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Bioactive Cyclometalated Phthalimides: Design, Synthesis and Kinase Inhibition

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Abstract

The regioselective cyclometalation of 4-(pyridin-2-yl)phthalimide was exploited for the economical design of organometallic protein kinase inhibitors. 4-(Pyridin-2-yl)phthalimide can be prepared from inexpensive 4-bromophthalimide in just three steps including one Pd-catalyzed *Stille* cross-coupling. The versatility of this new ligand was demonstrated with the synthesis of ruthenium(II) half-sandwich as well as octahedral ruthenium(II) and iridium(III) complexes. The regioselectivity of the C-H activation in the course of the cyclometalation can be influenced by the reaction conditions and the steric demand of the introduced metal complex fragment. The biological activity of this new class of metalated phthalimides was evaluated by profiling two representative members against a large panel of human protein kinases. A cocrystal structure of one metallo-phthalimide with the protein kinase Pim1 confirmed an ATP-competitive binding with the intended hydrogen bonding between the phthalimide moiety and the hinge region of the ATP-binding site.

Introduction

Metal complexes are highly versatile structural scaffolds for the molecular recognition of biomolecules such as nucleic acids and proteins.^{1–4} Over the last several years our laboratory contributed to this area of research with the design of substitutionally inert ruthenium(II),⁵ osmium(II),⁶ rhodium(III),⁷ iridium(III),⁸ and platinum(II)⁹ complexes as highly potent and selective ATP-competitive inhibitors of protein kinases and lipid kinases.¹⁰ Our previous design was mainly inspired by the natural product staurosporine with the maleimide moiety of pyridocarbazole metal complexes (Figure 1) undergoing hydrogen bonding with the hinge region of the ATP-binding site, while the pyridocarbazole heterocycle occupying the hydrophobic adenine binding cleft, and the remaining coordination sphere interacting with the region of the ribose-triphosphate binding site and thereby strongly contributing to binding affinity and selectivity.¹¹ However, the synthesis of the pyridocarbazole heterocycle is cumbersome and contains a photochemical step which is

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difficult to scale.¹² Furthermore, due to an intrinsic binding bias of the pyridocarbazole moiety we estimate that only a subset of the more than 500 human protein kinases are suitable targets for the metallo-pyridocarbazole scaffold.¹³ To address these limitations we recently introduced a new class of cyclometalated metal complexes with the ligand 3-(pyridin-2-yl)-1,8-naphthalimide and we demonstrated their suitability for the development of nanomolar protein kinase inhibitors.^{14,15} It turned out that a drawback of this scaffold is manifested by the steric interference between the ligand sphere of the metal complexes and the 5-position of the naphthalene moiety (highlighted in Figure 1), resulting in a distortion of the octahedral coordination geometry and thus rendering structure-based inhibitor design somewhat more complicated. Our recent studies have hence focused on a smaller, sterically less demanding ligand for cyclometalation and we developed 4-(pyridin-2-yl)phthalimide as novel ligand for the highly efficient design of cyclometalated metallo-phthalimide protein kinase inhibitors. In a preliminary report we found a ruthenium phthalimide complex as nanomolar inhibitor of the p21 activated kinase 1 (PAK1) and confirmed its ATP-competitive binding by an X-ray cocrystal structure.¹⁶ We here provide a full account on the design, synthesis, and kinase inhibition of cyclometalated pyridylphthalimide complexes and present a new cocrystal structure of a metallo-pyridylphthalimide bound to the ATP-binding site of the protein kinase Pim1.

Results and Discussion

Pyridylphthalimide ligand synthesis

N-Benzyl-4-bromophthalimide (**1a**) and *N*-(*tert*-butyldimethylsilyl)-4-bromophthalimide (**1b**) were converted to *N*-benzyl-4-(pyridin-2-yl)phthalimide (**2a**) and *N*-(*tert*-butyldimethylsilyl)-4-(pyridin-2-yl)phthalimide (**2b**) using palladium-mediated *Stille* cross-coupling with 2-(trimethylstannyl)pyridine and catalytic tetrakis(triphenylphosphine)palladium(0) in yields of 85% and 49%, respectively (Scheme 1). In a variation of this route, stannylation of **1b** with hexa-*n*-butylditin provided desilylated 4-(tri-*n*-butylstannyl)phthalimide (**3**) (47%) which was reacted with 2-bromopyridine using *Stille* cross-coupling conditions to obtain 4-(pyridin-2-yl)phthalimide (**2c**) in 65%. This latter synthesis is supposed to be especially suitable for the rapid synthesis of pyridylphthalimides with a variety of modifications at the pyridyl moiety.

Regioselectivity of cyclometalation

Cyclometalation with this new pyridylphthalimide ligand can afford two regioisomers depending on whether the C-H activation occurs at C-3 or C-5 of the phthalimide moiety. For initial experiments we reacted pyridylphthalimide **2a** with [Ru(η^5 -C₅H₅)(CO)(MeCN)₂][PF₆]¹⁷ in the presence of Et₃N in methanol at 55 °C and smoothly obtained the half-sandwich complex **4** as a single regioisomer (70%) (Scheme 2). A crystal structure of this complex is displayed in Figure 2 and confirms the cyclometalation as well as the bidentate binding of the ruthenium to the C-3 atom of the phthalimide moiety (Ru-C = 2.05 Å) and the pyridine nitrogen (Ru-N = 2.09 Å). The complex shows a pseudo-octahedral geometry at the ruthenium center with the CO ligand oriented perpendicular to the pyridylphthalimide ligand. Interestingly, when we instead reacted **2a** with [Ir(COD)Cl]₂ and triphenylphosphine, the octahedral iridium(III) complex **5** (67%) was obtained as a single

regioisomer but this time through cyclometalation with the carbon C-5 of the phthalimide moiety (Scheme 2). A crystal structure shown in Figure 3 confirms the coordination of the iridium to the pyridine ligand (Ir-N = 2.13 Å) and the formation of an iridium-carbon bond (Ir-C = 2.00 Å). The chloro ligand and the calculated position of the hydride are arranged within the plane of the pyridylphthalimide moiety *cis* and *trans* to the pyridine ligand, respectively, whereas the two bulky triphenylphosphines are coordinated at the axial positions. Despite the coordinated hydrido ligand, which is strongly shifted up-field in the ¹H-NMR to –16.58 ppm, as well as the carbon-iridium bond, the complex is very robust and can be easily handled under air. This is most likely due to the two bulky triphenylphosphine ligands shielding the metal center from further reactions.

It can be assumed that in these two reactions the regioselectivity of the C-H activation is strongly influenced by steric effects, with small metal fragments preferring a cyclometalation with C-3, probably directed by a transient coordination to the neighboring maleimide carbonyl group, whereas more bulky metal fragments prefer the sterically less congested cyclometalation with C-5.

Synthesis of bioactive metal complexes

Guided by simple molecular modeling experiments we concluded that the cyclometalation at the 5-position of the phthalimide moiety should be preferred for the generation of protein kinase inhibitors. We therefore decided to synthesize ruthenium pyridylphthalimide complexes containing the facial tridentate ligand 1,4,7-trithiacyclononane since it not only prevents the formation of diastereomers due to its high symmetry but we also expected that due to its bulkiness it would guide the cyclometalation to the C-5 position. Indeed, the reaction of the pyridylphthalimide ligands **2a** or **2b** with [Ru(MeCN)₃([9]aneS₃)](CF₃SO₃)₂ (**6**),¹⁸ ([9]aneS₃ = 1,4,7-trithiacyclononane) afforded the monoacetonitrile complexes **7a** (79%) or **7b** (74%), respectively, with high regioselectivity (Scheme 3).

