

Synthesis and Structure Activity Relationship of D-homo Cyclopamine Analogs: A-ring fused Heterocyclic Analogs

Michael J. Grogan, André Lescarbeau, Martin R. Tremblay, Grace Lin, Margit Hagel, Karen McGovern, and Alfredo C. Castro

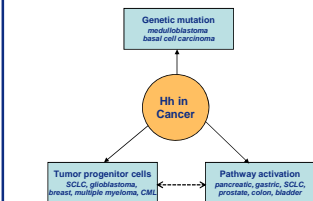
Infinity Pharmaceuticals Inc., 780 Memorial Drive, Cambridge, MA, USA

Abstract

Absent Hedgehog (Hh) signaling pathway has been implicated in several types of cancer. Cyclopamine, a plant Veratrum alkaloid natural product is an antagonist of the Hedgehog pathway and has shown promising anticancer activity. 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. Efforts to improve the biological activity and properties of this novel D-homo cyclopamine scaffold utilized the 3-ketone as a handle for synthetic manipulations. These efforts resulted in the discovery of novel A-ring fused heterocyclic 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 are reported.

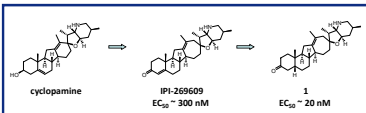
Background

Manifestations of the Hedgehog pathway in cancer¹⁻⁷



Study Design

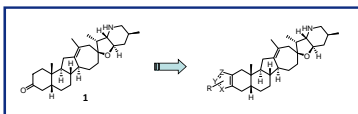
Rationale for heterocyclic cyclopamine analogs



We previously reported the development of D-homo cyclopamine analogs IPI-269609 that exhibited improved chemical stability and aqueous solubility relative to cyclopamine.⁸ IPI-269609 demonstrated in vivo efficacy in several mouse xenograft models.^{9,10} 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 C3-alcohols, which are subsequently glucuronidated.¹¹ 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.

Proposed A-ring modifications

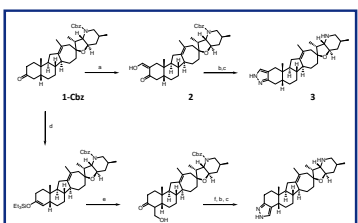


We considered various less reactive pharmacophores to incorporate onto the A-ring that could improve the metabolic stability and reduce in vivo clearance. Fusion of heterocycles to the A-ring of steroid 1 has successfully provided analogs with improved pharmacologic profiles. With this in mind, we designed and synthesized various analogs of compound 1 having heterocycles fused to the A-ring.

This work describes the design and synthesis of such analogs which were expected to shunt the metabolic fate of 1 while also retaining its improved potency. New analogs were evaluated for their ability to inhibit the hedgehog pathway using oxysterol-dependent differentiation of CH10T1/2 cells, as well as for their in vitro metabolic stability in human liver microsomes.

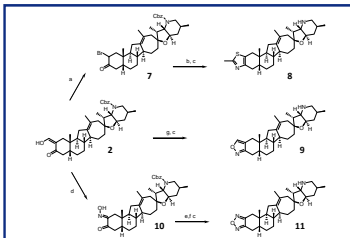
Synthesis

Pyrazoles fused to the cis-decalin



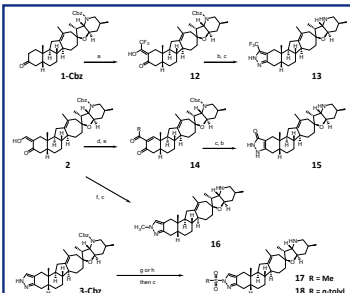
Reagents and Conditions (a) HCODEt, t-BuOK, t-BuOH, 25 °C (b) N₂H₄·H₂O, EtOH, 80 °C (c) H₂, Pd/C, 25 °C (d) KHMDS, Et₃SiH, -78 °C (e) HCHO, TBAC, CH₂Cl₂, 0 °C (f) Dess-Martin periodinane, CH₂Cl₂, 0 °C

Thiazole, oxazole, and oxadiazole steroid alkaloids



Reagents and Conditions (a) NBS, HOAc, NaOAc, wet dioxane, 25 °C (b) CH₂Cl₂/NH₂EtOH, reflux (c) H₂, Pd/C, 25 °C (d) NaNO₂, aq. CH₃CO₂H, 0 °C (e) HONH₂·HCl, NaOH, EtOH, 25 °C (f) KOH, dioxane, ethylene glycol, 120 °C (g) HONH₂·HCl, pyridine, 120 °C

