Research Article

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A Dy(III)–organic framework as a fluorescent probe for highly selective detection of picric acid and treatment activity on human lung cancer cells

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Abstract: A dysprosium(III) organic framework, $\{[\text{Dy}(\text{H}_2\text{O})(\text{BTCTB})] \cdot 2\text{H}_2\text{O}\}_n$ (**1**, H_3BTCTB = 3,3',3''-[1,3,5-benzenetriyltris(carbonylimino)]tris-benzoic acid), was synthesized through the hydrothermal reaction of $\text{Dy}(\text{NO}_3)_3$ with the C_3 -symmetric organic ligand H_3BTCTB at 160°C for 96 h. At the same time, the sensitivity of picric acid in water medium was tested with material **1** as the fluorescent sensor. The detection limit was $0.71\ \mu\text{M}$ and K_{SV} of this experiment was $8.55 \times 10^4\ \text{M}^{-1}$, which might be attributed to the presence of abundant amide groups in the framework of **1**. In addition, the treatment effect of compound **1** against the NCI-H292 lung cancer cells was evaluated. The Cell Counting Kit-8 (CCK-8) method was conducted to measure the viability of cancer cell after treated through the compound **1**. The DCFH-DA was applied for the determination of ROS. The relative expression of the inflammatory genes was measured with RT-PCR. The western blotting was conducted to detect the effect of the compound against MDM-2 levels in NCI-H292 lung cancer cells. The possible binding interactions in terms of binding poses are probed by performing molecular docking simulations.

Keywords: Dy–MOF, C_3 -symmetric organic ligand, picric acid sensor, lung cancer, molecular docking

1 Introduction

In modern medicine, metal ions and their complexes have been used for decades [1]. Cisplatin is an effective cancer drug and its discovery promotes the development of inorganic chemistry of drugs. In the current research and development system of anticancer drugs, platinum complexes have always been the focus of attention [2]. In the redox environment, there is a cell-level program to enhance the drug function. This discovery makes it possible to replace cisplatin with tunable metal complexes as a new anticancer drug. For example, research work on copper complexes has been completed, that is, endogenous metals are less harmful to normal cells except cancer cells [3]. In addition, Wilson and his team also claim that cobalt(III) complexes may contribute to the development of new anticancer drugs [4].

Metallic organic skeletons (MOFs or PCPs) are often referred to as porous coordination polymers. These materials have the advantages of controllable pore size and a strong functional pore structure, so they have attracted much attention in many fields such as gas storage, catalytic reaction, drug conduction, separation, proton flow, magnetic pole, optics, and conduction [5–10]. At the same time, because of the crystallinity, adjustable porosity, and special electronic structure of MOFs, they are widely used in the field of fluorescent sensors. Recently, a variety of MOFs have been combined with nitroaromatic hydrocarbons to produce luminous probes for the determination of picric acids (PAs) in water solutions [11–14]. In these MOFs, those compounds based on lanthanide metal centers have become a new research object due to their unique characteristics of long emission life, high color purity, and a large displacement of Stokes. Nevertheless, it is challenging to synthesize the Ln–MOFs because they possess a high number of coordinations and they have a tendency to coordinate with the ligand of oxygen donor that contains more flexibility and higher number of coordinations [15–17]. In addition, some Ln–MOFs could not keep their integrality framework in water, which restricts their further applications. The C_3 -symmetric ligand 3,3',3''-[1,3,5-benzenetriyltris(carbonylimino)]tris-benzoic acid

