Cell Death Targets and Potential Modulators in Alzheimer's Disease

Rui E. Castro*, Maria M. M. Santos, Paulo M.C. Glória, Carlos J.A. Ribeiro, Duarte M. S. Ferreira, Joana M. Xavier, Rui Moreira and Cecília M. P. Rodrigues

Research Institute for Medicines and Pharmaceutical Sciences, Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal

Abstract: Apoptosis is now recognized as a normal feature in the development of the nervous system and may also play a role in neurodegenerative disorders, such as Alzheimer's disease. Cell surface receptors, caspases, mitochondrial factors or p53 participate in the modulation and execution of cell death. Therefore, the ability to understand and manipulate the cell death machinery is an obvious goal of medical research. Potential therapeutic approaches to modulate disease by regulating apoptosis are being tested, and include the traditional use of small molecules to target specific players in the apoptosis cascade. As our understanding of apoptosis increases, further opportunities will arise for more specific therapies that will result in improved efficacy. This review focuses on molecular mechanisms of apoptosis in Alzheimer's disease and highlights the potential use of small molecule modulators to treat neurodegenerative disorders.

Keywords: Alzheimer's disease, Bcl-2 family, Caspases, Death receptors, Mitochondria, p53, Small molecules.

INTRODUCTION

The tight balance between cell death and proliferation represents one of the most critical factors regulating tissue and organ homeostasis. During development, apoptosis plays a key role in controlling the final numbers of neuronal and glial cells in both the central and peripheral nervous system. However, in the adult nervous system, neurons are terminally differentiated, postmitotic cells. Therefore, it is not surprising that apoptosis is often associated with physiological aging and several neurodegenerative disorders. Still, the exact role of apoptosis in neurodegenerative diseases remains scattered. Events such as synaptic loss or disruption of neuronal signaling are often considered hallmarks of many neurodegenerative diseases. Yet, they may precede or be a consequence of initial neurodegenerative processes. In addition, several insults including misfolded proteins, reactive oxygen species (ROS), excitotoxicity, trophic-factor withdrawal, among others, have been shown to trigger neuronal apoptosis. Therefore, the development of new therapeutic strategies for the treatment of neurodegenerative diseases would benefit from a better understanding of the molecular mechanisms underlying neuronal apoptosis.

In this review, we will summarize the most current knowledge on the mechanisms of cell death in the brain and its relevance for neurodegenerative diseases, particularly Alzheimer's disease (AD). Current and prospective small molecule strategies that target modulation of apoptotic pathways involved in neurodegeneration will be specifically addressed.

1. Basic Principles of Apoptosis

Cellular division and migration are crucial events during the normal development and homeostasis of multicellular organisms. In addition, animals often need to get rid of cells that are in excess or became potentially dangerous. Programmed cell death (PCD) is a mechanism that controls cell number and tissue size, while protecting the organism against harmful cells that threaten homeostasis [1]. Apoptosis is the classical type of PCD, characterized by morphological features such as nuclear pyknosis, chromatin condensation, membrane blebbing, and the formation of apoptotic bodies, which represent the package and removal of dying neighboring cells, without activating the immune system. Such controlled demolition of the cell is orchestrated by apoptotic modulators that act in synergy to drive cell death in a regulated fashion. The main modulators of apoptosis are caspases and the Bcl-2 family of proteins [2]. In fact, the morphological and histochemical changes associated with apoptosis are largely the result of activation of caspases, cysteine proteases that cleave the peptide bond C-terminal to aspartic acid residues [3]. The Bcl-2 family is a group of pro- and anti-apoptotic molecules, which converge at the mitochondrial membrane to regulate apoptosis [4].

There are two well-characterized apoptotic pathways in mammalian cells: the extrinsic and the intrinsic pathways (Fig. **1**). The extrinsic, or death-receptor pathway, is initiated by ligand-induced activation of death receptors at the plasma membrane, resulting in activation of caspase-8 or -10. The intrinsic, or mitochondrial pathway, responds to internal signals, and involves the mitochondrial release of cytochrome *c*, leading to caspase-9 activation [5]. Cross-talk and integration between death-receptor and mitochondrial pathways is provided by Bid, a pro-apoptotic Bcl-2 family member. Upon caspase-8-mediated cleavage, Bid greatly increases its pro-death activity after translocation to mitochondria, where it promotes cytochrome *c* release.

2. Cell Death in the Central Nervous System

Natural neuronal death is thought to mold the cellular structure and function of the nervous system. In fact, the physiological role of apoptosis in the nervous system is critical during development, when an excessive number of neuronal cells are produced. Almost one half of these neurons undergo apoptosis during the reshaping of the nervous system, a process which is highly conserved throughout most species [6]. It has been suggested that the developing brain overproduces neuronal cells to ensure that a correct and sufficient number of synaptic connections are formed. Neurons that fail to make those connections are removed by apoptosis [7]. Similarly, glial cells are also produced in high levels during development, and the excess number is removed by apoptosis [8]. In the rat brain, apoptosis has been shown to occur between postnatal day P7 and P9, in the cortex [9], hippocampus [10], and cerebellum [11]. This naturally occurring apoptosis is accompanied by decreased Bcl-2 to Bax ratio and increased expression of active caspase-3. In addition, cytochrome *c* and Apaf-1 are also required for the mitochondrial pathway of apoptosis during brain development [12, 13]. Interestingly enough, most pro-apoptotic proteins, including Apaf-1, caspase-3 and Bim, become dramatically downregulated after P10 in the mouse brain, possibly as part of a mechanism to protect the adult brain tissue from the loss of irreplaceable cells [14]. However,

*Address correspondence to this author at the Research Institute for Medicines and Pharmaceutical Sciences, Faculty of Pharmacy, University of Lisbon, Av. Prof. Gama Pinto, 1649-003 Lisbon, Portugal;
Tel: +351217946400; Fax: +351217946470;
E-mails: ruieduardocastro@ff.ul.pt; mariasantos@ff.ul.pt

1381-6128/10 $55.00+.00

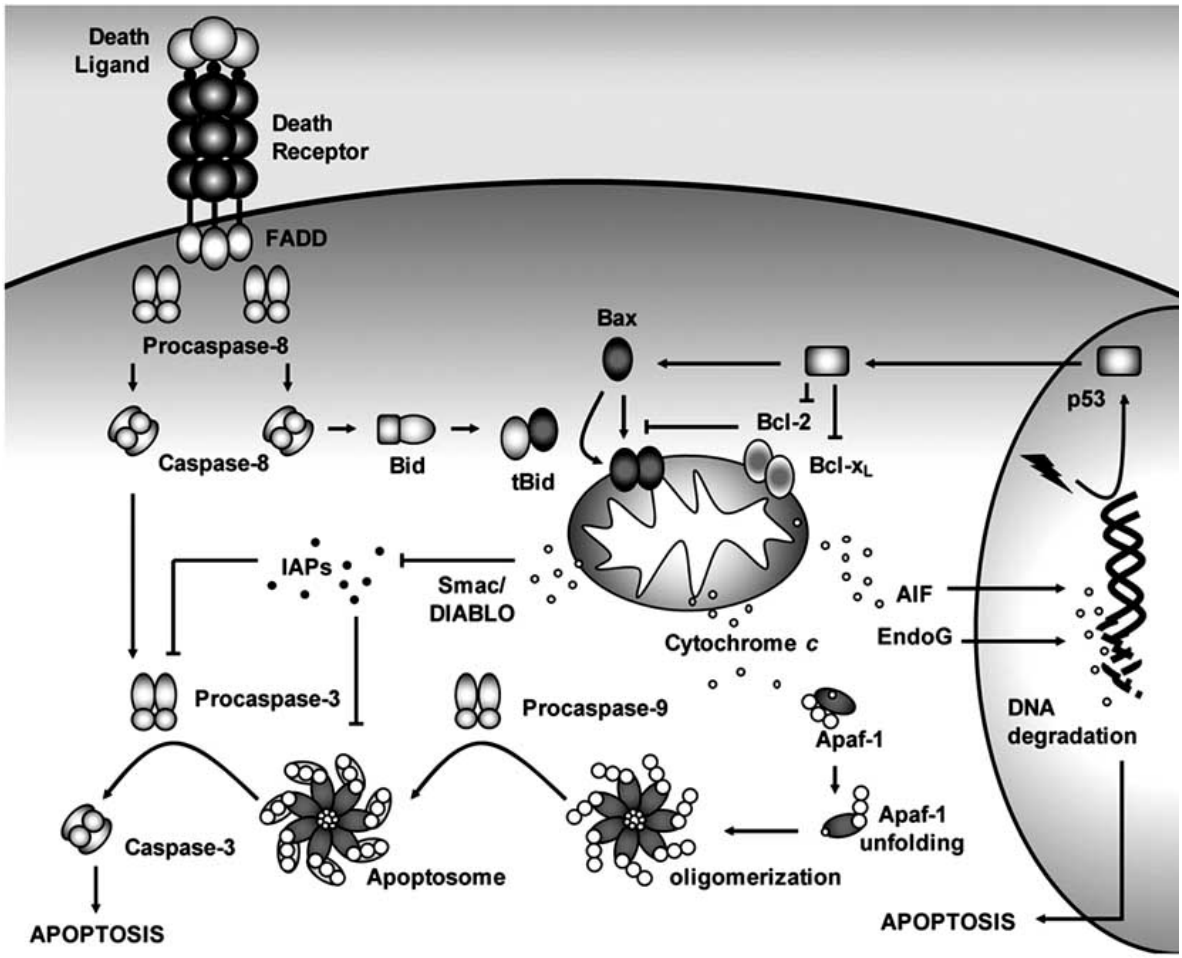


Fig. (1). Schematic overview of typical receptor- and mitochondria-mediated apoptosis. In the death receptor pathway, the interaction between the receptor and its cognate ligand results in recruitment of Fas-associated protein with death domain (FADD) and initiator procaspase-8, leading to caspase activation. Active initiator caspases then act on procaspase-3, which becomes functional and cleaves key substrates in the cell to execute apoptosis. In the mitochondrial pathway, death stimuli target mitochondria either directly or through transduction by pro-apoptotic members of the Bcl-2 family, such as Bax. In turn, Bax may be specifically activated by p53, transcribed upon DNA damage, or inhibited by action of Bcl-2 family anti-apoptotic members, such as Bcl-2 and Bcl-x_L. Upon targeting, mitochondria may then release cytochrome *c*, Smac/DIABLO, AIF, and EndoG. Cytochrome *c* induces oligomerization of Apaf-1, which recruits and activates procaspase-9. Caspase-9 then activates procaspase-3 to induce apoptosis. Smac/DIABLO functions to antagonize the action of inhibitors of apoptosis proteins (IAPs), which will liberate caspase inhibition and consequently cause apoptosis. Finally, mitochondrial regulators of caspase-independent cell death AIF and EndoG are translocated to the nucleus, inducing DNA degradation and, ultimately, apoptosis. Crosstalk between both pathways of apoptosis is mediated by Bid, which is truncated and activated by caspase-8.

this mechanism seems to be insufficient to compensate for all types of injury. Additional evidence from studies in primary cultures, cell lines and transgenic animals, further established the need for a proper balance between pro- and anti-apoptotic factors for both the normal development of the nervous system and its homeostasis in adults. In that sense, an increased anti- to pro-apoptotic protein ratio may also lead to several brain abnormalities, including nervous system hypertrophy and aberrant structural organization. In turn, a decreased ratio leads to reduced neuronal survival or exacerbation of apoptosis [15]. This is the case of neurodegenerative conditions, where death of neurons occurs through apoptosis or a closely related mechanism.

A number of distinct programmed cell death pathways have been proposed in neuronal cells, including apoptosis and autophagy, as well as atypical forms of cell death such as paraptosis, oncosis, calcium-mediated cell death, among others. These are usually distinguished based on morphological aspects, as well as their triggers and mediators, although it is still not clear if the specificity is determined by the stimulus, cell type, or both. Many cell death stimuli can induce more than one mode of cell death depending on the intensity and duration of the stress signal, as well as on the cell energy status. In fact, the crosstalk between different mechanisms of cell death in the nervous system is evident; 6-hydroxydopamine for instance, a mimetic of Parkinson's disease (PD), has been shown to induce both apoptosis and necrosis *in vitro* [16]. Recently, the crosstalk between apoptosis and autophagy and, particularly, endoplasmic reticulum (ER) stress-induced cell death have been shown to play a significant role in neurodegenerative diseases [17].

Classical apoptosis is the most studied form of PCD in the brain. This is not surprising, as it represents the prevalent form of cell death during developmental neurogenesis and in many acute and chronic pathologic conditions, including neurodegenerative and other chronic age-related disorders. Several specific stimuli for neuronal apoptosis have been largely described and include deprivation of neurotrophic factors, such as the nerve growth factor (NGF), basic fibroblast growth factor (bFGF), and brain-derived neurotrophic factor (BDNF), or even glutamate, nitric oxide, among others [18]. Nevertheless, the observation that common morphological and biochemical alterations occur independently of the

event that triggers apoptosis suggests that most apoptotic pathways converge on a restricted number of common effector pathways [19]. In fact, the biochemical alterations that occur during early stages of neuronal apoptosis usually induce mitochondrial or ER dysfunction. In addition, neuronal apoptosis may also occur through the death-receptor pathway of apoptosis [20]. Of note, p53 appears to be essential for apoptosis progression in the mature nervous system, as it is deregulated in several neurodegenerative disorders. Parallel to its transcriptional activation of pro-apoptotic genes, p53 may more directly trigger apoptosis by acting at the level of the mitochondria, a process known to occur in synapses [21].

