Chapter 5

Synthesis and Biological Evaluation of Some Thiazolylhydrazinomethylideneferrocenes as Antimicrobial Agents
CHAPTER 5. Synthesis and Biological Evaluation of Some Thiazolylhydrazinomethylideneferrocenes as Antimicrobial Agents

5.1. Motivation for the current work

Metal containing macromolecules form an integral part of the human system and participate as structural protein components as well as in enzymatic activities such as haemoglobin and transferrin (Fe), carbonic anhydrase (Zn), xanthine oxidase (Mb), hepatocuperin (Cu), etc. The introduction of metal ions in biologically active molecules is a continuing area of research for scientists and this innovative idea led to the development of a new field of science called bio-organometallic chemistry, forming a link between organometallic chemistry and biology, medicine and molecular biotechnology. Such organometallic medicinal compounds are widely used nowadays for curing various diseases, e.g. arsphenamine containing arsenic as antimalarial, silver sulfadiazine for treatment of severe burns, meralein containing mercury as antiseptic, etc. Thus ‘metal-based drugs’ are assuming increasing importance in the area of medicinal chemistry. During the last six decades after the discovery of ferrocene in 1951, the physico-chemistry as well as biochemistry of ferrocenes have attracted considerable attention of physicists, chemists and biologists due to their diverse applications in material sciences, catalysis and designing biologically active compounds. Therefore, they are now considered as “Legendary Magic Bullets” in organometallics.

From biochemistry point of view, ferrocene and its derivatives have wide range of biochemical applications. For example, avidin is a tetrameric glycoprotein found in the oviducts of reptiles, birds and amphibians that binds biotin with a very high affinity. Ferrocene derivatives can be coupled to the glycoprotein avidin through a flexible spacer molecule and results in the formulation of a conjugate with the reversible redox characteristic properties of ferrocenes and the biotin-binding properties of avidin. These ferrocene-avidin conjugates are potential elements in the electrochemical immunosensors employing enzyme fragments or redox enzymes as the electrochemical label for antibodies. These can also be employed for stable
immobilization of biotintylated redox enzymes on the electrodes to generate a large range of electrochemical enzyme sensors.

It has been reported that introduction of ferrocenyl moiety in a number of already in-use therapeutic drugs has considerably increased their bioactivities. A series of antibiotics conjugated with ferrocene\textsuperscript{17-19} that includes ferrocenyl-penicillin (2), ferrocenyl-cephalosporin (3) and ferrocenyl-hybrid of penicillin and cephalosporin (4) were synthesized in the 1970s. The introduction of ferrocene moiety in these drugs enhanced antibiotic activity many times to the original drugs. On the other hand, introduction of ferrocene moiety in established therapeutic antifungal compounds, fluconazole (5) and triadimenol (6) making the corresponding ferrocene-fluconazole\textsuperscript{20} (7) and ferrocene-triadimenol\textsuperscript{21} (8) gave no promising results as antifungal activity was either reduced or extinct.
The replacement of one of the phenyl rings in tamoxifen (9), a selective estrogen receptor modulator used against breast cancer, by ferrocene has given rise to a group of compounds called ferrocifens (10) which are the first molecules shown to be active against both hormone-dependent and hormone-independent breast cancer cells. Reported results support the hypothesis that tamoxifen-like framework in ferrocifens confers recognition for the estrogen receptor (estrogenic activity) while the presence of ferrocene induces damage to DNA (genotoxic), possibly via reactive oxygen species generated in a Fenton-like reaction involving the Fe$^{2+}$/Fe$^{3+}$ couple and hence exhibit greater anti-proliferative activity.

Some of the water soluble polyaspartamide-based ferrocene conjugates (11) synthesized by Neuse (11) were found to be potent anti-cancer agents. Some ferrocenium salts i.e. ferrocenium picrate (12) and ferrocenium trichloroacetate (13) were also found to possess good anti-proliferative activity due to better solubility and thus easy permeability through cell membrane.
Malaria is a disease caused by a parasite, *Plasmodium falciparum*. Several antimalarial drugs like chloroquine (14) are used against the malarial parasite, but an increasing resistance to the available drugs is a growing cause of concern. As the parasite needs iron for its development inside the red blood cells (RBCs), Brocard and his coworkers combined the poison (chloroquine) and bait (ferrocene) in the same molecule. They just inserted a ferrocenyl moiety into the side chain of chloroquine, producing a hybrid compound called ferroquine (15), which was found to be more potent than chloroquine.

Owing to various favorable properties associated with ferrocene such as stability, non toxicity, membrane permeability, favorable electrochemical properties as well as capability to exhibit various biomedicinal activities such as antimicrobial, anticancer, antiplasmodial, antitubercular, etc., chemists are trying for its further exploration and incorporation into various heterocyclic moieties.

