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

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Abstract: An important objective for the discovery of compounds with unique biological activities is the development of methods for the synthesis of molecular scaffolds with defined three-dimensional shapes. We are currently investigating the scope of using metal complexes to accomplish this goal. In these compounds, the metal center has the role of organizing the orientation of the organic ligands, thus defining the overall shape of the molecule. A strategy is presented that allows a rapid scanning of ligands around a ruthenium center in the search for ligand spheres that are complementary in shape and functional group presentation to ATP binding sites of protein kinases. Following this approach, we have identified octahedral ruthenium complexes as potent inhibitors for the protein kinases Pim1, MSK1, and GSK3α.

Introduction

Natural products display a high diversity of molecular skeletons, and many complex natural products adopt very distinctive three-dimensional conformations. Unquestionably, these defined structures are important for their unique biological properties. As a consequence, an important challenge for the future discovery of synthetic molecular probes with superior biological properties is the development of methods for the economical synthesis of molecules with defined three-dimensional shapes.

We recently started a program that aims in exploring small molecule chemical space with metal-containing compounds. In these molecules, the coordinative bonds are designed to be kinetically inert and thus stable in biological environments. Hence, such metal complexes are supposed to behave like purely organic compounds without displaying any metal-related cytotoxicities. We believe that the addition of a metal to an otherwise organic scaffold opens new opportunities for the design of bioactive molecules with novel properties.

First, it gives access to areas of the chemical space that may not be easily accessible with purely organic scaffolds. In this respect, we like to think of a chemically inert metal center as a “hypervalent carbon” with extended structural opportunities. Second, metal complexes are built from a central core and thus may have an advantage in building shape and functional group diversity in an economical fashion. We here demonstrate the power of this strategy with the discovery of novel octahedral ruthenium complexes as potent protein kinase inhibitors, all synthesized from a common precursor complex by a rapid ligand scanning protocol.

Our strategy for the design of metal complexes as protein kinase inhibitors uses the indolocarbazole alkaloid staurosporine as a lead structure (Figure 1). Staurosporine adopts a very defined globular structure with the carbohydrate moiety being oriented perpendicular to the plane of the indolocarbazole heterocycle. The indolo[2,3-α]carbazole moiety occupies the hydrophobic adenine binding cleft, with the lactam group mimicking the hydrogen bonding pattern of the adenine base by usually forming two canonical hydrogen bonds to the backbone of the hinge between the N-terminal and C-terminal domains.

The carbohydrate moiety forms hydrophobic contacts

(3) For chemical space, see: Dobson, C. M. Nature 2004, 432, 824−828.
and hydrogen bonds within the globular ribose binding site. Thus, staurosporine matches the shape of the ATP binding site perfectly, which makes it a highly potent, albeit unspecific inhibitor for protein kinases. This distinctive globular three-dimensional structure of staurosporine is in contrast to many synthetic protein kinase inhibitors which mainly fill the adenine binding site with planar scaffolds and flexible side chains protruding into other regions of the active site.\(^\text{(12)}\)

To match the shape of the ATP binding site of protein kinases in a fashion similar to staurosporine, but with less synthetic effort and more extended structural options, we replaced the indolocarbazole alkaloid scaffold with simple metal complexes in which the main features of the indolocarbazole aglycon are retained in the metal-chelating pyridocarbazole ligand (highlighted in red in Figure 1), thus targeting the metal complexes \(^1\) to the ATP binding site (Figure 1). This places the metal center within the ribose binding site and gives the opportunity to build defined globular shapes by assembling ligands around the metal center. Following this strategy, we recently reported an organoruthenium half-sandwich scaffold for the highly potent inhibition of glycogen synthase kinase 3 (GSK3).\(^\text{(3,6)}\)

To quickly scan for the most suitable ligand combination to fill up the coordination sphere around the ruthenium center, it would be desirable to have a compound which could serve as a common precursor for a large and diverse set of metal-containing compounds of type \(^1\). We here introduce such a metal complex \(^2\) (Figure 1), which has four leaving groups in addition to the pyridocarbazole aglycon and allows for rapid access to a diversity of novel structures just by simple ligand replacement chemistry. On the basis of this concept, we report the discovery of inhibitors for the protein kinases MSK1, Pim1, and GSK3α.

