APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. 2007; **21**: 613–625
Published online 11 April 2007 in Wiley InterScience
(www.interscience.wiley.com) DOI:10.1002/aoc.1202

Review

On the medicinal chemistry of ferrocene

Mohammad F. R. Fouda, Mokhles M. Abd-Elzaher*, Rafeek A. Abdelsamaia and Ammar A. Labib

Inorganic Chemistry Department, National Research Centre, PO 12622 Dokki, Cairo, Egypt

Received 1 November 2006; Revised 6 December 2006; Accepted 26 December 2006

Organometallic chemistry and biochemistry have been merged in the last two decades into a new field: bioorganometallic chemistry. This new research area was devoted to the synthesis of new organometallic compounds and their biological and medical effects against some types of diseases, such as cancer and malaria. For several years, the use of ferrocene in bioorganometallic chemistry has been growing rapidly, and several promising applications have been developed since ferrocene is a stable, nontoxic compound and has good redox properties. This review will focus on ferrocenyl compounds which have been biologically evaluated against certain diseases. This area has attracted many researchers due to the promising results of some ferrocene compounds in the medicinal applications. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: bioorganometallic; ferrocene; anticancer; antimalarial; HIV; DNA detection

INTRODUCTION

Different platinum derivatives {e.g. cisplatin [*cis*-diamminedichloroplatinum(II)], carboplatin [*cis*-diammine-1,1-cyclobutanedicarboxylatoplatinum(II)] and others} are well-established clinically used anticancer drugs.[1,2] Cisplatin is the most prominent member of this class.[3] This drug is known to be 70–80% effective in cases of testicular cancer and is also used in the treatment of ovarian cancer, although it has a high general toxicity and narrow spectrum of activity. Current research in the medical field is aimed at the design of new compounds which are active against a wider range of cancers, and have lesser side-effects. Metallocenes are also known to exhibit a wide range of biological activity.[4,5] Among them, ferrocene has attracted special attention since it is a neutral, chemically stable and nontoxic molecule.[6] It can be easily derivatized and functionalized or oxidized to ferricenium salts. Many ferrocenyl compounds display interesting cytotoxic,[7–9] antitumor,[10,11] antimalarial,[12] antifungal[13] and DNA-cleaving activity.[14] Several reviews have been directed to the chemistry of ferrocene: Dyson *et al*. focused their review on the properties of organometallic compounds that make them suitable for pharmaceutical applications;[15] Neuse devoted his review to the macromolecules containing ferrocene in the cancer research;[16] and Metzler-Nolte *et al*.[17] and also Fish *et al*.[18] directed their reviews to the bioorganometallic chemistry of ferrocene. Therefore, medicinal application of ferrocene is an active research area and many reports have shown that ferrocene derivatives have a highly promising activity *in vitro* and *in vivo* against several diseases. This review focuses on the promising results of the ferrocene derivatives including their effect against certain diseases in the last decade. Comparison of ferrocene and ruthenocene was carried out due to the simliraty between them with respect to structure and redox properties.

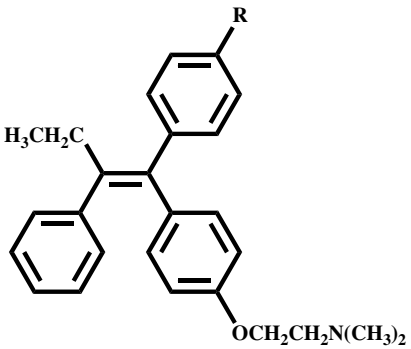
*Correspondence to: Mokhles M. Abd-Elzaher, Inorganic Chemistry Department, National Research Centre, PO 12622 Dokki, Cairo, Egypt.
E-mail: mokhlesm20@yahoo.com

FERROCENYL DERIVATIVES IN CANCER RESEARCH

Cancer is a class of diseases characterized by uncontrolled cell proliferation and the ability of these cells to invade other tissues. Cancer can be treated by several methods including chemotherapy, which is one of the main weapons in the fight against cancer. Chemotherapy is the treatment of cancer with drugs (anticancer drugs) that destroy cancer cells. In the last decade, a revolution in the cancer treatment has been enacted by organometallic chemists.[19,20] Many ferrocenyl derivatives show good results as antitumor agents, and some of them are now in clinical trials.[21]

Breast cancer

Breast cancer is the most common cancer among women; it affects about 1 in 8 women in the West.[22] In general, breast tumors can be divided into two groups that are distinguished by the presence [ER(+)] or absence [ER(−)] of the estrogen receptor. About two-thirds of all cases belong to the ER(+) type, which is susceptible to hormone therapy by selective estrogen receptor modulators (SERMs). In the ER(+) cell lines, there are two receptor subtypes, ERα and ERβ.[23] The primary drug used to treat this disease is tamoxifen **1** and hydroxytamoxifen **2**. Tamoxifen acts *in vivo* as a particularly well tolerated cytostatic agent. It should be noted that the molecule exists in both *Z* and *E* configurations, of which the *Z* isomer is the most strongly antiestrogenic. The antiproliferative action of tamoxifen arises from the competitive binding to ERα, thus repressing estradiol-mediated DNA transcription in the tumor tissue.[24] Tamoxifen has some undesirable side effects as resistance to the drug can develop during long-term therapy, and it increases the risk of blood clotting in the lungs; tamoxifen is also not effective against hormone-independent tumors.[25]

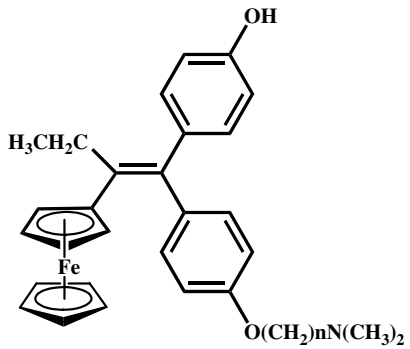


R = H, **1**; R = OH, **2**

Ferrocifen

Jaouen *et al*. prepared several ferrocenyl derivatives based on the structure of tamoxifen **1** and hydroxytamoxifen **2**. The series of ferrocifens was biologically examined *in vitro* and *in vivo*, and the results were surprising.[26,27] The effects of several hydroxy-substituted ferrocifens have been studied on the proliferation of two lines of breast cancer cells, one used for tumors mediated by the ERα receptor, and one used for tumors mediated by ERβ. The antiproliferative effect of ferrocifen on breast cancer cells has been measured using the MCF7 cell line, standard for studies of ERα tumors, and the MDA-MB231 cell line, the standard for ERα and ERβ breast cancer lines. Jaouen *et al*.[28] showed that with the MCF7 cell line all the ferrocifens studied have overall antiproliferative effects. Three of the ferrocifens **3** exhibited a strong antiproliferative effect in both cell lines: for $n = 2$, and especially for $n = 8$, the antiproliferative effects are weaker than those of hydroxytamoxifen; while for $n = 3–5$, the results are comparable to those of hydroxytamoxifen or even slightly better. Ferrocene by itself had no effect. The results showed that ferrocifens are the first molecules shown to be active against both hormone-dependent and hormone-independent breast cancer cells.[29]

Attaching the ferrocenyl moiety to the skeleton of tamoxifen could give several advantages, like ideally increasing the cytotoxicity of tamoxifen and hydroxytamoxifen.[30] Also, ferrocene has been reported to have antitumor activity due to metabolic formation of ferrocenium ions.[31,33]



n = 2–8

3

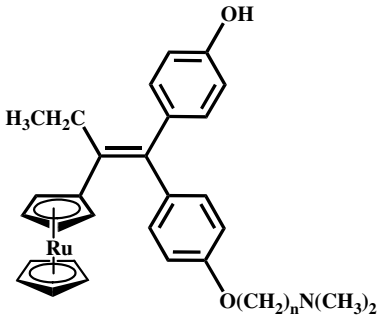
Ruthenocifen

Ruthenocene and some of its derivatives show good antitumor activity.[34–38] This may be due to the similarity between both ferrocene and ruthenocene in terms of structure and also in redox properties. Based on the structure of tamoxifen, Jaouen *et al*.[39] have prepared a Ru-analog of ferrocifen. They synthesized a series of ruthenocene derivatives, 1-{4-[O(CH$_2$)*n*N(CH$_3$)$_2$]phenyl}-1-(4-hydroxyphenyl)-2-ruthenocenylbut-1-ene, with $n = 2–5$, in high yield and tested their effectiveness toward ER(+) and ER(−) breast cancer cell lines. The results showed that the ruthenocifen derivatives **4** act as anti-estrogens toward the ER(+) MCF7 breast cancer cell line and have no cytotoxic effect on the ER(−) MDA-MB231 breast cancer cell line. This result is surprising in that it contrasts with the ferrocene derivatives, which show a cytotoxic effect in the ER(−) breast cancer cell line. The difference in the activity between ferrocene and ruthenocene derivatives may be due to the different redox properties for the two metallocenes.[40] Electrochemical studies show also that the ruthenium radical cation quickly decomposes after electron transfer, which is not the case for the stable ferrocenium radical.