It is known in the literature that thiazole and coumarin heterocyclic moieties, when present individually as well as together, exhibit diverse biological activities. It has recently been reported that ferrocene containing thiazole derivatives show significant antibacterial as well as antifungal activities. These considerations motivated us to synthesize some novel thiazolylhydrazinomethylideneferrocenes 16 and 17 bearing phenyl and coumarin moieties respectively for their evaluation as antibacterial and antifungal agents.
5.2. Synthetic discussion for thiazolylhydrazinomethylideneferrocenes (16 and 17)

Target thiazolylhydrazinomethylideneferrocenes 16 and 17 have been synthesized by the reaction of formylferrocene thiosemicarbazone 20 with various $p$-substituted phenacyl bromides (21) and 6-substituted-3-bromoacetylcoumarins (22) respectively according to Scheme 5.1. Treatment of ethanolic solution of ferrocene carbaldehyde 18 with thiosemicarbamide (19) dissolved in water in the presence of small amount of acetic acid afforded formylferrocene thiosemicarbazone 20 which on subsequent reaction with various $p$-substituted phenacyl bromides (21) and 6-substituted-3-bromoacetylcoumarins (22) in refluxing EtOH : THF in the presence of sodium acetate afforded the target thiazolylhydrazinomethylideneferrocenes 16 and 17 respectively. The synthetic details for each step are given in the following text.

### Scheme 5.1. Synthesis of some novel thiazolylhydrazinomethylideneferrocenes 16 and 17

<table>
<thead>
<tr>
<th>Compound 16 &amp; 21</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
<th>e</th>
<th>f</th>
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<tr>
<td>R</td>
<td>H</td>
<td>CH$_3$</td>
<td>F</td>
<td>Cl</td>
<td>Br</td>
<td>NO$_2$</td>
</tr>
<tr>
<td>Compound 17 &amp; 22</td>
<td>a</td>
<td>b</td>
<td>c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R$'$</td>
<td>H</td>
<td>Cl</td>
<td>Br</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
5.2.1. Synthesis of formylferrocene thiosemicarbazone (20)

Ferrocene carbaldehyde 18 was converted into formylferrocene thiosemicarbazone by dropwise addition of an ethanolic solution of 18 to a solution of thiosemicarbazide (19) in water. Small amount of glacial acetic acid was then added and the resulting reaction mixture was refluxed for 1 h. On completion of the reaction, the content was concentrated and allowed to cool to room temperature to obtain dark red precipitates which were filtered, washed with cold water, dried and crystallized from aqueous ethanol to afford formylferrocene thiosemicarbazone in good yield. The mechanism of the reaction (Scheme 5.2) involves an initial nucleophilic attack of NH of thiosemicarbazide (19) on aldehydic carbon of 18 to generate intermediate 23. Subsequent loss of H₂O molecule from 23 afforded the required formylferrocene thiosemicarbazone 20.

![Scheme 5.2. Mechanism for formylferrocene thiosemicarbazone synthesis](image)

The structure of formylferrocene thiosemicarbazone 20 was ascertained by analysis of its IR and ¹H NMR spectra. The IR spectrum of 20 showed two absorption bands at 3333 cm⁻¹ and 3201 cm⁻¹ for N-H stretchings and an absorption band at 3070 cm⁻¹ assigned for C-H stretching. The functional group region of the spectrum also exhibited absorption bands at 1597 cm⁻¹ (C=N stretching), 1543 cm⁻¹ (C=N stretching) and 1504 cm⁻¹ (N-H bending). The ¹H NMR spectrum of 20 in DMSO-δ₆ displayed an exchangeable singlet due to one proton at δ 11.19 that was assigned to NH connected to CH=N. Two more exchangeable singlets due to one proton each appeared at δ 8.00 and δ 7.59 which were assigned to the SH and NH protons indicating that in solution, CSNH₂ moiety may exist in its tautomeric form (HS-C=NH). A singlet due to one proton at δ 7.88 was assigned to N=CH. All ferrocene protons were assigned in the...
aliphatic region at δ 4.72, δ 4.40 and δ 4.19 appearing as three singlets in the relative proton ratio of 2:2:5.

5.2.2. Synthesis of thiazolylhydrazinomethylideneferrocenes (16 and 17)

Having formylferrocene thiosemicarbazone 20 in hand, we turned our attention to its reaction with various p-substituted phenacyl bromides 21 and 6-substituted-3-bromoacetylcoumarins 22 to afford thiazolylhydrazinomethylidene-ferrocenes 16 and 17 respectively (Scheme 5.1). For achieving the target, first of all starting materials, p-substituted phenacyl bromides53,54 21 and 6-substituted-3-bromoacetylcoumarins55,56 22, were synthesized following established literature procedures. The reaction of α-haloketones with a thioamide has been the most important method for the thiazole synthesis ever since it was introduced by Hantzsch and Weber.57 The mechanism of the reaction of formylferrocene thiosemicarbazone 20 with bromomethyl aryl ketone 24 is presumed to be on the lines of Hantzsch thiazole synthesis and is depicted in Scheme 5.3. The first step is the formation of an acyclic intermediate 25 by the nucleophilic attack of sulfur (20) on the bromine-carrying carbon of bromomethyl aryl ketone (24) resulting in the formation of the C-S-C bond via Walden inversion (SN2-reaction) mechanism. In the next step, an intramolecular attack by the lone pair of nitrogen atom at the carbonyl carbon in intermediate 25 yields a cyclic intermediate 26 which on elimination of water molecule leads to the formation of thiazolylhydrazinomethylideneferrocenes 16 and 17.