### Results and Discussion

As our common precursor \(^2\), we synthesized ruthenium complex \(^3\), bearing four leaving groups in addition to the pyridocarbazole ligand: three acetonitriles and one chloride (Figure 2).


individually, isolated, and characterized spectroscopically. All shown compounds are racemates due to metal-centered chirality. These examples demonstrate that we can use 3 as a precursor for synthesizing defined ligand spheres with mono-, bi-, and
trivalent ligands, having a variety of coordinating functional groups, and adopting distinct three-dimensional globular shapes.

This method opens an avenue for the rapid scanning of ligands around the ruthenium center, searching for three-dimensional structures that are complementary in shape and functional group presentation to the active site of individual protein kinases. In fact, some of the compounds from Figure 4 were initially identified by screening of in situ synthesized libraries from precursor 3 and combinations of different ligands. Figure 5 shows the activity of a set of selected compounds 10, 12, 13, and 16 at a concentration of 100 nM against a small panel of the protein kinases CDK2/CyclinA,16 MSK1,17 Pim1,18 and GSK3α,19 For example, we identified the racemic compound 10 as a subnanomolar inhibitor for the protein kinase Pim1 (black bars in Figure 5). At 100 nM of 10, the activity of Pim1 is reduced to less than 0.5%. Under the same conditions, the protein kinases GSK3α, MSK1, and CDK2/CyclinA still display activities of 19, 31, and 99%, respectively. The IC50 (concentration of compound at which 50% of the enzyme is inhibited) of 10 for Pim1 is 450 pM at 100 μM ATP and 10 is thus at least 2 orders of magnitude more potent against Pim1 than the unspecified inhibitor staurosporine (IC50 = 50 nM at 2 μM ATP; see Supporting Information). A Lineweaver–Burk kinetic analysis (Figure 6) confirms that 10 binds to Pim1 in an ATP competitive fashion. Curve fitting yields a remarkably low inhibition constant (Ki) of 90 ± 20 μM. In addition, methylation of the imide nitrogen of 10 reduces the activity by more than 2 orders of magnitude (IC50 = 120 nM at 2 μM ATP; see Supporting Information), reassuring that the imide N–H of 10 is involved in hydrogen bonding within the ATP binding site as designed.

To test the importance of the CO group in scaffold 10 for potency against Pim1, we synthesized compounds in which the CO group is exchanged for other monodentate ligands, such as cyanide (11), P(OCH3)3 (12), azide (13), NH3 (14), and DMSO (15). This fast positional scanning would not be possible without the precursor complex 3. It is noteworthy that, because of the cyclic nature of the tridentate ligand and the sp3 hybridization at the coordinating sulfur atoms, 1,4,7-trithiaclononane has to occupy both coordination sites within the plane of the pyridocarbazole ligand, thus leaving a position perpendicular to the pyridocarbazole plane for the coordination of a monodentate ligand (as an example, see Supporting Information for the crystal structure of N-benzylated 11). This combination of a tridentate and monodentate ligand thus allows one to control the position of functional groups perpendicular to the plane of the pyridocarbazole ligand.

Intriguingly, all tested compounds are at least 2 orders of magnitudes less potent inhibitors for Pim1, as demonstrated by the IC50 curves shown in Figure 6. For example, replacing the CO group of 10 for a cyanide (11) reduces the IC50 for Pim1

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.

Figure 6. (A) IC50 curves with Pim1 obtained by phosphorylation of S6 kinase/Rsk2 Substrate Peptide 2 with [γ-32P]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 (Vrel) against varying ATP concentrations in the presence of 0 (●), 100 (○), and 200 μM (▼) of 10.
by a factor of 360 (IC₅₀ = 160 nM). This is a remarkable modulation of activity by the change of just a single atom (including a change in overall charge). Similarly, exchanging the CO against azide (13) or NH₃ (14) reduces the activities by more than 2 orders of magnitude (IC₅₀ = 100 and 80 nM, respectively), and replacing the CO against P(OCH₃)₃ (12) (IC₅₀ = 800 nM) or DMSO (15) (IC₅₀ = 1 μM) even leads to complexes that are more than a factor of 1000 less potent against Pim1. In fact, we even tested more monodentate ligands but did not find any ligand that can replace the CO against Pim1. In fact, we tested more monodentate ligands but did not find any ligand that can replace the CO against Pim1 (data not shown). Interestingly, azide compound 13 is a quite potent inhibitor for the protein kinase MSK1 with an IC₅₀ of 70 nM (see Figure 5).

