Bioorg. Med. Chem. 132 (2026) 118453

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Selenoureido-*N*-alkyl-3,4,5-trihydroxypiperidines: probing their dual-target role in Gaucher disease

Debora Pratesi [a], Francesca Clemente [a,*], Camilla Matassini [a], Silvia Falliano [b], Amelia Morrone [b,c], Rebecca Sodano [d], Paolo Paoli [d], Andrea Goti [a], José G. Fernández-Bolanos [e], Óscar López [e,*], Francesca Cardona [a,*]

[a] Department of Chemistry "Ugo Schiff" (DICUS), University of Florence, Via della Lastruccia 3-13, 50019 Sesto F.no (FI), Italy
[b] Laboratory of Molecular Genetics of Neurometabolic Diseases, Department of Neuroscience and Medical Genetics, Meyer Children's Hospital IRCCS, Viale Pieraccini 24, 50139 Firenze, Italy
[c] Department of Neurosciences, Psychology, Drug Research and Child Health University of Florence, Viale Pieraccini 24, 50139 Firenze, Italy
[d] Department of Experimental and Clinical Biomedical Sciences, University of Florence, Viale Morgagni 50, 50134 Firenze, Italy
[e] Departamento de Química Organica, Facultad de Química, Universidad de Sevilla, Apartado 1203, 41071 Seville, Spain

ARTICLE INFO

Keywords:
GCase
Selenium
Iminosugars
Gaucher disease
Dual-target
ROS

ABSTRACT

The design and synthesis of a novel class of selenoureido-iminosugar compounds that uniquely combine antioxidant properties with pharmacological chaperone activity are reported. These derivatives feature a 3,4,5-trihydroxypiperidine iminosugar core linked *via* nine- or twelve-carbon alkyl chains to *N*-aryl selenoureido groups. By simultaneously targeting oxidative stress and lysosomal β-glucocerebrosidase (GCase) dysfunction, this dual-action strategy addresses two key pathological hallmarks of Gaucher disease. These compounds were evaluated for the first time on human GCase and on fibroblasts derived from Gaucher patients carrying clinically relevant *GBA1* variants. Notably, one of the newly synthesized compounds significantly restored GCase activity in patient-derived cells and induced a ~60% reduction in intracellular ROS levels, further supporting its bifunctional therapeutic potential. This study introduces an innovative dual-action molecular scaffold for addressing key pathological mechanisms in Gaucher disease and related neurodegenerative conditions.

1. Introduction

Selenium is an essential trace element with well-documented antioxidant properties, primarily due to its incorporation into selenoproteins, such as glutathione peroxidases and thioredoxin reductases, which protect cells from oxidative stress.[1–4] Organoselenium compounds, including selenoureas, have gained attention for their ability to mimic these antioxidant effects while offering enhanced chemical stability and bioavailability.[5–7] Selenoureas, characterized by the replacement of the carbonyl oxygen in ureas with selenium, exhibit unique redox properties that contribute to their potential as antioxidant agents. Numerous studies highlight their capacity to scavenge reactive oxygen species (ROS) and modulate oxidative pathways, making them promising candidates for therapeutic applications in oxidative stress-related diseases.[8–12] Iminosugars, carbohydrate analogues in which the endocyclic ring oxygen is replaced by nitrogen, act as pharmacological chaperones, stabilizing misfolded enzymes. Their binding to glycosidases prevents the degradation of mutant variants and facilitates lysosomal trafficking.[13] In the context of Gaucher disease (GD) and Parkinson's disease (PD), iminosugars have been investigated for their potential to restore the activity of glucocerebrosidase (GCase), a lysosomal enzyme encoded by the *GBA1* gene.[14–17] In GD, mutations in *GBA1* gene lead to deficient or dysfunctional GCase, resulting in the accumulation of glucosylceramide (GlcCer) and glucosylsphingosine (GlcSph), which disrupt lysosomal function and contribute to multi-organ pathology.[18] Beyond its role in GD, *GBA1* variants are also recognized as the most common genetic risk factor for PD. Among all *GBA1* gene variants, N370S and L444P are the most frequent worldwide in GD patients. These variants are also associated with an increased risk of developing PD, both in individuals with Gaucher disease and in heterozygous carriers.[19–22] Reduced GCase activity in neurons, even in heterozygous mutation carriers, leads to impaired degradation of α-synuclein, a protein that forms toxic aggregates in Parkinson's pathology.[23–27] The bidirectional relationship between GCase dysfunction and α-synuclein accumulation

* Corresponding authors.
E-mail addresses: francesca.clemente@unifi.it (F. Clemente), osc-lopez@us.es (Ó. López), francesca.cardona@unifi.it (F. Cardona).

https://doi.org/10.1016/j.bmc.2025.118453
Received 31 July 2025; Received in revised form 15 October 2025; Accepted 17 October 2025
Available online 19 October 2025

suggests that targeting this enzyme could have therapeutic benefits for both disorders. By enhancing GCase function, iminosugars help to reduce GlcCer accumulation, restore lysosomal homeostasis and mitigate α-synuclein aggregation, highlighting their potential as therapeutic agents in the treatment of Gaucher and Parkinson's diseases. The development of multifunctional molecules capable of interacting with several diversified biological targets represents an increasingly promising strategy for treating neurodegenerative disorders.[28,29] We have recently started to investigate this aspect with a promising description of the ability by some iminosugar derivatives to bind both GCase and α-synuclein.[30] Despite the potential of this approach, there are currently few examples of dual-target iminosugar compounds reported in the literature for other therapeutic applications.[31,32] It should be noticed that, in addition to GCase dysfunction, oxidative stress is another pathological hallmark common to both GD and PD.[18,26,27,33–39] In this context, selenoureido-iminosugars could provide a new class of multifaceted therapeutics able in principle to address both enzymatic dysfunction and oxidative stress. Some of us previously reported strong inhibition of commercially available β-glucosidase by an *N*-alkylated deoxynojirimycin (DNJ) derivative bearing a selenoureido motif spaced by a five-carbon atom linker (structures **1**, Fig. 1), showing promise in this direction.[40] Indeed, selenoureido-iminosugars combine the potential pharmacological chaperone activity of an iminosugar unit with the redox-regulating properties of selenium, enabling a dual-target approach that might both restore lysosomal balance and counteract oxidative damage. Based on this hypothesis, we present in this work the synthesis of a new class of selenoureido-iminosugar compounds **2a–c** and **3a–c** (Fig. 1). These molecules are based on a 3,4,5-trihydroxypiperidine iminosugar linked to an *N*-aryl selenoureido pendant *via* an alkyl chain of a nine- (**2a–c**, Fig. 1) or a twelve-carbon atoms (**3a–c**, Fig. 1). The aim is to further explore the therapeutic potential of selenoureido-iminosugars in the context of Gaucher disease. The length of the spacer was chosen on the basis of previously reported studies on *C*- and *N*-alkyl iminosugars whose affinity towards GCase resulted stronger for alkyl chains longer than eight-carbon atoms.[41–45]

Therefore, these new selenoureido-iminosugar compounds **2** and **3** differ from the previously reported *N*-alkylated deoxynojirimycin-containing selenoureas **1**[40] both in the iminosugar scaffold employed and in the length of the alkyl chain connecting the selenourea functionality. Compared to deoxynojirimycin and other C-6 hydroxymethyl iminosugars, 3,4,5-trihydroxypiperidines have been much less investigated for their biological properties, nonetheless they recently showed a very interesting biological profile.[46] This study presents, for the first time, a novel family of selenoureido-iminosugar compounds evaluated on the human lysosomal enzyme GCase. These molecules were assessed for their ability to enhance enzymatic activity in fibroblasts derived from Gaucher patients carrying a specific pathogenic mutation. Importantly, the most promising chaperone was also investigated for its capacity to reduce intracellular reactive oxygen species (ROS), thereby further supporting the therapeutic potential of these bifunctional compounds.

