



HAL
open science

The design of donecopride, a dual 5-HT₄R agonist/AChE inhibitor with potential interest for AD treatment

Cédric Lecoutey, Damien Hedou, Thomas Freret, Patrizia Giannoni, Florence Gaven, Marc Since, Valentine Bouet, Céline Ballandonne, Sophie Corvaisier, Aurélie Malzert-Fréon, et al.

► To cite this version:

Cédric Lecoutey, Damien Hedou, Thomas Freret, Patrizia Giannoni, Florence Gaven, et al.. The design of donecopride, a dual 5-HT₄R agonist/AChE inhibitor with potential interest for AD treatment. Proceedings of the National Academy of Sciences of the United States of America, 2014, 54, pp.75-86. 10.1016/j.ejmech.2012.04.029 . hal-01800439v1

HAL Id: hal-01800439

<https://hal.science/hal-01800439v1>

Submitted on 26 May 2018 (v1), last revised 3 Dec 2018 (v2)

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

The design of donecopride, a dual 5-HT₄R agonist/AChE inhibitor with potential interest for AD treatment

Cédric Lecoutey,^{1,2} Damien Hedou,^{1,2} Thomas Freret,^{1,3} Patrizia Giannoni,^{4,5,6} Florence Gaven,^{4,5,6} Marc Since,^{1,2} Valentine Bouet,^{1,3} Céline Ballandonne,^{1,2} Sophie Corvaisier,^{1,2,3} Aurélie Malzert-Fréon,^{1,2} Serge Mignani,^{1,2} Thierry Cresteil,⁷ Michel Boulouard,^{1,3} Sylvie Claeysen,^{4,5,6} Christophe Rochais,^{1,2} Patrick Dallemagne^{1,2}

¹ Normandie Univ, France

² UNICAEN, CERMN (Centre d'Etudes et de Recherche sur le Médicament de Normandie), F-14032 Caen, France

³ UNICAEN GMPc (Groupe Mémoire et Plasticité comportementale), F-14032 Caen, France

⁴ CNRS, UMR-5203, Institut de Génomique Fonctionnelle, F-34000 Montpellier, France

⁵ Inserm, U661, F-34000 Montpellier, France

⁶ Universités de Montpellier 1 & 2, UMR-5203, F-34000 Montpellier, France

⁷ ICSN - CNRS UPR 2301 Avenue de la terrasse F-91198 Gif sur Yvette, France

Corresponding authors:

Patrick Dallemagne, PhD, PharmD

Christophe Rochais, PhD

CERMN – EA4258

Boulevard Becquerel, 14032 Caen, France

+33 231 566 813

patrick.dallemagne@unicaen.fr

christophe.rochais@unicaen.fr

Abstract

RS67,333 is a partial 5-HT₄R agonist that has been widely studied for its procognitive effect. More recently it has been demonstrated that its ability to promote the non-amyloidogenic cleavage of the precursor of the neurotoxic peptide A β leads to the secretion of the neurotrophic protein sAPP α . This effect has generated great interest in RS67,333 as a potential treatment for Alzheimer's Disease (AD). We show herein that RS67,333 is also a submicromolar acetylcholinesterase (AChE) inhibitor and therefore could contribute, through this effect, to the restoration of the cholinergic neurotransmission that becomes altered in AD. We planned to pharmacomodulate RS67,333 in order to enhance its AChE inhibitory activity to take advantage of this pleiotropic pharmacological profile in the design of a novel multi-target directed ligand (MTDL) that is able to exert not only a symptomatic but also a disease-modifying effect against AD. These efforts allowed us to select donecopride as a valuable dual (*h*)5-HT₄R partial agonist (K_i = 10.4 nM; a 48.3% of control agonist response)/(*h*)AChEI (IC₅₀ = 16 nM) that further promotes sAPP α release (EC₅₀ = 11.3 nM). Donecopride, as a druggable lead, was assessed for its *in vivo* procognitive effects (0.1, 0.3, 1 and 3 mg/kg) with an improvement of memory performances observed at 0.3 and 1 mg/kg on the object recognition test. On the basis of these *in vitro* and *in vivo* activities, donecopride appears as a promising drug candidate for AD treatment.

\body

Significance Statement

Targeting more than one molecular cause implied in the pathogenesis of AD with a sole drug is considered a promising challenge, as it may address the numerous failures that recently occurred during clinical trials that were conducted in this area. Donecopride has been designed by us as a multi-target-directed ligand (MTDL), targeting both 5-HT₄R and acetylcholinesterase (AChE) with excellent *in vitro* activities. The latter appear able to not only restore the cholinergic neurotransmission altered in AD, but also to promote the secretion of a neurotrophic protein (sAPP α), which is detrimental to the neurotoxic A β peptide. With its excellent drugability, donecopride further displayed significant procognitive effects in mice and generated a promising lead for a novel approach in AD treatment.

