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Supporting Information

Red fluorescent probes for real-time imaging of cell cycle by dynamic monitoring of nucleolus and chromosome

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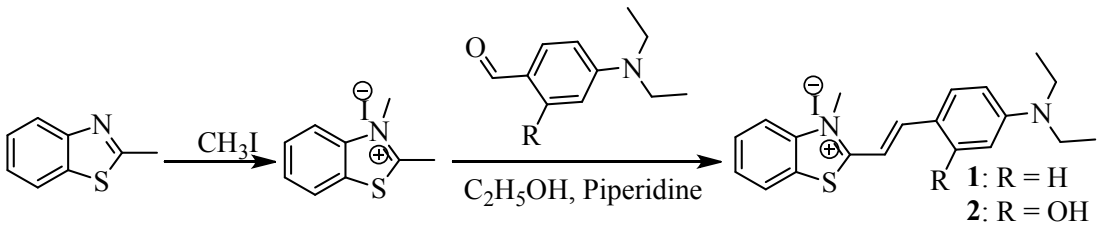
Experimental section

General materials and apparatus

All starting materials were used as received from commercial sources unless otherwise indicated. Solvents were purified and degassed by standard procedures. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma Aldrich), SYTO RNA-Select, Hoechst 33342 were all purchased from Life Technologies, USA. ct-DNA and RNA (Bovine Serum Albumin, from baker's yeast) were purchased from Sigma Aldrich.

NMR spectra were recorded on a Varian INOVA 500NB and a Mercury Plus 400 spectrometer. Shifts are referenced relative to the internal solvent signals. Fluorescence spectra and fluorescence lifetime was recorded on a combined fluorescence lifetime and steady state spectrometer FLS 920 (Edinburgh). UV–vis spectra were recorded on a Varian Cary 300 spectrophotometer. ESI-MS were recorded on a Thermo Finnigan LCQ DECA XP spectrometer (USA). The quoted m/z values represent the major peaks in the isotopic distribution. Fluorescence microscopy of cells was performed in Carl Zeiss LSM 710 (Göttingen, Germany).

Synthesis of Compounds



Scheme S1. Synthesis of compounds in this work.

The probes **1** and **2** were synthesized by the condensation reaction between the substituted 4-Diethylaminobenzaldehyde or its derivatives 4-(Diethylamino) salicylaldehyde and 2-Methyl-N-methylbenzothiazolium iodide. The raw materials were put into 100 ml flask with a stirrer and condenser, and then an amount of ethanol and 3 drops of piperidine were added in turn. The mixture was heated under reflux for 4 h. After cooling to room temperature, precipitate of compounds **1** and **2** were filtered and washed by ethanol to afford the products.

1 magenta solid (371 mg, yield 69.8%). ^{1}H NMR (400 MHz, DMSO) δ 10.75 (s, 1H), 8.19 (d, *J* = 7.9 Hz, 1H), 8.10 (d, *J* = 15.0 Hz, 1H), 8.00 (d, *J* = 8.4 Hz, 1H), 7.82 (d, *J* = 9.2 Hz, 1H), 7.72 (t, *J* = 7.8 Hz, 1H), 7.61 (t, *J* = 7.7 Hz, 1H), 7.45 (d, *J* = 15.1 Hz, 1H), 6.44 (d, *J* = 9.1 Hz, 1H), 6.19 (s, 1H), 4.11 (s, 3H), 3.44 (d, *J* = 14.0, 7.0 Hz, 4H), 1.16 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR (126 MHz, DMSO) δ 171.09 (s), 151.44 (s), 150.03 (s), 141.90 (s), 133.30 (s), 128.81 (s), 127.31 (s), 126.68 (s), 123.76 (s), 121.04 (s), 115.83 (s), 111.61 (s), 105.65 (s), 44.22 (s), 35.63 (s), 12.57 (s). For $[M-I]^+$ m/z 323.30. Found: $[M-I]^+$ m/z 323.16.

2 magenta solid (475 mg, yield 81%).^{1}H NMR (400 MHz, DMSO) δ 8.28 (d, *J* = 8.1 Hz, 1H), 8.09 – 8.03 (m, 1H), 7.98 (d, *J* = 14.4 Hz, 1H), 7.88 (d, *J* = 8.7 Hz, 2H), 7.79 – 7.70 (m, 1H), 7.66 (t, *J* = 7.4 Hz, 1H), 7.60 – 7.50 (m, 1H), 6.80 (d, *J* = 9.0 Hz, 2H), 4.21 (s, 3H), 3.56 – 3.38 (m, 4H), 1.15 (t, *J* = 5.9 Hz, 6H). ^{13}C NMR (126 MHz, DMSO) δ 171.09 (s), 151.44 (s), 150.03 (s), 141.90 (s), 133.30 (s), 128.81 (s), 127.31 (s), 126.68 (s), 123.76 (s), 121.04 (s), 115.83 (s), 111.61 (s), 105.65 (s), 44.22 (s), 35.63 (s), 12.57 (s). For $[M-I]^+$ m/z 339.28. Found: $[M-I]^+$ m/z 339.15.

Absorption and emission

The spectroscopic investigations were carried out in PBS (pH=7.4) and organic solvents used without further purification. The absorption spectra were recorded with a Varian Cary 300 spectrophotometer at 298K. The emission spectra were recorded on an Edinburgh FLS 920 Spectrometer at 298K. Decay curves of compounds were recorded by an Edinburgh FLS 920 Spectrometer at 298K. The analysis of the fluorescence decay profiles was accomplished with decay-analysis software provided by the manufacturer, and the quality of the fit was assessed with the χ^2 value close to unity and with the residuals regularly distributed along the time axis.

Determination of quantum yield

The relative fluorescence quantum yields were determined by using Rhodamine 6G in methanol (0.95) as standard[1] and were calculated through the following equation:

$$\phi_x=\phi_s*(F_x/F_s)*(A_s/A_x)*(n_x/n_s)^2$$

where ϕ represents quantum yield; F is integrated area under the corrected emission spectrum; A absorbance at the excitation wavelength; n the refractive index of the

solution; and the subscripts x and s refer to the unknown and the standard, respectively.

Cell culture

A549 cells were cultured in RPMI 1640 medium with 10% fetal bovine serum (FBS), Hela cells were cultured in DMEM medium with 10% FBS, all with 1% penicillin-streptomycin, at 37 °C under a 5% CO_2 atmosphere.

Confocal microscopy

For living cell staining experiments, cultures cells grown on confocal petri dish were stained with **1** or **2** in complete medium for 10 min at 37°C and then imaged with both one- and two-photon Microscopy (ex: 750 nm). Temperature dependence studies used cells that had been cooled at 4 °C and incubated with **1** or **2** at 4 °C for 10 min. For inhibitors, cells were previously treated with chloroquine at the stated concentrations for 30 minutes, and then incubated with **1** or **2** for 10 min before imaging. For cell counterstain experiment: A549 cells were stained with 10 μM **1** or **2** for 10 min. After rinsing with PBS twice, the same sample was stained with 2.5 μM SYTO RNA-Select for 10 min or Hoechst 33342 and then imaged by confocal microscopy immediately.

Digest test

For DNase and RNase digest test, A total of 1 mL clean PBS (as control experiment), 100 μg/mL DNase-Free RNase (GE) or 100 U DNase was added into three sets of prefixed A549 cells for 30 min, and then cells were stained with 10 μM **1** or **2** for 10 min. Cells were rinsed by clean PBS twice before imaging.

MTT assay for the cell cytotoxicity

This involves the reduction of MTT tetrazolium to MTT formazan pigment by the metabolic activity of living cells. Cells were seeded at a density of 1 *10^5 cells/ml in a 96-well plate. After 24 h of cell attachment, cells were treated with **1** or **2** for 24 h. Six replicate wells were used for each control and tested concentrations. After incubation for 24 h, the medium was removed and cells were washed with PBS twice.

MTT tetrazolium solution (100 mL of 0.5 mg/mL in PBS) was added to each well, and the cells were further incubated at 37 °C for 4 h in a 5% CO_2 humidified atmosphere. Excess MTT tetrazolium solution was then carefully removed and the colored formazan was dissolved in 100 ml dimethyl sulfoxide (DMSO). The plate was shaken for 10 min and the absorbance was measured at 570 using a microplate reader.

