Indian Journal of Chemistry
Vol. 61, January 2021, pp. 31-36

A new Ln(III) coordination polymer constructed from a hexadentate triazine ligand: Treatment activity and nursing value for ovarian cancer

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Received 30 November 2021; revised and accepted 24 December 2021

In this current paper, a 3D lanthanide (Ln) coordination polymer (CP), compound **1** with a chemical formula of $[(NH_2(Me)_2)]_6\cdot[Gd_3(TATAT)_2(\mu_2\text{-}O)_{1.5}]\cdot 3EtOH\cdot 7.5H_2O$ is synthesized with the reaction between $GdCl_3\cdot 6H_2O$ and 5,5′,5″-(1,3,5-triazine-2,4,6-triyltriimino)tris(azanediyl) triisophthalate (H_6TATAT). Its treatment activity and nursing value against ovarian cancer is tested and the associated mechanism is analyzed simultaneously. At first, the CCK-8 detection is implemented to determine the suppression activity of compound **1** against the cell viability of ovarian cancer after treated via our compound. Furthermore, in order to detect the relative expression for the estrogen receptor on cells of ovarian cancer, real time RT-PCR is carried out.

Keywords: Coordination polymer, Ovarian cancer, RT-PCR

Ovarian cancer is defined as the fifth major reason of death in women. On account of lack of the effective early detection indicators together with its location oncealment, it has the highest mortality rate in all of the gynecological tumors, which produces a great threat to lives of women[1]. The five-year survival rate for highly aggressive ovarian cancer is less than 30 percent, according to new research. Nonetheless, up to now, the ovarian cancer pathogenesis is not completely understood[2,3]. As a result, it is essential to further investigate the molecular mechanism of the ovarian cancer.

The architecture together with design of the supramolecular structures involving metal in accordance with crystal engineering is a research hotspot in the area of the coordination chemistry along with supramolecular chemistry. People are increasingly interested in the above area because of their valuable unit architectures, simultaneously because of their underlying application prospects in the biochemistry, catalysis as well as luminescence, in particular, in the area of the modern pharmaceutical chemistry[4-7]. Functional complexes have received significant interest among the prepared compounds because of their underlying medicinal value. As a result, for the clinical application, drug therapy as well as the architectural design, choosing biocompatible, safe and efficient ligands is regarded as a critical factor[8-11]. Heterocyclic ligands involving nitrogen or the polycarboxylic acids and other polydentate ligands have been widespread exploited in the proper design and controlled generation of the multifunctional compounds. In the last several years, N-heterocyclic carboxylic acid ligands have aroused great interest of the biologists along with chemists due to their rich functional properties as well as the coordination patterns, together with the H bond receptors and donors in solution[12-15]. In this current paper, a fresh 3D lanthanide coordination polymer (CP), with a chemical formula of $[(NH_2(Me)_2)]_6\cdot[Gd_3(TATAT)_2(\mu_2\text{-}O)_{1.5}]3EtOH\cdot 7.5H_2O$ (compound **1)** was generated with the reaction between $GdCl_3\cdot 6H_2O$ and H_6TATAT. This complex was well characterized through elemental analysis, IR spectroscopy, and single crystal X-ray diffraction (XRD), thermo gravimetric analysis (TGA) as well as powder XRD. The architectural analysis demonstrated that compound **1** is constructed from the binuclear building blocks of $[Gd_2(COO)_{12}(\mu_2\text{-}O)]$ linked through the triazine hexacarboxylic framework, and it displayed a fascinating 3-dimensional microporous architecture. CCK-8 detection and the real time RT-PCR was performed for measuring the clinical and nursing value of the compound for treating the ovarian cancer.

Experimental Details

Chemicals and measurements

The H_3TATAT ligand (97%, AR) was supplied by Jinan Henghua Chemical Reagent Company and was used as received. A Perkin-Elmer 240 C automatic analyzer (Waltham, USA) was employed for conducting the N, H together with C elemental analysis at the analysis center of Liaoning Normal University. The Advance-D8 with 5° < 2θ< 55° Cu-Kα radiation was applied for collecting the patterns of PXRD, at 2 s/step count time and 0.02° (2θ) step size. TGA was performed with the Perkin Elmer Diamond TG/DTA with 10 °C per min heating rate between RT and 800 °C. IR spectra were collected using Bruker AXS TENSOR-27 FT-IR spectrometer in 400 - 4000 cm^{-1} range and sample was prepared in the form of utilizing KBr pellets.

