CHAPTER TWENTY-SIX

Recent Advances in Wnt/β-Catenin Pathway Small-Molecule Inhibitors

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ABBREVIATIONS

APC adenomatosis polyposis coli
AXIN2 axis inhibition protein 2
CBP cAMP response element-binding protein-binding protein
CK1-α cyclin-dependent kinase 1α
CK1ε cyclin-dependent kinase 1ε
CREB cyclic AMP response element-binding protein
DVL dishevelled

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GSK3β glycogen synthase kinase 3β
HGF hepatocyte growth factor
LEF lymphoid enhancer factor; transcription factor
LGR5 leucine-rich repeat-containing, G protein-coupled receptor 5
LRP5/6 LDL receptor-related protein 5/6
MM multiple myeloma
PARP poly (ADP-ribose) polymerase
PKA protein kinase A
PORC porcupine
RNF146 poly (ADP-ribose)-directed E3 ligase
SAR structure–activity relationship
STF super TOPFlash reporter containing TCF/LEF-binding site
TCF T-cell factor; transcription factor
TNKS TRF-1-interacting ankyrin-related ADP-ribose polymerase
β-TrCP β-transducin repeat-containing protein

1. INTRODUCTION

Wnt/β-catenin signaling is a branch of a functional network that dates back to the first metazoans, and is involved in a broad range of biological systems, including stem cell biology, developmental biology, and adult organ systems. Simplified, the pathway may be described as Wnt protein binding to the cell surface LDL receptor-related protein 5/6 (LRP5/6)–Frizzled receptor complex and allowing β-catenin to travel to the nucleus where binding to T-cell factor/lymphoid enhancer factor (TCF/LEF) results in gene transcription. An overview of the Wnt pathway is shown in Fig. 26.1.

Specific organ systems that depend on Wnt/β-catenin signaling during their development and/or in their adult steady state include the cerebral cortex, hippocampus, eye, lens, spinal cord, limbs, bone, cartilage, somites, neural crest, skin, teeth, gut, lungs, heart, pancreas, liver, kidneys, mammary glands, the hematopoietic system, and the reproductive system. Deregulation of components of Wnt/β-catenin signaling is implied in a wide spectrum of diseases including degenerative diseases, metabolic diseases, and a number of cancers such as colon, breast, bladder, head and neck, nonsmall-cell lung, gastric, melanoma, prostate, leukemia, hepatocellular, pancreas adenocarcinoma, ovarian, and Wilms tumor.[1–4]

Serving several cellular functions, the key mediator of Wnt signaling, β-catenin, is found in a dynamic mode at multiple subcellular locations,

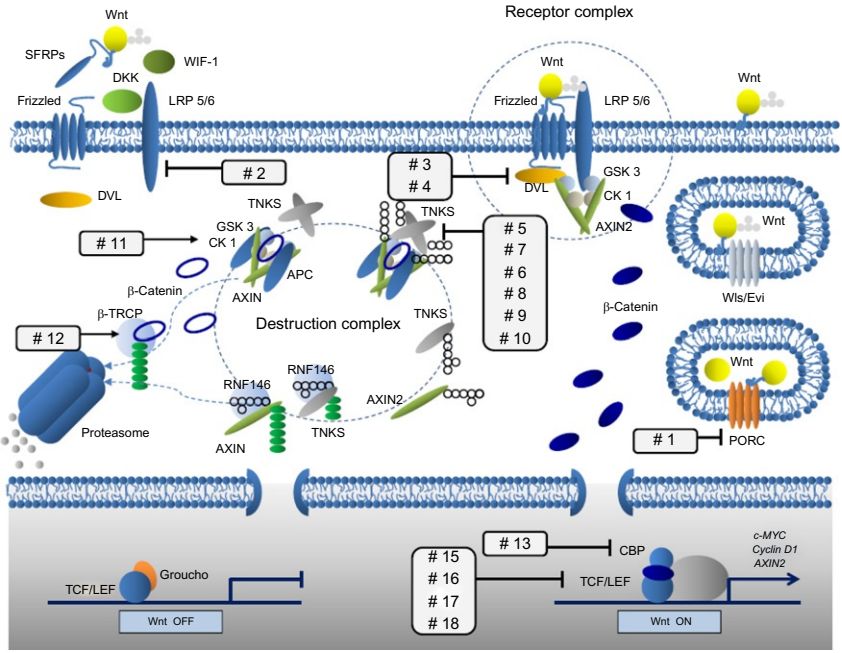


Figure 26.1 Model for Wnt signaling. Newly synthesized Wnt is palmitoylated by PORC and N-glycosylated (gray dots). Upon Wnt binding to the Frizzled/LRP5/6 receptor complex, β-catenin (blue circles) ceases to be degraded and enters the nucleus where it binds TCF/LEF. In the absence of active Wnt, β-catenin becomes phosphorylated (blue rings) in the destruction complex. Subsequently, it is ubiquitinated (green circles) and degraded in the proteasome. Destruction complex proteins are gradually poly-ADP-ribosylated (black rings) by TNKS. Poly-ADP-ribosylation of AXIN and TNKS leads to a destabilization of the destruction complex, followed by ubiquitination and degradation of AXIN and TNKS in the proteasome. Inhibitory substances are marked by a bar, while activating substances are marked by an arrow. The numbers (#) refer to the compounds in the text.

including junctions, where it contributes to the stabilization of cell–cell contacts, the cytoplasm where β-catenin thresholds are regulated by the destruction complex, and the nucleus where β-catenin is involved in transcriptional regulation and chromatin interactions. Wnt morphogens, cysteine-rich, secreted glycoproteins, are the central regulators of β-catenin thresholds. Through the LRP5/6–Frizzled receptor complex, Wnt morphogens regulate the location and activity of the destruction complex and, consequently, intracellular β-catenin levels. However, β-catenin thresholds are also influenced by multiple other factors, including hypoxia, hepatocyte growth factor, protein kinase A (PKA), and E-cadherin.

