CNS Drugs (2017) 31:19–32
DOI 10.1007/s40263-016-0399-3

LEADING ARTICLE

Serotonin 5-HT$_6$ Receptor Antagonists in Alzheimer's Disease: Therapeutic Rationale and Current Development Status

Hilda Ferrero[1] · Maite Solas[1] · Paul T. Francis[2] · Maria J. Ramirez[1]

Published online: 3 December 2016

Abstract Alzheimer's disease (AD) is the most common cause of dementia in elderly people. Because of the lack of effective treatments for this illness, research focused on identifying compounds that restore cognition and functional impairments in patients with AD is a very active field. Since its discovery in 1993, the serotonin 5-HT$_6$ receptor has received increasing attention, and a growing number of studies supported 5-HT$_6$ receptor antagonism as a target for improving cognitive dysfunction in AD. This article reviews the rationale behind investigations into the targeting of 5-HT$_6$ receptors as a symptomatic treatment for cognitive and/or behavioral symptoms of AD. In addition to describing the available clinical evidence, this article also describes the purported biochemical and neurochemical mechanisms of action by which 5-HT$_6$ receptor antagonists could influence cognition, and the preclinical data supporting this therapeutic approach to AD. A large number of publications describing the development of ligands for this receptor have come to light and preclinical data indicate the procognitive efficacy of 5-HT$_6$ receptor antagonists. Subsequently, the number of patents protecting 5-HT$_6$ chemical entities has continuously grown. Some of these compounds have successfully undergone phase I clinical studies and have been further evaluated in clinical phase II trials with variable success. Phase II studies have also revealed the potential of combining 5-HT$_6$ receptor antagonism and cholinesterase inhibition. Two of these antagonists, idalopirdine and RVT-101, have been further developed into ongoing phase III clinical trials. Overall, 5-HT$_6$ receptor antagonists can reasonably be regarded as potential drug candidates for the treatment of AD.

Key Points

Research focused on identifying compounds that restore cognition and functional impairment in Alzheimer's disease (AD) is a very active field of research.

Preclinical studies support the notion that serotonin 5-HT$_6$ receptor antagonists could improve memory and help to alleviate behavioral symptoms of the illness.

5-HT$_6$ receptor antagonists have successfully undergone phase I clinical studies (healthy volunteers), and no side effects have been reported.

Several compounds have shown promising results in phase II and III studies (patients).

5-HT$_6$ receptor antagonists can reasonably be regarded as potential drug candidates for the treatment of AD.

1 Introduction

Alzheimer's disease (AD), the most common cause of dementia among elderly people, is characterized by a progressive decline in memory function [1]. Because of the aging of populations worldwide, this disorder is reaching epidemic proportions, with an associated large human,

✉ Maria J. Ramirez
mariaja@unav.es

[1] Department of Pharmacology and Toxicology, University of Navarra, C/Irunlarrea, 31008 Pamplona, Spain

[2] Wolfson Centre for Age-Related Diseases, King's College London, London, UK

social, and economic burden. Senile plaques, neurofibrillary tangles, and cholinergic dysfunction are major hallmarks of the disease [1]. Clinical and preclinical studies point to neuronal and synaptic loss, as well as synaptic impairment and associated neurochemical alterations of several transmitter systems as the main factors underlying both cognitive and neuropsychiatric symptoms [2–7]. The currently used treatment for AD is symptomatic and is based on the use of acetylcholinesterase inhibitors such as donepezil, rivastigmine and galantamine, as well as the *N*-methyl-d-aspartate (NMDA) receptor antagonist memantine. These treatments are only moderately effective in stabilizing the illness for some months and do not target all symptoms associated with dementia. Therefore, there is an unmet need for disease-modifying therapies that halt or substantially slow disease progression. Furthermore, there is a need for improved symptomatics that will be required until disease-modifying drugs are widely available, and for those who develop dementia too late for early intervention

The serotonergic system has resurfaced as an important player in the field and is suggested to have promise in generating novel therapies. The 5-HT_6 receptor is the most recently identified of the serotonin (5-HT) receptor superfamily. Initially, interest in the 5-HT_6 receptors was triggered by evidence showing that certain antipsychotics are able to bind to these receptors; however, nowadays, there is substantial preclinical/clinical evidence supporting the procognitive efficacy of 5-HT_6 receptor antagonists [8–11]. In addition, 5-HT_6 receptor ligands are being subjected to study for future use as potential procognitive, antipsychotic or anti-obese drugs, and all these actions could be considered as interesting in the field of treatment of AD.

This article aims to provide an up-to-date explanation regarding the rationale behind testing 5-HT_6 receptor antagonist therapies for AD. In addition, to describe the newly synthesized compounds active on this receptor, this article will focus on preclinical and ongoing clinical works describing the purported efficacy of 5-HT_6 receptor compounds for the treatment of AD.

2 Pharmacology of 5-HT_6 Receptors

2.1 Structure and Localization of 5-HT_6 Receptors

The 5-HT_6 receptor belongs to the G-protein-coupled receptor (GPCR) family, displaying seven transmembrane domains. Initially cloned from striatal tissue [12], the rat 5-HT_6 receptor gene encodes a protein of 438 amino acids and shares 89% homology with the human form [13, 14]. In terms of structure, the 5-HT_6 receptor is the most different from all serotoninergic receptors (homology of less than 50%) and exhibits a unique pharmacological profile [15] as it is characterized by a short, third cytoplasmatic loop and a long C-terminal tail, and contains one intron located in the middle of the third cytoplasmatic loop. The development of fluorescent compounds such as [11C]GSK215083 has helped to improve the characterization of this GPCR [16]. The 5-HT_6 receptor has no known functional isoforms, although a non-functional truncated splice variant of this receptor has been identified but appears to have no physiological significance. Kohen et al. [14] identified a silent polymorphism at base pair 267 (C267T), and although there is evidence linking this polymorphism to several syndromes that affect cognition, including dementia, AD, and schizophrenia, their significance has not yet been determined.

5-HT_6 receptor expression is mainly restricted within the central nervous system (CNS), with the highest density found in the olfactory tubercle, followed by the frontal and entorhinal cortices, dorsal hippocampus (i.e. dentate gyrus and the CA1, CA2, and CA3 regions), nucleus accumbens, and striatum [17, 18]. Lower levels were observed in the hypothalamus, amygdala, substantia nigra, and several diencephalic nuclei [17, 18]. Therefore, 5-HT_6 receptors appear to be preferentially localized in the brain areas involved in learning and memory processes. Within these areas, the receptor has been demonstrated to be exclusively expressed in neurons [19]. A postsynaptic location of 5-HT_6 receptors is expected because rats subjected to a selective serotonergic lesion have shown that 5-HT_6 receptors are not present in serotonergic raphe neurons, but they could be located within 5-HT projection fields in non-serotonergic neurons [20]. The existence of a 5-HT_6 receptor-mediated positive feedback control of 5-HT neurons in the midbrain dorsal raphe nucleus has also been reported [21]. A purported localization of 5-HT_6 receptors on cholinergic neurons seems less likely as a selective cholinergic lesion, induced by injection of the immunotoxin 192-IgG-saporin, failed to alter the density of 5-HT_6 receptor messenger RNA (mRNA) or protein expression in the deafferentated frontal cortex [22]. On the other hand, and based on microdialysis studies showing that treatment with a 5-HT_6 receptor antagonist or atypical antipsychotics with high affinities for 5-HT_6 receptors, such as clozapine, enhance glutamate levels in the frontal cortex and hippocampus, it has been suggested that these serotonergic receptors could be located on glutamatergic neurons [23]. Electrophysiological recordings from medium spiny neurons of the striatum and layer V pyramidal neurons of the prefrontal cortex have shown that 5-HT_6 receptor activation by the novel agonist ST1936 reduced the frequency of spontaneous excitatory postsynaptic currents [24]. 5-HT_6 receptor activation also reduced the amplitude of spontaneous excitatory postsynaptic currents recorded from medium spiny neurons, suggesting a mechanism of action

involving postsynaptic 5-HT$_6$ receptors [24]. The inhibitory effect of ST1936 on glutamatergic transmission was prevented by the selective 5-HT$_6$ receptor antagonist SB258585 [24].

It has also been shown that 5-HT$_6$ receptors may be expressed on GABAergic spiny neurons of the striatum. The co-localization of glutamic acid decarboxylase and 5-HT$_6$ receptors in rat cerebral cortex and hippocampus has also been demonstrated, and almost 20% of 5-HT$_6$-like immunoreactive neurons have been shown to be GABAergic [25]. Based on all these data regarding localization of 5-HT$_6$ receptors, together with the fact that the effects of 5-HT$_6$ receptor antagonists on memory have been shown to be mediated, at least partially, by increased acetylcholine release [22, 23, 26], it could be suggested that 5-HT$_6$ receptor ligands modulate cholinergic and/or glutamatergic systems via disinhibition of GABAergic neurons (Fig. 1). Therefore, 5-HT$_6$ receptors, by means of their localization on GABAergic and glutamatergic principal neurons, are positioned to regulate the balance between excitatory and inhibitory signaling in the brain [27].

