NPR

Translating endoplasmic reticulum biology into clinic: a role for ER-targeted natural products?

Journal:	*Natural Product Reports*
Manuscript ID:	NP-REV-07-2014-000102.R2
Article Type:	Review Article
Date Submitted by the Author:	03-Feb-2015

Complete List of Authors:	Pereira, David; Faculty of Farmacy, University of Oporto Oporto, Portugal, Laboratory of Pharmacognosy Valentão, Patrícia; Faculty of Farmacy, University of Oporto Oporto, Portugal, Laboratory of Pharmacognosy Correia-da-Silva, Georgina; Faculty of Farmacy, University of Oporto Oporto, Portugal, Laboratory of Biochemistry Teixeira, Natércia; Faculty of Farmacy, University of Oporto Oporto, Portugal, Laboratory of Biochemistry Andrade, P. B.; ; Faculty of Farmacy, University of Oporto ,

Natural Products Reports

ARTICLE

Translating endoplasmic reticulum biology into clinic: a role for ER-targeted natural products?

Cite this: DOI: 10.1039/x0xx00000x

Received 31st July 2014,
Accepted xxxxx

DOI: 10.1039/x0xx00000x

www.rsc.org/

David M. Pereira[a]*, Patrícia Valentão[a], Georgina Correia-da-Silva[b,c], Natércia Teixeira[b,c], Paula B. Andrade[a]

ER stress has been identified as a hallmark, and sometimes trigger, of several pathologies, notably cancer, inflammation and neurodegenerative diseases like Alzheimer's and Parkinson. Among the molecules described in literature known to affect ER function, the majority are natural products, suggesting that natural molecules may constitute a significant arsenal of chemical entities for modulating this cellular target.

In this review, we will start by presenting the current knowledge of ER biology and the hallmarks of ER stress, thus paving the way for presenting the natural products that have been described as being ER modulators, either stress inducers or ER protectors. The chemistry, distribution and mechanism of action of these compounds will be presented and discussed.

Period covered: 1970-2014

[a] REQUIMTE/Laboratório de Farmacognosia, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira, nº 228, 4050-313 Porto, Portugal. Email: dpereira@ff.up.pt

[b] Laboratório de Bioquímica, Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira, nº 228, 4050-313 Porto, Portugal.

[c] IBMC –Instituto de Biologia Molecular e Celular, Universidade do Porto, 4150-180 Porto, Portugal.

Table of Contents

1. Introduction

The endoplasmic reticulum (ER) is an organelle constituted by a network of branching tubules and sacs that is present in all eukaryotic cells. From a functional point of view, the ER is mainly responsible for the synthesis, folding, modification and delivery of proteins to their target sites[1]. Once the synthesized proteins are folded into their native conformation and undergo post-translational modifications, like *N*-linked glycosylation and disulphide bonds formation, they are tagged to the Golgi complex and from there to lysosomes, plasma membrane or extracellular space. The ER also plays a pivotal role in Ca^{2+} homeostasis at cellular level, being one of the most important Ca^{2+} stores, its levels sometimes reaching 500 times those found in cytosol. For the control of Ca^{2+} gradients, the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) is one of the most relevant ATPases. Notably, some of the most potent SERCA inhibitors are natural products. In addition, ER is the site where synthesis of the majority of lipid molecules takes place.

Several signals can alter ER homeostasis, from physiological states that increase the demand for protein folding to *stimuli* that disrupt protein folding. This may originate accumulation of misfolded or unfolded proteins in the ER lumen and cause ER stress and ER stress response, a condition referred as unfolded protein response (UPR)[2-5]. Disruption of cellular lipid composition may also induce UPR, though the underlying mechanisms are not fully understood.

To this moment, three major proteins are known to act as stress sensors in ER: double-stranded RNA-dependent protein kinase PKR-like ER kinase (PERK), inositol-requiring 1α (IRE1α) and activating transcription factor 6 (ATF6) (Scheme 1). Each of these proteins displays an ER-luminal domain that senses unfolded proteins, a transmembrane domain and a cytosolic domain that transduces the signals to the transcriptional and translational machinery[3, 6, 7]. Two of these sensor proteins also have catalytic activity: PERK has a protein-kinase activity and phosphorylates eukaryotic translation-initiation factor 2α (eIF2α). IRE1α has both protein-kinase activity and endoribonuclease (RNase) activity and removes a 26-nucleotide intron from mRNA encoding the X box binding protein (XBP) (Scheme 1). In cells that are not undergoing stress, these ER stress sensors are in their inactive form *via* association with ER chaperones, such as immunoglobulin-heavy-chain-binding protein (BiP/HSPA5/GRP78), which has a *N*-terminal ATPase and a *C*-terminal substrate binding domain[4]. Dissociation of GRP78 from PERK triggers the homodimerization and subsequent autophosphorylation of the kinase, yielding the active form of PERK and phosphorylation of the translational factor, eIF2α, which inhibits protein synthesis (Scheme 1), as demonstrated by experiments with $PERK^{-/-}$ mouse embryonic fibroblasts that failed to inhibit protein translation following an ER stress elicitor[8]. Like PERK, suppression of GRP78 leads to activation of IRE1α through dimerization and phosphorylation. Activated IRE1α excises the intron from the XBP1 pre-mRNA originating the sXBP1 splice, which encodes a transcription factor that activates the transcription of UPR target genes. As it will be presented later, many natural products modulate the ER by interfering with these sensor proteins.

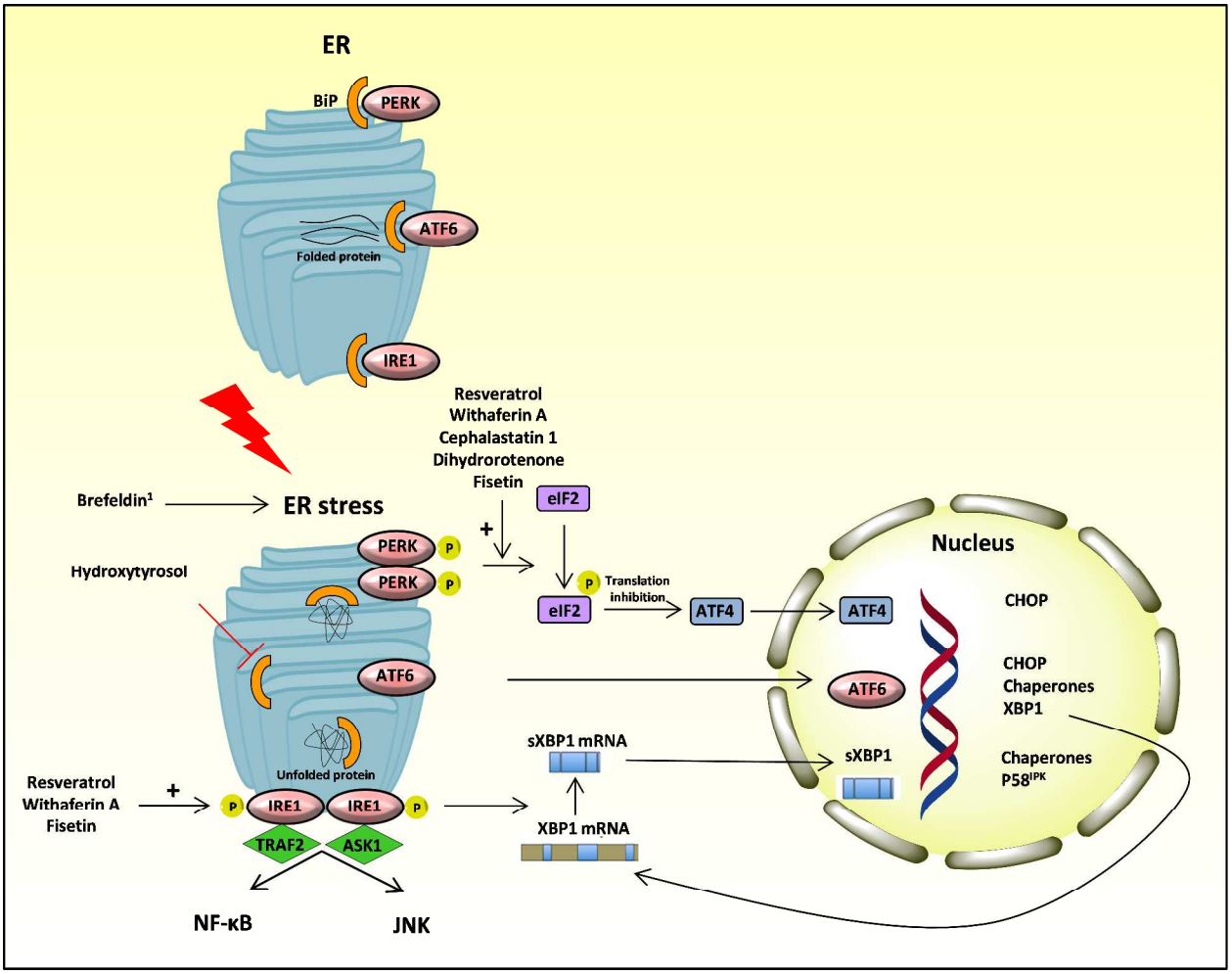
On the other hand, after GRP78 release, ATF6 is translocated to the Golgi complex and cleaved by the protease protein site (S1P) and S2P, giving rise to a functional fragment of ATF6, p50ATF6, in the cytosol that migrates to the nucleus and activates, like sXBP1, transcription of UPR genes[3].

When the UPR is activated, several emergency systems can be activated in order to cope with stressful and potentially harmful consequences of protein folding impairment, including induction of ER chaperones, degradation of unfolded proteins *via* ER degradation-enhancing α-mannosidase-like protein (EDEM), translational attenuation and NF-κB activation[3, 5, 6, 9, 10]. However, when these mechanisms are not sufficient to suppress the induced ER stress, cell death is triggered by apoptosis through the activation of proteases, kinases, apoptotic factors and transcription factors.

DNA-damage-inducible gene 153 (GADD153), also known as C/EBP homologous protein (CHOP, a member of the C/EBP transcription factor family that heterodimerizes with other C/EBPs) is induced and is responsible for several consequences of ER stress, from apoptosis to inflammation and cell cycle arrest[11]. In a general way, CHOP is ubiquitously expressed in most cells, albeit at very low levels[10]. ER stress causes the proteolytic degradation of the ER transmembrane protein ATF6, thus releasing p50ATF6, which is then transported into the nucleus where it binds to the ER stress responsive element (ERSE) of *CHOP* gene and, hence, up-regulates the levels of the protein[12, 13].

Another consequence of ER stress, frequently being responsible for the ER-triggered apoptosis (Scheme 2), is the activation of caspases, as it will be discussed in a subsequent section.

The UPR is also tightly linked to the activation of the mitogen-activated protein kinases (MAPK) pathway[14]. Following IRE1α



Scheme 1 - Major proteins involved in ER stress sensing and signal transduction and respective modulators of natural origin. [1] Brefeldin induces ER stress by inhibiting the ADP ribosylation factor (ARF), thus causing the disruption of the ER-Golgi vesicular transport, not depicted in this scheme.

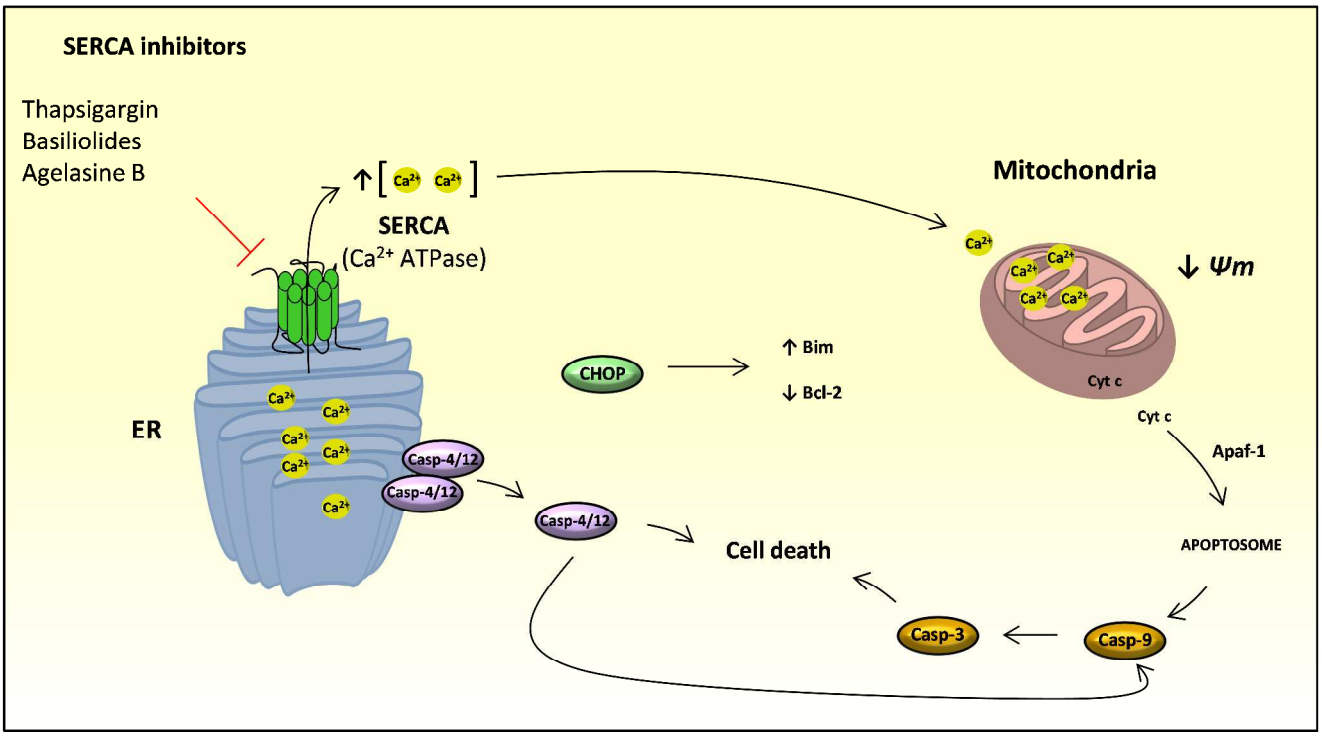
dimerization and phosphorylation, clusters of TNF receptors-associated factor 2 (TRAF2) are recruited, subsequently leading to *c*-Jun terminal kinase (JNK) phosphorylation, which, in turn, activate downstream transcription factors, such as *c*-jun and *c*-fos[15].

