Published in final edited form as:
Pharmacol Res. 2014 May ; 0: 3–9. doi:10.1016/j.phrs.2014.04.002.

Using pharmacological chaperones to restore proteostasis

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Abstract

Normal organismal physiology depends on the maintenance of proteostasis in each cellular compartment to achieve a delicate balance between protein synthesis, folding, trafficking, and degradation while minimizing misfolding and aggregation. Defective proteostasis leads to numerous protein misfolding diseases. Pharmacological chaperones are cell-permeant small molecules that promote the proper folding and trafficking of a protein via direct binding to that protein. They stabilize their target protein in a protein-pharmacological chaperone state, increasing the natively-folded protein population that can effectively engage trafficking machinery for transport to the final destination for function. Here, as regards the application of pharmacological chaperones, we focus on their capability to promote the folding and trafficking of lysosomal enzymes, G protein coupled receptors (GPCRs), and ion channels, each of which is presently an important drug target. Pharmacological chaperones hold great promise as potential therapeutics to ameliorate a variety of protein misfolding diseases.

Keywords

Pharmacological chaperone; proteostasis; chaperone; ERAD; protein misfolding disease; lysosomal storage disease; GPCR; ion channel

1. Proteostasis and proteostasis maintenance

1.1 Proteostasis in health and disease

Proteostasis represents an optimal state of the cellular proteome, in which a delicate balance between protein synthesis, folding, trafficking, aggregation and degradation is achieved for individual proteins that make up the proteome (1, 2). Normal organismal physiology

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Chemical compounds studied in this article
NN-DNJ (PubChem CID: 501640); E4031 (PubChem CID: 3185); nicotine (PubChem CID: 89594); GABA (PubChem CID: 119); SR121463 (PubChem CID: 9810773); naltrexone (PubChem CID: 5360515)

depends on the maintenance of proteostasis in each cellular compartment. Proteostasis maintenance is challenged by intrinsic stress, such as inherited misfolding-prone proteins (3), environment (4, 5), and aging (6, 7), leading to loss-of-function and gain-of-toxic-function diseases. Loss-of-function diseases include ion channel diseases, such as cystic fibrosis (8, 9), cardiac arrhythmias (10), and idiopathic epilepsy (11), as well as G-protein-coupled receptor (GPCR) conformational diseases (12, 13), and lysosomal storage diseases (14). Loss-of-function diseases often result from the inheritance of a misfolding-prone mutant protein, which triggers the collapse of proteostasis and leads to disease. Gain-of-toxic-function diseases, including Alzheimer's disease, Parkinson's disease, and Huntington's disease, are often associated with aggregation (15, 16).

Proteostasis deficiency can be corrected using two classes of mechanistically distinct small molecules: pharmacological chaperones and proteostasis regulators (17). Pharmacological chaperones bind to and stabilize a specific protein to enable its proper folding and trafficking, whereas proteostasis regulators increase the proteostasis network capacity in a general way to restore proteostasis (18, 19). In this review, we will focus on the application of pharmacological chaperones for lysosomal enzymes, ion channels, and GPCRs, which represent a potential therapeutic strategy to ameliorate a variety of protein misfolding diseases.

1.2 Proteostasis maintenance by the proteostasis network

The proteostasis network maintains the proteome integrity to achieve optimal concentrations, conformations, interactions, and locations of individual proteins in cells. This network is composed of a variety of subnetworks, including chaperone, degradation, and trafficking networks, as well as cellular signaling pathways. The signaling pathways include the heat shock response, which regulates cytosolic proteostasis (20); the unfolded protein response (UPR), which regulates proteostasis in the endoplasmic reticulum (ER) (21, 22); Ca^{2+}-sensitive folding pathways (23, 24); ER-associated degradation (25); autophagy (26); and many others (16, 27).

