Chinese Chemical Letters 26 (2015) 1307–1310

Contents lists available at ScienceDirect

Chinese Chemical Letters

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

Original article

Synthesis and antiproliferative activity of novel 4-substituted-phenoxy-benzamide derivatives

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ARTICLE INFO

Article history:
Received 24 March 2015
Received in revised form 13 April 2015
Accepted 29 May 2015
Available online 20 June 2015

Keywords:
Aryl cycloaliphatic amine
Antiproliferative activity
Hedgehog signaling

ABSTRACT

A series of novel 4-substituted-phenoxy-benzamide derivatives bearing an aryl cycloaliphatic amine moiety were synthesized and evaluated for antiproliferative activity against SW620, HT29 and MGC803 cancer cell lines *in vitro*. The pharmacological data demonstrated that the majority of target compounds exhibited moderate efficacy in HT29 and MGC803 cell lines. Compound **10c** showed promising inhibition of hedgehog (Hh) signaling pathway in an Hh-related assay. In addition, the superposition pattern of **10c** showed a good fit for a pharmacophoric model generated by Hh inhibitors and provided a basis for further structural optimization.

1. Introduction

The Hedgehog (Hh) protein family, which includes Sonic (Shh), Indian (Ihh), and Desert (Dhh) hedgehogs, regulated cell growth and migration during embryonic development [1–3]. Activation of the Hh signaling pathway is initiated by Shh ligands bound to its receptor Patched (Ptch), which relieves its inhibition of Smoothen (Smo) receptor. Smo activation triggers a series of intracellular events ultimately leads to specific gene expression mediated by the Gli family transcription factors [4,5]. Hh signaling pathway was normally silent in adult tissues, nevertheless aberrant activation of the Hh pathway was associated with certain cancers. Thus, the blockade of Hh pathway had been investigated as a novel strategy in cancer chemotherapy [6,7].

Inhibition of Smo activity has shown some promise in the treatment of cancers driven by activating mutations of the Hh pathway [8–10]. Furthermore, several Hh pathway antagonists have proceeded to clinical development, among which vismodegib (**1**, Fig. 1) has obtained marketing authorization in the United States in 2012 [11,12]. Sonidegib (**2**, Fig. 2), a clinical stage Hh inhibitor developed by Novartis, is awaiting for the registration in the U.S. for the treatment of patients with advanced basal cell carcinoma. Sonidegib (**2**) bearing a morpholinopyridine unit suppressed the growth of Hh pathway-dependent tumors by selective inhibition of the positive regulator smoothened (Smo) [13,14]. LEQ506 (**3a**), a second-generation Hh inhibitor, is currently being investigated in a Phase I clinical trial for patients with solid tumors. SAR studies had demonstrated the replacement of the benzylic methylene linker with an oxygen atom (**3b**) was well tolerated, whereas replacement with an NH group (**3c**) resulted in a 10-fold decrease in inhibition of the Hh pathway [15].

Inspired by the structural characteristics of Sonidegib (**2**) and LEQ506 (**3a**), we envisioned that the merging of these two bioactive components would afford a hybrid structure with the potential for antiproliferative activity. We therefore adopted the biaryl ether (Part A) active fragment from LEQ506 analog (**3b**) and morpholino pyridine (Part B) unit from Sonidegib (**2**) in target compounds, and a carbonyl group or an amide group was selected as the linker between the two parts. Additionally, the order of heteroaryl or aryl cycloaliphatic amine units in the target compounds attaching to the linker was reversed based on the

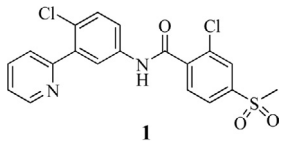

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Fig. 1. The structure of vismodegib (**1**).

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http://dx.doi.org/10.1016/j.cclet.2015.06.017

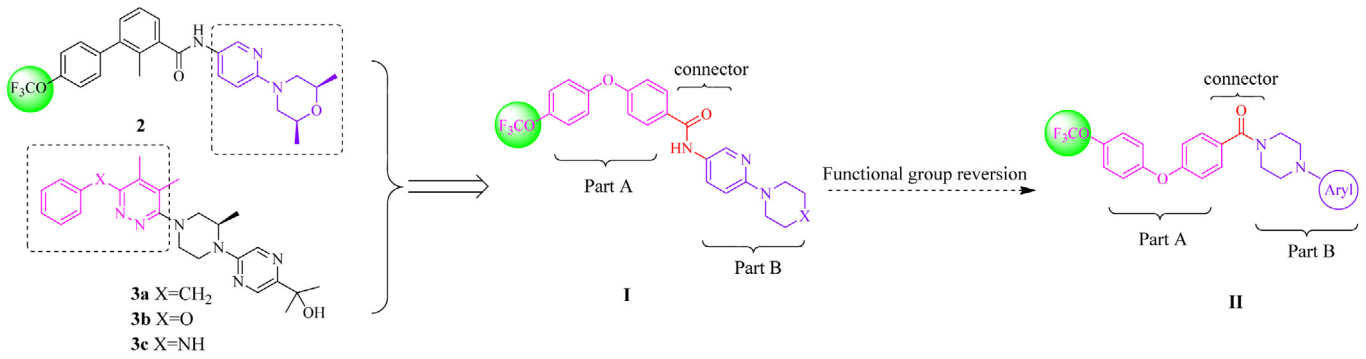


Fig. 2. Sonidegib (**2**), LEQ506 analogs (**3a–c**) and general structure of the target compounds (**I** and **II**).

functional group reversion principle. Eventually, a series of 4-substituted-phenoxy-benzamide derivatives (I, II Fig. 2) were obtained. In this paper, the synthesis of these benzamide derivatives was reported, and their biological activities were evaluated.

