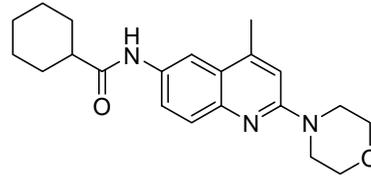


Project: Glucocerebrosidase Inhibitors (Chemotype 1)

Probe:

Chemotype 1:
N-(4-methyl-2-morpholinoquinolin-6-yl)cyclohexanecarboxamide

SID: 26753329
Internal IDs: NCGC00092410



PubChem Primary Bioassay Identifier (AID): 360

SID	IC50 (μM)	Antitarget	Selectivity*
26753329	0.035	Rice alpha glucosidase, Human alpha galactosidase Inactive @77uM	~2000

*Selectivity = antitarget IC50/target IC50

Assigned Assay Grant #: XO1-MH078932-01

Screening Center Name: NIH Chemical Genomics Center
Principal Investigator of Screening Center: Christopher Austin

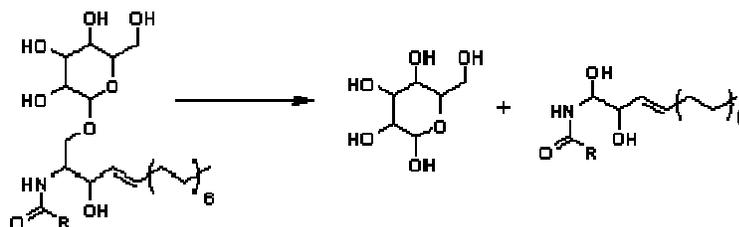
Assay Submitter & Institution: Ellen Sidransky, National Human Genome Research Institute

Assay or Pathway Target: Glucocerebrosidase enzyme assay

Assay provider information

Specific Aims: (1) Identification of inhibitors of glucocerebrosidase that may function as molecular chaperones to restore glucocerebrosidase activity. (2) Characterizing and optimizing probe compounds to enable therapeutic proof-of-concept studies in cells and animals.

Significance: Gaucher disease is caused by the inherited deficiency of a lysosomal enzyme, glucocerebrosidase (acid β-glucosidase, 1-N-acyl-sphingosyl-1-β-D-glucoside glucohydrolase, EC 3.2.1.45).



This enzyme hydrolyzes glucosylceramide, one type of sphingoglycolipid, to form ceramide and glucose. Glucosylceramide results from the lysosomal degradation of cell membranes (1;2). The deficiency of glucocerebrosidase activity in lysosomes results in the accumulation of glucosylceramide in macrophages and clinical manifestations including hepatosplenomegaly, anemia, bone lesions and thrombocytopenia. Based on the presence or absence of neuronopathic symptoms, Gaucher disease has been classified into three clinical types. Type-1 is nonneuronopathic Gaucher disease. Type-2 is acute neuronopathic Gaucher disease with an onset of a few months of age and rapidly progressing neurologic deterioration. Type-3 is chronic neuronopathic Gaucher disease with primarily visceral manifestations and some neurological findings, including abnormal eye movements, ataxia, seizures, myoclonus, or dementia (3;4).

Currently, two types of treatment options are available for the Gaucher disease. Enzyme replacement therapy is the supplementation of recombinant active enzyme, Cerezyme, (imiglucerase, Genzyme Co.). The intravenously infused enzyme reportedly is taken up into the cells via macrophage mannose receptors. The enzyme replacement therapy effectively improves the clinical symptoms of type-1 Gaucher disease and the quality of life of patients. However, the therapy is extremely costly (ranging from \$100,000 to \$750,000 per year for each patient), and is inconvenient due to bi-weekly intravenous infusions of the enzyme. Also there is no effect on neurological symptoms (inability to cross blood-brain barrier). Substrate reduction therapy uses a small molecule enzyme inhibitor, miglustat, to block the production of glucosylceramide, which is the substrate for glucocerebrosidase. The clinical efficacy and drug safety of substrate reduction therapy are still under investigation (5;6;7;8). Thus, new treatment options for Gaucher disease are still needed, especially cost effective treatments capable of addressing the neurological symptoms of the disease.

