Pharmacological Chaperones for β-Galactosidase Related to G\textsubscript{M1}-Gangliosidosis and Morquio B: Recent Advances

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Abstract: A short survey on selected β-galactosidase inhibitors as potential pharmacological chaperones for G\textsubscript{M1}-gangliosidosis and Morquio B associated mutants of human lysosomal β-galactosidase is provided highlighting recent developments in this particular area of lysosomal storage disorders and orphan diseases.

Keywords: Carbohydrates, Inhibitors, Lysosomal β-Galactosidase, Lysosomal storage disease, Pharmacological chaperone

1. Introduction

The lysosome is the waste management organelle of the cell and part of a larger network of autophagy-, endocytosis-, and salvage processes, the “greater lysosomal system”.\textsuperscript{[1]} It harbours a large number and variety of hydrolases which are responsible for the degradation of glycolipids, glycoproteins, as well as homo- and heteroglycans, just to mention carbohydrate containing structures. Mutations in genes encoding for these lysosomal carbohydrate processing enzymes may affect the formation of misfolded proteins in the endoplasmic reticulum (ER) that are consequently targeted for recycling by the cell’s quality control mechanisms. Hence, the misfolded proteins never arrive at the lysosome or may provide catalytically incompetent mutants which reach the lysosome but are unable of processing their substrates at physiologically necessary levels. Either results in substrate deposits in the lysosome and disturbed feed-forward cycles by the downstream lack of all other consecutive intermediates and final degradation products. Such enzyme deficiencies lead to diseases collectively coined as lysosomal storage disorders following a concept proposed in 1963.\textsuperscript{[2]} Despite the fact that due to their individual rarities (typically 1 in 40,000 to 1 in 1,000,000 live births), their combined incidence may range around 1 : 6,000 to 1 : 8,000.\textsuperscript{[3,4]}

Several different strategies for disease management have been developed and are available for selected forms of lysosomal storage diseases or undergo currently clinical trials in different stages. The most important ones a described below.

Enzyme replacement therapy (ERT)\textsuperscript{[5–7]} involves biweekly infusions of recombinant enzyme at various dosages, depending on the disease and side effects of this treatment. Bone disease symptoms such as in Gaucher disease are apparently not reversible and the blood-brain barrier has remained a problem for enzyme replacement therapy of lysosomal disorders with neuronopathic course.

Substrate reduction therapy (SRT)\textsuperscript{[8–10]} involves the application of reversible small molecule inhibitors to reduce the upstream formation of the incompetent enzyme’s substrate. Less frequently exploited are experimental treatment methods including bone marrow transplantation (BMT), haematopoietic stem cell transplantation (HSCT), induced pluripotent stem cells (iPSCs) and gene therapy (GT),\textsuperscript{[11–18]} the latter having already reached clinical stages.
Recently, so-called chaperone-mediated therapy (CMT) has become a highly promising area and, thus, a field of many intense research endeavours. Early hypotheses of “enzyme manipulation therapy” were proposed by Desnick and co-workers.\(^{[19,20]}\) The current concept of CMT was established by Fan\(^{[21]}\) two decades ago and has attracted tremendous interest since.

This therapeutic approach\(^{[22–43]}\) features the application of small, active site directed molecules (usually – but not in all cases – powerful competitive inhibitors of the enzyme under consideration). These, in sub-inhibitory concentrations, serve as templates for the correct folding of the therapy-susceptible freshly synthesized enzyme mutant and its transport from the ER to the lysosome bypassing the endoplasmic-reticulum-associated protein degradation (ERAD) shunt of quality control and degradation. Upon arrival, by diffusion or pH value change, most such inhibitors easily exit the active site which is now available for the respective substrate, albeit at lower turn-over numbers than in the corresponding “healthy” wild-type enzyme. As a rule of thumb, an activity increase of threefold and an activity of at least 10\% of the wild type are the thresholds for a positive chaperone effect as would be required at minimum for homeostasis and reduction of substrate deposits. Human lysosomal β-galactosidase (EC 3.2.1.23) is a retaining GH 35 exo-glycosidase which removes the outermost β-galactopyranosyl residue from the gangliosides \(G_{M1}\) and \(G_{A1}\), thus providing \(G_{M2}\) and \(G_{A2}\)-gangliosides as substrates for \(N\)-acetyl-hexosaminidases in the subsequent degradation step.\(^{[44]}\)

