

Synthesis and Structure Activity Relationship of D-homo Cyclopamine Analogs: 3-Substituted Analogs



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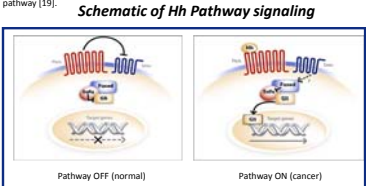
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Abstract

There is increasing evidence suggesting that blocking aberrant Hedgehog signaling can be a very promising and novel therapeutic avenue for the treatment 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 new Hedgehog pathway antagonists. A 7-membered D-ring semi-synthetic analog of cyclopamine, IPI-269609, was previously shown to have greater acid stability and better aqueous solubility relative to cyclopamine. The stereoselective reduction of the enone of IPI-269609 to the di-decane provides analogs with a 10-fold increase in potency relative to cyclopamine. Further synthetic manipulations of the resulting 3-ketone provided a novel series of analogs that potently inhibit the Hedgehog pathway. Synthetic transformations of the 3-ketone as well as the structure-activity relationship of the products will be reported. This work resulted in the discovery of IPI-926, a systemic Hedgehog antagonist currently under clinical evaluation.

Background

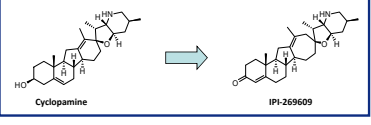
The hedgehog (Hh) signaling pathway (Fig. 1) is important in tissue growth and differentiation and plays a pivotal role in embryogenesis as well as tissue homeostasis [1]. Over the last decade, there have been an increasing number of reports documenting the implication of the Hh pathway in human diseases,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



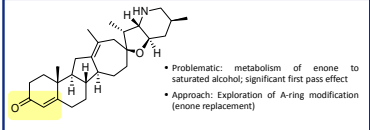
We previously reported D-homo cyclopamine analogue IPI-269609 as having improved chemical stability and aqueous solubility relative to cyclopamine [20]. IPI-269609 demonstrated in vivo efficacy in several mouse xenograft models [21,22]. However, development of IPI-269609 as a drug candidate was limited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

Further SAR studies on this novel class of cyclopamine derivatives focused on improving the potency and metabolic stability. We considered various approaches to modifying the enone moiety of IPI-269609, including reduction of the enone and functionalization of the C3 position. This work describes the synthesis of reduced IPI-269609 analogs as well as 3-substituted analogs that were expected to shunt the metabolic fate of IPI-269609 while also improving potency [23].

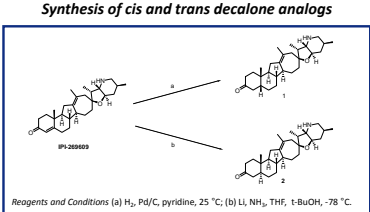
New analogues were evaluated for their ability to inhibit the hedgehog pathway using oxysterol-dependent differentiation of C3H10T1/2 cells, as well as for their in vitro metabolic stability in human liver microsomes.



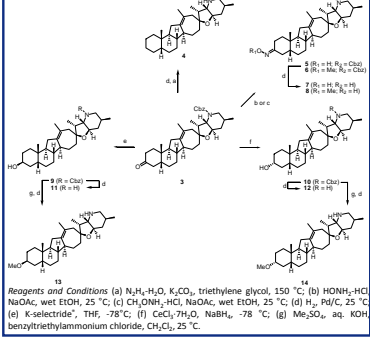
Study Design



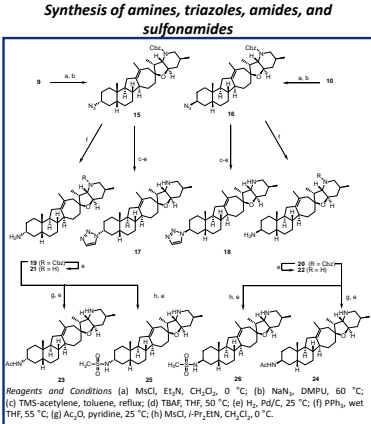
Chemistry



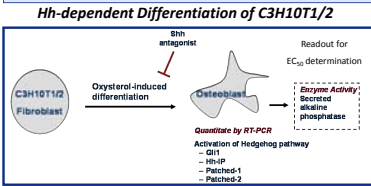
Synthesis of alcohols, ethers, and oximes



