Acta Pharmaceutica Sinica B 2021;11(10):2995–3014

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

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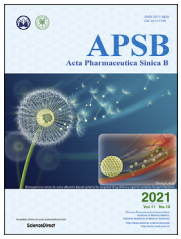

REVIEW

Natural compounds in the regulation of proteostatic pathways: An invincible artillery against stress, ageing, and diseases

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Received 2 September 2020; received in revised form 12 October 2020; accepted 3 November 2020

KEY WORDS

Proteostasis;
Proteinopathies;
Chaperones;
Ubiquitin proteasome system;
Autophagy;
Cancer;
Neurodegeneration;
Ageing;
Natural molecules;
Drug discovery

Abstract Cells have different sets of molecules for performing an array of physiological functions. Nucleic acids have stored and carried the information throughout evolution, whereas proteins have been attributed to performing most of the cellular functions. To perform these functions, proteins need to have a unique conformation and a definite lifespan. These attributes are achieved by a highly coordinated protein quality control (PQC) system comprising chaperones to fold the proteins in a proper three-dimensional structure, ubiquitin-proteasome system for selective degradation of proteins, and autophagy for bulk clearance of cell debris. Many kinds of stresses and perturbations may lead to the weakening of these protective cellular machinery, leading to the unfolding and aggregation of cellular proteins and the occurrence of numerous pathological conditions. However, modulating the expression and functional efficiency of molecular chaperones, E3 ubiquitin ligases, and autophagic proteins may diminish cellular proteotoxic load and mitigate various pathological effects. Natural medicine and small molecule-based therapies have been well-documented for their effectiveness in modulating these pathways and reestablishing the lost proteostasis inside the cells to combat disease conditions. The present article summarizes various similar reports and highlights the importance of the molecules obtained from natural sources in disease therapeutics.

Abbreviations: 17-AAG, 17-allylamino-geldanamycin; APC, anaphase-promoting complex; BAG, BCL2-associated athanogene; CAP, chaperone-assisted proteasomal degradation; CASA, chaperone-assisted selective autophagy; CMA, chaperone-mediated autophagy; CHIP, carboxy-terminus of HSC70 interacting protein; DUBs, deubiquitinases; EGCG, epigallocatechin-3-gallate; ESCRT, endosomal sorting complexes required for transport; HECT, homologous to the E6-AP carboxyl terminus; HSC70, heat shock cognate 70; HSF1, heat shock factor 1; HSP, heat shock protein; KFERQ, lysine-phenylalanine-glutamate-arginine-glutamine; LAMP2a, lysosome-associated membrane protein 2a; LC3, light chain 3; NBR1, next to BRCA1 gene 1; PQC, protein quality control; RING, really interesting new gene; Ub, ubiquitin; UPS, ubiquitin–proteasome system.

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Peer review under responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2021.01.006

1. Introduction

A eukaryotic cell represents a well-evolved architecture, made up of many small components working independently and coherently. A highly efficient and coordinated way of functioning of multiple subsystems towards the fitness and survival of individual cells and the organism is a highly complex biological phenomenon. It remains a great challenge to understand the intricacies and complexities of the living systems. For a very long period, the central dogma, *i.e.*, the idea of sequential flow of information from DNA to RNA, followed by its retrieval into the form of proteins, remained a mystery for the scientists. However, the improvements in the techniques and adaptations of the newer approaches to decipher the molecular arrangements have led to a higher understanding of the fine details of the cells' structure and functional arrangements. The involvement of biochemical and molecular biology approaches has led to the deduction of most of the metabolic pathways and their subsequent impact on the cellular physiology. At the same time, structural and computational biologists have played a critical role in providing crucial insights about the mysteries of the genetic codes, amino acid sequences, and structural plans of the proteins. Many other tools have also helped in devising various ways of visualizing and interpreting the intermolecular interactions involved in different cellular pathways and mechanisms.

With all the advancements and our current understanding of cellular architecture, we can believe that a functional proteome is a prerequisite for regulating the essential physiological pathways and maintaining good cellular health[1]. To preserve an advantageous proteome, the cells have a well-developed protein quality control (PQC) machinery that ensures a healthy set of protein repertoires to execute all the necessary cellular tasks[2,3]. First, it helps the nascent polypeptide chains to attain their native conformations. Second, it identifies any dysfunctional or aberrant protein that remains present into our cellular milieu; and third, it removes all such unwanted proteins from the system. However, under certain kinds of stress conditions, their inefficiency may lead to the generation of unwanted proteinaceous species inside the cytoplasm, leading to misfolding and aggregation[4]. These events of failure of proteostasis and formation of large molecular weight aggregates or cytoplasmic inclusion bodies underlie the causal mechanism behind several systemic and non-systemic diseases, as summarized in Table 1.

In the past many years, substantial efforts have been made to affect the functional efficiency of many components of the PQC systems to establish and maintain the homeostatic conditions inside cells[5,6]. Small molecules obtained from plants and other natural sources may have diverse medicinal values, as they can modulate many cellular proteins and affect several associated signaling pathways[7,8]. The primary sources of these molecules of immense medicinal values include bacterial or fungal isolates, extracts of marine animals or plant sources, and few specific mammalian tissue secretions, etc. The upcoming sections describe the significance of these crucial cellular subsystems in regulating multiple molecular networks. The article further provides a brief overview of the available reports describing various naturally-derived molecules with the proposed medicinal values.

2. Cellular protein quality control system

A battery of multifaceted enzymes is involved in the replication and transcriptional processes, exhibiting highly efficient proofreading activity to preserve the genomic contents of the cell[9−11]. Similarly, in association with an array of extremely proficient molecular chaperones, a well-organized ribosomal quality control (RQC) machinery maintains the robustness of the cellular proteome[12−14]. Additionally, a specialized pathway of quality assurance of newly synthesized polypeptides (called ERAD) operates inside the endoplasmic reticulum and associated secretory pathways[15]. Several molecular chaperones and additional proteins get involved in these QC pathways, regulating the folding and degradation processes inside the cells and maintain a healthy and functional cellular proteome[16,17]. All the cellular proteins have their unique turnover rate regulated by the ubiquitin−proteasome system (UPS) that involves a few hundred E3 ubiquitin ligase enzymes to provide the substrate specificity[18,19].

Under some physiological conditions, the E3 ubiquitin ligases, along with few other adapter proteins, may take part in identifying and redirecting aberrant or aggregated forms of intracellular proteins to another proteolytic pathway, called autophagy, which is not as specific as UPS and is chiefly take part in the degradation of the bulk of cellular debris[20,21]. Similarly, heat shock proteins (HSPs) or molecular chaperones also play crucial roles in the triage of polypeptides inside the cytoplasm by switching among different quality control pathways. Here, we are providing a very brief outline of these major QC pathways in this section. An intracellular overview of these significant components of the cellular QC pathways is presented in Fig. 1.

2.1. *Molecular chaperones*

Proteins are large (macro-) molecules inside the cells, which orchestrate most of the physiological and metabolic tasks and are inclusively involved in the structural organization of the cellular components. Therefore, the maintenance of their native conformations is a prerequisite for the cells to be healthy. Such a condition of a stable and healthy set of proteins is called proteostasis[22,23]. Chaperones are the first line of molecules that start their work immediately after the newly synthesized peptide exits from the ribosome[24]. Different classes of molecular chaperones have already been reported in various forms of life across different kingdoms, including prokaryotes and eukaryotes[25,26]. The *de novo* folding of nascent polypeptides is orchestrated by family chaperones and is accomplished by multiple cycles of 'binding and release' in an energy-dependent manner[27,28]. Folding of a proportion of proteins is governed by HSP70 and HSP40,

Table 1 Major amyloidosis and the associated aggregatory proteins. The table summarizes major systemic and non-systemic diseases associated with misfolding and aggregation of certain proteins.

Disease/pathological condition	Proteins misfolded/aggregated	Ref.
Alzheimer's disease	Amyloid β42, Tau protein	1,2
Amyotrophic lateral sclerosis	SOD1, TDP43,	3,4
Atrial amyloidosis	Atrial natriuretic factor	5
BriPP amyloidosis	Amyloid-Bri	6
Cancer	Tumor protein 53	7
Cataracts	Crystallins	8
Creutzfeldt Jakob disease	Prion	9
Cystic fibrosis	CFTR	10
Diabetes	Amylin, IAPP	11,12
Familial amyloid polyneuropathy I	Transthyretin	13
Familial amyloid polyneuropathy III	Apolipoprotein AI	14
Familial hypercholesterolemia	LDL receptor	15
Fibrinogen α-chain amyloidosis	Fibrinogen α-chain variants	16
Finnish hereditary systemic amyloidosis	Gelsolin	17
Fronto-temporal dementias	Tau protein	18
Haemodialysis-related amyloidosis	β2-Microglobulin	19
Hereditary cerebral amyloid angiopathy	Cystatin C	20
Lysozyme-related amyloidosis	Lysozyme	21
Hereditary renal amyloidosis	Fibrinogen α-A chain, lysozyme	16,22
Huntington's disease	Huntingtin	23
IBMPFD	Valosin-containing protein	24
Insulin-related amyloidopathy	Insulin	25
Leprechaunism	Insulin receptor	26
Marfan syndrome	Fibrillin	27
Medullary carcinoma of the thyroid	Calcitonin	28
Osteogenesis imperfecta	Type I procollagen pro α	29
Parkinson's disease/Lewy body dementia	α-Synuclein	30
Primary systemic amyloidosis	Ig light chains	31
Retinitis pigmentosa	Rhodopsin	32
Scrapie	Prion protein	33
Secondary systemic amyloidosis	Serum amyloid A	34
Senile systemic amyloidosis	Transthyretin	35
Spinal and bulbar muscular atrophy	Androgen receptor	36
Spinocerebellar ataxias	Ataxin proteins	37
Spinocerebellar ataxia 17	TATA box-binding protein	38
Spongiform encephalopathies	Prion protein	39
Tay-Sachs disease	β-Hexosaminidase	40
α1-Antitrypsin deficiency	α1-Antitrypsin	41

CFTR, cystic fibrosis transmembrane receptor; IBMPFD, inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia; IAPP, islet amyloid polypeptide.

whereas the rests of the proteins are transferred to HSP90 proteins[29].

