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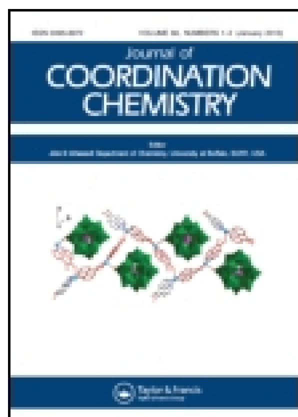

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New supramolecular ferrocenyl phenylguanidines as potent antimicrobial and DNA-binding agents

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New supramolecular ferrocenyl phenylguanidines as potent antimicrobial and DNA-binding agents

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Six new ferrocenyl phenylguanidines have been synthesized and characterized by elemental analysis, FT-IR, multinuclear (^{1}H and ^{13}C) NMR, and single crystal analysis. The latter showed a supramolecular structure for **2** mediated by O···H and π···H interactions. A subsequent DNA-binding study of these complexes proved them to be good DNA binders with the binding constant varying in the range of $1.2–5.6 \times 10^{5}\,M^{-1}$. These compounds were found to have moderate antibacterial and significant antifungal activities, especially for compounds having a chlorophenyl. These compounds may emerge as a new class of anticancer and antifungal agents alone or in combination with other drugs.

Keywords: Ferrocenyl phenylguanidine; DNA binding; Antibacterial; Antifungal activity

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1. Introduction

Compounds containing CN_3 named guanidines have a rich history in biological and bio-inspired molecular recognition due to their amphoteric nature [1–3]. For example, a guanidine is present in arginine side chains which not only involve in binding and recognition of ionic substrates, but also maintain a protein tertiary structure. The guanidinium within a variety of molecular architectures forms strong noncovalent interactions with anionic groups through hydrogen-bonding and charge-pairing interactions. Guanidine derivatives are being used as neurotransmitters (neuropeptide Y) [4], cardiovascular or antihypertensive drugs (amiloride, triamterene, doxazosin mesylate, *etc*.) [5], and as antibiotics (streptomycin, trimethoprim, and chlorhexidine) [6]. Some naturally occurring guanidines were screened for their nuclease activity and exhibited cytotoxic properties [7]. Guanidine is an inhibitor of urokinase, which is responsible for a large number of malignancies including breast, lung, bladder, stomach, cervix, kidney, and brain cancers [8–10].

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It has been established that polyaromatic guanidinium derivatives offer cytotoxic activities by forming stronger adducts with DNA [11].

Incorporation of ferrocenyl into an organic compound often enhances the biological action of the resulting products [12]. Various researchers have reported ferrocenic analogs of chloroquine, mefloquine, and quinine, with enhanced antimalarial activities [13, 14]. The substitution of the aromatic ring of the well-known anticancer drug tamoxifen with ferrocenyl group (called ferrocifen) showed pronounced activity against tamoxifen resistive breast cancer cells [15]. Similarly, the antibiotic activity of penicillin and cephalosporin was enhanced many fold on incorporation of the ferrocenyl moiety [16]. Low cytotoxicity in biological systems, lipophilicity, cytotoxicity of metabolites towards tumors, the *pi*-conjugated system, and the resulting electron-transfer ability of ferrocene derivatives make them candidates for investigation of their biological applications [17–21].

Here, we combine a ferrocenyl with phenyl guanidines to get electrochemistry and improved biological action. Study including DNA interaction and antimicrobial activity is not reported for ferrocenyl guanidine, and this contribution will provide a foundation for future work.

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2. Experimental

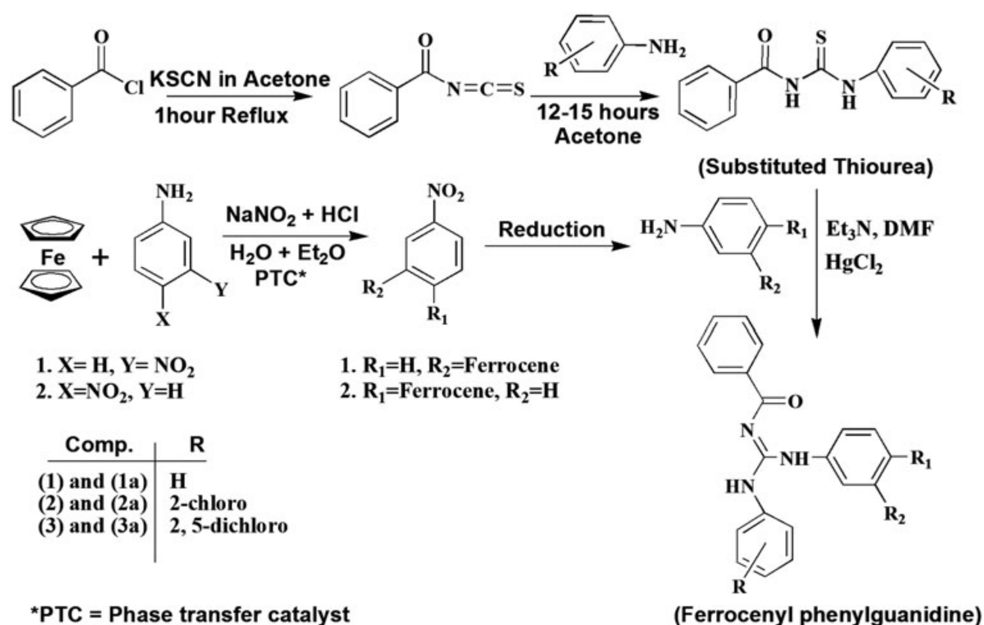
2.1. *Materials*

Ferrocene, 3- and 4-nitroaniline, sodium nitrate, hexadecyltrimethyl ammonium bromide, palladium on charcoal and hydrazine (for the synthesis of para and meta ferrocenyl aniline), benzoic acid, thionyl chloride, KSCN/NH_4SCN, simple and chloro substituted anilines (for the synthesis of thioureas), and mercuric chloride were obtained from Fluka, Switzerland. Solvents like diethyl ether, triethylamine, dimethylformamide (DMF), acetone, ethanol, and dimethyl sulfoxide (DMSO) were obtained from Merck, Germany and freshly dried using standard methods. Elemental analysis was performed using Fisons EA1108 CHNS analyzer and LECO-183 CHNS analyzer. Melting points were determined on a Bio Cote SMP10-UK using open capillary tubes. The solid state Fourier transform spectrum was recorded on Bio-Rad Excalibur FT-IR Model FTS 3000 MX and Bruker Tensor 37 spectrophotometers. ^{1}H and ^{13}C-NMR spectra were obtained on a Bruker 300 MHz NMR spectrophotometer in $CDCl_3$ using tetramethylsilane as internal reference. The data for the crystal structure analysis were collected on a Bruker Kappa APEX II CCD Diffractometer.

