

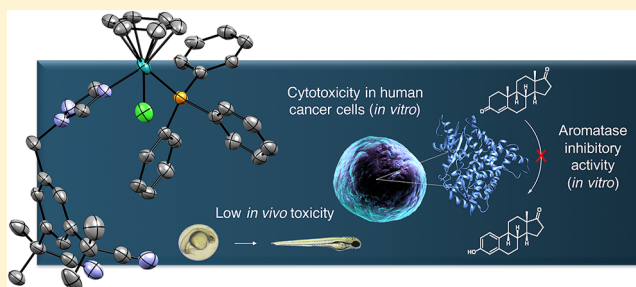
Organoruthenium(II) Complexes Bearing an Aromatase Inhibitor: Synthesis, Characterization, *in Vitro* Biological Activity and *in Vivo* Toxicity in Zebrafish Embryos

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Supporting Information

ABSTRACT: Third-generation aromatase inhibitors such as anastrozole (ATZ) and letrozole (LTZ) are widely used to treat estrogen receptor-positive (ER+) breast cancers in postmenopausal women. Investigating their ability to coordinate metals could lead to the emergence of a new category of anticancer drug candidates with a broader spectrum of pharmacological activities. In this study, a series of ruthenium(II) arene complexes bearing the aromatase inhibitor anastrozole was synthesized and characterized. Among these complexes, $[\text{Ru}(\eta^6\text{-C}_6\text{H}_6)(\text{PPh}_3)(\eta^1\text{-ATZ})\text{Cl}]\text{-BPh}_4$ (**3**) was found to be the most stable in cell culture media, to lead to the highest cellular uptake and *in vitro* cytotoxicity in two ER+ human breast cancer cell lines (MCF7 and T47D), and to induce a decrease in aromatase activity in H295R cells. Exposure of zebrafish embryos to complex **3** ($12.5 \mu\text{M}$) did not lead to noticeable signs of toxicity over 96 h, making it a suitable candidate for further *in vivo* investigations.



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INTRODUCTION

The coordination of biologically active molecules to metals is a promising strategy for the development of agents with a broader range of anticancer properties. Because metal complexes are widely studied for their ability to reduce the viability of cancer cells, the introduction of biologically active ligands within their structure can result in multitargeting drug candidates that could limit the emergence of cancer cell resistance mechanisms.^{1–3} For instance, numerous enzyme inhibitors are used to treat and/or prevent several types of cancers,^{4–6} making them promising ligands for the design of metal-based therapeutics. In addition, metal complexation greatly increases the structural possibilities to form enzyme inhibitors relative to purely organic molecules. Because they can adopt geometries other than linear, trigonal, or tetrahedral, metals can allow organic ligands (or enzyme inhibitors) to occupy a specific position in the active site of enzymes.^{7,8} A number of anticancer metal complexes including an enzyme inhibitor in their structure have been reported previously.^{9–11} However, metal complexes bearing aromatase inhibitors have so far been overlooked. Aromatase is the enzyme that catalyzes the final, rate-limiting step in estrogen synthesis from androgens.¹² More than two-thirds of breast tumors are estrogen receptor positive (ER+),¹³ and estrogens play a key role in initiating and promoting this type of hormone-dependent cancer.^{14–17} Currently, third-generation aromatase inhibitors such as the nonsteroidal triazole derivatives anastrozole (Arimidex) and letrozole (Femara) are found to

inhibit the aromatase activity in breast tissues. They are widely used to treat ER+ breast cancer, particularly in postmenopausal women who no longer produce ovarian estrogens and derive their estrogens mainly from adrenal androgens in extra ovarian tissues that have aromatase activity such as (breast) adipose.¹⁸ However, in about one-third of patients with metastatic ER+ breast cancer, endocrine therapies that involve aromatase inhibitors (or tamoxifen, known to inhibit ER+ cancer growth by blocking estrogen receptors) lead to the emergence of tumor cells that grow even in the absence of estrogens, resulting in a treatment-resistant cancer that is often incurable.¹⁹ Depriving ER+ cells of estrogens was also previously shown to sensitize them to cytotoxic agents.^{20,21} Thus, investigating the anticancer properties arising from the coordination of aromatase inhibitors to metals could lead to the development of efficient drug/prodrug candidates that display more than one mode of action, which could potentially circumvent the emergence of drug resistance mechanisms, a common cause of mortality in ER+ breast cancer patients. It was recently reported that the coordination of hydroquinoline, aminoquinoline, and uracyl ligands to copper can lead to cytotoxic complexes with an aromatase inhibitory activity.^{22,23} To our knowledge, only a few investigations from other groups involved the preparation of letrozole (Cu, Co, and Ni)^{24,25} or anastrozole (Pt)²⁶ metal complexes, and none of these studies

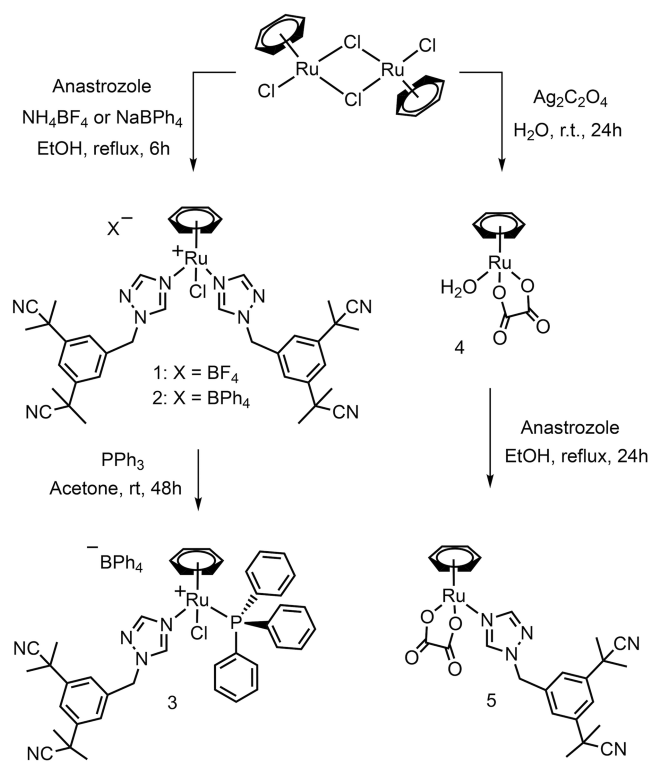
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reported an assessment of their aromatase inhibitory activity. Organoruthenium complexes are of particular interest for their activity against numerous types of cancer cells via multiple mechanisms and are often considered as interesting alternatives to currently used therapeutics.^{27–32} Maysinger et al. previously reported a preliminary account of the cytotoxicity of a series of ruthenium complexes bearing letrozole ligands.³³ Here, we report the synthesis, characterization, and biological activity of a similar class of ruthenium(II) complexes bearing the aromatase inhibitor anastrozole.

