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Ligand-Based Neutral Ruthenium(II) Arene Complex: Selective Anticancer Action

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Two new ruthenium(II) arene complexes, **2a** ($C_{24}H_{34}B_{10}FeRuS_2$) and **2b** ($C_{15}H_{26}B_{10}O_2RuS_2$), bearing a carborane unit and other different functional groups were synthesized, and their cytostatic effects on cancerous cells were evaluated. Our observations illustrate that a structural change from a ferrocene unit to a carboxyl group could lead to high selectivity toward cancer cells and facilitate the efficient inhibition of the proliferation of target cells, indicating that the tuning of the overall properties of the ruthenium(II) arene complex by appropriate ligand tagging is critical to creating a selective antineoplastic agent.

Enormous interest has been focused on the research of metallopharmaceuticals in order to find good alternatives to platinum drugs because of their significant clinical side effects and resistance that cause relapse of cisplatin.[1] Among them, different types of ruthenium-based complexes have been explored in the biomedical realm.[2] Because of the versatile synthetic chemistry and biological features of ruthenium complexes, they are recognized as the most promising anticancer prodrugs.[3] Especially, it is found that ruthenium-based anticancer drugs exhibit low general toxicity, specificity, and selectivity toward cancer cells.[4a,b] The ruthenium(II) arene fragment coordination with a multidrug resistance (MDR) modulator modified ligand (like anthracene) shows significant improvement of the cytotoxicity and P-glycoprotein inhibition behavior, demonstrating the promise of the ruthenium arene fragment in biomedical realm.[4c] Recently, potential biologically active moieties, such as carborane and ferrocene (Fc), have been extensively involved in new-type drug design because of their unique properties. A myriad of compounds containing single- or multiple-carborane clusters were synthesized and evaluated in both cellular and animal studies.[5] Meanwhile, Fc has been incorporated in penicillin, chloroquine, tamoxifen, and diphenols,[6a] thus modifying relative activities due to its small size, relative lipophilicity, ease of chemical modification, and accessible one-electron-oxidation potential. Some unconjugated ferrocenyl derivatives[6b] and Fc-containing bioconjugates[6c,d] have shown promising bioactivities like antineoplastic, antimalarial, or antibacterial activities. In addition, appropriate attachment of the carboxyl acid (COOH) in organometallic complexes could modulate the complex solubility, cell transport, and biological activity.[7]

We are interested in exploring whether the combination of an areneruthenium motif with some potent biologically

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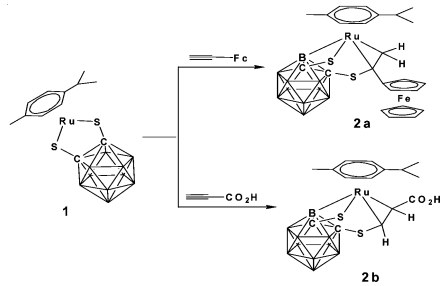
Scheme 1



active ligands would result in novel efficient chemotherapeutic agents against human cancer. In this contribution, we synthesized two organoruthenium complexes, **2a** ($C_{24}H_{34}B_{10}FeRuS_2$) and **2b** ($C_{15}H_{26}B_{10}O_2RuS_2$), containing carborane, Fc, or carboxyl moieties, and their cytotoxicity was tested. The results indicate that different ligands in the ruthenium(II) arene complexes are critical to their selective and efficient proliferative inhibition of target cancerous cells.

As shown in Scheme 1, the two organoruthenium complexes containing a carborane unit were synthesized by the reaction of ferrocenylacetylene (for **2a**, see SIFigure 1 in the Supporting Information) or propiolic acid (for **2b**) with the 16e complex (*p*-cymene)Ru($S_2C_2B_{10}H_{10}$) (**1**). The solid-state structure of **2b** (Figure 1) shows a characteristic Ru−B bond that is generated as a result of the sequence of reactions including the initial insertion of the alkyne into one of the Ru−S bonds, metal-induced activation of the B−H bond, and finally hydrogen transfer from boron via ruthenium to the adjacent carbon of the inserted alkyne.[8] **2a** and **2b** contain ferrocenyl and carboxyl groups, respectively, and the H atom from the carborane cage is transferred to the terminal or internal C atom, respectively. Additionally, the S-η^2-C(Fc)−C and C(1)−B(Ru) moieties in **2a** and the S-η^2-C−C(COOH) and C(1)−B(Ru) moieties in **2b** occupy a cisoid or transoid position, respectively.

