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A Fast Entry to Furanoditerpenoid-Based Hedgehog Signaling Inhibitors: Identifying Essential Structural Features

Maria Chatzopoulou,[†] Antonia Antoniou,[†] Emmanuel N. Pitsinos,*[,†] Marina Bantzi,[§] Sofia D. Koulocheri,[‡] Serkos A. Haroutounian,[‡] and Athanassios Giannis*[,§]

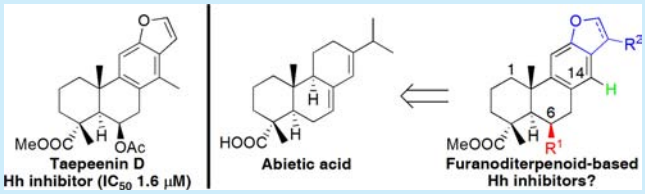
[†]NCSR "Demokritos", P.O. Box 60228, GR-153 10 Ag. Paraskevi, Athens, Greece

[‡]Agricultural University of Athens, Iera Odos 75, GR-118 55 Athens, Greece

[§]Institut für Organische Chemie, Universität Leipzig, Johannisallee 29, D-04103 Leipzig, Germany

Supporting Information

ABSTRACT: New, small molecule Hedgehog (Hh) pathway inhibitors, such as the furanoditerpenoid taepeenin D, are of high medicinal importance. To establish key structure−activity relationships (SARs) for this lead, a synthetic sequence has been developed for the expedient preparation of several derivatives and their evaluation as Hh inhibitors exploiting its structural similarity to abietic acid. While C(14) substitution is not essential for biological activity, the presence of a hydrogen bond acceptor at C(6) and an intact benzofuran moiety are.



The Hedgehog (Hh) signaling pathway is one of the pathways that control embryonic patterning and cellular proliferation and differentiation.[1] In adult organisms the Hh pathway is active for the homeostasis and regeneration of tissues such as skin and bone. However, its abnormal activation has been linked with the occurrence of basal cell carcinoma and meduloblastoma[2] while several other tumors (such as cancers of the skin,[3] brain,[4] lung,[5] pancreas,[6] digestive tract,[7] prostate[8]) are codependent on Hh signaling. Moreover, recent evidence suggests that Hh signaling is important for the self-renewal of cancer stem cells in pancreatic cancer,[9] glioblastoma,[4b] multiple myeloma, and chronic myeloid leukemia.[10] Thus, inhibition of the Hh pathway has become an attractive strategy in anticancer therapy,[11] and several related clinical trials are underway.[12]

Cyclopamine **1** is a naturally occurring alkaloid that was one of the first small-molecule inhibitors ($IC_{50} \approx 5\ \mu M$) of the Hh pathway to be discovered[10] and has attracted considerable attention both as a synthetic target and as a prototype for the design of related analogues.[13] This benchmark inhibitor, as well as the majority of small molecule Hh pathway inhibitors that are currently undergoing clinical trials, target Smoothened (Smo).[11b,14] However, Smo is a protein involved in the early steps of this signaling cascade. In several types of human tumors Hh signaling is constitutively activated due to mutations in Smo, and they are thus insensitive to Smo inhibition. Furthermore, investigations of GDC-0449 (a Smo antagonist used for treatment of medulloblastoma) have indicated that a single amino acid mutation of Smo can give rise to resistance without affecting Hh signaling.[15] Thus, new small molecule Hh inhibitors possessing novel pharmacophores that might be capable of overcoming acquired resistance and/or target signaling molecules downstream of the Smo receptor are highly desired.[10,14] In this context, taepeenin D (Figures 1, 2), a

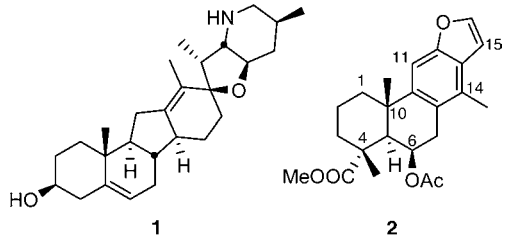


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Figure 1. Structures of cyclopamine, **1**, and taepeenin D, **2**.

cassane-type diterpenoid originally isolated from *Caesalpinia crista*,[16a] was identified as a constituent of *Acacia pennata* with significant Hh/Gli-mediated transcription inhibitory activity (IC_{50} 1.6 μM) and selective cytotoxicity against cancer cells with increased Hh signaling levels (IC_{50} 3.2−3.4 μM).[16b]

A program aiming to establish a convergent and versatile strategy toward the total synthesis of taepeenin D[17] and related furanoditerpenoids,[18] as well as to explore their medicinal potential, was recently initiated. In the latter context it was desirable to establish a fast and reliable synthetic entry to this scaffold and to identify essential structural features for the desired biological activity. To this end, several 14-desmethyl derivatives of taepeenin D have been targeted and their synthesis and evaluation as inhibitors of the Hh signaling cascade are reported herein.