RESULTS AND DISCUSSION

Cationic complex **1** was obtained from a previously reported procedure³³ that led to its letrozole analogue $[\text{Ru}(\eta^6\text{-C}_6\text{H}_6)(\eta^1\text{-LTZ})_2\text{Cl}]\text{BF}_4$ (Ru-LTZ) by refluxing an ethanol solution of $[\text{Ru}(\eta^6\text{-C}_6\text{H}_6)\text{Cl}_2]_2$ and anastrozole (4 equiv) with an excess of NH_4BF_4 (68% yield) (Scheme 1). The same

Scheme 1. Synthetic Route to Complexes 1–5



synthetic strategy was used to prepare complex **2**, using NaBPh_4 (60% yield). As the ability of the triphenylphosphine ligand to enhance the cytotoxicity of complexes by improving their lipophilicity and by providing them with a mitochondrial targeting ability is well-precedented,^{34–37} the synthesis of compound **3** was undertaken by allowing an acetone solution of **2** to react with an excess of PPh_3 (74% yield). During the preparation of this 18-electron complex, only one of its anastrozole ligands underwent a substitution reaction, most likely via a dissociative mechanism. Complexes **1–3** are air-stable, soluble in acetone, in chlorinated solvents and DMSO, but poorly soluble in water. In alcohols, complex **1** is highly soluble whereas complexes **2** and **3** have a relatively lower solubility. In addition to obtaining the right balance between their lipophilicity and hydrophilicity, improving the poor water solubility of drug candidates remains an essential challenge in

drug design.³⁸ It was previously reported that oxalate ligands could significantly enhance the water solubility of ruthenium arene complexes when included in their coordination sphere.³⁹ Complex **5**, bearing both an oxalate and an anastrozole ligand, was therefore prepared by refluxing an ethanol solution of anastrozole and the ruthenium oxalate precursor **4** (52% yield). The identity of complexes **1–5** was confirmed by high-resolution electrospray ionization mass spectrometry (HR-ESI-MS), elemental analysis, and NMR spectroscopy. As expected, in the ^1H NMR spectrum of complexes **1–3** and **5**, resonances corresponding to the triazole protons of anastrozole are observed at downfield chemical shifts compared to the corresponding resonances in the spectrum of the free ligand. In the case of compound **3**, the presence of a singlet at 35 ppm (acetone- d_6) observed by $^{31}\text{P}\{^1\text{H}\}$ NMR confirms the coordination of triphenylphosphine to the ruthenium.^{33,40–42}

Solid-state structures of complexes **2–5** were obtained from single-crystal X-ray diffraction analyses (Table S1 in the Supporting Information). ORTEP views of the complexes are shown in Figure 1. As expected, they revealed a piano-stool

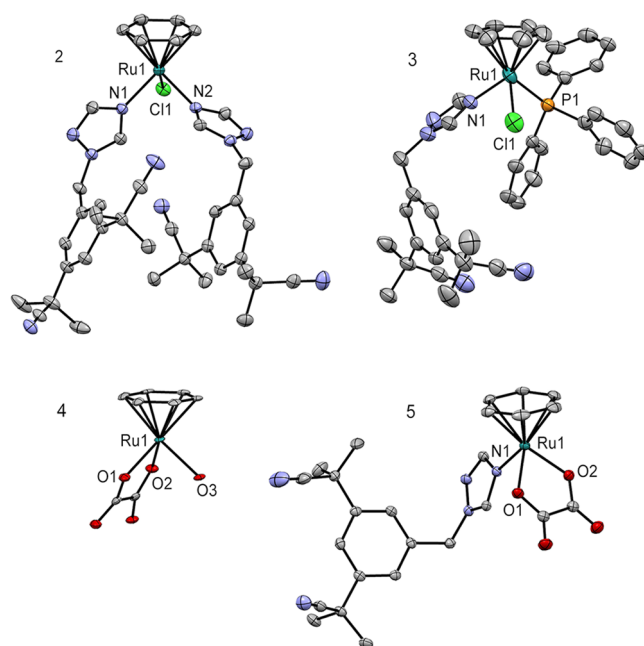


Figure 1. ORTEP diagrams (showing thermal ellipsoids at the 50% probability level) of complexes **2–5**. Note that in the case of **3**, only one site is shown for the disordered benzene, Cl and CN. **2**: Ru1–N1, 2.1024(12) Å; Ru1–N2, 2.1104(12) Å. **3**: Ru1–N1, 2.114(4); Ru1'–N1', 2.091(5). **5**: Ru1–N1, 2.1130(15) Å.

configuration, characteristic of ruthenium arene complexes.⁴³ Notably, similar Ru–N (anastrozole) bond lengths were noted for complexes **2**, **3**, and **5**, reflecting their comparable bond strength (Figure 1 and Table S2).

As metal-based drug candidates often display a limited solubility in cell culture media, DMSO is commonly used for the preparation of metal complex stock solutions for biological screenings. However, their solubility once diluted in culture medium and the lability of their ligand(s) in the presence of DMSO are often overlooked.⁴⁴ All complexes reported here were found to be soluble in cell culture medium (0.5% DMSO) at concentrations typically used for cytotoxicity screenings, as assessed by ultraviolet–visible (UV–vis) absorbance measurements. Their stability was evaluated in

three different conditions relevant to the biological experiments performed in this study: (i) in DMSO- d_6 , (ii) in water (0.5% DMSO), and (iii) in DMEM-F12 medium (0.1% DMSO). ^1H NMR analysis of all complexes in DMSO- d_6 revealed a high stability for all the complexes for which less than 5% of anastrozole dissociation was observed, except for **5**, for which a 15% anastrozole release was noted. Moreover, a limited anastrozole release (3–15%) was observed for all complexes when 150 μM aqueous solutions (0.5% DMSO) were separately incubated at 37 $^\circ\text{C}$ for 48 h (3 being the most stable) (Table S3), as assessed by ^1H NMR spectroscopy. The stability of each complex was also evaluated under conditions similar to that of the tritiated water-release assay. To this aim, a liquid/liquid extraction/HPLC-UV method was developed (Figure S1) to measure the amount of released anastrozole (or letrozole) when 10 μM solutions of complexes 1–3, **5**, and Ru-LTZ (previously reported letrozole analogue of **1**)³³ were incubated in DMEM/F-12 medium for 1.5 h (the duration of the tritiated water-release assay). The release of anastrozole (or letrozole) from most complexes was found to be considerable, except for complex **3**, for which only 4% of its aromatase inhibitor ligand was released (Table S4).

Because all ruthenium complexes reported in this study (except **4**) include at least one anastrozole ligand, their cytotoxicity was evaluated after 48 h in two ER+ human breast cancer cell lines, T47D and MCF7, using the sulforhodamine B (SRB) assay, and compared to clinically approved anticancer drugs *cis*-platin and anastrozole. As previously reported,³³ the cell growth inhibitor anastrozole did not display any noticeable cytotoxicity in MCF7 or T47D cells (results not shown). However, complexes 1–3 were each found to be active to various extents (Figure 2 and Table 1), whereas complexes **4** and **5** did not display any significant cytotoxicity (results not shown). It is noteworthy that because of the significant contribution of the tumor microenvironment to the implementation of the antitumor activity of similar types of Ru(II) species, the observed *in vitro* cytotoxicities of the complexes reported in this study are not necessarily indicative

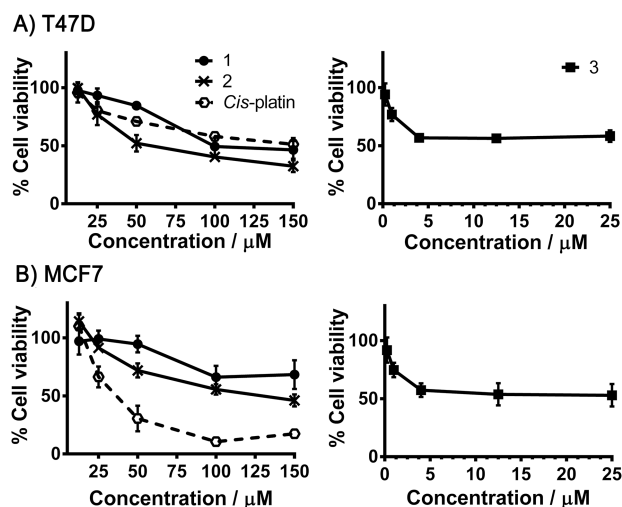


Figure 2. Cell viability determined by the SRB assay (48 h) in ER(+) breast cancer cells: T47D (A) and MCF7 (B), treated with **1**, **2**, and *cis*-platin (black dashed line) at the concentrations 12.5, 25, 50, 100, and 150 μM (left) and **3** at the concentrations 0.25, 1, 4, 12.5, and 25 μM (right). All values are expressed as means (from three independent experiments) \pm SD relative to the carrier.

