affinity was restored for **5d**, and to a certain extent **5e**. Compared to **3** (entry 11), all compounds **6a**,**d**,**e** showed slightly reduced affinity (entries 12-14).

**Table 3:** Binding affinities of new 5-$HT_4$ ligands



**1**,**4** (X = H, Y = C)
**2**,**5** (X = F, Y = C)
**3**,**6** (X = F, Y = N)

| Entry | Compd | R | % inh[a] ($10^{-6}$M/ $10^{-8}$M) | 5-$HT_4$[b] $K_i$ (nM) | h5-$HT_4$[c] $K_i$ (nM) |
|---|---|---|---|---|---|
| 1 | **1** | $CH_2CH_2CH_3$ | 100/11 | 51.5 | n.m.[d] |
| 2 | **4a** | $CH_2CHF_2$ | 58/0 | n.m. | n.m. |
| 3 | **4b** | $CH_2CF_3$ | 1/0 | n.m. | n.m. |
| 4 | **4c** | $CH_2CF_2CF_3$ | 1/0 | n.m. | n.m. |
| 5 | **2** | $CH_2CH_2CH_3$ | 100/63 | 20.1 | 3.1 |
| 6 | **5a** | $CH_2CHF_2$ | 93/0 | n.m. | n.m. |
| 7 | **5b** | $CH_2CF_3$ | 3/0 | n.m. | n.m. |
| 8 | **5c** | $CH_2CF_2CF_3$ | 4/0 | n.m. | n.m. |
| 9 | **5d** | $CH_2CH_2CF_3$ | 100/61 | 22.0 | n.m. |
| 10 | **5e** | $CH_2CH_2CH_2CF_3$ | 100/49 | 14.1 | n.m. |
| 11 | **3** | $CH_2CH_2CH_3$ | 100/94 | 2.2 | 0.04 |
| 12 | **6a** | $CH_2CHF_2$ | 100/26 | 91.4 | n.m. |
| 13 | **6d** | $CH_2CH_2CF_3$ | 100/80 | 9.0 | 0.11 |
| 14 | **6e** | $CH_2CH_2CH_2CF_3$ | 100/80 | 4.2 | 0.20 |

[a]Inhibition percentages were determined using guinea pig striatal membrane 5-$HT_4$R. [b]Guinea pig striatal membrane 5-$HT_4$R (n=3). [c]Human 5-$HT_4$R (n=3), $K_i$ determinations performed at CEREP- France. [d]n.m. = not measured.

Five compounds (**5d, 5e, 6a, 6d, 6e**) were selected for $K_i$ determination in 5-$HT_4$R Guinea Pig striatal membranes (Table 3, col B). Interestingly, compound **5e** (14.1 nM) showed a slightly better affinity compared to its parent **2** (20.1 nM; compare entries 10 with 5), and **6e** (4.2 nM), with the same side

chain, showed comparable affinity with **3** (2.2 nM; compare entries 14 with 11).

Next, **6d** and **6e** were selected for human 5-$HT_4$R $K_i$ determination (Table 3, col C). Both showed significantly better affinity for *h*-5$HT_4$R compared to Guinea Pig 5-$HT_4$R ($K_i$ values of 0.11 and 0.20 nM; entries 13,14).

**Table 4:** Intrinsic activity[a] and binding affinities[b] of **6d** and **6e** toward 5-HT receptors

| Entry | | 3[c] | 6d | 6e |
|---|---|---|---|---|
| 1 | $K_B$ | 0.025 | 0.079 | 0.199 |
| 2 | h-5$HT_4$ | 0.04 | 0.11 | 0.20 |
| 3 | h-5$HT_{1A}$ | 4987 | $> 10^4$ | $> 10^4$ |
| 4 | h-5$HT_{1B}$ | $>10^4$ | $> 10^4$ | $> 10^4$ |
| 5 | h-5$HT_{1D}$ | $>10^4$ | $> 10^4$ | $> 10^4$ |
| 6 | h-5$HT_{1E}$ | $>10^4$ | n.m. | n.m. |
| 7 | h-5$HT_{2A}$ | $>10^4$ | $> 10^4$ | 8700 |
| 8 | h-5$HT_{2B}$ | 136 | 230 | 190 |
| 9 | h-5$HT_{2C}$ | 492 | $> 10^4$ | 980 |
| 10 | h-5$HT_3$ | 641 | 1200 | 2600 |
| 11 | h-5$HT_{5A}$ | $>10^4$ | $> 10^4$ | $> 10^4$ |
| 12 | h-5$HT_6$ | $>10^4$ | $> 10^4$ | $> 10^4$ |
| 13 | h-5$HT_7$ | 1945 | $> 10^4$ | $> 10^4$ |

[a]Functional assays performed at CEREP- France. [b]Binding affinities performed at CEREP and expressed as $K_i$ (nM)- France. [c]Results reported in ref 15.

Based on these results, the binding affinities of **6d** and **6e** on a panel of other 5-HT receptors and their pharmacological profile were then determined (Table 4). As for the parent compound **3**, **6d** and **6e** showed a full antagonist profile with $K_B$ values in the same order of magnitude as the $K_i$ (entry 1). Interestingly, both compounds show a very good selectivity overall, and very comparable with the non-fluorinated parent **3**. Pleasingly, the introduction of fluorine atoms on **6d** and **6e**, with the accompanying modification of the lipophilicity and the basicity of the piperidine's nitrogen atom, maintained the affinity, selectivity and pharmacological profile compared to the parent compound **3**.

## 5. Conclusion

Eleven analogues of 5-$HT_4$R antagonists fluorinated on the alkyl lateral chain were synthesized. The results show a reduced affinity toward the 5-$HT_4$ receptor due to the diminished basicity of the piperidine nitrogen atom, while the increased lipophilicity has a lesser impact. Among the synthesized compounds, 3,3,3-trifluoropropyl and 4,4,4-trifluorobutyl derivatives **6d** and **6e**, which nitrogen basicities are the least affected, have kept their affinities toward the 5-$HT_4$R compared to the non-fluorinated ligand **3**. Moreover, their pharmacological profile and selectivity toward other 5-HTRs have also been maintained compared to **3**. Hence we have demonstrated the possibility to modify both basicity and lipophilicity on a 5-$HT_4$R antagonist without disturbing their affinity, selectivity and pharmacological profile, by introduction of fluorine atoms, which will be of interest for the development of compounds targeting the CNS. It is worth noting that, concerning the development of PET tracers targeting brain 5-$HT_4$R, $^{18}$F introduction on the azaphenanthridine ring of **6d** and **6e** could be achieved using previously established methodology from suitable precursors.[18]

## 6. Experimental

### *6.1. General methods*

All chemical reagents and solvents were purchased from commercial sources and used without further purification except THF which was distilled on Na/benzophenone. Starting materials **16**, **17** and **18** were prepared from known literature procedures.[15,34] 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. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker AV300 spectrometer or on a Jeol Lambda 400 spectrometer with chemical shifts expressed in parts per million (in DMSO-$d_6$ or $CDCl_3$), with compound numbering given in the supporting information. 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. The purities of all tested compounds were analyzed by LC-MS, with the purity all being higher than 95% using a Waters alliance 2695 as separating module using the following gradient: A (95%)/B (5%) to A (5%)/B (95%) in 10 min. This ratio was hold during 3 min before return to initial conditions in 1 min. Initial conditions were then maintained for 5 min (A: $H_2O$, B: MeCN; each containing HCOOH: 0.1 %; Column: C18 Xterra MSC118/2.1_50 mm). MS detection was performed with a Micromass ZMD 2000.

### *6.2. General procedure A for the synthesis of compounds **9a-c***

To the piperidine derivative (4.0–16.0 mmol, 1 equiv.) was added the corresponding acid anhydride (3.6 equiv.). The mixture was heated at reflux for 3 h and then concentrated in *vacuo*. The residue was diluted in $Et_2O$ and washed with saturated $Na_2CO_3$ solution. The aqueous layer was extracted with $Et_2O$. The combined organic layers were washed twice with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The product was purified by flash chromatography on silica gel to afford the desired *N*-acylpiperidine derivative.

**Methyl *N*-difluoroacetylisonipecotate 9a** Following general procedure A, starting form methyl isonipecotate (1.14 g, 7.98 mmol) and difluoroacetic anhydride, using petroleum ether/acetone 80/20 as the eluent for the flash chromatography to obtain **9a** (1.71 g, 97%). IR (neat) 1729, 1665, 1136, 1037 cm$^{-1}$. ). $^1$H NMR (400 MHz, $CDCl_3$) δ 6.09 (t, 1H, *J* 53.7 Hz, H-6), 4.20 (dtd, 1H, *J* 13.4, 4.2, 1.3, H-4eq), 3.97–3.87 (m, 1H, H-4'eq), [{$^{19}$F} 3.92 (dtd, 1H, *J* 14.0, 4.3, 1.4 Hz, H-4'eq)], 3.68 (s, 3H, H-7), 3.20 (ddd, 1H, *J* 14.1, 11.0, 3.0 Hz, H-4'ax), 3.03–2.94 (m, 1H, H-4ax), 2.61 (tt, 1H, *J* 10.5, 4.0 Hz, H-2), 2.03–1.94 (m, 2H, H-3eq and H-3'eq), 1.83–1.66 (m, 2H, H-3ax and H-3'ax). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 174.1 (*C*$O_2$Me), 160.6 (t, *J* 25.3 Hz, $CHF_2$*C*ON), 110.9 (t, *J* 254.6 Hz, *C*$HF_2$), 51.9 (*C*$H_3$), 43.9 (t, *J* 4.4, C-4'), 41.9 (C-4), 40.4 (*C*H), 28.3 (C-3'), 27.5 (C-3). $^{19}$F{$^1$H} NMR (282 MHz, $CDCl_3$) δ –121.4 (s, 2F, CH*F*$_2$). MS (ES) *m/z*: 244 $[M + Na]^+$. HRMS (ES) for $C_9H_{13}F_2NNaO_3$ $[M + Na]^+$ calcd 244.0756, found 244.0755 (0.4 ppm error).

