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Quinoline-*para*-quinones and metals: coordination-assisted formation of quinoline-*ortho*-quinones†

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The reaction of the *para*-quinone 6,7-dichloroquinoline-5,8-dione with various transition metal dimers led to the unexpected formation of quinoline-*ortho*-quinone metal complexes. Systematic variation of the reaction conditions helped identify the solvent as the source of the carbonyl oxygen.

Organometallic anticancer agents based on bioactive ligand systems have attracted a lot of attention due to their tuneable bioactivity against primary tumours or metastases and their general versatility in drug development.[1,2] Recently, we reported a series of naphthoquinone complexes featuring an *O*,*O*-bidentate coordination motif where metal coordination induced the formation of reactive oxygen species in cancer cells in dependence of the nature of the metal centre.[3,4] However, we found that these compounds undergo ligand exchange reactions in the presence of biological ligands which results to some extent in the cleavage of the ligand from the metal centre.[3] In order to overcome this issue, we turned our research efforts to *N*,*O*-chelating quinoline ligands, which were found to form extremely stable and anticancer-active complexes.[4,5] To combine the benefits of quinoline ligands with the interesting biological properties offered by quinone structures, we aimed to explore the coordination chemistry and biological activity of 5,8-quinolinediones. Moreover, a literature search showed that only a few metal complexes of such ligands have been reported.[6,7]

5,8-Quinolinedione derivatives are biologically active and exhibit antitumor, antibacterial and antimalarial effects.[8] This structural motif is part of the pharmacophore found in several antitumor antibiotics, such as streptonigrin and lavendamycin.[9] The cytotoxicity of quinolinediones results from its inhibition of the electron transport chain in mitochondria, effectively halting aerobic respiration and producing a semiquinone radical in a redox reaction.[8]

The 5,8-quinolinedione derivative 6,7-dichloro-5,8-quinolinedione (**DQQ**) was selected as the ligand and used to synthesize Ru^{II}-, Os^{II}-, Ir^{III}- and Rh^{III}-based metallopharmaceuticals. In order to prepare **DQQ**, 8-hydroxyquinoline was oxidized using sodium chlorate in HCl (Fig. 1).[10] The yield of this reaction after recrystallization was low (15–18%) which can be explained by the occurrence of several side reactions competing with the formation of the desired product, also noted by Shen *et al.*[11,12] The by-product 5,6,7-trichloro-2-alkoxy-8-hydroxyquinoline ($^{H}\mathbf{HQ_{Cl}}$) was isolated and characterized by NMR spectroscopy, as was its methoxy derivative $^{Me}\mathbf{HQ_{Cl}}$ by single crystal X-ray diffraction analysis (ESI†). The latter species surprisingly formed during recrystallization with methanol. Due to the reduced electron density of the 2-position of the pyridine ring proximal to the nitrogen, this position is amenable to oxidative nucleophilic substitution resulting in the formation of compounds $\mathbf{HQ_{Cl}}$ under the oxidative reaction conditions used.[13]

To improve the yield of **DQQ** and to minimize the side reactions occurring, methanol was replaced with ethanol,

