

# Supplementary Materials: Synthesis, Structure and Antimicrobial Activity of New Co(II) Complex with *bis*-Morpholino/Benzimidazole-*s*-Triazine Ligand

Saied M. Soliman <sup>1,\*</sup>, Eman M. Fathalla <sup>1</sup>, Mona M. Sharaf <sup>2</sup>, Ayman El-Faham <sup>1</sup>, Assem Barakat <sup>3</sup>, Matti Haukka <sup>4</sup>, Alexandra M. Z. Slawin <sup>5</sup>, John Derek Woollins <sup>5,6</sup> and Morsy A. M. Abu-Youssef <sup>1,\*</sup>

- <sup>1</sup> Department of Chemistry, Faculty of Science, Alexandria University, P.O. Box 426, Ibrahimia, Alexandria 21321, Egypt; eman.nameir@alexu.edu.eg (E.M.F.); ayman.elfaham@alexu.edu.eg (A.E.-F.)
- <sup>2</sup> Protein Research Department, Genetic Engineering and Biotechnology Research Institute, City of Scientific Research and Technological Applications, Alexandria, P.O. Box 21933, Egypt; sharafmona4@gmail.com
- <sup>3</sup> Department of Chemistry, College of Science, King Saud University, P. O. Box 2455, Riyadh 11451, Saudi Arabia; ambarakat@ksu.edu.sa
- <sup>4</sup> Department of Chemistry, University of Jyväskylä, P.O. Box 35, FI-40014 Jyväskylä, Finland; matti.o.haukka@jyu.fi
- <sup>5</sup> School of Chemistry, University of St Andrews, St Andrews KY16 9ST, UK; amzs@st-andrews.ac.uk (A.M.Z.S.); jdw3@st-andrews.ac.uk (J.D.W.)
- <sup>6</sup> Department of Chemistry, Khalifa University, Abu Dhabi, PO Box 127788, United Arab Emirates
- \* Correspondence: saeed.soliman@alexu.edu.eg (S.M.S.); morsy5@alexu.edu.eg (M.A.M.A.-Y.)

## Method S1. Evaluation of antimicrobial activity [38]

### a) Tested pathogenic microbes

The antimicrobial activity of the ligand (**DMPT**) and complexes **1-2** was evaluated against two Gram positive bacteria ((*S. aureus* (ATCC 25923) and *B. subtilis* (RCMB015(1)NRR LB-543)), two Gram negative 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 °C; 300 mL of each stock-culture was added to 3 mL of BHI broth. Overnight cultures were kept for 24 h at 37 °C ± 1 °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 10<sup>8</sup> 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 Ketoconazole was used standard antifungal agent.

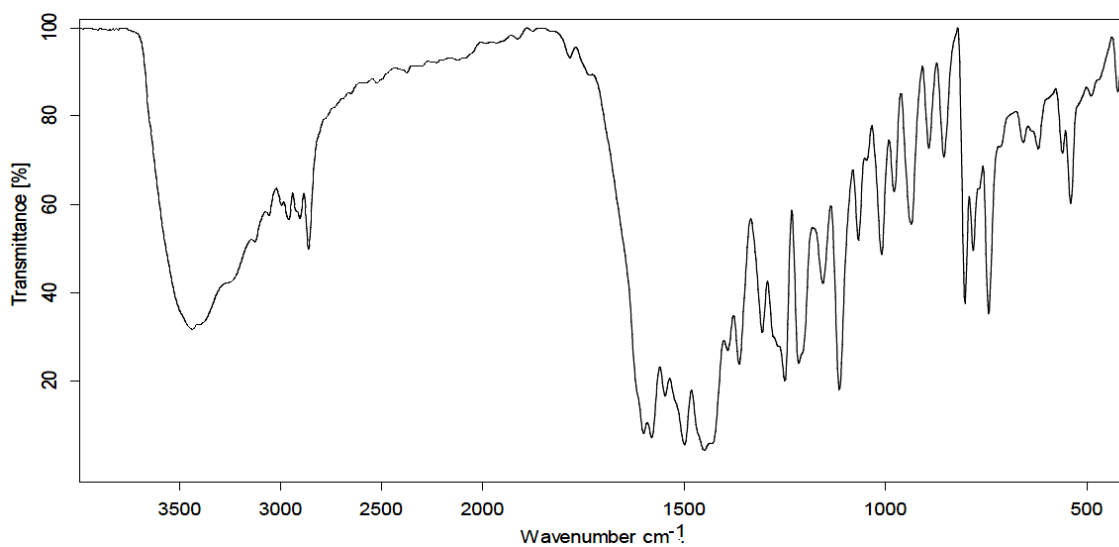
### b) Agar well diffusion method

Synthetic compound was 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 100 µL of the synthetic compound was poured in each well comparably with DMSO as control. The plates were incubated at 37 °C for 24–48 h (for bacteria) and at 28 °C for 48 h (for fungi). After incubation period; antimicrobial activity was determined by inhibition zones.

