implications for structural and molecular biology far beyond simple recognition events.

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CDK versus GSK-3 Inhibition: a Purple Haze No Longer?

The ubiquitous ATP binding site offers a global target for protein kinase inhibitors. The corollary is that molecular selectivity with such agents may be difficult to achieve and ascertain. A relevant example is discussed in terms of design and biomedical rationale.

Over the last two decades, the structural biology of protein kinases as well as the design of their inhibitors has become of great interest to both academic and pharmaceutical scientists. Because the ATP binding site in kinases is inherently amenable to blocking with drug-like small molecules, the discovery and development of kinase inhibitors is now being pursued for every imaginable therapeutic indication [1]. Furthermore, since the protein kinases, of which some 500 have been identified in the human genome [2], are the key players in the regulation of all biochemical pathways, they appear to represent excellent targets for a new generation of mechanism-based drugs. However, unpredictable pharmacology and, ultimately, unforeseen patient side effects might be expected with therapeutic agents targeting any conserved recognition site, i.e. the ATP binding pocket, which is highly conserved not only in kinases but also in many other mononucleotide binding proteins. The challenge with kinase inhibitors is thus how to achieve selectivity. A paper in this issue of *Chemistry & Biology* by Meijer et al. addresses this very point [3].

The authors take us on an historical excursion regarding the discovery of indirubins (Figure 1, **1**). These compounds confer the characteristic purple color to natural indigo dyes. According to legend, purple dye was first discovered by Herakles, when he observed that his dog's mouth was stained purple after chewing on snails along the Levantine coast. King Phoenix of Tyre is believed to have received a purple-dyed robe from Herakles and decreed that the rulers of Phoenicia should wear this color as a royal symbol, hence the name Tyrian purple. Pharmacological properties of indirubins have also long been known, and indirubin (Figure 1, **1a**) is a constituent of a traditional Chinese cancer medicine [8]. Their antiproliferative properties may in part be due to the fact that indirubins inhibit the phylogenetically and structurally closely related cyclin-dependent kinases (CDK) and the glycogen synthase kinases-3 (GSK-3). In fact, many compounds discovered as CDK inhibitors are known to block GSK-3 function as well [9].

Meijer et al. present crystal structures of indirubin-3′-oxime (Figure 1, **1b**) and its 6-bromo derivative Figure 1, **1c**) in complex with CDK5 and GSK-3β, respectively [3]. The latter compound is >16-fold selective for GSK-3 compared to CDKs 2 and 5 (which are structurally extremely similar [10]). The des-bromo compound (Figure 1, **1b**), on the other hand, is only about 5-fold selective for GSK-3, and the 5-sulfonate analog (Figure 1, **1d**) is about 4-fold selective for CDKs [11]. The structures presented show that the selectivity gain associated with the bromo substituent on C6 of the indirubin scaffold correlates with one of the main structural differences between GSK-3 and CDK2/5, viz. the so-called gatekeeper residue often exploited for the design of kinase selectivity in ligands [12]. In nine of the ten known CDK isoforms, this residue is Phe (F80 in CDK2), whereas Leu (L132) is found in GSK-3. The authors argue that because of the smaller aliphatic side chain in that position, GSK-3 is better able to accommodate the 6-bromo group and that indirubin C5/6 substitution studies may give rise to future analogs with increased selectivity [3].

In fact, another very recently published study with the thiazole-methoxybenzyl-thiourea compound **2** (Figure 1), which is truly selective for GSK-3 versus CDKs [13], confirms this hypothesis. In that case, it was found that the thiazole nitro substituent occupies the subsite adjacent to L132 in GSK-3, which is not accessible in CDKs because of the larger aromatic Phe side chain. Additionally, the anisole system in compound **2** was observed to bind at the entrance to the ATP binding site in GSK-3 in the vicinity of R141, which is salt bridged with E137. In CDK2 and probably also in CDK5, a corresponding

