Supplementary Materials for

Polyoxovanadate-IodoBodipy Supramolecular Assemblies: New Agents for High Efficiency Cancer Photochemotherapy

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Methods

X-ray crystallography.

Crystals with suitable size were selected for diffraction analysis. The single crystal X-ray data was collected on SuperNova, Dual, Cu at zero, AtlasS2 (Rigaku) using Mo/Kα (λ=0.71073 Å). The crystal was measured at 173 K. Data collection and reduction were performed in CrysAlisPro 1.171.38.43f. The structure solution and refinement were performed with progam package of SHLEX-97 25 and Olex-1.2 26. Multi-scan method was used as the absorption correction. The structures were solved by direct methods and refined against F^2 by full-matrix least-squares techniques. All non-H atoms were refined anisotropically. Hydrogen atoms were added in geometrically idealized position and constrained to ride on their parent atoms, with Uiso(H) = 1.2 Ueq (C) for methylene and Uiso (H) = 1.5 Ueq (O) for hydroxyl. The clear molecular structure of $(2I\text{-}BDP\text{-}C_6)_2V_6$ was given in Fig. S9.

Other instrument information.

UV-Vis spectra were measured by UH4150 spectrophotometer. ^1H NMR spectra were recorded on a JEOL JNM-ECA400 spectrometer. Electron Paramagnetic Resonance (EPR) characterization were performed on Bruker EMX-Plus EPR spectrometer. Scanning electron microscopy (SEM) was performed on a Hitachi SU-8010 electron microscope, and energy-dispersive X-ray spectrum (EDS) was collected at an accelerating voltage of 15 kV.

Light source

White light used for $^1O_2$ detection, PDT and photochemotherapy was provide by a mercury lamp with a band-pass filter from 400 nm to 700 nm. the whole band emission spectrum of mercury lamp was shown in **Fig. S11**. Besides, the power density of the light source was 38.7 mW/cm^2.

Preparation of solution for cell tests.

$(TBA)_2V_6$, $(TBA)_3V_{10}$, $(2I\text{-}BDP\text{-}C_6)Br$, $(2I\text{-}BDP\text{-}C_6)_2V_6$ and $(2I\text{-}BDP\text{-}C_6)_3V_{10}$ were dissolved in DMSO (cell culture grade) with 1 mM. Then the agents was diluted with DMEM medium to specified concentration: 50 μM, 25 μM, 10 μM, 5 μM, 2 μM, 1 μM, 0.5 μM, 0.25 μM. The concentration of $(TBA)_2V_6$ is half of the $(2I\text{-}BDP\text{-}C_6)Br$ and the concentration of $(TBA)_3V_{10}$ is a third of the $(2I\text{-}BDP\text{-}C_6)Br$.

Cell culture.

HepG2 and L02 cells were incubated in DMEM medium with 1 % penicillin/ streptomycin and 10 % FBS (fetal bovine serum) in an incubator with humid air containing 5 % CO_2 at 37 ℃.

Cytotoxicity tests.

HepG2 and L02 cells were seeded in 96-well plates with 5000 cells per well, respectively. The cells were incubated overnight followed by the cytotoxicity tests.

Cytotoxicity tests of $(TBA)_2V_6$ and $(TBA)_3V_{10}$: HepG2 cells were incubated with different concentration of $(TBA)_2V_6$ and $(TBA)_3V_{10}$ for 2 h and go through 24 h incubation times for dark toxicity test, respectively. HepG2 cells were incubated with $(TBA)_2V_6$ or $(TBA)_3V_{10}$ for 2 hours, photoirradiated 3 mins and go through 24 h incubation times for phototoxicity test, respectively. Then the old medium was replaced by fresh medium with 10 % CCK8 for one hour. The cell viability was evaluated by measuring the absorbance at 450 nm.

Safety tests of (2I-BDP-C_6)Br, (2I-BDP-C_6)$_2V_6$ and (2I-BDP-C_6)$_3V_{10}$ in dark: L02 cells were incubated with different concentration of (2I-BDP-C_6)Br, (2I-BDP-C_6)$_2V_6$ and (2I-BDP-C_6)$_3V_{10}$ for 2 h and go through 24 h incubation times for safety test, respectively. Then the old medium was replaced by fresh medium with 10 % CCK8 for one hour. The cell viability was evaluated by measuring the absorbance at 450 nm.

Photochemotherapy efficacy tests of (2I-BDP-C_6)Br, (2I-BDP-C_6)$_2V_6$ and (2I-BDP-C_6)$_3V_{10}$: HepG2 cells were incubated with different concentration of (2I-BDP-C_6)Br, (2I-BDP-C_6)$_2V_6$ and (2I-BDP-C_6)$_3V_{10}$ for 2 h, photoirradiated 3 mins and go through 24 h incubation times for phototoxicity test, respectively. Then the old medium was replaced by fresh medium with 10 % CCK8 for one hour. The cell viability was evaluated by measuring the absorbance at 450 nm.

