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# Novel multi target-directed ligands targeting 5-HT<sub>4</sub> receptors with *in cellulo* antioxidant properties as promising leads in Alzheimer's disease

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## KEYWORDS

Alzheimer's Disease; 5-HT<sub>4</sub> receptor; antioxidant; phenol; MTDL.

## ABSTRACT

Facing the complexity of Alzheimer's disease (AD), it is now currently admitted that a therapeutic **pleiotropic** intervention is needed to alter its progression. Among the major hallmarks of the disease, the amyloid pathology and the oxidative stress are closely related. We propose in this study to develop original Multi-Target Directed Ligands (MTDL) able to impact at the same time A $\beta$  protein accumulation and toxicity of Reactive Oxygen Species (ROS) in neuronal cells. Such MTDL were obtained by linking on a central piperidine two scaffolds of interest: a typical aminochlorobenzophenone present in numerous 5-HT<sub>4</sub>R agonists, and diverse antioxidant chemotypes. **Interestingly, the most active compound 9g possesses a  $K_i$  of 12.7 nM towards 5-HT<sub>4</sub>R and an antioxidant activity *in vitro* and *in cellulo*.**

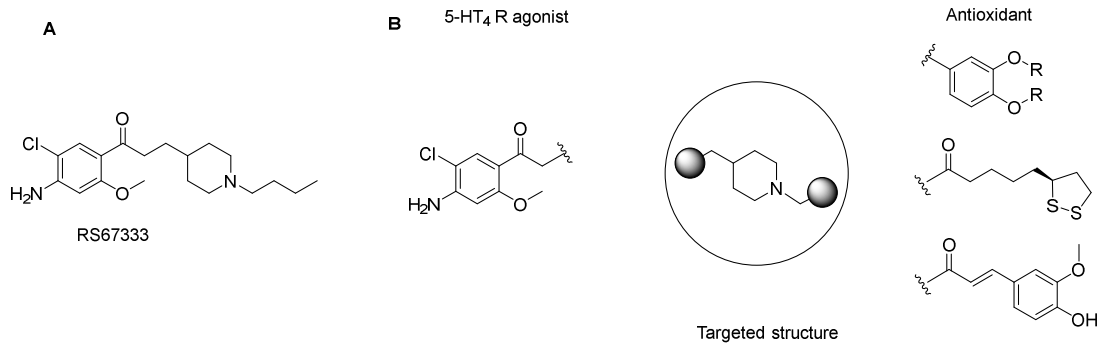
## Introduction

Alzheimer's disease (AD) is the most common form of dementia and affects more than 50 million people worldwide.<sup>1</sup> More than a century after its discovery, AD is now characterized by synaptic dysfunction and synapse loss correlated with cognitive decline.<sup>2</sup> These dysfunction are supposed to be due to the presence of two peptide deposits in and around neuronal cells. The accumulation and aggregation of A $\beta$  peptide is at the origin of the amyloid pathology whereas the neurofibrillary tangles are mainly composed of phosphorylated tau protein.<sup>3</sup> These lesions are generally associated with mitochondria dysfunction, an increase inflammatory response and the presence of oxidative stress.<sup>4</sup> The latter was further particularly linked to the presence of amyloid aggregates which can produce Reactive Oxygen Species (ROS) in the presence of metal ions and which ultimately leads to neuronal cell death.<sup>5</sup> The control of ROS production is therefore of particular interest in the prevention or the treatment of AD.

Despite these progresses, the discovery and development of treatments with disease-modifying effect on AD remain one of the greatest challenges in drug discovery.<sup>6</sup> After numerous failures in clinical trials with compounds targeting mainly amyloid<sup>7</sup> or tau pathologies,<sup>8</sup> it is now currently admitted that a pleiotropic approach is needed for this multifactorial neurodegenerative disease.<sup>9</sup> In order to impact several biological targets at the same time, single pleiotropic compounds, known as Multi-Target Directed Ligands (MTDLs),<sup>10,11,12</sup> have been developed during the last decade. Among them, many examples include compounds able to act on oxidative stress and on another target.<sup>13,14,15</sup> On the other hand, many examples have been described in order to limit amyloid pathology with numerous examples of MTDL acting on  $\beta$ -secretase inhibition,<sup>16</sup> or inhibiting A $\beta$  aggregation.<sup>14</sup> In addition to those classical targets, the serotonergic system appears

of particular interest, notably *via* the modulation of 5-HT<sub>4</sub> and 5-HT<sub>6</sub> receptors.<sup>17,18</sup> The activation of 5-HT<sub>4</sub> receptors (5-HT<sub>4</sub>R) with partial agonists, such as RS67333, is of particular interest as it can promote the non-amyloidogenic cleavage of Amyloid Protein Precursor (APP) and subsequently decrease the amyloid load in AD transgenic mice models.<sup>19,20</sup> In this context, we have in the past modulated the structure of RS67333 in order to introduce another target of choice for the treatment of AD such as acetylcholinesterase (AChE) inhibitory activity<sup>21,22</sup> or 5-HT<sub>6</sub>R antagonism.<sup>23</sup> These two studies demonstrated that the replacement of the butyl chain of RS67333 by diverse alkyl moieties has limited impact on both the affinity and the pharmacological profile towards 5-HT<sub>4</sub>R. A similar strategy has been recently followed in order to generate 5-HT<sub>6</sub>R antagonists bearing antioxidant activities.<sup>24</sup>

In this context, we would like to assess the possibility to introduce, on the central piperidine ring of the targeted structures (Figure 1), various chemical moieties known to exhibit antioxidant activities. We postulate that such MTDL would be of particular interest combining, for the first time in a unique compound, antioxidant properties as well as 5-HT<sub>4</sub>R agonism. Several antioxidant scaffolds were chosen for this proof of concept: polyphenol,<sup>25</sup> hydroxycinnamic acid<sup>26</sup> and lipoic acid.<sup>27,28</sup> Following this strategy, the two pharmacophores, will be connected through a permanent linker in order to optimize the central distribution of the antioxidant moiety. Indeed, the presence of the piperidine as a linker appears essential for the 5-HT<sub>4</sub>R activity and the generated compounds should possess physicochemical properties for CNS application.



**Figure 1.** Chemical structure of **RS67333** **A** and targeted structure of the MTDL **B**.

The influence of these substitutions on 5-HT<sub>4</sub>R affinity and the antioxidant activity of the resulting compounds could thus be assessed *in vitro* and *in cellulo*.

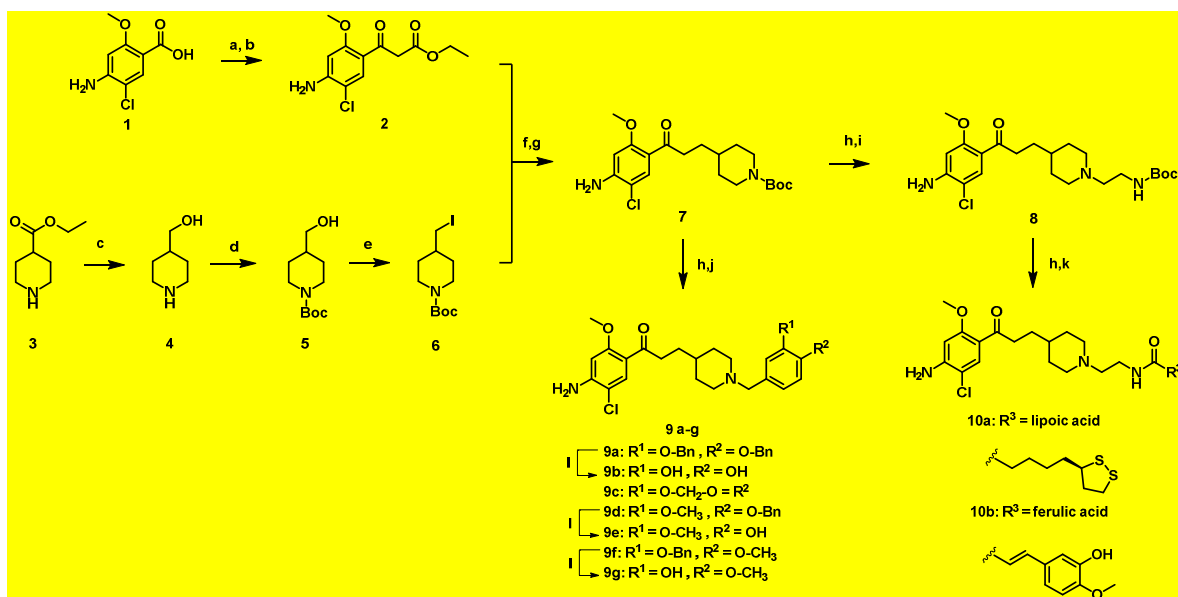
## Results

### Chemistry

According to our previous chemical studies, the precursor **7** appears as a central scaffold in the synthesis of RS67333.<sup>22,23</sup> Compound **7** was synthesized in a three-step sequence through formation of a  $\beta$ -keto ester **2** by reaction between acid **1**, activated *in situ* with CDI, and potassium 3-ethoxy-3-oxopropanoate.<sup>29,30</sup> Subsequent alkylation with a previously synthesized piperidine chain **6**, and followed by reaction with KOH in an EtOH/H<sub>2</sub>O mixture provided a saponification-decarboxylation reaction to afford **7**.<sup>22</sup> From this precursor **7**, different moieties were linked, such as catechol, vanillin or isovanillin widely known for their antioxidant properties.<sup>31,32</sup> The first compounds **9a**, **9c**, **9d**, and **9f** were obtained by alkylation with the corresponding bromobenzyl derivatives at room temperature. A final debenylation step was performed with HBr 33% in solution in acetic acid at 0°C to obtain free alcohols derivatives **9b**, **9e**, and **9g** respectively.

