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REVIEW

Chemical Chaperones to Inhibit Endoplasmic Reticulum Stress: Implications in Diseases

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Abstract: The endoplasmic reticulum (ER) is responsible for structural transformation or folding of de novo proteins for transport to the Golgi. When the folding capacity of the ER is exceeded or excessive accumulation of misfolded proteins occurs, the ER enters a stressed condition (ER stress) and unfolded protein responses (UPR) are triggered in order to rescue cells from the stress. Recovery of ER proceeds toward either survival or cell apoptosis. ER stress is implicated in many pathologies, such as diabetes, cardiovascular diseases, inflammatory diseases, neurodegeneration, and lysosomal storage diseases. As a survival or adaptation mechanism, chaperone molecules are upregulated to manage ER stress. Chemical versions of chaperone have been developed in search of drug candidates for ER stress-related diseases. In this review, synthetic or semi-synthetic chemical chaperones are categorized according to potential therapeutic area and listed along with their chemical structure and activity. Although only a few chemical chaperones have been approved as pharmaceutical drugs, a dramatic increase in literatures over the recent decades indicates enormous amount of efforts paid by many researchers. The efforts warrant clearer understanding of ER stress and the related diseases and consequently will offer a promising drug discovery platform with chaperone activity.

Keywords: endoplasmic reticulum stress, unfolded protein response, chemical chaperone, drug discovery, diabetes, cardiovascular disease, neurodegeneration, lysosomal storage disease

Introduction

The endoplasmic reticulum (ER) is a cellular organelle in which folding of de novo synthesized proteins occurs. The ER plays an important role in homeostasis of proteins and calcium.[1] Ribosomes on rough ER synthesize proteins based on the genetic information transferred by mRNA. Smooth ER does not have ribosomes but has neighboring Golgi bodies. The rough ER folds nascent proteins and transports them to the Golgi. Protein homeostasis is tightly controlled by various cellular mechanisms, and failure or error of these quality control systems results in cellular dysfunction. The ER enters a stressed condition when nascent proteins are misfolded or unfolded and abnormally accumulate in the lumen of the ER, leading to failure to transfer to the Golgi. Upon sensing misfolded proteins, a series of cellular events known as unfolded protein response (UPR) or ER stress response is triggered in order to adapt to the cellular damage caused by ER stress. The UPR consists of canonical cellular processes[2] such as a decrease in translation to prevent further production of misfolded proteins, upregulation of chaperones to assist the folding process, ER-associated degradation (ERAD), and apoptosis. A successful rescue process can result in cell survival; a failed rescue can drive cells to apoptosis in order to reduce the risk of wasting precious amino acids and energy (Figure 1). ER stress is especially important in cells in which a high level of protein synthesis constantly occurs. In this context, insulin-secreting pancreatic β-cells and cancer cells could be more susceptible to ER stress than other cells.

ER stress is implicated in various diseases such as diabetes, β-cell apoptosis, diabetic neuropathy, inflammation, cardiovascular disease, neurodegeneration, and lysosomal storage diseases.[3–6] A number of studies have suggested an intriguing role of ER stress to induce β-cell apoptosis for initiation and maintenance of diabetes.[3,7] ER stress is detected

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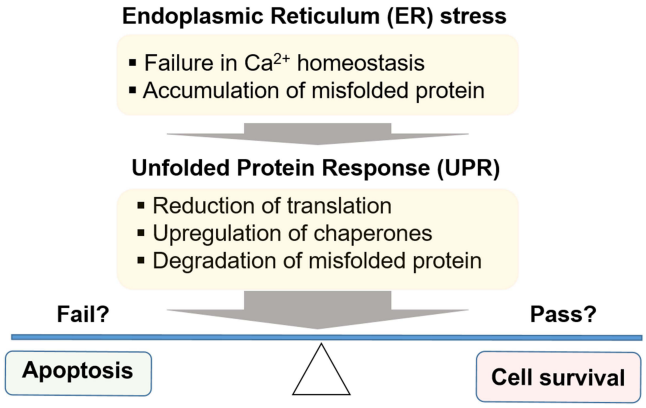

Figure 1 ER stress and UPR process to adapt stress condition: survival or cell death?

by three well-conserved ER-resident sensor molecules: protein kinase RNA-activated (PKR)-like ER kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring enzyme 1α (IRE1α).[8] These factors relay complex and interrelated downstream signaling pathways that decide cell 'survival' (adaptive signaling) or "suicide" (apoptotic signaling). The activated PERK dimerizes and phosphorylates eukaryotic translation initiation factor 2α (eIF2α), which attenuates protein synthesis and increases the expression of ATF4 to upregulate apoptosis-inducing factors such as C/EBP homology protein (CHOP). It is noteworthy that ATF4 in general relays an adaptive signal to upregulate genes that promote ER homeostasis and survival. ATF6 is also involved in activating the transcription of many UPR mediators including an ER chaperone protein, glucose-regulated protein (GRP78).[9] IRE1α processes X-box binding protein 1 (XBP1) mRNA to produce an active spliced form, which becomes a competent transcription factor for UPR-related genes. Chaperone molecules such as GRP78 (or BiP)[10] play an important role in UPR to aid proteins in achieving a functional folding conformation. It is believed that chaperones partially or fully bind to the unfolded protein polypeptides, preventing aggregation or incorrect folding.[11] Chemical versions of chaperones, chemical chaperones,[12] have been identified and implicated as potential treatments for ER stress-related pathologies. The best known examples of chemical chaperones are shown in Figure 2. 4-phenylbutyric acid (4-PBA, **1**) and tauro-ursodeoxycholic acid (t-UDCA, **2**) provided therapeutic value for several ER stress-related conditions such as type 2 diabetes (T2D). Both compounds protected against ER stress. They suppressed tunicamycin (Tm)-induced phosphorylation of PERK and eIF2α and JNK activation in cells. XBP-1 mRNA was also markedly reduced by both of them. Their reduction of ER stress and recovery of insulin sensitivity in animals were able to strongly support correlation between ER-stress and T2D, suggesting ER stress being pathological cause of T2D and therapeutic alternative.[13–15] Salubrinal (**3**) has also been found to protect cells from ER stress by protecting eIF2α from dephosphorylation, one of the hallmark events of UPR.[16] More recently, high throughput screening (HTS) cell-based assays have been developed to identify novel chemical chaperones[17–21] that prevent ER stress aggregation, proteotoxicity, and UPR. Consequently, several chemical chaperones such as compound **4** (IBT21) were identified.[17] In contrast to chaperones that reduce ER stress to rescue cells, there is a group of chemicals that interfere or inhibit chaperone salvaging activity and induce cell death. Representative examples