The proteostasis network adapts by modifying its components. To understand how proteostasis is maintained by the proteostasis network in cells, it is critical to identify cellular proteostasis network components. Each specific protein uses only a subset of cellular proteostasis network components in a crowded cellular environment. These include chaperones/co-chaperones, folding enzymes, degradation factors and trafficking machinery. Thus, the identification of proteostasis network components that are specific for each protein will be important for understanding how to restore its proteostasis. Identification of a protein's interactome in cells followed by bioinformatics analysis can effectively refine the potential proteostasis network components of the protein of interest. Examples include the identification of proteostasis network components for the cystic fibrosis transmembrane conductance regulator (CFTR) (28) and γ-aminobutyric acid type C ($GABA_C$) receptors (29). Modern affinity purification (AP) coupled with tandem mass spectrometry (AP-MS/MS) technologies (30) were used to obtain a comprehensive understanding of the proteostasis network components for physiologically important ion channel proteins, which also contributed to the identification of novel pathways that might be useful in the clinic to

ameliorate related ion channel diseases. Although it is certainly desirable to define each protein's interactome as a potentially useful route for restoring proteostasis, pharmacological chaperones do not rely on the identification of a complex network of components, but instead focus in a specific manner to restore native folding and trafficking in a way that does not have the potential to alter complex networks.

In this review we focus on pharmacological chaperones that target proteins synthesized in the ER. To function, proteins need to fold into their native structures and traffic efficiently to their final destination. Protein folding is a complicated process during which the polypeptide chain obtains its biologically active three-dimensional conformation. About 1/3 of the eukaryotic proteins are folded in the ER, including all membrane proteins, proteins in the secretory pathways, and proteins targeted to subcellular compartments such as the lysosome (Figure 1). Remarkably, over 10,000 different eukaryotic proteins are co-translationally translocated into the ER. Soluble proteins will enter the ER lumen, whereas membrane proteins, including ion channels and GPCRs, will reside on the ER membrane for folding.

Asparagine *N*-linked glycosylation is the most common protein modification in the ER and occurs co-translationally, serving as a recognition tag for glycoprotein maturation (31). (Figure 2A) (32). The pathway of glycoprotein folding in the ER has been extensively studied (33–35). A glycoprotein is initially attached with $Glc_3Man_9GlcNAc_2$ in its Asn residue in an Asn-X-Ser/Thr sequon (Figure 2A). N-glycan trimming serves as a crucial tag for protein maturation in the ER (Figure 2B). The two outermost glucose residues of a glycoprotein are cleaved by glucosidase I and glucosidase II before entering the calnexin/calreticulin folding cycles. The molecular chaperone lectins calnexin and calreticulin facilitate the folding of monoglucosylated glycocproteins. Additionally, folding enzymes such as protein disulfide bond isomerases (PDIases) catalyze disulfide bond formation, and peptidylprolyl isomerases (PPIases) assist proline isomerization. Removal of the terminal glucose residue by glucosidase II triggers the dissociation of the substrate from calnexin/calreticulin. At this point, this substrate can be reglucosylated by the folding sensor, UGGT1, and re-enter the calnexin/calreticulin folding cycles. Eventually, the substrate exits the calnexin/calreticulin cycles upon removal of mannose residues by ER-mannosidase I. When folded, the substrate will engage the trafficking machinery for export to the Golgi.

Beyond glycan-dependent, lectin-assisted chaperoning integral membrane proteins also undergo glycan-independent chaperoning by heat shock proteins (Hsp) (36). Hsp molecular chaperones play a central role in the maintenance of proteostasis (37, 38). They are universally present in all types of cells and in most of the cellular compartments, and they provide different pathways to assist peptide folding during different stages. Excellent reviews are present in the literature (39–41). The major Hsp chaperone machinery includes small Hsps, Hsp40, Hsp60, Hsp70, and Hsp90. During the protein folding process Hsps aid in disulfide bond formation, proline isomerization, and bind to hydrophobic patches of unfolded proteins to prevent aggregation.

After collaborative glycan-dependent and glycan-independent folding, the properly folded proteins exit the ER and traffic through the Golgi en route to their final destination. For protein trafficking, the COPII machinery is responsible for anterograde (forward) cargo

protein vesicle transport from the ER to the Golgi (42, 43), whereas the COPI machinery is responsible for retrograde (backward) retrieval of cargo proteins (44). Terminally misfolded proteins are subjected to ER-associated degradation (ERAD): they are recognized, ubiquitinated, retrotranslocated into the cytosol, and degraded by the proteasome (25).

