Recent Progress in Medicinal Organometallic Chemistry

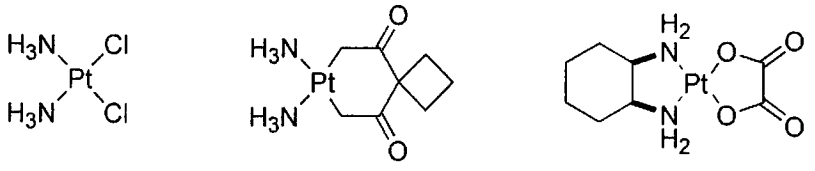
Introduction

Transition-metal-based drugs are increasing inportance in the therapy and other diseases. Probably the most prominent example is cisplatin, which is widely used in cancer chemotherapy.

In 1965, B. Rosenberg and coworkers discovered that electrolysis products from a platinum electrode inhibited binary fission in Escherichia coli (E. coli) bacteria. In the 1970s, a series of experiments were conducted to test the effects the cis-diamminedichloroplatinum(II). This study found that cis-diamminedichloroplatinum(II) was the most effective out of this group, which started the medicinal career of cisplatin.

Since the discovery of antitumor activity of cisplatin, medicinal inorganic chemistry has been developed. In this seminar I would like to show new approach in this field.

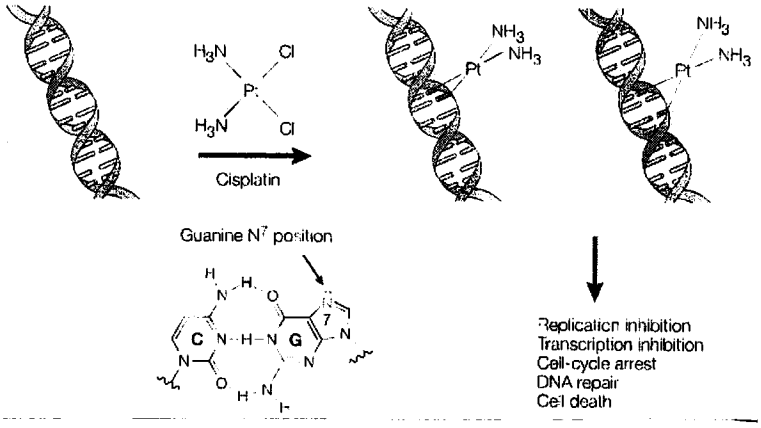
ref. Rosenberg et al. *Nature* **1965**, *205*, 698.



Cisplatin *Carboplatin* *Oxaliplatin*

Platinum-based anticancer drugs

Mechanistic Insights



The platinum atom of cisplatin binds covalently to the N7 position of purines to form 1,2- or 1,3-intrastrand crosslinks, and interstrand crosslinks. Cisplatin–DNA adducts cause various cellular responses, such as replication arrest, transcription inhibition, cell-cycle arrest, DNA repair and apoptosis.

ref. Wang, D.; Lippard, S. J. *Nature Rev. Drug. Discovery* **2005**, *4*, 307.

Metal center involves in interaction with the active site of target protein or DNA.

Recently, Meggers and co-workers developed ruthenium complex **1** that was a highly potent inhibitor for the protein kinase GSK-3 and Pim-1. Their concept is different from the way mentioned above.

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Organometallic and inorganic compounds
as ***structural scaffolds*** for enzyme inhibition

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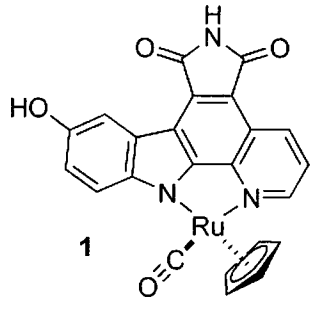
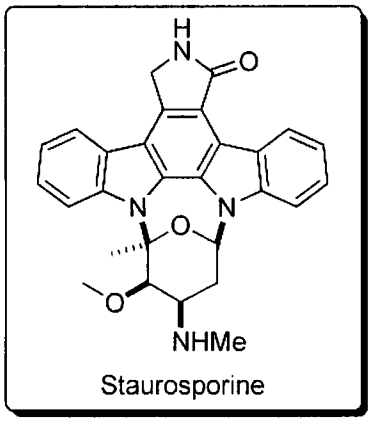
Metal-Ligand assemblies allow convergent synthetic approaches



1

A highly potent inhibitor for the protein kinase GSK-3, Pim-1

Staurosporine



Staurosporine

Isolated from *Streptomyces staurosporeus* in 1977.

ref. Ōmura et al. *J. Antibiotics* **1977**, *30*, 275.

Tamaoki et al. reported that staurosporine had the inhibitory activity against protein kinase C.

ref. Tamaoki et al. *Biochem. Biophys. Res. Commun.* **1986**, *135*, 397.

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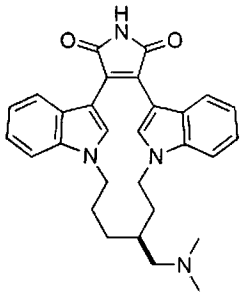
Staurosporine is a very potent but relatively nonspecific inhibitor of many protein kinases.

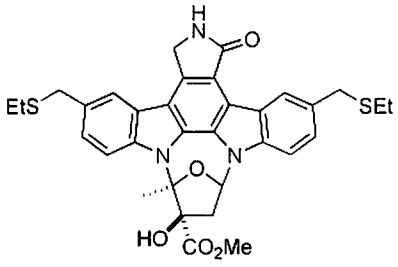
ref. Fabbro et al. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 399.