These chaperones are also implicated in refolding and disaggregating aberrantly folded polypeptides, or unfolding and degrading aggregated proteins[30,31]. In fact, a large number of chaperones and chaperonins are coherently involved in the folding, refolding, and disaggregation processes of all the cellular proteins[32,33]. Chaperones can guide the substrate proteins towards two well-established systems of proteins degradation, *i.e.*, UPS and autophagy[34]. They may interact with crucial proteins implicated in these two pathways, *e.g.*, sequestosome-1 (SQSTM1/P62), BCL2 associated athanogene 1 or 3 (BAG1/3), carboxy-terminus of heat shock cognate 70 (HSC70) interacting protein (CHIP), next to BRCA1 gene 1 (NBR1), and several E3 ubiquitin ligases[35,36]. The mechanisms that are driven by chaperones in concerted action with the other pathways are chaperone-mediated autophagy (CMA), chaperone-assisted selective autophagy (CASA), and chaperone-assisted proteasomal degradation (CAP)[37,38].

2.2. *Autophagy*

The idea of autophagy originated in the 1960s when Christian de Duve identified lysosome, an organelle that contains hydrolytic enzymes, and got involved in removing cytoplasmic waste materials[39,40]. Nobel Prize in Medicine to Christian De Duve in 1974, and Yoshinori Ohsumi in 2014 for the discovery of the lysosome and detailed investigation of this degradation pathway confirm the importance of the autophagy machinery for the cells. This lysosomal degradation process targets not only the damaged organelles but also different forms of cellular proteins, either ubiquitylated or non-ubiquitylated[41,42]. Multiple lysosomal degradation pathways have been identified in the past with different roles and specificities; for example, the formation of a double-membrane bound structure, called the autophagosome, is a characteristic of macroautophagy that engulfs a large amount of cellular debris along with bulky proteinaceous inclusions[43,44].

Aggrephagy is often used to describe selective targeting of bulky protein aggregates or inclusion bodies for degradation

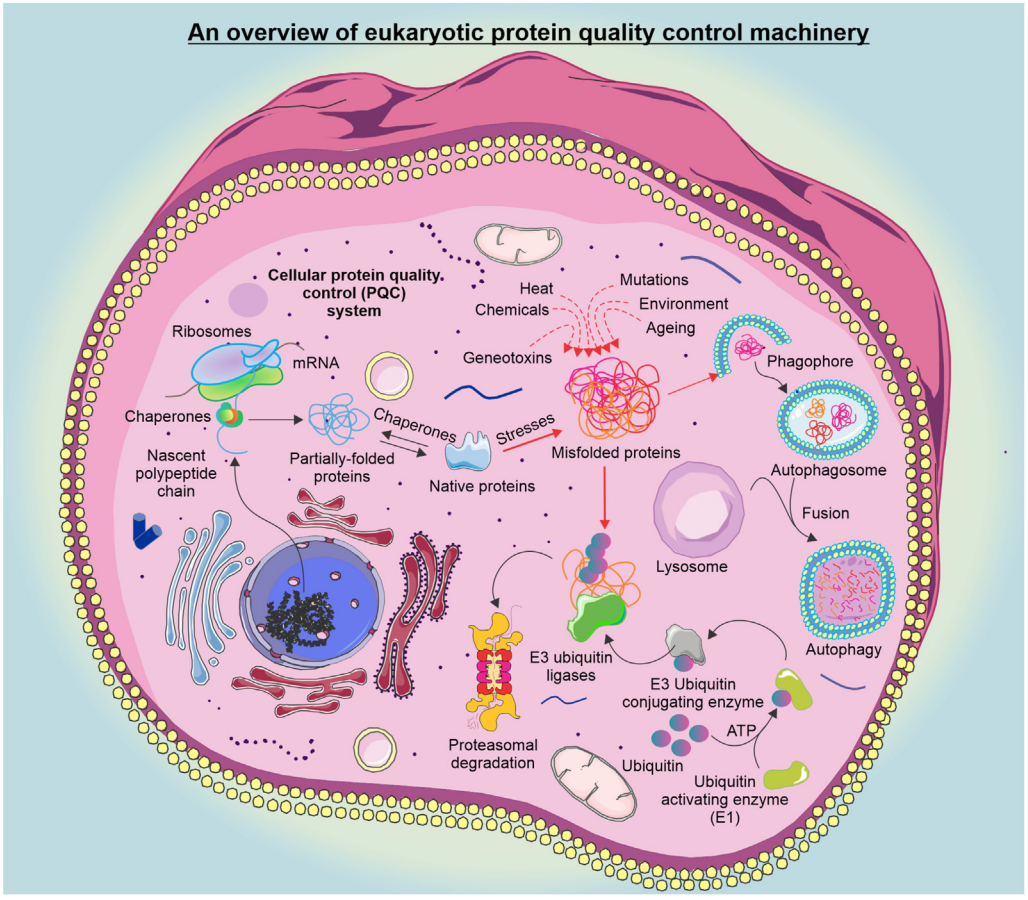

Figure 1 A eukaryotic cell showing the major cellular components constituting the cellular protein quality control machinery. Molecular chaperones are the immediate interactors of the newly synthesized proteins, which help them to achieve their natural active conformation and provide additional opportunities during lifetime to attain the same if they lose their native conformation. The UPS is comprised of three primary classes of enzymes: E1 ubiquitin-activating enzymes, in an ATP-dependent manner, activate small ubiquitin molecules that are later conjugated to E3 ubiquitin ligases by E3 conjugating enzymes. E3 ligases provide substrate specificity to transfer these conjugated ubiquitin molecules to the aberrant proteins, unfolded, misfolded, or aggregated inside the cytoplasm and other cell compartments. The ubiquitinated proteins are subjected to proteasomal degradation. Autophagy is a bulk degradation system in which a large amount of cytoplasmic waste material is packaged in membranous structures. The membrane-bound waste material is subjected to degradation by lysosomal proteases, which is accomplished by the fusion of the membranous vesicles to the lysosomes.

through macroautophagy in a process facilitated by adapter proteins, like P62 and NBR1 and light chain 3 (LC3), a phagophore membrane receptor[45,46]. A double-membrane structure called autophagosome is formed as a result of the closure of phagophore, which is followed by fusion with late endosomal vesicle or lysosomal sacs[47,48]. The contents within this newly formed structure, referred to as amphisome, are degraded by various lysosomal enzymes[49,50]. Similar to aggrephagy, few other pathways of selective degradation of cytoplasmic proteins are orchestrated by cytosolic chaperones HSC70 along with its regulatory co-chaperones[51,52]. For example, microautophagy involves selective transport of cytosolic proteins to vesicles using endosomal sorting complexes required for transport (ESCRT I and III) in the HSC70-dependent manner[53,54]. However, the microautophagy pathway involves invagination and tube formation, followed by vesicle expansion and degradation[55,56].

Another highly selective proteolytic pathway is CMA that could be defined as a process of selective identification of the KFERQ motif-containing cellular proteins by HSC70 and co-chaperones[57,58]. The HSC70-conjugated substrates are internalized after binding to LAMP-2a (a lysosome-associated membrane protein) and later degraded by membrane-bound proteases[59,60]. BAG3-mediated selective degradation pathway, CASA is also governed by chaperones HSC70 and HSPB8, in concerted action with CHIP (an E3 ligase) that mediates the ubiquitination of the proteins before their disposal to the lysosomal compartment in a P62-dependent manner[61,62].

2.3. *Ubiquitin–proteasome system (UPS)*

The ubiquitin–proteasomal pathway is a multistep process of protein degradation, in which a series of enzymes sequentially catalyze the substrate proteolysis inside a large barrel-shaped, cylindrical protein complex called proteasome[63,64]. The 26S proteasome is a multi-subunit complex containing a 20S core particle and one or two regulatory 19S sub-particles to regulate the entry of the ubiquitylated chains into the core[65,66]. The 20S core proteasome subunit contains three types of protease activities governing the cleavage of incoming polypeptides into smaller fragments[67,68]. Out of four heptameric rings forming the core, two

inner rings, termed β-rings, contain the proteolytic activities of different types: post-glutamyl peptide hydrolase ($\beta1$), trypsin ($\beta2$), and chymotrypsin ($\beta5$)[69,70]. In the first ATP-dependent step, an E1 ubiquitin-activating enzyme activates the small 8 kDa ubiquitin molecule (Ub) and forms a thioester bond[71,72]. A transacylation reaction transfers this ubiquitin to the thiol group present on another class of enzymes called E2 ubiquitin-conjugating enzyme[73,74]. These thiol esters (ubiquitin-E2 conjugates) provide ubiquitin molecules to the third class of enzymes called E3 ubiquitin ligases for tagging the substrate proteins[73,75]. The C-terminus glycine of the ubiquitin polypeptide forms an isopeptide bond with one of the lysine residues present on the cellular proteins[76].