Modifications to the 2,3-pyrazole



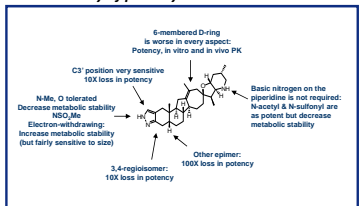
Reagents and Conditions (a) CF₃COEt, t-BuOK, t-BuOH, 25 °C (b) N₂H₄·H₂O, EtOH, 80 °C (c) H₂, Pd/C, 25 °C (d) DOD, toluene, 25 °C (e) PdC, MeOH, DMF, 25 °C (f) CH₃NHNH₂, EtOH, 80 °C (g) MsCl, pyridine, 25 °C (h) TsCl, pyridine, CH₂Cl₂, 25 °C

References & Acknowledgements

References: (1) Goodrich, L.V.; Scott, M.P. *Neuron*, 1998, 21, 1243; (2) Kubo, M.; Nakamura, M.; Tasaki, A.; Yamashita, N.; Kashiwagi, H.; Nomura, K.; Kuroki, S.; Katano, M.; Cancer Res., 2004, 64, 6071; (3) Bertram, D.M.; Karhadkar, S.S.; Maltra, A.; Montes de Oca, R.; Gerstenblith, M.R.; Briggs, K.; Parker, A.R.; Shimata, Y.; Eshleman, J.R.; Watkins, D.N.; Beachy, P.A. *Nature*, 2003, 425, 846; (4) Bertram, D.M.; Karhadkar, S.S.; Halahan, A.R.; Pritchard, L.; Oberhart, C.G.; Watkins, D.N.; Chen, K.; Cooper, M.K.; Taylor, J.; Olson, J.M.; Beachy, P.A. *Science* 2002, 297, 1155; (5) Thayer, S.P.; Di Magliano, M.P.; Helms, P.W.; Hansen, C.M.; Roberts, D.J.; Lawless, G.J.; Di, Y.P.; Guin, S.; Castillo, C.F.; Yanik, V.; Antoniou, B.; McMahon, M.; Warrshaw, A.L.; Hebrock, M. *Nature*, 2003, 425, 851; (6) Karhadkar, S.S.; Bova, S.; Abdallah, N.; Dhara, S.; Gardner, D.; Maltra, A.; Isaacs, J.T.; Bertram, D.M.; Beachy, P.A. *Nature*, 2004, 431, 707; (7) Watkins, D.N.; Bertram, D.M.; Burkholder, S.G.; Dhara, S.; Beachy, P.A. *Nature*, 2003, 422, 313; (8) Tremblay, M.R.; Nevalainen, M.; Nair, S.; Porter, J.R.; Castro, A.C.; Behr, L.H.; Yu, L.C.; Hagel, M.; Patis, K.; Grenier, L.; Campbell, M.J.; Cushing, J.; Woodward, C.N.; Hoyt, J.; Foley, M.A.; Read, M.A.; Sydor, J.; Ji, Tong, J.C.; Palombella, V.J.; McGovern, K.; Adams, J.J. *Mol. Chem. Commun.* 2008, 5, 6446; (9) Feldman, G.; Fendrich, V.; McGovern, K.; Bejo, D.; Bains, S.; Alvarez, M.; Koestler, J.B.; McHabb, K.; Karickhoff, C.; Mulvender, M.; Cabrerola, M.; Gaboritoni, K.; J.; Sharma, R.; Marzari, W.; Maltra, A. *Mol. Cancer Ther.* 2008, 7, 275; (10) McGovern, K.; J.; Piro, C.S.; Wright, J.L.; WOOD0212311; November 1, 2007; (11) Manna, J.D.; Alvarez-Diez, T.M.; Grogan, M.J.; Porter, J.R.; Tremblay, M.R.; Castro, A.C.; Sydor, J.R. *ASMS Conference*, Denver, CO, June 1-5, 2008; (12) Tremblay, M.R.; Lescarbeau, A.; Grogan, M.J.; Tan, L.; Lin, G.; Campbell, M.J.; Nair, S.; Ji, Tong, J.C.; Bernik, M.; White, K.; Conley, J.; Manna, J.; Alvarez-Diez, T.; Hoyt, J.; Pink, M.; MacDougall, J.; Campbell, M.J.; Cushing, J.; Ferguson, J.; Curtis, M.S.; McGovern, K.; Read, M.A.; Palombella, V.J.; Castro, A.C.; Adams, J. *manuscript submitted*. Acknowledgements: Formulation: Matthew Campbell, Jill Cushing, Jeanne Ferguson, Michael S. Curtis; DMPK: Jens R. Sydor, Jennifer Hoyt.