A crystal structure of complex **7b** is shown in Figure 4 and confirms the coordination of ruthenium to the pyridine ligand including a cyclometalation with the 5-position of the phthalimide. The structure also nicely reveals the ability of the maleimide moiety to form a donor and acceptor hydrogen bond with the imide NH and carbonyl CO, respectively. The semilabile acetonitrile ligand is prone to substitution against other anionic or neutral monodentate ligands as has been demonstrated in related systems.¹⁹ For example, the reaction of **7a** or **7b** with CO in DMF at 95 °C for 3 hours afforded the CO-complexes **8a** (85%) or **8b** (82%), respectively, whereas the reaction of **7b** with NaSCN, NaOCN, and KSeCN afforded the complexes **9b** (*N*-bound isomer, 47%), **10b** (*N*-bound isomer, 55%), and **11b** (*Se*-bound isomer, 96%), respectively. A crystal structure of complex **8a** is displayed in Figure 5 and demonstrates the expected almost perfectly octahedral coordination around the ruthenium, as compared to a strongly distorted octahedral coordination sphere around the ruthenium center in the related pyridylphthalimide complex (Figure 1).¹⁴

Protein kinase profiling

To gain insight into the protein kinase inhibition properties of pyridylphthalimide metal complexes, complexes **8b** and **9b** were profiled against the majority of the human protein kinases encoded in the human genome (human kinome).¹³ This was accomplished by using an active-site-directed competition binding assay with 442 different protein kinases (KINOMEscan, DiscoverX) which provides primary data (%ctrl = percent of control: 0% = highest affinity, 100% = no affinity) that correlate with binding constants (K_d).^{20,21} Interestingly, although these two complexes should be capable of forming hydrogen bonds between the maleimide moiety and all profiled protein kinases, the screening data demonstrated surprisingly high selectivities, with the CO-complex **8b** only binding strongly to 15 and the NCS-complex **9b** only to 5 protein kinases with %ctrl < 1% (Figure 6). The observed high selectivity is consistent with all our previous studies on the kinase inhibition properties using octahedral metal complexes and we hypothesize that this is due to the space-demanding, defined globular shapes of the octahedral inhibitors that are very sensitive even to small differences in the structure and dynamics of the kinase ATP-binding sites. Interestingly, the somewhat lower kinase selectivity of complex **9b** as compared to **8b** might be due to the rotational freedom of the coordinated NCS ligand which is absent in the CO-complex **8b**. It is also interesting to note that although **8b** and **9b** only differ by a single monodentate ligand, both complexes target completely different subsets of protein kinases as visualized by the kinase dendrograms shown in Figure 6.

Protein kinase binding

Whereas the binding of the NCS-complex **9b** to the protein kinase PAK1 was reported by us recently,¹⁶ we were interested in understanding the protein kinase binding mode of the more selective CO-complex **8b** and we therefore cocrystallized the racemate of **8b** with Pim1. The structure of the Pim1/**8b** complex was determined and refined to a resolution of 2.3 Å. The overall structure is shown in Figure 7 and reveals the typical two-lobe protein kinase architecture with the catalytic ATP-binding site positioned in a deep intervening cleft and occupied with the ruthenium complex **8b**. The maleimide moiety of **8b** forms one hydrogen bond between the NH and the backbone carbonyl group of Glu121, whereas a second hydrogen bond is prevented by Pro123 (Figure 8). A significant number of hydrophobic contacts are formed with the pyridylphthalimide, the 1,4,7-trithiacyclononane, and the CO ligand. The ruthenium-coordinated CO ligand points towards the flexible glycine-rich loop (P-loop) and fills a small hydrophobic pocket that is formed by induced fit. The same unusual interaction between coordinated CO and the glycine-rich loop has been frequently observed with the previously established metallo-pyridocarbazole kinase inhibitors.^{5,6,22} Interestingly, a comparison of this Pim1/**8b** structure with the cocrystal structure of complex **9b** and PAK1 (PDB ID: 4DAW)¹⁶ reveals some significant differences. Firstly, the binding orientation of the pyridylphthalimide within the ATP-binding sites differs with a preference for opposite metal-centered stereoisomers (Figure 9). Secondly, whereas the NCS ligand of **9b** points towards the interface of the glycine-rich loop and the adjacent β -sheet, the CO of **8b** is located right below the glycine-rich loop. Thus, although **8b** and **9b** differ only by the nature of a single monodentate ligand, their binding modes within the ATP-binding sites diverge, thus explaining the distinguished kinase profiling results of **8b** and **9b**.

Conclusions

In summary, we demonstrated the merit of 4-(pyridin-2-yl)phthalimide for the straightforward generation of metal-based protein kinase inhibitors. The imide moiety is designed to hydrogen bond with the hinge region of the ATP-binding site, whereas the metal coordination to the pyridine with concomitant regioselective cyclometalation to either C-3 or C-5 of the phthalimide moiety reduces the required number of coordinating heteroatoms. Thus, based on this simple pyridylphthalimide scaffold, selective protein kinase inhibitors can be synthesized in just a few steps in an economical fashion compared to the often tedious synthesis of our previously established staurosporine-inspired metallo-pyridocarbazoles. It is worth noting that the facile C-H activation and robustness of the formed metal-carbon bond can be attributed to the highly electron-deficient nature of the phthalimide moiety, being functionalized with electron-withdrawing pyridyl and maleimide substituents. Finally, we demonstrated the promising protein binding profiles of two pyridylphthalimide metal complexes and investigated the binding of one phthalimide complex to the ATP-binding site of the protein kinase Pim1. The investigation of the *in vitro* and *in vivo* properties of this novel class of bioactive metallo-phthalimides is underway.

Experimental Section

General methods

All reactions were carried out using oven-dried glassware and conducted under a positive pressure of nitrogen unless specified otherwise. Chemicals were used as received from standard suppliers. 2-(Trimethylstannyl)pyridine,²³ tetrakis (triphenylphosphine) palladium(0),²⁴ [Ir(COD)Cl]₂,²⁵ [Ru(η^5 -C₅H₅)(CO)(MeCN)₂]PF₆,¹⁷ and [Ru(MeCN)₃([9]aneS₃)](CF₃SO₃)₂ (**6**)¹⁸ were prepared according to literature procedures. Phthalimides **1a**, **1b** were synthesized according to modified literature procedures (see ESI for further details). All solvents for chromatography were distilled prior to use. Acetonitrile and DMF were dried by common methods and freshly distilled prior to use. NMR spectra were recorded on an Avance 300 (300 MHz), DRX 500 (500 MHz), Avance 500 (500 MHz), or DPX-250 (250 MHz) spectrometer. Infrared spectra were recorded on a Bruker Alpha FTIR. High resolution mass spectra were obtained with a Finnigan LTQ-FT instrument using either APCI or ESI.

N-(Benzyl)-4-(pyridin-2-yl)phthalimide (**2a**)

N-Benzyl-4-bromophthalimide (**1a**) (1.28 g, 4.04 mmol) and 2-(trimethylstannyl)pyridine (0.90 g, 3.69 mmol) were dissolved in *m*-xylene (35 mL) and the solution was purged with nitrogen for 20 minutes. Tetrakis-(triphenylphosphine)palladium(0) (473 mg, 0.41 mmol) was added and the solution heated to reflux under nitrogen for 48 h. The solution was cooled to ambient temperature, water (50 mL) was added, the organic phase was separated, and the aqueous phase was extracted with CH₂Cl₂ twice. The combined organic layers were washed with brine, dried using Na₂SO₄, filtered, and the solvent removed *in vacuo*. The crude product was subjected to silica gel chromatography with first CH₂Cl₂ and then CH₂Cl₂:MeOH 75:1. The combined product eluents were dried *in vacuo* and *N*-benzyl-4-(pyridin-2-yl)phthalimide (**2a**) was obtained as light yellow solid (980 mg, 85%). ¹H-NMR

(300 MHz, CDCl₃): δ (ppm) 8.74 (dt, J = 4.8, 1.4 Hz, 1H), 8.44–8.45 (m, 1H), 8.41 (dd, J = 7.8, 1.5 Hz, 1H), 7.93 (dd, J = 7.8, 0.6 Hz, 1H), 7.81–7.83 (m, 2H), 7.44–7.47 (m, 2H), 7.27–7.35 (m, 4H), 4.88 (s, 2H). ¹³C-NMR (75 MHz, CDCl₃): δ (ppm) 167.8, 155.1, 150.1, 145.3, 137.1, 136.4, 132.9, 132.5, 132.0, 128.7, 128.6, 127.8, 123.8, 123.5, 121.7, 121.0, 41.7. IR (film): ν (cm⁻¹) 3029, 2930, 1766, 1699, 1617, 1584, 1422, 1387, 1341, 1304, 1155, 1106, 1067, 949, 786, 740, 695, 623. HRMS calcd for C₂₀H₁₅N₂O₂ (M+H)⁺ 315.1128, found 315.1124.

