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Discovery of an Aurora kinase inhibitor through site-specific dynamic combinatorial chemistry

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ABSTRACT

We demonstrate a fragment-based lead discovery method that combines site-directed ligand discovery with dynamic combinatorial chemistry. Our technique targets dynamic combinatorial screening to a specified region of a protein by using reversible disulfide chemistry. We have used this technology to rapidly identify inhibitors of the drug target Aurora A that span the purine-binding site and the adaptive pocket of the kinase. The binding mode of a noncovalent inhibitor has been further characterized through crystallography.

Dynamic combinatorial chemistry (DCC) allows dimeric or multimeric compounds to assemble reversibly from constituent reactants.[1–5] Ideally, this assembly is guided by experimental conditions chosen to enrich a reaction mixture for molecules with desired characteristics, such as binding to a receptor. A potentially powerful use of DCC is fragment-based lead discovery, an approach that builds drug leads from smaller component molecules, or 'fragments'.[6–11] Despite the conceptual similarities, technical hurdles have, with a few notable exceptions,[12–19] stymied the use of DCC in fragment-based lead discovery. This letter describes a successful approach to apply DCC to overcome challenges of identifying and linking fragments.

A previously reported fragment-based discovery technology called Tethering[20] 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.[21] A later version of the technology, Tethering with extenders, identifies companion fragments in the presence of a known binding moiety, or 'extender'.[22,23] An extender can both irreversibly modify a target cysteine residue and reversibly capture companion fragments that bind to an adjacent site. However, that advance still requires two sequential rounds of protein modification, time-consuming steps which could disrupt a protein's delicate structure. Here we describe Tethering with dynamic extenders, where the irreversible electrophile of the extender is replaced with a disulfide. This disulfide enables both targeted and reversible cysteine modification. Another disulfide on the same extender reversibly captures fragments, allowing those with binding affinity to be detected. This new technique not only streamlines the methodology, but it also powerfully combines dynamic combinatorial chemistry with fragment-based lead discovery in a way that is generally applicable.

We demonstrate this methodology on a protein kinase, one of the most actively pursued target classes in drug discovery today. All kinases bind ATP, and structure-based design has been very effective at generating compounds that bind in the purine binding site. Inhibitors can also target a non-conserved adaptive region several angstroms from this site. Compounds that bind in this location have demonstrated high specificity for individual kinases, but systematically identifying such compounds has been difficult. We chose to demonstrate our new DCC technology to identify a fragment that binds in the adaptive pocket of Aurora A, a kinase which

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has recently attracted considerable attention for its critical role in regulating mitosis.[24]

