REVIEW

Procognitive Properties of Drugs with Single and Multitargeting H₃ Receptor Antagonist Activities

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SUMMARY

The histamine H₃ receptor (H₃R) is an important modulator of numerous central control mechanisms. Novel lead optimizations for H₃R antagonists/inverse agonists involved studies of structure–activity relationships, cross-affinities, and pharmacokinetic properties of promising ligands. Blockade of inhibitory histamine H₃ autoreceptors reinforces histaminergic transmission, while antagonism of H₃ heteroreceptors accelerates the corticolimbic liberation of acetylcholine, norepinephrine, glutamate, dopamine, serotonin and gamma-aminobutyric acid (GABA). The H₃R positioned at numerous neurotransmission crossroads indicates therapeutic applications of small-molecule H₃R modulators in a number of psychiatric and neurodegenerative diseases with various clinical candidates available. Dual target drugs displaying H₃R antagonism/inverse agonism with inhibition of acetylcholine esterase (AChE), histamine N-methyltransferase (HMT), or serotonin transporter (SERT) are novel class of procognitive agents. Main chemical diversities, pharmacophores, and pharmacological profiles of procognitive agents acting as H₃R antagonists/inverse agonists and dual H₃R antagonists/inverse agonists with inhibiting activity on AChE, HMT, or SERT are highlighted here.

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Introduction

Histamine, 2-(1*H*-imidazol-4-yl)ethanamine, is a biogenic amine implicated in various physiological and pathophysiological processes. It is a chemical mediator in immediate allergic response, inflammatory and immunomodulatory processes, and gastric acid secretion and also acts as a neurotransmitter in the central nervous system (CNS) [1]. Histamine is synthesized from L-histidine by cytoplasmatic L-histidine decarboxylase enzyme (HDC, E.C. 4.1.1.22) in the histaminergic neurons, stored in the vesicles and then released from the axon terminals [1,2]. In the brain, histamine is mainly inactivated by neuronal histamine N-methyltransferase enzyme (HMT, E.C. 2.1.1.8) [2,3] in the nearest glia cells [2] or by diamine oxidase (DAO, EC 1.4.3.6) into imidazole acetic acid (IAA), which acts as γ -aminobutyric acid (GABA)_A receptor agonist [4]. Histamine mediates its main biological activities by interaction with four distinct histamine receptor subtypes (H₁R–H₄R). All histamine receptor subtypes belong to the class A family of G protein-coupled receptors (GPCRs) [1,3]. Although H₁R and H₂R antagonists are crucial drugs in therapy of allergy and ulcer, respectively, the H₃R and H₄R antagonists are in clinical or discovery phase. The recently discovered H₄ receptors are involved in immunomodulatory and inflammatory processes [4–7], while H₃ receptors play a central role in neurotransmission of histamine and control release of many other neurotransmitters

[8,9]. The H₃R is pharmacologically discovered in 1983 as histamine presynaptic autoreceptor [3]. The H₃ autoreceptors are involved in a negative feedback modulation of histamine synthesis and inhibition of its release from histaminergic neurons [3]. The histaminergic neurons are mainly located in the posterior hypothalamus and extensively projected into all major areas of the CNS of humans and rodents (Figure 1) [7–9]. The histamine H₃ receptor is not only expressed on histaminergic neurons, but also on neighboring aminergic neurons, as a heteroreceptor. (In) Activation of H₃ heteroreceptor modulates the release of acetylcholine in cerebral cortex, hippocampus, nucleus accumbens, nucleus basalis magnocellularis, and striatum [10–13]; noradrenaline in cerebral cortex, hippocampus, hypothalamus, locus coeruleus, and para-ventricular nucleus [14,15]; dopamine in striatum [16–20]; GABA in cerebral cortex, striatum, substantia nigra, and ventral tegmental area [21–23]; and serotonin in cerebral cortex, hypothalamus, substantia nigra (part reticulate), and striatum [24–26]. While this extensive list is not complete, it clearly demonstrates the wide range of H₃R-mediated neuromodulation. Also, the H₃ receptor is indirectly involved in regulation of other neurotransmitter systems by modulating levels of histamine, which then activates postsynaptic receptors (Figure 1). Studies on the frontocortical histaminergic pathways proved that blockade of negative feedback mechanism of presynaptic H₃ autoreceptors augments histaminergic transmission, while antagonism of H₃

