Electronic Supplementary Information for

Bio-compatible fluorescent nano TiO materials prepared from titanium-oxo-cluster precursors

P.-Y. Liu, L.-N. Cui, L. Yang, X.-P. Shu, Q.-Y. Zhu* and J. Dai*

Experimental section

General materials and instruments.

All the analytically pure reagents were purchased commercially and used without further purification. UV-vis absorption spectra were obtained on a Shimadzu UV-2600 spectrophotometer. Elemental analyses of C, H and N were performed using a VARIDEL III elemental analyzer. FT-IR spectra were recorded on a Magna 550 spectrophotometer. Room-temperature X-ray diffraction (XRD) data were collected on a D/MAX-3C diffractometer using a Cu tube source (Cu-Kα, λ = 1.5406 Å). Fluorescence spectra were measured on a Hitachi F2500 apparatus. X-ray photoelectron spectroscopic (XPS) measurements were recorded on a Shimadzu Axis ULTRA spectrometer. The morphology and size of the NPs were determined by TEM (Hitachi, HT7700) at 120 kV. Thermogravimetric analysis (TGA) was performed using a SDT 2960 microanalyzer and the sample was heated under a nitrogen stream of 100 ml min^{-1} with a heating rate of 20 ºC min^{-1}. The cellular uptake was examined using a confocal laser scanning microscope (TCS SP5 II). Cellular uptake of nano solution based on Ti concentrations was determined by ICP-MS (iCAPTM Qc, Thermo Fisher scientific).

Syntheses of compounds 1−3.

$[Ti_4O_2(O^iPr)_4(L1)_2(C_9H_{15}O_3)_2]\cdot 2C_3H_6O$ (1).

Analytically pure $Ti(O^iPr)_4$ (0.10 mL, 0.34 mmol) and L1(8.5 mg, 0.033 mmol) were mixed in 0.3 mL of anhydrous acetone. The mixture was sealed in a thick glass tube after degassing by argon. The sealed tube was heated at 40 °C for 6 days, and then cooled to room temperature to yield orange crystals (25 % yield based on L1). The crystals were rinsed with acetone, dried in vacuum and were preserved in a desiccator. Anal. Calcd. for $C_{64}H_{98}N_2O_{22}Ti_4$ (MW 1438.93): C, 53.42; H, 6.86; N, 1.95. Found: C, 52.94; H, 7.21; N, 2.02. IR data (cm^{-1}): 2969(w), 1751(m), 1618(m), 1591(m), 1535(w), 1508(m), 1446(w), 1390(s), 1344(s), 1245(s), 1193(s), 1126(s), 986(s), 849(w), 769(w), 697(w), 626(s).

$[Ti_4O_2(O^iPr)_4(L2)_2(C_9H_{15}O_3)_2]\cdot 2C_3H_6O$ (2).

Compound **2** was prepared by the same manner as that for **1**. Analytically pure $Ti(O^iPr)_4$ (0.15 mL, 0.51 mmol) and L2 (7.9 mg, 0.025 mmol) were mixed in 0.3 mL of anhydrous acetone. The mixture was sealed in a thick glass tube after degassing by argon. The sealed tube was heated at 40 °C for 6 days, and then cooled to room temperature to yield yellow crystals (39 % yield based on TPA-AA). Anal. Calcd. for $C_{72}H_{94}N_2O_{16}Ti_4$ (MW 1547.12): C, 60.55; H, 6.65; N, 1.81. Found: C, 60.67; H, 7.44; N, 1.94. IR data (cm^{-1}): 2967(w), 1632(w), 1588(m), 1521(m), 1505(m), 1421(w), 1394(s), 1325(m), 1250(m), 1172(m), 1115(s), 979(s), 960(s), 836(w), 749(m), 694(m), 611(m).

$[Ti_4O_2(O^iPr)_4(L3)_2(C_9H_{15}O_3)_2]$ (3).

Compound **3** was prepared by the same manner as that for **1** except the L1 was replaced by L3 (6 mg, 0.027 mmol). The mixture was sealed in a thick glass tube and the sealed tube was heated at 40 °C for 5 days, and then cooled to room temperature to yield yellow crystals (26 % yield based on DEPA-AA). Anal. Calcd. for $C_{56}H_{92}N_2O_{16}Ti_4$ (MW 1240.82): C, 54.21; H, 7.47; N, 2.26. Found: C, 54.65; H, 7.57; N, 2.22. IR data (cm^{-1}): 2968(w), 1627(w), 1601(m), 1510(m), 1433(w), 1391(s), 1357(m), 1250(s), 1181(m), 1119(m), 984(s), 960(s), 853(w), 814(m), 736(w), 690(w), 621(m), 592(s).

X-ray crystallographic analysis.

The single crystal measurements of **1–3** were carried out on a Rigaku Mercury CCD diffractometer for **1** and **2** and Bruker APEX-II CCD diffractometer for **3** with graphite monochromated MoKα (λ = 0.71075 Å) radiation at room temperature. The structures were solved by direct methods using SHELXS-2016 and the refinements were performed against F^2 using SHELXL-2016. All the non-hydrogen atoms are refined anisotropically. The hydrogen atoms are positioned with idealized geometry and refined with fixed isotropic displacement parameters. Relevant crystal data, collection parameters, and refinement results can be found in Table S1.

