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GCase Activators

Novel β-Glucocerebrosidase Activators That Bind to a New Pocket at a Dimer Interface and Induce Dimerization

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Abstract: *Genetic, preclinical and clinical data link Parkinson's disease and Gaucher's disease and provide a rational entry point to disease modification therapy via enhancement of β-Glucocerebrosidase (GCase) activity. We discovered a new class of pyrrolo[2,3-b]pyrazine activators effecting both Vmax and Km. They bind to human GCase and increase substrate metabolism in the lysosome in a cellular assay. We obtained the first crystal structure for an activator and identified a novel non-inhibitory binding mode at the interface of a dimer, rationalizing the observed structure–activity relationship (SAR). The compound binds GCase inducing formation of a dimeric state at both endoplasmic reticulum (ER) and lysosomal pHs, as confirmed by analytical ultracentrifugation. Importantly, the pyrrolo[2,3-b]pyrazines have central nervous system (CNS) drug-like properties. Our findings are important for future drug discovery efforts in the field of GCase activation and provide a deeper mechanistic understanding of the requirements for enzymatic activation, pointing to the relevance of dimerization.*

Introduction

Aberrant processing of α-synuclein is thought to play a role in the development of neurodegenerative diseases such as Parkinson's disease (PD) and dementia with Lewy bodies (DLBs). Gaucher's disease (GD) is the most common lysosomal storage disorder caused by homozygous mutations in the β-Glucocerebrosidase gene (GBA1) which encodes GCase,[1,2] and patients or relatives have an increased risk of developing parkinsonism and α-synuclein pathology.[2,3] Heterozygous mutations in GBA1 are among the most common known genetic risk factors for PD and DLBs, however only a minority of GBA1 mutation carriers develop PD and DLBs.[4] Genetic loss of GCase function leads to accumulation of toxic α-synuclein in vitro and in vivo murine models as well as in iPS derived neurons from GD patients.[5] In addition, gain of GCase activity in the CNS (restoration, overexpression) leads to removal of α-synuclein in vitro and in vivo in both GD and PD models.[6]

Loss of function affects α-synuclein metabolism in the lysosome, however the exact molecular mechanism of this reciprocal relationship is still unclear.[5,7] In clinics, GCase dysfunction has been observed in PD patients with heterozygous mutations in particular brain areas.[8] Furthermore, reduced GCase activity has been found in post mortem samples of PD patients without GBA1 mutations, suggesting that a reduction in enzymatic activity, associated with impaired lysosomal function, have a key contribution to PD pathogenesis.[9] In addition, PD patients without GBA1 mutations were reported to have lower GCase activity as compared with healthy controls, indicating GBA1 is involved in the pathologic mechanisms of PD even in the absence of mutations.[10,11] Enzyme Replacement Therapy (ERT) (e.g Cerezyme) shows efficacy, however it can only be used clinically for non-neuropathic GD as it does not go to the brain.[12] Gene therapy, using adeno-associated virus (AAV) vectors to bring GCase in the brain, is currently being explored.[6a,b,13] Our understanding of the role of GCase in Parkinson pathogenesis remains incomplete,[14] nonetheless all available data suggest that therapeutics aiming at enhancing GCase activity are of high importance for the treatment of PD and DLBs.[6c,15] Altogether these data provide evidence that GCase enzyme dysfunction plays a key role in PD, and thus drug-like compounds that can pass through the blood–brain barrier restoring lysosomal GCase activity, would be highly relevant. Active site inhibitory chaperones or pharmacological chaperones (PCs), such as the iminosugar Isofagomine, bind to and stabilize the enzyme, allowing translocation of a correctly folded enzyme from the endoplasmic reticulum (ER) to the lysosome, releasing a catalytically active protein due to a reduced binding affinity at the lysosomal acidic pH.[16] To overcome the limitations of inhibitory chaperones

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and to balance against their functional inhibition,[16a] new allosteric modulators have been identified in several HTS campaigns.[17] Recently, non-inhibitory modulators tool compounds (NCGC00188758 & NCGC00241607) have been successfully studied across cellular models suggesting that they could be beneficial as treatments for PD and related synucleinopathies.[18]

Results and Discussion

In our drug discovery effort, we wanted to avoid the problems associated with an inhibitory chaperone in regard to the narrow balance between inhibition and activation that impacts the dose regimen.[16a] Therefore, we tried to identify non-inhibitory and brain penetrant modulators that could increase GCase lysosomal activity. We also thought that the understanding of the binding mode of activators was important for insights into their possible mechanism of action as modulators.

As previously shown for GCase modulators there can be a variability in potency across assays, in particular in cell-free assays.[17,19] Thus, we developed a cell-based activity assay to determine the increase in human GCase activity selectively. We confirmed afterwards in an orthogonal assay, whether putative cellular activators had the capability to bind to the enzyme.