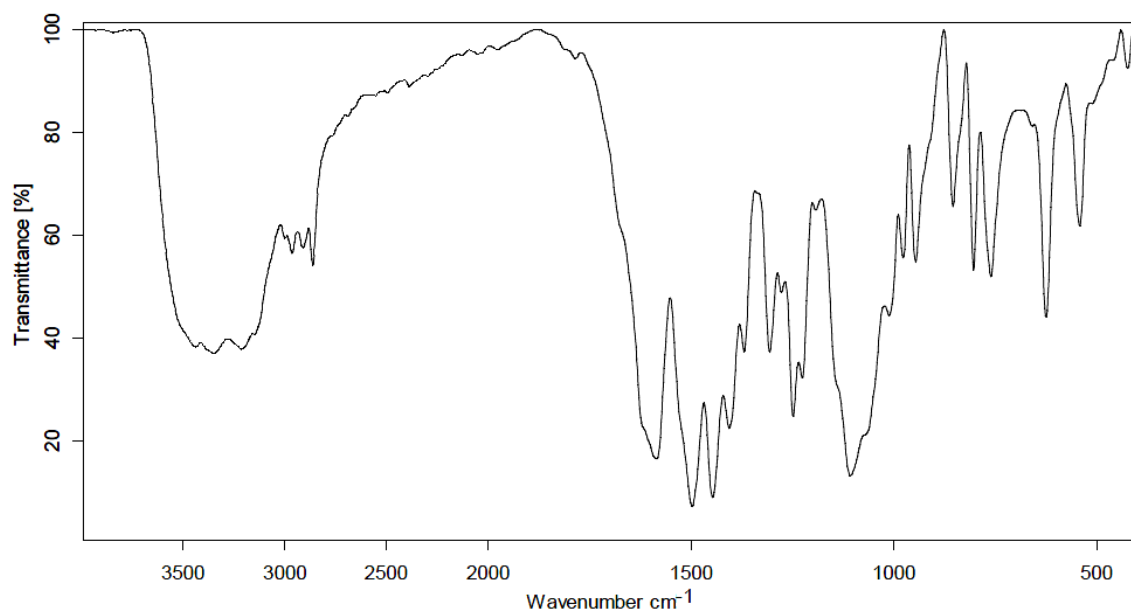
### c) Minimum Inhibitory Concentration (MIC)

Different dilutions of the compound are inoculated with tested pathogenic microbes. After incubation period of 96 well microplate, the results are measured using microplate

