Novel Synthetic Antagonists of Canonical Wnt Signaling Inhibit Colorectal Cancer Cell Growth

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Abstract

Canonical Wnt signaling is deregulated in several types of human cancer where it plays a central role in tumor cell growth and progression. Here we report the identification of 2 new small molecules that specifically inhibit canonical Wnt pathway at the level of the destruction complex. Specificity was verified in various cellular reporter systems, a Xenopus double-axis formation assay and a gene expression profile analysis. In human colorectal cancer (CRC) cells, the new compounds JW67 and JW74 rapidly reduced active β-catenin with a subsequent downregulation of Wnt target genes, including AXIN2, SP5, and NKD1. Notably, AXIN2 protein levels were strongly increased after compound exposure. Long-term treatment with JW74 inhibited the growth of tumor cells in both a mouse xenograft model of CRC and in ApcMin mice (multiple intestinal neoplasia, Min). Our findings rationalize further preclinical and clinical evaluation of these new compounds as novel modalities for cancer treatment. Cancer Res; 71(1); 197–205. ©2011 AACR.

Introduction

β-Catenin is a key component of the Wnt pathway that upon Wnt activation translocates to the nucleus where it activates gene transcription in association with TCF/LEF transcription factors. In the inactive state of Wnt signaling, β-catenin is located in the cytoplasm in a large protein complex with AXIN, APC, GSK-3β, and others that facilitates β-catenin phosphorylation and its subsequent degradation in the proteasome. Canonical Wnt signaling plays a crucial role in embryonic development, and in a deregulated stage, Wnt signaling is a common denominator in a variety of tumors (1–3). Wnt-activating mutations are present in a variety of cancers including colon cancer, gastric cancer, hepatocellular carcinoma, Wilms tumor of the kidney, medulloblastoma, melanoma, non–small cell lung cancer, ovarian endometrioid cancer, anaplastic thyroid cancer, pancreas adenocarcinoma, and prostate cancer (1). About 90% of sporadic colon cancers show aberrant Wnt signaling (2, 4, 5), whereas all pancreatic adenocarci predominas exhibit alterations in Wnt/Notch signaling (6). Frequently, mutations in the adenomatous polyposis coli (APC), β-catenin, or AXIN genes lead to the accumulation of nuclear β-catenin and contribute to tumor initiation and progression (5). Furthermore, alterations in extracellular proteins that silence Wnt signaling, including secreted frizzled related proteins (7), Dickkopf (DKK; ref. 8), and members of the Wnt inhibitor factor (WIF) family (9), may lead to an abnormal pathway activity (10).

Blocking canonical Wnt activity in colorectal and other Wnt-deregulated cancers has been shown to cause cell-cycle arrest in the G1 phase, which is likely a crucial step in tumor cell growth inhibition (11, 12). Because elevated levels of β-catenin in the nucleus seem to be a common feature of abnormal canonical Wnt signaling (1), downregulation of canonical Wnt activity by reducing the presence of nuclear β-catenin represents a promising therapeutic strategy. In recent years, several classes of small molecules have been shown to act as Wnt inhibitors that exert their inhibitory effects at various levels of the Wnt signaling pathway (13–21). Recently, 2 groups of chemical substances, IWR-1 and XAV939, were identified that stabilize the destruction complex (22, 23). Mathematical modeling prediction of destruction complex activity showed that AXIN2 functions as a critical rate limiting factor (24). By blocking the PARP domain of Tankyrase, XAV939 and IWR-1 are thought to alter the poly-ADP ribosylation (PARylation) and ubiquitination of AXIN2 that results in increased stability and in inhibition of the canonical Wnt signaling.

In this study, we present 2 novel small molecules that strongly and specifically reduce levels of active β-catenin in vitro and in vivo. Their specificity and tumor growth...
inhibition suggest that these chemical structures could be attractive candidates for further pharmaceutical development.