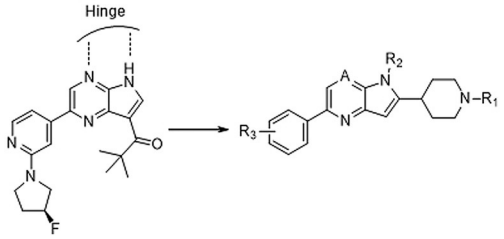
In the cellular assay we took advantage of a reported artificial cell-permeable lysosomal GCase substrate, 5-(pentafluorobenzoylamino) fluorescein di-β-D-glucopyranoside (PFB-FDGlu), measuring activity by its increased turnover in the acidic environment. In addition, two activators reported in the literature exhibited dose dependent increase of activity (see also supporting information).[17]

PFB-FDGlu has been used to monitor GCase activity in peripheral blood monocytes cells from Gaucher patients and to monitor efficacy after patient treatment with ERT.[20] In addition, it is used in cellular assays monitoring activity in living neurons.[21] Recently, it has been explored in PD patients as a possible biomarker by measuring wild type GCase activity in monocytes.[9a]

In a first round, 400 diverse heterocyclic compounds were selected to be tested on the cellular assay, using 3D shape similarity searches with published tool compounds and filtering by molecular properties.[22a] A subset was confirmed for specificity in the GBA1 knockout H4 cells. We observed that pyrrozolopyrazines reported in our labs as JAK and SYK inhibitors,[22b] were behaving as GCase specific activators. In order to avoid kinase activities, we performed a hit expansion eliminating the unwanted hinge binding motif needed for kinase inhibition, in a search for novel, selective and safer compounds.

From the initially tested compounds we observed SAR trends for compounds **1** to **3** (Table 1). The alternative non methylated core analog **3** (A = C, R_2 = H) appears to be slightly weaker than **2** (A = N, $R_2 = CH_3$) and the amide at R_1 on the piperidine (**2** vs. **1**) improves GCase activity in the enzymatic assay. Based on the observed trend we prepared a library of 150 compounds (Table 2).

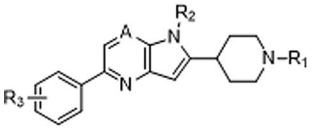
Table 1: Data from enzymatic assay.



Comp	R_3	A	R_1	R_2	EC_{50} (μM)[a]	EC_{20} (μM)
1	H	N	$-CH_3$	CH_3	45	13
2	H	N	$-COCH_3$	CH_3	9.5	2.3
3	H	C	$-COCH_3$	H	36	10

[a] EC_{50} concentration of a drug that gives half-maximal response.

Table 2: Data from enzymatic assay and key SAR for activators.



R_3 = Cl, F, CF_3, OCH_3,...
R_2 = CH_3, H (when A is C)
A= N, C
R_1 = -COR, SO_2R
R= CH_3, CH_2-CH_3, Pr, CH_2 cycloProp, CH-CH_2cycloProp

Cp	R_3	A	R_1	R_2	EC_{50} (μM)	EC_{20} (μM)[a]
4	o-Cl	N	H	CH_3	44	14
5	o-Cl	N	$-COCH_3$	CH_3	1.8	0.68
6	o-Cl	N	$-SO_2CH_3$	CH_3	2	0.6
7	H	N	$-SO_2CH_3$	CH_3	12.6	2.3
8	o-diCl	N	$-COCH_3$	CH_3	6.03	2.1
9	o-Cl	C	$-COCH_3$	H	4.7	1.3
10	o-Cl	C	$-COCH_3$	CH_3	3.3	0.8
11	o-Cl	N	$-CONCH_3$	CH_3	5.2	1.6
12	o-Cl	N	$-COCH_2CH_3$	CH_3	2.6	0.73
13	o-Cl	N	$-COCH_2$cyPro	CH_3	1.8	0.54
14	p-OCH_3	N	$-COCH_3$	CH_3	3.8	1.3
15	oCl,pOCH_3	N	$-COCH_3$	CH_3	2.4	0.5
16	o-p-diCl	N	$-COCH_3$	CH_3	1.2	0.35
17	oCl,pOCH_3	N	-COPropyl	CH_3	0.640[b]	0.147

[a] literature data indicates GBA-PD brain show a (≈ −40%) reduction of activity and idiopathic Parkinson's (20%), thus a < 50% increase of enzymatic activity is potentially enough for therapeutic effect.[23] [b] At high concentrations the compound precipitates.

In terms of aromatic substitution (R_3), initially the biggest potency improvement was observed for the mono ortho-chloro analogs (compounds **5** vs. **2** and **7** vs. **6**). However, the di-ortho-dichloro analog was detrimental to activity (**8** vs. **5**). The free piperidine amine (R_1 = H) is not active (**4**), as well as the methylated piperidine (**1**), however the R_1 amide group is important for enzymatic activity as well as a sulfone that follows closely in potency (**6** vs. **5**, **7** vs. **2**). A urea derivative (**11** vs. **5**) is less active, however still tolerated. The size of the alkyl group at R_1 is less predictable in term of improving potency (**5** vs. **12**,**13**) and can provide some contribution to activity (**15** vs. **17**). The core analogs for compounds **2**, **5** (**3**, **9**, **10**), in the subclasses with A = C, R_2 = H, or A = C, $R_2 = CH_3$, did not contribute to the enzymatic activity. With respect to the aromatic substitution at R_3 the p-methoxy group was well

tolerated (**14** vs. **2**), as well asthe combination of an ortho and para substitution (**15**, **16** vs. **2**).

Binding to GCase, employing high enzymatic concentration to ensure GCase dimerization, was confirmed for selected compounds using surface plasmon resonance (SPR) dose-response experiments at ER pH 7.2 and at lysosomal pH 5.2. Interestingly, competition experiments with the inhibitory chaperone Isofagomine confirmed binding, however not at the active site but at a different pocket (Table 3) (see also supporting information).