3. Modulation of Apoptosis in Alzheimer's Disease

Neurodegenerative diseases include a variety of progressive disorders that result in cognitive and/or motor deterioration. Interestingly, neuronal loss alone does not always fully explain the neurological deficits. In fact, decreased cell body size, atrophy of dendrites, and reductions in axonal terminal fields are also prominent features of neurodegenerative diseases. It is thought that a combination of progressive neuronal loss and neuronal dysfunction underlies neurodegeneration [22]. Furthermore, some neurons appear to compensate for the global neuronal loss by expanding their connective interactions and functional capacities [23]. This may explain the fact that neurological deficits are only clinically detectable when ~ 50 to 70% of neurons are lost in a given population. Therefore, strategies aimed at inhibiting neuronal death should be employed as early as possible.

The study of apoptosis in neurodegenerative diseases remains a challenging task. For years, the question on whether or not apoptosis occurs and plays a key role as the major form of cell death in neurodegenerative diseases has been largely discussed, especially because synaptic loss and electrophysiological abnormalities typically precede cell loss. In addition, given the slow progression of most neurodegenerative diseases, in contrast with the rapid progression of a cell through apoptosis, only a few cells exhibiting apoptotic features are found in postmortem human brain tissue. Therefore, the synchronous detection of a substantial number of apoptotic neurons at any given time point is, indeed, unlikely. In turn, apoptotic processes may be terminated or not yet begun by the time the tissue is available for examination. In fact, the collected tissue is usually representative of the end-stage disease, being removed much later than it would be ideal to treat patients and prevent neuronal cell death. Finally, the criteria used to classify the type of cell death as apoptosis is often based solely on morphological assessment and biochemical assays, which may also account for other forms of PCD. For example, the terminal transferase dUTP nick-end labeling (TUNEL) assay is usually used as a marker of apoptosis in human brain tissue. However, TUNEL can also label necrotic cells, which is why more than one assay should be considered when determining the type of cell death experimentally [24].

Despite these limitations, there is now a consensus for a major role of apoptosis in human neuronal cell death and neurodegenerative processes. Studies on the pathological mechanisms underlying neuronal apoptosis in hereditary forms of neurodegenerative diseases have provided important clues, by linking neuronal apoptosis with oxidative stress and mitochondrial dysfunction. Nevertheless, the most prominent facts underlying the role of apoptosis in neurodegenerative diseases came from studies using either *in vitro* cell culture systems or *in vivo* models of disease. Although with inherent limitations, as these models tend to examine the effect of only one toxin or genetic defect, usually involving acute exposure to neurological insults, they have greatly increased our fundamental knowledge. This knowledge may now be used for the development of new drugs that interfere with the apoptotic cascade in human neurodegenerative diseases.

AD is the most common form of dementia among the elderly population. It represents the fourth cause of death in industrialized countries and it is estimated that, by 2050, more than 100 million people worldwide will suffer from AD [25]. AD is characterized by a progressive impairment of cognitive functions and its hallmark pathological features consist of external amyloid plaques and internal neurofibrillary tangles (NFTs), which are composed primarily of amyloid β (Aβ) and tau proteins, respectively [26]. In parallel with these characteristic histological changes, decreased synaptic density and loss of neurons in the basal forebrain, hippocampus and cortex, are hallmarks of AD. In fact, it has been suggested that progressive loss of synapses and neurons in limbic and cortical areas is critical in leading to the manifestations of the clinical symptoms of AD. Nevertheless, this process of cell loss may start up to 40 years before the manifestation of the first symptoms [27].

In most cases, the causes of AD remain unclear. However, it is generally accepted that AD is a complex multifactorial disease in which both genetic and environmental factors are responsible for initiating and modulating the progression of the disease. Familial AD is caused by mutations in amyloid precursor protein (APP) or presenilin-1 or -2 [28]. These mutations are ultimately responsible for the deposition and oligomerization of altered Aβ peptides, forming plaques and fibrils [26]. Environmental factors may include head trauma or high calorie diet [29, 30].

Despite the various genetic and environmental factors that may lead to the manifestation of AD, several features of the neurodegenerative process are common to different experimental models, including increased levels of oxidative stress, metabolic compromise, perturbed calcium regulation, ER stress, mitochondrial dysfunction, DNA damage, activation of apoptotic biochemical cascades, autophagy and abnormalities in protein processing and degradation (Fig. **2**) [31]. Interestingly, both synaptic loss and neuritic dystrophy are affected early in AD, with no apparent cell loss. Although these events precede extensive neuronal loss, it is unknown whether the corresponding neuronal cell bodies are truly healthy. Conversely, it is also unclear whether early dendritic and axonal defects kill the corresponding neurons, or whether dendritic, axonal and neuronal deaths are each direct consequences of extracellular Aβ. Nevertheless, the pathogenic cascade of AD finally results in neuronal cell and synapse loss.

Discussion on the nature of neuronal loss in AD remains controversial. Although autophagy has been suggested to play a central role in AD neurodegeneration [32], it is still not clear whether it does play a causative role or, inversely, a protective role, or if it is a consequence of the disease process itself [33]. In contrast, the detection of cleaved caspases and the accumulation of their cleaved substrates in post-mortem AD brain tissue, represent a small part of the whole frame supporting the hypothesis that apoptosis plays a key role in AD [28]. In addition, biochemical and morphological hallmarks of apoptosis have been associated with neuropathological features found in AD brain tissue, including NFTs and amyloid plaques. This further underlies the role of neuronal apoptosis in the progression of the disease [31].

Finally, there is increasing evidence for a pivotal role of ER stress in AD-associated cell death. During ER stress, perturbed calcium homeostasis or altered protein processing leads to the accumulation of unfolded proteins in the ER, which activate the unfolded protein response (UPR). In turn, the UPR activates transcription factor C/EBP homologous protein (CHOP), which may induce apoptosis. In fact, CHOP has been suggested as the link between ER and mitochondria during apoptotic cell death induced by the Aβ peptide [34].

3.1. Caspases

Substantial evidence demonstrates that multiple caspases are detected in brains of patients with AD. In fact, caspases-1, -2, -3, -5, -6, -7, -8 and -9 have all been found to be transcriptionally increased in AD [35].

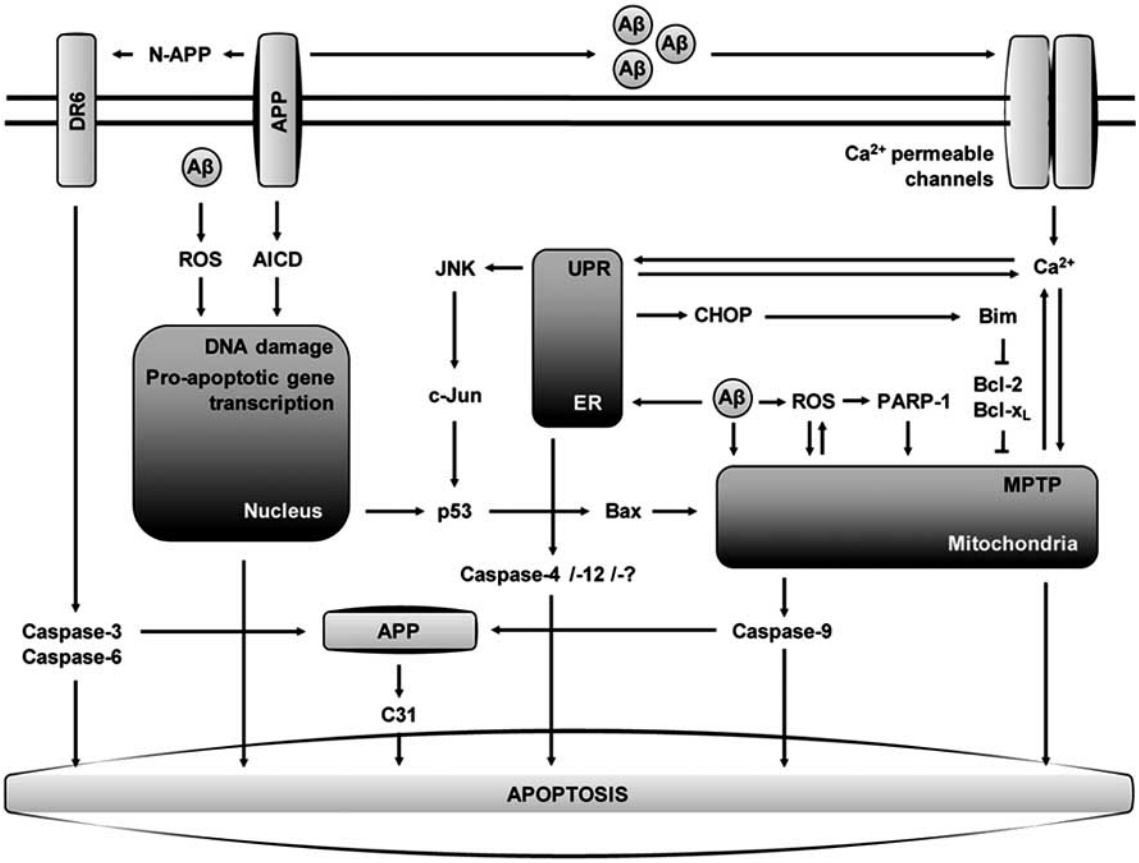


Fig. (2). Mechanisms linking AD pathogenesis to apoptosis. Sequential cleavage of APP generates Aβ, AICD and N-APP, among others. Of these, Aβ is a main player in inducing apoptosis. It enhances calcium (Ca^{2+}) influx into the cell by the formation and/or stimulation of Ca^{2+}-permeable channels. In addition, Aβ acts at the mitochondria and ER to further deregulate Ca^{2+} levels, ultimately resulting in opening of the mitochondrial permeability transition pore (MPTP) and activation of the UPR, including JNK and CHOP activation, respectively. Consequently, apoptosis occurs as a result of caspase activation. In particular, caspase-3 and -9 may also directly cleave APP originating a strong C-terminal pro-apoptotic peptide, C31. Aβ is also responsible for inducing ROS production, which exacerbates the apoptotic process, by mediating DNA damage and activating PARP-1 and p53. AICD may be translocated to the nucleus, where it also interacts with p53 and enhances its transcriptional and pro-apoptotic functions. Finally, by binding to DR6, N-APP was recently shown to activate a self-destruction program in neurons dependent on caspase-3/-6, thus contributing to AD.

Caspase-9 activity has been specifically implicated in the pathology of AD. It was demonstrated that neurons and dystrophic neuritis within plaque regions of AD hippocampal brain sections, display strong activation of caspase-9. Importantly, these studies suggest that caspase-9 activation precedes NFT formation [36]. Caspase-9 was also shown to directly cleave APP, originating the strong pro-apoptotic peptide C31 [37]. Finally, it appears that caspase-9 may induce apoptosis in AD brains independently of caspase-3 or Apaf-1. This would represent an alternative circuit to the classical intrinsic pathway of apoptosis in AD [7].

Active caspase-3 has been detected in neurons of AD brains, with a high degree of colocalization with NFTs and senile plaques [38]. It has recently been shown that caspase-3 is increased and preferentially located in the postsynaptic density fractions in AD, suggesting an important role for caspase-3 in synapse degeneration during disease progression [39]. In fact, in parallel with its role in apoptosis, caspase-3 may be also important in AD pathogenesis by cleaving tau in its C-terminal region; in AD brains, caspase-3-cleaved tau colocalizes with both intracellular Aβ and activated caspase-3, mainly in tangle bearing neurons. Finally, different caspases, including caspase-3, have also been shown to cleave APP, generating Aβ-containing peptides [40, 41], while amyloid plaques are enriched in caspase-cleaved APP [40]. This suggests that Aβ plaque formation and toxicity is likely amplified in a caspase-dependent manner. Nevertheless, by cleaving APP and presenilins, caspase-3 may give rise to C31, which induces cell death through a caspase-independent process and triggers selective increase of Aβ1-42 *in vitro* [42].

Caspase-6 is also capable of cleaving tau, thereby promoting its aggregation in NFTs [43]. Importantly, APP and death receptor 6 (DR6/TNFRSF21) were recently shown to activate a widespread caspase-6-dependent self-destruction program in neurons [44]. Specifically, a cleaved amino-terminal fragment of APP (N-APP) binds DR6 and triggers neurodegeneration through caspase-6, thus contributing to AD.