2. Synthesis of the selenoureido-iminosugars

In order to obtain the target *N*-alkyl-3,4,5-trihydroxypiperidine containing selenoureido functionalities **2** and **3**, we synthesized the trihydroxypiperidines **9** and **10** alkylated at nitrogen with an alkyl chain of different length and a terminal amine moiety for further functionalization (Scheme 1). The synthesis and characterization of all new compounds are reported in the ESI.

The procedure started with the synthesis of compound **4** from low-cost sugar D-mannose in 80% yield over six steps as previously

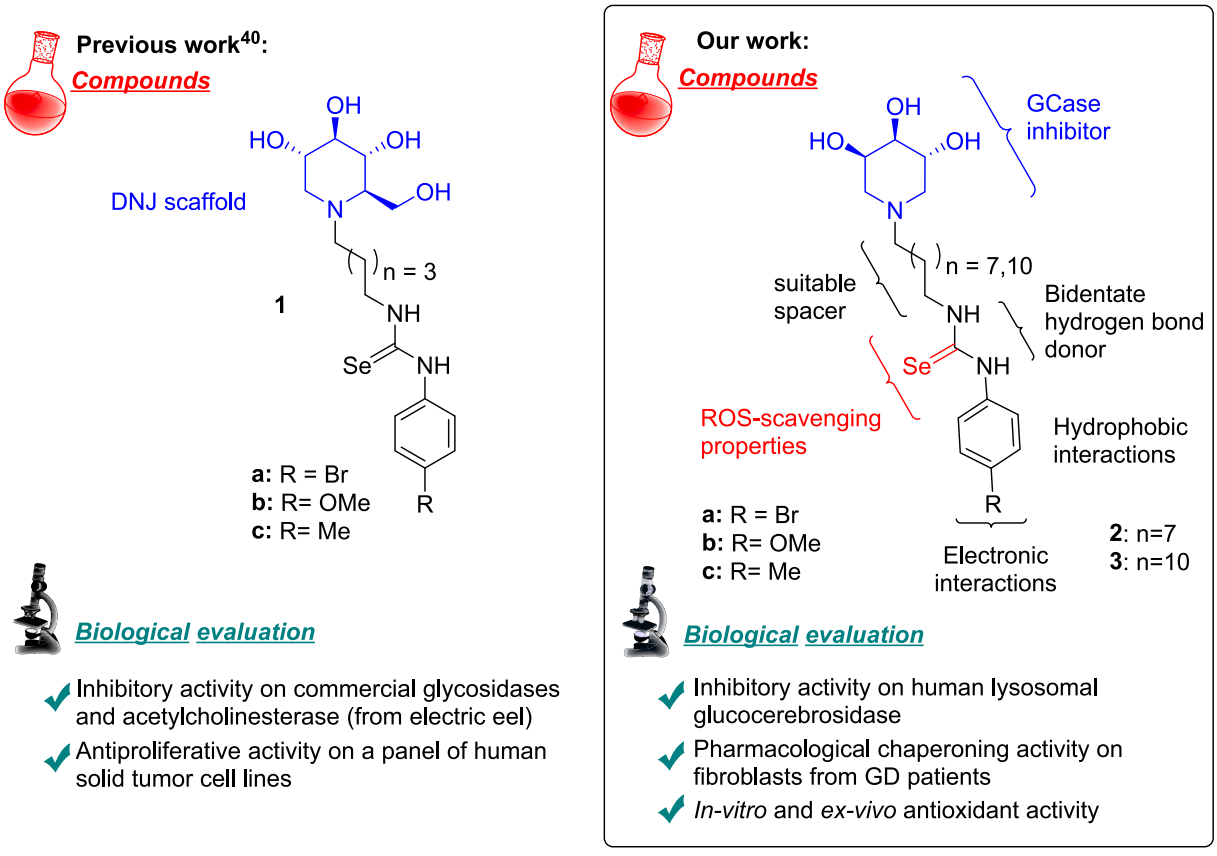
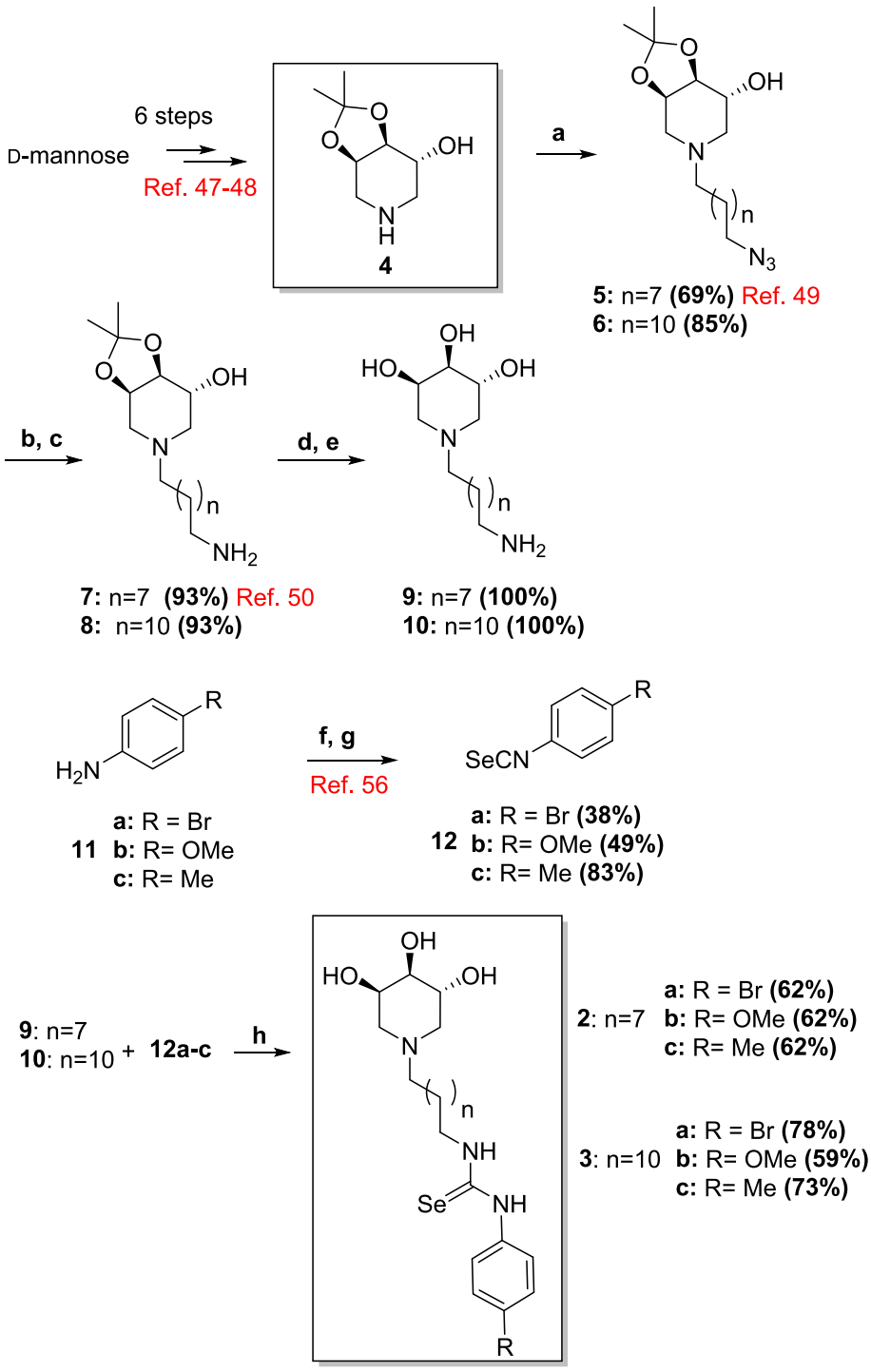