Table 3: SPR direct binding affinities (K_D) measured at pH 7.2 and 5.2 and competition data with Isofagomine.

Comp	KD ± σ (μM) (pH 7.2)	KD ± σ (μM) (pH 5.2)	Competition with isofagomine
5	3.4 ± 1.5	3.6 ± 0.89	No
14	3.8 ± 1.1	3.8 ± 1.7	No
16	1.3 ± 0.26	1.3 ± 0.20	No
17[a]	1.8 ± 0.53	1.6 ± 0.19	No

[a] this compound shows partially nonspecific binding (up to 20% of total resonance signal intensity).

To identify the binding site of activators and to characterize its interactions with human GCase we co-crystallized the protein with compound **14**.

The crystals of GCase with **14** belong to space group $P2_12_12_1$ and diffract to a resolution of 1.85 Å. The asymmetric unit of the crystal is assembled by four GCase monomers. Based on analysis with PISA[24] they form a pair of dimers with the active sites facing each other and located towards the dimer interface. The dimer organization resembles the interface I described by Gruschus.[25]

The dimer interface is mainly formed by loops 1, 2 and 4 of the TIM barrel (Figure 1, Supplementary Figure 14). These loops, surrounding the active site, are suggested to provide the binding surface of the "ceramide" part of the substrate.[26] Compound **14** binds close to the active site in the dimerization interface of human GCase (Figure 1 and Figure 3), inducing large changes in the conformation of loop1-3 of the GCase monomer A and loop1-2 of the monomer B. In detail, in monomer A of the GCase dimer, the backbone and side chains of amino acids 312–319 (loop1), 344–350 (loop2) and 394–399 (loop3), move with largest difference observed for Trp348, crucial for binding of **14**. In comparison to GCase pdb entry 1OGS there is a movement of ≈13 Å taking the position of the Trp348 Nε atom as reference (Supplementary Figure 15). This movement results in the positioning of Lys346 into the active site with a distance of 2.8 Å to the carboxyl group of the catalytic Glu235. Furthermore, Glu235 is hydrogen bonded to Tyr313 and His311. In monomer B, largest changes are observed for amino acids 343–352 (loop2) and 315–320 (loop1) with a difference of 11 Å for Asp315. The overall structure of GCase remains unchanged upon ligand binding and is highly similar to other previously published GCase structures[26,27] (Supplementary Figure 16). Due to the loop rearrangement and the binding contributions from compound **14** across the GCase dimer interface, only one ligand molecule per dimer can be bound.

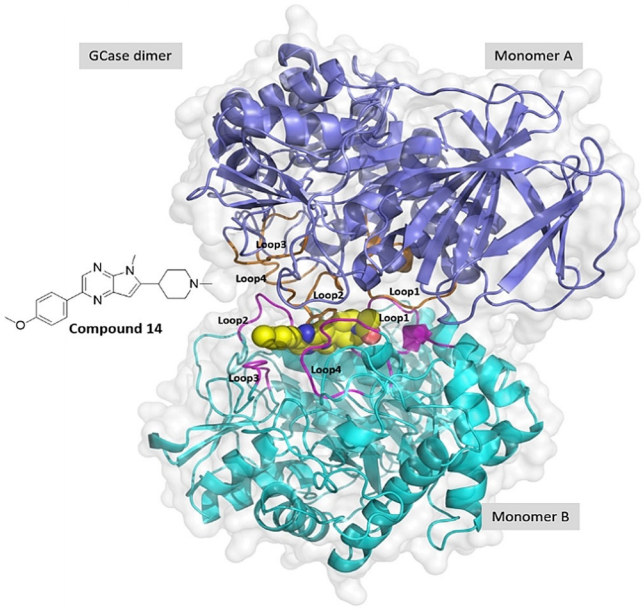


Figure 1. Activator 14 (in ball and stick) binding to the dimer interface of GCase monomer A (shown in blue) and monomer B (shown in cyan). Loops surrounding the active site are in orange and magenta (monomers A and B).

The activator **14** is well positioned in the binding pocket which is mainly hydrophobic (Figure 2) with some additional water-mediated polar interactions. In more detail, the compound binds to the transient pocket via a π-π stacking interaction of the central pyrrolo[2,3-b]pyrazine or 4,7-diazaindole ring, with the side chain of Trp348 of monomer A (Trp348A). This stacking interaction is stabilized by additional vdW interactions (in yellow and orange) between the N-methyl substituent of the central core motif and the side chain of Tyr313B as well as between the piperidine ring and the side chains of Trp348A, Gln284B and Leu314B.

