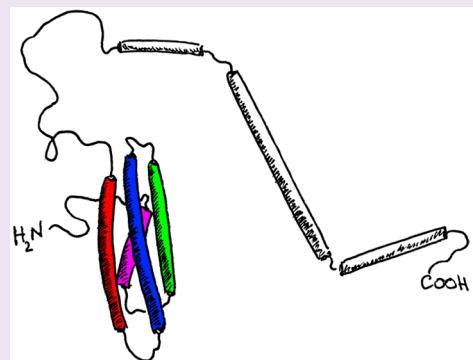


# Pharmacological Chaperones: Design and Development of New Therapeutic Strategies for the Treatment of Conformational Diseases

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**ABSTRACT:** Errors in protein folding may result in premature clearance of structurally aberrant proteins, or in the accumulation of toxic misfolded species or protein aggregates. These pathological events lead to a large range of conditions known as *conformational diseases*. Several research groups have presented possible therapeutic solutions for their treatment by developing novel compounds, known as *pharmacological chaperones*. These cell-permeable molecules selectively provide a molecular scaffold around which misfolded proteins can recover their native folding and, thus, their biological activities. Here, we review therapeutic strategies, clinical potentials, and cost-benefit impacts of several classes of pharmacological chaperones for the treatment of a series of conformational diseases.



Almost every biochemical process is sustained by the activity of proteins. The amino acid sequence of each protein determines its specific three-dimensional structure and, hence, its function and biological activity.<sup>1,2</sup> Therefore, folding in a nonaberrant structure is fundamental to ensuring correct protein activity; misfolded proteins are eliminated by activation of the cellular proteasome pathway. Furthermore, living organisms have developed a system for quality control at the level of the endoplasmic reticulum and Golgi apparatus consisting of several proteins, known as chaperones. Chaperones prevent protein aggregation, promote the folding process, and function as “correct-folding” markers. However, the efficacy of this cellular machinery can be compromised by the occurrence of point mutations in the *de novo* synthesized polypeptide chain that are responsible for the destabilization of the entire protein structure,<sup>3</sup> or by the presence of stable protein’s folding intermediates that elude the control system of protein trafficking and maturation.<sup>4–6</sup> As a consequence, two noxious scenarios emerge: either the accumulation of toxic protein aggregates<sup>6–8</sup> in the cell or the premature clearance of misfolded proteins.<sup>6–8</sup> Both pathological conditions are recognized as *conformational diseases*.<sup>6–8</sup>

Starting from the pioneering studies of Sato *et al.* and Brown *et al.* on the efficacy of osmolytes in the recovery of misfolded anion channels,<sup>8,9</sup> more and more evidence has been provided supporting the ability of small molecules to stabilize protein structures or to inhibit protein aggregation.<sup>9–14</sup> Such compounds, called *chemical chaperones*, are defined as membrane-permeable molecules able to nonselectively stabilize mutant proteins, facilitate their folding, and rescue their physiological functionality.<sup>6</sup> Chemicals such as glycerol, trimethylamine-N-oxide (TMAO), or dimethyl sulfoxide (DMSO) have been shown to effectively restore the activity of several pathological mutant proteins *in cell*.<sup>9,15–17</sup> However,

their clinical use is prevented by their poor druglikeness and lack of target specificity. Initiated by the work of the Clark<sup>18</sup> and January<sup>19</sup> research groups, the urgency of identifying compounds with better physicochemical properties and with high protein specificity has driven the development of a new class of molecules called *pharmacological chaperones* (PCs), also known as pharmacoperones. PCs are cell-permeable molecules able to selectively bind specific targets and provide a molecular scaffold around which mutant or misfolded proteins can correctly assume their three-dimensional structures.<sup>20,21</sup> The biological functionality of a chemical chaperone and PC is identical; yet the latter includes the pharmacological notion of protein specificity, which is pivotal for clinical treatment (Figure 1).

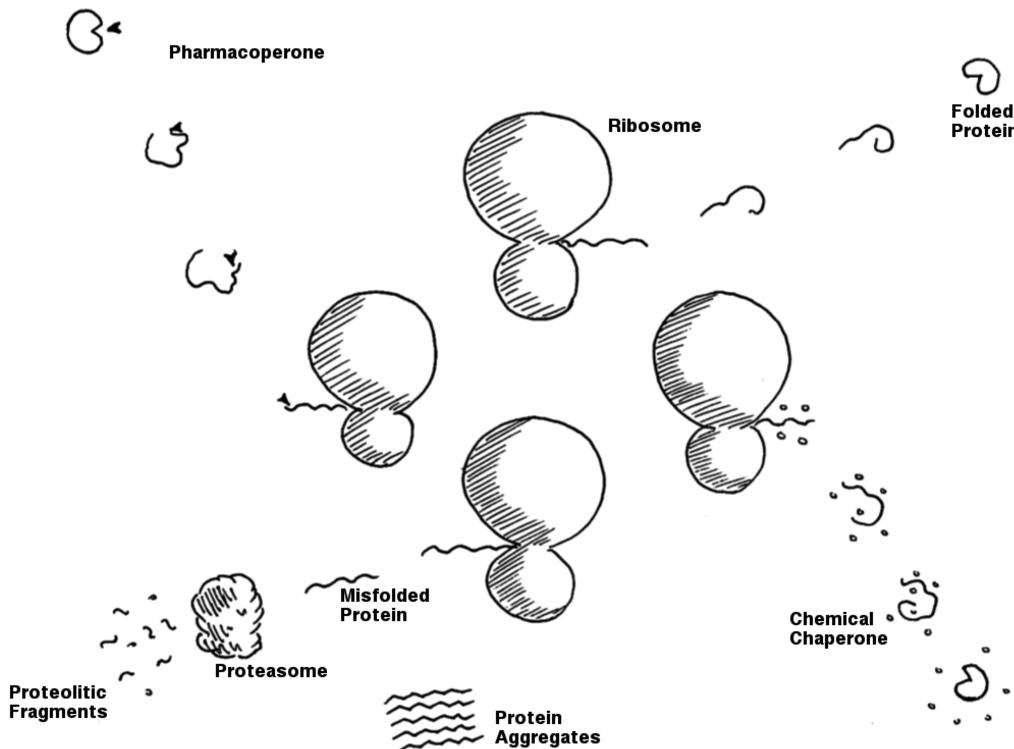
The characterization of protein–PC interactions may be challenging because classical structural biology techniques (i.e., crystallography or nuclear magnetic resonance) are unsuitable to study short-lived protein intermediate states, especially if the latter are not structurally stabilized by widely adopted biochemical approaches (e.g., cross-linkers, protein redesign, experimental conditions). Nevertheless, huge efforts have been invested in the development of PCs because of their target specificity over chemical chaperones. Although their mechanism of action is not clearly understood, a number of compounds have been proved to effectively revert the phenotypic outcomes of several pathological states. In this review, we will discuss a series of PCs that could provide new therapies for several conformational diseases, including

Received: March 1, 2016

Accepted: April 20, 2016

Published: April 20, 2016





**Figure 1.** Folding of a protein in a functional three-dimensional structure (top right), essential for its physiological activity. Aberrant nonfunctional protein conformations are quickly degraded by the cellular proteasome system or undergo a conformational change and aggregate in a toxic highly ordered supramolecular assembly (bottom left). Chemical chaperones and PCs are small membrane-permeable molecules able to bind proteins and facilitate their folding process (bottom right). The lack of selectivity of chemical chaperones is overcome by the development of pharmacological chaperones (also called PCs), which selectively bind specific targets and serve as molecular scaffolds to facilitate the correct protein folding.

lysosomal storage diseases, as well as aggregation- and degradation-dependent disorders.