2. Using pharmacological chaperones to restore proteostasis

2.1. Overview of pharmacological chaperones

Pharmacological chaperones are cell-permeant small molecules that bind to and stabilize a target protein. In many protein misfolding diseases, a mutation in a protein leads to its misfolding and extensive degradation. The consequence is that few proteins reach their final destination for function. Pharmacological chaperone treatment stabilizes the target protein in the ER and enables its exit from the ER and trafficking through the Golgi and onward to its final destination (Figure 1 shows an example for an ion channel protein after pharmacological chaperone treatment). The mechanism of pharmacological chaperones is illustrated by using a free energy diagram (Figure 3). Without treatment, only a limited number of molecules of a target protein can achieve their folded state for trafficking. Pharmacological chaperone treatment transitions the target from the folded state to the folded protein-pharmacological chaperone state, where the free energy is more favorable. This will increase the concentration of the natively-folded protein that can successfully be recruited to the ER exit sites. A prominent advantage of pharmacological chaperones is their specificity. However, off-target effects have been reported for low potency pharmacological chaperones.

Pharmacological chaperones have been applied to enhance proteostasis of different types of proteins, including lysosomal enzymes (45), GPCRs (46), ion channels (47), transporters (48), and aggregation-prone proteins (49). Other reviewers of this issue have provided prominent examples. Here, we focus on their application on lysosomal enzymes, ion channels and GPCRs (Table 1). The chemical structures of the described pharmacological chaperones in Table 1 are shown in Figure 4.

2.2. Application of pharmacological chaperones in lysosomal enzymes

The lysosome is an acidic organelle containing a variety of lysosomal enzymes that are responsible for the metabolic degradation of many complex molecules such as glycolipids, glycoproteins, and oligosaccharides. Defective lysosomal enzymes result in the accumulation of their substrates in the lysosome, causing lysosomal storage diseases (50, 51). To date, more than 50 lysosomal storage diseases have been described, including Gaucher disease, Fabry disease, and Pompe disease. In many cases, mutations in one lysosomal enzyme result in its degradation in the ER and thus its low concentrations in the lysosome, causing diseases (52). Application of pharmacological chaperones is expected to stabilize the target lysosome enzyme in the ER at neutral pH and enable its trafficking to the lysosome. Once the pharmacological chaperones reach the acidic lysosome, the binding between them and the lysosomal enzyme is weakened or released because of the acidic environment and the competition from natural substrates of the enzyme in the lysosome.

Currently, pharmacological chaperones are under clinical trial for the treatment of Fabry disease (http://www.clinicaltrials.gov) (53).

Degradation of glycosphingolipids occurs in the lysosomes by numerous enzymes in a stepwise fashion. Fabry disease is the result of a defect in lysosomal α-galactosidase A (54), whereas Gaucher disease, the most common lysosomal storage disease, results from a defect in acid β-glucosidase (55). The earliest documented application of pharmacological chaperones to rescue lysosomal enzyme trafficking was on mutant lysosomal α-galactosidase A associated with Fabry disease (56). Since then, many pharmacological chaperones have been synthesized and tested in numerous disease-associated lysosomal enzymes (45, 57, 58). Here, we focus on introducing the application of pharmacological chaperones on Fabry disease and Gaucher disease.

Application of DGJ (Compound **1**, Figure 4), a potent inhibitor of α-galactosidase A, promoted the trafficking of a Fabry disease-associated α-galactosidase A variant harboring the R301Q mutation in cells (56). Furthermore, administration of DGJ in a transgenic mouse restored the enzymatic activities of lysosomal α-galactosidase A (56). This discovery established a new paradigm for the amelioration of Fabry disease, and many other lysosomal storage diseases (45, 59). More potent pharmacological chaperones have been developed for α-galactosidase A (60). To confirm a direct stabilization effect of pharmacological chaperones on α-galactosidase A, a variety of biophysics assays, including urea-induced unfolding assays, have been developed (61, 62).

Application of NN-DNJ (Compound **2**, Figure 4), a potent inhibitor of acid β-glucosidase, promoted the trafficking of disease-causing acid β-glucosidase mutants in Gaucher patient-derived fibroblasts (63). Further mechanism studies confirmed an increased stability of acid β-glucosidase in the presence of pharmacological chaperones (64). Many pharmacological chaperones effectively increase the trafficking of acid β-glucosidase harboring the N370S mutation. However, it is more difficult to rescue acid β-glucosidase harboring the L444P mutation using pharmacological chaperones, possibly because the L444P mutation leads to much faster degradation of acid β-glucosidase than the N370S mutation. More potent and specific pharmacological chaperones have been developed to enhance the trafficking of Gaucher disease-associated acid β-glucosidase (65–67).

