Sulfur-Containing Ferrocenyl Alcohols and Oximes: New Promising Antistaphylococcal Agents

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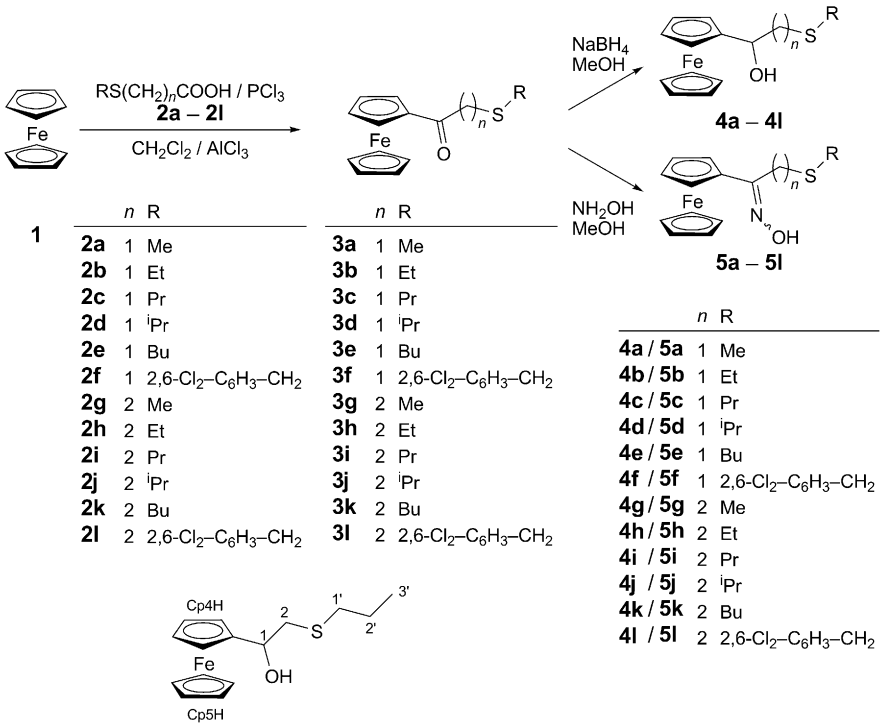
A small library containing four different series of new ferrocene derivatives, 2-(alkylsulfanyl)-1-ferrocenylethan-1-ols, 3-(alkylsulfanyl)-1-ferrocenylpropan-1-ols, (*E*)- and (*Z*)-2-(alkylsulfanyl)-1-ferrocenylethan-1-one oximes, and (*E*)- and (*Z*)-3-(alkylsulfanyl)-1-ferrocenylpropan-1-one oximes (36 different compounds in total) was synthesized starting from ferrocene and the corresponding sulfanyl acids. All compounds were spectrally (IR and NMR) and electrochemically characterized. In general, the obtained compounds were found to exhibit very strong antimicrobial activities (broth microdilution assay) against the tested microorganisms (six common human pathogens). For the majority of the tested compounds, the determined *MIC* values were either under the 10 μg/ml *MIC* limit recognized to delimit efficient antimicrobials or were comparable to/lower than those of the used positive controls (tetracycline/nystatin). The most susceptible organism was found to be *Staphylococcus aureus* with *MIC* values even reaching 0.001 μg/ml. The presence of –CH(OH)$(CH_2)_n$S– and –CH(=NOH)$(CH_2)_n$S– ($n=1$ or 2) structural fragments seems to be essential for the observed strong activity (introduction of hydroxyimino and alcohol functionalities, instead of the keto function, resulted in a more than 10^5-fold increase in antistaphylococcal activity in some instances). Nevertheless, a possible influence of the ferrocenyl-core redox chemistry (Fe^{2+}/Fe^{3+}) should not be disregarded. The studied alcohols exhibited a reversible one-electron redox couple at almost the same position as ferrocene, while the hydroxyimino group conjugated with cyclopentadienyl ring considerably shifted the redox potential of the ferrocene unit in oximes.

Introduction. – Over the past few decades, the number and extent of infections caused by multi-drug resistant (MDR) microorganisms has reached an alarming level. The rapidly developing drug resistance and drug side-effects limit the use of a large number of antimicrobial agents. All of this represents a serious challenge to the medical community [1]. Hence, the discovery and development of new antimicrobial agents became an important goal. Certain organometallic compounds offer a particular promise in this respect, because they are kinetically inert, often uncharged, and fairly lipophilic [2]. The metal centers exist in low oxidation states, which limit the danger of

oxidative damage inside cells [2]. Ferrocene (**1**; *Scheme 1*) and some of its derivatives (*e.g.*, ferrocifen) were found to have strong and useful pharmacological properties, and are considered as promising lead structures. Even ferrocene itself was found to have some antiproliferative activity. Its mechanism of action remains elusive despite several efforts; however, it is assumed by some researchers that the redox chemistry (Fe^{2+}/Fe^{3+}) is at the heart of its activity [2]. Synthesis of new ferrocene-containing antimicrobial/cytotoxic agents is one of the main objectives of our research program, and, up to now, we managed to obtain a considerable number of new ferrocene derivatives, some of which were shown to possess interesting biological/pharmacological properties [3–12]. In continuation of our work, we wanted to synthesize some additional (new) ferrocene derivatives that would contain moieties known to be potent pharmacophores.

Scheme 1



Many S- and N-containing organic compounds exhibit strong antimicrobial and antioxidant properties [10]. For example, the 2- and 3-(alkylsulfanyl)-1-hydroxy moiety is a common structural fragment in a vast group of natural products that were proven to have useful biological and pharmaceutical activities [13–18]. Leukotrienes LTC4 and LTD4 could serve as an example of the previously mentioned. On the other hand, we assumed, for several reasons, that the introduction of the hydroxyimino group could further enhance antimicrobial properties of the designed ferrocenyl derivatives. First of

all, it is a quite common and stable functional group frequently employed in current drugs [19]. Second, the oxime group has an electron lone pair of at the N-atom that can participate in H-bonding with the drug target. And finally, this functional group can be readily obtained from the corresponding ketone in a single synthetic step [20].

Thus, encouraged by all of the previously mentioned findings and with the aim of finding new antimicrobial agents, we have synthesized, and spectrally and electrochemically characterized four different, analogous series of ferrocene derivatives, 2-(alkylsulfanyl)-1-ferrocenylethan-1-ols, 3-(alkylsulfanyl)-1-ferrocenylpropan-1-ols, (*E*)- and (*Z*)-2-(alkylsulfanyl)-1-ferrocenylethan-1-one oximes, and (*E*)- and (*Z*)-3-(alkylsulfanyl)-1-ferrocenylpropan-1-one oximes (36 different compounds in total; *Scheme 1*). All of the synthesized compounds were assayed for antimicrobial activities (broth microdilution susceptibility test). The set of compounds prepared and tested varied in structure in such a manner as to additionally allow the examination of structure–activity relationships. By changing the the alkyl group at the S-atom, we anticipated that we could finetune the lipophilicity of the novel compounds in order to attain the highest potency and most favorable physical properties. Moreover, the electrochemical properties of synthesized compounds (that possibly could also be important with respect to the aims of this study) were investigated by cyclic voltammetry.

Results and Discussion. – *Library Design*. A series of S-containing acyl ferrocenes was previously studied and characterized in terms of their antimicrobial properties (broth microdilution assay) [3]. Despite the presence of keto, ferrocenyl, and sulfanyl moieties, at least some of which are considered to induce potentially strong biological activities in a number of different, previously studied molecules, these compounds have shown moderate-to-low antimicrobial activity [3]. One of the reasons for the obtained unexpectedly poor results of the performed activity assays could be possibly found in the fact that the electrophilicity of the keto group is significantly reduced by conjugation with the strongly electron-donating ferrocene core, directly attached to the CO C-atom. Regardless of the mentioned, somewhat discouraging (average) results, we decided to use this series of acylferrocenes as the starting point for the design of new compounds that would potentially have enhanced target properties. We assumed that simple chemical modifications of the mentioned ketones would result in significant improvement of the desired biological activity. Based on the fact that 2- and 3-(alkylsulfanyl)-1-hydroxy unit is a common structural motif in a number of biologically and pharmacologically active natural products [14–18], we decided to convert the CO function in the S-containing acylferrocenes to the alcohol group. On the other hand, for the already mentioned reason (a stable functional group frequently employed in current drugs, the presence of a N lone pair of electrons that can participate in H-bonding with the drug target), we supposed that the introduction of (hydr)oxyimino group could further enhance antimicrobial properties of the designed ferrocenyl derivatives [19]. Replacement of the CO (H-acceptor) by alcohol and hydroxyimino groups (H-acceptor/donor) could have a significant impact on the binding of these new analogs with the target biomolecule(s). A further assumption was that the sulfanyl and alcohol/oxime groups would not 'act' separately, as the distance between these two moieties (one or two CH_2 groups apart) could allow potential anchimeric assistance of

S in the reactions that would involve nucleophilic attack at the C-atom directly attached or part of the alcohol/oxime functionalities.

Alongside with the derivatives with alkyl groups (*normal* and branched chains; different number of C-atoms) on the S-atom, we decided to also include analogs bearing a (2,6-dichlorophenyl)methyl moiety. This was conducted to possibly test the influence of an additional aromatic core on the activity (corresponding additional binding interactions with the target(s) might be important for optimal docking).

As previously shown, the length and bulkiness of the side chain, as well as the position of the S-atom, were important issues that influenced the antimicrobial activities of the studied S-containing ferrocenyl ketones [3]. Our aim was to test if new derivatives would follow the same trends, or if the newly introduced functional groups, oxime and alcohol, would have a predominant impact on the expected activity.

Chemistry. The synthesis of S-containing ferrocenyl alcohols, **4a**–**4l**, and oximes, **5a**–**5l**, is outlined in *Scheme 1*. Ferrocenyl ketones **3a**–**3l** were obtained *via* the modified *Friedel–Crafts* acylation of ferrocene (**1**) with *in situ* prepared chlorides of the corresponding sulfanyl acids **2a**–**2l**, respectively, according to the previously published procedure [3][8][9]. Then, they were either reduced with $NaBH_4$ [6] or were refluxed in MeOH with $NH_2OH \cdot HCl$ [7]. In this way, a library of 24 different S-containing ferrocenyl derivatives (alcohols: series *I*, compounds **4a**–**4f**; series *II*, compounds **4g**–**4l**; and oximes: series *III*, compounds **5a**–**5f**; series *IV*, compounds **4g**–**4l**) was prepared. Nevertheless, the hydroxyimination of ketones gave mixtures of the corresponding (*E*)- and (*Z*)-isomers in quantitative yields. The ratio of these diastereoisomers was inferred from ^{1}H-NMR data of the mixtures and was found to be in the range from (*E*)/(*Z*) 2.08 to (*E*)/(*Z*) 6.85. Single-crystal X-ray-analysis of the pure isomers confirmed that the (*E*)-isomer is the one obtained in higher yield [7], while calculations performed on the semi-empirical level of theory (PM3 method; *Polak–Ribiere* (conjugate gradient) minimization method; energy convergence criterion of 0.01 kJ/mol) showed that, generally speaking, in the gas phase, the (*E*)-isomer is the one thermodynamically more favorable (*e.g.*, for compound **5b**, calculated equilibrium (*E*)/(*Z*) ratio was 40.33). In addition, it has been previously shown that (*E*)- and (*Z*)-isomers undergo interconversion on prolonged standing in solution [7]. Thus, since the dissolution of any of the pure isomers would eventually give an equilibrium mixture of these two isomers, they were not further separated from one another, and their mixtures (obtained after the appropriate workup of the corresponding reaction mixtures) were used for the antimicrobial assays (this equilibration is expected to go to completion in the nutrient broth during the antimicrobial tests).