The encoding gene (\(GLB1\)) is located on chromosome 3 (3p21.33).\(^{[45]}\) The protein exists in two isoforms (76 kDa, 677 amino acids; 60.5 kDa, 546 amino acids) and has seven possible \(N\)-glycosylation sites two of which are indeed glycosylated (N464, N555). The enzyme forms a complex with α-neuraminidase and lysosomal protective protein/cathepsin A.\(^{[46]}\)

Transportation-challenged or catalytically incompetent β-d-galactosidase mutants, of which more than 160 are known to date,\(^{[47]}\) are the cause of neurodegenerative \(G_{M1}\)-gangliosidosis and of bone-damaging Morquio B disease, a mucopolysaccharidosis.

\(G_{M1}\)-Gangliosidosis (Online Mendelian Incidence in Men (OMIM): Phenotype: #230500, type I, infantile; 230600, type II, juvenile; 230650, type III, adult) and Morquio B disease (OMIM #253010, one of eleven mucopolysaccharidoses) are caused by mutation of the \(GLB1\) gene at chromosome (3p21.23) and characterized by the absence of catalytically competent β-galactosidase. Type I is a neurosomatic disease with rapidly progressing neurologic deterioration, visceromegaly and generalized dysostosis. More attenuated juvenile and adult forms present progressive neurologic disease in childhood or early adulthood caused by predominant storage of glycolipids in neuronal tissues with minor or absent dysmorphic changes.\(^{[48]}\) Neuronal damages are not primarily caused by

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**Figure 1.** Human lysosomal β-galactosidase (EC 3.2.1.23) catalyzes the transformation from \(G_{M1}\) to \(G_{M2}\)-ganglioside by removal of the terminal α-galactose unit.
deposition of unprocessed G\textsubscript{M1}-ganglioside but by secondary effects to the unfolded protein resulting in the up-regulation of chaperones and apoptotic factors.\[49\]

Whereas G\textsubscript{M1}-gangliosidosis is a neurodegenerative disorder, Morquio B is caused by the lack/failure of this $\beta$-galactosidase in the degradation cycle of glycosaminoglycans and is mainly associated with bone damage (“skeletal phenotype”). In case of some mutations, it is currently still impossible to predict the phenotype - neuronal damage versus bone damage - from the genotype.\[50\]

G\textsubscript{M1}-Gangliosidosis occurs in 1 : 100,000 births with considerably higher incidence in geographically or culturally isolated communities, for example, in southern Brazil (1 : 13,000)\[51\] or Malta (1 : 3,700).\[52\] The rare Morquio B disease is prevalent in 250,000 to 1,000,000 live births.\[40\]

For both, Morquio B disease as well as G\textsubscript{M1}-gangliosidosis, there is currently no cure available. Traditional treatment includes attempted relief from pain and reducing symptoms as well as family counseling.\[53,54\]

In contrast, for non-neuronopathic disorders, for example, certain forms of Gaucher’s (catalytic incompetence of $\beta$-glucocerebrosidase) as well as Fabry’s disease ($\alpha$-galactosidase activity deficit), approved recombinant enzyme preparations are available.\[55\] Enzyme replacement therapy with recombinant human $\beta$-galactosidase occurs in 1 : 100,000 births with considerably higher incidence in geographically or culturally isolated communities, for example, in southern Brazil (1 : 13,000)\[51\] or Malta (1 : 3,700).\[52\] The rare Morquio B disease is prevalent in 250,000 to 1,000,000 live births.\[40\]

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Consequently, great hope is currently focused on the concept of chaperone mediated therapy.