Altogether, caspases play an active role at distinct levels of Aβ-induced neurotoxicity and AD. Apart from representing a terminal event in apoptosis associated with AD, activation of caspases appears to constitute also a proximal event that promotes the pathology underlying the disease. Nevertheless, caspase-independent pathways of apoptosis may be relevant to certain neurodegenerative diseases. Such pathways are mediated through the mitochondrial release of apoptosis-inducing factor (AIF) and/or endonuclease G (EndoG). AIF is normally confined to the mitochondrial intermembrane space, but translocates to the cytosol and to the nucleus after apoptotic insults. In particular, AIF has been associated with neuronal death in AD [45].

Ursodeoxycholic acid (UDCA), an endogenous bile acid, and its taurine conjugate tauroursodeoxycholic acid (TUDCA) are potent modulators of apoptosis [46, 47]. TUDCA inhibits apoptosis induced by several stimuli in neuronal cells both *in vitro* and *in*

vivo, acting as a pleiotropic agent, particularly in AD-related models [26]. In fact, TUDCA inhibits caspase-2 and -6 activities in AD mutant neuroblastoma cells [48], as well as caspase-3 activation in both primary cortical neurons [49] and PC12 cells [50] incubated with Aβ. Thus, therapeutics aimed at preventing the activation and execution of apoptosis through caspases may provide effective means of treating AD, and neurodegenerative diseases in general.

Caspase Inhibitors as Therapeutic Agents

Caspases represent one of the most specific protease families yet described, since they have an almost absolute requirement for an aspartic acid residue in the P_1 position of their substrate [51, 52]. A typical caspase inhibitor can be divided into three structural components: the warhead, the P_1 aspartic acid and the P_2-P_4 peptidomimetic [53]. The warhead targets the active cysteine residue of the caspase and depends on its substituents that lead either to reversible or irreversible caspase inhibitors. Although all caspases have a common stringent requirement for Asp in position P_1, their extended specificities are distinct. Group I (caspase-1, -4, and -5), group II (caspase-2, -3, and -7) and group III (caspase-6, -8, -9, and -10) prefer the sequences WEHD, DEXD and (LV)EXD, respectively [54].

The search for effective caspase inhibitors as possible therapeutic agents is an important area for the treatment of neurodegenerative disorders [55, 56]. The first caspase inhibitors developed were directed for interleukin-1β converting enzyme (ICE/caspase-1) and were based on the tetrapeptide Ac-TyrValAlaAsp-CHO. Reversible inhibitors such as peptidic aldehydes **1** and ketones **2**, and irreversible inhibitors such as peptidic acyloxy- **3**, aryloxy- **4**, pyrazoloxy- **5**, tetronoyloxy- **6**, and phosphinyloxy-methyl ketones **7** have been described as substrate based caspase-1 inhibitors (Fig. **3**) [57-63].

Since peptide-based ICE inhibitors were likely to suffer from poor oral bioavailability and rapid clearance, several single amino-acid inhibitors **8**-**9** [64-68], non-peptide inhibitors lacking an aspartic acid residue **10** [69] and peptidomimetic **11**-**16** caspase-1 inhibitors were developed (Fig. **4**) [70-78].

After the initial studies for caspase-1, several peptidic inhibitors were designed to target effector caspases, in particular, caspase-3 [79-93]. A class of NO donors, *N*-nitrosoaniline derivatives **17**-**18** (Fig. **5**), were shown to inhibit caspase-3 by *S*-nitrosylation of the enzyme [94]. Several non-peptide inhibitors **19**-**21** were also developed to inhibit caspases [95-103] (Fig. **5**). In particular, isatin sulfonamides inhibitors **19** were shown to inhibit selectively caspase-3 and -7 by interacting primarily with the S_2 subsite, without bind in the caspase primary aspartic acid binding pocket (S_1) [95, 96]. More recently, caspase inhibitors based on Michael acceptor scaffolds, such as aza-peptide and sulfonamide isatin Michael acceptors **22**-**23** were described as potent irreversible and selective caspase inhibitors (Fig. **5**) [104-108].

Of these inhibitors, only a few have entered preclinical studies with animal models of human diseases and their major disadvantage is the lack of selectivity. Up to now, only VX-740 (Pralnacasan) **24**, VX-765 **25**, IDN-6556 **26** and LB-84451 have reached clinical trials (Fig. **6**) [109, 110]. LB84451 is a pan-caspase inhibitor currently being evaluated in a Phase II clinical trial in patients chronically infected with the hepatitis C virus. IDN-6556, an oxamyl dipeptide irreversible and pan caspase inhibitor [111-113] was apparently discontinued in phase II clinical trials for the treatment of liver disease [126]. VX-765, a prodrug that requires esterase cleavage to yield the aldehyde functionality, has entered a phase II clinical study targeting psoriasis. Pralnacasan, a reversible ICE inhibitor [73], progressed into clinical trials for the potential treatment of inflammatory diseases [114], but the phase II program was discontinued due to liver abnormalities in dogs after a nine-month exposure to a high dose of Pralnacasan. The non-peptide VRT-018858 **27**, active metabolite of the pro-drug pralnacasan, was shown to be neuroprotective, which confirms that caspase-1 is an important target for intervention in acute central nervous system injury [115]. Also, the reduction of ischemic brain injury by Z-VAD-fmk, Ac-YVAD-cmk, Z-DEVD-fmk in mice [116], and Z-VAD-DCB in rats indicates that caspase inhibitors should provide a potential therapy for the treatment of neurodegenerative disorders. For example, M826, a small reversible inhibitor of caspase-3, shown to be neuroprotective, was 50-1000-fold more potent than the peptide-based caspase-3 inhibitors Z-DEVD-fmk, Z-VAD-fmk and Boc-D-fmk, in an enzyme assay system with brain tissue lysates [117, 118].

3.2. Bcl-2 Family Members

Apart from caspases, several other molecules involved in the apoptotic cascade have also been linked to AD. It has been shown, for instance, that Bcl-2 family members Bax and Bcl-2 co-localize in the frontal cortex of AD patients [119]. In hippocampal tissue, apoptotic proteins c-Fos, c-Jun, and Bak were shown to be increased [120]. *In vitro*, Aβ induces expression of pro-apoptotic Bcl-2 proteins, including Bax, and down regulation of anti-apoptotic members such as Bcl-2, Bcl-x_L and Bcl-w [121]. Increased Bax expression has also been associated with senile plaques in AD hippocampal brain tissue and neurons with tau-positive NFTs [122]. Consistently, Aβ fails to induce apoptosis in Bax-deficient neurons [123], whereas overexpression of Bcl-x_L and Bcl-2 attenuates Aβ-induced apoptosis [124, 125]. Aβ also induces Bim in cultured hippocampal and cortical neurons. Moreover, Bim was found to be up-regulated in entorhinal cortical neurons of postmortem AD human brains [126].

Bcl-2 Family Members as Therapeutic Targets

A successful therapeutic intervention would seem to entail an anti-apoptotic gain of function and/or a pro-apoptotic loss of function to promote mitochondrial function stabilization [127]. Therefore, Bax and Bcl-2 homology 3 (BH3) only proteins inhibitors can represent potential targets for therapeutic intervention in AD and other neurodegenerative disorders. However, while several small

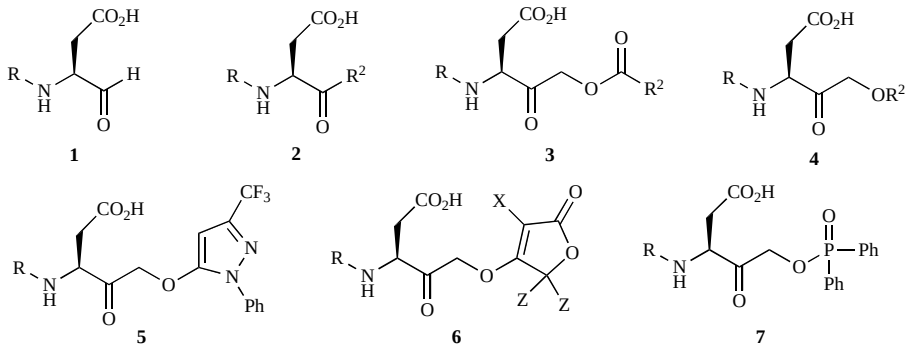


Fig. (3). Peptidic caspase-1 inhibitors.

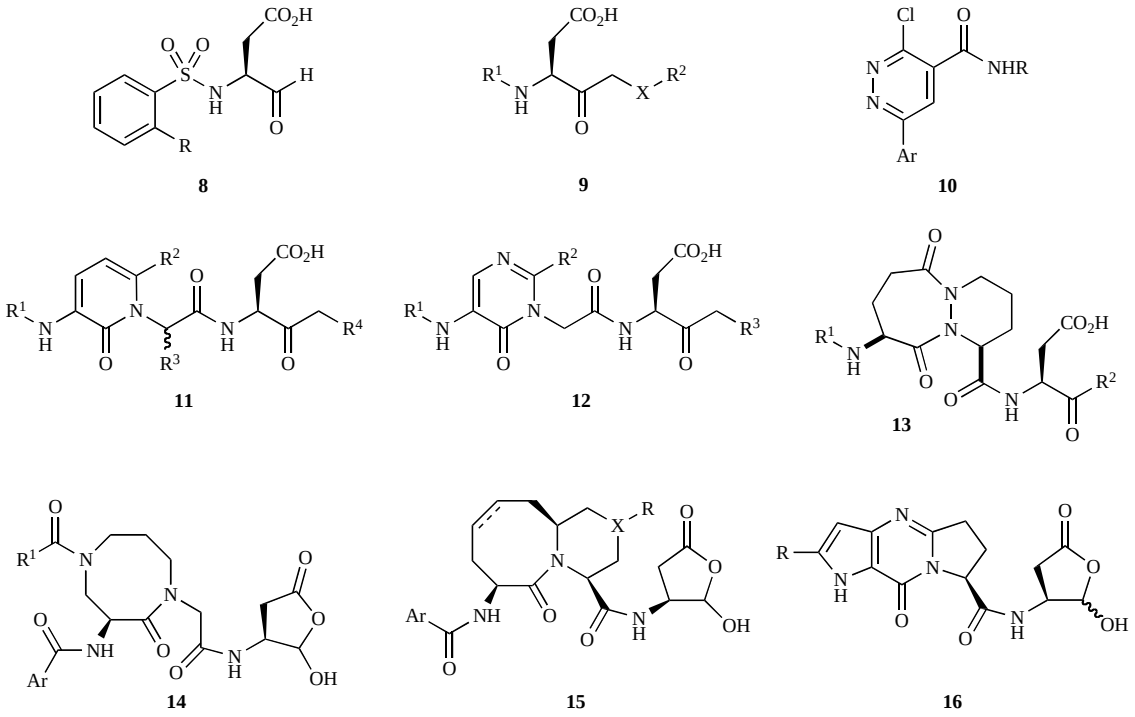


Fig. (4). Single amino acid, peptidomimetic and non-peptide caspase-1 inhibitors.

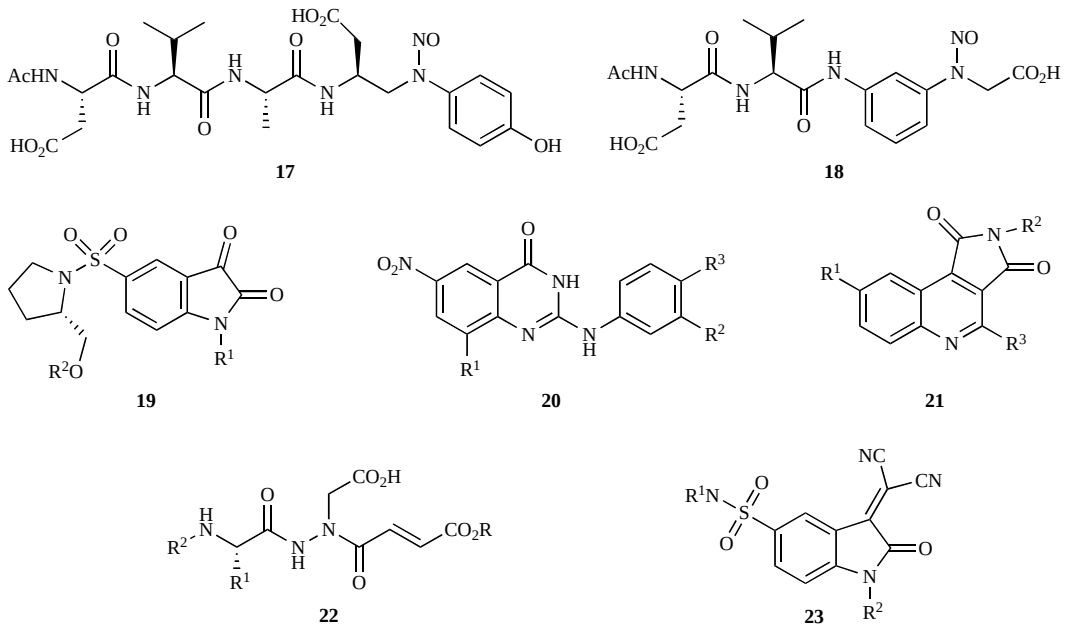


Fig. (5). Scaffolds used in the design of caspase inhibitors.

molecule inhibitors of anti-apoptotic members of the Bcl-2 family have been reported [128], only a few inhibitors of pro-apoptotic members are described.

