Selenoureido-iminosugars: A new family of multitarget drugs

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ABSTRACT

Herein we report the synthesis of N-alkylated deoxynojirimycin derivatives decorated with a selenoureido-motif at the hydrocarbon tether as an example of unprecedented multitarget agents. Title compounds were designed as dual drugs for tackling simultaneously the Gaucher disease (by selective inhibition of β-glucosidase, \( K_i = 1.6–5.5 \) μM, with improved potency and selectivity compared to deoxynojirimycin) and its neurological complications (by inhibiting AChE, \( K_i \) up to 5.8 μM). Moreover, an excellent mimicry of the selenoenzyme glutathione peroxidase was also found for the catalytic scavenging of H\(_2\)O\(_2\) (\( K_{cat}/K_{m} \) up to 640) using PhSH as a cofactor, with improved activity compared to known positive controls, like (PhSe)\(_2\) and ebselen; therefore, such compounds are also excellent scavengers of peroxides, an example of reactive oxygen species present at high concentrations in patients of Gaucher disease and neurological disorders.

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1. Introduction

Polyhydroxylated imino- and azasugars, that is, carbohydrate mimics where the endocyclic oxygen, or a carbon, respectively, has been replaced by a nitrogen atom, are attractive pharmacological agents due to their strong inhibitory properties against glycosidases and glycosyltransferases [1]. Although such enzymes exert pivotal functions in living beings, they also play a critical role in a series of pathologies [2], and thus, their selective inhibition has become a challenging research area [2,3]. In this context, glycosidase inhibitors have been claimed to be potentially useful in the treatment of lysosomal storage disorders (Gaucher, Fabry, Sandhoff or Tay-Sachs diseases) [4], and as anti-diabetic [5], antimicrobial [6], anti-cancer [7] and immunosuppressive [8] agents.

Among the vast arsenal of glycosidase inhibitors reported so far, polyhydroxylated piperidines are particularly remarkable; a notorious example is the natural iminosugar 1-deoxynojirimycin (DNJ) 1 [9]. Some N-alkylated deoxynojirimycin derivatives, like n-BuDNJ 2 (Zavesca®), a glucosylceramide synthase inhibitor) [10], and the 2-hydroxyethyl counterpart 4 (Diastobol®), an intestinal α-glucosidase inhibitor [11] have reached the market for the treatment of Gaucher disease and diabetes-type 2, respectively. It has been demonstrated that a lengthening of the hydrocarbon chain (e.g. 3) provides better selectivity towards α-glucosidase, whereas a significant increase of the size and hydrophobicity of the N-alkyl substituent furnishes more potent glucosylceramide synthase inhibitors (e.g. 5) [1c,12].

Gaucher disease, the most frequent lysosomal storage disorder, is a recessively inherited disease caused by mutations in the glucocerebrosidase (GBA1) gene, leading to the misfolding of the β-glucosidase responsible for the metabolic hydrolysis of glycoconjugates. As a result, the accumulation of partially-degraded metabolites in the lysosomes of several organs, including the brain, takes place causing severe damage to tissues and organs, and potentially, the death of the patient [13]. Currently, three possible treatments for Gaucher disease can be applied [1c]: enzyme replacement therapy (ERT), based on the administration of a recombinant glycosidase; substrate-reduction therapy (SRT), which uses glycosidase inhibitors to reduce the biosynthesis of

Abbreviations: AChE, acetylcholinesterase; CCT, chemical chaperone therapy; DNJ, 1-deoxynojirimycin; ERT, enzyme replacement therapy; GBA1, β-glucosidase acid 1; G0/S, growth inhibition 50%; GPx, glutathione peroxidase; ROS, reactive oxygen species; SRT, substrate-reduction therapy.
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glycosphingolipids; and the chemical chaperone therapy (CCT). The last approach is based on the fact that in some lysosomal storage disorders the defective glycosidase, although still keeps some of its catalytic activity, is misfolded, and thus, easily degraded by the endoplasmic reticulum quality-control system [1c]. Nevertheless, a high-affinity glycosidase inhibitor acting as a chemical chaperone can bind the mutant glycosidase at sub-inhibitory concentrations to induce its proper folding, and thus, to prevent its degradation. In this context, defective glucocerebrosidase, the enzyme responsible for the Gaucher disease, showed increased activity in cell cultures in the presence of 2 [14]. In fact, the efficiency of 2 for treating type 1 Gaucher has been claimed to be due to a combined action of inhibiting glucosylceramide synthase and a chaperone effect in misfolded glucocerebrosidase [15]. N-Bu-DNJ 2 also induced a pH-dependent stabilization of imiglucerase and velaglucerase alfa, recombinant glycosidases currently used in the ERT, supporting the role of 2 as a chaperone for pharmaceutical formulations in Gaucher disease [16].

