Enzyme Inhibitors

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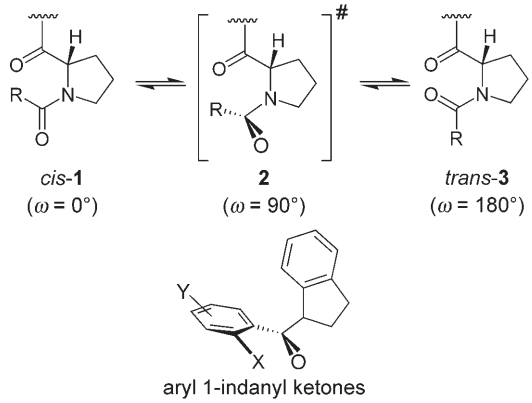
Aryl Indanyl Ketones: Efficient Inhibitors of the Human Peptidyl Prolyl *cis*/*trans* Isomerase Pin1**

*Sebastian Daum, Frank Erdmann, Gunter Fischer, Boris Féaux de Lacroix, Anahita Hessamian-Alinejad, Sabine Houben, Walter Frank, and Manfred Braun**

The rigidity of the amide bond is a feature that distinctly influences the structure of proteins. The rotation about the imidic peptide bond preceding proline has to surmount an activation barrier of 75 to 100 kJ mol^{-1} for the conversion of the *cis*-conformer **1** into *trans*-**3** (Scheme 1). Thereby, the torsion angle ω changes from 0 to 180°. In unfolded proteins, the *trans*-isomer **3** predominates; however, native proteins frequently contain a specific prolyl bond in the *cis* conformation **1**.[1]



Scheme 1. *Cis–trans* isomerization of peptidyl–prolyl bonds. Aryl 1-indanyl ketones as mimics of the twisted-amide transition state **2**.

Peptidyl prolyl *cis*/*trans* isomerases (PPIases) are enzymes that catalyze the *cis–trans* interconversion of prolyl bonds (**1**⇌**3**).[2] Discovered in 1984, they have been found to be an important group of enzymes with a strong impact on the slow processes involved in protein folding.[3] Concerning the mechanism of the enzymatic catalysis exhibited by PPIases, various hypotheses have been developed to rationalize how

[*] Dipl.-Chem. B. Féaux de Lacroix, Dr. A. Hessamian-Alinejad, S. Houben, Prof. Dr. W. Frank, Prof. Dr. M. Braun
Wissenschaftliche Einrichtung Chemie
Universität Düsseldorf
Universitätsstrasse 1, 40225 Düsseldorf (Germany)
Fax: (+49) 211-811-5079
E-mail: braunm@uni-duesseldorf.de
S. Daum, Dr. F. Erdmann, Prof. Dr. G. Fischer
Max-Planck-Forschungsstelle für Enzymologie der Proteinfaltung
Weinbergweg 22, 06120 Halle (Germany)

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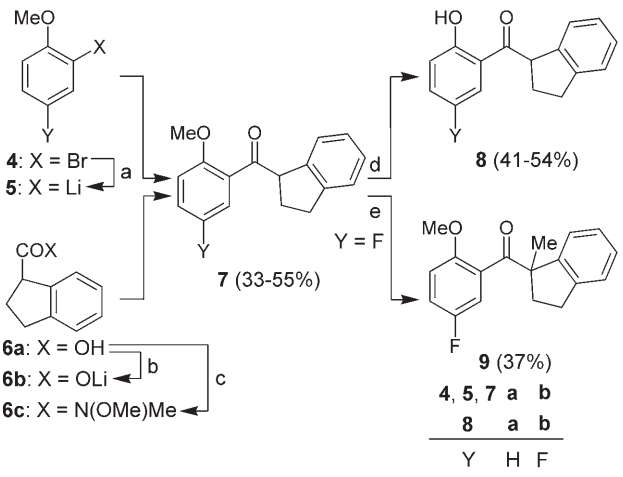
the energy of the transition state **2** in the uncatalyzed isomerization of *cis*-**1** and *trans*-**3** is lowered to the experimentally measured values of the enzyme catalyzed reaction.[1,2b]