As it has been presented before, any event that results in misfolded or aggregated proteins may result in ER stress. In this regard, it is important to discuss the 26S proteasome, a multicatalytic enzymatic complex responsible for one of the main protein quality control pathways that has been increasingly investigated as a target for natural molecules[16].

From a structural point of view, it is composed by two 19S end caps, which recognize the ubiquitylated proteins and prepare them for degradation, and the cylindrical 20S core, responsible by protein proteolysis[17, 18].

The 20S core comprises two rings of seven α subunits and two rings of seven β subunits, stacked in the order αββα. From a functional point of view, β5, β2 and β1subunits are responsible for three different catalytic activities: chymotrypsin-like, trypsin-like and peptidyl-glutamyl peptide hydrolysis (caspase-like), respectively[17-19]. At the cellular level this complex enforces the degradation of either damaged or misfolded proteins, which are marked as such by ubiquitin.

Protein ubiquitination relies on three enzymes, Ub-activating (E1), Ub-conjugating (E2) and Ub-ligating (E3). Briefly, E1 activates ubiquitin by forming a thiol ester in an ATP-requiring process. E2 transfers activated ubiquitin, *via* an additional high energy thiol-ester intermediate, to the substrate that is bound to E3,



Scheme 2 – Major pathways connecting ER stress and apoptosis.

ubiquitin being linked to a lysine residue of the target[20]. A simplified representation of this process can be found in Scheme 3, where we present some of the natural products that are described as proteasome inhibitors and that will be discussed later. This ubiquitin-proteasome degradation pathway is pivotal for the regulation of several cellular events, including angiogenesis, proliferation, cell cycle progression and apoptosis.

Several works have shown that inhibition of the proteasome leads to ER stress and trigger the UPR [21-23], with different mechanisms linking these two events.

Misfolded proteins in the ER lumen are recognized by ER quality control systems to promote protein folding. When these mechanism are not sufficient for properly refold proteins, activation of the UPR promotes ER-associated degradation (ERAD), which involves the retrograde translocation of the misfolded proteins out of the ER [4, 5] and subsequent degradation by cytosolic 26S proteasomes [24, 25].Thus, inhibition of proteasome function prevents this process, hence generating an overload of misfolded proteins that contribute to ER stress. On the other hand, in the event of proteasome inhibition, the short-lived proteins that are usually in the cytosol to be ubiquitinated and degraded can aggregate, thus eliciting a response that involves up-regulation of the GRP78 chaperone and attenuation of protein translation by activating several branches of the ER response, such as PERK/eIF2 [22].

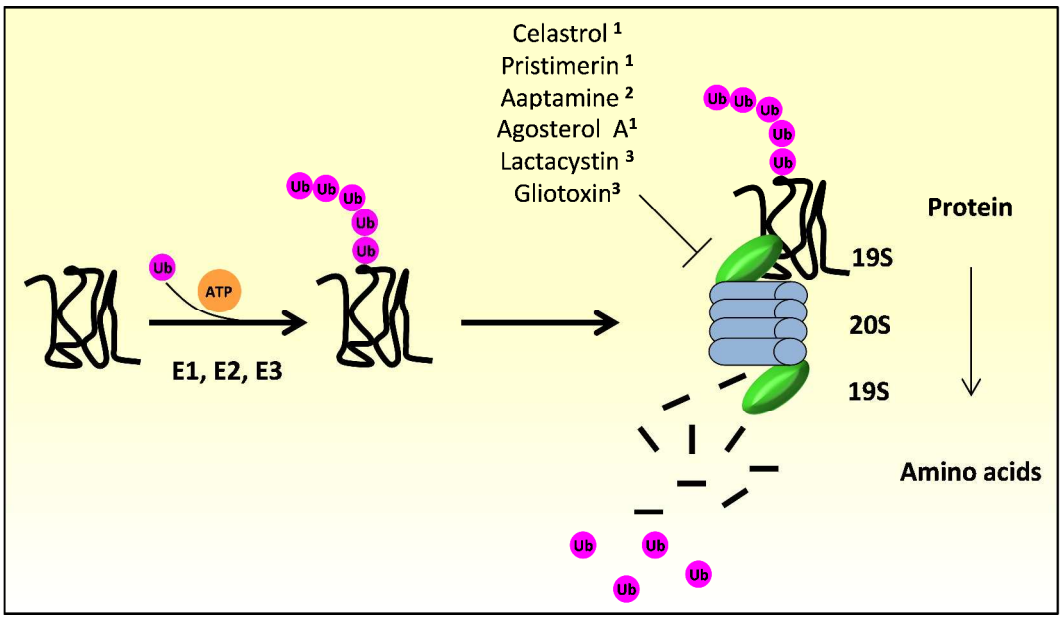
The discovery that cancer cells are more sensitive to proteasome inhibition, as a result of their higher metabolic activity and hence protein turnover has highlighted the potential role of this cellular target as an anticancer strategy. In fact, at least one proteasome inhibitor, bortezomib, has been recently approved by the FDA, as we will discuss later.

As it will be presented later in this review, there is an increasing number of natural molecules that have been found to inhibit proteasomal function, thus having a negative impact upon protein degradation.

1.1. ER stress in apoptosis

Nowadays, the role of ER stress in apoptosis is well established; however, the precise mechanisms linking these two events are not yet fully understood. Still, many natural products are known to cause apoptosis in cancer cells by triggering ER stress.

The characteristic attenuation of protein translation found in ER-stressed cells is not absolute, as some genes can bypass the eIF2-α-mediated translational attenuation. That is the case of ATF4, that include amino acid metabolism, stress response and protein



Scheme 3 - Protein ubiquitination and proteasomal degradation. [1] The compounds inhibit proteasomal function by inhibiting its chymotrypsin-like activity; [2] the compound inhibits the chymotrypsin- and caspase-like activities of the proteasome; [3] the compound inhibits the three peptidases activities of the proteasome

secretion. In addition, ATF4 is also involved in pro-apoptotic pathways via up-regulation of CHOP (Schemes 1 and 2) [11].

Other major UPR sensors, like XBP-1 and ATF6, and a cross talk between the PERK/eIF2a and the IRE1α/TRAF2/Ask1 pathways also induce transcription of the *CHOP* gene[3, 4, 10].

As presented before, IRE1α originates the sXBP1 splice form of XBP1. This molecules targets chaperones and $P58^{IPK}$, a HSP40 family member. After this point, $P58^{IPK}$ inhibits PERK and provides a negative feedback signal that attenuates PERK-mediated blockage of translation. At the moment, it is believed that up-regulation of P58IPK marks the end of the UPR. If this time-gap is enough to correct the factors that triggered UPR, then the cell survives. If, however, stress is maintained, then apoptotic cell death is triggered by activating death cascades that include CHOP, JNK and the Bcl2 family proteins [2, 10, 26, 27].

ER stress-mediated apoptosis can be inhibited by increased levels of Bcl-2 or of one of its homologues, thus indicating that this process is started by pro-apoptotic proteins[26]. Some studies refer Bim as the sole responsible for this process, without involvement of Puma or Bid, and a calcium-independent mechanism involving Bim dephosphorylation is proposed[27].

Differently, in studies using the oestrogen receptor positive human breast cancer cell line MCF-7, Puma was found to be the most important pro-apoptotic protein**,** while in the mouse embryonic fibroblast (MEF) cell line Puma and Noxa were the predominant pro-apoptotic proteins[28, 29]. Taken together, these results show that there is a cell type-specificity regarding ER stress induction of BH3-only proteins.

Regardless the proteins involved, in most cases Bim, Bid, Noxa or Puma are not enough to trigger apoptosis. Instead, they interact with other pro-apoptotic proteins, which, in turn, are responsible for the effective part of cell death. Among these proteins, Bax and Bak are pivotal to this process, as demonstrated by early reports in which deficient levels of these proteins conferred protection against ER-mediated apoptosis[30]. In resting cells, Bax and Bak proteins can be found in the ER-membrane, where they are inhibited by the anti-apoptotic protein Bcl-2.

When UPR is not sufficient to restore homeostasis, several events take place. Bcl-2 expression is suppressed by CHOP, which disturbs the equilibrium between pro- and anti-apoptotic proteins. As so, Bak and Bax are freed from the ER and translocate to the mitochondrial membrane, where they induce the formation of pores that lead to a loss of $\Delta\Psi$ and, subsequently, trigger the classical intrinsic apoptosis[8]. Although the role of Bcl2 family proteins proteins in ER-mediated apoptosis is clear, the precise link between the ER-insult and the apoptotic trigger remained elusive for a long time. Nowadays, CHOP and JNK are thought to be the link. When CHOP is expressed *via* the above-mentioned ER-stress sensors, it

represses Bcl2 gene family, thus favouring pro-apoptotic proteins. At the same time, JNK, which is critical for the phosphorylation of Bcl2 proteins, is activated by the IRE1α-TRAF2-ASK1 pathway of UPR. This results in the phosphorylation of Bcl2 which, in turn, allows Bax and Bak mitochondrial translocation and activation of the intrinsic apoptosis pathway[8].

Caspases are also major players in ER stress, especially caspase -12/4 in mice and humans, respectively, which belong to the caspase-1 subfamily and are bound to the ER membrane, being cleaved following ER stress[26, 31, 32]. The activation of these caspases is believed to take place *via* TRAF2 and a possible role of JNK in the activation of caspase-4 has also been described. However, this is probably not an universal mechanism, as some compounds were shown to activate caspase-4 even when JNK was inhibited[33-35]. Additionally, caspase-12 activation seems to be independent of the assembly of the apoptosome, as demonstrated by Apaf-1-deficient cells that were able to activate caspase-12[36, 37].

As will be presented later in this review, many of the natural molecules described as ER stress triggers also display pro-apoptotic activity, thus being potentially useful in pathological situations where apoptosis is desirable, such as cancer[38-44].

1.2. ER stress in inflammation

ER stress has been increasingly suggested as a potential source and aggravation of inflammatory processes. The crosstalk between ER stress and inflammatory response can be mediated by several distinct pathways, such JNK, production of radical oxygen species (ROS) and Ca^{2+} release, most of which result in the downstream activation of NF-κB[7, 45, 46].

NF-κB is a ubiquitous and inducible transcription factor. It is one of the most important regulators of the inflammatory response and immune system, being also involved in cell proliferation and apoptosis[47].

As it has been referred, increased levels of ROS and cytoplasmic Ca^{2+} found, for example, in response to excessive/defective protein folding in the ER can result in NF-κB activation. Differently, ER stress sensors can, *per si*, result in NF-κB activation. As mentioned before, the activation of PERK-eIF2α leads to protein translation attenuation. Given the fact that the half-life of the inhibitory protein IκB is shorter than that of NF-κB, activation of this ER stress pathway will result in NF-κB activation[48].

In addition to PERK, yet another ER stress sensor can link UPR with inflammation. Autophosphorylation of IRE1α triggers the recruitment of TRAF2. This IRE1α-TRAF2 complex has been shown to be able to recruit IκB kinase (IKK), thus causing IκB phosphorylation and NF-κB activation[15]. As can be found in Scheme 1, the IRE1α-TRAF2 complex also activates JNK. Once activated, JNK induces the expression of several inflammatory genes by phosphorylation of the transcription factor activator protein 1 (AP1)[49]. An increasing number of works link the anti-inflammatory activity of natural products with their ability to modulate ER stress pathways[50, 51].