To explore the cytotoxicity of these organoruthenium complexes, initially the real-time cell electronic sensing (RT-CES) assay was utilized for in vitro monitoring of dynamic changes induced by cell−ligand interaction. The cell status such as the cell number, viability, morphology, and adherence and even cell proliferation and apoptosis was monitored and quantified dynamically by measuring the electrical impedance, which is displayed as a cell index (CI),[9]

$$\mathrm{CI} = \max_{i=1,\ldots,N}[R_{\mathrm{cell}}(f_i)/R_{\mathrm{b}}(f_i) - 1]$$

where $R_{\mathrm{b(f)}}$ and $R_{\mathrm{cell(f)}}$ are frequency-dependent electrode resistances (a component of impedance) without or with cells, respectively, and N is the number of the frequency points where the impedance is measured. A normalized CI (NCI) at a given time point is calculated by dividing the CI at the time point by the CI at a reference time point.

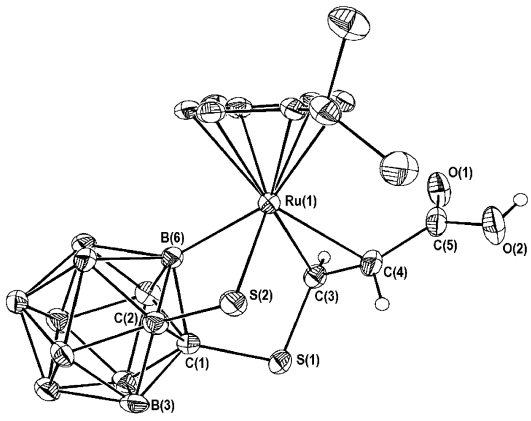


Figure 1. Molecular structure of **2b** (30% probability displacement ellipsoids). The H atoms are omitted for clarity. Selected bond lengths (Å) and angles (deg): C(1)−C(2) 1.760(5), C(1)−S(1) 1.749(4), C(2)−S(2) 1.766(4), S(1)−C(3) 1.795(4), C(3)−C(4) 1.421(5), Ru(1)−S(2) 2.4175(11), Ru(1)−C(3) 2.130(4), Ru(1)−C(4) 2.177(4), Ru(1)−B(6) 2.159(4), B(6)−Ru(1)−S(2) 70.03(13); B(6)−Ru(1)−C(3) 78.97(16), B(6)−Ru(1)−C(4) 112.92(16), S(2)−Ru(1)−C(3) 91.02(12), S(2)−Ru(1)−C(4) 86.16(12), C(3)−Ru(1)−C(4) 38.50(13).

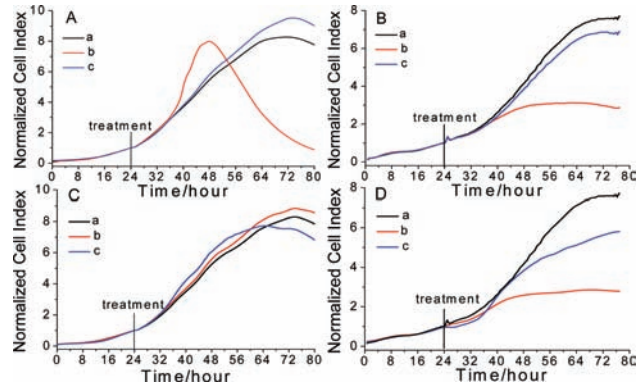


Figure 2. Dynamic monitoring cytotoxic response of HELF cells (A and C) and SMMC-7721 cells (B and D) to **2a** (A and B) and **2b** (C and D), where parts a−c of [**2a**] are 0, 4.69, and 0.47 μM and parts a−c of [**2b**] are 0, 7.31, and 0.42 μM. Data were normalized to the time of compound addition at 24 h of cell culture.

As shown in Figure 2, the typical NCI curves for human embryonic lung fibroblast (HELF) normal cells and for SMMC-7721 hepatocellular carcinoma cells without the addition of the complex (curve a) are observed to culminate to the maximum gradually and then begin to decrease after ~72 h of incubation. Upon the addition of **2a** and **2b**, the target cells display different NCI patterns. Similar NCI curves appear in the absence and presence of 0.47 μM **2a**, suggesting that at the relatively low concentration **2a** has no effect on the growth of both SMMC-7721 cancerous cells and HELF normal cells. However, at a higher concentration of **2a** (4.69 μM), the NCI for HELF (curve b, Figure 2A) increases to the maximum initially after treatment for ~22 h and then starts to decrease sharply. Compared with curve a in Figure 2A for a HELF cell untreated with **2a**, the results show that **2a**, at a relatively higher concentration, has a remarkable effect on the life cycles of HELF normal cells and quickly induces cell death. Dramatically different from HELF cells, the NCI curves for SMMC-7721 cancerous cells increase to a maximum at a slow growth rate after ~24 h of treatment

