

Pharmacological inhibitors of glycogen synthase kinase 3

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Three closely related forms of glycogen synthase kinase 3 (GSK-3 α , GSK-3 β and GSK-3 β 2) have a major role in Wnt and Hedgehog signaling pathways and regulate the cell-division cycle, stem-cell renewal and differentiation, apoptosis, circadian rhythm, transcription and insulin action. A large body of evidence supports speculation that pharmacological inhibitors of GSK-3 could be used to treat several diseases, including Alzheimer's disease and other neurodegenerative diseases, bipolar affective disorder, diabetes, and diseases caused by unicellular parasites that express GSK-3 homologues. The toxicity, associated side-effects and concerns regarding the absorption, distribution, metabolism and excretion of these inhibitors affect their clinical potential. More than 30 inhibitors of GSK-3 have been identified. Seven of these have been co-crystallized with GSK-3 β and all localize within the ATP-binding pocket of the enzyme. GSK-3, as part of a multi-protein complex that contains proteins such as axin, presenilin and β -catenin, contains many additional target sites for specific modulation of its activity.

Protein phosphorylation, the most common post-translational mechanism used by cells to regulate enzymes and structural proteins, is controlled by ~ 520 protein kinases and ~ 80 protein phosphatases. Because many diseases are associated with abnormalities of protein phosphorylation, pharmacological inhibitors of kinases and phosphatases have become a major interest in drug discovery [1,2].

Glycogen synthase kinase 3 (GSK-3) was one of the first kinases to be identified and studied, initially for its function in the regulation of glycogen synthase (reviewed in [3–5]). Interest in GSK-3 has grown far beyond glycogen metabolism during the past decade and GSK-3 is now known to occupy a central stage in many cellular and physiological events, including Wnt and Hedgehog signaling, transcription, insulin action, cell-division cycle, response to DNA damage, cell death, cell survival, patterning and axial orientation during development, differentiation, neuronal functions, circadian rhythm and others. The name GSK-3, thus, appears to be a rather limited tribute to the large diversity of its physiological effects.

The GSK-3 kinase family is highly conserved throughout evolution. In humans, two genes, which map to

19q13.2 and 3q13.3, encode two distinct but closely related GSK-3 forms, GSK-3 α (51 kDa) and GSK-3 β (47 kDa). They display 84% overall identity (98% within their catalytic domains) with the main difference being an extra Gly-rich stretch in the N-terminal domain of GSK-3 α . However, they are not interchangeable functionally, as demonstrated by the embryonic-lethal phenotype observed when the gene that encodes GSK-3 β is knocked out. Recently, GSK-3 β 2, an alternative splicing variant of GSK-3 β that contains a 13-amino-acid insertion in the catalytic domain, has been identified.

GSK-3 β has been crystallized recently [6,7]. The overall shape is shared by all kinases, with a small N-terminal lobe, which consists mostly of β -sheets and a large C-terminal lobe, which is formed essentially of α -helices [2]. The ATP-binding pocket is located between the two lobes. Arg96, Arg180 and Lys205 form a small pocket where the phosphate group of the primed substrate and the pseudo-substrate (see later) bind.

GSK-3 is regulated at multiple levels (Figure 1). First, GSK-3 β is regulated by post-translational phosphorylation of Ser9 (inhibitory) and Tyr216 (activating) (Ser21 and Tyr279, respectively, in GSK-3 α). Phosphorylated Ser9 in the N-terminal domain of GSK-3 β acts as a pseudo-substrate that blocks the access of substrates to the catalytic site. Unphosphorylated Tyr216 in the T-loop domain prevents access of substrates to the catalytic site, and phosphorylation releases this inhibition. Second, GSK-3 β is regulated by interactions with many other proteins. Axin and presenilin act as docking proteins that allow the substrates to make contact with the priming kinase [casein kinase (CK1) and protein kinase A, respectively] and GSK-3. Docking proteins might, thus, specify different GSK-3 functions in the cell. Third, GSK-3 action requires the priming phosphorylation of its substrates by another kinase on a serine residue located four amino acids C-terminal to the GSK-3 phosphorylation site. Fourth, GSK-3 is regulated through its intracellular distribution.

Pharmacological inhibitors: diversity of structures and mechanism of action

It was nearly 50 years after the discovery of the unique properties of lithium in manic-depression illness (bipolar affective disorder) that GSK-3 was identified as one of its main targets [8–10]. Since then, lithium has been used widely as a pharmacological inhibitor of GSK-3, despite

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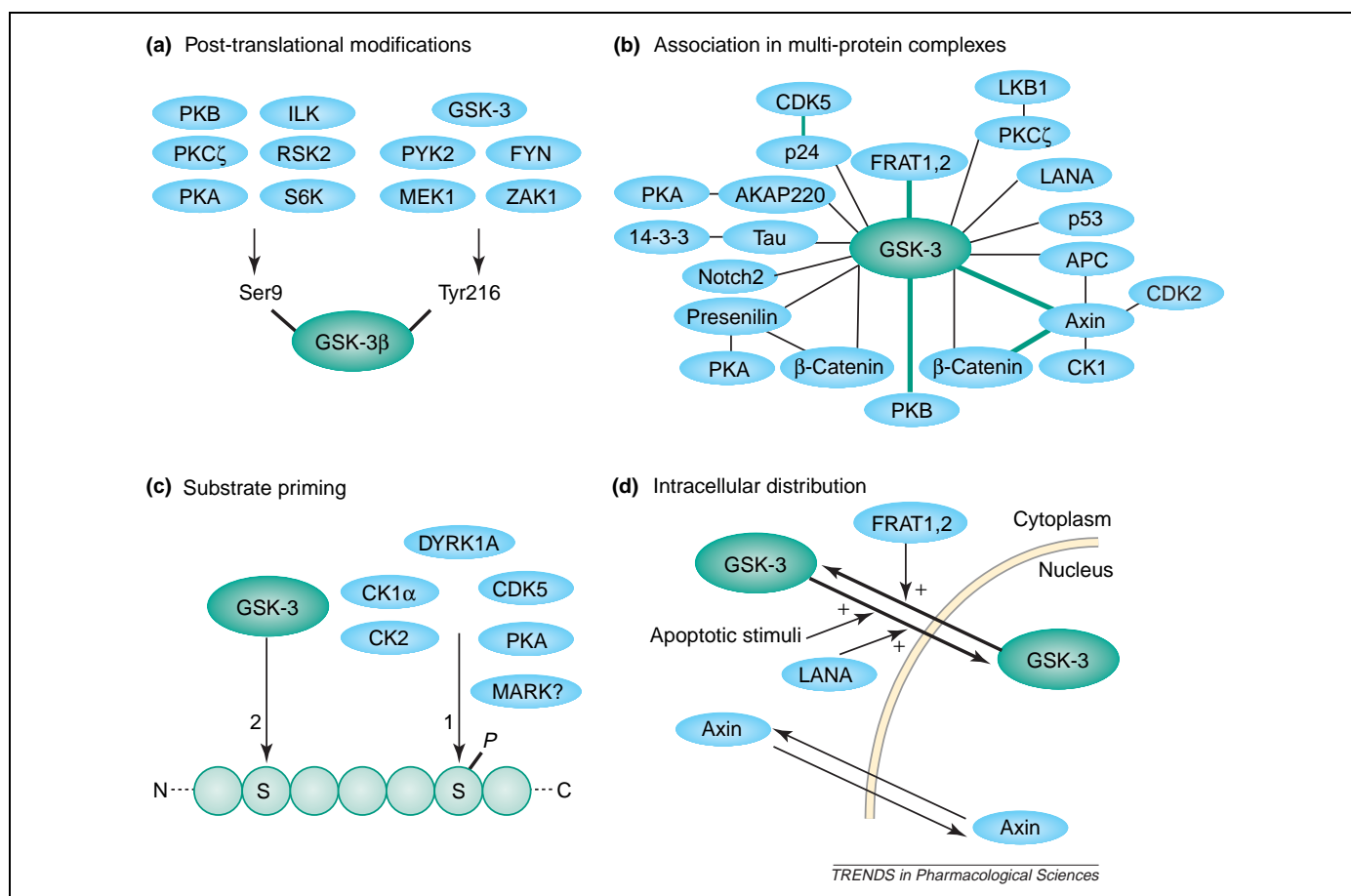


