A tale of two necessities: breakaway technology versus diabetes

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Diabetes is endemic in industrialized society. A lucrative market is waiting for companies that can find an effective remedy, but traditional HTS approaches are often ineffective in uncovering leads for the key targets. This, then, is a tale of two necessities: to find a more effective diabetes drug, and a novel discovery strategy to assist the search. Now, a new technology dubbed 'breakaway tethering' is being used to uncover molecules that block a key enzyme in the diabetes disease mechanism [1].

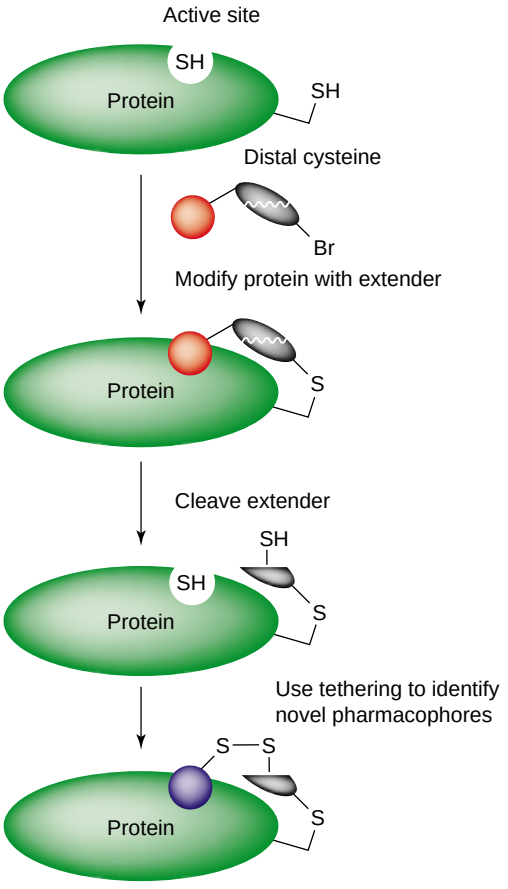

Figure 1. An overview of the breakaway tethering technique. A distal amino acid is mutated to cysteine, which is then attacked by a cleavable extender molecule. A moiety with affinity for the active site (shown in red) sterically protects the active-site cysteine of protein tyrosine phosphatase-1B (PTP1B) from alkylation by the extender. Once the protein has been modified, the extender is cleaved, re-exposing the active site and preparing the terminal portion of the extender for thiol exchange. Screening of a library of sulfur-containing compounds will then reveal groups that are able to bind with the nearby active site and would therefore make interesting leads for drug development. Figure provided by Daniel Erlanson of Sunesis Pharmaceuticals (http://www.sunesis.com/).

Establishing a target

Diabetes is a common disorder in which blood sugar is ineffectively processed. Type 2 diabetes is the most prolific form, representing ~90% of cases. The primary cause is either insufficient secretion or action of insulin, whose role is to facilitate the passage of glucose into cells. When insulin is scarce or ineffective, extracellular glucose accumulates, starving cells of their energy source and causing a diversity of symptoms that can include blindness and heart disease. Type 2 diabetes can often be controlled by careful lifestyle management, but insulin injections or medication often become necessary. Unfortunately, diabetes drugs often suffer from low efficacy as monotherapy and can cause side-effects; therefore, there is a large medical need for an effective treatment.

Protein tyrosine phosphatase-1B (PTP1B) is a well-validated target in type 2 diabetes drug research. This enzyme modulates the signalling cascade that is activated upon insulin binding to its cell-surface receptor. Specifically, PTP1B dephosphorylates tyrosine residues on the receptor, stopping insulin action. Knocking out PTP1B in mice improves insulin sensitivity over control mice [2]. By selectively blocking PTP1B in humans, therefore, this halting mechanism might prolong the effects of insulin. The PTP family of enzymes is large, however, and all are highly specific for the charged phosphotyrosine residue. Finding a selective small-molecule inhibitor of PTP1B is thus proving difficult. Numerous PTP1B candidates are undergoing trials, but big successes have yet to emerge.

