Biology-Oriented Synthesis

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Discovery of Inhibitors of the Wnt and Hedgehog Signaling Pathways through the Catalytic Enantioselective Synthesis of an Iridoid-Inspired Compound Collection**

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In biology-oriented synthesis (BIOS), biological relevance is employed as a key criterion to generate hypotheses for the design and synthesis of focused compound libraries.[1] In particular, the underlying scaffolds of natural product (NP) classes provide inspiration for BIOS since they define the areas of chemical space explored by nature, and therefore they can be regarded as "privileged". The use of NPs and NP-inspired compound collections has been particularly rewarding in the chemical-biological investigation of cellular signaling cascades.[1,2] For example, the Wnt[3] and Hedgehog[4] signaling pathways are of major importance for the regulation of differentiation, tissue regeneration, and stem-cell renewal, and are major pathways with relevance to the establishment of cancer. Modulators of Wnt-pathway signaling are efficient tools for the dissection of signal progression through the pathway.[2g,3,5] Aberrant activation of the Hedgehog pathway is involved in particular in skin cancer (basal-cell carcinoma) and brain tumors (medulloblastoma). Therapeutic strategies aimed at targeting the Hedgehog pathway are in high demand.[6] In light of the structural complexity and richness in stereogenic centers of natural products, and consequently of the compound collections inspired by their structure, the development of efficient enantioselective synthetic methods is at the heart of BIOS.[1,2,7]

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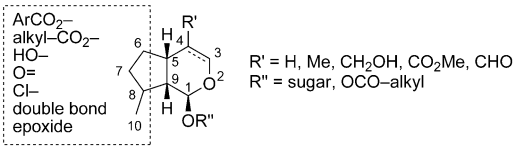
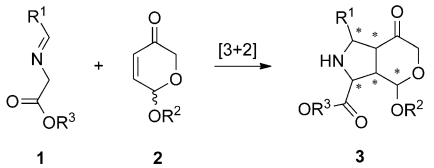
Iridoids are a large group of cyclopentano[*c*]pyran monoterpene secondary metabolites of terrestrial and marine flora and fauna.[8] Structurally, they are predominantly *cis*-fused bicycles decorated by various functional groups with multiple stereogenic centers (Scheme 1). Iridoids constitute



Scheme 1. Structural features of over 500 natural iridoids.

major components of traditional folk-medicinal plants and exhibit a wide range of pharmacological and biological properties.[8,9] Given this pronounced bioactivity and biological relevance, it is surprising that the synthesis of iridoid-inspired compound collections has essentially remained unexplored.[10] Herein, we describe the design and highly enantioselective synthesis of a novel iridoid-inspired compound collection and its investigation in cell-based assays for modulation of the Wnt and Hedgehog signal-transduction pathways.

For the synthesis of a compound collection that was similar in structure and complexity to the iridoids, we envisaged that a matching target scaffold might be accessed by the kinetic resolution of racemic 2*H*-pyran-3(6*H*)-ones **2** by means of an asymmetric [2+3] cycloaddition with azomethine ylides derived from amino acid ester imines **1** [Eq. (1)].[11–13] Such an *endo*-selective cycloaddition should



1 2 3 (1)

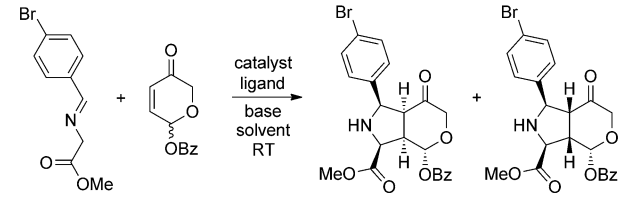
provide bicyclic compounds **3** with *cis*-fused five- and six-membered rings and five stereogenic centers. The target compounds **3** can be regarded as aza analogues of natural iridoids (Scheme 1). Racemic 2*H*-pyran-3(6*H*)-one derivatives are readily accessible by the Achmatowicz oxidation of furfuryl alcohols. Critical for the success of this strategy is the

development of an efficient method for the resolution of racemic derivatives **2** during the planned cycloaddition.

For the establishment of suitable reaction conditions, we selected *N*-4-bromobenzylideneglycine methyl ester (**1a**) and 5-oxo-5,6-dihydro-2*H*-pyran-2-yl benzoate (**2a**) as substrates. Initially, different salts of Ag^I and Cu^I were tested in the presence of the chiral P,N ferrocene ligand **4** and triethylamine as a base (Table 1, entries 1–5). Notably, the relative configuration of bicycle **3a** with its multiple stereocenters is identical to the relative configuration of natural iridoids. The highest enantioselectivity for the synthesis of **3a** was observed in the presence of $[Cu(CH_3CN)_4]BF_4$. Subsequently, a variety of ligands were tested (Table 1, entries 6–11; see the Supporting Information for additional details). An increase in enantioselectivity for **3a** was observed when (*R*)-Fesulphos (**7**) was employed as a ligand (Table 1, entry 11). For further optimization of the reaction conditions, we focused on the solvent, base, and catalyst loading (Table 1, entries 12–15; see also the Supporting Information). An increase in the *ee* value of **3a** to 93% was observed when DBU was employed as the base (Table 1, entry 12). The catalyst loading could be reduced to 5 mol% without compromising the yield and enantioselectivity for the formation of **3a** (Table 1, entry 14). Therefore, further cycloaddition reactions were carried out with $[Cu(CH_3CN)_4]BF_4$ (5 mol%), the chiral ligand **7** (6 mol%), and DBU (20 mol%) in dichloromethane (0.1M) at ambient temperature.

