Bioorganic & Medicinal Chemistry Letters 24 (2014) 983–988

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and evaluation of 4-(2-pyrimidinylamino) benzamides inhibitors of hedgehog signaling pathway

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ARTICLE INFO

Article history:
Received 18 September 2013
Revised 10 November 2013
Accepted 12 December 2013
Available online 17 December 2013

Keywords:
Hedgehog signaling pathway
Inhibitors
SAR
Pharmacokinetic properties

ABSTRACT

A novel series of hedgehog signaling pathway inhibitors has been designed based on the 4-(2-pyrimidinylamino) benzamides scaffold. The synthesis and SAR of these compounds are described. Optimization leads to the identification of compound **3c**, a potent and orally available agent with improved physicochemical and pharmacokinetic properties.

The Hedgehog (Hh) signaling pathway is receiving increasing attention because of its very role in malignant transformation of various cancers.[1] Genetic mutations associated with this signaling pathway such as Ptch-mutation, Smo-mutation, and Sufu-mutation result in tumorigenesis in several cancers such as basal cell carcinoma (BCC), medulloblastoma, and rhabdomyosarcoma. Additionally, strong evidences link overexpression of Hh ligands to tumor aggravation of various other cancers such as hepatocellular, pancreatic, gastric, colorectal, esophageal, lung, ovarian, prostate, melanoma, glioblastoma, leukemia, lymphoma.[2] Thus, inhibition of Hh signaling pathway is thought to have considerable potential for therapy of many cancer types.

To date, many classes of Hh pathway inhibitors have been described.[3] Vismodegib has been recently approved by FDA for metastatic BCC treatment.[4] Other molecules including sonidegib (NVP-LDE225, Phase III), LY-2940680 (Phase II), BMS-833923 (XL-139, Phase II), PF-04449913(Phase I), TAK-441 (Phase I), NVP-LEQ506 (Phase I) have been already studied in clinic for many tumors treatment (Fig. 1).[5] Besides, there are still some novel small molecules investigated in preclinical stage or drug discovery stage.[6]

In aspiration of interest in developing Hh signaling pathway inhibitors for treatment of many tumors, we recently detailed the discovery of a novel series of 4-(2-pyrimidinylamino) benzamides as potent Hh signaling pathway inhibitors, exemplified by **1** (Fig. 2), which inhibited the Hh signaling pathway in Gli-luciferase reporter assay with IC_{50} = 1.3 nM, more potent than control positive drug vismodegib. Initial SAR studies in this series showed that structural modification on A-ring of **1** seriously affected the inhibitory activity and the optimal substituents was conferred to 4-trifluoromethylphenyl, phenyl, 4-pyridinyl or others. Following SAR studies focused on D-ring modifications, especially at the position of phenyl group, demonstrating that methyl substitution at C-2 of the phenyl or several basic side chains functionalized at C-5 was favorable for high inhibitory activity.[7]

As a continuation of our study toward identification of potent Hh signaling pathway inhibitors, a further SAR study was conducted to explore various basic amine chains linked to D-ring, looking to identify potent inhibitors with improved physicochemical properties which was of importance for successfully developing drug candidate. Furthermore, considering that **1** exhibited unsatisfactory pharmacokinetic (PK) profile, efforts to improve the PK properties of this scaffold was also made by modification on both A-ring and D-ring. Herein, in this report we disclose the synthesis and SAR analysis of these analogs of Hh signaling pathway inhibitors, and also we report the PK evaluation in vivo.

On the basis of the previous SAR study, at the outset, our strategy focused on bearing various basic substitutions at C-5 of D-ring. A number of analogs incorporating basic amine side chains at C-5 of D-ring were summarized in Table 1. The Hh signaling pathway inhibitory activity of compounds **2a**–**2zj** were evaluated by a

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http://dx.doi.org/10.1016/j.bmcl.2013.12.050

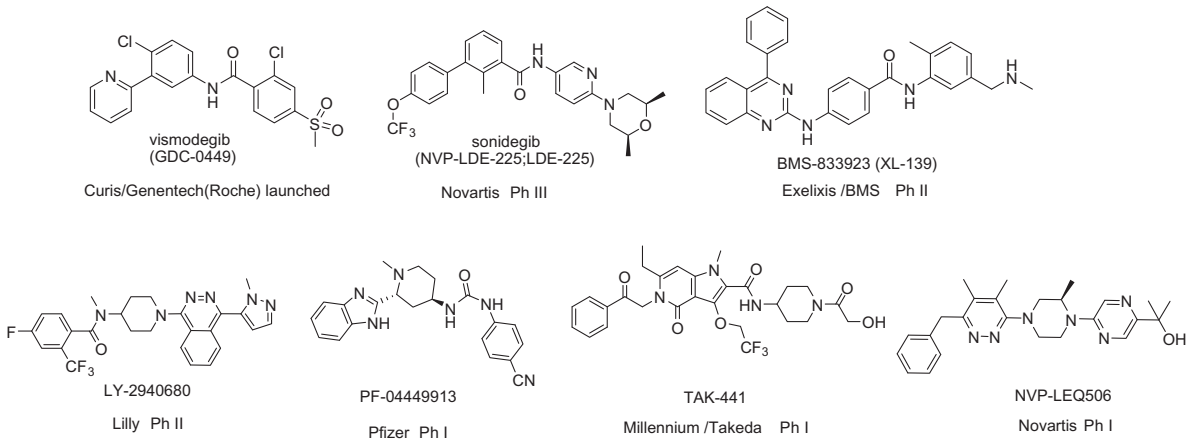

Figure 1. Chemical structures of clinical Hh signaling pathway inhibitors.