***N*-(*tert*-Butyldimethylsilyl)-4-(pyridin-2-yl)phthalimide (2b)**

N-(*tert*-Butyldimethylsilyl)-4-bromophthalimide (**1b**) (1.10 g, 2.98 mmol) and 2-(trimethylstannyl)pyridine (0.74 g, 3.06 mmol) were dissolved in *m*-xylene (10 mL) and the solution was purged with nitrogen for 20 minutes. Tetrakis-(triphenylphosphine)palladium(0) (40 mg, 35 μ mol) was added and the solution heated to reflux under nitrogen for 48 h. The solution was cooled to ambient temperature, water (50 mL) was added, the organic phase was separated, and the aqueous phase was extracted with CH₂Cl₂ twice. The combined organic layers were washed with brine, dried using Na₂SO₄, filtered, and the solvent removed *in vacuo*. The crude product was subjected to silica gel chromatography with first CH₂Cl₂ and then CH₂Cl₂:MeOH 50:1. The combined product eluents were dried *in vacuo* and *N*-(*tert*-butyldimethylsilyl)-4-(pyridin-2-yl)phthalimide (**2b**) was obtained as light yellow solid (490 mg, 49%). ¹H-NMR (300 MHz, CDCl₃): δ (ppm) 8.75 (dt, J = 4.8, 1.3 Hz, 1H), 8.43 (d, J = 7.8, 1.5 Hz, 1H), 8.37–8.38 (m, 1H), 7.90 (dd, J = 7.8, 0.6 Hz, 1H), 7.81–7.84 (m, 2H), 7.31–7.35 (m, 1H), 1.00 (s, 9H), 0.55 (s, 6H). ¹³C-NMR (75 MHz, CDCl₃): δ (ppm) 155.5, 150.3, 145.4, 137.3, 135.0, 134.1, 132.8, 123.6, 123.5, 121.5, 121.2, 26.5, 19.2, -4.1. IR (film): ν (cm⁻¹) 2951, 2926, 2880, 2854, 1759, 1693, 1620, 1585, 1465, 1418, 1350, 1296, 1249, 1155, 1057, 849, 838, 787, 746, 673. HRMS calcd for C₁₉H₂₃N₂O₂Si (M+H)⁺ 339.1523, found 339.1516.

4-(Tri-*n*-butylstannyl)phthalimide (3)

N-(*tert*-butyldimethylsilyl)-4-bromophthalimide (**2b**) (100 mg, 294 μ mol) was dissolved in toluene (5 mL) and the solution was degassed for 20 min. Hexa-*n*-butylditin (342 μ L, 676 μ mol) was added followed by the addition of tetrakis(triphenylphosphine)palladium(0) (102 mg, 88 μ mol) and the solution was heated to reflux for 5 days. The solvent was removed *in vacuo*, the crude product subjected to silica gel chromatography with first hexane and then hexane:EtOAc 10:1. The combined product eluents were dried *in vacuo* and 4-(tri-*n*-butylstannyl)phthalimide (**3**) was obtained as colourless oil (60 mg, 47%). ¹H-NMR (300 MHz, CDCl₃): δ (ppm) 8.27 (s, 1H), 7.96–7.97 (m, 1H), 7.86 (dd, J = 7.3, 0.5 Hz, 1H), 7.77 (dd, J = 7.2, 0.7 Hz, 1H), 1.47–1.56 (m, 6H), 1.28–1.36 (m, 6H), 1.10–1.15 (m, 6H), 0.88 (t, J = 7.3 Hz, 9H). ¹³C-NMR (63 MHz, CDCl₃): δ (ppm) 169.3, 168.9, 152.9, 142.2, 132.1, 131.2, 130.9, 122.2, 28.9, 27.3, 13.6, 9.9. IR (film): ν (cm⁻¹) 3253, 2955, 2921, 2853, 1773, 1720, 1459, 1411, 1338, 1294, 1105, 1060, 741, 674. HRMS calcd for C₂₀H₃₂NO₂Sn (M+H)⁺ 438.1453, found 438.1450.

4-(Pyridin-2-yl)phthalimide (2c)

4-(Tri-*n*-butylstannyl)phthalimide (**3**) (30 mg, 69 μmol) and 2-bromopyridine (7.4 μL , 76 μmol) were dissolved in 3 mL *m*-xylene and purged with nitrogen for 15 minutes. Tetrakis-(triphenylphosphine)palladium(0) (8 mg, 6.9 μmol) was added and the mixture heated to reflux for 48 h. The solvent was removed *in vacuo*, the crude product subjected to silica gel chromatography using a gradient of CH_2Cl_2 :MeOH 100:1 to 35:1. The combined product eluents were dried *in vacuo* and 4-(pyridin-2-yl)phthalimide (**2c**) was obtained as white solid (10 mg, 65%). $^1\text{H-NMR}$ (300 MHz, $\text{DMSO}-d_6$): δ (ppm) 11.43 (s, 1H), 8.74 (ddd, $J = 4.8$, 1.8, 0.9 Hz, 1H), 8.54 (dd, $J = 7.9$, 1.5 Hz, 1H), 8.47 (dd, $J = 1.5$, 0.6 Hz, 1H), 8.19 (dt, $J = 8.0$, 0.9 Hz, 1H), 7.98 (td, $J = 7.7$, 1.8 Hz, 1H), 7.93 (dd, $J = 7.8$, 0.6 Hz, 1H), 7.47 (ddd, $J = 7.5$, 4.8, 1.0 Hz, 1H). $^{13}\text{C-NMR}$ (63 MHz, $\text{DMSO}-d_6$): δ (ppm) 168.94, 168.91, 153.9, 149.8, 144.3, 137.6, 133.5, 132.6, 132.2, 123.9, 123.4, 121.3, 120.6. IR (film): ν (cm^{-1}) 2926, 2722, 1717, 1655, 1587, 1430, 1351, 1303, 1177, 1141, 1102, 1059, 996, 833, 783, 737, 687, 634. HRMS calcd for $\text{C}_{13}\text{H}_9\text{N}_2\text{O}_2$ ($\text{M}+\text{H}$) $^+$ 225.0659, found 225.0659.

Half-sandwich complex 4

N-Benzyl-4-(pyridin-2-yl)phthalimide (**2a**) (20 mg, 64 μmol) was dissolved in methanol (4 mL). Triethylamine (6.4 μL , 77 μmol) was added, followed by $[\text{Ru}(\eta^5\text{-C}_5\text{H}_5)(\text{CO})(\text{MeCN})_2]\text{PF}_6$ (40 mg, 96 μmol), and the solution was stirred for 2 h at 55 $^\circ\text{C}$. The solution was cooled to ambient temperature, CH_2Cl_2 (20 mL) was added, and the solution was washed with brine twice. The organic phase was dried using MgSO_4 , filtered, and evaporated to dryness *in vacuo*. The crude product was subjected to silica gel chromatography with CH_2Cl_2 . The combined product eluents were dried *in vacuo* and the half-sandwich complex **4** was obtained as orange solid (25 mg, 70%). $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ (ppm) 8.95 (ddd, $J = 5.7$, 1.5, 0.7 Hz, 1H), 7.94 (d, $J = 7.7$ Hz, 1H), 7.91–7.94 (m, 1H), 7.77 (td, $J = 7.8$, 1.6 Hz, 1H), 7.55 (d, $J = 7.5$ Hz, 1H), 7.46–7.48 (m, 2H), 7.27–7.36 (m, 3H), 6.99–7.04 (m, 1H), 5.10 (s, 5H), 4.87 (s, 2H). $^{13}\text{C-NMR}$ (75 MHz, CDCl_3): δ (ppm) 177.8, 170.8, 165.3, 157.6, 153.7, 137.4, 136.7, 132.8, 128.7, 128.6, 127.6, 127.0, 121.7, 120.1, 117.3, 84.8, 41.5. IR (film) ν (cm^{-1}) 3114, 2942, 1906, 1755, 1696, 1590, 1390, 1326, 1267, 1104, 943, 807, 779, 749, 699. HRMS calcd for $\text{C}_{26}\text{H}_{19}\text{N}_2\text{O}_3\text{Ru}$ ($\text{M}+\text{H}$) $^+$ 509.0441, found 509.0427.