Dark toxicity tests of (2I-BDP-C_6)Br, (2I-BDP-C_6)$_2V_6$ and (2I-BDP-C_6)$_3V_{10}$: HepG2 cells were incubated with different concentration of (2I-BDP-C_6)Br, (2I-BDP-C_6)$_2V_6$ and (2I-BDP-C_6)$_3V_{10}$ for 2 h and go through 24 h incubation times for test, respectively. Then the old medium was replaced by fresh medium with 10 % CCK8 for one hour. The cell viability was evaluated by measuring the absorbance at 450 nm.

Evaluation of photochemotherapy synergy coefficient

CDI (coefficient of drug interaction) was used to evaluate the synergistic effect of the two drugs. CDI formula calculation: $CDI = AB / (A \times B)$. AB is the cell viability (%) when using two drugs meanwhile. A or B is the cell viability (%) when using A or B alone.[1] CDI < 1, it means the two drugs have a synergistic effect; CDI < 0.7, it means the synergistic effect of the two drugs is very significant; CDI =1, it means the action properties of the two drugs are additive; CDI > 1, it means the action properties of the two drugs are antagonistic.

Herein, we use CDI to estimate the synergistic effect of anions and cations in (2I-BDP-C_6)$_3V_{10}$. AB refer to the (2I-BDP-C_6)$_3V_{10}$ assembly. A or B refer to (2I-BDP-C_6)Br and (TBA)$_3V_{10}$, respectively.

Synthesis of *BDP*

BDP was synthesized according to the reported procedures from 2,4- dimethyl 1-pyrrole and 4-pyridinecarboxaldehyde as reactants.[2] The 2,4- dimethyl-1-pyrrole and 4-pyridinecarboxaldehyde were purchased commercially and without further purification before using. The structure can be confirmed by ^{1}H NMR (400 MHz, $CDCl_3$, TMS) (Fig. S1). δ (ppm)= 1.40 (s, 6H, −CH3), 2.55 (s, 6H, −CH3), 6.00 (s, 2H, −CH), 7.29 (d, 2H, −CH), 8.78 (d, 2H,−CH).

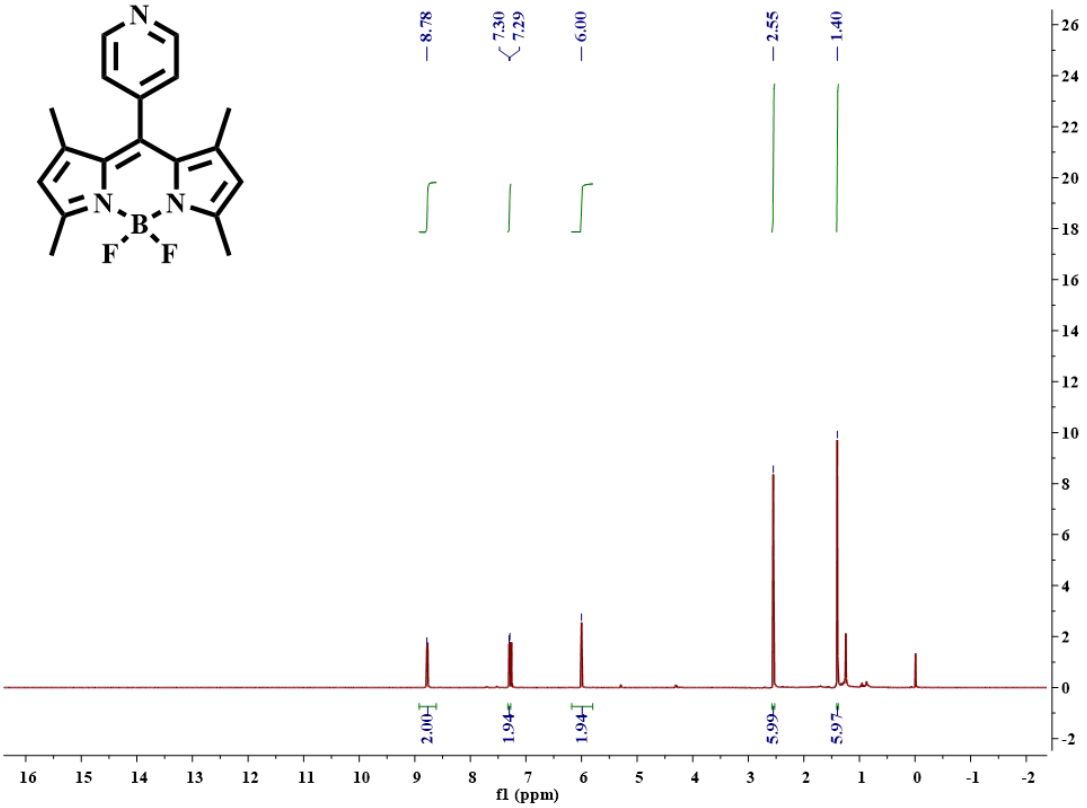

Fig.S1 ^{1}H NMR spectrum of *BDP*.

Synthesis of *2I-BDP*

BDP-2I was synthesized according to the reported procedures from BDP, I_2 and HIO_3 as reactants. [2] The I_2 and HIO_3 were purchased commercially and were used directly. The structure of 2I-BDP was confirmed by ^1H NMR (400 MHz, $CDCl_3$, TMS) (Fig. S2). δ (ppm)= δ (ppm)= 1.41 (s, 6H, −CH3), 2.64 (s, 6H, −CH3), 7.28 (d, 2H, −CH), 8.81 (d, 2H,−CH).

