Send Orders for Reprints to reprints@benthamscience.ae

Mini-Reviews in Medicinal Chemistry, 2017, *17*, 000-000

REVIEW ARTICLE

Recent Advances of Metallocenes for Medicinal Chemistry

Miguel M. Santos*, Pedro Bastos, Isabelle Catela, Karolina Zalewska, Luis C. Branco*

LAQV-REQUIMTE, Chemistry Department, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, Campus da FCT/UNL, Portugal

ARTICLE HISTORY

Received: January 19, 2016
Revised: June 10, 2016
Accepted: September 16, 2016

DOI: 10.2174/1389557516666161031141620

Abstract: The recent advances for the synthesis and application of different metallocenes for Medicinal Chemistry is reviewed. This manuscript presents the different metallocene scaffolds, with special emphasis on ferrocene derivatives, and their potential pharmaceutical application. Over the last years, the synthesis of new metallocene compounds and their biological and medicinal effects against some types of diseases (e.g. anti-tumoral, antibiotics, anti-viral) have been reported. From the medicinal point of view, the attractive properties of metallocene derivatives, such as their high stability, low toxicity and appealing redox behaviors are particularly relevant. This area has attracted many researchers as well as the pharmaceutical industry due to the promising results of some metallocenes, in particular ferrocene compounds, in breast cancer and malaria.

Keywords: Metallocenes, ferrocene derivatives; medicinal chemistry; anti-tumoral; anti-malaria; anti-HIV

1. INTRODUCTION

The metallocene unit is composed of a transition metal and two cyclopentadienyl ligands coordinated in a sandwich structure, where the two cyclopentadienyl anions are on parallel planes with equal bond lengths and strengths [1]. Ferrocene was the first discovered metallocene and has been largely studied throughout the last decades by the peculiar electronic properties and ease of functionalization [2]. Ferrocene derivatives have been applied in materials science [3] including electroactive and aerospace materials [4], sensors [5] and organometallic catalysts for different organic transformations [6]. The high stability of ferrocene in aqueous and aerobic media, the large variety of several derivatives as well as its favorable electrochemical properties have made ferrocenyl compounds very attractive scaffolds for biological [7] and pharmaceutical applications [8].

Recent reports have shown that some ferrocene derivatives are active *in vitro* and *in vivo* against different diseases including cancer (*anti-tumoral*), malaria (*anti-malaria*) and human immunodeficiency virus (*anti-HIV*) [9].

Other metallocene derivatives [10] such as titanocene, vanadocene, ruthenocene and zirconocene also exhibit catalytic properties, although these metallocenes are rarely used industrially.

*Address correspondence to this author at the LAQV-REQUIMTE, Chemistry Department, Faculty of Science and Technology, Universidade NOVA de Lisboa, P.O. Box: 2829-516, Portugal; Tel/Fax: +351-212-948-300 ext: 10986; E-mail: miguelmsantos@fct.unl.pt, l.branco@fct.unl.pt

According to the number of published reviews on the metallocene research topic [11, 12] we decided to focus this mini-review mainly on the reports from the last three years (from 2013), especially concerning ferrocene derivatives, for application in medicinal chemistry.

2. METALLOCENES AS ANTI-TUMORAL

Organometallic compounds as anticancer drugs have played a central role in the pharmaceutical industry since the 1970s. These consist mainly of platinum complexes [13], however many side effects such as nephrotoxicity and ineffectiveness against platinum-resistant tumors are severe disadvantages [14].

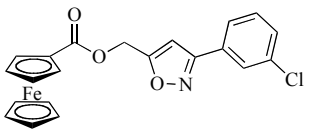
Over the last years, metallocene-based compounds have stepped into the spotlight as the future in metal-based scaffolds for medicinal chemistry. In the particular case of ferrocene its physicochemical properties, such as lipophilicity, redox profile and stability in aqueous and aerobic media, are very desirable as far as drug development is concerned. Furthermore, the ferrocene moiety is readily recognizable by amino acids, proteins, DNA and carbohydrates overly expressed in cancer tissues, which therefore may account for the typically enhanced targeting and efficiency observed for such compounds [15].

J.-P Monserrat *et al.* [16] reported the preparation and study of some ferrocene derivatives, such as ferrocenyl-modified flavonoids with the purpose of improving the cytotoxic and vascular disrupting activities of flavonoids. A variety of ferrocenyl flavonoids were synthesized and tested for antiproliferative effects on B16 murine melanoma and

1389-5575/17 $58.00+.00

potential vascular disrupting effects on EAhy 926 endothelial cell models. The study showed that the cytotoxic and morphological activities of the compounds tested were not actually correlated. The presence of a ferrocenyl group in the flavones led to a clear increase in cytotoxic activity on cancer cells but no activity relationship regarding the morphological effects on endothelial cells was observed. Ideally, the most promising drugs would possess both cytotoxic and antivascular properties. Nevertheless, it is expected that these ferrocene flavones will act as pure antivascular agents *in vivo*, destroying tumor vasculature without directly interfering with cancer cell growth or division.

J. Mólnar *et al.* [17] conducted a cancer research in which flavonoids were shown to inhibit several pathways regarding drug resistance, apoptosis, division and metastasis as well as being an important class of vascular disrupting agents. These experiments suggested that a fast destruction of the tumor vasculature occurs, thus preventing oxygen and nutrient flow to the tumor. Canzhong Lu *et al.* [18] studied the combination of a ferrocene with an isoxazole compound in order to test the activity of various structures based on the isoxazole moiety containing ferrocene derivatives against some types of cancer cells. This isoxazole moiety is usually introduced into drug molecules to improve their biological activity and therefore the possibility to combine with ferrocene, one of the most attractive pharmacophores for drug design and discovery, has been reported with particular interest. Seven novel structures were synthesized and their *in vitro* anticancer activity against several cancer cell lines, namely MCF-7 (breast), A549 (lung) and HCT116 (colorectal) was evaluated using the MTT method. The results indicated that introducing the isoxazole moiety to the ferrocene core greatly improves its anticancer activity. The most promising candidate for the development of anticancer drugs was ferrocene carboxylic acid 3-(2-chloro-phenyl)-isoxazol-5-yl-methyl ester (1), which showed IC_{50} values of 0.747 nM and 3.65 nM for the A549 and HCT116 cell lines, respectively.



1

Scheme (1).

Various types of cancer are treated through photochemical methods, such as PhotoDynamic Therapy (PDT), a recent technique which uses porphyrin dyes and requires a photosensitizing drug, light and oxygen to initiate a photo chemical reaction that results in the generation of highly reactive singlet oxygen ($^1O_2$) species. The administration of the drug into the tumor site generates the reactive oxygen species upon photo-activation, damaging the tumor cells. However, the approved drugs for this therapy show severe side effects such as skin-photosensitivity and hepatotoxicity. The use of biocompatible transition-metal complexes could overcome these issues by using photosensitizers that are active in the PDT spectral window. B. Balaji *et al.* [19] took into account the high biological stability and non-toxicity with reversible redox properties of ferrocene to develop new ferrocene-conjugated oxovanadium (IV) complexes capable of indicating photo-induced DNA damage activity and photocytotoxicity, remaining non-toxic in the absence of light. The reported results showed that the oxovanadium (IV) complexes composed of ferrocenylmethylbis-(2-pyridylmethylamine) linked to an acetylacetone unit presented the highest IC_{50} values in HeLa cells in the dark (IC_{50} > 100 μM) comparing to the other compounds studied and the second highest in visible light. Therefore, by having high photocytotoxic behavior, these complexes may be suitable for PDT applications.

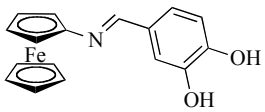
There have been a novel series of asymmetric poly(amidoamine) (PAMAM) dendrons containing a single ferrocene unit prepared and tested for anti-cancer activity by real-time cell analysis and cell viability techniques. It was observed that while cell viability decreased, anti-cancer activity increased gradually in a dose-dependent manner in one, two, and three generations of the ferrocene-PAMAM dendrons. Dendrons have been extensively used in drug delivery systems due to their highly branched, three dimensional, and its monodisperse structure as well as a suitable method of synthesis. In this context, PAMAMs have been extensively studied in drug delivery due to the ability to control their size and shape, ease of functionalization, low toxicity, as well as high availability. T. Sağir *et al.* [20] verified that the ferrocene-cored PAMAM dendrons with several generations presented a definite structure-activity relationship. In general, the size of the dendron linked to the ferrocene unit directly correlates with the increase of water solubility of the compounds. Consequently, this increase in water solubility seems to enhance the cytotoxic and cell death apoptotic effects by apoptosis and necrosis *in vitro*, concluding that the ferrocene compounds with the larger dendrons would more likely be soluble in the body generating the active ferrocene drug species.

The delivery of bioactive drugs in pharmacology through nanotechnology has been known to be an innovative revolution by developing drug nanocarriers or drug delivery systems (DDS) that can boost the bioavailability of drugs. It is required that these drug nanocarriers of DDS are “smart” which have to be constructed with well-defined structures to include therapy drugs as well as to have the ability to release the loaded drugs in response to environmental stimuli under specific conditions. The construction of supramolecular vesicles from novel supramolecular amphiphiles through host-guest interactions, particularly with stimuli-responsiveness, is of great interest and importance in biotechnology and biomedicine, especially drug delivery. L. Wang *et al.* [21] reported a novel supramolecular amphiphilic inclusion between water soluble pillararene (WP6) and a hydrophobic ferrocene derivative, *N*-1-decyl-ferrocenylmethylamine, in aqueous solution, which was further applied to create nanoscale supramolecular vesicles with pH responsiveness for drug delivery. Pillararenes are a new class of macrocyclic hosts in supramolecular chemistry and ferrocene was found to strongly bind WP6 in water to achieve a stable amphiphilic inclusion complex and thus form supramolecular vesicles in water by self-assembly.

The well-known electrochemical properties of ferrocene derivatives are also an added value in cancer therapy. L. Snegur *et al.* [22] studied redox-active ferrocene modified pyrimidines and adenine as potential antitumor agents. More precisely, several enantiopure ferrocenyl-(alkyl)pyrimidines and ferrocenyl(ethyl)adenine were tested for effects on the DNA synthesis in ovarian cancer cells. The results suggest that DNA may be one of the most probable primary cell targets for the ferrocene modified compounds exhibiting antitumor effects at low doses.

A. Badshah *et al.* [23] reported the cytotoxic effect against ovarian cancer cell lines of new ferrocene compounds incorporating *N,N′*-disubstituted thioureas. The observed results provided support to the idea that these compounds undergo electrostatic interactions with the anionic phosphate DNA backbone, thus suggesting that non-covalent interactions may be appropriate to induce cell death.

