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Research Article

Ferrocene Derivatives Carrying Urea, Thiourea, and Sulfonamide Moieties: Synthesis and Evaluation of Antibacterial and Antifungal Activities

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In the present study, some novel ferrocene derivatives carrying urea, thiourea, and sulfonamide groups were synthesized, and all compounds were characterized by spectral and elemental analyses. These compounds were screened for their antibacterial activities and also their minimum inhibitory concentration (MIC) against Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative bacteria (*Klebsiella pneumonia* and *Escherichia coli*) and antifungal activities against *Saccharomyces cerevisiae* and *Candida albicans*. Amongst the tested compounds, **4b**, **4c**, **5b**, and **6b** displayed excellent antimicrobial activity.

1. Introduction

Although there are many important developments in antimicrobial therapy infectious diseases caused by bacteria and fungi remain a major global health problem because of the rapid development of resistance to the existing antimicrobial drugs, in other words, the increasing use and misuse of the existing antimicrobial drugs have resulted in the development of resistant pathogens [1–3]. Therefore, novel antimicrobial agents are needed for effective treatment against infections caused by the pathogenic microbes.

Ferrocene and its derivatives find extensive applications in areas like homogeneous catalysis, material science, nonlinear optics, and molecular sensors [4–10]. Additionally, incorporation of a ferrocene fragment into a molecule of an organic compound often produces unexpected biological activity. Recently, some new ferrocenyl-substituted organic compounds as potential pharmaceuticals have been reported [11–15]. Many ferrocenyl compounds display interesting cytotoxic, antitumor, antimalarial, antifungal, antioxidant, and DNA-cleaving activity [16–18].

In addition, some urea, thiourea, and sulfonamide derivatives are known to be associated with a wide range of biological activities such as analgesic, antitumor, antioxidant, anticonvulsant, and anti-HIV properties [19–28]. However, antibacterial and antifungal activities of urea and thiourea derivatives have been less widely documented [29, 30]. In view of the above-mentioned facts and in an attempt to achieve potential antibacterial and antifungal agents, in the present study we hereby report the synthesis of some ferrocenyl substituted urea, thiourea, and sulfonamide derivatives and evaluation of their in vitro antimicrobial activities. The structural characterization and preliminary biological evaluation of these novel compounds could be interesting for screening potent drug.

2. Experimental

All the reagents for syntheses were commercially available and used without further purification or purified by standard methods prior to use. Melting points were determined using an *Electrothermal 9100* apparatus, uncorrected. All NMR spectra were recorded on a *Bruker* 400 (^{1}H: 400 MHz, ^{13}C: 100 MHz) NMR spectrometer, in $CDCl_3$. Chemical shift values were reported in ppm relative to those for TMS used as an internal reference standard, J in Hz. The elemental analyses for C, H, N, and S were done on LECO, CHNS-932. FTIR spectra were recorded on a *Mattson 1000* spectrometer

using KBr pellets. The progress of reactions was monitored by TLC using Silufol UV-254 plates. The compounds **2** and **3** were synthesized using a published method. All the physical and spectral data were in line with the previously reported results [31, 32].

*2.1. General Procedure for Synthesis of (**4a–4e**).* A stirred mixture of 4-ferrocenylaniline (**3**, 2.77 g, 10 mmol) and a mol-equivalent amount of the corresponding phenyl isocyanate in 20 mL ethanol is heated under reflux for 2 h (TLC control). At the end of this period, the reaction mixture was evaporated to dryness. This crude product was recrystallized from an appropriate solvent to afford the desired compound.

*2.1.1. 1-(4-Ferrocenylphenyl)-3-phenylurea (**4a**).* Crystallization from ethanol; orange powder, 83% yield; mp 181-182°C; IR (ν, cm^{-1}): 3054, 1639, 1611. ^{1}H-NMR (400 MHz, $CDCl_3$): 10.85 (1H, s, NH), 9.51 (1H, s, NH), 7.82 (2H, d, J = 8.6 Hz, Ar–H), 7.56 (2H, d, J = 8.6 Hz, Ar–H), 6.97–7.48 (5H, m, Ar–H), 5.01 (2H, s, C_5H_4), 4.48 (2H, s, C_5H_4), 4.02 (5H, s, C_5H_5). ^{13}C-NMR (100 MHz, $CDCl_3$): 64.3, 68.7, 69.4, 86.1, 121.3, 122.0, 128.3, 129.1, 131.6, 133.9, 138.4, 140.2, 156.4. Elemental analysis for $C_{23}H_{20}FeN_2O$: Calculated: C, 69.71; H, 5.09; N, 7.07%. Found: C, 69.81; H, 5.01; N, 7.19%.