2.3. Application of pharmacological chaperones in ion channels

Loss-of-function of ion channels leads to ion channel diseases called channelopathies (68). Misfolding of an ion channel leads to its reduced expression on the plasma membrane and thus loss of function. Pharmacological chaperones have been applied to promote the trafficking of numerous ion channels, including hERG (human ether-à-go-go-related gene) channels (69), CFTR (cystic fibrosis transmembrane conductance regulator) (70), and the Cys-loop superfamily of ligand-gated ion channels (71–73) (Table 1). For ion channel proteins, both agonists and antagonists are potential pharmacological chaperone candidates.

To our knowledge, the earliest documented application of pharmacological chaperones on ion channels was on trafficking-deficient mutant hERG channels (69). Loss of function of hERG channels causes type 2 long QT syndrome (LQT2), which is characterized by delayed

cardiac repolarization and prolonged QT intervals on the electrocardiogram, leading to ventricular arrhythmias and sudden death (10). Most clinically important hERG mutations lead to protein trafficking defect: an insufficient amount of mutant hERG channels are trafficked to the plasma membrane (74). It was shown that E4031 (Compound **3**, Figure 4), an antagonist of hERG channels, promoted the trafficking of hERG channels harboring the N470D mutation (69). Several other potent hERG channel blockers, such as astemizole and cisapride, were later reported to rescue the trafficking of LQT2-associated hERG channels harboring the G601S mutation (75). As a second example, cystic fibrosis results from loss of function of CFTR. In the literature, correctors are used to describe small molecules that promote the trafficking of CFTR, whereas potentiators are for small molecules that enhance CFTR channel function on the plasma membrane. Both correctors, including corr-4a, VRT-325, VRT-532 (76, 77), RDR1 (Compound **4**, Figure 4) (78), and potentiators, including MPB compounds (79), could be pharmacological chaperone candidates. It was reported that RDR1 binds to the first nucleotide binding domain of ΔF508 CFTR directly. Therefore, RDR1 acted as a pharmacological chaperone to stabilize and rescue partial function of ΔF508 CFTR (78). A recent review summarized the current progress for the application of correctors and potentiators on CFTR (70). Thirdly, the Cys-loop superfamily of neurotransmitter-gated ion channels, which plays an important role for brain function, includes acetylcholine receptors, serotonin receptors, glycine receptors, and $GABA_A$ (γ-aminobutyric acid type A) receptors (80–82). It was reported that nicotine (Compound **5**, Figure 4), an agonist of nicotinic acetylcholine receptors (nAChRs), acted as a pharmacological chaperone to increase the surface expression of wild-type nAChRs (72, 73). Furthermore, the nAChR upregulation by nicotine depends on specific subtypes, which possibly results from different nicotine binding affinity to nAChR subtypes (83, 84). Similar results were reported for $GABA_A$ receptors: GABA (Compound **6**, Figure 4), an agonist of $GABA_A$ receptors, enhanced the trafficking of wild-type $GABA_A$ receptors (71). Enhanced trafficking of wild-type ion channels upon pharmacological chaperone treatment might indicate that even wild-type ion channels do not traffic at their optimal efficiency.

2.4. Application of pharmacological chaperones in GPCRs

Pharmacological chaperones have also been identified to restore proteostasis of numerous GPCRs associated with a variety of diseases (12, 13, 46, 85). Mutant V2 vasopressin receptors are retained in the ER causing nephrogenic diabetes insipidus. Their function can be effectively recovered if the mutant receptors are successfully trafficked to the membrane. It was shown that a cell-permeant antagonist of the V2 vasopressin receptor, SR121463 (Compound **7**, Figure 4), promoted the trafficking and maturation of mutant receptors (86). Many other V2 vasopressin receptor antagonists, including VPA985, YM087, and OPC31260, were later used as pharmacological chaperones for V2 vasopressin receptors (87–89). Similarly, numerous mutations in gonadotropin-releasing hormone (GnRH) receptors are associated with congenital hypogonadotropic hypogonadism. Cell-permeant GnRH receptor antagonists, including IN3 (Compound **8**, Figure 4), rescued the trafficking deficiency of mutant GnRH receptors in cells (90). Recently, it was demonstrated that in a knock-in mouse model expressing E90K GnRH, IN3 application restored the plasma membrane expression and partial function of E90K GnRH (91). Other structurally distinct pharmacological chaperones were later reported for GnRH receptors, including A177775,