Iridium(III) complex 5

N-Benzyl-4-(pyridin-2-yl)phthalimide (**2a**) (31 mg, 99 μmol) and PPh_3 (103 mg, 395 μmol) were placed in a flask under nitrogen. $[\text{Ir}(\text{COD})\text{Cl}]_2$ (33 mg, 49 μmol) was added in the glovebox and 2-ethoxyethanol (4 mL) was added. The solution was heated to reflux for 18 h. Upon cooling to ambient temperature, a yellow precipitate was formed that was filtered off and washed three times with methanol. The resulting yellow solid was recrystallized from hexane: CHCl_3 10:1 to afford iridium(III) complex **5** as yellow powder (70 mg, 67%). $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ (ppm) 9.07 (d, $J = 5.4$ Hz, 1H), 7.63 (d, $J = 8.1$ Hz, 1H), 7.59 (s, 1H), 7.48–7.51 (m, 1H), 7.34–7.39 (m, 16H), 7.27–7.30 (m, 1H), 7.07–7.09 (m, 18H), 6.83–6.85 (m, 1H), 6.61 (s, 1H), 4.70 (s, 2H), –16.58 (t, $J = 16.5$ Hz, 1H). $^{13}\text{C-NMR}$ (125 MHz, CDCl_3): δ (ppm) 169.5, 168.0, 165.43, 165.37, 163.6, 150.5, 146.6, 138.3, 137.3, 136.4, 133.93, 133.89, 133.8, 131.6, 131.4, 131.3, 131.2, 129.3, 128.5, 128.3, 127.6, 127.51,

127.47, 127.4, 122.4, 122.2, 118.5, 116.3, 41.0. ^{31}P -NMR (121 MHz, CDCl_3): δ (ppm) 5.4. IR (film): ν (cm^{-1}) 3047, 2926, 2117, 1758, 1696, 1601, 1481, 1431, 1380, 1340, 1184, 1092, 820, 790, 744, 693. HRMS calcd for $\text{C}_{56}\text{H}_{44}\text{ClIrN}_2\text{O}_2\text{P}_2\text{Na}$ ($\text{M}+\text{Na}$) $^+$ 1089.2088, found 1089.2087.

Ruthenium acetonitrile complex 7a

N-(Benzyl)-4-(pyridin-2-yl)phthalimide (**2b**) (50 mg, 148 μmol) and $[\text{Ru}(\text{MeCN})_3([\text{9}]\text{aneS}_3)](\text{CF}_3\text{SO}_3)_2$ (**6**) (156 mg, 222 μmol) were dissolved in DMF (4 mL) and purged with nitrogen for 15 minutes. Triethylamine (26.8 μL , 192 μmol) was added and the solution heated to 90 $^\circ\text{C}$ for 16 h. The solvent was removed and the crude product subjected to silica gel chromatography with first acetonitrile and then acetonitrile:water:sat. KNO_3 solution 50:3:1. The combined product eluents were dried *in vacuo*, dissolved in a minimal amount of aqueous acetonitrile, and NH_4PF_6 was added. The solution was centrifuged, the resulting orange filter cake washed three times with water, and dried *in vacuo* to provide the monoacetonitrile complex **7a** as light orange solid (91 mg, 79%). ^1H -NMR (300 MHz, CD_3CN): δ (ppm) 8.74 (dd, $J = 5.6, 0.8$ Hz, 1H), 8.31 (s, 1H), 8.20 (d, $J = 8.1$ Hz, 1H), 8.17 (s, 1H), 7.90 (td, $J = 7.8, 1.5$ Hz, 1H), 7.26–7.37 (m, 6H), 4.79 (d, $J = 1.6$ Hz, 2H), 2.82–3.08 (m, 4H), 2.59–2.75 (m, 3H), 2.44–2.52 (m, 1H), 2.22–2.40 (m, 3H), 2.08–2.17 (m, 1H), 1.98 (s, 3H). ^{13}C -NMR (63 MHz, CD_3CN): δ (ppm) 196.0, 169.7, 169.1, 164.4, 152.9, 151.4, 137.2, 132.7, 130.7, 126.5, 123.8, 122.8, 120.3, 116.4, 35.0, 33.9, 33.3, 31.3, 30.2, 27.7, 2.8. IR (film): ν (cm^{-1}) 3250, 3028, 1762, 1699, 1599, 1477, 1385, 1353, 1273, 1077, 1014, 834, 748, 696. HRMS calcd for $\text{C}_{28}\text{H}_{28}\text{N}_3\text{O}_2\text{RuS}_3$ ($\text{M}-\text{PF}_6$) $^+$ 636.0387, found 636.0381.

Ruthenium acetonitrile complex 7b

N-(*tert*-Butyldimethylsilyl)-4-(pyridin-2-yl)phthalimide (**2b**) (50 mg, 148 μmol) and $[\text{Ru}(\text{MeCN})_3([\text{9}]\text{aneS}_3)](\text{CF}_3\text{SO}_3)_2$ (**6**) (156 mg, 222 μmol) were dissolved in DMF (5 mL) and purged with nitrogen for 15 minutes. Triethylamine (26.8 μL , 192 μmol) was added and the solution was heated to 90 $^\circ\text{C}$ for 16 h. The solvent was removed and the crude product subjected to silica gel chromatography with first acetonitrile and then acetonitrile:water:sat. KNO_3 solution 50:3:1. The combined product eluents were dried *in vacuo*, dissolved in a minimal amount of aqueous acetonitrile, and NH_4PF_6 was added. The solution was centrifuged, the resulting orange filter cake washed three times with water, and dried *in vacuo* to provide the monoacetonitrile complex **7b** as light orange solid (75 mg, 74%). ^1H -NMR (500 MHz, CD_3CN): δ (ppm) 8.75 (ddd, $J = 5.6, 1.5, 0.7$ Hz, 1H), 8.60 (s, 1H), 8.28 (s, 1H), 8.24 (d, $J = 8.1$ Hz, 1H), 8.17 (s, 1H), 7.92 (ddd, $J = 8.1, 6.7, 1.5$ Hz, 1H), 7.30 (ddd, $J = 7.4, 5.6, 1.4$ Hz, 1H), 2.92–3.06 (m, 3H), 2.83–2.88 (m, 1H), 2.59–2.74 (m, 3H), 2.45–2.50 (m, 1H), 2.33–2.39 (m, 1H), 2.25–2.31 (m, 2H), 2.10–2.16 (m, 1H). ^{13}C -NMR (125 MHz, CD_3CN): δ (ppm) 196.3, 170.0, 169.5, 164.7, 153.2, 151.8, 137.6, 133.0, 131.1, 126.9, 124.1, 120.6, 116.8, 35.4, 34.3, 33.7, 31.6, 30.6, 28.1. IR (film): ν (cm^{-1}) 3185, 3059, 1755, 1704, 1590, 1476, 1444, 1411, 1339, 1307, 1211, 1131, 1044, 835, 747, 677, 645. HRMS calcd for $\text{C}_{21}\text{H}_{22}\text{N}_3\text{O}_2\text{RuS}_3$ ($\text{M}-\text{PF}_6$) $^+$ 545.9916, found 545.9911.

