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Small Molecule Drugs for Treatment of Alzheimer's Diseases Developed on the Basis of Mechanistic Understanding of the Serotonin Receptors 4 and 6

Charlotte Uldahl Jansen and Katrine M. Qvortrup

Abstract

Alzheimer's disease (AD) is the most common form of dementia affecting millions of people worldwide and currently, the only possible treatment is the use of symptomatic drugs. Therefore, there is a need for new and disease-modifying approaches. Among the numbers of biological targets which are today explored in order to prevent or limit the progression of AD, the modulation of serotonin receptors the subtype 4 and 6 receptors (5-HT4R and 5-HT6R) has received increasing attention and has become a promising target for improving cognition and limit the amyloid pathology through modulation of the neurotransmitter system. A large number of publications describing the development of ligands for these serotonin receptors have emerged, and their pharmaceutical potential is now quite evident. However, 5-HT4R and 5-HT6R functionality is much more complex than initially defined. This chapter describes recent advances in the understanding of this modulation as well as the medicinal chemistry efforts towards development of selective 5-HT4R or 5-HT6R ligands.

Keywords: serotonin pathways, Alzheimer's, 5-HT4R and 5-HT6R modulators, structure–activity relationship

1. Introduction

Alzheimer's disease (AD) is a devastating but poorly treated disease. Therefore, there is an urgent need for new and efficient treatment strategies, emphasized by recent statistics from WHO predicting that AD will become the second-most prevalent cause of death within 20 years.

Mounting evidence accumulated over the past years indicates that the neurotransmitter serotonin plays a significant role in cognition and memory. The intimate anatomical and neurochemical association of the serotonergic system and brain areas affected in AD have inspired researchers to focus on this system as a major therapeutic drug target.

Based on the current knowledge of mechanisms involved in serotonin regulation, we here provide structural insight into chemical compounds that have

been developed for targeting of the serotonin receptors the subtype 4 and 6 receptors (5-HT4R and 5-HT6R) processes as potential treatments in AD.

2. The serotonergic system in Alzheimer's disease

Serotonin is a small molecule that functions both as a hormone in the periphery, and as neurotransmitter and neuromodulator in the central nervous system (CNS) [1]. In the CNS, it is produced by a small cluster of neurons located in the raphe nuclei of the midbrain. Through innervation of numerous brain regions, serotonin (5-hydroxytryptamine, 5-HT) modulates various physiological functions including circadian rhythms, mood, sleep, appetite, and learning and memory. The areas of the brain involved in learning and memory show high concentrations of 5-HT1AR, 5-HT4R, 5-HT6R and 5-HT7R, why modulation of these is of particular interest in for reversing the cognitive impairment associated with AD [2].

AD has been linked to a decrease of serotonergic neurons in the raphe nuclei, seemingly due to the accumulation of hyperphosphorylated Tau as well as deposits of amyloid beta in the projection sites of serotonergic neurons, causing retrograde degeneration of the neurons [3]. Furthermore, a significant decrease in the number of serotonin transporter (SERT) have also been reported [4]. Overall, this leads to a decrease in serotonin neurotransmission, suggesting that increasing serotonin level in the raphe nuclei can improve cognitive performance in AD patients. This is supported by studies showing that administration of selective serotonin reuptake inhibitors (SSRI) to mouse models of AD, reduced the production of toxic amyloid beta plaques [5, 6]. However, recent clinical trials concluded that treatment with amyloid beta lowering agents should be administered in the very early stages of the disease progression to have any impact on AD, limiting their use until better pre-symptomatic AD diagnostics have been developed.

Several 5-HT receptors (5-HTR) have been shown to influence processing of the amyloid protein precursor (APP), including 5-HT2AR, 5-HT2CR, and 5-HT4R [7, 8]. Among them, the 5-HT4R and 5-HT6R receptors have been of most interest. The 5-HT4R was identified as a most promising target, since activation of this receptor shift the equilibrium of APP cleavage towards formation of the soluble non-amyloidogenic form (sAPPα) fragment possessing neurotrophic and neuroprotective properties [7], while the 5-HT6R has caused much interest for potential roles in AD due to its modulatory effects on gamma-aminobytyric acid (GABA) and glutamate levels, [9] which facilitate the secondary release of other neurotransmitters including dopamine, noradrenaline and acetylcholine, all of which are compromised in AD. In addition, 5-HT6R are exclusively found in the CNS, indicating the possibility of selective CNS targeting to limit off-target toxic effects.

3. Serotonin subtype 4 receptor

Among the large family of 5-HTR, the 5-HT4R's are postsynaptic receptors. Although widely expressed throughout the body, the highest density is observed in the brain (olfactory tubercles, basal ganglia, substantia nigra, superior colliculi, hippocampus, and cortex). These are all CNS structures that are extensively involved in cognitive functions, suggesting that the 5-HT4R could be a therapeutic target for improving memory performance and hereby slowing memory deficits, such as those that occur in AD [10]. Moreover, it has been shown that 5-HT4R expression is reduced in AD patients. Furthermore, it has been shown

that activation of these receptors enhances the release of acetylcholine in the frontal cortex and hippocampus [11], increases long term potentiation in the hippocampus [12] and induces a rapid and sustained increase in basal firing of 5-HT cells in the dorsal raphe nucleus [13, 14]. 5-HT4R activation also stimulates hippocampal expression of plasticity/learning-related proteins such as brain-derived-neurotrophic-factor, AKT, CREB, as well as neurogenesis in the dentate gyrus [15].

In addition, and a major advantage of using 5-HT4R agonists in treatment of AD, is their ability to shift the equilibrium of APP processing pathway from production of the neurotoxic amyloid-beta-peptide towards formation of the sAPPα [16]. In contrast to amyloid-beta-peptide, the soluble form has putative neurotropic and neuroprotective properties, see Maillet et al. [17] for a review. The ability of 5-HT4R agonists to stimulate the amyloidogenic pathway leading to release of soluble form of APP has been demonstrated in various cell-based animal models [18].

In early years, 5-HT4R agonists attracted much attention as potential gastrointestinal drugs used in the therapy of functional bowel illnesses such as constipation, irritable bowel syndrome, gastroparesis, and gastroesophageal reflux disease [19]. The first generations of 5-HT4R agonists used in clinical medicine were Tegaserod (**1**), Cisapride (**2**) and Procalupride (**3**) (**Figure 1A**), which showed clinically effective in treatment of gastrointestinal motility disorders; however, adverse cardiovascular events have resulted in the restricted availability of these drugs [20]. Therefore, in order to develop clinically relevant 5-HT4R agonists, the ligands must be potent and highly selective, which is hampered by high similarity of subtype receptors. In 1998, the molecular structure and functional characterization of four splice variants of the human 5-HT4R were described [21] that differ in the carboxy terminal cytoplasmic domain while extracellular and transmembrane domains are absolutely conserved [22].
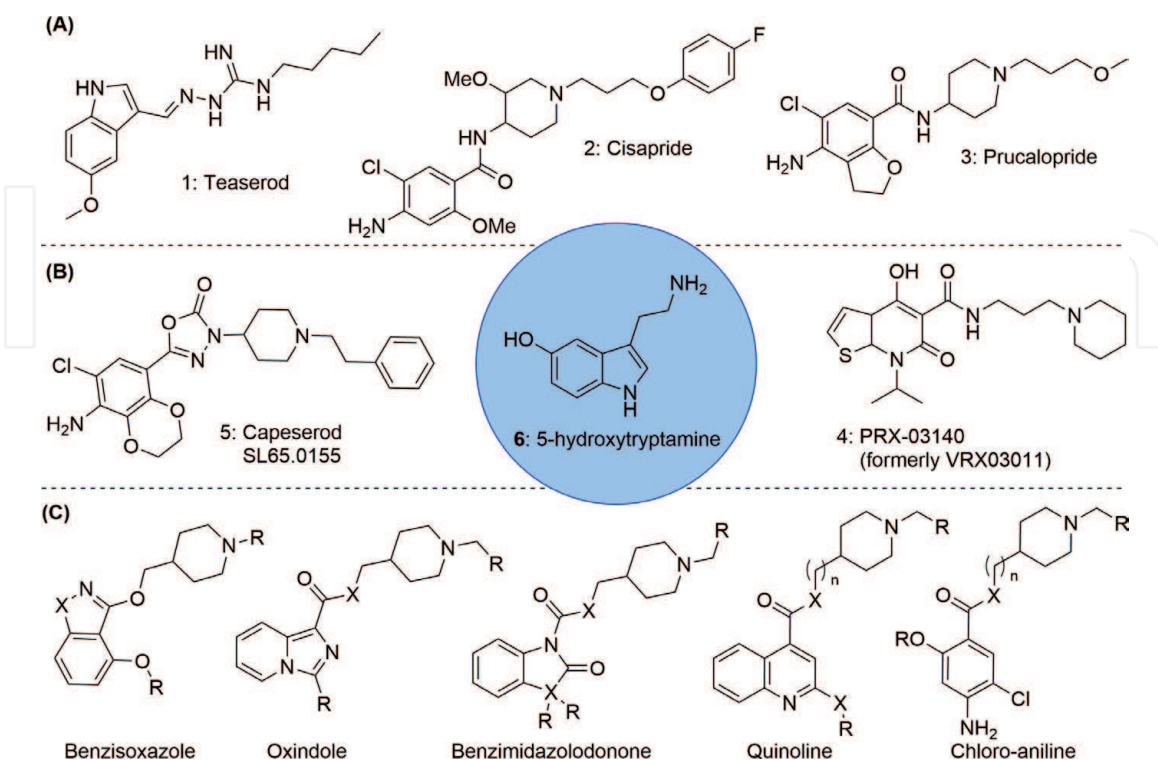
Figure 1.
5-HT4 receptor ligands (A) first generations of 5-HT4R agonists used in clinical medicine, (B) partial 5-HT4R for treatment of AD that reached Phase 2 studies, (C) Overview of structures studied for development of partial 5-HT4R for treatment of AD.

Another concern is the risk that prolonged or repeated exposure of the 5-HT4R to an agonist, may lead to receptor desensitization. The 5-HT4Rs are G-protein-coupled receptors (GPCRs), which can be desensitized following activation by agonists [23]. Agonist-induced desensitization of GPCRs is less common for partial agonists than strong agonists and therefore, most focus has been given to developing highly selective partial 5-HT4R agonists for treatment of AD. In order to being therapeutic useful in treatment of AD, the compound must therefore fulfill several requirements. In addition to being a selective partial 5-HT4R agonist, the target molecule must show good brain barrier penetration, which was also limited in the early generation agonists [24].

