HHS Public Access

Author manuscript

Bioorg Med Chem Lett. Author manuscript; available in PMC 2021 January 15.

Published in final edited form as:

Bioorg Med Chem Lett. 2020 January 15; 30(2): 126806. doi:10.1016/j.bmcl.2019.126806.

Novel compounds that reverse the disease phenotype in Type 2 Gaucher disease patient-derived cells

Wayne Childers[*], **Rong Fan**, **Rogelio Martinez**, **Dennis J. Colussi**, **Edward Melenski**, **Yuxiao Liu**, **John Gordon**, **Magid Abou-Gharbia**, **Marlene A. Jacobson**[*]

Moulder Center for Drug Discovery Research, Department of Pharmaceutical Sciences, Temple University School of Pharmacy, 3307 N. Broad Street, Philadelphia, PA, 19140, USA

Abstract

Gaucher disease (GD) results from inherited mutations in the lysosomal enzyme β-glucocerobrosidase (GCase). Currently available treatment options for Type 1 GD are not efficacious for treating neuronopathic Type 2 and 3 GD due to their inability to cross the blood-brain barrier. In an effort to identify small molecules which could be optimized for CNS penetration we identified tamoxifen from a high throughput phenotypic screen on Type 2 GD patient-derived fibroblasts which reversed the disease phenotype. Structure activity studies around this scaffold led to novel molecules that displayed improved potency, efficacy and reduced estrogenic/antiestrogenic activity compared to the original hits. Here we present the design, synthesis and structure activity relationships that led to the lead molecule Compound **31**.

Graphical Abstract

Tamoxifen
Gaucher Type 2 EC_{50} = 10 μM
Emax = 148%
% Estrogen Activity @ 10 uM = 100%

Compound 31
Gaucher's Type 2 EC_{50} = 3.1 μM
Emax = 240 %
% Estrogen Activity @ 10 uM = 0%

[*]Corresponding Authors: wayne.childers@temple.edu (W. Childers) marlene.jacobson@temple.edu (M. Jacobson).

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.

Supplementary Material

Supplementary material (synthetic procedures, biological procedures) associated with this article can be found in the online version.

Keywords

Gaucher disease; Lysosomal storage disease; Phenotypic screening; Diphenylethanono; Neuronopathic

Lysosomal storage disorders (LSDs) are inheritable metabolic diseases with deficiencies in enzymes that function within the glycosphingolipid biosynthetic and metabolic pathway. As a consequence, non-degraded substrates accumulate and normal lysosomal function is compromised, resulting in impaired autophagy, disruption of cell signaling and eventually cell death. A group of more than 70 diseases have been classified as LSDs, including Gaucher, Tay-Sachs, Sandhoff and Fabry disease, Niemann-Pick type C and GM1 gangliosidosis.[1,2] Two thirds of LSDs have central nervous system involvement resulting in a progressive neurodegeneration and the leading cause of patient death. The most common LSD is Gaucher disease, with a prevalence of 1 in 57,000 births.[3]

Gaucher disease (GD) is an autosomal recessive disorder resulting from mutations in the gene *GBA1*, encoding the lysosomal enzyme β-glucocerebrosidase (GCase). Reduced or loss of GCase activity leads to accumulation of glucosylceramide and glucosylsphingosine. GD is clinically defined into three types based on the age of disease onset and clinical manifestations of organ involvement.[4] Type 1 GD is non-neuronopathic while types 2 and 3 GD have CNS involvement.

Type 2 GD (acute neuronopathic) is the most severe, having rapid and progressive neurodegeneration during the first years of life. Type 3 GD also has neurological manifestations and exhibits a later onset and slower progression.

Current treatment options for GD patients include enzyme replacement therapy (ERT) with recombinant GCase (Cerezyme®, imiglucerase) and substrate reduction therapy (SRT) with small molecule inhibitors of glucosylceramide synthase (GCS) to reduce production of substrate which is not degraded due to the reduced GCase activity.[5] Two GCS inhibitors (Figure 1) are FDA approved for treatment of Type 1 GD, the sugar-based glucocerebrosidase inhibitor Zavesca™ (miglustat)[6] and the benzodioxan derived GCS inhibitor Cerdelga® (eliglustat).[7] While the availability of ERT and SRT has had significant impact on the treatment of GD, their effectiveness is limited to patients with non-neuronopathic Type 1 GD, as neither the enzyme nor the small molecules are able to cross the blood-brain barrier. Recently a GCS inhibitor, Venglustat (ibiglustat, Figure 1) with improved properties to penetrate the CNS has been reported with potential to overcome the limitations of existing SRT and is currently in clinical trials.[8,9]

The application of high throughput screening (HTS) approaches to identify small molecule treatments for GD have targeted GCase to either identify chaperones which improve or enhance enzyme activity or inhibitors of GCS to reduce substrate burden into the defective enzyme pathway.[10,11] With the goal to discover small molecule therapeutics for neuronopathic GD, we chose a phenotypic screening approach utilizing patient-derived cells to identify small molecules with activity to reverse the diseased lysosomal phenotype as an alternative to target-based screening strategies.[12] Disruption of calcium homeostasis and

impaired lysosomal function are measurable, phenotypic characteristics of LSD patient-derived cells, including GD.[13–16]