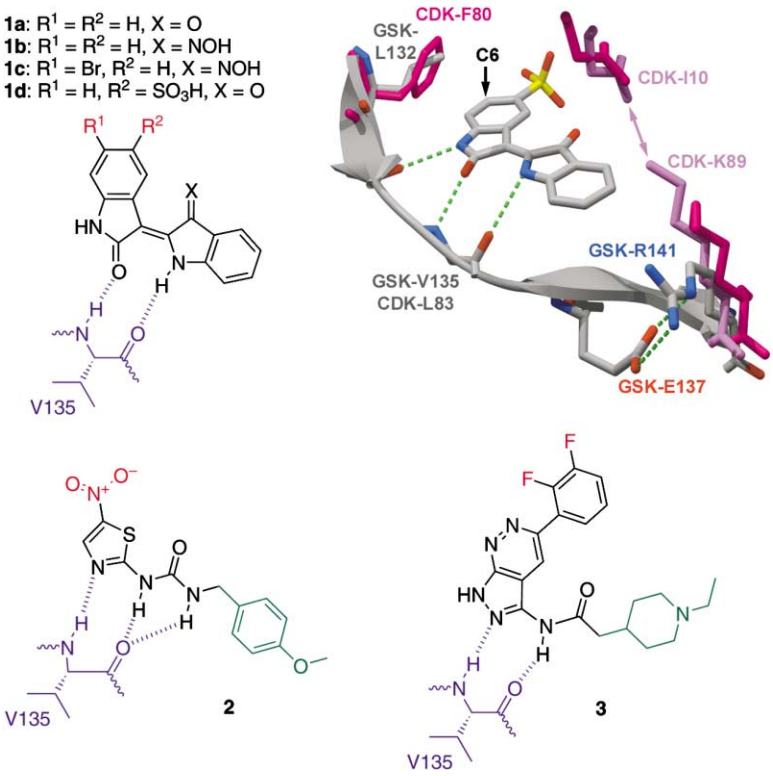

Figure 1. Structures and Kinase Binding Modes of CDK/GSK-3 Inhibitors

The positions of the conserved H-bond pair with the kinase hinge region, i.e., V135 (in GSK-3), are indicated. The 3D structure shows the binding mode of **1d** constructed from alignments between Protein Data Bank ID codes 1E9H (complex of **1d** with CDK2 [4]; purple side chains), 1I09 (GSK-3β [5]), 1QMZ (activated CDK2-cyclin A complex [6]; pink side chains), and 1Q41 (complex of **1b** with GSK-3β [7], not shown).

site is not present because the residue equivalent to R141, i.e., K89, points into the ATP pocket and in fact interacts with I10 in the Gly-rich loop of the N-terminal kinase lobe in the activated CDK2/cyclin A complex. Analysis of the SARs and structural studies with 5-aryl-pyrazolo[3,4-*b*]pyridazines provide a similar picture [14]. Here, the positions of the fluorine atoms in e.g., the highly GSK-3-selective analog compound **3** (Figure 1) were observed in the vicinity of the GSK-3 L132 side chain and are associated with both potency and selectivity for GSK-3. Again, an additional determinant for selectivity was seen; this concerns the acyl portion of the amide, i.e., CH_2-piperidine-ethyl in compound **3**. Presumably, this group occupies a similar subsite as the anisole portion in **2**. In Figure 2, which gives an overall comparison of the shape of the ATP binding sites in GSK-3 and CDK2, the major difference in the entrance to the ATP binding pocket is evident.

Yet another recent structural study using inhibitors of varying selectivity also concludes that there are numerous subsites of the GSK-3 ATP binding pocket that can be exploited for the design of selective inhibitors [7]. The fact that methylation or acetylation of the oxime function in compound **1c** further increased GSK-3 selectivity [3] is in line with this finding.

