Kinase Inhibition that Hinges on Halogen Bonds

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A major challenge for the discovery of protein kinase inhibitors is to identify potent, selective, and novel pharmacophores. In this issue, Fedorov et al. (2011) describes KH-CB19, an ATP-competitive inhibitor of cdc2-like kinase that interacts with the ATP hinge region through a halogen-bonding motif.

The cdc2-like kinase (CLK) family belongs to the CMGC class of protein kinases that also includes the cyclin-dependent kinases. CLK isoforms can phosphorylate the serine- and arginine-rich (SR) proteins that regulate the alternative pre-mRNA splicing process. Alternative splicing of pre-mRNA contributes to the diversity and complexity of proteins expressed within cells. A dysfunction in this splicing process can lead to the expression of mis-spliced or anomalous proteins and may contribute to the development of various diseases (Hagiwara, 2005), including disorders of the nervous system (Morikawa and Manabe, 2010). Inhibitors of CLK activity have been useful for elucidating the role of CLK in cellular signaling pathways and establishing that CLK can control the phosphorylation of SR proteins in mammalian cells (Nayler et al., 1998), and thereby modulate the in vivo expression of alternative splice variant proteins.

A number of diverse chemical classes of CLK inhibitors have been reported. The adenosine mimetic 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) is an inhibitor of CLK2, casein kinase II, and other kinases such as CDK9, a subunit of P-TEFb (Nayler et al., 1998, Baumli et al., 2010). DRB is an inhibitor of RNA synthesis and functions as a transcription blocker with activity against many gene promotors. One of the first potent and selective CLK inhibitors to be reported, TG003, was developed by the Hagiwara laboratory in Tokyo (Muraki et al., 2004). TG003 was identified from a series of benzothiazole derivatives with inhibitory activity against several CLK isoforms and selective toward CLKs when evaluated in a panel of biochemical assays for a small set of protein kinases. TG003 was able to inhibit CLK-mediated phosphorylation of SR proteins in cells and blocked the in vivo expression of proteins from a spliced variant in a mouse model (Muraki et al., 2004). Another inhibitor series of 6-arylquinazolin-4-amines was recently described by scientists at the NIH, with high selectivity toward the CLK1-4 isoforms as well as a related CMGC kinase, Dyrk1A (Mott et al., 2009). In this issue, Fedorov et al. (2011) describes the discovery of dichloroindolyl enaminonitrile derivatives as another chemical class of CLK inhibitors. One of these compounds, KH-CB19, was found to be a potent and selective inhibitor of CLK1 and CLK4 based on thermal shift analysis. This compound blocked the TNF-α stimulated phosphorylation of SR proteins and reduced the mRNA expression of tissue factor splice variants in endothelial cells, similar to TG003. Taken together, these reports demonstrate that the discovery and development of CLK inhibitors is still at an early, preclinical stage. What is noteworthy about this newest class of CLK inhibitors reported by Fedorov et al. (2011) is that they have achieved inhibitor potency and selectivity using a rather uncommon halogen-bonding interaction.

The ATP binding site in protein kinases is located between the small N-terminal lobe of the catalytic domain and the larger C-terminal segment. A hinge region connecting the two protein lobes forms key hydrogen bonds to bound ATP through backbone NH and CO. A second hinge CO, not satisfied by ATP, is also available as an acceptor site. The binding of the vast majority of kinase inhibitors occurs at the ATP site, where hydrogen bonds are made with at least one of these polar elements (Figure 1A). While this