One of the most important features concerning the development of new chemotherapy approaches is related with the mitigation and elimination of side effects. It is believed that their origin is due to the generation of the free radicals and the reactive oxidant species (ROS). Thus, the design of new and efficient chemotherapeutic drugs may reside on the combination of antioxidant and anticancer activities, by which the free radicals and ROS produced can be scavenged promptly thus limiting their action on healthy cells. Schiff-bases, particularly hydroxyl-substituted ones, have drawn significant attention due to their fine free radical scavenging and anticancer activities. Y.-N. Liu *et al.* [24] synthesized three new hydroxyl-substituted Schiff-bases containing ferrocenyl moieties and found that compound **2** was a capable candidate to achieve the double function of antioxidant and anticancer activities in cancer chemotherapy, and offered an alternative to conventional chemotherapeutic agents or the co-administration of antioxidants and anticancer drugs. *In vivo* experiments are currently being conducted to assess the biological activities of the Schiff-base compounds containing ferrocenyl moieties.

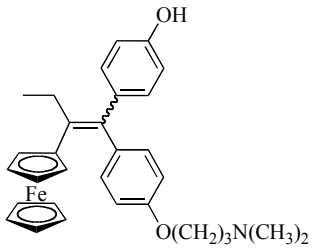


2

Scheme (2).

Furthermore, new ferrocenyl compounds containing 1,2,4-triazole units, as well as other known bioactive derivatives, have been recently investigated as anticancer agents against lung cancer, one of the most common malignant human tumors and a leading cause of death worldwide. Y. Li *et al.* [25] investigated the effects of various novel 1-ferrocenyl-2-(5-phenyl-1*H*-1,2,4-triazol-3-ylthio) ethanone derivatives on human lung cancer cells *in vitro* and found that the compounds exerted anti-cancer effect G1-phase cell cycle arrest. Much attention has also been focused on pyrazoles which display diverse biological activities including anti-cancer and whose structures have been found among naturally occurring compounds such as the antibiotic pyrazofurin, documented to possess potent anti-cancer and antimicrobial activities. B. Zhao *et al.* [26] reported the synthesis of several ferrocene derivatives based on pyrazoles with optical activity showing that some of the compounds exerted notable cytotoxicity and selectivity for lung cancer cells.

Breast cancer (BC) is the most frequently diagnosed cancer in women around the world and accounts for about 23% of the total cancer cases. [27] Surviving BC has been increasing since the early 1990´s thanks to important breakthroughs in medicinal therapies. Nevertheless, it still remains as the main cause of death from cancer among women, representing about 14% of global cancer deaths. The triple negative breast cancer (TNBC), defined by the absence of the estrogen receptor alpha (ERa), the progesterone receptor (PR) and the human epidermal growth factor receptor 2 (HER2), accounts for almost 20% of all breast cancer cases and is responsible for a high rate of mortality. TNBC usually occurs in younger women and denotes the most intense form associated with a high risk of reappearance, visceral and central nervous system metastases and early death. Tamoxifen has been the oldest and most prescribed drug against BC, however, over the years, its use for long periods of time led to the development of drug resistance phenomena. In this context, A.-L. Lainé *et al.* [28] divised a strategy of coupling ferrocene to hydroxytamoxifen (**3**) meant to supplement Tamoxifen with a redox entity in order to overcome the therapeutic limitation found with the organic analogue. The evaluation of this novel drug on a TNBC xenografted model confirmed that a marked delay in tumor growth was observed and consequently a significant decrease in tumor volume in comparison to the untreated group of mice was recorded. The results represented the first evidence of an *in vivo* effect of **3** and ferrocenyl derivatives in general on xenografted breast tumors.

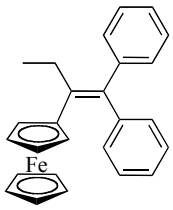


3

Scheme (3).

Although it isn't particularly active against breast cancer cells, the diphenylferrocenyl derivative **4** has already demonstrated to have a strong cytotoxic effect against SF-295 human glioblastoma, HCT-8 human colon cancer, and HL-60 acute promyelocytic leukemia cell lines with IC_{50} values below 2 μM. Its mechanism of biological action against HL-60 cells has been subsequently investigated by the group of E. A. Hillard. [29] Morphological analysis showed that the cell death was caused by apoptosis *via* intercalation of the DNA, as well as activation of caspases 3 and 7 and externalization of phosphatydilserine. In addition,

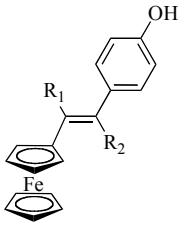
4 interfered with the cell cycle, leading to accumulation of cells in the G0/G1 phase.

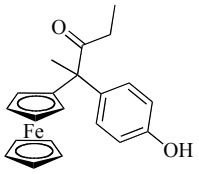


4

Scheme (4).

G. Jaouen, [30] reported on the hydroxylated ferrocenyl diethylstilbestrol derivatives **5-7** as cytotoxic agents for hormone-independent breast cancer cell line MDA-MB-231. These compounds showed to be very effective with IC_{50} values of 0.14 to 0.36 μM which are lower than the ones reported for ferrocidiphenol (**8**). As already reported for the family of ferrocenophenols, the presence of only one hydroxyl group has a negative effect in the cytotoxic effect, as compound **9** is four times less effective than the dihydroxyl analogue **6**. The substitution of the ethyl group in **8** by a methyl (**10**) or an ethyl ester (**11**) group increases the IC_{50} values to 1.09 and 1.16 μM, respectively, as well as removing the central double bond as in compound **12** (IC_{50} = 3.13 μM).





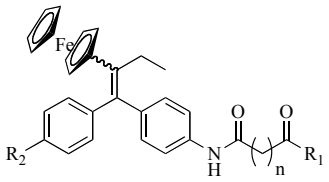
12

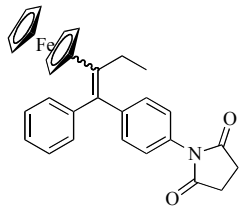
5 R_1 = EtOH, R_2 = Me
6 R_1 = EtOH, R_2 = Et
7 R_1 = EtOH, R_2 = Prop
8 R_1 = Et, R_2 = 4-OH-Ph
9 R_1 = R_2 = Et
10 R_1 = Me, R_2 = 4-OH-Ph
11 R_1 = CH_2COEt, R_2 = 4-OH-Ph

Scheme (5).

J. Cázeres-Marinero *et al.* [31] prepared a new series of compounds, following the previously reported ferrocifen derivatives **12** and **13**. With the introduction of a hydroxyl group at the tamoxifen moiety of these structures, **14** and **15**, the cytotoxicity towards the triple negative MDA-MB-231 and hormone-dependent MCF-7 breast cancer cells decreased between 1.9 and 4.2-fold. The carboxylic acid analogue (**16**) also presented a similar behavior. The only exception was the derivative **12** against MCF-7, which IC_{50} value lowered from 2.01 to 1.50 μM.

By varying the alkyl chain length (compounds **17-22**) IC_{50} values on the low micromolar range were obtained. [32] Primary amides once again showed to be the most effective towards MDA-MB-231 cells, with comparable IC_{50} values of 0.54 and 0.50 μM by **22** and **12**, respectively. On the other hand, the succinimide **23** was the most active against MCF-7 cells (IC_{50} = 1.06 μM) followed by compound **22** (IC_{50} = 1.30 μM).



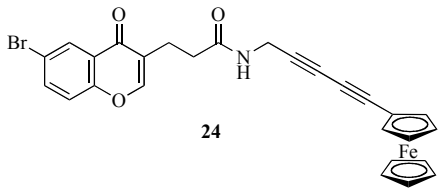


23

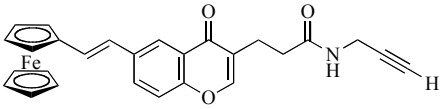
12 n = 6, R_1 = NH_2, R_2 = H
13 n = 6, R_1 = NHOH, R_2 = H
14 n = 6, R_1 = NH_2, R_2 = OH
15 n = 6, R_1 = NHOH, R_2 = OH
16 n = 6, R_1 = OH, R_2 = OH
17 n = 4, R_1 = OH, R_2 = H
18 n = 4, R_1 = NHOH, R_2 = H
19 n = 4, R_1 = NH_2, R_2 = H
20 n = 2, R_1 = OH, R_2 = H
21 n = 2, R_1 = NHOH, R_2 = H
22 n = 2, R_1 = NH_2, R_2 = H

Scheme (6).

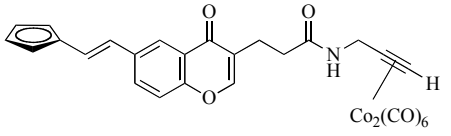
A small library of ferrocenylvinyl chromones was prepared by K. Kowalski and co-workers. [33] The compounds **24-26** (Scheme **7**) presented cytotoxic and antiproliferative activity towards hepatocellular carcinoma (Hep G2), ER+ (MCF-7) breast adenocarcinoma and leukemic (CCRF-CEM) human cancer cell lines with IC_{50} values ranging from 23.0-50.0 μM. Compound **25** has the broadest spectrum of activity and the lowest IC_{50}/IG_{50} values, and the introduction of the hexacarbonyl cobalt (II) moiety (**26**) reduced the activity. The mechanism of action relies on the production of ROS species that yields DNA scission and genotoxic effects, and ultimately induces apoptosis. Moreover, the cycle of the studied cancerous cells arrested in the G2/M phase, the same as for the currently used active principals docetaxel and doxorubicin.



24



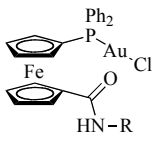
25



26

Scheme (7).

P. Stepnicka reported the ferrocene compounds containing Au-Cl moiety in their structure. [34] The compounds **27**-**32** were evaluated for their cytotoxic activity towards the A2780 and A2780R (cisplatin resistant) human ovarian cancer cell lines, to which all were found to be active in the low micromolar range.

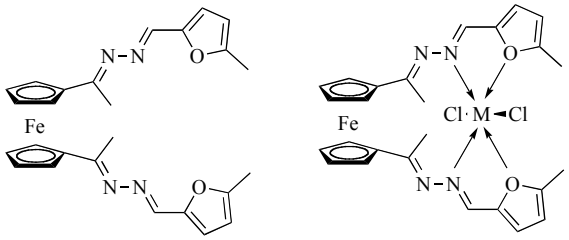


27 R = CH_2CO_2Me
28 R = CH_2CONH_2
29 R = CH_2CH_2OH
30 R = $CH(CH_2OH)_2$
31 R = $C(CH_2OH)_3$
32 R = $CH_2SO_3(HNEt_3)$

Scheme (8).

Compounds **27** and **29** were the most efficient with IC_{50} values of 0.27 and 0.60 μM for A2780 and 3.10 and 6.50 μM for A2780R, respectively. Comparing to Cisplatin and Auranofin, both these compounds are more efficient against the first type of cells, while Auranofin is at least twice more cytotoxic for the second type of cells. Despite the promising antiproliferative results, these compounds lack cell selectivity as healthy HEK cells were also affected, with IC_{50}(HEK/A2780) ranging from 1.6 (**32**) up till 5 (**28**).

