Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B

Supporting information

for

A Bright Red Fluorescent Cyanine Dye for Live-Cell Nucleic Acid Imaging, with High Photostability and Large Stokes Shift

Si Zhang,[a] Jiangli Fan,*[a] Zhiyong Li,[a] Naijia Hao,[a] Jianfang Cao,[a] Tong Wu,[a] Jingyun Wang,*[b] and Xiaojun Peng[a]

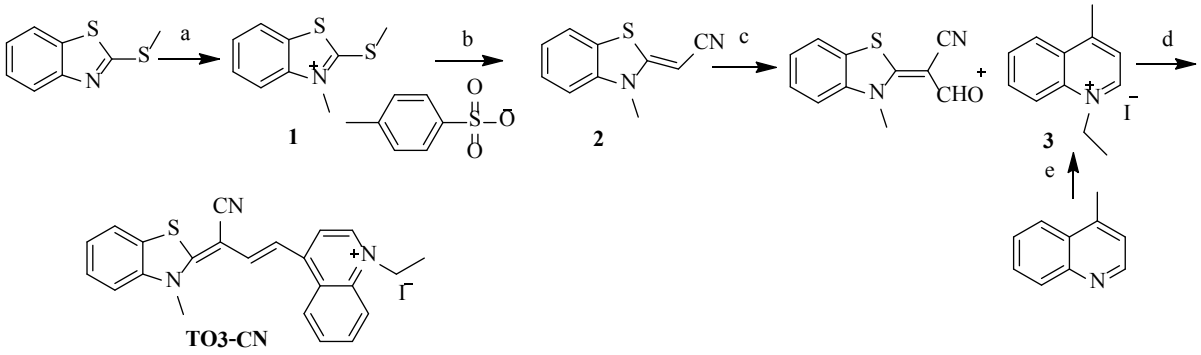
[a] State Key Laboratory of Fine Chemicals, [b] School of Life Science and Biotechnology, Dalian University of Technology, No. 2 Linggong Road, Hi-tech Zone, Dalian 116024, P.R. China

Jiangli Fan (fanjl@dlut.edu.cn); Jingyun Wang (wangjingyun@dl.cn)

Table of Contents:

Protocols for dye syntheses

Known compound **TO3** was synthesized by a published method [s1].



(a) $CH_3C_6H_4SO_3CH_3$, 120 °C, 1h; (b) $CNCH_2COOH$, Pyridine, Et_3N, rt., overnight; (c) DMF, $POCl_3$, 90 °C, 2h; (d) 210 °C, 30min; (e) CH_3CH_2I, toluene, reflux, 12h

Scheme S1. Synthetic route of dyes **TO3-CN**.

Synthesis of 3-methyl-2-(methylthio)-benzothiazolium tosylate (1) To 2.0 g (11 mmol) 2-(methylthio)-benzothiazole was added 3.0 g (16 mmol) methyl p-toluenesulfonate and heated to 130 ℃ for 1 h. After cooling to 70 ℃, acetone was added until a white precipitate appeared. Reflux was maintained for another 30min before cooling to room temperature. The precipitate was collected by filtration and dried under vacuum to yield **1** (3.02 g, 74.5%). ^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 8.22 (1 H, d, J = 8.2), 8.08 (d, J = 8.5, 1 H), 7.85 (t, J = 7.8, 1 H), 7.72 (t, J = 14.1, 6.3, 1 H), 7.68 (d, J = 8.1, 2 H), 7.21 (d, J = 8.1, 2 H), 4.15 (s, 1 H), 3.13 (s, 1 H), 2.35 (s, 1 H). HR-TOF-MS Exact mass calculated for $C_9H_{10}NS_2^+$ requires 196.0255. Found m/z 196.0261. HR-TOF-MS Exact mass calculated for $C_7H_7O_3S^-$ requires 171.0116. Found m/z 171.0116.

2-(3-methyl-2(3H)-benzothiazolylidene)acetonitrile (2) To a solution of 7.35 g (20 mmol) 3-methyl-2-(methylthio)-benzothiazolium tosylate (1) in 50 mL pyridine, 2.04 g (24 mmol) cyanoacetic acid was added, followed by 2.43 g (24 mmol) of triethylamine. After overnight stirring of the resulting reddish material under a nitrogen atmosphere, the mixture was concentrated under vacuum. Roughly 3 volumes of water were slowly added to the reaction flask with stirring during which time a homogeneous solution was obtained followed by the precipitation of the product. The solids were collected by filtration and washed thoroughly with water to yield **2** (2.57 g, 68.5%) ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.39 (d, *J* = 7.7, 1H),

7.27 (t, 1H, *J* = 7.8 Hz), 7.08 (t, *J* = 7.6, 1H), 6.93 (d, *J* = 8.1, 1H), 4.22 (s, 1H), 3.32 (s, 3H).

2-Formyl-2-(3-methyl-2(3H)-benzothiazolylidene)acetonitrile (3) A 2.3 g (15 mmol) amount of phosphorus oxychloride was added dropwise to 10 mL dimethylformamide (DMF) in an ice bath. After the addition was complete, the solution was allowed to warm to room temperature. A solution of 2.27 g (12 mmol) 2-(3-methyl-2(3H)-benzothiazolylidene)acetonitrile (2) in 15 mL DMF was added. The mixture was then stirred at 90ºC for 2 h under a nitrogen atmosphere. Then the mixture was cooled to room temperature and added to ice-water mixture. 1 M NaOH was slowly added to the reaction flask with stirring during which time a homogeneous solution was obtained followed by the precipitation of the product. The solids were collected by filtration and washed thoroughly with water to yield **3** (0.85g, 33%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.52 (s, 1H), 7.76 (d, *J* = 8.0, 1H), 7.57 (t, *J* = 7.8, 1H), 7.49 – 7.36 (m, 2H), 4.23 (s, 3H).