Photostability test

A549 cells were incubated in 1 ml culture solution consist of 10 μM probes **1**/**2** or 2.5 μM SYTO RNA-Select for 20 min and imaged by confocal microscope without wash. The laser powers 488 nm and 543 nm were used to irradiate the SYTO RNA-Select and probes **1**/**2** stained cells respectively. The initial intensity referred to the first scan of each dye.

References

1 Pure Appl. Chem. , Vol. 83, No. 12, pp. 2213–2228, 2011

Figures

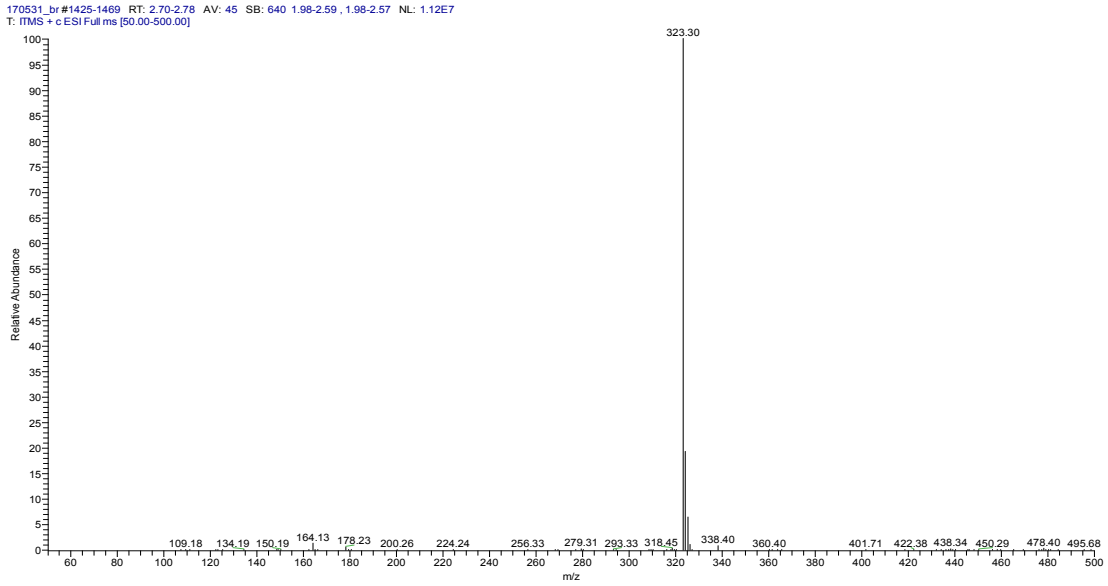


Fig. S1. The mass spectra of probe **1.**

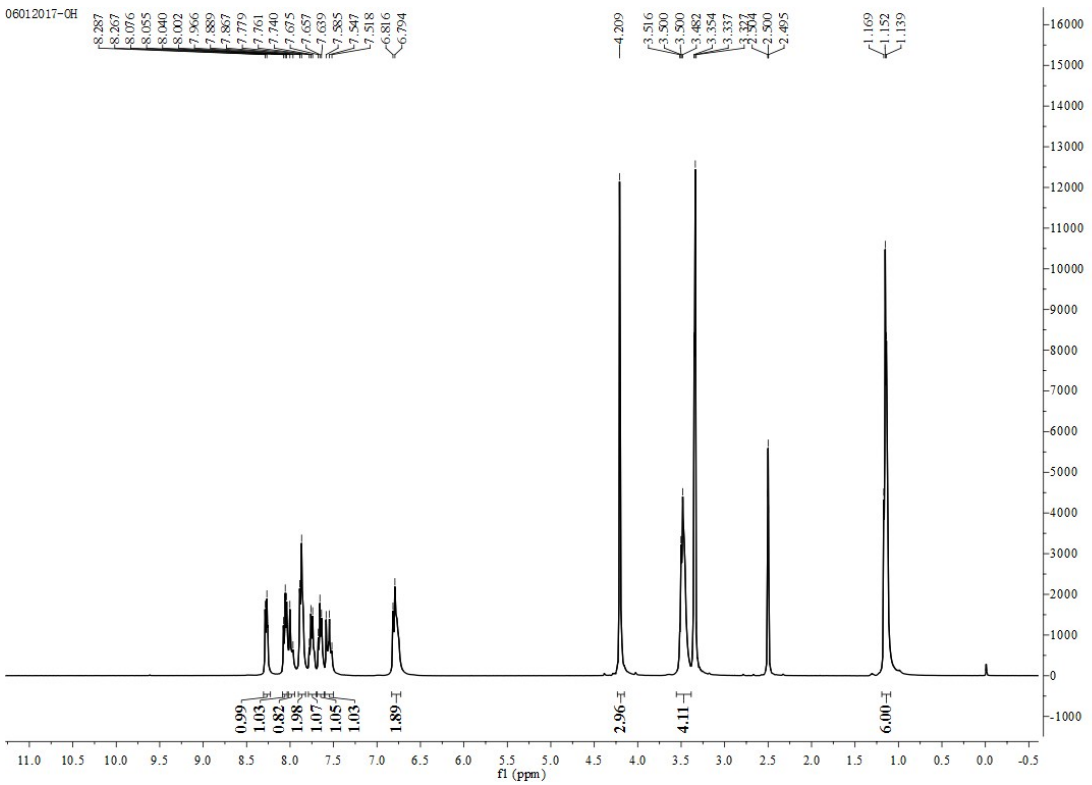


Fig. S2.^{1}H NMR spectrum of **1**.

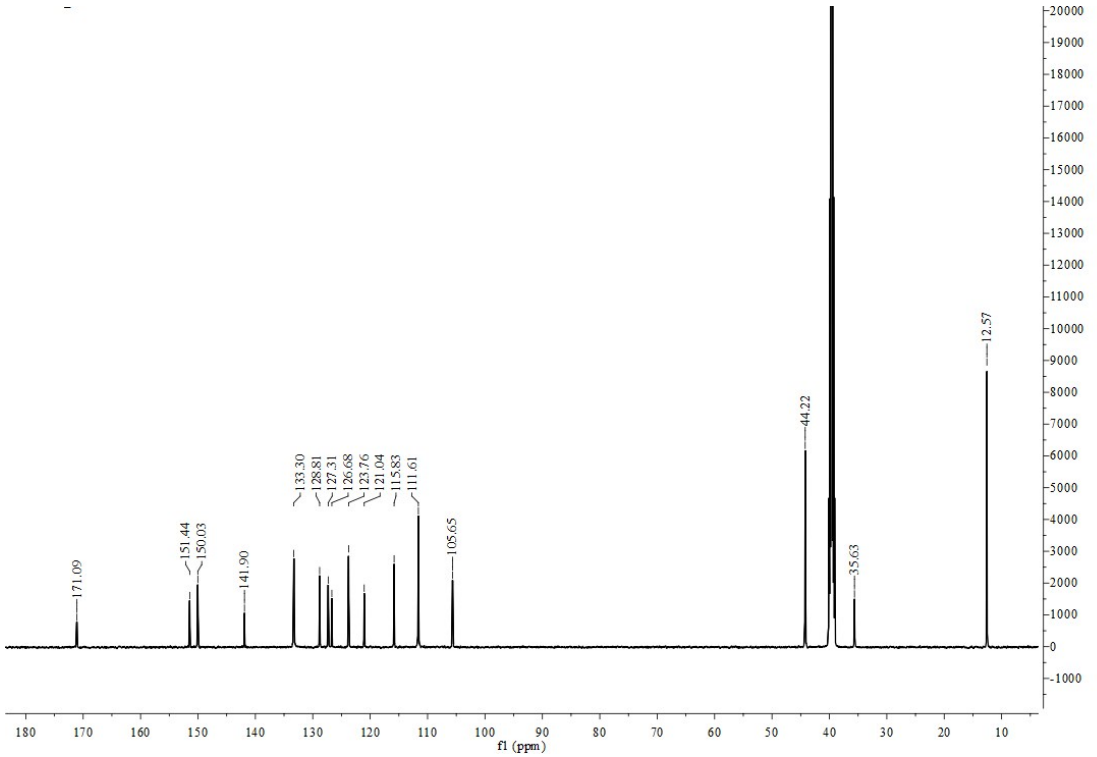


Fig. S3. ^{13}C NMR spectrum of **1**.