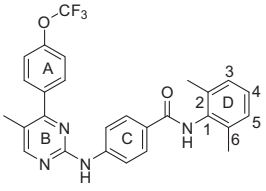

Figure 2. Structure of compound **1**.

luciferase reporter in NIH3T3 cell carrying a stably transfected Gli-reporter construct (Gli-luciferase reporter cell lines).[7,8] Table 1 outlined the in vitro IC_{50} for this series of various basic substitutions at C-5 of D-ring of 4-(2-pyrimidinylamino) benzamides[9]. Notably, all the analogs bearing basic amine on the molecules (**2a**–**2zj**) showed good Hh signaling inhibitory activity with IC_{50} varied from 0.23 to 35.35 nM. Of all the amines explored, some cyclic aliphatic amines, such as morpholine (**2a**), dimethyl morpholine (**2b**), piperidine (**2c**), thiomorpholine (**2d**), pyrrolidine (**2e**), were more effective than the lead compound **1** and 7–20 fold higher activity than GDC-0449. However the similar cyclic amine piperazine (**2f**) showed significantly decreased activity, perhaps due to relatively strong basicity of second basic center of NH. Other piperazine derivatives such as *N*-methyl piperazine (**2g**), *N*-ethyl piperazine (**2h**), *N*-phenyl piperazine (**2i**), *N*-cyclopropanecarbonyl piperazine (**2j**) and piperazin-3-one (**2k**) exhibited improved potency compared to **2f** because of relatively suitable basicity. A survey of substituted piperidines was investigated and led to some more favorable piperidine derivatives including 4-hydroxy piperidine (**2l**), piperidin-4-one (**2m**), tetramethyl piperidine (**2n**), and tetramethyl piperidin-4-one (**2o**). However, 4-piperidyl piperidine (**2p**) displayed lower activity, while the activity of piperidine fusing aromatic ring such as tetrahydroquinoline (**2q**) and tetrahydrothienopyridine (**2r**) almost retained. These results indicated that adding extra basicity was not favorable to the activity. The same case was accounted by pyrrolidine derivatives **2s** whose activity was weaker than **2e**. Additionally, two bicyclic amine derivatives, **2t** and **2u**, were also investigated, and it was found **2t** was 6-fold more potent than **2u**. After screening a variety of cyclic amine substituents, some acyclic amines were explored. It appeared a suitable length of acyclic amines was important for maintaining activity. For examples, **2v**–**2z** displayed subnanomolar inhibition. However, compounds **2za** and **2zb**, displayed much lower potency, with IC_{50} = 35.3 and 10.9 nM, respectively, mainly due to the larger size of amine chains. In fact, the extra basicity of these two compounds also strongly effected the decreased activity additively. Steric hindrance could also affect the activity, for instance compound **2zc** bearing a bulky dicyclohexylamine group, which showed moderate activity. Besides, secondary amines like ethylamine (**2zd**), hydroxyethylamine (**2ze**), *tert*-butylamine (**2zf**) or cyclopentylamine (**2zg**) were tolerated, displaying nearly comparable activity to lead compound **1**, except **2zh** that showing weak potency of Hh inhibitory activity, which was considered due to its length and extra basicity. In addition, interestingly, the inhibitory activity of the basic amine substituents containing aromatic groups such as furylmethamine (**2zi**) and phenylamine (**2zj**) were retained, and these results were consistent with the above reported ones **2q** and **2r**. In a word, this SAR study showed 30 compounds displayed better inhibitory activities than positive control GDC-0449, of which 20 exhibited superior activities to lead compound **1**.

To evaluate whether these compounds had an improved druggability compared to **1**, of our most potent inhibitors, two compounds **2a** and **2b** were further investigated by profiling iv and po pharmacokinetics in SD rat.[10] The results were illustrated in Table 3. After intravenous injection with 1 mg/kg in SD rats, the area-under-curve (AUC) of **2a** reached 1.3 h μg/mL, larger than that of **1** (0.92 h μg/mL). Such more plasma exposure is due part to the much smaller volume of distribution (7.4 L/kg) and lower systemic clearance (12.0 mL/min/kg). Furthermore, when orally administered at 5 mg/kg dose, **2a** showed higher oral exposure and lower systemic clearance. Consequently, compound **2a** showed superior oral bioavailability (F = 46%) to **1** (36%). However, unfortunately, **2b** bearing *N*-methylpiperizine group showed poor PK characteristics both by iv and po administration. Despite **2a** and **2b** contained much hydrophilic group morpholine and *N*-methylpiperizine in structures respectively, both molecules had already possessed higher lipophilicity (**2a**, $A\log P$ = 7.07 and **2b**, $A\log P$ = 7.34) compared to vismodegib ($A\log P$ = 4.265). It appeared attractive to attempt a structural modification to decrease the $A\log P$ value so as to further improve drug-like properties. Therefore, Three compounds (**3a**–**3c**) were synthesized (Table 2), beginning to replace of trifluoromethoxyphenyl of A-ring with phenyl, 4-pyridinyl and 1-methylpyrazolyl because these groups were previously reported to afford good to moderate inhibitory activity.[7] Of the three compounds, both compounds **3a** and **3b** showed nearly equipotent inhibition with **2a** in Hh signaling pathway and possessed a lower $A\log P$ values (**3a**, $A\log P$ = 4.95 and **3b**, $A\log P$ = 4.069), but did not improve the plasma exposure in an intravenous administration (Tables 2 and 3). Fortunately, at this point, **3c** with 1-methylpyrazolyl, showing equipotent potency with 1 and a much lower $A\log P$ (**3c**, $A\log P$ = 3.6), was found to