Hundreds of derivatives based on this structure have been produced.

Representative examples



LY333531
(Eli Lilly)
PKCβ selective inhibitor
Diabetic retinopathy
(糖尿病性網膜症)
Phase III



CEP-1347
(Cephalon)
MLK inhibitor
Neurodegeneration
(神経変性)
Phase II/III
Discontinuation in Parkinson's disease

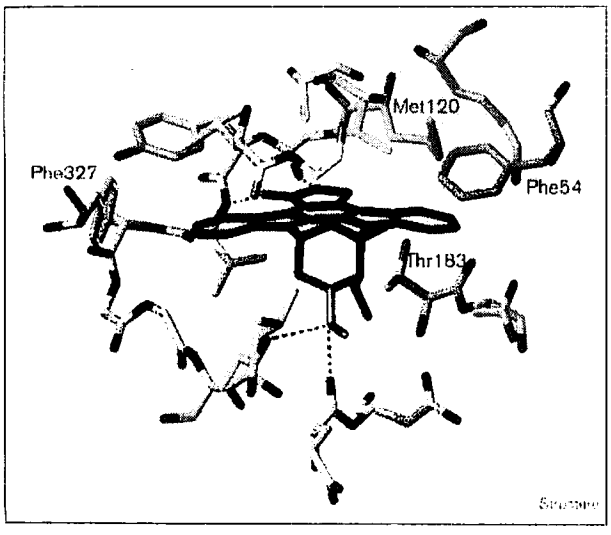
MLK: mixed lineage kinases

Structure of staurosporine bound to cAPK

cAPK: cAMP-dependent protein kinase

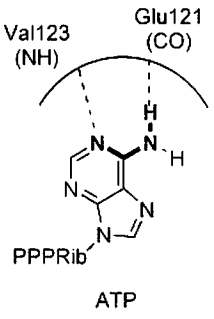
Lydon et al. *Structure* **1997**, *5*, 1551.

Figure 1

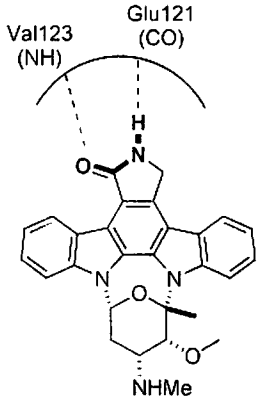


Binding of staurosporine to cAPK. Residues within a 4 Å radius of the staurosporine nucleus are displayed. The labeled residues (cyan) are those which display the most pronounced sidechain conformational changes and are discussed in the text. (For more details, please see figures in [14].)

Staurosporine occupies a site which overlaps with that of the adenosine moiety of ATP



ATP



Staurosporine

The carbohydrate moiety forms hydrophobic contacts and hydrogen bonds within the globular ribose binding site.

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This hydrophobic surface of the staurosporine nucleus is complemented by the large hydrophobic surface of the ATP-binding cleft

Ruthenium Complexes as Protein Kinase Inhibitors

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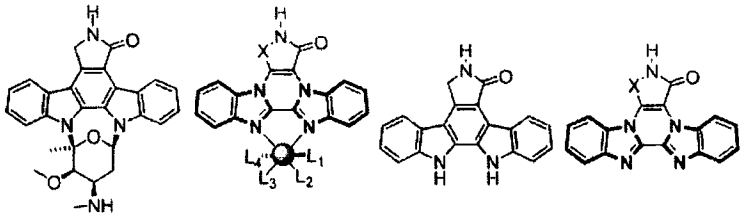
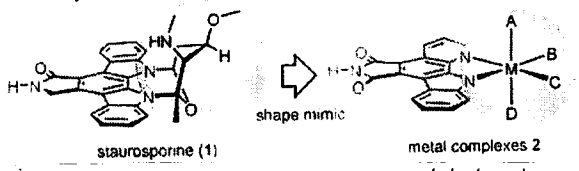


Concept



globular shape

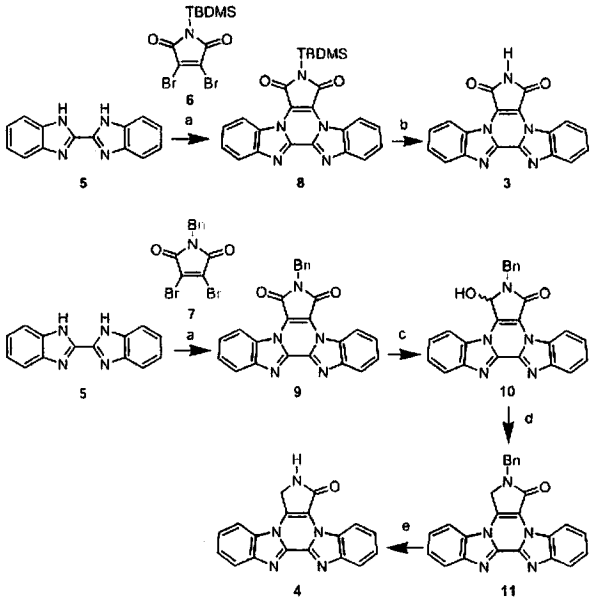
Modification is easy.
(by chaninging ligands)

Center-metal: Ru

1. Ru offers a hexavalent coordination sphere
2. Ru tends to form kinetically very inert coordinative bonds. (stable complex)

More extended structural options, but with less synthetic efforts

Scheme 1. Synthesis of Ligands **3** and **4**[a]



[a] Reagents and conditions: (a) Deprotonation of **5** with 2.1 equiv of NaH in DMF, followed by addition **6** or **7** (**8**, 33%; **9**, 35%). (b) TBAF, CH_2Cl_2 (71%). (c) $NaBH_4$, EtOH (90%). (d) First reflux in Ac_2O, then addition of Zn and reflux (89%). (e) TFA, H_2SO_4, anisole, reflux (76%).

