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ARTICLE TYPE

Facile Synthesis and Characterization of Bio-Organometallic Compounds and their Biological Activity Contour against Human Pathogens†

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Abstract: We have reported the synthesis of novel di & trisubstituted pyrroles and its biological activity. Most of the compounds show antibacterial activity towards gram positive and negative bacteria. The agar-diffusion method was used for prelude screening studies, which showed a hopeful broad range of antibacterial activity. The minimum inhibitory concentration (MIC) for bacteria was determined by the micro dilution method of selected active compounds. For the same compounds cytotoxicity assay also performed. The photophysical properties were studied for the selected compounds and its result was suitably supported by DFT computation.

1. Introduction

Metallocene derivatives of organic compounds show enhanced biological application in pharmaceutical industries.[1] In particular, the usage of ferrocene is a vibrant research area in medicinal applications. Ferrocene alone is not a toxic compound and gets degraded or hydroxylated enzymatically by cytochrome in liver.[2] Ferrocene derivatives have dynamic in *vitro* and *vivo* against infections caused by bacteria[3], fungus[4] and resistance against malaria [5-8], tumour[9-14a] and human immunodeficiency virus (HIV).[15] A Ferrocifen (Figure 1) derivative has been reported as effectively active against the estrogen-dependent tumor and breast cancer cells, ER(+)[14b,c] and good activity against *P.falciparum*, which causes malaria. The redox potential of ferrocene could be the key factor which affords anti-malarial activity[16] and it was exposed similar activity when compared with chloroquine, which is current antimalarial drug.[17] Ferrocene was the first organometallic compound to reported to have anti-proliferative properties.[18] The unique magnetic & electronic properties, stability in aqueous and aerobic medium were complimentary for the ferrocene molecule. These unique structural features encouraged us to focus on the synthesis of pyrrole heterocycles with ferrocene moiety.

Pyrroles are one of the most important and simple heterocyclic compounds. It's structural element present in numerous natural products, synthetic medicinal agents, chlorophyll, haemoglobin, vitamin B12, etc.[19] Functionalized pyrroles have been found for widespread applications in pharmaceuticals (e.g., Lipitor), synthetic building blocks, flavor additives, agrochemicals, anti-port agents, organoleptic, and materials with high-tech devices like molecular sensors.[20] Highly substituted pyrroles have the potential to inhibit the growth of tumour cells.[21] The construction of pyrrole ring has been done by a number of cycloaddition methods. Paal-Knorr condensation of 1,4-dicarbonyls with different amines was the pioneer strategy for the synthesis of pyrroles.[22] *N*-acyl-3,4-disubstituted pyrrole was reported by Piloty-Robinson.[23] Recently, we have reported the efficient method for the synthesis of substituted pyrroles[24] by involving *α*-aroylketene dithioacetal and *p*-toluenesulfonylmethyleneisocyanide (TosMIC) in excellent yields. Uniqueness of this work is ferrocene was attached through a carbonyl to the fourth carbon (C-4) and aryl groups are at the C-3 position of pyrrole. The novel di & tri-substituted pyrrole molecules with ferrocene and aryl ring could be prospectively the reason for enhanced biological activity.[25]

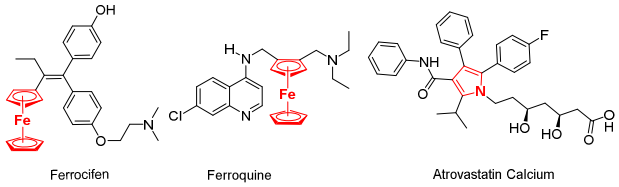


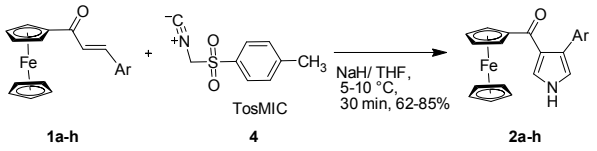
Figure 1. Active drugs containing ferrocene and pyrrole moiety.

Result and Discussion

(i) *Chemistry and its significance*

The reaction between *α,β*-unsaturated carbonyl compound and TosMIC is well known class of 1,3-dipolar cycloaddition reactions, for the formation of novel heterocyclic compounds.[26] TosMIC **4** is a one-carbon synthon which is having the unique properties of isocyanide and tosyl group. The tosyl is a very good leaving group and enhances acidity to *α*-protons and isocyanide

which favour the carbon atom to act as a dynamic force for multiple reactions. Taking advantage of structural features of **1a-h** and TosMIC **4**, we synthesized our target molecule 3-aryls-4-ferocenoylpyrroles **2a-h** in good yield (Scheme 1, Table 1).

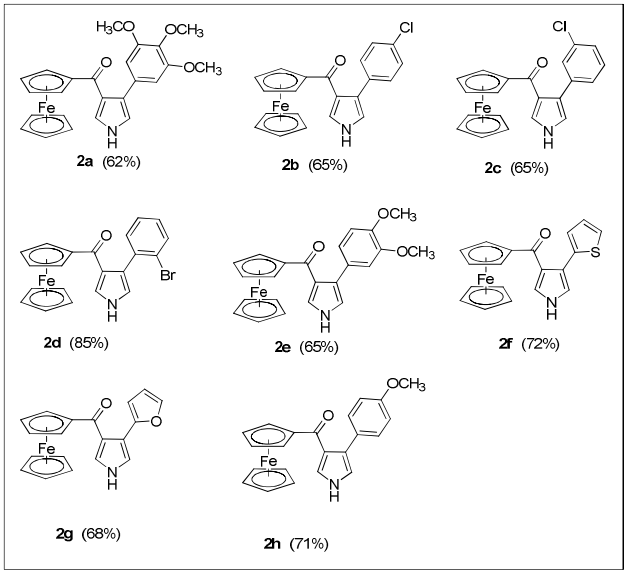


1a-h **4** **2a-h**

Scheme 1. Synthesis of 3,4-disubstituted pyrrole **2a-h**

The 1,3-dipolar cycloaddition involves two new C-C bonds formation followed by elimination of tosyl group to afford **2a-h**. NaH used as a base to generate the nucleophile by removing the acidic proton from active methylene carbon of **4**. This carbon nucleophile readily attack the electron deficient *β*-carbon of **1** and reverse flow of unpaired electron on oxygen atom leads to the further nucleophilic attack on the isocyanides of **4** and followed by the elimination of tosyl group (Scheme 3). All ferrocenoylpyrroles **2a-h** were characterized by NMR and mass spectroscopy techniques. In addition, the antibacterial activities also were screened (Table 4).

