

Molecular Targets Involved in the Neuroprotection Mediated by Terpenoids

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ABSTRACT

Natural products and their derivatives represent the most consistently successful source of drug leads. Terpenoids, a structurally diverse group, are secondary metabolites widely distributed in nature, endowed with a wide range of biological activities such as antibacterial, anti-inflammatory, antitumoral, or neuroprotective effects, which consolidate their therapeutic value. During the last decades, and taking into consideration the prevalence of aging-related diseases, research activity into the neuroprotective effects of these types of compounds has increased enormously. Several signaling pathways involved in neuroprotection are targets of their mechanism of action and mediate their pleiotropic protective activity in neuronal cell damage. In the present review, molecular basis of the neuroprotection exerted by terpenoids is presented, focusing on preclinical evidence of the therapeutic potential of diterpenoids and triterpenoids on neurodegenerative disorders. By acting on diverse mechanisms simultaneously, terpenoids have been emphasized as promising multitarget agents.

Introduction

Neurodegenerative diseases such as AD, PD, stroke, or, more recently, glaucoma are characterized by a progressive loss of structure or function of specialized cells. Taking into consideration the increase in the expectancy of life, these pathologies are the cause of rapidly growing disabilities, with profound social and economic implications throughout the world. Nowadays, there is no effective treatment for addressing the consequences of these neurological disorders, thus, the finding of new valuable medicines is one of the health-care system's priorities.

AD, caused by the aggregation of A β peptides and neurofibrillary tangles, is the cause of nearly 80% of neurodegenerative disorders that lead to disturbances of memory and cognitive functions. Its prevalence is around 5 million people at the age of 65

years or older, with an estimated increase up to 13.8 million by 2050 [1,2]. PD is the second most frequent neurodegenerative disease around the world and includes symptoms derived from degeneration of dopaminergic neurons, such as uncontrollable tremors, postural imbalance, and slowness of movement and rigidity. Its incidence is between 10 and 50/100 000 person per year, with a prevalence between 100 and 300/100 000 population. Both incidence and prevalence increase progressively after 60 years of age [3–5].

In the last years, glaucoma has been included as a neurodegenerative disease, as it causes the loss of functional retinal neurons called RGCs, optic nerve atrophy, and specific visual field defects, which leads to irreversible blindness and is mainly due to high intraocular pressure (IOP). Thus, neuroprotection in glaucoma is de-

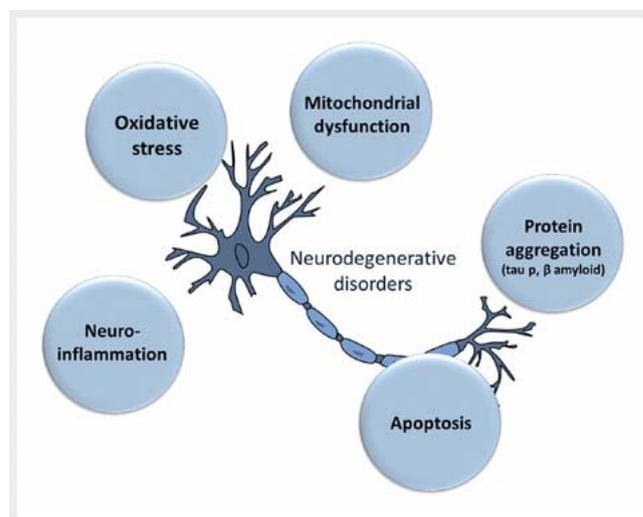
ABBREVIATIONS

6-OHDA	6-hydroxy dopamine
A β	amyloid- β
AD	Alzheimer's disease
AMPk	AMP-activated protein kinase
BDNF	brain-derived neurotrophic factor
BBB	blood-brain barrier
CNS	central nervous system
COX-2	cyclooxygenase 2
CREB	cyclic AMP-response element binding protein
Cyt c	cytochrome c
ERK1/2	extracellular signal regulated kinase
GDNF	glial cell line-derived neurotrophic factor
GSK-3 β	glycogen synthase kinase 3 β
HO-1	heme oxygenase-1
iNOS	inducible nitric oxide synthase
MAKPs	mitogen-activated protein kinases
MCAO	middle cerebral artery occlusion
MMP	mitochondrial membrane potential
MPP+	1-methyl-4-phenyl pyridinium
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NF- κ B	nuclear factor kappa B
NGF	nerve growth factor
Nrf2	nuclear factor E2-related factor
PD	Parkinson's disease
RGC	retina ganglion cell
ROS	reactive oxygen species
SCI	spinal cord injury
TBI	traumatic brain injury

fined as a new therapeutic approach independent of IOP lowering [6, 7].

Different pathological mechanisms are known to cause the neurodegeneration process, leading to a wide range of neurological disorders (► Fig. 1). The increase in the knowledge of the cellular and molecular events underlying the degenerative process has allowed for identifying new compounds capable of stopping or, at least, slowing the progress of neural deterioration. Identification of new agents pharmacologically active against different human diseases has largely depended on the screening of natural products as secondary metabolites from microbes, fungi, marine organism, higher plants, and animals. Due to their broad spectrum of pharmacological and biological activities, natural products might be possible candidates for the management of such multifactorial pathologies [8–11].

