MMP9 that has been observed in cerebral ischaemia and reperfusion.

The authors say that this NO-activated MMP mechanism "confers responsiveness of the extracellular matrix to nitrosative and oxidative stress", which are found in several conditions, including cerebral ischaemia and neurodegenerative diseases. The extracellular proteolytic cascades that are triggered by MMPs can disrupt the extracellular matrix, contribute to cell detachment and lead to anoikis (apoptosis due to cell detachment from the substrate). So, the authors conclude that preventing NO-activated MMP activity could be a novel way of tackling neurodegenerative diseases.

Simon Frantz

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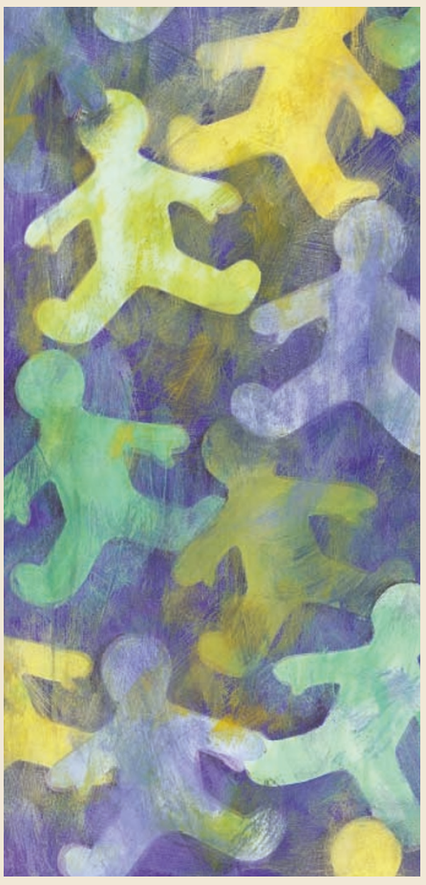
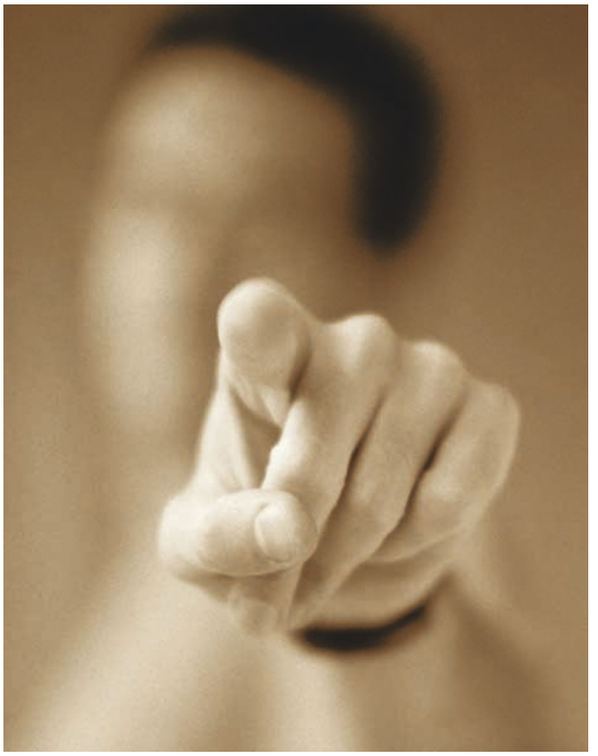


STRUCTURE-BASED DRUG DESIGN

Reiterating the point



The cyclin-dependent kinases (CDKs) have an important role in controlling the cell cycle. Aberrant CDK activity is a common defect in human tumours, which makes CDKs important targets for therapeutic intervention in cancer, and CDK inhibitors are in clinical trials at present. In the October issue of *Nature Structural Biology*, Davies *et al.* show that structure-based development and iterative biological evaluation can be used to optimize a CDK inhibitor rapidly, resulting in nanomolar potency that is 1,000-fold greater than the parent compound.

Knowledge of the structure of CDK2 has been key in driving the design and development of a large number of ATP competitive inhibitors. The ATP-binding site of CDK2 is located between the two domains of the kinase, and is best described as a hydrophobic 'slot'. Contained within this cleft are various sub-sites, which could be probed by inhibitors, some of which are not explored by ATP itself. The novel ATP-competitive, purine-based inhibitor O^6-cyclohexylmethyl guanine (NU2058) inhibits CDK1 and CDK2, but has no activity against CDK4. To optimize NU2058, the structure of the activated CDK2–cyclin A complex bound to NU2058 was determined. NU2058 forms a triplet of hydrogen bonds between its purine ring and the active site of CDK2. The purine ring also makes several van der Waals interactions and hydrophobic contacts with the ATP-binding cleft of CDK2. The O^6 group sits in the ATP-ribose binding site and forms highly complementary packing and hydrophobic interactions with an apolar pocket in a glycine-rich loop of CDK2.

Structure–activity relationships for the O^6 position show that a cyclic hydrophobic group, such as cyclohexylmethyl, is optimal, so this group was maintained. The binding of CDK inhibitors to CDK2, compared with the natural product indirubin, indicated that the addition of functional groups at the 2-amino (N2) position of NU2058 would increase potency. Groups added at this position would project out of the ATP-binding cleft and contact the 'specificity surface' of CDK2. The greater sequence variation of the specificity surface compared with other parts of the active site (which are highly conserved in all kinases) indicates that targeting it might afford inhibitor specificity as well as potency. NU6094 contained an anilino group at the N2 position, and had a tenfold increase in affinity for CDK2 over the parent, NU2058. To further increase the potency, a sulphonamide group was introduced at the anilino *para* position of NU6094, in an attempt to form an extra hydrogen bond. The resulting compound, NU6102, was a highly potent CDK2 inhibitor.

The crystal structure of activated CDK2–cyclin A complexed with NU6102 shows the interactions formed and explains the tight binding. The purine ring forms the usual triplet of hydrogen bonds with the CDK2 active site. However, although the anilino group packs closely to the specificity surface, the sulphonamide does not form the designed hydrogen bond with CDK2. Instead, the increased potency arises from the formation of two other hydrogen bonds, which facilitate optimum hydrophobic packing of the anilino group with the surface of CDK2.

In cellular studies, NU6102 inhibited growth of a human breast-carcinoma cell line. It also inhibited phosphorylation of downstream CDK target proteins, which is consistent with CDK1 and CDK2 inhibition. This is the first example of using an activated complex to direct iterative synthesis.

Melanie Brazil

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