



Structure–activity relationship studies of small-molecule inhibitors of Wnt response

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ABSTRACT

Suppression of oncogenic Wnt-mediated signaling holds promise as an anti-cancer therapeutic strategy. We previously reported a novel class of small molecules (IWR-1/2, inhibitors of Wnt response) that antagonize Wnt signaling by stabilizing the Axin destruction complex. Herein, we present the results of structure–activity relationship studies of these compounds.

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The Wnt signal transduction pathways play key roles in embryogenesis and tissue homeostasis,¹ and are frequently mis-activated in cancer.² Small molecules that antagonize the Wnt signaling pathways therefore hold promise as anti-cancer therapeutics.^{3,4} We recently reported two small molecules, IWR-1 (**1**) and IWR-2 (**2**) (Fig. 1) that suppressed Wnt signaling by stabilizing the Axin destruction complex, a negative regulator of canonical Wnt signaling.⁵ To understand the structure–activity relationship of **1** and **2**, we synthesized a series of analogs and compared their ability to suppress Wnt signaling in luciferase-based reporter assays as previously described.^{5,6} The results of these studies are presented in this Letter.

To facilitate our structure–activity relationship analyses, we considered **1** and **2** to be composed of three structural subunits, the norbornyl, spacer and amide regions (Fig. 1). The equal potency of **1** and **2** suggested that their amide region may accommodate structural modifications without suffering a significant loss in activity. We therefore focused our efforts on modifying the amide substitution groups (Table 1). Indeed, we found that introduction of a methyl group to the 7-position of the quinoline (**3**) only slightly decreased activity. However, partial saturation of the quinoline group (**4**) resulted in considerable reduction of activity. The limited access to 8-aminoquinoline derivatives prompted us to

examine the potential of substituting the quinoline with other aromatic groups.

We first sought to simplify the structure of the aromatic amide. We recognized that the anisoyl analog **5** was a less potent hit of the initial screen⁵ that yielded the discovery of **1** and **2**. Interestingly, its parental analog phenyl amide **6** was inactive at even 50 μ M, suggesting that fine-tuning the steric and electronic properties of **6** to be a feasible approach to discover new Wnt inhibitors. We therefore set out to evaluate the activity of a series of phenyl amide analogs (Table 1).

Significantly, introduction of halogen atoms to the 4-position of the phenyl group (**13–15**) was found to be effective, with the 4-bromophenyl derivative **15** being the most active compound. Other halogen derivatives (**7–11**) were found nearly inactive with the

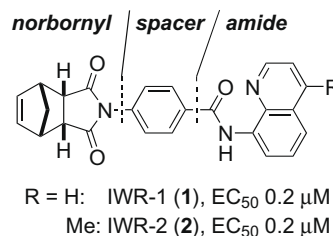


Figure 1. The structures of IWR-1 (**1**) and IWR-2 (**2**).

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Table 1
SAR studies on the IWR amide region

Compounds	R	EC ₅₀ (μM)
3	7-Methylquinolin-8-yl	0.8
4	5,6,7,8-Tetrahydroquinolin-8-yl	10
5	2-Methoxyphenyl	1
6	Phenyl	>25
7	2-Fluorophenyl	>25
8	2-Chlorophenyl	>25
9	2-Bromophenyl	>25
10	3-Fluorophenyl	>25
11	3-Chlorophenyl	>25
12	3-Bromophenyl	9
13	4-Fluorophenyl	4
14	4-Chlorophenyl	3
15	4-Bromophenyl	1
16	2,3-Difluorophenyl	>25
17	2,4-Difluorophenyl	9
18	2,4-Dichlorophenyl	10
19	2,4-Dibromophenyl	>25
20	2,5-Difluorophenyl	>25
21	2,6-Difluorophenyl	>25
22	3,4-Difluorophenyl	3
23	3,4-Dichlorophenyl	5
24	3,4-Dibromophenyl	10
25	3,5-Difluorophenyl	>25
26	2-(Trifluoromethyl)phenyl	>25
27	3-(Trifluoromethyl)phenyl	20
28	4-(Trifluoromethyl)phenyl	>25
29	Benzyl	20
30	(2-Pyridyl)methyl	>25
31	(3-Pyridyl)methyl	>25
32	(4-Pyridyl)methyl	10
33	<i>trans</i> -(2-Methoxy)cyclohexyl	2
34	<i>trans</i> -(2-Hydroxy)cyclohexyl	>25
35	Cyclohexyl	>25

exception of modest activity for the 3-bromophenyl derivative **12**. We next examined the effects of dihalogen substitutions. These compounds (**16–25**) were generally less potent than the mono-halogenated derivatives **7–15**. Only the 3,4-dihalophenyl derivatives (**22–24**) showed moderate activity. The trifluoromethylphenyl derivatives **26–28** were also found to be ineffective Wnt inhibitors.

