VIP Quinone Complexes

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Discovery, Structure, and Anticancer Activity of an Iridium Complex of Diselenobenzoquinone**

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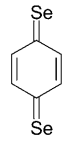
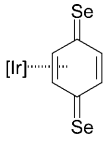
Quinones are a prominent class of compounds that play an important role in chemistry and biology,[1] with vitamin K being a notable example of a biologically relevant molecule that possesses a quinone function in its structure.[2] The biological action of quinones is often linked to their electron-transfer rates and redox behavior.[3]

In contrast to the chemistry of *p*-benzoquinone, which is well-documented,[5] no report has been published on the related diselenobenzoquinones. In fact, the replacement of the oxygen atoms in quinone by the heavier chalcogen atoms, sulfur or selenium, leads to highly reactive intermediates that cannot be isolated in pure form owing to the instability of the unnatural functional groups C=E (E = S, Se).[6] Thus, examples of isolated thioquinones are scarce and the parent compound has been generated and characterized spectroscopically only at low temperature (10 K) in an argon matrix.[7] These procedures illustrate the difficulty in isolating and stabilizing thioquinones. The related selenoquinones, such as **1**, are in contrast unstable and consequently do not



1

Unstable

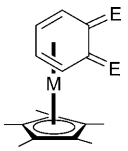


9

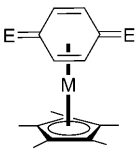
[Ir] = Cp*Ir

exist in nature, and thus their chemical and biological properties remain unknown. It is noteworthy, however, that selenium is a highly important element in organic chemistry, and several reviews describe its versatility in synthesis.[4] To the best of our knowledge, there is no report in the literature devoted to the preparation of selenoquinone or even an attempt to isolate such a molecule. Thus the work we describe herein is the first successful isolation of such a species as an iridium complex.

We have often used the {Cp*Ir} fragment (Cp* = pentamethylcyclopentadienyl) as a stabilizing entity to isolate reactive intermediates.[8] For example, in 1998 we reported the synthesis of the first stable *ortho*-quinone methide metal complex, in which the {Cp*Ir} unit is attached to the internal diene moiety by η^4 coordination.[9] In 2006 we described the synthesis of first stable iridium *p*-dithiobenzoquinone complex *p*-[Cp*Ir(η^4-$C_6H_4S_2$)] (**8**) and also the related *ortho* congener *o*-[Cp*Ir(η^4-$C_6H_4S_2$)] (**7**).[10] Both compounds were



3 (M = Rh, E = O)
5 (M = Ir, E = O)
7 (M = Ir, E = S)



4 (M = Rh, E = O)
6 (M = Ir, E = O)
8 (M = Ir, E = S)
9 (M = Ir, E = Se)

used as successful organometallic linkers to construct functional coordination assemblies.[11]

Continuing our research into the stabilization of reactive intermediates, we sought to prepare a metal complex of *p*-diselenobenzoquinone. As mentioned before, the parent molecule *p*-diselenobenzoquinone **1** is unknown and has never been characterized even as an intermediate. Selenium is an essential dietary component for many living systems, including humans, and there is increasing evidence for the efficacy of certain forms of selenium as cancer-chemopreventive compounds.[12] The parent diselenobenzoquinone **1** was obtained as an iridium complex [Cp*Ir(η^4-$C_6H_4Se_2$)] (**9**) and was fully characterized including a X-ray molecular structure. Furthermore, we describe the anticancer activity of a family of metalated quinones, thioquinones, and selenoquinones, and remarkably, the selenoquinone derivative has a cytotoxicity similar to that of cisplatin.