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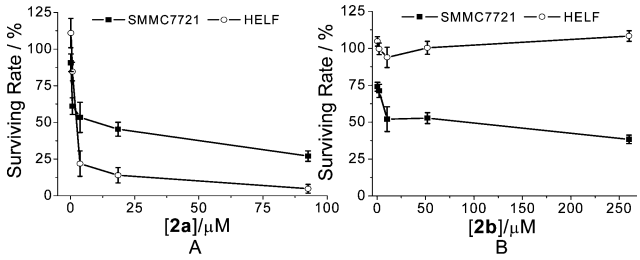

Figure 3. Surviving rate for SMMC-7721 and HELF cells treated with **2a** (A) and **2b** (B) for 48 h derived from the NCI curves.

of **2a** with a concentration of 4.69 μM (curve b, Figure 2B) and then remain constant with time. This indicates that **2a** (4.69 μM) can obviously restrain the growth of SMMC-7721 cancerous cells after about 24 h of treatment.

In comparison, the cytotoxicity of **2b** is apparently different from that of **2a**. As shown in Figure 2C, the NCI curves are nearly the same as those of HELF cells alone even at different concentrations of **2b**. This shows that **2b** has nearly no effect on the normal cells at relevant experimental conditions. However, the NCI values for SMMC-7721 cells increase slowly during the initial 22-h incubation period and then remain constant after ~20 h of treatment with **2b** (Figure 2D). To note, SMMC-7721 cells continue to proliferate in the absence of **2b** (curve a, Figure 2D) because the related NCI curve increases sharply during the incubation time. Thus, the considerable decrease of the NCI values for the cancer cells when treated with **2b** indicates the significant inhibition of SMMC-7721 cell growth resulting from the toxicity of the organoruthenium complex. Thus, **2b** has nearly no toxicity toward noncancerous cells but has high toxicity to cancerous cells.

On the basis of the above studies, the surviving rates of SMMC-7721 and HELF cells incubated with **2a** and **2b** for 48 h could be calculated from the NCI value[10] and is displayed in Figure 3A,B. The apparent IC_{50} values for **2a** (Figure 3A) are estimated as 7.9 μM for SMMC-7721 cancerous cells and 6.5 μM toward HELF normal cells, respectively. For **2b** (Figure 3B), it has little effect on the proliferation of HELF cells during the range of 0.42−260 μM but shows dose-dependent cytotoxicity for SMMC-7721 cancerous cells. Also, the apparent IC_{50} of **2b** is about 42.0 μM for SMMC-7721 cancerous cells. Additionally, our optical microscopy images (SIFigure 2 in the Supporting Information) and flow cytometric analyses (SIFigure 3 in the Supporting Information) also give direct evidence for the relevant distinctive activities toward target cancer cells after treatment with the new complexes.

It is well-known that the development of anticancer agents that are selective toward cancer cells is most important for drug design and cancer chemotherapy.[11] Impressively, the two neutral ruthenium complexes **2a** and **2b** show high in vitro antitumor activity. This is probably because of enhanced biomolecular recognition and transportation through the cell membrane because of the hydrophobic face provided by the arene ligands.[12a] When a ferrocenyl ligand is added to the organoruthenium complex, it is anticipated that higher affinity for cancerous cells due to the generation of oxygen active species could be achieved,[12b] but comparative results show that the facilitated toxicity of **2a** would inhibit the growth of both cancerous and noncancerous cells at relevant experimental conditions. Interestingly, **2b** has nearly no effect on noncancerous cells but a potent toxicity toward cancer cells. Considering the specific structure of **2a** and **2b**, it appears that the carboxyl moiety of **2b** may play a critical role for the highly selective antitumor activity toward target cancer cells so that **2b** could not easily penetrate the hydrophobic cellular membrane of noncancerous cells but could readily bind to certain biomolecules overexpressed on surfaces of the rapidly growing cancer cells. Thus, the relevant tuning between hydrophilic and hydrophobic properties of the new organoruthenium compounds may contribute to the enhancement of the selective binding affinity of the related anticancer agent to cancer cell lines.

In summary, in this contribution, we have synthesized two new organoruthenium complexes containing a carborane moiety and have explored the bioactivity and cellular effect of these organoruthenium complexes by using bioanalysis and the RT-CES assay. Complex **2a** shows a high proliferation inhibition efficiency toward both cancerous and noncancerous cells. While the Fc unit in **2a** is replaced by a carboxyl group, **2b** gains highly selective antitumor activity toward the target cancerous cells through a different pathway. It is evident that a structural change from a Fc unit to a carboxyl group could lead to high selectivity toward cancer cells and facilitate the efficient inhibition of the proliferation of target cells. This presents an innovative example to design new targeted multifunctional anticancer agents and evaluate their cytotoxicity toward different cell lines in the biomedical area.

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Supporting Information Available: Experimental details, optical microscopic images, and flow cytometric analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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