

# Dual Histamine H<sub>3</sub>R/Serotonin 5-HT<sub>4</sub>R Ligands with Antiamnesic Properties: Pharmacophore-Based Virtual Screening and Polypharmacology

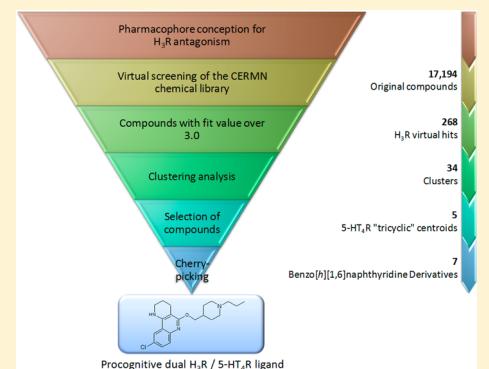
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## Supporting Information

**ABSTRACT:** In recent years, preclinical and clinical studies have generated considerable interest in the development of histamine H<sub>3</sub> receptor (H<sub>3</sub>R) antagonists as novel treatment for degenerative disorders associated with impaired cholinergic function. To identify novel scaffolds for H<sub>3</sub>R antagonism, a common feature-based pharmacophore model was developed and used to screen the 17,194 compounds of the CERMN (Centre d'Etudes et de Recherche sur le Médicament de Normandie) chemical library. Out of 268 virtual hits which have been gathered in 34 clusters, we were particularly interested in tricyclic derivatives also exhibiting a potent SHT<sub>4</sub>R affinity. Benzo[*h*][1,6]naphthyridine derivatives showed the highest H<sub>3</sub>R affinity, and compound 17 (H<sub>3</sub>R *K<sub>i</sub>* = 41.6 nM; 5-HT<sub>4</sub>R *K<sub>i</sub>* = 208 nM) completely reversed the amnesiant effect of scopolamine at 3 mg/kg in a spatial working memory experiment. For the first time we demonstrated the feasibility to combine H<sub>3</sub>R and 5-HT<sub>4</sub>R activities in a single molecule, raising the exciting possibility that dual H<sub>3</sub>R antagonist/SHT<sub>4</sub>R agonist have potential for the treatment of neurodegenerative diseases such as Alzheimer's disease.



## INTRODUCTION

Histamine is a biogenic amine that exerts its effects through interaction with four G-protein coupled receptor (GPCR) subtypes (H<sub>1–4</sub>R).<sup>1</sup> Originally described as presynaptic autoreceptors that modulate histamine release,<sup>2</sup> H<sub>3</sub> receptors (H<sub>3</sub>R) are also expressed as heteroreceptors that regulate the release of multiple neurotransmitters including acetylcholine (ACh),<sup>3</sup> norepinephrine (NE),<sup>4</sup> dopamine (DA),<sup>5</sup> and serotonin (5-HT).<sup>6</sup> Besides, H<sub>3</sub>R antagonists have been shown to evoke the release of ACh in the cerebral cortex and hippocampus,<sup>7,8</sup> two brain regions where H<sub>3</sub>R are highly expressed, and to enhance memory performance in preclinical models.<sup>9,10</sup> Because cholinergic transmission is well recognized as a major neurochemical modulator of cognitive processing (the so-called "cholinergic hypothesis"<sup>11</sup>), these preclinical studies have generated considerable interest in the development of H<sub>3</sub> antagonists as novel treatment for degenerative disorders associated with impaired cholinergic function such as Alzheimer's disease (AD).<sup>12–15</sup> However, patients who suffer from AD exhibit an excessive loss of cholinergic neurons, resulting in pathologically low levels of cholinergic transmission in the memory-associated regions of the brain.<sup>16,17</sup> Acetylcholinesterase inhibitors (AChEI), the primary therapeutic strategy that increases synaptic ACh levels by inhibiting the enzymatic

degradation of ACh, only provide modest symptomatic alleviation that declines with the progressive cholinergic cell loss associated with AD. With the intention of completing this symptomatic approach, it is necessary to develop therapeutics that may slow the AD pathological progression, i.e., that have disease-modifying effects. Drug discovery efforts toward such therapies have focused on two hallmarks of the AD, namely, the formation of amyloid plaques and neurofibrillary tangles. The latter result from the hyperphosphorylation of the microtubule-associated  $\tau$  protein.<sup>18</sup> Recently, Bitner and co-workers suggested that administration of ABT-239, a reference H<sub>3</sub>R antagonist, activates signaling pathways that inhibit the glycogen synthase kinase 3 $\beta$  (GSK3  $\beta$ ),<sup>19</sup> a primary  $\tau$  kinase in AD. Besides, Medhurst et al. demonstrated that H<sub>3</sub>R expression remains prevalent in the medial temporal cortex of patients diagnosed with AD, even in advanced stages of the disease.<sup>10</sup> These studies have generated considerable interest in the development of H<sub>3</sub>R antagonists for both symptomatic and disease-modifying effects in AD patients.<sup>20</sup>

In the present work, we describe our efforts to identify new histamine H<sub>3</sub>R antagonists by a pharmacophore-based virtual

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screening approach. The pharmacophore conception for H<sub>3</sub>R antagonism was carried out using the “Common Features Alignment” method of Catalyst.<sup>21,22</sup> The resulting pharmacophore was used as a three-dimensional (3D) search query for the virtual screening of our chemical library, presently including more than 17,000 compounds. In the search of new H<sub>3</sub>R antagonists, the enrichment of this chemical library in aminergic GPCR ligands is highly beneficial. Particularly, there are annotations with affinity data for 5-HT<sub>1A</sub>R, 5-HT<sub>4</sub>R, and 5-HT<sub>6</sub>R, three serotonin receptor subtypes targeted by emerging AD therapies.<sup>17,23–25</sup> Regarding the virtual screening hits, the analysis of the stored off-target activities highlighted the high 5-HT<sub>4</sub>R affinity of some series of tricyclic derivatives. We were particularly interested in this 5-HT<sub>4</sub>R activity since 5-HT<sub>4</sub>R agonists also represent a valuable pharmacological target for the treatment of AD. Lezoualc'h actually suggested that 5-HT<sub>4</sub>R agonists may provide both symptomatic alleviation of cognitive impairments as well as neuroprotection by reducing A $\beta$  generation and toxicity.<sup>26</sup> In parallel, several studies from our group showed that activation of 5-HT<sub>4</sub>R may improve cognitive processes such as learning and memory.<sup>27–30</sup>

Binding experiments confirmed the discovery of benzo[*h*][1,6]naphthyridine derivatives with high hH<sub>3</sub>R and 5-HT<sub>4</sub>R affinities. To assess the putative cognition-enhancing potential of the benzo[*h*][1,6]naphthyridine derivatives, their ability to reverse the scopolamine-induced cognitive impairment<sup>31</sup> in a spatial working memory task was evaluated. These results raised the exciting opportunity to combine H<sub>3</sub>R antagonism and 5-HT<sub>4</sub>R agonism in a single molecule, a concept commonly referred as polypharmacology.<sup>32</sup>

## ■ EXPERIMENTAL SECTION

**Chemistry.** For synthetic experimental details on the benzo[*h*][1,6]naphthyridine derivatives, the reader is invited to refer to the publication of Hinschberger et al.<sup>33</sup> As delineated in this previous work, all of the compounds were characterized by elemental analysis, IR spectra, and <sup>1</sup>H NMR spectra.

**Software Specifications.** Pharmacophore modeling and virtual screening experiments were performed using the Catalyst software implemented in Discovery Studio version 3.5 (Accelrys Inc., San Diego, CA, USA). Structural clustering of virtual hit compounds was calculated using the LibMCS clustering component (Chemaxon Ltd., Budapest, Hungary) integrated into Pipeline Pilot version 8.0 (Accelrys). Docking simulations were performed using Glide Induced Fit Docking workflow implemented in Maestro 9.2 (Schrödinger LLC, New York, NY, USA).

**Training Set Compilation and Preparation.** The six H<sub>3</sub>R antagonists of the training set were selected from the literature.<sup>34–39</sup> All compounds underwent 3D structure generation before the pharmacophore model development. The initial structure of each compound was built with the Sketch Molecules tool of Discovery Studio 3.5 and optimized using the modified parameter set of the CHARMM force field<sup>40</sup> devoid of electrostatic component. The conformational flexibility of each training set compound was modeled by generating multiple conformers (up to 250) using a stochastic search coupled to a poling method,<sup>41</sup> with a 20 kcal/mol energy cutoff.