Materials and Methods

High-content small molecule screen

A total of 8,000 ST-d1EGFP HEK293 cells were seeded in poly-L-lysine–coated clear bottomed 384-well plates with black walls (Corning). After 24 hours, cell culture media was robotically changed to 75% Wnt3a-CM containing compounds at 50 or 20 μmol/L concentrations (Sciclone ALH 3000 Liquid Handling Workstation; Caliper LifeSciences). After 24 hours, the cells were counterstained with Hoechst and assayed for d1EGFP expression with the automated ArrayScan VTi HCS Reader and Thermo Scientific BioApplications software (Thermo Scientific).

Other Materials and Methods

See Supplementary Materials and Methods.

Results

High-throughput screen for inhibitors of the canonical Wnt signaling

To screen for small molecule inhibitors of canonical Wnt signaling, a synthetic TCF-responsive promoter [SuperTOPFlash (ST-Luc); 7× TCF-binding sites; refs. 25, 26] was coupled to a destabilized d1EGFP gene (Fig. 1A). The construct was stably transfected into HEK293 cells. These cells were activated using conditioned media from L Wnt3a-expressing cells (27) and sorted by FACS to create the stable reporter cell line ST-d1EGFP HEK293. In the reporter line, the expression of d1EGFP peaked at 24 hours (35% d1EGFP-positive cells), and detectable reporter expression disappeared within 24 hours after the removal of Wnt-containing medium (Fig. 1B, top). The ST-d1EGFP HEK293 cell line was used for screening 37,000 compounds, selected on the basis of structural diversity, stability, and molecular mass. A hit cutoff was set to below IC90 (dotted line in Fig. 1B, bottom). The initial screen yielded 77 primary hits.

JW67 and JW74 are efficient and specific inhibitors of the canonical Wnt signaling in vitro and in vivo

In the following rescreening tests of the 77 primary hits, we used luciferase reporter assays, including Renilla expression as an internal reference, for 3 cell signaling pathways to examine the compound specificity: (i) HEK293 cells that were transiently transfected with Wnt signal inducible ST-Luc, (ii) Shh Light II cells (Gli1-Luc) were used as a stable reporter line for the Sonic hedgehog signaling pathway; and (iii) HEK293 cells with transiently transfected NF-kB luciferase reporter (NF-kB-Luc) were used to test the NF-kB pathway. The reporter cells were induced with 30% Wnt3a-conditioned media (Wnt3a-CM), 50% Shh-CM (Gli-Luc), or 10 ng/mL of TNFα (NF-kB-Luc), respectively, and incubated in the presence of various compound concentrations. A low effect in ST-Luc or unspecific repression of the Shh- or NF-kB pathways excluded most of the primary hits and yielded 2 lead compounds named JW67 and JW74 (Fig. 1D). Compounds JW67 and JW74 showed a reduction of canonical Wnt signaling in the ST-Luc assay with IC50 values of 1.17 μmol/L and 790 nmol/L, respectively (Fig. 2A and Supplementary Fig. S1A). JW67 or JW74, at concentrations 1 to 10 μmol/L, showed no significant inhibitory effects in the assays when measuring Shh-Luc (Fig. 2B and Supplementary Fig. S2A), NF-kB activity (Fig. 2C), or Renilla readout.

To examine the in vivo efficacy and specificity of the compounds JW67 and JW74 in obstructing canonical Wnt signaling, a Xenopus laevis axis duplication assay was carried out. Injection of ectopic X. laevis Wnt8 (XWnt8) mRNA into the ventral blastomeres of 4-cell stage embryos activates the canonical Wnt pathway and induces a secondary body axis. This assay thus provides a reliable method to test biological effects of compounds with potential effects on Wnt signaling. A coinjection of 10 pg of XWnt8 with 0.4 pmol of JW67 resulted in a 57% reduction of double-axis formation compared with DMSO controls (n = 123), whereas JW74 showed a reduction of 87% at 0.4 pmol (n = 335; Fig. 2D, top and bottom). In summary,
JW67 and JW74 increase concentration of AXIN2 and decrease the active form of β-catenin in SW480 colorectal cancer cells

