

## SUPPORTING INFORMATION

# A New Bromo-Mn(II) Complex with 1,3,5-Triazine Derivative: Synthesis, Crystal Structure, DFT and Biological Studies

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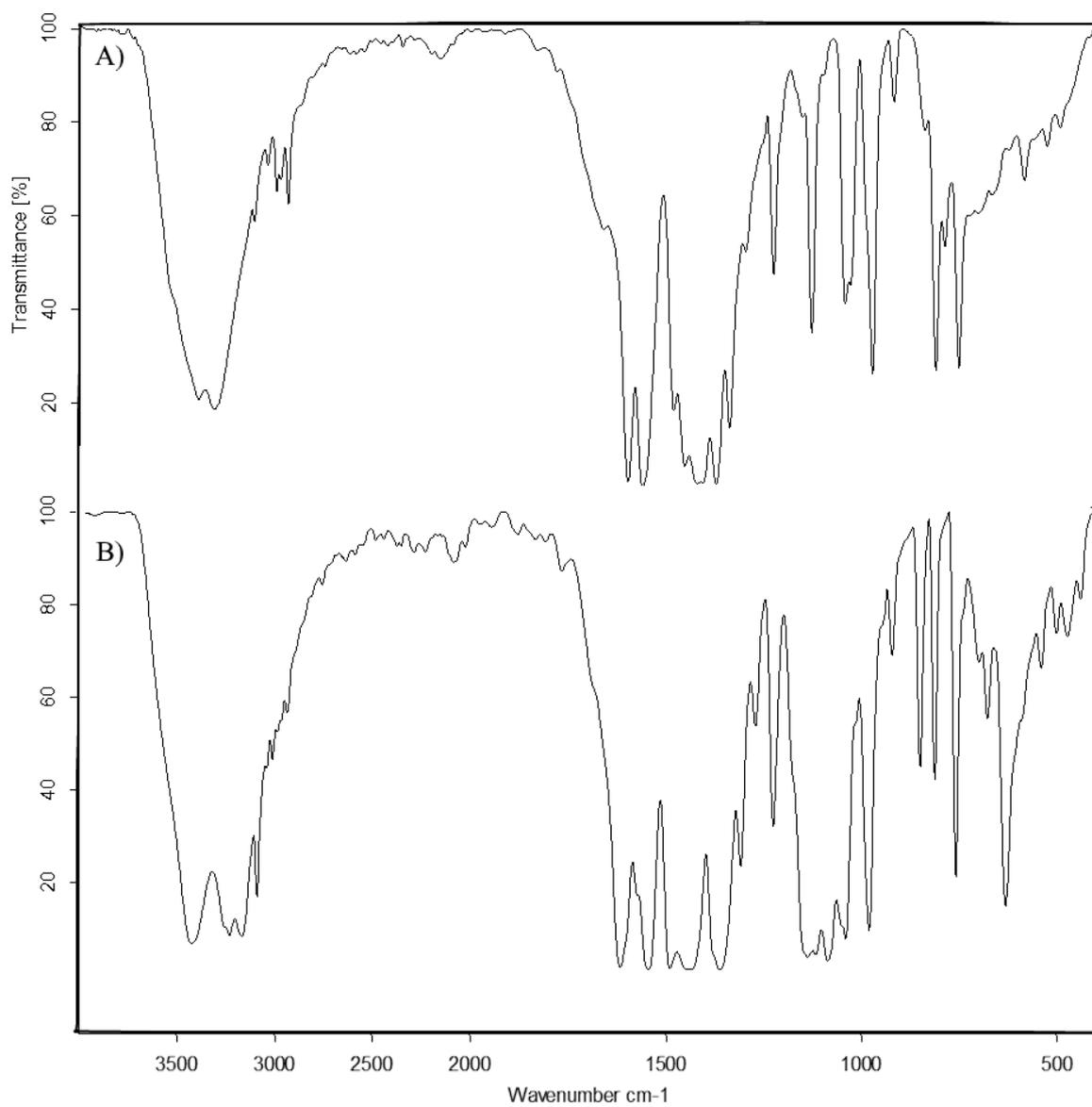
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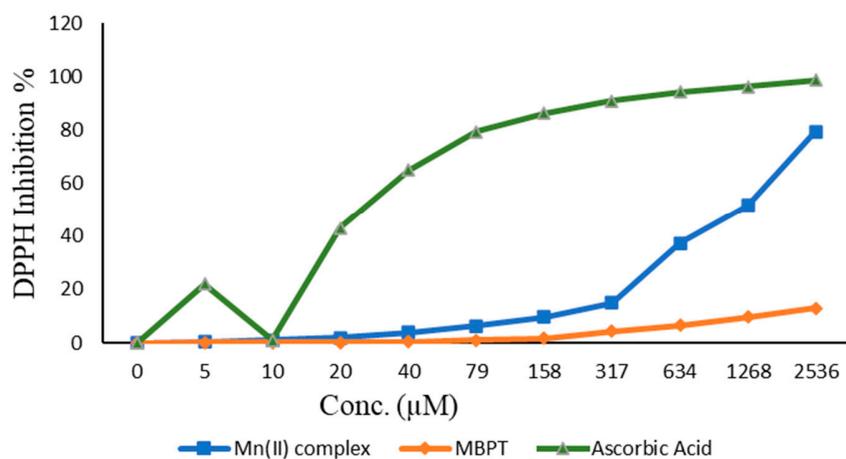