Introduction

Among the large family of serotonergic receptors (5-HTR), some of them are of particular interest in improving memory performance and, therefore, in decreasing memory deficits, such as those that occur in Alzheimer's disease (AD). This is the case for 5-HT₄R. In the central nervous system, they are located in structures that are primarily involved in cognitive functions like the olfactory tubercles, basal ganglia, septum, substantia nigra, superior colliculi, hippocampus, and cortex. Several compounds act as agonists to 5-HT₄R (BIMU1, BIM2, RS17017, SL65.0155, VRX-03011, prucalopride, RS67,333, and RS67506). One of the most affine (pK_i = 7.88) and selective *versus* other receptors is RS67,333, which acts as a partial agonist [1]. With respect to the potential therapeutic modulation of 5-HT₄R with RS67,333, and excluding its putative antidepressant-like activity [2-4], most studies focused on the promnesic or anti-amnesic actions of this compound. These effects on cognitive functions that concern learning and memory are probably due, in part, to the fact that the pharmacological stimulation of these receptors increases the release of acetylcholine (ACh) in the hippocampus and cortex, and it also increases serotonin, dopamine, and gamma-aminobutyric acid (GABA) release [5-13]. Concerning the selective aspects of memory functions, RS67,333 has been shown to improve object recognition in adult [14,15] and aged animals [16,17], or place recognition [10] in rodents. It also increases spatial learning on the Morris water maze task in rodents, and it even reverses the deleterious effect of atropine [18] or scopolamine during this same task.

Based on the structural analogy existing between RS67,333 and donepezil (Fig. 1), we postulated that RS67,333 could improve learning and memory not only by activating 5-HT₄R, but also by inhibiting acetylcholinesterase (AChE) activity. Indeed, several early studies reported that the inhibition of AChE improved cognition, and that this is the main reason for the initial use of donepezil, galantamine, and rivastigmine as cognitive enhancers in AD [19,20]. AChE inhibition has also been reported to improve performances in healthy rodents or in animal models of memory deficiency [21]. The hypothesis of an involvement of AChE inhibition by RS67,333 in memory improvement is an issue that has never been tested.

Furthermore, such a pharmacological profile could be also exploited to lead to novel pleiotropic compounds that are theoretically useful in AD treatment. Indeed, today it is well established that 5-HT₄R activation not only favors acetylcholine (ACh) release, but it is also involved in the non-amyloidogenic cleavage of amyloid precursor protein (APP) in the neurotrophic sAPP α fragment, whose secretion is detrimental to amyloid- β peptide (A β) production [22-24]. On the other hand, inhibiting the catalytic activity of AChE is widely used to restore cholinergic neurotransmission in AD, and interacting with the peripheral anionic site (PAS) of this enzyme could also reduce amyloid aggregation, for which AChE would be responsible [25]. These activities appear to be synergistic when they are associated in an AD animal model, as we have recently demonstrated in mice [26]. A second pharmacological approach bases on the fact that a single compound may be able to hit multiple targets is now emerging. This novel concept, called Multi-Target-Directed Ligands (MTDLs) [27,28], would have inherent advantages over combination of drugs called Multiple-Medication-Therapy (MMT). It would specially obviate the problems linked to the complexity of the pharmacokinetic profile of the combined drugs and the risk of drug-drug interactions. Moreover, MTDL could also alleviate compliance difficulties associated to MMT. It has been moreover demonstrated that MTDL generally show a higher synergistic effect than those observed with a combination of drugs. Numerous examples of MTDL against AD have been recently described [27]. Most of them associate an AChE inhibitory effect with another activity hitting another molecular target of AD such as antioxidant effect, MAO inhibition, calcium channel blocking effect, metal chelating activity, etc. However, none MTDL associating an inhibition of AChE and 5-HT₄R agonist effect has been hitherto described. Among the different ways to synthesize such MTDL, one of them is to merge the frameworks of two selective starting compounds, each one exerting an activity towards a sole target [28]. This goal is even more easily reached if the starting compounds are structurally close. This is the reason why we considered the structural analogy between donepezil and RS67333 as a good starting point in order to design MTDL displaying both activities (AChE inhibition and 5-HT₄R agonist activities). We will thus provide proof of this concept with the synthesis and biological evaluation of MR31147 (donecopride), as conceived from the pharmacomodulation of RS67,333.

Manuscript text

RS67,333 is a submicromolar (*h*)AChE inhibitor.

RS67,333 displayed remarkable inhibitory effects toward (*h*)AChE (IC₅₀ = 403 nM), which has not been described previously. This submicromolar activity appears to be highly linked to a structural family effect, since the compound RS67506 - another potent 5-HT₄R agonist (pK_i = 8.8) that is chemically similar to RS67,333 - also strongly inhibits (*h*)AChE (IC₅₀ = 211 nM). These compounds share common intracyclic nitrogen able to engage a Π -cation interaction with a Phe in the aromatic gorge of AChE [29]. Conversely, some other 5-HT₄R agonists belonging to other structural families are devoid of such activity (Table 1).