Among the three families of human PPIases, the parvulin-like Pin1 is unique in its substrate specificity which has made it possible to specifically accelerate the *cis–trans* isomerization of $PO_3H_2Ser(PO_3H_2Thr)$–Pro bonds in short peptides and proteins.[4] Therefore, Pin1 interacts with and regulates many key phosphoproteins of the eukaryotic cell cycle, such as cyclin D1, p53, or β-catenin.[5] In several human cancer cell lines, the depletion of Pin1 causes cell cycle arrest. Moreover, the enzyme has been found to be overexpressed in many tumor cell lines.[6] Pin1 has also been found to be involved in Alzheimer's disease.[7] To date, however, few compounds have been reported to act as inhibitors of Pin1. Among them, the natural naphthoquinone juglone inactivated Pin1 in an irreversible manner by minor structural alterations at the active site after the Michael addition of a thiol group.[8] Several polycyclic aromatic compounds[9] and natural peptide mimetics[10] have also been reported to inhibit Pin1.

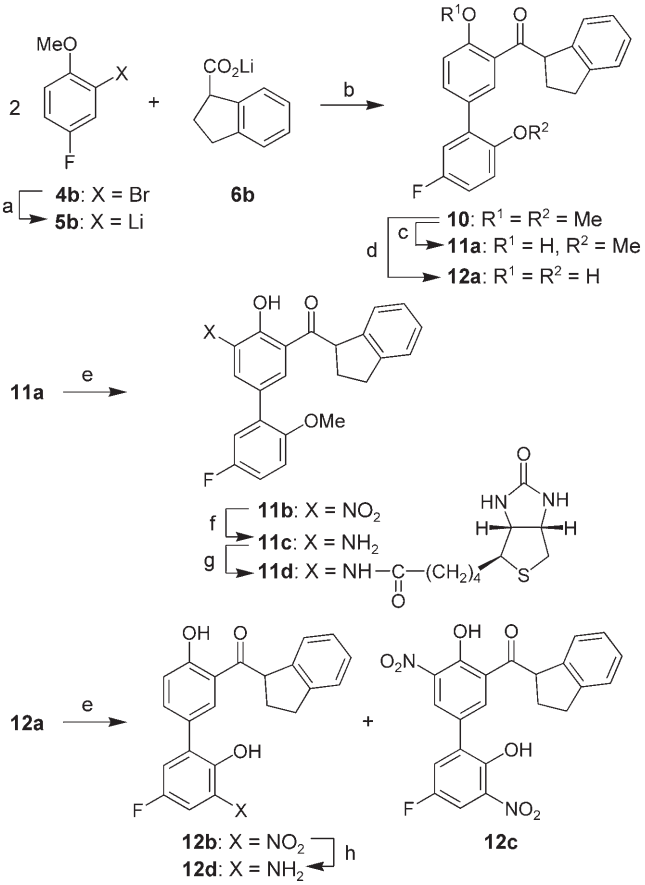
In our search for efficient reversible inhibitors of human Pin1 (hPin1), we were guided by the idea that the "twisted-amide" transition-state model **2** along with the concomitant change of hybridization about the ring nitrogen atom might be readily mimicked by aryl 1-indanyl ketones, because we anticipated them to have a twisted conformation in front of the five-membered ring. Its similarity to the "twisted amide" **2** is indicated in Scheme 1.