Ruthenium carbonyl complex **8a**

Monoacetonitrile complex **7a** (25 mg, 32 μmol) was dissolved in DMF (3 mL), purged with CO gas for 30 sec, and heated under CO atmosphere to 95 °C for 3 h. The solvent was removed and the crude product subjected to silica gel chromatography with first acetonitrile and then acetonitrile:water:sat. KNO_3 solution 50:3:1. The combined product eluents were dried *in vacuo*, dissolved in a minimal amount of aqueous acetonitrile, and NH_4PF_6 was added. The solution was centrifuged, the resulting orange filter cake washed three times with water, and dried *in vacuo* to provide the carbonyl complex **8a** as orange solid (21 mg, 85%). $^1\text{H-NMR}$ (300 MHz, CD_3CN): δ (ppm) 8.55 (dd, $J = 5.4, 0.6$ Hz, 1H), 8.31–8.34 (m, 2H), 8.12 (s, 1H), 8.06 (dt, $J = 7.5, 1.5$ Hz, 1H), 7.25–7.41 (m, 6H), 4.82 (s, 2H), 3.27–3.36 (m, 1H), 3.10–3.23 (m, 3H), 2.83–2.96 (m, 3H), 2.70–2.81 (m, 2H), 2.53–2.64 (m, 2H), 2.35–2.46 (m, 1H). $^{13}\text{C-NMR}$ (125 MHz, CD_3CN): δ (ppm) 194.2, 180.0, 168.7, 168.6, 163.9, 153.7, 151.0, 139.4, 137.2, 132.8, 131.5, 128.7, 128.4, 127.7, 127.5, 125.2, 121.9, 118.0, 41.1, 37.0, 34.9, 34.8, 33.2, 32.3, 29.8. IR (film): ν (cm^{-1}) 2949, 1978, 1763, 1703, 1600, 1477, 1381, 1271, 1190, 1107, 1032, 966, 829, 750, 722. HRMS calcd for $\text{C}_{27}\text{H}_{25}\text{N}_2\text{O}_3\text{RuS}_3$ (M-PF_6) $^+$ 623.0071, found 623.0064.

Ruthenium carbonyl complex **8b**

Monoacetonitrile complex **7b** (15 mg, 22 μmol) was dissolved in DMF (3 mL), purged with CO gas for 30 sec, and heated under CO atmosphere to 95 °C for 3 h. The solvent was removed and the crude product subjected to silica gel chromatography with first acetonitrile and then acetonitrile:water:sat. KNO_3 solution 50:3:1. The combined product eluents were dried *in vacuo*, dissolved in a minimal amount of aqueous acetonitrile, and NH_4PF_6 was added. The solution was centrifuged, the resulting orange filter cake washed three times with water, and dried *in vacuo* to provide the carbonyl complex **8b** as orange solid (12 mg, 82%). $^1\text{H-NMR}$ (500 MHz, CD_3CN): δ (ppm) 8.81 (s, 1H), 8.55 (ddd, $J = 5.6, 1.5, 0.8$ Hz, 1H), 8.34 (dd, $J = 7.3, 0.7$ Hz, 1H), 8.29 (s, 1H), 8.09 (s, 1H), 8.06 (ddd, $J = 8.1, 7.6, 1.5$ Hz, 1H), 7.39 (ddd, $J = 7.5, 5.6, 1.4$ Hz, 1H), 3.29–3.34 (m, 1H), 3.11–3.21 (m, 3H), 2.91–2.96 (m, 1H), 2.82–2.90 (m, 2H), 2.71–2.79 (m, 2H), 2.57–2.64 (m, 2H), 2.39–2.45 (m, 1H). $^{13}\text{C-NMR}$ (125 MHz, CD_3CN): δ (ppm) 194.3, 179.6, 169.1, 169.0, 163.9, 153.7, 151.1, 139.3, 132.7, 132.3, 129.3, 125.2, 122.0, 117.9, 36.9, 34.9, 34.8, 33.2, 32.3, 29.8. IR (film): ν (cm^{-1}) 3223, 1970, 1765, 1713, 1560, 1480, 1450, 1408, 1343, 1307, 1221, 1171, 1133, 1042, 831, 745, 645. HRMS calcd for $\text{C}_{20}\text{H}_{19}\text{N}_2\text{O}_3\text{RuS}_3$ (M-PF_6) $^+$ 532.9596, found 532.9606.

Ruthenium isothiocyanate complex **9b**

Monoacetonitrile complex **7b** (23 mg, 33 μmol) was dissolved in DMF (3 mL). NaSCN (4 mg, 50 μmol) dissolved in water (300 μL) was added and the solution heated to 90 °C for 5 h. The solvent was removed and the crude product subjected to silica gel chromatography with CH_2Cl_2 :MeOH 35:1 to 10:1. The combined product eluents were dried *in vacuo* and the *N*-bound isomer was obtained as red solid (8 mg, 47%). Small amounts of the *S*-bound isomer (2 mg, 9%) were obtained as a purple solid. The coordination mode of the ambidentate thiocyanate ligand was verified by X-ray crystallography of a benzylated derivative.¹⁶

N-bound isomer: $^1\text{H-NMR}$ (300 MHz, $\text{DMSO-}d_6$): δ (ppm) 10.88 (s, 1H), 8.79 (d, $J = 5.1$ Hz, 1H), 8.42 (d, $J = 8.1$ Hz, 1H), 8.20 (s, 1H), 8.18 (s, 1H), 7.91 (t, $J = 7.2$ Hz, 1H), 7.33 (t, $J = 6.3$ Hz, 1H), 2.79–3.02 (m, 4H), 2.42–2.68 (m, 7H), 2.03–2.10 (m, 1H). $^{13}\text{C-NMR}$ (75 MHz, $\text{DMSO-}d_6$): δ (ppm) 201.5, 170.9, 170.2, 164.4, 152.8, 151.3, 136.8, 132.5, 132.1, 130.1, 125.2, 123.7, 120.2, 116.5, 34.7, 34.5, 33.5, 30.9, 30.4, 27.8. IR (film): ν (cm^{-1}) 2961, 2922, 2099, 1752, 1706, 1591, 1558, 1474, 1339, 1305, 1210, 1128, 1035, 820, 790, 722. HRMS calcd for $\text{C}_{20}\text{H}_{19}\text{N}_3\text{O}_2\text{RuS}_4\text{Na}$ ($\text{M}+\text{Na}$) $^+$ 585.9297, found 585.9296.

S-bound isomer: $^1\text{H-NMR}$ (300 MHz, $\text{DMSO-}d_6$): δ (ppm) 10.85 (s, 1H), 8.72 (dd, $J = 5.7$, 0.9 Hz, 1H), 8.39 (d, $J = 8.1$ Hz, 1H), 8.17 (s, 1H), 8.08 (s, 1H), 7.84–7.90 (m, 1H), 7.30 (ddd, $J = 7.5$, 5.7, 1.2 Hz, 1H), 2.62–2.93 (m, 11H), 2.07–2.14 (m, 1H). HRMS calcd for $\text{C}_{20}\text{H}_{19}\text{N}_3\text{O}_2\text{RuS}_4\text{Na}$ ($\text{M}+\text{Na}$) $^+$ 585.9297, found 585.9293.

Ruthenium isocyanate complex 10b

Monoacetonitrile complex **7b** (23 mg, 33 μmol) was dissolved in DMF (3 mL). NaOCN (3 mg, 50 μmol) dissolved in water (300 μL) was added and the solution heated to 95 $^\circ\text{C}$ for 3 h. The solvent was removed and the crude product subjected to silica gel chromatography with CH_2Cl_2 :MeOH 35:1 to 10:1. The combined product eluents were dried *in vacuo* to provide the isocyanate complex **10b** as red solid (10 mg, 55%). $^1\text{H-NMR}$ (300 MHz, $\text{DMSO-}d_6$): δ (ppm) 10.82 (s, 1H), 8.80 (dd, $J = 5.6$, 0.8 Hz, 1H), 8.37 (d, $J = 8.3$ Hz, 1H), 8.19 (s, 1H), 8.15 (s, 1H), 7.84–7.90 (m, 1H), 7.27–7.31 (m, 1H), 2.73–2.95 (m, 5H), 2.56–2.64 (m, 3H), 2.34–2.45 (m, 3H), 1.99–2.08 (m, 1H). $^{13}\text{C-NMR}$ (125 MHz, $\text{DMSO-}d_6$): δ (ppm) 205.4, 171.6, 170.8, 164.9, 153.1, 151.8, 136.8, 132.9, 130.3, 125.0, 123.9, 120.4, 116.8, 35.4, 34.8, 34.3, 31.2, 30.7, 28.0. IR (film): ν (cm^{-1}) 3436, 3154, 3052, 2181, 1751, 1700, 1620, 1587, 1473, 1405, 1338, 1306, 1037, 898, 816, 743. HRMS calcd for $\text{C}_{20}\text{H}_{19}\text{N}_3\text{O}_3\text{RuS}_3\text{Na}$ ($\text{M}+\text{Na}$) $^+$ 569.9526, found 569.9520.