β-Catenin is a specialized member of the larger armadillo protein family that consists of three subfamilies: the p120 subfamily, the beta-subfamily (β-catenin and plakoglobin), and the more distant alpha subfamily.[5] The functional interplay between members of this protein family is not well understood, but an involvement of p120 in Wnt/β-catenin signaling was recently shown.[6] Further functional overlaps may exist, in particular, with plakoglobin.

The presence and stability of β-catenin in its various locations, as well as its shuffling through the cell, provide alternative intervention points for therapeutic reagents. The broad implications of Wnt/β-catenin signaling in development, the adult body, and in disease, render it a prime target for pharmacological research and development.

2. REGULATION OF β-CATENIN

2.1. Regulation of β-catenin stability by the destruction complex

The major gatekeeper for regulating β-catenin thresholds in the cell is the β-catenin destruction complex in the cytoplasm, a multiprotein complex containing several druggable biotargets. Although the precise molecular structure and composition of the destruction complex remains to be elucidated, the core of the destruction complex consists of axis inhibition protein 2 (AXIN2), adenomatous polyposis coli (APC), the priming kinase CK1-α, and the kinase glycogen synthase kinase 3β (GSK3β). The positively charged β-catenin associates with the destruction complex where cyclin-dependent kinase 1α (CK1-α) and GSK3β phosphorylate β-catenin at the N-terminal positions S45 and S33, and S37 and T41, respectively. The reduced positive charge on β-catenin causes its dissociation from the destruction complex. The β-transducin repeat-containing protein (β-TrCP), a part of the ubiquitin ligase complex, recognizes β-catenin, causing its ubiquitination, and β-catenin is subsequently degraded by the 26S proteasome. The stability of the destruction complex itself is regulated by tankyrase (TRF-1-interacting ankyrin-related ADP-ribose polymerase) and RNF146. Tankyrase (TNKS) belongs to the 22-member family of poly (ADP-ribose) polymerase (PARP) enzymes and plays a key role in the destabilization of the β-catenin destruction complex by adding negatively charged poly-ADP-ribose units to AXIN2 and to itself. TNKS exists in two highly homologous isoforms. Upon poly-ADP-ribosylation, TNKS 1/2 and AXIN2 presumably dissociate from the destruction complex and are recognized by the ubiquitin ligase RNF146, ubiquitinylated, and then degraded by the

proteasome. The degree of poly-ADP-ribosylation at the destruction complex is positively regulated by the availability of NAD^+ and negatively regulated by poly (ADP-ribose) glycohydrolase. Taken together, the tankyrase/destruction complex is a highly dynamic protein complex that centrally regulates cytoplasmic β-catenin thresholds.[3,4,7–9]

2.2. Regulation of β-catenin by the Wnt receptor complex

In the secretory pathway, Wnt morphogens have to be modified by palmitoylation and glycosylation to become mature signaling proteins. Evidence suggests that the membrane-bound *O*-acyltransferase Porcupine (PORC) may be involved in palmitoylation and secretion of Wnt proteins, a process that has recently been established as drug sensitive. Upon binding of the Wnt morphogen, a Frizzled-LRP5/6 receptor complex forms the basic unit of the Wnt signalosome. The secreted proteins, Wnt inhibitor factor-1, Cerberus, and soluble Frizzled-related proteins can interfere with Wnt binding to the Frizzled-LRP5/6 receptor, while a Dickkopf/Kremen complex prevents the formation of the Wnt signalosome by recruiting LRP5/6 and thus preventing it from forming a complex with Frizzled.

Nineteen isoforms of the Wnt morphogen together with 10 isoforms of the Frizzled receptor in humans allow some functional diversity and differential cellular response. In the canonical branch of Wnt signaling, components of the destruction complex are drawn to the Wnt signalosome. This disables the destruction complex from phosphorylating β-catenin and leads to an elevation of cytoplasmic and nuclear β-catenin. At the cytoplasmic side of the Wnt signalosome, phosphorylated Dishevelled (DVL) binds to Frizzled and enables the positioning of AXIN2 to LRP5/6. Blocking DVL attenuates the Wnt signaling cascade leading to reduced β-catenin levels. DVL has recently been described as a drug biotarget.[7]

Alternatively, Wnt binding to the Frizzled-LRP5/6 signalosome induces phosphorylation of the armadillo protein p120 by CK1ε followed by its release to the nucleus.[6]

2.3. β-Catenin in the nucleus

In the nucleus, β-catenin appears to have multiple functions. The best understood role of β-catenin is its binding to members of the TCF/LEF zinc finger family of transcription factors to act as transcriptional activators. In contrast, TCF3 is predominantly a transcriptional repressor. The histone acetyltransferase

cAMP response element-binding protein-binding protein (CBP) attenuates the complex and acts as a context-dependent β-catenin-binding transcriptional regulator.[9] Furthermore, p120 binding to the zinc finger transcription factor Kaiso releases its inhibition of the β-catenin–TCF4 transcriptional complex. Both β-catenin/TCF binding and β-catenin/CBP binding have been used as interference points for exploratory drugs.[7]

Also, β-catenin shuttling to and from the nucleus is regulated. A picture emerges where stability and nuclear uptake of β-catenin can be enhanced by the context-dependent C-terminal phosphorylation of β-catenin at S675 by PKA, while export of β-catenin from the nucleus is GSK3β dependent.[10] Both kinases are well-explored drug targets.

