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Comparative biological evaluation of two ethylene linked mixed binuclear ferrocene/ruthenium organometallic species

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ABSTRACT

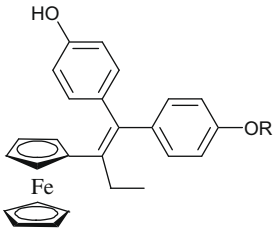
Two ethylene linked binuclear mixed ferrocene/ruthenium complexes were biologically investigated in comparison to structurally related mononuclear ferrocene or ruthenium species with styryl substituents or ligands. Results indicated that the electron distribution (but not the redox potential), cellular uptake and (to some minor extent) anti-estrogenic effects were the key contributors to antiproliferative effects observed in tumor cell lines.

In the search for new anticancer drugs transition metal complexes have been playing a prominent role based on the tremendous success of the platinum tumor therapeutics. Innovative strategies to overcome inherent disadvantages of the established platinum drugs (e.g., nephrotoxicity or resistance development) include the following strategies: exchange of the platinum atom against other metal centers, the development of multinuclear compounds, the targeting of protein targets instead of DNA, and the development of organometallic drugs.[1–4]

Recently, several ferrocene derivatives with promising biological properties have been described.[5–7] The presence of a mechanism related to cellular redox chemistry and/or the activation of reactive oxygen species (ROS) formation in tumor cells has been suggested for ferrocenes and may be a general property at least contributing to antiproliferative effects of this type of organometallic species.[8–10] Thus, Jaouen and co-workers reported about ferrocenyl phenols (also called ferrocifenes), which are ferrocene derivatives that are structurally related to the breast cancer therapeutic tamoxifene (see Fig. 1).[9,10] Tamoxifene is used due to its anti-estrogenic properties against breast cancer.[11] Ferrocene phenols, however, seem to trigger antiproliferative effects not exclusively by this mode of action but also based on their redox properties. With the complex Fc-diOH (in nanocapsule form) promising in vivo activity was observed in rats.[12]

Recently, some of us described the mixed ferrocene ruthenium organometallic species **1** and **2** (see Fig. 2) with the main focus on measuring the degree of electron delocalization in mixed-valent **2** and its medium dependence.[13] It was shown that both, the ferrocene and the vinyl ruthenium moieties of radical cation **2** carry a significant portion of the spin density and the overall unipositive charge, and that **2** is best described as $Fc^{\delta+}\{-(CH{=}CH)\ RuCl(CO)\text{-}(P^iPr_3)_2\}^{(1-\delta)+}$ ($Fc = (\eta^5\text{-}C_5H_5)Fe(\eta^5\text{-}C_5H_4)$) with $\delta \approx 0.6$ in solution and $\delta \approx 0.8$ in the solid state.

Based on these results and also on recent reports about the biological activity of other ruthenium complexes and of mixed ferrocene/ruthenium organometallic compounds[14,15] we evaluated the biological properties of the above mentioned compounds **1** and **2**



R = -O(CH$_2$)$_3$N(CH$_3$)$_2$ (E+Z)
- OH : Fc-diOH

Figure 1. Ferrocene phenol derivatives.

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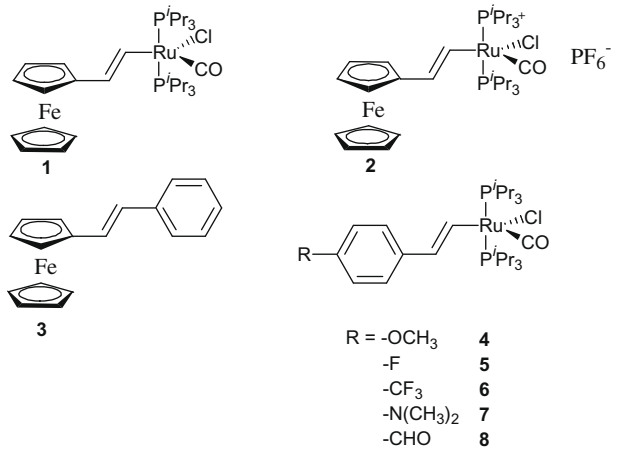

Figure 2. Compounds under study.

in comparison to the closely related styryl ferrocene analogue **3** and the styryl ruthenium complexes **4**–**8**. This series of eight complexes[16] (see Fig. 2) was investigated for their antiproliferative properties in two tumor cell lines in order to clarify if the ruthenium center, the ferrocene structure or a combination of both would lead to bioactive compounds. Additionally, the cellular uptake and hormonal activity of the mixed ferrocene ruthenium organometallic species was studied to give a more detailed picture on their biological profile.