Ruthenium selenocyanate complex 11b

Monoacetonitrile complex **7b** (13 mg, 19 μmol) was dissolved in DMF (3 mL). KSeCN (4 mg, 28 μmol) dissolved in water (300 μL) was added and the solution heated to 90 $^\circ\text{C}$ for 5 h. The solvent was removed and the crude product subjected to silica gel chromatography with CH_2Cl_2 :MeOH 35:1 to 10:1. The combined product eluents were dried *in vacuo* to provide the selenocyanate complex **11b** as red solid (11 mg, 96%). The coordination mode of the ambident selenocyanate ligand through the selenium atom was verified by the chemical shift in the $^{13}\text{C-NMR}$ spectrum. The signal at 108.2 ppm is in accordance with reported chemical shifts of coordinated selenocyanates.²⁶

$^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$): δ (ppm) 10.83 (s, 1H), 8.72 (d, $J = 5.0$ Hz, 1H), 8.38 (d, $J = 8.1$ Hz, 1H), 8.17 (s, 1H), 8.08 (s, 1H), 7.84–7.87 (m, 1H), 7.27–7.30 (m, 1H), 2.86–2.93 (m, 2H), 2.75–2.84 (m, 3H), 2.66–2.74 (m, 1H), 2.44–2.54 (m, 5H), 2.06–2.12 (m, 1H). $^{13}\text{C-NMR}$ (125 MHz, $\text{DMSO-}d_6$): δ (ppm) 201.8, 171.4, 170.7, 164.6, 153.2, 151.6, 136.7, 132.8, 130.6, 125.6, 124.0, 120.8, 116.9, 108.2, 35.3, 34.7, 33.4, 33.3, 32.4, 28.7. IR (film): ν (cm^{-1}) 3121, 3053, 2920, 2719, 2098, 1749, 1708, 1590, 1473, 1442, 1406, 1336, 1304, 1208, 1139, 1039, 1012, 949, 899, 817, 744. HRMS calcd for $\text{C}_{20}\text{H}_{19}\text{N}_3\text{O}_2\text{RuS}_3\text{SeNa}$ ($\text{M}+\text{Na}$) $^+$ 633.8747, found 633.8734.

Small molecule single crystal X-ray diffraction studies

Single crystals of complexes **4**, **5**, and **8a** were obtained upon slow diffusion of diethylether into dichloromethane at 6 °C. Single crystals of complex **7b** were obtained upon slow diffusion of diethylether into acetonitrile at 6 °C. The intensity data sets for all compounds were collected at 100 K using a STOE IPDS2 (**4**, **8a**) or IPDS-2T (**5**, **7b**) system. The data were corrected for absorption effects using multi scanned reflections.²⁷ The structure was solved using direct methods (SIR-92²⁸ for **7b** and **8a**, and SIR2008²⁹ for **4** and **5**) and refined using the full matrix least squares procedure implemented in SHELX-97.³⁰ Hydrogen atoms were included at calculated positions. In complex **5**, the position of the hydrogen atom bonded to iridium has been calculated using XHYDEX.³¹

Protein crystallography

The protein was expressed and purified as described previously.^{22a} To a solution of Pim1 (7 mg/mL) in 50 mM HEPES pH 7.5, 250 mM NaCl, 5 mM DTT, 5 % glycerol was added the racemic ruthenium complex **8b** (10 mM DMSO stock solution) to a concentration of 1 mM and the mixture was incubated on ice for 1 hour. Crystals of nonphosphorylated Pim1 were grown at 4 °C in 0.6 µL sitting drops using crystallization roboter Cartesian Microsys™ SQ 4004 (MarXTAL Laboratory, Philipps-University Marburg), where 0.3 µL of protein solution were mixed with 0.3 µL of the precipitate stock containing 200 mM sodium citrate, 100 mM HEPES, 30 % MPD pH 7.5 (JCSG Core Suite IV, Qiagen). The final concentration of complex **8b** was 0.5 mM and 5% DMSO resulting from the ruthenium stock solution. Crystals were obtained after 3–5 days and were soaked in the crystallization buffer supplemented with 25 % glycerol before being flash frozen in liquid nitrogen. The Pim1/**8b** complex was crystallized in spacegroup P6₅ with $\alpha = \beta = 90^\circ$, $\gamma = 120^\circ$ and $a = b = 99.2 \text{ \AA}$ and $c = 80.6 \text{ \AA}$. X-ray data were collected at beamline ID23-2 (14.20 keV, 0.8726 Å), European Synchrotron Radiation Facility (ESRF), Grenoble, France at 100 K from a single crystal with a MarMosaic 225 detector system. Data reduction was carried out using XDS and XSCALE.³² The structure was solved with Pim1 coordinates (PDB ID 3BWF)⁶ by molecular replacement using PHASER³³ of the CCP4³⁴ package. The Phaser solution with data up to 2.3 Å yielded one molecule per asymmetric unit with z scores of 24.0 for the rotation function and 55.9 for the translation. Further refinement was carried out with REFMAC5³⁵ and COOT³⁶ at 2.3 Å, which led to 2 outliers (2/273, 0.73%) and 7 residues in allowed regions (4/273, 2.6%) according to the Ramachandran plot. Final statistics reported R-factors of $R_{\text{work}} = 17.1\%$ and $R_{\text{free}} = 20.1\%$ for the Pim1/**8b** complex (Table 2).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Literature