Fig. 1. *N*-Alkylated deoxynojirimycin-containing selenoureas **1** previously reported in literature and new selenoureido-iminosugar compounds **2** and **3** synthesized in this work.



Scheme 1. Synthetic procedure for compounds **2** and **3**. Reagents and conditions: (a) $Br(CH_2)_nN_3$, Et_3N, CH_3CN, 80 °C, 18 h; (b) PPh_3, dry THF, 60 °C, 1 h; (c) H_2O, 50 °C, 17 h; (d) HCl 12 M, MeOH, r.t., 18 h; (e) Ambersep 900-OH resin, r.t., 6–18 h; (f) Acetoformic anhydride; (g) triphosgene, Et_3N, black Se; (h) dry Et_3N, dry MeOH, darkness, 23–26 h, r.t.

reported.[47,48] The trihydroxypiperidine **7** with a nine-carbon atom chain bearing the amino group, in turn obtained from azide **5**, was already reported and synthesized as described.[49,50] Following a similar synthetic strategy, its analogue **8** with a twelve-carbon atom chain was obtained in two steps as shown in Scheme 1. First, alkylation of the protected trihydroxypiperidine **4** with 1-azido-12-bromododecane[51] carried out in Et_3N and CH_3CN at 80 °C for 18 h afforded compound **6** in 85% yield. Then, a Staudinger reduction carried out by addition of PPh_3 in dry THF for 1 h at 60 °C followed by addition of water afforded compound **8** in 93% yield. Acetonide deprotection of compounds **7** and **8** was achieved by reaction with aqueous hydrochloric acid in MeOH at room temperature overnight, followed by treatment with the strongly basic resin Ambersep 900-OH to afford compounds **9** and **10** in quantitative yields as free amines. With the amino derivatives **9** and **10** in hands, the aromatic isoselenocyanates **12** were selected as the most suitable electrophiles to provide the target selenoureido-trihydroxypiperidines **2** and **3**. The presence of substituents with diverse electronic effects (R = Br, OMe, Me, Scheme 1) in the *para*-

position of the phenyl ring was also explored because it can modulate the biological properties of final selenoureas. Out of the numerous reported methods for the synthesis of isoselenocyanates,[52–54] the formation of *N*-alkyl and *N*-aryl formamides from the corresponding anilines followed by the one-pot dehydration with triphosgene and coupling of the transient isocyanide with black selenium powder,[55] optimized by some of us,[56] was selected. Following this strategy, isoselenocyanates **12a–c** were obtained starting from aryl amines **11a–c** in moderate to good yields (Scheme 1). Isoselenocyanates **12a–c** were subsequently employed in the coupling reaction with the trihydroxypiperidines **9** and **10**. We initially run the reaction of trihydroxypiperidine **9** and isoselenocyanate **12b** in the conditions reported (dry MeOH at room temperature in the dark for 24 h)[40] but no complete conversion of the starting material was observed. Since heating was preferably avoided, due to the possible reaction of highly reactive isoselenocyanate with MeOH to give the corresponding selenocarbamate, we tried to accelerate the reaction with the addition of Et_3N (0.4 equivalents) as the base, and to our delight the reaction went to completion in 26 h. Applying these optimized conditions also to the other substrates, afforded six new selenoureido-iminosugars with satisfactory to good yields (59–78%). The two series of new selenoureido iminosugars obtained, namely compounds **2a–c** and **3a–c**, differ in the length of the carbon chain separating the iminosugar and the selenoureido moiety and bear an electron-withdrawing group (Br, **2a** and **3a**), a strong electron donating group (OMe, **2b** and **3b**) and a moderate electron donating group at the *para* position of the phenyl substituent (Me, **2c** and **3c**), respectively. Conformers might exist in compounds **2** and **3** due to the partial C═N character in the selenoureido motif, a situation that is frequently found in sulfur analogues (thioureas). As a result, in their ^{13}C NMR spectra, a broadening of the carbon signals located next to the selenourea scaffold is usually observed, leading to small peaks, close to the baseline. A similar situation was found in the iminosugars-selenoureas hybrids prepared by some of us.[40]

3. Biological *in vitro* assays

3.1. Inhibitory activity against GCase

The six selenoureido-iminosugars **2** and **3** were evaluated as inhibitors of human lysosomal GCase at 1 mM concentration using human leukocyte homogenates from healthy donors. The assay was conducted following a well-established protocol in our group,[42–44] in which the compounds were incubated with the enzyme at pH 5.8 for 1 h. All the newly synthesized selenoureido-iminosugar derivatives **2** and **3** exhibited strong inhibitory activity against GCase at this concentration, with inhibition rates ranging from 91% to96 % (see ESI, Fig. S01). To further characterize their inhibitory potency, IC_{50} values were determined (Table 1; see ESI). Both series of new compounds **2** and **3** exhibited IC_{50} values in the low micromolar range, as shown in Table 1, suggesting that the presence of the selenourea moiety does not hamper the iminosugar activity as GCase inhibitor.[43–45] A general trend is observed, with compounds **3** bearing the longer twelve-carbon atom alkyl chain behaving as stronger inhibitors than compounds **2** featured by the shorter nine-carbon atom alkyl chain, which is in agreement with previous findings.[41–45] For both series of selenoureido-iminosugars, the slightly less active are those bearing the methyl group. However, the presence of electronically different substituents at the *para* position of the phenyl ring did not significantly affect the inhibitory potency, with all compounds maintaining IC_{50} values in the low-to medium micromolar range, thus showing promise for undergoing pharmacological chaperoning test.

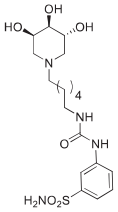
Table 1
IC_{50} values were determined by measuring GCase activity at different concentrations of each inhibitor.