Scheme 5.3. Mechanism for the synthesis of thiazolylhydrazinomethylideneferrocenes (16 and 17) following Hantzsch thiazole synthesis
Corresponding to the Hantzsch thiazole synthesis, the present synthesis of thiazolylhydrazinomethylideneferrocenes 16 and 17 consists of the condensation of appropriate p-substituted phenacyl bromide 21 or 6-substituted-3-bromoacetyl coumarin 22 with formylferrocene thiosemicarbazone 20 in refluxing EtOH : THF in the presence of sodium acetate (Scheme 5.1). The reaction of formylferrocene thiosemicarbazone 20 with phenacyl bromide (21a) was first to be investigated. To a solution of 20 in EtOH : THF was added phenacyl bromide (21a) followed by sodium acetate and refluxed the reaction mixture for 6-7 h whereupon solution was reduced to 1/4th of its original volume and cooled to room temperature. The solid that separated out was filtered, washed with water followed by cold ethanol, dried and crystallized from ethanol to afford ferrocene-1-carbaldehyde N-(4-phenyl-1,3-thiazol-2-yl)hydrazone (16a) in 90% yield. The structure of 16a was assigned on the basis of its IR, 1H NMR and 13C NMR spectral data and was confirmed on the basis of elemental analysis (C, H, N). The IR spectrum of 16a showed two absorption bands at 3171 cm⁻¹ and 3086 cm⁻¹ which were assigned to the N-H stretching and C-H stretching, respectively. The functional group region of the spectrum also exhibited absorption band at 1582 cm⁻¹ which was assigned to C=N stretching. 1H NMR of 16a in CDCl₃ displayed an exchangeable singlet for one proton at δ 10.65 ascribed to NH. Two singlets for one proton each at δ 7.15 and δ 6.85 were assigned to the thiazole C₅-H and CH=N respectively. Higher δ value for thiazole proton as compared to that of CH=N was assigned considering slight shielding of the later due to cyclopentadienyl ring of ferrocene but might be interchangeable. Three singlets in the aliphatic region at δ 4.32, δ 4.28 and δ 4.11 in the relative ratio of 2 : 2 : 5 were assigned to the ferrocene protons. Other aromatic protons were depicted in the aromatic region.

Other thiazolylhydrazinomethylideneferrocenes 16b-16f and 17a-17c were also synthesized following the same procedure and spectral characteristics similar to 16a were observed. Besides displaying other appropriate signals, in general, 1H NMR spectra of 16 or 17 displayed an exchangeable singlet due to one proton in the range of δ 10.62-10.68 in CDCl₃ (16b-16d) and δ 11.82-11.93 in DMSO-d₆ (16e, 16f, 17a-17c) which was clearly assigned to NH. A singlet in the range of δ 7.09-7.37 in CDCl₃ and δ 7.84-7.90 in DMSO-d₆ was attributed to C₅-proton of thiazole ring and another
one in the range of δ 6.78-6.85 in CDCl₃ and 7.33-7.75 in DMSO-d₆ was attributed to CH=N connected to ferrocene. Values suggest that all these three protons show solvent dependant shift in ¹H NMR. All ferrocene protons were clearly assigned in the range of δ 4.00-5.00 as two or three singlets in the relative ratio of either 4 : 5 (16b) or 2 : 2 : 5 (16c-16f and 17a-17c). The presence of a methyl group attached to aromatic ring in 16b was ascertained on the basis of its ¹H NMR and ¹³C NMR spectrum which displayed a singlet for three protons at δ 2.43 and a signal at δ 21.2 respectively. The presence of fluorine at the para position of the aromatic ring in 16c was confirmed by the presence of three doublets at δ 162.0 (d, ¹J_CF = 244.6 Hz), 127.9 (d, ²J_CF = 7.5 Hz) and 115.8 (d, ²J_CF = 21.8 Hz) in its ¹³C NMR spectrum. All coumarin thiazoles (17a-17c) were showing characteristic C=O stretch of lactone at 1705-1720 cm⁻¹ in FT-IR. Two singlets due to one proton each in the region δ 8.45-8.52 (17a-17c) and δ 7.99-8.11 (17b, 17c) in ¹H NMR spectra were assigned to coumarin C₄-H and C₅-H respectively.

5.3. Biological Testing Results

5.3.1. Antimicrobial studies

All the newly synthesized thiazolylhydrazinomethylideneferrocenes (16a-16f and 17a-17c) were evaluated for their in vitro antibacterial activity against two Gram-positive and two Gram-negative bacteria. In addition to this, these compounds were also evaluated for their in vitro antifungal activity against two fungi. All the microbial cultures used in the present study were procured from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, INDIA.