Table 1: List of Synthesized compounds 3,4-disubstituted pyrroles **2a-h**



[a] isolated yield

Based on the biological screening report of **2** (Table 4), we were passionate to introduce a simple alkyl group, by substituting a methyl group, in-order to protect the nitrogen atom. The labile hydrogen in pyrrole has possibility to form hydrogen bonding, which will be arrested by protecting nitrogen atom. As expected, the difference in polarity and electron density of compound **2** and **3** may cause substantial changes in the microbial activity between them. Thus we could compare the suitability of molecule as lyophilic or lyophobic and we could choose the substrate depending upon the appropriateness of a target.

The reaction condition was optimized to the *N*-methylation of **2** using various bases and solvents (Table 2). Initially, methylation of **2d** was tried using methyl iodide in the presence of K_2CO_3/acetone at 25 °C for 12 h to afford **3d** in 65% yield (Table 2, entry 1). Continuing the alkylation process using same solvent and base at 80 °C, the *N*-methylation was obtained in 70% yield (Table 2, entry 2). The yield of **3d** was reduced to 68%, when the reaction was executed in DMF solvent at 25 °C (Table 2, entry 3). Interestingly, the yield of **3d** was improved to 83%, when the reaction temperature was raised up to 80 °C (Table 2, entry 4). The product **3d** was obtained in 80% yield while using sodium hydroxide as a base, (Table 2, entry 5). The *N*-methylation of **2d** was not found, when the reactions were performed using organic base like DMAP in THF at RT – 90 °C for 12 h (Table 2, entries 6-7).

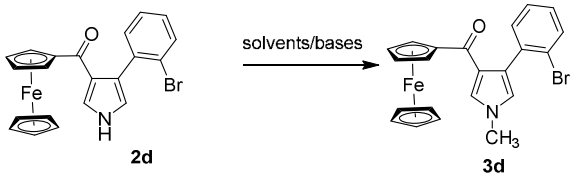
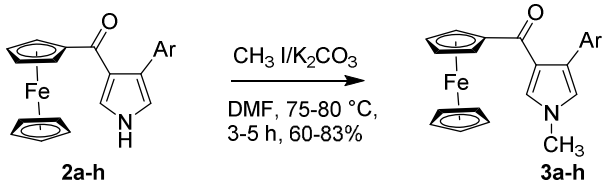


Table 2: Optimization chart for synthesizing the compound **3d**

Entry.	Solvent	Base	Temp. (°C)	Time (h)	Yield (%)
1	Acetone	K_2CO_3	25°C	12	65
2	Acetonitrile	K_2CO_3	80°C	4	70
3	DMF	K_2CO_3	25°C	12	68
4	DMF	K_2CO_3	80°C	3	83
5	DMF	NaOH	80°C	3	80
6	THF	DMAP	25°C	12	--[a]
7	THF	DMAP	90°C	12	--[a]

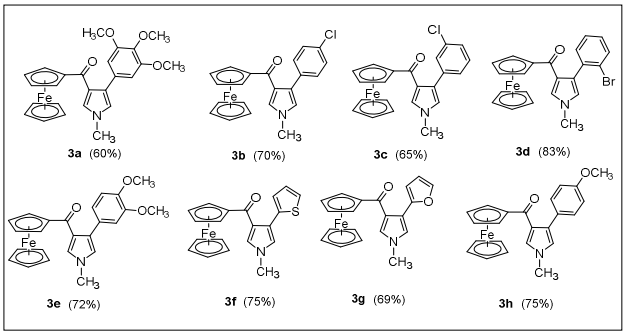
[a]Methylation did not take place and unreacted **2d** was recovered

The *N*-methylation was preceded smoothly in polar aprotic solvent rather than other solvents. Finally, the *N*-methylation of **2a** to afford **3a** (85% yield) was optimized in the presence of K_2CO_3/DMF at 80 °C for 3 h.



Scheme 2. Synthesis of 1,3,4-trisubstituted pyrroles **3a-h**

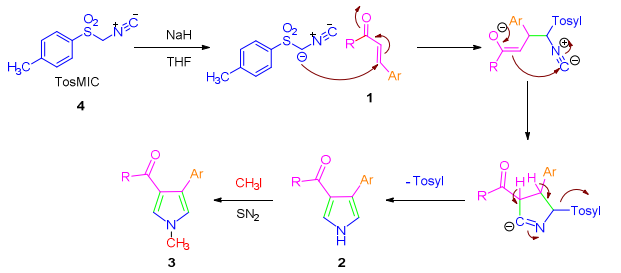
Table 3: List of synthesized compound 1,3,4-trisubstituted pyrroles **3a-h**



[a]Isolated yield

Following the optimized reaction conditions, the *N*-

methylation was extended to various pyrroles **2a-h** to afford **3a-h** in good yields (Scheme 2, Table 3). Electron donating group substituted phenyl ring of **2** gave a moderate yield (Table 3, entries 1 & 5). Halogen substituted aryl of *N*-methyl pyrroles gave better yields than methoxy substituted compound (Table 3, entries 2-4). The selective *N*-methylation was obtained; even thiophene was substituted instead of phenyl ring of **2** at C-3 (Table 3, entry 6). All the *N*-methylated pyrroles **3a-h** was well characterized by ^{1}H & ^{13}C NMR and Mass spectroscopy techniques.