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(H₃BTCTB) should be an excellent selection for generating the Ln–MOF sensors that contain outstanding detection properties and high skeleton stability. First, the stability of Ln–MOF sensors was improved by adding the rigid benzoic acid group bound to metal ions. Second, three amide bonds attached to ligands can be utilized at the hydrogen bond position and the Lewis base position to increase conductivity in aqueous media. Based on the above considerations, a novel Dy(III)–MOF has been fabricated hydrothermally, and its chemical formula is {[Dy(H₂O)(BTCTB)]·2H₂O}_n (**1**), which was detected in depth through the analysis of element, TGA, the diffraction of single-crystal X-ray as well as the PXRD. Due to its strong fluorescent emission and high water stability, complex **1** was studied as the fluorescent sensor to determine the PA in the water. In the bio-study, the inhibition of compound **1** against the NCI-H292 lung cancer cells was measured. First of all, the viability of NCI-H292 cells after treatment was assessed by the CCK-8 method, and the obtained results revealed that the cancer cell viability could be significantly reduced by the compound **1** exposure. In addition to this, the cell apoptosis and ROS accumulation were determined and the data indicated that the compound **1** has the ability to induce cancer cell apoptosis by triggering the ROS production. Next, the relative expression of the inflammatory genes revealed that the compound **1** could also inhibit the inflammatory response in the cancer cells. The MDM-2 levels in the NCI-H292 lung cancer cells were obviously decreased by the compound treatment. The possible binding poses are studied in detail by using molecular docking simulations, which could provide evidence for the experimental hypothesis from the molecular level.

2 Experimental

2.1 Chemicals and measurements

All the chemicals used in the experiment could be acquired from market and they were utilized without additional purification. The analysis of elements for nitrogen, hydrogen, and carbon was carried out using a PerkinElmer 240C analyzer. The infrared spectrum from 4,000 cm^{−1} to 400 cm^{−1} obtained by the solid reagent was recorded on a Bruker ALPHA spectrometer. Using a Bruker-D8-Advance X-ray diffractometer to collect the PXRD data, we find that λ is 1.5418 Å and the range of 2θ is 5° to 50°. In order to conduct the thermogravimetric analyses, set the LABSYS Evo thermal analyzer in

nitrogen environment which increases 5°C every minute, and the temperature is in the range of 25–800°C. The ultraviolet-visible absorption spectrum in the range of 200–800 nm was collected by a ultraviolet spectrophotometer. The photoluminescence spectrum was measured on the FLS1000 Photoluminescence Spectrophotometer of Edinburgh instrument.

2.2 Preparation and characterization for {[Dy(H₂O)(BTCTB)]·2H₂O}_n (**1**)

We mixed the 0.1 mmol and 45 mg of Dy(NO₃)₃·6H₂O, 0.05 mmol and 28.4 mg of H₃BTCTB, and 30 μ L and 0.01 mmol L^{−1} of water NaOH solution with doubly deionized 30 μ L and 0.01 mmol L^{−1} of water NaOH solution with the 10.0 mL doubly deionized water to generate a mixture, and stored the obtained mixture into the 23.0 mL autoclave lined with Teflon. Afterward, we heated the autoclave for four days at 160°C under autogenous pressure. After cooling the above mixture slowly at the rate of 3.0°C h^{−1} to the ambient temperature, we can directly acquire the colorless crystals with a block shape of the complex **1** which are fit for single-crystal X-ray diffraction, cleaned by utilizing water, and then dried in air with a yield of 87% (on the basis of H₃BTCTB). Anal. calcd for C₃₀H₂₄DyN₃O₁₂: N, 5.38%; H, 3.10 and C, 46.14. Found: N 5.44%; H 3.15 and C 46.76. FT-IR (KBr): $\tilde{\nu}$ = 513 (w), 588 (w), 676 (m), 731 (w), 771 (m), 829 (w), 910 (w), 1,081 (w), 1,165 (w), 1,242 (m), 1,292 (w), 1,396 (s), 1,435 (m), 1,534 (s), 1,583 (s), 1,623 (s), 1,669 (s), 3,361 (br) cm^{−1}.

