

An Organometallic Inhibitor for Glycogen Synthase Kinase 3

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The vast majority of specific enzyme inhibitors are small organic molecules that gain their specificity by a combination of weak interactions, including hydrogen bonding, electrostatic contacts, and hydrophobic interactions. In contrast, inorganic compounds find applications in medicinal chemistry predominately for their reactivity and imaging properties.¹ We started a research program that aims in exploring the versatility of organometallic and inorganic compounds as structural scaffolds for the design of specific enzyme inhibitors.^{2,3} It is noteworthy that coordinative bonds with transition metals such as ruthenium can reach kinetic stabilities that are comparable with those of covalent bonds.⁴ With this in mind, a ruthenium center may be considered as a virtual “hypervalent carbon” with unique structural opportunities.

We recently introduced a strategy for developing ruthenium complexes that target the ATP-binding site of protein kinases by copying structural features of small organic molecule inhibitors.⁵ The adenine base of ATP is lined with a cleft-forming set of conserved hydrophobic residues and forms two hydrogen bonds to the backbone of the hinge between the N-terminal and C-terminal domain.⁶ Small-molecule inhibitors usually copy this binding mode.⁷ For example, the protein kinase inhibitor staurosporine **1** contains the planar hydrophobic indolo[2,3-*a*]carbazole aglycon **2a** in which the lactam moiety mimics the hydrogen bonding pattern of the adenine base (Figure 1).⁸

We envisioned that replacing the indolocarbazole alkaloid scaffold with metal complexes in which the structural features of the indolocarbazole aglycon **2a** or the related arcyriaflavin A **2b** are retained in one of the ligands would allow metal complexes to be targeted to the ATP-binding site of protein kinases. Potent and specific inhibitors for a particular kinase could then be obtained by assembling elaborate structures around the metal center. As a demonstration of this concept, we here report the organometallic ruthenium compound **3** as an extremely potent inhibitor for the glycogen synthase kinase 3 (GSK-3).

The key component of our design is the novel pyridocarbazole ligand **4**, derived from arcyriaflavin A **2b** by just replacing one indole moiety with a pyridine (shown in red in Figure 1). An X-ray structure of the N-benzylated derivative of **3** proves that ligand **4** can in fact serve as a bidentate ligand for ruthenium, having one classical coordinative bond with the pyridine (Ru1–N19 = 2.13 Å) in addition to one covalent σ -bond with the indole nitrogen (Ru1–N2 = 2.11 Å) (indicated in green in Figure 1).⁹ The coordination sphere is further filled up by a cyclopentadienyl and CO group. This neutral half-sandwich 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.

Screening a small library of ruthenium complexes against a panel of protein kinases identified **3** as an extremely potent inhibitor for GSK-3. The concentration at which 50% of the enzyme is inhibited (IC₅₀) is 3 nM for GSK-3 α (α -isoform) and 10 nM for GSK-3 β (β -isoform). Ruthenium complex **3** also displays a high degree of

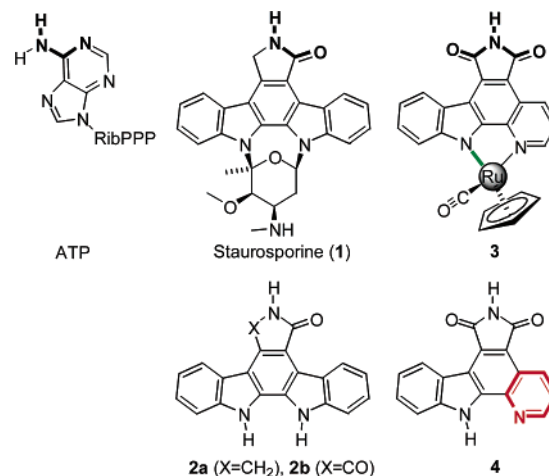


Figure 1. Mimicking indolocarbazole protein kinase inhibitors with metal complexes.

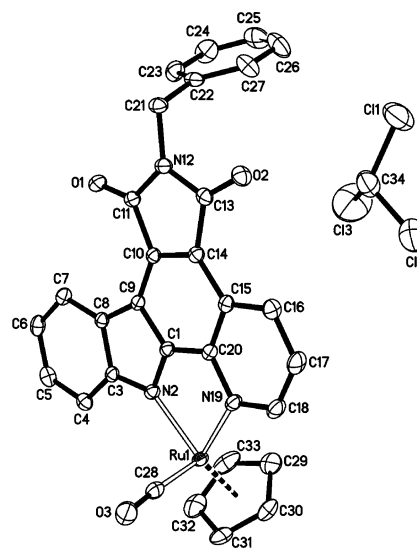


Figure 2. X-ray structure of the N-benzylated derivative of **3**. ORTEP drawing with 35% probability thermal ellipsoids.

selectivity. For example, Abl (IC₅₀ = 5 μ M), CDK2/Cyclin A (IC₅₀ = 3 μ M), CHK1 (IC₅₀ = 25 μ M), Lck (IC₅₀ = 3 μ M), MAPK1/Erk1 (IC₅₀ > 100 μ M), PKC α (100 μ M), c-Src (4 μ M), and ZAP-70 (IC₅₀ = 15 μ M) all yield just micromolar inhibition. Only RSK1 shows a submicromolar inhibition with an IC₅₀ of 100 nM.

The IC₅₀ curves of the racemic mixture of **3** and the corresponding pyridocarbazole ligand **4** against GSK-3 α are shown in Figure 3 (red and green curves, respectively). The pyridocarbazole ligand **4** itself is a very weak inhibitor for GSK-3 with an IC₅₀ of only 50 μ M. This means that upon formation of the metal complex **3**, the potency increases by a factor of more than 15000. Consequently, the activity of complex **3** requires the entire assembly, kept together by the central ruthenium atom.