1-Ethyl-4-methylquinolinium iodide (4) 1-Ethyl-4-methylquinolinium iodide 4a was synthesized by mixing lepidine (2.86 g, 20.0 mmol) with ethyl iodide (3.34 g, 24.0 mmol) and heating to reflux for 8 h in 20 mL toluene. After cooling, the solid was crushed, washed several times with ethyl ether and dried under vacuum to give crude product **4** (5.44 g, 91%). HR-TOF-MS Exact mass calculated for $C_{12}H_{14}N$ (M^+) requires 172.1126. Found m/z 172.1132.

TO3-CN 1-Ethyl-4-methylquinolinium iodide 4a (0.60g, 2 mmol) and 2-Formyl-2-(3-methyl-2(3H)-benzothiazolylidene)acetonitrile 3 (0.22g, 1 mmol) were heated to 210 ° C for 30 min and then allowed to cool. The mixture was purified by silica flash column chromatography (DCM/methanol=50:1) to yield the product **TO3-CN** (0.19 g, 38%). The HPLC purity of **TO3-CN** was determined to be 98.44%. ^{1}H NMR (400 MHz, DMSO) δ (ppm): 8.99 (d, *J* = 6.8, 1 H), 8.55 (d, *J* = 8.0, 1 H), 8.39 (d, *J* = 8.7, 1 H), 8.29 (d, *J* = 6.8, 1 H), 8.21 – 8.11 (2 H, m), 8.01 (d, *J* = 7.1, 1 H), 7.98 – 7.89 (1 H, m), 7.77 (d, *J* = 8.2, 1 H), 7.60 (t, *J* = 7.9, 1 H), 7.44 (t, *J* = 8.0, 1 H), 7.19 (d, *J* = 14.4, 1 H), 4.86 (q, *J* = 7.3, 2 H), 4.12 (2 H, s), 1.54 (t, *J* = 7.1, 3 H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 165.6, 145.5, 143.3, 141.9, 138.1, 137.0,

135.0, 129.0, 128.5, 125.9, 125.6, 124.9, 123.0, 119.3, 119.2, 118.1, 114.1, 114.1, 110.0, 75.9, 51.4, 36.7, 15.5. HR-TOF-MS Exact mass calculated for $C_{23}H_{20}N_3S$ requires 370.1378. Found m/z 370.1365.

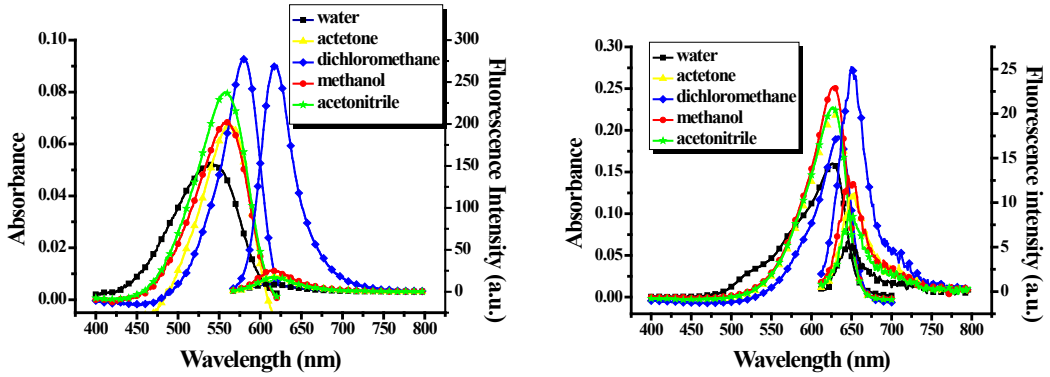
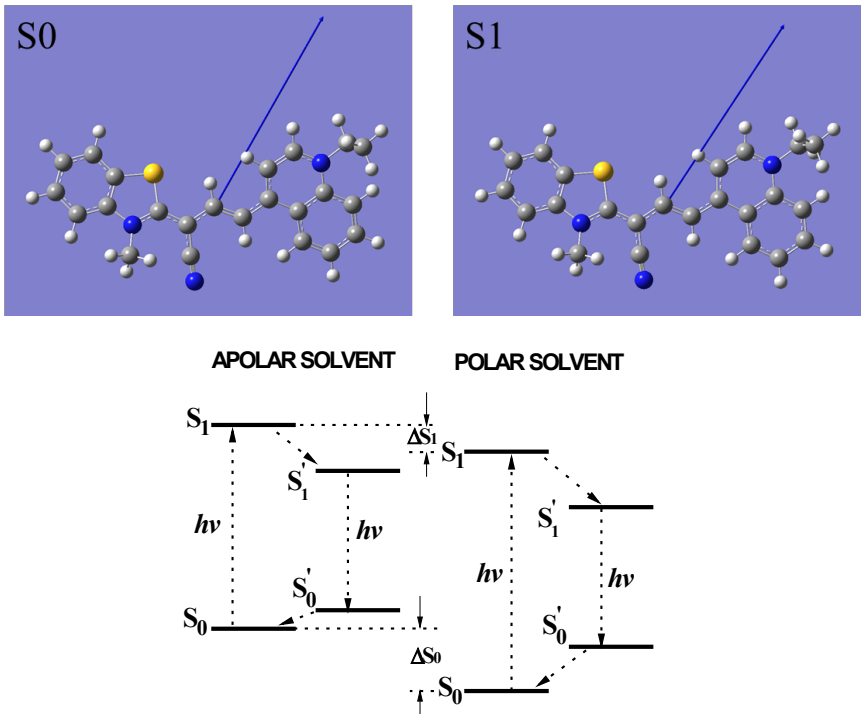


Figure S1. The absorption and fluorescent spectra of **TO3-CN** (left) and **TO3** (right) in different solvents.



