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Research paper

Inhibitor versus chaperone behaviour of D-fagomine, DAB and LAB sp^2-iminosugar conjugates against glycosidases: A structure–activity relationship study in Gaucher fibroblasts

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ABSTRACT

A library of sp^2-iminosugar conjugates derived from the piperidine iminosugar D-fagomine and the enantiomeric pyrrolidine iminosugars DAB and LAB has been generated in only two steps involving direct coupling of the fully unprotected polyhydroxylated heterocycles with isothiocyanates, to give monocyclic thiourea adducts, and further intramolecular nucleophilic displacement of the δ-located primary hydroxyl group by the thiocarbonyl sulphur atom, affording bicyclic isothioureas. These transformations led to a dramatic shift in the inhibitory selectivity from α- to β-glucosidases, with inhibition potencies that depended strongly on the nature of the aglycone-type moiety in the conjugates. Some of the new derivatives behaved as potent inhibitors of human β-glucocerebrosidase (GCase), the lysosomal enzyme whose dysfunction is responsible for Gaucher disease. Moreover, GCase inhibition was 10-fold weaker at pH 5 as compared to pH 7, which is generally considered as a good property for pharmacological chaperones. Surprisingly, most of the compounds strongly inhibited GCase in wild type fibroblasts at rather low concentrations, showing an unfavourable chaperone/inhibitor balance on disease-associated GCase mutants in cellulo. A structure–activity relationship analysis points to the need for keeping a contiguous triol system in the glycone moiety of the conjugates to elicit a chaperone effect. In any case, the results reported here represent a proof of concept of the utmost importance of implementing diversity-oriented strategies for the identification and optimization of potent and specific glycosidase inhibitors and chaperones.

1. Introduction

Iminosugars comprise the most attractive class of carbohydrate mimetics reported to date and are ideally positioned to take advantage of our increasing understanding of glycobiology in the search for new medicine [1]. Among them, D-fagomine (1,2-dideoxynojirimycin) **1,** the first iminosugar found in plants, is a mild glycosidase inhibitor [2] that has shown a short-term activity along the intestinal tract, inhibiting the adhesion of potentially deleterious bacteria to the intestinal mucosa [3]. Five-membered iminocyclitols, such as the polyhydroxylated pyrrolidine derivatives 1,4-dideoxy-1,4-imino-D- and L-arabinitol (DAB and LAB, respectively) **2** and **3**, also exhibit exceptional biological activity [4], behaving as strong inhibitors of α-glucosidases from different sources [5,6], including mice and rat intestinal glycosidases. DAB is also a potent inhibitor of glycogen phosphorylase [7–9]. Their potential as regulators of glycogen-degrading enzymes in addition to the reduction of the postprandial glycaemia in blood makes DAB and LAB promising therapeutic agents for the prevention and treatment of type-II diabetes [10,11].

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Unfortunately, first-generation iminosugars like **1**–**3** suffer from relatively low selectivities among different glycosidases, which represents a serious drawback for their translation into the clinic. Interestingly, some structural modifications of D-fagomine, DAB and LAB have been reported to lead to derivatives with improved activity and selectivity. Thus, *N*-alkylated derivatives of D-fagomine showed enhanced inhibitory activity against α-glucosidases as well as a significant cytotoxic activity in eight human cancer cell lines [12]. Chemical modification of DAB and LAB has also been proposed as a strategy to finely tune their biological activity, leading to the identification of drug candidates for the treatment of cancer, arteriosclerosis and some lysosomal storage diseases (LSDs) [13–15]. In all the reported examples the amine-type nature of the endocyclic nitrogen, a defining feature of iminosugar glycomimetics, was preserved. Some years ago, we found that a subtle change in the structure of iminosugars, by replacing the sp^3 ring nitrogen atom by a pseudoamide-type nitrogen (urea, thiourea, carbamate, thiocarbamate, isourea, isothiourea, guanidine) with substantial sp^2 character, led to a new family of glycomimetics with increased selectivity and potency as glycosidase inhibitors, namely sp^2-iminosugars [16–22]. Indeed, some sp^2-iminosugars have been also isolated from natural sources and showed remarkable glycosidase inhibitory properties, e.g. nagstatin (**4**) and kifunensine (**5**) [23,24]. Most interestingly, transformation of an iminosugar precursor into a series of sp^2-iminosugar derivatives is compatible with diversity-oriented and optimization strategies, speeding the identification of new leads [25,26]. Compounds encompassing a sp^2-iminosugar glycone-type moiety that mimics the natural substrate of the target enzyme and a hydrophobic aglycone-type substituent that interacts with amino acids at the vicinity of the active site, e.g. the 1-deoxygalactonojirimycin derivative **6** [27] or the calystegine B_2 analogue **7** [28], are currently under study in therapies against cancer [29,30], leishmaniasis [31] and the LSDs Gaucher [32–36], Fabry [37] and GM_1 gangliosidosis [38].