The carbonyl group of the terminal amide (R_1) is engaged in H-bond interactions to a water network connecting several charged and polar amino acids (Asp283B, Ser237B). The

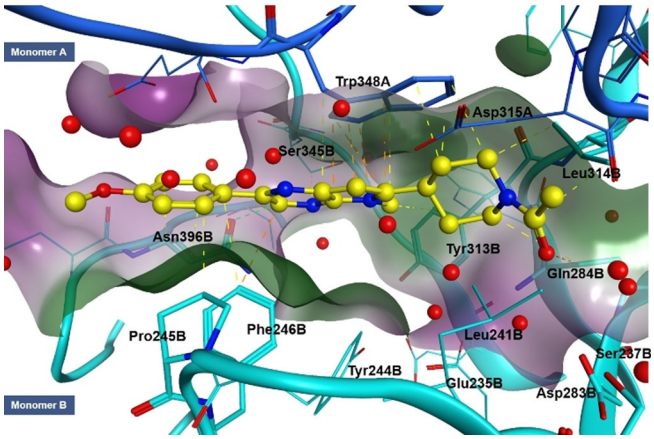


Figure 2. Amino acids involved in binding, hydrophobic (green) and hydrophilic (magenta) pocket binding surface with ligand and different monomers (shown with blue and cyan loops).

aromatic ring substituted by R_3, is engaged in vdW interactions with Asn396B, Pro245B and Phe246B with R_3 pointing towards the solvent (Figure 2).

At the central core the replacement of one nitrogen (A = C) at the same side of the indole is tolerated and releases a displaceable water. The nitrogen at the opposite side, however, cannot be replaced by a carbon atom, because the aromatic nitrogen is engaged in a water network (Figure 3A). The methyl substituent of the indole ring ($R_2 = CH_3$) occupies well the hydrophobic environment (in green) where the core sits and where there is no acceptor partner for a free NH.

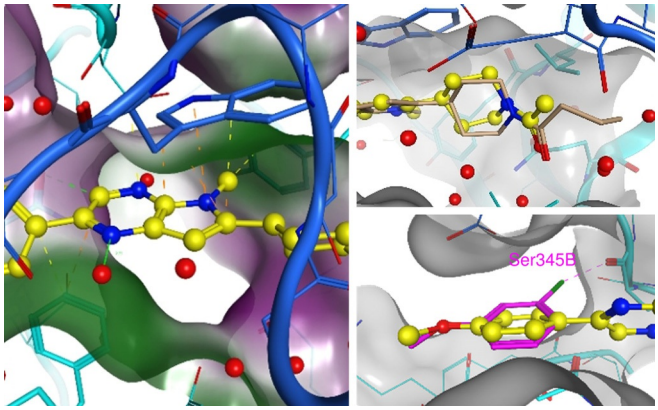


Figure 3. Left: Central core in an hydrophobic environment (green) and water network for nitrogen at opposite site of indole N-CH_3; Top right: piperidine R_1 alkyl groups (COMe vs. COCyPro) in yellow and ivory, respectively; Bottom right: superpositions of R_3 aromatic ring substitutions for **5** and **14**, halogen interaction with Ser 345.

For piperidine R_1 =-H, -CH_3, the interactions with the water network cannot be formed (**1** vs. **2**, **4** vs. **5**). The binding pocket around the piperidine moiety offers sufficient space for larger substituents than the amide group in the X-ray structure (Figure 3B). In fact, the amide group can be replaced by sulfonamide and urea substituents (**5**,**6**,**11**). Whereas the R_1 sulfonamide group is well tolerated, the urea substituent (R_1 =-$CONHCH_3$) is disfavored due to the exposure of a free NH without a H-bond acceptor in H-bonding distance. The extensions of the R_1 terminal amide with small alkyl groups (**12**,**13**,**17**) are likely to displace bound water molecules (Figure 3B).

The methoxy phenyl ring points (R_3) towards the solvent. R_3 variations, therefore, would primarily modulate physicochemical properties instead of potency. An exception is the o-Cl substituent (**5** vs. **2**) which induces a torsional twist between the aromatic rings and enables the chlorine atom to establish a halogen bond with the backbone carbonyl of Ser345B. A further increase of the torsion angle by an additional ortho substituent (**8**) results in steric strain and a loss of potency (Figure 3C).

Recently the inhibitory modulator JZ5029 was described and structurally characterized.[28] JZ5029 binds to the dimer interface (ratio of 2) closer to each active site compared to compound **14** (Figure 4). JZ5029 acts as inhibitor via hydrogen bonding with the active site residue Glu235. Compound **14** instead induces a different transient pocket and does not block the active site as shown by SPR competition experiments with isofagomine.

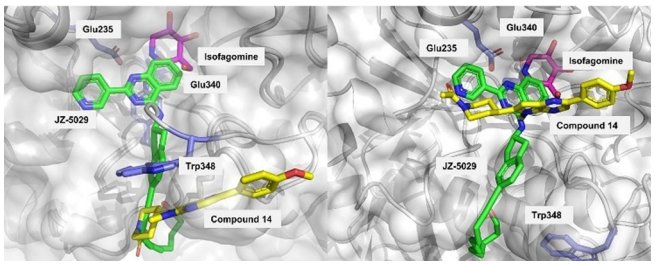


Figure 4. Overlay with inhibitory modulator JZ-5029 (green, pdb entry 5LVX). Isofagomine (magenta). View onto monomer A (left), and view onto monomer B (right). Isofagomine (magenta, pdb entry 2NSX) is shown to highlight the active site.

Published studies suggest that GCase can be more stable in vivo as a dimer and that unlike its cofactor Sap C, binding of α-synuclein to GCase does not affect GCase multimerization.[25] However, GCase is in a monomer-dimer equilibrium in vitro at a concentration of 8 μM.[25] Recent data shows that described GCase modulators or PCs also affect dimerization and increase Tm/stabilize GCase, however these compounds are characterized as inhibitors (Figure 4).[28,29] Considering the translocation of the enzyme from the ER to the lysosome, we wanted to explore if the activators induce dimerization.