Spectral Characterization of the Obtained Compounds. All synthesized compounds were characterized by means of IR, and ^{1}H- and ^{13}C-NMR spectroscopy. IR Spectra of the compounds of series *I* and *II*, *i.e.*, **4a**–**4l**, displayed broad bands between 3550 and 3200 cm^{-1} (OH), and strong adsorptions in the range of 970–1150 cm^{-1} (C–O), characteristic for alcohols. This, together with the absence of the C=O absorption, confirmed the transformation of the ketones to the corresponding alcohols. As expected, IR spectra of the compounds of the series *III* and *IV*, *i.e.*, **5a**–**5l**, were characterized by the presence of intensive bands in the ranges of 3600–2800 (OH) and 1640–1615 cm^{-1} (C=N), which corroborated the presence of the hydroxyimino moiety in the structures of these compounds. Once again, a band characteristic for ketones

(1760–1670 cm^{-1}) was not observed. Spectra of some of the studied compounds (especially oximes **5a**–**5l**) were further characterized by a strong band around 490 cm^{-1} that most probably corresponds to the ferrocene ring tilt.

In the ^{1}H-NMR spectra of all obtained alcohols, the signals of H-atoms of the ferrocene ring were observed at 4.17–4.28 ppm. Five H-atoms from Cp5H ring (cyclopentadienyl ring with 5 H-atoms) gave a broad *singlet* at *ca.* 4.20 ppm, while the signals of Cp4H (cyclopentadienyl with 4 H-atoms) H-atoms appeared as two broad *pseudo-triplet*s (in some cases, even more complex *multiplet*s) at *ca.* 4.18 and 4.25, with coupling constant of *ca.* 1.8 Hz. For compounds **4a**–**4f** (series *I*), signals of the two diastereotopic H-atoms (β-H atoms), observable at *ca.* 2.73 and 2.88, characterized by a strong geminal coupling, were well-defined *doublet*s of *doublet*s ($J_a \approx 13$ and $J_b \approx 4$ Hz, *i.e.*, $J_a \approx 13$ and $J_b \approx 8$ Hz), and they could be straightforwardly assigned. With the other alcohol series (series *II*, **4g**–**4l**), these H-atoms were a part of a much more complex spin system ($XCHCH_2CH_2Y$). Each of them was coupled with four other different H-atoms. It seems, from the shape and complexity of these and of the signals of other H-atoms from the same spin system, that some of the coupling constants had close, but still not completely equal values. For the mentioned reasons, detailed analyses of the mentioned *multiplet*s at *ca.* 1.85–2.00 (β-H atoms) were not possible. In some cases, the signal(s) corresponding to the γ-H-atoms (at *ca.* 2.66) had an appearance of a broad *triplet*, but in fact it was a *multiplet* that was not further analyzed. It could be interesting to note that the spectra of some of the alcohols (*e.g.*, **4f**, **4h**, **4j**, *etc.*) showed couplings of the α- and the H-atoms from the alcohol groups, with the value of *ca.* 3 Hz. After the addition of a few drops of D_2O to the mentioned samples, the signal assigned to the OH H-atom disappeared, and the structure of that corresponding to the α-H atom was simplified (*i.e.*, in some cases, the hard-to-interpret *multiplet*, at *ca.* 4.3 ppm, became a sharp *doublet* of *doublet*s). Worth mentioning is the finding that a low exchange rate on the NMR time scale of analogous exchangeable H-atoms (attached to heteroatoms) has previously been already noticed for some other ferrocene derivatives, while their phenyl analogs displayed a swift exchange [10][11].

As mentioned before, the hydroxyimination of ketones gave mixtures of (*E*)- and (*Z*)-isomers. Previously, it was shown that only the (*E*)-isomer is the one obtained in higher yield, and that the two isomers undergo interconversion easily on the prolong standing in solution [7]. Therefore, the isomers were not separated from one another prior to further analysis/assays. In general, NMR signals corresponding to (*E*)- and (*Z*)-isomers were well-resolved, and their intensities differed significantly. This enabled their (almost complete) assignments, even through the analysis of the NMR spectra of mixtures of isomers **5a**–**5l**. The ratio of the isomers was also estimated on the basis of ^{1}H-NMR. This was accomplished *via* the ratios of the integrals corresponding to the matching appropriate signals. In the case of the compounds belonging to the series *III*, *singlet*s corresponding to the H-atoms of the CH_2S moieties (at *ca.* 3.55 and 3.72) were used, while for those from the series *IV*, signals of the two H-atoms from the Cp4H ring (appearing at higher shifts, *ca.* 4.56 and 4.91) were chosen.

As in the case of alcohols, the shifts corresponding to the H-atoms from the repeating structural units of all of the compounds belonging to series *III* and *IV* were mutually comparable. Shifts of the analogous H-atoms from the ferrocene core differed only slightly. The same is true for β- (and γ-H-atoms) or those from the alkyl–S

substituents. *Multiplet*s corresponding to β- and γ-H-atoms of the compounds **5g**–**5l** (series *IV*; *ca.* 2.78–3.00) overlapped completely, and their complete assignment was not possible. As expected, it was much simpler to interpret the spectra of the compounds belonging to the series *III*.

The structures of the synthesized compounds were additionally corroborated by the analysis of their ^{13}C-NMR spectra. The ^{13}C-NMR spectra of the studied compounds contained groups of signals corresponding to the ferrocene ring (one signal that originated from five C-atoms of the Cp5 H ring and five signals of the C-atoms of Cp4 H; signals of the C-atoms of Cp4 H were not equivalent, due to the presence of α-stereogenic center, alkyl chains, and C-atoms of the oxime/carbinol functionalities, which could be easily distinguished and almost completely unambiguously assigned. In the case of compound **5l**, with the higher (*E*)/(*Z*)-ratio, some signals corresponding to the C-atoms of the (*Z*)-isomer were not observed, but the shifts of those detected in the spectrum had expected values, based on the structure and the spectra of other studied compounds.

Electrochemistry. As previously mentioned, it is assumed by some researchers that the redox chemistry (Fe^{2+}/Fe^{3+}) of the ferrocene core is the source of its biological/pharmacological activity [2]. Bearing this in mind, electrochemical investigations undertaken in the present study were aimed to evaluate redox features of the synthesized compounds appearing due to the presence of the ferrocene nucleus. For this purpose, cyclic voltammetry of the synthesized compounds in MeCN, containing 0.1M $LiClO_4$ as the supporting electrolyte, has been utilized. Cyclic voltammetry was the method of choice, as it is a very versatile electrochemical technique, which allows probing the mechanisms of redox reactions and transporting properties of a system in solution [21]. Since ferrocene itself exhibits, under given conditions (a Pt working electrode, scanning rate $\nu = 0.1\ V \cdot s^{-1}$), a reversible redox couple at $E_{1/2}(\nu) = 0.358$ V, we chose the potential window 0.000–1.000 V for our measurements. As the representative examples, we give here the voltammograms of alcohol **4h** and oxime **5h** (*Fig. 1*), whereas the data for all other alcohols and oximes are compiled in *Table 1*. It is observable from this data that all alcohols exhibit a reversible one-electron redox couple at almost the same position as ferrocene. However, as it can be expected from an electron-withdrawing group, the hydroxyimino functionality conjugated with the cyclopentadienyl ring considerably shifts the redox potential of the ferrocene unit in oximes (by *ca.* 0.100 V). This shift, however, is considerably less than that of the CO group in the same position (by *ca.* 0.300 V) [3]. The differences between anodic and cathodic peak potentials (*cf. Table 1*) are close to theoretical values, and independent of the scan rate ν. Both anodic and cathodic peak currents are proportional to the square root of the scan rate, and their ratio is independent of the scan rate, indicating a diffusion-controlled process.

Biological Activity. Antibacterial activities against two *Gram*-positive (*S. aureus* and *B. cereus*) and three *Gram*-negative bacteria (*E. coli*, *K. pneumoniae*, and *P. vulgaris*), as well as the antifungal activity against one fungal organism (*C. albicans*) were assessed for compounds **4a**–**4l** and **5a**–**5l**. The minimal inhibitory concentration (*MIC*; the lowest concentration of the tested compound in μg/ml which inhibited the growth of bacteria or fungi) values were determined using a microdilution method based on the recommendations of NCCLS [22]. Under the same conditions, solutions

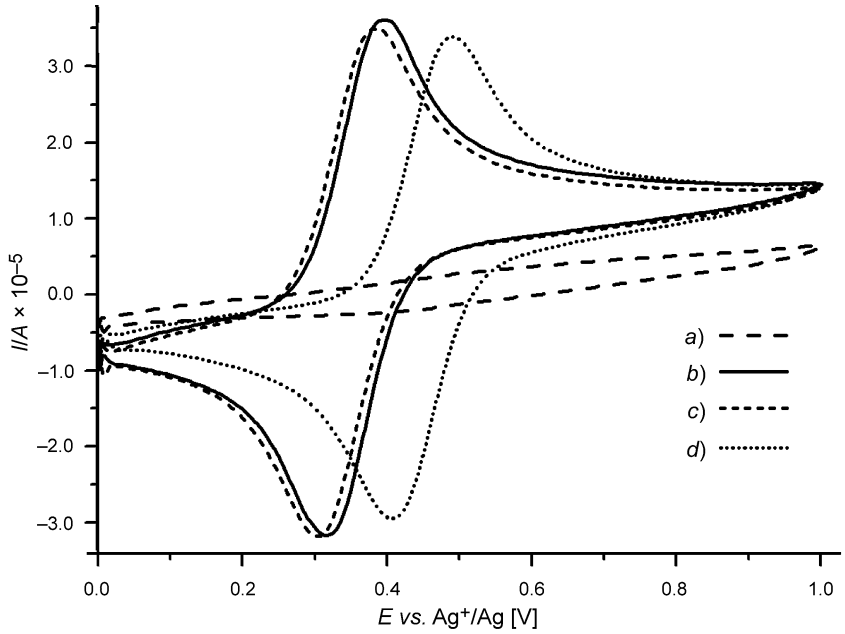


Fig. 1. *Cyclic Voltammograms of* a) *5 m*M ferrocene, b) *5 m*M **3b**, c) *5m*M **4h**, *and* d) *electrolyte at the Pt electrode* (2 mm diameter) *with a 0.1 Vs*$^{-1}$ *scan rate in a 0.1*M $LiClO_4$ *solution in MeCN*