The tethering technique

Drug discovery often uses HTS technology to screen an enzyme active site against thousands of molecules to find those that bind with highest affinity. For some targets, such as certain protein–protein interactions, small-molecule binders can be difficult to find. Using molecular fragments is one way around the problem, but the fragments usually have low binding

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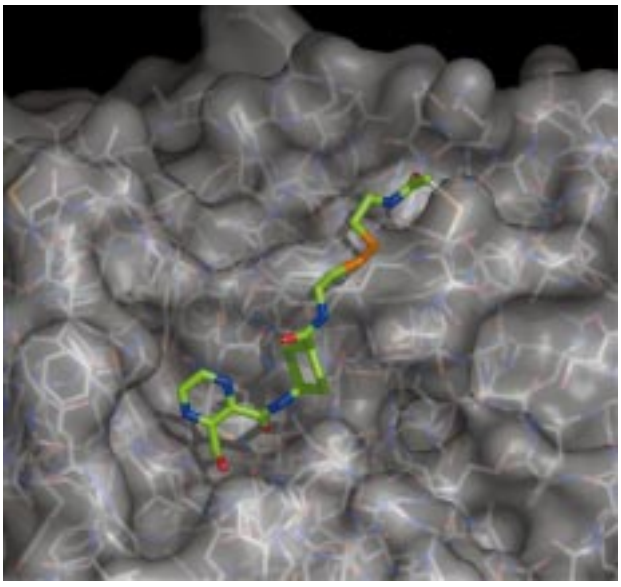

Figure 2. X-ray crystallographic structure of a novel pTyr mimetic bound to protein tyrosine phosphatase-1B (PTP1B) at 2.4 Å resolution. PDB code 1NWL. Figure provided by Daniel Erlanson of Sunesis Pharmaceuticals (http://www.sunesis.com/).

affinities and can be difficult to pick out against 'background noise'. To tackle this problem, Sunesis Pharmaceuticals (http://www.sunesis.com/) have developed a 'tethering' strategy, which uses simple, yet elegant, chemistry [3] and relies upon the formation of a disulfide bond between the ligand and a cysteine residue on the protein; this must be within or close to the active site and can be natural or introduced by mutation. The protein is then screened against a library of sulfur-containing compounds under partially reducing conditions. Rapid thiol exchange occurs at the cysteine site as each ligand forms a transient disulfide bond before being reduced. However, after disulfide formation, a few ligands will show affinity for the nearby active site. Even if this affinity is weak, the disulfide bond will be entropically stabilized and the tethered compound can be identified by MS. This technique is particularly good at finding low- and moderate-affinity binders that routine HTS would miss. Stitching together several such low-affinity binders might then lead to a higher-affinity and selective drug.

With PTP1B, however, the active site is deep and highly conserved; introducing cysteine mutations into the active site would probably disrupt the protein's function and lead to inaccurate binding studies. An ingenious way around this problem is to make the cysteine modification away from the active site. A bridging structure can then be used to reach round to the active site (Fig. 1). The bridge terminates with a thiol group, enabling screening with a library of sulfur-containing compounds. After screening a library of 15,000 disulfide-containing fragments, a high-potency compound was discovered that is chemically distinct from previous PTP1B inhibitors. The binding mode, as assessed by X-ray crystallography (Fig. 2), was also found to differ from those of other PTP1B inhibitors.

Principle proven, partnerships possible

The breakaway tethering technique is part of a suite of complementary technologies developed by Sunesis. The original methodology and other spin-offs have yielded several successes, including a highly potent molecule that blocks the IL-2/IL-2Rα protein–protein interaction. According to Daniel Erlanson, first author of the paper, the technique holds great promise for finding inhibitors of proteins that are intangible by traditional HTS. 'Besides the published examples, this approach has rapidly yielded compounds with potencies as high as single-digit-nanomolar against important therapeutic targets in in-house programs', he said. The novelty of their approach is generating interest among pharmaceutical companies. Sunesis are actively pursuing partnerships to develop the diabetes drug, and other inhibitors generated by their technique.

Rob Hooft van Huijsduijnen (Serono; http://www.serono.com/) an expert on PTP1B inhibitors who is not connected with this study, is impressed by the technology as a proof-of principle. He commented that covalently linking proteins to the enzyme substrate also helps co-crystallization and rational drug design. However, he was less enthusiastic about the inhibitors themselves. 'The compounds shown do not differ much from some of the competition,' he added. 'The road to drugs suitable for the clinic is still very long.'

References

1 Erlanson, D.A. *et al.* (2003) Discovery of a new phosphotyrosine mimetic for PTP1B using breakaway tethering. *J. Am. Chem. Soc.* 125, 5602–5603

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3 Erlanson, D.A. et al. (2000) Site-directed ligand discovery. *Proc. Natl. Acad. Sci. U. S. A.* 97, 9367–9372