Ligand **33** and its complexes with Co (II), Ni (II), Cu (II) and Zn (II) (**34**-**37**) were investigated as cytotoxic agents against cervical carcinoma (KB), ovarian carcinoma (SKOV-3), CNS cancer (SF-268), non-small lung cancer (NCl H460), colon adenocarcinoma (RKOP 27), leukemia (HL60, U937, K562), melanoma (G361,SKMEL-28), neuroblastoma (GOTO, NB-1), cervical cancer (HeLa), breast cancer (MCF-7), lung fibrosarcoma (HT1080) and hepatocellular liver carcinoma (HepG2) cells [35].



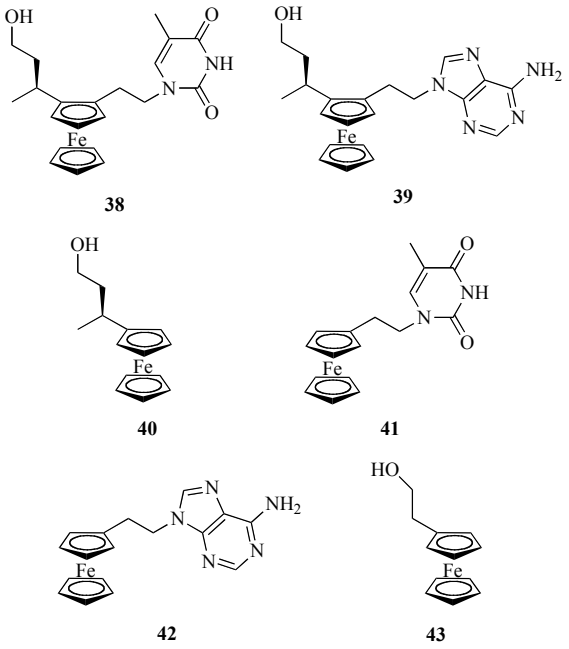
33

34 M = Co
35 M = Ni
36 M = Cu
37 M = Zn

Scheme (9).

All complexes performed better than the ligand itself, probably due to the enhanced lipophilic character of complexes *vs* the ligands, and the Zn-complex showed the best IC_{50} values with all cell lines. The ligand and the complexes exhibited IC_{50} values in the nano- and sub-nanomolar range, and they were ubiquitously more effective on all cell lines comparing to current standard drugs for such cancer cells, except in the cases of KB, GOTO and NB-1 cells. Further studies will be performed by the authors in order to assess the viability of these complexes as future anticancer drugs.

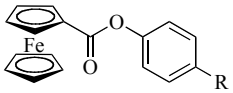
The ferronucleosides **38** and **39** containing a chiral hydroxylalkyl group were prepared and studied as cytotoxic and antiproliferative agents against murine leukemia (L1210), HeLa and human T-lymphocyte (CEM) cell lines. [36] Both compounds performed better than the derivatives **40**-**43**, showing that both nucleoside and hydroxyalkyl moieties are essential to the cytotoxic effects. All compounds showed IC_{50} values in the micromolar ou submicromolar range, with **38** being more effective towards L1210 cells and **39** for CEM and HeLa, with IC_{50} values of 0.78, 0.35 and 1.1, respectively. In general, compounds **38** and **39** performed with comparable performance to cisplatin and 5-fluorouracil. No evidences were observed for toxicity towards non-cancerous cells (IC_{50} > 50 μM). Moreover, compounds **38** and **39** are non-mutagenic, in addition to an antineoplasic activity comparable to 5-fluorouracil.



38 **39** **40** **41** **42** **43**

Scheme (10).

Six simple ferrocene phenyl ester derivatives substituted at *para*-position with halogen or pyrrolyl moieties (**44**-**49**) were prepared and tested against estrogen dependent MCF-7 breast cancer cell lines and MCF-10A normal breast cell lines [37].



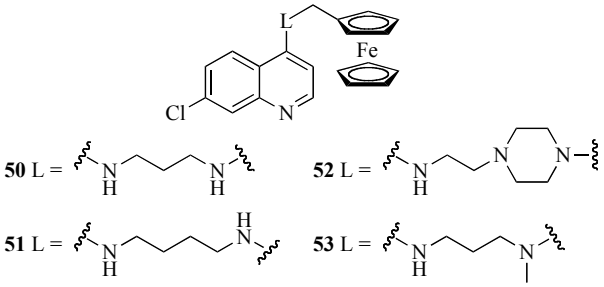
44 R = H **45** R = 1*H*-pyrrol-1-yl
46 R = F **47** R = Cl
48 R = Br **49** R = I

Scheme (11).

While 4-(1*H*-pyrrol-1-yl)phenyl ferrocenecarboxylate (**45**) gave the lowest IC_{50} value for MCF-7 lines (1.4 μM), it

also had a strong toxic effect over the normal breast cells (IC_{50} of 1.8 μM). Despite the IC_{50} values of 9.2 and 33 μM, the *p*-bromophenyl ferrocenecarboxylate (**48**) showed the highest IC_{50} (MCF-10A/MCF-7) ratio of 38, followed by the chloro derivative **47**, with 15, respectively. No cytotoxic effect was detected for the fluoro-compound **46**, and the iodo- derivative (**49**) was far more toxic to normal cells than to the cancerous ones. Docking and electrochemical studies revealed that the high selectivity of the bromo- compound is not ascribed to anti-estrogenic properties but to the appropriate combination of the ferrocene moiety and the electronegativity and charge distributions. [37]

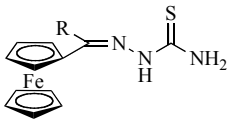
The quinolone-ferrocene hybrids **50**-**53** exhibited potent cytostatic effect, with GI_{50} values from 0.6-3.3 μM, as well good cytotoxicity, with LC_{50} values between 6-8 μM, against renal (TK10), melanoma (UACC62) and breast (MCF7) cancer cell lines. [38] Hybrids **52** and **53** exhibited the most potent cytostatic and cytotoxic effects on the proliferation of all three cancer cell lines, with LC_{50} and GI_{50} values ranging from 7.4 to 7.7 μM and 1.7 to 2.6 μM, respectively. Their mechanism of action may rely on the disruption of DNA transcription in the cancerous cells due to a possible optimal interaction with the negatively charged phosphate groups of the DNA backbone given by the three carbon atom spacing between two consecutive nitrogen atoms in the spacer of the hybrids. While compounds **50** and **51** showed to be the most antiproliferative of all, their high levels of toxicity towards Chinese Hamster Ovarian (CHO) cells (1.30 uM < IC_{50} < 2.25 μM) makes them unwanted candidates for future studies.



Scheme (12).

The ferrocenylthiosemicarbazones **54**-**63** were prepared by Cortez-Maya *et al.* [39] and screened as cytotoxic agents towards human glyoblastoma U251, human prostatic adenocarcinoma PC-3, human chronic myelogenous leukemia K562, human colorectal adenocarcinoma HCT-15, human mammary adenocarcinoma MCF-7 and human lung adenocarcinoma SKLU-1 cell lines.

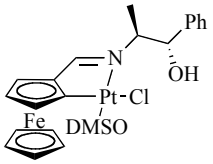
The compounds **58**-**63** inhibit the proliferation of all cell lines, with the *meta*- and *para*-chloro-derivatives **59** and **60** showing the lower IC_{50} values, ranging from 0.34 mM for **59** and MCF-7 cells to 22.3 mM for **60** and SKLU-1 cells. [39] These data for compound **59** are better than the ones recorded for cisplatin and, in addition, compound **59** did not show any activity against normal fibroblast NIH3T3 cells.



54 R = H **55** R = *i*Pr
56 R = Pr **57** R = Bn
58 R = 2-Cl-Ph **59** R = 3-Cl-Ph
60 R = 4-Cl-Ph **61** R = 2-F-Ph
62 R = 3-F-Ph **63** R = 4-F-Ph

Scheme (13).

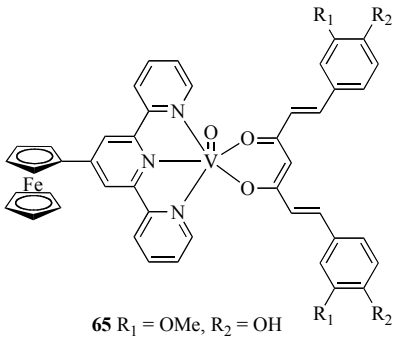
A follow-up study on the cycloplatinated complex **64**, previously prepared by M. Cascante [40,41], showed that it induces a phosphorylation of the tumor suppressor FOXO3a, leading to its the nuclear translocation and inhibition of upstream AKT phosphorylation. The cytotoxic activity in A549 cells is caused by the activation of intrinsic caspase pathway that leads to apoptosis. Moreover, it has low toxicity to healthy cells and it has a synergistic effect with cisplatin on A549 non-small lung cancer cell lines.



64

Scheme (14).

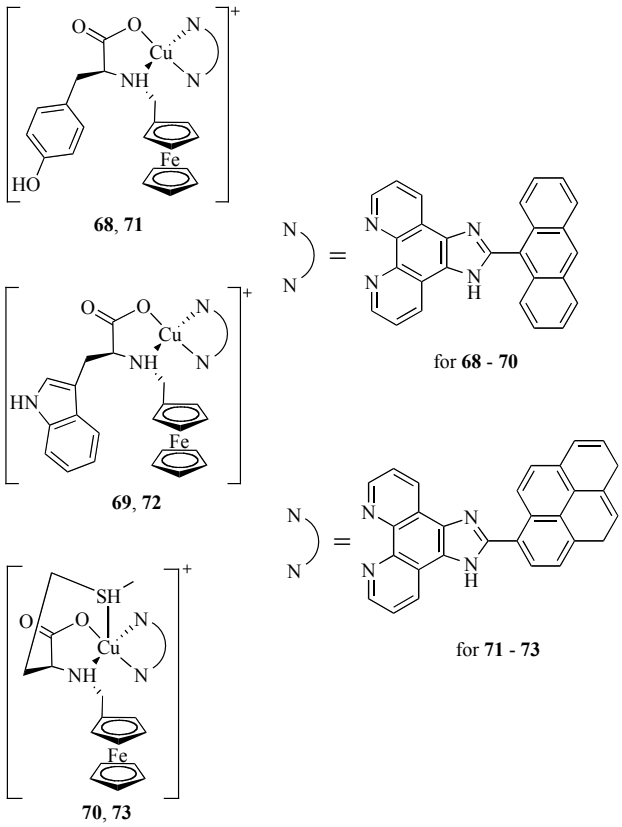
The ferrocenyl-terpyridine oxovanadium (IV) complexes of curcuminoids **65**-**67** showed remarkable PDT activity in HeLa cells with IC_{50} dark/light ratios of 13-25.[42] In addition, they presented 5 to 10-fold selectivity towards the cancer cells over normal 3T3 cells due to the formation of hydroxyl radicals under blue or red light excitation. The best results were obtained for compound **65**, showing the essential role of the methoxyl and hydroxyl groups of the curcumin moiety. Its photo-induced efficiency is indeed better than Photofrin®. The high cell uptake of **65** leads to its accumulation both in the cytosol and nucleus of the HeLa cells, and there are evidences that the cell death occurs via an apoptopic pathway.