Rationale: In Gaucher disease, the deficiency of glucocerebrosidase activity in lysosomes is due to genetic mutations. Certain mutations appear to cause misfolding, instability and mistrafficking of this enzyme protein in endoplasmic reticulum (ER), which results in the endosomal degradation of the premature protein before it reaches its functional site, the lysosome. The pharmacological chaperone approach is a potential new approach to protein conformational disorders. Small molecule antagonists of the V2 vasopressin receptor can dramatically increase cell surface abundance of mutant V2 receptors by promoting their proper folding and maturation (9). The pharmacological chaperone effect of small molecule antagonists were also reported recently for the mutated gonadotropin-releasing hormone receptor, δ -opioid receptor and rhodopsin (10;11). There is preliminary evidence that pharmacological chaperones could be useful in lysosomal storage disorders including Gaucher, Sandhoff, Fabry and Tay-Sachs diseases (12). N-nonyl-deoxynojirimycin, a broad spectrum inhibitor of several lysosomal glycosidases at high concentrations, has been shown to increase the cellular activity of N370S glucocerebrosidase as well as the activity of wild-type enzyme at sub-inhibitory levels (13;14). It is suggested that the pharmacological chaperone stabilizes the glucocerebrosidase conformation to prevent misfolding and premature degradation, and helps its trafficking from the ER to its functional site, the lysosome.

Therefore, the pharmacological chaperone approach has therapeutic potential for Gaucher disease. A small molecule enzyme inhibitor used as a pharmacological chaperone offers a better therapeutic alternative because it not only can bind to the enzyme to stabilize the conformation and help to improve protein trafficking, but it might also be designed to cross the blood-brain barrier to be used as a potential therapy for neuronopathic Gaucher disease, where currently no efficacious therapy is available. However, specific and potent inhibitors

and activators for glucocerebrosidase are not currently available, and existing iminosugar inhibitors of glucocerebrosidase may have significant toxicities.

Screening center information

Assay Implementation and Screening

PubChem Bioassay Name: Glucocerebrosidase

List of PubChem bioassay identifiers generated for this screening project (AIDs):

AID	Target	Concentration	Bioassay type
1393	Glucocerebrosidase		Summary
360	Glucocerebrosidase	77µM – 5 nM	Primary qHTS
348	Glucocerebrosidase-p2	77µM – 5 nM	Confirmation assay
957	Coffee Bean alpha-Galactosidase at pH 4.5	77µM – 5 nM	Counterscreen
992	Human alpha-Galactosidase at pH 4.5	77µM – 5 nM	Counterscreen
997	Rice alpha-Glucosidase at pH 5.0	77µM – 5 nM	Counterscreen
998	Coffee Bean alpha-Galactosidase at pH 5.9	77µM – 5 nM	Counterscreen
1382	Glucocerebrosidase	77µM – 5 nM	Confirmation assay

Primary Assay Description as defined in PubChem:

Overview:

Beta-glucocerebrosidase catalyzes the hydrolysis of beta-glucocerebroside to glucose and ceramide. The inherited deficiency of beta-glucocerebrosidase results in Gaucher disease, which is characterized by a wide variety of symptoms including hepatosplenomegaly, anemia, thrombocytopenia, bony lesions and bone marrow infiltration with characteristic storage cells, known as Gaucher cells. There are also forms of the disorder affecting the central nervous system. Patients with the same genotypes can manifest with diverse clinical presentations and it is believed that improper folding and trafficking of beta-glucocerebrosidase may contribute to the phenotypes observed.

Low molecular weight molecules, acting as chaperones, may potentially restore trafficking of misfolded beta-glucocerebrosidase from the endoplasmic reticulum to the lysosomes, thereby enhancing functional lysosomal beta-glucocerebrosidase activity.

Using normal beta-glucocerebrosidase, an assay was developed to screen for small molecule inhibitors that could potentially act as molecular chaperones on the mutant forms [developed with NIH investigator Dr. Ellen Sidransky's laboratory]. Two pro-fluorescent substrates were chosen for two screens against a small molecule library. One beta-glucocerebrosidase assay used Resorufin beta-D-Glucopyranoside (Marker Gene Technology Inc.), generating a red fluorescent product. The second assay used Fluorescein di-beta-D-Glucopyranoside, generating a blue fluorescent product. The Km for the Resorufin beta-D-Glucopyranoside and Fluorescein di-beta-D-Glucopyranoside were determined to be 71.2 µM

and 855 μM , respectively. The IC_{50} values of Conduritol B Epoxide, a known glucocerebrosidase inhibitor, were determined to be 87.5 μM and 117 μM , respectively. The use of two difference pro-fluorescent substrates in the screens helped to eliminate false positives. All compounds were screened in a titration series resulting in AC_{50} s (e.g., IC_{50} s or EC_{50} s).

