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FULL PAPER

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Novel Pyrazoline-based Organometallic Compounds Containing Ferrocenyl and Quinoline units: Synthesis, Characterization and Microbial susceptibilities

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Some novel pyrazoline-based organometallic compounds were synthesized as new leads in antimicrobial chemotherapy. The structures of compounds were elucidated by different spectroscopic techniques and elemental analyses. All compounds were investigated for *in vitro* antimicrobial studies against fifteen ATTC bacterial and fungal strains. The microbial susceptibility of these compounds revealed that all the tested compounds gave good minimum inhibitory concentration (MIC) values against the tested organisms that are either similar or even better than the reference drugs amoxicillin and fluconazole, which gave MIC values 8-64 μg/ml against bacterial and 64 μg/ml against fungal strains, respectively. Among all compounds, compound (**4d**) 1-(5-(4-chlorophenyl)-3-ferrocenyl-4,5-dihydropyrazol-1-yl)-2-quinolin-8-yloxy) ethanone, emerged out the most promising antimicrobial organometallic derivative with MIC values against all the strains ranging from 8-32 μg/ml. Other compounds gave a range of MIC values between 16-64 μg/ml against *S. bovis*, 16-32 μg/ml against *E. coli*, and *C. tropicalis* except compound (**4d)** which gave MIC 8 μg/ml against *S. bovis* and *E. coli*, whereas 32 μg/ml against *C. tropicalis*. Collectively, these compounds gave a lower MIC value between 32-64 μg/ml against both of the biofilm forming strains namely, *P. aeruginosa* and *S. mutans*. The results of microbial susceptibility concluded that these novel organometallic compounds are new leads in antimicrobial chemotherapy and can be very useful for further optimization work on microbial chemotherapy.

KEYWORDS
antibacterial studies, antifungal studies, ferrocenyl and quinoline units, new leads in microbial chemotherapy, novel pyrazoline-based organometallics

1 | INTRODUCTION

Recently, a considerable amount of research on biologically active organometallic compounds has been reported[1–6] and has attracted growing interest of medicinal chemists. It has been reported that the presence of metals influence the properties of a drug, which in turn increase or make significant changes in biological activity with respect to the core drug molecules.[7–13] Extensive literature survey on ferrocene has revealed that high

stability, formal oxidation potential, lipophilicity, low toxicity and iron redox activity of ferrocene have testified it an interesting pharmacophore for drug design to develop more potent and effective curative agents,[14] for example ferroquine (antimalarial drug) which is in phase II clinical trials,[15] ferrocifen (anticancer drug),[16] ferrocenyl conjugates of antiestrogen tamoxifen[17] and antiandrogen nilutamide.[18] Recently, some novel ferrocenyl quinolines have also been reported as useful scaffolds for bioactive molecule development.[3,19]

Moreover, pyrazoline and its derivatives have long been targeted for synthetic investigations as they possess a plethora of biological activities such as anti-inflammatory,[20–26] antimicrobial,[27–31] anti-fungal,[32] anticancer,[33] antitumor,[34] antidepressant[35,36] anticonvulsant,[37] antiamoebic[38,39] and antibacterial.[40]

On the basis of above observations and as a part of our ongoing research focused on the synthesis of biologically active heterocycles/organometallics,[41–45] we have synthesized some novel pyrazoline-based organometallic compounds containing ferrocenyl and quinoline units and evaluated for *in vitro* antimicrobial studies against fifteen ATCC bacterial and fungal stains to investigate pharmaceutical applications of these compounds.

