THE ODYSSEY OF CYCLOPAMINE

Reported by Howard S. Roth May 5, 2011

INTRODUCTION

In the late 1950s, farmers in Idaho observed a large number of newborn sheep displaying cyclopia, the presence of only one central eye. Scientists from the Department of Agriculture studied the sheep and observed that the appearance of birth defects corresponded with ingestion of the flower *Veratrum californicum.* The researchers isolated three *Veratrum* alkaloids that induced this phenotype, with cyclopamine (**1**, Figure 1) as the most potent of the three. Cyclopamine is a structurally unique steroidal alkaloid, with a C-*nor*-D-*homo* ring system. Early synthetic efforts were reported in the late 1960s and early 1970s, but these strategies were inefficient, low-yielding, and non-stereoselective.[1]

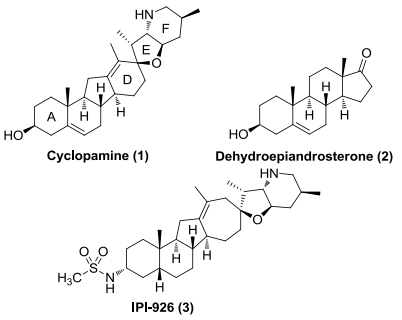

Figure 1. Cyclopamine (**1**), dehydroepiandrosterone (**2**), and semisynthetic cyclopamine analogue IPI-926 (**3**).

DIASTEREOSELECTIVE TOTAL SYNTHESIS OF CYCLOPAMINE

In 2009, Giannis and coworkers reported a diastereoselective total synthesis of cyclopamine from dehydroepiandrosterone (**2**, Figure 1). The synthesis began with a regioselective copper-mediated C-H activation/hydroxylation with molecular oxygen. The C-*nor*-D-*homo* ring structure was formed via a biomimetic Wagner-Meerwein rearrangement, with a 7:3 isomeric ratio for the exocyclic:endocyclic olefin. The exocyclic isomer was determined to be the best substrate for the subsequent tandem Horner-Wadsworth-Emmons reaction/conjugate addition, and a late-stage Alder-ene reaction gave the desired endocyclic product. The synthesis proceeded in 20 steps with a 1% overall yield.[2]

MECHANISM OF ACTION

The highly conserved hedgehog (Hh) signaling pathway is vital for cell growth, differentiation, and homeostasis. The pathway is associated with normal function in development and pathogenesis in diseases such as cancer.[3] The signaling cascade begins with the binding of the Hh protein to the transmembrane protein Patched (Ptch). Hh binding relieves the Ptch-mediated inhibition of the transmembrane protein Smoothened (Smo). Smo then activates the Gli transcription factors, leading to the expression of the Hh target genes, including growth promoters Bcl-2 and VEGF.[4] In 1998, Beachy and coworkers were searching for a small-molecule modulator of the Hh signaling pathway. Knowing

that Hh mutations often lead to cyclopia, they identified the Hh pathway as the target for cyclopamine and found that it acted specifically by inhibiting Smo.[5]

SEMISYNTHETIC CYCLOPAMINE ANALOGUES

The identification of the Hh signaling pathway as the target of cyclopamine has initiated efforts to use cyclopamine as an anticancer agent. However, cyclopamine is unstable to acid, as the D ring aromatizes to form the hemolytic *Veratrum* alkaloid veratramine, making the natural product a poor drug candidate. To improve the stability in acid, a semisynthetic analogue with a seven-membered D ring was designed. Synthesis of this analogue involved a Simmons-Smith cyclopropanation of the D-ring alkene, followed by a Lewis acid-catalyzed ring expansion. This analogue (D-homocyclopamine) no longer has the ability to aromatize and is stable to acid. Further studies identified the 4-en-3-one of D-homocyclopamine as an orally bioavailable cyclopamine analogue.[6] However, *in vivo* experiments showed that the ketone was easily reduced to the corresponding saturated alcohol, which was rapidly glucuronidated and cleared. A methanesulfonamide analogue, IPI-926 (**3**, Figure 1) was designed, and its clearance from the body is much slower.[7] Currently, Phase II clinical trials are underway for the use of IPI-926 in the treatment of metastatic pancreatic cancer.

SUMMARY

The identification of the Hh pathway as the target of cyclopamine has intensified interest in the natural product as an anticancer strategy. A diastereoselective total synthesis was completed, and efforts toward an improved synthesis are underway. A semisynthetic analogue, IPI-926, is in Phase II clinical trials for cancer treatment. Cyclopamine has proven to be a useful tool in the study of the Hh pathway and the development of novel anticancer drugs.

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