**Supplementary Material**

Electrochemical studies and potential anticancer activity in ferrocene derivatives

SARA REALISTA, SUSANA QUINTAL, PAULO N. MARTINHO\*, ANA I. MELATO, ADRIÀ GIL\*, TERESA ESTEVES, MARIA DE DEUS CARVALHO, LILIANA P. FERREIRA, PEDRO D. VAZ and MARIA JOSÉ CALHORDA

This Supplementary Material includes:

- *In vitro* cytotoxicity assays against A549 and A375 cells for all the studied ferrocene complexes

- isomer shift and quadrupole splitting parameters from Mössbauer experiments and DFT calculations for all the studied complexes

- the cell viability by the MTT assay of A375 cells in the presence of compound **6**

- cyclic voltammograms, ipa versus v1/2 plots of the analyzed complexes

- the plot of Ea1/2 versus Hammet constant () for the ferrocene derivatives exhibiting diffusion controlled electrochemical processes

**Contents**

|  |  |  |
| --- | --- | --- |
| Table S1 | *In vitro* cytotoxicity assays for ferrocene complexes **1**-**7** against A549 and A375 cells (mean ± SD). | **SI3** |
| Table S2 | Isomer shift (IS */* mm s-1) and quadrupole splitting (QS / mm s-1) determined from the Mössbauer spectra at room temperature (**Exp.)** of complexes **1**-**7**, electronic density at the nucleus (ρ / a.u.) and IS and QS from DFT calculations (**Calc**). | **SI4** |
| Figure S1 | Cell viability by the MTT assay of A375 cells, in the presence of compound **6**. | **SI5** |
| Figure S2 | Cyclic voltammograms of a Pt electrode in 0.1 M TBAPF6/CH2Cl2 solution containing 0.8 mM of complex **2** using different sweep scan rates. | **SI5** |
| Figure S3 | Cyclic voltammograms of a Pt electrode in 0.1 M TBAPF6/CH2Cl2 solution containing 0.8 mM of complex **5** using different sweep scan rates. | **SI5** |
| Figure S4 | ipa versus v1/2 for complex **1**. | **SI6** |
| Figure S5 | ipa versus v1/2 for complex **2**. | **SI6** |
| Figure S6 | ipa versus v1/2 for complex **5**. | **SI6** |
| Figure S7 | ipa versus v1/2 for complex **3**. | **SI7** |
| Figure S8 | ipa versus v1/2 for complex **4**. | **SI7** |
| Figure S9 | ipa versus v1/2 for complex **7**. | **SI7** |
| Figure S10 | Cyclic voltammograms of a Pt electrode in 0.1 M TBAPF6/CH2Cl2 solution containing 0.8 mM of complex **6** using different sweep scan rates. | **SI8** |
| Figure S11 | Cyclic voltammograms of a Pt electrode in 0.1 M TBAPF6/CH2Cl2 solution containing 0.8 mM of 2-aminobenzimidazoleat 100 mV s-1. | **SI8** |
| Figure S12 | Cyclic voltammograms of a Pt electrode in 0.1 M TBAPF6/CH2Cl2 solution containing 0.8 mM of complex **4** using different sweep scan rates. | **SI8** |
| Figure S13 | Cyclic voltammograms of a Pt electrode in 0.1 M TBAPF6/CH2Cl2 solution containing 0.8 mM of complex **7** using different sweep scan rates. | **SI9** |
| Figure S14 | Ea1/2 versus Hammet constant (σ) for ferrocene derivatives **1**, **2** and **5**. | **SI9** |

**Table S1**. *In vitro* cytotoxicity assays for ferrocene complexes **1**-**7** against A549 and A375 cells (mean ± SD).

