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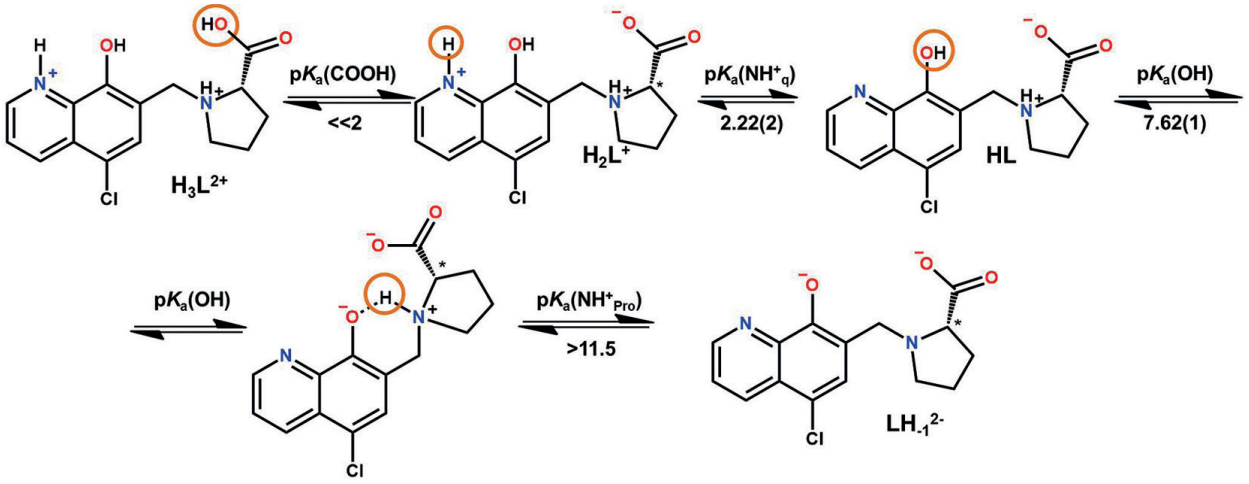
János P. Mészáros
Department of Inorganic and Analytical Chemistry
University of Szeged (Hungary)
meszaros.janos@chem.u-szeged.hu

AN ANTICANCER 8-QUINOLINOL-PROLINE HYBRID AND ITS HALF-SANDWICH COMPLEXES

Herein, the synthesis of an 8-hydroxyquinoline (HQ) derivative and its half-sandwich complexes is reported. Hybridization of HQ with L-proline improved water solubility. Our results revealed high complex stability. The ligand and its Rh complex show selective toxicity for resistant cancer cells.

Cancer is one of the most abundant diseases worldwide. One of the major problems is the multidrug resistance (MDR) of the cancer cells, which develops during treatment **[1]**. Resistance is connected to the increased amount of efflux pumps **[1]**. Potent pump inhibitors were explored, however, the use of them at the needed concentrations resulted in serious side effects **[1]**.
Szakács *et al.* found that 8-hydroxyquinolines functionalized at position 7 have higher cytotoxicity in MDR cancer cells than in non-resistant ones **[2]**. The combination of HQ-type ligands with half-sandwich Ru(η^6-arene) moiety increased the anticancer effect **[3-5]**. While halogenation of the bidentate HQ ligand improved cytotoxicity, the water solubility of these compounds is rather low **[4]**. We designed and synthesized a novel HQ-amino acid hybrid, namely (*S*)-5-chloro-7-((proline-1-yl) methyl)8-hydroxyquinoline (HQCl-Pro) combining the abovementioned properties (Scheme 1): halogen substitution, -CH_2-N- subunit in position 7 and a zwitterionic substituent to increase



Scheme 1 - Proton dissociation equilibria of the ligand HQCl-Pro (I = 0.2 M KNO_3)