Despite their relatively simple structure, only a few compounds containing the aryl 1-indanyl-ketone moiety have been synthesized.[11] Most of them served as intermediates for fredericamycin A[11c–g] and, in only a few cases, were nonracemic products synthesized.[11d,i] Thus, general routes to aryl indanyl ketones had to be developed to make the preparation of specifically substituted products possible. As shown in Scheme 2, monoaryl indanyl ketones **7a**,**b** were easily available by coupling lithiated arenes **5a**,**b**, generated by a bromine–lithium exchange from bromoarenes **4a**,**b**, with monodeprotonated indanecarboxylic acid **6a**.[12] As an alternative to the lithium salt **6b**, the Weinreb-type[13] amide **6c** was also found to react readily with aryllithium **5** to deliver the ketones **7**. Generally, this variant provided higher yields. The cleavage of the methyl ether group in compounds **7a**,**b** occurred upon treatment with aluminum chloride[14] to give the phenols **8a**,**b**. For the introduction of a methyl substituent in the α-carbonyl position, the enolate generated from ketone **7b** was treated with methyl iodide to give racemic product **9**.

A novel domino reaction led to the biaryl indanyl ketone **10**, as shown in Scheme 3. When two equivalents of aryllithium **5b**, generated by bromine–lithium exchange from **4b**, were allowed to react with the lithium salt **6b** of indane carboxylic acid, the biaryl indanyl ketone **10** resulted as the only product apart from protonated starting material **5b** (H instead of Li) and carboxylic acid **6a**. Clearly, not only the addition to the carboxylate **6b** had occurred, but also a substitution of the fluorine atom by the second equivalent of the aryllithium **5b** serving as a nucleophile. Although the



Scheme 2. Synthesis of monoaryl indanyl ketones **7**–**9**. Reagents and conditions: a) *n*BuLi, Et_2O, −78 °C; b) *n*BuLi, Et_2O, −78 to −10 °C; c) 1. $SOCl_2$, reflux; 2. HN(OMe)Me·HCl, pyridine, 0 °C to room temperature; d) $AlCl_3$, CH_2Cl_2, 0 °C; e) 1. LiN*i*Pr$_2$, THF, −78 to 0 °C, 2. MeI, −20 °C to room temperature.



Scheme 3. Domino reaction between aryllithium **5b** and carboxylate **6b**. Synthesis of ketones **11** and **12** derived from **10**. Reagents and conditions: a) *n*BuLi, Et_2O, −78 °C; b) −78 °C to reflux, 27%; c) $AlCl_3$, CH_2Cl_2, 0 °C to room temperature, 59%; d) BBr_3, CH_2Cl_2, 71%; e) HNO_3, HOAc, 10 °C, 79%; f) H_2/Pd/C, THF, 73%; g) biotinyl chloride, pyridine, 8%; h) H_2/Pd/C, EtOH, 95%.

question remains open whether this step follows an addition–elimination or an aryne pathway, the complete regiocontrol is remarkable. The domino reaction does not occur when, instead of the carboxylate **6b**, the amide **6c** is treated with two equivalents of **5b**.

An unambiguous confirmation of the structure of ketone **10**, which forms as a single isomer, comes from a crystal-structure analysis of phenolic ketone **11a** (Figure 1),[15] the

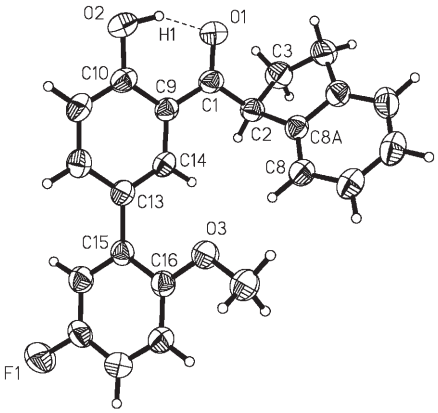


Figure 1. Molecular structure of ketone **11a**. Thermal ellipsoids are set at 30% probability; radii of hydrogen atoms are chosen arbitrarily. Selected interatomic distances [Å] and angles [°]: C1–O1 1.2443(15), C10–O2 1.3570(15), O2–H1 0.996(18), H1···O1 1.639(18), O1···O2 2.5570(15); O2-H1···O1 151.2(15), C3-C2-C8A 101.55(12); O1-C1-C9-C10 5.01(18), O1-C1-C2-C8A 92.15(15), O1-C1-C2-C3 −20.98(19), C2-C1-C9-C10 −174.96(12), C14-C13-C15-C16 57.77(17).