In comparison with the extensive knowledge on polyphenols, terpenoids, also referred as terpenes or isoprenoids, have revealed great interest as new potential therapeutic agents in neurological disorders. In recent years, the molecular mechanisms of these chemical multitarget molecules have been studied, focusing on their actions on neuroinflammation, apoptosis, oxidative stress, and neurofunctional regulation of survival pathways, among others. As multitarget drugs, they may contribute to regulating several alterations associated with a disease, thus helping to over-



► Fig. 1 Main pathological mechanisms in neurodegenerative disorders affected by terpenoids.

come the drawback of resistance associated with specific target drugs and reducing their range of side effects [12].

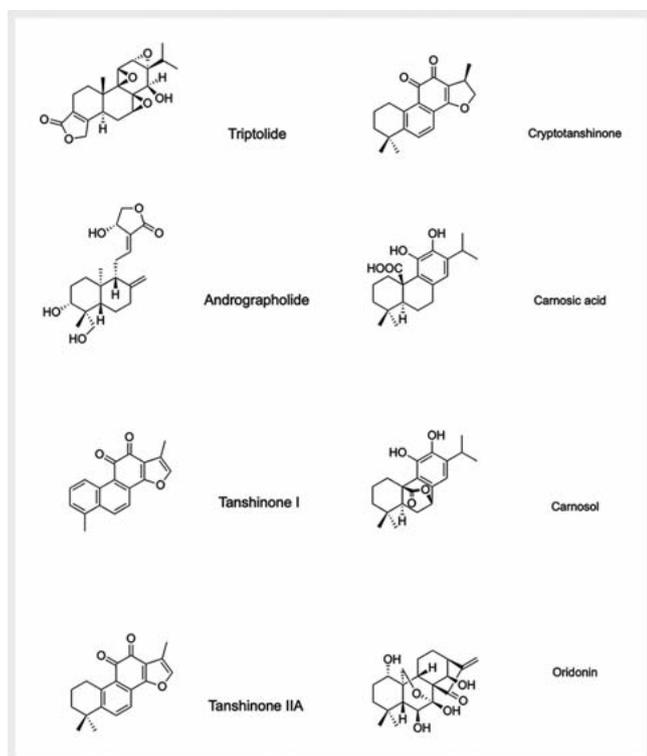
This review provides an overview of the most promising neuroprotective diterpenoids (► Fig. 2) and triterpenoids (► Fig. 3) with potential therapeutic value against neurodegenerative diseases. The current knowledge about the molecular mechanisms of action involved in their protective effects is critically discussed.

Molecular Mechanisms Involved in Neurodegeneration

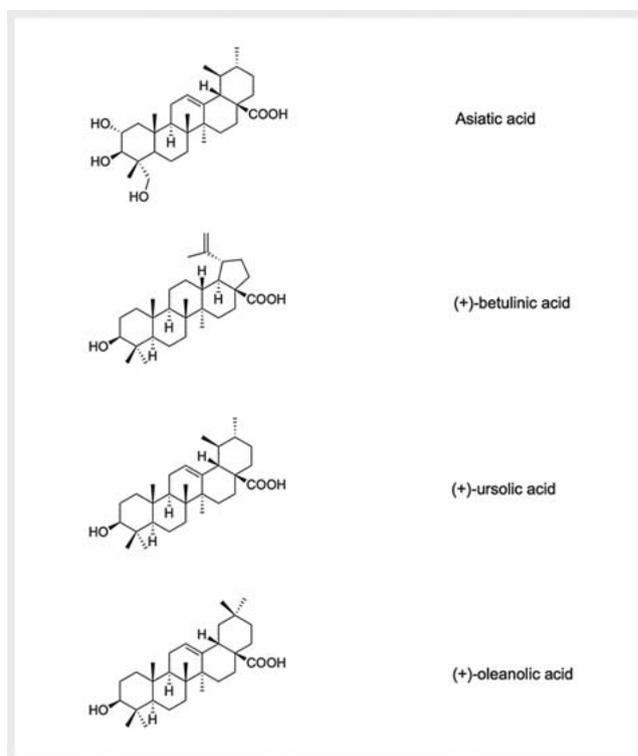
Neuroinflammation

Neuroinflammation consists of immune responses relevant to the central nervous system (CNS), carried out mainly by microglial cells but also astrocytes, which are in turn responsible for the neurodegenerative process [13–15]. Aging, metabolic diseases, and viral infections are sources of inflammation that can affect vessels and neurons, leading to neurodegeneration [16]. For all these inflammatory processes, the activation of NF- κ B is crucial, as it regulates the expression of multiple genes involved in inflammation, apoptotic cell death, cell survival and neuronal differentiation in the CNS. Microglia activation promotes NF- κ B activation, which triggers the transcription of inflammatory and oxidative stress-related genes such as iNOS and COX-2, thus inducing great amounts of proinflammatory mediators such as TNF- α , interleukin-6 (IL-6), interleukin-1 (IL-1), and ROS, which could lead to neuronal injury and death [17, 18].

Increasing evidence indicates that microglial activation in the CNS can be categorized in two opposite phenotypes: M1, or proinflammatory, and M2, or anti-inflammatory. Depending on the activated phenotype, microglia can produce either cytotoxic or neuroprotective effects [19]. Several MAPKs such as p38, c-Jun N-terminal kinase (JNK) and ERK are also involved in regulating the production of inflammatory mediators in activated microglia [20].



► **Fig. 2** Structures of diterpenoids with potential neuroprotective activity.



► **Fig. 3** Structures of triterpenoids with potential neuroprotective activity.

