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Redox-active Cationic Organoiron Complex: A Promising Lead Structure for Developing Antimicrobial Agents with Activity Against Gram-positive Pathogens including methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium*

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Abstract

We report a new class of antimicrobial agent, a redox-active, cationic organometallic, η^6-arene-η^5-cyclopentadienyliron(II) complex, with activity against Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium*. Structure-property relationship investigations revealed that the antimicrobial activity against these pathogens, especially methicillin-resistant *Staphylococcus aureus*, is tunable. The ability of this new class of antimicrobial agent to induce cellular oxidative stress was confirmed using a dichlorodihydrofluorescein assay. We attributed the induction of oxidative stress as a mechanism that contributes to the overall antimicrobial activity of these compounds. Generally, this antimicrobial agent was non-toxic to BJ fibroblast cell lines at < 128 μg/mL. The η^6-arene-η^5-cyclopentadienyliron(II) complex represent a potential lead structure for the development of a topical antimicrobial therapeutic to combat resistant strains of Gram-positive bacteria.

Introduction

Antimicrobial resistance poses an enormous threat to our society, as an annual 10 million losses in human lives and a 100 trillion USD cost to global economy is projected by 2050.[1] Consequently, there is an accelerated interest in the discovery of new antimicrobial agents that curb the virulence of antimicrobial-resistant microbes. Recent attention in the design of antimicrobial agents has been focused on organometallic molecules,[2-10] with the assumption that the functionality offered by the presence of a metal centre in the molecule will, possibly, impact a new mechanism of action that bypass resistance mechanisms in the drug-resistant bacterium.

An emerging class of organometallic compounds that are explored for overcoming resistance is the metallocene. This class of compounds is increasingly investigated[5-8] and some of these compounds are currently undergoing clinical trials as anticancer[11-15] and antimalarial drugs.[16-19] As an example, ferroquine, a ferrocene analogue of chloroquine, is effective against chloroquine-resistant strains of *Plasmodium falciparum*.[18] A subcellular probe of the *P. falciparum* after treatment with the ferroquine indicates increased reactive oxygen species (ROS), specifically the hydroxyl radical, which was hypothesized to cause oxidative damage to the cells and ultimately kills the parasite.[18] This mechanism of action is linked to the redox chemistry of ferrocene, which under physiological conditions oxidizes to a 17-electron ferrocenium cation that catalyzes the *in vivo* generation of ROS.[7]

The redox-active, cationic η^6-arene-η^5-cyclopentadienyliron(II) complex (Cp-Fe^{II}-arene) is a congener of ferrocene. Unlike ferrocene, which is in the forefront of organometallic medicinal chemistry, this complex is yet to be explored for its biological activity despite its rich redox chemistry. Cp-Fe^{II}-arene reduces to a 19-electron complex, Cp-Fe^{I}-arene, which via electron transfer to O_2 generate $O_2^{-\bullet}$, a ROS.[20,21] Under physiological conditions, $O_2^{-\bullet}$ reacts with thiol-containing proteins, heme peroxidases, or transition metals centres, generating secondary radicals that trigger cellular oxidative stress.[22] Although oxidative stress damages cells, it is a cellular defence strategy employed against a broad spectrum of pathogens.[23-26] Thus, it is plausible to consider Cp-Fe^{II}-arene complex as a potential antimicrobial agent.

In this study, we challenged two problematic drug-resistant Gram-positive bacteria, methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecium* (VRE), as well as other infection-causing microbes that included *Staphylococcus warnerii*, *Candida albicans*, *Pseudomonas aeruginosa*, and *Proteus vulgaris* with a series of Cp-Fe^{II}-arene complexes. The objective was to assess the antimicrobial activity of these complexes and to gain insight on their relative antimicrobial activity with respect to the well-exploited ferrocene. A structure-property relationship investigation was conducted with the objective of tuning the antimicrobial activity of the molecule. Using a dichlorodihydrofluorescein assay, we assayed for the induction of cellular oxidative stress in MRSA after treatment with Cp-Fe^{II}-arene complexes. The cytotoxicity of these complexes was investigated *in-vitro* by challenge with healthy BJ fibroblast cells. We also examined their activity against human breast adenocarcinoma cells (HTB-26). Compared to ferrocene, which was inactive; these complexes induced cellular oxidative stress and exhibited antimicrobial activity against the Gram-positive bacteria. Structure-property relationship studies revealed that the antimicrobial activity of the complexes is tunable. These results place Cp-Fe^{II}-arene complexes in the portfolio of antimicrobial agents with activity against antimicrobial-resistant strains of bacteria.

