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Original article

Design, synthesis, and structure–activity-relationship of tetrahydrothiazolopyridine derivatives as potent smoothened antagonists

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ABSTRACT

The Smoothened (Smo) receptor is an important component of the hedgehog (Hh) signaling pathway, which plays a critical role during embryonic development. In adults, Hh signaling is curtailed and has limited functions such as stem cell maintenance and tissue repair. However, aberrant activity of the Hh signaling in adults has been linked to numerous human cancers. Inhibition of Smo leads to the blockade of Hh signaling, and therefore represents a promising approach toward novel anticancer therapy. Through scaffold morphing of a few known Smo antagonists, a series of novel tetrahydrothiazolopyridine derivatives were developed. Compounds from this new scaffold demonstrated excellent Hh signaling inhibition which was comparable to or better than that of Vismodegib. Further, compound **30** exhibited a lower melting point and a moderately improved solubility compared with those of Vismodegib; compounds **11** and **30** showed good pharmacokinetic profiles with 34% and 77% oral bioavailability in rat, respectively. Collectively, these results strongly support further optimization of this novel scaffold to develop better Smo antagonists.

1. Introduction

The hedgehog (Hh) signaling pathway is a critical developmental pathway which regulates patterning, growth and cell migration during embryonic development. However, its role in adulthood is substantially curtailed to tissue maintenance and repair. Under normal conditions, the endogenous ligands Sonic hedgehog, Indian hedgehog and Desert hedgehog bind to their cellular membrane receptor Patched (Ptch), relieving the repression effect of Ptch on GPCR-like receptor Smoothened (Smo). Smo activation triggers a series of intracellular events ultimately lead to specific gene expression mediated by the Gli family transcription factors [1,2]. Aberrant Hh signaling has been linked to numerous human cancers. Mutational inactivation of the inhibitory pathway components such as Ptch (lost function of Ptch) or activation of Smo (gain function of Smo) leads to constitutive ligand-independent activation of the Hh signaling pathway, resulting in cancers such as basal cell carcinoma and medulloblastoma [3,4]. Ligand-dependent activation of Hh signaling is involved in lung, colorectal, prostate, pancreatic, breast and some blood cancers [5–7]. Thus, inhibition of the aberrant Hh signaling represents a promising approach for novel anticancer therapy [8–13].

Cyclopamine (Fig. 1), a naturally occurring alkaloid, was the first Hh signaling pathway inhibitor to be reported in the literature [14]. It was later identified as a Smo antagonist [15]. A derivative of

Abbreviations: AUC, area under curve; CE, collision energy; DCM, dichloromethane; DIPEA, *N*,*N*-diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; DP, declustering potential; EDTA, ethylene diamine tetraacetic acid; EtOAc, ethyl acetate; GPCR, G protein-coupled receptor; Hh, hedgehog; HPLC, high performance liquid chromatography; m.p., melting point; MRM, multiple reaction-monitoring; N-G-L, NIH3T3-GRE-Luc reporter gene assay; NMP, *N*-methyl-2-pyrrolidone; PBST, PBS buffer supplied with 0.05% Tween-20; PFA, paraformaldehyde; P.K., pharmacokinetics; Ptch, Patched; SEM, standard error measurement; Smo, Smoothened; SMO-BCB, BODIPY-cyclopamine binding assay; TLC, thin layer chromatography.

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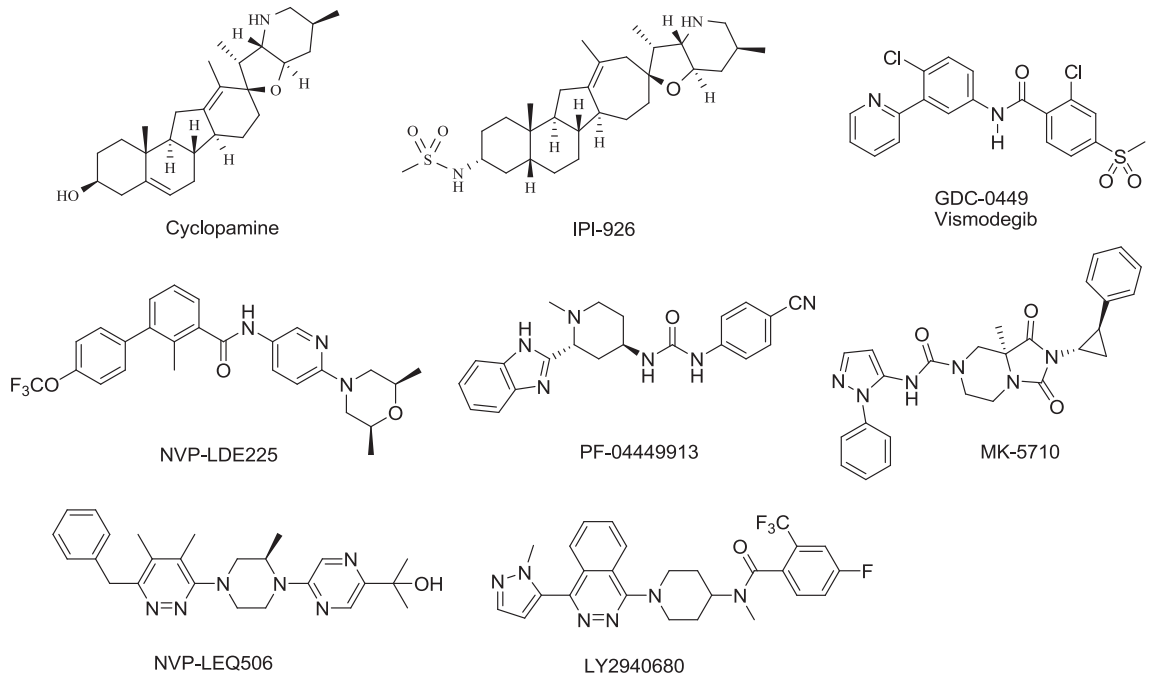