However, the potential for 5-HT4R partial agonists to offer clinical benefit for the treatment of AD has indeed been demonstrated. Data from a small Phase 2 study in patients with mild to moderate AD with the selective partial 5-HT4R agonist, PRX-03140 (**4**, EPIX Pharmaceuticals), [25] **Figure 1B**, showed a statistically significant improvement of cognitive processes after only two weeks of therapy [20]. Also, the partial agonist SL65.0155 (**5**, Sanofi-Aventis) reached phase II clinical trial for the treatment of AD [26]. However, these were both later discarded due to serious off-target effects.

This has stimulated much research aiming at designing and developing more selective 5-HT4R partial agonists.

3.1 Pharmacophore of the 5-HT4R ligand

In accordance with the natural ligand, 5-HT (**6**), the general pharmacophore of 5-HT4R agonists consists of an aromatic core connected via a chemical spacer to a basic amino moiety [27] to introduce affinity, while an extra hydrogen-bond donor-acceptor function (e.g., phenol in 5-HT) is required for high affinity ligands [28]. Furthermore, it is accepted that voluminous substituents of the basic nitrogen interact with a hydrophobic pocket in the 5-HT4R ligand recognition site [29]. Based on this pharmacophore framework, a broad range of substances has been investigated, aiming at introducing selectivity.

Suitable aromatic systems [30] include 4- amino-5-chloro-2-methoxy benzoic acid, indole, imidazopyridines and N-alkyl benzimidazolodonone among others (see **Figure 1C**). Several basic amines with voluminous substituents such as piperizines [31], and piperidines [30] have been used.

3.2 Agonists of 5-HT4R

Over the last years, a broad range of structural varied 5-HT4R agonists have been developed, which all share the common structural features presented by the pharmacophore described above. Below we have grouped them into 4 main groups and discuss the structural features for each of these groups.

3.2.1 Group 1: benzisoxazole, oxindole and benzimidazolodonone core

Much research aiming at developing new 5-HT4R agonists has focused on compounds possessing a benzyl ring linked to a 5 membered heterocycle, analogous to the indole ring in the natural 5-HTR ligand.

Inspired from early generations of 5-HT4R agonists (**1**–**3**, **Figure 1**), Brodney and coworkers [32] synthesized and studied a diverse set of structural varied chemical libraries aiming at identifying excellent CNS active 5-HT4R agonists. To improve chances of identifying compounds with brain barrier penetration, structures were selected based on a number of criteria: (1) reduced number of hydrogen
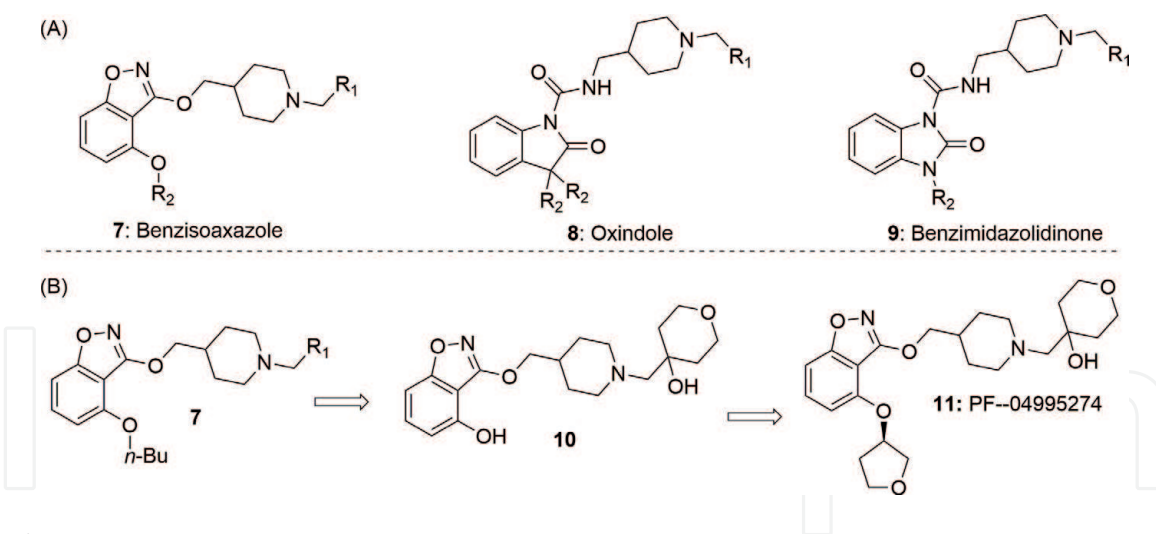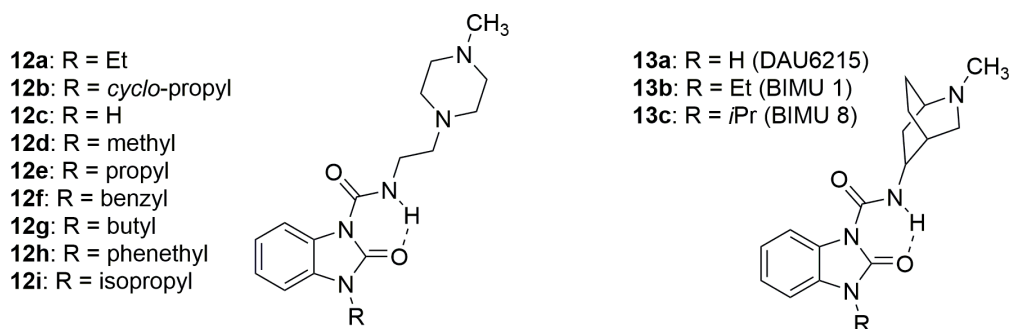
Figure 2.
SAR studies of (A) benzisoxazole (7), oxindole (8) and benzimidazolodonone (9) core stuctures, (B) provided 11 as a potent partial 5-HT4R agonist [32].

donors (≤ 1), (2) low molecular weight (<400) and (3) weakly basic amine centers (pKa < 8,5) [33]. Based on these criteria, the benzisoxazole (**7**), oxindole (**8**) and benzimidazolodonone (**9**) was chosen as core structural templates.

For benzisoaxazoles (**7**), the studies showed that manipulation of molecular size and shape of the R1 and R2 groups (structure 7, **Figure 2**) provided means to modulate intrinsic properties and ADME (adsorption-distribution-metabolism-excretion). Highly lipophilic compounds (R1 = n-butyl) resulted in high clearance from human liver and low passive permeability. Replacing the piperidine n-butyl group with hydroxyl-tetrahydropyran reduced the partition coefficient (ClogP), but analogue **10** still demonstrated high clearance. To further reduce ClogP, the isobytyl side chain was replaced with more polar groups. While the tertiary carbinol and tetrahydropyran analogues exhibited poor metabolic stability, the tetrahydrofyran **11** provided a potent partial agonist with low clearance.

Orjales and coworkers [33] provided a structure–activity-relationship (SAR) study of 2,3-dihydro-2-oxo-1H-benzimidazole-1-carboxamide derivatives bearing a piperazine moiety (**12a-h**, **Figure 3**). Both, the influence of the 3-substituent of the benzimidazole ring, the 4-substituent of the piperazine moiety, and the alkylene spacer was studied and especially the substituent in the 3-position was found to be critical for 5-HT4R affinity. While compounds with ethyl (**12a**), and cyclopropyl (**12b**) substituents showed moderate to high affinity for the receptor, derivatives having smaller alkyl substituents (H: **12c**, methyl: **12d**, propyl: **12e**) showed a significant drop in affinity. Also, introduction of large and bulky substituents (benzyl: **12f**, butyl: **12g** and phenylethyl: **12h**) severely reduced the affinity for the 5-HT4R. In addition to receptor affinity, also the 5-HT4R activity was dramatically influenced by substituents in the benzimidazolone 3-position. While ethyl- and cyclopropyl-functionalized derivatives (**12a**, **12b**) showed moderate antagonistic activity, the isopropyl derivatives (**12i** and **13c**) acted as partial agonists. The dramatic variation in the 5-HT4R pharmacological activity as a result of only small structural variations is in agreement with previous observations for benzoate derivatives [29].

A similar trend with benzimidazolone 5-HT4R ligands was reported by Langlois et al. [34] While the DAU-6215 ligand (**13a**) with no alkyl substituent on the nitrogen in the 3-position is in-active towards the 5-HT4R, the BIMU-1 (**13b**) and BIMU-8 (**13c**) compounds with ethyl and isopropyl substituents are potent 5-HT4R agonists [34, 35].

Figure 3.
Structures investigated in a SAR study of 2,3-dihydro-2-oxo-1H-benzimidazole-1-carboxamide derivatives bearing a piperazine moiety [33].
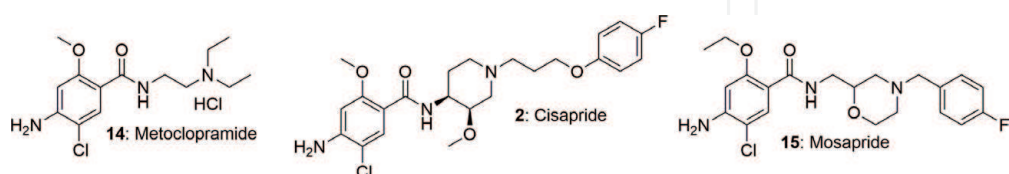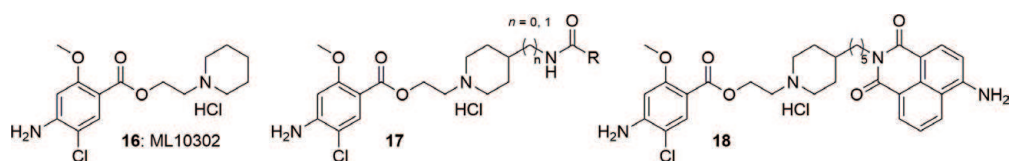

Figure 4.
Parent compounds of the chloro-aniline class of structures.

3.2.2 Group 2: chloro-aniline core

The parent compound of this class is metoclopramide [36, 37] (**14**, **Figure 4**), a drug well-known for its gastric prokinetic activity. Furthermore, compounds having the chloro-aniline core is already well known to confer 5-HT4R agonist activity, for example in Cisapride (**2**) [38] and Mosapride (**15**) [39]. Therefore, it is not surprising that several derivatives of 4-amino-5-chloro-2-methoxybenzoic acids have been investigates as 5-HT4R agonist in AD research.

Russo et al. [40] synthesized and studied a library of structures based on the 5-HT4R partial agonist, ML10302 (**16**, **Figure 5**) by introducing an amide group linked to the piperidine ring of ML10302 (**16**), **Figure 5**, hoping to introduce additional binding interactions. Displacements experiments with a 5-HT4R specific antagonist revealed that compounds in which the amide functionality was directly attached to the piperidine ring (**17**, n = 0) had weaker binding affinities compared to ML10302 (**16**). However, compounds where the amide moiety were attached to the piperidine ring through a methylene bridge, showed binding affinities that were globally better than ML10302 (**16**). Importantly, 4 compounds were identified that showed better functional properties than ML10302 (**16**) and induced up to 50% higher cyclic adenosinde monophosphate (cAMP) production. One compound (**18**) was further evaluated by in vivo biological tests, showing promising results for AD treatment.