Table 1. Estimated IC_{50} Values Illustrating the Effect of Complexes **1**–**3** and *cis*-Platin on the Viability of MCF7 and T47D Cancer Cells^a

	IC_{50} (μM) ^b	
	MCF7	T47D
1	>150	>150
2	139.4 (\pm 14.3)	53.5 (\pm 9.1)
3 ^c	\geq 4	\geq 4
<i>cis</i> -platin	37.0 (\pm 2.4)	>150

^aData extracted from Figure 2. ^bCytotoxicity was determined by exposure of cell lines to each complex for 48 h and expressed as the concentration required to inhibit cell viability by 50% (IC_{50}). Values in parentheses correspond to the standard deviation of three independent experiments. ^cAs seen in Figure 2, cell viability reached a plateau at concentrations above 4 μM .

of their potential *in vivo* antitumor activities.^{29,45} Although complexes **1** and **2** were less cytotoxic than *cis*-platin in MCF7 cancer cells, compounds **2** and **3** had a similar or even more effective cytotoxicity than that of the clinically approved drug in T47D cancer cells, which are known for their *cis*-platin resistance.⁴⁶ These results highlight the importance of developing such alternative complexes for breast cancer therapy. At concentrations below 12.5 μM , compound **3** was found to be the most cytotoxic of all complexes, reducing T47D and MCF7 cell viability by almost half at 4 μM . Cancer cells exposed to higher concentrations of the various complexes were especially susceptible to compound **2**, more significantly in the case of T47D cells, where the cell viability was inhibited by almost half at 50 μM . Interestingly, a significant difference was observed between the cytotoxicity of complexes **1** and **2** on both cell lines, which differ only in the nature of their counterion (BF_4^- vs BPh_4^-) (Table 1). The higher cytotoxicity of complexes **2** and **3** is not likely due to the sole contribution of the BPh_4^- counterion as both complexes induced a significantly higher cytotoxicity than NaBPh_4 in T47D cells at 25 μM (Figure S2).

Cellular levels of ruthenium were measured by inductively coupled plasma mass spectrometry (ICP-MS) after MCF7 cells were exposed to 4 μM solutions of all complexes for 48 h (Figure 3A). Compared to nontreated cells (control), a significant amount of ruthenium was observed in cells treated with each complex. Cellular ruthenium levels of each complex appeared to depend on their respective lipophilicity, in agreement with previous reports demonstrating that more

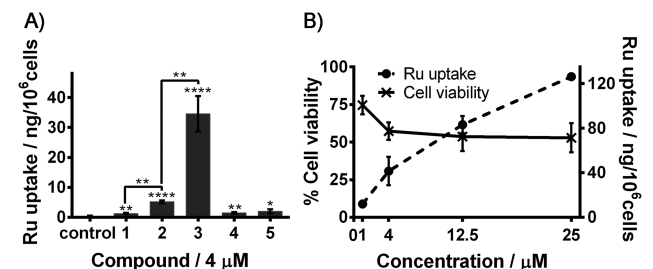


Figure 3. (A) Ruthenium cellular uptake (determined by ICP-MS) after exposure of MCF7 cells to 4 μM solutions of **1**–**5** (48 h); (B) ruthenium cellular uptake (determined by ICP-MS) and cell viability after exposure of MCF7 cells to **1**, **4**, 12.5, and 25 μM solutions of **3** (48 h). Error bars in the graph represent the standard deviation. Significant differences: * p < 0.05; ** p < 0.01; **** p < 0.0001.

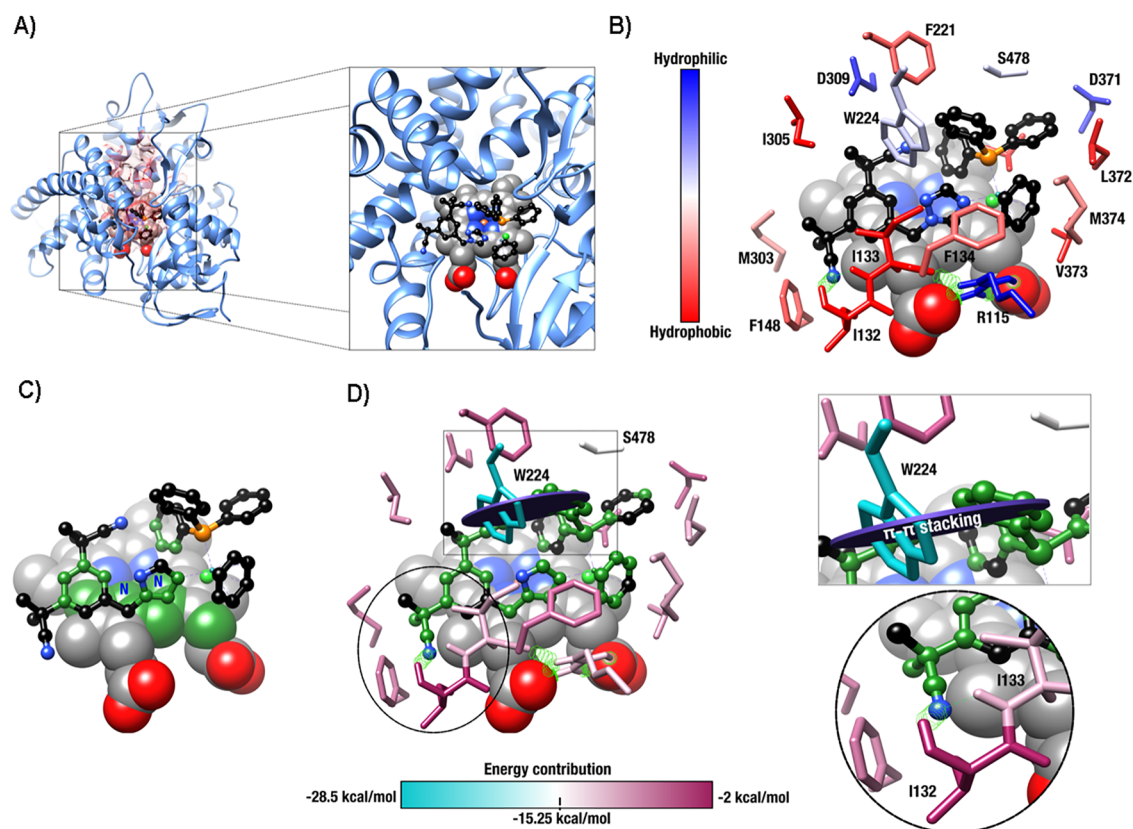


Figure 4. Ternary complex formation between compound 3, human aromatase, and a heme group as enzymatic cofactor. (A) Active-site pocket of aromatase⁶¹ highlighted by a red transparent surface with a zoomed view of the ligand interacting with the cofactor. (B) Ternary complex showing the most important stabilizing interactions between enzyme residues and the ruthenium complex. Amino acid (stick representation) identity is shown with single-letter code, and their hydrophobic profile is illustrated using the Kyte–Doolittle scale.⁶² Cofactor atoms (sphere representation) are color-coded as follows: carbon (gray), nitrogen (blue), iron (orange), and oxygen (red). Only hydrogen atoms participating in H-bonding interactions are shown (green strings and dotted lines). The ruthenium complex atoms (ball-and-stick representation) are shown using the same color coding, except for carbon (black) and phosphorus atoms (orange). The ruthenium and chlorine atoms are depicted in dark and light green, respectively. (C) Atomic interactions exhibiting higher energy values between the ruthenium-based ligand and cofactor in the ternary complex are highlighted in dark green. (D) Energy contributions for amino acids that stabilize the ternary complex are shown on a scale ranging from -2 to -28.5 kcal/mol. Trp224 is the most important energy contributor to this interaction, exhibiting a potential orthogonal π – π stacking energy value of -28.5 kcal/mol (black circle in the rectangle inset). Two alternative hydrogen bonding interactions were identified for Ile132 and Ile133 (circle inset). All panels show the same atomic orientation.