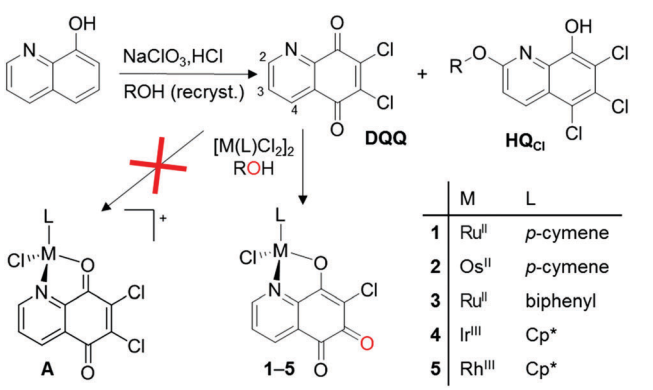

Fig. 1 Preparation of **DQQ**, $\mathbf{HQ_{Cl}}$, and the *ortho*-quinoid metal complexes **1–5**. R = H ($^{H}\mathbf{HQ_{Cl}}$), methyl ($^{Me}\mathbf{HQ_{Cl}}$), ethyl ($^{Et}\mathbf{HQ_{Cl}}$), *n*-propyl ($^{nPr}\mathbf{HQ_{Cl}}$), i-propyl ($^{iPr}\mathbf{HQ_{Cl}}$), *n*-butyl ($^{Bu}\mathbf{HQ_{Cl}}$).

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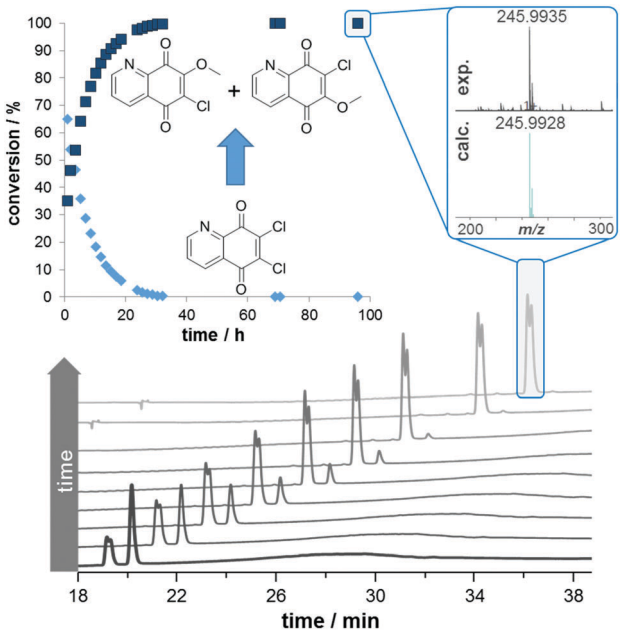

Fig. 2 (top left) Time course of the conversion of **DQQ** to the methoxy derivative in 25% H_2O/methanol. (bottom) Time-dependent chromatograms showing the growth of a second peak at lower retention time which was identified by high resolution ESI-MS as the methoxy derivative (top right).

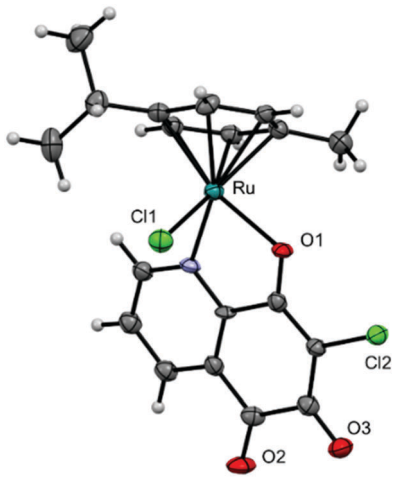

Fig. 3 Molecular structure of one enantiomer of **1** drawn at 50% probability level. Solvent molecules were deleted for clarity. Selected bond lengths are given in the ESI.†

n-propanol, i-propanol and *n*-butanol in the recrystallization step. While the yields of **DQQ** did not improve, the ethyl, *n*-propyl, i-propyl and *n*-butyl derivatives of 5,6,7-trichloro-2-alkoxy-8-hydroxyquinoline were isolated in up to 8% yield (ESI† for ^{1}H NMR spectra). Furthermore, HPLC analysis of **DQQ** dissolved in a mixture of MeOH/H_2O revealed the conversion of **DQQ** to 6- and 7-methoxy-5,8-quinolinedione derivatives by replacing one of the chloro substituents with a methoxy group (Fig. 2). This is indicated by the presence of two peaks with close retention times and the same accurate mass as determined by HPLC-ESI-QToF-MS, indicating isomer formation. The chloro/methoxy substitution reaction was found to be water content-dependent. While the substitution was suppressed in pure water, acetonitrile and dichloromethane, it was completed within 32 h in 25% H_2O/methanol and even accelerated in a 1/1 mixture to be complete in 10 h. In pure methanol, the reaction was to some extent suppressed and only 50% conversion could be observed after 95 h.

