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Synthesis and evaluation of novel benzylphthalazine derivatives as hedgehog signaling pathway inhibitors

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ABSTRACT

We report herein the design and synthesis of a series of novel benzylphthalazine derivatives as hedgehog signaling pathway inhibitors. Gli-luciferase assay demonstrated that changing piperazine ring of Anta XV to different four, five or six-membered heterocyclic building blocks afforded significant influences on Hh pathway inhibition. In particular, compound **10e** with piperidin-4-amine moiety was found to possess 12-fold higher Hh inhibitory activities comparing to the lead compound in vitro. In vivo efficacy of **10e** in a ptch$^{+/-}$p53$^{-/-}$ mouse medulloblastoma allograft model also indicated encouraging results.

Hedgehog (Hh) is a highly conserved developmental pathway involved in organogenesis, stem cell maintenance, and tissue repair/regeneration.[1] Hh signaling cascade is initiated by the binding of Hh protein ligand to its cellular membrane receptor Patched (Ptch), which relieves Ptch-mediated repression of the seven-transmembrane protein Smoothened (Smo). Activated Smo transduces the signal to the Gli family of transcription factors, which translocate to the nucleus to regulate numerous gene products involved in tissue patterning and cell differentiation.[2–4] Aberrant Hh pathway activation controls multiple aspects of tumorigenesis including basal cell carcinoma (BCC), leukemias and medulloblastoma, bladder, colorectal, lung, pancreatic, prostate, stomach cancer.[1,2] Furthermore, pharmacological inhibition of Hh signaling could impair the growth of imatinib-resistant mouse and human CML make this signaling pathway attractive to researcher because of the frequent drug-resistant in clinical tumor therapy.[5] Therefore, the development of small molecule inhibitors of Hh pathway represents a promising route toward novel anticancer therapeutics.[2,6]

Over a decade ago, Beachy reported the first Smo antagonist cyclopamine.[7] Subsequent work on this signaling pathway has made great achievements (Fig. 1). In 2012, the small molecule Hh signaling pathway inhibitor vismodegib (GDC-0449, **1**) was approved by FDA for its significantly clinical efficacy in phase II clinical evaluation of metastatic and locally advanced BCC.[8] Subsequently, another Hh pathway inhibitor sonidegib (NVP-LDE225, **2**) was approved by the FDA for treating basal cell carcinoma in July 2015.[9] Other Smo antagonists reported in different stages of development include LEQ-506(phase I, **3**),[10] LY-2940680 (phase II),[11] IPI-926 (phase II), XL-139(phase II) and PF-04449913 (phase II).[12]

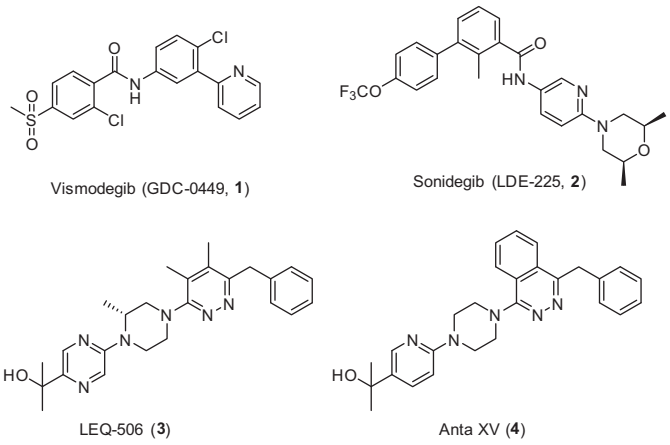

Figure 1. Chemical structure of several Hh signaling pathway inhibitors.