We investigated the scope of the [2+3] cycloaddition for the synthesis of an iridoid-inspired compound collection from various glycine ester imines **1** and pyranone **2a** under the optimized reaction conditions (Table 2, entries 1–8). Regardless of the electronic properties of the substituents introduced into the glycine ester imine, products **3** were obtained as single diastereomers in 60–71% yield with 88–99% *ee* (Table 2, entries 1–8). The use of *ortho*-, *meta*-, and *para*-substituted benzylideneglycine methyl esters led to similar results. Imines obtained from aliphatic aldehydes were not

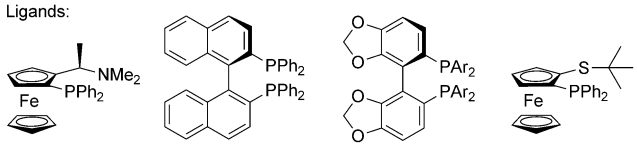
Table 1: Optimization of the reaction conditions.[a]



Entry	Catalyst	L	Solvent	Base	*T* [h]	Yield [%][b]	**3a**/**3a'**[c]	*ee* [%][d]
1	AgOTf	**4**	CH_2Cl_2	Et_3N	4	76	>20:1	0
2	AgOAc	**4**	CH_2Cl_2	Et_3N	4	68	>20:1	26
3	$AgOCOCF_3$	**4**	CH_2Cl_2	Et_3N	4	63	>20:1	20
4	$CuPF_6$	**4**	CH_2Cl_2	Et_3N	4	61	>20:1	53
5	$CuBF_4$	**4**	CH_2Cl_2	Et_3N	4	51	>20:1	65
6	$CuPF_6$	**5**	CH_2Cl_2	Et_3N	24	trace	–	–
7	$CuBF_4$	**5**	CH_2Cl_2	Et_3N	24	trace	–	–
8	$CuPF_6$	**6**	CH_2Cl_2	Et_3N	24	–	–	–
9	$CuBF_4$	**6**	CH_2Cl_2	Et_3N	24	–	–	–
10	$CuPF_6$	**7**	CH_2Cl_2	Et_3N	4	55	>20:1	64
11	$CuBF_4$	**7**	CH_2Cl_2	Et_3N	4	62	>20:1	70
12[e]	$CuBF_4$	**7**	CH_2Cl_2	DBU	4	65	>20:1	93
13[e]	$CuBF_4$	**7**	toluene	DBU	4	65	>20:1	83
14[e,f]	$CuBF_4$	**7**	CH_2Cl_2	DBU	4	69	>20:1	93
15[e,g]	$CuBF_4$	**7**	CH_2Cl_2	DBU	4	65	>20:1	85

[a] Reaction conditions: **1a** (1.0 equiv, 0.1 mmol), **2a** (2 equiv), Et_3N (1 equiv), catalyst (10 mol%), chiral ligand (12 mol%), solvent (0.1 M), room temperature. [b] Yield of the isolated product after column chromatography. [c] The diastereomeric ratio was determined by ^1H NMR spectroscopy. [d] The *ee* value was determined by HPLC analysis on a chiral stationary phase. [e] DBU (20 mol%) was used. [f] The reaction was carried out with 5 mol% of the catalyst and 6 mol% of **7**. [g] The reaction was carried out with 2 mol% of the catalyst and 2.4 mol% of **7**. Bz = benzoyl, $CuBF_4 = Cu(CH_3CN)_4BF_4$, $CuPF_6 = Cu(CH_3CN)_4PF_6$, DBU = 1,8-diazabicycloundec-7-ene, Tf = trifluoromethanesulfonyl.