The absorption blue shift is $\Delta S = \Delta S_0 - \Delta S_1$

Figure S2. The calculated dipole moments of the ground state (9.7727) and the excited state (9.5190) of **TO3-CN**.

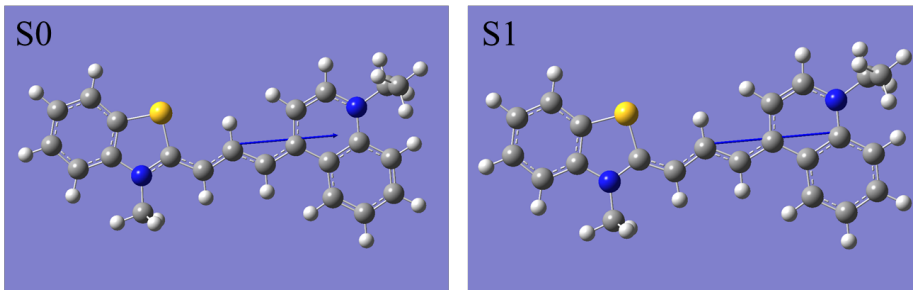


Figure S3. The calculated dipole moments of the ground state (3.9069) and the excited state (5.0688) of **TO3**.

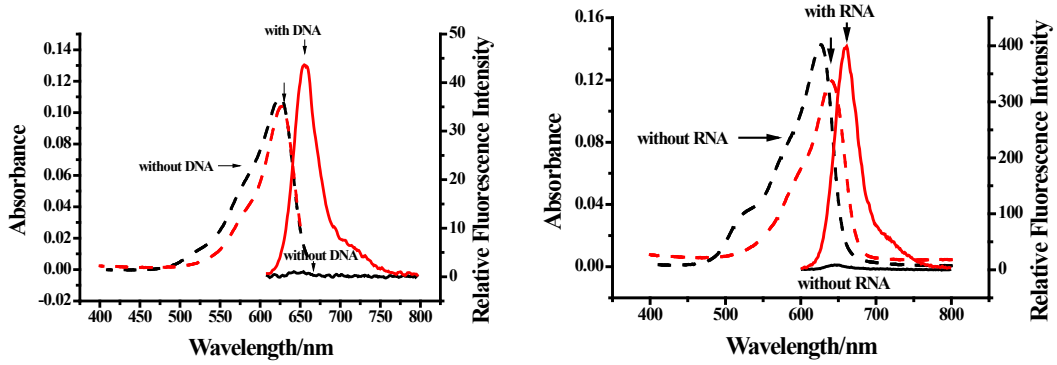


Figure S4. Absorbance and emission spectra of **TO3** in the absence and presence of CT DNA (left) or RNA (right) in buffer.

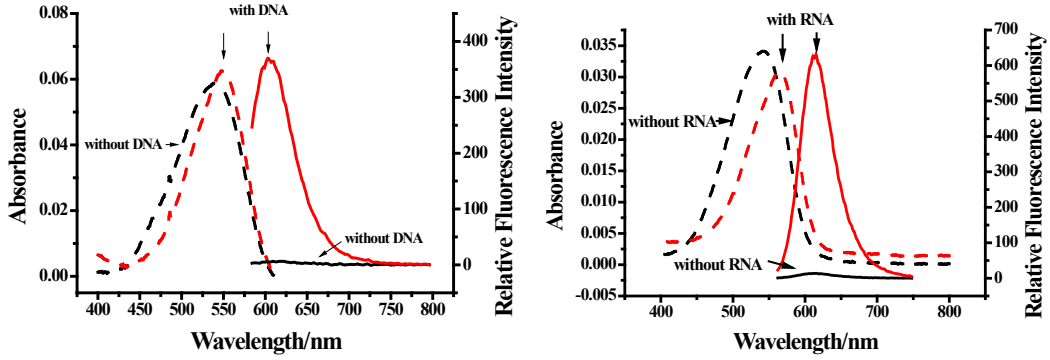


Figure S5. Absorbance and emission spectra of **TO3-CN** in the absence and presence of CT DNA (left) or RNA (right) in buffer.

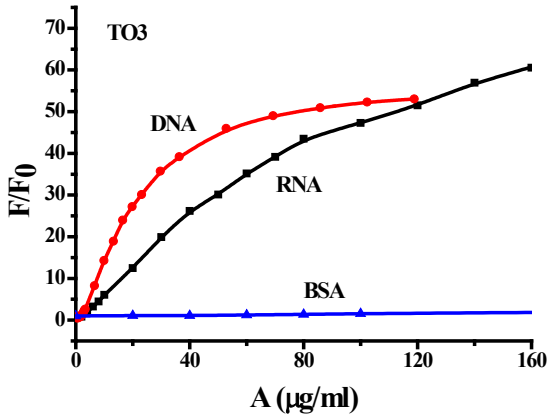


Figure S6. Fluorescence response of **TO3** (1 μM) to CT DNA (red line), tRNA (black line), and BSA (blue line) at different concentrations in Tric-HCl buffer (10 mM, pH = 7.4).