1.3. ER stress in neurodegenerative diseases

Many etiologically unrelated neurodegenerative disorders manifest a common trait: the accumulation of insoluble, misfolded protein aggregates in the form of filamentous deposits[52, 53]. These events disrupt several signalling pathways and neuronal connectivity and, for this reason, it is understandable that situations in which protein folding is impaired, such as ER stress, can lead to the accumulation of proteins and, hence, aggravation of the disease[54-57].

Parkinson's disease (PD) is a progressive degenerative disease characterized by the loss of dopaminergic neurons in the *substantia nigra pars compacta*. At the same time, intraneuronal cytoplasmic inclusion bodies, known as Lewy bodies, are found in neurons. Several mechanisms have been linked to neuronal death in PD, from excitotoxicity to impaired energy metabolism[58, 59]. In what concerns to ER, recent studies show that ER stress, in conjunction with abnormal protein degradation, can contribute to the pathophysiology of PD[52, 56]. In fact, it is believed that, although an early UPR response may constitute a mechanism of neuroprotection for dopaminergic neurons, sustained ER stress may trigger the up-regulation of gene products that induce neuronal cell death[56].

Alzheimer's disease (AD) is a neurodegenerative disease characterized by the progressive decline of cognitive functions as a result of the loss of neurons in different brain regions, particularly frontal cortex, hippocampus and basal forebrain[56]. Some pathological findings include extracellular senile plaques composed of amyloid β-peptide (Aβ) and neurofibrillary tangles (NFT), which are intracellular filamentous structures of tau protein[60]. Neurons that contain NFT showed markedly increased Ca^{2+} levels, either a cause or consequence of the referred structures[56, 61]. The involvement of the ER was first hypothesized taking into account that Aβ is synthesized and accumulates in the ER[62]. In addition, the role of ER in protein folding further strengthened the hypothesis of the

involvement of this organelle in AD, which hallmark is the presence of misfolded/aggregated proteins.

AD-linked mutations in presenilin 1 (PS1) and 2 (PS2), two ER-resident membrane proteins with γ-secretase activity and necessary for generating Aβ, have been shown to impair the ER stress response and to sensitize cells to ER stress-induced apoptosis[62]. In the case of PS-1 mutations, the mechanisms include deregulation of Ca^{2+} homeostasis, directly by preventing Ca^{2+} entry in the cell and indirectly by triggering Ca^{2+} release from ER *via* processing of amyloid precursor protein (APP) and generation of Aβ. This perturbation of Ca^{2+} homeostasis ultimately causes apoptosis, a process in which caspase-12 is involved[63].

Milhavet et al. also report that PS1 mutation interferes with IRE1α and PERK function by decreasing the expression of BiP[64]. Apart from its role in ER stress, GPR78 levels are very important as they bind the amyloid precursor protein (APP).

Yet another link between the onset of AD and ER stress is the fact that Aβ-mediated toxicity is believed to involve ER-resident caspases. In addition, caspase-12 knockout mice showed increased resistance to Aβ-induced cell death[65].

Despite the increasing body of proof linking ER with several neurodegenerative diseases, further studies are necessary to validate this organelle as a druggable target for the prevention/treatment of these conditions[66].

2. Natural products as modulators of the ER

2.1. ER STRESS TRIGGERS

2.1.1. SERCA inhibitors

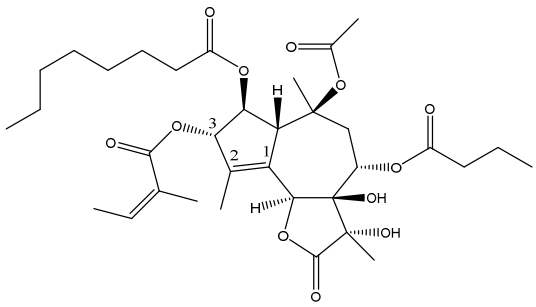
Thapsigargin

The Mediterranean umbelliferous plant *Thapsia garganica* is the source of the sesquiterpene lactone thapsigargin (**1**, Table 1), which, in addition to tunicamycin and brefeldin A, is one of the most widely used and recognized ER stress inducers, albeit through a different mechanism of action. From a biochemical point of view, thapsigargin is an irreversible SERCA inhibitor (**1**, Table 1), thus having a profound impact upon Ca^{2+} homeostasis.

Following thapsigargin incubation, Ca^{2+} is released from the ER, hence increasing its cytoplasmic concentration[43]. Part of this Ca^{2+} is taken by the mitochondria, leading to the dissipation of the $\Delta\Psi$, with release of cytochrome *c* and subsequent activation of the intrinsic pathway of apoptosis (Scheme 2).

The structural characteristics that result in binding to SERCA are fairly understood and several analogues have been synthesized[67].

The enantioselective total synthesis of thapsigargin has been described. With a ketoalcohol as a starting point, key steps involve regioselective introduction of the internal olefin at C_4–C_5, protecting group choice to allow chelation-controlled reduction at C_3, and chemoselective introduction of the angelate ester function at C_3-O, with an overall yield of 0.61% being reported[68].



1

Transtaganolides and basiliolides

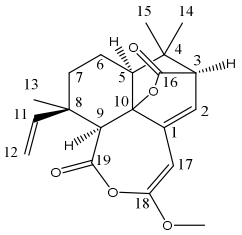
Species belonging to the genus *Thapsia*, the origin of the above-mentioned inhibitor thapsigargin, are also the source of another group of compounds that target the ER. These compounds, structurally unrelated to thapsigargin, are tetracycle C_{19} dilactones, sometimes addressed as tetrahomosesquiterpenoids, and are believed to be biosynthesized through a carbon dioxide-triggered electrophilic polyolefin cyclization route[69].

The chemical diversity of this small group of molecules resides mainly in the oxidation of the gem-dimethyl group at C4, which can result in different patterns of oxygen bridging.

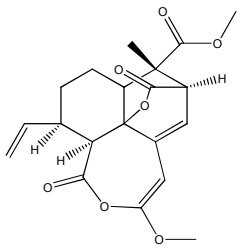
The first authors to describe these compounds, Appendino et al., coined the term transtaganolides for compounds having the 3-carboxy group oxygen bound to C_{15}, while basiliolides is reserved for molecules where the 3-carboxylate is oxygen bound to C_{10} (**2-4**, basiliolide A1, B and C, respectively)[69]. From a structural point of view, transtaganolide and basiliolide molecules exhibit a complex polycyclic ring system that comprises a transdecalin framework, a bridging lactone and a rare cyclic acyl ketone acetal[70, 71].

In mammalian cells basiliolide A1 (**2**, Table 1) leads to a rapid depletion of ER Ca^{2+} levels, with subsequent increase of cytoplasmic content, in a range comparable to that of thapsigargin[72]. Contrarily to thapsigargin, basiliolide A1 fails to elicit apoptosis, thus suggesting that its effect upon Ca^{2+} must follow a different pathway, as it is not able to trigger the ER-stress *stimulus* required to cause a pro-

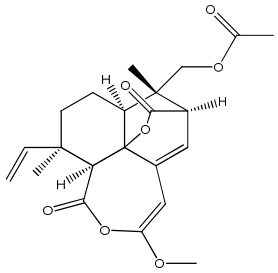
apoptotic effect. In addition, basiliolide A1 fails to trigger the pathways leading to the activation of NF-κB and activator protein 1 (AP-1), being once again distinct from thapsigargin. The compound is, however, able to synergize with both phorbol-myristate acetate (PMA) and ionomycin, inducing complete degradation of the NF-κB inhibitory protein IκB-α and increasing JNK1 and JNK2 phosphorylation status[72].



2



3



4

It has been suggested that the fact that basiliolide A1, despite being a SERCA inhibitor similarly to thapsigargin (Scheme 2), fails to trigger both apoptosis and NF-κB activation as a consequence of the different kinetics of its binding with the ATPase.

Thus, the kinetics displayed by basiliolide A1 would cause a slow Ca^{2+} release from ER, hence allowing reuptake of the ion to take place, leading to an equilibrium between cytosolic and ER Ca^{2+} concentrations. Differently, in the case of thapsigargin this process is much faster, which results in a fast increase of Ca^{2+}, thus bypassing the cell's ability to restore Ca^{2+} homeostasis, resulting in apoptosis[72].

The synthetic routes for basiliolides have been subjected to extensive investigations. Gordon et al. have obtained basiliolide C *via* an Ireland–Claisen/Diels–Alder cascade[73]. The use of a palladium-catalysed cross-coupling of methoxy alkynyl zinc reagents allowed the protecting group-free syntheses of transtaganolides C and D by generating three rings, two all-carbon quaternary centres, and four tertiary stereocentres from a monocyclic, achiral precursor[73]. The same compounds have been obtained by Larsson et al. following a biomimetic approach[74].

Other groups conducted cyclopropanation/ring opening strategy for establishing the stereogenic centers at C_8 and C_9, a biomimetic 2-pyrone Diels–Alder cycloaddition for the synthesis of the ABD ring system, with subsequent biomimetic intramolecular O-acylation for the C ring formation[75].

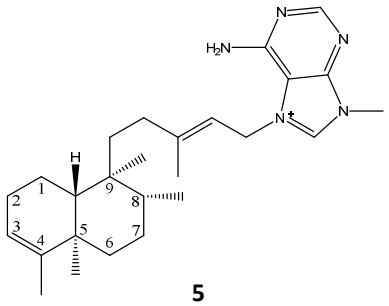
Agelasine B

Agelasine B is a *trans*-clerodane diterpene with a 9-methyladeninum substitution (**5**, Table 1) found in the marine sponge *Agelas clathrodes*.

In a work by Pimentel et al, agelasine B was shown to decrease the viability of several cancer cell lines, namely MCF-7, SKBr3 and PC-3[42]. The terpene causes significant release of Ca^{2+} from the ER, an effect that has been linked to its activity as a SERCA inhibitor. The cellular fate of Ca^{2+} can be tracked to mitochondrion, where its increasing concentration elicits the dissipation of the $\Delta\Psi$, with subsequent apoptosis. Thus, the effect caused in mitochondrion can be tracked back to the impact upon ER Ca^{2+} homeostasis.

In MCF-7 cells incubated with agelasine B, increased levels of active caspase-8 are found, while no effect in the effector caspase-7 can be detected. The mechanism of action behind the pro-apoptotic effect of agelasine B is associated to a reduction of Bcl-2 levels[42].

Agelasine B can be prepared from decalone in a route involving the stereoselective alkylation of nitrile derivatives and coupling of iodides in order to produce the clerodane skeleton, with subsequent electrochemical reduction yielding agelasine B[76].



5

2.1.2. Modulators of ER stress sensors

Resveratrol

Resveratrol (**6**) is one of the most widely studied natural products, being investigated for its potential applications in situations like oxidative stress, inflammation or cancer, among others. From a phytochemical point of view, resveratrol is a stilbene

(**6**, Table 1) that can be found in several species, notably the skins of red grapes. Resveratrol has been shown to cause accumulation and misfolded proteins in cancer cells, thus triggering ER-stress[77].

In human nasopharyngeal carcinoma cells exposed to resveratrol activation of the UPR and ER stress can be found, as ascribed by the expression levels of IRE1α, p-PERK, ATF-6 and CHOP. Furthermore, ER stress is accompanied by apoptosis, as shown by increased levels of cleaved PARP, and by autophagy, ascribed by TEM and LC3-II levels. Detailed TEM and confocal microscopy studies show that resveratrol induces ER-targeted autophagy, a process coined by Bernales et al. as ER-phagy[78]. This process is pointed as a defence mechanism of the cell, which is confirmed by the increase in apoptosis following silencing of ATG7, a pivotal autophagy-related protein. Interestingly, silencing of IRE1α and CHOP had no effect on resveratrol-mediated apoptosis, thus demonstrating that another pathway was linking ER-stress to the apoptotic process.

The role of the ER-resident caspases-12 and -4 has been investigated by using specific inhibitors of these enzymes, which, in the case of the former but not of the latter, markedly attenuates resveratrol-mediated activation of caspase-3 and -9 and hence the apoptotic process.

Given its rather simple structure, several synthetic routes have been described for this molecule[79-82].

Withaferin A

Withaferin A is a steroidal lactone (**7**, Table 1) isolated from *Withania somnifera* and constitutes the lead compound of the withanolide sub-class of natural products[83]. Several works point to the anticancer activity of this molecule against several cell types, an effect that has been shown to involve induction of Par-4, Bim and FOXO3a, activation of p38 pathway, as well as up-regulation of death receptor 5 (DR5) and attenuation of the NF-κB, Akt and Notch signalling pathways[84-88].