of differing concentration of tetracycline (antibacterial) and nystatin (antifungal) were used as positive controls. The results of the broth microdilution susceptibility assay are compiled in *Table 2*. As presumed during the library design, compounds **4a**–**4l** and **5a**–**5l** showed significantly higher antimicrobial activities than the corresponding starting ketones **3a**–**3l** [3]. The *MIC* values of the synthesized compounds ranged from 0.001 to 5000 μg/ml. Concentrations above 5000 μg/ml were not tested. Every compound tested showed a very high inhibitory activity against at least one microorganism. For some of the tested compounds, *MIC* values against all of the microbial strains (apart from for *E. coli*) were close to or under the 10-μg/ml *MIC* limit set for an efficient antimicrobial [3]. It must be stressed that the activity of some of them was of the same order of magnitude, or even better, compared to the positive controls that were used. Moreover, some of the compounds could be considered as being quite selective in their action (their activity varied significantly from one microorganism to another). For example, compound **5e** was much more active against *B. cereus* and *C. albicans* ($MIC \leq 0.400$ μg/ml) than against other tested strains ($MIC \geq 39.000$ μg/ml). Just the opposite could be said for compound **4b**. It was characterized with lower *MIC* values than tetracycline in the case of *S. aureus* and *K. pneumoniae*. It showed notable activities against all other considered microorganisms as well ($MIC \leq 1.000$ μg/ml, except against *E. coli*; see *Table 2*).

To better interpret the results of the biological assays, we performed agglomerative hierarchical clustering (AHC) and principal component analysis (PCA) on the mentioned samples (*Table 2*; the methods were applied utilizing the *MIC* values as

Table 1. *Electrochemical Data for Compounds* **4a**–**4l** *and* **5a**–**5l**

Compound	E_{pa} [a]) [V]	E_{pc} [a]) [V]	$E_{1/2}$ [b]) [V]	ΔE [c]) [mV]
Alcohols				
4a	0.385	0.314	0.350	71
4b	0.406	0.327	0.366	79
4c	0.394	0.320	0.357	74
4d	0.388	0.327	0.357	61
4e	0.388	0.320	0.354	68
4f	0.394	0.327	0.360	67
4g	0.381	0.311	0.346	70
4h	0.378	0.308	0.343	70
4i	0.381	0.311	0.346	70
4j	0.400	0.320	0.360	80
4k	0.385	0.314	0.350	71
4l	0.400	0.320	0.360	80
Oximes				
5a	0.479	0.406	0.442	73
5b	0.473	0.403	0.438	70
5c	0.479	0.403	0.441	76
5d	0.476	0.403	0.440	73
5e	0.476	0.403	0.440	73
5f	0.485	0.418	0.452	67
5g	0.479	0.409	0.444	70
5h	0.488	0.409	0.448	79
5i	0.485	0.409	0.447	76
5j	0.482	0.406	0.444	76
5k	0.485	0.406	0.446	79
5l	0.485	0.409	0.447	76

[a]) E_{pa} and E_{pc} are anodic and cathodic peak potentials, respectively, at 0.1 Vs^{-1}. [b]) $E_{1/2}=(E_{pa}+E_{pc})/2$. [c]) $\Delta E=E_{pa}-E_{pc}$.

original variables without any recalculation). The results of the statistical analyses showed that the level of activity of a great number of the tested compounds was not statistically different from that of the controls. In addition, compound **5f** was clearly separated from all others as the least active one. A possible explanation for this could be sought in the bulkiness of the particular *S*-substituent, which could have a negative impact on the **3f**/**5f** docking to the target biomolecule. In contrast to **5f**, compound **5l** was much more active against the tested strains. Once again, for this pair of compounds, this is in agreement with the previous results, which revealed that there are significant differences in the activity of 2- and 3-(alkylsulfanyl)acylferrocenes [3]. It could be interesting to note, however, that alcohols **4f** and **4l** were both active in rather low concentrations against the tested strains.

Multivariate statistical analyses performed using an inversed observation/variable table as the input data set clearly separated *E. coli*, the least susceptible strain, from all other microorganisms. This type of analysis also indicated that the studied active compounds demonstrate some level of selectivity towards bacteria or fungi. However,

Table 2. MIC *Values* [μg/ml] *of the Studied Compounds,* **4a**–**4l** *and* **5a**–**5l**, *Obtained from a Broth Microdilution Susceptibility Assay*

Compound	Bacteria					Fungus
	Gram-positive		*Gram*-negative			
	S. aureus	*B. cereus*	*E. coli*	*K. pneumoniae*	*P. vulgaris*	*C. albicans*
4a	310	156	1250	156	1.000	0.10
4b	39	156	2500	0.20	620	0.20
4c	0.070	0.30	>5000	0.90	70	0.40
4d	9.000	156	>5000	0.20	70	0.20
4e	0.001	0.003	>2500	0.003	0.90	0.90
4f	0.070	0.003	>2500	0.20	0.020	0.030
4g	0.050	0.003	1250	0.20	0.050	0.15
4h	0.001	0.780	>2500	0.003	1.0	0.90
4i	0.001	0.150	>5000	0.15	35	0.070
4j	9.0	156	>5000	156	70	0.070
4k	0.050	0.20	>2500	0.20	0.400	0.030
4l	0.050	0.003	>2500	0.20	0.020	0.030
5a	0.40	0.30	>5000	0.40	0.40	0.070
5b	19	156	>5000	0.070	560	0.15
5c	0.10	0.070	>5000	0.40	0.10	0.070
5d	19	156	>5000	0.070	310	0.15
5e	9.0	0.40	>5000	39	70	0.070
5f	1250	0.90	750	0.10	560	>2500
5g	0.001	0.030	>5000	0.10	70	0.070
5h	0.003	0.070	>2500	0.070	35	0.80
5i	9.0	0.40	>5000	0.20	70	0.030
5j	0.10	39	>5000	0.40	0.40	0.030
5k	19	156	>5000	0.10	310	0.070
5l	4.0	78	325	78	1.0	0.030
Control	0.003[a])	0.110[a])	0.230[a])	0.050[a])	0.110[a])	0.039[b])

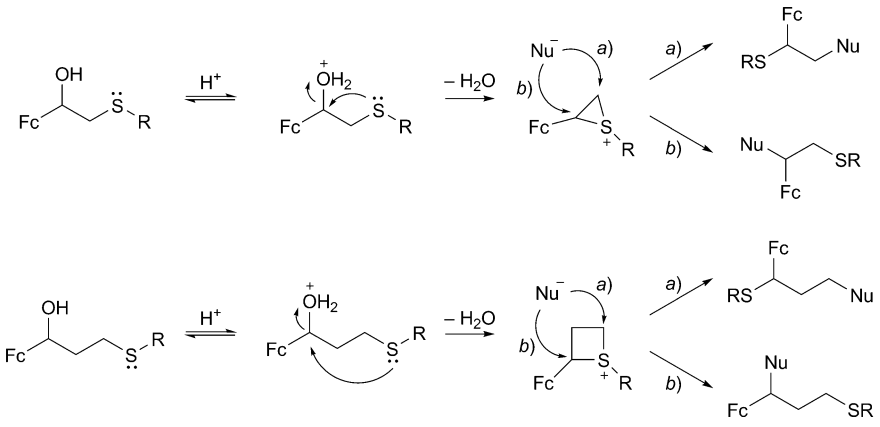
[a]) Tetracycline. [b]) Nystatin.

it seems that *Gram*-negative and *Gram*-positive strains were not differentiated by their susceptibility towards the synthesized compounds.

For all of the prepared compounds, log $P_{o/w}$ values were calculated (this was performed in the following way: log $P_{o/w}$ of the corresponding phenyl analogs was assessed using Chem3D ultra 10.0 Software (*CambridgeSoft*), and the obtained values were then corrected according to [22]). But, it seems that the hydrophilicity of the compounds does not play an important role, when antimicrobial activity of this set of ferrocenyl derivatives is in question. In addition, to check if there is any correlation between the redox properties of these ferrocene derivatives and the observed activity, we plotted the redox potentials (E_{pa}, E_{pc}, $E_{1/2}$, and ΔE) against log(1/*MIC*) and performed linear regression analyses (least-squares method). In all cases, the correlation coefficients (r^2) had values considerably less than 0.8 (maximal r^2 value found was 0.6). These results indicate that the redox properties were not essential for the observed differences in the activities (in any potential QSAR equation, the corresponding coefficients would probably have relatively low values).

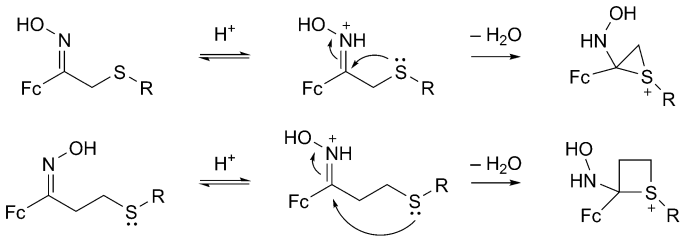
Based on all of the previously mentioned findings, it seems that the significant increase of activity for the alcohols and oximes, compared to the starting ketones, is not (predominantly) due to the changes in the hydro/lipophilicity and/or electrochemical properties, but that the newly introduced functional groups are directly involved in some novel interaction with the target biomolecules. Possible mechanisms of antimicrobial action of the herein studied series of compounds are depicted in *Schemes 2* and *3*. After the initial protonation of the OH groups, and a concomitant intramolecular attack of the S-atom (anchimeric aid), H_2O could be eliminated from the molecule to give the highly electrophilic substituted 1-alkylthiiranium or 1-alkylthietanium cations. The two reactive species could then react with a (bio)nucleophile and give two possible products (*Scheme 2*) that would represent covalently modified (inactive) enzymes for example. An analogous mechanism could be pictured for the oximes as well (*Scheme 3*): after the protonation, S (intramolecular nucleophile) could attack the oxime electrophilic C-atom and form a potent electrophilic species, *i.e.*, intramolecular tetrahedral intermediate. The obtained substituted 1-alkylthiiranium/1-alkylthietanium cations could then react with some available nucleophile (bacterial metabolite).