2 | RESULTS AND DISCUSSION

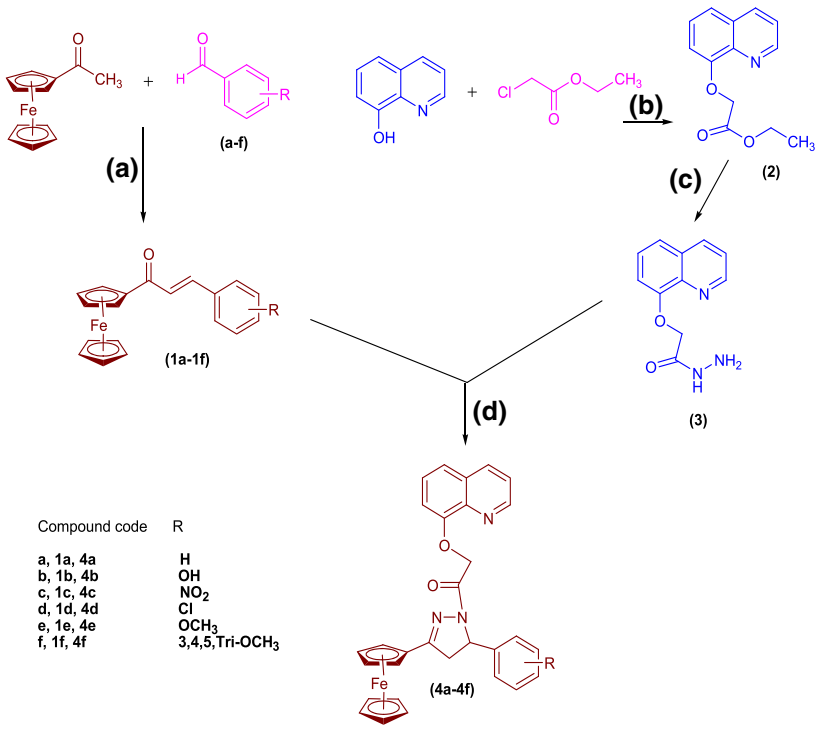
2.1 | Synthesis and characterization

The synthesis of novel pyrazoline-based organometallic compounds (**4a-4f**) was performed as illustrated in Scheme 1. The lead compounds (ferrocenyl chalcones **1a**-**1f**) were prepared by Claisen–Schmidt condensation of acetyl ferrocene with substituted aromatic aldehydes in the presence of potassium hydroxide and absolute ethanol, according to reported procedure.[46] Compound (**2**), ethyl (quinolin-8-yloxy) acetate was prepared by refluxing a mixture of 8- hydroxy quinoline, ethyl chloroacetate and anhydrous potassium carbonate in dry acetone, which was converted into 2-(quinolin-8-yloxy) acetohydrazide (**3**) by it with hydrazine hydrate in the presence of absolute ethanol.

The cyclization of ferrocenyl chalcones with 2-(quinolin-8-yloxy) acetohydrazide (**3**) in the presence of sodium hydroxide and absolute ethanol afforded novel pyrazoline-based organometallic compounds (**4a-4f**). The structures of all newly synthesized compounds (**4a-4f**) were established by different spectroscopic techniques such as Infra Red, Nuclear Magnetic Resonance (^{1}H-NMR and ^{13}C NMR spectra) and Mass spectrometry. The purity of compounds was confirmed by elemental analysis which was found in accordance with ± 0.3%.

The Infra-Red spectra of the target compounds **(4d)**, showed the characteristic band for (C=N) stretch at 1572 cm^{-1} which indicated the formation of ring. Other characteristic band was observed at 1257 cm^{-1} due to the (C-N) stretch vibrations which further confirmed the formation of the pyrazoline ring. The bands for (C-O-C) and (C=O) were appeared at 1121 cm^{-1} and 1632 cm^{-1}, respectively.

The structure of **(4d)** was further confirmed by its ^{1}H-NMR spectrum which provided diagnostic signals for the



SCHEME 1 General synthesis of pyrazoline-based organometallics (**4a-4f**) with ferrocenyl and quinoline units

positional elucidation of the protons. The assignments of all signals corresponding to individual H-atom and C-atom has been carried out on the basis of typical chemical shift (δ) values and coupling constants (J).

The C-4 protons (H_a and H_b) of pyrazoline ring are geminal protons and appeared

at δ 3.69 and δ 3.78 ppm, respectively, as doublet of doublets in compound (**4d**).

The C-5 proton (Hx) of the pyrazoline ring were was also appeared as doublet of doublets at δ 5.92 ppm due to vicinal coupling with two non equivalent geminal protons (H_a and H_b) at C-4 carbon, suggesting the formation of pyrazoline ring in organometallic compound **(4d)**. A singlet at δ 4.92 ppm was appeared and has been assigned to the -OCH_2 protons. In addition two singlets were appeared at δ 4.78 and δ 4.49 ppm, which were attributed to the four protons of monosubstituted Cp of ferrocenyl group of the compound **(4d)**. Another characteristic singlet at δ 4.19 ppm was assigned to the five protons of unsubstituted Cp of ferrocenyl group. All other aromatic and aliphatic protons were observed with expected chemical shift and integral values and are presented in the data given in the experimental section.

Further evidence regarding the formation of pyrazoline-based organometallic compound **(4d)** was obtained by its ^{13}C NMR spectrum. The fourth carbon (C-4) and fifth carbon (C-5) of the pyrazoline ring resonated at δ 44.2 and δ 58.9 ppm, respectively. All compounds also showed a signal at δ 150.9-154.5 ppm, attributed to third carbon (C-3) of pyrazoline ring. The carbons at ferrocenyl and aromatic rings resonated at their usual positions and their data are given in the experimental section.

