Bioorganic & Medicinal Chemistry 24 (2016) 5861–5872

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Design, synthesis, and evaluation of novel porcupine inhibitors featuring a fused 3-ring system based on the 'reversed' amide scaffold

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ARTICLE INFO

Article history:
Received 20 July 2016
Revised 1 September 2016
Accepted 16 September 2016
Available online 17 September 2016

Keywords:
Wnt signaling pathway
Porcupine
Antagonist
Cancer therapy
Scaffold hybridization

ABSTRACT

The Wnt signaling pathway is an essential signal transduction pathway which leads to the regulation of cellular processes such as proliferation, differentiation and migration. Aberrant Wnt signaling is known to have an association with multiple cancers. Porcupine is an enzyme that catalyses the addition of palmitoleate to a serine residue in Wnt proteins, a process which is required for the secretion of Wnt proteins. Here we report the synthesis and structure–activity-relationship of the novel porcupine inhibitors based on a 'reversed' amide scaffold. The leading compound **53** was as potent as the clinical compound LGK974 in a cell based STF reporter gene assay. Compound **53** potently inhibited the secretion of Wnt3A, therefore was confirmed to be a porcupine inhibitor. Furthermore, compound **53** showed excellent chemical and plasma stabilities. However, the clearance of compound **53** in liver microsomal tests was moderate to high, and the solubility of compound **53** was suboptimal. Collective efforts toward further optimization of this novel tricyclic template to develop better porcupine inhibitors will be subsequently undertaken and reported in due course.

1. Introduction

The Wnt signaling pathway plays a critical role in the regulation of cellular processes such as proliferation, differentiation and migration.[1–3] The canonical Wnt signaling pathway begins when Wnt ligands bind to the Frizzled and LRP families of cell surface receptors via the cytoplasmic protein Dishevelled (DSH), leading to an accumulation of cytoplasmic β-catenin and its translocation into the nucleus. Ultimately, β-catenin associates with the TCF/LEF family of DNA-binding proteins and activates the expression of β-catenin mediated genes downstream. In contrast, in the absence of Wnt ligand stimulation, β-catenin is phosphorylated and degraded by an intracellular β-catenin destruction complex, resulting in the inhibition of downstream gene expression.[4] Overexpression of Wnt ligands has been associated with numerous cancers.[5,6] Porcupine, a member of the membrane-bound *O*-acyltransferase family of proteins, adds palmitoleate to a serine residue in Wnt proteins—a process which is required for the secretion of Wnt proteins.[7] Porcupine inhibitors can thus block aberrant Wnt signaling and inhibit tumor growth.[8] Therefore, porcupine has emerged as a potential target for the treatment of cancer.

The IWP series of compounds (Fig. 1) identified in a high throughput screen were the first small molecule porcupine inhibitors reported by Chen et al.[9] Since then, other classes of porcupine inhibitors have also been investigated. LGK974, developed by Novartis in 2012, is a potent porcupine inhibitor which has been

Abbreviations: BPO, benzoyl peroxide; DCM, dichloromethane; DIPEA, *N*,*N*-diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; FaSSIF, fasted state simulated intestinal fluid; HATU, 1-[bis(dimethylamino) methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate; SEM, standard error measurement; SGF, simulated gastric fluid; STF, super-top flash; TFA, trifluoroacetic acid.

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http://dx.doi.org/10.1016/j.bmc.2016.09.041
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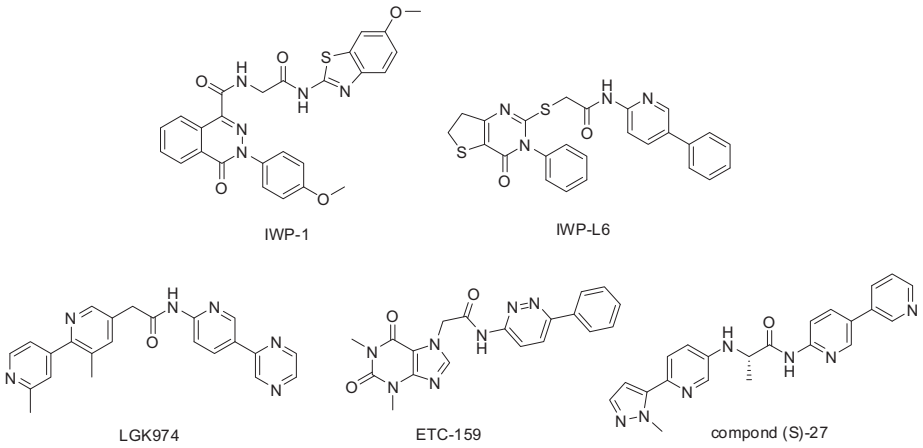

Figure 1. Reported porcupine inhibitors in the literature.

advanced into a phase I/II clinical trial.[10,11] Recently, Virshup and co-workers reported their work on porcupine inhibitors.[12,13] Among them, ETC-159 has been advanced into a phase I clinical trial[12] (Fig. 1).