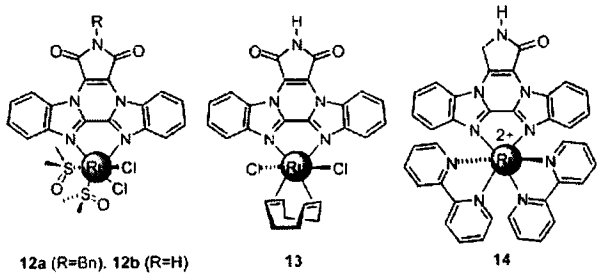


Figure 2. Synthesized ruthenium complexes **12**–**14**. See Supporting Information for experimental details of the synthesis.

8 + *cis*-$RuCl_2(DMSO)_4$ —TBAF→ **12b**
8 + $Ru(COD)(CH_3CN)_2Cl_2$ —TBAF→ **13**
4 + $Ru(bpy)_2(EtOH)_2^{2+}$ —reflux→ **14**

 # 12a, 12b, 13, 14

Air-stable (They can be stored on the bench for weeks without decomposition.)

 # 14

S-stable (**14** can withstand a 1mM methanolic solution of 2-mercaptoethanol for 3 h without decomposition)

Kinase Inhibition Assay

Table 1. Inhibition of Some Protein Kinases by Ligands **3** and **4** and the Ruthenium Complexes **12b**, **13**, and **14**[a]

compound	Abl	RSK1	Src	PKCα	ZAP70
staurosporine	2	<1	<1	<1	<1
3	25	30	>100	>100	>100
4	20	25	60	>100	50
12b	10	8	30	>100	40
13	2	8	40	>100	30
14	5	8	30	50	40

[a] Concentrations required for 50% inhibition (IC_{50}) in μM. Determined by phosphorylation of peptide or protein substrates with [γ-^{32}P]ATP in the presence of varying concentrations of inhibitors.

3, **4**: ligand

By introducing the ruthemnium complex, affinity and specificity become modulated.

14: the only compound that inhibits PKCα

13: the best inhibitor for Abl

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An Organometallic Inhibitor for Glycogen Synthase Kinase 3

(4/10)

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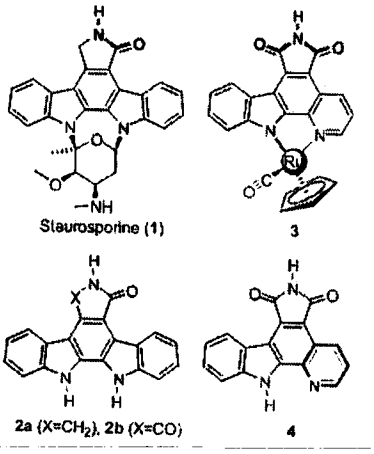
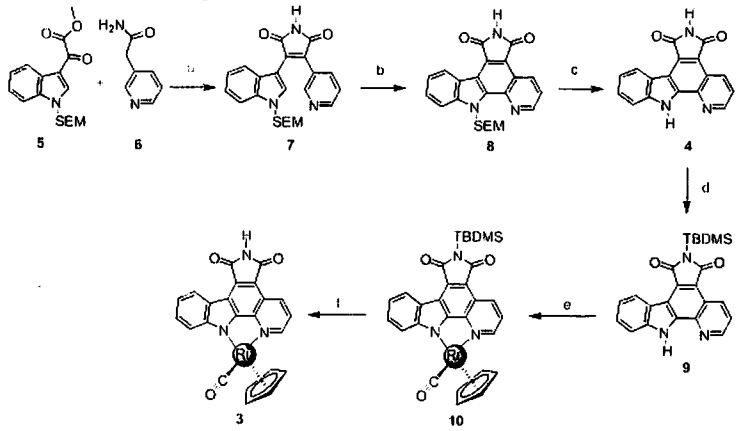
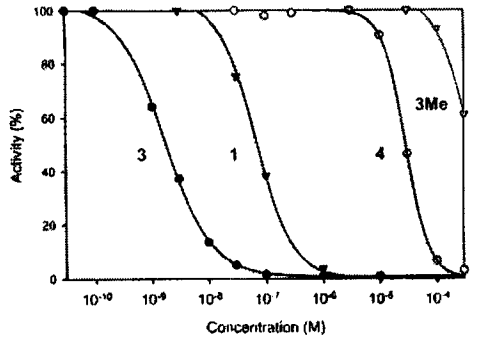




Scheme 1. Synthesis of pyridocarbazole 4 and ruthenium complex 3. (a) 3 equivalents of KO*t*Bu, DMF, 4 Å mol. sieves (50%). (b) Photolysis in MeCN with a medium pressure mercury lamp in presence of air and catalytic amounts of I_2 (63%). (c) $LiBF_4$, MeCN/H_2O, reflux (100%). (d) Reflux in MeCN with *tert*-butyldimethylsilyloxy-methoxyethene, (92.5%). (e) $[CpRu(CO)(MeCN)_2]^+PF_6^-$, 1 equiv of K_2CO_3, overnight in MeCN at 55°C (87%). (f) TBAF, CH_2Cl_2 (96%). SEM = $CH_2OCH_2CH_2Si(CH_3)_3$, TBDMS = *tert*-butyldimethylsilyl.