We discovered that Type 2 GD patient-derived cells (L444P/L444P genotype) display reduced calcium release from lysosomal acidic stores in response to Gly-Phe-β-napthylamide (GPN) compared to normal cells (WT) (Figure 2). GPN is a substrate of cathepsin C, which upon hydrolysis, produces osmotic lysis and release of calcium from lysosomal acidic stores into the cytosol.[17] On the basis of this phenotypic difference between GD and WT cells, we developed a high throughput fluorescence-based assay to screen for compounds with activity to reverse the lysosomal calcium release deficit found in GD patient-derived cells. We have previously described this HTS assay and its application to Tay-Sachs patient-derived cells.[18] Briefly, GPN-induced calcium release into the cytosol is detected with a fluorescent calcium indicator (Fluo-8AM) and monitored in real- time on a fluorescence kinetic plate reader (Hamamatsu FDSS μCell). A pilot screen on 1,200 known FDA approved drugs (Prestwick Chemical Library®), 10 μM screening concentration, was completed. One of the hits identified was tamoxifen (Figure 3), a selective estrogen receptor modulator (SERM) used to treat estrogen-sensitive breast cancers. Tamoxifen exhibited modest functional potency, EC_{50} = 10 μM and 148% activation over the basal response to GPN-induced lysosomal calcium release measured on GD cells in the absence of compound treatment (Figure 2). Subsequent testing of related analogs identified idoxifene (**2**, Figure 3), which showed similar activity as tamoxifen. The known drug-like properties of these molecules make them reasonable starting points for hit-to-lead activities. However, their potent estrogenic activity would be considered unacceptable as a chronic therapy, especially in the younger patient population affected by Type 2 GD. We therefore carried out structure-activity studies to enhance the potency and efficacy of this chemical scaffold while reducing the estrogen activity. Our results are presented below.

Published SAR studies suggested that the anti-estrogenic activity of the tamoxifen scaffold can be modulated by substitution on the aryl rings not containing the dimethylaminoethoxy group group.[19,20] The greatest effect was seen when 4-substitution was modified on the aryl ring occupying the 2-position of the double bond (carbon 26)[21]. Therefore, we initiated a short structure-activity relationship (SAR) study to examine the effects of aryl substitutions, ring deletions, positioning of the alkyl group and saturation of the double bond on the potency of the scaffold as an estrogen modulator versus potency in the GPN-induced lysosomal calcium release assay in Type 2 GD fibroblasts (Table 1). Detailed synthetic methods are provided in the Supplementary Material.

Removing one of the phenyl rings reduced estrogen receptor activity with a switch in functional activity from inhibitor to activator. However, most of the analogs lost potency in the calcium release assay. The one exception was compound **5**, which was somewhat more potent than and equally efficacious as tamoxifen in the calcium assay. Homologating the one phenyl ring to a benzyl group (compounds **8, 9**) did not enhance the desired potency and efficacy or reduce the estrogenic activity. Substitution on the aryl ring lacking the dimethylaminoethoxy group (**10–15**) reduced potency in the calcium assay in general. One structural difference between compound **5** and most other analogs is the ability of the distal aryl ring to more easily assume a skewed conformation relative to the conjugated system due

to a lack of the central double bond. Hypothesizing that the ability of this ring to assume a non-planar, unconjugated conformation might play a role in the improved efficacy on GD cells, we embarked on a series of novel di-phenylethanone derivatives (compounds **17–31**) that possessed substitution in the 2-position of the aryl ring that we felt needed to be perpendicular to the rest of the scaffold. The purpose of the ketone was to arrive at a novel chemical scaffold and to eliminate the extend conjugation that promotes planarity in tamoxifen and related analogs. The results are presented in Table 2.

The synthetic method for compounds **17–24** is depicted in Scheme 1. Phenyl acetic acid and 2-(chloroethoxy) benzene were condensed according to the method of Horton et al.[22] to give the desired diphenylethanone intermediate, which was then alkylated and aminated to provide the compounds. Some final targets were isolated as free bases, while the ones that were purified by reversed chromatography were obtained as trifluoroacetate salts. The one exception to this general method was the synthesis of compound **17** where 2-phenylbutanoic acid and 2-(chloroethoxy)benzene were condensed directly to give the ethyl intermediate which was then converted to the dimethylamine. Detailed experimental methods are provided in the Supplementary Material.

The general method for the syntheses of compounds **25–31** are depicted in Schemes 2. A similar sequence to that depicted in Scheme 1 was used to arrive at a common intermediate, which was then subjected to a Suzuki coupling to arrive at compounds **25** and **27**. Those compounds were then catalytically reduced to provide compounds, **26** and **28–31**. The order of some steps was altered for the synthesis of compounds **30** and **31** but the same series of reactions was used. Detailed experimental methods are provided in the Supplementary Material.

