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Preview

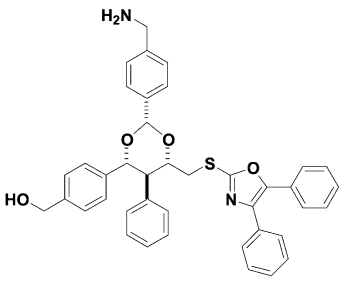
Protein Modulators Made to Order

Small molecules were created by diversity-oriented synthesis and subsequently subjected to microarray-based screening for their ability to bind a protein of interest. This general two-step method proved powerful in generating highly specific modulators of protein function.

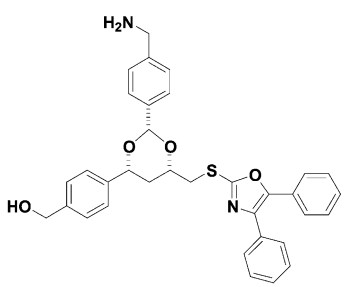
For several decades, natural and synthetic small molecules have provided powerful biological probes to delineate protein functions and regulatory mechanisms of physiological systems. However, small molecules with high specificity toward given proteins have been hard to come by, and the traditional approach of pursuing biologically active natural products often takes years to identify each cellular target. With the completion of the draft human genome sequence, the pressing need to study protein functions has put small molecules on center stage. In demand are more efficient and systematic methods to identify specific molecular probes. A recent report by Kuruvilla et al. in *Nature* [1] elegantly demonstrates the power of such a method. A library of small molecules was generated by diversity-oriented synthesis [2] and microarrayed on glass slides. Screening of this library led to the discovery of a specific inhibitor—named uretupamine —for the yeast protein Ure2p, a central regulator of nutrient-dependent gene expression. Initial screening of 3780 compounds for their ability to bind a fluorescently labeled Ure2p yielded eight candidates, of which one compound (uretupamine A) (Figure 1) specifically inhibited Ure2p function in cellular reporter assays. Upon further analyzing the structure and activity of this compound, the researchers were able to synthesize a more potent derivative (uretupamine B) (Figure 1) with increased solubility in cell media. The striking specificity of uretupamine was demonstrated by whole-genome transcriptional profiling; a subset of genes known to be suppressed by Ure2p was significantly upregulated by uretupamine, but they were unaffected by the compound in a yeast strain in which Ure2p was deleted.

Perhaps the most remarkable, and somewhat unexpected, property of uretupamine is its ability to inhibit a particular function of Ure2p without affecting the protein globally. Ure2p regulates gene expression in response to nitrogen and carbon source quality [3–5]. In the presence of high-quality nitrogen and carbon sources, Ure2p inhibits the transcriptional activators Gln3 and Nil1 that are responsible for the expression of genes regulating nitrogen and carbon metabolism. Upon glucose deprivation or a switch to a poor nitrogen source, Ure2p suppression is removed, which leads to Gln3 and Nil1 activation and subsequent gene expression. Interestingly, uretupamine treatment activated only a subset of genes that are controlled by Ure2p—the same set of genes that are upregulated upon glucose removal. Previously, the same authors proposed that Ure2p might differentially control responses to nitrogen and carbon source via Gln3 and Nil1, respectively [5, 6] (Figure 2). Now taking advantage of the selectivity of uretupamine, they are able to confirm this hypothesis by a combination of genetic manipulation, whole-genome profiling, and biochemical studies. Here, the power of chemical genetics is demonstrated at its fullest. Uretupamine is capable of modulating Ure2p function at a level that genetic deletion of Ure2p cannot, and it is comparable to functionally selective Ure2p mutants, which are not yet known.

This exquisite specificity of uretupamine is not so surprising if one recalls the case of the bacterial macrolide rapamycin, which has served as one of the paradigms of small-molecule probes [7]. Coincidentally, the targets of rapamycin (Tor1/2p) in yeast are upstream regulators of Ure2p, and rapamycin has been instrumental in helping dissect nutrient-response pathways (e.g., [3, 5, 6]). In addition to regulating transcription, Tor2p controls a multitude of cellular functions in response to nutrient availability; such functions include actin cytoskeleton reorganization [8]. Rapamycin, in complex with its cellular receptor FKBP, directly binds the Tor proteins and inhibits Tor1/2p's ability to regulate transcription via Ure2p and other proteins, but it has no effect on actin cytoskeleton reorganization [9], which is mediated by ROM2 and its effectors downstream of Tor2p ([8]; Figure 2). Thus, there is a clear parallel between rapamycin and uretupamine in their specificity. From a protein biochemistry point of view, such selectivity of small molecules



Uretupamine A



Uretupamine B

Figure 1. Structure of Uretupamine A and B

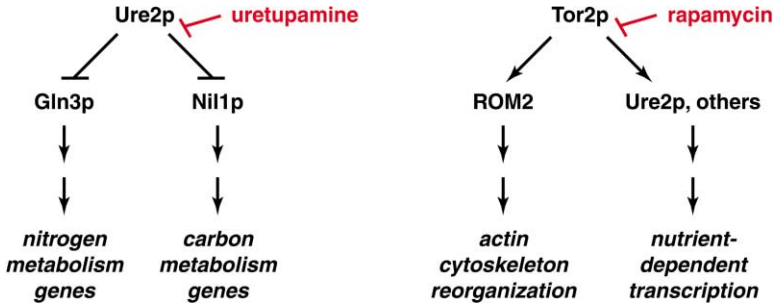


Figure 2. Selective Inhibitors

By directly binding to their targets, uretupamine and rapamycin inhibit only one of two functions of Ure2p and Tor2p, respectively. For simplicity, other pathways regulated by Tor2p are omitted.

is easily conceivable because a multifunctional protein may utilize distinct domains to modulate different downstream effectors.

It may be informative to consider the history of rapamycin development as a molecular probe. Originated from a strain of bacteria, rapamycin has gone through several stages of morphogenesis as an antifungal agent, an immunosuppressant, and now an anti-cancer drug candidate. In addition to the clinical importance, rapamycin represents one of the few highly specific inhibitors that have been invaluable in delineating cellular regulatory mechanisms. Despite intense research interest and effort, two decades passed after the initial isolation of rapamycin before its cellular target was identified. In comparison, the identification of uretupamine was achieved in a fraction of the time it took to characterize rapamycin, demonstrating the full advantage of reverse chemical genetics. The small-molecule library generated by Kuruvilla et al. is structurally complex and unbiased toward any particular protein target and is thus applicable for many proteins and does not require any structural information on the targets. With the microarray method offering desirable efficiency for high-throughput screening, this general strategy is bound to facilitate the development of molecular probes and boost our confidence in the possibility of achieving the ultimate goal of having one (or several) tailored small-molecule modulator for every protein expressed by the genome.

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Selected Reading

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