Synthesis and Structure Activity Relationship of D-homo Cyclopamine Hedgehog Antagonists: 7-Membered A-ring Lactam Analogs

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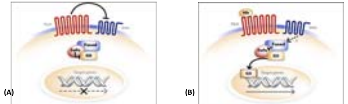
Abstract

Recent evidence suggests that blocking aberrant Hedgehog pathway signaling may be a potential therapeutic strategy for the treatment of several types of cancer. Cyclopamine, a plant *Veratrum* alkaloid natural product, is an antagonist of the Hedgehog pathway and was used as a starting point for the development of improved Hedgehog pathway antagonists. A 7-membered D-ring semi-synthetic analog of cyclopamine, IPI-269609, was shown to have greater acid stability and better aqueous solubility relative to cyclopamine while also having equivalent biological activity in the Hedgehog pathway. Further synthetic manipulations of the A-ring provided a novel series of analogs that potently inhibit the hedgehog pathway. Synthetic transformations of the A-ring into various lactams resulted in the discovery of novel 7-membered ring lactam analogs with a 10 fold improvement in biological activity relative to cyclopamine. The synthetic transformations that resulted in this potent series as well as the structure activity relationship of the products is reported.

Background

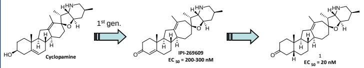
The hedgehog (Hh) signaling pathway is important in tissue growth and differentiation and plays a pivotal role in embryogenesis as well as tissue homeostasis [1]. Over the last decade, there have been an increasing number of reports documenting the implication of the Hh pathway in human diseases, such as cancers [2-15]. Cyclopamine, a steroidal alkaloid isolated from *Veratrum californicum* [16-18], is a potent antagonist of the Hh pathway that has shown tumor growth inhibitory effect in several mouse xenograft models [9-13]. Cyclopamine was shown to act on Smoothened (Smo), a key effector of the Hh pathway [19].

Schematic of the Hedgehog signaling pathway



(**A**) In the absence of ligand, which is typical for most cells in adulthood; (**B**) In several types of cancers, the pathway is activated by the presence of ligand and/or loss-of-function of Ptch and/or gain-of-function of Smo. Cyclopamine blocks the Hh pathway by inhibiting wild-type Smo.

1st generation cyclopamine derived Hh antagonist



Structure of 1st generation analog

We previously reported the development of D-homo cyclopamine analogue IPI-269609 that exhibited improved chemical stability and aqueous solubility relative to cyclopamine [20]. IPI-269609 demonstrated *in vivo* efficacy in several mouse xenograft models [21-22]. However, development of IPI-269609 as a drug candidate was limited by its moderate potency and the low metabolic stability of its A/B ring system.

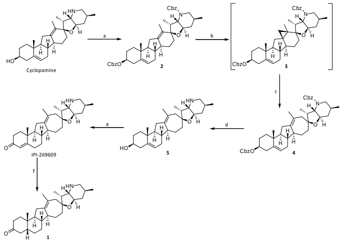
Further SAR studies led to the identification of *cis*-decalone compound **1** as having a >10 fold increase in potency compared to cyclopamine and IPI-269609. Unfortunately, this more potent decalin **1** still presented liabilities of high lipophilicity and rapid clearance. The ketone and the enone motifs on the A/B ring system of compound **1** and IPI-269609, respectively, are particularly prone to metabolic degradation. These analogs are rapidly metabolized *in vitro* by reduction to a C3-alcohol, which are subsequently glucuronidated [23]. Hence, we chose to focus on modifications to the A ring of the more potent decalin analog **1** as a way to improve pharmacological properties.

We considered various less reactive pharmacophores to incorporate into the A-ring that could improve the metabolic stability and reduce *in vivo* clearance. We designed and synthesized various analogs of compound **1** with lactams of various ring sizes as replacements for the A-ring. This work describes the synthesis of lactam analogs that were expected to shunt the metabolic fate of **1** while also retaining its improved potency.

New analogues were evaluated for their ability to inhibit the hedgehog pathway using oxysterol-dependent differentiation of C3H10T1/2 cells, as well as for their *in vitro* metabolic stability in human liver microsomes.

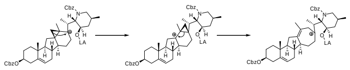
Chemistry background

Synthesis of the 1st generation analog



Reagents and Conditions (a) Cbz-OBt, Et_3N, DMAP, EtOAc, 40 °C; (b) Et_2Zn, $(ArO)_2P(O)OH$, CH_2I_2, DCM, 27 °C; (c) MsOH, -45 °C; (d) H_2, Pd/C, toluene/IPA; (e) Al(O*t*-Bu)$_3$, toluene/MEK, 75 °C; (f) H_2, Pd/C, pyridine.

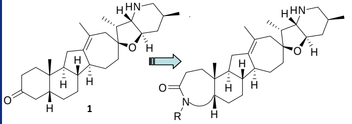
Ring expansion proposed mechanism



Proposed mechanism for the formation of compound **4**

Study design

Proposed structure modifications



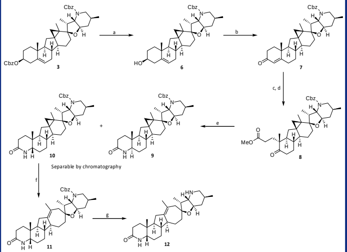
Planned A ring modifications of compound **1**

The ketone motif of the A ring system was specially prone to metabolic degradation. The hypothesis was to replace the ketone with a less reactive lactam and increase the metabolic stability. In addition, our goal was to explore the SAR by varying the size and the substitution pattern of the A ring.

