

Letters

Potent Inhibitors of the Hedgehog Signaling Pathway

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Abstract: A small family of phenyl quinazolinone ureas is reported as potent modulators of Hedgehog protein function. Preliminary SAR studies of the urea substituent led to a nanomolar Hedgehog antagonist.

The Hedgehog (Hh) proteins are essential in embryonic development.^{1–3} The protein–ligands for the receptor, Sonic (SHh), Indian (IHh), and Desert (DHh), as well as the other protein components of the Hedgehog cascade, show very high interspecies homology from species as diverse as *Drosophila* and human.

The three Hedgehog ligands, SHh, IHh, and DHh, are morphogens that have different roles in embryogenesis. Thus, IHh affects cartilage and bone development, SHh influences neuronal development in the CNS, and DHh regulates peripheral neuronal development.

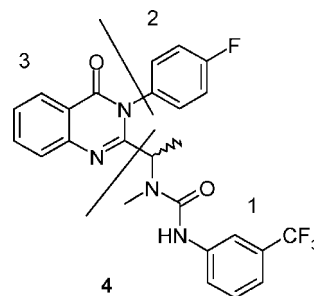
Inappropriate activation of the Hedgehog pathway occurs in several cancers such as basal cell carcinoma (BCC) and medulloblastoma.^{4–7}

The complex natural product cyclopamine, one of the jervine family of compounds isolated from the lily *Veratrum californicum*, acts upon the Hh pathway as an antagonist.⁸ Cyclopamine is a teratogenic compound that produces cyclopia, among other effects, in offspring of sheep that graze upon the *Veratrum* lily. Recently, modern cellular pharmacology experiments with cyclopamine have shown it to modify Hedgehog pathway activation in cell cultures.⁹ It has been reported that the proliferation of human tumor cell lines, including medulloblastomas¹⁰ and basal cell carcinomas (BCCs),¹¹ is inhibited in culture by cyclopamine. However, cyclopamine presents a poor lead structure for modification because it is a very complex, hydrophobic azasteroid with 10 chiral centers.

We therefore became interested in finding an alternative small molecule inhibitor of the Hh pathway that contained drug-like characteristics and would lend itself well to chemical manipulations.

As part of our screening work, we found a hit with weak antagonist activity, IC₅₀ ~1.4 μ M, whose structure was confirmed as racemic quinazolinone **4** (Chart 1) by resynthesis. A similar route was used to one that has been previously described¹² (Scheme 1, Route A). The first step involved the formation of an oxazinone by heating anthranilic acid with

Chart 1. Initial Hh Antagonist Screening Hit



propionic anhydride. This was opened to the bisamide by heating with 4-fluoroaniline in chloroform then cyclized to the quinazolinone by heating in ethylene glycol at 130–140 °C to give **1**. The ethyl side chain was brominated with bromine in acetic acid buffered with sodium acetate and converted to amine (**2**) using methylamine in ethanol. The final urea formation to give **4** was achieved using 3-trifluoromethylphenyl isocyanate in chloroform. Testing of racemic **4** in the Hh assay¹³ confirmed the IC₅₀ as 1.4 μ M. Intermediates **1** and **2** were also tested for activity but were considered to be much less active (IC₅₀ > 10 μ M).

The initial aims were to identify a compound with improved potency (<100 nM) over **4**. There were three obvious regions of **4** that could be investigated to reach this goal, as indicated in Chart 1: the urea group, the 4-fluorophenyl, and the quinazolinone ring. Here, we describe some preliminary SAR work that led to tractable, nanomolar potent Hh pathway antagonists.