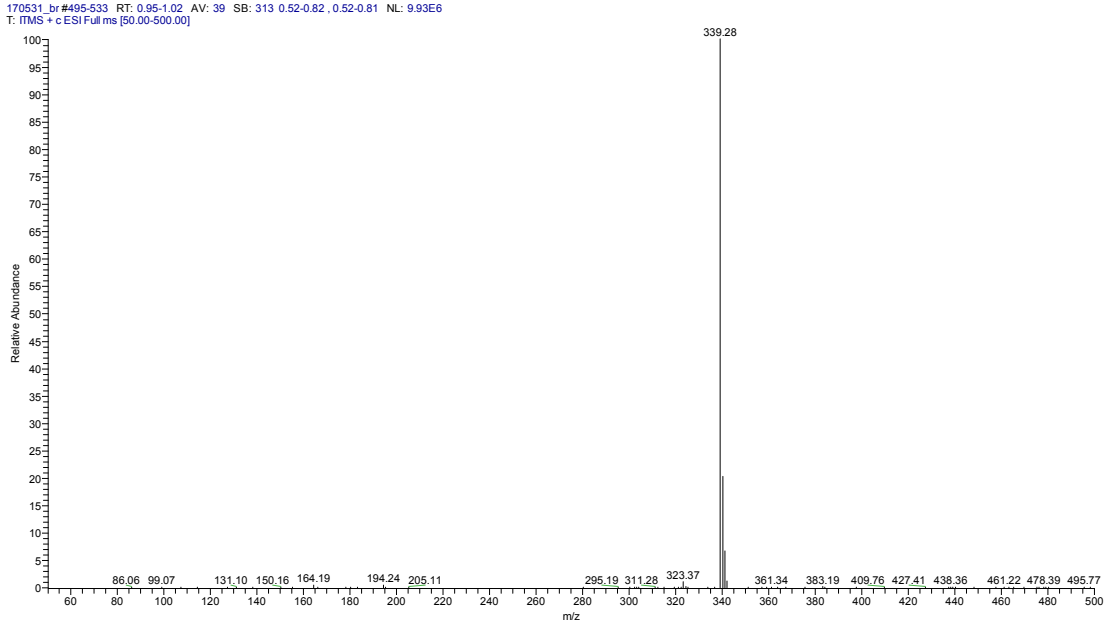


Fig. S4. The mass spectra of probe **2.**

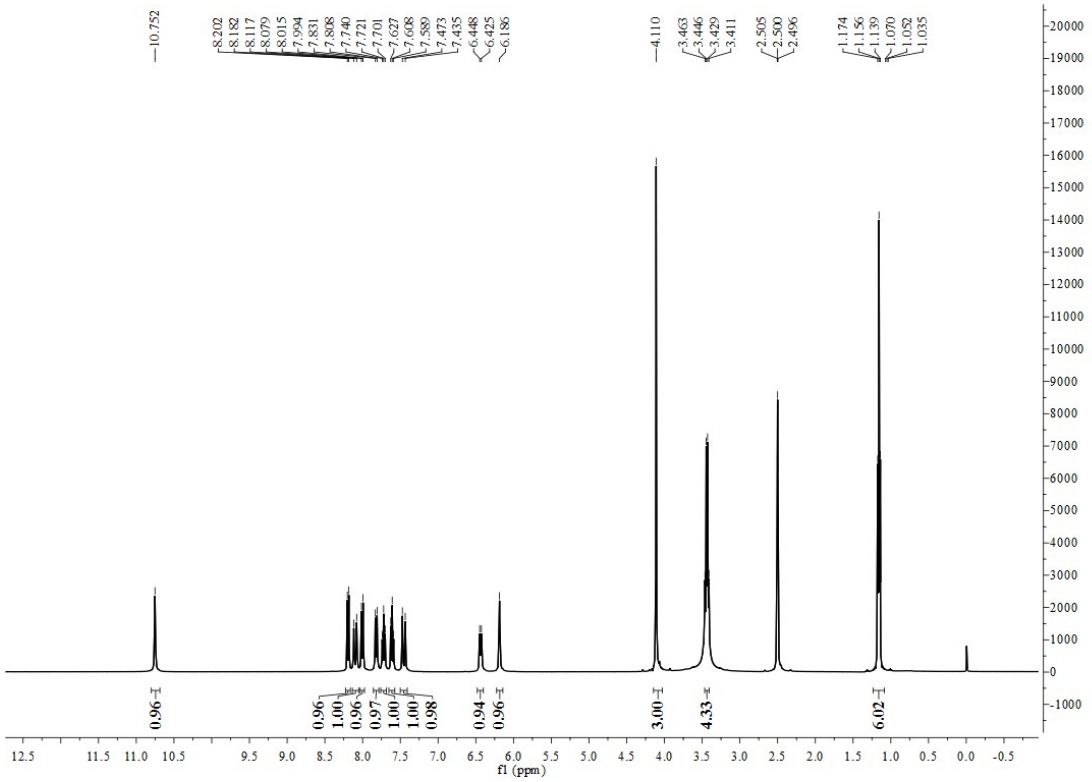


Fig. S5. ^{1}H NMR spectrum of **2**.

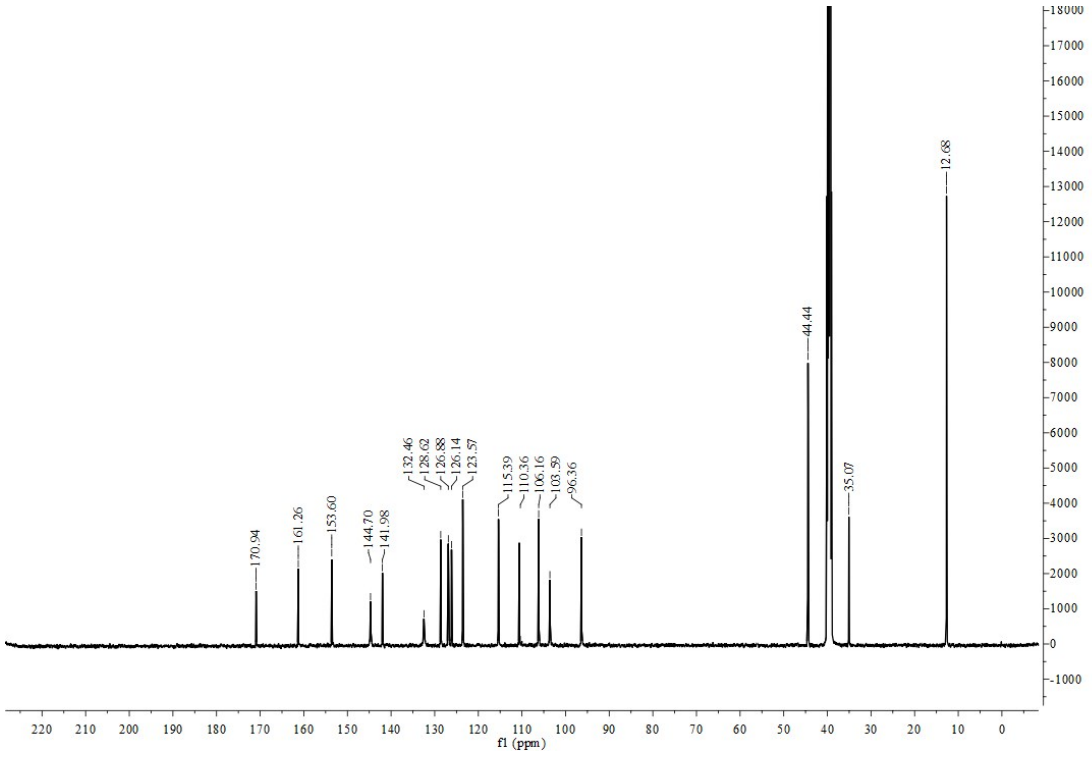


Fig. S6. ^{13}C NMR spectrum of **2**.

Table S1. Crystallographic data of probes 2.