For our initial SAR studies on the diphenylethanone series (Table 2) we held the dimethylaminoethoxy group constant, set R_2 = H and varied the alkyl substituent R_1 (compounds **17 – 24**). Compounds with linear alkyl groups such as ethyl (**17**), n-propyl (**18**), n-butyl (**21**) and n-hexyl (**24**) demonstrated little effect on lysosomal calcium release from GD patient-derived cells. Increasing lipophilic bulk in this region of the molecule by installing an isopropyl group (**19**) or gem-di-propyl moieties (**20**) provided analogs that demonstrated greater potency to that demonstrated by tamoxifen in the GPN-stimulated calcium release assay (EC_{50} = 4 – 4.4 μM vs. 10 μM, respectively) but these compounds did not increase the percent of calcium release over basal levels to the same percentage as tamoxifen (54 – 60% vs/148%, respectively). Shifting the lipophilic bulk to further out on the alkyl chain provided the isobutyl derivative **22**, which was 10-fold more potent but somewhat less efficacious than tamoxifen, and the isopentyl derivative **23** which was 3-fold more potent and roughly 30% more efficacious than tamoxifen. Estrogenic activity for compounds **23** and **24** was reduced compared to tamoxifen and they demonstrated partial agonist activity compared to tamoxifen, which demonstrated antagonist activity.

We then moved to test our hypothesis regarding the preference of a non-conjugated distal phenyl ring in the diphenylethanone scaffold by introducing 2-substitutents in this ring (compounds **25 – 31**, Table 2).

As previously discussed, the n-butyl derivative **21** showed no activity in the calcium release assay in GD patient-derived cells. However, introduction of a 2-substituent into that scaffold resulted in analogs that showed activity in that assay that was similar to (compounds **25** and **26**) or superior to (compounds **27** and **28**) that seen with tamoxifen. Greater potency was realized when the planarity of the substituent was eliminated. This is seen by comparing compound **25** to **26** (EC_{50} = 2.3 μM and 0.65 μM, respectively) and compound **27** to **28** (EC_{50} = 2.2 μM and 1.4 μM, respectively). A significant increase in efficacy was seen when the isopropyl group was introduced into the 2-position of the distal phenyl ring (compound **28**, Emax = 401% of the basal response to GPN). The presence of the branched alkyl group also had a beneficial effect on estrogenic activity. The ethenyl derivative **25** showed significant estrogen receptor activity at a concentration of 10 μM (100% of the EC_{100} response seen with β-estradiol) whereas the propenyl (**27**) and i-propyl (**28**) analogs were less potent in the estrogen receptor assay (35% and 45%, respectively). Interestingly, the ethyl derivative **26** showed similar potency in the estrogen receptor assay but was found to be an antagonist rather than an agonist like **25, 27** and **28**. The reasons for this switch in intrinsic activity are not clear, although it should be noted that the ethyl group is present in a comparable position in the tamoxifen/idoxifene scaffold so it is possible that the ethyl group is a privileged substituent in this position.

Similar results were seen when the i-butyl group was substituted for the n-butyl moiety. Compound **29**, which combined the i-butyl substitution on the ethanone chain and the i-propyl group in the 2-position of the distal phenyl ring, demonstrated reasonable potency, superior efficacy and reduced estrogenic activity compared to tamoxifen. Moving the i-propyl group to the 3-position of the distal phenyl ring (compound **30**) resulted in a compound with significantly reduced efficacy (80% compared to 406% for compound **29**). Constraining the i-propyl group to give the cyclopropyl analog **31** resulted in a molecule that showed similar potency and enhanced efficacy in the calcium release assay compared to tamoxifen and was devoid of estrogenic activity at a concentration of 10 μM.

Activity for the four most potent compounds (**27, 28, 29** and **31**) and tamoxifen in the lysosomal calcium assay in GD patient-derived cells and wild-type cells are summarized in Table 3. Full curves are provided in the Supplementary Material. All four analogs demonstrated superior potency and percent maximal activation of GPN-stimulated calcium release compared to tamoxifen. And while the EC_{50} values for activation of GPN-stimulated calcium release were slightly lower in WT cells compared to GD-patient cells, all four compounds showed significantly greater efficacy (maximal response) in GD-patient cells. Compounds **28** and **31** in particular demonstrated high maximal activation in GD-patient cells (227% and 240%, respectively) in comparison to the maximal activation in WT cells (56% and 50%, respectively).

To differentiate compounds **27, 28, 29** and **31** and prioritize one to advance to in vivo studies, we examined their in vitro drug-like properties in two assays, namely maximum aqueous solubility in 2% DMSO/phosphate buffered saline and stability in mouse liver microsomes in the presence of NADPH (an indicator of liability to oxidative metabolism). The results are summarized in Table 4.

All four phenylethanone derivatives showed superior aqueous solubility compared to tamoxifen. All four phenylethanone derivatives also showed moderate stability in mouse and human liver microsomes. Compound **29** was somewhat more stable in mouse liver microsomes than the other three. However, compound **31** stood out from the others because of its lack of any estrogenic activity at 10 μM (Table 2). For this reason we selected compound **31** for advancement.

