APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. 2006; **20**: 112–116
Bioorganometallic Chemistry AOC
Published online 21 November 2005 in Wiley InterScience (www.interscience.wiley.com). DOI:10.1002/aoc.1018

Antibacterial and antifungal ferrocene incorporated dithiothione and dithioketone compounds

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Received 14 September 2005; Accepted 14 October 2005

Two antibacterial and antifungal ferrocene incorporated compounds have been synthesized, characterized and screened for their *in vitro* antibacterial activity against *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi, Shigella dysenteriae, Bacillus cereus, Corynebacterium diphtheriae, Staphylococcus aureus* and *Streptococcus pyogenes* bacterial strains and for *in vitro* antifungal activity against *Trichophyton longifusus, Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani* and *Candida glaberata*. Results show that these compounds have significant activity against tested bacterial and fungal strains and thus introduce a novel class of ferrocene incorporating antibacterial and antifungal compounds. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: antibacterial; antifungal; ferrocenes; dithiothione; dithioketone

INTRODUCTION

The development and design of novel compounds having potentials for use as antibacterial and antifungal agents has gained renewed interest, especially within the growing area[1–3] of research into 'metal-based drugs'. Sulfur donor ligands as organic donor molecules, mainly derived from tetrathiafulvalene and dithiolates, are known[4,5] to have potential as pesticides and herbicides, when these are interacted with suitable metals or metal-containing moieties forming planar compounds.

In order to increase the potentiality of drugs, our previous studies have indicated[6–11] that incorporation of metals or some metal-based systems into the molecules of antibacterial or antifungal drugs not only enhances the bactericidal or fungicidal properties, but those compounds which have less potential are also improved. A few reports have already highlighted[12–15] the use of ferrocene and its derivatives in the design of biologically active compounds,[16–20] but it has not been investigated on a larger scale. Some reports have indicated[21–23] that, if the aromatic group in penicillin and cephalosporin antibiotics is replaced by the ferrocenyl moiety, the bactericidal property of the obtained compound is significantly increased. However, the versatility of ferrocene-containing compounds has led to their recognition[24–26] in medicinal chemistry. These considerations attracted our attention to combining the chemistry of ferrocene with dithiothione (dtt) and dithioketone (dtk) moieties by preparing compounds **1** and **2** (Fig. 1) and studying their further antibacterial/antifungal properties in this new area of ferrocene-incorporated dithiolates.

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EXPERIMENTAL

Chemical preparation and analysis

^{1}H-NMR spectra were obtained on a Bruker 250 MHz instrument. Ten-second pulse delays were utilized in the acquisition of the ^{13}C-NMR. IR spectra were recorded on Phillips analytical PU900 and Nicolet 205 Fourier-transform instruments. Butterworth Laboratories Ltd, Middlesex, carried out CHN analysis. Melting points were measured using a Kofler hot-stage microscope and are uncorrected. Zincate salts, $[Net_4]_2[Zn(dmio)_2]$ and $[Net_4]_2[Zn(dmit)_2]$, were obtained by published procedures[27–29] and had physical properties in agreement with the published values.

*4,5-(1,1′-Ferrocenyldimethylthio)-1,3-dithioketone; $Fc(CH_2)_2$-dtk (**1**)*

A mixture of 1,1′-ferrocenedimethanol (0.65, 3.0 mmol), triethylamine (0.60 g, 6.0 mmol) and dichloromethane (20 cm^3) were cooled in an ice-bath. Thionyl chloride (0.71 g, 6.0 mmol) in dry dichloromethane (20 cm^3) was added into this mixture

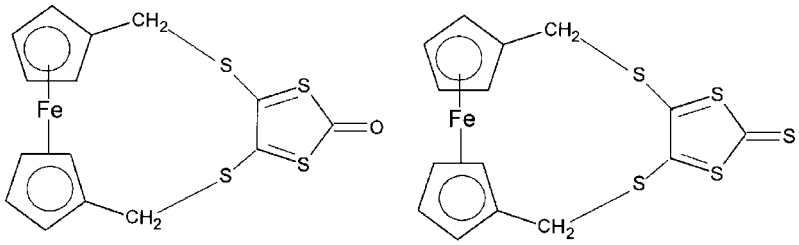


Figure 1. Structure of dithioketone (dtk) and dithiothione (dtt).

