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Synthesis and Anti-Biofilm Activity of New Ferrocene Schiff Bases

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Abstract—Four new Schiff bases have been synthesized. The effectiveness of ferrocene systems in inhibiting formation of *Staphylococcus aureus* biofilms is tested. One of studied ferrocene Schiff bases efficiently inhibits formation of methicillin-sensitive *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) biofilms.

Keywords: ferrocene, Schiff bases, staphylococcal biofilms, antibiofilm activity

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INTRODUCTION

Ferrocene-substituted Schiff bases demonstrate high free radical scavenging and anticancer activities [1]. Most of ferrocene derivatives synthesized over recent years have been investigated in terms of antitumor [2–4] and anti-infective [3–5] properties, and there is a limited number of papers devoted to their potential of inhibiting bacterial biofilms [6, 7]. Biofilms are surface attached communities of microorganisms that are embedded in a matrix of extracellular polymeric substances and play a significant role in supporting bacterial infections. Formation of biofilms makes bacteria more resistant to antibiotics and the host immune response. It is estimated that such microorganism communities could be responsible for about 80% of bacterial infections [8]. So far there are no drugs clinically used that target specifically bacterial biofilms. Therefore, the current study has been focused on designing and testing anti-biofilm compounds that could effectively minimize and eradicate biofilm supported infections.

Ferrocenylcarboxaldehyde is a suitable precursor in Schiff base chemistry for which reason it has been used in synthesis of ferrocenyl derivatives containing imine and amine functional groups. Structures of the new ferrocene substituted Schiff bases were supported by ^{1}H NMR. Electrochemical properties of all compounds were determined by cyclic voltammetry (CV).

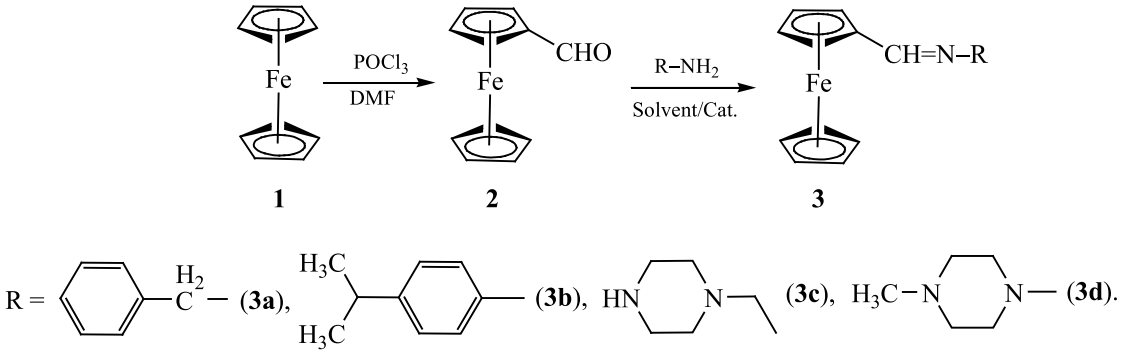
EXPERIMENTAL

Unless otherwise noted, all chemicals of analytical grade were obtained from commercial suppliers and used without further purification. Chloroform was refluxed with calcium hydride and distilled under atmospheric pressure. TLC was performed on silica gel plates, and column chromatography was carried out using silica gel (200–300 mesh). ^{1}H and ^{13}C NMR spectra were measured on a Bruker 500 MHz spectrometer in $CDCl_3$ using TMS as the internal reference. UV-Vis spectra were recorded on a Shimadzu UV-1700 spectrophotometer.

Synthesis of ferrocenecarboxaldehyde (2a). DMF (15 mL) was added to freshly distilled $POCl_3$ (6.44 g, 42.0 mmol) under the atmosphere of N_2 and cooled down from –5 to –10°C. The reaction mixture was stirred to the point of its complete conversion into a glassy solid. Then a solution of ferrocene (5.3 g, 28.0 mmol) in chloroform (60 mL) was added dropwise, the mixture was stirred at room temperature overnight, then poured into 100 mL ice cold water and filtered. The filtrate was neutralized to pH 8–9 by NaOH (10%, w/v) and then extracted with ether. The organic extracts were washed with water and dried over anhydrous $MgSO_4$. After evaporation of the solvent, the crude product was recrystallized from *n*-hexane to afford pure compound **2a** as a crimson solid, yield 79%. ^{1}H NMR spectrum, δ, ppm: 9.96 s (1H, HC=O), 4.80 d (2H, Cp-rings), 4.61 d (2H, Cp-rings), 4.28 s (5H, Cp-rings).

