Journal of Organometallic Chemistry 696 (2011) 1038–1048

Contents lists available at ScienceDirect

Journal of Organometallic Chemistry

journal homepage: www.elsevier.com/locate/jorganchem

Evaluation of bactericidal and fungicidal activity of ferrocenyl or phenyl derivatives in the diphenyl butene series

Mehdi El Arbi [a,b], Pascal Pigeon [a], Siden Top [a,*], Ali Rhouma [b], Sami Aifa [c], Ahmed Rebai [c,**], Anne Vessières [a], Marie-Aude Plamont [a], Gérard Jaouen [a]

[a] Chimie ParisTech (Ecole Nationale Supérieure de Chimie de Paris), Laboratoire Charles Friedel, UMR CNRS 7223, 11 rue Pierre et Marie Curie, 75231 Paris Cedex 05, France
[b] Unité de Recherche Protection des Plantes Cultivées et Environnement, Institut de l'olivier de Sfax, Route de Sokra Km 1,5 – 3003 Sfax, Tunisia
[c] Centre de Biotechnologie de Sfax, Route de Sidi Mansour Km 6 – BP 1177, 3018 Sfax, Tunisia

ARTICLE INFO

Article history:
Received 19 July 2010
Received in revised form
2 September 2010
Accepted 3 September 2010

Keywords:
Ferrocene
Iron
Bioorganometallic
Antibacterial
Antifungal

ABSTRACT

Some ferrocene compounds, such as tamoxifen derivatives hydroxyferrocifen **1** and ferrociphenol **2**, show strong antiproliferative activity on hormone-dependent and hormone-independent breast cancer cells. In order to evaluate their antimicrobial activity, they were tested, together with their purely organic analogs, on the bacteria *Pseudomonas aeruginosa* and *Staphylococcus aureus* and the fungus *Candida albicans*. It has been found that the compounds bearing alkylamino chains are active, and in these cases the antimicrobial activity increases for compounds bearing two amino chains. These dialkyamino compounds are equally as active as doxycycline on *P. aeruginosa* and *S. aureus* but superior to it on *C. albicans*. The results show that there are no general correlation between the antitumoral activity and the bactericidal and fungicidal activities of these compounds. The ferrocene derivatives and their organic analogs have similar activity on bacteria and fungus. This bactericidal and fungicidal behaviour is a novel area of activity for these entities.

1. Introduction

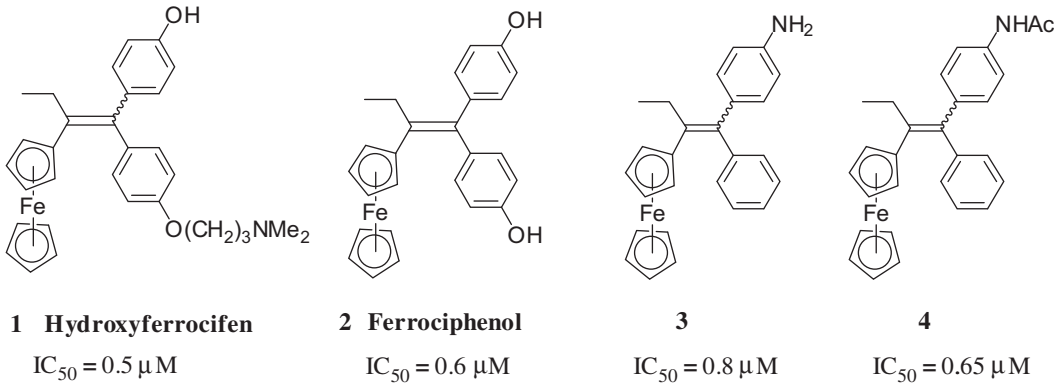
Bioorganometallic chemistry, defined as the discipline that combines study of organometallic molecules with that of their biological applications, has seen exponential growth in recent years [1–10]. Of the transition metal complexes studied, ferrocene derivatives are amongst the most interesting. This is particularly due to the redox potential of ferrocene, as well as its stability and lack of toxicity. These complexes have numerous applications. For example, ferrocene analogs of tamoxifen are very active on hormone-dependent cancer cells, but unlike tamoxifen are also active on hormone-independent breast cancer cells [11–15]. Modification of the chloroquine molecule by substitution of a part of the molecule with a ferrocene group resulted in a new molecule, ferroquine, active on malarial strains that are resistant to chloroquine [16–20]. Clinical trials for this product have now reached phase II. Ferrocene derivatives of quinoxaline have also been studied for their antimalarial activity [21]. In addition, ferrocene derivatives can be used as "electrochemical sensors" for biological substances [22].

The idea of using a ferrocene group to enhance the activity of antibiotics was suggested by Edwards et al. in 1976 [23]. These authors found that incorporation of a ferrocene group into the penicillin molecule increased its antibiotic activity. In recent years other articles on the use of transition metals as antibacterials and antifungals have been published. Chelates formed by complexation of hydrazone, ceftriaxone or oxalyldihydrazide have been found to improve antibacterial activity of the relevant species [24,25]. Ferrocene derivatives of ethambutol have been prepared and tested against *Mycobacterium tuberculosis* [26,27]; the best compounds are less active than ethambutol itself. Ferrocenyl derivatives of dihydropyrazole have also been tested as antibacterials [28,29]. Ferrocenyl derivatives of thiazoloacylhydrazone [30] and of various peptides[31] have been found to be active on *Staphylococcus aureus*, *Esherichia coli* and *Pseudomonas aeruginosa*. Ferrocenyl aroylhydrazones have shown moderate activity on bacteria and fungi [32,33]. A ferrocenyl analog of the antifungal fluconaxole has also been synthesized and its activity is modest compared to that of the corresponding organic compound [34].

In the course of our work on the activity of organometallic analogs of tamoxifen on breast and prostate cancer, a number of

* Corresponding author. Tel.: +33 1 44 27 66 99; fax: +33 143260061.
** Corresponding author.
E-mail addresses: siden-top@chimie-paristech.fr (S. Top), ahmed.rebai@cbs.rnrt.tn (A. Rebai).

0022-328X/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jorganchem.2010.09.015



1 Hydroxyferrocifen $IC_{50} = 0.5\ \mu M$

2 Ferrociphenol $IC_{50} = 0.6\ \mu M$

3 $IC_{50} = 0.8\ \mu M$

4 $IC_{50} = 0.65\ \mu M$

Chart 1. Ferrocenyl derivatives and their IC_{50} values on the hormone independent cancer cells (MDA-MB-231).

complexes have been synthesized. Some compounds demonstrated excellent cytotoxic activity on both hormone-dependent and hormone-independent cancer cells. For example, compounds **1** (hydroxyferrocifen) [12], **2** (ferrociphenol) [12], **3** [14] and **4** [14] are very active on hormone-independent MDA-MB-231 cancer cells, with IC_{50} values ranging from 0.5 μM to 0.8 μM (chart 1).

The idea of using antibiotics to treat cancers has also been pointed out. For example, the well-known antibiotic doxycycline has been found to be active on breast and prostate cancer [35–37]. In addition, tamoxifen has been found to display good antifungal activity by interfering with calcium homeostasis [38,39]. These results suggest that there may be a correlation between antitumoral activity and antimicrobial activity. Accordingly, we decided to test the activity of our ferrocene derivatives on pathogenic bacteria and fungi. For this study we selected two bacterial pathogens, *P. aeruginosa* and *S. aureus*, and the pathological fungal strain *Candida albicans*. Doxycycline, often used in tests to establish antimicroorganism activity [40–42] was chosen as the standard antibiotic for our tests. Chart 2 shows the chemical structure of the 27 products selected for this study.

Compounds **1**–**16**, **24**, **26** and **27** are ferrocene complexes. Complexes **26** and **27** are citric salts of **1** and **10** respectively. The purely organic compounds **17**–**23** and a ketone, **25**, were chosen for purposes of comparison. Compounds **1** [43], **2** [43], **3** [44], **4** [14], **5** [45], **6** [46], **11** [46], **12** [47], **13** [47], **16** [48], **17** [49], **18** [12], **19** [47], **21** [50], **22** [51], **24** [52], have already been reported as part of a study on their antiproliferative activity on breast cancer cells. Here we focus on the bactericidal and fungicidal activity of all these compounds.

2. Results and discussion

2.1. Synthesis

4,4′-Bis-dimethylaminopropoxy-benzophenone **25** was prepared by reacting the dimethylamine with the 4,4′-bis-bromopropoxy-benzophenone, **28** [53], at 60 °C for 24 h (Scheme 1).

1,1-Bis[4-(3-dimethylaminopropoxy)-phenyl]-2-ferrocenyl-but-1-ene, **10**, was prepared from ferrociphenol, **2** (Scheme 2). The reaction of the bromopropane with the sodium phenolate, generated by reacting the sodium ethanolate with **2**, gives first the brominated compound **29**. Heating this latter compound with dimethylamine in methanol at 60 °C produced **10** in 57% overall yield.

Amido compounds **7**, **8** and **9** were prepared by alkylation of amino compound **3** by an appropriated acid chloride (Scheme 3). **7**, **8** and **9** were obtained in 92%, 76% and 63% yield, respectively.

The reaction of the sodium salt of ferrociphenol **2** with the pivaloyl chloride gives pivaloate derivatives **14** and **15**. Palmitate compound **23** was prepared in the same way from **18** (Scheme 4).

Citric salts **26** and **27** were prepared by addition of citric acid to **1** and **10**, respectively (Scheme 5).

2.2. Biological studies

Gram negative strain *P. aeruginosa*, gram positive strain *S. aureus* and fungus *C. albicans*. were used for inhibitory tests using doxycycline as a positive control. The minimal inhibitory concentrations (MIC, μg/mL) were estimated by the two fold broth dilution technique in liquid plate count agar (PCA) [54]. Compounds were dissolved in ethanol and diluted in the media to concentrations ranging from 200 μg/mL to 12.5 μg/mL. Inoculants of 10^5 bacteria or fungi/mL were used. After incubation at 30 °C for 24 h, the minimal inhibitory concentrations (MIC, μg/mL and μM) were recorded as the lowest concentration of compound in the medium that showed no microbial growth by visual observation. Solvent, media and positive growth controls were also run simultaneously. The bactericidal and fungicidal activities were detected after incubation at 30 °C for 24 h of PCA medium sowing from each clear sample.