heteroreceptors facilitates the corticolimbic liberation of acetylcholine, noradrenaline, dopamine, glutamate, GABA, and serotonin [27–30]. Recent pharmacological and (pre)clinical studies confirmed that the H₃R antagonists/inverse agonists are effective in the treatment of a sleep disorders (narcolepsy), cognitive impairment, pain/itch, stroke, depression, schizophrenia, Alzheimer's disease (AD), attention deficit hyperactivity disorder (ADHD), dementia, and neurodegenerative disorders [31–41].

Molecular aspects and signaling of the H₃R [42–45], structure–activity relationships of H₃R antagonists/inverse agonists [35,46–54], and H₃R antagonists/inverse agonists pharmacology have recently been discussed in several comprehensive reviews [4,32,34,36,52,55–58].

The location of H₃R at the neurotransmission crossroads indicated potential therapeutic applications of ligands that are better refined to therapeutic needs using a less selective pharmacological profile by multiple targeting. In this respect, dual H₃R antagonism and inhibitory potency on acetylcholine esterase (AChE), catabolic HMT, or serotonin transporter (SERT) have been described as novel classes of procognitive agents for treatment of wide range of central disorders. In this manuscript, pharmacophores of H₃R antagonists/inverse agonists and H₃R antagonists/inverse agonists with dual HMT or SERT inhibiting activity are reviewed. The

influence of structural variations in peripheral scaffolds on potency and the pharmacokinetic profile of drug candidates are discussed.

Histamin H₃R Antagonists/Inverse Agonists

Early synthetic H₃R ligands were imidazole derivatives, as analogs of histamine [59,60], showing in numerous cases some affinity at other aminergic receptors, species selectivity, low oral bioavailability, or rapid metabolism. Similarly, first-generation H₃R antagonists, thioperamide (**1**), clobenpropite (**2**), ciproxifan (**3**), and FUB181 (**4**) (Figure 2), were derivatives of 4-monosubstituted-imidazole [47,59,61–64]. The imidazole-containing H₃R ligands (**1–4**, Figure 2) have displayed low H₃R selectivity and intensive hepatic metabolism [47,65].

The vast majority of H₃R antagonists were pharmacologically classified as inverse agonists because of their negative effects on the intrinsic activity of the H₃R with mostly high levels of constitutive activity [45,66,67]. Nearly all H₃R antagonists/inverse agonists have structurally similar basic moiety with an aliphatic tertiary amine substituted by a linking alkyl chain [68]. This basic skeletal moiety is crucial for ionic interactions between protonated

Figure 1 Physiological role of H₃R in central nervous system (CNS): HA, histamine; ACh, acetylcholine; NT, neurotransmitters (e.g., noradrenaline, serotonin, dopamine, GABA); TMN, tuberomammillary nucleus; HMT, histamine N-methyltransferase; DAO, diamine oxidase; NMH, N^m-methyl-histamine; IAA, imidazole acetic acid; IP₃, inositol trisphosphate; DAG, diacylglycerol; cAMP, cyclic adenosine triphosphate; ctx, cerebral cortex; hip, hippocampus; hyp, hypothalamus; na, nucleus accumbens; str, striatum; gp, globus pallidus; amg, amygdala.