Activity of polyphenolic ferrocenyl derivatives as anticancer

Polyphenolic compounds such as stilbenes, flavonoids, proanthocyanidines and their derivatives are one of the most studied classes of phytochemicals due to their antioxidant potency against free radicals, which have been associated with diseases related to aging (certain cancers, cardiac, ocular and

DOI: 10.1002/aoc



n = 2–5

4

degenerative problems, etc).[41–43] They are found throughout the vegetable world (for example, in grapes, green tea and cocoa).[44]

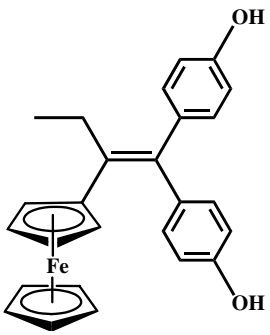
Jaouen and coworkers prepared several derivatives of polyphenolic compounds containing ferrocene moiety and evaluated them as anticancer agents using the standard breast cancer cell lines.[45,46] The results showed that the diphenolic compound 1,1-bis(4′-hydroxyphenyl)-2-ferrocenyl-but-1-ene **5** has good antiproliferative effects on both hormone-dependent (MCF7) and -independent (MDA-MB231) breast cancer cells. Surprisingly, **6** [1,2-bis(4′-hydroxyphenyl)-2-ferrocenyl-but-1-ene], the regioisomer of **5**, shows only a modest effect on these cell lines. This antiproliferative effect of **5** was even stronger than that observed for 4-hydroxytamoxifen. The high effect of **5** may lead to the generation of a potent cytotoxic compound. The strong antiproliferative effect of **5** is owing to the presence of a ferrocene moiety and its position plays an important role in increasing the anticancer activity. It should be noted that the increased activity of **5** cannot be solely attributed to higher receptor affinity, as the values for **5** and **6** are very similar for ERα, while the relative binding affinity (RBA) is actually higher for **6** than **5** for ERα. There are two notable structural differences between **5** and **6**. First, one of the two phenol groups is necessarily always oriented *trans* to the ferrocene group in **5**, while there is a *cis* relationship between the ferrocene and phenol in **6**. Second, the two phenol groups share the same carbon atom in **5**, while in **6**, one phenol group resides on each of the alkene carbon atoms.

Also, Jaouen *et al*. have prepared a series of simple unconjugated ferrocenyl diphenol complexes (*ortho, para; meta, para; para, para* **7, 8, 9**). These compounds retain a reasonable to good affinity for both estrogen receptor types, with higher values for the **8** form, and superior binding for the *para, para* diphenol complex. *In vitro* these complexes exhibit significant cytotoxic effects on hormone-independent prostate (PC3) and breast cancer cell lines (MDA-MB231). This effect is more marked with PC3, the *ortho, para* diphenol complex proving the most effective. Electrochemical studies show that the cytotoxic effect of the complexes correlates with the ease of oxidation of the ferrocene group. All these complexes are much less cytotoxic than the ferrocenyl diphenol butene derivative **5**.

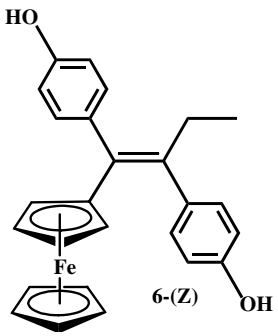
Jaouen *et al*.[47] also reacted ferrocene with methoxy-substituted benzyl and benzhydryl alcohols in the presence of trifluoroacetic acid, to afford methoxybenzyl or benzhydryl-ferrocenes. Demethylation of these compound leads to the ferrocenyl phenols and bisphenols. The results showed that the bisphenol derivatives of ferrocene **10** exhibit a high affinity for the two forms of estrogen receptor ERα and ERβ.

Water-soluble ferrocenyl derivatives as anticancer agents

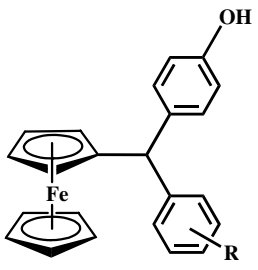
Preparation of water-soluble ferrocenyl derivatives that have activity as anticancer agents attracted much attention.[48–50] Some results showed that these compounds are effective as anticancer agents, and in some cases they were more potent than the water-insoluble molecules.[16,51] This effect



5



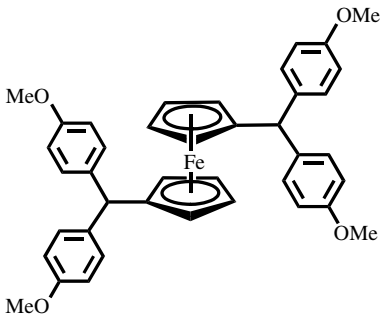
6



R = *o*-OH, **7;** R = *m*-OH, **8**;

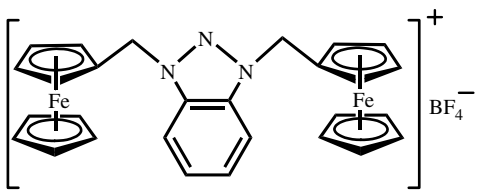
R = *p*-OH, **9**

DOI: 10.1002/aoc

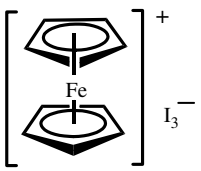


10

may arise from the solubility difference between the soluble and insoluble compounds. In this context, it is important to mention that Koepf-Maier is one of the pioneers in this field. He synthesized several biologically active ferrocenyl compounds in the 1980s.[52–54] These compounds were covered by Dyson in his review.[15] Some examples of the most important ferrocenyl compounds that have good activity as anticancer agents are ferrocenium tetraflouroborate salt **11**[55] and ferricenium tri-iodide **12**.[56]



11



12

Ferrocene conjugates in colon cancer

Neuse *et al*.[57–64] focused their research on the synthesis of ferrocene conjugates in which the bioactive (ferrocene) unit is covalently bound (anchored) to polyaspartamide (water-soluble carrier polymers). The polymer was designed in accordance with requisite biomedical specifications. The anchoring link in most of these conjugates has been an aliphatic spacer containing the biofissionable amide or ester group (Fig. 1). The carrier is equipped with variously spaced amide or hydroxyl side groups, to which the ferrocenylation agent, 4-ferrocenylbutanoic acid, is connected through amidation[57] or esterification.[58]

For example; Neuse *et al*.[62] prepared several ferrocenyl conjugates **13–21**, which are derived from carriers **13-C** to **21-C** based on a linear polyaspartamide structure (Fig. 2).

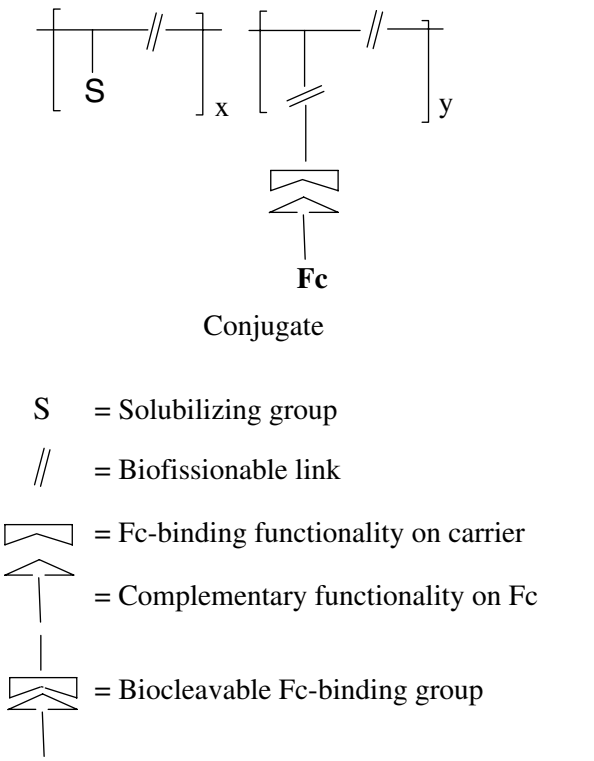


Figure 1. General representation of ferrocenyl conjugates structure.