The complexes were investigated for their antiproliferative effects in HT-29 colon carcinoma and MCF-7 breast cancer cells (Table 1).[17] For the mixed binuclear derivatives **1** and **2** indeed promising IC_{50} values between 4.8 μM and 16.8 μM were determined. This is within the range of established cytostatics such as cisplatin or 5-fluorouracil investigated in the same assay (IC_{50} values in the range of 1–10 μM[18,19]). The higher activity of **2** compared to **1** agrees well with the previously reported high degree of charge delocalization of this complex, which might lead to an increased cellular ROS formation.[13]

The inactivity in the HT-29 cell line and the minor activity in the MCF-7 cell line displayed by the ferrocene analogue **3** indicated that the presence of the ruthenium center might be important for the triggering of cell growth inhibitory effects. However, this conclusion could not be confirmed by ruthenium complexes **5** to **7**, which were devoid of any activity in both tumor cell lines.

Interestingly, the methoxy and formyl substituted mononuclear ruthenium complexes **4** and **8** again exhibited antiproliferative effects close to **1** and **2**. This result might be related to differences in the electron distribution of the respective species. Thus, the delocalization of electrons in **4** and **8** is supposedly facilitated if compared to **5** and **6**, which carry different substituents that likely not contribute to electron delocalisation. For **7** supposedly resonance stabilisation effects play an important role. In order to study

Table 1
Antiproliferative effects of compounds **1**–**8**

	IC_{50} (μM) HT-29	IC_{50} (μM) MCF-7
1	16.8 (±7.0)	16.5 (±1.2)
2	9.2 (±0.4)	4.8 (±0.2)
3	>100	54.8 (±14.5)
4	16.7 (±3.1)	12.9 (±1.7)
5	>100	>100
6	>100	>100
7	>100	>100
8	21.6 (±1.5)	14.2 (±1.5)
Cisplatin[18,19]	7.0	2.0

Table 2
Voltammetric data for compounds **1**–**8**

Compound	$E_{1/2}$ (V)[a]
1[13]	−0.235
2[13]	+0.580
3	+0.025
4	+0.065
5	+0.275
6	+0.400
7	−0.190
8	+0.420

[a] Data in CH_2Cl_2/NBu_4PF_6 (0.1 M) at rt; potentials are referenced to the $Cp_2Fe/Cp_2Fe^{0/+}$ couple.

the possible influence of the redox potentials of the compounds on their bioactivity the complexes **1**–**8** were subjected to voltammetric analysis.[13] However, these experiments did not reveal any immediate relation between the redox potentials of **1–8** with cytotoxic activity (see Table 2).

Besides the direct interaction with cellular molecular targets the activity of drugs is also influenced by their uptake into tissues and the subsequent biodistribution. Comparing the structures of **1** and **2** the most relevant difference affecting these parameters is the cationic character of **2** in comparison to the neutral nature of **1**. In this context it should be noted that we had observed significant differences in cellular accumulation for certain ruthenium polypyridyl complexes indicating a significant influence of complex overall charge and lipophilicity on cell growth inhibitory efficacy.[19] Consequently, we studied here the cellular uptake of **1** and **2** and used **4** as a reference.

Cellular uptake was determined by measurement of the ruthenium content of cells exposed to the complexes by atomic absorption spectroscopy (see Fig. 3).[17,19,20] Unexpectedly, the cellular ruthenium levels obtained with the cationic **2** exceeded those of neutral **1** and were comparable to those found with **4**. Thus, it can be speculated that for these complexes the positive charge might enable an enhanced membrane passage probably related to the presence of active transporter systems, which are crucial factors for cellular metal biodistribution. For example, proteins relevant in copper or iron homeostasis also play a role in the transport of platinum and ruthenium metallodrugs and might therefore be involved in the distribution of the presented complexes in a similar manner.[21–23]

Finally, we evaluated **1**, **2**, **3** and **4** for their hormonal activities based on their structural analogy to hormonal stilbene derivatives and the ferrocifenes. In the assay for estrogenic activity the hormonal activity is reflected by the ability of a compound to trigger luciferase expression in stably transfected MCF-7-2a cells contain-

