Bioorganic & Medicinal Chemistry 24 (2016) 1079–1088

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Synthesis and pharmacological evaluation of trifluoromethyl containing 4-(2-pyrimidinylamino)benzamides as Hedgehog signaling pathway inhibitors

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ARTICLE INFO

Article history:
Received 8 December 2015
Revised 14 January 2016
Accepted 18 January 2016
Available online 19 January 2016

Keywords:
Hedgehog signaling pathway inhibitors
4-(2-Pyrimidinylamino)benzamide
Trifluoromethyl
SAR
In vivo

ABSTRACT

In present study, a series of novel containing trifluoromethyl 4-(2-pyrimidinylamino)benzamide derivatives were designed by the fluorine scan strategy. Their Hh signaling inhibitory activities were evaluated by Gli-luciferase reporter method. The comprehensive SAR was discussed and several derivatives were found to display more potent Hh signaling inhibitory activity than positive drug vismodegib. Compound **13d** was the most potent compound with IC_{50} of 1.44 nM against Hh signaling pathway and also exhibited optimal PK properties in the in vivo PK properties study, deserved as an ideal lead compound for further study in future.

1. Introduction

Cancer is so far the second major worldwide fatal ailment. In spite of significant progress of therapeutic techniques against this disease, there is still a long way to go for the victory of the war. With improved understanding of the substantially pathological mechanisms of tumor initiation and progression, cancer therapeutic strategy has evolved from cytotoxic to targeted therapy. It is clearer that targeted therapy can achieve significant benefit due to its selectively anchoring the tumor cells. During past years, many targeted drugs such as crizotinib, vemurafenib, and ibrutinib have been developed and have exhibited great efficacy in clinic.[1] Recently, Hedgehog (Hh) signaling pathway is identified as an attractive target in oncology community.[2] Compounds targeted Hh signaling pathway are considered to have promising potential for treatment of many human tumors, such as basal cell carcinoma (BCC), medulloblastoma, rhabdomysarcoma, lymphoma, leukemia, lung, pancreatic, hepatocellular, gastric, esophageal, colorectal, ovarian, prostate, melanoma and glioblastoma.[3] Until now, two Hh signaling pathway inhibitors, vismodegib (GDC-0449, **1**) and sonidegib (LDE-225, **2**), have been already approved by FDA for treatment of locally advanced BCC.[4] In addition, there have many Hh inhibitors investigated in clinical and preclinical stages, such as taladegib (LY-2940680, **3**) and TAK-441 (**4**)[5,6] (Fig. 1).

On the basis of our accumulated knowledge of the reported structures of Hh inhibitors, we recently disclosed a comprehensive understanding of Hh signaling pathway inhibitors composed of 4-(2-pyrimidinylamino)benzamides.[7,8] In an effort to obtain more potent and orally available Hh inhibitors, we directed our work to the exploration of systematic structure–activity relationship (SAR) of 4-(2-pyrimidinylamino)benzamides and the expansion of novel heterocyclic scaffolds.[9–11] In several cases, it was found that methyl or fluoro substitution on C-5 position of pyrimidine of B-ring resulted in potent activity, such as compound **5** and **6**.[12,13] In our further druglike optimization, it is found that the C5-fluoro substitution compounds show better pharmacokinetic (PK) profiles than corresponding C5-methyl substituted ones. Considering this useful SAR information of 5-methyl and 5-fluoro, our novel drug design attention was turned to focus on the rational fluorine scan on C-5 position. In fact, the introduction of fluorine atoms into bioactive compounds is very popular in drug design and discovery.[14] The C–F bond is found to possess specificity due to its special size and electronegativity, and fluorine-compounds containing a fluorine atom or trifluoromethyl group generally

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http://dx.doi.org/10.1016/j.bmc.2016.01.034
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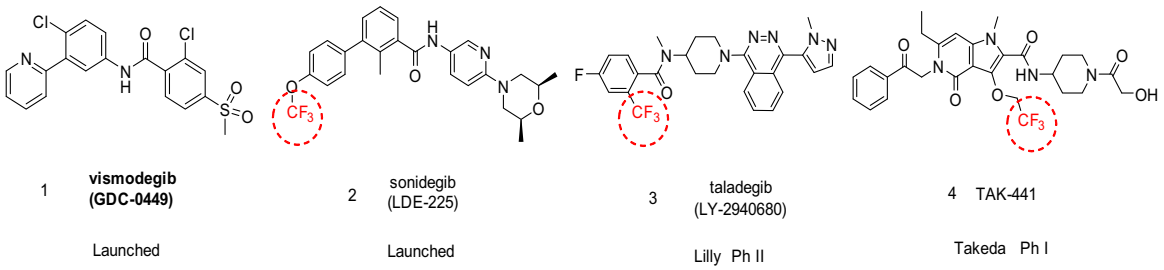


Figure 1. Representative structures of Hh signaling pathway inhibitors.

show more stability and membrane permeability compared to corresponding hydrocarbons.[15] Many approved drugs structures contain fluorine atom or trifluoromethyl group, such as gefitinib, sorafinib sunitinib and nilotinib. Especially, it is noticed that Hh inhibitors such as sonidegib, taladegib, and TAK-441 also contain the trifluoromethyl fragment. Based on these findings and connection to our drug design program, utilizing the rational fluorine scan strategy, we intend to make the further structural derivatization by introducing a trifluoromethyl to the C5 position of the 4-(2-pyrimidinylamino)benzamide skeleton to search for novel Hh signaling pathway inhibitors. Herein, we describe the synthesis, biological activities evaluation of novel 4-(5-trifluoromethyl substituted 2-pyrimidinylamino)benzamide derivatives (Fig. 2).