Experimental section

Materials

General. All chemicals were purchased from Sigma Aldrich or Alfa Aesar and used without purification. Cyclic voltammetry (CV) was performed using a Princeton Applied Research/EG&G potentiostat/galvanostat Model 263A equipped with a glassy carbon working electrode, platinum counter electrode, and Ag reference electrode. The CV experiments were performed under nitrogen at room temperature.

Representative Synthesis of 2–7. The syntheses of these complexes followed a procedure reported previously.[27-32] The NMR spectra of the complexes agreed with previous reports.[27-32]

Representative Synthesis of 5–7. Syntheses of these bimetallic complexes (**5** and **6**) as well as the oligomer (**7**) were based on a procedure previously reported.[33] The characteristic NMR peaks were observed and agreed with previous reports.[33]

Antibacterial Activity. The antimicrobial activity of the complexes was assayed against methicillin-resistant *Staphylococcus aureus* ATCC 33591 (MRSA), vancomycin-resistant *Enterococcus faecium* EF379 (VRE) as well as *Staphylococcus warnerii* ATCC 17917, *Pseudomonas aeruginosa* ATCC 14210, *Proteus vulgaris* ATCC 12454, and *Candida albicans* ATCC 14035. The assay was carried out in 96-well plates using the Clinical Laboratory Standards Institute microbroth antimicrobial testing protocol.[34,35] Assays were carried out at twelve different concentrations obtained by serial dilution of the initial concentration, 128 μg/mL, to a final concentration, 0.0625 μg/mL, in 2% DMSO. In each plate were eight uninoculated positive controls (media + 2% DMSO), eight untreated negative controls (media + 2% aqueous DMSO + microorganism), and one column containing a concentration range of a control antibiotic. In this assay, vancomycin was used as positive control form MRSA and *S. warneri* while rifampicin, gentamycin, ciprofloxacin and nystatin were used as positive controls for VRE, *P. aeruginosa*, *P. vulgaris*, and *C. albicans*, respectively. The optical density of the plate was recorded using a Thermo Scientific Varioskan Flash plate reader at 600 nm before and after incubation of the plates at 37 °C for 22 hours. For MRSA, bactericidal activity was inferred using AlamarBlue. Twenty-four hours after treatment, AlamarBlue was added to each well at 10% of the culture volume (11 μL in 100 μL). Fluorescent emission at 590 nm was monitored using a Thermo Scientific Varioskan Flash plate reader after excitation at 560 nm. The emission was monitored before Alamar blue was added and 4 hours later.

Cytotoxicity Assay. The toxicity of the compounds against human foreskin BJ fibroblast cells (ATCC CRL-2522) and human breast adenocarcinoma cells (ATCC HTB-26) was carried out as previously reported.[35] Prior to the cytotoxicity assays, the cells were grown to 80% confluency as previously described.[35] At 80% confluency, the cells were counted, diluted and plated into 96 well-treated cell culture plates. The compounds were dissolved in 1% sterile DMSO and a dilution series was prepared for each cell line using

cell culture growth medium of which 10 μL were added to the respective assay plate well to give eight final concentrations that ranged from 128 μg/mL to 1 μg/mL per well that had a final volume of 100 μL. In each plate were four uninoculated positive controls (media + 1% DMSO), four untreated negative controls (Media + 1% DMSO + cells), and one column containing zinc pyrithione or doxorubicin as positive control for BJ fibroblast or HTB-26 cell lines, respectively. The plate that contained the BJ fibroblast were incubated at 37 °C in a humidified atmosphere of 5% CO_2 for 24 hours, while those that contained the HTB-26 cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2 for 72 hours. Twenty-four hours after treatment, AlamarBlue was added to each well at 10% of the culture volume (11 μL in 100 μL). Fluorescent emission at 590 nm was monitored using a Thermo Scientific Varioskan Flash plate reader after excitation at 560 nm. The emission was monitored before AlamarBlue was added and 4 hours later.

Oxidative Stress Assay. The oxidative stress assay, which was based on previously reported protocol,[36-38] was carried out in 96 well plates with the same inoculum density generated using the antimicrobial assay protocol. Prior to plate inoculation, the MRSA inoculum was split by transferring equal volumes into two 50 mL conical centrifuge tubes and both tubes were centrifuged at 10 000 RPM for 5 minutes. The supernatant was discarded and the bacterial pellets resuspended in 10 mL of assay buffer (4.2 g MOPS, 80 mg NH_4NO_3, 4 mg K_2HPO_4 in 1L sterile deionized H_2O), one of which included dichlorodihydrofluorescein (H_2DCF, 4.87 mg/L), and incubated for 30 minutes at room temperature. Bacterial cells were then centrifuged, the cell pellet washed twice with assay buffer, and then resuspended to the original volume in pre-warmed CAMHB media (37°C), of which 90 μL of the H_2DCF treated and untreated bacterial cells were dispensed into 96 well assay plates containing test compounds, a vancomycin dilution series (as per antimicrobial assays), and a 200–25 μM dilution series of H_2O_2. Fluorescent emission at 535 nm was monitored using a Thermo Scientific Varioskan Flash plate reader after excitation at 485 nm. Plates were incubated at 37°C and fluorescence measurements were taken at 0, 0.5, 4, and 24 hrs. The assay results were correct for baseline fluorescence (measurements of untreated controls were subtracted from treated controls) and expressed as a percentage of maximal oxidative stress response relative to H_2O_2 controls.