5.3.2. In vitro antibacterial assay

The in vitro antibacterial activity of the newly synthesized compounds (16a-16f and 17a-17c) was evaluated by agar well diffusion method. All the microbial cultures were adjusted to 0.5 McFarland standards, which is visually comparable to a microbial suspension of approximately 1.5 × 10⁸ cfu/mL. 20 mL of Mueller Hinton agar media was poured into each petri plate and the agar plates were swabbed with 100 μL inocula of the test microorganisms and kept for 15 min for adsorption. Using sterile cork borer of 8 mm diameter, wells were bored into the seeded agar plates and
these were loaded with a 100 μL volume with concentration of 4.0 mg/mL of each compound reconstituted in dimethylsulphoxide (DMSO). All the plates were incubated at 37 °C for 24 h. Antibacterial activity, indicated by an inhibition zone surrounding the well containing the compounds, was recorded if the zone of inhibition was greater than 8 mm. The experiments were performed in triplicate. DMSO was used as a negative control whereas ciprofloxacin was used as a positive control.

5.3.3. Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation. MIC of various compounds against bacterial strains was tested through a macrodilution tube method as recommended by National Committee for Clinical Laboratory Standards (NCCLS). In this method, various test concentrations of compounds were made from 128 to 0.25 μg/mL in sterile tubes numbered 1 to 10. 100 μL sterile Mueller Hinton Broth (MHB) was poured in each sterile tube followed by addition of 200 μL solution of test compound in tube 1. Two fold serial dilutions were carried out from the tube 1 to the tube 10 and excess broth (100 μL) was discarded from the last tube No. 10. To each tube, 100 μL of standard inoculum (1.5 × 10⁸ cfu/mL) was added. Ciprofloxacin was used as control. Turbidity was observed after incubating the inoculated tubes at 37 °C for 24 h.

5.3.4. In vitro antifungal assay

The in vitro antifungal activity of compounds (16a-f & 17a-c) was evaluated by poisoned food method. All the test molds were grown on Saburaud Dextrose Agar (SDA) at 25 °C for 7 days. One week old culture of the mold was used as inocula for evaluating antifungal activity of chemical compounds.

The molten SDA (45 °C) was poisoned by the addition of 100 μL volume having concentration of 4.0 mg/mL of each compound reconstituted in the DMSO, poured into the sterile petri plates and allowed to solidify at room temperature. The prepared SDA plates containing the test compounds were inoculated with fungal plugs (8 mm diameter) obtained from the actively growing margins of the fungal plates. Plates were incubated at 25 °C for 7 days. DMSO was used as a negative control whereas fluconazole was used as positive control. The experiments were performed in
triplicates. Diameter of the fungal colonies was measured and expressed as percentage mycelial inhibition determined by the formula given below:

\[
\% \text{ inhibition} = \frac{dc - dt}{dc} \times 100
\]

Where

dc = average diameter of fungal colony in negative control plates
dt = average diameter of fungal colony in experimental plates

5.3.5. Results and discussion

5.3.5.1. In vitro antibacterial activity

Results revealed that in general, all the tested compounds (16a-16f and 17a-17c) possessed moderate antibacterial activity against Gram-positive bacteria (S. aureus, B. subtilis). However, none of the compounds was found to be effective against any of the Gram-negative bacteria (E. coli, P. aeruginosa). On the basis of zone of inhibition against the test bacterium, compound 16e was found to be the most effective against S. aureus showing the maximum zone of inhibition of 18.3 mm and compound 16a against B. subtilis producing 19.6 mm zone of inhibition (Table 5.1) as compared with the standard drug ciprofloxacin which showed the zone of inhibition 27.6 mm against S. aureus and 26.3 mm against B. subtilis.

### Table 5.1. In vitro antibacterial activity and MIC of compounds 16 and 17

<table>
<thead>
<tr>
<th>Compound</th>
<th>Diameter of growth of inhibition zone (mm)</th>
<th>Minimum inhibitory concentration (MIC) (μg/ml)</th>
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<tbody>
<tr>
<td></td>
<td>S. aureus</td>
<td>B. subtilis</td>
</tr>
<tr>
<td>16a</td>
<td>17.3</td>
<td>19.6</td>
</tr>
<tr>
<td>16b</td>
<td>14.6</td>
<td>15.3</td>
</tr>
<tr>
<td>16c</td>
<td>14.3</td>
<td>15.0</td>
</tr>
<tr>
<td>16d</td>
<td>16.6</td>
<td>18.3</td>
</tr>
<tr>
<td>16e</td>
<td>18.3</td>
<td>18.6</td>
</tr>
<tr>
<td>16f</td>
<td>16.6</td>
<td>19.3</td>
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<tr>
<td>17a</td>
<td>15.3</td>
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<tr>
<td>17b</td>
<td>15.0</td>
<td>16.6</td>
</tr>
<tr>
<td>17c</td>
<td>13.6</td>
<td>14.3</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>27.6</td>
<td>26.3</td>
</tr>
</tbody>
</table>

– No activity.

*Concentration 4.0 mg/mL.