On the basis of these results, RS67,333 was chosen as a hit in order to perform a hit-to-lead chemistry study. Numerous derivatives of RS67,333 were synthesized. Among them, the compound MR31147 (donecopride) was selected for its outstanding *in vitro* activity towards both (*h*)5-HT_{4e}R and (*h*)AChE. The cyclohexyl moiety, introduced into the structure of donecopride, appears as a compromise between the *N*-butyl group of RS67,333 and the bulky benzyl group of donepezil, able to potentiate both activities.

Donecopride is a potent, selective and partial (*h*)5-HT_{4e}R agonist

Donecopride (Fig. 2) is a (*h*)5-HT_{4e}R ligand that is as potent as RS67,333 (Table 2). It behaves like a partial agonist with its intrinsic activity of 48% versus 49% for RS67,333 (Fig. 3). This profile is in accordance with our objective, since prolonged exposure of G protein-coupled receptors (GPCR) to an agonist can result in receptor desensitization, which is often dependent upon the degree of intrinsic activity [30]. On the other hand, the selectivity of donecopride towards 5-HT₄R is quite good, since among a panel of 42 common neurotransmitter receptors and transporters, only 5-HT_{2b}R and σ ₂R are more sensitive than 5-HT₄R (Table 2). Concerning 5-HT_{2b}R, donecopride behaves like an inverse agonist, thus avoiding the potential risk of valvulopathy that the activation of these receptors could provoke. Concerning σ ₂R, they are found in many peripheral tissues where their role remains unclear. On the other hand, they are overexpressed in a wide variety of human and murine tumor cell lines and solid tumors and appear as valuable biomarkers of cell proliferation [31]. This σ ₂ profile for donecopride should not theoretically interfere with its central activity.

Donecopride is a potent, selective, mixed type competitive (h)AChE inhibitor

Donecopride is also a potent catalytic (h)AChE inhibitor, with the same order of magnitude as donepezil. It appears further selective towards this enzyme *versus* (eq)BuChE (Table 3). Furthermore, it also appears to be able to interact with the PAS of AChE, as was suggested by its significant ability to displace propidium iodide from the latter (% inhibition of the fluorescence of propidium bound to AChE = 24% at 10 μ M), donepezil being used as a control (21.5% at 10 μ M) (Table 3) [32]. This result was confirmed by the mixed type competitive AChE inhibitor profile of donecopride (Fig. 4) and accounts for its potential inhibitory effect against AChE-induced A β aggregation.

Donecopride promotes the sAPP α secretion in an *in vitro* cellular model

5-HT₄R activation induces the non-amyloidogenic processing of APP and sAPP α release *in vivo* and *in vitro* [34,35]. Interestingly, we have recently demonstrated that the early and chronic administration of RS67,333 is able to reduce amyloid plaque formation and it also prevents cognitive deficits in a transgenic mouse model of AD [36]. The *in vitro* ability of donecopride to promote sAPP α secretion was evaluated on COS-7 cells that were transiently expressing 5-HT₄R. Similar to RS 67333, donecopride induced a clear dose-dependent sAPP α release (Table 4 and Fig. 5).

Donecopride exhibits good drugability parameters

Before performing an *in vivo* study of donecopride, several drugability parameters were established, including its water solubility and blood-brain barrier (BBB) cross-membrane penetration.

The thermodynamic water solubility of donecopride at 48 hours was determined at various pH levels according to the classic shake-flask method and miniaturized ultraviolet (UV) spectrometric quantification (Table 5) [37].

Particular attention has been paid to the ability of the fumarate salt of donecopride (chosen among a series of salts) to cross the BBB. According to a parallel artificial membrane permeability assay (PAMPA) experiment [38], the drug (MW = 508 g.mol⁻¹) was classified among the compounds as having “good brain penetration” with logPe = -4.43 \pm 0.10 (effective permeability). This result was fully confirmed after performing a cellular test using bovine brain capillary endothelial cells and rat glial cells with Pe > 2 \times 10⁻³ cm/min (Fig. 6). This good permeability appears in accordance with the calculated log P of donecopride (log P = 4.4, MarvinSketch 5.11.1).

Since the poor brain penetration of 5-HT₄R ligands is often linked to P-glycoprotein (P-gp)-mediated efflux [39], we evaluated the ability of donecopride to inhibit the P-gp on NCI/ADR-res cells at 1 μ M and 10 μ M. The results confirmed that donecopride inhibits rhodamine 123 efflux to a lesser degree than cyclosporin (\approx 140 *versus* 400%). Cyclosporin is a reference substrate of P-gp and markedly promoted the accumulation of rhodamine 123 into cells, whereas donecopride had only a modest effect at concentration ranged 1-10 μ M. This clearly suggested that donecopride was not a potent substrate for P-gp and therefore is not actively effluxed from cells (Table 6).

Some other toxicological parameters have been also established for donecopride including mutagenicity, cytotoxicity and HERG affinity. It did not exhibit mutagenicity, cytotoxicity or significant HERG affinity (Table 7).

All of these parameters account for a suitable drugability that enabled the preliminary study of donecopride *in vivo*.