2.2 Biochemical Mechanisms Mediating 5-HT$_6$ Receptor Function

The 5-HT$_6$ receptor is positively coupled to adenylate cyclase activity, meaning that upon agonist activation, cyclic adenosine monophosphate (cAMP) formation is increased. 5-HT$_6$ receptor coupling to Gαs has been widely described, but coupling of 5-HT$_6$ receptors to other Gα protein subunits (Gαi/o or Gαq) or to Ca^{2+} signaling has also been reported [28, 29]. Using a yeast two-hybrid assay, it has been reported that the carboxyl-terminal region of the 5-HT$_6$ receptor interacts with the Fyn tyrosine kinase, a member of the Src family of non-receptor protein tyrosine kinases [30], and this mechanism could be responsible for activation of the extracellular signal-regulated kinase 1/2 (ERK1/2). These findings suggest that Fyn plays an important role in 5-HT$_6$ receptor-mediated signaling pathways in the CNS. A physical interaction between 5-HT$_6$ receptors and the Jun activation domain-binding protein 1 (Jab1), using different experimental approaches, has also been described (Fig. 1), suggesting another signal transduction pathway for these receptors [31].

2.3 5-HT$_6$ Receptor Ligands: Drug Development

Since the initial discovery of the first ligands in the late 1990s, a growing number of scientific publications and patent applications have appeared, mainly focusing on the relationship between this receptor and cognition [32]. As the 5-HT$_6$ receptor has not yet been crystallized, several homology models, such as the 3D-QSAR method, molecular docking and membrane-embedded molecular dynamic simulations, have been helpful in the development of novel and powerful 5-HT$_6$ receptor ligands [33, 34]. An increasing number and diversity of novel, highly selective 5-HT$_6$ receptor ligands of all functional types has been reported, although the principal efforts have been focused on antagonism (Fig. 2).

2.3.1 5-HT$_6$ Receptor Ligands

2.3.1.1 5-HT$_6$ Receptor Antagonists Over the years, numerous groups have developed potent and selective 5-HT$_6$ receptor antagonists based on different structures. The first 5-HT$_6$ selective ligands that were identified in 1998 as antagonist, by high-throughput screening, were Ro 04-06790 and Ro 63-0563 from Roche [35]. Both were bisaryl sulfonamides with a basic amino group. Due to structural analogy between ligands, it has been proposed as a pharmacophore model for 5-HT$_6$ receptor antagonists, which was initially based on indolylsulfonamide derivatives [36]. The pharmacophore includes four common key structural elements: a positive ionizable atom (piperidine, piperazine, or diethylamine group) and a central aromatic group (indole or isostere) linked to a hydrophobic group by a hydrogen bond acceptor group (sulfonyl group). The positive ionizable atom, a protonated nitrogen atom, is usually a piperazine or a (dimethylamino) ethyl fragment. Indole, or indole-like cores, are occupied in the central aromatic group region, including a number of mono- or bisaryl π-electron donor aromatic or heteroaromatic systems. The hydrogen bond acceptor group is commonly a sulfone or sulfonamide group, and the hydrophobic group is occupied by diverse hydrophobic aromatic rings, such as benzene, naphthalene, benzothiophene, or imidazo[2,1-b]thiazole. Following this pharmocophore structure, new antagonists have been developed [37, 38]. The different combinations formed by binding the four pharmacophore elements result in different 5-HT$_6$ receptor antagonists that can be classified into four structural classes: indoles, indole-like derivatives, bisaryl sulphonamides and non-sulfonyl compounds [39].

Indoles derived as shown were the first based on serotonin and are now the most studied and recurring chemical motifs present in many 5-HT$_6$ receptor ligands. As a recent example, eight *N*-arylsulfonylindole compounds have been synthesized, and exhibited moderate to high binding affinities and displayed antagonist profile [40]. Otherwise, numerous indole derivative studies have proposed that the indole central core is only a scaffold to maintain the proper orientation of pharmacophore elements to effectively interact with the receptor. For instance, different scaffolds

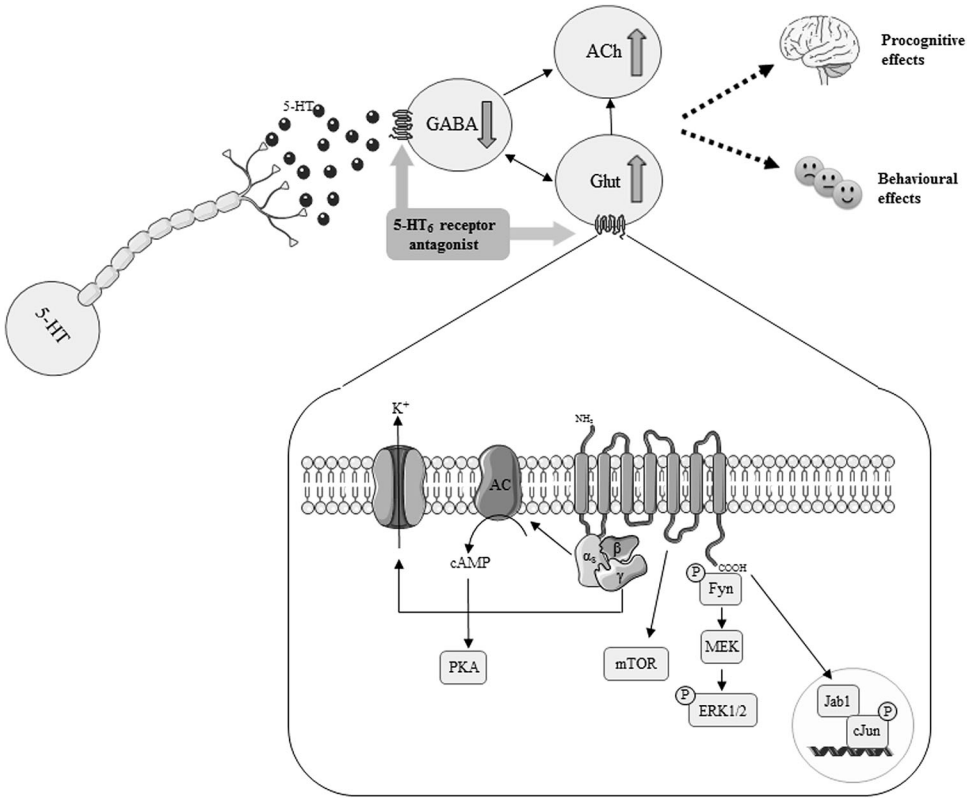


Fig. 1 Neurochemical and biochemical mechanisms mediating 5-HT_6 receptor functions. The proposed neurochemical circuitry activated after 5-HT_6 receptor blockade to influence cognition and behavior involves modulation of cholinergic and/or glutamatergic activity through GABAergic interneurons. The 5-HT_6 receptor is positively coupled to adenylate cyclase activity and activates the ERK1/2 via the Fyn-dependent pathway. An interaction between the 5-HT_6 receptor and Jab1 is also described. *AC* adenylate cyclase, *ACh* acetylcholine, *cAMP* cyclic adenosine monophosphate, *ERK1/2* extracellular signal-regulated kinase 1/2, *GABA* gamma-aminobutyric acid, *Glut* glutamate, *Jab1* Jun activation domain-binding protein 1, *mTOR* mammalian target of rapamycin, *PKA* protein kinase A, *MEK* mitogen-activated protein kinase kinase, *c-Jun* c-Jun

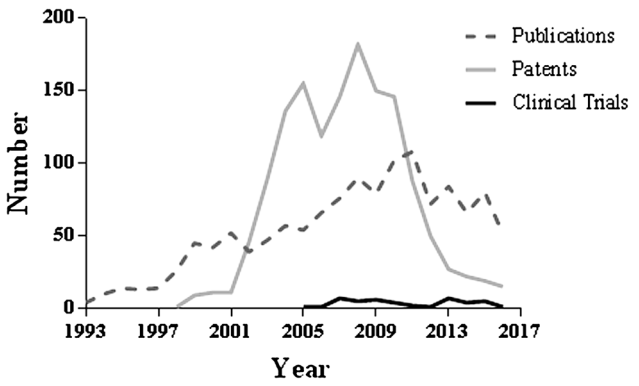
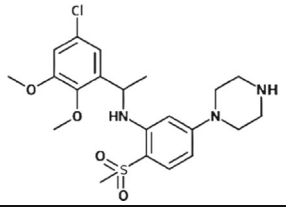
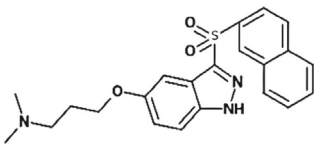
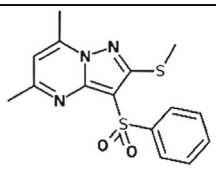
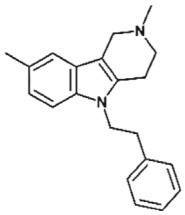
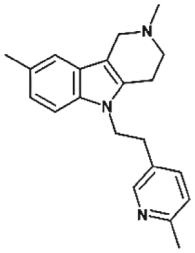
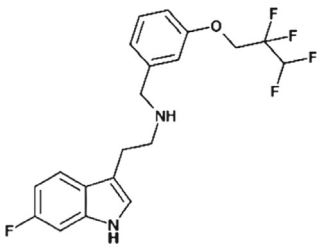
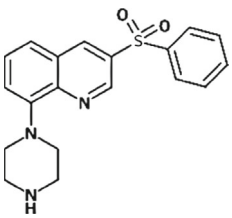


Fig. 2 Number of scientific publications, patent applications and clinical trials focused on 5-HT_6 receptors. Over the past 20 years, there have been almost 600 published studies that directly or indirectly focused on these receptors, studying them from either a pharmacological, physiological, behavioral, or biochemical point of view. 1422 documents protect 5-HT_6 chemical entities. Source: Medline search for '5-HT_6 receptors', Patent Cooperation Treaty database, and ClinicalTrials.gov webpage

that maintain similar topographical orientations of both the arylsulfonyl group and the basic amino group have been proclaimed as the indole-like derivative antagonist class. SB-742457, an indole-like derivate, has been further studied as a potent and effective antagonist [41]. Other antagonist class is bisaryl sulfonamide, an example of which is *N*′-(sulfonyl)pyrazoline-1-carboxamide, which has been developed as a new singular core [42]. Futhermore, some novel sulfonamides have been conceived and synthesized as a 5-HT_6 receptor antagonist, and also act as a partial agonist for the 5-HT_4 receptor [43]. Finally, non-sulfonyl compounds are a class where the hydrogen bond acceptor group of pharmacophores contains a carbonyl or alkoxy instead of having a sulfonamide or sulfone. One of the best known molecules is LU AE58054, also known as idalopirdine, which has been reported to be highly selective over 5-HT_6 receptors as an antagonist [44]. Table 1 shows the structure of some of the molecules that have been further developed into clinical trials (phase II or III).