We observed by analytical ultracentrifugation (AUC) that our activator **14** induces GCase dimerization in a manner dependent on compound concentration at the relevant lysosomal pH 5.2 (Figure 5). In addition, we also confirmed compound induced dimerization at ER pH 7.2 (see full data in supporting information).

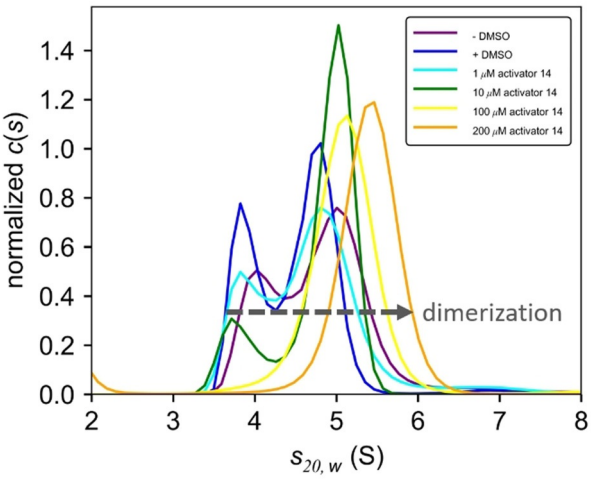


Figure 5. GCase dimerization induced by activator **14** (pH 5.2) as monitored by AUC in sedimentation velocity mode with absorbance detection (280 nm). Continuous sedimentation coefficient distribution $c(s_{20,w})$ normalized to peak area are shown for GCase at 10 μM without (purple) or with 2% (v/v) DMSO (blue) and in the presence of activator **14** at the indicated concentrations. All experiments with activator **14** contained 2% (v/v) DMSO.

We also rationalized the SAR trend for our novel activators in the cellular assay at the observed pocket at the GCase dimer interface. Thus, with our X-ray structure, the biophysical data (mainly the AUC, supported by SPR) and the observed SAR in the cellular assay, we hypothesize that dimerization could be an important process for GCase activation. The AUC data also points to dimeric GCase being present at neutral pH in trafficking vesicles.

A detailed characterization of the mode of action of compound **14** was performed with enzyme kinetics experiments. Using a biochemical assay with the fluorogenic substrate resorufin-β-glucopyranoside (Res-β-Glc), we confirmed a robust activation of GCase by compound **14** (two fold at pH 6) (Supplementary Figure 9). The enzyme kinetics experiments revealed that **14** has a dual mode of action causing a decrease in the Michaelis constant K_M and an increase in the catalytic rate k_{cat} for the turnover of Res-β-Glc by GCase. This results in a 2.5-fold increase of the catalytic efficiency k_{cat}/K_M in the presence of the activator (Figure 6 and Table 4). Thus, the activation of GCase by compound **14** is in fact both K-type (better affinity for substrate) and V-type (acceleration of catalytic rate) (for full experimental details see supporting information). This is in line with compound **14** being a non-essential activator of GCase.

A similar effect was described previously for small molecule activators of the glycoside hydrolase BtGH84.[30] Of note, similarly to the published non-essential activators of BtGH84, compound **14** does not stabilize GCase either in monomeric or in dimeric form (see thermal stability data in supporting information). This observation together with our enzyme kinetics data, show that **14** is a bona fide activator of GCase that directly activates the enzyme, as opposed to pharmacological chaperones, such as the inhibitory modulator JZ-4109 and Isofagomine, which stabilize and inhibit dimeric or monomeric Gcase, respectively.[28,29]

The novel GCase activators have good CNS drug-like profile: low MW and excellent physicochemical properties that predict brain penetration (Table 5) such as polar properties (or polar surface area < 60–70 Å^2), adequate number of H-bond donors (typically HBD (< 3), fewer groups able to function as H-bond acceptors HBA (< 7), and a suitable lipophilicity or logD.[31] Importantly, they also have very low metabolic clearance or rate of elimination from the body. In comparison the elongated propyl analog at R_1 (compound **17**, Table 5) has a higher lipophilicity and also increased clearance, thus the metabolic stability in human and mouse gets compromised by the elongated and hydrophobic alkyl chain.

Table 5: Physicochemical parameters to assess brain penetration and metabolic clearance (in human and mouse).

Modulator	EC_{50}	PSA[a]	Solubility μg mL^{-1}	LogD[b]	CL μL/min/mg(h/m)
14	3.8	49.9	133	3.07	< 10/12 (low/low)
5	1.8	40.6	76	3.4	< 10/ < 10 (low/low)
16	1.2	38.7	7.7	3.7	< 10/ < 10 (low/low)
17	0.6	45.9	3.4	4.0	28/40 (med/med))

[a] PSA: Polar surface area for CNS penetration < 70 Å^2; [b] Log D = logarithm of the octanol-water distribution coefficient at pH 7.4; CL (h/m): human/mouse microsomal clearance. In general MW < 450 Da, HBD (NHs + OHs) (0-1) and HBA (Ns + Os) < 3 for the activators.