After having discovered the opportunity to influence inhibitor selectivity by manipulating a single coordination site, we next turned our attention to the rest of the ligand sphere. The bar diagram in Figure 5 demonstrates that the CO compound 10 is most potent against Pim1, but also inhibits GSK3α to a significant extent at 100 nM (19% activity, IC₅₀ = 14 nM at 100 μM ATP). Apparently, the CO ligand in the plane perpendicular to the pyridocarbazole chelate is an important pharmacophore both for Pim1 and GSK3, but not for most other kinases. We were therefore wondering if we could now modulate the selectivity between the two kinases by the rest of the ligand sphere that complements the CO. Accordingly, scanning ligands starting again from precursor complex 3, we discovered complex 16, which differs from 10 by the replacement of the 1,4,7-trithiacyclononane ligand for an 2-aminomethylpyridine and chloride (Figure 4). The stereochemical identity of this compound was elucidated by X-ray crystal structure analysis of a benzylated derivative of 16 (see Supporting Information). Whereas 10 prefers Pim1 over GSK3α, by a factor of 30 at 100 μM ATP, compound 16 displays selectivity for GSK3α, as visualized in the bar diagram of Figure 5. The IC₅₀ of 16 for GSK3α is 8 nM at 100 μM ATP, and with this, 16 is by an order of magnitude more potent for GSK3α compared to Pim1 (IC₅₀ = 95 nM).

The space-filling model of 16 (Figure 7) illustrates that the ruthenium center is buried and not accessible for direct interactions with the target protein. This is consistent with our model that the metal center has a solely structural role in organizing the orientation of the organic ligands and determining the overall shape of the molecule. In this regard, it is noteworthy that most of the discussed organometallic scaffolds are highly rigid. For example, ruthenium complex 16 cannot change its three-dimensional shape because of minimal rotational freedom. We assume that this rigidity plays an important role in generating highly selective protein kinase inhibitors because the molecule is not able to accommodate a different active site by adjusting the position of functional groups. We think that this hypothesis is manifested in the high selectivity of 16 for GSK3α. For example, 16 is significantly more potent for the α-isoform of GSK-3 (IC₅₀ = 8 nM) over the β-isoform (IC₅₀ = 50 nM). This is astonishing since GSK-3α and GSK-3β show 97% sequence identity within the ATP binding site.

In conclusion, we here introduced a method which opens an avenue for the rapid scanning of ligands around a ruthenium center, searching for three-dimensional structures that are complementary in shape and functional group presentation to the active site of individual protein kinases. It is likely that inhibitor structures 10, 13, and 16 cannot be easily mimicked by an organic scaffold, and we believe that we are herewith accessing an area of the chemical space that is mostly unexplored.

**Experimental Section**

**General Procedures and Reagents.** NMR spectra were recorded on a Bruker AM-500 (500 MHz) or DMX-360 (360 MHz) spectrometer. Low-resolution mass spectra were obtained on an LC platform from Micromass using ESI technique. ES-TOF spectra were measured by Waters Micromass MS Technologies. High-resolution mass spectra were obtained with a Micromass AutoSpec instrument using either CI or ES ionization. Infrared spectra were recorded on a Perkin-Elmer 1600 series FTIR spectrometer. Solvents and reagents were used as supplied from Aldrich, Acros, or Strem. Reactions were performed under an atmosphere of argon unless otherwise specified. TBS-protected pyridocarbazole 4 was synthesized as published recently. Protein kinases (human) and substrates were purchased from Upstate Biotechnology USA.

**Compound 5.** A suspension of 4 (1.84 g, 4.3 mmol), K₂CO₃ (654 mg, 4.73 mmol), and [Ru(benzene)Cl₂] (1.1 g, 2.2 mmol) in CH₂CN (173 mL) was purged with argon and stirred overnight at ambient temperature. The resulting dark red suspension was dried in vacuo and the crude material subjected to silica gel chromatography with methylene chloride:methanol (100:1, then 20:1) as the eluting solvent. The product 5 was isolated as a red solid (1.84 g, 69%). ¹H NMR (500 MHz, CDCl₃): δ (ppm) 9.27 (d, J = 8.2 Hz, 1H), 1.99 (d, J = 4.6 Hz, 1H), 8.91 (d, J = 7.8 Hz, 1H), 7.83 (d, J = 8.1 Hz, 1H), 7.62–7.57 (m, 2H), 7.40 (t, J = 7.4 Hz, 1H), 5.94 (s, 6H), 1.04 (s, 9H), 0.62 (s, 3H), 0.61 (s, 3H). ¹³C NMR (125 MHz, CDCl₃): δ (ppm) 175.4, 174.8, 153.0, 151.2, 142.6, 134.9, 132.6, 126.4, 125.1, 124.3, 122.8, 121.5, 119.9, 114.77, 114.74, 114.5, 83.4, 26.1, 18.8, –4.4. IR (film): ν (cm⁻¹) 3072, 2931, 2861, 1743, 1684, 1520, 1502, 1472, 1414, 1337, 1276, 1267, 1232, 1049, 826. HRMS calcd for C₅₇H₄₆N₂O₂SiRu (M – Cl + CH₃CN)⁺: 621.1260, found (M – Cl + CH₃CN)⁺: 621.1276.