Moreover, neuronal manifestations have also been found in a significant number of Gaucher disease patients [17]. Recently, it has been demonstrated that mutation in the GBA1 gene increases up to 30% the risk of developing synucleinopathies, mainly Parkinson and the Lewy body variant of Alzheimer’s disease, both in Gaucher patients and carriers [17], therefore leading also to parkinsonian symptoms.

Furthermore, some of the physiological manifestations of dementia-type diseases are directly connected to acetylcholinesterase (AChE), either due to significant low levels of the neurotransmitter acetylcholine via its increased hydrolytic cleavage, or by promoting the deposit of β-amyloid plaques [18]; subsequently, AChE inhibitors have been suggested as therapeutic agents for improving the cognitive functionality in such kind of pathologies (cholinergic hypothesis) [19].

Herein our main target has been the design of a new family of dual drugs for tackling simultaneously the Gaucher disease and its neurological complications; for that purpose, we incorporated pharmacophores that afforded good β-glycosidase and acetylcholinesterase inhibition, leading to a privileged structure that might be useful for the treatment of such pathology.

2. Results and discussion

2.1. Chemistry

We propose the general structure 6 (Fig. 1) as a hitherto unknown example of dual multitarget drugs designed for inhibiting simultaneously β-glucosidase, responsible for the Gaucher disease, and acetylcholinesterase, involved in the progressive loss of the cognitive function associated to the neurological complications of Gaucher disease.

We hypothesize that the incorporation of an arylalkyl fragment on the nitrogen would increase its selectivity towards β-glucosidase, in a similar way as found for the azafagomine alkylation on the same position [20].

Moreover, concerning AChE, the resolution of its crystal structure revealed [21] the presence of some critical regions located in the bottom of a gorge of roughly 20 Å; such regions must be taken into consideration when designing inhibitors. In the proximity of the catalytic triad, comprised of Ser, His and Glu, there is a region called the catalytic anionic site, responsible for binding the quaternary ammonium residue of acetylcholine, through cation-π interactions involving a Trp residue [21]. We propose that the deoxynojirimycin residue of 6, partially protonated at physiological pH values, could mimic the iminium cation of acetylcholine. Moreover, roughly 15 Å away from the catalytic anionic site there is a region known as the peripheral anionic site, capable of establishing strong interactions with planar and aromatic motifs, due to its high content of aromatic aminoacids [22]. The interaction with this domain, besides enhancing the binding of the inhibitor with the enzyme can also reduce the capacity of AChE of starting the degradation of β-amyloid plaques [23], a common feature of pathologies associated to Alzheimer’s disease. For that purpose, we suggest the incorporation of an N-aryl selenourea appendage; the selenoureido motif would also eliminate deleterious Reactive Oxygen species (ROS), present at high concentration levels in both, neurodegenerative disorders [24] and Gaucher disease; in fact, in the latter, the severity of the disease is determined by the degree of oxidative stress in the endoplasmic reticulum, and antioxidants have shown protective effects [25].

The synthesis of targeted structure 6 was accomplished following the synthetic methodology depicted in Scheme 1. The key template was O-protected DNJ 9, which can be accessed starting from methyl α-D-glucopyranoside 7 in a 5-step procedure: per-O-benzylation, glycoside hydrolysis, reduction of reducing sugar 8, Swern oxidation and cyclization of the corresponding transient and unstable hexosulose mediated by ammonium formate and sodium cyanoborohydride.

Overkleeft and co-workers claimed [26] that Swern oxidation was a more reproducible procedure for preparing the key dicarbonyl compound in the synthesis of 9 than the Pfitzer–Moffat oxidation originally reported by Matos et al. [27].