Results and Discussion

Synthesis of η^6-Arene-η^5-cyclopentadienyliron(II) Complex.

The increasing prevalence of antimicrobial resistance coupled with the decreasing number of effective antibiotics highlights a need for new treatment options. In an effort to identify new antimicrobial agents, organometallic molecules and macromolecules have been explored[2-5,7,8,13,39,40] and metallocene such as ferrocene (**1a**) are front-line candidates.[5,7]

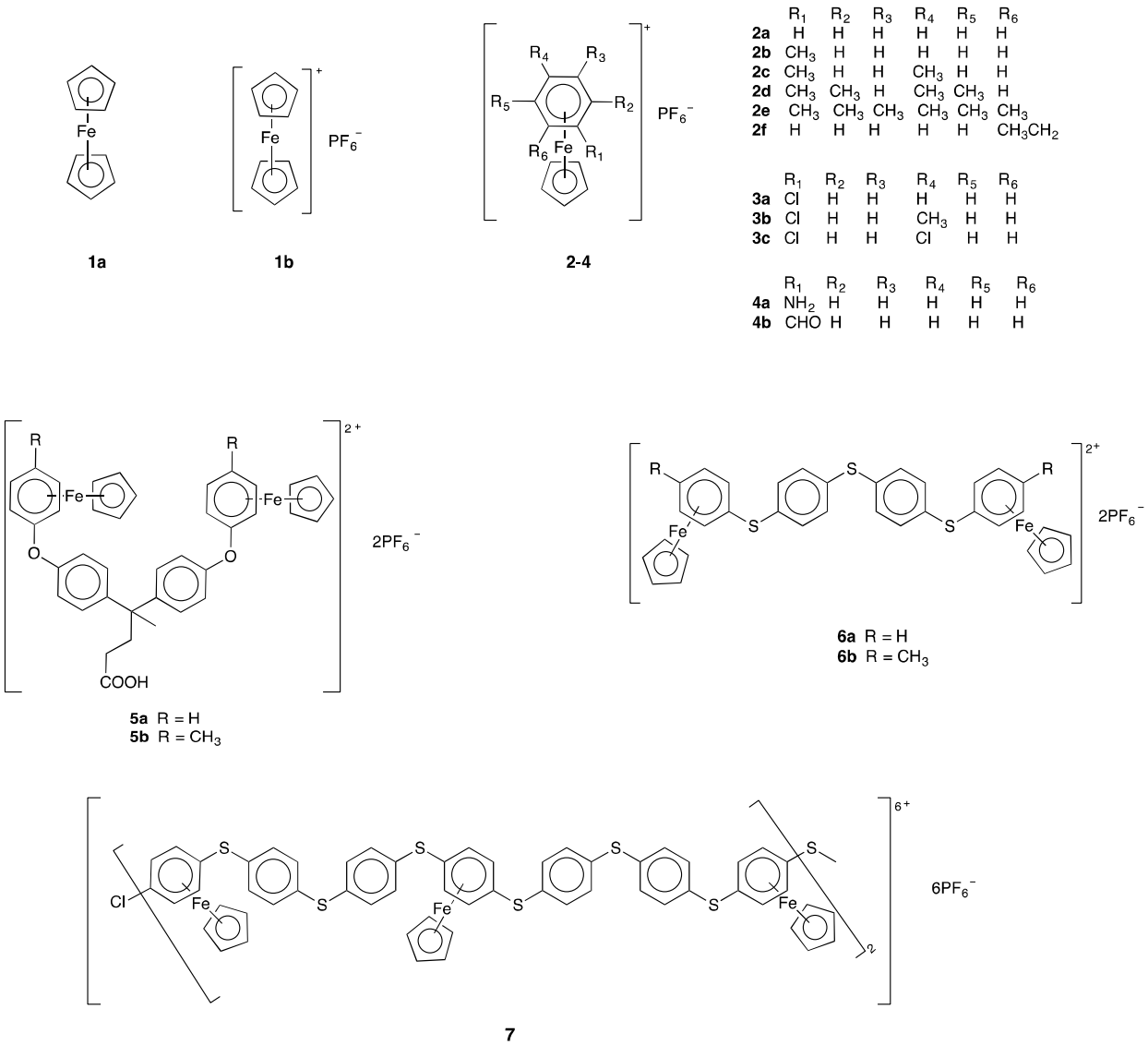

Figure 1. Schematic Representation of Compounds **1–7**.