## ■ ALZHEIMER'S DISEASE AND APOLIPOPROTEIN E4 STRUCTURE CORRECTORS

Alzheimer's Disease (AD) is the most common form of dementia.<sup>22,23</sup> It is a progressive neurodegenerative disease characterized by the intra- and extracellular accumulation of protein aggregates due to misfolding and aggregation of tau protein ( $\tau$ ) and  $\beta$ -amyloid peptide ( $A\beta$ ), respectively.<sup>24,25</sup> The major genetic risk factor for AD is apolipoprotein E (ApoE), specifically the isoform E4 (ApoE4).<sup>26–28</sup> ApoE is a 299-amino-acid apolipoprotein responsible for lipid transportation. It consists of an N-terminal receptor-binding domain and a C-terminal lipid-binding domain connected by a hinge region.<sup>29</sup> It exists in three isoforms, namely ApoE2, ApoE3, and ApoE4, which differ by the presence of a cysteine or arginine at position 112 or 158 in the amino acid sequence (Table 1) and differentially contribute to the risk of development of AD (Table 2). Specifically, ApoE4 is associated with the highest risk of AD onset compared with the risk-neutral ApoE3 isoform and

**Table 2. Role of ApoE4 in AD Frequency and Age of AD Onset<sup>a</sup>**

	ApoE4 noncarrier	ApoE4 heterozygous	ApoE4 homozygous
AD frequency (%)	20	47	91
Mean age of AD onset (years)	84	76	68

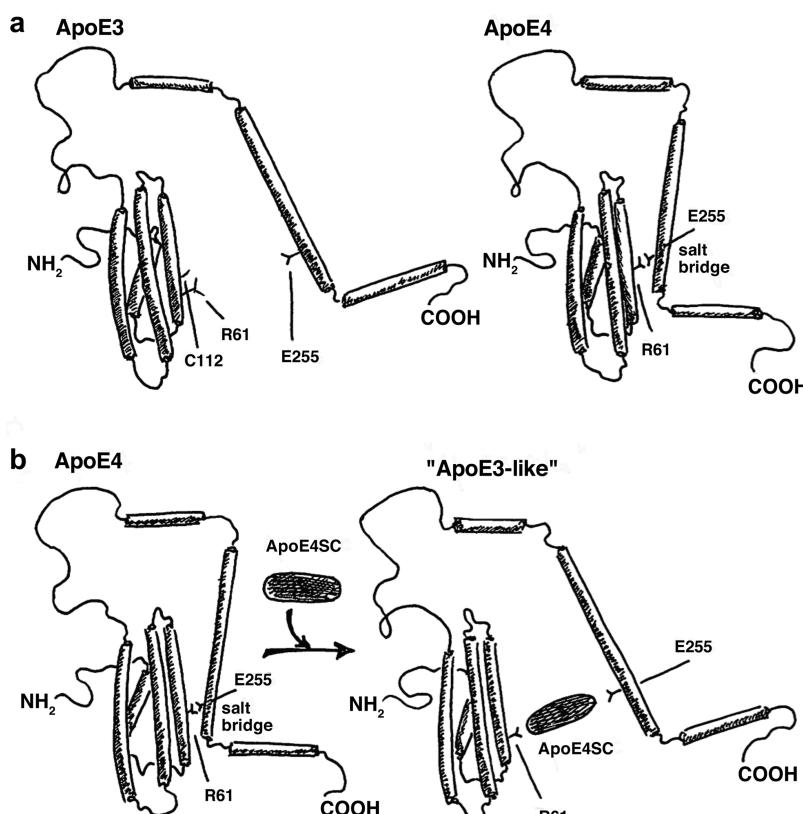
<sup>a</sup>Table adapted from ref 30.

the ApoE2 variant that is, instead, considered protective.<sup>30</sup> The expression of ApoE4 correlates with increased  $A\beta$  synthesis and fibrillization, as well as deficient clearance of monomeric  $A\beta$ , leading to the formation of extracellular neurotoxic oligomers.<sup>31–34</sup> Moreover, the intracellular proteolytic cleavage of ApoE4 is associated with a gain in  $\tau$  phosphorylation, which is the first step in the accumulation of intracellular  $\tau$  neurofibrillary tangles found in AD patients' neurons.<sup>35</sup> The pathological consequences mediated by ApoE4 arise from the interactions taking place between the two structural domains of this isoform that lead to the formation of a misfolded and nonfunctional protein conformation. In fact, because of the C112R mutation occurring in ApoE4 (Table 1), arginine 61 (R61) in the N-terminal domain can assume a solvent-exposed conformation that forms a salt bridge with the glutamate 255 (E255) in the C-terminal domain. The resulting domain-domain interaction causes the rearrangement of the ApoE4 conformation in a stable folding intermediate (Figure 2, panel a),<sup>36,37</sup> which Mahely and colleagues speculate is responsible for hindering the normal protein trafficking through the intracellular compartments.<sup>36</sup> Consequently, the misfolded conformation of ApoE4 becomes more prone to proteolytic

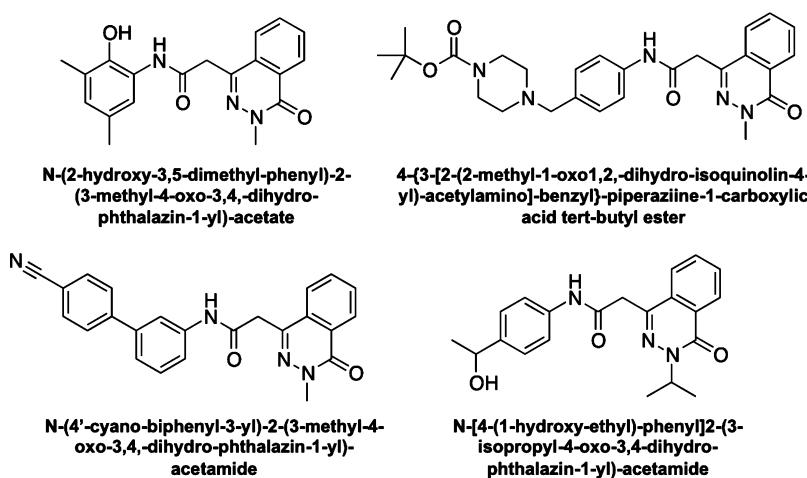
**Table 1. ApoE Isoform-Specific Amino Acid Differences<sup>a</sup>**

ApoE isoform	isoform-specific amino acid difference	
	112	158
E2	Cys	Cys
E3	Cys	Arg
E4	Arg	Arg

<sup>a</sup>Table adapted from ref 30.



**Figure 2.** Structural models of ApoE3 and ApoE4 isoforms. (a) The R61-E255 salt bridge is responsible for the domain–domain interaction between N-terminal and C-terminal domains in ApoE4 isoform. The misfolded protein structure mediates several pathological outcomes observed in AD. (b) Domain–domain interaction in the ApoE4 isoform can be disrupted by the presence of ApoE4 structure correctors (ApoE4SCs) that selectively bind to the N-terminal portion of ApoE4 and hamper the formation of the R61-E225 salt bridge. The pharmacological result is the recovery of an “ApoE3-like” structure for the ApoE4 isoform and the rescue of its physiological function.

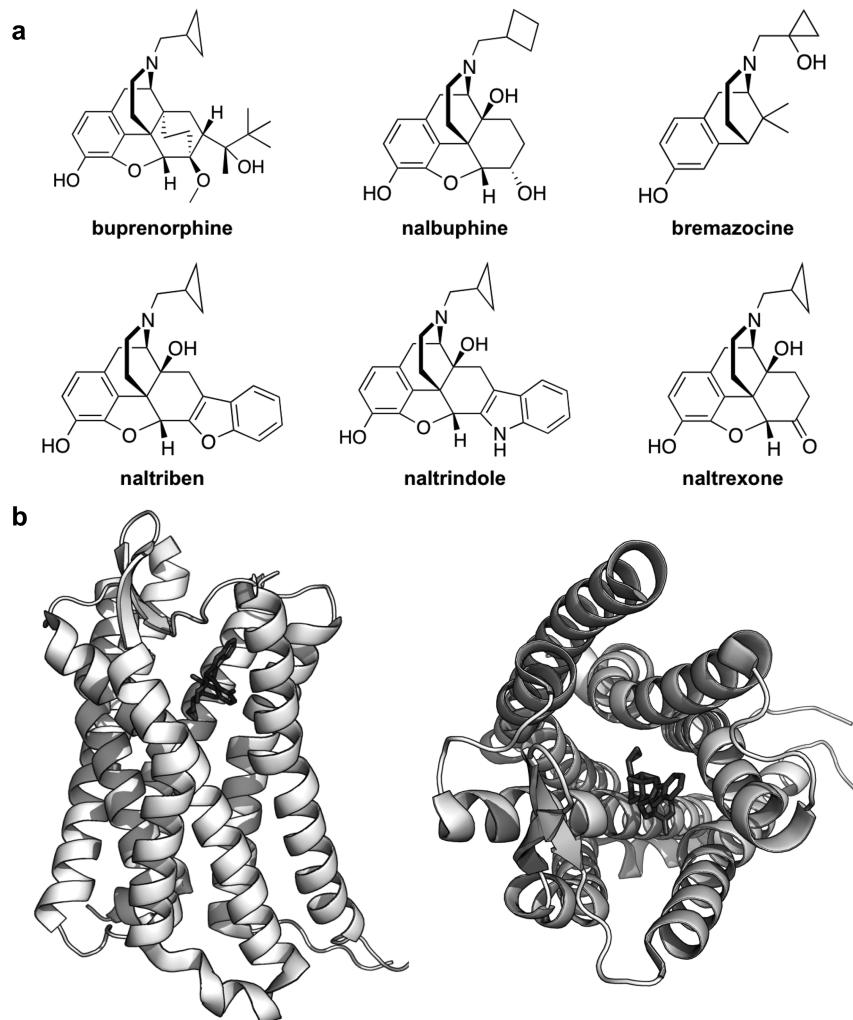


**Figure 3.** Chemical structures of highly potent phthalazinones able to rescue the domain–domain interaction in ApoE4.

cleavage by intracellular chymotrypsin serine proteases. The newly generated ApoE4 protein fragments alter the mitochondria activity in cells and are responsible for many of the above cited pathological outcomes observed in AD.<sup>36-39</sup>