TAK-013, and Q89 (92). Another important example is the opsin variant harboring the P23H mutation, which is associated with autosomal dominant retinitis pigmentosa (93). Pro23 is located in the extracellular N-terminal domain, and P23H mutation resulted in the ER retention of P23H-opsin. The application of an inverse agonist, 11-cis-7-ring retinal (Compound **9**, Figure 4), quantitatively rescued the P23H opsin trafficking deficiency and restored its transport to the plasma membrane (94). Other retinoids, including 9-cis-retinal and 11-cis-retinal, also rescued the trafficking of P23H opsin (95). Less misfolded opsin variants were more susceptible for pharmacological chaperoning (96). It is worth noting that pharmacological chaperones can also stabilize wild-type GPCRs and increase their surface expression. For example, membrane-permeant opioid antagonists, such as naltrexone (Compound **10**, Figure 4), facilitated the transport of wild-type δ-opioid receptors (97). This indicates that for complicated GPCRs, even the wild-type proteins may not traffic at their optimal efficiency, which was also seen in ion channel protein cases.

3. Concluding remarks

The pharmacological chaperoning strategy is a promising one to rescue misfolded proteins in a specific manner. The approach has been applied to many protein targets both *in vitro* and in a few cases, *in vivo*, including for lysosomal enzymes, GPCRs, and ion channels. While pharmacological chaperones increase the pool of natively-folded proteins, proteostasis regulators function by increasing the proteostasis network capacity. Because of their distinct mechanisms, we expect that co-application of pharmacological chaperones and proteostasis regulators may yield additive or synergistic effects to restore proteostasis. Indeed, a synergistic restoration of mutant acid β-glucosidase function in Gaucher patient-derived fibroblasts was shown when a proteostasis regulator, MG-132, and a pharmacological chaperone, NN-DNJ, were co-applied (18). Because pharmacological chaperone treatment decreases the population of misfolded proteins in the ER, it is expected that they may also reduce ER stress under circumstances in which abnormally folded protein would accumulate in the ER. The interplay between pharmacological chaperones and proteostasis regulators may provide a powerful approach for treating protein folding disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the Research Start-up Fund from Case Western Reserve University School of Medicine, Epilepsy Foundation of America (225243), and the Clinical Translational Science Collaborative of Cleveland CTSA (UL1RR024989) from the National Center for Research Resources and the National Center for Advancing Translational Sciences of the National Institutes of Health.

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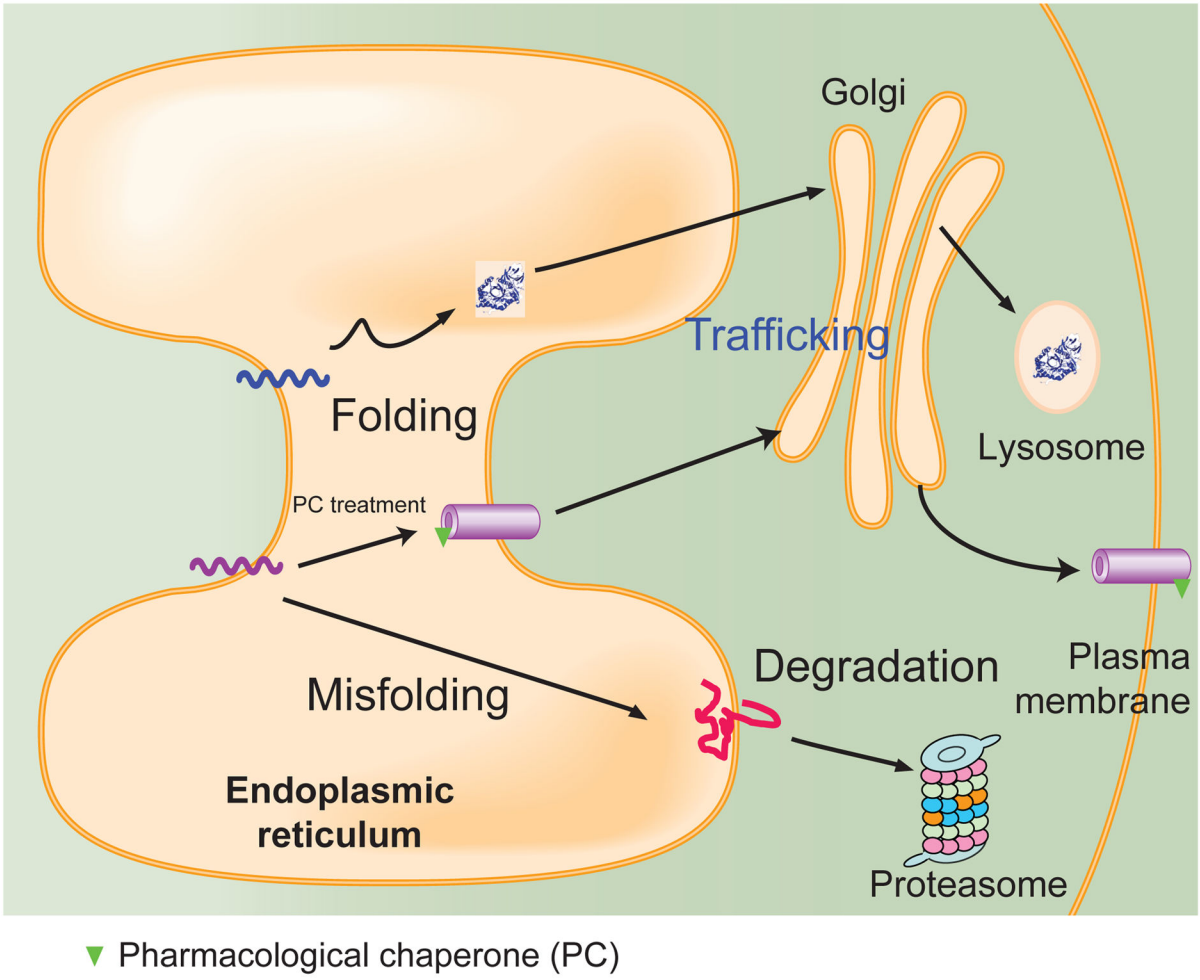