65 R_1 = OMe, R_2 = OH
66 R_1 = OMe, R_2 = H
67 R_1 = H, R_2 = OH

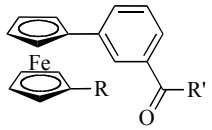
Scheme (15).

The ferrocenyl-L-amino acid copper(II) complexes **68**-**73** (Scheme **15**) by A. R. Chakravarty *et al.* [43] showed notable photocytotoxicity in HeLa and MCF-7 cancer cells with low dark toxicity ($5.5 < IC_{50}$(dark/light) < 13.2), mainly because of the redox inactive copper(II) center. Both types of cells were mostly affected by compound **73**, with IC_{50} values of 2.3 and 3.2 μM, respectively, under visible light. The compounds were mainly localized in the endoplasmatic reticulum of the HeLa cells, which, once the compounds can bind to DNA, can avoid the occurrence of mutations on nuclear DNA upon photoactivation.



Scheme (16).

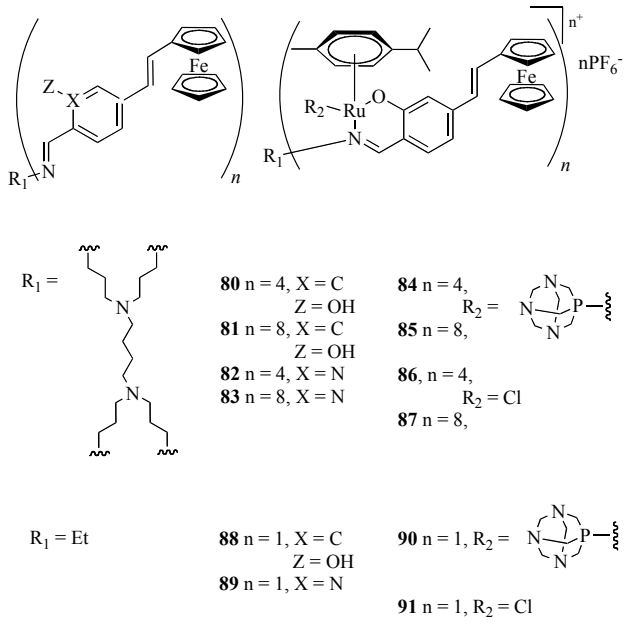
P. Kenny and co-workers [44] reported on the 1-alkyl-1'-*N*-*meta*- and 1-alkyl-1'-*N*-*para*-(ferrocenyl)benzoyl dipeptide esters **74**-**79** that, from a wide family of 23 compounds, showed micromolar activity against the H1299 NSCLC cell line, with IC_{50} values in the range 2.6-20.1 μM. The best result was observed for compound **74**, however cisplatin shows a lower IC_{50} value of 1.5 μM. Linking the ferrocene moiety at the *ortho* position of benzoyl group decreases the activity of the compounds. In addition, the cytotoxicity of the derivatives increases with a decrease of the size of the alkyl group on the previously reported unsubstituted cyclopentadiene ring. However, better results have previously been obtained without any alkylation of the cyclopentadiene ring in the *orto*- and *meta*- families of compounds, but in the *para*- positions this strategy enhanced the cytotoxic activity of the compounds.



74 R = CH_3, R' = Gly-Gly(OEt)
75 R = CH_3, R' = Gly-L-Ala(OEt)
76 R = CH_2CH_3, R' = Gly-Gly(OEt)
77 R = CH_2CH_3, R' = Gly-L-Ala(OEt)
78 R = $CH_2CH_2CH_3$, R' = Gly-Gly(OEt)
79 R = $CH_2CH_2CH_3$, R' = Gly-L-Ala(OEt)

Scheme (17).

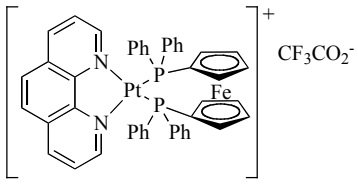
G.S. Smith [45] performed preliminary *in vitro* proliferation studies of the ferrocenyl-derived ligands (**80**-**91**) and the corresponding *p*-cymene-Ru(II) complexes ([**84**][PF_6]$_4$-[**87**][PF_6]$_8$, [**90**][PF_6], and [**91**][PF_6]) towards five distinct cancer cell lines: A2780 and A2780cisR human ovarian, the SISO human cervix, the LCLC-103H human lung, and the 5637 human bladder. In general, these compounds showed to be very specific for ovarian cancer cells, with nine of them inhibiting the growth of both the A2780 and A2780cisR cell lines in more than 50% at a concentration of 5 μM. Quite strong antiproliferative effects were also observed for the 5637 bladder cancer line, while both the SISO and LCLC-103H lines were generally more resistant.



Scheme (18).

The first- and second-generation ferrocenyl-derived *N*,*O*-*p*-cymene-ruthenium-PTA metallodendrimers have shown to be the most active of the series. Further studies will be carried on by the authors, considering that adequate chemical transformations will have to be made taking into account the poor solubility of the compounds in the testing media.

The simple fluorescent Pt complex **92**, prepared by R. Yousefi and co-workers [46], showed very strong antiproliferative effect on human T-cell lymphoblast Jurkat cell line, and, to a smaller extent, also on human breast adenocarcinoma MCF-7 cell line. After 24 and 48h of incubation **92** showed lower IC_{50} values than the standard cisplatin, more precisely 1.46 and 0.46 μM for the former, and 18.21 and 8.71 M for the latter. The complex actuates through a mixed-binding mode, comprising partial intercalation, electrostatic binding and coordination to hsDNA that ultimately leads to apoptosis.



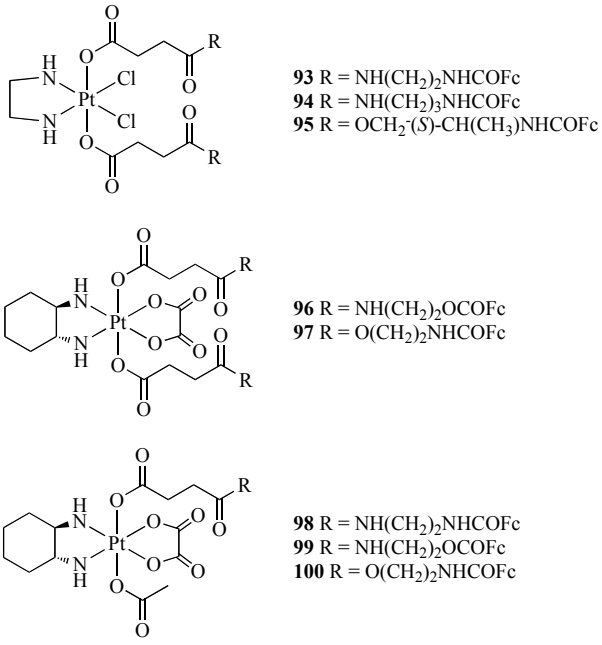
92

Scheme (19).

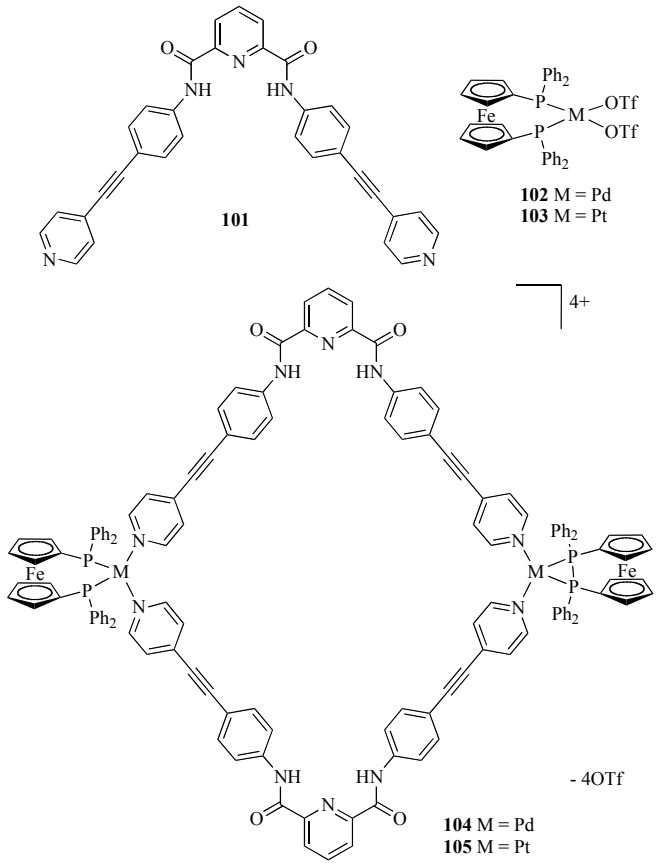
The dichlorido(ethane-1,2-diamine)platinum(IV) complexes **93-95** and oxaliplatin derivatives **96-100** were found to be very cytotoxic towards cisplatin-sensitive ovarian carcinoma CH1 cell lines, with IC_{50} values ranging from 0.84–2.3 μM with no particular distinction between the two kinds of compounds. [47] On another hand, the oxaliplatin derivatives **96-100** showed to be far more cytotoxic towards the intrinsically cisplatin-resistant SW480 colon carcinoma cell lines than the cisplatin-like complexes **93-95**, with IC_{50} values ranging from 2.7-5.6 μM and 32-46 μM, respectively. Finally, low to moderate cytotoxic effect was addressed to non-small cell lung carcinoma A549 cancer cells by both types of complexes (IC_{50} > 24 μM). However, none of the studied complexes outperformed oxaliplatin in any of the cancer cell lines. Overall, no particular relation between the cytotoxic effect and the chain lengths or the linking fragments can be established.

The hetero-bimetallacycles containing Pd (II) and Pt (II) **104** and **105**, respectively, were found to be more efficient than cisplatin against T98G brain cancer, KB head and neck cancer and SNU80 thyroid cancer cells, as well as less toxic to normal human HEK-293 cells at effective concentrations between 0.5 and 16 μM. [48] As expected, the complexes also were also more cytotoxic than the monomers **101-103**. The IC_{50} values ranged from 4.5-12.0 μM and 5.2-18.3 μM for **105** and **104**, respectively, for the cancerous cells, with particular selectivity for the T98G cells. The cellular uptake of **105** in these cells is also more efficient than **104**'s, and its genotoxicity promotes oxidative stress that ultimately leads to cell death probably by an apoptotic pathway.

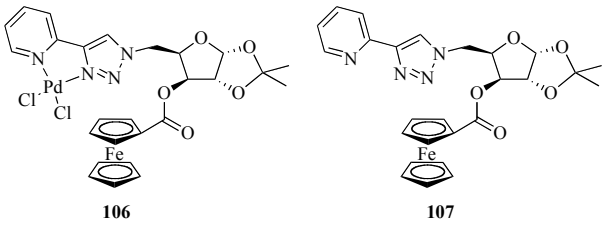
The carbohydrate Pd (II) complex **106** bearing a ferrocenyl ester moiety prepared by R. Trivedi and co-workers [49] was studied as cytotoxic agents against A549, HeLa, MDA-MB-231 and MCF-7 cell lines. However, it only presented activity towards the first cell line with an IC_{50} value of 13.18 μM, which was higher than the 5.73 μM found for its less bulky precursor **107** (5.73 μM), as well as doxorubicin (0.46 μM) and cisplatin (0.15 μM).