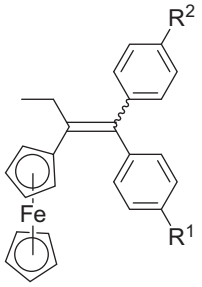
Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC, μg/mL and μM) or minimum fungicidal concentration (MFC, μg/mL and μM) values for the compounds are shown in Table 1. We also show IC_{50} values for the MDA-MB-231 hormone-independent cancer cells, and lipophilicity, expressed as log $P_{o/w}$, for most of the compounds.

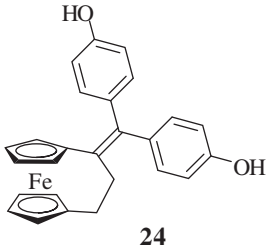
The antiproliferative activity on breast cancer cells of most of the compounds has already been reported. The IC_{50} values of **7**, **8**, **9**, **11**, **16** and **20** are shown in the table and that of **10**, **12**, **14**, **15**, **21**, **23**, **25**, **26** and **27** will be determined in the future. Compound **24** is the only ferrocenophane derivative presented in the table; it is the most active compound with an IC_{50} value of 0.09 μM [52]. All tested organic compounds **18**–**20**, **22** and **23** are not active while most of the ferrocene analogs show good activity. For example, the dihydroxy **18** is non-toxic while its analog **2** is very active with an IC_{50} value of 0.6 μM. The same behaviour was observed for the **22**/**1** and **20**/**4** couples. In the case of the amido compounds, the size of the substituent is important; the antiproliferative activities dramatically decrease from acetamido **4** ($IC_{50} = 0.65\ \mu M$) to pivaloylamino **9** (non-toxic). The presence of two palmitate chains in **16** also inhibits its activity. On the other hand compound **11** with an amino chain longer than that of ferrocifen **1** is as active as **1**. To summarize, the best compounds are ferrocene derivatives bearing two free phenols (**2** and **24**) or one phenol and one amino chain (**1** and **11**).

The MIC and MBC values for doxycycline were found to be <12.5 μg/mL and 25 μg/mL respectively on *P. aeruginosa*, <12.5 μg/mL and 12.5 μg/mL on *S. aureus*, and 100 μg and 200 μg/mL on *C. albicans*.. The compounds tested can be grouped into three categories. The first one contains the compounds possessing an activity

Ferrocenyl derivatives

1 $R^1 = OH$; $R^2 = O(CH_2)_3NMe_2$
2 $R^1 = R^2 = OH$
3 $R^1 = H$; $R^2 = NH_2$
4 $R^1 = H$; $R^2 = NHAc$
5 $R^1 = R^2 = H$
6 $R^1 = H$; $R^2 = OH$
7 $R^1 = H$; $R^2 = NHCOEt$
8 $R^1 = H$; $R^2 = NHCO^iPr$
9 $R^1 = H$; $R^2 = NHCO^tBu$
10 $R^1 = R^2 = O(CH_2)_3NMe_2$
11 $R^1 = OH$; $R^2 = O(CH_2)_4NMe_2$
12 $R^1 = H$; $R^2 = Br$
13 $R^1 = H$; $R^2 = CN$
14 $R^1 = OH$; $R^2 = OCO^tBu$
15 $R^1 = R^2 = OCO^tBu$
16 $R^1 = R^2 = OCO(CH_2)_{14}CH_3$

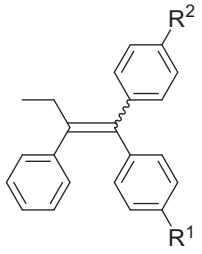


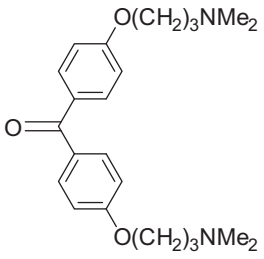


24

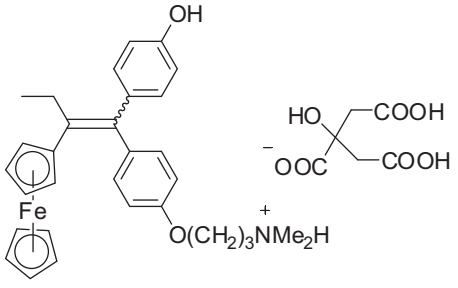
Organic derivatives

17 $R^1 = H$; $R^2 = OH$
18 $R^1 = R^2 = OH$
19 $R^1 = H$; $R^2 = NH_2$
20 $R^1 = H$; $R^2 = NHAc$
21 $R^1 = R^2 = O(CH_2)_3NMe_2$
22 $R^1 = OH$; $R^2 = O(CH_2)_3NMe_2$
23 $R^1 = R^2 = OCO(CH_2)_{14}CH_3$

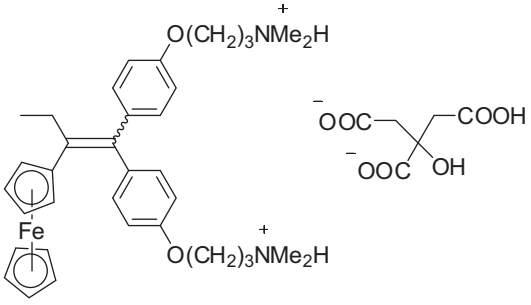




25



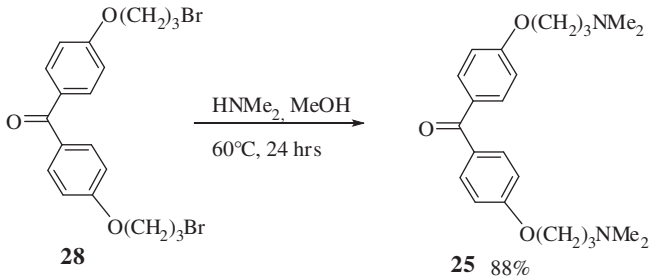
26



27

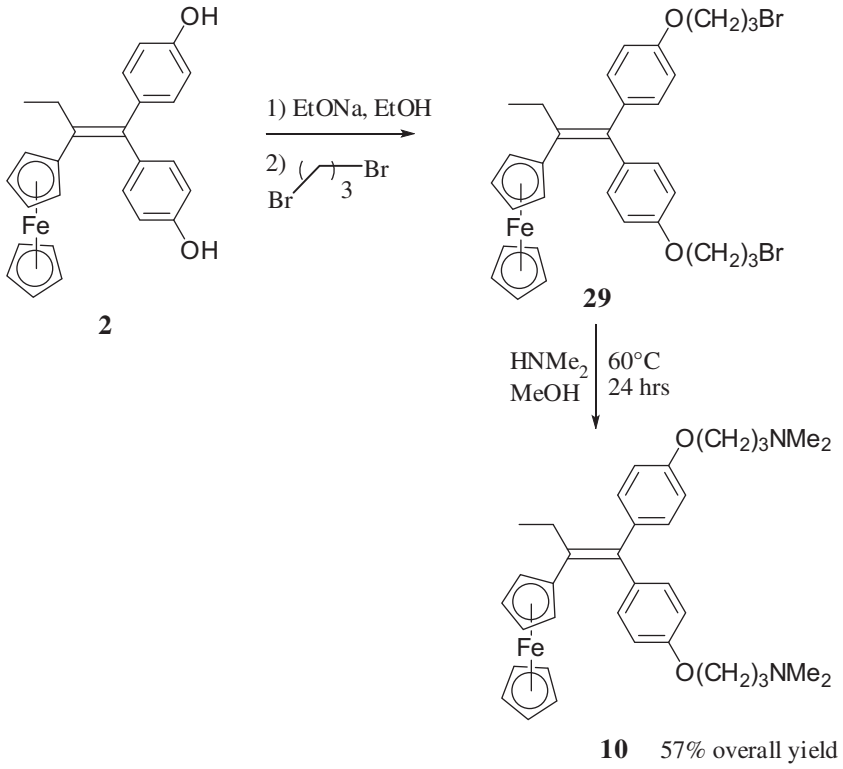
Chart 2. Compounds selected for this study.

similar or superior to that of doxycycline. These are the purely organic compounds **21** and **22**, which bear one or two amino chains on the molecule. They are in fact more active than doxycycline on *C. albicans*.. The second category is that of compounds slightly less active than doxycycline. In this category are found the ferrocene derivatives **1**, **10** and **11**, which are ferrocenyl analogs of compounds **21** and **22**. The final category includes all the remaining products, with MIC and MBC values around 100 μM and 200 μM, respectively. The first observation to be made from this result is that the presence of the dimethylamino alkyl chain is instrumental for the activity of the compound. This is certainly the case for compounds **21**, **22**, **1**, **10** and **11**. OH, NH_2, NHCOR, Br or CN substituents in para position play essentially no role, since the products bearing these functions have the same activity as the unsubstituted compound **5**. Examination of the antimicrobial activity of the organic compound/ferrocene analog pairs **17**/**6**, **18**/**2**, **19**/**3**, **20**/**4**, **22**/**1** and **21**/**10** clearly show that the aromatic ferrocenyl group behaves like a phenyl, indeed it produces a slight deactivation in certain cases since it is more bulky than a flat arene. This behaviour is opposite to the one we observed for breast cancer cells where the redox effect of ferrocene is triggered. In fact the organic compounds **18**, **19**, **20** and

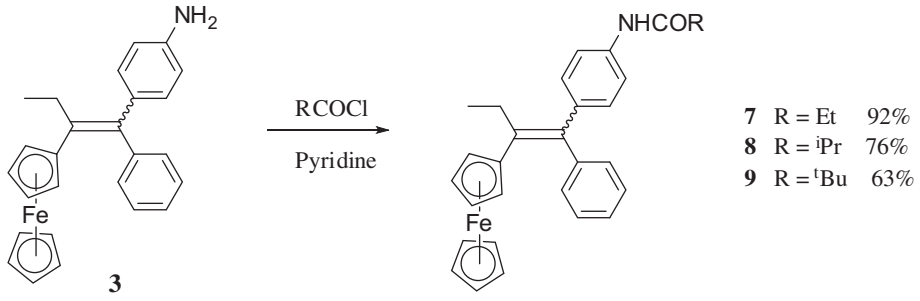


28 **25** 88%

Scheme 1. Synthesis of 4,4′-bis-dimethylaminopropoxy-benzophenone **25**.