Figure 2 Early synthetic hH₃R antagonists/inverse agonists (**1–4**). Basic scaffold of the hH₃R antagonists/inverse agonists pharmacophore is marked in blue color.

ammonium functionality of the H₃R antagonists/inverse agonists and Asp114 in transmembrane domain III of the H₃R [69]. Due to the risks of low selectivity, rapid metabolism, and cytochrome P₄₅₀ interactions of imidazole-containing H₃R ligands (**1–4**, Figure 2), this moiety was biosterically replaced with pyrrolidine (**5–8**, Figure 3, **20, 21** Figure 4), piperidine (**9, 10**, Figure 3, **18–20**, Figure 4), azepane (**11, 22** Figure 4), piperazine (**12, 13**, Figure 4), diazepane (**14, 15**, Figure 4), tetrahydrobenzo[d]azepine, and related systems (**16, 17**, Figure 4) that have been investigated. The imidazole exchange resulted in a significant increase in affinities at human H₃R (hH₃R) and with optimized substitution pattern in improvement of pharmacokinetic properties in various lead compounds (**5–22**, Figures 3 and 4) [53,54,70,71]. Recently developed hH₃R antagonists/inverse agonists have demonstrated efficacy in diverse preclinical models of cognitive deficits, suggesting the potential value of the ligands as cognition enhancers in many CNS diseases [72].

The highly potent and selective benzofuran/methylpyrrolidine hH₃R antagonist ABT-239 (**5**, Figure 3) was in preclinical development for the potential treatment of cognitive disorders and schizophrenia [32,52] but was not advanced for clinical evaluation due to hERG channel affinity and QT prolongation in monkey [55]. ABT-239 displayed an acceptable rat pharmacokinetic profile (53% oral BA, *t*_{1/2} = 5.3 h) and CNS penetration (B/P ratio

>20). The naphthalene/methylpyrrolidine derivative Abbott (**6**, Figure 3) also showed strong hH₃R antagonistic activity, good rat pharmacokinetic properties (55% oral BA, *t*_{1/2} = 5.5 h), but significantly higher CNS penetration (B/P ratio = 72) than the methylpyrrolidine/benzofuran analog ABT-239 (**5**, Figure 3) [73]. Furthermore, phenoxypropyldimethylpyrrolidine derivatives, such as iradabisant (**7**, Figure 3), demonstrated potent hH₃R antagonistic activity, acceptable rat pharmacokinetic profile (83% oral BA) and CNS penetration (B/P ratio = 2.3), and favorable behavioral effects in studies using several preclinical rodent models of centrally occurring disorders such as schizophrenia, AD, and ADHD [74]. A recent preclinical study on ABT-239 showed that H₃R antagonism may deliver symptomatic treatment in AD and also possesses disease-modifying efficacy [75]. Very recently developed 5-{4-[3-(R)-2-methylpyrrolidin-1-yl]propoxy}phenyl-2-pyridin-2-yl-2H-pyridazin-3-one showed high affinity for hH₃R (pKi(hH₃R): 8.8) with very high selectivity over the other histamine receptor subtypes (Table 1). The compound displayed excellent drug-like properties, such as satisfactory pharmacokinetic properties (oral BA = 78% rat, 92% dog, 96% monkey, *t*_{1/2} = 4.5 h rat, 0.8 h dog, 6.5 h monkey), low binding to human plasma proteins, weakly inhibited cytochrome P450 isoforms, and acceptable brain exposure (B/P ratio = 1.1 rat). The ligand showed potent H₃R antagonistic activity and enhancement of cognitive

Figure 3 The hH₃R antagonists/inverse agonists for cognition with pyrrolidine/piperidine ring as a basic moiety (**4–10**). Basic scaffold of the hH₃R antagonists/inverse agonists pharmacophore is marked in blue color.

Figure 4 The hH₃R antagonists/inverse agonists for cognition with azepane/piperazine/diazepane as a basic moiety (**11–22**). Basic scaffold of the hH₃R antagonists/inverse agonists pharmacophore is marked in blue color.

function in rat models, but further development was discontinued because of detected genotoxicity [76].