**Methyl *N*-trifluoroacetylisonipecotate 9b** Following general procedure A, starting from methyl isonipecotate (2.98 g, 20.8 mmol) and trifluoroacetic anhydride, using petroleum ether/acetone 90/10 as the eluent for the flash chromatography to

obtain **9b** (3.30 g, 66%). IR (neat) 2957, 1732, 1687, 1168, 1138 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$) δ 4.27 (dtd, 1H, *J* 13.5, 4.3, 1.3, H-4eq), 3.95–3.87 (m, 1H, H-4'eq), [{$^{19}F$} 3.91 (dtd, 1H, *J* 14.0, 4.1, 1.4 Hz, H-4'eq)], 3.69 (s, 3H, H-7), 3.26 (ddd, 1H, *J* 14.0, 10.9, 2.9 Hz, H-4'ax), 3.06 (ddd, 1H, *J* 13.6, 10.7, 3.3 Hz, H-4ax), 2.62 (tt, 1H, *J* 10.3, 4.2 Hz, H-2), 2.04–1.95 (m, 2H, H-3eq + H-3'eq), 1.83–1.69 (m, 2H, H-3ax + H-3'ax);. $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 173.9 (*C*1), 155.3 (q, *J* 35.9 Hz, $CF_3CO$), 116.4 (q, *J* 288.4 Hz, $CF_3$), 51.9 (C7), 44.8 (q, *J* 3.9 Hz, C-4'), 42.7 (C-4), 40.2 (*C*H), 28.2 (C-3'), 27.4 (C-3). $^{19}F\{^1H\}$ NMR (282 MHz, $CDCl_3$) δ -69.18 (s, 3F, $CF_3$).

**Methyl *N*-pentafluoropropionylisonipecotate 9c** Following general procedure A, starting from methyl isonipecotate (2.12 g, 14.8 mmol) and pentafluoropropionic anhydride, using petroleum ether/acetone 90/10 as the eluent for the flash chromatography to obtain **9c** (3.73g, 87%). IR (neat) 1733, 1677, 1199, 1157, 1122 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) δ 4.35-4.25 (m, 1H, H-4eq), 4.10–3.98 (m, 1H, H-4'eq), 3.72 (s, 3H, H-8), 3.31 (ddd, 1H, *J* 13.9, 11.0, 2.9 Hz, H-4'ax), 3.15–3.00 (m, 1H, H-4ax), 2.65 (tt, 1H, *J* 10.2, 4.4 Hz, H-2), 2.10-1.95 (m, 2H, H-3eq and H-3'eq), 1.90–1.70 (m, 2H, H-3ax and H-3'ax); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 173.9 (C1), 156.2 (t, *J* 25.3 Hz, C5), 118.0 (qt, *J* 285.7, 34.0 Hz, C7), 108.5 (tq, *J* 270.2, 36.0 Hz, C6), 52.0 ($CH_3$), 44.7 (t, *J* 5.8, C-4'), 42.7 (C-4), 40.2 (*C*H), 28.3 (C-3'), 27.5 (C-3). $^{19}F$ NMR (282 MHz, $CDCl_3$) δ -82.39 ($CF_3$), -115.07 (d, *J* 8.6 Hz, $CF_2$). MS (ES) *m/z*: 312 $[M + Na]^+$. HRMS (ES) for $C_{10}H_{12}F_5NNaO_3$ $[M + Na]^+$ calcd 312.0630, found 312.0632 (0.6 ppm error).

*6.3. General procedure B for the synthesis of compounds **7a-c***

To the obtained *N*-acylpiperidine derivative (1.3–14.0 mmol, 1 equiv.) in dry THF (12.5 mL/mmol) under $N_2$ was added the $BH_3$•THF (1M) solution (7-10 equiv.) at 0 °C *via* syringe. The reaction mixture was stirred at room temperature overnight, heated at 55 °C for 3 h and then stirred again at room temperature for 12 h. After that was carefully added methanol (50 mL/mmol) at 0 °C and the reaction mixture was concentrated. To the residue was added methanol (70 mL/mmol) and the mixture heated to reflux overnight and then concentrated *in vacuo*. The product was purified by flash chromatography on silica gel to afford the desired *N*-alkylpiperidine derivatives.

**[1-(2,2-difluoroethyl)piperidin-4-yl]methanol 7a.** Following general procedure B, methyl *N*-difluoroacetylisonipecotate **9a** (295 mg, 1.33 mmol) was reduced with borane (8 equiv.), and *n*-hexane/acetone 65/35 as the eluent for the chromatography, to afford **10c** (186 mg, 78%) as a pale yellow oil. IR (neat) 3331 (OH), 2920, 1123, 1037, 1013 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$) δ 5.89 (tt, 1H, *J* 56.0, 4.4 Hz, H-6), 3.46 (d, 2H, *J* 6.5 Hz, H-1), 3.02–2.88 (m, 2H, H-4eq), 2.73 (td, 2H, *J* 15.1, 4.5 Hz, H-5), 2.19 (td, 2H, *J* 11.7, 2.2 Hz, H-4ax), 2.01 (br. s, 1H, OH), 1.78–1.63 (m, 2H, H-3eq), 1.55–1.40 (m, 1H, H-2), 1.30 (dtd, 2H, *J* 12.2, 12.2, 3.8 Hz, H-3ax); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 116 (t, *J* 241 Hz, C6), 67.9 (C1), 60.9 (t, *J* 24.8 Hz, C5), 54.7 (C4), 38.4 (C2), 29.0 (C3). $^{19}F$ NMR (282 MHz, $CDCl_3$) δ -118.47 (dt, 2F, *J* 57.0, 15.0 Hz, $CHF_2$). HRMS/ESI calcd. for $C_8H_{16}F_2NO$ $[M+H]^+$ 180.1200, found 180.1196.

**[1-(2,2,2-trifluoroethyl)piperidin-4-yl]methanol 7b.** Following general procedure B, methyl *N*-trifluoroacetylisonipecotate **9b** (359 mg, 1.50 mmol) was reduced with $BH_3$ (8 equiv.), and *n*-hexane/acetone 6/4 as the eluent for the chromatography, **7b** was obtained as a pale yellow oil (211 mg, 71 %). IR (neat) 3332 (OH), 2921, 1269, 1138, 1093 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$) δ 3.49 (d, 2H, *J* 6.2 Hz, H-1), 3.07–2.92 (m, 4H, H-4eq and H-5), 2.37 (td, 2H, *J* 11.3, 1.5 Hz, H-4ax), 1.80–1.65 (m, 2H, H-3eq), 1.59–1.44 (m, 1H, H-2), 1.34 (dtd, 2H, *J* 12.1, 12.1, 3.8 Hz, H-3ax); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 125.5 (q, *J* 280 Hz, C6), 67.6 (C1), 58.6 (q, *J* 31.0 Hz, C5), 54.0 (C4), 37.8 (C2), 28.7 (C3). $^{19}F$ NMR (282 MHz, $CDCl_3$) δ -69.18 (t, 3F, *J* 8.6 Hz, $CF_3$). HRMS/ESI calcd. for $C_8H_{15}F_3NO$ $[M+H]^+$ 198.1106, found 198.1100.

**[1-(2,2,3,3,3-pentafluoropropyl)piperidin-4-yl]methanol 7c.** Following general procedure B, methyl *N*-pentafluoropropionylisonipecotate **9c** (1.6 g, 5.5 mmol) was reduced with borane (9 equiv.), and *n*-hexane/acetone 8/2 as the eluent for the column chromatography, to afford **7c** (1.01 g, 74%) as a colourless oil. IR (neat) 3329 (OH), 2920, 1183, 1095, 1039 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$) δ 3.46 (d, 2H, *J* 6.2 Hz, H-1), 3.05–2.85 (m, 4H, H-4eq and H-5), 2.32 (td, 2H, *J* 11.4, 1.9 Hz, H-4ax), 2.02 (br. s, 1H, OH), 1.77–1.61 (m, 2H, H-3eq), 1.56–1.38 (m, 1H, H-2), 1.29 (dtd, 2H, *J* 12.1, 12.1, 3.9 Hz, H-3ax);. $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 119.0 (qt, *J* 286, 35.4 Hz, C7), 114.8 (tq, *J* 254, 35.4 Hz, C6), 67.5 (C1), 56.9 (t, *J* 22.1 Hz, C5), 54.5 (C4), 37.8 (C2), 28.7 (C3). $^{19}F$ NMR (282 MHz, $CDCl_3$, $\{^1H\}$) δ –84.32 (s, 3F, $CF_3$), –119.46 (s, 2F, $CF_2$). HRMS/ESI calcd. for $C_9H_{15}F_5NO$ $[M+H]^+$ 248.1074, found 248.1068.

*6.4. Synthesis of compounds **7d**,**e***

**Synthesis of 3,3,3-trifluoro-1-bromopropane 15d.** To a round-bottom flask with $CBr_4$ (4.51 g, 13.61 mmol, 1.2 equiv.) were added $Ph_3P$ (3.57 g, 13.61 mmol, 1.2 equiv.) and 3,3,3-trifluoropropanol **10** (1 mL, 11.34 mmol, 1 equiv.) at 0 °C. Short-path condenser with pre-weighed receiver was immediately attached to the neck of the flask. The ice bath was removed and the mixture was warmed slowly to room temperature. The reaction mixture was then heated with vigorous stirring until liquid refluxed in the neck of flask then refluxed for 1 h. The temperature was increased to distill the product, with receiver cooled to 0 °C. The product was washed with aqueous saturated $NaHCO_3$ solution and dried over $MgSO_4$ to obtain the desired **15d** (0.969 g) and the crude was directly used for the further reaction. IR (neat) 2935, 2853, 1370, 1266, 1230, 1206, 1130, 1078, 950, 604 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) δ 3.46 (t, 2H, *J* 7.9 Hz, $CH_2Br$), 2.59–2.82 (m, 2H, $CF_3CH_2$). $^{13}C$ NMR (75 MHz, $CDCl_3$) δ 125.3 (q, *J* 280.8 Hz, $CF_3$), 37.5 (q, *J* 29.5 Hz, $CF_3CH_2$), 21.0 (q, *J* 4.4 Hz, $CH_2Br$). $^{19}F$ NMR (282 MHz, $CDCl_3$) δ -66.4 (t, 3F, *J* 10.7 Hz, $CF_3$). Data corresponded to literature.[35]

**Synthesis of Methyl *N*-3, 3, 3-trifluoropropylisonipecotate 13d** To a round-bottom flask, acetonitrile (5.0 mL), **8** (0.21 mL, 1.54 mmol, 1 equiv.), **15d** (820 mg, 4.63 mmol, 3 equiv.) and potassium carbonate (852 mg, 6.16 mmol, 4 equiv.) were added. The reaction mixture was stirred and heated to 60 °C for 16 h. The reaction mixture was cooled to room temperature and diluted with EtOAc (15 mL), washed with water (20 mL) and brine (2 × 20 mL). Organic phase was dried over anhydrous $Na_2SO_4$, then filtered and concentrated. After flash chromatography using petroleum ether/acetone 65/35 as the eluent, the desired compound **13d** (347 mg, 94%) was obtained as colorless oil. IR (neat) 2954, 1732, 1253, 1152, 1124 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) δ 3.69 (s, 3H, H-8), 2.90–2.80 (m, 2H, H-4eq), 2.63–2.55 (m, 2H, H-5), 2.40–2.25 (m, 3H, H-2 and H-6), 2.08 (td, 2H, *J* 11.6, 2.8 Hz, H-4ax), 1.96–1.86 (m, 2H, H-3eq), 1.84–1.68 (m, 2H, H-3ax). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 175.2 (C1), 126.6 (q, J 276 Hz, C7), 52.7 (C4), 51.6 (C8), 50.9 (d, br, *J* 2.21 Hz, C5), 40.7 (C2), 31.8 (q, *J* 27.6 Hz, C6), 28.1 (C3). $^{19}F$ NMR (282 MHz, $CDCl_3$) δ -66.53 (t, 3F, *J* 8.5 Hz, $CF_3$). MS (ES) *m/z*: 240 $[M + H]^+$. HRMS (ES) for $C_{10}H_{17}F_3NO_2$ $[M + H]^+$ calcd 240.1206, found 240.1210 (1.7 ppm error).