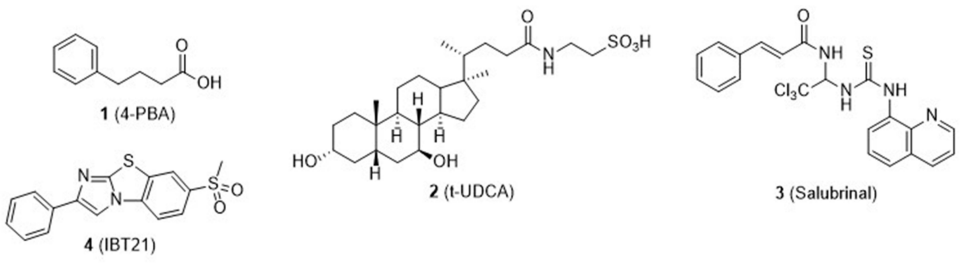

Figure 2 Structures of representative chemical chaperones found in literature. These compounds reduced ER stress and alleviated ER stress-related pathologies such as metabolic diseases (4-PBA, t-UDCA, and Salubrinal) and proteotoxicity induced by chemical (tunicamycin) and proteotoxin (mutant prion proteins) (IBT29).

of this class include heat shock protein (HSP) inhibitors, which inhibit chaperone activity of HSP70[22,23] and HSP 90,[24–26] for use as anticancer agents.[27,28]

In this review, chemical chaperones related to pathologies caused by ER stress are described along with their structures and activities. A major part of the review covers synthetic compounds and synthetic derivatives of natural compounds as summarized in Table 1. It should be noted that Table 1 also displays the chaperone activity of those compounds to regulate ER stress signaling molecules.

Table 1 Activity of Compounds as Chemical Chaperones in Various Pathologies

Cpd No	Common Name	Potential Therapeutic Area	Activity as Chemical Chaperone	EC_{50} (or IC_{50})	Ref
1	4-PBA	Diabetes, Anti-inflammatory	GRP78, p-PERK, p-IRE1 ATF6, CHOP, ATF4, p-eIF2alpha NF-kB ↓	IC_{50} = 63.2 μM	[13–15,58]
2	t-UDCA	Diabetes	p-PERK, p-IRE-1, p-c-Jun, p-IRS-1 ↓ recover insulin resistance blood glucose↓	*ob/ob* mouse, 500 mg/kg, P.O.	[13]
5	_	Diabetes	CHOP, ATF4, XBP1u +XBP1s, GRP94 ↓ STZ-induced diabetic mouse, 5 mg/kg, IP	EC_{50} = 32 nM β-cell protection Tm-induced INS-1 cell viability	[38]
6	_	Diabetes	Cleaved PARP Cleaved Caspase3 GRP94, ATF4, CHOP ↓	EC_{50} = 0.56 microM INS-1 cell viability. β-cell protection	[39]
7	Vildagliptin	Diabetes	DPP4 inhibitor, Bip, p-IRE1, p-PERK xBP-1s, p-eIF2alpha, CHOP mRNA ↓	IC_{50} = 2.3 nM	[40]
8	RH01687	Diabetes	CHOP mRNA ↓	EC_{50} = 8.1 μM	[20]
9	Telithromycin	Diabetes	CHOP mRNA ↓, protect β-cell	EC_{50} = 1.6 μM	[20]
10	1-HNA	Diabetes	p-PERK, p-IRE1 GRP78, CHOP, XBP1-s ↓	EC_{50} = 460 μM Tm-induced GRP78-driven reporter assay	[19]
11	3-HNA	Diabetes	p-PERK, p-eIF2alpha, p-IRE1 GRP78, CHOP, XBP1-s, p-JNK ↓	EC_{50} = 45 μM Tm-induced GRP78 reporter assay *ob/ob* mouse, 150 mg/kg, P.O.	[21]
12	KM04794	Diabetes	XBP1-s, BiP, Herpud1, ATF4 ↓ Enhances insulin production	EC_{50} = 9 ~16 μM Tm-induced UPRE-, AARE-, and ERSE-driven assay	[44]
14	Azoramide	Diabetes	CHOP, GRP78, ↓ Improve insulin secretion and survival in β-cells	EC_{50} = 9 ~16 μM Tm-induced assay *ob/ob* mouse	[45]
15	_	Diabetes	CHOP mRNA, cleaved PARP, cleaved caspase 3 ↓ blood glucose ↓	EC_{50} = 2.8 μM CHOP reporter assay. STZ-induced diabetic mouse	[46]
16	Berberine	Anti-inflammatory, Hypoglycemic activity, Alzheimer's disease, Anti-atherosclerosis	FNF-alpha, IL-6, IL-1beta MCP-1, CHOP, ATF4, XBP-1s ↓	IC_{50} = 6.6 μM	[55]
19	Ar9273	Anti-inflammation sEH inhibitor Gastrointestinal diseases	IL-6, TNF-α, CHOP TRB3, IL-1beta mRNA, MCP-1 mRNA, ATF3 ↓	IC_{50} = 197 nM	[62]
20	Diflunisal	Anti-inflammation, NSAIDs	p-PERK, p-JNK ↓	EC_{50} = 58 μM Tm-induced GRP78 reporter assay	[63]
22	Valsartan	Angiotensin receptor blocker	caspase 3, GRP78, PERK, IRE1-alpha, ATF-6, eIF2α, ATF-4, CHOP ↓	IC_{50} = 2.7 nM	[68]

