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Development of a triazole class of highly potent Porcn inhibitors

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ABSTRACT

The acyltransferase Porcupine (Porcn) is essential for the secretion of Wnt proteins which contribute to embryonic development, tissue regeneration, and tumorigenesis. We have previously discovered four molecular scaffolds harboring Porcn-inhibitory activity. Comparison of their structures led to the identification of a general scaffold that can be readily assembled by modular synthesis. We report herein the development of a triazole version of this new class of Porcn inhibitors. This study yielded IWP-O1, a Porcn inhibitor with an EC_{50} value of 80 pM in a cultured cell reporter assay of Wnt signaling. Additionally, IWP-O1 has significantly improved metabolic stability over our previously reported Porcn inhibitors.

Secreted Wnt proteins play essential roles in embryonic development and adult tissue homeostasis.[1–3] Although aberrant Wnt signaling is frequently associated with the formation and metastasis of tumors, there is no drug targeting this cellular signaling pathway approved for clinical use. We previously identified the Wnt acyltransferase Porcupine (Porcn) that supports Wnt secretion[4] to be highly druggable.[3] We describe herein the development of a new class of small-molecule Porcn inhibitors[5–13] that is highly active in a cultured cell reporter assay of Wnt signaling.

We have previously identified four classes of small-molecule Porcn inhibitors (e.g., **1**–**4**) from a high-throughput screen (HTS) (Fig. 1).[5,6] A close examination of their structures led to the identification of a common structural feature wherein an aryl amide (aryl ketone for **4**) is attached to a heteroaromatic ring through a heteroatom. In particular, general structure **5** serves as a privileged scaffold for developing Porcn inhibitors (Fig. 2). Our previous studies focused on the molecular scaffold of IWP-2 (**1**).[7] A key finding there is that biaryl amide helps provide high potency. For example, IWP-L6 (**6**) is 60-fold more potent than **1** in L-Wnt-STF cells.[7] We now disclose that the same modification also significantly improves the potency of **3** and the aryl group of **5** is important to its activity against Porcn. For example, whereas IWP-L1 (**7**) is inactive at low micromolar concentrations, IWP-L2 (**8**) suppressed Wnt signaling with an EC_{50} value of 0.3 nM in L-Wnt-STF cells.

The observation that **4** has a shorter linker yet high potency made us believe that removal of the X-atom from the linker of **5** would improve activity because of reduced rotational degrees of freedom. We further envisioned that replacement of 1,2,4-triazole with 1,2,3-triazole would support module-based synthesis of new IWPs.

Therefore, we set **9** as the general structure of interest (Fig. 3). Its assembly can be easily achieved by Huisgen 1,3-dipolar cycloaddition, triazole C—H arylation, and amidation. Synthetically, coupling of aryl alkyne **10** with azide **11** proceeded smoothly to provide triazole **12**. The palladium-catalyzed C—H arylation of **12** under our newly modified conditions[14] gave 1,4,5-trisubstituted triazole **13** in good yields except for a few sterically hindered substrates. Subsequent treatment of **13** with trifluoroacetic acid afforded the corresponding carboxylic acid uneventfully. However, the following amidation was surprisingly difficult. We did not observe any amidation product when using the acid chloride, PyBOP, HATU, or TBTU coupling method. Although a small amount of **14** could be obtained from EDC/HOBt coupling, purification was proved challenging. In our hands, activation of the carboxylic acid as an acyl mesylate[15] was the only effective way to prepare **14**.

With a suitable synthetic route in hand, we prepared a collection of new IWPs (**15**) using 2-amino-5-phenylpyridine as the standard biaryl group in the initial studies (Table 1). We tested the ability of **15** to suppress Wnt signaling in L-Wnt-STF cells using a previously reported protocol.[7] Among the mono-arylated triazoles (Ar^2 = H), only the 4-pyridyl derivative show good potency (Table 1,

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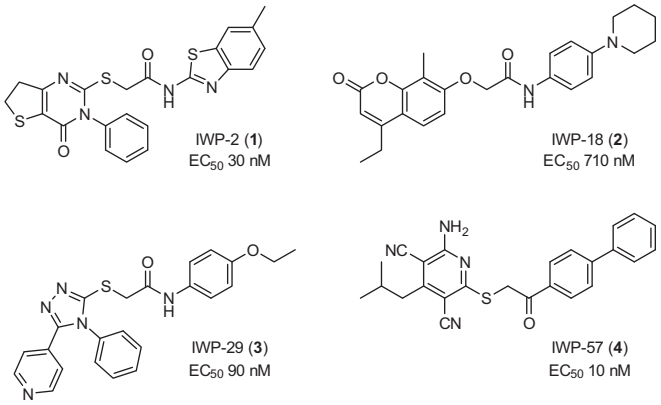