As mentioned above, there are only a few 5,8-quinolinedione complexes known.[6,7] In order to prepare complexes of the general structure **A** (Fig. 1) from the 5,8-quinolinedione ligand **DQQ**, it was reacted with dimeric $[M(L)Cl_2]_2$ precursors (M = Ru, Os, Rh, Ir; L = η^6-*p*-cymene [cym], η^6-biphenyl, η^5-pentamethylcyclopentadienyl [Cp*]) in alcoholic solution under reflux for 1 h (Scheme 1), which resulted in the formation of purple precipitates. While NMR spectroscopy suggested the formation of **A**, X-ray diffraction analysis of single crystals of **1**, **2**, **4** and **5** surprisingly revealed the formation of the *ortho*-quinone complexes (Fig. 3; ESI†). All complexes featured the pseudo-tetrahedral piano stool structure with the metal centre coordinated to the pyridine nitrogen and the adjacent oxygen in a bidentate fashion. The first coordination sphere is completed by chlorido and *p*-cymene (**1** and **2**) or Cp* (**4** and **5**) ligands. The conversion of **DQQ** to an *ortho*-quinone was confirmed by high resolution ESI-MS indicating the absence of a chloro substituent after complexation and substitution with an oxygen atom. The ^{1}H NMR spectral shifts observed for the pyridine proton H2 were found downfield for the Ru and Os complexes **1–3** as compared to **DQQ**, whereas the Ir and Rh congeners showed a slight upfield shift for the proton adjacent to the nitrogen atom.

A similar reaction was observed by Corey and König who showed that anhydrides form from metal carboxylates, such as Cu(acetate)$_2$, in the presence of **DQQ**,[14] while in the presence of alcohols esters were obtained. In both cases a metal-coordinated *ortho*-quinone formed. It was suggested that the addition of water to the intermediate yields a polymeric structure but elemental analysis data indicated the formation of a Cu(*ortho*-quinone)$_2$ complex,[14] which we confirmed by ESI-MS ($[M + Na]^+$ m/z_{exp} 501.882, m/z_{theor} 501.879). These Cu(*ortho*-quinone)$_2$ species may feature the same coordination mode as observed in **1–5** for the formed *ortho*-quinone and the metal centres used. Using pseudo-octahedral organometallic moieties in our experiments where the ligands cannot take part in the reaction, avoided the formation of polymers or higher metal-to-ligand ratio complexes.

To distinguish between the solvent or dissolved oxygen as the source of the oxygen atom introduced in position 6 of the *ortho*-quinone, we performed the reaction between **DQQ** and $[M(L)Cl_2]_2$ in dry protic and aprotic, deoxygenated solvents and under different conditions, *i.e.*, reflux, microwave-assisted and at room temperature (Table 1). ^{1}H NMR spectroscopy experiments with **DQQ** and $[Ru(cym)Cl_2]_2$ in $CDCl_3$ showed neither conversion of **DQQ** into the *ortho*-quinone nor complex formation, even when refluxing the reaction mixture. Addition of MeOD to the mixture on the other hand led almost instantaneously to the formation of Ru compound **1**. These studies demonstrate that the *ortho*-quinone–metal complex only forms in the presence of protic solvents. As the use of dry degassed solvents had no

Table 1 Reaction conditions used to study the formation of the *ortho*-quinone during the reaction between **DQQ** and $[Ru(cym)Cl_2]_2$ in various solvents