Compound **22** is derived from a polyamide carrier **22-C**, which is obtained by a Michael-type addition polymerization of methylenebisacrylamide with ethylenediamine and 4,7,10-trioxa-1,13-tridecanediamine as co-monomers (Fig. 3). Compounds **13–18** contain *tert*-amine side chain functionality for achieving water solubility whereas **19–21** are characterized by hydroxyl side groups as solubilizers, and the solubility of **22** is provided by intrachain-type oligo(ethylene oxide) segments.

Conjugates **13–22** were evaluated in cell culture tests for antiproliferative activity against the Colo line and, for comparison, also against HeLa line. The values for IC50 represent the mean polymer concentration required to achieve a 50% cell growth inhibition. The results revealed an excellent performance for most of the conjugates against both cell lines. Conjugates **13–18**, as well as **22**, are the most active, giving IC50 values in the range of 0.2–2 mg Fe/ml.

Anticancer agents and topoisomerase

Topoisomerases, which are further classified as types I and II, are enzymes responsible for maintaining the topology of DNA. Inhibition or poisoning of human topoisomerases has been implicated in the mechanism of activity of several antitumor drugs[65,66] and, since tumors utilize an increase in topoisomerase activity, the inhibition of topoisomerases becomes an important target in achieving antitumor activity.[67–69] This area of research

DOI: 10.1002/aoc

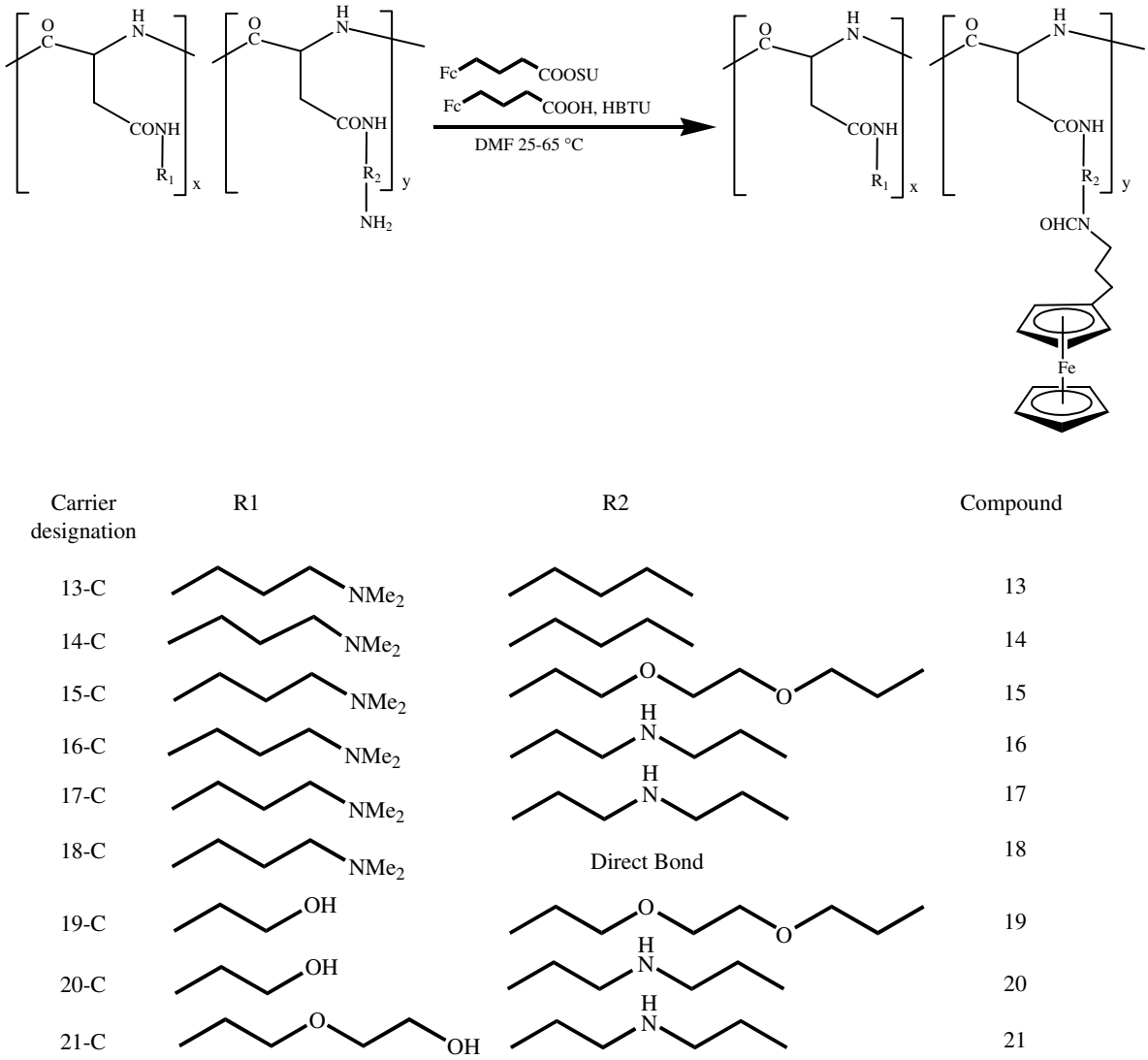


Figure 2. Synthesis of ferrocenyl polyaspartamide conjugates.

has attracted Kondapi and coworkers,[70,71] who prepared several ferrocenyl derivatives. They found that compound **23** has antiproliferative activity against several human cancer cell lines, notably the Colo 205 colon adenocarcinoma; in addition, they found that azalactone (IC50 = 100 nM; **24**) and thiomorpholideamidomethylferrocene; IC50 = 50 nM; **25**) have higher activity than other compounds prepared by them.

Enzyme complexation with **23**, as proposed by the authors, could involve the hetero atoms in the carboxaldoxime substituent, leading to nitrogen and oxygen donor interaction with the enzyme. The authors suggest that azalactone ferrocene inhibits DNA passage activity of enzyme leading to the formation of cleavable complex (DNA + topo II + ferrocenyl derivative), while thiomorpholideamidomethyl ferrocene competes with ATP binding resulting in the inhibition of catalytic activity of the enzyme; i.e. thiomorpholideamidomethyl ferrocene and azalactone ferrocene show distinctly different mechanisms in inhibition of catalytic activity of topoisomerase II. The role of the ferrocene moiety is not explained by the authors.

Miscellaneous ferrocenyl derivatives as anticancer

Rajput *et al.*[1] prepared a series of ferrocenyl nitrogen donor ligands including ferrocenylpyridines, ferrocenylphenylpyridines and 1,10-di(2-pyridyl)ferrocene. Coordination studies of the substituted pyridines were carried out with platinum, palladium, rhodium and iridium. They prepared the following types of complexes: $[MCl(CO)_2(L)]$ and $[M(cod)(L)_2]ClO_4$, where M = Rh or Ir, cod = 1,5-cyclooctadiene; $[M'Cl_2(L)_2]$ where M′ = Pt or Pd. Several of the complexes displayed significant cytotoxic activity against the cancer cell line WHOCO1, especially complexes **26** and **27**.[1]

DOI: 10.1002/aoc

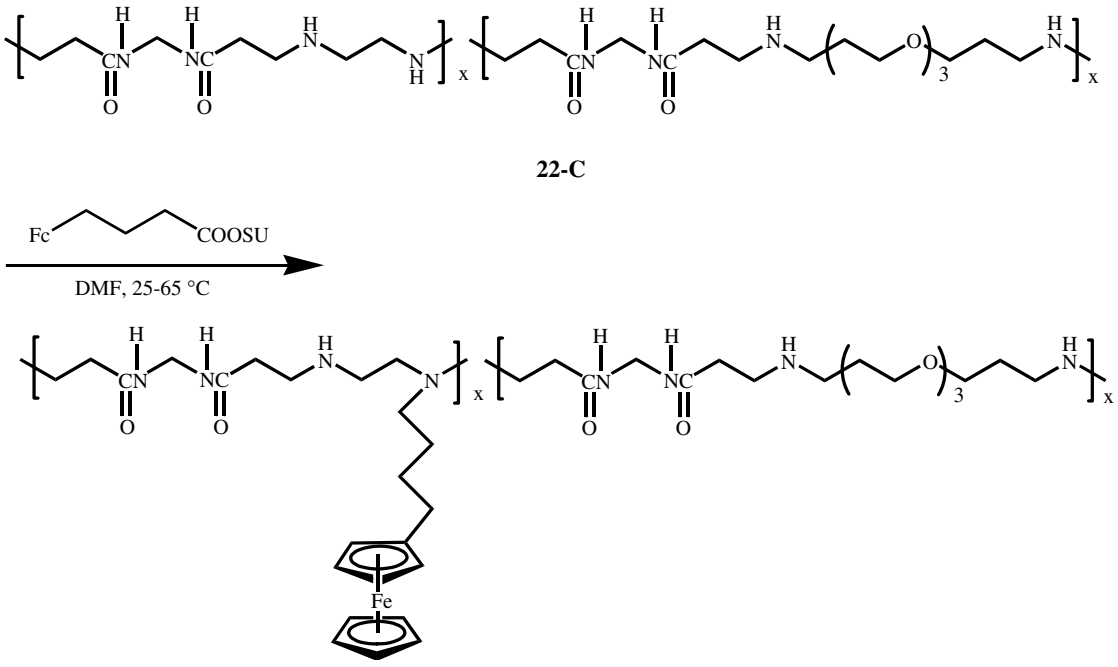
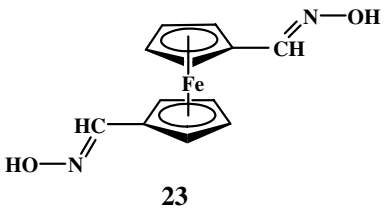
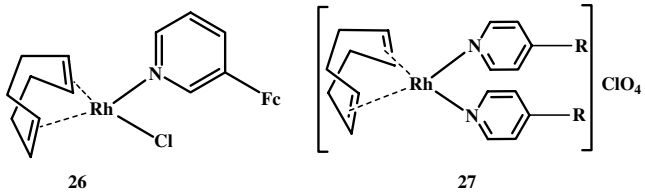
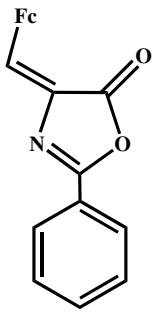


