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Design and Synthesis of Inhibitors of Hedgehog Signaling Based on the Alkaloid Cyclopamine

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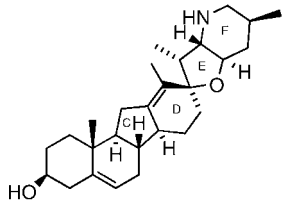
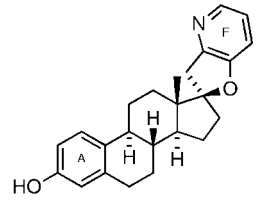
ABSTRACT



Cyclopamine 1



Estrone-Derived Analog 5

The synthesis and biological evaluation of structurally simplified, metabolically stable cyclopamine-like Sonic Hedgehog (SHH) signaling inhibitors, i.e., 5, is described in four chemical steps from commercially available steroidal precursors. Biological evaluation of this cyclopamine analogue in two different systems establishes the high potency of 5 as a SHH signaling inhibitor. This approach provides important new lead structures for the development of new cancer chemotherapeutic agents based on the inhibition on SHH signaling.

In 1966, Binns, Keeler and co-workers established that the alkaloid cyclopamine **1** was responsible for the catastrophic birth defects observed in calves from livestock that were fed diets rich in the corn lily, *Veratrum californicum* (Figure 1a).[1,2] The observed phenotype included anophthalmia, cyclopia, and profound craniafacial effects (Figure 1b).

Figure 1. (a) *Veratrum californicum* (California corn lily); (b) cyclopia as a consequence of cyclopamine ingestion.

It was subsequently established that cyclopamine **1** suppresses the Sonic Hedgehog (SHH) cellular signaling pathway, which is important for tissue growth and differentiation, thus playing a pivotal role in embryogenesis.[3,4] During development, activation of the SHH-signal transduction

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(1) Binns, W.; James, L. F.; Keeler, R. F.; Balls, L. D. Effects of teratogenic agents in range plants. *Cancer Res.* **1968**, *28*, 2323–2326.
(2) Keeler, R. F.; Binns, W. Teratogenic compounds of Veratrum californicum (Durand). II. Production of ovine fetal cyclopia by fractions and alkaloid preparations. *Can. J. Biochem.* **1966**, *44*, 829–838.
(3) Cooper, M. K.; Porter, J. A.; Young, K. E.; Beachy, P. A. Teratogen-mediated inhibition of target tissue response to Shh signaling. *Science* **1998**, *280*, 1603–1607.
(4) Incardona, J. P.; Gaffield, W.; Kapur, R. P.; Roelink, H. The teratogenic Veratrum alkaloid cyclopamine inhibits sonic hedgehog signal transduction. *Development* **1998**, *125*, 3553–3562.
(5) Hooper, J. E.; Scott, M. P. Communicating with Hedgehogs. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 306–317.
(6) Ingham, P. W.; McMahon, A. P. Hedgehog signaling in animal development: paradigms and principles. *Genes Dev.* **2001**, *15*, 3059–3087.

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pathway is initiated by the binding of the SHH ligand to the cellular membrane receptor Patched (PTC), which relieves the PTC-mediated inhibition of the transmembrane protein Smoothened (SMO) (Figure 2).[5,6] Activated SMO transduces

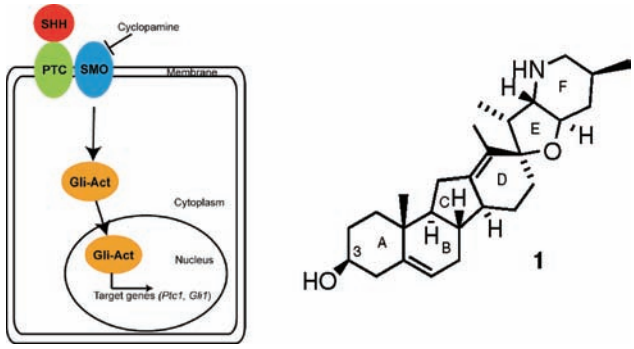
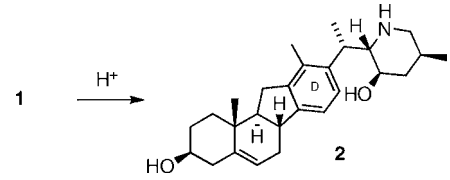


Figure 2. Hedgehog signaling pathway and the structure of cyclopamine **1**.

the signal to the nucleus to regulate gene expression via Gli transcription factors. Beachy and co-workers have established that cyclopamine **1** disrupts this pathway by inhibition of SMO.[7] The teratogenicity described above has not hampered interest in the development of this class of compounds.[8,9] Treatment of cancer cells with cyclopamine **1** induces a decrease in proliferation, an increase of apoptosis, and/or a decrease of metastasis.[10−13] Therefore, cyclopamine and its related structures hold great promise in cancer chemotherapy.