Scheme 2. *Possible Mechanism of Antimicrobial Action of the Studied Alcohols through Anchimeric Assistance* (Nu: nucleophile)



Although further studies to test the correctness of this potential mechanism of action were not carried out, we have performed a simple test which showed that anchimeric assistance of the S-atoms in these molecules is quite probable. Compound **4b** (*m*/*z* 290 (M^+)), dissolved in Et_2O, was allowed to stand in the presence of $BuNH_2$ for 1 h. Then, the reaction mixture was directly injected into a GC/MS, and the concomitant analysis showed that the dominantly produced compound was the corresponding elimination product (ethyl(2-ferrocenylvinyl)sulfane (*m*/*z* 272 (M^+)); identification of this compound was based on the MS fragmentation pattern (*Fig. 2*), and the correspondence of the experimentally determined and expected values of the retention indices. It seems that there are no other reasonable mechanisms, except for

Scheme 3. *Possible Anchimeric Mechanism of Antimicrobial Action of the Studied Oximes* (Nu: nucleophile)



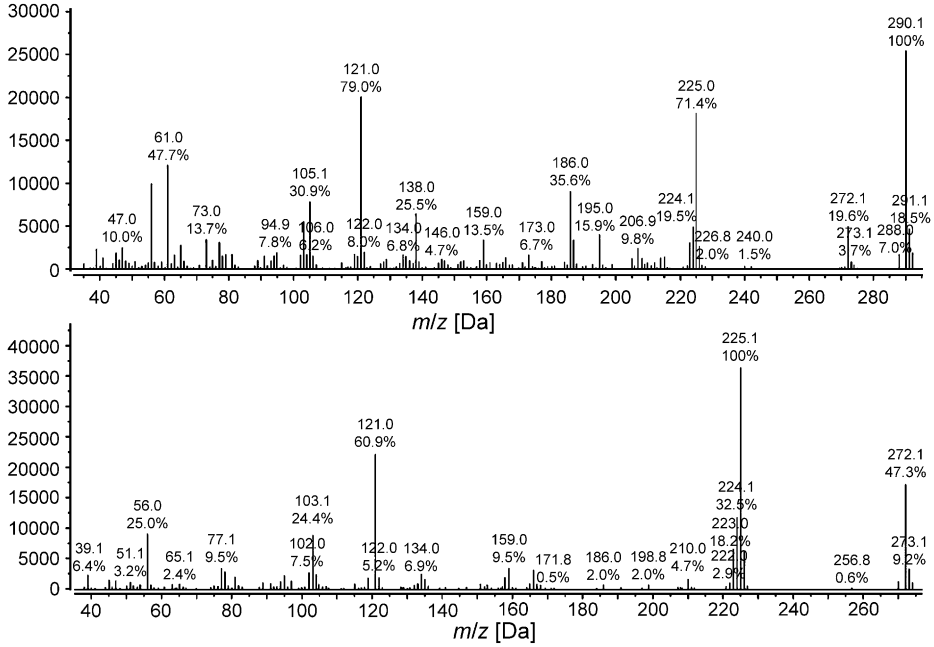


Fig. 2. *Mass spectra of compounds* **4b** (top) *and ethyl(2-ferrocenylethenyl)sulfane* (bottom)

that involving the anchimeric assistance of the S-atom that could explain the elimination of H_2O from the alcohol (**4b**) under basic conditions.

It is interesting to note that, except **4l**/**4f** and **5l**/**5f** (log $P_{o/w} > 5$), all other herein studied ferrocene derivatives are in agreement with the *Lipinski*'s rule of five, related to orally active drugs. This rule gives only rough guidelines regarding molecules considered for oral pharmaceuticals. Nevertheless, the finding that the studied series of biologically active molecules fulfills at least these basic general requirements could be considered as a justification for some further, more in-depth studies.

Conclusions. – To summarize, taking into account the properties of hydroxyimino and hydroxy functional groups and the results of a previous study focused on the

biological activity of a series of S-containing acyl ferrocenes [3], we have designed a small library comprising four different, but analogous groups of compounds. All of the obtained compounds were spectrally and electrochemically fully characterized, and their antimicrobial activities against some of the common human pathogens were determined (broth microdilution assay). The results of the antimicrobial assays showed that the studied compounds are strong and promising antimicrobial agents (except in the case of *E. coli*). Also, the results of the activity studies, as well as the calculated log $P_{o/w}$ values, indicated that the introduction of alcohol/oxime groups was essential for the onset of activity of the studied ferrocenyl derivatives, leading to a more than 10^5-fold increase in antistaphylococcal activity in some instances, and that correct assumptions were used during the library design. Furthermore, as can be seen from *Table 2*, although some correlation between the activity of compounds and the position of the S-atom could be found, it seems that this is not as important for the observed antimicrobial properties as in the case of the corresponding ketones. However, similarly as for starting acyl ferrocenes, the effect of bulkiness of the side chains could be easily deduced (compare the activities of compounds with Pr and ^{i}Pr groups attached to the S-atom (*Table 2*)). The finding that the studied compounds were generally active in very low concentrations (*e.g.*, *MIC* values against *S. aureus* were only 0.001 μg/ml for compounds **4e**, **4h**, **4j**, and **5g**), which were either under the 10 μg/ml *MIC* limit or were comparable/lower than those determined for the used positive controls (tetracycline and nystatin), renders them as candidates for further studies, even as potential oral antimicrobial drugs especially against *S. aureus*.

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Experimental Part

General. All chemicals were commercially available and used as received, except that the solvents were purified by distillation. The protected sulfanyl acids **2a**–**2l** (*Scheme 1*) were synthesized by the known procedure from the corresponding sulfanyl acids (thioglycolic and 3-sulfanylpropanoic acid) and org. halides [9]. Column chromatography (CC): silica gel *60* (*Merck*, 230–400 mesh ASTM). TLC: silica gel *60* on Al plates, layer thickness 0.2 mm (*Merck*). M.p. (uncorrected): *Mel-Temp* cap. melting-points apparatus, model *1001*. IR Spectra: *Perkin-Elmer FTIR 31725-X* spectrophotometer. ^{1}H- and ^{13}C-NMR spectra: in $CDCl_3$, *Varian Gemini* (200 MHz) spectrometer; chemical shifts (δ) [ppm] rel. to the residual solvent H-atoms $CHCl_3$ (7.26 ppm) for ^{1}H, and $^{13}CDCl_3$ (77.0 ppm) for ^{13}C as the internal standards. Cyclic voltammetry experiments: at r.t., under Ar in a three-electrode cell using an *Autolab* potentiostat (*PGSTAT 302N*). The working electrode was a Pt disk (2 mm diameter). The counter electrode was a Pt wire, and a Ag/AgCl electrode was used as the reference electrode.

Synthesis of Ketones **3a**–**3l** [3][8][9]. A mixture of the corresponding carboxylic acid (3 mmol), PCl_3 (0.3 ml; *ca.* 1 mmol), and ferrocene (558 mg; 3 mmol) in 50 ml of CH_2Cl_2 was stirred 3 h at r.t. under Ar, then anh. $AlCl_3$ (600 mg) was added. The obtained dark-violet mixture was stirred for 3 h, and the reaction was quenched with 50 ml of cold 2M KOH soln. The org. layer was separated, and the H_2O phase was extracted with CH_2Cl_2 (two 30-ml portions). The collected org. layers were washed with H_2O and brine, and dried (Na_2SO_4) overnight. After the evaporation of the solvent, the residue was purified by CC (SiO_2; petroleum ether (PE)/AcOEt 9:1 (*v*/*v*)). A small amount of the unchanged ferrocene eluted as the first component, followed by the corresponding ketones **3a**–**3k** (36–86%) [3][8][9].

Synthesis of Alcohols **4a**–**4l** [6]. To a soln. of the corresponding ketone **3a**–**3k** (1 mmol) in MeOH (30 ml), an excess of $NaBH_4$ was added in several portions, and the resulting mixture was stirred at r.t., monitoring the reaction progress by TLC. After the reaction was completed (*ca.* 2 h), the solvent was

evaporated, and H_2O (30 ml) was added to the residue. The mixture was extracted with CH_2Cl_2 (two 30 ml portions), the collected org. layers washed with H_2O and brine, and dried (anh. Na_2SO_4) overnight. After the evaporation of the solvent, the crude product was subjected to CC (SiO_2; toluene/AcOEt 9 : 1 (*v*/*v*)). The starting ketone was eluted first, then the corresponding alcohols **4a**–**4k**, followed by small amounts of 1-ferrocenylethanol (in the case of ketones **3a**–**3f**) or 1-ferrocenylpropan-1-ol (in the case of ketones **3g**–**3l**).

1-Ferrocenyl-2-(methylsulfanyl)ethanol (**4a**). Yield: 72%. IR (KBr): 3442, 3092, 2915, 1410, 1224, 1105, 1049, 1000, 817. ^{1}H-NMR (200 MHz): 2.11 (*s*, Me); 2.70 (br., OH); 2.71 (*dd*, *J* = 13.7, 8.1, 1 H of CH_2S); 2.82 (*dd*, *J* = 13.7, 4.5, 1 H of CH_2S); 4.17 (br., 2 H of Cp4 H); 4.19 (*s*, Cp5 H); 4.27 (br., 2 H of Cp4 H); 4.49 (br. *dd*, *J* = 8.1, 4.5, C*H*OH). ^{13}C-NMR: 16.0 (C(1′)); 42.5 (C(2)); 65.6 (1 C of Cp4 H); 66.7 (1 C of Cp4 H); 67.8 (1 C of Cp4 H); 67.9 (1 C of Cp4 H); 68.0 (1 C of Cp4 H); 68.4 (Cp5 H); 91.3 (C(1)). Anal. calc. for $C_{13}H_{16}FeOS$ (276.18): C 56.54, H 5.84; found: C 56.58, H 5.86.

2-(Ethylsulfanyl)-1-ferrocenylethanol (**4b**). Yield: 94%. IR (KBr): 3438, 3093, 2963, 2924, 2870, 1451, 1410, 1265, 1224, 1105, 1045, 1022, 1000, 816. ^{1}H-NMR (200 MHz): 1.26 (br. *t*, *J* = 7.3, Me); 2.56 (br. *q*, *J* = 7.3, MeC*H*$_2$); 2.68 (br., OH); 2.73 (*dd*, *J* = 13.6, 8.2, 1 H of CH_2S); 2.88 (*dd*, *J* = 13.6, 4.2, 1 H of CH_2S); 4.18 (br., 2 H of Cp4 H); 4.20 (*s*, Cp5 H); 4.28 (br., 2 H of Cp4 H); 4.44–4.50 (*m*, C*H*OH). ^{13}C-NMR: 14.7 (C(2′)); 26.4 (C(1′)); 40.0 (C(2)); 65.7 (1 C of Cp4 H); 66.8 (1 C of Cp4 H); 67.8 (1 C of Cp4 H); 67.9 (1 C of Cp4 H); 68.3 (1 C of Cp4 H); 68.4 (Cp5 H); 91.5 (C(1)). Anal. calc. for $C_{14}H_{18}FeOS$ (290.20): C 57.94, H 6.25; found: C 57.97, H 6.23.