RS67333 (**19**) [41] is a very affine 5-HT4R partial agonist, which also have high selectivity vs. other receptors. Its therapeutic relevance for treatment of AD is evident as it was shown to improve both object and place recognition in adult [42, 43] and aged animals [44, 45]. On this basis, Dallemagne and coworkers synthesized a series of analogues of RS6733 (**19**) [46], aiming at identifying a multitarget-directed ligand (MTDL) having both 5HT4R agonist and acetylcholinesterase (AChE) inhibitor activities. Among them, the compound donecopride (**20**) was designed: The cyclohexyl moiety was introduced to be a compromise between the N-butyl group of RS67333 (**19**) and the bulky benzyl group of the potent AChE inhibitor, donepezil (**21**). Donecopride (**20**) showed outstanding in vitro activity and was able to potentiate both the 5-HT4R partial agonist activities as well as the

Figure 5.
Structures investigated in a SAR study of analogues of the 5-HT4R partial agonist, ML10302 [40].
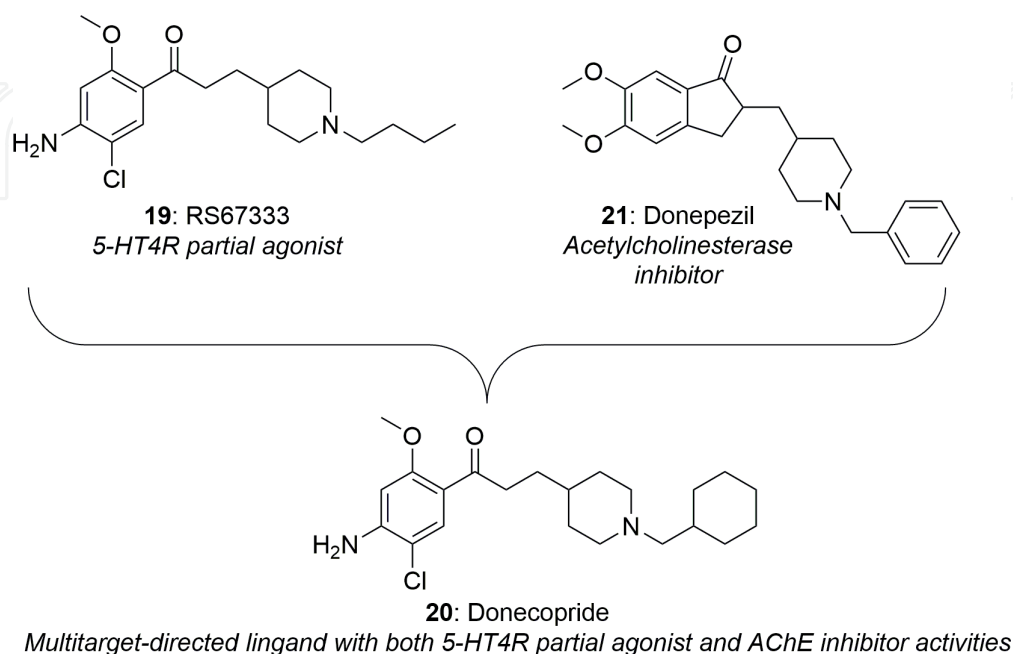
Figure 6.
A multitarget-directed ligand Donecopride (20) was developed to have both 5HT4R agonist and acetylcholinesterase inhibitor activities [46].
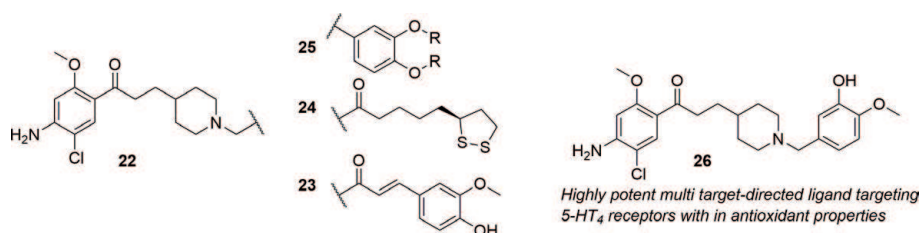
Figure 7.
Compound 26 was developed by combining the 5-HT4R chloro-aniline pharmacophore (22) and structures (23–25) having antioxidant properties [48].

inhibition of AChE, resulting in the alleviation of both amyloid aggregation and tau hyperphosphorylation, [47] which are known to be two major features in AD (**Figures 6** and **7**).

In 2019, Lanthier et al. [48] generated a MTDL targeting both activation of the 5-HT4R while also bearing antioxidant activities; hereby being able to both control Ab protein accumulation and prevent toxicity of reactive oxygen species (ROS) in neuronal cells [48].

The chloro-aniline core connected via a chemical spacer to a basic piperidine ring (structure **22**) was introduced as the 5-HT4R binding moiety of the MDLT. As replacement of the butyl chain of RS67333 (**19**) by diverse alkyl moieties has been shown to have limited impact on both the affinity and the pharmacological profile towards the 5-HT4R [46], the chemical moiety having antioxidant activities was introduced as substituent on the central piperidine ring. A variety of structures was investigated, varying in spacer between the two pharmacophores. Also, various

chemical structures known to exhibit antioxidant activities were investigated, including polyphenol [49], hydroxycinnamic acid (**23**) [50], lipoic acid (**24**) [51, 52], vanillin or isovanillin (**25**) [53, 54]. Hereby, Lanthier et al. were able to identify a potent 5-HT4R ligand (**26**) with promising antioxidant activity for future preclinical tests.

A similar approach was investigated by Yahiaoui et al., who reported the design of the dual compound **27** with 5-HT4R agonist and 5-HT6R antagonist effects (**Figure 8**) [55]. The dual 5-HT4R/5-HT6R ligand was designed through modulation of the 5-HT4R partial agonist, RS67333 (**19**) by introducing a 5-HT6R antagonist (**28,29**) pharmacophore (a positive ionizable atom, a hydrogen bond acceptor group, a hydrophobic site, and an aromatic-ring hydrophobic site) [56–60]. Yahiaoui et al. synthesized and tested a library of structures consisting of RS67333 (**19**) modulated with various arylsulfonyl groups (sulfonamides and sulfones) attached to the piperidine moiety through a variable number of methylene groups [61]. These studies resulted in identification of the compound **27** having nanomolar and submicromolar affinities towards 5-HT4R and 5-HT6R, acting as a partial agonist and antagonist, respectively.

To further elaborate on these studies, Hatat et al. [61] designing an antiamnesic MTDL with balanced 5-HT4R agonist, 5-HT6R antagonist and AChE inhibitory activities. Starting from the dual MTDL **30** and the benzyl analog of Donecopride (**35**) [62], various analogues were designed and tested. However, counteracting requirements within the scaffold made the design difficult. While an unsubstituted benzyl group was the best substituent on the piperidine for affinity towards AChE and 5-HT4R, the 5-HT4R affinity appeared linked to substitution of the benzyl group. However, the analogue with a methyl group in the benzyl meta-position (**32**) showed balanced activities towards all three targets (**Figure 9**).
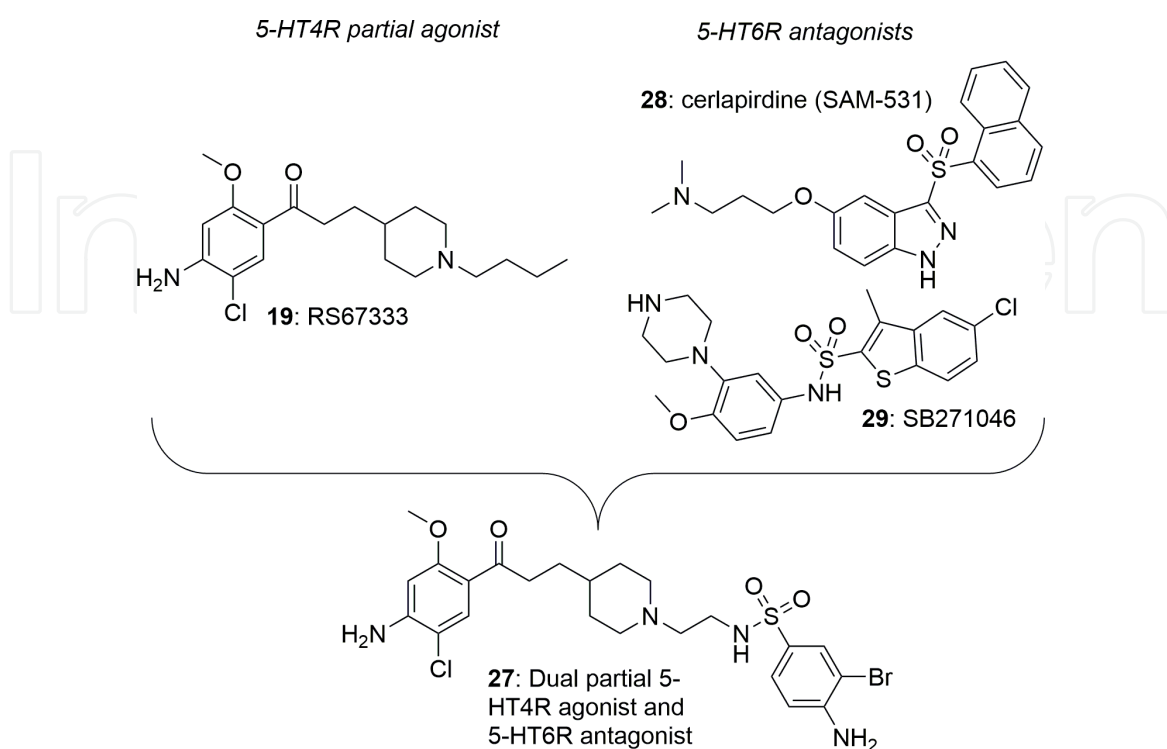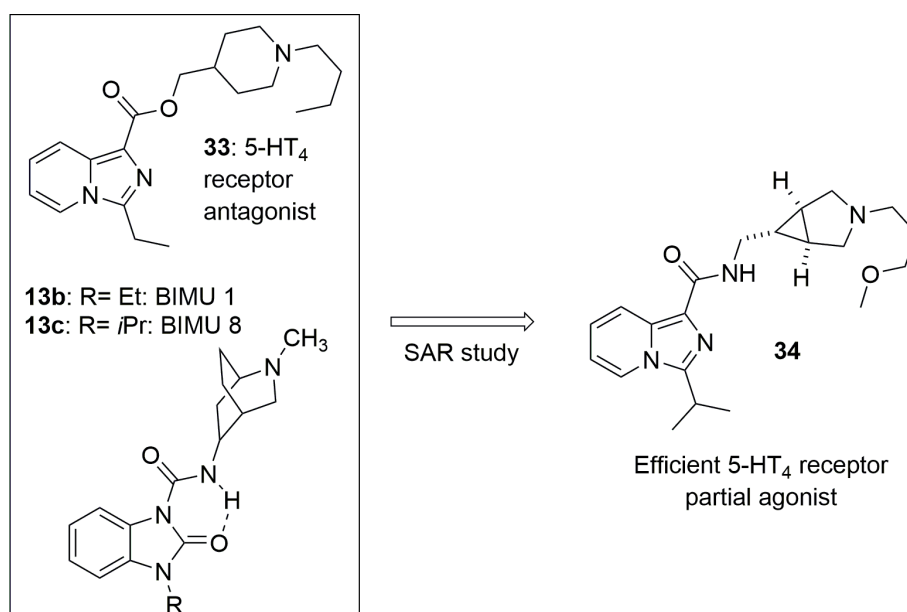