Synthesis of aqueous solution of nanoparticles

Aqueous nanoparticle solution of **1-a** was prepared by adding a THF solution of **1** (1×10^{-4} mol·dm^{-3}, 0.1 mL) drop by drop under high speed stirring to 5 mL water. In order to remove THF, the solution was stirred continuously at 35 ºC and a clear and yellow aqueous nanoparticle solution was obtained. Aqueous nanoparticle solution of **2-a** was prepared from **2** in a similar procedure for that of **1-a**. The nanoparticle solutions were stable for days in ambient condition.

Cell culture

HepG2 (HB-806, ATCC) were maintained in Dulbecco's Modified Eagle's Medium, high glucose (SH30022.01, Hyclone), containing 10% FBS (SH30071, Thermo Fisher, USA), 10% penicillin/streptomycin/fungizone (15240-062, Gibco, USA), in an atmosphere of 95% air and 5% CO_2 at 37°C.

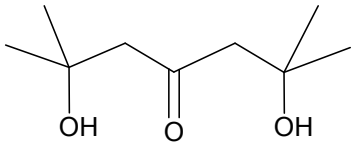
Cellular uptake

The cellular uptake of nano solution of **1-a** by HepG2 cells was examined using a confocal laser scanning microscope (TCS SP5 II). HepG2 cells (5×10^4 per well) were plated in 14 mm glass coverslips (801010, Nest) in a 24-well culture plate and grown for 12 h at 37°C before uptake study. And then the cells were incubated with nano solution of **1-a** (0.5 mL, 70 μM) at 37°C for different times (4, 8, 12 h), followed by rinsing with PBS buffer three times. Finally, the harvested cells were fixed with formaldehyde (4%, 1 mL/well) at room temperature for 10 min and blocked with immunostaining blocking buffer (P0102, Beyotime) for 1 h. Actin was stained with Rhodamine Phalloidin (R415, Thermo Fisher). Cellular uptake of nano solution based on Ti

concentrations was determined by ICP-MS.

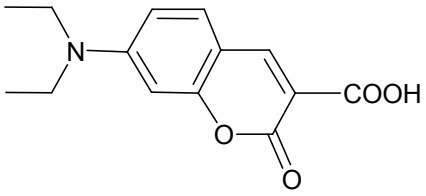
In vitro cytotoxicity of 1-a

HepG2 cells were seeded on 96-well plates at a density of 5.0×10^4 /mL cells and further cultured for 12 h with CO_2 (5%) at 37°C. Nano solution of **1-a** was diluted by DMEM high-glucose containing 10% FBS to certain concentration of 5, 10, 20, 40, 50, 60, 70 μM and continued to incubate cells for 12 h at 37°C in 5% CO_2. One set of blank control (6 wells) was left with culture only. Next, 10 μL of MTT solution (5 mg/mL) (C0009, Beyotime) was added to each well and the cells were incubated for another 4 h at the same condition. Finally, Formazan (0.2 mL) was added into each well and incubated for 3 h until formazan crystals dissolve completely. The optical absorbance of each well at 570 nm was recorded using a micro-plate reader.



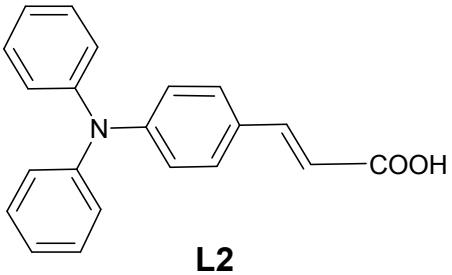
$C_9H_{18}O_3$

2,6-dihydroxy-2,6-dimethylheptan-4-one



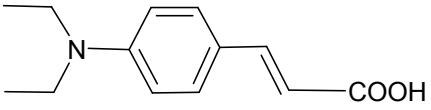
L1

7-(diethylamino)-2-oxochroman-3-carboxylic acid



L2

3-(4-(diphenylamino)phenyl)acrylic acid



L3

3-(4-(diethylamino)phenyl)acrylic acid

Chart 1. Structures of the ligands used in this work.