According to the long-standing notion, attachment of single ubiquitin (monoubiquitination) generally does not target substrate proteins for proteolytic pathways; however, recent advancements also oppose this belief[77]. In addition, more than one ubiquitin molecules might get attached to the substrate proteins, independently (multi-monoubiquitination) or one over the other (polyubiquitination) through lysine residues present in the already conjugated ubiquitin or the N terminal methionine residue of the ubiquitin[78]. This may result in an array of signals, and ubiquitin codes interpreted and dealt in different manners by cellular subsystems[79,80]. The patterns of attachment of subsequent ubiquitin moieties may govern differential fates of the targeted proteins. For example, a Lys-63 linked ubiquitin chain preferably directs the proteins towards autophagic degradation[81,82]. Contrarily, highly abundant K-48 linked polyubiquitin chains are majorly targeted for proteasomal degradation[83]. Other ubiquitin chains formed with K6, K11, K27, K29, and K33 linkages form different kinds of signals and regulate multiple physiological processes, including cell cycle control, cellular transport, and DNA repair[84–86].

Altogether, the involvement of the UPS has been reported in immune pathways, hormonal signaling, cellular metabolism, apoptosis, etc.[19,87]. Considering the coexistence of all these proteolytic processes inside the eukaryotic cells, we can assume that maintenance of proteostasis requires a very tightly regulated coordination between different components and arms of the cellular protein quality control[88,89]. Their involvement in the pathologies of cancer, neurodegeneration, and aging processes has led scientists to identify their therapeutic potential and devise methods or ways to modulate them for exploitation for remedial purposes. Natural molecules have remained a primary therapeutic tool over the years showing enormous potential to modulate crucial regulatory proteins inside the cells. Several reports over the past few years, as shown in Table 2, have been published describing various kinds of possible regulation of different UPS components, which ultimately govern many disease-associated pathways.

3. Pathological conditions affected by altered protein quality control

Aging, neurodegeneration, and cancer have always remained significant challenges before the scientific community. Many theories and hypotheses have been formulated and postulated to explain these pathologies, but none has succeeded in understanding why these pathological changes occur. Genetic, environmental, infections and metabolic alterations are among the many possible reasons behind most proteopathies[90,91]. However, none of these could solely be held responsible for pathological conditions; instead, a blend of multiple factors contribute towards a highly diverse disease condition. This diversity among the individual cases of these pathologies further complicates the research processes and leads to failure of treatment options[92–94]. However, in the past few decades, tremendous progress is observed in our understanding of many of these pathologies. At the same time, these advancements have led to the evolution of multiple lines of research methodologies and approaches to understand a given problem. This has given rise to speculations and multiple lines of theories behind the origin, development, sustenance, and progression of these pathologies.

The declined competence of cellular defense mechanisms and pathways are suggested to be one such notion that has attained wide acceptance in recent decades[4,5]. Inefficient functions of quality control systems that regularly monitor the well-being of the genomic and proteomic repertoire of the cells could be a possible cause of instigating multiple pathways leading towards aging[1]. The compromised capacity of molecular chaperones to fold the nascent polypeptides into the proper three-dimensional shape and deficient functioning of autophagy and the proteasomal system could be credited for over-burdening the cytoplasmic milieu with misfolded proteins[95,96]. Aggregation of multiple types of aberrant proteins could lead to the formation of large perinuclear/cytoplasmic inclusion bodies that may further mount a heightened reaction by initiating immunological responses[97]. The increased burden of the aggregates may lead to increased neuronal deaths, as observed in many disease models of neurodegeneration[98,99].

Aging encompasses several other attributes or hallmarks, which may include but is not limited to the genomic instability, mitochondrial loss, telomere shortening, metabolic alterations, etc.[91,100]. These pathways and alterations in their physiological conditions are also among the crucial factors responsible for most types of cancers[101,102]. Altogether, the conditions discussed above have many common features. One of the similarities is the compromised proteostasis caused due to the inefficient protein folding and degradation in cells[103,104]. Many other diseases, like diabetes, cataract, cystic fibrosis, myopathies, etc. are directly affected by the aggregation of one or more proteins[22,105]. An imbalanced proteostasis may directly or indirectly link with many other life-threatening diseases associated with lungs, heart, liver, kidneys, etc.[19,106]. Based on the recommendations made by the International Society of Amyloidosis, a depiction of various amyloidogenic proteins, their aggregatory forms, and the affected organs in many associated diseases is presented in Fig. 2[107–109]. However, drawing a common line across all these diseases would be difficult at the present state of our understanding of these intracellular systems. Based on their common connecting links, *i.e.*, perturbed proteostasis and the cellular PQC machinery, various strategies have been postulated in the past, while some are currently under trial.

4. Small natural molecules: An effective therapeutic armory targeting severe pathological conditions

Humans have learned the art of extraction and effectively utilizing naturally occurring bioactive components and chemical molecules towards medical purposes for centuries. Many groundbreaking discoveries about the inherent medicinal properties of natural compounds against numerous life-threatening diseases have been awarded Nobel Prizes in the past. The mid-nineteenth century discoveries of antibiotics penicillin and

Table 2 Small natural compounds having chaperone-modulating activities. A broad array of natural molecules have been identified over the years, which can enhance or suppress the cellular chaperoning activity by elevating the expression or interfering with the functioning of major chaperones belonging to HSP70, HSP90, small HSPs or co-chaperones.

Compound	Source	Target protein	Target disease	Model system	Ref.
Inducers of chaperone machinery					
Actinomycin D	*Streptomyces parvullus*	HSP70	Huntington's disease	*S. cerevisiae*	42
Celastrol	*Tripterygium wilfordii*	HSF1, SSA3/4	Stress response	*S. cerevisiae*	43
Compound A	Salsola tuberculatiformis	HSP70	Inflammation	A549 cells	44
Curcumin	*Curcuma longa*	HSF1, HSP70	Stress response	C6 cells, rats	45
Geldanamycin	*Streptomyces* spp.	HSP70	Neurodegeneration	H4 cells	46
Glycyrrhizin	*Glycyrrhiza glabra*	HSP70	Stress response	HeLa cells	47
Handelin	*Handelia trichophylla*	HSP70	Neuroinflammation	BV2, HEK293T	48
Lanosterol	Metabolic intermediate	CHIP	Neurodegeneration	Cos7 cells	49
Myricetin	Fruits and berries	HSP70, HSF1	Neurodegeneration	Cos7 cells	50
Paeoniflorin	*Paeonia lactiflora*	HSF1, HSP70	Stress response	HeLa cells	47
Prostaglandins	Human	HSF1, HSP70	Stress response	C6 cells	51
Withaferin A	*Withania somnifera*	HSP25, HSP70	ALS	Mice	52
HSP90 inhibitors					
Argenteoside A	*Tabebuia argentea*	HSP90	Epithelial carcinoma	HeLa cells	53
Celastrol	*Tripterygium wilfordii*	HSP90	Prostate cancer	LNCaP cells	54
Clorobiocin	*Streptomyces* spp.	HSP90	Breast cancer	SKBR3, MCF7	55
Coumermycin A1	*Streptomyces* spp.	HSP90	Breast cancer	SKBR3, MCF7	55
Cruentaran A	*Byssovorax cruenta*	HSP90	Lung, breast cancer	A549, MCF-7	56
Curcumin	*Curcuma longa*	HSP90	Viral infection	HELF cells	57
Deguelin	*Derris trifoliata*	HSP90	Cancer	Mice	58
Derrubone	*Derris robusta*	HSP90	Breast cancer	SKBR3, MCF-7	59
EGCG	*Camellia sinensis*	HSP90	Hepatoma	HePa, HspG2	60
Gambogic acid	*Garcinia harburyi*	HSP90	Cancer	SKBR3, MCF7	61
Gedunin	*Azadirachta indica*	HSP90	Prostate cancer	LNCaP cells	54
Geldanamycin	*Streptomyces* spp.	HSP90, HSF1	Cancer	3T3 cells	62
Herbimycin A	*Streptomyces* spp.	HSP90	Cancer	3T3 cells	62
Hypericin	*Hypericum* spp.	HSP90	Squamous carcinoma	SQ2 cells	63
Kotschyn D	*Pseudrocedrela kotschyi*	HSP90	Prostate cancer	PC-3 cells	64
Lentiginosine	*Astragalus lentiginosus*	HSP90	Cancer	*In silico*	65
Macbecin	*Actinomyces* spp.	HSP90	Prostate, lung cancer	DU145, H460	66
Monocillin I	*Monocillium nordinii*	HSP90	Breast cancer	MCF-7 cells	67
Novobiocin	*Streptomyces niveus*	HSP90	Breast cancer	SKBR3, MCF-7	55
Pochonins	*Pochonia chlamydosporia*	HSP90	Cancer	*In vitro*	68
Radicicol	*Monosporium bonorden*	HSP90	Cancer	NIH3T3 cells	69
Sansalvamide A	*Fusarium* spp.	HSP90	Colon cancer	HCT-116	70
Withanolides	*Withania somnifera*	HSP90, HSF1	Thyroid cancer	DRO, NPA cells	71
Quercetin	Fruits and berries	HSP90, HSF1	Breast cancer	HeLa	72
Triptolide	*Triptergium wilfordii*	HSP90	Cancers	HeLa cells	73
HSP70 inhibitors					
Apidaecin	Insect peptides	DNAK, GROEL	Microbial infection	*E. coli*	74
Cantharidin	*Epicauta funebris*	HSP70	Colorectal cancer	HCT-116 cells	75
Drosocin	Insect peptides	DNAK, GROEL	Microbial infection	*E. coli*	74
Fisetin	Fruits and berries	HSP70, HSF1	Colorectal cancer	HCT-116 cells	76
Myricetin	Fruits and berries	DNAK	Proteostasis	*E. coli*	77
Novolactone	Fungal metabolites	HSP70	Proteostasis	HCT-116 cells	78
Pyrrhocoricin	Insect peptides	DNAK, GROEL	Microbial infection	*E. coli*	74
Quercetin	Fruits and berries	HSP70	Lung cancer	A549, H460 cells	79
adaSGC	Human	HSP70	Proteostasis	BHK cells	80
Spergualin	*Bacillus subtilis*	HSC70	Immune reaction	Jurkat cells	81
Triptolide	*Triptergium wilfordii*	HSF1, HSP70	Cancers	HeLa, HEK293T	82
Tubocapsenolide A	*Tubocapsicum anomalum*	HSP90-HSP70	Breast cancer	MDA-MB-231	83

streptomycin have thoroughly changed the idea of drug discovery and accelerated the pursuit of more such compounds for other medicinal purposes in the following decades. Technological advancements, the inclusion of computational approaches, and the reincarnation of the vast literature of ancient Indian and Chinese medicine have substantially assisted and overwhelmed the field of drug discovery. The recent Nobel Prize for recognizing the medical importance of avermectins and artemisinin has again pressed upon the hidden potential of the small molecule-based drug substances.