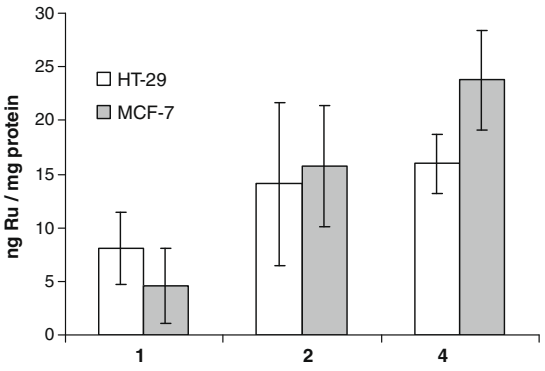

Figure 3. Cellular uptake of compounds **1**, **2**, and **4** into HT-29 and MCF-7 cells (exposure concentration: 10 μM, exposure period: 6 h).

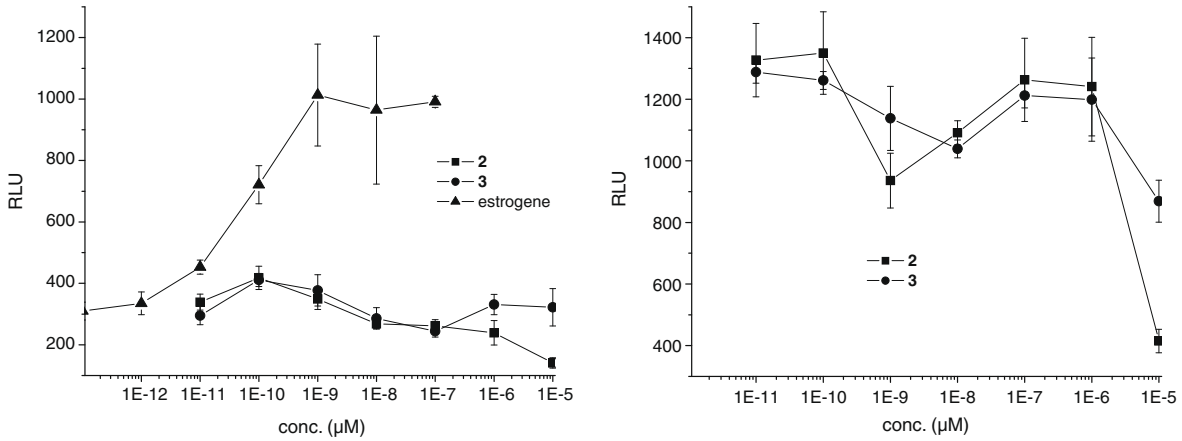

Figure 4. Left: estrogenic activity, right: anti-estrogenic activity; RLU: relative light units.

ing an estrogen receptor response element. In the assay for anti-estrogenic activity those compounds are termed as anti-estrogenic, which reverse the effect of the natural ligand estrogene.[17,24]

In an initial screening, **1** did not show any effects in both assays whereas minor effects were noted for the other complexes, which were investigated in a concentration dependent manner (see Fig. 4). Thus, **2** and **3** displayed some minor estrogenic activity, which was however significantly lower than that of the positive reference estrogen (see Fig. 4, left). Anti-estrogenic effects could be noted in the highest investigated concentration corresponding to 10 μM (see Fig. 4, right). This needs to be interpreted carefully as this concentration was already sufficient to trigger antiproliferative effects as described above. However, the hormonal assay uses shorter exposure periods and is (in contrast to the proliferation assay) performed in an almost confluent cell layer. Indeed, an evaluation of the cytotoxic effect of **2** and **3** at 10 μM against MCF-7-2a cells cultured under the same experimental conditions as in the setup for anti-estrogenic activity revealed that the cell biomass was reduced only by less than 5% (data not shown). This indicates that the observed effects can not be explained by a termination of MCF-7-2a cells.

In conclusion the mixed ferrocene/ruthenium bioorganometallics **1** and **2** displayed promising antiproliferative effects in two tumor cell lines. Comparison with structurally related mononuclear ruthenium complexes and a related ferrocene derivative indicated that an enhanced electron delocalisation (but not the redox potential) might be of high relevance for the bioactivity. Further influences on bioactivity were related to the cellular uptake and some anti-estrogenic effects at higher dosage.