Figure 1. Glycogen synthase kinase 3 (GSK-3) regulation. **(a)** Post-translational modifications. GSK-3 β is regulated by post-translational phosphorylation of Ser9 and Tyr216. Phosphorylation of Ser9 can be carried out by p70^{S6K}, p90^{RSK}, protein kinase A (PKA), PKB (AKT), PKC isoforms and integrin-linked kinase (ILK). The src-like FYN kinase, the Ca²⁺-sensitive praline-rich tyrosine kinase 2 (PYK2), a putative homolog of the *Dictyostelium* zaphod kinase 1 (ZAK1) tyrosine kinase and mitogen-activated protein kinase kinase 1 (MEK1) might be responsible for Tyr216 phosphorylation. **(b)** Association with partners. GSK-3 β is regulated by interactions with many proteins. Interactions that have been characterized by crystallography are indicated by green lines. **(c)** Priming of substrates. The substrate-recognition site of GSK-3 is S-X-X-X-S-P, where S-P is a priming, pre-phosphorylated serine residue. The priming kinase can be casein kinase 2 (CK2) (for glycogen synthase), CK1 α (for β -catenin), dual-specificity tyrosine-phosphorylation regulated kinase 1A (DYRK1A) (for eukaryotic protein synthesis initiation factor 2B), PKA (for *Cubitus interruptus*) or cyclin-dependent kinase 5 (CDK5)/p25 (for Tau). **(d)** Intracellular distribution. GSK-3 β is predominantly cytoplasmic, but it is also present in the nucleus and in mitochondria. The activity of nuclear GSK-3 is higher than cytoplasmic GSK-3, and the nuclear pool is stimulated further in apoptotic cells or by p53. GSK-3 β is localized predominantly in the neuronal soma and processes whereas GSK-3 β 2 is localized in the soma. FRAT1,2 (frequently rearranged in advanced T-cell lymphomas 1,2) promotes the nuclear efflux of GSK-3 β whereas latent nuclear antigen (LANA), a Kaposi virus protein, sequesters GSK-3 β in the nucleus and mimics Wnt signaling. Abbreviations: AKAP220, A-kinase anchoring protein; APC, adenomatous polyposis coli; LKB1, gene denomination of serine/threonine kinase 11; MARK, microtubule affinity-regulating kinase; RSK, ribosomal S6 kinase; S6K S6 kinase.

the millimolar concentrations that are required to affect GSK-3 in living cells.

The definitive mood-stabilizing properties of lithium, the insulin-mimetic properties of GSK-3 inhibition and the GSK-3-dependent abnormal phosphorylation of Tau and production of amyloid- β in Alzheimer's disease have stimulated the search for potent, selective inhibitors of GSK-3. At present >30 inhibitors have been described, some with IC₅₀ values in the nanomolar range (Table 1 and Figure 2) (reviewed in [11–15]). Soon after the crystallization of GSK-3 β [6,7], the enzyme was co-crystallized with some inhibitors, which provided an exquisite understanding of their mechanism of interaction within the ATP-binding pocket. Despite wide chemical diversity, most pharmacological inhibitors of GSK-3 share common properties: (i) they have a low molecular weight (<600); (ii) they are rather flat, hydrophobic heterocycles; (iii) most, but not all [16,17], act by competing with ATP in the ATP-binding site of the kinase (lithium acts directly by competing with magnesium, but also indirectly by

increasing the inhibitory phosphorylation of Ser9 and Ser21 of GSK-3 β and GSK-3 α , respectively [8]); (iv) like inhibitors of cyclin-dependent kinases (CDKs), they essentially bind through hydrophobic interactions and 2–3 hydrogen bonds with the kinase; and (v) the backbone carbonyl and amino side-chains of Val135 act as an H-bond acceptor and H-bond donor, respectively, to the inhibitors, whereas the backbone carbonyl of Asp133 often acts as an H-bond acceptor [18–23]. These interactions are similar to those described for CDK inhibitors [24], which is consistent with the fact that the primary structures of GSK-3 and CDK families are closely related.

Because GSK-3 is embedded in multi-protein complexes (Figure 1b), small molecules designed to target the interaction sites between GSK-3 and other proteins are attractive options to modulate its physiological functions (Figure 3). Although identifying such molecules is a major challenge in drug discovery, it has attracted more attention following recent successes. For example, Nutlin, modulates the interaction between MDM2 (mouse double

Table 1. Pharmacological inhibitors of GSK-3^a

Inhibitor ^b	Class	IC ₅₀ (μM)		Refs
		GSK-α and GSK-3β ^c	CDK1–cyclin B complex	
Hymenialdisine	Pyrroloazepine	0.010 (β)	0.022	[68]
Flavopiridol	Flavone	0.450	0.400	[33]
Kenpauillone	Benzazepinone	0.023 (β)	0.400	[29,32]
Alsterpauillone	Benzazepinone	0.004 (α); 0.004 (β)	0.035	[29,32]
Azakenpauillone	Benzazepinone	0.018 (β)	2.000	[69]
Indirubin-3'-oxime	Bis-Indole	0.022 (β)	0.018	[33]
6-Bromoindirubin-3'-oxime (BIO)	Bis-Indole	0.005	0.320	[20,21]
6-Bromoindirubin-3'-acetoxime	Bis-Indole	0.010	63.000	[20,21]
Aloisine A	Pyrrolopyrazine	0.650	0.150	[70]
Aloisine B	Pyrrolopyrazine	0.750	0.850	[70]
TDZD8	Thiadiazolidinone	2.000 (β); 7.000 (α/β) ^d	> 100; > 10 ^d	[16]
Compound 12	Pyridyloxadiazole	0.390 (β); 8.000 (α/β) ^d	> 10 ^d	[71]
Pyrazolopyridine 18	Pyrazolopyridine	0.018 (α)	Inhibits CDK2–cyclin A (95% at 10 μM)	[72]
Pyrazolopyridine 9	Pyrazolopyridazine	0.022 (α)	Inhibits CDK2–cyclin A (90% at 10 μM)	[22]
Pyrazolopyridine 34	Pyrazolopyridine	0.007 (α)	> 10 (CDK2–cyclin A)	[73,74]
CHIR98014	Aminopyrimidine	0.00065 (α); 0.00058 (β)	3.700	[54]
CHIR99021 (CT99021)	Aminopyrimidine	0.010 (α); 0.007 (β)	8.800	[54]
CT20026	Aminopyridine	0.004 (α/β)	?	[14]
Compound 1	Pyrazoloquinoline	1.000	0.600	[75]
SU9516	Oxindole (indolinone)	0.330; 0.35 (α/β) ^d	0.040; 0.022 ^d	[76]
ARA014418	Thiazole	0.104 (β)	> 100 (CDK2 and CDK5)	[19]
Staurosporine	Bisindolylmaleimide	0.015; 0.089	0.006; 0.008	[77]
Compound 5a	Bisindolylmaleimide	0.018 (β)	0.24	[77]
Compound 29	Azaindolylmaleimide	0.034 (β)	> 10	[78]
Compound 46	Azaindolylmaleimide	0.036 (β)	> 10	[79]
GF109203x (bisindolylmaleimide I)	Bisindolylmaleimide	0.190 (β)	2.300 ^d	[80]
Ro318220 (bisindolylmaleimide IX)	Bisindolylmaleimide	0.003–0.038 (β)	?	[28,80]
SB216763	Arylindolemaleimide	0.034 (α); 0.075 (α/β) ^d	0.550 ^d	[53]
SB415286	Anilinomaleimide	0.078 (α); 0.13 (α/β) ^d	0.900 ^d	[53,81]
I5	Anilinoarylmaleimide	0.076 (α); 0.160 (β)	> 10 (CDK2–cyclin A)	[18]
CGP60474	Phenylaminopyrimidine	0.010 ^d	0.017; 0.0006 ^d	[82]
Compound 8b	Triazole	0.280 (β)	> 250 (CDK2–cyclin A)	[83]
TWS119	Pyrrolopyrimidine	0.030 (β)	?	[65]
Compound 1A	Pyrazolopyrimidine	0.016 (β)	?	[84]
Compound 17	Chloromethyl thienyl ketone	1.00 (β)	?	[17]
Lithium	Atom (competition with Mg ²⁺)	2000.0	No effect	[8,9]
Beryllium	Atom (competition with Mg ²⁺ and ATP)	6.00	Inhibits CDK1	[9]
Zinc	Atom (uncompetitive)	15.00	No effect	[85]

^aAbbreviations: CDK1, cyclin-dependent kinase 1; GSK-3, glycogen synthase kinase 3.