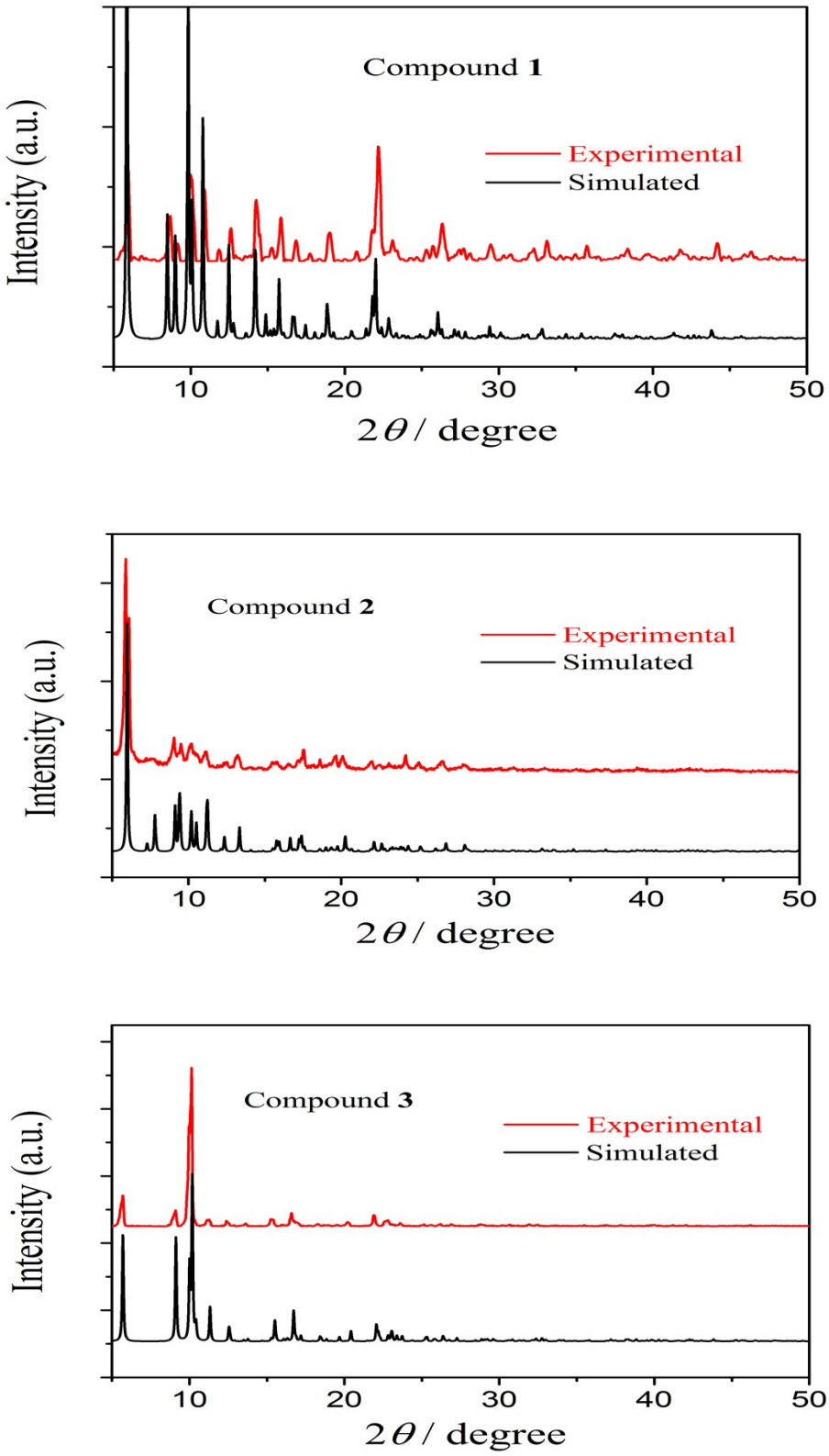


Fig. S1. Experimental and simulated XRD spectra of compounds **1–3**. The XRD patterns of the powdered microcrystals are in agreement with those simulated from the data of single-crystal analysis.