The ruthenium complex **3** is stable under air and in water and can even withstand the presence of millimolar concentrations of thiols as determined by ^1H NMR spectroscopy.

Activity



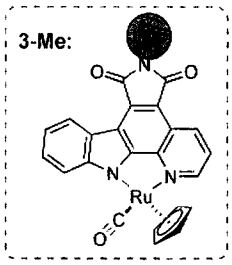


Figure 3. IC_{50} curves with GSK-3α obtained by phosphorylation of a substrate with [γ-^{32}P]ATP: red, racemic complex **3** (IC_{50} = 3 nM); blue, staurosporine **1** (IC_{50} = 50 nM); green, pyridocarbazole **4** (IC_{50} = 50 μM); pink, 3Me, the N-methylated derivative of **3** (IC_{50} > 300 μM).

Ruthenium complex **3** is more potent than staurosporine **1**.

Interaction with GSK-3β

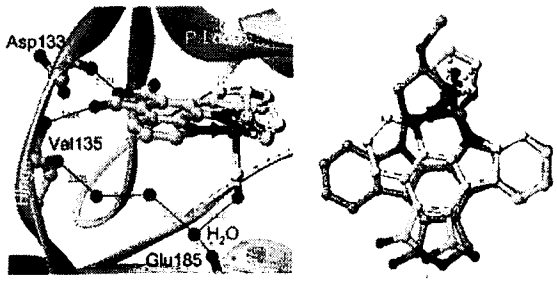


Figure 5. Molecular modeling (CAChe, Fujitsu). Left: Interactions of 3-R_{Ru} with the ATP binding site of GSK-3β (PDB code 1Q3D). Right: Overlay of the cocrystallized position of staurosporine in GSK-3β with the docked position of 3-R_{Ru}.

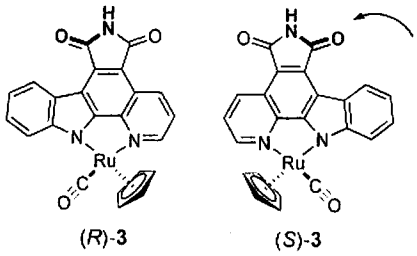
Hydrogen bonding

imide N-H · · · · · Asp133 C=O
imide C=O · · · · · Val135 N-H
CO ligand · · · H_2O · · · Gln185 COOH

This ordered H_2O is unique for GSK-3

Chirality

3 possesses metal-centered chirality. The activities of the individual enantiomers differ only 2-fold (IC_{50} = 2 and 4 nM).



Symmetrical imide group might allow for the same orientation of the CO and Cp ligands.

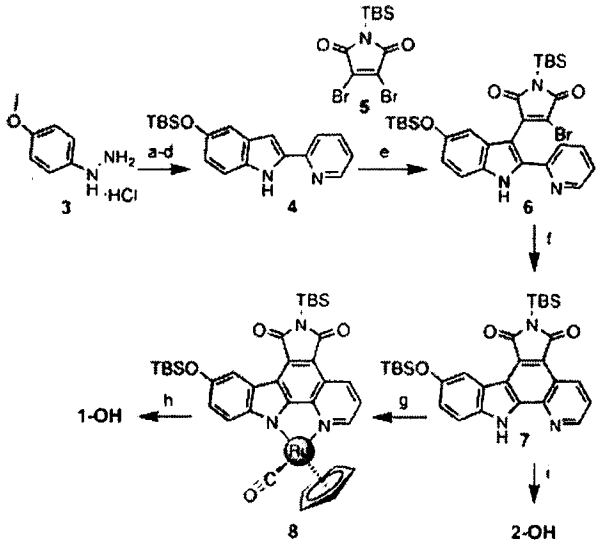
Angew. Chem. Int. Ed. 2005, 44, 1984–1987

Enzyme Inhibitors

Switching on a Signaling Pathway with an Organoruthenium Complex**

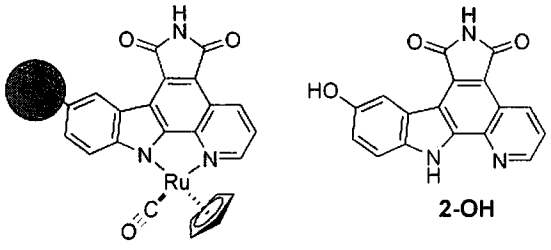
*Douglas S. Williams, G. Ekin Atilla, Howard Bregman, Arpine Arzoumanian, Peter S. Klein, and Eric Meggers**

(5/10)



Scheme 2. Synthesis of ruthenium complex **1-OH** and pyridocarbazole **2-OH**. a) 2-Acetylpyridine, *t*BuOH, 4 h reflux (100%). b) Trimethylsilyl polyphosphate, 115 °C, overnight (63%). c) BBr_3, CH_2Cl_2, −60 °C, then RT overnight (87%). d) DIEA, DMF, 0 °C, 40 min, then TBS triflate, 0 °C, 1 h (71%). e) Li hexamethyldisilazide, THF, −15 °C, 45 min, then **5** in THF, −15 °C, 15 min, then RT, 45 min (58%). f) *hν*, Pyrex filter, MeCN, 3 h, (78%). g) $[Ru(Cp)(CH_3CN)_2(CO)]PF_6$, K_2CO_3, MeCN, 55 °C, overnight (86%). h) TBAF, CH_2Cl_2, RT, 30 min (87%). i) TBAF, CH_2Cl_2, RT, 30 min (80%). DIEA = *N*,*N*-diisopropylethylamine, TBAF = tetrabutylammonium fluoride, TBS = *tert*-butyldimethylsilyl.