FIGURE 1.

The proteostasis network maintains proteostasis in cells. About 1/3 of the eukaryotic proteins are folded in the ER, including all membrane proteins, proteins in the secretory pathways, and proteins targeted to subcellular compartments such as the lysosome. As illustrated examples, membrane proteins (purple line and purple cylinders) fold on the ER membrane, traffic through the Golgi, and reach the plasma membrane. Lysosomal enzymes (blue line and folds) fold in the ER lumen, traffic through the Golgi, and reach the lysosome. Misfolded proteins (red line) are subject to ER-associated degradation (ERAD), being retrotranslocated to the cytosol and degraded by the proteasome. The proteostasis network maintains the proteome integrity to achieve optimal concentrations, conformations, interactions, and locations of individual proteins in cells. Pharmacological chaperones (green triangles) are cell-permeant small molecules that bind to and stabilize a target protein. Pharmacological chaperone treatment stabilizes that membrane protein on the ER membrane, enabling more efficient trafficking from the ER through the Golgi and onward to the plasma membrane.

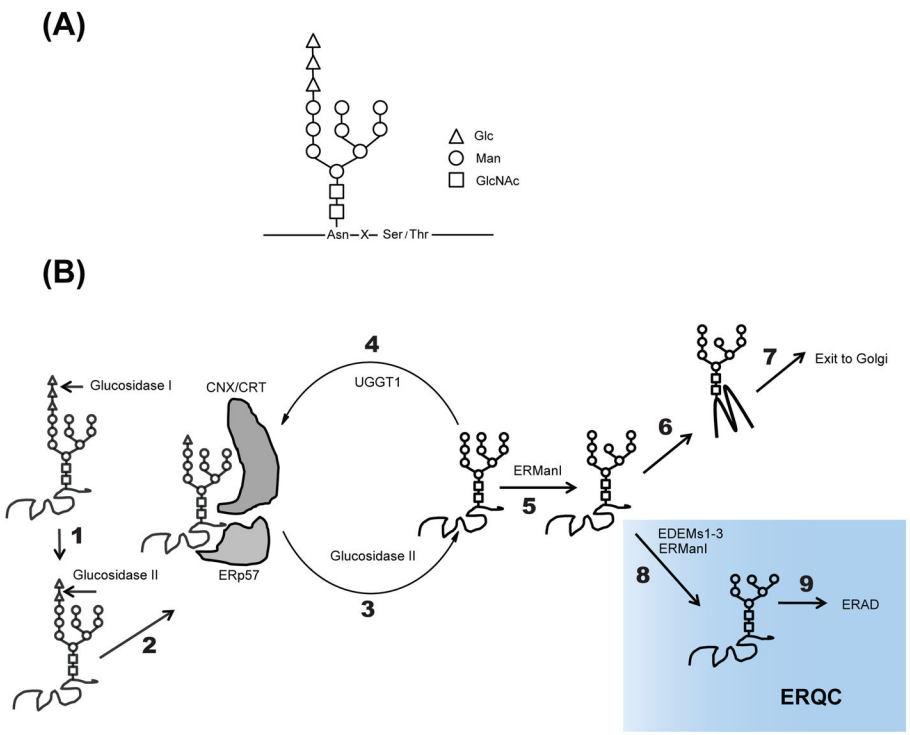

FIGURE 2.