**Pharmacophore Modeling.** The Common Features Alignment method of Catalyst<sup>21,22</sup> is an automated tool that models drug–receptor interactions using information derived only from active drug's structures. These arrangements are

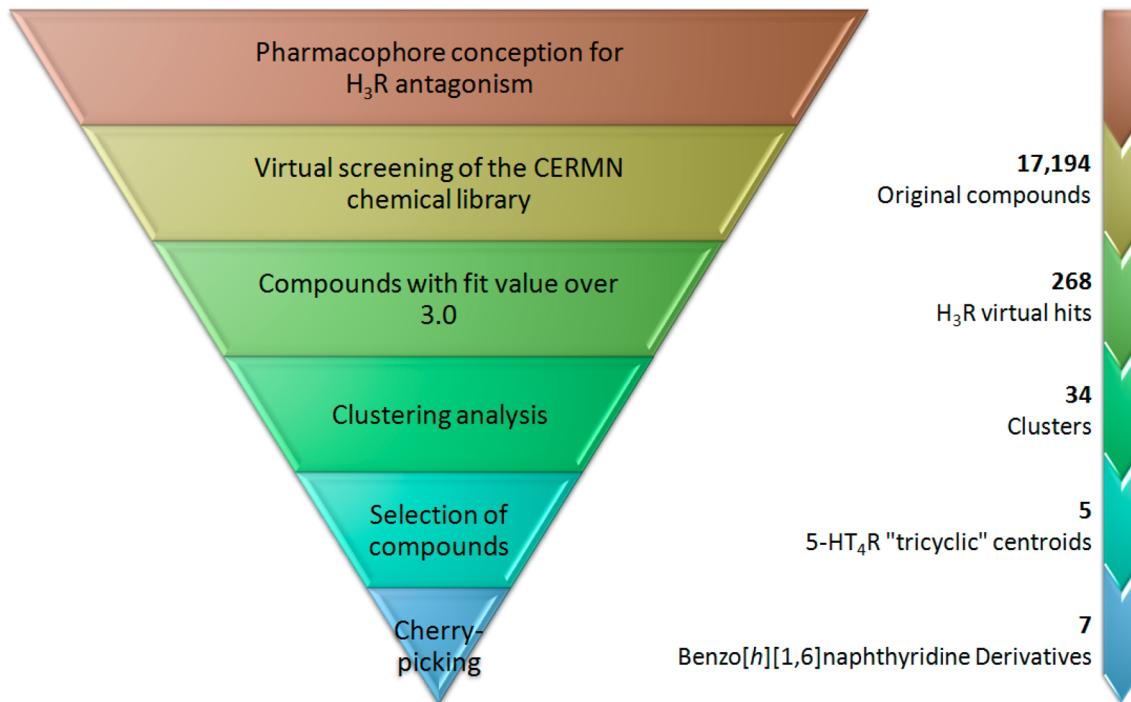
identified by a pruned exhaustive search, starting from small sets of features and extending them until no larger common arrangement is found. The chemical features considered in this study were hydrogen bond donors (HBD) and acceptors (HBA), hydrophobic groups (H), aromatic rings (R), and positive ionizable groups (P). The “Principal” and “Max-OmitFeat” parameters for the training set compounds were set to 2 and 0, respectively, meaning that each compound had a similar influence in the model building phase and that partial mapping was not allowed. The spacing parameter, which controls the minimum allowed interfeature distance in the resulting hypotheses, was set to 2.0 Å. Regarding the remaining parameters, the default values were used. The hypothesis generation step returned 10 ranked pharmacophore hypotheses. The ranking is a measure of how well the active training molecules map onto the proposed pharmacophores, as well as the uniqueness and/or selectivity of each hypothesis. In other words, the rank shows the rarity of the pharmacophore model. If a particular pharmacophore is “rare”, then it will be less likely to map to an inactive compound and therefore it will be given a higher rank. Finally, the best hypothesis was determined by calculating the fitting scores, which are computed from the displacement of the features of a molecule from the center of the location constraint. The maximum fitting score is equal to the number of features of the pharmacophore model.

**Virtual Screening.** The CERMN chemical library (<http://www.cermn.unicaen.fr>), which presently includes 17,194 compounds, was converted into a 3D multiconformational database using the catDB module of Catalyst. For each compound, a maximum of 100 conformers was computed in Fast mode. The selected hypothesis was used as a 3D search query for the virtual screening of the database using the “Best Flexible Search” option. This option allows a limited flexibility of the conformational models stored in the database to map to the query.

**Structural Clustering of the Virtual Hits.** The virtual hits were clustered using LibMCS (<http://www.chemaxon.com/jchem/doc/user/LibMCS.html>) which groups together a set of chemical structures in a hierarchical manner based on the concept of maximum common substructures (or MCS).<sup>42</sup> A MCS is defined for two chemical structures, and it refers to the largest substructure common in both structures. In this study, we used a value of 15 atoms to specify the minimum required MCS size. The LibMCS algorithm considers the outliers as singletons in order to not deteriorate the maximal common substructures.

**Homology Modeling.** The sequence of the human H<sub>3</sub>R was retrieved from the UniProt knowledge base<sup>43</sup> (Entry No. Q9Y5N1). The @tome server<sup>44</sup> identified the crystal structure of the human H<sub>1</sub>R complexed with the Doxepin antagonist as the better 3D experimental template (PDB entry, 3RZE;<sup>45</sup> resolution, 3.1 Å). Before the construction step, the alignment of the two sequences was manually optimized and evaluated using the TITO program.<sup>46</sup> The disulfide bond Cys107-Cys188 between TM3 and ECL2 of human H<sub>3</sub>R was conserved. The sequence identity is 31% by omitting the intracellular loop ICL3 which fits the T4-lyzozyme part. Once the 3D model was built with MODELER,<sup>47</sup> its folding quality was evaluated using the 3D evaluation tools Verify3D<sup>48</sup> and Eval23D.<sup>49</sup> The figure corresponding to the sequence alignment, provided as Supporting Information, was generated with ESPript.<sup>50</sup>

**Docking Experiment.** Docking calculations were performed using the Glide Induced-Fit Docking (IFD) workflow<sup>51</sup>



**Figure 1.** Workflow overview of the study including pharmacophore modeling, virtual screening, clustering analysis, and selection of compounds.

in order to account for both compound and receptor flexibility. The IFD protocol is a multistep workflow that consist of (i) a temporary mutation to alanine of the side chains from selected active-site residues, presently Tyr115, Cys118, and Trp371, to provide more room for ligand docking; (ii) an initial Glide SP docking using a softened potential (scaling of van der Waals radii to 0.7 and 0.5 for receptor and ligand heavy atoms, respectively), a docking region (grid) defined by the centroid of Asp114 and Glu206 with default box sizes, and two constraints on highly conserved H-bonds with Asp114 and Glu206;<sup>52–54</sup> (iii) a restoration of the temporary mutated side chains of the selected active-site residues; (iv) a Prime refinement (side-chain prediction and minimization) for all residues within 5 Å of any ligand pose; and (v) a Glide SP redocking into each refined receptor structure using the default potential (van der Waals radii scaling of 1.0 and 0.8 for receptor and ligand, respectively).

**Binding Experiments.** CHO-hH3 cells were amplified in HAM F12 Glutamax + 10% FCS culture medium. Cells were collected in phosphate-buffered saline (PBS) and pelleted before freezing at –80 °C. Membranes were prepared by cell disruption with a polytron (3 × 10 s) in 37.8 mM Na<sub>2</sub>HPO<sub>4</sub> + 12.2 mM KH<sub>2</sub>PO<sub>4</sub> buffer, pH 7.4, followed by slow speed centrifugation (140 g, 10 min, 4 °C) to eliminate fragments. Supernatant was submitted to ultracentrifugation (23000g, 30 min, 4 °C), and microsomal pellet was resuspended in 10% (v/w) buffer. Protein concentration was estimated by the Lowry method.  $K_d$  and  $B_{max}$  were determined by Scatchard plot analysis of [<sup>125</sup>I]-Iodoproxifan (PerkinElmer) saturation binding experiments. For competition assays, compounds were diluted logarithmically from 10<sup>–4</sup> to 10<sup>–10</sup> M in 10% dimethyl sulfoxide (DMSO). Duplicates of compounds in dilution were mixed to [<sup>125</sup>I]-Iodoproxifan (final concentration of 25 pM) and 4 fmol/mL membranes in buffer (200 μL) and incubated in 96-well polypropylene plates (Costar) for 1 h. Nonspecific binding was estimated in the presence of clobenproptop.

dibromhydrate (Tocris) at 1 μM. Incubation plates were filtrated on 96-well, GF-B plates (PerkinElmer) coated with 0.1% PEI and rinsed twice with 1 mL of 50 mM Tris-HCl pH 7.4. Bound radioactivity was counted on a TopCount luminescent counter (Packard) after addition of 30 μL/well Microscint 20 (PerkinElmer). IC<sub>50</sub> values were estimated with Prism software, and  $K_i$  was calculated as follow:  $K_i = IC_{50}/(1 + ([^{125}IP_x]/K_d))$  and then transformed to p $K_i$  (– log( $K_i$ )). Results were reproduced until SEM(p $K_i$ ) < 0.3 (with SEM = standard error/n (number of replicates)). Experiments were done in duplicates, and proofs of dose–response behavior for the three lead compounds are provided as Supporting Information.

**Functional Experiments.** Antagonist potency was quantified for the selected compounds through their ability to reduce the histamine-elicited production of cAMP by CHO-hH3 cells, as originally described by Lim et al.<sup>55</sup> and as derived in the time-resolved fluorescence resonance energy transfer (TR-FRET) LANCE cAMP 384 assay (PerkinElmer). In brief, cells were incubated in 384-well plates for 10 min at 37 °C with 300 nM histamine as an agonist at the hH3R in Hank's balanced salt solution (HBSS), containing 5 mM N-(2-hydroxyethyl)-piperazine-N'-ethanesulfonic acid (HEPES) buffer (Invitrogen), 0.01% bovine serum albumin (BSA), and 0.5 mM 3-isobutyl-1-methylxanthine (IBMX; pH 7.4) and the fluorescent cAMP antibody, in the absence or presence of increasing concentrations of the compounds to test. Detection buffer containing EU-labeled streptavidin and biotin-cAMP was added, mixed to the cell preparations, and further incubated for another 20 min at room temperature in the dark with gentle shaking to achieve the reaction equilibrium. The time-gated fluorescence signal was measured at 615 nm using 340 nm excitation wavelength, 400 μs delay, and 400 μs integration times with a Victor2 1420 multilabel counter (PerkinElmer).  $K_B$  values were derived from the IC<sub>50</sub> data for each compound.<sup>56</sup>