(*Continued*)

Table 1 (Continued).

Cpd No	Common Name	Potential Therapeutic Area	Activity as Chemical Chaperone	EC_{50} (or IC_{50})	Ref
23	Metformin	Diabetes (T2D) drug cardioprotection	p-IRE1α, p-PERK ATF6, GRP78 ↓	EC_{50} = 8.6 nM	[70]
24	Guanabenz	α2-adrenergic agonist Antihypertension Antiprion	p-eIF2α, ATF-4, BIP GRP94, CHOP	IC_{50} = 4.85 mM	[73,89]
25	Fasudil	Rho-kinase inhibitor Neurodegenerative Diseases	GRP78 BMPR2 ↓	IC_{50} = 10.7 mM	[81]
28	_	IRE1α kinase Inhibitor Rhodopsin protection	p-IRE1α, m-RNA splicing	IC_{50} = 160 nM	[91]

Type 2 Diabetes (T2D)

One of the key features of T2D is increased insulin resistance. A high incidence of T2D is observed in the obese population whose elevated level of free fatty acids is attributed to the pathology. Although the precise mechanism is unclear, presence of free fatty acids in obesity patients is a well-established risk factor of ER stress and insulin resistance.[10,29–33] These fatty acids cause the accumulation of misfolded proteins in the ER and induce UPR, leading to insulin resistance (Figure 3).