The second goal was to link ferulic and lipoic acid (LA) described as powerful antioxidants in the literature.<sup>33,34,35</sup> In order to link LA and ferulic acid while maintaining the basicity of the nitrogen of the piperidine, an ethylamine linker was added. According to literature,<sup>23</sup> an ethylamine linker has no influence on the 5-HT<sub>4</sub>R affinity and allowed the availability of a free terminal amine for peptidic coupling. Once the intermediate **8** was obtained, several coupling reactions were performed with EDCI, HOBt as activators, to synthesize coupled products **10a** and **10b**. Due to the sensitivity of LA to air, light, and heat, reactions were performed in dry, cold and dark conditions.

**Scheme 1.** Synthesis of the final derivatives **9-10**



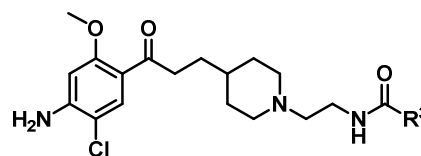
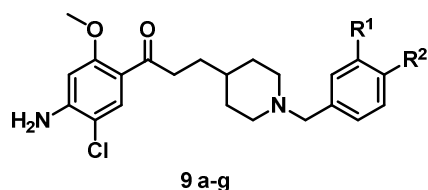
\*Reagents, conditions: (a) CDI, THF; (b) KO<sub>2</sub>CCH<sub>2</sub>CO<sub>2</sub>Et, MgCl<sub>2</sub>, THF, 40° C; (c) LiAlH<sub>4</sub>, Et<sub>2</sub>O, reflux; (d) (Boc)<sub>2</sub>O, DCM; (e) PPh<sub>3</sub>, I<sub>2</sub>, Imidazole, THF; (f) K<sub>2</sub>CO<sub>3</sub>, DMF; (g) KOH, EtOH/H<sub>2</sub>O; (h) TFA, DCM; (i) tert - butyl N - (2 - bromoethyl)carbamate, K<sub>2</sub>CO<sub>3</sub>, ACN ; (j) Bromobenzylalcohol, K<sub>2</sub>CO<sub>3</sub>, DCM ; (k) EDCI, HOBT, Et<sub>3</sub>N, 0° C to rt, in the dark ; (l) HBr 33% in AcOH, DCM, 0° C..

### *In vitro* evaluation

As previously mentioned, the main goal of our study was to discover a hit that will have sufficient antioxidant properties, as well as a good affinity toward 5-HT<sub>4</sub>R. All the synthesized compounds, were tested on 5-HT<sub>4</sub>R according to the Grossman et al method.<sup>36</sup> The affinity toward the receptor was evaluated using a displacement assay of the tritiated ligand [<sup>3</sup>H]-GR113808, a specific and highly potent 5-HT<sub>4</sub>R antagonist used to label specific binding sites in human brain.

**Table 1.** Binding to h5-HT<sub>4</sub>R and *in vitro* antioxidant activities of final derivatives





10a: R<sup>3</sup> = lipoic acid  
10b: R<sup>3</sup> = ferulic acid

Cmpd	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	cLogP*	<i>h5-HT<sub>4</sub>R</i>				<i>DPPH</i>	<i>ORAC</i>
					% inhibition at 10 <sup>-8</sup> M	% inhibition at 10 <sup>-6</sup> M	K <sub>i</sub> (nM)	IC <sub>50</sub> (nM)	EC <sub>50</sub> at 120 min (μM)	(μM <sub>compound</sub> / μM Trolox®)
<b>9a</b>	O-Bn	O-Bn	-	6.78	3%	89%	-	-	>250	< 0.2
<b>9b</b>	OH	OH	-	3.48	76%	100%	<b>5.9 ± 0.7</b>	<b>7.5 ± 0.7</b>	<b>19.6 ± 0.8</b>	<b>3.40 ± 0.02</b>
<b>9c</b>	O-CH <sub>2</sub> -O		-	3.84	22%	100%	-	-	>250	< 0.2
<b>9d</b>	O-CH <sub>3</sub>	O-Bn	-	5.55	0%	97%	-	-	>250	< 0.2
<b>9e</b>	O-CH <sub>3</sub>	OH	-	3.92	7%	95%	-	-	<b>180.3 ± 25.5</b>	<b>3.88 ± 0.55</b>
<b>9f</b>	O-Bn	O-CH <sub>3</sub>	-	5.62	7%	88%	-	-	>250	< 0.2
<b>9g</b>	OH	O-CH <sub>3</sub>	-	3.94	64%	100%	<b>12.7 ± 2.1</b>	<b>14.5 ± 5.8</b>	<b>85.5 ± 2.0</b>	<b>3.41 ± 0.59</b>
<b>10a</b>	-	-	Lipoic acid	4.98	26%	99%	-	-	>250	0.76 ± 0.11
<b>10b</b>	-	-	Ferulic acid	3.65	39%	100%	<b>101 ± 8.0</b>	<b>101 ± 3.6</b>	<b>45.5 ± 2.0</b>	<b>1.47 ± 0.25</b>
<b>Serotonin</b>	-	-	-	-	0%	65%	<b>246 ± 31</b>	<b>323 ± 25</b>	-	-
<b>Trolox</b>	-	-	-	-	-	-	-	-	<b>22.9 ± 0.9</b>	1
<b>Quercetin</b>	-	-	-	-	-	-	-	-	<b>9.3 ± 1.5</b>	<b>6.50 ± 0.73</b>

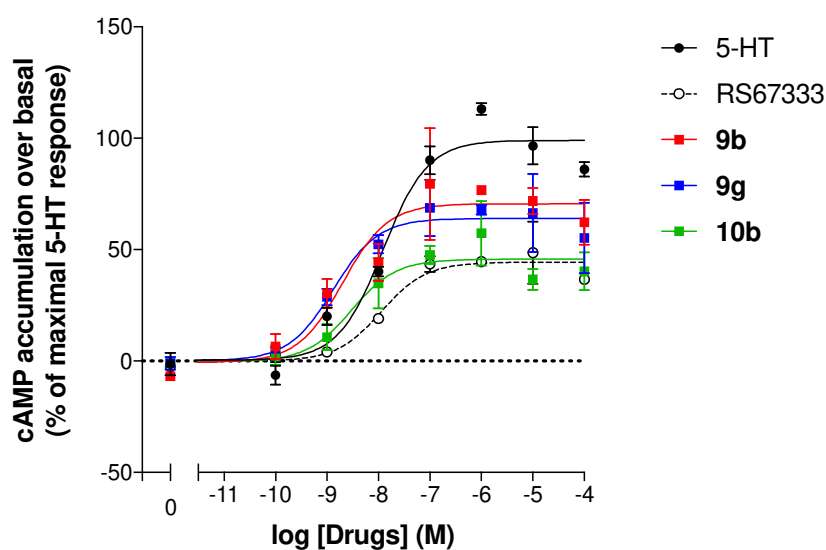
\* calculated logP with ALOGPS 2.1.

Those compounds were also developed for their antioxidant properties and preliminary antioxidant *in vitro* test was also performed. Our attention was focused on two tests, DPPH, and ORAC.<sup>37</sup> The ORAC<sub>Fl</sub> assay was conducted in an aqueous medium according to well-described procedure. It evaluates the antioxidant activity via a Hydrogen Atom Transfer (HAT) mechanism. Calculating the AUC of the fluorescence decrease for 1.5 hours, quantitative and kinetic aspects are both taken into account. Here, phenol gifted compounds showed a moderate antioxidant activity compared with a TEAC ranged between Trolox<sup>®</sup> (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic Acid), the reference, and quercetin (Table 1), a natural polyphenol antioxidant. The DPPH assay was performed in an organic solvent and enabled the evaluation of antioxidant activity following a Single-Electron-Transfer (SET) mechanism. According to recent literature, our initial procedure was completed here by slight modifications to ensure a complete evaluation of our compounds. First calculation of the specific absorbance of DPPH subtracting the absorbance of others components in the solution was realized. The maximum effect was normalized using a high dose of Trolox<sup>®</sup> (375  $\mu$ M) to get the absolute EC<sub>50</sub>.<sup>38</sup> The kinetic analysis to reach the plateau of % DPPH RSA at infinite time was determined and EC<sub>50</sub> were calculated using linear or non-linear regression ( $R^2 > 0.98$ ) from the dose-effect curves.