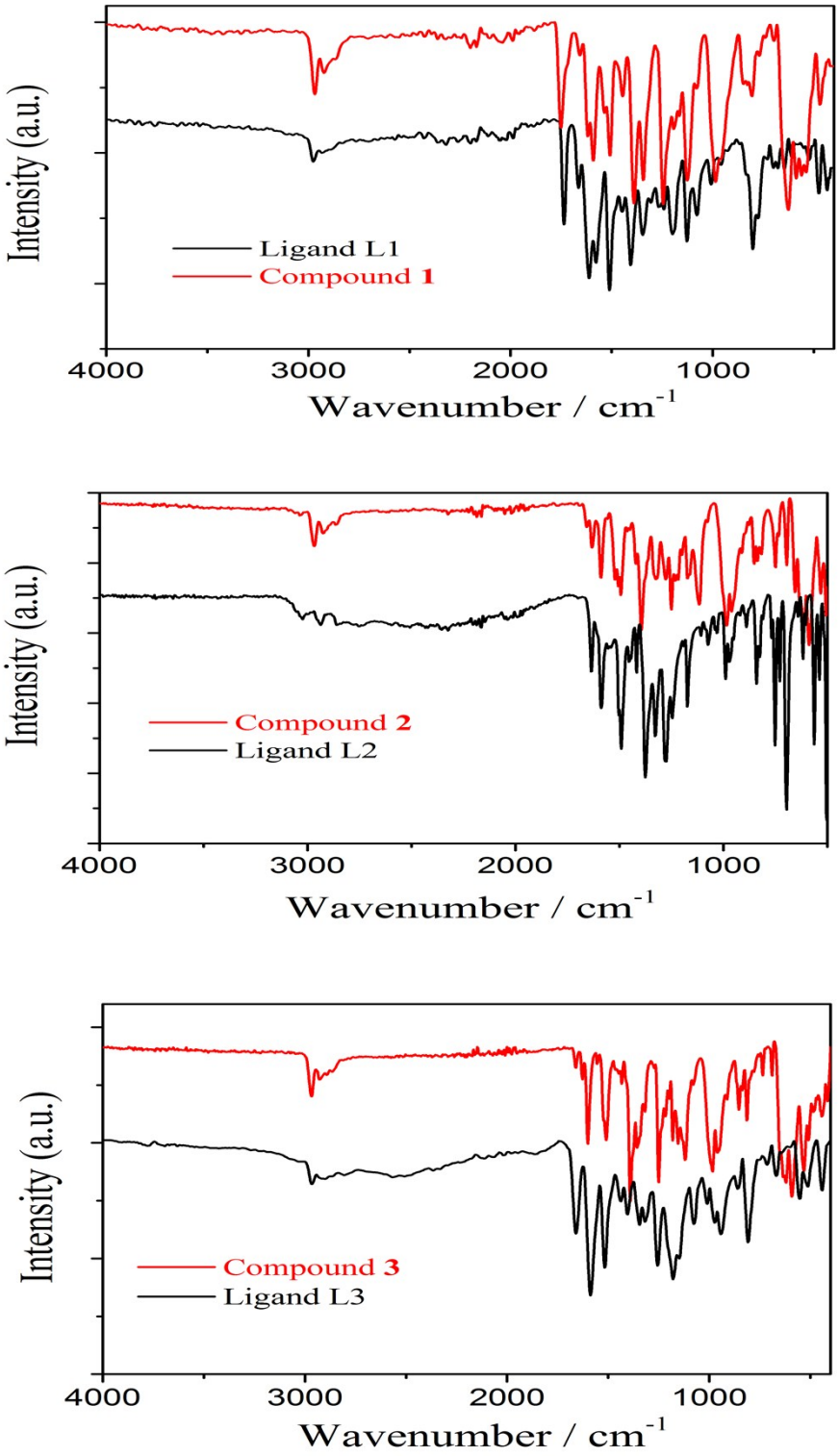


Fig. S2. IR spectra of compounds **1–3** along with their dye ligands L1–L3. For each compound, the FTIR stretches are similar with those of their ligands L1–L3. Isopropoxy groups and the $C_9H_{15}O_3$ are detected by the ν_{C-H} (between 2970 and 2850 cm^{-1}) and ν_{Ti-O-C} (around 980 cm^{-1}) vibrations. The intense bands below 650 cm^{-1} are attributed to the Ti–O–Ti vibrations (Fig S2). The band at 1750 cm^{-1} for **1** is assigned to the characteristic band of the keto group of the ligand L1.