Assay principle and protocol:

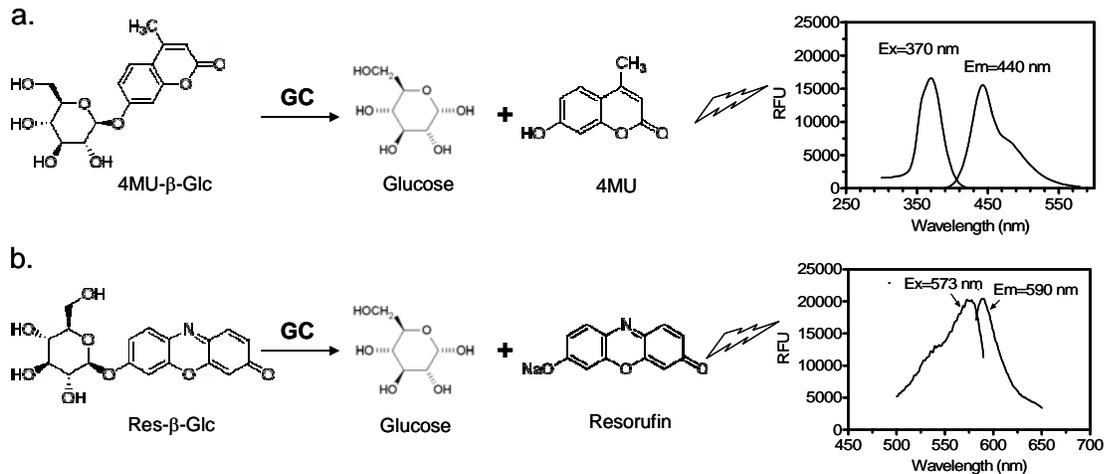


Figure 1: Principles of enzyme reactions and product spectrums of two GC enzyme assays. (a) The “Red” GC enzyme assay. The profluorescence substrate Res- β - glucopyranoside is hydrolyzed to form two products, glucose and resorufin with an excitation peak of 573 nm and an emission peak of 590 nm. This assay is used for the primary screen. (b) The “Blue” GC enzyme assay. The profluorescence substrate 4MU- β -Glc is hydrolyzed to form two products, glucose and 4MU with an excitation peak of 365 nm and an emission peak of 440 nm.

Using a purified human glucocerebrosidase (GC), a red fluorogenic enzyme assay was developed to screen for small molecule inhibitors. The enzyme hydrolyzes a substrate, resorufin beta-D-glucopyranoside (Marker Gene Technology Inc.), generating a red fluorescent product (Fig. 1a). The K_m for the Resorufin beta-D-Glucopyranoside was determined to be 35 μM . The IC_{50} value of Conduritol B Epoxide, a known glucocerebrosidase inhibitor, was determined to be 17.5 μM in this assay. The assay was miniaturized in 1536well plate format for primary screen (Table 1). A similar blue substrate GC enzyme assay was also optimized as a counter screen in compound confirmation stage to eliminate the interference of quenching effect of self-fluorescence of compounds.

Table 1: qHTS protocol (1536-well plate) for glucocerebrosidase assay.

Glucocerebrosidase red pro-fluorescence substrate Assay Protocol			
Step	Parameter	Value	Description
1	Reagent	2 uL	0.2 nM GC (final concentration)
2	Compounds	23 nL	5nM - 77uM in final concentrations (titration)
3	Reagent	1uL	30 uM Reso-Glu (final concentration)
4	Incubation	20 min	Ambient temp.
5	Detector	Ex 573 Em 600 nm	Viewlux plate reader

Step	Notes
1	FRD dispensing, buffer with 50 mM citric acid, 176 mM K ₂ HPO ₄ , pH 5.9, 0.01% Tween-20 and 10 mM sodium taurocholate
2	Pintool dispensing, 7 concentration titration in DMSO solutions
3	FRD dispensing, 90 uM resorufin beta-D-glucopyranoside
4	Enzyme reaction at room temperature
5	Viewlux kinetic read 30 sec intervals; fluorescence intensity

Center Summary of the Primary Screen:

The primary screen was performed with 7-concentration titration for a collection of approximately 53,000 compounds. A total of 603 active compounds were identified, 121 as activators and 482 as inhibitors. Of these, 255 met the cut-off criteria of AC50 < 10uM, Hill coef.>0.75 and maximal activity>50%. The hit rate was 0.48% (Table 2).

Table 2: Summary of active compounds from qHTS

AC50 value		# of compounds	Hit rate
< 1µM	Inhibitor	27	0.05%
	Activator	0	0
1-5 µM	Inhibitor	105	0.18%
	Activator	3	0.005%
5-10 µM	Inhibitor	123	0.21 %
	Activator	14	0.023%

Identification of Active Clusters: Following the qHTS, the concentration response curves (CRCs) data are subjected to a classification scheme to rank the quality of the CRCs as described in Inglese and co-workers¹⁸ (Figure 2, see also scheme 1) Briefly, CRCs are placed into four classes. Class 1 contains complete concentration-response curves showing both upper and lower asymptotes and r² values > 0.9. Class 2 contains incomplete CRCs lacking the lower asymptote and shows r² values greater than 0.9. Class 3 curves are of the lowest confidence because they are defined by a single concentration point where the minimal acceptable activity is set at 3 SD of the mean activity calculated as described above. Finally, class 4 contains compounds that do not show any CRCs and are therefore classified as inactive. Once an active set of compounds was identified, hierarchical agglomerative clustering with a 0.7 Tanimoto cutoff was performed by using Leadscape (Leadscape Inc., Columbus, OH) fingerprints. For each cluster, maximal common

substructures (MCS) were extracted, a manual step of MCS trimming was performed to create a list of scaffolds, and any overlapping scaffolds were abridged to a canonical set. Each scaffold was then represented as a precise definition to indicate descriptors such as the number of attachment points or the ring size variability. All filters were then relaxed to include the entire negative (class 4) assay data.

