GSK3 Inhibitors and Disease

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Abstract: This review describes, briefly, the characteristics and regulation of glycogen synthase kinase 3 (GSK3) together with the role of GSK3 dysfunctions in different pathologies, and GSK3 as target for therapeutic treatment in different diseases. Several GSK3 inhibitors acting at different levels are described in this work, ranging from cations like lithium to small compounds developed by different pharmaceutical companies. Also, the use of specific interference RNA (iRNA) for the specific inhibition of the expression of the different GSK3 isoforms is discussed.

Key Words: GSK3, lithium, Alzheimer, neurodegeneration.

1. INTRODUCTION

In 1988, Ishiguro and his colleagues identified a protein kinase (activity) associated with brain microtubules which phosphorylated tau in more than one residue [1] modifying its electrophoretic mobility. This kinase was designated as tau protein kinase [E.C.2.7.1.135], but when it was further characterized, two different proteins were observed in the original protein fraction. These kinases were called tau protein kinase I (TPKI) and tau protein kinase II (TPKII) [2], further identified as GSK3β and CDK5 [3-4].

In this review, we will focus on TPKI and other GSK3 isoforms. Glycogen synthase kinase 3 (GSK3) was first identified as one of the kinases able to modify glycogen synthase [5]. The crystal structure of GSK3β [6-7] has revealed a typical serine /threonine kinase fold with a small N-terminal domain and a larger C-terminal domain being located the ATP binding site at the interface of both domains. In mammals, GSK3 is encoded by two genes, gsk3α and gsk3β, expressing the proteins GSK3α (51KDa) and GSK3β (47KDa) and sharing almost a complete sequence identity between their catalytic domains [8]. The presence of a glycine rich region present in GSK3α close to the N-terminal region and absent in the GSK3β isoform, is the most obvious structural difference between both isoforms. In addition, different isoforms could arise from alternative splicing of GSK3β RNA [9].

2. GSK3 REGULATION

It has been suggested that GSK3 could be constitutively activated *in vivo* and, mainly, negatively regulated by its phosphorylation in its N-terminal region (serine residue 21 in GSK3α and at serine 9 in GSK3β) [10]. The modification at those serine residues can be achieved by different kinases like PKA, PKB (Akt) or PKC [10,11]. More recently, the action of other kinases like p38 [12] has been suggested, although p38 inactivates GSK3β by direct phosphorylation at its C-terminus. Curiously, only GSK3β but not GSK3α was phosphorylated by p38 *in vitro* [12].

On the other hand, newly synthesized GSK3 is modified at tyrosine 279 (GSK3α) or tyrosine 216 (GSK3β), probably due to the fact that during the folding process an intramolecular autophosphorylation takes place [13]. However, it has been observed that after pharmacological inhibition of GSK-3 activity, a total correlation of enzymatic activity inhibition with a decrease in tyrosine phosphorylation at residues 216/279 does not exist. This suggests that other tyrosine kinases and/or phosphatases may also regulate phosphorylation of these residues [14].

As indicated, phosphorylation at serine 21(GSK3α) or serine 9 (GSK3β) could result in the inhibition of GSK3 activity. On the other hand, dephosphorylation of those phosphoserine residues could activate GSK3. The regulation of these dephosphorylations has been attributed to the actions of protein phosphatase 2A (PP2A) [15] and protein phosphatase 1 (PP1) [16]. The holoenzyme PP1 contains a catalytic subunit which when bound to its inhibitor-2, (I-2), abolishes its activity [16]. A way to regulate PP1 activity is through I-2 phosphorylation mediated by GSK3, since phosphorylated I-2 inhibitor is unable to inhibit PP1 activity [17, 18].

Negative regulation of GSK3 takes places through different pathways like insulin [11] or wnt [19]. Wnt proteins bind to the frizzled receptor, activating the disheveled protein, which in turn inhibits GSK3 activity by disrupting this multiprotein complex [19]. Also, GSK3 activity could depend on its subcellular localization, since GSK3 can be found in the cytoplasm, nucleus and mitochondria [20]. Finally, a novel way of regulating GSK3 activity involves the removal of a fragment from the N-terminal region of GSK3, including regulatory serines 21/9 [21], by calpain.

3. GSK3 SUBSTRATES AND REQUIREMENT OF PRIMING KINASES

In addition to tau and the PP1 inhibitor (I-2), GSK3 can modify many proteins present in different subcellular com-

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partments. Some substrates of GSK3, like tau and amyloid precursor protein [22], are present in the cytoplasm, whereas others, like some transcription factors, are present in the nucleus. Indeed, it was reported the presence of GSK3 associated with the nucleus envelope [23] and inside the nucleus [24] has been reported. Therefore, it has been suggested that GSK3 activity could be also regulated by its intracellular localization [20].

Many GSK3 substrates require a previous (priming) phosphorylation by other kinase (priming kinase) at a residue located four aminoacids towards the C-terminal of the residue to be modified by GSK3. Among these priming kinases are PKA [25]; PKC [26]; casein kinase I [27]; cdk5 [28], or PAR1 [29]. Thus, theses kinases could regulate the phosphorylation of different substrates by GSK3.

4. GSK3 AND DISEASE

Dysregulation (usually increase) of GSK3 activity is believed to play a role in different and important disorders like cancer [30], heart disease [31], neurodegenerative disorders [32], diabetes [33] or viral infections [34]. More recently, the requirement of GSK3 for a type of human leukemia has been reported, suggesting GSK3 as a candidate drug target for the treatment of this cancer [35]. Among neurodegenerative disorders, GSK3 dysregulation has been related to Alzheimer disease, bipolar disorder, or Huntington disease [10-32]. Recently, an association of a GSK3β polymorphism with Alzheimer disease and with frontotemporal dementia has been reported [36].