Here, we screened congeners of ferrocene, η^6-arene-η^5-cyclopentadienyliron(II) (Cp-Fe^{II}-arene) complexes, for their activity against a panel of infection-causing microbes that included resistance strains, MRSA and VRE, with the objective of adding new antimicrobial agents to the current portfolio. This series of Cp-Fe^{II}-arene complexes (**2**–**4**) were obtained through the well-established ligand exchange reaction of the appropriate arene with ferrocene.[27] The chemistry of these complexes is rich and tunable, allowing access to several functional molecules as well as macromolecules.[41] We also screened the antimicrobial activity of the Cp-Fe^{II}-arene complexes at the macromolecular scale using compounds **5**–**7**, which were obtained via nucleophilic aromatic substitution.[33] The cationic nature of **7** prevented gel permeation chromatographic determination of its molecular weight,[33] thus, we used ^{1}H NMR to calculate its number-average molecular weight, which was 3364.79 g/mol.

Antimicrobial Activity.

Antimicrobial activity of the complexes (**2**–**7**) were assayed against two antimicrobial-resistant strains of Gram-positive bacteria, MRSA and VRE, as well as other common microbes, *S. warneri*, *C. albicans*, *P. aeruginosa*, *P. vulgaris*. As a reference, ferrocene (**1a**) and its cationic 17-electron species (**1b**) were also tested. Under these conditions, **1a** and **b** were inactive against all tested microbes (Table 1). Previously, molecular ferrocene was reported to be inactive against pathogens**;** however, it contributed to antimicrobial activity via oxidative damage caused by reactive oxygen species (ROS) that are generated by the redox chemistry of the iron centre.[7] The efficiency of ROS generation is linked with the reduction potential (E_{pc}) of the iron centre.[20,22] For instance, with the Cp-Fe^{II}-arene, the greater the difference between the E_{pc} of the Cp-Fe^{II}-arene/Cp-Fe^{I}-arene couple and that of the $O_2/O_2^{-\bullet}$ couple, the more efficient the generation of ROS.[20] Indeed, the redox chemistry of ferrocene and the Cp-Fe^{II}-arene is well established,[21,30-32,42] being significantly different as confirmed by our CV experiments (Table 1). It is plausible to assume that this difference will affect their antimicrobial activity. Clearly, the inactive ferrocene (**1a**) and its cationic analogue (**1b**) exhibited a positive potential whereas the complexes (**2**–**7**), which had negative potentials, were generally active, being more active against the Gram-positive bacteria including MRSA and VRE (Table 1).

Table 1. Reduction Potential and Antimicrobial Activity of Compounds[a]

Compounds	[b]E_{pc} (V)	IC_{50} /MIC (μM)					
		MRSA	VRE	*S. warnerii*	*P.aeruginosa*	*P.vulgaris*	*C.albicans*
1a	+0.27	-	-	-	-	-	-
1b	+0.22	-	-	-	-	-	-
2a	−1.59	294	161/186	-	-	-	-
2b	−1.64	176/358	165/357	307/358	-	-	-
2c	−1.70	95/172	201/344	93.4/172	-	-	-
2d	−1.72	6.6/10.0	161/320	7.27/10.0	-	-	-
2e	−1.79	174/299	34.4/74.7	257/299	-	299	63.9
2f	−1.65	76.8/172	108/172	139/172	-	-	342
3a	−1.38	10.5/21.1	-	10.3/21.1	-	-	-
3b	−1.44	46.3/81.5	-	25.9/81.5	-	-	-
3c	−1.21	28.9/77.5	-	38.8/77.5	-	-	-
4a	−1.75	11.6/22.3	-	10.1/22.3	-	-	-
4b	−1.64	53.0/86.0	257/344	68.4/86.0	-	-	-
5a	−1.56	93.2/132	-	51.8/132	-	-	-
5b	−1.57	18.0/32.1	122	17.1/32.1	-	-	-
6a	−1.47	29.8/68.5	-	23.7/68.5	72.9	19.3	13.0/17.1
6b	−1.50	11.4/16.6	-	11.3/16.6	-	-	7.04/16.6
7	−1.27	15.3/38.0	1.1/2.3	9.24/19.0	-	-	7.79

[a]The compounds were tested at twelve different concentrations obtained by serial dilution of the initial concentration, 128 μg/mL, to a final concentration, 0.0625 μg/mL, in 2% DMSO. Non-active compounds (-) did not show activity at ≤ 128 μg/mL. [b]Cyclic voltammetry was conducted using nitrogen-purged 6 mM solution of compound in propylene carbonate at room temperature; working electrode, glassy carbon; reference electrode, Ag; counter electrode, Pt; scan rate, 0.1 V/s; supporting electrolyte, 0.1 M [n-Bu_4N][PF_6]; E vs ferrocene (external)