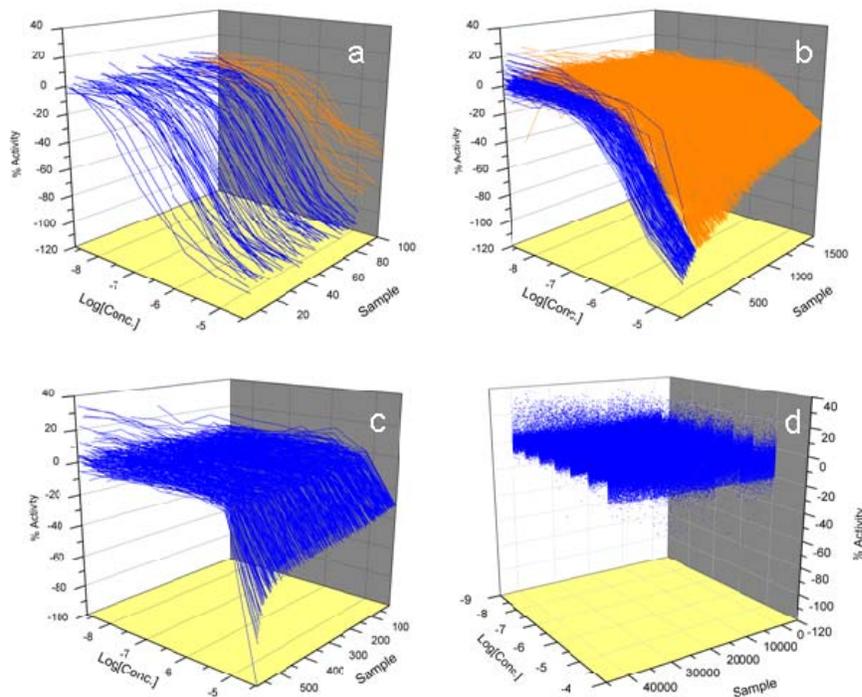
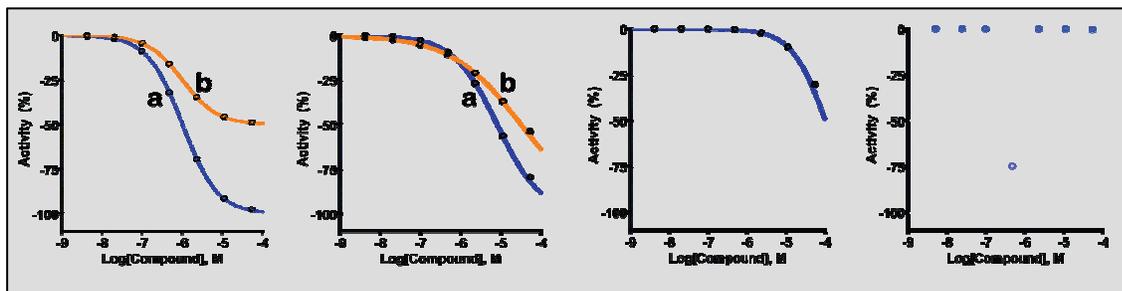


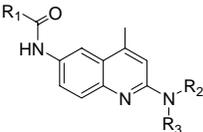
Figure 2. qHTS curve-fit data from AID 662 binned into curve classifications 1-4 (panels a-c) based classification criteria. Sub-classifications, e.g. 1a vs 1b, are color coded as blue and orange respectively, also see scheme 1 for additional details on qHTS curve classifications.



Scheme 1: Example classification scheme for assignment of resulting curve-fit data into classes. Curves fitting the following classification criteria are then used to establish SAR: Class 1 curves display two asymptotes, an inflection point, and $r^2 \geq 0.9$; subclasses 1a vs. 1b are differentiated by full ($>80\%$) vs. partial ($\leq 80\%$) response. Class 2 curves display a single left-hand asymptote and inflection point; subclasses 2a and 2b are differentiated by a max response and r^2 , $>80\%$ and >0.9 or $<80\%$ and <0.9 , respectively. Class 3 curves have a single left-hand asymptote, no inflection point, and a response $>3SD$ the mean activity of the sample field. Class 4 defines those samples showing no activity across the concentration range.