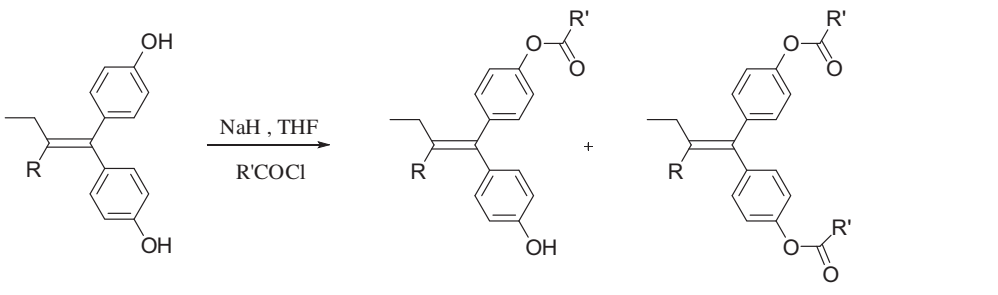


Scheme 2. Synthesis of ferrocenyl diamino compound **10**.

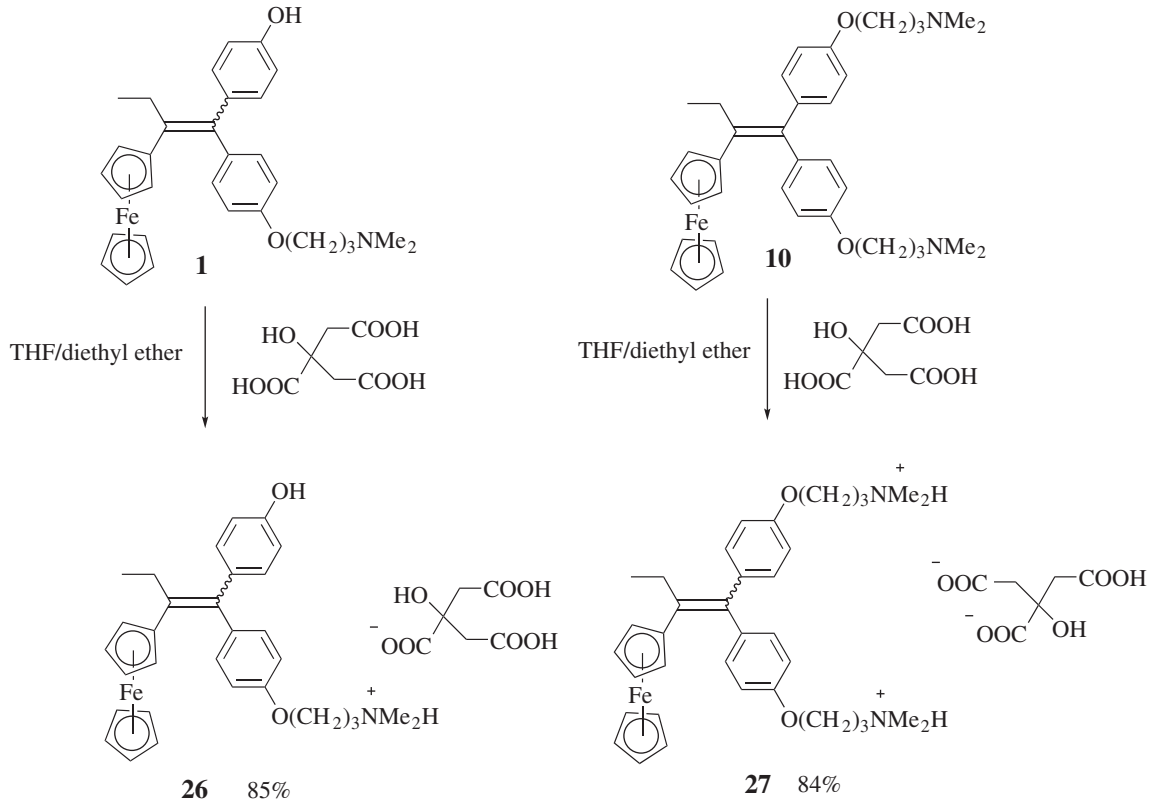


Scheme 3. Synthesis of amido compounds **7**, **8** and **9**.

22 are inactive on hormone-independent MDA-MB-231 breast cancer cells, while their ferrocenyl analogs **2**, **3**, **4** and **1** are amongst the most effective, with IC_{50} values between 0.5 μM and 0.8 μM. This difference is confirmed with compound **24**, which is very active on MDA-MB-231 cancer cells, with an IC_{50} value of 0.09 μM, but less active on microorganisms. The only shared characteristic between *P. aeruginosa, S. aureus, C. albicans*. and MDA-MB-231 cancer cells seems to be their sensitivity towards compounds bearing amino alkyl chains. The monoamine compounds **22** and **1** are active on the three microorganisms, with MIC values ranging



Scheme 4. Synthesis of pivaloate derivatives **14** and **15** and palmitate derivative **23**.



Scheme 5. Synthesis of citric salts **26** and **27**.

Table 1
Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC), IC_{50} and log $P_{o/w}$ values.

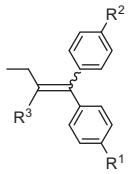
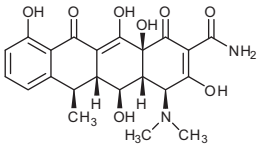
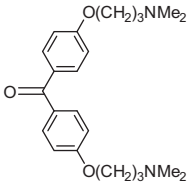
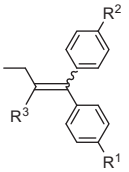
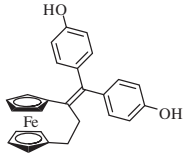
Cpd. Num.			*Pseudomonas aeruginosa* (μg/mL (μM))	*Staphylococcus aureus* (μg/mL (μM))	*Candida albicans* (μg/mL (μM))	IC_{50} on MDA-MB-231 (μM)	log Po/w
	 Doxycycline	MIC	<12.5 (<28.1)	<12.5 (<28.1)	100 (225)	–	–
		MBC	25 (56.2)	12.5 (28.1)	200[a] (450)		
25		MIC	25 (65.0)	50 (130.0)	50 (130.0)	–	–
		MBC	50 (130.0)	200 (520.1)	100[a] (260.0)		
5	$R^1 = R^2 = H$	MIC	100 (254.9)	100 (254.9)	100 (254.9)	7.54	6.43
	R^3 = Fc	MBC	200 (509.8)	200(509.8)	200[a] (509.8)		
17	R^1 = H; R^2 = OH	MIC	25 (83.2)	25 (83.2)	100 (332.9)	–	–
	R^3 = Ph	MBC	100 (332.9)	100 (332.9)	200[a](665.8)		
6	R^1 = H; R^2 = OH	MIC	100 (244.9)	100 (244.9)	100 (244.9)	1.13[b]	6.17[b]
	R^3 = Fc	MBC	200 (489.8)	200 (489.8)	200[a] (489.8)		
18	R^1 = OH; R^2 = OH	MIC	100 (316.1)	100 (316.1)	100 (316.1)	n.tox[c]	4.4[c]
	R^3 = Ph	MBC	200 (632.1)	200 (632.1)	200[a] (632.1)		

Table 1 (*continued*)