Figure 8.
The dual 5-HT4R/5-HT6R ligand was designed through modulation of the 5-HT4R partial agonist, RS67333 by introduction of a 5-HT6R antagonist pharmacophore [55].
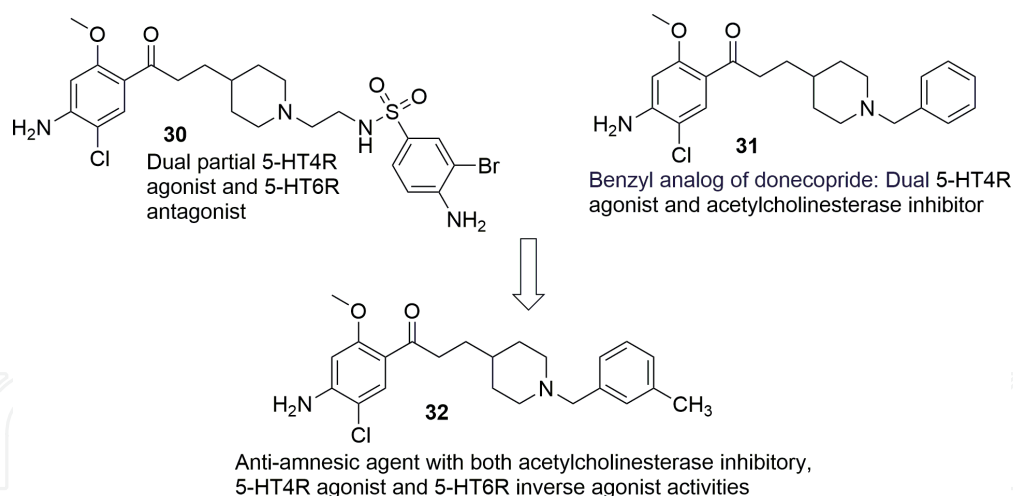
Figure 9.
Antiamnesic MTDL (32) with balanced 5-HT4R agonist, 5-HT6R antagonist and AChE inhibitory activities [61].

Figure 10.
SAR studies of imidazo[1,5-a]pyridine structures provided 34 as an efficient 5-HT4R partial agonist [66].

3.2.3 Group 3: imidazo[1,2-a]pyridine, imidazo[1,5-a]pyridine, imidazo[4,5-b] pyridine and imidazo[4,5-c]pyridine core

Compounds based on the imidazo[1,5-a]pyridine [63, 64] core were initially reported as dual mediators of 5-HT3R and 5-HT4R [65], see **Figure 10** (**33**). However, work from Nirogi et al. provided an understanding of the substitution patterns on both the imidazo[1,5-a]pyridine ring and piperidine ring, allowing for development of 5-HT4R partial agonists based on this core [66]. Derivatives were designed as bioisosteric analogues of the potent 5-HT4R agonists BIMU-1 and BIMU-8 (**Figure 10**, compounds **13b** and **13c**) [34] by replacing the benzimid-azolone core with an imidazo[1,5-a] pyridine while preserving an alicyclic amine moiety, in accordance with the 5-HT4R pharmacophore (see section 3.1). Structural optimization was focused on modification of the alkyl substituent at the imid-azopyridine ring, as well as the type of alicyclic amine. Also, a SAR iteration was carried out to understand the role of a methylene spacer between the amide group with the piperidine moiety, in addition to the effect of length and structure of the

N-alkyl/heteroalkyl chain. This process resulted in the discovery of a highly potent and selective partial 5-HT4R agonist **34** with pro-cognitive efficacy in rats and adequate ADME properties [66].

3.2.4 Group 4: quinoline core

Compounds containing the quinoline bicyclic aromatic core have attracted much attention in the design of 5-HT4R ligands [67–70]. To provide medicinal chemistry understanding of the quinoline 5-HT receptor ligands, Castriconi et al. [71] synthesized and studied binding affinity of potential 5-HT4R agonists with reference to the 5-HT4R ligands ML10302 (**16**) and PRX-03140 (**4**) by replacing the aromatic moieties with 2-methoxyquinoline. Interestingly, the flexible quinoline derivatives **35**, **36** showed remarkable differences in 5-HT4R affinity. In fact, while the ester derivative **35** showed a K_i value in the nanomolar range, the corresponding secondary amide **36** was at least two orders of magnitude less active. This was ascribed to the chemical nature of the amid bond linking the side chain with the quinoline moiety in **36** affecting the preferred conformation (**Figure 11**). To test this hypothesis, the flexible compounds **36** were transformed into the conformationally constrained derivatives **37**, **38** (**Figure 11**). This enhanced 5-HT4R affinity to the nanomolar range, suggesting that the conformationally constrained derivatives **37**, **38** represented the bioactive conformation of ester **35**, which cannot be populated by amide derivative **36** for steric reasons. The higher affinity of **38** compared to **37** suggests a secondary role of the second carbonyl group in the interaction with 5-HT4R binding site.

In later efforts, Cappelli and coworkers published a more comprehensive SAR study of receptor ligands with 2-methoxyquinoline as the aromatic system [72]. A series of piperidine containing functionalities were investigated, demonstrating N-butyl-4-piperidinylmethyl (also present in RS67333 (**19**), see **Figure 12**) to be a most promising basic moiety. Substituting the quinoline methoxy-substituent with a chloro- or cyclopropyl-substituent, did not have any significant effect on the activity (**39a-d**).
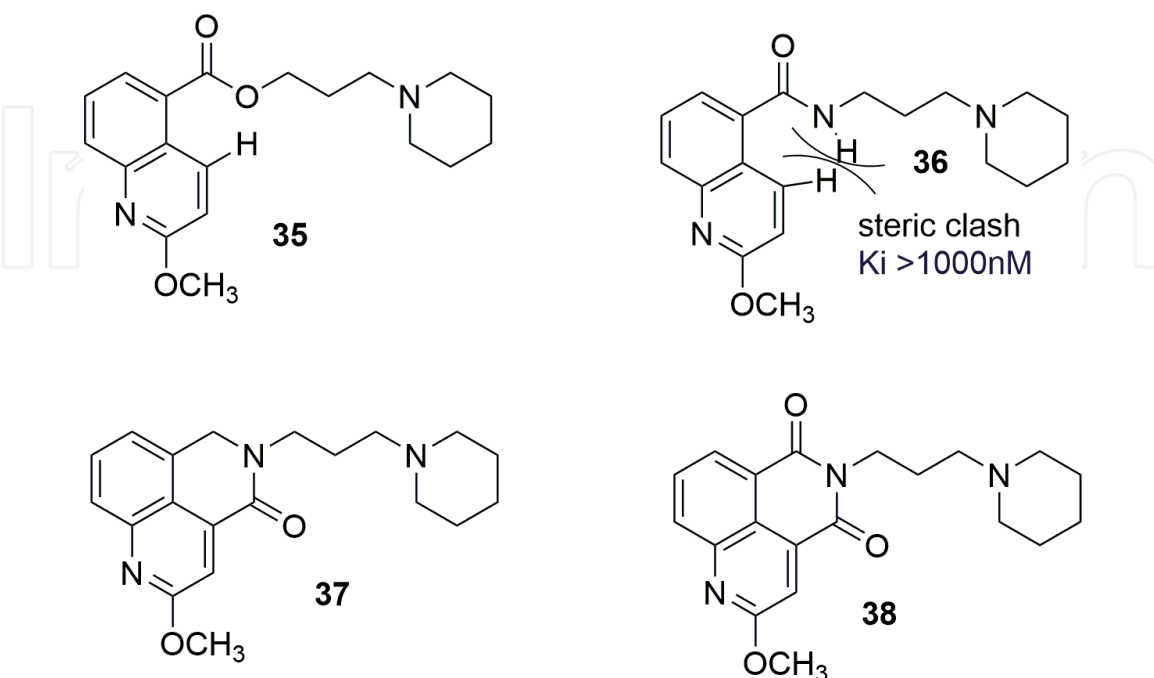
Figure 11.
Quinoline 5-HT$_4$ receptor ligands. Conformationally constrained derivatives 37, 38 showed improved affinity compared to the flexible compounds 35, 36 [71].
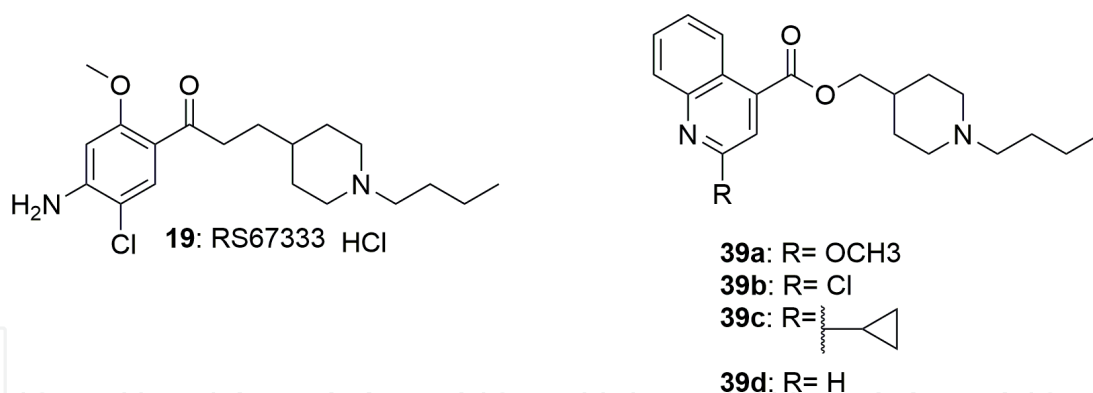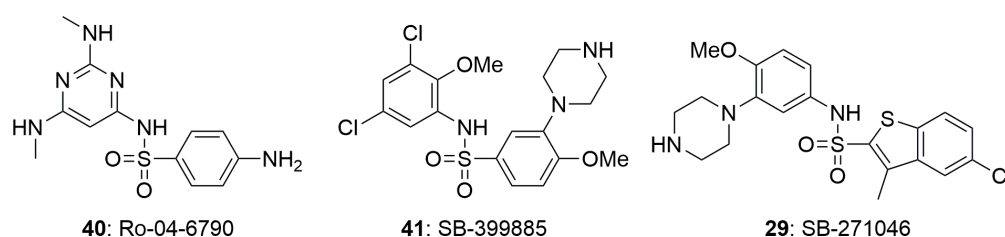
Figure 12.
A comprehensive SAR study of receptor ligands with 2-methoxyquinoline core structure provided 39a as an efficient 5-HT6R partial agonist [72].

