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Stereoselective Synthesis of F-Ring Saturated Estrone-Derived Inhibitors of Hedgehog Signaling Based on Cyclopamine

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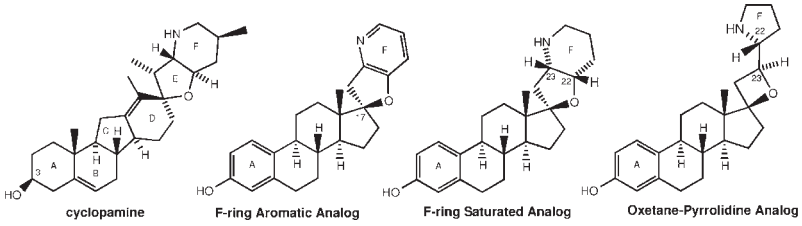
ABSTRACT



Previous work in this laboratory established that the readily available F-ring aromatic analog of cyclopamine is a highly potent inhibitor of Hedgehog signaling. The synthesis and biological evaluation of two F-ring saturated analogs that are more potent than the F-ring aromatic structure are reported.

Some time ago it was found that ingestion of the California corn lily, *veratrum californicum*, by pregnant sheep could induce cyclopia and other profound developmental defects in the offspring. Keeler and co-workers established that the active constituent in the plant is the alkaloid cyclopamine **1** (Figure 1),[1] and it was later found that cyclopamine mediates this effect by interfering with Sonic Hedgehog (SHH) signaling,[2] specifically via the inhibition of the transmembrane protein Smoothened (SMO).[3] SHH signaling is active in the majority of sporadic basal cell carcinomas[4] and also in brain tumors, including medulloblastomas and gliomas.[5] This signaling pathway has also been linked to melanoma,[6] lung adenocarcinoma,[7] as well as prostate,[8] small cell lung,[9] and pancreatic cancer.[10] As a result, the development of inhibitors of the SHH cellular signaling pathway has emerged as an important goal in medicinal chemistry. The most common strategy in the pharmaceutical industry

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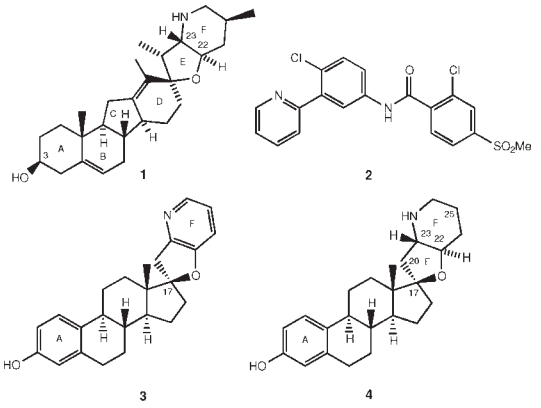

Figure 1. Structures of cyclopamine **1**, GDC-0449 **2**, estrone analog **3**, and saturated analog **4**.

has involved the screening of libraries of diverse chemical structures in the hope of discovering drug-like structures that will interfere with SHH signaling. The most noteworthy success to date using this approach is GDC-0449 **2** (Figure 1), a compound that is currently in phase II clinical trials.[11]

Using a conceptually different approach, we have recently shown that designed, structurally simplified, cyclopamine-like structures such as the estrone-derived analog **3** (Figure 1) are potent inhibitors of Hedgehog signaling.[12] These analogs offer two important advantages over cyclopamine **1**: (1) they do not contain the allylic ether present in **1** that confers metabolic, that is, acid, instability and (2) they are easily prepared (four chemical steps) from commercially available steroids, that is, estrone.

There are several important structural differences between the estrone-derived lead structure **3** and cyclopamine **1**, notably the presence of the aromatic A and F rings in **3** that are not present in **1** (Figure 1). We report herein the design and synthesis of F-ring saturated analogs of **3** that are related to **4**, which contains the same relative stereochemistry at C-22 and C-23 as cyclopamine **1**, and that the SHH signaling inhibitory activity of these new analogs is greater than that of cyclopamine **1** in medulloblastoma cell viability assays.

While the direct reduction of the pyridine ring in **3** would appear to be the most direct approach to the synthesis of F-ring saturated analogs, that is, **4**, we have found the pyridine ring in **3** resistant to direct reduction without extensive decomposition.