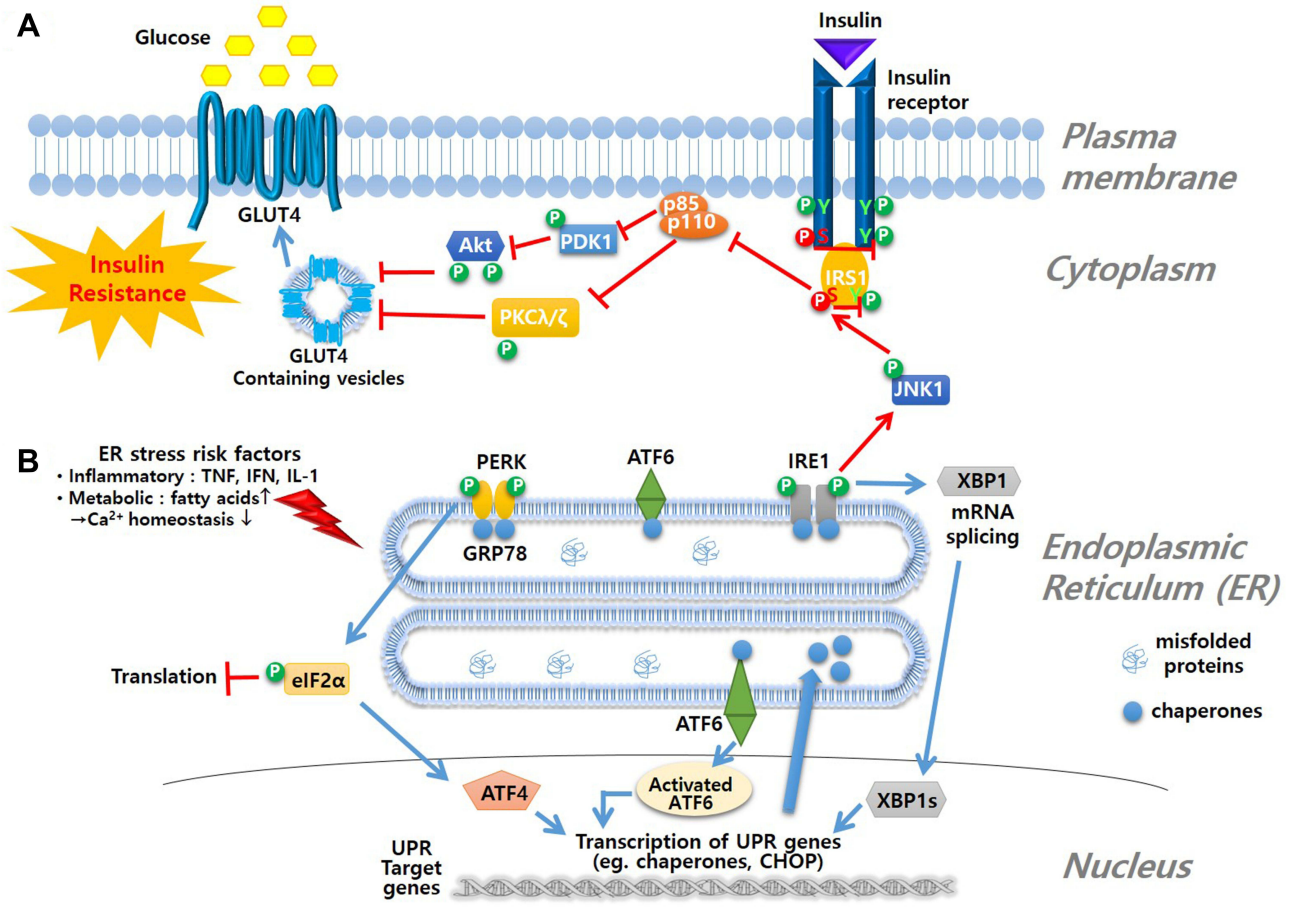

Figure 3 Mechanism of ER stress and insulin resistance. (**A**) insulin signal pathway is blocked due to ER stress-induced phosphorylation of JNK1 followed by phosphorylation of serine moiety of IRS1 to inhibit glucose influx as a result (**B**) ER stress is triggered by various risk factors that interrupt Ca^{2+} homeostasis. Accumulation of misfolded proteins in lumen of ER induces ER-resident membrane sensor molecules, such as PERK, ATF6 and IRE1, to initiate the UPR signal starting with dimerization and autophosphorylation of the sensing molecules. UPR signals lead to inhibition of translation, activation of UPR genes such as chaperones and CHOP, or degradation of misfolded protein by ER-associated protein degradation (ERAD) process.

Under normal conditions, binding of insulin to its receptor initiates a signaling pathway via autophosphorylation of tyrosine residues of the receptor and consequent phosphorylation of tyrosine residues of the insulin receptor substrate (IRS1). These initial events result in relocation of cytosolic glucose transporter 4 (GLUT4) to the cell membrane where it transports plasma glucose into cells through complex signaling. Under ER stress, however, IRE1 phosphorylation caused by UPR induces phosphorylation of serine moieties of IRS1 via c-Jun N-terminal kinase 1 (JNK1) phosphorylation, which inhibits the phosphorylation of tyrosine residues of IRS1 (IRE1-JNK-IRS signaling axis). This sequence leads to blockage of the signaling pathway, causing failure of glucose influx, namely insulin resistance (Figure 3A). With increased insulin resistance, pancreatic β-cells produce additional insulin beyond the folding capacity of the ER, leading to ER stress. The pancreatic β-cell function is diminished in the T1D condition mainly via an autoimmune process, but ER stress is also implicated in deterioration of β-cell function, and some chemical chaperones have been shown to protect β-cells.[8,14,34,35] There is a group of ER stress reducing chemicals that have shown anti-diabetic indications such as protection against insulin resistance and of β-cells (Figure 3).

Figure 4 lists compounds with chaperone activity as well as anti-diabetic activity. Salubrinal (**3**) is a well-known ER stress inhibitor and eIF2α-dephosphorylation inhibitor[16] and extends inhibition of translation. However, its activity is controversial and cell-type dependent. It was reported that **1** protects pheochromocytoma PC12 cells against ER stress-induced apoptosis but triggers apoptosis in pancreatic β-cells.[27,36] Duan et al synthesized a series of benzamide derivatives and screened for potential ER stress inhibiting activity.[37] Of the derivatives, a benzamide **5** was found to have EC_{50} = 32 nM in protecting INS-1 cells from Tm-induced ER stress. **5** showed remarkable activity to protect β-cells from Tm-induced ER stress and downregulated ER stress markers including ATF4, CHOP, XBP1s, and BiP. In addition, **5** significantly lowered blood glucose and increased β-cell survival in a streptozotocin (STZ)-induced diabetic mouse model. Another report by that group disclosed a 2,4-diaminoquinazoline **6** with an EC_{50} value in the micromolar range. **6** also downregulated ER stress markers such as ATF4, CHOP, XBP1s, and BiP and cleaved PARP and caspase 3.[38] Compound **7** (Vildagliptin) is a marketed drug that acts as a dipeptidyl peptidase-4 (DPP-4) inhibitor in diabetes. It