Scheme (20).



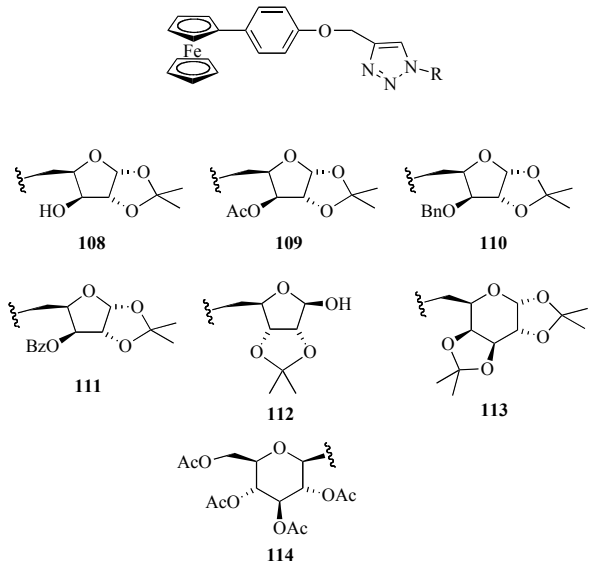
Scheme (21).



106 **107**

Scheme (22).

The (4-ferrocenylphenyl)propargyl ether derived carbohydrate triazoles **108-114** were tested as cytotoxic agents against HeLa, MDA-MB-231 and MCF-7 cell lines. [50] Only **113** and **114** showed activity, with moderate (9.0 μM) to high (61.79 μM) IC_{50} values being obtained. The reason for the poor performance of these compounds may lie on the hydrophobic character of the phenoxymethyl triazole moiety, as previously prepared amide-triazole derivatives demonstrated much higher activity.



108 **109** **110**

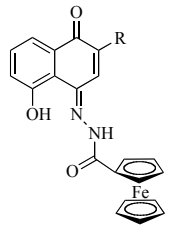
111 **112** **113**

114

Scheme (23).

The introduction of a ferrocene moiety in the structure of the natural compound plumbagin (**115**) enhanced its inherent cytotoxic activity towards cells of human 518A2 melanoma, HCT-116 colon carcinoma and also the multi-drug resistant KB-V1/Vbl cervix carcinoma, with IC_{50} values of 0.5, 4.7 and 1.1 μM versus 1.1, 5.8 and 26.2 μM, for the derivative and parent compounds, respectively.

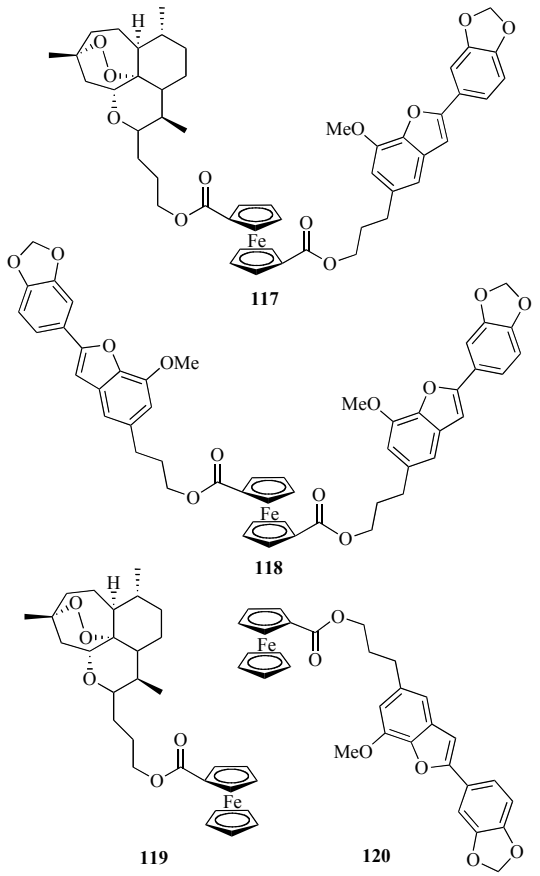
In addition, the growth of chicken heart fibroblasts was not affected by **115**, showing a good selectivity towards cancer cells [51]. The cell cycle stopped at the G1-S phase in the presence of the ferrocenyl derivative, and significant dose and time-dependent increases of dying cancer cells (sub-G0/G1 fraction) were observed after treatment. In agreement, **115** precipitated a more extensive change of the shape of linear DNA probably due to the intercalation of the naphthoquinone unit bearing a large orthogonal metal complex fragment running alongside the DNA or/and of additional ROS-induced lesions. Finally, the authors correlate the high efficacy of **115** against the multi-drug resistant KB-V1/Vbl cervix carcinoma with the inhibition of the drug efflux Pgp-type pumps which are overexpressed in this particular cell line. A similar synthetic approach was conducted with the natural analogue Juglone, which is particularly effective against the human 518A2 melanoma and HCT-116 colon carcinoma cell lines. However, the inherent cytotoxic effect was lost with the introduction of the ferrocenyl moiety-containing **116**, showing the detrimental effect that simple and small chemical groups can have on the biological activity of compounds.



115 R = CH_3
116 R = H

Scheme (24).

The natural compound egonol has also been functionalized with several ferrocene moieties, yielding compounds **117-120** [52].



117

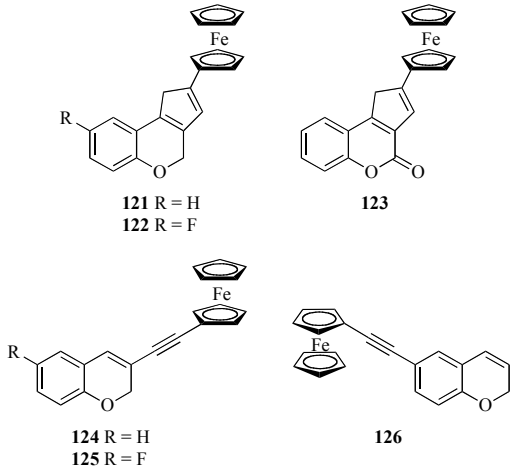
118

119 **120**

Scheme (25).

These compounds were tested as cytotoxic agents for wild-type CCRF-CEM and multidrug-resistant P-glycoprotein-over-expressing CEM/ADR5000 human leukemia cells, with **117** showing very strong selectivity towards the first cell line, given by the IC_{50} values of 0.07 and 147.27 μM, respectively. In another hand, compound **119**, which encompasses the anti-malaria active 1,2,4-trioxane moiety linked to ferrocene by an ester group, has a very strong effect in both types of cells with IC_{50} values of 0.25 and 0.57 μM, respectively. Finally, the dimeric egonol-ferrocene aggregate **118** and its monomer **120** were only active towards CCFR-CEM cells, with IC_{50} values of 3.17 and 36.15 μM. Despite lower than doxorubicin for the first cell line, the high efficiency of compounds **117** and **119** may actually be related to the cooperative and synergistic effect of the endoperoxide bridge and the ferrocene unit, given the lower activity observed for analogous compounds without these units.

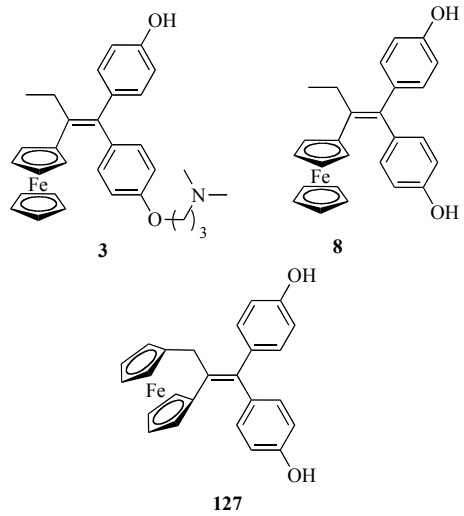
The ferrocenyl derivatives of the natural compounds pterocarpene and coumestan, and other chromones **121-126** were screened as cytotoxic agents against MCF-7 and MDA-MB-231 mammary carcinoma cells and HT-29 colon carcinoma cells. [53] Compounds **123** and **126** did not present any cytotoxic activity towards the studied cell lines. The lower IC_{50} values were recorded for compounds **124** and **125**, which were active against all cell lines. However a higher toxicity towards human RC-124 epithelial kidney cells than for the cancerous ones was observed. Both complexes **121** and **122** were more selective towards the HT29 cancer cells, with IC_{50} values of 30 μM, than the healthy ones. Compound **122** was also effective against MCF-7 cells showing an IC_{50} value of 46.3 μM.



Scheme (26).

2.1. Mechanistic Studies

C. Amatore and co-workers [54] reported that the previously prepared ferrocifen derivatives **3**, **8** and **127** actuate by distinct mechanistic pathways. In general, their mechanism of action relies on the formation of a quinone methide derivative upon oxidation of the ferrocene moiety, which is not attainable by **127** due to structural features. Moreover, despite their similar structures, ROS/RNS production in a stressful environment is enhanced by the last two compounds in opposition to the former.



Scheme (27).

2.2. Other Organometallic Complexes

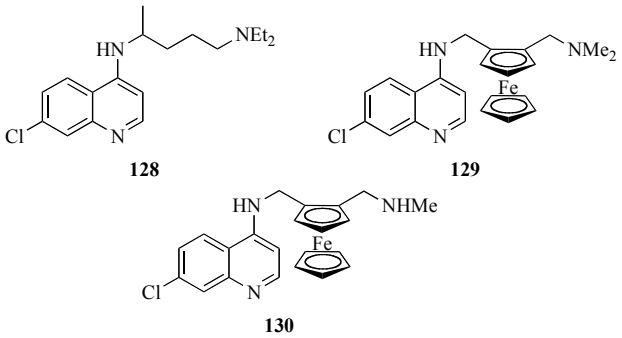
Several other organometallic complexes containing Au [55,56], Rh [57], Ru [58, 59], Ir [60], Ti [61, 62] and Os [63] in their structure have also been reported to exhibit interesting anti-cancer activities.

3. METALLOCENES AS ANTI-MALARIA

The parasite disease malaria still remains the most common among tropical regions and affects more than 200 million people, lethal in 627.000 cases in 2013. [64,65] The most dangerous of the five single cell protozoan parasites of the *Plasmodium* species is the *P. falciparum*, accounting for about 90% of all deaths from malaria. [66] The quinoline-based compound, Chloroquine (**128**) has been known to have lost its efficacy, encountering chemo-resistance issues, creating the urgent need to discover novel anti-malarial agents. The fact that using metal complexes enhances the biological activity of compounds has evolved into an important research strategy in biological communities as well as in organometallic chemistry. The ferrocene-based drug ferroquine (**129**) has been the most successful example of a new antimalarial which is highly active against chloroquine-resistant strains, currently developed by Sanofi-Aventis [67].