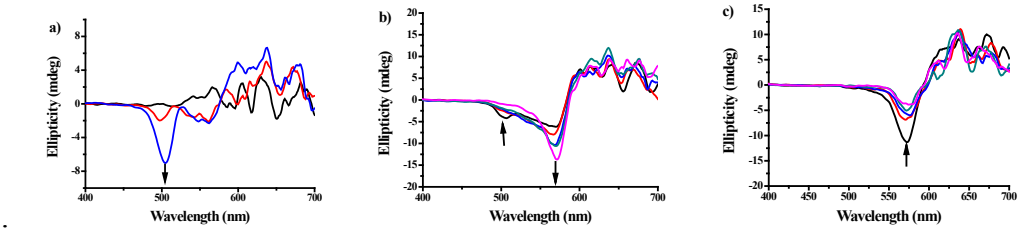


Figure S7. CD spectra during the titration of a 10.5 μM solution of **TO3** with CT DNA at 20 °C in buffer. The [base pair]/[**TO3**] molar ratios are 0.33, 0.67 in (a), 1, 1.33, 1.67, 2, 4 in (b) and 8, 16, 20, 30 and 40 in (c). The black dashed line refers to **TO3** without CT DNA. The arrows indicate how the CD bands respond to the increases in the CT DNA concentration.

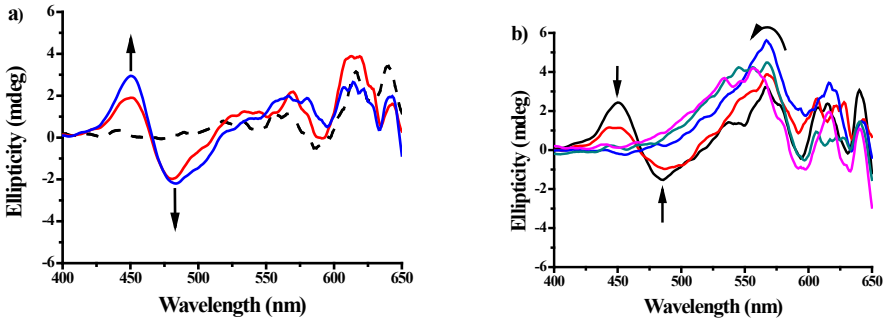


Figure S8. CD spectra during the titration of a 21 μM solution of **TO3-CN** with CT

DNA at 20 °C in buffer. The [base pair]/[**TO3-CN**] molar ratios are 0.33, 0.67 in (a) and 2, 4, 8, 20 and 40 in (b). The black dashed line refers to **TO3-CN** without CT DNA. The arrows indicate how the CD bands respond to the increases in the CT DNA concentration.

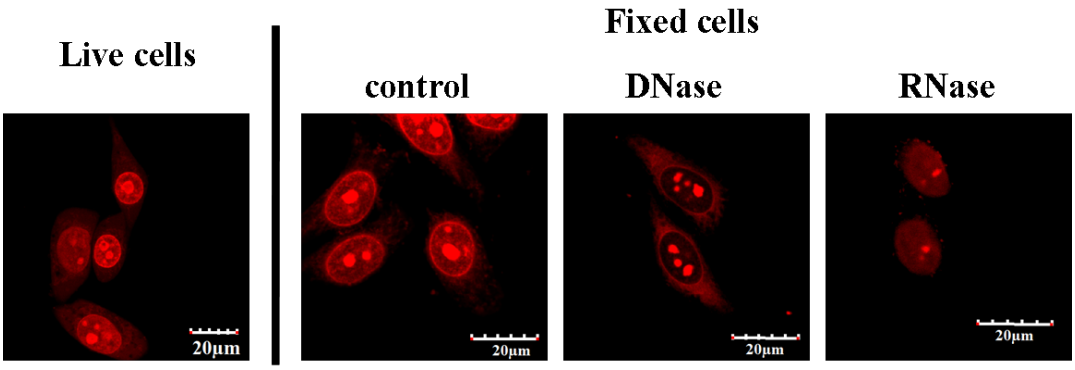


Figure S9. Live-cell staining and DNase and RNase digest experiments with **TO3-CN** in HeLa cells. **TO3-CN** (excited at 559 nm and collected at 575 nm to 620 nm) was cultured at 2 μM concentration for 45 min. The scale bar represents 20 μm.

