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Original article

Conformationally-locked *N*-glycosides: Exploiting long-range non-glycone interactions in the design of pharmacological chaperones for Gaucher disease

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ABSTRACT

Pyranoid-type glycomimetics having a *cis*-1,2-fused glucopyranose–2-alkylsulfanyl-1,3-oxazoline (Glc-PSO) structure exhibit an unprecedented specificity as inhibitors of mammalian β-glucosidase. Notably, their inhibitory potency against human β-glucocerebrosidase (GCase) was found to be strongly dependent on the nature of aglycone-type moieties attached at the sulfur atom. In the particular case of ω-substituted hexadecyl chains, an amazing influence of the terminal group was observed. A comparative study on a series of Glc-PSO derivatives suggests that hydrogen bond acceptor functionalities, e.g. fluoro or methyloxycarbonyl, significantly stabilize the Glc-PSO:GCase complex. The S-(16-fluorohexadecyl)-PSO glycomimetic turned out to be a more potent GCase competitive inhibitor than ambroxol, a non glycomimetic drug currently in pilot trials as a pharmacological chaperone for Gaucher disease. Moreover, the inhibition constant increased by one order of magnitude when shifting from neutral (pH 7) to acidic (pH 5) media, a favorable characteristic for a chaperone candidate. Indeed, the fluoro-PSO derivative also proved superior to ambroxol in mutant GCase activity enhancement assays in N370S/N370S Gaucher fibroblasts. The results presented here represent a proof of concept of the potential of exploiting long-range non-glycone interactions for the optimization of glycosidase inhibitors with chaperone activity.

1. Introduction

The search for compounds capable of modulating the activity of glycosidases, the enzymes that catalyze the hydrolysis of the glycosidic bond in polysaccharides and glycoconjugates, represents one of the more active research fields in glycobiology [1]. Glycosidase inhibitors are fundamental tools to interrogate biological processes involving biosynthesis, metabolism and recognition of carbohydrates [2] and bear strong potential for the development of drugs against associated pathologies [3], including cancer [4–8], diabetes [9,10], infection [11–13], ischemia [14] or neurodegenerative diseases [15,16]. On the other hand, compounds stabilizing the proper folding of trafficking-incompetent mutant glycosidases at the endoplasmic reticulum (ER), thereby rescuing them from degradation by the quality control system of the cell, show high promise as pharmacological chaperones for the treatment of lysosomal storage disorders [17–21] such as Gaucher [22–27], Fabry [28–30] or G_{M1} gangliosidosis [31–34], formally acting as effectors of the corresponding dysfunctional enzyme. Someway counterintuitively, the glycosidase inhibitory and chaperoning activities often coexist, the balance between them being a function of concentration and relative binding affinities at neutral (ER) and acidic pH (lysosome) [35].

With few exceptions [36–40], most naturally occurring or de novo synthesized glycosidase inhibitors/chaperones are

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carbohydrate-like derivatives (glycomimetics) in which the acetal group characteristic of glycosides has been modified while preserving a hydroxylation pattern of stereochemical complementarity with the aglycone moiety of the putative glycosidase substrate. Yet, recent work has shown the importance of implementing non-glycone interactions to achieve glycosidase selectivity levels within isoenzymes compatible with clinical applications [41–47]. Most of the work in this sense has focused on nitrogen- (iminosugars [48,49], sp^2-iminosugars [50–58], azasugars [59,60]) and carbon-in-the-ring (carbasugars [61,62], cyclitols [63,64]) carbohydrate mimics. Surprisingly, aglycon effects for glycomimetics keeping the pyranose core intact have been much less studied [65–68], even though modifications at the glycosidic region has the potential to be compatible with molecular diversity-oriented strategies with a relatively low synthetic cost [69].

In a previous report [70], we developed a new family of pyranoid-type glycomimetics having a glucopyranoso-based 2-alkylsulfanyl-1,3-oxazoline structure (Glc-PSO, Fig. 1) behaving as selective β-glucosidase inhibitors. PSO derivatives can be formally considered as conformationally locked *N*-glycosides, which warrant chemical and enzymatic stability. Their fused six-membered—five-membered bicyclic skeleton, analogous to that of the potent *O*-(*N*-acetylglucosaminidase) inhibitors NAG-thiazoline, NButGT and thiamet-G [71] (Fig. 1), imposes a skew-boat conformation to the pyranose ring, which has been found to impart glycosidase transition state mimic character [72]. Structure-chaperone activity relationship studies on fibroblasts from Gaucher disease patients evidenced a strong impact of the nature of exocyclic *S*-substituents of Glc-PSO glycomimetics on the mutant lysosomal β-glucosidase (β-glucocerebrosidase; GCase) effector abilities. Notably, the *S*-(16-hydroxyhexadecyl) derivative (Glc-PSO-HHD) was as efficient as the non-glycomimetic chaperone candidate ambroxol (ABX), currently in pilot trials in humans [73], for the N370S homozygous GCase mutation, the most prevalent for this lysosomal storage disorder. The possibility of hydrogen bonding involvement of the terminal hydroxyl group, once the pyranoid ring sits in the active site of the enzyme, with an amino acid residue located at an appropriate distance was advanced. To test this hypothesis, we have now expanded the PSO family with the preparation of a series of analogues keeping the hexadecyl chain in the aglycone but modifying the terminal group or the configuration of the core. The synthetic strategy and the evaluation of the new compounds as glycosidase inhibitors and chaperone candidates are reported.

