The dimeric Nuphar alkaloids are a structurally unique family of natural products containing an unsymmetrical thiaspirane. These sulfur-containing alkaloids were first isolated from the fresh water plant Nuphar lutea by Achmatowicz in the early 1960s. The most potent member of the Nuphar dimer family (6-hydroxythiobinuphraidine, Figure 1) is a rapid inducer of apoptosis, exhibiting in vivo antitumor activity. The structural basis for the bioactivity of this class of compounds is not well-understood, but it has been shown that the presence of a hemiaminal adjacent to the thioether is required for activity. In the ACS Central Science article by Shenvi and co-workers, the authors propose that activation of a dormant electrophilic sulfur atom embedded in the Nuphar dimers might account for their bioactivity.

In 2013, Shenvi described the first total synthesis of a Nuphar dimer via a biomimetic approach involving a dihydroxydimer intermediate. Of note, the dihydroxydimer intermediate itself proved difficult to isolate. The synthesis of the dihydroxydimer was recently accomplished using alternate methods as reported by MacMillan, Eastman and Wu enabling the study of its bioactivity. Shenvi and co-workers suggest that prior isolation difficulties stemmed from S-electrophilicity of the thiaspirane resulting in retrodimerization due the use of sulfur-based reagents in the presence of acid. While it is surprising retrodimerization had not previously been reported—especially in the extensive isolation literature—it is unlikely that dihydroxydimers would have been subjected simultaneously to an acid and thiol concoction during the isolation process. Enlightened by this fortuitous observation, the authors set out to demonstrate their thiol-triggered retrodimerization hypothesis which they believed was also linked to the mechanism of action, and therefore bioactivity, of this class of compounds.

Shenvi and co-workers focused their initial proof-of-concept studies around unadorned monomeric iminium thiaspirane systems in order to cleanly study the reactivity of this pharmacophore with nucleophiles. While the monomers are stable to alcohols such as methanol, they proved reactive to thiophenol. And in the presence of a mild reducing agent, a tertiary amine containing disulfide was obtained. This demonstrated for the first time that the iminium thiaspirane can function as a sulfur electrophile and selectively reacts with thiols over water or alcohol (Figure 2a).

While subsequent studies around a small library of spirocyclic iminium analogues revealed reactivity trends in line with a standard Hammett analysis, the monomer did not prove to be an ideal model system because the reaction was reversible in the absence of reductant preventing the isolation of the elusive ring-opened disulfide iminium species. Furthermore, as the reaction only occurred at high and thus biologically irrelevant concentrations of thiol (1−5 M), the thio-triggered activation appeared incongruous to the proposed biological mode of action. For this reason, the authors then turned their attention to producing simplified dimer analogues of the Nuphar scaffold, analogues

Hidden electrophilicity in Nuphar alkaloids is responsible for their impressive bioactivity, report Shenvi and colleagues.

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believed—based on the culmination of their efforts—to be capable of forming the thioether imine through a thiol induced thiaspirane ring opening mechanism.

Simplified Nuphar dimers (Figure 2b), prepared from a tetrathiol-mediated dimerization strategy, were found to afford iminium thioether adducts in the presence of thiols at a cellularly relevant concentration (5 mM). The authors postulate a retrodimerization reaction is initiated through nucleophilic attack at the electrophilic thiaspirane sulfur and then proceeds in part through an unsaturated iminium “warhead” which captures thiols at the β-position to form a stable iminium thioether.

In order to evaluate the cellular relevance of this sulfur electrophilicity and its release of an unsaturated iminium warhead, the authors measured the potency and rate of killing of the various Nuphar analogues. It was found that dimers promote rapid apoptosis at single digit micromolar concentrations, whereas monomeric iminiums lead to less pronounced cell death and only at high concentrations (25 μM).

In an attempt to assess whether formation of the unsaturated iminium warhead occurs in a “native biological environment” isoTOP-activity-based protein profiling was performed. In this method, developed by coauthor Ben Cravatt, cells or cell lysates are treated with the electrophile of interest (such as the thiaspiranes), and proteinaceous cysteine residues that react with the electrophile can be detected by mass spectrometry. Using this approach, the simplified Nuphar dimer and the unsaturated iminium warhead were found to display similarly strong isoTOP-ABPP profiles, whereas the monomers reacted with cysteine residues only modestly.

As stated by the authors, it is important to note that the varying biological activities displayed by Nuphar dimers cannot be solely attributed to retrodimerization. Some Nuphar monohydroxy dimers cannot retrodimerize due to the position of the hydroxyl group, yet maintain biological activity. Whether an electrophilic carbon or electrophilic sulfur underlies the mechanism of these monohydroxy Nuphar dimers in a biological setting remains unknown.

In summary, Shenvi and co-workers have demonstrated that the thiaspirane pharmacophore of the Nuphar alkaloids contains an electrophilic sulfur atom which reacts with nucleophilic thiols. The ensuing reaction results in the unraveling of the tricyclic system to an iminium thioether proposed to proceed in part through an unsaturated iminium warhead. Extension of this concept to the dihydroxythiaspirane dimeric natural products suggest they are “prodrugs” for a highly reactive iminium covalent binder. The “caged iminium thioether” of the Nuphar alkaloids represents an interesting pharmacophore for further investigation, both in the context of chemical probes for thiol capture and the design of therapeutics. More generally, it raises the question of how many other hidden warheads crouch behind elemental features of natural products either previously thought to be well-understood, or otherwise innocuous.

In summary, Shenvi and co-workers have demonstrated that the thiaspirane pharmacophore of the Nuphar alkaloids contains an electrophilic sulfur atom which reacts with nucleophilic thiols.

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REFERENCES
(1) Achmatowicz, O.; Bellen, Z. Alkaloids of Nuphar luteum (L) sm. Isolation of alkaloids containing sulphur. Tetrahedron Lett. 1962, 3, 1121–1124.
(2) Matsuda, H.; Morikawa, T.; Oda, M.; Asao, Y.; Yoshikawa, M. Potent anti-metastatic activity of dimeric sesquiterpene thioalkaloids.
from the rhizome of Nuphar pumilum. *Bioorg. Med. Chem. Lett.* 2003, 13, 4445−4449.

(3) Korotkov, A.; Li, H.; Chapman, C. W.; Xue, H.; MacMillan, J. B.; Eastman, A.; Wu, J. Total syntheses and biological evaluation of both enantiomers of several hydroxylated dimeric Nuphar alkaloids. *Angew. Chem., Int. Ed.* 2015, 54, 10604−10607.

(4) Tada, N.; Jansen, D. J.; Mower, M. P.; Blewett, M. M.; Umotoy, J. C.; Cravatt, B. F.; Wolan, D. W.; Shenvi, R. A. Synthesis and sulfur electrophilicity of the Nuphar thiaspirane pharmacophore. *ACS Cent. Sci.* 2016, DOI: 10.1021/acscentsci.6b00113.

(5) Jansen, D. J.; Shenvi, R. A. Synthesis of (−)-neothiobinupharidine. *J. Am. Chem. Soc.* 2013, 135, 1209−1212.

(6) Weerapana, E.; Speers, A. E.; Cravatt, B. F. Tandem orthogonal proteolysis-activity-based protein profiling (TOP-ABPP)—a general method for mapping sites of probe modification in proteomes. *Nat. Protoc.* 2007, 2, 1414−1425.