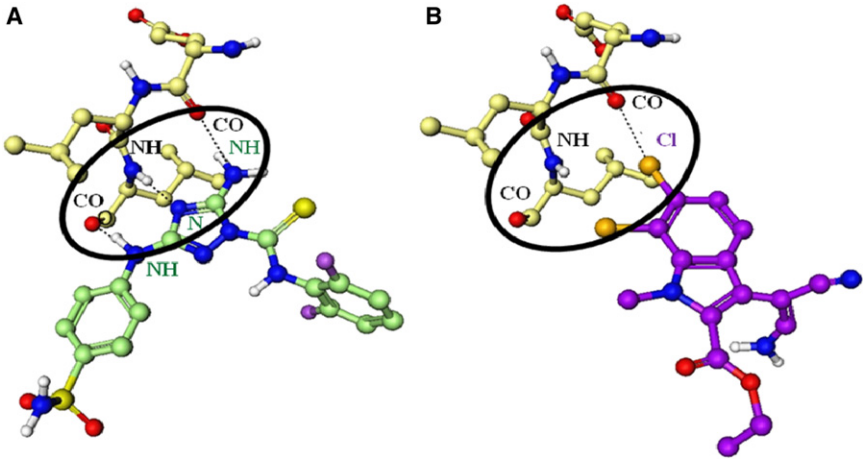

Figure 1. Comparison of Inhibitors K0546 and KH-CB19 Bound to CLK3
(A) X-ray structure of the triazole diamine K0546 (green) bound to CLK3 (yellow) shows an example of canonical interactions with the hinge (hashed black lines), in this case a donor-acceptor-donor motif.
(B) X-ray complex of KH-CB19 (magenta) bound to CLK3 (yellow) reveals the halogen interaction with the hinge CO (dashed black line).

type of ATP-competitive inhibitor can achieve potent affinity levels for a target kinase, maintaining selectivity against the other protein kinase family members remains a major challenge (Grant, 2009). Fedorov et al. (2011) have shown that KH-CB19, an inhibitor with a noncanonical halogen interaction with the hinge backbone CO, can attain potent and selective affinity for members of the CLK family (Figure 1B). Contributing to the affinity is an inward binding of Phe172 in the P loop, which partly defines the pocket. Other pharmacophores with a halogen interaction to the kinase hinge region have been reported by other groups (De Moliner et al., 2003), including DRB, which binds to CDK9 through two chlorines. (Baumli et al., 2010).

It remains to be seen whether or not the replacement of the standard kinase inhibitor core with a novel halogenated ring system is a strategy that can be applied more broadly toward the elaboration of potent and selective kinase ligands. Yet, the results reported here by Fedorov et al. (2011) enhance the attractiveness of such an approach and highlight a potential emerging area of chemical space for the design of kinase inhibitors.

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A Jump-Start for Planarian Head Regeneration

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Planaria are simple flatworms with an extraordinary ability to regenerate missing body parts. This makes them a unique model system for the study of regeneration. Extending an earlier chemical screen, Beane et al. (2011) now reveal a role for H^+/K^+ ATPase and membrane depolarization in anterior regeneration in planaria.

Biologists have known for decades that vital events in early embryonic development are accompanied by changes in ion flow, leading to biophysical signaling events such as membrane depolarization, spatial patterns of membrane current, or alterations in gap junctional communication. Tissues undergoing wound healing or regeneration show similar biophysical signals, which arise from changes in the activity of ion channels or transporters. Understanding how these biophysical signals promote the establishment of embryonic pattern, the specification of cell fate, or wound healing however, has been a major challenge. The chief difficulty has been in defining a functional link between altered ion channel or transporter activity and the changes in gene regulation that underlie developmental processes. Now Beane et al. (2011) have demonstrated that H^+/K^+ ATPase activity is essential for planarian head regeneration and identified a mechanism by which H^+/K^+ ATPase activity could activate expression of genes associated with head formation.

Planaria, free-living arrow-headed flatworms, are simple invertebrates with a surprisingly complex nervous system and an unparalleled capacity for regeneration. Amputation of either the head or the tail region leads to regrowth of the lost structures within several days. As with regeneration of the limbs or tail in lower vertebrates, regeneration proceeds by formation of an undifferentiated cell mass referred to as a blastema, which then undergoes coordinated differentiation to reconstitute the missing structures. While a vertebrate blastema forms via dedifferentiation and proliferation of the cells near the amputation site, the planarian blastema arises from the migration and proliferation of neoblasts, a highly pluripotent stem cell population that constitutes 30% of the cells of the adult flatworm. Blastema formation is followed by the establishment of regional identity along the anteroposterior axis. During anterior regeneration, the emergence of the brain rudiment, neuronal differentiation, and the establishment of neuronal connectivity rapidly follow, and specific behavioral responses are reestablished within five days after amputation. Planarian regeneration has emerged