Compound	2
CDCC no.	1584307
formula	$C_{21}H_{27}IN_2O_4S$
molecular weight	530.41
description	block, fuchsia
temperature (K)	150
crystal size (mm)	0.20 × 0.20 × 0.1
λ (Å)	1.54184
crystal system	monoclinic
space group	$P2_1/c$
a (Å)	7.9761(3)
b (Å)	18.6330(6)
c (Å)	15.9637(4)
α (°)	90.00
β (°)	90.406(3)
γ (°)	90.00
volume, Å	2372.44(13)
Z	4
absorption coefficient (mm^{-1})	0.05658
F(000)	1072.0
θ range (deg)	7.3 – 123.3
reflections collected/unique	23391
final R indices[$I > 2\sigma(I)$][a]	R_1 = 0.0583, wR_2 = 0.1816
R indices (all data)	R_1 = 0.0628, wR_2 = 0.1853
GOF[b]	1.194

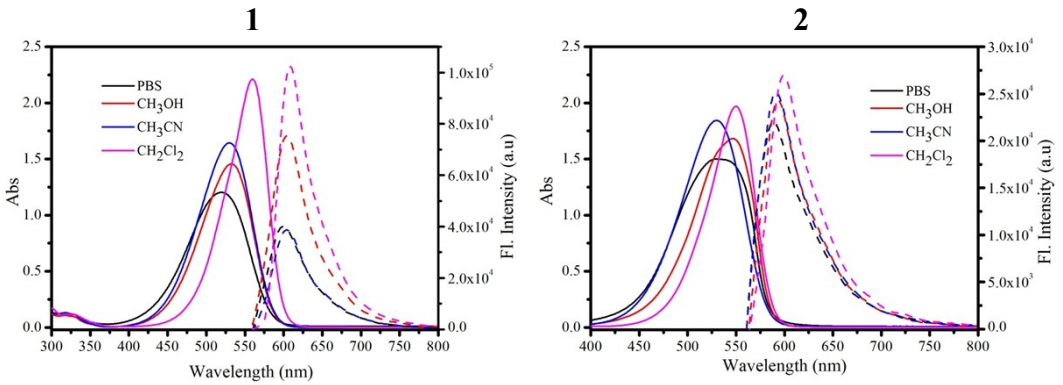


Fig. S7. Absorption and emission spectra of probes **1** and **2** in different solvents.

Table S2. Photophysical data of compounds 1 and 2 at room temperature

Compounds		λ_{max}^{abs} (nm)	$\varepsilon(10^4 cm^{-1}M^{-1})$	λ_{max}^{fluo} (nm)	Φ
1	PBS	520	6.02	598	0.0078
	CH_3OH	533	7.28	600	0.0093
	CH_3CN	530	8.22	604	0.0069
	CH_2Cl_2	560	11.06	609	0.0192
2	PBS	529	7.50	588	0.0046
	CH_3OH	549	8.39	593	0.0067
	CH_3CN	530	9.22	591	0.0055
	CH_2Cl_2	553	14.07	599	0.0110

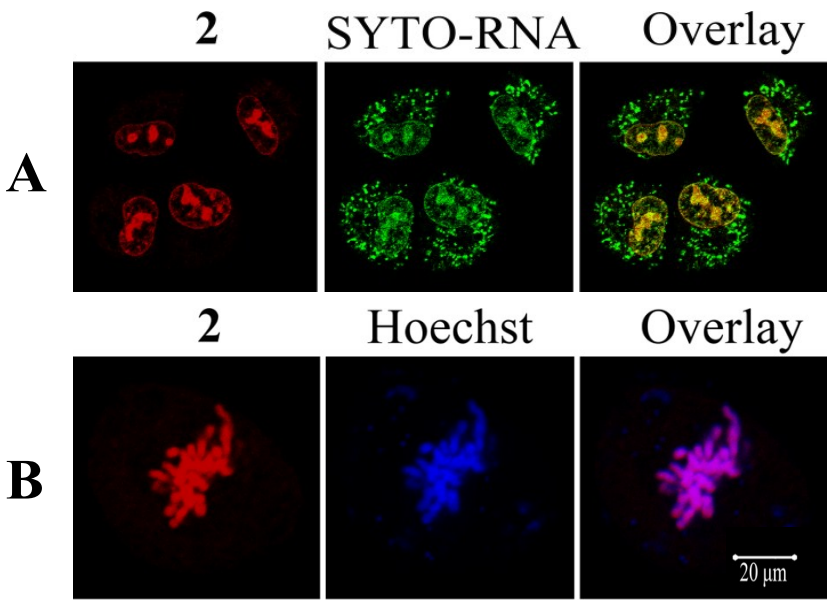


Fig. S8. Co-stained with SYTO-RNA Select (A) and DNA dye Hoechst 33342 (B) to indicate nucleolus and chromosome localization of probe **2**. Images were obtained with excitation of 543 nm and emission of 600 ± 20 nm for probe **2**; excitation of 405 nm and emission of 460 ± 20 nm for Hoechst 33342; excitation of 488 nm and emission of 530 ± 20 nm for SYTO-RNA Select.

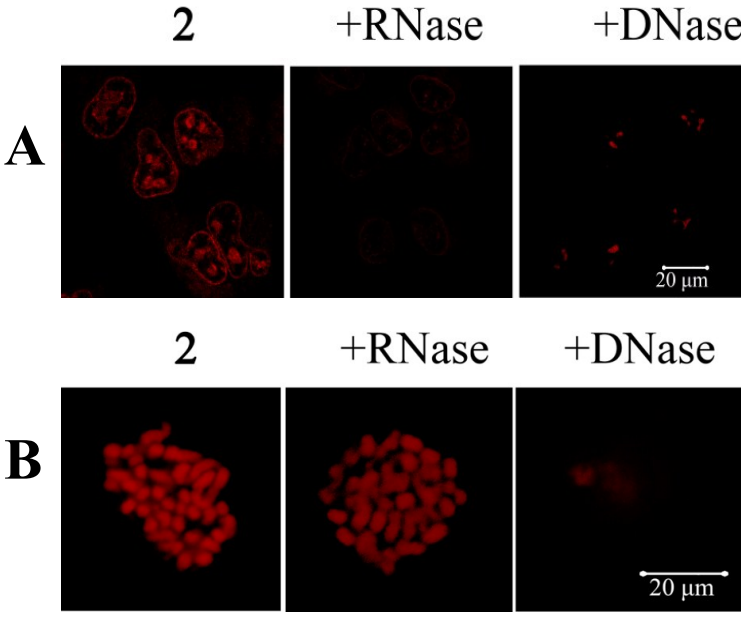


Fig. S9. Digest experiments (A) indicate nucleolus localization in interphase and (B) chromosome localization during mitosis. Images were obtained with excitation of 543 nm and emission of 600 ± 20 nm for probe **2**.