In addition to this mechanism of action, withaferin A causes a peak of ATF4, CHOP and GRP78 expression levels, with splicing of XBP1 and phosphorylation of eIF-2α (Scheme 1)[83]. This process involves oxidative stress, as ascribed by the fact that it is inhibited by pre-incubation with the antioxidant *n*-acetylcysteine. Likewise, inhibition of caspase-4 attenuates the apoptotic process.

Using an *in silico* docking approach, Yang et al. hypothesized that withaferin A could be a novel proteasome inhibitor, predicting that C_1 and C_{24} would be susceptible towards a nucleophilic attack by the hydroxyl group of *N*-terminal threonine from the proteasome chymotrypsin subunit. In subsequent experiments using human prostate cancer cells this activity was confirmed[89].

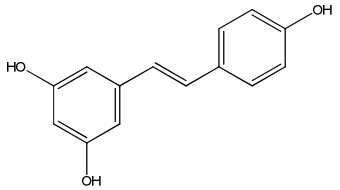
The stereoselective synthesis of withaferin A or the related 27-deoxywithaferin A can be accomplished by introduction of the desired substituent at C_{25} and steroselective construction of the A:B rings by a facile allyl sulfoxide—sulfenate rearrangement[90].

Dihydrorotenone

Dihydrorotenone (**8**, Table 1) is a derivative of rotenone, the major natural pesticide of derris and *Lonchocarpus* (Fabaceae), being used in fishing, gardening (organic) and farming. Dihydrorotenone is the result of rotenone reduction by the action of soil bacteria, which saturate the side chain of the parent compound[91, 92].

Zhang et al. showed that dihydrorotenone was able to trigger apoptosis, as a consequence of the loss of mithocondrial ΔΨ that follows the inhibition of the complex I in respiratory chain. Thus, the mechanism of action is distinct from that of most of the compounds discussed above, whose effect upon ΔΨ involved changes in Ca^{2+} levels[44]. Dihydrorotenone also causes an increase in the expression levels of GRP78, ATF4 and CHOP, thus showing its ability to trigger the UPR and ER-stress[42].

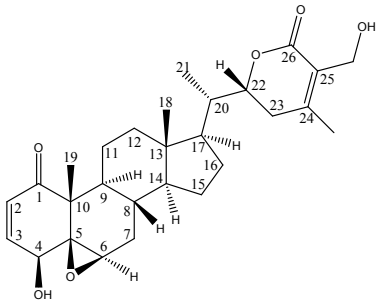
The effect of this natural pesticide on kinases usually associated with ER stress has been evaluated in several cell lines, showing an increase of the phosphorylation of p38, but not of JNK[44]. This is in opposition to previous reports on rotenone, in which both kinases were activated. The involvement of p38 in dihydrorotenone-induced apoptosis was demonstrated by the fact that SB203580, a specific inhibitor of p38, attenuated the cleavage of PARP and activation of caspase-3[44]. In a general way, dihydrorotenone is synthesized by hydrogenation of other rotenoids[93].



6

Cephalostatin 1

Cephalostatin 1 is a marine bis-steroidal pyrazine molecule with remarkable chemical complexity, being made up of two C_{27}



7

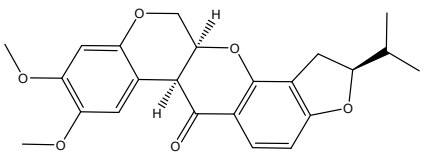
steroids that are substituted isomers of hecogenin (**9**, Table 1).

This tube worm-derived molecule has attracted several researchers, as it displays subnanomolar anticancer activity. It was the first compound described to trigger apoptosis without activation of caspase-8 or release of cytochrome *c* from the mitochondria and apoptosome formation, while activating caspase-9[39]. This mechanism of action is so distinct from classical apoptotic pathways that some authors refer to the "cephalostatin pathway of apoptosis"[94].

Cephalostatin 1 increases the expression of GRP78 and CHOP and also the phosphorylation of eIF-2 (Scheme 1). The involvement of ASK1-JNK pathway and activation of caspase-4 is also known. From a temporal point of view, activation of caspase-4 preceded that of caspase-9[33].

Cephalostatin 1 is a natural product of remarkable chemical complexity, its synthesis being a considerable challenge. The detailed discussion of its synthetic routes is beyond the scope of this work. Nevertheless, a few considerations can be established.

It is frequent to address cephalostatin 1 by considering its two major components, the western and eastern halves. Fortner and colleagues have described their approach to this compound, with key steps being a methyl group selective allylic oxidation, directed C−H hydroxylation of a sterol at C_{12}, Au(I)-catalysed 5-endo-dig cyclization and a kinetic spiroketalization[95]. LaCour et al. described the convergent total synthesis of cephalostatin 1 and two hybrid analogues, ritterostatins GN1N and GN1S[96].



8

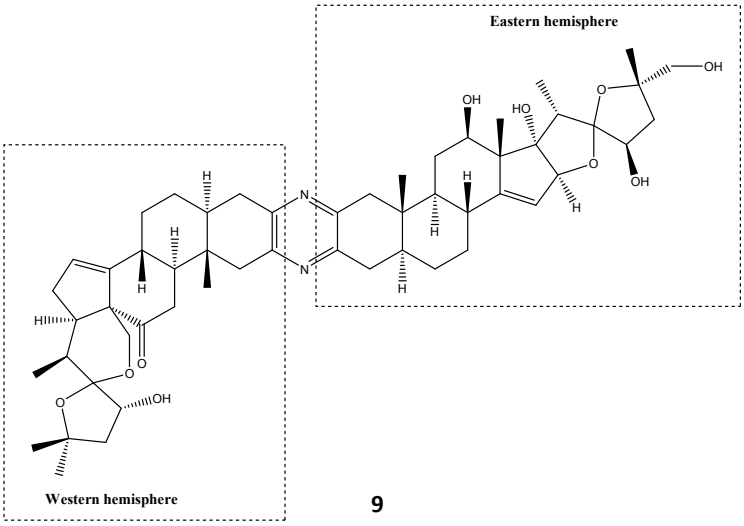
Fisetin

Fisetin is a flavonol (**10**, Table 1) that can be found in many plants and fruits, especially strawberries and mangoes, and that has been investigated for several biological activities, from nerve differentiation to apoptosis[97-101].

In the human melanoma cell lines A375 and 451Lu, fisetin elicits ER stress through several pathways, namely IRE1α, sXBP1, ATF4 and GRP78. Interestingly, both the intrinsic and extrinsic pathways of apoptosis are activated, the latter being mediated by TNFR-1 and TNFR-2, but not DR-3, DR-4 and DR-5, contrarily to other molecules referred earlier in this work, which triggered the extrinsic pathway of apoptosis[102].

This flavonol causes phosphorylation and activation of AMPK-activated protein kinase (AMPK), which is involved in pathways related to apoptosis and cell cycle regulation[102].

From a chemical synthesis point of view, several routes can be used to obtain fisetin, from aroylation of *o*-hydroxyacetophenone[103] to the 7-hydroxyflavonol synthesis method proceeding from resacetophenone and benzaldehyde[104].



9

2.1.3. Proteasome inhibitors

Celastrol

Celastrol is a quinone methide pentacyclic triterpene (**11**, Table 1) isolated for the first time from *Tripterygium wilfordii*, also known as the Thunder God vine[105]. Following initial results that showed the pro-apoptotic effect of this molecule upon cancer cells, Yang et al. revisited the mechanism of action of this triterpene by evaluating its ability to inhibit the proteasome[106]. The authors showed that celastrol inhibited the chymotrypsin-like activity of a purified 20S proteasome (IC_{50} = 2.5 μM) and human prostate cancer

cellular 26S proteasome (at 1-5 μM) (Scheme 3). Among the several proteins up-regulated after proteasome inhibition, the authors identified Bax and p27 as probable players in the apoptotic effect [106]. Semi-synthesis of several biotinylated[107] and C_{28} ester and amide[108] analogues can be found in literature.

Lactacystin

Lactacystin (**12**, Table 1) is a natural product obtained from some *Streptomyces* species.

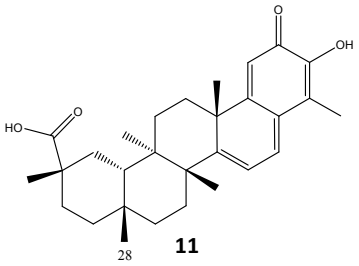
Earlier studies on the biological activity of this lactam revealed its ability to inhibit cell growth and to induce neurite outgrowth in the murine neuroblastoma cell line Neuro-2a[109]. Even before the molecular target was identified, structure-activity relationship (SAR) studies were performed using lactacystin analogues, showing that an electrophilic carbonyl at the C_4 position was required for activity[110]. It was also shown that reactive electrophiles were required in the molecule, thus implying that the unknown target was prone to nucleophilic attack[110, 111].

Nowadays it is known that lactacystin is a proteasome inhibitor, in particular it inhibits all three peptidase activities of the proteasome, chymotrypsin-like, trypsin-like and caspase-like, the first two irreversibly. The lactacystin-induced ER stress that leads to apoptosis has been widely described in literature in several cell lines[112, 113] and is a consequence of the activation of ER stress sensor proteins that follows proteasome inhibition, as discussed above. Lactacystin is, in fact, a pro-drug, as it undergoes spontaneous intramolecular formation of a lactone ring by attack of the β-hydroxy function of the γ-lactam ring to cleave the thioester bond, with concomitant loss of *N*-acetylcysteine. The resulting clasto-lactacystine-β-lactone (**13**, omuralide) is the biologically active molecule[114].

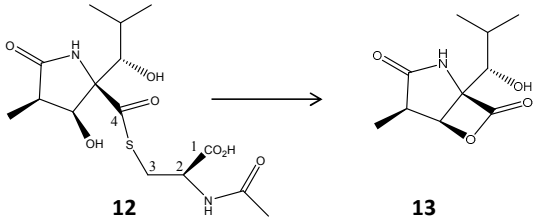
The first total synthesis of lactacystin was achieved by Corey et al. in 1992, starting from *N*-benzylserine methyl ester[115]. Since then, several distinct synthesis have been described, many of which using different starting molecules, such as glutamate[116], (2*R*,3*S*)-3-hydroxyleucine[117]and glucose[118]. Many other synthesis are available [119-121] and an excellent review regarding the synthesis of lactacystin and related compounds is available[122].

Pristimerin

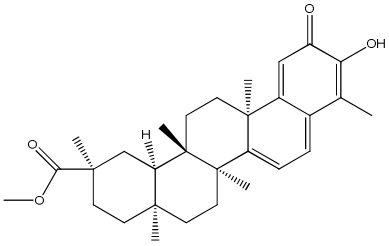
Pristimerin, like the above mentioned celastrol, is a quinone methide pentacyclic triterpene (14, Table 1) and can be obtained from several species from Celastraceae and Hippocrateaceae.



11



12 **13**



14

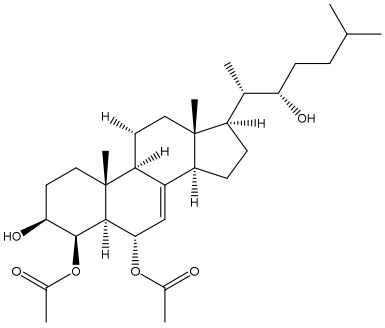
Earlier studies demonstrated the ability of this compound to cause cytotoxicity in several cancer cell lines and Wu et al. addressed the mechanism of action in the human breast cancer cell line MDA-MB-231[123]. Overall, the compound triggers apoptosis, as shown by the loss of mitochondrial ΔΨ, cytochrome *c* release, as well as caspase activation, DNA fragmentation and morphological changes. Interestingly, the levels of Bcl-2, Bcl-X_L and Bax are not altered.

Bcl-2 overexpression fails to prevent pristimerin-induced apoptosis, thus suggesting a non-classical mechanism of action[123]. Yang et al. confirmed this mechanism in the prostate cancer cell line PC-3[124]. In addition, it was shown that an inhibition around 30% of the proteasomal chymotrypsin-like activity (Scheme 3) and also accumulation of polyubiquitinated proteins take place. Further works were conducted on the interaction of pristimerin and the proteasome, as well as kinetic studies[124]. Synthetic routes for pristimerin derivatives are essentially similar to those of the closely related celastrol, discussed above.