Plasmodium vivax is responsible for about half of the malaria cases located in south-east Asia and in the Pacific coast. An *in vitro* evaluation of the efficacy of **129** *versus* other reference anti-malaria agents on blood isolates infected with *P. Vivax* was thoroughly conducted through the invention of Fraisse& Struxiano [68] on the use of **129** or its *N*-demethylated metabolite compound **130** in the treatment and/or prevention of malaria. This invention demonstrated that compound **129** was always active throughout the various

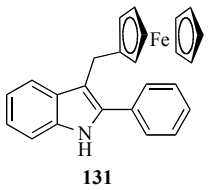
parasite growth stages, whereas **128** proved to be 10 to 20 times less active in mature stages. Furthermore, due to its promising results, a unit form of administration of **129** in tablet form was suggested.



Scheme (28).

Through the work of A. Patti *et al.* [69] other ferrocenylquinolines have also been synthesized and prepared as a potentially useful class of bioactive organometallics and evaluated against malaria. A new class of substituted quinolines bearing a ferrocene unit connected to the heterocycle in the side chain has been screened for their activity in the growth inhibition of chloroquine-susceptible D10 and chloroquine resistant W2 strains of *P. falciparum* using chloroquine as reference drug. Although the obtained products were found not active against the parasite, the authors acknowledged the given contributions of the ferrocenyl and dimethylamino substituents used.

J. Quirante *et al.* [67] reported other antimalarial ferrocene based compounds containing indole moieties. Indole molecules themselves have been extensively studied and proven to be attractive as lead compounds due to their low cost and straightforward synthesis. From the several ferrocene-indole hybrids prepared, compound **131** showed to be the most active one against *P. Facliparium*.



Scheme (29).

4. METALLOCENES AS ANTI-HIV AGENTS

C. Pereira *et al.* [70] conducted one of the many studies regarding the applications of ferrocene in medical research consisting on the preparation of some adducts through the incorporation of the ferrocenemethyl moiety into a heterocyclic base. This attracted some attention to the evaluation of these compounds against the human immunodeficiency virus (HIV) as well as other viruses such as hepatitis B (HBV) and bovine viral diarrhea virus (BVDV). M. Fouda *et al.* [71] observed that only compounds bearing thymine showed significant cytotoxicity against MT-4 cells. It was shown that the ferrocenyl derivatives of 3'-deoxy-3'-azidothymidine were active against HIV-1. However, they proved to less potent than 3'-α-azidothymidine(AZT) used as the reference drug.

Other applications regarding the use of ferrocene derivatives against the activity of HIV are related to electrochemical examination of HIV enzymes using ferrocene-conjugated peptides. The main idea was to test the possibility of developing inhibitors that target different steps in the HIV life cycle by blocking the function of HIV-related proteins such as HIV-1 integrase (HIV-1 IN), HIV-1 reverse transcriptase (HIV-1 RT) and HIV-1 protease (HIV-1 PR). These are vital proteins that control the HIV's ability to infect cells as well as the production of new copies of the virus.

H. Kraatz *et al.* [72] demonstrated for the first time the detection of these enzymes at a nanomolar level using ferrocene (Fc)-conjugated peptides on gold microelectrodes. The interaction between the Fc-conjugated peptides and the enzymes was studied through cyclic voltammetry. It was shown that the electrochemical behaviour of the surface-bound Fc- bioconjugate changed as the protein concentration increased, suggesting that the HIV protein was binding to the peptide film and encapsulating the Fc redox center on its surface.

5. CONCLUSION

Application of metallocenes especially ferrocene derivatives in medicine and pharmaceutical fields is a very attractive research area.

The high potential of some ferrocenyl derivatives for cancer therapeutics is well known in academia as significant *in vitro* cytotoxicity against several tumor cell lines, including prostate, lung, colon, leukemia and breast cancer cell lines, has been recorded in many cases.

Recent studies reported the importance of attaching specific units to ferrocene scaffolds for medicinal applications.

The use of metallocene derivatives especially ferrocene scaffolds (ferrocifens, ferrocenium and ferricenium salts and ferrocenyl complexes) as anti-tumoral, anti-malaria and anti-HIV agents is very promising both in the academic and industrial points of view.

Combination of the redox properties of ferrocene and its high stability have also been explored in order to prepare electrochemical sensors for very different substrates such as DNA, proteins and environmental pollutants.

It would be of great interest to pursue the design of novel metallocene derivatives which can improve water solubility and blood circulation life-time of standard drugs, and enhance selectivity towards cancer tissues. In parallel, the use of multifunctional drug delivery carriers would allow the potential combination between metallocene compounds and imaging agents and/or other drugs, opening future perspectives to enlarge the application of such compounds in theranostics.

ACKNOWLEDGEMENTS

This work was supported by LAQV-REQUIMTE; Fundação para a Ciência e Tecnologia (FCT, Portugal), through projects and the IF researcher consolidator contract (IF/0041/2013/CP1161/CT005), and Solchemar Company.

REFERENCES

[1] a) Rosenblum, M.; *Chemistry of the Iron-Group Metallocenes*, *Part I*, Interscience: New York, **1965**. b) Togni, A.; Halterman, R. L. (Eds), Metallocenes: *Synthesis Reactivity Application*s, Wiley-VCH Weinheim, **2008**.

[2] a) Woodward, R. B.; Rosenblum, M.; Whiting, M. C.; A New Aromatic System; *J. Am. Chem. Soc.*, **1952**, 74, 3458-3459. b) Togni A.; Hayashi, T.; *Ferrocenes*, Wiley-VCH, Weinheim, **1994**. c) Heinze, K.; Ferrocene-Beauty and Function *Organometallics* **2013**, 32, 5623-5625.

[3] a) Adams, R. D.; Metallocenes-Special Issue; *J. Organomet. Chem.* **2001**, 637-639, 1-875. b) Hendry, S. P.; Cardosi, M. F.; Turner, A. P. F.; Neuse, E. W.; Polyferrocenes as mediators in amperometric biosensors for glucose; *Anal. Chim. Acta* **1993**, 281, 453-459. c) Labande, A.; Astruc, D.; Colloids as redox sensors: recognition of $H_2PO_4^-$ and HSO_4^- by amidoferrocenylalkylthiol-gold nanoparticles; *Chem. Commun,* **2000**, 1007-1008.

[4] a) Astruc, D.; Ornelas, C.; Ruiz, J.; Metallocenyl Dendrimers and Their Applications in Molecular Electronics, Sensing, and Catalysis; *Acc. Chem. Res.* **2008**, 41, 841-856. b) Ornelas, C.; Ruiz, J. ; Belin, C. ; Astruc, D.; Giant Dendritic Molecular Electrochrome Batteries with Ferrocenyl and Pentamethylferrocenyl Termini; *J. Am. Chem. Soc.* **2008**, 131, 590-601. c) Wang, A.; Ornelas, C.; Astruc, D.; Hapiot, P.; Electronic Communication between Immobilized Ferrocenyl-Terminated Dendrimers; *J. Am. Chem. Soc.* **2009**, 131, 6652-6653.

[5] a) Labande, A.; Ruiz, J.; Astruc, D.; Supramolecular Gold Nanoparticles for the Redox Recognition of Oxoanions: Syntheses, Titrations, Stereoelectronic Effects, and Selectivity; *J. Am. Chem. Soc.* **2002**, 124, 1782-1789. b) M. C. Daniel, J. Ruiz and D. Astruc, Supramolecular H-Bonded Assemblies of Redox-Active Metallodendrimers and Positive and Unusual Dendritic Effects on the Recognition of $H_2PO_4^-$ *J. Am. Chem. Soc.* **2003**,125, 1150-1151.

[6] Togni, A.; Hayashi, T. (Eds); Ferrocenes Homogeneous Catalysis Organic Synthesis Material Science, VCH, Weinheim, **1995**.

[7] a) Van Staveren, D. R. ; Metzler-Nolte, N.; Bioorganometallic Chemistry of Ferrocene; *Chem. Rev.* **2004**, 104, 5931-5986. b) Allardyce, C. S.; Dorcier, A.; Scolaro, C.; Dyson, P. J.; Development of organometallic (organo-transition metal) pharmaceuticals; *Appl. Organomet. Chem.* **2005**, 19, 1-10.

[8] a) Fouda, M. F. R.; Abd-Elzaher, M. M.; Abdelsamaia, R. A.; Labib, A. A.; On the medicinal chemistry of ferrocene; *Appl. Organomet. Chem.* **2007**, 21, 613-625. b) Nguyen, A.; Vessières, A.; Hillard, E. A.; Top, S.; Pigeon, P.; Jaouen, G.; Ferrocifens and Ferrocifenols as New Potential Weapons against Breast Cancer; *Chimia* **2007**, 61, 716-724.

[9] a) Biot, C.; Glorian, G.; Maciejewski, L. A.; Brocard, J. S.; Domarle, O.; Blampain, G.; Millet, P.; Georges, A. J.; Abessolo, H.; Dive, D.; Lebibi, J.; Synthesis and Antimalarial Activity *in Vitro* and *in Vivo* of a New Ferrocene−Chloroquine Analogue; *J. Med. Chem.* **1997**, 40, 3715-3718. b) Delhaes, L.; Biot, C.; Berry, L.; Maciejewski, L. A.; Camus, D.; Brocard, J. S.; Dive, D.; Novel ferrocenic artemisinin derivatives: synthesis, *in vitro* antimalarial activity and affinity of binding with ferroprotoporphyrin IX; *Bioorg. Med. Chem.* **2000**, 8, 2739-2745.

[10] Gasser, G.; Ott, I.; Metzler-Nolte, N.; Organometallic Anticancer Compounds, *J. Med. Chem.* **2011**, 54(1), 3-25.

[11] Fouda, M. F. R.; Abd-Elzaher, M. M.; Abdelsamaia, R. A.; Labib, A. A.; On the medicinal chemistry of ferrocene; *Appl. Organometal. Chem.* **2007**, 21, 613-625

[12] Ornelas, C.; Application of ferrocene and its derivatives in cancer research, *New J. Chem.* **2011**, 35, 1973-1985.

[13] Dysona, P.; Sava, G.; Metal-based antitumour drugs in the post genomic era, *Dalton Trans*. **2006**, 16, 1929-1933.

[14] Gasser, G.; Ott, I.; Metzler-Nolte, N.; Organometallic anticancer compounds, *J. Med. Chem*. **2011**, 54, 3-25.

[15] a) Joksović, M. D.; Marković, V.; Juranić, Z. D.; Stanojković, T., Jovanović, L. S.; Damljanović, I.S.; Szecscenyi, K.M.; Todorović, N.; Trifunović, S.; Vukicević, R.D.; Synthesis, characterization and antitumor activity of novel N-substituted α-amino acids containing ferrocenyl pyrazole-moiety. *J. Organomet. Chem.* **2009**, 694, 3935-3942. b) Schatzschneider, U.; Metzler-Nolte, N.; New Principles in Medicinal Organometallic Chemistry, *Angew. Chem. Int. Ed.* **2006**, 45, 1504-1507. c) Bruijnincx, P.C.A.; Sadler, P.J.; New trends for metal complexes with anticancer activity, *Curr. Opin. Chem. Biol.* **2008**, 12, 197-206.