In conclusion, a phenotypic screen identified tamoxifen as a hit with modest functional potency to reverse the lysosomal calcium deficits in GD patient-derived cells. SAR aimed at reducing the planar conformation of tamoxifen led to the identification of a novel series of phenylethanone derivatives, ultimately resulting in the selection of compound **31** for further evaluation. In the current studies compound **31** was tested as a racemic mixture. Despite the realatively high pKa predicted for the enolic proton of **31** (calculated pKa[24] for the proton adjacent to the carbonyl = 15.66), data demonstrate that drugs containing enolic hydrogens can undergo some racemization at physiological pH, especially in the presence of phosphate buffers.[25,26] It will be important to understand the role of chirality on in vitro and in vivo activity in this series of compounds and understand the propensity for them to undergo partial or full racemization at physiological pH. These and other studies are currently in progress and will be the subject of future publications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Dr. Michael Denison for the BG1Luc4E2 (VM7Luc4E2) cell line. This research was partially supported by the Officer of the Director, National Institutes of Health under award number S10OD018195-01 to M.A.J.

References and notes

1. Platt FM, D'Azzo A, Davidson BL et al., Nat. Rev. Dis. Primers, 2018, 4, 27. [PubMed: 30275469]
2. Marques ARA and Saftig PJ Cell Sci, 2019, 132,
3. Meikle PJ, Hopwoodd JJ, Clauge WF, Carery WF JAMA, 1999, 281, 249. [PubMed: 9918480]
4. Grabowki GA, Hemat. Am. Soc. Hematol. Ed. Program, 2012, 13.
5. Platt FM Nat Rev Drug Discov, 2017, 17, 133. [PubMed: 29147032]
6. McCormack PL, Goa KL, Drugs, 2003, 63, 2427. [PubMed: 14609352]
7. Shayman JA, Drugs of the Future, 2010, 35, 613. [PubMed: 22563139]
8. Marshall J, Sun Y, Bangari DS, et al., Mol Ther. 2016 24,1019. [PubMed: 26948439]
9. https://clinicalttials.gov/ct2/show/NCT01674036. Last accessed 10/25/2019.
10. Zheng W, Padia J, Urban DJ, et al., Natl. Acad. Sci. USA, 2007, 104, 13192.
11. Koltun E, Richards S, Chan V, et al., Bioorg. Med. Chem. Lett, 2011, 21, 6773. [PubMed: 21974949]
12. Swinney DC, J. Biomol. Screen, 2013, 18, 1186. [PubMed: 23983234]
13. Lloyd-Evans E, Platt FM, Cell Calcium 2011, 50, 200. [PubMed: 21724254]
14. Kiselyov K, Yamaguchi S, Lyons CW, Muallem S, Cell Calcium 2010, 47, 103. [PubMed: 20053447]
15. Vitner EB, Platt FM, Futerman AH, J. Biol. Chem 2010, 285, 20423. [PubMed: 20430897]

16. Kilpatrick BS, Magalhaes J, Beavan MS, McNeill A et al., Cell Calcium 2016, 59, 12. [PubMed: 26691915]
17. Berg TO, Stromhaug E, Lourdal T, et al. Biochem. J 1994, 300, 229. [PubMed: 8198538]
18. A manuscript on the Gaucher patient-derived cell HTS is in preparation. A similar assay protocol employing Tay Sachs patient-derived cells is available in: Jacobson MA; Colussi DJ, SLAS Disc., 2019, 24, 295.
19. McCague R, Leclercq G, Legros N, et al., J. Med. Chem, 1989, 32, 2527. [PubMed: 2585441]
20. Lloyd DG, Hughes RB, Zisterer M, et al., J. Med. Chem, 2004, 47, 5612. [PubMed: 15509159]
21. Shahrokh K, Cheatham TE III, Yost GS, Biochem. Biophys. Acta, 2012, 1820, 1605. [PubMed: 22677141]
22. Horton MN, Jarmin M, Potter GA, J. Lab. Comp. Radiopharm 1994 34 767.
23. Sanford Burnham Center for Chemical Genomics, Record # SID56323502, https://pubchem.ncbi.nlm.nih.gov/bioassay/1996#section=Data=Table. Last accessed 07/25/2019.
24. Calculated based on the partial charge distribution method using the pKa calculator available from ChemAxon in the free application MarvinSketch (https://docs.chemaxon.com/display/docs/pKa+calculation). Last accessed 10/25/2019.
25. Ballard A, Narduolo S, Ahmad HO, et al., Exp. Opin. Drug Disc, 2019, 29, 527.
26. Ballard A, Ahmad HO, Narduolo S, et al., Ang. Chem. Int. Ed. Eng, 2018, 57, 982.