New analogues were evaluated for the ability to inhibit the hedgehog pathway using oxysterol-dependent differentiation of C3H10T1/2 cells, as well as for their metabolic stability in human liver microsomes.

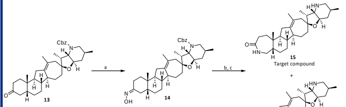
Chemistry

Synthesis of 6-member ring lactam



Reagents and Conditions: (a) NaOH, $MeOH/H_2O$/THF, 55 °C; (b) Al(O*t*-Bu)$_3$, toluene/MEK, 75 °C; (c) $KMnO_4$, $NaIO_4$, Na_2CO_3, *t*-BuOH/H_2O, 80 °C; (d) $TMSCHN_2$ in hexanes, toluene/MeOH; (e) $NaCNBH_3$, NH_4OAc, MeOH, 50 °C; (f) BF_3-OEt_2, DCM, -78 °C; (g) H_2, Pd/C, EtOAc.

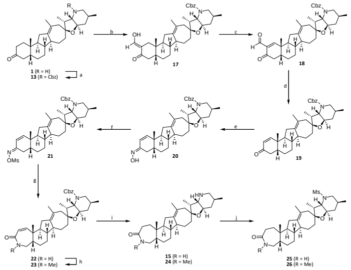
Synthesis of 7-member ring lactam mixture



Reagents and Conditions: (a) $HONH_2$-HCl, NaOAc, EtOH, 25 °C; (b) MsCl, pyridine; (c) H_2, Pd/C, EtOAc.

The unselective Beckmann rearrangement led to an unseparable mixture of regioisomers. A new synthetic approach to compound **15** was elaborated.

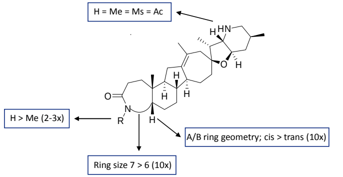
Synthesis of 7-member ring lactam



Reagents and Conditions: (a) CbzCl, Et_3N, 2-MeTHF; (b) HCOOEt, *t*-BuOK, *t*-BuOH, 25 °C; (c) DDQ, toluene, 25 °C; (d) $(PPh_3)_3RhCl$, toluene, 80 °C; (e) $HONH_2$-HCl, NaOAc, EtOH, 25 °C; (f) MsCl, pyridine, 5 °C; (g) HCl, MeOH, toluene, 60 °C; (h) KHMDS, CH_3I, THF, -78 °C to 25 °C; (i) H_2, Pd/C, alcohol; (j) MsCl, Et_3N, DCM.

Results

SAR: Relative potency

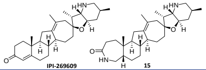


Relative potency (EC50) against C3H10 differentiation assay

The geometry of the AB ring is very important to preserve the potency. Substitution on the nitrogen of the A or F ring are usually tolerated to retain potency (basic nitrogen on the F ring is not necessary), but these substituted compounds are less metabolically stabile. Finally the spatial positioning of the lactam plays an important role; the 7-membered favored.

Results

Comparison of potency and pharmacokinetics for IPI-269609 and compound 15



Potency		
C3H10 (EC50)	200-300 nM	30-40 nM
DMPK		
HLM stability ($T_{1/2}$)	75 min	65 min
F oral		
CD-1 mouse (5 mg/kg, PO)	79%	~100%
Sprague Dawley rat (5 mg/kg, PO)	13%	~100%
Beagle dog (4 mg/kg, PO)	7%	97%
Cynomolgus monkey (4 mg/kg, PO)	69%	~100%
Half-life ($T_{1/2}$)		
CD-1 mouse (5 mg/kg, PO)	3.5 hr	3 hr
Sprague Dawley rat (5 mg/kg, PO)	1.7 hr	5.4 hr
Beagle dog (4 mg/kg, PO)	2.2 hr	6.5 hr
Cynomolgus monkey (4 mg/kg, PO)	2.4 hr	3.8 hr
Volume of distribution (V_d)		
CD-1 mouse (5 mg/kg, PO)	18 L/kg	2 L/kg
Sprague Dawley rat (5 mg/kg, PO)	28 L/kg	4.9 L/kg
Beagle dog (4 mg/kg, PO)	13.9 L/kg	3.0 L/kg
Cynomolgus monkey (4 mg/kg, PO)	21.3 L/kg	4.3 L/kg
Clearance (Cl)		
CD-1 mouse (5 mg/kg, PO)	3.6 L/hr/kg	0.5 L/hr/kg
Sprague Dawley rat (5 mg/kg, PO)	12.4 L/hr/kg	0.63 L/hr/kg
Beagle dog (4 mg/kg, PO)	4.7 L/hr/kg	0.33 L/hr/kg
Cynomolgus monkey (4 mg/kg, PO)	6.2 L/hr/kg	0.8 L/hr/kg

Parameters obtained from the concentration-time profile after IV administration (1 mg/kg); T_{last} = 24 hours in all studies

Conclusions

A number of A-ring lactam cyclopamine derivatives were designed and synthesized with the aim of improving the metabolic stability while retaining potency. The previously observed increase in potency of cyclopamine analogs upon introduction of the cis A/B ring system was also observed in this series. The size of the A ring lactam and the orientation of the amide in the ring were found to be important for optimal Hh antagonistic activity. Compound **14**, having a 7-membered ring lactam and the cis A/B ring system proved to be the best combination of potency and optimal pharmacokinetic profile. Lactam analog **14** stood out among these analogs as a potent and metabolically more stable analog of *cis* decalin **1**. As reported here, the pharmacokinetic profile of lactam **14** is superior to that of IPI-269609 across most species evaluated and is significantly more potent than IPI-269609.

References & Acknowledgements

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