We utilized the Oxford XcaliburE diffractometer to get the X-ray data. Statistical analysis of various strength data was performed using CrysAlisPro software and the results were converted into the HKL format. The SHELXS program was used to create the model directly, and the least square method was used to adjust the model. All of the non-hydrogen atoms contain a number of different parameters of the opposite sex. With the AFIX command, the H atom is pinned to the C atom to which it is attached. According to Table 1, we can see the experimental details of complex **1** and the final crystal data.

2.3 Cell counting assay

Taking the NCI-H292 lung cancer cells as an example, the CCK-8 method was used to test the antiproliferation

Table 1: The crystallographic parameters and the refinement for the complex **1**

Empirical formula	C ₃₀ H ₂₄ DyN ₃ O ₁₂
Formula weight	781.02
Temperature/K	293(2)
Crystal system	Triclinic
Space group	<i>P</i> $\bar{1}$
<i>a</i> /Å	8.69540(16)
<i>b</i> /Å	11.1362(2)
<i>c</i> /Å	15.69870(14)
α /°	70.5695(12)
β /°	78.012(3)
γ /°	77.3710(13)
Volume/Å ³	1383.77(4)
<i>Z</i>	2
ρ_{calc} (g/cm ³)	1.874
μ /mm ⁻¹	15.115
Data/restraints/parameters	4,929/0/415
Goodness-of-fit on <i>F</i> ²	1.023
Final <i>R</i> indexes [<i>I</i> ≥ 2σ(<i>I</i>)]	<i>R</i> ₁ = 0.0332, ωR_2 = 0.0766
Final <i>R</i> indexes [all data]	<i>R</i> ₁ = 0.0374, ωR_2 = 0.0792
Largest diff. peak/hole/e Å ⁻³	1.62/−1.14
CCDC	1985897

effect of the complex, and appropriate modifications were made at the same time. NCI-H292 cells in the growing stage were placed in the 100 μL of DMEM at 1×10^3 cells each well concentration. The culture medium was added with 10% FBS and 1% solution of penicillin–streptomycin. The culture medium was placed at 37°C with a 5% carbon dioxide content overnight. When 80% of the cell population was mixed, the compounds with different concentrations of 1, 2, 4, 8, 10, 20, 40, and 80 μM were left to remain idle for 24 h. Then, the original medium was replaced by 10 μL of CCK-8 solution and placed in a dark environment at 37°C for 1 h. Finally, BioTek ELx808 Absorbance Microplate Reader was used to measure the absorbance at 450 nm. According to the results of the experiment, the cell survival rate can be obtained. The cell survival rate is equal to the result of OD treatment minus OD blank divided by the OD control result minus the blank of OD.

2.4 Intracellular ROS measurement

The ROS content in the NCI-H292 lung cancer cells was determined by using the 2',7'-dichlorofluorescein diacetate test kit. First of all, the NCI-H292 cells were kept in a 6-well plate for 6 h and then a certain concentration of compounds was added for treatment. Then 20 μM DCFH-DA solution was poured in and placed in a dark

environment at 37°C for half an hour. After cleaning the cells with PBS, flow cytometry was utilized for the analysis of cell absorbance in different experimental groups.

2.5 ELISA

After treated through compound **1**, the inflammatory response level in the NCI-H292 lung cancer cells was determined by the ELISA test of the TNF-α and IL-1β content. This assay was performed strictly according to the instructions. In short, the NCI-H292 cells in the logarithmic growth stage were inoculated into 6-well plates for half a day, and after that, we added compound **1** for cell treatment at the indicated concentration. After treatment, the cell supernatant was harvested and the TNF-α and IL-1β content was measured with the ELISA test kit. This test is needed to be implemented three times or more.