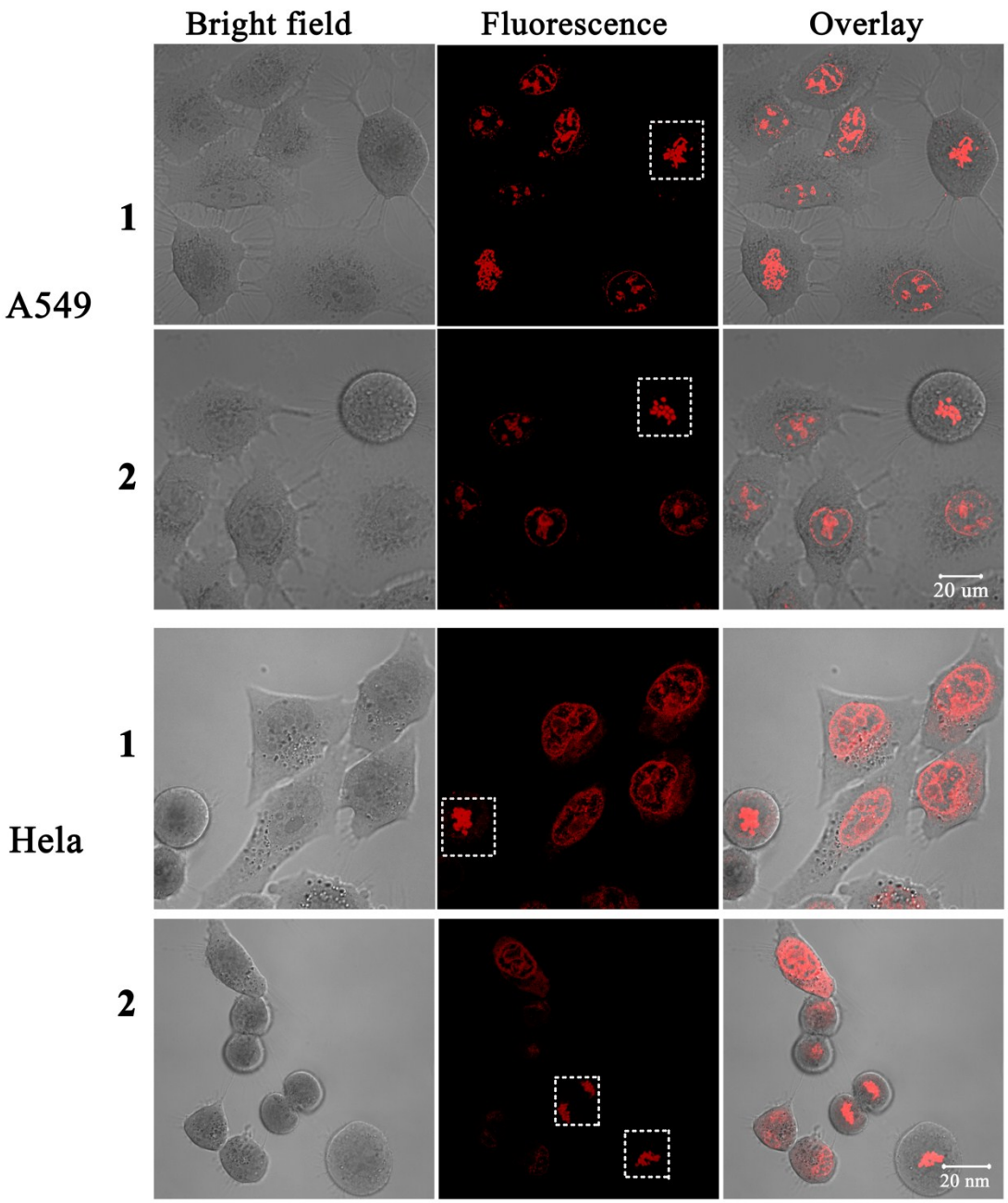


Fig. S10. Nucleolus and chromosome stain in A549 cells and Hela cells. The cells in the square are cell undergoing mitosis with assembled chromosome.

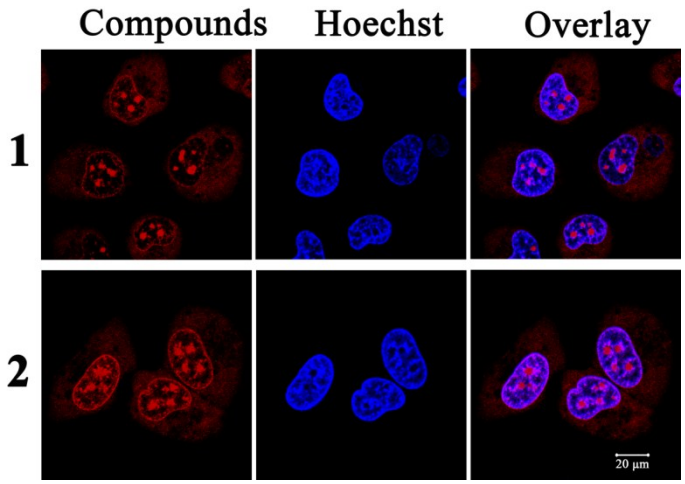
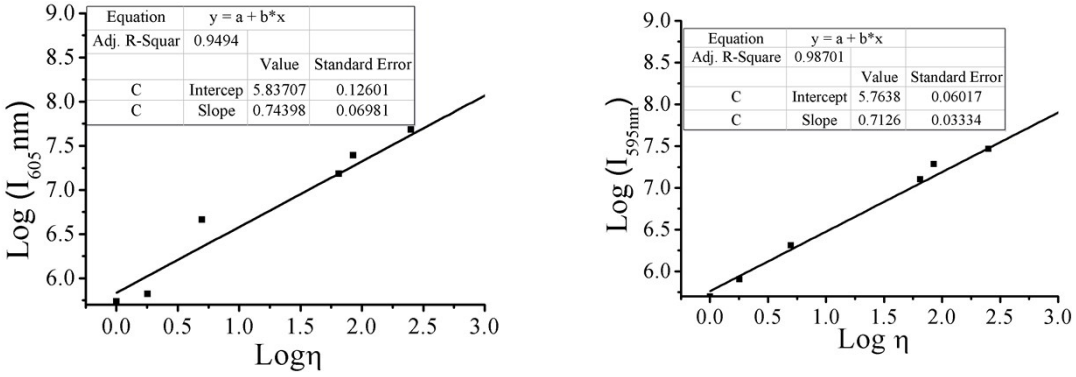


Fig.S11. Live cell counter-stain of probes **1** and **2** with Hoechst 33342 (2 μg/ml). Images were obtained with excitation of 543 nm and emission of 600 ± 20 nm for probes **1** and **2**; excitation of 405 nm and emission of 460 ± 20 nm for Hoechst 33342.



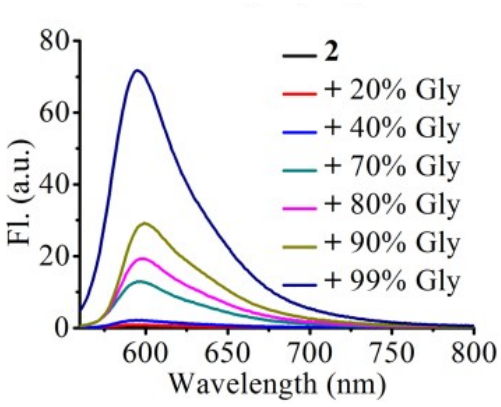


Fig. S12. The height vs the viscosity on a double logarithmic plot of probe **1** (A) and **2** (B) in the presence of glycerol in PBS. And emission spectra of probe **2** upon subsequent addition of glycerol in PBS (C).

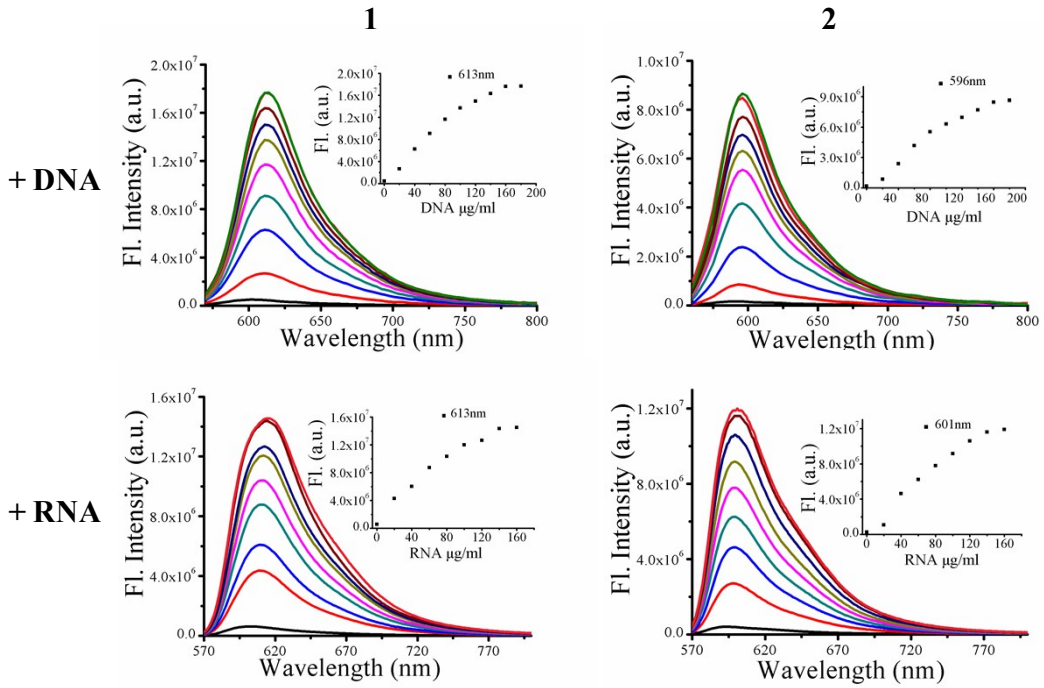


Fig. S13. Emission spectra of compounds **1** and **2** upon titration of nucleic acid (DNA and RNA) with excitation at 530 nm.

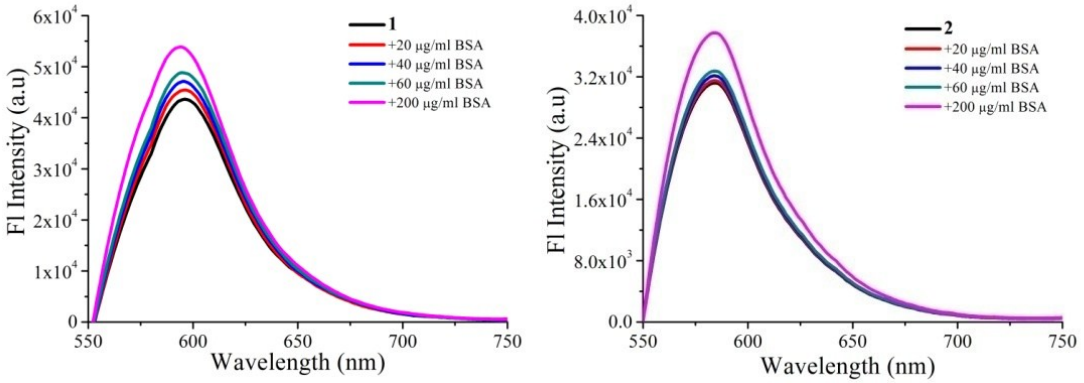


Fig. S14. Emission spectra of compounds **1** and **2** upon titration of BSA with excitation at 530 nm.