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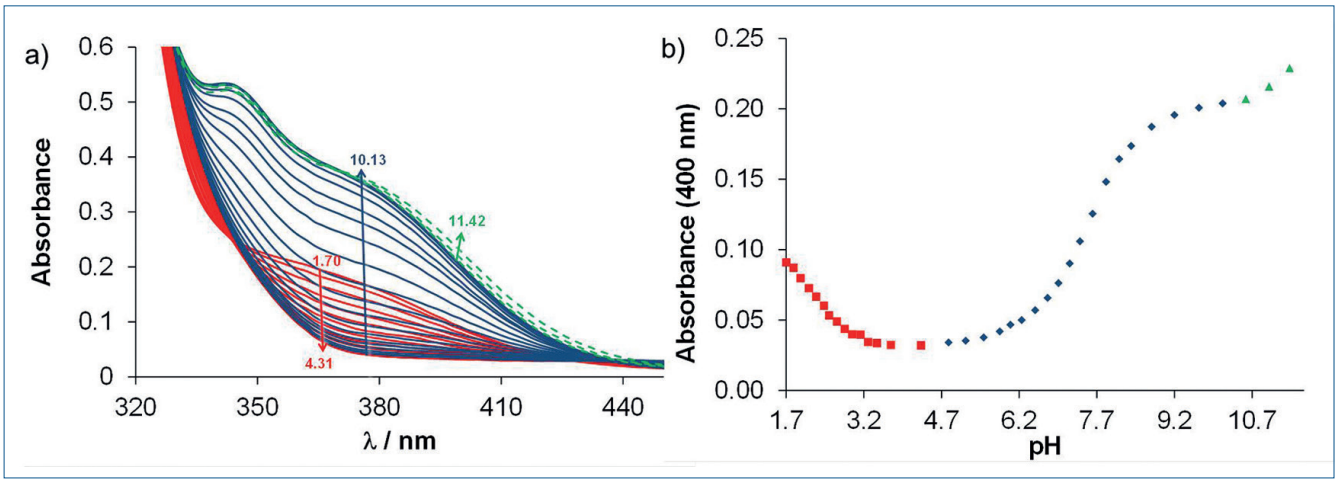

Fig. 1 - a) UV-Vis absorption spectra of HQCl-Pro at pH = 1.7-11.4; b) absorbance values at 400 nm as a function of pH (■, ♦, ▲), {*c*(HQCl-Pro) = 130 μM; *I* = 0.20 M (KNO_3); T=25.0 °C; ℓ = 1 cm}

water solubility. Synthesis of the ligand was performed via a modified Mannich reaction between 5-chloro-8-hydroxyquinoline and *L*-proline (yield = 62%). pH-potentiometry, ^{1}H NMR and UV-visible (UV-vis) spectrophotometry were used to reveal the proton dissociation processes of HQCl-Pro ligand in aqueous solution and showed in Scheme 1. Fig. 1 shows the changing absorption spectra of HQCl-Pro at different pH values.

HQCl-Pro has four deprotonation steps. Deprotonation of the carboxyl group occurs at acidic pH (pH<<2), while the deprotonation of proline-amino group starts at pH>11,5. The pK_a value of the latter process is high which can be explained with the formation of an H-bond between the hydroxylate group and the protonated amino group (Scheme 1, L^-).

Complex formation of HQCl-Pro with the half-sandwich [Ru(η^6-*p*-cymene)$(H_2O)_3]^{2+}$, [Ru(η^6-toluene)$(H_2O)_3]^{2+}$ and [Rh(η^5-pentamethylcyclopentadienyl)$(H_2O)_3]^{2+}$ (abbreviated as [Ru(η^6-*p*-cym)$(H_2O)_3]^{2+}$, [Ru(η^6-tol)$(H_2O)_3]^{2+}$ and [Rh(η^5-C_5Me_5)$(H_2O)_3]^{2+}$) was followed by UV-vis spectrophotometry. [Rh(η^5-C_5Me_5)$(H_2O)_3]^{2+}$ forms a complex very fast, while with [Ru(arene)$(H_2O)_3]^{2+}$ cations reacted much slower. Only mono complex formation occurs in water with the suggested structural formula [M(arene)(L)$(H_2O)]^+$, where L^- is (L^- = HQCl-ProH_{-1}) (Fig. 2). The synthesis of these complexes in the chlorinated form [M(arene)(L)Cl] was achieved with the simple mixing of the ligand and the organometallic precursors ([M(arene)$Cl_2]_2$) in methanol (yield = 84-90%).