Cpd. Num.			*Pseudomonas aeruginosa* (μg/mL (μM))	*Staphylococcus aureus* (μg/mL (μM))	*Candida albicans* (μg/mL (μM))	IC_{50} on MDA-MB-231 (μM)	log Po/w
2	R^1 = OH; R^2 = OH	MIC	100 (235.67)	100 (235.67)	100 (235.67)	0.6[c]	5.0[c]
	R^3 = Fc	MBC	200 (471.35)	200 (471.35)	200[a] (471.35)		
19	R^1 = H; R^2 = NH_2	MIC	50 (167)	50 (167)	50 (167)	> 1 μM[d]	4.86[d]
	R^3 = Ph	MBC	200 (678)	200 (678)	200[a] (678)		
3	R^1 = H; R^2 = NH_2	MIC	25 (61.4)	100 (245.5)	100 (245.5)	0.8[d]	5.75[d]
	R^3 = Fc	MBC	50 (122.7)	200 (491)	200[a] (491)		
20	R^1 = H; R^2 = NHAc	MIC	100 (292.9)	100 (292.9)	100 (292.9)	55.4	–
	R^3 = Ph	MBC	200 (585.7)	200 (585.7)	200[a] (585.7)		
4	R^1 = H; R^2 = NHAc	MIC	100 (222.5)	100 (222.5)	100 (222.5)	0.65[d]	5.92[d]
	R^3 = Fc	MBC	200 (445.1)	200 (445.1)	200[a] (445.1)		
7	R^1 = H; R^2 = NHCOEt	MIC	100 (215.80)	100 (215.80)	100 (215.80)	1.2	5.89
	R^3 = Fc	MBC	200 (431.6)	200 (431.6)	200[a] (431.6)		
8	R^1 = H;	MIC	100 (209.5)	100 (209.5)	100 (209.5)	2.02	6.13
	R^2 = NHCOiPr	MBC	200 (418.9)	200 (418.9)	200[a] (418.9)		
	R^3 = Fc						
9	R^1 = H; R^2 = $NHCO^tBu$	MIC	100 (203.5)	100 (203.5)	100 (203.5)	> 1 μM	6.35
	R^3 = Fc	MBC	200 (407)	200 (407)	200[a] (407)		
22	R^1 = OH	MIC	<12.5 (<31.1)	12.5 (31.1)	25 (62.3)	n.tox	5.75
	R^2 = $O(CH_2)_3NMe_2$	MBC	<12.5 (<31.1)	25 (62.3)	50[a] (124.5)		
	R^3 = Ph						
1	R^1 = OH	MIC	25 (49.1)	25 (49.1)	50 (98.1)	0.5[c]	4.40[c]
	R^2 = $O(CH_2)_3NMe_2$	MBC	50 (98.1)	50 (98.1)	100[a] (196.3)		
	R^3 = Fc						
21	R^1 = $O(CH_2)_3NMe_2$	MIC	<12.5 (<25.7)	<12.5 (<25.7)	<12.5 (<25.7)	nd	nd
	R^2 = $O(CH_2)_3NMe_2$;	MBC	<12.5 (<25.7)	25 (51.4)	<12.5[a] (<25.7)		
	R^3 = Ph						
10	R^1 = $O(CH_2)_3NMe_2$	MIC	<12.5 (<21.02)	<12.5 (<21.02)	<12.5 (<21.02)	nd	nd
	R^2 = $O(CH_2)_3NMe_2$	MBC	50 (84.1)	25 (42.0)	50[a] (84.1)		
	R^3 = Fc						
11	R^1 = OH	MIC	50 (95.5)	50 (95.5)	25 (47.7)	≅ 0.5	4.06
	R^2 = $O(CH_2)_4NMe_2$; R^3 = Fc	MBC	100 (191.0)	100 (191.0)	100[a] (191.0)		
12	R^1 = H; R^2 = Br	MIC	100 (212.2)	100 (212.2)	100 (212.2)	≫1 μM[e]	nd
	R^3 = Fc	MBC	200 (424.4)	200 (424.4)	200[a] (424.4)		
13	R^1 = H; R^2 = CN	MIC	50 (119.8)	100 (239.6)	100 (239.6)	11[e]	6.37[e]
	R^3 = Fc (Z isomer)	MBC	200 (479.2)	200 (479.2)	200[a] (479.2)		
14	R^1 = OH;	MIC	50 (98.3)	100 (196.7)	100 (196.7)	nd	nd
	R^2 = OCO^tBu	MBC	200 (393.4)	200 (393.4)	200[a] (393.4)		
	R^3 = Fc						
15	R^1 = OCO^tBu	MIC	100 (168.7)	100 (168.7)	50 (168.7)	nd	nd
	R^2 = OCO^tBu;	MBC	200 (337.5)	200 (337.5)	200[a] (337.5)		
	R^3 = Fc						
16	R^1 = $OCOC_{15}H_{31}$	MIC	50 (55.5)	100 (111.0)	100 (111.0)	n.tox	nd
	R^2 = $OCOC_{15}H_{31}$	MBC	200 (221.9)	200 (221.9)	200[a] (221.94)		
	R^3 = Fc						
23	R^1 = $OCOC_{15}H_{31}$	MIC	50 (63.0)	50 (63.0)	100 (126.0)	nd	nd
	R^2 = $OCOC_{15}H_{31}$	MBC	200 (252.1)	200 (252.1)	200[a] (252.1)		
	R^3 = Ph						
26	R^1 = $O(CH_2)_3NMe_2H^+$	MIC	25 (35.6)	50 (71.3)	25 (35.6)	nd	nd
	R^2 = OH; R^3 = Fc	MBC	50 (71.3)	100 (142.5)	100[a] (142.5)		
27	R^1 = $O(CH_2)_3NMe_2H^+$	MIC	25 (31.1)	25 (31.1)	<12.5 (<15.5)	nd	nd
	R^2 = $O(CH_2)_3NMe_2H^+$	MBC	200 (248.5)	200 (248.5)	>200[a] (>248.5)		
	R^3 = Fc						
		MIC	100 (236.8)	100 (236.8)	100 (236.8)		
		MBC	200 (473.6)	200 (473.6)	200[a] (473.6)		
24						0.09[f]	4.60

[a] MFC value; n.tox: non-toxic at 10 μM; nd: not determined.
[b] Values from Ref. [45].
[c] Values from Ref. [12].
[d] Values from Ref. [14].
[e] Values from Ref. [47].
[f] Values from Ref. [52].

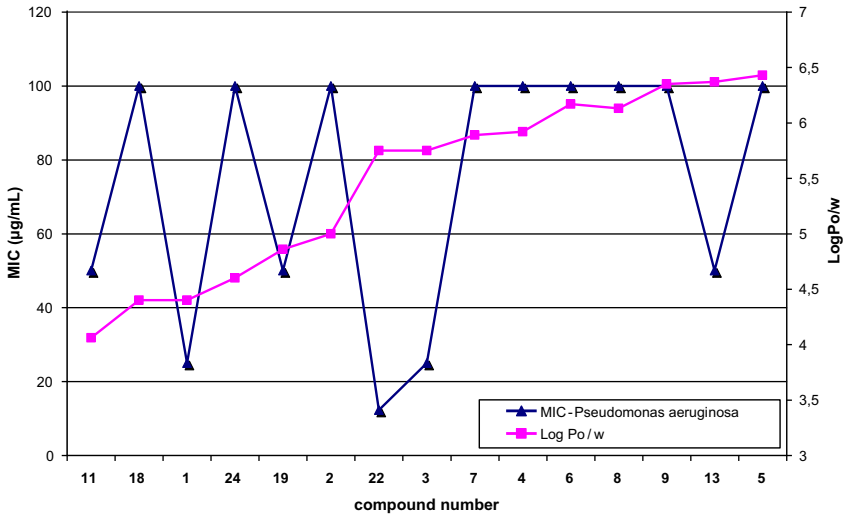


Fig. 1. Log $P_{o/w}$ and MIC values (µg/mL) of selected compounds.

from <12.5 to 50 µg/mL. The compound with a longer alkylamino chain is slightly less active. Antimicrobial activity becomes very pronounced when the molecules have two amino chains. This is the case for the organic diamino compound **21** and the ferrocenyl diamino compound **10**, with MIC values of <12.5 µg/mL and MBC values of <12.5–50 µg/mL. They are thus as active as doxycycline but become superior to the latter on the fungus *C. albicans*.. Even the diamino ketone **25** is slightly active. Interestingly, tamoxifen, which also contains alkyamino chain, exhibits antifungal activity with MIC values ranging from 8 to 64 µg/mL [38,39]. It may be noted that, compounds **1** and **11**, which are slightly active, are ones of the most hydrophilic compounds in the list (log $P_{o/w} = 4.40$ and 4.06, respectively). However, it seems that the hydrophilicity of compounds does not play an important role because both the low lipophilic compound **18** (log $P_{o/w} = 4.4$) and the most lipophilic compound **5** (log $P_{o/w} = 6.43$) have the same antimicrobial activity. Fig. 1 shows clearly the lack of correlation between lipophilicity and microbial activity on *P. aeruginosa*.

It may be noted that cobaltifen, a cobalt complex analog of hydroxytamoxifen, which bears two amino chains, is active on MDA-MB-231 cancer cells ($IC_{50} = 2.5 \pm 0.4$ µM) [50]. It also seems to show that the amino chain, presented in the molecules, is responsible for their antimicrobial activity. We have previously suggested that ferrocifen **1** might target calmodulin [11]. Dolan et al. have recently investigated the antifungal activity of tamoxifen, they conclude that the ability of tamoxifen to interfere with calmodulin is part of its mechanism of action [38]. As the basic chain could complex Ca^{++}, the involvement of calmodulin in the antimicrobial activity of our amino compounds should be envisaged as one of the possible mechanisms. The transformation of compounds **1** and **10** into citric salts **26** and **27** decreases their activity slightly but improves their solubility in water. The amides **4**, **20**, **7**, **8** and **9** and the esters **23**, **14**, **15**, **16** are amongst the least active compounds, all with similar MIC and MBC values.

3. Conclusion

Twenty seven ferrocenyl or phenyl derivatives in the diphenyl butene series were tested on the bacteria *P. aeruginosa* and *S. aureus* and on the fungus *C. albicans*.. The results obtained appear to show that there are no general correlation between the cytotoxic activity on cancer cells and the bactericidal or fungicidal activity of the compounds. The ferrocene derivatives and their organic analogs show similar levels of activity on the bacteria and the fungus. Only the compounds bearing alkylamino chains are very active, and in these cases the antimicrobial activity increases for compounds bearing two amino chains. They are equally as active as doxycycline on *P. aeruginosa* and *S. aureus* but superior to it on *C. albicans*.. Consequently we intend to concentrate our future studies on this type of compound and will attempt to elucidate the reasons for this interesting activity which opens up new directions in research into active antibiotics. This is an important goal of current anti-infective research [38].

4. Experimental

4.1. Synthesis and characterization of compounds

The synthesis of all compounds was performed under an argon atmosphere, using standard Schlenk techniques. Anhydrous THF was obtained by distillation from sodium/benzophenone. Thin layer chromatography was performed on silica gel 60 GF254. Infrared spectra were obtained on IR-FT BOMEM Michelson-100 and FT/IR-4100 JASCO spectrometers. ^1H and ^{13}C NMR spectra were recorded on a 300 MHz Bruker spectrometer. Mass spectrometry was performed with a Nermag R 10-10C spectrometer. Elemental analyses were performed by the microanalysis service of CNRS at Gif sur Yvette. The preparative HPLC separations were performed on a Shimadzu apparatus with a Nucleodur C18 column (length of 25 cm, diameter of 2.5 cm, and particle size of 10 µm).