2.2. *Apparatus and procedure*

2.2.1. Synthesis of ferrocenylaniline and thioureas. Two anilines (*p*-ferrocenylaniline and *m*-ferrocenylaniline) and three thioureas–(1-benzoyl-3-phenyl) thiourea, 1-benzoyl-3-(2-chlorophenyl) thiourea, and 1-benzoyl-3-(2,5-dichlorophenyl) thiourea, were synthesized by the reported method [22, 23].

2.2.2. Synthesis of ferrocenyl phenylguanidine. All guanidines were synthesized by the reported method [24]. The thioureas were mixed with ferrocenylaniline in DMF in



Scheme 1. Synthesis of ferrocenyl phenylguanidine.

equimolar ratios with two equivalents of triethylamine. The temperature was maintained below 5 °C using an ice bath and one equivalent of mercuric chloride was added to the reaction mixture with vigorous stirring. The ice bath was removed after 30 min and the mixture was stirred overnight. Progress of the reaction was monitored by TLC till completion. Chloroform (20 mL) was added to the reaction mixture and the suspension was filtered through a sintered glass funnel to remove HgS. The solvent from the filtrate was evaporated under reduced pressure and the residue was redissolved in CH_2Cl_2 (20 mL), washed with water (4 × 50 mL), and the organic phase, dried over anhydrous $MgSO_4$. The solvent was evaporated; the residue was purified by column chromatography and recrystallized in ethanol (scheme 1). Crystals were obtained for **2** only.

2.3. *X-ray crystallography*

For **2**, X-ray data were collected on a Bruker kappa APEXII CCD diffractometer using graphite-monochromated Mo-K_α radiation (wavelength = 71,073 Å). The structure was solved using SHELXS-97. Final refinement on F^2 was carried out by full-matrix least-squares using SHELXL-97. The disordered cyclopentadienyl was refined in two groups as regular pentagons of 1.39 and 1.44 Å. The anisotropic temperature factors of the disordered carbons were restrained to be nearly isotropic.

2.4. *Cyclic voltammeter and UV–Vis spectrophotometer*

Cyclic voltammetric experiments were performed using a Bio-Logic SP-300 voltameter. Tetrabutylammonium perchlorate (TBAP) (Fluka, 99% purity) was further purified by recrystallization using methanol. Measurements were carried out in a conventional three electrode

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cell consisting of Ag/AgCl as a reference electrode, a thin Pt wire of thickness 0.5 mm with an exposed end of 10 mm as the counter electrode, and a platinum disk as the working electrode. For electrochemical measurements, a known concentration of the test solution was kept in an electrochemical cell and the voltammogram was recorded in the absence of DNA. The procedure was then repeated for systems with a constant concentration of the drug and varying concentration of DNA. The working electrode was cleaned after every electrochemical assay. Ct-DNA stock solution was prepared in doubly-distilled water and stored at 4 °C. The concentration of the stock solution of DNA was determined by UV absorbance at 260 nm using the molar extinction coefficient (ε) of 6600 M^{-1} cm^{-1} [25].

Absorption spectra were measured with a Shimadzu 1800 UV–Vis spectrometer. The electronic absorption spectrum of a known concentration of the drug was obtained without DNA. The spectroscopic response of the same amount of the drug was then monitored by addition of small aliquots of DNA solution. All samples were allowed to equilibrate for 15 min prior to every spectroscopic measurement.

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2.5. *Biological studies*

2.5.1. Antibacterial assay. All six guanidines were studied against five bacterial strains, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Klebsiella pneumoneae,* and *Escherchia coli*. The agar diffusion method [26, 27] was used for determination of the inhibition zone. A single colony from each bacterial culture plate was transferred to nutrient broth (pH 7) and incubated at 37 °C for 24 h. About 2 mg/mL concentration of each compound was prepared. Briefly, 0.75 mL of broth culture containing 10^6 colony forming units per millimeter of test strain was added to the 75 mL of nutrient agar medium, mixed well, and then poured into a 14 cm sterile agar plate. The reaction was performed in triplicate. Wells were prepared using an 8 mm sterilized metallic borer, sealed with media, and filled with 100 µL of respective concentration of each compound. Penicillin (1 mg/mL) was used as standard drug, while DMSO was used as negative control. Plates were incubated at 37 °C aerobically and the zone of inhibition was measured after 24 h. Experiments were run in triplicates.

2.5.2. Antifungal assay. Activity was tested against three different fungal strains, *Fusarium moniliforme*, *Aspergillus fumigates,* and *Aspergillus flavus*, according to the reported method [26, 27]. The samples for antifungal assay were prepared from an initial stock of 2 mg of the compound in 1 mL of DMSO. Culture media were prepared by dissolving 6.5 mg of Sabouraud dextrose agar per 100 mL distilled water and pH was adjusted to 5.6. The Sabouraud dextrose agar (MERCK) was dispensed as 10 mL volume into screw capped tubes or cotton plugged test tubes and was autoclaved at 121 °C for 21 min. Tubes were allowed to cool to 50 °C and Sabouraud dextrose agar was loaded with 67 µL from the stock solution. Tubes were then allowed to solidify in a slanting position at room temperature. Three slants of the test sample were prepared for each fungus. Tubes containing solidified media and test compounds were inoculated with a 4 mm diameter piece of inoculum, taken from a seven day old culture of fungus. One sample of each compound was prepared, which was used for positive control. Slants without test sample were used for negative control. The test tubes were incubated at 28 °C for seven days. Cultures were examined twice weekly during the incubation. Reading was taken by measuring the linear length of fungus in slant by measuring growth (mm) and growth inhibition was calculated

with reference to the negative control. Percentage inhibition of fungal growth for each concentration of the compound was determined as:

$$\text{Percentage inhibition of fungal growth} = \frac{1 - \text{Linear growth (cm) in test sample}}{\text{Linear growth (cm) in control}} \times 100$$