*2.1.2. 1-(4-Chlorophenyl)-3-(4-ferrocenylphenyl)urea (**4b**).* Crystallization from ethanol; yellow powder, 87% yield; mp 166-167°C; IR (ν, cm^{-1}): 3067, 1632, 1604. ^{1}H-NMR (400 MHz, $CDCl_3$): 10.93 (1H, s, NH), 9.43 (1H, s, NH), 7.73 (2H, d, J = 8.4 Hz, Ar–H), 7.59 (2H, d, J = 8.0 Hz, Ar–H), 7.56 (2H, d, J = 8.4 Hz, Ar–H), 7.45 (2H, d, J = 8.0 Hz, Ar–H), 4.93 (2H, s, C_5H_4), 4.45 (2H, s, C_5H_4), 4.01 (5H, s, C_5H_5). ^{13}C-NMR (100 MHz, $CDCl_3$): 65.9, 69.3, 70.7, 87.2, 115.9, 122.5, 127.1, 129.0, 135.5, 139.2, 139.9, 141.1, 156.1. Elemental analysis for $C_{23}H_{19}ClFeN_2O$: Calculated: C, 64.14; H, 4.45; N, 6.50%. Found: C, 64.04; H, 4.41; N, 6.45%.

*2.1.3. 1-(4-Bromophenyl)-3-(4-ferrocenylphenyl)urea (**4c**).* Crystallization from butanol; yellow powder, 80% yield; mp 201-202°C; IR (ν, cm^{-1}): 3084, 1629, 1601. ^{1}H-NMR (400 MHz, $CDCl_3$): 10.73 (1H, s, NH), 9.62 (1H, s, NH), 7.92 (2H, d, J = 8.3 Hz, Ar–H), 7.61 (2H, d, J = 8.3 Hz, Ar–H), 7.54 (2H, d, J = 8.6 Hz, Ar–H), 7.46 (2H, d, J = 8.6 Hz, Ar–H), 4.96 (2H, s, C_5H_4), 4.44 (2H, s, C_5H_4), 4.02 (5H, s, C_5H_5). ^{13}C-NMR (100 MHz, $CDCl_3$): 64.3, 69.0, 69.5, 86.3, 118.4, 121.6, 122.1, 124.9, 135.3, 139.0, 139.4, 141.3, 155.0. Elemental analysis for $C_{23}H_{19}BrFeN_2O$: Calculated: C, 58.14; H, 4.03; N, 5.90%. Found: C, 58.04; H, 4.00; N, 6.02%.

*2.1.4. 1-(4-Ferrocenylphenyl)-3-p-tolylurea (**4d**).* Crystallization from ethanol; orange powder, 74% yield; mp 146-147°C; IR (ν, cm^{-1}): 3053, 2987, 1632, 1609. ^{1}H-NMR (400 MHz, $CDCl_3$): 10.84 (1H, s, NH), 9.49 (1H, s, NH), 7.80 (2H, d, J = 8.5 Hz, Ar–H), 7.55 (2H, d, J = 8.5 Hz, Ar–H), 7.30 (2H, d, J = 8.2 Hz, Ar–H), 7.11 (2H, d, J = 8.2 Hz, Ar–H), 4.94 (2H, s, C_5H_4), 4.45 (2H, s, C_5H_4), 4.01 (5H, s, C_5H_5), 2.32 (3H, s, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$): 22.2, 64.1, 68.8, 69.3, 86.0, 118.1, 119.3, 124.8, 127.6, 133.2, 136.9, 138.8, 141.2, 155.8. Elemental analysis for $C_{24}H_{22}FeN_2O$: Calculated: C, 70.26; H, 5.40; N, 6.83%. Found: C, 70.34; H, 5.46; N, 6.75%.