For chaperone design or optimization, the structures of the respective free enzyme as well as of enzyme-inhibitor complexes are most helpful tools. Of human lysosomal $\beta$-galactosidase, there are several crystal structures available: In 2012, Shimizu and co-workers provided the structures of the protein complex with $\alpha$-galactose (PDB 3THC) as well as of the complex with the powerful $\alpha$-galactosidase inhibitor 1-deoxy-$\alpha$-galactonojirimycin, (DGJ), 1,5-dideoxy-1,5-imino-$\alpha$-galactitol, 3, PDB 3THD).\[64\] (Figure 2)

Together with their Spanish collaborators, the structures of the enzyme with the benchmark chaperone carbasugar NOEV [$N$-(n-octyl)-4-epi-valienamine, 4, PDB 3WEZ] with iminosugars 6S-NBI-DGJ [5N,6S-(N'-butylimethylidene)-6-thio-1-deoxygalactonojirimycin, 5, PDB 3WF0], 6S-NBI-GJ [5N,6S-(N'-butylimethylidene)-6-thiogalactonojirimycin, 6, PDB 3WF1] and with NBTDGJ [N-(N'-butylthiocarbamoyl)-1-deoxy-galactonojirimycin, 7, PDB 3WF2] have been published in 2014.\[65\] (structure of compounds 4–7 in Figure 3) Furthermore, crystal structures of the enzyme mutant I51T (which is frequent amongst G\textsubscript{M1}-gangliosidosis patients in Japan) in complex with compound 5 (PDB 3WF4) and with $\alpha$-galactose (PDB 3WF3) have been reported.\[65\] Additionally, a bacterial GH35 $\beta$-galactosidase...
model featuring sufficient homology and similarity of the active site has recently been shown highly useful.\[66\]

2. Pharmacological Chaperones for G\(_{M1}\)-Gangliosidosis – Recent Developments

Quite a few \(\alpha\)-galactosidase inhibitors have been evaluated as potential pharmacological chaperones for G\(_{M1}\)-gangliosidosis and Morquio B related mutants. In particular, two main structural types, a carbasugar motif as well as a series of DGJ (3) related iminosugar derivatives have initially been investigated.

Based on historical development, cases of lysosomal storage diseases in the area as well as the considerable efforts of clinicians and families of patients, the pediatric unit of Graz General Hospital has become a hotspot for diagnosis, treatment and research, in particular in context with G\(_{M1}\)-gangliosidosis and Morquio B disease. In 2007, in a strategic meeting, gene therapy was defined as a long term goal for effective treatment beyond 2020 whereas on medium term, chaperone mediated therapy was chosen as interdisciplinary research project. To start with the latter, proven \(\alpha\)-galactosidase inhibitors derived from 1-deoxy-\(\alpha\)-galactonojirimycin (3) already available in Graz were screened for their potential as pharmacological chaperones for selected G\(_{M1}\)-gangliosidosis or Morquio B affected cell lines of resident patients and initial results were found encouraging enough to embark a synthesis project to provide leads and candidate molecules for pharmacological chaperoning of the more frequent human \(\beta\)-galactosidase mutants.

In 2001, Nanba and co-workers had already found that DGJ, 3 as well as its \(N\)-(\(n\)-butyl) derivative 2 at concentrations of 500 \(\mu\)M increased \(\beta\)-galactosidase activities in mouse fibroblasts.\[67\] In mutant reference cell lines R201C (juvenile) and in R457Q (adult) these increases were 5.4- to 6-fold for compound 3 and 4.8-5.4-fold for 2 at 500 \(\mu\)M. In addition, the latter inhibitor provided a 6.1-fold increase of enzymatic activity at 500 \(\mu\)M in the I51T (adult) cell line, a frequently occurring mutation in Japan. Increases of \(\beta\)-galactosidase activity were also noted with human cell lines. (Table 1, Supporting Information)

When the Graz project was initiated, the benchmark to be matched for G\(_{M1}\)-gangliosidosis chaperones, though, was the carbasugar and highly potent \(\beta\)-galactosidase inhibitor, NOEV 4, (\(IC_{50}=0.3\) \(\mu\)M with human \(\beta\)-galactosidase\[68,69\]) introduced as pharmacological chaperone by Suzuki\[70\] in 2003. (Synthesis Scheme 1, Supporting Information)

This compound also exhibited blood-brain-barrier permeability\[71\] which is a conditio sine qua non for any therapeutic agent designed for gangliosidosis treatment.