Table 1 Structure of 5-HT$_6$ receptor ligands that have been further developed into clinical trials

Compound	Structure	Structural family	Company
PRX-07034		Indole like derivate	Epix Pharmaceuticals
SAM-531 (cerlapirdine)		Indole like derivate	Wyeth/Pfizer
AVN-211		Indole like derivate	Avineuro Pharmaceuticals
AVN-101		Non-sulfonyl compound	Avineuro Pharmaceuticals
Dimebon (latrepirdine)		Non-sulfonyl compound	Pfizer
Lu-AE58054 (idalopirdine)		Non-sulfonyl compound	H. Lundbeck A/S
RVT-101 (SB-742457)		Indole like derivate	Axovant Sciences GlaxoSmithKline

The development of a positron emission tomography (PET) radioligand tracer for imaging 5-HT$_6$ receptors in the brain would enable in vivo imaging of this target, along with assessment of its involvement in disease pathophysiology. Based on the aforementioned, the development of *N*-[3,5-dichloro-2-(methoxy)phenyl]-4-(methoxy)-3-(1-piperazinyl)benzenesulfonamide (SB399885), a selective and high affinity (pK(i) = 9.11) 5-HT$_6$ receptor antagonist, radiolabeled with carbon-11 by *O*-methylation of the corresponding desmethyl analog with [(11)C]MeOT, has been described. PET studies with [(11)C]SB399885 in baboons showed fast uptake followed by rapid clearance in the brain [45]. Poor brain entry and inconsistent brain uptake of [(11)C]SB399885, compared with known 5-HT$_6$ receptor distribution, limits its usefulness [46]. The evaluation of another compound, GSK215083 (GlaxoSmithKline) radiolabeled with (11)C via methylation, showed that this compound readily entered the brain, leading to a heterogeneous distribution of the receptor (striatum > cortex > cerebellum) that is consistent with reported 5-HT$_6$ receptor densities and distribution determined by tissue-section autoradiography in humans [43]. Subsequently, a structurally related tracer, (11)C-LuAE60157, was developed [47]. The first 5-HT$_6$ (18)F-labeled radiotracer, [18]F-2FNQ1P, has been tested in preclinical trials [48], and the tritrated version of LuAE60157 has also been developed for binding studies in rodents [49].

2.3.1.2 Agonists versus Antagonists Efforts have been made to design novel agonists acting on 5-HT$_6$ receptors [50], most of which are indole-derived. Over the years, new 5-HT$_6$ receptor agonists have been synthetized by rigidifying the dimethyltryptamine side chain [51] or the piperidine ring of the indole core [52]. Another example of the new synthesized class of drugs are *N*-heteroarylsulfonylindole derivatives [53], azaindole derivatives (WAY-208466) and indene derivatives (E-15136). Moreover, *N*-benzenesulfonylindole derivatives have been developed as partial agonists of 5-HT$_6$ receptors [54]. Another compound, 5-chloro-2-methyl-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole hydrochloride (EMD386088) is also considered as a partial agonist [55].

Interestingly, it has been suggested that both 5-HT$_6$ receptor agonists and antagonists may have procognitive activities [56], implying that both activation and inhibition of this receptor could lead to similar biochemical responses. The selective 5-HT$_6$ receptor agonist LY-586713 caused a bell-shaped dose–response curve on hippocampal BDNF mRNA expression (a potential procognitive pathway). It also increased the Arc mRNA levels, an effect that was blocked by the 5-HT$_6$ receptor antagonist SB-271046. However, in some brain regions the antagonist was not able to block the agonist effect and, in fact, it induced an increase in Arc expression [57] consistent with a potential differential mechanism. The mechanism for paradoxically similar effects of agonist/antagonists on cognition could be related to either the presence of alternative biochemical pathways activated by 5-HT$_6$ receptors or the hypothesis that compounds that are being used as 5-HT$_6$ receptor ligands could display 5-HT$_6$ receptor-independent effects. The latter possibility was supported when investigating the effects of EMD386088, a 5-HT$_6$ receptor agonist, on cell viability. It was found that the cytotoxic effects of EMD386088, regardless of the presence of 5-HT$_6$ receptors, were mediated by the downregulation of ERK1/2 activities [58].

2.3.2 Patents

As shown in Fig. 2, the first 5-HT$_6$ receptor antagonist was patented in 1998 [59, 60], with more than 1400 5-HT$_6$ receptor ligands being patented since then [59, 60]. A detailed review (1998–September 2016) in the Patent Cooperation Treaty database identified that 1422 documents protect 5-HT$_6$ chemical entities or processes for manufacturing and therapeutic indications. Patent filing activity increased dramatically in 2002, with 49 patent documents. The number of published patent documents per

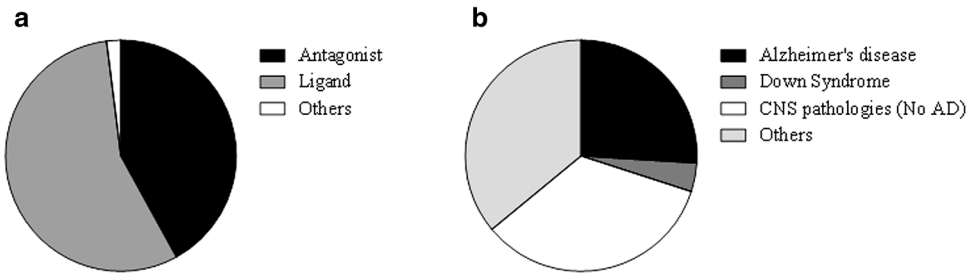


Fig. 3 **a** Patent applications and **b** clinical trials focused on 5-HT$_6$ receptors. In **a** almost half of the patented compounds are antagonists, and a small percentage designed as 'others', mainly 5-HT$_{2A}$ and 5-HT$_6$ combined receptor antagonists. According to the therapeutic indication described (**b**), the main focus of attention is CNS disorders (mainly AD). 'Others' refers to non-specific labeling of various disorders related to 5-HT$_6$ receptors. Source: Patent Cooperation Treaty database and ClinicalTrials.gov webpage. *AD* Alzheimer's disease, *CNS* central nervous system

annum has risen continually year after year, reaching 186 registered patents in 2008; however, since 2010, the number of published patents has been gradually decreasing. Furthermore, this analysis revealed that the field attracts global interest as these patent documents were filed in several countries, on all five continents with patent offices.

Analysing the activity of the compounds, almost half (42%) were described as antagonists and a small percentage (2%) designed as 5-HT_{2A} and 5-HT_6 combined receptor antagonists (Fig. 2). On the other hand, according to the therapeutic indication described, the main focus of attention is CNS, mainly AD (26% of 5-HT_6 receptor-related patents). However, a high number of all patents are dedicated to the non-specific use of treatment of various disorders related to 5-HT_6 receptors (Fig. 3) [59, 60].

3 Experimental Approaches to the Role of 5-HT_6 Receptors in Neurophysiology

Although the mechanisms associated with 5-HT_6 receptor activation/blockade are not completely understood, a progressively increasing focus on the 5-HT_6 receptor as a potential target for cognitive disorders has been clearly noted in the past 10 years. The localization of 5-HT_6 receptors in brain areas involved in learning and memory processes appears to be the initial hypothesis that identified this receptor as a putative target for the treatment of cognitive dysfunction in AD. In addition, ligands acting on 5-HT_6 receptors have demonstrated possible utility as antidepressant and anxiolytic agents in affective and psychotic disorders, as well as for the treatment of obesity and related metabolic syndrome [61, 62], all of which could be of relevance for the treatment of AD.

