ABSTRACT

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Title of doctoral thesis: Amaryllidaceae alkaloids of haemanthamine structural type and their semisynthetic derivatives as potential drugs in the treatment of Alzheimer´s disease.

To deepen the knowledge about the Amaryllidaceae alkaloid haemanthamine, which was isolated at our workplace as part of previous phytochemical studies, derivatives of this alkaloid were synthesized. First, series of aliphatic (**3-12**) and aromatic ester derivatives (**13-66**) were prepared, and then, to compare the structure-activity relationship, ether derivatives (**67-80**) were prepared from the most active substituents. All synthesized compounds were identified using the following structural analysis methods: NMR, HPLC/MS, and HRMS, including testing physical properties such as optical rotatability. After structure confirmation, all derivatives were subjected to screening studies for their inhibitory potential against *h*AChE and *h*BuChE. The selected derivatives were tested for their inhibitory potential against another enzyme, GSK-3β, which plays a significant role in the pathogenesis of AD. In cooperation with the Faculty of Medicine in Hradec Králové, Charles University, most of the derivatives were also subjected to cytotoxic screening on a panel of tumor and non-tumor cell lines. Docking studies were performed on promising derivatives to elucidate the structural aspects responsible for the inhibitory activity.

From the obtained data, it was found that the derivative 11-*O*-(2-methylbenzoyl)haemanthamine with IC_{50} values of 18.18 ± 1.30 μM for *h*AChE and 6.59 ± 1.19 μM for *h*BuChE proved to be a good inhibitor within *h*AChE/*h*BuChE. Another substance inhibiting both enzymes was 11-*O*-(2-chlorobenzoyl)haemanthamine with IC_{50} values 13.7 ± 0.76 μM for *h*AChE and 5.61 ± 0.62 μM for *h*BuChE. Both mentioned derivatives were selected for more detailed pharmacokinetic studies in order to describe the mechanism of action of inhibition. Derivatives with a nitro group together with a chlorine or methyl group on the aromatic core of the substituent turned out to be very good selective *h*AChE inhibitors. Specifically, the substances 11-*O*-(2-chloro-5-nitrobenzoyl)haemanthamine (IC_{50} = 0.17 ±

0.01μM), 11-*O*-(4-chloro-3-nitrobenzoyl)haemanthamine (IC_{50} = 0.12 ± 0 .01 μM) and 11-*O*-(4-methyl-3-nitrobenzoyl)haemanthamine (IC_{50} = 0.17 ± 0.01 μM) with IC_{50} in nanomolar values against *h*AChE showed an even tenfold greater inhibition compared to the standard galantamine used. For a better understanding of the relationship between structure vs. effect, ether derivatives were prepared from the most active substances, where the inhibitory activity against *h*BuChE deepened, on the contrary, almost no inhibitory effect was detected against *h*AChE. The mentioned 11-*O*-(4-chloro-3-nitrobenzoyl)haemanthamine also showed a very interesting antiproliferative effect, where the average growth percentage (GP) value was 5% compared to haemanthamine (GP = 25%). This haemanthamine derivative has been subjected to detailed studies in order to clarify its cytotoxic action (influence of the cell cycle, induction of apoptosis, induction of caspases, etc.).

Key words: haemanthamin, Amaryllidaceae alkaloids, Alzheimer's disease, acetylcholinesterase, butyrylcholinesterase, cytotoxicity.