In the case of bipolar disorders, patients with an increased copy number of the gene encoding GSK3β [37] have been shown. Also, GSK3 inhibitors like lithium [38] have been used for the treatment of this disorder. On the other hand, GSK3β can also play a role in the abnormality of circadian rhythm [39,40] which is shortened when the GSK3 activity increases [41]. Thus, it has been found that lithium (GSK3 inhibitor) increases the circadian rhythm [42]. It will be of interest to know if other GSK3 inhibitors play a similar function than lithium in this process [43].

On the other hand, the role of wnt signaling, (which results in a decrease of GSK3 activity), in the development of the blood-brain barrier (BBB) has been reported. Breakdown of BBB has been involved in the pathology of some central nervous disorders like stroke [44]; and in the future it should be tested if GSK3 inhibitors may facilitate the repair of BBB.

5. GSK3 INHIBITORS AND NEURODEGENERATION

The potential role of GSK3 inhibitors as therapeutical tools in different disorders has been studied. Some of these inhibitors could act in an indirect way by regulating phosphatases or priming kinases acting on GSK3 substrates. Examples of compounds regulating GSK3 activity, as discussed above, are kinases like PKB, PKC, PKA, p38, or phosphatases like PP2A or PP1 [32].

On the other hand, compounds can act on the expression of GSK3 isoforms. In this way, hairpin siRNA expression vectors have been used to inhibit GSK3α and GSK3β expression, although a further good design for these iRNA will be needed, in order to have a specific, but not simultaneous, inhibition of both GSK3 isoforms [45].

Another approach to decrease GSK3 activity has been the use of small molecules, as highly specific inhibitors for GSK3, like lithium [46-49]. Thus, several GSK3 inhibitors like indirubines [50], maleimides [51], 3-amino pyrazoles (that are mainly decreasing GSK3α activity) [52], paullones [53], thiazoles [54] or Thiadizolidinones [55] have been indicated as GSK3 inhibitors. It should be mentioned that paullones are not only GSK3 (TPKI) but also cdk5 (TPKII) inhibitors, a characteristic which share other compounds like hymenaldisine [56]. Another point that should be always taken into account is that essentially there are no specific inhibitors able to inhibit exclusively GSK3α or GSK3β isoforms, although, as previously indicated [52], it has been suggested that 3-aminopyrazoles are mainly decreasing GSK3α activity. Also, lithium can preferentially inhibit GSK3β over GSK3α (see below). More recently, a method to design selective GSK3β inhibitors using virtual screening methods [57] has been described.

A variety of analyses could be required to explain the mechanism for the inhibition mediated by small molecules on GSK3 activity. One of these analysis is to obtain the X-ray crystal structure of GSK3β complexed with its inhibitor [6, 7]. GSK3 structure is similar to that of other kinases, except at their N-terminal and C-terminal regions. The ATP-binding pocket has been identified and there are compounds that compete with ATP at the active centre of the enzyme (ATP competitors), while others, as lithium, are non-ATP competitive inhibitors. In some cases the inhibition correlates with a further phosphorylation of GSK3 at serine 21(GSK3α)/9(GSK3β) and, in other cases, self phosphorylation of GSK3 at its tyrosine 279(α)/216(β) is prevented. An example of a specific and well known inhibitor is lithium. Lithium does not only inhibit GSK3 but also inhibits several other enzymes being inositol monophosphatase (IMPase), inositol polyphosphatase 1-phosphatase, fructose 1, 6-bisphosphatase, bisphosphatase nucleotidase, and phosphoglucomutase as the best studied [58], although other additional enzymes have been described as targets of lithium [58-59]. Lithium is a non-competitive inhibitor with respect to ATP, but a competitive inhibitor of magnesium [17], cation which is required for GSK3 enzymatic activity. More recently, it has been suggested that lithium may destabilize a complex where PKB (Akt) and PP2A are present. As a consequence of this destabilization, Akt is not dephosphorylated, retaining its activity and promoting GSK3 inhibition [60]. In addition, GSK3β engages in a positive feedback loop with protein phosphatase 1 (PP1) [61]. This takes place through GSK3 phosphorylation of PP1 I2 (inhibitor 2 of the phosphatase) (I-2) which results in PP1 activation. This increase in phosphatase activity results in the dephosphorylation of GSK3β at ser 9 and the additional activation of the kinase. Recent data suggested a preferential phosphorylation of GSK3β over GSK3α in the presence of lithium, suggesting that PP1 inhibitor could probably act in a better way on the inhibition of GSK3β dephosphorylation than on the dephosphorylation of GSK3α [34]. Curiously, SB415286 (an ATP competitor), another GSK3 inhibitor, facilitated the phosphorylation of ser 21 of GSK3α isoform over the phosphorylation of serine 9 of GSK3β [62]. The possible mechanism for this difference is under study. No such a difference in modifying ser 9 (GSK3β) or ser 21 (GSK3α) was detected for other GSK3

inhibitors (Hernández F. *et al.* to be published), like the non ATP competitive TDZ compounds [55]. On the other hand, preliminary studies suggest a different behavior for ATP competitive and non competitive compounds. For ATP competitive molecules, a decrease in the phosphorylation of Tyr 279 (GSK3α) or Tyr 216 (GSK3β) was found, whereas no such decrease was observed for non competitive GSK3 inhibitors, like lithium or thiadiazolidinone (NP-12).

