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Isosorbide-based cholinesterase inhibitors; replacement of 5-ester groups leading to increased stability

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ABSTRACT

Isosorbide-2-carbamate-5-esters are highly potent and selective butyrylcholinesterase inhibitors with potential utility in the treatment of Alzheimer's Disease (AD). They are stable in human plasma but in mouse plasma they undergo hydrolysis at the 5-ester group potentially attenuating in vivo potency. In this paper we explore the role of the 5-position in modulating potency. The focus of the study was to increase metabolic stability while preserving potency and selectivity. Dicarbamates and 5-keto derivatives were markedly less potent than the ester class. The 2-benzylcarbamate-5-benzyl ether was found to be potent (IC_{50} 52 nM) and stable in the presence of mouse plasma and liver homogenate. The compound produces sustained moderate inhibition of mouse butyrylcholinesterase at 1 mg/kg, IP.

1. Introduction

The cholinesterases are well established targets now for drugs used to treat glaucoma, myasthenia gravis and Alzheimer's Disease (AD).[1–3] Cholinesterase inhibitors also find application in chemoprotection from organophosphate poisoning.[4] There are two types of cholinestesterase-AChE (3.1.1.7) and BuChE (3.1.1.8). Greatest attention has been paid to AChE due to its classical actions in regulating synaptic acetylcholine and because of less clearly defined, non-enzymatic or non-classical actions. The cholinesterase inhibition strategy in AD treatment arises from the recognition that the cognitive deficits arising in the disease correlate with diminished cholinergic neurotransmission. By inhibiting AChE the functional pool of AChE in the cholinergic neurons is augmented.

BuChE has generally received less attention mainly because its biological function is not as clearly defined as is that of AChE and although it is more ubiquitous than AChE its distribution in the CNS has not traditionally been recognized as consistent with an important role in neurotransmission, at least in the healthy brain. The several drugs on the market for AD are therefore more or less selective for AChE. There has been a surge in interest in BuChE as a potential target for AD treatment in recent years due to a number of factors: (i) the nullizygote (AChE −/−) mouse while having a clear phenotype appears to have normal CNS function, indicating at least a compensatory role for BuChE in the CNS[5]; (ii) while synaptic AChE levels decrease markedly in AD progression, there is a corresponding increase in BuChE activity[6]; (iii) post mortem tissue analysis on AD patients shows a high level of BuChE in the hallmark lesions of AD.[7] In rats, the selective BChE inhibitor cymserine causes elevation of acetylcholine and augments long term potentiation and learning.[8]

Isosorbide-2-carbamate-5-esters (Fig. 1) are nanomolar BuChE inhibitors with very high selectivity over AChE (up 60,000-fold).[9] Although they possess a 5-ester group, they are stable in human plasma because inhibitory interactions with BuChE (arising as a result of enzymatic interactions with the 2-carbamate functionality) are preferred over substrate (5-ester) interactions. However, during in vivo experiments in a mouse model of memory and learning it became apparent that compound **1a** (R′ = Ph) was undergoing hydrolysis in plasma and liver tissue at the 5-ester position generating the less potent isosorbide-2-carbamate and benzoic acid.[10] Mouse plasma possesses carboxylesterases which are absent in human plasma, and the undesirable degradation of the esters could be attributed to these enzymes. In this paper we investigate the relationship between 5-substituent and esterase inhibition in order to better understand the SAR for the class and to find a more

Abbreviations: AChE, acetylcholinesterase; ATCI, acetylthiocholine iodide; BTCI, butyrylthiocholine iodide; BuChE, butyrylcholinesterase; DCC, dicyclohexylcarbodiimide; DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid); *hu*BuCHE, human butyrylcholinesterase; ISMN, isosorbide-5-mononitrate; ISMNA, isosorbide mononitrate aspirinate; MAO, monoamino oxidase; SAR, structure–activity relationship, TBAF, tetrabutylammonium fluoride; TBDMS, tertbutyldimethylsilyl; TLC, thin layer chromatography; TMS, tetramethylsilane.

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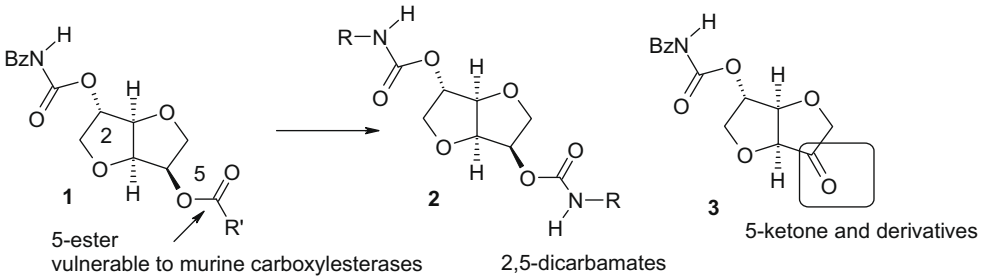


Figure 1. General strategy for increasing metabolic stability towards rodent carboxylesterases.

stable but potent and selective BuChE inhibitor that could be used to probe the role of the enzyme in the mouse.