Instead, we have developed an alternative approach to the synthesis of F-ring saturated analogs, that leads, *inter alia*, to the synthesis of novel structural analogs lacking the E-ring tetrahydrofuran present in both **1** and in **3**. These new compounds are highly potent inhibitors of SHH signaling, suggesting that the tetrahydrofuran E ring of cyclopamine is not required for biological function.

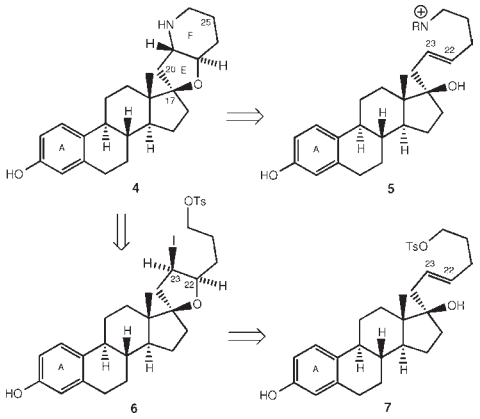

Figure 2. Complementary approaches to the synthesis of the F-ring saturated analog **4**.

We envisioned that the EF heterocyclic moiety of the target structure **4** could be prepared via nitrenium ion mediated bicyclization of **5** (Figure 2). Elegant studies by Wardrop[13] have suggested that such an approach should be feasible. We have initiated a more incremental approach to the synthesis of the EF heterobicyclic ring system, via iodoetherification of **7** to give **6**, which on treatment with ammonia would generate the requisite EF heterobicyclic ring system. The synthesis and reaction of the key iodoetherification substrate **10** is outlined in Scheme 1.

Reaction of the known epoxide **8**[14] (Scheme 1) with the anion formed from the *t*-butyldiphenylsilyl ether of 4-pentynol and removal of the silyl protecting group with TBAF provided alkynol **9**. Reduction of **9** with $LiAlH_4$ stereoselectively provided the (*E*)-alkenol that was reacted with TsCl to generate **10**. In analogy to the work of Knight[15] and Lipshutz[16] on the iodocyclization of homoallylic alcohols, exposure of **10** to iodine and sodium bicarbonate efficiently led to the anticipated *trans*-β-iodotetrahydrofuran **11** as a single diastereomer.[17]

The stereochemical outcome of the reaction is consistent with the model proposed by Knight, in which conformation A, which leads to the formation of the observed product **11**, is favored over conformation B, which would lead to the formation of the diastereomeric *trans*-disubstituted tetrahydrofuran **13** (only the hydrindane moiety of the estrone framework is shown; Figure 3).

Reaction of **11** with liquid ammonia afforded a piperidine product via displacement of both the iodide and tosylate moieties present in **11**, followed by reductive

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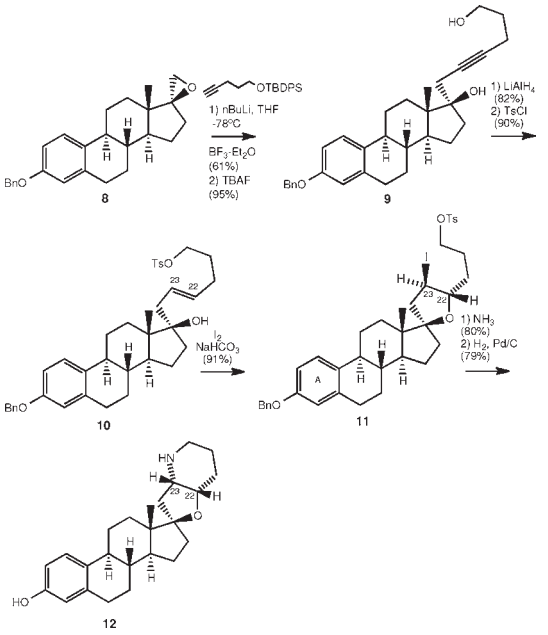
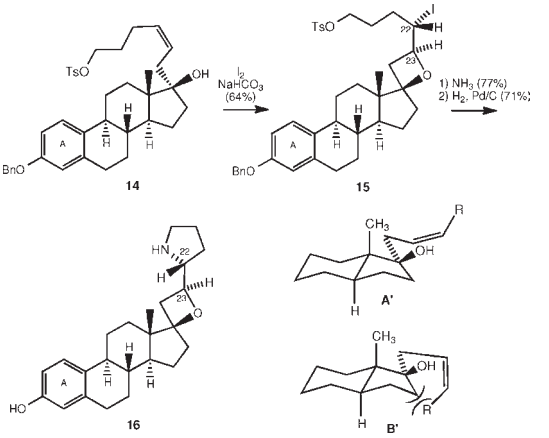
Scheme 1. Synthesis and Cyclization of *E*-Alkene Substrate **10** to Give **12**



