# *In silico* evolution of substrate selectivity: comparison of organometallic ruthenium complexes with the anticancer drug cisplatin†‡

**Dirk V. Deubel* and Justin Kai-Chi Lau**

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**A comparative quantum chemical approach helps to clarify how the selectivity of anticancer metallopharmaceuticals towards potential biological targets can be controlled by metal and ligands.**

The success of the anticancer drug cisplatin (**1-Cl**, *cis*-$[Pt(NH_3)_2Cl_2]$) has stimulated the search for new metallopharmaceuticals.[2] Various ruthenium complexes have attracted attention.[3] Organometallic ruthenium(II) anticancer complexes of the type $[Ru(Ar)(en)Cl]^+$ (**6-Cl**, Ar = *e.g.*, $\eta^6$-benzene; en = 1,2-diaminoethane) are of current interest.[4] Despite many experimental studies, the discussion of similarities and differences in the anticancer chemistry of cisplatin and Ru(II) complexes has been controversial. In a recent book chapter, the authors summarized ruthenium anticancer complexes in the section entitled: "*Ru complexes that mimic cisplatin*",[3d] whereas others stated that "*Ru complexes probably function in a different manner than cisplatin*".[3b] We believe that this discussion can be rationalized by considering three steps: (i) the binding of metal complexes to biologically relevant functional groups, (ii) the binding of metal complexes to biological macromolecles, and (iii) the effect of metal complexes on entire cells and organisms. While many recent experiments have focused on the biochemistry of the title complexes,[4] quantum chemical calculations could provide the best access to transition structures and predict the trends in the reactivity and selectivity of anticancer complexes towards potential biological targets. In view of 80 quantum chemical studies and molecular simulations of platinum anticancer drugs,[5] it is surprising that the promising developments of $[Ru(Ar)(en)Cl]^+$ complexes have been ignored by theoreticians, with few recent exceptions.[6–8] In one study,[6] the authors compare the hydrolysis of arene–en anticancer complexes that contain leaving groups other than chloride. The other study[7] includes a comparison of calculated and experimental activation and reaction free energies for the hydrolysis of $[Ru(\eta^6\text{-benzene})(en)Cl]^+$.

We wish to report a combined density functional theory (DFT) and continuum dielectric model (CDM) study at the B3LYP level,[8] aiming to compare in a logical manner pharmaceutically relevant reactions of platinum and ruthenium(II) anticancer complexes. As shown in Fig. 1, the archetypal anticancer drug cisplatin (**1-Cl**) has been mutated successively, obtaining after five generations the organometallic anticancer complex (**6-Cl**). The chloro complexes are likely activated upon intracellular hydrolysis of metal–chloro bonds, yielding the aqua complexes *cis*-$[Pt(NH_3)_2(OH_2)Cl]^+$ (**1-OH$_2$**)–$[Ru(Ar)(en)(OH_2)]^{2+}$ (**6-OH$_2$**). For each generation, we have investigated the reaction of the aqua complexes with a library of substrates L shown in Fig. 1.[10,11] Fig. 2 displays the activation free energies ($\Delta G_a$) and reaction free energies ($\Delta G_r$) for the substitution reactions in eqn (1). The discussion shall not overemphasize the absolute *numbers*, for the prediction of which we have achieved an accuracy of approximately 4 kcal mol$^{-1}$.[7] We rather focus on the selectivity *trends* when comparing one generation of metal complexes with the next, starting with **1-OH$_2$** and ending with **6-OH$_2$** (Fig. 1).

$$\mathbf{X\text{-}OH_2} + L \rightarrow \mathbf{X\text{-}L} + H_2O \ (\mathbf{X} = \mathbf{1} \Rightarrow \mathbf{6}) \qquad (1)$$