*2.5.2.1. Synthesis of N-(4-ferrocenylphenyl-N'-phenyl-N"-benzoylguanidine) (**1**).* Yield: 2.59 g, 72%; m.p. 151-152 °C; FT-IR (KBr, cm^{-1}): 3340, 3278, 3117, 2995, 2950, 2917, 1673, 1583, 1517, 1465, 1449, 1394, 1331, 1243, 1102, 1078, 1033, 877, 860, 800, 730, 480, 448, 420; ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ 4.8 (*s*, 5H), 4.91 (*t*, 2H, J= 1.9 Hz, C_5H_4), 5.2 (*t*, 2H, J= 1.9 Hz, C_5H_4), 7.2 (*d*, 2H, J=8.2 Hz), 7.3 (*d*, 2H, J=8.1 Hz), 7.4–7.91 (*m*, 10H), 10.3 (*s*, H, N–H), 11.3 (*s*, H, N–H); ^{13}C NMR (75.47 MHz, $CDCl_3$, 25 ° C): δ 66.1, 67.01, 67.5, 83.1, 121.2, 122.3, 122.9, 129.2, 129.8, 130.2, 130.6, 134.1, 134.5, 135.5, 140.1, 140.4, 156.8 (CN_3), 176 (C=O); Anal. Calcd for $C_{30}H_{25}N_3FeO$ (499.13): C, 72.15; H, 5.05; N, 8.41. Found: C, 72.4; H, 5.13; N, 7.9%.

*2.5.2.2. Synthesis of N-(3-ferrocenylphenyl-N'-phenyl -N"-benzoylguanidine) (**1a**).* Yield: 2.1 g (71%), m.p. 151–152 °C; FT-IR (KBr, cm^{-1}): 3342, 3279, 3118, 2991, 2952, 2917, 1675, 1610, 1583, 1515, 1465, 1448, 1394, 1333, 1243, 1102, 1078, 1033, 876, 860, 801, 730, 497, 482, 455, 422; ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ 4.8 (*s*, 5H), 4.9 (*t*, 2H, J= 1.9 Hz, C_5H_4), 5.2 (*t*, 2H, J= 1.9 Hz, C_5H_4), 7.25–7.35 (*m*, 3H), 7.38 (*s*, H), 7.4–7.9 (*m*, 10H), 10.2 (*s*, H, N–H), 11.3 (*s*, H, N–H); ^{13}C NMR (75.47 MHz, $CDCl_3$, 25 °C): δ 66.0, 67.2, 67.6, 83.1, 121.4, 122, 122.3, 122.9, 124, 129.1, 129.8, 130.4, 130.6, 134.2, 134.5, 135.5, 140.1, 140.6, 156.8 (CN_3), 176 (C=O); Anal. Calcd for $C_{30}H_{25}N_3FeO$ (499.13): C, 72.15; H, 5.05; N, 8.41. Found: C, 71.5; H, 5.2; N, 7.1%.

*2.5.2.3. Synthesis of N-(4-ferrocenylphenyl-N'-(2-chlorophenyl)-N"-benzoylguanidine) (**2**).* Yield: 2.6 g, 74%; m.p. 157–158 °C; FT-IR (KBr, cm^{-1}): FT-IR (KBr, cm^{-1}): 3315, 3158, 3059, 2953, 1670, 1596, 1559, 1509, 1455, 1398, 1382, 1307, 1267, 1230, 1185, 1101, 1075, 1012, 833, 725, 625, 609, 588, 569, 520, 505, 476, 447, 407; ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ 4.9 (*s*, 5H), 4.97 (*t*, 2H, J= 1.9 Hz, C_5H_4), 5.3 (*t*, 2H, J= 1.9 Hz, C_5H_4), 7.26 (*d*, 2H, J=8.1 Hz), 7.35 (*d*, 2H, J=8.1 Hz), 7.4–8.0 (*m*, 9H), 10.3 (*s*, H, N–H), 11.4 (*s*, H, N–H); ^{13}C NMR (75.47 MHz, $CDCl_3$, 25 °C): δ 66.8, 67.4, 67.5, 83.6, 121.5, 122.7, 124.5, 124.8, 129.6, 130.2, 131, 133.7, 134.7, 135.5, 136.4, 139.7, 141.2, 141.9, 160.5 (CN_3), 179.3 (C=O); Anal. Calcd for $C_{30}H_{24}N_3ClFeO$ (533.8): C, 67.58; H, 4.53; N, 7.87. Found: C, 67.2; H, 4.1; N, 7.2%.

*2.5.2.4. Synthesis of N-(3-ferrocenylphenyl-N'-(2-chlorophenyl)-N"-benzoylguanidine) (**2a**).* Yield: 2.3 g, 73%; m.p. 157–158 °C; FT-IR (KBr, cm^{-1}): 3308, 3158, 3060, 2954, 1670, 1624, 1594, 1558, 1506, 1456, 1398, 1385, 1307, 1263, 1230, 1182, 1101, 1070, 1012, 833, 725, 628, 609, 584, 569, 543, 520, 505, 476, 447, 433, 412, 403; ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ 4.89 (*s*, 5H), 4.95 (*t*, 2H, J= 1.9 Hz, C_5H_4), 5.25 (*t*, 2H, J= 1.9 Hz, C_5H_4), 7.2–7.3 (*m*, 3H), 7.4 (*s*, H), 7.6–8.0 (*m*, 9H), 10.3 (*s*, H, N–H), 11.4 (*s*, H, N–H); ^{13}C NMR (75.47 MHz, $CDCl_3$, 25 °C): δ 66.6, 67.3, 67.5, 83.4, 121.3 122.7, 123.1, 124.2, 124.7, 129.1, 129.7, 130.2, 130.8, 133.5, 134.7, 135, 136.5, 139.2, 141.1,

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142, 160.8 (CN_3), 179.4 (C=O); Anal. Calcd. for $C_{30}H_{24}N_3ClFeO$ (533.8): C, 67.58; H, 4.53; N, 7.87. Found: C, 67.01; H, 4.30; N, 7.6%.