In previous sections, we have discussed how the formation of inclusion bodies follows an aberrant protein aggregation. The inefficiency of cellular QC mechanisms to fight back and address the loss of proteostasis-like conditions may lead to an array of systemic

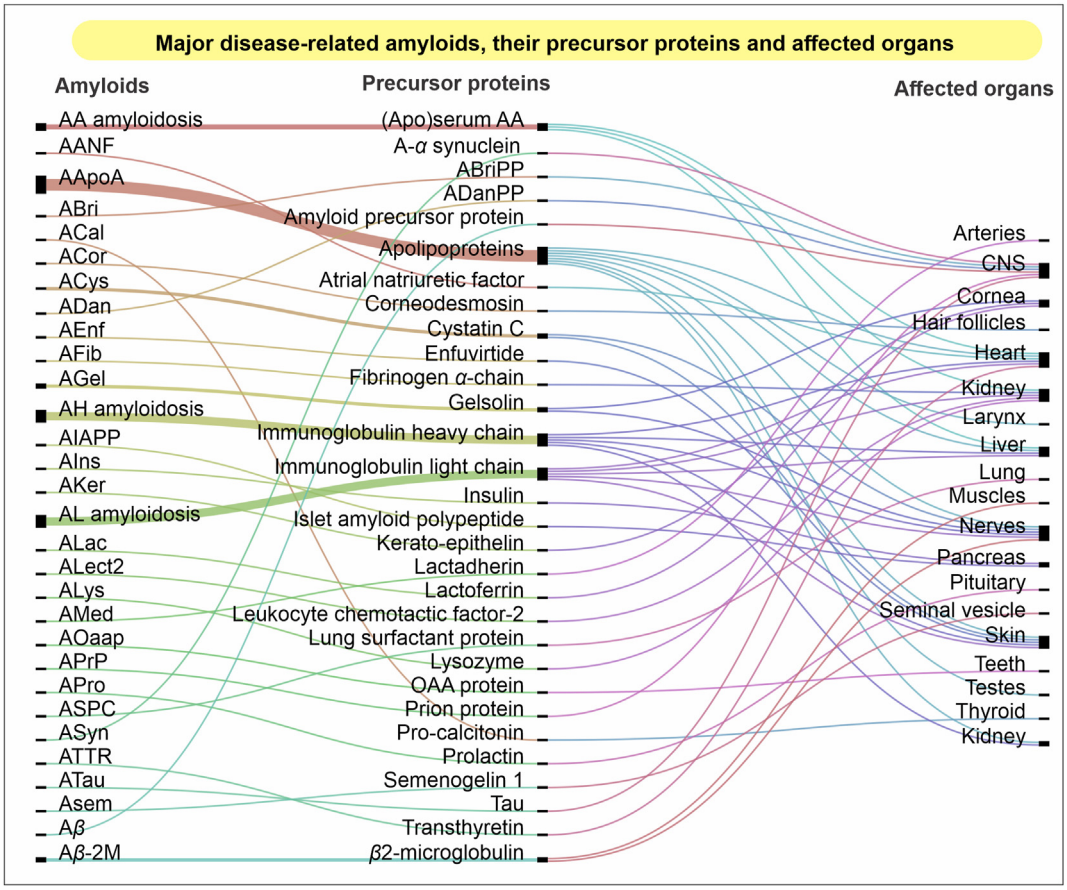

Figure 2 An overview of amyloidosis. Various amyloid-forming proteins (left), their normal precursor protein forms (middle), and tissues or organs affected in one or more similar diseases caused by individual proteins[106,107]. The left column shows a list of amyloidic forms of various proteins shown in the center as precursors. These proteins may aggregate in such amyloidic structures in their full, cleaved, or modified forms, while several mutations contribute to their amyloidogenicity. The structural modification of these proteins may lead to abnormal metabolic or signaling alterations at the molecular level in different tissues and organs. These changes may lead to a possible functional loss or decline, causing multiple pathological conditions. Many proteins are found to be involved in multiple diseases of different organs, whereas some diseases may have several proteins involved together in the pathogenesis. The figure was prepared using RAWGraphs, an open source platform for data representation (http://rawgraphs.io)[108].

and non-systemic diseases[103]. With the available knowledge and experimental evidence, it could be understood that reestablishing the lost activities of these pathways, by inducing chaperone function or enhancing the activities of proteolytic machinery (proteasome and autophagy), etc., may exhibit the tremendous potential to delay the onset of pathologies or aging-associated changes inside the organisms[110]. Results from many studies converge towards the consensus advocating for small molecule-based therapies as beneficial and low-cost strategic tools to suppress the aggregation of most kinds of disease-associated amyloidogenic aggregates[111]. Notably, many recently recognized molecules, termed as pharmacological chaperones, have a strong potential of precisely facilitating the folding and stabilization of aberrant proteins, thereby assist in restoring their native functions[112,113].

The bulk degradation pathway of the cells, termed as 'autophagy' soon after its discovery by Christian de Duve, derived its name from Greek words meaning self-eating[114]. In later years, it was found that the autophagic degradation, which was initially considered a non-specific degradation system of the cells, could also be a part of much-targeted protein degradation pathways in association with chaperones or UPS components[115,116]. It joins hands with the UPS and plays a balancing act of degradation with the protein synthesis and folding machinery concurrently working in the cells[89,117]. Many studies also suggest that autophagy and proteasome pathways may also compensate each other under different stress conditions; therefore, a few drugs suppressing UPS activity, *e.g.*, MG132 and lactacystin, may lead to activation of autophagic responses[118–120]. Contrarily, inhibition of autophagy overwhelms the cells with accumulating protein inclusions causing impairment of proteasomal degradation[121]. In truth, a clear understanding of how the two systems balance each other while protecting the cells from proteotoxic stresses is not known. Here, a comprehensive overview is provided for those molecules or drug candidates, which can bind and modulate the activity or functions of one or multiple cellular PQC machinery components.

4.1. Modulating cellular chaperoning potential: Provides additional buffer against stress conditions

Chaperones are essential regulators of cell homeostasis, and their anomalous functioning can lead to perturbance of many normal and stress-related pathways. The primary functions that the HSP family proteins perform inside the cells are recognizing any unusual change in the cellular homeostasis, encountering spontaneous stress condition, and providing a piece of machinery to

Table 3 Natural molecules affecting the activities and functions of the proteasome. These molecules have been identified as the putative modulators, including inducers and inhibitors, of the three known protease activities, including chymotrypsin, trypsin, and caspase-like specificity of the proteasome subunits. The associated diseases and studied model systems are also presented in adjacent columns.