In the case of [Ru(arene)(L)$(H_2O)]^+$, the excess of HQCl-Pro causes an intense colour change. A wide band above 500 nm suggests oxidation of the Ru center and the loss of half-sandwich structure and formation of the tris complex ([Ru(III)$(L)_3$]), as it was published for [Ru$(HQH^{-1})_3$] **[6]**. Oxidation of the Ru(II) centre was also proved by EPR spectroscopic method **[7]**. The loss of arene ligand was also induced upon the interaction with the (N,N) donor 1,10-phenanthrolin (phen) and the binding of 1 equiv. of phen is followed by the ox-

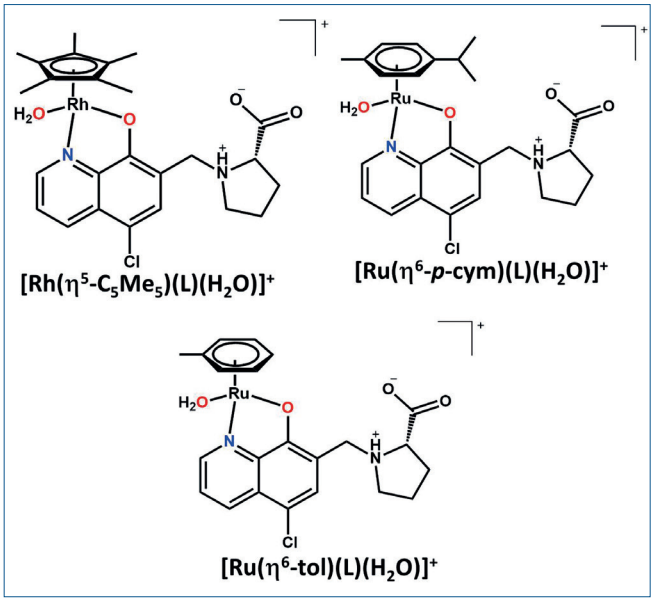

Fig. 2 - The proposed structures of the mono complexes [M(arene)(L)$(H_2O)]^+$ formed with HQCl-Pro and the various organometallic cations