Donecopride is procognitive in mice

The aim of this study was to draw a dose–effect curve of donecopride on the behavioral performances observed during an object recognition task. Therefore, we examined the impact of the intraperitoneal administration of donecopride (given 30 min just before an acquisition session) on long-term memory performances, which were evaluated after 72 hours. The required time to reach the time criterion of objects exploration during training did not significantly differ between the different groups. After a 72-hour inter-trial interval, two-way analysis of variance (ANOVA) showed an objects effect and a group \times object interaction (respectively, $F_{(1, 44)} = 12.944$, $P = 0.0008$ and $F_{(4, 44)} = 3.547$, $P = 0.0136$). Indeed, only mice treated with either 0.3 mg/kg or 1 mg/kg of donecopride showed an objects effect (respectively, one-way ANOVA: $F_{(1, 9)} = 7.263$, $P = 0.0246$ and $F_{(1, 8)} = 12.244$, $P = 0.0081$). Besides, post hoc analysis using the Student–Newman–Keuls test ($\alpha = 0.05$) indicated that mice treated with either 0.3 mg/kg or 1 mg/kg of donecopride showed a significantly higher

discrimination index ($DI = 6.300 \pm 1.647$ and 8.222 ± 2.350 , respectively) than did the control group ($DI = -0.222 \pm 1.839$) (Fig. 7). However, the absence of procognitive effect at the highest dose (3 mg/kg) may easily be attributed to the appearance of a cholinergic side effect, classically observed while using high doses of AChE inhibitors [40].

Discussion

The discovery of the AChE inhibitory activity of RS67,333 appears to be of a great interest since this compound has been widely studied for its *in vivo* procognitive effect. The latter was hitherto only imputed to the 5-HT₄R agonist activity and, therefore, to the resulting 5-HT₄R related ACh (and, perhaps, sAPP α) release enhancement. The influence of AChE inhibition and, therefore, the contribution of the resulting increase in synaptic ACh concentration, on this procognitive effect will have to be assessed. Further, RS67,333 can be considered as a hit in order to design pleiotropic agents with a dual 5-HT₄R agonist/AChE inhibition activity. With this goal in mind, we performed the synthesis of a series of novel analogs of RS67,333. Our first objective was to confirm whether modulation of the chemical structure of RS67,333 could enhance its activity towards both targets. For the synthesized compound, we were pleased to identify donecopride, which validates our hypothesis. First, this compound conserves its affinity towards 5-HT₄R *in vivo*, with a K_i of 10.4 nM, but it also retains the partial agonist activity of RS67,333. We have further proven that donecopride is able to promote the secretion of the neurotrophic protein, sAPP α , in an *in vitro* cellular model. In addition, more interestingly, we have also shown that a simple chemical modification, such as the replacement of the *n*-butyl chain of RS67,333 by a methylenecyclohexyl group, dramatically increases the potency of our compound toward AChE. Indeed, donecopride inhibits the catalytic active site (CAS) of AChE with an IC_{50} of 16 nM, which is comparable to donepezil, and which is 25-fold better than RS67,333. Donecopride is also able to display a DBS inhibitory effect on AChE. This MTDL profile seems to confer to donecopride the ability to display both improvements in cholinergic neurotransmission (through the catalytic inhibition of AChE and the release of ACh), and in the inhibition of A β accumulation and aggregation. This second effect would be possible through the non-amyloidogenic 5-HT₄R-dependant cleavage of the precursor of A β , as well as through the interaction of donecopride with the peripheral anionic site of AChE, which is able to counteract the chaperone role played by the latter in A β aggregation. Finally, these complementary activities could exert both symptomatic and disease-modifying effects for AD treatment (Fig. 8).

Interest in such a dual effect has been recently demonstrated with the synergistic co-administration of sub-active doses of donepezil and RS67,333, which resulted in a significantly procognitive effect [26]. Donecopride, which combines the activities of donepezil and RS67,333 in a single compound, possesses excellent drugability parameters with a good bioavailability; it also has the ability to cross the BBB and features a non-toxic profile. Finally, donecopride exerted a significant procognitive effect in mice *in vivo*. It will be interesting to compare the *in vivo* effects of drugs bearing only one activity (ACh inhibition or 5-HT₄R stimulation) with donecopride in AD mouse models. Considering that chronic treatments with RS67,333 have already demonstrated their ability to slow down AD pathology and prevent cognitive deficits in mice [36], donecopride opens the door to a new class of compounds that could take advantage of the well-known properties of AChE inhibitors, and it could also enhance the serotonergic transmission that appears to be more and more relevant for a cure for AD [41]. Undeniably, serotonin signaling is associated with a lowering of A β levels, either in soluble form or in deposits, a decrease that has a positive outcome on cognitive performance in mice and that may prevent deleterious amyloid accumulation in AD [41-43]. On the other side, activation of 5-HTR (5-HT_{2A}, 5-HT_{2C} and 5-HT₄) has been demonstrated to also induce an increase in soluble form of APP α [34,36,44]. Whereas the precise identification of the soluble fragment is still pending for 5-HT_{2A/C}R, 5-HT₄ agonists undoubtedly induce the secretion of the neuroprotective sAPP α fragment and the activation on the non-amyloidogenic processing of APP [36]. Thus, to the symptomatic cholinergic effects, donecopride and related MTDLs should add capacities to impact APP metabolism by activating 5-HT₄R. Further *in vivo* experiments are required to precisely examine the beneficial effect of this two-target combination and whether it could slow down AD progression.