Folding and degradation of glycoproteins in the ER. (A) Structure of N-linked glycans. Upon entering the ER, 14-monosaccharide residues $Glc_3Man_9GlcNAc_2$ (Glc: glucose, Man: mannose, GlcNAc: N-acetylglucosamine) are attached to an Asn residue contained in an Asn-X-Ser/Thr sequon (where X is any residue except proline). (B) N-glycan trimming serves as a crucial tag for protein maturation in the ER. The two outermost glucose residues of a glycoprotein are cleaved by glucosidase I (step 1) and glucosidase II (step 2) before entering the calnexin (CNX)/calreticulin (CRT) folding cycles. CNX and CRT facilitate the folding of monoglucosylated glycocproteins. Removal of the terminal glucose residue by glucosidase II triggers the dissociation of the substrate from CNX/CRT (step 3). At this point, this substrate can be reglucosylated by the folding sensor, UGGT1 (step 4), and re-enter the CNX/CRT folding cycles. Eventually, the substrate exits the CNX/CRT cycles upon removal of mannose residues by ER-mannosidase I (ERManI) (step 5). When folded, the substrate will engage the trafficking machinery for export to the Golgi (step 6 to 7). Terminally misfolded glycoproteins will be further trimmed by ERManI and EDEM family proteins (step 8) and delivered to the ERAD pathway (step 9). Terminally misfolded proteins are segregated into a domain called the ER-derived quality-control compartment (ERQC), which is tightly linked to ERAD. The ERQC compartment is shadowed in cyan.

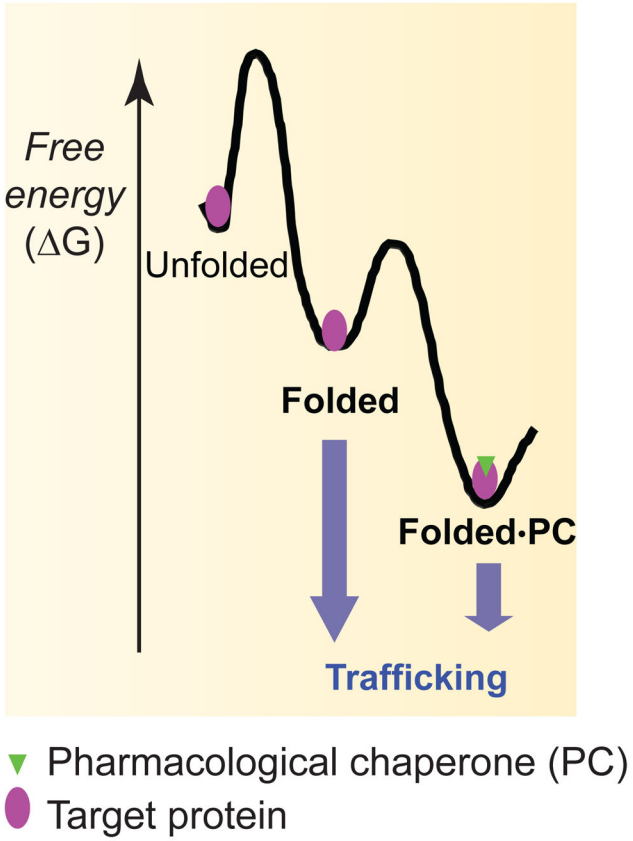

FIGURE 3.

A free energy diagram illustrates the mechanism of using pharmacological chaperones to restore proteostasis. Pharmacological chaperone (green triangle) treatment pulls the target protein (purple eclipse) from the folded state to the folded protein-pharmacological chaperone state, in which the free energy is more favorable. This will increase the folded protein population that can engage the trafficking machinery for transport to the final destination.