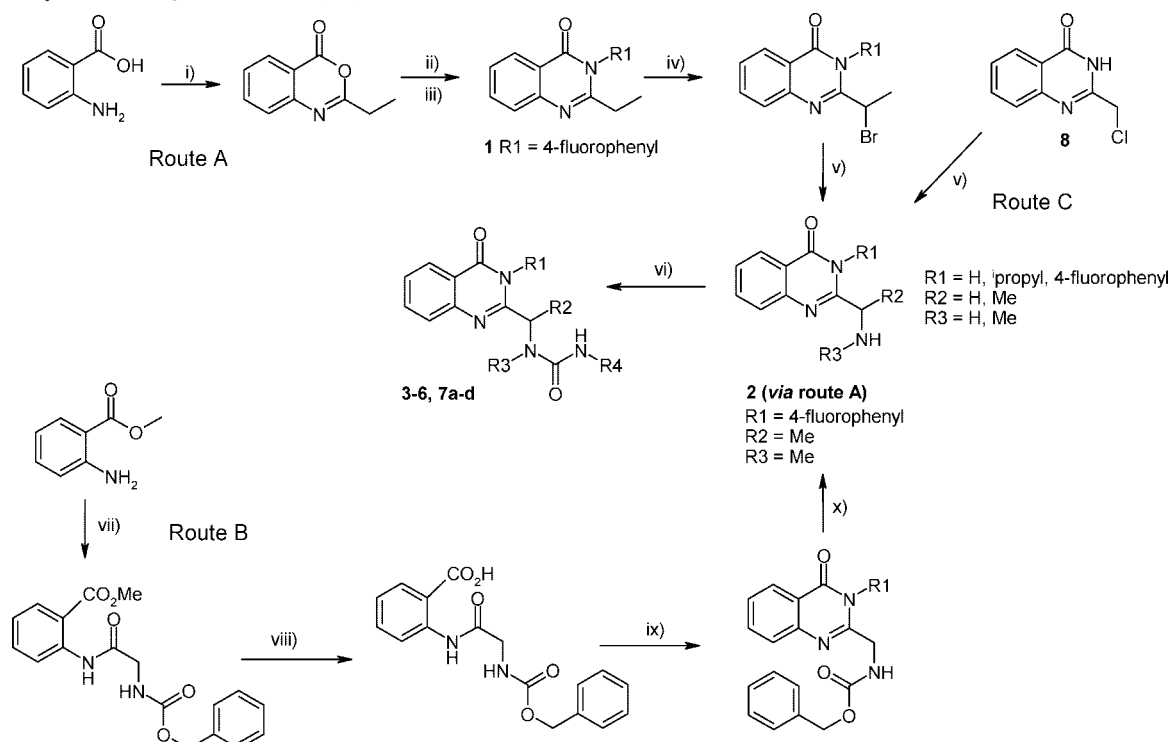
Initially, we investigated the importance of the two methyl groups in **4** for activity and prepared the bis-desmethyl analogue (**7a**) starting from methyl anthranilate (Scheme 1, Route B). A coupling reaction with *N*-Cbz glycine using CDI^a in THF gave an amide, which was saponified with lithium hydroxide in aqueous dioxane. The resulting acid was coupled with 4-fluoroaniline then cyclized to the quinazolinone using CDI in THF. The Cbz protecting group was removed by hydrogenation, and the resulting amine was treated with 3-trifluoromethylphenyl isocyanate to give **7a**. Testing of this compound established that both methyl groups were not required for activity because a 2-fold increase in activity was obtained over **4** (Table 1).

A monomethyl derivative **5** was also prepared (Scheme 1, Route A) following the same route as described for **4** except that ammonium chloride was used as a source of ammonia to form the primary amine, followed by treatment with 3-trifluoromethyl isocyanate to give the final product. Interestingly, **5** proved to be ~3-fold less active than the parent compound (**4**) and ~7-fold less active than the bis-desmethyl compound (**7a**). With that result in mind, three further bis-desmethyl ureas (**7b–d**) were prepared (Scheme 1, Route B) to explore SAR around that region. The final compounds were prepared starting from methyl anthranilate as described for **7a**. Compound **7d**