3.1 Effects on Cognition

The first indirect evidence of 5-HT_6 receptor involvement in memory was obtained by using antisense oligonucleotides [63]. Following the discovery of 5-HT_6 receptor ligands with good brain penetration, there is general agreement and preclinical evidence that supports the use of 5-HT_6 receptor antagonism for treating cognitive dysfunction [64, 65]. Further support came from studies based on how learning paradigms decrease 5-HT_6 receptor expression [66, 67], while 5-HT_6 receptor overexpression of 5-HT_6 receptors in the striatum, achieved by targeted gene delivery, led to cognition impairments, in a reward-based instrumental learning task, a striatum-dependent learning model [68]. Cognitive training, administration of the 5-HT6 receptor antagonist SB-399885 and amnesic drugs modulated 5-HT_6 receptor mRNA expression in the prefrontal cortex, hippocampus and striatum [67]. The procognitive effect of 5-HT_6 antagonists has been extensively described in numerous animal models and cognitive paradigms [69, 70], including water maze [71], inhibitory avoidance [26, 72], autoshaping [73–76], and novel object recognition [25, 76–78]. At this point, it is worth mentioning that there is no single experimental test that recapitulates cognitive impairment in AD and, therefore, that can be used to evaluate the purported therapeutical efficacy of a new drug. Moreover, despite large investments in drug development, the overall success rate of drugs during clinical development remains low. One prominent explanation is flawed preclinical research, in which the use and outcome of animal models is pivotal to bridge the translational gap to the clinic. Over the last decade, different behavioral paradigms have been developed for the evaluation of cognitive functions in animal models, such as the Morris water maze, Barnes maze, passive avoidance, radial arm tests, fear conditioning or novel object recognition tests. All these paradigms have caveats, mainly in the light of replicability and reproducibility. Therefore, the selection of a validated and predictive animal model is essential to address the clinical question [61, 64, 65, 68, 74, 79]. However, considering the variety of tests in which 5-HT_6 receptor antagonists have shown procognitive effects, the translation from preclinical to clinical impact is more likely. Furthermore, several authors propose that 5-HT_6 receptors also seem to be useful as neural markers involved in the processing of information regarding memory and/or amnesia [79].

Unsurprisingly, it appears that 5-HT_6 receptor blockade is more consistently effective in alleviating memory deficits rather than increasing memory in normally functioning animals [80, 81]. 5-HT_6 receptor antagonists have been shown to reverse cognitive deficits induced either by pharmacological interventions that attenuate cholinergic [82] or glutamatergic mechanisms [44], or those associated with the aging process [71, 81]. Interestingly, the coadministration of a 5-HT_6 receptor antagonist with an acetylcholinesterase inhibitor to age-impaired rats resulted in additive or synergistic effects. The combination of 5-HT_6 receptor antagonists with donepezil demonstrated significantly improved performance in water maze, passive avoidance cognition, and/or novel object recognition models, compared with animals treated with either substance alone [80, 83]. These findings led to the recent first report on the design, synthesis and biological evaluation of a novel class of multifunctional ligands that combine a 5-HT_6 receptor antagonist with a cholinesterase inhibitor. These novel multi-target-directed ligands (MTDLs) were designed by combining pharmacophores directed against the 5-HT_6 receptor [1-(phenylsulfonyl)-4-(piperazin-1-yl)-1H-indole] and cholinesterases (tacrine or *N*-benzylpiperidine analogs) [84]. Moreover, combination with

galantamine was able to reverse scopolamine-induced impairment [80], suggesting additional opportunities for the clinical use of 5-HT_6 receptor antagonists as an add-on therapy to established agents in AD.

Several reports indicate that beneficial effects on cognition arise from the blockade of 5-HT_6 receptors via mechanisms that may not involve primary Gs coupling (Fig. 1). Administration of 5-HT_6 receptor antagonists to rodents improves cognitive performance in numerous behavioral tests through stimulation of glutamate, acetylcholine, or catecholamine release in cortical and limbic areas. Indeed, 5-HT_6 receptor antagonists reversed cognitive impairments induced by scopolamine (a cholinergic muscarinic antagonist), dizocilpine or phencyclidine (both glutamatergic antagonists), apomorphine (a dopaminergic agonist), or tryptophan depletion (a precursor of serotonin) [68, 85, 86]. Moreover, inhibition of the mTOR pathway or stimulation of neurite outgrowth may also be involved in the positive action of 5-HT_6 antagonists on cognitive processes (Fig. 1).

Intriguingly, Fone [87] and Kendall et al. [88] reported that selective 5-HT_6 receptor agonists appear to restore memory impairments in the novel object discrimination paradigm. Furthermore, the 5-HT_6 receptor agonist E-6801, at a non-active dose [88], was able to synergistically improve the activity of non-active doses of donepezil, memantine or the 5-HT_6 receptor antagonist SB-271046. Thus, both 5-HT_6 receptor agonist and antagonist compounds show procognitive activity in preclinical studies, although the explanation for their paradoxical analogous effect is currently unclear.

3.2 Non-Cognitive Effects of 5-HT_6 Ligands of Relevance for Alzheimer's Disease (AD)

Initially, interest in 5-HT_6 receptors was triggered by evidence showing that certain antipsychotics are able to bind to these receptors. The 5-HT_6 receptor has been implicated in affective disorders, anxiety and depression, epilepsy and obesity [89]. The dense expression of 5-HT_6 receptors within dopaminergic terminal regions of CNS (striatum and nucleus accumbens), as well as expression in the hippocampal and cortical regions, is compatible with the idea that this 5-HT receptor subtype may be involved in manifestation of psychosis symptoms and cognitive impairments observed in patients with schizophrenia [25, 78, 90, 91].

Preclinical data suggest that 5-HT_6 receptor agonists and/or antagonists could establish a new class of therapeutic agents in the treatment of anxiodepressive disorders. This is particularly interesting, taking into account that these mood disorders are found in AD. 5-HT_6 receptor antagonists have shown to induce antidepressant-like activity in rodents [92, 93]. Two selective 5-HT_6 antagonists (SB-399885 and SB-271046), as well as donepezil, were evaluated in the rat forced swimming test, widely used to identify drugs with antidepressant activity. Systemic administration of the 5-HT_6 receptor antagonist produced a significant reduction in immobility time in the rat forced swimming test, with a similar profile in terms of 5-HT_6 receptor occupancy, measured by binding assay. These data suggest that 5-HT_6 antagonists, at doses corresponding to those that occupy central 5-HT_6 receptors, could have an antidepressive effect in humans. This may differentiate 5-HT_6 antagonists from acetylcholinesterase inhibitors with respect to mood control in the symptomatic treatment of AD [94]. However, similar to the effects on cognition, both 5-HT_6 receptor agonists and antagonists may evoke identical responses in animal models of depression and anxiety. Antidepressant-like effects were also observed when testing agonists in the tail suspension or forced swim tests in mice or rats [95–97]. Moreover, the antidepressant- or anxiolytic-like effects of 5-HT_6 receptor agonists were reversed by selective 5-HT_6 receptor antagonists. Interestingly, those 5-HT_6 receptor agonists also induced anxiolytic-like effects in different rat models [98]. Further research is warranted to try and untangle this apparent paradox.

4 5-HT_6 Receptors and AD

Significant reductions in 5-HT_6 receptor density in the cortical areas of AD patients have been found, although the reductions in 5-HT_6 receptor density were unrelated to cognitive status prior to death [99]. Since 5-HT_6 receptor blockade induces acetylcholine release, reductions in 5-HT_6 receptors may represent an effort to restore acetylcholine levels in a deteriorated cholinergic system. However, as both GABAergic and glutamatergic systems are also affected by 5-HT_6 receptor blockade (or decreased expression), a purported role for these receptors in the pathogenesis of AD cannot be ruled out.

On the other hand, it has also been reported that dysregulation of 5-HT_6 receptor activation by 5-HT in the temporal cortex may be related to behavioral symptoms in AD [80]. Overall, these data support preclinical data on the non-cognitive effects of 5-HT_6 receptor ligands (see Sect. 3.2).

In addition, a limited set of preclinical data also points at the potential disease-modifying effects of 5-HT_6 antagonists. It was reported that treatment with SB-271046 or SB-SB-399885, two different 5-HT_6 receptor antagonists, enhances expression of the polysialylated neural cell adhesion molecule (PSA-NCAM), an effect that could be related to synaptic remodeling [100]. Another line of

evidence associates 5-HT_6 receptors with neuronal activity; aberrant neuronal activity is one of the mechanisms implicated in the pathophysiology of AD. There is also some evidence that 5-HT_6 receptor antagonists may reduce neuronal hyperexcitability as several 5-HT_6 receptor antagonists were found to have in conventionally-induced preclinical seizures [101]. However, at this point, it is worth mentioning a purported relationship between Fyn and Tau. Tau is a microtubule-associated protein and, in a hyperphosphorylated state, a main component of neurofibrillary tangles, one of the pathologic hallmarks of AD. Most of the Tau phosphorylation sites that have been routinely characterized are serine and threonine residues, but recent reports state that Tau can be phosphorylated at tyrosine residues by kinases, including Fyn. In addition, proline-rich, extensin-like receptor kinase-1 (pERK1) is one of the kinases involved in tau phosphorylation [30, 102]. Therefore, it is possible to suggest that modulation of 5-HT_6 receptors might lead to increased tau phosphorylation. In other words, it is even possible to speculate that 5-HT_6 receptor modulation might, in the short-term, improve cognitive function but, over a longer-term, enhance the neurodegenerative processes in AD; however there is no evidence for such a postulate from studies conducted to date.

5 Clinical Data for 5-HT_6 Receptor Antagonists

Based on the available preclinical data, several drug companies (Avineuro, Pfizer, Lundbeck, Otsuka Pharmaceuticals, EPIX Pharmaceutical, Roivant, GlaxoSimthKline, Suven Life Sciences, Biotie Therapies) have succeeded in developing candidates for clinical testing (reviewed by Wicke et al. [11]). 5-HT_6 receptor antagonists that have been advanced into clinical phases of development are reported to have good CNS penetration and receptor selectivity profiles towards other serotonergic receptor subtypes. As described in Table 2, a number of 5-HT_6 receptor antagonists have successfully undergone phase I clinical studies (healthy volunteers) and some have been evaluated in clinical phase II and III studies (patients) for the treatment of Alzheimer's disease. Overall, favorable phase I studies indicate no target-related side effects, and have supported the development of phase II and III studies. Based on the analysis of information in the ClinicalTrials webpage (http://www.clinicaltrials.gov) and the National Library of Medicine's PubMed database (date of last search, 22 September 2016), several compounds have reached clinical trials.