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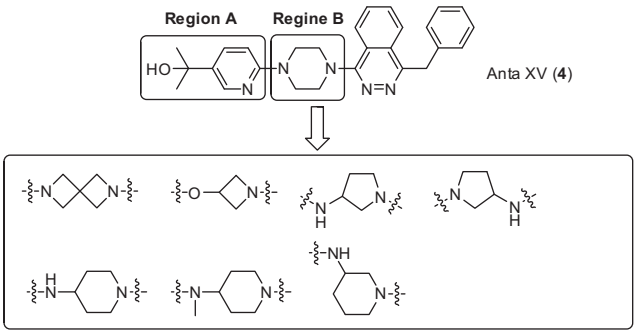


Figure 2. Design concept of novel benzylphthalazine derivatives.

The benzylphthalazine derivative Anta XV (**4**) was reported to be a potent hedgehog pathway inhibitor with low nanomolar affinity for Smo and good efficacy in Hh-related allograft model.[13] Further optimization of this compound led to the clinical candidate LEQ-506 (**3**).[10] The first co-crystal structures of the transmembrane domain of the human Smo receptor bound to the LY2940680 was reported by Stevens in 2013,[14] and then in 2014 co-crystal structure with Anta XV(**4**) was reported by the same group,[15] which have provided a rich set of structural information for drug discovery efforts on Hedgehog pathway inhibitors.

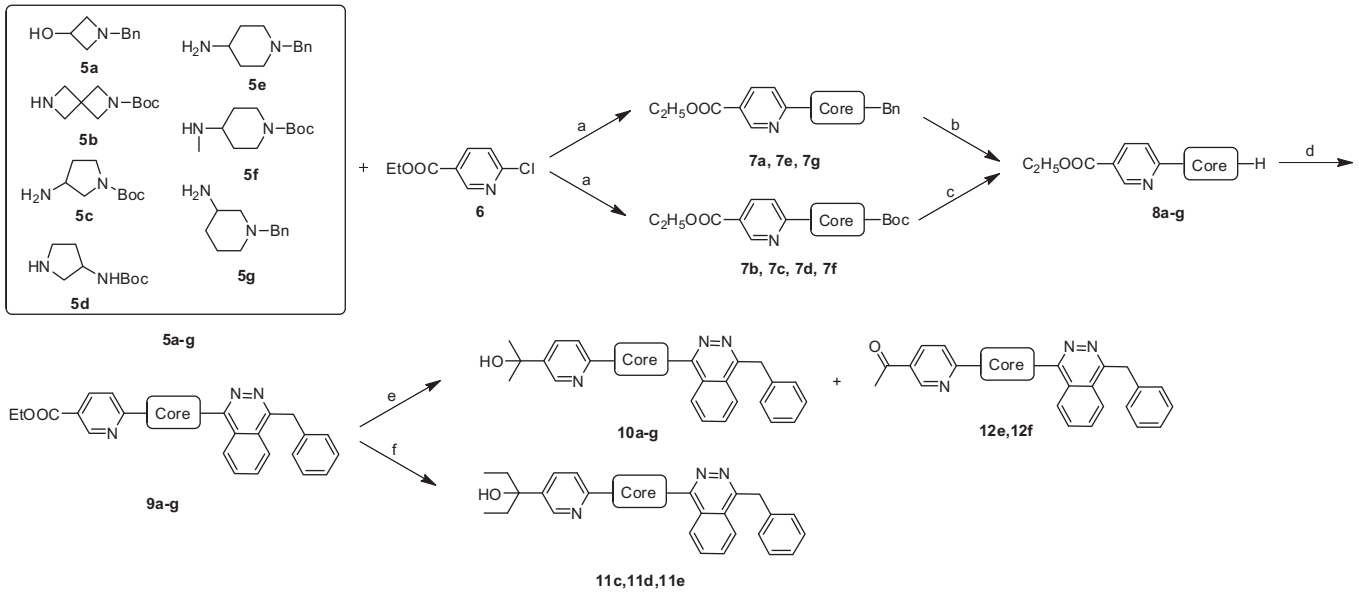
Based on the solved co-crystal structure, Anta XV forms three critical hydrogen bonds binding with Smo protein: R400 hydrogen bonds with the phthalazine core, N219 form hydrogen bonds to the pyridine N atom and the third hydrogen bond forms between K395 and the hydroxyl O atom. The piperazine ring of Anta XV plays an important role as a linker which can adjust the orientation of pharmacophores phthalazine and pyridin-3-propanol.[15]

