A novel centrosymmetric Fe(III) complex with anionic *bis*-pyrazolyl-*s*-triazine ligand; synthesis, structural investigations and antimicrobial evaluations

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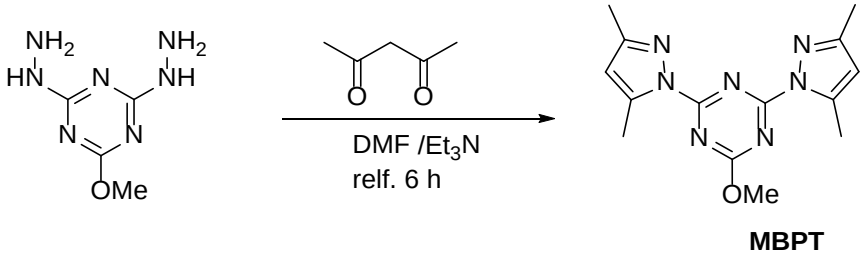
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Method S1: Synthesis of MBPT

At room temperature, 2,4-dihydrazinyl-6-methoxy-1,3,5-triazine (10 mmol in 20 mL DMF) [31] was mixed with acetylacetone (25 mmol) followed by addition of triethylamine (8 mmol in 10 mL DMF) with stirring then refluxed for 6 h (**Scheme 1)**.. After completion of the reaction, the mixture is left to cool till room temperature, and then ice-cold water was added (100 mL). The reaction mixture was kept at 0^{o}C for 2 h, white solid powder of **MBPT** is precipitated. The product is filtered, washed with cold water (3 x 20 mL), and then dried.

Yield; $C_{14}N_7H_{17}O$ (**MBPT**) 86%; mp 175-176 °C. Anal. Calc. C, 56.18; H, 5.72; N, 32.76%. Found: C, 56.16; H, 5.74; N, 32.77%; IR (KBr, cm^{-1}): 3041, 2978, 2926, 1593, 1555; (**Fig. S1**, Supplementary data). ^{1}H NMR ($CDCl_3$) δ: 2.30 (s, 6H, $2CH_3$), 2.72 (s, 6H, $2CH_3$), 4.12 (s, 3H, OCH_3),6.05 (s, 2H, CH) ppm (**Fig. S2**, Supplementary data); ^{13}C NMR ($CDCl_3$): δ 13.8, 17.8, 55.7, 111.9, 144.4, 153.3, 164.8, 172.3 ppm (**Fig. S2**, Supplementary data).



Scheme S1 Synthesis of the ligand (**MBPT**)**.**

Method S2: Antimicrobial studies

a) Tested pathogenic microbes

The antibacterial activity of the studied ligands and their Co(II) complexes were evaluated against two Gram positive bacteria ((*S. aureus* (ATCC 25923) and *B. subtilis* (RCMB015(1)NRR LB-543)), two Gram negatvie bacteria ((*E. coli* (ATCC 25922) and *P. vulgaris* (RCMB 004(1)ATCC 13315)) and two fungi ((*A. fumigatus* (RCMB 002008) and *C. albicans* (RCMB 005003(1) ATCC 10231)). Gentamycin was used as standard antibacterial agent. The samples maintained in Brain heart infusion (BHI) at 20^{o}C; 300 mL of each stock–culture was added to 3 mL of BHI broth. Overnight cultures were kept for 24 h at 37 ^{o}C ± 1^{o}C and the purity of cultures was checked after 24 h of incubation. After 24 h of incubation, bacterial suspension was diluted with sterile physiological solution, for the diffusion and indirect

bioautographic tests, to 108 CFU/mL (turbidity = McFarland barium sulfate standard 0.5). In case of fungi *A. fumigatus* (RCMB 002008) and *C. albicans* (RCMB 005003(1) ATCC 10231), the used medium in antagonistic activity against tested fungi is Potato Dextrose Agar, where Fluconazole was used standard antifungal agent.

b) Agar well diffusion method

Synthetic compounds were prepared at concentration 10 mg/mL dissolved in DMSO as stock solutions. Preparation of sterilized Mueller Hinton agar plates seeded with tested pathogenic bacteria occurred. The wells are done by sterilized cork borer in size 6 mm and hence 200 μg of the synthetic compound was poured in each well comparably with DMSO as control. The plates were incubated at 37°C for 24 h. after incubation period; antimicrobial activity was determined by inhibition zones.

c) Minimum Inhibitory Concentration (MIC)

Different dilutions of the compounds are inoculated with tested pathogenic microbes. After incubation period of 96 well microplate, the results are measured using microplate reader. To determine at what level the MIC endpoint is established; subculture of test samples at different concentrations occurred in nutrient agar plates.