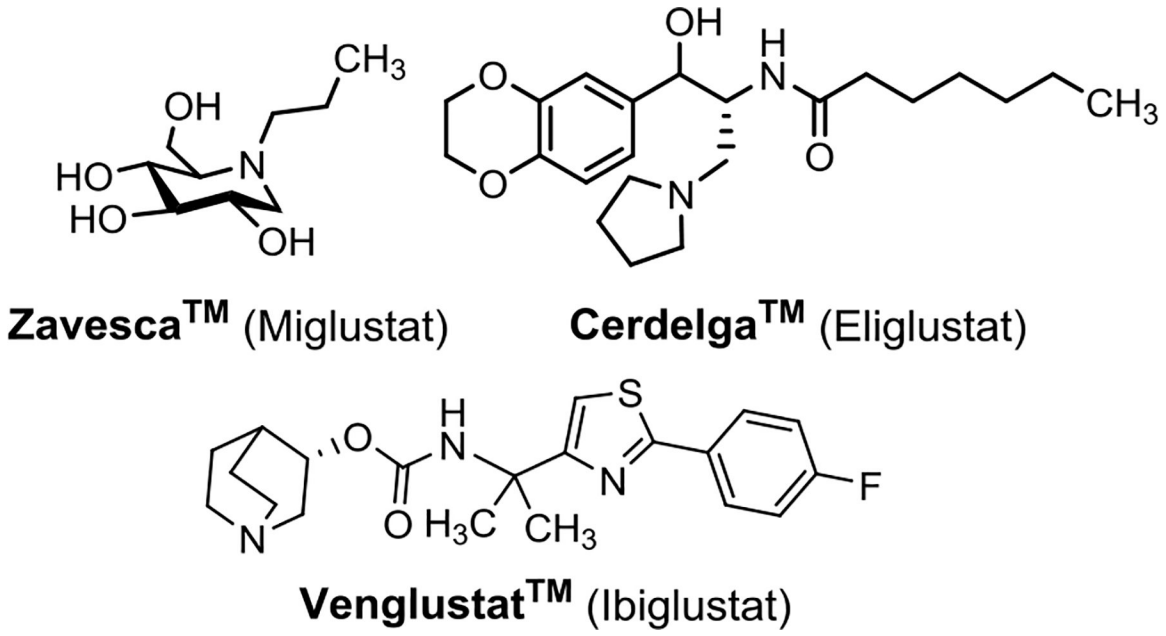


Figure 1.
Small molecules for substrate reduction therapy.

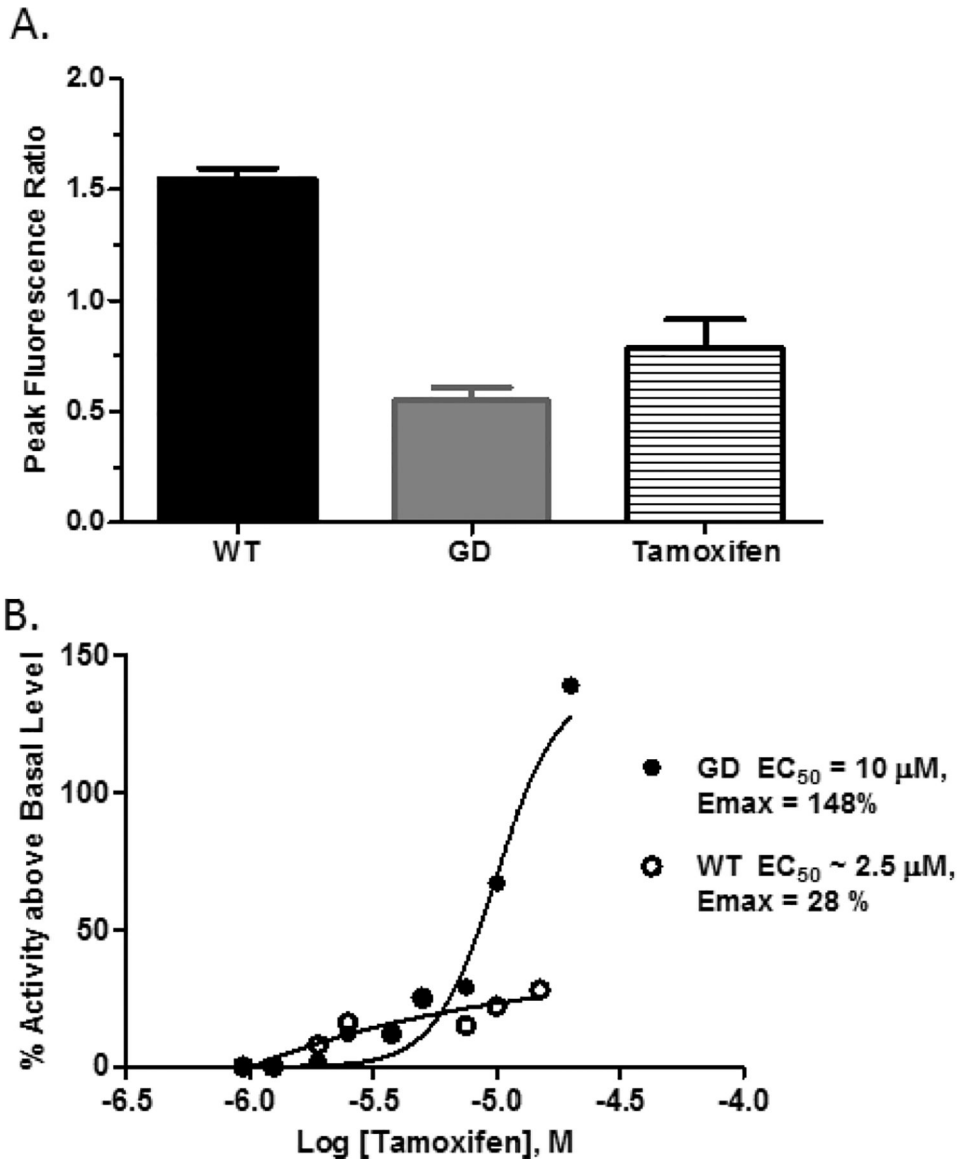


Figure 2.