*2.5.2.5. Synthesis of N-(4-ferrocenylphenyl-N'-(2,5-dichlorophenyl)-N''-benzoylguanidine) (**3**).* Yield: 2.6 g (74%), m.p. 157–158 °C, FT-IR (KBr, cm^{-1}): 3305, 3162, 3091, 2960, 2925, 2855, 1670, 1615, 1594, 1555, 1506, 1458, 1398, 1389, 1307, 1263, 1236, 1187, 1101, 1077, 1012, 833, 726, 628, 607, 586, 569, 545, 520, 508, 476, 446, 438, 412, 408; ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ 4.8 (*s*, 5H), 4.9 (*t*, 2H, *J* = 1.9 Hz, C_5H_4), 5.2 (*t*, 2H, *J* = 1.9 Hz, C_5H_4), 7.25 (*d*, H, *J* = 8.1 Hz, Ar–H), 7.35 (*d*, H, *J* = 8.2 Hz, Ar–H), 7.41 (*s*, H), 7.63 (*d*, H, *J* = 8.2 Hz), 7.71 (*d*, H, *J* = 8.2 Hz), 7.8–8.0 (*m*, 5H), 10.4 (*s*, H, N–H), 11.5 (*s*, H, N–H); ^{13}C NMR (75.47 MHz, $CDCl_3$, 25 °C): δ 66.8, 67.6, 67.7, 83.3 121.1, 121.7, 122.7, 124.8, 129.6, 130.4, 131.1, 133.7, 134.7, 135.5, 136.1, 139.7, 141.2, 160.7 (CN_3), 179.1 (C=O); Anal. Calcd for $C_{30}H_{23}N_3Cl_2FeO$ (568.2): C, 63.4; H, 4.08; N, 7.39. Found: C, 62.5; H, 3.7; N, 7.2%.

*2.5.2.6. Synthesis of N-(3-ferrocenylphenyl-N'-(2,5-dichlorophenyl)-N''-benzoylguanidine) (**3a**).* Yield: 2.2 g (72%), m.p. 157–158 °C, FT-IR (KBr, cm^{-1}): 3301, 3156, 3092, 2955, 1966, 1673, 1605, 1594, 1558, 1506, 1456, 1398, 1385, 1310, 1263, 1230, 1185, 1105, 1073, 1012, 833, 726, 627, 607, 586, 572, 545, 520, 508, 477, 445, 417, 405; ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ 4.89 (*s*, 5H), 4.99 (*t*, 2H, *J* = 1.9 Hz, C_5H_4), 5.3 (*t*, 2H, *J* = 1.9 Hz, C_5H_4), 7.28–7.38 (*m*, 3H), 7.4 (*s*, H), 7.55 (*s*, H), 7.6 (*d*, H, *J* = 8.2 Hz), 7.7 (*d*, H, *J* = 8.2 Hz), 7.8–8.0 (*m*, 5H), 10.4 (*s*, H, N–H), 11.51 (*s*, H, N–H); ^{13}C NMR (75.47 MHz, $CDCl_3$, 25 °C): δ 66.8, 67.6, 67.7, 83.2, 121.5, 121.6, 122.7, 124.5, 124.8, 129.2, 129.7, 130.3, 131.1, 133.7, 134.7, 135.5, 136, 139.7, 141.5, 142.1, 160.8 (CN_3), 179.3 (C=O); Anal. Calcd for $C_{30}H_{23}N_3Cl_2FeO$ (568.2): C, 63.4; H, 4.08; N, 7.39. Found: C, 62.8; H, 4.50; N, 7.1%.

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3. Results and discussion

All ferrocenyl phenylguanidines were synthesized in good yield and characterized by different techniques. Elemental analyses of all the compounds were in agreement with calculated values. In FT-IR spectra two peaks, a sharp and a weak, were observed for N–H bonds at 3392-3245 cm^{-1}. The C=N stretch in all compounds was observed at 1544-1586 cm^{-1}, intermediate between single and double bond, an indication of conjugation between all three nitrogens of the guanidine [28]. A sharp C=O stretch was also observed at 1601–1665 cm^{-1} for all the compounds. ^{1}H and ^{13}C-NMR spectra were recorded relative to TMS as reference in chloroform. Both N–H protons appeared downfield at 8.2–9 ppm due to the presence of aromatic groups on nitrogen. Aromatic protons were not well resolved and appeared as complex patterns. In ^{13}C-NMR, the carbonyl carbon was at 176–178 ppm while CN_3 carbon was at 156–158 ppm as expected for guanidine [29].

3.1. *Single crystal X-ray analysis of 2*

Crystals of **2**, suitable for crystallographic analysis, were grown from ethanol by slow evaporation. The molecular structure determination was carried out using X-ray crystallographic analyses (figure 1). Crystals were monoclinic P2(1)/c space group. The C–O bond

length indicates full double bond character while the C–N bond length showed partial double bond character, an indication of resonance in the molecule. Geometrical parameters are summarized in table 1 while selected bond lengths and angles are given in table 2.

The packing diagram showed supramolecular structure for **2** mediated by O···H and π···H interactions, connecting each molecule with five other molecules resulting in a supramolecular structure (figure 2). The O···H interaction involving O-1 and H-13 is 2.519 Å connecting two molecules resulted in a cyclic structure with 18-membered ring of composition $C_8N_4O_2$.

Ferrocene moieties are in opposite directions to minimize repulsion; 2-chlorophenyl of the dimer moves upward and downward for the same reason. H-27 of benzoyl of the dimer is involved in noncovalent interaction with the chloro substituted phenyl ring (2.519 Å). Ferrocene is also involved in H-bonding with H-1a of the guanidine (2.857 Å). These noncovalent interactions result in the supramolecular structure as depicted in figure 3 with cavities along the *b*-axis (figure 4). These compounds may find applications in separation, storage, and transport and also in catalysis [30].