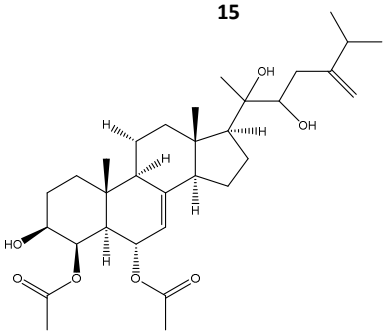
Agosterols

Agosterols are polyhydroxylated sterol acetates isolated from *Spongia* sp.[125] and, later, from *Acanthodentrilla* sp.[126]. Initial studies

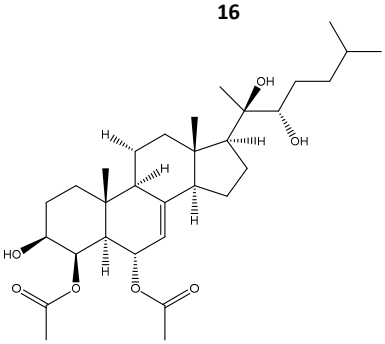
showed the ability of agosterol A to reverse P-glycoprotein (P-gp)-mediated multidrug resistance in tumour cells[125]. Tsukamoto et al. evaluated the proteasome-inhibiting capacity of ten compounds from this series, expressed as chymotrypsin-like inhibition. The most potent compounds were agosterol C (**15**), C_7 (**16**) and C_2 (**17**), with IC_{50} of 10, 20 and 23 μg/mL, respectively[126]. The total synthesis of agosterol A proceding from ergosterol has been described, relying in a regioselective epoxy-cleavage reaction and regioselective dehydroxylation as key reactions[127].



15



16



17

Flavonoids

Several flavonoids have been described as proteasome inhibitors, namely apigenin (**18**), quercetin (**19**), myricetin (**20**) and kaempferol (**21**), which displayed IC_{50} values towards 20S proteasome of 1.8, 3.5, 10.0 and 10.5 μM, respectively[128].

The presence, number and position of hydroxyl groups in the B-ring are believe to play a relevant role in the activity displayed.

In another study by the same authors, chrysin (**22**), luteolin (**23**), naringenin (**24**) and eriodictyol (**25**) were evaluated, yielding 20S proteasome IC_{50} values of 4.9, 1.5, 48.9 and 16.2, respectively[129].

Thus, the order of potency for this class of natural products is luteolin>apigenin>quercetin>chrysin>myricetin>kaempferol>eriodic tyol.

Gliotoxin

Gliotoxin (**26**, Table 1) was first described in 1943 by Johnson et al.,[130] but its structure was only revealed in 1966 in a work by Beecham et al.[131], which placed this compound in the class of epipolythiodioxopiperazines, characterized by a heterobicyclic moiety containing a polysulphide bridge with 2–4 atoms of sulphur. Several studies suggest that the sulphide bridge is pivotal for the biological effect, as shown by the loss of activity against virus and bacterial proliferation when it is disrupted[132-134].

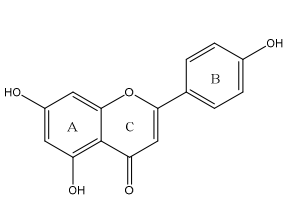
In the specific case of the proteasome, all proteolytic activities of purified proteasome are inhibited in a non-competitive manner, though the effect is more pronounced towards the chymotrypsin-like, reaching 95% of inhibition at 100 μM.

The reducing agent dithiothreitol, which converts gliotoxin to the corresponding thiol, reverses this inhibition, thus confirming the relevance of the sulphide bridge to the activity of this fungal metabolite [132]. Several synthetic routes for gliotoxin are available, both for racemic and optically active gliotoxin[135, 136].

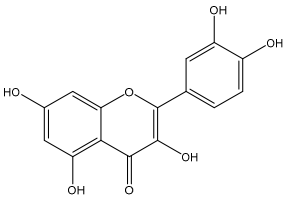
Aaptamines

Aaptamines are a class of 1*H*-benzo[*de*][1,6]-naphthyridine alkaloids that were first isolated by Nakamura et al., from the sponge *Aaptos aaptos*[137]. Initial studies showed that they exhibited antineoplastic and cancer cell growth inhibitory activity[138, 139]. Recently, the ability of several aaptamines for inhibiting the proteasome were described[140]. Aaptamine, isoaaptamine and demethylaaptamine (**27-29**, respectively) were evaluated for their capacity for inhibiting the chymotrypsin- and caspase-like activity of a 20S proteasome preparation from human erythrocytes, with IC_{50} values around 19, 7 and 10 μM, respectively.

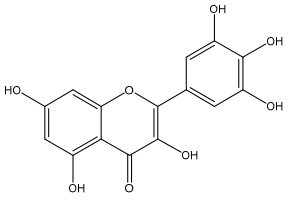
The majority of synthesis processes published for aaptamine exploit either the isoquinoline (AB moiety[141, 142]) or quinoline (AC moiety [143]) components of the benzo[de][1,6]-naphthyridine ring as a platform for obtaining the third ring. Other routes, starting from simpler molecules, such as veratrol[144] and nitrovanillin[145], have also been described.



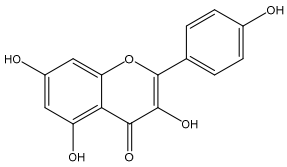
18



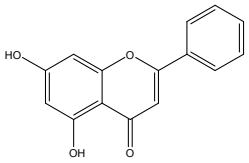
19



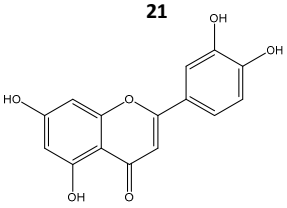
20



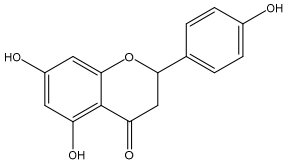
21



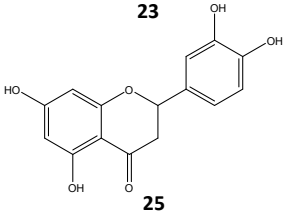
22



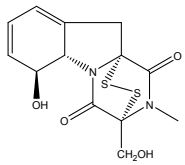
23



24



25



26

2.1.4. Other mechanisms of action

Tunicamycin

Tunicamycin (**30**, Table 1) is mixture of nucleoside homologues originally isolated from a new species of the genus *Streptomyces*, *Streptomyces lysosuperificus*, and characterized in 1971[146]. Just a few years later, its ability to inhibit *N*-linked protein glycosylation was described, thus paving its way to other potential biological applications[147-149].

From a chemical point of view, tunicamycins contain *N*-acetyl glucosamine, uracil, a 11-carbon aminodialdose named tunicamine, as well as a fatty acid linked to the amino group. The difference of the fatty acid molecule results in the several homologues of which A, B, C and D are the most relevant for their biological properties[146, 150, 151].

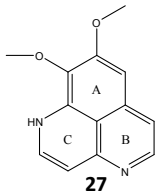
Tunicamycin is an inhibitor of both bacterial and eukaryote *N*-acetylglucosamine transferases, thus preventing the formation of *N*-acetylglucosamine lipid intermediates, as well as glycosylation of newly synthesized glycoproteins[152]. Nowadays, tunicamycin is one of the most widely used compounds for eliciting ER-stress, being found in most works in this area as a positive control. Commercial tunycamin is often a mixture of several homologues.

In 1984, Suami et al. described the synthesis of eight analogues of tunicamycins by condensation of a *N*-acetyl-D-glucosamine derivative with an anomeric chloride of tunicaminyl uracil, followed by deprotections and *N*-acylation[153]. In 1989, the fully synthetic stereoselective routes to the differentially protected subunits of the tunicamycins has also been described[154].

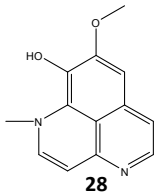
Brefeldin A

Brefeldin A (**31**, Table 1) is a 16-membered macrocyclic lactone found in *Penicillium brefeldianum*, whose biosynthesis proceeds from palmitate as its chemistry suggests. This fungal metabolite causes a reversible dissociation of a Golgi-associated peripheral membrane protein that is identical to one of the subunits of the coat of Golgi-derived (non-clathrin) coated vesicles, β-COP, thus implying that brefeldin A prevents protein traffic by blocking the assembly of protein coats and hence the budding of enclosed vesicles. Inhibition of ARF also takes place, which compromises the Golgi-ER vesicular transport, a phenomenon that results in Golgi and ER stress[155-157].

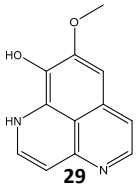
Several groups have taken the task of synthesizing this biologically relevant molecule and, in a general way, with good results[158-163]. Brefeldin has been obtained through several synthetic routes, starting from mannitol+glutamic acid[164], a β-lactone-based cyclopentane derivative, among others.



27



28



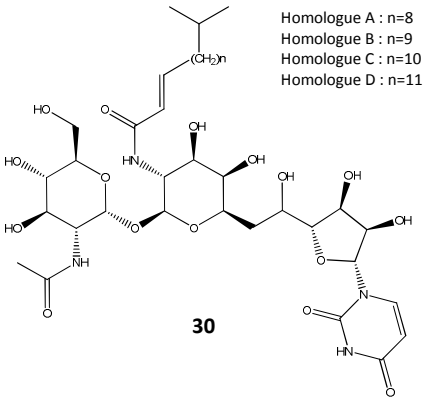
29

2.2. ER protectors

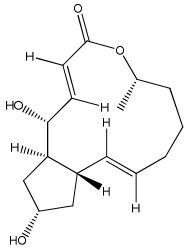
Berberine

Berberine is a benzyl-tetrahydro isoquinoline alkaloid (**32**, Table 1) present in several species, notably *Berberis* sp.

Initial studies focusing in its effect upon HIV therapeutics and insulin signal transduction suggested that the mechanism of action of this molecule might include negative modulation of ER-stress, thus displaying a biological effect distinct from other molecules discussed earlier in this work[51, 165].



30



31

In the colon cancer cell line Caco-2, berberine protects cells from IFN-γ plus TNF-α-induced apoptosis[166]. This molecule also proved to be efficient in preventing the tunicamycin or IFN-γ plus TNF-α-induced up-regulation of GRP78 expression and the splicing of XBP1 mRNA[166]. In addition, co-incubation of berberine with pro-inflammatory cytokines attenuates caspase-3/12 expression and activity.

Over a hundred berberine derivatives, and their respective (semi)-synthetic routes, can be found in literature. These derivatives can be grouped according to the substitution pattern. 9-*O*-Substituted derivatives include either aza-aromatic terminal groups[167, 168], alkylated[169, 170] and glycosylated[171]. 8-Alkyl derivatives, albeit less frequent, have also been described[172].

Hydroxytyrosol

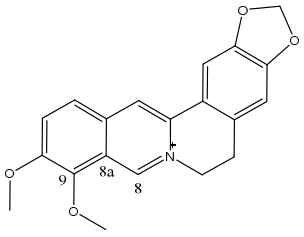
Hydroxytyrosol (**33**, Table 1) is a rather simple phenolic compound that can be found in several nutritionally-relevant plant species and foodstuffs, notably olive oil, in which it is a major compound[173].

Hydroxytyrosol has been investigated for its ability to attenuate tunicamycin-induced ER stress in human hepatocarcinoma cells. In resting cells this polyphenol reduces the basal mRNA levels of CHOP and GRP78[174]. The same activity is observed in tunicamycin plus hydroxytyrosol-treated cells, in which Bcl2 levels being restored to control levels.

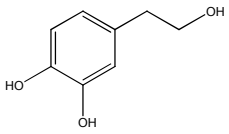
While hydroxytyrosol-treated cells display no change in phosphorylated-eIF2α when compared with the controls, co-incubation of the polyphenol with tunicamycin successfully lowers phosphorylated-eIF2α protein levels, thus showing that the effect of hydroxytyrosol occurs *via* modulation of the PERK branch of UPR (Scheme 1)[174]. Hydroxytyrosol is one of the most simple phenols and, for this reason , many strategies for its synthesis can be found in the literature, with starting materials like tyrosol, homovanillyl alcohol or dihydroxybenzaldehyde[175, 176].

Vaticanol B

Vaticanol B (**34**, **Table 1**) is a resveratrol tetramer that can be found in the bark of *Vatica rassak* [177]. In concentrations in the 10-20 μM range, vaticanol B successfully attenuates both tunicamycin and thapsigargin-triggered ER stress-induced cell death in F9 embryonal carcinoma cells.



32

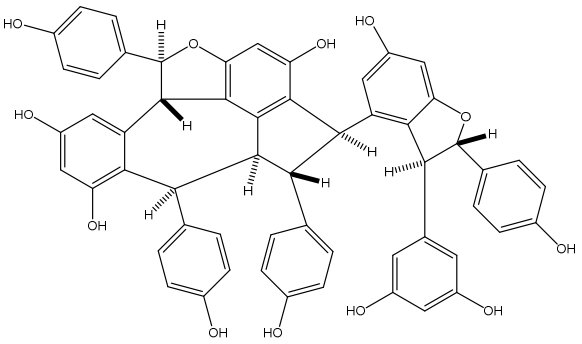


33

This compound prevents the up-regulation of GRP78 and CHOP, the target genes of ATF6/IRE1α-XBP1 and PERK-eIF2α pathways, respectively. Thus, the effect of vaticanol B is not related with the activation of the UPR[178].