hydrophobic systems have a greater affinity for the cell membrane.⁴⁷ For instance, lipophilic counterion-containing compounds 2 and 3 (BPh_4^-) displayed the highest ruthenium cellular uptake ($3 > 2 > 1, 4, 5$). As previously suggested,⁴⁸ hydrophobic interactions between the arene ligands of organoruthenium cations and the phenyl groups of their BPh_4^- counterion might lead to strong ion-pairing, which might modulate drug uptake and consequently have an impact on their cytotoxicity. More specifically, complex 3 produced 7-fold higher cellular ruthenium levels than complex 2 (at $4 \mu\text{M}$), most likely because of the high lipophilicity of its PPh_3 ligand. Interestingly, we found that the steady cytotoxicity of complex 3 at concentrations greater than $1 \mu\text{M}$ was not a consequence of its limited cellular uptake at those concentrations (Figure 3B), as higher concentrations did result in higher ruthenium levels in MCF7 cells. A solubility assessment by UV–vis absorbance measurements also revealed that solutions of the complexes were not saturated at the tested concentrations. This suggests that complex 3 might act via a distinct mechanism of action. Whereas complex 3 was found to display the highest ruthenium cellular uptake, complexes 4 and 5 resulted in a very low ruthenium cellular uptake. It is

noteworthy that further studies revealed that 4 and 5 can inhibit the migration of MCF7 breast cancer cells (Figure S3), for which extracellular modes of action are often known to take place.⁴⁹

Because complex 3 showed negligible anastrozole ligand lability in DMSO and DMSO/media, we evaluated, theoretically and experimentally, the potential of this compound to act as an aromatase inhibitor. Docking simulations have been previously used to study the plausible interactions between transition-metal complexes and proteins or DNA.^{50–52} Here, we report a theoretical investigation of the potential interaction between a ruthenium complex (compound 3) and the aromatase enzyme using a docking simulation, based on the crystal structure of human placental aromatase cytochrome P450 (CYP19A1). Indeed, unlike suggested from previous docking studies for free anastrozole,^{53,54} the binding of anastrozole to the heme iron of CYP19 via its N1 triazole nitrogen atom (see Figure 1) is not possible in this system because of its involvement in the ruthenium coordination sphere. Nevertheless, results from this docking calculation (Figure 4) suggest that the interaction between the aromatase

protein and the inhibitor-containing ruthenium complex is energetically highly favorable.

The tritiated water-release assay was then selected to measure aromatase activity because it is a rapid and simple technique with high sensitivity and reproducibility.^{55,56} Given the low levels of aromatase in MCF7 cells,⁵⁷ human H295R adrenocortical carcinoma cells were selected for this study. Notably, H295R cells express numerous steroidogenic enzymes, including aromatase, making them very useful to examine compounds for their potential to interfere with the activity and/or expression of several key cytochrome P450 (CYP) enzymes involved in the biosynthesis of steroid hormones.^{58–60} Moreover, H295R cells were found to be less sensitive than MCF7 cells to the cytotoxicity of the ruthenium complexes we report here, allowing their use for this assay (Figure S4).

To determine the level of inhibition of the catalytic activity of aromatase in the presence of each complex (all complexes were studied for comparison purposes), H295R cells were coincubated with 1β -³H-androstenedione and each ruthenium complex for 1.5 h. Aromatase activity was assessed by quantifying the radioactivity of the tritium oxide produced from the aromatization reaction of the labeled androstenedione. Exposure of H295R cells to all complexes at concentrations greater than 1 nM resulted in a statistically significant, concentration-dependent reduction of aromatase activity (Figure 5), except for complex 4 (data not shown),

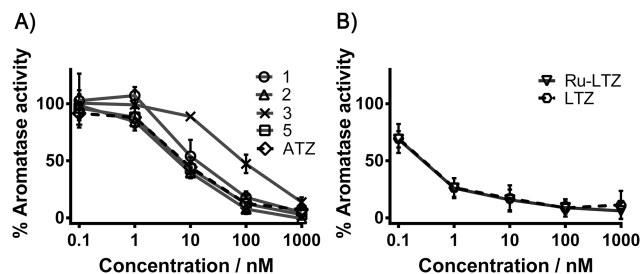


Figure 5. Effects of the exposure of H295R cells with (A) anastrozole (ATZ) (black dashed line), 1, 2, 3, and 5 and (B) letrozole (LTZ) (black dashed line) and Ru-LTZ on aromatase activity. Cells were treated for 1.5 h with the indicated concentrations of the compounds. Values represent the mean \pm SD. Significant differences for aromatase activity are reported relative to the controls. Significant differences ($p < 0.001$): $14 \pm 4\%$ for 3 at 1000 nM vs $34 \pm 7\%$ for ATZ at 40 nM; $47 \pm 3\%$ for 3 at 100 nM vs $74 \pm 3\%$ for ATZ at 4 nM (ATZ results obtained from interpolation).

confirming the crucial role of anastrozole in the structure of the ruthenium complex. The aromatase inhibitory activity observed for complexes 1, 2, 5, and Ru-LTZ was not significantly different from that of the corresponding aromatase inhibitor alone, an observation which is consistent with the considerable lability of the enzyme inhibitor ligands under these conditions (*vide supra*). Interestingly, complex 3 was found to be a less potent inhibitor of aromatase activity than anastrozole alone but to have a significantly higher activity than the free anastrozole levels expected from stability studies (Figure 5A). The observed activity for 3 suggests a supplementary contribution from the intact complex, as supported by docking studies (Figure 4), and/or an intracellular substitution of the anastrozole ligand. Notably, during this short period of incubation (1.5 h), a relatively high level of ruthenium in H295R cells treated with 3 was revealed by ICP-

MS (Figure S5), which confirms the internalization of the complex.

