

Synthesis of novel, chemically stable D-homo-cyclopamine analogs *via* a cyclopropanation/ring-expansion sequence



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Introduction

The hedgehog (Hh) signaling pathway (Fig. 1A) is important in tissue growth and differentiation and plays a pivotal role in embryogenesis as well as tissue homeostasis.¹ Over the last decade, there have been an increasing number of reports documenting the implication of the Hh pathway in human diseases, such as cancers.²⁻⁵ Indeed, aberrant Hh signaling has been described in numerous primary tumor tissues including basal cell carcinoma,⁶⁻⁷ breast,⁸ esophagus,⁹ gastric,⁹ medulloblastoma,¹⁰ pancreatic adenocarcinoma,¹¹ prostate¹² and small cell lung cancers.¹³ Cyclopamine, a steroidal alkaloid isolated from *Veratrum californicum*,¹⁴⁻¹⁶ is a potent antagonist of the hedgehog (Hh) pathway (Fig. 1B). Cyclopamine was shown to act on Smoothened (Smo), a key effector of the Hh pathway.¹⁷

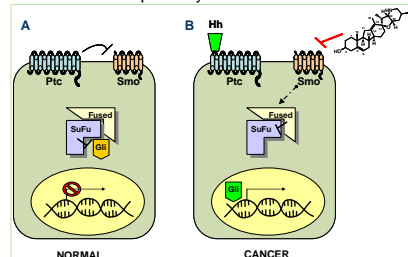


Figure 1. Schematic of the Hedgehog signaling pathway. (A) In the absence of ligand, which represents the situation on most normal cells during adulthood; (B) In several types of cancer cells, the pathway is activated by the presence of ligand and/or loss-of-function of Ptc and/or gain-of-function of Smo. Blockade of the Hh pathway can be achieved by cyclopamine

Cyclopamine has been a valuable pharmacological tool used to validate the Hh pathway as a promising target for the development of anti-tumor agents. However, there are some drawbacks to the use of cyclopamine as a viable therapeutic agent: availability of raw material, poor aqueous solubility (~5µg/ml), and chemical instability in acid (Fig 2).

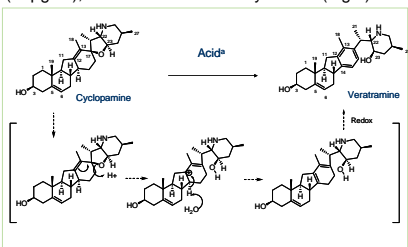


Figure 2. Acid instability of cyclopamine.

Results

A method for extraction and isolation of cyclopamine from *Veratrum californicum* was described several years ago by Keeler and co-workers.¹⁴ This published procedure required some modifications in order to access multi-gram quantities of cyclopamine in a robust and reliable manner. Figure 3 depicts the process by which 100 g of cyclopamine was extracted and isolated from plants harvested in western United States.

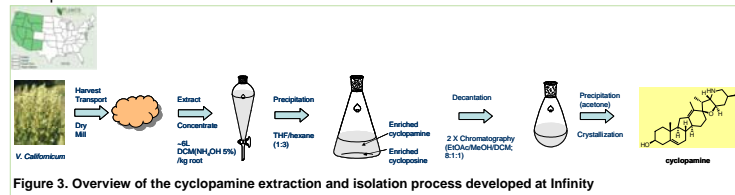


Figure 3. Overview of the cyclopamine extraction and isolation process developed at Infinity

To increase the solubility of cyclopamine we evaluated the possibility of forming an ammonium salt. This proved to be a challenging task due to the acid instability of cyclopamine. Ultimately, it was found that hydrochloride salt of cyclopamine could be generated and isolated in high yield using anhydrous HCl in ethyl acetate (Table 1). The addition of 2-hydroxypropyl-β-cyclodextrin, a common steroid-complexing agent, significantly enhances the solubility of cyclopamine hydrochloride to a point where it could be delivered *in vivo* using various modes of administration.

Table 1. Combination of salting and cyclodextrin formulation enhances the solubility of cyclopamine and allows for iv and po dosing in animal studies.