Bombrun *et al*. described molecules that inhibit cytochrome *c* release from mitochondria and suggested that this action was mediated by inhibition of Bax channel-forming activity (Bax channel blockers) [129]. They synthesized and evaluated a series of 3,6-dibromocarbazole piperazine derivatives of 2-propanol, starting with compound **28** (Fig. **7**). It was found that both enantiomers of compound **28** have the same activity. Modifications in the 2-propanol spacer by substituting the hydroxyl group by a pure hydrogen bond acceptor (keto) or donor (amino), as well as monofluoro and difluoro groups, resulted in a decrease of activity. The structure-activity relationship (SAR) studies also suggested the importance of the piperazine basic nitrogen; therefore, introduction of an aliphatic group on the piperazine showed good activity. Similar activity was also found when aromatic groups, particularly those bearing small substituents with little lipophilic and electronic effects, were connected to the piperazine by a methyl group.

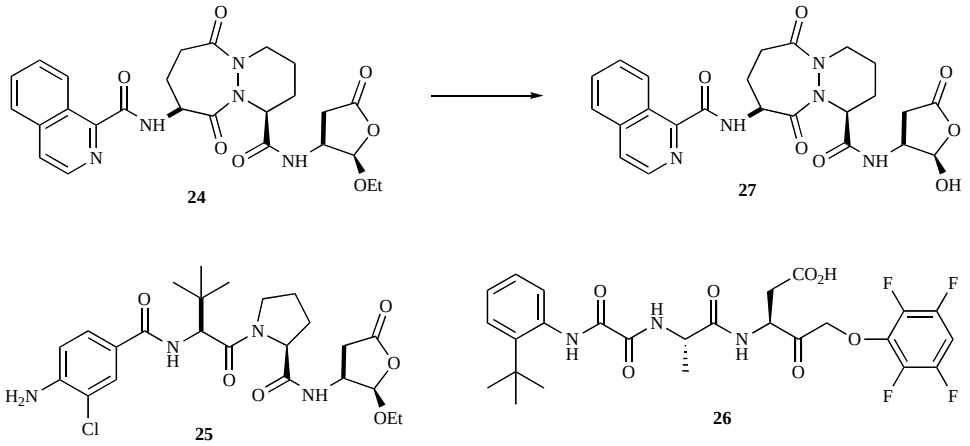


Fig. (6). Caspase inhibitors that reached clinical trials.

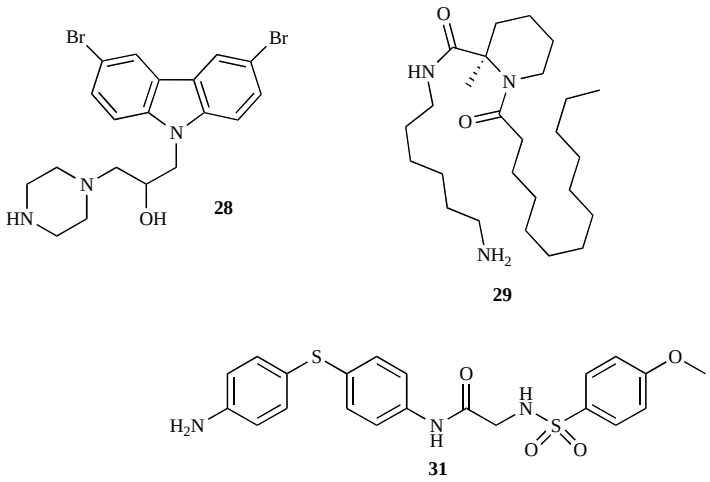


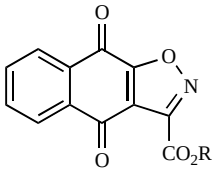
Fig. (7). Chemical structures of Bax inhibitors and Bid inhibitors.

Hetz *et al.* later described and evaluated two novel Bax channel inhibitors, Bci1 **29** and Bci2 **30** (Fig. **7**) [130]. It was demonstrated that these compounds prevent activation of mitochondria-induced apoptosis, augmenting protection against ischemia-induced tissue damage. Becattini *et al.* [131], using nuclear magnetic ressonance (NMR) based strategies, designed a series of 4-phenylsulfanyl-phenylamine derivatives that bind and suppress the BH3-only protein Bid. Eight compounds were synthesized, and BI-6C9 **31** (Fig. **7**) was found to be the strongest Bid binder. The compound occupies a hydrophobic crevice on the surface of Bid or truncated Bid (tBid) and, as a result, the authors speculated that it either interferes with exposure of the BH3 domain or blocks tBid insertion into membranes by maintaining Bid in an inactive conformation.

Another approach to pro-apoptotic multidomain proteins suppression entails the identification of compounds that mimic the peptides designed from the Ku70 Bax-inhibiting domain. Ku70 is a 70 kDa subunit of the Ku complex, which has an important role in DNA double-strand break repair in the nucleus and interacts with pro-apoptotic protein Bax in the cytosol preventing its mitochondrial translocation, thus inhibiting Bax-mediated apoptosis [132]. Sawada *et al*. [133] developed pentapeptides from the Bax-binding domain of Ku70, designated Bax-inhibiting peptides (BIP), such as VPMLK (V5) and PMLKE (P5) that inhibit Bax mediated apoptosis. Interestingly, TUDCA was also shown to inhibit translocation of Bax to the mitochondria in isolated cortical neurons incubated with Aβ [49], as well as Bax expression in PC12 cells challenged with Aβ [50]. Dibucaine, a local anesthetic, and propranolol, a β-adrenergic blocking agent, are amphiphilic membrane-active cationic drugs and inhibit Bax-induced cytochrome *c* release downstream of Bax mitochondrial insertion, likely through a direct interaction with the lipid membrane, blocking Bax-induced changes in lipid structure [134].

Humanin (HN), an endogenous peptide of 26 amino-acids length, and HN derivatives, possess neuroprotective activity and play an important role against AD-related neuronal cell death [135]. Although its mechanism of action is not completely elucidated, some studies report that HN inhibits Bax translocation to mitochondria by binding to the inactive form of Bax in the cytosol. This prevents activation from BH3-only proteins, contributing at least partially to its neuroprotective effect [136]. Compounds that mimic the HN peptide would be of therapeutic relevance.

BH1-4 proteins Bcl-2 and Bcl-x_L inhibit mitochondrial permeability transition and strengthen antioxidant defenses [137]. Therefore, they are also attractive targets for neuroprotection. Delivery of Bcl-x_L directly to the brain was already accomplished by creating a fusion protein containing a protein transduction domain derived from the human immunodeficiency TAT protein [138]. Finally, naphtho[2,3-d]isoxazole-4,9-dione-3-carboxylates **32** [139] exert a general potent protective role against apoptotic cell death (Fig. **8**). They significantly increase cell viability, while reducing nuclear fragmentation, caspase-3, -8 and -9 activation, and cytochrome *c* release induced by camptothecin in primary rat hepatocytes. In addition, compounds **32** up-regulate Bcl-x_L. Importantly, similar



32

Fig. (8). Chemical structure of naphtho[2,3-d]isoxazole-4,9-dione-3-carboxylates.

protective effects were seen in HuH-7 and PC12 cells incubated with distinct apoptotic stimuli, such as camptothecin, TGF-β1, or rotenone [140].

A further optimization of the compounds cited above may provide a starting point for development of potential drug candidates for human illnesses associated with uncontrolled cell death like neurodegenerative disorders, in particular AD. However, the requirement of some Bcl-2 family proteins for normal tissue homeostasis raises concerns about potential cancer development upon chronic suppression of these anti-apoptotic proteins, and might limit their use to acute situations [141].

3.3. Mitochondria

Mitochondrial function is compromised in brain cells of AD patients. Impairment of tricarboxylic acid (TCA) cycle enzymes of mitochondria was found to correlate with the clinical state of AD [142]. Recently, it was demonstrated that Aβ induces apoptosis in primary hippocampal neurons, which derives, at least in part, from mitochondria dysfunction, as assessed by depolarized membrane potential, decreased cytochrome *c* oxidase activity and ATP levels, and cytochrome *c* release [143]. These results indicate that Aβ induces neuronal death by activating an apoptotic pathway involving impaired mitochondria function and cellular homeostasis. Aβ is also involved in the disruption of calcium homeostasis, through ER-mediated stress, thereby inducing mitochondrial dysfunction [144]. Furthermore, Aβ-mediated mitochondrial dysfunction also enhances generation of ROS. In addition to membrane lipid or protein damage, ROS can mediate DNA damage, which activates fatal apoptotic signaling through the tumor suppressor p53 and poly(ADP-ribose) polymerase (PARP) [31]. Interestingly, UDCA and, more efficiently, TUDCA, have also been shown to inhibit Aβ-induced mitochondrial membrane permeabilization and subsequent cytochrome *c* release in isolated neuronal mitochondria [145], as well as Aβ-driven modifications in mitochondrial membrane redox status, lipid polarity and protein order [146].

Modulators of the Apoptosome as Therapeutic Agents

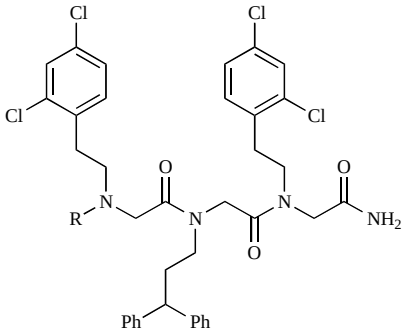
The apoptosome links mitochondrial disfunction with activation of effector caspases and, therefore, is of great interest for the development of apoptotic modulators. Perez-Paya *et al.* [147] identified compounds that inhibit the apoptosome-mediated activation of procaspase-9, from the screening of a diversity-oriented chemical library of *N*-alkylglycines (oligomers of *N*-alkylglycines, known as peptoids) (Fig. **9**). These non-natural molecules are attractive for the drug discovery process due to their broad variety of biological activities and proteolytic stability. The active compounds rescued from the library were chemically optimized to obtain molecules that bind to both recombinant and human endogenous Apaf-1, and decrease the apoptotic phenotype in mitochondrial-mediated models of cellular apoptosis. Peptoid **33** is the most active compound and directly binds to Apaf-1 and induces a nonactive apoptosome complex.

Despite its potency, **33** exhibited low membrane permeability and modest efficacy in arresting cellular apoptosis. Therefore, this new first-in-class apoptosome inhibitor has been further improved by modifications directed to enhance its cellular penetration by conjugation with known cell penetrating peptides, such as penetratin (PEN), yielding PEN-1 **34** (Fig. **9**). Enhanced efficacy was probably related to an efficient lysosomotropic drug release in the cytosol, when a polymeric carrier was combined with **33** to yield compounds that decrease cell death, like PGA-1 **35**. In addition, restriction of the conformational mobility of peptoid **33** through backbone cyclization (compound **36**) decreased the unspecific toxicity of **33** and increased its anti-apoptotic activity [148].

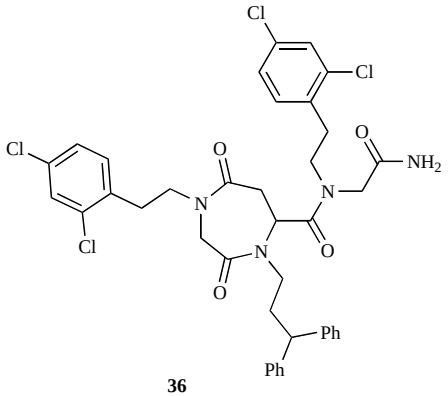
3.4. PARP and p53

DNA-damage-induced PARP-1 and p53 have both been implicated in AD [21, 149]. Oxidative stress appears to be the main responsible for the elevated PARP-1 activity and protein levels observed in the frontal and temporal lobe of AD patient brains [150]. Activated PARP-1 may lead to release of AIF from mitochondria, and ultimately induce apoptosis in the rat brain [45]. Furthermore, targeting nuclear factor kappaB (NF-κB) dependent glial activation with pharmacological inhibitors of PARP-1 enzymatic activity reduces expression of inflammatory mediators and APP, thereby reducing the neurotoxic potential of activated glia *in vitro* [151]. Therefore, inhibition of PARP-1 may attenuate apoptotic signaling in AD and simultaneously reduce inflammatory processes.