Site-directed mutagenesis experiments have demonstrated that ApoE4-mediated adverse effects can be completely reversed by preventing the occurrence of the domain–domain interaction in ApoE4 through the disruption of the R61-E255 salt bridge.<sup>40,41</sup> Hence, several efforts have been made to identify small molecules that preferentially bind the ApoE4

isoform and disrupt the R61-E255 interaction. Mahley and colleagues have provided a pivotal contribution through the development of a series of PCs, called ApoE4 structure correctors (ApoE4SC), which are able to recover ApoE4's correct conformation (Figure 2, panel b).<sup>26,36</sup> ApoE4SCs constitute a series of highly potent phthalazinones (representative structures are reported in Figure 3), which selectively bind ApoE4 at nanomolar concentrations and prevent the generation of stable misfolded intermediates by blocking R61-E255 salt bridge and the subsequent domain–domain interaction.<sup>26,36</sup>



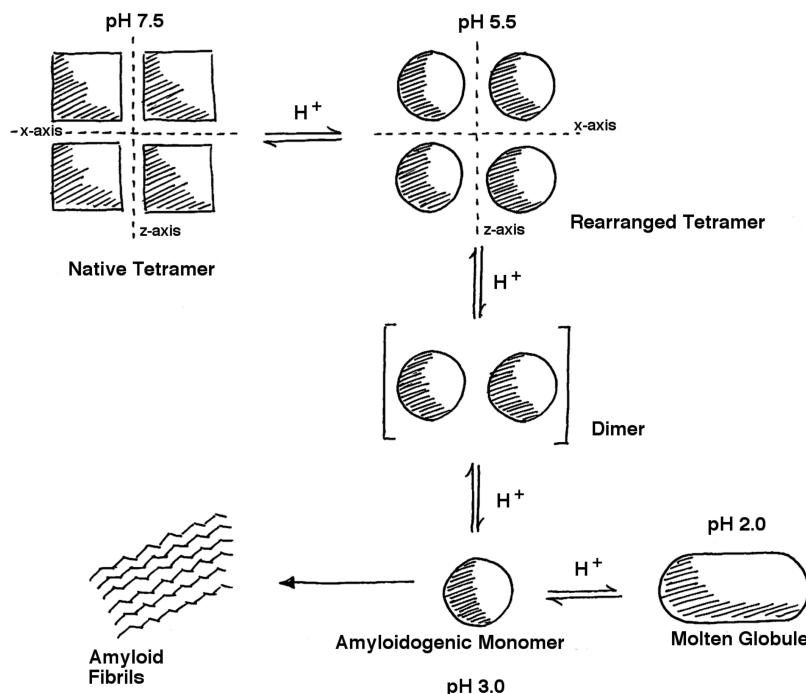
**Figure 4.** (a) Chemical structures of OR agonist (top) and OR antagonist (bottom) morphinan opioids with chaperone activity toward  $\delta$ -OR. (b) Side (left) and top (right) view of crystallographic coordinates (PDB-id: 4ej4) of  $\delta$ -OR in complex with the antagonist naltrindole (dark gray).

The global pharmacological result is the suppression of ApoE4-mediated neurotoxic effects due to the recovery of a functional ApoE3-like protein conformation (Figure 2, panel b). Thereby, ApoE4SCs rescue the intracellular trafficking of ApoE4 at the endoplasmic reticulum and Golgi apparatus, reduce the proteolytic cleavage of misfolded protein, and restore the cellular mitochondrial activity.<sup>26,36</sup> The encouraging results obtained with ApoE4SCs pave the way to the development of new PCs able to enhance the conformational stability of ApoE4 and, thus, are potentially useful for the treatment of AD.

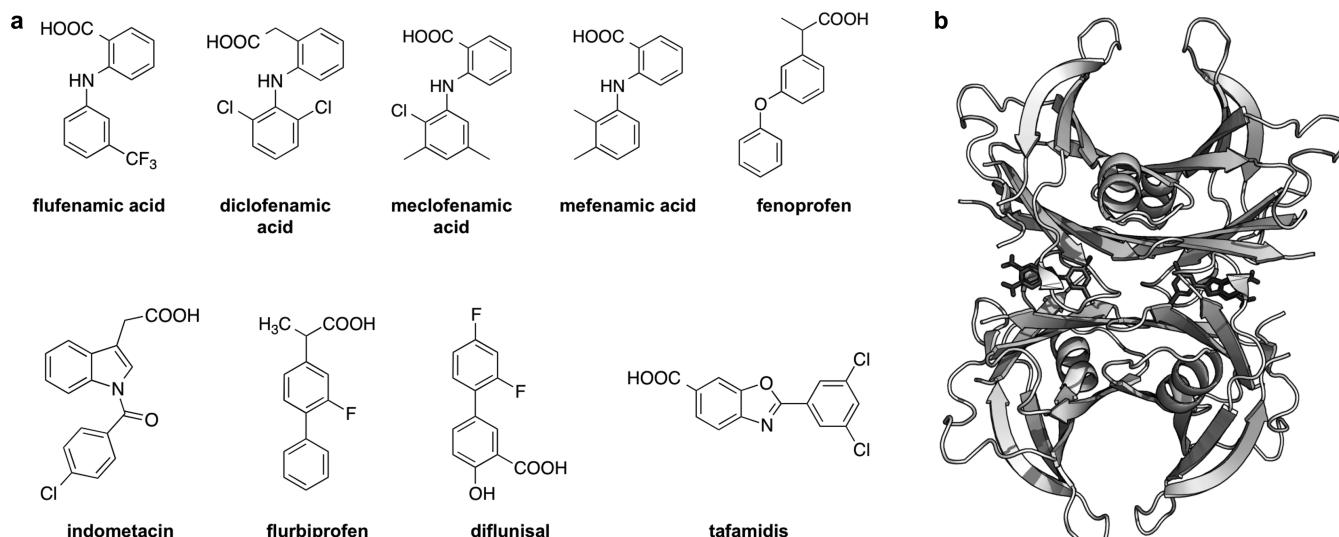
## PAIN AND PCs ACTING ON $\delta$ -OPIOID RECEPTOR

The family of opioid receptors (ORs) plays a fundamental role in the perception of pain. ORs exist in three isoforms, namely  $\mu$ -,  $\delta$ -, and  $\kappa$ -OR, which are generally identified on the basis of their biological response to the exposure of endogenous endorphins or exogenous opioids.<sup>42,43</sup> The activation of  $\mu$ - or  $\kappa$ -OR results in an inhibition of acute noxious stimuli, but it is also related to the onset of severe side effects, like addictive behaviors or hallucinogenic events for  $\mu$ -OR or  $\kappa$ -OR, respectively.<sup>44–46</sup>  $\delta$ -OR activation is associated with the inhibition of chronic pain<sup>47,48</sup> and the improvement of emotional conditions related to pain perception.<sup>49,50</sup> Those

receptor-specific pharmacological aspects make  $\delta$ -OR a more appealing therapeutic target. However,  $\delta$ -OR is very inefficiently converted in its mature form in the endoplasmic reticulum, and only around 40% of the newly expressed protein migrates to the cell surface.<sup>51</sup> Interestingly, Bouvier and colleagues have discovered that several membrane permeable opioids (Figure 4) can promote the migration of  $\delta$ -OR to the cell surface.<sup>52</sup> They have observed that, upon administration of membrane-permeable morphinan opioids, the population of mature  $\delta$ -OR increases at the level of the plasma membrane in a concentration-dependent fashion. They also found that the permanence of the receptor at the membrane is unaltered. On the contrary, the administration of membrane-impermeable peptides (i.e., enkephalin) does not significantly affect  $\delta$ -OR maturation and trafficking.<sup>52</sup> Those findings are consistent with a chaperone activity of morphinan opioids, which are able to stabilize functional conformations of  $\delta$ -OR and, ultimately, promote its trafficking to the cell surface. The chaperone activity of opioids toward  $\delta$ -OR is receptor specific. In fact, the administration of such compounds does not interfere with the folding process of other GPCRs, and similarly, unrelated GPCR binders do not affect the maturation of  $\delta$ -OR.<sup>52</sup> Moreover, both agonists and antagonists exhibit a PC behavior on  $\delta$ -OR, indicating that the receptor exists in a folding competent form



**Figure 5.** pH-dependent denaturation pathway of TTR. In the figure, *x*- and *z*-axes are indicated; T4 hormone and TTR stabilizers bind at the dimer–dimer interface along the *z*-axis of TTR tetramer.