We have also examined the activity of alkyl amide analogs. The benzyl amide **29** was a weak inhibitor and incorporation of a nitrogen atom to the 4-position of the phenyl group (**32**) slightly improved the activity. Other pyridyl derivatives (**30** and **31**) were found to be inactive. Saturation of the phenyl group of **5** led to a twofold decrease in potency (**33**). Further truncation of the structure (**34** and **35**) abolished activity.

To explore the structural requirement of the spacer region of IWR's, we replaced the central aromatic ring of **1** with five different spacers (**Table 2, a–e**). Introduction of a chloro, methyl or methoxyl group to the 2-position of the center aromatic ring (**36–38**) significantly twisted the conformation and abolished the activity. On the other hand, the 3-position of the spacer tolerates certain modification (**39–41**). The chloro and methyl derivatives (**39** and **40**) are moderately active. We also found that extension of the spacer by one nitrogen atom (**R = b**) to form an aromatic urea linker (**42**) rendered the compound inactive. Interestingly, saturation of this spacer (**R = c**) resulted in a slight loss of activity (**43–45**). Incorporation of a nitrogen atom to the saturated spacer (**R = d**) to form a urea linker (**46**) also reduced the activity significantly. Consistent with the observation with **42** (**R = b**), extension of the saturated

Table 2
SAR studies on the IWR spacer region

Compounds	R	R'	Ar	EC ₅₀ (μM)
36	a	2-Chloro	Quinolin-8-yl	>25
37	a	2-Methyl	Quinolin-8-yl	>25
38	a	2-Methoxy	Quinolin-8-yl	>25
39	a	3-Chloro	Quinolin-8-yl	1
40	a	3-Methyl	Quinolin-8-yl	2
41	a	3-Methoxy	Quinolin-8-yl	>25
42	b	—	Quinolin-8-yl	>25
43	c	—	Quinolin-8-yl	0.4
44	c	—	4-Methylquinolin-8-yl	1
45	c	—	7-Methylquinolin-8-yl	1
46	d	—	Quinolin-8-yl	10
47	e	—	Quinolin-8-yl	>25
48	e	—	4-Methylquinolin-8-yl	>25
49	e	—	7-Methylquinolin-8-yl	10

spacer by one carbon atom (**R = e**) led to drastic decrease in activity (**47–49**). We further found that introduction of an *N*-alkyl group to the amide abolished the activity. *N*-Me-IWR (**50**) was not active even at 50 μM (**Fig. 2**). These results underlined the importance of the amide functional group and the conformation of IWR.

We previously reported that *exo*-IWR **51** was only active at high concentration.⁵ Quantitatively, **51** was 25-fold less active than *endo*-IWR **1** (**Fig. 2**). Interestingly, saturation of the olefin did not affect the activity. *Sat*-IWR **52** and **1** were equally potent in the *in vitro* assays (**Fig. 2**). These results indicated that the norbornyl region of **1** could only tolerate subtle steric perturbation.

We have also tested the *in vivo* activity of IWR's and found that IWR-1 (**1**) effectively inhibited zebrafish tail fin regeneration.⁵ We show herein that the minimum inhibitory concentration of **1** is 0.5 μM (**Fig. 3**). We further demonstrated that the *in vivo* activity

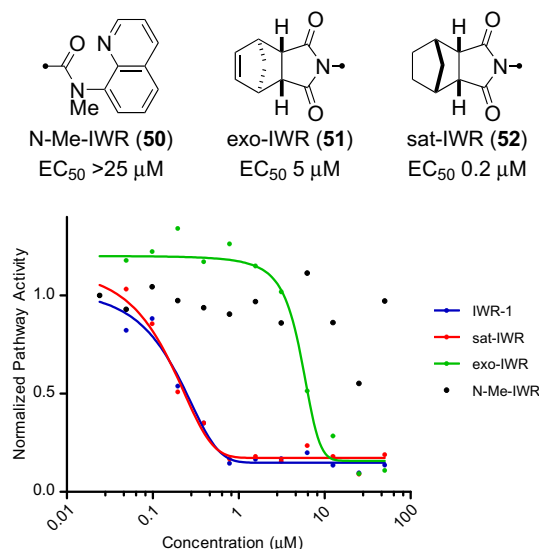


Figure 2. The structural deviation of **50–52** from **1** and their dose–response curves.