4. Serotonin subtype 6 receptor

The 5-HT6R was discovered in 1993 by Monsma et al. [73–76]. These receptors are GPCRs, which are located postsynaptically to serotonergic neurons [77]. Since its identification, significant efforts have led to a better understanding of the biology of this receptor. The $5\text{-}HT_6$ receptors are present in regions of the brain regions responsible for learning and memory, making them of high interest in AD research. Furthermore, blockade of 5-HT6R function was shown to increase acetylcholine- and glutamate-related neurotransmission, which enhances learning and memory [78, 79]. Evidence indicates that blockade of this receptor improve both cholinergic and glutamatergic system [79]. Furthermore, blockade of 5-HT6R alleviates memory deficits, such as age-related decline in cholinergic or glutamatergic neurons, [79, 80]. Studies conducted by Kotańska et al. [74] revealed that antagonism of the 5-HT6R enhanced neuroplasticity, helped maintain neurite growth and provided a neuroprotective effect against amyloid beta neurotoxicity [81, 82].

This has led to a high interest in this receptor in treatment of the cognitive decline associated with AD [75, 78, 79, 83]. In addition, the receptor is exclusively expressed in the CNS, primarily in the striatal, hippocampal and cortical areas of the brain [75] and therefore, could potentially provide therapeutics with limited peripheral side-effects [80].

Among the first reported selective antagonists are Ro-04-6790 (**40**) reported in 1998 [82, 84], SB-271046 (**29**) in 1999 [82, 85, 86] and SB-399885 (**41**) in 2002 [87] (see **Figure 13**) showed a 200-fold selectivity for the 5-HT6R. Although selective antagonists have been developed, no 5-HT6R antagonist has reached the pharmacological market to date. Thus, the search for new 5-HT6R agents is still of high focus in medicinal chemistry research. In this context, a better SAR understanding of the 5-HT6R pharmacophore is needed.

Figure 13.
*Illustrations of the some of the first selective 5-HT6R antagonists; Ro-04-6790 (**40**), SB-399885 (**41**) and SB-271046 (**29**).*
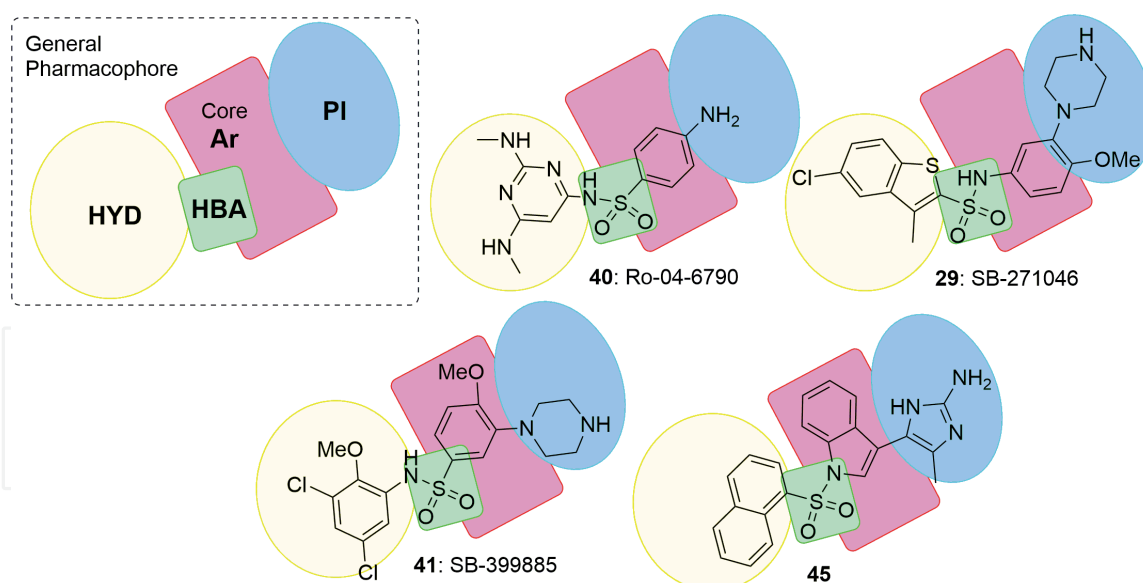
Figure 14.
*Schematic illustration of the pharmacophore (stripped box) along with overlays with using the first selective antagonist (**40, 29, 41**) and compound **45** from section 4.1. The figure is adapted from [88].*

4.1 Pharmacophore of the 5-HT6R ligand

From 1998 until today a fair number of studies have been conducted on 5-HT6R antagonists, which led to a good understanding of the pharmacophore. There are four main features responsible for interaction with the receptor: a polar positively ionizable (PI) group, a hydrogen bond acceptor (HBA), an aromatic area (AR) and a hydrophobic site (HYD) [75, 79] (see **Figure 14**).

In 2017, González-Vera and coworkers published [88] a SAR study regarding the hydrophobic moiety (HYD). In total 18 compounds were synthesized, all containing a sulfonamide as the HBA moiety. This study revealed that aromatic halogens in the HYD part of the structure increased affinity.

Based on this pharmacophore framework, a broad range of substances has been investigated as ligands for the 5-HT6R, aiming at introducing selectivity.

4.2 Antagonists for 5-HT6R

A comprehensive review was published by López-Rodríguez and colleagues in 2014 [79], which discussed the structural key features of 5-HT6R antagonists. In this review, they grouped them into 4 overall groups of structures that had been investigated for antagonism of 5-HT6R (see **Figure 15**). They made some general conclusions, which are summarized in **Figure 15**.

For more details about specific compounds, please refer to [79]. This chapter will focus on studies made since 2014, while the reader is referred to the following excellent reviews [75, 79] for investigations before 2014.

A SAR study published by Zajdel et al. in 2016 [89] studied analogues of the natural substrate 5-HT (**6**) for the receptor. Aiming to increase affinity by constraining the ligand into its preferred conformation, a substituent (R1) was introduced to the tryptamine core, hoping to obtain a more constrained basic amine (**Figure 16**). Furthermore, an aryl arylsulfonyl moiety was introduced in the N1 position of the indole moiety. A total of 28 compounds were tested, which provided two compounds, **42** and **43**, with both high affinity and selectivity for the 5-HT6R. The affinity data showed that R2 substituents were unfavored, while substituents in the indole C5 position improved properties
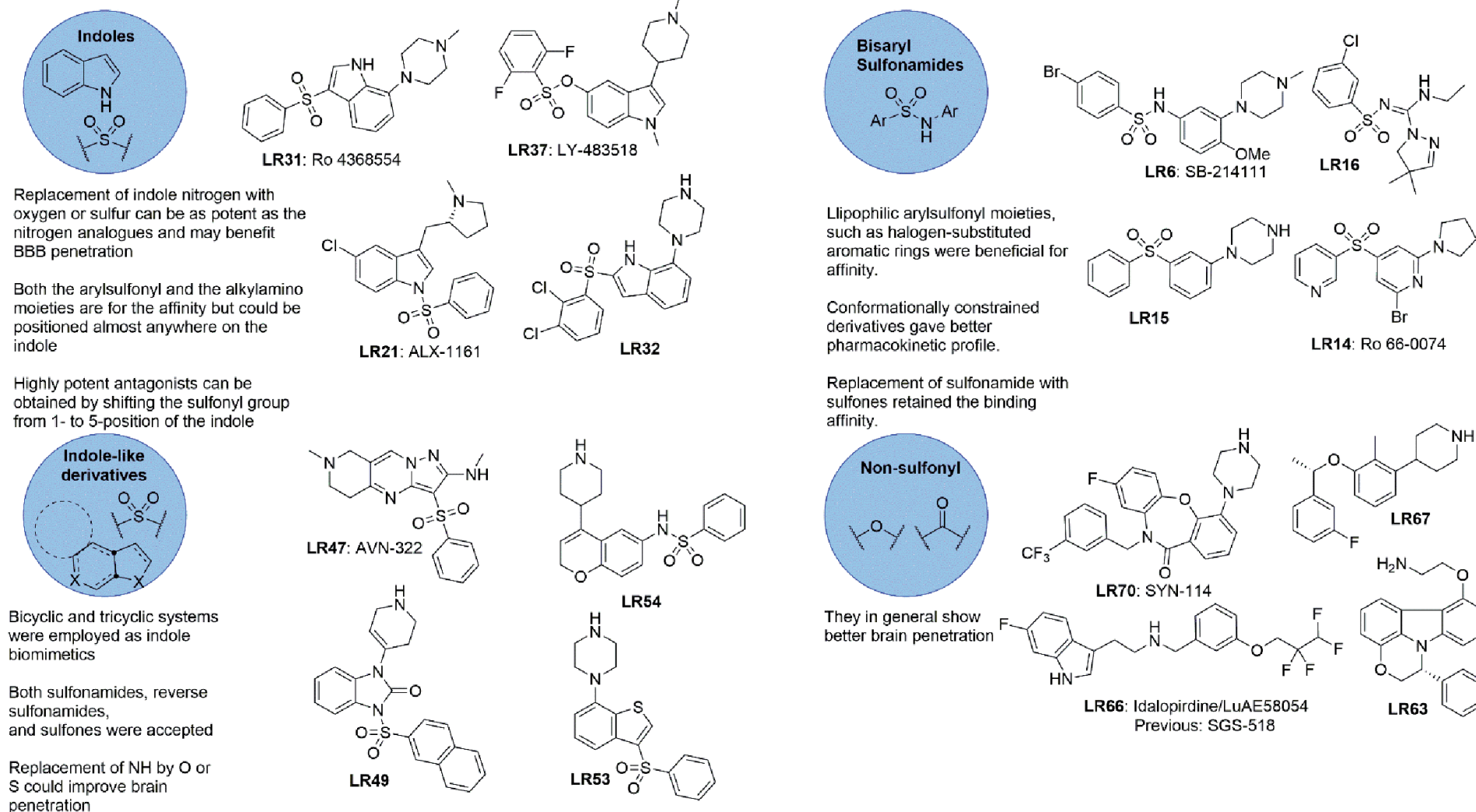
Figure 15.
Representative examples from review by López-Rodríguez and colleagues [79]. The compound notation/numbering correlates with that of the review.
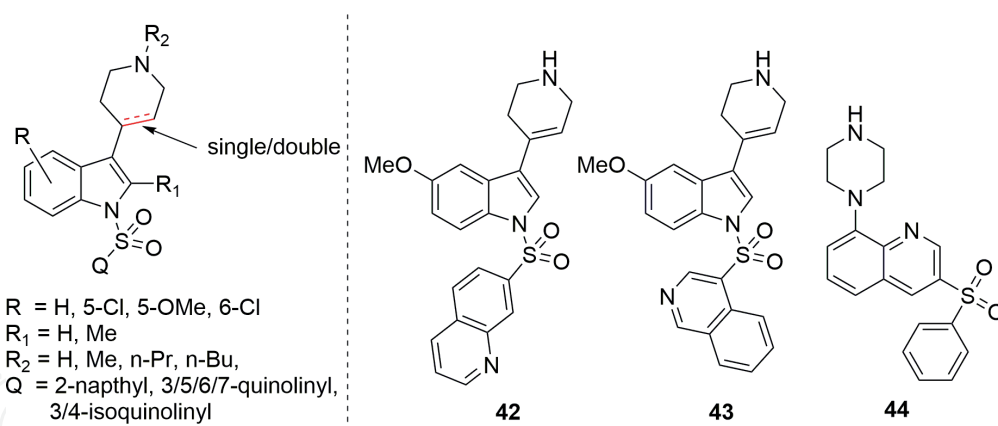
Figure 16.
Summarizing the structures from the SAR, followed by two potential antagonists (42 and 43), along with reference compound SB-742457 (44). [89].