Next, we questioned which level of activation of canonical Wnt signaling was specifically targeted by the 2 compounds. Downstream of the receptor level, the activity of GSK-3β can be inhibited by LiCl, resulting in inefficient phosphorylation of β-catenin and its subsequent degradation that ultimately leads to the activation of Wnt target genes (28, 29). HEK293 cells were transiently transfected with ST-Luc, induced with 25 mmol/L of LiCl and exposed to various doses of the compounds. Both compounds, JW67 and JW74, antagonized LiCl-induced activation of the canonical Wnt signaling with IC<sub>50</sub> values of 690 and 420 mmol/L, respectively (Fig. 3A and Supplementary Fig. S1B). These results indicated that the 2 compounds exerted their effects at the level of the β-catenin destruction complex (GSK-3β/AXIN/APC) or downstream of it. Downstream of the destruction complex, Wnt signaling can be readily activated by the overexpression of normal full-length β-catenin or by β-catenin carrying stabilizing alanine point mutations at the N-terminal phosphorylation sites (S33, S37, T41, S45; dominant, active β-catenin, da-Cat). HEK293 cells were transiently transfected with ST-Luc/Renilla and with plasmids encoding full-length β-catenin or da-Cat. Plasmid amounts used for transfections were diminished to nanograms per well in a 48-well plate to obtain reporter induction in the range of 10 to 20 fold. The activity of ST-Luc after overexpression of normal full-length β-catenin was significantly reduced by both compounds at 10 mmol/L (Shapiro–Wilk test failed, Mann–Whitney rank-sum test: JW67, <i>P</i> = 0.001; JW74, <i>P</i> = < 0.001; Fig. 3B, left, and Supplementary Fig. S2A). However, Wnt activation induced by da-Cat was not significantly antagonized by the compounds (Fig. 3B, right; Shapiro–Wilk tests failed, Mann–Whitney: JW67, <i>P</i> = 0.126; JW74, <i>P</i> = 0.928; Supplementary Fig. S2B). Thus, both compounds may affect the steady-state level of β-catenin through N-terminal phosphorylation, most likely through activity by the GSK-3β/AXIN/APC destruction complex.

Next, we tested JW67 and JW74 in the colorectal cancer (CRC) cell lines SW480 and DLD-1 that contain mutations in the APC gene that leads to an elevated level of nuclear β-catenin and aberrant canonical Wnt signaling. SW480 cells were treated for 24 hours with the compounds at various concentrations and alterations in central components of the canonical Wnt pathway were examined using Western blot analysis. Antibodies against AXIN2, total β-catenin or by active non-phosphorylated form of β-catenin (ABC), revealed that upon compound treatment the active form of β-catenin in the nuclear and cytoplasmic compartments was reduced, whereas cytoplasmic AXIN2 accumulated remarkably (Fig. 3C and Supplementary Fig. S3A–C). JW67 increased the steady-state expression of AXIN2 at a minimum of 1 mmol/L, whereas JW74 increased AXIN2 at 0.1 mmol/L. JW67 and JW74 decreased ABC levels at 1 mmol/L, and total β-catenin was mildly reduced by JW67 but strongly decreased by JW74 (both 5 and 10 mmol/L). At the same time, the concentration of phosphorylated β-catenin (p-β-catenin), which represents its degradable form, was elevated.
Overall reduction of the nuclear and cytoplasmic \(\beta\)-catenin was further confirmed by immunofluorescence in SW480 cells, using an anti-\(\beta\)-catenin antibody after 48-hour incubations with 5 or 10 \(\mu\)mol/L of compounds (equal shutter speed; Supplementary Fig. S5). Moreover, confocal microscopy (different automatic shutter speeds) revealed that the remaining \(\beta\)-catenin was found to be concentrated in adherence junctions (Fig. 4, arrowheads). AXIN2 was simultaneously reduced in the nucleus and elevated in the cytoplasm when compared with the controls. Strikingly, the confocal imaging of double-stained cells revealed aberrant foci in the cytoplasm containing abundant \(\beta\)-catenin and AXIN2 (arrows in Fig. 4). JW74 treatment yielded most of such foci that may represent clustered destruction complexes. Taken together, our data may suggest that JW67 and JW74 primarily act on the stabilization of AXIN2 directly or through other components leading to enhanced \(\beta\)-catenin degradation in APC mutant CRCs (Fig. 3D).

