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Discovery of a potent and highly selective PDK1 inhibitor via fragment-based drug discovery

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ABSTRACT

We report the use of a fragment-based lead discovery method, Tethering with extenders, to discover a pyridinone fragment that binds in an adaptive site of the protein PDK1. With subsequent medicinal chemistry, this led to the discovery of a potent and highly selective inhibitor of PDK1, which binds in the 'DFG-out' conformation.

The phosphatidylinositol-3 kinase (PI3K) signalling pathway could provide a compelling intervention point to treat cancer. When the pathway is activated, PI3K generates phosphatidylinositol 3,4,5-triphosphate, which causes protein 3-phosphoinositide-dependent protein kinase-1 (PDK1) to localize to the plasma membrane, where it first autophosphorylates and subsequently phosphorylates the protein Akt. This activates Akt, which in turn phosphorylates a number of substrates ultimately resulting in increased cell survival and resistance to apoptosis. Because the pathway is activated in most tumors, blocking the pathway may sensitize tumors to other anticancer agents.[1]

The importance of the PI3K signalling pathway is reflected in the number of programs targeting pathway members. However, although there have been considerable drug discovery efforts on PI3K and Akt, PDK1 has been relatively ignored. Indeed, when we first started working on the target, there were almost no inhibitors reported in the literature. Since then there have been some reports,[2–6] but most of these compounds are not specific for PDK1 over other kinases. In terms of validating the target, a potent and highly specific inhibitor would be a useful tool compound.

We used fragment-based drug discovery to search for such compounds. Fragment-based drug discovery builds drug leads from smaller component molecules, or 'fragments'.[7–12] Screening fragments rather than fully elaborated molecules can increase the efficiency of sampling chemical space, and can potentially increase the novelty of resulting molecules.

A previously reported fragment-based discovery technology called Tethering[13] uses reversible disulfide bonds between a cysteine residue in a protein and a thiol-containing fragment to enable the capture and identification of weak binding fragments by mass spectrometry.[14] A later version of the technology, Tethering with extenders, identifies companion fragments in the presence of a known binding moiety, or 'extender'.[15,16] An extender can both irreversibly modify a target cysteine residue and reversibly capture companion fragments that bind to an adjacent site. We have also reported a variant of this technology, Tethering with dynamic extenders, and used it to discover Aurora A inhibitors.[17] Here, we report the use of Tethering with extenders to discover highly selective PDK1 inhibitors.

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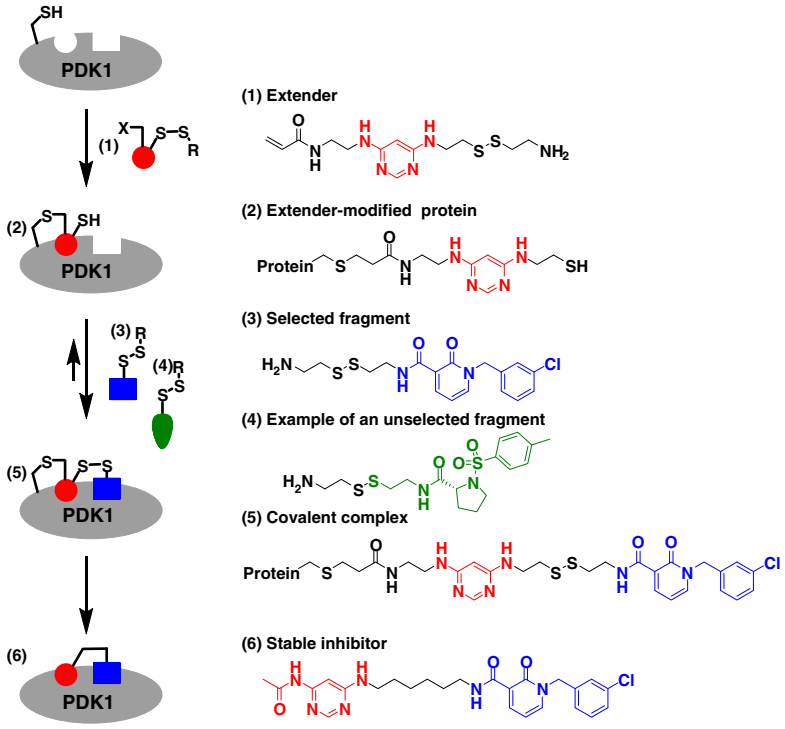

Scheme 1. Tethering with extenders applied to PDK1.