There are several compounds that have shown promising results in phase I studies. For SYN120, a wide therapeutic margin was established, enabling doses that are well tolerated [103] and, beyond blocking the 5-HT_6 receptor, will also robustly antagonize 5-HT_{2A} receptors in the CNS. Phase I studies reported on SUVN-502 also suggest that the compound was well tolerated by the subjects at all dose levels [104]. A phase II study of this compound in mild-to-moderate AD patients is ongoing, and results are expected at the end of 2017. For PRX-0734 [41], at least two phase I trials identified no dose-limiting toxicity. There are no reported data relating to two phase II studies on the use of this compound for cognitive deficits.

Several phase I trials for SAM-531 (cerlapirdine, Pfizer) [103], assessing its pharmacokinetics, pharmacodynamics, safety and tolerability in healthy subjects, have been carried out successfully with no adverse effects and suitable plasma concentrations; however, this agent revealed no efficacy at any dose level in two phase II trials [105]. A follow-up to cerlapirdine, SAM-760 (PF 5212377) has been also described to be safe and well tolerated in healthy young and elderly subjects. A new phase II study with this compound has been performed in AD patients with neuropsychiatric symptoms, but the results have not yet been released (Table 2).

Lu AE58054 (idalopirdine) is being developed as a symptomatic adjunct to cholinesterase inhibitor treatment in AD. It was licensed for clinical development in cognitive impairment in disorders such as schizophrenia. In phase I studies, high 5-HT_6 receptor occupancy was observed following oral doses of idalopirdine. Clinical studies demonstrated this compound to be safe and well tolerated, and the clinical effects of idalopirdine have been investigated in both schizophrenia patients and AD patients. Phase II studies on AD revealed a significant impact of this agent, on top of donepezil, on cognition, as measured by the Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog) [60]. The sponsor company (Lundbeck) launched a global phase III program in AD that consists of four trials planned to enrol a total of approximately 3000 patients (Table 2). All four phase III trials in this program are enrolling patients with mild to moderate AD who are already taking a stable dose of 10 mg/day of an acetylcholinesterase inhibitor; two studies require that patients be taking donepezil, one allows any of the three available drugs of that mechanism. Secondary outcomes will assess various aspects of global clinical function and behavior. In the STARSHINE study, idalopirdine showed a weak efficacy profile as neither of the two doses used in the study met the primary endpoint of a reduction in the ADAS-cog total score when added to donepezil. In addition, the secondary endpoints also did not show separation from placebo. The overall safety profile for idalopirdine showed that idalopirdine was safe and well tolerated. Further analysis of the data is ongoing. The two remaining studies in the phase III program that are

Table 2 Summary of clinical trials of 5-HT_6 receptor antagonists in phase I, II and III

Compound	Phase	Other intervention	Period	Trial identifier	Key notes
ABT-354	I	Atypical antipsychotic	2011–2012	NCT01545310	Open-label
	I	Placebo, AChEI	2013–2013	NCT01908010	Randomized, double-blind
LU AE58054	I	Placebo	2013–2013	NCT01975779	Randomized, double-blind
Idalopirdine	I		2013–2014	NCT02019394	Open-label
	I	Itraconazole	2014–2014	NCT02122692	Open-label
	I		2014–2015	NCT02231450	Non-randomized, open-label
	I		2015–2015	NCT02340195	Non-randomized, open-label
	I		2015–2015	NCT02371707	Randomized, open-label
	I	Placebo, moxifloxacin	2015–2015	NCT02436486	Randomized, double-blind
	I		2015–2016	NCT02415907	Open-label
	I		2016–2016[a]	NCT02894515	Randomized, open-label
	II	Placebo, risperidone	2008–2012	NCT00810667	Randomized, double-blind
	II	Placebo, donepezil	2009–2012	NCT01019421	Randomized, double-blind
	III	Placebo, donepezil	2013–2016[a]	NCT01955161	Randomized, double-blind
	III	Placebo, donepezil	2013–2017[a]	NCT02006641	Randomized, double-blind
	III	Placebo, AChEI	2013–2017[a]	NCT02006654	Randomized, double-blind
	III	Memantine, donepezil	2014–2017[a]	NCT02079246	Non-randomized, open-label
RVT-101	I		2007–2008	NCT00551772	Randomized, open-label
SB-742457	II	Placebo	2005–2009	NCT00224497	Randomized, double-blind
	II	Placebo, donepezil	2006–2009	NCT00348192	Randomized, double-blind
	II	Placebo, donepezil	2008–2012	NCT00708552	Randomized, double-blind
	II	Placebo, donepezil	2008–2012	NCT00710684	Randomized, double-blind
	II	Placebo, AChEI	2014–2017[a]	NCT02258152	Randomized, double-blind
	II	Placebo, AChEI	2016–2017[a]	NCT02910102	Randomized, double-blind
	II	Placebo, AChEI, memantine	2016–2017[a]	NCT02669433	Randomized, double-blind
	III	Placebo, donepezil	2015–2017[a]	NCT02585934	Randomized, double-blind
	III	Memantine, AChEI	2015–2018[a]	NCT02586909	Open-label
SAM-531	I	Placebo	2007–2007	NCT00479700	Randomized, double-blind
Cerlapirdine	I	Placebo	2007–2007	NCT00480818	Randomized, double-blind
	I	Placebo, donepezil	2007–2008	NCT00519298	Randomized, double-blind
	I	Placebo	2007–2009	NCT00479297	Randomized, double-blind
	I		2007–2009	NCT00479349	Randomized, double-blind
	I	Placebo	2008–2009	NCT00726115	Randomized, double-blind
	I		2008–2010	NCT00745576	Non-randomized, open-label
	I		2009–2009	NCT00906191	Non-randomized, open-label
	I	Gemfibrozil	2009–2010	NCT00966966	Non-randomized, open-label
	I		2010–2011	NCT01253655	Non-randomized, open-label
	II	Placebo	2007–2009	NCT00481520	Randomized, double-blind
	II	Placebo, donepezil	2009–2012	NCT00895895	Randomized, double-blind
SAM-760	I	Placebo, ketoconazole	2009–2013	NCT00948662	Randomized, double-blind
	I	Placebo	2010–2011	NCT01159496	Randomized, double-blind
	I	Placebo, scopolamine, donepezil	2010–2012	NCT01213355	Randomized, double-blind
	I		2011–2011	NCT01258751	Non-randomized, open-label
	I		2013–2014	NCT02005991	Non-randomized, open-label
	II	Placebo, donepezil	2012–2016	NCT01712074	Randomized, double-blind
SUVN-502	II	Placebo, donepezil, memantine	2015–2017[a]	NCT02580305	Randomized, double-blind

Only compounds with available data are included

Trial identifier from ClinicalTrials (http://www.clinicaltrials.gov)

Sponsors: ABT-354, Abbott, AbbVie; LU AE58054, H. Lundbeck A/S; RVT-101, Axovant Sciences; SB742457, GlaxoSmithKline; SAM-531, Wyeth/Pfizer; SAM-760, Pfizer; SUVN-502, Suven Life Sciences

AChEI acetylcholinesterase inhibitors

[a] Open studies

currently ongoing (STARBEAM and STARBRIGHT) will continue as planned, and data are expected in the first quarter of 2017.

For RVT-101 (previously known as GSK742457, SB-742457), several phase I studies have shown that the compound was well tolerated. The sponsor company (GlaxoSmithKline or Axovant) has performed several phase II studies with this compound in AD patients, some of which were dose-ranging trials comparing RVT-101 with placebo, a comparative study using the RVT-101 and donepezil arms, or RVT-101 as adjunct therapy to patients already taking donepezil. In these studies, the primary endpoints were cognition and function, but secondary endpoints also covered behavioral symptoms, activities of daily living and caregiver burden. Even though no clear results have been found in these outcomes, in a combination study in addition to donepezil, RVT-101 therapy was reported to be associated with improvements in cognition and function [41]. In 2015, the company started an ongoing phase III trial (with a 12-month, open-label extension) of a once-daily dose of RVT-101 35 mg added to stable donepezil therapy in 1150 patients with mild to moderate AD, with a standard co-primary outcome of ADAS-cog and Alzheimer's Disease Cooperative Study-Activities of Daily Living (ADCS-ADL).

AVN-322, AVN-211 and AVN-101 [106] were also reported to be well tolerated in a wide range of doses, with no adverse events observed. After two completed phase II studies for the treatment of other CNS pathologies (i.e. schizophrenia), the sponsor company (Avineuro) has planned another trial in AD patients with AVN-211, and another for AVN-101, for the treatment of AD and anxiety.

On the other hand, not all compounds have shown such promising results. Dimebon (latrepirdine, also known as dimebolin), was originally developed as an antihistamine drug (Table 1). This compound showed good affinity for 5-HT_6 receptors. Dimebon received widespread publicity as a potential therapy for AD following a very positive phase II study [107], but a phase III study showed no improvements [108].

6 Concluding Remarks

A growing number of preclinical/clinical studies support the use of 5-HT_6 receptor antagonism to treat not only cognitive dysfunction but also behavioral alterations in AD. Currently, 5-HT_6 receptors have obvious pharmaceutical potential in terms of the synthesis of new molecules and related patents. These 5-HT_6 receptor antagonists display an excellent pharmacological profile in terms of potency and selectivity, and good excellent CNS penetration. Currently, several antagonists have successfully undergone phase I trials, and some of these antagonists are now being further developed into phase II and III trials.