Scheme 2. Synthesis and Cyclization of *Z*-Alkene Substrate **14** to Give Pyrrolidino-Oxetane **16**



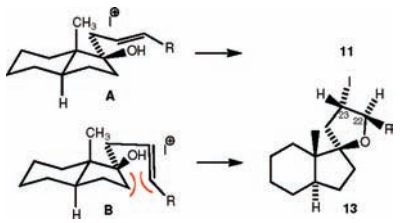


Figure 3. Rationale for the observed formation of **11** and not **13** in the cyclization of *E*-alkene **10**.

debenzylation to generate **12** (in nine chemical steps and 23% overall yield from estrone), the structure of which was confirmed by X-ray crystallographic analysis of the derived para-bromobenzamide. We note that **12** is epimeric with the target structure **4** (Figure 1) at C-22, that is, *cis*- instead of *trans*-EF ring fusion.

Although the work of Knight had demonstrated that the iodoetherification of *Z*-homoallylic alcohols was slower than that of the E-alkene substrates, we envisioned that the trans EF ring fusion stereochemistry found in cyclopamine and in **4** could result from analogous reaction of **14**, the *Z*-alkene corresponding to **10**, as outlined in Scheme 2.

In the event, Lindlar reduction of **9** (Scheme 1) afforded **14**, which on exposure to iodine-mediated cyclization conditions afforded not the anticipated tetrahydrofuran product analogous to **11** (Scheme 1) but instead the oxetane **15** in 64% yield. Reaction of bis-electrophile **15** with ammonia afforded the oxetane-pyrrolidine **16** in 55% overall yield after O-debenzylation. The stereochemistry of **16** was unequivocally established by X-ray crystallographic analysis of the derived 3,5-dinitrobenzamide, confirming that the cyclization occurred with the analogous orientation of the *Z*-alkene (A′ in Scheme 2) as shown in A for the *E*-alkene cyclization substrate **10** (Scheme 1). However, instead of the anticipated tetrahydrofuran product (the result of 5-endo cyclization), based on the work of Knight, we observed the exclusive formation of the oxetane product **15**, the result of 4-exo cyclization of **14**. The basis for this surprising regiochemical outcome is currently under study in our laboratory.

Biological evaluation of **12** and **16** reveals that both are highly potent inhibitors of Hedgehog signaling, comparable to cyclopamine **1** in potency.[18] We first tested **12** and **16** for their activity on the SHH signaling pathway by using the SHH-Light2 cells: this cell line is a 3T3 clone that stably expresses a GLI-dependent reporter.[19] Treatment of these cells with recombinant SHH activates GLI-dependent firefly luciferase expression and this SHH-induced activation is inhibited when cells are also treated with cyclopamine **1**.[18] In this assay, SHH-light2 cells were treated with SHH (600 ng/mL, R&D) alone or in combination with cyclopamine **1**, **12** or **16** (all at 5 μM). As illustrated in Figure 4, **12** and **16** both lead to a strong inhibition of SHH signaling activity comparable to that of cyclopamine.

Granule neuron precursors (GNPs) represent a physiological system for the testing of SHH inhibition, as SHH is a potent mitogen for postnatal mouse cerebellar GNPs,[19] and cyclopamine **1** is known to block their proliferation.[20] As illustrated in Figure 5, GNP proliferation in response to SHH signaling activation is blocked with **1** as well as with compounds **12** and **16**.