Compound	Source	Subunit	Pathway/disease	Model system	Ref.
Proteasomal inducers					
Betulinic acid	*Betula* sp.	β5	Neurodegeneration	MT4 cells	84
Canthin-6-one	*Ailanthus altissima*	β5	Parkinson's disease	Mice	85
Fatty acids	Animal sources	β1	Ageing	Rats	86
Harmine	*Peganum harmala*	β1, β2, β5	Parkinson's disease	Mice	87
Heparin	Animal sources	β2	Ageing	Human erythrocytes	88
Lysophospholipids	Animal sources	β5	Acrosome formation	Sea urchin sperm	89
Oleuropein	*Olea europea*	β1, β2, β5	Ageing	IMR90, WI38 cells	90
Oxyphylla A	*Alpinia oxyphylla*	β5	Parkinson's disease	Mice	91
Sulfatides	Animal sources	β5	Ageing	Human erythrocytes	88
Sulforaphane	*Brassica oleracea*	β1, β2, β5	Neurodegeneration	Mice	92
Zerumbone	*Zingiber zerumbet*	β5	Neurodegeneration	Hepa1c1c7 cells	93
Proteasomal inhibitors					
Microbial sources					
Aaptamine	*Aaptos suberitoides*	β1, β5	Cancer	HeLa cells	94
Aclarubicin	*Streptomyces galilaeus*	β5	Cancer	Bovine pituitary	95
Agosterol C	*Spongia* sp.	β5	Cervical carcinoma	HeLa cells	96
Antiprotealide	*Salinispora tropica*	β5	Multiple myeloma	RPMI 8226 cells	97
Argyrin A	*Archangium gephyra*	β1, β2, β5	Cancer	HeLa, SW480 cells, mice	98
Belactosin A/C	*Streptomyces* sp.	β5	Muscle wasting	Rats	99
Carmaphycin-17	*Symploca* sp.	β1, β5	Trichomoniasis	*Trichomonas vaginalis*	100
Ciclosporine A	*Tolypocladium inflatum*	β5	Inflammation	RAW, murine brain	101
Cinnabaramides	*Streptomyces* sp.	β5	Cancer	PBMC cells	102
Cystargolide A	*Kitasatospora cystarginea*	β5	Cancer	Purified 20S proteasome	103
Dibromophakellin	*Phakellia flabellata*	β1, β5	Cancer	HeLa cells	104
Eponemycin	*Streptomyces* sp.	β5	Murine thymoma	EL4 cells	105
Epoximycin	*Actinomycetes* sp.	β2, β5	Inflammation	HUVEC cells	106
Fellutamide B	*Penicillium fellutanum*	β5	Nerve injury	Mouse fibroblasts	107
Glidobactins	*Polyangium brachysporum*	β2, β5	Cancer	*Phaseolus vulgaris*	108
Gliotoxin	*Aspergillus fumigatus*	β5	Cancer	HeLa cells	109
Halicyclamine B	*Haliclona* sp.	β1, β2, β5	Cancer	HeLa cells	110
Heteronemin	*Hyrtios* sp.	β2, β5	Leukemia	K562, Jurkat T cells	111
Lactacystin	*Streptomyces lactacystinicus*	β1, β2, β5	Neuroblastoma	Neuro2a	112
Lovastatin	*Pleurotus ostreatus*	β5	Breast cancer	MDA-MB-157 cells	113
Marizomib	*Salinospora* sp.	β5	Colon carcinoma	HCT-116	114
Mevastatin	*Penicillium citinium*	β5	Neuroblastoma	NBP2 cells	115
Mycalolides	*Mycale* sp.	β5	Melanoma	B-16 cells	116
Omuralide	*Streptomyces* sp.	β5	Neuroblastoma	Neuro2a	112
Palau'amine	*Stylotella agminata*	β5	Cancer	HeLa cells	104
Petrosaspongiolide M	*Petrosaspongia nigra*	β1, β5	Inflammation	THP cells	117
Rhabdastrellic acid-A	*Rhabdastrella globostellata*	β2, β5	Leukemia	HL-60 cells	118
Syringolins	*Pseudomonas syringae*	β1, β2, β5	Cancer	*Phaseolus vulgaris*	108
TMC-95	*Apiospora montagnei*	β1, β2, β5	Cancer	HCT-116, HL-60 cells	119
Tetradehydrohalicyclamine B	*Acanthostrongylophora ingens*	β1, β2, β5	Cancer	HeLa cells	110
Tyropeptin A	*Kitasatospora* sp.	β2, β5	Cancer	PC-12 cells	120
Plant products					
Ajoene	*Allium sativum*	β2, β5	Leukemia	HL-60 cells	121
Apigenin	*Portulaca oleracea*	β5	Breast cancer	MDA-MB-231, mice	122
Bisbibenzyls	Bryophytes	β5	Prostate cancer	LNCaP cells	123
Capsaicin	*Capsicum annuum*	β1, β2, β5	Prostate cancer	PC-3 cells	124
Celestrol	*Tripterygium wilfordii*	β5	Prostate cancer	PC-3 cells, mice	125
Chrysin	*Passiflora caerulea*	β2, β5	Cancer	HepG2, HL-60, A549	126
Curcumin	*Curcuma longa*	β1, β2, β5	Cancer	Neuro 2a cells	127
Catechin-gallate	*Camellia sinensis*	β5	Cancer	Jurkat T cells	128
Emodin	*Rheum palmatum*	β1, β2, β5	Cancer	HeLa cells, mice	129
Fangchinoline	*Stephania tetrandra*	β1	Prostate cancer	LNCaP, PC-3 cells	130
Genistein	*Glycine max*	β5	Cancer	LNCaP, MCF-7 cells	131
Ginsenosides	*Panax ginseng*	β5	Cancer	Pig RBCs	132
Isoginkgetin	*Ginkgo biloba*	β1, β2, β5	Cancer	HeLa cells	133
Kaempferol	Fruits and vegetables	β5	Leukemia	Jurkat T cells	134
Luteolin	*Cichorium endivia*	β2, β5	Cancer	HepG2, HL-60, A549	126
Marchantin M	*Marchantia* sp.	β1, β5	Prostate cancer	PC-3 cells	135

Table 3 (*continued*)

Compound	Source	Subunit	Pathway/disease	Model system	Ref.
Myricetin	Fruits and vegetables	β5	Leukemia	Jurkat T cells	134
Pectolinarin	*Cirsium chanroenicum*	β1, β5	Tuberculosis	*M. tuberculosis*	136
Physalin B	*Physalis angulata*	β1, β2, β5	Colon cancer	DLD-1 cells	137
PMI5011	*Artemisia dracunculus*	β1, β5	Diabetes	C2C12 cells, mice	138
Pristimerin	*Maytenus ilicifolia*	β5	Prostate cancer	PC-3 cells, mice	139
Quercetin	*Aesculus indica*	β1, β2, β5	Atherosclerosis	Rabbits	140
Resveratrol	*Vitis viniferae*	β5	Neurodegeneration	N27 cells	141
Tannic acid	*Caesalpinia spinosa*	β5	Cancer	Jurkat T cells	142
Vinblastine	*Vinca rosea*	β1, β2, β5	Leukemia	HL-60 cells	143
Withaferin A	*Withania somnifera*	β5	Prostate cancer	LNCaP cells, mice	144
Other natural compounds					
Arenobufagin	Toad venom	β1, β2, β5	Cervical carcinoma	HeLa cells	145

monitor and establish the structures and functioning of other cellular proteins. The term 'chemical chaperone' has been widely used in the past decade for a group of potentially active molecules that can stabilize cellular proteins in a non-specific way and help in reversing the mislocalization or aggregation[122,123]. These molecules mostly act on the proteins' active domains or sites, providing them an increased opportunity to form hydrogen, electrostatic, and van der Waals interactions and potentially stabilize the overall structure of the proteins[124]. Additionally, an array of naturally occurring substances and their derivatives have shown modulatory potential over inherent chaperoning capacity inside the cells[125].

These bioactive chemical molecules can bind and alter the structure, activity, and overall functions of the most active HSP70 and HSP90 chaperone complexes, along with many of their co-chaperones and accessory factors[126,127]. They also provide cushion for structural rearrangements of unfolded or misfolded proteins inside the cytosol, thus help in ameliorating the accumulation of aberrant proteins. However, the initial attempts to exploit chaperones for therapeutic purposes started with identifying the inhibitory activity of radicicol against HSP90 ATP-binding pockets[128,129]. It was initially used against malignant fibroblasts. Although promising, the drug failed in delivering the promises because of several pharmacokinetic challenges. The other prominent molecule in this category is a bacterial isolate geldanamycin that was later proved to be toxic to the liver[130]. In later years, advancements in the medicinal chemistry tools have led to the synthesis of many derivatives of these less successful drug candidates, *e.g.*, monocillin I, pochonins, 17-allylaminogeldanamycin (17-AAG), etc.[131−133].

Small molecules can shatter the interaction of major chaperones with their co-chaperones, thereby affecting chaperoning activities. For example, celestrol, a triterpene, and gambogic acid, a xanthonoid, can interfere with the interaction of HSP90 with its co-chaperone CDC37; while curcumin blocks HSP90−P23 binding, leading to the induction of cell death signaling pathways[134,135]. Other drug candidates with similar cell death-inducing effects are herbimycin A and derrubone[130,136]. Quercetin, one of the most studied flavonoids, shows an upstream regulation of heat-shock response inside the cells by suppressing the heat shock factor (HSF1), the major transcription factor that regulates the intracellular levels of most of the chaperones[137]. A green tree extracted molecule, epigallocatechin-3-gallate (EGCG), can also inhibit multiple chaperones, including HSP90, HSP70, and ER-resident GRP78, and suppress the growth of cancer cells[138,139]. Interestingly, other mechanisms of functional suppression of HSP90 are increased ubiquitination (by hypericin), destruction of chaperone cycle (by sansalvamide A), and oxidation (by tubocapsenolide A) of HSP90 itself[140−142]. All these can interfere with the turnover of the substrate proteins of the chaperones, thus deregulating the proteostasis balance of the cell.

Modulation of HSP70 functions by myricetin and spergualin may also help suppress cancerous cells' growth, possibly by inhibiting the ATPase activity of the chaperone[143,144]. Few reports further suggest the possible activation of upstream regulator HSF1 in response to drug-mediated suppression of one or the other molecular chaperones; however, more work is required to understand the feedback mechanisms involved in this mechanism[145]. A few studies have shown that geldanamycin-mediated HSP90 inhibition may, in turn, upregulate the activities of HSP70 and HSP40, which could be helpful and may benefit the neuronal cells under different stress or pathology conditions, *e.g.*, HD, ALS, cerebral ischemia, etc.[146−150]. Similarly, treatment of curcumin and withaferin A may also exert neuroprotective effects on the cells and mouse models; the effects could be due to improved activities of HSP70, HSP27, and α-crystallin chaperones[151,152]. A summarized overview of various such kinds of molecules of natural origin that can help in reestablishing the proteostasis inside the cells by modulating the inherent chaperoning capacity of the cell has been presented in Table 3.

4.2. Regulating the UPS components: Playing with the fine balance

UPS is the next line of defense in most subcellular compartments and works continuously to regulate the proteostasis inside these organelles[75]. As described previously, ubiquitination and proteasomal degradation are a kind of intracellular regulatory mechanisms that often is crucial for many cellular pathways. Therefore, any disturbances in these systems may have deleterious effects on cellular health[153]. The proteasomal system comprises several components that could be regulated by different mechanisms and may exert varying effects on cellular physiology. For example, regulating the activities of proteasomal subunits has been shown to have a direct effect on the overall cellular protein degradation scheme and the overall proteostasis[19]. Many proteasome modulators have been proposed, and a few of them are under clinical trials for diseases like cancer and neurodegeneration[154,155]. A plethora of naturally-derived chemicals has been reported over the years, which have shown a substantial modulation of the activities of various enzymes of the pathway. Thus, their use may enhance or suppress the proteostasis provided by these enzymes[19,156].