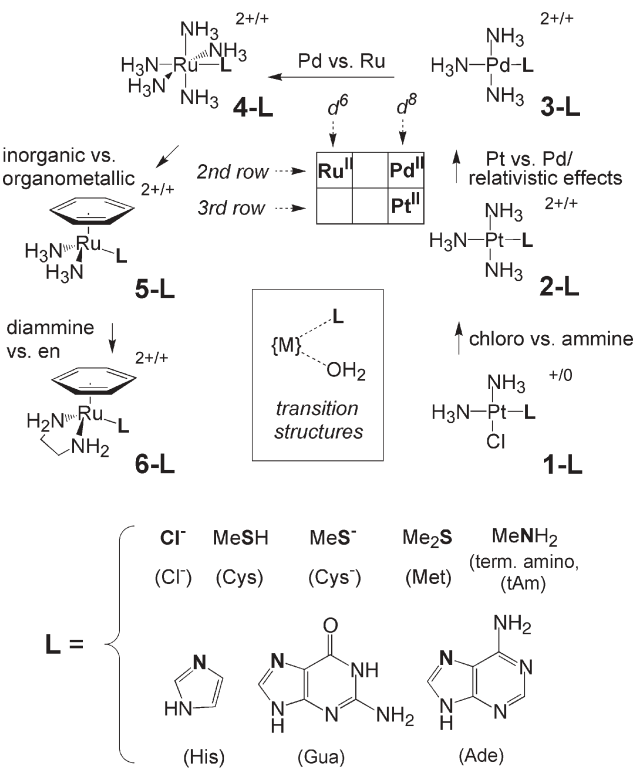


**Fig. 1** Metal complexes (top) and library of substrates L (bottom). In parentheses: biological relevance.

*ETH Zurich, USI Campus, Computational Science, Via Guiseppe Buffi 13, CH-6900, Lugano, Switzerland.*
*E-mail: metals-in-medicine@phys.chem.ethz.ch*

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‡ Electronic supplementary information (ESI) available: Partial charges, analysis of inorganic reaction mechanisms, selected transition structures, structure of NAMI-A and KP1019, computational details. See DOI: 10.1039/b601590e

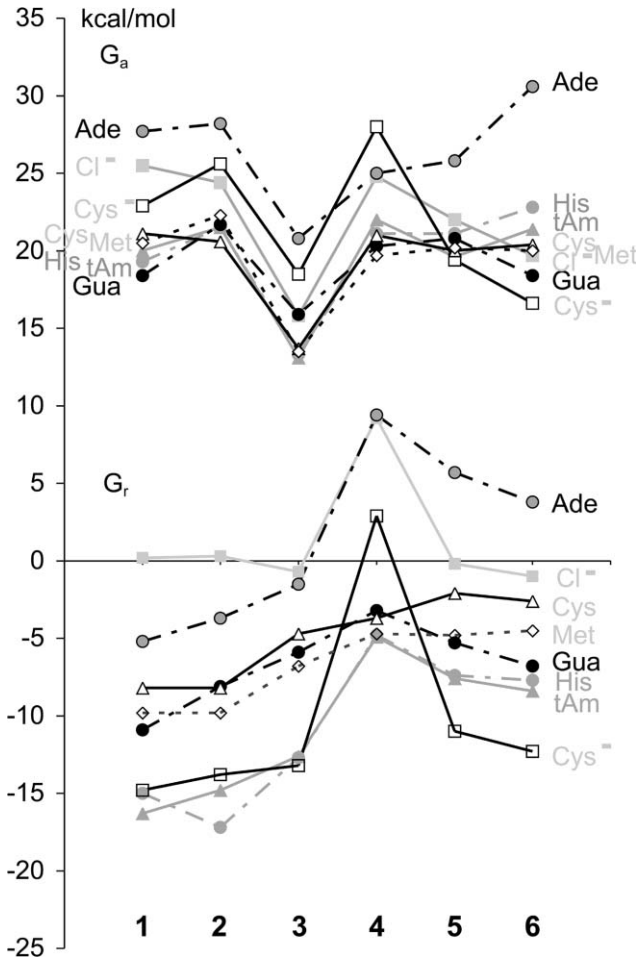


**Fig. 2** Predicted $\Delta G_a$ (top) and $\Delta G_r$ (bottom) of the reactions in eqn (1).

Generation **1** → **2**: Replacing the chloro ligand with an ammine ligand causes relatively little changes in $\Delta G_a$ and $\Delta G_r$ (Fig. 2).