4.2. 4,4′-Bis-dimethylaminopropoxy-benzophenone, *25*

4,4′-Bis-bromopropoxy-benzophenone **28** [53] (0.365 g, 0.8 mmol) was added to a solution of dimethylamine in methanol (2 M, 4.8 mL, 9.6 mmol) placed in a pressure tube. The mixture was heated with stirring at 60 °C for 24 h. After cooling, the mixture was concentrated under reduced pressure, dissolved in dichloromethane, washed with an aqueous solution of sodium hydrogenocarbonate, then with water. The organic phase was dried ($MgSO_4$), filtered and concentrated. The crude product was recrystallized from an ether/petroleum ether solution to yield **25** in 88% yield. mp: 75 °C. ^1H NMR ($CDCl_3$) : δ 1.80–2.00 (m, 4H, CH_2), 2.19 (s, 12H, NMe_2), 2.39 (t, $J = 7.1$ Hz, 4H, CH_2N), 4.02 (t, $J = 6.4$ Hz, 4H, CH_2O), 6.88 (d, $J = 8.8$ Hz, 4H, C_6H_4), 7.69 (d, $J = 8.8$ Hz, 4H, C_6H_4). ^{13}C NMR ($CDCl_3$): δ 26.4 (2 CH_2), 44.5 (2 NMe_2), 55.2 (2 CH_2N), 65.4 (2 CH_2O), 112.9 (2 × 2 CH C_6H_4), 129.6 (2C, C_6H_4), 131.2 (2 × 2 CH, C_6H_4), 161.3 (2C, C_6H_4), 193.5 (CO). IR (KBr, ν cm^{-1}): 3035, 2951, 2866, 2812, 2762 (CH_2, CH_3). MS (CI, NH_3) m/z : 385 $[M + H]^+$. Anal. Calcd. for

$C_{23}H_{32}N_2O_3$: C, 71.84; H, 8.38; N, 7.28. Found: C, 71.81; H, 8.41; N, 7.29.

4.3. 1,1-Bis[4-(3-dimethylaminopropoxy)phenyl]-2-ferrocenyl-but-1-ene, **10**

1,1-Bis(4-hydroxyphenyl)-2-ferrocenyl-but-1-ene **2** (3.00 g, 7.7 mmol) was added to a solution of sodium ethanolate prepared by addition of sodium (0.325 g, 14.1 mmol) to ethanol (20 mL). After the mixture had been stirred at reflux for 1 h, dibromopropane (8.560 g, 42.4 mmol) was added. After 1 h at reflux, the solution was left to cool to room temperature and was hydrolysed with water (100 mL). The product was extracted with dichloromethane. The organic phase was washed with water, dried over magnesium sulfate, filtered, and the mixture was concentrated under reduced pressure. The crude mixture of products was chromatographed on a silica gel column with petroleum ether as an eluent. The first colored band mainly contained the dibromo product **29**. The crude product was used without further purification in the second step: The compound was transferred into a pressure tube and a 2 M solution of dimethylamine in methanol (30 mL, 60 mmol) was added. The pressure tube was heated at 60 °C for 24 h and then was cooled to room temperature. The solution was concentrated under reduced pressure, dissolved in dichloromethane, washed with a solution of saturated sodium hydrogenocarbonate and water, dried on magnesium sulfate and concentrated under reduced pressure. The crude mixture was chromatographed on silica gel column. Acetone was first used as an eluent to remove the by-products, it was followed by a solution of triethylamine at 10% in acetone to elute the pure compound **10**. It was obtained as an oil with a yield of 57%. ^{1}H NMR ($CDCl_3$) : δ 0.94 (t, J = 7.4 Hz, 3H, CH_3), 1.79–1.95 (m, 4H, 2 CH_2), 2.18 (s, 6H, NMe_2), 2.19 (s, 6H, NMe_2), 2.37 (t, J = 7.2 Hz, 2H, CH_2N), 2.40 (t, J = 7.2 Hz, 2H, CH_2N), 2.50 (q, J = 7.4 Hz, 2H, CH_2), 3.83 (t, J = 1.9 Hz, 2H, C_5H_4), 3.89 (t, J = 6.6 Hz, 2H, CH_2O), 3.91 (t, J = 6.6 Hz, 2H, CH_2O), 3.98 (t, J = 1.9 Hz, 2H, C_5H_4), 4.02 (s, 5H, Cp), 6.66 (d, J = 8.7 Hz, 2H, C_6H_4), 6.77 (d, J = 8.7 Hz, 2H, C_6H_4), 6.86 (d, J = 8.7 Hz, 2H, C_6H_4), 7.02 (d, J = 8.7 Hz, 2H, C_6H_4). ^{13}C NMR ($CDCl_3$): δ 15.5 (CH_3), 27.5 (CH_2), 27.6 (CH_2), 27.9 (CH_2), 45.5 (2 NMe_2), 56.5 (2 CH_2N), 66.1 (2 CH_2O), 67.9 (2 CH C_5H_4), 69.1 (5 CH, Cp), 69.3 (2 CH, C_5H_4), 87.2 (C_{ipso}), 114.1 (2 CH, C_6H_4), 114.2 (2 CH, C_6H_4), 130.4 (2 CH, C_6H_4), 130.9 (2 CH, C_6H_4), 136.5 (C), 137.2 (C), 137.3 (C), 137.4 (C), 157.3 (2C). IR (KBr, ν cm^{-1}): 3093, 3032, 2947, 2866, 2816, 2765 (CH_2, CH_3). MS (EI, 70 eV) m/z: 594 $[M]^{+\cdot}$, 121 $[CpFe]^+$, 86 $[CH_2CH_2CH_2NMe_2]^+$, 58 $[CH_2NMe_2]^+$. HRMS (ESI, $C_{36}H_{47}FeN_2O_2$: $[M + H]^+$) calcd.: 595.29815, found: 595.29681.

4.4. General procedure for the synthesis of amido compounds **7**, **8** and **9**

In a Schlenk flask under argon, 2-ferrocenyl-1-(4-aminophenyl)-1-phenyl-but-1-ene **3** [44] was dissolved in anhydrous THF. Acid chloride and pyridine were added and the reaction mixture was stirred for 3 h. Water was added and the product was extracted with dichloromethane. The organic phase was washed with water, dried over magnesium sulfate, filtered, and the solvent was evaporated. The product was purified on a silica gel column with ether/pentane (1/1) as an eluent.

4.5. 1-(4-Propionylaminophenyl)-1-phenyl-2-ferrocenyl-but-1-ene, **7**

2-Ferrocenyl-1-(4-aminophenyl)-1-phenyl-but-1-ene **3** (810 mg, 2 mmol); anhydrous THF (30 mL); propionyl chloride (204 mg, 2.2 mmol); pyridine (174 mg, 2.2 mmol). Compound **7** was obtained as a yellow solid (890 mg, 92% yield) consisting of undeterminated 55/45 mixture of Z and E isomers. ^{1}H NMR ($CDCl_3$): δ 0.91 and 0.92 (t, J = 7.3 Hz, 3H, CH_3), 1.12 and 1.14 (t, J = 7.3 Hz, 3H, CH_3), 2.25 and 2.28 (q, J = 7.3 Hz, 2H, CH_2), 2.45 and 2.47 (q, J = 7.3 Hz, 2H, CH_2), 3.79 and 3.85 (s, 2H, H C_5H_4), 3.98 and 4.00 (s, 2H, H C_5H_4), 4.02 and 4.03 (s, 5H, Cp), 6.91 and 6.95 (d, J = 8.0 Hz, 2H, H_{arom}), 7.00–7.43 (m, 7H, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 7.8 (CH_3), 13.5 (CH_3), 25.9 and 26.1 (CH_2), 28.9 (CH_2), 66.5 and 66.7 (2 CH C_5H_4), 67.5 and 67.6 (2CH C_5H_4), 67.7 and 67.8 (5 CH Cp), 85.2 and 85.5 (C_{ipso}), 117.5 and 117.7 (2 CH_{arom}), 124.3 (CH_{arom}), 126.2 and 126.3 (2 CH_{arom}), 127.5 and 128.0 (2 CH_{arom}), 128.1 and 128.7 (2 CH_{arom}), 134.2 (C), 135.5 (C), 135.6 (C), 138.5 and 138.6 (C), 142.4 and 142.7 (C), 169.9 and 170.0 (CON). IR (KBr, ν cm^{-1}): 3451, 3272 (NH), 3094, 2967, 2930, 2872 (CH_2, CH_3), 1650 (CON). MS (EI, 70 eV) m/z : 463 $[M]^{+\cdot}$, 398, 397, 326, 121 $[CpFe]^+$. HRMS (ESI, $C_{29}H_{29}FeNO$: $[M]^{+\cdot}$) calcd.: 463.15931, found: 463.15884. Anal. Calcd. for $C_{29}H_{29}FeNO$: C, 75.16; H, 6.3; N, 3.02. Found: C, 74.78; H, 6.41; N, 2.73.

4.6. 1-(4-Isobutyrylaminophenyl)-1-phenyl-2-ferrocenyl-but-1-ene, **8**

2-Ferrocenyl-1-(4-aminophenyl)-1-phenyl-but-1-ene **3** (410 mg, 1 mmol); anhydrous THF (15 mL); isobutyryl chloride (117 mg, 1.1 mmol); pyridine (87 mg, 1.1 mmol). Compound **8** was obtained as a yellow solid (362 mg, 76% yield) consisting of undeterminated 55/45 mixture of Z and E isomers. ^{1}H NMR ($CDCl_3$): δ 0.91 and 0.92 (t, J = 7.3 Hz, 3H, CH_3), 1.13 and 1.15 (d, J = 6.2 Hz, 6H, 2 CH_3), 2.30–2.53 (m, 3H, CH_2 + CH), 3.80 and 3.88 (s, 2H, C_5H_4), 3.99 and 4.00 (s, 2H, C_5H_4), 4.03 and 4.04 (s, 5H, Cp), 6.92 and 6.96 (d, J = 8.1 Hz, 2H, H_{arom}), 6.99–7.44 (m, 7H, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 15.4 and 15.5 (CH_3), 19.7 (2 CH_3), 27.9 and 28.0 (CH_2), 36.8 (CH), 68.6 and 68.9 (2 CH, C_5H_4), 69.5 and 69.7 (2 CH, C_5H_4), 69.7 and 69.9 (5 CH, Cp), 86.0 (C_{ipso}), 119.4 and 119.6 (2 CH_{arom}), 126.2 (CH_{arom}), 128.1 and 128.2 (2 CH_{arom}), 129.5 and 129.9 (2 CH_{arom}), 130.0 and 130.7 (2 CH_{arom}), 136.2 (C), 137.4 and 137.6 (C), 137.6 and 137.7 (C), 140.4 and 140.5 (C), 144.3 and 144.6 (C), 175.0 and 175.1 (CON). IR (KBr, ν cm^{-1}): 3451, 3262 (NH), 3094, 2967, 2930, 2871 (CH, CH_2, CH_3), 1652 (CON). MS (EI, 70 eV) m/z: 477 $[M]^+$, 412, 342, 326, 121 $[CpFe]^+$. HRMS (ESI, $C_{30}H_{31}FeNO$: $[M]^+$) calcd.: 477.17496, found: 477.17429.

