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Cyclic acyl guanidines bearing carbamate moieties allow potent and dirigible cholinesterase inhibition of either acetyl- or butyrylcholinesterase

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ABSTRACT

A series of cyclic acyl guanidine with carbamate moieties have been synthesized and evaluated in vitro for their AChE and BChE inhibitory activities. Structure–activity relationships identified compound **23** as a nanomolar and selective BChE inhibitor, while compound **32** exhibited nanomolar and selective AChE inhibition, selectivity depending on both the structure of the carbamate substituent as well as the position of guanidines-*N* substitution. The velocity of enzyme carbamoylation was analyzed and showed similar behavior to physostigmine. Phenolic compounds formed after carbamate transfer to the active site of cholinesterases showed additional neuroprotective properties on a hippocampal neuronal cell line (HT-22) after glutamate-induced intracellular reactive oxygen species generation.

1. Introduction

Alzheimer's disease (AD) is the most common dementia occurring among elderly people. It is a progressive and neurodegenerative disorder affecting regions of the brain that control cognition, memory, language, speech and awareness.[1,2] The major pathological hallmarks of AD are formation of beta-amyloid aggregates that form senile plaques, neurofibrillary tangles of hyperphosphorylated τ-protein and progressive loss of cholinergic neural transmission.[3,4]

Acetylcholinesterase (AChE) hydrolyzes the neurotransmitter acetylcholine (ACh); the second cholinesterase in the brain of mammals is butyrylcholinesterase (BChE, or pseudo-cholinesterase), which is less substrate specific than AChE. Its expression level is more abundant in the peripheral system. However, both cholinesterases are found in neurons and glial cells as well as in neuritic plaques and tangles in AD patients.[5]

Both AChE and BChE are involved in the breakdown of acetylcholine in the brain. It is demonstrated that AChE has a key role in the acceleration of Aβ-peptide deposition and promoting the formation of Aβ-plaques in Alzheimer's brain.[6] Recent studies suggest that BChE is present in key brain areas and may also influence the aggregation of neuritic β-amyloid (Aβ) plaques.[7,8] For clinical purposes, it is particularly important to consider the fact that while brain AChE activity continuously declines, BChE activity might stay the same or even increase during disease progression.[9–11]

To date, only four drugs have been approved and licensed to treat AD. The NMDA antagonist memantine, and three AChE inhibitors (rivastigmine, donepezil and galanthamine). Although these drugs share the same therapeutic target, they differ in their molecular mode of action and pharmacokinetics and possess different degrees of side effects. While galanthamine and donepezil are reversible ChE inhibitors, rivastigmine (**1**, Fig. 1)—a compound derived from the alkaloid physostigmine (**2**, Fig. 1)—represents an irreversible inhibitor transferring a carbamate moiety to the serine unit in the catalytic active site (CAS) of AChE and BChE. The carbamates' mode of action is termed more exactly pseudoirreversible since the carbamate moiety slowly hydrolyses off the enzyme, yielding the active enzyme again, in contrast to irreversibly inhibiting organophosphates. Rivastigmine is of special interest because in clinical and pharmacological investigations it was shown to be of high effectiveness, which some authors attributed to its ability to inhibit BChE also with high efficiency (Fig. 1).[12,13]

The guanidine group possesses chemical and physicochemical properties relevant for many compounds of medicinal interest and guanidine-containing derivatives constitute an important class of therapeutic agents suitable for the treatment of a wide spectrum of diseases.[14] Recently, acyl guanidine derivatives were applied to

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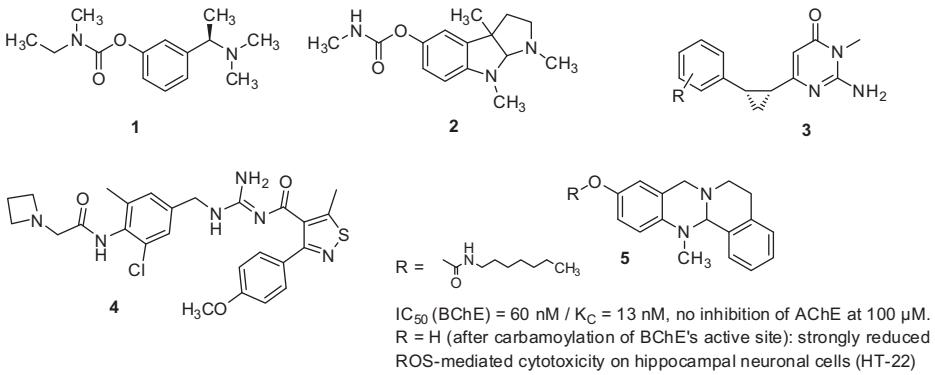

Figure 1. Structures of AD drug rivastigmine **1**, the ChE inhibiting alkaloid physostigmine **2**, acyl guanidine inhibitors of β-secretase (BACE-1) **3** and **4**, and the tetracyclic pseudoirreversible BChE inhibitor lead structure **5**.[15–17]

target AD as β-secretase (BACE-1) inhibitors (compounds **3** and **4**, Fig. 1).[15,16]