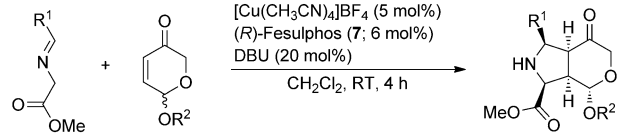
Ligands:



4 (*R*)-binap (**5**) **6** (*R*)-Fesulphos (**7**)

(Ar = 4-MeO-3,5-$tBu_2C_6H_2$)

Table 2: Scope of the asymmetric [2+3] cycloaddition.[a]



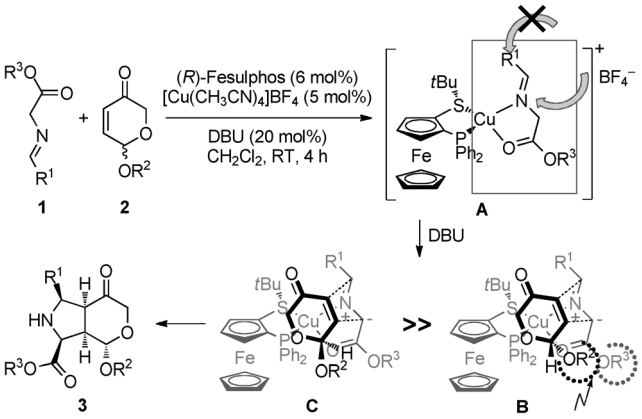
1 **2** **3**

Entry	R^1	R^2	Product	Yield [%][b]	*ee* [%][c]
1	Ph	Bz	(+)-**3b**	69	92
2	*p*-$MeOC_6H_4$	Bz	(+)-**3c**	71	94
3	*p*-MeC_6H_4	Bz	(+)-**3d**	61	92
4	*m*-MeC_6H_4	Bz	(+)-**3e**	63	88
5	*o*-MeC_6H_4	Bz	(+)-**3f**	62	94
6	*p*-FC_6H_4	Bz	(+)-**3g**	66	95
7	*m*-FC_6H_4	Bz	(+)-**3h**	60	90
8	2-furanyl	Bz	(+)-**3i**	60	99
9	*p*-BrC_6H_4	Piv	(+)-**3j**	68	88
10	Ph	Piv	(+)-**3k**	65	92
11	*p*-$MeOC_6H_4$	Piv	(+)-**3l**	60	85
12	*p*-FC_6H_4	Piv	(+)-**3m**	62	95
13	*m*-FC_6H_4	Piv	(+)-**3n**	65	90
14	*o*-FC_6H_4	Piv	(+)-**3o**	68	96
15	2-naphthyl	Piv	(+)-**3p**	58	94
16	*p*-BrC_6H_4	Ac	(+)-**3q**	58	84
17	*p*-$MeOC_6H_4$	Ac	(+)-**3r**	55	93
18	*p*-FC_6H_4	Ac	(+)-**3s**	55	91
19	*m*-FC_6H_4	Ac	(+)-**3t**	56	91
20	*o*-FC_6H_4	Ac	(+)-**3u**	51	85
21	2-naphthyl	Ac	(+)-**3v**	56	91
22	*p*-MeC_6H_4	methoxyacetyl	(+)-**3w**	61	94
23	*p*-FC_6H_4	methoxyacetyl	(+)-**3x**	68	88
24	*m*-FC_6H_4	methoxyacetyl	(+)-**3y**	65	94
25	2-naphthyl	methoxyacetyl	(+)-**3z**	66	94
26	*p*-BrC_6H_4	4-pentenoyl	(+)-**3aa**	73	93
27	2-naphthyl	4-pentenoyl	(+)-**3ab**	70	92

[a] Reaction conditions: imine **1** (1.0 equiv, 0.1 mmol), oxodihydropyran **2** (2 equiv), DBU (20 mol%), $[Cu(CH_3CN)_4]BF_4$ (5 mol%), **7** (6 mol%), CH_2Cl_2 (0.1 M), room temperature, 4 h. [b] Yield of the isolated product after column chromatography. [c] The *ee* value was determined by HPLC analysis on a chiral stationary phase. Piv = pivaloyl.

reactive under these reaction conditions. In subsequent experiments, the scope of the [2+3] cycloaddition with respect to the oxodihydropyran **2** was examined (Table 2, entries 9–27). Various esters obtained from 6-hydroxy-2*H*-pyran-3(6*H*)-one by acylation with aliphatic acids smoothly underwent the cycloaddition. The desired products were obtained with 84–96% *ee* and in 51–73% yield as single diastereomers, regardless of the electronic and steric properties of the substituents on the substrates. In total, a collection of 115 compounds was synthesized.

The absolute configuration of cycloadduct (+)-**3j** was determined by X-ray crystal-structure analysis (see the Supporting Information for details). The absolute configuration of all other compounds was assigned by analogy on the basis of this crystal structure. The high selectivity in the kinetic resolution of racemic derivatives **2** arises from a steric interaction in the 1,3-dipolar cycloaddition (Scheme 2). A



Scheme 2. Proposed model of kinetic resolution by 1,3-dipolar cycloaddition.

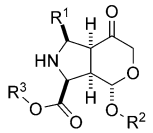
complex **A** is formed by coordination of the copper salt with the chiral ligand **5e** and the glycine ester imine **1**. Deprotonation by DBU leads to the formation of an azomethine ylide, which undergoes 1,3-dipolar cycloaddition with derivatives **2**. The cycloaddition occurs in such a way as to avoid unfavorable interactions between the bulky R^2 group and the azomethine ylide. The transition state **C** is preferred over **B** owing to the occurrence of minimal steric interactions and thus defines the absolute configuration of all stereogenic centers of products **3**.