Synthesis and characterization for $[(NH_2(Me)_2)]_6\cdot[Gd_3(TATAT)_2(\mu_2\text{-}O)_{1.5}]\cdot 3EtOH\cdot 7.5H_2O$ (1)

The mixture synthesized by 0.0187 g of $GdCl_3\cdot 6H_2O$ (0.05 mmol) and 0.0145 g of H_6TATAT (0.025 mmol) were lysed in ethanol (2 mL, EtOH) and *N*,*N*-dimethylacetamide (4 mL, DMA) mixed solution. The created mixture was subsequently stirred at RT for half an hour, and the solution was added with NaOH (2 mL, 0.1 mol/L). Afterwards, its pH was adjusted as 6–7 through utilizing the dilute hydrochloric acid. Then, after stirring it for half an hour, the solution was put into a polytetrafluoroethylene reactor (20 mL) and kept in an oven at a temperature of 160 °C in order to heat for 7 days. Afterthat, solution was cooled to RT, which were then cleaned using DMA to gather the light yellow transparent massive crystals. Molecular formula: $C_{54}H_{24}N_{12}O_{25.5}Gd_3$ (1725.65), with a yield of 59% (in the light of H_6TATAT). Elemental analysis: calc (%): N, 1.98, C, 55.92 and H, 4.24; found (%): N, 1.95, C, 55.88 and H, 4.19. IR (KBr pellet, cm^{-1}): 535(s), 580(w), 618(w), 689(s), 710(m), 756(s), 776(s), 836(m), 925(s), 1005(w), 1026(m), 1108(s), 1150(m), 1162(m), 1233(s), 1312(s), 1338(s), 1364(m), 1380(m), 1451(s), 1489(s), 1563(s), 1593(s), 2783(m), 2934(m), 3063(m), 3423(m).

The Oxford Xcalibur E was used for acquiring the data of X-ray. CrysAlisPro was used for analyzing the strength data, afterwards, the data was converted to the HKL files. The SHELXS according to direct approach as well as the SHELXL-2014 software according to least-squares strategy were employed, respectively, for the synthesis and refinement of original architectural modes. After the use of entire non-H atoms, we can fix the anisotropic parameters. Ultimately, the whole H-atoms were next fixed on the carbon atoms, which they are bridged with AFIX commands in the geometry. The optimizations of the as-prepared compound along with its parameters of crystallography were shown in the Table 1.

Cell Counting Kit-8 assay

With the aim of testing the inhibitory effect of as-synthesized compound on the viability of ovarian cancer cells, the Cell Counting Kit-8 was used for the detection. This implementation was finished totally adhere to the instructions after minor refinement. Generally, the cells of ovarian cancer in a stage of logical growth were gathered, which were next inoculated into the plates (96 well, 5000 cells in each well), the above cells were next stored in the incubator with 5% CO_2 under a temperature of 37 °C for half a day. Afterwards, the wells were treated with the compound in order to accomplish treatment at a variety of concentrations (namely, 1, 2, 4, 8, 10, 20, 40 and 80 μM). 5-FU together with the solvent was employed as the positive and negative control. After finishing the above treatment, discarding the medium

Table 1 — Optimization details and crystallography parameters of compound **1**

Empirical formula	$C_{54}H_{24}Gd_3N_{12}O_{25.5}$
Formula weight	1720.62
Temperature (K)	296.15
Crystal system	cubic
Space group	I23
a (Å)	27.5296(7)
b (Å)	27.5296(7)
c (Å)	27.5296(7)
α (°)	90
β (°)	90
γ (°)	90
Volume ($Å^3$)	20864.1(16)
Z	7.99992
ρ_{calc} (g/cm^3)	1.096
μ (mm^{-1})	1.940
Reflections collected	68127
Independent reflections	8636 [R_{int} = 0.0584, R_{sigma} = 0.0398]
Data/restraints/parameters	8636/18/285
Goodness-of-fit on F^2	1.047
Final R indexes [I>=2σ (I)]	R_1 = 0.0263, ωR_2 = 0.0595
Final R indexes [all data]	R_1 = 0.0307, ωR_2 = 0.0641
Largest diff. peak/hole / e $Å^{-3}$	0.74/-0.37
Flack parameter	-0.010(7)
CCDC	2124858

of culture, next the fresh medium with the reagent of CCK-8 was added to conduct deep incubation for two days. Eventually, at 450 nm, the absorbance in each well could be tested. This study was carried out for 3 times, and all the results were expressed with mean±standard deviation.