Scheme 3. Plausible mechanism for the cycloaddition reaction and *N*-methylation

Biological Data

(i). Assesment of Cytotoxicity

For the cytotoxicity assay[27], human gastric cancer cell line AGS was seeded in 96 well cell culture plates (5×10^3 cells per well) in the culture medium DMEM (HiMedia) with 10% Fetal Bovine Serum (Sigma). After 24 hours, the chemical compounds were treated at various concentrations with the culture medium and incubated for 48 hours. After incubation, 1µM resazurin (Sigma) was treated for 4 hours and then the developed resorufin was measured in fluorimetry with 550nm excitation and emission at 590 nm. The background noise was adjusted by using blank (without cells) in the experiment. The percentage of viable cells was calculated with reference to the untreated control and plotted in the graph. This experiment was done twice with triplicates and similar results were observed in both the times.

All the compounds **2a-d** and **3a-d** were taken in ethanol medium and then further diluted to various concentrations. The fluorimetric analysis provided an idea about the cytotoxicity of these compounds. All the compounds **2a-d** and **3a-d** did not showed any inhibition of cellular proliferation, except the compound **2c,** which showed the dosage dependent cytotoxicity on AGS cells. The compound **2b** seems also an exception, but not as **2c**. It was well known that the ferrocene was non-toxic and highly stable. The chloro substitution at *meta* position of aryl ring may accountable for this inhibition activity. The dead cells were not seen in most of the cases, which substantiate the compounds were not toxic to the cells. The cytotoxicity of AGS cells was clearly represented in the graph (Figure 2).

(ii). Disc diffusion method

The synthesized compounds were accessed for their antimicrobial activity against selected gram positive and negative bacteria *viz*., *Staphylococcus aureus, Bacillus subtilis* and *Klebsiella aerogenes, Serratia sp, Escherichia coli* respectively using disc diffusion assay.[28] These bacteria individually responsible for the various infections and disorders like toxic shock syndrome[29], haemolytic uremic syndrome[30], infections of the bloodstream, urinary tract infection, septicaemia, meningitis, diarrhoea[31], pneumonia, lower respiratory tract infection and rarely cause anthrax like respiratory illness[32] and food poisoning.

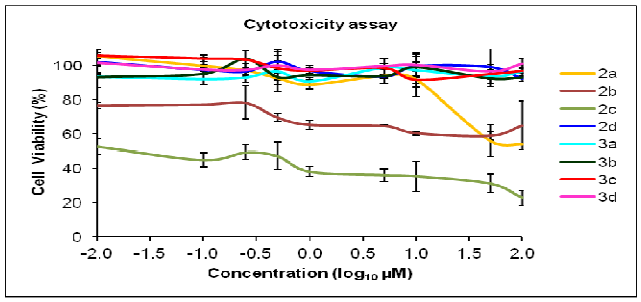


Figure 2. Minimum Inhibitory concentration of selected compounds

Briefly, a Muller Hinton agar plate was prepared and the overnight grown cultures were swapped on the plates to make lawn of the bacterial cultures. Stock solutions of the synthesized compounds were prepared in DMSO. The sterile paper discs (Himedia lab) were impregnated with filter sterilized compounds approximately 20µl/disc. The paper discs were placed on the agar plates and incubated at 30 °C for 24 hours. After 24 hours of incubation, the zone of clearance around the discs was observed and measured (Table 4).

Table 4: Screening activity profile of pyrroles **2a-h** & **3a-h**

Entry	Sample Code	#*Gram –ve*			#*Gram +ve*	
		Serratia sp	Escherichia coli	Klebsiella aerogenes	Staphylococcus aureus	Bacillus subtilis
1	**2a**	6±0.25	-	-	-	-
2	**2b**	8±0.10	6±0.30	-	5±0.25	-
3	**2c**	7±0.20	6±0.25	7±0.20	5±0.10	6±0.10
4	**2d**	-	8±0.10	-	-	-
5	**2e**	-	-	-	-	-
6	**2f**	-	-	-	-	-
7	**2g**	-	-	-	-	-
8	**2h**	-	-	-	-	-
9	**3a**	7±0.15	-	5±0.50	5±0.30	-
10	**3b**	-	4±0.25	-	-	-
11	**3c**	-	-	-	4±0.15	-
12	**3d**	5±0.10	5±0.10	5±0.15	7±0.10	6±0.15
13	**3e**	-	-	-	-	-
14	**3f**	-	-	-	-	-
15	**3g**	-	-	-	-	-
16	**3h**	-	-	-	-	-

#values represent the activity of compounds against the bacteria, the average diameter of the active zone in mm. - No inhibition

(iii). Minimum Inhibitory Concentration (MIC)

For the determination of MIC[33], selected cultures and tenfold serially diluted compound (up to 5 concentrations) were dispensed in the wells containing nutrient broth to the final volume of 200µl/well. The plates were sealed tightly with sterile adhesive and incubated for 2 days in an orbital shaker at 90rpm. The growth of the cultures was measured using Optical Density (OD) at 600nm absorption for every 24 hours of incubation and also by the visual appearance of turbidity.

Table 5: MIC values (µg/mL)[#] against selective human pathogens

Entry	Sample Code	*Serratia sp*	*Escherichia coli*	*Staphylococcus aureus*	*Klebsiella aerogenes*	*Bacillus subtilis*
1	**2a**	45	-	-	-	-
2	**2b**	40	5.0	5.0	-	-
3	**2c**	4.0	4.0	40	40	40
4	**2d**	-	4.0	-	-	-
5	**3a**	46	-	5.0	5.0	-
6	**3b**	-	5.0	-	-	-
7	**3c**	-	-	40	-	-
8	**3d**	45	4.5	4.5	45	45

[#] Values show considerable activity, µg/mL is required to confine the bacterial growth; - No Inhibition.