and the mixture stirred for 2 h under a slow stream of N_2. The solvents were removed on a rotary evaporator. The solid was redissolved in dry dichloromethane (20 ml) and $[NEt_4]_2[Zn(dmio)_2]$ (1.06 g, 1.25 mmol) in dry dichloromethane (20 ml) was added. The resultant mixture was refluxed overnight under a slow stream of N_2. The reaction mixture was cooled to room temperature, the solvent was evaporated and oil was obtained. The oil was chromatographed on a silica gel column using petroleum ether (b.p 40–60 °C): dichloromethane (70 : 30) as eluent. After removing the solvent on a rotary evaporator, a red-brown solid was obtained which was recrystallized from dichloromethane; m.p 196–197 °C; yield 0. 42 g (62%). IR (KBr, cm^{-1}) 1665, 1610, 1518, 1465, 1432, 1259, 1244, 1102, 1037, 1022, 995, 830, 735, 685, 505. ^{1}H-NMR ($CDCl_3$, 250 MHz) δ 3.8 [s, 4H, CH_2S], 4.6 [d, 2H, ferrocenyl], 4.7 [d, 2H, ferrocenyl]. ^{13}C-NMR ($CDCl_3$, 63 MHz) δ 38.2 [CH_2], 68.8, 69.5, 83.7 [ferrocenyl], 130.2 [C═C], 190.3 [C═O]. Analysis: found: C, 45.7; H, 3.2. Calculated for $C_{15}H_{12}FeS_4O$: C, 45.9; H, 3.1%.

*4,5-(1,1′-Ferrocenyldimethylthio)-1,3-dithiothione; $Fc(CH_2)_2$-dtt (**2**)*

A mixture of 1,1′-bis(hydroxymethyl)ferrocene (0.65 g, 3.0 mmol), triethylamine (0.6 g, 6.0 mmol) and dichloromethane (20 cm^3) was cooled in an ice-bath. Thionyl chloride (0.71 g, 6.0 mmol) in dry dichloromethane (15 cm^3) was added into this mixture under N_2 at such a rate as to keep the temperature between 15 and 20 °C. After complete addition, the reaction mixture was kept at 20 °C for 30 min and then stirred for another 30 min at 40 °C. Then $[NEt_4]_2[Zn(dmit)_2]$ (0.36 g, 0.0005 mol) in dichloromethane (20 cm^3) was added to it. The reaction mixture was refluxed overnight under N_2. After allowing to cool to room temperature solvent evaporated to give a red-orange solid which was chromatographed over silica gel column using dichloromethane/petroleum ether (b.p. 40–60 °C; 70 : 30) as eluent. After removing the solvent, orange red crystals were obtained which were recrystallized from dichloromethane; m.p 182–185 °C; yield 0.36 g (60%). IR (KBr, cm^{-1}) 2919, 1518, 1432, 1345, 1238, 1214, 1104, 1086, 1060, 1026, 995, 803, 859, 819, 584, 499, 480, 451. ^{1}H-NMR ($CDCl_3$, 250 MHz) δ 3.7 [s, 4H, CH_2S], 4.6 [d, 2H, ferrocenyl], 4.7 [d, 2H, ferrocenyl]. ^{13}C-NMR ($CDCl_3$, 63 MHz) δ 38.4 [CH_2], 69.9, 70.4, 84.5 [ferrocenyl], 139.2 [C═C], 212.7 [C═S]. Analysis: found: C, 43.; H, 3.1. Calculated for $C_{15}H_{12}FeS_5$: C, 43.1; H, 2.9%.

Biological activity: antibacterial *in vitro* bioassay

The two ferrocene compounds were tested against *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi, Shigella dysenteriae, Bacillus cereus, Corynebacterium diphtheriae, Staphylococcus aureus* and *Streptococcus pyogenes* bacterial strains using the agar well diffusion method[30] according to literature protocol.[31,32] Two to eight hour-old bacterial inoculums containing approximately 10^4–10^6 colony-forming units (CFU)/ml were used in these assays. The wells were dug in the media with the help of a sterile metallic borer with centers at least 24 mm. Recommended concentration (100 µl) of the test sample (1 mg/ml in DMSO) was introduced in the respective wells. Other wells supplemented with DMSO and reference antibiotic drug, Imipenum (Fischer Scientific International Inc.), served as negative and positive controls, respectively. The plates were incubated immediately at 37 °C for 20 h. Activity was determined by measuring the diameter of zones showing complete inhibition (mm). Growth inhibition was compared with the standard antibacterial drug, Imipenum. In order to clarify any participating role of DMSO in the biological screening, separate studies were carried out with the solutions alone of DMSO. and they showed no activity against any bacterial strains.

The minimum inhibitory concentration (MIC) of the selected compound, which showed significant activity against selected bacterial strains, was determined using the disc diffusion method.[32] MIC was the lowest concentration of a substance at which the inhibition of the growth occurred.