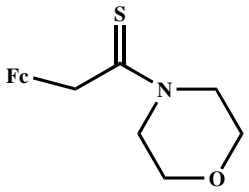
Figure 3. Synthesis of ferrocenyl polyaspartamide using Michael addition polymerization.





R = (C=N)(C_6H_4)Fc





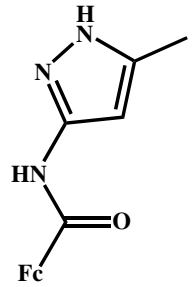
Fc = Ferrocenyl moiety

Another series of ferrocenyl derivatives were prepared and evaluated as anticancer agents by Kraatz,[72] who synthesized ferrocenyl pyrazole ligand (3-Fc-AMP) **28**. This ligand readily coordinates to a variety of transition metal ions. Kraatz described the structural characterization of iron and cobalt complexes of Fc-AMP, and the cytotoxicity profiles of the prepared compounds comparing his results with the carboplatin *in vitro*.

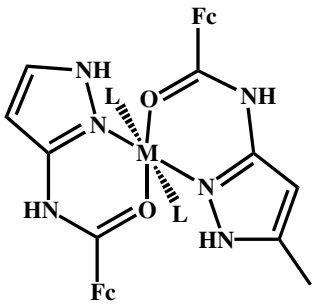
Kraatz and coworkers evaluated the bioactivity of the free ligand **28**, and the metal complexes **29, 30** and **31**. They reported the induced dose-dependent cytotoxicity in human mammary adenocarcinoma MCF-7 cells and the results showed that MCF-7 cells were sensitive to all four compounds (**28, 29, 30** and **31**) at varying concentrations after treatment for six days. The Co-complex **30** showed the highest toxicity. It was also noticed that the observed cytotoxicity of the complexes appeared to follow the inverse order to the E1/2, and as the redox potential increased, the toxicity decreased. The activity followed the order Co > Ni > Fe, while the E1/2 followed the order of Co < Ni < Fe. Thus, the redox potential plays an important role in the compounds activities.

Some new glycosides of 3-ferrocenyl-1-(4′-hydroxyphenyl)-prop-2-en-1-one were prepared and transformed into the corresponding pyrazoline and pyrazole derivatives.[73] The *in vitro* antitumor activity of the substances was investigated against human leukemia (HL-60) cells. Among these new compounds some chalcone derivatives compounds (**32**–**35**) showed good antitumor effects.

DOI: 10.1002/aoc

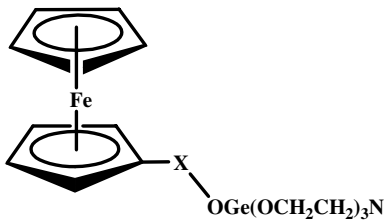


28



M = Ni, **29**; M = Co, **30**; M = Fe, **31**
L = MeOH

Chen *et al*.[74] prepared some ferrocenyl compounds, **36–38**, and the antitumor activities of compounds **36** and **38** were determined *in vitro* against KB cells and Bel-7402 cells. The data indicate that compounds **36** and **38** possess potential antitumor activity against KB cells.



X = -CH_2-, **36;** X = -$CH(CH_3)$-, **37;**
X = *p*-C_6H_4-CH_2-, **38**

Mechanism of ferrocenes in cancer treatment

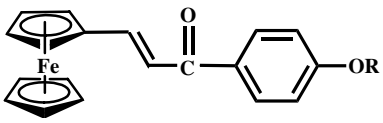
The mechanism of action of the ferrocene derivatives in the treatment of cancer has been studied by several authors.[10,16,21,31,33,52,53] The results show that the activity of ferrocenyl compound is depend on the oxidation state of iron in the ferrocene moiety. Some results confirmed that the Fe(II) ferrocenyl compound is more active than Fe(III) ones. The mechanism of ferrocifen as one of the Fe(II) compounds has been studied and the results[31] indicate that the ferrocifens act by changing the conformation of the receptor protein. In addition, when ferrocifen binds to ERβ, an 'oxidant/antioxidant' mechanism may occur. The ferrocifen–ERβ complex is thought to dimerize and attach itself to a particular region of DNA, and Fe^{2+} complexes are known to be oxidized to Fe^{3+} by O_2, leading to the generation of highly reactive $^{\bullet}OH$ radicals.[21] These radicals[33] could damage the DNA strand close to the binding site, thus explaining the observed antiproliferative effect in connection with the ERβ receptor. DNA damage produced by exposure to ferrocifens was observed.[29,30]

Another view of the mechanism was given by Osella *et al*.,[32] who suggested that the reduction of ferrocenium ions *in vivo* generates active oxygen radicals such as hydroxyl responsible for its anticancer activity through the formation of radical metabolites that are responsible for biological damage in the cancer cell.

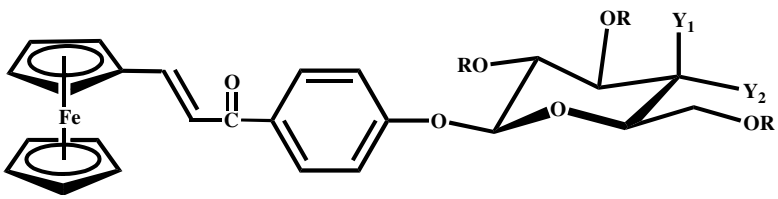
In other words, the good redox properties of ferrocene and formation of OH radicals are the key for the high activity ferrocene compounds as anticancer agent in the two mechanisms.

EFFECTIVENESS OF FERROCENE DERIVATIVES AS ANTIMALARIAL AGENTS

Malaria is one of the most problematic parasitic infections in the world. It ranks among the major developmental



R = H, **32;** R = AC, **33**



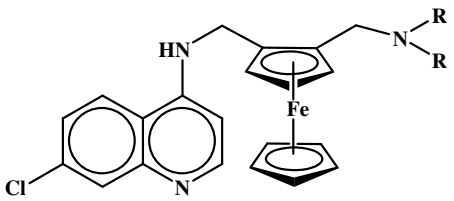
Y_1 = H, Y_2 = OR, **34;** Y_1 = OR, Y_2 = H, **35**
R = Ac

DOI: 10.1002/aoc

challenges facing large parts of the world, including some of the poorest countries. It is currently the first priority tropical disease of the World Health Organization (WHO). According to recent estimates, malaria affects more than 2400 million people, and approximately 40% of the world's population, in more than 100 countries are at risk.

There are four parasite species that cause human malaria, *Plasmodium falciparum*, *P. vivax*, *P. ovale* and *P. malariae*, and they are distributed globally, especially in Africa.

Chloroquine (CQ), mefloquine and quinine are the most effective drugs against malaria.[75–77] The most dangerous parasite, *Plasmodium falciparum*, is however becoming resistant to these drugs. Therefore, synthesis of newly ferrocenyl antimalarial agents has attracted many authors.[78–87] Ferroquine (FQ) derivatives **39, 40, 41** and **42** are novel antimalarial compounds currently in phase I clinical trials.[88] FQ is a unique ferrocenyl compound designed to overcome the CQ resistance.[87,89] Indeed, FQ was more potent than CQ in the inhibition of growth of *P. falciparum in vitro* and on *P. berghei in vivo*.[81,82,90] Also, FQ showed 22-fold higher activity than chloroquine *in vivo* in mice infected with *P. berghei* and *P. yoelii NS*, and no significant evidence of toxicity of ferrocenes was detected.[83] Synthesis of FQ was based on incorporation of ferrocenyl moiety with chloroquine to give FQ.