Our synthetic procedure involves the synthesis of the precursor metal complex [Cp*Ir(η^6-$C_6H_4Cl_2$)][BF_4]$_2$ (**2**) and subsequent substitution of the chlorides by an excess of dried Na_2Se in CH_3CN, providing **9** in high yield. The dihaloarene metal complex **2** was formed quantitatively as a white

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microcrystalline solid by treatment of an excess of 1,4-dichlorobenzene with the solvated iridium complex [Cp*Ir(acetone)$_3$][OTf]$_2$ in BF_3·2 H_2O solution.[10b]

Following purification, the neutral η^4-*p*-diselenobenzoquinone complex **9** was obtained as an orange microcrystalline material in analytically pure form. The infrared spectrum displayed a band at 1024 cm^{-1} that was tentatively attributed to the C=Se vibration. Similar results were obtained for the metalated selenocarbonyl compounds.[13] This selenocarbonyl stretching frequency is lower than that observed in other C=Se compounds. Such a trend has been observed for the C=S and C=O vibrations in the free monothio-1,4-benzoquinone[7b] compared to the metalated 1,4-benzoquinone and 1,4-dithiobenzoquinone compounds reported recently.[10,14] The ^{1}H NMR spectrum recorded in CD_2Cl_2 contains a singlet at $\delta = 1.89$ ppm attributed to the Cp* methyl protons and another singlet at $\delta = 6.26$ ppm attributed to the protons of the η^4-diene ring. Furthermore, the ^{77}Se NMR spectrum recorded in CD_2Cl_2 exhibits a singlet at $\delta = 296.5$ ppm relative to the dimethylselenide reference.

The structure of the diselenobenzoquinone complex [Cp*Ir(η^4-$C_6H_4Se_2$)] (**9**) was confirmed by a single-crystal X-ray diffraction study. Crystals of **9** were grown by vapor diffusion of diethyl ether into a solution of the complex in MeOH.[15] The structure of **9** (Figure 1) clearly shows the Cp*Ir moiety coordinated to only four diene carbons of the π-selenoquinone ligand. The Ir–C=Se bond lengths are 2.370 (5) and 2.349(5) Å, which are longer than those of the Ir–C=C diene subunit (average bond distance of 2.232(5) Å). Furthermore, the η^4-selenoquinone ligand adopts a boat-like conformation, with the selenoquinoid carbons bent out the diene plane by $\theta = 7.0$ (5)° and $\theta = 6.0(4)$°. These angles are slightly smaller than those reported for the analogous iridium thioquinone ($\theta = 9$°)[10] and iridium benzoquinone ($\theta = 16$°) complexes.[14a] The C–Se bond distances for **9** are 1.865(5) and 1.876(5) Å, which are indicative of double-bond character. These bond lengths are shorter than that reported for diselenocin, which has a C–Se single bond of 1.924(8) Å,[16] and are closer in value to the C=Se double bond of 1.857(9) Å reported for 2-selenoxoperhydro-1,3-selenazin-4-one.[17] The C=Se bonds in **9** are slightly longer than that reported for selenoacrylamide, with a C=Se double bond distance of 1.837(4) Å,[18] and 4,4′-dimethoxy-selenobenzophenone, with a C=Se bond length of 1.79 Å.[19] However, the reported bond length for a selenoaldehyde tungsten complex is 1.854 Å, which is comparable to that found in **9**.[20] Consequently, the free C=Se bond is expected to be shorter than that present in a metal complex.

Inspection of the crystal packing in **9** shows that the individual diselenoquinone molecules exhibit π–π interactions (3.5 Å) between the η^5-{Cp*Ir} moiety and the Se=C unsaturated bonds in the η^4-(Se–C_6H_4–Se) unit of another selenoquinone complex, thus providing a one-dimensional supramolecular chain along *a* axis (Figure 1b). These columns undergo another weak non-covalent hydrogen-bond-like C–H···Se interaction ($d_{Se-H} = 2.97$ and 3.03 Å) to give a three-dimensional organometallic assembly (Figure 1c). A similar non-covalent interaction has been reported by Tomoda et al. for a diselenocin molecule with $d_{Se-H} = 2.92$ Å.[16].