Solubility	Cyclopamine	Cyclopamine-HCl
Saline	< 1 mg/mL	<1.5 mg/mL
Water (5.5% cyclodextrin)	< 1 mg/mL	10 mg/mL

As depicted in Figure 2, the acid instability of cyclopamine arises from the chemical reactivity of the spirofuran ring adjacent to the endocyclic double bond of the steroid D-ring, therefore we sought a more stable surrogate to the spiro allylic ether. It was hypothesized that reactivity of the allylic ether could be taken advantage of to selectively derivative cyclopamine to a more stable

analog. Towards this end, it was found that a cyclopropane ring could be installed selectively on the β-face of the steroid by zinc carbenoids (Fig. 4). In addition, such cyclopropyl derivatives of cyclopamine (e.g. IPI-329) readily undergo a Lewis acid catalyzed rearrangement to give D-homo cyclopamine derivatives. Presumably, this rearrangement occurs through the formation of a stabilized cyclopropylcarbinyl cation and subsequent rearrangement.¹⁸ Overall this two step process inserts a methylene unit between the double bond and the spirofuran with retention of stereochemistry.

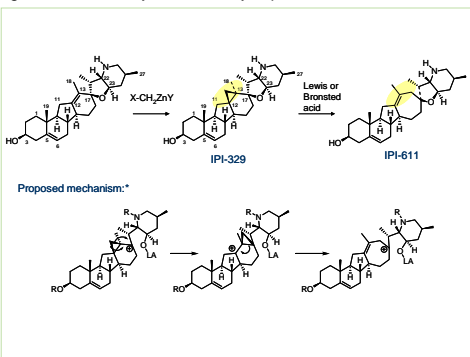


Figure 4. Overview of the synthetic approach to D-homo cyclopamine analogs. Sequence of cyclopropanation followed by acid-catalyzed ring-expansion. *Mechanism based on ref. 18.

The newly created D-homo cyclopamine analog (IPI-611) was anticipated to be more stable to acid-catalyzed degradation than the natural product because it no longer has the allylic spirofuran. Indeed, IPI-611 was found to be more stable than cyclopamine at low pH (Fig. 6). Unfortunately, the aqueous solubility of the hydrochloride salt of IPI-611 was less than that of cyclopamine. With a more stable ring system in hand, we were able to further derivative this analog and found that changing the A/B-ring of IPI-611 to the corresponding conjugated enone led to a compound (IPI-609) with enhanced solubility and stability relative to cyclopamine (Fig. 5).

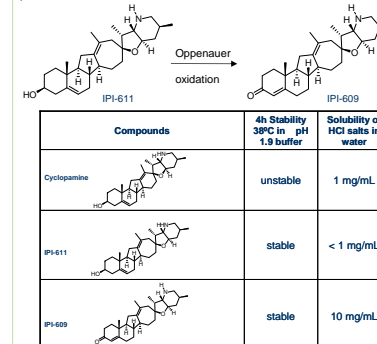


Figure 5. Change in A/B ring oxidation state and installation of D-homo ring system greatly improve solubility and stability at low pH.

A representative synthetic route to this compound is illustrated below (Fig. 6).¹⁹

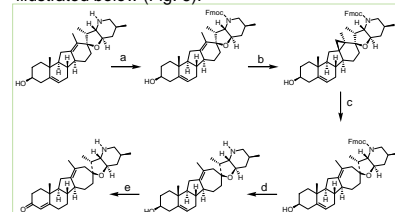


Figure 6. Typical synthesis of IPI-609 from Cyclopamine Reagents and Conditions: (a) Fmoc-OSu, CH₂Cl₂, 25°C (70-70%); (b) Et₂Zn, CH₂Cl₂, 0°C to 25°C (60-70%); (c) BF₃·OEt₂, CH₂Cl₂, 0°C (60-70%); (d) Et₃NH, CH₂Cl₂, 25°C (70%); (e) Al(OiPr)₃, cyclohexanone, toluene, 80°C (70-80%).

Conclusion

A new class of cyclopamine derivatives with better pharmaceutical properties was discovered. These studies enable exploration of the full potential of this natural pharmacophore as an anticancer agent.

References

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