**Synthesis of Methyl *N*-4, 4, 4-trifluorobutylisonipecotate 13e.** To a round-bottom flask, acetonitrile (3.0 mL), **8** (0.127 mL, 0.94 mmol, 1 equiv.), **15e** (778 mg, 4.07 mmol, 4.3 equiv.) and potassium carbonate (650 mg, 4.70 mmol, 5 equiv.) were added. The reaction mixture was stirred and heated to 60 °C for 16 h. The reaction mixture was cooled to room temperature and diluted with EtOAc (15 mL) and washed with water (12.5 mL) and saturated brine (2×12.5 mL). Organic phase was dried over anhydrous $Na_2SO_4$, then filtered and concentrated. After flash chromatography using petroleum ether/acetone (85/15) as the eluent, the desired compound **13e** (234 mg, 98%) was obtained as pale yellow oil. IR (neat) 2953, 1735, 1255, 1173, 1129 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) δ 3.67 (s, 3H, H-9), 2.90–2.75 (m, 2H, H-4eq), 2.35 (t, 2H, *J* 7.1 Hz, H-5), 2.30–2.24 (m, 1H, H-2), 2.16-2.05 (m, 2H, H7), 2.04-1.96 (td, 2H, *J* 11.4, 2.5 Hz, H-4ax), 1.94–1.84 (m, 2H, H-3eq), 1.80-1.67 (m, 4H, H6 + H-3ax). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 175.5 (C1), 127.1 (q, *J* 276 Hz, C8), 57.0 (C5), 52.8 (C4), 51.6 (C9), 41.1 (C2), 31.6 (q, *J* 27.6 Hz, C7), 28.3 (C3), 19.5 (C6). $^{19}F$ NMR (282 MHz, $CDCl_3$) δ -66.5 (t, 3F, *J* 10.7 Hz, *CF*$_3$). MS (ES) *m/z*: 254.2 $[M + H]^+$.

**[1-(3,3,3-trifluoropropyl)piperidin-4-yl]methanol 7d**. The ester **13d** (345 mg, 1.44 mmol, 1 equiv.) is dissolved in dry THF (17 mL) and then $LiAlH_4$ (in THF, 1M) solution (4.33 mL, 4.33 mmol, 3 equiv.) was added at 0 °C. The reaction mixture was warmed to room temperature and stirred for a further 4 h. Then water (0.2 mL) was added slowly, followed by 10% aqueous NaOH solution (0.25 mL) then water (0.42 mL). Then the mixture was stirred for one more hour then filtered and concentrated *in vacuo* and purified by flash chromatography to obtain the desired **7d** (213 mg, 70%). IR (neat) 3330 (OH), 2921, 1253, 1153, 1127 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) δ 3.50 (d, 2H, *J* 6.4 Hz, H-1), 2.97–2.85 (m, 2H, H-4eq), 2.65–2.55 (m, 2H, H-5), 2.40–2.20 (m, 2H, H-6), 2.01 (td, 2H, *J* 11.6, 2.5 Hz, H-4ax), 1.83–1.70 (m, 2H, H-3eq), 1.60–1.42 (m, 2H, H-2 and OH), 1.35-1.20 (m, 2H, H-3ax). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 126.6 (q, *J* 276 Hz, C7), 67.5 (C2), 53.3 (C4), 51.0 (d, br, *J* 3.32 Hz, C5), 38.3 (C2), 31.8 (q, *J* 27.6 Hz, C6), 28.7 (C3). $^{19}F$ NMR (282 MHz, $CDCl_3$) δ –65.65 (t, 3F, *J* 8.5 Hz, C*F*$_3$). HRMS/ESI calcd. for $C_9H_{17}F_3NO$ $[M+H]^+$ 212.1262, found 212.1257.

**[1-(4,4,4-trifluorobutyl)piperidin-4-yl]methanol 7e**. The obtained methyl *N*-alkylisonipecotate (145 mg, 0.57 mmol) was dissolved in dry THF (10 mL) and then $LiAlH_4$ in THF (1M) (1.72 mL, 1.72 mmol) was added at 0 °C. The reaction mixture was then warm to room temperature and stirred for 4 h. Water (1 mL) was added slowly, followed by 10% aqueous NaOH solution (1.5 mL) and then water (2.5 mL). The mixture was stirred for one hour, filtered and concentrated in *vacuo* to obtain the desired **7e** (135 mg) as a colorless oil used without further purification. (estimated yield: 88 %). IR (neat) 3353 (OH), 2941, 2926, 1372, 1259, 1134, 1039, 903, 729 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) δ 3.50 (d, 2H, *J* 6.4 Hz), 2.96-2.82 (m, 2H), 2.37 (t, 2H, *J* 7.3 Hz), 2.16-2.03 (m, 2H), 1.94 (td, 2H, *J* 11.7 Hz, 2.4 Hz), 1.82-1.68 (m, 4H), 1.60-1.44 (m, 2H), 1.35-1.18 (m, 2H). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 127.2 (q, *J* 276 Hz), 67.9 (s), 57.2 (s), 53.4 (s), 38.6 (s), 31.9 (q, *J* 27.6 Hz), 28.8 (s), 19.5 (s). $^{19}F$ NMR (282 MHz, $CDCl_3$) δ -66.5 (t, *J* 8.5 Hz, C*F*$_3$). HRMS/ESI calcd. for $C_{10}H_{19}F_3NO$ $[M+H]^+$ 226.1419, found 226.1413.

**Methyl *N*-methanesulfonylisonipecotate 14**. IR (neat) 2954, 1724, 1437, 1319, 1139 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) δ 3.75–3.60 (m, 5H, H-4eq and H-6), 2.92–2.81 (m, 2H, H-4ax), 2.78 (s, 3H, H-5), 2.52–2.40 (m, 1H, H-2), 2.08–1.97 (m, 2H, H-3eq), 1.92–1.77 (m, 2H, H-3ax). $^{13}C$ NMR (75 MHz, $CDCl_3$) δ 174.2 (*C*$O_2$Me), 51.9 (O*C*$H_3$), 45.1 ($CH_2$*C*$H_2$N), 39.9 (*C*H), 35.0 (*C*$H_3SO_2$), 28.1 (CH*C*$H_2$CH$_2$N). MS (ES) *m/z* (%): 244 ((M + Na)$^+$ 100), 222 ((M + H)$^+$ 16).

***6.5.** General procedure C for the synthesis of compounds **4-6***

The chosen (aza)phenanthridin-6(*5H*)-one (**2a-c**) and $POCl_3$ (5 mL/mmol) 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 then carefully adjusted to 12 using a 28% ammonia solution. The product was then extracted using EtOAc (3 times). The organic phase was then dried with $MgSO_4$, filtered and evaporated. The crude material was then added at 0 °C to a solution of **7a-e** (1 equiv.) and NaH (4 equiv.) in anhydrous DMF (10 mL/mmol). 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 $MgSO_4$, filtered, evaporated and purified by silica gel chromatography.

**6-[1-(2,2-difluoroethyl)piperidin-4-yl]methyloxyphenanthridine 4a**. Starting from **16** (51 mg, 0.26 mmol), following general procedure C and using *n*-hexane/ethyl acetate 9/1 as the eluent for the column chromatography, **4a** was obtained as a yellow crystals (75 mg, 80 %). Mp 83–85 °C. IR (neat) 3074, 2940, 1315, 1124, 1086 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) δ 8.50 (d, 1H, *J* 8.4 Hz, $H_{Ar}$), 8.40 (dd, 2H, *J* 11.3, 8.0 Hz, $H_{Ar}$), 7.90 (d, 1H, *J* 8.1 Hz, $H_{Ar}$), 7.85–7.76 (m, 1H, $H_{Ar}$), 7.64 (t, 2H, *J* 7.7 Hz, $H_{Ar}$), 7.54–7.44 (m, 1H, $H_{Ar}$), 5.93 (tt,1H, *J* 56.0, 4.4 Hz, H-6), 4.52 (d, 2H, *J* 5.9 Hz, H-2), 3.10–2.96 (m, 2H, H-4eq), 2.78 (dt, 2H, *J* 15.1, 4.2 Hz, H-5), 2.35–2.20 (m, 2H, H-4ax), 2.07–1.88 (m, 3H, H-3eq + H-2), 1.69–1.51 (m, 2H, H-3ax). $^{13}C$ NMR (75 MHz, $CDCl_3$) δ 158.7 (s, *C*NO), 143.3 ($C_{Ar}$), 134.7 ($C_{Ar}$), 130.8 ($C_{Ar}$), 128.7 ($C_{Ar}$), 127.7 ($C_{Ar}$), 127.1 ($C_{Ar}$), 124.9 ($C_{Ar}$), 124.3 ($C_{Ar}$), 122.4 ($C_{Ar}$), 122.0 ($C_{Ar}$), 121.8 ($C_{Ar}$), 120.1 ($C_{Ar}$), 115.8 (t, *J* 241.0 Hz, *C*$HF_2$), 70.1 (*C*$H_2$O), 60.6 (t, *J* 24.3 Hz, *C*$H_2$CHF$_2$), 54.3 (*C*$H_2$N), 35.3 (*C*HCH$_2$O), 29.2 (*C*$H_2$CH$_2$N). $^{19}F$ NMR (282 MHz, $CDCl_3$) δ –118.3 (dt, 2F, *J* 55.9, 17.2 Hz, *CHF*$_2$). MS (ESI+) *m/z* (%) 357.2 ((M + H)$^+$, 35), 379.2 ((M + Na)$^+$, 11). LC-MS (ESI): $t_R$ = 5.27 min; $[M+H]^+$: 357.29. HRMS/ESI calcd. for $C_{21}H_{23}F_2N_2O$ $[M+H]^+$ 357.1773, found 357.1772.