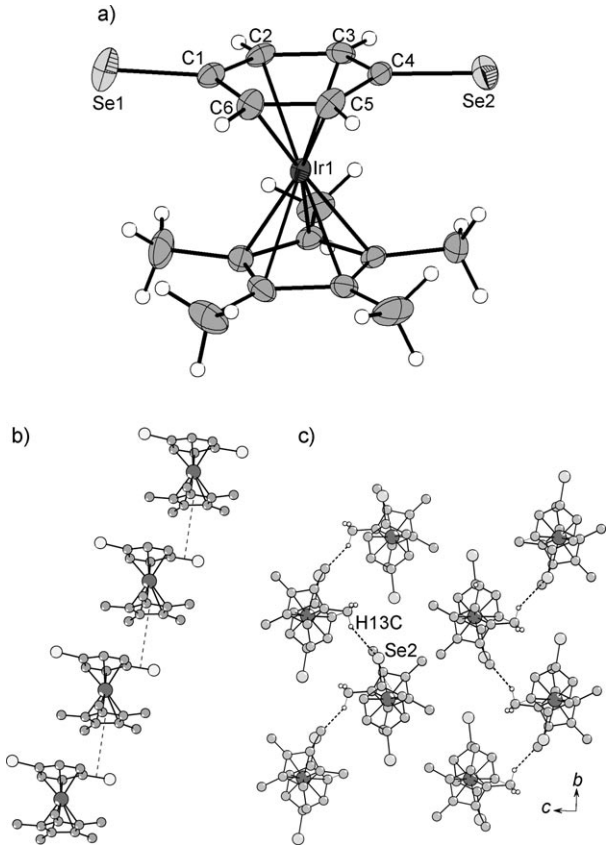
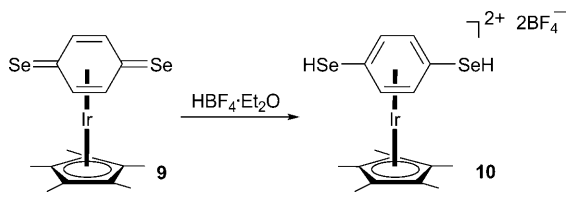


Figure 1. a) X-ray molecular structure of [Cp*Ir(η^4-$C_6H_4Se_2$)] (**9**). b) The one-dimensional supramolecular chain formed through π–π contacts between individual molecules. c) View along the *a* axis of the three-dimensional organometallic assembly formed by C–H···Se interactions between adjacent columns. Selected interatomic distances [Å] and angles [°] for **9**, molecule 1: Ir1–C1 2.370(5), Ir1–C2 2.229(5), Ir1–C3 2.236(5), Ir1–C4 2.349(5), Ir1–C5 2.226(5) , Ir1–C6 2.239(5), C1–Se1 1.876 (5), C4–Se2 1.865(5); C2-C1-C6 113.8(5), C3-C4-C5 113.7(4).

Protonation of a solution of **9** in CH_2Cl_2 with an excess of HBF_4·Et_2O led to a rapid color change, giving a light yellow precipitate, and following purification the related diselenohydroquinone complex [Cp*Ir(η^6-HSe-C_6H_4-SeH)][BF_4]$_2$ (**10**) was obtained in 93 % yield as a pale yellow microcrystalline solid (Scheme 1). Compound **10** was characterized by spectroscopy (^{1}H NMR and IR), and of note, the ^{1}H NMR of **10** recorded in CD_3CN contains a singlet at $\delta = 2.11$ ppm attributable to the methyl protons of η^5-{Cp*Ir} and another singlet at $\delta = 7.15$ ppm that may be assigned to four aromatic protons.



Scheme 1. Formation of diselenohydroquinone complex **10** by protonation of diselenobenzoquinone complex **9**.