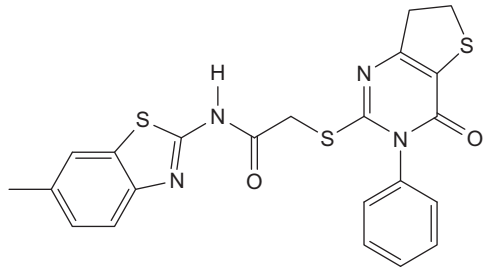
3. SELECTIVE SMALL-MOLECULE ANTAGONISTS OF WNT/β-CATENIN SIGNALING

Wnt/β-catenin signaling in tumorigenesis has provided a prime rationale for mapping druggable intervention points in the pathway. While an inhibition or normalization of the pathway is predominantly desired in the cancer arena, a controlled attenuation or increase of Wnt/β-catenin signaling may also be sought for certain aspects of regenerative medicine.

3.1. PORC inhibitor

PORC is involved in the palmitoylation and secretion of Wnt proteins. A PORC inhibitor would reduce secretion and maturation of the Wnt morphogen, thereby inhibiting paracrine Wnt signaling.

Compound **1** inhibits the Wnt pathway (IC_{50}: 27 nM, L-Wnt STF cells) by competitively inhibiting the function of PORC. Inhibition of LRP6 and DVL phosphorylation and cytoplasmic β-catenin accumulation was also observed upon exposure of **1** at 10 μM to L-Wnt super TOPFlash reporter containing TCF/LEF-binding site (STF) cells.[11]



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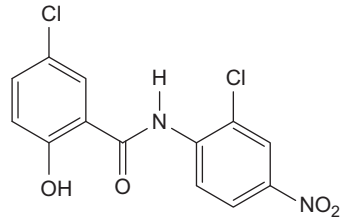
3.2. LRP6 inhibitor

The transmembrane proteins LRP5/6 and Frizzled form the core of the Wnt signalosome. An induced degradation of LRP5/6 would sever the cell's responsiveness to an incoming Wnt signal.

Niclosamide (**2**) has been shown to reduce cancer cell growth by inducing LRP6 degradation.[12] Niclosamide was also shown to suppress LRP6 expression and phosphorylation in HEK293 human embryonic kidney cells.



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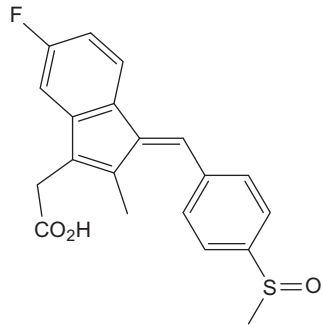
Niclosamide is also an inhibitor of multiple other pathways (NF-κB, Wnt, NOTCH, ROS, mTORC1, STAT3), most of which are implicated in cancer metastasis. Compound **2** inhibited cell growth (GI_{50}s) in human prostate (PC-3, DU145) and breast cancer (MDA-MB-231, T-47D) cell lines at 0.7–0.3 μM. It also inhibited S100A4 protein expression, cell proliferation, migration, and invasion in colon cancer cell lines.[13]

3.3. DVL inhibitors

DVL interacts with the membrane-bound Wnt Frizzled receptor. A DVL inhibitor would, among other things, reduce the interplay between the Wnt signalosome and the destruction complex and thus inhibit canonical Wnt signaling.

Sulindac (**3**), a nonsteroidal anti-inflammatory drug, was shown to block the PDZ domain of DVL (IC_{50}: 10.7 μM) and cause decreased nuclear accumulation of β-catenin and reduced expression of the metastatic mediator protein S100A4 in a human colon cancer xenograft model.[14]

Compound **4** exhibited a K_d of 10.6 μM toward the DVL PDZ domain in an assay system using fluorescence anisotropy.[15] At an IC_{50} of 12.5 μM, **4** reduced cell growth and β-catenin levels in the PC-3 prostate cell line.



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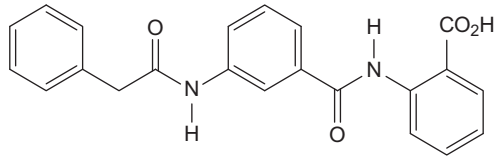

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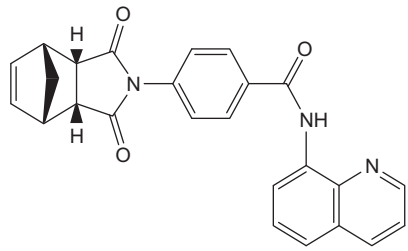
3.4. Tankyrase inhibitors

Tankyrase (TNKS) 1 and 2 have multiple cellular functions including marking destruction complex proteins by poly-ADP-ribosylation for ubiquitination and degradation. Since TNKS 1/2 are druggable modulators of Wnt/β-catenin signaling, they have recently received substantial attention. However, obtaining selectivity of small molecules between the PARP family members and the TNKS isoforms can be been challenging. Blocking the PARP domain of TNKS 1/2 can lead to a context-dependent inhibition or normalization of Wnt/β-catenin signaling.