1. Metal complexes for medicine and the life sciences:(a) Thompson KH, Orvig C. *Science*. 2003; 300:936–939. [PubMed: 12738851] (b) Ang WH, Dyson PJ. *Eur J Inorg Chem*. 2006:4003–4018. (c) Hambley TW. *Dalton Trans*. 2007:4929–4937. [PubMed: 17992277] (d) Hambley TW. *Science*. 2007; 318:1392–1393. [PubMed: 18048674] (e) Mukherjee A, Sadler PJ. *Wiley Encycl Chem Biol*. 2009:1–47.(f) Haas KL, Franz KJ. *Chem Rev*. 2009; 109:4921–4960. [PubMed: 19715312] (g) Levina A, Mitra A, Lay PA. *Metallomics*. 2009; 1:458–470. [PubMed: 21305154] (h) Hillard EA, Jaouen G. *Organometallics*. 2011; 30:20–27.(i) Gasser G, Ott I, Metzler-Nolte N. *J Med Chem*. 2011; 54:3–25. [PubMed: 21077686]
2. (a) Meggers E. *Chem Commun*. 2009:1001–1010.(b) Davies CL, Dux EL, Duhme-Klair AK. *Dalton Trans*. 2009:10141–10154. [PubMed: 19921045] (c) Che CM, Siu FM. *Curr Opin Chem Biol*. 2010; 14:255–261. [PubMed: 20018553]
3. For recent examples of metal-based enzyme inhibitors, see:(a) Can D, Spingler B, Schmutz P, Mendes F, Raposinho P, Fernandes C, Carta F, Innocenti A, Santos I, Supuran CT, Alberto R. *Angew Chem Int Ed*. 2012; 51:3354–3357.(b) Salmon AJ, Williams ML, Hofmann A, Poulsen SA. *Chem Commun*. 2012; 48:2328–2330.(c) Spencer J, Amin J, Wang M, Packham G, Alwi SSS, Tizzard GJ, Coles SJ, Paranal RM, Bradner JE, Heightman TD. *ACS Med Chem Lett*. 2011; 2:358–362. [PubMed: 21572592] (d) Monnard FW, Heinisch T, Nogueira ES, Schirmer T, Ward TR. *Chem Commun*. 2011; 47:8238–8240.
4. (a) Zeglis BM, Pierre VC, Barton JK. *Chem Commun*. 2007:4565–4579.(b) Keene FR, Smith JA, Collins JG. *Coord Chem Rev*. 2009; 253:2021–2035.(c) Hannon MJ. *Chem Soc Rev*. 2007; 36:280–295. [PubMed: 17264930] (d) Liu HK, Sadler PJ. *Acc Chem Res*. 2011; 44:349–359. [PubMed: 21446672]
5. (a) Feng L, Geisselbrecht Y, Blanck S, Wilbuer A, Atilla-Gokcumen GE, Filippakopoulos P, Kräling K, Celik MA, Harms K, Maksimoska J, Marmorstein R, Frenking G, Knapp S, Essen LO, Meggers E. *J Am Chem Soc*. 2011; 133:5976–5986. [PubMed: 21446733] (b) Maksimoska J, Feng L, Harms K, Yi C, Kissil J, Marmorstein R, Meggers E. *J Am Chem Soc*. 2008; 130:15764–15765. [PubMed: 18973295]
6. Maksimoska J, Williams DS, Atilla-Gokcumen GE, Smalley KSM, Carroll PJ, Webster RD, Filippakopoulos P, Knapp S, Herlyn M, Meggers E. *Chem Eur J*. 2008; 14:4816–4822. [PubMed: 18425743]
7. Dieckmann S, Riedel R, Harms K, Meggers E. *Eur J Inorg Chem*. 2012:813–821.
8. Wilbuer A, Vlecken DH, Schmitz DJ, Kräling K, Harms K, Bagowski CP, Meggers E. *Angew Chem Int Ed*. 2010; 49:3839–3842.
9. Williams DS, Carroll PJ, Meggers E. *Inorg Chem*. 2007; 46:2944–2946. [PubMed: 17375909]
10. For the design of metal-containing protein kinase inhibitors, see also:(a) Spencer J, Mendham AP, Kotha AK, Richardson SCW, Hillard EA, Jaouen G, Male L, Hursthouse MB. *Dalton Trans*. 2009:918–921. [PubMed: 19173072] (b) Biersack B, Zoldakova M, Effenberger K, Schobert R. *Eur J Med Chem*. 2010; 45:1972–1975. [PubMed: 20149940]
11. For the structure of the catalytic cleft, see:(a) Zhang J, Yang PL, Gray NS. *Nature Rev Cancer*. 2009; 9:28–39. [PubMed: 19104514] (b) Liao JLL. *J Med Chem*. 2007; 50:409–424. [PubMed: 17266192]
12. (a) Bregman H, Williams DS, Meggers E. *Synthesis*. 2005:1521–1527.(b) Pagano N, Maksimoska J, Bregman H, Williams DS, Webster RD, Xue F, Meggers E. *Org Biomol Chem*. 2007; 5:1218–1227. [PubMed: 17406720]
13. Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S. *Science*. 2002; 298:1912–1934. [PubMed: 12471243]
14. Blanck S, Cruchter T, Vultur A, Riedel R, Harms K, Herlyn M, Meggers E. *Organometallics*. 2011; 30:4598–4606. [PubMed: 21918590]
15. For cyclometalated metal complexes with biological activity, see:(a) Djukic JP, Sortais JB, Barloy L, Pfeffer M. *Eur J Inorg Chem*. 2009:817–853.(b) Meng X, Leyva ML, Jenny M, Gross I, Benosman S, Fricker B, Harlepp S, Hébraud P, Boos A, Wlosik P, Bischoff P, Sirlin C, Pfeffer M, Loeffler JP, Gaiddon C. *Cancer Res*. 2009; 69:5458–5466. [PubMed: 19549908]

16. Blanck S, Maksimoska J, Baumeister J, Harms K, Marmorstein R, Meggers E. *Angew Chem Int Ed.* 2012; 51:5244–5246.
17. Gill TP, Mann KR. *Organometallics.* 1982; 1:485–488.
18. Landgrafe C, Sheldrick WS. *Dalton Trans.* 1994:1885–1893.
19. See, for example:(a) Ryabov AD, Sukharev VS, Alexandrova L, Le Lagadec R, Pfeffer M. *Inorg Chem.* 2001; 40:6529–6532. [PubMed: 11720516] (b) Bregman H, Carroll PJ, Meggers E. *J Am Chem Soc.* 2006; 128:877–884. [PubMed: 16417378] (c) Mulcahy SP, Gründler K, Frias C, Wagner L, Prokop A, Meggers E. *Dalton Trans.* 2010; 39:8177–8182. [PubMed: 20689887]
20. Fabian MA, Biggs WH III, Treiber DK, Atteridge CE, Azimioara MD, Benedetti MG, Carter TA, Ciceri P, Edeen PT, Floyd M, Ford JM, Galvin M, Gerlach JL, Grotzfeld RM, Herrgard S, Insko DE, Lai AG, Lélías J-M, Mehta SA, Milanov ZV, Velasco AM, Wodicka LM, Patel HK, Zarrinkar PP, Lockhardt DJ. *Nat Biotechnol.* 2005; 23:329–336. [PubMed: 15711537]
21. Karaman MW, Herrgard S, Treiber DK, Gallant P, Atteridge CE, Campbell BT, Chan KW, Ciceri P, Davis MI, Edeen PT, Faraoni R, Floyd M, Hunt JP, Lockhardt DJ, Milanov ZV, Morrison MJ, Pallares G, Patel HK, Pritchard S, Wodicka LM, Zarrinkar PP. *Nat Biotechnol.* 2008; 26:127–132. [PubMed: 18183025]
22. (a) Debreczeni JÉ, Bullock AN, Atilla GE, Williams DS, Bregman H, Knapp S, Meggers E. *Angew Chem Int Ed.* 2006; 45:1580–1585.(b) Bullock AN, Russo S, Amos A, Pagano N, Bregman H, Debreczeni JÉ, Lee WH, von Delft F, Meggers E, Knapp S. *PLoS One.* 2009; 4:e7112. [PubMed: 19841674] (c) Atilla-Gokcumen GE, Di Costanzo L, Meggers E. *J Biol Inorg Chem.* 2011; 16:45–50. [PubMed: 20821241]
23. Brotschi C, Mathis G, Leumann CJ. *Chem Eur J.* 2005; 11:1911–1923. [PubMed: 15685710]
24. Ranger M, Rondeau D, Leclerc M. *Macromolecules.* 1997; 30:7686–7691.
25. Yang D, Long Y, Wang H, Zhang Z. *Org Lett.* 2008; 10:4723–4726. [PubMed: 18834138]
26. (a) Poleschner H, Radeaglia R, Kuprat M, Richter AM, Fanghänel E. *J Organomet Chem.* 1987; 327:7–15.(b) Bellama JM, Tandon SK. *Inorg Chim Acta.* 1985; 102:23–27.
27. Spek, AL. *PLATON, A Multipurpose Crystallographic Tool.* Utrecht University; Utrecht, The Netherlands: 1998.
28. Altomare A, Cascarano G, Giacovazzo C, Guagliardi A. *J Appl Cryst.* 1993; 26:343–350.
29. Burla MC, Caliendo R, Camalli M, Carrozzini B, Cascarano GL, De Caro L, Giacovazzo C, Polidori G, Siliqi D, Spagna R. *J Appl Cryst.* 2007; 40:609–613.
30. Sheldrick GM. *Acta Cryst A.* 2008; 64:112–122. [PubMed: 18156677]
31. Orpen AG. *J Chem Soc Dalton Trans.* 1980:2509–2516.
32. Kabsch W. *Acta Cryst D.* 2010; 66:125–132. [PubMed: 20124692]
33. McCoy AJ, Grosse-Kunstleve RW, Adams PD, Winn MD, Storoni LC, Read RJ. *J Appl Cryst.* 2007; 40:658–674. [PubMed: 19461840]
34. Collaborative computational project, number 4. *Acta Cryst D.* 1994; 50:760–763. [PubMed: 15299374]
35. Murshudov GN, Vagin AA, Dodson EJ. *Acta Cryst D.* 1997; 53:240–255. [PubMed: 15299926]
36. Emsley P, Lohkamp B, Scott WG, Cowtan K. *Acta Cryst D.* 2010; 66:486–501. [PubMed: 20383002]

Figure 1.