4.7. 1-(4-Pivaloylaminophenyl)-1-phenyl-2-ferrocenyl-but-1-ene, **9**

2-Ferrocenyl-1-(4-aminophenyl)-1-phenyl-but-1-ene **3** (410 mg, 1 mmol); anhydrous THF (15 mL); Trimethyl acetyl chloride (132 mg, 1.1 mmol); pyridine (87 mg, 1.1 mmol). Compound **9** was obtained as a yellow solid (309 mg, 63% yield) consisting of undeterminated 60/40 mixture of Z and E isomers. ^{1}H NMR ($CDCl_3$): δ 0.91 and 0.92 (t, J = 7.3 Hz, 3H, CH_3), 1.20 and 1.22 (s, 9H, ^{t}Bu), 2.45 and 2.47 (q, J = 7.3 Hz, 2H, CH_2), 3.79 and 3.86 (s, 2H, C_5H_4), 3.98 and 4.00 (s, 2H, C_5H_4), 4.02 and 4.03 (s, 5H, Cp), 6.87–7.44 (m, 9H, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 15.4 (CH_3), 27.7 (3 CH_3, ^{t}Bu), 28.0 (CH_2), 39.6 (C ^{t}Bu), 68.8 and 69.1 (2 CH, C_5H_4), 69.6 and 69.8 (2 CH, C_5H_4), 69.9 and 70.1 (5 CH, Cp), 87.9 (C_{ipso}), 119.6 and 119.8 (2 CH_{arom}), 126.2 (CH_{arom}), 128.1 and 128.2 (2 CH_{arom}), 129.5 and 130.0 (2 CH_{arom}), 130.0 and 130.7 (2 CH_{arom}), 136.2 (C), 137.4 and 137.5 (C), 137.6 and 137.7 (C), 140.4 and 140.6 I, 144.2 and 144.5 (C), 176.4 and 176.5 (CON). IR (KBr, ν cm^{-1}): 3432 (NH), 3094, 2965, 2929, 2871 (CH_2, CH_3), 1655 (CON). MS (EI, 70 eV) m/z: 491 $[M]^{+\cdot}$, 426, 377, 343, 326, 121 $[CpFe]^+$. HRMS (ESI, $C_{31}H_{33}FeNO$: $[M]^+$) calcd.: 491.19061, found: 491.18981. Anal. Calcd. for $C_{31}H_{33}FeNO$: C, 75.76; H, 6.76; N, 2.85. Found: C, 75.59; H, 6.83; N, 2.82.

4.8. 1-(4-Hydroxyphenyl)-1-(4-pivaloyloxyphenyl)-2-ferrocenyl-but-1-ene **14** and 1,1-Bis(4-pivaloyloxyphenyl)-2-ferrocenyl-but-1-ene, **15**

In a Schlenk tube, ferrociphenol **2** (1.272 g, 3 mmol) was dissolved in anhydrous THF, then sodium hydride (0.16 g, 4 mmol, 60%

suspension in oil) was added. After 10 min under stirring, trimethylacetyl chloride (0.362 g, 0.37 mL, 3 mmol) was added and the mixture was stirred for 3 h. The mixture was poured into water, extracted twice with dichloromethane and concentrated under reduced pressure. Then the residue was chromatographed on a silica gel column. The compounds were first eluted by dichloromethane/petroleum ether 1/1, and after by dichloromethane. 1-(4-Hydroxyphenyl)-1-(4-pivaloyloxyphenyl)-2-ferrocenyl-but-1-ene **14** (undeterminated 55/45 mixture of *Z* and *E* isomers) and 1,1-bis-(4-pivaloyloxyphenyl)-2-ferrocenyl-but-1-ene **15** were obtained in 34% and 28% yields, respectively, after recrystallization from diethyl ether/pentane. **14**: ^{1}H NMR ($CDCl_3$): δ 0.93 and 0.94 (t, $J = 7.4$ Hz, 3H, CH_3), 1.26 and 1.28 (s, 9H, ^{t}Bu), 2.48 and 2.52 (q, $J = 7.4$ Hz, 2H, CH_2), 3.83 and 3.84 (t, $J = 1.9$ Hz, 2H, C_5H_4), 3.98 and 3.99 (t, $J = 1.9$ Hz, 2H, C_5H_4), 4.01 and 4.02 (s, 5H, Cp), 5.80 and 5.90 (s, 1H, OH), 6.57 and 6.68 (d, $J = 8.5$ Hz, 2H, C_6H_4), 6.81 (d, $J = 8.5$ Hz, 2H, C_6H_4), 6.94 and 7.12 (d, $J = 8.5$ Hz, 2H, C_6H_4), 6.96 (d, $J = 8.5$ Hz, 2H, C_6H_4). ^{13}C NMR ($CDCl_3$): δ 15.4 and 15.5 (CH_3), 27.2 (3 CH_3, ^{t}Bu), 27.9 and 28.1 (CH_2), 39.1 (C ^{t}Bu), 68.0 and 68.1 (2 CH, C_5H_4), 68.1 and 68.2 (2 CH, C_5H_4), 69.2 and 69.3 (5 CH, Cp), 86.8 and 86.9 (C_{ipso}), 115.1 and 115.2 (2 CH, C_6H_4), 121.0 and 121.2 (2 CH, C_6H_4), 130.3 and 130.7 (2 CH, C_6H_4), 130.9 and 131.2 (2 CH, C_6H_4), 136.5 and 136.7 (C), 137.3 and 137.6 (C), 142.2 and 142.4 (C), 149.2 and 149.3 (C), 154.4 (2C), 177.4 (CO). IR (KBr, ν cm^{-1}): 3437 (OH), 3093, 3035, 2970, 2927, 2873 (CH_2, CH_3), 1732 (CO). MS (EI, 70 eV) *m/z*: 508 $[M]^+$, 443, 359, 121 $[CpFe]^+$, 57 $[^tBu]^+$. Anal. Calcd. for $C_{31}H_{32}FeO_3$ $(H_2O)_{0.2}$: C, 72.72; H, 6.38. Found: C, 72.68; H, 6.33. 1,1-bis-(4-pivaloyloxyphenyl)-2-ferrocenyl-but-1-ene, **15**: mp: 191 °C ^{1}H NMR ($CDCl_3$): δ 0.94 (t, $J = 7.2$ Hz, 3H, CH_3), 1.26 (s, 9H, ^{t}Bu), 1.29 (s, 9H, ^{t}Bu), 2.47 (q, $J = 7.2$ Hz, 2H, CH_2), 3.90 (s, 2H, C_5H_4), 4.07 (s, 7H, C_5H_4 + Cp), 6.83 (d, $J = 8.5$ Hz, 2H, C_6H_4), 6.95 (d, $J = 8.5$ Hz, 2H, C_6H_4), 6.97 (d, $J = 8.5$ Hz, 2H, C_6H_4), 7.11 (d, $J = 8.5$ Hz, 2H, C_6H_4). ^{13}C NMR ($CDCl_3$): δ 15.4 (CH_3), 27.1 (6 CH_3, ^{t}Bu), 28.1 (CH_2), 39.1 (2C), 68.6 (2 CH, C_5H_4), 69.5 (2 CH, C_5H_4 + 5 CH, Cp), 87.2 (C_{ipso}), 121.1 (2 CH, C_6H_4), 121.3 (2 CH, C_6H_4), 130.4 (2 CH, C_6H_4), 131.0 (2 CH, C_6H_4), 136.2 (C), 138.4 (C), 141.5 (C), 141.7 (C), 149.4 (C), 149.5 (C), 177.0 (2 CO). IR (KBr, ν cm^{-1}): 3093, 2970, 2931, 2873 (CH_2, CH_3), 1751 (CO). MS (EI, 70 eV) *m/z*: 592 $[M]^{+\cdot}$, 527, 443, 121 $[CpFe]^+$, 57 $[^tBu]^+$. Anal. Calcd. for $C_{36}H_{40}FeO_4$: C, 72.97; H, 6.8. Found: C, 73.36; H, 7.01.

4.9. 1,1-Bis-(4-palmitoyloxyphenyl)-2-phenyl-but-1-ene, **23**

In a Schlenk tube, diphenol **18** (0.95 g, 3 mmol) was dissolved in anhydrous THF, then sodium hydride (0.30 g, 7.55 mmol, 60% suspension in oil) was added. After 10 min under stirring, palmitoyl chloride (1.814 g, 2 mL, 6.6 mmol) was added and the mixture was stirred for 3 h. 5 mL of ethanol was added and the stirring was continued for 1 h in order to destroy the excess of palmitoyl chloride. The mixture was poured into water, extracted twice with dichloromethane and concentrated under reduced pressure. Then the residue was chromatographed on silica gel column using dichloromethane/petroleum ether 1/1 as an eluent. The product was recrystallized from diethyl ether to yield **23** in a 96% yield as a white solid. Mp: 75 °C ^{1}H NMR ($CDCl_3$): δ 0.81 (t, $J = 7.0$ Hz, 6H, CH_3), 0.85 (t, $J = 7.4$ Hz, 3H, CH_3), 1.09–1.41 (m, 48H, CH_2), 1.53–1.76 (m, 4H, CH_2), 2.32–2.55 (m, 6H, CH_2), 6.65 (d, $J = 8.7$ Hz, 2H, C_6H_4), 6.78 (d, $J = 8.7$ Hz, 2H, C_6H_4), 6.96–7.20 (m, 9H, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 13.5 (CH_3), 14.1 (2 CH_3), 22.7 (2 CH_2), 25.0 (2 CH_2), 29.1 (2 CH_2), 29.2 (CH_2), 29.3 (2 CH_2), 29.4 (2 CH_2), 29.5 (2 CH_2), 29.7 (6 × 2 CH_2), 31.9 (2 CH_2), 34.4 (2 CH_2), 120.4 (2 CH, C_6H_4), 121.2 (2 CH, C_6H_4), 126.3 (CH, C_6H_5), 127.9 (2 CH_{arom}), 129.6 (2 CH_{arom}), 130.5 (2 CH_{arom}), 131.7 (2 CH_{arom}), 137.0 (C), 140.2 (C), 140.6 (C), 141.8 (C), 143.0 (C), 148.7 (C), 149.5 (C), 172.1 (CO), 172.3 (CO). IR (KBr, ν cm^{-1}): 3047, 2916, 2850 (CH_2, CH_3), 1759 (CO). MS (CI, NH_3) *m/z*: 792 $[M]^+$, 810 $[M + NH_4]^+$. Anal. Calcd. for $C_{54}H_{80}O_4$: C, 81.76; H, 10.16. Found: C, 81.51; H, 10.37.