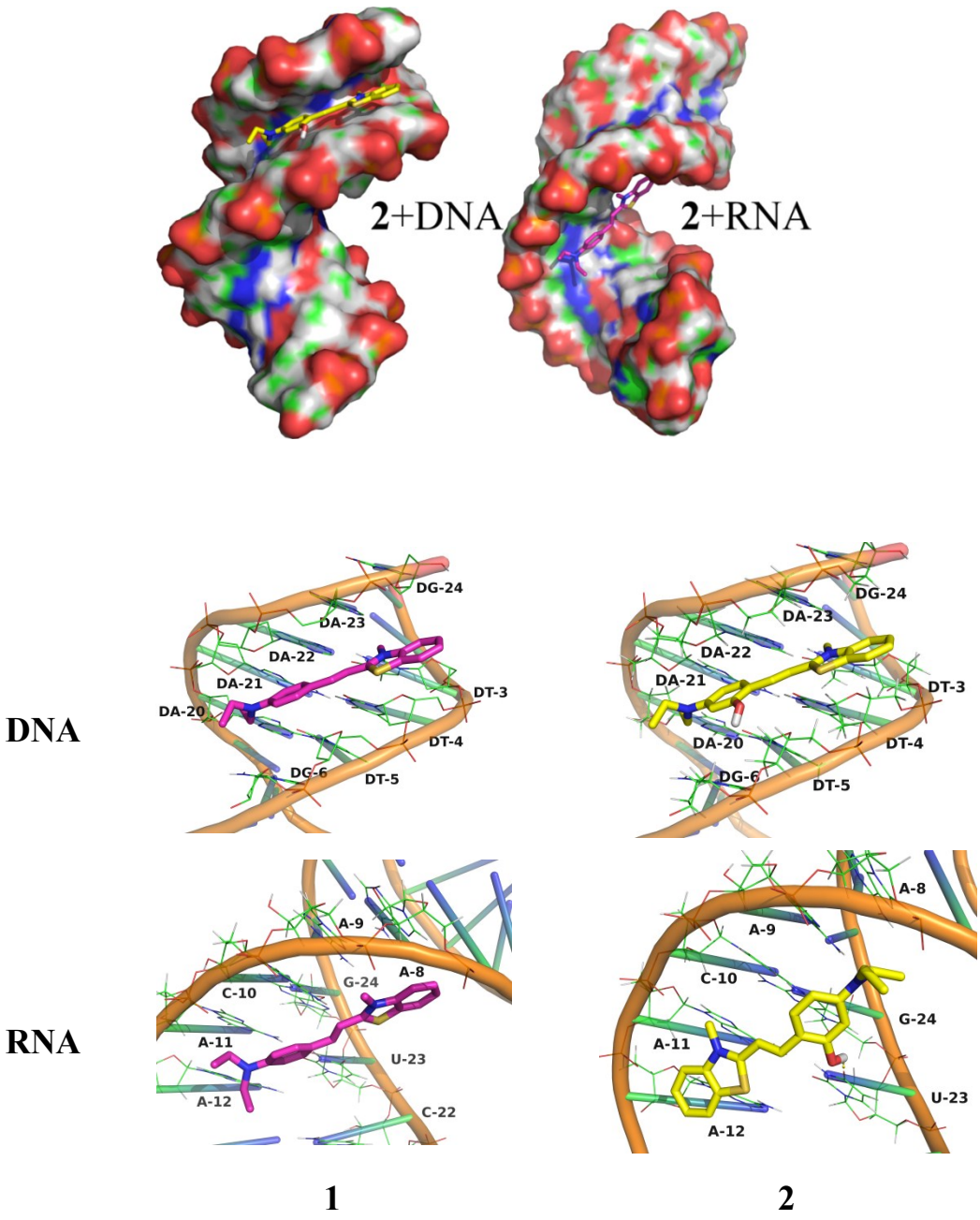


Fig. S15. Models obtained after molecular modeling of the interaction of **1** and **2** with DNA and RNA fragment.

Table S3. The CDOCKER energy of probes 1 and 2 with nucleic acid.

1 for DNA	**2** for DNA	**1** for RNA	**2** for RNA
Cdocker Energy			
-36.3601	-37.8862	-22.6233	-22.5081
-36.3337	-36.9875	-22.2002	-22.0786
-36.1577	-36.9486	-21.8791	-21.3162
-36.126	-36.9155	-21.7491	-21.0578
-36.0937	-36.7827	-21.7451	-20.5638
-35.9161	-36.7307	-21.4932	-20.504
-35.5676	-36.6688	-21.3936	-19.6503
-35.4325	-36.5796	-21.3281	-19.4498
-35.2226	-35.5684	-21.1489	-19.2514
-35.083	-36.4897	-21.1097	-19.1736

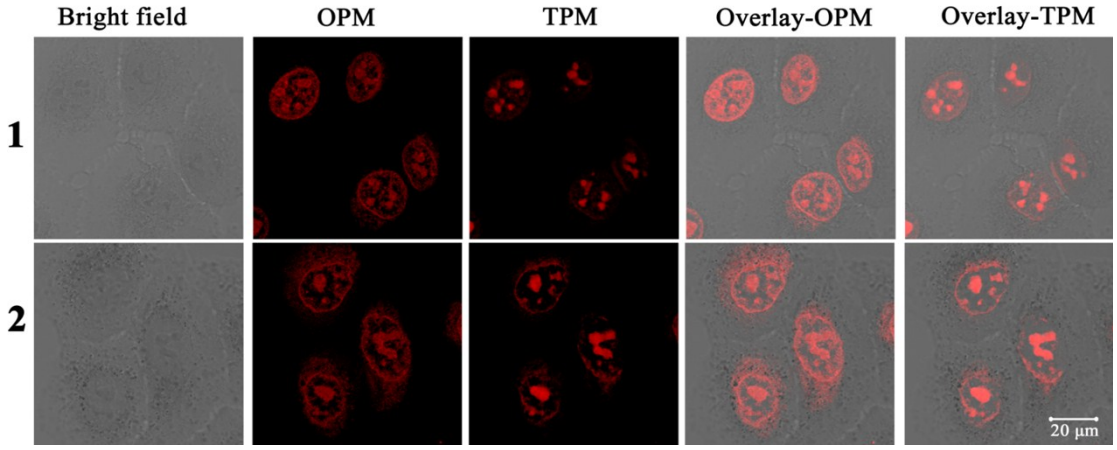
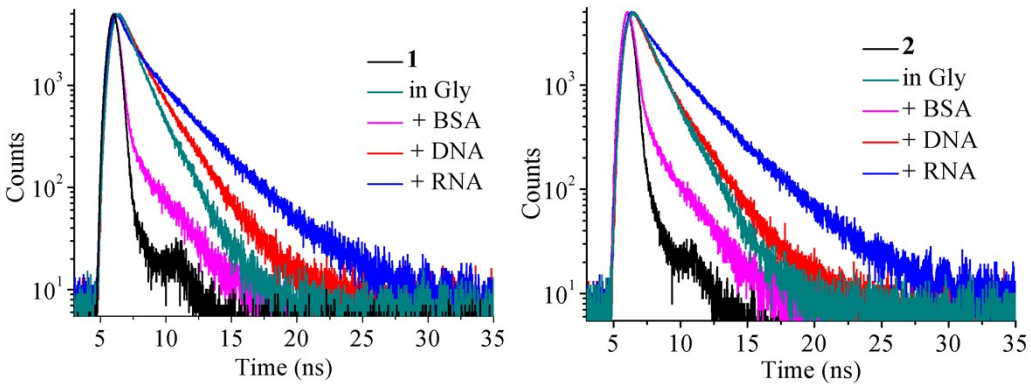


Fig. S16. Cellular uptake of compounds **1** and **2** in both one- and two-photon microscopy. A549 cells treated with 10 μM probes **1** and **2** for 10 min. OPM: One-Photon Microscopy (λ_{ex} = 543 nm); TPM: Two-Photon Microscopy (λ_{ex} = 750 nm).