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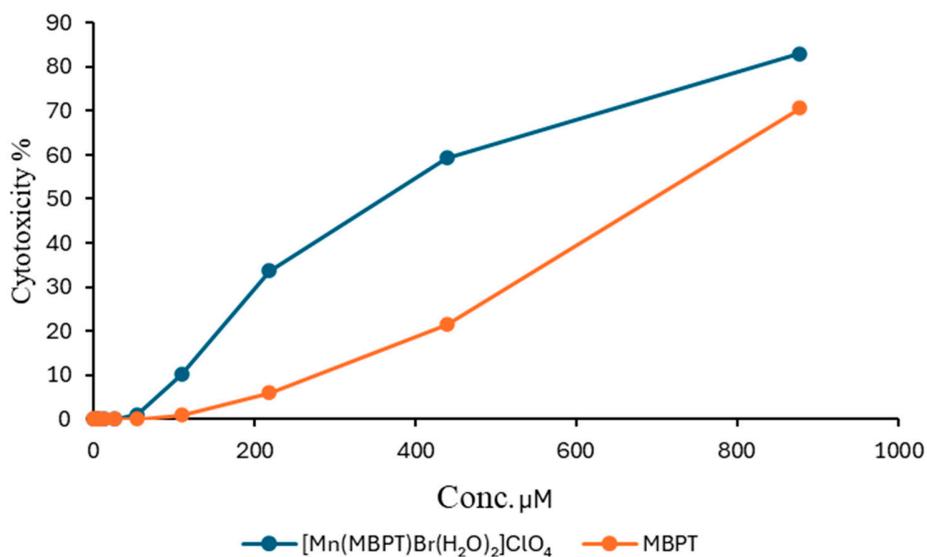
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**Figure S1.** FTIR spectra of the ligand **MBPT** (A) and  $[\text{Mn}(\text{MBPT})(\text{H}_2\text{O})_2\text{Br}]\text{ClO}_4$  complex (B).