2.6 Western blotting detection

The western blotting was performed in this current study to assess the MDM-2 protein expression levels in the NCI-H292 lung cancer cells, as well as the influence of the compound on the MDM-2 relative expression. All the performances in this research were accomplished under manufacturer's guidance with slight changes [18,19]. In short, the NCI-H292 lung cancer cells were harvested and then inoculated in the 6-well plates with cells' ultimate density of 1×10^6 cells each well concentration. The plates were kept in the cell incubator at 5% CO₂ and 37°C environment. After culturing for 12 h, the compound was added for treatment with $1 \times \text{IC}_{50}$ and $3 \times \text{IC}_{50}$. Next, the cells were harvested and then lysed by utilizing RIPA buffer for the overall extraction of protein. The BCA Protein Assay kit (23225, Pierce, USA) was recommended to evaluate the protein samples' quality and quantity. After that, the protein samples were divided into equal amounts and loaded onto 10% SDS-PAGE denaturing gels, followed by transfer onto PVDF membranes. All of the PVDF membranes were sealed with non-fat milk of 5% for 120 min under ambient temperature and then inoculated at the 4°C overnight utility with the proper primary antibody toward MDM2 or glyceraldehyde-3-phosphate dehydrogenase. Subsequently, the secondary antibody bound to horseradish peroxidase was added for

incubation for another 60 min. Ultimately, the MDM-2 proteins were visualized by utilizing the increased ECL test kit and quantified with the ImageJ software (BIO-RAD).

2.7 Simulation details

To perform the molecular docking simulation, the complex and the probe protein were prepared by AutoDockTools, after which the simulation was performed by AutoDock4. The MDM-2 protein was selected as the probe protein, and the protein structure was downloaded from the protein database. The PDB ID of MDM-2 protein is 4UD7. On the other hand, the complex contains the organic moiety as well as the Eu metal core. The complex has been treated as the semiflexible ligand. The coordinate of the grid center is $-4.166, 2.034, 19.229$.

The length of the grid box is 80. The unit of length is angstrom. The maximum allowed number of poses for scoring is 20. The visualizations have been complemented by PyMol.

Ethical approval: The conducted research work is not related to either human or animal use.

3 Results and discussion

3.1 Molecular structure

With sodium hydroxide as the pH regulator, the Dy(NO_3) $_3 \cdot 6\text{H}_2\text{O}$ reacts with H_3BTCTB for four days in water to afford white crystals of **1** with a yield of 87%. The addition of NaOH could not only control the pH value of the reaction system, but could also facilitate the deprotonation of the H_3BTCTB ligand. The single-crystal X-ray analysis reflects that the complex **1** is crystallized in the triclinic space group of $P\bar{1}$ (Table 1), displaying a (4,8)-linked skeleton that contains the paddle wheel subunits $\{\text{Dy}_2(\text{COO})_4\}$ extended through the 4-linked linkages of BTCTB^{3-} . The complex **1**'s asymmetric unit contains a Dy(III) ion, a triple H-removed anion of BTCTB^{3-} for the compensation of charge, two lattice molecules of water as well as a terminal aqua ligand. As illustrated in Figure 1a, the Dy(III) ion local coordination surroundings are detected through six carboxylic acid oxygens (O9E, O8E, O6D, O5C, O2A, and O1), a coordinated molecule of water (O12W), and an amide oxygen donor (of O4B) in five deprotonated ligands of BTCTB^{3-} . The lengths of the Dy(III)–O bond vary from 2.292 to 2.553 Å. The distinctive Dy(III) ions are octacoordinated. CShM = 1.241 is calculated through the SHAPE software, and the mode of coordination is the micro distorted double triangular prism. By contrast, the sole ligand of BTCTB^{3-} in the complex **1** uses the pattern of

Figure 1: (a) The coordination environment of the Dy(III) ion view in the complex **1**. (b) The coordination fashion for ligand. (c) The complex **1**'s three-dimensional network. (d) The complex **1**'s 4,8-linked skeleton.