**Spatial Working Memory.** In all studies, male NMRI mice (20–24 g, CER Janvier, France) were used. All compounds

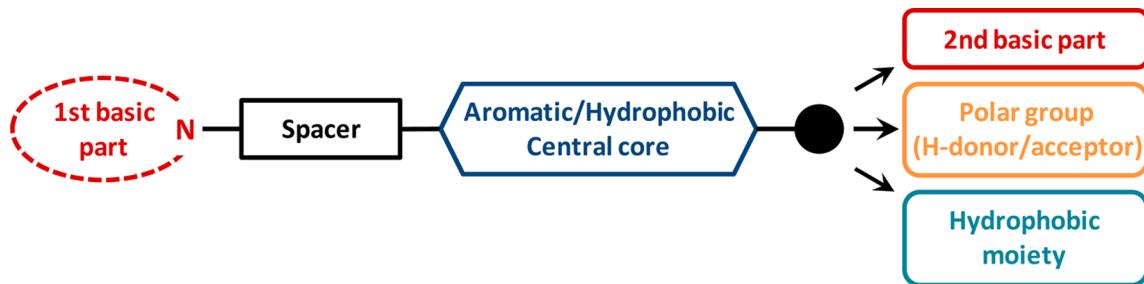
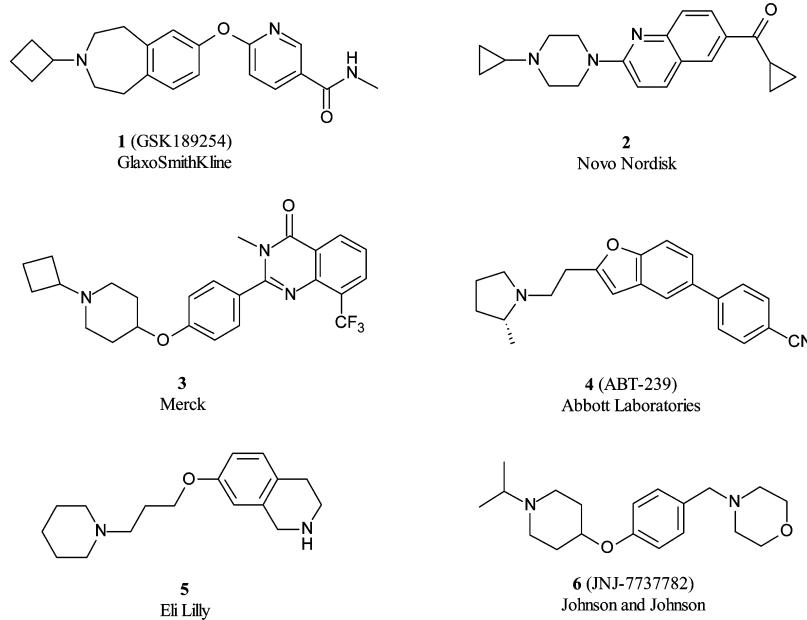


Figure 2. Accepted pharmacophore model for H<sub>3</sub>R antagonists. Adapted with permission from ref 61. Copyright 2005 Elsevier.

Chart 1. Training Set Compounds Used for the Generation of the Pharmacophore Model for H<sub>3</sub>R Antagonism



tested were dissolved in saline solution and administered intraperitoneally (10 mL/kg). The antiamnesic activity of tested compounds was evaluated through the measurement of the capacity of tested compounds to reverse the scopolamine-induced deficit on spontaneous alternation behavior in the Y maze test.<sup>57</sup> The black wooden maze consisted of three equally spaced arms (22 cm long and 6.5 cm wide with walls 10 cm high). The mouse was placed at the end of one of the arms and allowed to move freely through the maze during a 5 min session while the sequence of arm entries was recorded by an observer. An arm entry was scored when all four feet crossed into the arm. An alternation was defined as entries into all three arms on a consecutive occasion. The number of possible alternations is thus the total number of arm entries minus two; the percentage of alternation was calculated as (number of actual alternations/number of possible alternations) × 100. The percentage of alternation of scopolamine-treated mice (0.25 mg/kg)<sup>57</sup> was significantly reduced in comparison to control mice (52% vs 66%, respectively; *p* = 0.0049; ANOVA and PLSD of Fisher). Compounds 16 and 17 were tested at 0.3, 1, and 3 mg/kg. For comparison purpose, pharmacological references were also tested: (i) the H<sub>3</sub>R antagonist thioperamide at 2.5 mg/kg and (ii) the 5-HT<sub>4</sub>R partial agonist RS 67333 at 1 mg/kg.<sup>28</sup> At these doses, preliminary studies demonstrated that the above-mentioned compounds failed to exert promnesic activities *per se*. Regarding statistical analyses, one-way analyses of variance (ANOVA) were performed on the percentage of alternation

(“treatment” factor) and PLSD of Fischer test post hoc analyses were performed when significant principal effects and interactions between factors were detected. Student’s *t* tests were also used to compare the percentage of alternation to a random choice level of exploration (50% value).

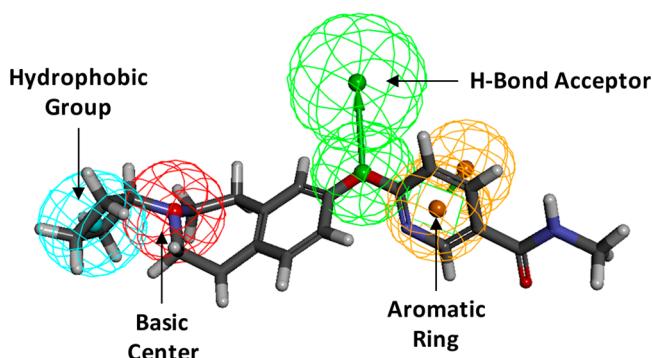
## RESULTS AND DISCUSSION

A workflow overview of this study including pharmacophore modeling, virtual screening, clustering analysis, and selection of compounds is provided in Figure 1.

**Pharmacophore Modeling.** The first aim of our study was to retrieve novel scaffolds for H<sub>3</sub>R antagonists. The pharmacophore modeling approach is suitable for this task due to its well-known scaffold hopping potential.<sup>58</sup> Although some studies have suggested that the optimal H<sub>3</sub>R molecular framework consists of an aromatic/hydrophobic linker flanked by two basic amines both participating in salt bridges,<sup>59,60</sup> numerous other high-affinity chemical series have been discovered that possess only a single basic amine. Thus, the histamine H<sub>3</sub>R seems to be fairly accommodating. It is generally accepted that a basic tertiary amine linked to an aromatic/hydrophobic central core that is connected to either (i) a second basic part, (ii) a polar group, or (iii) a hydrophobic moiety (Figure 2) will yield high-affinity H<sub>3</sub>R antagonists.<sup>13,61</sup>

We developed our own pharmacophore model for H<sub>3</sub>R antagonism, and to take into account the above-mentioned

variability of the chemical structures of  $\text{H}_3\text{R}$  antagonists, model generation was based on the structural information on six compounds developed by six different pharmaceutical companies (Chart 1) and reported in the literature.<sup>34–39</sup> The highest ranked hypothesis consisted of four features: one hydrophobic group, one basic center, one H-bond acceptor, and one aromatic ring (Figure 3). As expected, this pharmacophore



**Figure 3.** Compound 1 (GSK189254) mapped onto the pharmacophore model for  $\text{H}_3\text{R}$  antagonists.

model fixed the nature and 3D spatial arrangement of the minimum structural requirements for a high  $\text{H}_3\text{R}$  affinity, *i.e.*, a basic tertiary amine linked to an aromatic/hydrophobic central core. The fit values, which show how well the training molecules map onto the pharmacophore, are displayed in Table 1.

**Table 1.  $\text{H}_3$  Receptor Affinity and Fit Values for the Training Set Compounds**

compd	human $\text{H}_3\text{R}$ $K_i$ (nM)	fit value	ref
1	0.12	3.99	36
2	1.80	2.73	34
3	0.22	2.47	35
4	0.40	2.23	37
5	0.60	2.30	38
6	2.14	2.17	39

**Virtual Screening.** The 17,194 compounds of the CERMN chemical library were screened utilizing our designed pharmacophore for  $\text{H}_3\text{R}$  antagonists as query. The Best Flexible Search of Catalyst captured 952 compounds that matched all of the features of the pharmacophore model. We observed the top-ranked virtual hits were already annotated with affinity data for receptors of interest in the treatment of AD. Since we searched for ligands acting on multiple targets, we only considered the 268 compounds with a fitting score greater than 3 during the following step. The ranking and the structures of all of the 268 filtered compounds are provided as Supporting Information.

**Structural Clustering of the Virtual Hits.** The 268 virtual hits were classified into 65 clusters which included 31 singletons. Since we intended to discover novel scaffolds exemplified by several compounds, all singletons were removed and only 34 scaffolds were taken up for further analysis. The 34 centroids of these clusters, *i.e.*, the most representative structures of the groups, are represented in Table 2.