3.2. *DNA binding studies of ferrocenyl phenylguanidines*

3.2.1. By cyclic voltammetry. Cyclic voltammogram of **1** is shown in figure 5 and comparison of **1**, **2,** and **3** at different concentration is given in Supplementary material. All ferrocenyl phenylguanidines show similar voltammetric behavior, with a couple of well-defined and stable redox peaks in the potential range of ± 1.0 V. As no other prominent peaks were observed, all compounds were further studied from 0.0–0.8 V.

The anodic peaks of **1**, **2,** and **3** are at 0.491, 0.551, and 0.521 V with corresponding cathodic peaks at 0.363, 0.397, and 0.391 V, respectively. For simple ferrocene, oxidation was observed at 0.518 V under the same conditions. The electrochemical behavior of the oxidizing moiety of ferrocene can be modulated by

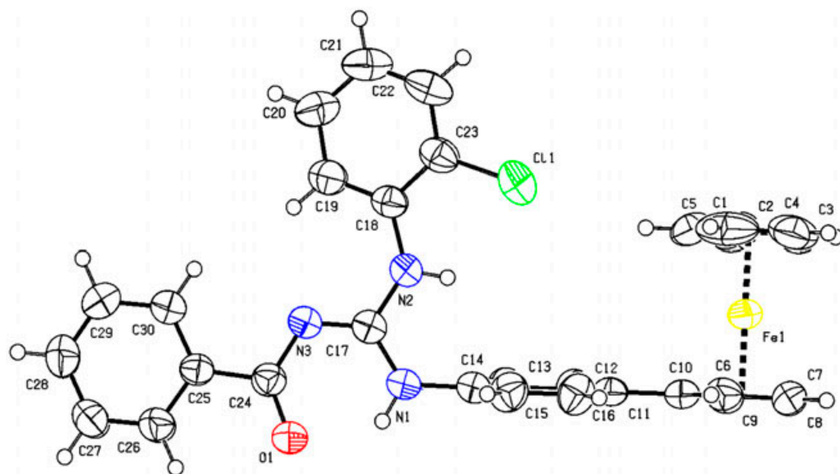

Figure 1. Crystal structure of **2**.

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Table 1. Crystallographic data for **2**.

Empirical formula	$C_{30}H_{24}ClFeN_3O$
Formula weight	533.82
Temperature (*K*)	296(2)
Wavelength (Å)	1.54178
Crystal system	Monoclinic
Space group	P2(1)/c
Unit cell dimensions	
a (Å)	11.1555(6)
b (Å)	9.4859(4)
c (Å)	23.1419(12)
α (°)	90.00
β (°)	92.978(2)
γ (°)	90.00
Cell volume	2445.6(2)
Density (calcd) (Mg/m^3)	1.450
Crystal size (mm^3)	0.40 × 0.30 × 0.20
Index ranges	$-15 <= h <= 15$
	$-13 <= k <= 13$
	$-12 <= l <= 12$
F (0 0 0)	1104
Total reflections	19,031
Refinement method	Full-matrix least-squares on F^2
Independent reflections	1544 [*R* (int) = 0.0607]
R indices (all data)	$R1 = 0.0691$, $wR2 = 0.2367$
Final *R* indices [$I > 2\sigma(I)$]	$R1 = 0.0735$, $wR2 = 0.2311$
Goodness-of-fit	1.060
θ Range for data collection (°)	1.83 – 25.25

Table 2. Selected bond lengths (Å) and angles (°) for **2**.

Fe1–C1	2.022(4)	C1–Fe1–C2	39.8(2)
O1–C24	1.239(3)	C1–Fe1–C3	67.1(2)
N1–C14	1.433(3)	N1–C17–N2	114.7(2)
N1–C17	1.245(3)	N1–C17–N3	125.7(2)
N2–C17	1.359(3)	N2–C17–N3	119.6(2)
N2–C18	1.399(3)	O1–C24–N3	126.6(2)
N3–C17	1.319(3)	O1–C24–C25	118.5(2)
N3–C24	1.366(3)	Cl1–C23–C22	119.0(2)
C24–C25	1.494(4)	Cl1–C23–C18	119.6(2)
C23–Cl1	1.738(3)	N3–C24–C25	114.9(2)

changing the electronic properties of the cyclopentadienyl ring. The slight change in the redox behavior of the compounds to pure ferrocene is attributed to the electron donating effect of NH on ring-A (scheme 2(a)). This group makes oxidation slightly easier than ferrocene as evidenced by a negative shift for **1**. For electron withdrawing chloro at ring-B, a slight positive shift was observed due to a decrease in the donating effect of NH of ring-A. In general, the inductive effect works up to three or four bonds, However, the minor change in the position of redox peak due to chloro (**2**, **2a**, **3,** and **3a**) suggests that the three nitrogens are in conjugation with each other (scheme 2(b)) [28]. The effect is minimized when a second chloro on ring-B is present at *para* position to the first one (**3**, **3a**) and as a consequence, the two chloro groups cancel the inductive effect of each other (scheme 2(c)).

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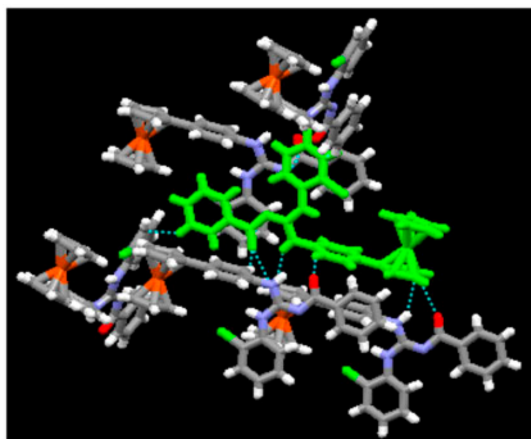

Figure 2. Connection of a reference molecule (light green) with five other molecules (**2**) (see http://dx.doi.org/10.1080/00206814.2013.796371 for color version).