*Values, including diameter of the well (8 mm), are means of three replicates.
Besides 16e, no other compound was found possessing significant antibacterial activity against  *S. aureus* with zone of inhibition >18.0 mm while compounds 16d, 16e and 16f showed significant antibacterial activity against  *B. subtilis* with zone of inhibition >18.0. However, in terms of MIC, none of the compounds was found to possess appreciable antibacterial activity. Amongst all the compounds, the MIC ranged between 64 (16a, 16d, 16e, 16f) and 256 μg/mL against Gram-positive bacteria (Table 5.1).

A comparison between the two series of compounds (16 and 17) indicates that in general, there is an appreciable decrease in activity against both Gram-positive bacteria when phenyl analogues (16a, 16d, 16e) are replaced by coumarin analogues (17a, 17b, 17c). Within the individual series, no correlation between the antibacterial activity and the substituent on phenyl or coumarin ring is observed.

### 5.3.5.2. In vitro antifungal activity

Of all the tested compounds, only 16e was found to possess moderate antifungal activity with >55% inhibition of mycelial growth against both the fungal strains (*A. niger* and *A. flavus*) as compared to the standard drug fluconazole (75.3% & 74.6% inhibition against *A. niger* and *A. flavus* respectively) (Table 5.2). Other compounds were found to possess no appreciable antifungal activity. The same trend was observed here also that there is slight to appreciable decrease in activity against both fungi when phenyl analogues (16a, 16d, 16e) are replaced by coumarin analogues (17a, 17b, 17c).

**Table 5.2. In vitro antifungal activity of compounds 16 and 17 through poisoned food method**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mycelial growth inhibition (%)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td><em>A. niger</em></td>
<td><em>A. flavus</em></td>
</tr>
<tr>
<td>16a</td>
<td>50.0</td>
<td>51.1</td>
<td></td>
</tr>
<tr>
<td>16b</td>
<td>48.8</td>
<td>50.0</td>
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<tr>
<td>16c</td>
<td>51.1</td>
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</tr>
<tr>
<td>16d</td>
<td>52.2</td>
<td>51.1</td>
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</tr>
<tr>
<td>16e</td>
<td>58.8</td>
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<td></td>
</tr>
<tr>
<td>16f</td>
<td>53.3</td>
<td>52.2</td>
<td></td>
</tr>
<tr>
<td>17a</td>
<td>44.4</td>
<td>50.0</td>
<td></td>
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</table>
In the present study, nine novel thiazolylhydrazinomethylideneferrocenes 16a-16f and 17a-17c bearing phenyl and coumarin analogues respectively at position-4 of 1,3-thiazole were synthesized and screened for their \textit{in vitro} antibacterial activity and antifungal activity. No appreciable results were obtained by combining the biochemistry of ferrocene with thiazole and coumarins. A general decrease in antibacterial as well as antifungal activity was found when phenyl analogues were replaced by coumarin analogues. Out of the tested compounds, 16a, 16d, 16e and 16f exhibited moderate antibacterial activity against Gram-positive bacteria and 16e exhibited moderate antifungal activity against the tested fungi. However, none of the newly synthesized compounds was found to be superior over the reference drugs.

\begin{table}[h]
\begin{tabular}{lcc}
   & 17b & 48.4 & 51.1 \\
17c & 53.3 & 52.2 \\
Fluconazole & 75.3 & 74.6 \\
\end{tabular}
\end{table}

\textsuperscript{a}Concentration 4.0 mg/mL.

\section*{5.4. Conclusions}

In the present study, nine novel thiazolylhydrazinomethylideneferrocenes 16a-16f and 17a-17c bearing phenyl and coumarin analogues respectively at position-4 of 1,3-thiazole were synthesized and screened for their \textit{in vitro} antibacterial activity and antifungal activity. No appreciable results were obtained by combining the biochemistry of ferrocene with thiazole and coumarins. A general decrease in antibacterial as well as antifungal activity was found when phenyl analogues were replaced by coumarin analogues. Out of the tested compounds, 16a, 16d, 16e and 16f exhibited moderate antibacterial activity against Gram-positive bacteria and 16e exhibited moderate antifungal activity against the tested fungi. However, none of the newly synthesized compounds was found to be superior over the reference drugs.
5.5. Experimental section

All reactions were carried out under atmospheric pressure. Melting points were determined in open capillaries in an electrical melting point apparatus and are uncorrected. IR spectra were recorded on ABB MB 3000 DTGS IR instrument using the KBr pellet technique. The $^1$H NMR and $^{13}$C NMR spectra were recorded either in pure CDCl$_3$ or DMSO-$d_6$ or in CDCl$_3$/DMSO-$d_6$ mixture on Bruker NMR spectrometer at 300 MHz and 75.5 MHz, respectively. The $\delta$ values are given in ppm relative to tetramethylsilane (TMS) as internal standard (for $^1$H and $^{13}$C NMR). Elemental analyses were performed on a varioMICRO V1.7.0 Elementar Analysensysteme GmbH instrument. The purity of the compounds was ascertained by $^1$H NMR and thin layer chromatography (TLC) on silica gel plates using a mixture of petroleum ether and ethyl acetate as eluent. Iodine or UV lamp was used as a visualizing agent. Abbreviations ‘s’ for singlet, ‘d’ for doublet, ‘m’ for multiplet, ‘ex’ for exchangeable proton (detected by disappearance of signal upon D$_2$O addition) are used for NMR assignments and ‘s’ for strong, ‘m’ for medium for IR assignments. ‘d’ stands for decomposition in melting point data.