The $A\beta$ deposits, one of the causative factors of AD, together with Tau hypothesis, cholinergic and inflammation hypothesis, are considered part of the neuroinflammation process. In this context, direct injection of $A\beta$ into the brain causes activation of microglial cells and neuronal loss [21]. Cytokines stimulate the surrounding astrocyte-neurons to further produce $A\beta_{42}$ oligomers, forming insoluble and pathological aggregates involving microglia to form clusters at sites of $A\beta$ deposition [22, 23]. Moreover, $A\beta$ -induced neurotoxicity has been linked to NF- κ B activation [24, 25]. Therefore, a promising therapeutic approach to attenuate the progression of neurodegenerative disorders might be the inhibition of microglia activation or the reduction of proinflammatory mediator release. Promoting M2 microglia polarization and inhibiting the M1 phenotype could be another strategy to treat neuroinflammation.

Apoptosis

Apoptosis is a highly regulated programmed cell death pathway. Uncontrolled apoptosis is the main mechanism implicated in neuronal death and the consequent tissue damage in patients with neurodegenerative disorders such as AD, PD, or glaucoma [26, 27].

Two main signaling pathways have been described for the apoptotic process, the *extrinsic* (death-receptor mediated) and *intrinsic* (mitochondria-mediated) pathways. Both converge on the activation of death effectors enzymes such as caspases -3, -6, and/or -7. Bc-2 family proteins are key components in the regulation of the *intrinsic* pathway via monitoring mitochondrial mem-

brane permeability, which leads to the loss of the MMP, and the release of Cyt c, a proapoptotic factor that promotes downstream caspases activation [27–30].

NF- κ B is also involved in the regulation of apoptosis. It can promote cell survival through the upregulation of the expression of Bcl-2 antiapoptotic factors [31–33].

Survival signaling pathways

Modulation of neuronal survival/death signal transduction pathways constitutes another approach in the search for effective therapies in neurodegenerative diseases. Experimental evidence indicated that protein kinase B (Akt) is a pivotal survival signal molecule mediating anti-apoptosis and cell survival. Two mitochondrial proteins are phosphorylated following stimulation of mitochondrial Akt, the β -subunit of ATP synthase and GSK-3 β , an important and well-known protein involved in the direct induction of tau phosphorylation in AD, which leads to cell death [34]. In addition, the survival phosphatidylinositol 3-kinase (PI3K/Akt) pathway modulates mammalian target of rapamycin (mTOR) kinase activity, which is also altered in neurodegenerative diseases accompanied by cognitive deficits (AD, PD) due to its implication in the regulation of a broad range of cellular processes, including survival, proliferation, growth, and migration of cells [34, 35]. The fact that the activation of mTOR is related to the presence of soluble $A\beta$ and tau protein reveals the role of mTOR as one of the mechanisms involved in $A\beta$ -induced toxicity in AD and indicates the potential of mTOR modulators as effective agents in the prevention of these pathologies [36].

Autophagy is an intracellular degradative process in which damaged organelles and long-lived proteins are degraded for maintaining normal cellular homeostasis by forming autophagosomes, a double-membrane vesicle that fuses with lysosomes for the degradation of its contents [37]. Constitutive autophagy clears cytoplasmic components that are useless for cells and is considered a critical cytoprotective pathway. Induced autophagy has been associated with protection against neurodegenerative diseases [38]. The autophagic pathway can be overstimulated by multiple forms of stress, such as hypoxia and ROS, oxygen-glucose deprivation, and cerebral ischemia. A balanced level of autophagy is of paramount importance in neuronal function. Thus, autophagy in the CNS is involved in both survival and death, and its modulation constitutes a new drug target. Signaling pathways and proteins involved are the mTOR kinase complex, AMPk, and Beclin-1 [37, 39, 40].

The PI3K/Akt signaling pathway is essential for the growth and survival of neurons after cerebral ischemia. Activation of this pathway also plays a key role in synaptic activity and normal function, and can promote survival, inhibition of autophagy, and, finally, neuronal protection. The activation of Akt by controlling multiple intracellular signals is able to inhibit neuronal exacerbated autophagy and promote neuronal proliferation and survival [41]. Moreover, MAPKs also regulate various cellular activities, including proliferation, differentiation, apoptosis, survival, inflammation, and innate immunity [42, 43]. Compromised MAPK signaling pathways have also been found to play key roles in the pathogenesis of neurodegeneration by oxidative stress and cell cycle control, while increased levels of ERK have been found to be related to memory impairment in AD. In this case, JNK is localized with DNA damage and is associated with neurofibrillary pathology, probably its activation being a response to cellular stress. Nonetheless, simultaneous activation of ERK and JNK leads to the initial development of AD [20, 44].

Various studies revealed the involvement of ERK and one of its downstream effectors, CREB, in neuronal functions by mediating synaptic events involved in learning, and regulating neuronal plasticity via regulation of protein synthesis, thus playing a major role in memory and cognition [44–46].

Mitochondrial dysfunction and oxidative stress

Mitochondria are of paramount importance in various essential cellular functions, including metabolism and apoptosis. Due to their high metabolism rate, mitochondria are a source of ROS production and a major target for ROS-induced cellular injury, especially in the brain, which is not adequately able to neutralize ROS effects. Its antioxidant system is characterized by moderate activity of the enzymatic defense constituted mainly by superoxide dismutase (SOD) and glutathione (GSH) [47].