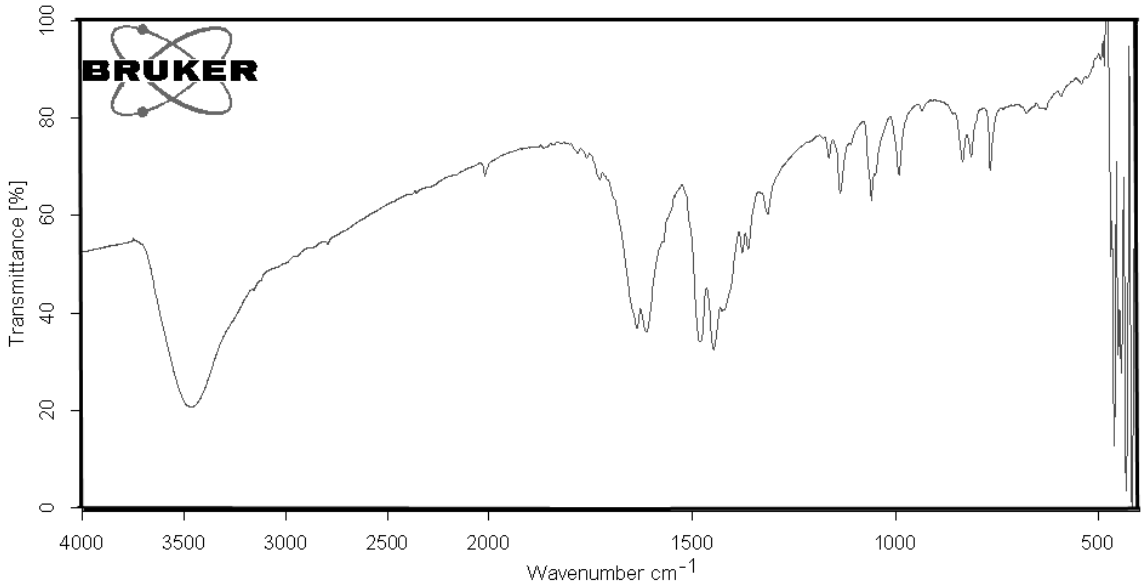


Fig. S1 FTIR spectra of the [Fe(BPT)(CH_3OH)Cl_2] complex.