Next, we tested whether the increase of AXIN2 and the phosphorylated \(\beta\)-catenin resulted from altered activities of major kinases or phosphatases. A diverse panel of phosphatases and kinases was exposed to 10 \(\mu\)mol/L of JW74 in vitro. None of the tested enzyme activities were considerably influenced by JW74 (Supplementary Fig. S4).

**JW67 and JW74 affect the expression of Wnt target genes and reduce cell growth of colon cancer cell lines**

To measure the effect of JW67 and JW74 on Wnt target genes, a real-time RT-PCR analysis was carried out on the colorectal cell lines SW480 and DLD-1. A dose-dependent reduction of \(\alpha\)IN2, SP5, and NKD1 was seen after 72-hour incubation with 10 or 25 \(\mu\)mol/L of the compounds (Fig. 5A). JW67 reduced expression of \(\alpha\)IN2 (32%) in SW480 cells (25 \(\mu\)mol/L) and \(\alpha\)IN2 (65%), SP5 (37%), and NKD1 (52%) in DLD-1 cells (10 \(\mu\)mol/L), JW74 reduced the expression of \(\alpha\)IN2 (40% and 64%), SP5 (26% and 37%), and NKD1 (24% and 46%) in SW480 cells (10 and 25 \(\mu\)mol/L, respectively), whereas 10 \(\mu\)mol/L of JW74 reduced the expression of \(\alpha\)IN2 (72%), SP5 (37%), and NKD1 (61%) in DLD-1 cells. At a 10-\(\mu\)mol/L dose, the effect of both compounds was stronger in DLD-1 than in SW480 cells, which may be caused by a more extensive deletion of the APC gene in SW480 than in DLD-1 cells (30).

To gain a general overview of affected gene expression, an Illumina array was carried out in biological triplicates. Alterations of gene expression were assayed in SW480 cells by using 10 or 25 \(\mu\)mol/L of JW74. JW67 at 10 \(\mu\)mol/L was tested in a similar setting by using DLD-1 cells. Known Wnt target genes, as listed on the Wnt homepage (31), were affected by the 2 compounds, including, for example, in the case of JW74-treated cells: \(\alpha\)IN2, SP5, NKD1, FZD2, FZD4, DKK1, ILS, EGF, CYR61, EFNBI, FOSL1, PTG1, SNAI2, SALL4, MET, PLAUR, WISP3, WIF, PITX2, RUNX2, MMP7, ID2, TCF7, BIRC5, CD44, and CDH13 (Supplementary Table 1). As expected for a specific inhibitor of canonical Wnt signaling, some of the altered genes were found to be differentially expressed in Ls174T CRC cell line expressing an inducible dominant, negative form of TCF4 (11). Interestingly, JW74 treatment in SW480 cells strongly reduced the expression of several
genes in the SPANX family that correlate significantly with liver metastasis of CRC (32–34).