Over the past few years, the development of zebrafish embryos has become a prominent *in vivo* model for drug discovery and toxicity assessment because of their high degree of genetic conservation with humans, rapid embryogenesis, short reproductive cycle, high transparency, and low cost.^{63,64} Also, the zebrafish embryo assay has previously been reported to be a suitable phenotype-based screening method to assess the *in vivo* toxicity of ruthenium complexes.^{47,65–67} Because of their greater *in vitro* cytotoxicity in T47D cells compared to that of the currently used chemotherapeutic agent *cis*-platin, complexes 2 and 3 were selected for an *in vivo* toxicity assessment using the zebrafish embryo assay. Hatching rates, survival rates, and phenotype changes of the zebrafish embryos treated with $12.5 \mu\text{M}$ of each compound were determined at 24, 48, 72, and 96 h post fertilization (hpf) (Figure 6). Given

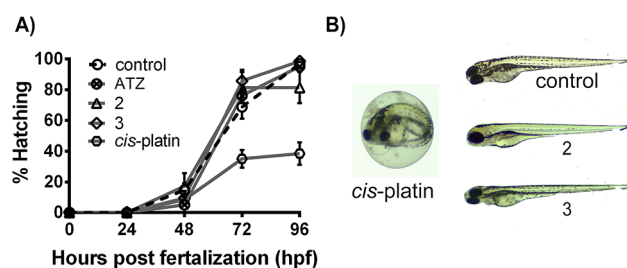


Figure 6. (A) Effect of 2, 3, anastrozole (ATZ), and *cis*-platin on the hatching rate of developing zebrafish embryos. Hatching rates were assessed at $12.5 \mu\text{M}$ over 4 days postfertilization (96 hpf). Control is shown as a black dashed line. (B) Gross morphological phenotypes in zebrafish embryos: untreated (control) and treated with $12.5 \mu\text{M}$ 2, 3, and *cis*-platin. Data are expressed as means \pm standard deviation from three independent experiments (a total of 60 embryos).

the poor solubility of the complexes in fish medium, concentrations higher than $12.5 \mu\text{M}$ were not tested. Specifically, *cis*-platin was more toxic to the embryos than the other compounds. Until 96 hpf, no significant mortality of zebrafish embryos was observed for any of the ruthenium complexes. However, a 72 h exposure to *cis*-platin dramatically decreased hatching rates, whereas no significant difference was observed between the hatching rate of embryos treated with complexes 2, 3, and anastrozole and that of nontreated embryos. Moreover, at 96 hpf, a significant number of phenotype abnormalities such as edema ($25\% \pm 5\%$) was detected in *cis*-platin treated embryos.

CONCLUSION

A series of ruthenium complexes bearing anastrozole ligands were prepared and characterized. Among these complexes, 3 was found to be the most stable in cell culture media and to lead to the highest cellular uptake in ER+ human breast cancer cells. In contrast to anastrozole alone, considerable *in vitro* cytotoxicity was observed in two ER+ human breast cancer cell lines (MCF7 and T47D) treated with 3. In addition, complex 3 was found to induce a decrease in aromatase activity in H295R cells. Exposure of zebrafish embryos to complex 3 ($12.5 \mu\text{M}$) did not lead to noticeable signs of toxicity over 96 h, making it a suitable candidate for further *in vivo* investigations. This study opens the door to the development of a novel class of anastrozole-containing organometallic

anticancer drug candidates with a broader spectrum of pharmacological activities.

EXPERIMENTAL SECTION

General Comments. All chemicals were obtained from commercial sources and were used as received. Anastrozole and letrozole were purchased from Triplebond and AK scientific, respectively. $\text{RuCl}_3 \cdot x\text{H}_2\text{O}$, ammonium tetrafluoroborate, sodium tetraphenylborate, triphenylphosphine, silver nitrate, hydrocortisone, and oxalic acid were purchased from Sigma-Aldrich. $[\text{Ru}(\eta^6\text{-C}_6\text{H}_6)_2\text{Cl}_2]_2$ dimer, silver oxalate, and Ru-LTZ were prepared from previously reported procedures.^{33,68,69} Experiments were performed under a nitrogen atmosphere, and solvents were dried using a solvent purification system (Pure Process Technology). All NMR spectra were recorded at room temperature on a 400 MHz (or 600 MHz) Varian spectrometer and were referenced to solvent resonances. Chemical shifts and coupling constants are reported in parts per million and Hertz, respectively. Mass spectral data was obtained from high-resolution and high accuracy mass analysis (HR-ESI-MS) using an Exactive Orbitrap spectrometer from ThermoFisher Scientific (Department of Chemistry, McGill University). A PerkinElmer Nexion 300X ICP mass spectrometer was used for the determination of ruthenium in biological samples (Department of Chemistry, Université de Montréal). The purity of all ruthenium complexes (>95%) was assessed by elemental analyses (Laboratoire d'Analyse Élémentaire, Department of Chemistry, Université de Montréal) and HPLC-MS, using a Waters 2795 separation module coupled to a Waters Micromass Quattro Premier XE tandem quadrupole mass spectrometer.

Complex Synthesis and Characterization. $[\text{Ru}(\eta^6\text{-C}_6\text{H}_6)(\eta^1\text{-ATZ})_2\text{Cl}]_2\text{BF}_4$ **1**. Anastrozole (0.235 g, 0.80 mmol), $[\text{Ru}(\eta^6\text{-C}_6\text{H}_6)_2\text{Cl}_2]_2$ (0.100 g, 0.20 mmol), and NH_4BF_4 (0.104 g, 1.00 mmol) were dissolved in degassed ethanol (20 mL). The mixture was heated under reflux for 6 h, allowed to cool to room temperature, filtered, and the precipitate was washed with a minimum of ethanol. The filtrate was then collected and dried under vacuum. The residue was dissolved in dichloromethane (10 mL), filtered, and concentrated to a minimum. Compound **1** was precipitated with diethyl ether, washed with hexane, and dried under vacuum. The final product was obtained as a yellow powder (0.240 g, 68%). ^1H NMR (CDCl_3 , 400 MHz): δ 1.75 (d, $J = 4.9$ Hz, CH_3 , 24H), 5.40 (m, CH_2 , 4H), 5.9 (s, C_6H_6 , 6H), 7.43 (d, $J = 1.6$ Hz, ArH, 4H), 7.54 (t, $J = 1.5$ Hz, ArH, 2H), 8.27 (s, H_{triazole} , 2H), 9.35 (s, H_{triazole} , 2H). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 100 MHz): δ 28.8 (CH_3 , 8C), 37.3 (CCN, 4C), 54.2 (CH_2 , 2C), 85.5 (C_6H_6 , 6C), 122.3 (CHC_{Ar} , 2C), 124.0 (CN, 4C), 125.0 (CHC_{Ar} , 4C), 135.4 (C_{Ar} , 2C), 143.4 (C_{Ar} , 4C), 146.7 ($\text{C}_{\text{triazole}}$, 2C), 151.6 ($\text{C}_{\text{triazole}}$, 2C). Found (%): C, 54.65; H, 5.18; N, 16.05. $\text{C}_{40}\text{H}_{44}\text{B}_1\text{Cl}_1\text{F}_4\text{N}_{10}\text{Ru}_1 \cdot 1/16 \text{C}_6\text{H}_{14}$ requires C, 54.28; H, 5.06; N, 15.67. HR-ESI-MS m/z (+): found 801.25 M^+ ($[\text{Ru}(\eta^6\text{-C}_6\text{H}_6)(\eta^1\text{-ATZ})_2\text{Cl}]^+$) (calcd 801.25), 508.08 [$\text{M}^+ \text{-ATZ}$] $^+$ (calcd 508.08).