The structural analogy between taepeenin D and abietic acid (**3**, Scheme 1), a readily available resin acid with a rich and well established chemistry,[19] prompted its exploitation as a chiral starting material. Thus, abietic acid was transformed, as

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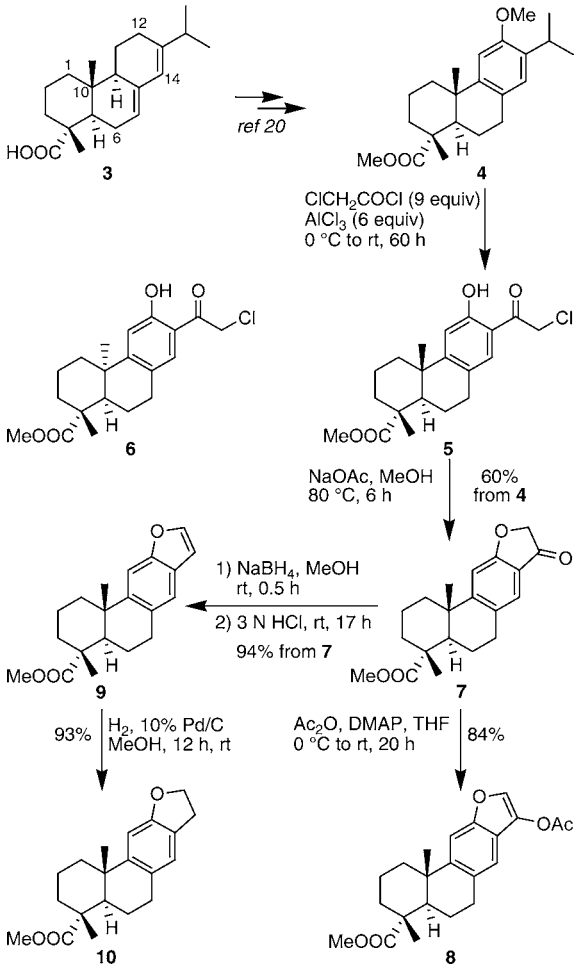
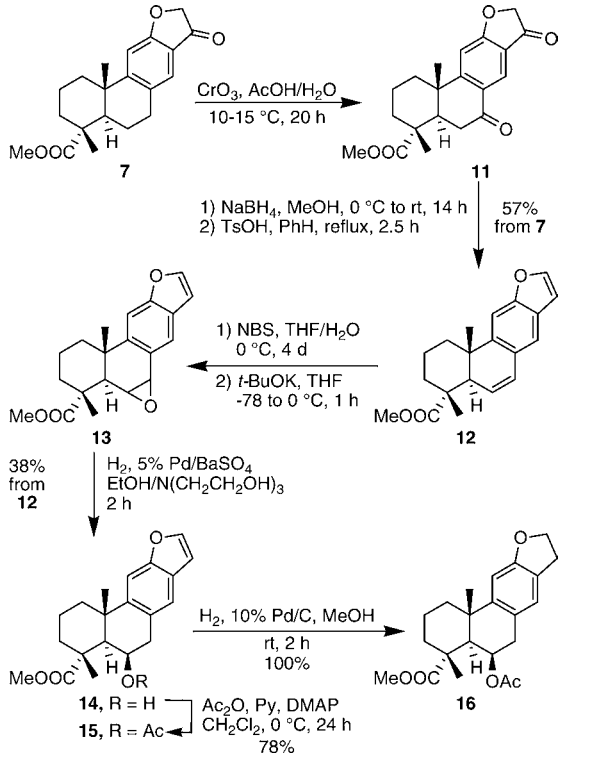
Scheme 1. Synthesis of 6-Deoxy-14-desmethyl Derivatives 7−10



Scheme 2. Synthesis of 14-Desmethyl Derivatives 14−16



previously described,[20] to the 12-methoxy-dehydroabietate derivative **4**. Dealkylation of dehydroabietate derivatives under Friedel−Crafts conditions through *ipso*-substitution is well documented,[21] as are complications that occasionally arise due to concomitant epimerization of the C(10) stereocenter.[21a,e] Indeed, direct conversion of dehydroabietate derivative **4** to phenol **5** with very good yield (87%) has been reported.[21c] Unfortunately, initial attempts to duplicate this report were plagued by low conversion and partial phenol demethylation. More disturbing was the observation that the desired product **5** was contaminated by a varying amount of the epimeric at C(10) isomer **6**.

Eventually, these obstacles were overcome by the use of excess chloroacetyl chloride (9 mol equiv) and aluminum chloride (6 mol equiv) and a prolonged reaction time. Treatment of phenol **5** with a mild base secured benzofuranone derivative **7**, which was subsequently transformed either to acetoxybenzofuran **8** or, through reduction and acid catalyzed dehydration, to benzofuran **9**. Hydrogenation of the latter over 10% Pd/C furnished the dihydrobenzofuran derivative **10**.

In order to gain access to derivatives functionalized at C(6), benzofuranone **7** was subjected to chromic acid oxidation in acetic acid and the diketo derivative **11** thus obtained was transformed to benzofuran **12**, through reduction to the corresponding diols and acid catalyzed double dehydration (Scheme 2).

