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Cholinesterase inhibitory activity of chlorophenoxy derivatives—Histamine H_3 receptor ligands

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ABSTRACT

In recent years, multitarget-directed ligands have become an interesting strategy in a search for a new treatment of Alzheimer's disease. Combination of both: a histamine H_3 receptor antagonist/inverse agonist and a cholinesterases inhibitor in one molecule could provide a new therapeutic opportunity. Here, we present biological evaluation of histamine H_3 receptor ligands—chlorophenoxyalkylamine derivatives against cholinesterases: acetyl- and butyrylcholinesterase. The target compounds showed cholinesterase inhibitory activity in a low micromolar range. The most potent in this group was 1-(7-(4-chlorophenoxy) heptyl)homopiperidine (**18**) inhibiting the both enzymes (*Ee*AChE IC_{50} = 1.93 μM and *Eq*BuChE IC_{50} = 1.64 μM). Molecular modeling studies were performed to explain the binding mode of **18** with histamine H_3 receptor as well as with cholinesterases.

Alzheimer's disease (AD) is a progressive neurodegenerative brain disorder and the most common form of dementia. It is estimated, that about 35 million people in the world suffer from AD nowadays.[1] The loss of cognitive functions in AD patients is mainly connected with cholinergic neurotransmission decline in the brain. To reverse this decline and increase acetylcholine (ACh) level in the brain cholinesterase inhibitors are used. Cholinesterases: acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) are enzymes which hydrolyze ACh. Both enzymes are present in the brain, but AChE predominates in the healthy people whereas BuChE activity progressively increases in patients with AD.[2–4]

In a current therapy of AD only 4 drugs are approved: 3 cholinesterase inhibitors (donepezil, galantamine, and rivastigmine), and an NMDA antagonist—memantine. These drugs can only offer a symptomatic treatment, temporarily improving cognitive functions, but not reversing, stopping, or even slowing the neurodegenerative process. Thus, there is an urgent need to find new drugs for AD. However, due to the complex pathology of the disease a selective ligand for a given target might not be sufficient to produce the desired efficacy. One of the new pharmacological approach is to use multitarget-directed ligands (MTDLs).[5–7] This strategy, based on designing of a single molecule that modulates multiple targets simultaneously, has been developed by scientists both from academy and industry. The most interesting MTDLs, which can be beneficial for AD treatment, combine cholinesterase inhibition with additional properties, e.g.: Aβ anti-aggregating activity, BACE1 inhibition, cannabinoid CB_1 receptor antagonism, NMDA receptor antagonism or histamine H_3 receptor antagonism.[5–7]

Histamine H_3 receptors (H_3Rs) are widely expressed in the brain, particularly in the area involved in a cognitive process and arousal. Blockade of these receptors with selective antagonists/inverse agonists can increase the release of neurotransmitters such as acetylcholine, dopamine or serotonin. Effectiveness of H_3R antagonists/inverse agonists in patients with AD have been investigated in clinical studies for several compounds[8–11], e.g., ABT-288 (**1**)[12], GSK-239512 (**2**)[13] or MK-3134 (**3**)[14] (Fig. 1). Early results from these studies have been recently published, showing that ABT-288 (**1**) as a monotherapy[9] did not demonstrate efficacy in the symptomatic treatment of AD, whereas GSK-239512 (**2**) improved episodic memory in patients with mild-to-moderate AD.[7] Clinical trials (single-dose approach) with MK-3134 (**3**) demonstrated a cognitive improvement both in a monotherapy and an adjunctive therapy with donepezil.[11]

Enhanced cholinergic neurotransmission in the brain cortex can be also achieved through the combination of histamine H_3R antagonism and AChE inhibition in a single molecule using the MTDLs approach. Recently, some interesting structures combining molecular elements important for the H_3R interactions (e.g., a piperidinyl-alkoxy part) and AChE inhibition (e.g., a quinoxaline or a pyrroloquinazoline moiety), have been published (Fig. 2).[15,16]

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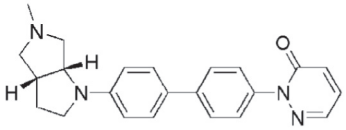
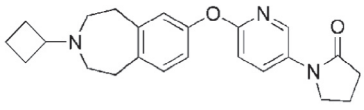


