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# Dual functional hybrid-polyoxometalate as a new approach for multidrug delivery

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ABSTRACT

Herein, a new antitumor active polyoxometalate, $(TBA)_4H_3[GeW_9V_3O_{40}]$, has been introduced as an inorganic drug with enormous influence on brain cancer cells and its outstanding results against U87 cells have been described. Post-functionalization of this polyoxometalate produces a drug delivery vehicle which comprises both kinds of inorganic and organic drugs. This system is consisted of mesoporous silica nanoparticles as organic drug carrier, along with redox-responsive disulfide bonds for drug release into the cell at the same time. Moreover, a fluorescence dye has been attached to the polyoxometalate that allows for tracking the drug into the cell environment. This dual-functionalized polyoxometalate was utilized in a designed multidrug delivery system, and worked greatly against U87 cells and knocked down these cancer cells up to near 70% in 48 h. Due to its unique properties, this multidrug delivery vehicle is potent to be developed and used for various applications in cancer therapy.



## 1. Introduction

Polyoxometalates (POMs) are molecular nano-sized early transition metal oxides that have been considered in many fields such as catalysis, analytical chemistry, material science, pharmaceutical, medicine and biosensors because of their diversity in molecular structure, composition, solubility, electrical properties and reactivity [1–3]. The anticancer, antibacterial and antiviral activities of these compounds have been shed more lights on the development of low-cost, tunable and novel inorganic drugs [4–7]. Substitution of transition metals and post functionalization with different organic groups modulate the bioactivity of POMs, enhance the targeting of bio macromolecules and facilitate their usage as drug [4].

Recently, a number of POMs such as PM-17, PM-26, PM-32, and organotitanium substituted heteropolytungstates were found to exhibit antitumor activities in various cancer cells [8–11]. In addition, it was shown that POMs could inhibit some enzymes such as protein kinase CK2 [12], sulfotransferases [13], DNA polymerases [14], HIV-1 protease [7], alkaline phosphatase, catalase [15] and NTPDase [6]. Furthermore, several researches have been undertaken to demonstrate the mechanism of POMs antitumor effect through induction of cell apoptosis and inhibition of ATP generation [16,17]. Furthermore, it has been shown that POMs could interact with basic fibroblast growth factor [18].

As mentioned above, many efforts have been done to use the POMs as an antitumor agent [16,19,20]. But all of them suffered from the lack of comprehensive procedures to utilize POMs as a drug which allows us to track the drug into the cells and at the same time enables us to conjugate POM to the other drugs or biological molecules to exhibit synergistic effects. On the other hand, using nanoparticles in drug delivery systems (DDSs) have shown many advantages. Among them mesoporous silica nanoparticles (MSNs) has been taken more attention due to its porous structure, biocompatibility and ease of surface functionalization [21].

Glioblastoma multiforme (GBM) is known as one of the most invasive brain tumors which is malignant and is nearly hard to achieve the successful treatment [22]. Regarding this fact, there is a need for combining the advantageous of different therapeutic compounds to enhance the efficacy of the cancer treatment and boost the influence of anticancer drugs. Additionally, because of the molecular heterogeneity of tumors, and as it is reported in the literature [23], it is beneficial to utilize multiple therapeutic modalities for co-delivering different anticancer drugs to achieve cooperative therapeutic effects.

To address these issues, a novel multifunctional POM, $(TBA)_4H_3[GeW_9V_3O_{40}]$ ($GeW_9V_3$), with outstanding anticancer

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effects in delivery to the human U87 glioblastoma cells was produced. This POM is not only functionalized to conjugate with the DOX-loadable mesoporous silica nanoparticle (MSNs), but also is attached to a fluorescence dye molecule to make the POM traceable in the cell. To the best of our knowledge, this is the first system that contains POM as inorganic drug and has the potential to carry various organic drugs at the same time in one delivery vehicle.