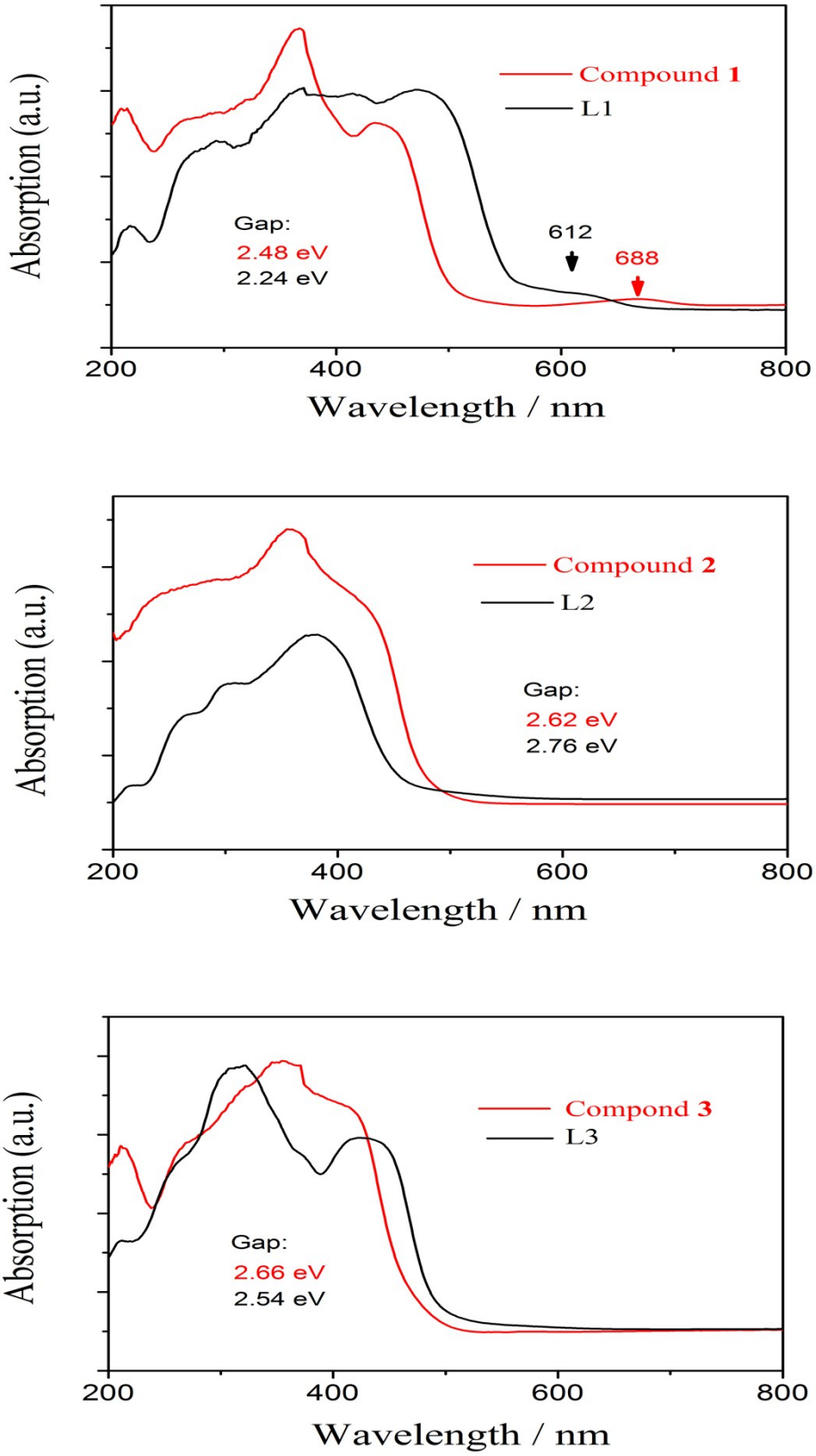


Fig. S3. Solid state UV-vis spectra of **1**–**3** with estimated energy gaps.

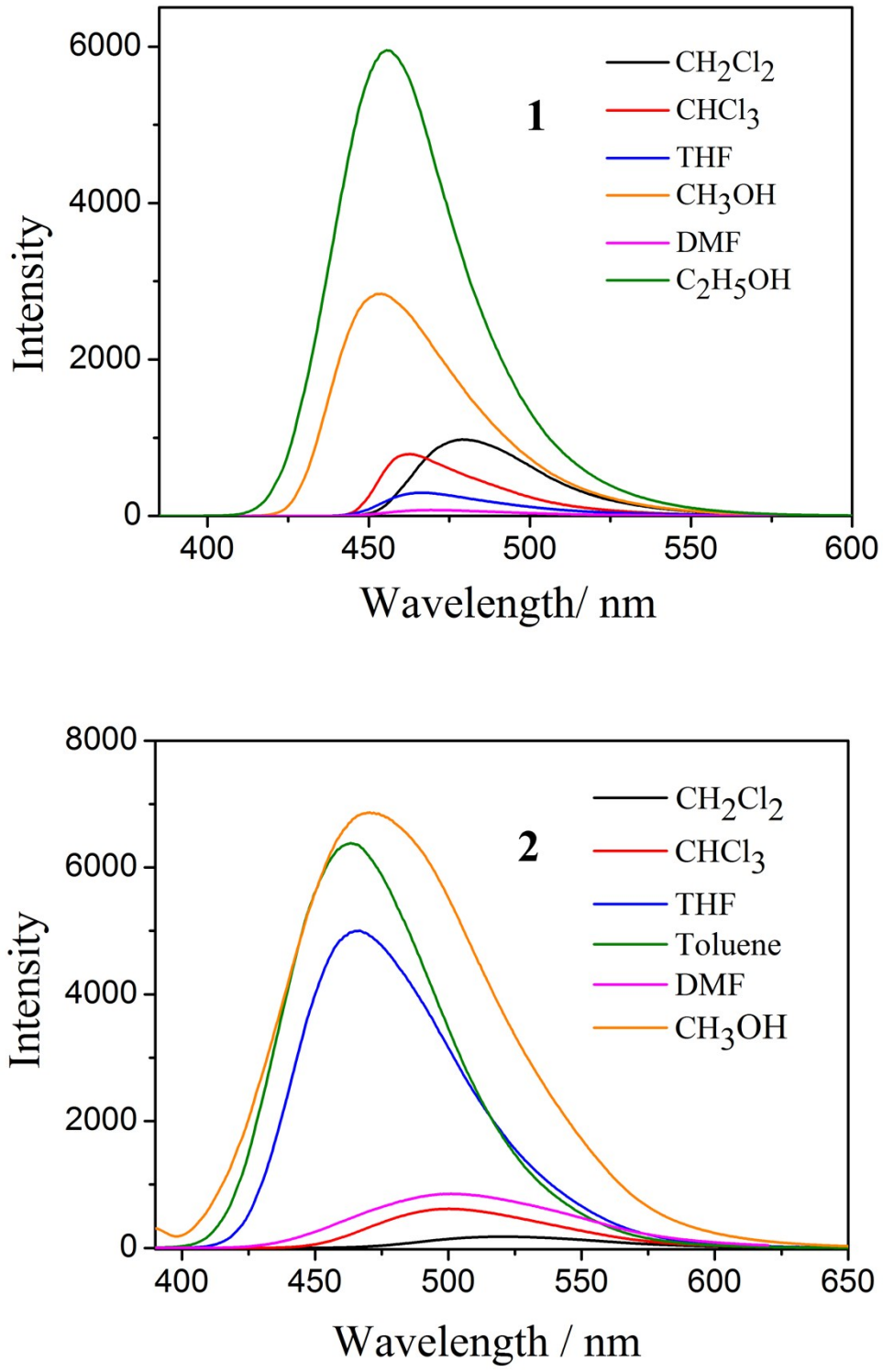


Fig. S4. Emission spectra of compounds **1** and **2** in different organic solvents (1.0×10^{-4} M).