*2.1.5. 1-(4-Ferrocenylphenyl)-3-(4-methoxyphenyl)urea (**4e**).* Crystallization from ethanol; orange powder, 86% yield; mp 173-174°C; IR (ν, cm^{-1}): 3078, 2993, 1639, 1612. ^{1}H-NMR (400 MHz, $CDCl_3$): 10.91 (1H, s, NH), 9.43 (1H, s, NH), 7.84 (2H, d, J = 8.4 Hz, Ar–H), 7.60 (2H, d, J = 8.4 Hz, Ar–H), 7.50 (2H, d, J = 8.9 Hz, Ar–H), 9.93 (2H, d, J = 8.9 Hz, Ar–H), 4.96 (2H, s, C_5H_4), 4.43 (2H, s, C_5H_4), 4.02 (5H, s, C_5H_5), 3.32 (3H, s, OCH_3). ^{13}C-NMR (100 MHz, $CDCl_3$): 55.2, 64.4, 69.0, 69.6, 86.1, 113.2, 118.3, 119.9, 124.6, 130.0, 138.8, 141.1, 154.2, 155.9. Elemental analysis for $C_{24}H_{22}FeN_2O_2$: Calculated: C, 67.62; H, 5.20; N, 6.57%. Found: C, 67.55; H, 5.12; N, 6.49%.

*2.2. General Procedure for Synthesis of (**5a–5e**).* A stirred mixture of 4-ferrocenylaniline (2.77 g, 10 mmol) and a mol-equivalent amount of the corresponding phenyl isocyanate in 20 mL ethanol is heated under reflux for 2 h (TLC control). At the end of this period, the reaction mixture was evaporated to dryness. This crude product was recrystallized from an appropriate solvent to afford the desired compound.

*2.2.1. 1-(4-Ferrocenylphenyl)-3-phenylthiourea (**5a**).* Crystallization from methanol; brown powder, 79% yield; mp 167-168°C; IR (ν, cm^{-1}): 3072. ^{1}H-NMR (400 MHz, $CDCl_3$): 10.93 (1H, s, NH), 9.82 (1H, s, NH), 7.20-7.73 (7H, m, Ar–H), 6.80 (2H, d, J = 8.6 Hz, Ar–H), 5.02 (2H, s, C_5H_4), 4.47 (2H, s, C_5H_4), 4.00 (5H, s, C_5H_5). ^{13}C-NMR (100 MHz, $CDCl_3$): 63.7, 68.7, 69.3, 85.5, 119.7, 122.6, 124.3, 127.7, 130.3, 138.1, 138.4, 143.2, 180.2. Elemental analysis for $C_{23}H_{20}FeN_2S$: Calculated: C, 67.00; H, 4.89; N, 6.79; S, 7.78%. Found: C, 66.91; H, 4.84; N, 6.73; S 7.64%.

*2.2.2. 1-(4-Chlorophenyl)-3-(4-ferrocenylphenyl)thiourea (**5b**).* Crystallization from butanol; yellow powder, 81% yield; mp 157-158°C; IR (ν, cm^{-1}): 3081. ^{1}H-NMR (400 MHz, $CDCl_3$): 11.01 (1H, s, NH), 9.85 (1H, s, NH), 7.67 (2H, d, J = 8.5 Hz, Ar–H), 7.16 (2H, d, J = 8.2 Hz, Ar–H), 6.93 (2H, d, J = 8.5 Hz, Ar–H), 6.69 (2H, d, J = 8.2 Hz, Ar–H), 5.03 (2H, s, C_5H_4), 4.49 (2H, s, C_5H_4), 4.04 (5H, s, C_5H_5). ^{13}C-NMR (100 MHz, $CDCl_3$): 63.9, 68.6, 69.4, 85.9, 119.5, 122.5, 124.3, 127.7, 130.3, 138.1, 138.3, 143.2, 179.2. Elemental analysis for $C_{23}H_{19}ClFeN_2S$: Calculated: C, 61.83; H, 4.29; N, 6.27; S, 7.18%. Found: C, 61.96; H, 4.25; N, 6.24; S, 7.10%.

*2.2.3. 1-(4-Bromophenyl)-3-(4-ferrocenylphenyl)thiourea (**5c**).* Crystallization from ethanol; orange powder, 89% yield; mp 189-190°C; IR (ν, cm^{-1}): 3046. ^{1}H-NMR (400 MHz, $CDCl_3$): 11.03 (s, 1H, NH), 9.94 (s, 1H, NH), 7.70 (2H, d, J = 8.5 Hz, Ar–H), 7.42 (2H, d, J = 8.8 Hz, Ar–H), 6.92 (2H, d, J = 8.5 Hz, Ar–H), 6.73 (2H, d, J = 8.8 Hz, Ar–H), 5.03 (2H, s, C_5H_4), 4.47 (2H, s, C_5H_4), 4.01 (5H, s, C_5H_5). ^{13}C-NMR (100 MHz, $CDCl_3$): 64.2, 68.9, 69.7, 86.3, 117.3, 122.1, 125.2, 132.8, 133.5, 137.2, 139.4, 140.3, 179.3. Elemental analysis for

$C_{23}H_{19}BrFeN_2S$: Calculated: C, 56.24; H, 3.90; N, 5.70; S, 6.53%. Found: C, 56.14; H, 3.83; N, 5.63; S, 6.45%.