The proteasomal system is very specific in its activity and takes part in the precise regulation of the majority of physiological pathways; therefore, very tightly-controlled modulation is needed in order to exploit it for therapeutic purposes[157,158]. Bortezomib was the initial drug having the proteasomal inhibitory potential and has been widely used as an anticancer drug for long[159]. Later, another synthetic molecule, carfilzomib, was also approved by the U.S. Food and Drug Administration (FDA) for anti-cancer therapy[160]. Following the identification of these two FDA approved drugs, many other drugs with similar inhibitory activity against different proteolytic subunits (β1, β2, and β5) of 20S proteasome have been identified and thoroughly investigated for their therapeutic applications in many diseases[161,162]. Lactacystin is the most well-known natural molecule of this class that was initially reported to be effective against neuroblastoma cells and is currently one of the widely used drugs in the research[163]. Eponemycin and epoximycin specifically target chymotrypsin-like activity containing β5 subunits of the 20S core and help in suppressing the inflammation in cancer cells[164,165]. Mevastatin, belactosin A, and fellutamide B are other similar bacterial isolates that have been presented with the anti-protease activity of the proteasome in different experimental model systems[166–168].

Fungi and marine animals are other prominent sources of many biologically active molecules having critical therapeutic properties. Many proteasomal inhibitors have been isolated from these animals also. For example, gliotoxin and cyclosporine A from fungal sources and agosterol C and aaptamine from sponges are prominent inhibitors of 20S proteases[169–172]. These molecules could affect one or the multiple protease subunits of the 20S core particle of the proteasome. Interestingly, the toad venom contains a compound called arenobufagin that has the potential to inhibit all three activities simultaneously[173]. An exhaustive list of such natural molecules obtained from various biological sources has been presented in the form of Table 3. Plant-based molecules have specifically dragged lots of attention for their proteasome-modulatory activity and have been widely covered in other descriptive reviews[156,174].

Flavonoids make the most comprehensively explored class and have shown tremendous potential to be used in therapeutics against many diverse kinds of diseases. For example, genistein, EGCG, and physalin B have anti-cancerous roles, while pectolinarin has positive effects on tuberculosis due to its anti-inflammatory potential[175–178]. Apigenin, myricetin, quercetin, and luteolin are anti-atherogenic and may also help suppress tumor growth[179–182]. PMI5011 is an ethanolic preparation obtained from a herb, *Artemisia dracunculus*, and shows pathological improvements in diabetes mice[183]. Polyphenols like vinblastine, capsaicin, resveratrol, tannic acid, and curcumin[184–188], along with some well-known terpenoids, *e.g.*, celestrol, pristimerin, etc., further adds up to the list[189,190]. The compounds like anthraquinones, saponins, sulfur-derivatives, and plant-derived lactones come next into this long list (Table 3) of compounds with different types of inhibitory potential against β1, β2, or β5 activities of proteasome.

Contrary to proteasomal suppression, which is widely exploited in cancer therapeutics, enhancing the proteasomal activities could be useful in many stressful conditions and in the diseases associated with protein misfolding and aggregation. Two widely explored terpenoids, zerumbone and betullinic acid, have activated the β5 activities and thus presented neuroprotective effects[191,192]. Myricetin, oleuropein, and sulforaphane are other plant-derived molecules representing the proteasomal activators that may upregulate one or multiple 20S core subunits[193,194]. Few other molecules were identified that might delay the aging and neurodegeneration processes by increasing proteasomal degradation of the substrate proteins. These are heparin, sulfatides, and lysophospholipids, a few metabolic byproducts or those obtained from other animal sources[195,196]. Unlike proteasome inhibition, the effects of proteasome activation are not widely explored and need a more rigorous investigation to identify new molecules with a positive effect on proteasome functioning and their downstream impact on protein clearance.

A few recent studies have given clear insights into Parkinson's disease models that activation of proteasome function by hermine, oxyphylla A, and canthin-6-one can significantly upregulate the clearance of alpha-synuclein, the major constituent of the Lewy bodies formed in the substantia nigra[197–199]. Apart from protease subunits of 20S particle, many other components involved in protein ubiquitination have been looked for their applicability as a possible drug target in aging, neurodegeneration, and many other diseases. Modulation of the major enzymes involved in the ubiquitination process, *e.g.*, E1, E2s, E3s, and deubiquitinases (DUBs), could be a vital strategy to regulating several critical signaling and metabolism pathways[19,200]. E1 ubiquitin-activating enzyme is a unique protein required for the ubiquitination of all the possible cellular substrate proteins. Therefore, interfering with its activity may compromise the whole UPS and may have devastating effects[71]. However, this observation can be utilized in anticancer therapeutics as previously exemplified by hyrtioreticulins largazole, himeic acid A and panepophenanthrin[201–204].

The next line of drug targets is E2 ubiquitin-conjugating enzymes, which transfer ubiquitin molecules from the E1 enzymes to the E3 ligases. Not too many drugs have been identified, which can interfere with the enzymatic activities of E2; however, a few known naturally-occurring compounds are vitexin, a polyphenolic extract from *Byrsonima crassifolia*, and a few poriferan-derived leucettamol A, manadosterols A and B, etc.[205–207]. Deubiquitinases (DUBs) are a group of enzymes that are crucial for breaking down the ubiquitin chains, replenishing the ubiquitin pool of the cells, and playing regulatory roles in many biological pathways[208,209]. Betulinic acid and one curcumin analog are a few known inhibitors of this class of enzymes, which have shown tremendous promises as anti-cancer molecules[210,211]. Cruciferous vegetables have a group of compounds called isothiocyanates, which are prominent inhibitors of DUBs, and have shown significant anti-tumor properties[212]. A diterpenoid candidate, 15-oxospiramilactone, is another DUB inhibiting molecule that has a positive effect on the restoration of the mitochondrial network[213].

Interestingly, the molecules that have the potency to modulate the most diverse class of enzymes of this pathway, the E3 ubiquitin ligases, has widely been explored for specific regulation of substrates and related pathways[214]. However, some molecules may inhibit multiple E3s simultaneously. Heclin is a recently developed molecule that can suppress many HECT domain-containing E3 ligases. Additionally, a few ubiquitin variants were prepared, which have shown tremendous inhibitory potential against RING and U-box domains of the E3 ligases[215–217]. A line of studies proposes several natural molecules as probable drug candidates against many life-threatening diseases. Inhibition of Mdm2 by matrine at the RNA level and by berberine *via* self-ubiquitination mechanism are prominent examples of regulating the turnover of P53, the primary tumor suppressor protein[218,219]. Oroxylin-A, apigenin, and genistein are plant flavonoids that may initiate a high apoptotic response in cancerous cells[220–222]. Many terpenoids (*e.g.*, triptolide, inulanolide, etc.), saponins, chalcones, and polyphenols extracted from

Table 4 Small natural molecules affecting the cellular autophagy pathway. A concise representation of the potential candidates that can alter the autophagic flux, increase the protein degradation or interfere with different steps of autophagosome biogenesis or lysosome fusion, therefore can target specific molecular targets and pathways that are involved in many harmful diseases.