To increase the affinity for GSK-3 & To increase water solubility



1-OH

1-OH has favorable solubility in water.
(it can be dissolved in 3% DMSO/water at concentration of 1mM)

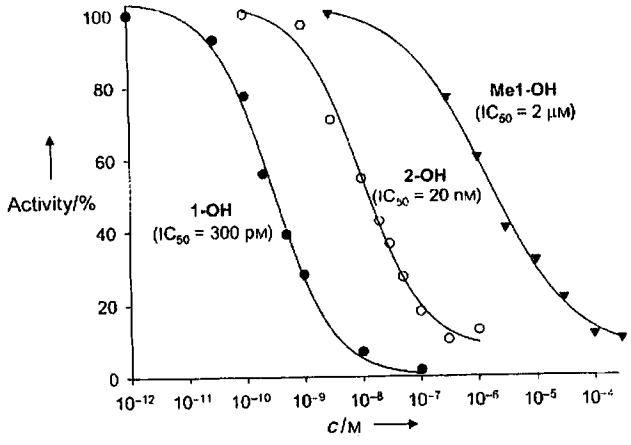


Figure 1. IC_{50} curves with GSK-3α obtained by phosphorylation of phosphoglycogen synthase peptide-2 with [γ-32P]ATP: ruthenium complex **1-OH** (●), ligand **2-OH** (○), and methylated ruthenium complex **Me1-OH** (▼).

⇒ Staurosporine: IC_{50} = 40 nM (GSK-3α)

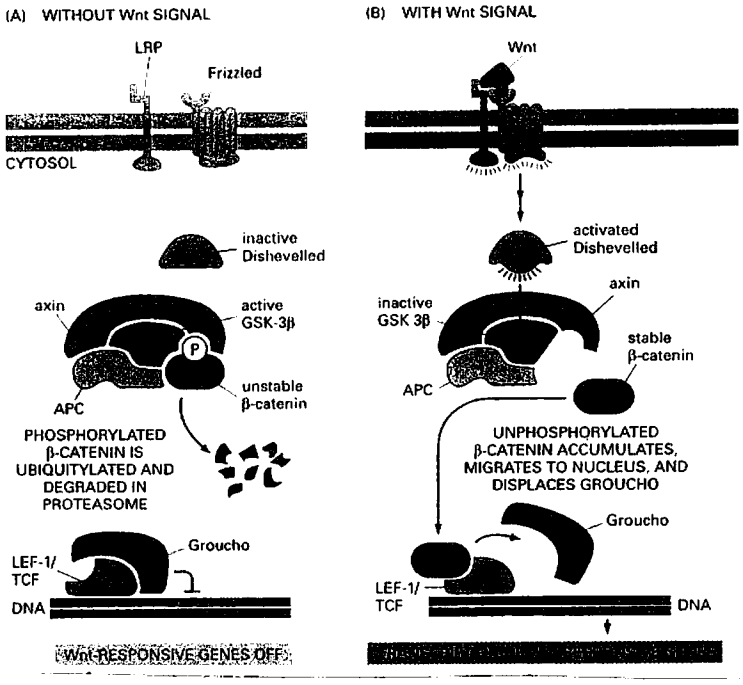
Inhibition of other protein kineses

	Ab1	CHK-1	Lck	MAPK-1	RSK-1	Src	Zap-70
IC_{50}	1 μM	2 μM	500 nM	> 3 μM	25 nM	>10 μM	4 μM

1-OH shows high selectivity.

GSK-3

GSK-3 is a negative regulator of the wnt signal transduction pathway that phosphorylates β-catenin.



Wnt signaling *pathway*

ON *β*-catenin ⇧
(GSK-3 inactive)

OFF *β*-catenin ⇩
(GSK-3 active)

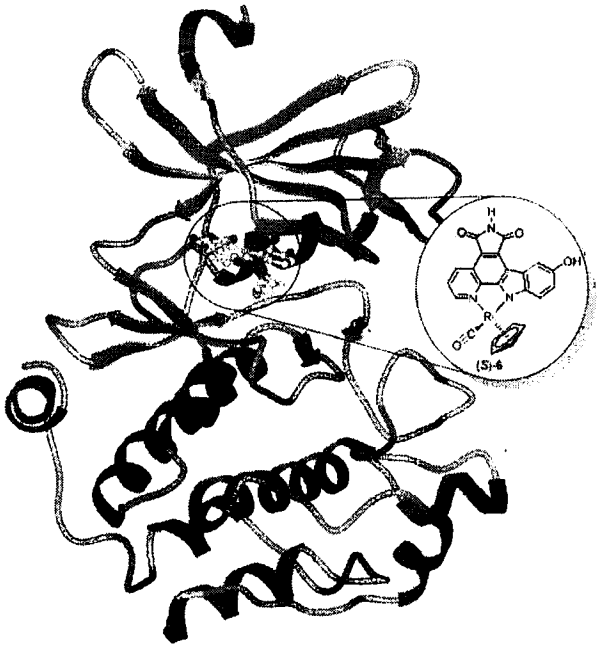


Figure 3. Crystal structure of Pim-1 with the enantiomerically pure ruthenium complex (*S*)-**6** bound to the ATP-binding site.