The structure of novel organometallic compound **(4d)** was further confirmed by mass spectrometry, which showed a particular molecular ion [M^++1] peak at m/z 550.83 corresponding to its molecular weight.

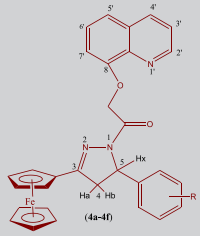
2.2 | *In vitro* antimicrobial assay

All novel organometallics **(4a-4f)** were evaluated for antimicrobial activity against fifteen ATCC bacterial (*Pseudomonas aeruginosa- ATCC® 15692™*, *Streptococcus bovis- ATCC® 33317™, Enterococcus faecalis- ATCC® 19433™, Klebsiella pneumoniae- ATCC®13883, Escherichia coli- ATCC® 11775™, Enterobacter cloacae ATCC® 13047™,* Methicillin-resistant *Staphylococcus aureus- ATCC® 33591* and *Streptococcus mutans- ATCC® 25175™)* and fungal (*Candida albicans- ATCC® 18804, Candida dubliniensis- ATCC® MYA-646, Candida glabrata- ATCC® 2001, Candida parapsilosis- ATCC® 22019, Candida tropicalis- ATCC® 750, Candida kefyr- ATCC® 8553* and *Candida krusei- ATCC® 14243*) strains by broth microdilution method.[47,48] For antibacterial studies, 4 Gram-positive bacteria (*S. bovis*, *E. faecalis*, Methicillin-resistant *S. aureus* and *S. mutans)* and 4 Gram-negative bacteria (*P. aeruginosa*, *K. pneumoniae*, *E. coli*, and *E. cloacae*) were used. Amoxicillin was used as a reference drug to compare antibacterial effect of compounds. It has been noted that amoxicillin gave a minimum inhibitory concentration (MIC) in the range of 8-64 μg/ml against the tested bacterial strains. The results of antibacterial activity revealed that all compounds gave good minimum inhibitory concentration (MIC) values against the tested organisms. Among all compounds, compound (**4d**) emerged as the most promising antimicrobial organometallic derivative with very low MIC values against all the bacterial strains ranging from 8-32 μg/ml. Other compounds gave a range of MIC value between 16-64 μg/ml against *S. bovis*, 16-32 μg/ml against *E. coli*, and *C. tropicalis* except (**4d)** which gave MIC 8 μg/ml against *S. bovis* and *E. coli* (similar to amoxicillin), whereas 32 μg/ml against *C. tropicalis*. Collectively, these compounds gave a lower MIC value between 32-64 μg/ml against both of the biofilm forming strains namely, *P. aeruginosa* and *S. mutans*. The results of antibacterial assay are mentioned in Table 1.

2.3 | *In vitro* antifungal activity

The antifungal activity of target compounds **(4a-4f)** was screened against seven fungal (*Candida albicans- ATCC® 18804, Candida dubliniensis- ATCC® MYA-646, Candida glabrata- ATCC® 2001, Candida parapsilosis- ATCC® 22019, Candida tropicalis- ATCC® 750, Candida kefyr- ATCC® 8553* and *Candida krusei- ATCC® 14243*) strains. Fluconazole was used as reference drug (widely used for the treatment of *Candida* infections) to compare antifungal effect of compounds. It has been observed that, fluconazole gave a minimum inhibitory concentration (MIC) value 64 μg/mL against all the tested fungal strains. The results of antifungal studies suggested that all the tested compounds gave MIC value either similar or even better than the reference drug fluconazole, for example, all compounds showed MIC value 64 μg/ml against *C. dubliniensis*, which is similar to fluconazole. On the other hand, Compounds **(4a**-**4c**, **4e)** and **(4f)** exhibited MIC value 32 μg/ml against *C. albicans* and *C. parapsilosis*, which is better than fluconazole. In case of other fungal strains, all compounds showed MIC value in the range of 16-32 μg/ml except compound **(4d)** which showed most promising that is much better antifungal activity (MIC value 16 μg/mL) against *C. albicans*, *C. dubliniensis, C. parapsilosis, C. kefyr* and *C. krusei* than the reference drug fluconazole. The results of antifungal activity are mentioned in Table 2.

TABLE 1 MIC (μg/ml) values of novel compounds (**4a-4f**) on the tested ATCC bacterial strains



4a: R=H, **4b**: R=OH, **4c**: R=NO_2, **4d**: R=Cl, **4e**: R=OCH_3, **4f**: R=3,4,5-TriOCH_3.