Structure–activity relationship studies and X-ray data evidenced the critical effect of the nature and the orientation of the aglycone-type moiety in the biological properties of sp^2-iminosugar conjugates [39–43]. Comparatively, the influence of the glycone-type scaffold in the activity of this family of glycomimetics has been much less studied, most of the available data corresponding to six-membered piperidine- (e.g. **6**) or *nor*-tropane-related conjugates (e.g. **7**) [44–47]. Five-membered ring analogues on record are very limited [48–50], mostly corresponding to 2,5-dideoxy-2,5-imino-D-mannitol (DMDP, **8**) derivatives. Remarkable specificities in glycosidase inhibition were achieved depending not only on the nature of the pseudoaglyconic side chain but also on the monocyclic (e.g. **9**) or bicyclic character (e.g. **10**) of the heterocyclic framework [48]. While there is a clear interest at broadening the range of sp^2-iminosugar structures for drug discovery, the difficulties associated to iminosugar synthesis represents an important limitation for these channels. Taken advantage of the recent development of a chemoenzymatic synthesis of iminocyclitols **1**–**3** from readily available achiral starting materials [51,52], we have now undertaken their transformation into sp^2-iminosugar analogues and conducted a comparative study on their glycosidase inhibitory profiles. Selected candidates have been further assayed as pharmacological chaperones for the treatment of Gaucher disease.

2. Results

2.1. Synthesis

To test the impact of converting iminosugars **1**–**3** into mono or bicyclic sp^2-iminosugar analogues, their reaction with isothiocyanates to afford thiourea adducts and the subsequent intramolecular cyclization into bicyclic isothioureas was investigated. The incorporation of *n*-butyl, *n*-octyl and ω-hydroxyhexadecyl aglycone-type substituents was considered keeping in mind that amphiphilic glycomimetic conjugates bearing such hydrophobic appendages have shown a high selectivity in the inhibition of lysosomal glycosidases of therapeutic interest, which translated into pharmacological chaperone behaviour against disease-associated dysfunctional mutant variants [53,54]. Thus, D-fagomine thioureas **11a**–**c** were accessed in high yield by direct coupling of the parent iminosugar **1** with *n*-butyl, *n*-octyl or 16-acetoxyhexadecyl isothiocyanate, respectively, in pyridine in the presence of a catalytic amount of triethylamine. The latter was subsequently deacetylated by standard Zemplén transesterification with catalytic sodium methoxide in methanol to give the long chain alcohol conjugate **11d** (Scheme 1). However, attempts to promote cyclization of the thioureas **11a**,**b**,**d** to the corresponding bicyclic 2-iminothiazolidines by activation with trifluoromethanesulfonic anhydride [55] or methanesulfonyl chloride [40] in *N*,*N*-dimethylformamide led to extensive hydrolysis of the sulfonylating reagents. Interestingly, direct treatment with hydrochloric acid in methanol at room temperature afforded the desired piperidine-iminothiazolidine bicyclic derivatives **12a**,**b**,**d** in virtually quantitative yield with no need of any additional promotor. The strong potential of this straightforward two-step procedure for sp^2-iminosugar library generation was further corroborated by the preparation of the homologous DAB and LAB thioureas **13a**–**d** and **15a**–**d** and their transformation into the corresponding bicyclic isothioureas **14a**,**b**,**d** and **16a**,**b**,**d**. In this case, the later reaction required heating at 70 °C for 72 h, probably due to the higher ring strain in the pyrrolizidine-type core.

The structure and purity of all the prepared compounds were confirmed by spectroscopic and analytical techniques. The NMR spectra of the thiourea derivatives exhibited the characteristic temperature-dependent line broadening associated to slow rotation about the nitrogen—(C=S) bonds [56]. The presence of the thiocarbonyl group was evidenced by the diagnostic carbon resonance at 185.7–181.2 ppm. The ^{13}C NMR spectra of the bicyclic derivatives showed instead a signal at 172.3–162.3 ppm corresponding to the imino double bond. The high field shift of the C-6 (piperidine) or C-5 (pyrrolidine) resonance on going from the monocyclic thioureas (63.5–62.1 ppm) to the bicyclic isothioureas (36.1–31.8 ppm) unequivocally confirmed the replacement of oxygen into sulphur at this position.

2.2. Evaluation of enzyme inhibitory properties

The inhibitory properties of the whole library of D-fagomine, DAB and LAB thioureas (**11a**–**d**, **13a**–**d**, **15a**–**d**) and isothiourea derivatives (**12a**,**b**,**d**, **14a**,**b**,**d** and **16a**,**b**,**d**, respectively) were first screened against a panel of commercial glycosidases including α-glucosidase (α-Glcase; yeast), isomaltase (yeast), amyloglucosidase (*Aspergillus niger*), β-glucosidase (β-Glcase; almonds and bovine liver, cytosolic), α-mannosidase (Jack bean), β-mannosidase (*Helix pomatia*), naringinase (β-glucosidase/α-L-rhamnosidase, *Penicillium decumbens*), α-galactosidase (green coffee beans) and β-galactosidase (*Escherichia coli*). The corresponding inhibition constant values (K_i, μM) are collected in Table 1; data for the parent iminosugars **1**–**3** are included for comparative purposes.

The results points to a dramatic impact of the transformation of the amine functionality in iminosugars **1**–**3** into linear thiourea or cyclic isothiourea groups, as well as a strong influence of the nature of the aglycone-type substituent, in the inhibition potency and selectivity against glycosidases. Whereas the selectivity for enzymes processing *gluco*-configured substrates was maintained,

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