A



B

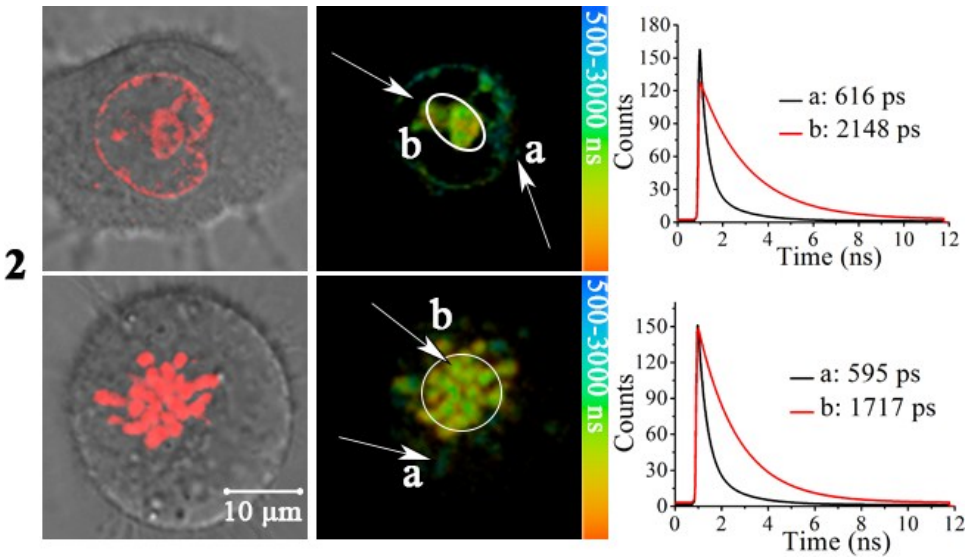


Fig. S17. Fluorescence and fluorescence lifetime imaging of probes **1** and **2** in nucleus. A, fluorescence lifetime of probes **1** (**1**: t = 81 ps; **1** in Gly: t = 1420 ps; **1** + DNA: t = 1790 ps; **1** + RNA: t = 2497 ps) and **2** (**2**: t = 122.7 ps; **2** in Gly: t = 1589 ps; **2** + DNA: t = 1764 ps; **2** = RNA: t = 2148 ps) with different substances in solution. Laser: 472 nm; B, fluorescence (left) and corresponding lifetime map (middle), (right) kinetic traces from a and b on lifetime map.

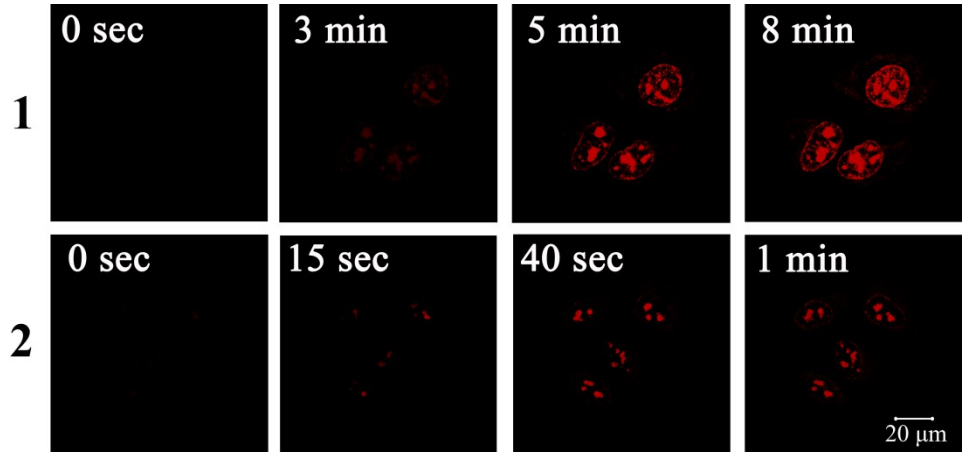


Fig. S18. Time-lapse imaging of nucleolus in A549 cells with 10 μM probes **1** and **2**. Images were obtained with excitation of 543 nm and emission of 600 ± 20 nm.

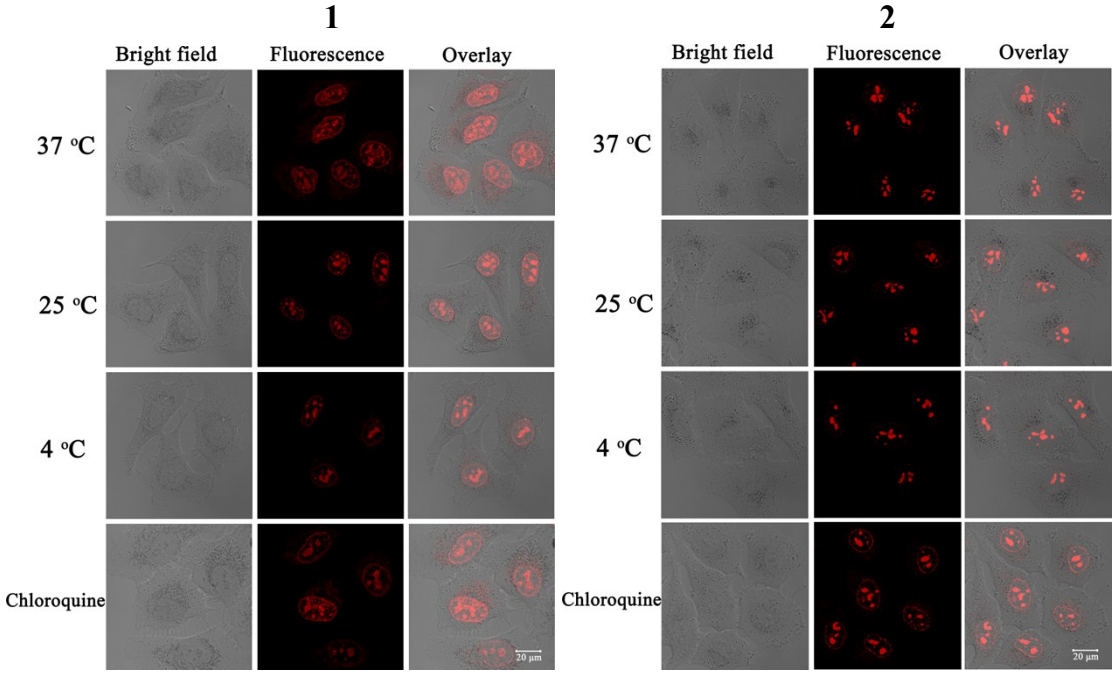


Fig. S19. Mechanism of cellular uptake of compounds **1** and **2**. Temperature-dependence and endocytosis inhibition studies for cellular uptake of compounds **1** and **2** (10 μM) for 10 min by confocal. Endocytosis inhibition treatment with chloroquine (50 μM).

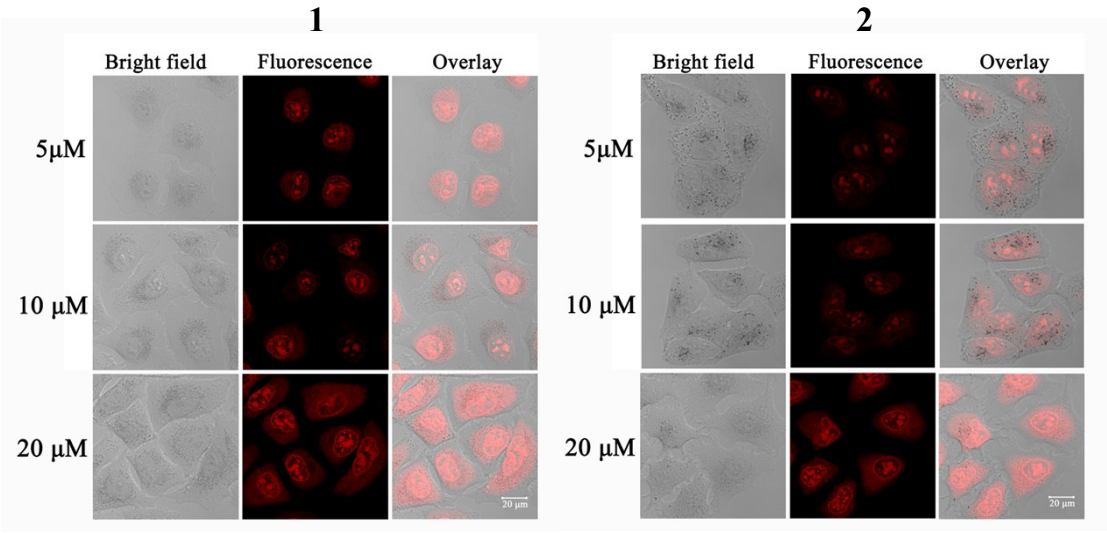


Fig. S20. Concentration-dependent cellular uptake of compounds **1** and **2** in A549 cells. Cells treated with probes **1** and **2** for 10 min.