Other benzene/pyrrolidine derivatives such as PF-03654746 (**8**, Figure 3), showed good hH₃R antagonistic activity, modest oral bioavailability (26%), acceptable CNS penetration (B/P ratio = 2.1), and procognitive performance in rodents [77]. Compound **8** has successfully completed human safety and efficacy studies in adults with ADHD [54,77]. However, PF-03654746 has failed to show efficacy superior to standard of care in clinical studies of excessive daytime sleepiness associated with narcolepsy

(EDS), cognitive impairment in schizophrenia (CIAS), AD, ADHD, and allergic rhinitis (AR) [http://www.ncats.nih.gov/files/PF-03654746.pdf], and clinical development was halted.

Exchange of imidazole ring in FUB 181 (**4**, Figure 2) resulted in formation of the piperidine analog pitolisant (**9**, formerly known as BF2.649 and tiprolisant, Figure 3), which showed strong hH₃R antagonistic potency and good rat pharmacokinetic properties (84% oral BA, *t*_{1/2} = 2 h) [78]. Pitolisant, which has been shown to have some precognitive effects, is currently in late stage of clinical development on therapy in Parkinson's disease and narcolepsy

Table 1 Pharmacodynamic and pharmacokinetic properties for hH₃R antagonists with procognitive effects

Compound	Name	pK _i (hH ₃ R)	Oral BA [%]	C(Brain)/ C(Plasma) ratio	t _{1/2} [h]	Therapeutic application	Clinical development
5	ABT-239	9.4	53	>20	5.3	Cognitive disorders, schizophrenia	
6	Abbott	9.6	55	72	5.5	Cognitive disorders	
7	Iradabisant	9.3	83	2.6		Cognitive disorders	Phase I
8	PF-03654746	8.6	26	2.1		Cognitive disorders, allergy	Terminated in Phase II
9	Pitolisant	7.1	84		2.0	Cognitive disorders, narcolepsy	Phase III
10	JNJ-5207852	9.2	85–107		>13	Cognitive disorders, narcolepsy	
15	GSK-334429	9.5	91		2.1	Cognitive disorders, neuropathic pain	
16	GSK-189254	9.9	83		1.6	Cognitive disorders, narcolepsy, neuropathic pain	Terminated in Phase II
21	ABT-288	8.7	37	1.5	1.3	Cognitive deficit in AD and schizophrenia	Failed to show efficacy in Phase II AD trials
22	GSK-239512	10.0	51	1.8	1.2	Cognitive deficit in AD	Tolerated in Phase II with positive effects on attention/memory in AD
	AZD-5213	9.3				Cognitive deficit in AD	Phase II for Tourette's syndrome and neuropathic pain

Values are obtained for rat species. AD, Alzheimer's disease.

as one of most promising H₃R inverse agonist [54,71,79]. Recently obtained results in an early Phase II study of the human photosensitivity model of epilepsy suggest that pitolisant might be helpful in chronic seizure treatment of both partial and generalized epilepsies [80].

