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Research paper

Converting maslinic acid into an effective inhibitor of acylcholinesterases

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ABSTRACT

During the last decade, maslinic acid has been evaluated for many biological properties, *e.g.* as an anti-tumor or an anti-viral agent but also as a nutraceutical. The potential of maslinic acid and related derivatives to act as inhibitors of acetyl- or butyryl-cholinesterase was examined in this communication in more detail. Cholinesterases do still represent an interesting group of target enzymes with respect to the investigation and treatment of the Alzheimer's disease and other dementia illnesses as well. Although other triterpenoic acids have successfully been tested for their ability to act as inhibitors of cholinesterases, up to now maslinic acid has not been part of such studies.

For this reason, three series of maslinic acid derivatives possessing modifications at different centers were synthesized and subjected to Ellman's assay to determine their inhibitory strength and type of inhibitory action. While parent compound maslinic acid was no inhibitor in these assays, some of the compounds exhibited an inhibition of acetylcholinesterase in the single-digit micro-molar range. Two compounds were identified as inhibitors of butyrylcholinesterase showing inhibition constants comparable to those of galantamine, a drug often used in the treatment of Alzheimer's disease. Furthermore, additional selectivity as well as cytotoxicity studies were performed underlining the potential of several derivatives and qualifying them for further investigations. Docking studies revealed that the different kinetic behavior within the same compound series may be explained by the ability of the compounds to enter the active site gorge of AChE.

1. Introduction

Many people living in the western hemisphere, and especially those of an advanced age, are afraid of spending their last years suffering from serious diseases, *e.g.* from cancer or a stroke. Dementia illnesses – like the Alzheimer disease (AD) – considerably contribute to uncertainty among the population due to their impact on cognitive abilities. Worldwide approximately 35 million individuals, *i.e.* one in 200, currently suffer from some kind of dementia; according to some estimates within the next 30 years this number might double [1,2]. 20 million of these persons are affected by AD, and the prevalence of AD increases with age starting from 10% at the age of 65 to nearly 50% at 85 years [1]. Therefore, there is a scientific and economic demand for further investigations for a better understanding as well as for finding effective treatments.

Although AD is not completely understood, several hypotheses exist and are foundations or at least starting points of current therapies. One of these theories ("amyloid hypothesis") deals with the neurotoxic effect of a β-amyloid being formed by the action of α-, β- and γ-secretases [3,4]. Although this is one of the most prominent theories, there are some deficits: For instance, approximately 30 per cent of healthy, middle-aged people possess equal amounts of β-amyloid plaques being usually found in AD brains [5,6]. Most of the therapies aiming to decrease the concentration of the plaques, however, did not result in permanent increase of cognitive abilities or their at least their restauration [7–10]. Other attempts to find possible treatments focus on the investigation of inflammatory processes, mitochondrial disorders or the τ-protein [11–13].

Impairment in the cholinergic function, however, is of critical importance in AD ("cholinergic hypothesis") [1,14]. Thus, our

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present study focusses on the neurotransmitter acetylcholine (ACh), whose concentration is reduced during AD leading to typical symptoms like amnesia or behavioral disorder [14–17]. The hydrolytic enzymes acetylcholinesterase (AChE, E.C. 3.1.1.7) and butyrylcholinesterase (BChE, E.C. 3.1.1.8) are responsible for the hydrolysis of ACh, thus, controlling the concentration of this neurotransmitter in different tissues of an organism. Therein, BChE serves as a coregulator of the cholinergic transmission, and although it is mainly present in other parts of the body, BChE is able to compensate a reduced AChE activity in the brain [1,18,19]. Furthermore, the AChE/BChE ratio in the brain alters from 0.2 in normal brain to 11 during AD [14,20,21]. For this reason, both enzymes represent interesting targets for the development of AD therapies or tools for a deeper insight into this disease.

The only treatments with clinical evidence [14] to AD patients are the cholinesterase inhibitors galantamine, donepezil and rivastigmine. Several triterpenes have also been shown to act as inhibitors of AChE; this includes several hopanes [22], lanostanes [23] and lupanes [24]. Pentacyclic triterpenoic acids and their derivatives have been shown to be potent cholinesterase inhibitors in micro-molar range [25] with compounds of the α- or β-amyrin type being most active. Ursolic acid, for instance, acts as mixed-type inhibitor on AChE in the same magnitude as tacrine, a well-established drug [26]. Oleanolic acid [27,28] and structurally related compounds, *e.g.* taraxerol [29], echinocystic acid [30] or glycyrrhetinic acid [31], possessed IC_{50} values and inhibition constants K_i comparable to those of standard remedies like galantamine or donepezil.

Several studies an anti-tumor [32,33], anti-inflammatory [34] or an anti-viral [35] activity of maslinic acid and derivatives have been performed but the ability of these compounds to act as inhibitors of cholinesterases has not been investigated so far. Thus we prepared a series of maslinic acid derivatives differing in the substitution pattern at positions C-2, C-3 and C-28. All of these derivatives were screened for their ability to inhibit AChE and BChE; they were tested employing Ellman's assay, and their inhibitory constants (K_i and K_i') as well as the type of inhibition was determined. Furthermore, seven representative compounds were selected and investigated for a selectivity towards others enzymes [lipase from *Candida antarctica* (a serine hydrolase), papain (a sulfhydryl enzyme) and carbonic anhydrase II (a metalloenzyme)]. Additionally, some preliminary toxicity studies for selected derivatives were performed employing murine embryonic fibroblasts (NiH 3T3) in a photometric sulforhodamine B (SRB) assay.