General procedure for synthesis of formylferrocene thiosemicarbazone (20)

An ethanolic solution (25 mL) of ferrocene carbaldehyde (18, 4.7 mmol) was added dropwise to a solution of thiosemicarbazide (19, 5.2 mmol) in water (40 mL). 1.0 mL of glacial acetic acid was then added and the resulting reaction mixture was then refluxed for 1h. On completion of the reaction, solution was concentrated and cooled to room temperature. Dark red precipitates separated out which were filtered, washed with cold water (100 mL), dried and crystallized from aqueous ethanol to afford the target formylferrocene thiosemicarbazone 20. Yield: 85%; m. p. 187-188 °C; Lit. m. p. 190 °C; IR (KBr) cm$^{-1}$: 3333 and 3201 (m, N-H stretch), 3070 (m, C-H stretch), 1597 (s, C=N stretch), 1543 (s, C=N stretch), 1504 (m, N-H bend); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 10.19 (s, ex, 1H, NH), 8.00 (s, ex, 1H, SH/=NH), 7.88 (s, 1H, CH=N), 7.59 (s, ex, 1H, SH/=NH), 4.72 (s, 2H, ferrocene-H), 4.40 (s, 2H, ferrocene-H), 4.19 (s, 5H, ferrocene-H).
Representative protocol for the synthesis of thiazolylhydrazinomethylideneferrocenes (16a-f and 17a-c)

To a solution of formylferrocene thiosemicarbazone (20, 2 mmol) in EtOH : THF (60 mL, 2 : 1 v/v) was added appropriate phenacyl bromide (21) or 3-bromoacetyl coumarin (22) (2.2 mmol) followed by anhydrous sodium acetate (2.2 mmol). The resulting reaction mixture was refluxed for 6-7 h. After completion of the reaction, solution was reduced to 1/4th of its volume and cooled to room temperature. The solid separated out was filtered, washed with water (100 mL) followed by cold ethanol (10 mL) and crystallized from ethanol to afford the target thiazolylhydrazinomethylideneferrocenes 16 or 17 as reddish brown or black solid.

**Ferrocene-1-carbaldehyde N-(4-phenyl-1,3-thiazol-2-yl)hydrazone (16a)**

Yield: 90%; m. p. 138-140 °C (d); IR (KBr) cm⁻¹: 3171 (m, N-H stretch), 3086 (m, C-H stretch), 1582 (s, C=N stretch), 1481 (m, N-H bend), 1427; ¹H NMR (300 MHz, CDCl₃): δ 10.65 (s, ex, 1H, NH), 7.90 (d, 2H, J = 7.2 Hz, Ar-H), 7.47-7.51 (m, 2H, Ar-H), 7.41 (d, 1H, J = 6.3 Hz, Ar-H), 7.15 (s, 1H, thiazole C₅-H), 6.85 (s, 1H, CH=N), 4.32 (s, 2H, ferrocene-H), 4.28 (s, 2H, ferrocene-H), 4.11 (s, 5H, ferrocene-H); ¹³C NMR (75.5 MHz, CDCl₃): δ 169.9, 150.9, 142.7, 135.0, 128.8, 127.9, 126.3, 103.0, 79.0, 69.7, 69.1, 67.1. Anal. calc. for C₂₀H₁₇FeN₃S (%): C, 62.03; H, 4.42; N, 10.85; found (%): C, 62.30; H, 4.53; N, 10.70.

**Ferrocene-1-carbaldehyde N-[4-(4-methylphenyl)-1,3-thiazol-2-yl]hydrazone (16b)**

Yield: 88%; m. p. 150-152 °C (d); IR (KBr) cm⁻¹: 3155 (m, N-H stretch), 3070 (m, C-H stretch), 1566 (s, C=N stretch), 1489 (m, N-H bend), 1427; ¹H NMR (300 MHz, CDCl₃): δ 10.62 (s, ex, 1H, NH), 7.80 (d, 2H, J = 7.8 Hz, Ar-H), 7.29 (d, 2H, J = 7.8 Hz, Ar-H), 7.09 (s, 1H, thiazole C₅-H), 6.78 (s, 1H, CH=N), 4.27 (s, 4H, ferrocene-H), 4.08 (s, 5H, ferrocene-H), 2.43 (s, 3H, CH₃); ¹³C NMR (75.5 MHz, DMSO-d₆): δ 168.6, 150.9, 142.4, 137.1, 132.6, 129.5, 125.9, 102.5, 80.1, 70.1, 69.3, 67.3, 21.2 (CH₃). Anal. calc. for C₂₁H₁₉FeN₃S (%): C, 62.85; H, 4.77; N, 10.47; found (%): C, 63.02; H, 4.89; N, 10.31.
Ferrocene-1-carbaldehyde N-[4-(4-fluorophenyl)-1,3-thiazol-2-yl]hydrazone (16c)