At concentrations up to 2 \(\mu\)M, this notable compound gave activity enhancements of 5.1-fold for R201C and of 4.5-fold for R201H cell lines (Table 1, Supporting Information) and proved three orders of magnitude more efficient than compounds 3 and 2. In extended studies, more than 20% of 94 screened mutants were found responsive to NOEV 4 chaperoning.\[72\] Various pathogenic proteins were found reduced after NOEV 4 treatment along with extended survival times.\[73-75\] (Table 1, Supporting Information)

One of the early promising compounds of the Graz group, a compound coined DLHex-DGJ, 8 (\(K_{i}=0.6\) \(\mu\)M), showed significant activity enhancements (5–6 fold) with chaperone-sensitive R201C as well as R201H cell lines at 20 \(\mu\)M and up to 18-fold increases at 500 \(\mu\)M.\[76\] (Table 1, Supporting Information) For its synthesis, a crafty approach by Steiner and co-workers was exploited based on the reductive amination/cyclization of 5-ulo-L-arabinohexose derivatives with \(N\)\(^2\)-protected lysine derivatives.\[77\]

A derivative, 9 (\(K_{i}=0.7\) \(\mu\)M), bearing the dansyl moiety at the terminus of the extended spacer arm gave nearly 6-fold activity increase with R201C at 10 \(\mu\)M and 9- to well over 10-fold increase between 20 and 500 \(\mu\)M.\[78\] (Table 1, Supporting Information)

By various modifications of the spacer arm and the terminal substituent, considerable activity enhancements could be achieved. For example, compounds with fluorous substituents such as 10 and 11 (\(K_{i}=0.8\) \(\mu\)M, each) showed similar effects at 5–20 \(\mu\)M (4- to 5-fold increase of activity with R201C). Furthermore, R201H and C230R mutants reacted favorably to both compounds with increases of 9- to 10-fold at 10 \(\mu\)M.\[79\] Noteworthy in this context was the unfortunate pronounced onset of inhibition at concentrations higher than 50 \(\mu\)M pointing to a much wider “therapeutic window” of compounds in the dansyl capped series. (Figure 4) (Table 1, Supporting Information)

Mahuran and co-workers demonstrated powerful effects for \(N\)-(\(n\)-nonyl)-DGJ, 12, (\(K_{i}=0.19\) \(\mu\)M) in two R201H cell lines that showed increases of 5-fold and 7-fold, respectively, at a concentration as low as 1 \(\mu\)M.\[80\] Accordingly, a feline G\(_{M1}\) model was responsive at concentrations between 0.7 and 2.1 \(\mu\)M. (Figure 4) (Table 1, Supporting Information)

Other researchers such as Siriwardena reported iminoalditol derived compounds including chain-extended iminoribitol 13, which also increased the activity of R201C mutants (6-fold at 400 \(\mu\)M).\[81\] Another compound of this type, provided by Martin and collaborators,\[82\] compound 14 (\(IC_{50}=0.8\) \(\mu\)M, bovine liver, 2.1-fold at 10 \(\mu\)M with R201C), behave similarly to isoimino sugar and potent inhibitor 4-\(\epsilon\)-isofagomine 15 (\(IC_{50}=0.4\) \(\mu\)M, \(\beta\)-galactosidase from human leucocyte lysate, 2.7-fold increase at 10 \(\mu\)M with R201C) and, thus, may be suspected to bind in the “isoimino sugar mode”\[83\] (Figure 5) (Table 1, Supporting Information)
Searching for NOEV (4) derivatives featuring improved pharmacological properties, Kuno and co-workers synthesized the 6-deoxy derivative 16 from (+)-proto-quercitol, which turned out a stronger inhibitor (IC$_{50}$ = 0.2 μM, bovine liver) than NOEV (4) itself (IC$_{50}$ = 2.6 μM, bovine liver). More recently, from the same starting material, a range of analogs lacking the hydroxymethyl side chain, for example compounds 17 (IC$_{50}$ = 120 μM), 18 (IC$_{50}$ = 15 μM), and 19 (IC$_{50}$ = 60 μM), were prepared. Despite IC$_{50}$ values that range one to two orders of magnitude higher than NOEV’s (2.6 μM), improved chaperoning properties were found with 19 in the lead (8.5-fold at 20 μM with R201C), albeit at 10-fold higher concentration than had to be applied for the parent compound 4. (Figure 6) (Table 1, Supporting Information)