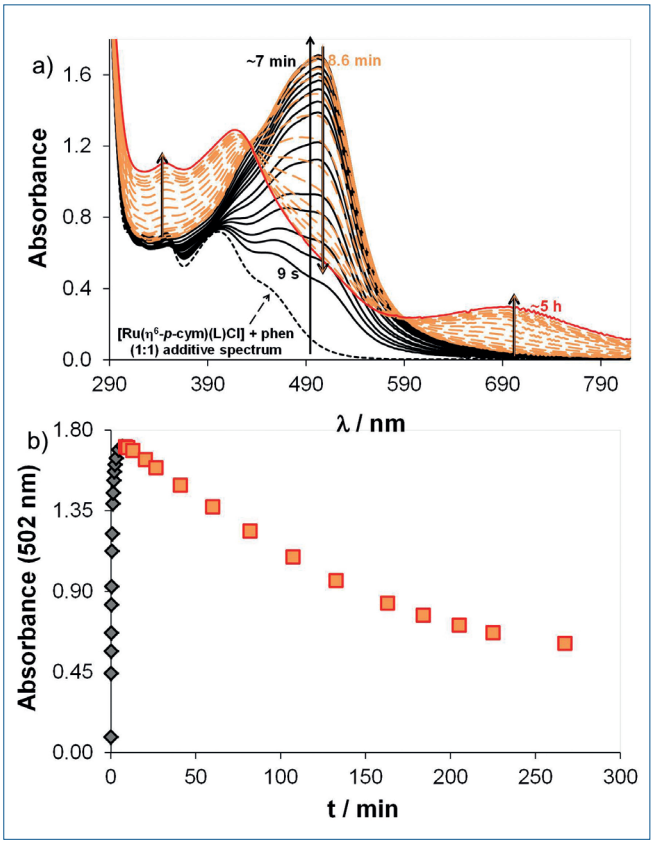

Fig. 3 - a) Time-dependent UV-Vis absorption spectra of the $[Ru(\eta^6\text{-}p\text{-cym})(HQCl\text{-}ProH_{-1})(H_2O)]^+$ - phen (1:1) system at pH=7.40 under aerobic conditions. Dashed curve shows the additive spectrum of the reactants; b) absorbance values at 502 nm in the function of time. {c($[Ru(\eta^6\text{-}p\text{-cym})(HQCl\text{-}ProH_{-1})(H_2O)]^+$) = 200 μM; c(phen) = 200 μM; pH=7.40 (20 mM phosphate); *c*(KCl) = 0.10 M; T=25.0 °C; ℓ = 1 cm}

idation of the Ru center (λ_{max} = 694 nm) (Fig. 3). However, the aqueous solutions of the mono complexes (metal-to-ligand ratio: 1:1) were stable during a week based on 1H NMR spectra. Three main reactions occur in the solution of this type of complexes **[7, 8]**: i) complex formation, ii) deprotonation of the coordinated water molecule and iii) substitution of the coordinated H_2O to Cl^- (Tab. 1). These complexes show high thermodynamic stability, neither the free ligand nor the unbound organometallic cations are observed at pH=2. Ligand competition experiments were performed to determine the stability constants of $[M(arene)(L)(H_2O)]^+$ complexes in chloride-free medium (I = 0.2 M (KNO_3)) using 2-picolylamine (pin) as competitor ligand. Arene loss occurred in the case of the Ru-complexes. Using ^{1}H NMR spectroscopy (Fig. 4) and the stability constant of $[Rh(\eta^5\text{-}C_5Me_5)(pin)(H_2O)]^{2+}$ **[8]**, the stability of the Rh complex could be determined (Tab. 1).

Increase of the pH in the solution of mono complexes causes a sigmoidal shift of peaks in the ^{1}H NMR spectra. From this change pK_a $[M(arene)(L)(H_2O)]^+$ values could be calculated (Tab. 1). At physiological pH, less than 10% of the complexes are found in their deprotonated form. The biofluids contain Cl^- in different concentrations (blood plasma: 100 mM, cytoplasm: 24 mM; nucleus: 4 mM **[9]**) affecting the actual ratio of the chlorinated and the aquated complexes, which has an important role in the bioactivity. Spectral change is detected when KCl solution was added to the solution of the complexes (20 mM phosphate, pH ~5.5). The water-chloride exchange constants (log *K'* (H_2O/Cl^-)) were determined by the deconvolution of UV-vis spectra (Tab. 1). Both the pK_a $[M(arene)(L)(H_2O)]^+$ and the log *K'* (H_2O/Cl^-) values show the same tendency: $Rh(\eta^5\text{-}C_5Me_5)$>>$Ru(\eta^6\text{-}p\text{-cym})$>$Ru(\eta^6\text{-tol})$. More than 50% of the complexes are in chlorinated form at $c(Cl^-)$ = 100 mM (Fig. 4), which drops to ~10% at $c(Cl^-)$ = 4 mM. The change in the ratio of the chlorinated and aqua complexes has influence on the charge and the lipophilicity.

The aim of the development of HQCl-Pro ligand was to obtain a compound which shows selectivity to resistant cells. Therefore, cytotoxicity of HQCl-Pro and its complexes was measured in doxorubicin-resistant Colo320 and in the parental Colo205 cell lines. The results are expressed as the IC_{50} values, which are the concentrations that reduce by 50% the number of the cancer cells.

	$Ru(\eta^6\text{-}p\text{-cym})$	$Ru(\eta^6\text{-tol})$	$Rh(\eta^5\text{-}C_5Me_5)$
log *K* [M(arene)(L)]	-	-	13.41 ± 0.02[a]
pK_a [M(arene)(L)][b]	8.62±0.04	8.45±0.03	9.62±0.04
log *K'* (H_2O/Cl^-)[c]	1.21±0.01	1.09±0.01	1.57±0.01

[a]Determined by ligand displacement studies followed by ^{1}H NMR spectroscopy
[b]Determined by UV-Vis spectrophotometric titration, pH=2.0-11.5
[c]Determined by UV-Vis spectrophotometry at pH = 5.5, (KCl)=0-0.2 M