4.10. 1-[4-(3-Dimethylamoniumpropoxy)phenyl]-1-(4-hydroxyphenyl)-2-ferrocenyl-but-1-ene citrate, **26**

1 (0.509 g, 1 mmol) was dissolved into 10 mL of THF. 40 mL of diethyl ether was added. A solution of citric acid (0.189 g, 0.9 mmol), in THF (5 mL) was added dropwise into the first solution. A yellow precipitate was immediately formed. After stirring for 20 min, the solution was stripped off by syringe and the yellow solid was washed with 10 mL of diethyl ether and dried under vacuum. 0.550 g of **26** were obtained (85% yield). ^{1}H NMR (CD_3OD) ($Z/E \cong 50/50$): δ 0.98 (t, $J = 7.3$ Hz, 3H, CH_3 for one isomer), 1.00 (t, $J = 7.3$ Hz, 3H, CH_3 for one isomer), 2.21 (broad m, 2H, CH_2), 2.60 (q, $J = 7.3$ Hz, 2H, CH_2 for one isomer), 2.74 (d, $J = 15.3$ Hz, 2H, CH_2), 2.79 (d, $J = 15.3$ Hz, 2H, CH_2), 2.85 and 2.86 (2s, 6H, $NMe2H^+$), 3.24 (broad m, 2H, CH_2N), 3.87 (t, $J = 1.9$ Hz, 2H, C_5H_4 for one isomer), 3.89 (t, $J = 1.9$ Hz, 2H, C_5H_4 for one isomer), 4.04 (m, 4H, CH_2O + C_5H_4), 4.09 (s, 5H, Cp), 6.61 (d, 2H, H_{arom} for one isomer), 6.73 (d, 2H, H_{arom} for one isomer), 6.76 (d, 2H, H_{arom} for one isomer), 6.79 (d, 2H, H_{arom} for one isomer), 6.90 (d, 4H, H_{arom}), 6.98 (d, 2H, H_{arom} for one isomer), 7.10 (d, 2H, H_{arom} for one isomer). ^{13}C NMR (CD_3OD): δ 15.9 (CH_3), 25.7 (CH_2), 29.1 (CH_2), 43.6 ($NMe2H^+$), 45.3 (CH_2), 56.7 (CH_2N), 66.1 (CH_2O), 68.9 (2 CH, C_5H_4), 70.1 (5 CH, C_p), 70.3 (2 CH, C_5H_4), 74.6 (C_q, citrate), 88.7 (C_{ipso} C_5H_4), 115.1 (2 CH, C_6H_4 for one isomer), 115.3 (2 CH, C_6H_4 for one isomer), 115.9 (2 CH for one isomer), 116.0 (2 CH, 115.1 2 CH for one isomer), 131.6 (2 CH, C_6H_4), 132.2 (2 CH, C_6H_4), 137.7 I, 137.8 I, 138.8 I, 139.2 I, 156.9 I, 158.3 I, 175.9 (2 × CO), 180.3 (CO). IR (KBr, ν cm^{-1}): 3421 (OH), 3093, 3032, 2966, 2873 (CH_2, CH_3), 1720 (CO). **26** contains traces of solvents (THF and diethyl ether).

4.11. 1,1-Bis-[4-(3-Dimethylamoniumpropoxy)phenyl]-phenyl]-2-ferrocenyl-but-1-ene citrate **27**

10 (0.594 g, 1 mmol) was dissolved into 15 mL of THF and 15 mL of diethyl ether. A solution of citric acid (0.105 g, 0.5 mmol), in THF (5 mL) was added dropwise into the first solution. An orange precipitate was immediately formed. After stirring for 20 min, the mixture was filtered under argon and the orange solid obtained was washed with 3 × 5 mL of diethyl ether and dried under vacuum. 0.338 g of **27** were obtained (84% yield). ^{1}H NMR (CD_3OD): δ 0.99 (t, $J = 7.4$ Hz, 3H, CH_3), 1.86 (broad m, 4H, 2 CH_2), 2.61 (q, $J = 7.4$ Hz, 2H, CH_2), 2.66 (d, $J = 15.3$ Hz, 2H, CH_2), 2.75 (d, $J = 15.3$ Hz, 2H, CH_2), 2.86 and 2.87 (2 s, 12H, 2 NMe_2H^+), 3.30 (broad m, 4H, 2 CH_2N), 3.87 (m, 2H, C_5H_4), 4.02 (m, 6H, 2 CH_2O + C_5H_4), 4.08 (s, 5H, Cp), 6.75 (d, $J = 8.1$ Hz, 2H, C_6H_4), 6.90 (m, $J = 8.1$ Hz, 4H, C_6H_4), 7.08 (d, $J = 8.1$ Hz, 2H, C_6H_4). ^{13}C NMR (CD_3SOCD_3): δ 15.3 (CH_3), 24.3 (2 CH_2), 42.8 (2 NMe_2H^+), 44.3 (2 CH_2 citrate), 54.5 (2 CH_2N), 66.9 (2 CH_2O), 67.8 (2 CH, C_5H_4), 68.6 (2 CH, C_5H_4), 69.0 (5 CH Cp), 71.4 (C_q, citrate), 86.0 (C_{ipso} C_5H_4), 114.1 (2 × 2 CH, C_6H_4), 129.9 (2 CH, C_6H_4), 130.3 (2 CH, C_6H_4), 136.2 I, 136.4 I, 136.9 I, 137.1 I, 156.6 (2C), 171.5 (2 × CO), 176.9 (CO). IR (KBr, ν cm^{-1}): 3421 (OH), 3032, 2962, 2877, (CH_2, CH_3), 1720 (CO). **27** crystals contain traces of solvents (THF and diethyl ether).

4.12. In vitro antibacterial activity

Three microorganisms, obtained from "Biotechnology Center of Sfax", were used: *P. aeruginosa* ATCC 15442, *S. aureus* ATCC 9144 and *C. albicans*. ATCC 10231. Antimicrobial activity was determined by the tube dilution method. Two fold dilutions of the compound were prepared in the Plate count agar liquid. A suspension of the standard microorganisms, prepared from 24 h cultures of bacteria in the liquid plate count agar at a concentration of 10^5 CFU/mL (Colony Forming Unit.mL^{-1}), were added to each dilution in a 1:1 ratio. Growth (or lack thereof) of the microorganisms was determined visually after incubation for 24 h at 30 °C. The lowest

concentration at which there was no visible growth (turbidity) was taken as the MIC. Then from each tube, one loopful was cultured on Plate count agar and incubated for 24 h at 30 °C. The lowest concentration of the compound supporting no colony formation was defined as the MBC. Doxycycline was used as standard antibiotic. Solutions of the test compounds and doxycycline were prepared in ethanol at concentration of 2 mg mL^{-1}. The two fold dilutions of the compounds were prepared (200, 100, 50, 25, 12.5 μg mL^{-1}). The microorganism suspensions at 10^5 CFU/mL concentrations were inoculated into the corresponding test tube.

4.13. Measurement of octanol/water partition coefficient (log $P_{o/w}$)

The log $P_{o/w}$ values of the compounds were determined by reverse-phase HPLC on a C-8 column (Kromasil C8, from Macherey Nagel, France) according to the method previously described by Minick [55] and Pomper log $P_{o/w}$. Measurement of the chromatographic capacity factors (k0) for each compounds were done at various concentrations in the range 95–70% methanol (containing 0.25% octanol) and an aqueous phase consisting of 0.15% n-decylamine in 0.02 M MOPS (3-morpholinopropanesulfonic acid) buffer pH 7.4 (prepared in 1-octanol-saturated water). These capacity factors (k′) are extrapolated to 100% of the aqueous component given the value of k'_w. log $P_{o/w}$ (y) is then obtained by the formula: $\log \log P_{o/w} = 0.13418 + 0.98452 \times \log k'w$.

4.14. Antiproliferative tests on hormone-independent breast cancer cells MDA-MB-231

Stock solutions (1×10^{-2} M) and serial dilutions of the compounds to be tested were prepared in DMSO just prior to use. Dulbecco's modified eagle medium (DMEM), fetal calf serum, glutamine and kanamycine were obtained from Invitrogen. MDA-MB-231 cells were from ATCC LGC standards. Cells were maintained in a monolayer culture in DMEM with phenol red/Glutamax I supplemented with 9% fetal bovine serum at 37 °C in a 5% CO_2/air-humidified incubator. For proliferation assays, MDA-MB-231 cells were plated in 1 mL of DMEM without phenol red, supplemented with 9% decomplemented and hormone-depleted fetal bovine serum, 0.9% kanamycin, 0.9% Glutamax I and incubated. The following day (D0), 1 mL of the same medium containing the compounds to be tested was added to the plates. After 3 days (D3) the incubation medium was removed and 2 mL of the fresh medium containing the compounds was added. After 5 days the total protein content of the plate was analyzed as follows: cell monolayers were fixed for 1 h at room temperature with methylene blue (1 mg mL^{-1} in 50:50 water/MeOH mixture), then washed with water. After addition of HCl (0.1 M, 2 mL), the plate was incubated for 1 h at 37 °C and then the absorbance of each well (three wells for each concentration) was measured at 655 nm with a Biorad microplate reader. The results are expressed as the percentage of proteins vs. the control. Experiments were performed at least in duplicate.