To investigate whether the iridoid-inspired compound collection contained members that modulate different biological processes, we subjected it to different cell-based assays, including a Wnt-pathway reporter-gene assay. Upon Wnt activation, β-catenin, the central player in the pathway, accumulates in the cytoplasm and enters the nucleus. Nuclear β-catenin associates with transcription factors of the TCF/LEF family and recruits transcriptional coactivators and chromatin remodeling complexes, which in concert drive the expression of target genes.[14] In the absence of a Wnt signal, the β-catenin level is low, and the TCF/LEF factors function as transcriptional repressors by interaction with histone deacetylases. In many epithelial cancers, the Wnt pathway is constitutively active as a result of mutations in different components of the pathway.

We used an HEK293 reporter cell line that is highly sensitive to stimulation by the protein Wnt3a owing to the presence of additional copies of the Frizzled receptor and the resulting 10–20-fold induction of a luciferase reporter to screen the library at a 30 μM concentration.[15] Compounds for which cell viability remained above 80% with respect to control experiments were used in a primary screening. Dose-response analyses were carried out for hit compounds. To rule out the inhibition of the luciferase protein by the compounds or their interference with transcription or translation, we also carried out dose-response analyses by using an HEK293 cell line with constitutive luciferase expression. The results of the assays are summarized in Table 3. Much to our delight, the compound collection contained potent inhibitors of the Wnt signaling pathway.

The delineation of a structure–activity relationship from the screening results revealed that the R^2 group plays an important role in the bioactivity of the compounds (Table 3; see the Supporting Information for details). The introduction

Table 3: Representative results of the evaluation of the compound collection for inhibition of the Wnt signaling pathway.[a]



115-membered compound collection

R^1 = (Het)Ar
R^2 = Ac, Bz, Piv, $COCH_2OMe$, $COCH_2OPh$, 3-methylbutanoyl, 4-pentenoyl, 2-furanoyl, Me, allyl
R^3 = Me, allyl, Bn, *t*Bu

Entry	R^1	R^2	R^3	**3**	Wnt IC_{50} [μM][b]	Luciferase IC_{50} [μM][c]
1	*p*-BrC_6H_4	Bz	Me	**3a**	25.8 ± 4.2	inactive
2	*m*-MeC_6H_4	Bz	Me	**3e**	27.1 ± 1.5	inactive
3	*m*-FC_6H_4	Bz	Me	**3h**	23.0 ± 6.5	inactive
4	***p*-BrC_6H_4**	**Ac**	**Me**	**3q**	**4.4 ± 0.5**	**inactive**
5	Ph	Ac	Me	**3ac**	7.2 ± 0.8	> 30
6	*p*-$MeOC_6H_4$	Ac	Me	**3r**	13.0 ± 1.9	inactive
7	*p*-MeC_6H_4	Ac	Me	**3ad**	10.1 ± 1.4	inactive
8	*m*-MeC_6H_4	Ac	Me	**3ae**	8.2 ± 0.4	inactive
9	*o*-MeC_6H_4	Ac	Me	**3af**	7.1 ± 0.4	> 30
10	*p*-FC_6H_4	Ac	Me	**3s**	10.8 ± 0.8	inactive
11	*m*-FC_6H_4	Ac	Me	**3t**	10.2 ± 1.0	inactive
12	*o*-FC_6H_4	Ac	Me	**3u**	7.5 ± 0.9	inactive
13	2-naphthyl	Ac	Me	**3v**	5.8 ± 0.9	16.5 ± 2.6
14	2-furanyl	Ac	Me	**3ag**	14.8 ± 4.3	inactive
15	*p*-MeC_6H_4	$MeOCH_2CO$	Me	**3w**	12.2 ± 3.3	inactive
16	*m*-MeC_6H_4	$MeOCH_2CO$	Me	**3ah**	8.0 ± 0.5	inactive
17	*p*-FC_6H_4	$MeOCH_2CO$	Me	**3x**	11.0 ± 1.7	inactive
18	*m*-FC_6H_4	$MeOCH_2CO$	Me	**3y**	10.2 ± 1.0	inactive
19	**2-naphthyl**	**$PhOCH_2CO$**	**Me**	**3ai**	**3.1 ± 0.6**	**inactive**
20	1-naphthyl	4-pentenoyl	Bn	**3aj**	8.7 ± 2.6	inactive

[a] See the Supporting Information for biological methods. [b] Mean IC_{50} value for the inhibition of the Wnt pathway as determined in a Wnt reporter-gene assay. [c] Mean IC_{50} value for the inhibition of luciferase activity as determined in a recombinant luciferase assay. Compounds referred to as "inactive" showed no inhibition of luciferase activity at 30 μM. Bn = benzyl.