Synthesis of ferrocenyl Schiff bases 3a–3d. The mixture of ferrocenecarboxaldehyde **2a** (0.214 g, 1.0 mmol) with an aromatic amine (1.0 mmol) was suspended in xylene (50 mL). Ferrocenecarboxaldehyde dissolved completely upon stirring. Basic Al_2O_3

Scheme 1. Synthetic route to ferrocenyl Schiff base derivatives **3a**–**3d**.



(0.1 g, 0.98 mmol) was added, and the reaction mixture was stirred at 100°C for 10 h and then cooled down to room temperature. The procedure was monitored by TLC. The reaction mixture was filtered, the solvent was evaporated from the filtrate under vacuum, and the residue was washed by ethanol to give the corresponding pure compound **3a**–**3d**.

(*E*)-*N*-Ferrocenylidenebenzylamine (3a). Red solid, yield 61.5%, mp 169–170°C. ^{1}H NMR spectrum, δ, ppm: 8.27 s (1H, CH=N), 7.35 t (2H, Ar-H), 7.34 m (1H, Ar-H), 7.33 d (2H, Ar-H), 4.68 s (2H, C_5H_4), 4.66 s (2H, C_5H_4), 4.38 s (5H, C_5H_5).

(*E*)-*N*-Ferrocenylidene-4-isopropylbenzenamine (3b). Dark red solid, yield 33.2%, mp 177–178°C. ^{1}H NMR spectrum, δ, ppm: 8.41 s (1H, CH=N), 7.25 d (2H, Ar-H), 7.11 d (2H, Ar-H), 4.80 s (2H, C_5H_4), 4.53 s (2H, C_5H_4), 4.25 s (5H, C_5H_5), 2.89 m (1H, CH), 1.22 s (6H, CH_3).

(*E*)-*N*-Ferrocenylidene-ethylpiperazinamine (3c). Dark red solid, yield 25.5%, mp 172–173°C. ^{1}H NMR spectrum, δ, ppm: 8.12 s (1H, CH=N), 4.61 s (2H, C_5H_4), 4.35 s (2H, C_5H_4), 4.17 s (5H, C_5H_5), 2.89 t (2H, CH_2), 2.62 t (2H, CH_2), 1.23 m (8H, CH_2).

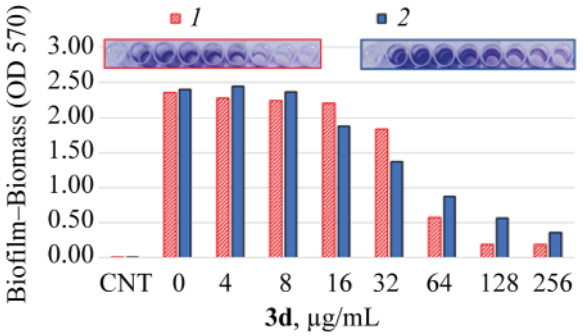


Fig. 1. Inhibition of biofilms formation by ferrocene Schiff base **3d** by reducing the biomass of (*1*) MRSA and (*2*) MSSA.

(*E*)-*N*-Ferrocenylidene-4-methylpiperazinamine (3d). Dark red solid, yield 21.3%, mp 175–176°C. ^{1}H NMR spectrum, δ, ppm: 8.22 s (1H, CH=N), 4.50 s (2H, C_5H_4), 4.20 s (2H, C_5H_4), 4.10 s (5H, C_5H_5), 3.04 d (4H, CH_2), 2.50 d (4H, CH_2), 1.23 s (3H, CH_3).

Bacterial strains and growth media. For determining antibacterial and antibiofilm potential of the ferrocene derivatives, broth micro dilution and microtiter crystal violet staining method assay were used. Two standard bacterial strains that gave biofilms, methicillin-sensitive *Staphylococcus aureus* (MSSA) ATCC 29213 and methicillin-resistant S*taphylococcus aureus* (MRSA) ATCC 43300, obtained from the microorganism culture collection of Vocational School of Health Services (Selcuk University) were used in the study. The strains were grown in Mueller–Hinton agar (MHA, Merck) at 37°C for 24 h, after which, bacterial culture was prepared by inoculating one single colony from a pure culture in Mueller-Hinton Broth (MHB, Merck) for MIC determination, tryptic soy broth (TSB, Sigma) for biofilm assay. 96-Well polystyrene flat bottom microtitration plates were purchased from Isolab, Clarithromycin (Klacid 500 mg I.V.) (Abbott).