$\mu_6\text{-}\eta^1\text{:}\eta^1\text{:}\eta^1\text{:}\eta^1\text{:}\eta^1\text{:}\eta^1\text{:}\eta^0\text{:}\eta^0$ to complex to six Dy(III) ions via an amide oxygen donor and six carboxylic acids (Figure 1b), displaying an uncommon heptadentate ligand. The BTCTB³⁻ ligand with six-coordination pattern makes the central phenyl ring not coplanar with three benzoyl groups, and the dihedral angles of the central phenyl ring and each carboxylic acid group, respectively, are 6.7°, 26.5°, and 74.8°. Two pairs of carboxylic acid groups in four symmetrically related ligands of BTCTB³⁻ aggregated two independent Dy(III) ions into the subunit {Dy₂(COO)₄} with a paddle wheel-shape, and the distance from metal to metal is 4.3362(2) Å (Figure 1c). Each of the subunits {Dy₂(COO)₄} is surrounded periodically by eight anions of $\mu_6\text{-BTCTB}^{3-}$, acting as the 8-linked node in topology. On the contrary, because of the extra coordination of the amide oxygen atom with the Dy(III) ion, the C₃-symmetric ligand of BTCTB³⁻ links with four subunits {Dy₂(COO)₄} unexpectedly, which leads to the tripod linker evolve to the 4-linked node. The 8-linked subunits {Dy₂(COO)₄} and 4-linked ligands BTCTB³⁻ are interlinked, generating a fresh 4,8-linked skeleton (Figure 1d).

The purity of the complex **1** can be checked by X-ray diffraction patterns. According to Figure 2a, the experimental results and PXRD simulation results have been consistent, indicating that the final crystal is a perfect reactant. The difference between the two results may depend on the quality of the experimental samples. In order to test the degradation of PA in the water, it is essential to detect complex **1**'s stability in the water. Compared with the original sample, the difference between the two results reflects its stability in water. The results show that complex **1** is quite stable. At the same time, we put **1** in a 25–800°C environment, increased 10°C every minute and carried out

thermogravimetric analysis in nitrogen environment to judge its thermal stability. Figure 2b shows that the first weightlessness occurs between 20 and 220°C, when coordination water molecules and lattice water molecules are released. Then, place **1** in the environment below 500°C. Further heating, we can see that **1** is in the second weightlessness process, structure collapse, and gradual decomposition of ligands. The experimental results show that the stability of **1** is very good, which meets the important preconditions for its application. In the infrared spectrum, the wide absorption band centered at 3,360 cm⁻¹ is attributed to the O–H stretching vibration, suggesting the presence of water molecules. There is no peak at 1,735 cm⁻¹, which means that the organic ligands are deprotonated completely. Accordingly, there are several strong bands of symmetric and asymmetric stretching vibrations for the carboxylic acid group at 1,621, 1,670, 1,534 and 1,582, 1,396, and 1,434 cm⁻¹. These data mentioned above proved that there is an effective coordination behavior between the Dy(III) ion and the triply deprotonated ligand of BTCTB³⁻.

3.2 Fluorescence behavior and selective detection of nitroaromatic compounds

The luminescent MOF based on Dy(III) has an excellent luminous performance. As shown in Figures 1 and 3a, it shows a strong characteristic transition at 393, 467, and 561 nm of the fluorescence spectrum. The coordination of Dy(III) ions leads to π to π^* transition, which leads to

Figure 2: (a) The complex **1**'s diagrams of PXRD. (b) The TGA curve for the complex **1**.

393 nm emission spectrum. $^4F_{9/2}$ to $^6H_{15/2}$ leads to 467 nm emission spectrum. $^4F_{9/2}$ to $^6H_{13/2}$ leads to 561 nm emission spectrum. In order to determine the PA content, fluorescence quenching titration was used. In order to study the sensing ability of **1**, PA, 1,4-dinitrobenzene (1,4-DNB), 4-nitrotoluene (4-NT) and 3-nitrotoluene (3-NT), 3-chloronitrobenzene (Cl-NB), nitrobenzene (NB) as well as 2,4-dinitrotoluene (2,4-DNT) were selected for analysis. In order to investigate the effect of the complex **1** against the aromatic nitro group, the luminescent performances of other compounds were tested. By continuously increasing the concentration of PA and recording the emission spectrum, we can study the influence of **1** on the conductivity of PA in water. As the PA amount changed from 20 to 200 μL (1.0 mM), 95.5% of **1** was quenched by ultraviolet-visible emission, and the quenching efficiency of all substances, such as NB,