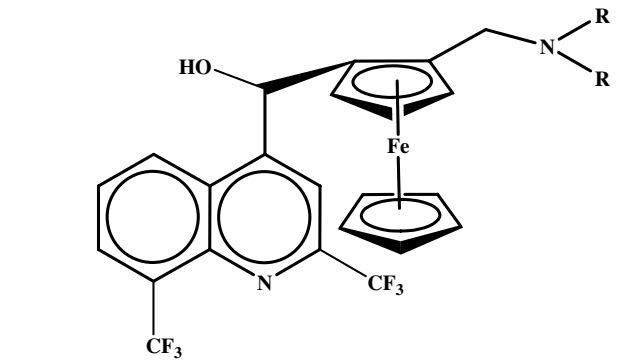


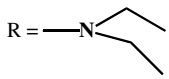
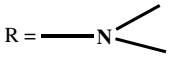
R = H, **39;** R = Me**, 40;** R = Et, **41;** R = t-Bu**, 42**

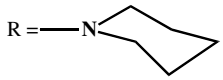
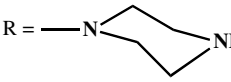
Biot *et al*.[84] prepared a series of ferrocenyl mefloquine and quinine analogs, which are close structural and stereochemical mimics of the parent drugs. These compounds were tested on *Plasmodium falciparum* strains, sensitive (HB3) or chloroquine, mefloquine-resistant (Dd2), and the results showed that the ferrocenyl compounds **43–48** exhibited a lower antimalarial activity than mefloquine or quinine themselves.

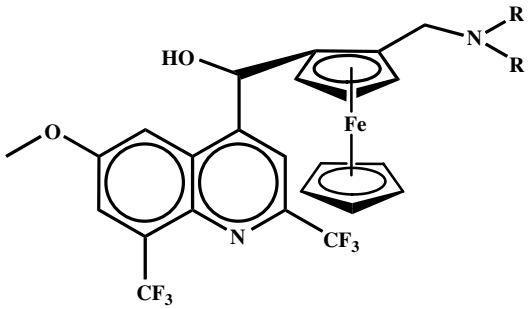
Other ferrocenyl derivatives have been prepared from triazacyclononane and evaluated against chloroquine sensitive (HB3) and chloroquine-resistant (Dd2) *Plasmodium falciparum*.[91] The most effective one was 7-chloro-4-[4-(7-chloro-4-quinolyl)-7-ferrocenylmethyl-1,4,7-triazacyclononan-1-yl]quinoline **49**. It showed potent antimalarial activity *in vitro* against the chloroquine-resistant strain Dd2.[91]

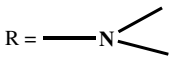
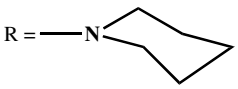
Go *et al*.[92,93] prepared a series of ferrocenyl chalcones and evaluated them *in vitro* against *Plasmodium falciparum*. The most active compound was 1-ferrocenyl-3-(4-nitrophenyl)prop-2-en-1-one, **50**. The results showed that the location of ferrocene and the polarity of the carbonyl linkage influenced the ease of oxidation of Fe^{2+} in ferrocene,

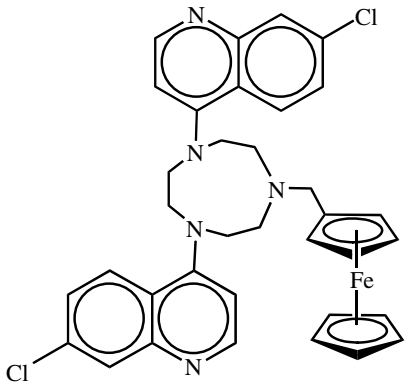


R =  , **43;** R =  , **44;**

R =  , **45;** R =  , **46**



R =  , **47;** R =  , **48**

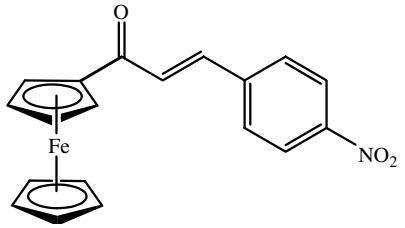


49

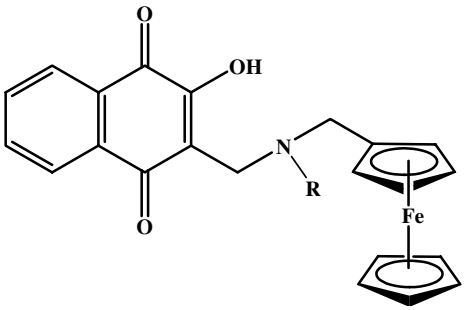
enhancing the antiplasmodial activity. The incorporation of ferrocene in the compound was found to enhance its role in processes that involved the quenching and generation of free radicals. Thus, ferrocene may participate in redox cycling and this process may contribute to the antiplasmodial activity of ferrocenyl chalcones. However, the extent to which this

DOI: 10.1002/aoc

property is manifested is also influenced by other physicochemical properties (lipophilicity, polarity, and planarity) of the compound.



50



R = $(CH_2)_nCH_3$ n = 5-8

51

On the other hand, a recent article by Brocard *et al*.[94] described the preparation of 14 ferrocenyl aminohydroxynaphthoquinones, analogs of atovaquone, from the hydroxynaphthoquinone core. These novel atovaquone derivatives were tested for their *in vitro* activity against two apicomplexan parasites of medical importance, *Toxoplasma gondii* and *Plasmodium falciparum*, including resistant strains to atovaquone (*T. gondii*) and chloroquine (*P. falciparum*). Three of these ferrocenic atovaquone derivatives **51**, composed of the hydroxynaphthoquinone core plus an amino-ferrocenic group and an aliphatic chain with six to eight carbon atoms, were found to be significantly active against *T. gondii*. Moreover, these novel compounds were also effective against the atovaquone-resistant strain of *T. gondii* (A to R).

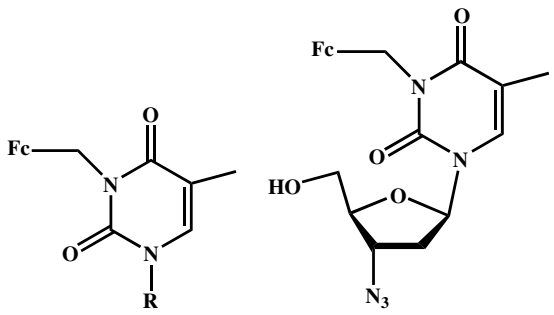
Mechanism of the ferrocenyl antimalarial agents

The mechanism of the action of ferroquine as one of the most active antimalarial agents was studied.[95] It was found that the mechanism of action of FQ is likely to be similar to that of CQ and probably involves hematin as the drug target and inhibition of hemozoin formation. However, both the basicity and lipophilicity of FQ are significantly different from those of CQ. The lipophilicity of FQ and CQ are similar when protonated at the putative food vacuole pH of 5.2 but differ markedly at pH 7.4.

In addition, the pK_a values of FQ are lower than those of CQ. This suggests that there will be somewhat less vacuolar accumulation of FQ compared with CQ. Single crystal structure determination of FQ shows the presence of a strong internal hydrogen bond between the 4-amino group and the terminal N atom. This, together with the electron donating properties of the ferrocene moiety, probably explains the decreased pK_a. Interestingly, the decreased accumulation arising from the less basic behavior of this compound is partly compensated for by its stronger ß-hematin inhibition. Increased lipophilicity, differences in geometric and electronic structure, and changes in the N–N distances in FQ compared with CQ probably explain its activity against CQ-resistant parasites.

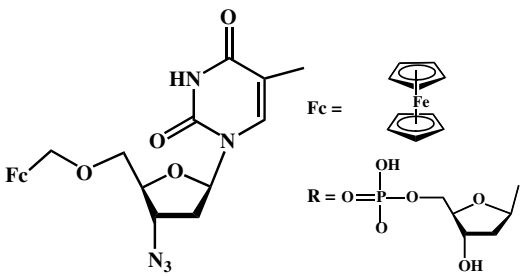
ACTIVITY OF FERROCENYL DERIVATIVES AGAINST HIV

The applications of ferrocene in medical research attracted Champdore *et al*.[96] to prepare some adducts by incorporating the ferrocenemethyl moiety into a heterocyclic base, which



52

53



54

DOI: 10.1002/aoc

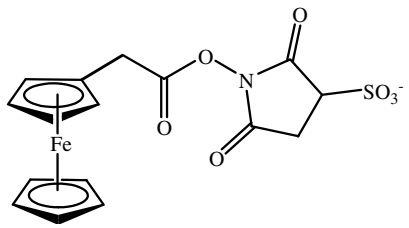
were evaluated against HIV-1, HBV, YFV, BVDV and several bacteria. Only compounds bearing thymine **52**–**54** showed significant cytotoxicity against MT-4 cells. The ferrocenyl-derivatives of 3′-deoxy-3′-aazidothymidine **53** and **54** were the sole compounds active against HIV-1. However, they proved to be 10- to 300-fold less potent than 3′-α-azidothymidine (AZT) used as the reference drug.

