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Neuroprotective Mechanisms Mediated by CDK5 Inhibition

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Abstract

Cyclin-dependent kinase 5 (CDK5) is a proline-directed serine/threonine kinase belonging to the family of cyclin-dependent kinases. In addition to maintaining the neuronal architecture, CDK5 plays an important role in the regulation of synaptic plasticity, neurotransmitter release, neuron migration and neurite outgrowth. Although various reports have shown links between neurodegeneration and deregulation of cyclin-dependent kinases, the specific role of CDK5 inhibition in causing neuroprotection in cases of neuronal insult or in neurodegenerative diseases is not well-understood. This article discusses current evidence for the involvement of CDK5 deregulation in neurodegenerative disorders and neurodegeneration associated with stroke through various mechanisms. These include upregulation of cyclin D1 and overactivation of CDK5 mediated neuronal cell death pathways, aberrant hyperphosphorylation of human tau proteins and/or neurofilament proteins, formation of neurofibrillary lesions, excitotoxicity, cytoskeletal disruption, motor neuron death (due to abnormally high levels of CDK5/p25) and colchicine-induced apoptosis in cerebellar granule neurons. A better understanding of the role of CDK5 inhibition in neuroprotective mechanisms will help scientists and researchers to develop selective, safe and efficacious pharmacological inhibitors of CDK5 for therapeutic use against human neurodegenerative disorders, such as Alzheimer's disease, amyotrophic lateral sclerosis and neuronal loss associated with stroke.

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

Keywords

CDK5 inhibitors; neuroprotection; neurodegeneration; β -amyloid; Alzheimer's disease; tau hyperphosphorylation; ischemic stroke; synaptic; excitotoxicity; amyotrophic lateral sclerosis

INTRODUCTION

Cyclin-dependent kinase 5 (CDK5) is a proline-directed serine/ threonine kinase belonging to the family of cyclin-dependent kinases. CDK5 is found within the nervous system and it is known to interact with numerous substrates [1]. Indeed, CDK5 plays a pivotal role in the development of the mammalian nervous system during embryogenesis by modulating cortical lamination and morphology through phosphorylation [2]. Additionally, CDK5 is necessary in the modulation of synaptic plasticity, neurite outgrowth, neuron migration, and maintenance of neuronal architecture in the adult [3–5]. Communication among neurons depends on the activity of the presynaptic terminal. In this regard, CDK5 serves as a major control point in the regulation of neurotransmitter release, and the inhibition of CDK5 activity results in significant potentiation of presynaptic function, including the uncovering of silent synapses [6]. The pivotal role that CDK5 plays in the presynaptic terminal is further confirmed by the finding that CDK5 and its physiological activator p35 are abundant in synaptosomes. A significant increase in neurotransmitter release from the synaptosomes was observed in response to membrane depolarization in rat hippocampal slices when roscovitine, a specific inhibitor of CDK5 in neurons, was administered [7].

CDK5 also regulates various signaling pathways within dendritic spines that are involved in plasticity and learning. For instance, postsynaptic density 95 (PSD-95) is a scaffolding protein that structurally connects signaling complexes and receptors at synapses. The N-terminal domain of PSD-95 comprises of three concurrent phosphorylation sites for CDK5. *In vitro* and *in vivo* experiments suggest that CDK5-dependent phosphorylation of PSD-95 prevents multimerization and clustering with ion channels [8], implicating CDK5 in maintaining the structural integrity of the postsynaptic terminal. Likewise, CDK5 is involved in the modulation of excitatory ionotropic glutamate receptors that are crucial in learning. The N-methyl-D-aspartic acid (NMDA) receptor plays an important role in learning and memory [9]. CDK5 facilitates interactions between calpain and the NR2B subunit of the calpain-mediated NMDA receptor [10]. Plattner *et al.* have shown that CDK5 phosphorylates the NR2B subunit of the NMDA receptor, and disrupting NR2B-CDK5 interactions through a small interfering peptide can result in enhanced memory formation *in vivo* and improved synaptic transmission [11]. In addition, *in vivo* animal studies have demonstrated that conditional knockout of CDK5 in adult mice causes improvement in performance in hippocampal long-term potentiation, neural plasticity and spatial learning tasks consequent to elevated levels of NR2B at the synapse caused by the breaking of the calpain/NR2B complex [12]. Furthermore, it was observed that increased basal excitability along with improvement in cognition and plasticity induced audiogenic seizures and epileptic activity in mice, suggesting a role of CDK5 in regulating synaptic plasticity and controlling neuronal and behavioral stimulus-induced excitability in neurons [13].