In these conditions, Trolox<sup>®</sup> and quercetine reached their maximal antioxidant activity very quickly (immediately and few minutes respectively) compared to other active compounds ( $T_{1/2}$  about 30 min). Their dose-effect curve was a linear ( $R^2 > 0.99$ ). In our phenol series, when DPPH EC<sub>50</sub> was calculable, EC<sub>50</sub> values obtained after 120 min were very spread from 19.6 to >250  $\mu$ M, varying as a terminal chemical pattern.

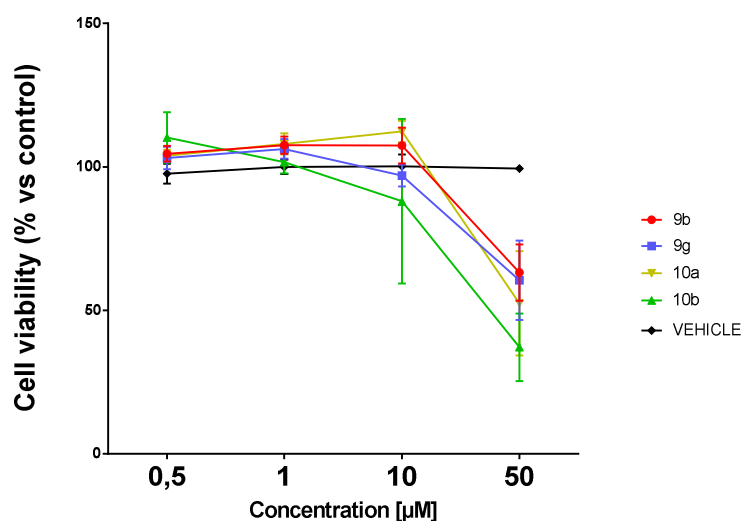
### *In cellulo* evaluation

The pharmacological profile of the selected compounds was assayed towards h5-HT<sub>4</sub>R transiently expressed in COS-7 cells (Figure 2). Three compounds behaved as partial agonists of the receptor with EC<sub>50</sub> of 2.8 ± 1.3 nM, 1.4 ± 0.8 nM and 4.5 ± 2.1 nM for **9b**, **9g** and **10b**, respectively (Figure 4). Compound **10a** also behaved as a partial agonist of the 5-HT<sub>4</sub>R, but its irregular profile suggested that it may engage other targets in the COS-7 and no clear pharmacological profile was established. Compound **10b** presented an E<sub>max</sub> of 47 ± 8% that is similar to the one of RS67333, the 5-HT<sub>4</sub>R agonist of reference. Compounds **9b** and **9g** showed greater efficacy than the reference to induce cAMP production with E<sub>max</sub> of 72 ± 10% and 57 ± 3%, respectively.

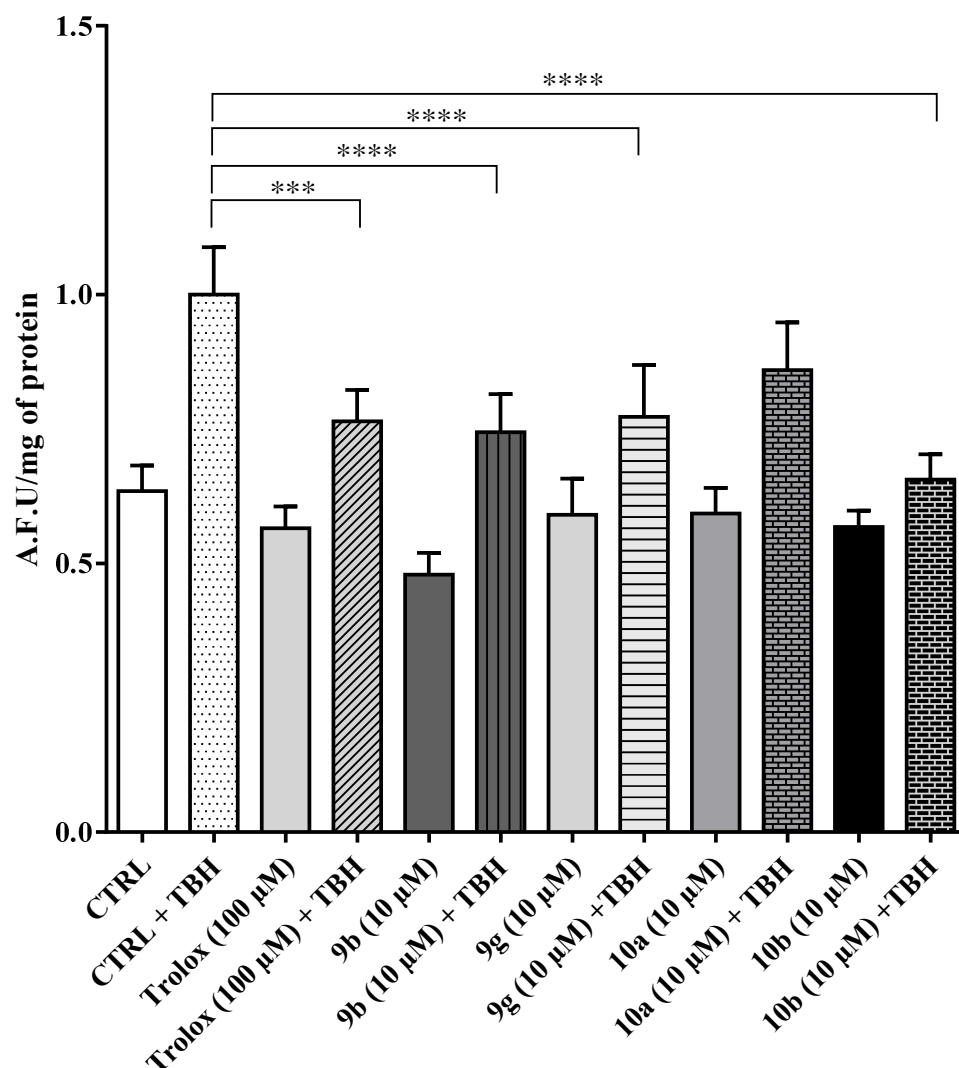


**Figure 2.** Determination of the 5-HT<sub>4</sub>R pharmacological profiles for compounds **9b**, **9g** and **10b**, by quantification of cAMP accumulation. The agonism activity of the compounds was determined with serotonin (5-HT) and the partial agonist RS67333 as references. Data presented are the mean of three independent experiments performed in duplicate.

The first *in vitro* antioxidant assays allowed us to select **9b**, **9g**, **10a-b**, to complete their antioxidant evaluation in a cellular context. To define the suitable concentration, the cytotoxicity of **9b**, **9g**, **10a-b** was determined by MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyl tetrazolium bromide) assay<sup>39</sup> in a neuroblastoma cell line, SH-SY5Y. As seen on Figure 3, treatment of the cells with compounds **9b**, **9g**, **10a** (0.5 to 10  $\mu$ M) did not show cytotoxicity. A little decrease of 12% of viability compared to control cells was seen with compound **10b**. While at a dose of 50  $\mu$ M all compounds seem to show a small cytotoxicity with a decreasing of 37%, 40%, 48%, 68% for **9b**, **9g**, **10a**, **10b** respectively. Therefore, a single dose of 10  $\mu$ M was chosen to perform the *in cellulo* antioxidant assay.



**Figure 3** Figure 3 Cell viability determined by MTT assay. Cells were grown into 96-well plates and treated with **9b**, **9g**, **10a** and **10b** for 24 hours at the concentration of 0.5, 1, 10 and 50  $\mu$ M or vehicle (DMSO). Data are presented as percentage of viable cells in comparison with vehicle treated controls. Error bars indicate  $\pm$  SEM.