**Figure 6.** (a) Chemical structures of TTR stabilizers. (b) Crystallographic coordinates of the TTR tetramer in complex with Tafamidis (dark gray). Both orientations of carboxyl group are solved in the X-ray structure (PDB-id: 3tct).

that can be directed toward the active or inactive conformation by the binding of ligands.<sup>52</sup> The chaperone activity hypothesis, initially formulated by Clark and Loo,<sup>18,53</sup> is consistent with a scenario in which  $\delta$ -OR PCs are able to penetrate through the cell membrane and bind the active site of  $\delta$ -OR at the level of the endoplasmic reticulum. The subsequent formation of the receptor–ligand complex promotes the stabilization of  $\delta$ -OR helices and shifts the thermodynamic equilibrium of the protein toward one specific functional conformation that migrates to the cell surface in order to fulfill its biological role.<sup>52</sup> The described mechanism of action is also compatible with very recent findings about the possible PC activity of morphine versus a newly discovered spliced isoform of  $\mu$ -OR,<sup>54–57</sup>

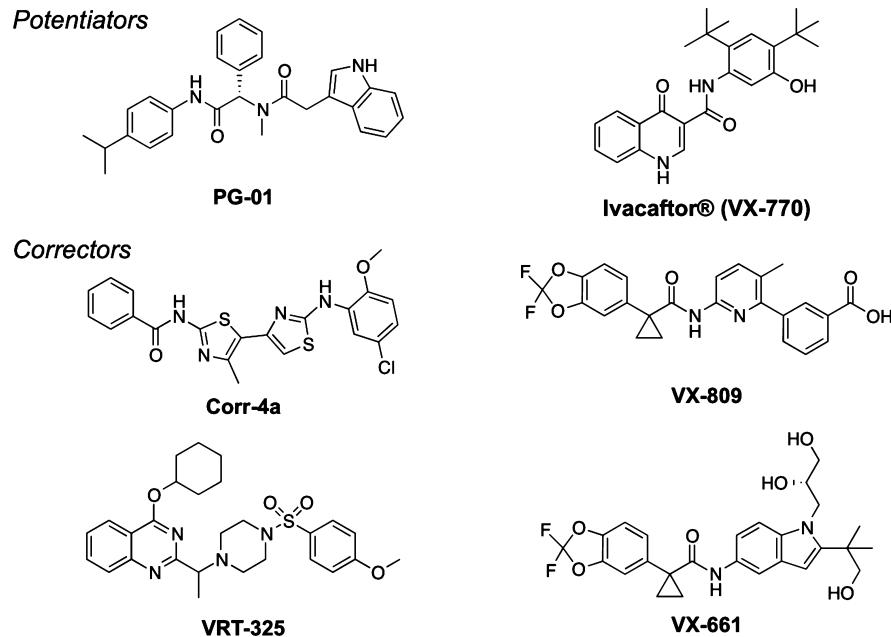
suggesting that several opioids can play a yet underestimated role in modulating the biological response of this class of receptors. Therefore, the tuning of the pharmacological and the chaperone activity of novel OR ligands can be a new therapeutic strategy for the development of more effective drugs to be used in the treatment of chronic pain conditions.

## ■ AMYLOIDOSIS AND PCs ACTING ON TRANSTHYRETIN

The medical term amyloidosis indicates a pathological condition characterized by the deposition of toxic, insoluble, supramolecular assemblies of soluble proteins. The insoluble aggregates, known as amyloids, adopt a  $\beta$ -sheet-rich structure.

Table 3. Different Types of Mutations Leading to Cystic Fibrosis<sup>a</sup>

class	effect on CFTR	examples of mutations
I	defective or no CFTR production	nonsense G542X, R553X, W1282X, R1162X, 621–1 > T, 1717–1G > A
II	defective CFTR trafficking	$\Delta$ F508, $\Delta$ I507, N1303K, S549N, L1065P
III	defective protein regulation (diminished ATP binding and hydrolysis)	G551D, R560T/S, V520F, S492F, R553G
IV	defective chloride conductance	missense R117H/C, R334W, G85E, R347P, L227R, D1152H
V	reduced transcription and synthesis of CFTR	3849 + 10kbC > T, 2789 + 5G > A, A455E

<sup>a</sup><http://www.cftrscience.com/common-CFTR-mutations>.Figure 7. Chemical structures of *potentiators* and *correctors* for rescuing the function of disease causative CFTR mutant channels.

Their occurrence is the characteristic hallmark of certain neurodegenerative medical conditions including Parkinson's and Alzheimer's disease.<sup>58</sup> Among more than 30 proteins that misfold and aggregate in amyloid structures, transthyretin (TTR) is responsible for several progressive neuropathies and cardiomyopathies with fatal outcomes.<sup>59–65</sup> TTR binds and transports holo-retinol binding protein and thyroid hormone T4 in blood and cerebrospinal fluid.<sup>66,67</sup> TTR is a 127-residue-long protein assembled in tetrameric units.<sup>68</sup> The T4 hormone binding sites are located along its z-axis dimer–dimer interface (Figure 5).<sup>69</sup> These clefts within the tetrameric assembly are largely unoccupied under physiological conditions because of the presence of thyroid binding globulin and albumin, which normally sequester the T4 hormone in the bloodstream.<sup>70</sup> By means of mutagenesis and biophysical experiments, Kelly and colleagues have reconstructed the pH-dependent denaturation pathway of TTR, showing that the destabilization of the physiological tetrameric unit is the main event leading to the formation of monomeric aggregation-prone intermediate species (Figure 5).<sup>59,71,72</sup> Mutations that destabilize the dimer–dimer interface of the TTR tetramer increase the rate of amyloidosis' onset.<sup>73–75</sup> Therefore, a possible therapeutic approach consists in increasing the stability of the TTR tetrameric unit by stabilizing the protein's dimer–dimer interface.<sup>73,76,77</sup>

This approach can be easily implemented with the use of small molecules that can bind to the T4 hormone's binding pocket at the TTR dimer–dimer interface and stabilize the native tetrameric structure of TTR. Following this hypothesis,

flufenamic acid was identified as a potent inhibitor of TTR aggregation by the pioneering work of Peterson and Kelly (Figure 5).<sup>78</sup> Later, many other nonsteroidal anti-inflammatory drugs (i.e., NSAID summarized in Figure 6) were identified to be stabilizers of the TTR tetramer by binding at the T4 hormone's binding clefts and, thus, inhibiting TTR aggregation.<sup>79</sup>

Many other chemical scaffolds that share the same mechanism of action have been identified as TTR stabilizers,<sup>80–93</sup> but the research efforts have mainly focused on the benzoxazole scaffold because of its favorable pharmacodynamic and pharmacokinetic properties.<sup>80</sup> In particular, Tafamidis (Figure 6),<sup>93</sup> discovered in Kelly's research group and now commercialized by Pfizer (Vyndaqel), has been approved for the treatment of TTR-related amyloidosis.

## ■ CYSTIC FIBROSIS: CORRECTORS AND POTENTIATORS

Cystic fibrosis (CF) is a life threatening genetic disease in the Caucasian population. The disease is triggered by nonsense mutations or single amino acid deletions (Table 3) in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene that impair the CFTR protein located at the apical pole of all epithelial cells.<sup>94,95</sup> The CFTR protein is an ABC transporter class, ATP-binding chloride ion channel that regulates the fluid and electrolyte exchange across the epithelial cell membrane.<sup>96</sup> Mutated CFTR protein disrupts the physiological balance of chloride, sodium ions, and water

molecules in the epithelial cells. Defective channel production, ineffective trafficking to the cell surface, incorrect protein regulation, deficient channel gating, and compromised stability or disabled chloride conductance can lead to the CF disease.<sup>97–100</sup> This pathological condition results in dehydration of the cell surface, production of thick mucus in epithelial cells, and airway obstruction that eventually leads to chronic infection and inflammation in various organs (lungs, intestine, pancreas, sweat glands, and genital tract), and ultimately to death.<sup>101</sup> The most prevalent mutation in CF disease is the deletion of the phenylalanine 508 ( $\Delta F508$ ) in the nucleotide-binding domain 1 (NBD1) of the CFTR protein that causes defective CFTR trafficking to the cell membrane.<sup>94,95</sup>

Numerous research groups have demonstrated that by applying site directed mutagenesis, it is possible to rescue CFTR channel function in cells by aiding the folding process, enabling membrane trafficking, or regulating ATP hydrolysis and channel gating.<sup>95,102–105</sup> These results suggest that small molecules, able to target the underlying defects of CFTR in a site-specific manner, can be applied for rescuing CFTR function. Such compounds, known as CFTR modulators, are functionally categorized into *potentiators* and *correctors*. Qualitatively speaking, *correctors* promote the exit of  $\Delta F508$ -CFTR from the endoplasmic reticulum and trafficking to the plasma membrane while *potentiators* help normalize CFTR channel gating. However, the precise mechanism by which these *correctors* and *potentiators* rescue the CFTR channel activity is unknown. They can act as PCs by either binding directly to the mutant protein, stabilizing the native protein like conformation, or acting as *proteostasis regulators* that alter mutation-specific recognition and processing by controlling the proteostasis networks responsible for biogenesis, protein folding, and trafficking.