In an effort to tune the antimicrobial activity of these complexes, we investigated their structure-antimicrobial activity relationship. In an approach, electron-donating alkyl group(s) was introduced to the arene nucleus to alter properties such as the redox chemistry of the iron centre as well as the hydrophilic/hydrophobic balance. Indeed, the addition of alkyl groups is a strategy employed in changing

the hydrophilic/hydrophobic balance of molecules with increasing alkyl groups resulting in increase in hydrophobicity.[43] Further, our CV measurements indicated that the addition of alkyl groups changed redox chemistry of the iron centres as shown by their E_{pc} (Table 1). Although interplay of hydrophilic/hydrophobic balance and redox chemistry could alter the antimicrobial activity, which changed after substitutions on the arene nucleus (Table 1), it seems hydrophilic/hydrophobic balance was more critical in tuning the antimicrobial activity of this complexes, especially against MRSA and *S. warnerii*. For instance, **2c** and **2d** had similar E_{pc} but an increase in activity against MRSA and *S. warnerii* was observed for the latter, probably due to the difference in their hydrophilic/hydrophobic balance (Table 1). Further, the more hydrophobic, ethyl-substituted analogue, **2f**, (E_{pc} = −1.65 V; IC_{50} = 76.8 μM) had improved activity against MRSA compared to its methyl analogue, **2b**, (E_{pc} = −1.64 V; IC_{50} = 176 μM). Interestingly, the more hydrophobic complexes, **2e** and **2f**, had a broader spectrum of activity, being active against the Gram-negative bacterium, *P. vulgaris,* as well as the fungus, *C. albicans* (Table 1).

Alkyl groups enhance the hydrophobicity of drugs, increasing their membrane permeability and thus their efficacy. However, they are metabolically susceptible to oxidation, leading to rapid excretion, and possibly, low efficacy.[43] Replacing such susceptible groups with less susceptible groups like the chloro group affords a means to optimizing the efficacy of drugs.[43] To this end, we replaced the methyl group of **2b** with a chloro group (**3a**) (Figure 1). The substitution increased the E_{pc} of **3a** (E_{pc} = −1.34 V) relative to **2b** (E_{pc} = −1.64 V) (Table 1) due to the electron withdrawing nature of the chloro group. It also decreased the lipophicity.[43] After a switch from a methyl group to a chloro group, a noticeable improvement in the antimicrobial activity against MRSA and *S. warnerii* was observed (Table 1). Similarly, **3b** and **c** exhibited increased activity against MRSA and S*. warnerii* compared to **2a** and **c** (Table 1).

We also investigated the effect of other polar functional groups, such as the amino group (**4a**) and aldehyde group (**4b**) on the antimicrobial activity of Cp-Fe^{II}-arene complexes. The presence of polar functional groups enhances hydrophilicity, leading to increased solubility in aqueous media.[43] In addition, the amino group is electron donating, pushing the E_{pc} toward a more negative potential (Table 1). The synergistic effect of decreased E_{pc} and enhanced hydrophilicity may be responsible for the increased activity observed for **4a** against MRSA and *S. warnerii* compared with its methyl-substituted analogue, **2b**

(Table 1). Extending a molecule to the macromolecular scale, changes its structural as well as functional properties. This effect was probed using bimetallic **5** and **6** as well as oligomer **7** as a platform. Generally, the activities were improved and the presence of the heteroatoms impacted not only on the antimicrobial activity but also on the spectrum of activity. For instance, the sulphur-containing **6** and **7** were active against the fungus, *C.albicans*. Cellular respiration of MRSA was absent at MIC concentrations for all the Cp-Fe^{II}-arene complexes as inferred by the lack of reduction of AlamarBlue; confirming that these complexes exerted a bactericidal effect on MRSA.

Oxidative Stress.