The first small-molecule inhibitors of TNKS 1/2 were disclosed in 2009 (**5** and **6**). IWR-1 (**5**) exhibited good potency against TNKS 1/2 with IC_{50}s of 131 and 56 nM, respectively, while not affecting PARP-1 and 2.[11] IWR-1 inhibited Wnt-stimulated transcription activity (IC_{50}: 180 nM) and increased AXIN2 and phosphorylated β-catenin levels in DLD-1 colorectal carcinoma cells at 1 μM.



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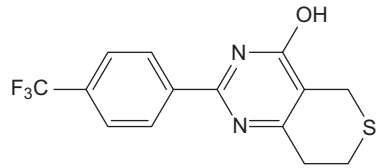

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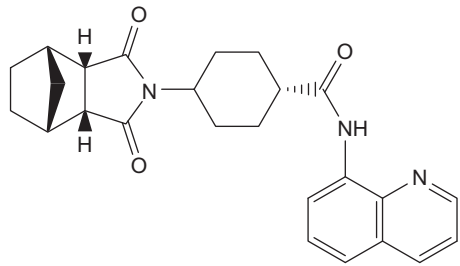
IWR-1 demonstrated *in vivo* activity by inhibiting zebra fish tail fin regeneration at 10 μM. IWR-1 suffers from instability in mouse liver microsomes ($T_{1/2} \sim 20$ min). Early structure–activity relationship (SAR) works to find IWR-1 analogs that exhibited improved potency and stability were met with modest success.[16] Compound **5** was obtained from a high-content screen searching for stimulators of cardiomyogenesis.[17] Optimization of **5** led to **7**, which exhibited an IC_{50} of 4 nM against the Wnt pathway in HEK293T cells, and demonstrated 45% greater efficacy in inducing cardiogenesis than **5**. Reduction of the double bond of the bicyclic ring and incorporation of a *trans*-cyclohexyl ring in place of the phenyl ring spacer of **7** led to a series that exhibited robust SAR.



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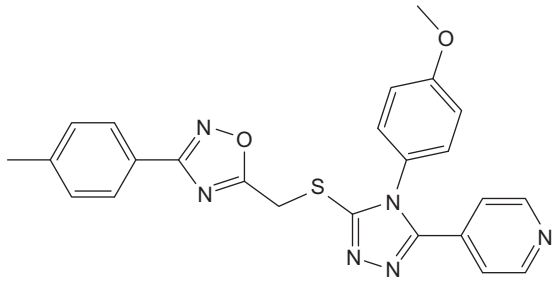
A recent X-ray crystal structure of **5** complexed with TNKS 2[18] illustrates that IWR-1 binds to the adenosine pocket of the PARP domain, which may account for its specificity to TNKS 1/2.

XAV939 (**6**) is a potent TNKS inhibitor (IC_{50}s: TNKS1: 11 nM, TNKS2: 4 nM (HEK293 STF cells)) that, in addition, inhibits PARP1 and 2 (IC_{50}: 2200 and 114 nM, respectively).[19] XAV939 increased AXIN2 and TNKS 1/2 protein levels and promoted the degradation of β-catenin in SW480 cells at 1 μM. XAV939 has been shown to reduce the growth of β-catenin-dependent DLD-1 colon carcinoma cells by 95% at 3.3 μM. An X-ray cocrystal structure of XAV939 bound to TNKS 2 showed that **6** binds in the nicotinamide pocket of the TNKS 2 PARP domain.[20,21] Along with **6**'s anticancer properties, it has also been shown to accelerate repair of oligodendrocyte progenitor cells from the brain and spinal cord after hypoxic and demyelinating injury.[22]

Recently, a structural analysis of PARP and TNKS inhibitors was conducted[21] to provide insight into the selectivity of TNKS inhibitors as compared to PARP 1–4 inhibitors. It was shown that the binding site near the NAD^+ pocket of PARPs is lined with hydrophilic residues, whereas in TNKS2, the residues are generally hydrophobic. Furthermore, TNKS2 has a more elongated binding site than PARPs 1–4, providing rationale to design TNKS-specific inhibitors.

The 1,2,4-triazole-containing compound (**8**) was identified as a selective Wnt pathway inhibitor (IC_{50}: 0.79 μM).[23] The biological target of **8** has recently been described as TNKS.[9] Compound **8** specifically inhibited induced Wnt signaling in a *Xenopus laevis* axis duplication assay at 0.8 pM, and was effective in stabilizing AXIN2 and reducing the active form of β-catenin in SW480 colon carcinoma cells. In SW480 cells, **8** reduced growth (GI_{50}) at 5 μM. Although **8** was found to be extremely labile to liver microsomes (human and mouse: 3 min), it reduced tumor growth of SW480 cells in a CB17/SCID xenograft model when dosed orally QD at 150 mg/kg for 21 days. It was also demonstrated that **8** inhibited tumor formation and growth in the small intestine and colon of APC^{min} mice by 48% when dosed at 150 mg/kg.