**Compound 6.** A suspension of 5 (235 mg, 0.38 mmol) was purged with argon for 15 min and then irradiated with a mercury medium pressure lamp (uranium filter) for 4 h while argon was bubbled through the solution. The resulting dark solution was dried in vacuo and the crude material subjected to silica gel chromatography with methylene chloride:methanol (20:1) yielding 6 (150 mg, 60%). Small quantities of a minor diastereoisomer were also isolated (17%). ¹H NMR (500 MHz, CDCl₃): δ (ppm) 9.61 (d, J = 4.1 Hz, 1H), 1.95 (d, J = 8.1 Hz, 1H), 8.93 (d, J = 8.0 Hz, 1H), 7.80 (br, 1H), 7.60 (dd, J = 8.4, 5.1 Hz).
Hz, 1H), 7.55 (t, J = 7.6 Hz, 1H), 7.35 (br, 1H), 2.81 (s, 3H), 2.08 (s, 6H), 1.07 (s, 9H), 0.63 (s, 6H). IR (film): ν (cm⁻¹) 2928, 2855, 2326, 2271, 1748, 1684, 1579, 1520, 1415, 1319, 1291, 1269, 1228, 1132, 1046, 827, 749. HRMS calcd for C₃₂H₃₂N₆O₄SiClRu (M⁺) 660.1010, found (M⁺) 660.1000.

**Compound 3.** To a stirred dark green solution of 6 (230 mg, 0.35 mmol) in CH₂CN (23 mL) was added tetraethylammonium fluoride (523 µL, 1 M in THF, 0.522 mmol), and the solution was stirred at room temperature for 10 min. To the resulting dark pink solution was added glacial acetic acid (30 µL, 0.522 mmol), and it was stirred for 5 min, during which time a color change to green was observed. The solution was dried in vacuo and the resulting crude material subjected to silica gel chromatography with acetonitrile:water:saturated aqueous KNO₃ (50:3:1). The combined product eluents were concentrated to dryness, and the resulting material was dissolved in minimal acetonitrile/water. The product was precipitated by the addition of excess solid NH₄PF₆. The precipitate was centrifuged and the pellet washed twice with water. The material was then dried under high vacuum to yield 7 (9 mg, 0.012 mmol) in DMF (2 mL) was purged with CO gas for 30 s, then stirred under an atmosphere of CO at 95 °C for 2 h. The resulting pink solution was dried in vacuo and coevaporated once with acetonitrile. The crude material was subjected to silica gel chromatography with acetonitrile:water:saturated aqueous KNO₃ (50:3:1). The combined product eluents were concentrated to dryness, and the resulting material was dissolved in minimal acetonitrile/water. The product was precipitated by the addition of excess solid NH₄PF₆. The precipitate was centrifuged and the pellet washed twice with water. The material was then dried under high vacuum to yield 10 (7 mg, 79%). 1H NMR (500 MHz, acetonitrile-d₃): δ (ppm) 9.41 (dd, J = 8.2, 0.9 Hz, 1H), 9.17 (dd, J = 5.1, 1.0 Hz, 1H), 8.91 (dd, J = 8.3 Hz, 1H), 7.96 (br s, 1H), 7.94 (dd, J = 8.4, 5.1 Hz, 1H), 7.74 (d, J = 8.4 Hz, 1H), 7.60 (d, J = 7.6 Hz, 1H), 7.41 (t, J = 7.5 Hz, 1H), 3.80–2.80 (m, 12H). 13C NMR (75 MHz, DMSO-d₆): δ (ppm) 157.3, 154.8, 153.9, 151.4, 143.9, 143.0, 135.9, 131.8, 127.8, 125.6, 124.8, 122.2, 120.6, 116.1, 115.1, 114.7, 83.9, 37.7, 36.8, 33.1, 32.4, 32.0, 23.8. IR (film): ν (cm⁻¹) 3389, 2014, 1702, 1584, 1414, 1343, 1226, 844. HRMS calcd for C₃₂H₃₀N₆O₄SiClRu (M⁺) 795.9770, found (M⁺) 795.9734.