2. Results and discussion

2.1. Chemistry

Dicarbamates (**2a**–**2f**) were prepared by heating isosorbide (**4**) in dry pyridine in the presence of an excess of the appropriate isocyanate for 1–2 h at 100 °C (Scheme 1). The excess isocyanate was removed by adding methanol and the mixture poured into ice-water. The target dicarbamates precipitated over a couple of hours.

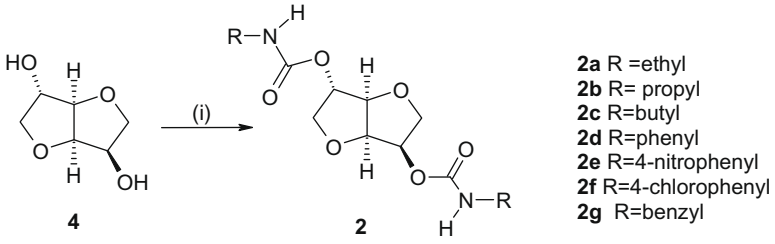
The clinically used isosorbide mononitrate (ISMN) **5** is a convenient material for exploring isosorbide chemistry because the difficult step of introducing regioselectivity has already been achieved and the nitrate is easily removed by reduction after elaboration of the 2-position. As reported previously isosorbide benzyl carbamates have optimal potency and selectivity for BuChE. Therefore the 2-position of ISMN (**5**) was carbamylated using benzyl isocyanate. Selective removal of the 5-nitrate was effected using Pd/C over H_2 and the triflate ester introduced using triflic anhydride. The alkene **7** was obtained by elimination of the intermediate triflate using DBU (Scheme 2). The 5,6-ene was distinguished from the potential isomeric 4,5-ene compound by the collapse of the 6-methylene group in the proton NMR and by the presence of signals for the 5- and 6-methine hydrogens.

The 5-ketone **3** was synthesised in order to determine the effect of flattening the isosorbide ring on ligand–BuChE interactions, and because the ketone is not susceptible to esterase-mediated metabolism. The ketone (**3**) was produced in good yield from isosorbide-2-benzylcarbamate-5-OH (**6**) using PCC in DCM with silica gel catalysis (Scheme 3). A number of classical or established ketone transformations were then attempted (Scheme 3). The 5-oxime (**8**) was generated by stirring with hydroxylamine hydrochloride in aqueous solution at high temperature. The oxime benzoate ester (**9**) was produced following acylation of **8** with benzoyl chloride and DMAP catalysis. Baeyer Villiger oxidation was accomplished by stirring **3** with *m*CPBA in DCM generating lactone **10**. Rearrangements of isosorbide-based ketones proceed with the expected migration of the more substituted alpha 4-carbon. The analogous Beckmann rearrangement was also performed on **3** using *N*-methylhydroxylamine in the presence of sodium acetate generating lactam **11** (the intermediate methyl ketoxime arising carried through without purification). Wittig reaction on **3** using the commercially available ylide, carbethyloxymethylene triphenylphosphorane yielded the alkenyl ester (**12**). The beta-amide **16** (Scheme 4) was accessed via a different strategy. Isoiodide (**14**) was generated from the commercially available isomannide (**13**) by bistosylation followed by SN_2 substitution with sodium acetate and hydrolysis. Monotosylation of isoiodide **14** was accomplished by treating the diol with a single equivalent of tosyl chloride at low temperature. The monotosylate was isolated by chromatography and treated with sodium azide to afford the 5-azide, isosorbide-5-deoxyazide (**15**). The azide, which was easier to handle than the corresponding amine was carbamylated at the 2-position using benzyl isocyanate and a tertiary base. The 5-azido group was then reduced to beta-amine and acylated with benzoyl chloride to afford the beta-amide **16** (Scheme 5).

Lastly, a number of 5-ether compounds were prepared as the ether functionality is resistant to esterase-induced hydrolysis. Treatment of **6** with benzyl or phenylethyl bromide yielded insufficient target ether because of concomitant alkylation of the 2-carbamate nitrogen. Therefore, ISMN was TBDMS protected, the 5-nitrate removed by reduction yielding **17** and the 5-OH group alkylated. The TBDMS protection was then removed and the 2-position converted to benzylcarbamate resulting in the benzyl and phenylpropyl ethers **18a** and **18b**.

2.2. Cholinesterase inhibition

Cholinesterase inhibition was determined using the Ellman method[11] with human plasma BuChE or electric eel AChE with butyryl- or acetyl-thiocholine as substrates as appropriate Table 1. The test compounds were incubated in the presence of enzyme for 30 min at 100 μM prior to addition of substrate. The inhibition experiments were repeated at successively lower concentration to determine the 30 min IC_{50} only where there was significant inhibition (>90%) at 100 μM. This corresponded to the solubility limit for the compounds in the assay medium. The seven dicarbamates (**2**) were tested first as these were the easiest to make. The diethyl compound **2a** was a moderately potent AChE inhibitor (IC_{50}, 6.5 μM) whereas the dibutyl compound **2c** was a moderately po-



Scheme 1. Preparation of the dicarbamates **2a–g**: (i) isocyanate, pyr, 100 °C, 1–2 h, 50–70%.