6. GSK3 INHIBITORS AND AXONAL REGENERATION

In addition to its possible relevance to prevent neurodegeneration, GSK3 inhibitors may also be useful to foster other forms of neuronal repair, including axon regeneration. Thus, systemic application of GSK-3 inhibitors including a clinical dose of lithium to rats with thoracic spinal cord transection or contusion injuries induce a significant descending corticospinal and serotonergic axon sprouting in caudal spinal cord which promotes locomotor functional recovery [63]. This observation agrees well with previous works showing that overexpression of insulin like growth factor1 (IGF-1) (which should inhibit GSK3 activity), provides neuroprotection after spinal cord injuring in rats [64], or that GSK3 inhibition reduces damage in spinal cord trauma [65]. Also, lithium reinforces the axonal regeneration promoted by chondriotinase ABC, after spinal cord injury [66]. These results are consistent with previous ones demonstrating that inhibition of GSK-3 results in enhanced neurite growth in rat cerebella granule neurons, DRG neurons and hippocampal neurons [67-69]. However, there is also some evidence that strong suppression of GSK-3 activity profoundly disrupts axon growth [70, 71]. Indeed, Munoz-Montano *et al.* (1999) first observed that the percentage of cerebella neurons with countable neurites was increased with lithium treatment at low concentration while it was decreased with high concentration of lithium [67]. The interesting effect observed with partial GSK-3 inhibition is consistent with the therapeutic roles for GSK-3 inhibitors over a certain concentration range.

With these caveats in mind, GSK-3 is still a possible therapeutic target for promoting functional recovery of adult CNS injuries. Therefore the administration of GSK-3 inhibitors at low or moderate doses may facilitate the effective treatment to CNS injuries including spinal cord trauma.

7. GSK3 INHIBITORS FOR THE TREATMENT OF DIFFERENT DISEASES-CLINICAL TRIALS

Previously, it has been suggested that the dysregulation of GSK3 activity may be important for the pathology of cancer, heart disease, diabetes, viral infections or neurodegenerative disorders like Alzheimer disease. In this way, GSK3

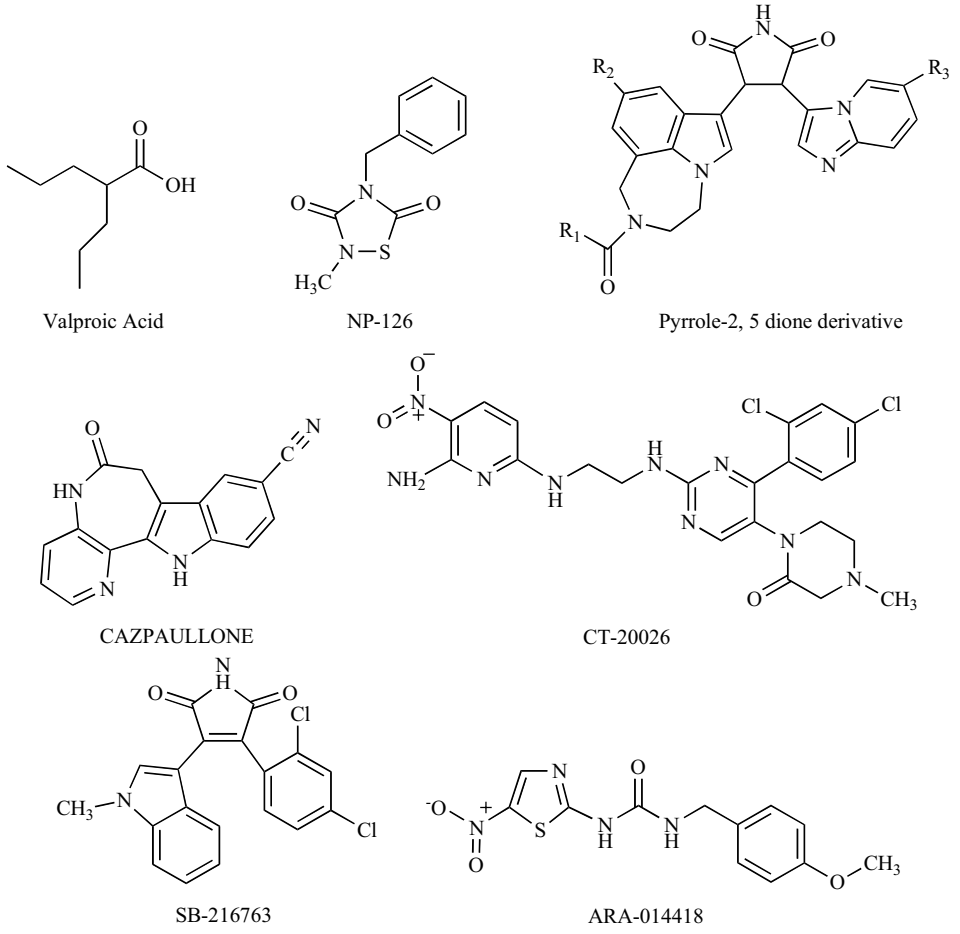

Fig. (1). GSK3 inhibitors: Structure of some of the GSK3 inhibitors discussed in this work.

inhibitors, (like lithium), have been patented for the treatment of viral infections by the respiratory syncitial virus [34] , or for the treatment of diabetes or Alzheimer disease. Also, lithium has been suggested to be used for the treatment of Alzheimer disease (AD) pathology. Indeed, in [http://clinicaltrials.gov/ct2/results?term=Alzheimer+disease] the use of lithium and valproate is indicated as possible GSK3 inhibitors [72] for the treatment of the disease. In a more indirect way, there is a study of administration of nasal insulin as a potential GSK3 inhibitor [10-73].

There are also small compounds like thiazoles, thiadiazolidinones, maleimides, 3-aminopyrazoles or purine derivates, used as GSK3 inhibitors. Some of these inhibitors, developed by several pharmaceutical companies, have reached the clinic [74].