Another transcription factor responsible for activating the expression of genes with antioxidant activity as a response to oxidative stress from the cytoplasm into the cell nucleus is the Nrf2, which in turn could induce many cytoprotective proteins such as HO-1 [48]. In AD, an upregulation of Nrf2 expression in neurons is detected due to oxidative damage, with the purpose of preventing neuronal toxicity caused by A β 42 peptides. Moreover, the ac-

tivation of Nrf2 in macrophages and microglia led to a downregulation of the NF- κ B-related inflammatory response [49].

The activation and overexpression of the sirtuin protein SIRT1, a nicotinamide adenine dinucleotide (NAD)(+)-dependent histone and protein deacetylase, has been reported to be related to neuroprotection in both acute CNS injuries and neurodegenerative diseases. The AMPK leads to an increase in the NAD⁺ level, which in turns enhances SIRT activity through modulation of peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 α), the latter regulating mitochondrial biogenesis. This axis explains the relationship between AMPK/SIRT1/PGC-1 α and the mitochondrial function and energy/redox status in mammalian cells [50].

Other targets: neurotrophic factors and neurogenesis

Neurotrophic factors (NTFs) or neurotrophins, such as BDNF, ciliary neurotrophic factor (CNTF), GDNF, insulin-like growth factor 1 (IGF-1), and NGF, play key roles in the development and survival of neurons, and maintain axonal and dendritic networks and synaptic plasticity, which is crucial in processes such as learning and memory. The neurotrophins manifest their effects by binding to two discrete receptor subtypes, the Trk (tropomyosin receptor kinase) family of RTKs (receptor tyrosine kinases) and the p75NTR (p75 neurotrophin receptor). It is well known that a deficiency in NTFs exists in classic neurodegenerative disorders [51–53].

In the last years, the role of neurogenesis as a therapeutic strategy for neurodegenerative diseases has been investigated [5, 54]. The adult brain is capable of generating new neurons from self-renewing and multipotent adult neuronal stem cells in the dentate gyrus in order to replace lost or damaged neuronal cells. In this sense, the Wingless-type MMTV integration site (Wnt) signaling cascade plays a key role by regulating multiple processes in developing the adult brain. In patients suffering neurodegenerative diseases, neurogenesis is impaired. Aging and neurotoxicant exposure strongly antagonize Wnt/ β -catenin signaling in mid-brain dopaminergic neurons and the subventricular zone (SVZ) and lead to the decline of SVZ plasticity and the limited nigrostriatal dopaminergic self-repair in PD. Thus, the finding of a clear mechanism involved in the improvement of endogenous neurogenesis appears to be an ideal approach for the treatment of neurological diseases [54].

Diterpenoids and Triterpenoids as Potential Neuroprotective Agents

Diterpenoids

Triptolide

Triptolide, the main bioactive compound in the Chinese herb *Tripterygium wilfordii* Hook F (Celastraceae), has attracted extensive attention due to its unique structure as a diterpenoid triepoxide with the ability to cross the BBB and its broad spectrum of biological actions, including neurotrophic and neuroprotective effects after systemic administration [55–57].

Neuroprotection exerted by triptolide has been related to its anti-inflammatory properties, which have been confirmed in ex-

perimental models of brain and spinal cord injuries. This diterpenoid improved neurological dysfunction in terms of deficit scores and reduced brain infarcted volume in an MCAO rat ischemia-reperfusion model [58, 59]. Several studies demonstrated that neuroinflammation was reduced by triptolide through the downregulation of NF- κ B and MAPK (p38, ERK1/2) signaling pathways [60, 61].

Additional protective mechanisms such as inhibition of apoptosis through the suppression of NF- κ B and activation of the PI3k/Akt/mTOR signaling pathways have also been confirmed in MCAO models after triptolide administration, resulting in attenuation of cerebral ischemia [59, 61, 62]. This diterpenoid has also emerged as a modulator of autophagy through the upregulation of Beclin-1 [63].

Furthermore, triptolide also demonstrated an *in vivo* therapeutic effect for TBI. It suppressed the increase of TBI induced in contusion volume, cell apoptosis, edema, and the levels of various proinflammatory mediators in the brain [64], with improvement of neurobehavioral outcomes regarding motor, sensory, reflex, and balance function.

Neuronal cytotoxicity induced by the abnormal deposition of proteins such as α -synuclein and amyloid beta-peptide ($A\beta$ 25–35), in PD and AD experimental models, respectively, was protected by triptolide by acting as a potent autophagy inducer in neuronal cells [60, 65]. Attenuation of immune-inflammatory responses of this diterpenoid on dendritic spines was observed after bilateral microinjection of aggregated beta-amyloid protein Ab1-40 into the hippocampus in a rat model of AD [66].

As previously mentioned, inflammatory response following activated microglia is also reported to occur in diseases as PD and glaucoma. Protective effects of triptolide on dopaminergic neurons damage were mediated by the inhibition of proinflammatory mediators from activated microglia, as demonstrated in MPP⁺-induced hemiparkinsonian rats [67], with an improvement in behavioral disabilities. Additionally, preclinical glaucoma studies have demonstrated that this diterpenoid improved RGC survival through the suppression of microglia activation and proliferation in glaucoma rat models [68, 69]. Besides having protective effects via the microglia, triptolide exhibited *in vitro* neurotrophic activity via stimulation of the production and release of NGF in astrocytes [70].

