

Ruthenium Half-Sandwich Complexes as Protein Kinase Inhibitors: An *N*-Succinimidyl Ester for Rapid Derivatizations of the Cyclopentadienyl Moiety

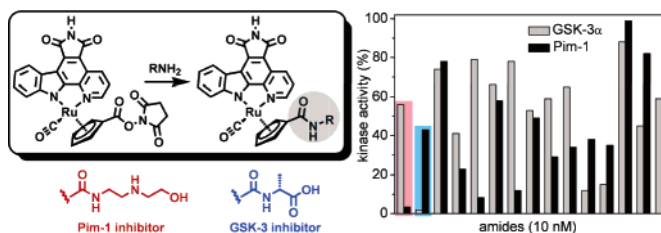
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ABSTRACT



Cyclopentadienyl half-sandwich ruthenium complexes have been demonstrated to be promising scaffolds as protein kinase inhibitors. In order to rapidly identify derivatives which display modified pharmacological properties, we developed the synthesis of an organoruthenium compound bearing an *N*-succinimidyl ester at the cyclopentadienyl moiety. The quenching of this activated ester with a library of primary amines, followed by testing of the resulting amide library, led to the identification of organometallic Pim-1 and GSK-3 inhibitors with improved potencies and kinase selectivities.

We recently introduced a new strategy for the design of enzyme inhibitors by using substitutionally inert organometallic scaffolds.^{1,2} It is our hypothesis that complementing organic elements with a metal center will provide new opportunities for building three-dimensional structures with

unique and defined shapes. This access to unexplored chemical space may lead to the discovery of molecules with unprecedented properties.³

Along these lines, we are currently designing organometallic inhibitors for protein kinases by using the class of ATP-competitive indolocarbazole alkaloids (e.g., staurosporine, Figure 1) as a lead structure.⁴ For this, we replaced the indolocarbazole alkaloid scaffold with simple metal complexes such as **1a,b** in which the main features of the

(1) For bioorganometallic and medicinal organometallic chemistry, see: (a) Severin, K.; Bergs, R.; Beck, W. *Angew. Chem., Int. Ed.* **1998**, *37*, 1634–1654. (b) Grotjahn, D. B. *Coord. Chem. Rev.* **1999**, *190*, 1125–1141. (c) Jaouen, G., Ed. *J. Organomet. Chem.* **1999**, *589*, 1–126. (d) Metzler-Nolte, N. *Angew. Chem., Int. Ed.* **2001**, *40*, 1040–1043. (e) Fish, R. H.; Jaouen, G. *Organometallics* **2003**, *22*, 2166–2177. (f) Stodt, R.; Gencaslan, S.; Müller, I. M.; Sheldrick, W. S. *Eur. J. Inorg. Chem.* **2003**, 1873–1882. (g) Schlawe, D.; Majdalani, A.; Velcicky, J.; Hessler, E.; Wieder, T.; Prokop, A.; Schmalz, H.-G. *Angew. Chem., Int. Ed.* **2004**, *43*, 1731–1734. (h) Van Staveren, D. R.; Metzler-Nolte, N. *Chem. Rev.* **2004**, *104*, 5931–5985. (i) Ott, I.; Kircher, B.; Gust, R. *J. Inorg. Biochem.* **2004**, *485*–489. (j) Jaouen, G., Ed. *Bioorganometallics*; Wiley-VCH: Weinheim, 2005. (k) Yan, Y. K.; Melchart, M.; Habtemariam, A.; Sadler, P. J. *Chem. Commun.* **2005**, 4764–4776. (l) Dyson, P. J.; Sava, G. *Dalton Trans.* **2006**, 1929–1933. (m) Schatzschneider, U.; Metzler-Nolte, N. *Angew. Chem., Int. Ed.* **2006**, *45*, 1504–1507.