On the other hand, recent results showed that topoisomerase (I and II) plays an important role in maintenance of topological changes during DNA replication and recombination. It has also been shown that topoisomerase II activity is required for HIV-1 replication and the enzyme is phosphorylated at early time points of HIV-1 replication. In a recent article, Kondapi *et al*.[97] studied the molecular action of topoisomerase II inhibitors, azalactone ferrocene (AzaFecp), thiomorpholide amido methyl ferrocene (ThioFecp) and ruthenium benzene amino pyridine [Ru(ben)Apy] on cell proliferation and also on various events of HIV-1 replication cycle. The topoisomerase II β over-expressing neuroblastoma cell line shows a higher sensitivity to these compounds compared with the Sup-T1 cell line. All the three topoisomerase II inhibitors show significant anti-HIV activity at nanomolar concentrations against an Indian isolate of HIV-193IN101 in the Sup-T1 cell line. The results showed that the compounds inhibit proviral DNA synthesis as well as the formation of pre-integration complexes completely. Further investigation using polymerase chain reaction and western blot showed that both the topoisomerase isoforms are presented in the pre-integration complexes, suggesting their significant role in HIV-1 replication.

FERROCENYL DERIVATIVES IN DNA DETECTION

One of the recent fields of research in organometallic chemistry is the synthesis and development of DNA detection sensing systems.[98,99] Such systems (chips) enable quick, simple, sensitive and low-cost gene diagnosis by the electrochemical detection method.[100–103] To construct such a system, it is important to develop a reproducible method to immobilize a capture DNA probe on the gold surface, and many kinds of immobilization methods have been reported.[104,105] Electrochemical systems for DNA detection are potentially cheaper and more reliable than the conventional fluorescence spectroscopy. Ferrocene and its derivatives are often used in such devices because of their favorable electrochemical properties.[106,107] Several reviews have been published recently covering this topic.[108,109]

In a recent paper, Liepold *et al*.[110] described a new and simple electrochemical approach for hybridization detection without the need for labeling the target DNA. The EDDA (electrically detected displacement assay) method uses a solution of short redox-labeled signaling oligonucleotides (oligonucleotides carrying a covalently attached redox active compound like ferrocene) **55** to characterize the hybridization state of label-free capture probe DNA immobilized on gold electrodes. The number of capture probes associated with signaling oligonucleotides is determined by chronocoulometry. This technique allows separation of the electrochemical response of capture probe-associated signal probes from the response of freely diffusing signaling probes. In the absence of the complementary target sequences the redox-labeled signaling probes at the surface give rise to an instantaneous increase of the detection signal, while freely diffusing signaling probes show a significantly delayed response. Hybridization with targets complementary to the capture probe displaces the loosely associated signaling probes, thereby decreasing the instantaneous signal. In other words they introduced EDDA technology, a potential candidate for a EC-based cost-effective DNA microarray system technology with low complexity (no target labeling) that can be improved to a system suitable for the detection of SNPs in a bench-top format for point-of-care or near-patient testing.

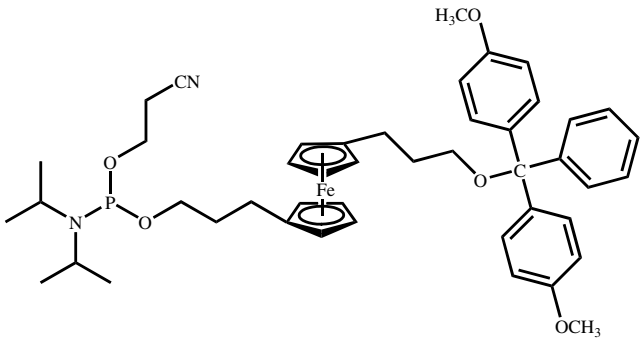


55

Brisset *et al*.[111] reported the synthesis and the characterization of the first electroactive ferrocene-labeled oligonucleotide phosphorothioate 1[3-*O*-dimethoxytritylpropyl]-1-[-3-o(2-cyanoethyl-*N*,*N*-diisopropyl phosphoramidityl) propyl]ferrocene (ODN-Fc-Ps **56**) probe obtained by automated synthesis. The electrochemical response of the modified electrode was analyzed in aqueous media before and after hybridization with the oligodeoxynucleutide (ODN) target. The hybridization with ODN target induces a large conformational change in the surface-confined DNA structure monitored by cyclic voltammetry of the ferrocene marker, which confirms the potential of ferrocene-labeled oligonucleotide phosphorothioate to develop electrochemical DNA chips.

Suye *et al*.[112] proposed an amperometric DNA sensing system based on the combination of sandwich hybridization of a reporter probe, a capture probe and target DNA. *Inv*A gene of *Salmonella typhimurium* was used for target DNA and glucose-6-phosphate dehydrogenase (G6PDH) was used for subsequent enzymatic electrochemical detection as a reporter probe. The DNA sensor was constructed as follows: a gold electrode was modified with mercaptopropionic acid, and then PEI-Fc (ferrocene immobilized polyethylenimine)/alginic acid, diaphorase/PEI, and PEI/streptavidin layers were formed on the surface of electrode by layer-by-layer adsorption. Finally, the capture probe was immobilized

DOI: 10.1002/aoc



56

on the electrode via streptavidin. The hybridization product was immobilized on the DNA sensor surface by the biotin–avidin bond. The detection limit of the present DNA sensor was femtomol order of target DNA. The proposed method could be applicable to the measurement of DNA from various organisms for medical analysis, food analysis, etc. In addition, further studies are necessary to establish the direct hybridization of target DNA and both probes on the electrode. Preparation of a thermostable enzyme-labeled reporter probe for this purpose is in progress.[112]

Peptide nucleic acid (PNA) oligomers are synthetic DNA analogs that have gained considerable attention because of their good properties, such as high stability in biological media and sequence-selective binding to RNA and DNA.[113–116] Many authors reported the synthesis and electrochemical characterization of PNA oligomers/monomer, containing at least one ferrocenyl moiety, and this new area of research is promising and will be of great interest in the near future.[116–118]

CONCLUSIONS

Ferrocene is nontoxic and has a unique structure as well as an excellent redox property, allowing wide applications in medicinal chemistry. Attaching ferrocenyl moiety in a well-established drug increases the biological activity and generally improves its broad spectrum. Ferrocenyl derivatives such as ferrocifens show good results *in vitro* and *in vivo* as anticancer. Ferrocifens have been studied on the proliferation of breast cancer cells and ER_α and $ER\beta$ receptors, and the results are promising. Also, some soluble ferrocenium salts (e.g. ferrocenium tetraflouroborate salt) have been prepared and they have good activity as anticancer compounds. Ferrocenyl complexes, which have antimalarial activity, e.g. ferroquine and ferrocenyl-mefloquine, have been developed. No significant evidence of toxicity of ferroquine and its derivatives has been detected. Also, the redox properties of ferrocene have been exploited to prepare different types of electrochemical sensors such as DNA, proteins, environmental pollutants and food sensors. This area is an interesting area of research and is likely to grow rapidly. The possibilities for using ferrocene compounds are endless and ferrocenyl derivatives will be used in the medical market in the near future.