Considering that the recent failures that have occurred in numerous clinical trials conducted with novel drugs for AD treatment were sometimes attributed to their great selectivity towards a unique target [45,46], the development of a MTDL, such as donecopride, could be viewed as a promising challenge.

Material and methods

Details describing the synthesis and characterization of donecopride; acetyl and butyrylcholinesterase assays; propidium competition assay; serotonin and other receptors assays; sAPP α production; PAMPA- and *in vitro*-BBB assay; water solubility determination; cytotoxicity assay; P-gp efflux pump inhibition; hERG assay; mutagenicity assay; Novel Object Recognition task can be found in *SI Text*.

ACKNOWLEDGMENTS

This work was supported by funding from the Conseil Régional de Basse Normandie, France (Dispositif de Soutien aux Projets de Recherche Emergents), the French Agence Nationale de la Recherche (project MALAD ANR-12-JS007-0012-01 and project ADAMGUARD ANR-12-BSV4-008-01) and The Ligue Européenne Contre la Maladie d'Alzheimer (LECMA, grant number #12721).

References

1. Eglén RM, et al. (1995) Pharmacological characterization of two novel and potent 5-HT₄ receptor agonists, RS67333 and RS67506, *in vitro* and *in vivo*. *Br J Pharmacol* 115: 1387-1392.
2. Gomez-Lazaro E, et al. (2012) Effects of a putative antidepressant with a rapid onset of action in defeated mice with different coping strategies. *Prog Neuropsychopharmacol Biol Psychiatry* 38(2):317-327.
3. Lucas G, et al. (2010) Selective serotonin reuptake inhibitors potentiate the rapid antidepressant-like effects of serotonin-4 receptor agonists in the rat. *PLoS One* 5(2):e9253.
4. Lucas G, et al. (2007) Serotonin₄ (5-HT₄) receptor agonists are putative antidepressants with a rapid onset of action. *Neuron* 55(5):712-725.
5. Kilbinger H, Wolf D (1992) Effects of 5-HT₄ receptor stimulation on basal and electrically evoked release of acetylcholine from guinea-pig myenteric plexus. *Naunyn Schmiedeberg's Arch Pharmacol* 345(3):270-275.
6. Consolo S, et al. (1994) 5-HT₄ receptor stimulation facilitates acetylcholine release in rat frontal cortex. *Neuroreport* 5(10):1230-1232.
7. Ge J, Barnes NM (1996) 5-HT₄ receptor-mediated modulation of 5-HT release in the rat hippocampus *in vivo*. *Br J Pharmacol* 117(7):1475-1480.
8. Lucas G, et al. (2001) Neurochemical and electrophysiological evidence that 5-HT₄ receptors exert a state-dependent facilitatory control *in vivo* on nigrostriatal, but not mesoaccumbal, dopaminergic function. *Eur J Neurosci* 13(5):889-898.
9. Matsumoto M, et al. (2001) Evidence for involvement of central 5-HT₄ receptors in cholinergic function associated with cognitive processes: behavioral, electrophysiological, and neurochemical studies. *J Pharmacol Exp Ther* 296(3):676-82.
10. Bianchi C, et al. (2002) Dual effects of 5-HT₄ receptor activation on GABA release from guinea pig hippocampal slices. *Neuroreport* 13(17):2177-2180.
11. Orsetti M, et al. (2003) Acquisition, retention, and recall of memory after injection of RS67333, a 5-HT₄ receptor agonist, into the nucleus basalis magnocellularis of the rat. *Learn Mem* 10(5):420-426.
12. Licht CL, et al. (2010) Effects of the 5-HT₄ receptor agonist RS67333 and paroxetine on hippocampal extracellular 5-HT levels. *Neurosci Lett* 476(2):58-61.
13. Steward LJ, et al. (1996) Ability of 5-HT₄ receptor ligands to modulate rat striatal dopamine release *in vitro* and *in vivo*. *Br J Pharmacol* 117(1):55-62.
14. Hotte M, et al. (2012) A biphasic and brain-region selective down-regulation of cyclic adenosine monophosphate concentrations supports object recognition in the rat. *PLoS One* 7(2):e32244.
15. Levallet G, et al. (2009) Increased particulate phosphodiesterase 4 in the prefrontal cortex supports 5-HT₄ receptor-induced improvement of object recognition memory in the rat. *Psychopharmacology (Berl)* 202(1-3):125-139.
16. Lamirault L, Simon H (2001) Enhancement of place and object recognition memory in young adult and old rats by RS 67333, a partial agonist of 5-HT₄ receptors. *Neuro-pharmacology* 41(7):844-853.
17. Lamirault L, et al. (2003) Combined treatment with galanthaminium bromide, a new cholinesterase inhibitor, and RS 67333, a partial agonist of 5-HT₄ receptors, enhances place and object recognition in young adult and old rats. *Prog Neuropsychopharmacol Biol Psychiatry* 27(1):185-195.
18. Fontana DJ, et al. (1997) The effects of novel, selective 5-hydroxytryptamine (5-HT)₄ receptor ligands in rat spatial navigation. *Neuropharmacology* 36(4-5):689-696.
19. Musial A, et al. (2007) Recent developments in cholinesterases inhibitors for Alzheimer's disease treatment. *Current Medicinal Chemistry* 14(25):2654-2679.