Compound	GCase inhibition IC_{50} (μM)
2a	12 ± 3
2b	10.0 ± 0.2
2c	30 ± 3
3a	6 ± 2
3b	6 ± 1
3c	9 ± 3

3.2. Antioxidant activity

Once the ability to interact with the target enzyme GCase was confirmed, we assessed the potential benefits of incorporating an antioxidant moiety into the iminosugars by evaluating the antioxidant activity of selenoureido-iminosugars **2** and **3** using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay.[57] DPPH is a stable free radical with a strong absorption at 515 nm. When reacting with antioxidant species, *via* a single electron or hydrogen atom transfer, a strong decay in the absorbance at λ_{max} is observed (change from deep purple to yellowish solutions).[57] DPPH concentration was fixed at 58.5 μM, and EC_{50} values were determined after 30 min of incubation (concentration of the antioxidant required for diminishing the DPPH concentration to 50% of its initial value). Higher DPPH concentrations than the recommended range (25–70 μM) should be avoided due to the loss of spectrometric accuracy.[58] All tested compounds exhibited an EC_{50} around 30 μM (Table 2), in the same range as the reference compound, L-ascorbic acid (EC_{50} 26.0 ± 1.5 μM), a potent natural free radical scavenger. Notably, the antioxidant activity appeared to be independent of both the alkyl chain length and the nature of the phenyl substituent. To further confirm the role of the selenium moiety in the antioxidant activity, compounds **9** and **10**, lacking the selenium functionality, were also tested and no antioxidant activity was observed. Additionally, the ureido-iminosugar (compound **13**) depicted in Table 2, synthesized previously in our group,[59] was also included in the study. The complete absence of activity in the latter compound when tested at a 250 μM concentration proved the essential role of the selenoureido scaffold in the antiradical activity. These findings highlight the crucial contribution of selenium in imparting the antioxidant properties of iminosugars.

Table 2
EC_{50} values were determined using the DPPH assay.

Compound	DPPH Antioxidant tests EC_{50} (μM)
2a	29.9 ± 1.1
2b	27.9 ± 1.7
2c	33.6 ± 3.4
3a	31.3 ± 1.2
3b	34.0 ± 1.8
3c	27.6 ± 2.4
9	>1000
10	>1000
 **13**	>250

3.3. GPx-like activity

We have also tested the capacity of selenoureas **2** and **3** to scavenge H_2O_2 by mimicking the activity of glutathione peroxidase (GPx). GPx are a group of selenoenzymes that keep redox homeostasis and thus behave as a natural defence for mitigating the effects of oxidative stress.[60] Through a catalytic cycle involving a selenocysteine residue at the active site, and the tripeptide glutathione as the cofactor, GPx reduce deleterious H_2O_2 and alkyl peroxides into water and alcohols, respectively. Herein, we have followed the methodology developed by Mugesh et al.[61] for analysing the GPx-like activity of selenoureido-iminosugar hybrids **2** and **3**. In this model, selenoureas **2** and **3** were used in substoichiometric amounts (0.1 and 0.41 mM) in the presence of an excess of H_2O_2 (41.6 mM) as a model ROS, and PhSH (10 mM), as the cofactor. The reduction of H_2O_2 was quantified in an indirect way, by monitoring the subsequent formation of $(PhS)_2$ (increase of absorbance at 305 nm). Compounds **2** and **3** proved to behave as excellent GPx mimics by reducing H_2O_2 efficiently in a catalytic load as low as 0.24% and 1% with respect to the H_2O_2 content (Table 3). Uncatalyzed reaction (background reaction) and $(PhSe)_2$ as a reference antioxidant control were also included. In this model, the selenoureas **2**/**3** and PhSH behave as mimetics of GPx and glutathione, respectively, and are capable of eliminating the excess of the ROS using just substoichoimetric amounts. For explaining this activity, some of us previously reported a catalytic cycle for steroid-derived selenoureas.[10,11] It was postulated that H_2O_2-promoted oxidation of the selenoureas furnishes a transient selenoxide, stabilized by resonance, which in turn reacts with the thiol (herein PhSH) to furnish a selenyl-sulfide adduct. Further reaction with a second molecule of PhSH affords diphenyl disulfide and regenerates the starting selenourea, which can further participate in subsequent catalytic cycles. Using Hammet plots, it was postulated that the first step, leading to the formation of the selenoxide, is the rate-determining step.[10] Compounds **2a** and **3a**, containing a *p*-bromophenyl appendage turned out to be the most potent compounds. Remarkably, at a ratio of just 0.24%, derivative **2a** accelerated the reduction of H_2O_2 by a factor of 4.3 compared to the non-catalyzed conditions (background) and exhibited a 2.9-fold improvement compared to the reference compound, $(PhSe)_2$. Shifting the enzyme mimetic ratio to 1% furnished a considerable increase in the reaction rate, up to 23-fold, leading to half-time reaction values ($t_{1/2}$) ranging from just 27.3–40.3 min.

In order to demonstrate an enzyme-type kinetics of the scavenging reaction exerted by selenoureas **2** and **3**, a Hanes plot ([S]/V *vs.* [S]) was performed, by modifying the thiol concentration (1.14–9.1 mM), while keeping constant the H_2O_2 concentration (41.6 mM). Selenourea **3c** (0.2 mM) was chosen as a model compound. Linearity found in Hanes plot of **3c** (see ESI, Fig. S02) demonstrates the saturation-type kinetics of this family of compounds, thus behaving as a GPx-mimetic. Plot of V *vs.* [PhSH] (see ESI, Fig. S03, background correction) further demonstrates this observation. Using the latter plot, and the software GraphPad Prism 8.01 (non-linear regression), the kinetic parameters were obtained: $V_{max} = 0.097$ Abs/min; $K_M = 5.15$ mM.

Table 3
[a]0.5% DMSO in MeOH; [b]2% DMSO in MeOH.

Compound	[Mimetic] = 0.1 mM[a]		[Mimetic] = 0.41 mM[b]	
	V (μM/min)	$t_{1/2}$ (min)	V (μM/min)	$t_{1/2}$ (min)
Background	6.7 ± 1.9	749.6	14.0 ± 2.7	357.1
2a	29.0 ± 3.5	172.4	183.0 ± 0.8	27.3
2b	9.6 ± 1.3	520.8	160 ± 4	31.3
2c	5.4 ± 0.2	925.9	124.0 ± 2.4	40.3
3a	18.0 ± 3.7	277.8	158.0 ± 6.3	31.6
3b	13.0 ± 2.5	384.6	166	30.1
3c	13.0 ± 0.1	384.6	145 ± 41	34.5
$(PhSe)_2$	10.0 ± 0.1	500.0	28.0 ± 0.6	178.6