2. Experimental

The key intermediates **7a–d** were synthesized according to the routes outlined in Scheme 1. Aryl iodides were synthesized from aryl amines by a diazotization reaction. The generated diazonium salts were iodinated with KI to give compounds **5a–d** [16]. The substituted iodobenzenes **5a–d** were coupled to methylparaben to afford compounds **6a–d**, *via* an *N*,*N*-dimethyl glycine-promoted Ullmann coupling. Compounds **6a–d** were further hydrolyzed to compounds **7a–d** in excellent yields under reflux [17].

Compounds **10a–f** were synthesized according to the routes outlined in Scheme 2. 2-Chloro-5-nitropyridine was converted to compounds **8a** and **b** in the presence of morpholine or piperidine [18]. The resulting nitro compounds **8a** and **b** were reduced to the amino compounds **9a** and **b** using stannous chloride dihydrate and hydrochloric acid in aqueous ethanol, and then alkalized with sodium hydroxide solution [19]. **7a–d** were treated with thionyl chloride to produce the corresponding acyl chloride. **9a** and **b** reacted with acyl chloride to give the target compounds **10a–f** [20].

Compounds **14a–n**, **15a–b**, **16a–j** were synthesized according to the routes outlined in Scheme 3. Compound **11** was synthesized from 6-chloronicotinic acid *via* an esterification reaction and then converted to compound **12** using a substitution reaction [21]. Compound **12** was deprotected to give compound **13** under acid conditions [22]. Intermediate **13** was reacted with the corresponding acyl chlorides **7a–d** to give the target compounds **14a–d,** which were further hydrolyzed to compounds **15a** and **b**. Similarly, compounds **16a–j** were obtained by the reaction of the acyl chloride **7c** and various aryl piperazine [23].

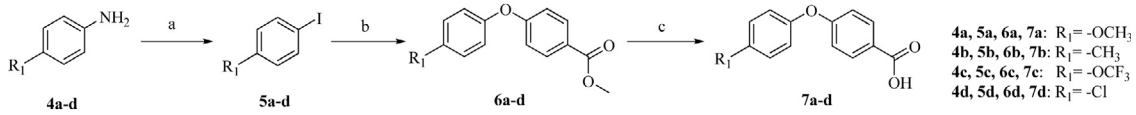
General procedure for preparation of target compounds was given in the Supporting information.

3. Results and discussion

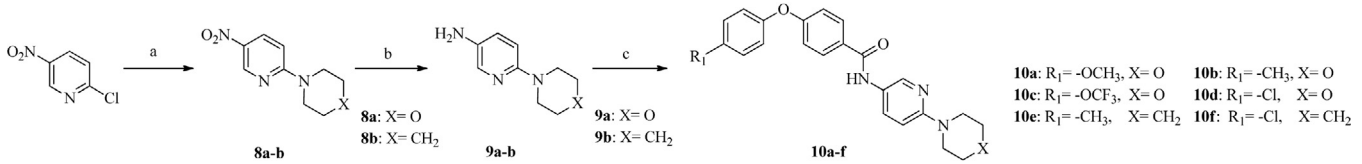
All the target compounds (**10a–f**, **14a–d**, **15a–b** and **16a–j**) were evaluated for their antiproliferative activity against human colorectal cancer cell lines (SW620 and HT29), and human gastric cancer cell line (MGC803) using the MTT assay with vismodegib as a positive control. The results expressed as half maximal inhibitory concentration (IC_{50}) values are summarized in Table 1.

Initially, target compounds were divided into two regions: diaryl ether (Part A), aryl cycloaliphatic amine moiety (Part B). In general, most of them displayed high efficacy in HT29 and MGC803 cell lines. At the outset, our focus was on the modifications of Part A, including the substitutions on the *para* position of phenyl. The *para*-trifluoromethoxy substituent **10c** (IC_{50} = 1.15 μmol/L [HT29], IC_{50} = 0.56 μmol/L [MGC803]) showed decent activity, however, *para*-methoxy and *para*-chlorine led to a significant loss of activity (**10c** *vs* **10a**, **10d** or **14a** *vs* **14b**, **14d**) against MGC803 cells, confirming the beneficial impact of trifluoromethoxy in the R_1 position.

Further investigations focusing on Part B on the antiproliferative activity were performed. On comparing **10d** with **10f**, it was found that morpholino pyridine surrogates were superior to piperidyl pyridine surrogates. Through functional group reversion, pyridyl piperazidine analog **16c** (IC_{50} = 4.33 μmol/L [HT29], IC_{50} = 5.35 μmol/L [MGC803]) was obtained and exhibited moderate potencies. Addition of a polar methoxycarbonyl (**14a**, IC_{50} = 2.36 μmol/L [HT29]) group to pyridine increased the activity while its hydrolysate **15a** lost potency by nearly two-fold. When



Scheme 1. Reagents and conditions: (a) (1) $NaNO_2$, H_2SO_4, 0 °C, 2 h; (2) KI, dichloromethane, 0 °C, 6 h; (b) 4-methyl-1-iodobenzene, CuI, *N*,*N*-dimethylglycine, Cs_2CO_3, 1,4-dioxane, reflux under nitrogen atmosphere, 24 h; (c) NaOH, ethanol solution, reflux, 3 h.



Scheme 2. Reagents and conditions: (a) morpholine or piperidine, K_2CO_3, THF, reflux, 4 h; (b) $SnCl_2{\cdot}2H_2O$, ethanol, HCl, reflux, 8 h; (c) acyl chloride of **7a–d**, Et_3N, dichloromethane, 0 °C, 12 h.