**Figure 4** Reactive oxygen species determination in live SH-SY5Y cells. Cells were grown in 96 wells plates and incubated for 24 hours with compounds **9b**, **9g**, **10a** and **10b**, Trolox or vehicle (CTRL). Oxidative stress was induced by treating the cells with 100μM TBH for 30 minutes and detected by staining the cells with the fluorogenic probe H<sub>2</sub>DCF-DA. Data are presented as Arbitrary Fluorescence Units normalized on protein content measured by Lowry method. Error bars indicate ± SEM. \*\*\* P < 0.001, \*\*\*\* P < 0.0001

The antioxidant activity of selected compounds was evaluated on live SH-SY5Y cells using the fluorogenic probe H<sub>2</sub>DFCDA. The cell-permeant H<sub>2</sub>DFCDA passively diffuses into cells and is retained in the intracellular level after cleavage by intracellular esterases. Upon oxidation by

ROS, the nonfluorescent H<sub>2</sub>DCF is converted to the highly fluorescent 2',7'-dichlorofluorescein (DCF) as described by Wang in 1999.<sup>40</sup> The level of oxidative stress is directly correlated with the intensity of DCF fluorescence.

To evaluate the antioxidant activity, cells were incubated for 24 hours with the selected compounds, Trolox or vehicle. Then cells were incubated with 10 $\mu$ M H<sub>2</sub>DCFDA probe for 30 minutes. After this time the cells were carefully washed with PBS and the oxidative stress was induced by 30 minutes treatment with tert-butyl hydroperoxide (t-BuOOH or TBH). The fluorescence intensity was normalized on protein content (Figure 4).

## Discussion

We have demonstrated in our previous study<sup>22</sup> that the introduction of a non-substituted benzyl group instead of the butyl group present in RS67333 has little impact on affinity towards the 5-HT<sub>4</sub>R and on Acetylcholinesterase inhibition. Based on these results a first series of hydroxyl or methoxy substituted benzylated derivatives was generated. In a previous study, we have demonstrated that the replacement of the butyl substituent of the piperidine of RS67333 by benzyl or methylenecyclohexyl moiety leads to potent AChE inhibitors with good physicochemical properties and in vivo promnesiant activities.<sup>22</sup> In order to verify the impact of these modulations on drugability parameters, cLogP were determined. If the protected benzylated compounds **9a**, **9d** and **9f** appear particularly lipophilic, all final compounds are compatible with optimal CNS parameters.<sup>41</sup> If benzyl piperidine scaffolds are well known pharmacophores for cholinesterase interaction,<sup>42</sup> we have verified that the RS67333 analogs were selective towards butyrylcholinesterase.<sup>22</sup> Docking studies in AChE binding site revealed that the orientation of those ligands was similar to Donepezil and reproduced its interactions. In particular, the benzyl

moiety is located at the bottom of the aromatic gorge of AChE and engaged in a hydrophobic interaction with Trp86. We postulate that the introduction of polar or bulky substituents on the benzyl group of our final compounds will be detrimental for their activities. In order to verify this hypothesis, all final compounds **9a-g** and **10a-b** were evaluated as inhibitors of human AChE. As expected, these modulations were particularly detrimental for the affinity of the ligands towards AChE (See Table S1 in Supporting Informations). Concerning the impact of these modulations on 5-HT<sub>4</sub>R activity, as shown in Table 1, all compounds exhibited good inhibition rates at 10<sup>-6</sup> M. However, the benzyl protecting group present on the scaffold of compounds **9a**, **9d** and **9f** has detrimental impact on affinity towards 5-HT<sub>4</sub>R receptors probably due to its steric hindrance. On the contrary, smaller substituents lead to enhanced activity. In this assay only **9b**, **9g**, and **10b** exhibited more than 35% of inhibition rate at 10<sup>-8</sup> M allowing us to calculate further data such as IC<sub>50</sub> and K<sub>i</sub>. Surprisingly the vanillin derivative **9e** showed a decreased potency compared to the isovanillin analog **9g**. On the contrary, **9b** and **9g** are really promising with K<sub>i</sub> values under 15 nM. According to our hypothesis, compounds **9b**, **9g** and **10a** behave as partial agonist for the 5-HT<sub>4</sub>R, the first two being able to induce cAMP production with a greater efficiency than the reference RS67333 (Figure 2).

All compounds were then engaged in two complementary *in vitro* antioxidant biological assays. In the DPPH assay, a SET mechanism test, hydroxylated compounds **9b**, **9e**, **9g** and **10b** were the only one to exert antioxidant activity, the catechol **9b** being the most potent. In this assay, the lipoic acid derivative **10a** did show limited antioxidant activity. In the ORAC evaluation, the antioxidant activities of all compounds were compared to Trolox<sup>®</sup>, a widely used antioxidant reference compound. The same compounds **9b**, **9e** and **9g** appeared to be 3-fold more potent than Trolox<sup>®</sup>, whereas ferulic derivative **10g** demonstrated similar activity than the reference. Both

test confirm that the *in vitro* activity is clearly linked to the introduction of a phenol group as all benzylated compounds are inactive in this assay.

Based on the results we have chosen to test the *in cellulo* antioxidant activity of these compounds **9b**, **9g**, **10a** and **10b**. Compound **9e** was not selected because of its lower affinity towards 5-HT<sub>4</sub>R. As already demonstrated for prucalopride, another 5-HT<sub>4</sub>R agonist, we have chosen to evaluate this antioxidant and neuroprotective activities in SH-SY5Y neuroblastoma cell line.<sup>43</sup> Lipoic acid antioxidant activity is dependent of mitochondrial activity<sup>44</sup> and therefore cannot be highlighted by an *in vitro* assay. For that reason, **10a** was also selected despite no sign of *in vitro* antioxidant effect.

A non-toxic dose of 10µM was chosen for the *in cellulo* antioxidant activity assay, after a general MTT viability assay. Oxidative stress was generated using TBH and the ability of the compounds to limit ROS induction was compared with the well-known antioxidant compound Trolox®.<sup>45</sup>

Trolox® was used at 100 µM and, as expected, exerts a significant reduction of ROS production. Used at a concentration of 10µM in this assay, compounds **9b**, **9g** and **10b** used at a concentration of 10µM significantly decreased ROS production by at least 50% compared to the control group. However, no activity was obtained in this assay with the lipoic acid substituted compound **10a**.

These results are in accordance with the pharmacological profile of the compounds on 5-HT<sub>4</sub>R, towards which no clear profile was established for compound **10a**. The three other compounds demonstrated clear agonism toward the receptor with the catechol substituted compound **9b** and the isovanillin substituted compound **9g** being interesting candidates to further *in vivo* evaluation.

## Conclusion



This study demonstrates for the time the possibility to generate a series of potent 5-HT<sub>4</sub>R ligands with promising antioxidant activity. Among them compound **9g** appears as the best compromise with a K<sub>i</sub> of 12.7 nM toward 5-HT<sub>4</sub>R and an antioxidant activity *in cellulo*. If the balance of activity between the *in cellulo* or *in vitro* antioxidant assay (micro molar range) and the *in vitro* assay for 5-HT<sub>4</sub>R affinity (nanomolar range) might be improved, these series of compound show great promises in more challenging models. In the experiment, the amount of oxidative stress needs to be high enough to be visible with DCF-DA. In the brain, the level of oxidative stress caused by AD is not properly quantified and is lower than the one induced by t-BuOOH. On the base of the obtained data, it seems plausible that, with a suitable pharmacokinetic and a proper Blood Brain Barrier crossing according to its adequate LogP, **9g** could have an antioxidant effect at a nanomolar concentration and will be tested in a near future in preclinical animal models of AD.

## Experimental section

**Chemistry.** All commercially available compounds were used without further purification. Melting points were determined on a Stuart melting point apparatus (SMP50). Analytical thin-layer chromatography (TLC) was performed on silica gel 60 F<sub>254</sub> on aluminium plates (Merck) and visualized with UV light (254 nm). Flash chromatography was conducted on a VWR SPOT II Essential instrument with silica gel 60 (40-63 $\mu$ m). Column size and flow rate followed the manufacturer's recommendations. NMR spectra were recorded at 295K, at 400 or 500 MHz (Bruker Avance III 400/500 MHz) for <sup>1</sup>H NMR and at 100 or 126 MHz for <sup>13</sup>C NMR in chloroform-*d*, methanol-*d*<sub>4</sub> or DMSO-*d*<sub>6</sub> with chemical shifts ( $\delta$ ) given in parts per million (ppm) relative to TMS as internal standard and recorded. The following abbreviations are used to describe peak splitting patterns when appropriate: br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, dt = doublet of triplet. Coupling constants *J* are reported in hertz units (Hz). Infrared spectra (IR) were obtained on a PERKIN-ELMER FT-IR spectrometer and are reported in terms of frequency of absorption (cm<sup>-1</sup>) using KBr discs. High-resolution mass spectra (HRMS) were obtained by electrospray (ESI, sampling cone 50V, capillary 0.3kV) on a Xevo G2-XS QToF WATERS mass spectrometer. LC-MS (ESI) analyses were realized with Waters Alliance 2695 as separating module using the following gradients: A (95%)/B (5%) to A (5%)/B (95%) in 4.00min. This ratio was hold during 1.50 min before return to initial conditions in 0.50 min. Initial conditions were then maintained for 2.00 min (A = H<sub>2</sub>O, B = CH<sub>3</sub>CN; each containing HCOOH : 0.1 %; column XBridge BEH C18 2.5  $\mu$ m/3.0 x 50 mm; flow rate 0.8 mL/min). MS were obtained on a SQ detector by positive ESI. Mass spectrum data are reported as m/z.