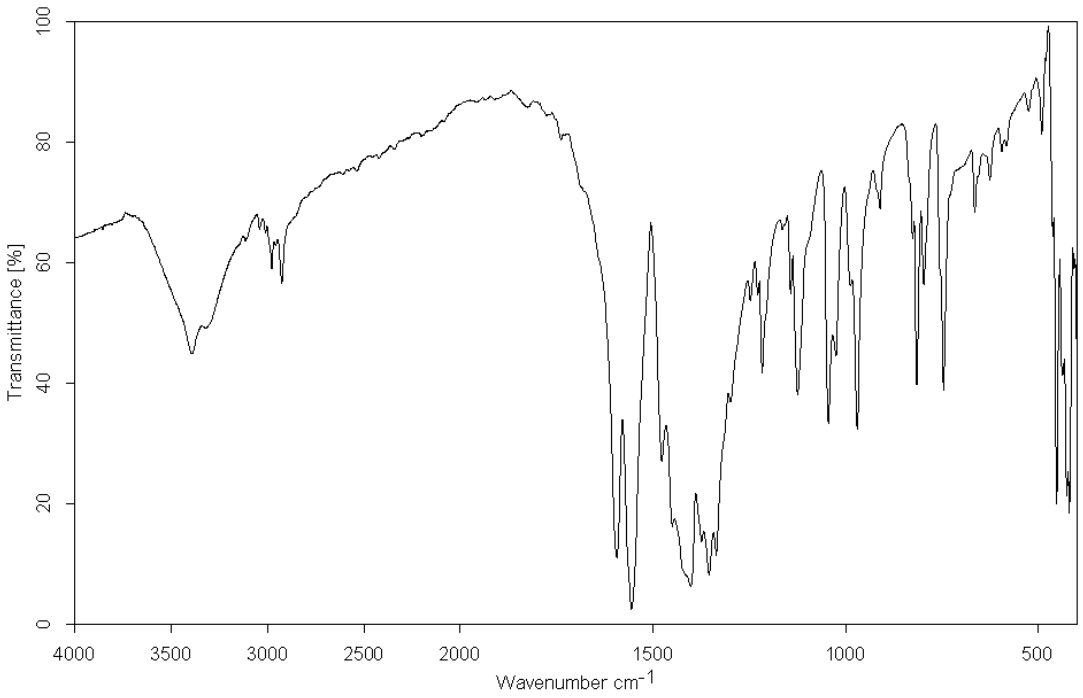
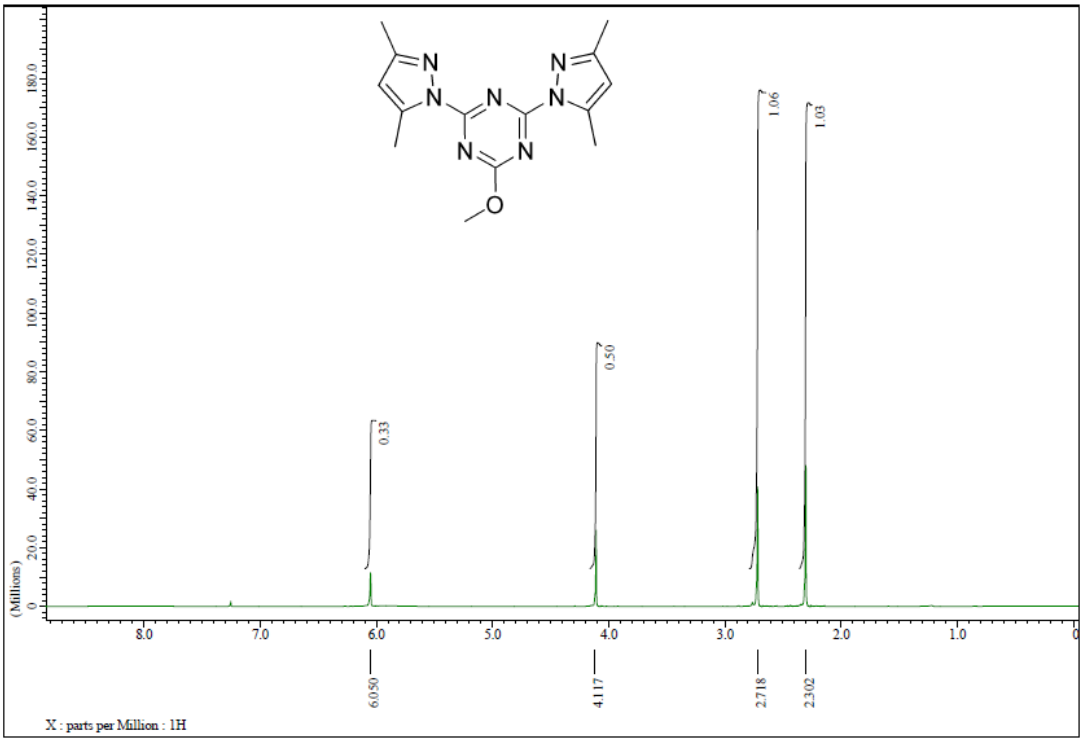


Fig. S2 FTIR spectra of the ligand (**MBPT**).