Despite the attractive pharmacological profile against a number of cancer xenografts, in vivo evaluation of cyclopamine has been hampered by its poor aqueous solubility (ca. 5 μg/mL) and acid lability. Cyclopamine readily converts to veratramine **2** via acid-catalyzed ring-opening of the spirotetrahydrofuran ring, followed by aromatization of the D ring (Scheme 1).[14] Unlike cyclopamine, **2** does not act as

Scheme 1. Acid-Mediated Conversion of Cyclopamine **1** to Veratramine **2**



an SHH antagonist and causes hemolysis by targeting other receptors.[15,16]

Two strategies have been reported to address the issues of water solubility and acid lability of **1**: (1) covalent modification of naturally occurring cyclopamine **1** to generate structurally related and metabolically stable lead structures,[9] as pioneered by Adams and co-workers at Infinity Pharmaceuticals, and (2) identification of small-molecule SHH antagonists through high-throughput screening.[17] The first approach, however, relies on the availability of **1**, which is prohibitively expensive ($1990/gram), while preliminary data suggests limited success with the Curis and Genentech structures.[18] Consequently, there is an urgent need to identify readily available potent inhibitors of SHH as lead structures for the development of new cancer chemotherapies.

We have opted to explore a third approach, which is not dependent on the availability of **1** and yet generates new lead compounds that closely resemble **1** in both structure and function. The advantage of cyclopamine-like structures is underscored by the observation that cyclopamine crosses the blood−brain barrier, a critical property for the development of clinical candidates for the treatment of brain malignancies.[12]

The difference in teratogenicity between cyclopamine **1** and the close structural analogue tomatidine **3** (Figure 3; nonteratogenic) has been attributed to the difference in the orientation of the nitrogen atom relative to the steroid plane in **1** and **3**. The C-nor-D-homo framework of **1** can thus be viewed as a scaffold that orients the E/F heterobicyclic moiety orthogonal to the steroidal ring system, with the F-ring nitrogen atom on the α-face of the steroid plane

(7) Chen, J. K.; Taipale, J.; Cooper, M. K.; Beachy, P. A. Inhibition of Hedgehog signaling by direct binding of cyclopamine to Smoothened. *Genes Dev.* **2002**, *16*, 2743–2748.

(8) Janardanannair, S.; Adams, J.; Ripka, A. S.; Hospital, M. R.; Tremblay, M. Methods for preparation cyclopamine analogs and use thereof in treating cancers. 2005-US30406; 2006026430, 20050826, 2006.

(9) Tremblay, M. R.; Nevalainen, M.; Nair, S. J.; Porter, J. R.; Castro, A. C.; Behnke, M. L.; Yu, L.-C.; Hagel, M.; White, K.; Faia, K.; Grenier, L.; Campbell, M. J.; Cushing, J.; Woodward, C. N.; Hoyt, J.; Foley, M. A.; Read, M. A.; Sydor, J. R.; Tong, J. K.; Palombella, V. J.; McGovern, K.; Adams, J. Semisynthetic Cyclopamine Analogues as Potent and Orally Bioavailable Hedgehog Pathway Antagonists. *J. Med. Chem.* **2008**, *51*, 6646–6649.

(10) Berman, D. M.; Karhadkar, S. S.; Hallahan, A. R.; Pritchard, J. I.; Eberhart, C. G.; Watkins, D. N.; Chen, J. K.; Cooper, M. K.; Taipale, J.; Olson, J. M.; Beachy, P. A. Medulloblastoma growth inhibition by hedgehog pathway blockade. *Science* **2002**, *297*, 1559–1561.

(11) Dahmane, N.; Sanchez, P.; Gitton, Y.; Palma, V.; Sun, T.; Beyna, M.; Weiner, H.; Ruiz i Altaba, A. The Sonic Hedgehog-Gli pathway regulates dorsal brain growth and tumorigenesis. *Development* **2001**, *128*, 5201–5212.

(12) Sanchez, P.; Ruiz i Altaba, A. In vivo inhibition of endogenous brain tumors through systemic interference of Hedgehog signaling in mice. *Mech. Dev.* **2005**, *122*, 223–230.

(13) Stecca, B.; Mas, C.; Clement, V.; Zbinden, M.; Correa, R.; Piguet, V.; Beermann, F.; Ruiz, I. A. A. Melanomas require HEDGEHOG-GLI signaling regulated by interactions between GLI1 and the RAS-MEK/AKT pathways. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 5895–5900.

(14) Keeler, R. F. Teratogenic compounds in Veratrum californicum. IX. Structure−activity relation. *Teratology* **1970**, *3*, 169–173.

(15) Nagata, R.; Izumi, K. Veratramine-induced behavior associated with serotonergic hyperfunction in mice. *Jpn. J. Pharmacol.* **1991**, *55*, 129–137.