product of a selective demethylation of the methoxy substituent in the position *ortho* to the carbonyl group. Although the yield of the domino reaction is moderate (25–30%), it opens an easy way to the ketone **10** that in turn serves as a starting material for further derivatives. Thus, a regioselective *ortho*-nitration of the phenol **11a** was possible and gave the product **11b** as a single isomer. Catalytic hydrogenation afforded the aromatic amine **11c** that was coupled with biotin-derived acid chloride to give the amide **11d**. A complete demethylation of biaryl indanyl ketone **10** by means of boron tribromide[14] led to the formation of bisphenolic ketone **12a**. Upon treatment with nitric acid in acetic acid, a mixture of nitro compounds **12b** and **12c** was obtained. After separation, the former was reduced to the aromatic amine **12d**.

Whereas all the aryl indanyl ketones described herein were obtained as racemic mixtures, the α-methyl-substituted ketone **9** was chosen to be prepared in an enantiomerically pure form. For this purpose, enantiomeric (*R*)- and (*S*)-1-methyl-1-indanecarboxylic acids[16,17] served as starting materials. Thus, reaction of the corresponding Weinreb-type amides with **5b** delivered (*R*)- and (*S*)-**9**, respectively, in 45 to 48% overall yield.

The inhibition of hPin1 by the aryl indanyl ketones prepared as described above was determined in a protease-free PPIase assay with Suc-Ala-Glu-Pro-Phe-pNA as the substrate.[18] The compounds exhibit substantial inhibition of hPin1. As shown in Table 1, most of the aryl indanyl ketones

Table 1: Inhibition constants of aryl indanyl ketones **7**–**9**, **11**, and **12**.

Entry	Ketone	K_i [μM]	Entry	Ketone	K_i [μM]
1	**7a**	6.2±0.4	9	**11d**	1.4±0.9
2	**7b**	8.7±0.2	10	**12a**	3.1±0.6
3	**8a**	5.1±0.8	11	**12b**	0.5±0.1
4	**8b**	15.0±3.0	12	**12c**	0.2±0.1
5	**9**	19.0±4.9	13	**12d**	0.4±0.1
6	**11a**	1.7±0.2	14	(*R*)-**9**	5.6±0.9
7	**11b**	1.1±0.2	15	(*S*)-**9**	51.0±9.0
8	**11c**	4.9±0.7			

(entries 1–10) have K_i values in the micromolar range. The hydrogen-bonded hydroxy group adjacent to the carbonyl group is not crucial to Pin1 inhibition, although the phenolic derivatives are superior to the phenol ethers. A significant improvement in Pin1 inhibition originates from the introduction of nitro groups. Thus, mono- and dinitro-substituted ketones **12b** and **12c** (Table 1; entries 11 and 12) led to a strong inhibition with K_i values in the sub-micromolar range. Remarkably, the replacement of a nitro group in **12b** by an amino substituent in **12d** did not influence Pin1 inhibition to a major extent (Table 1; entry 13).

Ketone **11d** with a biotin side chain allowed the reversibility of Pin1 inhibition to be demonstrated. For this experiment the activity assay was performed at a fixed concentration of **11d**, leading to 80% inhibition of the Pin1 PPIase activity. The addition of increasing concentrations of streptavidin (a stronger competitive binder for **11d**) resulted in the full return of Pin1 activity whereas the addition of streptavidin presaturated with biotin had no effect (Figure 2). This