(A) GPN-induced calcium release is reduced in Gaucher (GD) patient-derived cells compared with normal (WT) patients; Tamoxifen (10 μM) partially restores lysosomal calcium in GD patient-derived cells. (B) Tamoxifen dose response on GD and WT patient-derived cells.

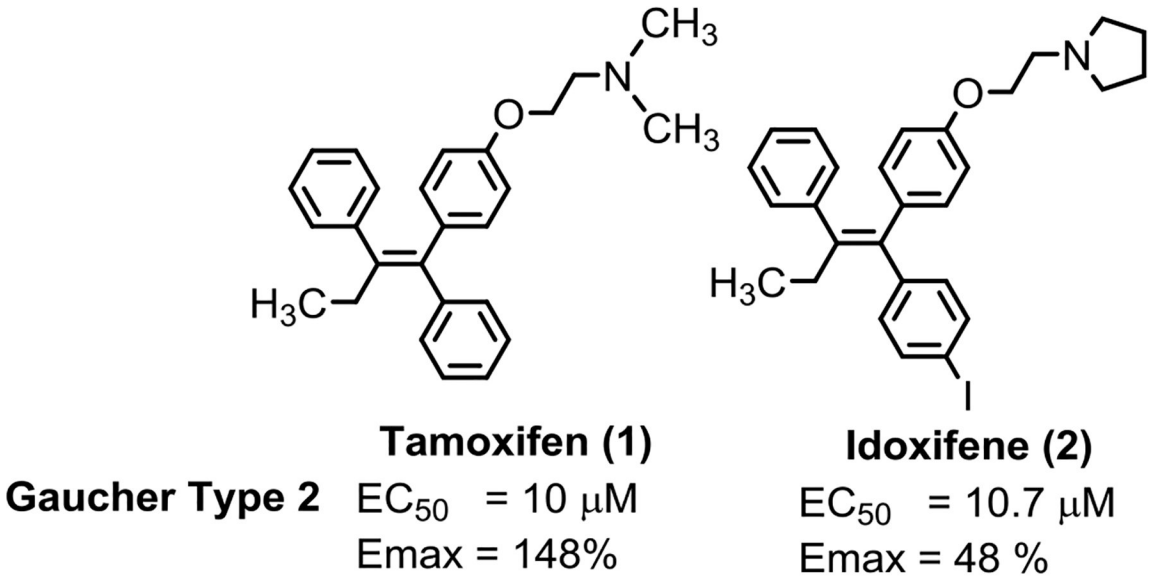