Chiron Corp. (now Novartis AG) developed some purine derivatives (like CT-98023) that were proposed to be useful for type 2 diabetes [75]. Astra Zeneca was investigating thiazoles (like ARA-014418) [54] (Fig. **1**) as GSK3 inhibitors for the treatment of Alzheimer disease, however it appears that this study (Phase 1) has been discontinued at the beginning of 2008. Kemia is investigating GSK3 inhibitors for the treatment of type 2 diabetes and neurodegenerative disorders. Glaxo-Smith-Kline (GSK) have identified pyrazolopyridine compounds as potent GSK3 inhibitors. Also, since malemide core is a common motif for GSK3 inhibitor, GSK has isolated several compounds (like SB-216763) with this motif. Roche-Holding AG is also using maleimides as GSK3 inhibitors for osteoporosis treatment. In addition, Eli Lilly and Co. has developed pyrrole-2, 5-dione derivates for the treatment of type 2 diabetes [76]. DeveloGen AG has isolated cazpaullone, a 1-azakenpaullone derivate, as a GSK3 inhibitor [77]. Mitsubishi-Pharma and Sanofi-Aventis started to investigate new GSK3 inhibitors, but there is no development reported since 2007. Novo Nordisk is testing the use of aminothiazoles for the treatment of diabetes. Also, Crystal Genomics, Amphora Discovery Corp. and Cyclacel Pharmaceuticals have developed GSK3 inhibitors for the treatment of diabetes, whereas Deciphera Pharma is looking for GSK3 inhibitors compounds against leukemia.

Finally, Neuropharma (now Noscira) has in Clinic phase 1 a GSK3 inhibitor thiadiazolidinone (NP-12) [78]. The main feature of this compound, compared to the other ones is the nature of its mechanism of inhibition, since NP-12 is a non ATP competitive inhibitor.

Among marine natural products, new GSK3 inhibitors have been found and are now under study [79], but they are far from clinical studies. These inhibitors of marine origin are the manzamines, β-carboline alkaloids, isolated from sponges [80], that are non competitive inhibitors of ATP binding on GSK3 [7]. The structure-activity relationship (SAR) of these compounds on GSK3 activity has been analyzed [79]. This SAR for these and other GSK3 inhibitors has not been commented in this short review, since there is a recent review on the subject [74].

In summary, dysregulation of GSK3 may play a role in several human disorders, like metabolic disorders, diabetes, viral infections or neurodegenerative diseases, including Alzheimer disease, as GSK3 activity is increased in these pathologies. GSK3 inhibitors should correct the activity status of this enzyme. Some of these GSK3 inhibitors have been briefly reviewed in this work.

REFERENCES

[1] Ishiguro, K.; Ihara, Y.; Uchida, T.; Imahori, K. A novel tubulin-dependent protein kinase forming a paired helical filament epitope on tau. *J. Biochem.,* **1988**, *104*, 319-21.

[2] Ishiguro, K.; Takamatsu, M.; Tomizawa, K.; Omori, A.; Takahashi, M.; Arioka, M.; Uchida, T.; Imahori, K. Tau protein kinase I converts normal tau protein into A68-like component of paired helical filaments. *J. Biol Chem.,* **1992**, *267*, 10897-901.

[3] Ishiguro, K.; Kobayashi, S.; Omori, A.; Takamatsu, M.; Yonekura, S.; Anzai, K.; Imahori, K.; Uchida, T. Identification of the 23 kDa subunit of tau protein kinase II as a putative activator of cdk5 in bovine brain. *FEBS Lett.,* **1994**, *342*, 203-8.

[4] Ishiguro, K.; Shiratsuchi, A.; Sato, S.; Omori, A.; Arioka, M.; Kobayashi, S.; Uchida, T.; Imahori, K. Glycogen synthase kinase 3 beta is identical to tau protein kinase I generating several epitopes of paired helical filaments. *FEBS Lett.,* **1993**, *325*, 167-72.

[5] Embi, N.; Rylatt, D. B.; Cohen, P. Glycogen synthase kinase-3 from rabbit skeletal muscle. Separation from cyclic-AMP-dependent protein kinase and phosphorylase kinase. *Eur. J. Biochem.,* **1980**, *107*, 519-27.

[6] ter Haar, E.; Coll, J. T.; Austen, D. A.; Hsiao, H. M.; Swenson, L.; Jain, J. Structure of GSK3beta reveals a primed phosphorylation mechanism. *Nat. Struct. Biol.,* **2001**, *8*, 593-6.

[7] Dajani, R.; Fraser, E.; Roe, S. M.; Young, N.; Good, V.; Dale, T. C.; Pearl, L. H. Crystal structure of glycogen synthase kinase 3 beta: structural basis for phosphate-primed substrate specificity and autoinhibition. *Cell,* **2001**, *105*, 721-32.

[8] Woodgett, J. R. Molecular cloning and expression of glycogen synthase kinase-3/factor A. *EMBO J.,* **1990**, *9*, 2431-8.

[9] Mukai, F.; Ishiguro, K.; Sano, Y.; Fujita, S. C. Alternative splicing isoform of tau protein kinase I/glycogen synthase kinase 3beta. *J. Neurochem.,* **2002**, *81*, 1073-83.

[10] Jope, R. S.; Johnson, G. V. The glamour and gloom of glycogen synthase kinase-3. *Trends Biochem. Sci.,* **2004**, *29*, 95-102.

[11] Cross, D. A.; Alessi, D. R.; Cohen, P.; Andjelkovich, M.; Hemmings, B. A. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature,* **1995**, *378*, 785-9.