The highly productive Seville group, following up on their own innovative work on the “sp$^2$-iminosugars” of the d-gluco series, provided structures clearly suitable as pharmacological chaperones. Ortiz Mellet and Garcia Fernandez prepared novel iminosugar-isothiourea hybrids such as compound 5, which was found to effect a 6-fold enhancement of β-galactosidase activity in the R201C cell line favourably comparing to NOEV (4) but above concentrations of 100 μM and without toxic side effects up to 640 μM. (Scheme 3, Table 1, Supporting Information)

Chaperone 5 was found a considerably weaker inhibitor than NOEV (4), however was active with an impressively broader spectrum of G$_{M1}$-gangliosidosis mutants than the former including 24 of 88 screened human mutants, in particular with I51T, a frequent mutation in Japan, as well as with G438T which both are known to be not responsive to NOEV (4). Gratifyingly, this chaperone was also capable of crossing the blood-brain barrier. An in-depth investigation into the differences between NOEV (4), and 6S-NBI-DGJ, 5 has recently been conducted revealing a possibly advantageous, more flexible binding mode of the latter, lacking interaction with Tyr133 in the catalytic site. At Graz, in early 2012 it became clear that despite all efforts and many years of experience in the iminosugar area, none of the DGJ-analogs prepared and screened by us thus far were able to match the reported properties of NOEV (4) or would be a viable lead into that direction. Consequently, structural requirements for compounds had to be re-defined. Of many possible choices, Fan’s finding that C-5α-chain extended derivatives of the powerful β-glucosidase inhibitor isofagomine exhibited highly superior inhibitory activities when compared with the parent compound, caught our attention. Mainly based on “educated guess” it was envisaged that 5α-modified 4-epi-isofagomines may also prove powerful reversible β-galactosidase inhibitors possibly suitable as pharmacological chaperones. So, it was decided to attempt introducing this particular structural feature, i.e., terminally functionalized medium chain lengths alkyl substituents into the 5α-position of d-galacto related 4-epi-isofagomine.
Due to synthetic difficulties and completely unexpected preparative pit-falls, work turned out frustrating and tedious. In 2014, endurance and preparative resilience were finally met with success and the first examples of 5α-chain extended derivatives of the powerful D-galactosidase inhibitor 4-epi-isofagomine (15) could be presented at the 97th Canadian Chemical Conference in Vancouver, BC.[93]

Contemporaneously, Martin and collaborators filed an important patent of related compounds[90] and subsequently, our innovative French colleagues as well as ourselves could present a range of very encouraging results. Indeed, this novel class of compounds has since provided some of the most powerful D-galactosidase inhibitors and pharmacological chaperones for Gaucher’s disease and Morquio B cell lines.