The minimum quantity of compound required for restricting the bacterial growth up to the maximum of 99% was specified (Table 5 and Figure 3). Interestingly, the activity of the compound varies depending on the group attached to the pyrrole molecule in its 1,3,4 positions. The halogens substituted aryls of **2** exhibited good biological activities. Among the halogens, chloro (Table 4, entry 3) substituted showed a wide range of activity. Whereas, the bromo and trimethoxy substituted compounds were active in gram negative bacteria only. In contradictory, the chloro substituted *N*-methylated compound of **3** exhibited inadequate activities towards gram positive bacteria (Table 4, entry 11), but the bromo and trimethoxy substituted compounds were active towards most of the selected bacteria. Thus, the presence of electron donating methyl group on the *N*-atom modifies the activity of the compounds towards these pathogens.

The consequence of report ensures that presence of ferrocene in the pyrrole moiety and along with chloro, bromo and trimethoxy substituent induces the antimicrobial activity. Based on the antimicrobial activity, we intended to introduce a five membered heterocycles such as 2-furan and 2-thiophene (Table 4, entries 6-7 & 14-15) instead of aryl group and examined its activity towards the human pathogens. The consequence showed that it does not have any impact on this system

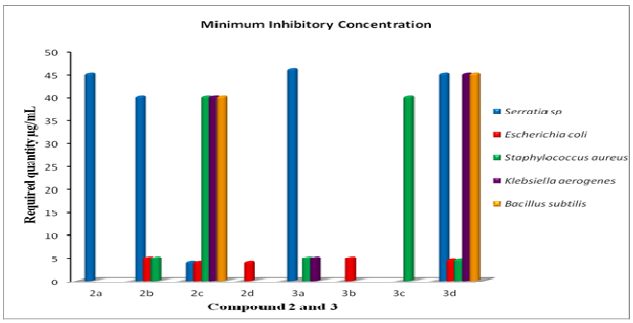


Figure 3. Minimum Inhibitory concentration of selected compounds

Photophysical and Computational Studies

It is well known that the molecules capable to emit light naturally or by external sources should be suitable for various applications like bio-imaging. We paid attention to find out the photo-physical/luminescence property for the above synthesized molecules. Biologically active di & tri substituted pyrroles **2a-d** and **3a-d** were further analyzed, its absorption and emission spectra were recorded separately in dilute EtOH solution ($2x10^{-5}$M) at room temperature (Figures 4, 5, 6 & 7). The absorption spectrum of **2a-d** displayed peaks at 216 nm and 273 nm, whereas **3a-d** shows at 204nm and 262nm. After *N*-methylation, the change in the absorption spectrum of **3a-d** in terms of peak intensity as well as wavelength (λ_{max}) towards lower energy region was observed. Similarly, the emission spectrum of **2a-d** & **3a-d** also were recorded using dilute EtOH solution ($2x10^{-5}$M) at room temperature (Figures 4, 5).

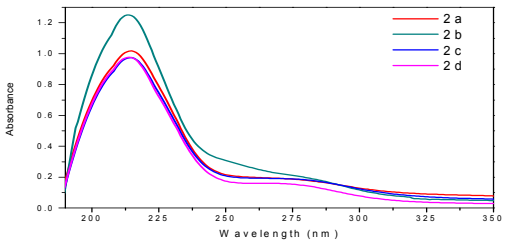


Figure 4. Absorbance spectrum of compound **2a-d** in EtOH ($2x10^{-5}$M solution)

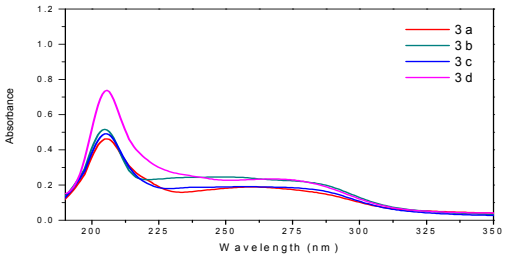


Figure 5. Absorbance spectrum of compound **3a-d** in EtOH ($2x10^{-5}$M solution)

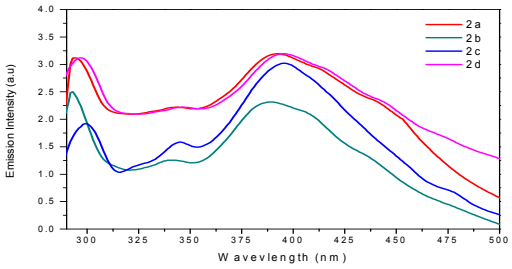


Figure 6. Emission spectrum ($2x10^{-5}$M solution) of NH pyrrole **2a-d**

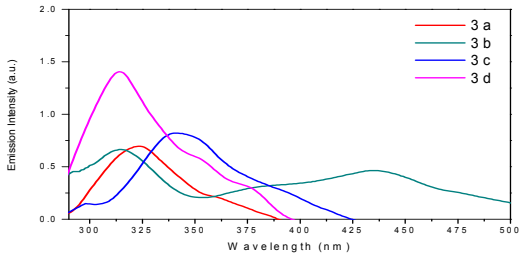


Figure 7. Emission spectrum ($2x10^{-5}$M solution) of *N*-methylated pyrrole **3a-d**

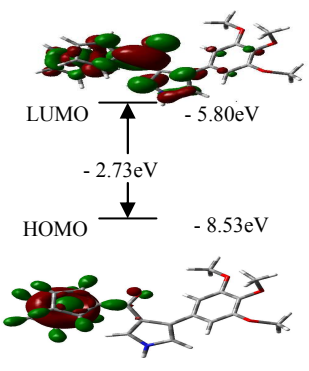
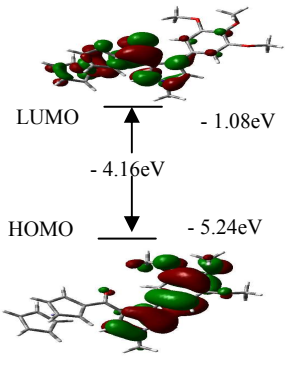
Interestingly, the emission spectrum of **2a-d** showed peaks

around 300 and 400nm (Figure 6), whereas, the fluoresence was quenched and could not find the peak around 400 nm for *N*-methylated pyrroles **3a-d** (Figure 7). The methyl group substitution at *N*-atom cause the improved electron density around the pyrrole ring than the ferrocene and also, which might be responsible for the prominent blue shift.[34]