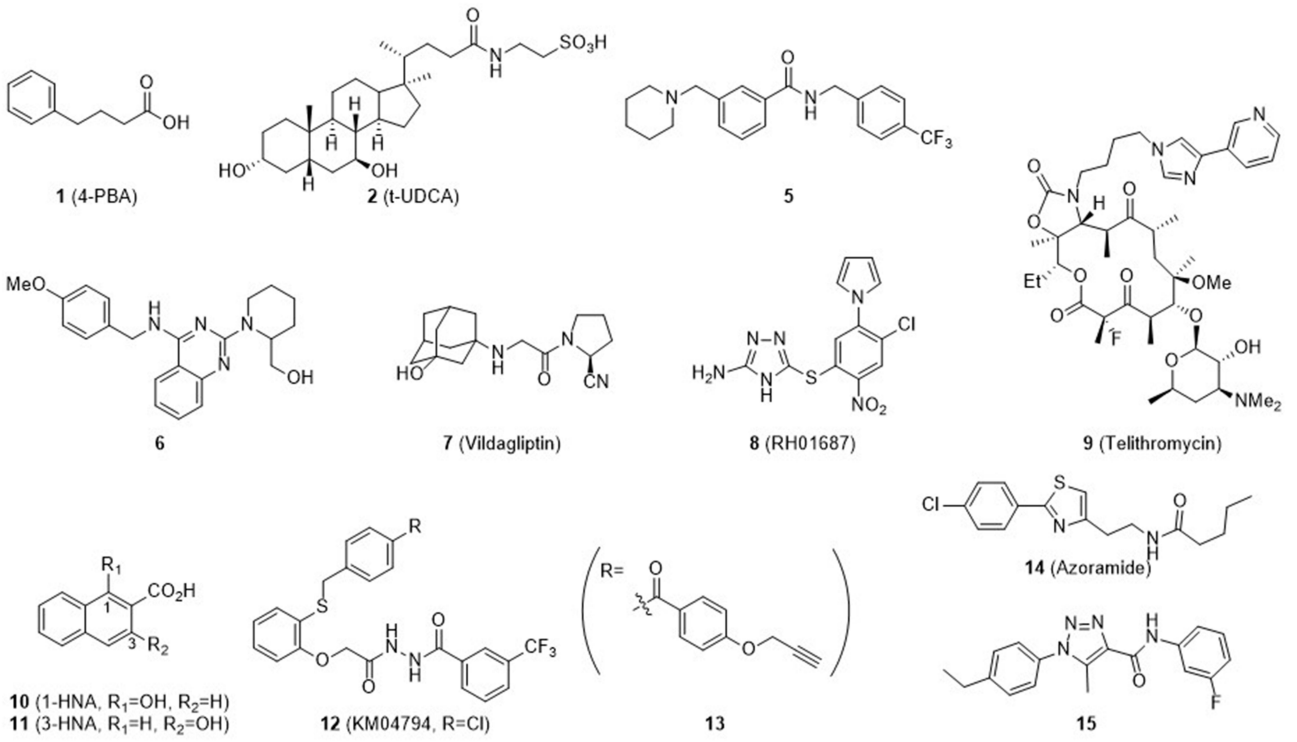

Figure 4 Chemical structures of compounds that attenuated ER stress as chemical chaperone and showed anti-diabetic activity. Many of them demonstrated anti-diabetic activity in vivo (4-PBA, t-UDCA, Azoramide, compound 5, 3-HNA, KM04794, compound 15). Note that Vildagliptin (7), a marketed anti-diabetic drug and DPP-4 inhibitor, showed chaperone activity to reduce ER stress.

inhibits the DPP-4 enzyme, increases GLP-1 activity, and stimulates insulin release. Thapsigargin (Tg)-induced ER stress in the liver was alleviated by Vildagliptin.[39] Chemical chaperones that protect pancreatic β-cells from ER stress-induced apoptosis were searched through an HTS campaign based on the viability of the mouse insulinoma β cell line (βTC6) upon treatment with Tm. Of 17,600 compounds screened, **8** (RH01687) and **9** (Telithromycin) were identified as active hits.[20] Both compounds modulated ER stress induced by Tm and protected β-cells, with EC_{50} = 8.1 and 1.6 μM, respectively. Telithromycin (**9**) is an erythromycin analogue antibiotics known to be an effective treatment for pneumonia.[40] Another HTS assay consists of the rLuc reporter driven by the human GRP78 promoter harboring three consecutive ER stress response elements. Using the cell-based assay system, Jeong et al identified a series of hydroxynaphthoic acids as chemical chaperones.[19] Compound **10** (1-hydroxy-3-naphthoic acid, 1-HNA) inhibited Tm- and palmitate-induced ER stress, with EC_{50} = 460 μM and 60 μM, respectively. ER stress markers such as p-PERK, p-IRE1, GRP78, CHOP, and XBP1-s were decreased accordingly. The anti-diabetic activity of compound **11** (3-HNA) was further studied by Park et al.[21] The compound showed Tm- and palmitate-induced ER stress inhibition, with EC_{50} = 570 μM and 45 μM, respectively, and downregulated ER stress markers. Oral administration of **11** (150 mg/kg) to ob/ob mice resulted in resolution of insulin resistance and protection of β-cells from apoptosis induced by glucolipotoxicity. t-UDCA (**2**) is a bile acid that has proven to have medicinal effects in T2D, various heart diseases, and neurodegenerative diseases (Alzheimer's and Amyotrophic lateral sclerosis (ALS)).[41–43] Compounds **1** (4-PBA) and **2** (t-UDCA) showed anti-diabetic activities such as i) recovery of insulin sensitivity and ii) decreases in blood glucose and insulin secretion levels after oral administration to ob/ob mice at a dose of 500 mg/kg.[13–15]

Miyake et al established a series of cell-based assays to identify ER stress inhibitors.[44] They used ER stress response element (ERSE)-, unfolded protein response element (UPRE)-, and amino acid-response element (AARE)-based assay systems for parallel monitoring of three major ER stress signaling pathways: IRE1, PERK, and ATF6 pathways. Compound **12** (KM04794) was identified as an ER proteostasis modulator that inhibited UPR signaling caused by ER stress induced by diverse chemicals. KM04794 (**12**) alleviated protein aggregation and enhanced insulin production in pancreatic β-cells. In addition, compound **13** was prepared by replacing the terminal 4-chlorophenyl group of the parent compound, **12**, with a pull-down moiety. Benzophenone and propynyl groups were tethered onto **13** as a photoaffinity tag and click reaction tag, respectively. Using **12** as a competitor molecule, a pull-down experiment was carried out and revealed BiP as the most probable binding partner of compound **13**. BiP is one of the most important chaperones and seems to directly bind to **12** to produce an improvement of ER proteostasis. Another HTS assay consisting of cLuc activity driven by ATF6α identified **14** (Azoramide) as a modulator of UPR with anti-diabetic activity.[45] **14** decreased CHOP and GRP78 under Tm-induced ER stress. In ob/ob mice and high fat diet-induced obese mice, administration of **14** improved insulin secretion and survival of β-cells. Duan et al reported a 1,2,3-triazole derivative (**15**) that protected pancreatic β cells against endoplasmic reticulum stress-mediated dysfunction and death through inhibition of C/EBP-homologous protein expression.[46]

Inflammatory Diseases[92]