*2.2.4. 1-(4-Ferrocenylphenyl)-3-p-tolylthiourea (**5d**).* Crystallization from ethanol; brown powder, 80% yield; mp 111-112°C; IR (ν, cm^{-1}): 3061. ^{1}H-NMR (400 MHz, $CDCl_3$): 10.95 (1H, s, NH), 9.86 (1H, s, NH), 7.65 (2H, d, J = 8.6 Hz, Ar–H), 7.02 (2H, d, J = 8.3 Hz, Ar–H), 6.88 (2H, d, J = 8.6 Hz, Ar–H), 6.79 (2H, d, J = 8.3 Hz, Ar–H), 5.04 (2H, s, C_5H_4), 4.45 (2H, s, C_5H_4), 4.02 (5H, s, C_5H_5), 2.27 (3H, s, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$): 21.5, 63.9, 68.6, 69.6, 85.8, 117.8, 123.7, 127.2, 133.4, 135.1, 138.8, 139.6, 142.5, 180.5. Elemental analysis for $C_{24}H_{22}FeN_2S$: Calculated: C, 67.61; H, 5.20; N, 6.57; S, 7.52%. Found: C, 67.46; H, 5.14; N, 6.49; S, 7.41%.

*2.2.5. 1-(4-Ferrocenylphenyl)-3-(4-methoxyphenyl)thiourea (**5e**).* Crystallization from butanol; brown powder, 83% yield; mp 182-183°C; IR (ν, cm^{-1}): 3059. ^{1}H-NMR (400 MHz, $CDCl_3$): 10.92 (1H, s, NH), 9.83 (1H, s, NH), 7.72 (2H, d, J = 8.4 Hz, Ar–H), 6.89 (2H, d, J = 8.4 Hz, Ar–H), 6.83 (2H, d, J = 8.8 Hz, Ar–H), 6.77 (2H, d, J = 8.8 Hz, Ar–H), 5.02 (2H, s, C_5H_4), 4.43 (2H, s, C_5H_4), 4.01 (5H, s, C_5H_5), 3.21 (3H, s, OCH_3). ^{13}C-NMR (100 MHz, $CDCl_3$): 53.2, 64.2, 68.8, 69.7, 85.9, 114.6, 118.2, 122.2, 125.3, 132.1, 139.3, 141.5, 155.3, 179.7. Elemental analysis for $C_{24}H_{22}FeN_2OS$: Calculated: C, 65.16; H, 5.01; N, 6.33; S, 7.25%. Found: C, 65.10; H, 5.03; N, 6.26; S, 7.33%.

*2.3. General Procedure for Synthesis of (**6a–6d**).* A mixture of 4-ferrocenylaniline (2.77 g, 10 mmol), dicyclohexylcarbodiimide (2.06 g, 10 mmol DCC), and 4-*N,N′*-dimethylaminopyridine (1.22 g, 10 mmol DMAP) in dichloromethane was stirred at 0°C for 3 h. Then the corresponding sulfonic acid derivatives were added to the mixture. After 2 h, the temperature of the solution was allowed to increase to room temperature, and the solution was stirred at this temperature for 12 h. The precipitated *N,N′*-dicyclohexylurea was removed by filtration, with the filtrate being extracted with 10% $NaHCO_3$. The organic phase evaporated, and the precipitate that occurred was filtrated and crystallized from an appropriate solvent to afford the desired compound.

*2.3.1. N-(4-Ferrocenylphenyl)methanesulfonamide (**6a**).* Crystallization from methanol; yellow powder, 71% yield; mp 109-110°C; IR (ν, cm^{-1}): 3039, 2983, 1613, 1030. ^{1}H-NMR (400 MHz, $CDCl_3$): 11.34 (1H, s, NH), 7.22 (2H, d, J = 8.4 Hz, Ar–H), 6.97 (2H, d, J = 8.4 Hz, Ar–H), 4.96 (2H, s, C_5H_4), 4.39 (2H, s, C_5H_4), 4.04 (5H, s, C_5H_5), 3.22 (3H, s, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$): 34.6, 67.2, 70.1, 70.4, 84.3, 114.3, 127.1, 129.6, 138.4. Elemental analysis for $C_{17}H_{17}FeNO_2S$: Calculated: C, 57.48; H, 4.82; N, 3.94; S, 9.03%. Found: C, 57.41; H, 4.89; N, 3.85; S, 9.11%.