**Figure. S2.** DPPH radical scavenging activity of Mn(II) complex , free ligand (MBPT) and ascorbic acid.



**Figure S3.** Safety assay of [Mn(MBPT)Br(H<sub>2</sub>O)<sub>2</sub>]ClO<sub>4</sub> and MBPT on the non-cancerous WI-38 cell line.

**Table S1** Zone of Inhibition (mm) for the  $[\text{Mn}(\text{MBPT})\text{Br}(\text{H}_2\text{O})_2]\text{ClO}_4$ .

Microbe	$[\text{Mn}(\text{MBPT})\text{Br}(\text{H}_2\text{O})_2]\text{ClO}_4$	Control
<b>Gram-positive bacteria</b>		
<i>S. aureus</i>	14	24 <sup>a</sup>
<i>B. subtilis</i>	19	26 <sup>a</sup>
<b>Gram-negative bacteria</b>		
<i>E. coli</i>	NA <sup>c</sup>	30 <sup>a</sup>
<i>P. vulgaris</i>	16	25 <sup>a</sup>
<b>Fungi</b>		
<i>A. fumigatus</i>	NA <sup>c</sup>	17 <sup>b</sup>
<i>C. albicans</i>	NA <sup>c</sup>	20 <sup>b</sup>

<sup>a</sup>Gentamycin, <sup>b</sup>Ketoconazole and <sup>c</sup>No Activity.

**Table S2.** IC<sub>50</sub> values ( $\mu\text{M}$ ) of the studied systems.

Cell line	MBPT	$[\text{Mn}(\text{MBPT})\text{Br}(\text{H}_2\text{O})_2]\text{ClO}_4$
A-549	1245.41 $\pm$ 45.57	557.75 $\pm$ 20.15
MCF-7	1113.59 $\pm$ 29.77	50.05 $\pm$ 2.16
HeLa	1198.58 $\pm$ 31.87	216.35 $\pm$ 5.34
HepG-2	671.47 $\pm$ 21.41	31.11 $\pm$ 2.04
WI-38	1320.22 $\pm$ 31.64	359.10 $\pm$ 8.72

### Materials and physical characterization

All solvents and chemicals were purchased from Sigma-Aldrich Chemical Company Inc. (St. Louis, MO, USA). Using FTIR instrument (Bruker Tensor 37, Waltham, MA, USA), the FTIR spectra were recorded in the KBr pellets at 4000–400  $\text{cm}^{-1}$ . To analyze CHN, a PerkinElmer 2400 Elemental Analyzer (PerkinElmer Inc.940 Winter Street, Waltham, MA, USA) was utilized. An atomic absorption spectrophotometer (AA-7000 series, Shimadzu, Ltd., Tokyo, Japan) was used to measure the Mn-content.

## **Method S1**

### **Crystal structure determination**

For X-Ray diffraction analysis, suitable crystals were selected, and data collection was performed on a Bruker diffractometer equipped with a graphite-monochromatic Mo-K $\alpha$  radiation at 296 K. The structures were solved by direct methods using SHELXT-2018 [42] and refined by full-matrix least-squares methods on F<sup>2</sup> using SHELXL-2018 [43] from within the WINGX [44] suite of software. Bruker APEX2 [45] was used for data collection, while molecular diagrams were created using MERCURY [46].

### **Biological activity methods**

#### **Method S2: Antimicrobial studies [49]**

##### **a) Tested pathogenic microbes**

The antibacterial activity of the studied ligand and its Mn(II) complex were 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  $\pm$  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 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  $\mu$ g 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.

### **Method S3**

#### **Safety assay [50,51]**

**WI-38 cell line** was used to evaluate the safety pattern for  $[\text{Mn}(\text{MBPT})\text{Br}(\text{H}_2\text{O})_2]\text{ClO}_4$  which is (human lung fibroblast normal cell line) were obtained from the American Type Culture Collection (ATCC, Rockville, MD).

#### **Cell line Propagation**

The cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50 $\mu\text{g}/\text{mL}$  Gentamycin. All cells were maintained at 37°C in a humidified atmosphere with 5%  $\text{CO}_2$  and were subcultured two times a week.