Oxidative stress results when an imbalance in the generation of reactive species such as ROS and the defence mechanism of antioxidants leads to cellular damage.[36] Although oxidative stress is implicated in a number of diseases[22] it is a defence strategy against microbes.[23-26] Indeed, various studies suggest that the generation of ROS by some organometallic therapeutics induces oxidative stress, which act in synergy with other bioactive molecules to enhance the biological activity.[7,18] To assess the level of cellular oxidative stress induced by our organometallic complexes, dichlorodihydrofluorescein (H_2DCF) assay was used. Cellular oxidation of the non fluorescent H_2DCF by hydrogen peroxide and various ROS to the fluorescent dichlorofluorescein (DCF) provides an index of oxidative stress within cells.[36-38] Using, a negative control, and hydrogen peroxide as a positive control, we assayed the level of cellular oxidative stress induced by these complexes on MRSA. As a reference, we also assayed vancomycin-treated cells for oxidative stress, as this antibiotic is known to induce stress on cells.[44] The microbe was incubated with H_2DCF at 37 °C for 30 minutes, followed by the addition of the test compounds, incubation at 37 °C for 24 hours, and quantification of oxidative stress using fluorescent plate reader. From the experiments, cellular oxidative stress was detected in cells treated with several of the complexes, hydrogen peroxide and vancomycin (Figure 1). Previously, ferrocene was reported to increase generation of ROS;[7,18] however, in this studies, **1a** did not induce cellular oxidative stress. We attributed this contrasting result to differences in assay protocol with previous assays monitoring specific ROS[7,18] whereas our assay

estimated oxidative stress in general.[37,38] Further, as **1a** was inactive, we attributed the induction of cellular oxidative stress to be an important mechanism of the antimicrobial activity of these complexes.

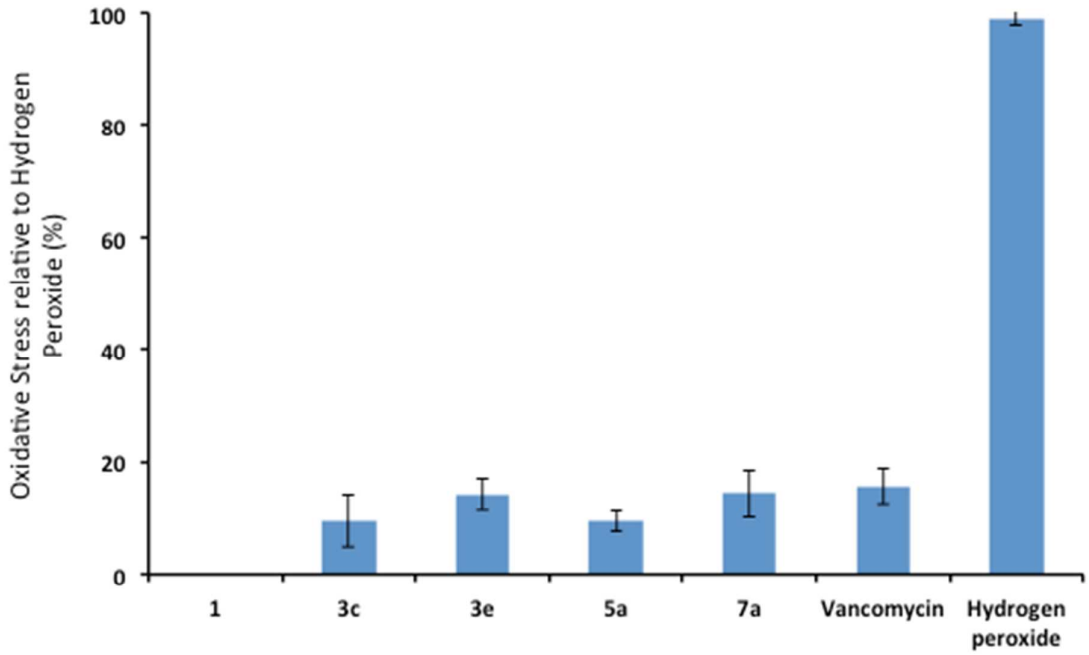

Figure 1. Cellular Oxidative Stress Induced by Representative Complexes

Biocompatibility

While oxidative stress provides a line of defence against microbes, it is also damaging to healthy cells. Moreover, the cationic charge, which is present on these complexes, interacts with the negatively charged mammalian cell membrane, disrupting membrane-dependent functions, and eventually causing cell death.[45,46] Thus, assessing the biocompatibility of these complexes is critical if they are to be used as antimicrobial agents. We assessed the biocompatibility of these complexes using the stress-sensitive skin cells, BJ fibroblast cells, to mine information on potential application of these complexes in topical treatment of microbial skin infections. We also use human breast adenocarcinoma cells (HTB-26) to test the anticancer activity of these complexes. All complexes except **6a, b** and **7** were inactive against BJ fibroblast cells up to 128 μg/mL, the initial concentration (Table 2). It is worth noting that **6a**, **b** and **7** are the sulphur-containing macromolecules. Similarly, these sulphur-containing complexes also exhibited

activity against HTB-26, positioning these complexes as potential anticancer agents. In particular, the oligomer **7** exhibited potent activity against HTB-26 (IC_{50} = 7.67 μM) (Table 2). In addition, **2c–e**, which had 2, 4, and 6 methyl groups, respectively (Table 1), were also active against HTB-26. The presence of the methyl group increases hydrophobicity and membrane permeability, leading to cellular accumulation. The results suggest that similar to antimicrobial activity, the biocompatibility, at least with these cell lines, is tunable.