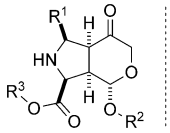
of bulky carboxylic residues, such as benzoic, pivalic, and *iso*-pentanoic acid, as the R^2 group led to less active inhibitors. Active compounds most frequently contained an acetic acid ester ($R^2 = Ac$). Nevertheless, aliphatic acid residues of different chain lengths without substituents in the α or β position were tolerated. Importantly, the use of more labile compounds, such as mixed acetals, in which the carboxylic acid residue was replaced with an alkyl group, did not lead to active inhibitors ($R^2 = Me$, allyl). Methyl esters ($R^3 = Me$) scored well in the inhibition of the Wnt signaling pathway. An increase in the size of the group at this position led to active compounds in the luciferase control assay. Notably, R^1 could be varied widely without loss of activity; *ortho*-, *meta*-, and *para*-substituted phenyl groups were tolerated at this position. However, bulky R^1 groups, such as 2-naphthyl, may lead to false-positive results owing to the inhibition of luciferase activity. Finally, protection of the nitrogen atom of compounds **3** with various carboxylic acid derivatives led to inactive compounds.

Protein structures and natural product scaffold structures are conserved in evolution, and the number of fold types and ligand-sensing cores with similar 3D structures yet different amino acid sequencers and precise ligand-recognition motifs is fairly limited. Thus, ligand-binding pockets with similar structures and sequences occur in various proteins with differing biological functions. Therefore, NP-inspired compound collections based on BIOS may contain members that hit different targets and thereby modulate different biological phenomena.[1,16,17] On the basis of this insight, we investigated the iridod-inspired compound collection for possible modulation of the Hedgehog pathway.

For assaying signal transduction through the Hedgehog signaling pathway, mouse embryonic mesoderm fibroblast C3H10T1/2 cells were used. These multipotent mesenchymal progenitor cells can differentiate into osteoblasts upon treatment with the Smoothened agonist. During differentiation, osteoblast-specific genes, such as alkaline phosphatase, which plays an essential role in bone formation, are highly expressed. The activity of alkaline phosphatase can be monitored directly by following substrate hydrolysis to yield a highly luminescent product.[6a,18] Inhibition of the Hedgehog pathway results in a reduction in luminescence. Hits showed reduction in the luminescence signal without alteration of the cell viability. Dose-response analysis was performed for hit compounds. Representative results of the assay are summarized in Table 4 (see the Supporting Information for details). To our delight, several iridoid-inspired compounds that were not identified as valid hits in the Wnt assay selectively inhibited the Hedgehog signaling pathway in the low micromolar range. This finding confirms that the application of NPs and NP-inspired compound collections is a powerful method for the identification of modulators and probes with diverse bioactivity from one given compound collection.

In conclusion, we developed a novel asymmetric catalytic method for the synthesis of an iridoid-inspired compound collection by the resolution of 2*H*-pyran-3(6*H*)-one derivatives. The desired products were formed efficiently and with high diastereo- and enantioselectivity. Evaluation of the obtained compound collection led to the discovery of a novel class of inhibitors of the Wnt and Hedgehog signaling pathways.

Table 4: Representative results of the evaluation of the compound collection for inhibition of the Hedgehog signaling pathway.[a]



R^1 = (Het)Ar
R^2 = Ac, Bz, Piv, $COCH_2OMe$,$COCH_2OPh$, 3-methylbutanoyl, 4-pentenoyl, 2-furanoyl, Me, allyl
R^3 = Me, allyl, Bn, *t*Bu

Entry	R^1	R^2	R^3	**3**	IC_{50} [μM][b]	Viability[c]
1	1-naphthyl	4-pentenoyl	Me	**3 al**	4.3 ± 1.8	inactive
2	2-naphthyl	$MeOCH_2CO$	Me	**3 z**	5.0 ± 1.3	inactive
3	2-naphthyl	$MeOCH_2CO$	Bn	**3 ap**	5.9 ± 1.0	inactive
4	*o*-MeC_6H_4	4-pentenoyl	Me	**3 aq**	6.0 ± 2.0	inactive
5	2-furanyl	4-pentenoyl	Bn	**3 ar**	7.0 ± 1.2	inactive
6	*p*-BrC_6H_4	Ac	allyl	**3 as**	9.8 ± 0.2	inactive

[a] See the Supporting Information for biological methods. [b] Mean IC_{50} value for the inhibition of the Hedgehog signaling pathway. [c] Compounds referred to as "inactive" showed more than 80% cell viability at 10 μM.

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[1] a) K. Kumar, H. Waldmann, *Angew. Chem.* **2009**, *121*, 3272–3290; *Angew. Chem. Int. Ed.* **2009**, *48*, 3224–3242; b) M. Kaiser, S. Wetzel, K. Kumar, H. Waldmann, *Cell. Mol. Life Sci.* **2008**, *65*, 1186–1201; c) R. S. Bon, H. Waldmann, *Acc. Chem. Res.* **2010**, *43*, 1103–1114; d) S. Wetzel, R. S. Bon, K. Kumar, H. Waldmann, *Angew. Chem.* **2011**, *123*, 10990–11018; *Angew. Chem. Int. Ed.* **2011**, *50*, 10800–10826.