Several *potentiators* like PG-01 or Ivacaftor (Vertex Pharmaceuticals)<sup>108–111</sup> have been produced via high throughput screening campaigns that can identify compounds able to rectify defective channel gating in the CF-causing mutant G551D-CFTR (Figure 7). Ivacaftor has recently been approved to be used as a drug for treating CF patients carrying the G551D mutation.<sup>112–114</sup> Additional studies are engaged in testing VX770's efficacy versus other gating/conductance mutations.<sup>115</sup> However, despite VX770's efficacy in the treatment of CF patients with G551D substitution, VX770 alone failed to rescue channel activity in CF patients with the  $\Delta F508$  mutation. In fact, several *potentiator*-based studies showed that *potentiator* efficacy requires a high concentration of cAMP agonist (e.g., in case of PG-01) followed by low temperature or *corrector* treatment for rescuing  $\Delta F508$ -CFTR. These observations suggest that very little CFTR is expressed in the membrane, and the rescued  $\Delta F508$ -CFTR remains in a non-native conformation in the membrane.<sup>95,107</sup>

To find suitable *correctors*, high throughput screening studies have been employed which produced several classes of small molecule  $\Delta F508$ -CFTR *correctors*.<sup>116</sup> Some studies suggested that bithiazole Corr-4a (Figure 7) directly interacts with  $\Delta F508$ -CFTR in the endoplasmic reticulum, promotes its folding efficiency, and increases chloride conductance.<sup>117–120</sup> Corr-4a, however, is not able to rescue other mutant membrane proteins for which pharmacological or low-temperature rescue is possible.<sup>95</sup> Another *corrector*, namely VRT-325 (Figure 7), was reported to have direct interaction with  $\Delta F508$ -CFTR altering CFTR ATPase activity and protease susceptibility.<sup>118–120</sup>

Vertex Pharmaceuticals Inc. has yielded several *correctors*, including Lumacaftor (VX809)<sup>109</sup> and VX661 (Figure 7). In a phase II clinical trial of experimental drug Lumacaftor, CF patients with  $\Delta F508$  mutation showed an increase of 7.4% lung function when used in combination with Ivacaftor.<sup>121</sup> It is possible that VX-809 promotes  $\Delta F508$ -CFTR conformational maturation at the endoplasmic reticulum and, hence, restores ~15% of non-CF channel activity in primary respiratory epithelia.<sup>95,107,109,122</sup> However, such mechanisms of action need to be further investigated. Another *corrector*, VX661, when coadministered with Ivacaftor, shows significant improvements in lung functions (~9%) of CF patients.<sup>118–120</sup> Several phase II clinical trials are underway to determine whether VX661 coadministered with VX770 will aid in alleviating CF disease without causing side effects.<sup>123</sup>

Similar small-scale screening efforts yielded additional candidate *correctors* such as glafanine and the phenylhydrazone RDR1 (Figure 7) that had only low efficacy.<sup>95,124–126</sup> Differential scanning fluorimetry data suggest that RDR1 stabilizes the conformation of mouse  $\Delta F508$  NBD1 by binding to the  $\Delta F508$  CFTR.<sup>126</sup> Similar studies may be utilized to understand the molecular mechanisms of the *correctors* and to assess if they directly bind and conformationally stabilize the mutant channel. These findings imply that *in silico* drug discovery efforts involving docking simulations hold promise for identifying *correctors* and *potentiators*. Successful retrieval of compounds, however, relies on a dependable 3D structure of  $\Delta F508$  CFTR, which is currently unavailable. In order to address this issue, numerous avenues of investigation are being pursued to model 3D structures of CFTR protein based on integrated computer modeling and experimental approaches. In fact, our lab pioneered the development of a CFTR model in its outward facing state.<sup>127</sup> We extensively use our *in house* developed MedusaDock<sup>128–130</sup> docking algorithm targeting the NBD1-ICL4 interface of the CFTR structure (this interface is validated by cross-linking experiments) to identify small molecules that could potentially be optimized as drugs to treat CF. This interface is specifically chosen as a target, as the  $\Delta F508$  deletion, which causes CF disease in 80% patients, resides in the NBD1-ICL4 interface in wild type CFTR.

From the current research status, it can be hypothesized that it may not be possible to design one compound that can competently restore the complex folding defect introduced by  $\Delta F508$ -CFTR and rescue CFTR channel function. Independent studies are being carried out to design *corrector-potentiator* hybrids. One such study yielded a bithiazole-phenylglycine hybrid which breaks into an active bithiazole *corrector* and phenylglycine *potentiator* when cleaved by intestinal enzymes.<sup>131</sup> The cyanoquinoline class of  $\Delta F508$  CFTR *correctors* also exhibits independent *potentiator* action,<sup>132</sup> with their *corrector* and *potentiator* efficacies respectively comparable to those of Corr-4a and genistein (Figure 7), although with EC<sub>50</sub>'s at micromolar rather than nanomolar concentrations. To further test this hypothesis and to find effective therapeutics for CF disease, several independent basic science studies<sup>133,134</sup> as well as clinical trials<sup>106,107</sup> are in progress. In fact, Vertex has recently launched phase III clinical trials with their newest FDA-approved drug Orkambi (a combination of Ivacaftor and Lumacaftor) to treat patients with two copies of  $\Delta F508$  alleles. Clinical data suggest that the administration of Orkambi leads to modestly increased lung function (2.6–4%).<sup>135</sup> Currently, numerous studies are ongoing to find more potent therapies toward CF. In this light, delineating the mechanism of actions

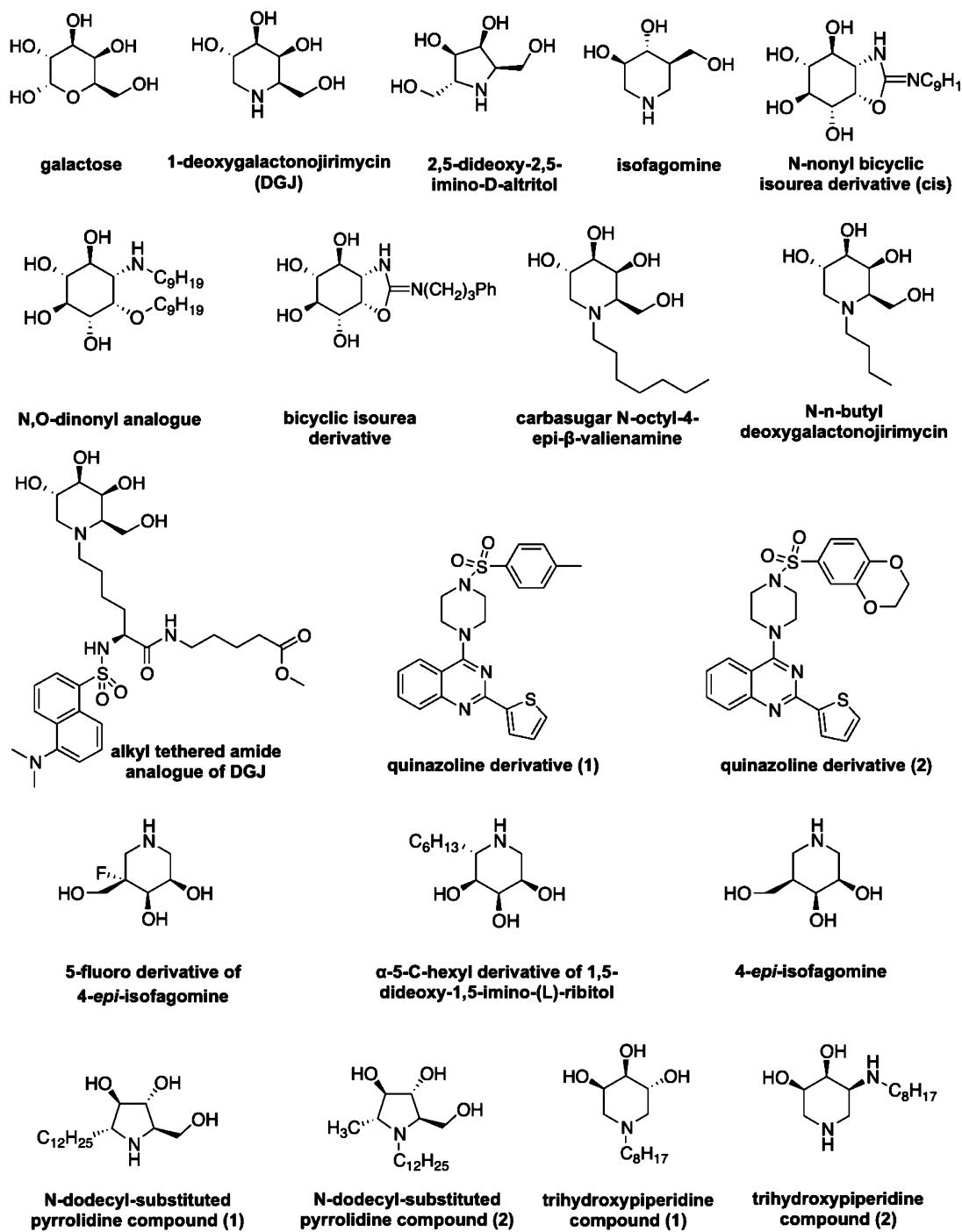


Figure 8. Chemical structures of PCs for the treatment of lysosomal storage disease.

of the *potentiators* and *correctors* by applying molecular modeling tools is crucial as it can provide insights to designing new molecules for rescuing  $\Delta F 508$ -CFTR function.