From 1972 when the first bioactive POM demonstrated some therapeutic effects [24–26], many efforts have been done to develop these inorganic clusters as novel anticancer and antiviral drugs. Although these attempts revealed good results in the case of antiviral and antibacterial effects, the antitumor aspect of these compounds have remained poor in the POM library. On the other hand, a need to find an efficient drug delivery system with the ability in simultaneous delivery of more than one drug, is essential for enhancing the efficacy of cancer cell knock downing. The scope of this study is the synthesis of an effective anti-tumor POM, which could be post-functionalized to form an outstanding vehicle for multidrug delivery purposes.

## 2. Experimental section

### 2.1. Material and methods

All materials and chemicals were provided from Merck and Sigma chemical companies without any purification. The synthesis of trivacant POMs $K_6Na_4[GeW_9O_{34}].13H_2O$ and $K_6Na_4[SiW_9O_{34}].13H_2O$ were performed according to the literature [27,28]. Infrared (FT-IR) spectra were recorded on a JASCO 6300 spectrophotometer. UV–vis spectra were recorded on JASCO V-670 spectrophotometer. Raman spectra were carried on Renishaw inVia Raman microscope. Transmission electron microscopy (TEM) images were obtained using a Topcon 002B electron microscope at 100 kV. The $N_2$ adsorption-desorption measurements were undertaken using Micromeritics Tristar-3000 surface area analyzer at −196 °C. Fluorescent images were obtained on a Nikon T2500 inverted epifluorescence microscope.

### 2.2. Synthetic procedures

#### 2.2.1. Thiolated mesoporous silica nanoparticles (MSNs-SH)

At first, 30 mg of sodium hydroxide and 90 mg of cetyl trimethyammonium bromide (CTAB) were dissolved into 60 mL of water at 90 °C to obtain a clear solution. Then, 0.6 mL of tetraethyl orthosilicate (TEOS) was added dropwise and the mixture was stirred for 4 h. Afterwards, 25 μL of (3-mercaptopropyl) triethoxysilane was added and vigorously stirred for 24 h. The product was collected using centrifuge, and surfactant was removed by refluxing the obtained product in a mixture of ethanol and hydrochloric acid (60 mL/0.5 mL) for 10 h. The final product was obtained by centrifuging, washing with methanol and drying overnight at 120 °C in vacuum.

#### 2.2.2. $(TBA)_4H_3[GeW_9V_3O_{40}]$ (**1**)

Sodium metavanadate (0.55 g, 4.3 mmol) was dissolved in 75 mL of hot water and cooled to room temperature. Addition of 0.7 mL (8.4 mmol) 12 M HCl to this colorless and homogeneous solution resulted in a pale yellow solution (pH ≈ 1.5). This was followed by addition of solid $K_6Na_4[GeW_9O_{34}].13H_2O$ (1.4 mmol) to the vigorously stirred solution. As the $GeW_9O_{34}$ was dissolved the color of the solution rapidly changed to a deep, cherry-red. The resulting homogeneous solution was re-acidified with 0.25 mL (2.8 mmol) of 12 M HCl. Next, 33.5 mmol of solid *tetra*-butylammonium bromide (TBABr) was added. After several hours stirring, the light orange precipitate was formed and collected on the fine frit. IR (KBr, $cm^{-1}$): 3448 (w), 2960 (m), 2935 (m), 2873 (m), 1637 (w), 1483 (m), 949 (vs), 879 (vs), 815 (s), 781 (vs), 534 (m).

Synthesis of $(TBA)_4H_3[SiW_9V_3O_{40}]$ was carried out according to the above procedure for Ge-containing one, with the exception of using $K_6Na_4[SiW_9O_{34}]$ instead of $K_6Na_4[GeW_9O_{34}]$. Other POMs, $(TBA)_n[XW_9M_3O_{40}]$ (X = Si, Ge and M = W, Mo) were also produced according to the literature [29].