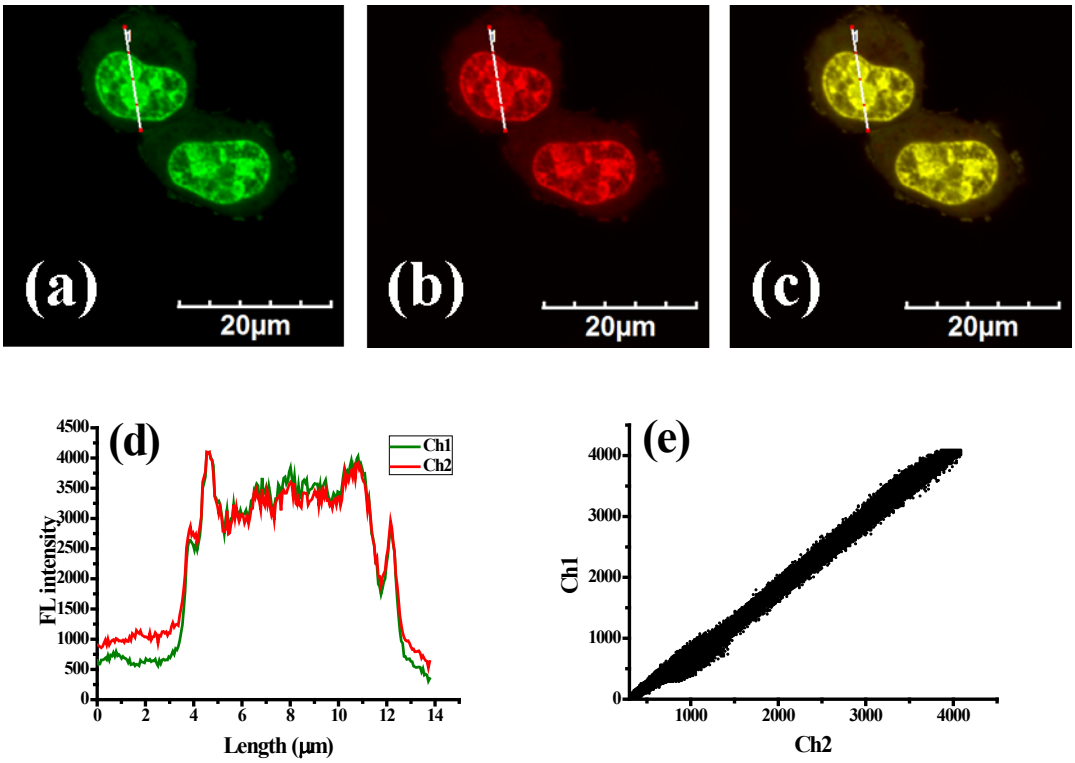


Figure S10. Colocalization imaging of HeLa cells stained with 3.0 μM SYTO 9 and

2.0 μM TO3-CN for 45 min at 37 °C. (a) Confocal image from SYTO 9 on channel 1 (495—535 nm, λ_{ex} = 488 nm). (b) Confocal image from **TO3-CN** on channel 2 (575—620 nm, λ_{ex} = 559 nm). (c) Merged image of channels 1 and 2. (d) Intensity profile of ROIs across MCF-7 cells. (e) Correlation plot of SYTO 9 and **TO3-CN** intensities.

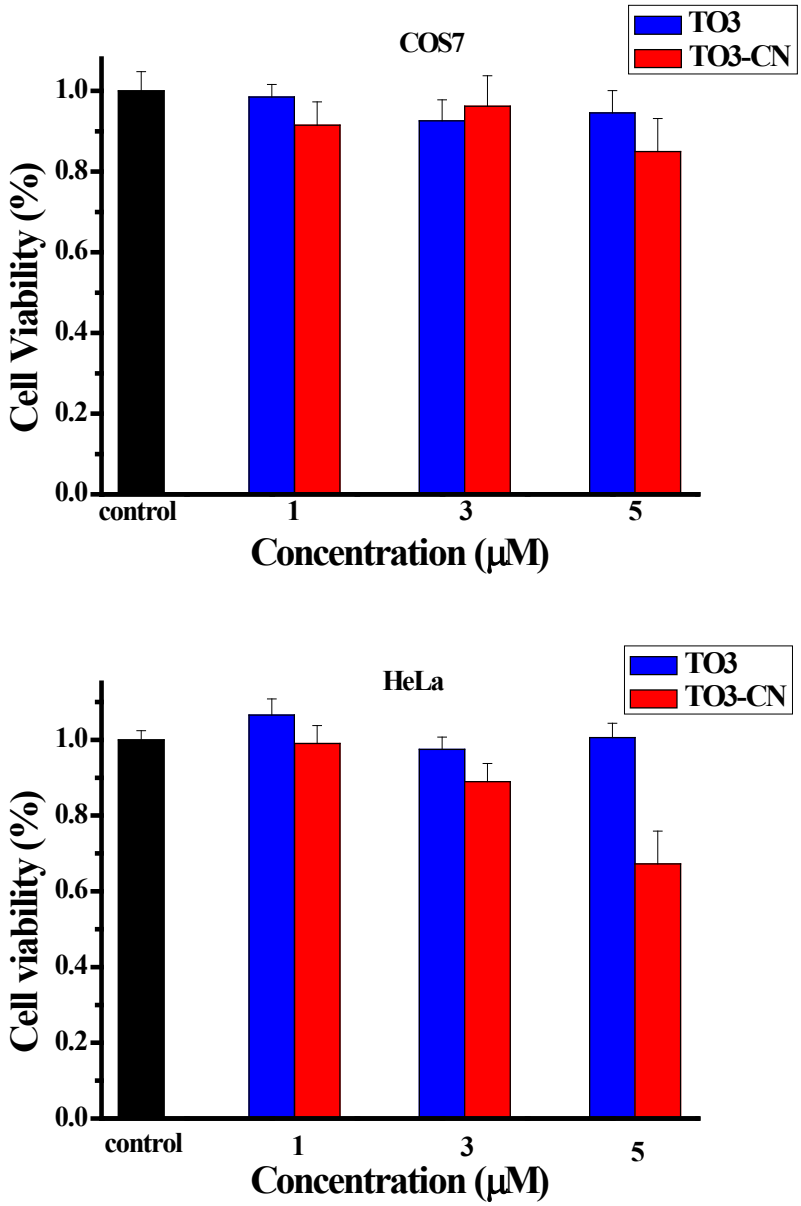
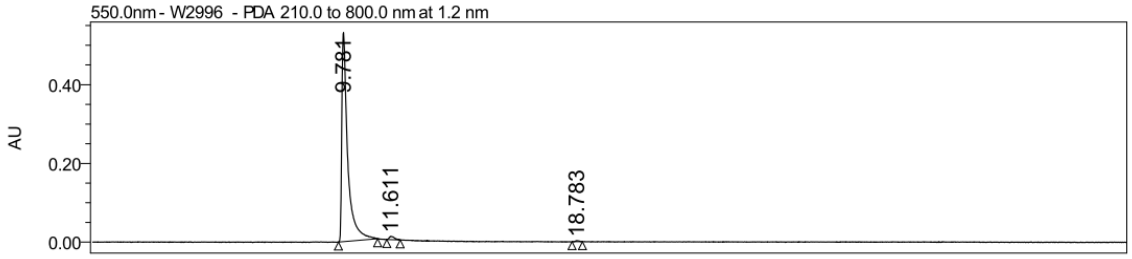