Active series: The cluster and SAR analysis of 238 inhibitors was carried as described above. The cluster analysis produced 42 clusters and 52 singletons. A total of 30 inhibitors were selected for confirmation and SAR analysis. The initial analysis for one of three inhibitor classes, the aminoquinoline series is presented (Table 3).

Table 3: Aminoquinoline series

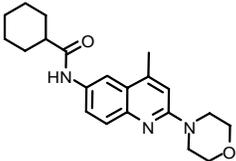
Cluster Number	Structure	Activity Range (μM)	No. Active compounds ($<10 \mu\text{M}$)	No. Compounds in NCGC collection
2 Quinoline		0.069 – 6.8	10	19

Probe Characterization

Prior Art for glucocerebrosidase: It has been demonstrated that the ability of iminosugars to act as chemical chaperones is dependent upon the nature of the specific mutant protein (13). Further, Kelly and coworkers have shown that temperature alterations have differential effects on the cellular trafficking of selected GC variants (14). These studies suggest that differing mutant forms of GC likely result in distinctive protein misfolding. Taken together with the vast number of clinically relevant GC mutations, these findings emphasize the value of developing divergent small molecule chemotypes to target alternate folded proteins.

Initial Probes for glucocerebrosidase: We purchased analogs of three lead structure series for the initial optimization of these active series (see additional probe reports for other chemotypes). The powder samples of active inhibitors and 21 analogs were purchased for confirmation and probe optimization. The chemical purity of these compounds was greater than 90% as verified by LC-MS. One purchased analog, SID 26753329 in the aminoquinoline series was determined to have an IC_{50} of 37 nM which was more potent than SID 46518504, the original lead compound. In this report, we focus on SID 26753329. Please refer to the attached appendix for more details.

Table 4: Initial glucocerebrosidase probes and confirmation data.

PubChem SID Internal ID	STRUCTURE	IC_{50} (M)	%Purity (PDA)	Ki (M)	CLogP	Supplier - ID
		E _{max}	%Purity (ELSD)			
		Hill Coef.	NMR			
SID 26753329 NCGC00092410		3.50E-09	~100	3.7E-08	4.204	ChemDi v- C095- 0200
		96%	~100			
		0.67	Yes			

Mechanism of inhibition of probe: The effect of GC inhibitor SID 26753329 on enzyme kinetics was studied in the enzyme assay to identify the mechanism of inhibition. SID 26753329 exhibited mixed inhibition (see following Lineweaver-Burk plot, Figure 3).

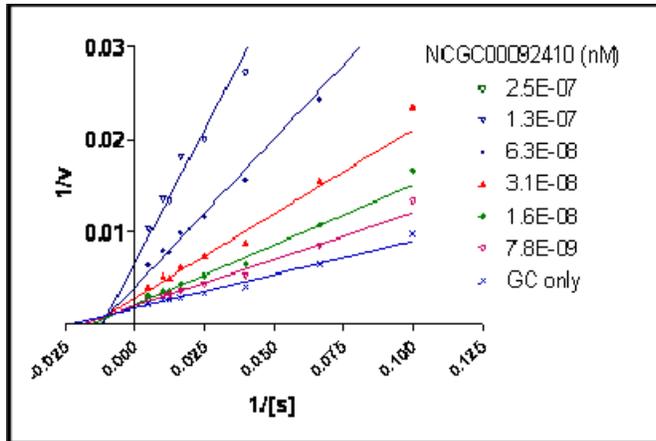


Figure 3: Inhibition mechanism of SID 26753329 (NCGC00092410)

Comparative data showing probe specificity for target: The selectivity of SID 26753329 was measured in three other hydrolases including α -glucosidase, α -galactosidase, and β -N-acetylglucosaminidase (β -N-acetylhexosaminidase, HEX). These enzymes are all lipid hydrolases and share the same metabolic pathway as GC. Deficiencies in α -glucosidase, α -

galactosidase and β -hexosaminidase result in Pompe disease, Fabry disease and Tay-Sachs or Sandhoff disease, respectively, all genetic disorders of lysosomal lipid metabolism similar to Gaucher disease (15). Substrates of these three enzymes labeled with the blue fluorophore 4-methylumbelliferone were used with a GC enzyme assay using the substrate 4-methylumbelliferyl- β -D-glucopyranoside (4MU- β -Glc) as control. These four enzyme assays were performed in parallel with the 1536-well plates (16).

Results showed that SID 26753329 did not inhibit the activities of α -glucosidase, α -galactosidase, or β -hexosaminidase at concentrations up to 77 μ M (following figure 4), demonstrating high selectivity to GC. In contrast, the iminosugar nonyl-DNJ was found to inhibit both GC and α -glucosidase, with IC_{50} values of 0.103 and 0.050 μ M, respectively (following figure). The IC_{50} values of the compounds in the GC enzyme assay using the blue fluorogenic substrate 4MU- β -Glc were similar to those using fluorogenic substrate Res- β -Glc (data not shown).