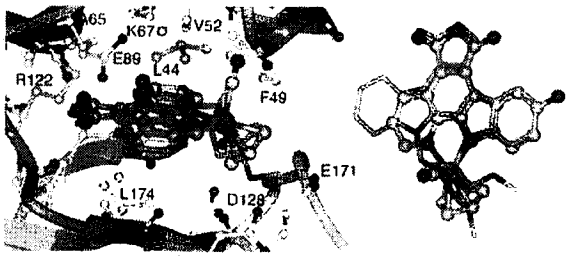


Figure 4. Superimposed cocrystal structures of ruthenium compound (*S*)-**6** and staurosporine with Pim-1.

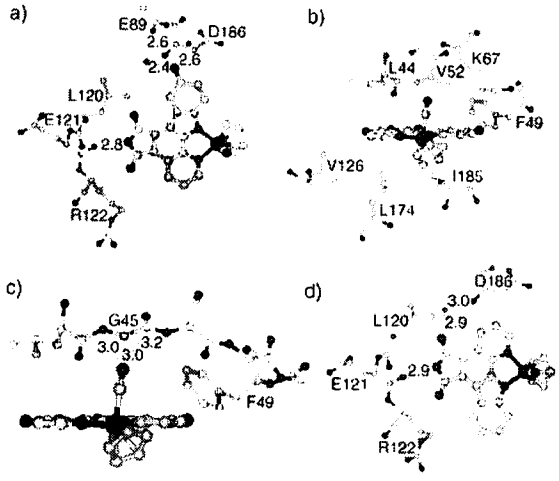


Figure 5. Interactions of (*S*)-**6** (a–c) and (*R*)-**5** (d) within the active site of Pim-1 (distance indicated in Å). a) Hydrogen bond between the maleimide NH group of (*S*)-**6** and the backbone amide carbonyl group of Glu121, and water-mediated contacts of the hydroxy group. b) The most important hydrophobic interactions with (*S*)-**6**. c) Highlighting the close contact of the CO ligand of (*S*)-**6** with Gly45. d) Hydrogen bonding of (*R*)-**5** within the active site of Pim-1.

hydrogen bonds (Figure 5a)

imide NH - - - - - - - - - Glu121 C=O

OH of (*S*)-**6** - - - - - ordered H_2O (Figure 5a)

- - - - - ordered H_2O

van der Waals contacts (Figure 5b)

N-terminal lobe (Figure 5b upper side)
Leu44, Phe49, Val52, Ala65

C-terminal lobe (Figure 5b under side)
Ile104, Val126, Leu174, Ile185

→ **hydrophobic pocket**
(for pyridocarbazole moiety)

CO ligand does not form any hydrogen bonds. (Figure 5c)

⇩

but, the distance of Gly45 is very short.
(dipolar interaction of the CO ligand with polarized methylene and CO group of Gly45 ?)

How does enantiomer bound to Pim-1? (Figure 5d)

A pyridocarbazole moiety has been flipped by 180 °C
(CO group and cyclopentadienyl ring are in very similar positions.)

Anyway...

The metal center is not involved in any direct interactions with the active site of Pim-1.

(**5** and **6** are highly rigid and cannot change their three-dimensional shapes.)

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JACS ARTICLES

Rapid Access to Unexplored Chemical Space by Ligand Scanning around a Ruthenium Center: Discovery of Potent and Selective Protein Kinase Inhibitors

(8/10)

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More extended structural options but with less synthetic effort

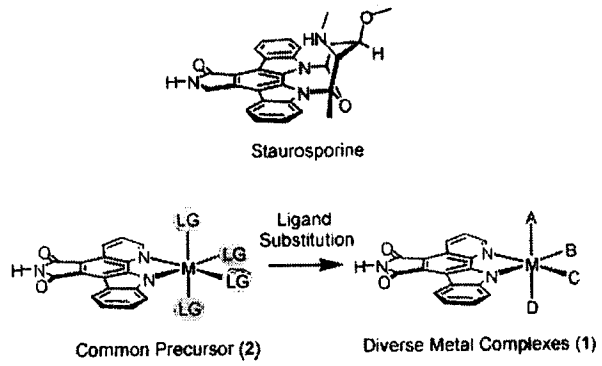


Figure 1. Mimicking the protein kinase inhibitor staurosporine with simple octahedral metal complexes. Indicated in blue are the globular domains of these compounds.