$[\text{Ru}(\eta^6\text{-C}_6\text{H}_6)(\eta^1\text{-ATZ})_2\text{Cl}]_2\text{BPh}_4$ **2**. Degassed ethanol (20 mL) was added to anastrozole (0.235 g, 0.80 mmol), $[\text{Ru}(\eta^6\text{-C}_6\text{H}_6)_2\text{Cl}_2]_2$ (0.100 g, 0.20 mmol), and NaBPh_4 (0.342 g, 1.00 mmol). The mixture was heated under reflux for 6 h until a yellow precipitate appeared and was then cooled to room temperature and filtered. The precipitate was washed with a minimum amount of ethanol before being dissolved in acetone (5 mL). The resulting solution was filtered on a Celite pad, and the filtrate was concentrated under reduced pressure. Compound **2** was precipitated with diethyl ether, washed with hexane, and dried under vacuum. The final product was obtained as a yellow powder (0.270 g, 60%). ^1H NMR ($(\text{CD}_3)_2\text{CO}$, 400 MHz): δ 1.73 (s, CH_3 , 24H), 5.60 (m, CH_2 , 4H), 6.07 (s, C_6H_6 , 6H), 6.77 (t, $J = 7.2$ Hz, H_{BPh_4} , 4H), 6.91 (t, $J = 7.4$ Hz, H_{BPh_4} , 8H), 7.33 (m, H_{BPh_4} , 8H), 7.45 (d, $J = 1.7$ Hz, ArH, 4H), 7.67 (t, $J = 1.8$ Hz, ArH, 2H), 8.50 (s, H_{triazole} , 2H), 9.08 (s, H_{triazole} , 2H). $^{13}\text{C}\{^1\text{H}\}$ NMR ($(\text{CD}_3)_2\text{CO}$, 100 MHz): δ 28.1 (CH_3 , 8C), 37.2 (CCN, 4C), 53.6 (CH_2 , 2C), 85.3 (C_6H_6 , 6C), 121.3 (C_{BPh_4} , 4C), 122.0 (CHC_{Ar} , 2C), 123.8 (CN, 4C), 124.7 (CHC_{Ar} , 4C), 125.1 (C_{BPh_4} , 8C), 136.1 (C_{BPh_4} , 8C), 136.4 (C_{Ar} , 2C), 143.5 (C_{Ar} , 4C), 146.4 ($\text{C}_{\text{triazole}}$, 2C), 153.0

($\text{C}_{\text{triazole}}$, 2C), 163.3 (C_{BPh_4} , 4C). Found (%): C, 69.27; H, 6.13; N, 11.70. $\text{C}_{64}\text{H}_{64}\text{B}_1\text{Cl}_1\text{N}_{10}\text{Ru}_1 \cdot 1/2\text{C}_6\text{H}_{14}$ requires C, 69.16; H, 6.15; N, 12.03. HR-ESI-MS m/z (+): 801.25 M^+ ($[\text{Ru}(\eta^6\text{-C}_6\text{H}_6)(\eta^1\text{-ATZ})_2\text{Cl}]^+$) (calcd 801.25), 508.08 [$\text{M}^+ \text{-ATZ}$] $^+$ (calcd 508.08), 1922.66 [$2\text{M}^+ \text{+BPh}_4$] $^+$ (calcd 1922.66).

$[\text{Ru}(\eta^6\text{-C}_6\text{H}_6)(\eta^1\text{-ATZ})(\text{PPh}_3)\text{Cl}]\text{BPh}_4$ **3**. Triphenylphosphine (0.032 g, 0.12 mmol) was added to a solution of **2** (0.112 g, 0.10 mmol) in acetone (8 mL), and the mixture was stirred at ambient temperature for 48 h. The solvent was then concentrated, and the product precipitated with diethyl ether. After the powder was washed with diethyl ether, compound **3** was collected as a yellow powder (0.081 g, 74%). ^1H NMR ($(\text{CD}_3)_2\text{CO}$, 400 MHz): δ 1.77 (s, CH_3 , 12H), 5.44 (m, CH_2 , 2H), 6.01 (s, C_6H_6 , 6H), 6.77 (t, $J = 7.2$ Hz, H_{BPh_4} , 4H), 6.91 (t, $J = 7.4$ Hz, H_{BPh_4} , 8H), 7.33 (m, H_{BPh_4} , 8H), 7.39–7.51 (m, H_{PPh_3} , 15H), 7.62 (d, $J = 1.8$ Hz, ArH, 2H), 7.75 (t, $J = 1.8$ Hz, ArH, 1H), 8.35 (s, H_{triazole} , 1H), 9.03 (s, H_{triazole} , 1H). $^{13}\text{C}\{^1\text{H}\}$ NMR ($(\text{CD}_3)_2\text{CO}$, 100 MHz): δ 28.2 (CH_3 , 4C), 37.2 (CCN, 2C), 53.5 (CH_2 , 1C), 90.7 (C_6H_6 , 6C), 121.3 (C_{BPh_4} , 4C), 122.3 (CHC_{Ar} , 1C), 123.9 (CN, 2C), 125.1 (C_{BPh_4} , 8C), 125.5 (CHC_{Ar} , 2C), 128.5 (C_{PPh_3}), 128.6 (C_{PPh_3}), 131.1 (C_{PPh_3}), 133.7 (C_{PPh_3}), 133.8 (C_{PPh_3}), 135.9 (C_{Ar} , 1C), 136.1 (C_{BPh_4} , 8C), 143.5 (C_{Ar} , 2C), 146.4 ($\text{C}_{\text{triazole}}$, 1C), 154.2 ($\text{C}_{\text{triazole}}$, 1C), 164.2 (C_{BPh_4} , 4C). $^{31}\text{P}\{^1\text{H}\}$ NMR ($(\text{CD}_3)_2\text{CO}$, 200 MHz): δ 35.4. Found (%): C, 70.45; H, 5.45; N, 7.16. $\text{C}_{65}\text{H}_{60}\text{B}_1\text{Cl}_1\text{N}_5\text{P}_1\text{Ru}_1 \cdot \text{H}_2\text{O}$ requires C, 70.49; H, 5.64; N, 6.32. [Although the elemental analysis value of N is outside the range viewed as establishing analytical purity, it is provided to illustrate the best value obtained to date. NMR spectra are provided in the Supporting Information as evidence of bulk purity (Figures S6–S8).] HR-ESI-MS m/z (+): 770.17 M^+ ($[\text{Ru}(\eta^6\text{-C}_6\text{H}_6)(\eta^1\text{-ATZ})(\text{PPh}_3)\text{Cl}]^+$) (calcd 770.17).

$[\text{Ru}(\eta^6\text{-C}_6\text{H}_6)\text{oxalate}(\text{H}_2\text{O})]$ **4**. A mixture of silver oxalate (0.303 g, 1.00 mmol) and $[\text{Ru}(\eta^6\text{-C}_6\text{H}_6)_2\text{Cl}_2]_2$ (0.200 g, 0.40 mmol) was stirred in degassed water (60 mL) at ambient temperature for 24 h. The reaction mixture was filtered on a Celite pad and washed with water (30 mL), and the filtrate was dried under reduced pressure. After adding dichloromethane, the product precipitated as an orange powder (0.157 g, 65%). ^1H NMR (D_2O , 400 MHz): δ 5.75 (s, C_6H_6 , 6H). $^{13}\text{C}\{^1\text{H}\}$ NMR (D_2O , 100 MHz): δ 77.9 (C_6H_6 , 6C), 163.8 ($\text{C}_{\text{oxalate}}$, 2C). Found (%): C, 32.40; H, 2.58. $\text{C}_8\text{H}_8\text{O}_5\text{Ru}_1 \cdot 1/4\text{CH}_2\text{Cl}_2$ requires C, 32.34; H, 2.80. HR-ESI-MS m/z (–): 284.93 [M-H] $^-$ (calcd 284.93), 552.87 [$2\text{M-H}_2\text{O-H}$] $^-$ (calcd 552.86), 820.80 [$3\text{M-H}_2\text{O-H}$] $^-$ (calcd 820.80).