Many advances have recently been made in terms of developing novel chemical entities to inhibit the functions of different kinases. Of these, likely the most notable is the development of imatinib (brand name: Gleevec) as a potent Abl-tyrosine-kinase inhibitor to treat multiple cancers, and in particular chronic myelogenous leukemia [14]. Imatinib selectively targets the inactive conformation of Abl-tyrosine-kinase; thereby, inhibiting the ability of the Abl-tyrosine kinase enzyme to phosphorylate subsequent proteins and initiate the signaling cascade. Thus, the mechanism of inhibition that imatinib exercises on Abl-tyrosine-kinase provides an example of how specificity can be achieved by targeting the inactive conformation of a given kinase when the inactive state is more distinctive and biologically exploitable than the active conformation [15]. Hence, development of selective and potent inhibitors targeting CDK5 can potentially aid in the recognition and characterization of important targets in neuroprotection and the treatment of those human neurological disorders in which the activity of the CDK family of proteins is dysregulated [16,17].

Role of CDK5 Deregulation in the Pathogenesis of AD

Neuronal circuits create and house memories in the brain and they are capable of altering the strength of the synapses that connect them [18]. When these neuronal circuits undergo neurodegeneration due to neurotoxicity or for other reasons, cognitive deficits and memory impairment can result [19]. Deregulation of CDK5 activity has neurotoxic effects and has been shown to contribute to the pathogenesis of neurodegenerative disorders, such as Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Parkinson's disease and Niemann-Pick type C disease, as well as to ischemia [20–24]. Among all neurodegenerative diseases, AD is the most prevalent memory disorder and one of the major diseases afflicting the aging population. It has been estimated that nearly 46.8 million people are living with dementia worldwide [25]. Accumulated β -amyloid deposits, neurofibrillary tangles and neuronal deterioration within the brain are the pathological hallmarks of AD [26,27]. The presence and elevated levels of neurofibrillary tangles have been shown to be directly associated with the degree of dementia [28]. The abnormal buildup of β -amyloid peptide, and in particular soluble oligomeric forms, within the brain causes aberrant synaptic function [29]. *In vivo* animal studies have demonstrated that abnormally elevated levels of circulating β -amyloid, as seen in the early stages of AD, cause cerebral vessel dysfunction, leading to constriction of blood vessels, ischemia and, eventually, neuronal cell death in the surrounding brain tissues of rats [30]. In addition, β -amyloid plaque accumulation in the brain has also been shown to induce vascular endothelial damage and neurodegeneration through generation of superoxide free radicals [31–33]. CDK5 is a kinase that is involved in the phosphorylation of tau proteins. Dysregulation of CDK5 in AD induces hyperphosphorylation of tau proteins resulting in neuronal dysfunction and cell death [91]. Tau proteins are particularly abundant within neurons of the central nervous system (CNS) where they function to stabilize microtubules. When not properly regulated, tau proteins can become hyperphosphorylated and this can result in self-assembly of tangles of paired helical filaments and straight filaments, thereby contributing to the formation of neurofibrillary lesions in AD brain [34]. CDKs that hyperphosphorylate tau have hence been suggested as important targets for inhibition in order to delay AD progression, although the consequences

of selective inhibition of CDKs during the pathogenesis of AD have yet to be elucidated [35–37].