Enhanced p53 expression has been detected in damaged neurons of AD patients brain tissue [152], as well as in brains of transgenic mice overexpressing Aβ [153]. In addition, familial AD mutations increase p53 expression and activity in human brains [154]. However, most evidences for a critical role of p53 in AD-



33 R=H
34 R=CPP (cell penetrating peptide)
35 R=$COCH_2NHCOCH_2NHCOCH_2PC$ (PC=polymeric carrier)



36

Fig. (9). Peptoids that inhibit the apoptosome-mediated activation of procaspase-9.

associated neuronal death were provided by data from cell culture models. In PC12 cells, for instance, exposure to Aβ resulted in increased p53 levels, which were sufficient to increase Bax protein and nuclear fragmentation [50]. Similar results were obtained in neuroblastoma cells after inducing the endogenous expression of Aβ [48]. Interestingly, intracellular Aβ1-42 may cause p53-dependent neuronal apoptosis through activation of the p53-promoter, thus providing an alternative pathway of cell death in AD [155]. In turn, inhibition of p53 attenuates Aβ-induced apoptosis, as a result of a preserved mitochondrial function and reduced caspase-3 activation [156]. Finally, upregulation of p53 is also associated with indirect induction of tau abnormal phosphorylation in HEK293a cells [157], while p53-dependent mechanisms are involved in apoptosis induced by APP-derived cleavage product designated amyloid precursor protein intracellular domain (AICD) [154].

Interestingly, p53 is emerging as a prime target of TUDCA during modulation of apoptosis [158]. In fact, TUDCA inhibits E2F-1 induction and p53 stabilization in PC12 cells challenged with Aβ and protects against p53- and Bax-dependent apoptosis induced by E2F-1 and p53 overexpression, respectively [50]. The role of p53 in mediating the effects of TUDCA was further confirmed using an *in vitro* model of familial AD [48]. Although it is still not clear how TUDCA interferes with p53 expression and activation, it appears that nuclear steroid receptors may play a role [159]. It was also recently demonstrated that UDCA reduces p53 transcriptional activity and its ability to bind DNA in primary rat hepatocytes [160].

p53 as a Therapeutic Target

In an attempt to demonstrate that p53 suppression can decrease the side effects of antitumor therapy, pifithrin-α (PFT-α) **37** (Fig. **10**), an antihelminthic compound, was identified as a p53 inhibitor in a screening of 10000 random compounds [161]. Meanwhile, the implication of p53 in neuronal death suggested that inhibition of p53 might prove effective in reducing or preventing the neurodegenerative process. Several studies assessed and verified the neuronal protection conferred by PFT-α [162] and PFT-α methyl analogue Z-1-117 **38** (Fig. **10**) [163].

Ever since PFT-α-mediated p53 inhibition was recognized, compounds have been developed around the 2-imino-2,3,4,5,6,7-hexa-hydrobenzothiazole scaffold [164]. Zhu *et al.* [165] synthesized tetrahydrobenzothiazole analogues, and assessed and confirmed their value as neuroprotective agents. SAR studies revealed the importance of the N-substituent group in the iminothiazole hetero-cycle for biological activity and that substitution on the aryl ring influences activity, favoring *p*-chloro, *p*-methoxy, or a lack of substitution. Furthermore, an oxazole instead of a thiazole ring increases potency. This study opened a new strategy to reduce or prevent neuronal death *via* p53-dependent apoptosis, with potential use in AD and other neurodegenerative disorders.

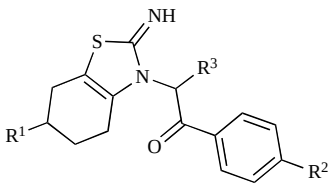
Pietrancosta *et al*. also synthesized PFT-α analogues to evaluate their capacity as p53 inactivators, and initially found compounds **39**-**40** (Fig. **10**) to be one log more potent than PFT-α [166]. To unveil its mechanism of action, PFT-α derivatives (opened PFT, cyclic dehydrated PFT and oxime derivative analogues) were evaluated *in vitro* and *in vivo* [167]. *In vitro* assays demonstrated that the tricyclic analogue **40** (Fig. **10**) was the most active. SAR studies revealed the importance of the cyclohexene ring, since the inhibitory effect was abolished when this ring was substituted with larger ones. Compound **41** (Fig. **10**) showed an inhibitory activity similar to PFT-α, whereas its oxime derivative was found to be inactive. *In vivo* evaluation showed that **41** was 2 log values more active than PFT-α. The authors concluded that **41** is transformed *in situ* into its cyclic form (compound **40**) by dehydration, increasing its inhibition effect. The importance of cyclization in activity was corroborated by the fact that the noncyclizable oxime analogue of **41** is inactive. The cyclization also occurs in PFT-α, forming PTF-β **42** (Fig. **10**) [168]. In addition, the authors established that these compounds act as p53 posttranscriptional activity inhibitors and are specific to the p53-dependent pathway.

Barchéchath *et al.* described analogues to PFT-α and PFT-β bearing an aryl ring instead of a saturated ring [169]. Only aromatic analogues of PFT-β were found to possess improved cytoprotective activity. They described compound **43** and **44** (Fig. **10**) as the most promising protective agents for potential clinical development. PFT-α and PFT-β inhibit both apoptosis and growth arrest [170]. More recently, a new analogue, PFT-μ **45** (Fig. **10**), was found to be a selective inhibitor of the mitochondrial branch of the p53 pathway, not showing activity on p53-dependent transactivation with important implication in cancer treatment [171].

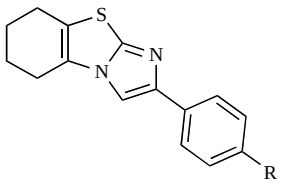
PFT-α and related derivatives can be promising drugs to treat acute conditions like stroke, brain injury, or other conditions characterized by sudden brain trauma. Once safety is established, their use could be extended for long-term diseases.

3.5. Death Receptors

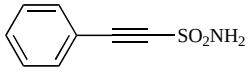
Key apoptosis regulator Fas ligand (FasL) may participate in both neuronal and immune cell apoptosis in AD. In fact, Fas-regulated apoptosis has been linked to neurodegenerative lesions in the brain of patients with AD [152]. In addition, transgenic mice overexpressing the wild-type human APP gene display increased expression of Fas [172]. In particular, FasL appears to play an important role in Aβ-mediated neurotoxicity. Exposure of cortical neurons to Aβ activates c-Jun N-terminal kinase (JNK). JNK is required for the activation of the c-Jun transcription factor, which in turn stimulates the transcription of several key target genes, including the death inducer FasL. The binding of FasL to its receptor Fas then induces a cascade of events, leading to caspase activation and, ultimately, apoptosis [173]. Disruption of Fas/FasL signaling using a fusion protein containing the ligand binding domain of Fas and the Fc domain of IgG (FasFc), has been found to protect primary



37 R^1=H, R^2=Me, R^3=H
38 R^1=Me, R^2=Me, R^3=H
39 R^1=H, R^2=OMe, R^3=CN
41 R^1=H, R^2=NO_2, R^3=H
43 R^1=H, R^2=pyrrolidine, R^3=H



40 R=NO_2
42 R=Me
44 R=pyrrolidine



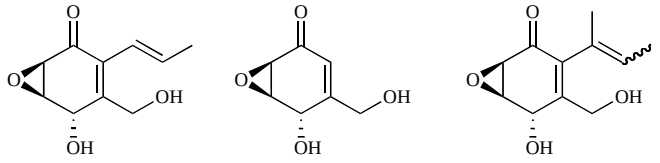
45

Fig. (10). Chemical structure of p53 inhibitors.

cortical and cerebellar neurons from Aβ neurotoxicity [174]. Similarly, neuronal cultures derived from mice carrying inactivating mutations in Fas (Faslpr) or FasL (Fasgld) exhibit protection from Aβ(1-42)-induced cell death [175]. In parallel, tumor necrosis factor alpha (TNFα), tumor necrosis factor receptor 1 (TNF-R1) and TNF-R1-associated death domain protein (TRADD) have all been found to be increased in AD. These changes appear to translate into increased degenerative potential, as the downstream effector caspase-3 and product of the TNF pathway was also found to be increased in the same study [176]. In addition, TRAIL was shown to contribute substantially to Aβ-induced neurotoxicity in a human SH-SY5Y neuronal cell line [177].

Modulators of the Death Receptor Pathway

To find specific inhibitors of Fas-mediated apoptosis, Osada *et al.* [178] have screened a library of microbial secondary metabolites. This study identified epoxycyclohexenone (ECH) **46** that selectively inhibited Fas-mediated apoptosis by preventing the activation of pro-caspase-8 at the death-inducing signaling complex (DISC). In a later study, the same group designed, synthesized and performed SAR of several ECH-related molecules, showing that RKTS-33 **47** and RKTS-34 **48** exhibited selective inhibitory activity toward Fas-mediated apoptosis (Fig. **11**) [179].



46 **47** **48**

Fig. (11). ECH derivatives with selective inhibitory activity toward Fas-mediated apoptosis.

CONCLUDING REMARKS

Although many of the key apoptotic proteins activated during AD and other neurodegenerative diseases have already been identified, our understanding of the complex underlying mechanisms remains scattered. In some circumstances, this is due to the lack of appropriate models. For AD, animal models available still do not provide an accurate model of the human disease, and very few of them display significant levels of cell death.

Targeting the cause of neuronal cell death in different neurodegenerative diseases, by the use of chaperone-related strategies and/or protein degradation-enhancing drugs represents a challenging task. In turn, targeting the apoptotic response itself, once the initiating events in neurodegeneration have been triggered, may constitute a broader and more consistent approach. In this regard, the pre-mitochondrial events in the apoptotic cascade constitute the most desirable targets, as the mitochondrial membrane permeabilization is usually assumed as the "point-of-no-return" during the intrinsic pathway of apoptosis. Molecules aimed at preventing mitochondrial dysfunction, particularly Bcl-2 family modulators are, therefore, excellent candidates for blocking the release of multiple proapoptotic proteins and simultaneously preserving energy metabolism for optimal neuroprotection. Nevertheless, interventions that block the post-mitochondrial phase of apoptosis are still relevant in early phases of neurodegeneration and, more importantly, within the few hours after neuronal injury by ischemia/reperfusion or trauma. Therefore, in particular cases, the inhibition of caspases and other late apoptotic effectors may also afford significant neuroprotection.

Inhibition of apoptosis may be effective in the treatment of neurodegenerative diseases. However, the inhibitors of apoptosis must be selective enough to avoid blocking normal apoptosis. Much still remains to be elucidated about the specific pathways that intervene in neurodegeneration and neuroprotection.

ACKNOWLEDGEMENTS

Supported by grants PTDC/SAU-FCF/67912/2006 and PTDC/BIA-BCM/67922/2006 from Fundação para a Ciência e Tecnologia (FCT), Lisbon, Portugal. PMCG and DMSF were recipients of fellowships SFRH/BPD/22631/2005 and SFRH/BD/60521/2009, respectively, from FCT.

ABBREVIATIONS

Ac	=	Acetyl
AD	=	Alzheimer's disease
AIF	=	Apoptosis-inducing factor
AICD	=	Amyloid precursor protein intracellular domain
APP	=	Amyloid precursor protein
Aβ	=	Amyloid β
BH	=	Bcl-2 homology
CHOP	=	Transcription factor C/EBP homologous protein
cmk	=	Chloromethylketone
DR	=	Death receptor
EndoG	=	Endonuclease G
ER	=	Endoplasmic reticulum
FasL	=	Fas ligand
fmk	=	Fluoromethylketone
HN	=	Humanin
IAP	=	Inhibitor of apoptosis protein
ICE	=	Interleukin-1β converting enzyme
JNK	=	c-Jun N-terminal kinase
NFT	=	Neurofibrillary tangle
PARP	=	Poly(ADP-ribose) polymerase
PCD	=	Programmed cell death
PD	=	Parkinson's disease
PFT	=	Pifithrin
ROS	=	Reactive oxygen species
SAR	=	Structure-activity relationship
tBid	=	Truncated Bid
UDCA	=	Ursodeoxycholic acid
UPR	=	Unfolded protein response
TUDCA	=	Tauroursodeoxycholic acid
Z	=	Benzyloxycarbonyl

REFERENCES

[1] Hengartner MO. The biochemistry of apoptosis. Nature 2000; 407: 770-6.
[2] Degterev A, Yuan J. Expansion and evolution of cell death programmes. Nat Rev Mol Cell Biol 2008; 9: 378-90.
[3] Rupinder SK, Gurpreet AK, Manjeet S. Cell suicide and caspases. Vascul Pharmacol 2007; 46: 383-93.
[4] Green DR. Apoptotic pathways: ten minutes to dead. Cell 2005; 121: 671-4.
[5] Riedl SJ, Shi Y. Molecular mechanisms of caspase regulation during apoptosis. Nat Rev Mol Cell Biol 2004; 5: 897-907.
[6] Okouchi M, Ekshyyan O, Maracine M, Aw TY. Neuronal apoptosis in neurodegeneration. Antioxid Redox Signal 2007; 9: 1059-96.
[7] Madden SD, Cotter TG. Cell death in brain development and degeneration: control of caspase expression may be key! Mol Neurobiol 2008; 37: 1-6.