Cl-NB, PA, 3-NT, 2,4-DNT, 1,4-DNB, and 4-NT, ranged from high to low (Figure 3b). It is found that the aromatic compounds have a unique effect on the fluorescence intensity of **1** in water. After the addition of other substances, the fluorescence of **1** in water shows no change. After the addition of PA, the fluorescence is greatly weakened, which shows that **1** is very easy to perceive the electron defective analyte (Figure 3c). In conclusion, **1** can be used as a nitroaromatic sensor. The uniqueness of PA compared with those of other substances is very important in practical applications. By adding 4-NT, 3-NT, PA, 2,4-DNT and 1,4-DNB, NB as well as Cl-NB, the linearity of quenching can be observed by comparing PA with other nitroaromatics. When the amount of analyte added is consistent with the PA solution, the fluorescence intensity is immediately quenched.

Figure 3: (a) The luminescent quenching of the complex **1** dispersed in the water solution, after adding the PA to the water solution. (b) The quenching rates of the compound **1** in the presence of some other analytes. (c) The luminescent quenching of the complex **1** dispersed in the water solution, after adding some other analytes and adding the PA. (d) The SV diagram for a variety of nitro-analytes.

In order to determine the complex **1**'s quenching rate, we use the SV equation of $I_0/I = K_{SV}[Q] + 1$, in which the I_0 refers to the fluorescence strength in the absence of the analyte, the I refers to the fluorescence intensity when the analyte is added, the K_{SV} represents the quenching constant, and the $[Q]$ represents the analyte's molar concentration. According to the SV diagram in Figure 3d, PA is low concentration state and inclines upward when it reaches the critical point, while other analytes are always linear. The unique performance of PA may be due to the energy transfer between the PA and the complex or its own absorption of energy. The quenching constant of PA is $8.55 \times 10^4 \text{ M}^{-1}$, which shows that **1** is very strong for PA (Figure 4a). At the same time, the quenching constant of PA in water is higher than the original value [20–22]. In order to detect the sensitivity of **1**, the low concentration PA was quenched. Use $3\sigma/\text{slope}$ to calculate the detection limit (Figure 4b). The σ was acquired from the fluorescence strength test without PA. According to the fitting curve for the concentration of PA and fluorescence strength, the slope can be obtained. The calculation shows that the LOD of the sensor is $0.71 \mu\text{M}$, so the sensor is very sensitive to PA in water.

3.3 Compound **1** inhibits the viability of NCI-H292 lung cancer cells

After synthesizing the compound **1** with a fresh structure, the antiviability effect of the compound **1**

Figure 5: The viability of the NCI-H292 cells was decreased with the dose-dependent pattern after treated through the compound **1**. The NCI-H292 cells were treated through the indicated compound **1**'s high dilutions for one day. After this treatment, the absorbance was determined and the diagram of the cell viability was plotted and the compound's IC_{50} value was calculated. This research study is needed to be implemented three times or more.

toward the NCI-H292 lung cancer cells was measured through the CCK-8 method. According to the results illustrated in Figure 5, after the exposure of the compound **1** for one day, the viability results of the lung cancer cells were detected and then plotted. We find that the compound **1** inhibited the NCI-H292 lung cancer cells with the dose-dependent pattern. The complex **1**'s IC_{50} value against the NCI-H292 cells was $2.78 \pm 0.09 \mu\text{g/mL}$, which was calculated based on the results of CCK-8. The obtained results suggested that the compound **1** may be a good candidate anticancer drug for the treatment of lung cancer.