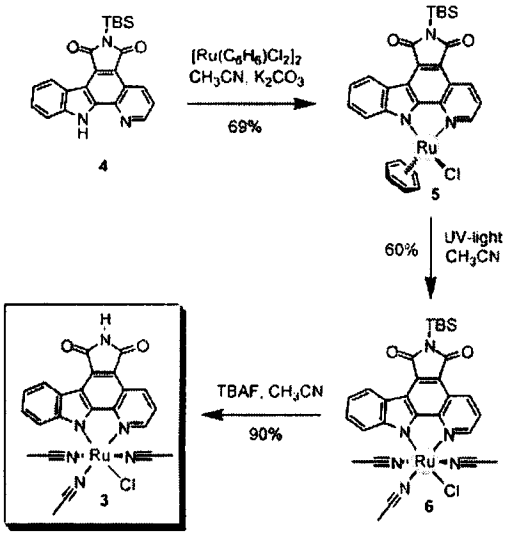
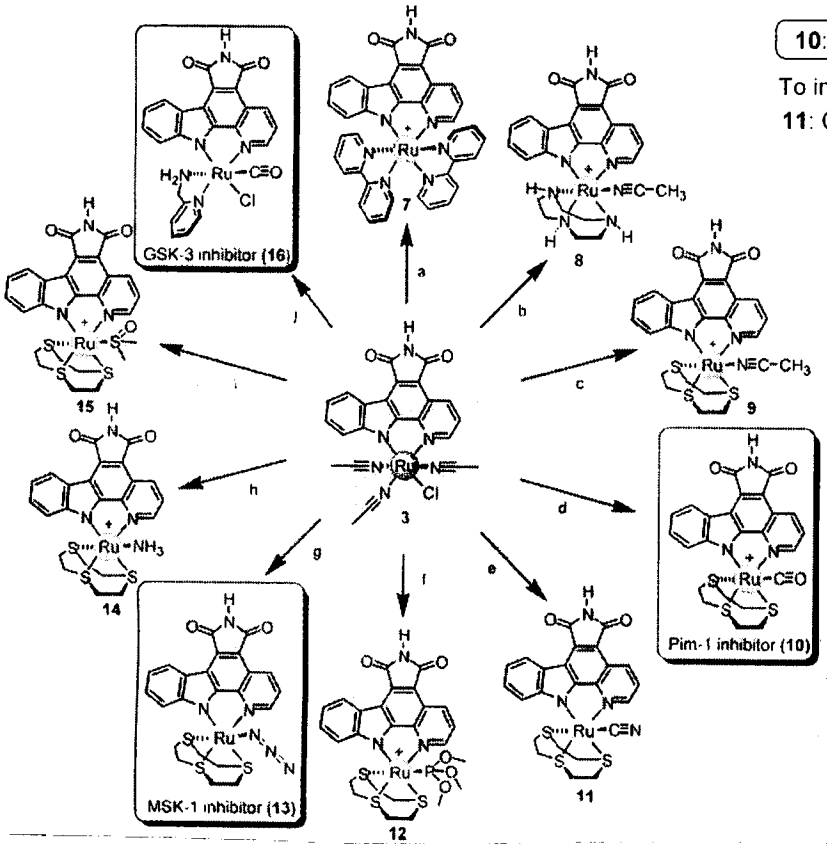


Figure 2. Synthesis of the precursor complex 3 for rapid ligand scanning.



10: Pim-1 inhibitor

To investigate CO effects...

11: CN, **12**: $P(OMe)_3$, **13**: N_3, **14**: NH_3, **15**: DMSO

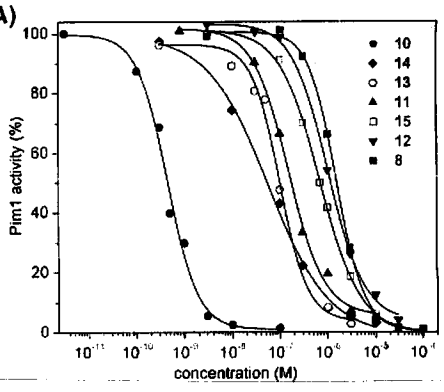


Figure 6. (A) IC_{50} curves with Pim1 obtained by phosphorylation of S6 kinase/Rsk2 Substrate Peptide 2 with [γ-^{32}P]ATP in the presence of 100 μM ATP and different concentrations of ruthenium complexes **8**, and **10**−**15**. (B) Double-reciprocal plots of relative initial velocities (V_{rel}) against varying ATP concentrations in the presence of 0 (●), 100 (○), and 200 pM (▼) of **10**.

All compounds are less inhibitors for Pim-1.

Figure 4. Ruthenium complex syntheses from precursor **3**. All cations were isolated as their PF_6 salts: (a) 2.2 equiv of 2,2′-bipyridine, EtOH, reflux, 1 h (93%); (b) 1.2 equiv of 1,4,7-triazacyclononane, reflux in EtOH for 2 h (65%); (c) 1.0 equiv of 1,4,7-trithiacyclononane, DMF, 80 °C for 45 min (50%). (d) From **9** by heating for 2 h in CO-saturated DMF to 95 °C (79%). (e) First, 1.0 equiv of 1,4,7-trithiacyclononane, DMF, 75 °C for 1 h, then addition of 1 equiv of NaCN and another hour at 90 °C (15%). (f) From **9** by heating with 2 equiv of $P(OMe)_3$ at 75 °C for 1 h (60%). (g) First, 1 equiv of 1,4,7-trithiacyclononane, DMF, 80 °C for 1 h, then addition of 1 equiv of NaN_3 and stirring at 90 °C for another hour (46%). (h) From **9** by heating in the presence of 0.17 M ammonia in DMF:dioxane (2:1) in a closed vessel for 1 h at 85 °C (41%). (i) First, 1.0 equiv of 1,4,7-trithiacyclononane, DMF, 85 °C for 1 h, then addition of 3.6 equiv of DMSO and stirring at 110 °C for another hour (65%). (j) First reaction in CO-saturated DMF at 75 °C for 1.5 h, then addition of 1 equiv of 2-aminomethylpyridine and heating for another 1.5 h at 95 °C (32%).