Other phenoxypropylpiperidine preclinical candidate JNJ-5207852 (**10**, Figure 3) displayed high and selective hH₃R antagonism, high oral bioavailability (>85 %), but rather long half-life time (t_{1/2} > 13 h) and very high risk for phospholipidosis development during long-term therapy [35,81]. The observed risk of phospholipidosis was assumably attributed to its dibasic and cationic amphiphilic nature [55,81]. Compound **10** showed *in vivo* efficacy in rodent arousal models and thus represents a new pharmacological tool for the treatment of conditions associated with EDS [82]. Phenoxypropylazepane derivative (**11**, Figure 4) displayed potent and selective antagonistic/inverse agonistic activity on hH₃R [54,74], while phenoxypropylpiperazine analog (**12**, Figure 4) showed modest hH₃R antagonism, high selectivity over H₁ and H₂ receptors, good oral bioavailability, and procognitive effects in rodent models [83]. In addition, phenoxypropylpiperazine derivative (**13**, S38761-1, Figure 4) displayed potent and selective antagonistic/inverse agonistic activity on hH₃R with possible therapeutic application as procognitive agent [54,84]. Also, phenoxypropyldiazepane analog (**14**, A-320436, Figure 4) showed good binding to hH₃R, but poor brain penetration and lack of procognitive activity *in vivo* [85]. An improved pharmacokinetic profile (91% oral BA, t_{1/2}: 2.1 h) was achieved with the novel diazepine derivative (**15**, GSK-334429, Figure 4) that demonstrated positive effects in rat models for cognitive disorders, neuropathic pain, and memory impairment [86]. Use of the tetrahydrobenzo[d]azepine system as basic moiety of the hH₃R antagonist/inverse agonist resulted in development of the clinical candidate for cognitive disorders [**16**, GSK-189254, Figure 4]. GSK-189254 showed potent and selective hH₃R antagonistic activity, good pharmacokinetic profile (83% oral BA, t_{1/2}: 1.6 h),

CNS penetration, and favorable procognitive activity in preclinical models of cognitive impairment, neuropathic pain, and narcolepsy [49,87]. Compound **16** displayed promising preclinical results in cognition models [88], but the development of this compound was terminated in Phase II [87,89]. The tetrahydrobenzo[d]azepine and phenoxypropylpiperidine moieties have been combined by GlaxoSmithKline in dibasic antagonist (**17**, Figure 4), which provide potential flip-flop binding modes in hH₃R [90]. The cyclobutylpiperidinyloxy side chain was used as a moiety in a series represented by potent and selective hH₃R inverse agonists (**18** and **19**, Figure 4) [91,92]. A process patent has been applied for compound **18** [93], whereas **19** was optimized to overcome adrenergic activity and hERG liability of lead compounds [92]. Dibasic biphenyl series of hH₃R antagonists, characterized by a biphenyl core and two basic centers, is exemplified by very potent hH₃R antagonist (**20**, Figure 4) [90]. The high binding affinity shown by these biaryl compounds supporting the hypothesis that the two regions described within the hH₃ receptor binding site can be simultaneously occupied by an dibasic biphenyl hH₃R ligand. A related multimodal binding mode is possible for several other dibasic hH₃R antagonists, such as **10** and **17**.

The human H₃R antagonists AZD-5213 (structure undisclosed) [94] and SAR-110894 (structure undisclosed) [95] are in Phase I clinical studies for AD (NCT-ID: NCT01548287 and NCT01266525, respectively) with AZD-5213 advancing to Phase II for Tourette's syndrome (<http://clinicaltrials.gov/ct2/show/NCT01904773>) and neuropathic pain (<http://clinicaltrials.gov/ct2/show/NCT01928381>). AZD-5213 displayed efficacy in rodent behavioral models of cognition and in humans was safe and well tolerated following repeated doses with a short half-life [94].

The hH₃R antagonists ABT-288 (**21**, Figure 4) and GSK-239512 (**22**, Figure 4) are in Phase II trials for AD (NCT-ID: NCT01018875 and NCT01009255, respectively). Human H₃R antagonist ABT-288 (pK_i(hH₃R) = 8.7)) enhances the release of acetylcholine and dopamine in rat prefrontal cortex and shows efficacy in several

animal cognition models. ABT-288 demonstrated good pharmacokinetics (37% oral BA, $t_{1/2}$: 1.3 h) and CNS penetration (B/P ratio = 1.5) in rat models, a safe preclinical profile in rodent and dog studies and has advanced to clinical studies for the treatment of cognitive deficits in AD and schizophrenia [89,96]. However, a recent report indicated that while safe and well tolerated in humans, ABT-288 failed to show procognitive effects in AD and schizophrenic patients [97,98]. The GSK-239512 (pK_i (hH₃R) = 10.0) displayed acceptable pharmacokinetics (51% oral BA, $t_{1/2}$: 1.2 h) and CNS penetration (B/P ratio = 1.8) in rats [99] and in patients with AD, exhibited evidence for positive effects on attention and memory [100]. Furthermore, for hH₃R inverse agonist, MK-0249 (structure undisclosed) [101] has been completed Phase II study for AD (NCT-ID NCT00420420).