2. Results and discussion

2.1. Chemistry

The first group of compounds (**1**–**27**, Scheme 1), representing several esters of maslinic acid, could be obtained from maslinic acid (**MA**) by reaction of **MA** with alkyl bromides in the presence of powdered K_2CO_3 in dry DMF [36,37].

The second group of compounds (Scheme 2) consists of matching pairs of maslinic acid derivatives (**28**, **29**), its methyl ester (**30**, **31**) and amides (**32**–**43**) possessing either free or acetylated hydroxyl groups at positions C-2 and C-3. Acetylations were carried out with acetic anhydride in pyridine [37] while the sulfamates **30** and **31** were obtained from the methyl ester **1** and sodium hydride in THF, followed by the addition of sulfamoyl chloride [39]. The synthesis of amides started from 2,3-diacetyl maslinic acid that was allowed to react with thionyl chloride followed by the addition of an amine [37].

A third group of compounds included augustic acid (**44**) and derivatives thereof. Augustic acid (**44**, Scheme 3) was synthesized *via* a four step chromatography-free synthesis starting from oleanolic acid as previously reported [39]. Its derivatizations were performed using well-established reactions as described above for the synthesis of derivatives of **MA**.

2.2. Biology

All compounds **1**–**48**, including maslinic acid (**MA**) and augustic acid (**44**), were subjected to Ellman's assays to determine their inhibitory activity (expressed as inhibition constants K_i and K_i') for the enzymes AChE and BChE. The results of these measurements are compiled in Table 1.

In summary, inhibitions constants for 24 compounds towards AChE and for 3 compounds towards BChE were determined. Two compounds, **18** and **19**, however, were not soluble under the conditions of the assay. For parent compound maslinic acid no inhibition constants below 100 μM could be obtained. Hence, maslinic acid does not significantly inhibit the cholinesterases; higher concentration could not be applied due to solubility reasons. Also, augustic acid (**44**) is no inhibitor of AChE, but – in contrast to maslinic acid – for BChE a mixed-type inhibition was observed (inhibition constants: K_i = 35.64 ± 5.73 μM and K_i' = 10.58 ± 1.95 μM). The uncompetitive part of the mixed-type inhibition (as expressed by K_i') is predominant. This indicates that augustic acid deploys its inhibitory action predominantly by binding to the enzyme–substrate complex rather than by binding to the free enzyme.

The first group of compounds (the esters **1**–**27**) were inhibitors of AChE, with the 1-chloro-butylester **13** as the most active compound of this series. This compound is a competitive inhibitor of AChE (K_i = 1.68 ± 0.30 μM). In comparison, for the esters **1**–**12** and **14**–**27** inhibition constants between 2.03 and 34.85 μM were determined. Especially, those esters having alkyl groups with more than three carbons showed K_i < 10 μM. While compounds **13** and **27** were competitive inhibitors, all other compounds of this series gave a mixed-type inhibition. Thus, the propyl ester **4** and the heptyl ester **17** gave an almost non-competitive inhibition (similar K_i and K_i') while a mixed-type inhibition with a dominating competitive part (K_i < K_i') could be determined for the ethyl ester **2** or the 1′-butinyl ester **12**. The 1-chloro-propyl ester **8** and the cyclohexyl ester **20** represent examples for mixed-type inhibitors with a dominating uncompetitive part (K_i > K_i').

From the second group [representing 2,3-substituted maslinic acid esters (**28**–**31**) and amides (**32**–**43**)] four compounds (**29**, **31**, **40** and **43**) exhibited an activity towards AChE, only – albeit in moderate micro-molar magnitude. Thus, **29** and **31** showed a moderate mixed-type inhibition (K_i and K_i' between 10 and 40 μM) while the 2,3-diacetylated propargyl amide **40** was determined to act as a competitive inhibitior of AChE, and the amide **32** is a competitive inhibitor of BChE with an inhibition constant of K_i = 18.11 ± 3.43 μM. Out of this series, two compounds (**32** and **42**) were identified as inhibitors of BChE. Their inhibitory activity is slightly lower than that of standard galantamine hydrobromide (K_i = 9.37 ± 0.67 μM).

As far as the last group of compounds [consisting of augustic acid (**44**) and related derivatives **44**–**48**] is concerned, parent augustic acid turned out to be a mixed-type inhibitor of BChE with a dominating uncompetitive part (K_i = 35.64 ± 5.73 μM, K_i' = 10.58 ± 1.95 μM); this compound is no inhibitor of AChE. Compounds **45** and **46** did not show any activity for AChE or BChE, while 2,3-dichloroacetyl-substituted **47** and **48** act as AChE inhibitors. Both compounds are mixed-type inhibitors in the single-digit micro-molar range.

Another important criterion is the selectivity of the compounds concerning one of the cholinesterases on one hand and concerning