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Aminocyclitols as Pharmacological Chaperones for Glucocerebrosidase, a Defective Enzyme in Gaucher Disease

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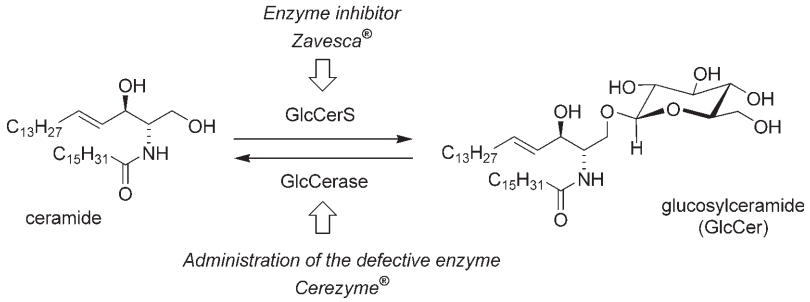
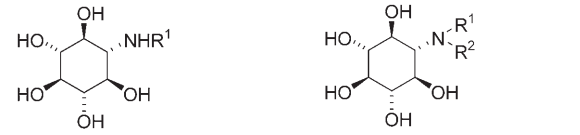
Some genetic pathologies are associated to mutations that produce minor changes in a protein structure. Of particular interest are those mutations responsible for the misfolding of certain lysosomal enzymes, which are prematurely degraded in the endoplasmic reticulum (ER) as a result of their inability to pass the quality control imposed by the ER for further processing.[1] Over the last years, chemical chaperones have emerged as a kind of structurally nonspecific, low molecular weight compound able to stabilize the conformation of defective proteins to allow for their correct folding and transport.[2] More recently, chemical chaperones acting as active-site specific ligands of a variety of defective lysosomal enzymes have been described as pharmacological tools for the rescue of mutant protein function. These so-called pharmacological chaperones are generally competitive inhibitors of the target enzyme that assist the proper folding of the defective protein at subinhibitory concentrations.[3–5] Gaucher disease is a type of lysosomal storage disorder caused by a deficiency of lysosomal glucocerebrosidase (GlcCerase), a β-glucosidase that degrades glucosylceramide (GlcCer) into glucose and ceramide (Cer).[6] Accumulation of undegraded GlcCer in macrophages is responsible for the symptoms of this disease.[7,8] Current therapeutic approaches to Gaucher disease aim at reducing GlcCer levels either by inhibiting its biosynthesis with a GlcCer synthase inhibitor (*N*-butyl-1-deoxynojirimycin (NBDNJ), miglustat, Zavesca®),[9] by administration of Cerezyme®, a recombinant GlcCerase,[10] or by a combination of both strategies (Scheme 1). In all cases, treatments are extremely expensive and only applicable to non-neuronopathic types of the disease.[11,12]

Over the last few years, several research groups have tried to apply the concept of pharmacological chaperones to the design of high-affinity low molecular weight ligands able to specifically target GlcCerase.[13] In this context, we recently reported on a series of aminocyclitol derivatives as specific GlcCerase inhibitors with potential applications as pharmacological chaperones.[14,15] In this paper we wish to present our preliminary results along this line. The aminocyclitols described



Scheme 1. Current therapeutic approaches to Gaucher disease.

in this study (compounds **1a**–**1o**) are shown in Figure 1. They have been synthesized following our previously reported regio and stereoselective opening of enantiopure conduritol B epoxide **2**[14,16] (Scheme 2) and are representative of three different structural types: a) *N*-alkyl substituted aminocyclitols (**1a**–**1g**), b) *N*-arylalkyl substituted aminocyclitols (**1h**–**1k**), and c) *N*,*N*-disubstituted aminocyclitols (**1l**–**1o**).



a: R^1= H
b: R^1= C_4H_9
c: R^1= C_7H_{15}
d: R^1= C_8H_{17}
e: R^1= C_9H_{19}
f: R^1= $C_{10}H_{21}$
g: R^1= $C_{16}H_{33}$
h: R^1= $(CH_2)_2Ph$
i: R^1= $(CH_2)_4Ph$
j: R^1= CH_2CHPh_2
k: R^1= $(CH_2)_2CHPh_2$
l: R^1=R^2= C_8H_{17}
m: R^1= C_8H_{17}; R^2= $(CH_2)_2Ph$;
n: R^1=R^2= $(CH_2)_2Ph$
o: R^1= $(CH_2)_2Ph$; R^2= COC_7H_{15};

Figure 1. Aminocyclitols described in this study.

Compounds were evaluated as inhibitors of recombinant GlcCerase (Imiglucerase, Cerezyme®, from Genzyme), as shown in Table 1.