**Compound 11.** To a solution of 3 (19.6 mg, 0.036 mmol) in DMF (1.15 mL) was added 1,4,7-triaicyclosilane (359 µL, 100 mM in DMF). The solution was purged with argon for 5 min and then heated to 75 °C for 1 h. To the resulting purple solution was added NaN₅ (359 µL, 100 mM in water). The reaction was stirred at 90 °C for 1 h, after which time the purple solution was dried in vacuo. The crude material was subjected to silica gel chromatography with methylene chloride:methanol (10:1), later changing to 5:1 and 3:1. The combined product eluents were dried to provide 11 (3.1 mg, 15%). 1H NMR (500 MHz, DMSO-d₆): δ (ppm) 11.04 (s, 1H), 9.08 (dd, J = 8.3, 1.1 Hz, 1H), 8.96 (dd, J = 5.0, 1.1 Hz, 1H), 8.72 (d, J = 7.8 Hz, 1H), 7.74 (dd, J = 8.4, 5.1 Hz, 1H), 7.67 (d, J = 8.4 Hz, 1H), 7.50 (t, J = 7.6 Hz, 1H), 7.29 (t, J = 7.4 Hz, 1H), 3.10–0.23 (m, 12H). 13C NMR (125 MHz, DMSO-d₆): δ (ppm) 171.0, 170.9, 153.4, 152.1, 150.4, 143.2, 139.8, 131.8, 130.2, 125.7, 124.1, 123.7, 123.3, 121.1, 118.8, 114.5, 111.6, 36.2, 35.0, 33.5, 31.5, 30.3, 31.0. IR (film): ν (cm⁻¹) 2922, 2925, 2068, 1744, 1700, 1543, 1523, 1454, 1287, 1268. HRMS calcd for C₃₂H₃₀N₆O₄SiClRu (MH⁺) 794.9870, found (MH⁺) 794.9900.
The product was precipitated by the addition of excess solid KPF6 and the resulting material was dissolved in minimal acetonitrile/water. The combined product eluents were concentrated to dryness, and the resulting purple solution was added sodium azide (230 °C, 1 h), purged with argon for 5 min and then heated to 85 °C for 1 h. The resulting purple solution was dried in vacuo and coevaporated with ethanol. The crude material was subjected to silica gel chromatography with methylene chloride:methanol 20:1 and later with 10:1 (6.6 mg, 46%). 1H NMR (500 MHz, DMSO-d6): δ (ppm) 10.99 (s, 1H), 9.09 (dd, J = 5.1, 1.2 Hz, 1H), 8.73 (d, J = 7.8 Hz, 1H), 7.77 (m, 2H), 7.50 (t, J = 7.6 Hz, 1H), 7.29 (t, J = 7.5 Hz, 1H), 3.07–2.19 (m, 12H). 13C NMR (90 MHz, DMSO-d6): δ (ppm) 171.0, 170.9, 153.9, 150.5, 143.4, 132.2, 130.2, 125.6, 123.8, 123.5, 121.1, 118.7, 115.0, 114.7, 111.7, 34.6, 33.9, 33.4, 32.6, 31.7, 29.4 (IR film): ν (cm⁻¹) 3206 (N2), 1740, 1697, 1658, 1562, 1415, 1337, 1285, 1229, 934, 873, 752, 696, 678. ES-ToF (M +>Hello 609.0.