$[\text{Ru}(\eta^6\text{-C}_6\text{H}_6)\text{oxalate}(\eta^1\text{-ATZ})]$ **5**. Silver oxalate (0.303 g, 1.00 mmol) was added to a solution of $[\text{Ru}(\eta^6\text{-C}_6\text{H}_6)_2\text{Cl}_2]_2$ (0.200 g, 0.40 mmol) in degassed water (60 mL), and the suspension was stirred at ambient temperature for 24 h. The reaction mixture was filtered on a Celite pad, washed with water (30 mL), and the filtrate was dried under reduced pressure. The resulting intermediate was dissolved in ethanol (60 mL), and anastrozole (0.469 g, 1.60 mmol) was added to the solution. The mixture was then heated under reflux for 24 h. The reaction mixture was concentrated under reduced pressure to dryness, and the product was purified by column chromatography (silica gel) using dichloromethane/methanol (7:1 v/v). Compound **5** was obtained as a light yellow powder (0.235 g, 52%). ^1H NMR (CD_3OD , 400 MHz): δ 1.71 (s, CH_3 , 12H), 5.54 (s, CH_2 , 2H), 5.88 (s, C_6H_6 , 6H), 7.44 (d, $J = 1.8$ Hz, ArH, 2H), 7.64 (t, $J = 1.8$ Hz, ArH, 1H), 8.21 (s, H_{triazole} , 1H), 8.88 (s, H_{triazole} , 1H). $^{13}\text{C}\{^1\text{H}\}$ NMR ($(\text{CD}_3)_2\text{CO}$, 100 MHz): δ 27.7 (CH_3 , 4C), 37.2 (CCN, 2C), 53.4 (CH_2 , 1C), 82.5 (C_6H_6 , 6C), 122.1 (CHC_{Ar} , 1C), 123.7 (CN, 2C), 124.3 (CHC_{Ar} , 2C), 136.2 (C_{Ar} , 1C), 143.5 (C_{Ar} , 2C), 145.2 ($\text{C}_{\text{triazole}}$, 1C), 151.7 ($\text{C}_{\text{triazole}}$, 1C), 165.7 ($\text{C}_{\text{oxalate}}$, 2C). Found (%): C, 52.76; H, 4.62; N, 12.16. $\text{C}_{25}\text{H}_{25}\text{N}_5\text{O}_5\text{Ru}_1 \cdot 1/8\text{CH}_2\text{Cl}_2$ requires C, 52.84; H, 4.46; N, 12.26. HR-ESI-MS m/z (+): 584.08 [$\text{M} + \text{Na}$] $^+$ (calcd 584.08), 852.01 [$2\text{M-ATZ} + \text{Na}$] $^+$ (calcd 852.01), 1145.18 [$2\text{M} + \text{Na}$] $^+$ (calcd 1145.18).

Solubility in DMSO/Media. UV–vis spectroscopy was used to evaluate the solubility of the ruthenium complexes. Accordingly, a 2 mL solution of each complex was prepared in full RPMI growth media (phenol red free) at the concentrations used for cellular proliferation studies (DMSO final concentration: 0.5%). After incubation for 48 h

at 37 °C, the solution was filtered on a Celite pad, and the absorbance was recorded using a Cary 300 Bio UV–vis spectrometer. The concentration of saturation of the complexes in growth media was assessed by determining the concentration at which a maximum intensity in UV absorbance (274–278 nm) was observed.

X-ray Diffraction. Four ruthenium(II) complexes were structurally characterized by single-crystal X-ray analysis. Suitable crystals were obtained by slow evaporation of the solutions of compounds 2, 3, 4, and 5 in ethanol/acetone, acetone/diethyl ether, water, and methanol, respectively. Data were collected at 100 K using a Bruker Smart APEX diffractometer. Structures were solved with the XT structure solution program and refined with the XL refinement package using least squares minimizations.^{70–72}

Cell Culture. Protocols used for biological studies were approved by the Institutional Research Ethics Committee of INRS - Institut Armand-Frappier. Estrogen receptor positive (ER+) breast cancer cells MCF7 and T47D were respectively provided by Prof. Chatenet and Prof. Plante (INRS). The H295R human adrenocortical carcinoma cell line that expresses CYP19 (aromatase)⁵⁹ were obtained from Prof. Sanderson's collection (INRS). Human MCF7 breast cancer cells were grown in RPMI 1640 containing fetal bovine serum (10%). The growth medium for T47D breast cancer cells was RPMI 1640 supplemented with HEPES (2.38 g/L), sodium pyruvate (0.11 g/L), glucose (2.5 g/L), insulin bovine (10 µg/mL), and fetal bovine serum (10%). H295R cells were cultured in DMEM/F-12 supplemented with Nu Serum (2.5%) and ITS (1%). All growth media were supplemented with penicillin/streptomycin. All cell culture products were obtained from commercial sources, including Gibco, Sigma-Aldrich, Corning, and Invitrogen.

Cytotoxicity. Cell viability was examined by slightly modified standard methods using the SRB colorimetric assay described by Vichai and Kirtikara.⁷³ Briefly, for all experiments, cells were seeded in 96-well plates (Sarstedt) at a density of 1×10^4 cells/well (MCF7 and T47D) or 3×10^4 cells/well (H295R) maintained at 37 °C, 5% CO₂ in a humidified atmosphere and were grown in serum-containing media for 24 h before treatment. Stock solutions of the compounds were prepared in DMSO, and the final concentration of DMSO was kept constant (nontoxic concentration) depending on the cell line: 0.5% for MFC-7 and T47D and 0.25% for H295R. To reach final concentrations of 1, 4, 12.5, 25, 50, 100, and 150 µM, 1 µL (or 0.5 µL) of each stock solution (200 or 400 times more concentrated than the corresponding final concentration) was added to each well containing 200 µL of fresh and complete growth medium. Also, cancer cells were exposed to complete growth medium alone, growth medium containing 0.5% or 0.25% DMSO (negative control), and growth medium containing 25% DMSO (positive control). After incubation for 48 h, without removing the cell culture supernatant, cells in each well were fixed with 100 µL of cold trichloroacetic acid (TCA) 10% w/v at 4 °C for 1 h. After fixation, TCA was discarded, and wells were washed four times with slow-running tap water and then air-dried. An SRB solution (0.057% w/v) was added to the wells, and plates were kept for 30 min at room temperature. Unbound SRB was removed by washing the wells four times with 1% acetic acid. Plates were air-dried. To dissolve the protein-bound dye, cells from each well were exposed to 200 µL of 10 mM Tris base solution (pH 10.5) for 30 min. Absorbance was measured at 510 nm using a microplate reader. The viability of the cancer cells versus different concentrations of each complex was reported. This assay was carried out in two or three independent sets of experiments, each experiment with four or five replicates per concentration level.

Stability in DMSO/Water. Stock solutions of the test compounds in DMSO (150 µL) were diluted with distilled water (40 mL) to give a final concentration of 150 µM (maximum DMSO content did not exceed 0.5%). After incubation of the samples for 48 h at 37 °C, solutions were dried by vacuum. An NMR sample of each compound was prepared in an appropriate deuterated solvent (the same solvents as the ones used for ¹H NMR characterizations) in which both the complex and the aromatase inhibitor were highly soluble. For each complex, the percentage of released aromatase inhibitor was calculated by comparing the signal intensity of one of the protons

of anastrozole or letrozole with that of corresponding signal in the ¹H NMR spectrum of their complex. This experiment was done in triplicate.