### General procedure for deprotection/alkylation of the piperidine moiety A

To a stirred solution of tert-butyl piperidine-1-carboxylate derivative (1.0 eq.) in DCM (20 mL/mmol) was added TFA (2 mL/mmol). The resulting mixture was stirred at room temperature for 1h. Removal of the solvent under vacuum afforded the crude product, which was directly engaged in the next step. The residue obtained (1.0 eq.) was dissolved in DCM or ACN (10 mL/mmol) and bromo-benzyl-alcohol derivatives (1.1-1.5 eq.) and K<sub>2</sub>CO<sub>3</sub> (10.0 eq.) were added. The resulting mixture was stirred at RT or 70°C depending on the product for 2-72h, then concentrated *in vacuo*. The crude was directly purified by chromatography on silica gel column and concentrated under reduced pressure to afford alkylated compound (isolated yields).

### **The general procedure to obtain unprotected catechol B**

Appropriate benzylated product (1 eq) was stirred in bromhydric acid 33 % diluted in acetic acid (10 mL.mmol<sup>-1</sup>, 0.10M) and DCM (50 mL.mmol<sup>-1</sup>, 0.02M) at 0°C for 1 to 4h. The mixture was evaporated in vacuo at rt. Then the crude was directly purified on silica gel column (DCM/MeOH; from 100:0 to 80:20).

### **Ethyl 3-(4-amino-5-chloro-2-methoxyphenyl)-3-oxopropanoate (2)**

To a solution of 4-amino-5-chloro-2-methoxybenzoic acid (5.00 g, 24.8 mmol, 1.0 eq.) in THF (200 mL, 0.12 M) was added CDI (4.42 g, 27.3 mmol, 1.1 eq.) portionwise. The mixture was stirred at rt for 4h. Then, ethyl potassium malonate (5.06 g, 29.7 mmol, 1.2 eq.) and MgCl<sub>2</sub> (2.83 g, 29.7 mmol, 1.2 eq.) were added portionwisely. The resulting mixture was stirred 16h at 40°C.

Solvent were evaporated under vacuum. The residue was diluted with water and then extracted with DCM three times. Organics layers were combined and washed with NaHCO<sub>3</sub> sat. solution, then brine, and dried over MgSO<sub>4</sub>. Solvent was evaporated under vaccum to afford a crude product which was purified on silica gel column (Cyclohexane/EtOAc from 100:0 to 60:40). The

compound was obtained as a light yellow powder (3.76 g, 60% yield). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.92 (s, 1H), 6.23 (s, 1H), 4.55 (s, 2H), 4.17 (q, *J* = 7.1 Hz, 2H), 3.87 (s, 2H), 3.82 (s, 3H), 1.23 (t, *J* = 7.1 Hz, 3H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 189.6, 168.6, 159.9, 148.7, 132.5, 117.3, 111.7, 97.0, 60.8, 55.4, 50.5, 14.2 MS *m/z* [M+H]<sup>+</sup> 272.51 IR (neat, cm<sup>-1</sup>) ν 3463, 3363, 3223, 2983, 1725, 1648, 1622, 1573, 1479, 1457 1326, 1220, 837, 654 Mp 117°C. Spectral and analytical data matched with literature.<sup>21</sup>

#### **(piperidin-4-yl)methanol (4)**

To a solution of ethyl isonipecotate (2 mL, 13.0 mmol, 1.0 eq.) in Et<sub>2</sub>O (150 mL) was added portion-wise LiAlH<sub>4</sub> (1.48 g, 39.0 mmol, 3.0 eq.) at 0°C and the reaction mixture was refluxed for 7h. The mixture was then cooled to 0°C and the reaction was then slowly quenched with 1.5 mL of water, 1.5 mL of 15% NaOH solution followed by 4.5 mL of water to afford a granular inorganic precipitate. The white solid was filtered and washed several times with EtOAc. Evaporation of the filtrate under reduced pressure provided 1.38 g (92% yield) of compound (1) as a colorless oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 3.45 (d, *J* = 5.9 Hz, 2H), 3.08 (m, 2H), 2.59 (dt, *J* = 12.0 Hz, *J* = 3.0 Hz, 2H), 1.96 (br s, 1H), 1.71 (m, 2H), 1.60-1.55 (m, 1H), 1.17-1.07 (m, 2H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 66.9, 45.9 (2C), 38.8, 29.7 (2C); IR (neat, cm<sup>-1</sup>) ν 3369 , 2923, 2855, 1636, 1531, 1425, 1266, 1036. Spectral and analytical data matched with literature.<sup>46</sup>

#### ***Tert*-butyl 4-(hydroxymethyl)piperidine-1-carboxylate (5)**

To a solution of (1) (1.14 g, 9.96 mmol, 1.0 eq.) in DCM (40 mL) was added di-*tert*-butyldicarbonate (2.39 g, 10.9 mmol, 1.1 eq.). The solution was stirred for 15h at room temperature. The mixture was then diluted with water and the aqueous layer was extracted several times with DCM. The organic layer was dried over MgSO<sub>4</sub> and concentrated in vacuo.

The crude product was purified by chromatography on silica gel column (cyclohexane/EtOAc, gradient 100:0 to 70:30) to give 1.34 g (62% yield) of compound (**2**) as white solid.

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  4.10 (m, 2H), 3.48 (d,  $J = 6.1$  Hz, 2H), 2.68 (m, 2H), 1.80 (br s, 1H, OH), 1.69 (m, 2H), 1.63 (m, 1H), 1.44 (s, 9H), 1.12 (m, 2H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  155.0, 79.5, 67.6, 43.9 (2C), 38.9, 28.7 (2C), 28.6 (3C); MS  $m/z$   $[\text{M}+\text{H}]^+$  216.47;  $[\text{M}-\text{Boc}+\text{H}]^+$  116.47;  $[\text{M}-t\text{-butyl}+\text{H}]^+$  160.47; IR (neat,  $\text{cm}^{-1}$ )  $\nu$  3470, 2926, 2859, 1672, 1422, 1168, 1045, 572; Mp 85°C.

#### ***Tert*-butyl 4-(iodomethyl)piperidine-1-carboxylate (**6**)**

To a solution of (**2**) (2.25 g, 10.5 mmol, 1.0 eq.), imidazole (858 mg, 12.6 mmol, 1.2 eq.) and triphenylphosphine (3.30 g, 12.6 mmol, 1.2 eq.) in dry THF (30 mL) was added drop-wise a solution of iodine (3.20 g, 12.6 mmol, 1.2 eq.) in dry THF (30 mL) at 0°C. The reaction mixture was stirred at room temperature for 14h. The mixture was quenched with a saturated  $\text{Na}_2\text{S}_2\text{O}_3$  solution. The aqueous layer was extracted with EtOAc. The organic layer was washed with water, dried over  $\text{MgSO}_4$ , and concentrated in vacuo. The crude was several times diluted with cold  $\text{Et}_2\text{O}$ , and white crystals were filtered. After concentration of filtrate in vacuo, 3.13 g of the compound (**3**) was obtained as a colorless oil (92% yield).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  4.11 (m, 2H), 3.10 (d,  $J = 5.8$  Hz, 2H), 2.68 (m, 2H), 1.83 (m, 2H), 1.64-1.61 (m, 1H), 1.46 (s, 9H), 1.19-1.09 (m, 2H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  154.6, 79.5, 43.4 (2C), 38.6, 32.5 (2C), 28.4 (3C), 13.5; IR (neat,  $\text{cm}^{-1}$ )  $\nu$  2975, 2932, 2850, 1692, 1422, 1243, 1157, 965. Spectral and analytical data matched with literature.<sup>47</sup>

***Tert*-butyl 4-[3-(4-amino-5-chloro-2-methoxyphenyl)-3-oxopropyl]piperidine-1-carboxylate (7)**

To a solution of ethyl 3-(4-amino-5-chloro-2-methoxyphenyl)-3-oxopropanoate (**4**) (3.14 g, 11.6 mmol, 1.0 eq.) in DMF (30 mL) and *tert*-butyl 4-(iodomethyl)piperidine-1-carboxylate (**3**) (4.14 g, 12.7 mmol, 1.1 eq.) was added K<sub>2</sub>CO<sub>3</sub> (3.19 g, 23.10 mmol, 2 eq.). The mixture was stirred 72h at rt. The reaction mixture was diluted with water, and product was extracted with EtOAc. Combined organic layers were washed with water and brine and then dried over MgSO<sub>4</sub>. Evaporation of the solvent gave a crude mixture which was directly dissolved in EtOH (500 mL), H<sub>2</sub>O (100 mL) and KOH (3.23 g, 57.5 mmol, 5.5 eq.) were added. The reaction mixture was refluxed for 3h, EtOH was evaporated. The mixture was diluted with H<sub>2</sub>O and extracted with EtOAc. The organic layer was washed with H<sub>2</sub>O and brine. Evaporation of the solvent provided a crude product, which was purified by column on silica gel, eluting on DCM/EtOAc from 100:0 to 60:40 to give the product **5** as a colorless solid (3.11 g, 67% isolated yield).