Comparison of different metal-containing structural scaffolds for the design of ATP-competitive inhibitors of protein kinases. Shown are the intended interactions with the hinge region of the ATP-binding site. Note that (a) not all protein kinases form two hydrogen bonds from the hinge region to the adenine base of ATP and (b) a second binding orientation of the maleimide inhibitors is feasible.

Figure 2. Crystal structure of ruthenium half-sandwich complex **4**. ORTEP drawing with 50% probability thermal ellipsoids. Selected bond distances (Å): C1-Ru1 = 2.048(4), N11-Ru1 = 2.089(3), C100-Ru1 = 1.827(4), C23-Ru1 = 2.264(4).

Figure 3. Crystal structure of iridium(III) complex **5**. ORTEP drawing with 50% probability thermal ellipsoids. Selected bond distances (Å): C1-Ir1 = 2.001(8), N11-Ir1 = 2.126(7), Cl1-Ir1 = 2.483(2), P1-Ir1 = 2.3417(18), P2-Ir1 = 2.3300(17).

Figure 4.

Crystal structure of ruthenium acetonitrile complex **7b**. A solvent molecule of diethyl ether and a hexafluorophosphate counter ion are omitted for clarity. ORTEP drawing with 50% probability thermal ellipsoids. Selected bond distances (Å): C1-Ru1 = 2.039(4), N11-Ru1 = 2.093(3), N1-Ru1 = 2.055(4), S1-Ru1 = 2.2988(10), S2-Ru1 = 2.2988(10), S3-Ru1 = 2.4108(9).

Figure 5. Crystal structure of the *N*-benzylated complex **8a**. The hexafluorophosphate counter ion is omitted for clarity. ORTEP drawing with 50% probability thermal ellipsoids. Selected bond distances (Å): C1–Ru1 = 2.055(4), N10–Ru1 = 2.105(3), C29–Ru1 = 1.859(4), S1–Ru1 = 2.3297(11), S2–Ru1 = 2.4235(10), S3–Ru1 = 2.4102(11).

Figure 6.

Binding selectivities of complexes **8b** and **9b** within the human protein kinase dendrogram which displays the protein kinase families and the evolutionary relationships between the individual kinases (wild type). Determined by an active-site-directed affinity screening (KINOMEscan, DiscoveRx) against 442 human protein kinases. Shown are hits with %ctrl < 1% (below 1% of control; 0% = highest affinity, 100% = no affinity) at compound concentrations of 10 μ M. See ESI for additional data.

Figure 7. Cocrystal structure of protein kinase Pim1 with one enantiomer of phthalimide **8b** bound to the ATP-binding site. SIGMAA-weighted $2F_{\text{obs}} - F_{\text{calc}}$ difference electron density map of the ruthenium inhibitor was contoured at 1σ . Coordinates of the structure have been deposited in the Protein Data Bank (PDB ID: 4AS0).

Figure 8. Interactions of phthalimide **8b** (PDB ID: 4AS0, PDB ligand identifier **RPS**) within the active site of Pim1.

Figure 9. Relative binding modes of **8b** (light brown, PDB ID: 4AS0, PDB ligand identifier **RPS**) and **9b** (white, PDB ID: 4DAW, PDB ligand identifier **OH2**) within the ATP-binding sites of Pim1 and PAK1, respectively. Superimposed with the PyMOL Molecular Graphics System, Version 1.3, Schrödinger, LLC.

Scheme 1.

Synthesis of the pyridylphthalimides **2a–c**. TBS = *tert*-butyldimethylsilyl.

Scheme 2.

Regioselective C-H activation of ligand **2a**. Synthesis of the pseudo-octahedral ruthenium half-sandwich complex **4** and the octahedral iridium complex **5**.

Scheme 3.

Synthesis of a library of bioactive octahedral metal complexes **8a,b** and **9b–11b** via regioselective C-H-activation at the 5-position of the phthalimide moiety followed by a ligand substitution reaction. MX = NaOCN, NaSCN, or KSeCN.

Table 1Crystallographic data for **4**, **5**, **7b**, and **8a**.^a

	4	5	7b	8a
formula	C ₂₆ H ₁₈ N ₂ O ₃ Ru	C ₅₆ H ₄₄ ClIr N ₂ O ₂ P ₂ , 1.63 CHCl ₃	C ₂₅ H ₃₂ N ₃ O ₃ RuS ₃ PF ₆	C ₂₇ H ₂₅ N ₂ O ₃ RuS ₃ PF ₆
fw	507.49	1260.49	764.76	767.71
a(Å)	10.9398(5)	9.3488(3)	25.2192(9)	9.1961(5)
b(Å)	11.2895(4)	24.1361(8)	10.9005(5)	12.0944(7)
c(Å)	16.6190(6)	49.146(2)	24.1706(9)	13.5948(8)
α(°)	90	90	90	70.913(4)
β(°)	90	90	115.242(3)	75.580(5)
γ(°)	90	90	90	83.035(5)
V(Å ³)	2052.53(14)	11089.6(7)	6010.1(4)	1382.58(14)
Z	4	8	8	2
space group	P 2 ₁ 2 ₁ 2 ₁	P b c a	C 2/c	P –1
d _{calcd} (Mg/m ³)	1.642	1.510	1.690	1.844
μ(mm ⁻¹)	0.796	2.792	0.854	0.928
θ range(°)	2.18 – 25.0	2.49 – 25.0	4.54 – 25.0	1.63 – 25.0
no. of indep. reflections	3614	9746	5248	4749
no. of parameters	289	651	442	388
wR2 (all data) ^b	0.0462	0.1111	0.1028	0.0865
R1 (I > 2σ(I)) ^b	0.0268	0.0526	0.0383	0.0368
CCDC no. ^c	861334	861335	861336	861337

^a MoKα radiation (λ = 0.71073 Å).^b $R1 = \sum ||F_o| - |F_c|| / \sum |F_o|$; $wR2 = [\sum w(F_o^2 - F_c^2)^2 / \sum w(F_o^2)^2]^{1/2}$.^c Crystallographic data (excluding structure factors) have been deposited in the Cambridge Crystallographic Data Center. CIF files can be obtained from the CCDC free of charge via http://www.ccdc.cam.ac.uk/data_request/cif.

Table 2Data collection and refinement statistics for the complex Pim1/**8b**.

<i>Data collection & processing</i>	
X-ray source	ESRF, Grenoble, France
detector	MarMOSAIC 225
wavelength (Å)	0.8726 Å
space group	<i>P</i> 6 ₅
cell dimensions (<i>a, b, c</i> Å / α, β, γ °)	99.2, 99.2, 80.6 90.0, 90.0, 120.0
resolution (Å) *	24.8–2.3 (2.4–2.3)
total reflections *	128610 (15302)
multiplicity *	6.4 (6.4)
unique reflections *	20098 (2386)
<i>R</i> _{merge} (%) *	12.8 (51.5)
completeness (%) *	99.8 (100.0)
<i>I</i> / σ (<i>I</i>) *	16.6 (4.81)
Wilson B-factor (Å ²)	28.3
<i>refinement statistics</i>	
resolution (Å)	24.8–2.3
<i>R</i> _{factor} , <i>R</i> _{free} (%)	17.1, 20.1
reflections (working, test set)	19383 (711)
completeness for range (%)	99.90
r.m.s.d. from ideal:	
bond lengths (Å)	0.014
bond angles (°)	1.44
total number of non-hydrogen atoms	2383
mean <i>B</i> value (Å ²)	24.0

* in parenthesis is given the value for the highest resolution shell.