[16] Monserrat, J.; Tiwari, K.; Quentin, L.; Pigeon, P.; Jaouen, G.; Vessières, A.; Chabot, G.; Hillard, A.; Ferrocenyl flavonoid-induced morphological modifications of endothelial cells and cytotoxicity against B16 murine melanoma cells, *Journal of Organometallic Chemistry* **2013**, 734, 78-85.

[17] Molnár, J.; Engi, H.; Hohmann, J.; Molnár, P.; Deli, J.; Wesolowska, O.; Michalak, K.; Wang, Q.; Reversal of multidrug resistance by natural substances from plants, *Curr. Top. Med. Chem*. **2010**, 10, 1757-1768.

[18] Yong, J.; Lu, C.; Wu, X.; Synthesis of isoxazole moiety containing ferrocene derivatives and preliminarily *in vitro* anticancer activity, *Med. Chem. Commun*., **2014**, 5, 968-972.

[19] Balaji, B.; Somyajit, K.; Banik, B.; Nagaraju, G.; Chakravarty, A.; Photoactivated DNA cleavage and anticancer activity of oxovanadium(IV) complexes of curcumin, *Inorganica Chimica Acta* **2013**, 400, 142-150.

[20] Sağır, T.; Işık, S.; Şenel, M.; Ferrocene incorporated PAMAM dendrons: synthesis, characterization, and anti-cancer activity against AGS cell line, *Med Chem Res* **2013**, 22, 4867-4876.

[21] Duan, Q.; Cao, Y.; Li, Y.; Hu, X.; Xiao, T.; Lin, C.; Pan, Y.; Wang, L.; pH-Responsive Supramolecular Vesicles Based on Water-Soluble Pillar[6]arene and Ferrocene Derivative for Drug Delivery, *J. Am. Chem. Soc*. **2013**, 135, 10542-10549.

[22] Snegur, L.; Zykov, S.; Simenel, A.; Nekrasov, Y.; Starikova, Z.; Peregudova, S.; Il'in, M.; Kachala, V.; Sviridova, I.; Sergeeva, N.; Redox active ferrocenemodified pyrimidines and adenine as antitumor agents:structure, separation of enantiomers, and inhibition of the DNA synthesis in tumor cells, *Russian Chemical Bulletin* **2013**, 62, 2056-2064.

[23] Lal, B.; Badshah, A.; A Altaf, A.; Tahir, M.; Ullah, S.; Huq, F.; Study of New Ferrocene Incorporated N,N9-Disubstituted Thioureas as Potential Antitumour Agents, *Aust. J. Chem.* **2013**, *66*, 1352-1360.

[24] Chen, W.; Ou, W.; Wang, L.; Hao, Y.; Cheng, J.; Li, J.; Liu, Y-N.; Synthesis and biological evaluation of hydroxyl substituted Schiff-bases containing ferrocenyl moieties, *Dalton Trans*. **2013**, 42, 15678-15686.

[25] Li, Y.; Ma, H.; Han, L.; Liu, W.; Zhao, B.; Zhang, S.; Miao, J.; Novel ferrocenyl derivatives exert anti-cancer effect in human lung cancer cells *in vitro* via inducing G1-phase arrest and senescence, *Acta Pharmacologica Sinica* **2013**, 34, 960-968.

[26] Shen, S.; Shao, J.; Luo, J.; Liu, J.; Miao, J.; Zhao, B.; Novel chiral ferrocenylpyrazolo[1,5-a][1,4]diazepin-4-one derivatives-Synthesis, characterization and inhibition against lung cancer cells, *Eur. J. Med. Chem.* **2013**, 63, 256-268.

[27] Jemal, A.; Bray, F.; Center, M.M.; Ferlay, J.; Ward, E.; Forman, D.; Global cancer statistics, *CA. Cancer J. Clin.* **2011**, 61, 69-90.

[28] Lainé, A-L.; Adriaenssens, E.; Vessières, A.; Jaouen, G.; Corbet, C.; Desruelles, E.; Pigeon, P.; Toillon, R-A.; Passirani, C.; The *in vivo* performance of ferrocenyl tamoxifen lipid nanocapsules in xenografted triple negative breast cancer, *Biomaterials* **2013**, 34, 6949-6956.

[29] a) Hillard, E. A.; Pigeon, P.; Vessires, A.; Amatore, C.; Jaouen, G.; The influence of phenolic hydroxy substitution on the electron transfer and anti-cancer properties of compounds based on the 2-ferrocenyl-1-phenyl-but-1-ene motif, *Dalton Trans.* **2007**, 5073-5081. b) Oliveira, A. C.; Hillard, E. A.; Pigeon, P.; Rocha, D. D.; Rodrigues, F. A. R.; Montenegro, R. C.; Costa-Lotufo, L. V.; Goulart, M. O. F.; Jaouen, G.; Biological evaluation of twenty-eight ferrocenyl tetrasubstituted olefins: Cancer cell growth inhibition, ROS production and hemolytic activity, *Eur. J. Med. Chem.* **2011**, 46, 3778-3787. c) Oliveira, A. C.; Silva, E. G.; Rocha, D. D.; Hillard, E. A.; Pigeon, P.; Jaouen, G.; Rodrigues, F. A. R.;

Abreu, F. C.; Ferreira, F. R.; Goulart, M. O. F.; Costa-Lotufo, L. V.; Molecular Mechanism of Action of 2-Ferrocenyl-1,1-diphenylbut-1-ene on HL-60 Leukemia Cells, *ChemMedChem.* **2014**, 9, 2580-2586.

[30] a) Pigeon, P.; Görmen, M.; Kowalski, K.; Müller-Bunz, H.; McGlinchey, M. C.; Top, S.; Jaouen, G.; Atypical McMurry Cross-Coupling Reactions Leading to a New Series of Potent Antiproliferative Compounds Bearing the Key [Ferrocenyl-Ene-Phenol] Motif, *Molecules* **2014**, 19, 10350-10369. b) Hillard, E.A.; Pigeon, P.; Vessières, A.; Amatore, C.; Jaouen, G. The influence of phenolic hydroxy substitution on the electron transfer and anti-cancer properties of compounds based on the 2-ferrocenyl-1-phenyl-but-1-ene motif, *Dalton Trans.* **2007**, 43, 5073-5081.

[31] Cázares-Marinero, J. J.; Top, S.; Vessières, A.; Jaouen, G.; Synthesis and antiproliferative activity of hydroxyferrocifen hybrids against triple-negative breast cancer cells, *Dalton Trans.* **2014**, 43, 817-830.

[32] a) Cázares-Marinero, J. J.; Top, S.; Jaouen, G.; Synthesis and characterization of new ferrocenyl compounds with different alkyl chain lengths and functional groups to target breast cancer cells, *J. Organomet. Chem.* **2014**, 751, 610-619. b) Cázares-Marinero, J. J.; Lapierre, M.; Cavaillès, V.; Saint-Fort, R.; Vessières, A.; Top, S.; Jaouen, G.; Efficient new constructs against triple negative breast cancer cells: synthesis and preliminary biological study of ferrocifen-SAHA hybrids and related species, *Dalton Trans.* **2013**, 42, 15489-15501.

[33] Kowalski, K.; Hikisz, P.; Szczupak, L.; Therrien, B.; Koceva-Chyla, A.;Ferrocenyl and dicobalt hexacarbonyl chromones-New organometallics inducing oxidative stress and arresting human cancer cells in G2/M phase, *Eur. J. Med. Chem*. **2014**, 81, 289-300.

[34] Schulz, J.; Tauchman, J.; Císarová, I.; Riedel, T.; Dyson, P. J.; Stepnicka, P.; Synthesis, structural characterization and cytotoxicity of bimetallic chlorogold(I) phosphine complexes employing functionalized phosphinoferrocene carboxamides, *J. Organomet. Chem.* **2014**, 751, 604-609.

[35] Abd-Elzaher, M. M.; Labib, A. A.; Mousa, H. A.; Moustafa, S. A.; Abdallah, M. M.; Synthesis, characterization and cytotoxic activity of ferrocenyl hydrazone complexes containing a furan moiety, *Res. Chem. Intermed.* **2014**, 40, 1923-1936.

[36] Nguyen, H. V.; Sallustrau, A.; Balzarini, J.; Bedford, M. R.; Eden, J. C.; Georgousi, N.; Hodges, N. J.; Kedge, J.; Mehellou, Y.; Tselepis, C.; Tucker, J. H. R.; Organometallic Nucleoside Analogues with Ferrocenyl Linker Groups: Synthesis and Cancer Cell Line Studies, *J. Med. Chem.* **2014**, 57, 5817-5822.

[37] Vera, J. L.; Rullán, J.; Santos, N.; Jiménez, J.; Rivera, J.; Santana, A.; Briggs, J.; Rheingold, A. L.; Matta, J.; Meléndez, E.; Functionalized ferrocenes: The role of the para substituent on the phenoxy pendant group, *J. Organomet. Chem.* **2014**, 749, 204-214.

[38] N'Da, D. D.; Smith, P. J; Synthesis, *in vitro* antiplasmodial and antiproliferative activities of a series of quinoline-ferrocene hybrids, *Med. Chem. Res.* **2014**, 23, 1214-1224.

[39] Cortez-Maya, S.; Klimova, E.; Flores-Alamo, M., Martínez-Klimova E., Ramírez-Ramírez A., Ramírez, T. A; Martínez-García M.; Anticancer Activity of Ferrocenylthiosemicarbazones, *Anti-Cancer Agents in Medicinal Chemistry* **2014**, 14, 459-465.

[40] Cortés, R.; Tarrado-Castellarnau, M.; Talancón, D. López, C.; Link, W.; Ruiz,, D.; Centelles, J. J.; Quirante, J.; Cascante, M.; A novel cyclometallated Pt(II)-ferrocene complex induces nuclear FOXO3a localization and apoptosis and synergizes with cisplatin to inhibit lung cancer cell proliferation, *Metallomics* **2014**, 6, 622-633.

[41] Talancón, D.; López, C.; Font-Bardía, F.; Calvet, C; Quirante, J.; Calvis, C.; Messeguer, R.; Cortés, R.; Cascante, M.; Baldomà, L.; Badia, J.; *J. Inorg. Biochem.* **2013**, 118, 1-12.

[42] Balaji, B.; Balakrishnan, B.; Perumalla, S.; Karande, A. A.; Chakravarty, A. R.; Photoactivated cytotoxicity of ferrocenyl-terpyridine oxovanadium(IV) complexes of curcuminoids, *Eur. J. Med. Chem.* **2014**, 85, 458-467.

[43] Goswami, T. K.; Gadadhar S.; Balaji, B.; Gole, B.; Karande, A. A.; Chakravarty, A. R.; Ferrocenyl-L-amino acid copper(II) complexes showing remarkable photo-induced anticancer activity in visible light, *Dalton Trans.* **2014**, 43, 11988-1199.