Interestingly, this molecule also displays anti-inflammatory activity in LPS-activated macrophages. The mechanism of action is believed to involve the ER, as shown by the ability of vaticanol B to prevent LPS-triggered loss of ER membrane integrity that results from the altered distribution of ER luminal proteins. This effect is likely to be associated with the regulation of cytosolic phospholipase

A2 ($cPLA_2$) activation and protein synthesis. To the best of our knowledge, no synthetic routes for vaticanol B have been described.



34

2.3. Molecules in clinical use/evaluation

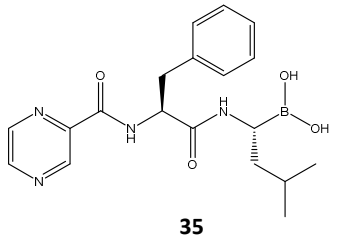
As far as we know, proteasome inhibitors are the most representative class of the compounds discussed in this work currently used in clinic or undergoing clinical trials.

Boronate peptides are synthetic molecules that are structurally related to the naturally-occurring peptide aldehydes, of which the natural products tyropeptin and fellutamide B, isolated from *Kitasatospora* sp and *Penicillium fellutanum*, respectively, are a good example. Bortezomib (**35**), a boronate peptide, is one of the most widely used drugs for the treatment of multiple myeloma and mantle cell lymphoma, being the first-in-class proteasome inhibitor in clinic. It has been included in clinical trials in 1999 and was approved by the FDA in 2003 and in Europe in 2004. Its citrate boronic ester derivative, MLN-9708 (**36**), has improved pharmacokinetics, thus being administered orally as a pro-drug, being enrolled in over a dozen clinical studies. Recently, it has entered phase III trials by the Japanese company Takeda[179].

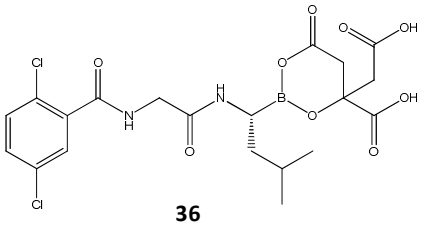
Delanzomib (**37**), another peptide boronate inhibitor, has successfully concluded phase I trials[180], phase II trials being currently undergoing.

Carfilzomib (PR-171, **38**) was developed by Proteolix Inc., later continued under Onyx Pharmaceuticals, and over 10 clinical trials are active at the moment. On June 2012 the FDA's Oncologic Drugs Advisory Committee concluded that carfilzomib, under the name Kyprolis, had a favourable benefit–risk assessment for the use in patients with relapsed and/or refractory multiple myeloma who have received at least two prior lines of therapy (proteasome inhibitor and immunomodulatory agent).

Salinosporamide A (**39**) is a molecule isolated in 2003 from the bacteria *Salinospora tropica*[181] and, like the active metabolite of lactocystin, it displays a γ-lactam-β-lactone moiety, albeit with a different substitution pattern that includes an unusual cyclohexenyl substituent, a methyl group situated at the β-lactone system and a chloroethyl side chain at the α position.



35



36

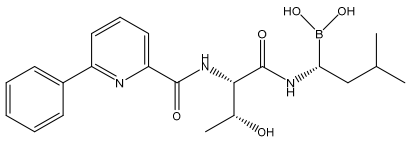
Owing to its remarkable potency at the nanomolar range, this molecule has entered phase I clinical trials only 3 years after its discovery and is currently under evaluation for several applications.

In 2013, orphan drug designation was assigned in the U.S. for the treatment of multiple myeloma. Several synthesis for salinosporamide A have been described, including those starting from 4-pentenoic acid[182], a pyroglutamate derivative or a β-keto amide[183]. However, the first synthesis described, which would inspire other research groups would be that of Reddy et al., starting with a (S)-threonine derivative and yielding some impressive 16.5%[184].

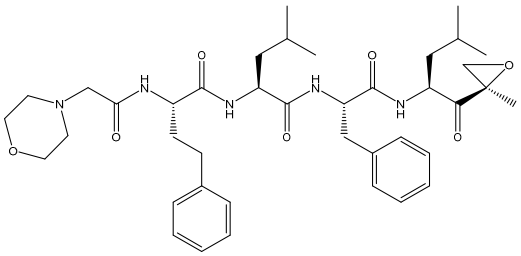
3. Future directions

The role of ER in health and disease is a topic that has received increasing attention in the last few years, with the number of works addressing this organelle growing steadily.

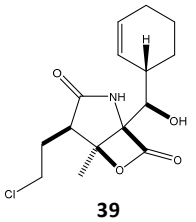
While there seems to be no doubt that ER dysfunction is found in many pathological conditions, such as inflammatory diseases, Parkinson's or Alzheimer's, in many cases it is not clear if this trait is either the cause or consequence of such pathologies. Remarkable advances have been reported recently and in the next few years we expect the remaining points to be elucidated. This may ultimately result in the establishment of the ER as a drug target for the pharmacotherapy of the above-mentioned conditions.



37



38



39

In this regard, natural products are expected to play a pivotal role in this approach, either *per si* or as starting point for semi-synthesis, as they are among the most potent molecules that are known to modulate this organelle. As so, ER stressors may be helpful as pro-apoptotic molecules with potential application in cancer, while compounds that ameliorate or prevent ER stress can be useful in situations when ER stress is the putative cause of disease, such as in inflammatory conditions.

Regardless of the case, further studies are required in this field, particularly those concerning to the precise mechanism of action of these compounds.

Table 1

#	Compound	Natural source	Class	Synthetic route	Target	Target reference
1	**Thapsigargin**	*Thapsia garganica*	Sesquiterpene lactone	68	SERCA	43
2	**Basiliolide A1**	*Thapsia* sp.	Tetracyclic C_{19} dilactone	73, 75, 185	SERCA	78
5	**Agelasine B**	*Agelas clathrodes*	*trans*-Clerodane diterpene	*76*	SERCA	42
6	**Resveratrol**	Several	Stilbene	79-82	IRE1α/PERK/ATF6	38
7	**Withaferin A**	*Withania somnifera*	Steroidal lactone	90	PERK/IRE1α /Proteasome inhibitor	81
8	**Dihydrorotenone**	*Derris* sp. / *Lonchocarpus* sp.	Rotenoid	93	Mitochondrial ΔΨ / ATF4	44
9	**Cephalostatin 1**	*Cephalodiscus gilchristi*	Bis-steroidal pyrazine	95, 96, 186	PERK/ASK1-JNK	33
10	**Fisetin**	Several	Flavonol	103, 104	IRE1α / ATF4	103
11	**Celastrol**	*Tripterygium wilfordii*	Quinone methide pentacyclic triterpene	107, 108 [1]	Proteasome inhibitor	105
12	**Lactacystin**	*Streptomyces* sp.	Lactam	115-122	Proteasome inhibitor	107,108
14	**Pristimerin**	Celastraceae / Hippocrateaceae	Quinone methide pentacyclic triterpene	107, 108 [1]	Proteasome inhibitor	112
15-17	**Agosterols**	*Spongia* sp / *Acanthodentrilla* sp.	Polyhydroxylated sterol acetates	127	Proteasome inhibitor	126
18-25	**Several flavonoids**	Several	Flavanols	103, 104	Proteasome inhibitor	128, 129
26	**Gliotoxin**	Several fungi	(Epipolythiodioxo)piperazine	135, 136	Proteasome inhibitor	132
27-29	**Aaptamine(s)**	*Aaptos* sp.	1*H*-benzo[*de*][1,6]-naphthyridine alkaloids	137, 141-144, 187-189	Proteasome inhibitor	140
30	**Tunicamycin**	*Streptomyces* sp.	Nucleoside derivative	153, 154	*N*-linked glycosylation	68-70
31	**Brefeldin A**	*Penicillium brefeldianum*	Macrocyclic lactone	158-163	ARF / Proteasome inhibitor	92-94
32	**Berberine**	*Rhizoma coptidis*	Benzyl-tetrahydro-isoquinoline alkaloid	167-172 [1]	Prevents ER stress	89
53	**Hydroxytyrosol**	Several	Phenolic compound	175, 176	Prevents ER stress	97
34	**Vaticanol B**	*Vatica rassak*	Oligostilbenoid	NA	Prevents ER stress/anti-inflammatory	178

[1] – Refers to analogues of the compound presented in the table.

NA – Not available

References

1. R. J. Kaufman, *Gene Dev*, 1999, **13**, 1211-1233.
2. J. E. Chambers and S. J. Marciniak, *Am J Physiol-Cell Physiol*, 2014.
3. M. Schroder and R. J. Kaufman, *Annu Rev Biochem*, 2005, **74**, 739-789.
4. M. Schröder and R. J. Kaufman, *Mutat Res*, 2005, **569**, 29-63.
5. H. Yoshida, *FEBS J*, 2007, **274**, 630-658.
6. D. Ron and P. Walter, *Nat Rev Mol Cell Biol*, 2007, **8**, 519-529.
7. K. Zhang and R. J. Kaufman, *Nature*, 2008, **454**, 455-462.
8. E. Szegezdi, S. E. Logue, A. M. Gorman and A. Samali, *EMBO Rep*, 2006, **7**, 880-885.
9. H. P. Harding, M. Calfon, F. Urano, I. Novoa and D. Ron, *Annu Rev Cell Dev Bi*, 2002, **18**, 575-599.
10. S. Oyadomari and M. Mori, *Cell Death Differ*, 2004, **11**, 381-389.
11. M. Endo, S. Oyadomari, M. Suga, M. Mori and T. Gotoh, *J Biochem*, 2005, **138**, 501-507.
12. K. Haze, T. Okada, H. Yoshida, H. Yanagi, T. Yura, M. Negishi and K. Mori, *Biochem J*, 2001, **355**, 19-21.
13. H. Yoshida, T. Okada, K. Haze, H. Yanagi, T. Yura, M. Negishi and K. Mori, *Mol Cel Biol*, 2000, **20**, 6755-6767.
14. N. J. Darling and S. J. Cook, *Biochim Biophys Acta Mol Cell Res*, 2014, **1843**, 2150-2163.
15. F. Urano, X. Wang, A. Bertolotti, Y. Zhang, P. Chung, H. P. Harding and D. Ron, *Science*, 2000, **287**, 664-666.
16. H. Yang, K. Landis-Piwowar, D. Chen, V. Milacic and Q. Dou, *Curr Protein Pept Sci*, 2008, **9**, 227-239.
17. J. Adams, *Cancer Treat Rev* 2003, **29**, 3-9.
18. D. S. Leggett, J. Hanna, A. Borodovsky, B. Crosas, M. Schmidt, R. T. Baker, T. Walz, H. Ploegh and D. Finley, *Mol Cell* 2002, **10**, 495-507.
19. K. R. Landis-Piwowar, V. Milacic, D. Chen, H. Yang, Y. Zhao, T. H. Chan, B. Yan and Q. P. Dou, *Drug Resist Update*, 2006, **9**, 263-273.
20. G. Nalepa, M. Rolfe and J. W. Harper, *Nat Rev Drug Discovery*, 2006, **5**, 596-613.
21. A. Fribley, Q. Zeng and C.-Y. Wang, *Molecular and cellular biology*, 2004, **24**, 9695-9704.
22. S. T. Nawrocki, J. S. Carew, K. Dunner, L. H. Boise, P. J. Chiao, P. Huang, J. L. Abbruzzese and D. J. McConkey, *Cancer research*, 2005, **65**, 11510-11519.
23. E. A. Obeng, L. M. Carlson, D. M. Gutman, W. J. Harrington, K. P. Lee and L. H. Boise, *Blood*, 2006, **107**, 4907-4916.
24. Z. Kostova and D. H. Wolf, *The EMBO journal*, 2003, **22**, 2309-2317.
25. B. Tsai, Y. Ye and T. A. Rapoport, *Nature Reviews Molecular Cell Biology*, 2002, **3**, 246-255.
26. J. Hitomi, T. Katayama, Y. Eguchi, T. Kudo, M. Taniguchi, Y. Koyama, T. Manabe, S. Yamagishi, Y. Bando and K. Imaizumi, *J Cell Biol*, 2004, **165**, 347-356.
27. H. Puthalakath, L. A. O'Reilly, P. Gunn, L. Lee, P. N. Kelly, N. D. Huntington, P. D. Hughes, E. M. Michalak, J. McKimm-Breschkin and N. Motoyama, *Cell*, 2007, **129**, 1337-1349.
28. J. Li, B. Lee and A. S. Lee, *J Biol Chem*, 2006, **281**, 7260-7270.
29. C. Reimertz, D. Kögel, A. Rami, T. Chittenden and J. H. Prehn, *J Cell Biol*, 2003, **162**, 587-597.
30. C. W. Distelhorst and T. S. McCormick, *Cell Calcium*, 1996, **19**, 473-483.
31. M. Saleh, J. P. Vaillancourt, R. K. Graham, M. Huyck, S. M. Srinivasula, E. S. Alnemri, M. H. Steinberg, V. Nolan, C. T. Baldwin and R. S. Hotchkiss, *Nature*, 2004, **429**, 75-79.
32. M. Yavari, G. Brinkley, K. Klapstein, W. Hartwig, R. Rao and E. Hermel, *Int J Immugenet*, 2012, **39**, 389-393.
33. N. López-Antón, A. Rudy, N. Barth, L. M. Schmitz, G. R. Pettit, K. Schulze-Osthoff, V. M. Dirsch and A. M. Vollmar, *J Biol Chem*, 2006, **281**, 33078-33086.
34. S. T. Nawrocki, J. S. Carew, M. S. Pino, R. A. Highshaw, R. H. Andtbacka, K. Dunner, A. Pal, W. G. Bornmann, P. J. Chiao and P. Huang, *Cancer Res*, 2006, **66**, 3773-3781.
35. T. Yoneda, K. Imaizumi, K. Oono, D. Yui, F. Gomi, T. Katayama and M. Tohyama, *J Biol Chem*, 2001, **276**, 13935-13940.
36. N. Morishima, K. Nakanishi, H. Takenouchi, T. Shibata and Y. Yasuhiko, *J Biol Chem*, 2002, **277**, 34287-34294.
37. R. V. Rao, S. Castro-Obregon, H. Frankowski, M. Schuler, V. Stoka, G. del Rio, D. E. Bredesen and H. M. Ellerby, *J Biol Chem*, 2002, **277**, 21836-21842.