**6-[(1-(2,2,2-trifluoroethyl)piperidin-4-yl]methyloxyphenanthridine 4b**. Starting from **16** (55 mg, 0.28 mmol), following general procedure C and using *n*-hexane/ethyl acetate 9/1 as the eluent for the column chromatography, **4b** was obtained as a white crystals (72 mg, 68 %). Mp 94–96 °C. IR (neat) 3077, 2940, 1120, 1093 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) δ 8.51 (d, 1H, *J* 8.1 Hz, $H_{Ar}$), 8.41 (dd, 2H, *J* 14.3, 8.0 Hz, $H_{Ar}$), 7.89 (d, 1H, *J* 8.1 Hz, $H_{Ar}$), 7.82 (t, 1H, *J* 7.3 Hz, $H_{Ar}$), 7.70–7.58 (m, 2H, $H_{Ar}$), 7.49 (t, 1H, *J* 7.3 Hz, $H_{Ar}$), 4.52 (d, 2H, *J* 5.9 Hz, H-1), 3.12–2.96 (m, 4H, H-4eq + H-5), 2.51–2.39 (m, 2H, H-4ax), 2.08–1.88 (m, 3H, H-3eq + H-2), 1.69–1.52 (m, 2H, H-3ax). $^{13}C$ NMR (75 MHz, $CDCl_3$) δ 158.8 ($C_{Ar}$),143.3 ($C_{Ar}$), 134.8 ($C_{Ar}$), 130.8 ($C_{Ar}$), 128.7 ($C_{Ar}$), 127.7 ($C_{Ar}$), 127.2 ($C_{Ar}$), 125.6 (q, $^1J$ 280.8 Hz, *C*F$_3$), 124.9 ($C_{Ar}$), 124.3 ($C_{Ar}$), 122.4 ($C_{Ar}$), 122.1 ($C_{Ar}$), 121.8 ($C_{Ar}$), 120.1 ($C_{Ar}$), 70.1 (*C*$H_2$O), 58.8 (q, $^2J$ 29.9 Hz, *C*$H_2$CF$_3$), 54.0 (*C*$H_2$N), 35.2 (*C*HCH$_2$O), 29.3 (*C*$H_2$CH$_2$N). $^{19}F$ NMR (282 MHz, $CDCl_3$) δ –69.0 (t, 3F, $^3J_{H-F}$ = 8.6 Hz, C*F*$_3$). LC-MS (ESI): $t_R$ 8.50 min; $[M+H]^+$: 375.27. HRMS/ESI calcd. for $C_{21}H_{21}F_3N_2O$ $[M+H]^+$ 375.1679, found 375.1674.

**6-[(1-(2,2,3,3,3-pentafluoropropyl)piperidin-4-yl]methyloxyphenanthridine 4c**. Starting from **16** (51 mg, 0.26 mmol), following general procedure C and using *n*-hexane/ethyl acetate 9/1 as the eluent for the column chromatography, **4c** was

obtained as a white crystals (56 mg, 50 %). Mp 75–77 °C. IR (neat) 3076, 2940, 1187, 1120, 1093 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) δ 8.52 (d, 1H, *J* 8.1 Hz, $H_{Ar}$), 8.41 (dd, 2H, *J* 15.7, 8.1 Hz, $H_{Ar}$), 7.89 (d, 1H, *J* 8.1 Hz, $H_{Ar}$), 7.82 (t, 2H, *J* 7.7 Hz, $H_{Ar}$), 7.71–7.59 (m, 2H, $H_{Ar}$), 7.54–7.45 (m, 1H, $H_{Ar}$), 4.52 (d, 2H, *J* 6.2 Hz, H-1), 3.14–2.93 (m, 4H, H-5 + H-4eq), 2.43 (t, 2H, *J* 11.0 Hz, H-4ax), 2.08–1.86 (m, 3H, H-3eq + H-2), 1.71–1.50 (m, 2H, H-3ax). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 158.8 (*C*NO), 143.3 ($C_{Ar}$), 134.8 ($C_{Ar}$), 130.8 ($C_{Ar}$), 128.7 ($C_{Ar}$), 127.8 ($C_{Ar}$), 127.2 ($C_{Ar}$), 124.9 ($C_{Ar}$), 124.3 ($C_{Ar}$), 122.4 ($C_{Ar}$), 122.1 ($C_{Ar}$), 121.9 ($C_{Ar}$), 120.1 ($C_{Ar}$), 119.1 (qt, *J* 286.7, 36.0 Hz, $CF_2CF_3$), 114.9 (tq, *J* 254.6, 36.0 Hz, $CF_2CF_3$), 70.2 ($CH_2O$), 57.0 (t, *J* 22.4 Hz, $CH_2CF_2$), 54.6 ($CH_2N$), 35.2 ($CHCH_2O$), 29.3 ($CH_2CH_2N$). $^{19}F$ NMR (282 MHz, $CDCl_3$) δ –84.1 (s, 3F, $CF_3$), –119.3 (t, 2F, *J* 17.2 Hz, $CF_2$). LC-MS (ESI): $t_R$ 9.65 min; $[M+H]^+$: 425.30. HRMS/ESI calcd. for $C_{22}H_{22}F_5N_2O$ $[M+H]^+$ 425.1647, found 425.1646.

**4-Fluoro-6-[1-(2,2-difluoroethyl)piperidin-4-yl]methyloxyphenanthridine 5a**. Starting from **17** (57 mg, 0.27 mmol), following general procedure C and using *n*-hexane/ethyl acetate 9/1 as the eluent for the column chromatography, **5a** was obtained as yellow crystals (79 mg, 79 %). Mp 128-130 °C. IR (neat) 2940, 1590, 1314, 1128, 1049 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) δ 8.47 (d, 1H, *J* 8.0 Hz, $H_{Ar}$), 8.39 (d, 1H, *J* 8.1 Hz, $H_{Ar}$), 8.18 (dd, 1H, *J* 7.0, 1.5 Hz, $H_{Ar}$), 7.87–7.78 (m, 1H, $H_{Ar}$), 7.72–7.63 (m, 1H, $H_{Ar}$), 7.45–7.30 (m, 2H, $H_{Ar}$), 5.93 (tt, 1H, *J* 56.0, 4.4 Hz, H-6), 4.56 (d, 2H, *J* 5.9 Hz, H-1), 3.09–2.98 (m, 2H, H-4eq), 2.78 (td, 2H, *J* 15.0, 4.4 Hz, H-5), 2.36–2.22 (m, 2H,H-4ax), 2.08–1.87 (m, 3H, H-3eq + H-2), 1.69–1.51 (m, 2H, H-3ax). $^{13}C$ NMR (75 MHz, $CDCl_3$) δ 158.9 (*C*NO), 157.4 (d, $^1J$ 252.1 Hz, $C_{Ar}F$), 134.3 (d, *J* 3.3 Hz, $C_{Ar}$), 132.7 (d, *J* 10.0 Hz, $C_{Ar}$), 131.2 ($C_{Ar}$), 127.7 ($C_{Ar}$), 125.1 ($C_{Ar}$), 124.5 (d, *J* 2.2 Hz, $C_{Ar}$), 123.9 (d, *J* 7.7 Hz, $C_{Ar}$), 122.2 ($C_{Ar}$), 120.2 ($C_{Ar}$), 117.6 (d, *J* 4.4 Hz, $C_{Ar}$), 115.8 (t, $^1J$ 241.0 Hz, $CHF_2$), 114.0 (d, *J* 18.8 Hz, $C_{Ar}$), 70.5 ($CH_2O$), 60.6 (t, $^2J$ 25.4 Hz, $CH_2CHF_2$), 54.3 ($CH_2N$), 35.3 ($CHCH_2O$), 29.2 ($CH_2CH_2N$). $^{19}F$ NMR (282 MHz, $CDCl_3$) δ –118.3 (dt, 2F, *J* 55.9, 17.2 Hz, $CHF_2$), –126.7 (br. s, 1F, $C_{Ar}F$). LC-MS (ESI): $t_R$ 5.41 min; $[M+H]^+$: 375.46. HRMS/ESI calcd. for $C_{21}H_{21}F_3N_2ONa$ $[M+Na]^+$ 397.1498, found 397.1493.

**4-Fluoro-6-[1-(2,2,2-trifluoroethyl)piperidin-4-yl]methyloxyphenanthridine 5b**. Starting from **17** (60 mg, 0.28 mmol), following general procedure C and using *n*-hexane/ethyl acetate 9/1 as the eluent for the column chromatography, **5b** was obtained as white crystals (77 mg, 69 %). Mp 144–146 °C. IR (neat) 3078, 2940, 1125, 1094 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) δ 8.47 (d, 1H, *J* 8.1 Hz, $H_{Ar}$), 8.39 (d, 1H, *J* 8.0 Hz, $H_{Ar}$), 8.18 (d, 1H, *J* 7.0 Hz, $H_{Ar}$), 7.83 (td, 1H, *J* 8.1, 0.7 Hz, $H_{Ar}$), 7.68 (t, 1H, *J* 7.7 Hz, $H_{Ar}$), 7.45–7.31 (m, 2H, $H_{Ar}$), 4.56 (d, 2H, *J* 6.2 Hz, H-1) 3.12–2.96 (m, 4H, H-4eq + H-5), 2.51–2.39 (m, 2H, H-4ax), 2.08–1.88 (m, 3H, H-3eq + H-2), 1.69–1.52 (m, 2H, H-3ax). $^{13}C$ NMR (75 MHz, $CDCl_3$) δ 158.9 (s, *C*NO), 157.4 (d, *J* 251.0 Hz, $C_{Ar}F$), 134.3 (d, *J* 3.3 Hz, $C_{Ar}$), 132.7 (d, *J* 11.1 Hz, $C_{Ar}$), 131.2 ($C_{Ar}$), 127.8 ($C_{Ar}$), 125.6 (q, *J* 280.9 Hz, $CF_3$), 125.1 ($C_{Ar}$), 124.5 (d, *J* 2.2 Hz, $C_{Ar}$), 123.9 (d, *J* 7.7 Hz, $C_{Ar}$), 122.2 ($C_{Ar}$), 120.2 ($C_{Ar}$), 117.6 (d, *J* 4.4 Hz, $C_{Ar}$), 114.0 (d, *J* 18.8 Hz, $C_{Ar}$), 70.4 ($CH_2O$), 58.8 (q, *J* 31.0 Hz, $CH_2CF_3$), 54.0 ($CH_2N$), 35.2 ($CHCH_2O$), 29.3 ($CH_2CH_2N$). $^{19}F$ NMR (282 MHz, $CDCl_3$) δ –69.0 (t, 3F, *J* 8.6 Hz, $CF_3$), –126.7 (br. s, 1F, $C_{Ar}F$). LC-MS (ESI): $t_R$ 8.41 min; $[M+H]^+$: 393.29. HRMS/ESI calcd. for $C_{21}H_{21}F_4N_2O$ $[M+H]^+$ 393.1585, found 393.1591.