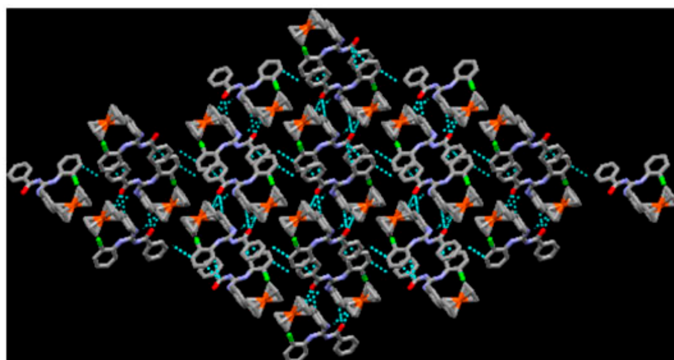

Figure 3. Supramolecular structure of **2**.

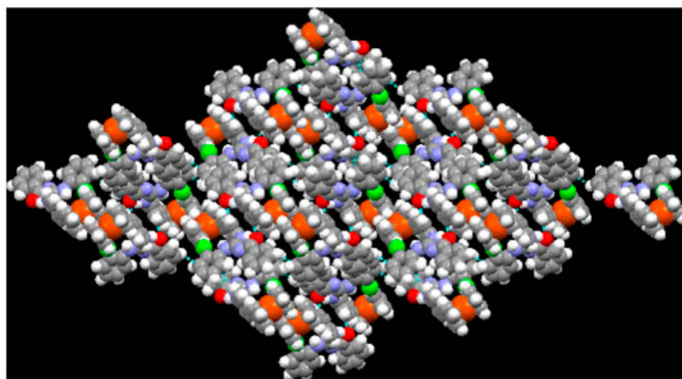

Figure 4. Space filling diagram of **2** showing cavities along the *b*-axis.

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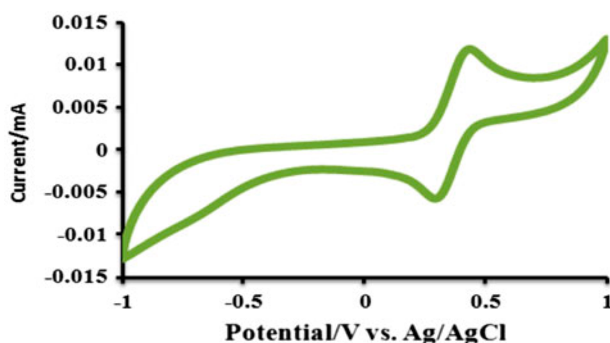
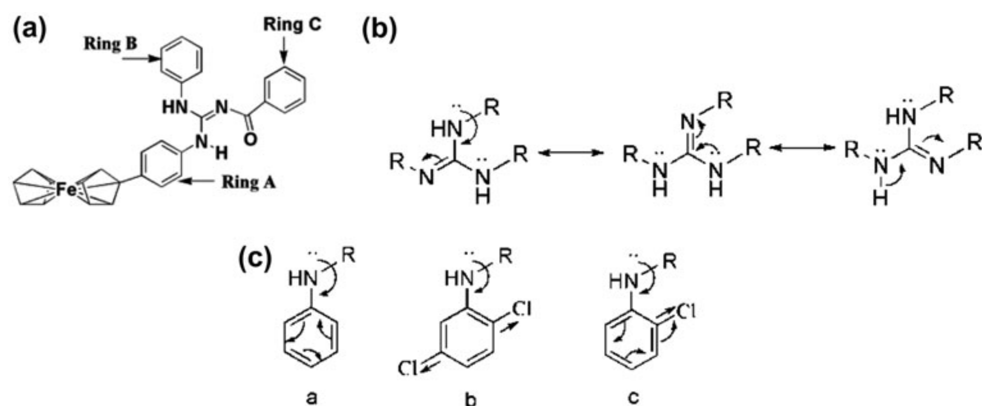


Figure 5. Cyclic voltammogram of **1**.



Scheme 2. (a) Ferrocenyl phenylguanidine skeleton. (b) Resonating structures of guanidine and (c) effect of electronegative substituent on basicity.

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In the presence of DNA, the anodic peak potential of the compound was slightly shifted cathodically (Supplementary material) along with decrease in I_{pa}. The substantial diminution in peak current is attributed to the formation of slowly diffusing compound-DNA supramolecular complex, diminishing free compound (mainly responsible for the transfer of current) is lowered. The mode of drug–DNA interaction can be judged from variation in formal potential. Positive peak potential shift indicates intercalation of the drug into DNA, negative peak potential shift is suggestive of electrostatic interaction and groove binding is witnessed by decrease in peak current accompanied by no shift in peak position. The negative shift in signal remaining at the same position demonstrates the interaction of ferrocenyl phenylguanidines with DNA by electrostatic followed by groove binding.

The cyclic voltammetric behavior of guanidine without ferrocene, N-N'-diphenyl-N''-benzoyl guanidine in the absence and presence of 30-90 μM DNA was also studied. The voltammogram without DNA featured two irreversible oxidation peaks and no reduction peak in the negative potential domain. By addition of DNA, a shift to less positive potentials and a decrease in peak current indicates the same mode of interaction with DNA.

Based upon the decay in peak current of all compounds by addition of different concentrations of DNA (30–60 μM, B-E), the binding constant values were calculated according to the equation [31]:

$$\frac{1}{[\mathrm{DNA}]} = \frac{K(1-A)}{1-(I/I_0)}$$

where K is binding constant, I and I_0 are the peak currents with and without DNA and A is proportionality constant. The plot of 1/[DNA] versus $1/(1-I/I_0)$ yielded binding constant values.