We had previously synthesized a series of novel tri- and tetracyclic *N*-bridgehead ChE inhibiting structures based on a quinazolinone moiety.[18] In 2012, we modified one lead structure by introducing a phenolic hydroxyl group in the *para* position to the tertiary anilinic amine, and in the *meta* position to the basic *N*-bridgehead atom (compound **5**, Fig. 1) and used it as a starting point for the development of novel BChE selective inhibitors. The cholinesterase inhibitory activities and their selectivity towards BChE were dramatically improved by introducing a carbamate group at the phenolic hydroxy group (compound **5**, Fig. 1). The enzyme carbamoylation process of this class of inhibitors were studied kinetically, and the hydrolyzed inhibitor released after carbamate transfer to the serine residue at the catalytic active site of the ChEs is a phenolic compound with pronounced neuroprotective properties on a hippocampal neuronal cell line (HT-22), in which reactive oxygen species (ROS) are formed intracellularly after extracellular glutamate challenge (see the neuroprotectivity chapter for more details). The phenolic compounds showed also antioxidant capacities ranging from 1.3 to 1.6 trolox equivalents, meaning that they are more potent than the positive control trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) a water soluble vitamin E derivative.[17] Recently, we identified another neuroprotective and BChE-selective inhibitor derived from the natural quinazolinone alkaloid evodiamine by connecting the carbonyl reduced analog of evodiamine with heptyl carbamate. The heptyl carbamate heterocycle exhibited an IC_{50} at BChE of 77 nM with no inhibition of AChE at 10 μM. The heptyl carbamate and its corresponding phenolic structure showed again pronounced antioxidant capacity and noticeable neuroprotective properties on HT-22 cells.[19]

These properties might become important for future AD therapeutics since it has been proven experimentally that dysregulation of the redox state strongly participates in an early stage of AD by stimulating and activating multiple cell signaling pathways that contribute to the initial progression of the neurodegenerative process.[20] Additionally, it was observed that ROS and reactive nitrogen species (RNS) are mediators of injury in AD patients' brains.[21] Numerous studies showed that levels of oxidative markers of proteins, lipids, carbohydrates, and nucleic acids are increased in AD.[22–24] Furthermore, levels of antioxidant enzymes were found to be altered in brain regions of AD patients.[25] All of the aforementioned findings support the 'oxidative stress hypothesis' of AD at least as one component of neuronal cell damage in AD.[26,27]

Low basicity of the quinazolinone moiety besides its very poor water solubility and low to moderate inhibitory activities made structural modifications of the heterocycle necessary for further development. Modifications of quinazolinones e. g. to mono- and bivalent quinazolinimines and *N*-bridgehead tri- and tetracyclic moieties proved to yield more potent ChE inhibitors.[18,28,29] The quinazolinones' modifications were based on an increase of the basicity of the heterocycle by (a) reduction of the amide carbonyl group to the corresponding quinazoline (Fig. 2),[18] (b) imine formation by Schiff-base reaction of thioquinazolinones with different aliphatic and aromatic amines in presence of heavy metal salts.[29,30]

Quinazolinone modification presented in this work was based on increasing the basicity of the core ring by introducing a nitrogen atom in the fused aliphatic cycle to the core ring of the lead quinazolinone to yield tricyclic acyl guanidine structures. Besides, introduction of suitable carbamate groups to the same phenolic hydroxyl group had been described before to improve inhibitory activities at BChE and to increase in BChE selectivity (Fig. 2).[17]

Here, several sets of cyclic acyl guanidine compounds connected with different carbamates groups were synthesized. SARs were investigated with regards to the guanidine group by introducing different substituents at its two unsubstituted nitrogen atoms. Inhibitory activities of the synthesized pseudoirreversible inhibitors were evaluated at both cholinesterases and the carbamoylation process studied kinetically for the most potent inhibitors.

1.1. Chemistry

6-(Benzyloxy)-1*H*-benzo[*d*][1,3]oxazine-2,4-dione (**6**) was synthesized in four steps with almost quantitative overall yield, starting from 2-amino-5-hydroxybenzoic acid.[31,32] This benzylated isatoic anhydride was used as a key intermediate for synthesis of all target compounds, the benzylated phenolic group helped to avoid the solubility problem of the isatoic heterocycle. 2-(Methylthio)-1,4,5,6-tetrahydropyrimidine was prepared according to a literature procedure starting from 1,4,5,6-tetrahydropyrimidine-2-thiol in quantitative yield (Scheme 1).[33]

The unsubstituted (*N*H)-cyclic acyl guanidine **7** (8-(benzyloxy)-3,4-dihydro-1*H*-pyrimido[2,1-*b*]quinazolin-6(2*H*)-one) was employed as a synthetic precursor for different substituted cyclic guanidine moieties and it was obtained by fusion reaction of the benzylated isatoic anhydride **6** with the with 2-(methylthio)-1,4,5,6-tetrahydropyrimidine in *N*,*N*-dimethylformamide (DMF) at 100 °C (Scheme 1A).

Alkylation of the *N*H group of the resulting cyclic acyl guanidine with iodomethane, benzylbromide, or 1-(3-bromopropyl)-piperidine, respectively, using sodium hydride in tetrahydrofurane (THF) gave the corresponding alkylated cyclic acyl guanidine compounds **8**, **9**, and **10** (Scheme 1A). Quantitative debenzylation by catalytic hydrogenation using Pd/C in ethanol at room temperature gave the corresponding phenolic cyclic acyl guanidine compounds