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.79 (s, 1H), 6.26 (s, 1H), 4.46 (s, 2H), 4.18 – 3.98 (m, 2H), 3.85 (s, 3H), 2.91 (dd, *J* = 8.3, 6.9 Hz, 2H), 2.67 (t, *J* = 12.4 Hz, 2H), 1.72 – 1.64 (m, 2H), 1.63 – 1.58 (m, 2H), 1.58 – 1.54 (m, 1H), 1.45 (s, 9H), 1.10 (qd, *J* = 12.4, 4.3 Hz, 2H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100.4 MHz) δ 198.7, 159.5, 154.9, 147.7, 132.2, 118.9, 111.3, 97.5, 79.2, 55.7, 44.0, 40.6, 35.8, 32.1, 31.1, 28.5. MS *m/z* [M+H]<sup>+</sup> 397.60 ; [M-Boc+H]<sup>+</sup> 297.51; [M-*t*-butyl+H]<sup>+</sup> 341.55; IR (neat, cm<sup>-1</sup>) ν 3470, 3353, 2975, 2930, 2851, 1673, 1624, 1588, 1421, 1366, 1311, 1250, 1216, 1173, 967, 738; Mp 136°C. Spectral and analytical data matched with literature.<sup>21</sup>

***Tert*-butyl *N*-(2-{4-[3-(4-amino-5-chloro-2-methoxyphenyl)-3-oxopropyl]piperidin-1-yl}ethyl)carbamate (8)**

Following procedure A with *tert*-butyl 4-[3-(4-amino-5-chloro-2-methoxyphenyl)-3-oxopropyl]piperidine-1-carboxylate (**7**) (2.37 g, 5.98 mmol, 1 eq.) and *tert*-butyl *N*-(2-bromoethyl)carbamate (1.61 g, 7.18 mmol, 1.2 eq.) synthesized following the literature, the reaction was stirred at rt for 16h. After a purification by chromatography on silica gel column (Cyclohexane/EtOAc, gradient 100:0 to 0:100), the compound (**8**) was obtained as a light yellow solid (780 mg, 30% isolated yield).

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.71 (s, 1H), 6.20 (s, 1H), 4.98 (br s, 1H, NH), 4.49 (s, 2H, NH<sub>2</sub>), 3.77 (s, 3H), 3.14 (t, *J* = 6.0 Hz, 2H), 2.86 – 2.72 (m, 4H), 2.35 (t, *J* = 6.0 Hz, 2H), 1.92 – 1.76 (m, 2H), 1.67 – 1.54 (m, 2H), 1.56 – 1.44 (m, 2H), 1.38 (s, 9H), 1.24 – 1.08 (m, 3H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100.0 MHz) δ 199.0, 159.4, 156.0, 147.7, 132.2, 118.9, 111.2, 97.5, 79.1, 57.5, 55.6, 53.8 (2C), 40.9, 37.4, 35.6, 32.2 (2C), 31.3, 28.5 (3C); MS *m/z* [M+H]<sup>+</sup> 441.8; IR (neat, cm<sup>-1</sup>) ν 3486, 3376, 3325, 2980, 2925, 2855, 1690, 1625, 1573, 1467, 1367, 1311, 1175, 1216, 1114, 969, 610; Mp 119°C; HRMS/ESI: *m/z* calcd. for C<sub>22</sub>H<sub>34</sub>ClN<sub>3</sub>O<sub>4</sub> [M+H]<sup>+</sup> 440.2316, found 440.2324;

**1-(4-amino-5-chloro-2-methoxy-phenyl)-3-[1-[(3,4-dibenzyloxyphenyl)methyl]-4-piperidyl]propan-1-one (9a)**

Following procedure A with *tert*-butyl 4-[3-(4-amino-5-chloro-2-methoxyphenyl)-3-oxopropyl]piperidine-1-carboxylate (**7**) (200 mg, 0.50 mmol, 1 eq.) and 1,2-bis(benzyloxy)-4-(bromomethyl)benzene (230 mg, 0.60 mmol, 1.2 eq.) synthesized following the literature, the reaction was stirred at RT for 10h. After a purification by chromatography on silica gel column (DCM/MeOH, gradient 100:0 to 90:10), the compound (**9a**) was obtained as a light yellow solid (141 mg, 47% isolated yield).

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) 7.78 (s, 1H), 7.49 – 7.40 (m, 4H), 7.40 – 7.24 (m, 6H), 7.01 (d, *J* = 2.0 Hz, 1H), 6.87 (d, *J* = 8.2 Hz, 1H), 6.81 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.25 (s, 1H), 5.18 (s, 2H), 5.14 (s, 2H), 4.51 (s, 2H), 3.82 (s, 3H), 3.49 (s, 2H), 2.95 – 2.79 (m, 4H), 1.97 (t, *J* = 10.5 Hz, 2H), 1.66 (d, *J* = 10.5 Hz, 2H), 1.64 – 1.51 (m, 2H), 1.47 – 1.20 (m, 4H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 198.9, 159.54, 148.6, 148.4, 147.8, 137.3, 137.3, 132.2, 129.5, 128.5 (2C), 128.4 (2C), 127.8 (2C), 127.7 (2C), 127.5, 127.3, 122.7, 118.7, 116.6, 114.6, 111.2, 97.5, 71.3, 71.2, 62.5, 55.6, 53.3 (2C), 40.7, 35.0, 31.5 (2C), 31.0; MS *m/z* [M+H]<sup>+</sup> 599.7; IR (neat, cm<sup>-1</sup>) ν 3482, 3368, 3220, 2921, 2847, 2798, 1618, 1585, 1508, 1453, 1419, 1257, 1214, 1184, 1015, 735, 695; Mp 104.5°C. HRMS/ESI: *m/z* calcd. for C<sub>36</sub>H<sub>39</sub>ClN<sub>2</sub>O<sub>4</sub> [M+H]<sup>+</sup> 599.2677, found 599.2673;

**1-(4-amino-5-chloro-2-methoxy-phenyl)-3-[1-[(3,4-dihydroxyphenyl)methyl]-4-piperidyl]propan-1-one (9b)**

From general procedure B with 1-(4-amino-5-chloro-2-methoxy-phenyl)-3-[1-[(3,4-dibenzoyloxyphenyl)methyl]-4-piperidyl]propan-1-one **9a** (125 mg, 0.21 mmol) to afford 71 mg of 1-(4-amino-5-chloro-2-methoxy-phenyl)-3-[1-[(3,4-dihydroxyphenyl)methyl]-4-piperidyl]propan-1-one **9b** as a pale yellow powder with a yield of 81%. <sup>1</sup>H NMR (MeOD, 400 MHz) δ 7.66 (s, 1H), 6.92 (s, 1H), 6.85 – 6.80 (m, 2H), 6.45 (s, 1H), 4.06 (s, 2H), 3.86 (s, 3H), 3.39 (d, *J* = 11.8 Hz, 2H), 2.97 – 2.81 (m, 4H), 1.95 (m, 2H), 1.66 – 1.52 (m, 3H), 1.51 – 1.36 (m, 2H). <sup>13</sup>C NMR (MeOD, 100 MHz) δ 198.78, 160.32, 150.43, 146.74, 145.57, 131.46, 122.57, 120.61, 117.59, 116.27, 115.16, 110.26, 96.64, 60.35, 54.75, 51.86 (2C), 39.86, 33.28, 30.16, 29.05 (2C); MS *m/z* [M+H]<sup>+</sup> 419.8; IR (neat, cm<sup>-1</sup>) ν 3432, 3366, 3206, 2938, 2725, 1619, 1584,



1453, 1419, 1295, 1261, 1216, 1184. Mp 115.9°C. HRMS/ESI: m/z calcd. for C<sub>22</sub>H<sub>28</sub>ClN<sub>2</sub>O<sub>4</sub> [M+H]<sup>+</sup> 419.1738, found 419.1737;

**1-(4-amino-5-chloro-2-methoxy-phenyl)-3-[1-(1,3-benzodioxol-5-ylmethyl)-4-piperidyl]propan-1-one (9c)**

*tert*-butyl 4-(3-(4-amino-5-chloro-2-methoxyphenyl)-3-oxopropyl)piperidine-1-carboxylate (200 mg, 0.50 mmol, 1 eq.) was dissolved in 4 mL of DCM, then 1 mL of TFA was added. The solution was stirred 15 min at RT, then the solvent was reduced under pressure. The resulting yellow oil was dissolved in DMF (10 mL), then K<sub>2</sub>CO<sub>3</sub> (691.0 mg, 5.00 mmol, 10 eq.) and 5-(bromomethyl)benzo[*d*][1,3]dioxole (129.4 mg, 0.60 mmol, 1.2 eq.) were added dropwisely. The reaction mixture was warmed at 110°C for 3h.