As shown in Figure 5, there are a number of compounds that possess chaperone activity as well as anti-inflammatory activity. Compound **16** (Berberine) is a naturally occurring compound that has various physiological effects such as hypoglycemic, anti-microbial, anti-inflammatory, anti-Alzheimer, and anti-atherosclerosis activities.[47–52] Among its derivatives, **17** was reported to have anti-breast cancer activity, which also displayed ER stress inhibition.[53] Study of ER stress reduction on mitochondrial dysfunction and inflammation have been reported.[54] Berberine (**16**) inhibited palmitate (PA)- and lipopolysaccharide (LPS)–induced inflammation through modulation of ER stress.[55]

Compound **1** (4-PBA) is an inhibitor of histone deacetylase (HDAC),[56] which is involved in various diseases such as metabolic syndrome (obesity, T2D), misfolding diseases (cystic fibrosis), inflammatory disorder (diabetic nephropathy), neurological disorder (Parkinson's), tissue diseases (fibrosis), and cancers (gastric carcinoma, colon cancer). HDAC is also known to inhibit platelet aggregation and is associated with β-globin disorders.[57] ER stress suppression was examined in male C57BL/6J mouse hyperoxia-exposed lung epithelial cells. Hyperoxia-induced lung injury was attenuated by treatment with 4-PBA (**1**), which modulated inflammation-related markers (IkB-α, NF-kB).[58] Soluble epoxide hydrogenase (sEH) converts epoxyeicosatrienoic acid (EET) to dihydroxytrienoic acids (DiHETrES), which

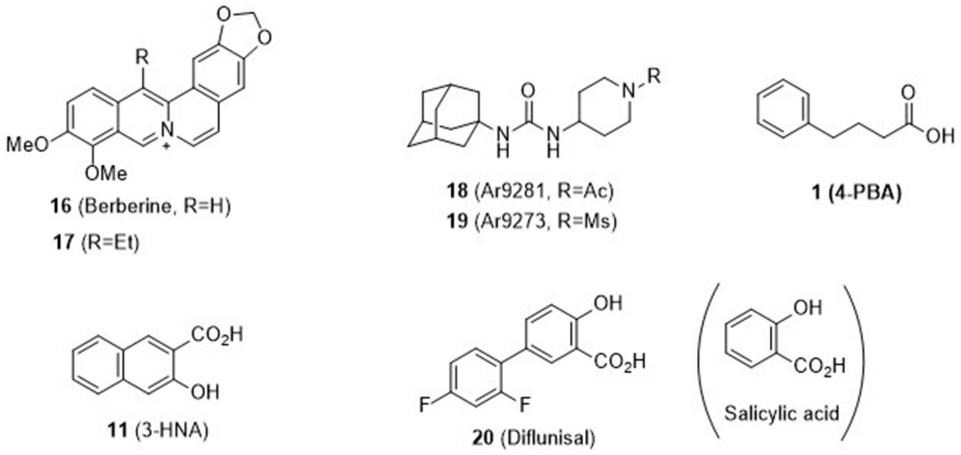

Figure 5 Chemical chaperones to relieve the ER stress with anti-inflammatory activity.

triggers the inflammation cascade.[59] Compound **18** (Ar9281)[60,61] has a potent IC_{50} = 8.0 nM against sEH; however, due to its low water solubility, a more hydrophilic analogue was synthesized (**19**, AR9273). Although the IC_{50} of **19** was lower than that of **18** (197 nM), water solubility, microsomal stability, sEH enzyme selectivity, and blood-brain barrier (BBB) penetration were improved. ER stress reduction was observed in the Ce (cerulean)-induced pancreatitis AR42J cell model.[62] In a continuing study on salicylate-related ER stress chaperones, our group found that 3-HNA (**11**), which contains a naphthoic acid scaffold, improves glucose lowering activity through ER stress amelioration.[21] Compared to the parent salicylate (EC_{50}=5.07 mM),[19] a standard anti-inflammatory drug, extended aromaticity provided by the naphthalene moiety of 3-HNA (**11**) was credited with its remarkable anti-ER stress activity (EC_{50}=0.57 mM).[21] In addition, salicylate analogues with a biphenyl scaffold were synthesized. Of these, **20** (Diflunisal), an anti-inflammatory drug with a biphenyl ring harboring a fluorine substituent, exhibited the best anti-ER stress activity. The EC_{50} value (EC_{50} = 58 μM) of Diflunisal (**20**)[63] was approximately 6- and 90-fold higher than 3-HNA (**11**) (EC_{50} = 328 μM) and t-UDCA (**2**) (EC_{50} = 5.2 mM),[64] respectively. Moreover, Diflunisal (**20**) ameliorated palmitate-induced ER stress and decreased UPR markers.[63]

Cardiovascular Diseases[65,66]

Chemical chaperones that inhibit ER stress and provide cardioprotective activity are shown in Figure 6. Doxorubicin (Dox) is an anticancer drug, but it induces cardiotoxicity, resulting in apoptosis of cardiomyocytes, which has been

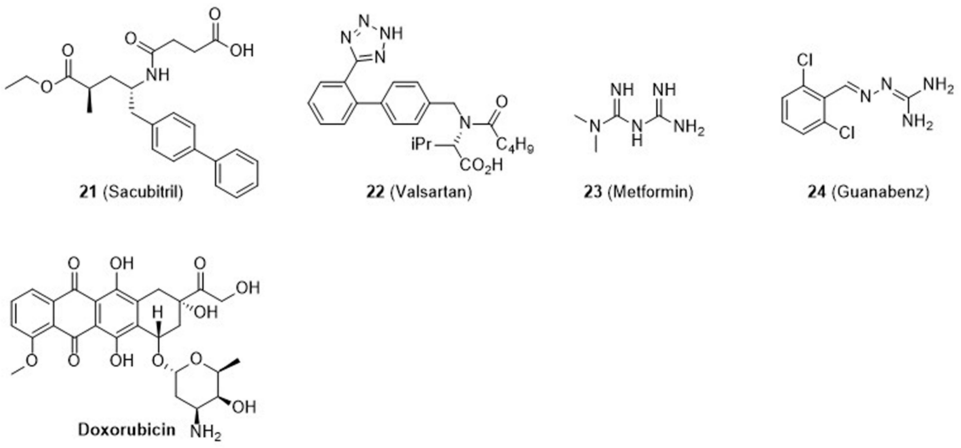

Figure 6 Chemical chaperones to relieve the ER stress with cardioprotection activity.