Taking into account the beneficial cognitive effects reported for numerous H₃R antagonists/inverse agonists, an additive or maybe synergistic effect can be speculated with the combination of additional procognitive pharmacological properties in one molecule.

Dual Properties of H₃R Antagonists with Procognitive Effect

Modern medicinal chemistry has applied multitarget directed ligands (MTDLs) design to find multipotent compounds effective in treatment of CNS disorders such as AD, PD, ADHD, schizophrenia, and depression [102,103]. In the last few years, multiple-targeted compounds have been designed using natural products as lead resources that have shown multiple pharmacological activities in CNS diseases [104]. Novel dual-activity hH₃R antagonists/inverse agonists concomitantly interact with at least one further

target, such as AChE, HMT, SERT, dopamine D₂-like receptors (D₂ and D₃), (5-HT₃), and monoamine oxidase [102,103,105]. Dual-activity hH₃R antagonists with additional acetylcholinesterase inhibitory activity have been identified that lead to cognitive synergy with hH₃R antagonism, as both pharmacological actions are able to enhance cholinergic neurotransmission. Based on the structure of acetylcholinesterase inhibitor tacrine, ligands with dual hH₃R antagonistic/inverse agonistic and AChE inhibitory activity (**23**, FUB-833, Figure 5) [106] were designed. Indeed, this compound class showed additional inhibition of HMT and butyrylcholinesterase (BuChE), simultaneously targeting four different procognitive pathways [106]. Based on the dual-acting tetrahydroaminoacridine hybrids [106], 1,1'-octa-, nona-, and decamethylene-bis-piperidine derivatives and novel tetrahydroaminoacridine-piperidine hybrids were developed that displayed potent and selective hH₃R antagonism with remarkable anticholinesterase activity [107].

The hybrid compounds, such as ligand with (sub)nanomolar activities on the both hH₃R and HMT targets (**24**, Figure 5), could increase intersynaptic histamine levels in the CNS and lead to beneficial procognitive effects in psychiatric and neurodegenerative diseases [33,106,108–111]. Multitarget ligands displaying dual hH₃R antagonistic/inverse agonistic and HMT-inhibiting properties are able to greatly enhance histaminergic neurotransmission via hH₃ autoreceptors blockade and reduced catabolic rate for histamine inactivation via HMT inhibition [106,110,111]. Also, group of dibasic dual hH₃R antagonists/inverse agonists-AChE inhibitors (**25**, **26**, Figure 5) have been proposed for therapy of AD [112,113]. Merging the piperidinylpropoxy phenyl hH₃R pharmacophore into heterocyclic moiety of AChE inhibitors yielded reversible and competitive AChE inhibitors with balanced

Figure 5 Dual-activity hH₃R antagonists/inverse agonists with procognitive effect (**23**–**28**). Basic scaffold of the hH₃R antagonists/inverse agonists pharmacophore is marked in blue color.

nanomolar affinities at both targets (**27**, Figure 5) and high selectivity over the other histamine receptor subtypes [114]. Based on the hH₃R antagonists/inverse agonists pharmacophore, the AChE inhibitor BYYT-25 that possesses β -secretase (BACE 1) inhibitor activity, a set of quinoxaline-based compounds acting on hH₃R/AChE/BACE 1 were designed as novel MTDLs for treatment of AD. The most effective compound (**28**, Figure 5) displayed high selectivity over the other histamine receptor subtypes [115].

Results obtained from our previous theoretical studies, pharmacophore modeling, design [116–131], synthesis, and biological evaluation of novel compounds [33,106,108–111] should be further used in discovery of novel dual-activity hH₃R antagonists/inverse agonists with procognitive effect.