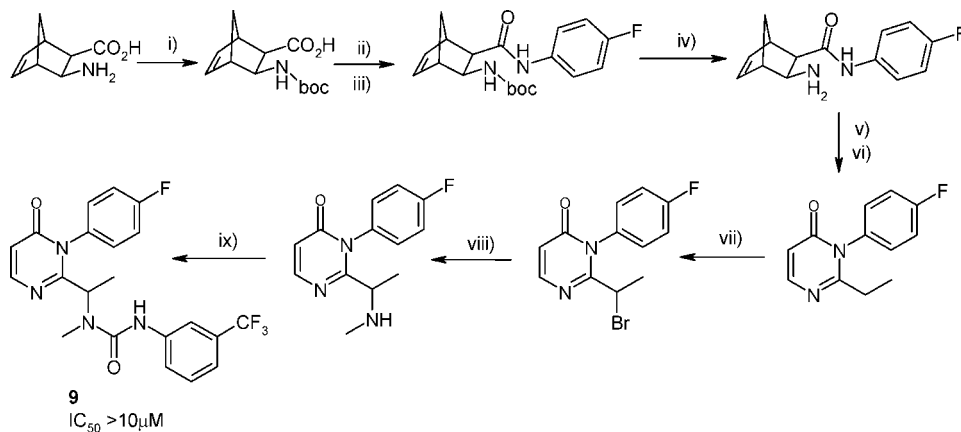
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^a Abbreviations: CDI, 1,1'-carbonyl diimidazole; Cbz, benzyl oxycarbonyl; Boc, *tert*-butoxycarbonyl; TFA, trifluoroacetic acid; DCM, dichloromethane.

Scheme 1. Synthesis of Quinazolinones (1-7)^a

^a Reagents and conditions: (i) (R₂CH₂CO)₂O, reflux, 4 h, 90%; (ii) R₁NH₂, CHCl₃, reflux, 6 h; (iii) NaOH, ethylene glycol, 130–140 °C, 5 h, quantitative; (iv) bromine, AcOH/NaOAc, 40 °C, 5 h, 96%; (v) R₃NH₂, EtOH, 40 °C, 1 h, 62% quantitative; (vi) R₄NCO, CHCl₃, rt, 1 h, 11% quantitative; (vii) *N*-Cbz-amino acid, CDI, THF, rt, 22 h, 14%; (viii) LiOH, dioxane, water, rt, 16 h, 96%; (ix) R₁NH₂, CDI, THF, reflux, 20 h, 48%; (x) H₂, 10% Pd/C, EtOH, rt, 2 h, 95%.

Scheme 2. Synthesis of pyrimidinone analogue (9)^a

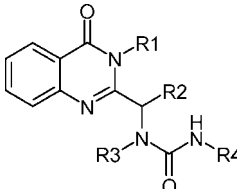
^a Reagents and conditions: (i) (Boc)₂O, dioxan, 1 N NaOH; rt, 6 h, 85%; (ii) *i*-BuOCOCl, Et₃N, THF, –20 °C, 20 min; (iii) 4-fluoroaniline, THF, <–7 °C, 5 h then rt, 18 h, quantitative; (iv) TFA, CH₂Cl₂, rt, 4 h, 64%; (v) triethyl orthopropionate, 100 °C, 7 h; (vi) toluene, reflux, 26 h, quantitative; (vii) bromine, AcOH/NaOAc, 40 °C, 2 h, 96%; (viii) MeNH₂, EtOH, rt, 3 h, 76%; (ix) R₄NCO, CHCl₃, rt, 1 h, 66%.

proved to be the most potent derivative from this series, with an IC₅₀ of 70 nM, providing a 20-fold increase in activity over **4**.

The requirement of the 4-fluorophenyl group for activity was next investigated and the NH analog **3** was prepared starting from the commercially available quinazolinone **8** (Route C). Treatment of **8** with methylamine followed by 3-trifluoromethylphenyl isocyanate gave the target compound **3**, which proved to be much less active (> 10 μM). We next explored alkyl replacement of the 4-fluorophenyl and prepared the *N*-isopropyl analogue **6** from methyl anthranilate (Scheme 1, Route B), as described for **7a**, except that isopropyl amine was used in the quinazolinone forming step. This compound resulted

in a 3-fold decrease in activity compared to **4**, suggesting that the phenyl ring may be required for activity.