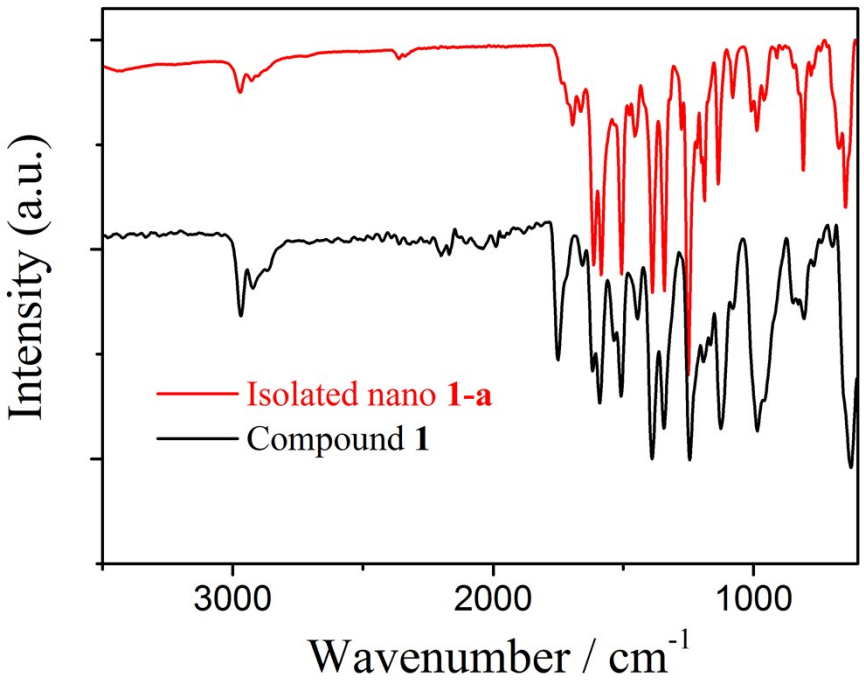


Fig. S5. IR spectra of compounds **1** and the isolated **1-a** particles.

The IR vibrations of the nano **1**-a are similar with those of compound **1** though there are same changes in intensity. The ν_{C-H} (between 2970 and 2850 cm^{-1}) and ν_{Ti-O-C} (about 980 cm^{-1}) vibrations clearly weakened due to the loss of the isopropoxy groups in the cluster condensation. The vibrations of carboxyl anion at 1585 to 1620 cm^{-1} are still very strong. The intense bands below 650 cm^{-1} are attributed to the Ti–O–Ti vibrations (Fig S2).