Many clinical studies report that patients with depression often also suffer cognitive impairment [132]. Selective serotonin reuptake inhibitors (SSRIs) are the most frequently prescribed antidepressant drugs, but they do not improve cognitive function of depressive patients [133,134]. Therefore, drugs displaying dual

hH₃R antagonistic/inverse agonistic and serotonin reuptake inhibiting properties may represent novel multitarget agents for the improved treatment of depressive states [135].

The combination of potent and selective hH₃R antagonists/inverse agonists 1-[4-(3-piperidin-1-ylpropoxy)benzyl]piperidine with tetrahydroisoquinoline-derived SERT inhibitors gave potent dual hH₃R antagonists/inverse agonists SERT inhibitors. Structural modification of the hH₃R pharmacophore in the pendant amine portion of the molecule did not show significant influence on the SERT affinity of the compounds while binding affinity at hH₃R varied significantly. Potent compounds were obtained by introducing morpholine and substituted piperidines (**29**, **30**, Figure 6). Compounds **29** and **30** were also tested for functional activity at the hH₃R and were found to be potent antagonists ($pA_2 = 8.6$ and 9.6 , respectively). Further examination in the 5-hydroxy-tryptophan (5-HTP)-induced head twitch model in mice and in blood-brain barrier (BBB) experiments demonstrated a good correlation of behavioral pharmacology of

Figure 6 Ligands with dual hH₃ antagonistic/inverse agonistic and serotonin transporter (SERT) inhibiting properties for cognition (**29–40**). Basic scaffold of the hH₃R antagonists/inverse agonists pharmacophore is marked in blue color.

29 and **30** compounds with their brain concentration [136]. The compounds with the optimized hH₃R pharmacophore (**29**, **30**, Figure 6) were used for further optimization of the SERT pharmacophore by changing at the 4-aryl position. The modification in the aryl ring of the compound **30**, such as replacement of the 4-methoxy group or monosubstitution at 2 or 3 position, had little effect on the affinity for the hH₃R. Also, for several compounds, characterization of enantiomerically pure products was performed. Compounds with 4-thiomethyl substituent (**31**, **32** (JNJ-28583867), Figure 6) or 4-methoxy substituent (**33**, Figure 6) were selected as ligands with high SERT affinity. Screening the **31**, **32**, and **33** for affinity at 50 receptors, ion channels and transporters showed that **31** and **33** have moderate selectivity for the SERT over human norepinephrine transporter (NET) and the dopamine transporter (DAT), while **32** displayed 30-fold selectivity for the SERT over the NET and DAT. The highest bioavailability in rat and high exposure in the brain after oral administration have been shown for **31** and **32**. Following subcutaneous administration, compounds **32** and **33** showed 100% receptor occupancy in the rat brain at both SERT and hH₃R [137]. An *in vivo* functional activity of **32** at the hH₃R was confirmed using the blockade of imetit-induced drinking model. In the mouse tail suspension tests, this compound exhibited antidepressant-like effect. The **32** was very effective at suppressing REM sleep from the dose of 1 mg/kg onward [138].

Attachment of a piperidinylpropoxy side chain to the pyrrolidino-tetrahydroisoquinoline scaffold also gave potent dual hH₃R antagonists/inverse agonists SERT inhibitors (**34**, Figure 6). Substitutions in the 3 or 4 position of aryl ring provided compounds with higher affinity for SERT than their 2-substituted counterparts. Both electron-donating and electron-withdrawing groups were tolerated with exceptions including two compounds (**35**, **36**, Figure 6) of special interest, as these moieties are found in selective SSRIs citalopram (4-CN) and fluoxetine (4-CF₃). The most potent pyrrolidino-tetrahydroisoquinolines dual hH₃R antagonists/inverse agonists-SERT inhibitor **34** exhibited moderate bioavailability, relatively high brain concentrations and beneficial pharmacological effects in both 5-hydroxytryptophan potentiated (5-HTP) head twitch model and microdialysis experiments [139]. Combination of 4-aryl-2,6-tetrahydronaphthyridine scaffold and various hH₃R pharmacophores resulted in the potent dual hH₃R antagonists/inverse agonists SERT inhibitors. (**37**, Figure 6). In this group, substitution in the 3 or 4 position of aryl moiety was obligatory for SERT activity. The position of nitrogen on the tetrahydronaphthyridine core was also important for hH₃R antagonistic and SERT inhibiting activity. The 4-aryl-2,5-tetrahydronaphthyridines demonstrated significantly lower affinity for both targets indicating that 2,5-tetrahydronaphthyridine core is not favored particularly with respect to SERT affinity [140].