Next, we tested a panel of CRC cell lines with different mutations in the Wnt pathway stably transfected with ST-Luc and Renilla: APC truncated SW480 cells (mutated in codon 1,338), HCT-15 cells (mutated in codon 1,417), and 2 cell lines with a point mutation in the CK1α-dependent phosphorylation site S45 of β-catenin (HCT116 with 1 mutated allele and Ls174T with 2 mutated alleles; ref. 35). After 48-hour incubation with various concentrations of JW67 or JW74, dose-dependent reductions of the Wnt dependent ST-Luc activities were quantified (Fig. 5A, right). Corresponding to the real-time RT-PCR experiment, JW67 affected the reporter signal in SW480 only at the concentration 25 μmol/L whereas JW74 was effective in the range 2.5 to 10 μmol/L. A more potent effect was observed in HCT116 cells.

Upon inhibition of canonical Wnt signaling, an arrest in the G1 phase of the cell cycle can be observed in CRC cell lines (11). Therefore, the effect of JW67 and JW74 on proliferation was tested in SW480 cells in an MTS assay. A concentration-dependent reduction in proliferation was shown after treatment with JW67 and JW74 with GI50 values of 7.8 and 11.8 μmol/L, respectively (Fig. 5B). Control SW480 cells underwent 1.3 cell doublings (mean = Abs490t72/Abs490t0) within 72 hours. A plateau on cell growth at higher concentrations (10–25 μmol/L) may be explained by the hydrophobic properties of the 2 compounds and their poor solubility. JW67: clog P = 1.8, and JW74: clog P = 6.1 (ChemBioDraw; CambridgeSoft). Plain precipitation of both compounds was observed at 25 μmol/L in aqueous solution (PBS). Only a minor growth reduction was observed in control cells lacking endogenous canonical Wnt activity such as HeLa and the human hepatocyte cell line THLE2. In addition, reduced proliferation in the presence of 5 μmol/L of JW67 or JW74 was monitored over several passages (1:4 splitting) in SW480 cells. The reduced growth rate of treated cells persisted during the 9 passages and resulted in increasingly diluted cell numbers (Fig. 5C). Abnormal quantities of detached cells, indicating apoptosis or necrosis, were not seen in any of the proliferation assays.

Next, cell-cycle progression after compound exposure was measured by bromodeoxyuridine (BrdU) labeling of cultured cells that were counterstained with propidium iodide (PI). FACS analysis of double-labeled SW480 cells after a 72-hour treatment with 25 or 10 μmol/L of either JW67 or JW74 showed that the percentage of cells in the S phase decreased whereas the G1 compartment increased compared with the DMSO control (Fig. 5D). A 25 μmol/L solution of JW74 nearly halved the percentage of S-phase cells from 28.4% to 16.2% and
increased G1-phase cells from 37.3% to 46.4%. Taken together, these data indicate that compounds JW67 and JW74 reduce growth of SW480 CRC cells in vitro by inhibiting cell-cycle progression at the G1/S phase as expected for an antagonist of canonical Wnt signaling.

JW74 reduces tumor growth of SW480 cells in a CB17/SCID xenograft model

Following cell culture experiments, the in vivo efficacy of the compound JW74 was tested using SW480 cell xenografts. JW67 was not included in the xenograft experiment because of a lower effect in real-time RT-PCR and the reporter assays. A total of 10⁷ SW480 cells were injected subcutaneously into CB17/SCID mice and intraperitoneal compound injections were initiated after 7 days when palpable tumors were detected in ~50% of the mice. A relatively high dose of JW74 (150 or 300 mg/kg) was used because of a rapid compound degradation in the organism as indicated in the human liver microsome analysis (t1/2 = 2.5 minutes) and in pharmacokinetic analyses (after oral injections: t1/2 = 30 minutes and intravenous injections: t1/2 = 15 minutes; Supplementary Fig. S7A). Animals were sacrificed after 29 days. Blood plasma was harvested and isolated tumors were measured for size and weight. A reduction of 33% and 35% in mean tumor size was observed at doses 150 and 300 mg/kg, respectively (Supplementary Fig. S6). The statistical significance was calculated only when the 2 groups (150 and 300 mg/kg) were pooled together (Mann–Whitney: P = 0.045; Supplementary Fig. S2D).
No obvious adverse side effects, such as weight loss, were observed during treatment (Supplementary Fig. S7E). The presence of JW74 in tumors and plasma was identified by mass spectrometry (ref. 36; Supplementary Fig. S7A and B). The compound concentration in tumors was in the range 4.2 to 72.1 μmol/kg for JW74150 mg/kg, 1.9 to 11.1 μmol/kg for JW74300 mg/kg, and 2.8 μmol/L in plasma for both doses. Next, RNA was isolated from representative tumors (n = 5), and real-time RT-PCR revealed that SP5 mRNA, a target gene of canonical Wnt signaling, was downregulated in tumors treated with JW74 (Supplementary Fig. S7D). Altogether, the xenograft experiment indicated that JW74 reduced growth of SW480 CRC cells by in vivo inhibition of Wnt signaling.