Real time RT-PCR

The real time RT-PCR was accomplished in the current investigation for measuring the estrogen receptor relative expression levels on the cells of ovarian cancer after treating utilizing compound **1**. This research was implemented completely abide by the protocols after a little change. Briefly, in a stage of logical growth, ovarian cancer cells were collected, which were subsequently inoculated into the plates (24 well, 100000 cells in each well), the above cells were next stored in the incubator with 5% CO_2 under a temperature of 37 °C for half a day. Afterwards, the wells were added with compound **1** in order to accomplish treatment at a variety of concentrations (namely, 1, 2, 4, 8, 10, 20, 40 and 80 μM). 5-FU together with the solvent was employed as the positive and negative control. Afterwards, we gathered the cells and extracted the overall RNA existed in cells through employing reagent of TRIZOL. After testing its quantity and quality, it could next be reverse transcript into a cDNA. Eventually, the estrogen receptor relative expression on the cells of ovarian cancer after treating utilizing our compound was examined via real time RT-PCR, in which *gapdh* was exploited as the internal control. This present work was performed at least 3 times, and the obtained data were represented with mean±standard deviation.

Results and Discussion

Structural characterization

The architectural exploration of X-ray single crystal suggests that the compound **1** belongs to a cubic *I*23 of space group. Gd1 is 8-coordinated via carboxyl O1, $O1^{\#1}$, and O2, O4, O5, O6 as well as O8 atoms provided by five TATAT together with a linking O3 atom to produce the dodecahedral geometry (Fig. 1a) and this is fully different from that reported in literature. In which, O5, O4, and O2 are coordinated through the Gd1 atom with a bidentate chelate fashion, O8 and O6 are coordinated to the Gd1 atom with a monodentate pattern, O1 together with $O1^{\#1}$ are coordinated to Gd1 atom with a three-bridge fashion. The neighbouring Gd atoms are connected via μ_2-O and linked with a carboxyl group derived from the TATAT to generate $[Gd_2(COO)_{12}(\mu_2\text{-}O)]$, a binuclear architectural unit, which is bridged with 8 ligands. There exist two torsion angles between benzene and triazine rings of

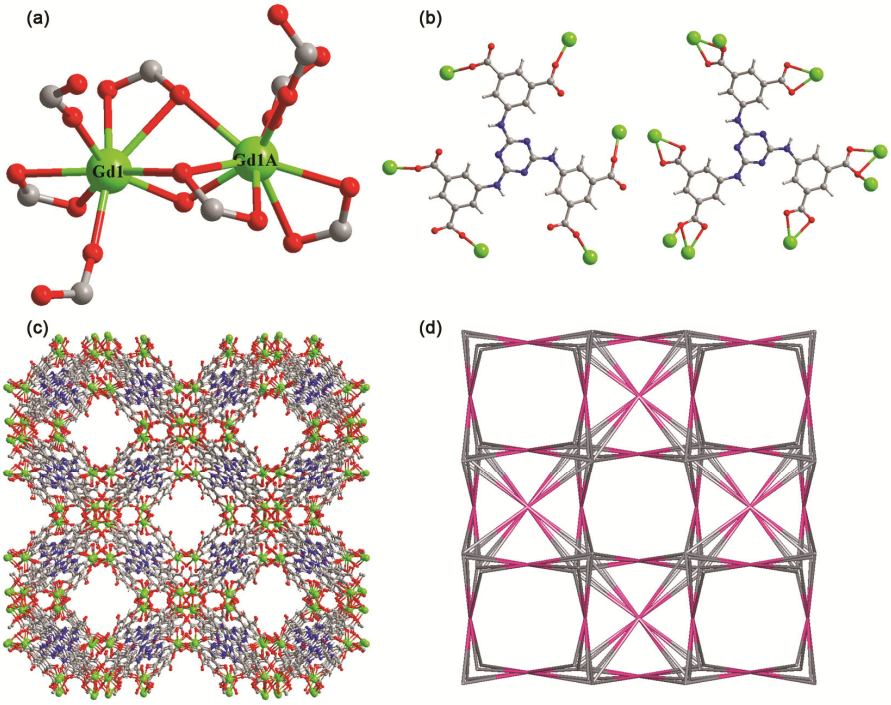

Fig. 1 —(a) Gd2 cluster, (b) coordination manner of ligand, (c) 3-dimensional net and (d) 16-linked net of compound **1**