Chemistry

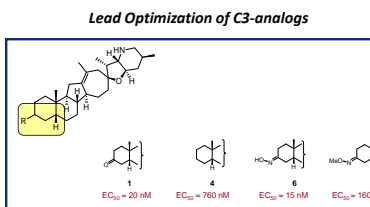
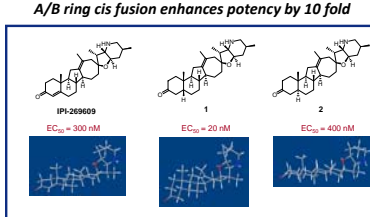


Biological Assays



References: (1) Altaba, A.R.L.; Sánchez, P.; Dahmane, N. *Nature Rev. Cancer*, **2002**, *2*, 361; (2) Lum, L.; Beachy, P.A. *Science*, **2004**, *304*, 1755; (3) Di Magliano, M.P.; Hebrink, M. *Nature Rev. Cancer*, **2003**, *3*, 903; (4) Beachy, P.A.; Karhadkar, S.S.; Berman, D.M. *Nature*, **2004**, *432*, 324; (5) Dahmane, N.; Lee, J.; Robins, P.; *Nature*, **1997**, *389*, 876; (6) Hutchins, M.E.; Knapper, M.S.; Grachtchouk, M.; Wang, Z.; Wei, L.; Cummings, D.; Liu, J.; Michael, L.; Glick, A.; Dhogooti, A.A.; Geres, A.; *Genes Dev.*, **2004**, *18*, 2143; (7) Goodwin, L.V.; Scott, M.P.; *Neuron*, **1998**, *21*, 1243; (8) Kubo, M.; Nakamura, M.; Tazaki, A.; Yamazaki, N.; Nakahama, N.; Nomura, M.; Kuraki, I.; Katano, M.; *Cell*, **2004**, *118*, 1047; (9) Karhadkar, S.S.; Marra, A.; Montes del Oso, R.; Greenblatt, M.B.; Brigg, K.; Parker, A.B.; Sharma, T.; *Science*, **2003**, *425*, 846; (10) Berman, D.M.; Karhadkar, S.S.; Hallahan, A.P.; Hebrink, M.; *Nature*, **2003**, *425*, 846; (11) Berman, D.M.; Karhadkar, S.S.; Hallahan, A.P.; Hebrink, M.; *Nature*, **2003**, *425*, 846; (12) Karhadkar, S.S.; Bova, G.S.; Abdallah, N.; Dhara, S.; Gardner, D.; Matrisa, A.; Innes, J.T.; Berman, D.M.; Beachy, P.A. *Nature*, **2004**, *432*, 707; (13) Watkins, D.N.; Berman, D.M.; Burdickson, S.; Wang, B.; Beachy, P.A.; *Nature*, **2004**, *432*, 131; (14) Rubin, L.S.; Le Sauvage, F.J. *Nature Reviews Cancer*, **2006**, *6*, 1026; (15) Yu, R.; Guo, S.; Tang, Y.; Tian, H.; Ahn, C.; Marhabat, D.; Fu, J.; Jia, J.; Jia, J.; *Nature*, **2008**, *455*, 406; (16) Keeler, R.F. *Phytochemistry*, **1968**, *7*, 301; (17) Keeler, R.F. *Phytochemistry*, **1969**, *8*, 223; (18) Keeler, R.F.; *Wolstenholme*, **1968**, *2*, 5; (19) Chen, K.J.; Tapale, J.; Cooper, M.K.; Beachy, P.A.; *Genes Dev.*, **2002**, *16*, 2743; (20) Tremblay, M.R.; Nevelainen, M.; Nair, S.; Porter, J.R.; Castro, A.C.; Behne, M.; Lu, L.; Yu, L.C.; Hagi, M.; White, K.; Fala, K.; Grenier, L.; Campbell, M.; Coaling, J.; Woodward, C. N.; Hoyt, J.; Foley, M.A.; Reed, M.A.; Sydor, J.; R.; Tong, J.; Palombella, V.J.; McGovern, K.; Adams, J.; *J. Med. Chem.*, **2008**, *51*, 6646; (21) Feldmann, G.; Fendrich, V.; McGovern, K.; Bedja, D.; Bluh, S.; Alvarez, N.; Korostoff, J.; M.; Habbe, N.; Karikari, C.; Mulholland, M.; Gabrielson, K. L.; Sharma, R.; Mattoo, V.; Mitra, A. *Mol. Cancer Ther.*, **2008**, *7*, 2735; (22) McGovern, K.; Piro, C. S.; Wright, J. L. WO2007123511, November 1, 2007; (23) Marra, J. D.; Alvarez-Duez, T. M.; Grogan, M. J.; Porter, J. R.; Castro, A. C.; Sydor, J. R. ASMC Conference, Denver, CO, June 5-8, 2008. **Acknowledgements:** Cyclopamine sourcing (Infinity) Joseph McPherson, Charles Johannes, David Mann; Cyclopamine sourcing (collaborator) US Forestry Service USDA and Steve Mosen; Formulation: Matthew Campbell, Jill Cushing, Jeanne Ferguson, Michael S. Curtis; DMPF: Jens R. Sydor, Jennifer Hoyt.