*2.3.2. N-(4-Ferrocenylphenyl)ethanesulfonamide (**6b**).* Crystallization from methanol; yellow powder, 74% yield; mp 132-133°C; IR (ν, cm^{-1}): 3056, 2989, 1619, 1023. ^{1}H-NMR (400 MHz, $CDCl_3$): 11.23 (1H, s, NH), 7.45 (2H, d, J = 8.4 Hz, Ar–H), 6.81 (2H, d, J = 8.4 Hz, Ar–H), 5.01 (2H, s, C_5H_4), 4.42 (2H, s, C_5H_4), 4.01 (5H, s, C_5H_5), 3.40 (2H, q, J = 7.6 Hz, CH_2), 1.52 (3H, t, J = 7.6 Hz, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$): 23.1, 48.3, 67.6, 70.2, 70.5, 84.5, 119.7, 128.5, 133.9, 137.5. Elemental analysis for $C_{18}H_{19}FeNO_2S$: Calculated: C, 58.55; H, 5.19; N, 3.79; S, 8.68%. Found: C, 58.69; H, 5.16; N, 3.71; S, 8.56%.

*2.3.3. N-(4-Ferrocenylphenyl)benzenesulfonamide (**6c**).* Crystallization from ethanol; orange powder, 68% yield; mp 174-175°C; IR (ν, cm^{-1}): 3087, 1612, 1015. ^{1}H-NMR (400 MHz, $CDCl_3$): 11.31 (1H, s, NH), 7.25–7.61 (7H, m, Ar–H), 6.96 (2H, d, J = 8.6 Hz, Ar–H), 4.94 (2H, s, C_5H_4), 4.40 (2H, s, C_5H_4), 4.03 (5H, s, C_5H_5). ^{13}C-NMR (100 MHz, $CDCl_3$): 67.4, 70.1, 70.4, 84.2, 119.3, 125.1, 127.4, 121.1, 133.4, 134.2, 140.9. Elemental analysis for $C_{22}H_{19}FeNO_2S$: Calculated: C, 63.32; H, 4.59; N, 3.36; S, 7.68%. Found: C, 63.24; H, 4.52; N, 3.69; S, 7.75%.

*2.3.4. N-(4-Ferrocenylphenyl)-4-methylbenzensulfonamide (**6d**).* Crystallization from ethanol; yellow powder, 68% yield; mp 169-170°C; IR (ν, cm^{-1}): 3045, 2986, 1621, 1017. ^{1}H-NMR (400 MHz, $CDCl_3$): 11.27 (1H, s, NH), 7.81 (2H, d, J = 8.2 Hz, Ar–H), 7.52 (2H, d, J = 8.5 Hz, Ar–H), 7.28 (2H, d, J = 8.2 Hz, Ar–H), 7.01 (2H, d, J = 8.5 Hz, Ar–H), 4.70 (2H, s, C_5H_4), 4.41 (2H, s, C_5H_4), 4.02 (5H, s, C_5H_5), 2.26 (3H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$): 24.3, 67.2, 69.4, 70.3, 84.4, 117.7, 122.6, 126.5, 128.2, 137.3, 137.4, 138.8, 140.4. Elemental analysis for $C_{23}H_{21}FeNO_2S$: Calculated: C, 64.05; H, 4.91; N, 3.25; S, 7.43%. Found: C, 64.14; H, 4.82; N, 3.19; S, 7.32%.

3. Antimicrobial Evaluation

Chemical compounds were individually tested against a panel of Gram-positive and Gram-negative bacterial pathogens, yeast, and fungi. Antimicrobial tests were carried out by the agar well diffusion method [33] using 100 μL of suspension containing 1×10^6 CFU/mL of pathological tested bacteria and 1×10^6/mL of yeast spread on nutrient agar (NA) and Sabouraud dextrose agar (SDA), respectively. After the media had cooled and solidified, wells (10 mm in diameter) were made in the solidified agar and loaded with 100 μL of tested compound solution prepared by dissolving 100 mg of the chemical compound in one mL of dimethyl sulfoxide (DMSO). The inculcated plates were then incubated for 24 h at 37°C for bacteria and 48 h at 28°C for fungi. Negative controls were prepared using DMSO employed for dissolving the tested compound. Ciprofloxacin (50 μg/mL) and Ketoconazole (50 μg/mL) were used as standard for antibacterial and antifungal activities respectively. After incubation time, antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms and compared with that of the standard. Antimicrobial activities were expressed as inhibition diameter zones in millimeters (mm). The experiment was carried out in triplicate, and the average zone of inhibition was calculated. Compounds that showed significant growth inhibition zones (>14 mm) using the twofold

serial dilution technique were further evaluated for their minimal inhibitory concentrations (MIC).

