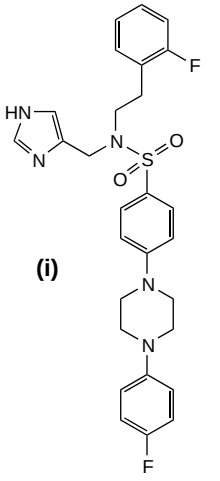
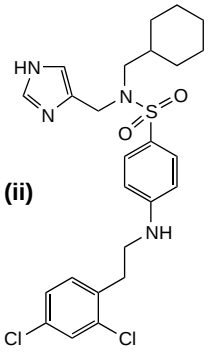
Combinatorial chemistry

Novel antifungals

Current available therapy in treating fungal infections can suffer from drug-related toxicity, hazardous drug–drug interactions, non-optimal pharmacokinetics, and development of drug resistance. Enzymes in the ergosterol-biosynthesis pathway, specifically the lanosterol 14-α demethylase, are the targets for successful marketed antifungal drugs. 1*H*-imidazole 4-methaneamine sulphonamides are a class of imidazole-based antifungal agents which inhibit fungal ergosterol synthesis in much the same manner as triazole-containing antifungal drugs. Efforts have been made to try to understand how imidazole-based antifungal agents interact at the target enzyme via the demethylase active site. An understanding of such interaction should help in the design of more potent ergostreol synthesis inhibitors[1]. A library of 29 individual compounds, each purified by HPLC, was synthesized on 2-chlorotrityl chloride polystyrene solid-phase resin. Screening of these compounds against eight isolates of *Candida* spp., revealed several active compounds. One of the most potent compounds discovered was **(i)**, which possessed an IC_{50} value of 3 nM against ergosterol, and 163-fold selectivity for inhibition of yeast sterol versus mammalian cholesterol synthesis. This analogue was inactive against mould strains, making it uniquely yeast active. A second active compound **(ii)** demonstrated activity against the azole resistant strain *Candida albicans* 1, and possessed an IC_{50} of 15 nM against ergosterol. This library has been successful by the continued optimization of 4-substituted imidazole antifungals by high-speed synthesis methods, leading to highly yeast-selective as well as potent broad spectrum antifungal agents. These molecules and their study may provide stimulus for further research towards the discovery of clinically successful new antifungal drugs.





1 Saha, A.K. *et al.* (2000) Novel antifungals based on 4-substituted imidazole: solid-phase synthesis of substituted aryl sulfonamides towards optimization of *in vitro* activity. *Bioorg. Med. Chem. Lett.* 10, 2135–2139

Cholesterol-ester transfer-protein-mRNA ligands

Several RNA oligonucleotide–ligand interactions have been characterized and described. A 17-amino acid peptide containing the arginine-rich region of the HIV Rev protein binds to Rev response-element RNA. Also, basic peptides from the carboxy terminus of the HIV type 1 (HIV-1) Tat protein bind to the stem-loop region of transactivation response region (TAR) RNA and inhibit HIV-1 replication *in vivo*. Tripeptides that bind to the TAR RNA and inhibit gene expression could be isolated from randomized pools of combinatorial libraries[2].

A library of 625 compounds was synthesized in mixtures of 25 on an M-Mal–PEG solid-phase resin. Small-peptide ligands were found that bind to a 23-nt RNA oligonucleotide from the cholesterol-ester transfer-protein mRNA. A 27-nt RNA oligonucleotide from the HIV type-1 TAR RNA was used to control the binding specificity. Gel-shift affinity screening was used to extract the peptides with the best RNA-binding properties. Following deconvolution of the library, the peptide showing the most visible affinity for the 23-nt RNA was **(iii)**, which gave a binding constant K_d of 32 μM. This work has demonstrated that it is possible to obtain peptide ligands for different RNA targets using a gel-shift assay. Also, elucidation of the minimum pharmacophoric elements requirements for binding has been achieved, namely, basic and hydrophobic residues. This work lays the foundation for the design of more potent inhibitors in the future.