Dibasic hH₃R antagonists with serotonin reuptake inhibition were designed utilizing structure of the fluoxetine, known as SSRI. Starting from the known hH₃R pharmacophore blueprint consisting of two basic functional groups attached to the central lipophilic core that contains an aromatic ring, fluoxetine structure was combined. Type and position of the hH₃R side chain and substitution on the phenoxy ring significantly affected the affinity for SERT. The most potent ligands with highest hH₃R

and SERT affinity (**38**, Figure 6) were obtained by the introduction of the 3-piperidinylpropoxy side chain in the *para* position of the phenyl ring (unsubstituted phenyl ring of fluoxetine). Replacement of this side chain with other hH₃R components led to the derivatives with higher hH₃R affinity but lower SERT inhibiting activity. All examined 3-piperidinylpropoxy derivatives possess very high hH₃R antagonistic activity, while SERT affinity is affected by type and position of substituents in phenyl ring [141]. The 5-ethynyl-2-aryloxybenzylamine-based dual hH₃R antagonists/inverse agonists SERT inhibitors were designed modifying the structure of compound **30** with goal to decrease complexity of ligands and improve their physical properties associated with good absorption and distribution into the brain. When position C-3 of tetrahydroisoquinoline ring of **30** is removed, resulted structure was benzyl amine compounds. Besides, replacement of C-4 position of tetrahydroisoquinoline ring by oxygen led to the ligands without optical activity. Combination of resulting aryloxybenzyl amine structure having SERT affinity with piperidine alkyne-based hH₃R pharmacophore gave compounds with high affinity for both hH₃R and SERT (**39**, Figure 6). Substitution in 4 position of piperidine with fluorine or piperidine replacement by morpholine (**40**, Figure 6) resulted in decrease in affinity for hH₃R and retained high SERT affinity. The **40** have good selectivity over human NET and DAT [142].

For few dibasic dual hH₃R antagonists/inverse agonists SERT inhibitors, **38–40** could be proposed flip-flop binding modes in hH₃R [90].

Conclusion

Based on preclinical data, human H₃R antagonists/inverse agonists display beneficial procognitive and arousal effects for the potential treatment of a broad spectrum of centrally occurring disorders such as AD, PD, ADHD, schizophrenia, and depression. This wide spectrum of potential therapeutic indications of hH₃R antagonists/inverse agonists defined the hH₃R as a highly significant drug target. The pharmacophore of hH₃R antagonists/inverse agonists consists of a basic moiety linked by an alkyl spacer to an aromatic and then polar scaffold. As many hH₃R antagonists/inverse agonists have failed in the first or second phase of clinical research, further lead optimization and pharmacokinetic screening as well as an increased understanding of the role of H₃Rs in human CNS diseases are warranted significant steps in early-stage H₃R antagonist/inverse agonist drug discovery. Drugs displaying dual hH₃R blockade and inhibition of AChE, HMT, or SERT represent a novel class of agents potentially capable of additively or synergistically augmenting the procognitive effects of hH₃R antagonism.

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Conflict of Interest

The authors declare no conflict of interest.

Disclosure

One of the authors (HS) is co-inventor of pitolisant.

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