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Ruthenium Complexes as Protein Kinase Inhibitors

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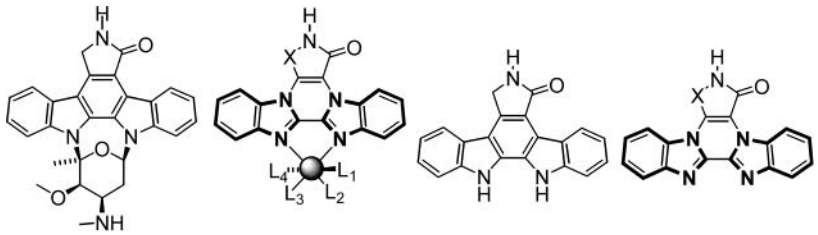
ABSTRACT



Replacing complex natural products with simple metal complexes could lead to a new class of metallopharmaceuticals in which the metal center plays mainly a structural role. A strategy is introduced for the creation of ruthenium complex-based protein kinase inhibitors 1 (X = CO or CH_2), morphed out of the class of indolocarbazole inhibitors with the alkaloid staurosporine as its most prominent member.

The development of small molecules that perturb specific protein functions is of great importance for probing biological processes and ultimately for the generation of potent and safe drugs. Medicinal chemistry is predominately focused on the design of organic molecules, whereas the incorporation of inorganic components into drugs is much less investigated.[1] We are interested in exploring organometallic and inorganic compounds as structural scaffolds for enzyme inhibition.[2,3] Such metal−ligand assemblies allow convergent synthetic approaches and give access to structural motifs that differ from purely organic molecules. Our efforts are focused on ruthenium complex scaffolds because ruthenium offers a hexavalent coordination sphere that cannot be easily obtained by any organic element. In addition, ruthenium tends to form kinetically very inert coordinative bonds, making it possible to obtain compounds that display stabilities that are comparable to purely organic molecules. We here introduce a strategy that uses a class of natural products as a lead for a ruthenium complex scaffold.

Protein kinases regulate most aspects of cellular life and are one of the main drug targets.[4−6] The microbial alkaloid staurosporine is a very potent but relatively nonspecific inhibitor of many protein kinases (for the structure, see Abstract).[7] Many staurosporine derivatives and related organic compounds with modulated specificities have been

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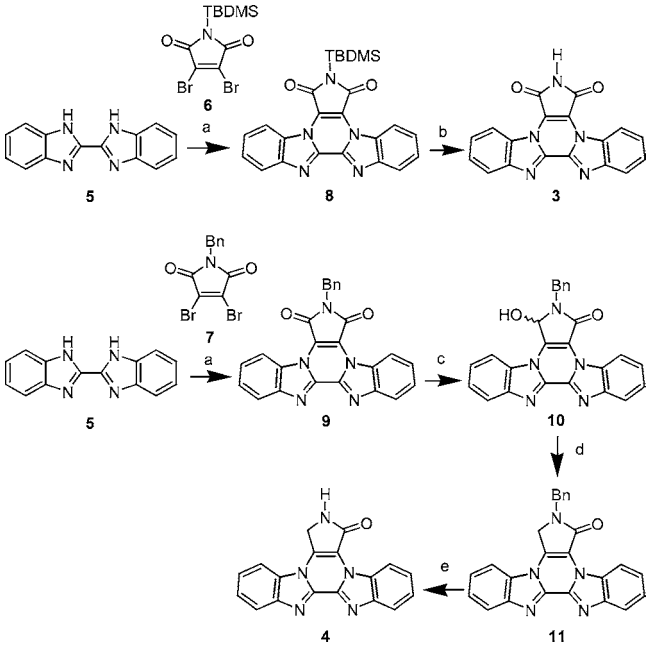
Scheme 1. Synthesis of Ligands **3** and **4**[a]



[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%).

developed, and several are in clinical trials as anticancer drugs.[5] They all share the indolo[2,3-a]carbazole aglycon **2**, which binds to the ATP binding site and can hydrogen bond with two conserved amino acids.[8] For this class of inhibitors, specificity for a particular protein kinase can be achieved by the moiety that is attached to the indole nitrogen atoms.