Olivier Martin and collaborators designed and synthesized both, (5αS)-5-C-pentyl-4-epi-isofagomine (20) as well as (5αR)-5-C-pentyl-4-epi-isofagomine (21, aka DO-1).[92,91] (Figure 7) (Scheme 4 and Scheme 5, Supporting Information) Their elegant approach was based on a 1-nitrohexane addition to the carbonyl group in benzyl 2,3-O-isopropylidene-4-ulo-β-D-erythopiranose and subsequent conversion of this intermediate into the desired iminosugar 20. The (5αR)-epimer 21 was found to be a particularly superior pharmacological chaperone for a large range of β-galactosidase mutants. This compound 21, a highly potent inhibitor of human lysosomal β-D-galactosidase ($IC_{50} = 8$ nM) enhanced the residual activities of 15 out of 23 screened patient cell lines (including R201C/R201C 15-fold at 10 μM, R201C/H281Y 18-fold at 10 μM, Q255H/K578R 20-fold at 10 μM) significantly to dramatically with up to more than 90% (H281Y/splicing, 35-fold enhancement at 10 μM) of wild type efficacy.[91] (Table 1, Supporting Information) Such impressive biological activities were also found with the compounds prepared in Graz.[93,94]

In the (5αR)-series, 5α-C-dansylaminobutyl-4-epi-isofagomine 24 (Figure 7) turned out as powerful inhibitor of β-D-galactosidas showing an $IC_{50}$ value of 0.21 μM with human lysosomal β-galactosidase. A preliminary report on its chaperoning properties revealed a 6-fold activity increase in the R201C cell line at 0.04 μM and a maximum effect of 15-fold increase at 5 μM. At higher chaperone concentrations, inhibitory effects reduced the enhancement gradually to a level of 11-fold at 25 μM.[93] In the corresponding (5αS)-series, epimer 22 was a weaker inhibitor by one order of magnitude ($IC_{50} = 2.67$ μM) and gave 9-fold enhancement in a concentration range between 20 and 100 μM. Interestingly, 5αS-analog 23 featuring a two carbons longer spacer arm, inhibited at a level comparable to 5αR-compound 24 with $IC_{50} = 0.38$ μM. As a chaperone compound 23 showed a 3.5-fold activity increase at 0.02 μM and reached a maximum of 10-fold at 2.5 μM. Similar to the latter, dose-dependent onset of inhibition of 23 gradually reduced the beneficial effect to 9-fold at 12.5 μM.[94] Additionally, the (5αR) configured 5α-C-dansylaminobutyl derivative 25 was found as most active inhibitor in this series with an $IC_{50}$ value of 0.094 μM against human β-galactosidase and maximum chaperoning effect of 10.7-fold increased activity in the R201C cell line at 0.5 μM.[96] In keeping with the trend in this series of compounds, the corresponding (5αS)-derivative 20 did not match the pronounced biological activity of its (5αR)-epimer. (Table 1, Scheme 6, Supporting Information).

Employing a Morita-Baylis-Hillman based protocol, synthetic access to chaperones with simple N-alkyl side chains such as 21 (DO-1) could be considerably improved[95] in terms of steps and time consumption. (Scheme 7, Supporting Information)

It should be noted that these compounds exhibit excellent selectivities without noteworthy side activities for lysosomal α-D-galactosidase and β-glucocerebrosidase.

These most encouraging data would clearly merit further research efforts but, despite all such efforts thus far, synthetic access has remained a challenge, in particular, considering necessary larger amounts of pure compounds for in-depth biochemical, toxicological and medical studies.

As a viable “plan B”, a very interesting but rarely cited group of highly functionalized cyclopentane derivatives, i.e., “carbasugars” was also studied in parallel to the isofagomines. At the turn of the millennium, pioneered by Vasella,[96] Jäger, as well as Reymond[97] and their respective co-workers, some very powerful glycosidase inhibitors were discovered in this compound class. In particular, β-D-galacto configured

![Figure 7. Structure of compounds 20–25.](Image)
amine 26 featuring a 4-bromobenzyl substituent at the nitrogen has been reported an outstanding β-galactosidase inhibitor.\(^{[97]}\) (Figure 8) A reasonably simple synthetic route based on Vasella's (2 + 3)-cycladdition method\(^{[96]}\) paired with high expectations of potent inhibitors and – hopefully – powerful chaperones prompted this most recent field of interest. Known amino(hydroxymethyl)cyclopentanetriol 27\(^{[97]}\) could easily be prepared and its amino group chemoselectively addressed employing conventional N-alkylation or reductive amination conditions to give compounds 26, 28–30.\(^{[66,99]}\) (Figure 8) (Scheme 8, Supporting Information)