attributed to ER stress.[66,67] Compounds **21** (Sacubitril) and **22** (Valsartan) have been used as a combination therapy for treatment of heart failure. Sacubitril is a prodrug and a neprilysin inhibitor, and valsartan is an angiotensin II receptor blocker (ARB). Administration of the Sac/Val combination suppressed ER stress induced by treatment with Dox; UPR markers caspase 3, GRP78, PERK, IRE1α, ATF-6, eIF2α, ATF-4, and CHOP were all decreased.[68] Another study investigated the anti-ER stress activity of Valsartan (**22**) in tubular epithelial cells of diabetic cardiomyopathy rats.[69]

Compound **23** (Metformin) is effective in T2D treatment, especially when co-administered with sulfonylureas, and is especially beneficial for obese diabetic patients. It has also been shown to be effective in the treatment of cardiovascular disease (CVD).[70] The cardioprotective effect of Metformin (**23**) through ER stress reduction on human coronary artery endothelial cells (HCAEC) has been reported. Metformin (**23**) suppressed Tm- and high dextrose-induced ER stress by regulating p-IRE1α, p-PERK, and ATF6 activities, leading to cardioprotection.[70] Compound **24** (Guanabenz) is an α-2 adrenergic receptor agonist that is used as an antihypertensive drug. Albeit somewhat controversial, it is believed that Guanabenz (**24**) interferes with dephosphorylation of elF2α-P' by disrupting the PPPIR15-PP1 complex.[71,72] Guanabenz (**24**) did not show any noticeable effect alone, but when used with ER stress inducer (tunicamycin), it provided lower levels of ER stress markers such as p-eIF2α, ATF4, BiP, GRP94, and CHOP and eventual cell protection.[73]

Neurodegeneration and Neuroprotection[74,75]

Figure 7 shows chemical chaperones that possess neuroprotective activity and anti-neurodegenerative activity. Compound **25** (Fasudil), a Rho-kinase inhibitor, is used as a treatment for cerebral vasospasm, pulmonary hypertension, cardiovascular diseases, age-related neurodegenerative memory loss, corneal neovascularization, and other conditions.[75–80] It improves motor function and has been approved in Japan for treatment of cerebral vasospasm following subarachnoid hemorrhage.[81] Several ER stress modulating functions of Fasudil (**25**) have been reported; i) ischemia/reperfusion injury through SERCA activity,[82] ii) inhibition of vascular cellular adhesion molecule (VCAM-1) expression by modulating UPR,[83] and iii) inhibition of leukocyte-endothelial interaction through modulation of GRP78 and BMPR2 expression.[84] Compound **26** is an HSP70 agonist showing chaperone activity. It produced a clear reduction in α-synuclein aggregation in neuroglioma cells, which is a hallmark of Parkinson's disease.[85–87] Oxindole **27** suppressed protein aggregate accumulation in vitro and in hippocampal HT22 neuronal cells and prevented ER stress-induced cell death as a chemical chaperone.[88] Guanabenz (**24**), a antihypertensive drug, promoted ovine PrPsc clearance in a cell-based assay, increasing the survival of treated mice significantly. Interestingly, other α2-adrenergic agonists did not show such anti-prion activity.[89]

Miscellaneous Diseases

In addition to the diseases discussed above, ER stress is involved in other pathologies, and compounds with chaperone activity have been developed and characterized (Figure 8). It would be rather appropriate to consider IRE1 kinase as a core UPR component whose inhibitors can make a huge subset collection of kinase inhibitors. It will be beyond the scope of this review to describe the vast list of IRE1 kinase inhibitors. We here would like to provide a few examples of IRE1 kinase inhibitors that showed ER stress inhibition. Inhibitors of IRE1α kinase, a major signaling molecule of UPR, were developed to treat neurodegenerative cancer, diabetes, lipidemia, and inflammatory diseases.[8,90] Compound **28** was

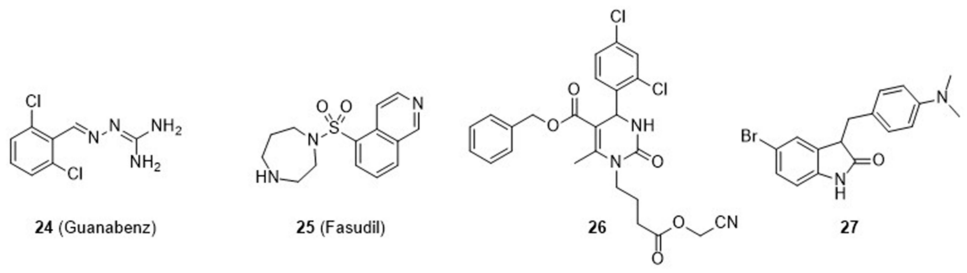

Figure 7 Chemical chaperones to inhibit ER stress with neuroprotection activity.