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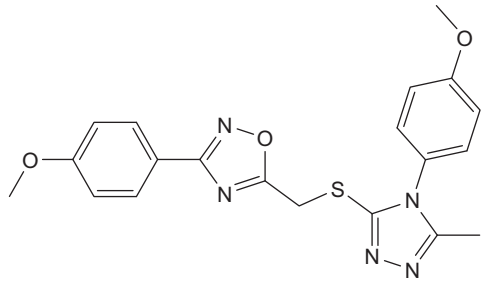
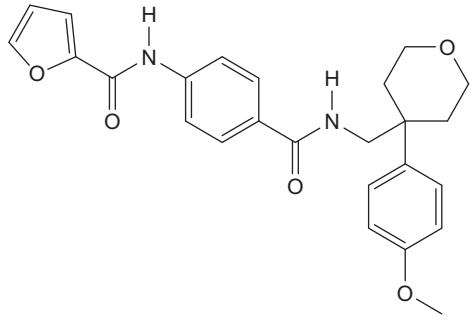


9

Compound **8** was independently identified and found to be a modest TNKS inhibitor (TNKS1, IC_{50}: 2.55 μM; TNKS2, IC_{50}: 0.65 μM (HEK293 STF cells)).[24] Early SAR development of **8** resulted in **9**. Compound **9** has an IC_{50} of 33 nM against TNKS2 and IC_{50}s greater than 19 μM for PARP1 and 2. By installing a methyl group in place of the pyridyl group of **8**, diminished P450 isozyme inhibition was observed. X-ray cocrystal structure of **9** in TNKS1 showed **9** bound in the adenosine and diphosphate linker portion of the NAD^+ donor site. Additional interactions of **9** in the hydrophobic section surrounding the diphosphate linker portion were also observed.



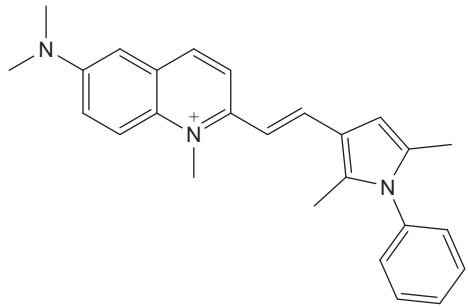
10

Compound **10** was shown to be a potent inhibitor of the Wnt pathway (IC_{50} in HEK293 reporter cells: 0.470 μM) and a selective inhibitor of TNKS (biochemical TNKS1 inhibition, IC_{50}: 1.9 μM; biochemical TNKS2 inhibition, IC_{50}: 0.83 μM) as compared to PARP1 ($IC_{50} > 20$ μM).[9] Similar to compound **6**, compound **10** stabilized AXIN2 protein levels; however, in contrast, **10** reduced TNKS protein levels in SW480 colorectal carcinoma cells. Compound **10** when exposed to SW480 cells displayed a general reduction of total β-catenin at 1 and 5 μM. It also reduced the expression of the Wnt target genes AXIN2, SP5, and NκD1 at 10 μM in SW480 and DLD1 colorectal cell lines. Compound **10** was administered orally at

100 mg/kg to $APC^{cko/cko}$ Lgr5-CreERT2^{+} mice and found to reduce mean total tumor area in the small intestine.

3.5. Casein kinase 1-α activity enhancer

The priming kinase CK1-α is part of the destruction complex, which phosphorylates β-catenin at serine 45. A drug that induces increased CK1-α/γ activity would regulate negatively the stability of β-catenin in the cytoplasm.

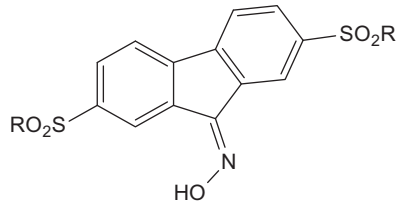


11

Compound **11** is an FDA-approved anthelmintic compound that potently (EC_{50}: 10 nM) inhibits Wnt gene transcription in HEK293 cells.[25] **11** binds to all casein kinase 1 family members and selectively enhances CK1-α activity, thus lowering cytoplasmic β-catenin levels. Furthermore, **11** promoted the degradation of the Pygopus PHD-finger protein within the nucleus and subsequently reduced Wnt-directed transcription. Compound **11** displayed toxicity when dosed to mice at 200 nM IV or IP, but at lower doses exhibited wound repair and postmyocardial infarction remodeling potential in mice.[26]

3.6. Wnt/β-catenin inhibitors enhancing β-TrCP/β-catenin interaction

A compound with the general structure **12** has been reported to decrease c-Myc, cyclin D1, and survivin expression in a multiple myeloma (MM) mouse model, and also prolonged survival of mice with MM.[27] **12** inhibited the proliferation of MM cells in a time- and dose-dependent manner (with IC_{50}s ranging from 11 to 82 nM[28]) by enhancing the β-TrCP/β-catenin interaction. Ubiquitination of cellular β-catenin was shown to be increased, and nuclear β-catenin levels reduced.