The anticancer activity of **9** towards human A2780 ovarian cancer cells was investigated. For comparison purposes, and to establish structure–activity relationships, we also studied the activity of a series of related compounds, namely, the metalated quinones *ortho*- and *para*-[Cp*M(η^4-$C_6H_4O_2$)] (M = Rh: **3**, **4**; M = Ir: **5**, **6**), and the metalated thioquinones *ortho*- and *para*-[Cp*Ir(η^4-$C_6H_4S_2$)] (**7**, **8**). Both *ortho*- and *para*-isomers were examined, except in case of the *ortho*-selenoquinone compound which was unstable in solution.

As shown in Table 1, **9** was by far the most active of the series by one to two orders of magnitude compared to the metalated thioquinone or to the naturally occurring benzo-quinone metal derivatives. The cytotoxicity of **9** is comparable to that of the benchmark metal-based drug cisplatin. Compared to other iridium-based organometallic compounds that have been evaluated in vitro as anticancer agents, the cytotoxicity of **9** compares very favorably,[21] although the mode of action of **9** is probably quite distinct. Indeed, the high cytotoxicity of **9** illustrates the role of selenium as an important element to combat cancer, and paves the way for the preparation of other functionalized selenoquinone metal complexes with medicinal properties.

Table 1: Cytotoxicity of **9** and related compounds in A2780 human ovarian cancer cells.

Compound	IC_{50} [μM][a] A2780
p-[Cp*Rh(η^4-$C_6H_4O_2$)] (**4**)	> 400
o-[Cp*Rh(η^4-$C_6H_4O_2$)] (**3**)	> 400
p-[Cp*Ir(η^4-$C_6H_4O_2$)] (**6**)	93
o-[Cp*Ir(η^4-$C_6H_4O_2$)] (**5**)	> 400
p-[Cp*Ir(η^4-$C_6H_4S_2$)] (**8**)	154
o-[Cp*Ir(η^4-$C_6H_4S_2$)] (**7**)	49
p-[Cp*Ir(η^4-$C_6H_4Se_2$) (**9**)	5
cis-[Pt(NH_3)$_2$$Cl_2$]	3

[a] IC_{50} is the drug concentration necessary for 50% inhibition of cell viability.

In conclusion, we report the first isolation and complete characterization of diselenobenzoquinone as a metal complex in which the {Cp*Ir} moiety stabilizes the elusive intermediate by the formation of an η^4 complex. Spectroscopic and X-ray data supports stabilization of this reactive species by metal-to-ligand π backbonding. Furthermore, the selenoquinone complex exhibited strong anticancer behavior compared to the related quinone and thioquinone complexes, thus illustrating the biological role of selenium in these compounds.

Experimental Section

All synthetic manipulations were carried out under argon using Schlenk techniques. ^{1}H, ^{13}C, and ^{77}Se NMR spectra were recorded in CD_2Cl_2 and CD_3CN using a Bruker Avance 400 NMR spectrometer. IR spectra were recorded on a Bruker Tensor 27 FT-IR spectrometer equipped with a Harricks ATR unit. The metalated *o*-, *p*-quinones [Cp*M(η^4-$C_6H_4O_2$)] M = Rh (**3**–**4**), M = Ir (**5**–**6**), and *o*-, *p*-thioquinones [Cp*Ir(η^4-$C_6H_4S_2$)] (**7**, **8**) and the halogenated metal–arene compounds were prepared according to literature procedures.[10–11,14]

Complex **9** was obtained as an orange microcrystalline powder (255 mg, 0.48 mmol). Yield: 96%. M.p. 127°C (decomp.) Anal. calcd. (%) for $C_{16}H_{19}IrSe_2$•CH_2Cl_2 (561.47 g mol^{-1}): C 31.85, H 3.59, Se 24,94; found: C 31.59, H 3.27, Se 24,43. ^{1}H NMR (400.13 MHz, CD_2Cl_2): δ = 1.89 (s, 15H, Cp*), 6.26 ppm (s, 4H, aromatic). ^{13}C{^{1}H} NMR (100.61 MHz, CD_2Cl_2): δ = 6.9 (s, -CH_3, Cp*), 96.8 (s, C=C, Cp*), 97 (s, C-H, aromatic), 133 ppm (s, C=Se, aromatic) ^{77}Se NMR (76.31 MHz, CD_2Cl_2): δ = 296.5 ppm. IR (ATR): $\tilde{\nu}$ = (C=Se) 1024 cm^{-1}.