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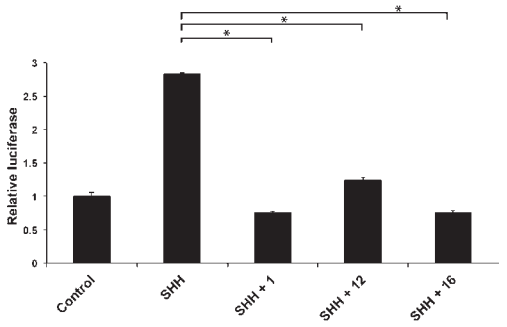

Figure 4. **12** and **16** are potent inhibitors of the SHH-induced GLI-luciferase activity in SHH-Light2 cells. The asterisk indicates *p*-value $p < 0.001$.

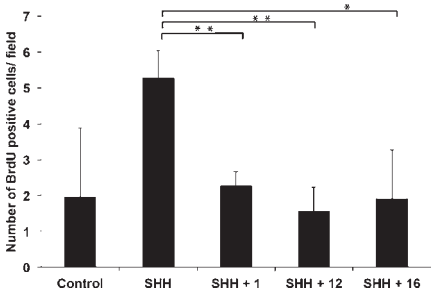

Figure 5. **12** and **16** block SHH-induced granule neuron precursor (GNP) proliferation. In this event, GNPs were purified from postnatal day 5 (P5) mouse cerebella and treated with either SHH alone or in combination with **1**, **12** or **16** (all at 5 μM). BrdU was added to the media 6 h before fixation. Percentage of brdU positive cells were determined by counting with a fluorescence microscope using a 20× objective (Axioskop, Zeiss). At least five independent fields for each culture condition were counted. The analysis was performed on three independent experiments. Statistical analysis was performed with the Student *t* test. The asterisk indicates *p*-value $*p < 0.05$, $**p < 0.01$.

Abnormal activation of the SHH signaling pathway has been linked to various cancers including medulloblastoma, a malignancy of the cerebellum.[21] A subset of these tumors is thought to arise from deregulation of granule neuron precursor (GNP) development in the cerebellum and require SHH signaling for their growth. We thus tested whether compounds **12** and **16** were also efficient in reducing the viability of medulloblastoma cells using human medulloblastoma DAOY cells. DAOY cells were treated for 72 h with either cyclopamine **1**, **12** or **16** before cell survival was assayed with a colorimetric MTT assay, in which absorbance at 570 nm is directly proportional to the number of living cells. As illustrated in Figure 6, there is a significant decrease in cell survival when the cells are treated with either cyclopamine **1**, **12**, or **16**, and that both **12** and **16** are more active in reducing DAOY cell viability than **1**.

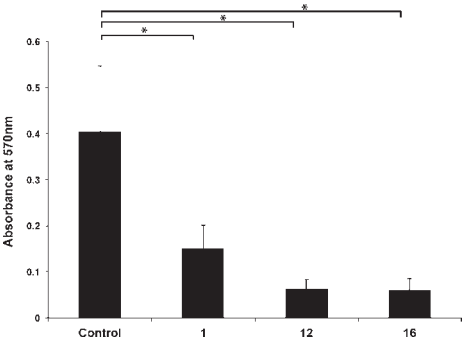

Figure 6. Effect of **12** and **16** on cell survival of human medulloblastoma DAOY cells. The asterisk indicates *p*-value $p < 0.05$.

The significance of the structures of **12** and **16** for observed potency in these diverse biological systems is underscored by the recent disclosure of the synthesis and biological activity of desmethylveramiline **17**, which represents a des-E analog of cyclopamine **1**, by Mann and co-workers (Figure 7).[22] They report that **17** is ca. 50% less potent than **1** in the same SHH-light2 assay described in Figure 4, in which **16** is equipotent with **1**, suggesting that the rigidity imparted by the oxetane ring is important to the observed potency of **16**.

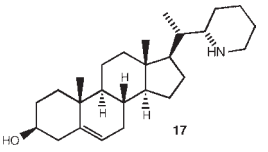

Figure 7. Desmethylveramiline.

We have developed structurally novel analogs **12** and **16** of cyclopamine **1** that exhibit enhanced potency relative to **1** in both GNP proliferation and DAOY medulloblastoma cellular assays. Further studies on stereoisomeric analogs of **12** and **16** and related structures are currently underway in our laboratory, and our results will be reported in due course.

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Supporting Information Available. Full experimental details, characterization data and NMR spectra of all compounds, and crystallographic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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