Then water was added and the solution was extracted three times with EtOAc, then dried over MgSO<sub>4</sub>, concentrated under vacuum to afford a crude product which was purified on neutral alumine column with a 100% DCM isocratic gradient. The product was obtained as a yellowish powder (65 mg, 30% isolated yield) <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.77 (s, 1H), 6.84 (s, 1H), 6.74 (d, *J* = 7.5 Hz, 1H), 6.72 (d, *J* = 7.5 Hz, 1H), 6.24 (s, 1H), 5.93 (s, 2H), 4.46 (br s, 2H), 3.82 (s, 3H), 3.38 (s, 2H), 2.93 – 2.80 (m, 4H), 1.90 (m, 2H), 1.87 (m, 1H), 1.66 (d, *J* = 9.2 Hz, 2H), 1.58 (dd, *J* = 7.2, 6.4 Hz, 2H), 1.31 – 1.15 (m, 2H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 199.1, 159.5, 147.7, 147.5, 146.4, 132.5, 132.2, 122.3, 118.9, 111.2, 109.6, 107.8, 100.8, 97.5, 63.3, 55.6, 53.8 (2C), 40.9, 35.6, 32.3 (2C), 31.3; MS m/z [M+H]<sup>+</sup> 431.6; IR (neat, cm<sup>-1</sup>) ν 3470, 3358, 3216, 3013, 2922, 2873, 2854, 2810, 2775, 1715, 1642, 1619, 1573, 1440, 1421, 1332, 1312, 1261, 1214, 1178, 986, 932, 738, 567; Mp 124°C. HRMS/ESI: m/z calcd. for C<sub>23</sub>H<sub>28</sub>ClN<sub>2</sub>O<sub>4</sub> [M+H]<sup>+</sup> 431.1738, found 431.1747;

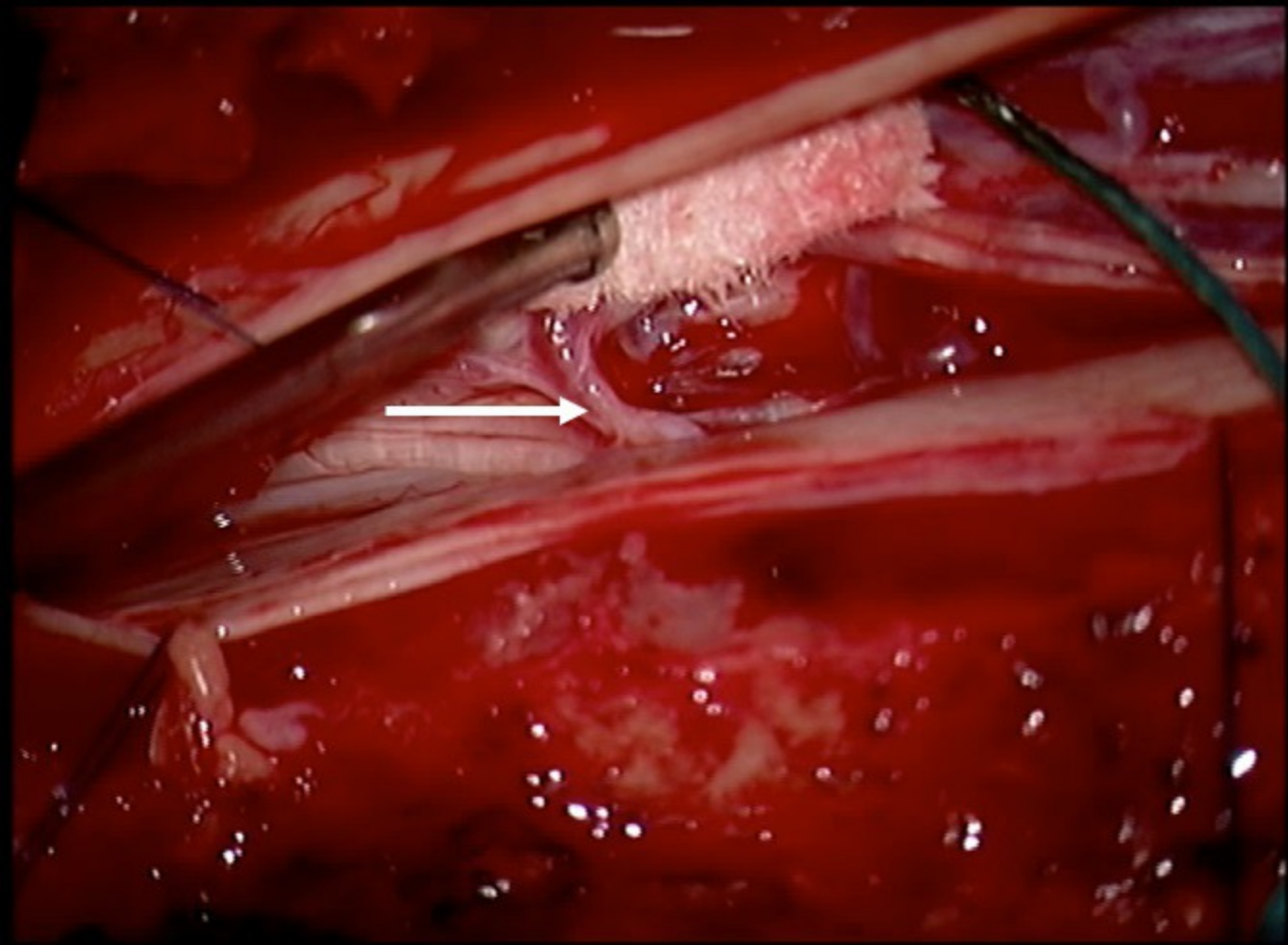
**1-(4-amino-5-chloro-2-methoxyphenyl)-3-(1-([4-(benzyloxy)-3-methoxyphenyl]methyl)piperidin-4-yl)propan-1-one (9d)**

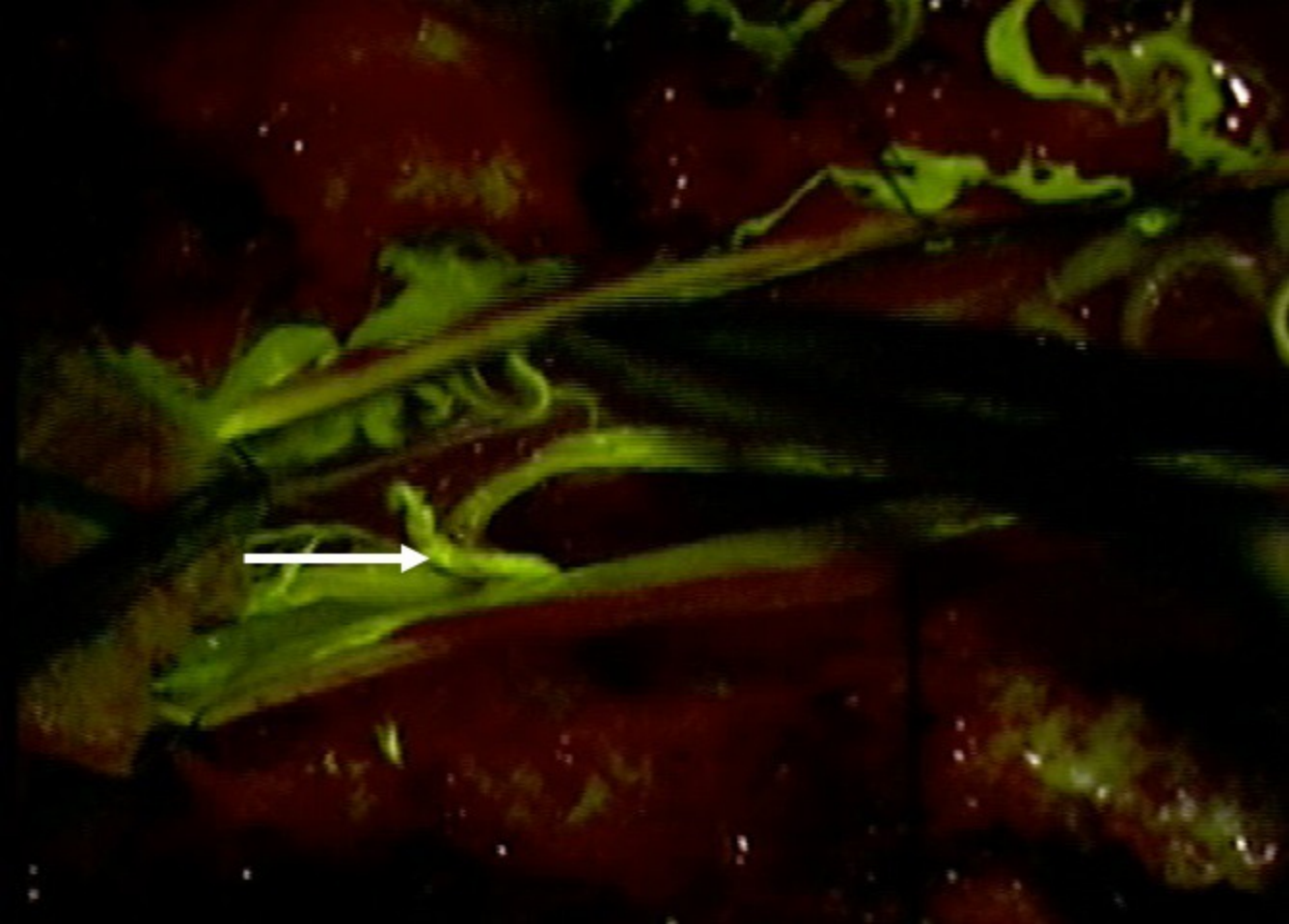
Following procedure A with **7** (200 mg, 0.50 mmol, 1 eq.) and 1-(benzyloxy)-4-(bromomethyl)-2-methoxybenzene (185 mg, 0.60 mmol, 1.2 eq.), the reaction was stirred at RT for 16h. After a purification by chromatography on silica gel column (DCM/MeOH, gradient 100:0 to 80:20), the compound (**9d**) was obtained as a light yellow oil (74 mg, 28% isolated yield).