4. Biological *ex vitro* assays

4.1. Cell viability

Cytotoxicity screening in wild-type fibroblasts was used to select non-toxic working concentrations *via* two representative compounds. To evaluate the impact of selenoureido-iminosugar compounds **2** and **3** on cell viability, a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed using human wild-type (WT) fibroblasts. Two representative compounds, **2a** and **3c**, were selected as model compounds, differing in their alkyl chain lengths (nine and twelve carbon atoms, respectively) and the substitution pattern at the Ph residue (Br and Me, respectively). The results demonstrated a significant reduction in cell viability at the highest tested concentrations, indicating a dose-dependent cytotoxic effect for both compounds (see ESI, Fig. S04). Notably, the compound **3c** with the longer twelve-carbon alkyl chain exhibited greater toxicity, consistent with previous findings observed for other alkylated iminosugars.[44]

4.2. Chaperoning assay

The chaperone assay provides valuable insights into the ability of these compounds to stabilize GCase enzyme and protect it from degradation. To assess their potential, we evaluated the ability of selenoureido-iminosugar compounds to enhance GCase activity directly in intact GD fibroblasts carrying the L444P and N370S variants. The experiment involved the direct administration of 4-methylumbelliferyl-β-D-glucoside to intact cells, followed by measurement of the GCase activity (see ESI, Fig. S05–S07).[62] This assay was performed on compounds **3**, which demonstrated higher affinity towards GCase and increased stability over time. These compounds were tested on both cell lines at six different nominal concentrations (from 50 nM to 20 μM) selected on the basis of a cell viability assay. Within the exposure range used for the experiments, no reduction in viability was observed relative to vehicle controls. For comparison, isofagomine (**IFG**), an iminosugar PC, and ambroxol (**ABX**), a non-iminosugar PC were tested as controls. These compounds reached clinical trials for GD treatment and **ABX** also for GD related PD. In our hands, **IFG** and **ABX** gave results which were consistent with previous data in literature,[62–64] demonstrating that these compounds effectively enhanced GCase activity in N370S fibroblasts but lost efficacy in L444P fibroblasts when tested in intact cells. Additionally, the parent compound *N*-dodecyl-3,4,5-trihydroxypiperidine, previously synthesized by our group,[45] was re-evaluated using the test in intact Gaucher fibroblasts employed in this work, which is more reliable for evaluating chaperoning activity.[62] This allowed a direct comparison of this compound with the newly synthesized selenoureido-iminosugar derivatives (see ESI, Fig. S08). Under these experimental conditions, the compound did not enhance GCase activity. Likewise, derivatives **3b** and **3c** showed no detectable increase in enzyme activity in either of the tested cell lines. In contrast, compound **3a** significantly increased mutant GCase activity by approximately 30% at 10 μM nominal concentration, but only in N370S fibroblasts (Fig. 2 and ESI, Fig. S05A). Interestingly, the only compound that exhibited chaperoning activity was **3a**, which contains a bromine functional group, a structural feature that it shares with **ABX**. Even more intriguingly, bromine also characterizes another iminosugar derivative recently synthesized by some of us and described both as a GCase enhancer and α-synuclein binder.[30] This suggests a potential structure-activity relationship (SAR) that may contribute to the observed chaperone effect in the N370S cell line and requires future investigation through targeted SAR studies. Building on its promising chaperone profile, compound **3a** was further investigated for its ability to reduce intracellular ROS levels. Remarkably, at the same concentration at which it demonstrated chaperone activity, **3a** induced an approximate 60% reduction in ROS levels (Fig. 3) in N370S fibroblasts, underscoring a pronounced antioxidant effect. This dual activity, rescuing enzymatic function and mitigating oxidative stress, strongly

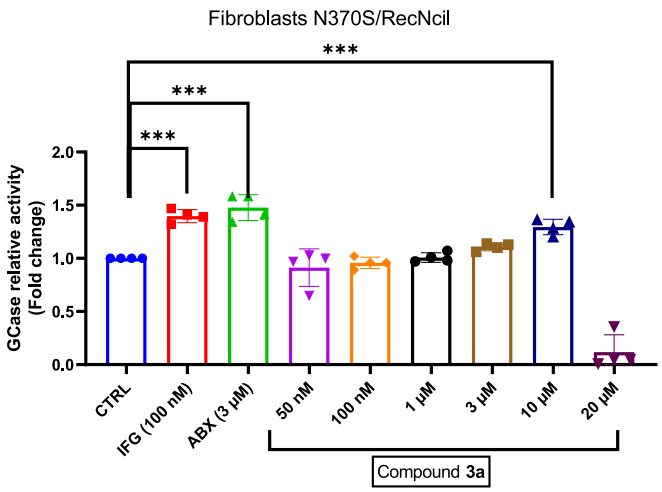


Fig. 2. GCase relative activity is reported as the ratio between the GCase activity measured in human fibroblasts derived from GD patients with N370S/RecNcil mutations after 4 days of incubation with compound **3a** and the GCase activity of the corresponding control without compound, using IFG (100 nM) and ABX (3 μM) as control. Statistical significance was determined using Student's *t*-test: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ and **** $p \leq 0.0001$. Each data point represents the analysis of quadruplicates for the CTRL group and duplicates for the samples, based on $N = 4$ independent experiments.

underscores the therapeutic potential of compound **3a** as a bifunctional agent for the treatment of GD.

5. Conclusions

In this work, we describe a straightforward approach for the synthesis of selenoureido-iminosugars **2** and **3**, bifunctional compounds designed for both GCase enhancing and ROS-scavenging. Specifically, two amino-ending *N*-alkyl-3,4,5-trihydroxypiperidine iminosugars (compounds **9** and **10**), differing in alkyl chain length (nine *vs.* twelve carbon atoms), were synthesized and subsequently coupled with three different aromatic isoselenocyanates (**12a–c**). The antioxidant activity of the six new selenoureido-iminosugars was confirmed using the DPPH radical scavenging assay, with all compounds showing an EC_{50} around 30 μM, similar to L-ascorbic acid. Moreover, such compounds, when tested in substoichiometric amounts were found to exhibit prominent GPx-like activity in the removal of peroxides using PhSH as a cofactor; they showed a significant improvement compared to $(PhSe)_2$, an antioxidant agent included herein as a reference compound. Enzyme-like kinetics were found in their mode of action. *In vitro* evaluation towards human GCase from leukocyte extract demonstrated high inhibitory activity for the whole set of compounds. We than investigated for the first time the ability of these compounds to act as pharmacological chaperones towards human fibroblasts bearing the selected Gaucher mutation. Chaperone assays on the most stable over time selenoureido-iminosugars **3a–c** revealed that compound **3a** effectively stabilized GCase and enhanced its activity in N370S fibroblasts, leading to a 30% increase in enzymatic activity at 10 μM nominal concentration. This result highlights its potential as lead compound for further clinical development. Importantly, compound **3a** also demonstrated a strong antioxidant effect, reducing intracellular ROS levels by approximately 60% at the same concentration. This dual mode of action, enhancing lysosomal GCase activity and mitigating oxidative stress, addresses two major pathological hallmarks of Gaucher disease. In conclusion, this study identifies compound **3a** as the first selenoureido-iminosugar with confirmed bifunctional activity, offering a promising basis for the development of novel therapeutic strategies targeting both lysosomal dysfunction and oxidative stress in Gaucher disease and related neurodegenerative disorders.