[8] Buss RR, Oppenheim RW. Role of programmed cell death in normal neuronal development and function. Anat Sci Int 2004; 79: 191-7.
[9] Ferrer I, Bernet E, Soriano E, del Rio T, Fonseca M. Naturally occurring cell death in the cerebral cortex of the rat and removal of dead cells by transitory phagocytes. Neuroscience 1990; 39: 451-8.
[10] Ferrer I, Serrano T, Soriano E. Naturally occurring cell death in the subicular complex and hippocampus in the rat during development. Neurosci Res 1990; 8: 60-6.
[11] Tanaka M, Marunouchi T. Immunohistochemical analysis of developmental stage of external granular layer neurons which undergo apoptosis in postnatal rat cerebellum. Neurosci Lett 1998; 242: 85-8.
[12] Yoshida H, Kong YY, Yoshida R, *et al.* Apaf1 is required for mitochondrial pathways of apoptosis and brain development. Cell 1998; 94: 739-50.
[13] Kuida K, Zheng TS, Na S, *et al*. Decreased apoptosis in the brain and premature lethality in CPP32-deficient mice. Nature 1996; 384: 368-72.
[14] Madden SD, Donovan M, Cotter TG. Key apoptosis regulating proteins are down-regulated during postnatal tissue development. Int J Dev Biol 2007; 51: 415-23.
[15] Lossi L, Merighi A. *In vivo* cellular and molecular mechanisms of neuronal apoptosis in the mammalian CNS. Prog Neurobiol 2003; 69: 287-312.
[16] Gorman AM, Szegezdi E, Quigney DJ, Samali A. Hsp27 inhibits 6-hydroxydopamine-induced cytochrome c release and apoptosis in PC12 cells. Biochem Biophys Res Commun 2005; 327: 801-10.
[17] Gorman AM. Neuronal cell death in neurodegenerative diseases: recurring themes around protein handling. J Cell Mol Med 2008; 12: 2263-80.
[18] Mattson MP. Neuronal life-and-death signaling, apoptosis, and neurodegenerative disorders. Antioxid Redox Signal 2006; 8: 1997-2006.
[19] Sastry PS, Rao KS. Apoptosis and the nervous system. J Neurochem 2000; 74: 1-20.
[20] Lorz C, Mehmet H. The role of death receptors in neural injury. Front Biosci 2009; 14: 583-95.
[21] Culmsee C, Mattson MP. p53 in neuronal apoptosis. Biochem Biophys Res Commun 2005; 331: 761-77.
[22] Kruttgen A, Saxena S, Evangelopoulos ME, Weis J. Neurotrophins and neurodegenerative diseases: receptors stuck in traffic? J Neuropathol Exp Neurol 2003; 62: 340-50.
[23] Cotman CW, Anderson KJ. Synaptic plasticity and functional stabilization in the hippocampal formation: possible role in Alzheimer's disease. Adv Neurol 1988; 47: 313-35.
[24] Galluzzi L, Aaronson SA, Abrams J, *et al.* Guidelines for the use and interpretation of assays for monitoring cell death in higher eukaryotes. Cell Death Differ 2009; 16: 1093-107.
[25] Turner RS. Alzheimer's disease. Semin Neurol 2006; 26: 499-506.
[26] Ramalho RM, Viana RJ, Low WC, Steer CJ, Rodrigues CM. Bile acids and apoptosis modulation: an emerging role in experimental Alzheimer's disease. Trends Mol Med 2008; 14: 54-62.
[27] Khachaturian ZS. Toward a comprehensive theory of Alzheimer's disease--challenges, caveats, and parameters. Ann N Y Acad Sci 2000; 924: 184-93.
[28] Ribe EM, Serrano-Saiz E, Akpan N, Troy CM. Mechanisms of neuronal death in disease: defining the models and the players. Biochem J 2008; 415: 165-82.
[29] Mattson MP. Pathways towards and away from Alzheimer's disease. Nature 2004; 430: 631-9.
[30] Selkoe DJ. Alzheimer's disease: genes, proteins, and therapy. Physiol Rev 2001; 81: 741-66.
[31] Culmsee C, Landshamer S. Molecular insights into mechanisms of the cell death program: role in the progression of neuro-degenerative disorders. Curr Alzheimer Res 2006; 3: 269-83.
[32] Ling D, Salvaterra PM. A central role for autophagy in Alzheimer-type neurodegeneration. Autophagy 2009; 5: 738-40.
[33] Hung SY, Huang WP, Liou HC, Fu WM. Autophagy protects neuron from Abeta-induced cytotoxicity. Autophagy 2009; 5: 502-10.
[34] Costa RO, Ferreiro E, Cardoso SM, Oliveira CR, Pereira CM. ER Stress-mediated apoptotic pathway induced by amyloid-beta. J Alzheimers Dis 2010.
[35] Pompl PN, Yemul S, Xiang Z, *et al*. Caspase gene expression in the brain as a function of the clinical progression of Alzheimer disease. Arch Neurol 2003; 60: 369-76.
[36] Rohn TT, Rissman RA, Davis MC, Kim YE, Cotman CW, Head E. Caspase-9 activation and caspase cleavage of tau in the Alzheimer's disease brain. Neurobiol Dis 2002; 11: 341-54.
[37] Lu DC, Rabizadeh S, Chandra S, *et al.* A second cytotoxic proteolytic peptide derived from amyloid beta-protein precursor. Nat Med 2000; 6: 397-404.
[38] Su JH, Zhao M, Anderson AJ, Srinivasan A, Cotman CW. Activated caspase-3 expression in Alzheimer's and aged control brain: correlation with Alzheimer pathology. Brain Res 2001; 898: 350-7.
[39] Louneva N, Cohen JW, Han LY, *et al.* Caspase-3 is enriched in postsynaptic densities and increased in Alzheimer's disease. Am J Pathol 2008; 173: 1488-95.
[40] Gervais FG, Xu D, Robertson GS, *et al.* Involvement of caspases in proteolytic cleavage of Alzheimer's amyloid-beta precursor protein and amyloidogenic A beta peptide formation. Cell 1999; 97: 395-406.
[41] LeBlanc A, Liu H, Goodyer C, Bergeron C, Hammond J. Caspase-6 role in apoptosis of human neurons, amyloidogenesis, and Alzheimer's disease. J Biol Chem 1999; 274: 23426-36.
[42] Dumanchin-Njock C, Alves da Costa CA, Mercken L, Pradier L, Checler F. The caspase-derived C-terminal fragment of betaAPP induces caspase-independent toxicity and triggers selective increase of Abeta42 in mammalian cells. J Neurochem 2001; 78: 1153-61.
[43] Guo H, Albrecht S, Bourdeau M, Petzke T, Bergeron C, LeBlanc AC. Active caspase-6 and caspase-6-cleaved tau in neuropil threads, neuritic plaques, and neurofibrillary tangles of Alzheimer's disease. Am J Pathol 2004; 165: 523-31.
[44] Nikolaev A, McLaughlin T, O'Leary DD, Tessier-Lavigne M. APP binds DR6 to trigger axon pruning and neuron death *via* distinct caspases. Nature 2009; 457: 981-9.
[45] Adamczyk A, Czapski GA, Jesko H, Strosznajder RP. Non A beta component of Alzheimer's disease amyloid and amyloid beta peptides evoked poly(ADP-ribose) polymerase-dependent release of apoptosis-inducing factor from rat brain mitochondria. J Physiol Pharmacol 2005; 56 (Suppl 2): 5-13.
[46] Amaral JD, Viana RJ, Ramalho RM, Steer CJ, Rodrigues CM. Bile acids: regulation of apoptosis by ursodeoxycholic acid. J Lipid Res 2009; 50: 1721-34.
[47] Castro RE, Solá S, Steer CJ, Rodrigues CMP. Hepatotoxicity: from genomics to *in-vitro* and *in-vivo*. In: Sahu SC, Ed. USA: John Wiley & Sons, Ltd. 2007; pp. 391-419.
[48] Ramalho RM, Borralho PM, Castro RE, Sola S, Steer CJ, Rodrigues CM. Tauroursodeoxycholic acid modulates p53-mediated apoptosis in Alzheimer's disease mutant neuroblastoma cells. J Neurochem 2006; 98: 1610-8.
[49] Sola S, Castro RE, Laires PA, Steer CJ, Rodrigues CM. Tauroursodeoxycholic acid prevents amyloid-beta peptide-induced neuronal death *via* a phosphatidylinositol 3-kinase-dependent signaling pathway. Mol Med 2003; 9: 226-34.
[50] Ramalho RM, Ribeiro PS, Sola S, Castro RE, Steer CJ, Rodrigues CM. Inhibition of the E2F-1/p53/Bax pathway by tauroursodeoxy-cholic acid in amyloid beta-peptide-induced apoptosis of PC12 cells. J Neurochem 2004; 90: 567-75.
[51] Fuentes-Prior P, Salvesen GS. The protein structures that shape caspase activity, specificity, activation and inhibition. Biochem J 2004; 384: 201-32.
[52] Talanian RV, Brady KD, Cryns VL. Caspases as targets for anti-inflammatory and anti-apoptotic drug discovery. J Med Chem 2000; 43: 3351-71.
[53] Fischer U, Schulze-Osthoff K. Apoptosis-based therapies and drug targets. Cell Death Differ 2005; 12: 942-61.
[54] Thornberry NA, Ranon TA, Pieterson EP, *et al.* A combinatorial approach defines specificities of members of the caspase family and granzyme B - Functional, relationships established for key mediators of apoptosis. J Biol Chem 1997; 272: 17907-11.
[55] Friedlander RM. Mechanisms of disease: Apoptosis and caspases in neurodegenerative diseases. New Engl J Med 2003; 348: 1365-75.
[56] Howley B, Fearnhead HO. Caspases as therapeutic targets. J Cell Mol Med 2008; 12: 1502-16.
[57] Thornberry NA, Peterson EP, Zhao JJ, Howard AD, Griffin PR, Chapman KT. Inactivation of interleukin-1-beta converting-enzyme

by peptide (acyloxy)methyl ketones. Biochemistry 1994; 33: 3934-40.

[58] Dolle RE, Hoyer D, Prasad CVC, *et al.* P-1 aspartate-based peptide alpha-((2,6-dichlorobenzoyl)oxy)methyl ketones as potent time-dependent inhibitors of interleukin-1-beta-converting enzyme. J Med Chem 1994; 37: 563-4.

[59] Dolle RE, Singh J, Rinker J, *et al.* Aspartyl alpha-((1-phenyl-3-(trifluoromethyl)pyrazol-5-yl)oxy)methyl ketones as interleukin-1-beta converting-enzyme inhibitors - significance of the p-1 and p-3 amido nitrogens for enzyme-peptide inhibitor binding. J Med Chem 1994; 37: 3863-6.

[60] Dolle RE, Singh J, Whipple D, *et al.* Aspartyl alpha-((diphenylphosphinyl)oxy)methyl ketones as novel inhibitors of interleukin-1-beta converting-enzyme - utility of the diphenylphosphinic acid leaving group for the inhibition of cysteine proteases. J Med Chem 1995; 38: 220-2.

[61] Prasad CVC, Prouty CP, Hoyer D, *et al.* Structural and stereochemical requirements of time-dependent inactivators of the interleukin-1-beta converting-enzyme. Bioorg Med Chem Lett 1995; 5: 315-8.

[62] Graybill TL, Prouty CP, Speier GJ, *et al.* alpha-((tetronoyl)oxy)- and alpha-((tetramoyl)oxy)methyl ketone inhibitors of the interleukin-1 beta converting enzyme (ICE). Bioorg Med Chem Lett 1997; 7: 41-6.

[63] Revesz L, Briswalter C, Heng R, Leutwiler A, Mueller R, Wuethrich HJ. Synthesis of p1 aspartate-based peptide acyloxymethyl and fluoromethyl ketones as inhibitors of interleukin-1-beta-converting enzyme. Tetrahedron Lett 1994; 35: 9693-6.

[64] Mjalli AMM, Chapman KT, Zhao JJ, Thornberry NA, Peterson EP, Maccoss M. Inhibition of interleukin-1-beta converting-enzyme by n-acyl-aspartic acid ketones. Bioorg Med Chem Lett 1995; 5: 1405-8.

[65] Mjalli AMM, Zhao JJ, Chapman KT, *et al.* Inhibition of interleukin-1-beta converting-enzyme by n-acyl-aspartyl aryloxymethyl ketones. Bioorg Med Chem Lett 1995; 5: 1409-14.

[66] Shahripour AB, Plummer MS, Lunney EA, *et al.* Structure-based design of caspase-1 inhibitor containing a diphenyl ether sulfonamide. Bioorg Med Chem Lett 2001; 11: 2779-82.

[67] Shahripour AB, Plummer MS, Lunney EA, *et al.* Structure-based design of nonpeptide inhibitors of interleukin-1 beta converting enzyme (ICE, caspase-1). Bioorg Med Chem 2002; 10: 31-40.

[68] Harter WG, Albrect H, Brady K, *et al.* The design and synthesis of sulfonamides as caspase-1 inhibitors. Bioorg Med Chem Lett 2004; 14: 809-12.

[69] Dolle RE, Hoyer D, Rinker JM, *et al.* 3-chloro-4-carboxamido-6-arylpyridazines as a non-peptide class of interleukin-1 beta converting enzyme inhibitor. Bioorg Med Chem Lett 1997; 7: 1003-6.