The most active inhibitors in this system were found among aliphatic *N*-alkyl aminocyclitols (**1c**–**1g**), where a clear correlation between lipophilicity (chain length)[17] and inhibitory activity was observed (Table 1, see also Figure 2a).[18] Inhibitory ac-

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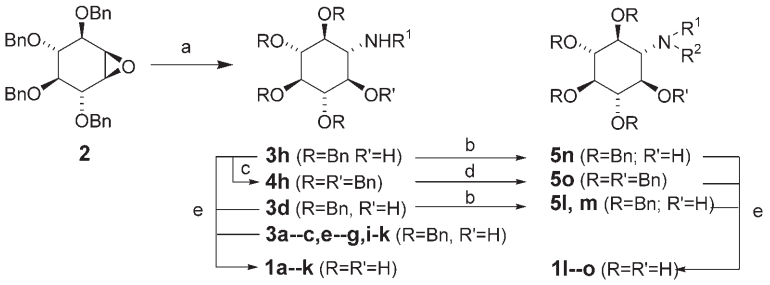
Supporting information for this article is available on the WWW under http://www.chemmedchem.org or from the author.



Scheme 2. Synthesis of aminocyclitols **1**: a) R^1NH_2, 2 N $LiClO_4$, CH_3CN (73–90%); b) $PhCH_2CHO$ (for **5n** and **5m**) or $C_7H_{15}CHO$ (for **5l**), $NaBH_3CN$, AcOH, MeOH (87–91%); c) BnBr, THF, NaH (95%); d) $C_7H_{15}COCl$, DCM, RT (82–87%); e) 1 M BCl_3 heptane, DCM, −78 °C (quant). See Figure 1 for R^1 and R^2 groups.

Table 1. Inhibitory activity of aminocyclitols over Imiglucerase.[a]

Compd	% activity (1 mM)	Imiglucerase IC_{50} [μM]	Imiglucerase K_i [μM]
1a	95	–	–
1b	21	99.4	39.4
1c	11	29.1	15.5
1d	3	18.2	10.4
1e	3	3.9	1.9
1f	2	1.8	0.3
1g	3	1.2	0.4
1h	18	76.8	24.1
1i	3	27.6	8.5
1j	6	30.7	14.4
1k	3	17.1	7.2
1l	5	41	17.2
1m	4	75	14.1
1n	6	52	15.6
1o	10	70.5	20.7

[a] Cerezyme®, Genzyme.

tivity for amines **1e**, **1f**, and **1g** was remarkable, with K_i values in the low μM range.[19] The presence of a distal aromatic moiety (compounds **1h**–**1k**) as well as *N,N*-disubstituted aminocyclitols (compounds **1l**–**1n**) also led to acceptable inhibitors. Interestingly, tertiary amide **1o** showed a K_i similar to that of the structurally related tertiary amine **1m** (Table 1 and Figure 2a). In all cases, active aminocyclitols showed a competitive inhibition pattern, as illustrated in Figure 3 for **1e**. A similar inhibition profile was obtained using rat liver lysosomes, although with higher IC_{50} values (see Supporting Information).

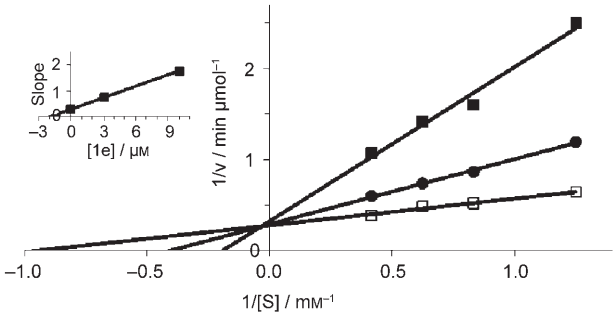


Figure 3. Lineweaver–Burk plot for **1e** with Imiglucerase. Empty symbols (control), full circles (3 μM), full squares (10 μM).