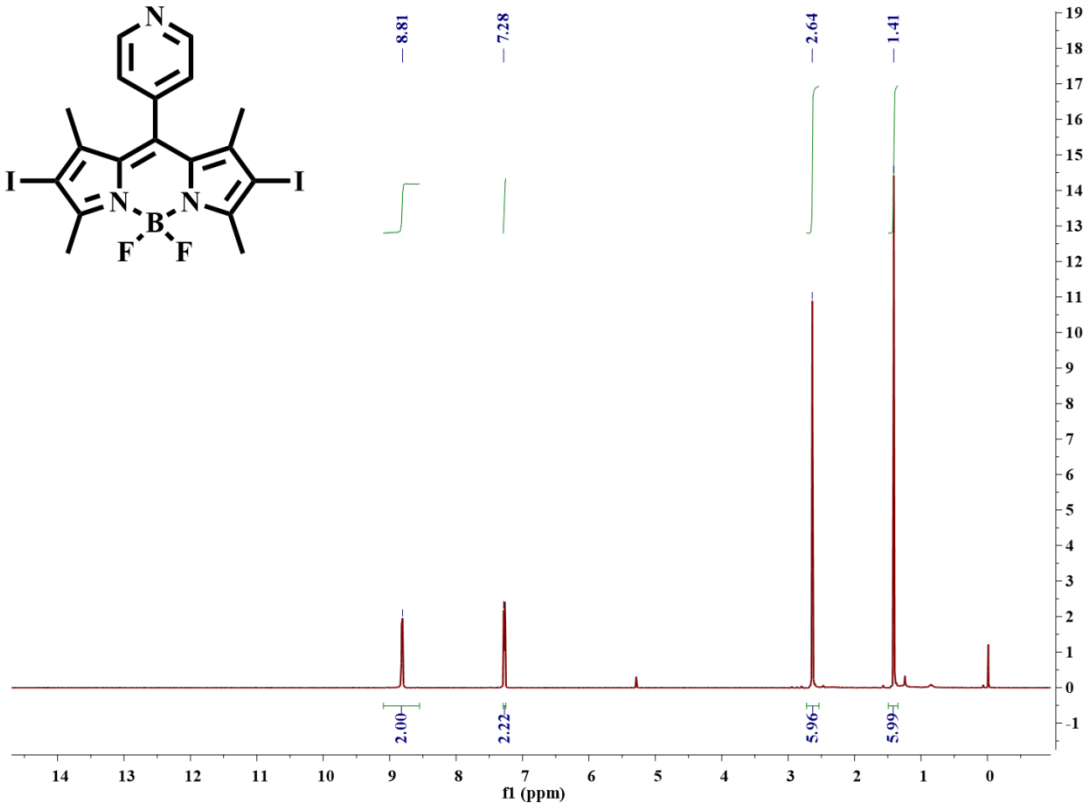

Fig.S2 ^1H NMR spectrum of *2I-BDP*.

Synthesis of (*2I-BDP-C$_6$)Br*

Mixing BDP-2I (577 mg, 1 mmol) and 1-bromohexane (825 mg, 5 mmol) in 10 mL DMF. The mixture was stirred at 80℃ for 16 hours. After cooling to room temperature, the solution was added to a large amount of diethyl ether (100 mL). The resulting precipitates were dissolved in 30 ml CH_2Cl_2 and filtered to remove the insolubles. The filtrate was concentrated and the products were isolated by column chromatography. (*2I-BDP-C$_6$)Br* was obtained as purple solid. It was characterized by ^{1}H NMR(400 MHz, DMSO-d6, TMS) (Fig. S3). δ (ppm)= 0.86 (t, 3H, −CH3),1.29 (m, 6H, −CH2), 1.41 (s, 6H, −CH2), 1.99 (m, 2H, −CH2), 2.58 (s, 6H, −CH3), 4.74 (t, 1H, −CH2), 8.48 (d, 1H, −CH), 9.36 (d, 1H, −CH).