Figure S11. Comparisons of the cytotoxicity of **TO3** and **TO3-CN** at various concentrations (1, 3, and 5 μM) in living cos7 or HeLa cells for 6 h.

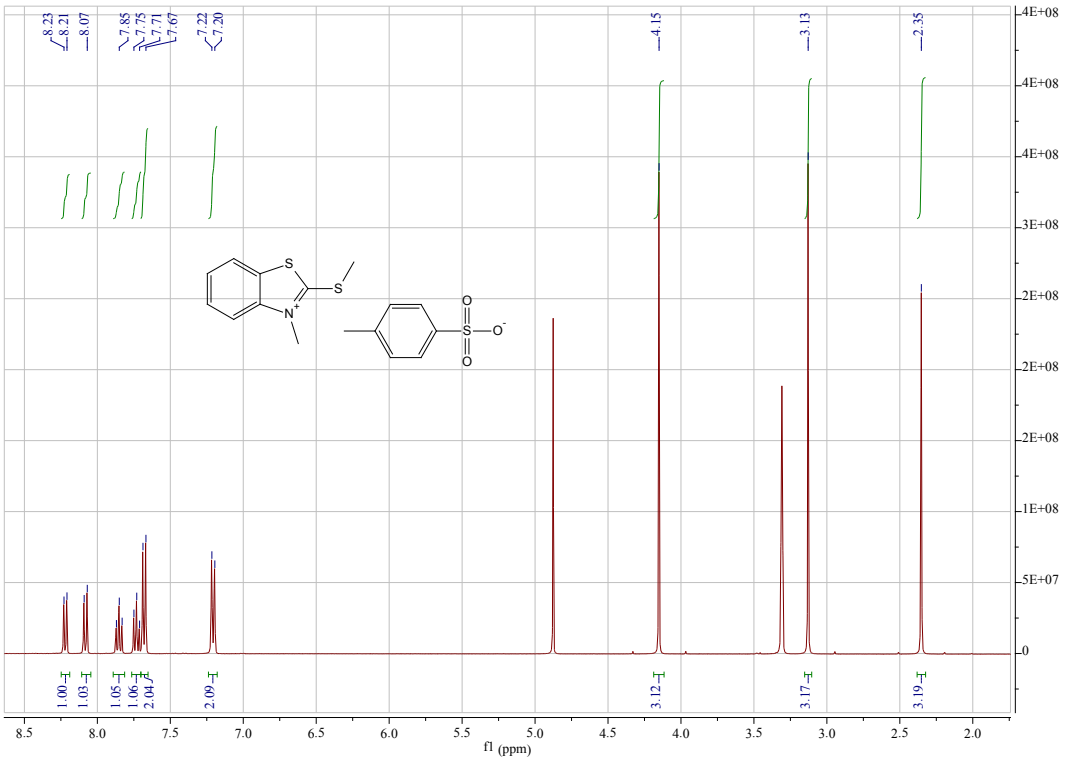


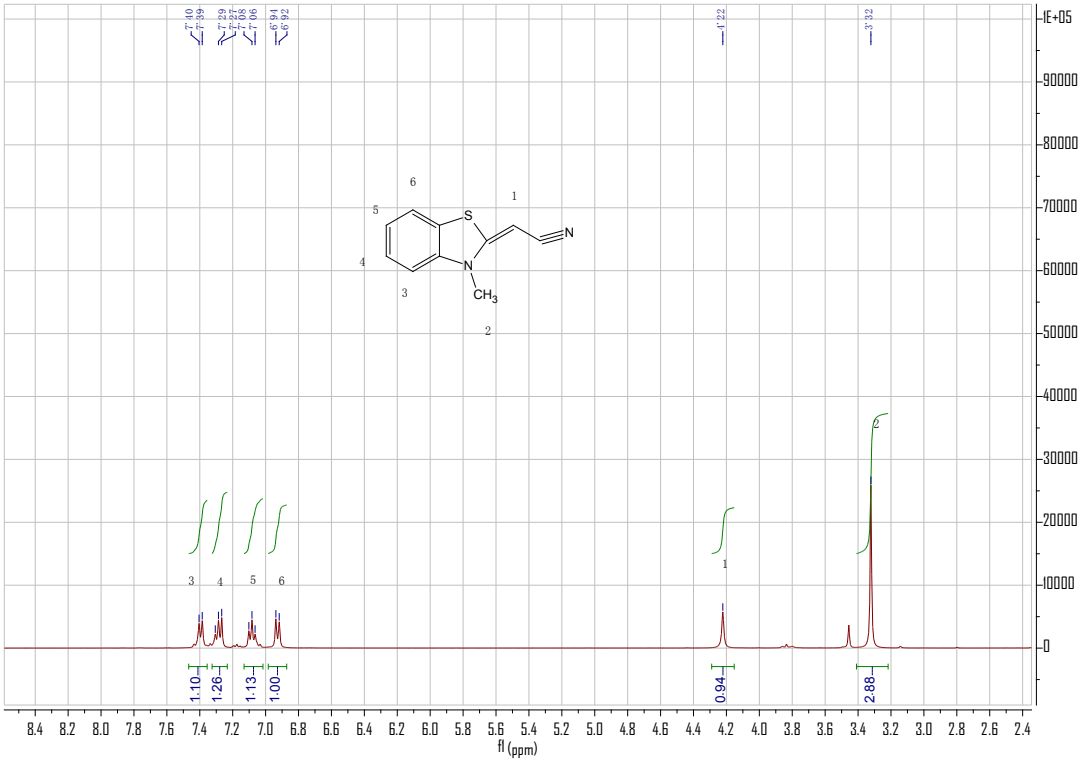