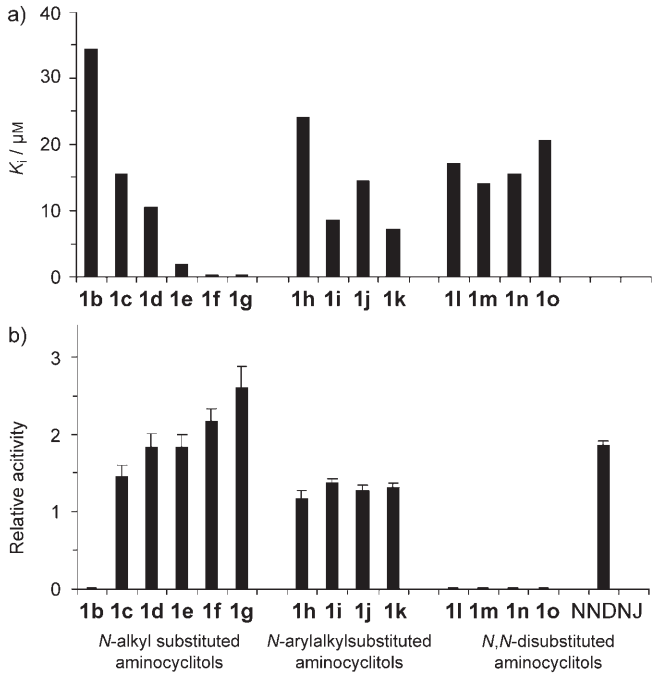


Figure 2. a) Activity of aminocyclitols on Imiglucerase (K_i values). b) Chaperone effect at 100 μM of aminocyclitols **1a**–**1o** and NNDNJ after thermal denaturation for 60 min.

Interestingly, GlcCerase inhibition by the above aminocyclitols proved to be specific as they were devoid of activity on commercial yeast α-glucosidase and sweet almond β-glucosidase as well as lysosomal acidic α-glucosidase (see data in Supporting Information). Compounds were not active against microsomal GlcCer synthase, except **1i**, **1j**, **1k**, and **1l** that inhibited this enzyme at high concentrations, exhibiting IC_{50} values of 880, 1170, 580, and 139 μM, respectively.

Aminocyclitols **1b**–**1o** were evaluated as pharmacological chaperones on the basis of their ability to induce the recovery of recombinant GlcCerase activity after thermal[20] or urea-induced enzyme denaturation.[21] In our hands, both experiments afforded comparable results (see Supporting Information) and, in all cases, the chaperone activity of the aminocyclitols was challenged against that of *N*-(*n*-nonyl)deoxynojirimycin (NNDNJ), a well-known pharmacological chaperone for GlcCerase.[20] Recovery of Imiglucerase activity was measured in the presence of increasing concentrations of aminocyclitol at different time incubations under thermal denaturation conditions, as illustrated in Figure 4 for compound **1e**.

This chaperone effect was also observed for other aliphatic *N*-alkyl substituted aminocyclitols described in this study. Some of our compounds showed a potent chaperone effect, comparable to or even higher than that of NNDNJ[20] (Figure 2b). In-

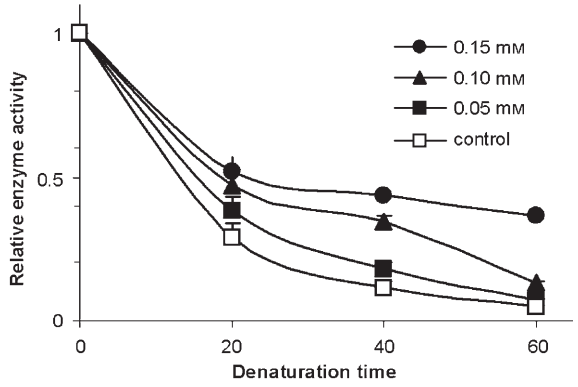


Figure 4. Imiglucersase activity after thermal denaturation (48 °C) in the presence of increasing concentrations of **1e**. Enzyme activity is reported relative to unheated enzyme.

terestingly, this chaperone effect increased gradually with the length of the aromatic chain. However, contrary to the inhibition pattern shown above, all *N*-arylalkyl substituted aminocyclitols tested (**1h**–**1k**) caused a similar chaperone effect (around 1.2 times), whereas *N*,*N*-disubstituted aminocyclitols (**1l**–**1o**) did not protect the enzyme against thermal inactivation. These results are indicative of different structural requirements for GlcCerase inhibition and the chaperone effect for this kind of aminocyclitol. Finally, the cytotoxicity of *N*-alkyl substituted aminocyclitols in A549 cells paralleled their lipophilicity. Thus, compounds **1a**–**1e** were nontoxic at concentrations up to 200 μM, whereas **1f** and **1g** were toxic (LD_{50}) at 75 and 25 μM, respectively. A similar toxicity range was also found for *N*-arylalkyl substituted aminocyclitols. Thus, **1h**–**1j** were cytotoxic at 70–75 μM and **1k** at 25 μM.

In summary, the aminocyclitols described herein represent new structural types of compounds with promising chaperone-like properties on GlcCerase, a defective enzyme associated with Gaucher disease. Further studies to improve cell tolerance, disclose the key structural motifs required for the chaperone activity, and confer selectivity against different point mutations of the defective enzyme are currently underway in our group and will be reported in due course.

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Keywords: chaperones · cyclitols · gaucher · glycolipids · glucocerebrosidase

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