REFERENCES

1. Rajput JR, Moss AT, Hutton DT, Hendricks CE, Imrie AC. *J. Organomet. Chem.* 2004; **689**: 1553.
2. Guo Z, Sadler PJ. *Angew. Chem. Int. Edn* 1999; **38**: 512.
3. Wheate NJ, Collins JG. *Coord. Chem. Rev.* 2003; **241**: 133.
4. Champdore M, Fabio G, Messere A, Montesarchio D, Piccialli G, Loddo R, Collad M. *Tetrahedon* 2004; **60**: 6555.
5. Harding MM, Mokdsi G. *Curr. Med. Chem.* 2000; **7**: 1289.
6. Dombrowski KE, Baldwin W, Sheats JE. *J. Organomet. Chem.* 1986; **302**: 281.
7. Meunier P, Ouattara I, Gautheron B, Tirouflet J, Camboli D, Besancon J. *Eur. J. Med. Chem.* 1991; **26**: 351.
8. Top S, Tang J, Vessieres A, Carrez D, Provot C, Jaouen G. *Chem. Commun.* 1996; 955.
9. Dori Z, Gershon D, Scharf Y, Fr. Demande FR, 2,598,616 (Cl. A61K31/12), 20 Nov. 1987, US Appl. 862,804, 13 May 1986; p. 13.
10. Koepf-Maier P, Koepf H, Neuse EW. *Angew. Chem., Int.Edn Engl.* 1984; **23**: 456.
11. Koepf-Maier P, Koepf H. *Drugs Future* 1986; **11**: 297.
12. Biot C, Francois N, Maciejewski L, Brocard J, Poulain D. *Bioorg. Med. Chem. Lett.* 2000; **10**: 839.
13. Itoh T, Shirakami S, Ishida N, Yamashita Y, Yoshida T, Kim H-S, Wataya Y. *Bioorg. Med. Chem. Lett.* 2000; **10**: 1657.
14. Baldoli C, Maiorana S, Licandro E, Zinzalla G, Perdicchia D. *Org. Lett.* 2002; **4**: 4341.
15. Allardyce CS, Dorcier A, Scolaro C, Dyson P. *Appl. Organometal. Chem.* 2005; **19**: 1.
16. Neuse EW. *J. Inorg. Organomet. Polym. Mater.* 2005; **15**: 1.
17. van Staveren DR, Metzler-Nolte N. *Chem. Rev.* 2004; **104**: 5931.
18. Fish RH, Jaouen G. *Organometallics* 2003; **22**: 2166.
19. Dyson PJ, Sava G. *Dalton Trans.*, 2006; 1929.
20. Huxham LA, Cheu ELS, Patrick BO, James BR. *Inorg. Chim. Acta* 2003; **352**: 238.
21. Top S, Vessieres A, Cabestaing C, Laios I, Leclercq G, Provot C, Jaouen G. *J. Organometal. Chem.* 2001; **637**: 500.
22. Warner E, Plewes DB, Shumak RS, Catzavelos GC, Di Prospero LS, Yaffe MJ, Goel V, Ramsay E, Chart PL, Cole DEC, Taylor GA, Cutrara M, Samuels TH, Murphy JP, Murphy JM, Narod SA. *J. Clin. Oncol.* 2001; **19**: 3524.
23. Nagel SC, Hagelbarger JL, Mcdonnell DP. *Endocrinology* 2001; **142**: 4721.
24. Huynh H, Yang X, Pollak M. *J. Biol. Chem.* 1996; **271**: 1016.
25. Cuzick J, Powles T, Veronesi U, Forbes J, Edwards R, Ashley S, Boyle P. *Lancet* 2003; **361**: 296.
26. Top S, Tang J, Vessieres A, Carrez D, Provot C, Jaouen G. *Chem. Commun.* 1996; 955.
27. Top S, Dauer B, Vaisserman J, Jaouen G. *J. Organomet. Chem.* 1997; **541**: 355.
28. Jaouen G, Top S, Vessieres A, Leclercq G, Quivy J, Jin L, Croisy A. *C. R. Acad. Sci. Paris Ser. IIc* 2000; **3**: 89.
29. Vessieres A, Top S, Beck W, Hillarda E, Jaouen G. *Dalton Trans.* 2006; 529.
30. Top S, Vessieres A, Leclercq G, Quivy J, Tang J, Vaissermann J, Huche M, Jaouen G. *Chem. Eur. J.* 2003; **9**: 5223.
31. Osella D, Mahboobi H, Colangelo D, Cavigiolio G, Vessie'res A, Jaouen G. *Inorg. Chim. Acta* 2005; **358**: 1993.
32. Osella D, Ferrali M, Zanello P, Laschi F, Fontani M, Nervi C, Cavigiolio G. *Inorg Chim. Acta* 2000; **306**: 42.

