Rapid covalent-probe discovery by electrophile fragment screening


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Covalent probes can display unmatched potency, selectivity and duration of action, however, their discovery is challenging. In principle, fragments that can irreversibly bind their target can overcome the low affinity that limits reversible fragment screening. Such electrophilic fragments were considered non-selective and were rarely screened. We hypothesized that mild electrophiles might overcome the selectivity challenge, and constructed a library of 993 mildly electrophilic fragments. We characterized this library by a new high-throughput thiol-reactivity assay and screened them against ten cysteine-containing proteins. Highly reactive and promiscuous fragments were rare and could be easily eliminated. By contrast, we found selective hits for most targets. Combination with high-throughput crystallography allowed rapid progression to potent and selective probes for two enzymes, the deubiquitinase OTUB2, and the pyrophosphatase NUDT7. No inhibitors were previously known for either. This study highlights the potential of electrophile fragment screening as a practical and efficient tool for covalent ligand discovery.