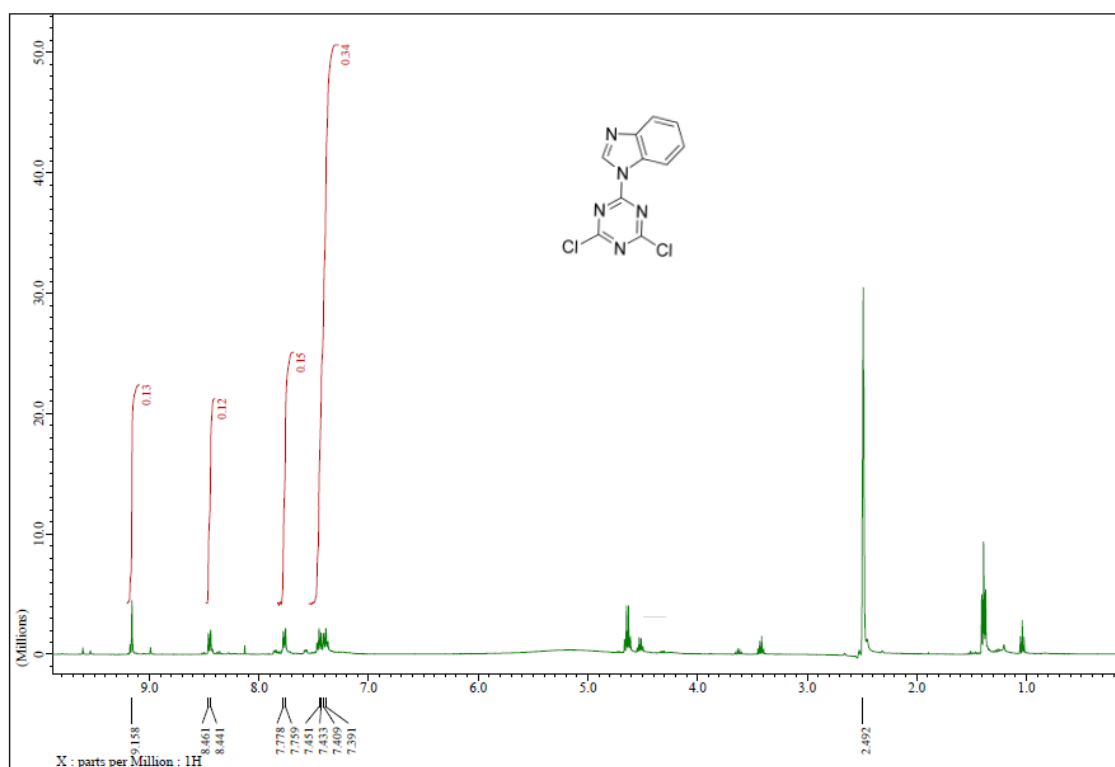
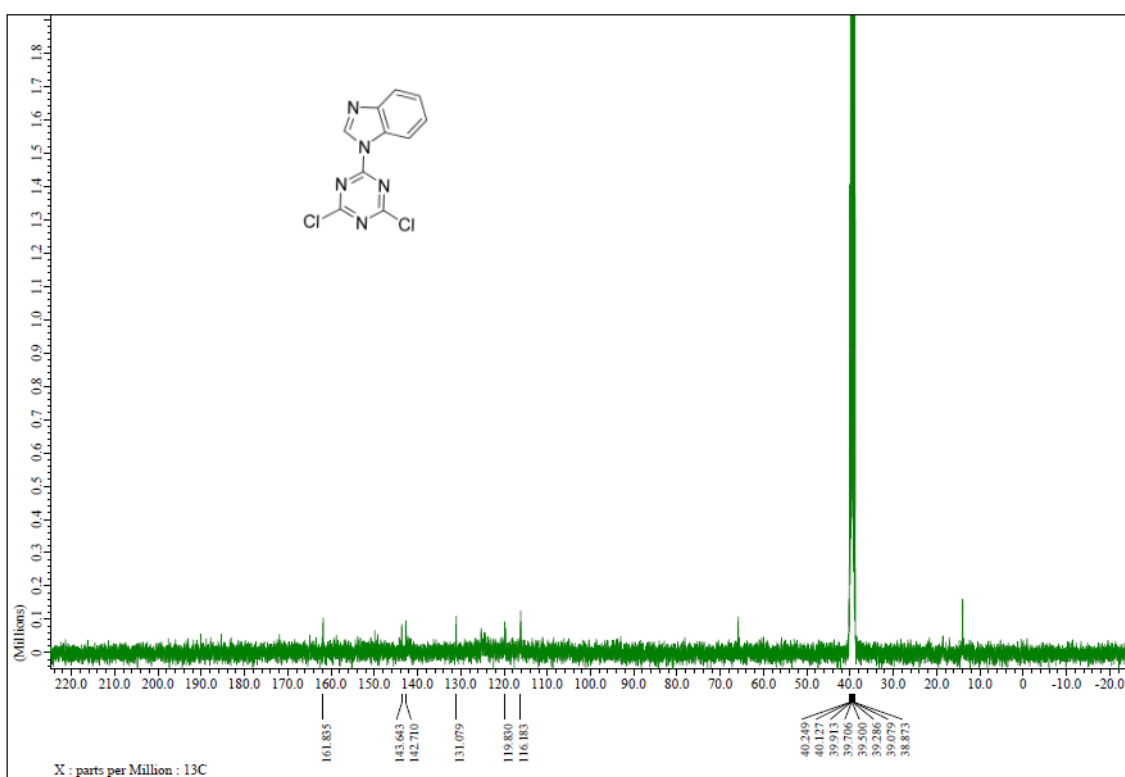
reader. To determine at what level the MIC endpoint is established; subculture of test samples at different concentrations occurred in nutrient agar plates.



**Figure S1.** FTIR spectra of **BMBIT**.



**Figure S2.** FTIR spectra of  $[\text{Co}(\text{BMBIT})_2(\text{H}_2\text{O})_4](\text{ClO}_4)_2 \cdot \text{H}_2\text{O}$  complex.

Figure S3. <sup>1</sup>H NMR spectra of 3.Figure S4. <sup>13</sup>C NMR spectra of 3.