CRediT authorship contribution statement

Debora Pratesi: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Francesca Clemente:** Writing – original draft, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **Camilla Matassini:** Writing – review & editing, Supervision. **Silvia Falliano:** Investigation. **Amelia Morrone:** Writing – review & editing, Supervision. **Rebecca Sodano:** Investigation. **Paolo**

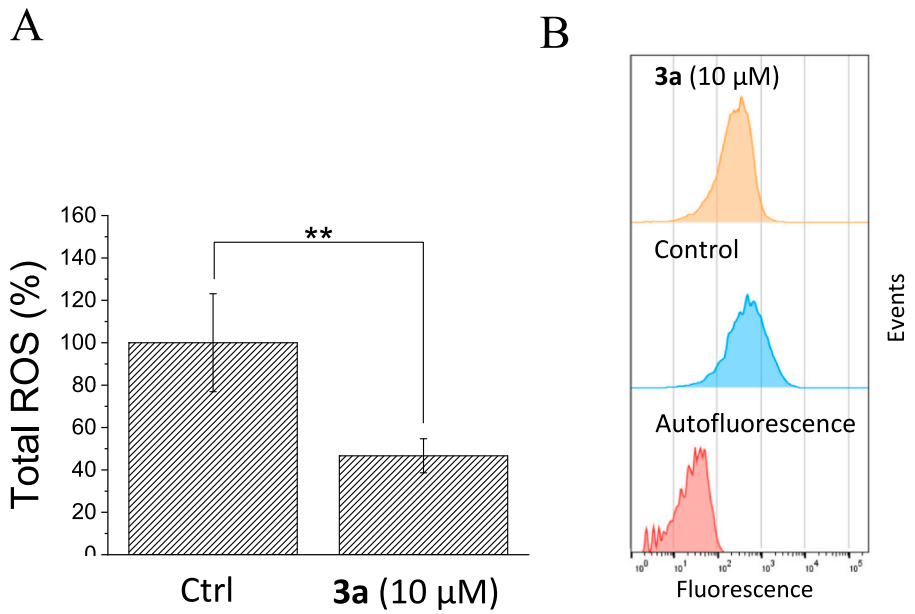


Fig. 3. Effect of **3a** on basal ROS level on fibroblasts derived from GD patients bearing N370S/RecNcil mutation. Confluent fibroblasts were treated or not with 10 μM **3a** for 24 h. After this time, intracellular ROS levels were detected using Total ROS/Superoxide Detection Kit (Enzo Life Sciences). The analyses of ROS was carried out using a flow cytometer. For each sample, at least 10,000 events were acquired. Data were analysed using FlowJo software. Data shown in the Figure (A) represent the mean ± SD. (B), fluorescence distribution of cells analysed by the flow cytometer (representative histograms). Statistical analysis of the data was performed with OriginPro 2024b (OriginLab Corporation) using Student's *t*-test ($n = 5$);** $p < 0.01$.

Paoli: Writing – review & editing, Supervision. **Andrea Goti:** Writing – review & editing, Supervision. **José G. Fernández-Bolanos:** Writing – review & editing, Investigation, Formal analysis. **Óscar López:** Writing – original draft, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **Francesca Cardona:** Writing – original draft, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

F. Cardona, F. Clemente and A. Morrone thank #NEXTGENERATIONEU (NGEU) funded by the Ministry of University and Research (MUR), National Recovery and Resilience Plan (NRRP), project MNESYS (PE0000006) – A Multiscale integrated approach to the study of the nervous system in health and disease (DN. 1553 11.10.2022). F. Cardona thanks #NEXTGENERATIONEU (NGEU) funded by the Ministry of University and Research (MUR) (PRIN: PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE – Bando 2022, Prot. 2022N9E847) for the project: MULTIFUNCTIONAL COMPOUNDS FOR A MULTI-TARGET APPROACH AGAINST NEURODEGENERATIVE DISORDERS (MULTIFUN). Ó. López thanks Spanish MICINN (grant PID2023-147401OB-I00, funded by MICIU/AEI/10.13039/501100011033 and FEDER, UE). J.G. Fernández-Bolaños and Ó. also thank Junta de Andalucía (FQM134) and VII Plan Propio de Investigación (University of Seville). A. Morrone gratefully thanks the AMMeC ONLUS for their continuing support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmc.2025.118453.

Data availability

Supplementary data to this article can be found online at (link)