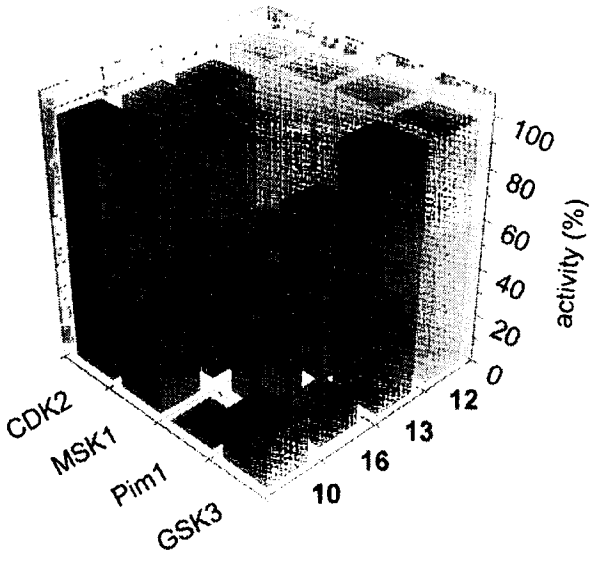


Figure 5. Activity of the organoruthenium compounds **10**, **12**, **13**, and **16** at a concentration of 100 nM against the protein kinases GSK3α, Pim1, MSK1, and CDK2/CyclinA. ATP concentration was 100 μM.

(9/10)

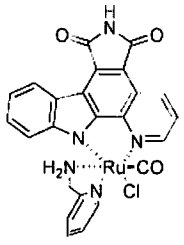
10 is most potent against Pim-1, but also inhibits GSK-3α.

⇩

CO ligand is important pharmacophore both Pim-1 and GSK-3α?

⇩ further screening

16 shows selectivity for GSK-3α.



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Appendix 1

Representative reviews for medicinal organometallic chemistry

Guo, Z.; Sadler, P. J. *Angew. Chem. Int. Ed.* **1999**, *38*, 1512.
Special issue on *Medicinal Inoranic Chemistry* (Eds.: Orvig, C.; Abrams, M. J.): *Chem. Rev.* **1999**, *99*.
Yan, Y. K.; Melchart, M.; Habtemariam, A.; Sadler, P. J. *Chem. Commun.* **2005**, 4764.

Appendix 2

Table 7.1 Toxic Effects of Some Nonbiological Metals

Metal (Class)	Effect of Excess	Comments
Aluminum (hard, Al^{3+})	Implicated in Alzheimers disease	May interact with phosphates, may cross-link proteins.
Cadmium (soft, Cd^{2+})	Renal toxicity	Blocks sulfhydryl groups in enzymes and competes with zinc. Stimulates metallothionein synthesis and interferes with Cu(II) and Zn(II) metabolism.
Mercury (soft, Hg_2^{2+}, Hg^{2+})	Damage to central nervous system, neuropsychiatric disorders	CH_3Hg^+ compounds are lipid-soluble.
Lead (soft, Pb^{2+})	Injuries to peripheral nervous system, disturbs heme synthesis and affects kidneys	Pb^{2+} may replace Ca^{2+} with loss of functional and structural integrity. Reacts with sulfhydryl groups, replaces Zn^{2+} in δ-aminolevulinic acid dehydratase.
Thallium (soft, Tl^+)	Poisonous to nervous systems, enters cells via K^+ channels	Although similar to K^+, Tl^+ binds more tightly to N and S ligands.

Table 7.2 Some Examples of Inorganic Elements and Compounds with Medicinal Purposes

Element	Example of a Product Name	Active Compound in the Product	Medicinal Usage
Li	Camcolit	Li_2CO_3	Manic depression
N	Laughing gas	N_2O (nitrous oxide)	Anesthetic
F		SnF_2	Tooth protectant
Mg	Magnesia	MgO	Antacid, laxative
Fe		Fe(II) fumarate, succinate	Dietary iron supplement
Co	Cobaltamin S	Coenzyme vitamin B_{12}	Dietary vitamin supplement
Zn	Calamine	ZnO	Skin ointment
Zn		Zn undecanoate	Antifungal (athlete's foot)
Br		NaBr	Sedative
Tc	TechneScan PYP	^{99m}Tc-pyrophosphate	Bone scanning
Sb	Triostam	NaSb(V) gluconate	Antileishmanial (antiprotozoal)
I		I_2	Antiinfective, disinfectant
Ba	Baridol	$BaSO_4$	X-ray contrast medium
Gd	Magnevist™	$[Gd(III)(DTPA)(H_2O)]^{2-}$ DTPA = diethylenetriamine pentaacetic acid	MRI contrast agent
Pt	Cisplatin, platinol, cisDDP	cis-$[Pt(NH_3)_2Cl_2]$	Anticancer agent
Pt	Carboplatin	$[Pt(NH_3)_2(CBDCA)]$ CBDCA = cyclobutanedicarboxylic acid	Anticancer agent
Au	Auranofin	$Au(I)(PEt_3)$ (acetylthioglucose)	Antiarthritic
Bi	De-Nol	$K_3[Bi(III)(citrate)_2]$	Antacid, antiulcer