Fig. 1. Representative structures of the four classes of IWPs (**1–4**) identified from HTS

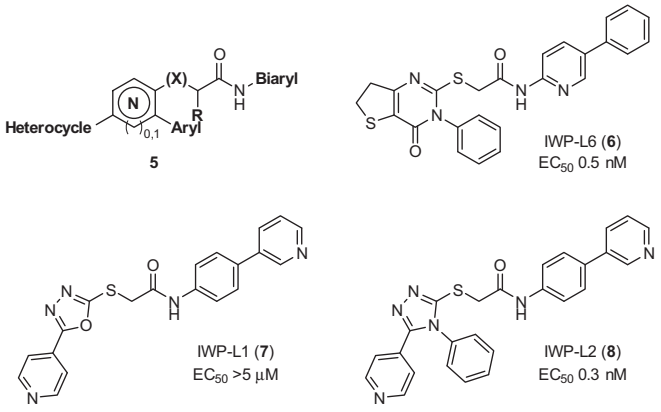


Fig. 2. The general structure of IWP (**5**) and the effects of the biaryl and phenyl groups (**6–8**)

entries 1–4). Deleting or moving the position of the nitrogen atom of the pyridyl group led to dramatically reduced activity. However, removal of the sulfur atom in the linker indeed improved potency. Compared to IWP-L1 (**7**) that showed no activity at 1 μM concentration, the corresponding triazole analog IWP-N3 (**16**) is a potent Porcn inhibitor (EC_{50} 9 nM).

Introduction of a phenyl group to triazole further improved the potency. Even the 4,5-diphenyl substituted triazole **15** (Ar^1, Ar^2 = -Ph) showed weak Porcn inhibitory activity (entry 5). Adding a trifluoromethyl group to the 2-position of the 4-phenyl group had little effect (entry 6), but introducing a hydrogen bond acceptor to the 4-position was beneficial (entry 7). The activity of the pyridyl substituted triazoles **15** (Ar^1 = pyridyl) was also significantly improved after incorporation of a 5-phenyl group (Ar^2 = Ph). The EC_{50} values for the 2-, 3-, and 4-pyridyl derivatives are 2.5 μM, 5 nM, and 80 pM, respectively (entries 8–10). In particular, the 4-pyridyl derivative IWP-O1 (**17**) is 2.5 times more potent than LGK974[5a,b] (0.2 nM), one of two Porcn inhibitors that has advanced to clinical studies.[1] Introduction of an α-methyl or ethyl group to amide **17** (**15**, Ar^1 = 4-pyridyl, Ar^2 = Ph, R = Me or Et) reduced the activity likely due to disfavored ligand conformations (entries 11 and 12).

Consistent with our experience with IWP-2 (**1**),[5–7] the phenyl group (Ar^2) of **17** could tolerate a range of structural modifications. The presence of a hydrogen bond donor or acceptor at the 4-position of the phenyl group only resulted in slightly reduced activity (entries 13–17). A methyl group at the 3-position was also compatible (entry 18). However, substitution at the 2-position had more significant impact on the activity (entries 19–21). Finally, introduction of a fluoro, methyl or trifluoromethyl group to the 2-position of the pyridyl group slightly attenuated the activity (entries 22–24).

With an optimized triazole group in hand, we next studied if the potency can be further improved by varying the structure of the aryl amide group of **18** (Table 2). Removal of the nitrogen atom from **17** led to a 5-fold decrease of potency (entry 1). Change of the substitution position of the biphenyl group led to further loss of activity (entry 2), consistent with what was observed with the

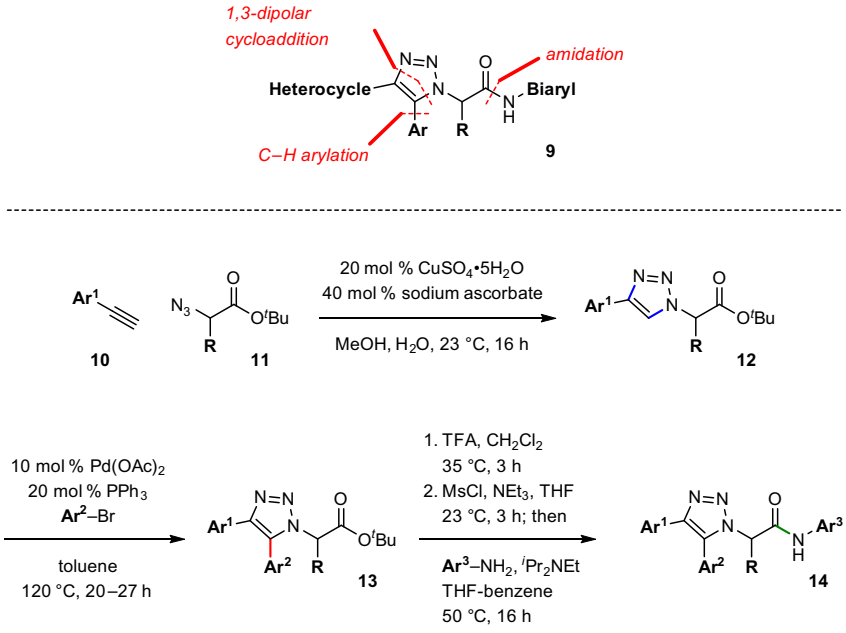
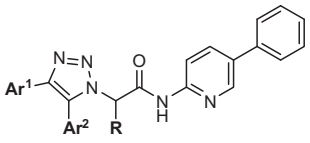