**Selection of Compounds for Biological Testing.** As we work for several years on the serotonergic system, our

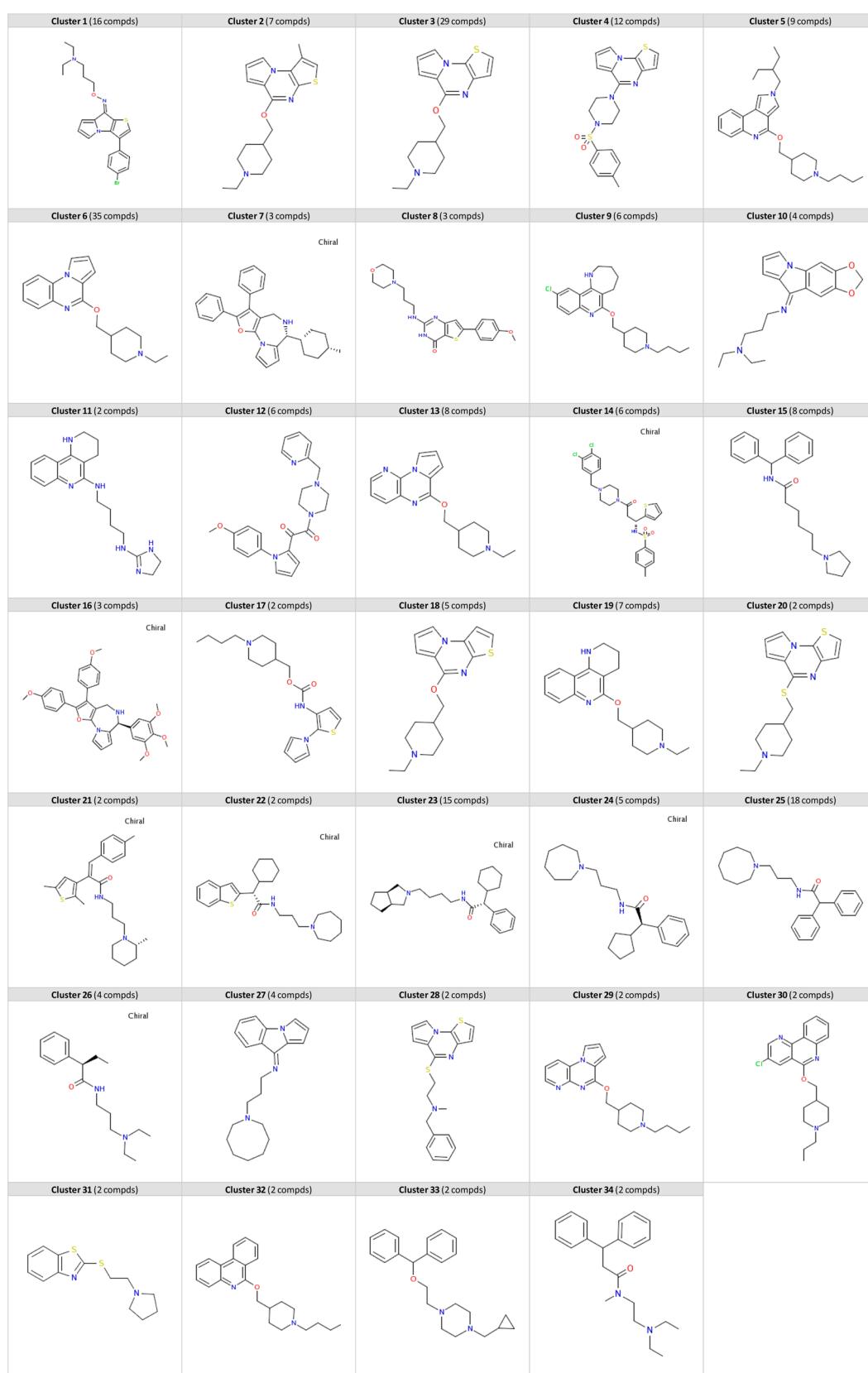
chemical library is mainly GPCR-oriented. Particularly, numerous medicinal chemistry programs focused on a serotonin (5-HT) “privileged structure”, *i.e.*, on a scaffold that preferentially binds to the 5-HT target family. This concept of “privileged structure” was introduced by Evans et al. in 1988<sup>62</sup> and has gained particular attention over the years in the drug discovery process.<sup>63–65</sup> For our part, we demonstrated that chemical modification of a tricyclic molecular framework linked to an aminoalkyl side chain may result in active ligands for one or more 5-HT receptor subtypes.<sup>33,57,66–70</sup> Since the  $\text{H}_3\text{R}$  is part of the aminergic GPCR family, half of the clusters captured by the pharmacophore exhibited such a tricyclic scaffold (Table 2). We were particularly interested in clusters 2, 3, 6, 13, and 19 due to their annotation with affinity data for three serotonin receptor subtypes targeted by emerging AD therapies, namely, 5-HT<sub>1A</sub>R, 5-HT<sub>4</sub>R, and 5-HT<sub>6</sub>R.<sup>17,23–25</sup> The affinity data stored in our chemical library clearly showed that these tricyclic compounds bind preferentially to the 5-HT<sub>4</sub>R (Table 3), and affinities for 5-HT<sub>1A</sub>R and 5-HT<sub>6</sub>R were insignificant (data not shown). It raised the exciting possibility to combine for the first time  $\text{H}_3\text{R}$  and 5-HT<sub>4</sub>R affinities in a single molecule. Indeed, dual  $\text{H}_3\text{R}$ /5-HT<sub>4</sub>R ligands have potential for both symptomatic and disease-modifying effects in Alzheimer’s disease since  $\text{H}_3\text{R}$  antagonists and 5-HT<sub>4</sub>R agonists both enhance the release of acetylcholine,<sup>28,71</sup>  $\text{H}_3\text{R}$  antagonists activate signaling pathways that inhibit the GSK3  $\beta$ ,<sup>19</sup> a primary  $\tau$  kinase in AD, and 5-HT<sub>4</sub>R agonists control the maturation of the amyloid precursor protein (APP), leading to an enhancement of the non-amyloidogenic neuroprotective pathway.<sup>26</sup>

**Binding Experiments.** Results from the competition experiments for the five selected compounds toward binding of [<sup>125</sup>I]-Iodoproxifan ( $K_i$  values) are displayed in Table 3. All five compounds showed a submicromolar affinity for  $\text{H}_3\text{R}$ . Among these results, we developed a particular interest for the benzo[*h*][1,6]naphthyridine derivatives of cluster 19, not only based on the highest  $\text{H}_3\text{R}$  affinity for their centroid but also based on the presence of two basic amines on both sides of an aromatic central core. As discussed previously, even if numerous chemical series with high  $\text{H}_3\text{R}$  affinity possess only a single basic amine, a second basic site which significantly enhances activity is present in many instances.<sup>59,60</sup> Our results seem to perfectly fit with this statement.

From these initial biological results, we decided to “cherry-pick” other members of the benzo[*h*][1,6]naphthyridine derivatives also developed within the context of a research program on 5-HT<sub>4</sub>R ligands. The  $\text{H}_3\text{R}$  binding affinities ( $K_i$  values) and the functional antagonism ( $K_B$  values) measured for the six additional compounds are recapitulated in Table 4. Varying the length of the alkyl chain in the R<sub>1</sub> position showed that an ethyl or propyl substituent is best suited to  $\text{H}_3\text{R}$  affinity. Indeed, the ethylated (11;  $K_i = 72$  nM) and propylated (14;  $K_i = 152$  nM) compounds were more potent than their methylated (13;  $K_i = 233$  nM) and butylated (15;  $K_i = 496$  nM) counterparts. It should also be noted that compound 12, with a hydrogen instead of an alkyl chain in the R<sub>1</sub> position, led to a complete loss of activity ( $K_i > 10,000$  nM). The addition of a chlorine atom in the R<sub>2</sub> position was well tolerated and even had a slightly improved affinity, as exemplified by compounds 16 and 17, which respectively exhibited  $K_i$  values of 96.9 and 41.6 nM. Figure 4C shows the mapping of 17 onto the designed pharmacophore (fit value = 3.18).

**Homology Modeling and Docking Experiment.** To complete the molecular modeling studies, docking experiments

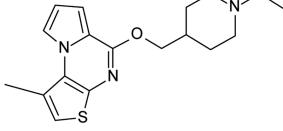
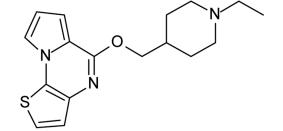
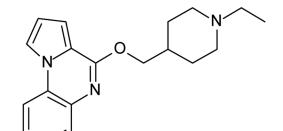
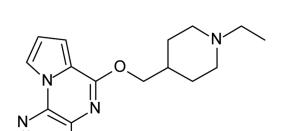
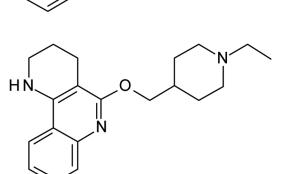
**Table 2.** Centroids of the 34 Clusters Resulting from the Pharmacophore-Based Virtual Screening (Number of Compounds per Cluster is Indicated in Brackets)



were carried out to link the pharmacophoric features to interactions with key binding site residues. Despite the recent advances in X-ray determination of GPCRs structure, we are

still waiting for the H<sub>3</sub>R structure to be solved. The crystal structure of the human H<sub>1</sub>R, a phylogenetically close neighbor of H<sub>3</sub>R, was recently determined,<sup>45</sup> and we used it as a template

**Table 3.** Experimental 5-HT<sub>4</sub>R and H<sub>3</sub>R Binding Affinities Measured for the Six Selected Compounds after the Pharmacophore-Based Virtual Screening and Clustering Steps

	compd		human 5-HT <sub>4</sub> R % inhibition @ 10 <sup>-6</sup> /10 <sup>-8</sup> M	human H <sub>3</sub> R <i>K<sub>i</sub></i> (nM)
cluster 2	7		100/30	262
cluster 3	8		84/42	271
cluster 6	9		100/30	243
cluster 13	10		95/19	202
cluster 19	11		100/55	72

**Table 4.** Experimental 5-HT<sub>4</sub>R and H<sub>3</sub>R Binding Affinities (*K<sub>i</sub>* Values) and Functional Antagonism (*K<sub>B</sub>* Values) Measured for Benzo[*h*][1,6]naphthyridine Derivatives after the “Cherry-Picking” Selection Process

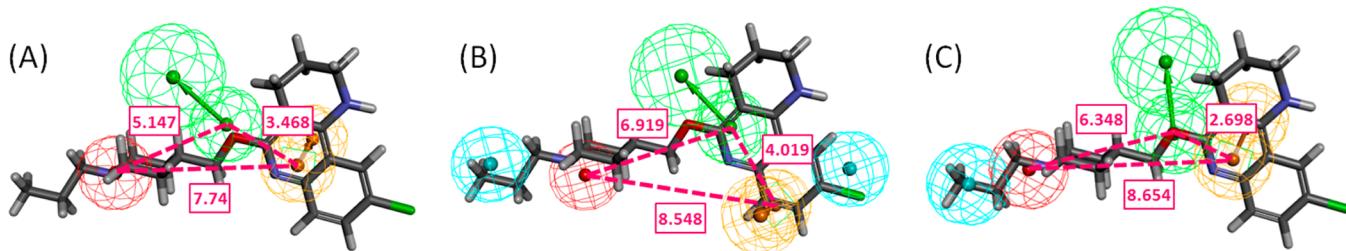
compd	R <sub>1</sub>	R <sub>2</sub>	human 5-HT <sub>4</sub> R <i>K<sub>i</sub></i> (nM)	human H <sub>3</sub> R <i>K<sub>i</sub></i> (nM)	human H <sub>3</sub> R <i>K<sub>B</sub></i> (nM)
12	H	H	96/24 <sup>a</sup>	>10000	>10000
13	CH <sub>3</sub>	H	79	233	178
11	CH <sub>2</sub> CH <sub>3</sub>	H	15	72	56.4
14	(CH <sub>2</sub> ) <sub>2</sub> CH <sub>3</sub>	H	4.5	152	119
15	(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub>	H	10	496	257
16	CH <sub>2</sub> CH <sub>3</sub>	Cl	78	96.9	30.9
17	(CH <sub>2</sub> ) <sub>2</sub> CH <sub>3</sub>	Cl	208	41.6	54.6

<sup>a</sup>% inhibition @ 10<sup>-6</sup>/10<sup>-8</sup> M.