We envisioned that by replacing the indolocarbazole alkaloid scaffold with metal complex **1**, elaborate structures could be assembled in an efficient manner by variation of the ligands. Key components of our design are ligands **3** and **4**, derived from the indolocarbazole aglycon **2** by just replacing two carbon against two nitrogen atoms. This transformation does not change the shape of the ligand but generates two benzimidazole moieties (bold in the structure in Abstract) that can function as coordination sites for the ruthenium center. The remaining four coordination sites at the ruthenium can become filled by ligands L_1-L_4 and substitute for the carbohydrate moiety, with the metal center serving as a "glue" for holding all parts together.

Bisbenzimidazolomaleimide **3** was synthesized in an economical fashion from readily available precursors in two steps by condensing deprotonated bisbenzimidazole **5** with *N*-TBDMS-2,3-dibromomaleimide **6** followed by deprotection with TBAF to afford **3** (Scheme 1). Lactam **4** was obtained by condensing deprotonated bisbenzimidazole **5** with *N*-benzyl-2,3-dibromomaleimide **7**, yielding **9**, followed by a reduction and deprotection sequence as shown in Scheme 1. Accordingly, one carbonyl group of **9** was first reduced to the alcohol **10** with $NaBH_4$, followed by acetylation of the alcohol with acetic anhydride and reductive elimination to **11** after addition of zinc dust.[9] Finally, deprotection of the benzyl group under acidic conditions yielded the lactam **4**.[10]

Despite their unique geometry, having nitrogen donor ligands from two five-membered rings annulated to a central six-membered ring, **3** and **4** are able to serve as bidentate ligands. For example, refluxing the benzyl derivative **9** with *cis*-$RuCl_2(DMSO)_4$ in toluene yielded diastereoselectively the cis(Cl),cis(DMSO) complex **12a**, which isomerized to the cis(Cl),trans(DMSO) isomer upon crystallization from chloroform (Figure 1). The structure reveals that the two

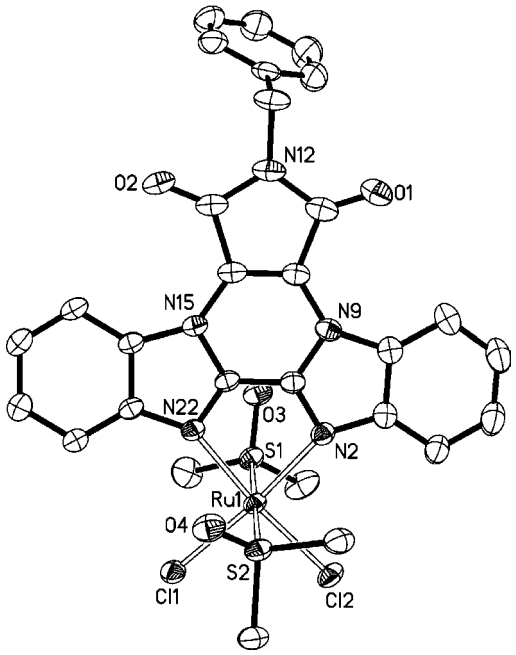


Figure 1. ORTEP drawing with 35% probability thermal elipsoids of the X-ray structure obtained upon crystallization of complex **12a**. The complex isomerized to the cis(Cl),trans(DMSO) isomer.

benzimidazoles can indeed serve as coordination sites for the ruthenium and that the ligand **9** is, as expected, almost superimposable to the indolocarbazole **2**. The ruthenium−nitrogen bonds are remarkably long with distances of 2.15 and 2.16 Å, respectively, supposedly a consequence of both

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the open biting angle of the two nitrogens and their large distance apart. Interestingly, a comparison with the crystal structure of the free ligand **9** reveals that, upon complexation, the distance between the two coordinating nitrogen atoms decreases from 3.05 to 2.80 Å, a remarkable change in length of 8.2%.