Acknowledgements

We thank the Ministère de la Recherche and the Centre National de la Recherche Scientifique for financial support, Mrs Barbara McGlinchey for helpful discussions. M. El Arbi thanks the Ministère de l'Enseignement Supérieur et de la Recherche Scientifique of Tunisia for financial support and ISET-Sfax for technical support.

References

[1] G. Jaouen, A. Vessières, I.S. Butler, Acc. Chem. Res. 26 (1993) 361–369.
[2] N. Metzler-Nolte, Angew. Chem. Int. Ed. Engl. 40 (2001) 1040–1043.
[3] R.H. Fish, G. Jaouen, Organometallics 22 (2003) 2166–2177.
[4] G. Jaouen, S. Top, A. Vessières, G. Leclercq, M.J. McGlinchey, Curr. Med. Chem. 11 (2004) 2505–2517.
[5] A. Vessières, S. Top, W. Beck, E.A. Hillard, G. Jaouen, Dalton Trans. 4 (2006) 529–541.
[6] G. Jaouen, S. Top, A. Vessières, in: G. Jaouen (Ed.), Bioorganometallics, Wiley-VCH, Weinheim, 2006, pp. 65–95.
[7] D.R. van Staveren, N. Metzler-Nolte, Chem. Rev. 104 (2004) 5931–5985.
[8] C.G. Hartinger, P.J. Dyson, Chem. Soc. Rev. 38 (2009) 391–401.
[9] M.F.R. Fouda, M.M. Abd-Elzaher, R.A. Abdelsamaia, A.A. Labib, Appl. Organomet. Chem. 21 (2007) 613–625.
[10] N. Chavain, C. Biot, Curr. Med. Chem. 17 (2010) 2729–2745.
[11] S. Top, A. Vessières, G. Leclercq, J. Quivy, J. Tang, J. Vaissermann, M. Huché, G. Jaouen, Chem. Eur. J. 9 (2003) 5223–5236.
[12] A. Vessières, S. Top, P. Pigeon, E.A. Hillard, L. Boubeker, D. Spera, G. Jaouen, J. Med. Chem. 48 (2005) 3937–3940.
[13] A. Nguyen, E.A. Hillard, A. Vessières, S. Top, P. Pigeon, G. Jaouen, Chimia 61 (2007) 716–725.
[14] P. Pigeon, S. Top, O. Zekri, E.A. Hillard, A. Vessières, M.-A. Plamont, O. Buriez, E. Labbé, M. Huché, S. Boutamine, C. Amatore, G. Jaouen, J. Organomet. Chem. 694 (2009) 895–901.
[15] M. Gormen, D. Plazuk, P. Pigeon, E.A. Hillard, M.-A. Plamont, S. Top, A. Vessières, G. Jaouen, Tetrahedron Lett. 51 (2010) 118–120.
[16] C. Biot, G. Glorian, L.A. Maciejewski, J.S. Brocard, J. Med. Chem. 40 (1997) 3715–3718.
[17] C. Biot, L. Delhaes, C.M. N'Diaye, L.A. Maciejewski, D. Camus, D. Dive, J.S. Brocard, Bioorg. Med. Chem. 7 (1999) 2843–2847.
[18] C. Biot, L. Delhaes, L. Maciejewski, M. Mortuaire, D. Camus, D. Dive, J. Brocard, Eur. J. Med. Chem. 35 (2000) 707.
[19] C. Biot, Anti-Infect. Agents. Curr. Med. Chem. 3 (2004) 135–147.
[20] D. Dive, C. Biot, ChemBioChem 3 (2008) 383–391.
[21] J. Guillon, S. Moreau, E. Mouray, V. Sinou, I. Forfar, S.B. Fabre, V. Desplat, P. Millet, D. Parzy, C. Jarry, P. Grellier, Bioorg. Med. Chem. 16 (2008) 9133–9144.
[22] N. Hüsken, G. Gasser, S.D. Köster, N. Metzler-Nolte, Bioconjug. Chem. 20 (2009) 1578–1586.
[23] E.I. Edwards, R. Epton, G. Marr, J. Organomet. Chem. 107 (1976) 351–357.
[24] B. Murukan, K. Mohanan, Transition Met. Chem. 31 (2006) 441–446.
[25] J.R. Anacona, A. Rodriguez, Transition Met. Chem. 30 (2005) 897–901.
[26] D. Razafimahefa, D.A. Ralambomanana, L. Hammouche, L. Pélinski, S. Lauvagie, C. Bebear, J. Brocard, J. Maugein, Bioorg. Med. Chem. Lett. 15 (2005) 2301–2303.
[27] D.A. Ralambomanana, D. Razafimahefa-Ramilison, A.C. Rakotohova, J. Maugein, L. Pélinski, Bioorg. Med. Chem. 16 (2008) 9546–9553.
[28] J. Fang, Z. Jin, Z. Li, W. Liu, J. Organomet. Chem. 674 (2003) 1–9.
[29] I. Damljanovic, M. Vukicevic, N. Radulovic, R. Palic, E. Ellmerer, Z. Ratkovic, M.D. Joksovic, R.D. Vukicevic, Bioorg. Med. Chem. Lett. 19 (2009) 1093–1096.
[30] J. Zhang, Appl. Organomet. Chem. 22 (2008) 6–11.
[31] J.T. Chantson, M.V.V. Falzacappa, S. Crovella, N. Metzler-Nolte, J. Organomet. Chem. 690 (2005) 4564–4572.
[32] R. Malhotra, J. Mehta, J.K. Puri, Cent. Eur. J. Chem. 5 (2007) 858–867.
[33] S.K. Sengupta, O.P. Pandey, A.K. Srivastava, A. Bhatt, K.N. Mishra, Transition Met. Chem. 24 (1999) 703–707.
[34] C. Biot, N. Francois, L. Maciejewski, J. Brocard, D. Poulain, Bioorg. Med. Chem. Lett. 10 (2000) 839–841.
[35] M. Kothari, S.R. Simon, Cytokine 35 (2006) 115–125.
[36] R.R. Chhipa, S. Singh, S.V. Surve, M.V. Vijayakumar, M.K. Bhat, Toxicol. Appl. Pharmacol. 202 (2005) 268–277.
[37] R.S. Fife, G.W. Sledge, B.J. Roth, C. Proctor, Cancer Lett. 127 (1998) 37–41.
[38] K. Dolan, S. Montgomery, B. Buchheit, L. DiDone, M. Wellington, D.J. Krysan, Antimicrob. Agents Chemother. 53 (2009) 3337–3346.
[39] W.H. Beggs, Res. Commun. Chem. Pathol. Pharmacol. 80 (1993) 125–128.
[40] F. Timurkaynak, F. Can, ÖK. Azap, M. Demirbilek, H. Arslan, S. Ö Karaman, Int. J. Antimicrob. Agents 27 (2006) 224–228.
[41] M.A. Pfaller, M. Castanheira, H.S. Sader, R.N. Jones, Int. J. Antimicrob. Agents 35 (2010) 282–287.
[42] L. Ates, C. Hanssen-Hübner, D.E. Norris, D. Richter, P. Kraiczy, K.-P. Hunfeld, Ticks Tick Borne. Dis. 1 (2010) 30–34.
[43] G. Jaouen, S. Top, A. Vessières, G. Leclercq, J. Quivy, L. Jin, A. Croisy, C. R. Acad. Sci. Paris, Série IIc (2000) 89–93.
[44] O. Buriez, E. Labbé, P. Pigeon, G. Jaouen, C. Amatore, J. Electroanal. Chem. 619–620 (2008) 169–175.
[45] E.A. Hillard, P. Pigeon, A. Vessières, C. Amatore, G. Jaouen, Dalton Trans. (2007) 5073–5081.
[46] S. Top, A. Vessières, C. Cabestaing, I. Laios, G. Leclercq, C. Provot, G. Jaouen, J. Organomet. Chem. 637 (2001) 500–506.
[47] O. Zekri, E.A. Hillard, S. Top, A. Vessières, P. Pigeon, M.-A. Plamont, M. Huché, S. Boutamine, M.J. McGlinchey, H. Müller-Bunz, G. Jaouen, Dalton Trans. (2009) 4318–4326.
[48] E. Allard, N.T. Huynh, A. Vessières, P. Pigeon, G. Jaouen, J.P. Benoit, C. Passirani, Int. J. Pharm. 379 (2009) 317–323.
[49] P.L. Coe, C.E. Scriven, J. Chem. Soc. Perkin 1 (1986) 475–477.
[50] K. Nikitin, Y. Ortin, H. Müller-Bunz, M.-A. Plamont, G. Jaouen, A. Vessières, M.J. McGlinchey, J. Organomet. Chem. 695 (2010) 595–608.
[51] S. Top, A. Vessieres, G. Jaouen, R.H. Fish, Organometallics 25 (2006) 3293–3296.

[52] D. Plazuk, A. Vessières, E.A. Hillard, O. Buriez, E. Labbé, P. Pigeon, M.-A. Plamont, C. Amatore, J. Zakrzewski, G. Jaouen, J. Med. Chem. 52 (2009) 4964–4967.
[53] S. Top, A. Vessières, P. Pigeon, M.N. Rager, M. Huché, E. Salomon, C. Cabestaing, J. Vaissermann, G. Jaouen, ChemBioChem 5 (2004) 1104–1113.
[54] J.H. Jorgensen, in: P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, R.H. Yolken (Eds.), Manual of Clinical Microbiology, ASM Press, Washington DC, 1999, pp. 1467–1662.
[55] D.J. Minick, J.H. Frenz, M.A. Patrick, D.A. Brent, J. Med. Chem. 31 (1988) 1923–1933.