[12] Thornton, T. M.; Pedraza-Alva, G.; Deng, B.; Wood, C. D.; Aronshtam, A.; Clements, J. L.; Sabio, G.; Davis, R. J.; Matthews, D. E.; Doble, B.; Rincon, M. Phosphorylation by p38 MAPK as an alternative pathway for GSK3beta inactivation. *Science,* **2008**, *320*, 667-70.

[13] Lochhead, P. A.; Kinstrie, R.; Sibbet, G.; Rawjee, T.; Morrice, N.; Cleghon, V. A chaperone-dependent GSK3beta transitional intermediate mediates activation-loop autophosphorylation. *Mol. Cell.,* **2006**, *24*, 627-33.

[14] Simon, D.; Benitez, M. J.; Gimenez-Cassina, A.; Garrido, J. J.; Bhat, R. V.; Diaz-Nido, J.; Wandosell, F. Pharmacological inhibition of GSK-3 is not strictly correlated with a decrease in tyrosine phosphorylation of residues 216/279. *J. Neurosci. Res.,* **2008**, *86*, 668-74.

[15] Planel, E.; Yasutake, K.; Fujita, S. C.; Ishiguro, K. Inhibition of protein phosphatase 2A overrides tau protein kinase I/glycogen synthase kinase 3 beta and cyclin-dependent kinase 5 inhibition and results in tau hyperphosphorylation in the hippocampus of starved mouse. *J. Biol. Chem.,* **2001**, *276*, 34298-306.

[16] King, T. D.; Gandy, J. C.; Bijur, G. N. The protein phosphatase-1/inhibitor-2 complex differentially regulates GSK3 dephosphorylation and increases sarcoplasmic/endoplasmic reticulum calcium ATPase 2 levels. *Exp. Cell Res.,* **2006**, *312*, 3693-700.

[17] Ryves, W. J.; Dajani, R.; Pearl, L.; Harwood, A. J. Glycogen synthase kinase-3 inhibition by lithium and beryllium suggests the presence of two magnesium binding sites. *Biochem. Biophys. Res. Commun.,* **2002**, *290*, 967-72.

[18] Jope, R. S. Inhibition of glycogen synthase kinase-3: a potential therapeutic target of lithium. *Clin. Neurosci. Res.,* **2004**, *4*, 171-179.

[19] Cadigan, K. M.; Liu, Y. I. Wnt signaling: complexity at the surface. *J. Cell Sci.,* **2006**, *119*, 395-402.

[20] Grimes, C. A.; Jope, R. S. The multifaceted roles of glycogen synthase kinase 3beta in cellular signaling. *Prog. Neurobiol.,* **2001**, *65*, 391-426.

[21] Goni-Oliver, P.; Lucas, J. J.; Avila, J.; Hernandez, F. N-terminal cleavage of GSK-3 by calpain: a new form of GSK-3 regulation. *J. Biol. Chem.,* **2007**, *282*, 22406-13.

[22] Phiel, C. J.; Wilson, C. A.; Lee, V. M.; Klein, P. S. GSK-3alpha regulates production of Alzheimer's disease amyloid-beta peptides. *Nature,* **2003**, *423*, 435-9.

[23] Ragano-Caracciolo, M.; Berlin, W. K.; Miller, M. W.; Hanover, J. A. Nuclear glycogen and glycogen synthase kinase 3. *Biochem. Biophys. Res. Commun.,* **1998**, *249*, 422-7.

[24] Diehl, J. A.; Cheng, M.; Roussel, M. F.; Sherr, C. J. Glycogen synthase kinase-3beta regulates cyclin D1 proteolysis and subcellular localization. *Genes Dev.,* **1998**, *12*, 3499-511.

[25] Singh, T. J.; Zaidi, T.; Grundke-Iqbal, I.; Iqbal, K. Modulation of GSK-3-catalyzed phosphorylation of microtubule-associated protein tau by non-proline-dependent protein kinases. *FEBS Lett.,* **1995**, *358*, 4-8.

[26] Liu, S. J.; Zhang, A. H.; Li, H. L.; Wang, Q.; Deng, H. M.; Netzer, W. J.; Xu, H.; Wang, J. Z. Overactivation of glycogen synthase kinase-3 by inhibition of phosphoinositol-3 kinase and protein kinase C leads to hyperphosphorylation of tau and impairment of spatial memory. *J. Neurochem.,* **2003**, *87*, 1333-44.

[27] Amit, S.; Hatzubai, A.; Birman, Y.; Andersen, J. S.; Ben-Shushan, E.; Mann, M.; Ben-Neriah, Y.; Alkalay, I. Axin-mediated CKI phosphorylation of beta-catenin at Ser 45: a molecular switch for the Wnt pathway. *Genes Dev.,* **2002**, *16*, 1066-76.

[28] Li, T.; Hawkes, C.; Qureshi, H. Y.; Kar, S.; Paudel, H. K. Cyclin-dependent protein kinase 5 primes microtubule-associated protein tau site-specifically for glycogen synthase kinase 3beta. *Biochemistry,* **2006**, *45*, 3134-45.

[29] Nishimura, I.; Yang, Y.; Lu, B. PAR-1 kinase plays an initiator role in a temporally ordered phosphorylation process that confers tau toxicity in Drosophila. *Cell,* **2004**, *116*, 671-82.

[30] Manoukian, A. S.; Woodgett, J. R. Role of glycogen synthase kinase-3 in cancer: regulation by Wnts and other signaling pathways. *Adv. Cancer Res.,* **2002**, *84*, 203-29.

[31] Hardt, S. E.; Sadoshima, J. Glycogen synthase kinase-3beta: a novel regulator of cardiac hypertrophy and development. *Circ. Res.,* **2002**, *90*, 1055-63.