Figure 4: (a) Fluorescence quenching of compound **1** through a variety of PA concentrations, where the I and I_0 , respectively, represent the fluorescence signal strengths for sensing material in the presence and absence of analyte. $I_0/I = K_{SV}[\text{PA}] + 1$ (correlation coefficient, $R^2 = 0.983$). (b) The relation between the fluorescence intensity and PA added into the suspension of **1** and their linear fit curve for the estimation of LOD ($R^2 = 0.987$).

3.4 Compound 1 induces ROS accumulation and inflammatory cytokine release in NCI-H292 lung cancer cells

The inflammatory response level of cells is crucial for the survival of cells, and the inflammatory response level is commonly displayed as the ROS accumulation level and the release of inflammatory cytokines. Therefore, DCFH-DA is needed to measure the ROS in the NCI-H292 cells. According to the results, we can see that the higher the compound **1** dose, the higher the ROS content in NCI-H292 cells (Figure 6a). When the concentration of the compound **1** was $1 \times IC_{50}$, the ROS positive cells were 12.74%, but when the concentration of the compound **1** was $3 \times IC_{50}$, the ROS positive cells increased to 83.67%, suggesting that there was a dose-response relationship between the compound treatment and ROS accumulation in the cancer cells. In addition to this, ELISA was further exploited to determine the TNF- α and IL-1 β level released from the NCI-H292 cells after compound **1** treatment. In Figure 6b, we can see that compound **1** treatment obviously reduced the TNF- α and IL-1 β content with the dose-dependent pattern. This result is in accordance with the above-mentioned results of ROS, suggesting that the compound **1** has an outstanding antitumor activity on the NCI-H292 lung cancer cells.

3.5 Compound 1 regulates the expression levels of the MDM-2 protein in NCI-H292 lung cancer cells

In the former research work, we have confirmed that the compound **1** has an outstanding inhibition against the proliferation of the NCI-H292 lung cancer cells and promotes the ROS accumulation in the cells. In this section, a further study was performed on the exploration of an anticancer mechanism. The MDM-2 is highly expressed in the NCI-H292 lung cancer cells, and its expression level is correlated with clinical symptoms, which also participate in the occurrence, growth, invasion, and lymph node metastasis of NCI-H292 lung cancer. The expression levels of MDM-2 have been widely used as indicator symptoms of lung cancer development. According to the results illustrated in Figure 7, we find that there was an obviously higher level of the MDM-2 levels in the NCI-H292 lung cancer, in comparison with the BEAS-2B human normal lung epithelial cells. After treated through the compound at

the concentrations of $3 \times IC_{50}$ and $1 \times IC_{50}$, the MDM-2 expression levels in the NCI-H292 lung cancer cells were decreased significantly and dose-dependently (Figure 7a). The quantification of the protein expression is shown in Figure 7b.

3.6 Molecular docking

In order to understand if the tumor antiproliferation were due to suppression of MDM-2 gene expression alone or due to a combination with MDM-2 inhibition, a molecular docking study was carried out. A major challenge in the identification of the binding site is because of the protein flexibility. It has been shown in a previous study that the MDM-2 protein has two novel binding sites, that is why it has been chosen as the probe protein in the current study. It is obvious that the Dy(III) complex has multiple “-NH-CO-” functional groups, which have been suggested from the experimental results that these functional groups could be the source of the binding interactions. In order to explain the experimental hypothesis, the energy favorable binding poses have been shown in Figure 8. From here, we can see that the complex indeed has multiple interactions to the protein, and almost all of the identified interactions are formed by the “-NH-CO-” functional groups from the complex. The corresponding binding energies are -8.63 (7a), -8.13 (7b), -8.07 (7c), and -7.95 (7d) kcal/mol.