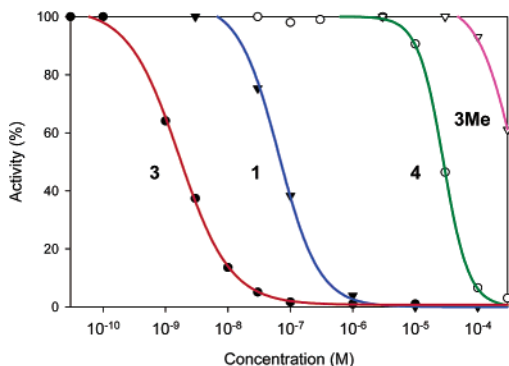


Figure 3. IC_{50} curves with GSK-3 α obtained by phosphorylation of a substrate with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$: red, racemic complex **3** ($IC_{50} = 3$ nM); blue, staurosporine **1** ($IC_{50} = 50$ nM); green, pyridocarbazole **4** ($IC_{50} = 50$ μM); pink, **3Me**, the N-methylated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

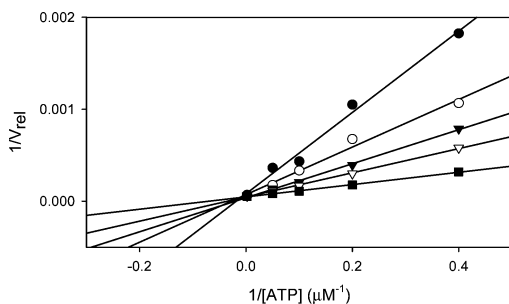


Figure 4. Double-reciprocal plots of relative initial velocities (V_{rel}) against varying ATP concentrations in the presence of 0 (■), 1 nM (▼), 2 nM (▽), 4 nM (○), and 8 nM (●) concentrations of **3**. The plots intersect at the $1/V_{\text{rel}}$ axis, confirming that **3** binds competitively with respect to ATP.

To test if **3** does, as designed, bind to the ATP site, we synthesized a derivative of **3** with the imide hydrogen replaced by a methyl group (**3Me**). This methylation abolishes the activity completely ($IC_{50} > 300$ μM , see pink curve in Figure 3), consistent with the assumption that the imide hydrogen is involved in hydrogen bonding with the adenine binding cleft. Additionally, a Lineweaver–Burk analysis (Figure 4) of relative initial velocities of GSK-3 α at different concentrations of ATP and **3** reaffirms ATP competitive binding and yields an inhibition constant (K_i) of 0.98 ± 0.1 nM.

Ruthenium complex **3** is pseudotetrahedral and possesses metal-centered chirality.¹⁰ Interestingly, the activities of the individual enantiomers differ only 2-fold ($IC_{50} = 2$ and 4 nM). To gain insight into the binding mode of **3** within the ATP-binding site of GSK-3, we modeled **3** into the active site of a cocrystallized structure of GSK-3 β with staurosporine.¹¹ It is likely that both enantiomers bind similarly due to their symmetrical imide group, which allows for the same orientation of the CO and cyclopentadienyl ligands in the active site just by rotation of 180° around the pyridocarbazole. Figure 5 shows the interactions of the **3**- R_{Ru} enantiomer with the active site of GSK-3 β . As designed, the imide-NH undergoes hydrogen bonding with the backbone carbonyl of Asp133 and one imide carbonyl group of **3**- R_{Ru} undergoes a hydrogen bond with the backbone amide-NH of Val135. A water-mediated contact is observed between the carbonyl ligand and the carboxylate of Gln185. This ordered water molecule appears to be unique for GSK-3 and may at least be in part responsible for the observed specificity.^{8,11}

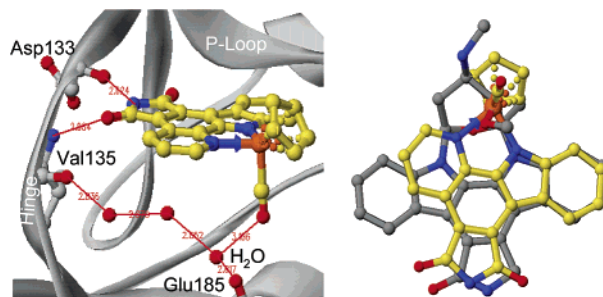


Figure 5. Molecular modeling (CACE, Fujitsu). Left: Interactions of **3**- R_{Ru} with the ATP binding site of GSK-3 β (PDB code 1Q3D). Right: Overlay of the cocrystallized position of staurosporine in GSK-3 β with the docked position of **3**- R_{Ru} .

An overlay of the cocrystallized position of staurosporine with the docked binding position of **3** demonstrates how closely ruthenium complex **3** copies the binding mode of staurosporine (Figure 5). The pyridocarbazole occupies the binding site of the indolocarbazole moiety, and the cyclopentadienyl and CO ligand replace the glycosidic ring in the ribose binding site. The ruthenium center is not involved in any direct interactions and serves entirely as an innocent bystander, helping to organize the positions of the ligands in the receptor space.

In conclusion, we have described a new strategy for the design of ruthenium complexes as protein kinase inhibitors by mimicking the structure of organic indolocarbazoles. Ruthenium complex **3** is an order of magnitude more potent than staurosporine (IC_{50} of 50 nM against GSK-3 α , see blue curve in Figure 3) and compares well in terms of potency and selectivity with the best published organic GSK-3 inhibitors to date.¹² Such an unprecedented property of an organometallic compound indicates that our approach may lead to a novel class of metallotherapeutics.

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Supporting Information Available: Experimental details, spectroscopic data, and crystallographic data (PDF, CIF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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