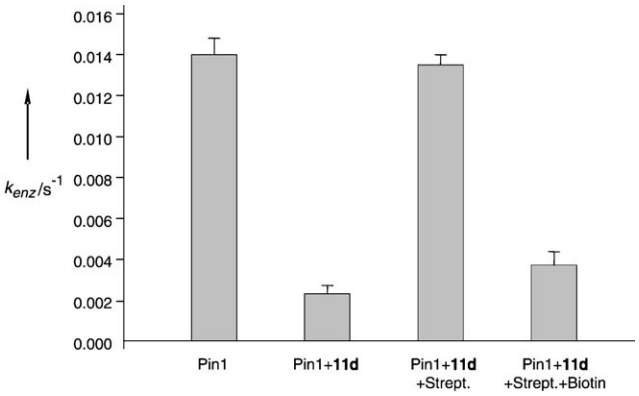


Figure 2. Reversibility of Pin1/**11d** complex formation; PPIase activity of 5 nM Pin1 in the absence and presence of 5 μM **11d**, 10 μM Streptavidin, 30 μM Biotin.

behavior indicates that the inhibitor is noncovalently attached to Pin1 and the concentration-dependent formation of inactive enzyme is not due to irreversible Pin1 denaturation and aggregation.[10c]

As expected, a substantial difference in the inhibition of the enzyme occurred when the two enantiomers of ketone **9** were tested, and the corresponding K_i values differed by an order of magnitude from each other (Table 1; entries 14 and 15). The different behavior of the enantiomers underlines the

idea of the similarity between the structure of aryl indanyl ketones and the "twisted-amide" transition state, because the binding sites in the transition state of the active center must have a stereochemical component. More support for the hypothesis comes from the crystal structure of ketone **11a** (Figure 1). The torsion angle between the aromatic-carbonyl and the indanyl moiety (O1-C1-C2-C8A) of 92° is in accord with the features of the twisted amide **2**. In addition, homologues of aryl indanyl ketones with four- or six-membered rings instead of a five-membered one do not display substantial Pin1 inhibition, a fact that supports the "twisted-amide" hypothesis (data not shown).

Next we asked whether application of selected compounds of Table 1 would give rise to the same effects in cells as those obtained after the depletion of Pin1 in cells by genetic means. A luciferase reporter gene assay with a p53 response element in MCF-7 cells (breast cancer cell line) was performed. In cells, the phosphorylated form of tumor-suppressor protein p53 interacts with endogenous Pin1 after DNA damage by chemotherapeutic drugs or irradiation. In this pathway, Pin1 induces conformational changes which lead to increased transactivation activity and higher proteolytic stability of p53.[19]

As shown in Figure 3, the compounds (*S*)-**9**, (*R*)-**9**, and *rac*-**9** are able to reduce the stimulated activity of the p53 reporter gene in etoposid treated MCF-7 cells in a concentration-dependent manner and according to their inhibitory potencies. Similarly, cotransfection of Pin1-negative mouse-embryo fibroblasts with the expression construct for an inactive Pin1 variant failed to increase the stability and transactivation activity of p53.[19c]

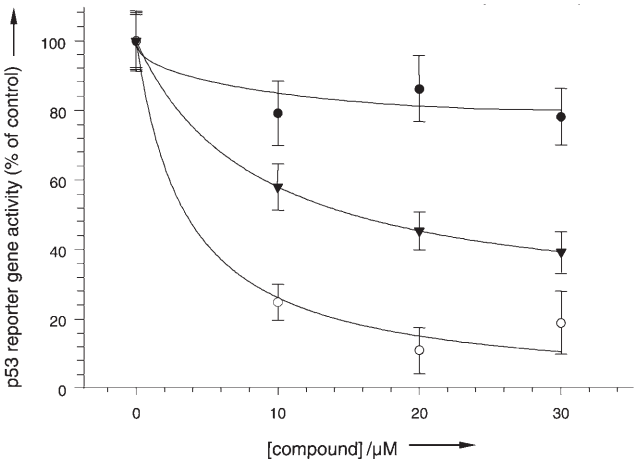


Figure 3. Influence of (*R*)-**9** (○), (*S*)-**9** (●), and *rac*-**9** (▼) on p53 reporter gene activity in etoposid-treated MCF-7 cells. Data are mean values of three independent measurements (± standard deviation).