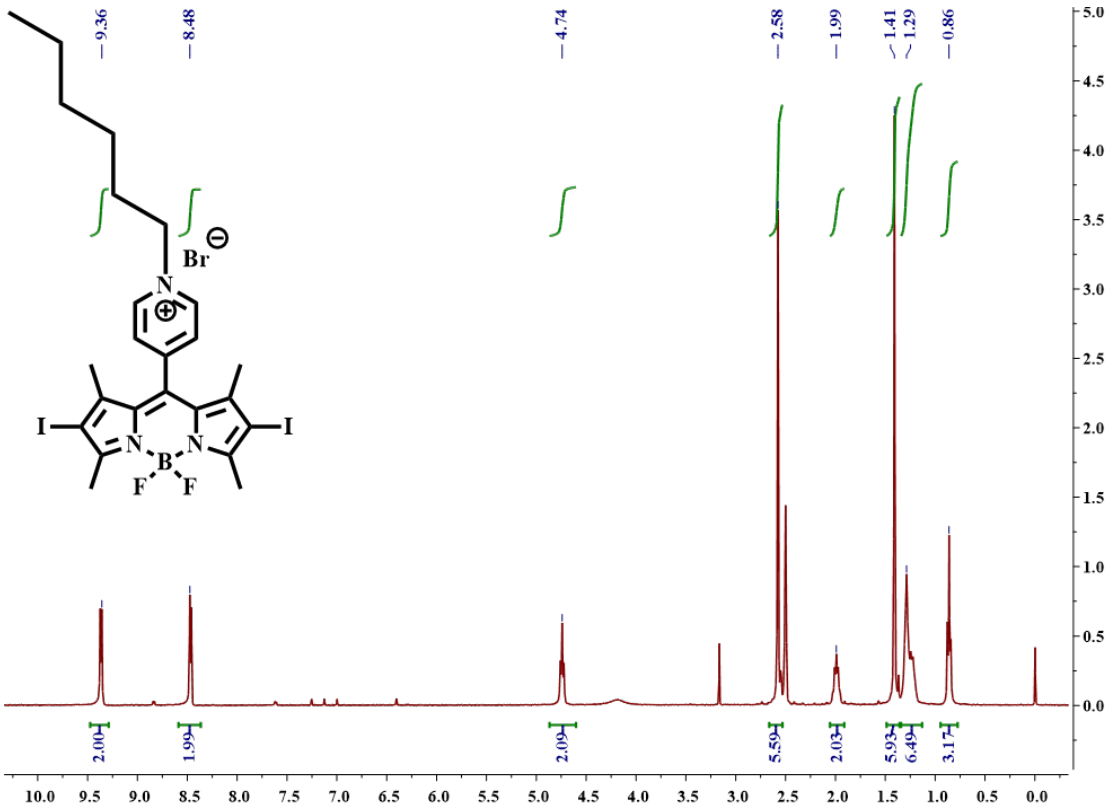

Fig.S3 ^{1}H NMR spectrum of (*2I-BDP-C$_6$)Br*.

Synthesis of *(2I-BDP-C_6)$_2$$V_6$*

A solution of $Na_2[V_6O_{13}\{(OCH_2)_3CCH_2OH\}_2]$(0.5 mmol, 413 mg) was dropwise added into the aqueous solution of (2I-BDP-C_6)Br (1 mmol, 742 mg). Purple precipitates formed immediately and can be isolated by filtration, then washed with acetonitrile and deionized water several times, dried in oven at last. It characterized by ^{1}H NMR (400 MHz, DMSO-d6, TMS) (Fig. S4). δ (ppm)= 0.86 (t, 6H, −CH3), 1.29 (t, 12H, −CH2), 1.41 (s, 12H, -CH3), 2.01 (t, 4H, −CH2), 2.58(s, 12H, −CH3), 3.27 (d, 4H, -CH2), 4.65(t, 2H, −OH), 4.75 (t, 4H, -CH2), 4.87(s, 12H, −CH2), 8.47 (d, 2H,−CH), 9.36 (d, 2H, −CH).