Fig. 1. Smoothened antagonists in advanced development.

cyclopamine, IPI-926 (Fig. 1), which demonstrated better potency, chemical and metabolic stability, and other pharmaceutical properties than those of cyclopamine, had entered clinical development [16–18]. Unfortunately, on June 18, 2012, Infinity Pharmaceuticals reported disappointing results of a phase II study of IPI-926 in patients with metastatic chondrosarcoma and the company subsequently suspended the trials for myelofibrosis [19]. In contrast, a synthetic Smo antagonist NVP-LDE225 demonstrated promising results in recent trial reports for advanced basal cell carcinoma [20,21]. The mixed results prompted debate whether blockage of ligand dependent Hh pathway activation can be effective in cancer treatment [12,13]. Other synthetic Smo antagonists had been reported in recent years [[22–35], Fig. 1]. The most advanced in the class, GDC-0449 (Vismodegib, Fig. 1), was approved by FDA in January 2012 for patients with locally advanced or metastatic basal cell carcinoma which was not suitable for operation [36]. This approval highlighted the first embryonic pathway inhibitor for the treatment of human cancer. However, emerging evidence showed resistance with different mechanisms can be developed including Smo mutation for Vismodegib [37], Gli2 amplification for NVP-LDE225 [38]. There is still an urgent need to develop potent Hh inhibitors with distinct chemotype.

2. Design

We have investigated a number of templates in pursuit of novel Smo receptor antagonists under the guidance of the pharmacological model developed by Manetti [33] and the recently solved Smo crystal structure [39]. Both GDC-0449 [24] and NVP-LDE225 [29] were the results of optimization campaigns from initial screening hits. In these two molecules, the central amide bonds were reversed, yet both molecules demonstrated excellent potency (Fig. 2, the amide and the "reversed" amide were highlighted in red). This observation suggested that the central amides were place-holders, unlikely to have participated in significant interactions with the Smo receptor. This assumption was reinforced by Pfizer compound **1** [40,41] and SEN794 from Siena Biotech [31] (Fig. 2). GDC-0449 skeleton was composed by uniformly sp2-hybridized carbons, leading to a high melting point (264 °C) and poor solubility (9.5 μg/mL). This reported solubility was achieved by adding an ortho–chloro group to the right side ring to introduce tilt and reduce planarity of the aryl amide [24]. It is well documented in the literature that planarity and molecular topology have significant impacts on solubility, absorption, metabolism and toxicity [42,43]. In our own experience, we had to abandon a molecule because of its totally flat structure and thus extremely poor solubility, despite the fact that it demonstrated excellent potency and selectivity in receptor binding assays, and remarkable efficacy in animal models [44,45]. In order to improve the physical–chemical properties, we proposed maintaining the saturation ring as the place-holder (introducing sp3-hybridized carbons, reducing planarity), as featured in compound **1** and SEN794. We also proposed tying the tail part in compound **1** and SEN794 to decrease rotatable bonds as a way to enhance absorption and metabolic stability [46]. We had applied this strategy to obtain a novel series of compounds based on tetrahydroimidazo[1,2-*a*]pyrazine [47]. Here we report a further development of our tetrahydroimidazo[1,2-*a*]pyrazine template base on tetrohydrothiazolo [5,4-*c*]pyridine as shown in Fig. 2.

3. Chemistry

The general synthetic route used to prepare compounds **8**–**19** was outlined in Scheme 1. Suzuki coupling of 2-bromo-3,5-dimethyl pyridine with 2,5-dichloropyridin-4-ylboronic acid afforded bipyridine **4** in moderate yields. Commercially available *tert*-butyl 4-oxopiperidine-1-carboxylate **5** was reacted with cyanamide and S_8 in pyridine at 130 °C to give key intermediate **6**. Removal of BOC of intermediate **6** by HCl/EtOAc afforded intermediate **7**, which was used to displace the 2-position chlorine of bipyridine **4** to yield compound **8**. The poor yield of the displacement reaction was due to the low reactivity of the amine **7**, which was unchanged even after 36 h heating. A few standard palladium mediated coupling reaction conditions did not improve the results.