(**Figure 16**). When reducing the double bond in compound **42** and **43**, a slight decrease in affinity was observed, probably due to higher flexibility.

The two compounds were further tested for their functional activity *in vitro* and compared with reference compound SB-742457 (**44**). The compounds behaved as potent antagonist in a cAMP assay, while only displaying weak affinity for off-target receptors. Preliminary pharmacokinetic profiling of **43** showed good blood–brain-barrier (BBB) penetration, improved recognition of novel objects in mice, in addition to a putative antidepressant activity in lower doses compared to the reference compound. Furthermore, **43** did not show any anxiolytic activity.

In 2019 Hogendorf et al. [90] published a comprehensive SAR study involving more than 50 compounds, all containing an aminoimidazole moiety as a new bioisoster of the classical PI amino-group. These were found to form strong hydrogen bonds with electronegative acceptors, such as carbonyls, in the enzyme active site. In total six different series were investigated, but only the two series that were found to be most important for activity are included in **Figure 17**.

Based on affinity, physiochemical properties (ClogP, pKa, topological polar surface area, water solubility, etc.), metabolic stability, mutagenic/toxicity and BBB permeation, compound **45**, **Figure 17**, was chosen as the lead structure. The compound showed high selectivity and displayed ability to reverse scopolamine-induced cognitive impairment. Crystallographic studies of the 2-aminoimidazole-based antagonists revealed that the high receptor affinity was reached due to a resonance driven conformational change [90] upon protonation of the imidazole fragment.

AD is more than just an imbalance in the cholinergic or glutamatergic systems and therefore, it was speculated whether better therapeutics could be achieved by using MTDLs affecting several neurotransmitter pathways [77]. In recent years, various MTDLs have been studied [81].

Marcinkowska et al. [81] designed MTDLs combining 5-HT6R antagonism, inhibitory effect against butyrylcholinesterase (BuChE) [91] and antioxidant properties [81], aiming at achieving both cognition-enhancing properties and neuroprotective activity. Both series were based on typical the 5-HT6R antagonist scaffold with a N-substituted 4-(piperazin-1-yl)-1H-indole core [79]. The indole 1-position was occupied with a benzyl group for series 1 and a 2-chloro benzene moiety for series 2 (marked with blue in **Figure 18**). The piperazine was N-substituted with an alkyl-phthalimide moiety with variation of the length of the alkyl chain (see **Figure 18**). The phthalimide group (marked with yellow in **Figure 18**) is known for interacting with the hydrophobic pocket in BuChE [92]. The indole moiety (marked with red in **Figure 18**), in addition to being important for 5-HT6R antagonism, had

antioxidant properties: Indoles are known for their ability to capture free radicals and protect biological systems against peroxidation [93]. From their screen they found that longer alkyl chains, connecting the phthalimide to the piperazine ring, decreased affinity for the 5-HT6R, while substituents on the benzyl group did not alter affinity significantly.

However, counteracting requirements within the scaffold complicated the design. From the BuChE inhibitor (BuChEI) screen they concluded that generally the unsubstituted benzyl analogues showed highest activity, with compound **46** (R = H, n = 1) being the best inhibitor, while best 5-HT6R affinity was obtained with substrates having chloride in meta position on the benzyl moiety (**47**). Taking both targets into account, compound **48** (R = Cl, n = 1) showed the best overall properties, with good affinity for 5-HT6R, promising BuChE inhibitory effect and satisfying antioxidant properties. This SAR study demonstrated promise for MTDLs as AD therapeutics and inspired further research in the field.

Zajdel et al. recently [94] investigated MTDLs combining 5-HT6R inverse agonist activities and a monoamine oxidase B (MAO-B) inhibitory effect. The
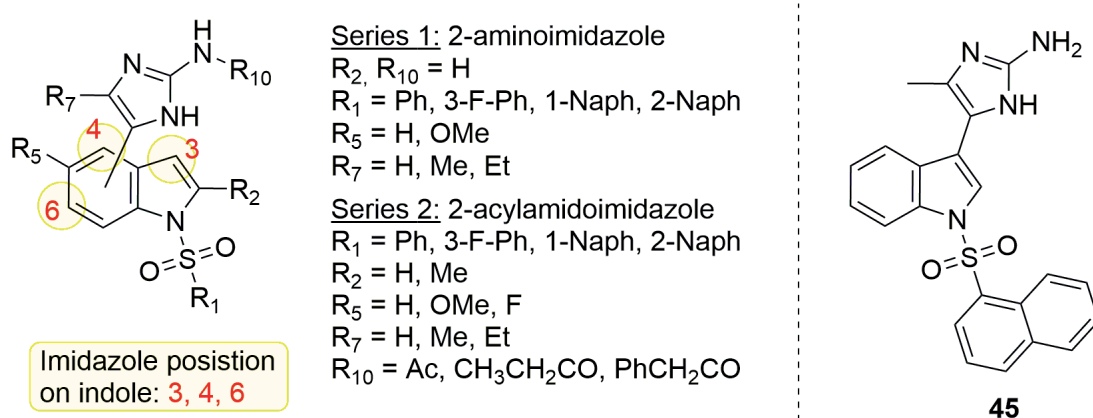
Figure 17.
SAR illustration covering two out of six series along with lead compound 45. [90].
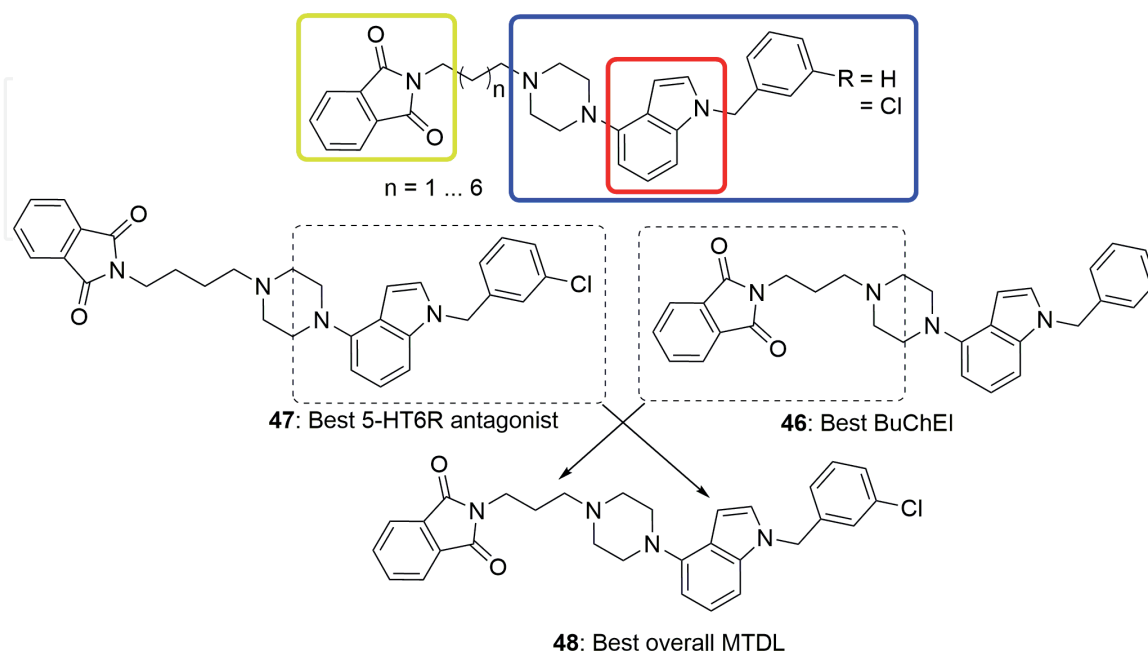
Figure 18.
Schematic illustration of the model compound highlighting important feature for the MTDL. Compound 47 was the best 5-HT6R antagonist from the study, compound 46 the best BuChE inhibitor and lead compound 48 with the best overall results. [81].

design included a 5-HT6R antagonist scaffold and fragments of either a reversible or an irreversible MAO-B inhibitor (see **Figure 19**) attached through an alkyl spacer of different lengths.

Their results indicate that the sulfone group was crucial for affinity to the 5-HT6R, while non-substituted phenyl groups (R = H) seemed to result in the best 5-HT6R binding. However, it is relevant to mention that no compounds containing the sulfone group together with the chloride-substituent were tested, making it difficult to make an overall conclusion. Among the 18 synthesized compounds, the very promising lead compound **49** was discovered. This compound displayed moderate metabolic stability, good artificial membrane permeability as well as good distribution in the brain. Furthermore, the compound showed glioprotective properties and fully reversed scopolamine-induced memory deficits.