Finally, the requirements of the quinazolinone ring for activity were explored by preparing the truncated pyrimidinone derivative **9** (Scheme 2). Aminonorborene carboxylic acid was protected with Boc anhydride in dioxane with 1 N NaOH. The acid was coupled with 4-fluoroaniline via the mixed anhydride from *iso*-butyl chloroformate. The Boc protecting group was removed with TFA in DCM and the annulated pyrimidinone formed by heating with triethyl orthopropionate. A retro Diels–Alder reaction was then performed in refluxing toluene overnight to leave the pyrimidinone. This was brominated, reacted with methylamine and finally treated with 3-trifluoro-

Table 1. Quinazolinone SAR


compd	R1	R2	R3	R4	IC ₅₀ , ^d nM
3 ^c	H	H	methyl	3-F ₃ C phenyl	>10000
4 ^a	4-fluorophenyl	methyl	methyl	3-F ₃ C phenyl	1400 ± 300
5 ^a	4-fluorophenyl	methyl	H	3-F ₃ C phenyl	4700
6 ^b	isopropyl	H	H	3-F ₃ C phenyl	4300
7a ^b	4-fluorophenyl	H	H	3-F ₃ C phenyl	700
7b ^b	4-fluorophenyl	H	H	3-F ₃ C, 5-OMe phenyl	250
7c ^b	4-fluorophenyl	H	H	3,5-di Cl, 4-pyridyl	110
7d ^b	4-fluorophenyl	H	H	3-F ₃ C, 4-Cl phenyl	70 ± 20

^a Route A. ^b Route B. ^c Route C. ^d See ref 13 and Supporting Information for assay details.

phenyl isocyanate to give the target urea, as described for Route A (Scheme 1). This compound proved to be much less active (>10 μ M), which suggests that the quinazolinone ring is important for activity.

In conclusion, we have described the optimization of a micromolar hit **4** to afford compound **7d**, a nanomolar potent Hh antagonist. As such, **7d** and its structural analogs may have potential as novel cancer therapeutics.

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Supporting Information Available: Experimental procedures and data for all final compounds, and experimental details for the "Gli-Luc" assay. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) Chiang, C.; Litingtung, Y.; Lee, E.; Young, K. E.; Corden, J. L.; Westphal, H.; Beachy, P. A. Cyclopia and defective axial patterning in mice lacking *Sonic hedgehog* gene function. *Nature* **1996**, *383*, 407.
- (2) St-Jacques, B.; Hammerschmidt, M.; McMahon, A. P. Indian hedgehog signaling regulates proliferation and differentiation of chondrocytes and is essential for bone formation. *Genes Dev.* **1999**, *13*, 2072.
- (3) Ingham, P. W.; McMahon, A. P. Hedgehog signaling in animal development: paradigms and principles. *Genes Dev.* **2001**, *15*, 3059.
- (4) Hahn, H.; Wicking, C.; Zaphiropoulos, P. G.; Gailani, M. R.; Shanley, S.; Chidambaram, A.; Vorechovsky, I.; Holmberg, E.; Uden, A. B.; Gilles, S.; Negus, K.; Smyth, I.; Pressman, C.; Leffell, D. J.; Gerrard, B.; Goldstein, A. M.; Dean, M.; Toftgard, R.; Chenevix-Trench, G.; Wainwright, B.; Bale, A. E. Mutations of the human homolog of

Drosophila patched in the nevoid basal cell carcinoma syndrome. *Cell* **1996**, *85*, 841.