JW74 inhibit tumor formation and growth in the small intestine and colon of ApcMin mice

ApcMin mice (multiple intestinal neoplasia, Min) harbor mutations in one allele of the Apc tumor suppressor gene and these mice develop polyposis and colon adenocarcinoma because of frequent spontaneous mutations in the second allele (37). Thus, the ApcMin mouse line provides an excellent animal model for human colon cancer. We tested the effect of JW74 on tumor formation in these mice after combined subcutaneous (age = 5–21 days) and oral applications (21 days to 9 weeks; 150 mg/kg of JW74 in 1% Tween-80 or DMSO, respectively). The small intestine and colon was dissected at 9 weeks and investigated by microscopy. The frequency, distribution, and size of the tumors were quantified. Effects on body weight (Supplementary Fig. S8B) or other considerable discomforts were not noticed in the animals. As no significant difference in tumor frequency in the small intestine could be seen between sexes (Supplementary Fig. S2E), the data were combined in 1 group. A significant reduction of tumor number in the small intestine was detected after injections with 150 mg/kg of JW74 (mean = 36.1; and median = 35) when compared with the control group (Fig. 6A, left and right; mean = 74.8, median = 45; Shapiro–Wilk tests failed, Mann–Whitney: P = 0.031; Supplementary Fig. S2E). When divided by sex, only the female group achieved significance (control vs. treatment; Shapiro–Wilk tests failed, Mann–Whitney: P = 0.036; Supplementary Fig. S2E). When divided by sex, only the female group achieved significant difference. Instead, the female group showed a consistent reduction in tumor number (Fig. 6A, right; mean = 74.8, median = 45; Shapiro–Wilk tests failed, Mann–Whitney: P = 0.036; Supplementary Fig. S2E). The small intestine was divided into several sections according to the distance from the ventricle (in cm). In mice treated with JW74, the last two third of the small intestine had substantially fewer total tumors than the control (Fig. 6B). Furthermore, the tumors could be sorted by size into size classes (in mm²). The tumors frequencies, especially among those between 0.10 and 3.20 mm², were drastically reduced after JW74 treatment (Fig. 6C). In addition, when tumor frequency and size were combined as a total tumor load (sum of all tumors in mm²/n), the inhibition of formation and growth of tumors in the small intestine by JW74 was again clearly documented (control: 38.1 mm²; and JW74: 16.4 mm²; Supplementary Fig. S8A). Next, the occurrence of tumors was examined in the colon. A statistically significant reduction of the number of tumors in the colon was accomplished after JW74 treatment (mean = 0.42; and median = 0) when compared with the control (mean = 1.78; and median = 1; Shapiro–Wilk tests failed, Mann–Whitney: P = 0.003; Fig. 6D and Supplementary Fig. S2F). The results from the ApcMin experiments proved that JW74 has the capacity to reduce the development of intestinal cancer.