It is interesting to note that the neuropharmacological/biochemical profile of 5-HT_6 receptor antagonists is different from that of currently used AD medications (acetylcholinesterase inhibitors or memantine), and the potential of 5-HT_6 receptor antagonists for the treatment of AD when administered as add-on to cholinesterase inhibitors has been demonstrated in preclinical studies and clinical trials.

However, 5-HT_6 receptor functionality is being revealed to be much more complex than initially defined, and the full characterization of the functional profile of the 5-HT_6 receptor is still pending. Based on the existing data, and depending on the drug used, different cellular pathways may be activated. Not only that, but agonists acting on this receptor have shown similar effects on cognition and behavior than the antagonists. It is expected that, in the near future, the drug discovery process will benefit from the complexity of functional responses associated with 5-HT_6 receptors, and new molecules generated could be considered candidates for the treatment of AD.

Acknowledgements Hilda Ferrero is the recipient of a fellowship from the Ministerio de Educación y Ciencia (FPU).

Compliance with Ethical Standards

Conflict of interest Maria J. Ramirez and Paul T. Francis have received speaker fees and honoraria for attending advisory boards for pharmaceutical companies (Lundbeck or AbbVie). Hilda Ferrero and Maite Solas declare no conflicts of interest.

Funding The authors have no funding sources to declare.

References

1. Querfurth HW, LaFerla FM. Alzheimer's disease. N Engl J Med. 2010;362:329–44.
2. Perry EK, Johnson M, Kerwin JM, Piggott MA, Court JA, Shaw PJ, et al. Convergent cholinergic activities in aging and Alzheimer's disease. Neurobiol Aging. 1992;13:393–400.
3. Perry E, Walker M, Grace J, Perry R. Acetylcholine in mind: a neurotransmitter correlate of consciousness? Trends Neurosci. 1999;22:273–80.
4. IPA. Behavioral and psychological signs and symptoms in dementia (BPSSD): implications for research and treatment. Int Psychogeriatr. 1996;8:215–552.
5. Levy ML, Cummings JL, Kahn-Rose R. Neuropsychiatric symptoms and cholinergic therapy for Alzheimer's disease. Gerontology. 1999;45:15–22.
6. Martinson IM, Muwaswes M, Gilliss CL, Doyle GC, Zimmerman S. The frequency and troublesomeness of symptoms associated with Alzheimer's disease. J Community Health Nurs. 1995;12:47–57.
7. Parnetti L, Amici S, Lanari A, Gallai V. Pharmacological treatment of non-cognitive disturbances in dementia disorders. Mech Ageing Dev. 2001;122:2063–9.

8. Codony X, Vela JM, Ramírez MJ. 5-HT6 receptor and cognition. Curr Opin Pharmacol. 2011;11:94–100.
9. Meneses A, Pérez-García G, Ponce-Lopez T, Castillo C. 5-HT6 receptor memory and amnesia: behavioral pharmacology: learning and memory processes. Int Rev Neurobiol. 2011;96:27–47.
10. Ramírez MJ. 5-HT6 receptors and Alzheimer's disease. Alzheimer's Res Ther. 2013;5:15.
11. Wicke K, Haupt A, Bespalov A. Investigational drugs targeting 5-HT6 receptors for the treatment of Alzheimer's disease. Expert Opin Investig Drugs. 2015;24:1515–28.
12. Monsma FJJ, Shen Y, Ward RP, Hamblin MW, Sibley DR. Cloning and expression of a novel serotonin receptor with high affinity for tricyclic psychotropic drugs. Mol Pharmacol. 1993;43:320–7.
13. Ruat M, Traiffort E, Arrang JM, Tardivel-Lacombe J, Diaz J, Leurs R, et al. A novel rat serotonin (5-HT6) receptor: molecular cloning, localization and stimulation of cAMP accumulation. Biochem Biophys Res Commun. 1993;193(1):268–76.
14. Kohen R, Metcalf MA, Khan N, Druck T, Huebner K, Lachowicz JE, et al. Cloning, characterization, and chromosomal localization of a human 5-HT6 serotonin receptor. J Neurochem. 1996;66:47–56.
15. Wacker D, Wang C, Katritch V, Han GW, Huang X-P, Vardy E, et al. Structural features for functional selectivity at serotonin receptors. Science. 2013;340:615–9.
16. Comley RA, Salinas C, Mizrahi R, Vitcu I, Ng A, Hallett W, et al. Biodistribution and radiation dosimetry of the serotonin 5-HT6 ligand [11C]GSK215083 determined from human whole-body PET. Mol Imaging Biol. 2012;14:517–21.
17. Gerard C, Martres M, Lefevre K, Verge D, Lanfumey L, Doucet E, et al. Immuno-localization of serotonin 5-HT 6 receptor-like material in the rat central nervous system. Brain Res. 1997;746(1–2):207–19.
18. Hamon M, Doucet E, Lefèvre K, Miquel MC, Lanfumey L, Insausti R, et al. Antibodies and antisense oligonucleotide for probing the distribution and putative functions of central 5-HT6 receptors. Neuropsychopharmacology. 1999;21(2 Suppl):68S–76S.
19. de Jong I, Helboe L. Distribution of serotonine receptor 5-HT6 mRNA in selected neuronal populations in rat brain: a double-labelling in situ hybridization study. Alzheimer's Dement. 2014;10:P925–6.
20. Gérard C, El Mestikawy S, Lebrand C, Adrien J, Ruat M, Traiffort E, et al. Quantitative RT-PCR distribution of serotonin 5-HT6 receptor mRNA in the central nervous system of control or 5,7-dihydroxytryptamine-treated rats. Synapse. 1996;23:164–73.
21. Brouard JT, Schweimer JV, Houlton R, Burnham KE, Quérée P, Sharp T. Pharmacological evidence for 5-HT6 receptor modulation of 5-HT neuron firing in vivo. ACS Chem Neurosci. 2015;6:1241–7.
22. Marcos B, Gil-Bea FJ, Hirst WD, García-Alloza M, Ramírez MJ. Lack of localization of 5-HT6 receptors on cholinergic neurons: implication of multiple neurotransmitter systems in 5-HT6 receptor-mediated acetylcholine release. Eur J Neurosci. 2006;24:1299–306.
23. Dawson L, Nguyen HQ, Li P. The 5-HT6 receptor antagonist SB-271046 selectively enhances excitatory neurotransmission in the rat frontal cortex and hippocampus. Neuropsychopharmacology. 2001;25:662–8.
24. Tassone A, Madeo G, Schirinzi T, Vita D, Puglisi F, Ponterio G, et al. Activation of 5-HT6 receptors inhibits corticostriatal glutamatergic transmission. Neuropharmacology. 2011;61:632–7.
25. Woolley ML, Marsden CA, Fone KC. 5-ht6 receptors. Curr Drug Targets CNS Neurol Disord. 2004;3:59–79.
26. Riemer C, Borroni E, Levet-trafit B, Martin JR, Poli S, Porter RHP, et al. Influence of the 5-HT6 receptor on acetylcholine release in the cortex: pharmacological characterization of 4-(2-Bromo-6-pyrrolidin-1-ylpyridine-4-sulfonyl)phenylamine, a potent and selective 5-HT6 receptor antagonist. J Med Chem. 2003;46:1273–6.
27. Helboe L, Egebjerg J, de Jong IEM. Distribution of serotonin receptor 5-HT6 mRNA in rat neuronal subpopulations: a double in situ hybridization study. Neuroscience. 2015;310:442–54.
28. Dupuis DS, la Cour CM, Chaput C, Verrièle L, Lavielle G, Millan MJ. Actions of novel agonists, antagonists and antipsychotic agents at recombinant rat 5-HT6 receptors: a comparative study of coupling to Gαs. Eur J Pharmacol. 2008;588:170–7.
29. Zhang JY, Nawoschik S, Kowal D, Smith D, Spangler T, Ochalski R, et al. Characterization of the 5-HT6 receptor coupled to Ca^{2+} signaling using an enabling chimeric G-protein. Eur J Pharmacol. 2003;472:33–8.
30. Yun H-M, Kim S, Kim H-J, Kostenis E, Kim JI, Seong JY, et al. The novel cellular mechanism of human 5-HT6 receptor through an interaction with Fyn. J Biol Chem. 2007;282:5496–505.
31. Yun H-M, Baik J-H, Kang I, Jin C, Rhim H. Physical interaction of Jab1 with Human serotonin 6 G-protein-coupled receptor and their possible roles in cell survival. J Biol Chem. 2010;285:10016–29.
32. Ivachtchenko AV, Ivanenkov YA, Tkachenko SE. 5-Hydroxytryptamine subtype 6 receptor modulators: a patent survey. Expert Opin Ther Pat. 2010;20:1171–96.
33. Hirst WD, Abrahamsen B, Blaney FE, Calver AR, Aloj L, Price GW, et al. Differences in the central nervous system distribution and pharmacology of the mouse 5-hydroxytryptamine-6 receptor compared with rat and human receptors investigated by radioligand binding, site-directed mutagenesis, and molecular modeling. Mol Pharmacol. 2003;64:1295–308.
34. Kumari M, Chandra S, Tiwari N, Subbarao N. 3D QSAR, pharmacophore and molecular docking studies of known inhibitors and designing of novel inhibitors for M18 aspartyl aminopeptidase of *Plasmodium falciparum*. BMC Struct Biol. 2016;16:12.
35. Sleight AJ, Boess FG, Bös M, Levet-Trafit B, Riemer C, Bourson A. Characterization of Ro 04-6790 and Ro 63-0563: potent and selective antagonists at human and rat 5-HT6 receptors. Br J Pharmacol. 1998;124:556–62.
36. López-Rodríguez ML, Benhamú B, de la Fuente T, Sanz A, Pardo L, Campillo M. A three-dimensional pharmacophore model for 5-hydroxytryptamine6 (5-HT6) receptor antagonists. J Med Chem. 2005;48:4216–9.
37. Rossé G, Schaffhauser H. 5-HT6 receptor antagonists as potential therapeutics for cognitive impairment. Curr Top Med Chem. 2010;10:207–21.
38. Witty D, Ahmed M, Chuang TT. Advances in the design of 5-HT6 receptor ligands with therapeutic potential. Prog Med Chem. 2009;48:163–224.
39. Benhamú B, Martín-Fontecha M, Vázquez-Villa H, Pardo L, López-Rodríguez ML. Serotonin 5-HT 6 receptor antagonists for the treatment of cognitive deficiency in Alzheimer's disease. J Med Chem. 2014;57:7160–81.
40. Vera G, Lagos CF, Almendras S, Hebel D, Flores F, Valle-Corvalán G, et al. Extended *N*-arylsulfonylindoles as 5-HT_6 receptor antagonists: design, synthesis & biological evaluation. Molecules. 2016;21:E1070 **(pii)**.
41. Upton N, Chuang TT, Hunter AJ, Virley DJ. 5-HT6 receptor antagonists as novel cognitive enhancing agents for Alzheimer's disease. Neurotherapeutics. 2008;5:458–69.