these ligands, (i) 14.554°, 14.540° and 14.547° for pink-marked ligand and (ii) 10.668°, 10.681° and 10.659° for blue-marked ligand, as reflected in Fig. 1b. Moreover, the TATAT carboxylic acid groups utilize three new patterns, which is different from the patterns reported in literature for the linkage with the coordination center of Gd^{3+}. In mode I, the entire ligand is linked to metal Gd^{3+} with a μ_9-$\eta^2\eta^2\eta^1\eta^2\eta^2\eta^1\eta^2\eta^2\eta^1$ fashion, where the three of six carboxylic acid groups are linked by the bidentate chelate linkage manner and the other three carboxylic acid groups are linked with the Gd^{3+} by a three-bridge manner; In the mode II, the six carboxylic acid groups in TATAT are coordinated to central metal of Gd^{3+} by the monodentate fashion, and the detailed linkage manner is μ_6-$\eta^1\eta^1\eta^1\eta^1\eta^1\eta^1$; In the mode III, the detailed linkage fashion is μ_8-$\eta^1\eta^2\eta^2\eta^2\eta^2\eta^1\eta^2\eta^2$, where the four of six carboxylic acid groups in TATAT employ a bidentate chelate pattern for the coordination with the central metal of Gd^{3+}, and the other two carboxylic acid groups utilize a three-bridge manner for the coordination with central metal of Gd^{3+}. Every two bilayer ligand bridges $[Gd_2(COO)_{12}(\mu_2\text{-}O)]$, a six binuclear architectural unit (Fig. 1c) to produce a 3-D porous structure, and for each square channel, the size is 13.8 Å × 14.3 Å. In topology, two consecutive metals are considered to be a linking node, which employs eight-coordinated vertices, and each of the organic connector utilizes as a 6-coordinated node in each frame. Therefore, the compound **1** exhibits an unprecedented 16-connected uninodal network architecture containing $\{3^{40}\cdot4^{66}\cdot5^{14}\}$ symbol, and it has not been reported (Fig. 1d).

For the exploration of the phase purity of compound **1**, the study of PXRD for the as-prepared compound was performed (Fig. 2a). Between the PXRD patterns peak positions of the simulation and experiment, there exist a well accordance, and this result suggests that the crystal architectures is a real representation of massive crystal products. The strength differences are probably resulted from crystal samples preferred selection. Simultaneously, for exploring the thermal stability of the compound, the TGA was performed under the N_2 flow under a temperature from 30 to 800 °C with 10 °C per min increasing rate. The TGA plot of compound **1** is shown in Fig. 2b. Here its weight loss was happened in two stages. The first loss appears in the temperature range between RT and 600 °C, which is probably owing to the loss of 7.5 free H_2O molecules, physical H_2O molecules, three molecules of EtOH as well as six $(NH_2(Me)_2^+)$ ions generated from DMA decomposition (with the theoretical and measured values of 39.68% and 39.91%). The second weight loss appears between 600 and 800 °C, and it is on account of the loss of TATAT moiety (with the theoretical and measured values of 22.97% and 22.81%). Ultimately, the molecular structure of the compound got collapsed, and the residue was metal oxide.

Viability reduction of ovarian cancer cells

Bio-activity of compound **1** against the treatment of ovarian cancer was tested via measuring the cell viability of ovarian cancer cells using CCK-8 kit. On the basis of Fig. 3, different from control group, our

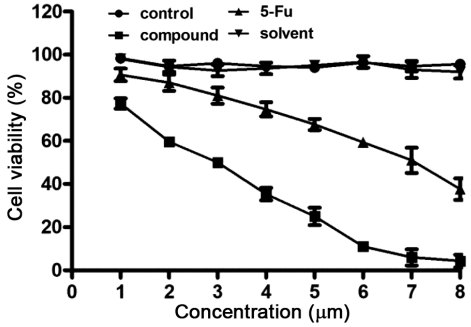

Fig. 3 — Plots for cells viability of the ovarian cancer (tested using CCK-8) with different concentrations of compound **1**. 5-Fu and the solvent were employed as the positive and negative control