^bSee Figure 2.

^c(α) or (β) cited after individual values indicates which specific isoform was tested; (α/β) cited after individual values indicates that a mixture of isoforms was tested. The absence of (α), (β) or (α/β) indicates that the study cited did not specify which isoform(s) was tested.

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minute 2) and p53 by binding to the p53-binding pocket of MDM2 and, thereby, activates the p53 pathway [25]. The well-characterized interaction between GSK-3 and axin [26] provides an essential physiological target. It is expected that inhibitors of this interaction will prevent the interaction between GSK-3 and some of its substrates and priming kinases, and reorientate the overall cellular GSK-3 activity towards other substrates. In this context, a viral protein (LANA) that competes with axin for binding to GSK-3 and, consequently, targets GSK-3 to the nucleus [27], indicates that GSK-3 nuclear targeting compounds could be identified. In general, compounds that interfere with the intracellular localization of GSK-3 might constitute alternative ways to modulate the physiological functions of GSK-3.

The selectivity of inhibitors

Selectivity is a key issue when GSK-3 inhibitors are used as pharmacological tools to demonstrate the involvement

of GSK-3 in a cellular process. By contrast, absolute selectivity is not necessarily the best approach when considering GSK-3 inhibitors as potential treatments for complex diseases in which multiple pathways are deregulated, because high selectivity could lead rapidly to resistance.

Because the ATP-binding pockets of GSK-3α and GSK-3β are similar, inhibitors that target these sites are unlikely to differentiate between the two isoforms. Such selectivity might only be achieved by drugs that act at other sites on the kinases, by alternative molecular approaches (Table 2), and when either the intracellular distribution or the expression is different for the two isoforms.

The selectivity of most of the available GSK-3 inhibitors is poorly known and, essentially, based on their evaluation with limited panels of kinases [28,29] and purification of potential targets on immobilized inhibitors using affinity chromatography [20,30]. Lithium has alternative targets

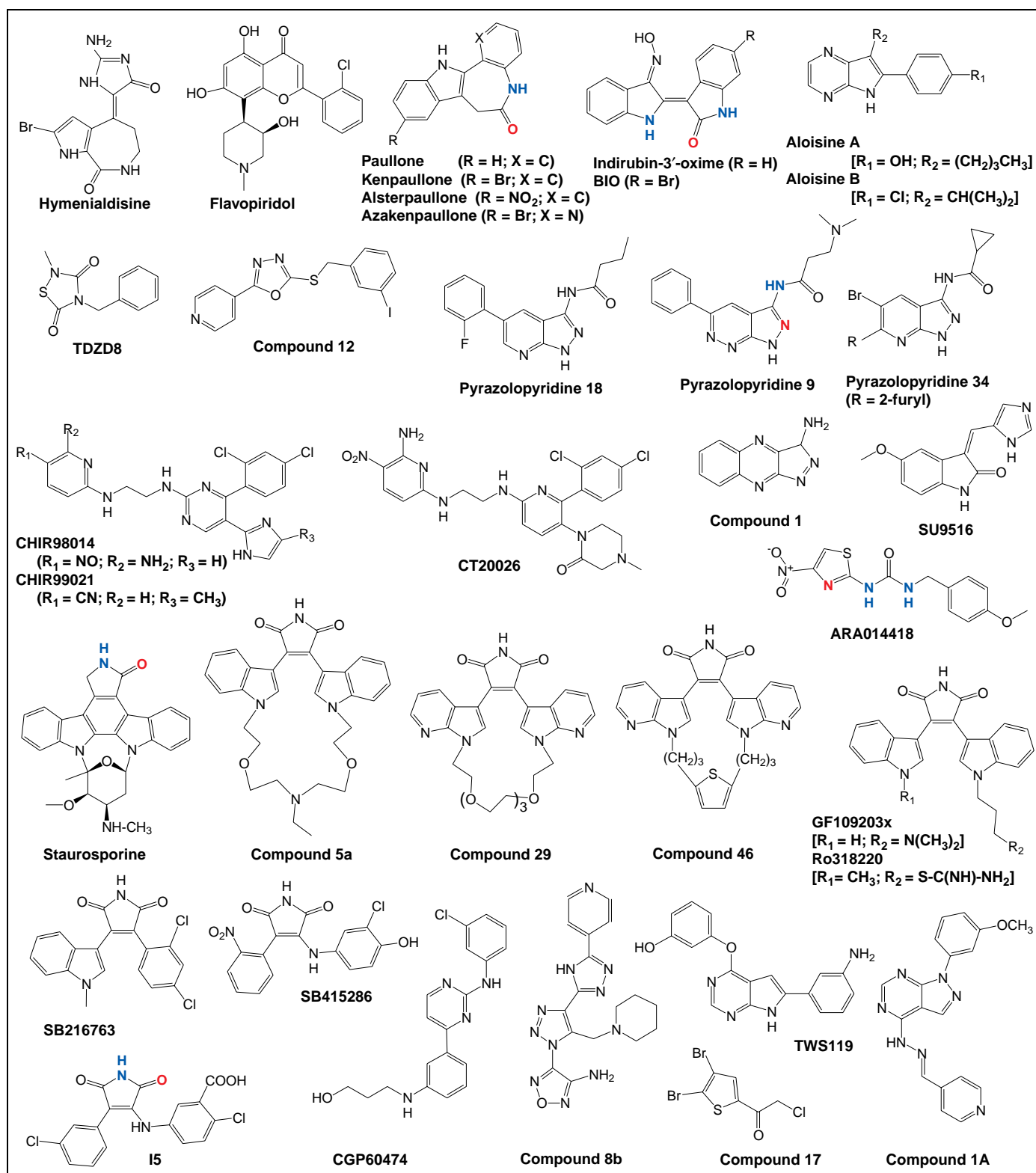


Figure 2. Structures of some of the most studied pharmacological inhibitors of glycogen synthase kinase 3 (GSK-3). The inhibitors are grouped in chemical families. For molecules that have been co-crystallized with GSK-3 β , the atoms that interact with the side-chains of Val135 and Asp133 are shown in red (hydrogen acceptors) and blue (hydrogen donors).

such as inositol-phosphate phosphatases [31]. Paullones, especially kenpaullone [29,32], 6-bromo-substituted indirubins [20,21,33] and the azaindolylmaleimide compound 46 (Figure 2) [29], appear to be among the most selective inhibitors identified so far. However, because

many GSK-3 inhibitors also affect CDKs (Table 1) [33], control experiments should be carried out using CDK inhibitors that do not target GSK-3 (such as roscovitine and purvalanol) [29]. In some circumstances, CDK5-GSK-3 dual specificity could be an advantage, as illustrated later.