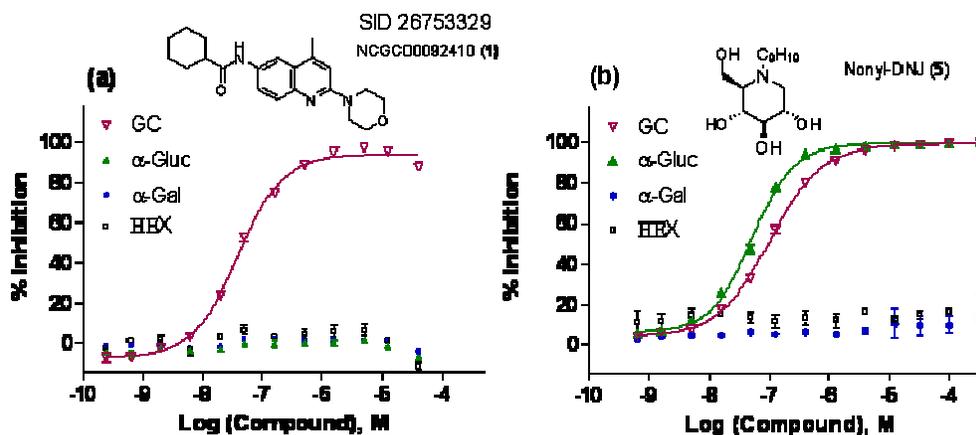


Figure 4: Selectivity of inhibitors with related hydrolases. Inhibitors were tested on GC, α -glucosidase (α -Glc), α -galactosidase (α -Gal), and β -N-acetylglucosaminidase (HEX). Data represent the results experiments performed with three replicates per sample.

Increase of glucocerebrosidase activity in Gaucher fibroblasts: Primary cells from Gaucher patients with N370S mutations were treated with 4.4, 13.3 and 40 μ M of SID 26753329, for 2 days in comparison with the wt. cells. A treatment of 40 μ M inhibitor resulted in a 90% increase of the mutant enzyme (N370S) activity in fibroblasts from Gaucher patients while the increase of normal enzyme activity was much smaller. This result indicates that these inhibitors may stabilize the mutant enzyme protein, help its proper folding/trafficking and thus increase its activity in the cell-based assay.

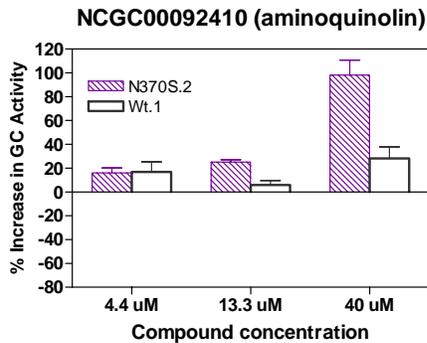
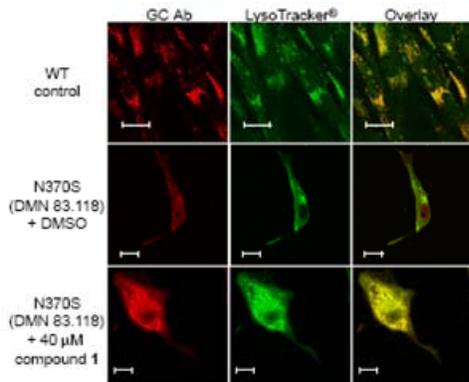


Figure 5: Effects of GC inhibitors on enzyme activity and trafficking in fibroblasts from patients with Gaucher disease (genotype N370S/N370S) and from control subjects (WT). Percentage change in GC activity. Fibroblast lines DMN 87.30 (N370S) and GM5659 (WT) treated with compounds at either 13.3 or 40 μ M were assayed for GC activity. Data represent three independent experiments performed with three replicates per sample. Error bars are SEM.

Increase of lysosomal glucocerebrosidase amount in Gaucher fibroblasts: The effects of the SID 26753329 (compound 1 below) and SID 847960 (compound 3 below; described in detail in separate probe report), an inactive aminoquinoline analog (compound 22 below; see ref 17) and the known iminosugar inhibitor nonyl-DNJ (compound 5) on the mutant GC trafficking were studied in fibroblast cell lines from patients with genotype N370S/N370S with a wild-type cell line as control. Dual labeling with polyclonal GC antibody and a lysosomal marker revealed that GC was localized to the lysosomes in the wild-type cells, whereas only a limited amount of enzyme reached the lysosome in a N370S mutant line in the absence of compounds (left panel in following figure). Treatment of two different N370S mutant fibroblasts with SID 26753329 (compound 1), and, to a lesser extent, SID 847960 (compound 3), resulted in increased co-localization (an increase of GC protein in lysosome), suggesting improvement of GC trafficking. This change was not seen in mutant cells treated with DMSO alone, compound 22, an inactive aminoquinoline analog, or with the known inhibitor nonyl-DNJ (compound 5) (right panel in following figure 6).