Codes of compounds	ATCC Bacterial strains *P. aeruginosa*	*S. bovis*	*E. faecalis*	*K. pneumoniae*	*E. coli*	*E. cloacae*	MRSA	*S. mutans*
4a	32	32	16	32	32	32	64	32
4b	32	32	32	32	16	32	32	32
4c	32	32	16	32	16	64	32	32
4d	16	8	16	32	8	16	32	16
4e	32	16	16	32	32	32	32	32
4f	64	32	32	32	32	32	64	32
Amoxicillin	64	8	8	16	8	16	16	64

TABLE 2 MIC (μg/ml) values of novel compounds (**4a-4f**) on the tested ATCC fungal strains

Codes of compounds	ATCC Fungal strains *C. albicans*	C. dubliniensis	*C. glabrata*	*C. parapsilosis*	*C. tropicalis*	C. kefyr	*C. krusei*
4a	32	32	16	32	32	32	32
4b	32	32	32	32	16	32	32
4c	32	32	16	32	16	64	32
4d	16	8	16	32	8	16	16
4e	32	16	16	32	32	32	64
4f	64	32	32	32	32	32	64
Fluconazole	64	64	64	64	64	64	64

3 | CONCLUSION

Some novel pyrazoline-based organometallic compounds containing ferrocenyl and quinoline units were synthesized as new leads in antimicrobial chemotherapy. *In vitro* antimicrobial activity of compounds was examined by broth microdilution method against fifteen ATTC (Bacterial: *Pseudomonas aeruginosa*, *Streptococcus bovis, Enterococcus faecalis, Klebsiella pneumoniae, Escherichia coli, Enterobacter cloacae, Methicillin-resistant Staphylococcus aureus* (MRSA) and *Streptococcus mutans*; fungal: *Candida albicans, Candida dubliniensis, Candida glabrata, Candida parapsilosis, Candida tropicalis, Candida kefyr* and *Candida krusei*) strains. The results of antimicrobial studies suggested that all these organometallics are new leads in antimicrobial chemotherapy as they exhibited an extra ordinary minimum inhibitory concentration (MIC) values against the tested organisms that are either similar or even better than the reference drugs amoxicillin (MIC values 8-64 μg/mL against the tested bacterial strains) and fluconazole (MIC values 64 μg/ml against the tested fungal strains). Compound (**4d**) was emerged as the most promising antimicrobial organometallic derivative with very interesting MIC value against all the strains ranging from 8-32 μg/ml. Other compounds gave a range of MIC value between 16-64 μg/ml against *S. bovis*, 16-32 μg/ml against *E. coli*, and *C. tropicalis* except (**4d**) which gave MIC 8 μg/ml against *S. bovis* and *E. coli*, whereas 32 μg/ml against *C. tropicalis*. Collectively, these compounds gave a lower MIC value between 32-64 μg/ml against both of the biofilm forming strains namely, *P. aeruginosa* and *S. mutans*. These results concluded that these novel organometallics can be employed as a two in one formulation to develop potent microbial agents and

can be very useful for further optimization work in microbial chemotherapy.

4 | EXPERIMENTAL

Acetylferrocene, substituted aromatic aldehydes, other chemicals and reagents were purchased from Sigma Aldrich (St. Louis, MO, USA). Thin-layer chromatography (TLC) was performed on precoated aluminum sheets (silica gel 60 F_{254}, Merck Germany) and spots were visualized under UV light. Elemental analyses were performed on Heraeus Vario EL III analyzer. The results were within ± 0.3% of the theoretical values. Melting points were determined on Thomas Hoover capillary melting apparatus and are uncorrected. IR spectra were recorded on Perkin-Elmer model 1600 FT-IR RX1 spectrophotometer as KBr discs. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AVANCE 400 spectrometer using $CDCl_3$ as solvent with tetramethylsilane (TMS) as internal standard. Splitting patterns are designated as follows; s, singlet; d, doublet; m, multiplet. Chemical shift (δ) values are given in ppm. ESI-MS was recorded on a MICROMASS QUATTRO II triple quadrupole mass spectrometer.

4.1 | General procedure for the synthesis of ferrocenyl chalcones (1a-1f)

Ferrocenyl chalcones (**1a-1f)** were synthesized according to our reported method[46] and their data has been found in agreement with the reported ones.[46] A mixture of acetyl ferrocene (6 mmol) and potassium hydroxide (0.4 g) was dissolved in absolute ethanol (50 ml) and stirred for 15 minutes at room temperature, then a solution of substituted aromatic aldehydes (prepared in absolute ethanol) was added drop wise to the reaction mixture and was further stirred at room temperature. The reaction was monitored by TLC on precoated silica gel sheets. After complete conversion the reaction mixture was neutralized by 2 M hydrochloric acid (HCl), leading to the formation of a deep red precipitate which was filtered and first washed with water alone several times then with cold ethanol-water mixture, dried under reduced pressure to obtain pure respective ferrocenyl chalcone.