References

1.. Tinggi U. Selenium: its role as antioxidant in human health. *Environ Health Prev Med*. 2008;13:102–108. https://doi.org/10.1007/s12199-007-0019-4.
2.. Hariharan S, Dharmaraj S. Selenium and selenoproteins: it's role in regulation of inflammation. *Inflammopharmacol*. 2020;28:667–695. https://doi.org/10.1007/s10787-020-00690-x.
3.. Bjørklund G, Shanaida M, Lysiuk R, et al. Selenium: an antioxidant with a critical role in anti-aging. *Molecules*. 2022;27:6613–6629. https://doi.org/10.3390/molecules27196613.
4.. Kieliszek M, Bano I, Zare H. A comprehensive review on selenium and its effects on human health and distribution in middle eastern countries. *Biol Trace Elem Res*. 2022;200:971–987. https://doi.org/10.1007/s12011-021-02716-z.
5. Gallo-Rodriguez C, Rodriguez JB. Organoselenium compounds in medicinal chemistry. *ChemMedChem*. 2024;19, e202400063. https://doi.org/10.1002/cmdc.202400063.
6. Sonego JM, de Diego SI, Szajnman SH, Gallo-Rodriguez C, Rodriguez JB. Organoselenium compounds: chemistry and applications in organic synthesis. *Chem Eur J*. 2023;29, e202300030. https://doi.org/10.1002/chem.202300030.
7. Anghinoni JM, Birmann PT, da Rocha MJ, et al. Recent advances in the synthesis and antioxidant activity of low molecular mass Organoselenium molecules. *Molecules*. 2023;28:7349–7394. https://doi.org/10.3390/molecules28217349.
8.. Calvo-Martín G, Plano D, Encío I, Sanmartín C. Novel *N,N'*-disubstituted selenoureas as potential antioxidant and cytotoxic agents. *Antioxidants*. 2021;10:777–791. https://doi.org/10.3390/antiox10050777.
9. Roldán-Peña JM, Alejandre-Ramos D, López Ó, et al. New tacrine dimers with antioxidant linkers as dual drugs: anti-Alzheimer's and antiproliferative agents. *Eur J Med Chem*. 2017;138:761–773. https://doi.org/10.1016/j.ejmech.2017.06.048.
10. Fuentes-Aguilar A, Romero-Hernández LL, Arenas-González A, et al. New selenosteroids as antiproliferative agents. *Org Biomol Chem*. 2017;15:5041–5054. https://doi.org/10.1039/C7OB00458C.
11. Romero-Hernández LL, Merino-Montiel P, Montiel-Smith S, et al. Diosgenin-based thio(seleno)ureas and triazolyl glycoconjugates as hybrid drugs. Antioxidant and antiproliferative profile. *Eur J Med Chem*. 2015;99:67–81. https://doi.org/10.1016/j.ejmech.2015.05.018.
12. Hussain RA, Badshah A, Shah A. Synthesis and biological applications of selenoureas. *Appl Organomet Chem*. 2014;28:61–73. https://doi.org/10.1002/aoc.3093.
13. Pereira DM, Valentão P, Andrade PB. Tuning protein folding in lysosomal storage diseases: the chemistry behind pharmacological chaperones. *Chem Sci*. 2018;9:1740–1752. https://doi.org/10.1039/C7SC04712F.
14. Yu Z, Sawkar AR, Kelly JW. Pharmacologic chaperoning as a strategy to treat Gaucher disease. *FEBS J*. 2007;274:4944–4950. https://doi.org/10.1111/j.1742-4658.2007.06042.x.
15. Boyd RE, Lee G, Rybczynski P, et al. Pharmacological chaperones as therapeutics for lysosomal storage diseases. *J Med Chem*. 2013;56:2705–2725. https://doi.org/10.1021/jm301557k.
16. Sánchez-Fernández EM, García Fernández JM, Ortiz Mellet C. Glycomimetic-based pharmacological chaperones for lysosomal storage disorders: lessons from Gaucher, G_{M1}-gangliosidosis and Fabry diseases. *Chem Commun*. 2016;52:5497–5515. https://doi.org/10.1039/C6CC01564F.
17.. Martínez-Bailén M, Clemente F, Matassini C, Cardona F. GCase enhancers: a potential therapeutic option for Gaucher disease and other neurological disorders. *Pharmaceuticals*. 2022;15:823–849. https://doi.org/10.3390/ph15070823.
18. Grabowski GA, ed. *Advances in Gaucher Disease: Gaucher Disease: Basic and Clinical Perspectives, Future Medicine Ltd, Unitec House, 2 Albert Place*. London N3 1QB: UK; 2013.
19.. Avenali M, Blandini F, Cerri S. Glucocerebrosidase defects as a major risk factor for Parkinson's disease. *Front Aging Neurosci*. 2020;12:97. https://doi.org/10.3389/fnagi.2020.00097.
20. Smith L, Schapira AHV. *GBA* variants and Parkinson disease: mechanisms and treatments. *Cells*. 2022;11:1261–1284. https://doi.org/10.3390/cells11081261.
21. Höglinger G, Schulte C, Jost WH, et al. *GBA*-associated PD: chances and obstacles for targeted treatment strategies. *J Neural Transm*. 2022;129:1219–1233. https://doi.org/10.1007/s00702-022-02511-7.
22. Huh YE, Usnich T, Scherzer CR, Klein C, Chung SJ. *GBA1* variants and Parkinson's disease: paving the way for targeted therapy. *J Mov Disord*. 2023;16:261–278. https://doi.org/10.14802/jmd.23023.
23.. Davis MY, Trinh K, Thomas RE, et al. Glucocerebrosidase deficiency in *Drosophila* results in α-synuclein-independent protein aggregation and neurodegeneration. *PLoS Genet*. 2016;12, e1005944. https://doi.org/10.1371/journal.pgen.1005944.
24.. Rockenstein E, Clarke J, Viel C, et al. Glucocerebrosidase modulates cognitive and motor activities in murine models of Parkinson's disease. *Hum Mol Genet*. 2016;25:2645–2660. https://doi.org/10.1093/hmg/ddw124.
25.. Silveira CRA, MacKinley J, Coleman K, et al. Ambroxol as a novel disease-modifying treatment for Parkinson's disease dementia: protocol for a single-Centre, randomized, double-blind, placebo-controlled trial. *BMC Neurol*. 2019;19:20. https://doi.org/10.1186/s12883-019-1252-3.
26. Kuo SH, Tasset I, Cheng MM, et al. Mutant glucocerebrosidase impairs α-synuclein degradation by blockade of chaperone-mediated autophagy. *Sci Adv*. 2022;11, eabm6393. https://doi.org/10.1126/sciadv.abm6393.
27.. Hertz E, Chen Y, Sidransky E. Gaucher disease provides a unique window into Parkinson disease pathogenesis. *Nat Rev Neurol*. 2024;20:526–540. https://doi.org/10.1038/s41582-024-00999-z.
28. Bolognesi ML, Cavalli A. Multitarget drug discovery and Polypharmacology. *ChemMedChem*. 2016;11:1190–1192. https://doi.org/10.1002/cmdc.201600161.
29. Ramsay RR, Popovic-Nikolic MR, Nikolic K, Uliassi E, Bolognesi ML. A perspective on multi-target drug discovery and design for complex diseases. *Clin Transl Med*. 2018;7:3. https://doi.org/10.1186/s40169-017-0181-2.
30. Tagliaferro G, Davighi MG, Clemente F, et al. Evidence of α-Synuclein/Glucocerebrosidase dual targeting by Iminosugar derivatives. *ACS Chem Neurosci*. 2025;16:1251–1257. https://doi.org/10.1021/acschemneuro.4c00618.
31. Ferhati X, Matassini C, Fabbrini MG, et al. Dual targeting of PTP1B and glucosidases with new bifunctional iminosugar inhibitors to address type 2 diabetes. *Bioorg Chem*. 2019;87:534–549. https://doi.org/10.1016/j.bioorg.2019.03.053.
32. Wennekes T, Meijer AJ, Groen AK, et al. Dual-action lipophilic Iminosugar improves glycemic control in obese rodents by reduction of visceral glycosphingolipids and buffering of carbohydrate assimilation. *J Med Chem*. 2010;53:689–698. https://doi.org/10.1021/jm901281m.
33.. Mozafari H, Khatami S, Kiani A, et al. Oxidative stress parameters, trace elements, and lipid profile in Iranian patients with Gaucher disease. *Biol Trace Elem Res*. 2020;193:130–137. https://doi.org/10.1007/s12011-019-01709-3.
34. Kartha RV, Terluk MR, Brown R, et al. Patients with Gaucher disease display systemic oxidative stress dependent on therapy status. *Mol Genet Metab Rep*. 2020;25, 100667. https://doi.org/10.1016/j.ymgmr.2020.100667.
35. Sahasrabudhe SA, Terluk MR, Rudser KD, Cloyd JC, Kartha RV. Biological variation in peripheral inflammation and oxidative stress biomarkers in individuals with Gaucher disease. *Int J Mol Sci*. 2022;23:9189–9203. https://doi.org/10.3390/ijms23169189.
36. Roh J, Subramanian S, Weinreb NJ, Kartha RV. Gaucher disease – more than just a rare lipid storage disease. *Int J Mol Med*. 2022;100:499–518. https://doi.org/10.1007/s00109-021-02174-z.
37.. Cabasso O, Kuppuramalingam A, Lelieveld L, et al. Animal models for the study of Gaucher disease. *Int J Mol Sci*. 2023;24:16035–16056. https://doi.org/10.3390/ijms242216035.