1-Ferrocenyl-2-(propylsulfanyl)ethanol (**4c**). Yield: 79.5%. IR (KBr): 3445, 3094, 2960, 2920, 2871, 1456, 1410, 1382, 1223, 1105, 1046, 1022, 1000, 816. ^{1}H-NMR (200 MHz): 0.98 (*t*, *J* = 7.3, Me); 1.60 (br. *qt*, *J* = 7.3, MeC*H*$_2$); 2.50 (br. *t*, *J* = 7.3, CH_2CH_2S); 2.71 (*dd*, *J* = 13.5, 8.3, 1 H of CH_2S); 2.76 (br., OH); 2.85 (*dd*, *J* = 13.5, 4.3, 1 H of CH_2S); 4.15 (br. *t*, *J* = 1.6, 2 H of Cp4 H); 4.18 (*s*, Cp5 H); 4.27 (br. *t*, *J* = 1.6, 2 H of Cp4 H); 4.46 (br. *dd*, *J* = 8.3, 4.3, C*H*OH). ^{13}C-NMR: 13.3 (C(3′)); 22.8 (C(2′)); 34.5 (C(1′)); 40.4 (C(2)); 65.6 (1 C of Cp4 H); 66.6 (1 C of Cp4 H); 67.7 (1 C of Cp4 H); 67.8 (1 C of Cp4 H); 68.4 (Cp4 H); 68.4 (5 C of Cp5 H); 91.3 (C(1)). Anal. calc. for $C_{15}H_{20}FeOS$ (304.23): C 59.22, H 6.63; found: C 59.25, H 6.66.

1-Ferrocenyl-2-[(1-methylethyl)sulfanyl]ethanol (**4d**). Yield: 78.4%. IR (KBr): 3445, 3094, 2959, 2923, 2865, 1453, 1410, 1383, 1365, 1223, 1105, 1046, 1000, 816. ^{1}H-NMR (200 MHz): 1.27 (br. *d*, *J* = 6.8, 2 Me); 2.69 (br., OH); 2.74 (*dd*, *J* = 13.2, 8.1, 1 H of CH_2S); 2.89 (*dd*, *J* = 13.2, 4.2, 1 H of CH_2S); 2.94 (*sept.*, *J* = 6.8, Me_2CH); 4.17 (br., 2 H of Cp4 H); 4.20 (*s*, Cp5 H); 4.29 (br., 2 H of Cp4 H); 4.45 (*dd*, *J* = 8.1, 4.2, C*H*OH). ^{13}C-NMR: 23.4 (C(2′)); 23.4 (C(2′)); 35.2 (C(1′)); 38.9 (C(2)); 65.7 (1 C of Cp4 H); 66.8 (1 C of Cp4 H); 67.9 (1 C of Cp4 H); 68.4 (Cp5 H); 68.5 (1 C of Cp4 H); 68.6 (1 C of Cp4 H); 91.6 (C(1)). Anal. calc. for $C_{15}H_{20}FeOS$ (304.23): C 59.22, H 6.63; found: C 59.24, H 6.67.

2-(Butylsulfanyl)-1-ferrocenylethanol (**4e**). Yield: 99%. IR (KBr): 3447, 3094, 2956, 2927, 2871, 1464, 1411, 1273, 1221, 1106, 1047, 1022, 1000, 816. ^{1}H-NMR (200 MHz): 0.91 (*t*, *J* = 7.2, Me); 1.24–1.47 (*m*, MeC*H*$_2$); 1.48–1.66 (*m*, CH_2CH_2Me); 2.53 (*t*, *J* = 7.2, CH_2S); 2.66 (br., OH); 2.72 (*dd*, *J* = 13.4, 8.2, 1 H of CH_2S); 2.86 (*dd*, *J* = 13.2, 4.2, 1 H of CH_2S); 4.17 (br., 2 H of Cp4 H); 4.20 (*s*, Cp5 H); 4.28 (br., 2 H of Cp4 H); 4.46 (br. *dd*, *J* = 8.1, 4.2, C*H*OH). ^{13}C-NMR: 13.6 (C(4′)); 21.9 (C(3′)); 31.7 (C(2′)); 32.3 (C(1′)); 40.5 (C(2)); 65.7 (1 C of Cp4 H); 66.9 (1 C of Cp4 H); 67.8 (1 C of Cp4 H); 67.9 (1 C of Cp4 H); 68.5 (Cp5 H); 68.5 (1 C of Cp4 H); 91.5 (C(1)). Anal. calc. for $C_{16}H_{22}FeOS$ (318.26): C 60.38, H 6.97; found: C 60.40, H 6.96.

2-{[(2,6-Dichlorophenyl)methyl]sulfanyl}-1-ferrocenylethanol (**4f**). Yield: 79.6%. IR (KBr): 3447, 3092, 2920, 1580, 1560, 1436, 1219, 1105, 1087, 1047, 1000, 819, 778, 759. ^{1}H-NMR (200 MHz): 2.60 (*d*, *J* = 3.0, OH), 2.83 (*dd*, *J* = 13.6, 8.4, 1 H of CH_2S); 2.98 (*dd*, *J* = 13.6, 4.2, 1 H of CH_2S); 4.06 (*s*, ArC*H*$_2$S); 4.17 (br., 2 H of Cp4 H); 4.18 (*s*, Cp5 H); 4.21 (br., 2 H of Cp4 H); 4.45–4.55 (*m*, C*H*OH); 7.11 (*dd*, *J* = 7.2, 8.8, 1 arom. H); 7.30 (br. *d*, *J* ≈ 7.6, 2 arom. H). ^{13}C-NMR: 32.0 (C(1′)); 40.8 (C(2)); 65.7 (1 C of Cp4 H); 66.9 (1 C of Cp4 H); 67.8 (1 C of Cp4 H); 67.9 (1 C of Cp4 H); 68.5 (Cp5 H); 68.6 (1 C of Cp4 H); 91.5 (C(1)); 128.5; 128.6; 135.2; 135.4. Anal. calc. for $C_{19}H_{18}Cl_2FeOS$ (421.16): C 54.18, H 4.31; found: C 54.22, H 4.34.

1-Ferrocenyl-3-(methylsulfanyl)propan-1-ol (**4g**). Yield: 96.3%. IR (KBr): 3420, 3092, 2915, 1426, 1411, 1276, 1262, 1236, 1105, 1046, 1023, 1000, 960, 882, 815. ^{1}H-NMR (200 MHz): 1.87–2.00 (*m*, CH_2CH_2S); 2.09 (br., OH); 2.11 (*s*, Me); 2.57–2.67 (*m*, CH_2S); 4.15–4.19 (*m*, 2 H of Cp4 H); 4.20 (br. *s*,

Cp5 H); 4.22–4.26 (*m*, 2 H of Cp4 H); 4.43–4.54 (*m*, C*H*OH). ^{13}C-NMR: 15.5 (C(1′)); 30.7 (C(3)); 37.1 (C(2)); 65.3 (1 C of Cp4 H); 66.9 (1 C of Cp4 H); 67.8 (1 C of Cp4 H); 68.0 (1 C of Cp4 H); 68.3 (Cp5 H); 68.4 (1 C of Cp4 H); 93.4 (C(1)). Anal. calc. for $C_{15}H_{20}FeOS$ (290.20): C 57.94, H 6.25; found: C 57.98, H 6.22.

3-(Ethylsulfanyl)-1-ferrocenylpropan-1-ol (**4h**). Yield: 79.4%. IR (KBr): 3420, 3093, 2961, 2922, 1449, 1411, 1375, 1262, 1105, 1044, 1019, 1000, 889, 815. ^{1}H-NMR (200 MHz): 1.26 (*t*, *J* = 7.3, Me); 1.87–1.99 (*m*, CH_2CH$_2$S); 2.09 (*d*, *J* = 3.2, OH), 2.54 (br. *t*, *J* = 7.3, MeCH_2S); 2.60–2.70 (*m*, CH$_2$CH_2S); 4.17 (br. *t*, *J* = 1.6, Cp4 H); 4.20 (*s*, Cp5 H); 4.24 (br. *t*, *J* = 1.6, Cp4 H); 4.43–4.53 (*m*, C*H*OH). ^{13}C-NMR: 14.7 (C(2′)); 25.9 (C(1′)); 28.1 (C(3)); 37.5 (C(2)); 65.3 (1 C of Cp4 H); 66.9 (1 C of Cp4 H); 67.8 (1 C of Cp4 H); 68.0 (1 C of Cp4 H); 68.3 (Cp5 H); 68.4 (1 C of Cp4 H); 93.4 (C(1)). Anal. calc. for $C_{15}H_{20}FeOS$ (304.23): C 59.22, H 6.63; found: C 59.23, H 6.67.

1-Ferrocenyl-3-(propylsulfanyl)propan-1-ol (**4i**). Yield: 83.6%. IR (KBr): 3420, 3093, 2959, 2928, 2871, 1412, 1377, 1289, 1238, 1105, 1044, 1021, 1000, 888, 815. ^{1}H-NMR (200 MHz): 0.99 (*t*, *J* = 7.3, Me); 1.64 (br. *qt*, *J* = 7.3, MeCH$_2$); 1.87–2.00 (*m*, CH_2CH$_2$S); 2.09 (*d*, *J* = 3.2, OH); 2.51 (br. *t*, *J* = 7.3, MeCH$_2$CH_2S); 2.59–2.68 (*m*, CH(OH)CH$_2$CH_2S); 4.16 (br. *t*, *J* = 1.8, 2 H of Cp4 H); 4.20 (*s*, Cp5 H); 4.24 (br. *t*, *J* = 1.6, 2 H of Cp4 H); 4.43–4.54 (*m*, C*H*OH). ^{13}C-NMR: 13.4 (C(3′)); 22.84 (C(2′)); 28.5 (C(3)); 34.1 (C(1′)); 37.5 (C(2)); 65.3 (1 C of Cp4 H); 66.9 (1 C of Cp4 H); 67.8 (1 C of Cp4 H); 67.9 (1 C of Cp4 H); 68.3 (Cp5 H); 68.4 (1 C of Cp4 H); 93.4 (C(1)). Anal. calc. for $C_{16}H_{22}FeOS$ (318.26): C 60.38, H 6.97; found: C 60.41, H 6.94.

1-Ferrocenyl-3-[(1-methylethyl)sulfanyl]propan-1-ol (**4j**). Yield: 70.6%. IR (KBr): 3447, 3094, 2956, 2927.32, 2871, 1464, 1411, 1273, 1221, 1106, 1047, 1022, 1000, 816. ^{1}H-NMR (200 MHz): 1.26 (*d*, *J* = 6.8, Me); 1.27 (*d*, *J* = 6.8, Me); 1.85–1.99 (*m*, CH_2CH$_2$S); 2.08 (*d*, *J* = 3.2, OH), 2.61–2.71 (*m*, CH$_2$S); 2.94 (*sept.*, *J* = 6.8, Me$_2$C*H*) 4.16 (br. *t*, *J* = 1.6, 2 H Cp4 H); 4.20 (*s*, Cp5 H); 4.24 (br. *t*, *J* = 1.6, 2 H of Cp4 H); 4.43–4.54 (*m*, C*H*OH). ^{13}C-NMR: 23.3 (C(2′)); 27.0 (C(3)); 34.8 (C(1′)); 37.7 (C(2)); 65.4 (1 C of Cp4 H); 66.9 (1 C of Cp4 H); 67.8 (1 C of Cp4 H); 67.9 (1 C of Cp4 H); 68.3 (Cp5 H); 68.5 (1 C of Cp4 H); 93.4 (C(1)). Anal. calc. for $C_{16}H_{22}FeOS$ (318.26): C 60.38, H 6.97; found: C 60.40, H 6.98.