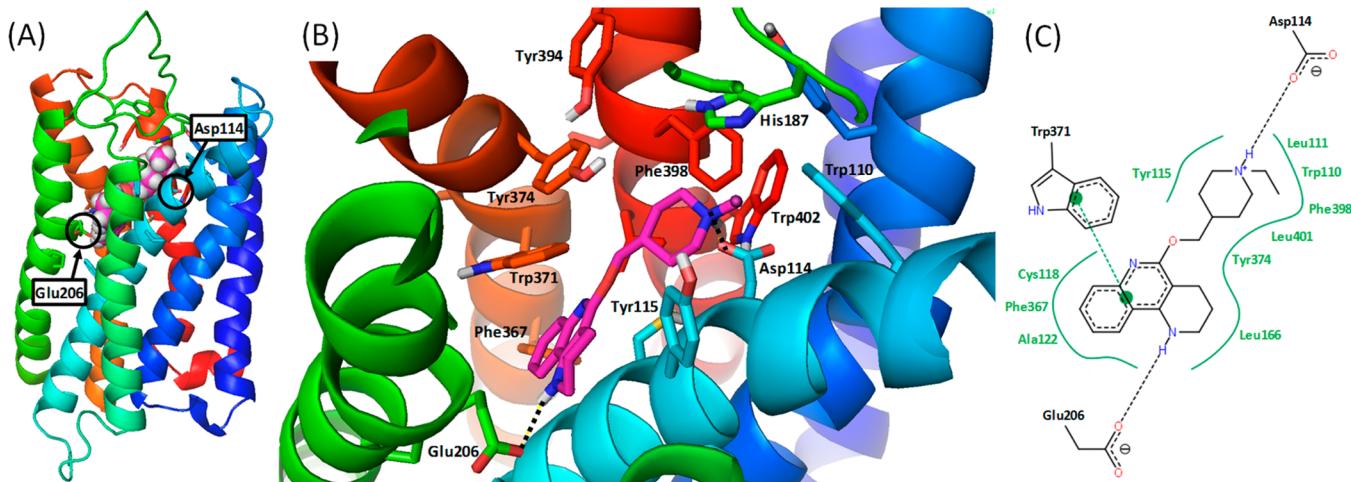
to build a homology model. Docking simulations of the centroid of cluster 19 (compound 11) into the H<sub>3</sub>R model were performed using Glide. Parts A and B of Figure 5 show the proposed binding mode of 11, and Figure 5C shows a summary of all of the surrounding residues. The docking solution is accommodated in the active-site cavity formed by

TM2, TM3, TM5, TM6, and TM7. It should be mentioned that the H<sub>3</sub> receptor-binding conformation of 11 is in agreement with the stable forms of the benzo[*h*][1,6]-naphthyridine derivatives fitting the pharmacophore (Figure 4C). Straight correspondences were found between the pharmacophoric features and the residues interacting with the docking solution (Figure 5): (i) the basic center representing the protonated amine of the piperidine group forms a salt bridge with Asp114 in TM3, (ii) the aromatic system makes a π-π interaction with the indole ring of Trp371 in TM6, (iii) the hydrophobic group is surrounded by Leu111, Trp110 in TM3, and Phe398 in TM7, and (iv) the H-bond acceptor can be linked to the hydroxyl group of Tyr374 in TM6, probably through a water-mediated H-bond. Concerning the NH of the tricyclic system, which was not considered by the pharmacophore, it serves as a hydrogen donor and forms a hydrogen bond with the side chain of Glu206 in TMS. These results are consistent with the usually accepted optimal H<sub>3</sub>R molecular framework which consists of an aromatic/hydrophobic linker flanked by two basic amines both participating in salt bridges.<sup>59,60,54</sup> It should be noted that other H<sub>3</sub>R ligands also possessing two positive ionizable groups (imidazole, alkylamine) are sometimes supposed to interact with Asp80 in TM2 or with Glu175 and Glu191 from the extracellular loops.<sup>72</sup>

**Spatial Working Memory.** Scopolamine is a nonselective muscarinic antagonist that causes alteration in a wide range of cognitive functions including attention, learning, and memory by blocking, at least in part, the cholinergic neurotransmis-



**Figure 4.** Compound 17 mapped onto the pharmacophore models [Red: basic center, green: H-bond acceptor, orange: aromatic ring, blue: hydrophobic group. Interfeature distances in angstroms.] for 5-HT<sub>4</sub>R agonism (A), 5-HT<sub>4</sub>R antagonism (B), and H<sub>3</sub>R antagonism (C).



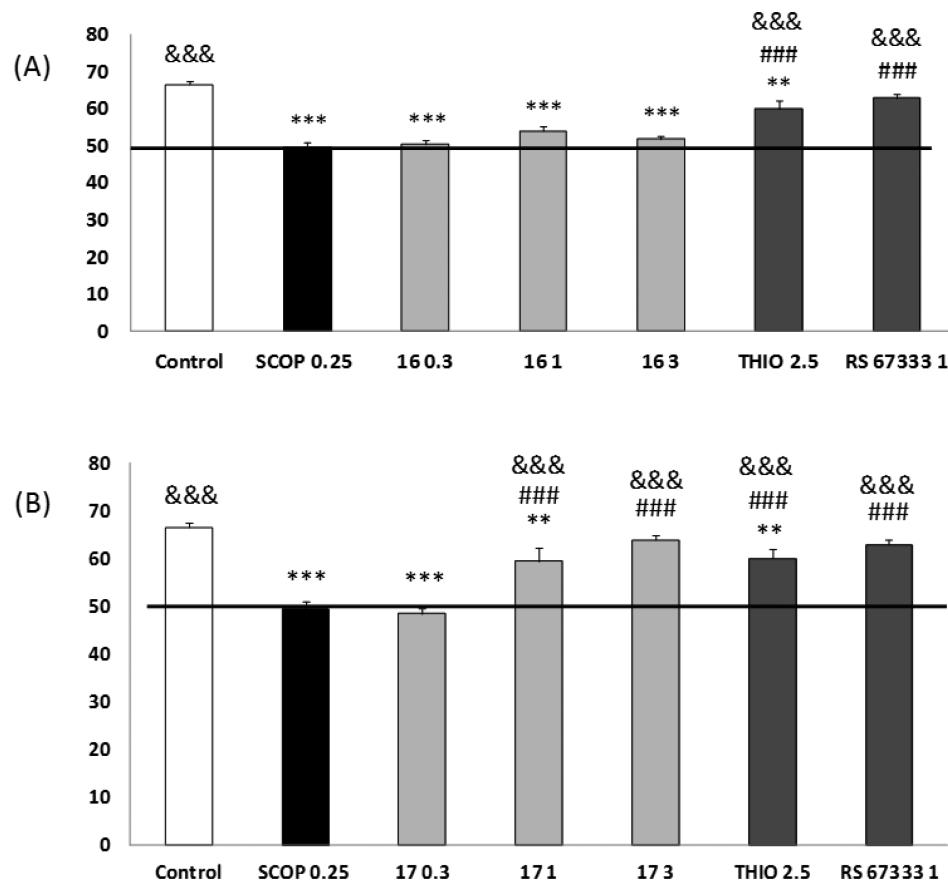
**Figure 5.** (A) General overview of the docking mode of the H<sub>3</sub>R antagonist 11 into the hH<sub>3</sub>R homology model. (B) Predicted best model of 11 bound to the hH<sub>3</sub>R homology model. The H-bonds are represented by dotted lines. (C) Schematic representation of the interactions between 11 and the hH<sub>3</sub>R homology model (diagram generated with PoseView<sup>82</sup>).

sion.<sup>22</sup> For that reason, the scopolamine-induced cognitive impairment test has been widely used to examine the cognitive enhancing effects of experimental compounds that are hypothesized to act through the modulation of cholinergic neurotransmission.<sup>73,74</sup> Particularly, H<sub>3</sub>R antagonists have been shown to improve performances in various rodent cognition models following a pharmacological challenge with scopolamine.<sup>10,75–77</sup>

The capacity of compound 14 to modulate such central action has already been evaluated, and 14 did not exhibit any action on the scopolamine-induced deficit.<sup>33</sup> Here, we selected compounds 16 and 17 for characterizing their antiamnesic activity through the measurement of their capacity to reverse the scopolamine-induced deficit on spontaneous alternation behavior in the Y maze test.<sup>57</sup> While compound 16 had no effect on spontaneous alternation performance at the tested doses (Figure 6A), compound 17, at the respective doses of 1 and 3 mg/kg, partially and completely reversed the amnesia effect of scopolamine (Figure 6B). This effect, superior to the one observed after the coadministration of scopolamine and thioperamide (thioperamide only partially reversing the scopolamine-induced deficit), highlights the therapeutic potential of compound 17 for the treatment of degenerative disorders associated with impaired cholinergic function.