[2] a) F. E. Koehn, G. T. Carter, *Nat. Rev. Drug Discovery* **2005**, *4*, 206–220; b) K. S. Lam, *Trends Microbiol.* **2007**, *15*, 279–289; c) D. J. Newman, G. M. Cragg, *J. Nat. Prod.* **2007**, *70*, 461–477; d) A. Ganesan, *Curr. Opin. Chem. Biol.* **2008**, *12*, 306–317; e) A. L. Harvey, *Drug Discovery Today* **2008**, *13*, 894–901; f) J. W. H. Li, J. C. Vederas, *Science* **2009**, *325*, 161–165; g) T. F. Molinski, D. S. Dalisay, S. L. Lievens, J. P. Saludes, *Nat. Rev. Drug Discovery* **2009**, *8*, 69–85; for previous studies by our research group, see: h) S. Basu, B. Ellinger, S. Rizzo, C. Deraeve, M. Schürmann, H. Preut, H.-D. Arndt, H. Waldmann, *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 6805–6810; i) H. Dückert, V. Pries, V. Khedkar, S. Menninger, H. Bruss, A. W. Bird, Z. Maliga, A. Brockmeyer, P. Janning, A. Hyman, S. Grimme, M. Schürmann, H. Preut, K. Hubel, S. Ziegler, K. Kumar, H. Waldmann, *Nat. Chem. Biol.* **2012**, *8*, 179–184; j) T. Voigt, C. Gerding-Reimers, T. N. T. Tuyen, S. Bergmann, H. Lachance, B. Schölermann, A. Brockmeyer, P. Janning, S. Ziegler, H. Waldmann, *Angew. Chem.* **2013**, *125*, 428–432; *Angew. Chem. Int. Ed.* **2013**, *52*, 410–414; k) V. Eschenbrenner-Lux, H. Dückert, V. Khedkar, H. Bruss, H. Waldmann, K. Kumar, *Chem. Eur. J.* **2013**, *19*, 2294–2304; l) T. Knoth, K. Warburg, C. Katzka, A. Rai, A. Wolf, A. Brockmeyer, P. Janning, T. F. Reubold, S. Eschenburg, D. J. Manstein, K. Hübel, M. Kaiser, H. Waldmann, *Angew. Chem.* **2009**, *121*, 7376–7381; *Angew. Chem. Int. Ed.* **2009**, *48*, 7240–7245.

[3] a) H. Clevers, R. Nusse, *Cell* **2012**, *149*, 1192–1205; b) B. T. MacDonald, K. Tamai, X. He, *Dev. Cell* **2009**, *17*, 9–26; c) A.

Klaus, W. Birchmeier, *Nat. Rev. Cancer* **2008**, *8*, 387 – 398; d) N. Barker, H. Clevers, *Nat. Rev. Drug Discovery* **2006**, *5*, 997 – 1014; e) C. Y. Logan, R. Nusse, *Annu. Rev. Cell Dev. Biol.* **2004**, *20*, 781 – 810; f) A. Kochegarov, *Expert Opin. Ther. Pat.* **2009**, *19*, 275 – 281; g) F. Osakada, Z. B. Jin, Y. Hirami, H. Ikeda, T. Danjyo, K. Watanabe, Y. Sasai, M. Takahashi, *J. Cell Sci.* **2009**, *122*, 3169 – 3179; h) T. Reya, H. Clevers, *Nature* **2005**, *434*, 843 – 850; i) D. L. Zhang, L. J. Gu, L. Liu, C. Y. Wang, B. S. Sun, Z. Li, C. K. Sung, *Biochem. Biophys. Res. Commun.* **2009**, *378*, 149 – 151; j) C. Fathke, L. Wilson, K. Shah, B. Kim, A. Hocking, R. Moon, F. Isik, *BMC Cell Biol.* **2006**, *7*, 4; k) D. Silkstone, H. Hong, B. A. Alman, *Nat. Clin. Pract. Rheumatol.* **2008**, *4*, 413 – 419.

[4] For reviews, see: a) L. L. Rubin, F. J. de Sauvage, *Nat. Rev. Drug Discovery* **2006**, *5*, 1026 – 1033; b) J. M. Y. Ng, T. Curran, *Nat. Rev. Cancer* **2011**, *11*, 493 – 501; c) S. Peukert, K. Miller-Moslin, *ChemMedChem* **2010**, *5*, 500 – 512; d) M. R. Tremblay, K. McGovern, M. A. Read, A. C. Castro, *Curr. Opin. Chem. Biol.* **2010**, *14*, 428 – 435; e) B. Z. Stanton, L. F. Peng, *Mol. BioSyst.* **2010**, *6*, 44 – 54; f) N. Mahindroo, C. Punchihewa, N. Fujii, *J. Med. Chem.* **2009**, *52*, 3829 – 3845; g) P. Heretsch, L. Tzagkaroulaki, A. Giannis, *Bioorg. Med. Chem.* **2010**, *18*, 6613 – 6624.