Table 2. Cytotoxicity of Compounds Against BJ Fibroblast and Human Breast Adenocarcinoma Cells[a]

Compounds	IC_{50} (μM)		Selectivity[b]
	BJ fibroblast	HTB-26	
2c	-	163	-
2d	-	262	-
2e	-	121	-
6a	86.2	32.6	2.9
6b	100.4	53.4	8.8
7	31.7	7.67	2.1

[a]Only cytotoxic compounds are shown; non-toxic compounds did not show activity at ≤ 128 μg/mL. [b]Selectivity = BJ fibroblast IC_{50}/MRSA MIC

Conclusion

Unlike ferrocene, Cp-Fe^{II}-arene complexes were active against drug-resistant MRSA and VRE as well as *S. warnerii*. Structure-activity relationship investigations suggest that activity, especially against MRSA and *S.warnerii*, was tunable. Also of interest is the ability to broaden the spectrum of activity of these complexes by control of the hydrophilic/hydrophobic balance and introduction of certain heteroatoms such as sulphur. Generally, the complexes, except the sulphur-containing ones, were non-cytotoxic as shown by their activity against BJ fibroblast cell line. Overall, our results suggest that by judicious control of parameters such as the nature of heteroatoms and the hydrophilic/hydrophobic balance, the cytotoxicity can be reduced, antimicrobial

activity increased, and the spectrum of activity broadened. The complexes are also potentially active as anticancer platforms. In summary, Cp-Fe^{II}-arene complexes are a new lead structure for the design of bioactive organometallic compounds with tunable activity against, especially antimicrobial-resistant bacteria such as MRSA.

Acknowledgements

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Reference

1. L. J. Shallcross, S. J. Howard, T. Fowler and S. C. Davies, *Philos. Trans. R. Soc. B.* 2015, **370**, 20140082.

2. M. Patra, G. Gasser and N. Metzler-Nolte, *Dalton Trans.,* 2012, **41**, 6350-6358.

3. M. Patra, M. Wenzel, P. Prochnow, V. Pierroz, G. Gasser, J. E. Bandow and N. Metzler-Nolte, *Chem. Sci.,* 2015, **6**, 214-224.

4. P. Lam, G. Lu, K. Hon, K. Lee, C. Ho, X. Wang, J. Tang, K. Lam, R. Wong and S. Kok, *Dalton Trans.,* 2014, **43**, 3949-3957.

5. W. Chen, W. Ou, L. Wang, Y. Hao, J. Cheng, J. Li and Y. Liu, *Dalton Trans.,* 2013, **42**, 15678-15686.

6. H. B. Albada, P. Prochnow, S. Bobersky, J. E. Bandow and N. Metzler-Nolte, *Chem. Sci.,* 2014, **5**, 4453-4459.

7. M. Wenzel, M. Patra, C. H. R. Senges, I. Ott, J. J. Stepanek, A. Pinto, P. Prochnow, C. Vuong, S. Langklotz and N. Metzler-Nolte, *ACS Chem. Biol.,* 2013, **8**, 1442-1450.

8. J. Zhang, Y. P. Chen, K. P. Miller, M. S. Ganewatta, M. Bam, Y. Yan, M. Nagarkatti, A. W. Decho and C. Tang, *J. Am. Chem. Soc.,* 2014, **136**, 4873-4876.

9. D. Nguyen, T. Nguyen, S. A. Rice and C. Boyer, *Biomacromolecules,* 2015, .

10. F. Paladini, M. Pollini, A. Sannino and L. Ambrosio, *Biomacromolecules,* 2015, **16**, 1873-1885.

11. W. Kandioller, E. Balsano, S. M. Meier, U. Jungwirth, S. Göschl, A. Roller, M. A. Jakupec, W. Berger, B. K. Keppler and C. G. Hartinger, *Chem. Commun.,* 2013, **49**, 3348-3350.

12. A. A. Nazarov, D. Gardini, M. Baquié, L. Juillerat-Jeanneret, T. P. Serkova, E. P. Shevtsova, R. Scopelliti and P. J. Dyson, *Dalton Trans.,* 2013, **42**, 2347-2350.

13. A. F. Peacock and P. J. Sadler, *Chem Asian J.,* 2008, **3**, 1890-1899.

14. G. Gasser, I. Ott and N. Metzler-Nolte, *J. Med. Chem.,* 2010, **54**, 3-25.

15. A. C. Fernandes, P. Florindo, D. M. Pereira, P. M. Borralho, C. M. Rodrigues and Minas da Piedade, Maria Fátima, *J. Med. Chem.,* 2015, .