3.1. Minimal Inhibitory Concentration (MIC) Measurement. The microdilution susceptibility test in Müeller Hinton Broth (Oxoid) was used for the determination of antibacterial activity, and Sabouraud Liquid Medium (Oxoid) was used for the determination of antifungal activity. Stock solutions of the tested compounds, Ciprofloxacin, and Ketoconazole were prepared in DMF at concentration of 1000 mg/mL. Twofold serial dilutions of the tested compounds solutions were prepared using the proper nutrient broth. The final concentration of the solutions was 132, 66, 33, 16.5, and 8.25 μg/mL. The tubes were then inoculated with the test organisms, grown in their suitable broth at 37°C for 24 h for bacteria (about 1×10^6 CFU/mL). each 5 mL received 0.1 mL of the previous inoculum and incubated at 37°C for 24 h. The lowest concentration showing no growth was taken as the minimum inhibitory concentration (MIC). Control experiments with DMF and uninoculated media were run parallel to the test compounds under the same conditions. The MIC (mg/mL) and inhibition zone diameters values are recorded in Table 1.

4. Results and Discussion

4.1. Chemistry. The synthetic strategies adopted for the synthesis of the intermediates and target compounds are depicted in Scheme 1. The starting material compound **2** (4-nitrophenylferrocene) was synthesized through arylation of ferrocene by a diazonium salt under phase transfer conditions, according to the literature [31]. The reduction of compound **2** with tin in acidic condition gives compound **3** (4-ferrocenylaniline) [32].

The urea (**4a**–**4e**) and thiourea derivatives (**4f**–**4j**) were obtained by the reaction of 4-ferrocenylaniline with the appropriate isocyanates and isothiocyanates, respectively, in ethanol. The sulfonamide derivatives (**5a**–**5d**) were synthesized by the reaction of 4-ferrocenylaniline with the appropriate sulfonic acid in the presence of dicyclohexylcarbodiimide/dimethylaminopyridine (DCC/DMAP).

The chemical structures of the title compounds were elucidated by ^{1}H-NMR, ^{13}C-NMR, and FT-IR spectra and elemental analysis. Elemental analysis data of all products were in good agreement with the calculated values. IR spectra of all compounds showed all characteristic peaks. The absence of N–H at 3500–3300 cm^{-1} confirmed the formation of urea, thiourea, and sulfonamide. ^{1}H-NMR and ^{13}C-NMR spectra of the synthesized compounds were recorded in $CDCl_3$ relative to TMS as reference. The ^{1}H-NMR spectrum showed signals in the region at 6.69–7.73 ppm corresponding to the aromatic phenyl protons. The benzene ring directly attached to ferrocene contained two types of protons which appeared as a doublet with coupling constant of 8.4–8.6 Hz. Substituted cyclopentadiene contained two types of protons, which appeared as two singlets at 4.39–4.48 and 4.93–5.04 ppm, respectively. All five protons of unsubstituted cyclopentadiene were chemically equivalent and appeared in ^{1}H-NMR spectra as singlets at 4.00–4.04 ppm. In the ^{1}H-NMR spectra, the N–H protons of the urea (**4a**–**4e**) and thiourea (**5a**–**5e**) derivatives were observed as two separate singlets at 9.43–9.94 and 10.73-11.03 ppm, respectively. The N-H protons of the sulfonamide derivatives (**6a**–**6d**) were observed as singlet at 11.27–11.34. In the ^{13}C-NMR spectra, the compounds **4a**–**4e** showed a signal at 155.0–156.1 ppm due to (C=O) of urea derivatives. Thiourea derivatives **5a**–**5e** showed a signal at 179.2–180.5 ppm due to (C=S).