(16) Thron, C. D.; McCann, F. V. Pharmacological tests of the mechanism of the periodic rhythm caused by veratramine in the sinoatrial node of the guinea pig. *Gen. Pharmacol.* **1998**, *32*, 81–89.

(17) Williams, J. A.; Guicherit, O. M.; Zaharian, B. I.; Xu, Y.; Chai, L.; Wichterle, H.; Kon, C.; Gatchalian, C.; Porter, J. A.; Rubin, L. L.; Wang, F. Y. Identification of a small molecule inhibitor of the hedgehog signaling pathway: effects on basal cell carcinoma-like lesions. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 4616–4621.

(18) Hosoya, T.; Arai, M. A.; Koyano, T.; Kowithayakorn, T.; Ishibashi, M. Naturally occurring small-molecule inhibitors of hedgehog/GLI-mediated transcription. *ChemBioChem* **2008**, *9*, 1082–1092.

(19) Keeler, R. F. Cyclopamine and related steroidal alkaloid teratogens: their occurrence, structural relationship, and biologic effects. *Lipids* **1978**, *13*, 708–715.

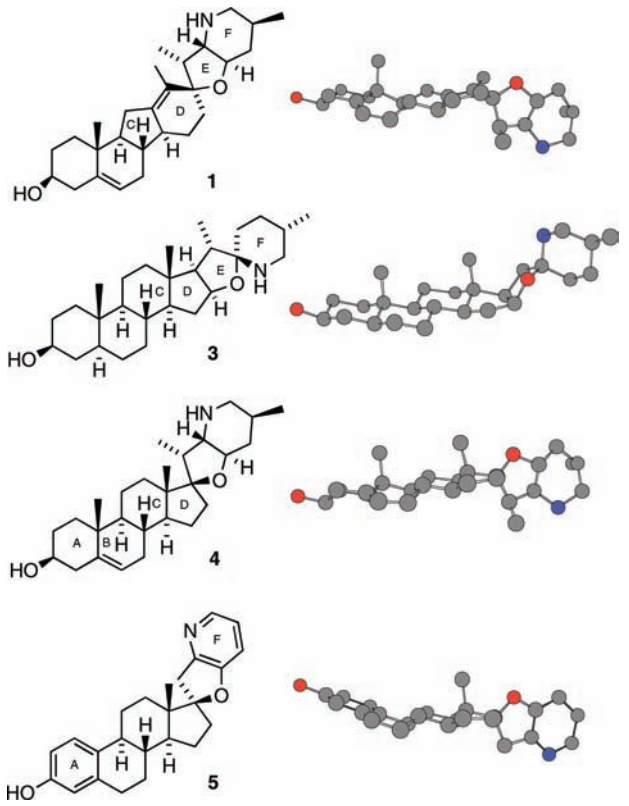


Figure 3. Structures and three-dimensional models of cyclopamine **1**, tomatidine **3**, androstrane analogue **4**, and estrone analogue **5**.

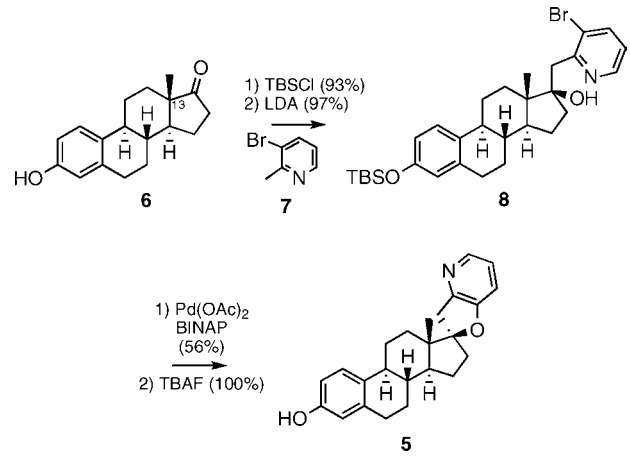
(Figure 3).[19] In contrast, the tetrahydrofuran ring of **3** lies in the steroid plane, and the nitrogen atom is on the α-face of the steroid plane.

We propose that the C-nor-D-homo steroidal ring system of **1** functions as a scaffold for the orientation of the heterobicyclic framework of the EF rings relative to the C-3β oxygen functionality in **1**. Replacement of the C-nor-D-homo steroidal system with the androstane ring system generates **4**. Energy minimization of **4** (MM2), which does not contain the C-nor-D-homo ring system of **1**, leads to a structure that exhibits significant homology to that of **1** (Figure 3). Further stereochemical simplification of **4** can be achieved by aromatization of rings A and F to generate **5**. While the three-dimensional overlap of **5** was not as good as that of **4** with **1** (Figure 3), the relative ease of synthesis of **5** (Scheme 2) led us to first examine the synthesis and biological activity of bis-aromatic analogue **5**.