Lys-Tyr-Lys-Leu-Tyr-Lys-Cys-NH_2

(iii)

2 Griesinger, C. *et al.* (2001) Combinatorial synthesis of cholesterol-ester transfer-protein mRNA ligands and screening by non-denaturating gel-electrophoresis. *J. Med. Chem.* 44, 2172–2177

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Profile

GSK-3 inhibitors: potential drugs for neurodegenerative disorders

Protein kinases, the enzymes that phosphorylate protein substrates, are key players in the signalling of extracellular events to the cytoplasm and the nucleus, and take part in practically any event relating to the life and death of cells, including mitosis, differentiation and apoptosis. As such, protein kinases have long been favourable drug targets.

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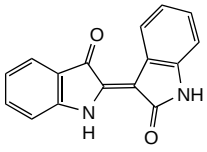
However, they have presented a problem in that the inhibition of many kinases could lead to cell death, because their activity is crucial to the well-being of the cell. Although this is a desirable effect for anticancer drugs, it is a major drawback for most other therapeutics. Now, a relatively less familiar member of the family of protein kinases, glycogen synthase kinase-3 (GSK-3), could become a genuine drug target among the protein kinases[1].

GSK-3 is a cytoplasmic serine–threonine kinase that is involved in insulin signalling and metabolic regulation, as well as in Wnt signalling and the scheme of cell fate during embryonic development. Two similar isoforms of the enzyme, termed GSK-3α and GSK-3β, have been identified. The assertion that GSK-3 is a favourable drug target among the protein kinase family is based on the fact that unlike other protein kinases, which are typically activated by signalling pathways, GSK-3 is normally activated in resting cells, and its activity is attenuated by the activation of certain signalling pathways such as after the binding of insulin to its cell-surface receptor. Activation of the insulin receptor leads to the activation of protein kinase B (PKB, also called Akt), which in turn phosphorylates GSK-3, thereby inactivating it. The inhibition of GSK-3 presumably leads to the activation of glycogen synthesis. The intricate insulin-signalling pathway is further complicated by negative-feedback regulation of insulin signalling by GSK-3 itself, which phosphorylates insulin-receptor substrate-1 on serine residues[2].

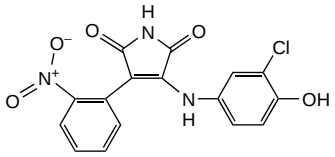
Therefore, synthetic GSK-3 inhibitors might mimic the action of certain hormones and growth factors, such as insulin, which use the GSK-3 pathway. In certain pathological situations, this scheme might permit the bypassing of a defective receptor, or another faulty component of the signalling machinery, so that the biological signal will take effect even when some upstream players of the signalling cascade are at fault, such as in non-insulin-independent type 2 diabetes. Other diseases for which GSK-3 might become a promising drug target are neurodegenerative disorders such as Alzheimer's disease, as well as affective disorders. This notion is based on the observation that lithium ions, commonly used for treating affective disorders, inhibit GSK-3 at the therapeutic concentration range employed in the clinic[3]. However, lithium ions are non-selective inhibitors of GSK-3, and chronic lithium therapy might cause renal toxicity.

The design of specific GSK-3 inhibitors will become easier following the recent publication of the crystal structure of GSK-3β (Refs 4,5). Meanwhile, several synthetic GSK-3 inhibitors have been published, and these could potentially become lead compounds in the design of yet more selective drugs. An interesting example is indirubin **(i)**, an active ingredient of *Danggui Longhui Wan*, the traditional Chinese medicine recipe used against chronic myelocytic leukaemia. The cell-permeable analogue of this drug, indirubin-3'-monoxime, was recently shown to arrest the growth of HBL-100 myeloma cells[6]. However, indirubin is not a selective GSK-3 inhibitor, and also inhibits CDK-2 (Ref. 7).