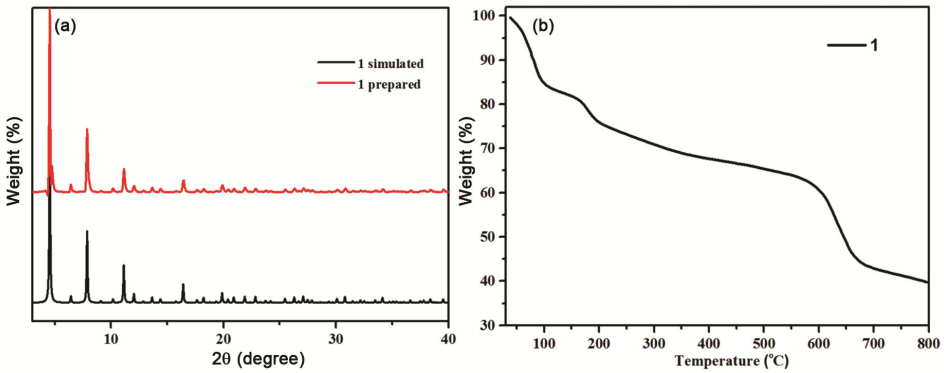

Fig. 2 — (a) PXRD and (b) TGA plots of compound **1**

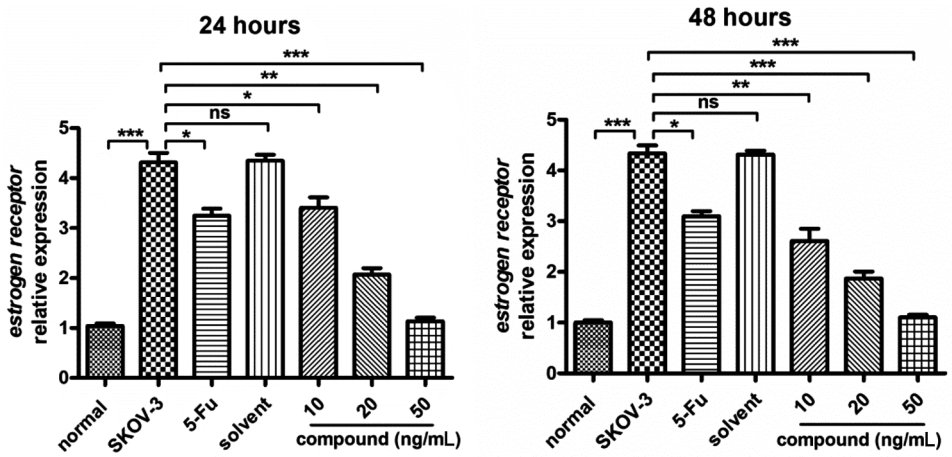

Fig. 4 — Real time RT-PCR plots for estrogen receptor relative expression levels on the cells of ovarian cancer after treating compound **1**. 5-Fu and the solvent were employed as the positive and negative control

compound could remarkably decrease the cells viability of ovarian cancer. Simultaneously, the solution exhibited that there is no bio-activity on the cell viability of ovarian cancer. After finishing this study, it can be observed that the bio-activity of compound **1** was much superior than 5-Fu, a positive drug, which indicating the superb application prospects on treating ovarian cancer.

Inhibition of estrogen receptor expression on the ovarian cancer cells

In the previous work, we have confirmed that compound **1** could remarkably decrease the cells viability of ovarian cancer, which is much superior than 5-Fu. Except for this, the estrogen receptor exerts a principal effect in ovarian cancer cells proliferation together with their apoptosis, hence, on the cells of ovarian cancer, the estrogen receptor relative expression was also measured via employing real time RT-PCR detection. According to Fig. 4, there exists an evidently enhanced estrogen receptor expression on the cells of ovarian cancer, which is different from control group. Between the above two groups, there exist an evidently difference, where P is less than 0.005. After treating through compound **1**, the degrees of the estrogen receptor on cells of ovarian cancer were declined in a dose dependent fashion. The solution suggested that there is no bio-activity on the cell viability of ovarian cancer. From the plots, it can be observed that the bio-activity of compound **1** is much superior than 5-Fu.

Conclusion

In conclusion, we have synthesized a coordination polymer compound **1** with the reaction between $GdCl_3 \cdot 6H_2O$ and H_6TATAT. The architectural analysis demonstrated that compound **1** is constructed from the binuclear building blocks of $[Gd_2(COO)_{12}(\mu_2\text{-}O)]$ linked through the triazine hexacarboxylic framework, which displayed a fascinating 3-dimensional microporous architecture. The detection of CCK-8 indicated that our compound could remarkably decrease the cells viability of ovarian cancer. Except for this, the estrogen receptor levels on the cells of ovarian cancer were down-regulated by the dose dependent fashion, which is superior than 5-Fu. Eventually, it can be concluded that our compound could be a superior candidate for treating the ovarian cancer with the decrease of estrogen receptor on the cells of ovarian cancer.

Acknowledgement

The research was supported by Ningbo Public Welfare Science and Technology Project (No.2021S158).

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