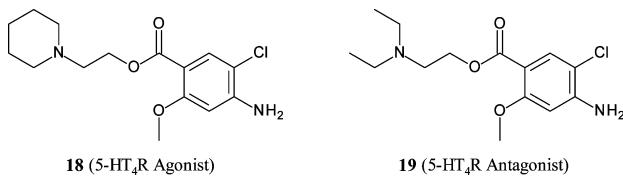
**Antiamnesic Properties of Compound 17.** One purpose of polypharmacology is to study the binding of one drug to multiple targets within a regulatory network process that is altered in the disease state. In the case of complex neurodegenerative diseases, such as AD which is related to the abnormality and dysfunction of multisystems, polypharma-

cology seems to be ideally suited to the search of new treatments.<sup>78</sup> However, drugs that are active at more than one target can lead to various outcomes, both beneficial and harmful. While studies suggested that activation of 5-HT<sub>4</sub>R may improve cognitive processes such as learning and memory,<sup>28</sup> marked impairments were observed after injection of 5-HT<sub>4</sub>R antagonists.<sup>79</sup> Therefore, the beneficial effect of our H<sub>3</sub>R antagonists on cognitive performances seemed to be counteracted by a behavior as 5-HT<sub>4</sub>R antagonists, as observed for compound 16. The antiamnesic effect observed with compound 17 in the spatial working memory experiment is more interesting. At first glance, the cross-analysis of the data reported in Table 4 showed that compound 17 exhibited the highest H<sub>3</sub>R affinity ( $K_i = 41.6$  nM) and a correct 5-HT<sub>4</sub>R affinity ( $K_i = 208$  nM). The explanation for the difference observed in the spatial working memory experiments should be related to a difference in the 5-HT<sub>4</sub>R profile itself and not only to the lowest 5-HT<sub>4</sub>R affinity of 17 compared to 16. For the 5-HT<sub>4</sub>R, it would not be the first time that very close chemical structures have been found to be agonists, partial agonists, inverse agonists, or antagonists.<sup>80</sup> As an example, slight modification around the basic function of reference compounds 18 and 19 drastically influenced their pharmacological profiles since 18 is a 5-HT<sub>4</sub>R agonist and 19 a 5-HT<sub>4</sub>R antagonist (Chart 2). This scenario also corroborates the studies relating to the high similarity between the pharmacophores for 5-HT<sub>4</sub>R antagonism and 5-HT<sub>4</sub>R agonism.<sup>81</sup> Figure 4 illustrates the common characteristics of the 5-HT<sub>4</sub>R agonist pharmacophore not only with the 5-HT<sub>4</sub>R antagonist pharmacophore, but also with the H<sub>3</sub>R antagonist pharmacophore. All of them display a



**Figure 6.** Effects of compound 16 (A) or 17 (B) (0.3, 1, and 3 mg kg<sup>-1</sup>, i.p.), thioperamide (THIO, 2.5 mg kg<sup>-1</sup>, i.p.), and RS 67333 (1 mg kg<sup>-1</sup> ip) in combination with scopolamine (SCOP, 0.25 mg kg<sup>-1</sup>, s.c.) on working memory in the spontaneous alternation test. Mice were injected by compound 16, compound 17, thioperamide, or RS 67333 just after scopolamine administration, 30 min before the Y-maze test. Data represent the mean ( $\pm$ SEM) percentage of alternation of 10 mice per group.

#### Chart 2. Example of Close 5-HT<sub>4</sub>R Reference Compounds with Opposite Pharmacological Profiles



basic center, an aromatic ring, and a hydrogen bond acceptor. For explaining variations between the pharmacophores, shorter distances were observed between the basic center and the two remaining characteristics for several 5-HT<sub>4</sub>R agonists. By the way, the mapping of the benzo[*h*][1,6]naphthyridine derivative 17 on all of these pharmacophores explains its dual H<sub>3</sub>R/5-HT<sub>4</sub>R affinities.

#### CONCLUSION

Within this study, we described the high H<sub>3</sub>R affinity of benzo[*h*][1,6]naphthyridine derivatives. These ligands were previously reported as 5-HT<sub>4</sub>R antagonists/partial agonists. Such dual-acting ligands may be of therapeutic interest for the treatment of degenerative disorders associated with impaired cholinergic function, such as Alzheimer's disease, and could work synergistically to have both symptomatic and disease-modifying effects. In a spatial working memory experiment, encouraging results were observed since compound 17

completely reversed the cognitive impairment effect of scopolamine at 3 mg/kg, denoting its antiamnesic properties. Therefore, we proved for the first time the possibility to combine 5-HT<sub>4</sub>R and H<sub>3</sub>R activities in a single molecule.

#### ASSOCIATED CONTENT

##### S Supporting Information

Figures showing the ranking and the structures of all of the 268 filtered compounds, proofs of the dose-response behavior for the three lead compounds, and sequence alignment. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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##### Notes