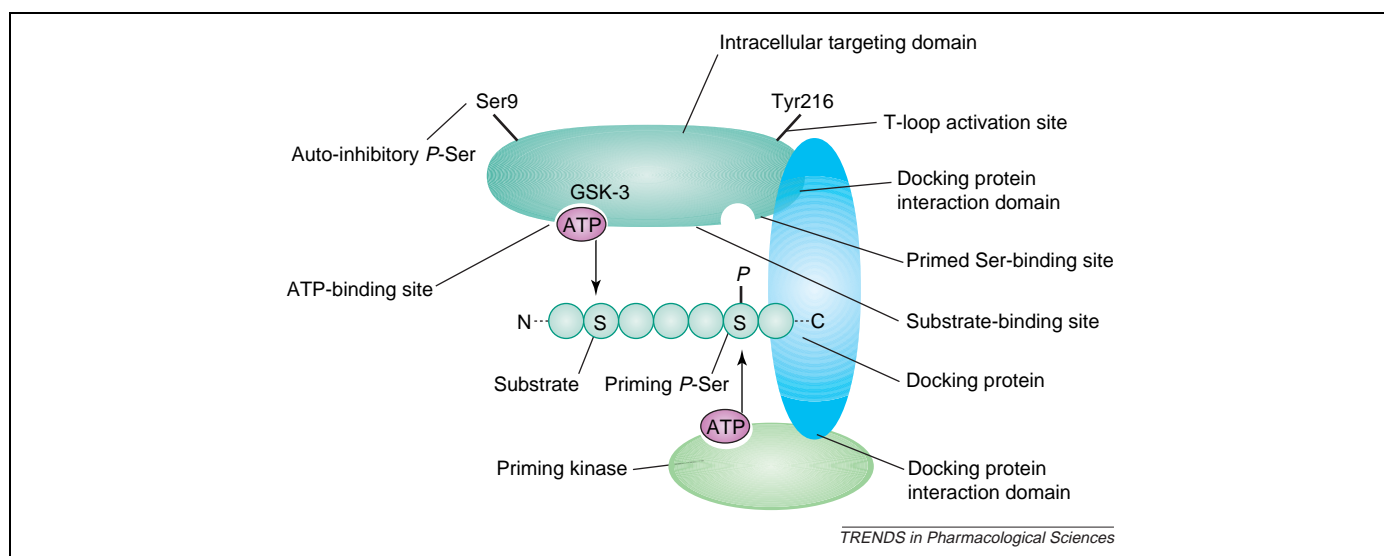


Figure 3. Potential sites for inhibition of glycogen synthase kinase 3 (GSK-3). Most kinase inhibitors act by competition with either ATP or metal-binding sites that are involved directly in the catalytic process. However, small-molecular-weight compounds might regulate GSK-3 activity by inhibiting the protein–protein interactions that are necessary for binding of substrate [the primed phosphorylated serine binding area and the docking protein (axin and presenilin)], by modulating the Tyr216 (GSK-3 β) and Tyr279 (GSK-3 α) activation sites and the Ser9 (GSK-3 β) and Ser21 (GSK-3 α) inhibition sites, and by interfering with the intracellular targeting domain of GSK-3. Inhibition of the interaction between the docking protein and the priming kinase might change the substrate specificity of GSK-3.

GSK-3 inhibitors: diversity of applications

Based on knowledge of the literature, several therapeutic areas might benefit from the development of GSK-3 inhibitors (Figure 4). Obviously many of our suggestions are highly speculative. In addition, the potential toxicities and absorption, distribution, metabolism and excretion (ADME) properties of GSK-3 inhibitors could mitigate against their clinical use.

Nervous system disorders

The beneficial effect of lithium in the treatment of bipolar affective disorder might result from GSK-3 inhibition but it might also rely on inhibition of a set of additional targets. Despite its undisputed efficacy, lithium has side-effects and more-potent GSK-3 inhibitors possibly represent an alternative treatment for bipolar disorder [31].

Alzheimer's disease (AD) is characterized by three essential events (reviewed in [34]): (i) in familial AD, mutation of any one of the genes that encode presenilin-1, presenilin-2 and the β -amyloid precursor protein (β -APP) results in \sim 100% penetrance; (ii) the extracellular accumulation of amyloid- β (40–42 amino-acid peptides derived from proteolytic cleavage of β -APP by several enzyme complexes, including β - and γ -secretases); and (iii) the intracellular aggregation of hyperphosphorylated forms of the microtubule-binding protein Tau. GSK-3 has been implicated in each of these processes. For example,

the activities of protein kinase B (AKT) and GSK-3 β kinase decrease and increase, respectively, in cells from familial AD patients that contain mutated presenilin-1/2 or β -APP, compared with control cells [35]. Furthermore, the activity of GSK-3 α is required for the production of amyloid- β [36] and amyloid- β toxicity is mediated by increased GSK-3 activity. Last, GSK-3, with CDK5 and microtubule affinity-regulating kinase (MARK), is one of the major kinases that is involved in abnormal Tau phosphorylation on AD-specific sites. Presenilin-1 interacts directly with GSK-3, which favors the interaction of GSK-3 with substrates such as Tau. Together, these data indicate the potential benefits of GSK-3 inhibition in counteracting the three major AD events, presenilin action, amyloid- β peptide production and Tau hyperphosphorylation.

GSK-3 is involved in neuronal cell death and there are numerous examples of the neuroprotection provided by GSK-3 inhibitors following different insults [20]. Two animal models illustrate the link between GSK-3 activation and neuronal cell death. The first is that conditional-transgenic mice that overexpress GSK-3 in brain during adulthood show signs of neurodegeneration and spatial-learning deficits [37]. The second example is provided by *Drosophila*, in which overexpression of *shaggy*, the GSK-3 β homolog, enhances neurodegeneration induced by the expression of human Tau, whereas expression of a

Table 2. Alternative approaches to inhibit GSK-3^a

Inhibitors	Class	Mechanism of action	Refs
FRAT1, FRAT2 (GBP)	Protein	Prevents interaction with axin	[86]
Adenoviral FRAT1 overexpression	Protein	Prevents interaction with axin	[87]
FRATtide (FRAT1 _{188–226} peptide)	FRAT-derived peptide	Prevents interaction with axin	[88]
p24	Protein	Unknown	[89]
siRNA	Hairpin siRNA	Inhibits GSK-3 expression	[63,90]
GID _{320–429} and GID _{380–404} peptides	Axin-derived peptide	Prevents interaction with axin and substrates?	[91]

^aAbbreviations: FRAT1, frequently rearranged in advanced T-cell lymphomas 1; GBP, GSK-3 binding protein; GID, GSK-3 inhibitory domain; GSK-3, glycogen synthase kinase 3; siRNA, small-interfering RNA.

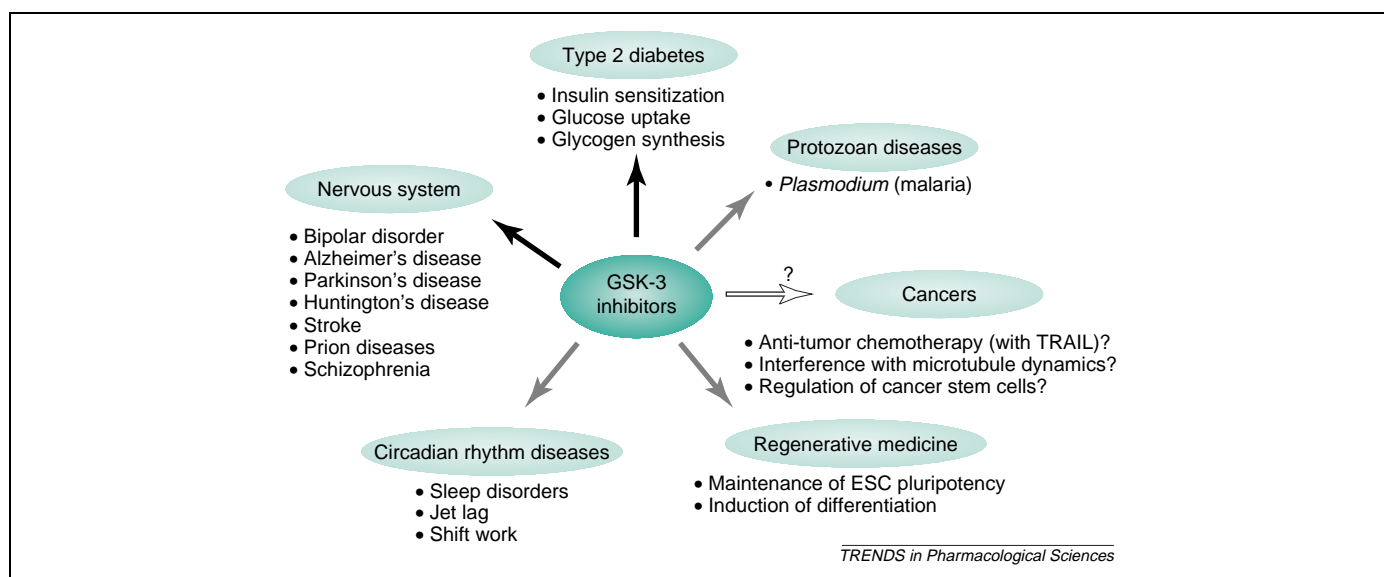


Figure 4. Potential applications of pharmacological inhibitors of glycogen synthase kinase 3 (GSK-3). There are reasons to investigate the potential of pharmacological inhibitors of GSK-3 in many therapeutic areas; some areas are supported by strong data (black arrow), support for some is tentative (grey arrows), and one area is problematic (white arrow). Abbreviations: ESC, embryonic stem cell; TRAIL, tumor necrosis factor-related apoptosis inducing ligand.