Stability in DMSO/Media. A HPLC-UV method was developed and consisted of a simple liquid/liquid extraction after incubation of the complexes in media. The method was found to be reproducible and linear over the range of concentrations used for the aromatase activity assessment. **Preparation of standards.** Solutions of anastrozole (or letrozole) in 20 mL of phenol red free DMEM/F-12 at 0.1, 1, 5, 10, and 15 µM (DMSO final concentration: 0.1%) were incubated at 37 °C for 1.5 h (conditions used for the tritiated water-release assay). After incubation, anastrozole or letrozole was retrieved from the media solution by liquid/liquid extraction using diethyl ether (3 × 10 mL), which has previously been reported as an adequate solvent to recover anastrozole from human plasma.⁷⁴ The diethyl ether solution was then dried under vacuum, and the residue was dissolved in 2 mL of acetone and loaded on a thin layer of silica. Acetone (20 mL) was used as a mobile phase to completely recover anastrozole (or letrozole) from silica and minimize the amount of media residue in the final HPLC samples. Final HPLC samples were prepared by evaporating the acetone solution to dryness and dissolving the residue in 1 mL of HPLC grade acetone containing 100 µM hydrocortisone as an external standard. Standard curves of anastrozole and letrozole are shown in Figure S1. **HPLC-UV method.** The chromatography was performed on an Agilent UHPLC system (1260 Infinity GPC/SEC). An Agilent Poroshell 120 EC-C18 column (4.6 × 100 mm, 2.7 µm) was used, and the column temperature was maintained at room temperature. Chromatographic separation was obtained through a 13 min gradient delivery of a mixture of acetonitrile and water at a flow rate of 2 mL/min: (a) 0–1 min, water, 100%; (b) 1–4 min, acetonitrile, 0–30%; (c) 4–10 min, acetonitrile, 30%; (d) 10–11 min, acetonitrile, 30–100%; (e) 11–13 min, acetonitrile, 100%. UV absorbance at both 254 and 215 nm was recorded, but only the data acquired at 215 nm was used. **Sample preparation.** Stock solutions (10⁴ µM) of anastrozole, letrozole and all complexes (except 4, which has no aromatase inhibitor ligand) were prepared in DMSO. The 10 µM solutions of the compounds were prepared by adding 20 µL of a stock solution to 20 µL of DMEM/F12 (DMSO final concentration: 0.1%). Solutions were incubated at 37 °C for 1.5 h, and further steps were as described for the standards' preparation. The experiment was carried out in triplicate.

Antimigratory Activity. MCF7 cells were cultured in RPMI 1640 supplemented with 10% FBS and penicillin-streptomycin. 100 000 cells/well were seeded in 24 well plates (Sarstedt) and incubated at 37 °C and 5% CO₂ to reach confluency. Scratches were created using a pipet tip on the confluent monolayer and washed with full growth medium (1 × 500 µL) to remove cellular debris. The fresh medium (500 µL) supplemented with 0.5% FBS containing 10 µM of the synthesized complexes (except compound 3 which was cytotoxic at 10 µM) was individually added into each well and incubated for 48 h to allow the wound closure. The RPMI-1640 medium containing 0.5% FBS and 0.1% DMSO (vehicle) was used as a control. The migration of the treated and untreated cells (vehicle) into the wound area at incubation 0 and 48 h were compared by capturing the images with a Nikon Eclipse Ti microscope (equipped with a Nikon DS-Ri2 camera) at 10× magnification. Cell migration was analyzed using ImageJ software and the MRI wound healing tool plugin (NIH, Bethesda, MD) and computed into a percentage of control (means ± SEM; n = 8) using untreated wells at 100%.

Cellular Uptake. MCF7 cells were grown in 6-well plates (200 000 cells/well) and incubated for 24 h. Stock solutions (20 mM) of the complexes in DMSO were freshly prepared and diluted with cell culture medium to the desired concentration, for which no cytotoxicity was expected to maintain nearly complete cell survival (final complex concentration was 4 µM, except compound 3, which was tested at four concentrations: 1, 4, 12.5, and 25 µM). The cell culture medium of each well was replaced with 2 mL of the cell culture medium solutions containing the complexes, and the plates were incubated at 37 °C under 5% CO₂ for 48 h. The culture medium was removed, and the cell layer of each well was washed with 2 mL of

phosphate buffered saline (PBS). Then cells in each well were trypsinized (300 μL) and resuspended in 1700 μL of growth media. The number of cells in each well was counted, and cell pellets were isolated by centrifugation (3000g for 10 min at room temperature). Each pellet was digested for 3 days at room temperature in HNO_3 (70%, Sigma-Aldrich), and the resulting solutions were diluted to 25 mL using Milli-Q water (final concentration of 2.8% nitric acid). The amount of Ru cellular uptake was evaluated by ICP-MS. The experiment was carried out in triplicate.

Aromatase Inhibition. The H295R cell line was selected for this study because it expresses CYP19 enzyme, making it a suitable model for the study of aromatase activity.⁵⁸ Aromatase activity was measured using a tritiated water-release assay as described previously.^{59,75} Briefly, H295R cells were cultured in 24-well plates (100 000 cells/well) containing 1 mL of the appropriate culture medium. After 24 h, the medium was removed and cells were washed once with 500 μL PBS. Then, a volume of 250 μL of culture medium containing 54 nM $1\beta\text{-}^3\text{H}$ -androstenedione and different concentrations of anastrozole, letrozole, or each complex (0.1, 1, 10, 100, and 1000 nM) was added to each well, and cells were incubated for 1.5 h at 37°C (5% CO_2). Further steps were as described previously.⁵⁹ Tritiated water was counted in 24-well plates containing a liquid scintillation cocktail using a Microbeta Trilux (PerkinElmer, Waltham, MA). Incubations in the absence of cells (blanks) and in the presence of DMSO 0.1% (which was the concentration used to dissolve the complexes in the growth media for this study) were included as negative and positive controls, respectively.

Virtual Docking of Compound 3 with the Human Aromatase Enzyme. Formation of the ternary complex between human aromatase, compound 3, and the heme group was simulated using a ligand-imprinted docking procedure.¹² A Nelder–Mead simplex iteration was applied during the energy minimization steps, and the steric interactions, hydrogen bonding, and electrostatic forces were calculated by piecewise linear potentials and Coulomb potentials, respectively.¹³ The crystal structure of 3 was generated with the XT structure solution program and refined with the XL refinement package using least squares minimization against the crystallographically resolved aromatase template (PDB entry SJI6).⁶¹ The MolDock scoring function was used in combination with a cavity prediction algorithm using the Molegro Virtual Docker 6.0 suite without the inclusion of water molecules.¹³ Twenty rounds of docking simulations were performed to maintain search robustness, with the best conformations emerging from a group of up to 4 000 000 combinations.

Zebrafish Embryo Assay. Wild-type zebrafish (*Danio rerio*) embryos were raised at 28.5 °C and staged as previously described.⁷⁶ Embryos at the 4-cell stage were separately exposed to 2, 3, anastrozole, and *cis*-platin solutions (12.5 μM), prepared by diluting the DMSO stock solution of each compound in the fish medium (DMSO final concentration = 0.1%). The medium (containing the compound to be tested) was refreshed after 48 h for each experiment. A no-treatment control was also included. Experiments were performed in triplicate, and a total of 60 embryos from the pooling of three different crosses have been used per each treatment. The mortality, gross morphology, and hatching rates of the embryos in each system were observed every 24 h for a period of 96 h under a stereo microscope (Leika S6E). Zebrafish experiments were performed following a protocol approved by the Canadian Council for Animal Care (CCAC) and our local animal care committee.

■ ASSOCIATED CONTENT

● Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.organomet.8b00897.

(PDF)

■ Accession Codes

CCDC 1840870–1840873 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

The authors declare no competing financial interest.

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