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## ■ REFERENCES

- (1) Brown, R. E.; Stevens, D. R.; Haas, H. L. The physiology of brain histamine. *Prog. Neurobiol.* **2001**, *63*, 637–672.
- (2) Arrang, J.-M.; Garbarg, M.; Schwartz, J.-C. Auto-inhibition of brain histamine release mediated by a novel class (H3) of histamine receptor. *Nature* **1983**, *302*, 832–837.
- (3) Clapham, J.; Kilpatrick, G. J. Histamine H3 receptors modulate the release of [<sup>3</sup>H]-acetylcholine from slices of rat entorhinal cortex: evidence for the possible existence of H3 receptor subtypes. *Br. J. Pharmacol.* **1992**, *107*, 919–923.
- (4) Schlicker, E.; Fink, K.; Hinterthaner, M.; Göthert, M. Inhibition of noradrenaline release in the rat brain cortex via presynaptic H3 receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1989**, *340*, 633–638.
- (5) Schlicker, E.; Fink, K.; Detzner, M.; Göthert, M. Histamine inhibits dopamine release in the mouse striatum via presynaptic H3 receptors. *J. Neural Transm.: Gen. Sect.* **1993**, *93*, 1–10.
- (6) Schlicker, E.; Betz, R.; Göthert, M. Histamine H3 receptor-mediated inhibition of serotonin release in the rat brain cortex. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1988**, *337*, 588–590.
- (7) Martinez-Mir, M. I.; Pollard, H.; Moreau, J.; Arrang, J.-M.; Ruat, M.; Traiffort, E.; Schwartz, J.-C.; Palacios, J. M. Three histamine receptors (H1, H2 and H3) visualized in the brain of human and non-human primates. *Brain Res.* **1990**, *526*, 322–327.
- (8) Pollard, H.; Moreau, J.; Arrang, J.-M.; Schwartz, J.-C. A detailed autoradiographic mapping of histamine H3 receptors in rat brain areas. *Neuroscience* **1993**, *52*, 169–189.
- (9) Fox, G. B.; Esbenshade, T. A.; Pan, J. B.; Radek, R. J.; Krueger, K. M.; Yao, B. B.; Brownman, K. E.; Buckley, M. J.; Ballard, M. E.; Komater, V. A.; Miner, H.; Zhang, M.; Faghah, R.; Rueter, L. E.; Bitner, S. R.; Drescher, K. U.; Wetter, J.; Marsh, K. C.; Lemaire, M.; Porsolt, R. D.; Bennani, Y. L.; Sullivan, J. P.; Cowart, M. D.; Decker, M. W.; Hancock, A. A. Pharmacological properties of ABT-239 [4-(2-{2-[2R)-2-Methylpyrrolidinyl]ethyl}-benzofuran-5-yl)benzonitrile]: II. Neurophysiological characterization and broad preclinical efficacy in cognition and schizophrenia of a potent and selective histamine H3 receptor. *J. Pharmacol. Exp. Ther.* **2005**, *313*, 176–190.
- (10) Medhurst, A. D.; Atkins, A. R.; Beresford, I. J.; Brackenborough, K.; Briggs, M. A.; Calver, A. R.; Cilia, J.; Cluderay, J. E.; Crook, B.; Davis, J. B.; Davis, R. K.; Davis, R. P.; Dawson, L. A.; Foley, A. G.; Gartlon, J.; Gonzalez, M. I.; Heslop, T.; Hirst, W. D.; Jennings, C.; Jones, D. N. C.; Lacroix, L. P.; Martyn, A.; Ociepka, S.; Ray, A.; Regan, C. M.; Roberts, J. C.; Schogger, J.; Southam, E.; Stean, T. O.; Trail, B. K.; Upton, N.; Wadsworth, G.; Wald, J. A.; White, T.; Witherington, J.; Woolley, M. L.; Worby, A.; Wilson, D. M. GSK189254, a novel H3 receptor antagonist that binds to histamine H3 receptors in Alzheimer's disease brain and improves cognitive performance in preclinical models. *J. Pharmacol. Exp. Ther.* **2007**, *321*, 1032–1045.
- (11) Contestabile, A. The history of the cholinergic hypothesis. *Behav. Brain Res.* **2011**, *221*, 334–340.
- (12) Esbenshade, T. A.; Brownman, K. E.; Bitner, S. R.; Strakhova, M.; Cowart, M. D.; Brioni, J. D. The histamine H3 receptor: an attractive target for the treatment of cognitive disorders. *Br. J. Pharmacol.* **2008**, *154*, 1166–1181.
- (13) Gemkow, M. J.; Davenport, A. J.; Harich, S.; Ellenbroek, B. a.; Cesura, A.; Hallett, D. The histamine H3 receptor as a therapeutic drug target for CNS disorders. *Drug Discovery Today* **2009**, *14*, 509–515.
- (14) Sander, K.; Kottke, T.; Stark, H. Histamine H3 receptor antagonists go to clinics. *Biol. Pharm. Bull.* **2008**, *31*, 2163–2181.
- (15) Singh, M.; Jadhav, H. R. Histamine H3 receptor function and ligands: recent developments. *Mini-Rev. Med. Chem.* **2013**, *13*, 47–57.
- (16) Auld, D. S.; Korneckook, T. J.; Bastianetto, S.; Quirion, R. Alzheimer's disease and the basal forebrain cholinergic system: relations to beta-amyloid peptides, cognition, and treatment strategies. *Prog. Neurobiol.* **2002**, *68*, 209–245.
- (17) Mangialasche, F.; Solomon, A.; Winblad, B.; Mecocci, P.; Kivipelto, M. Alzheimer's disease: clinical trials and drug development. *Lancet Neurol.* **2010**, *9*, 702–716.
- (18) Goedert, M.; Klug, A.; Crowther, R. A. Tau protein, the paired helical filament and Alzheimer's disease. *J. Alzheimer's Dis.* **2006**, *9*, 195–207.
- (19) Bitner, S. R.; Markosyan, S.; Nikkel, A. L.; Brioni, J. D. In-vivo histamine H3 receptor antagonism activates cellular signaling suggestive of symptomatic and disease modifying efficacy in Alzheimer's disease. *Neuropharmacology* **2011**, *60*, 460–466.
- (20) Brioni, J. D.; Esbenshade, T. A.; Garrison, T. R.; Bitner, S. R.; Cowart, M. D. Discovery of histamine H3 antagonists for the treatment of cognitive disorders and Alzheimer's disease. *J. Pharmacol. Exp. Ther.* **2011**, *336*, 38–46.
- (21) Clement, O. O.; Mehl, A. T. HipHop: Pharmacophores based on multiple common-feature alignments. In *Pharmacophore Perception, Development, and Use in Drug Design*; Guner, F. O., Ed.; IUL Biotechnology Series: La Jolla, CA, USA, 2000; pp 69–84.
- (22) Barnum, D.; Greene, J.; Smellie, A.; Sprague, P. Identification of common functional configurations among molecules. *J. Chem. Inf. Comput. Sci.* **1996**, *36*, 563–571.
- (23) Geldenhuys, W. J.; Van der Schyf, C. J. Role of serotonin in Alzheimer's disease: A new therapeutic target? *CNS Drugs* **2011**, *25*, 765–781.
- (24) Herrmann, N.; Chau, S. A.; Kircanski, I.; Lanctôt, K. L. Current and emerging drug treatment options for Alzheimer's disease: a systematic review. *Drugs* **2011**, *71*, 2031–2065.
- (25) Sabbagh, M. N. Drug development for Alzheimer's disease: Where are we now and where are we headed? *Am. J. Geriatr. Pharmacother.* **2009**, *7*, 167–185.
- (26) Lezoualc'h, F. 5-HT4 receptor and Alzheimer's disease: The amyloid connection. *Exp. Neurol.* **2007**, *205*, 325–329.
- (27) Lelong, V.; Dauphin, F.; Boulouard, M. RS 67333 and D-cycloserine accelerate learning acquisition in the rat. *Neuropharmacology* **2001**, *41*, 517–522.
- (28) Lelong, V.; Lhonneur, L.; Dauphin, F.; Boulouard, M. BIMU 1 and RS 67333, two 5-HT4 receptor agonists, modulate spontaneous alternation deficits induced by scopolamine in the mouse. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2003**, *367*, 621–628.
- (29) Hotte, M.; Dauphin, F.; Freret, T.; Boulouard, M.; Levallet, G. A biphasic and brain-region selective down-regulation of cyclic adenosine monophosphate concentrations supports object recognition in the rat. *PLoS One* **2012**, *7*, e32244.
- (30) Levallet, G.; Hotte, M.; Boulouard, M.; Dauphin, F. Increased particulate phosphodiesterase 4 in the prefrontal cortex supports 5-HT4 receptor-induced improvement of object recognition memory in the rat. *Psychopharmacology (Berlin, Ger.)* **2009**, *202*, 125–139.
- (31) Klinenberg, I.; Blokland, A. The validity of scopolamine as a pharmacological model for cognitive impairment: A review of animal behavioral studies. *Neurosci. Biobehav. Rev.* **2010**, *34*, 1307–1350.
- (32) Peters, J.-U. Polypharmacology - Foe or friend? *J. Med. Chem.* **2013**, *56*, 8955–8971.
- (33) Hinschberger, A.; Butt, S.; Lelong, V.; Boulouard, M.; Dumuis, A.; Dauphin, F.; Bureau, R.; Pfeiffer, B.; Renard, P.; Rault, S. New benzo[h][1,6]naphthyridine and azepino[3,2-c]quinoline derivatives as selective antagonists of 5-HT4 receptors: Binding profile and pharmacological characterization. *J. Med. Chem.* **2003**, *46*, 138–147.
- (34) Zaragoza, F.; Stephensen, H.; Peschke, B.; Rimvall, K. 2-(4-alkylpiperazin-1-yl)quinolines as a new class of imidazole-free histamine H3 receptor antagonists. *J. Med. Chem.* **2005**, *48*, 306–311.
- (35) Levoine, N.; Labeeuw, O.; Calmels, T.; Poupartin-Olivier, O.; Berrebi-Bertrand, I.; Lecomte, J.-M.; Schwartz, J.-C.; Capet, M. Novel and highly potent histamine H3 receptor ligands. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5378–5383.
- (36) Heightman, T. D.; Wilson, D. M. Benzo[d]azepine derivatives for the treatment of neurological and psychiatric disorders. WO2004035544, 2004.
- (37) Cowart, M. D.; Faghah, R.; Curtis, M. P.; Gfesser, G. A.; Bennani, Y. L.; Black, L. A.; Pan, L.; Marsh, K. C.; Sullivan, J. P.; Esbenshade, T. A.; Fox, G. B.; Hancock, A. A. 4-(2-[2-(2(R)-Methylpyrrolidin-1-yl)ethyl]benzofuran-5-yl)benzonitrile and related