3.2.2. By UV–Vis spectrophotometer. The interaction of ferrocenyl phenylguanidines with DNA was also probed by UV–Vis spectrophotometry. The representative spectrum of **1** is given in Supplementary material. All the compounds had three strong bands at 205–285 nm. In the visible region, a weak signal at 450 nm was noticed. The strong bands in the UV–region of the spectrum can be assigned to π–π^* transition of aromatic phenyl ring and C=N. It has been reported that UV–Vis spectra of ferrocene derivatives give two absorptions originating from ferrocene. The following three spin-allowed ligand field transitions are expected: $^1A_{1g} \rightarrow a^1E_{1g}$, $^1A_{1g} \rightarrow {}^1E_{2g}$, and $^1A_{1g} \rightarrow b^1E_{1g}$. The first two transitions are unresolved and give the band at 450 nm and the third transition is responsible for the signal at 325 nm. Both bands are weak owing to the Laporte-forbidden *d–d* character of ligand field transitions [32]. Thus, the weak bands in the compounds may be assigned to ferrocene based *d–d* transitions. The UV–visible spectrum of only guanidine having no ferrocene possesses three peaks and the peak corresponding to *d–d* transition of ferrocene was missing.

Peaks **1** and **4** were not affected by addition of DNA. Peak **2** showed a slight red shift and **3** exhibited a blue shift. Both peaks showed pronounced hyperchromic effect (figure 6) by the incremental addition of DNA. A small bathochromic effect is associated with a decrease in the energy gap between the highest unoccupied and lowest occupied molecular orbitals after interaction with DNA via groove binding mode. Minor groove binders should be crescent in shape; the L-shaped structure of our compounds indicates that they can bind in major grooves [33]. The hyperchromic effect and slight blue shift of **3** suggest electrostatic interaction with DNA [34]. Thus, the compounds interact with DNA by mixed binding mode. The guanidines without ferrocene showed the same behavior on addition of DNA. The binding constants for all compounds were calculated by the following equation [33]:

$$\frac{A_0}{A-A_0} = \frac{\varepsilon_G}{\varepsilon_{H-G}-\varepsilon_G} + \frac{\varepsilon_G}{\varepsilon_{H-G}-\varepsilon_G}\frac{1}{K[\mathrm{DNA}]}$$

where K is the binding constant, A_0 and A are absorbance of the free drug and the apparent drug-DNA adduct, while ε_G and ε_{H-G} are their corresponding absorption coefficients. The slope to intercept ratio of the plot between $A_0/(A-A_0)$ vs. 1/[DNA] gave the binding constant with values very close to those obtained from cyclic voltammetry, 1.1×10^5–$5.6\times10^5\,\mathrm{M}^{-1}$ (table 3). The binding constant values calculated for the ferrocenyl phenylguanidines were greater with the guanidines having no ferrocene. Hence, the presence of ferrocene enhances the DNA binding activity of guanidines.

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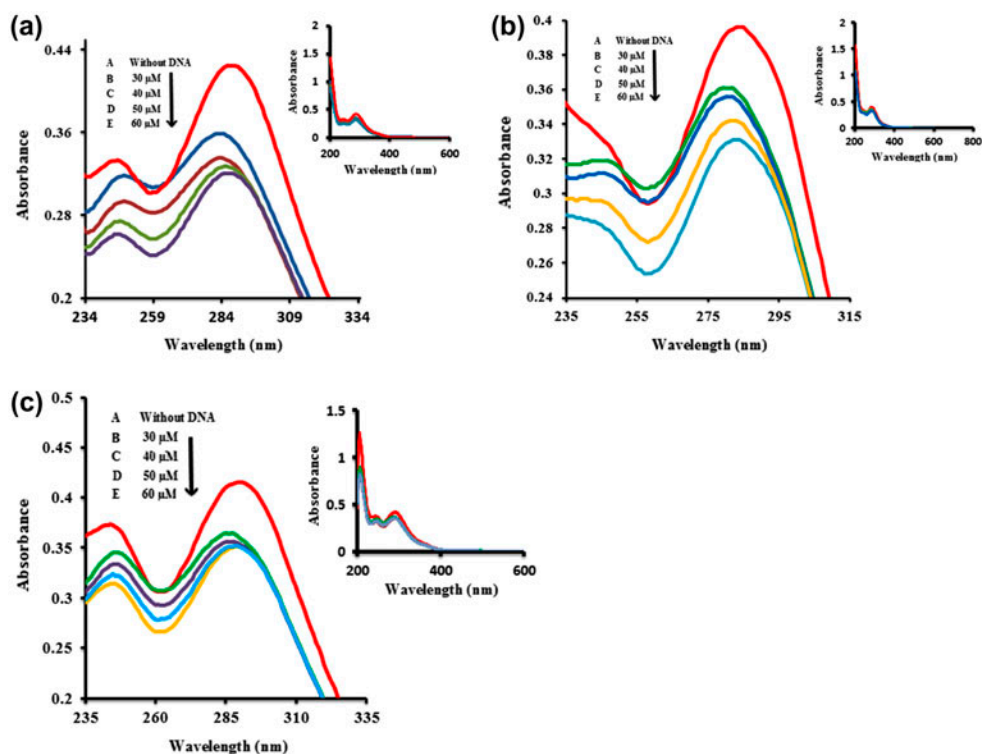

Figure 6. UV–Vis absorption spectra of 25 µM of **1** (a), **2** (b) and **3** (c) in the absence of DNA (A) and presence of 30–60 µM DNA (B-E) in 20% aqueous ethanol.

3.3. *Biological studies*

All six compounds were screened for antifungal and antibacterial activities (tables 4 and 5), showing moderate to significant antibacterial and antifungal activities, better than phenylguanidines having no ferrocene [24]. Compounds **2**, **2a**, **3,** and **3a** having chlorophenyl showed slightly better antibacterial and significantly better antifungal activity than **1** and **1a**, especially against *Aspergillusflavus* and *Fusariummoniliforme* fungi. Antifungal activity of the latter class was more than the standard drug, attributed to the presence of chloro on the phenyl ring (ring-B, scheme 2(a)). Due to its electronegativity, a decrease in the basicity

Table 3. Binding constant values.