Recently, the loss of CFTR function has been associated with aberrant dynamics of the mutant channel.<sup>136</sup> Dokholyan's research group has demonstrated that the expression and function of the pathogenic CFTR could be restored *via* rescue mutations distributed throughout the NBD1 domain.<sup>136</sup> Indeed, these rescue mutations can be exploited to localize novel allosteric drug binding pockets.<sup>137–139</sup> These results will add a whole new dimension toward the development of *allosteric PCs* that would recover CFTR activity and pave the

way to the next-generation of individualized medicines for CF patients.

## ■ LYSOSOMAL STORAGE DISORDERS AND PC THERAPY

Numerous glycosidic and proteolytic enzymes present in the lysosome are required for metabolizing a wide variety of molecules such as proteins, glycoproteins, glycolipids, and oligosaccharides. This process is critical for cellular homeostasis, i.e., the degradation of complex molecules into simple ones that essentially facilitates the expulsion of toxic material from the cell or recycling into a biosynthetic pathway.

Proteolytic enzymes are synthesized in the endoplasmic reticulum at neutral pH and are transferred to the lysosome via the Golgi apparatus.<sup>140–142</sup> They are characterized by the ability to function in the acidic environment (pH 4.5–5.5) of lysosome, which is necessary for metabolic degradation processes. The loss of enzymatic activities due to missense or nonsense deletion mutations causes the accumulation of one or more metabolic intermediates and leads to pathological conditions known as lysosomal storage disorders (LSDs).<sup>140,141</sup> These lysosomal dysfunctions comprise approximately 50 different types of pathologies<sup>140,141,143</sup> depending on several factors, such as the type of the accumulated substrate, where the material accumulates, and also the degree of compromised enzymatic activity. Note that while different types of LSDs are rare, altogether they affect a considerable portion of population, and cause death within a few years from the onset of the disease.

Current therapeutic approaches for LSD comprise substrate reduction therapy<sup>144</sup> and enzyme replacement therapy.<sup>145,146</sup> These therapeutic approaches are primarily used for correcting the loss-of-function diseases by restoring the enzymatic activity at cellular and tissue levels by replenishing the deficiency of required lysosomal enzymes. Substrate reduction therapy includes the inhibition of the enzyme associated with production of pathological storage products and is only available for the treatment of type I Gaucher disease (GD) and Niemann-Pick type C disease. On the other hand, enzyme replacement therapy involves regular infusions of recombinant enzymes and is widely used for numerous LSDs. However, the effectiveness of enzyme replacement therapy is hindered by a number of limitations, which include the production of antiprotein antibodies leading to hypersensitive reactions, inactivation and premature clearance of infused enzymes, and anaphylactic shock. Additionally, currently available enzyme replacement therapies are not able to penetrate the blood–brain barrier and therefore cannot be used to treat central nervous system- (CNS-)related pathologies associated with some LSD types.<sup>147–149</sup> To overcome this issue, new enzymes are being designed that can penetrate the blood–brain barrier. This may prove to be an effective therapeutic strategy for CNS-related LSDs in the future.<sup>150</sup> However, it is noteworthy that enzyme replacement therapy may not only induce immune response but is also highly expensive.

Therefore, as an alternative therapeutic approach, PC therapy has gained considerable attention in regard to the LSD treatment.<sup>140,151</sup> Many LSDs are caused by mutations in the genes that encode lysosomal enzymes.<sup>152</sup> While many of these mutations involve large insertions, deletions, and premature stop codons that lead to no protein synthesis or production of inactive forms, various single mutations are fairly subtle, leading to the production of misfolded proteins. These aberrant proteins, while functionally active, are unstable due to their misfolded conformations. They often get accumulated in the endoplasmic reticulum or other organelles and form unwanted aggregates, as they cannot be transported to the intended cellular organelle. In lysosomes, catalytically active proteins can maintain functionality for a long time after the PCs are dissociated from its catalytic site.<sup>153–155</sup> Here, we will focus on PC therapy applied to three major LSDs, namely, Fabry disease, GM1-gangliosidosis, and Gaucher diseases.

**α-Galactosidase A or Fabry Disease.** Fabry disease (FD) is a rare inherited (X-linked recessive) disorder and is caused by a wide variety of mutations in the galactosidase- $\alpha$  (GLA) gene

that encodes the  $\alpha$ -galactosidase enzyme.<sup>156,157</sup> The  $\alpha$ -galactosidase enzyme, residing in lysosomes, catabolizes the  $\alpha$ -galactosyl moieties from glycolipids and glycoproteins. Missense mutations in the GLA gene fail to stabilize and fold  $\alpha$ -galactosidase correctly and eventually degrade in lysosome. Due to the deficit of these enzymes in cells, a glycosphingolipid with terminal  $\alpha$ -linked galactosyl moieties called globotriaosylceramide (GL-3) and specifically globotriaosylceramides (Gb3) accumulate in the vascular endothelium, skin, and heart. Pathological conditions associated with this accumulation include acroparesthesias, angiokeratomas, cardiomyopathy, stroke, and renal failure.

Currently, few enzyme replacement treatments are available for FD.<sup>158</sup> However, numerous efforts are underway to develop PCs as a therapeutic strategy for FD. Since galactose has been shown to (Figure 8) behave like a PC, it was administered to Fabry patient-derived lymphoblasts in an early study. Using cell-based assays, increased  $\alpha$ -Gal A activity was observed.<sup>159</sup> Later studies showed that another natural substrate mimetic, 1-deoxygalactonojirimycin (DGJ, Figure 8), can potentially be a PC<sup>154</sup> in FD. DGJ showed a concentration-dependent (in the range between 0.2 and 20  $\mu$ M) increase in  $\alpha$ -Gal A activity in FD patient-derived lymphoblast cultures.<sup>154</sup> *In vivo* studies using a transgenic mouse model expressing a mutant form of human R301Q (found in both classic and late-onset patients) of  $\alpha$ -Gal A demonstrated that oral administration of DGJ can increase  $\alpha$ -Gal A activity and decrease levels of GL-3 in disease-relevant tissues of an FD mouse model.<sup>155,160</sup> Following these studies, the investigational drug, migalastat hydrochloride (Figure 8; also known as DGJ), is currently in phase III clinical trials to evaluate its safety and efficacy as a potential treatment for FD.<sup>161</sup> In other studies, several additional iminosugars from the roots of *A. tryphilla* were tested in an attempt to develop new drugs for FD as these PCs bind to various glycosidases.<sup>162</sup> Treatment of FD patient lymphoblast cell cultures with one of these analogues, 2,5-dideoxy-2,5-imino-D-altritol at 50–500  $\mu$ M concentration, (Figure 8) showed a significant increase (2- to 9-fold) in  $\alpha$ -Gal A activity. Although this PC appeared to be a promising lead for a new series of drugs for FD, further work has yet to yield new drugs. Lately, Withers's research group has designed and synthesized 5-fluoro derivatives of 4-epi-isofagomine (a strong inhibitor of  $\beta$ -galactosidase); these compounds (Figure 8) demonstrated considerably improved activity as well as selectivity toward  $\alpha$ -galactosidase enzyme. These findings suggest that these PCs can pave the way to discovering new therapeutics for FD.<sup>163</sup>

**β-Galactosidase or GM1-Gangliosidosis.** Certain autosomal recessive diseases such as GM1-gangliosidosis and a closely related LSD known as Morquio Syndrome B (MSB) are caused by the deficiency of lysosomal human D- $\beta$ -galactosidase enzyme ( $\beta$ -Gal) and the subsequent buildup of two glycolipids, monosialotetrahexosylganglioside (GM1) and asialo-GM1 ganglioside (GA1), in lysosome. Physiologically functional  $\beta$ -Gal is located in the lysosome and catalyzes the breaking down and removal of terminal  $\beta$ -linked galactose in GM1 ganglioside and keratan sulfate. Mutations in the galactosidase- $\beta$ 1 (GLB1) gene lead to misfolding of these hydrolases and consequent degradation. Without a sufficient amount of functional  $\beta$ -Gal, GM1-ganglioside molecules accumulate to toxic levels in several tissues including nerve tissues. Unlike other LSDs, GM1-gangliosidosis manifests skeletal abnormalities, profound intellectual disability, gingival hypertrophy, and cardiomyopathy but shows little to no CNS impairment.<sup>140</sup>