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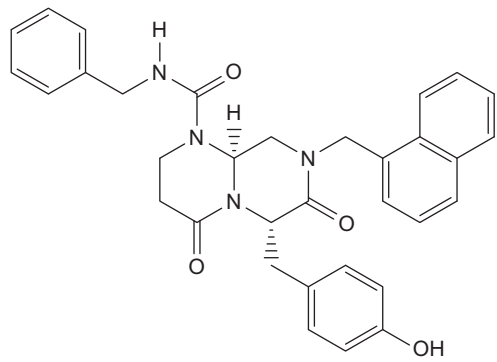
3.7. Inhibitors of β-catenin/TCF-LEF and β-catenin/CBP binding

Interference between β-catenin and its nuclear targets has long been a prominent target for drug discovery. Both binding between β-catenin and members of the TCF/LEF family and binding between β-catenin and CBP can be inhibited by small molecules. Such an inhibition would not lower β-catenin thresholds in the cytoplasm and nucleus, but would alter the transcription of Wnt/β-catenin downstream genes such as c-Myc, cyclin D1, and survivin.



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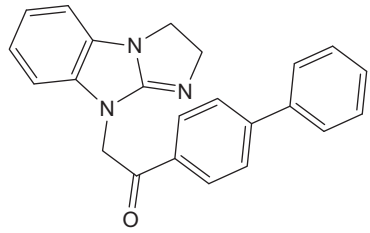
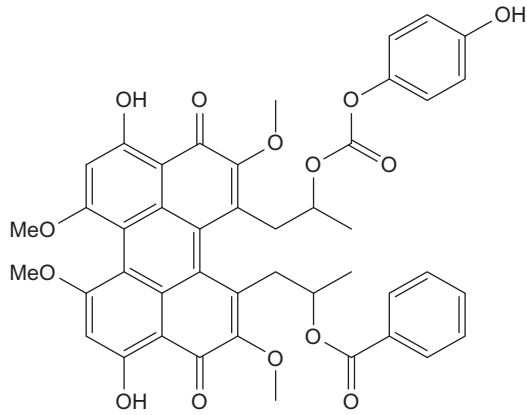
Compound **13** interacts with CBP, a context-dependent coactivator for Wnt/β-catenin transcription.[9,29] Compound 13 is a modest inhibitor (IC_{50}: 3 μM (SW480 TOPFlash cells)) of the Wnt/β-catenin signaling pathway and selective for CBP, but not its close homolog p300. β-Catenin can switch from interacting with CBP to interacting with p300, which causes cells to become less differentiated and behave more like stem cells. Compound **13** has been claimed to eliminate cancer stem cells without affecting normal somatic stem cells.[30] Recently, a nondisclosed homolog of **13** that also inhibits the β-catenin/CBP interaction (IC_{50}: 200 nM) has entered phase I trials for patients with solid tumors.[31]



14

A cell-based, small-molecule screen for regulators of TCF-dependent transcription yielded **14**.[32] Compound **14** blocked nuclear and cytosolic β-catenin accumulation in mouse L-3 cells when incubated with a GSK3 inhibitor. Additional mechanistic studies suggest that **14** interacts through multiple molecular targets.



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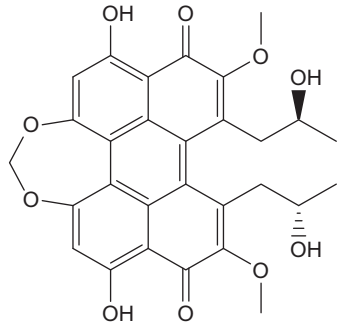

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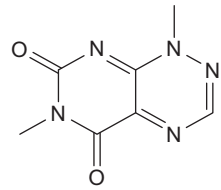
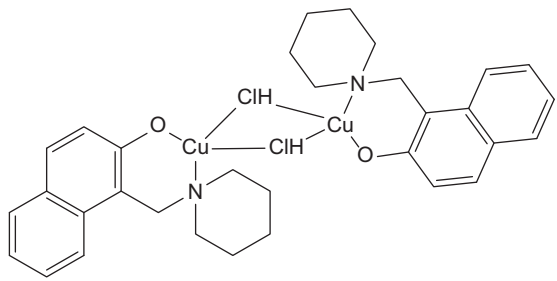


17

Compounds **15**–**17** inhibit the β-catenin/TCF/LEF interaction and transcriptional activity.[33] All three compounds were found to be cytotoxic to hepatoma cells (IC_{50}s 0.26–0.98 μM) but not to normal hepatocytes. Compound **15** was most active against the hepatoma cell lines (Hep40, Huh7, HepG2) tested. Compounds **15** and **16** were also efficient in selectively killing chronic lymphocytic leukemia (CLL) cells (LC_{50}s: 0.7–0.9 μM) over B cells,[34] and demonstrated *in vivo* efficacy in CLL mouse model when dosed at 25 mg/kg/day for 12 days.



18

Compound **18** was obtained from a virtual screen of agonist putative binding sites of TCF4 with β-catenin.[35] **18** displayed competitive binding to GST-TCF4 with an IC_{50} of 5 μM. **18** also decreased HCT116 colon cancer cell viability (IC_{50}: 15 μM) and at 5 μM blocked 80% of the colony-forming capabilities of HCT116 cells as well as downstream target genes c-Myc and cyclin D1.