Direct oxidation of this substrate's C(6)−C(7) double bond employing *m*-CPBA, dimethyldioxirane, or osmium tetroxide based protocols were unsuccessful, presumably due to the lability of the benzofuran moiety under these conditions.[22] Selective oxidation was accomplished with NBS in THF/H_2O to produce a mixture of bromohydrins that upon treatment with potassium *tert*-butoxide at low temperature furnished epoxide **13**.[23] Although the ^{1}H NMR spectrum of the crude epoxide indicated a single diastereomer, its sensitivity hindered rigorous purification/characterization and necessitated immediate use. Thus, subsequent chemoselective hydrogenation over 5% Pd/$BaSO_4$ in the presence of triethanolamine[24] provided the 6-hydroxy-derivative **14** as a single diastereomer;[25] apparently at the bromohydroxylation step, the axial methyl group at C(10) favored formation of the intermediate bromonium ion on the least hindered face of the alkene followed by *anti*-attack of water at the benzylic position. Finally, acetylation furnished 14-desmethyl-taepeenin D (**15**) and the corresponding dihydrobenzofuran derivative **16** was obtained upon hydrogenation over 10% Pd/C.

The ability of derivatives **7**−**10** and **14**−**16** at different concentrations (50−1 μM) to block Hh pathway activation by SAG (100 nM) in Shh-LIGHTII cells, a clonal mouse fibroblast cell line (NIH 3T3) stably transfected with Gli-dependent firefly luciferase and constitutive *Renilla* luciferase reporters,[13c,26] was evaluated.

Derivatives **14** and **15**, which lack the C(14) methyl substitution but bear respectively a hydroxy (hydrogen bond acceptor/donor) or an acetoxy (hydrogen bond acceptor) substituent at C(6), inhibited Gli-mediated transcriptional activity (Figures 2−3). However, no inhibition was observed with compounds **7**−**10** (up to 50 μM), which lack C(6) substitution. Intriguingly, no inhibition was observed with the dihydrobenzofuran derivative **16** (up to 50 μM) despite the

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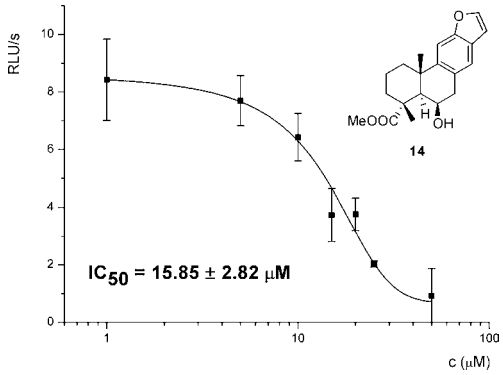

Figure 2. Determination of IC_{50} value for Hh inhibition by compound **14**.

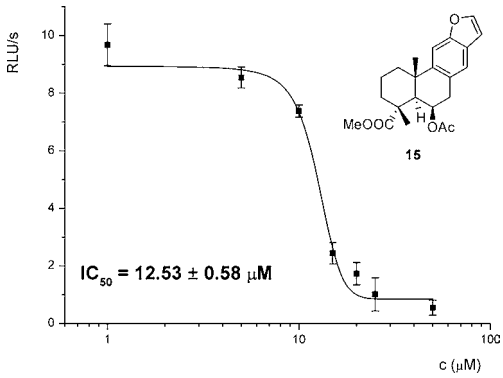

Figure 3. Determination of IC_{50} value for Hh inhibition by compound **15**.

presence of a 6-acetoxy moiety. This is an unexpected result that highlights the importance of an intact benzofuran moiety and justifies further study of taepeenin D derivatives with different electronic/steric characteristics at the aromatic region. Interestingly, derivatives **14** and **15** exhibit comparable potency with cyclopamine ($IC_{50} \approx 16$ and 13 μM respectively for **14** and **15** vs $IC_{50} \approx 5$ μM for cyclopamine[10,13c]).

The above results indicate that, although substitution at C(14) is not a prerequisite for inhibition of Hh signaling by furanoditerpenoid-based inhibitors, the presence of a hydrogen bond acceptor at C(6) is crucial. Furthermore, an intact benzofuran ring appears to be a critical structural feature.

Omission of the C(14) methyl substitution is a significant structural simplification of taepeenin D since it allows an expedient entry to related analogues exploiting the chemistry of abietic and/or podocarpic acid. The retention of Hh inhibitory activity by these derivatives encourages the synthesis and biological evaluation of additional related structures that are currently underway in our laboratories.

■ ASSOCIATED CONTENT

Supporting Information

Spectral data and experimental procedures for compounds **7**−**10**, **12**, and **14**−**16** as well as the biological assay. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: pitsinos@chem.demokritos.gr.
*E-mail: giannis@uni-leipzig.de.

Notes

The authors declare no competing financial interest.

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(25) (a) No epimeric at C(6) alcohol could be identified in the reaction mixture. (b) The stereochemistry of alcohol **14** was deduced by comparison of its ^{1}H NMR spectrum with the one reported (ref 16a) for the closely related taepeenin C; see Table 1 in Supporting Information.

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