(2) (a) Bregman, H.; Williams, D. S.; Atilla, G. E.; Carroll, P. J.; Meggers, E. *J. Am. Chem. Soc.* **2004**, *126*, 13594–13595. (b) Williams, D. S.; Atilla, G. E.; Bregman, H.; Arzoumanian, A.; Klein, P. S.; Meggers, E. *Angew. Chem., Int. Ed.* **2005**, *44*, 1984–1987. (c) Bregman, H.; Williams, D. S.; Meggers, E. *Synthesis* **2005**, 1521–1527. (d) Bregman, H.; Carroll, P. J.; Meggers, E. *J. Am. Chem. Soc.* **2006**, *128*, 877–884. (e) Debreczeni, J. E.; Bullock, A. N.; Atilla, G. E.; Williams, D. S.; Bregman, H.; Knapp, S.; Meggers, E. *Angew. Chem., Int. Ed.* **2006**, *45*, 1580–1585.

(3) For chemical space, see: Dobson, C. M. *Nature* **2004**, *432*, 824–828.

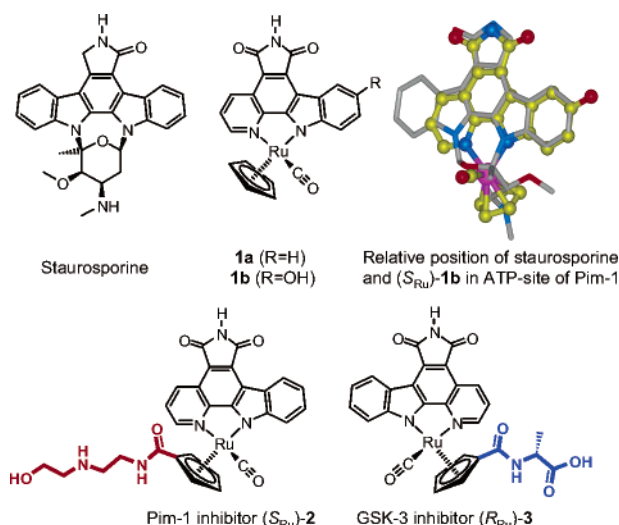
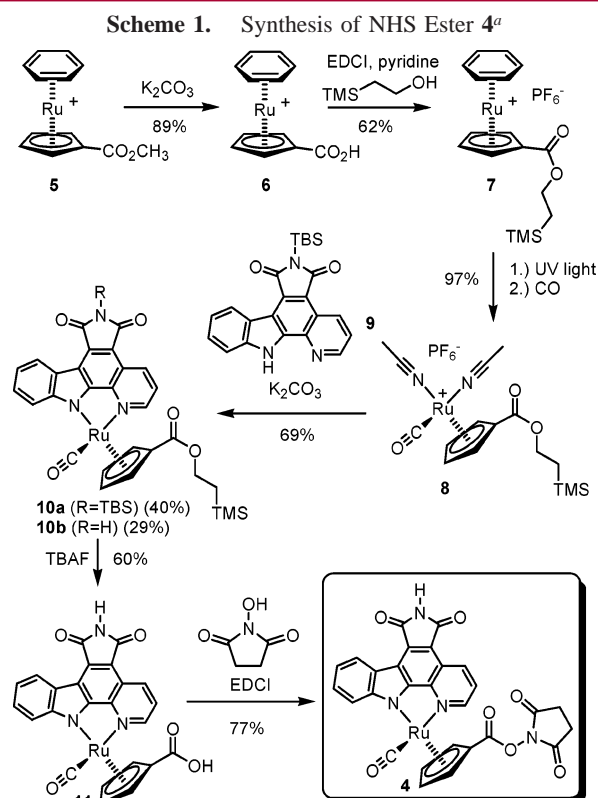


Figure 1. Comparison of molecular structures and kinase binding of staurosporine and the ruthenium half-sandwich scaffold **1**. Organometallic **1a** can be converted into the superior inhibitors (*S*_{Ru})-**2** and (*R*_{Ru})-**3** for Pim-1 and GSK-3, respectively.