These highly functionalized cyclopentane derivatives may be regarded products of formal homolytic extrusion of the corresponding hexose's ring oxygen and subsequent C–C-bond formation between C-1 and C-5. Consequently, all relative stereochemical relationships (cis/trans) between substituents-functional groups remain identical – d-galacto remains “d-galacto” – (naturally, there are changes of configurations of stereogenic centers according to CIP-rules) but interatomic distances of functional groups may vary dramatically from the pyranose chair's. Depending on the conformation, the distance between N-1 and O-2, for example, may be altered between +20% (N axial, hydroxymethyl equatorial) and more than −5% (N equatorial, hydroxymethyl axial) when compared to the pyranose form. The distance between O-2 and O-3 is increased between 30 and 40% relative to the situation in the pyranose, the N-1/O-3 distance is reduced between more than 40% (N axial) and around 5% (N equatorial) and the N-1/O-4 distance may vary between −40 % and around +2 %. These considerable ranges would be constricted by N-substituents larger than the adjacent hydroxymethyl group forcing the nitrogen into equatorial orientation but interactions with the active site, on the other hand, may overrule such thermodynamically favored situation.

With human lysosomal β-galactosidase, some of the new N-alkylated derivatives of 27 exhibited inhibitory activities in the range of previously known\(^{[97]}\) bromobenzyl derivative 26 (IC\(_{50}\) = 0.47 μM) and NOEV (4, IC\(_{50}\) = 0.51 μM). For example, N-hexyl as well as N-3-(phenyl)propyl analogs 28 and 29 had IC\(_{50}\) = 1.44 μM,\(^{[99]}\) with dansylaminohexyl derivative 30 being the most active compound from this series, thus far (IC\(_{50}\) = 0.4 μM). Consequently, inhibitor 30 was taken forward into cell based screening. In terms of chaperoning activities, this chaperone, with the R201C/R201C mutant, exhibited more than 4-fold activity enhancement at 0.1 μM and more than 6-fold at 1 μM with a maximum enhancement of 7.4-fold at 10 μM. Despite trailing isofagomines, these data were fairly encouraging for a starting point.\(^{[99]}\) (Figure 8) (Table 1, Supporting Information)

With R201H/H281Y (adult G\(_{M1}\)-gangliosidosis), both structural types, 24 as well as 30, show β-galactosidase activity increases of 6- to 7-fold at 0.1 μM and 9.1- to 10-fold at 1 μM with slight advantages for the isofagomine derivative.\(^{[66]}\) Interestingly, with Y333H/Y333H (juvenile G\(_{M1}\)-gangliosidosis), carbacycle 30 is the most potent chaperone with a maximum activity of 8.7-fold at 10 μM, followed by NOEV (4, 7.5-fold at 25 μM) and 24 (5.0-fold at 25 μM).\(^{[66]}\) (Table 1, Supporting Information)

3. Conclusion and Outlook

Over the past two decades, quite a few research groups have produced a noteworthy number of experimental pharmacological chaperones featuring high activities with a considerable variety of G\(_{M1}\)-gangliosidosis and Morquio B related β-galactosidase mutants. In particular, bicyclic neutral 1-DGJ derivatives such as 5 as well as several chain-extended 4-epi-isofagomines have been demonstrated worthwhile towards respective drugs. Highly functionalized cyclopentane derivatives were also shown efficient chaperones in vitro but screened representatives of this compound class unfortunately exhibit prohibitive side effects as potent β-glucocerebrosidase inhibitors. This might be attributed to their relatively high structural flexibility (vide supra). Provided modifications featuring more favorable selectivities can be discovered and methodology for their preparation can be improved, a possibly useful drug type might become available, as one redeeming feature of these compounds is their low cell toxicity.

New, improved routes of access will also be necessary for the isofagomine type chaperones. Such improved availability provided, next-generation compounds may result in effective drug manufacturing and viable agents in the not too distant future, filling the time gap until superior therapies such as gene therapy will become more widely available.

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