Since DGJ (Figure 8) worked remarkably well for FD patients in early studies, it was also tested in the patient derived cell lines and showed increased enzymatic activity.<sup>164</sup> DGJ and N-n-butyl deoxygalactonojirimycin<sup>165</sup> (Figure 8) also showed an overall increase in enzymatic activity for GM1-gangliosidosis and MSB related mutations at high concentrations (500  $\mu\text{M}$ ) when administered in mouse cell lines transfected with mutant forms of human  $\beta$ -galactosidase associated with GM1-gangliosidosis and MSB. Followed by these studies, a variety of DGJ analogues was derived by Wrodnigg's research group for identifying potential drugs.<sup>166–168</sup> One of these alkyl tethered amide analogues (Figure 8) showed increased  $\beta$ -galactosidase activity at a 10–100  $\mu\text{M}$  concentration for several mutant forms obtained from adult GM-1 and MSB patients. However, this PC had little to no efficacy to the severe, infantile-onset mutant forms.<sup>168</sup> A similar series of drugs was designed and evaluated by Fröhlich's research group.<sup>169</sup> From those compounds, carbasugar N-octyl-4-epi- $\beta$ -valienamine (Figure 8) was evaluated as the most potent drug and showed enhanced enzymatic activity at low concentrations (0.2–2  $\mu\text{M}$ ) in 27 different cell lines with various mutations corresponding to infantile and juvenile GM-1 gangliosidosis.<sup>170,171</sup> Upon administration of this drug, transgenic mice that were transfected with human GM-1 gangliosidosis mutants showed a moderate decrease in accumulated storage products in the brain.<sup>172</sup> Due to its accessibility to the brain and interaction with the targeted enzyme, this drug shows great promise. However, further biochemical analyses and optimization are required for maximizing its efficacy. In a more recent work by Martin's research group, two compounds were found to be potent inhibitors of human  $\beta$ -galactosidase: 4-*epi*-isofagomine and a 5-C-hexyl derivative of 1,5-dideoxy-1,5-imino-(L)-ribitol (Figure 8). Both molecules have been shown to enhance the enzymatic activity (at 10  $\mu\text{M}$  concentrations) up to ~2.7-fold upon administration to the GM-1 gangliosidosis R301Q mutant cell line. New investigations are being pursued to assess and optimize these PCs.<sup>173</sup>

**Acid  $\beta$ -Glucosidase or Gaucher Disease.** Gaucher disease (GD) is one of the most prevalent diseases among all LSDs. It is caused by the deficiency or the absence of the functional enzyme acid  $\beta$ -glucosidase or  $\beta$ -glucocerebrosidase (GCase),<sup>140</sup> leading to the storage of glucocerbroside (GLC) in the macrophages of the liver, spleen, and bone marrow. Pathological manifestations of GD include anemia, thrombocytopenia, hepatosplenomegaly, and skeletal deformities. Additionally, some patients also exhibit neurological impairment. Unlike in other LSDs, enzyme replacement therapy is widely used for treating patients with GD. There are several FDA and/or EMEA approved enzymes, namely imiglucerase, velaglucerase alfa, and taliglucerase alfa.<sup>140</sup> Despite nearly 200 mutations leading to GD, approximately 80% of patients carry either the L444P or the N370S mutation in GCase. In particular, the N370S mutant is mainly connected to non-neuronopathic GD, which can be treated with enzyme replacement therapy. However, patients with the L444P mutation often manifest CNS impairment. Enzyme replacement therapy cannot be applied for treating patients with L444P mutation, as these enzymes cannot cross the blood–brain barrier.<sup>174</sup> Therefore, development of a PC therapy is especially promising for the pathological condition related to L444P mutation.

Several high throughput screenings (HTS) have been carried out to identify novel GD therapeutics. These studies yielded a number of diverse PCs that can be categorized into two

subtypes: carbohydrate mimetics (iminosugars, azasugars, and carbasugars) and noncarbohydrate compounds. Carbohydrate mimetics obtained from HTS were evaluated in a cell-based enhancement assay. These compounds, while capable of increasing the GCase activity of other less severe mutant forms, had moderate or null effect on N370S cell lines and no effect on L444P cell lines.<sup>170,174–180</sup> Fan's research group pursued some iminosugar and azasugar compounds for evaluating their chaperone properties in N370S patient cell lines.<sup>178</sup> From these studies, isofagomine (Figure 8) was identified to be a promising new candidate. Administration of isofagomine showed a concentration dependent increase in GCase activity in both N370S and L444P cell lines.<sup>181</sup> Furthermore, *in vivo* studies with isofagomine and L444P mutant mice also revealed improved GCase activity in liver, spleen, lung, and brain.<sup>181</sup> Numerous clinical trials were pursued followed by the promising results of isofagomine's efficacy against GD. During phase I studies, isofagomine showed a dose-dependent increment of GCase levels (up to 3.5 fold) in white blood cells (WBC). In a subsequent 4-week-long phase II study conducted in 26 subjects with different GBA1 mutations (including N370S and L444P) previously treated with enzyme replacement therapy (<http://ClinicalTrials.gov> ID NCT00433147), WBC GCase activity increased in 20 patients while the levels of disease markers including platelet, hemoglobin, glucosylceramide, and chitotriosidase levels remained unaltered. In another 6-month phase II study, all patients with no history of enzyme replacement therapy (<http://ClinicalTrials.gov> ID NCT00446550) exhibited only incremental change in WBC GCase levels, except one (attaining improvements in clinical measures).<sup>151,182</sup> In summary, despite the promising indications of isofagomine's efficacy against GD, it failed during clinical trials. Another PC, Ambraxol (chemical structure not shown), also failed to alleviate GD related pathology during clinical trials.<sup>151</sup>

Simultaneous independent studies also yielded multiple novel drugs that showed increased enzymatic activity in either N370S, L444P, or both cell lines at nanomolar concentrations.<sup>183–185</sup> Recently, Trapero's research group reported an N,O-dinonyl analogue (Figure 8) that increases GCase activity by 1.9-fold in an N370S cell line at 1 nM and a 1.4-fold increase in an L444P cell line at 0.01 nM.<sup>186</sup> Also, molecules shown in Figure 8 (discovered by Trapero's research group<sup>186</sup>) showed similar enhanced GCase activity in N370S and L444P cell lines at 1 nM.<sup>186,187</sup>

As mentioned earlier, in addition to the carbohydrate mimetics, candidate noncarbohydrate PCs were identified through numerous HTS and subjected to evaluation for their potency to enhance GCase activity. These compounds showed very low to moderate efficacy when compared to isofagomine in N370S and L444P cell lines.<sup>188–191</sup> Importantly, in another effort, Marugan et al. discovered two quinazoline derivatives (Figures 8) that can stabilize GCase and enhance trafficking with a significantly greater activity relative to isofagomine.<sup>192</sup> Further studies are in progress to better understand the pharmacology of this series of compounds for identifying new drugs and optimizing existing ones. A new study performed by Cardonna's research group aimed at identifying inhibitors of GCase from different classes of iminosugars: pyrrolidine, piperidine, and pyrrolizidine. They discovered two submicromolar range *n*-dodecyl-substituted pyrrolidine molecules through the biological evaluation for GCase inhibition (Figure 8). They also reported two new compounds from the

**Table 4.** List of Additional Disorders Associated with Protein Misfolding

condition	associated gene	associated protein
nephrogenic diabetes insipidus (hereditary form)	AVPR2 (~90% patients) and AQP2 (~10% patients)	vasopressin or antidiuretic hormone <sup>a</sup>
permanent neonatal diabetes mellitus	ABCC8	SUR1 subunit of ATP sensitive potassium channel
Niemann Pick disease	SMPD1	acid sphingomyelinase
Pompe	GAA	acid alpha-glucosidase
Parkinson's	LRRK2, PARK2, PARK7, PINK1, or SNCA	Parkin, DJ-1, ubiquitin C-terminal hydrolase isozyme L1 (UCH-L1), nuclear receptor-related factor 1, and alpha-synuclein
amyotrophic lateral sclerosis	SOD1	Cu/Zn superoxide dismutase
Huntington's	HTT	Huntingtin
spinocerebellar ataxia type 1/2/3	ATXN1/2/3	ataxin-1/2/3
amyloidosis	TTR	transthyretin
alpha-thalassemia	HBA1	hemoglobin, alpha subunit
beta-thalassemia	HBB	hemoglobin, beta subunit
Lesch–Nyhan syndrome	HPRT1	hypoxanthine phosphoribosyltransferase
hemolytic anemia	PKLR	human erythrocyte pyruvate kinase
phenylketonuria	PAH	phenylalanine hydroxylase
mucopolysaccharidosis VI	ARSB	arylsulfatase B
hyperammonemia	OTC	ornithine carbamoyltransferase
Hyper-IgM syndrome	CD40LG	CD40 Ligand
porphyria	UROD	uroporphyrinogen decarboxylase
various prion diseases	PRNP	prion protein
dopa-responsive dystonia	GCH1	GTP cyclohydrolase I
methemoglobinemia	CYB5R3	cytochrome b5 reductase 3
gyrate atrophy	OAT	ornithine aminotransferase
mucopolysaccharidosis VII	GUSB	$\beta$ -glucuronidase
lactic acidosis	PDHA1	pyruvate dehydrogenase (alpha subunit)
aniridia	PAX6	paired box 6
muscular dystrophy; cardiomyopathy	LMNA	lamin A/C (globular domain)
various hereditary cancers (including breast, ovarian, liver, bone, lung, Li–Fraumeni syndrome, head and neck squamous cell carcinoma)	TP53	tumor protein (p53)
cardiovascular diseases	HERG	HERG potassium channel <sup>b</sup>
autosomal dominant congenital cataract	CRYAA	crystallin
oculocutaneous albinism	TYR	tyrosinase
Menkes disease	ATP7A, ATP7B	ATPase copper transporting alpha polypeptide, ATPase copper transporting beta polypeptide
sickle cell anemia	HBB	$\beta$ -globin <sup>c</sup>