indolocarbazole aglycon are retained in the metal-chelating pyridocarbazole ligand, whereas the carbohydrate is replaced by a ruthenium fragment. The crystal structures of (*R*_{Ru})-**1a** and (*S*_{Ru})-**1b** bound to Pim-1 verify the structural and functional mimicry of these organometallic scaffolds (see also Figure 1).^{2e,5} However, at the same time, these new scaffolds have properties which are clearly distinct from their parent indolocarbazole alkaloids. For example, whereas staurosporine is a nanomolar inhibitor for most protein kinases, the racemic mixtures of ruthenium half-sandwich compounds **1a** and **1b** show a remarkable preference for just a few kinases, especially GSK-3 and Pim-1.^{2a,b,e}

To further explore chemical space by derivatization of the cyclopentadienyl moiety, we here disclose the synthesis of compound **4** (framed in Scheme 1),⁶ having an *N*-hydroxy-succinimide (NHS) ester functionality at the cyclopentadienyl ring. This activated ester enables us to rapidly synthesize amide libraries, simply by the reaction with amines in the last step, therefore preventing the tedious and time-consuming individual total synthesis of every new compound.⁷ Based



^a Only one enantiomer is shown for the racemic ruthenium compounds **10**, **11**, and **4**.

on this strategy, we discovered (*S*_{Ru})-**2** and (*R*_{Ru})-**3** which show improved potencies and selectivities for Pim-1 and GSK-3, respectively.

For the synthesis of **4**, the methyl ester in sandwich compound **5**⁸ was hydrolyzed with K₂CO₃ to the carboxylic acid **6** (89%) and subsequently protected as the 2-(trimethylsilyl)ethyl ester **7** by EDCI-coupling with 2-trimethylsilylethanol (62%) (Scheme 1).⁹ This was followed by the photochemical replacement of benzene by three acetonitrile ligands and a subsequent substitution of one acetonitrile ligand by CO to provide **8** (97% over two steps).¹⁰ The reaction of **8** with TBS-protected pyridocarbazole **9**^{2c} in the presence of K₂CO₃ yields half sandwich complex **10a** (R = TBS) in 49% yield and **10b** (R = H) in 20% yield, both of which can be carried on to the next step. For example, TBAF treatment of **10a** leads to the deprotection of the imide and carboxylic acid, providing **11** in 60% yield. In the final step, **11** was converted into the NHS ester **4** by reaction with *N*-hydroxysuccinimide in the presence of EDCI (77%). Compound **4** can be purified using standard silica gel chromatography and stored in the freezer until used.

(4) For co-crystal structures of staurosporine with protein kinases, see, for example: (a) Toledo, L. M.; Lydon, N. B. *Structure* **1997**, *5*, 1551–1556. (b) Lawrie, A. M.; Noble, M. E. M.; Tunnah, P.; Brown, N. R.; Johnson, L. N.; Endicott, J. A. *Nature Struct. Biol.* **1997**, *4*, 796–801. (c) Prade, L.; Engh, R. A.; Girod, A.; Kinzel, V.; Huber, R.; Bossemeyer, D. *Structure* **1997**, *5*, 1627–1637.

(5) The absolute configuration at the ruthenium has been assigned according to the priority order of the ligands being η⁵-C₅H₅ > pyridine [N(C, C, C)] > indole [N(C, C, lone pair)] > CO.

(6) NHS esters of organometallic compounds have been used for labeling biomolecules. See, for example: (a) Salmain, M.; Vessieres, A.; Butler, I. S.; Jaouen, G. *Bioconjugate Chem.* **1991**, *2*, 13–15. (b) Gorfli, A.; Salmain, M.; Jaouen, G.; McGlinchey, M. J.; Bennouna, A.; Mousser, A. *Organometallics* **1996**, *15*, 142–151. (c) Osella, D.; Ravera, M.; Vincenti, M.; Salmain, M.; Jaouen, G. *Organometallics* **1996**, *15*, 3037–3041. (d) Rudolf, B.; Zakrzewski, J.; Salmain, M.; Jaouen, G. *Tetrahedron Lett.* **1998**, *39*, 4281–4282. (e) Salmain, M.; Fischer-Durand, N.; Cavalier, L.; Rudolf, B.; Zakrzewski, J.; Jaouen, G. *Bioconjugate Chem.* **2002**, *13*, 693–698.