Antibiofilm activity by the crystal violet assay. The microtiter plate based crystal violet assay [9, 10] is widely used as the standard test for the detection of biofilm formation. Biofilm inhibition study was carried out on two staphylococcus strains that produced biofilms [11, 12].

RESULTS AND DISCUSSION

Syntheses of ferrocene Schiff bases **3a**–**3d** (Scheme 1) involved the condensation reaction of equimolar amounts of ferrocenecarboxyaldehyde [5] with substituted amines in xylene upon refluxing.

Table 1. Electrochemical data for compounds **3a**–**3d**

Compound	E_{pc}[a], mV	E_{pa}[a], mV	$E_{1/2}$[b] or E_p, mV	ΔE_p[c], mV
Ferrocene	85.71	-3.279	41	89
3a	447.8	373.8	411	74
3c	739.8	641.5	691	98
3d	577.3	503.5	540	74
3f	571.9	497.9	535	74

[a] E_{pa} and E_{pc} anodic and cathodic peak potentials, respectively, at 0.1 V/s.
[b] $E_{1/2} = (E_{pa} + E_{pc})/2$.
[c] $\Delta E_p = E_{pa}E_{pc}$.

Table 2. Biofilms inhibiting values for ferrocene Schiff bases (%)

Comp. no.	MSSA (ATCC 29213)			MRSA (ATCC 43300)		
	concentration, μg/mL					
	256	128	64	256	128	64
3a	73.27	33.90	7.50	31.28	9.48	6.29
3b	44.05	7.53	2.91	54.70	24.26	13.19
3c	32.35	5.64	4.71	26.03	18.82	5.35
3d	84.48	75.09	61.48	93.32	93.28	78.53

Formation of the products **3a**–**3d** was confirmed by a singlet of the imine proton in the range between 8.12 and 8.41 ppm in their ^{1}H NMR spectra. Signals of the ferrocenyl Cp protons were measured in the respective range of 4.10–4.80 ppm [13]. In FT-IR spectra of the products **3a**–**3d** a new band at 1622–1627 cm^{-1} attributed to azomethine (CH=N) group was recorded [14]. The band recorded at 810 cm^{-1} was attributed to ferrocenyl C–H bending vibration. A band typical for Cp–Fe–Cp stretching vibration of ferrocenyl moiety was recorded in the range of 476–518 cm^{-1} [15]. The effect of different substitutes in aromatic rings on charge-transfer (CT) absorption properties of the compounds, UV–Vis spectra were recorded in CH_3CN medium. All compounds exhibited absorption peaks between 200 and 750 nm. The spectra contained one major absorption band characteristic for the high energy intra-ligand charge transfer, and ligand-to-ligand charge transfer transition was recorded by the corresponding peaks at 234–255 nm. Cyclic voltammetry has been used in dichloromethane containing 0.1 mol/L TBAP as a supporting electrolyte for the evaluation of electrochemical properties of the synthesized compounds. All ferrocene-containing Schiff bases exhibited a reversible one-electron redox process at the similar potential, oxidation waves appeared at 373.8–641.5 mV and the reduction ones at 447.8–739.8 V (Table 1).

Anti-biofilm activity of ferrocene derivatives. The effect of ferrocene derivatives **3a**–**3d** on biofilm formation by MSSA (ATCC 29213) and MRSA (ATCC 43300) was analyzed in terms of total biomass with the crystal violet microtitration assay. Bacterial cells were incubated with the compounds at sub-MIC doses during the whole period of biofilm formation (24 h) on a polystyrene microtitration plate. According to the percent inhibition values (Table 2) for ferrocene derivatives action on staphylococcus biofilms, the most active product **3d** was singled out. Treatment of biofilms by **3d** in the doses of 4 to 256 μg/mL for 24 h resulted in significant reduction of the biofilms biomass in comparison with the control biofilm of the bacteria only (Fig. 1). The tests indicated that **3d** was more effective against MRSA biofilms. The other synthesized compounds had very low anti-biofilm potential with inhibition values below 50%.

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CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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