10: This compound is air- and moisture-sensitive, and therefore accurate elemental analysis could not be obtained. ^{1}H NMR (300 MHz, CD_3CN): δ = 2.11 (s, 15H, Cp*), 7.15 ppm (s, 4H, aromatic C-H). IR (ATR): $\tilde{\nu}$ = (B-F) s, br, 1021 cm^{-1}.

Cytotoxicity study: The human A2780 ovarian cancer cell line was obtained from the European Collection of Cell Cultures (Salisbury, UK). Cells were grown routinely in RPMI medium containing glucose, 5% foetal calf serum (FCS), and antibiotics at 37°C and 5% CO_2. Cytotoxicity was determined using the MTT assay (MTT = 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide). Cells were seeded in 96-well plates as monolayers with 100 μL of cell solution (approximately 20000 cells) per well and pre-incubated for 24 h in medium supplemented with 10% FCS. Compounds were prepared as DMSO solution then dissolved in the culture medium and serially diluted to the appropriate concentration, to give a final DMSO concentration of 0.5%. Drug solution (100 μL) was added to each well and the plates were incubated for another 72 h. MTT (5 mg mL^{-1} solution) was then added to the cells and the plates were incubated for a further 2 h. The culture medium was aspirated, and the purple formazan crystals formed by the mitochondrial dehydrogenase activity of vital cells were dissolved in DMSO. The optical density, which is directly proportional to the number of surviving cells, was quantified at 540 nm using a multiwell plate reader, and the fraction of surviving cells was calculated from the absorbance of untreated control cells.

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[15] Crystal data for **9**: red crystals, $C_{16}H_{19}IrSe_2$, monoclinic, $P2_1/c$, $a = 7.6161(9)$, $b = 10.1745(11)$, $c = 20.9365(17)$ Å, $\beta = 90.837(9)°$, $V = 1622.2(3)$ Å^3, $Z = 4$, $T = 200(2)$ K, $\mu = 12.703$ mm^{-1}, 17567 reflections measured, 4701 independent ($R_{int} = 0.0499$), 3652 observed [$I > 2\sigma(I)$], 177 parameters, final R indices R_1 [$I > 2\sigma(I)$] = 0.0340 and wR_2 (all data) = 0.0769, GOF on $F^2 = 1.041$, max/min residual electron density = 2.37/−2.14 e Å^{-3}. A single crystal of compound **9** was selected, mounted onto a glass fiber, and transferred in a cold nitrogen gas stream. Intensity data were collected with a Bruker-Nonius Kappa-CCD with graphite-monochromated $Mo_{K\alpha}$ radiation. Unit-cell parameter determination, data collection strategy, and integration were carried out with the Nonius EVAL-14 suite of programs (A. J. M. Duisenberg, L. M. J. Kroon-Batenburg, A. M. M. Schreurs, *J. Appl. Crystallogr.* **2003**, *36*, 220). Multiscan absorption correction was applied (R. H. Blessing, *Acta Crystallogr. Sect. A* **1995**, *51*, 33). The structure was solved by direct methods using the SIR92 program (A. Altomare, G. Cascarano, C. Giacovazzo and A. Guagliardi, *J. Appl. Crystallogr.* **1993**, *26*, 343) and refined anisotropically by full-matrix least-squares methods using the SHELXL-97 software package (G. M. Sheldrick, *Acta Crystallogr. Sect. A* **2008**, *64*, 112). CCDC 771866 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
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