2. Design

We have investigated a novel series of porcupine inhibitors by a scaffold hopping strategy from a known porcupine antagonist LGK974.[14] DC-9 was the result of optimization campaigns in a recently published Novartis patent.[15] Although the central amide bonds were reversed, both LGK974 and DC-9 showed excellent potency. Encouraged by this result, we decided to introduce the tricyclic element into the 'reversed' amide porcupine inhibitor framework. Here we report the synthesis and structure–activity-relationship of the novel porcupine inhibitors based on the 'reversed' amide scaffold as shown in Figure 2.

3. Chemistry

The synthetic route used to prepare compounds **5**, **6** and **10** is outlined in Scheme 1. The synthesis of compounds **3** and **7** was described in our previously published paper.[14] Commercially available 4-boronobenzoic acid was coupled with 2-chloropyrazine to produce aromatic acid **2**. Nitrile **3** was reduced with H_2 and Pd/C to give amine **4**, which was reacted with corresponding aromatic acids in the presence of HATU in DMF to give the final compounds **5** and **6**, respectively. Compound **7** was converted to nitrile **8** in the presence of $Zn(CN)_2$ and $Pd(Ph_3P)_4$. Nitrile **8** was reduced with $LiAlH_4$ to give amine **9**, which was reacted with aromatic acid **2** to give the final compound **10**.

Target compounds **19**–**24** were prepared via the synthetic route depicted in Scheme 2. The synthesis of aryl iodide **11**, 9,9-difluoro-9*H*-fluorene-2-carboxylic acid, 9*H*-carbazole-2-carboxylic acid and 9-methyl-9*H*-carbazole-2-carboxylic acid was also described in our previously published paper.[14] Compound **11** was converted to nitrile **12** in the presence of $Zn(CN)_2$ and $Pd(Ph_3P)_4$. Nitrile **12**, **3** and **8** were hydrolyzed to give carboxylic acids **13**–**15**, respectively. Commercially available 4-bromo-2-methylpyridine was borylated to provide aryl boronate **17**, which was coupled with (4-bromophenyl)methanamine to give intermediate **18**. Condensation of amine **18** with corresponding aromatic acids in the presence of HATU in DMF afforded the target compounds **19**–**24**, respectively.

Scheme 3 shows the synthetic approaches to the target compounds **27**, **28**, **34** and **35**. Compound **25** was reacted with (4-bromophenyl)methanamine to give intermediate **26**, which was coupled with corresponding aryl boronic acid to produce the desired compounds **27** and **28**, respectively. Commercially available 4-bromobenzonitrile **29** was coupled with imidazole or 4-methyl-1*H*-imidazole to give intermediates **30** and **31**, which were reduced with $LiAlH_4$ to yield amines **32** and **33**, respectively. The amide coupling was carried out by treatment of carboxylic acid **25** with aromatic amines **32** or **33** in the presence of HATU in DMF, which led to the desired compounds **34** and **35**, respectively.

The synthetic pathways used to prepare the target compounds **50**–**53** are summarized in Scheme 4. Suzuki coupling of commercially available 5-(aminomethyl)-2-chloropyridine **36** with aryl boronate **17** produced amine **37**. Aldehydes **38** and **42** were reacted with *tert*-butyl carbamate in the presence of TFA and Et_3SiH to give compounds **39** and **43**, respectively. Removal of Boc in **39** and **43** by TFA/DCM provided amines **40** and **44**, which were coupled with aryl boronate **17** to give intermediates **41** and **45**, respectively. Bromination of 2-chloro-3-fluoro-5-methylpyridine using NBS in CH_3CN provided compound **47**, which was reacted with NaN_3 and subsequently reduced with PPh_3 to give amine **48**. Compound **48** was coupled with aryl boronate **17** to give intermediate **49**. Condensation of aromatic acid **25** with corresponding amines gave the target compounds **50**–**53**, respectively.

The synthesis of the target compounds **57** and **61**–**66** is summarized in Scheme 5. Commercially available 6-bromoisoquinoline **54** was converted to nitrile **55** in the presence of $Zn(CN)_2$ and $Pd(Ph_3P)_4$. Compound **55** was reduced with H_2 and Pd/C to give amine **56**, which was reacted with aromatic acid **25** to yield the target compound **57**. 6-Hydroxy-2-naphthonitrile **58** was readily converted to the triflate intermediate **59**. Compound **59** was coupled with $MeNO_2$ in the presence of $Pd(dba)_2$, K_3PO_4 and Xphos in dioxane and subsequently reduced by Zn in AcOH to give amine **60**, which was reacted with aromatic acid **25** to provide the target compound **61**. Condensation of amine **49** with corresponding aromatic acids gave the target compounds **62**–**66**, respectively.

4. Results and discussion

4.1. Evaluation of pharmacological activity

A cell based STF (super-top flash) reporter gene assay was employed to test Wnt signaling inhibition of the target compounds. We first confirmed that LGK974 was active in this assay (LGK974, 0.9 nM, Table 1), and its IC_{50} number was consistent with the number reported in the literature (LGK974, 0.4 nM).[10,11] The structure–activity relationship is summarized in Table 1.