4.2. Antimicrobial Activity. Fourteen of the newly synthesized target compounds were evaluated for their in vitro antibacterial activities at 100 μg/mL concentration against *Staphylococcus aureus* ATCC 29213 and *Bacillus subtilis* ATCC 6633 as examples of Gram-positive bacteria and *Klebsiella pneumonia* ATCC13883 and *Escherichia coli* ATCC 25922 as examples of Gram-negative bacteria. They were also evaluated for their in vitro antifungal potential against *Saccharomyces cerevisiae* and *Candida albicans* NRRL Y-477 fungal strains. Agar-diffusion method was used for the determination of the preliminary antibacterial and antifungal activities. Ciprofloxacin and Ketoconazole were used as reference drugs. The results were recorded for each tested compound as the average diameter of inhibition zones (IZ) of bacterial or fungal growth around the disks in mm. The results depicted in Table 1 revealed that most of tested compounds displayed variable inhibitory effects on the growth of the tested Gram-positive and Gram-negative bacterial strains and also against antifungal strains.

In general, most of the urea derivatives revealed better activity against the Gram-positive rather than the Gram-negative bacteria. Most of sulfonamide derivatives have superior significant antifungal potency than antibacterial potency. It would be also noticed that compounds belonging to the urea and thiourea groups exhibited better antibacterial potentials than members of the sulfonamide groups. Compounds **4b**, **4c**, **5b**, and **6b** exhibited the highest potency against tested organisms with respect to reference drugs. Compound **4b** inhibited the growth of *S. aureus* ATCC 29213, *B. subtilis* ATCC6633, and *K. pneumonia* ATCC13883 with inhibition zones 33, 31, and 32 mm, respectively. While compounds **5b** and **5e** showed excellent activity against *K. pneumonia* ATCC13883 and *E. coli* ATCC 25922 of Gram-negative bacteria, also compounds **6b** and **6d** showed highest activity against *S. cerevisiae* and *C. albicans* NRRL Y-477 fungal strains.

The minimum inhibitory concentration (MIC) of the synthesized compounds against highly inhibited organisms is reported in Table 2. Compound **4b** exhibited low MIC (8.25 μg/mL) against *S. aureus* ATCC 29213, *B. subtilis* ATCC 6633, and *K. pneumonia* ATCC13883. On the other hand, compound **4b** revealed high MIC (132 μg/mL) against *C. albicans* NRRL Y-477 fungal strains. Compounds **5b** and **5e** showed MIC 8.25 μg/mL against *K. pneumonia* ATCC13883 and *E. coli* ATCC 25922 of Gram-negative bacteria. Additionally, compounds **5d**, **6b,** and **6d** exhibited MIC 8.25 μg/mL against *S. cerevisiae,* and also **6a** and **6d** showed MIC 8.25 μg/mL against *C. albicans* NRRL Y-477 fungal strains.

Table 1: Antimicrobial activity expressed as inhibition diameter zones in millimeters (mm) of chemical compounds against the pathological strains based on well diffusion assay.

Compound	Zone of inhibition (mm)					
	Bacteria				Fungi	
	Gram-positive		Gram-negative			
	S. aureus	*B. subtilis*	*K. pneumoniae*	*E. coli*	*S. cerevisiae*	*C. albicans*
4a	22	18	18	14	19	16
4b	33	31	32	27	16	15
4c	30	32	27	32	20	21
4d	20	19	24	27	18	16
4e	23	18	18	19	14	19
5a	28	26	24	24	26	29
5b	25	27	30	31	24	28
5c	24	28	28	24	21	32
5d	23	30	26	28	29	26
5e	26	22	32	33	24	26
6a	24	28	24	25	26	30
6b	22	25	29	28	32	34
6c	28	24	17	22	24	18
6d	22	23	26	28	30	30
Ciprofloxacin	30	30	29	24	NT	NT
Ketoconazole	NT	NT	NT	NT	30	31

The experiment was carried out in triplicate, and the average zone of inhibition was calculated.
NT: not tested.