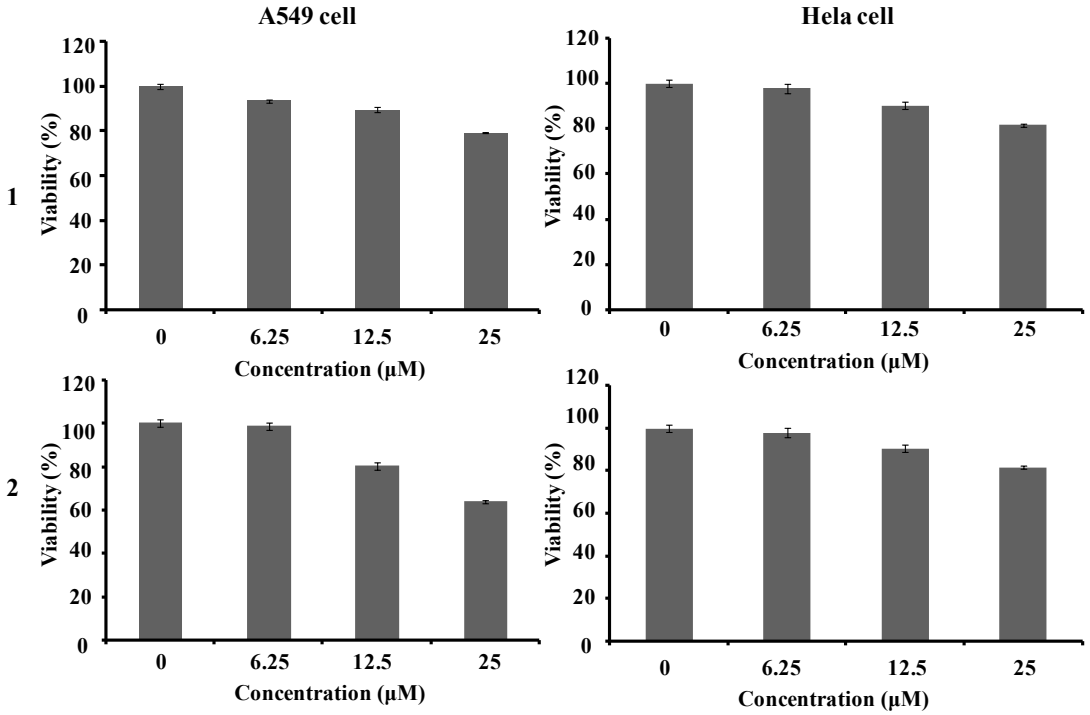


Fig. S21. Cell viability in A549 cells and Hela cells after treated with **1** and **2** for 24 h measured by MTT assay.

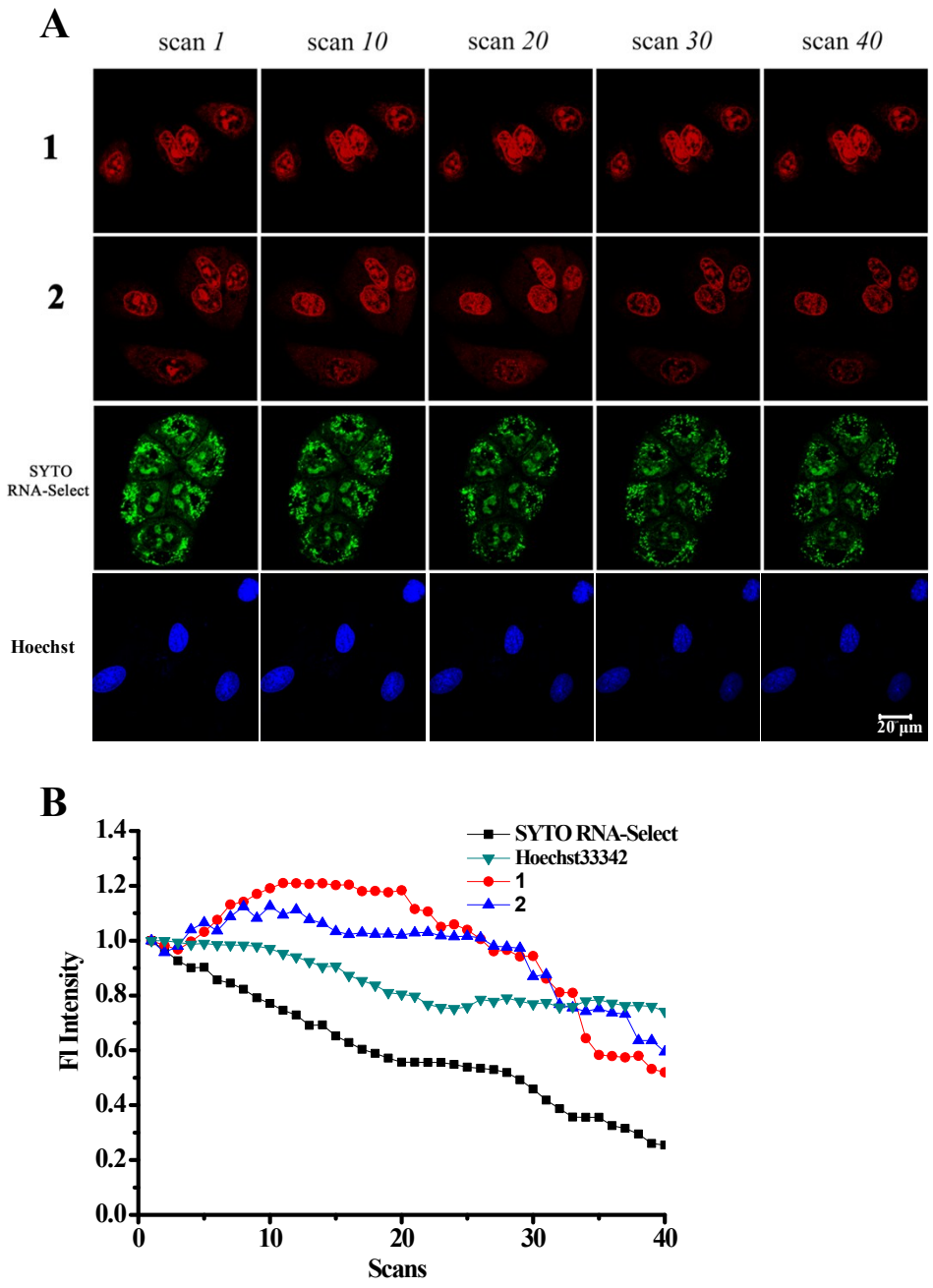


Fig. S22. Comparison of photobleaching of compounds **1**, **2** and SYTO RNA-Select and Hoechst 33342 by confocal. **A**, Confocal images of the living A549 cells incubated with **1** and **2** (10 μM) for 10 min. For RNA-Select dye, cells were fixed and then treated with 3 μM for 10 min. Continuous excitation with 488 nm for 10 min. For Hoechst 33342, cells were fixed and then treated with 2 μg/ml for 10 min. Continuous excitation with 405 nm for 10 min. **B**, Quantitative analysis of the changes in fluorescence intensities of the three dyes in panel A.

Table S4. Comparison with commercial dye SYTO RNA -Select and Hoechst 33342.

	SYTO-Select RNA	Hoechst33342	Probes 1 and 2 in this study
Exct/em Max (nm)	**488 / 530**	**405 /460**	**543 / 610**
Stokes shift	**~ 40**	**~ 55**	**~ 70**
Living cell / Fixed cell	+ / +	+ / +	+ / +
Permeability	+	+	+ +
Photostability	**Weak**	**Relative High**	**High**
Selectivity	**Weak**	**High**	**High**
Chemical structure	**Unknown**	**Known**	**Easily accessible**