#### 2.2.3. $(TBA)_4[GeW_9V_3((CH_2O)_3CNH_2)O_{37}]$ (**2**)

Amine-terminated POM was synthesized according to the Hill's method [30] with some modifications: 30 mL of N,N-dimethylacetamide (DMA) was added to the mixture of tris(hydroxymethyl)aminomethane (3 mmol) and $(TBA)_4H_3[GeW_9V_3O_{40}]$ (1 mmol). The resulting orange solution was stirred at 80–90 °C under $N_2$ atmosphere for 72 h. The reaction mixture was cooled to room temperature and the resulting deep brown solution was filtered. A dark brown precipitate was formed by drop wise addition of filtrate to 90 mL of diethyl ether. Calcd. elemental analysis for $((n\text{-}C_4H_9)_4N)_4[GeW_9V_3((CH_2O)_3CNH_2)O_{37}]$ (%): C 22.94, H 4.30, N 1.97 Found: C 22.90, H 4.26, N 1.95.

#### 2.2.4. $(TBA)_4[GeW_9V_3((CH_2O)_3CNHCOCHNHBocCH_2SH)O_{37}]$ (**3**)

1 mmol of N-Boc-Cys was added to the solution of 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) (1.5 mmol) in refluxing $CH_3CN$. After stirring for 10 min, the $(TBA)_4[GeW_9V_3((CH_2O)_3CNH_2)O_{37}]$ (0.5 mmol) was added to the reaction mixture and kept stirred overnight under $N_2$ atmosphere at reflux. After cooling the mixture to room temperature, the solution was filtered and filtrate added drop wise to the 70 mL of $Et_2O$. The formed deep brown precipitate was collected and dried under vacuum. IR (KBr, $cm^{-1}$): 3422 (m), 2960 (m), 2933 (m), 2873 (m), 1663 (s), 1541 (m), 1491 (m), 1438 (m), 1389 (m), 1255 (m), 1101 (m), 952 (s), 878 (s), 808 (s), 771 (s), 662 (m). Calcd. elemental analysis for $((n\text{-}C_4H_9)_4N)_4[GeW_9V_3((CH_2O)_3CNHCOCHNH((CH_3)_3C_2O_2)CH_2SH)O_{37}]$ (%): C 24.26, H 4.42, N 2.23, S 0.85. Found: C 24.23, H 4.38, N 2.21, and S 0.82.

#### 2.2.5. $(TBA)_4[GeW_9V_3((CH_2O)_3CNHCOCHNHBocCH_2SS(MSNs))O_{37}]$ (**4**)

A solution of 90 mg MSNs-SH in 5 mL methanol was made and added drop wise to a solution of 0.2 g 2,2′-dithiodipyridine in 5 mL methanol. The mixture was stirred for 6 h at room temperature and nanoparticles were collected using centrifuge and washed three times with methanol. At the next step, a solution of 0.2 mmol $(TBA)_4[GeW_9V_3((CH_2O)_3CNHCOCHNHBocCH_2SH)O_{37}]$ in 10 mL DMF was added to the solution of dispersed nanoparticles in 5 mL DMF. The mixture was stirred overnight at room temperature under $N_2$ atmosphere. Finally, the POM-MSNs were collected using centrifuge, washed thrice with DMF and dried under vacuum (yield 72%).

#### 2.2.6. $(TBA)_4[GeW_9V_3((CH_2O)_3CNHCOCHNH(C_{21}H_{11}NO_5S)CH_2SSMSNs)O_{37}]$ (**5**)

Two mL trifluoroacetic acid (TFA) was added to a cooled (0 °C) solution of 0.1 mmol $(TBA)_4[GeW_9V_3((CH_2O)_3\text{-}CNHCOCHNHBocCH_2SS(MSNs))O_{37}]$ in 5 mL anhydrous DMF. The mixture was stirred firstly at 0 °C for 1 h and then at room temperature for 3 h. The obtained TFA-treated POM-MSNs were collected by centrifuge, washed with DMF and dried under vacuum. In the next step, a solution of 0.05 mmol TFA-treated POM-MSNs, 25 mg fluorescein 5(6)-isotiocyanate and 5 mg of 4-(dimethylamino)pyridine in 5 mL DMF was prepared. The solution was stirred overnight at room temperature under $N_2$ atmosphere. The final product was collected using centrifuge and washed several times with DMF and then dried under vacuum (yield 68%).