DOI: 10.1002/aoc

33. Tamura H, Miwa M. *Chem. Lett.* 1997; **11**: 1177.
34. Dorcier A, Dyson PJ, Gossens C, Rothlisberger U, Scopelliti R, Tavernelli I. *Organometallics* 2005; **24**: 2114.
35. Scolaro C, Geldbach TJ, Rochat S, Dorcier A, Gossens C, Bergamo A, Cocchietto M, Tavernelli I, Sava G, Rothlisberger U, Dyson PJ. *Organometallics* 2006; **25**: 756.
36. Reisner E, Arion VB, Guedes MFC, Lichtenecker R, Eichinger A, Keppler BK, Kukushkin VY, Pombeiro AJL. *Inorg. Chem.* 2004; **43**: 7083.
37. Dorcier A, Ang WA, Bolaño S, Gonsalvi L, Juillerat-Jeannerat L, Laurenczy G, Peruzzini M, Phillips AD, Zanobini F, Dyson PJ. *Organometallics*, 2006; **25**: 4090.
38. Maillard S, Gauduchon J, Marsaud V, Gouilleux F, Connault E, Opolon P, Fattal E, Sola B, Renoir JK. *J. Steroid Biochem. Mol. Biol.* 2006; **100**: 67.
39. Top S, El Hafa H, Vessieres A, Huche M, Vaissermann J, Jaouen G. *Chem. Eur. J.* 2002; **8**: 5241.
40. Schatzschneider U, Metzler-Nolte N. *Angew. Chem. Int. Edn* 2006; 45.
41. Corder R, Douthwaite JA, Lees DM, Khan NQ, Visendos-Santos AC, Wood EE, Carrier MJ. *Nature* 2001; **414**: 863.
42. Fre'mont L. *Life Sci.* 2000; **66**: 663.
43. Quideau S. *Chem. Biochem.* 2004; 427.
44. Santos-Buelga C, Williamson G. *Methods in Polyphenol Analysis*. RSC: Cambridge, 2003.
45. Vessieres A, Top S, Pigeon P, Hillard E, Boubeker L, Spera D, Jaouen G. *J. Med. Chem.* 2005; **48**: 3937.
46. Hillard E, Vessieres A, Le Bideau F, Plazuk D, Spera D, Huch M, Jaouen G. *Chem. Med. Chem.* 2006; **1**: 551.
47. Plazuk D, Vessieres A, Le Bideau F, Jaouen G, Zakrzewskib J. *Tetrahedron Lett.* 2004; **45**: 5425.
48. Neuse EW, Khan FBD, Meirim MG. *Appl. Organomet. Chem.* 1987; **2**: 129.
49. Neuse EW, Meirim MG, Blom NF. *Organometallics* 1988; **7**: 2562.
50. Weissfloch L, Wagner M, Probst T, Senekowitsch-Schmidtke R, Tempel K, Molls M. *BioMetals* 2001; **14**: 43.
51. Koepf-Maier P, Koepf H, Neuse EW. *Angew. Chem.* 1984; **96**: 446.
52. Koepf Maier P, Koepf H, Neuse EW. *J. Cancer Res. Clin. Oncol.* 1984; **108**: 336.
53. Koepf-Maier P. *Eur. J. Clin. Pharm.* 1994; **47**: 1.
54. Koepf-Maier P. *Z. Natur-forsch. C: Biosci.* 1985; **40C**: 843.
55. Koepf-Maier P, Koepf H. *Chem. Rev.* 1987; **87**: 1137.
56. Popova LV, Babin VN, Belousov YA, Nekrasov YS, Snegireva AE, Borodina NP, Shaposhnikova GM, Bychenko OB, Raevskii PM. *Appl. Organomet. Chem.* 1993; **7**: 85.
57. Meirim MG, Neuse EW, Caldwell GA. *J. Inorg. Organomet. Polym.* 1997; **7**: 71.
58. Meirim MG, Neuse EW, Caldwell GA. *J. Inorg. Organomet. Polym.* 1998; **8**: 225.
59. Neuse EW, Meirim MG, N''Da DD, Caldwell GA. *J. Inorg. Organomet. Polym.* 1999; **9**: 221.
60. Caldwell GA, Meirim MG, Neuse EW, Beloussow K, Shen W. *J. Inorg. Organomet. Polym.* 2000; **10**: 93.
61. Schechter B, Caldwell G, Neuse EW. *J. Inorg. Organomet.c Polym.* 2000; **10**: 177.
62. Johnson MT, EKreft E, N''Da DD, Neuse EW, J. van Rensburg CE. *J. Inorg. Organomet. Polym.* 2003; **13**: 255.
63. Neuse EW. *Macromol Symp.* 2001; **172**: 127.
64. Neuse EW. *Polym. Adv. Technol.* 1998; **9**: 786.
65. Malonne H, Atassi G. *Anticancer Drugs.* 1997; **8**: 811.
66. Fiorani P, Bjornsti M. *Ann. NY Acad. Sci.* 2000; **922**: 65.
67. Gong Y, Firestone GL, Bjeldanes LF. *Mol. Pharmac.* 2006; **69**: 1320.
68. Das BB, Sen N, Roy A, Dasgupta SB, Ganguly A, Mohanta BC, Dinda B, Majumder HK. *Nucl. Acids Res.* 2006; **34**: 1121.
69. Lage H, Aki-Sener E, Yalcin I. *Int. J. Cancer* 2005; **119**: 213.
70. Gopal YNV, Jayaraju D, Kondapi AK. *Arch. Biochem. Biophys.* 2000; **376**: 229.
71. Krishna ADS, Panda G, Kondapi AK. *Arch. Biochem. Biophys.* 2005; **438**: 206.
72. Duivenvoorden WCM, Liu Y, Schatte G, Kraatz H. *Inorg. Chim. Acta* 2005; **358**: 3183.
73. Zsoldos-Mády V, Csámpai A, Szabó R, Mészáros-Alapi E, Pásztor J, Hudecz F, Sohár P. *Chem. Med. Chem.* 2006; **1**: 1119.
74. Chen L, Chen J, Sun L, Xie Q. *Appl. Organometal. Chem.* 2005; **19**: 1038.
75. WHO. *Weekly Epidemiol. Rep.* 1996; **3**: 17.
76. WHO. *Weekly Epidemiol. Rep.* 1996; **4**: 25.
77. WHO. *Weekly Epidemiol. Rep.* 1996; **5**: 37.
78. Biot C, Glorian G, Maciejewski LA, Brocard JS, Domarle O, Blampain G, Millet P, Georges AJ, Abessolo H, Dive D, Lebibi J. *J. Med. Chem.* 1997; **40**: 3715.
79. Biot C, Caron S, Maciejewski LA, Brocard JS. *J. Labelled Compd Radiopharm.* 1998; **51**: 911.
80. Domarle O, Blampain G, Agnaniet H, Nzadiyabi T, Lebibi J, Brocard JS, Maciejewski LA, Biot C, Georges AJ, Millet P. *Antimicrob. Agents Chemother.* 1998; **42**: 540.
81. Biot C, Delhaes L, Abessolo H, Domarle O, Maciejewski LA, Mortuaire M, Delcourt P, Deloron P, Camus D, Dive D, Brocard JS. *J. Organomet. Chem.* 1999; **589**: 59.
82. Biot C, Delhaes L, N'Diaye CM, Maciejewski LA, Camus D, Dive D, Brocard JS. *Bioorg. Med. Chem.* 1999; **7**: 2843.
83. Delhaes L, Abessolo H, Biot C, Berry L, Delcourt P, Maciejewski LA, Brocard JS, Camus D, Dive D. *Parasitol. Res.* 2001; **87**: 239.
84. Biot C, Delhaes L, Maciejewski LA, Mortuaire M, Camus D, Dive D, Brocard JS. *Eur. J. Med. Chem.* 2000; **35**: 707.
85. Delhaes L, Biot C, Berry L, Maciejewski LA, Camus D, Brocard JS, Dive D. *Bioorg. Med. Chem.* 2000; **8**: 2739.
86. Delhaes L, Biot C, Berry L, Delcourt P, Maciejewski LA, Camus D, Brocard JS, Dive D. *Chem. Biol. Chem* 2002; **3**: 418.
87. Atteke C, Ndong JMM, Aubouy A, Maciejewski LA, Brocard JS, Lebibi J, Deloron P. *J. Antimicrob. Chemother.* 2003; **51**: 1021.
88. Daher W, Biot C, Fandeur T, Jouin H, Pelinski L, Viscogliosi E, Fraisse L, Pradines B, Brocard J, Khalife J, Dive D. *Malaria J.* 2006; **5**: 11.
89. Biot C. *Curr. Med. Chem. Anti-Infective Agents* 2004; **3**: 135.
90. Pradines B, Tall A, Rogier C, Spiegel A, Mosnier J, Marrama L, Fusai T, Millet P, Panconi E, Trape JF, Parzy D. *Trop. Med. Int. Health* 2002; **7**: 265.
91. Biot C, Dessolin J, Ricard I, Dive D. *J. Organometal. Chem.* 2004; **689**: 4678.
92. Wu X, Wilairat P, Go ML. *Bioorg. Med. Chem. Lett.* 2002; **12**: 2299.
93. Agnes TLC, Beng KS, Go M. *Eur. J. Pharm. Sci.* 2006; **27**: 175.
94. Baramee A, Coppin A, Mortuaire M, Pelinski L, Tomavoc S, Brocard J. *Bioorg. Med. Chem.* 2006; **14**: 1294.
95. Biot C, Taramelli D, Forfar-Bares I, Maciejewski LA, Boyce M, Nowogrocki G, Brocard JS, Basilico N, Olliaro P, Egan TJ. *Mol. Pharm.* 2005; **2**: 185.
96. De Champdoré M, Di Fabio G, Messere A, Montesarchio D, Piccialli G, Loddo R, La Colla M, La Colla P. *Tetrahedron* 2004; **60**: 6555.
97. Kondapi AK, Satyanarayana N, Saikrishna AD. *Arch. Biochem. Biophys.* 2006; **450**: 123.
98. Sato S, Nojima T, Takenaka S. *J. Organometal. Chem.* 2004; **689**: 4722.
99. Mukumoto K, Ohtsuka K, Nojima T, Takenaka S. *Anal. Sci.* 2006; **22**: 349.
100. Drummond TG, Hill MG, Barton JK. *Nat. Biotechnol.* 2003; **21**: 1192.
101. Umek RM, Lin SW, Vielmetter J, Terbrueggen RH, Irvine B, Yu CJ, Kayyem JF, Yowanto H, Blackburn GF, Farkas DH, Chen YP. *J. Mol. Diag.* 2001; **3**: 74.

DOI: 10.1002/aoc

102. Patolsky F, Weizmann Y, Willner I. *J. Am. Chem. Soc.* 2002; **124**: 770.
103. Fan C, Plaxco KW, Heeger AJ. *Proc. Natl Acad. Sci. USA* 2003; **100**: 9134.
104. Wang Y, Zhu X, Hu Z, Wang J, Zhouc F. *Electroanalysis* 2005; **17**: 2163.
105. Liao JC, Mastali M, Gau V, Suchard MA, Møller AK, Bruckner DA, Babbitt JT, Li Y, Gornbein J, Landaw EM, McCabe ERB, Churchill BM, Haake DA. *J. Clin. Microb.* 2006; **44**: 561.
106. Baldoli C, Licandro E, Maiorana S, Resemini D, Rigamonti C, Falciola L, Longhi M, Mussini PR. *J. Electroanal. Chem.* 2005; **585**: 197.
107. Zocchi G. *ChemPhysChem* 2006; **7**: 555.
108. Szunerits S, Bouffier L, Calemczuk R, Corso B, Demeunynck M, Descamps E, Defontaine Y, Fiche J, Fortin E, Livache T, Mailley P, Roget A, Vieila E. *Electroanalysis* 2005; **17**: 2001.
109. Lee TM-H, Hsing I-M. *Anal. Chim. Acta* 2006; **556**: 25.
110. Liepold p, Wieder H, Hillebrandt H, Friebel A, Hartwicht G. *Bioelectrochemistry* 2005; **67**: 143.
111. Brisset H, Navarroc A-E, Spinelli N, Chaix C, Mandrand B. *Biotechnol. J.* 2006; **1**: 95.
112. Suye S, Matsuura T, Kimura T, Zheng H, Hori T, Amano Y, Katayama H. *Microelectron. Engng* 2005; **81**: 441.
113. Nielsen PE, Egholm M, Berg RH, Buchardt O. *Science* 1991; **254**: 1497.
114. Egholm M, Buchardt O, Christensen L, Behrens C, Freier SM, Driver DA, Berg RH, Kim SK, Norden B, Nielsen PE. *Nature* 1993; **365**: 566.
115. Demidov V, Potaman VN, Frank-Kamenetskii MD, Buchardt O, Egholm M, Nielsen PE. *Biochem. Pharmac.* 1994; **48**: 1309.
116. Maurer A, Kraatz H-B, Metzler-Nolte N. *Eur. J. Inorg. Chem.* 2005; 3207.
117. Hamzavi R, Happ T, Weitershaus K, Metzler-Nolte N. *J. Organomet. Chem.* 2004; **689**: 4745.
118. Maiorana S, Licandro E, Perdicchia D, Baldoli C, Vandoni B, Giannini C, Salmain C. *J. Mol. Catal. A* 2003; **204–205**: 165.

DOI: 10.1002/aoc