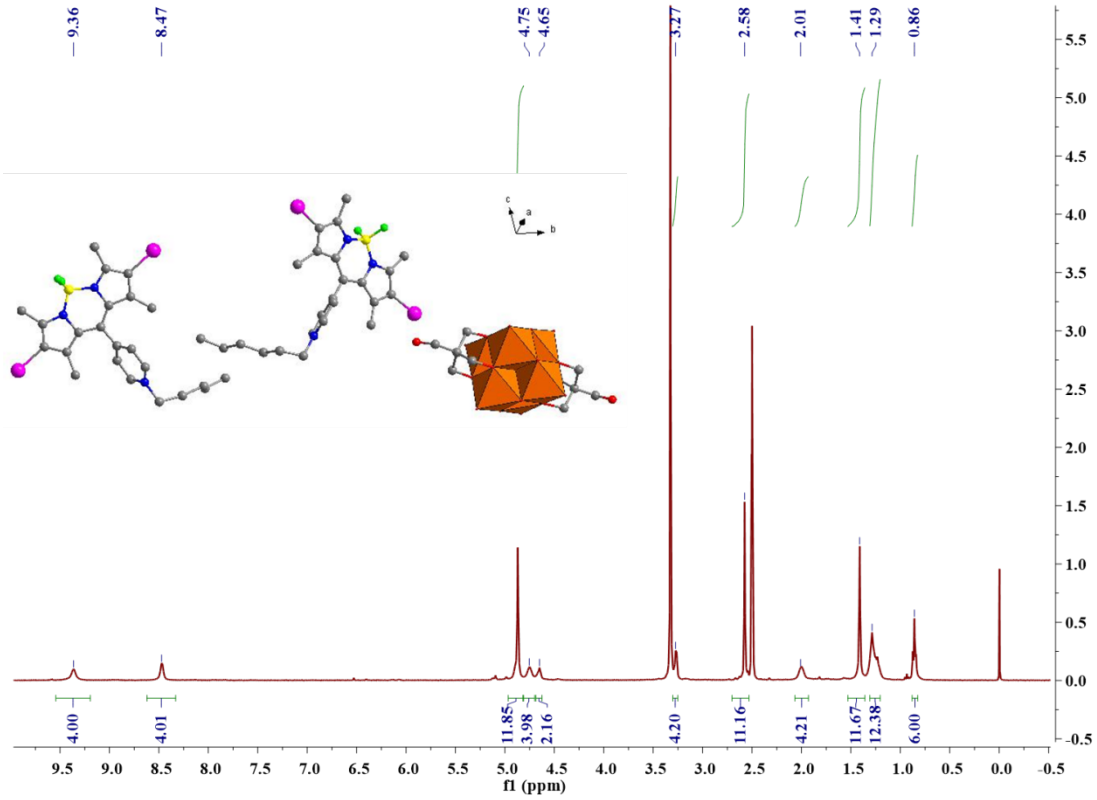

Fig. S4 ^{1}H NMR spectrum of dried *(2I-BDP-C_6)$_2$$V_6$*

Synthesis of *(2I-BDP-C_6)$_3V_{10}$*

The acetonitrilesolution of $[(C_4H_9)_4N]_3[H_3V_{10}O_{28}]$ (0.33 mmol, 563 mg) was dropwise added into the acetonitrile solution of (2I-BDP-C_6)Br (1 mmol, 742 mg). Purple precipitates formed immediately and can be isolated by filtration, then washed with acetonitrile and deionized water several times, dried in oven at last.

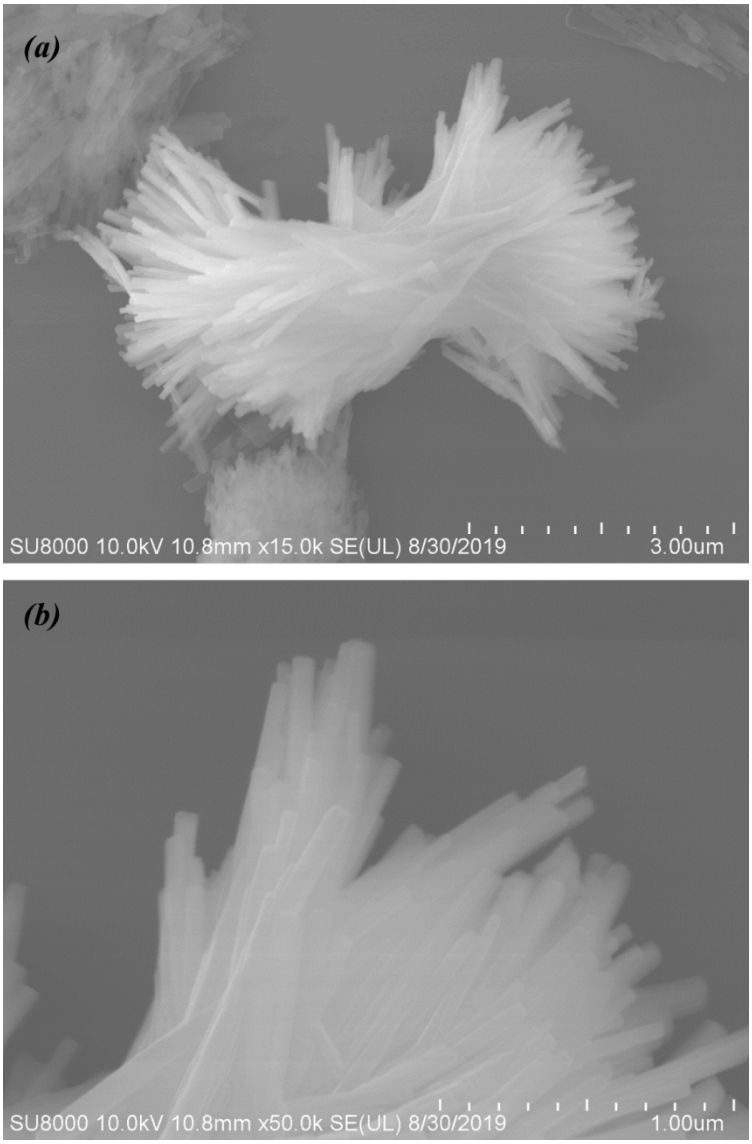

Fig. S5 SEM image of (2I-BDP-C_6)$_3$$V_{10}$ with different magnification.