loss-of-function mutant of *shaggy* prevents this neurodegeneration [38]. There is experimental evidence that reducing GSK-3 activity by small-interfering RNS (siRNA), lithium and kenpaullone in cell lines, and by lithium in mice, reduces amyloid- β production [36]. Together, these and other data strongly encourage the evaluation of GSK-3 inhibitors as neuroprotective agents in AD. Because CDK5 is also involved in neurodegeneration [39], and mitotic CDK1 and its upstream activators are expressed in the brains of AD patients but not of normal individuals, CDK-GSK-3 dual-specificity inhibitors might have advantages over GSK-3-selective compounds. However, the consequences of GSK-3 inhibition (presumably over long durations) on a late-diagnosed neurodegenerative disease such as AD are speculative.

Acute neuronal-cell death during stroke might represent a more suitable therapeutic application for GSK-3 inhibitors in which they would, presumably, be used transiently. Ischemic insults lead to the death of specific neurons (e.g. CA1 pyramidal neurons of the hippocampus) through a phenomenon known as excitotoxicity. This glutamate-induced, Ca^{2+} -dependent process is thought to be mediated by NMDA receptors, but the intracellular mechanisms are only beginning to be uncovered. These include activation of GSK-3 [40] and CDK5 [41], and expression of several mitotic markers such as cyclin D1, cyclin B1 and CDK2. Lithium is reported to protect neuronal cells in culture from excitotoxicity. Lithium reduces the neurological deficits and decreases the brain-infarct size when given before or after middle-cerebral-artery occlusion in a rat model of stroke [42,43]. Again, GSK-3-CDK5 dual-specificity inhibitors might provide a therapeutic advantage in the treatment of stroke over GSK-3-selective compounds.

Parkinson's disease (PD) is a neurodegenerative disease that is characterized by the specific loss of dopamine-containing neurons that project from the substantia nigra to the caudate-putamen (striatum). PD-like

neurodegeneration in the substantia nigra and striatum can be induced in animal models and humans by administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). 1-Methyl-4-phenylpyridinium (MPP^+), the active metabolite of MPTP, triggers neuronal cell death in culture through a caspase-3-dependent apoptotic pathway. This event is facilitated by overexpression of GSK-3 β and attenuated by lithium [44]. Recently, CDK5 has been demonstrated to be instrumental in the death of dopamine-containing neurons in an animal model of PD [45]. Dual CDK-GSK-3 inhibitors might, thus, be more appropriate to control nigral cell death in PD.

Huntington's disease is a neurodegenerative disease caused by a polyglutamine extension of the huntingtin protein. The modified protein is toxic and forms intracellular aggregates. Decreased β -catenin levels and reduced transcription in cells that express mutant huntingtin indicates that GSK-3 modulates cellular toxicity. Indeed, GSK-3 inhibitors and overexpression of a dominant-negative GSK-3 β protect cells from the toxicity of mutant huntingtin [46]. GSK-3 inhibitors, thus, warrant evaluation in the treatment of Huntington's disease.

Transmissible spongiform encephalopathies (TSEs, also known as prion diseases) are marked by the accumulation of an amyloidogenic, insoluble form of the prion protein. The pathological prion protein induces neuronal cell death, a process that is reduced significantly in culture by treatment with either lithium or insulin (which inhibits GSK-3) and by overexpression of a dominant-negative mutant of GSK-3 [47]. Although preventing transformation of normal prion protein to its pathological form is an obvious priority in the strategies to control TSEs, GSK-3 inhibitors might constitute a valuable tool to investigate and, possibly, prevent the toxic effects of TSE prions.

The recent discovery of reduced AKT1 concentrations and decreased phosphorylation of Ser9 of GSK-3 β (and,

presumably, increased GSK-3 β activity) in the brains of patients with schizophrenia, and the compensatory effect induced by the antipsychotic haloperidol indicate that alteration in AKT1 and GSK-3 β contributes to schizophrenia [48]. Sustained stimulation of dopamine receptors leads to GSK-3 activation, and several GSK-3 inhibitors appear to partially compensate dopamine-dependent hyperactivity in mice [49]. It might, thus, be worth investigating whether GSK-3 β inhibitors affect schizophrenia.

The genetic basis of circadian-clock regulation is starting to be better understood [50,51]. In *Drosophila*, casein kinase 1 δ (CK1 δ), CK1 ϵ and GSK-3 contribute to fine-tuning the rhythm by regulating the transcription factors Period and Timeless. Because GSK-3 appears to have a similar function in mammals, GSK-3 inhibitors might modulate forced alterations of the circadian rhythm (jet lag and shift work) and pathological situations that are linked to unbalanced circadian rhythms.

Type 2 diabetes

One of the main functions of insulin is to stimulate the uptake, metabolism and storage of glucose in adipocytes, myocytes and hepatocytes. Defective insulin secretion and insulin resistance result in diabetes. Type 1 diabetes originates from a lack of insulin production by pancreatic β -cells. By contrast, type 2 diabetes (90% of cases) results from resistance to insulin. Insulin decreases the phosphorylation and increases the activity of glycogen synthase by inhibiting GSK-3. Chemical inhibitors of GSK-3 that act downstream of hormone binding might, thus, represent a promising opportunity to circumvent insulin resistance and to treat type 2 diabetes (reviewed in [13,15]). Lithium, a pseudosubstrate peptidic inhibitor [52] and pharmacological inhibitors of GSK-3 [15,53–56] have been demonstrated to mimic insulin-induced glycogen synthase activation, glycogen synthesis, suppression of gluconeogenesis and increased glucose uptake in several cell cultures. Insulin-like effects are also observed *in vivo* [15,57,58]. Pharmacological inhibition of GSK-3 potentiates insulin action in the skeletal muscle of insulin-resistant rats [57]. Together, these results indicate that GSK-3 inhibition might improve glucose utilization and insulin sensitivity therapeutically. Because the priming phosphorylation of glycogen synthase by CK2 is a prerequisite for phosphorylation by GSK-3, the combination of CK2 and GSK-3 inhibitors and the use of CK2–GSK-3 dual-specificity inhibitors might offer a therapeutic advantage.

Similar to amyloid aggregates in AD brains, the deposition of amylin in insulin-producing Langerhans islets is frequently observed in the pancreas of patients with type 2 diabetes. Interestingly the expression of p35, the activating subunit of CDK5, is stimulated strongly in pancreatic β -cells following glucose stimulation. Active CDK5/p35 is required for hyperglycemia-dependent stimulation of insulin synthesis [59]. It is possible that glucose-induced dysregulation of CDK5/p35 is a pathophysiological mechanism that is involved in β -cell dysfunction and in the progression of type 2 diabetes. Evaluation of the treatment of type 2 diabetes with

GSK-3 inhibitors should explore the possible benefits of additional CDK inhibition.

Cancer

Many cancers, especially colorectal cancers, have defects in elements of the Wnt pathway that result in the abnormal activation of Wnt signaling and accumulation of β -catenin [60], a cotranscription factor that controls numerous genes involved in carcinogenesis. Furthermore, GSK-3 inhibition stabilizes three cell-cycle regulators, namely cyclin D1, cyclin E and c-Myc, the overexpression of which is linked with tumorigenesis. Because GSK-3 inhibition is expected to mimic Wnt signaling and stabilize oncogenic proteins, there is concern that GSK-3 inhibitors might be potent inducers of cancer. Addressing this concern is necessary for each GSK-3 inhibitor. However, available epidemiological data shows that long-term use of lithium at concentrations that inhibit GSK-3 is not associated with increased cancer morbidity in patients with bipolar disorder [61]. Furthermore, lithium treatment does not significantly increase the number of tumors in a mutant adenomatous polyposis coli mouse model [62].