**Compound 14.** To a solution of 9 (10 mg, 0.013 mmol) in DMF (1 mL) was added ammonia (0.5 M in dioxane) (500 µL, 0.25 mmol). The reaction vessel was closed with a septum and heated to 85 °C for 1 h, and the purple solution was dried in vacuo. The crude material was subjected to silica gel chromatography with acetonitrile:water:saturated aqueous KNO3 20:1, and later with 35:1, yielding 15 (17 mg, 0.031 mmol) in addition to 32% of a diastereomeric product. 1H NMR (500 MHz, acetone-d6): δ (ppm) 8.07 (d, 1J = 7.8 Hz, 1H), 7.74 (d, J = 8.4, 5.1 Hz, 1H), 9.07 (dd, J = 7.8, 0.9 Hz, 1H), 7.16 (dd, J = 8.3, 7.0, 1.4 Hz, 1H), 6.11 (d, J = 8.1 Hz, 1H), 4.88 (m, 2H), 4.76 (m, 2H), 4.21 (m, 1H). 13C NMR (90 MHz, acetone-d6): δ (ppm) 200.4, 171.0, 170.7, 164.6, 155.7, 154.3, 151.2, 151.1, 144.1, 137.8, 133.1, 130.4, 125.9, 124.6, 124.1, 123.4, 122.8, 121.8, 120.8, 118.9, 114.4, 112.9, 112.8, 49.2. IR (film): ν (cm⁻¹) 3216, 2925, 1744, 1699, 1581, 1526, 1494, 1476, 1421, 1344, 1226, 1130, 1017. HRMS (ESI-ToF) calcd for C23H18N4S3O2Ru (M−H) 557.9907, found (M−H) 557.9916.

**Protein Kinase Assays.** Various concentrations of inhibitors were incubated at room temperature in 20 mM MOPS, 30 mM MgCl2, 0.8 µg/mL BSA, 5% DMSO (resulting from the inhibitor stock solution), pH 7.0, in the presence of substrate [(Pim1 = 50 μM S6 kinase/Rsk2 Substrate Peptide 2; MSK1 = 30 μM crosstide; GSK3α = 20 μM phosphoglyceronate synthase-2; CDK2/CyclinA = 0.1 μg/mL histone H1) and kinase (Pim1 = 0.02 ng/µL for IC50, and 0.4 ng/µL for the determination of activities at 100 nM inhibitor concentration (bar diagram in Figure 4); MSK1 = 0.4 ng/µL; GSK3α = 0.4 ng/µL; CdK2/CyclinA = 0.8 ng/µL). After 15 min, the reaction was initiated by adding ATP to a final concentration of 100 µM, including approximately 0.2 Ci/µL. [32P]ATP. Reactions were performed in a total volume of 25 µL. After 30 min, the reaction was terminated by spotting 17.5 µL on a circular P81 phosphocellulose paper (diameter = 2.1 cm, Whatman), followed by washing four times (5 min each wash) with 0.75% phosphoric acid and once with acetone. The dried P81 papers were transferred to a scintillation vial, and 5 mL of scintillation cocktail was added, and the counts per minute (CPM) were determined with a Beckmann 6000 scintillation counter. IC50 values were defined to be the concentration of inhibitor at which the CPM was 50% of the control sample, corrected by the background.

**Lineweaver–Burk Kinetics.** Various concentrations of ATP and inhibitor 10 were incubated at room temperature for 1 h in 20 mM MOPS, 30 mM MgCl2, 0.8 µg/mL BSA, 5% DMSO (resulting from the inhibitor stock solution), pH 7.0, in the presence of S6 kinases/Rsk2 Substrate Peptide 2 (50 µM) and Pim1 human, (0.02 ng/µL). The dual reaction volume was 25 µL. Each ATP solution contained the same ratio of ATP to radioactive [32P]ATP and were as follows: 250 µM ATP with 0.40 µCi/µL; [32P]ATP, 100 µM ATP with 0.08 µCi/µL; [32P]ATP, 20 µM ATP with 16 nCi/µL; [32P]ATP, and 4 µM ATP with 3.2 nCi/µL. The inhibitor concentrations used were 0, 0.1, 0.2, 0.5, and 1 nM. Prior to the addition of ATP, the inhibitor 10 substrate, and protein kinase were preincubated for 20 min in the reaction buffer. After 1 h, the reactions were stopped by spotting 17.5 µL of the reaction solution on a circular P81 phosphocellulose paper (diameter = 2.1 cm, Whatman) followed by washing four times (5 min each wash) with 0.75% phosphoric acid and once with acetone. After the papers dried, they were transferred to scintillation vials, and the counts per minute (CPM) were measured with a Beckmann 6000 scintillation
counter. CPM values were treated as relative initial velocities. All reactions were performed at least in duplicate. The inhibition constant ($K_i$) of 10 was calculated by nonlinear regression using the software GraphPad Prism (version 4.0).

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**Supporting Information Available:** IC$_{50}$ curves, crystallographic data, $^1$H NMR spectrum of precursor 3, an example of the synthesis of an in situ ruthenium complex library, and complete ref 10h. This material is available free of charge via the Internet at http://pubs.acs.org.

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