In 2016 Grychowska et al. [83] published a study for a new core design based on a scaffold-hopping approach, with swapping of carbon and nitrogen atom in the indole ring [95], starting from the SSRI 6-nitroquipazine (**50**) to achieve the 1H-pyrrolo [3,2-c] quinoline core (**51**). They afterwards studied alternating substitution patterns on the arylsulfonyl fragment, which resulted in identification of **52**, see **Figure 20**, as their lead structure for further studies. The SAR revealed that substituents in the meta-position of the phenyl were beneficial. However, it is difficult to make any general conclusions of this effect, as both electron-withdrawing and -donating substituents resulted in compounds with good affinity for the receptor. Of all the compounds tested, compounds containing a chloro-substituent were found to induce strong antagonistic properties. In general, the S enantiomers of the amino-pyrrolidine were more favored than the R enantiomers and compound **52** showed to be the best candidate with high selectivity for the 5-HT6R over the 5-HT1AR, 5-HT2AR, 5-HT2CR, 5-HT2BR, 5-HT7R as well as dopamine (D2), adrenergic (alpha1A), histamine (H1), muscarinic acetylcholine (M1) receptors and SERT.

From a GPCR signaling assay it was determined that compound **52** behaved as neutral antagonist, whereas the reference SB-742457 (**44**) behaved as an inverse agonist. *In vivo* studies demonstrated that both **52** and SB-742457 (**44**) have pro-cognitive properties, as they were able to reverse pharmacological-induced memory deficits in rats and improve recognition of novel objects. Additionally, **52** showed antidepressant properties. Compound **52** was later used as scaffold for development of a MTDL with both neuroprotective and precognitive activities (5-HT6R and dopamine subtype 3 receptor (D3R) antagonism).

In an attempt to obtain the desired MTDL, Grychowska et al. [96] designed a series of 11 compounds combining structural elements of the 5-HT6R antagonist **52**, (blue box in **Figure 21**) with structural elements of a D3R antagonist (Eticlopride [97] and Nafadotride [98]), red box in **Figure 21**. All compounds synthesized in the study showed moderate-to-high affinity for 5-HT6R; however, only four compounds showed acceptable affinity for D3R. An elongation in the length of the alkyl chain (R, **Figure 21**) provided a better binding to D3R, however, decreased the affinity for the 5-HT6R. A chloride-substituted phenyl (X = Cl) showed significantly higher affinity for the 5-HT6R compared to the unsubstituted phenyl (X = H), in accordance with the previous mentioned importance of a halogen substituent (see section 4.1). Rewardingly, the S enantiomers were favored over the R enantiomers for both receptors. Based on all results, **53** showed overall good properties and its selectivity for the 5-HT6R compared to other receptors was studied. Fortunately, it did not bind to 5-HT1AR, 5-HT2AR or 5-HT7R and also showed a 10-fold selectivity over D2Rs. Furthermore, compound **53** proved to be a neutral antagonist, as it did not significantly affect the cAMP level. Its therapeutic potential was nicely demonstrated from animal studies, where both a neuroprotective effect and reversal of pharmacological-induced memory decline was observed.
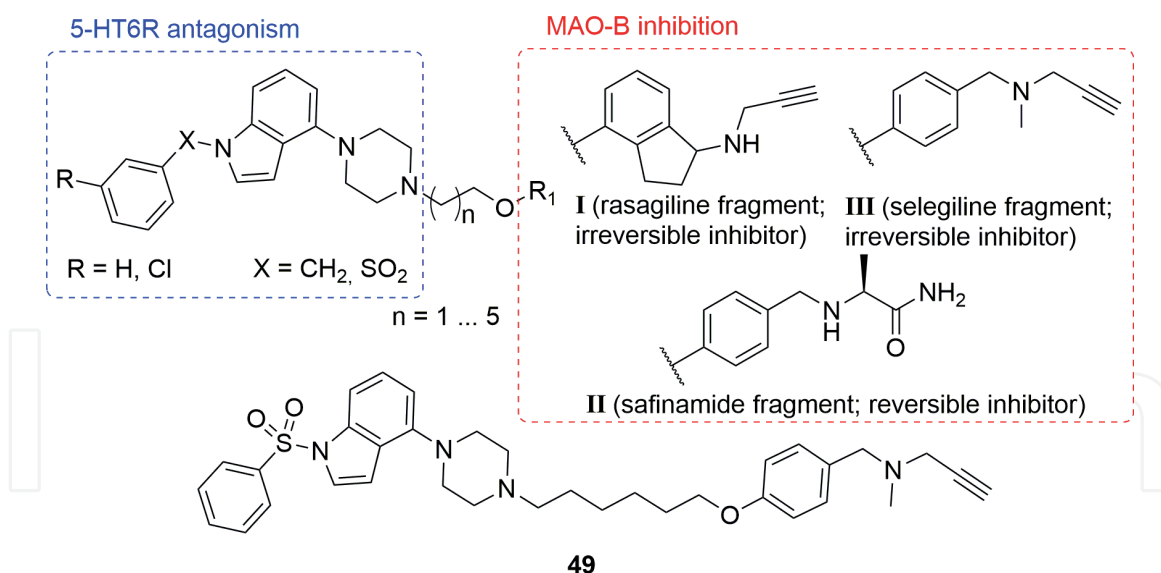
Figure 19.
*Schematic illstration outlining the scope of the SAR study and potential MTDL compound **49**. [94].*
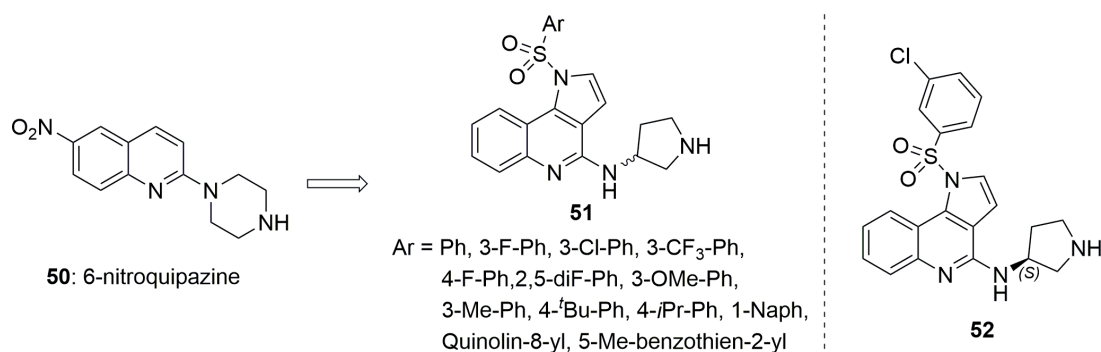
Figure 20.
*SAR scaffold-hopping approach from SSRI (**50**) to 1H-pyrrolo[3,2-c] quinoline core (**51**) to lead compound **52**. [83].*
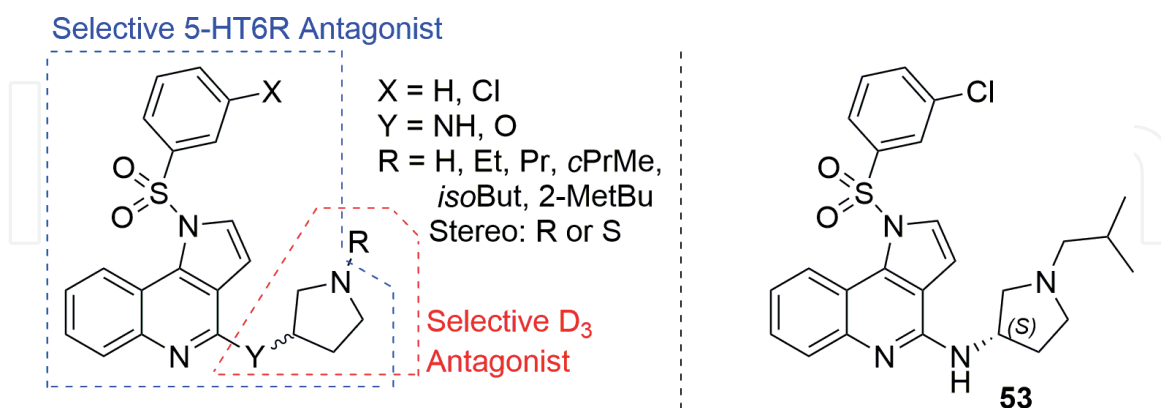
Figure 21.
*Schematic illustration from MTDL SAR combining 5-HT6R antagonist core with selective D3R antagonist fragment, to give lead compound **53**. [96].*

Structure **54** (see **Figure 22**) was identified through a drug discovery strategy based on a virtual screening platform [75, 99]. In total 45 compounds, all possessing the aryl-sulfonamide, was held up against the knowledge of the binding pocket and 5-HT6R ligand pharmacophore, while also adding knowledge from the GPCR ligand database.

For several of the antagonists developed over the years, clinical trials seemed promising until the late studies. One example is the well-known non-sulfonyl
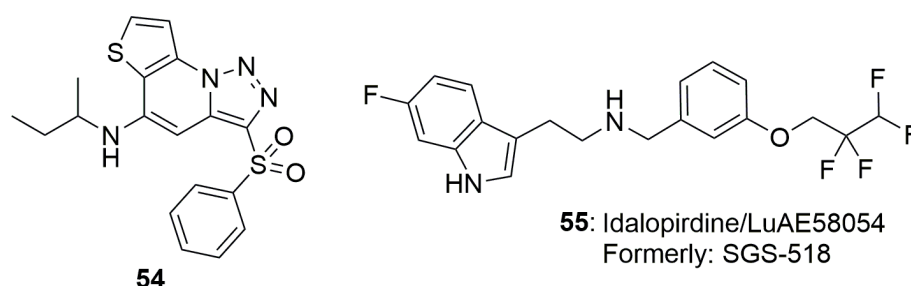
Figure 22.
(left) Compound 54 obtained from a virtual screening platform [75, 99]. (right) Idalopirdine/LuAE58054 (55) [100].

compound Idalopirdine or Lu AE58054 (**55**, **Figure 22**), discovered by Lilly. Idalopirdine was found to have high affinity for the 5-HT6R (>50-fold) compared to more than 70 targets studied [79] and reversed pharmacological-induced cognitive impairment. Lilly licensed the compound to Saegis for clinical development. Phase I was started in 2005 by Saegis and phase II in 2009 by Lundbeck (Lundbeck acquired Saegis in 2006) [100]. In phase II, Idalopirdine (**55**) was given to AD patients already receiving donepezil (**21**, an AChE inhibitor). It was found that Idalopirdine (**55**) provided an inhibitory effect on CYP206, which is involved in the metabolism of donepezil (**21**), therefore, it cannot be ruled out that the initial positive results originated from an increase in donepezil bioavailability [85]. Three Phase III studies with idalopirdine were initiated in 2013 involving patients with mild to moderate AD. Patients were treated with idalopirdine in combination with either donepezil (two of the studies; NCT02006641 and NCT01955161) or an unspecified AChE inhibitor (the third study; NCT02006654). Idalopirdine seemed to be highly tolerated with very few side effects. However, all three studies did not meet the necessary efficacy and Idalopirdine was therefore removed from the pipeline in 2017. (for more detail on the clinical trials the reader is referred to [101, 102]).