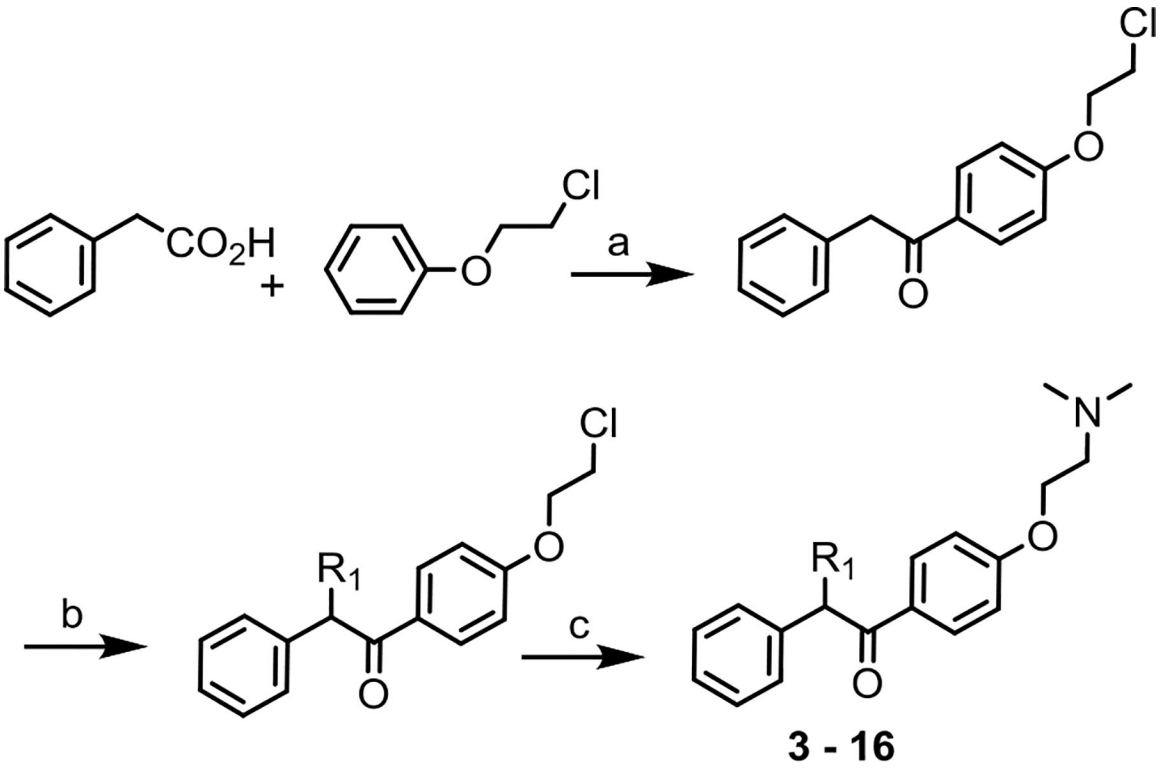
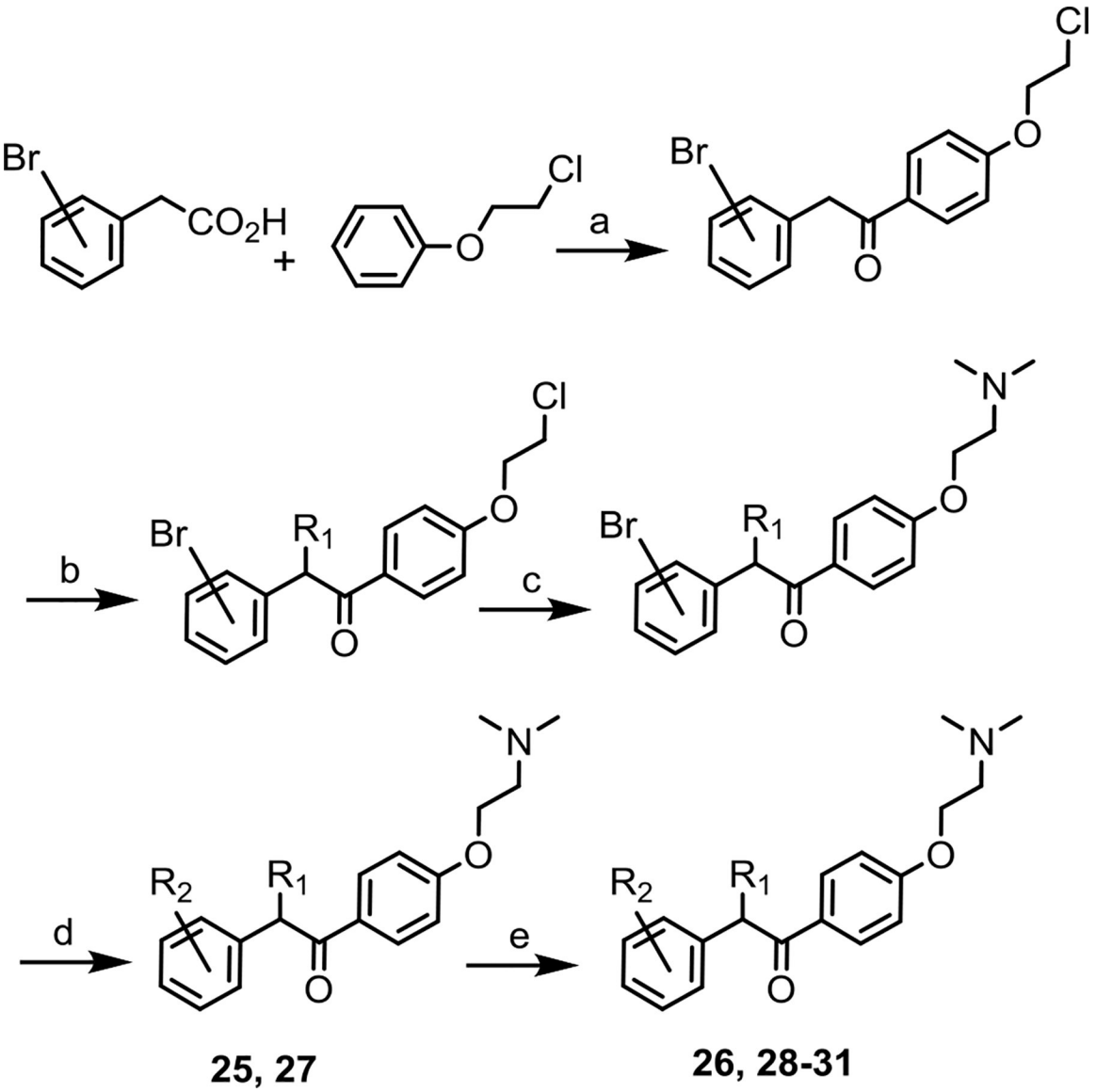


Figure 3.
Structures and activity of hits tamoxifen and idoxifene.



Scheme 1.

Synthesis of compounds **17–24**. *Reagents and Conditions*: (a) Trifluoroacetic acid, r.t., overnight; (b) NaH, R_1X; (c) aq. 40% dimethylamine, reflux.