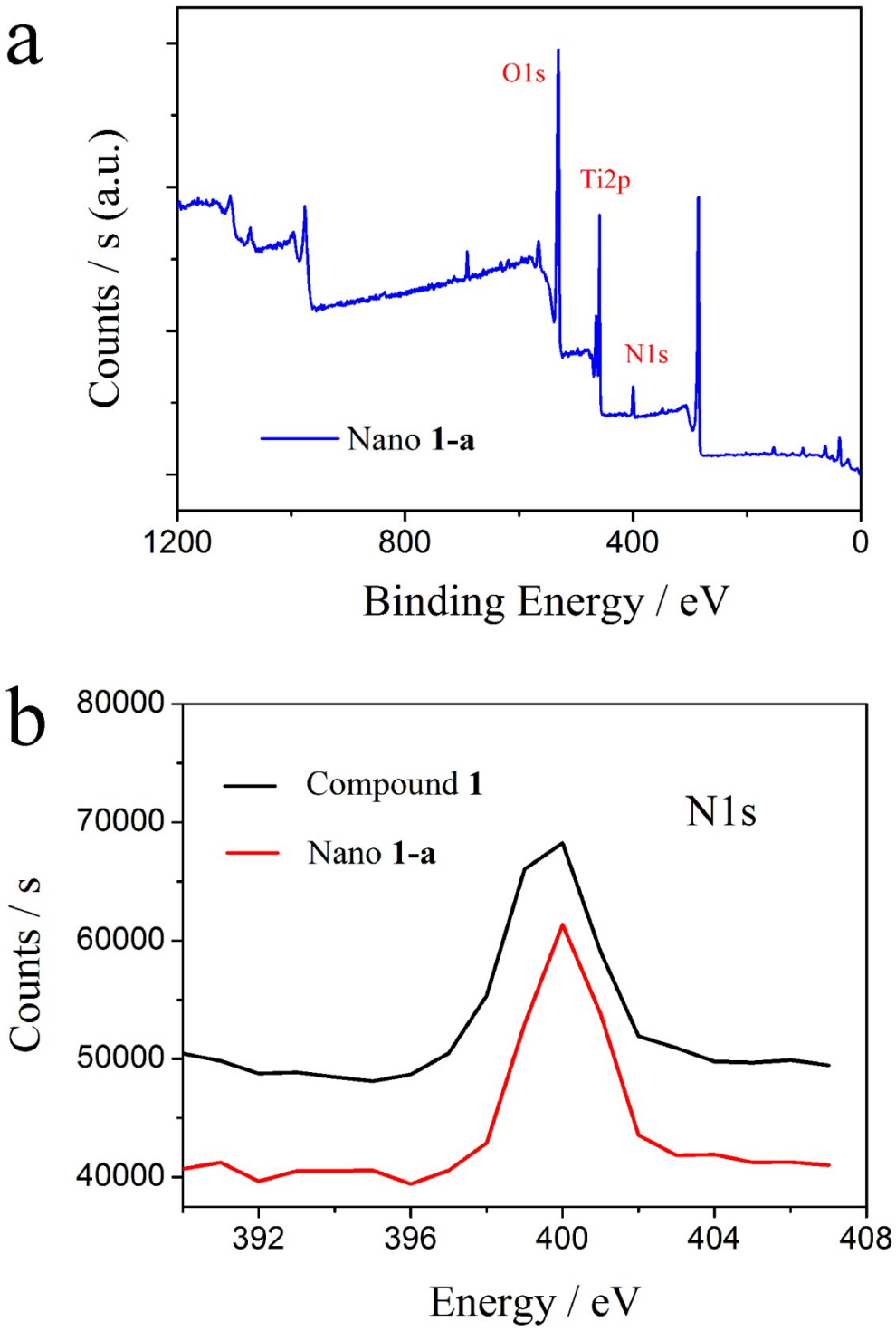


Fig. S6. (a) XPS survey of nano **1-a**. (b) XPS spectra of compound **1** and nano **1-a**.

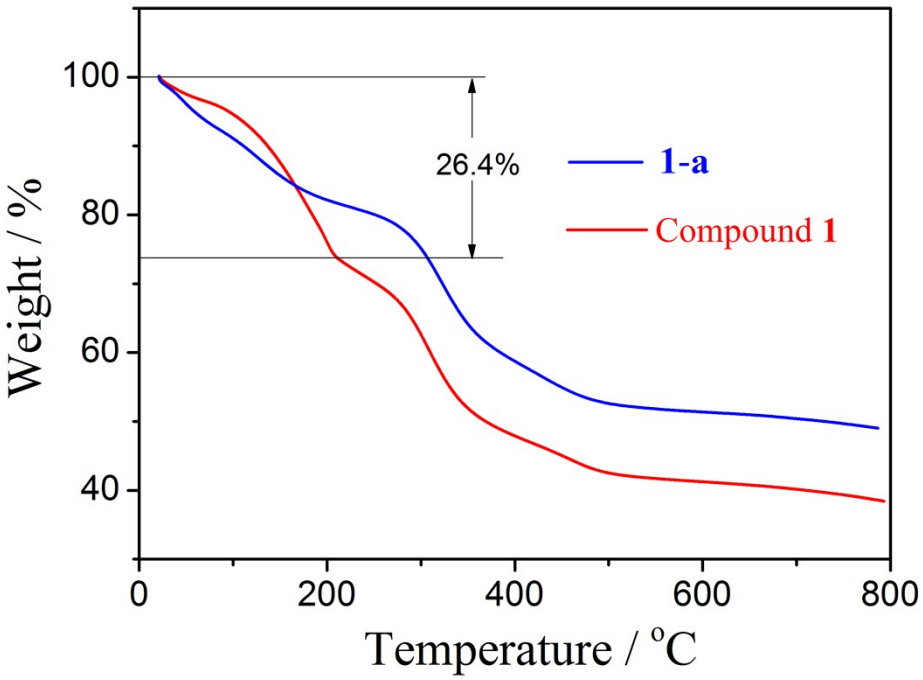


Fig. S7. Thermogravimetric plots of **1** and **1-a**.

The TG curves are similar to each other except in the range from room temperature to 250 °C. Isopropoxy groups are lost easy in this range. The calculated percentage of isopropoxy groups is 24.5 % for **1** that is close to 26.4 % found. Because part of the isopropoxy groups has lost already in the cluster condensation, no obvious stage appears in this range for 1-a in compared with that of 1.