Addition of the conjugate base of **7** to the C-3 silyl ether of estrone **6** led to the exclusive formation of **8**, in which the anion added to the C-17 ketone from the sterically less hindered α-face. Hartwig−Buchwald-mediated cyclization then afforded the desired dihydrofuran, which upon removal of the C-3 silyl protecting group with TBAF led to **5**.[20,21]

(20) Anderson, K. W.; Ikawa, T.; Tundel, R. E.; Buchwald, S. L. The Selective Reaction of Aryl Halides with KOH: Synthesis of Phenols, Aromatic Ethers, and Benzofurans. *J. Am. Chem. Soc.* **2006**, *128*, 10694–10695.

Scheme 2. Synthesis of Estrone-Derived Analogue **5**



Preliminary biological evaluation of **5** revealed that the simplified structure is a micromolar inhibitor of SHH signaling, as measured by both (1) inhibition of SHH-induced proliferation of mouse granule neuron precursors (GNPs;

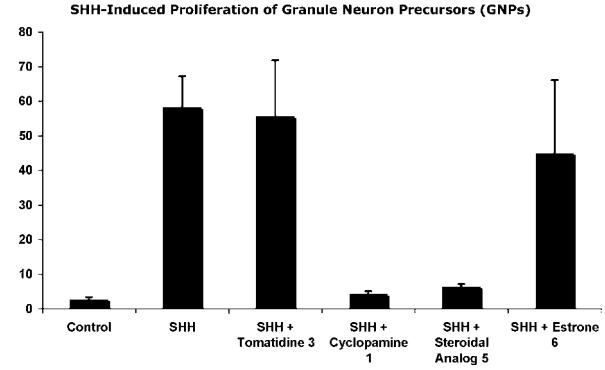


Figure 4. Compound **5** inhibits SHH-induced proliferation of granule neuron precursors (GNPs).

Figure 4) and (2) inhibition of ligand-induced SHH signaling activity in a luciferase-based assay (Figure 5).

Purified mouse P5 GNPs were treated with SHH (600 ng/mL), alone or in combination with cyclopamine **1** (10 μM), tomatidine (10 μM) **3** (cf. a cyclopamine analogue that does not inhibit Hh signaling), steroidal analogue **5** (10 μM), or estrone **6** (10 μM). SHH alone enhances cell proliferation. Addition of cyclopamine **1** significantly decreases cell proliferation for cells treated with SHH while tomatidine **2** has no effect. At 10 μM, compound **5** is equipotent with cyclopamine **1** in inhibiting SHH-induced GNP proliferation. Estrone **6** does not significantly modify the effects of SHH.

We also tested estrone analogue **5** for SHH inhibitory activity using the well-established cell line Light2, which is

(21) Hartwig, J. F. Discovery and understanding of transition-metal-catalyzed aromatic substitution reactions. *Synlett* **2006**, 1283–1294.

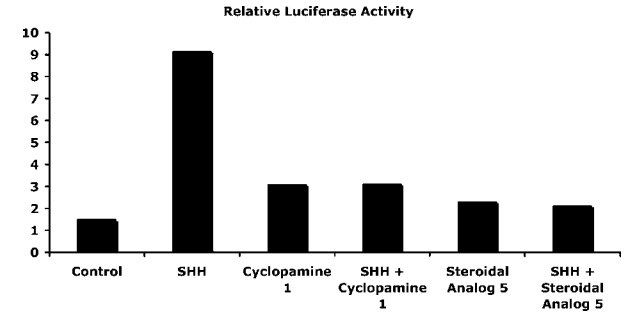


Figure 5. Luciferase-based assay for SHH activity.

a mouse 3T3 cell line clone that stably expresses a Gli-dependent firefly luciferase.[22] Treatment of these cells with recombinant SHH activates GLI-dependent luciferase expression. Treatment of SHH-Light2 cells with recombinant SHH resulted in the strong induction of reporter activity, which was largely blocked by cotreatment with cyclopamine **1** or **5** (10 μM).

These results demonstrate that structurally simple steroid-derived analogues of cyclopamine can function as micromolar inhibitors of Hedgehog signaling in two distinct assays. The systematic analysis of the SAR of **5** is the subject of study in our laboratories, and these results will be reported in due course.

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Supporting Information Available: Experimental procedures and spectral data for estrone analogue **5** and intermediate **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(22) Taipale, J.; Chen, J. K.; Cooper, M. K.; Wang, B.; Mann, R. K.; Milenkovic, L.; Scott, M. P.; Beachy, P. A. Effects of oncogenic mutations in Smoothened and Patched can be reversed by cyclopamine. *Nature* **2000**, *406*, 1005–1009.