Compound	Source	Target pathway	Physiological condition	Model system	Ref.
Autophagy inducers					
Marine/microbial products					
Actinonin	*Streptomyces* sp.	AMPK, mtRNA	Cancers	HeLa cells	146
Araguspongine C	*Xestospongia* sp.	PI3K/AKT/mTOR	Breast cancer	BT-474 cells	147
Chromomycin A2	*Streptomyces* sp.	LC3	Melanoma	MALME-3M cells	148
Clionamine B	*Cliona celata*	LC3	Breast cancer	MCF-7 cells	149
Coibamide A	*Leptolyngbya* sp.	LC3	Glioblastoma	U87-MG cells	150
Hirsutanol A	*Chondrostereum* sp.	LC3	Hepatic carcinoma	Hep3B cells	151
Ilimaquinone	*Hippospongia* sp.	p53	Colon cancer	RKO cells	152
Isoaaptamine	*Aaptos* sp.	LC3	Breast cancer	T-47D cells	153
Monanchocin D	*Monanhora pulchra*	P38, ERK	Germ cell tumors	NCCIT cells	154
Ovothiol A	*Paracentrotus lividus*	Beclin-1, LC3	Hepatic carcinoma	HepG2 cells	155
Papuamine	*Haliclona* sp.	LC3, JNK	Breast cancer	MCF-7 cells	156
Psammaplin A	*Psammaplysilla* sp.	P73	Glioblastoma	U87-MG cells	157
Rapamycin	*Streptomyces hygropicus*	mTOR	Polyglutamine diseases	PC12, Cos7 cells	158
Rhabdastrellic acid A	*Rhabdastrella* sp.	AKT	Various human cancers	Hep3B, A549 cells	159
Salinosporamide A	*Salinospora tropica*	eIF2α	Prostate cancer	LNCaP-Pro5	160
Stellettin B	*Jaspis stellifera*	PI3K/AKT/mTOR	Lung cancer	A549 cells	161
SD118-xanthocilin-X	*Penicillium commune*	MEK/ERK	Hepatic carcinoma	HepG2 cells	162
Trehalose	*Streptomyces cerevisiae*	mTOR	Neurodegeneration	SK-N-SH, PC12 cells	163
Urolithin A	Gut microbiome	AMPK	Ageing	*C. elegans*	164
Xestospongin B	*Xestospongia exigua*	IP_3R	Cervical adenocarcin	HeLa cells	165
Plant products					
Terpenes					
Bigelovin	*Inula helianthus*	AKT/mTOR/S6K	Liver cancer	HepG2, mice	166
Eriocalyxin B	*Isodon eriocalyx*	AKT/mTOR/S6K	Breast cancer	MCF-7, MDA-MB-231	167
Gossypol	*Gossypium* sp.	Beclin-1, ATG5,	Breast adenocarcinoma	MCF-7, HeLa cells	168
Grifolin	*Albatrellus confluence*	AKT/mTOR/S6K	Ovarian cancer	A2780, SKOV3 cells	169
Oridonin	*Rabdosia rubescens*	P21	Prostate cancer	PC-3, LNCaP cells	170
Platycodin-D	*Platycodon grandiflorum*	PI3K/AKT/mTOR	Lung cancer	NCI−H460, A549 cells	171
Triptolide	*Tripterygium wilfordii*	SQSTM1, LC3	Parkinson's disease	MN9D cells, rats	172
Ursolic acid	*Ocimum sanctum*	JNK, BCL-2	Colorectal carcinomas	HCT-15 cells, mice	173
Flavonoids					
Ampelopsin	*Ampelopsis* sp.	AKT/mTOR/S6K	Breast cancer	MDA-MB-231, MCF-7	174
Apigenin	Fruits, vegetables	mTOR, S6	Leukemia	HL60, TF1 cells	175
Curcumin	*Curcuma longa*	FOXO1, beclin-1	Oxidative stress	HUVEC cells	176
Delicaflavone	*Selaginella doederleinii*	AKT/mTOR/S6K	Lung cancer	A549, PC-9	177
5-Demethylnobiletin	*Sideritis tragoriganum*	JNK	Lung cancer	A549 and CL1-5 cells	178
Galangin	*Alpinia officinarum*	P53	Hepatic carcinoma	HepG2 cells	179
Glabridin	*Glycyrrhiza glabra*	JNK1/2, P38, ERK	Hepatoma	Huh7 cells	180
Juglanin	*Juglans mandshurica*	JNK	Breast cancer	MCF-7 cells, mice	181
Kaempferol	Fruits and berries	AMPK, AKT	Hepatic cancer	SK-HEP-1 cells	182
Licochalcone A	*Glycyrrhiza* sp.	PI3K/AKT/mTOR	Cervical cancer	SiHa cells	183
Luteoloside	*Gentiana macrophylla*	AKT/mTOR/S6K	Lung cancer	A549, H292 cells	184
Myricetin	Fruits, vegetables	mTOR	Hepatic carcinoma	HepG2 cells	185
Quercetin	Fruits and berries	PI3K, beclin-1	Leukemia	P39 cells, mice	186
Resveratrol	*Vitis viniferae*	SIRT1, RAB7	Oxidative stress	Mice	187
Alkaloids					
Berberine	Coptidis Rhizoma	AKT/mTOR, beclin-1	Hepatic carcinoma	HepG2, MHCC97-L cells	188
Capsaicin	*Capsicum annuum*	Beclin-1, LC3	Hepatic carcinoma	HepG2 cells	189
Corynoxine B	*Uncaria rhynchophylla*	Beclin-1	Parkinson's disease	N2a,SHSY-5Y cells	190
Fangchinoline	*Stephania tetrandra*	Sestrin2	Hepatic carcinoma	HepG2 cells	191
Harmol	*Peganum harmala*	Survivin	Glioma	U251MG cells	192
Isorhynchophylline	*Uncaria rhynchophylla*	Beclin-1	Parkinson's disease	N2a, PC12, SH-SY5Y	193
Matrine	*Sophora flavescens*	mTOR, P53	Hepatic carcinoma	HepG2, SMMC-7721	194
Piperlongumine	*Piper longum*	AKT/mTOR	Various cancers	786-O, PC-3, MCF7	195
Vinblastine	*Vinca rosea*	Cathepsin D	Stress conditions	Rat hepatocytes	196
Other natural molecules					
Arenobufagin	Toad venom	PI3K/AKT/mTOR	Hepatic carcinoma	HepG2 cells	197
Benzyl isothiocynate	*Lepidium sativum*	AKT, mTOR	Prostate cancer	Rv-1, PC-12 cells	198
Bisbibenzyls	Bryophytes	LC-3	Prostate cancer	LNCaP cells	123

(*continued on next page*)

Table 4 (*continued*)

Compound	Source	Target pathway	Physiological condition	Model system	Ref.
Bufalin	*Bufo gargarizans*	JNK, ATG5, beclin-1	Colorectal cancer	HT-29 and Caco-2 cells	199
Cinobufagin	*Bufo gargarizans*	PARP, JNK/P38	Osteosarcoma	U2OS cells	200
Concanavalin A	*Canavalia ensiformis*	LC3, BNIP3, AKT	Hepatoma	ML-1 cells	201
Daucosterol	*Smilax glabra* Roxb.	Beclin-1, LC-3	Breast cancer	MCF-7 cells	202
Docosahexaenoic acid	Metabolic intermediate	NFE2L2	Neurodegeneration	ARPE-19	203
Embelin	*Embelia ribes*	ATG-5, ATG-12	Oral cancer	Ca9-22 cells	204
Lanosterol	Metabolic intermediate	CHIP	Neurodegeneration	Cos-7	49
Noggin	*Xenopus*	LC3, beclin-1	Acute pancreatitis	AR42J cells, mice	205
Ophiopogonin B	*Radix ophiopogon* var.	PI3K/AKT/mTOR	Lung cancer	NCI-H157, NCI-H460	206
Polyphyllin G	*Paris yunnanensis*	AKT, MAPK	Nasopharyngeal carcinoma	HONE-1 and NPC-039	207
Rottlerin	*Mallotus philippinensis*	PI3K/AKT/mTOR	Pancreatic cancer	Cancer stem cells	208
6-Shogaol	*Zingiber officinale*	AKT/mTOR	Lung cancer	A549	209
Sitosterol	Plant sterols	P38	Sitosterolemia	Mice macrophages	210
Spermidine	Natural polyamine	ATG7	Ageing	Yeast, fly, worm, PBMC	211
Sulforaphane	*Brassica oleracea*	ERK	Huntington's disease	Mice	212
Autophagy inhibitors					
Aspargine	Natural amino acid	Lysosome fusion	Proteopathies	Rat hepatocytes	213
Cytochalasins	*Aspergillus* sp.	Microfilaments	Proteopathies	Rat kidney cells	214
Emodin	*Fallopia japonica*	LC3, beclin-1	Acute pancreatitis	Rats	215
Estrogen	Natural hormone	CXCL12	Endometriosis	Endometrial stromal cells	216
Leupeptin	*Streptomyces* sp.	Serine proteases	Proteopathies	Rat hepatocytes	217
3-Methyladenine	Metabolic intermediate	PI3K	Proteopathies	Hepatocytes	218
Pepstatin A	*Streptomyces* sp.	Aspartyl peptidases	Proteopathies	Rat livers, hearts	219
Vinblastine	*Catharanthus rosea*	Microtubules	Proteopathies	Rat fibroblasts	220
Vincristine	*Catharanthus rosea*	Microtubules	Proteopathies	Rat fibroblasts	220
Wortmannin	*Penicillium* sp.	PI3K	Acute pancreatitis	Rats	221

plants and other natural sources have also shown promising effects against cancerous cells by inhibiting the MDM2−P53 interaction and degradation of the tumor suppressor[191,223,224].

Enhancing the functions of the anaphase-promoting complex (APC) by crosslinking CDC27 also exerts a similar effect by acting at the spindle assembly checkpoint of the proliferating cells[225]. Similarly, inducing the functioning of crucial E3 ligases like CHIP by lanosterol, a sterol molecule, as we found in our previous study, may help ameliorate the wide-spread proteotoxicity and related cellular deaths[226]. Enhancing the E3 ligase activities may elevate the clearance of accumulated proteins inside the cells, which could be a promising strategy against neurodegeneration. Recently, we found a similar effect of myricetin on the E6-AP and HSP70-mediated clearance of the substrate proteins[227]. Trehalose, an autophagy inducer, has also been shown to improve the clinical deficits caused by mutated CHIP in ataxia patient-derived fibroblasts[228]. Other studies from our group and possibly from many other labs are undergoing to identify other similar molecules with potency to modulate different E3 ubiquitin ligases so that specific molecular pathways could be targeted for disease therapeutics and drug development.

4.3. Natural modulators of autophagic pathway: Boosting the cellular stress response

The autophagic pathway was initially identified as an intracellular lysosomal degradation mechanism that targets consumed, unusable, or toxic cell material using protease enzymes present within membrane-bound organelles[114]. Autophagic clearance pathways may have many variants that select and degrade cellular proteins and debris differentially through varying mechanisms using multiple selections and targeting mechanisms using several adapters and membrane-bound receptor proteins[55,229]. In a way, this leads to a variety of opportunities to regulate these pathways of degradation at various points. An array of reports has shown that autophagy regulation using small natural molecules could also be achieved and used for drug discovery purposes[229,230]. Modulation of autophagic pathways is proposed for therapeutics against cancer and neurodegeneration in a large number of studies[231,232]. As shown in Table 4, different types of proteinopathies, neurodegenerative disorders, cancers, and several systemic diseases could be targeted by derivatives of natural molecules with modulatory effects on various effectors of the autophagy pathway. Several reports could still not be included in the present article due to space restrictions. The most prominent members of this class of natural autophagy inducers are resveratrol and trehalose[233,234]. Both these inducers have shown the tremendous potential of relieving neurons from various stresses by reducing free radicals and degrading protein aggregates[234,235].