Results



Comparison of potency and pharmacokinetics for IPI-269609 and compound 25

Parameter	IPI-269609	25 (IPI-926)
IC ₅₀ (EC ₅₀)	200-300 nM	7-15 nM
DMPE		
In vitro HLM stability (T _{1/2})	75 min	85 min
F oral		
CD-3 mouse (5 mg/kg, PO)	79%	>100%
Sprague Dawley rat (5 mg/kg, PO)	13%	>100%
Beagle dog (4 mg/kg, PO)	7%	50%
Cynomolgus monkey (4 mg/kg, PO)	69%	74%
Half-life (T _{1/2})		
CD-3 mouse (5 mg/kg, PO)	3.5 hr	10.5 hr
Sprague Dawley rat (5 mg/kg, PO)	1.7 hr	> 24 hr
Beagle dog (4 mg/kg, PO)	2.2 hr	< 15.5 hr
Cynomolgus monkey (4 mg/kg, PO)	2.4 hr	8.2 hr
Volume of distribution (V _d)		
CD-3 mouse (5 mg/kg, PO)	18 L/kg	11 L/kg
Sprague Dawley rat (5 mg/kg, PO)	28 L/kg	30 L/kg
Beagle dog (4 mg/kg, PO)	13.9 L/kg	15.3 L/kg
Cynomolgus monkey (4 mg/kg, PO)	21.1 L/kg	9.5 L/kg
Clearence (Cl)		
CD-3 mouse (5 mg/kg, PO)	3.6 L/hr/mouse	0.74 L/hr/mouse
Sprague Dawley rat (5 mg/kg, PO)	12.4 L/hr/mouse	0.21 L/hr/mouse
Beagle dog (4 mg/kg, PO)	4.7 L/hr/mouse	0.66 L/hr/mouse
Cynomolgus monkey (4 mg/kg, PO)	6.2 L/hr/mouse	0.5 L/hr/mouse

References & Acknowledgements

Conclusions

The cis conformation of the A/B ring system of cyclopamine analogs was found to have a profound impact on the Hh antagonistic activity of these compounds. In addition, we found polar substituents with an R configuration at the C3 position to be important for maintaining potency. Compound 25 (IPI-926), having a methyl sulfonamide group at C3 with the R configuration and the cis A/B ring system is 20-30 fold more potent and has a favorable pharmacokinetic profile across species relative to IPI-269609.