Generation **2** → **3**: Replacing Pt by Pd lowers all activation barriers systematically, indicating an increase in *reactivity* but no change in kinetic *selectivity* (Fig. 2). All reactions of the Pd complex (**3-OH$_2$**), except for the anation, are thermodynamically slightly less favorable than those of the Pt complex (**2-OH$_2$**). To elucidate these findings, we have performed relativistic and non-relativistic calculations of the reactants, transition states, and products for L = tAm (Fig. 3).[12] The calculations confirm the well-known[13] trend that the relativistic bond stabilization is stronger for third-row transition metal complexes (Pt) than for second-row transition metal complexes (Pd). The non-relativistic free energy profiles of the Pd and Pt complexes are found to be virtually identical (Fig. 3). Importantly, the calculations show that the relativistic bond stabilization decreases in the order: *one strong bond* (M–N bond in the products) > *one weak bond* (M–O bond in the reactants) ≫ *two partial bonds* (M–O and M–N in the TS). These results explain why the reactions become kinetically much more favorable but thermodynamically slightly less favorable upon replacing Pt by Pd.

Generation **3** → **4**: Moving from $\{Pd(NH_3)_3\}^{2+}$ to $\{Ru(NH_3)_5\}^{2+}$ makes all substitution reactions of the aqua complexes both kinetically and thermodynamically less favorable (Fig. 2). This trend cannot be attributed to steric effects in **4-OH$_2$**,

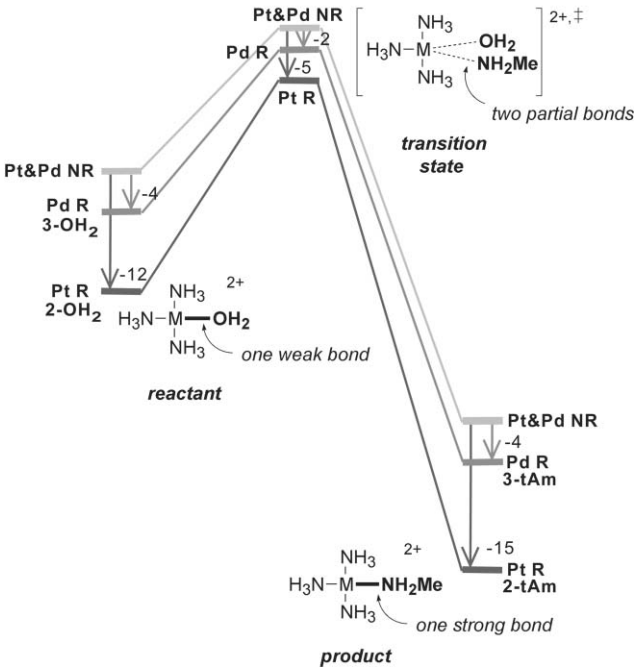


**Fig. 3** Non-relativistic (NR) and relativistic (R) free energy profiles for the reaction of **2-OH$_2$** (Pt) and **3-OH$_2$** (Pd) with $MeNH_2$ ("tAm"). Arrows: relativistic stabilization of the bond between $\{M(NH_3)_3\}^{2+}$ and $OH_2$ and/or $MeNH_2$ (values in kcal mol$^{-1}$).

because the metalations of the thiol ("Cys") and the larger thioether ("Met") residues have similar $\Delta G_a$ and $\Delta G_r$ values. The strongest change from **3** to **4** is observed for the thermodynamics of the metalation of the anionic L ($Cys^-$ and $Cl^-$). To elucidate this result, we have calculated the amount of charge that is transferred from L to the metal in the transition states and products (for numbers, see ESI‡). The charge transfer from L to the metal increases in the following order: (i) reactions of **4-OH$_2$** < reactions of the other aqua complexes, (ii) reactions of neutral L < reactions of anionic L ($Cys^-$ and $Cl^-$), and (iii) transition states < products. It becomes clear that, in the products of anionic L, there is the greatest *demand* for a charge transfer, but the *ability* of $\{Ru(NH_3)_5\}^{2+}$ (**4**) to accept charges is weakest. This result explains why the reactions of **4-OH$_2$** with anionic L become thermodynamically so unfavorable in comparison with those of **3-OH$_2$**.