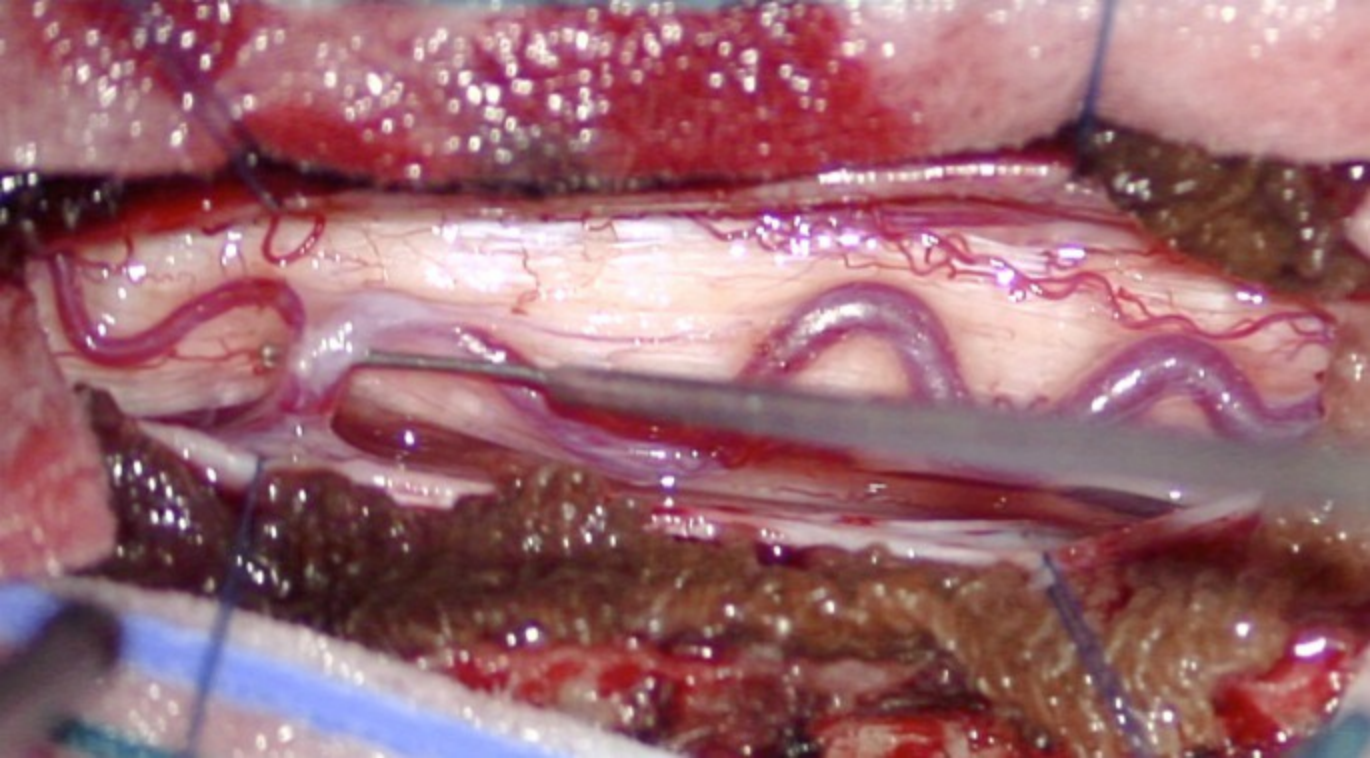
<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.78 (s, 1H), 7.49 – 7.28 (m, 5H), 6.96 – 6.88 (m, 1H), 6.81 (d, *J* = 8.2 Hz, 1H), 6.75 (dd, *J* = 8.2, 1.9 Hz, 1H), 6.24 (s, 1H), 5.13 (s, 2H), 4.46 (s, 2H), 3.89 (s, 3H), 3.82 (s, 3H), 3.43 (s, 2H), 2.98 – 2.78 (m, 4H), 1.92 (s, 2H), 1.67 (m, 2H), 1.59 (m, 2H), 1.28 (s, 3H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 199.1, 159.5, 149.4, 147.7, 147.2, 137.3, 132.2, 129.5, 128.5(2C), 127.78, 127.23 (2C), 121.4, 118.9, 113.5, 112.9, 111.2, 97.5, 71.1, 63.2, 56.1, 55.6, 53.8 (2C), 40.9, 35.6, 32.2 (2C), 31.3. MS *m/z* [M+H]<sup>+</sup> 523.6; IR (neat, cm<sup>-1</sup>) ν 3.449, 2.919, 2.853, 2.788, 1.616, 1.586, 1.463, 1.450, 1.414, 1.261, 1.216, 1.177, 735; HRMS/ESI: *m/z* calcd. for C<sub>30</sub>H<sub>36</sub>ClN<sub>2</sub>O<sub>4</sub> [M+H]<sup>+</sup> 523.2364, found 523.2363;

**1-(4-amino-5-chloro-2-methoxyphenyl)-3-{1-[(4-hydroxy-3-methoxyphenyl)methyl]piperidin-4-yl}propan-1-one (9e)**

From general procedure B with **9d** (80 mg, 0.16 mmol, 1.0 eq.) to afford **9e** as yellow powder with a yield of 85%. <sup>1</sup>H NMR (MeOD, 400 MHz) δ 7.66 (s, 1H), 7.10 (d, *J* = 2.0 Hz, 1H), 6.91 (dd, *J* = 8.1, 2.0 Hz, 1H), 6.85 (d, *J* = 8.1 Hz, 1H), 6.45 (s, 1H), 4.13 (s, 2H), 3.90 (s, 3H), 3.86 (s,











**Table 1** Patient characteristics. Aminoff-Logue score is defined by gait (G) and miction (M) disturbances

Patient, Age/sex	Diagnostic circumstance	Pre-operative Aminoff-Logue score (G+M)	Level of arterial afference	Operative time (mn)	Occlusion	Aminoff-Logue score at last follow-up	Follow-up (months)	Post-operative complications
1, 64/M	Progressive Sensitive and motor deficit of the lower limbs	5 (4+1)	Left T4	96	Success	3 (2+1)	6	None
2, 62/M	Progressive motor deficit and radicular pain of the lower limbs	5 (4+1)	Right T9	144	Success	5 (4+1)	10	None
3, 40/M	Progressive motor deficit of the lower left limb	3 (2+1)	Left T7	80	Success	1 (0+1)	6	None
4, 73/M	Progressive motor deficit and radicular pain of the lower limbs	6 (4+2)	Left T10	73	Success	4 (2+2)	6	None
5, 71/M	Progressive Sensitive and motor deficit of the lower limbs, retention of urine	6 (3+3)	Right L1	143	Success	7 (4+3)	29	Cerebrospinal fluid leakage
6, 57/M	Progressive motor deficit of	8 (5+3)	Left T4	80	Success	7 (4+3)	12	None

the lower  
limbs

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