4.3 Neutral antagonists and inverse agonists for the 5-HT6R

An important feature of the 5-HT6R is its ability to exist in different conformational states depending on the ligand bound, which can lead to initiation of different signal transduction pathways. The engagement of the 5-HT6R in several pathways has now been demonstrated. In addition to the canonical Gs adenylyl cyclase signaling pathway implicated in the control of neuronal migration [103], the 5-HT6R is also involved in pathways engaged in brain development of synaptic plasticity, more specifically the rapamycin [104] and cyclin-dependent kinase 5 (Cdk5) signaling pathways [105]. Another relevant feature is the high level of constitutive activity of the 5-HT6R. The 5-HT6R has different pathways that can be activated upon different antagonistic and agonistic approaches, and the above-mentioned problems during clinical trials, stimulated interest for investigating other mechanistic approaches against the receptor.

It is worth having in mind that Cdk5-dependent neurite growth has been found to involve the 5-HT6Rs [105] and being agonist dependent. An inverse agonist of this signaling system, like SB-258585 (**56**, **Figure 23**) prevents neurite growth, neuronal migration and dendritic spine morphogenesis [96, 105]. For this purpose, both neutral antagonists and inverse agonists have been investigated.

Utilizing a scaffold-hopping approach based on swapping one carbon with a nitrogen atom in the indole ring, Vanda et al. [95] synthesized 33 compounds varying in both the position of the nitrogen, alkyl-substituents on the C2 position (R-group, **Figure 23**) and substituents on the benzyl group and based on biological

testing, they made some general conclusions. Localization of the nitrogen was crucial for 5-HT6R affinity and compounds with the imidazole[4,5-b]pyridine fragment were in general the best binders. Elongation of the benzyl to a phenethyl group decreased affinity. Furthermore, while bulky and aromatic substituents were not tolerated in the C2 position, small alkyl substituents was in general accepted, with the ethyl-group being the most favored. Furthermore, substituents in the benzyl 3-position were generally preferred, while substituents in the 2- and 4-position lowered affinity compared to the non-substituted analogue. The studies resulted in identification of compound **57** as a new and potent 5-HT6R partial inverse agonist at the Gs signaling pathway, while being a neutral antagonist in the Cdk5 pathway.

4.4 Agonists for the 5-HT6R

Interestingly, it has been suggested that not only 5-HT6R antagonists but also 5-HT6R agonists may have pro-cognitive activities [107]. The 5-HT6R agonist WAY-181187 (**58**) was shown to enhance GABA concentrations, which may potentially
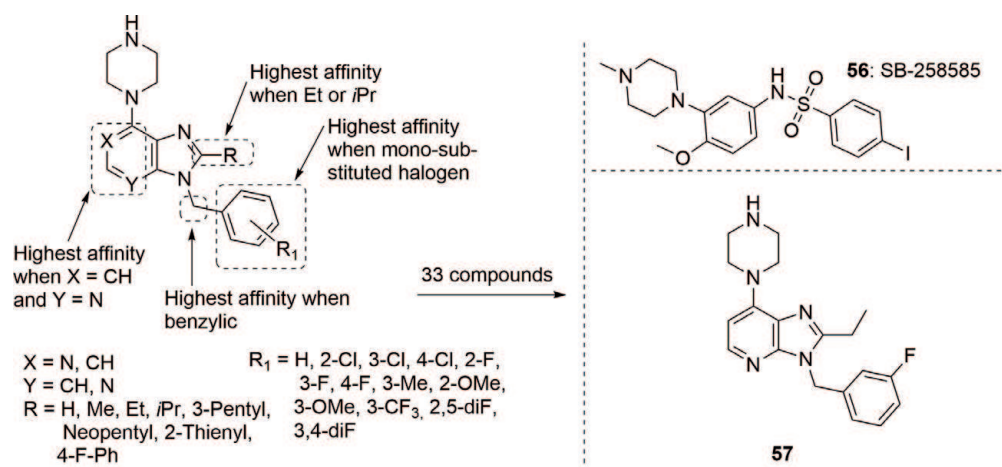
Figure 23.
*Illustration of conducted SAR study adapted from [106] and the obtained lead compound (**57**), along with compound **56**.*
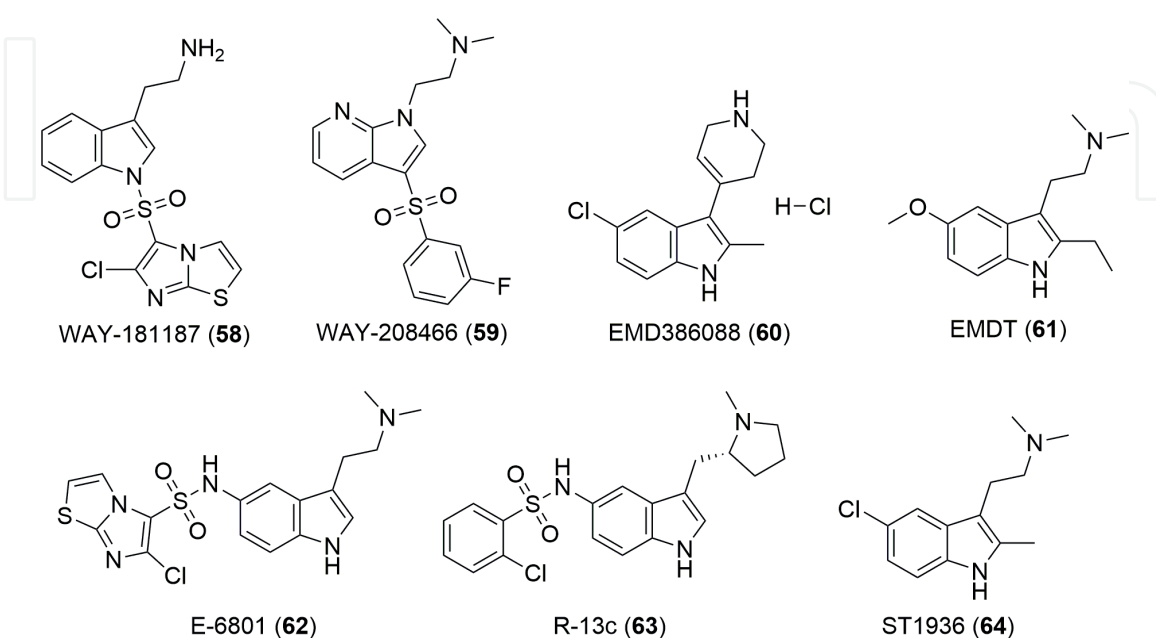
Figure 24.
*5-HT6R agonists; WAY-181187 (**58**) [108, 109], WAY-208466 (**59**) [109], EMDT (**60**) [110], EMD386088 (**61**) [111], E-6801 (**62**) [112], R-13c (**63**) [113], ST1936 (**64**) [114].*

have a positive effect on the neuronal plasticity [76], likewise enhancing cholinergic and glutamatergic mechanisms [80], indicating that both activation and inhibition of this receptor evoke similar responses. Although, the mechanism behind these paradoxical similar effects of 5-HT6R agonists and antagonists is not fully understood, it has been suggested that they could be acting on receptors located on distinct neuronal populations. Further discussing of these paradoxical effects can be found in [80], where also other possible explanations are presented.

Several 5-HT6R agonists have been identified, some of them are summarized in **Figure 24** [80].

The effectiveness of the antagonist vs. the agonist approach is further discussed in reviews by Meneses et al. [115] and Fone [80].

5. Conclusion

Alzheimer's disease is increasingly being recognized as one of the most challenging medical and social challenging health concerns in older people. To date, only treatments offering symptomatic relief to patients exist for this disease, limiting benefit to patients. As there is no curable medical treatment available, much effort has been focusing on identifying novel potential targets for drug development. The rich involvement of serotonin (5-HT) in both cognition and memory; some of the most symptomatic areas being affected in AD, has directed current drug discovery programs to focus on this system as a major therapeutic drug target.

Thus, serotonin receptor modulators offer an attractive option for a future treatment of AD patients and modulation of 5-HT4R has indeed demonstrated to improve neurotransmission and enhance the release of acetylcholine resulting in the memory formation. Furthermore, in various cell based and animal models, partial 5-HT4R agonists were demonstrated to promote the release of sAPPα and block the release of amyloid beta peptide. Remarkably, 5-HT4R agonists were also reported to induce neurogenesis in hippocampus as well as enteric system through the activation of cyclic AMP response element binding protein in rodents.

During the past 20 years, also the 5-HT6R has received increasing attention and is now a promising target for improving cognition. However, 5-HT6R functionality is much more complex. Several studies with structurally different compounds have shown that not only antagonists but also 5-HT6R agonists improve learning and memory in animal models. This paradoxical effect may explain why several compounds that reached phase III clinical trials failed to replicate the positive impact on cognition [76, 94]. Therefore, even though preclinical and clinical trials show that the 5-HT6R is a promising target for treatment of neurodegenerative diseases such as AD, there is an urgent need for a better understanding of the pathways involved in modulation of the receptor. However, there is hope that with the recent advances in molecular biological techniques, including improved cloning and sequencing methods, strategies for the development of in silico GPCR models, will advance our understanding of the molecular mechanisms underlying the impact of serotoninergic signaling in AD to provide beneficial treatments for AD.

Taken together, 5-HT4R and 5-HT6R modulators address all major facets of AD. However, although important progress has been made with developing relevant modulators to improve both cognition and memory, crucial challenges still need to be overcome before a promising cure to AD has been found. Most importantly, an in depth understanding of the pathways involved in modulation of the serotonin receptors is urgently needed. Also, to limit side-effects the identification of CNS specific molecules is crucial.

Abbreviations

AChE	acetylcholinesterase
AD	Alzheimer's disease
ADME	absorption, distribution, metabolism, and excretion
alpha1A	adrenergic
APP	amyloid protein precursor
AR	aromatic area
BBB	blood–brain barrier
BuChE	butyrylcholinesterase
cAMP	cyclic adenosinde monophosphate
Cdk5	cyclin dependent kinase 5
ClogP	calculated logarithm of octanol/water partition coefficient
CNS	central nervous system
D2	dopamine
D3R	dopamine subtype 3 receptor
GABA	gamma-aminobytyric acid
GPCR	G protein-coupled receptor
H1	histamine
HBA	hydrogen bond acceptor
HYD	hydrophobic site
M1	muscarinic acetylcholine
MAO-B	monoamine oxidase B
MTDL	multitarget-directed ligands
PI	positively ionizable
ROS	reactive oxygen species
sAPPα	soluble non-amyloidogenic form
SAR	structure–activity relationship
SERT	serotonin transporter
SSRI	selective serotonin reuptake inhibitors
5-HT	5-hydroxytryptamine
5-HT4R	5-hydroxytryptamine receptor 4
5-HT6R	5-hydroxytryptamine receptor 6
5-HTR	5-hydroxytryptamine receptor

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