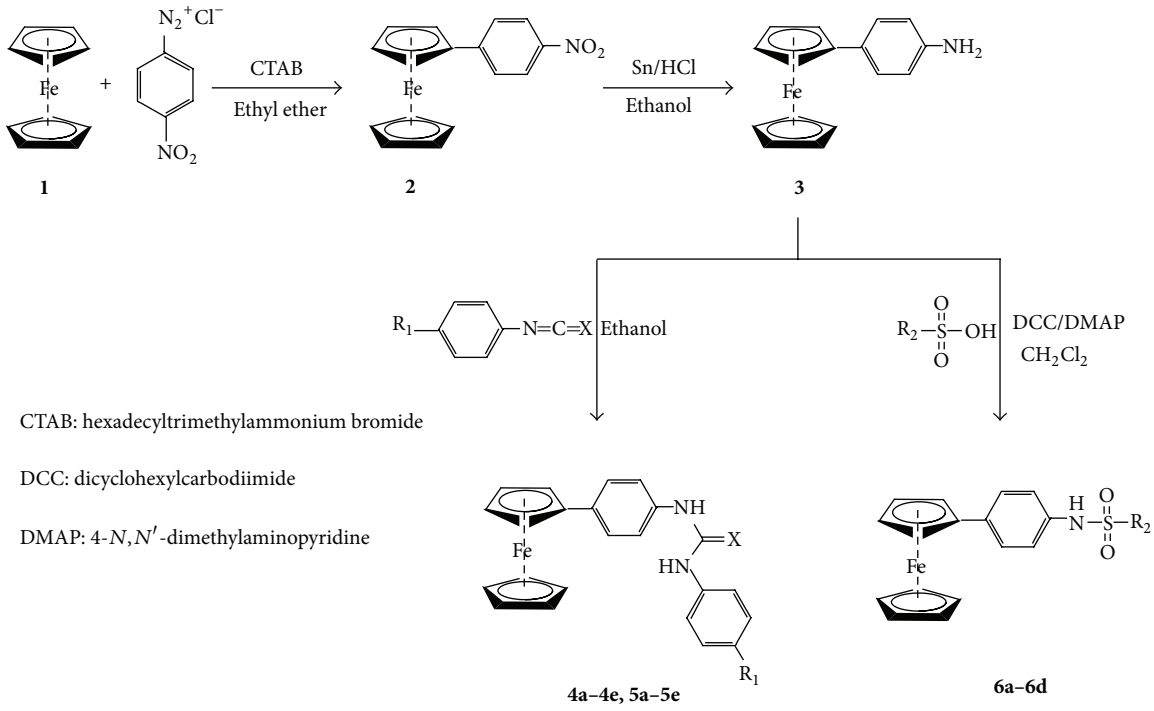
Table 2: Minimum inhibitory concentration (μg/mL) against the pathological strains based on twofold serial dilution technique.

Compound	MIC in μg/mL					
	Bacteria				Fungi	
	Gram-positive		Gram-negative			
	S. aureus	*B. subtilis*	*K. pneumoniae*	*E. coli*	*S. cerevisiae*	*C. albicans*
4a	33	66	66	132	66	132
4b	8.25	8.25	8.25	16.5	132	132
4c	16.5	8.25	16.5	8.25	66	66
4d	66	33	16.5	16.5	66	132
4e	33	66	66	66	132	66
5a	16.5	16.5	16.5	33	16.5	8.25
5b	33	16.5	8.25	8.25	33	16.5
5c	33	16.5	16.5	33	16.5	8.25
5d	33	8.25	33	16.5	8.25	33
5e	16.5	33	8.25	8.25	33	33
6a	33	16.5	33	33	16.5	8.25
6b	33	33	16.5	16.5	8.25	16.5
6c	16.5	33	66	33	33	66
6d	33	66	33	16.5	8.25	8.25
Ciprofloxacin	8.25	8.25	8.25	16.5	NT	NT
Ketoconazole	NT	NT	NT	NT	8.25	8.25

NT: not tested.

5. Conclusion

In conclusion, the objective of the present study was to synthesize and investigate the antimicrobial activities of some novel ferrocene derivatives carrying urea, thiourea, and sulfonamide groups with the hope of discovering new structure leads serving as potent antimicrobial agents. Amongst the tested compounds, **4b**, **4c**, **5b**, and **6b** displayed excellent



1 **2** **3**

4a–4e, 5a–5e **6a–6d**

	X	R_1		X	R_1
4a	O	–H	**5a**	S	–H
4b	O	–Cl	**5b**	S	–Cl
4c	O	–Br	**5c**	S	–Br
4d	O	$–CH_3$	**5d**	S	$–CH_3$
4e	O	$–OCH_3$	**5e**	S	$–OCH_3$

	R_2
6a	$–CH_3$
6b	$–C_2H_5$
6c	
6d	

Scheme 1: Synthesis of ferrocene derivatives carrying urea, thiourea and sulfonamide moieties.

antimicrobial activity. These preliminary results are beneficial for further studies in developing new urea, thiourea, and sulfamide substituted ferrocene derivatives as potential antimicrobial agents.

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