Biological activity: antifungal *in vitro* bioassay

The antifungal screening of all compounds was carried out against six fungal strains (*Trichophyton longifusus, Candida albican, Aspergillus flavus, Microsporum canis, Fusarium solani* and *Candida glaberate*) according to the literature protocol.[32] Sabouraud dextrose agar (Oxoid, Hampshire, UK) was seeded with 10^5 (cfu) ml^{-1} fungal spore suspension and transferred to Petri plates. Discs soaked in 20 ml (10 µg/ml in DMSO) of each compound were placed at different positions on the

agar surface. The plates were incubated at 32 °C for 7 days. The results were recorded as zones of inhibition in mm and compared with standard antifungal drugs Miconazole and Amphotericin B (Fischer Scientific International Inc.).

RESULTS AND DISCUSSION

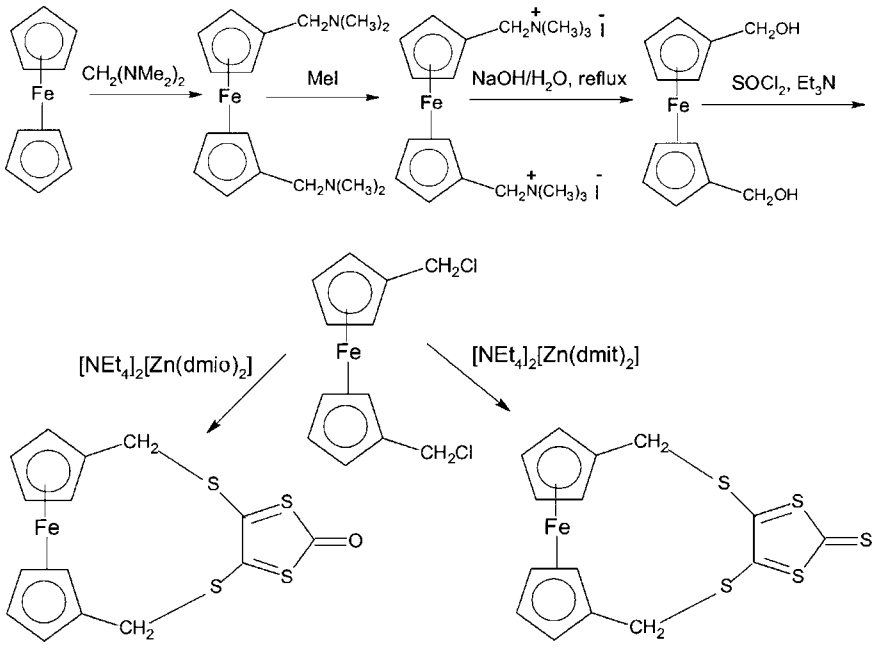
Chemistry

In the past few years, a number of studies have highlighted the use of ferrocene containing molecules in many applications.[33–35] The addition of ferrocene as an extra electron donor site onto the dtk and dtt moieties might not only serve to enhance its potential as antibacterial and antifungal agent but may be of considerable interest within the expanding area of metal-based drug chemistry. Towards these objectives, a successful attempt has been made to incorporate one ferrocene with one dtk and dtt moiety, respectively, by making potential use of both cyclopentadienyl rings of ferrocene, as shown in Scheme 1.

For this purpose, 1,1′-ferrocenedimethanol was prepared from 1,1-bis(ferrocenylmethyl)trimethylammonium iodide by a previously reported method.[36] 1,1′-Ferrocenedimethanol was converted to its bis(chloromethyl) derivative using thionyl chloride in triethylamine. This was followed by *in situ* reaction with the dtk or dtt source[37] $[NEt_4]_2[Zn(dmio)_2]$ or $[NEt_4]_2[Zn(dmit)_2]$. Products obtained were (1,1′-ferrocenyldimethylthio)-1,3-dithioketone and (1,1′-ferrocenyldimethylthio)-1,3-dithiothiole, respectively (Scheme 1). The products were obtained as dark orange needles after purification by washing with dichloromethane through a silica gel column and recrystallizing from dichloromethane. Oxalyl chloride was initially used instead of thionyl chloride as a chlorinating agent for the preparation of chloromethylferrocene, but a mixture of products was achieved which could not be separated. The replacement of the hydroxyl group by chloride in hydroxymethylferrocene using thionyl chloride proved to be more facile and successful.

Compounds **1** and **2** are microcrystalline species, which are soluble in chloroform, acetone and dichloromethane. They are stable in air and light and can be stored at room temperature for indefinite period of time. The structures of these air-stable compounds, **1** and **2**, were characterized by their IR, ^{1}H-NMR, ^{13}C-NMR spectral and elemental analysis data.