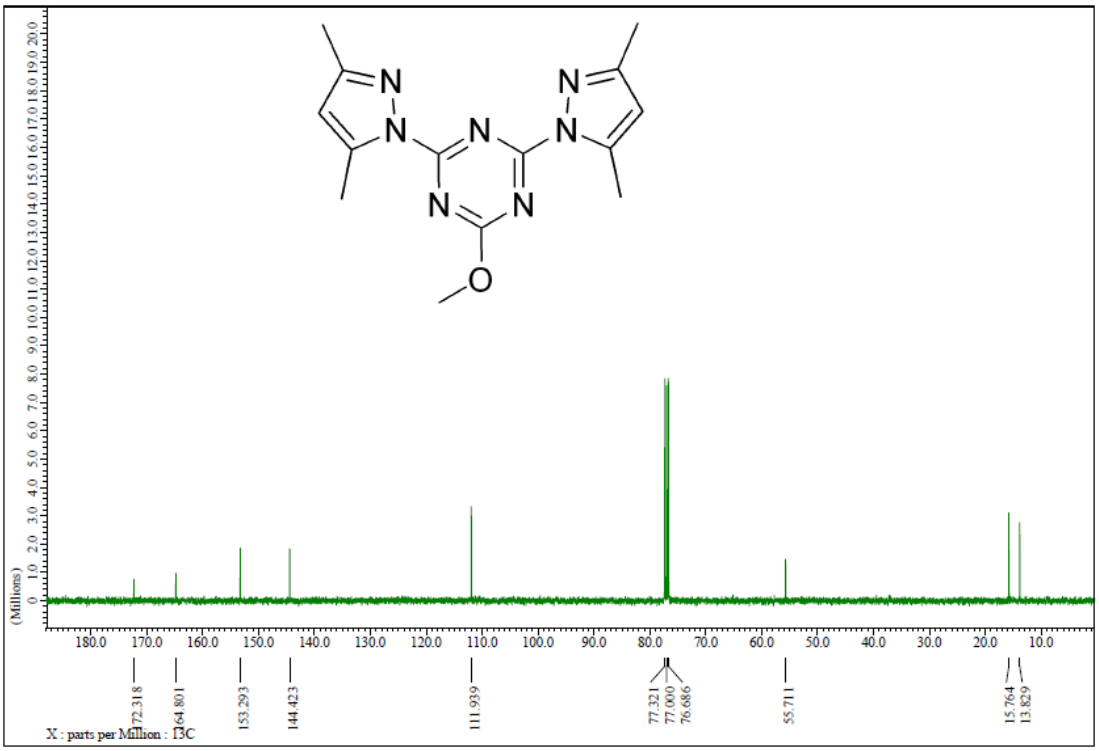


Fig. S3 ^{1}H and ^{13}C NMR spectra of the ligand (**MBPT**). Chemical shifts are reported in parts per million (ppm).

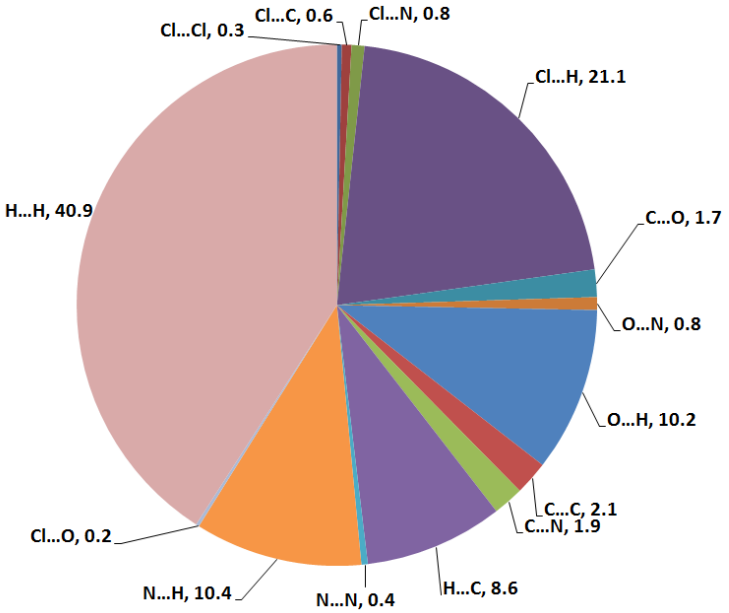


Fig. S4 Summary of the intermolecular interactions and their percentages.