[70] Dolle RE, Prouty CP, Prasad CVC, *et al.* First examples of peptidomimetic inhibitors of interleukin-1 beta converting enzyme. J Med Chem 1996; 39: 2438-40.

[71] Semple G, Ashworth DM, Baker GR, *et al.* Pyridone-based peptidomimetic inhibitors of interleukin-1 beta-converting enzyme (ICE). Bioorg Med Chem Lett 1997; 7: 1337-42.

[72] Golec JMC, Mullican MD, Murcko MA, *et al.* Structure-based design of non-peptidic pyridone aldehydes as inhibitors of interleukin-1 beta converting enzyme. Bioorg Med Chem Lett 1997; 7: 2181-6.

[73] Dolle RE, Prasad CVC, Prouty CP, *et al.* Pyridazinodiazepines as a high-affinity, P-2-P-3 peptidomimetic class of interleukin-1 beta-converting enzyme inhibitor. J Med Chem 1997; 40: 1941-6.

[74] Semple G, Ashworth DM, Batt AR, *et al.* Peptidomimetic aminomethylene ketone inhibitors of Interleukin-1 beta-converting enzyme (ICE). Bioorg Med Chem Lett 1998; 8: 959-64.

[75] Oppong KA, Ellis CD, Laufersweiler MC, *et al.* Discovery of novel conformationally restricted diazocan peptidomimetics as inhibitors of interleukin-1 beta synthesis. Bioorg Med Chem Lett 2005; 15: 4291-4.

[76] O'Neil SV, Wang YL, Laufersweiler MC, *et al.* Synthesis and evaluation of novel 8,6-fused bicyclic peptidomimetic compounds as interleukin-1 beta converting enzyme inhibitors. Bioorg Med Chem Lett 2005; 15: 5434-8.

[77] Wang YL, O'Neil SV, Wos JA, *et al.* Synthesis and evaluation of unsaturated caprolactams as interleukin-1 beta converting enzyme (ICE) inhibitors. Bioorg Med Chem 2007; 15: 1311-22.

[78] Laufersweiler MC, Wang YL, Soper DL, *et al.* Synthesis and evaluation of tricyclic pyrrolopyrimidinones as dipeptide mimetics: Inhibition of interleukin-1 beta-converting enzyme. Bioorg Med Chem Lett 2005; 15: 4322-6.

[79] Karanewsky DS, Bai X, Linton SD, *et al.* Conformationally constrained inhibitors of caspase-1 (interleukin-1 beta converting enzyme) and of the human CED-3 homologue caspase-3 (CPP32, apopain). Bioorg Med Chem Lett 1998; 8: 2757-62.

[80] Garcia-Calvo M, Peterson EP, Leiting B, Ruel R, Nicholson DW, Thornberry NA. Inhibition of human caspases by peptide-based and macromolecular inhibitors. J Biol Chem 1998; 273: 32608-13.

[81] Choong IC, Lew W, Lee D, *et al.* Identification of potent and selective small-molecule inhibitors of caspase-3 through the use of extended tethering and structure-based drug design. J Med Chem 2002; 45: 5005-22.

[82] Allen DA, Pham P, Choong IC, *et al.* Identification of potent and novel small-molecule inhibitors of caspase-3. Bioorg Med Chem Lett 2003; 13: 3651-5.

[83] Isabel E, Black WC, Bayly CI, *et al.* Nicotinyl aspartyl ketones as inhibitors of caspase-3. Bioorg Med Chem Lett 2003; 13: 2137-40.

[84] Micale N, Vairagoundar R, Yakovlev AG, Kozikowski AP. Design and synthesis of a potent and selective peptidomimetic inhibitor of caspase-3. J Med Chem 2004; 47: 6455-8.

[85] Linton SD, Karanewsky DS, Ternansky RJ, *et al.* Acyl Dipeptides as reversible caspase inhibitors. Part 2: Further optimization. Bioorg Med Chem Lett 2002; 12: 2973-5.

[86] Ullman BR, Aja T, Deckwerth TL, *et al.* Structure-activity relationships within a series of caspase inhibitors: Effect of leaving group modifications. Bioorg Med Chem Lett 2003; 13: 3623-6.

[87] Cai SX, Guan LF, Jia SJ, *et al.* Dipeptidyl aspartyl fluoromethylketones as potent caspase inhibitors: SAR of the N-protecting group. Bioorg Med Chem Lett 2004; 14: 5295-300.

[88] Wang Y, Huang JC, Zhou ZL, *et al.* Dipeptidyl aspartyl fluoromethylketones as potent caspase-3 inhibitors: SAR of the P-2 amino acid. Bioorg Med Chem Lett 2004; 14: 1269-72.

[89] Yang W, Guastella J, Huang JC, *et al.* MX1013, a dipeptide caspase inhibitor with potent *in vivo* antiapoptotic activity. Brit J Pharmacol 2003; 140: 402-12.

[90] Wang Y, Guan LF, Jia SJ, Tseng B, Drewe J, Cai SX. Dipeptidyl aspartyl fluoromethylketones as potent caspase inhibitors: peptidomimetic replacement of the P-2 alpha-amino acid by a alpha-hydroxy acid. Bioorg Med Chem Lett 2005; 15: 1379-83.

[91] Wang Y, Jia SJ, Tseng B, Drewe J, Cai SX. Dipeptidyl aspartyl fluoromethylketones as potent caspase inhibitors: Peptidomimetic replacement of the P-2 amino acid by 2-aminoaryl acids and other non-natural amino acids. Bioorg Med Chem Lett 2007; 17: 6178-82.

[92] Asgian JL, James KE, Li ZZ, *et al.* Aza-peptide epoxides: A new class of inhibitors selective for clan CD cysteine proteases. J Med Chem 2002; 45: 4958-60.

[93] James KE, Asgian JL, Li ZZ, *et al.* Design, synthesis, and evaluation of aza-peptide epoxides as selective and potent inhibitors of caspases-1,-3,-6, and-8. J Med Chem 2004; 47: 1553-74.

[94] Guo ZM, Xian M, Zhang W, McGill A, Wang PG. N-nitrosoanilines: A new class of caspase-3 inhibitors. Bioorg Med Chem 2001; 9: 99-106.

[95] Lee D, Long SA, Adams JL, *et al.* Potent and selective nonpeptide inhibitors of caspases 3 and 7 inhibit apoptosis and maintain cell functionality. J Biol Chem 2000; 275: 16007-14.

[96] Lee D, Long SA, Murray JH, *et al.* Potent and selective nonpeptide inhibitors of caspases 3 and 7. J Med Chem 2001; 44: 2015-26.

[97] Podichetty AK, Faust A, Kopka K, *et al.* Fluorinated isatin derivatives. Part 1: Synthesis of new N-substituted (S)-5- 1-(2-methoxymethylpyrrolidinyl) sulfonyl isatins as potent caspase-3 and-7 inhibitors. Bioorg Med Chem 2009; 17: 2680-8.

[98] Podichetty AK, Wagner S, Schroer S, *et al.* Fluorinated Isatin Derivatives. Part 2. New N-substituted 5-pyrrolidinylsulfonyl isatins as potential tools for molecular imaging of caspases in apoptosis. J Med Chem 2009; 52: 3484-95.

[99] Scott CW, Sobotka-Briner C, Wilkins DE, *et al.* Novel small molecule inhibitors of caspase-3 block cellular and biochemical features of apoptosis. J Pharmacol Exp Ther 2003; 304: 433-40.

[100] Kravchenko DV, Kysil VM, Tkachenko SE, Maliarchouk S, Okun IM, Ivachtchenko AV. Pyrrolo 3,4-c quinoline-1,3-diones as potent caspase-3 inhibitors. Synthesis and SAR of 2-substituted 4-methyl-

8-(morpholine-4-sulfonyl)-pyrrolo 3,4-c quinoline-1,3-diones. Eur J Med Chem 2005; 40: 1377-83.

[101] Kravchenko DV, Kysil VV, Ilyn AP, *et al.* 1,3-dioxo-4-methyl-2,3-dihydro-1H-pyrrolo 3,4-c quinolines as potent caspase-3 inhibitors. Bioorg Med Chem Lett 2005; 15: 1841-5.

[102] Kravchenko DV, Kuzovkova YA, Kysil VM, *et al.* Synthesis and structure-activity relationship of 4-substituted 2-(2-acetyloxyethyl)-8-(morpholine-4-sulfonyl)pyrrolo 3,4-c quinoline-1, 3-diones as potent caspase-3 inhibitors. J Med Chem 2005; 48: 3680-3.

[103] Havran LM, Chong DC, Childers WE, *et al.* 3,4-Dihydro-pyrimido(1,2-a)indol-10(2H)-ones as potent non-peptidic inhibitors of caspase-3. Bioorg Med Chem 2009; 17: 7755-68.

[104] Ekici OD, Gotz MG, James KE, *et al.* Aza-peptide Michael acceptors: A new class of inhibitors specific for caspases and other clan CD cysteine proteases. J Med Chem 2004; 47: 1889-92.

[105] Ekici OD, Li ZZ, Campbell AJ, *et al.* Design, synthesis, and evaluation of aza-peptide Michael acceptors as selective and potent inhibitors of caspases-2, -3, -6, -7, -8, -9, and -10. J Med Chem 2006; 49: 5728-49.

[106] Chu WH, Rothfuss J, d'Avignon A, *et al.* Isatin sulfonamide analogs containing a michael addition acceptor: A new class of caspase 3/7 inhibitors. J Med Chem 2007; 50: 3751-5.

[107] Chu WH, Rothfuss J, Chu YX, Zhou D, Mach RH. Synthesis and *in vitro* evaluation of sulfonamide isatin michael acceptors as small molecule inhibitors of caspase-6. J Med Chem 2009; 52: 2188-91.

[108] Santos MMM, Moreira R. Michael acceptors as cysteine protease inhibitors. Mini-Rev Med Chem 2007; 7: 1040-50.

[109] Eda H. In: Design of caspase inhibitors as potential clinical agents, Therapeutic potential for caspase inhibitors present and future. USA: CRC Press 2009; pp. 251-287.

[110] Cornelis S, Kersse K, Festjens N, Lamkanfi M, Vandenabeelel P. Inflammatory caspases: Targets for novel therapies. Curr Pharm Design 2007; 13: 367-85.

[111] Linton SD, Aja T, Armstrong RA, *et al.* First-in-class pan caspase inhibitor developed for the treatment of liver disease. J Med Chem 2005; 48: 6779-82.

[112] Hoglen NC, Chen LS, Fisher CD, Hirakawa BP, Groessl T, Contreras PC. Characterization of IDN-6556(3-{2-(2-tert-butyl-phenylaminooxalyl)amino]-propionylamino}-4-ox o-5-(2,3,5,6-tetrafluoro-phenoxy)-pentanoic Acid): a liver-targeted caspase inhibitor. J Pharmacol Exp Ther 2004; 309: 634-40.

[113] Baskin-Bey ES, Washburn K, Feng S, *et al.* Clinical trial of the pan-caspase inhibitor, IDN-6556, in human liver preservation injury. Am J Transplant 2007; 7: 218-25.

[114] Siegmund B, Zeitz M. Pralnacasan vertex pharmaceuticals. Idrugs 2003; 6: 154-8.

[115] Ross J, Brough D, Gibson RM, Loddick SA, Rothwell NJ. A selective, non-peptide caspase-1 inhibitor, VRT-018858, markedly reduces brain damage induced by transient ischemia in the rat. Neuropharmacology 2007; 53: 638-42.

[116] Hara H, Friedlander RM, Gagliardini V, *et al.* Inhibition of interleukin 1 beta converting enzyme family proteases reduces ischemic and excitotoxic neuronal damage. Proc Natl Acad Sci USA 1997; 94: 2007-12.

[117] Han BH, Xu DG, Choi JJ, *et al.* Selective, reversible caspase-3 inhibitor is neuroprotective and reveals distinct pathways of cell death after neonatal hypoxic-ischemic brain injury. J Biol Chem 2002; 277: 30128-36.

[118] Toulmond S, Tang K, Bureau Y, *et al.* Neuroprotective effects of M826, a reversible caspase-3 inhibitor, in the rat malonate model of Huntington's disease. Brit J Pharmacol 2004; 141: 689-97.

[119] Lu G, Kwong WH, Li Q, Wang X, Feng Z, Yew DT. bcl2, bax, and nestin in the brains of patients with neurodegeneration and those of normal aging. J Mol Neurosci 2005; 27: 167-74.

[120] Sajan FD, Martiniuk F, Marcus DL, *et al.* Apoptotic gene expression in Alzheimer's disease hippocampal tissue. Am J Alzheimers Dis Other Demen 2007; 22: 319-28.

[121] Yao M, Nguyen TV, Pike CJ. Beta-amyloid-induced neuronal apoptosis involves c-Jun N-terminal kinase-dependent downregulation of Bcl-w. J Neurosci 2005; 25: 1149-58.