[44] a) Harry, A. G.; Butler, W. E.; Manton, J. C.; Pryce, M. T.; O'Donovan, N.; Crown, J.; Rai, D. K., Kenny, P. T. M.; The synthesis, structural characterization and *in vitro* anticancer activity of novel 1-alkyl-10-*N*-meta-(ferrocenyl) benzoyl dipeptide esters and novel 1-alkyl-10-*N*-ortho-(ferrocenyl) benzoyl dipeptide esters, *J. Organomet. Chem.* **2014**, 766, 1-12. b) Harry, A.G.; Butler, W.E.; Manton, J.C.; Pryce, M.T.; O'Donovan, N.; Crown, J.; Rai, D. K.; Kenny, P.T.M.; The synthesis, structural characterization and *in vitro* anti-cancer activity of novel 1-alkyl-10-*N*-*para*-(ferrocenyl) benzoyl dipeptide esters, *J. Organomet. Chem.* **2014**, 757, 28-35. c) Harry, A.G.; Murphy, J.; Butler, W.E; Tiedt, R.; Mooney, A.; Manton, J. C.; Pryce, M.T.; O'Donovan N.; Walsh, N.; Crown, J.; Rai, D.K; Kenny, P.T.M.; *J. Organomet. Chem.* **2013**, 734, 86-92.

[45] Govender, P.; Lemmerhirt, H.; Hutton, A. T.; Therrien, B.; Bednarski, P. J.; Smith, G. S.; First- and Second-Generation Heterometallic Dendrimers Containing Ferrocenyl−Ruthenium (II)−Arene Motifs: Synthesis, Structure, Electrochemistry, and Preliminary Cell Proliferation Studies, *Organometallics* **2014**, 33, 5535-5545.

[46] Jamshidia, M.; Yousefi, R.; Nabavizadeh, S. M.; Rashidi, M.; Haghighi, M. G.; Niazic, A.; Moosavi-Movahedi, A. A.; Anticancer activity and DNA-binding properties of novel cationicPt(II) complexes, *Int. J. Biol. Macromol.* **2014**, 66, 86-96.

[47] Banfic, J.; Legin, A. A.; Jakupec, M. A.; Galanski, M.; Keppler, B. K.; Platinum(IV) Complexes Featuring One or Two Axial Ferrocene Bearing Ligands-Synthesis, Characterization, and Cytotoxicity, *Eur. J. Inorg. Chem.* **2014**, 3, 484-492.

[48] Mishra, A.; Lee, S. C.; Kaushik, N.; Cook, T. R.; Choi, E. H.; Kaushik, N. K.; Stang, P. J.; Chi, K.-W.; Self-Assembled Supramolecular Hetero-Bimetallacycles for Anticancer Potency by Intracellular Release, *Chem. Eur. J.* **2014**, 20, 14410-14420.

[49] Deepthi S. B.; Trivedi, R.; Giribabu L.; Sujitha, P.; Kumar, C. G.; Palladium(II) carbohydrate complexes of alkyl, aryl and ferrocenyl esters and their cytotoxic activities, *Inorganica Chimica Acta* **2014**, 416, 164-170.

[50] Deepthi, S. B.; Trivedi, R.; Giribabu, L.; Sujitha, P.; Kumar, C. G.; Sridharc, B; (4-Ferrocenylphenyl)propargyl ether derived carbohydrate triazoles: influence of a hydrophobic linker on the electrochemical and cytotoxic properties, *New J .Chem.* **2014**, 38, 22-27.

[51] Spoerlein-Guettler, C.; Mahal, K.;Schobert, R.; Biersack, B.; Ferrocene and (arene)ruthenium(II) complexes of the natural anticancer naphthoquinone plumbagin with enhanced efficacy against resistant cancer cells and a genuine mode of action, *J. Inorg. Biochem.* **2014**, 138, 64-72.

[52] Reiter, C.; Karagöz, A. C.; Fröhlich, T.; Klein, V.; Zeino, M.; Viertel, K.; Held, J.; Mordmüller, B.; Öztürk, E. S.; Anıl, H.; Efferth, T.; Tsogoeva, S. B.; Synthesis and study of cytotoxic activity of 1,2,4-trioxane- and egonol-derived hybrid molecules against Plasmodium falciparum and multidrug-resistant human leukemia cells, *Eur. J. Med. Chem.* **2014**, 75, 403-412.

[53] Kowalski, K.; Szczupak, L.; Oehninger, L.; Ott, I.; Hikisz, P.; Koceva-Chyła, A.; Therrien, B.; Ferrocenyl derivatives of pterocarpene and coumestan: Synthesis, structure and anticancer activity studies, *J. Organomet. Chem.* **2014**, 772-773, 49-59.

[54] a) Lu, C.; Heldt, J.-M.; Guille-Collignon,M.; Lematre, F.; Jaouen, G.; Vessires, A.; Amatore, C.; Quantitative Analyses of ROS and RNS Production in Breast Cancer Cell Lines Incubated with Ferrocifens, *ChemMedChem* **2014**, 9, 1286-1293. b) Messina, P.; Labbe, E.; Buriez, O.; Hillard, E. A.; Vessires, A.; Hamels, D.; Top, S.; Jaouen, G.; Frapart Y. M.; Mansuy, D.; Amatore, C.; *Chem. Eur. J.* **2012**, 18, 6581-6587. c) Tan, Y. L. K.; Pigeon, P.; Top, S.; Labbe, E.; Buriez, O.; Hillard, E. A.; Vessires, A.; Amatore, C.; Leong, W. K.; Jaouen, G.; *Dalton Trans.* **2012**, 41, 7537-549.

[55] Wetzel, C.; Kunz, P.; Kassack, M.; Hamacher, A.; Böhler, P.; Watjen, W.; Ott, I.; Rubbiani, R.; Spingler, B.; Gold(I) complexes of water-soluble diphos-type ligands: synthesis, anticancer activity, apoptosis and thioredoxin reductase inhibition, *Dalton Trans*. **2011**, 40, 9212-9220.

[56] Köster, S.; Alborzinia, H.; Can, S.; Kitanovic, I.; Wölfl, S.; Rubbiani, R.; Ott, I.; Riesterer, P.; Prokop, A.; Merza, K.; Metzler-Nolte, N.; A spontaneous gold(I)- azide alkyne cycloaddition reaction yields gold-peptide bioconjugates whichovercome cisplatin resistance in a p53-mutant cancer cell line, *Chem. Sci*. **2012**, 3, 2062-2072.

[57] Geldmacher, Y.; Splith, K.; Kitanovic, I.; Alborzinia, H.; Can, S.; Rubbiani, R.; Nazif, M.; Wefelmeier, P.; Prokop, A.; Ott, I.; Wölfl, S.; Neundorf, I.; Sheldrick, W.; Cellular impact and selectivity of half-sandwich organorhodium(III) anticancer complexes and their organoiridium(III) and richloridorhodium(III) counterparts, *J. Biol. Inorg. Chem*. **2012**, 17, 631-646.

[58] Pelletier, F.; Comte, V.; Massard, A.; Wenzel, M.; Toulot, S.; Richard, P.; Picquet, M.; Gendre, P.; Zava, O.; Edafe, F.; Casini, A.; Dyson, P.; Development of bimetallic titanocene-ruthenium-arene complexes as anticancer agents: relationships between structural and biological properties, *J. Med. Chem*. **2010**, 53, 6923-6933.

[59] Hanif, M.; Nazarov, A.; Legin, A.; Groessl, M.; Arion, V.; Jakupec, M.; Tsybin, Y.; Dyson, P.; Kepplera, B.; Hartinger, C.; Maleimide-functionalised organoruthenium anticancer agents and their binding to thiol-containing biomolecules, *Chem. Commun*. **2012**, 48, 1475-1477.

[60] Amouri, H.; Moussa, J.; Renfrew, A.; Dyson, P.; Rager, M.; Chamoreau, L.-M.; Discovery, structure, and anticancer activity of an iridium complex of diselenobenzoquinone, *Angew. Chem. Int. Ed*. **2010**, 49, 7530-7533.

[61] Pérez-Quintanilla, D.; Gómez-Ruiz, S.; Žižak, Ž, Sierra, I.; Prashar, S.; I. del Hierro, Fajardo, M.; Juranić, Z.; Kaluđerović, G.; A new generation of anticancerdrugs: mesoporous materials modified with titanocene complexes, *Chem. Eur.J*. **2009**, 15, 5588-5597.

[62] Tzubery, A.; Tshuva, E.; Cytotoxicity and hydrolysis of trans-Ti(IV) complexes of salen ligands: structure-activity relationship studies, *Inorg. Chem*. **2012**, 51, 1796-1804.

[63] Van Rijt, S.; Kostrhunova, H.; Brabec, V.; Sadler, P.; Functionalization of osmium arene anticancer complexes with (poly)arginine: effect on cellular uptake, internalization, and cytotoxicity, *Bioconjug. Chem*. **2011**, 22, 218-226.

[64] Maguene, M. G.; Lekana-Douki, J.; Mouray, E.; Bousquet, T.; Grellier, P.; Pellegrini, S.; Ndouo, F.; Lebibi, J.; Pélinski, L.; Synthesis and *in vitro* antiplasmodial activity of ferrocenyl aminoquinoline derivatives, *Eur. J. Med. Chem.* **2015**, 90, 519-525.

[65] Hay, S.; Guerra, C.; Tatern, A.; Noor, A.; Snow, R.; The global distribution and population at risk of malaria: past, present, and future, *Lancet Infect. Dis.* **2004**, 4, 327-336.

[66] Dubar, F.; Khalife, J; Brocard, J. Dive, D.; Biot, C.; Ferroquine, an Ingenious Antimalarial Drug -Thoughts on the Mechanism of Action, *Molecules* **2008**, 13, 2900-2907.

[67] Quirante, J.; Dubar, F.; González, A.; Lopez, C.; Cascante, M.; Cortés, R.; Forfar, I.; Pradines, B.; Biot, C.; Ferrocene-indole hybrids for cancer and malaria therapy, *J. Organomet. Chem.* **2011**, 696, 1011-1017.

[68] Fraisse, L.; Struxiano, A.; Use of ferroquine in the treatment or prevention of malaria, *US 2012/0270851 A1*, **2012**.

[69] Patti, A.; Pedotti, S.; Grassi, T.; Idolo, A.; Guido, M.; Donno, A.; Synthesis of 2-ferrocenylquinoline derivatives and evaluation of their antimalarial activity, *J. Organomet. Chem.* **2012**, 716, 216-221.

[70] Pereira, C.; Venzke, D.; Síntese de heterociclos bioativos derivados do ferroceno, *Quim. Nova* **2013**, 36, 143-152.

[71] Fouda, M.; Abd-Elzaher, M.; Abdelsamaia, R.; Labib, A.; On the medicinal chemistry of ferrocene, *Appl. Organometal. Chem.* **2007**, 21, 613-625.

[72] Kerman, K.; Kraatz, H.; Electrochemical probing of HIV enzymes using ferrocene-conjugated peptideson surfaces, *Analyst* **2009**, 134, 2400-2404.