Processed Channel Descr.: PDA 550.0 nm

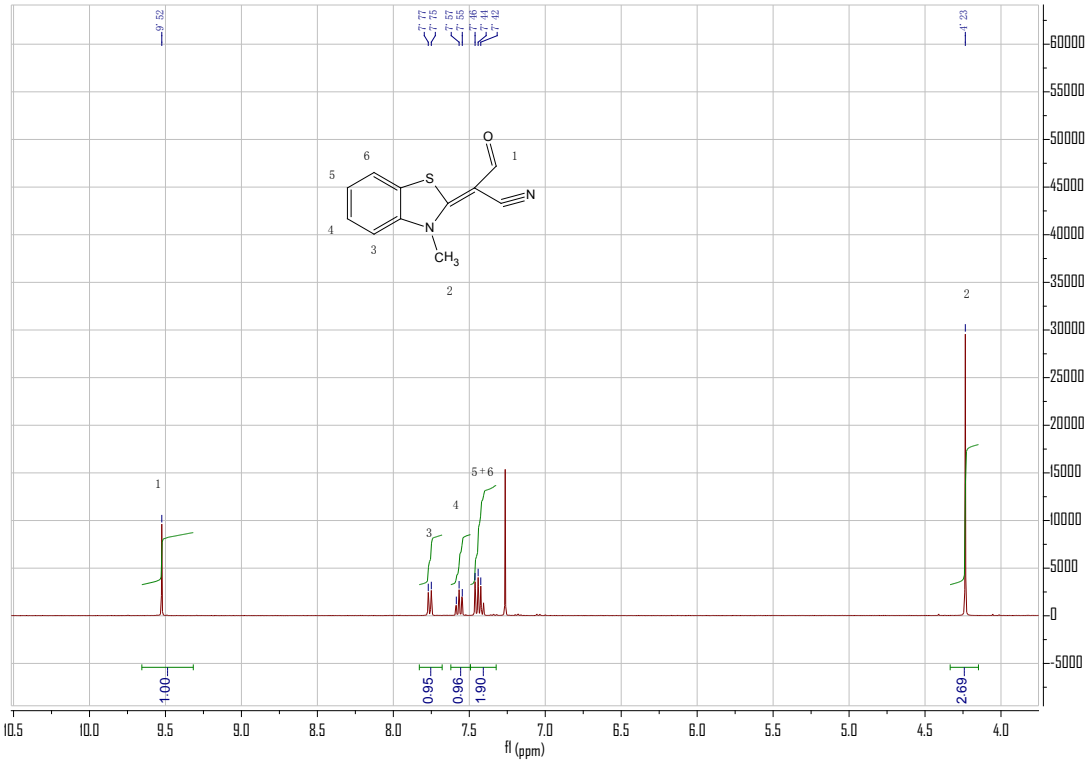
	Processed Channel Descr.	RT	Area	% Area	Height
1	PDA 550.0 nm	9.781	8390728	98.44	531836
2	PDA 550.0 nm	11.611	111276	1.31	7989
3	PDA 550.0 nm	18.783	22076	0.26	2197