(7) For a related strategy, see, for example: Liang, P.-H.; Cheng, W.-C.; Lee, Y.-L.; Yu, H.-P.; Wu, Y.-T.; Lin, Y.-L.; Wong, C.-H. *ChemBioChem* **2006**, *7*, 165–173.

(8) For the synthesis of **5**, see: Atilla-Gokcumen, G. E.; Williams, D. S.; Bregman, H.; Pagano, N.; Meggers E. *ChemBioChem* **2006**, *7*, 1443–1450.

(9) (a) Sieber, P. *Helv. Chim. Acta* **1977**, *60*, 2711–2716. (b) Gerlach, H. *Helv. Chim. Acta* **1977**, *60*, 3039–3044.

(10) Gill, T. P.; Mann, K. R. *Organometallics* **1982**, *1*, 485–488.

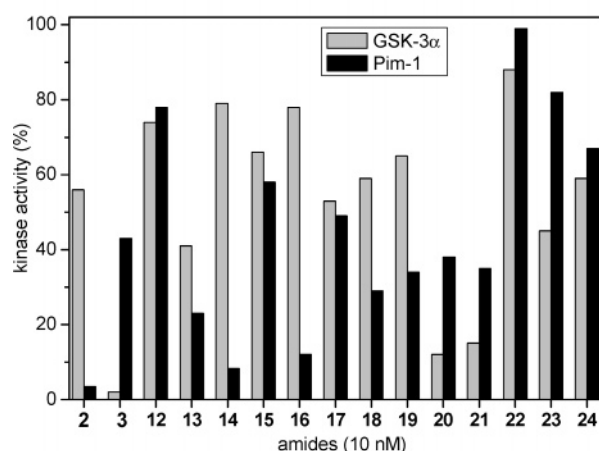
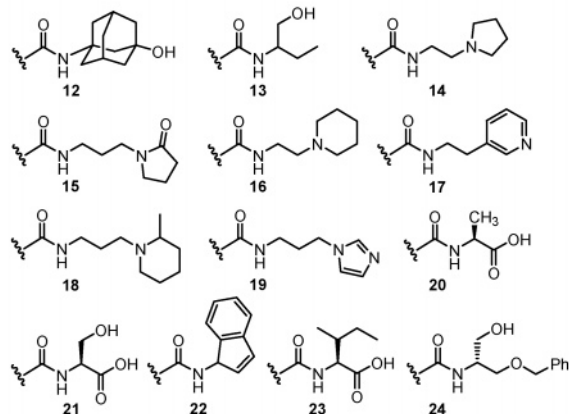
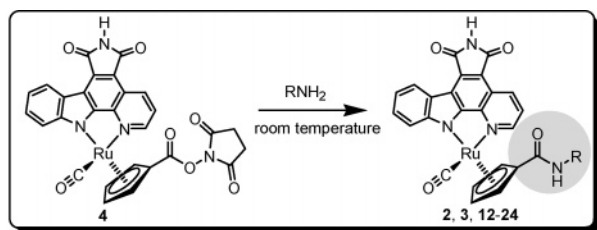


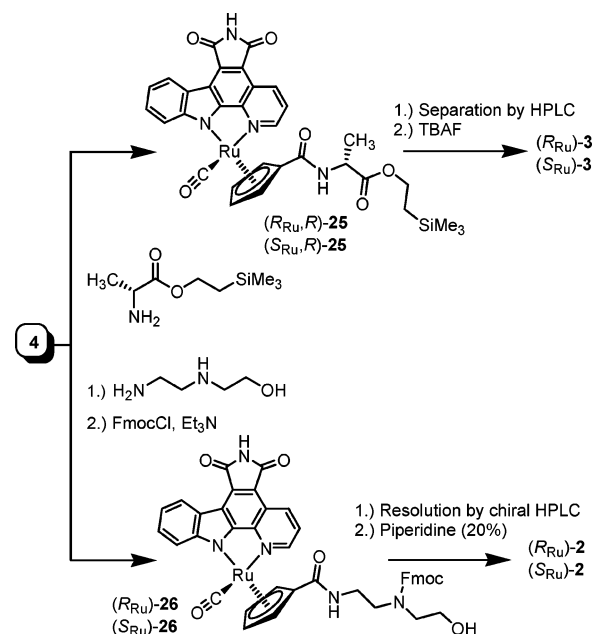
Figure 2. Synthesis of an amide library by reacting the activated ester **4** with a selection of primary amines. The crude amides were screened for their ability to inhibit Pim-1 and GSK-3 α at concentrations of around 10 nM (100 μ M ATP).