Under some circumstances, GSK-3 inhibitors might be useful in treating cancer. Indeed, resistance to TRAIL, an apoptosis-inducing ligand that is being evaluated as an anti-cancer agent, involves activation of GSK-3 β [63]. GSK-3 β suppression, by lithium, SB216673 and siRNA, dramatically enhances TRAIL-induced apoptosis in prostate cancer cell lines. Because CDK inhibition also contributes to the anti-tumor effect of TRAIL, CDK–GSK-3 dual-specificity inhibitors might synergize with TRAIL in prostate cancer treatment.

GSK-3 plays a role in the dynamics of the mitotic spindle. GSK-3 inhibitors prevent chromosome movements and lead to the stabilization of microtubules and a prometaphase-like arrest that is similar to that observed with low doses of taxol [64].

GSK-3 inhibitors should, thus, be examined for potential use in cancer therapy. An alternative way to circumvent the potential Wnt-mimetic activity of GSK-3 inhibitors is to design inhibitors that either target the phosphorylation of non-Wnt pathway substrates or inhibit GSK-3 activity only in a specific cell compartment.

Stem-cell biology and regenerative medicine

There are considerable expectations associated with the use of stem cells and their differentiated products to either replace or repopulate diseased and damaged tissues (i.e. stem-cell therapeutics).

Although the Wnt pathway is a major regulator in multipotent stem cells (hematopoietic, neural, skin and embryonic), activation of Wnt leads to different outcomes, probably depending on the particular state of a given cell. In one example, GSK-3 inhibition by TWS119 was found to lead to neuronal differentiation of embryonic stem cells (ESCs) [65]. Thus, GSK-3 inhibitors might be used to orientate the differentiation of stem cells into well-defined, committed cell lineages. Cancers might result from mutations in genes that control the normal renewal of stem cells, leading to deregulated stem-cell renewal. The use of kinase inhibitors to force cancer stem cells to

differentiate into non-tumorigenic cancer cells is one of the most attractive potential applications of these molecules.

By contrast, inhibition of GSK-3 by 6-bromo-indirubin-3'-oxime supports the renewal of human and mouse ESCs and the maintenance of their pluripotency in the absence of feeder cells [66]. In this situation, GSK-3 inhibitors might have a practical application in regenerative medicine, by allowing the maintenance of undifferentiated ESC lines in the absence of feeder cells in a chemically defined medium. This use of a GSK-3 inhibitor might circumvent some of the ethical issues and technical problems raised by the generation and use of ESCs.

GSK-3 inhibitors have diverse but clear effects on ESCs. Variability might be caused by the different state of the initial cells, and the selectivity, intracellular distribution and metabolism of the different GSK-3 inhibitors used.

Unicellular parasites

There is an increasing interest in the evaluation of kinases from unicellular parasites as targets for potential new anti-parasitic drugs. The evolutionary difference between unicellular kinases and their human homologues might be sufficient to allow the design of parasite-specific inhibitors. The *Plasmodium falciparum* genome contains 65 genes that encode kinases, including three forms of GSK-3. An initial study showed that *P. falciparum* exports PfGSK-3 to the cytoplasm of host erythrocytes (which are devoid of GSK-3), where it colocalizes with parasite-generated membrane structures known as Maurer's clefts [67]. The function of PfGSK-3 is unknown, but the presence of PfCK1, a CK1 homolog, in infected red blood cells supports the hypothesis that both kinases play a role in regulating the strong circadian rhythm of the parasite, which is responsible for the circadian fevers that are characteristic of this infectious disease. The sensitivity of PfGSK-3 to pharmacological inhibitors does not overlap completely with that of mammalian GSK-3, which indicates that PfGSK-3-selective inhibitors might be identified for therapeutic evaluation against the proliferation of the parasite and, possibly, its differentiation into gametocytes.

Concluding remarks

The existence of three closely related, non-interchangeable isoforms of GSK-3 (GSK-3 α , GSK-3 β and GSK-3 β 2), the multiple, sometimes apparently opposing, functions of GSK-3, and the poorly characterized selectivity, cell permeability and stability of pharmacological inhibitors of GSK-3 means that the development of therapeutically useful anti-GSK-3 drugs will not be easy. However, the partial success of lithium, the clear connection between dysregulation of GSK-3 and major human diseases, the promising results from cellular and animal models in several therapeutic areas, the wide chemical diversity of possible inhibitors, and the existence of multiple sites for potential inhibition constitute strong encouragement to pursue the development and evaluation of GSK-3 inhibitors as potential drugs. As our knowledge of the function and regulation of GSK-3 progresses, we become more

aware of the limitations and the potential benefits of inhibiting this class of kinases.

In addition to direct inhibitors of GSK-3, compounds that regulate the intracellular localization of GSK-3, and that prevent the interaction of GSK-3 with particular scaffold proteins and substrates are also needed. Because most substrates of GSK-3 require priming phosphorylation by specific kinases, chemical inhibitors of these upstream, priming kinases might provide the opportunity to mimic a subset of the effects of GSK-3 inhibition.

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References

- Cohen, P. (2002) Protein kinases – the major drug targets of the twenty-first century? *Nat. Rev. Drug Discov.* 1, 309–315
- Noble, M.E. *et al.* (2004) Protein kinase inhibitors: insights into drug design from structure. *Science* 303, 1800–1805
- Frame, S. and Cohen, P. (2001) GSK3 takes centre stage more than 20 years after its discovery. *Biochem. J.* 359, 1–16
- Doble, B.W. and Woodgett, J.R. (2003) GSK-3: tricks of the trade for a multi-tasking kinase. *J. Cell Sci.* 116, 1175–1186
- Jope, R.S. and Johnson, G.V.W. (2004) The glamour and gloom of glycogen synthase kinase-3. *Trends Biochem. Sci.* 29, 95–102
- Dajani, R. *et al.* (2001) Crystal structure of glycogen synthase kinase 3 β : structural basis for phosphate-primed substrate specificity and autoinhibition. *Cell* 105, 721–732
- ter Haar, E. *et al.* (2001) Structure of GSK3 β reveals a primed phosphorylation mechanism. *Nat. Struct. Biol.* 8, 593–596
- Klein, P.S. and Melton, D.A. (1996) A molecular mechanism for the effect of lithium on development. *Proc. Natl. Acad. Sci. U. S. A.* 93, 8455–8459
- Phiel, C.J. and Klein, P.S. (2001) Molecular targets of lithium action. *Annu. Rev. Pharmacol. Toxicol.* 41, 789–813
- Jope, R.S. (2003) Lithium and GSK-3: one inhibitor, two inhibitory actions, multiple outcomes. *Trends Pharmacol. Sci.* 24, 441–443
- Martinez, A. *et al.* (2002) Glycogen synthase kinase 3 (GSK-3) inhibitors as new promising drugs for diabetes, neurodegeneration, cancer, and inflammation. *Med. Res. Rev.* 22, 373–384
- Eldar-Finkelman, H. and Ilouz, R. (2003) Challenges and opportunities with glycogen synthase kinase-3 inhibitors for insulin resistance and type 2 diabetes treatment. *Expert Opin. Investig. Drugs* 12, 1–9
- Van Wauwe, J. and Haefner, B. (2003) Glycogen synthase kinase-3 as drug target: from wallflower to center of attention. *Drug News Perspect.* 16, 557–565
- Wagman, A.S. *et al.* (2004) Discovery and development of GSK3 inhibitors for the treatment of Type 2 diabetes. *Curr. Pharmacol. Des.* 10, 1105–1137
- Cohen, P. and Goedert, M. (2004) GSK3 inhibitors: development and therapeutic potential. *Nat. Rev. Drug Discov.* 3, 479–487
- Martinez, A. *et al.* (2002) First non-ATP competitive glycogen synthase kinase 3 β (GSK-3 β) inhibitors: thiazolidinones (TDZD) as potential drugs for the treatment of Alzheimer's disease. *J. Med. Chem.* 45, 1292–1299
- Conde, S. *et al.* (2003) Thienyl and phenyl alpha-halomethyl ketones: new inhibitors of glycogen synthase kinase (GSK-3 β) from a library of compound searching. *J. Med. Chem.* 46, 4631–4633
- Bertrand, J.A. *et al.* (2003) Structural characterization of the GSK-3 β active site using selective and non-selective ATP-mimetic inhibitors. *J. Mol. Biol.* 333, 393–407
- Bhat, R. *et al.* (2003) Structural insights and biological effects of glycogen synthase kinase 3-specific inhibitor AR-A014418. *J. Biol. Chem.* 278, 45937–45945