Yield: 92%; m. p. 172-174 °C (d); IR (KBr) cm\(^{-1}\): 3263 (m, N-H stretch), 3086 (m, C-H stretch), 1551 (s, C=N stretch), 1481 (m, N-H bend), 1412; \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 10.68 (s, ex, 1H, NH), 7.85 (dd, 2H, \(J_{HF} = 5.4\) Hz, \(J_{HH} = 8.7\) Hz, Ar-H), 7.23 (s, 1H, thiazole C\(_5\)-H), 7.16 (t, 2H, \(J = 8.7\) Hz, Ar-H), 6.79 (s, 1H, CH=N), 4.38 (s, 2H, ferrocene-H), 4.32 (s, 2H, ferrocene-H), 4.14 (s, 5H, ferrocene-H); \(^13\)C NMR (75.5 MHz, CDCl\(_3\)): \(\delta\) 168.9, 149.8, 142.2, 134.0, 132.4, 128.7, 127.3, 103.1, 80.0, 70.1, 69.3, 67.3. Anal. calc. for C\(_{20}\)H\(_{16}\)FFeN\(_3\)S (%): C, 59.27; H, 3.98; N, 10.37; found (%): C, 59.44; H, 4.11; N, 10.20.

Ferrocene-1-carbaldehyde N-[4-(4-chlorophenyl)-1,3-thiazol-2-yl]hydrazone (16d)

Yield: 78%; m. p. 168-170 °C (d); IR (KBr) cm\(^{-1}\): 3263 (m, N-H stretch), 3101 (m, C-H stretch), 1551 (s, C=N stretch), 1474 (m, N-H bend), 1404; \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 10.67 (s, ex, 1H, NH), 7.79 (d, 2H, \(J = 8.4\) Hz, Ar-H), 7.43 (d, 2H, \(J = 8.4\) Hz, Ar-H), 7.37 (s, 1H, thiazole C\(_5\)-H), 6.83 (s, 1H, CH=N), 4.44 (s, 2H, ferrocene-H), 4.35 (s, 2H, ferrocene-H), 4.16 (s, 5H, ferrocene-H); \(^13\)C NMR (75.5 MHz, CDCl\(_3\)/DMSO-d\(_6\)): \(\delta\) 168.9, 149.8, 142.2, 134.0, 132.4, 128.7, 127.3, 103.5, 80.0, 70.1, 69.2, 67.3. Anal. calc. for C\(_{20}\)H\(_{16}\)ClFeN\(_3\)S (%): C, 56.96; H, 3.82; N, 9.96; found (%): C, 56.88; H, 3.75; N, 10.12.

Ferrocene-1-carbaldehyde N-[4-(4-bromophenyl)-1,3-thiazol-2-yl]hydrazone (16e)

Yield: 85%; m. p. 174-176 °C (d); IR (KBr) cm\(^{-1}\): 3448 (m, N-H stretch), 3109 (m, C-H stretch), 1643 (s, C=N stretch), 1558 (s, C=N stretch), 1474 (m, N-H bend), 1396; \(^1\)H NMR (300 MHz, DMSO-d\(_6\)): \(\delta\) 11.85 (s, ex, 1H, NH), 7.86 (s, 1H, thiazole C\(_5\)-H), 7.79 (d, 2H, \(J = 8.4\) Hz, Ar-H), 7.58 (d, 2H, \(J = 8.4\) Hz, Ar-H), 7.33 (s, 1H, CH=N), 4.59 (s, 2H, ferrocene-H), 4.38 (s, 2H, ferrocene-H), 4.18 (s, 5H, ferrocene-H); \(^13\)C NMR (75.5 MHz, CDCl\(_3\)/DMSO-d\(_6\)): \(\delta\) 168.9,
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149.7, 142.3, 134.3, 131.6, 127.6, 120.8, 103.6, 79.9, 69.9, 69.2, 67.2. Anal. calc. for C$_{20}$H$_{16}$BrFeN$_3$S (%): C, 51.53; H, 3.46; N, 9.01; found (%): C, 51.70; H, 3.58; N, 8.91.

**Ferrocene-1-carbaldehyde N-[4-(4-nitrophenyl)-1,3-thiazol-2-yl]hydrazone (16f)**

Yield: 90%; m. p. 200-202 °C (d); IR (KBr) cm$^{-1}$: 3310 (m, N-H stretch), 3109 (m, C-H stretch), 1574 (s, C=N stretch), 1504 (m, N-H bend), 1420; $^1$H NMR (300 MHz, DMSO-$d_6$): δ 11.93 (s, ex, 1H, NH), 8.27 (d, 2H, $J$ = 8.7 Hz, Ar-H), 8.09 (d, 2H, $J$ = 8.7 Hz, Ar-H), 7.88 (s, 1H, thiazole C$_5$-H), 7.66 (s, 1H, CH=N), 4.61 (s, 2H, ferrocene-H), 4.42 (s, 2H, ferrocene-H), 4.21 (s, 5H, ferrocene-H); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$): δ 169.1, 148.9, 146.6, 143.2, 141.2, 126.7, 124.5, 108.3, 79.8, 70.2, 69.4, 67.4. Anal. calc. for C$_{20}$H$_{16}$FeN$_3$O$_2$S (%): C, 55.57; H, 3.73; N, 12.96; found (%): C, 55.68; H, 3.88; N, 12.77.