16. F. Dubar, T. J. Egan, B. Pradines, D. Kuter, K. K. Ncokazi, D. Forge, J. Paul, C. Pierrot, H. Kalamou and J. Khalife, *ACS Chem. Biol.,* 2011, **6**, 275-287.

17. C. Biot, F. Nosten, L. Fraisse, D. Ter-Minassian, J. Khalife and D. Dive, *Parasite,* 2011, **18**, 207-214.

18. F. Dubar, C. Slomianny, J. Khalife, D. Dive, H. Kalamou, Y. Guérardel, P. Grellier and C. Biot, *Angew. Chem. Int. Ed.,* 2013, **52**, 7690-7693.

19. D. Dive and C. Biot, *Curr. Top. Med. Chem.,* 2014, **14**, 1684-1692.

20. D. Astruc, J. R. Hamon, E. Roman and P. Michaud, *J. Am. Chem. Soc.,* 1981, **103**, 7502-7514.

21. A. Rapakousiou, Y. Wang, R. Ciganda, J. Lasnier and D. Astruc, *Organometallics,* 2014, **33**, 3583-3590.

22. C. C. Winterbourn, *Nat. Chem. Biol.,* 2008, **4**, 278-286.

23. C. Deffert, J. Cachat and K. Krause, *Cell. Microbiol.,* 2014, **16**, 1168-1178.

24. H. J. Kim, C. Kim, J. Ryu, M. Kim, C. Y. Park, J. M. Lee, M. J. Holtzman and J. Yoon, *Am. J. Respir. Cell Mol. Biol.,* 2013, **49**, 855-865.

25. B. Rada and T. L. Leto, *Contrib. Microbiol.,* 2008, **15**, 164-187.

26. A. P. West, G. S. Shadel and S. Ghosh, *Nat. Rev. Immunol.,* 2011, **11**, 389-402.

27. I. Khand, P. Pauson and W. Watts, *J. Chem. Soc.: C,* 1968, 2257-2260.

28. I. Khand, P. Pauson and W. Watts, *J. Chem. Soc.: C* 1968, 2261-2265.

29. I. Khand, P. Pauson and W. Watts, *J. Chem. Soc.: C* 1969, 116-119.

30. A. S. Abd-El-Aziz, C. R. D. Denus, K. M. Epp, S. Smith, R. J. Jaeger and D. T. Pierce, *Can. J. Chem.,* 1996, **74**, 650-657.

31. A. S. Abd-El-Aziz, K. Winkler and A. S. Baranski, *Inorg. Chim. Acta,* 1992, **194**, 207-212.

32. A. Abd-El-Aziz, A. Baranski, A. Piorko and R. Sutherland, *Inorg. Chim. Acta,* 1988, **147**, 77-85.

33. A. S. Abd-El-Aziz, E. K. Todd, R. M. Okasha, P. O. Shipman and T. E. Wood, *Macromolecules,* 2005, **38**, 9411-9419.

34. National Committee for Clinical Laboratory Standards, Approved standard M7–A6 6th ed.

35. D. P. Overy, F. Berrue, H. Correa, N. Hanif, K. Hay, M. Lanteigne, K. Mquilian, S. Duffy, P. Boland and R. Jagannathan, *Mycology,* 2014, **5**, 130-144.

36. B. Halliwell and M. Whiteman, *Br. J. Pharmacol.,* 2004, **142**, 231-255.

37. W. Jakubowski and G. Bartosz, *Cell Biol. Int.,* 2000, **24**, 757-760.

38. H. Wang and J. A. Joseph, *Free Radic. Biol. Med.,* 1999, **27**, 612-616.

39. F. Li, J. G. Collins and F. R. Keene, *Chem. Soc. Rev.,* 2015, **44**, 2529-2542.

40. F. Li, E. J. Harry, A. L. Bottomley, M. D. Edstein, G. W. Birrell, C. E. Woodward, F. R. Keene and J. G. Collins, *Chem. Sci.,* 2014, **5**, 685-693.

41. A. S. Abd-El-Aziz, C. Agatemor, N. Etkin and R. Bissessur, *Macromol. Chem. Phys.,* 2015, **216**, 369-379.

42. D. Astruc and J. Ruiz, *J. Inorg. Organomet. Polym. Mater.* 2015, **25**, 330-338.

43. G. L. Patrick, An Introduction to Medicinal Chemistry, Oxford University Press, 2013.

44. M. H. Ahmida, *Exp. Toxicol. Pathol.,* 2012, **64**, 149-153.

45. J. G. Hurdle, A. J. O'Neill, I. Chopra and R. E. Lee, *Nat. Rev. Microbiol.,* 2011, **9**, 62-75..

46. D. S. Uppu, P. Akkapeddi, G. B. Manjunath, V. Yarlagadda, J. Hoque and J. Haldar, *Chem. Commun.,* 2013, **49**, 9389-9391.