Additionally, the DFT calculation and energy level diagram of frontier orbital gain further and provided valuable information for the above results. The photo excitation of molecule leads to intramolecular charge transfer (ICT) at the excited state. ICT normally reduces the quantum yield due to more pronounced relaxation and non-radioactive decay in the excited state.[35] The ICT process in ferrocene was responsible for the decrease of energy & electron density in the exited state.[36]

The HOMO of **2a** was exclusively located only on ferrocene ring, while the LUMO of this molecule was observed in the carbonyl group attached with ferrocene and also on the pyrrole ring (Table 6). The HOMO of compound **3a** found on aryl ring and pyrrole ring, whereas the LUMO of this compound was observed in ferrocenoyl and pyrrole ring. The HOMO-LUMO transition of **3a** shows intramolecular charge transition (ICT) from aryl ring to ferrocenoyl group. Due to this ICT, the wavelength (λ_{max}) moved 10nm towards the blue region in the absorption spectra of **3a** (Figure 5). While examining the HOMO of **2a** & **3a**, the electron donating methyl group substituted at *N*-atom of pyrrole shifted from left to right side of the molecule i.e., from ferrocene to aryl group was observed. The HOMO-LUMO energy gap was calculated as 2.73eV and 4.16eV corresponds to **2a** and **3a** respectively. The increase of energy gap was clearly evident to the decrease of intensity in the emission spectrum of **3a-d**.

Table 6: Frontier orbital diagrams of **2a** and **3a** calculated at the **B3LYP/6–31G** (d) level

N-H Pyrrole **2a**	N-Methyl Pyrrole **3a**
	
2a	**3a**

Conclusion

A series of di & trisubstituted pyrroles were synthesized in a convenient method from easily available raw materials with moderate to good yield. Most of them exhibit a significant amount of antibacterial activity. The presence of ferrocene and different aryl substituents on the pyrrole ring plays a vital role in enhancing antibacterial activity. Interestingly, the *N*-alkylation of pyrrole molecule modifies the antibacterial activity towards the selective pathogens. The Chloro substituted compound **2b** & **2c** and Bromo substitution of compound **3d** shows better antibacterial activity than other compounds. Fascinatingly, many of these compounds were not having any cytotoxic effect at the concentration in which they showed antimicrobial activity. Thus, the compounds have potential to extend further application and constructive towards the clinical studies. The study of photophysical property revealed that *N*-methylation of pyrrole ring shifts the absorption towards the blue region and consequently the emission intensity was diminished. The molecule should possess luminescence character to make them pertinent in bio-imaging. Based on our results, further we plan to improve according to that requirement. The DFT studies also accomplished to sustain the above result. All the synthesized compounds and their structures were characterized and confirmed by ^{1}H & ^{13}C NMR and Mass spectral techniques.

Experimental Data

General methods

Solvents and Chemicals were obtained from commercial sources and used without further purification. The ^{1}H and ^{13}CNMR spectra of the new compounds were measured at 400 & 300MHz and 75MHz respectively, using Bruker NMR instrument in DMSO-d_6, $CDCl_3$ and the chemical shifts are reported as δ values (ppm) relative to tetramethylsilane. Mass analysis was done in Agilent LC-Ms instruments and spectra were recorded. Elemental analysis recorded on Thermofinnigan flash 2000 organic elemental CHNS analyzer. The melting points reported on the work are uncorrected. Petroleum ether employed in column chromatography purification refers to the fraction which boils at 60-80 °C.

General procedure for the preparation of 3,4-disubstituted pyrrole (2a-h).

To a stirred and cleaned suspension of NaH (60% suspension in oil) 0.11g, 0.0044mol) in dry THF (5 ml) at 0 °C was added a mixture of TosMIC **4** (0.68g, 0.0035mol) and a chalcone **1a** (1g, 0.0029mol) in dry THF for 30 min. The reaction mixture was then allowed to stir at room temperature for 24 hours. After completion of the reaction (confirmed by the absence of chalcone in TLC), the mixture was carefully acidified using acetic acid (up to pH 6.5) and diluted with ethyl acetate (15 ml). The organic layer was washed with water (3x15 ml), brine solution (15 ml) and dried over anhydrous Na_2SO_4. Evaporation of the solvent under reduced pressure furnished ferrocenyl(4-(3,4,5-trimethoxyphenyl)-1*H*-pyrrol-3-yl)methanone **2a**. The crude product was subjected to column chromatography on silica gel (60-120) and ethyl acetate / pet ether (30:70) as eluent to obtain the expected product.

(i). Spectral Characterization for representative example

The compound **2h** was taken as a representative example and its NMR chemical shift values were discussed. The ^{1}H NMR exhibit sharp singlet at δ 3.79 ppm for OMe group and two doublets at δ 6.83 and 7.36 ppm with coupling constant J = 9 Hz was mutually coupled with two CH protons of the phenyl group. Three singlets were appearing at δ 4.17, 4.45 and 4.83 ppm for corresponds nine protons of ferrocene ring. Two singlets at δ 6.80

and 7.47 ppm for two CH protons and a broad singlet at δ 10.85 ppm was observed for the corresponding N*H*- proton of pyrrole ring. The mass spectrum value m/z 386 (M^+1) also was confirmed the formation of product **2h**.