[32] Avila, J.; Lucas, J. J.; Perez, M.; Hernandez, F. Role of tau protein in both physiological and pathological conditions. *Physiol. Rev.,* **2004**, *84*, 361-84.

[33] Eldar-Finkelman, H. Glycogen synthase kinase 3: an emerging therapeutic target. *Trends. Mol. Med.,* **2002**, *8*, 126-32.

[34] Asenjo, A.; Mendieta, J.; Gomez-Puertas, P.; Villanueva, N. Residues in human respiratory syncytial virus P protein that are essential for its activity on RNA viral synthesis. *Virus Res.,* **2008**, *132*, 160-73.

[35] Wang, Z.; Smith, K. S.; Murphy, M.; Piloto, O.; Somervaille, T. C.; Cleary, M. L. Glycogen synthase kinase 3 in MLL leukaemia maintenance and targeted therapy. *Nature,* **2008**, *455*, 1205-9.

[36] Schaffer, B. A.; Bertram, L.; Miller, B. L.; Mullin, K.; Weintraub, S.; Johnson, N.; Bigio, E. H.; Mesulam, M.; Wiedau-Pazos, M.; Jackson, G. R.; Cummings, J. L.; Cantor, R. M.; Levey, A. I.; Tanzi, R. E.; Geschwind, D. H. Association of GSK3B with Alzheimer disease and frontotemporal dementia. *Arch. Neurol.,* **2008**, *65*, 1368-74.

[37] Lachman, H. M.; Pedrosa, E.; Petruolo, O. A.; Cockerham, M.; Papolos, A.; Novak, T.; Papolos, D. F.; Stopkova, P. Increase in GSK3beta gene copy number variation in bipolar disorder. *Am. J. Med. Genet. B. Neuropsychiatr. Genet,* **2007**, *144B*, 259-65.

[38] Klein, P. S.; Melton, D. A. A molecular mechanism for the effect of lithium on development. *Proc. Natl. Acad. Sci. USA,* **1996**, *93*, 8455-9.

[39] Kato, T. Molecular neurobiology of bipolar disorder: a disease of 'mood-stabilizing neurons'? *Trends Neurosci.,* **2008**, *31*, 495-503.

[40] Prickaerts, J.; Moechars, D.; Cryns, K.; Lenaerts, I.; van Craenendonck, H.; Goris, I.; Daneels, G.; Bouwknecht, J. A.; Steckler, T. Transgenic mice overexpressing glycogen synthase kinase 3beta: a putative model of hyperactivity and mania. *J. Neurosci.,* **2006**, *26*, 9022-9.

[41] Dokucu, M. E.; Yu, L.; Taghert, P. H. Lithium- and valproate-induced alterations in circadian locomotor behavior in Drosophila. *Neuropsychopharmacology,* **2005**, *30*, 2216-24.

[42] Yin, L.; Wang, J.; Klein, P. S.; Lazar, M. A. Nuclear receptor Rev-erbalpha is a critical lithium-sensitive component of the circadian clock. *Science,* **2006**, *311*, 1002-5.

[43] Liebner, S.; Corada, M.; Bangsow, T.; Babbage, J.; Taddei, A.; Czupalla, C. J.; Reis, M.; Felici, A.; Wolburg, H.; Fruttiger, M.; Taketo, M. M.; von Melchner, H.; Plate, K. H.; Gerhardt, H.; Dejana, E. Wnt/{beta}-catenin signaling controls development of the blood-brain barrier. *J. Cell Biol.,* **2008**, *183*, 409-17.

[44] Zlokovic, B. V. The blood-brain barrier in health and chronic neurodegenerative disorders. *Neuron,* **2008**, *57*, 178-201.

[45] Yu, J. Y.; Taylor, J.; DeRuiter, S. L.; Vojtek, A. B.; Turner, D. L. Simultaneous inhibition of GSK3alpha and GSK3beta using hairpin siRNA expression vectors. *Mol. Ther.,* **2003**, *7*, 228-36.

[46] Noble, W.; Planel, E.; Zehr, C.; Olm, V.; Meyerson, J.; Suleman, F.; Gaynor, K.; Wang, L.; LaFrancois, J.; Feinstein, B.; Burns, M.; Krishnamurthy, P.; Wen, Y.; Bhat, R.; Lewis, J.; Dickson, D.; Duff, K. Inhibition of glycogen synthase kinase-3 by lithium correlates with reduced tauopathy and degeneration *in vivo*. *Proc. Natl. Acad. Sci. USA,* **2005**, *102*, 6990-5.

[47] Engel, T.; Goni-Oliver, P.; Lucas, J. J.; Avila, J.; Hernandez, F. Chronic lithium administration to FTDP-17 tau and GSK-3beta overexpressing mice prevents tau hyperphosphorylation and neurofibrillary tangle formation, but pre-formed neurofibrillary tangles do not revert. *J. Neurochem.,* **2006**, *99*, 1445-55.

[48] Churcher, I. Tau therapeutic strategies for the treatment of Alzheimer's disease. *Curr. Top. Med. Chem.,* **2006**, *6*, 579-95.

[49] Bhat, R. V.; Budd Haeberlein, S. L.; Avila, J. Glycogen synthase kinase 3: a drug target for CNS therapies. *J. Neurochem.,* **2004**, *89*, 1313-7.

[50] Leclerc, S.; Garnier, M.; Hoessel, R.; Marko, D.; Bibb, J. A.; Snyder, G. L.; Greengard, P.; Biernat, J.; Wu, Y. Z.; Mandelkow, E. M.; Eisenbrand, G.; Meijer, L. Indirubins inhibit glycogen synthase kinase-3 beta and CDK5/p25, two protein kinases involved in abnormal tau phosphorylation in Alzheimer's disease. A property common to most cyclin-dependent kinase inhibitors?. *J. Biol. Chem.,* **2001**, *276*, 251-60.

