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Discovery and structure-activity relationship study of phthalimide-phenylpyridine conjugate as inhibitor of Wnt pathway

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ABSTRACT

Aberrant Wnt signaling has been implicated in a variety of disease. Inhibition of the Wnt pathway is an attractive approach for developing new therapeutics for the treatment of various types of fibrosis and cancers. We have discovered the phthalimide-phenylpyridine conjugate as a novel hit compound for the Wnt pathway inhibitors from cellular screening. The structure-activity relationship of these compounds suggested both of the substituent group on the phthalimide fragment and the structure of the linker were critical to the inhibitory activity. The most potent compound was about 10-folds more potent than the hit compound, with IC_{50} value of 0.28 ± 0.01 μM.

Introduction

The Wnt signaling pathway is a critical developmental pathway which controls cellular functions such as proliferation and differentiation.[1,2,3] Extracellular Wnt can trigger the canonical Wnt/β-catenin dependent pathway, the noncanonical Wnt/Ca^{2+} and Planar Cell Polarity pathway (PCP). The β-catenin dependent signaling pathway is activated by the binding of Wnt ligand to the low-density lipoprotein receptor (LRP-5/6 receptors) and Frizzled receptors. This in turn activates Disheveled protein (Dvl), which inhibits Axin-mediated β–catenin phosphorylation, resulting in accumulation of cytoplasmic β–catenin. The β–catenin migrates to the nucleus, interacting there with T cell-specific factor (TCF)/lymphoid enhancer binding factor (LEF) and co-activators, to turn on the Wnt target genes such as c-Myc, cyclin D1 and Cdkn1.

Aberrant Wnt signaling has been implicated in a variety of disease, such as degenerative diseases, metabolic diseases and cancer.[4] In several types of malignancy, Wnt signaling contribute to the maintenance of the cancer stem cell (CSC) population.[5] Inhibition of the Wnt pathway is an attractive approach for developing new therapeutics for the treatment of various types of fibrosis and cancers.[6,7] Several types of Wnt-signaling inhibitors are under ongoing development as anticancer therapies, including Porcupine inhibitors.[8,9], β-Catenin-destruction complex inhibitors such as Tankyrase inhibitors.[10,11] and Disheveled inhibitors.[12], TCF/β-catenin transcription complex inhibitors.[13] and CREB-binding protein (CBP)/β-catenin antagonist.[14]. The Porcupine inhibitors LGK974[8] and CBP/β-catenin antagonist PRI-724 are now in clinical trails for the treatment of cancers (Fig 1).

Herein, we describe the identification of phthalimide-phenylpyridine conjugate as a novel Wnt inhibitor from cellular screening. With the L-Wnt3A cell line that contains a luciferase reporter for β-catenin mediated transcriptional activation reported by Lum and co-workers,[6] we screened the synthetic small-molecule library in our lab. Fortunately, one compound (**1**) showed modest inhibitory activity (34%) at 10 μM in the assay (Fig 2), which has a phthalimide and phenylpyridine fragment, linked by a carbon chain. There are few similar compounds and biological activity reported, so it is worthy to study their structure-activity relationship.

At first, the modifications on the phthalimide fragment were carried out (Scheme 1). The derivatives of the compound **1** were synthesized via a two–step amide coupling reactions from the commercially available precursor phthalic anhydrides. In the first step, the phthalic anhydrides coupled with 1, 4- aminobutyric acid in the presence of the acetic acid at 120 °C to afford the immediate III. This compound was

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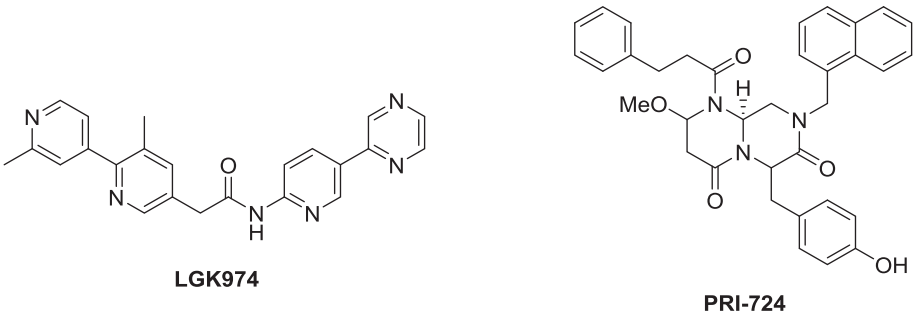


Figure 1. The structure of LGK974 and PRI-724.

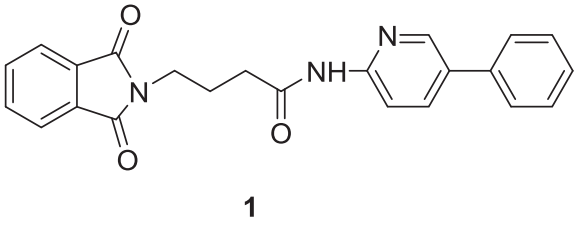


Figure 2. The structure of hit compound 1.

pre-activated by the HATU, followed by the coupling reaction with 2-amino-5-phenylpyridine, giving the finial phthalimide-phenylpyridine conjugates in 29～67% yields.