Similarly, **3a** was taken as a representative example and analyzed its ^{1}H NMR spectrum. The ^{1}H NMR spectrum of **3a** exhibit three singlets at δ 3.78, 3.80 and 3.81 p.m. for three methoxy groups which were attached to the phenyl ring of **3a**. Two aryl protons were appearing as a singlet at δ 6.61ppm. Three sharp singlets at δ 4.18, 4.45 and 4.83 ppm for ferrocenyl protons has been observed. Two singlets at δ 6.74 and 7.07 ppm was observed for CH protons of pyrrole ring. Another sharp peak as singlet at δ 3.75 ppm was observed for *N*-methyl protons of pyrrole ring. No peak was observed for N*H*- proton around at δ 10-11 ppm, which confirms the *N*-methylation of pyrrole. The mass spectrum value m/z 460 (M^+1) also was confirmed the formation of the product **3a**.

Ferrocenyl(4-(3,4,5-trimethoxyphenyl)-1*H*-pyrrol-3-yl)methanone (2a)

Brown solid; m.p. 188 ^{o}C; Yield: 62%; ^{1}H NMR (400 MHz, DMSO-d_6): δ, 3.57 (s, 3H), 3.66 (s, 6H), 4.12 (s, 5H), 4.42 (s,2H), 4.71 (s, 2H), 6.64 (s, 2H), 6.99 (s, 1H), 7.69 (s, 1H) 11.46 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ, 55.5, 55.5, 60.0, 80.8, 112.7, 113.7, 119.5, 122.4, 123.8, 125.5, 126.9, 139.1, 148.0, 149.1, 194.4; LC-MS (m/z): 446 (M^{+1}); Anal. Calcd (%) for $C_{24}H_{23}FeNO_4$: C, 64.74; H, 5.21; N, 3.15. Found: C, 64.67; H, 5.18; N, 3.11.

Ferrocenyl(4-(4-chlorophenyl)-1*H*-pyrrol-3-yl)methanone (2b)

Brown colored solid; M.p. 216 ^{o}C; Yield: 65%; ^{1}H NMR (300 MHz, DMSO-d_6): δ, 4.18 (s, 5H), 4.47 (s, 2H), 4.81 (s, 2H), 6.89 (s, 1H), 7.24 (d, J = 8.1 Hz, 2H), 7.39 (d, J = 8.1 Hz, 2H), 7.52 (s, 1H), 11.26 (bs, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ, 68.1, 69.2, 69.7, 79.6, 117.3, 120.5, 122.2, 123.3, 126.1, 128.3, 129.2, 133.1, 191.1; LC-MS (m/z): 390 (M^{+1}); Anal. Calcd (%) for $C_{21}H_{16}ClFeNO$: C, 64.73; H, 4.14; N, 3.59. Found: C, 64.71; H, 4.08; N, 3.53.

Ferrocenyl(4-(3-chlorophenyl)-1*H*-pyrrol-3-yl)methanone (2c)

Dark brown solid; m.p. 198-200 ^{o}C; Yield: 65%; ^{1}H NMR (400 MHz, DMSO-d_6): δ, 4.14 (s, 5H), 4.46 (s, 2H), 4.75 (s, 2H), 7.05-7.50 (m, 6H), 11.45 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ, 68.1, 69.2, 69.7, 79.6, 117.3, 120.5, 122.2, 123.3, 126.1, 128.3, 129.2, 133.1, 137.0, 137.9, 191.1; LC-MS (m/z): 390 (M^{+1}); Anal. Calcd (%) for $C_{21}H_{16}ClFeNO$: C, 64.73; H, 4.14; N, 3.59. Found: C, 64.70; H, 4.11; N, 3.53.

Ferrocenyl(4-(2-bromophenyl)-1*H*-pyrrol-3-yl)methanone (2d)

Yellow colored solid; m.p. 192-194 ^{o}C; Yield: 85%; ^{1}H NMR (300 MHz, DMSO-d_6): δ, 4.19 (s, 5H), 4.48 (s, 2H), 4.78 (s, 2H), 6.84 (s, 1H), 7.16 (s, 1H), 7.29 (t, 2H), 7.55 (d, 1H), 7.66 (s, 1H), 11.46 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ, 70.3, 72.4, 72.7, 80.2, 111.2, 118.2, 119.5, 121.6, 122.4, 128.2, 129.7, 131.0, 134.4, 136.5, 182.0. LC-MS (m/z): 435 (M^{+1}); Anal. Calcd (%) for $C_{21}H_{16}BrFeNO$: C, 58.10; H, 3.72; N, 3.23. Found: C, 58.05; H, 3.69; N, 3.18.

Ferrocenyl(4-(3,4-dimethoxphenyl)-1*H*-pyrrol-3-yl)methanone (2e)

Pale brown solid; m.p. 174 ^{o}C; Yield: 65%; ^{1}H NMR (300 MHz, DMSO-d_6): δ, 3.76 (s, 6H), 4.09 (s, 5H), 4.35 (s, 2H), 4.75 (s, 2H), 6.75 (s, 2H), 6.95 (s, 2H), 7.38 (s, 1H), 10.49 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ, 55.5, 55.5, 69.5, 70.7, 71.0, 80.8, 110.7, 112.3, 117.7, 120.4, 122.4, 123.8, 124.9, 128.3, 147.0, 147.9, 193.6; LC-MS (m/z): 416 (M^{+1}); Anal. Calcd (%) for $C_{23}H_{21}FeNO_3$: C, 66.52; H, 5.10; N, 3.37. Found: C, 66.49; H, 5.04; N, 3.32.