38. Teleanu DM, Niculescu AG, Lungu II, et al. An overview of oxidative stress, Neuroinflammation, and neurodegenerative diseases. *Int J Mol Sci*. 2022;23: 5938–5960. https://doi.org/10.3390/ijms23115938.
39. Tansey MG, Wallings RL, Houser MC, Herrick MK, Keating CE, Joers V. Inflammation and immune dysfunction in Parkinson disease. *Nat Rev Immunol*. 2022;22:657–673. https://doi.org/10.1038/s41577-022-00684-6.
40. Olsen JI, Plata GB, Padrón JM, López Ó, Bols M, Fernández-Bolaños JG. Selenoureido-iminosugars: a new family of multitarget drugs. *Eur J Med Chem*. 2016; 123:155–160. https://doi.org/10.1016/j.ejmech.2016.07.021.
41. Compain P, Martin OR, Boucheron C, et al. Design and synthesis of highly potent and selective pharmacological chaperones for the treatment of Gaucher's disease. *ChemBiochem*. 2006;7:1356–1359. https://doi.org/10.1002/cbic.200600217.
42.. Parmeggiani C, Catarzi S, Matassini C, et al. Human acid β-glucosidase inhibition by carbohydrate derived Iminosugars: towards new pharmacological chaperones for Gaucher disease. *ChemBioChem*. 2015;16:2054–2064. https://doi.org/10.1002/cbic.201500292.
43.. Clemente F, Matassini C, Goti A, Morrone A, Paoli P, Cardona F. Stereoselective synthesis of C-2 alkylated Trihydroxypiperidines: novel pharmacological chaperones for Gaucher disease. *ACS Med Chem Lett*. 2019;10:621–626. https://doi.org/10.1021/acsmedchemlett.8b00602.
44.. Clemente F, Matassini C, Faggi C, et al. Glucocerebrosidase (GCase) activity modulation by 2-alkyl trihydroxypiperidines: inhibition and pharmacological chaperoning. *Bioorg Chem*. 2020;98, 103740. https://doi.org/10.1016/j.bioorg.2020.103740.
45.. Vanni C, Cardona F, Clemente F, et al. Amphiphilic Iminosugar pharmacological chaperones towards β-Glucocerebrosidase: self-assembly and biological activity. *Eur J Org Chem*. 2023;26, e202200911. https://doi.org/10.1002/ejoc.202200911.
46. Prichard K, Campkin D, O'Brien N, Kato A, Fleet GWJ, Simone MI. Biological activities of 3,4,5-trihydroxypiperidines and their *N*- and *O*-derivatives. *Chem Biol Drug Des*. 2018;92:1171–1197. https://doi.org/10.1111/cbdd.13182.
47.. Matassini C, Mirabella S, Goti A, Cardona F. Double reductive amination and selective Strecker reaction of a D-Lyxaric aldehyde: synthesis of diversely functionalized 3,4,5-Trihydroxypiperidines. *Eur J Org Chem*. 2012:3920–3924. https://doi.org/10.1002/ejoc.201200587.
48.. Matassini C, Mirabella S, Ferhati X, et al. Polyhydroxyamino-Piperidine-type Iminosugars and Pipecolic acid analogues from a D-mannose-derived aldehyde. *Eur J Org Chem*. 2014:5419–5432. https://doi.org/10.1002/ejoc.201402427.
49.. Vanni C, Clemente F, Paoli P, et al. 3,4,5-Trihydroxypiperidine based multivalent Glucocerebrosidase (GCase) enhancers. *ChemBioChem*. 2022;23, e202200077. https://doi.org/10.1002/cbic.202200077.
50. Clemente F, Davighi MG, Matassini C, et al. Light-triggered control of Glucocerebrosidase inhibitors: towards Photoswitchable pharmacological chaperones. *Chem Eur J*. 2023;29, e202203841. https://doi.org/10.1002/chem.202203841.
51.. Romuald C, Cazals G, Enjalbal C, Coutrot F. Straightforward synthesis of a double-lasso macrocycle from a nonsymmetrical [c2]daisy chain. *Org Lett*. 2013;15: 184–187. https://doi.org/10.1021/ol303186j.
52.. Koketsu M, Suzuki N, Ishihara H. Preparation of isoselenocyanate and synthesis of carbodiimide by oxidation of selenourea. *J Org Chem*. 1999;64:6473–6475. https://doi.org/10.1021/jo990096j.
53.. Suzuki H, Usuki M, Hanafusa T. A photochemical route to some substituted benzyl Isoselenocyanates. *Synthesis*. 1979:705–707. https://doi.org/10.1055/s-1979-28803.
54.. Henriksen L, Ehrbar U. One-step synthesis of alkyl and aryl Isoselenocyanates from primary amines. *Synthesis*. 1976:519–521. https://doi.org/10.1055/s-1976-24102.
55. Barton DHR, Parekh SI, Tajbakhsh M, Theodorakis EA, Tse C-L. A convenient and high yielding procedure for the preparation of isoselenocyanates. Synthesis and reactivity of *O*-alkylselenocarbamates. *Tetrahedron*. 1994;50:639–654. https://doi.org/10.1016/S0040-4020(01)80783-3.
56. López Ó, Maza S, Ulgar V, Maya I, Fernández-Bolanos JG. Synthesis of sugar-derived isoselenocyanates, selenoureas, and selenazoles. *Tetrahedron*. 2009;65:2556–2566. https://doi.org/10.1016/j.tet.2009.01.038.
57. Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J Agric Food Chem*. 2005;53:4290–4302. https://doi.org/10.1021/jf0502698.
58. Sharma OP, Bhat TK. DPPH antioxidant assay revisited. *Food Chem*. 2009;113: 1202–1205. https://doi.org/10.1016/j.foodchem.2008.08.008.
59.. Pratesi D, Brenzini Biagioni F, Matassini C, et al. Studies on Azasugar-based sulfonamides: a new class of carbonic anhydrase inhibitors. *Bioorg Chem*. 2025;164, 108857. https://doi.org/10.1016/j.bioorg.2025.108857.
60.. Pei J, Pan X, Pan X, Wei G, Hua Y. Research progress of glutathione peroxidase family (GPX) in redoxidation. *Front Pharmacol*. 2023;14, 1147414. https://doi.org/10.3389/fphar.2023.1147414.
61. Mugesh G, Panda A, Singh HB, Punekar NS, Butcher RJ. Glutathione peroxidase-like antioxidant activity of Diaryl Diselenides: a mechanistic study. *J Am Chem Soc*. 2001;123:839–850. https://doi.org/10.1021/ja994467p.
62.. Li H-Y, Chen W-A, Lin H-Y, et al. A practical synthesis of nitrone-derived C5a-functionalized isofagomines as protein stabilizers to treat Gaucher disease. *Commun Chem*. 2024;7:91–100. https://doi.org/10.1038/s42004-024-01164-9.
63. Sawkar AR, Cheng W-C, Beutler E, Wong C-H, Balch WE, Kelly JW. Chemical chaperones increase the cellular activity of N370S β-glucosidase: a therapeutic strategy for Gaucher disease. *Proc Natl Acad Sci*. 2002;99:15428–15433. https://doi.org/10.1073/pnas.192582899.
64.. Luana Z, Lia L, Higaki K, Nanbab E, Suzukic Y, Ohnoa K. The chaperone activity and toxicity of ambroxol on Gaucher cells and normal mice. *Brain Dev*. 2013;35: 317–322. https://doi.org/10.1016/j.braindev.2012.05.008.