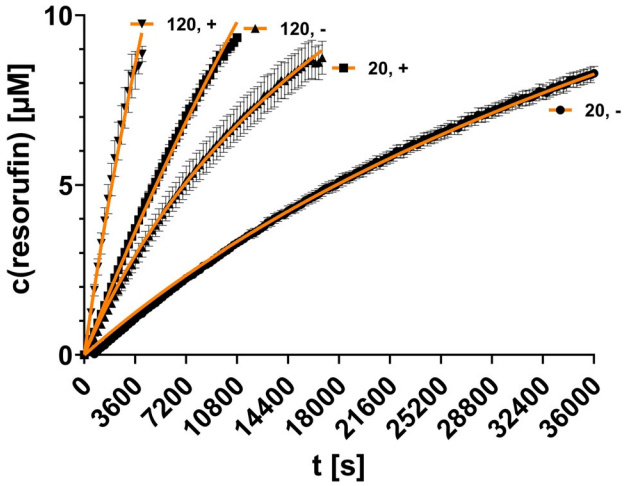


Figure 6. Progress curves of the turnover of 20 μM and 120 μM Res-β-Glc substrate to resorufin as product in absence (−) and presence (+) of 100 μM activator **14**. Data and error bars are means and standard deviations of 4 replicates; the fits of the integrated rate equation to the data are shown in orange.

Table 4: Enzyme kinetics parameters for the turnover of Res-β-Glc by GCase.[a]

c(cp14) [μM]	K_M [μM]	V_{max} [μM/s]	k_{cat} [s^{-1}]	k_{cat}/K_m [$M^{-1}s^{-1}$]
0	108 ± 49	0.0026 ± 0.0013	0.105 ± 0.050	959 ± 26
100	79 ± 6	0.0050 ± 0.0004	0.199 ± 0.017	2506 ± 14

[a] Values are mean ± standard deviation of two experiments carried out with four replicates each.

Conclusion

These results indicate that it is possible to identify highly relevant GCase activators with CNS drug-like properties, as disease modifiers for the treatment of neuropathic GD, PD or other synucleopathies. Binding and activation of GCase by compound **14** was confirmed by X-ray crystallography, biophysical, cellular and enzyme kinetics data. We observed compound **14** induced GCase dimerization by AUC. It was also possible to establish and rationalize an SAR trend for the novel series of activators at the observed pocket, confirming that our compounds do not bind to the GCase catalytic site but at the dimer interface, as supported by X-ray and by competition experiments in SPR. Because of this, compound dissociation for substrate turnover is not required.

The mode of activation of GCase by **14** was shown to be both K-type and V-type and binding of **14** leads to two-fold increase in the catalytic efficiency of the turnover of the substrate Res-β-Glc by GCase. In addition GCase dimer stabilization seemed not to be a prerequisite for activation. A similar behavior has been described for non-essential BtGH84 activators, with very similar mode of action, that destabilized this glycoside hydrolase[30] indicating that stabilization is not a prerequisite for activation. The enzyme kinetic data corroborates GCase activation and the X-ray crystallography, the SAR in the cellular assay and the biophysical data supports GCase dimerization. However, without a full mechanistic understanding, the connection between GCase activation and induction of dimerization remains speculative. We also cannot rule out that compound **14** binds and creates a conformational fit in the monomer which induces dimer formation. The reported findings reveal new mechanistic alternatives for GCase activation. For a more extensive understanding further experimentation will be needed, especially as GCase is a biochemically complex target, with many physical interactions.[14] Our results give an important mechanistic insight on the field of GCase activation and suggest that dimerization plays a significant role at both ER and lysosomal pHs. We also provide valuable insights for future drug discovery efforts in the field of GCase activation and contribute to the area of enzymatic activation, with further evidence for considering dimerization.[32]

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Conflict of interest

The authors declare no conflict of interest. All authors are employees of F.Hoffmann-La Roche Ltd.

Keywords: activators · β-glucocerebrosidase · dimerization · non inhibitory modulator binding site · structure elucidation

[1] a) K. S. Hruska, M. E. LaMarca, C. R. Scott, E. Sidransky, *Hum. Mutat.* **2008**, *29*, 567 – 583; b) E. Beutler, G. A. Grabowski, in *Metabolic and Molecular Bases of Inherited Disease*, *Vol. 3* (Eds.: C. R. Scriver, W. S. Sly, D. Valle), McGraw Hill, New York, **2001**, pp. 3635 – 3667.

[2] J. Stirnemann, N. Belmatoug, F. Camou, C. Serratrice, R. Froissart, C. Caillaud, T. Levade, L. Astudillo, J. Serratrice, A. Brassier, C. Rose, T. Billette de Villemeur, M. G. Berger, *Int. J. Mol. Sci.* **2017**, *18*, 441.

[3] a) E. Aflaki, W. Westbroek, E. Sidransky, *Neuron* **2017**, *93*, 737 – 746; b) K. S. Hruska, O. Goker-Alpan, E. Sidransky, *J. Biomed. Biotechnol.* **2006**, 78549.

[4] a) M. Avenali, F. Blandini, S. Cerri, *Front. Aging Neurosci.* **2020**, *12*, 97; b) A. Velayati, W. H. Yu, E. Sidransky, *Curr. Neurol. Neurosci. Rep.* **2010**, *10*, 190 – 198; c) S. Lesage, M. Anheim, C. Condroyer, P. Pollak, F. Durif, C. Dupuits, F. Viallet, E. Lohmann, J. C. Corvol, A. Honore, S. Rivaud, M. Vidailhet, A. Durr, A. Brice, G. French, *Hum. Mol. Genet.* **2011**, *20*, 202 – 210.