[5] a) M. Lepourcelet, Y. N. P. Chen, D. S. France, H. Wang, P. Crews, F. Petersen, C. Bruseo, A. W. Wood, R. A. Shivdasani, *Cancer Cell* **2004**, *5*, 91 – 102; b) N. Chung, S. Marine, E. A. Smith, R. Liehr, S. T. Smith, L. Locco, E. Hudak, A. Kreamer, A. Rush, B. Roberts, M. B. Major, R. T. Moon, W. Arthur, M. Cleary, B. Strulovici, M. Ferrer, *Assay Drug Dev. Technol.* **2010**, *8*, 286 – 294; c) K. H. Emami, C. Nguyen, H. Ma, D. H. Kim, K. W. Jeong, M. Eguchi, R. T. Moon, J. L. Teo, H. Y. Kim, S. H. Moon, J. R. Ha, M. Kahn, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 12682 – 12687; d) B. Chen, M. E. Dodge, W. Tang, J. Lu, Z. Ma, C. W. Fan, S. Wei, W. Hao, J. Kilgore, N. S. Williams, M. G. Roth, J. F. Amatruda, C. Chen, L. Lum, *Nat. Chem. Biol.* **2009**, *5*, 100 – 107.

[6] For selected examples of small-molecule Hedgehog-pathway modulators, see: a) S. Sinha, J. K. Chen, *Nat. Chem. Biol.* **2006**, *2*, 29 – 30; b) J. K. Chen, J. Taipale, M. K. Cooper, P. A. Beachy, *Genes Dev.* **2002**, *16*, 2743 – 2748; c) J. K. Chen, J. Taipale, K. E. Young, T. Maiti, P. A. Beachy, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 14071 – 14076; d) M. F. Strand, S. R. Wilson, J. L. Dembinski, D. D. Holsworth, A. Khvat, I. Okun, D. Petersen, S. Krauss, *PLOS one* **2011**, *6*, e19904; e) A. Büttner, K. Seifert, T. Cottin, V. Sarli, L. Tzagkaroulaki, S. Scholz, A. Giannis, *Bioorg. Med. Chem.* **2009**, *17*, 4943 – 4954; f) K. Seifert, A. Büttner, S. Rigol, N. Eilert, E. Wandel, A. Giannis, *Bioorg. Med. Chem.* **2012**, *20*, 6465 – 6481.

[7] a) H. Waldmann, V. Khedkar, H. Dückert, M. Schürmann, I. M. Oppel, K. Kumar, *Angew. Chem.* **2008**, *120*, 6975 – 6978; *Angew. Chem. Int. Ed.* **2008**, *47*, 6869 – 6872; b) A. P. Antonchick, C. Gerding-Reimers, M. Catarinella, M. Schürmann, H. Preut, S. Ziegler, D. Rauh, H. Waldmann, *Nat. Chem.* **2010**, *2*, 735 – 740; c) A. P. Antonchick, S. López-Tosco, J. Parga, S. Sievers, M. Schürmann, H. Preut, S. Höing, H. R. Schöler, J. Sterneckert, D. Rauh, H. Waldmann, *Chem. Biol.* **2013**, *20*, 500 – 509; d) P.-Y. Dakas, J. A. Parga, S. Höing, H. R. Schöler, J. Sterneckert, K. Kumar, H. Waldmann, *Angew. Chem.* **2013**, *125*, 9755 – 9760; *Angew. Chem. Int. Ed.* **2013**, *52*, 9576 – 9581.

[8] a) B. Dinda, D. R. Chowdhury, B. C. Mohanta, *Chem. Pharm. Bull.* **2009**, *57*, 765 – 796; b) B. Dinda, S. Debnath, R. Banik, *Chem. Pharm. Bull.* **2011**, *59*, 803 – 833; c) B. Dinda, S. Debnath, Y. Harigaya, *Chem. Pharm. Bull.* **2007**, *55*, 689 – 728; d) B. Dinda, S. Debnath, Y. Harigaya, *Chem. Pharm. Bull.* **2007**, *55*, 159 – 222.

[9] a) E. L. Ghisalberti, *Phytomedicine* **1998**, *5*, 147 – 163; b) R. Tundis, M. R. Loizzo, F. Menichini, G. A. Statti, F. Menichini, *Mini-Rev. Med. Chem.* **2008**, *8*, 399 – 420; c) A. Viljoen, N. Mncwangi, I. Vermaak, *Curr. Med. Chem.* **2012**, *19*, 2104 – 2127.

[10] For the synthesis and investigation of an iridoid-inspired compound collection with a different scaffold structure, see Ref. [7d].