3-(Butylsulfanyl)-1-ferrocenylpropan-1-ol (**4k**). Yield: 80.5%. IR (KBr): 3433, 3094, 2956, 2927, 2871, 1465, 1412, 1382, 1271, 1227, 1105, 1044, 1022, 1000, 816. ^{1}H-NMR (200 MHz): 0.92 (*t*, *J* = 7.2, Me); 1.30–1.46 (*m*, MeCH_2); 1.47–1.65 (*m*, CH_2CH$_2$Me); 1.85–2.00 (*m*, CH(OH)CH_2CH$_2$S); 2.09 (*d*, *J* = 3.4, OH); 2.53 (*t*, *J* = 7.6, MeCH$_2$CH$_2$CH_2S); 2.60–2.69 (*m*, CH$_2$S); 4.14–4.19 (*m*, 2 H of Cp4 H); 4.22–4.25 (*s*, Cp5 H); 4.24 (*m*, 2 H of Cp4 H); 4.43–4.54 (*m*, C*H*OH). ^{13}C-NMR: 13.6 (C(4′)); 21.6 (C(3′)); 28.6 (C(3)); 31.7 (C(2′)); 31.8 (C(1′)); 37.6 (C(2)); 65.3 (1 C of Cp4 H); 66.9 (1 C of Cp4 H); 67.8 (1 C of Cp4 H); 67.9 (1 C of Cp4 H); 68.3 (Cp5 H); 68.4 (1 C of Cp4 H); 93.4 (C(1)). Anal. calc. for $C_{17}H_{24}FeOS$ (332.28): C 61.45, H 7.28; found: C 61.44, H 7.26.

3-[(2,6-Dichlorophenyl)methylsulfanyl]-1-ferrocenylpropan-1-ol (**4l**). Yield: 83.6%. IR (KBr): 3427, 3091, 2923, 1580, 1560, 1435, 1105, 1087, 1051, 1022, 1000, 891, 817, 777, 760, 692. ^{1}H-NMR (200 MHz): 1.89–2.02 (*m*, CH_2CH$_2$S, OH); 2.70–2.80 (*m*, CH(OH)CH$_2$CH_2S); 4.04 (br. *s*, ArCH_2S); 4.15–4.17 (*m*, 2 H of Cp4 H); 4.19 (*s*, Cp5 H); 4.20–4.23 (*m*, 2 H of Cp4 H); 4.42–4.53 (*m*, C*H*OH); 7.11 (*dd*, *J* = 7.2, 8.8, 1 arom. H); 7.30 (br. *d*, *J ca.* 7.6, 2 arom. H). ^{13}C-NMR: 29.1 (C(3)); 31.6 (C(1′)); 37.7 (C(2)); 65.3 (1 C of Cp4 H); 67.0 (1 C of Cp4 H); 67.8 (1 C of Cp4 H); 67.9 (1 C of Cp4 H); 68.3 (Cp5 H); 68.4 (1 C of Cp4 H); 93.4 (C(1)); 128.4; 128.5; 135.3; 135.4. Anal. calc. for $C_{20}H_{20}Cl_2FeOS$ (435.19): C 55.20, H 4.63; found: C 55.23, H 4.62.

Synthesis of Oximes **5a**–**5l** [7]. The soln. of 1 mmol of the corresponding ketone (**2a**–**2l**) in 20 ml of MeOH was added to a soln. of 820 mg (10 mmol) of AcONa and 695 mg (10 mmol) of NH$_2$OH·HCl in 10 ml of H$_2$O. The mixture was refluxed for 2 h, cooled to r.t., and then MeOH was evaporated *in vacuo*. The residue was extracted with CH$_2$Cl$_2$ (two 30-ml portions), org. layers were washed with H$_2$O and brine, and dried (Na$_2$SO$_4$) overnight. After the evaporation of the solvent, the solid was filtered through a short pad (5 g of SiO$_2$; toluene/AcOEt 9 : 1 (*v*/*v*)) to obtain mixture of (*Z*)- and (*E*)-oximes **5a**–**5k**. The ratios of the obtained stereoisomers were determined by using the integrals of appropriate corresponding non-overlapping ^{1}H-NMR signals.

*(*E*)- and (*Z*)-1-Ferrocenyl-2-(methylsulfanyl)ethan-1-one Oxime* (**5a**). Yield: quant. Spectral data in accordance with those previously published for the same compound(s) [7]. (*E*)/(*Z*) 67.7 : 32.3. Anal. calc. for $C_{13}H_{16}FeNOS$ (289.02): C 53.99, H 5.23; found: C 53.98, H 5.25.

*(*E*)- and (*Z*)-2-(Ethylsulfanyl)-1-ferrocenylethanone Oxime* (**5b**). Yield: quant. (*E*)/(*Z*) 67.5 : 32.5. IR (KBr): 3205, 2965, 2929, 1634, 1442, 1404, 1298, 1230, 1106, 1030, 1021, 1002, 976, 940, 877, 824, 768, 707, 576, 486.

(*E*)-Isomer. ^{1}H-NMR (200 MHz): 1.31 (*t*, *J* = 7.4, Me); 2.69 (*q*, *J* = 7.4, MeC*H*$_2$); 3.74 (*s*, CH$_2$S); 4.19 (*s*, Cp5 H); 4.34 (*t*, *J* = 1.9, 2 H of Cp4 H); 4.64 (*t*, *J* = 1.9, 2 H of Cp4 H); 9.78 (*s*, OH). ^{13}C-NMR: 14.7 (C(2′)); 25.2 (C(1′)); 26.9 (C(2)); 66.9 (2 C of Cp4 H); 69.6 (2 C of Cp4 H); 69.4 (5 C of Cp5 H); 79.3 (1 C of Cp4 H); 156.5 (C(1)).

(*Z*)-Isomer. ^{1}H-NMR (200 MHz): 1.24 (*t*, *J* = 7.3, Me); 2.58 (*q*, *J* = 7.3, MeC*H*$_2$); 3.54 (*s*, CH$_2$S); 4.18 (*s*, Cp5 H); 4.39 (*t*, *J* = 1.9, 2 H of Cp4 H); 5.0 (*t*, *J* = 1.9, 2 H of Cp4 H); 9.78 (*s*, OH). ^{13}C-NMR: 14.3 (C(2′)); 25.9 (C(1′)); 34.4 (C(2)); 69.5 (5 C of Cp5 H); 70.1 (2 C of Cp4 H); 71.0 (2 C of Cp4 H); 72.8 (1 C of Cp4 H); 153.4 (C(1)). Anal. calc. for $C_{14}H_{17}FeNOS$ (303.20): C 55.46, H 5.65; found: C 55.48, H 5.66.

*(*E*)- and (*Z*)-1-Ferrocenyl-2-(propylsulfanyl)ethanone Oxime* (**5c**). Yield: quant. (*E*)/(*Z*) 67.9 : 32.1. IR (KBr): 3420, 3165, 2961, 2925, 1619, 1454, 1403, 1382, 1298, 1106, 1029, 1000, 956, 883, 821, 514, 504, 486.

(*E*)-Isomer. ^{1}H-NMR (200 MHz): 0.98 (*t*, *J* = 7.3, Me); 1.67 (br. *qt*, *J* = 7.3, MeC*H*$_2$); 2.64 (*t*, *J* = 7.3, MeCH$_2$C*H*$_2$S); 3.72 (*s*, C(NOH)C*H*$_2$S); 4.18 (*s*, Cp5 H); 4.32 (*t*, *J* = 1.8, 2 H of Cp4 H); 4.64 (*t*, *J* = 1.8, 2 H of Cp4 H); 10.07 (*s*, OH). ^{13}C-NMR: 13.4 C(3′)); 22.8 (C(2′)); 25.4 (C(2)); 34.9 (C(1′)); 66.8 (2 C of Cp4 H); 69.5 (2 C of Cp4 H); 69.3 (Cp5 H); 79.2 (1 C of Cp4 H); 156.4 (C(1)).

(*Z*)-Isomer. ^{1}H-NMR (200 MHz): 0.97 (*t*, *J* = 7.3, Me); 1.64 (br. *qt*, *J* = 7.3, MeC*H*$_2$); 2.56 (*t*, *J* = 7.3, MeCH$_2$C*H*$_2$S); 3.55 (*s*, C(NOH)C*H*$_2$S); 4.17 (*s*, Cp5 H); 4.37 (*t*, *J* = 1.8, 2 H of Cp4 H); 5.0 (*t*, *J* = 1.8, 2 H of Cp4 H); 10.07 (*s*, OH). ^{13}C-NMR: 15.1 (C(3′)); 22.4 (C(2′)); 34.0 (C(1′)); 34.6 (C(2)); 69.4 (Cp5 H); 70.0 (2 C of Cp4 H); 71.0 (2 C of Cp4 H); 72.7 (1 C of Cp4 H); 153.4 (C(1)). Anal. calc. for $C_{15}H_{19}FeNOS$ (317.23): C 56.79, H 6.04; found: C 56.82, H 6.04.

*(*E*)- and (*Z*)-1-Ferrocenyl-2-[(1-methylethyl)sulfanyl]ethan-1-one Oxime* (**5d**). Yield: quant. (*E*)/(*Z*) 70.1 : 29.9. IR (KBr): 3202, 2959, 2924, 2865, 1623, 1452, 1411, 1382, 1296, 1106, 1030, 1001, 952, 881, 820, 502, 487.

(*E*)-Isomer. ^{1}H-NMR (200 MHz): 1.31 (*t*, *J* = 6.8, 2 Me); 3.1 (*sept.*, *J* = 6.8, Me$_2$C*H*); 3.73 (*s*, CH$_2$S); 4.19 (*s*, Cp5 H); 4.32 (*t*, *J* = 1.9, 2 H of Cp4 H); 4.61 (*t*, *J* = 1.9, 2 H of Cp4 H); 10.08 (*s*, OH). ^{13}C-NMR: 23.0 (C(2′)); 36.1 (C(1′)); 24.6 (C(2)); 66.8 (2 C of Cp4 H); 69.5 (2 C of Cp4 H); 69.3 (Cp5 H); 79.3 (1 C of Cp4 H); 156.6 (C(1)).