- (5) Johnson, R. L.; Rothman, A. L.; Xie, J.; Goodrich, L. V.; Bare, J. W.; Bonifas, J. M.; Quinn, A. G.; Myers, R. M.; Cox, D. R.; Epstein, E. H., Jr.; Scott, M. P. Human homolog of patched, a candidate gene for the basal cell nevus syndrome. *Science* **1996**, *272*, 1668.
- (6) Raffel, C.; Jenkins, R. B.; Frederick, L.; Hebrink, D.; Alderete, B.; Fults, D. W.; James, C. D. Sporadic medulloblastomas contain PTCH mutations. *Cancer Res.* **1997**, *57*, 842.
- (7) Xie, J.; Murone, M.; Luoh, S. M.; Ryan, A.; Gu, Q.; Zhang, C.; Bonifas, J. M.; Lam, C. W.; Hynes, M.; Goddard, A.; Epstein, E. H., Jr.; de Sauvage, F. J. Activating Smoothened mutations in sporadic basal-cell carcinoma. *Nature* **1998**, *391*, 90.
- (8) (a) Keeler, R. F.; Binns, W. Teratogenic compounds of *Veratrum californicum* V. Comparison of cyclopean effects of steroidal alkaloids from the plant and structurally related compounds from other sources. *Teratology* **1968**, *1*, 5. (b) The Hh pathway relies on a number of complex ligand-protein and protein-protein interactions that are not yet completely elucidated. Chen, J. K.; Taipale, J.; Cooper, M. K.; Beachy, P. A. Inhibition of hedgehog signaling by direct binding of cyclopamine to Smoothened. *Genes Dev.* **2002**, *16*, 2743.
- (9) Cooper, M. K.; Porter, J. A.; Young, K. E.; Beachy, P. A. Teratogen-mediated inhibition of target tissue response to Shh signaling. *Science* **1998**, *280*, 1603.
- (10) Berman, D. M.; Karhadkar, S. S.; Hallahan, A. R.; Pritchard, J. I.; Eberhart, C. G.; Watkins, D. N.; Chen, J. K.; Cooper, M. K.; Taipale, J.; Olson, J. M.; Beachy, P. A. Medulloblastoma growth inhibition by hedgehog pathway blockade. *Science* **2002**, *297*, 1559.
- (11) Williams, J. A.; Guicherit, O. M.; Zaharian, B. I.; Xu, Y.; Chai, L.; Wichterle, H.; Kon, C.; Gatachalian, C.; Porter, J. A.; Rubin, L. A.; Wang, F. Y. Identification of a small molecule inhibitor of the hedgehog signaling pathway: Effects on basal cell carcinoma-like lesions. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 4616.
- (12) (a) Storelli, S.; Verdijk, P.; Verzijl, D.; Timmerman, H.; Van de Stolpe, A. C.; Tensen, C. P.; Smit, M. J.; De Esch, I. J. P.; Leurs, R. Synthesis and structure-activity relationship of 3-phenyl-3H-quinazolin-4-one derivatives as CXCR3 chemokine receptor antagonists. *Bioorg. Med. Chem. Lett.* **2005**, *15* (11), 2910–2913. (b) Baxter, A. D.; Boyd, E. A.; Guicherit, O. M.; Price, S.; Rubin, L. Preparation of 3-aryl-2-aryleureidoalkylquinazolin-4-ones and related compounds as mediators of hedgehog signaling pathways. *PCT Int. Appl.* WO 2001019800 A2, 2001.
- (13) The induction of cells by Hh proteins sets in motion a cascade involving the activation and inhibition of downstream effectors, the ultimate consequence of which is a detectable change in the transcription or translation of a gene (e.g., Gli). The Hh assay is a reporter gene assay that measures the end stage of this cascade (i.e., transcriptional modulation). A reporter gene construct, which utilizes Luciferase, is inserted into the reagent cell to generate a detection signal, and the amount of transcription from the reporter gene can be measured. The amount of expression from the reporter gene is compared to the amount of expression from the same cell in the absence of the test compound. Any decrease in the amount of transcription indicates that the test compound is a potential Hh antagonist. This "Gli-Luc" assay can be applied to both HTS and lead compound discovery platforms, see: Frank-Kamenetsky, M.; Zhang, X. M.; Bottega, S.; Guicherit, O.; Wichterle, H.; Dudek, H.; Bumcrot, D.; Wang, F. Y.; Jones, S.; Shulok, J.; Rubin, L.; Porter, J. Small-molecule modulators of Hedgehog signaling: Identification and characterization of Smoothened agonists and antagonists. *J. Biol.* **2002**, *1*, 10.

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