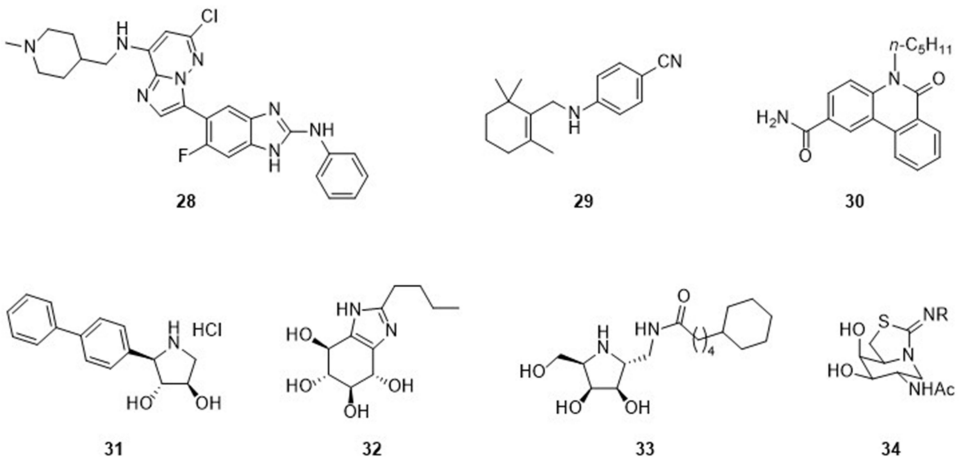

Figure 8 Chemical chaperones to inhibit ER stress with therapeutic implications in other diseases.

discovered through docking studies and has IC_{50} = 160 nM and 80 nM against IRE1α kinase and IRE1α RNAse, respectively. It is highly selective for IRE1α kinase over the IRE1β isoform, although interference of kinase activity by compound **28** was marginal in a kinome assay.[91] ER stress can occur in photoreceptors as well. Ocular protein conformational diseases such as retinitis pigmentosa can be caused by misfolded or mistrafficked rhodopsin, a complex of opsin protein and retinal, which aggregate and accumulate in the ER, leading to photoreceptor cell death. Compound **29** binds to opsin, a visual pigment, as a chemical chaperone, assisting its proper folding and trafficking to the outer cell membrane and preventing the loss of photoreceptors under ER stress conditions.[93]

Chemical chaperones have further been implicated in lysosomal storage disorders such as Gaucher disease,[94] Fabry disease, and Tay-Sachs disease, in which trafficking of proteins or lipids is disrupted. Compound 25 was synthesized and tested in Niemann-Pick disease type C1[95] (NPC1), a disease characterized by abnormal accumulation of lipids and cholesterol in lysosomal and late endosomal compartments. Compound **30** alleviated the folding defect of the NPC1 protein I1061T mutant, resulting in transport of misfolded mutant NPC1 to late endosomes similar to normal NPC1. Compound **31** is an imino sugar analogue that inhibited β-glucosidase or β-glucocerebrosidase (GCase) that cleaves glucose from gluco(syl)ceramide (sphingolipid). Defects of this enzyme cause accumulation of glucoceramide, the genesis of Gaucher disease. Compound **31**-assisted folding of mutant enzyme prevents its degradation by the ER proteostasis function.[96] Compound **32** also strongly inhibited β-glucosidase.[97] Similarly, a defect in lysosomal α-galactosidase A (α-Gal A) causes Fabry disease due to accumulation of neutral glycosphingolipids bearing a terminal α-galactosyl residue. Compound **33** showed a chaperone effect for several α-Gal A mutants in COS7 cells and lymphocytes of the N215S Fabry patient-derived cell line.[98]

In the late-onset form of Tay-Sachs disease, defects were found in *N*-acetyl-β-hexosaminidase A (HexA) that catalyzes the removal of terminal, non-reducing *N*-acetyl-β-D-glucosamine (GlcNAc) or galactosamine (GalNAc). Mutational defects impair folding of the enzyme, resulting in its normal trafficking to lysosome. Compound **34** was found to be a competitive inhibitor of HexA and rescued disease-causing mutant HexA, showing potential as a chemical chaperone. It increased the activity of lysosomal HexA in Tay-Sachs patient fibroblasts containing the G269S mutation, the most prevalent mutation in late-onset Tay-Sachs disease.[99]

Conclusion and Perspectives

ER stress is implicated in various diseases and pathologies. We listed in this review chemical versions of chaperone molecules and categorized them into potential therapeutic areas such as diabetes, inflammatory diseases, cardiovascular diseases, and neurodegeneration. In addition, lysosomal storage diseases have shed new light on the therapeutic value of chemical chaperones. Many compounds with chaperone activity have shown therapeutic potential in rescuing misfolded proteins caused by ER stress or genetic defects. Various HTS assays are currently available, which may inspire

researchers to conduct phenotypic screening using large volume chemical libraries. Target-based medicinal chemistry efforts can also be undertaken against specific targets. ER stress sensing molecules such as PERK inhibitors have been developed and examined clinically.[100,101] Recent studies have shown that PERK signaling plays a critical role in immunosuppression in macrophages, opening a new era into the role of chemical chaperone inhibitors in the immune system.[102] It was also reported that STING-PERK signaling can be an alternative innate immune pathway, which plays a critical role in fibrotic diseases.[103] Other prevalent targets of chemical chaperones include HSP's as mentioned in this review. Inhibitors of HSP's have mostly been pursued for their therapeutic value as anticancer agents. Targets including GRP78 or BiP could also be novel candidates. BiP was identified as a direct binding partner of a chemical chaperone during a target identification effort using the pull-down probe, compound **13**. An increasing number of chemical chaperones is being developed, and we hope this review and concise list of currently identified structures provides structural inspiration for new chemical chaperones.

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Disclosure

The authors report no conflicts of interest in this work.

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