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Preparation, in vitro evaluation and molecular modelling of pyridinium–quinolinium/isoquinolinium non-symmetrical bisquaternary cholinesterase inhibitors

Marketa Komloova [a,b], Anna Horova [c], Martina Hrabinova [c], Daniel Jun [c,e], Martin Dolezal [d] Jarmila Vinsova [a], Kamil Kuca [e,f], Kamil Musilek [f,*]

[a] Charles University in Prague, Faculty of Pharmacy in Hradec Kralove, Department of Inorganic and Organic Chemistry, Heyrovskeho 1203, 500 05 Hradec Kralove, Czech Republic
[b] University of Jan Evangelista Purkynje, Faculty of Science, Department of Chemistry, Ceske mladeze 8, 400 96 Usti nad Labem, Czech Republic
[c] University of Defence, Faculty of Military Health Sciences, Department of Toxicology and Center of Advanced Studies, Trebesska 1575, 500 01 Hradec Kralove, Czech Republic
[d] Charles University in Prague, Faculty of Pharmacy in Hradec Kralove, Department of Pharmaceutical Chemistry and Drug Control, Heyrovskeho 1203, 500 05 Hradec Kralove, Czech Republic
[e] University Hospital, Biomedical Research Centre, Sokolska 581, 500 05 Hradec Kralove, Czech Republic
[f] University of Hradec Kralove, Faculty of Science, Department of Chemistry, Rokitanskeho 62, 50003 Hradec Kralove, Czech Republic

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ABSTRACT

Two series of non-symmetrical bisquaternary pyridinium–quinolinium and pyridinium–isoquinolinium compounds were prepared as molecules potentially applicable in myasthenia gravis treatment. Their inhibitory ability towards human recombinant acetylcholinesterase and human plasmatic butyrylcholinesterase was determined and the results were compared to the known effective inhibitors such as ambenonium dichloride, edrophonium bromide and experimental compound BW284C51.

Two compounds, 1-(10-(pyridinium-1-yl)decyl)quinolinium dibromide and 1-(12-(pyridinium-1-yl)dodecyl)quinolinium dibromide, showed very promising affinity for acetylcholinesterase with their IC_{50} values reaching nM inhibition of acetylcholinesterase. These most active compounds also showed satisfactory selectivity towards acetylcholinesterase and they seem to be very promising as leading structures for further modifications and optimization. Two of the most promising compounds were examined in the molecular modelling study in order to find the possible interactions between the ligand and tested enzyme.

Myasthenia gravis (MG) is a very rare disease, caused by immunologically-mediated destruction of postsynaptic membrane in the neuromuscular junction.[1] In most cases, antibodies bind to the nicotinic acetylcholine receptors (nAChR) which makes them inaccessible for neurotransmitter acetylcholine (ACh). Binding of the antibody also initiates an immunological reaction leading to the destruction of the receptors and reduction of their number in the synaptic cleft.[2,3] In other cases, different structures on postsynaptic membrane can be targeted as well. For example, most of the so called seronegative types of MG are characterized by the presence of antibodies targeting the muscle—specific tyrosine kinase (MuSK), a protein important in the development of the neuromuscular junction.[4] Generally, all these destructive processes result in simplification of the normally highly folded membrane and consequently in impaired cholinergic transmission. MG usually manifests by fatigue and weakness of striated muscles.[5]

Up-to-date, the pharmacological strategy of the MG treatment is based on the use of immunosuppressants (e.g., glucocorticoids, azathioprine and cyclosporine) in combination with peripherally acting inhibitors of acetylcholinesterase (AChEI). In the initial stages or mild forms of MG AChEI can be used alone.[1] Since acetylcholinesterase (AChE) is an enzyme responsible for the degradation of ACh, its inhibition leads to increased level of this neurotransmitter in the synaptic cleft. Higher availability of ACh enhances the cholinergic transmission despite the depletion of receptors, presuming that there are still some nAChR present.[6] ACHEI currently used in the therapy are carbamate inhibitors neostigmine bromide (Prostigmin®, Vagostigmin®), pyridostigmine bromide (Mestinon®) and distigmine dibromide (Ubretid®), with prolonged time of action (Fig. 1). Another option is a bisquaternary non-carbamate compound ambenonium dichloride (Fig. 1, Mytelase®), which is characterized by fewer side effects and longer pharmacological effect.[7]

The application of AChEI is often followed by side effects, for example gastrointestinal discomfort, increased salivation, lacrimation and bronchial secretion.[1,8] These side effects are related to

* Corresponding author. Tel.: +420 493331167.
E-mail address: kamil.musilek@gmail.com (K. Musilek).

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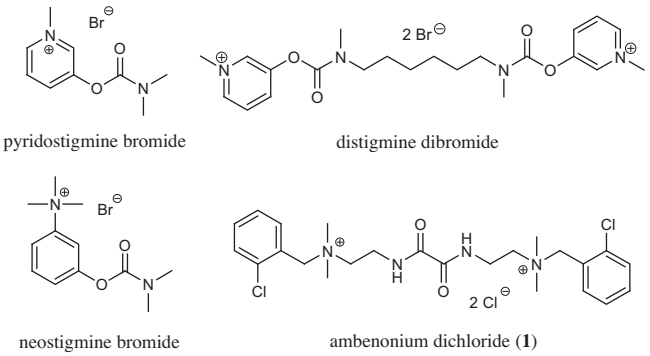


Figure 1. Structure of commercially available AChEI used for MG treatment.