20. Anand P, Singh B (2013) A review on cholinesterase inhibitors for Alzheimer's disease. *Archives of pharmacol research* 36(4):375-399.
21. Yuede CM, et al. (2007) Anti-dementia drugs and hippocampal-dependent memory in rodents. *Behavioural Pharmacology* 18(5-6):347-363.
22. Cho S, Hu Y (2007) Activation of 5-HT₄ receptors inhibits secretion of β -amyloid peptides and increases neuronal survival. *Exp Neurol* 203:274-278.
23. Lezoualc'h F (2007) 5-HT₄ receptor and Alzheimer's disease: the amyloid connection. *Exp Neurol* 205:325-329.
24. Russo O, et al. (2009) Design, synthesis, and biological evaluation of new 5-HT₄ receptor agonists: application as amyloid cascade modulators and potential therapeutic utility in Alzheimer's disease. *J Med Chem* 52:2214-2225.
25. Inestrosa NC, et al. (1996) Acetylcholinesterase accelerates assembly of amyloid- β -peptides into alzheimer's fibrils: possible role of the peripheral site of the enzyme. *Neuron* 16:881-891.
26. Freret T, et al. (2012) Synergistic effect of acetylcholinesterase inhibition (donepezil) and 5-HT₄ receptor activation (RS67333) on object recognition in mice. *Behavioural Brain Research* 230:304-308.
27. Cavalli A, et al. (2008) Multi-target-directed ligands to combat neurodegenerative diseases. *J Med Chem* 51:347-72.
28. Morphy R, Rankovic Z. (2006) The physicochemical challenges of designing multiple ligands. *J Med Chem* 49:4961-4970.
29. Sopkova-de Oliveira Santos J, et al. (2010) Virtual screening discovery of new acetylcholinesterase inhibitors. *J Chem Inf Model* 50:422-428.
30. Brodney MA, et al. (2012) Identification of multiple 5-HT₄ partial agonist clinical candidates for the treatment of Alzheimer's disease. *J Med Chem* 55:9240-9254.
31. Bai S, et al. (2014) Synthesis and structure-activity relationship studies of conformationally flexible tetrahydroisoquinolinyl triazole carboxamide and triazole substituted benzamide analogues as σ 2 receptor ligands. *J Med Chem* 4,(57):4239-4251.
32. T.L. Rosenberry, WD. et al (1999) A steric blockade model for inhibition of acetylcholinesterase by peripheral site ligands and substrate. *Chem Biol Interact.* 119-120:85-97.
33. Sugimoto H, et al. (2000) Donepezil hydrochloride (E2020) and other acetylcholinesterase inhibitors. *Cur Med Chem* 7:303-339.
34. Maillot M, et al. (2003). Crosstalk between Rap1 and Rac regulates secretion of sAPP α . *Nat Cell Biol* 5(7):633-639.
35. Cochet M, et al. (2013) 5-HT₄ Receptors Constitutively Promote the Non-Amyloidogenic Pathway of APP Cleavage and Interact with ADAM10. *ACS Chem Neurosci* 4(1):130-140.
36. Giannoni P, et al. (2013) Early administration of RS 67333, a specific 5-HT₄ receptor agonist, prevents amyloidogenesis and behavioral deficits in the 5XFAD mouse model of Alzheimer's disease. *Front Aging Neurosci* 5, 96. doi: 10.3389/fnagi.2013.00096.
37. Bard B, et al. (2008) High throughput UV method for the estimation of thermodynamic solubility and the determination of the solubility in biorelevant media. *Eur J Pharm Sci* 33:230-240.
38. Avdeef A, et al. (2007) PAMPA-Critical factors for better predictions of absorption. *J Pharm Sci* 96(11):2893-2909.
39. Brodney MA, et al. (2012) Identification of multiple 5-HT₄ partial agonist clinical candidates for the treatment of Alzheimer's disease. *J Med Chem* 55:9240-9254.
40. Moser PC et al., (2002) SL65.0155, A novel 5-hydroxytryptamine-4 receptor partial agonist with potent cognition-enhancing properties. *J Pharmacol Exp Ther* 302(2):731-41.
41. Sheline YI, et al. (2014) An Antidepressant Decreases CSF A β Production in Healthy Individuals and in Transgenic AD Mice. *Sci Transl Med* 14:6(236):236re4.
42. Cirrito JR, et al. (2011) Serotonin signaling is associated with lower amyloid- β levels and plaques in transgenic mice and humans. *Proc Natl Acad Sci U S A* 108(36):14968-73.
43. Fowler SW, et al. (2014) Genetic modulation of soluble A β rescues cognitive and synaptic impairment in a mouse model of Alzheimer's disease. *J Neurosci.* 34(23):7871-85.
44. Nitsch RM, et al. (1996) Serotonin 5-HT_{2a} and 5-HT_{2c} receptors stimulate amyloid precursor protein ectodomain secretion. *J Biol Chem* 271(8):4188-94.
45. Rosini M, et al. (2014) Multi-Target Design strategies in the context of Alzheimer's disease: acetylcholinesterase Inhibition and NMDA receptor antagonism as the driving forces. *Neurochem Res* DOI 10.1007/s11064-014-1250-1.
46. Citron M (2010) Alzheimer's disease: strategies for disease modification. *Nature Reviews Drug Discovery* 9: 387-398.