CDK5 activity depends upon its direct association with p35 or p39, the two non-cyclin cofactors that play an important role in regulating CDK5 activity [38,39]. These cofactors, predominantly expressed in the cerebral cortex and cerebellum, respectively, are cleaved by a non-lysosomal protease enzyme, calpain. In the occurrence of neuronal injury or neurotoxic insult, there is a subsequent increase in intracellular calcium levels; thus causing p35 to convert to its N-terminal truncated product p25, and p39 to convert to p27. Studies have shown that the formation of CDK5/p25 or CDK5/p27 complexes deregulates CDK5 activity, resulting in neuronal cell dysfunction and death, which may promote neurodegeneration [40]. Indeed, CDK5 dysregulation and conversion of CDK5/p35 to CDK5/p25 have been shown to be neurotoxic and have been associated with neurodegenerative disorders, including AD and Parkinson's disease [41–43]. When phosphorylation of the longest form of human tau by CDK5 and its activators was studied using recombinant proteins in an *in vitro* study, it was found that CDK5/p25 complex causes abnormal hyperphosphorylation of human tau protein and may play a crucial role in neuronal cell death evident in AD [44]. Such CDK5 complexes with p35 or p39 or their cleavage products generate a heterodimer, causing CDK5 activation. These N-terminal truncation products are biologically more stable than p35 and p39, and thus can overexcite CDK 5 signaling, to potentially exert pathological changes. Hence, unlike p35, p25 does not degrade rapidly and remains bound to CDK5; thereby activating CDK5 kinase activity and changing its substrate specificity. When CDK5/p25 complex was expressed in cultured primary neurons, it was reported to induce cytoskeletal disruption, morphological degeneration and apoptosis; thereby implicating a role for the CDK5/p25 complex in neurodegenerative disease pathogenesis [45]. Thus, CDK5/p25 may contribute to the chronic demise of neurons in AD even though it may not be taking the lead.

Role of CDK5 Deregulation in the Pathogenesis of ALS

ALS is further progressive and fatal neurodegenerative disorder that impacts upper and lower motor neurons within the brain and the spinal cord. Affected motor neurons develop protein-rich inclusions within their cell bodies and axons that contain ubiquitin and generally incorporate one or more ALS-associated proteins (i.e., superoxide dismutase 1 (SOD1) and TAR DNA-binding protein 43 (TDP-43)), and their progressive degeneration leads to abnormal functioning of muscle tissues. This causes myasthenia, dysphagia, atrophy and, eventually, loss of control of all muscles responsible for voluntary movements. Approximately 5% of ALS patients may also develop frontotemporal dementia, whereas the majority of ALS patients undergo subtle cognitive changes [46]. ALS ultimately results in total paralysis of patients; with respiratory failure being the most common cause of death, and occurring within five years of the onset of ALS symptoms [47]. Dysregulation of CDK5 in ALS patients has been shown to induce hyperphosphorylation of neurofilament proteins leading to cellular apoptosis and neuronal cell death [48].

Neurofilament proteins constitute the cytoskeletal elements that maintain the shape and structure of neurons. Neurofilament proteins in mammals are categorized into three distinct

types or subunits that are based on their apparent molecular weight as evaluated by gel electrophoresis: light neurofilament (NF-L) that runs at ~70 kilodalton, medium neurofilament (NF-M) that runs at ~150 kilodalton, and heavy neurofilament (NF-H) that runs at ~220 kilodalton. Neurofilament proteins have been implicated in the pathogenesis and progression of ALS. This view is supported by studies showing that genetically modified mice with neurofilament abnormalities have phenotypes that resemble certain neuropathological aspects of ALS, epitomized by a retarded conduction speed in peripheral nerves and decreased axonal caliber. Moreover, spatial learning abilities were found to be deteriorated in transgenic mice expressing transgenes for NF-H and NF-M, which had parallels to memory deficits noted in ALS patients [49]. CDK5 is considered the major neurofilament kinase that is involved in signal transduction pathways, such as myelin-associated glycoprotein and mitogen-activated protein kinase pathways that, in turn, affect phosphorylation of neurofilaments and other cytoskeletal proteins [50].