38. S.-E. Chow, C.-H. Kao, Y.-T. A. Liu, M.-L. Cheng, Y.-W. Yang, Y.-K. Huang, C.-C. Hsu and J.-S. Wang, *Apoptosis*, 2013, **19**, 527-541.
39. V. M. Dirsch, I. M. Müller, S. T. Eichhorst, G. R. Pettit, Y. Kamano, M. Inoue, J.-P. Xu, Y. Ichihara, G. Wanner and A. M. Vollmar, *Cancer Res* 2003, **63**, 8869-8876.
40. S. Park, B. Sanders and K. Kline, *Breast Cancer Res Tr*, 2010, **124**, 361-375.
41. D. M. Pereira, G. Correia-da-Silva, P. Valentão, N. Teixeira and P. B. Andrade, *Mar Drugs*, 2013, **12**, 54-68.
42. A. Pimentel, P. Felibertt, F. Sojo, L. Colman, A. Mayora, M. Silva, H. Rojas, R. Dipolo, A. Suarez, R. Compagnone, F. Arvelo, I. Galindo-Castro, J. Sanctis, P. Chirino and G. Benaim, *Cancer Chemoth Pharm*, 2012, **69**, 71-83.
43. O. Thastrup, P. J. Cullen, B. Drøbak, M. R. Hanley and A. P. Dawson, *P Natl Acad Sci USA*, 1990, **87**, 2466-2470.
44. J. Zhang, J. Tang, B. Cao, Z. Zhang, J. Li, A. D. Schimmer, S. He and X. Mao, *PloS One*, 2013, **8**, e69911.
45. A. Deniaud, *Oncogene*, 2007, **27**, 285-299.
46. H. L. Pahl and P. A. Baeuerle, *FEBS Lett*, 1996, **392**, 129-136.
47. X. Dolcet, D. Llobet, J. Pallares and X. Matias-Guiu, *Virchows Archiv*, 2005, **446**, 475-482.
48. J. Deng, P. D. Lu, Y. Zhang, D. Scheuner, R. J. Kaufman, N. Sonenberg, H. P. Harding and D. Ron, *Mol Cel Biol*, 2004, **24**, 10161-10168.
49. R. J. Davis, *Cell*, 2000, **103**, 239-252.
50. D. M. Pereira, G. Correia-da-Silva, P. Valentão, N. Teixeira and P. B. Andrade, *PloS One*, 2014, **9**, e88341.
51. W. Zha, G. Liang, J. Xiao, E. J. Studer, P. B. Hylemon, W. M. Pandak Jr, G. Wang, X. Li and H. Zhou, *PLoS One*, 2010, **5**, e9069.
52. M. S. Forman, V. M. Y. Lee and J. Q. Trojanowski, *Trends Neurosci*, 2003, **26**, 407-410.
53. J. Q. Trojanowski, *Ann Neurol*, 2002, **52**, 263-265.
54. A. Ciechanover and P. Brundin, *Neuron*, 2003, **40**, 427-446.
55. Y. Imai, M. Soda, H. Inoue, N. Hattori, Y. Mizuno and R. Takahashi, *Cell*, 2001, **105**, 891-902.
56. D. Lindholm, H. Wootz and L. Korhonen, *Cell Death Diff*, 2006, **13**, 385-392.
57. C. Soto, *Nat Rev Neurosci*, 2003, **4**, 49-60.
58. J. T. Greenamyre and T. G. Hastings, *Science*, 2004, **304**, 1120-1122.
59. V. M.-Y. Lee and J. Q. Trojanowski, *Neuron*, 2006, **52**, 33-38.
60. D. J. Selkoe, *Physiol Rev*, 2001, **81**, 741-766.
61. F. M. LaFerla, *Nat Rev Neurosci*, 2002, **3**, 862-872.
62. K. Takuma, S. S. Yan, D. M. Stern and K. Yamada, *J Pharmacol Sci*, 2005, **97**, 312-316.
63. S. L. Chan, C. Culmsee, N. Haughey, W. Klapper and M. P. Mattson, *Neurobiol Dis*, 2002, **11**, 2-19.
64. O. Milhavet, J. L. Martindale, S. Camandola, S. L. Chan, D. S. Gary, A. Cheng, N. J. Holbrook and M. P. Mattson, *J Neurochem*, 2002, **83**, 673-681.
65. T. Nakagawa and J. Yuan, *J Cell Biol*, 2000, **150**, 887-894.
66. A. I. Plácido, C. M. F. Pereira, A. I. Duarte, E. Candeias, S. C. Correia, R. X. Santos, C. Carvalho, S. Cardoso, C. R. Oliveira and P. I. Moreira, *Biochim Biophys Acta*, 2014, **1842**, 1444-1453.
67. A.-M. L. Winther, H. Liu, Y. Sonntag, C. Olesen, M. le Maire, H. Soehoel, C.-E. Olsen, S. B. Christensen, P. Nissen and J. V. Møller, *J Biol Chem*, 2010, **285**, 28883-28892.
68. M. Ball, S. P. Andrews, F. Wierschem, E. Cleator, M. D. Smith and S. V. Ley, *Org Lett*, 2007, **9**, 663-666.
69. G. Appendino, S. Prosperini, C. Valdivia, M. Ballero, G. Colombano, R. A. Billington, A. A. Genazzani and O. Sterner, *J Nat Prod*, 2005, **68**, 1213-1217.
70. G. Li, S. Kusari and M. Spiteller, *Nat Prod Rep*, 2014, **31**, 1175-1201.
71. H. M. Nelson, K. Murakami, S. C. Virgil and B. M. Stoltz, *Angew Chem Int Edit*, 2011, **50**, 3688-3691.
72. C. Navarrete, R. Sancho, F. J. Caballero, F. Pollastro, B. L. Fiebich, O. Sterner, G. Appendino and E. Muñoz, *J Pharmacol Exp Ther*, 2006, **319**, 422-430.
73. J. R. Gordon, H. M. Nelson, S. C. Virgil and B. M. Stoltz, *J Org Chem*, 2014.
74. R. Larsson, O. Sterner and M. Johansson, *Org Lett*, 2009, **11**, 657-660.
75. L. Min, Y. Zhang, X. Liang, J. Huang, W. Bao and C. S. Lee, *Angew Chem Int Edit*, 2014.
76. E. Piers and J. Y. Roberge, *Tetrahedron Lett*, 1992, **33**, 6923-6926.

77. F.-M. Wang, D. L. Galson, G. D. Roodman and H. Ouyang, *Exp Hematol*, 2011, **39**, 999-1006.
78. S. Bernales, S. Schuck and P. Walter, *Autophagy*, 2007, **3**, 285-287.
79. D. A. Alonso, C. Nájera and M. Varea, *Tetrahedron Lett*, 2004, **45**, 573-577.
80. M. B. Andrus, J. Liu, E. L. Meredith and E. Nartey, *Tetrahedron Lett*, 2003, **44**, 4819-4822.
81. M. Guiso, C. Marra and A. Farina, *Tetrahedron Lett*, 2002, **43**, 597-598.
82. A. V. Moro, F. S. P. Cardoso and C. R. D. Correia, *Tetrahedron Lett*, 2008, **49**, 5668-5671.
83. M. J. Choi, E. J. Park, K. J. Min, J.-W. Park and T. K. Kwon, *Toxicol in Vitro*, 2011, **25**, 692-698.
84. S. Koduru, R. Kumar, S. Srinivasan, M. B. Evers and C. Damodaran, *Mol Cancer Ther*, 2010, **9**, 202-210.
85. C. Mandal, A. Dutta, A. Mallick, S. Chandra, L. Misra, R. S. Sangwan and C. Mandal, *Apoptosis*, 2008, **13**, 1450-1464.
86. J. H. Oh, T.-J. Lee, S. H. Kim, Y. H. Choi, S. H. Lee, J. M. Lee, Y.-H. Kim, J.-W. Park and T. K. Kwon, *Apoptosis*, 2008, **13**, 1494-1504.
87. S. Srinivasan, R. S. Ranga, R. Burikhanov, S.-S. Han and D. Chendil, *Cancer Res*, 2007, **67**, 246-253.
88. S. D. Stan, E.-R. Hahm, R. Warin and S. V. Singh, *Cancer Res*, 2008, **68**, 7661-7669.
89. H. Yang, G. Shi and Q. P. Dou, *Mol Pharmacol*, 2007, **71**, 426-437.
90. M. Hirayama, K. Gamoh and N. Ikekawa, *Tetrahedron Lett*, 1982, **23**, 4725-4728.
91. F. S. Sariaslani and J. Rosazza, *Appl Environ Microb*, 1983, **45**, 616-621.
92. X. Xu, J. Zhang, K. Han, Z. Zhang, G. Chen, J. Zhang, X. Mao and B. Cao, *J Biochem Mol Toxic*, 2014, **28**, 232-238.
93. S. Takahashi, H. Fukami and M. Nakajima, *J Agric Chem Soc Jap*, 1960, **24**, 123-126.
94. A. Rudy, N. López-Antón, V. M. Dirsch and A. M. Vollmar, *J Nat Prod*, 2008, **71**, 482-486.
95. K. C. Fortner, D. Kato, Y. Tanaka and M. D. Shair, *J Am Chem Soc*, 2009, **132**, 275-280.
96. T. G. LaCour, C. Guo, S. Bhandaru, M. R. Boyd and P. Fuchs, *J Am Chem Soc*, 1998, **120**, 692-707.
97. K. Y. Jang, S.-J. Jeong, S.-H. Kim, J. H. Jung, J.-H. Kim, W. Koh, C.-Y. Chen and S.-H. Kim, *Cancer Lett*, 2012, **319**, 197-202.
98. N. Khan, F. Afaq, D. N. Syed and H. Mukhtar, *Carcinogenesis*, 2008, **29**, 1049-1056.
99. Y. Sagara, J. Vanhnasy and P. Maher, *J. Neurochem.*, 2004, **90**, 1144-1155.
100. Y. Suh, F. Afaq, J. J. Johnson and H. Mukhtar, *Carcinogenesis*, 2009, **30**, 300-307.
101. E. Szliszka, K. J. Helewski, E. Mizgala and W. Krol, *International journal of oncology*, 2011, **39**, 771.
102. D. N. Syed, R. K. Lall, J. C. Chamcheu, O. Haidar and H. Mukhtar, *Arch Biochim Biophys*, 2014, **In press**.
103. J. Allan and R. Robinson, *J. Chem. Soc.*, 1926, **129**, 2334-2336.
104. N. Maftuhah, 2013.
105. J. P. Kutney, G. M. Hewitt, T. Kurihara, P. J. Salisbury, R. D. Sindelar, K. L. Stuart, P. M. Townsley, W. T. Chalmers and G. G. Jacoli, *Can J Chem*, 1981, **59**, 2677-2683.
106. A. Salminen, M. Lehtonen, T. Paimela and K. Kaarniranta, *Biochem Bioph Res Co*, 2010, **394**, 439-442.
107. L. Klaić, R. I. Morimoto and R. B. Silverman, *ACS Chem Biol*, 2012, **7**, 928-937.
108. H. Sun, L. Xu, P. Yu, J. Jiang, G. Zhang and Y. Wang, *Bioorg Med Chem Lett*, 2010, **20**, 3844-3847.
109. S. Omura, *J Antibiot*, 1991, **44**, 113-116.
110. G. Fenteany, R. F. Standaert, G. A. Reichard, E. Corey and S. L. Schreiber, *P Natl Acad Sci USA*, 1994, **91**, 3358-3362.
111. G. Fenteany and S. L. Schreiber, *J Biol Chem*, 1998, **273**, 8545-8548.
112. N. Cheung, M. Choy, B. Halliwell, T. Teo, B. Bay, A.-W. Lee, R. Qi, V. Koh, M. Whiteman and E.-C. Koay, *Cell Mol Life Sci*, 2004, **61**, 1926-1934.
113. E. H. J. Yew, N. S. Cheung, M. S. Choy, R. Z. Qi, A. Y. W. Lee, Z. F. Peng, A. J. Melendez, J. Manikandan, E. S. C. Koay and L. L. Chiu, *J Neurochem*, 2005, **94**, 943-956.
114. G. Fenteany, R. F. Standaert, W. S. Lane, S. Choi, E. Corey and S. L. Schreiber, *Science*, 1995, **268**, 726-731.
115. E. J. Corey and G. A. Reichard, *J Am Chem Soc*, 1992, **114**, 10677-10678.
116. H. Uno, J. E. Baldwin and A. T. Russell, *J Am Chem Soc*, 1994, **116**, 2139-2140.