[51] Smith, D. G.; Buffet, M.; Fenwick, A. E.; Haigh, D.; Ife, R. J.; Saunders, M.; Slingsby, B. P.; Stacey, R.; Ward, R. W. 3-Anilino-4-arylmaleimides: potent and selective inhibitors of glycogen synthase kinase-3 (GSK-3). *Bioorg. Med. Chem. Lett.,* **2001**, *11*, 635-9.

[52] Witherington, J.; Bordas, V.; Haigh, D.; Hickey, D. M.; Ife, R. J.; Rawlings, A. D.; Slingsby, B. P.; Smith, D. G.; Ward, R. W. 5-arylpyrazolo[3,4-b]pyridazines: potent inhibitors of glycogen synthase kinase-3 (GSK-3). *Bioorg. Med. Chem. Lett.,* **2003**, *13*, 1581-4.

[53] Leost, M.; Schultz, C.; Link, A.; Wu, Y. Z.; Biernat, J.; Mandelkow, E. M.; Bibb, J. A.; Snyder, G. L.; Greengard, P.; Zaharevitz, D. W.; Gussio, R.; Senderowicz, A. M.; Sausville, E. A.; Kunick, C.; Meijer, L. Paullones are potent inhibitors of glycogen synthase kinase-3beta and cyclin-dependent kinase 5/p25. *Eur. J. Biochem.,* **2000**, *267*, 5983-94.

[54] Bhat, R.; Xue, Y.; Berg, S.; Hellberg, S.; Ormo, M.; Nilsson, Y.; Radesater, A. C.; Jerning, E.; Markgren, P. O.; Borgegard, T.; Nylof, M.; Gimenez-Cassina, A.; Hernandez, F.; Lucas, J. J.; Diaz-Nido, J.; Avila, J. Structural insights and biological effects of glycogen synthase kinase 3-specific inhibitor AR-A014418. *J. Biol. Chem.,* **2003**, *278*, 45937-45.

[55] Martinez, A.; Alonso, M.; Castro, A.; Perez, C.; Moreno, F. J. First non-ATP competitive glycogen synthase kinase 3 beta (GSK-3beta) inhibitors: thiadiazolidinones (TDZD) as potential drugs for the treatment of Alzheimer's disease. *J. Med. Chem.,* **2002**, *45*, 1292-9.

[56] Meijer, L.; Thunnissen, A. M.; White, A. W.; Garnier, M.; Nikolic, M.; Tsai, L. H.; Walter, J.; Cleverley, K. E.; Salinas, P. C.; Wu, Y. Z.; Biernat, J.; Mandelkow, E. M.; Kim, S. H.; Pettit, G. R. Inhibition of cyclin-dependent kinases, GSK-3beta and CK1 by hymenialdisine, a marine sponge constituent. *Chem. Biol.,* **2000**, *7*, 51-63.

[57] Vadivelan, S.; Sinha, B. N.; Tajne, S.; Jagarlapudi, S. A. Fragment and knowledge-based design of selective GSK-3beta inhibitors using virtual screening models. *Eur. J. Med. Chem.,* **2009**, doi:10.1016/j.ejmech.2009.02.031.

[58] Phiel, C. J.; Klein, P. S. Molecular targets of lithium action. *Annu. Rev. Pharmacol Toxicol.,* **2001**, *41*, 789-813.

[59] Quiroz, J. A.; Gould, T. D.; Manji, H. K. Molecular effects of lithium. *Mol. Interv.,* **2004**, *4*, 259-72.

[60] Beaulieu, J. M.; Marion, S.; Rodriguiz, R. M.; Medvedev, I. O.; Sotnikova, T. D.; Ghisi, V.; Wetsel, W. C.; Lefkowitz, R. J.; Gainetdinov, R. R.; Caron, M. G. A beta-arrestin 2 signaling complex mediates lithium action on behavior. *Cell,* **2008**, *132*, 125-36.

[61] Jope, R. S. Lithium and GSK-3: one inhibitor, two inhibitory actions, multiple outcomes. *Trends Pharmacol. Sci.,* **2003**, *24*, 441-3.

[62] Asenjo, A.; Gonzalez-Armas, J. C.; Villanueva, N. Phosphorylation of human respiratory syncytial virus P protein at serine 54 regulates viral uncoating. *Virology,* **2008**, *380*, 26-33.

[63] Dill, J.; Wang, H.; Zhou, F.; Li, S. Inactivation of glycogen synthase kinase 3 promotes axonal growth and recovery in the CNS. *J. Neurosci.,* **2008**, *28*, 8914-28.

[64] Hung, K. S.; Tsai, S. H.; Lee, T. C.; Lin, J. W.; Chang, C. K.; Chiu, W. T. Gene transfer of insulin-like growth factor-I providing neuroprotection after spinal cord injury in rats. *J. Neurosurg. Spine,* **2007**, *6*, 35-46.

[65] Cuzzocrea, S.; Genovese, T.; Mazzon, E.; Crisafulli, C.; Di Paola, R.; Muia, C.; Collin, M.; Esposito, E.; Bramanti, P.; Thiemermann, C. Glycogen synthase kinase-3 beta inhibition reduces secondary damage in experimental spinal cord trauma. *J. Pharmacol. Exp. Ther.,* **2006**, *318*, 79-89.

[66] Yick, L. W.; So, K. F.; Cheung, P. T.; Wu, W. T. Lithium chloride reinforces the regeneration-promoting effect of chondroitinase ABC on rubrospinal neurons after spinal cord injury. *J. Neurotrauma.,* **2004**, *21*, 932-43.