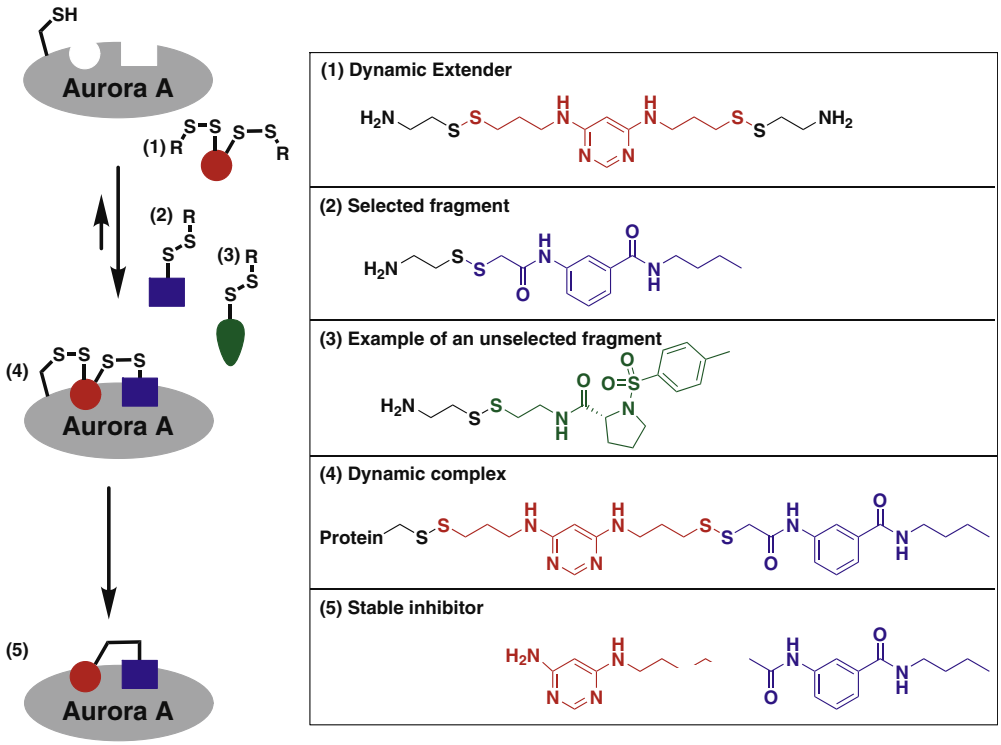
Our approach is shown schematically in Scheme 1. We used a construct of Aurora A designed to facilitate expression and crystallography.[25] We first introduced a cysteine residue near the ATP binding site (T217C) through site-directed mutagenesis of our modified Aurora A. Next, the protein was screened against a library of ~4500 disulfide-containing fragments under partially reducing conditions in the presence of dynamic extender **1**, whose synthesis is shown in Scheme 2.

This extender is based on a diaminopyrimidine (DAP), known to bind in the purine binding site.[26] We added two disulfide-containing appendages to this moiety. One arm anchors the extender to the protein in the purine binding site through the introduced cysteine residue; the other is poised to capture disulfide-containing fragments. Those fragments that bind in the adaptive region of the protein in close proximity to the extender form a stabilized disulfide bond with the extender, and these stabilized complexes are easily identified by using electrospray ionization mass spectrometry to detect any potential modification of the protein's mass.[27] To increase the screening throughput, we screened fragments in pools of roughly 10 compounds in which each compound has a unique mass; an example pool is shown in Figure 1. Because of the stringent requirement for two disulfides to exist simultaneously, very few hits were identified overall; an example of a hit that was identified is fragment **2** (Scheme 1). Its identification is shown in Figure 2, which shows the mass-deconvoluted spectrum of a mixture containing extender **1**, fragment **2**, and our cysteine-modified Aurora A.

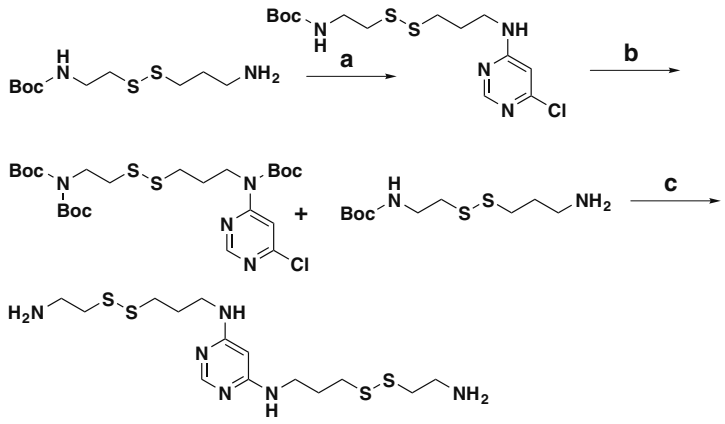
Converting the identified extender-fragment combination (complex **4** in Scheme 1) into a soluble inhibitor was straightforward: the disulfide between the extender and the fragment was replaced with a flexible alkyl linker and the other disulfide was removed from the DAP to generate compound **5**, which inhibited Aurora A with an IC_{50} of 17 μM. Replacing the DAP moiety with a purine produced a more potent compound (IC_{50} = 3.1 μM, not shown), and shortening the linker by one methylene produced compound **6**, with an IC_{50} of 2.9 μM (Fig. 3). Moreover, fragment **2** (which was identified from the dynamic selection) contributed significantly to the total binding affinity, as demonstrated by the fact that the alkyl-substituted purine **7** was inactive. Although compound **6** has only low micromolar activity, it does represent a new chemotype that is well suited to further optimization, and represents a proof of concept of the technology.