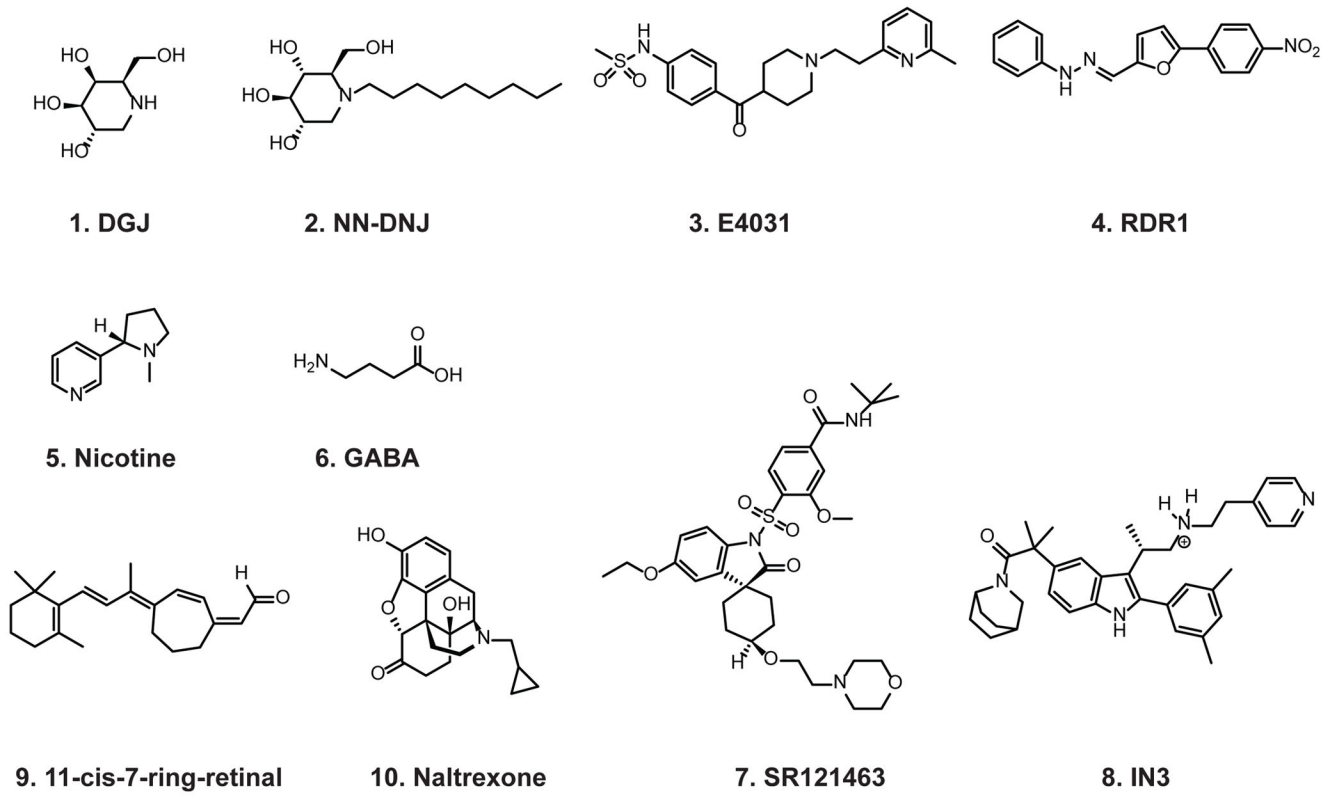

FIGURE 4.

Chemical structures of select pharmacological chaperones, described in Table 1.

Table 1

Select pharmacological chaperones used to enhance proteostasis of lysosomal enzymes, GPCRs and ion channels. See Figure 4 for chemical structures.

Protein type	Deficient protein	Disease	Pharmacological chaperones
Lysosomal enzymes	α-galactosidase A	Fabry disease	DGJ (56)
	Acid β-glucosidase	Gaucher disease	NN-DNJ (63)
Ion channels	hERG channel	Long QT syndrome	E4031 (69)
	CFTR	Cystic fibrosis	RDR1 (78)
	nAChR	Many such as epilepsy	Nicotine (72, 73)
	$GABA_A$ receptor	Epilepsy	GABA (71)
GPCRs	V_2 vasopressin receptor	Nephrogenic diabetes insipidus	SR121463 (86)
	GnRH receptor	Hypogonadotropic hypogonadsm	IN3 (90)
	Opsin	Retinitis pigmentosa	11-cis-7-ring-retinal (94)
	δ-opioid receptor	Pain	Naltrexone (97)