Tab. 1 - Stability (*K* [M(arene)(L)]), proton dissociation (K_a [M(arene)(L)]) and water-chloride exchange constants (*K'* (H_2O/Cl^-)) of complexes of HQCl-Pro {T=25.0 °C; I = 0.20 M (KNO_3)}

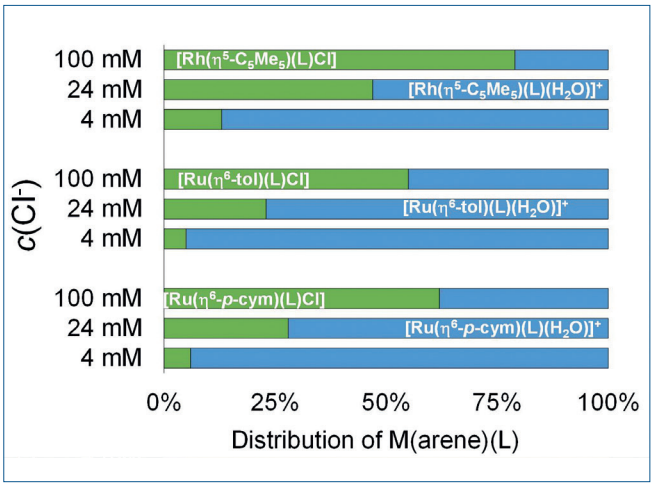

Fig. 4 - The calculated ratio of aquated ([M(arene)(L)(H_2O)]$^+$) (blue) and chlorinated ([M(arene)(L)Cl]) (green) forms of the HQCl-Pro complexes at different Cl^- concentrations, {*c*(M(arene)(H_2O)$_3$]$^{2+}$) = *c*(HQCl-Pro) = 100 μM; *c*(Cl^-) = 4, 24 and 100 mM; T=25.0 °C}

	Colo205	Colo320
HQCl-Pro	42.5±7.4	17.4±2.5
[Ru(η^6-tol)(L)(H_2O)]$^+$	72.6±4.8	60.9±8.2
[Ru(η^6-*p*-cym)(L)(H_2O)]$^+$	>100	>100
[Rh(η^5-C_5Me_5)(L)(H_2O)]$^+$	81.5±3.3	24.1±3.7
doxorubicin	1.56±0.03	6.45±0.19

Tab. 2 - *In vitro* cytotoxic effects (24 h) (IC_{50} values in μM) of HQCl-Pro and its complexes in Colo205 and in Colo320 (doxorubicin-resistant) human colonic adenocarcinoma cell lines

HQCl-Pro and its Rh-complex showed promising results after one day incubation time (Tab. 2). The accumulation of Rh and Ru in Colo205 cells was measured by total-reflection X-ray fluorescence (TXRF). After one day, more Rh was detected in the cells (Rh: 510±64 ng *vs* Ru: 222±9 ng in 1 million cells). All in all, the Ru-complexes have decreased toxicity which can be explained with the decomposition process and the less ability to enter into the cells.

Conclusions

In this work the newly synthesized HQCl-Pro ligand and its half-sandwich Ru and Rh complexes were investigated thoroughly. The developed ligand showed the designed properties, namely it has an increased water-solubility compared to HQ, its complexes have high stability and it has a higher activity in doxorubicin-resistant cancer cell line compared with the parental one. Ru complexes showed loss of activity and selectivity in resistant cells, while the ligand itself and the Rh complex showed promising results.

Acknowledgements

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Un ibrido anticancro dell'8-idrossichinolina e dei suoi complessi a semi-sandwich

Il presente lavoro descrive la sintesi di un derivato dell'8-idrossichinolina (HQ) e dei suoi complessi a semi-sandwich. L'ibridazione di HQ con *L*-prolina ha migliorato la solubilità in acqua. I risultati ottenuti hanno rivelato un'elevata stabilità dei complessi formati. Il legante e il suo complesso con rodio mostrano una tossicità selettiva per cellule tumorali resistenti.