<sup>a</sup>Vasopressin or antidiuretic hormone is a peptide consisting of nine amino acids. <sup>b</sup>Little is known about the nature of specific genetic risk factors related to cardiovascular diseases. <sup>c</sup>Mutations in  $\beta$ -globin result in different versions of abnormal hemoglobin (hemoglobin S, C, and E).

piperidine series (Figure 8) that exhibited up to ~1.5-fold GCase activity increase in N307/RecNcil human fibroblast cells at 100  $\mu$ M concentration.<sup>193</sup>

The above studies indicate that PC therapy represents a new and promising alternative to overcoming the limitations associated with enzyme replacement therapy for the treatment of LSDs. Nonetheless, despite their higher efficacy and ability to cross the blood–brain barrier, when compared to complementary treatments, the PC therapy poses certain challenges. The applicability is restricted to patients with responsive mutations, moreover, the dose optimization and administration procedures of PCs associated with maximum substrate turn over still remains a great challenge. In this light, it is noteworthy that most PCs demonstrating clinical efficacy versus numerous LSDs bind to the active sites of the enzymes and function as inhibitors. Unfortunately, not all LSD patients respond to active-site PCs. It is reasonable to speculate that deleterious mutations that affect stability, folding, and/or catalytic activity of the target enzyme might be unresponsive or show insignificant clinical response upon administration of active-site PCs. To address this limitation, current research is

aiming at identifying and designing PCs that bind to the allosteric sites of the targeted enzyme (and not the catalytic site) thus fostering progress in finding novel PCs.<sup>151,190,194–196</sup> Furthermore, a combined enzyme replacement-PC approach may offer a more effective therapeutic strategy, and in fact studies are ongoing to investigate this novel synergistic approach.<sup>197–199</sup>

## GENERAL CONSIDERATIONS AND CONCLUSIONS

Despite the plethora of diseases (examples are summarized in Table 4) related to protein misfolding, the few examples reported in this review demonstrate that PCs constitute a valid therapeutic approach for several aggregation- and degradation-dependent conformational diseases.

The availability of appropriate structural biology and/or biochemical and biophysical assays is at the basis of successful development of effective PCs. For instance, structural information (examples are summarized in Table 5) certainly facilitated the discovery and development of TTR stabilizers (Figure 6).<sup>93</sup> Similarly, the development of an appropriate intracellular Förster resonance energy transfer (FRET) assay to

**Table 5. Structural Information of the Described Misfolding Disease-Associated Targets**

protein	structural information available
ApoE	X-ray and NMR coordinates available in the Protein Data Bank
$\delta$ -OR	X-ray coordinates available in the Protein Data Bank
TTR	X-ray coordinates available in the Protein Data Bank
CFTR	Homology model coordinates of full-length protein are available freely and/or upon request from the Dokholyan, Callebaut, Villa-Freixa, Dawson, McCarty, Sakurai, and Tielemans research groups
$\alpha$ -galactosidase	X-ray coordinates available in the Protein Data Bank
$\beta$ -galactosidase	X-ray coordinates available in the Protein Data Bank
acid $\beta$ -glucosidase	X-ray coordinates available in the Protein Data Bank

biophysically monitor the intramolecular domain interactions of ApoE4 critically contributed to the identification of ApoE4 correctors.<sup>36</sup> The use of immunoprecipitation techniques has fostered identification of pain killers interacting with the  $\delta$ -opioid receptor.<sup>52,200</sup> For finding effective PCs associated with varied LSDs and continual progress in developing new therapies, cell-based *in vitro* HTS assays, inhibition, and/or thermal stability assays have been of great importance.<sup>140</sup> Likewise, cell-based HTS assays derived from fluorescence-based assays of membrane potential and halide efflux, as well as reporter assays, played a crucial role in the successful identification of CFTR potentiators and correctors from hundreds of thousands of chemical compounds.<sup>123</sup> Despite the efforts of many research groups on determining CFTR structure *via* electron microscopy, or crystallography, the lack of crystal coordinates of CFTR has significantly slowed down a rational, structure-based drug design for PCs' development.

Although, there is lack of a unified picture about the mechanism of actions of these classes of molecules, it is clear that PCs are characterized by small variations on defined molecular scaffolds (i.e., morphinans, phthalazinones, fenantrenes derivatives discussed above), which imply the existence of specific interactions with distinct locations of the targeted proteins. These structural features evidently distinguish PCs from macromolecular (proteins) or chemical chaperones (osmolytes), which instead facilitate protein folding by establishing nonspecific interactions with large protein interfaces or by changing local environment conditions (i.e., pH, ionic environment, membrane potential, solvation), respectively. In this regard, many PCs bind the active sites of the targeted proteins (i.e., morphinans to opioid receptors,<sup>200</sup> or DGJ to  $\alpha$ -Gal A, or isofagamine for GCase enzymes<sup>151,201,202</sup>), thus, interfering with the binding of the natural substrates. Hence, the resulting increased cellular protein activities after PC treatment may appear contradictory. In reality, because of the lower affinity interactions with immature protein intermediates along with the complications to access the intracellular compartments, the chaperoning efficacy (expressed as EC<sub>50</sub>) of these compounds is generally much higher than what it is required for functional protein activation or inhibition.<sup>201</sup> Additionally, the relative abundance and higher affinities of natural substrates may facilitate the cognate ligand binding and turnover (in case of catalytic enzymes) in lieu of PC-mediated protein inhibition.<sup>151,201</sup> Obviously, the occurrence of mutations in the active sites of proteins may equally diminish or abolish the binding of natural

substrates as well as PCs.<sup>203–205</sup> In this regard, an alternative strategy currently explored is the identification of PCs designed to specifically interact with allosteric regions of the target proteins and promote protein stabilization without hampering the binding of the natural substrate.<sup>141,151,201,206</sup> This novel approach has two main advantages: (i) avoiding PCs' interactions with the binding sites of folded, functional forms of the target proteins<sup>200</sup> and (ii) expanding the spectrum of activity of these compounds to several protein mutants that could be unresponsive to the usage of classic PCs targeting protein's active sites.<sup>141,151,201,206</sup> In conclusion, we have provided a broad picture on how PCs can effectively stabilize protein structure, enhance trafficking from the intracellular compartments to the cell surface, restore protein functions, and/or prevent their aggregation. Despite some limitations, discussed above, a huge economical effort has been committed for both the development of these molecules and their use in therapy (i.e., the cost of cystic fibrosis treatment with Ivacaftor is estimated around \$300,000/year per patient). PCs represent a valid complementary approach to the enzyme replacement treatment in terms of cost, efficacy, and patient's compliance to therapy.<sup>141</sup> Moreover, novel combinations with compounds able to interact with the macromolecular chaperones machinery and networks may represent a promising therapeutic approach applicable to a large range of pathologies.<sup>123,141,207</sup> Although to this day, it is difficult to formulate general considerations on the use of PCs in the clinical practice, the successful case of Ivacaftor shows that the further development of these compounds constitutes a promising research avenue in medicinal chemistry.

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### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

We thank Benfeard Williams for his help with this review. This work was supported by National Institutes of Health grant R01GM080742.

## ■ KEYWORDS

**Pharmacological chaperones:** Molecules able to stabilize protein structures or inhibit protein aggregation by serving as a molecular scaffold for proper protein folding

**Pharmacoperones:** Same as pharmacological chaperones

**Protein folding:** A process by which protein adopts its functional three-dimensional structure

**Protein misfolding:** A process by which protein folds in an unusual or incorrect three-dimensional structure

**Conformational diseases:** Class of pathologies in which specific proteins become structurally unstable, undergo a misfolding process, and lose their physiological function

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