42. Van Loevezijn A, Venhorst J, Iwema Bakker WI, De Korte CG, De Looff W, Verhoog S, et al. *N′*-(Arylsulfonyl)pyrazoline-1-carboxamidines as novel, neutral 5-hydroxytryptamine 6 receptor (5-HT6R) antagonists with unique structural features. J Med Chem. 2011;54:7030–54.
43. Yahiaoui S, Hamidouche K, Ballandonne C, Davis A, de Oliveira Santos JS, Freret T, et al. Design, synthesis, and pharmacological evaluation of multitarget-directed ligands with both serotonergic subtype 4 receptor (5-HT4R) partial agonist and 5-HT6R antagonist activities, as potential treatment of Alzheimer's disease. Eur J Med Chem. 2016;121:283–93.
44. Arnt J, Bang-Andersen B, Grayson B, Bymaster FP, Cohen MP, DeLapp NW, et al. Lu AE58054, a 5-HT6 antagonist, reverses cognitive impairment induced by subchronic phencyclidine in a novel object recognition test in rats. Int J Neuropsychopharmacol. 2010;13:1021–33.
45. Liu F, Majo VJ, Prabhakaran J, Milak MS, John Mann J, Parsey RV, et al. Synthesis and in vivo evaluation of [*O*-methyl-11C] N-[3,5-dichloro-2-(methoxy)phenyl]-4-(methoxy)-3-(1-piperazinyl)benzenesulfonamide as an imaging probe for 5-HT6 receptors. Bioorg Med Chem. 2011;19:5255–9.
46. Liu KG, Robichaud AJ. 5-HT6 medicinal chemistry. Int Rev Neurobiol. 2010;94:1–34.
47. Schmidt E, Kuwabara H, Areberg J, Zaidi E, Chamroonat W, Mathur A, et al. A clinical positron emission tomography (PET) study investigating occupancy at the 5-HT6 receptor after multiple oral doses of LU AE58054 to healthy men. Alzheimer's Dement. 2014;10:P925.
48. Becker G, Colomb J, Sgambato-Faure V, Tremblay L, Billard T, Zimmer L. Preclinical evaluation of [18F]2FNQ1P as the first fluorinated serotonin 5-HT6 radioligand for PET imaging. Eur J Nucl Med Mol Imaging. 2015;42:495–502.
49. Witten L, Bang-Andersen B, Nielsen SM, Miller S, Christoffersen CT, Stensbøl TB, et al. Characterization of [^{3}H]Lu AE60157 ([^{3}H]8-(4-methylpiperazin-1-yl)-3-phenylsulfonylquinoline) binding to 5-hydroxytryptamine$_6$ (5-HT$_6$) receptors in vivo. Eur J Pharmacol. 2012;676:6–11.
50. Glennon RA, Lee M, Rangisetty JB, Dukat M, Roth BL, Savage JE, et al. 2-Substituted tryptamines: agents with selectivity for 5-HT(6) serotonin receptors. J Med Chem. 2000;43(5):1011–8.
51. Cole DC, Lennox WJ, Lombardi S, Ellingboe JW, Bernotas RC, Tawa GJ, et al. Discovery of 5-arylsulfonamido-3-(pyrrolidin-2-ylmethyl)-1H-indole derivatives as potent, selective 5-HT 6 receptor agonists and antagonists. J Med Chem. 2005;48:353–6.
52. Mattsson C, Sonesson C, Sandahl A, Greiner HE, Gassen M, Plaschke J, et al. 2-Alkyl-3-(1,2,3,6-tetrahydropyridin-4-yl)-1H-indoles as novel 5-HT6 receptor agonists. Bioorg Med Chem Lett. 2005;15:4230–4.
53. Cole DC, Stock JR, Lennox WJ, Bernotas RC, Ellingboe JW, Boikess S, et al. Discovery of N1-(6-Chloroimidazo[2,1-b][1,3]thiazole-5-sulfonyl)tryptamine as a potent, selective, and orally active 5-HT 6 receptor agonist. J Med Chem. 2007;50:5535–8.
54. Russell MGN, Baker RJ, Barden L, Beer MS, Bristow L, Broughton HB, et al. *N*-Arylsulfonylindole derivatives as serotonin 5-HT(6) receptor ligands. J Med Chem. 2001;44(23):3881–95.
55. Jastrzêbska-Wiêsek M, Siwek A, Kazek G, Nawieśniak B, Partyka A, Marcinkowska M, et al. Partial agonist efficacy of EMD386088, a 5-HT6 receptor ligand, in functional in vitro assays. Pharmacol Rep. 2013;65(4):998–1005.
56. Pereira M, Martynhak BJ, Andreatini R, Svenningsson P. 5-HT6 receptor agonism facilitates emotional learning. Front Pharmacol. 2015;6:200.
57. de Foubert G, O'Neill MJ, Zetterström TSC. Acute onset by 5-HT6-receptor activation on rat brain brain-derived neurotrophic factor and activity-regulated cytoskeletal-associated protein mRNA expression. Neuroscience. 2007;147:778–85.
58. Yun H-M, Rhim H. 5-HT6 receptor ligands, EMD386088 and SB258585, differentially regulate 5-HT6 receptor-independent events. Toxicol In Vitro. 2011;25:2035–40.
59. Ivachtchenko AV, Ivanenkov YA. 5HT(6) receptor antagonists: a patent update. Part 1. Sulfonyl derivatives. Expert Opin Ther Pat. 2012;22:917–64.
60. Ivachtchenko AV, Ivanenkov YA, Skorenko AV. 5-HT(6) receptor modulators: a patent update. Part 2. Diversity in heterocyclic scaffolds. Expert Opin Ther Pat. 2012;22:1123–68.
61. Cavallaro S. Genomic analysis of serotonin receptors in learning and memory. Behav Brain Res. 2008;195:2–6.
62. Chen KH, Reese EA, Kim H-W, Rapoport SI, Rao JS. Disturbed neurotransmitter transporter expression in Alzheimer's disease brain. J Alzheimer's Dis. 2011;26:755–66.
63. Bourson A, Borroni E, Austin RH, Monsma FJ Jr, Sleight AJ. Determination of the role of the 5-ht6 receptor in the rat brain: a study using antisense oligonucleotides. J Pharmacol Exp Ther. 1995;274:173–80.
64. Lindner MD, Hodges DB, Hogan JB, Orie AF, Corsa JA, Barten DM, et al. An assessment of the effects of serotonin 6 (5-HT6) receptor antagonists in rodent models of learning. J Pharmacol Exp Ther. 2003;307(2):682–91.
65. Russell M, Dias R. Memories are made of this (perhaps): a review of serotonin 5-HT6 receptor ligands and their biological functions. Curr Top Med Chem. 2002;2:643–54.
66. Marcos B, Cabero M, Solas M, Aisa B, Ramirez MJ. Signalling pathways associated with 5-HT6 receptors: relevance for cognitive effects. Int J Neuropsychopharmacol. 2010;13:775–84.
67. Huerta-Rivas A, Pérez-García G, González-Espinosa C, Meneses A. Time-course of 5-HT6 receptor mRNA expression during memory consolidation and amnesia. Neurobiol Learn Mem. 2010;93:99–110.
68. Mitchell ES, Sexton T, Neumaier JF. Increased expression of 5-HT6 receptors in the rat dorsomedial striatum impairs instrumental learning. Neuropsychopharmacology. 2007;32:1520–30.
69. de Bruin NMWJ, van Loevezijn A, Wicke KM, de Haan M, Venhorst J, Lange JHM, et al. The selective 5-HT6 receptor antagonist SLV has putative cognitive- and social interaction enhancing properties in rodent models of cognitive impairment. Neurobiol Learn Mem. 2016;133:100–17.
70. Peele DB, Vincent A. Strategies for assessing learning and memory, 1978–1987: a comparison of behavioral toxicology, psychopharmacology, and neurobiology. Neurosci Biobehav Rev. 1989;13:317–22.
71. Hirst WD, Stean TO, Rogers DC, Sunter D, Pugh P, Moss SF, et al. SB-399885 is a potent, selective 5-HT6 receptor antagonist with cognitive enhancing properties in aged rat water maze and novel object recognition models. Eur J Pharmacol. 2006;553:109–19.
72. Foley AG, Murphy KJ, Hirst WD, Gallagher HC, Hagan JJ, Upton N, et al. The 5-HT(6) receptor antagonist SB-271046 reverses scopolamine-disrupted consolidation of a passive avoidance task and ameliorates spatial task deficits in aged rats. Neuropsychopharmacology. 2004;29:93–100.
73. Meneses A. Effects of the 5-HT(6) receptor antagonist Ro 04-6790 on learning consolidation. Behav Brain Res. 2001;118:107–10.
74. Meneses A. Role of 5-HT6 receptors in memory formation. Drug News Perspect. 2001;14:396–400.
75. Perez-García GS, Meneses A. Effects of the potential 5-HT7 receptor agonist AS 19 in an autoshaping learning task. Behav Brain Res. 2005;163:136–40.