Next, the subsequent N-alkylation of 9 via nucleophilic substitution on alkyl halides proved to be more complicated than previously anticipated; thus, attempts to accomplish such alkylation reaction with either 5-bromopentanol or monosubstitution on 1,5-dibromopentane failed, as no product formation was observed.
Probably the strong steric hindrance exerted by the benzyl ethers, particularly the nearby hydroxymethyl one, precluded the endocyclic nitrogen atom to efficiently act as a nucleophile. Nevertheless, catalytic hydrogenolysis reaction to give DNJ 1, followed by nucleophilic displacement on 1-azido-5-iodopentane took place with acceptable yield (45% for the two steps). Reduction of the azido moiety, followed by coupling with readily-available aromatic isoselenocyanates [28], furnished title compounds 6a–c in a 4.5–8.1% overall yield for the 9 steps (Scheme 1).

The three targeted compounds chosen as representative examples of the hitherto unknown family of selenoureidoiminosugars bear either an electron-withdrawing group (Br, 6a), a strong electron-donating group (OMe, 6b), and a moderate electron-donating group (Me, 6c).

### 2.2. Biological assessment

Compounds 6a–c were tested as potential glycosidase inhibitors against a panel of seven glycosidases of medical relevance (α/β-glucosidases, α/β-galactosidases, α/β-mannosidases). Lineweaver-Burk plots (double reciprocal plot, 1/v vs. 1/[S]) allowed the determination of the inhibition type, together with the $K_i$ values (e.g. Fig. 2).

Compounds 6a–c, in particular the p-bromo derivative 6a, were found to be strong and selective competitive β-glucosidase inhibitors, with $K_i$ in the low micromolar range (Table 1, 1.6–5.5 μM). It is noteworthy mentioning that a remarkable increase in α/β-glucosidase selectivity was found (up to 36-fold) when compared with parent DNJ ($K_i$, μM: 25 (α-glucosidase), 47 (β-glucosidase), 270 (α-mannosidase)) [29]. Surprisingly, a reversed selectivity was observed compared to DNJ, and particularly to analogues 2–3, which show preference for α-glucosidase. These results are presumably due to a combination of favourable π-stacking interactions involving the aromatic substituent of the selenoureido scaffold, and effective hydrogen bonding with polar residues of the β-glucosidase active site. Concerning the rest of glycosidases, negligible or weak activity was observed, a desirable effect for therapeutic applications.

Derivatives 6a–c were also tested against acetylcholinesterase (AChE from Electrophorus electricus); such enzyme catalyses [30] the breakdown of the cholinergic neurotransmitter acetylcholine. A well-known symptomatic feature of Alzheimer’s disease is the profound decline in the cholinergic function. Therefore, one of the targets for treating this neurodegenerative disease in the early stages is the development of acetylcholinesterase inhibitors [31] which might increase both, the level and the duration of the neurotransmitter action.

p-Bromophenyl selenourea 6a was found to be a strong mixed AChE inhibitor (Table 1, $K_{ia} = 5.8$ μM, $K_{ib} = 11.2$ μM); therefore, the p-bromophenyl moiety is again a crucial structural motif within the selenoureido-iminosugar to exert strong enzymatic inhibition. Until now, there is only one precedent [32] for the inhibition of AChE by imino- or azasugars.

The capacity of compounds 6 to act as glutathione peroxidase
Inhibitory properties of compounds 6a–c ([Kₐ, μM]).

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>6a</th>
<th>6b</th>
<th>6c</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Glucosidase (Bakers’ yeast)</td>
<td>30.7 ± 10.7</td>
<td>36.0 ± 8</td>
<td>35.0 ± 11</td>
</tr>
<tr>
<td>β-Glucosidase (Almonds)</td>
<td>1.6 ± 0.2</td>
<td>5.5 ± 0.8</td>
<td>3.7 ± 0.8</td>
</tr>
<tr>
<td>α-Galactosidase (Green coffee beans)</td>
<td>7%</td>
<td>20%</td>
<td>25%</td>
</tr>
<tr>
<td>β-Galactosidase (Asp. Oryze)</td>
<td>42%</td>
<td>29%</td>
<td>20%</td>
</tr>
<tr>
<td>β-Mannosidase (E. coli)</td>
<td>12%</td>
<td>15%</td>
<td>31%</td>
</tr>
<tr>
<td>β-Mannosidase (Jack bean)</td>
<td>12%</td>
<td>15%</td>
<td>31%</td>
</tr>
<tr>
<td>β-Mannosidase (Helix pomatia)</td>
<td>18%</td>
<td>7%</td>
<td>16%</td>
</tr>
<tr>
<td>Acetylcholin-esterase (electric eel)</td>
<td>18%</td>
<td>7%</td>
<td>16%</td>
</tr>
</tbody>
</table>

a Percentage of inhibition at 0.5 mM concentration.
b Inhibition constant for the E-I binding.
c Inhibition constant for the ES-I binding.