4. CONCLUSIONS

The search for viable inhibitors at multiple points along the Wnt/β-catenin signaling pathway has received considerable attention from pharmaceutical and academic groups. Drugs and druggable biotargets have been identified that inhibit Wnt/β-catenin signaling at the Wnt secretory pathway, at the Wnt signalosome, at the destruction complex, and at the nuclear β-catenin/TCF and β-catenin/CBP interface. Identification of additional

chemotypes that possess drug-like properties, and mapping their precise biotarget, will be needed to fully validate druggable interference points within the Wnt/β-catenin complex pathway. Validation of Wnt/β-catenin inhibitors as viable anticancer reagents *in vitro*, in animal models, and in clinical trials is still at an early stage.

REFERENCES

(1) Tanaka, S.S.; Kojima, Y.; Yamaguchi, Y.L.; Nishinakamura, R.; Tam, P.P. *Dev. Growth Differ.* **2011**, *53*, 843.
(2) Grigoryan, T.; Wend, P.; Klaus, A.; Birchmeier, W. *Genes Dev.* **2008**, *22*, 2308.
(3) Camilli, T.C.; Weeraratna, A.T. *Biochem. Pharmacol.* **2010**, *80*, 705.
(4) Polakis, P. *Cold Spring Harb. Perspect. Biol.* **2012**, http://dx.doi.org/10.1101/cshperspect.a008052.
(5) Zhao, Z.M.; Reynolds, A.B.; Gaucher, E.A. *BMC Evol. Biol.* **2011**, *11*, 198.
(6) Casagolda, D.; Del Valle-Pérez, B.; Valls, G.; Lugilde, E.; Vinyoles, M.; Casado-Vela, J.; Solanas, G.; Batlle, E.; Reynolds, A.B.; Casal, J.I.; de Herreros, A.G.; Duñach, M.A. *J. Cell Sci.* **2010**, *123*, 2621.
(7) MacDonald, B.T.; Tamai, K.; He, X. *Dev. Cell* **2009**, *17*, 9.
(8) Callow, M.G.; Tran, H.; Phu, L.; Lau, T.; Lee, J.; Sandoval, W.N.; Liu, P.S.; Bheddah, S.; Tao, J.; Lill, J.R.; Hongo, J.A.; Davis, D.; Kirkpatrick, D.S.; Polakis, P.; Costa, M. *PLoS One* **2011**, *6*, e22595.
(9) Waaler, J.; Machon, O.; Tumova, L.; Dinh, H.; Korinek, V.; Wilson, S.R.; Paulsen, J.E.; Pedersen, N.M.; Eide, T.J.; Machonova, O.; Gardl, D.; Von Kries, J.P.; Krauss, S. *Cancer Res.* **2012**, *72*, 2695.
(10) Li, J.; Sutter, C.; Parker, D.S.; Blauwkamp, T.; Fang, M.; Cadigan, K.M. *EMBO J.* **2007**, *26*, 2284.
(11) Chen, B.; Dodge, M.E.; Tang, W.; Lu, J.; Ma, Z.; Fan, C.-W.; Wei, S.; Hao, W.; Kilgore, J.; Williams, N.S.; Roth, M.G.; Amatruda, J.F.; Chen, C.; Lum, L. *Nat. Chem. Biol.* **2009**, *5*, 100.
(12) Lu, W.; Lin, C.; Roberts, M.J.; Waud, W.R.; Piazza, G.A.; Li, Y. *PLoS One* **2011**, *6*, e29290.
(13) Sack, U.; Walther, W.; Scudiero, D.; Selby, M.; Kobelt, D.; Lemm, M.; Fichtner, I.; Schlag, P.M.; Shoemaker, R.H.; Stein, U. *J. Natl. Cancer Inst.* **2011**, *103*, 1018.
(14) Stein, U.; Arlt, F.; Smith, J.; Sack, U.; Hermann, P.; Walther, W.; Lemm, M.; Fichtner, I.; Shoemaker, R.H.; Schlag, P.M. *Neoplasia* **2011**, *13*, 131.
(15) Gandy, D.; Shan, J.; Zhang, X.; Rao, S.; Akunuru, S.; Li, H.; Zhang, Y.; Alpatov, I.; Zhang, X.A.; Lang, R.A.; Shi, D.-L.; Zheng, J.J. *J. Biol. Chem.* **2009**, *24*, 16256.
(16) Lu, J.; Ma, Z.; Hsieh, J.-C.; Fan, C.-W.; Chen, B.; Longgood, J.C.; Williams, N.S.; Amatruda, J.F.; Lum, L.; Chen, C. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3825.
(17) Lanier, M.; Schade, D.; Willems, E.; Tsuda, M.; Spiering, S.; Kalisiak, J.; Mercola, M.; Cashman, J.R. *J. Med. Chem.* **2012**, *55*, 697.
(18) Narwal, M.; Venkannagari, H.; Lehtio, L. *J. Med. Chem.* **2012**, *55*, 1360.
(19) Huang, S.-M.A.; Mishina, Y.M.; Liu, S.; Cheung, A.; Stegmeier, F.; Michaud, G.A.; Charlat, O.; Wiellette, E.; Zhang, Y.; Wiessner, S.; Hild, M.; Shi, X.; Wilson, C.J.; Mickanin, C.; Myer, B.; Fazal, A.; Tomlinson, R.; Serluca, F.; Shao, W.; Cheng, H.; Schultz, M.; Rau, C.; Schirle, M.; Schlegl, J.; Ghidelli, S.; Fawell, S.; Lu, C.; Curtis, D.; Kirschner, M.W.; Lengauer, C.; Finan, P.M.; Tallarico, J.A.; Bouwmeester, T.; Porter, J.A.; Bauer, A.; Cong, F. *Nature* **2009**, *461*, 614.
(20) Karlberg, T.; Markova, N.; Johansson, I.; Hammarstrom, M.; Schutz, P.; Weigelt, J.; Schuler, H. *J. Med. Chem.* **2010**, *53*, 5352.