- 20 Meijer, L. *et al.* (2003) GSK-3 selective inhibitors derived from Tyrian purple indirubins. *Chem. Biol.* 10, 1255–1266
- 21 Polychronopoulos, P. *et al.* (2004) Structural basis for the synthesis of indirubins as potent and selective inhibitors of glycogen synthase kinase-3 and cyclin-dependent kinases. *J. Med. Chem.* 47, 935–946
- 22 Witherington, J. *et al.* (2003) 5-Aryl-pyrazolo[3,4-b]pyridazines: potent inhibitors of glycogen synthase kinase-3 (GSK-3). *Bioorg. Med. Chem. Lett.* 13, 1581–1584
- 23 Fischer, P.M. (2003) CDK versus GSK-3 inhibition: a purple haze no longer? *Chem. Biol.* 10, 1144–1146
- 24 Knockaert, M. *et al.* (2002) Pharmacological inhibitors of cyclin-dependent kinases. *Trends Pharmacol. Sci.* 23, 417–425
- 25 Vassilev, L.T. *et al.* (2004) *In vivo* activation of the p53 pathway by small-molecule antagonists of MDM2. *Science* 303, 844–848
- 26 Dajani, R. *et al.* (2003) Structural basis for recruitment of glycogen synthase kinase 3beta to the axin-APC scaffold complex. *EMBO J.* 22, 494–501
- 27 Fujimuro, M. *et al.* (2003) A novel viral mechanism for dysregulation of beta-catenin in Kaposi's sarcoma-associated herpesvirus latency. *Nat. Med.* 9, 300–306
- 28 Davies, S.P. *et al.* (2000) Specificity and mechanism of action of some commonly used protein kinase inhibitors. *Biochem. J.* 351, 95–105
- 29 Bain, J. *et al.* (2003) The specificities of protein kinase inhibitors: an update. *Biochem. J.* 371, 199–204
- 30 Knockaert, M. *et al.* (2002) Intracellular targets of paullones. Identification following affinity purification on immobilized inhibitor. *J. Biol. Chem.* 277, 25493–25501
- 31 Gould, T.D. *et al.* (2004) Glycogen synthase kinase-3: a target for novel bipolar disorder treatments. *J. Clin. Psychiatry* 65, 10–21
- 32 Leost, M. *et al.* (2000) Paullones are potent inhibitors of glycogen synthase kinase-3β and cyclin-dependent kinase 5/p25. *Eur. J. Biochem.* 267, 5983–5994
- 33 Leclerc, S. *et al.* (2001) Indirubins inhibit glycogen synthase kinase-3β and CDK5/p25, two kinases involved in abnormal tau phosphorylation in Alzheimer's disease – A property common to most CDK inhibitors? *J. Biol. Chem.* 276, 251–260
- 34 De Strooper, B. and Woodgett, J. (2003) Alzheimer's disease: Mental plaque removal. *Nature* 423, 392–393
- 35 Ryder, J. *et al.* (2004) Akt/GSK3β serine/threonine kinases: evidence for a signaling pathway mediated by familial Alzheimer's disease mutations. *Cell. Signal.* 16, 187–200
- 36 Phiel, C.J. *et al.* (2003) GSK-3α regulates production of Alzheimer's disease amyloid-beta peptides. *Nature* 423, 435–439
- 37 Lucas, J.J. *et al.* (2001) Decreased nuclear beta-catenin, tau hyperphosphorylation and neurodegeneration in GSK-3beta conditional transgenic mice. *EMBO J.* 20, 27–39
- 38 Jackson, G.R. *et al.* (2002) Human wild-type tau interacts with wingless pathway components and produces neurofibrillary pathology in *Drosophila*. *Neuron* 34, 509–519
- 39 Noble, W. *et al.* (2003) Cdk5 is a key factor in tau aggregation and tangle formation *in vivo*. *Neuron* 38, 555–565
- 40 Bhat, R.V. *et al.* (2000) Regulation and localization of tyrosine216 phosphorylation of glycogen synthase kinase-3beta in cellular and animal models of neuronal degeneration. *Proc. Natl. Acad. Sci. U. S. A.* 97, 11074–11079
- 41 Wang, J. *et al.* (2003) Cdk5 activation induces hippocampal CA1 cell death by directly phosphorylating NMDA receptors. *Nat. Neurosci.* 6, 1039–1047
- 42 Nonaka, S. and Chuang, D.M. (1998) Neuroprotective effects of chronic lithium on focal cerebral ischemia in rats. *NeuroReport* 9, 2081–2084
- 43 Ren, M. *et al.* (2003) Postinsult treatment with lithium reduces brain damage and facilitates neurological recovery in a rat ischemia/reperfusion model. *Proc. Natl. Acad. Sci. U. S. A.* 100, 6210–6215
- 44 King, T.D. *et al.* (2001) Caspase-3 activation induced by inhibition of mitochondrial complex I is facilitated by glycogen synthase kinase-3β and attenuated by lithium. *Brain Res.* 919, 106–114
- 45 Smith, P.D. *et al.* (2003) Cyclin-dependent kinase 5 is a mediator of dopaminergic neuron loss in a mouse model of Parkinson's disease. *Proc. Natl. Acad. Sci. U. S. A.* 100, 13650–13655
- 46 Carmichael, J. *et al.* (2002) GSK-3β inhibitors prevent cellular polyglutamine toxicity caused by the Huntington's disease mutation. *J. Biol. Chem.* 277, 33791–33798
- 47 Perez, M. *et al.* (2003) Prion peptide induces neuronal cell death through a pathway involving glycogen synthase kinase 3. *Biochem. J.* 372, 129–136
- 48 Emamian, E.S. *et al.* (2004) Convergent evidence for impaired AKT1–GSK3β signaling in schizophrenia. *Nat. Genet.* 36, 131–137
- 49 Beaulieu, J.M. *et al.* (2004) Lithium antagonizes dopamine-dependent behaviors mediated by an AKT/glycogen synthase kinase 3 signaling cascade. *Proc. Natl. Acad. Sci. U. S. A.* 101, 5099–5104
- 50 Iwahana, E. *et al.* (2004) Effect of lithium on the circadian rhythms of locomotor activity and glycogen synthase kinase-3 protein expression in the mouse suprachiasmatic nuclei. *Eur. J. Neurosci.* 19, 2281–2287
- 51 Harms, E. *et al.* (2003) CK1 and GSK3 in the *Drosophila* and mammalian circadian clock. *Novartis Found. Symp.* 253, 267–277
- 52 Plotkin, B. *et al.* (2003) Insulin mimetic action of synthetic phosphorylated peptide inhibitors of glycogen synthase kinase-3. *J. Pharmacol. Exp. Ther.* 305, 974–980
- 53 Coghlan, M.P. *et al.* (2000) Selective small molecule inhibitors of glycogen synthase kinase-3 modulate glycogen metabolism and gene transcription. *Chem. Biol.* 7, 793–803
- 54 Ring, D.B. *et al.* (2003) Selective glycogen synthase kinase 3 inhibitors potentiate insulin activation of glucose transport and utilization *in vitro* and *in vivo*. *Diabetes* 52, 588–595
- 55 Nikoulina, S.E. *et al.* (2002) Inhibition of glycogen synthase kinase 3 improves insulin action and glucose metabolism in human skeletal muscle. *Diabetes* 51, 2190–2198
- 56 MacAulay, K. *et al.* (2003) Use of lithium and SB-415286 to explore the role of glycogen synthase kinase-3 in the regulation of glucose transport and glycogen synthase. *Eur. J. Biochem.* 270, 3829–3838
- 57 Henriksen, E.J. *et al.* (2003) Modulation of insulin resistance by selective inhibition of GSK-3 in Zucker diabetic fatty rats. *Am. J. Physiol. Endocrinol. Metab.* 284, E892–E900
- 58 Cline, G.W. *et al.* (2002) Effects of a novel glycogen synthase kinase 3 inhibitor on insulin-stimulated glucose metabolism in Zucker diabetic fatty (fa/fa) rats. *Diabetes* 51, 2903–2910
- 59 Ubeda, M. *et al.* (2004) Glucose-induced expression of the CDK5 activator p35 involved in Alzheimer's disease regulates insulin gene transcription in pancreatic β-cells. *Endocrinology* 145, 3023–3031
- 60 Lustig, B. and Behrens, J. (2003) The Wnt signalling pathway and its role in tumor development. *J. Cancer Res. Clin. Oncol.* 129, 199–221
- 61 Cohen, Y. *et al.* (1998) Cancer morbidity in psychiatric patients: influence of lithium carbonate treatment. *Med. Oncol.* 15, 32–36
- 62 Gould, T.D. *et al.* (2003) Effects of glycogen synthase kinase-3 inhibitor, lithium, in adenomatous polyposis coli mutant mice. *Pharmacol. Res.* 48, 49–53
- 63 Liao, X. *et al.* (2003) Glycogen synthase kinase-3β suppression eliminates tumor necrosis factor-related apoptosis-inducing ligand resistance in prostate cancer. *Mol. Cancer Ther.* 2, 1215–1222
- 64 Wakefield, J.G. *et al.* (2003) A role for glycogen synthase kinase-3 in mitotic spindle dynamics and chromosome alignment. *J. Cell Sci.* 116, 637–646
- 65 Ding, S. *et al.* (2003) Synthetic small molecules that control stem cell fate. *Proc. Natl. Acad. Sci. U. S. A.* 100, 7632–7637
- 66 Sato, N. *et al.* (2004) Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3 specific inhibitor. *Nat. Med.* 10, 55–63
- 67 Droucheau, E. *et al.* (2004) *Plasmodium falciparum* glycogen synthase kinase-3, molecular model, expression, intracellular localisation and selective inhibitors. *Biochim. Biophys. Acta* 1697, 181–196
- 68 Meijer, L. *et al.* (2000) Inhibition of cyclin-dependent kinases, GSK-3β and casein kinase 1 by hymenialdisine, a marine sponge constituent. *Chem. Biol.* 7, 51–63
- 69 Kunick, C. *et al.* (2004) 1-Azakenpaullone is a selective inhibitor of glycogen synthase kinase-3β. *Bioorg. Med. Chem. Lett.* 14, 413–416
- 70 Mettey, Y. *et al.* (2003) Aloisines, a new family of CDK/GSK-3 inhibitors. SAR study, crystal structure in complex with CDK2, enzyme selectivity, and cellular effects. *J. Med. Chem.* 46, 222–236
- 71 Naerum, L. *et al.* (2002) Scaffold hopping and optimization towards libraries of glycogen synthase kinase-3 inhibitors. *Bioorg. Med. Chem. Lett.* 12, 1525–1528
- 72 Witherington, J. *et al.* (2003) 5-Aryl-pyrazolo[3,4-b]pyridines: potent inhibitors of glycogen synthase kinase-3 (GSK-3). *Bioorg. Med. Chem. Lett.* 13, 1577–1580