Despite the promising effects of triptolide on neurodegeneration, poor aqueous solubility and systemic toxicity were the main causes that limited preclinical development and clinical translation of the compound. The synthesis of new derivatives with enhanced water solubility and reduced toxicity has focused a great interest in this area. Key pharmacophores such as the epoxy and C-14-hydroxyl groups and their role on its *in vitro* neuroprotective action have been explored. Epoxy groups, but not the 14- β -OH group, were confirmed to be essential for the anti-neuroinflammatory action of triptolide derivatives [71].

Andrographolide

Andrographolide is a natural labdane diterpenoid from the aerial parts (mainly leaves) and roots of *Andrographis paniculata* (Burm. f.) Nees (Acanthaceae) [72, 73].

Andrographolide was able to target several mechanisms involved in cerebral ischemia. Its neuroprotective activity was linked to its ability to inhibit neuroinflammation and oxidative stress [73–75]. Both microglial activation and the expression of proinflammatory factors were significantly inhibited by this diterpenoid via attenuation of PI3K/AKT-dependent NF- κ B and hypoxia-inducible factor 1- α activation [74]. Andrographolide also provided protection against oxidative injury and neurological deficits via increasing Nrf2-HO-1 expression through p38 and ERK-MAPK regulation [76, 77], as confirmed in rat primary astrocytes [78]. Therefore, these findings suggested that this diterpenoid could improve neurobehavioral function by inhibiting NF- κ B and MAPK signaling pathways.

Additional protective mechanisms such as regulation of cell apoptosis, autophagy, and S100B expression (a compound produced mainly by astrocytes with a neurotrophic effect, which in turns reduces hypoxia injury) have been reported for andrographolide. *In vitro*, this diterpenoid attenuated the apoptotic process in hypoxia-injured astrocytes by reducing proapoptotic proteins such as caspase-3 and -9 and Bax and increasing Bcl-2 expression. Andrographolide increased Beclin-1 expression, together with other markers of autophagy such as LC3II, and, in parallel, decreased the formation of autophagosomes. As for the autophagy regulation, the neurotrophic effect was mediated by the JNK pathway [79].

The *in vivo* therapeutic potential of andrographolide has been confirmed in animal models of AD [80, 81]. When tested in the *Octodon degus* (degu) model associated with age, this diterpenoid exerted several beneficial effects, like recovery of spatial memory and learning performance, recovery of synaptic basal transmission, partial or complete protection of certain synaptic proteins, and a specific neuroprotective effect, including the reduction of the phosphorylated Tau protein and amyloid beta aggregate maturation [80]. Andrographolide inhibited certain pathways related to inflammation and apoptosis, including Akt, NF- κ B, and MAPK signaling. Beneficial effects were also demonstrated in synaptic transmission and recovery of memory along with a reduction in Tau phosphorylation in an AD transgenic mouse model with $A\beta$ PP and PS-1 mutant transgenes ($A\beta$ PP/PS1) [81].

On the other hand, a reduction in the dopaminergic neurodegeneration mediated by the inhibition of microglia activation, a mechanism involved in PD, has been described for this labdane diterpenoid [82]. Andrographolide stimulated neurogenesis in the adult hippocampus by acting as a competitive inhibitor of GSK-3 β , a key enzyme of the Wnt/ β -catenin signaling cascade [83].

Apart from the proven beneficial activities shown by this diterpenoid, some andrographolide derivatives, such as the andrographolide-lipoic acid conjugate AL-1 or andrographolide sulfonate have also demonstrated marked neuroprotective effects in animal models of PD and AD [84–86].

Tanshinones

Tanshinones are abietane diterpenoids isolated from different *Salvia* species, especially *Salvia miltiorrhiza* Bunge (Lamiaceae) (red sage, Chinese sage, tan shen or Danshen [87]), with tanshinone I, tanshinone IIA, and cryptotanshinone being their major bioactive constituents. Tanshinones have been reported to exert beneficial

activity in AD, cerebral ischemia/reperfusion injury, and even glioma [87].

Neuroprotective effects of tanshinone I have been mainly related to its antioxidant activity through the Nrf2/ARE signaling pathway. For instance, tanshinone I treatment suppressed hypoxic-ischemic brain damage (HIBD)-induced neuronal death and oxidative stress, thereby ameliorating myodynamia and motor abilities, as well as spatial learning and memory in neonatal rats after HIBD, an animal model of neonatal hypoxic-ischemic encephalopathy [88]. Protection against ischemic damage causing neuronal death in the gerbil hippocampal CA1 region has also been described via the maintenance of antioxidant status and the increase of neurotrophic factors (BDNF and IGF-I) [89].

Activation of the expression of the nuclear level of Nrf2 and its transcriptional activity by tanshinone I have also been reported in PD animal models [90]. This diterpenoid protected SH-SY5Y cells against 6-OHDA-induced neurotoxicity, thus ameliorating dopaminergic neurodegeneration in the *striatum* in mice. Furthermore, tanshinone I was able to modulate the immune response of microglia through inhibition of the expression of M1 proinflammatory factors (NO, TNF- α , IL-6, IL-1 β), but not the expression of M2 anti-inflammatory factors, and also prevented nigrostriatal dopaminergic neurodegeneration in an MPTP mouse model of PD [91].