<table>
<thead>
<tr>
<th>Compound</th>
<th>V (μM/min)¹</th>
<th>t½ (min)</th>
<th>Relative rate to control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (MeOH)</td>
<td>12.3 ± 2.3</td>
<td>351.9</td>
<td>1.0</td>
</tr>
<tr>
<td>Control (DMSO)</td>
<td>18.9 ± 4.4</td>
<td>214.0</td>
<td>1.0</td>
</tr>
<tr>
<td>9a (MeOH)</td>
<td>290.1 ± 3.2</td>
<td>147.7</td>
<td>23.6</td>
</tr>
<tr>
<td>9b (MeOH)</td>
<td>567.4 ± 25.7</td>
<td>8.3</td>
<td>46.1</td>
</tr>
<tr>
<td>(PhSe)₂ (MeOH)</td>
<td>31.3 ± 1.5</td>
<td>102.0</td>
<td>2.5</td>
</tr>
<tr>
<td>Ebselen (DMSO)</td>
<td>10.5 ± 4.4</td>
<td>148</td>
<td>0.6</td>
</tr>
</tbody>
</table>

¹ Experimental conditions: [PhSH]₀ = 10.0 mM; [H₂O₂]₀ = 41.6 mM; [Cat] = 0.1 mM, solvent: MeOH for 6a, 6b, (PhSe)₂, and 99:1 MeOH–DMSO for ebselen.

The kinetic parameters (Kₐ, Vₘₐₓ) of the GPx-like activity were calculated by using different PhSH concentrations (Table 3), while keeping constant the concentrations of the catalyst and H₂O₂. Hanes-Woolf plot of tested compounds demonstrated that these organoselenium derivatives fulfill Michaelis-type kinetics. Remarkably, for p-methoxy derivative 6b the Kcat/Kₐcat ratio was found to be 640, clearly indicating excellent H₂O₂ scavenging properties via a catalytic cycle; these results constitute a significant improvement compared to stoichiometric antioxidants.

**3. Conclusions**

In conclusion, N-alkylated deoxynojirimycin derivatives bearing a selenoureido motif have proved to be privileged structures that could be useful in the therapeutic treatment of Gaucher disease and its neurological complications. Thus, this prototype affords strong β-glucosidase and AChE inhibition, together with excellent catalytic scavenging properties against deleterious peroxides.

**4. Experimental section**

**4.1. N-(S’-Azidotopentyl)-1-deoxynojirimycin (13)**

To a solution of per-O-benzyl-1-deoxynojirimycin 9 (1.1 g, 2.1 mmol) in dry MeOH (45 mL) was added 20% Pd(OH)₂/C (0.15 g), and the suspension was acidified with 2 M aq. HCl, and then stirred under a H₂ atmosphere for 14 h. The mixture was then filtered through a Celite® pad and the filtrate was concentrated in vacuo to give crude DNJ 1 (0.54 g). Compound 1 was dissolved in dry DMF.
To a solution of compound 16 (66 mg, 0.23 mmol) in MeOH (10 mL) was added a catalytic amount of Raney-Ni. The mixture was then stirred for 1 h under H2 atmosphere. After that it was filtered through a Celite® pad and concentrated in vacuo to afford amine 14. To a solution of crude 14 (56 mg, 0.23 mmol) in dry MeOH (10 mL) was added the corresponding isoselenocyanate 16a–c (0.46 mmol), and the reaction was stirred at rt and in the dark for 24 h. Concentration to dryness and purification by column chromatography using the eluant indicated in each case afforded selenoureas 6a–c.

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### Appendix A. Supplementary data

Supplementary data related to this article can be found at [http://dx.doi.org/10.1016/j.ejmech.2016.07.021](http://dx.doi.org/10.1016/j.ejmech.2016.07.021).

### References