**4-Fluoro-6-[1-(2,2,3,3,3-pentafluoropropyl)piperidin-4-yl]methyloxyphenanthridine 5c**. Starting from **17** (128 mg, 0.6 mmol), following general procedure C and using *n*-hexane/ethyl acetate 9/1 as the eluent for the column chromatography, **5c** was obtained as white crystals (190 mg, 71 %). Mp 100–104 °C. IR (neat) 2940, 1315, 1189, 1154, 1098 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) δ 8.47 (d, 1H, *J* 8.1 Hz, $H_{Ar}$), 8.39 (d, 1H, *J* 8.1 Hz, $H_{Ar}$), 8.18 (d, 1H, *J* 7.7 Hz, $H_{Ar}$), 7.88–7.79 (m, 1H, $H_{Ar}$), 7.73–7.64 (m, 1H, $H_{Ar}$), 7.45–7.31 (m, 2H, $H_{Ar}$), 4.56 (d, 2H, *J* 6.2 Hz, H-1), 3.12–2.94 (m, 4H, H-5 + H-4eq), 2.50–2.36 (m, 2H, H-4ax), 2.07–1.86 (m, 3H, H-3eq + H-2), 1.70–1.52 (m, 2H, H-3ax). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 158.9 (*C*NO), 157.5 (d, *J* 251.7 Hz, $C_{Ar}F$), 134.2 (d, *J* 2.9 Hz, $C_{Ar}$), 132.7 (d, *J* 10.7 Hz, $C_{Ar}$), 131.1 ($C_{Ar}$), 127.7 ($C_{Ar}$), 125.1 ($C_{Ar}$), 124.5 (d, *J* 1.9 Hz, $C_{Ar}$), 123.9 (d, *J* 7.8 Hz, $C_{Ar}$), 122.2 ($C_{Ar}$), 120.2 ($C_{Ar}$), 119.1 (qt, *J* 286.7, 35.0 Hz, $CF_2CF_3$), 117.5 (d, *J* 3.9 Hz, $C_{Ar}$), 114.9 (tq, *J* 254.6, 36.0 Hz, $CF_2CF_3$), 113.9 (d, *J* 19.4 Hz, $C_{Ar}$), 70.4 ($CH_2O$), 57.0 (t, *J* 22.4 Hz, $CH_2CF_2$), 54.6 ($CH_2N$), 35.2 ($CHCH_2O$), 29.3 ($CH_2CH_2N$). $^{19}F$ NMR (282 MHz, $CDCl_3$) δ –84.2 (s, 3F, $CF_3$), –119.3 (t, 2F, *J* 17.2 Hz, $CF_2$), –126.8 - –126.6 (m, 1F, $C_{Ar}F$). LC-MS (ESI): $t_R$ 9.55 min; $[M+H]^+$: 443.31. HRMS/ESI calcd. for $C_{22}H_{21}F_6N_2O$ $[M+H]^+$ 443.1553, found 443.1555.

**4-Fluoro-6-[1-(3,3,3-trifluoropropyl)piperidin-4-yl]methyloxyphenanthridine 5d**. Starting from **17** (66 mg, 0.31 mmol), following general procedure C and using *n*-hexane/acetone 85/15 as the eluent for the column chromatography, **5d** was obtained as yellow crystals (68 mg, 54 %). Mp 178–181 °C. IR (neat) 2937, 2784, 2745, 1662, 1591, 1317, 1223, 1153 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) δ 8.44 (d, 1H, *J* 3.0 Hz, $H_{Ar}$), 8.40-8.36 (m, 1H, $H_{Ar}$), 8.20-8.10 (m, 1H, $H_{Ar}$), 7.81 (ddd, 1H, *J* 8.3, 7.1, 1.4 Hz, $H_{Ar}$), 7.66 (ddd, 1H, *J* 8.1, 7.1, 1.1 Hz, $H_{Ar}$), 7.41-7.30 (m, 2H, $H_{Ar}$), 4.55 (d, 2H, *J* 6.1 Hz, H1), 3.04-2.86 (m, 2H, H-4eq), 2.70-2.60 (m, 2H, H5), 2.48-2.27 (m, 2H, H6), 2.09 (td, 2H, *J* 11.7, 2.2 Hz, H4-ax), 2.04-1.88 (m, 3H, H2 + H-3eq), 1.70-1.50 (m ,2H, H-3ax). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 158.9 (CNO), 157.5 (d, *J* 246 Hz, $C_{Ar}F$), 134.3 (d, *J* 3 Hz, $C_{Ar}$), 132.7 (d, *J* 10 Hz, $C_{Ar}$), 131.2 ($C_{Ar}$), 127.7 ($C_{Ar}$), 126.7 (q, *J* 275 Hz, $CF_3$), 125.2 ($C_{Ar}$), 124.5 ($C_{Ar}$), 123.9 (d, *J* 7 Hz, $C_{Ar}$), 122.2 ($C_{Ar}$), 120.2 ($C_{Ar}$), 117.6 (d, *J* 3 Hz, $C_{Ar}$), 114.0 (d, *J* 19 Hz, $C_{Ar}$), 70.5 (C1), 53.5 (C4), 51.1 (d, *J* 3 Hz, C5), 35.7 (C2), 31.9 (q, *J* 28 Hz, C6), 29.2 (C3). $^{19}F$ NMR (282 MHz, $CDCl_3$) δ -65.6 (t, *J* 8.5 Hz, $CF_3$), -126.7 (m, $C_{Ar}F$). LC-MS (ESI): $t_R$ 5.54 min; $[M+H]^+$: 407.34. HRMS/ESI calcd. for $C_{22}H_{23}F_4N_2O$ $[M+H]^+$ 407.1747, found 407.1741.

**4-Fluoro-6-[1-(4,4,4-trifluorobutyl)piperidin-4-yl]methyloxyphenanthridine 5e**. Starting from **17** (55 mg, 0.26 mmol), following general procedure C and using *n*-hexane/acetone 3/1 as the eluent for the column chromatography, **5e** was obtained as a yellow oil (45 mg, 41 %). IR (neat) 2939, 2820, 2771, 2359, 1591, 1318, 1247, 1108 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) δ 8.46 (d, 1H, *J* 8.3 Hz, $H_{Ar}$), 8.40 (dd, 1H, *J* 8.1, 1.0 Hz, $H_{Ar}$), 8.17 (d, 1H, *J* 7.3 Hz, $H_{Ar}$), 7.82 (ddd, 1H, *J* 8.3, 7.1, 1.3 Hz, $H_{Ar}$), 7.72-7.65 (m, 1H, $H_{Ar}$), 7.46-7.32 (m, 2H, $H_{Ar}$), 4.55 (d, 2H, *J* 6.1 Hz, H1), 3.03-2.92 (m ,2H, H-4eq), 2.45-2.35 (m, 2H, H5), 2.25-2.08 (m, 2H, H7), 2.06-1.90 (m, 5H, H4-ax + H2 + H-3eq), 1.85-1.72 (m, 2H, H6), 1.65-1.50 (m ,2H, H-3ax). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 159.0 (*C*NO), 157.5 (d, *J* 251 Hz, $C_{Ar}F$), 134.3 (d, *J* 3 Hz, $C_{Ar}$), 132.7 (d, *J* 10 Hz, $C_{Ar}$), 131.2 ($C_{Ar}$), 127.8 ($C_{Ar}$), 127.4 (q, *J* 275 Hz, $CF_3$), 125.2 ($C_{Ar}$), 124.5 ($C_{Ar}$), 123.9 (d, *J* 8 Hz, $C_{Ar}$), 122.3 ($C_{Ar}$), 120.3 ($C_{Ar}$), 117.6 (d, *J* 5 Hz, $C_{Ar}$), 114.0 (d, *J* 19 Hz, $C_{Ar}$), 70.7 (C1), 57.4 (C5), 53.5 (C4), 35.9 (C2), 31.8 (q, *J* 29 Hz, C7), 29.3 (C3), 19.7 (C6). $^{19}F$ NMR (282 MHz, $CDCl_3$) δ -66.5 (t, *J* 11.3 Hz, $CF_3$), -126.7 (m, $C_{Ar}F$). LC-MS (ESI): $t_R$ 5.65 min; $[M+H]^+$: 421.42. HRMS/ESI calcd. for $C_{23}H_{25}F_4N_2O$ $[M+H]^+$ 421.1903, found 421.1898.

**7-Fluoro-5-[1-(2,2-difluoroethyl)piperidin-4-yl]methyloxybenzo[*h*]-1,6-naphthyridine 6a**. Starting from **18** (99 mg, 0.46 mmol), following general procedure C and using *n*-

hexane/ethyl acetate 7/3 as the eluent for the column chromatography, **6a** was obtained as a yellow crystals (71 mg, 41 %). Mp 167–170 °C. IR (neat) 2941, 2790, 1613, 1312, 1234, 1052 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) δ 9.10 (dd, 1H, *J* 4.5, 1.8 Hz, $H_{Ar}$), 8.75-8.68 (m, 1H, $H_{Ar}$), 8.59 (dd, 1H, *J* 8.1, 1.8 Hz, $H_{Ar}$), 7.58 (dd, 1H, *J* 8.1, 4.5 Hz, $H_{Ar}$), 7.50-7.40 (m, 2H, $H_{Ar}$), 5.92 (tt, 1H, *J* 12.0, 3.0 Hz, H6), 4.55 (d, 2H, J 6.2 Hz, H1), 3.08-2.97 (m , 2H, H-4eq), 2.77 (td, 2H, *J* 15.1, 4.4 Hz, H5), 2.35-2.22 (m, 2H, H-4ax), 2.06-1.85 (m, 3H, H2 + H-3eq), 1.68-1.51 (m ,2H, H-3ax). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 158.5 (*C*NO), 157.0 (d, *J* 250 Hz, $C_{Ar}F$), 153.3 ($C_{Ar}$), 150.3 (d, *J* 3 Hz, $C_{Ar}$), 134.1 (d, *J* 10 Hz, $C_{Ar}$), 133.0 ($C_{Ar}$), 125.6 ($C_{Ar}$), 124.4 (d, *J* 7 Hz, $C_{Ar}$), 122.8 ($C_{Ar}$), 119.3 (d, *J* 5 Hz, $C_{Ar}$), 115.8 (t, *J* 240 Hz, $CHF_2$), 115.6 (d, *J* 19 Hz, $C_{Ar}$), 115.5 (s, $C_{Ar}$),70.9 (C1), 60.6 (t, *J* 24 Hz, C5), 54.3 (C4), 35.3 (C2), 29.2 (C3). $^{19}F$ NMR (282 MHz, $CDCl_3$) δ -118.4 (dt, *J* 56.4, 12.7 Hz, $CHF_2$), -127.5 (m, $C_{Ar}F$). LC-MS (ESI): $t_R$ 4.97 min; $[M+H]^+$: 376.29. HRMS/ESI calcd. for $C_{20}H_{21}F_3N_3O$ $[M+H]^+$ 376.1637, found 376.1637.