Initial test reactions demonstrated that the activated ester **4** reacts with primary amines reliably and in high yields in a few minutes under air and at room temperature. In contrast, secondary amines need more vigorous reaction conditions or extended reaction times. We therefore next synthesized a small library of amides from a diverse collection of primary amines. This was accomplished by mixing **4** with an excess of the individual amines (5 equiv) in DMF or DMF/water mixtures, depending on the solubility of the amine, at room temperature in small tubes or microplates. With this protocol, using **4** at concentrations of 8 mM and having a final volume of 40 μ L, 50 amides can be synthesized economically with just 10 mg of the NHS ester **4**. The reactions were subsequently diluted into DMSO/water 1:1 and used for kinase assays without any workup.

Following this method, we created a 68 member library and screened it against Pim-1 and GSK-3 α at a concentration of approximately 10 nM in the presence of 100 μ M ATP. The results of 15 representative members (**2**, **3**, **12–24**) are displayed in Figure 2, and they reveal that the substitutions at the cyclopentadienyl moiety have a strong influence on the binding properties. Within this small library, organometallic **2** (see Figure 1 for the S_{Ru} -enantiomer), obtained by the reaction of **4** with *N*-(2-hydroxyethyl)ethylenediamine, is the strongest inhibitor for Pim-1 but not for GSK-3 α in this assay. In contrast, organometallic **3** (see Figure 1 for the R_{Ru} -diastereomer), obtained by the reaction of **4** with D-alanine, is much more potent against GSK-3 α than Pim-1.

Since the ruthenium coordination sphere is pseudo-tetrahedral (the cyclopentadienyl moiety occupies three coordination sites of an octahedral ligand sphere),⁵ ruthenium compound **2** is racemic and **3** is a mixture of diastereomers due to the chirality of the D-alanine moiety.¹¹ Obtaining enantiomerically pure (R_{Ru})-**2** and (S_{Ru})-**2** was achieved by derivatization of the secondary amine in racemic **2** with an Fmoc group to **26** (Scheme 2), followed by a resolution of

Scheme 2. Synthesis of the Isomerically Pure Organometallics (R_{Ru})-**2**, (S_{Ru})-**2**, (R_{Ru})-**3**, and (S_{Ru})-**3**



the racemate on a chiral HPLC column (Daicel ChiralPak 1B column with ethanol/hexanes 20:1), and a subsequent Fmoc deprotection of the individual enantiomers with piperidine. In a similar fashion, the individual diastereomers of ruthenium complex **3** were obtained by first reacting NHS ester **4** with the (trimethylsilyl)ethyl ester of D-alanine to form **25** (Scheme 2). The diastereomeric mixture was then separated with a silica gel HPLC column (ethyl acetate:

(11) For optically active organometallic compounds, see: Brunner, H. *Angew. Chem., Int. Ed.* **1999**, *38*, 1194–1208.

hexanes ca. 1:3), followed by a TBAF mediated deprotection of the individual diastereomers which provided the pure diastereomers (R_{Ru})-**3** and (S_{Ru})-**3**. The absolute configurations of all isomers were determined by correlation with the circular dichroism (CD) spectra of the enantiomers of **1a** and **1b**.^{2e}

With these isomerically pure compounds in hand, we next measured the concentrations required for 50% inhibition (IC_{50}) of Pim-1 and GSK-3 α (Table 1). Ruthenium com-

Table 1. Concentrations (nM) Required for 50% Inhibition (IC_{50}) of GSK-3 α and Pim-1 by the Organometallics **1a**, **2**, and **3**^a

	(R_{Ru})- 1a	(S_{Ru})- 1a	(R_{Ru})- 2	(S_{Ru})- 2	(R_{Ru})- 3	(S_{Ru})- 3
Pim-1	20	2.5	3	0.5	200	4
GSK-3 α	15	25	20	15	0.8	0.2