Missense mutations in the Cu/Zn SOD1 enzyme are implicated in progressive motor neuron death and paralysis, and are evident in nearly one-fifth of familial ALS patients [51]. Recent studies have revealed that deregulation of CDK5 activity is associated with the pathogenesis of mutant SOD1-mediated disease and that the inhibition of this activity may enhance motor neuron survival [52]. Furthermore, genetically modified mice carrying a mutant SOD1 gene have been described to exhibit a higher than normal p25 to p35 ratio, in addition to hyperactivation and aberrant localization of CDK5. Indeed, CDK5/p25 causes hyperphosphorylation and abnormal buildup of the neurofilament protein NF-H, a hallmark feature of ALS. An overexpression of NF-H in mutant SOD1 mice results in a prolongation of their life span, which implies that NF-H might act as a competitive substrate for CDK5 [53]. This accentuates the potential of CDK5 inhibition as a strategy to potentially treat ALS.

Role of CDK5 Deregulation in Stroke and Ischemic Insults

Stroke is the third major cause of death in industrialized countries. Those who survive ischemic injury can develop cognitive deficits, neurological disabilities and an elevated risk to progress into a neurodegenerative disorder. A stroke refers to the loss of brain function due to insufficient blood reaching the brain, causing a shortage of oxygen and glucose, which may eventually result in neuronal cell death. Stroke may occur due to lack of blood flow (ischemia) or occurrence of hemorrhage [54]. Ischemic strokes account for nearly 67–80% of all stroke cases [55]. Such ischemia initiates a cascade of pathological events within the brain, resulting in neuronal deterioration consequent to apoptosis and other cellular injury mechanisms. Studies have shown that immediate cell swelling and irreversible membrane depolarization ensues in neurons in the event of severe ischemia [56,57]. On the other hand, neurons undergo slow excitotoxicity and neurodegeneration in cases of mild ischemia [58]. Ischemia induces disruption of the ion exchange balance across the cell membrane, adversely affecting cellular mechanisms and, in particular, oxidative phosphorylation needed for respiration, to eventually cause depolarization of the cell membrane [59]. Rodent studies have demonstrated that aberrant activity of CDK5 is a primary cause of neuronal death during stroke [60]. Following focal cerebral ischemia, there is delayed neuronal death consequent to a gradual loss of natural CDK inhibition, followed

by an upregulation of cyclin D1 and activation of CDKs, which eventually leads to cytoskeletal disintegration [61]. Studies on rodents have shown that defective functioning of CDK5 protein kinase is a key cause of stroke-induced neuronal cell death [85]. Furthermore, based on a recent study, inhibition of the interaction between CDK5 and p25 can decrease spinal injury, as described in the treatment of acute ischemia/reperfusion-induced spinal injury by puerarin in rats [62]. Hence, developing pharmacological inhibitors aimed at cell cycle regulatory mechanisms may prove useful in offering neuroprotection in cases of ischemic insults.

Neuroprotection by Inhibition of CDK5 Activity

Despite the diversity of chemical structures, almost all known CDK inhibitors share certain common properties. All of the reported CDK inhibitors developed, thus far, are small molecule drugs with molecular weights less than 600 Daltons (see Fig. 1 showing structures of selected CDK5 inhibitors). Possessing flat, hydrophobic and heterocyclic structures, they appear to bind with the kinase enzyme mostly via hydrophobic interactions/hydrogen bonds and they work by competing with ATP for binding inside the kinase ATP-binding site [63]. Within the CDK family of kinases, no CDK inhibitor has been discovered, to date, that is highly selective for a single CDK form (although some CDK inhibitors may inhibit one CDK type more strongly than the other CDKs). This finding has been attributed to the conservation of the amino acid sequence that constitutes the lining of the CDK ATP-binding pocket [64]. Acute CDK5 inhibition has a high potential therapeutic value to prevent neuronal injury in the events of stroke or brain injury, or during high-risk surgeries, such as neurovascular or cardiovascular surgeries, or potentially during prolonged, complicated labors [10]. Pharmacological inhibition of CDK5 has been shown to protect neurons under a range of different stressful conditions [65]. Weishaupt *et al.* demonstrated in their studies that focal cerebral ischemia is followed by CDK5 overactivation; thereby activating neuronal cell death pathways upstream of mitochondrial dysfunction [66]. Studies conducted on rat hypoxia/ischemia injury models have demonstrated that inhibition of CDK5 activity after hypoxia/ ischemic insult enhances functional recovery in neonatal rats [67].