	Compound name	K (M^{-1})
1	N-(4-ferrocenylphenyl-N'-phenyl-N″-benzoylguanidine) **1**	1.22×10^5
2	N-(3-ferrocenylphenyl-N'-phenyl-N″-benzoylguanidine) **1a**	1.25×10^5
3	N-(4-ferrocenylphenyl-N'-(2-chlorophenyl)-N″-benzoylguanidine) **2**	2.51×10^5
4	N-(3-ferrocenylphenyl-N'-(2-chlorophenyl)-N″-benzoylguanidine) **2a**	2.11×10^5
5	N-(4-ferrocenylphenyl-N'-(2,5-dichlorophenyl-N″-benzoylguanidine) **3**	5.60×10^5
6	N-(3-ferrocenylphenyl-N'-(2,5-dichlorophenyl)-N″-benzoylguanidine) **3a**	5.00×10^5
A	N,N'-diphenyl-N″-benzoylguanidine	1.6×10^3
B	N-phenyl-N'-(2-chlorophenyl)-N″-benzoylguanidine	6.3×10^3

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Table 4. *In vitro* antibacterial assay of ferrocenyl phenylguanidines.

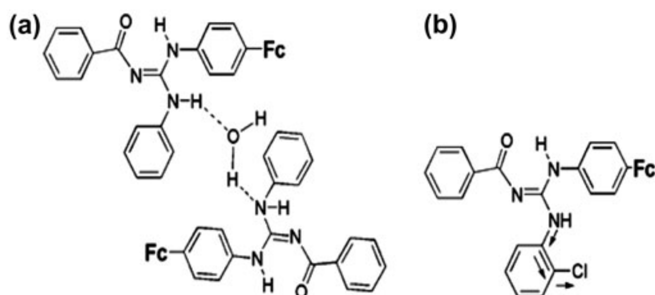
	Staphylococcus aureus	*Pseudomonas aeruginosa*	*Bacilus subtilis*	*Klebsiella pneumonie*	*Escherchia coli*
1	13.3 ± 0.3*	10.3 ± 0.3	13.3 ± 0.3	12.0 ± 0.6	10.7 ± 0.3
1a	13.3 ± 0.3	12.0 ± 0.6	12.7 ± 0.3	11.3 ± 0.3	10.0 ± 0.0
2	15.0 ± 0.6	14.0 ± 0.6	14.3 ± 0.3	11.6 ± 0.3	11.7 ± 0.3
2a	15.7 ± 0.3	14.3 ± 0.3	15.3 ± 0.7	12.3 ± 0.3	11.3 ± 0.3
3	18.3 ± 0.7	15.7 ± 0.3	15.7 ± 0.3	13.0 ± 0.0	11.7 ± 0.9
3a	16.7 ± 0.3	14.3 ± 0.3	14.3 ± 0.3	12.7 ± 0.3	11.0 ± 0.6
A	–	7.0 ± 0.0	7.70 ± 0.6	9.91 ± 0. 6	–
B	10.7 ± 0.6	7.70 ± 0.6	8.7 ± 0.6	10.0 ± 0.0	–
PC	26.7 ± 0.3	26.3 ± 0.3	27.3 ± 0.3	23.0 ± 0.6	20.3 ± 0.7

PC = Positive control, zone of inhibition (mm)*, (–) no zone of inhibition observed, penicillin (1 mg/mL) was used as standard drugs, while DMSO was used as negative control. **A** = N,N'-diphenyl-N′″-benzoylguanidine, **B** = N-phenyl-N'-(2-chlorophenyl)-N″-benzoylguanidine.

Table 5. *In vitro* antifungal assay of ferrocenyl phenylguanidines.

	Fusarium moniliforme		*Aspergillus fumigates*		*Aspergillus flavus*	
1	7.43 ± 0.03*	28#	7.87 ± 0.09	26	6.5 ± 0.1	33
1a	7.43 ± 0.03	28	6.8 ± 0.06	35	5.33 ± 0.07	46
2	2.37 ± 0.33	77	6.93 ± 0.09	36	0.53 ± 0.03	95
2a	2.47 ± 0.03	77	2.47 ± 0.03	77	0.37 ± 0.03	96
3	4.57 ± 0.07	56	9.33 ± 0.03	13	0.7 ± 0.1	93
3a	3.63 ± 0.07	65	8.4 ± 0.1	21	0.37 ± 0.07	96
A	–		7.43 ± 0.03	28	–	
B	5.57 ± 0.03	49	5.33 ± 0.03	46	6.5 ± 0.03	33
PC	0.77 ± 0.07	92	0.89 ± 0.03	92	0.83 ± 0.01	92
NC	10.3 ± 0.3	0	10.7 ± 0.03	0	10.3 ± 0.3	0

PC = Positive control (Terbinafin), NC = Negative control, ± shows the standard errors of mean values, (–) no zone of inhibition observed, fungal growth (cm)*, growth inhibition (%)#.



Scheme 3. (a) Hydrophobicity of guanidine. (b) Decrease in hydrophobicity and increase in lipophilicity by chloro group.

of NH (scheme 2(c)) and increase in lipophilicity may occur. The decrease in basicity is due to less availability of the lone pair of electron to H-bond with water in the living cell (scheme 3(a) and (b)).

4. Conclusion

This paper describes the synthesis of six new ferrocenyl phenylguanidines and their structural characterization. They are good DNA binders with binding constant varying in the sequence **3** ∼ **3a** > **2** ∼ **2a** > **1** ∼ **1a**. Antimicrobial study, proved them to be good antifungal agents and they may emerge as environmental friendly fungicides after clinical trials. Compound **2** has a supramolecular structure with channels or cavities when viewed along the *b*-axis. These compounds may find applications in selective trapping of reactive species and for conducting chemical reactions by using the cavities as reaction vessels.

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Supplementary material

Single crystal X-ray diffraction data for the structural analysis have been deposited with the Cambridge Crystallographic Data Center, CCDC No. 901,848 (**2**). The copy of this information may be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge, CBZ 1EZ, UK (Fax:+44 1223 336,033; E-mail: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk). DNA binding study for N,N'-diphenyl-N"-benzoylguanidine by UV–Vis spectrophotometer and cyclic voltammeter (UV–Vis spectrum and voltammogram).

Acknowledgments

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