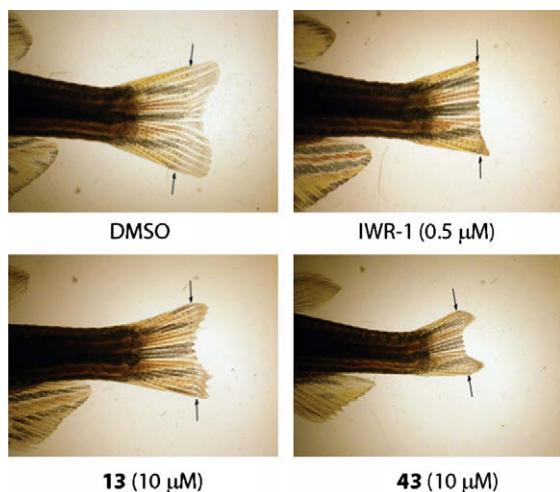


Figure 3. Inhibition of fin regeneration in zebrafish by IWR's. Arrows indicate the points of resection.

of IWR's correlated with their in vitro activity. For example, only partial inhibition of fin regeneration was observed with moderate inhibitors **13** and **43**. The weak inhibitor **17** only retarded the growth of the tail fin (picture not shown).

Preliminary metabolic stability studies⁷ indicated enzymatic cleavage of the amide bond to be the primary degradation pathway of **1** (Fig. 4). Loss of the parent compound **1** and subsequent appearance of acid **53** and 8-aminoquinoline (**54**) were noted when **1** was incubated in murine whole blood. The half-life in blood was 60 min. A similar pattern of cleavage was noted upon incubation of **1** with murine liver S9 fractions ($t_{1/2}$ = 20 min for Phase I); however, a decade of **54** was also noted, suggestive of a secondary metabolism pathway. The half-life of **1** in intact murine hepatocytes was 20 min and those of the less potent inhibitors **5** and **13** were 10 and 60 min, respectively.

In summary, we have conducted focused structure-activity relationship studies on IWR-1/2 (**1** and **2**). The norbornyl, spacer and amide regions can all be modified with compromised activity. The in vivo activity of the IWR's correlated with their in vitro activity. Further investigation will be centered on altering the steric and electronic properties of the norbornyl and spacer regions and modifying the amide linkage to improve the activity and pharmacokinetic properties.

Acknowledgments

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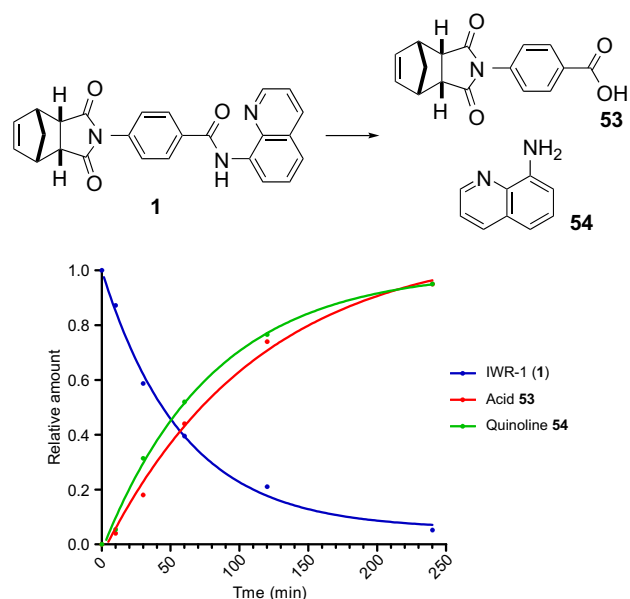


Figure 4. The murine whole blood metabolism experiments of **1**.

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- Wnt/ β -catenin pathway response was measured in L-cells expressing Wnt3A and harboring control and Wnt-responsive reporters.
- Metabolic stability studies were conducted in murine whole blood by incubating 2 μ M of **1** in 150 μ L of blood collected from C57BL/6 mice. Stability in the presence of S9 fractions (Celsis/InVitro Technologies) was conducted with 2 μ M of **1**, 1 mg of S9 protein, and an NADPH-regenerating system (1.7 mg/mL NADP, 7.8 mg/mL glucose-6-phosphate, 6 U/mL glucose-6-phosphate dehydrogenase in 2% w/v NaHCO_3 /10 mM MgCl_2) also containing uridine 5'-diphospho- α -D-glucuronic acid (1.9 mg/mL) and 3'-phosphoadenosine-5'-phosphosulphate (100 mg/mL) for both Phase I and Phase II metabolic reactions. Hepatocyte metabolism was conducted by incubating 2 μ M of **1** in the presence of 10^5 cryopreserved murine CD1 hepatocytes (Celsis/InVitro Technologies). Incubation was terminated at the indicated time points by addition of methanol. Reactions conducted in saline or buffer served as controls and no degradation was observed. Parent compounds and fragments were detected by LC/MS/MS.