[122] MacGibbon GA, Lawlor PA, Sirimanne ES, *et al.* Bax expression in mammalian neurons undergoing apoptosis, and in Alzheimer's disease hippocampus. Brain Res 1997; 750: 223-34.

[123] Selznick LA, Zheng TS, Flavell RA, Rakic P, Roth KA. Amyloid beta-induced neuronal death is bax-dependent but caspase-independent. J Neuropathol Exp Neurol 2000; 59: 271-9.

[124] Song YS, Park HJ, Kim SY, *et al*. Protective role of Bcl-2 on beta-amyloid-induced cell death of differentiated PC12 cells: reduction of NF-kappaB and p38 MAP kinase activation. Neurosci Res 2004; 49: 69-80.

[125] Tan J, Town T, Placzek A, Kundtz A, Yu H, Mullan M. Bcl-X(L) inhibits apoptosis and necrosis produced by Alzheimer's beta-amyloid1-40 peptide in PC12 cells. Neurosci Lett 1999; 272: 5-8.

[126] Biswas SC, Shi Y, Vonsattel JP, Leung CL, Troy CM, Greene LA. Bim is elevated in Alzheimer's disease neurons and is required for beta-amyloid-induced neuronal apoptosis. J Neurosci 2007; 27: 893-900.

[127] Letai A. Pharmacological manipulation of Bcl-2 family members to control cell death. J Clin Invest 2005; 115: 2648-55.

[128] Jana S, Paliwal J. Apoptosis: Potential therapeutic targets for new drug discovery. Curr Med Chem 2007; 14: 2369-79.

[129] Bombrun A, Gerber P, Casi G, *et al.* 3,6-dibromocarbazole piperazine derivates of 2-propanol as first inhibitors of cytochrome c release via Bax channel modulation. J Med Chem 2003; 46: 4365-8.

[130] Hetz C, Vitte P-A, Bombrun A, *et al.* Bax channel inhibitors prevent mitochondrion-mediated apoptosis and protect neurons in a model of global brain ischemia. J Biol Chem 2005; 280: 42960-70.

[131] Becattini B, Sareth S, Zhai D, *et al.* Targeting apoptosis via chemical design: inhibition of Bid-induced cell death by small organic molecules. Chem Biol 2004; 11: 1107-17.

[132] Sawada M, Sun W, Hayes P, *et al*. Ku70 suppresses the apoptotic translocation of Bax to mitochondria. Nat Cell Biol 2003; 5: 320-9.

[133] Sawada M, Hayes P, Matsuyama S. Cytoprotective membrane-permeable peptides designed from the Bax-binding domain of Ku70. Nat Cell Biol 2003; 5: 352-7.

[134] Polster BMM, Basanez G, Young M, *et al*. Inhibition of bax-induced cytochrome c release from neural cell and brain mitochondria by dibucaine and propranolol. J Neurosci 2003; 23: 2735-43.

[135] Arakawa T, Kita Y, Niikura T. A rescue factor for Alzheimer's diseases: discovery, activity, structure, and mechanism. Curr Med Chem 2008; 15: 2086-98.

[136] Zhai D, Luciano F, Zhu X, *et al*. Humanin binds and nullifies bid activity by blocking its activation of Bax and Bak. J Biol Chem 2005; 280: 15815-24.

[137] Susnow N, Zeng L, Margineantu D, *et al*. Bcl-2 family proteins as regulators of oxidative stress. Semin Cancer Biol 2009; 19: 42-9.

[138] Cao G, Pei W, Ge H, *et al. In vivo* delivery of a Bcl-xL fusion protein containing the TAT protein transduction domain protects against ischemic brain injury and neuronal apoptosis. J Neurosci 2002; 22: 5423-31.

[139] Santos MMM, Faria N, Iley J, *et al*. Reaction of naphthoquinones with substituted nitromethanes. Facile synthesis and antifungal activity of naphtho 2,3-d isoxazole-4,9-diones. Bioorg Med Chem Lett 2010; 20: 193-5.

[140] Santos DM, Santos MMM, Viana RJS, Castro RE, Moreira R, Rodrigues CMR. Naphtho[2,3-d]isoxazole-4,9-dione-3-carboxy-lates: Potent, non-cytotoxic, antiapoptotic agents. Chem-Biol Interact 2009; 180: 175-82.

[141] Reed J. Proapoptotic multidomain Bcl-2/Bax-family proteins: mechanisms, physiological roles, and therapeutic opportunities. Cell Death Differ 2006; 13: 1378-86.

[142] Bubber P, Haroutunian V, Fisch G, Blass JP, Gibson GE. Mitochondrial abnormalities in Alzheimer brain: mechanistic implications. Ann Neurol 2005; 57: 695-703.

[143] Yang TT, Hsu CT, Kuo YM. Cell-derived soluble oligomers of human amyloid-beta peptides disturb cellular homeostasis and induce apoptosis in primary hippocampal neurons. J Neural Transm 2009.

[144] Pereira C, Ferreiro E, Cardoso SM, de Oliveira CR. Cell degeneration induced by amyloid-beta peptides: implications for Alzheimer's disease. J Mol Neurosci 2004; 23: 97-104.

[145] Rodrigues CM, Sola S, Silva R, Brites D. Bilirubin and amyloid-beta peptide induce cytochrome c release through mitochondrial membrane permeabilization. Mol Med 2000; 6: 936-46.

[146] Rodrigues CM, Sola S, Brito MA, Brondino CD, Brites D, Moura JJ. Amyloid beta-peptide disrupts mitochondrial membrane lipid and protein structure: protective role of tauroursodeoxycholate. Biochem Biophys Res Commun 2001; 281: 468-74.

[147] Malet G, Martin AG, Orzaez M, *et al.* Small molecule inhibitors of Apaf-1-related caspase-3/-9 activation that control mitochondrial-dependent apoptosis. Cell Death Differ 2006; 13: 1523-32.

[148] Mondragon L, Orzaez M, Sanclimens G, *et al.* Modulation of cellular apoptosis with apoptotic protease-activating factor 1 (Apaf-1) inhibitors. J Med Chem 2008; 51: 521-9.

[149] de la Lastra CA, Villegas I, Sanchez-Fidalgo S. Poly(ADP-ribose) polymerase inhibitors: new pharmacological functions and potential clinical implications. Curr Pharm Des 2007; 13: 933-62.

[150] Love S, Barber R, Wilcock GK. Increased poly(ADP-ribosyl)ation of nuclear proteins in Alzheimer's disease. Brain 1999; 122 (Pt 2): 247-53.

[151] Chiarugi A, Moskowitz MA. Poly(ADP-ribose) polymerase-1 activity promotes NF-kappaB-driven transcription and microglial activation: implication for neurodegenerative disorders. J Neurochem 2003; 85: 306-17.

[152] de la Monte SM, Sohn YK, Wands JR. Correlates of p53- and Fas (CD95)-mediated apoptosis in Alzheimer's disease. J Neurol Sci 1997; 152: 73-83.

[153] LaFerla FM, Hall CK, Ngo L, Jay G. Extracellular deposition of beta-amyloid upon p53-dependent neuronal cell death in transgenic mice. J Clin Invest 1996; 98: 1626-32.

[154] Alves da Costa C, Sunyach C, Pardossi-Piquard R, *et al.* Presenilin-dependent gamma-secretase-mediated control of p53-associated cell death in Alzheimer's disease. J Neurosci 2006; 26: 6377-85.

[155] Ohyagi Y, Asahara H, Chui DH, *et al.* Intracellular Abeta42 activates p53 promoter: a pathway to neurodegeneration in Alzheimer's disease. FASEB J 2005; 19: 255-7.

[156] Culmsee C, Zhu X, Yu QS, *et al.* A synthetic inhibitor of p53 protects neurons against death induced by ischemic and excitotoxic insults, and amyloid beta-peptide. J Neurochem 2001; 77: 220-8.

[157] Hooper C, Meimaridou E, Tavassoli M, Melino G, Lovestone S, Killick R. p53 is upregulated in Alzheimer's disease and induces tau phosphorylation in HEK293a cells. Neurosci Lett 2007; 418: 34-7.

[158] Amaral JD, Castro RE, Steer CJ, Rodrigues CM. p53 and the regulation of hepatocyte apoptosis: implications for disease pathogenesis. Trends Mol Med 2009; 15: 531-41.

[159] Sola S, Amaral JD, Borralho PM, *et al.* Functional modulation of nuclear steroid receptors by tauroursodeoxycholic acid reduces amyloid beta-peptide-induced apoptosis. Mol Endocrinol 2006; 20: 2292-303.

[160] Amaral JD, Castro RE, Sola S, Steer CJ, Rodrigues CM. p53 is a key molecular target of ursodeoxycholic acid in regulating apoptosis. J Biol Chem 2007; 282: 34250-9.

[161] Komarova EA, Gudkov AV. Suppression of p53: a new approach to overcome side effects of antitumor therapy. Biochemistry-Moscow 2000: 41-8.

[162] Culmsee C, Zhu X, Yu Q-S, *et al.* A synthetic inhibitor of p53 protects neurons against death induced by ischemic and excitotoxic insults, and amyloid beta-peptide. J Neurochem 2001; 77: 220-8.

[163] Duan W, Zhu X, Ladenheim B, *et al.* p53 inhibitors preserve dopamine neurons and motor function experimental Parkinsonism. Ann Neurol 2002; 52: 597–606.

[164] Pietrancosta N, Garino C, Laras Y, *et al.* Are p53 inhibitors potentially useful therapeutics? Drug Dev Res 2005; 65: 43-9.

[165] Zhu X, Yu Q-S, Cutler RG, *et al.* Novel p53 inactivators with neuroprotective action: syntheses and pharmacological evaluation of 2- imino-2,3,4,5,6,7-hexahydrobenzothiazole and 2-imino-2,3,4,5,6,7-hexahydrobenzoxazole derivatives. J Med Chem 2002; 45: 5090-7.

[166] Pietrancosta N, Maina F, Dono R, *et al.* Novel cyclized pifithrin-alpha p53 inactivators: synthesis and biological studies. Bioorg Med Chem Lett 2005; 15: 1561-4.

[167] Pietrancosta N, Moumen A, Dono R, *et al.* Imino-tetrahydro-benzothiazole derivatives as p53 inhibitors: discovery of a highly potent in vivo inhibitor and its action mechanism. J Med Chem 2006; 49: 3645-52.

[168] Gary RK, Jensen DA. The p53 inhibitor pifithrin-alpha forms a sparingly soluble derivative via intramolecular cyclization under physiological conditions. Mol Pharm 2005; 6: 462-74.

[169] Barchéchath SD, Tawatao RI, Corr M, *et al.* Inhibitors of apoptosis in lymphocytes: Synthesis and biological evaluation of Compounds Related to Pifithrin-alpha. J Med Chem 2005; 48: 6409-22.

[170] Beretta GL, Gatti L, Benedetti V, *et al.* Small molecules targeting p53 to improve antitumor therapy. Mini-Rev Med Chem 2009; 8: 856-68.

[171] Strom E, Sathe S, Komarov PG, *et al.* Small-molecule inhibitor of p53 binding to mitochondria protects mice from gamma radiation. Nat Chem Biol 2006; 2: 474-9.

[172] Chen Z, Duan RS, Lepecheur M, Paly E, London J, Zhu J. SOD-1 inhibits FAS expression in cortex of APP transgenic mice. Apoptosis 2005; 10: 499-502.

[173] Morishima Y, Gotoh Y, Zieg J, *et al.* Beta-amyloid induces neuronal apoptosis *via* a mechanism that involves the c-Jun N-terminal kinase pathway and the induction of Fas ligand. J Neurosci 2001; 21: 7551-60.

[174] Ethell DW, Buhler LA. Fas ligand-mediated apoptosis in degenerative disorders of the brain. J Clin Immunol 2003; 23: 439-46.

[175] Su JH, Anderson AJ, Cribbs DH, *et al.* Fas and Fas ligand are associated with neuritic degeneration in the AD brain and participate in beta-amyloid-induced neuronal death. Neurobiol Dis 2003; 12: 182-93.

[176] Zhao M, Cribbs DH, Anderson AJ, *et al.* The induction of the TNFalpha death domain signaling pathway in Alzheimer's disease brain. Neurochem Res 2003; 28: 307-18.

[177] Cantarella G, Uberti D, Carsana T, Lombardo G, Bernardini R, Memo M. Neutralization of TRAIL death pathway protects human neuronal cell line from beta-amyloid toxicity. Cell Death Differ 2003; 10: 134-41.

[178] Miyake Y, Kakeya H, Kataoka T, Osada H. Epoxycyclohexenone inhibits Fas-mediated apoptosis by blocking activation of pro-caspase-8 in the death-inducing signaling complex. J Biol Chem 2003; 278: 11213-20.

[179] Kakeya H, Miyake Y, Shoji M, *et al.* Novel non-peptide inhibitors targeting death receptor-mediated apoptosis. Bioorg Med Chem Lett 2003; 13: 3743-6.

Received: July 9, 2010 Accepted: July 21, 2010