**7-Fluoro-5-[1-(3,3,3-trifluoropropyl)piperidin-4-yl]methyloxybenzo[*h*]-1,6-naphthyridine 6d**. Starting from **18** (100 mg, 0.46 mmol), following general procedure C and using *n*-hexane/acetone 8/2 as the eluent for the column chromatography, **6d** was obtained as a yellow crystals (84 mg, 44 %). Mp 138–140 °C. IR (neat) 2935, 2782, 2360, 1592, 1330, 1148, 1107 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) δ 9.09 (dd, 1H, *J* 4.4 Hz, 1.8 Hz, $H_{Ar}$), 8.73-8.67 (m, 1H, $H_{Ar}$), 8.58 (dd, 1H, *J* 8.1 Hz, 1.8 Hz, $H_{Ar}$), 7.56 (dd, 1H, *J* 8.1 Hz, 4.5 Hz, $H_{Ar}$), 7.48-7.38 (m, 2H, $H_{Ar}$), 4.54 (d, 2H, *J* 6.1 Hz, H1), 3.00-2.92 (m ,2H, H-4eq), 2.68-2.60 (m, 2H, H5), 2.40-2.28 (m, 2H, H6), 2.08 (td, 2H, *J* 11.7 Hz, 2.1 Hz, H4-ax), 2.03-1.88 (m, 3H, H2 + H-3eq), 1.62-1.50 (m ,2H, H-3ax). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 158.5 (*C*NO), 157.0 (d, *J* 250 Hz, $C_{Ar}F$), 153.3 ($C_{Ar}$), 150.3 (d, *J* 5 Hz, $C_{Ar}$), 134.1 (d, *J* 11 Hz, $C_{Ar}$), 133.0 ($C_{Ar}$), 126.7 (q, *J* 275 Hz, $CF_3$), 125.5 ($C_{Ar}$), 124.4 (d, *J* 9 Hz, $C_{Ar}$), 122.8 ($C_{Ar}$), 119.3 (d, *J* 3 Hz, $C_{Ar}$), 115.6 (d, *J* 19 Hz, $C_{Ar}$),115.5 (s, $C_{Ar}$), 70.9 (C1), 53.4 (C4), 51.1 (C5), 35.6 (C2), 31.9 (q, *J* 25 Hz, C6), 29.2 (C3). $^{19}F$ NMR (282 MHz, $CDCl_3$) δ -65.6 (t, *J* 11.3 Hz, $CF_3$), -127.4 (m, $C_{Ar}F$). LC-MS (ESI): $t_R$ 5.12 min; $[M+H]^+$: 408.52. HRMS/ESI calcd. for $C_{21}H_{22}F_4N_3O$ $[M+H]^+$ 408.1699, found 408.1694.

**7-Fluoro-5-[1-(4,4,4-trifluorobutyl)piperidin-4-yl]methyloxybenzo[*h*]-1,6-naphthyridine 6e**. Starting from **18** (52 mg, 0.24 mmol), following general procedure C and using *n*-hexane/acetone 7/3 as the eluent for the column chromatography, **6e** was obtained as a yellow crystals (68 mg, 67 %). Mp 129–131 °C. IR (neat) 2940, 2890, 2762, 1694, 1608, 1331, 1150 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) δ 9.13 (dd, 1H, *J* 4.4, 1.7 Hz, $H_{Ar}$), 8.74 (dd, 1H, *J* 6.2, 2.5 Hz, $H_{Ar}$), 8.65 (dd, 1H, *J* 8.2, 1.7 Hz, $H_{Ar}$), 7.61 (dd, 1H, *J* 8.1, 4.5 Hz, $H_{Ar}$), 7.50-7.42 (m, 2H, $H_{Ar}$), 4.58 (d, 2H, *J* 6.0 Hz, H1), 3.04-2.92 (m ,2H, H-4eq), 2.47-2.36 (m, 2H, H5), 2.25-2.10 (m, 2H, H7), 2.09-1.87 (m, 5H, H4-ax + H2 + H-3eq), 1.85-1.72 (m, 2H, H6), 1.65-1.48 (m, 2H, H-3ax). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 158.6 (*C*NO), 157.1 (d, *J* 250 Hz, $C_{Ar}F$), 153.3 ($C_{Ar}$), 150.4 (d, *J* 3 Hz, $C_{Ar}$), 134.2 (d, *J* 10 Hz, $C_{Ar}$), 133.2 ($C_{Ar}$), 125.6 ($C_{Ar}$), 124.7 (q, *J* 274 Hz, $CF_3$), 124.5 (d, *J* 6 Hz, $C_{Ar}$), 122.8 ($C_{Ar}$), 119.3 (d, *J* 4 Hz, $C_{Ar}$), 115.7 (d, *J* 19 Hz, $C_{Ar}$), 115.7 (s, $C_{Ar}$),71.0 (C1), 57.3(C5), 53.4 (C4), 35.9 (C2), 31.8 (q, *J* 29 Hz, C7), 29.3 (C3), 19.6 (C6). $^{19}F$ NMR (282 MHz, $CDCl_3$) δ -66.5 (t, *J* 11.3 Hz, $CF_3$), -127.4 (m, $C_{Ar}F$). LC-MS (ESI): $t_R$ 5.33 min; $[M+H]^+$: 422.48. HRMS/ESI calcd. for $C_{22}H_{24}F_4N_3O$ $[M+H]^+$ 422.1856, found 422.1850.

### *6.6. Pharmacological Assay and Screen*

Binding of compounds **4a-c**, **5a-e** and **6a,d,e** to native 5-$HT_4$R from guinea pig was determined using the method of Grossman.[36] 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 x *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 Lowry[37] 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 [$^3$H]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-presoaked Whatman GF/B filters (Alpha Biotech) using a Brandel Cell Harvester. Filters were subsequently washed three times with 4 ml of HEPES buffer 50 mM pH 7.4 at 4 °C. The method was validated from saturation studies: 6 concentrations of [$^3$H]GR 113808 were used to give final concentrations of 0.02-0.8 nM, non-specific binding of [$^3$H]GR 113808 was defined in the presence of 30 µM serotonin to determine the $K_d$ and the Bmax. For competition studies, [$^3$H]GR 113808 was used to give a final concentration of 0.1 nM. Percentages of inhibition of the binding of [$^3$H]GR 113808 were obtained for concentrations of $10^{-6}$ and $10^{-8}$ M of the ligands tested. For some of these compounds, affinity constants were calculated from 5-point inhibition curves using the EBDA-Ligand software, and expressed as $K_i$ ± SD.

Ligands **6d** and **6e** were evaluated for binding to human 5-$HT_4$ and other serotonin receptors (5-$HT_{1A-E}$, 5-$HT_{2A-C}$, 5-$HT_3$, 5-$HT_{5A}$, 5-$HT_6$, 5-$HT_7$) as well as for intrinsic activity at CEREP. Detailed assay protocols are available at the CEREP website (http://www.cerep.com).

## Acknowledgements

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## Supplementary Material

Copies of $^1H$, $^{13}C$, $^{19}F$ NMR spectra, and photographs of the Thin Layer Chromatography experiments.

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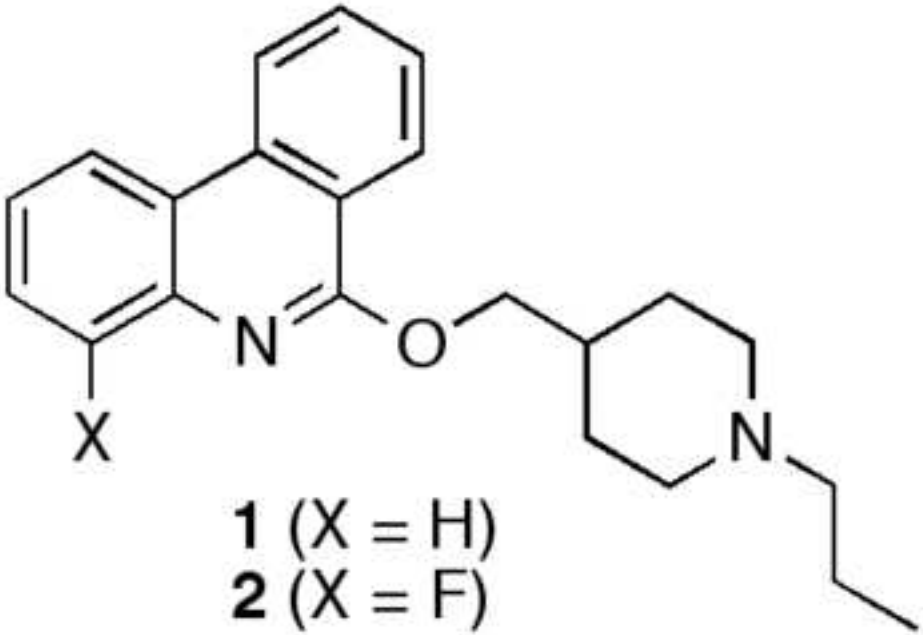
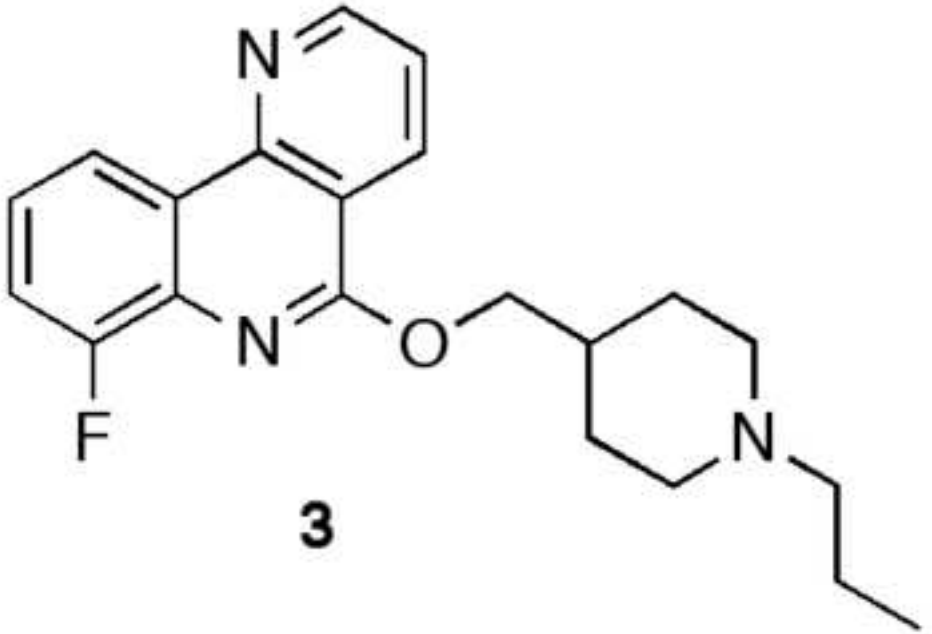