Figure Legends

Fig. 1. RS67,333 and donepezil are chemically close.

Fig. 2. Donecopride (MR31147) is issued from RS67,333 through a hit-to-lead chemistry process.

Fig. 3. RS67,333 and donecopride act as (*h*)5-HT_{4e}R partial agonists.

Fig. 4. Lineweaver Burk plots of inhibition kinetics show that donecopride acts as a non-competitive AChE inhibitor.

Fig. 5. Donecopride promotes sAPP α secretion in an *in vitro* cellular model to a similar degree as RS67,333.

Fig. 6. Donecopride fumarate exhibits a good brain penetration according to a cellular *in vitro* test.

Fig. 7. Donecopride exhibits a significant procognitive effect during the object recognition task. Significant differences between Novel and Familiar exploration times (Post-hoc Student Newman Keuls test: * for $p < 0.05$ and ** for $p < 0.01$).

Fig. 8. Pleiotropic interest of DBS AChEI/5-HT₄R agonists.

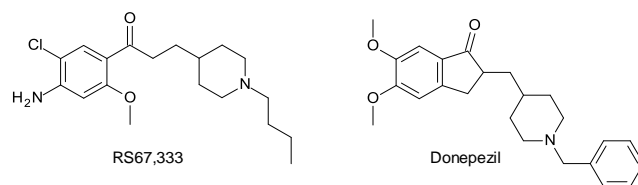
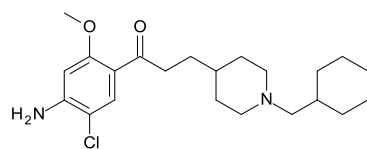


Fig. 1. RS67,333 and Donepezil are structurally close.



Donecopride (MR31147)

Fig. 2. Donecopride (MR31147) is issued from RS67,333 through a hit-to-lead chemistry process.

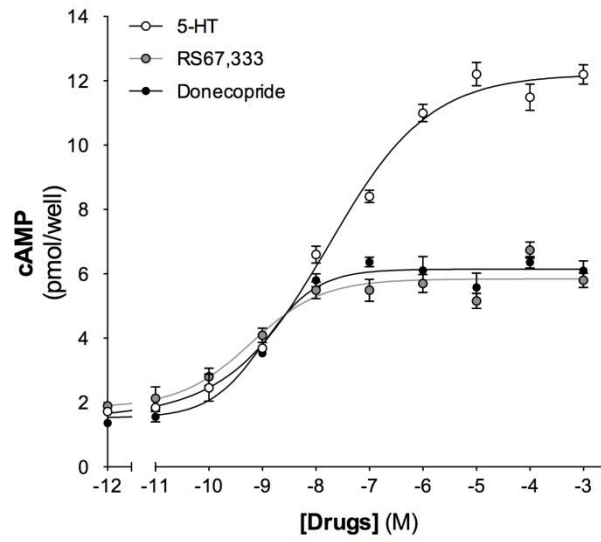


Fig. 3. RS67,333 and Donecopride act as 5-HT_{4e(h)}R partial agonists.

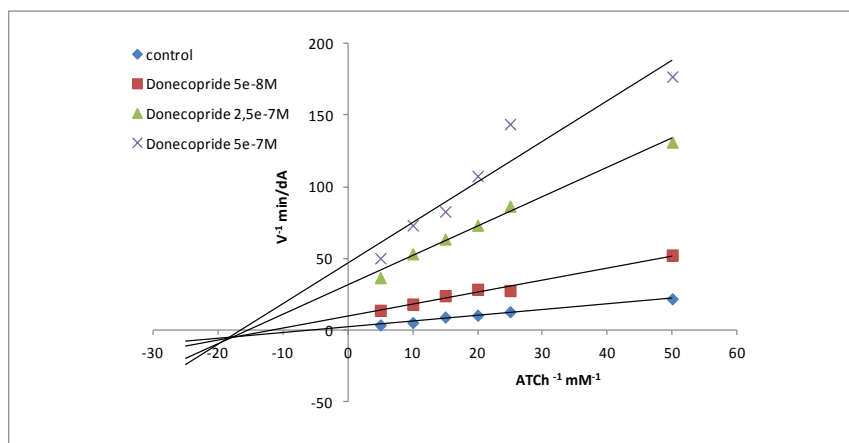


Fig. 4. Lineweaver-Burk plots of inhibition kinetics show that Donecopride acts as a non-competitive AChE inhibitor.