Ferrocenyl(4-(thiophen-2-yl)-1*H*-pyrrol-3-yl)methanone (2f)

Reddish brown solid; m.p. 154-156 ^{o}C; Yield: 72%; ^{1}H NMR (400 MHz, DMSO-d_6): δ, 4.18 (s, 5H), 4.51 (s, 2H), 4.83 (s, 2H), 6.97 (d, J = 3.7 Hz, 1H), 7.09 (s, 1H), 7.24-7.28 (m, 1H), 7.52 (s, 1H), 7.86 (d, J = 8 Hz, 1H), 11.45 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ, 69.4, 70.4, 71.2, 80.8, 117.3, 118.7, 121.5, 123.1, 124.4, 126.6, 128.8, 129.9, 192.3; LC-MS (m/z): 362 (M^{+1}); Anal. Calcd (%) for $C_{19}H_{15}FeNOS$: C, 63.17; H, 4.19; N, 3.88; S, 8.87. Found: C, 63.12; H, 4.15; N, 3.85; S, 8.80.

Ferrocenyl(4-(furane-2-yl)-1*H*-pyrrol-3-yl)methanone (2g)

Dark brown solid; m.p. 176-178 ^{o}C; Yield: 65%; ^{1}H NMR (300 MHz, DMSO-d_6): δ, 4.10 (s, 5H), 4.41 (s, 2H), 4.80 (s, 2H), 6.31 (s, 1H), 6.81 (d, J = 3 Hz, 1H), 7.07 (s, 1H), 7.26 (s, 1H), 7.36 (s, 1H), 10.95 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ, 69.0, 70.1, 70.8, 80.2, 105.5, 110.3, 114.5, 117.2, 120.3, 123.9, 139.2, 149.3, 192.2; LC-MS (m/z): 345 (M^{+1}); Anal. Calcd (%) for $C_{19}H_{15}FeNO_2$: C, 66.11; H, 4.38; N, 4.06. Found: C, 66.07; H, 4.32; N, 4.01.

Ferrocenyl(4-(4-methoxphenyl)-1*H*-pyrrol-3-yl)methanone (2h)

Reddish brown solid; m.p. 164-166 ^{o}C; Yield: 65%; ^{1}H NMR (300 MHz, DMSO-d_6): δ, 3.79 (s, 3H), 4.17 (s, 5H), 4.45 (s, 2H), 4.83 (s, 2H), 6.80 (s, 1H), 6.83 (d, J = 9 Hz, 2H), 7.36 (d, J = 9 Hz, 2H), 7.47 (s, 1H), 10.85 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ, 54.0, 68.7, 69.8, 70.2, 80.2, 112.2, 116.9, 121.1, 123.3, 123.9, 127.1, 128.5, 192.2; LC-MS (m/z): 386 (M^{+1}); Anal. Calcd (%) for $C_{22}H_{19}FeNO_2$: C, 68.59; H, 4.97; N, 3.64. Found: C, 68.56; H, 4.91; N, 3.59.

General procedure for the preparation of 1,3,4-trisubstituted Pyrrole (3a-h).

The compound ferrocenyl(4-(3,4,5-trimethoxyphenyl)-1*H*-pyrrol-3-yl)methanone **2a** (100mg, 0.0003mmol) was dissolved in DMF and the temperature was maintained at 5-10°C. K_2CO_3 (0.0039mmol) was charged into the reaction mixture and stirred well for 5 min. Methyl iodide (0.33mmol) was added dropwise into the reaction mixture and stirred well. The reaction mixture was maintained at 75-80 °C for 3-5 h. TLC was checked, after completion of the reaction, the mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine and treated with sodium sulfate. The organic layer was evaporated to afford 60 % yield of required product (1-methyl-4-(3,4,5-trimethoxyphenyl)-1*H*-pyrrol-3-yl)(ferrocenyl)methanone **3a**. The obtained solid was recrystallized from ethanol.

(1-methyl-4-(3,4,5-trimethoxyphenyl)-1*H*-pyrrol-3-yl)(ferrocenyl)methanone (3a)

Orange solid; m.p. 164-166 °C; Yield: 60%; ^{1}H NMR (300 MHz, $CDCl_3$): δ, 3.72 (s, 3H), 3.78 (s, 6H), 3.81 (s, 3H), 4.18 (s, 5H), 4.45 (s, 2H), 4.83 (s, 2H), 6.61 (s, 2H), 6.74 (s, 1H), 7.07 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ, 35.0, 56.1, 59.2, 59.2, 68.7, 69.8, 70.2, 80.2, 112.3, 116.9, 121.1, 123.3, 123.9, 127.1, 128.5, 156.7, 192.2; LC-MS (m/z): 460 (M^{+1}); Anal. Calcd (%) for $C_{25}H_{25}FeNO_4$: C, 65.37; H, 5.49; N, 3.05. Found: C, 65.31; H, 5.45; N, 3.01.

(1-methyl-4-(4-chlorophenyl)-1*H*-pyrrol-3-yl)(ferrocenyl)methanone (3b)

Pale brown solid; m.p. 150-152 °C; Yield: 70%; ^{1}H NMR (300 MHz, $CDCl_3$): δ, 3.76 (s, 3H), 4.12 (s, 5H), 4.42 (s, 2H), 4.76 (s, 2H), 6.84 (s, 1H), 7.19 (d, J = 8.1 Hz, 2H), 7.33 (d, J = 8.1 Hz, 2H), 7.47 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ, 37.8, 68.3, 69.3, 69.9, 79.8, 117.4, 120.6, 122.4, 123.4, 126.2, 128.4, 129.3, 133.2, 191.3; LC-MS (m/z): 404 (M^{+1}); Anal. Calcd (%) for $C_{22}H_{18}ClFeNO$: C, 65.46; H, 4.49; N, 3.47. Found: C, 65.40; H, 4.44; N, 3.41.