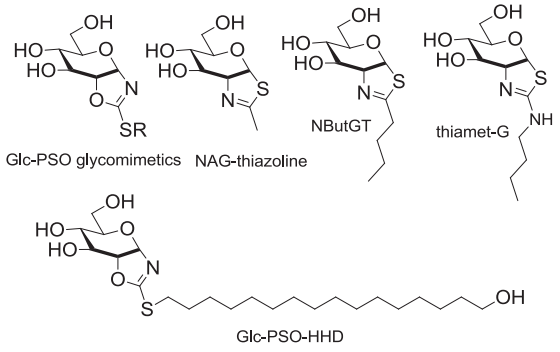

Fig. 1. General structure of Glc-PSO glycomimetics, structures of the related glycosidase inhibitors NAG-thiazoline, NBuGT and thiamet-G and structure of the previously reported Glc-PSO derivative Glc-PSO-HHD, which exhibited pharmacological chaperone abilities towards human glucocerebrosidase in Gaucher disease fibroblasts.

2. Results

2.1. Synthesis

The preparation of PSO glycomimetics with a hydroxylation profile matching that of D-glucose and differing in the S-linked aglycon portion relies on the *S*-alkylation reaction of the pivotal glucopyranoso-based 1,3-oxazolidine-2-thione intermediate **1**. Taking into consideration that the target enzyme GCase has been shown to exhibit low discriminating capabilities between active site-binding ligands differing in the configuration at C-4 for some glycomimetic families [74], the corresponding D-galacto configured epimer **2** was also initially considered. The methodology used for the synthesis of **1** and **2** started from tri-*O*-acetyl-D-glucal (**3**) or -D-galactal (**5**), respectively, and involved epoxidation of the double bond with *in situ* generated dimethyldioxirane. In the first case, the reaction afforded a mixture of the α-D-*gluco*-and β-D-*manno*-configured tri-*O*-acetyl-1,2-anhydrosugars **4** (D-*gluco*/D-*manno* ratio 7:1) in 90% yield [75]. Epoxidation of **5** provided instead exclusively the α-epoxide **6** (α-D-*galacto* configuration) in an almost quantitative yield [75]. Reaction of **4** and **6** with potassium thiocyanate and catalytic amounts of $TiO(CH_3CO_2)_2$ led to the requested thionocarbamates **1** and **2** in 87 and 79% yield, respectively [76,77] (Scheme 1).

In view of the good GCase chaperon properties observed for the D-*gluco*-PSO derivative bearing an *S*-(ω-hydroxyhexadecyl) aglycon moiety Glc-PSO-HHD (Fig. 1) and the significant contribution of the terminal hydroxyl to this behavior [70], in this work we have chosen to examine the incorporation of a series of ω-substituted hexadecyl chains bearing different terminal groups, including ester, carboxylic acid, iodo and fluoro. These compounds were prepared using the corresponding distal iodo derivatives as the alkylating agents. Methyl 16-iodohexadecanoate and 16-iodohexadecanoic acid were prepared from the corresponding commercially available 16-bromo derivatives by reaction with sodium iodide in acetone (see Supporting Information). Given the ambident character of the thionocarbamate functionality [78], the reaction conditions for the alkylation step had to be carefully adjusted in order to warrant the alkylation reaction regioselectively at the sulfur atom. Thus, compound **1** was treated with a series of different alkyl iodides in dichloromethane in the presence of triethylamine and catalytic amounts of *N*,*N*-dimethylaminopyridine (DMAP) or triazabicyclodecene (TBD) following the work by Rollin et al. [79–82]. Reactions carried out under these soft conditions afforded the expected acetyl-protected PSO derivatives **7**–**10** in 52–87 % yields (Table 1). Interestingly, reaction of **1** with 1,16-diiodohexadecane not only afforded the expected S-(16-iodohexadecyl)sulfanyl derivative **8**, but also the dialkylation product **9**, isolated in 32% yield (Table 1, entry 2). We also prepared the corresponding D-*galacto*-PSO derivative **11**, bearing the S-(16-hydroxyhexadecyl) antenna, to test the effect of the configurational change on the inihibitory/chaperone properties.

The S-alkyl character of compounds **7**–**11** was confirmed by ^{13}C NMR spectroscopy: the chemical shift for the quaternary sp^2 carbon atom at position 2 in the five-membered heterocycle varied from roughly 190 ppm (–N–C=S in 1,3-oxazolidine-2-thiones **1** and **2**) to approximately 170 ppm (–N=C–SR in PSO derivatives **7**–**11**). This data is in accordance with analogous thionocarbamates already reported in the literature [70].

The target fully-unprotected PSOA-glycomimetics **12**–**16** were obtained in 81–100 % yield by final removal of the acetyl protecting groups using methanol under standard NaOMe-catalyzed conditions. In order to obtain derivative **17**, precursor **7** was treated with a solution of sodium hydroxide in methanol under similar reactions conditions (Table 1, entry 2). The vicinal proton–proton coupling constants about the pyranose ring both for the acetylated (**7**–**11**) and the unprotected (**12**–**17**) PSO derivatives were in agreement