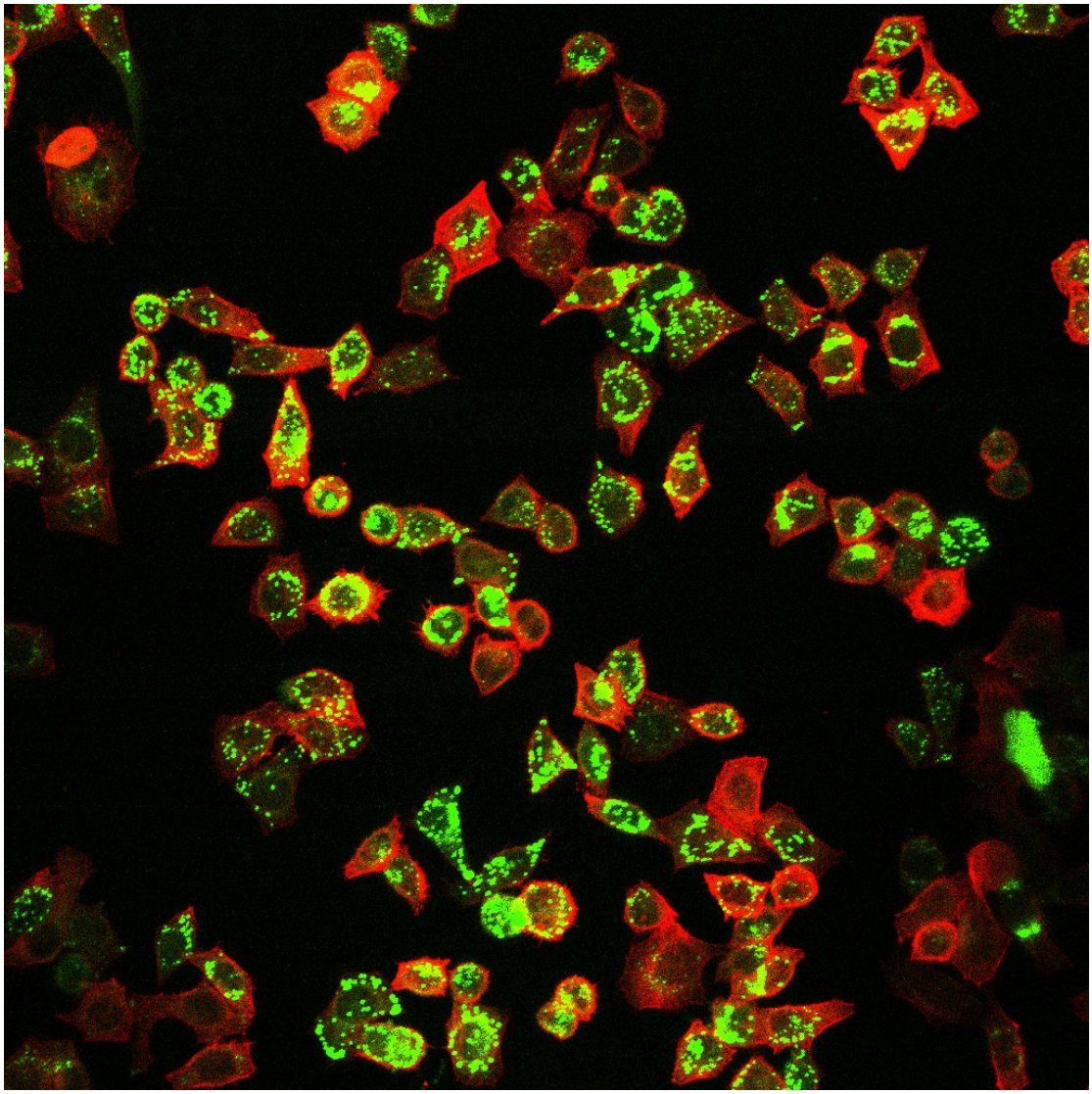


Fig. S8. Bio-imaging of the cellular uptake of nano solution of **1-a** by HepG2 cells (12 h) taken by fluorescent confocal microscopy (red, cells; green, nano TiO particles).

Table S1. Crystal data and structural refinement parameters for compounds **1**–**3**.

	1	**2**	**3**
formula	$C_{64}H_{102}N_2O_{22}Ti_4$	$C_{78}H_{108}N_2O_{18}Ti_4$	$C_{56}H_{92}N_2O_{16}Ti_4$
fw	1442.96	1553.16	1240.82
cryst size (mm^3)	0.20×0.15×0.10	0.30×0.25×0.25	0.25×0.25×0.10
cryst syst	triclinic	triclinic	monoclinic
space group	P-1	P -1	$P2_1/c$
a (Å)	12.740(3)	11.383(2)	15.836(2)
b (Å)	12.982(3)	12.183(2)	11.5845(14)
c (Å)	13.026(3)	14.906(3)	18.080(2)
α (deg)	64.27(3)	90.01(3)	90.00
β (deg)	79.31(3)	98.41(3)	102.343(4)
γ (deg)	78.76(3)	95.11(3)	90.00
V ($Å^3$)	1891.1(9)	2036.5(7)	3240.1(7)
Z	1	1	2
ρ_{calcd} (g cm^{-3})	1.225	1.980	1.270
F(000)	732	814	1312
μ (mm^{-1})	0.472	0.443	0.537
T (K)	293(2)	293(2)	293(2)
reflns collected	14406	15793	69449
unique reflns	6507	7121	5569
observed reflns	5076	6306	3703
no. params	397	470	370
GOF on F^2	1.119	1.108	1.064
R_1 [I>2σ(*I*)]	0.0871	0.0613	0.0646
$_wR_2$	0.2157	0.1868	0.1979