**1** (X = H)
**2** (X = F)

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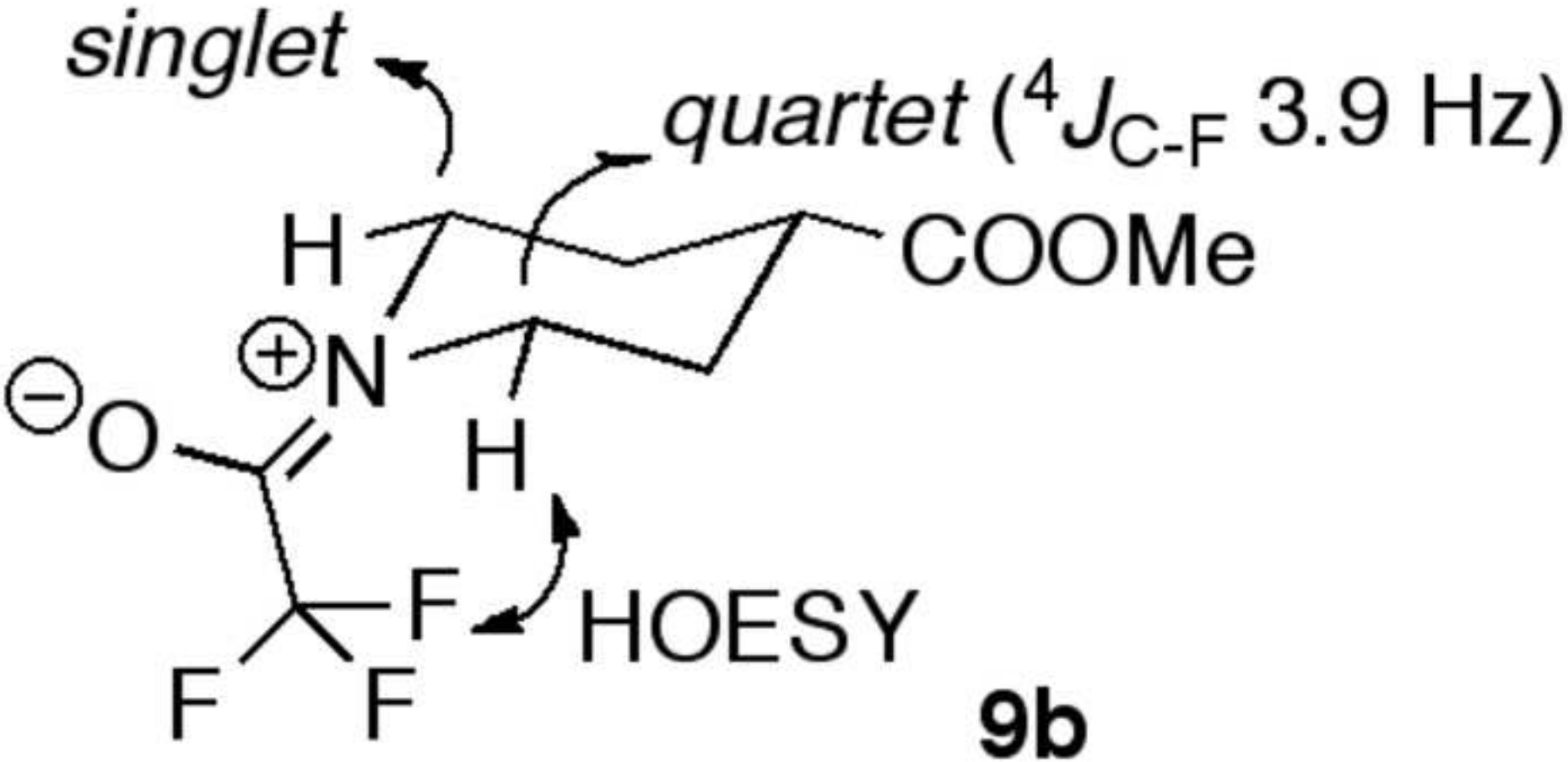
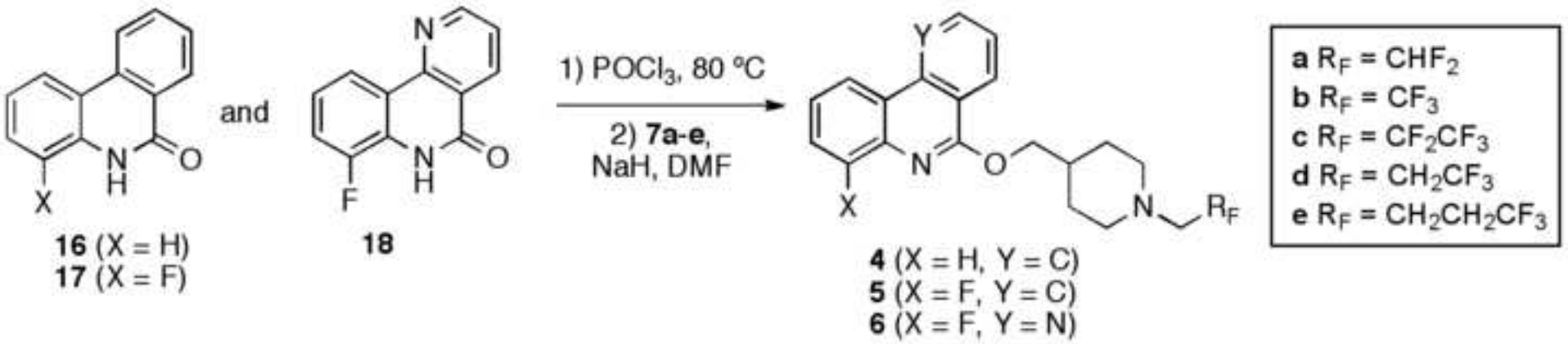



**9b**

Figure(s)





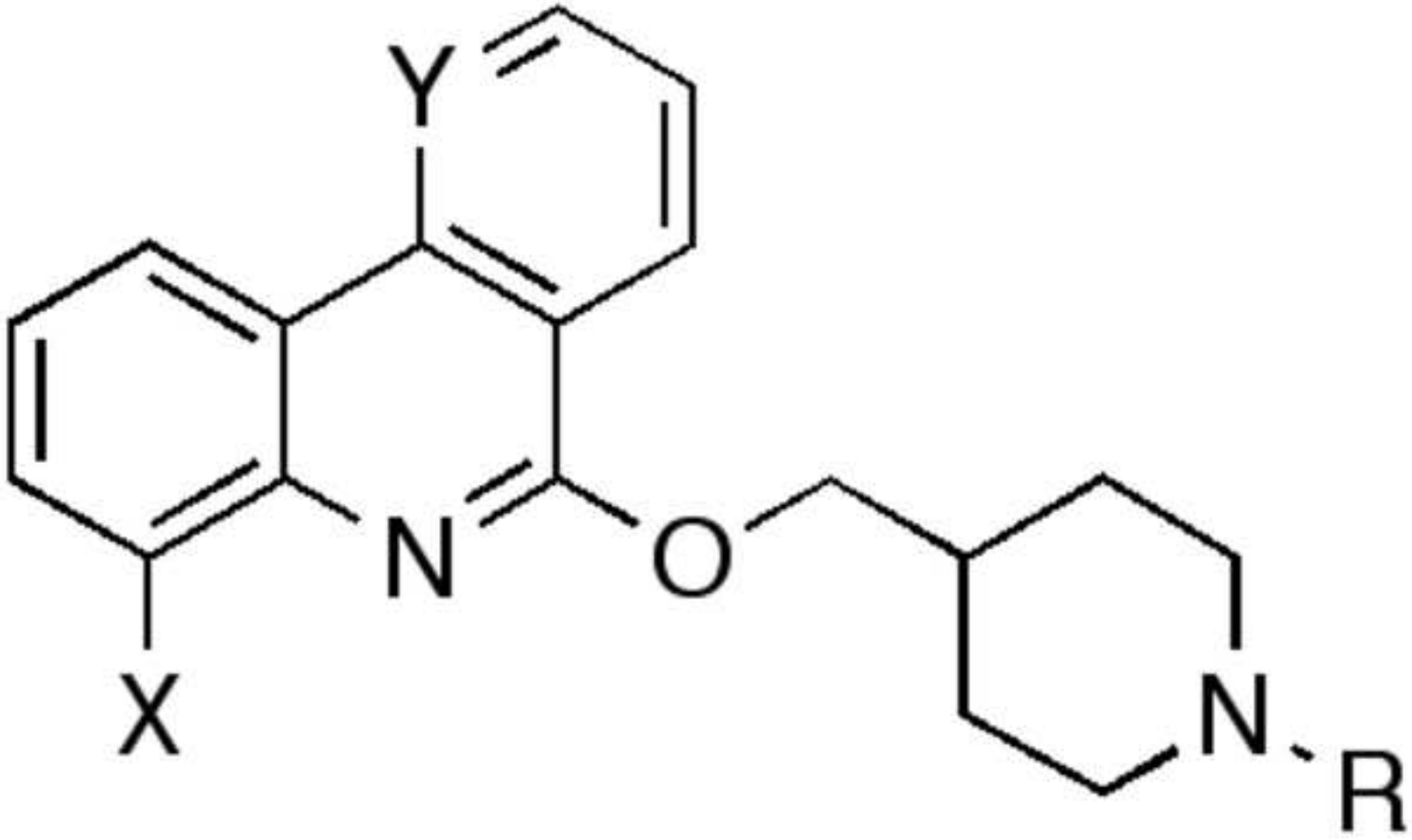

**16** (X = H)
**17** (X = F)

**18**

**4** (X = H, Y = C)
**5** (X = F, Y = C)
**6** (X = F, Y = N)

Figure(s)