A.



B.

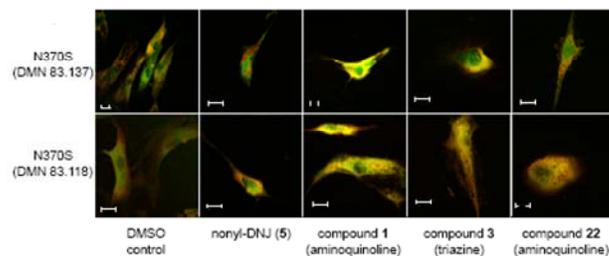


Figure 6: (A) Dual labeling with polyclonal GC antibody (GC Ab, red) and a lysosomal marker (LysoTracker, green) in untreated WT (GM 3348) and N370S (DMN 87.30) fibroblasts and in the N370S line treated with 40 μ M compound 1. Overlay images demonstrating colocalization (yellow) of

GC Ab with the lysosomal marker indicate potential improvement in GC trafficking. (B) Immunofluorescence staining of two N370S mutant fibroblast lines, DMN 83.137 and DMN 87.30, grown with 40 μ M compounds. Cells were costained with GC Ab and LysoTracker as in B. Overlay images are shown for cells treated with DMSO (control), **5** (nonyl-DNJ), active compounds **1**, **2**, and **3**, and **22**, an inactive compound. Although there is some lysosomal colocalization in both cell lines, compound **1** and, to a lesser extent, compound **3**, which significantly increased GC activity in the cell-based assay, show increased yellow fluorescence, demonstrating an increase of GC in the lysosomal compartment.

Recommendations for the scientific use of probe as research tool: SID 26753329 is a potent and selective inhibitor of glucocerebrosidase. The results indicate that it binds to a site/sites on the enzyme that may be different from the substrate binding site. For the chaperone experiment in cells, a "pulse-chase" experiment procedure should be used in order to see the increase in glucocerebrosidase activity. For example, the cells are treated with compounds for 2-3 days followed by a cell wash and incubation in medium for 3-5 hour to allow inhibitor dissociation from the enzyme before the measurement of enzyme activity.

Known probe properties:

Center summary of probe properties (solubility, absorbance/fluorescence, reactivity, toxicity, etc.) and recommendations for the scientific use of probe as research tool:

SID 26753329

Molecular Weight	353.45798 [g/mol]
Molecular Formula	C ₂₁ H ₂₇ N ₃ O ₂
XLogP	4.3
H-Bond Donor	1
H-Bond Acceptor	4
Rotatable Bond Count	3
Tautomer Count	2
Exact Mass	353.210327
MonoIsotopic Mass	353.210327
Topological Polar Surface Area	54.5
Heavy Atom Count	26

Has this compound been provided to the MLSMR:

SID 26753329 is in the process of being submitted. A close analog of SID 26753329 is available as MLS000044220.

Canonical SMILES:

CC1=CC(=NC2=C1C=C(C=C2)NC(=O)C3CCCCC3)N4CCOCC4

InChI:

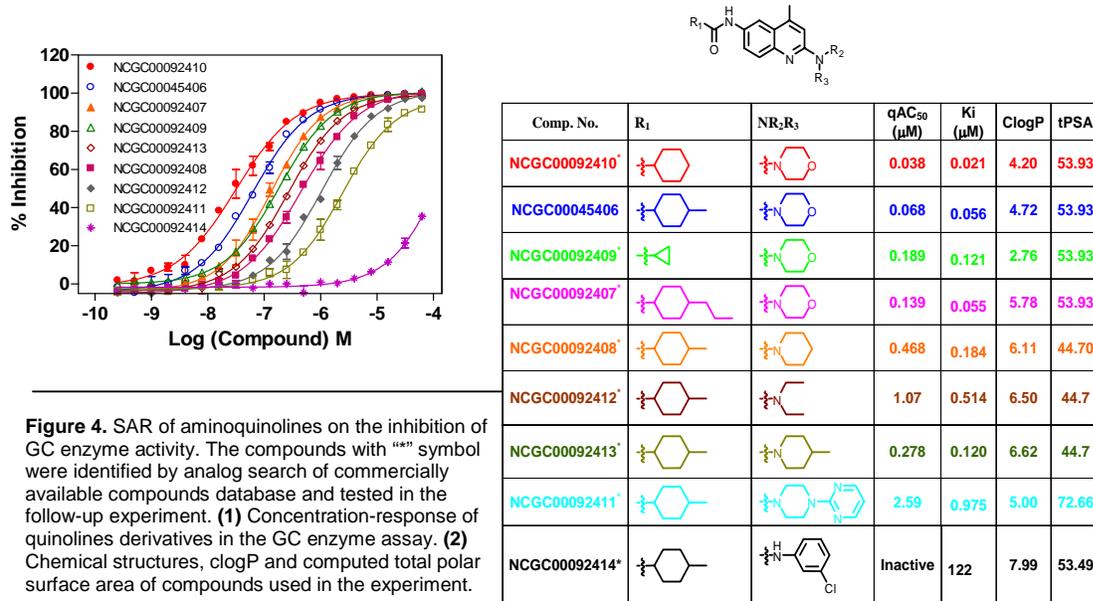
InChI=1/C21H27N3O2/c1-15-13-20(24-9-11-26-12-10-24)23-19-8-7-17(14-18(15)19)22-21(25)16-5-3-2-4-6-16/h7-8,13-14,16H,2-6,9-12H2,1H3,(H,22,25)/f/h22H