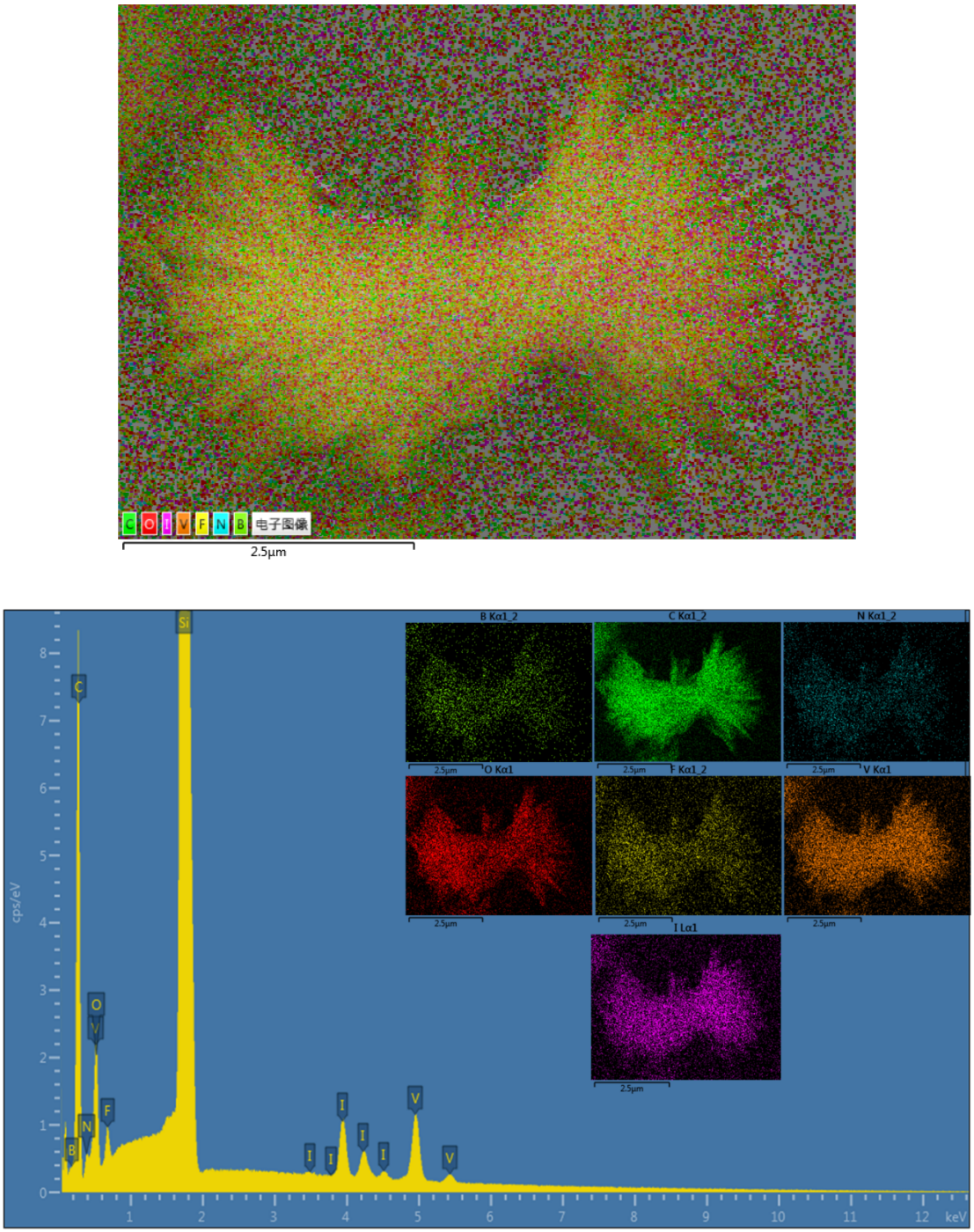

Fig. S6 EDS analysis spectra of $(2I\text{-}BDP\text{-}C_6)_3V_{10}$

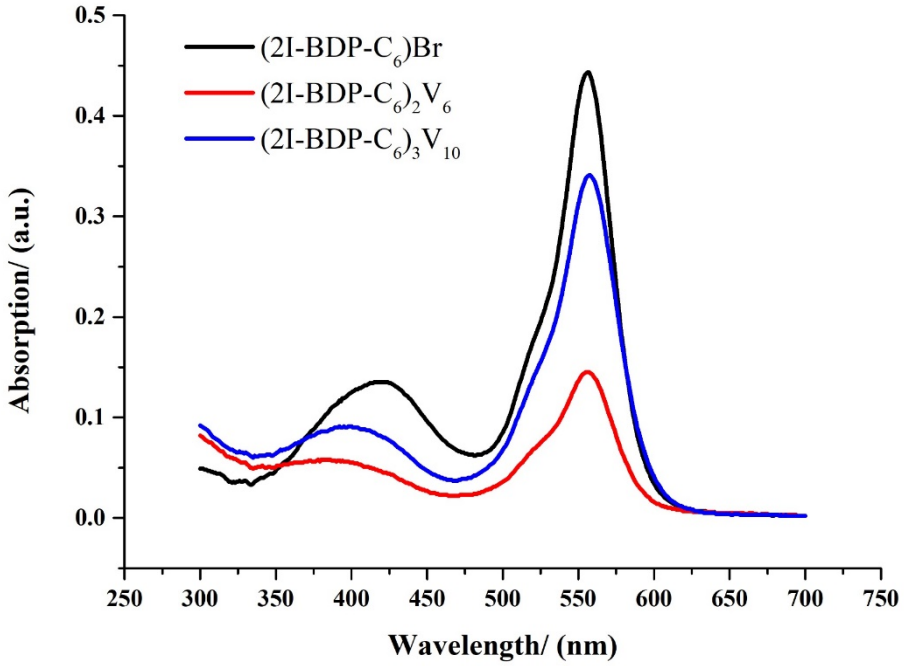

Fig. S7 UV-Vis spectra of (2I-BDP-C_6)Br, (2I-BDP-C_6)$_2$$V_6$ and (2I-BDP-C_6)$_3$$V_{10}$ in DMF(10^{-5}M).