In a second experiment, we investigated the effect of (*S*)-**9**, (*R*)-**9** and *rac*-**9** on the oncogenic transcriptional activator β-catenin. Several studies could demonstrate that Pin1 regulates the β-catenin turnover in tumor cells. Moreover, the β-catenin level directly correlates with the PPIase activity of Pin1, as shown by using a set of point-mutated Pin1 variants.[20] Cells of the tumor cell line SH-SY5Y were incubated with the compounds and the intracellular β-catenin level was determined by Western blotting with a specific anti-β-catenin antibody and subsequent densitometric analysis (Figure 4). The application of (*R*)-**9** and *rac*-**9** leads to a decreased β-catenin content in the cells, whereas (*S*)-**9** has only a minor effect.

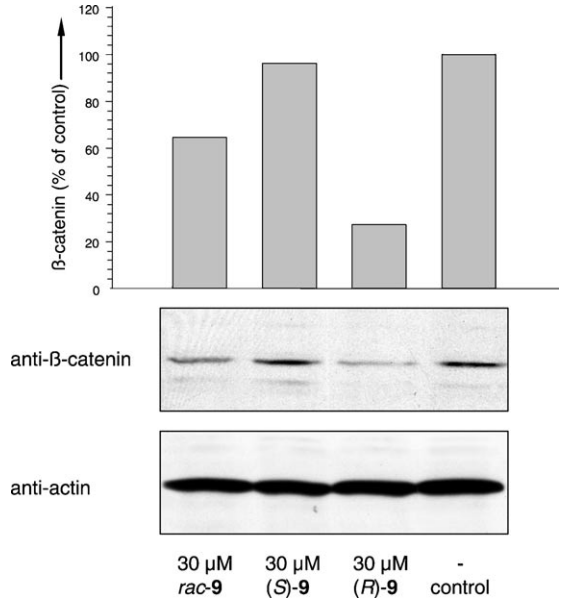


Figure 4. (*S*)-**9**, (*R*)-**9**, and *rac*-**9** decrease the intracellular β-catenin level in SH-SY5Y cells by Pin1 inhibition.

In summary, a new class of efficient, cell-penetrating, reversible inhibitors of human Pin1 has been developed that are based on the structural motif of aryl 1-indanyl ketones. They not only display inhibition constants in sub-micromolar range, but have shown biological activities and seem to be promising candidates for the development of anticancer drugs.

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[15] $C_{23}H_{19}FO_3$, $M_r = 362.38$, monoclinic, space group $P2_1/n$, $a = 13.3598(10)$, $b = 7.1470(4)$, $c = 18.9363(17)$ Å, $\beta = 97.202(10)°$, $V = 1793.8(2)$ Å^3, $Z = 4$, $\rho = 1.342$ g cm^{-3}, $\mu = 0.095$ mm^{-1}, $T = 291$ K, crystal dimensions: 0.25 mm × 0.3 mm × 0.3 mm, STOE-IPDS, $Mo_{K\alpha}$ radiation ($\lambda = 0.71073$ Å), $\theta_{max} = 25.65°$, 13715 measured, 3372 unique, and 1924 observed reflections with $I > 2\sigma(I)$, Lorentz polarization correction, direct methods and ΔF synthesis, minimization of $\Sigma w(F_o^2 - F_c^2)^2$, 320 refined parameters, $(\Delta/\sigma)_{max} = 0.000$, $R_1[F_o^2 > 2\sigma(F_o^2)] = 0.029$, $wR_2 = 0.059$ (all data), $w = 1/[\sigma^2(F_o^2) + (0.0215P)^2]$ where $P = (F_o^2 + 2F_c^2)/3$, $S = 1.028$, $\Delta\rho_{max}/\Delta\rho_{min} = 0.122/-0.106$ e Å^{-3}. CCDC-604159 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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