[11] For reviews on the cycloaddition of azomethine ylides, see: a) J. Adrio, J. C. Carretero, *Chem. Commun.* **2011**, *47*, 6784 – 6794; b) C. Nájera, J. M. Sansano, M. Yus, *J. Braz. Chem. Soc.* **2010**, *21*, 377 – 412; c) L. M. Stanley, M. P. Sibi, *Chem. Rev.* **2008**, *108*, 2887 – 2902; d) H. Pellissier, *Tetrahedron* **2007**, *63*, 3235 – 3285; e) V. Nair, T. D. Suja, *Tetrahedron* **2007**, *63*, 12247 – 12275; f) G. Pandey, P. Banerjee, S. R. Gadre, *Chem. Rev.* **2006**, *106*, 4484 – 4517.

[12] For selected recent examples of [2+3] cycloaddition reactions of azomethine ylides, see: a) B. M. Trost, S. M. Silverman, *J. Am. Chem. Soc.* **2012**, *134*, 4941 – 4954; b) S. Reboredo, E. Reyes, J. L. Vicario, D. Badía, L. Carrillo, A. De Cózar, F. P. Cossío, *Chem. Eur. J.* **2012**, *18*, 7179 – 7188; c) E. Conde, D. Bello, A. de Cózar, M. Sánchez, M. A. Vázquez, F. P. Cossío, *Chem. Sci.* **2012**, *3*, 1486 – 1491; d) M. González-Esguevillas, J. Adrio, J. C. Carretero, *Chem. Commun.* **2012**, *48*, 2149 – 2151; e) Z.-L. He, H.-L. Teng, C.-J. Wang, *Angew. Chem.* **2013**, *125*, 3006 – 3010; *Angew. Chem. Int. Ed.* **2013**, *52*, 2934 – 2938; f) M. González-Esguevillas, J. Adrio, J. C. Carretero, *Chem. Commun.* **2013**, *49*, 4649 – 4651; g) J. Hernández-Toribio, S. Padilla, J. Adrio, J. C. Carretero, *Angew. Chem.* **2012**, *124*, 8984 – 8988; *Angew. Chem. Int. Ed.* **2012**, *51*, 8854 – 8858; h) X. Jing, C. He, D. P. Dong, L. L. Yang, C. Y. Duan, *Angew. Chem.* **2012**, *124*, 10274 – 10278; *Angew. Chem. Int. Ed.* **2012**, *51*, 10127 – 10131.

[13] For examples from our research group, see: a) M. Potowski, J. O. Bauer, C. Strohmann, A. P. Antonchick, H. Waldmann, *Angew. Chem.* **2012**, *124*, 9650 – 9654; *Angew. Chem. Int. Ed.* **2012**, *51*, 9512 – 9516; b) M. Potowski, M. Schürmann, H. Preut, A. P. Antonchick, H. Waldmann, *Nat. Chem. Biol.* **2012**, *8*, 428 – 430; c) A. P. Antonchick, H. Schuster, H. Bruss, M. Schürmann, H. Preut, D. Rauh, H. Waldmann, *Tetrahedron* **2011**, *67*, 10195 – 10202; d) H. Waldmann, E. Blaser, M. Jansen, H. P. Letschert, *Angew. Chem.* **1994**, *106*, 717 – 719; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 683 – 685.

[14] K. Willert, K. A. Jones, *Genes Dev.* **2006**, *20*, 1394 – 1404.

[15] S. Park, J. Gwak, M. Cho, T. Song, J. Won, D. E. Kim, J. G. Shin, S. Oh, *Mol. Pharmacol.* **2006**, *70*, 960 – 966.

[16] a) M. A. Koch, L. O. Wittenberg, S. Basu, D. A. Jeyaraj, E. Gourzoulidou, K. Reinecke, A. Odermatt, H. Waldmann, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 16721 – 21676; b) M. A. Koch, A. Schuffenhauer, M. Scheck, S. Wetzel, M. Casaulta, A. Odermatt, P. Ertl, H. Waldmann, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 17272 – 17277; c) A. Nören-Müller, I. Reis-Corrêa, Jr., H. Prinz, C. Rosenbaum, K. Saxena, H. J. Schwalbe, D. Vestweber, G. Cagna, S. Schunk, O. Schwarz, H. Schiewe, H. Waldmann, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 10606 – 10611.

[17] J. Skolnick, M. Gao, *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 9344 – 9349.

[18] a) X. Wu, S. Ding, Q. Ding, N. S. Gray, P. G. Schultz, *J. Am. Chem. Soc.* **2002**, *124*, 14520 – 14521; b) M. M. Beloti, L. S. Bellesini, A. L. Rosa, *Cell Biol. Int.* **2005**, *29*, 537 – 541; c) X. Wu, J. Walker, J. Zhang, S. Ding, P. G. Schultz, *Chem. Biol.* **2004**, *11*, 1229 – 1238; d) X.-J. Li, B.-Y. Hu, S. A. Jones, Y.-S. Zhang, Y. Sha, T. Lavaute, Z.-W. Du, *Stem Cells* **2008**, *26*, 886 – 893.