#### **Cytotoxicity evaluation using viability assay**

For cytotoxicity assay, the cells were seeded in 96-well plate at a cell concentration of  $1 \times 10^4$  cells per well in 100 $\mu\text{l}$  of growth medium. Fresh medium containing different concentrations of the test sample in DMSO was added after 24 h of seeding. Serial two-fold dilutions of the tested chemical compound were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37°C in a humidified incubator with 5%  $\text{CO}_2$  for a period of 24 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells for at 37°C, for 24 h, the viable cells yield was determined by the MTT test. Briefly, the media was removed from the 96 well plate and replaced with 100  $\mu\text{L}$  of fresh culture DMEM medium without phenol red then 10  $\mu\text{L}$  of the 12 mM MTT stock solution (5 mg of MTT in 1 mL of PBS) to each well including the untreated controls. The 96 well plates were then incubated at 37°C and 5%  $\text{CO}_2$  for 4 hours. An 85  $\mu\text{L}$  aliquot of the media was removed from the wells, and 50  $\mu\text{L}$  of DMSO was added to each well and mixed thoroughly with the pipette and incubated at 37°C for 10 min. Then, the optical density was measured at 590 nm with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as  $[(\text{ODt}/\text{ODc})] \times 100\%$  where ODt is the mean optical density of wells treated with the tested

sample and ODC is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration (IC<sub>50</sub>), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each conc. using Graphpad Prism software (San Diego, CA. USA).

#### **Method S4**

##### **Evaluation of Cytotoxic activities [50-52]**

The following cell lines were used to examine the cytotoxic activity of the [Mn(MBPT)Br(H<sub>2</sub>O)<sub>2</sub>]ClO<sub>4</sub> complex, which are **HepG-2** cells (human hepatocellular carcinoma), **MCF-7** (Breast carcinoma), **HeLa** (Cervical carcinoma cells), and **A-549** (Lung carcinoma cells) that all were obtained from the American Type Culture Collection (ATCC, Rockville, MD).

##### **Cell line Propagation**

The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50µg/ml gentamycin. The cells were maintained at 37°C in a humidified atmosphere with 5% CO<sub>2</sub> and were subcultured two to three times a week.

##### **Cytotoxicity evaluation using viability assay:**

For antitumor assays, the tumor cell lines were suspended in medium at concentration 5x10<sup>4</sup> cell/well in Corning® 96-well tissue culture plates, then incubated for 24 hr. The tested compounds were then added into 96-well plates (three replicates) to achieve twelve concentrations for each compound. Six vehicle controls with media or 0.5 % DMSO were run for each 96 well plate as a control. After incubating for 24 h, the numbers of viable cells were determined by the MTT test. Briefly, the media was removed from the 96 well plate and replaced with 100 µl of fresh culture RPMI 1640 medium without phenol red then 10 µl of the 12 mM MTT stock solution (5 mg of MTT in 1 mL of PBS) to each well including the untreated controls. The 96 well plates were then incubated at 37°C and 5% CO<sub>2</sub> for 4 hours. An 85 µl aliquot of the media was removed from the wells, and 50 µl of DMSO was added to each well and mixed thoroughly with the pipette and incubated at 37°C for 10 min. Then, the optical density was measured at 590 nm with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as [(ODt/ODc)]x100% where ODt is the mean optical density of wells treated with the tested

sample and  $OD_c$  is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound.

The 50% inhibitory concentration ( $IC_{50}$ ), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each conc. using Graphpad Prism software (San Diego, CA. USA)

## Method S5

### DPPH Radical Scavenging Activity [53]

Freshly prepared (0.004%w/v) methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was prepared and stored at 10 °C in dark place. A methanolic solution of the test compound was prepared using the same procedure. A 40  $\mu$ L aliquot of the methanol solution was added to 3mL of DPPH solution. Absorbance measurements were recorded immediately with a UV-visible spectrophotometer (Milton Roy, Spectronic 1201). The decrease in absorbance at 515 nm was determined continuously, with data being recorded at 1 min intervals until the absorbance stabilized (16 min). The absorbance of the DPPH radical without antioxidant (control) and the reference compound ascorbic acid were also measured. All the determinations were performed in three replicates and averaged. The percentage inhibition (PI) of the DPPH radical was calculated according to the formula:

$$PI = \left[ \frac{(AC - AT)}{AC} \right] \times 100 \quad (1)$$

Where  $AC$  = Absorbance of the control at  $t = 0$  min and  $AT$  = absorbance of the sample+DPPH at  $t = 16$  min. The 50% inhibitory concentration ( $IC_{50}$ ), the concentration required to inhibit DPPH radical by 50%, was estimated from graphic plots of the dose response curve.

## References

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