4.2 | General procedure for the synthesis of ethyl (quinolin-8-yloxy) acetate (2)

Ethyl (quinolin-8-yloxy) acetate (**2**) was prepared by following reported procedure,[49] compared its data with the reported data and was found in agreement as reported earlier.[49] A mixture of 8- hydroxy quinoline (20 mmol), ethyl chloroacetate (20 mmol) and anhydrous potassium carbonate (20 mmol) in dry acetone (65 ml) was refluxed for 10 hours which led to the formation of compound (**2**). The resulting solid was filtered, dried under reduced pressure and crystallized by using ethanol to yield pure compound (**2**).

4.3 | General procedure for the synthesis of 2-(quinolin-8-yloxy) acetohydrazide (3)

The synthesis of 2-(quinolin-8-yloxy) acetohydrazide (**3**) was performed according to reported procedure.[49] In a round bottom flask a mixture of ethyl (quinolin-8-yloxy) acetate (**2**) (10 mmol), hydrazine hydrate (20 mmol), and absolute ethanol (100 ml) was refluxed for 5-6 hours. The progress of reaction was monitored by TLC. The resulting solid product was filtered, washed first with water then with cold ethanol, dried under

4.3.1 | 2-(Quinolin-8-yloxy) acetohydrazide (3)

Yield 85%; m.pt: 140 °C; yellow solid. Analysis calculated for $C_{11}H_{11}N_3O_2$: C 60.80, H 5.12, N 19.28%. Found: C 60.71, H 5.08, N 19.36 %. IR ν_{max} (cm^{-1}): 3465 (NH, CONH), 3036 (Ar–H), 2929 (C-H), 1670 (C=O), 1440 (C=C), 1180 (N–N), 1065 (C-O-C); ^{1}H NMR ($CDCl_3$) δ (ppm): 10.6 (s, 1H, CON*H*), 8.85 (dd, 1H, *H-2′* quinoline, $J_{2'\ 3'}$ = 4 Hz, $J_{2'4'}$= 1.6 Hz), 8.18 (dd, 1H, H-4′ quinoline, $J_{3'4'}$=8.4 Hz, $J_{2'4'}$=1.6 Hz), 7.18-7.53 (m, 4H, quinoline ring), 4.91 (s, 2H, OCH_2), 4.83 (2H, CO NH_2); ^{13}C-NMR ($CDCl_3$) δ(ppm): 168.4 (C=O), 157.3-109.6 (Ar-C), 69.8 (OCH_2); ESI-MS m/z: [M^++1] 218.09.

4.4 | General procedure for the synthesis of novel pyrazoline-based organometallic compounds (4a-4f)

A mixture of ferrocenyl chalcone (**1a-1f**) (4.28 mmol), 2-(quinolin-8-yloxy) acetohydrazide (**3)** (4.28 mmol) and sodium hydroxide (10 mmol) in ethanol (100 ml), was refluxed for 12 hours and progress of reaction was checked by TLC on precoated silica gel sheets. Afer completion of reaction, the mixture was cooled and poured on chilled water leading to the formation of solid mass which was extracted with petroleum ether (3 × 50 ml). The organic layer was dried over anhydrous sodium sulphate. Evaporation of organic solvent under reduced pressure gave gummy red colored mass which was recrystallized with acetone and hexane to afford pure compounds (**4a-4f**).

4.4.1 | 1-(5-Phenyl)-3-ferrocenyl-4,5-dihydropyrazol-1-yl)-2-quinolin-8-yloxy) ethanone (4a)