Regarding tanshinone IIA, the major constituent of Danshen, several studies demonstrated the therapeutic efficacy of this diterpenoid against cerebral ischemia/reperfusion injury and traumatic injury of the spinal cord in rats, with marked improvement of neurological deficits [92,93]. Tanshinone IIA exerted anti-inflammatory, antiapoptotic, and antioxidant actions and promoted neuronal survival [94]. Tanshinone IIA reduced the brain infarct area and BBB permeability in rat models of MCAO. Early protective effects of this terpenoid in acute ischemic stroke in rats were associated with the induction of the CREB signaling pathway, which plays a pivotal role in neuronal survival [95]. Protective effects were also partly mediated by its anti-inflammatory effect on macrophage migration inhibition factor (MIF) expression, NF- κ B activity, the release of proinflammatory cytokines (TNF- α and IL-6), and the reduction of neutrophil infiltration together with antiapoptotic effects by the reduction of caspase-3 expression and increased B-cell lymphoma 2 (bcl-2) protein expression in the ischemic cortex [92]. Nrf2 activation by tanshinone IIA treatment increased the content of antioxidant enzymes and reduced the generation of oxidative products after focal brain ischemia [96]. All these findings suggest great potential of tanshinone IIA in ischemic brain damage.

In addition, tanshinone IIA also proved to be an effective treatment for SCI, as it provided neuronal protection against apoptosis and attenuated the inflammatory and oxidative stress responses in rats [93,97]. Synergistic neuroprotective effects in combination with methylprednisolone have also been demonstrated with this diterpenoid, leading to improved motor function after SCI.

The therapeutic potential of this diterpenoid in AD and PD was confirmed by *in vivo* assays [98,99]. Both tanshinone IIA and cryptotanshinone alleviated memory decline in a nongenetic mouse model of A β 1–42-induced AD. Suppression of reactive gliosis and neuroinflammation was evidenced by reduction of inflammatory

markers (COX-2 and iNOS) as well as inhibition of the NF- κ B signaling pathway. Furthermore, promotion of survival of nigrostriatal dopaminergic neurons in the MPTP mouse model of PD was observed with both compounds through suppression of microglial activation and reduced expression of NADPH oxidase and iNOS [99].

Other Diterpenoids

Oridonin

Oridonin is an *ent*-kaurane diterpenoid isolated from the Chinese herb *Isodon rubescens* (Hemsl.) H. Hara (Lamiaceae). The therapeutic potential of this diterpenoid in neuroprotection has been recently reviewed [100]. It was able to interact with target proteins and signaling pathways that regulate several cellular responses, including apoptosis, autophagy, inflammation, and neuroinflammation [101]. Oridonin reduced microglia activation and A β 1–42 induced neuroinflammation through inhibition of the NF- κ B pathway and activation of the BDNF/TrkB/CREB and Nrf2 signaling pathways. Additionally, this diterpenoid upregulated the expression of the neurotrophic factor NGF. After oral administration of oridonin, a significant attenuation in memory and cognitive deficits was observed in a mice model with A β 1–42-induced AD.

Carnosic acid and carnosol

Carnosic acid and carnosol are the major abietane diterpenoids found in *Rosmarinus officinalis* L. (rosemary) (Lamiaceae). Protective effects upon neuronal cells have been linked with the intrinsic antioxidant capacity of both diterpenoids by activation of signaling pathways that modulate antioxidant defenses in brain cells, such as the Nrf2/ARE pathway [102]. *In vitro*, carnosic acid prevented apoptosis by activation of the PI3K/Akt/NF- κ B axis and reduction of phosphorylation of MAPKs. In addition, carnosic acid downregulated the expression of inflammatory markers such as COX-2 in hypoxic PC12 cells. An indirect antioxidant effect by regulating A β peptide synthesis was also demonstrated.

It was shown that carnosic acid penetrated the BBB and exerted protective effects by acting on redox balance and neuroinflammation. It was demonstrated to be an effective treatment against ischemic injury in an experimental MCAO mice model, preventing an increase in infarct volume. This diterpenoid also protected the rat hippocampus from amyloid- β -induced lesions in an experimental model of AD. Additionally, an improvement in the behavior was reported after oral administration in a rat model of PD induced by 6-OHDA [102,103].

Triterpenoids

Asiatic acid

Asiatic acid is a pentacyclic triterpenoid isolated from *Centella asiatica* (L.) Urb. (Apiaceae). Mechanisms involved in neuroprotection exerted by this terpenoid have been revised and clearly demonstrated asiatic acid acting as a multitarget agent [104,105].

Asiatic acid exerted beneficial effects in stroke models. It improved cell viability and MMP in HT-22 hippocampal neuronal cells exposed to oxygen-glucose deprivation. *In vivo* studies in several models of brain stroke, including the classical MCAO, have shown that oral administration of asiatic acid significantly reduced infarct

volume and improved neurological functions through reduction of BBB permeability and inhibition of mitochondrial Cyt c release [106]. Furthermore, these effects on mitochondrial dysfunction amelioration were confirmed after intravenous administration of asiatic acid [107]. Synergistic effects with a low dose of tissue plasminogen activator (t-PA) led to a reduction of the infarct volume and to an improvement in neurological outcomes [108].

Asiatic acid is also a potential agent for treating AD, dementia, and cognitive deficits. Antiapoptotic and antioxidant effects were shown *in vitro*, with the ability to attenuate cognitive deficits in a dementia model of monosodium glutamate-treated neonatal mice. Antioxidant effects were related to an increase in PGC-1 α and SIRT1 protein expression and the restoration of MMP, SOD activity, and glutathione levels [109].