Interestingly, autophagy plays very crucial roles in the clearance of many infectious agents, including HIV, *Mycobacterium*, or other parasites[236]. Triggering this pathway by vitamin D or starvation mechanisms have shown improvements in various pathological conditions, ranging from viral/bacterial infections to tuberculosis and malaria[237−240]. Autophagy also performs vital roles in cell metabolism and signaling, as evidenced by multiple lines of studies, which are covered in detail in several previous articles[241,242]. The influence of autophagy induction has been investigated in many metabolism-related disorders, including diabetes, glucose intolerance, obesity, and atherosclerosis[115]. It was evident from the past studies that modulation of autophagy may have enormous potential to counter the stress conditions and protect from several incurable diseases[243−245]. Likewise, the autophagy inducers, *e.g.*, bigelovin, oridonin, and stellettin B may accelerate the apoptotic pathways in various types of cancer cells[246−248]. The majority of molecules (*e.g.*, cinobufagin,

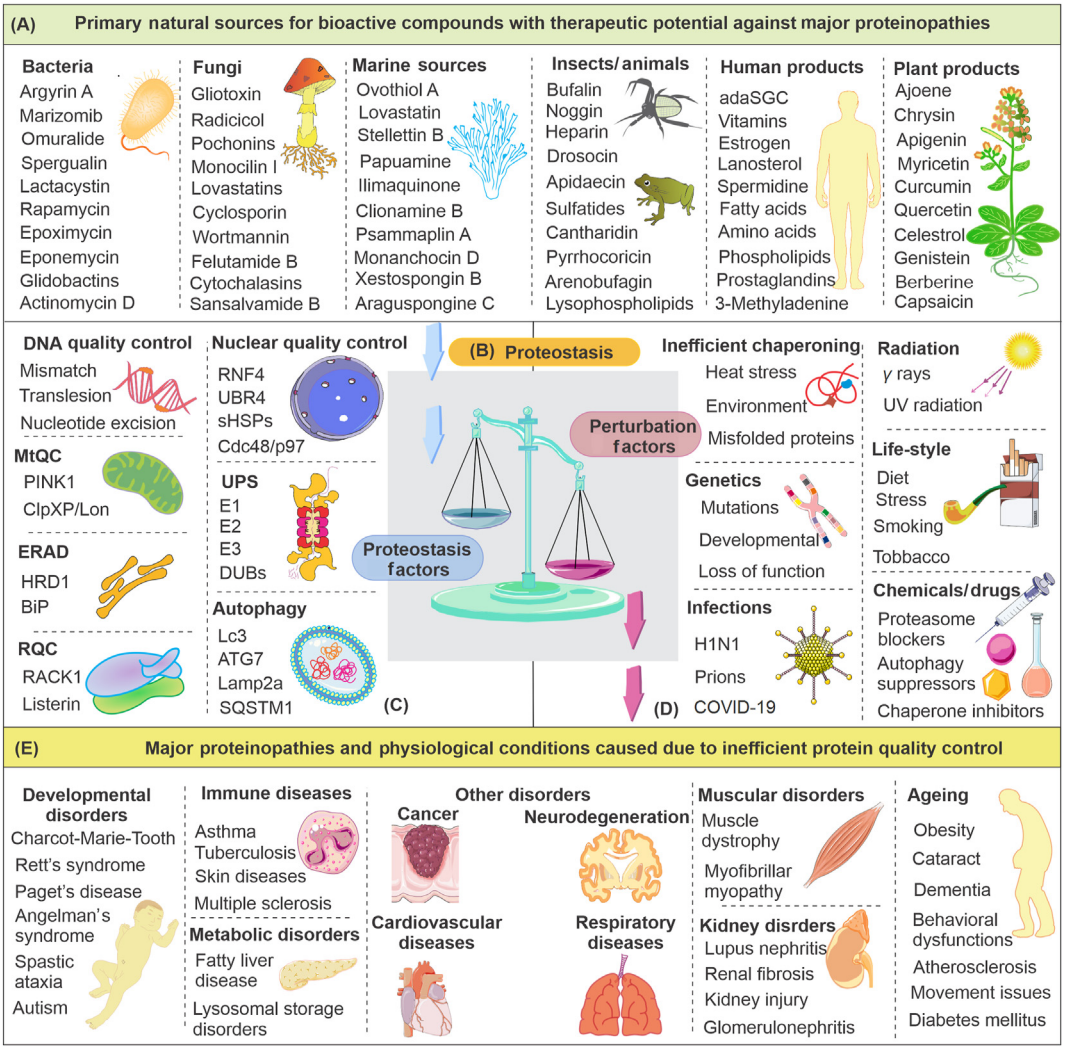

Figure 3 An overview of intricate association of natural compounds and proteostasis machinery. (A) A summary of important natural sources of bioactive compounds that can affect the activities and functioning of chief players of protein quality control machinery inside the cells; examples of small molecules from each source are also presented in respective boxes. (B) A central block shows how crucial is the precise balance of protein synthesis, maintenance, trafficking and degradation of cellular proteins for the maintenance of healthy proteome. Some internal or external perturbance factors may alter the homeostasis of the cells, while multiple key actors of cellular proteostasis machinery continuously maintain the health of the proteome. (C) Multiple kinds of intracellular regulatory subsystems are identified, which are required to synthesize and maintain the healthy set of proteins in various cellular compartments to perform all kinds of cellular functions. The key proteins of each subcellular pathway are mentioned in each box. (D) Many intra- and extracellular factors and stressors could be responsible for generating different kinds of stresses during the lifetime of the organisms, which are counter-balanced by factors described in (C). (E) A number of proteinopathies, which are directly and indirectly linked with perturbance in cellular proteostasis machinery, have been reported. These diseases could be associated with one or multiple pathways, tissues, and organs specified in each box.

juglanin, ursolic acid, ampelopsin, etc.) act on the target proteins, like PI3K, AKT, mTOR, S6K, MAPK, JNK, P38, ERK, etc., which are explicitly involved in the autophagy regulation[249–252]. For the past many decades attempts to upregulate the autophagic degradation of large aggregates of proteins have been made, and considerable success has been achieved.

The research on exogenous autophagy induction using exercise/starvation like lifestyle changes or natural molecule-based food habits has shown enormous promises to deliver in many stress-related changes like neurodegeneration and aging[253,254]. Use of curcumin and triptolide in oxidative stress conditions in cells and Parkinson's disease animal models have shown neuroprotective effects of these drugs *via* the upregulation of autophagy[255,256]. Docosahexaenoic acid, sulforaphane, and lanosterol are other natural inducers of autophagy, which have shown multifactorial effects in ameliorating the stress conditions of the cells and alleviate the degenerative conditions in the brain[194,226,257]. Although a vast literature is available on the induction of autophagy by small molecules, there are limited reports of inhibitors that can demonstrate beneficial effects on disease conditions. Emodin, wortmannin, and 3-methyladenine are few known autophagy suppressors with disease modulating potential[258–260]. A comprehensive list of naturally derived inducers and inhibitors of the autophagy pathway is prepared in Table 4.

5. Conclusions and future perspectives

Aberrant protein's accumulation inside cells is very well-described as a leading factor of aging, neurodegeneration, and multiple other pathologies, including cancer, diabetes, cystic fibrosis, etc. Researchers and clinicians have made multiple efforts to understand the underlying causes and mechanisms for these diseases. The molecular mechanisms whose failures can lead to inappropriate protein folding events and the common features across all these pathologies are still unclear. Some unique features across all these diseases and a noticeable genetic diversity in various conditions have prevented the scientific community from reaching a common conclusion and devising possible solutions for these life-threatening diseases[93,261]. However, continuous efforts are made worldwide to identify underlying causes, including the most common genetic mutations and contributing environmental or lifestyle associated factors. A comprehensive picture of all these factors, associated changes, and the pathological conditions caused by them is presented in Fig. 3. Unfortunately, none of the hypotheses and explanations addressing the mechanisms and causes behind such detrimental changes leading to the age-associated decline in the efficiency of physiological systems have led us to develop a proper understanding and possible solutions to these conditions.

In the past, many attempts, both successful and unsuccessful, have been made for devising novel therapeutic approaches against various diseases. Numerous molecules have been proposed for their efficacy for mitigating the proteotoxicity generated by intracellular protein aggregates or inclusion bodies[106,110,111]. One consensus that most of the studies meet is that natural products could be medicinally very active and useful. They were used for centuries in ancient traditional natural medicinal approaches in old-world countries. Based on all the observations mentioned above, it could be stated that targeting cellular PQC machinery by modulating their activities using small molecules may have vast potential. Plant extracts were used by ancient researchers and physicians to cure deadly infections and diseases, described in many primitive Indian, Unani, and Chinese literature[262,263]. Most natural molecules posit lesser toxicity and side effects than synthetic chemicals when administered to cells or animals in laboratory tests[264]. This makes them preferred choices over costly synthetic chemicals in experimental studies. Many less-invaded human territories, like the Himalayas, are the homeland of such medicinally rich natural resources and are yet to be explored and utilized for treatments of life-threatening diseases in these regions. A thorough and well-managed exploration could be done in order to identify and delve into more effective and easily derived drugs.

Several naturally extracted drugs obtained from microbial and fungal isolates, marine and land animals, many aquatic and terrestrial plants, are currently in research allowing us to identify and investigate more such drugs. These small natural molecules may have several unexplored applications that need further studies. The primary benefit of these naturally derived molecules is a low-cost therapeutic alternative to many treatment strategies, which are in the pipeline against these diseases and may need a much higher cost, although many challenges remain unaddressed[265]. The identification, isolation, purification, and characterization of new molecules are a highly tedious and lengthy process, requiring lots of hard work, funds, and time[19,266]. Many times, designing or synthesizing some derivatives of already known drugs seems a more straightforward and cost-effective strategy in comparison to looking for new molecules. Repurposing older drugs could also be a beneficial drug discovery model to save much time, effort, and cost. Additionally, these small plant-based molecules could be used as food supplements to reduce the overall risk of many diseases.

Acknowledgments

The author apologizes to various groups and scientists whose work could not have been cited due to space and time constraints. Author thanks the Central University of Rajasthan for providing space and resources while the manuscript was prepared. The author also appreciates Servier (http://smart.servier.com/) for providing templates for the preparation of medical illustrations under Creative Commons Attribution 3.0 Unported License.

Author contributions

Arun Upadhyay is responsible for all work of this review.

Conflicts of interest

The author has no conflict of interest to declare.

Supporting information

Supporting data to this article can be found online at https://doi.org/10.1016/j.apsb.2021.01.006.

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