Yield 72%; m.pt: 119 °C; red solid. Analysis calculated for $C_{30}H_{25}FeN_3O_2$: C 69.91, H 4.89, N 8.15%. Found: C 69.85, H 4.73, N 8.22 %. IR ν_{max} (cm^{-1}): 3032 (Ar–H), 2926 (C-H), 1630 (C=O), 1563 (C=N), 1443 (C=C), 1254 (C–N), 1120 (C-O-C); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.85 (dd, 1H, *H-2′* quinoline, $J_{2'3'}$=4 Hz, $J_{2'4'}$= 1.6 Hz), 8.18 (d, 1H, *H-4′* quinoline, $J_{3'4'}$=8.4 Hz), 7.18-7.74 (m, 9H, Ar-*H*), 5.92 (dd, 1H, *Hx*, J_{ax}= 4.8, J_{bx}=11.2 Hz, pyrazoline), 4.93 (s, 2H, OCH_2), 4.76 (s, 2H, ferrocene), 4.49 (s, 2H, ferrocene), 4.19 (s, 5H, ferrocene), 3.57 (dd, 1H, H_b, J_{ab}=17.4, J_{bx}=11.2 Hz, pyrazoline), 3.66 (dd, 1H, H_a, J_{ab}=17.4, J_{ax}= 4.8 Hz, pyrazoline); ^{13}C-NMR ($CDCl_3$) δ (ppm): 166.3 (C=O), 151.4 (C-3, pyrazoline), 148-110.3 (Ar-C), 80.8 (ipso-C_5H_4), 77.3, 77.0 (meta-C_5H_4), 76.7, 72.4 (C_5H_5), 70.9, 70.0 (ortho-C_5H_4), 68.7 (CH_2), 60.2, 44.1 (C-5 and C-4, pyrazoline) ESI-MS m/z: [M^++1] 516.13.

4.4.2 | 1-(5-(4-Hydroxyphenyl)-3-ferrocenyl-4,5-dihydropyrazol-1-yl)-2-quinolin-8-yloxy) ethanone (4b)

Yield 78%; m.pt: 224 °C; Maroon solid. Analysis calculated for $C_{30}H_{25}FeN_3O_3$: C 67.81, H 4.74, N 7.91%. Found: C 67.85, H 4.4.86, N 7.98 %. IR ν_{max} (cm^{-1}): 3036 (Ar–H), 2924 (C-H), 1634 (C=O), 1571 (C=N), 1448 (C=C), 1257 (C–N), 1114 (C-O-C); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.87 (dd, 1H, *H-2′* quinoline, $J_{2'3'}$= 3.9 Hz, $J_{2'4'}$= 1.6 Hz), 8.19 (d, 1H, *H-4′* quinoline, $J_{3'4'}$=8.0 Hz), 7.24-7.70 (m, 8H, Ar-*H*), 6.31 (dd, 1H, *Hx*, J_{ax}= 4.2, J_{bx}=11.2 Hz, pyrazoline), 4.95 (s, 2H, OCH_2), 4.72 (s, 2H, ferrocene), 4.69 (s, 1H, O*H*), 4.42 (s, 2H, ferrocene), 4.19 (s, 5H, ferrocene), 3.74 (dd, 1H, H_b, J_{ab}=17.4, J_{bx}=11.2 Hz, pyrazoline), 3.65 (dd, 1H, H_a, J_{ab}=17.4, J_{ax}= 4.2 Hz, pyrazoline); ^{13}C-NMR ($CDCl_3$) δ(ppm): 165.9 (C=O), 151.2 (C-3, pyrazoline), 148.2-116.4 (Ar-C), 80.5 (ipso-C_5H_4), 77.2, 77.0 (meta-C_5H_4), 76.7, 72.8 (C_5H_5), 70.1, 69.7 (ortho-C_5H_4), 68.2 (CH_2), 61.2, 44.1 (C-5 and C-4, pyrazoline) ESI-MS m/z: [M^++1] 532.12.

4.4.3 | 1-(5-(4-Nitrophenyl)-3-ferrocenyl-4,5-dihydropyrazol-1-yl)-2-quinolin-8-yloxy) ethanone (4c)

Yield 61%; m.pt: 255 °C; Dark red solid. Analysis calculated for $C_{30}H_{24}FeN_4O_4$: C 64.30, H 4.32, N 10.01 %. Found: C 63.78, H 4.44, N 10.15 %. IR ν_{max} (cm^{-1}): 3031 (Ar–H), 2929 (C-H), 1630 (C=O), 1575 (C=N), 1441 (C=C), 1255 (C–N), 1119 (C-O-C); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.88 (dd, 1H, *H-2′* quinoline, $J_{2'3'}$ = 3.6 Hz, $J_{2'4'}$= 1.6 Hz), 8.22 (d, 1H, *H-4′* quinoline, $J_{3'4'}$=8.0 Hz), 7.23-7.71 (m, 8H, Ar-*H*), 6.02 (dd, 1H, *Hx*, J_{ax}= 4.8, J_{bx}=11.2 Hz, pyrazoline), 4.96 (s, 2H, OCH_2), 4.75 (s, 2H, ferrocene), 4.41 (s, 2H, ferrocene), 4.21 (s, 5H, ferrocene), 3.81 (dd, 1H, H_b, J_{ab}=16.8, J_{bx}=11.2 Hz, pyrazoline), 3.62 (dd, 1H, H_a, J_{ab}=16.8, J_{ax}= 4.8 Hz, pyrazoline); ^{13}C-NMR ($CDCl_3$) δ(ppm): 166.2 (C=O), 150.9 (C-3, pyrazoline), 148.0-118.5 (Ar-C), 80.5 (ipso-C_5H_4), 77.3, 77.0 (meta-C_5H_4), 76.7, 72.4 (C_5H_5), 70.9, 70.0 (ortho-C_5H_4), 69.6 (CH_2), 60.7, 44.1 (C-5 and C-4, pyrazoline) ESI-MS m/z: [M^++1] 561.11.