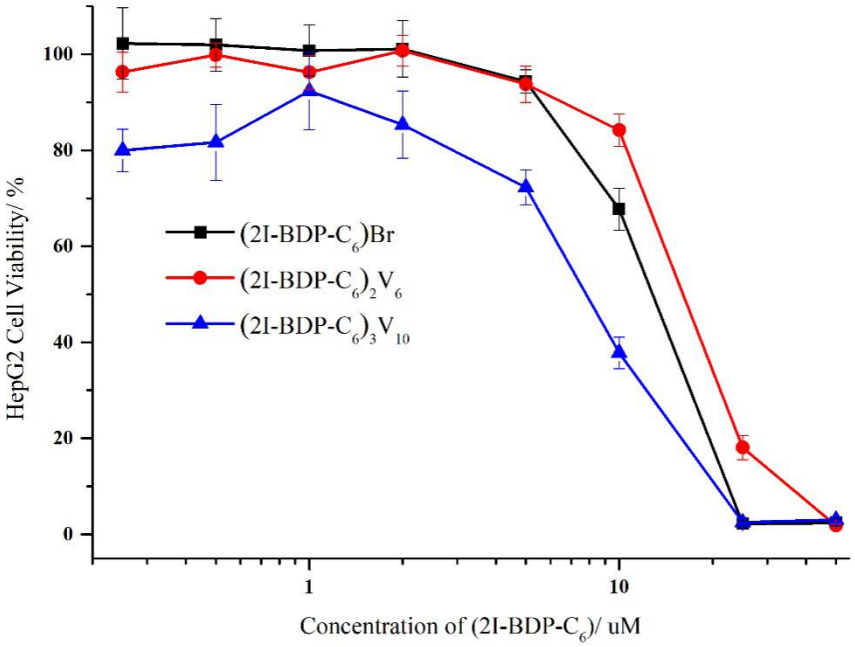

Fig. S8 The dark toxicity of (2I-BDP-C_6)Br, (2I-BDP-C_6)$_2$$V_6$ and (2I-BDP-C_6)$_3$$V_{10}$ against HepG2 cells

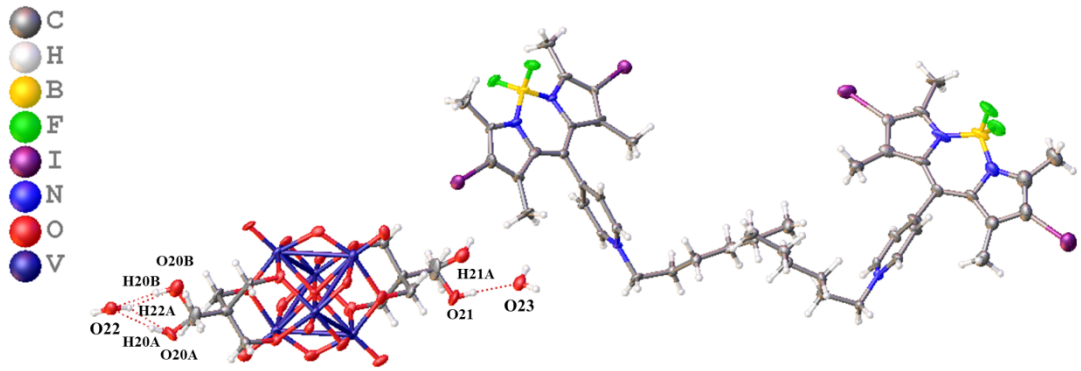

Fig. S9 The molecular structure and the existed H-bond of (2I-BDP-C_6)Br.