- 2-aminoethylbenzofuran H3 receptor antagonists potently enhance cognition and attention. *J. Med. Chem.* **2005**, *48*, 38–55.
- (38) Jesudason, C. D.; Beavers, L. S.; Cramer, J. W.; Dill, J.; Finley, D. R.; Lindsley, C. W.; Stevens, F. C.; Gadski, R. A.; Oldham, S. W.; Pickard, R. T.; Siedem, C. S.; Sindelar, D. K.; Singh, A.; Watson, B. M.; Hipskind, P. A. Synthesis and SAR of novel histamine H3 receptor antagonists. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3415–3418.
- (39) Dvorak, C. A.; Apodaca, R.; Barbier, A. J.; Berridge, C. W.; Wilson, S. J.; Boggs, J. D.; Xiao, W.; Lovenberg, T. W.; Carruthers, N. I. 4-Phenoxyperidines: Potent, conformationally restricted, non-imidazole histamine H3 antagonists. *J. Med. Chem.* **2005**, *48*, 2229–2238.
- (40) MacKerell, A. D.; Brooks, C. L.; Nilsson, L.; Roux, B.; Won, Y.; Karplus, M. CHARMM: The Energy Function and Its Parameterization with an Overview of the Program. In Schleyer, P. v. R., et al., Eds.; John Wiley & Sons: Chichester, U.K., 1998; Vol. 1, pp 271–277.
- (41) Smellie, A.; Teig, S. L.; Towbin, P. Poling: Promoting conformational variation. *J. Comput. Chem.* **1995**, *16*, 171–187.
- (42) Hagadone, T. R. Molecular substructure similarity searching: Efficient retrieval in two-dimensional structure databases. *J. Chem. Inf. Model.* **1992**, *32*, 515–521.
- (43) Jain, E.; Bairoch, A.; Duvaud, S.; Phan, I.; Redaschi, N.; Suzek, B. E.; Martin, M. J.; McGarvey, P.; Gasteiger, E. Infrastructure for the life sciences: Design and implementation of the UniProt website. *BMC Bioinf.* **2009**, *10*, 136.
- (44) Pons, J.-L.; Labesse, G. @TOME-2: A new pipeline for comparative modeling of protein-ligand complexes. *Nucleic Acids Res.* **2009**, *37*, W485–W491.
- (45) Shimamura, T.; Shiroishi, M.; Weyand, S.; Tsujimoto, H.; Winter, G.; Katritch, V.; Abagyan, R.; Cherezov, V.; Liu, W.; Han, G. W.; Kobayashi, T.; Stevens, R. C.; Iwata, S. Structure of the human histamine H1 receptor complex with doxepin. *Nature* **2011**, *475*, 65–70.
- (46) Labesse, G.; Mornon, J. Incremental threading optimization (TITO) to help alignment and modelling of remote homologues. *Bioinformatics* **1998**, *14*, 206–211.
- (47) Eswar, N.; Eramian, D.; Webb, B.; Shen, M.-Y.; Sali, A. Protein structure modeling with MODELLER. *Methods Mol. Biol.* **2008**, *426*, 145–159.
- (48) Eisenberg, D.; Lüthy, R.; Bowie, J. U. VERIFY3D: Assessment of protein models with three-dimensional profiles. *Methods Enzymol.* **1997**, *277*, 396–404.
- (49) Gracy, J.; Chiche, L.; Sallantin, J. Learning and alignment methods applied to protein structure prediction. *Biochimie* **1993**, *75*, 353–361.
- (50) Gouet, P.; Robert, X.; Courcelle, E. ESPript/ENDscript: Extracting and rendering sequence and 3D information from atomic structures of proteins. *Nucleic Acids Res.* **2003**, *31*, 3320–3323.
- (51) Sherman, W.; Day, T.; Jacobson, M. P.; Friesner, R. A.; Farid, R. Novel procedure for modeling ligand/receptor induced fit effects. *J. Med. Chem.* **2006**, *49*, 534–553.
- (52) Uveges, A. J.; Kowal, D.; Zhang, Y.; Spangler, T. B.; Dunlop, J.; Semus, S.; Jones, P. G. The role of transmembrane helix 5 in agonist binding to the human H3 receptor. *J. Pharmacol. Exp. Ther.* **2002**, *301*, 451–458.
- (53) Shin, N.; Coates, E.; Murgolo, N. J.; Morse, K. L.; Bayne, M.; Strader, C. D.; Monsma, F. J. Molecular modeling and site-specific mutagenesis of the histamine-binding site of the histamine H4 receptor. *Mol. Pharmacol.* **2002**, *62*, 38–47.
- (54) Kim, S.-K.; Fristrup, P.; Abrol, R.; Goddard, W. A. Structure-based prediction of subtype selectivity of histamine H3 receptor selective antagonists in clinical trials. *J. Chem. Inf. Model.* **2011**, *51*, 3262–3274.
- (55) Lim, H. D.; van Rijn, R. M.; Ling, P.; Bakker, R. A.; Thurmond, R. L.; Leurs, R. Evaluation of histamine H1-, H2-, and H3-receptor ligands at the human histamine H4 receptor: identification of 4-methylhistamine as the first potent and selective H4 receptor agonist. *J. Pharmacol. Exp. Ther.* **2005**, *314*, 1310–21.
- (56) Cheng, H. C. The power issue: Determination of KB or Ki from IC<sub>50</sub>. A closer look at the Cheng-Prusoff equation, the Schild plot and related power equations. *J. Pharmacol. Toxicol. Methods* **2001**, *46*, 61–71.
- (57) Lemaître, S.; Lepailleur, A.; Bureau, R.; Butt-Gueulle, S.; Lelong, V.; Duchatelle, P.; Bouloard, M.; Dumuis, A.; Daveu, C.; Lezoualc'h, F.; Pfeiffer, B.; Dauphin, F.; Rault, S. Novel antagonists of serotonin-4 receptors: Synthesis and biological evaluation of pyrrolothienopyrazines. *Bioorg. Med. Chem.* **2009**, *17*, 2607–2622.
- (58) Hessler, G.; Baringhaus, K.-H. The scaffold hopping potential of pharmacophores. *Drug Discovery Today Technol.* **2010**, *7*, e263–e269.
- (59) Apodaca, R.; Dvorak, C. A.; Xiao, W.; Barbier, A. J.; Boggs, J. D.; Wilson, S. J.; Lovenberg, T. W.; Carruthers, N. I. A new class of diamine-based human histamine H3 receptor antagonists: 4-(aminoalkoxy)benzylamines. *J. Med. Chem.* **2003**, *46*, 3938–3944.
- (60) Axe, F. U.; Bembeneck, S. D.; Szalma, S. Three-dimensional models of histamine H3 receptor antagonist complexes and their pharmacophore. *J. Mol. Graph. Modell.* **2006**, *24*, 456–464.
- (61) Celanire, S.; Wijtmans, M.; Talaga, P.; Leurs, R.; de Esch, I. J. P. Keynote review: Histamine H3 receptor antagonists reach out for the clinic. *Drug Discovery Today* **2005**, *10*, 1613–1627.
- (62) Evans, B. E.; Rittle, K. E.; Bock, M. G.; DiPardo, R. M.; Freidinger, R. M.; Whitter, W. L.; Lundell, G. F.; Veber, D. F.; Anderson, P. S.; Chang, R. S. Methods for drug discovery: Development of potent, selective, orally effective cholecystokinin antagonists. *J. Med. Chem.* **1988**, *31*, 2235–2246.
- (63) Müller, G. Medicinal chemistry of target family-directed masterkeys. *Drug Discovery Today* **2003**, *8*, 681–691.
- (64) Costantino, L.; Barlocchio, D. Privileged structures as leads in medicinal chemistry. *Curr. Med. Chem.* **2006**, *13*, 65–85.
- (65) Klabunde, T.; Hessler, G. Drug design strategies for targeting G-protein-coupled receptors. *ChemBioChem* **2002**, *3*, 928–944.
- (66) Rault, S.; Lancelot, J.-C.; Prunier, H.; Robba, M.; Renard, P.; Delagrange, P.; Pfeiffer, B.; Caignard, D. H.; Guardiola-Lemaitre, B.; Hamon, M. Novel selective and partial agonists of 5-HT3 receptors. Part 1. Synthesis and biological evaluation of piperazinopyrrolothienopyrazines. *J. Med. Chem.* **1996**, *39*, 2068–2080.
- (67) Prunier, H.; Rault, S.; Lancelot, J.-C.; Robba, M.; Renard, P.; Delagrange, P.; Pfeiffer, B.; Caignard, D. H.; Misslin, R.; Guardiola-Lemaitre, B.; Hamon, M. Novel and selective partial agonists of 5-HT3 receptors. 2. Synthesis and biological evaluation of piperazinopyridopyrrolopyrazines, piperazinopyrroloquinoloxalines, and piperazinopyridopyrroloquinoloxalines. *J. Med. Chem.* **1997**, *40*, 1808–1819.
- (68) Bureau, R.; Daveu, C.; Lancelot, J.-C.; Rault, S. Molecular design based on 3D-pharmacophore. Application to 5-HT subtypes receptors. *J. Chem. Inf. Comput. Sci.* **2002**, *42*, 429–436.
- (69) Bureau, R.; Daveu, C.; Lemaître, S.; Dauphin, F.; Landelle, H.; Lancelot, J.-C.; Rault, S. Molecular design based on 3D-pharmacophore. Application to 5-HT4 receptor. *J. Chem. Inf. Comput. Sci.* **2002**, *42*, 962–967.
- (70) Lepailleur, A.; Bureau, R.; Lemaître, S.; Dauphin, F.; Lancelot, J.-C.; Contesse, V.; Lenglet, S.; Delarue, C.; Vaudry, H.; Rault, S. Molecular design based on 3D pharmacophores. Applications to 5-HT7 receptors. *J. Chem. Inf. Comput. Sci.* **2004**, *44*, 1148–1152.
- (71) Consolo, S.; Arnaboldi, S.; Giorgi, S.; Russi, G.; Ladinsky, H. S. 5-HT4 receptor stimulation facilitates acetylcholine release in rat frontal cortex. *Neuroreport* **1994**, *5*, 1230–1232.
- (72) Levoin, N.; Labeeuw, O.; Krief, S.; Calmels, T.; Poupartdin-Olivier, O.; Berrebi-Bertrand, I.; Lecomte, J.-M.; Schwartz, J.-C.; Capet, M. Determination of the binding mode and interacting amino-acids for dibasic H3 receptor antagonists. *Bioorg. Med. Chem.* **2013**, *21*, 4526–4529.
- (73) Snyder, P. J.; Bednar, M. M.; Cromer, J. R.; Maruff, P. Reversal of scopolamine-induced deficits with a single dose of donepezil, an acetylcholinesterase inhibitor. *Alzheimer's Dementia* **2005**, *1*, 126–135.
- (74) Fredrickson, A.; Snyder, P. J.; Cromer, J. R.; Thomas, E.; Lewis, M.; Maruff, P. The use of effect sizes to characterize the nature of cognitive change in psychopharmacological studies: an example with scopolamine. *Hum. Psychopharmacol.* **2008**, *23*, 425–436.

- (75) Griebel, G.; Pichat, P.; Pruniaux, M.-P.; Beeské, S.; Lopez-Grancha, M.; Genet, E.; Terranova, J.-P.; Castro, A.; Sánchez, J. A.; Black, M.; Varty, G. B.; Weiner, I.; Arad, M.; Barak, S.; De Levie, A.; Guillot, E. SAR110894, a potent histamine H3-receptor antagonist, displays procognitive effects in rodents. *Pharmacol., Biochem. Behav.* **2012**, *102*, 203–214.
- (76) Hancock, A. A.; Fox, G. B. Perspectives on cognitive domains, H3 receptor ligands and neurological disease. *Expert Opin. Invest. Drugs* **2004**, *13*, 1237–1248.
- (77) Witkin, J. M.; Nelson, D. L. Selective histamine H3 receptor antagonists for treatment of cognitive deficiencies and other disorders of the central nervous system. *Pharmacol. Ther.* **2004**, *103*, 1–20.
- (78) Cavalli, A.; Bolognesi, M. L.; Minarini, A.; Rosini, M.; Tumiatti, V.; Recanatini, M.; Melchiorre, C. Multi-target-directed ligands to combat neurodegenerative diseases. *J. Med. Chem.* **2008**, *51*, 347–372.
- (79) Marchetti, E.; Dumuis, A.; Bockaert, J.; Soumireu-Mourat, B.; Roman, F. S. Differential modulation of the 5-HT4 receptor agonists and antagonist on rat learning and memory. *Neuropharmacology* **2000**, *39*, 2017–2027.
- (80) Bureau, R.; Boulouard, M.; Dauphin, F.; Lezoualc'h, F.; Rault, S. Review of 5-HT4R Ligands: State of Art and Clinical Applications. *Curr. Top. Med. Chem.* **2010**, *10*, 527–553.
- (81) Bureau, R.; Varin, T.; Lepailleur, A.; Daveu, C.; Lemaitre, S.; Lancelot, J.-C.; Lesnard, A.; Butt-Gueulle, S.; Dauphin, F.; Rault, S. Pharmacophores of 5-HT4 Receptor Ligands: Experience of CERMN and Implications for Drug Design. *Curr. Comput.-Aided Drug Des.* **2008**, *4*, 199–208.
- (82) Stierand, K.; Rarey, M. From modeling to medicinal chemistry: Automatic generation of two-dimensional complex diagrams. *Chem-MedChem.* **2007**, *2*, 853–860.