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16. *Chemistry*: Mononuclear styryl derivatives **4**–**8** were prepared by treating the hydride complex $RuClH(CO)(P^iPr_3)_2$ with 1 equiv of the appropriate 4-substituted phenylethyne, which results in the regio- and stereoselective insertion of the terminal alkyne into the Ru–H bond. A representative procedure for complex **4** along with the characterization data is given below. Further details on complexes **5**–**8** will be provided in a forthcoming report (Maurer, J.; Mücke, P.; Zabel, M.; Záliš, S. manuscript in preparation). A solution of $RuClH(CO)(P^iPr_3)_2$ (120 mg, 0.24 mmol) in 6 mL CH_2Cl_2 was treated with a solution of 32 mg (0.24 mmol) of 4-ethynylanisole in 5 mL of CH_2Cl_2 under a nitrogen atmosphere. A color change from orange to deep purple was observed within a few seconds after addition. The solution was stirred at room temperature for 30 min. The solvent was then evaporated under dynamic vacuum, which left a purple solid. Further purification was achieved by precipitating the complex with methanol from a concentrated CH_2Cl_2 solution (three times). After drying in vacuum, complex **4** was obtained in quantitative yield (148 mg, 0.24 mmol). ^{1}H NMR (δ, CD_2Cl_2, 300.1 MHz): 8.25 (d, 1H, $^3J_{H-H}$ = 13.44 Hz, Ru–C*H*), 6.95 (d, 2H, $^3J_{H-H}$ = 8.78 Hz, $CH_{meta\ (arene)}$), 6.73 (d, 2H, $^3J_{H-H}$ = ! 8.78 Hz, $CH_{ortho\ (arene)}$), 5.90 (dt, 1H, $^3J_{H-H}$ = 13.44 Hz, $^4J_{H-P}$ = 2.19 Hz, Ru–CH=C*H*), 3.73 (s, 3H, OCH_3), 2.81–2.67 (m, 6H, $PCH(CH_3)_2$, 1.35–1.21 (dd, 36 H, $PCH(CH_3)_2$. ^{13}C NMR (δ, CD_2Cl_2, 150.9 MHz): 209.4 (t, $^2J_{P-C}$ = 13.1 Hz, Ru–CO), 157.0 (s, $C_{ipso\ (arene)}$), 146.5 (t, $^2J_{P-C}$ = 10.9 Hz, Ru–CH), 133.8 (s, Ru–CH=CH), 132.8 (s, $C_{ipso'\ (arene)}$), 125.1 (s, $C_{meta\ (arene)}$), 114.0 $C_{ortho\ (arene)}$), 55.5 (s, OCH_3), 24.8 (t, J_{P-C} = 9.9 Hz, $PCH(CH_3)_2$), 20.0 (d, $^2J_{P-C}$ = 25.3 Hz, $PCH(CH_3)_2$). ^{31}P NMR (δ, CD_2Cl_2, 121.5 MHz): 38.3 (s). Elemental analysis for $C_{28}H_{51}P_2O_2ClRu$ (calcd/found): C (54.40/54.16), H (8.32/8.21).
17. *Biological activity*: Dimethylformamide (DMF) was used as a solvent for preparing stock solutions of the compounds and diluted with the respective media (final DMF concentration: 0.1% v/v). Studies on the antiproliferative effects were performed in HT-29 and MCF-7 cells. For this purpose cells were grown for 48 h (HT-29) or 72 h (MCF-7) and then exposed to media containing the drugs in graded concentrations for 72 h (HT-29) or 96 h (MCF-7). The biomass was estimated by crystal violet staining and the IC_{50} values were calculated as that concentration reducing the cell growth to 50%. Further details on this assay are given in Refs. 18,19. For cellular uptake measurements at least 70% confluent cell layers were exposed to 10 μM of the agents for 6 h. Afterwards cells were isolated, lysed and the obtained lysates were investigated for their ruthenium content by atomic absorption spectroscopy and for their protein content by the Bradford method. Accordingly, the amount of complex taken up into the cells was expressed as ng ruthenium per mg cellular protein. Further details on this assay can be found in Refs. 19,20. Hormonal activity measurements were essentially performed as described in Ref. 24.
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