Bis-indole indirubin is an active ingredient of a traditional Chinese medicine, Danggui Longhui Wan, used in the treatment of chronic diseases such as leukemia (Table 1). It has been found that indirubins act as dual inhibitors due to their ability to inhibit not only CDK5 and CDK1 but also glycogen synthase kinase-3 β (GSK-3 β) [68]. GSK-3 β is a serine/threonine protein kinase that facilitates the phosphorylation of proteins. GSK-3 β plays an active role in numerous intracellular signaling pathways that include cellular proliferation, migration, glucose regulation, and apoptosis [69,70]. Crystallographic studies have revealed that indirubins bind to GSK-3 β 's ATP binding pocket in a way similar to their binding to CDKs [71]. Deregulation of both CDK5 and GSK-3 β has been implicated in abnormal hyperphosphorylation of the microtubulebinding tau protein in AD. *In vitro* studies on a recombinant form of human brain tau have demonstrated that CDK5 and GSK-3 β promote aberrant hyperphosphorylation and glycosylation of tau in AD; thereby promoting neurofibrillary degeneration [72]. *In vitro* and *in vivo* studies have shown that indirubin-3'-monoxime has the potential to inhibit tau phosphorylation at AD-specific sites [73]. Hence, due to their ability to act as dual inhibitors of CDK5 and GSK-3 β , the use of indirubins may

have important implications in the treatment of neurodegenerative disorders, and represent agents worthy of further evaluation.

Butyrolactone-I (as shown in Table 1) is a CDK5 inhibitor that may directly impact and lower the aggregation of β -amyloid in AD. The reduced clearance and resulting brain buildup of initially soluble and then misfolded aggregated forms of β -amyloid peptide over multiple years likely initiates the AD process, which then instigates a self-propagating pathological cascade that leads to tau hyperphosphorylation and neuroinflammation [74–76]. β -amyloid appears to additionally be directly neurotoxic following its oligomerization, which induces synaptic dysfunction and loss, neuronal apoptosis, inflammation and, over decades, culminates in gradual cognitive impairment and an AD-like pathology [77–80]. Human CDK1 and CDK5 are reported to play an important role in the phosphorylation of β -amyloid in the brain, which can potentially occur at serine residues at positions 26 and 8, and at tyrosine in position 10 within the peptide [81–83]. The generation of such neurotoxic, fibrillary β -amyloid forms involves a conformational change within the peptide that involves a transition from a α -helix to a β -sheet state, which is a general characteristic of amyloid deposits [84,85]. There appear to be critical regions within the structure of β -amyloid that favor the adoption of a β -strand to generate a β -hairpin within the peptide and thereby support the intramolecular backbone hydrogen bonding between β -strand segments that underpins the self-aggregation of this peptide. The phosphorylation of β -amyloid at the described residues appears to drive this process, and augment the nucleation and seeding phases involved in the development of β -amyloid fibril formation, oligomerization and eventual deposition as plaques [84–87]. In addition, phosphorylation at serine residue 8 is described to reduce the clearance of β -amyloid to further promote levels in brain [83]. Interestingly, when rat hippocampal cells and human NT2 neuronal cells were co-incubated with β -amyloid as well as the CDK5 inhibitor butyrolactone-I, a significant reduction in the neurotoxic actions of β -amyloid was evident [81,88], which, in large part, mirrored the reduced cytotoxic response of a mutated β -amyloid peptide derivative lacking a CDK phosphorylation site [81].