^a Determined by the phosphorylation of phosphoglycogen synthase peptide 2 (GSK-3 α) or S6 kinase/Rsk-2 substrate peptide 2 (Pim-1) with [γ -³²P]ATP in the presence of varying concentrations of inhibitors and 100 μ M ATP. Every datapoint was determined from at least two independent measurements, and the error bar is \pm 25%.

pound (S_{Ru})-**2**, but not (R_{Ru})-**2**, displays a subnanomolar IC_{50} of 0.5 nM for Pim-1, which is an improvement by a factor of 5 for the *S*-isomer. More impressively, (R_{Ru})-**3**, having not only a significantly improved IC_{50} of 0.8 nM for GSK-3 α , shows a dramatically improved selectivity versus Pim-1 compared to the plain cyclopentadienyl half-sandwich complex (R_{Ru})-**1a**. Based on sequence alignment data (Protein Kinase Resource)¹² and cocrystal structural data^{2e,13} of three ruthenium half-sandwich compounds with Pim-1, we suggest that the cationic protonated secondary amine in the Pim-1 inhibitor (S_{Ru})-**2** interacts with the anionic carboxylate group of aspartate 131 (Asp131) located in the substrate binding site of Pim-1.¹⁴ In contrast, GSK-3 α has a positively charged arginine at the equivalent position (Arg141), which may explain the affinity to the negatively charged carboxylate group of GSK-3 inhibitor **3** and at the same time the discrimination against Pim-1 due to electrostatic repulsion between two carboxylate groups.

Finally, it is noteworthy to reemphasize how substantially the ligand sphere around the ruthenium center influences

(12) Smith, C. M.; Shindyalov, I. N.; Veretnik, S.; Gribskov, M.; Taylor, S. S.; Ten Eyck, L. F.; Bourne, P. E. *Trends Biochem. Sci.* **1997**, *22*, 444–446.

(13) See also protein data bank with the code 2BZJ.

(14) For co-crystal structures of Pim-1 with a substrate peptide, see the protein data bank with the codes 2BZK and 2BIL.

kinase binding affinities. For example, the plain pyridocarbazole ligand (obtained by TBS deprotection of **9**, see Scheme 1), devoid of any ruthenium, displays an IC_{50} of 150 μ M against GSK-3 α (Figure 3). Adding the cyclop-

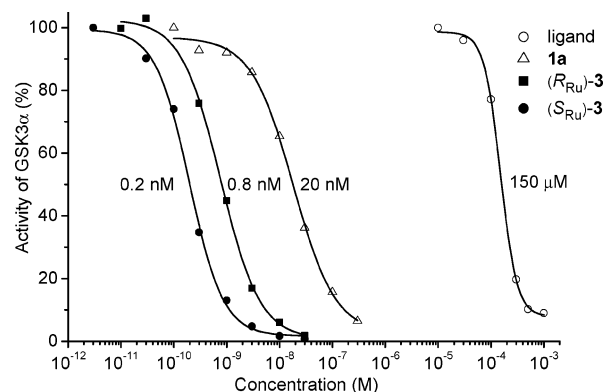


Figure 3. IC_{50} curves against GSK-3 α (100 μ M ATP).

tadienyl-CO fragment decreases the IC_{50} for racemic (*R*)-**1a** by almost 5 orders of magnitude to 20 nM. Modifying now the cyclopentadienyl moiety itself reduces the IC_{50} values further to 0.2 and 0.8 nM for (S_{Ru})-**3** and (R_{Ru})-**3** (Figure 3), respectively, thus demonstrating the power of using organometallic fragments for the design of enzyme inhibitors.

In conclusion, we have developed an efficient and economical synthetic strategy for the rapid modification of the cyclopentadienyl moiety of ruthenium half-sandwich protein kinase inhibitors. This methodology has led to the identification of Pim-1 and GSK-3 α inhibitors with improved potencies and selectivities. The generation of much larger libraries and its screening against other protein kinases is in progress.

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Supporting Information Available: Experimental procedures and analytical data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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