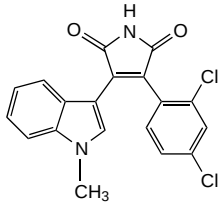


(i)

Another innovative example of small-molecule inhibitors of GSK-3 are the two structurally distinct maleimides, SB415286 **(ii)** and SB216763 **(iii)**, patented by GlaxoSmithKline (Harlow, UK)[8]. These compounds, which demonstrated *in vitro* inhibition of GSK-3 with K_i values of 31 nM and 9 nM, respectively, protected cultured neurons from apoptotic death. Other small-molecule GSK-3 inhibitors were recently patented by Chiron (Emeryville CA, USA), and these were claimed to be plausible drugs for the treatment of type 2 diabetes and Alzheimer's disease[9].



(ii)



(iii)

Notably, insulin itself was shown to rescue neurons from apoptotic cell death by a mechanism attenuating caspase-3 activation[10], and thus it is plausible that insulin-mediated attenuation of GSK-3 activity is implicated in this rescue. Indeed, overexpression of GSK-3 potentiated heat-shock-induced activation of caspase-3 in cultured neuroblastoma cells, an effect blocked by lithium ions[11], thus implying that GSK-3 inhibition could attenuate caspase-3-mediated apoptosis. In addition, conditional transgenic mice[12] overexpressing GSK-3 in their brains during adulthood exhibit increased brain expression of caspase-3 and associated neuronal degeneration[12]. Together, such observations imply that selective GSK-3 inhibitors could potentially become valuable drugs for treating neurodegenerative disorders and possibly also cerebral stroke, in which massive neuronal cell death follows the initial ischaemic insult.

1 Weston, C.R. and Davis, R.J. (2001) Signal transduction: signaling specificity – a complex affair. *Science* 292, 2439–2440

2 Eldar-Finkelman, H. and Krebs, E.G. (1997) Phosphorylation of insulin receptor substrate 1 by glycogen synthase kinase 3 impairs insulin action. *Proc. Natl. Acad. Sci. U. S. A.* 94, 9660–9664

3 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

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4 Dajani, R. *et al.* (2001) Crystal structure of glycogen synthase kinase 3β: structural basis for phosphate-primed substrate specificity and autoinhibition. *Cell* 105, 721–732
5 ter Haar, E. *et al.* (2001) Structure of GSK3β reveals a primed phosphorylation mechanism. *Nat. Struct. Biol.* 8, 593–596
6 Damiens, E. *et al.* (2001) Anti-mitotic properties of indirubin-3'-monoxime, a CDK/GSK-3 inhibitor: induction of endoreplication following prophase arrest. *Oncogene* 20, 3786–3797
7 Hoessel, R. *et al.* (1999) Indirubin, the active constituent of a Chinese antileukaemia medicine, inhibits cyclin-dependent kinases. *Nat. Cell Biol.* 1, 60–67
8 Cross, D.A. *et al.* (2001) Selective small-molecule inhibitors of glycogen synthase kinase-3 activity protect primary neurones from death. *J. Neurochem.* 77, 94–102
9 Schultz, P. *et al.* (2000) Inhibitors of glycogen synthase-3 kinase. US6,153,618
10 Barber, A.J. *et al.* (2001) Insulin rescues retinal neurons from apoptosis by a PI-3 kinase/Akt-mediated mechanism that reduces the activation of caspase-3. *J. Biol. Chem.* 276, 32814–32821
11 Bijur, G.N. *et al.* (2000) Glycogen synthase kinase-3β facilitates staurosporine- and heat-shock-induced apoptosis. Protection by lithium. *J. Biol. Chem.* 275, 7583–7590
12 Lucas, J.J. *et al.* (2001) Decreased nuclear β-catenin, tau hyperphosphorylation and neurodegeneration in GSK-3β conditional transgenic mice. *EMBO J.* 20, 27–39

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