76. Schreiber R, Vivian J, Hedley L, Szczepanski K, Secchi RL, Zuzow M, et al. Effects of the novel 5-HT(6) receptor antagonist RO4368554 in rat models for cognition and sensorimotor gating. Eur Neuropsychopharmacol. 2007;17:277–88.
77. Fone KCF. Selective 5-HT6 compounds as a novel approach to the treatment of Alzheimer disease. J Pharmacol Sci. 2006;101:53.
78. Mitchell ES, Neumaier JF. 5-HT6 receptors: a novel target for cognitive enhancement. Pharmacol Ther. 2005;108:320–33.
79. Meneses A. Serotonin, neural markers, and memory. Front Pharmacol. 2015;6:1–7.
80. Marcos B, Chuang TT, Gil-Bea FJ, Ramirez MJ. Effects of 5-HT6 receptor antagonism and cholinesterase inhibition in models of cognitive impairment in the rat. Br J Pharmacol. 2008;155:434–40.
81. Da Silva Costa-Aze V, Quiedeville A, Boulouard M, Dauphin F. 5-HT6 receptor blockade differentially affects scopolamine-induced deficits of working memory, recognition memory and aversive learning in mice. Psychopharmacology. 2012;222:99–115.
82. de Bruin NMWJ, Prickaerts J, van Loevezijn A, Venhorst J, de Groote L, Houba P, et al. Two novel 5-HT6 receptor antagonists ameliorate scopolamine-induced memory deficits in the object recognition and object location tasks in Wistar rats. Neurobiol Learn Mem. 2011;96:392–402.
83. King MV, Fone KCF, Shacham S, Fichman M, Melendez R, Orbach P, et al. Combination of sub-threshold doses of aricept and PRX-07034, a novel 5-HT6 receptor antagonist, enhances novel object discrimination (NOD) memory. J Pharmacol Sci. 2006;101:150.
84. Więckowska A, Kołaczkowski M, Bucki A, Godyń J, Marcinkowska M, Więckowski K, et al. Novel multi-target-directed ligands for Alzheimer's disease: combining cholinesterase inhibitors and 5-HT6 receptor antagonists. Design, synthesis and biological evaluation. Eur J Med Chem. 2016;124:63–81.
85. King MV, Marsden CA, Fone KCF. A role for the 5-HT(1A), 5-HT4 and 5-HT6 receptors in learning and memory. Trends Pharmacol Sci. 2008;29:482–92.
86. Wilson C, Terry AV. Enhancing cognition in neurological disorders: potential usefulness of 5-HT6 antagonists. Drugs Future. 2009;34:969–75.
87. Fone KCF. An update on the role of the 5-hydroxytryptamine6 receptor in cognitive function. Neuropharmacology. 2008;55:1015–22.
88. Kendall I, Slotten HA, Codony X, Burgueño J, Pauwels PJ, Vela JM, et al. E-6801, a 5-HT6 receptor agonist, improves recognition memory by combined modulation of cholinergic and glutamatergic neurotransmission in the rat. Psychopharmacology. 2011;213:413–30.
89. Heal DJ, Smith SL, Fisas A, Codony X, Buschmann H. Selective 5-HT6 receptor ligands: progress in the development of a novel pharmacological approach to the treatment of obesity and related metabolic disorders. Pharmacol Ther. 2008;117:207–31.
90. Nikiforuk A. The procognitive effects of 5-HT6 receptor ligands in animal models of schizophrenia. Rev Neurosci. 2014;25:367–82.
91. Roberts JC, Reavill C, East SZ, Harrison PJ, Patel S, Routledge C, et al. The distribution of 5-HT(6) receptors in rat brain: an autoradiographic binding study using the radiolabelled 5-HT(6) receptor antagonist [(125)I]SB-258585. Brain Res. 2002;934:49–57.
92. Zajdel P, Marciniec K, Satała G, Canale V, Kos T, Partyka A, et al. N1-azinylsulfonyl-1H-indoles: 5-HT6 receptor antagonists with procognitive and antidepressant-like properties. ACS Med Chem Lett. 2016;7:618–22.
93. Wesołowska A, Nikiforuk A. Effects of the brain-penetrant and selective 5-HT6 receptor antagonist SB-399885 in animal models of anxiety and depression. Neuropharmacology. 2007;52:1274–83.
94. Hirano K, Piers TM, Searle KL, Miller ND, Rutter AR, Chapman PF. Procognitive 5-HT6 antagonists in the rat forced swimming test: potential therapeutic utility in mood disorders associated with Alzheimer's disease. Life Sci. 2009;84:558–62.
95. Nikiforuk A, Kos T, Wesołowska A. The 5-HT6 receptor agonist EMD 386088 produces antidepressant and anxiolytic effects in rats after intrahippocampal administration. Psychopharmacology. 2011;217:411–8.
96. Carr GV, Schechter LE, Lucki I. Antidepressant and anxiolytic effects of selective 5-HT6 receptor agonists in rats. Psychopharmacology. 2011;213:499–507.
97. Svenningsson P, Tzavara ET, Qi H, Carruthers R, Witkin JM, Nomikos GG, et al. Biochemical and behavioral evidence for antidepressant-like effects of 5-HT6 receptor stimulation. J Neurosci. 2007;27:4201–9.
98. Jastrzębska-Więsek M, Siwek A, Partyka A, Kubacka M, Mogilski S, Wasik A, et al. Pharmacological evaluation of the anxiolytic-like effects of EMD 386088, a partial 5-HT6 receptor agonist, in the rat elevated plus-maze and Vogel conflict tests. Neuropharmacology. 2014;85:253–62.
99. Garcia-Alloza M, Hirst WD, Chen CPL-H, Lasheras B, Francis PT, Ramírez MJ. Differential involvement of 5-HT(1B/1D) and 5-HT6 receptors in cognitive and non-cognitive symptoms in Alzheimer's disease. Neuropsychopharmacology. 2004;29:410–6.
100. Foley AG, Hirst WD, Gallagher HC, Barry C, Hagan JJ, Upton N, et al. The selective 5-HT6 receptor antagonists SB-271046 and SB-399885 potentiate NCAM PSA immunolabeling of dentate granule cells, but not neurogenesis, in the hippocampal formation of mature Wistar rats. Neuropharmacology. 2008;54:1166–74.
101. Routledge C, Bromidge SM, Moss SF, Price GW, Hirst W, Newman H, et al. Characterization of SB-271046: a potent, selective and orally active 5-HT(6) receptor antagonist. Br J Pharmacol. 2000;130:1606–12.
102. Riccioni T, Bordi F, Minetti P, Spadoni G, Yun HM, Im BH, et al. ST1936 stimulates cAMP, Ca^{2+}, ERK1/2 and Fyn kinase through a full activation of cloned human 5-HT6 receptors. Eur J Pharmacol. 2011;661:8–14.
103. Mitchell ES. 5-HT6 receptor ligands as antidementia drugs. In: Borsini F, editor. International review of neurobiology. pharmacology of 5-HT6 receptors, Part II. Pomezia: Academic Press; 2011. p. 163–87.
104. Nirogi R, Kandikere V, Mudigonda K, Bhyrapuneni GJV. Safety, tolerability and pharmacokinetics of SUVN-502: a 5-HT6 receptor antagonist, first in human phase-1 single ascending dose (SAD) study. Alzheimers Dement. 2009;5:1–251.
105. Brisard C, Safirstein B, Booth K, Hua L, Brault Y, Raje S, et al. Safety, tolerability, and preliminary efficacy of SAM-531, a 5HT-6 antagonist, in subjects with mild-to-moderate Alzheimer's disease: results from a phase 2a study. Alzheimers Dement. 2010;6:1–457.
106. Ivachtchenko AV, Lavrovsky Y, Okun I. AVN-101: a multi-target drug candidate for the treatment of CNS disorders. J Alzheimers Dis. 2016;53:583–620.
107. Doody RS, Gavrilova SI, Sano M, Thomas RG, Aisen PS, Bachurin SO, et al. Effect of dimebon on cognition, activities of daily living, behaviour, and global function in patients with mild-to-moderate Alzheimer's disease: a randomised, double-blind, placebo-controlled study. Lancet. 2008;372:207–15.
108. Jones RW. Dimebon disappointment. Alzheimers Res Ther. 2010;2:25.