(*Z*)-Isomer. ^{1}H-NMR (200 MHz): 1.30 (*t*, *J* = 6.8, 2 Me); 3.02 (*sept.*, *J* = 6.8, Me$_2$C*H*); 3.60 (*s*, CH$_2$S); 4.17 (*s*, Cp5 H); 4.37 (*t*, *J* = 1.9, 2 H of Cp4 H); 5.0 (*t*, *J* = 1.9, 2 H of Cp4 H); 10.08 (*s*, OH). ^{13}C-NMR: 23.3 (C(2′)); 33.6 (C(1′)); 34.9 (C(2)); 69.4 (Cp5 H); 70.0 (2 C of Cp4 H); 71.0 (2 C of Cp4 H); 72.6 (1 C of Cp4 H); 153.6 (C(1)). Anal. calc. for $C_{15}H_{19}FeNOS$ (317.05): C 56.79, H 6.04; found: C 56.81, H 6.02.

*(*E*)- and (*Z*)-2-(Butylsulfanyl)-1-ferrocenylethanone Oxime* (**5e**). Yield: quant. (*E*)/(*Z*) 68.4 : 31.6. IR (KBr): 3206, 2957, 2929, 1627, 1456, 1412, 1381, 1297, 1225, 1107, 1030, 1001, 951, 881, 820, 503. 488.

(*E*)-Isomer. ^{1}H-NMR (200 MHz): 0.90 (*t*, *J* = 7.4, Me); 1.40 (br. *qt*, *J* = 7.4, MeC*H*$_2$); 1.63 (*tt*, *J* = 7.4, C*H*$_2$CH$_2$Me); 2.66 (*t*, *J* = 7.4, MeCH$_2$CH$_2$C*H*$_2$S); 3.72 (*s*, C(NOH)C*H*$_2$S); 4.18 (*s*, Cp5 H); 4.32 (br., 2 H of Cp4 H); 4.64 (br., 2 H of Cp4 H); 10.03 (*s*, OH). ^{13}C-NMR: 13.6 (C(4′)); 21.9 C(3′)); 25.4 (C(2)); 31.5 (C(2′)); 32.6 (C(1′)); 66.8 (2 C of Cp4 H); 69.3 (Cp5 H); 69.5 (2 C of Cp4 H); 79.2 (1 C of Cp4 H); 156.4 (C(1)).

(*Z*)-Isomer. ^{1}H-NMR (200 MHz): 0.90 (*t*, *J* = 7.4, Me); 1.40 (br. *qt*, *J* = 7.4, MeC*H*$_2$); 1.63 (*tt*, *J* = 7.4, CH$_2$C*H*$_2$CH$_2$Me); 2.58 (*t*, *J* = 7.4, MeCH$_2$CH$_2$C*H*$_2$S); 3.55 (*s*, C(NOH)C*H*$_2$S); 4.17 (*s*, Cp5 H); 4.37 (br., 2 H of Cp4 H); 5.0 (br., 2 H of Cp4 H); 10.03 (*s*, OH). ^{13}C-NMR: 13.6 (C(4′)); 21.9 C(3′)); 31.2 (C(2′)); 31.6 (C(1′)); 34.6 (C(2)); 69.4 (Cp5 H); 69.9 (2 C of Cp4 H); 71.0 (2 C of Cp4 H); 73.7 (1 C of Cp4 H); 153.3 (C(1)). Anal. calc. for $C_{16}H_{21}FeNOS$ (331.24): C 58.01, H 6.39; found: C 58.00, H 6.40.

*(*E*)- and (*Z*)-2-{[(2,6-Dichlorophenyl)methyl]sulfanyl}-1-ferrocenylethan-1-one Oxime* (**5f**). Yield: quant. (*E*)/(*Z*) 68.9 : 31.1. IR (KBr): 3202, 2937, 1638, 1579, 1559, 1436, 1299, 1106, 1088, 1026, 1003, 983, 953, 892, 876, 814, 776, 760, 512, 499, 488.

(*E*)-Isomer. ^{1}H-NMR (200 MHz): 3.88 (*s*, C(NOH)C*H*$_2$S); 4.25 (*s*, ArC*H*$_2$S); 4.17 (*s*, Cp5 H); 4.30 (*t*, *J* = 1.8, 2 H of Cp4 H); 4.53 (*t*, *J* = 1.8, 2 H of Cp4 H); 9.40 (*s*, OH); 7.11 (*dd*, *J* = 7.2, 8.8, 1 arom. H); 7.30 (br. *d*, *J ca.* 7.6, 2 arom. H). ^{13}C-NMR: 26.8 (C(2)); 33.6 (C(1′)); 66.8 (2 C of Cp4 H); 69.3 (Cp5 H); 69.6 (2 C of Cp4 H); 79.3 (1 C of Cp4 H); 155.8 (C(1)).

(*Z*)-Isomer. ^{1}H-NMR (200 MHz): 3.74 (*s*, C(NOH)CH_2S); 4.13 (*s*, ArCH_2S); 4.16 (*s*, Cp5 H); 4.36 (*t*, *J* = 1.8, 2 H of Cp4 H); 4.92 (*t*, *J* = 1.8, 2 H of Cp4 H); 9.40 (*s*, OH); 7.11 (*dd*, *J* = 7.2, 8.8, 1 arom. H); 7.30 (br. *d*, *J* ≈ 7.6, 2 arom. H). ^{13}C-NMR: 32.2 (C(1′)); 35.8 (C(2)); 69.5 (Cp5 H); 70.0 (2 C of Cp4 H); 70.8 (2 C of Cp4 H); 72.9 (1 C of Cp4 H); 153.1 (C(1)). Anal. calc. for $C_{19}H_{17}Cl_2FeNOS$ (434.16): C 52.56, H 3.95; found: C 52.57, H 3.94.

*(*E*)- and (*Z*)-1-Ferrocenyl-3-(methylsulfanyl)propan-1-one Oxime* (**5g**). Yield: quant. (*E*)/(*Z*) 77.5 : 22.5. IR (KBr): 3220, 3111, 2916, 1632, 1446, 1383, 1318, 1296, 1106, 1035, 1022, 999, 959, 934, 888, 809, 518, 503, 486.

(*E*)-Isomer. ^{1}H-NMR (200 MHz): 2.23 (*s*, Me); 2.76–3.01 (*m*, CH_2CH_2); 4.19 (*s*, Cp5 H); 4.34 (*t*, *J* = 1.8, 2 H of Cp4 H); 4.56 (*t*, *J* = 1.8, 2 H of Cp4 H); 9.26 (*s*, OH). ^{13}C-NMR: 15.6 (C(1′)); 28.2 (C(3)); 30.9 (C(2)); 66.6 (2 C of Cp4 H); 69.3 (Cp5 H); 69.8 (2 C of Cp4 H); 80.2 (1 C of Cp4 H); 157.9 (C(1)).

(*Z*)-Isomer. ^{1}H-NMR (200 MHz): 2.19 (*s*, Me); 2.76–3.01 (*m*, CH_2CH_2); 4.18 (*s*, Cp5 H); 4.38 (*t*, *J* = 1.8, 2 H of Cp4 H); 4.91 (*t*, *J* = 1.8, 2 H of Cp4 H); 9.26 (*s*, OH). ^{13}C-NMR: 15.7 (C(1′)); 32.3 (C(3)); 34.2 (C(2)); 69.5 (5 C of Cp5 H); 70.1 (2 C of Cp4 H); 70.7 (2 C of Cp4 H); 73.5 (1 C of Cp4 H); 150.3 (C(1)). Anal. calc. for $C_{14}H_{17}FeNOS$ (303.20): C 55.46, H 5.65; found: C 55.45, H 5.69.

*(*E*)- and (*Z*)-3-(Ethylsulfanyl)-1-ferrocenylpropan-1-one Oxime* (**5h**). Yield: quant. (*E*)/(*Z*) 77.9 : 22.1. IR (KBr): 3415, 3197, 3140, 2923, 1641, 1407, 1105, 1026, 1000, 971, 937, 886, 820, 774, 520, 506, 490.

(*E*)-Isomer. ^{1}H-NMR (200 MHz): 1.33 (*t*, *J* = 7.4, Me); 2.67 (*q*, *J* = 7.4, MeCH_2); 2.78–3.00 (*m*, CH_2CH_2); 4.19 (*s*, Cp5 H); 4.34 (*t*, *J* = 1.8, 2 H of Cp4 H); 4.56 (*t*, *J* = 1.8, 2 H of Cp4 H); 9.26 (*s*, OH). ^{13}C-NMR: 14.7 (C(2′)); 26.0 (C(1′)); 28.2 (C(3)); 28.7 (C(2)); 66.6 (2 C of Cp4 H); 69.3 (Cp5 H); 69.5 (2 C of Cp4 H); 80.2 (1 C of Cp4 H); 157.9 (C(1)).

(*Z*)-Isomer. ^{1}H-NMR (200 MHz): 1.30 (*t*, *J* = 7.4, Me); 2.63 (*q*, *J* = 7.4, MeCH_2); 2.78–3.00 (*m*, CH_2CH_2); 4.18 (*s*, Cp5 H); 4.34 (*t*, *J* = 1.8, 2 H of Cp4 H); 4.91 (*t*, *J* = 1.8, 2 H of Cp4 H); 9.26 (*s*, OH). ^{13}C-NMR: 14.7 (C(2′)); 26.2 (C(1′)); 29.7 (C(3)); 30.9 (C(2)); 69.8 (Cp5 H); 70.1 (2 C of Cp4 H); 70.7 (2 C of Cp4 H); 73.6 (1 C of Cp4 H); 150.3 (C(1)). Anal. calc. for $C_{15}H_{19}FeNOS$ (317.23): C 56.79, H 6.04; found: C 56.82, H 6.00.

*(*E*)- and (*Z*)-1-Ferrocenyl-3-(propylsulfanyl)propan-1-one Oxime* (**5i**). Yield: quant. (*E*)/(*Z*) 77.2 : 22.8. IR (KBr): 3197, 2925, 2871, 1636, 1452, 1409, 1382, 1106, 1025, 1000, 936, 883, 819, 518, 506, 490.

(*E*)-Isomer. ^{1}H-NMR (200 MHz): 1.01 (*t*, *J* = 7.2, Me); 1.65 (br. *qt*, *J* = 7.2, MeCH_2); 2.61 (*t*, *J* = 7.2, CH_2S); 2.75–3.00 (*m*, CH_2CH_2S); 4.17 (*s*, Cp5 H); 4.32 (br., 2 H of Cp4 H); 4.56 (br., 2 H of Cp4 H); 9.90 (*s*, OH). ^{13}C-NMR: 13.4 (C(3′)); 22.7 (C(2′)); 28.5 (C(3)); 28.7 (C(2)); 34.0 (C(1′)); 66.5 (2 C of Cp4 H); 69.1 (Cp5 H); 69.6 (2 C of Cp4 H); 80.0 (1 C of Cp4 H); 157.9 (C(1)).