Solvent	Conditions	**1**[a]
Methanol (abs), under N_2	Reflux 1 h	Yes
	M.W. 30 min, 80 °C	Yes
Ethanol (abs), under N_2	Reflux 1 h	Yes
	M.W. 30 min, 80 °C	Yes
Isopropanol (abs), under N_2	M.W. 30 min, 80 °C	Yes
n-Butanol (abs), under N_2	M.W. 30 min, 80 °C	Yes
Water	Reflux 1 h	Yes
	M.W. 30 min, 80 °C	Yes
THF (abs), under N_2	Reflux 1 h	No
	Reflux 36 h	No
	R.T. 5 d	No
	M.W. 30 min, 80 °C	No
Acetonitrile (abs), under N_2	Reflux 21 h	No
	R.T. 24 h	No
Chloroform (abs), under N_2	Reflux 4 h	No
	M.W. 30 min, 80 °C	No
Chloroform/methanol	Reflux 5 min	Yes
DCM (abs), under N_2	Reflux 4 h	No

[a] **1** was detected by ^{1}H NMR spectroscopy but not isolated. M.W., microwave.

influence on the reaction, the solvent should be the source of the oxygen atom in position 6 of the *ortho*-quinone. To further verify this assumption, ^{18}O-labelled water was used as a solvent in the reaction. Under these conditions, the formation of **1** was confirmed by ESI-MS. The mass spectrum featured a signal at *m*/*z* 507.9641 as the base peak. This ion was identified to contain three ^{18}O atoms ($[M + Na]^+$ m/z_{theor} 507.9648), which is evidence that the protic solvents used were the source of the oxygen atom. Performing the same reactions but with Os^{II}, Rh^{III} and Ir^{III} precursors supported this conclusion. This is also in line with previous reports that other Lewis acids can be used in the derivatization of **DQQ**.[15,16]

Based on these observations, the following mechanism for the formation of *ortho*-quinones is suggested (Fig. 4): similar to Corey and König,[14] the initial step involves coordination of the metal centre to **DQQ** with **DQQ** acting as a bidentate ligand, where the metal acts as a Lewis acid. The coordination of the metal centre activates C_6 to a nucleophilic attack by ROH into the conjugated π-electron system. Subsequent decomposition

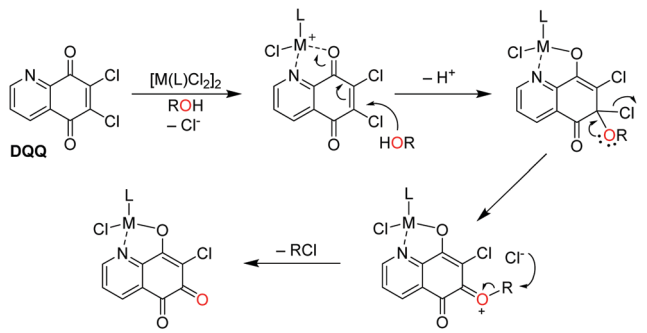

Fig. 4 Proposed reaction mechanism for the conversion of the quinoline-5,8-dione **DQQ** to an *ortho*-quinone derivative upon complexation to metal centres in protic solvents.

Table 2 50% inhibitory concentration (IC_{50}) of **1–5** and **DQQ** in HCT116 human colorectal, NCI-H460 non-small cell lung and SiHa cervical carcinoma cells

	IC_{50} values[a]/μM		
Compound	HCT116	NCI-H460	SiHa
DQQ	3.7 ± 0.8	3.7 ± 0.6	15 ± 2
1	≥50	≥50	≥50
2	1.8 ± 0.7	3.9 ± 0.8	4.4 ± 0.6
3	≥30	≥30	≥30
4	≥30	≥30	≥30
5	>40	>40	>40

[a] SRB assay, 72 h incubation.

of the formed tetrahedral intermediate affords an oxonium ion, to which the R–O bond is cleaved to yield the *ortho*-quinone–metal complex in a metal coordination-assisted reaction and RCl, as determined for the reaction with *n*-butanol by ESI-MS.