117. T. Nagamitsu, T. Sunazuka, H. Tanaka, S. Ōmura, P. A. Sprengeler and A. B. Smith, *J Am Chem Soc*, 1996, **118**, 3584-3590.
118. N. Chida, J. Takeoka, K. Ando, N. Tsutsumi and S. Ogawa, *Tetrahedron*, 1997, **53**, 16287-16298.
119. E. P. Balskus and E. N. Jacobsen, *J Am Chem Soc*, 2006, **128**, 6810-6812.
120. E. J. Corey, G. A. Reichard and R. Kania, *Tetrahedron Lett*, 1993, **34**, 6977-6980.
121. N. Fukuda, K. Sasaki, T. V. R. S. Sastry, M. Kanai and M. Shibasaki, *J Org Chem*, 2006, **71**, 1220-1225.
122. M. Shibasaki, M. Kanai and N. Fukuda, *Chem-Asian J*, 2007, **2**, 20-38.
123. C.-C. Wu, M.-L. Chan, W.-Y. Chen, C.-Y. Tsai, F.-R. Chang and Y.-C. Wu, *Mol Cancer Ther*, 2005, **4**, 1277-1285.
124. H. Yang, K. R. Landis-Piwowar, D. Lu, P. Yuan, L. Li, G. Reddy, X. Yuan and Q. P. Dou, *J Cell Biochem*, 2008, **103**, 234-244.
125. S. Aoki, Y. Yoshioka, Y. Miyamoto, K. Higuchi, A. Setiawan, N. Murakami, Z.-S. Chen, T. Sumizawa, S.-i. Akiyama and M. Kobayashi, *Tetrahedron Lett*, 1998, **39**, 6303-6306.
126. S. Tsukamoto, M. Tatsuno, R. W. van Soest, H. Yokosawa and T. Ohta, *J Nat Prod*, 2003, **66**, 1181-1185.
127. N. Murakami, M. Sugimoto, M. Morita and M. Kobayashi, *Chem-Eur J*, 2001, **7**, 2663-2670.
128. D. Chen, K. G. Daniel, M. S. Chen, D. J. Kuhn, K. R. Landis-Piwowar and Q. P. Dou, *Biochem Pharmacol*, 2005, **69**, 1421-1432.
129. D. Chen, M. S. Chen, Q. C. Cui, H. Yang and Q. P. Dou, *Frontiers in bioscience: a journal and virtual library*, 2006, **12**, 1935-1945.
130. J. R. Johnson, W. F. Bruce and J. D. Dutcher, *J Am Chem Soc*, 1943, **65**, 2005-2009.
131. A. Beecham, J. Fridrichsons and A. M. Mathieson, *Tetrahedron Lett*, 1966, **7**, 3131-3138.
132. M. Kroll, F. Arenzana-Seisdedos, F. Bachelerie, D. Thomas, B. Friguet and M. Conconi, *Chem Biol*, 1999, **6**, 689-698.
133. P. Waring, R. D. Eichner and A. Müllbacher, *Med Res Rev*, 1988, **8**, 499-524.
134. P. Waring, R. D. Eichner, A. Müllbacher and A. Sjaarda, *J Biol Chem*, 1988, **263**, 18493-18499.
135. T. Fukuyama and Y. Kishi, *J Am Chem Soc*, 1976, **98**, 6723-6724.
136. T. Fukuyama, S.-I. Nakatsuka and Y. Kishi, *Tetrahedron*, 1981, **37**, 2045-2078.
137. H. Nakamura, J. i. Kobayashi, Y. Ohizumi and Y. Hirata, *Tetrahedron Lett*, 1982, **23**, 5555-5558.
138. H. Nakamura, J. i. Kobayashi, Y. Ohizumi and Y. Hirata, *J Chem Soc*, 1987, 173-176.
139. K. Shaari, K. C. Ling, Z. Mat Rashid, T. P. Jean, F. Abas, S. M. Raof, Z. Zainal, N. H. Lajis, H. Mohamad and A. M. Ali, *Mar Drugs*, 2008, **7**, 1-8.
140. S. Tsukamoto, R. Yamanokuchi, M. Yoshitomi, K. Sato, T. Ikeda, H. Rotinsulu, R. E. Mangindaan, N. J. de Voogd, R. W. van Soest and H. Yokosawa, *Bioorg Med Chem Lett*, 2010, **20**, 3341-3343.
141. P. Balczewski, M. K. J. Mallon, J. D. Street and J. A. Joule, *Tetrahedron Lett*, 1990, **31**, 569-572.
142. J. C. Pelletier and M. P. Cava, *Tetrahedron Lett*, 1985, **26**, 1259-1260.
143. E. L. Larghi, B. V. Obrist and T. S. Kaufman, *Tetrahedron*, 2008, **64**, 5236-5245.
144. T. Ross Kelly and M. P. Maguire, *Tetrahedron*, 1985, **41**, 3033-3036.
145. R. G. Andrew and R. A. Raphael, *Tetrahedron*, 1987, **43**, 4803-4816.
146. A. Takatsuki, K. Arima and G. Tamura, *J Antibiot*, 1971, **24**, 215-223.
147. S.-C. Kuo and J. Lampen, *Biochem Bioph Res Commun*, 1974, **58**, 287-295.
148. K. Olden, R. M. Pratt and K. M. Yamada, *Cell*, 1978, **13**, 461-473.
149. E. G. Schneider, H. T. Nguyen and W. J. Lennarz, *J Biol Chem*, 1978, **253**, 2348-2355.
150. D. Duksin and W. Mahoney, *J Biol Chem*, 1982, **257**, 3105-3109.
151. T. Ito, A. Takatsuki, K. Kawamura, K. Sato and G. Tamura, *Agric Biol Chem*, 1980, **44**, 695-698.
152. I. A. King and A. Tabiowo, *Biochem J*, 1981, **198**, 331-338.
153. T. Suami, H. Sasai, K. Matsuno, N. Suzuki, Y. Fukuda and O. Sakanaka, *Tetrahedron Lett*, 1984, **25**, 4533-4536.

154. S. J. Danishefsky, S. L. DeNinno, S. H. Chen, L. Boisvert and M. Barbachyn, *J Am Chem Soc*, 1989, **111**, 5810-5818.
155. J. G. Donaldson, D. Cassel, R. A. Kahn and R. D. Klausner, *Proc Natl Acad Sci USA*, 1992, **89**, 6408-6412.
156. R. D. Klausner, J. G. Donaldson and J. Lippincott-Schwartz, *J Cell Biol*, 1992, **116**, 1071-1080.
157. J. L. Moon, S. Y. Kim, S. W. Shin and J.-W. Park, *Biochem Bioph Res Commun*, 2012, **417**, 760-764.
158. P. A. Bartlett and F. R. Green III, *J Am Chem Soc*, 1978, **100**, 4858-4865.
159. E. Corey and R. H. Wollenberg, *Tetrahedron Lett*, 1976, **17**, 4705-4708.
160. A. E. Greene, C. Le Drian and P. Crabbe, *J Am Chem Soc*, 1980, **102**, 7583-7584.
161. D. F. Taber, L. J. Silverberg and E. D. Robinson, *J Am Chem Soc*, 1991, **113**, 6639-6645.
162. B. M. Trost and M. L. Crawley, *J Am Chem Soc*, 2002, **124**, 9328-9329.
163. Y. Wang and D. Romo, *Org Lett*, 2002, **4**, 3231-3234.
164. T. Kitahara and K. Mori, *Tetrahedron*, 1984, **40**, 2935-2944.
165. Z.-s. Wang, F.-e. Lu, L.-j. Xu and H. Dong, *Acta Pharmacol Sin*, 2010, **31**, 578-584.
166. X. Hao, A. Yao, J. Gong, W. Zhu, N. Li and J. Li, *Inflammation*, 2012, **35**, 841-849.
167. Y. Ma, T.-M. Ou, J.-H. Tan, J.-Q. Hou, S.-L. Huang, L.-Q. Gu and Z.-S. Huang, *Bioorg Med Chem Lett*, 2009, **19**, 3414-3417.
168. A. Shi, L. Huang, C. Lu, F. He and X. Li, *Bioorg Med Chem*, 2011, **19**, 2298-2305.
169. J.-Y. Pang, Y. Qin, W.-H. Chen, G.-A. Luo and Z.-H. Jiang, *Bioorg Med Chem*, 2005, **13**, 5835-5840.
170. P. Yang, D.-Q. Song, Y.-H. Li, W.-J. Kong, Y.-X. Wang, L.-M. Gao, S.-Y. Liu, R.-Q. Cao and J.-D. Jiang, *Bioorg Med Chem Lett*, 2008, **18**, 4675-4677.
171. Z. Chen, X. Ye, J. Yi, X. Chen and X. Li, *Med Chem Res*, 2012, **21**, 1641-1646.
172. X. Ye, K. He, X. Zhu, B. Zhang, X. Chen, J. Yi and X. Li, *Med Chem Res*, 2012, **21**, 1353-1362.
173. K. L. Tuck and P. J. Hayball, *J Nutr Biochem*, 2002, **13**, 636-644.
174. E. Giordano, A. Davalos, N. Nicod and F. Visioli, *Mol Nutr Food Res*, 2013, **58**, 954-952.
175. R. Bernini, E. Mincione, M. Barontini and F. Crisante, *J Agric Food Chem*, 2008, **56**, 8897-8904.
176. Z.-L. Zhang, J. Chen, Q. Xu, C. Rao and C. Qiao, *Synthetic Commun* 2012, **42**, 794-798.
177. T. Tanaka, T. Ito, K.-i. Nakaya, M. Iinuma and S. Riswan, *Phytochemistry*, 2000, **54**, 63-69.
178. Y. Tabata, K. Takano, T. Ito, M. Iinuma, T. Yoshimoto, H. Miura, Y. Kitao, S. Ogawa and O. Hori, *Am J Physiol-Cell Physiol*, 2007, **293**, C411-C418.
179. Takeda, https://www.takeda.com/news/2013/20131119_6050.html 2013.
180. E. Gallerani, M. Zucchetti, D. Brunelli, E. Marangon, C. Noberasco, D. Hess, A. Delmonte, G. Martinelli, S. Böhm and C. Driessen, *Eur J Cancer*, 2013, **49**, 290-296.
181. R. H. Feling, G. O. Buchanan, T. J. Mincer, C. A. Kauffman, P. R. Jensen and W. Fenical, *Angew Chem Int Edit*, 2003, **42**, 355-357.
182. N. Satoh, S. Yokoshima and T. Fukuyama, *Org Lett*, 2011, **13**, 3028-3031.
183. H. Nguyen, G. Ma and D. Romo, *Chem Commun*, 2010, **46**, 4803-4805.
184. L. R. Reddy, P. Saravanan and E. Corey, *J Am Chem Soc*, 2004, **126**, 6230-6231.
185. J. R. Gordon, K. Murakami, H. Nelson, B. M. Stoltz and S. C. Virgil, Google Patents2012.
186. Y. Shi, L. Jia, Q. Xiao, Q. Lan, X. Tang, D. Wang, M. Li, Y. Ji, T. Zhou and W. Tian, *Chem-Asian J*, 2011, **6**, 786-790.
187. A. Bassoli, G. Maddinelli, B. Rindone, S. Tollari and F. Chioccara, *J Chem Soc-Chem Commun*, 1987, 150-151.
188. E. L. Larghi, M. L. Bohn and T. S. Kaufman, *Tetrahedron*, 2009, **65**, 4257-4282.
189. J. C. Pelletier and M. P. Cava, *J Org Chem*, 1987, **52**, 616-622.