(1-methyl-4-(3-chlorophenyl)-1*H*-pyrrol-3-yl)(ferrocenyl)methanone (3c)

Pale brown solid; m.p. 122-124 °C; Yield: 65%; ^{1}H NMR (300 MHz, $CDCl_3$): δ, 3.75 (s, 3H), 4.19 (s, 5H), 4.44 (s, 2H), 4.83 (s, 2H), 6.72 (s, 1H), 7.19-7.27 (m, 5H), 7.41 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ, 36.6, 69.9, 71.0, 71.4, 80.6, 122.3, 123.4, 126.1, 126.9, 127.3, 128.5, 129.1, 133.7, 137.1, 192.9; LC-MS (m/z): 404 (M^{+1}); Anal. Calcd (%) for $C_{22}H_{18}ClFeNO$: C, 65.46; H, 4.49; N, 3.47. Found: C, 65.43; H, 4.42; N, 3.43.

(1-methyl-4-(2-bromophenyl)-1*H*-pyrrol-3-yl)(ferrocenyl)methanone (3d)

Pale yellow solid; m.p. 165 °C; Yield: 83%; ^{1}H NMR (300 MHz, DMSO-d_6): δ, 3.40 (s, 3H), 4.22 (s, 5H), 4.51 (s, 2H), 4.81 (s, 2H), 6.88 (s, 1H), 7.20 (d, J = 6 Hz, 1H), 7.32 (t, J = 6 Hz, 2H), 7.59 (d, J = 9 Hz, 1H), 7.70 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ, 36.2, 68.3, 69.5, 70.0, 89.6, 113.3, 119.7, 123.7, 123.9, 125.8, 127.9, 132.1, 132.4, 134.5, 139.2, 193.2; LC-MS (m/z): 448 (M^{+1}); Anal. Calcd (%) for $C_{22}H_{18}BrFeNO$: C, 58.96; H, 4.05; N, 3.13. Found: C, 58.90; H, 3.99; N, 3.07.

(1-methyl-4-(3,4-dimethoxyphenyl)-1*H*-pyrrol-3-yl)(ferrocenyl)methanone (3e)

Pale brown solid; m.p. 120-122 °C; Yield: 72%; ^{1}H NMR (300 MHz, DMSO-d_6): δ, 3.60 (s,3H), 3.70 (s, 3H), 3.72 (s, 3H), 4.18 (s, 5H), 4.47 (s, 2H), 4.81 (s, 2H), 6.71 (d, J = 9 Hz, 1H), 6.86 (s, 1H), 6.91 (s, 1H), 7.04 (d, J = 9 Hz, 1H), 7.13 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ, 36.4, 55.8, 55.9, 69.3, 70.5, 70.8, 80.7, 110.0, 112.1, 117.5, 120.3, 122.2, 123.7, 124.7, 128.1, 146.8, 147.7, 193.4; LC-MS (m/z): 430 (M^{+1}); Anal. Calcd (%) for $C_{24}H_{23}FeNO_3$: C, 67.15; H, 5.40; N, 3.26. Found: C, 67.11; H, 5.34; N, 3.23.

(1-methyl-4-(thiophen-2-yl)-1*H*-pyrrol-3-yl)(ferrocenyl)methanone (3f)

Pale red solid; m.p. 117-119 °C; Yield: 75%; ^{1}H NMR (300 MHz, DMSO-d_6): δ, 3.49 (s, 3H), 4.07 (s, 5H), 4.45 (s, 2H), 4.76 (s, 2H), 6.57 (s, 1H), 6.89 (d, J = 15 Hz, 1H), 7.20 (s, 1H), 7.42 (s, 1H), 7.88 (d, J = 18 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ, 36.5, 70.3, 72.4, 72.5, 81.6, 115.1, 121.3, 122.2, 125.3, 128.9, 130.5, 131.5, 144.2, 191.0; LC-MS (m/z): 376 (M^{+1}); Anal. Calcd (%) for $C_{20}H_{17}FeNOS$: C, 64.01; H, 4.57; N, 3.73; S, 8.54. Found: C, 63.98; H, 4.53; N, 3.67; S, 8.50.

(1-methyl-4-(furane-2-yl)-1*H*-pyrrol-3-yl)(ferrocenyl)methanone (3g)

Dark Red solid; m.p. 140-142 °C; Yield: 69%; ^{1}H NMR (300 MHz, $CDCl_3$): δ, 3.72 (s, 3H), 4.20 (s, 5H), 4.48 (s, 2H), 4.90 (s, 2H), 6.41 (t, J = 3 Hz 1H), 6.94 (d, J = 3 Hz, 1H), 7.01 (d, J = 3 Hz, 1H), 7.22 (s, 1H), 7.34 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ, 29.67, 69.9, 70.9, 71.5, 81.2, 107.1, 111.2, 116.6, 121.4, 122.0, 127.3, 140.2, 149.5, 192.3; LC-MS (m/z): 360 (M^{+1}); Anal. Calcd (%) for $C_{20}H_{17}FeNO_2$: C, 66.92; H, 4.77; N, 3.90. Found: C, 66.92; H, 4.74; N, 3.85

(1-methyl-4-(4-methoxyphenyl)-1*H*-pyrrol-3-yl)(ferrocenyl)methanone (3h)

Red solid; m.p. 115-117 °C; Yield: 75%; ^{1}H NMR (300 MHz, $CDCl_3$): δ, 3.72 (s, 3H), 3.79 (s, 3H), 4.16 (s, 5H), 4.41 (s, 2H), 4.82 (s, 2H), 6.65 (s, 1H), 6.85 (d, J = 9 Hz, 2H), 7.26 (s, 1H), 7.35 (d, J = 9 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ, 36.5, 55.2, 69.9, 71.0, 71.3, 81.1, 113.4, 121.6, 123.1, 126.3, 127.0, 127.6, 129.7, 158.1, 193.2; LC-MS (m/z): 400 (M^{+1}); Anal. Calcd (%) for $C_{23}H_{21}FeNO_2$: C, 69.19; H, 5.30; N, 3.51. Found: C, 69.15; H, 5.27; N, 3.48.

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† Electronic Supplementary Information (ESI) available: All the ^{1}H & ^{13}C NMR and Mass spectrums of **2a-h** and **3a-h** are available. See DOI: 10.1039/b000000x/

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