4.4.4 | 1-(5-(4-Chlorophenyl)-3-ferrocenyl-4,5-dihydropyrazol-1-yl)-2-quinolin-8-yloxy) ethanone (4d)

Yield 81%; m.pt: 189 °C; Red solid. Analysis calculated for $C_{30}H_{24}ClFeN_3O_3$: C 65.53, H 4.40, N 7.64%. Found: C 65.48, H 4.44, N 7.58 %. IR ν_{max} (cm^{-1}): 3032 (Ar–H), 2927 (C-H), 1632 (C=O), 1572 (C=N), 1442 (C=C), 1257 (C–N), 1121 (C-O-C); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.92 (dd, 1H, *H-2′* quinoline, $J_{2'3'}$=4.0 Hz, $J_{2'4'}$= 1.6 Hz), 8.21 (d, 1H, *H-4′* quinoline, $J_{3'4'}$=8.4 Hz), 7.24-7.75 (m, 8H, Ar-*H*), 5.92 (dd, 1H, *Hx*, J_{ax}= 4.8, J_{bx}=11.7 Hz, pyrazoline), 4.91 (s, 2H, OCH_2), 4.78 (s, 2H, ferrocene), 4.49 (s, 2H, ferrocene), 4.19 (s, 5H, ferrocene), 3.78 (dd, 1H, H_b, J_{ab}=16.8, J_{bx}=11.7 Hz, pyrazoline), 3.69 (dd, 1H, H_a, J_{ab}=16.8, J_{ax}= 4.8 Hz, pyrazoline); ^{13}C-NMR ($CDCl_3$) δ(ppm): 165.5 (C=O), 154.2 (C-3, pyrazoline), 147.9-106.9 (Ar-C), 80.6 (ipso-C_5H_4), 77.3, 77.0 (meta-C_5H_4), 76.7, 72.7 (C_5H_5), 70.1, 69.7 (ortho-C_5H_4), 66.5 (CH_2), 58.9, 44.2 (C-5 and C-4, pyrazoline) ESI-MS m/z: [M^++1] 550.83.

4.4.5 | 1-(5-(4-Methoxyphenyl)-3-ferrocenyl-4,5-dihydropyrazol-1-yl)-2-quinolin-8-yloxy) ethanone (4e)

Yield 72%; m.pt: 210 °C; Deep red solid. Analysis calculated for $C_{31}H_{27}FeN_3O_3$: C 68.27, H 4.99, N 7.70 %. Found: C 68.21, H 4.90, N 7.68 %. IR ν_{max} (cm^{-1}): 3034 (Ar–H), 2924 (C-H), 1636 (C=O), 1571 (C=N), 1445 (C=C), 1253 (C–N), 1120 (C-O-C); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.91 (dd, 1H, *H-2′* quinoline, $J_{2'3'}$=3.6 Hz, $J_{2'4'}$= 1.6 Hz), 8.23 (d, 1H, *H-4′* quinoline, $J_{3'4'}$=8.4 Hz), 7.22-7.74 (m, 8H, Ar-*H*), 5.99 (dd, 1H, *Hx*, J_{ax}= 4.2, J_{bx}=11.7 Hz, pyrazoline), 4.92 (s, 2H, OCH_2), 4.79 (s, 2H, ferrocene), 4.45 (s, 2H, ferrocene), 4.21 (s, 5H, ferrocene), 3.86 (s, 3H, OCH_3), 3.75 (dd, 1H, H_b, J_{ab}=18.0, J_{bx}=11.7 Hz, pyrazoline), 3.67 (dd, 1H, H_a, J_{ab}=18.0, J_{ax}= 4.2 Hz, pyrazoline); ^{13}C-NMR ($CDCl_3$) δ(ppm): 161.3 (C=O), 152.9 (C-3, pyrazoline), 147.9-115.2 (Ar-C), 80.2 (ipso-

C_5H_4), 77.3, 77.0 (meta-C_5H_4), 76.9, 71.7 (C_5H_5), 70.9, 70.2 (ortho-C_5H_4), 69.0 (CH_2), 60.6 (C-5 pyrazoline), 55.5 (O- CH_3), 43.9 (C-4, pyrazoline) ESI-MS m/z: $[M^+ + 1]$ 546.41.

