Unravelling the Mode of Action of Furanoheliangolides through Total Synthesis and Chemical Proteomics

Cite This: ACS Cent. Sci. 2021, 7, 923−925

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Streamlined synthesis of goyazensolide and related natural products, coupled to chemical proteomics, identifies the target of these important and highly bioactive natural products.

Harnessing complex natural products (NPs) for their application as therapeutics or tool compounds has historically been a successful strategy in drug discovery and chemical biology. However, using electrophilic NPs to explore human biology, as showcased in the striking work by Winssinger et al. in ACS Central Science, has recently been experiencing a renaissance.

NPs that act through a covalent mechanism have unknowingly been used for decades; however, in many cases their specific targets have only been systematically elucidated after the advent of modern chemical proteomics technologies, including activity-based proteome profiling (ABPP). The latter emerged as a valuable method to either deconvolute the functional proteome state of a whole cell or to assess the role of a specific enzyme in biological systems. In this context, iodoacetamide-bearing, activity-based probes have been used to investigate the ligandable cysteines across the entire proteome. For example, the NP nimbolide was recently identified as a covalent binder of the E3 ligase RNF114 using isotopic tandem orthogonal proteolysis-enabled ABPP (isoTOP-ABPP), which resulted in the preparation of covalent proteolysis targeting chimeras (PROTACs). Another recent application of this technology to the NP parthenolide, coincidentally also a sesquiterpene, revealed that it covalently targets the focal adhesion kinase (FAK) in breast cancer cells.

Furanoheliangolides including goyazensolide are comprised of a complex, three-dimensional structure bearing varying numbers of α,β-unsaturated carbonyls as potentially

Published: May 27, 2021

https://doi.org/10.1021/acscentsci.1c00542
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NPs that act through a covalent mechanism have unknowingly been used for decades; however, in many cases their specific targets have only been systematically elucidated after the advent of modern chemical proteomics technologies, including activity-based proteome profiling (ABPP). They display a diverse array of bioactivities including promising anticancer and antiviral activity; however, their specific target(s) have remained elusive until now. Winssinger and co-workers combine an innovative total synthesis strategy with chemical proteomics and in-depth biological analysis to show that the NP goyazensolide covalently and selectively binds importin-5 (IPO5), a transport protein of the karyopherin superfamily, which explains its reported antiviral and cytotoxic activities.6

Although furanoheliangolides are available from natural sources, they can typically only be isolated in minute amounts. This makes direct functionalization to explore structure−activity relationships and obtain probes for target ID challenging. The authors solved this significant synthetic challenge by developing an approach based on the build/couple/pair strategy, originally described in the context of diversity-oriented synthesis (Figure 1).7 Two key intermediates were assembled in 3–5 steps in the “build” phase and were subsequently coupled via a Sonogashira reaction. The strained 10-membered germacrene framework was formed by a challenging Barbier-type macrocyclization in impressive yields. Diversification was carried out by employing a gold-catalyzed transannulation as the key step. Through this general synthetic sequence, access to not only goyazensolide but also 15 related NPs and two alkyne probes was achieved on a reasonable scale. Target identification was carried out with clickable fluorophore and biotin probes for visualization and pulldown, respectively (Figure 2). Although the in gel digestion of a band identified after competition with natural goyazensolide successfully identified IPO5 as the molecular target, further quantitative chemical proteomic strategies including isoTOP-ABPP may be applied in the future to conclusively determine target selectivity and the precise site of covalent labeling of goyazensolide's target protein(s).

Following its identification, validation of IPO5 as the primary target was carried out in a series of clear follow-up experiments. IPO5 is known to play a role in the transport of viral proteins to the nucleus by interacting with specific nuclear localization sequences (NLS). IPO5 is also involved in the transport of RAS protein activator like-2 (RASAL2) from the cytoplasm to the nucleus. The authors were able to show that goyazensolide is able to inhibit both interactions efficiently and in a dose-dependent manner, providing tantalizing clues for the antiviral and antitumor activities.
of the NP. The inhibition between RASAL2 and IPO5 was further analyzed in-depth via confocal microscopy and immunoblotting techniques. A clear increase in cytoplasmic RASAL2 and a consequent downstream effect in the lower abundance of pAKT is reported upon goyazensolide treatment, which further validates its mechanism of action (MoA).

In summary, by establishing a general build/couple/pair sequence, the synthetically challenging furanoheliangolide framework was rapidly accessed.

In summary, by establishing a general build/couple/pair sequence, the synthetically challenging furanoheliangolide framework was rapidly accessed. By completing the total synthesis of a natural product family, the authors provide the basis for expanding their work to investigate additional, structurally related NPs. This may involve growing the accessible compound space beyond NPs toward synthetic analogues that show improved activity. As also shown by the authors, only one other NP synthesized herein targets IPO5, although a number of different biological activities are reported for these compounds. With the potential to rapidly synthesize these NPs, their respective MoA may also be resolved by applying similar chemoproteomic techniques in the future. Finally, the value of IPO-5 as a drug target remains to be seen; however, it will be significantly facilitated by the use of goyazensolide and the work presented here.

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Notes
The authors declare no competing financial interest.

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