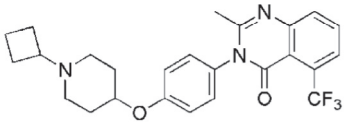
1 **ABT-288**
hH$_3$R K_i = 1.9 nM[12]

Abbott



2 **GSK-239512**
hH$_3$R K_i = 0.12 nM[13]

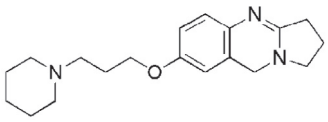
GlaxoSmithKline



3 **MK-3134**
hH$_3$R K_i = 3.6 nM[14]

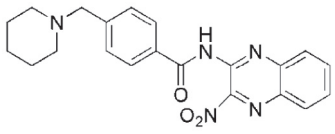
Merck

Figure 1. Structures and human H$_3$R (hH$_3$R) affinities of some clinically tested H$_3$R ligands in AD.[12–14]



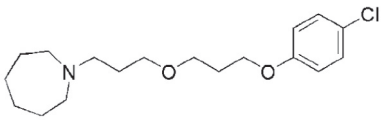
4
hH$_3$R K_i = 76 nM[15]
*Ee*AChE IC$_{50}$ = 67 nM[15]
*Eq*BuChE IC$_{50}$ = 8200 nM[15]

University of Regensburg & University of Wurzburg



5
hH$_3$R IC$_{50}$ = 280 nM[16]
AChE^{RC} IC$_{50}$ = 483 nM[16]

Zhejiang University



6
hH$_3$R K_i = 3.5 nM[18]
*Ee*AChE IC$_{50}$ = 7910 nM[17]
*Eq*BuChE IC$_{50}$ = 4970 nM[17]

Jagiellonian University

Figure 2. Selected structures of MTDLs targeting the human H$_3$R (hH$_3$R) and cholinesterases. (*Ee*AChE—AChE from *Electrophorus electricus*; AChE$^{R.C.}$—rat cortex homogenate as a source of AChE; *Eq*BuChE—BuChE from horse serum).

Previously, we reported the inhibitory activity against AChE and BuChE of diether derivatives of homo- or substituted piperidines.[17] The most interesting compound **6** (Fig. 2) displayed high affinity to hH$_3$R (K_i = 3.5 nM)[18] and a moderate inhibitory activity against both enzymes (AChE IC$_{50}$ = 7.91 μM; BuChE IC$_{50}$ = 4.97 μM).[17] As a continuation of this work we synthesized a new series of 3-chloro- and 4-chlorophenoxy derivatives of homo- or substituted piperidines and at first evaluated their binding properties at the human histamine H$_3$ receptor (hH$_3$R).[19] Later we assessed the compounds for inhibitory activities on acetyl- and butyrylcholinesterase. Herein we present the results of this evaluation against cholinesterases together with molecular modeling studies against both targets.

All compounds were tested against AChE from *Electrophorus electricus* (*Ee*AChE) and BuChE from horse serum (*Eq*BuChE). First they were tested at 10 μM screening concentration and then IC$_{50}$ values were determined for compounds with a percentage of inhibition higher than 50% at 10 μM. Structures of the tested compounds and the results of in vitro screening against cholinesterases and hH$_3$R affinities are presented in Table 1.

The tested compounds displayed an inhibitory activity towards AChE with the IC$_{50}$ values in the micromolar range (*Ee*AChE IC$_{50}$ = 1.93 to 23.61 μM). SAR analysis point out to a length of a linker that connects the phenoxy group with the heterocyclic moiety, as a major factor that influence the activity of these compounds. With an elongation of the linker the potency increased from not active or the least active compounds with the five carbon atoms linker to the most active with the seven carbon atom linker. There was a little, but an observable effect of a heterocyclic moiety on the activity that decreased in the following order: homopiperidine > 3-methylpiperidine > 4-methylpiperidine ⩾ piperidine. The influence of a position of a chlorine substituent at the phenoxy group is not clear but with a small advantage of the position 4.

The tested compounds are inhibitors of BuChE with the IC$_{50}$ values in the micromolar range (*Eq*BuChE IC$_{50}$ = 0.9–5.43 μM). The analysis of SAR led us to the similar conclusions as in the case of