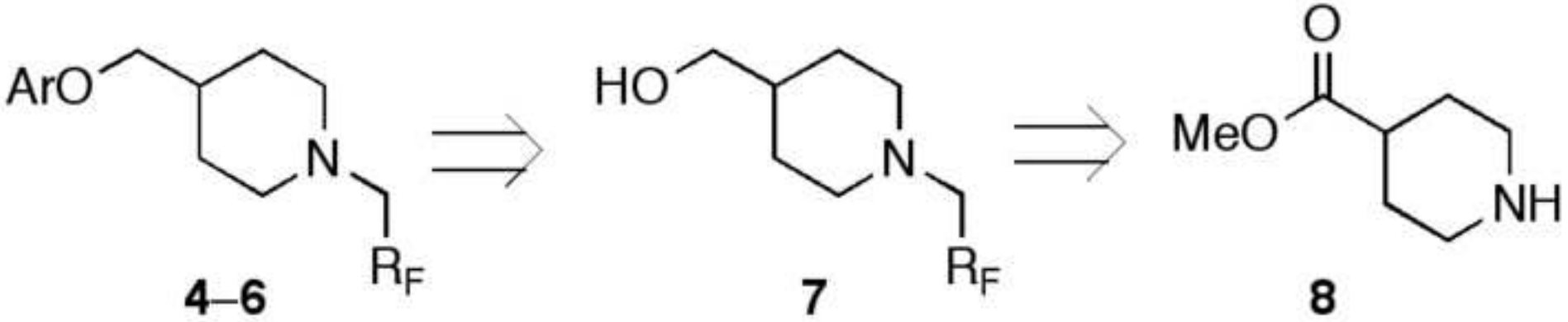




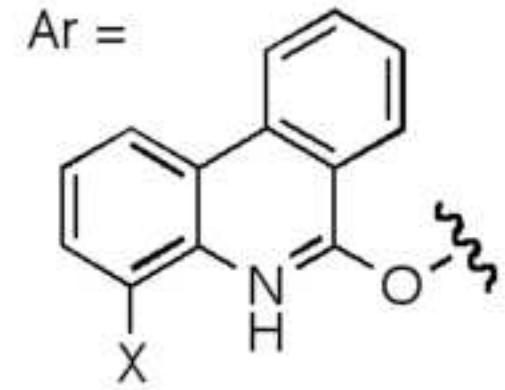

**1,4** (X = H, Y = C)
**2,5** (X = F, Y = C)
**3,6** (X = F, Y = N)

Figure(s)

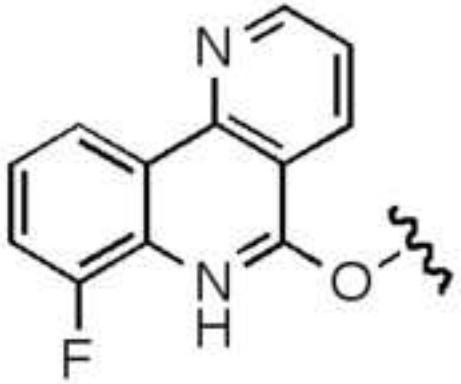





**4–6** **7** **8**




**4** (X = H); **5** (X = F)

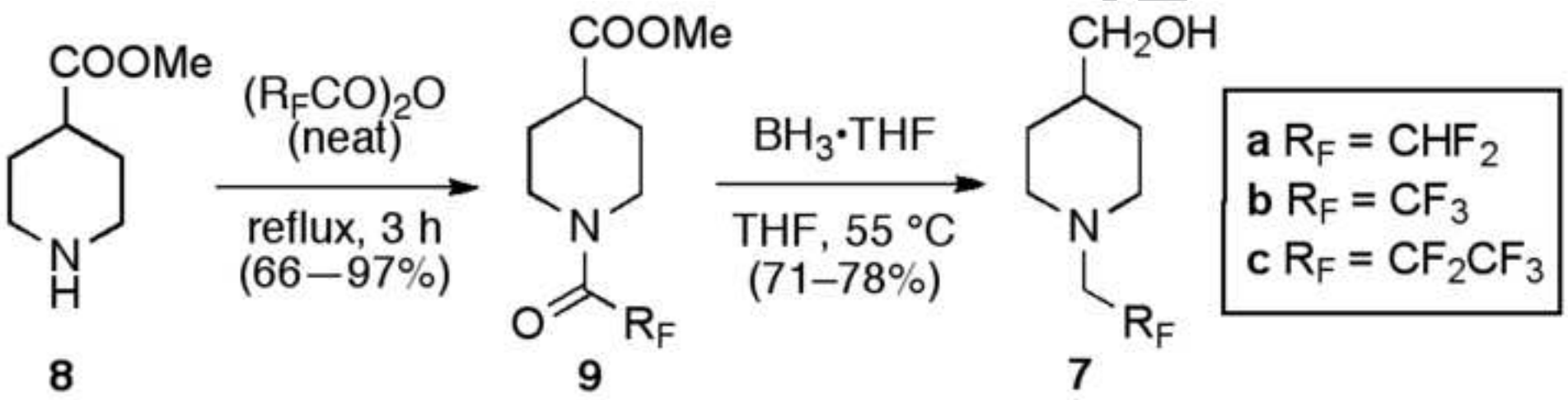



**6**

**a** $R_F = CHF_2$
**b** $R_F = CF_3$
**c** $R_F = CF_2CF_3$
**d** $R_F = CH_2CF_3$
**e** $R_F = CH_2CH_2CF_3$

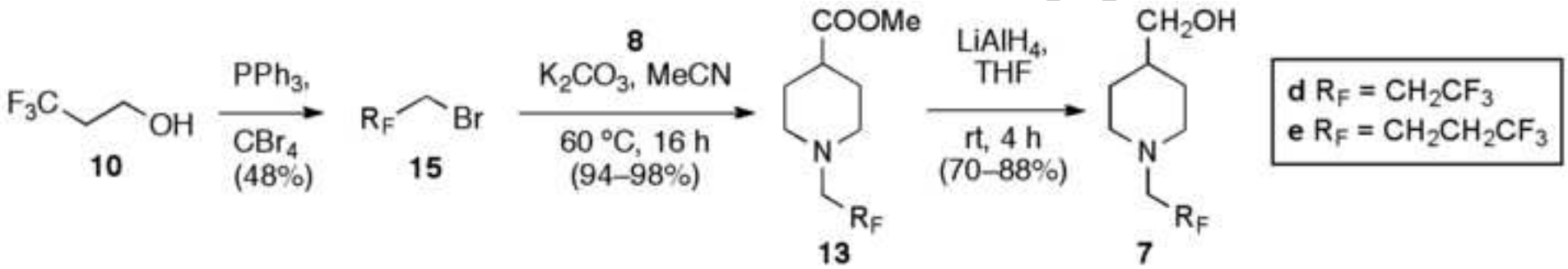
Figure(s)

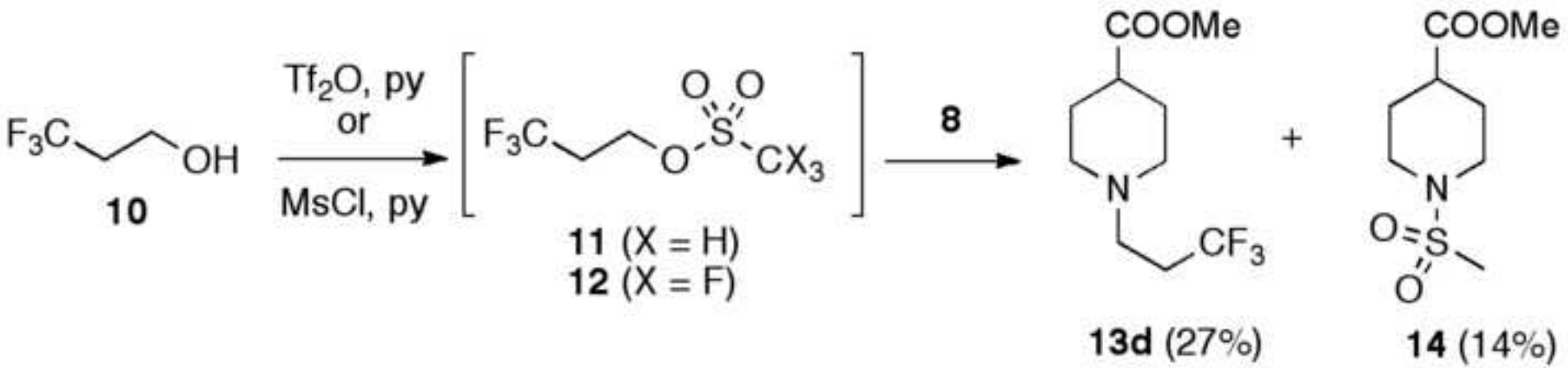




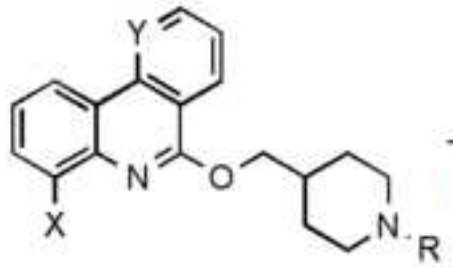
Figure(s)





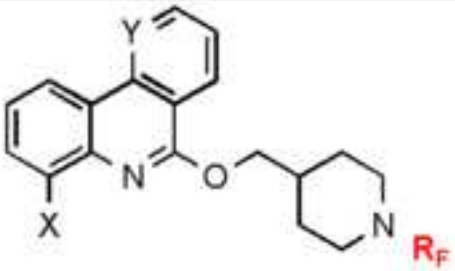


With
X = H, Y = CH
or
X = F, Y = CH
or
X = F, Y = N
and
R = Et, *n*-Pr, *n*-Bu




5-$HT_4$R selective antagonists

**Fluorine Introduction**




Basicity ? Liphophilicity ?
5-$HT_4$R binding Affinity and Selectivity ?
Pharmacological Profile ?