increased muscarinic activity and can be counteract with antimuscarinic drugs (e.g., propantheline bromide, diphenoxylate hydrochloride).[9] High dosing of AChEI in severe forms of MG also increase the risk of cholinergic crisis, a depolarization blockade of neuromuscular junction leading to even greater muscle weakness, and other symptoms containing diarrhea, bradycardia, and excessive oropharyngeal and bronchial secretion.[10]

In order to avoid some of the central side effects it is advantageous to employ compounds with reduced blood–brain barrier penetration. All of the compounds from AChEI family used in MG treatment contain a positive charge in their structure that provides mostly peripheral effect. However, studies suggest that some of the central effects can occur due to the stress-induced BBB penetration.[11–13] Compounds designed for the therapy of MG should also be selective towards AChE and leave the non-specific esterase (butyrylcholinesterase, BChE) intact. Though the main function of BChE in the human organism still remains unclear, it is a well-known fact that BChE plays an important role in metabolism of exogenous ester-based compounds (e.g., local anaesthetics, muscle relaxant succinylcholine).[14] The selectivity of the new inhibitors toward AChE is therefore another important factor in order to minimize the risk of development of the side effects and interactions with other drugs.

Formerly, three series of symmetrical bisquaternary inhibitors were prepared. Each series consisted of two identical heteroaromatic moieties (pyridinium,[15] quinolinium,[16] isoquinolinium[17]) bound together by variety of connecting linkers (Fig. 2).

The spatial orientation and the distance of the two charged nitrogen heteroaromatic moieties play a significant role in the formation of non-covalent interactions between the inhibitor and the enzyme. In the SAR study of the three former series, we observed the influence of the different linker length as well as different possibilities in bond rotation, the distribution of electrons in the linkers with heteroatoms and double bonds or aromatic moieties on the affinity towards the enzyme. Compounds with 7–12 methylene units in the connecting linker resulted with the highest inhibitory ability. Based both on in vitro results and molecular modelling data, it was assumed, that the distance between the two charged

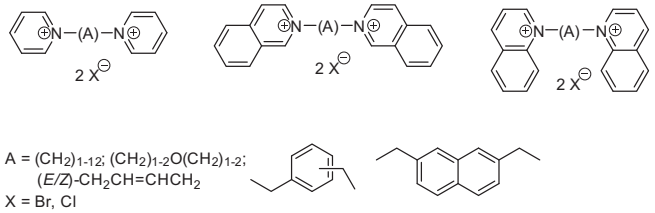


Figure 2. General structures of formerly prepared AChE inhibitors.

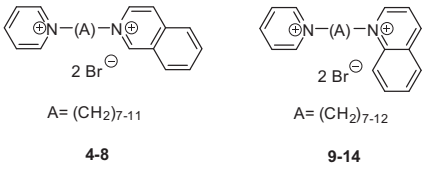


Figure 3. Structure of the prepared non-symmetrical cholinesterase inhibitors.

nitrogen in these molecules corresponds with the distance of important aromatic amino acid residues in the AChE gorge and is optimal for the formation of cation-π interactions between the inhibitor and the enzyme.

This Letter describes a preparation of non-symmetrical bisquaternary compounds, containing isoquinolinium or quinolinium moiety connected to pyridinium moiety using linkers with 7–12 methylene units (Fig. 3).

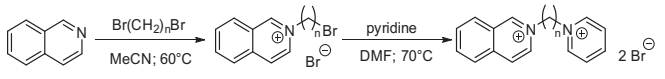
The new compounds were prepared via two step synthetic strategy (Scheme 1). The mixture of isoquinoline/quinoline and a excess of corresponding alkylating agent in MeCN were stirred at 60 °C for several hours. The monoquaternary semi-product was separated and crystallized. Purified monoquaternary compound was mixed with pyridine and stirred at 70 °C in DMF. The bisquaternary product was collected by filtration and crystallized. NMR, ESI–MS and elementary analysis were used for identification and determination of the purity of all compounds (for further details see Supplementary data).

The isoquinolinium–pyridinium and quinolinium-pyridinium bisquaternary compounds were investigated in vitro against human recombinant AChE (hAChE) and human plasmatic BChE (hBChE) using modified Ellman's procedure.[16] The inhibitory activity was expressed as IC_{50} and the preference for AChE as the selectivity index (SI; a ratio between the IC_{50} of hBChE and hAChE). The IC_{50} values and SI of all prepared compounds are summarised in the Table 1.

The results were compared to IC_{50} values of three standards: ambenonium dichloride (Fig. 1), edrophonium bromide and BW284C51 (Fig. 4).

Ambenonium dichloride is a compound with one of the highest affinities towards hAChE.[7] Edrophonium is a monoquaternary inhibitor with very short duration of action, which makes it inconvenient for pharmacological treatment. However, its rapid onset makes it a very useful tool for MG diagnosis. Third compound BW284C51 was chosen as the experimental standard with very high selectivity for AChE. The anticholinergic activity determined for the standard compounds confirmed the expected results (Table 1). Ambenonium showed tremendous affinity (0.7 nM) and very satisfactory selectivity towards hAChE. BW284C51 was found slightly worse to ambenonium in the hAChE inhibition, but more selective for hAChE. Edrophonium demonstrated only weak hAChE inhibition and selectivity. Additional kinetic experiments confirmed competitive character of inhibition for edrophonium, while other two standards acted as non-competitive inhibitors.

The novel bisquaternary compounds can be divided into two groups according to their structure: isoquinolinium–pyridinium (**4–8**) and quinolinium–pyridinium (**9–14**) group. Generally, isoquinolinium–pyridinium compounds showed a slightly weaker



Scheme 1. Synthetic pathway used in the preparation of isoquinolinium–pyridinium type of inhibitors.