To demonstrate that the methodology is specifically probing the Aurora A adaptive pocket and to further understand the inhibitor's molecular interactions, we obtained a co-crystal structure of compound **6** bound to Aurora A without the T217C mutation, as shown in Figure 4. As expected, the purine moiety nestles in the ATP-binding pocket while the fragment identified through Tethering with the dynamic extender binds in the adaptive binding site. The distal amide makes a hydrogen bond with the backbone of residue Phe 144 and the phenyl ring of the inhibitor makes a π-cation interaction with the side chain nitrogen of Lys162. The activation loop (A loop; residues 274–297) of the protein adopts a completely different conformation from the structure of unphosphorylated Aurora A in complex with ADP.[28] In the compound **6** complex, the backbone of the DFG loop has undergone a ~180° rotation, moving the side chain of Asp274 out of the active site while that of Phe275 points toward the active site. This so-called 'DFG-out' conformation is reminiscent of a structure reported by AstraZeneca of Aurora A and a pyrimidinoquinazoline inhibitor.[29] Interestingly, the A loop is able to adopt an inactive conformation despite the presence of the activating mutation T287D, demonstrating that the inhibitor stabilizes a 'DFG-out' conformation and prevents the A loop from adopting an active conformation. Overall, the co-crystal structure supports the notion that the methodology discovers fragments that are able to interact with the kinase adaptive pocket.

In summary, we have demonstrated a new hit identification strategy, which targets dynamic combinatorial chemistry to a



Scheme 1. Tethering with dynamic extenders applied to Aurora A.



Scheme 2. Synthesis of dynamic extender **1**. Reagents and conditions: (a) 4,6-dichloropyrimidine, triethylamine, ethanol, reflux, 4.5 h, 76%; (b) carbonic acid di-*tert*-butyl ester, DMAP, THF, reflux, 4 h, 94%; (c) DIEA, *n*-butanol, 85 °C, 16 h, then 50% TFA in DCM, 30 min.

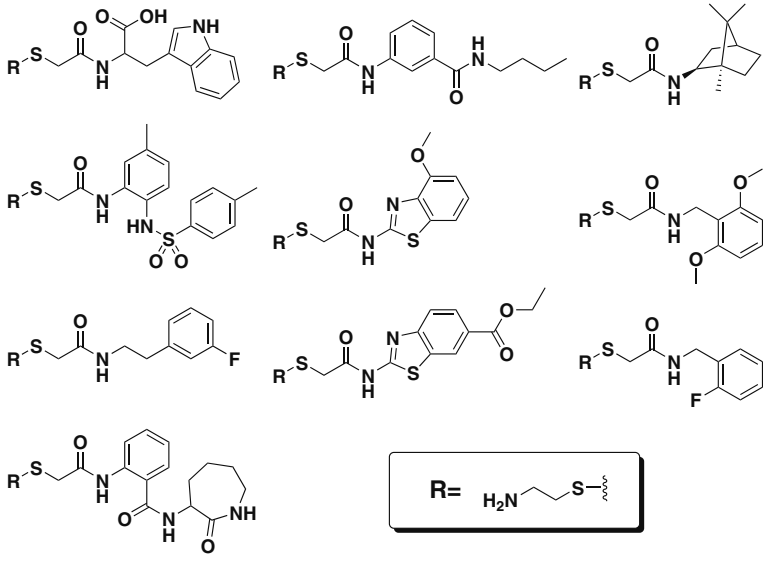


Figure 1. Example disulfide fragment pool.