CDK activation has been shown to occur in mature adult neurons during focal stroke *in vivo* and this signal is necessary for neuronal death after reperfusion injury [89], in accord with its reported involvement in the death of cultured neurons exposed to trophic factor withdrawal, DNA damage and β -amyloid toxicity. CDK/cyclin D1 levels and phosphorylation of their substrate, retinoblastoma protein, become elevated after stroke [89]. Osuga *et al.* demonstrated in rodent studies that the direct brain administration of flavopiridol (Table 1), a CDK inhibitor, blocks retinoblastoma protein phosphorylation and significantly mitigates neuronal cell death by 80%; hence making CDKs an attractive therapeutic target in the potential treatment of reperfusion injury following ischemia [89]. In a parallel manner, Buyang Huanwu Decoction is a traditional Chinese medicine prescription that combines 7 Chinese herbal preparations and has been used for some 300 years for its neuroprotective properties during post ischemic stroke rehabilitation [90,91] (Table 1). Studies on rodents have demonstrated that Buyang Huanwu Decoction administration prevents ischemic reperfusion-induced spinal injury in rats by causing a reduction in CDK5 [90], amongst other actions [91]. Likewise, the potential therapeutic use of flavopiridol has

also been suggested in neurological disorders (such as AD) in which cytoskeleton alteration induced by CDK5 activation and Par-4 expression has been demonstrated. Experimental evidence reveals that colchicine-induced apoptosis in cerebellar granule neurons is correlated with a substantial increase in the expression of CDK5 and Par-4, and that this increase is efficiently prevented by treatment with flavopiridol; reinforcing the suggested neuroprotective action of flavopiridol as a CDK inhibitor [92].

Inhibition of CDK5 by the small molecular weight molecule indolinone-A (Table 1) has been described to offer long-term rescue to injured neurons. This relatively selective inhibitor of CDK5 appears to have the potential to protect neurons from a variety of different insults, and is reported to lack unwanted cytostatic effects associated with the cross-inhibition of mitotic CDKs. CDK5 inhibition by indolinone-A activates stress responsive proteins that may protect neurons against further neuronal damage [93]. Phosphoproteome and transcriptome analysis have revealed that the compound promotes both neuronal survival and neurite outgrowth, albeit synaptic function of cultured neurons also appears to be affected by it [94]. Hence, targeting abnormal activity of CDK5 by acute CDK5 inhibition may provide a treatment strategy for stroke as well as other disorders.

The two stereoisomers of roscovitine, namely (S)-roscovitine and (R)-roscovitine, are described to be potent inhibitors of CDK5 and mitotic CDKs. (R)-roscovitine is reported to cross the bloodbrain barrier to provide brain levels of about 30% plasma ones, and to exhibit *in vitro* and *in vivo* neuroprotective effects in models of neuronal death [95–99] (Table 1). One recent study characterized neuroprotective effects of (R)-roscovitine in a transient model of focal cerebral ischemia in rats [100]. In this *in vivo* study, the direct intracerebroventricular administration of (R)-roscovitine prior to middle carotid artery occlusion resulted in the mitigation of microglial proliferation, cytokine production and a decline in the levels of cell-cycle proteins that included cyclin A, cyclin B, and cyclin E. This resulted in a reduction in neuronal apoptosis and infarct volume and, thereby, neuroprotection [100]. Menn *et al.*, in broadly parallel studies, demonstrated in a mouse and rat model of stroke that (S)-roscovitine is neuroprotective in a dose-dependent manner after both direct intracerebroventricular as well as systemic delivery [101], whether administered before or following the insult. Ischemia induced a cerebral increase in CDK5/p25 activity 3 hours postneuronal-insult in the mouse permanent focal ischemia stroke model, and (S)-roscovitine systemic administration prevented this enhanced CDK5/p25 activity. This provided direct evidence linking (S)-roscovitine neuroprotection *in vivo* to CDK5-dependent mechanisms [101].

CONCLUSION

CDK5 is a critical constitutively active protein kinase that contributes to brain development, synaptic remodeling, neurogenesis, synaptic vesicle cycling, learning, addiction, mental illness and numerous neuropsychiatric and neurological disorders. CDK5 is also being increasingly recognized as having a growing role in physiological processes and diseases outside the CNS. Dysregulation of CDK5 has been shown to be involved in neurodegenerative disorders, epitomized by AD and ALS, as well as in the neuronal death resulting from ischemic stroke through mechanisms that induce the overactivation of CDK5