Dithiolenes generally display an intense electronic transitions in the near-IR region, the origin of which was discussed by Mueller-Westerhoff *et al*.[38] These intense near-IR absorptions arise from a transition between the highest occupied (HOMO) and lowest unoccupied molecular orbital (LUMO) states. IR of the present ferrocene-incorporated dithiolene compounds (dtk or dtt) showed[39] the absence of bands at 3225 cm^{-1} assigned to OH, demonstrating the conversion of dimethanol groups into dichloromethyl by reacting them with thionyl chloride. *In situ* reaction of dichloromethyl ferrocene with $[Zn(dmio)_2][Net_4]_2$ and $[Zn(dmit)_2][Net_4]_2$ salts, respectively, indicated[40] the presence of carbonyl stretching (C═O) at 1660 cm^{-1} in compound **1** and C═S at 1065 cm^{-1} in **2**, thus confirming[41] that the starting dimethanol or dichloro derivatives no longer existed and had been converted into the expected compounds (**1** and **2**). Moreover, other frequencies[42] at 1410 and 995 cm^{-1} assigned to C═C and C–S and at 1518, 1432 and 803 cm^{-1} due to ferrocene confirmed the formation of **1** and **2**. ^{1}H NMR spectra indicated the values as



Scheme 1. Synthesis of ferrocene incorporated dtt and dtk compounds.

expected,[43] but the ^{13}C NMR spectra showed that C═C values were slightly upfield of the values found for the related DMIO and DMIT compounds. The C═O and C═S values also varied very little.

Antibacterial activity

The results (Table 1) were compared with those of the standard drug Imipenum, which showed severe antibacterial activity against all the bacterial strains assayed. Compound **1** was found to be significantly active against bacterial strains *a*, *b*, *d*, *j* and *k* and, inactive against *c*, *e*, *f*, *g* and *h*. Compound **2** was also found to be significantly active against bacterial species *a*, *b*, *d*, *j* and *k*, weakly active against *e* and *h* and, inactive against *c*, *f* and *g*.

DMSO solutions used as negative controls showed no activity against any bacterial strains. The MIC of these compounds varied from 10 to 100 μg/ml. The results as shown in Table 3 indicate that compound **1** proved to be the most active by inhibiting the growth of the tested organism *j* and **2** against *a* at 10 μg/ml concentration.

Antifungal activity

These results from antifungal screening of the two compounds illustrated in Table 2 indicate that both the synthesized compounds were prominently active against all fungal species, like the standard drugs Miconazole and Amphotericin B.

CONCLUSIONS

The biological activity data of the ferrocene-incorporated dtt and dtk compounds exhibited marked antifungal and antibacterial activities against all the tested bacterial/fungal strains. The compounds generally showed moderate antibacterial activity against two or four species and insignificant activity against one or two species. However, they showed good antifungal activity against most of the species.

Table 1. *In-vitro* antibacterial activity data of 1 and 2

Compound	Diameter of zones showing complete inhibition of growth (mm)									
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*j*	*k*
1	28	23	—	28	—	—	—	—	34	30
2	25	22	—	31	06	—	—	05	32	33
Imipenum	30	30	25	30	32	30	30	25	30	32

15 mm = significant activity; 7–14 mm = moderate activity; <7 mm = weak activity.
a, *Escherichia coli*; *b*, *Klebsiella pneumoniae*; *c*, *Proteus mirabilis*; *d*, *Pseudomonas aeruginosa*; *e*, *Salmonella typhi*; *f*, *Shigella dysenteriae*; *g*, *Bacillus cereus*; *h*, *Corynebacterium diphtheriae*; *j*, *Streptococcus pyogenes*; *k*, *Staphylococcus aureus*. Imipenum = standard drug.

Table 2. *In vitro* antifungal activity data of 1 and 2

Compound	Diameter of zones showing complete inhibition of growth (mm)					
	a	*b*	*c*	*d*	*e*	*f*
1	32	28	32	26	30	32
2	34	25	28	24	32	31
Miconazole	30	20	25	25	30	25
Amphotericin B	30	25	30	25	30	30

>14 mm = significant activity; 7–13 mm = moderate activity; <7 mm = weak activity.
a, *Trichophyton longifusus*; *b*, *Candida albicans*; *c*, *Aspergillus flavus*; *d*, *Microsporum canis*; *e*, *Fusarium solani*; *f*, *Candida glaberata.*
Miconazole and Amphotericin B = standard drugs.

Table 3. Minimum inhibitory concentration (μg/ml) against selected bacterial strains

Compound	*a*	*b*	*d*	*j*	*k*
1	>100	>100	>100	10	>100
2	10	>100	>100	>100	>100

a, *Escherichia coli*; *b*, *Klebsiella pneumoniae*; *d*, *Pseudomonas aeruginosa*; *j*, *Streptococcus pyogenes*; *k*, *Staphylococcus aureus.*

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