CDK-inhibitory drugs are sought predominantly as an-

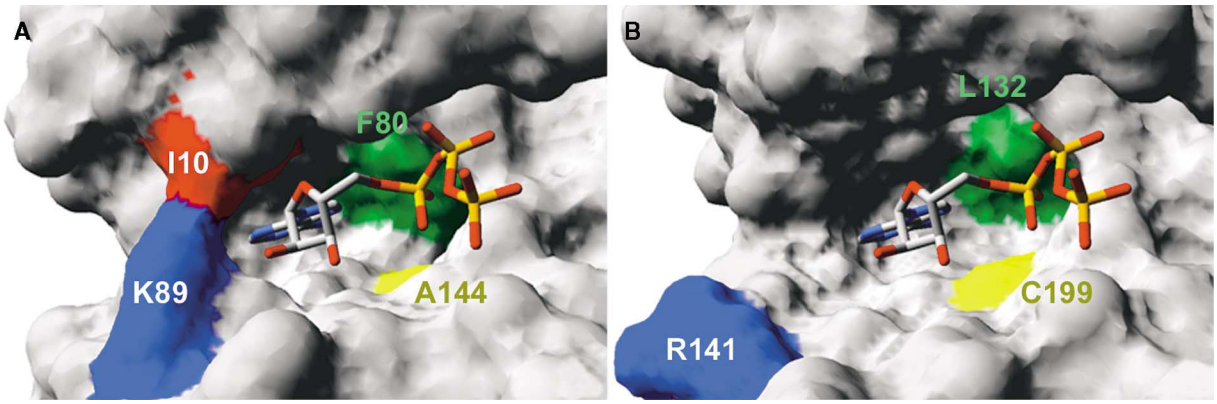

Figure 2. ATP Binding Sites in CDK2 and GSK-3

A view into the ATP binding pockets of CDK2 (A) and GSK-3β (B) constructed from alignments between Protein Data Bank ID codes 1QMZ and 1I09.

ticancer agents and for therapeutic use in other proliferative diseases [15] because of the central roles certain CDKs (especially isoforms 1, 2, 4, and 6) play in cell cycle regulation, which is frequently overridden in transformed cells. Other CDKs (at least isoforms 7, 8, and 9) are implicated in regulation of transcription at the level of RNA elongation, where they phosphorylate the C-terminal domain of RNA polymerase-2. Many viruses depend on host cell CDKs, often by recruiting these through virus-encoded cyclins, for their replication, and this has now led to a biomedical rationale for the application of CDK inhibitors as antiviral agents [16]. Many parasitic microorganisms also possess CDKs or CDK-like proteins; their selective inhibition may give rise to drugs against some of the most widespread human diseases, including malaria [17]. Of particular interest in connection with the Meijer et al. paper is CDK5. This kinase is highly expressed in the nervous system, where it acts on many different substrates. Of these, the microtubule binding protein tau, the β-amyloid peptides, and the neurofilament protein NF-H are of special interest because their hyperphosphorylated forms are associated with various neurodegenerative disorders, including Alzheimer's disease. Activity on such substrates appears to be due to both CDK5 and GSK-3. In fact, there exists a general association of CDK and GSK-3 activities with neuronal cell death. For application in neurodegenerative diseases, one might therefore expect neuroprotective effects with dual CDK/GSK-3 inhibitors [9].

GSK-3, of which humans have two nonredundant isoforms, α and β, that are practically identical in the kinase domain, has many other functions, notably those arising from its role in the Wnt signaling pathway. Meijer et al. use biological readouts from this pathway to demonstrate that 6-bromoindirubins do in fact behave as GSK-3 inhibitors in vitro and in vivo [3]. An additional important physiological function of GSK-3 is in the regulation of glucose to glycogen conversion. It has been demonstrated that GSK-3 inhibitors may be useful as drugs in the treatment of type II diabetes [18]. For this and other therapeutic applications of GSK-3 inhibitors, it will be important to develop selective compounds that do not possess the antiproliferative properties of CDK inhibitors.

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RNA as Multitude/RNA as One

In the configurations formed by RNA and its ions there are structural possibilities not yet realized; some are hinted at in new work on the binding of an amino acid analog.

RNA surely means different things to different biologists, but molecules are usually thought of as unitary objects, as explicit as a mountain or a building. We have been trained to think of structures in this way from the motionless, indispensable polychromes that illustrate the conclusions of structural biologists' experiments. However, the huge energetic penalty rendered for unpaired charges implies that RNA molecules are always electrically neutral. Therefore, RNAs are inevitably accompanied by ions, about one ionic charge per nucleotide phosphate. So, for many purposes, RNA is better illustrated as a fluctuating crowd of particles. Magnesium is more influential than potassium because the electrical neutrality generated by localizing a single magnesium ion frees two potassium ions, yielding an entropic ad-