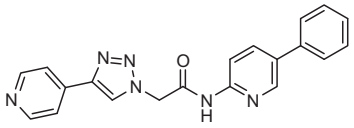
Fig. 3. The molecular scaffold (**9**) of interest in this study and the synthetic route for this triazole class of IWP molecules

Table 1
Porcn-inhibitory activity of the triazole-class IWPs.

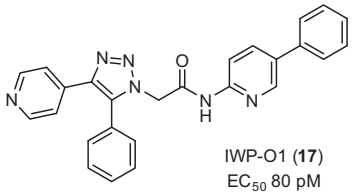


15

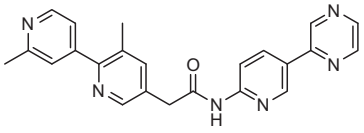
Entry	Ar^1	Ar^2	R	EC_{50}
1	Phenyl	H	H	>5 μM
2	2-pyridyl	H	H	>5 μM
3	3-pyridyl	H	H	100 nM
4	4-pyridyl	H	H	9 nM
5	Phenyl	Phenyl	H	400 nM
6	2-F3C-phenyl	Phenyl	H	500 nM
7	4-MeO-phenyl	Phenyl	H	18 nM
8	2-Pyridyl	Phenyl	H	2500 nM
9	3-Pyridyl	Phenyl	H	5 nM
10	**4-Pyridyl**	**Phenyl**	**H**	**0.08 nM**
11	4-Pyridyl	Phenyl	Me	3 nM
12	4-Pyridyl	Phenyl	Et	12 nM
13	4-Pyridyl	4-MeO-phenyl	H	0.18 nM
14	4-Pyridyl	4-EtO2C-phenyl	H	0.7 nM
15	4-Pyridyl	4-NC-phenyl	H	0.48 nM
16	4-Pyridyl	4-F3C-phenyl	H	0.3 nM
17	4-Pyridyl	4-F-phenyl	H	0.2 nM
18	4-Pyridyl	3-Me-phenyl	H	0.4 nM
19	4-Pyridyl	2-Me-phenyl	H	40 nM
20	4-Pyridyl	2-MeO-phenyl	H	9 nM
21	4-Pyridyl	1-Naphthyl	H	6.5 nM
22	2-F-4-pyridyl	Phenyl	H	0.13 nM
23	2-Me-4-pyridyl	Phenyl	H	0.14 nM
24	2-F3C-4-pyridyl	Phenyl	H	0.6 nM
25	Phenyl	H	H	>5 μM



IWP-N3 (**16**)
EC_{50} 9 nM

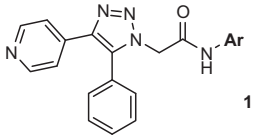


IWP-O1 (**17**)
EC_{50} 80 pM



LGK974
EC_{50} 0.2 nM

Table 2
Optimization of the aryl amide group.



18

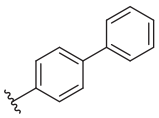
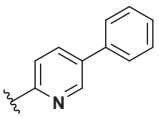
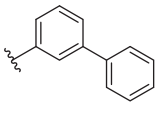
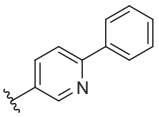
Entry	Ar	EC_{50}	Entry	Ar	EC_{50}
1		0.43 nM	**10**		**0.08 nM**
2		0.27 μM	11		0.2 nM

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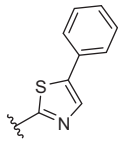
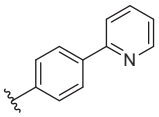
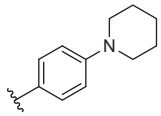
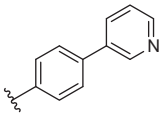
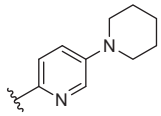
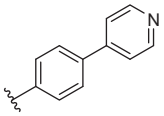
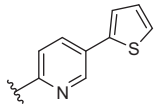
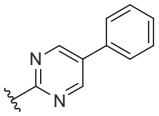
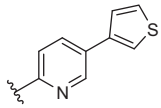
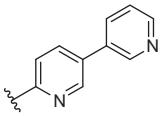
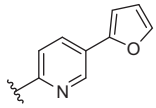
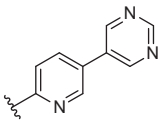
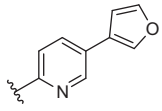
Table 2 (*continued*)