Generation **4** → **5**: Creating an organometallic Ru(II) complex by replacing three ammine ligands by one $\eta^6$-benzene ligand reverses the **3** → **4** trends in $\Delta G_a$ and $\Delta G_r$ fully or partially for some of the reactions (Fig. 2). Overall, the trends are (i) *fully* reversed for the thermodynamics of the reactions with anionic L, (ii) *partially* reversed for the kinetics of the reactions with anionic L, (iii) *partially* reversed for the thermodynamics of the reactions with neutral L, and (iv) *not* reversed for the kinetics of the reactions with neutral L. These results show that the $\eta^6$-benzene ligand functions as a mediator that adapts its π-acceptor properties to the stereoelectronic requirements in the metal complexes and transition states.

Generation **5** → **6**: Replacing the two remaining ammines by the en chelating ligand causes little change in $\Delta G_a$ and $\Delta G_r$ for most reactions (Fig. 2). Importantly, Gua metalation with **6-OH$_2$**

 

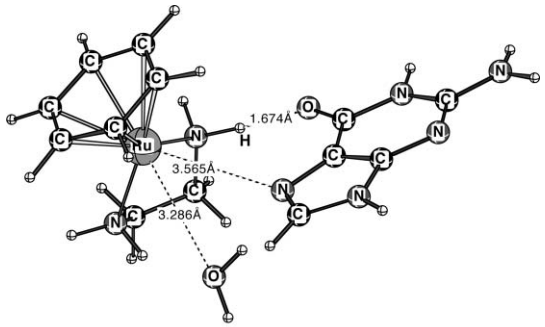


**Fig. 4** Structure of the transition state for the reaction of **6-OH$_2$** with Gua.

becomes more favorable, whereas Ade metalation becomes kinetically less favorable. The enhancement of the selectivity to Gua in the en complex can be partially attributed to a *conformational activation of the reactant*. The transition structure for the reaction of **6-OH$_2$** with Gua shows a strong N–H···O$^6$ hydrogen bond (Fig. 4). In the reactant [Ru(Ar)(en)(OH$_2$)]$^{2+}$ (**6-OH$_2$**), an N–H bond of the en ligand already points in the direction where Gua–*O*$^6$ will be located in the TS. In contrast, **5-OH$_2$** is not conformationally activated (see ESI‡). Remarkably, the metal–nucleophile and metal–leaving group distances in the transition structures for *all* Ru(II) complexes considered herein (**4-OH$_2$**, **5-OH$_2$** and **6-OH$_2$**) are much longer than those in the reactions of the Group 10 complexes. This result is interesting in light of former work, because experimental activation parameters[4f,14] suggested ligand substitution reactions of organometallic arene–en Ru(II) complexes to be more associative than those of the inorganic Ru(II) complexes. In the ESI,‡ we have defined a new protocol for analyzing transition structures of ligand-substitution reactions.

We conclude that, regarding the selectivity towards biologically relevant functional groups (Fig. 2), organometallic Ru(II) anticancer complexes are more similar to cisplatin than to inorganic Ru(II).[15] Both cisplatin and en–arene Ru(II) complexes strongly bind to Gua sites of genomic DNA. The latter complexes do not bind to Ade, and they form monofunctional DNA adducts that are recognized and repaired in the cell in a manner different from the bifunctional DNA adducts of cisplatin.[4i] Nevertheless, the formation and processing of DNA adducts leads in both cases to cell death,[4b,j] which arises partly from the selectivity of the complexes towards Gua. Given the difference in the chemical structure of cisplatin and organometallic [Ru(Ar)(en)Cl]$^+$ complexes and the striking similarity in their selectivity to biomolecules, we believe that the *in silico* evolution of substrate selectivity has a promising potential in the virtual screening of new metallopharmaceuticals.

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