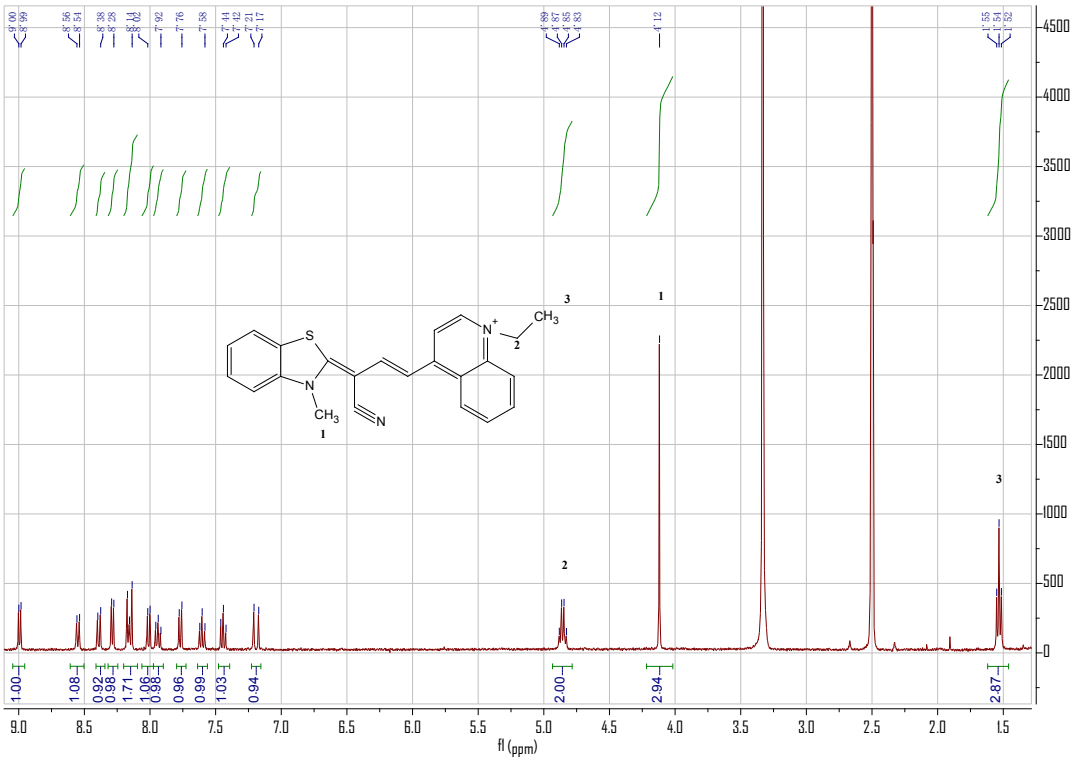
Figure S12. The HPLC purity of **TO3-CN**

^{1}H and ^{13}C NMR spectra

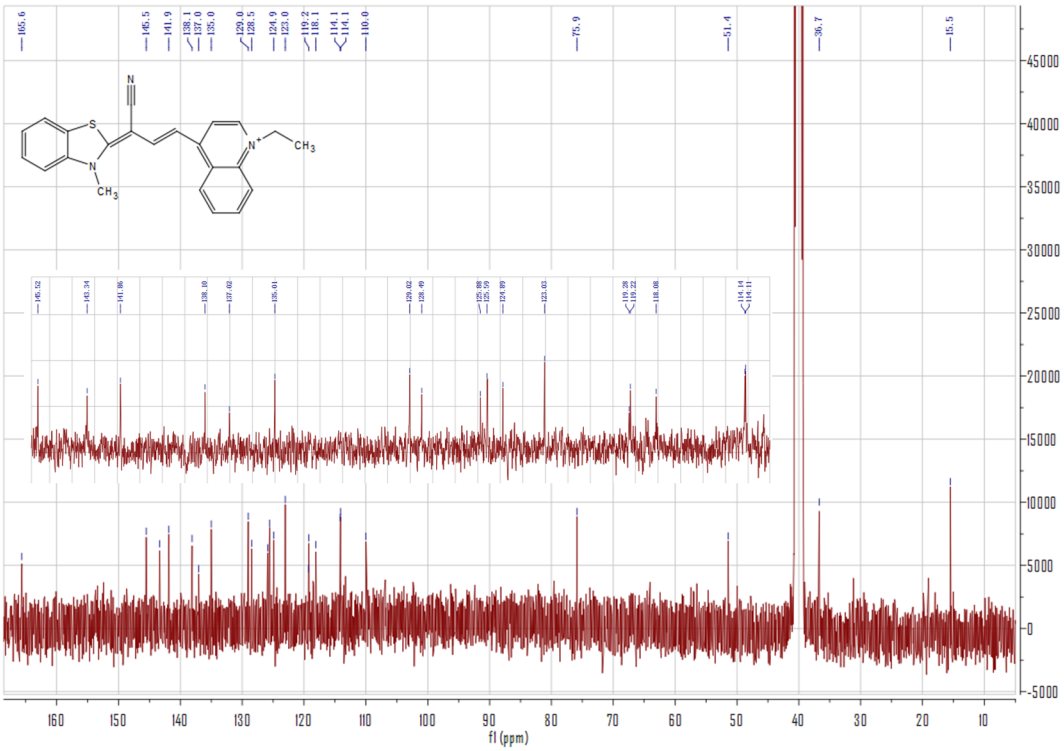



7.10
7.09
7.07
7.06
6.91
6.92
4.22
3.32
1E+05
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
S
N
CH3
1.10
1.26
1.13
1.00
0.94
2.88
f1 (ppm)


9.52
7.77
7.75
7.57
7.55
7.46
7.44
7.42
4.23
60000
55000
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
-5000
O
S
N
CH3
5+6
1.00
0.95
0.96
1.90
2.69
f1 (ppm)



^{1}H NMR spectrum of **TO3-CN**



^{13}C NMR spectrum of **TO3-CN**

[s1] G. H. K. L. G. S. Brooker, W. W. Williams, J. Am. Chem. Soc. **1942**, *64*, 199-210.