(*Z*)-Isomer. ^{1}H-NMR (200 MHz): 1.00 (*t*, *J* = 7.2, Me); 1.64 (br. *qt*, *J* = 7.2, MeCH_2); 2.57 (*t*, *J* = 7.2, CH_2S); 2.75–3.00 (*m*, CH_2CH_2S); 4.17 (*s*, Cp5 H); 4.36 (br., 2 H of Cp4 H); 4.91 (br., 2 H of Cp4 H); 9.90 (*s*, OH). ^{13}C-NMR: 13.4 (C(3′)); 22.8 (C(2′)); 30.1 (C(3)); 34.2 (C(2)); 34.5 (C(1′)); 69.3 (Cp5 H); 69.9 (2 C of Cp4 H); 70.6 (2 C of Cp4 H); 73.5 (1 C of Cp4 H); 154.7 (C(1)). Anal. calc. for $C_{16}H_{21}FeNOS$ (331.25): C 58.01, H 6.39; found: C 58.00, H 6.43.

*(*E*)- and (*Z*)-1-Ferrocenyl-3-[(1-methylethyl)sulfanyl]propan-1-one Oxime* (**5j**). Yield: quant. (*E*)/(*Z*) 77.4 : 22.6. IR (KBr): 3416, 3205, 2956, 2923, 1639, 1442, 1409, 1382, 1154, 1105, 1025, 937, 883, 820, 746, 520, 508, 494.

(*E*)-Isomer. ^{1}H-NMR (200 MHz): 1.33 (*d*, *J* = 6.6, 2 Me); 2.78–2.96 (*m*, CH_2CH_2); 3.11 (*sept.*, *J* = 6.6, Me_2C*H*); 4.19 (*s*, Cp5 H); 4.34 (*t*, *J* = 1.8, 2 H of Cp4 H); 4.55 (*t*, *J* = 1.8, 2 H of Cp4 H); 9.12 (*s*, OH). ^{13}C-NMR: 23.4 (C(2′)); 27.2 (C(3)); 28.9 (C(2)); 34.8 (C(1′)); 66.6 (2 C of Cp4 H); 69.3 (Cp5 H); 69.5 (2 C of Cp4 H); 80.2 (1 C of Cp4 H); 158.0 (C(1)).

(*Z*)-Isomer. ^{1}H-NMR (200 MHz): 1.31 (*d*, *J* = 6.6, 2 Me); 2.78–2.96 (*m*, CH_2CH_2); 3.11 (*sept.*, *J* = 6.6, Me_2C*H*); 4.18 (*s*, Cp5 H); 4.38 (*t*, *J* = 1.8, 2 H of Cp4 H); 4.91 (*t*, *J* = 1.8, 2 H of Cp4 H); 9.12 (*s*, OH). ^{13}C-NMR: 23.5 (C(2′)); 30.2 (C(3)); 34.7 (C(2)); 35.1 (C(1′)); 69.8 (2 C of Cp4 H); 70.1 (Cp5 H); 70.7 (2 C of Cp4 H); 73.6 (1 C of Cp4 H); 150.3 (C(1)). Anal. calc. for $C_{16}H_{21}FeNOS$ (331.25): C 58.01, H 6.39; found: C 58.03, H 6.42.

*(*E*)- and (*Z*)-3-(Butylsulfanyl)-1-ferrocenylpropan-1-one Oxime* (**5k**). Yield: quant. (*E*)/(*Z*) 79.0 : 21.0. IR (KBr): 3223, 2952, 1637, 1437, 1411, 1381, 1320, 1105, 1033, 1006, 983, 937, 889, 836, 817, 585, 520, 504.

(*E*)-Isomer. ^{1}H-NMR (200 MHz): 0.94 (*t*, *J* = 7.2, Me); 1.44 (br. *qt*, *J* = 7.2, MeC*H*$_2$); 1.64 (*tt*, *J* = 7.2, C*H*$_2$CH$_2$Me); 2.64 (*t*, *J* = 7.2, CH$_2$S); 2.78–2.99 (*m*, C*H*$_2$C*H*$_2$S); 4.19 (*s*, Cp5 H); 4.34 (*t*, *J* = 1.8, 2 H of Cp4 H); 4.55 (*t*, *J* = 1.8, 2 H of Cp4 H); 9.40 (br., OH). ^{13}C-NMR: 13.7 (C(4′)); 22.0 (C(3′)); 28.7 (C(3)); 28.7 (C(2)); 31.7 (C(2′)); 31.8 C(1′)); 66.6 (2 C of Cp4 H); 69.3 (Cp5 H); 69.8 (2 C of Cp4 H); 80.2 (1 C of Cp4 H); 157.92 (C(1)).

(*Z*)-Isomer. ^{1}H-NMR (200 MHz): 0.94 (*t*, *J* = 7.2, Me); 1.44 (br. *qt*, *J* = 7.2, MeC*H*$_2$); 1.63 (*tt*, *J* = 7.2, C*H*$_2$CH$_2$Me); 2.61 (*t*, *J* = 7.2, CH$_2$S); 2.78–2.99 (*m*, C*H*$_2$C*H*$_2$S); 4.18 (*s*, Cp5 H); 4.37 (*t*, *J* = 1.8, 2 H of Cp4 H); 4.91 (*t*, *J* = 1.8, 2 H of Cp4 H); 9.40 (br., OH). ^{13}C-NMR: 13.7 (C(4′)); 22.0 (C(3′)); 30.2 (C(3)); 31.8 (C(2′)); 32.1 C(1′)); 34.6 (C(1)); 69.5 (Cp5 H); 70.1 (2 C of Cp4 H); 70.7 (2 C of Cp4 H); 73.6 (1 C of Cp4 H); 150.3 (C(1)). Anal. calc. for $C_{17}H_{23}FeNOS$ (345.28): C 59.14, H 6.71; found: C 59.17, H 6.74.

*(*E*)- and (*Z*)-3-{[(2,6-Dichlorophenyl)methyl]sulfanyl}-1-ferrocenylpropan-1-one Oxime* (**5l**). Yield: quant. (*E*)/(*Z*) 87.2 : 12.8. IR (KBr): 3436, 3204, 2931, 1629, 1435, 1420, 1410, 1284, 1106, 1028, 999, 939, 867, 833, 816, 777, 760, 580, 512, 502, 486.

(*E*)-Isomer. ^{1}H-NMR (200 MHz): 2.93–3.03 (*m*, CH$_2$CH$_2$); 4.21 (*s*, CH$_2$S); 4.17 (*s*, Cp5 H); 4.34 (*t*, *J* = 1.8, 2 H of Cp4 H); 4.54 (*t*, *J* = 1.8, 2 H of Cp4 H); 7.11 (*dd*, *J* = 7.2, 8.8, 1 arom. H); 7.30 (br. *d*, *J ca.* 7.6, 2 arom. H); 9.11 (*s*, OH). ^{13}C-NMR: 28.6 (C(3)); 29.8 (C(2)); 32.2 C(1′)); 66.6 (2 C of Cp4 H); 69.3 (Cp5 H); 69.8 (2 C of Cp4 H); 80.2 (Cp4 H); 128.4; 128.5; 135.3; 135.5; 157.9 (C(1)).

(*Z*)-Isomer. ^{1}H-NMR (200 MHz): 2.93–3.03 (*m*, CH$_2$CH$_2$); 4.11 (*s*, CH$_2$S); 4.16 (*s*, Cp5 H); 4.36 (*t*, *J* = 1.8, 2 H of Cp4 H); 4.88 (*t*, *J* = 1.8, 2 H of Cp4 H); 7.11 (*dd*, *J* = 7.2, 8.8, 1 arom. H); 7.30 (br. *d*, *J* ≈ 7.6, 2 arom. H); 9.11 (*s*, OH). ^{13}C-NMR: 69.5 (Cp5 H); 70.0 (2 C of Cp4 H); 70.7 (2 C of Cp4 H); 73.6 (1 C of Cp4 H); 128.4; 128.5; 135.3; 135.5; 150.3 (C(1)). Anal. calc. for $C_{20}H_{19}Cl_2FeNOS$ (448.19): C 53.60, H 4.27; found: C 53.59, H 4.28.

Electrochemical Measurements. Electrochemical measurements were performed at r.t. (*ca.* 25°) with an *Autolab potentiostat* (*PGSTAT 302N*). A standard three-electrode cell (5 ml) equipped with a Pt wire and a Ag/AgCl electrode immersed in 0.1M $LiClO_4$ soln. in MeCN as the counter and reference electrode, resp. A Pt disk (*d* = 2 mm) was used as the working electrode.

Antimicrobial Assay. The *in vitro* antimicrobial activities of **4a**–**4l** and **5a**–**5l** were tested against a panel of laboratory control strains (food isolates or those belonging to the *American Type Culture Collection*, Maryland, USA). Antibacterial activities were evaluated against two *Gram*-positive and three *Gram*-negative bacteria. The following *Gram*-positive bacteria used were *Bacillus cereus* (food isolate) and *Staphylococcus aureus* (ATCC 6538). The *Gram*-negative bacteria utilized in the assays were *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 10031), and *Proteus vulgaris* (ATCC 8427). The antifungal activity was tested against *Candida albicans* (ATCC 10231). Bacterial isolates were obtained from the Institute of Public Health, Niš, Serbia, and are stored in the microbiological collection at the Microbiology Laboratory (Department of Biology, Faculty of Science and Mathematics, University of Niš, Niš, Serbia). Broth microdilution susceptibility assay was used, as recommended by NCCLS, for the determination of *MIC* values (NCCLS [23]). All tests were performed in *Mueller–Hinton* broth (MHB; BBL) supplemented with *Tween 80* detergent (final concentration of 0.5% (*v*/*v*)), with the exception of the fungal organism (*Sabouraud* dextrose broth-SDB + *Tween 80*), and with 2×10^5 colony-forming units (CFU) ml^{-1} of the bacteria/fungi in the exponential phase. Test compounds, **4a**–**4l** and **5a**–**5l**, were dissolved in DMSO, and this stock soln. was used to prepare the exact agar dilutions. Final concentrations of the compounds in the broth ranged from 0.001 to 5000 μg/ml, and these were prepared in a 96-well microtiter plate. The dilutions were based on a geometrical order (factor 2). To obtain more accurate *MIC* values, further dilutions were additionally prepared in the concentration range between the first well without visible growth and the one next to it with lower test compound concentration. Plates were incubated at 37° for 24 h for bacteria, and at 30° for 48 h for the yeasts. Each test was performed in duplicate and repeated twice. Tetracycline and nystatin were used as positive controls, while DMSO + *Tween 80* (in the form of a blank) were the negative control.

Statistical Analysis. Principal component analysis (PCA) and agglomerative hierarchical clustering (AHC) were performed using the Excel program plug-in XLSTAT version 2011.3.02 [24]. Both methods were applied utilizing the *MIC* values as original variables without any recalculation. AHC was determined using *Pearson* dissimilarity, where the aggregation criterion were simple linkage, unweighted pair-group average and complete linkage, and *Euclidean* distance where the aggregation criterion were

weighted pair-group average, unweighted pair-group average, and *Ward*'s method. PCA of the *Pearson* (n) type was performed.

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