Although from Figure 8 the binding interactions can be observed, it is relatively unclear as to what are the interacting residues and non-interacting residues that are surrounding the complex. In order to see the binding interactions in a more detailed way, the binding poses that have been obtained from the molecular docking simulation have been converted to 2-dimensional presentations by using the ligplus. The results are shown in Figure 9. In Figure 9a, the complex is shown to have three binding interactions, all of which are from the nitrogen atoms on the complex, and the involved residues are Ala21 (3.17), Asn111 (2.75), and Thr26 (2.81). In Figure 9b, not only is the nitrogen atom found to form the binding interaction, but the oxygen atom on “-NH-CO-” functional group is also found to form the binding interaction. The interacting residues are Gln24 (2.94) and Thr26 (2.93). There are other binding interactions in Figure 9b, which are mainly from the oxygen atoms near the metal core, which shows less importance. In Figure 9c, Ser17 (2.39) and Thr26 (2.81) are found to be

Figure 6: Reduced ROS accumulation and inflammatory cytokine release in NCI-H292 cells after treated through the compound 1. The NCI-H292 cells were treated with a series of indicated complex 1's dilutions ($3 \times IC_{50}$ and $1 \times IC_{50}$) for one day. (a) The ROS level in the NCI-H292 cells was measured by the detection kit. (b) The TNF- α and IL-1 β content in the NCI-H292 cells was measured by the ELISA test kit.

Figure 7: The inhibited MDM2 protein expression levels in the NCI-H292 lung cancer cells after compound treatment. The NCI-H292 cells were treated through the indicated distinct complex **1**'s concentrations ($3\times IC_{50}$ and $1\times IC_{50}$) for one day. The expression level of the MDM2 protein was measured with the western blotting assay (a). The quantification of figure a (b).

the interacting residues. In Figure 9d, three binding interactions are formed and only Thr26 is involved. As such, the experimental observations have been

confirmed and the interacting forms are shown at the molecular level. The molecular docking studies support the hypothesis that compound **1** also inhibits MDM-2.

Figure 8: The energy favorable binding poses indicate multiple binding interactions between the complex and the probe protein, and the binding energies are -8.63 (1a), -8.13 (1b), -8.07 (1c), and -7.95 (1d) kcal/mol. The cyan moieties indicate the synthesized Eu complex, whereas the green moieties present the interacting residues.

Figure 9: 2-Dimensional binding poses that have been presented in Figure 8, from which the interacting and non-interacting residues can be seen clearly. Their residue names as well as the binding distances are shown to guide the eye.

The *in vitro* studies are in progress and will be reported in due course.

4 Conclusion

In conclusion, we have synthesized a Dy(III)-MOF by hydrothermal reaction of Dy(NO₃)₃ with the C₃-

symmetric organic ligand H₃BTCTB at 160°C for 96 h. The crystal structure analysis of **1** by single-crystal X-ray diffraction reflects that the complex **1** is a three-dimensional skeleton structure on the basis of the subunits {Dy₂(COO)₄} with a paddle wheel-shape and displays the 4,8-linked network. At the same time, the sensitivity of PA in water medium was tested with material **1** as a fluorescent sensor. The detection limit was 0.71 μM and *K*_{SV} of this experiment was 8.55 ×

10^4 M^{-1} , which might be attributed to the presence of abundant amide groups in the framework of **1**. In the bio-research, the inhibition of the complex **1** against the NCI-H292 lung cancer cells was assessed. First of all, the CCK-8 method was conducted and the data showed that the viability for the NCI-H292 cells was decreased obviously after treated through the compound **1**. Afterward, the DCFH-DA results reflected that the ROS production in the NCI-H292 cells was significantly inhibited. The inflammatory genes' relative expression was also inhibited by the compound **1** exposure, which is confirmed by the RT-PCR assay, and the MDM-2 levels in the NCI-H292 lung cancer cells were obviously decreased via the compound treatment. The molecular docking study also proved the interaction between the MDM-2 and the synthetic compound **1**.

Conflict of interest: The authors declare that there is no conflict of interest regarding the publication of this paper.

Data availability: The data used to support the findings of this study are included within the article.

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