Entry	Ar	EC_{50}	Entry	Ar	EC_{50}
3		80 μM	12		1.8 nM
4		8 nM	13		0.2 μM
5		>1 μM	14		7 nM
6		0.26 nM	15		8 nM
7		0.65 nM	16		>1 μM
8		0.4 nM	17		1.8 nM
9		0.6 nM			

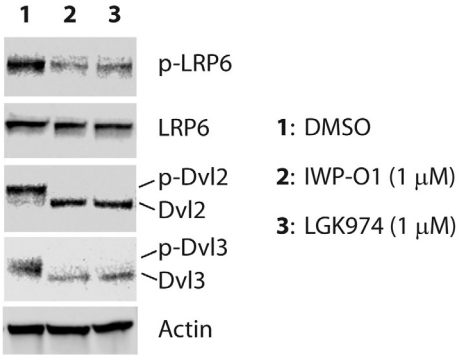


Fig. 4. Biochemical characterization of IWP-O1 (**17**). HeLa cells which exhibit high levels of cell autonomous Wnt signaling,[16] were incubated for 24 h with DMSO, **17**, or LGK974. Cell lysates were then subjected to Western blot analysis for biochemical markers of Wnt signaling (LRP6 and Dvl2/3 phosphorylation)

molecular scaffold of IWP-L6.[7] However, replacement of the 5-phenylpyridyl group with a 3-phenylthiazolyl group, an effective biaryl substitute in IWP-L6 (**6**), resulted in significant reduction of activity (entry 3). Substitution of the terminal phenyl group with a piperidine group also led to significantly reduced activity (entry 4). Surprisingly, restoration of the nitrogen atom yielded an essentially inactive compound (entry 5). Replacement of the phenyl group of 2-amino-5-phenylpyridine with a thiophenyl or a furyl group also attenuated activity (entries 6–9). Among all phenylpyridyl amides, 2-amino-5-phenylpyridine remained to be the best amino group (entry 10–14). Furthermore, incorporation of additional nitrogen atoms to the biaryl amide resulted in weakened Porcn inhibitors (entries 15–17).

We have biochemically confirmed that IWP-O1 (**17**) functions by preventing the secretion of Wnt proteins. Dishevelled (Dvl) phosphorylation is associated with both β-catenin dependent and independent Wnt signaling pathways. **17** effectively suppressed the phosphorylation of Dvl2/3 in HeLa cells (Fig. 4). At the same time, the phosphorylation of low density lipoprotein receptor-related protein 6 (LRP6), a hallmark of the Wnt/β-catenin pathway activity, was also suppressed by the chemical treatment.

We have previously found that IWP-L6 (**6**) with a thienopyrimidinone core has good stability in human but not mouse plasma, imposing challenges for model studies in mice.[7] We now show that the 1,2,3-triazole class of IWP molecules possesses improved metabolic stability. For example, the half-life time of **6** in murine liver S9 fractions and plasma is 26 min and <5 min, respectively (Table 3).[7] In contrast, IWP-N3 (**16**) and IWP-O1 (**17**), with a 1,2,3-triazole core, are much more stable in murine liver S9 frac-

Table 3
Comparison of the metabolic stability of different IWP scaffolds.

Half-life	IWP-L6 (**6**)	IWP-L2 (**8**)	IWP-N3 (**16**)	IWP-O1 (**17**)
murine liver S9 fractions	26 min	15 min	200 min	stable over 4 h
murine plasma	<5 min	<5 min	100 min	130 min

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tions and plasma. In particular, we did not observe any degradation with **17** after incubation with murine liver S9 fractions for 4 h. The low metabolic stability of **6** may be partially due to the presence of a sulfur atom as IWP-L2 (**8**) bearing a 1,2,4-triazole core also shows low stability in murine liver S9 fractions and plasma.

In summary, biaryl amide-bearing triazoles having a general structure of **5** wherein a hydrogen bond acceptor is present at the 4-position of the appending heterocycle comprise a group of highly potent Porcn inhibitors. The aryl group of **5** has significant contribution to its activity. Particularly, IWP-O1 (**17**) suppresses Wnt signaling in L-Wnt-STF cells with an EC_{50} value of 80 pM, 2.5 times more active than the investigational drug LGK974. With significantly improved metabolic stability, **17** is more suitable for model studies in mice.

Acknowledgments

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.11.012.

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