Regarding AD, experimental evidence supports neuroprotection by asiatic acid acting on multiple targets (amyloid- β formation, apoptosis, oxidative stress, cholinergic deficit). In primary rat cortical neurons, asiatic acid modulated numerous enzymes associated with the formation of A β [110] and also reduced mitochondria-dependent apoptosis by decreasing ROS production, maintaining MMP levels, and, finally, downregulating proapoptotic proteins and dephosphorylation of ERK1/2 [111]. In addition, activation of the AKT/GSK-3 β signaling pathway has also been reported [112]. Further evaluation by an aluminium chloride-induced model of AD revealed asiatic acid as a protective agent against A β formation and neuroinflammation [113].

Moreover, asiatic acid was able to enhance Notch1 and doublecortin (DCX) levels, which were strongly correlated with increased neurogenesis in the hippocampal dentate gyrus and, thus, a marked improvement in the spatial working memory in adult rats [114].

Antioxidant and antiapoptotic effects were also involved in the therapeutic potential of asiatic acid in PD. These effects were observed in rotenone-induced apoptosis in SH-SY5Y cells [115, 116] and further confirmed in MPTP-treated mice, as an *in vivo* model of PD [117]. After oral administration of asiatic acid, an increase in striatal glutathione levels, decrease in ROS production, and modulation of the apoptotic process, together with anti-inflammatory effects, were observed. These effects could be explained by the suppression in the striatal expression of toll-like receptors (TLRs) and NF- κ B [117]. Asiatic acid also improved dopamine striatal levels, thus ameliorating dopaminergic neurodegeneration, along with a marked neurotrophic effect by increasing the neurotrophins BDNF and glial cell line-derived GDNF [117] and its tyrosine kinase receptors (TrKB) [118]. Survival signaling pathways such as MAPKs and PI3K/Akt/mTOR were also involved in the neuroprotective effect offered by asiatic acid on dopaminergic neurons in this PD model.

Interestingly, a recent study pointed out asiatic acid as an anti-glaucoma agent due to its antiapoptotic properties [119]. The loss of RGCs and the apoptotic markers (Bcl-2, Bax, and caspase-3) were reduced after intravitreal injection of asiatic acid in glaucomatous rats, which lead to an amelioration of retinal dysfunction.

Betulinic acid

Betulinic acid, a lupane-type pentacyclic triterpenoid, is found mainly in the bark of *Betula* species (birch trees) (Betulaceae), but is widely present in the plant kingdom. It has shown several biological properties [120, 121]. As reported for other pentacyclic triterpenoids, multiple molecular mechanisms are involved in their neuroprotective effect, such as the reduction of neuroinflammation, oxidative stress, and apoptosis, the activation of neuronal survival signaling pathways, and the stimulation of neurogenesis.

Treatment with betulinic acid protected against cerebral ischemia-reperfusion injury in the MCAO model by the downregulation of NADPH oxidase 2 (NOX2), iNOS expression, and different isoforms of neuronal nitric oxide synthase (nNOS). It also enhanced blood flow by upregulating endothelial nitric oxide synthase (eNOS) expression and decreasing ROS production [122]. In addition, recent data indicated that the downregulation of NADPH oxidase 4 (NOX4) expression in the ischemic hemisphere is also involved in the reduction of infarct volume and neurological deficit carried out by betulinic acid [123]. Antioxidant effects were also confirmed in both rat hippocampal neuron culture and rat models of AD and vascular dementia [124–126].

Moreover, betulinic acid also influenced neuroinflammation by the reduction of proinflammatory cytokine levels in the hippocampus, with improvement of memory damage and cognitive decline [125]. Interestingly, it has also been shown to promote M2 anti-inflammatory phenotype polarization of lipopolysaccharide (LPS)-stimulated BV-2 microglial cells and to inhibit M1 proinflammatory phenotype. This study was carried out via calmodulin-dependent protein kinase β (CaMKK β)-dependent AMPK activation, which was further confirmed in mice brains treated with betulinic acid [127].

In addition, this triterpenoid has been shown to increase survival in hippocampal neuronal rat cells through the activation of the PI3K/Akt pathway and reduction of apoptosis [124]. Finally, betulinic acid administration significantly ameliorated cognitive deficits in vascular dementia by increasing cAMP/cGMP and BDNF hippocampal levels through CREB phosphorylation [126].