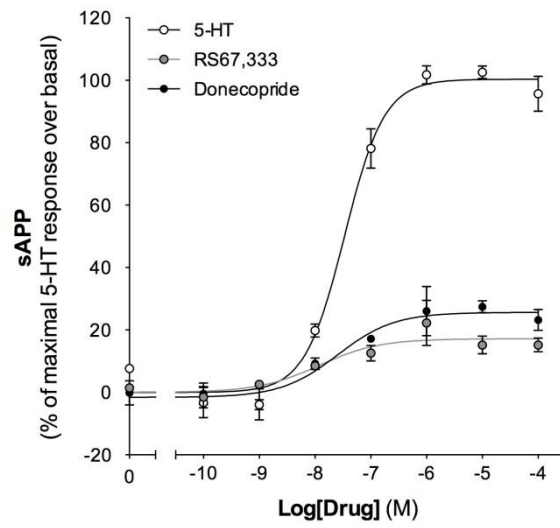


Fig. 5. Donecpride promotes the sAPP α secretion, in an *in vitro* cellular model, in a higher manner than RS67,333.

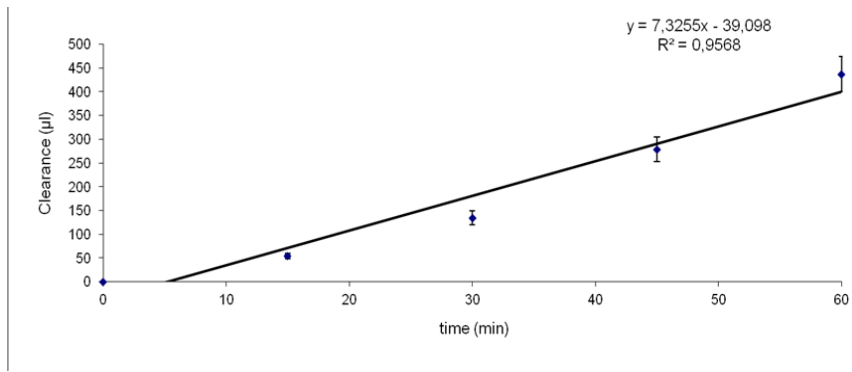


Fig. 6. Donecopride fumarate exhibits a good brain penetration according to a cellular *in vitro* test.

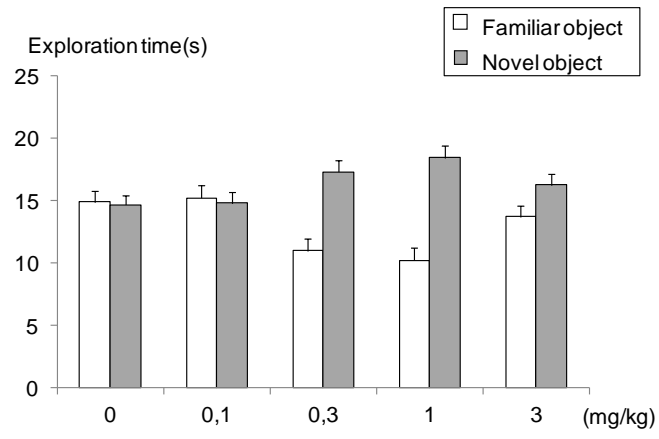


Fig. 7. Donecopride exhibits a significant procognitive effect in the object recognition task.

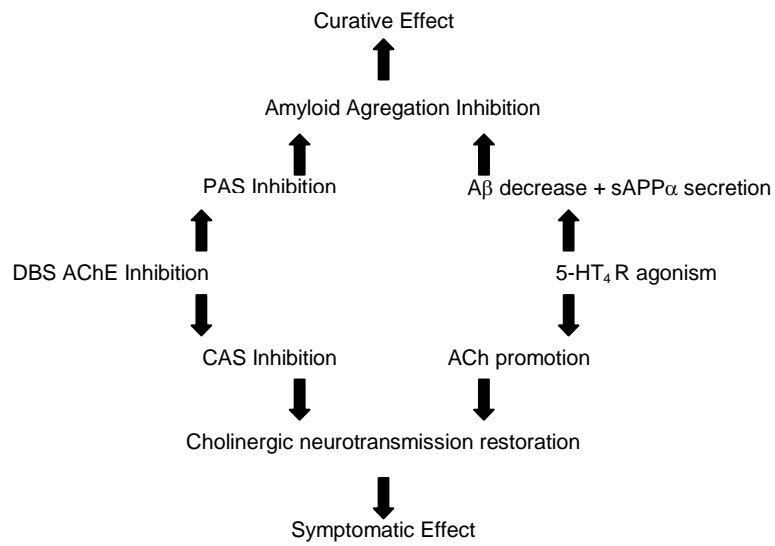


Fig. 8. Pleiotropic interest of DBS AChEI/5-HT₄R agonists.