[5] J. R. Mazzulli, Y. H. Xu, Y. Sun, A. L. Knight, P. J. McLean, G. A. Caldwell, E. Sidransky, G. A. Grabowski, D. Krainc, *Cell* **2011**, *146*, 37 – 52.

[6] a) S. P. Sardi, J. Clarke, C. Kinnecom, T. J. Tamsett, L. Li, L. M. Stanek, M. A. Passini, G. A. Grabowski, M. G. Schlossmacher, R. L. Sidman, S. H. Cheng, L. S. Shihabuddin, *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 12101 – 12106; b) S. P. Sardi, J. Clarke, C. Viel, M. Chan, T. J. Tamsett, C. M. Treleaven, J. Bu, L. Sweet, M. A. Passini, J. C. Dodge, W. H. Yu, R. L. Sidman, S. H. Cheng, L. S. Shihabuddin, *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 3537 – 3542; c) E. Rockenstein, J. Clarke, C. Viel, N. Panarello, C. M. Treleaven, C. Kim, B. Spencer, A. Adame, H. Park, J. C. Dodge, S. H. Cheng, L. S. Shihabuddin, E. Masliah, S. P. Sardi, *Hum. Mol. Genet.* **2016**, *25*, 2645 – 2660.

[7] a) K. E. Murphy, G. M. Halliday, *Autophagy* **2014**, *10*, 1350 – 1351; b) T. L. Yap, J. M. Gruschus, A. Velayati, W. Westbroek, E. Goldin, N. Moaven, E. Sidransky, J. C. Lee, *J. Biol. Chem.* **2011**, *286*, 28080 – 28088.

[8] M. E. Gegg, D. Burke, S. J. Heales, J. M. Cooper, J. Hardy, N. W. Wood, A. H. Schapira, *Ann. Neurol.* **2012**, *72*, 455 – 463.

[9] a) F. Atashrazm, D. Hammond, G. Perera, C. Dobson-Stone, N. Mueller, R. Pickford, W. S. Kim, J. B. Kwok, S. J. G. Lewis, G. M. Halliday, N. Dzamko, *Sci. Rep.* **2018**, *8*, 15446; b) E. M. Rocha, G. A. Smith, E. Park, H. Cao, E. Brown, P. Hallett, O. Isacson, *Ann. Clin. Transl. Neurol.* **2015**, *2*, 433 – 438.

[10] a) R. N. Alcalay, O. A. Levy, C. C. Waters, S. Fahn, B. Ford, S. H. Kuo, P. Mazzoni, M. W. Pauciulo, W. C. Nichols, Z. Gan-Or, G. A. Rouleau, W. K. Chung, P. Wolf, P. Oliva, J. Keutzer, K. Marder, X. Zhang, *Brain* **2015**, *138*, 2648 – 2658; b) D. Chiasserini, S. Paciotti, P. Eusebi, E. Persichetti, A. Tasegian, M. Kurzawa-Akanbi, P. F. Chinnery, C. M. Morris, P. Calabresi, L. Parnetti, T. Beccari, *Mol. Neurodegener.* **2015**, *10*, 15.

[11] K. E. Murphy, A. M. Gysbers, S. K. Abbott, N. Tayebi, W. S. Kim, E. Sidransky, A. Cooper, B. Garner, G. M. Halliday, *Brain* **2014**, *137*, 834 – 848.

[12] a) E. Gras-Colomer, M. A. Martinez-Gomez, M. Climente-Marti, M. Fernandez-Zarzoso, M. Almela-Tejedo, V. Giner-Galvan, J. A. Marcos-Rodriguez, A. Rodriguez-Fernandez, M. A. Torralba-Cabeza, M. Merino-Sanjuan, *Basic Clin. Pharmacol. Toxicol.* **2018**, *123*, 65 – 71; b) C. P. Phenix, B. P. Rempel, K. Colobong, D. J. Doudet, M. J. Adam, L. A. Clarke, S. G. Withers, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 10842 – 10847.

[13] E. M. Rocha, G. A. Smith, E. Park, H. Cao, E. Brown, M. A. Hayes, J. Beagan, J. R. McLean, S. C. Izen, E. Perez-Torres, P. J. Hallett, O. Isacson, *Neurobiol. Dis.* **2015**, *82*, 495 – 503.

[14] J. Do, C. McKinney, P. Sharma, E. Sidransky, *Mol. Neurodegener.* **2019**, *14*, 36.

[15] S. P. Sardi, J. M. Cedarbaum, P. Brundin, *Mov. Disord.* **2018**, *33*, 684 – 696.

[16] a) R. E. Boyd, G. Lee, P. Rybczynski, E. R. Benjamin, R. Khanna, B. A. Wustman, K. J. Valenzano, *J. Med. Chem.* **2013**, *56*, 2705 – 2725; b) T. Mena-Barragán, A. Narita, D. Matias, G. Tiscornia, E. Nanba, K. Ohno, Y. Suzuki, K. Higaki, J. M. Garcia Fernández, C. Ortiz Mellet, *Angew. Chem. Int. Ed.* **2015**, *54*, 11696 – 11700; *Angew. Chem.* **2015**, *127*, 11862 – 11866.