With the LGK 974 as the positive control agent, these compound were assayed in the L-Wnt3A-luciferase reporter system. Some derivatives showed modest inhibition ratio at 10 μM in this assay (Table 1). It is clear that the substitutions and their positions on the phthalimide fragment had great effects on the biological activity. The conjugates with halogen and methyl group at 3-position (**3**, **7**, **14**) were more potent than that with substitutions at 4-group (**2**, **6**, **13**, **15**, **16**). By contrast, both of the derivatives with the strong electron-withdraw nitro group at 3- or 4-position (**11** or **12**) showed weaker biological activity. Similarly, the extra halogen-substitutions of the 3- or 4-halogenated compounds (**4**, **5**, **8**, **9**) decreased the inhibitory activity of the Wnt signaling pathway. Among these phthalimide-phenylpyridine conjugates, the electron-donating 3-methyl derivatives (**14**) showed the best inhibitory activity, with IC_{50} value of 2.79 ± 0.01 μM in the cell assay. These results suggested that the electrical property of the phthalimide fragment played the important effect on the Wnt pathway inhibitory activity, possibly by the adjustment the electronegativity of the 1-carbonyl group which may provide a hydrogen-honding acceptor to the target. In additions, the stereo-hindrance effect also had great influence, thus compound **16** with Phenylacetylenyl group on 4-position showed weaker inhibitory rate than the methyl and butyl derivatives **13** and **15**.

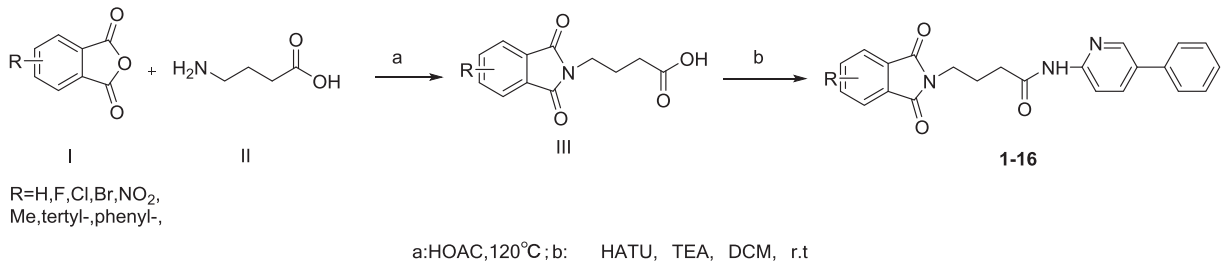
The structure of linker between the phthalimide and phenylpyridine fragments was then explored. With the similar synthetic methods as compounds **1**, the modified derivatives of the lead compound **14** with

Table 1
Wnt signaling pathway inhibitory activity of synthesized compounds.

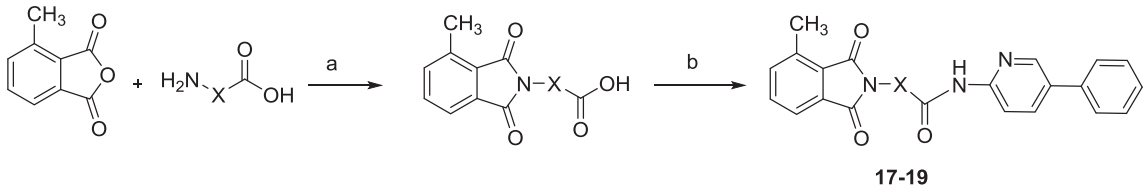
Comp	R	Yield/%	Inhibition ratio/%
1	H	68	34
2	4-F	40	20
3	3-F	40	44
4	3,4-F	41	21
5	3,6-F	40	37
6	4-Cl	55	22
7	3-Cl	52	46
8	4,5-Cl	68	−8
9	2,3,4,5-Cl	69	−5
10	4-Br	55	18
11	4-NO_2	58	19
12	3-NO_2	57	17
13	4-Me	50	20
14	3-Me	52	61 (IC_{50} = 2.79 ± 0.01 μM)
15	4-butyl	57	23
16	4-Phenylacetylenyl	34	7
LGK 974			93 (IC_{50} = 0.57 ± 0.28 nM)

different linkers were prepared (Scheme 2). As expected, the length of the linker had great influences on the biological activities (Table 2). By the decrease of the number of the carbon atoms from 3 to 2, the conjugate **17** showed much less inhibitory activity. By contrast, compound **18** with a one carbon-linker was about 10-folds more potent than conjugate **14**, with IC_{50} value of 0.28 ± 0.01 μM. However, when the linker was replaced by ethylidene, the inhibitory rate of the compound **19** was reduced significantly. The difference of the biological activity of these conjugates with different linker possibly because of the change of the conformation of the molecules. Assumedly, the compound **18** may adopt the relatively appropriated conformation to bind with the target, but the conformation of the compound **17** is different. For compound **19**, the linker has a chirality center, leading to each mesomer adopts the opposite conformation, so they showed about half inhibitory rate of compound **18**.

In conclusion, we have discovered the phthalimide-phenylpyridine conjugate as a promising new chemotype for the Wnt pathway inhibitors. The structure-activity relationship of these compounds



Scheme 1. The synthetic route of compounds.



Scheme 2. The synthetic route of compounds.

Table 2
Wnt signaling pathway inhibition activity of synthesized compounds.

Comp	X	Yield/%	inhibition ratio/%	IC_{50}/μM
17	$-CH_2CH_2-$	54	29	> 10
18	$-CH_2-$	52	76	0.28 ± 0.01[a]
19	$-CH(CH_3)-$	48	39	> 10

[a] IC_{50} = Mean ± S.E.(n = 3).

suggested both of the substituent group on the phthalimide fragment and the structure of the linker were critical to the inhibitory activity. Derivative **18** was the most potent compound to inhibit the Wnt signaling, which will be studied and evaluated in the future.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2019.02.009.

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