Having identified the solvent choice as essential for the formation of the *ortho*-quinone from **DQQ**, the preparation of the originally anticipated quinolinedione complexes was attempted by using a silver salt to activate $[Ru(cym)Cl_2]_2$ in acetonitrile to form $[Ru(cym)(CH_3CN)_2Cl]PF_6$. NMR spectroscopy and MS experiments indicated the presence of the desired products in the reaction mixture but we were not successful in isolating them. As the compounds were designed as anticancer agents with redox active ligand systems and this requires stability in aqueous solution, this route was not further pursued, since in biological medium the **DQQ** complexes would be converted into *ortho*-quinone compounds *in situ*. Instead, the *ortho*-quinone complexes **1–5** were evaluated on their cytotoxic properties. Surprisingly only the osmium complex showed promising activity in HCT116 human colorectal, NCI-H460 non-small cell lung and SiHa cervical carcinoma cells (Table 2) with IC_{50} values in the low μM range.

The cytotoxicity of quinone-based compounds has been suggested to be related to them undergoing redox reactions.[8] In order to study the impact of metal complexation on the redox properties of the *ortho*-quinone system, pulse radiolysis data was collected (Fig. 5 for **1**). As the free *ortho*-quinone ligand of **1–5** is not accessible, the redox properties of the complexes were compared with that of **DQQ**. While the one-electron reduction potential *E*(1) of **DQQ** is found in the positive range at 96 mV, the metal complexes feature around −100 mV (ESI†), which is similar to that of the anticancer drug candidate KP1019 whose mode-of-action is based on the activation by reduction mechanism.[17] Compared to *ortho*- and *para*-naphthoquinones the *E*(1) of **DQQ** is significantly higher, while the *E*(1) of *para*-naphthoquinone is lower than that of the *ortho* derivative.[18] When comparing the *para*-quinone **DQQ** with the complexes, where the ligand is present in an *ortho*-quinoid structure, the difference in *E*(1) is significantly larger. This indirectly demonstrates an impact of the metal centre on the *E*(1) of the ligands, although no direct coordination occurs to either of the oxygen atoms of the *ortho*-quinone. This can be explained by the conjugated electron system being present in the ligands and involving the metal centre.

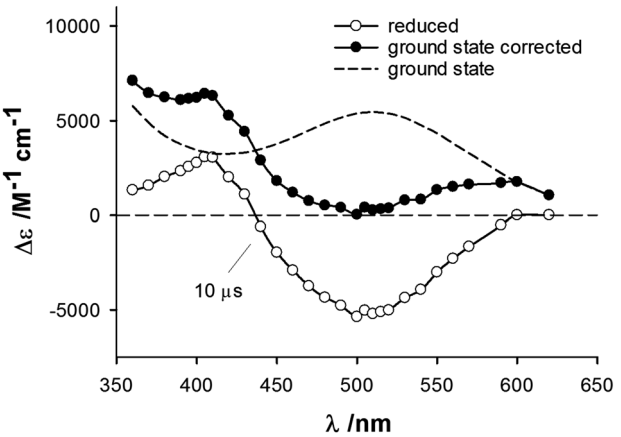

Fig. 5 Changes in the absorption spectrum recorded for **1** following a one-electron reduction, ○. Spectrum corrected for ground-state absorption (dashed line), ●.

In summary, when aiming to prepare organometallic compounds from **DQQ** we observed the surprising activation of the C–Cl bond in *meta*-position to the quinone oxygen aimed for coordination to the metal centre. Variation of the reaction conditions helped to identify the solvent as the source of the oxygen atom introduced to form a metal–quinoline-*ortho*-quinone complex. The reaction mechanism to form this complex involves bidentate coordination of **DQQ** to the metal centre, and thereby conversion of the *para*-quinone **DQQ** into a quinolinolato ligand featuring an *ortho*-quinone. Pulse radiolysis studies revealed an impact of metal coordination on the redox potentials of the ligands and the Os complex **2** was identified as the most cytotoxic derivative with IC_{50} values in the range of 1.8–4.4 μM in human cancer cells.

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Conflicts of interest

There are no conflicts to declare.

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