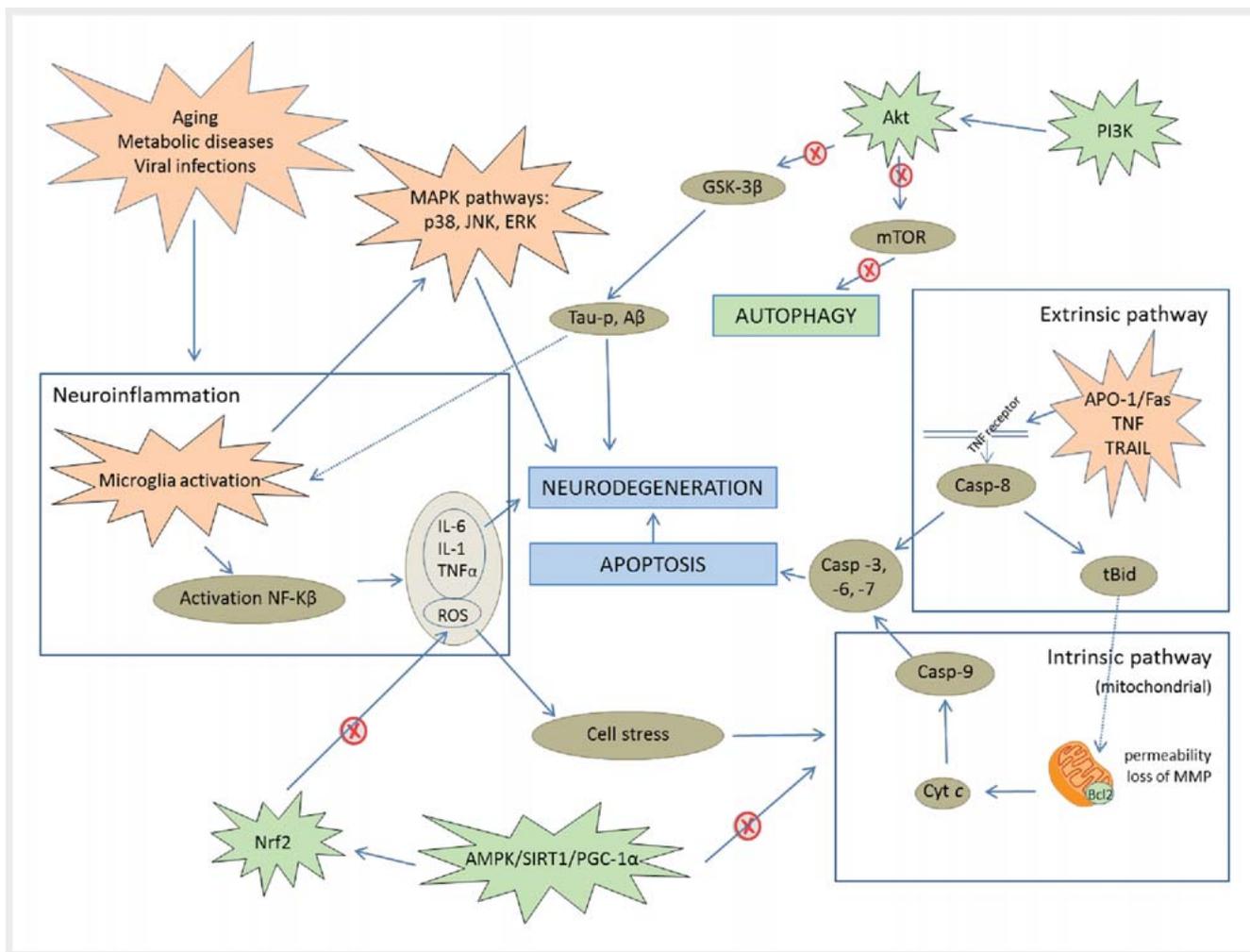
Other Triterpenoids

Ursolic acid

Ursolic acid, a natural pentacyclic triterpenoid acid found in several species of the Oleaceae family, protected against ischemic injury by activating the nuclear translocation of the Nrf2 protein and increasing the expression of HO-1 and AKT in the mouse brain [128, 129].

Oleanolic acid

Oleanolic acid is a ubiquitous triterpenoid found in medicinal plants such as *Olea europaea* L. (Oleaceae), *Calendula officinalis* L. (Asteraceae), and *Viscum album* L. (Viscaceae). The protective role of oleanolic acid and its derivatives has been studied using different *in vitro* and *in vivo* models of cerebral ischemia, such as oxygen-glucose deprivation in neuronal primary cultures and global or focal ischemia in rodents. Protective effects have been associated with a reduction of the cellular oxidative stress response via the upregulation of Nrf2 and inhibition of NF- κ B [130].



► **Fig. 4** Molecular mechanisms involved in the terpenoids neuroprotection.

Summary and Future Perspectives

Neuroprotective agents represent an exciting area of potential future interventions for many diseases. Currently, no pharmacologically effective treatment is available to block or cure the progression of neurodegenerative diseases. In this context, the discovery of new neuroprotective drugs is an area for extensive research.

Natural products and, among them, terpenoids have emerged as a promising group of potential agents in neuroprotection, supported by their structure diversity and results from preclinical studies. Terpenoids protect against neuronal cell damage and death, acting on multiple molecular targets that regulate several cellular responses. In this review, different protein targets and signaling pathways modulated by these natural products have been discussed (► **Fig. 4**).

Traditionally, the clinical use and efficacy of terpenoids has been hampered by several limitations that have greatly hindered their development as drug candidates due to poor aqueous solubility, a narrow therapeutic window, systemic toxicity, and low target efficacy. These factors are the main limitations with regard to the development of new formulations for clinical use. To solve

these obstacles, various approaches have been made in terpenoid research during the last decades. Advancements were done via the structural modifications of lead candidates to obtain more polar derivatives, yielding a great amount of information regarding the structure-activity relationship. Apart from structure modifications, the exploration of new nanoscale drug delivery systems that would enhance penetration of terpenoids into the brain have also been explored. Recently, novel nanoformulations of diterpenoids and triterpenoids based on suitable drug delivery systems have been developed with successful brain-targeted delivery, thus offering a significant improvement in the future application of terpenoids in neurological diseases.

In conclusion, in view of the pleiotropic effects on cell death and survival, terpenoids represent a promising chemical group with potential beneficial effects in neurological diseases. However, further studies, including clinical trials, should be conducted to develop in-depth knowledge of these compounds that would likely help for translational outcomes.

Conflict of Interest

The authors declare that they have no conflict of interest.

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