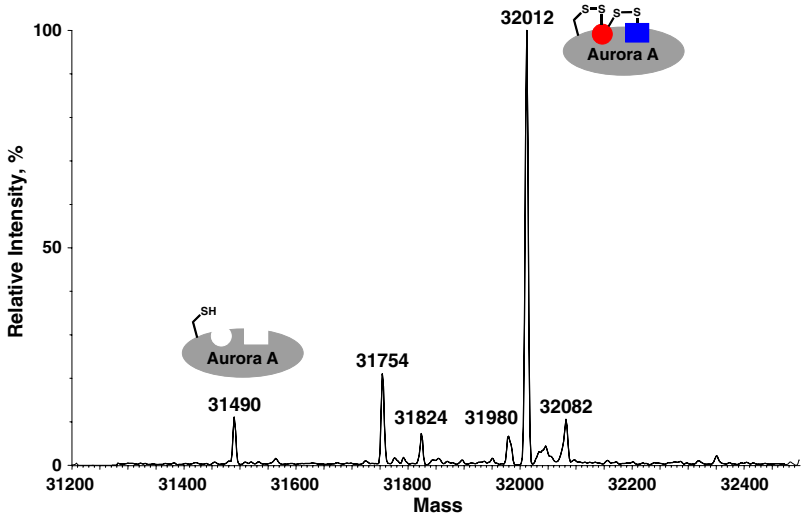


Figure 2. Deconvoluted, zero charge state mass-spectrum demonstrating a hit from Tethering with dynamic extenders. The dominant peak corresponds to Aurora A linked to extender **1**, which is in turn linked to fragment **2**.

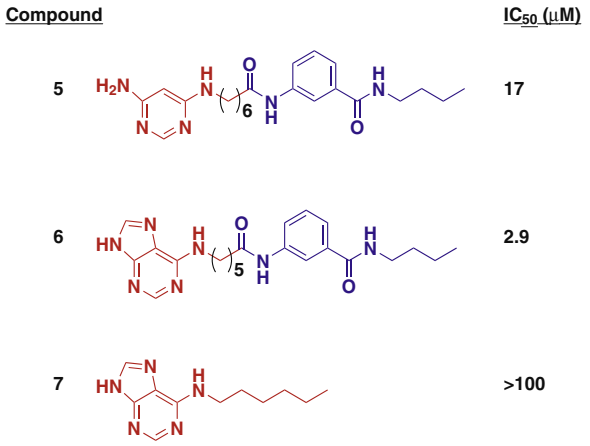


Figure 3. Enzymatic activity of compounds described.

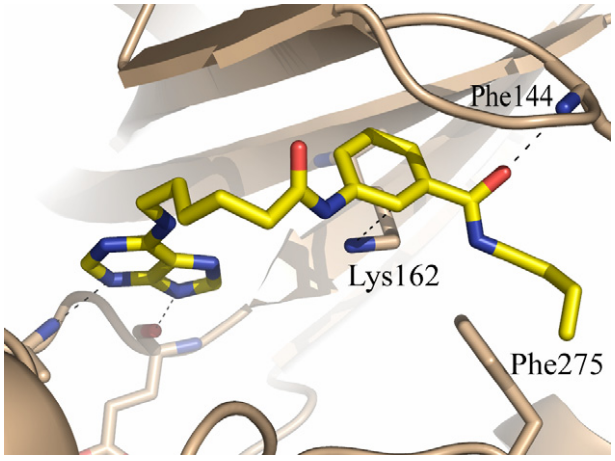


Figure 4. Co-crystal structure of Compound **6** bound to Aurora A, showing the interactions between the compound (yellow) and the protein. Figure made using PyMOL.[30] Coordinates have been deposited with the protein data bank (3DAJ.)

specified cysteine on a protein ('site-directed dynamic combinatorial chemistry') and have used this technology to identify inhibitors of Aurora A. Although there is no shortage of kinase inhibitors, this technique allows combinatorial chemistry to explore beyond the purine binding site and target the adaptive region of kinases specifically. We believe that this work illustrates the potential of the technique to rapidly identify ligands that could serve as starting points for a medicinal chemistry program. Moreover, the approach is general and could be applied to any kinase.

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