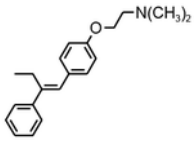
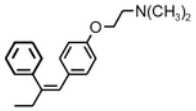
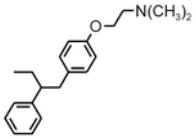
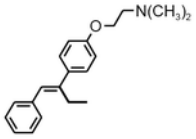
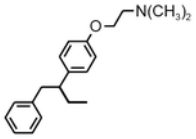
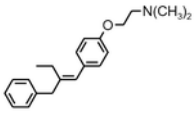
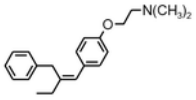
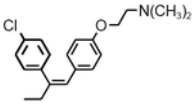
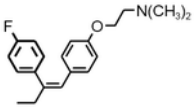


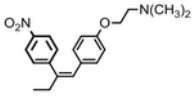
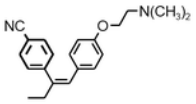
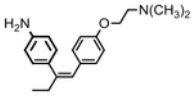
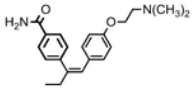
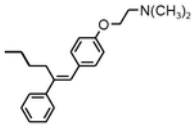
Scheme 2.

Synthesis of compounds **25–29**. *Reagents and Conditions*: (a) Trifluoroacetic acid, r.t., overnight; (b) NaH, R-X; (c) aq. 40% dimethylamine, reflux; (d) R_2-tetramethyl-dioxoboralane, K_2CO_3, $Pd(PPh_3)_4$, 120°C; (e) 1 atm. H_2,10% Pd/C.

Table 1.

SAR of tamoxifen scaffold.

Cd #		Lysosomal Ca^{+2} Assay*		Estrogen† Receptor
		EC_{50}, μM	Emax, %	% Activity @ 10 μM
3		5.8	73%	19%
4		15.5	80%	35%
5		4.3	154%	43%
6		3.0	53%	43%
7		> 20	0	75%
8		3.1	67%	69%
9		2.3	40%	47%
10		5.4	70%	70%
11		> 20	0	46%

Cd #		Lysosomal Ca^{+2} Assay*		Estrogen† Receptor
		EC_{50}, μM	Emax, %	% Activity @ 10 μM
12		> 20	0	23%††
13		4.3	47%	32%
14		> 20	0	25%
15		> 20	0	46%† (8%††)
16		7.2	56%	98%
1		10	148%	100%††

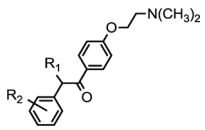
*EC_{50} units - μM; Emax = percent activation of basal GPN-induced calcium response measured in the absence of compound.

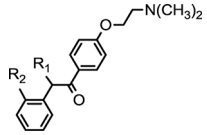
†Percent agonist response relative to the response with β-estradiol EC_{100}.

††Percent inhibition of EC_{100} response to β-estradiol.

Table 2.

SAR of diphenylethanone scaffold.





Cd #	R_1	R_2	Lysosomal Ca^{+2} Assay EC_{50}, µM	Lysosomal Ca^{+2} Assay Emax, %	Estrogen† Receptor % Activity @ 10 µM	Cd #	R_1	R_2	Lysosomal Ca^{+2} Assay EC_{50}, µM	Lysosomal Ca^{+2} Assay Emax, %	Estrogen† Receptor % Activity @ 10 µM
17	Et	H	0	0	ND	**25**	n-Bu		2.3	177%	100%
18	n-Pr	H	0	0	ND	**26**	n-Bu	Et	0.65	138%	87%††
19	i-Pr	H	4.4	54%	ND	**27**	n-Bu		2.2	257%	35%
20	gem-di-n-Pr	H	4.0	60%	25%	**28**	n-Bu	i-Pr	1.4	401%	45%
21	n-Bu	H	> 20	0	ND	**29**		i-Pr	1.7	406%	39%
22		H	0.9	108%	40%	**30**		3-i-Pr	1.5	80%	ND
23		H	3.6	191%	35%	**31**			3.1	240%	0%
24		H	> 20	0	5%	**1**			10	148%	100%††

*EC_{50} units - µM; Emax = percent activation of basal GPN-induced calcium response measured in the absence of compound.

†Percent agonist response relative to the response with β-estradiol EC_{100}.

††Percent inhibition of EC_{100} response to β -estradiol.

*ND = Not Determined.

Table 3.

Activity of selected compounds in lysosomal calcium assay.

Cd #	Lysosomal Ca^{+2} Assay			
	GD-patient cells		WT cells	
	EC_{50}, μM	Emax, %	EC_{50}, μM	Emax, %
27	2.2	257%	1.1	109%
28	1.48	227%	0.7	56%
29	1.8	449%	1.1	148%
31	3.1	240%	1.1	50%
1	10	148%	2.5	28%

Table 4.

Drug-like properties of selected diphenylethanones.

Cd. #	Max. Aq. Solubility (μM)	Microsomal Stability ($t_{1/2}$, min.)	
		Mouse	Human
27	45.2	9.1	24.4
28	49.4	7.9	21.6
29	40.0	22.3	23.7
31	31.7	7.7	26.7
1	6.9*	8.9	> 60

*Value reported in reference 23.