- 73 Witherington, J. *et al.* (2003) 6-Aryl-pyrazolo[3,4-b]pyridines: potent inhibitors of glycogen synthase kinase-3 (GSK-3). *Bioorg. Med. Chem. Lett.* 13, 3055–3057
- 74 Witherington, J. *et al.* (2003) 6-Heteroaryl-pyrazolo[3,4-b]pyridines: potent and selective inhibitors of glycogen synthase kinase-3 (GSK-3). *Bioorg. Med. Chem. Lett.* 13, 3059–3062
- 75 Ortega, M.A. *et al.* (2002) Pyrazolo[3,4-b]quinoxalines. a new class of cyclin-dependent kinases inhibitors. *Bioorg. Med. Chem.* 10, 2177–2184
- 76 Lane, M.E. *et al.* (2001) A novel cdk2-selective inhibitor, SU9516, induces apoptosis in colon carcinoma cells. *Cancer Res.* 61, 6170–6177
- 77 Zhang, H.C. *et al.* (2003) Macrocytic bisindolymaleimides as inhibitors of protein kinase C and glycogen synthase kinase-3. *Bioorg. Med. Chem. Lett.* 13, 3049–3053
- 78 Kuo, G-H. *et al.* (2003) Synthesis and discovery of macrocyclic polyoxygenated bis-7-azaindolymaleimides as a novel series of potent and highly selective glycogen synthase kinase-3 β inhibitors. *J. Med. Chem.* 46, 4021–4031
- 79 Shen, L. *et al.* (2004) Synthesis and biological evaluation of novel macrocyclic bis-7-azaindolymaleimides as potent and highly selective glycogen synthase kinase-3 beta (GSK-3 beta) inhibitors. *Bioorg. Med. Chem.* 12, 1239–1255
- 80 Hers, I. *et al.* (1999) The protein kinase C inhibitors bisindolymaleimide I (GF 109203X) and IX (Ro 31-8220) are potent inhibitors of glycogen kinase-3 activity. *FEBS Lett.* 460, 433–436
- 81 Smith, D.G. *et al.* (2001) 3-Anilino-4-arylmaleimides: potent and selective inhibitors of glycogen synthase kinase-3 (GSK-3). *Bioorg. Med. Chem. Lett.* 11, 635–639
- 82 Ruetz Ruetz, S. *et al.* (2003) Chemical and biological profile of dual cdk1 and cdk2 Inhibitors. *Curr. Med. Chem. Anti-Canc. Agents* 3, 1–14
- 83 Olesen, P.H. *et al.* (2003) Synthesis and *in vitro* characterization of 1-(4-aminofurazan-3-yl)-5-dialkylaminomethyl-1H-[1,2,3]triazole-4-carboxylic acid derivatives. A new class of selective GSK-3 inhibitors. *J. Med. Chem.* 46, 3333–3341
- 84 Peat, A.J. *et al.* (2004) Novel GSK-3 inhibitors with improved cellular activity. *Bioorg. Med. Chem. Lett.* 14, 2127–2130
- 85 Ilouz, R. *et al.* (2002) Inhibition of glycogen synthase kinase-3 β by bivalent zinc ions: insight into the insulin-mimetic action of zinc. *Biochim. Biophys. Res. Commun.* 295, 102–106
- 86 Yost, C. *et al.* (1998) GBP, an inhibitor of GSK-3, is implicated in *Xenopus* development and oncogenesis. *Cell* 93, 1031–1041
- 87 Culbert, A.A. *et al.* (2001) GSK-3 inhibition by adenoviral FRAT1 overexpression is neuroprotective and induces Tau dephosphorylation and β -catenin stabilisation without elevation of glycogen synthase activity. *FEBS Lett.* 507, 288–294
- 88 Thomas, G.M. *et al.* (1999) A GSK3-binding peptide from FRAT1 selectively inhibits the GSK3-catalysed phosphorylation of axin and beta-catenin. *FEBS Lett.* 458, 247–251
- 89 Martin, C.P. *et al.* (2002) P24, a glycogen synthase kinase (GSK 3) inhibitor. *Biochim. Biophys. Acta* 1586, 113–122
- 90 Yu, J-Y. *et al.* (2003) Simultaneous inhibition of GSK3 α and GSK3 β using hairpin siRNA expression vectors. *Mol. Ther.* 7, 228–236
- 91 Zhang, F. *et al.* (2003) Inhibitory phosphorylation of glycogen synthase kinase-3 (GSK-3) in response to lithium: evidence for autoregulation of GSK-3. *J. Biol. Chem.* 278, 33067–33077

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