|  |  |
| --- | --- |
| **Complexes** | **IC50 (µM)** |
| **A549** | **A375** |
| **1** | >200 | 157.4 ± 1.16 |
| **2** | >200 | >200 |
| **3** | >200 | 99.12 ± 1.05 |
| **4** | >200 | >200 |
| **5** | >200 | >200 |
| **6** | >200 | 28.16 ± 1.22 |
| **7** | >200 | 102.0 ± 1.10 |

**Table S2.** Isomer Shift (IS** mm s-1) and quadrupole splitting (QS / mm s-1) determined from the Mössbauer spectra at room temperature (**Exp.)** of complexes **1**-**7**, electronic density at the nucleus (ρ / a.u.) and IS and QS from DFT calculations (**Calc**).

|  |  |  |
| --- | --- | --- |
| **Complex** | **IS** | **QS** |
| **Exp.** | **** | **Calc.** | **Exp.** | **Calc.** |
| **1** | 0.422(1) | 11816.04150 | 0.667 | 2.324(3) | 1.528 |
| **2** | 0.438(1) | 11816.02986 | 0.671 | 2.260(1) | 1.510 |
| **3** | 0.446(1) | 11816.00327 | 0.681 | 2.305(1) | 1.512 |
| **4** | 0.431(1) | 11816.04011 | 0.667 | 2.263(2) | 1.543 |
| **5** | 0.433(1) | 11816.0330311816.04000 | 0.6700.667 | 2.279(2) | 1.491/1.469 |
| **6** | 0.439(1) | 11816.04097 | 0.667 | 2.267(1) | 1.504 |
| **7** | 0.424(1) | 11816.0424111816.03054 | 0.6670.671 | 2.243(2) | 1.516/1.486 |

DFT calculations were also performed in order to rationalize the Mössbauer parameters. The electronic density at the nucleus (ρ), were converted in isomer shifts (IS) using the approach of Neese [83, 84] and the ORCA program. Taking into account that the experimental results were obtained at room temperature, it can be considered that the calculated isomer shifts are close to the experimental ones, (shifted ~0.2mm s-1), while the QS values have a larger difference. Contrary to what has been found for other families of Fe(II) complexes, the trend of the experimental values are not well reproduced, most probably due to the very small influence of the substituents.

**Figure S1**. Cell viability by the MTT assay of A375cells, in the presence of compound **6**.



**Figure S2**. Cyclic voltammograms of a Pt electrode in 0.1 M TBAPF6/CH2Cl2 solution containing 0.8 mM of complex **2** using different sweep scan rates.



**Figure S3**. Cyclic voltammograms of a Pt electrode in 0.1 M TBAPF6/CH2Cl2 solution containing 0.8 mM of complex **5** using different sweep scan rates.



**Figure S4**. ipa versus v1/2 for complex **1**.



**Figure S5**. ipa versus v1/2 for complex **2**.

****

**Figure S6**. ipa versus v1/2 for complex **5**.



**Figure S7**. ipa versus v1/2 for complex **3**.

****

**Figure S8**. ipa versus v1/2 for complex **4**.

****

**Figure S9**. ipa versus v1/2 for complex **7**.

****

**Figure S10**. Cyclic voltammograms of a Pt electrode in 0.1 M TBAPF6/CH2Cl2 solution containing 0.8 mM of complex **6** using different sweep scan rates.



**Figure S11**. Cyclic voltammograms of a Pt electrode in 0.1 M TBAPF6/CH2Cl2 solution containing 0.8 mM of 2-aminobenzimidazoleat 100 mV s-1.



**Figure S12**. Cyclic voltammograms of a Pt electrode in 0.1 M TBAPF6/CH2Cl2 solution containing 0.8 mM of complex **4** using different sweep scan rates.



**Figure S13**. Cyclic voltammograms of a Pt electrode in 0.1 M TBAPF6/CH2Cl2 solution containing 0.8 mM of complex **7** using different sweep scan rates.



**Figure S14**. Ea1/2 versus Hammet constant (σ) for ferrocene derivatives **1**, **2** and **5**.