[17] S. Patnaik, W. Zheng, J. H. Choi, O. Motabar, N. Southall, W. Westbroek, W. A. Lea, A. Velayati, E. Goldin, E. Sidransky, W. Leister, J. J. Marugan, *J. Med. Chem.* **2012**, *55*, 5734 – 5748.

[18] a) J. R. Mazzulli, F. Zunke, T. Tsunemi, N. J. Toker, S. Jeon, L. F. Burbulla, S. Patnaik, E. Sidransky, J. J. Marugan, C. M. Sue, D. Krainc, *J. Neurosci.* **2016**, *36*, 7693 – 7706; b) E. Aflaki, D. K. Borger, N. Moaven, B. K. Stubblefield, S. A. Rogers, S. Patnaik, F. J. Schoenen, W. Westbroek, W. Zheng, P. Sullivan, H.

Fujiwara, R. Sidhu, Z. M. Khaliq, G. J. Lopez, D. S. Goldstein, D. S. Ory, J. Marugan, E. Sidransky, *J. Neurosci.* **2016**, *36*, 7441 – 7452.
[19] Z. Berger, S. Perkins, C. Ambroise, C. Oborski, M. Calabrese, S. Noell, D. Riddell, W. D. Hirst, *PLoS One* **2015**, *10*, e0119141.
[20] H. J. Kim, M. J. Ha, H. W. Kang, M. S. Yang, H. S. Kim, H. C. Kim, *Genet. Med.* **1999**, *1*, 42.
[21] J. R. Mazzulli, F. Zunke, O. Isacson, L. Studer, D. Krainc, *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 1931 – 1936.
[22] a) Using reported GCase tool compounds such as compounds **13** and **40** (used as controls on the GCase specific enzymatic assay and mentioned on Supplementary Figure 1), 3D shape similarity searches were performed with ROCS (ROCS 3.3.1.2: OpenEye Scientific Software, Santa Fe, NM. http://www.eyesopen.com.) within the Roche compound deck. After filtering out compounds with unwanted properties (e.g solubility) or reactive substructures, 400 compounds with the highest 3D similarities were selected; b) M. B. J. T. Bamberg, D. J. DuBois, T. R. Elworthy, R. T. Hendricks, J. C. Hermann, R. K. Kondru, R. Lemoine, Y. Lou, T. D. Owens, J. Park, D. B. Smith, M. Soth, H. Yang, C. W. Yee, (Ed.: R. PA), US20090215750, **2009**.
[23] T. E. Moors, S. Paciotti, A. Ingrassia, M. Quadri, G. Breedveld, A. Tasegian, D. Chiasserini, P. Eusebi, G. Duran-Pacheco, T. Kremer, P. Calabresi, V. Bonifati, L. Parnetti, T. Beccari, W. D. J. van de Berg, *Mol. Neurobiol.* **2019**, *56*, 1344 – 1355.
[24] a) "Protein interfaces, surfaces and assemblies' service PISA at the European Bioinformatics Institute (http://www.ebi.ac.uk/pdbe/prot int/pistart.html); b) E. Krissinel, K. Henrick, *J. Mol. Biol.* **2007**, *372*, 774 – 797.
[25] J. M. Gruschus, Z. Jiang, T. L. Yap, S. A. Hill, A. Grishaev, G. Piszczek, E. Sidransky, J. C. Lee, *Biochem. Biophys. Res. Commun.* **2015**, *457*, 561 – 566.
[26] R. L. Lieberman, B. A. Wustman, P. Huertas, A. C. Powe, Jr., C. W. Pine, R. Khanna, M. G. Schlossmacher, D. Ringe, G. A. Petsko, *Nat. Chem. Biol.* **2007**, *3*, 101 – 107.
[27] H. Dvir, M. Harel, A. A. McCarthy, L. Toker, I. Silman, A. H. Futerman, J. L. Sussman, *EMBO Rep.* **2003**, *4*, 704 – 709.
[28] J. Zheng, L. Chen, O. S. Skinner, D. Ysselstein, J. Remis, P. Lansbury, R. Skerlj, M. Mrosek, U. Heunisch, S. Krapp, J. Charrow, M. Schwake, N. L. Kelleher, R. B. Silverman, D. Krainc, *J. Am. Chem. Soc.* **2018**, *140*, 5914 – 5924.
[29] G. J. Kornhaber, M. B. Tropak, G. H. Maegawa, S. J. Tuske, S. J. Coales, D. J. Mahuran, Y. Hamuro, *ChemBioChem* **2008**, *9*, 2643 – 2649.
[30] J. F. Darby, J. Landstrom, C. Roth, Y. He, G. J. Davies, R. E. Hubbard, *Angew. Chem. Int. Ed.* **2014**, *53*, 13419 – 13423; *Angew. Chem.* **2014**, *126*, 13637 – 13641.
[31] H. Pajouhesh, G. R. Lenz, *NeuroRx* **2005**, *2*, 541 – 553.
[32] a) H. J. Snijder, I. Ubarretxena-Belandia, M. Blaauw, K. H. Kalk, H. M. Verheij, M. R. Egmond, N. Dekker, B. W. Dijkstra, *Nature* **1999**, *401*, 717 – 721; b) N. J. Marianayagam, M. Sunde, J. M. Matthews, *Trends Biochem. Sci.* **2004**, *29*, 618 – 625.

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