(21) Wahlberg, E.; Karlberg, T.; Kouznetsova, E.; Markova, N.; Macchiarulo, A.; Thorsell, A.-G.; Pol, E.; Frostell, A.; Ekbald, T.; Oncu, D.; Kull, B.; Robertson, G.M.; Pelliciari, R.; Schuller, H.; Weigelt, J. *Nat. Biotechnol.* **2012**, *30*, 283.
(22) Fancy, S.P.; Harrington, E.P.; Yuen, T.J.; Silbereis, J.C.; Zhao, C.; Baranzini, S.E.; Bruce, C.C.; Otero, J.J.; Huang, E.J.; Nusse, R.; Franklin, R.J.; Rowitch, D.H. *Nat. Neurosci.* **2011**, *14*, 1009.
(23) Waaler, J.; Machon, O.; Von Kries, J.P.; Wilson, S.R.; Lundenes, E.; Wedlich, D.; Gradl, D.; Paulson, J.E.; Machonova, O.; Dembinski, J.L.; Dinh, H.; Krauss, S. *Cancer Res.* **2011**, *71*, 197.
(24) Shultz, M.D.; Kirby, C.A.; Stams, T.; Chin, D.N.; Blank, J.; Charlat, O.; Cheng, H.; Cheung, A.; Cong, F.; Feng, Y.; Fortin, P.D.; Hood, T.; Tyagi, V.; Xu, M.; Zhang, B.; Shao, W. *J. Med. Chem.* **2012**, *55*, 1127.
(25) Thorne, C.A.; Hanson, A.J.; Schneider, J.; Tahinci, E.; Orton, D.; Cselenyi, C.S.; Jernigan, K.K.; Meyers, K.C.; Hang, B.I.; Waterson, A.G.; Kim, K.; Melancon, B.; Ghidu, V.P.; Sulikowski, G.A.; LaFleur, B.; Salic, A.; Lee, L.A.; Miller, D.M.; Lee, E. *Nat. Chem. Biol.* **2010**, *6*, 829.
(26) Saraswati, S.; Alfaro, M.P.; Thorne, C.A.; Atkinson, J.; Lee, E.; Young, P.P. *PLoS One* **2010**, *5*, e15521.
(27) Ashihara, H.Y.; Strovel, J.W.; Nakagawa, Y.; Kuroda, J.; Nagao, R.; Yokota, A.; Takeuchi, M.; Hayashi, Y.; Shimazaki, C.; Taniwaki, M.; Strand, K.; Padia, J.; Hirai, H.; Kimura, S.; Maekawa, T. *Blood Cancer J.* **2011**, *1*, e43.
(28) Hisayuki, Y.; Ashihara, E.; Nagao, R.; Kimura, S.; Hirai, H.; Strovel, J. W.; Padia, J.; Cholody, W. M.; Maekawa, T. *Abstract 2866*, 51st ASH Annual Meeting, New Orleans, LA, December **2009**.
(29) Emami, K.H.; Nguyen, C.; Ma, H.; Kim, D.H.; Jeong, K.W.; Eguchi, M.; Moon, R.T.; Teo, J.-L.; Oh, S.W.; Kim, H.Y.; Moon, S.W.; Ha, J.R.; Kahn, M. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 12682.
(30) Kahn, M. *Am. Soc. Clin. Oncol.* **2011**, 435.
(31) Cha, J. Y.; Jung, J. -E.; Lee, K. -H.; Briaud, I.; Tenzin, F.; Pyon, Y.; Lee, D.; Chung, J. U.; Lee, J. H.; Oh, S. -W.; Jung, K. Y.; Pai, J. K.; Emami, K. *Abstract 3038*, 52nd ASH Annual Meeting, Orlando, FL, December **2010**.
(32) Ewan, K.; Pajak, B.; Stubbs, M.; Todd, H.; Barbeau, O.; Quevedo, C.; Botfield, H.; Young, R.; Ruddle, R.; Samuel, L.; Battersby, A.; Raynaud, F.; Allen, N.; Wilson, S.; Latinkic, B.; Workman, P.; McDonald, E.; Blagg, J.; Aherne, W.; Dale, T. *Cancer Res.* **2010**, *70*, 5963.
(33) Wei, W.; Chua, M.-S.; Grepper, S.; So, S. *Int. J. Cancer* **2009**, *126*, 2426.
(34) Gandhirajan, R.K.; Staib, P.A.; Minke, K.; Gehrke, I.; Plickert, G.; Schlosser, A.; Schmitt, E.K.; Hallek, M.; Kreuzer, K.-A. *Neoplasia* **2010**, *12*, 326.
(35) Tian, W.; Han, X.; Yan, M.; Xu, Y.; Duggineni, S.; Lin, N.; Luo, G.; Li, Y.M.; Han, X.; Huang, X.; An, J. *Biochemistry* **2012**, *51*, 724.