In recent years, four-membered heterocycles as "compact modules" have attracted much interest in organic chemistry and drug discovery.[16] Incorporation of four-membered heterocycles into drug like scaffolds provides an opportunity to uniquely tune the biochemical and physicochemical properties of the parent compound. As typical examples, azaspiro[3,3]heptanes were considered as surrogates for piperazines, morpholine, thiomorpholine, and piperidine.[17] We have replaced the morpholine side chain of gefitinib with four-membered building blocks to generate new anilinoquinazoline derivatives, which possess higher EGFR inhibitory activities comparing to the lead compound with improved water solubility.[18] But in most reported cases, these new building blocks were used as side chain groups.

In this study, we choose Anta XV as our lead compound for the development of novel Hh signaling pathway inhibitors by modification of the piperazine moiety with different heterocyclics including four-membered heterocycle and Azaspirocycle. Therefore a series of structural modified benzylphthalazine compounds by changing region B from a piperazine to different four, five or six-membered heterocyclic building blocks were prepared (Fig. 2). Herein, the syntheses and preliminary in vitro and in vivo biological evaluation results are communicated.

The designed compounds were synthesized as shown in Scheme 1. Ethyl 6-chloro-nicotinate (**6**) was condensed with *N*-Boc or *N*-Bn protected four to six-membered heterocyclic building blocks **5a–g** in NMP under microwave condition. The obtained intermediates **7a–g** was deprotected by trifluoroacetic acid or by catalytic hydrogenation to afford the amine **8a–g**, respectively. Compounds **8a–g** was then condensed with 1-benzyl-4-chlorophthalazine assisted by microwave irradiation at 150 °C for 30 min to give **9a–g**. The target products **10a–g** and **11c–e** were then obtained through Grignard reaction with methylmagnesium iodide or ethylmagnesium bromide, respectively. The ketones **12e** and **12f** were obtained as side products (about 6% yields) in the preparation of **10e** and **10f**.

The twelve newly synthesized Anta XV derivatives (**10a–g**, **11c–e)** as well as three ester precursors (**9c**, **9d**, **9g**) were evaluated for their ability to inhibit the Hh signaling pathway using a luciferase reporter in NIH3T3 cell carrying a stably transfected Gli-reporter construct (Gli-luciferase reporter cell lines).[19] The in vitro IC_{50} values were illustrated in Table 1. Both Vismodegib (GDC-0449, **1**) and Anta XV were used as positive controls. Ten of the fifteen synthesized compounds exhibit potent Hedgehog signaling pathway inhibition with IC_{50} values ranging from 45.74 nM to 0.58 nM. As expected, changing region B from a piperazine to different four, five or six-membered heterocyclic building blocks afforded significant



Scheme 1. *Reagents and conditions:* (a) Et_3N, NMP, Microwave 180°C, 1 h, 55.6–84%; (b) H_2, 10% Pd–C, MeOH, rt, 12 h, 87.6–99%; (c) CF_3COOH, DCM, rt, 2 h, 85.8–99%; (d) 1-benzyl-4-chlorophthalazine, K_2CO_3, DMF, Microwave 150°C, 30 min, 45.2–66%; (e) CH_3MgI, THF, 0 °C–rt, 2 h, 17.2–48.4% for **10a–g**, 6.2% for **12e** and 6.8% for **12f**; (f) CH_2CH_3MgI, THF, 0 °C –rt, 2 h, 25.9% for **11c**, 16.2% for **11d** and 16.2% for **11e**.