Compound preparation: Compounds are prepared in DMSO at 10 mM stock concentration.

Probe availability: Aliquots of SID 26753329 are available from the NCGC upon request.

Appendices-A

SARs of aminoquinoline series. The structure activity relationships (SAR) of SID 26753329 series was expanded with the purchased analogs.



Appendices-B

Bibliography

- (1) Cox TM. Gaucher disease: understanding the molecular pathogenesis of sphingolipidoses. *J Inherit Metab Dis.* 24:106-21 (2001).
- (2) Dvir H, Harel M, McCarthy AA, Toker L, Silman I, Futerman AH, Sussman JL. X-ray structure of human acid-beta-glucosidase, the defective enzyme in Gaucher disease. *EMBO Rep.* 4:704-9 (2003).
- (3) Sidransky E. Gaucher disease: complexity in a "simple" disorder. *Mol Genet Metab.* 83:6-15 (2004).
- (4) Grabowski GA. Recent clinical progress in Gaucher disease. *Curr Opin Pediatr.* 17:519-24 (2005).
- (5) Radin NS. Treatment of Gaucher disease with an enzyme inhibitor. *Glycoconj J.* 13:153-7 (1996).
- (6) Pastores, G.M. & Barnett, N.L. Current and emerging therapies for the lysosomal storage disorders. *Expert Opin Emerg Drugs* 10, 891-902 (2005).
- (7) Zimran A, Elstein D. Gaucher disease and the clinical experience with substrate reduction therapy. *Philos Trans R Soc Lond B Biol Sci.* 358:961-6 (2003).
- (8) Cox, T.M. Substrate reduction therapy for lysosomal storage diseases. *Acta Paediatr Suppl* 94, 69-75; discussion 57 (2005).
- (9) Morello, J.P. et al. Pharmacological chaperones rescue cell-surface expression and function of misfolded V2 vasopressin receptor mutants. *J Clin Invest* 105, 887-895 (2000).
- (10) Bernier, V., Bichet, D.G. & Bouvier, M. Pharmacological chaperone action on G-protein-coupled receptors. *Curr Opin Pharmacol* 4, 528-533 (2004).
- (11) Wiseman, R.L. & Balch, W.E. A new pharmacology--drugging stressed folding pathways. *Trends Mol Med* 11, 347-350 (2005).
- (12) Fan, J.Q. A contradictory treatment for lysosomal storage disorders: inhibitors enhance mutant enzyme activity. *Trends Pharmacol Sci* 24, 355-360 (2003).
- (13) Sawkar, A.R. et al. Chemical chaperones increase the cellular activity of N370S beta-glucosidase: a therapeutic strategy for Gaucher disease. *Proc Natl Acad Sci U S A* 99, 15428-15433 (2002).
- (14) Sawkar, A.R. et al. Gaucher disease-associated glucocerebrosidases show mutation-dependent chemical chaperoning profiles. *Chem Biol* 12, 1235-1244 (2005).
- (15) Vellodi, A. Lysosomal storage disorders. *Br J Haematol* 128, 413-431 (2005).
- (16) Urban, DJ et al. Development and Validation of Two Miniaturized Glucocerebrosidase Enzyme Assays for High-Throughput Screening. *Combi Chem High Thr Screen.* in press (2008).
- (17) Zheng et al. Three classes of glucocerebrosidase inhibitors identified by quantitative high-throughput screening are chaperone leads for Gaucher disease. *PNAS* 104(32): 13192-13197 (2007).
- (18) Inglese, J. et al. Quantitative high-throughput screening: A titration-based approach that efficiently identifies biological activities in large chemical libraries. *Proc Natl Acad Sci U S A* **103**, 11473-11478 (2006).