2. Results and discussion

2.1. Chemistry

The novel 4-(2-pyrimidinylamino)benzamides containing trifluoromethyl were synthesized, as illustrated in Table 1. The synthetic routes for all the target compounds were outlined in Schemes 1 and 2.

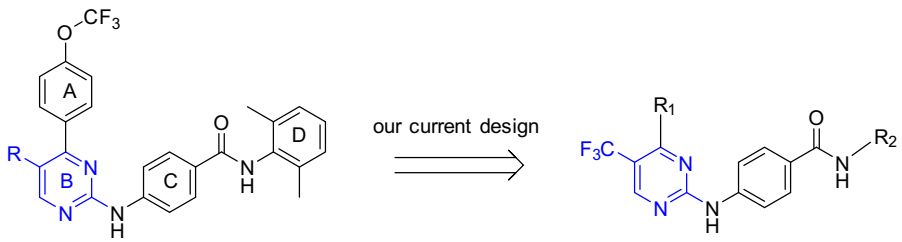
As shown in Scheme 1, the commercially available 5-trifluoromethyl 2,4-dichloropyrimidine (**7**) was initiatively reacted with methyl 4-aminobenzoate to afford intermediate **8**. Suzuki coupling of **8** with commercially available boronic reagents using Pd reagents as catalyst gave intermediate **9a**–**9d** and **9f**–**9h**, and **9e** was obtained by Stille coupling reaction under microwave heating condition due to the Suzuki coupling condition unsuccessful. **9** was subsequently hydrolyzed under basic condition to provide free acid **10**, which was followed by condensation with 3-amino-4-methylbenzyl alcohol to produce intermediate **11**, respectively. Subsequently, intermediate **11** was chlorinated to yield **12**, followed by nucleophilic substitution with corresponding secondary amines to afford target compounds **13a**–**13o** (Scheme 1).

Compounds **16** and **20** were prepared according to Scheme 2. The above intermediate **10h** were treated with 3-hydropiperidine to give **14**, which was subsequently reacted with methanesulfonyl chloride and followed by nucleophilic substitution with morpholine to afford the compound **16**. Likewise, condensation of **10h** with 1-Boc-4-aminopiperidine gave **17**, following by the removal of Boc group with TFA to generate **18**. Acylation of **18** with acetoxylacetic acid afforded **19**, which further underwent deprotection in presence of NaOH to yield compound **20** (Scheme 2).

2.2. Hh signaling inhibitory activity assay

The Hh signaling pathway inhibitory activities of newly synthesized trifluoromethyl containing 4-(2-pyrimidinylamino)benzamides were evaluated by applying a luciferase reporter in NIH3T3 cell carrying a stably transfected Gli-reporter construct (Gli-luciferase reporter cell lines). The Hh inhibitor vismodegib was used as the positive controls and the IC_{50} values were summarized in Table 1.

It was found that most of compounds exhibited good Hh signaling pathway inhibitory activity with IC_{50} values varied from 1.44 nM to 102.1 nM, except of compounds **16** and **20** which displayed no inhibitory activities with IC_{50} >500 nM. Although the side chain of **20** was same to TAK-441, it seems to have little contribution for the bioactivity. These data indicated that the aromatic C-ring is essential for maintaining the Hh signaling pathway inhibitory activities for this novel series of 4-(2-pyrimidinylamino) benzamides. Considering the high lipophilicity in the structures, the introduction of a hydrophilic substituent of R^2 at D-ring position was initiatively investigated. The IC_{50} data revealed the *N*-methyl piperazine (**13b**, 5.19 nM), piperidine (**13c**, 3.12 nM), morpholine (**13d**, 1.44 nM) and pyrrolidine (**13e**, 4.23 nM) afforded more potent activity compared to the control drug vismodegib (7.2 nM), while *N*-ethyl piperazine (**13a**, 62.78 nM) and dimethylamino (**13f**, 18.22 nM) displayed less potency. Especially, the morpholine (**13d**) yielded the most potent activity in this class, which was even 5-fold higher than vismodegib. In addition, several substituents of R^1 at A-ring position were also explored. The 4-cyanphenyl group (**13g**, 4.24 nM) showed higher potency than vismodegib, while the phenyl group (**13h** and **13i**) demonstrated reduced inhibitory activity regardless of R^2 position of which were



5 R = Me, IC_{50} = 1.3 nM
6 R = F, IC_{50} = 1.6 nM

Figure 2. Current design of trifluoromethyl containing 4-(2-pyrimidinylamino)benzamides.