The synthesis of ruthenium complexes with unprotected maleimide nitrogens was accomplished by using the TBDMS-modified ligand **8**. Accordingly, reaction of **8** with *cis*-$RuCl_2(DMSO)_4$ yielded diastereoselectively the unprotected ruthenium complex **12b** in one step (Figure 2). Reaction of

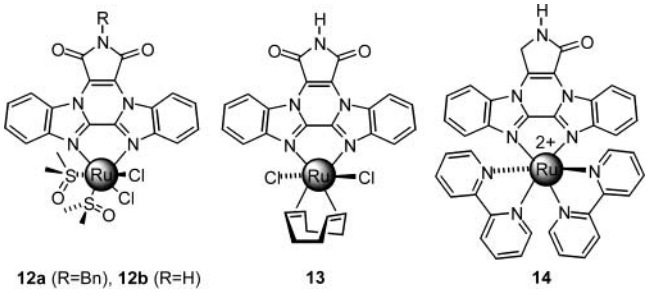

Figure 2. Synthesized ruthenium complexes **12**−**14**. See Supporting Information for experimental details of the synthesis.

8 with $Ru(COD)(CH_3CN)_2Cl_2$ followed by treatment with TBAF yielded **13**. Refluxing the lactam **4** in ethanol with $Ru(bpy)_2(EtOH)_2^{2+}$, generated from $Ru(bpy)_2Cl_2$ and $AgOSO_2CF_3$ in situ, yielded the ionic complex **14**.

All three metal complex scaffolds are air-stable and can be stored on the bench for weeks without any signs of decomposition. Bipyridine complex **14** is completely stable in a 1:1 water/DMSO solution for 12 h and can even withstand a 1 mM methanolic solution of 2-mercaptoethanol for 3 h without any decomposition. Time-dependent ^1H NMR measurements show that the compounds **12b** and **13** slowly release ligand **3** in 1:1 water/DMSO mixtures, with half-lives of 8 and 3 h, respectively.[11] However, their stability was sufficient for examining their potential as protein kinase inhibitors.

We examined the potency of ruthenium complexes **12b**, **13**, and **14** against a small panel of protein kinases and compared the results to the potency of the free ligands **3** and **4**. Table 1 gives the concentrations of compounds required for 50% inhibition (IC_{50}). As expected, **3** and **4** have some affinity against most of the tested kinases. The important observation is that upon formation of the ruthenium complexes, affinity and specificity become modulated. For example, the bipyridine complex **14** is the only compound that inhibits protein kinase C α (PKCα) below 100 μM. On the other hand, the COD complex **13** is the best inhibitor

(11) Stability of ruthenium complexes with **3** and **4** is coupled to the electronic nature of the other ligands. Ligand **3** seems to prefer an electron-rich center, whereas **4** prefers a more electron-poor ruthenium center.

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.

for the Abelson tyrosine kinase (Abl) with an IC_{50} of 2 μM, which is more than 10 times lower than the IC_{50} of the corresponding free ligand **3**. Additionally, the precursor $Ru(COD)(CH_3CN)_2Cl_2$ does not show any signs of inhibition against Abl even at 100 μM. Consequently, the activity of compound **13** requires the entire assembly, kept together by the central ruthenium ion. To test if **13** does, as designed, bind to the ATP site, we synthesized a derivative of **13** with the imide hydrogen replaced by a benzyl group. This derivative shows a potency that is strongly reduced by a factor of 25, consistent with the assumption that the imide hydrogen is involved in hydrogen bonding within the adenine binding cleft. Additionally, a Lineweaver−Burk analysis of the initial velocities with Abl at different ATP concentrations and fixed concentrations of ruthenium compound **14** reaffirms ATP competitive binding (see Supporting Information for details). It is also noteworthy that the specificities of the COD complex **13** and bipyridine complex **14** are different from that of staurosporine. In our assays, staurosporine is a nanomolar inhibitor for all tested kinases, except for Abl, against which it has an IC_{50} of 2 μM.

To our knowledge, this is the first report of ruthenium complexes as protein kinase inhibitors.[12] The scaffold **14** is chemically very robust and conformationally rigid and thus may be a promising lead structure for the development of potent and specific inhibitors of Abl by derivatizing the bipyridine ligands.[13]

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

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