**Ferrocene-1-carbaldehyde N-[4-(2-oxo-2H-chromen-3-yl)-1,3-thiazol-2-yl]hydrazone (17a)**

Yield: 90%; m. p. 204-206 °C (d); IR (KBr) cm$^{-1}$: 3232 (m, N-H stretch), 3132 (m, C-H stretch), 1705 (s, lactone C=O stretch), 1574 (s, C=N stretch), 1481 (m, N-H bend), 1435, 1381; $^1$H NMR (300 MHz, DMSO-$d_6$): δ 11.82 (s, ex, 1H, NH), 8.52 (s, 1H, coumarin C$_4$-H), 7.84-7.89 (m, 2H, thiazole C$_5$-H, coumarin), 7.72 (s, 1H, CH=N), 7.60-7.63 (m, 1H, coumarin), 7.39-7.46 (m, 2H, coumarin), 4.61 (s, 2H, ferrocene-H), 4.41 (s, 2H, ferrocene-H), 4.21 (s, 5H, ferrocene-H); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$): δ 168.1, 152.7, 143.2, 138.5, 132.1, 129.2, 126.7, 125.1, 119.6, 116.3, 110.5, 79.9, 70.2, 69.4, 67.4. Anal. calc. for C$_{23}$H$_{17}$FeN$_3$O$_2$S (%): C, 60.67; H, 3.76; N, 9.23; found (%): C, 60.82; H, 3.91; N, 9.08.

**Ferrocene-1-carbaldehyde N-[4-(6-chloro-2-oxo-2H-chromen-3-yl)-1,3-thiazol-2-yl]hydrazone (17b)**

Yield: 74%; m. p. 194-196 °C (d); IR (KBr) cm$^{-1}$: 3248 (m, N-H stretch), 3117 (m, C-H stretch), 1720 (s, lactone C=O stretch), 1574 (s, C=N stretch), 1481 (m, N-H
bend), 1427; $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$
11.88 (s, ex, 1H, NH), 8.45 (s, 1H, coumarin C$_4$-H), 7.99 (s, 1H, coumarin C$_5$-H), 7.89 (s, 1H, thiazole C$_3$-H, coumarin), 7.74 (s, 1H, CH=H), 7.63 (d, 1H, $J = 8.7$ Hz, coumarin C$_7$-H/C$_8$-H), 7.47 (d, 1H, $J = 8.7$ Hz, coumarin C$_7$-H/C$_8$-H), 4.61 (s, 2H, ferrocene-H), 4.40 (s, 2H, ferrocene-H), 4.21 (s, 5H, ferrocene-H); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$): $\delta$ 168.1, 158.8, 151.7, 143.4, 137.0, 131.0, 121.9, 121.6, 118.5, 116.8, 111.4, 79.8, 70.3, 69.4, 67.4. Anal. calc. for C$_{23}$H$_{16}$ClFeN$_3$O$_2$S (%): C, 56.40; H, 3.29; N, 8.58; found (%): C, 56.59; H, 3.43; N, 8.41.

**Ferrocene-1-carbaldehyde $N$-[4-(6-bromo-2-oxo-2H-chromen-3-yl)-1,3-thiazol-2-yl]hydrazone (17c)**

Yield: 76%; m. p. 198-200 °C (d); IR (KBr) cm$^{-1}$: 3209 (m, N-H stretch), 3132 (m, C-H stretch), 3094 (m, C-H stretch), 1720 (s, lactone C=O stretch), 1574 (s, C=N stretch), 1435; $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 11.86 (s, ex, 1H, NH), 8.45 (s, 1H, coumarin C$_4$-H), 8.11 (s, 1H, coumarin C$_5$-H), 7.90 (s, 1H, thiazole C$_3$-H), 7.75 (d, 2H, $J = 8.7$ Hz, CH=H, coumarin C$_7$-H/C$_8$-H), 7.41 (d, 1H, $J = 8.7$ Hz, coumarin C$_7$-H/C$_8$-H), 4.61 (s, 2H, ferrocene-H), 4.41 (s, 2H, ferrocene-H), 4.21 (s, 5H, ferrocene-H); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$): $\delta$ 168.1, 158.8, 151.7, 143.4, 137.0, 134.3, 131.0, 121.9, 121.6, 118.5, 116.8, 111.4, 79.8, 70.3, 69.4, 67.4. Anal. calc. for C$_{23}$H$_{16}$BrFeN$_3$O$_2$S (%): C, 51.71; H, 3.02; N, 7.87; found (%): C, 51.65; H, 2.95; N, 8.01.
5.6. References

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58 Ahmad, I.; Beg, A. J. *J. Ethnopharmacol.* **2001**, *74*, 113-123.


60 Villanova, P. A. *National Committee for Clinical Standards, Method for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically, Approved Standards*, 5th Edn., NCCLS Document M7-A5, **2000**.