Results

SAR summary of potency and metabolism



Conversion of the 3-ketone of 1 to a 2,3-fused heterocycle shows retention of potency for pyrazole and isoxazole. Heterocycles with a heteroatom bound to the A-ring C2 give considerable loss in potency, as do substitutions on C3' of the pyrazole. The heterocycles range in microsomal stabilities, beyond the $t_{1/2}$ of ketone 1 (70 min).

Hh-dependent cellular activity and in vitro metabolic stability

	EC ₅₀ (nM)	Differentiation of C3H10 cells	Half-life (min)	Stability against human liver microsomes
3	13	120		
6	220	27		
8	270	100		
9	62	8		
11	230	8		
15	170	100		
13	170	200		
16	54	35		
17 R=Me	140	80		
18 R=p-tolyl	900	65		

Results

Compound profile (IPI-269609 and the pyrazole 3)

Potency	200-300 nM	13 nM
CD110 (EC50)		
DMPK		
HLM stability (T _{1/2})	75 min	120 min
F oral		
CD-1 mouse (5 mg/kg, PO)	79%	52%
Sprague Dawley rat (5 mg/kg, PO)	13%	34%
Beagle dog (4 mg/kg, PO)	6%	74%
Cynomolgus monkey (4 mg/kg, PO)	79%	83%
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	1.2 hr
Beagle dog (4 mg/kg, PO)	2.4 hr	6.8 hr
Cynomolgus monkey (4 mg/kg, PO)	2.2 hr	5.6 hr
Volume of distribution (V _d)		
CD-1 mouse (5 mg/kg, PO)	18 L/kg	9 L/kg
Sprague Dawley rat (5 mg/kg, PO)	28 L/kg	13 L/kg
Beagle dog (4 mg/kg, PO)	13.3 L/kg	6 L/kg
Cynomolgus monkey (4 mg/kg, PO)	21.3 L/kg	13 L/kg
Clearance (Cl)		
CD-1 mouse (5 mg/kg, PO)	3.6 L/hr/kg	2 L/hr/kg
Sprague Dawley rat (5 mg/kg, PO)	12.4 L/hr/kg	7.5 L/hr/kg
Beagle dog (4 mg/kg, PO)	4.7 L/hr/kg	0.6 L/hr/kg
Cynomolgus monkey (4 mg/kg, PO)	6.2 L/hr/kg	1.8 L/hr/kg

Conclusions

Although IPI-269609 has favorable pharmacological properties relative to cyclopamine, it was only moderately potent in the Hh pathway and its A/B ring enone was extensively metabolized in multiple species after oral administration.¹¹ Consequently, IPI-269609 demonstrated modest efficacy in certain tumor xenograft models^{9,10} and no efficacy in the B337A medulloblastoma allograft model when administered orally at doses approaching the MTD. Further medicinal chemistry on D-homo cyclopamine analogs focused on improving potency and metabolic stability of IPI-269609. A significant improvement in potency was realized upon reduction of the enone to the cis-decalin A/B ring system, yet it was still prone to metabolism and rapid in vivo clearance. A number of fused heterocyclic D-homo cyclopamine analogs were designed and synthesized with the aim of improving the metabolic stability while retaining potency. Pyrazole analog 3 stood out among these analogs as a potent and metabolically more stable analog of compound 1. As reported here, the pharmacologic profile of pyrazole 3 is superior to that of IPI-269609 in non-rodents and is significantly more potent than IPI-269609. The work guided the identification of IPI-026 as a clinical drug candidate in cancer. In addition to the clinical investigation of IPI-026, studies are on-going to fully characterize the mode of action and other potential applications of these novel Hedgehog pathway antagonists.¹²