4.4.6 | 1-(5-(3,4,5-Trimethoxyphenyl)-3-ferrocenyl-4,5-dihydropyrazol-1-yl)-2-quinolin-8-yloxy) ethanone (4f)

Yield 69%; m.pt: 212 °C; reddish solid. Analysis calculated for $C_{33}H_{31}FeN_3O_5$: C 65.46, H 5.16, N 6.94 %. Found: C 65.39, H 5.22, N 6.97 %. IR ν_{max} (cm^{-1}): 3030 (Ar–H), 2922 (C-H), 1635 (C=O), 1574 (C=N), 1448 (C=C), 1255(C–N), 1120 (C-O-C); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.90 (dd, 1H, *H-2′* quinoline, $J_{2'3'}$=4.0 Hz, $J_{2'4'}$= 1.6 Hz), 8.24 (d, 1H, *H-4′* quinoline, $J_{3'4'}$=8.4 Hz), 7.24-7.68 (m, 6H, Ar-*H*), 6.17 (dd, 1H, *Hx*, J_{ax}= 4.8, J_{bx}=11.7 Hz, pyrazoline), 4.94 (s, 2H, OCH_2), 4.78 (s, 2H, ferrocene), 4.41 (s, 2H, ferrocene), 4.22 (s, 5H, ferrocene), 3.87 (s, 6H, 2 × OCH_3), 3.82 (s, 3H, OCH_3) 3.68 (dd, 1H, H_b, J_{ab}=18.0, J_{bx}=11.7 Hz, pyrazoline), 3.52 (dd, 1H, H_a, J_{ab}=18.0, J_{ax}= 4.8 Hz, pyrazoline); ^{13}C-NMR ($CDCl_3$) δ(ppm): 162.8 (C=O), 152.4 (C-3, pyrazoline), 147.9-119.5 (Ar-C), 80.6 (ipso-C_5H_4), 77.3, 77.1(meta-C_5H_4), 76.8, 72.7 (C_5H_5), 70.1, 69.8 (ortho-C_5H_4), 696.2 (CH_2), 59.5 (C-5 pyrazoline), 55.8 (3×O-CH_3), 44.6 (C-4, pyrazoline) ESI-MS m/z: $[M^+ + 1]$ 606.46.

5 | *IN VITRO* ANTIMICROBIAL ASSAY

All novel organometallics **(4a-4f)** were investigated for antimicrobial studies against eight bacterial (*Pseudomonas aeruginosa*, *Streptococcus bovis, Enterococcus faecalis, Klebsiella pneumoniae, Escherichia coli, Enterobacter cloacae,* Methicillin-resistant *Staphylococcus aureus* and *Streptococcus mutans)* and seven fungal (*Candida albicans, Candida dubliniensis, Candida glabrata, Candida parapsilosis, Candida tropicalis, Candida kefyr* and *Candida krusei*)) strains by broth microdilution method.[48] Inocula were prepared from an overnight nutrient broth culture plate of the bacterial test strains and yeast peptone dextrose (YPD) culture plate for fungal test organisms (Himedia Labs, Mumbai, India). Inoculum for the minimum inhibitory concentration (MIC) test was prepared by taking two or three considerably isolated colonies from respective agar culture plate. These colonies were picked up by a sterile loop and were transferred into a tube containing 4 to 5 ml of respective sterile liquid medium. This broth was incubated at 37 °C until it attained a desired turbidity.[47]

5.1 | Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was determined by using a broth microdilution method.[48] Stock standard solutions at 2 mg/ml were prepared in DMSO for all the compounds to be tested. Working solutions were prepared by dilution in microtiter plates at concentrations between 512 μg/ml and 0.244 μg/mL using nutrient medium as the diluent. DMSO (50 μL) was used as control and did not show any inhibitory activity. The bacterial suspension for each strain was added in the wells of the microtiter plate at the concentration of 10^5-10^6 cfu/ml (colony forming units/ml). Each inoculum was prepared in its respective medium and was diluted to 1:100 for the broth micro dilution procedure. The plates were incubated at 37 °C with bacterial strains and at 30 °C for fungal stains and the minimum inhibitory concentration (MIC) was recorded after 24 hours. Bacterial and fungal growth was shown by the presence of turbidity in the wells. The MIC was determined as the lowest concentration of the compound that completely inhibited the visible bacterial/fungal growth (turbidity) of the organism after 24 hour of incubation. All these determinations represent the mean of three independent experiments. The results of antimicrobial activity are summarized in Table 1 and Table 2**.**

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