[67] Munoz-Montano, J. R.; Lim, F.; Moreno, F. J.; Avila, J.; Diaz-Nido, J. Glycogen Synthase Kinase-3 Modulates Neurite Outgrowth in Cultured Neurons: Possible Implications for Neurite Pathology in Alzheimer's Disease. *J. Alzheimers Dis.,* **1999**, *1*, 361-378.

[68] Jones, D. M.; Tucker, B. A.; Rahimtula, M.; Mearow, K. M. The synergistic effects of NGF and IGF-1 on neurite growth in adult sensory neurons: convergence on the PI 3-kinase signaling pathway. *J. Neurochem.,* **2003**, *86*, 1116-28.

[69] Yoshimura, T.; Kawano, Y.; Arimura, N.; Kawabata, S.; Kikuchi, A.; Kaibuchi, K. GSK-3beta regulates phosphorylation of CRMP-2 and neuronal polarity. *Cell,* **2005**, *120*, 137-49.

[70] Kim, W. Y.; Zhou, F. Q.; Zhou, J.; Yokota, Y.; Wang, Y. M.; Yoshimura, T.; Kaibuchi, K.; Woodgett, J. R.; Anton, E. S.; Snider, W. D. Essential roles for GSK-3s and GSK-3-primed substrates in neurotrophin-induced and hippocampal axon growth. *Neuron,* **2006**, *52*, 981-96.

[71] Garrido, J. J.; Simon, D.; Varea, O.; Wandosell, F. GSK3 alpha and GSK3 beta are necessary for axon formation. *FEBS Lett.,* **2007**, *581*, 1579-86.

[72] Kim, A. J.; Shi, Y.; Austin, R. C.; Werstuck, G. H. Valproate protects cells from ER stress-induced lipid accumulation and apoptosis by inhibiting glycogen synthase kinase-3. *J. Cell Sci.,* **2005**, *118*, 89-99.

[73] Planel, E.; Tatebayashi, Y.; Miyasaka, T.; Liu, L.; Wang, L.; Herman, M.; Yu, W. H.; Luchsinger, J. A.; Wadzinski, B.; Duff, K. E.; Takashima, A. Insulin dysfunction induces *in vivo* tau hyperphosphorylation through distinct mechanisms. *J. Neurosci.,* **2007**, *27*, 13635-48.

[74] Medina, M.; Castro, A. Glycogen synthase kinase-3 (GSK-3) inhibitors reach the clinic. *Curr. Opin. Drug Discov. Dev.,* **2008**, *11*, 533-43.

[75] Wagman, A. S.; Johnson, K. W.; Bussiere, D. E. Discovery and development of GSK3 inhibitors for the treatment of type 2 diabetes. *Curr. Pharm. Des.,* **2004**, *10*, 1105-37.

[76] Engler, T. A.; Henry, J. R.; Malhotra, S.; Cunningham, B.; Furness, K.; Brozinick, J.; Burkholder, T. P.; Clay, M. P.; Clayton, J.; Diefenbacher, C.; Hawkins, E.; Iversen, P. W.; Li, Y.; Lindstrom, T. D.; Marquart, A. L.; McLean, J.; Mendel, D.; Misener, E.; Briere, D.; O'Toole, J. C.; Porter, W. J.; Queener, S.; Reel, J. K.; Owens, R. A.; Brier, R. A.; Eessalu, T. E.; Wagner, J. R.; Campbell, R. M.; Vaughn, R. Substituted 3-imidazo[1,2-a]pyridin-3-yl-4-(1,2,3,4-tetrahydro-[1,4]diazepino-[6,7,1-hi]indol-7-yl)pyrrole-2,5-dion es as highly selective and potent inhibitors of glycogen synthase kinase-3. *J. Med. Chem.,* **2004**, *47*, 3934-7.

[77] Stukenbrock, H.; Mussmann, R.; Geese, M.; Ferandin, Y.; Lozach, O.; Lemcke, T.; Kegel, S.; Lomow, A.; Burk, U.; Dohrmann, C.; Meijer, L.; Austen, M.; Kunick, C. 9-cyano-1-azapaullone (cazpaullone), a glycogen synthase kinase-3 (GSK-3) inhibitor activating pancreatic beta cell protection and replication. *J. Med. Chem.,* **2008**, *51*, 2196-207.

[78] Luna-Medina, R.; Cortes-Canteli, M.; Sanchez-Galiano, S.; Morales-Garcia, J. A.; Martinez, A.; Santos, A.; Perez-Castillo, A. NP031112, a thiadiazolidinone compound, prevents inflammation and neurodegeneration under excitotoxic conditions: potential therapeutic role in brain disorders. *J. Neurosci.,* **2007**, *27*, 5766-76.

[79] Hamann, M.; Alonso, D.; Martin-Aparicio, E.; Fuertes, A.; Perez-Puerto, M. J.; Castro, A.; Morales, S.; Navarro, M. L.; Del Monte-Millan, M.; Medina, M.; Pennaka, H.; Balaiah, A.; Peng, J.; Cook, J.; Wahyuono, S.; Martinez, A. Glycogen synthase kinase-3 (GSK-3) inhibitory activity and structure-activity relationship (SAR) studies of the manzamine alkaloids. Potential for Alzheimer's disease. *J. Nat. Prod.,* **2007**, *70*, 1397-405.

[80] Sakai, R. R.; Nicolaidis, S.; Epstein, A. N. Salt appetite is suppressed by interference with angiotensin II and aldosterone. *Am. J. Physiol.,* **1986**, *251*, R762-8.

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