Our approach is shown schematically in Scheme 1. We first introduced a cysteine residue into PDK1 at position 166 (E166C). Next, we reacted the protein with an extender based on diaminopyrimidine (DAP), a moiety known to bind in the purine binding site, or hinge region.[17,18] The extender contains an acrylamide moiety that reacts with the introduced cysteine residue, as well as a disulfide linker. Under the labeling conditions, this disulfide is reduced to reveal a free thiol positioned for probing the adaptive binding site. We were able to obtain a crystal structure that reveals the extender binding as expected (Fig. 1), with the kinase in the active 'DFG-in' conformation.

We next performed a Tethering screen against a library of roughly 3000 compounds. Of these, one of the strongest hits was the pyridinone shown in Scheme 1. We used an unphosphorylated form of the protein for the initial screen; interestingly, when we repeated the screen with phosphorylated (active) PDK1, selection of the pyridinone was much weaker.

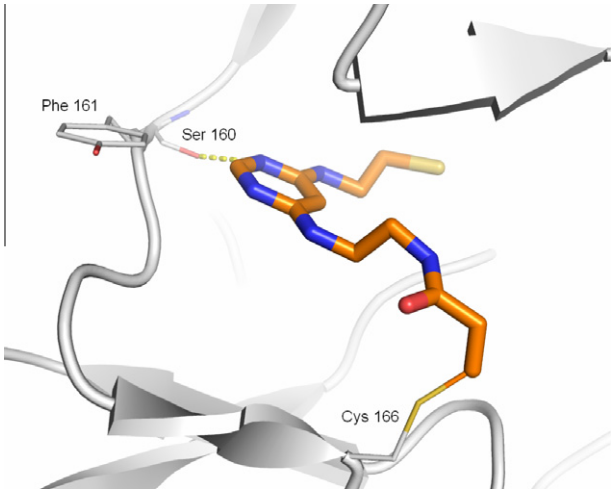

Figure 1. Crystal structure of complex **2**, (orange) extender-modified PDK1 (gray). Co-ordinates have been deposited with the protein data bank (PDB code: 3PWY).

Subsequently, we replaced the disulfide linkage between the DAP moiety and the pyridinone with a simple alkyl carbon chain. We were pleased to find that the resulting compound **6** inhibited a cascade PDK1-Akt assay with an IC_{50} of 0.2 μM. Under the conditions of the assay, PDK1 autophosphorylates; when we repeated the assay, this time with pre-phosphorylated PDK1, we found that compound **6** was less potent, with an IC_{50} of 2.9 μM. This is consistent with the Tethering results, and both suggest that the inhibitor binds to the inactive form of the protein. Control experiments revealed that the compounds were completely inactive against Akt (data not shown).

We were interested to probe the linker-length dependence of this chemotype. We hypothesized that if the pyridinone is binding specifically to the protein, a linker, that is, either too long or too short would cause a loss in activity. Table 1 shows that there is in fact a pronounced linker length dependence, with 5 or 6 methylene units being optimal (compounds **10** and **6**), which is consistent with the linker length expected from Tethering. Furthermore, aware of the potential for artifacts among low-affinity binders, we tested some of our compounds at a high ATP concentration to ensure they were ATP-competitive. All the inhibitors are ATP-competitive, suggesting that the DAP element is contributing to the affinity.

Having established the optimal linker length, we next sought to improve the potency of our molecules by exploring replacements for the DAP. We made a small library of roughly 50 different purine mimetics, many of which had previously been reported as fragments of kinase inhibitors (Table 2).

We chose the five-methylene linker rather than the slightly more potent six-methylene linker to try to keep the molecules as compact as possible. Removing the DAP moiety (compound **12**) led to a complete loss of activity, and most amide (compound **13**) or sulfonamide (compound **14**) linked compounds were also inactive or only weakly active. On the other hand, a number of amino-heterocycles were tolerated, with compound **18** being slightly