Discussion

In this work, 2 chemotypes, JW67 and JW74, were identified that specifically inhibit canonical Wnt signaling, decreased Wnt-dependent transcription in CRC cell lines that resulted in a reduced cell growth in vitro and in vivo, and inhibited polyp formation in ApcMin mice.

Although a detailed mechanism of action will be investigated in further studies, the inhibitory effect of JW67 and JW74 in LiCl-activated cells and in APC mutant cells indicates that the compounds may influence the multiprotein complex consisting of β-catenin/GSK-3β/AXIN/APC /CK1 that controls the degradation of β-catenin. Indeed, submicromolar concentrations of JW67 or JW74 in CRC APC mutant cells resulted in the reduction of nuclear active β-catenin that was accompanied with an increased steady-state level of cytoplasmic AXIN2 and phosphorylated β-catenin. Large foci with colocalized AXIN2/β-catenin in the cytoplasm, which formed even in the presence of truncated APC, may therefore represent abnormal β-catenin destruction complexes. Moreover, the compound treatment not only increased the phosphorylation of β-catenin and inhibited cell growth in CRC APC mutant cell lines such as SW480 but also reduced canonical Wnt-signaling in CRC cell lines with point mutations in the β-catenin gene. The ST-Luc activity was reduced in the HCT116 line carrying a single point mutation at S45 (CK1α phosphorylation site) in one β-catenin allele and in LS174T line with the same mutation in both alleles. Both these lines thus retain β-catenin that can be phosphorylated in other phosphorylation sites (e.g. S33, S37, T41; ref. 38). We further note that Wnt induction by overexpression of wild-type β-catenin was counteracted by JW67 or JW74 in ST-Luc assays. In striking contrast, the compounds had insignificant effect upon transiently transfected β-catenin with all mutated phosphorylation sites (S33, S37, T41, and S45). The 2 compounds thus may affect phosphorylation of β-catenin by several mechanisms, including altered phosphatase activity or an increased GSK-3β activity, stabilization of the β-catenin destruction complex, or an augmented negative regulatory feedback loop involving AXIN2. We hypothesize that the observed high amount of AXIN2 increases the formation of the multiprotein degradation complex as illustrated in the scheme (Fig. 3D). At present, we conduct several experiments with the aim to identify the compounds’ direct targets. Potential targets may include proteins such as AXIN2, components that affect AXIN2 stability or other hitherto unknown proteins interacting with the destruction complex.

Two recent studies present novel small molecule inhibitors of the canonical Wnt pathway that increase AXIN2 levels despite APC mutations (22, 23). XAV939 inhibits the PARP domain of Tankyrase 1 and 2 that destabilizes AXIN2 by PARylation (23). IWR-1 also stabilizes endogenous TNKS1 and TNKS2 proteins and increases AXIN2 protein, although the precise mechanism remains elusive (22). Neither JW67 nor
JW74 is structurally similar to IWR-1 or XAV939 and shows efficacy in submicromolar concentrations in similar assays. The decreased rate of polyp formation in ApcMin mice, particularly in the colon after treatment with JW74, classifies this compound among the small molecules that are plausible candidates for future human therapy of CRC. The dose of 150 mg/kg of JW74 in ApcMin model reduced the small intestinal adenoma by 48% (calculated from mean values in Fig. 6A) and is comparable, for example, with celecoxib (150 or 500 mg/kg: 71%; ref. 39) or rofecoxib, an analog of celecoxib (75 mg/kg: 45%; ref. 40). It is noteworthy that JW74 becomes rapidly cleared from the bloodstream because of poor in vivo stability (Supplementary Fig. 7A). At present, we test a large panel of structural analogues that may yield a stabilized structure and thus an increased efficacy in vivo. Investigation of the chemical space that influences the β-catenin destruction complex seems to be an increasingly important entry point for pharmacologic interventions of the canonical Wnt signaling.

Disclosure of Potential Conflicts of Interest

The described chemical compounds may have commercial value.

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