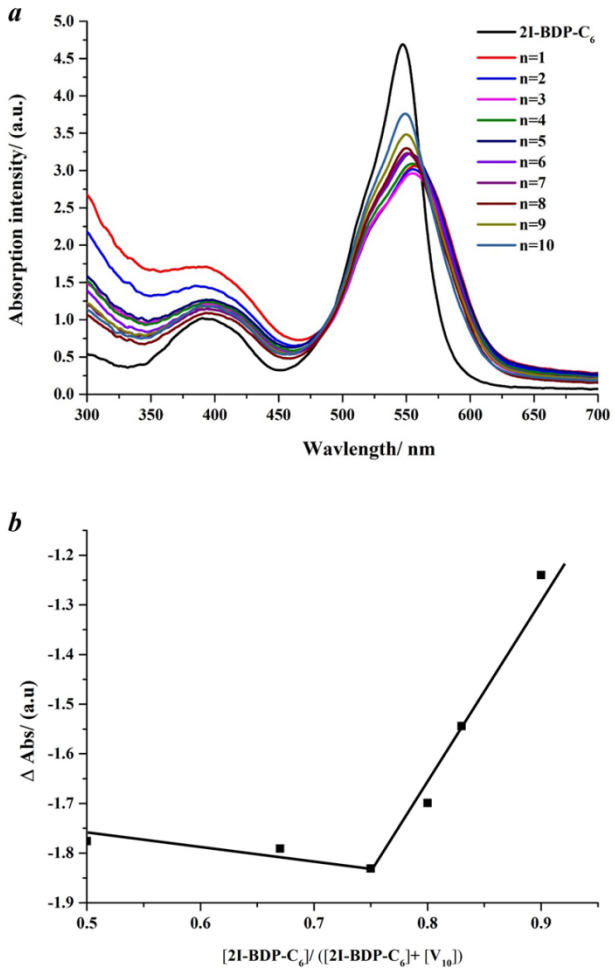

Fig. S10. ***a***, UV-Vis titration of (2I-BDP-C_6)Br (10 μM) in H_2O with $(TBA)_3V_{10}$ (1 mM) in mixture of DMSO and H_2O (v:v =1:9), n is the molar ratio of (2I-BDP-C_6)Br and $(TBA)_3V_{10}$; ***b***, Job's plot for the association equilibrium of 2I-BDP-C_6 and V_{10}.

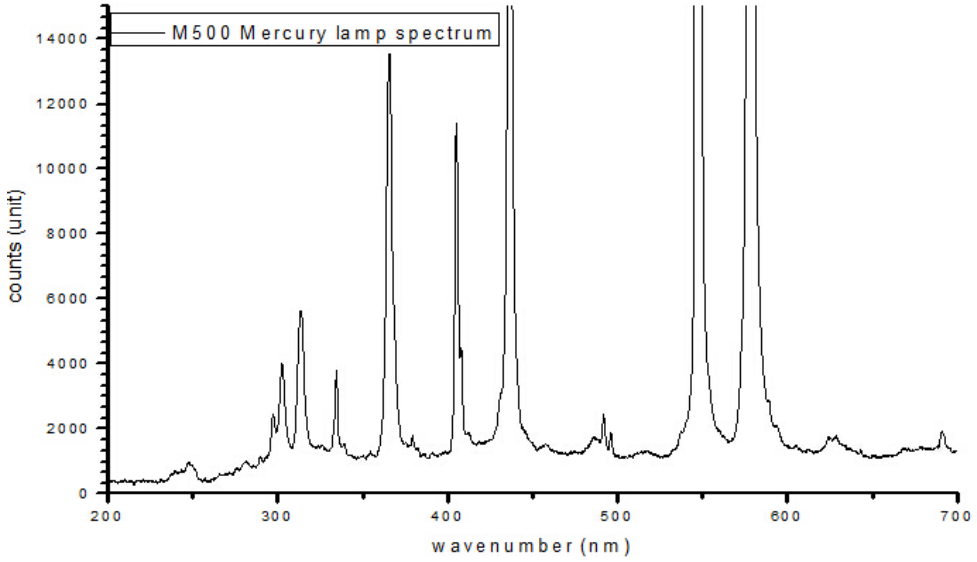

Fig. S11 The emission spectra of mercury lamp.

Tab. S1. Crystal data and structure refinement for $(2I\text{-}BDP\text{-}C_6)_2V_6$

Identification code	$(2I\text{-}BDP\text{-}C_6)_2V_6$
CCDC number	1968987
Empirical formula	$C_{60}H_{80}B_2F_4I_4N_4O_{23}V_6$
Formula weight	2136.14
Temperature/K	109.5(7)
Crystal system	monoclinic
Space group	$P2_1$
a/Å	10.02302(17)
b/Å	22.8571(4)
c/Å	15.9505(3)
α/°	90
β/°	91.5838(17)
γ/°	90
Volume/Å^3	3652.80(12)
Z	2
ρ_{calc}g/cm^3	1.942
μ/mm^{-1}	20.204
F(000)	2096.0
Crystal size/mm^3	0.2 × 0.15 × 0.1
Radiation	CuKα (λ = 1.54184)
2Θ range for data collection/°	7.736 to 152.296
Index ranges	$-12 \le h \le 7$, $-25 \le k \le 28$, $-19 \le l \le 20$
Reflections collected	15800
Independent reflections	10673 [R_{int} = 0.0607, R_{sigma} = 0.0906]
Data/restraints/parameters	10673/89/964
Goodness-of-fit on F^2	0.895
Final R indexes [I>=2σ (I)]	R_1 = 0.0693, wR_2 = 0.1930
Final R indexes [all data]	R_1 = 0.0792, wR_2 = 0.2118
Largest diff. peak/hole / e Å^{-3}	1.59/-2.35
Flack parameter	0.633(14)

Tab. S2

D-H	d(D-H)	d(H..A)	<DHA	d(D..A)	A
O20B-H20B	0.820	1.914	169.108	2.724	O22
O20A-H20A	0.820	1.995	169.252	2.805	O22
O22-H22A	0.850	2.071	133.175	2.724	O20B
O22-H22A	0.850	2.060	145.953	2.805	O20A

Reference

[1] S. Cao, Y. Zhen. *Cancer Chemother Pharmacol*, 1989, 24, 181- 186

[2] J. Bartelmess, A. J. Francis, K. A. E. Roz, F. N. Castellano, W. W. Weare, R. D. Sommer. *Inorg. Chem.* 2014, 53, 4527- 4534