mediated neuronal cell death pathways, disruption of pre-and post-synaptic neurotransmission and field potentials, enhanced excitotoxicity, neuronal apoptosis, hyperphosphorylation of tau and/or neurofilament proteins, cytoskeletal disruption, neuronal deterioration and apoptosis. Cell culture and *in vivo* animal studies have demonstrated that inhibitors of CDK5 offer neuroprotection and prevent neuronal loss. However, currently available CDK5 inhibitors may not have the ideal properties as clinically translatable drugs to fully evaluate the potential of this treatment strategy in human neurological disorders. As a consequence, the need exists for the design and development of safe, selective and efficacious CDK5 inhibitors that possess favorable drug-like features that include a pharmacokinetic profile to support rapid brain entry, an extended pharmacological action at their target coupled with a tolerable side-effects profile. As with all pharmaceuticals for long-term degenerative disorders, particularly in the elderly, it is of paramount importance to conduct long-term preclinical and human clinical studies to evaluate neuroprotective effects of CDK5 inhibitors as well as potential adverse/off target effects. Continued efforts to better understand the mechanisms of CDK5 inhibition, the down-stream processes instigated by CDK dysregulation and, importantly, the window of opportunity critical to the target, CDK5, within the disease process being evaluated will provide the necessary knowledge and mechanistic grounding [75] to guide researchers in developing future treatments targeting neurodegeneration associated with AD, stroke and potentially other human neurological disorders at the molecular level, and provide the translational basis [102] to support effective preclinical and clinical development of CDK5 inhibitors.

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LIST OF ABBREVIATIONS

AD

Alzheimer's disease

ALS	Amyotrophic lateral sclerosis
CNS	Central nervous system
CDK5	Cyclin-dependent kinase 5
GSK-3β	Glycogen synthase kinase-3beta
NMDA receptor	N-methyl-D-aspartic acid receptor
PSD-95	Postsynaptic density 95

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Indirubin

Butyrolactone-1

Flavopiridol

Indolinone-A

1-(2,6-Dichlorophenyl)-2Indolinone

(R)-roscovitine

(S)-roscovitine

Buyang huanwu decoction: A preparation of several defined Chinese herbs

Fig. (1).
Structures of selected CDK5 inhibitors.

Table 1

Synopsis of available CDK5 inhibitors.

Compound/Drug Name	Description	Mechanism of Neuroprotection	Reference(s)
Indirubins	Active ingredient of a traditional Chinese medicine, Danggui Longhui Wan	This class of compounds act as dual inhibitors due to their ability to inhibit not only CDK5 and CDK1 but also glycogen synthase kinase-3 β (GSK-3 β)	[68, 71–73]
Butyrolactone-I	An exogenous inhibitor of the CDK family of kinases	Co-incubation of rat hippocampal cells with β -amyloid and the CDK5 inhibitor butyrolactone-I results in significant reduction of neurotoxic effects of β -amyloid on primary culture cells	[81, 88]
Flavopiridol	A flavonoid derived from an indigenous plant from India	Rodent studies in the treatment of reperfusion injury following ischemia have shown that administration of flavopiridol blocks retinoblastoma protein phosphorylation and significantly reduces neuronal cell death by 80%	[89]
Buyang Huanwu Decoction	Traditional Chinese medicine with neuro-protective properties	Administration of this herbal preparation prevents ischemic-reperfusion-induced spinal injury in rats by causing a reduction in CDK5	[90–92]
Indolinone-A	A potent and selective small molecule inhibitor of CDK5	This CDK5-selective inhibitor works by cross inhibition of mitotic CDKs. Phosphoproteome and transcriptome analysis have revealed that the compound promotes both neuronal survival and neurite outgrowth	[93, 94]
(R)-roscovitine	Two stereoisomers of roscovitine that are potent inhibitors of CDK5 and mitotic CDKs	(R)-roscovitine can enter brain and exhibits neuroprotective effects in cellular and <i>in vivo</i> models of neuronal death	[95–100]
(S)-roscovitine		Systemic administration of (S)-roscovitine to mice with permanent focal ischemia has been reported to prevent enhanced CDK5/p25 activity; offering neuroprotection in a dose-dependent manner	[101]