Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Development of nucleus staining fluorescent probe for dynamic mitosis imaging in live cells

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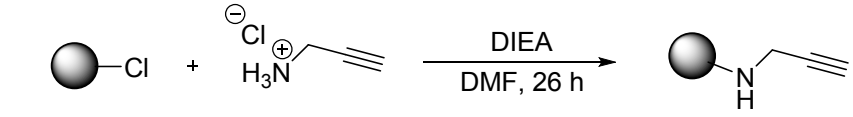
List of Information:

Materials and Methods

All the chemicals including entire acid chloride building blocks and solvents were purchased from Sigma Aldrich, Alfa Aesar, Fluka, MERCK or Across, and used without further purification. 2-Chlorotrityl alcohol resin (1.37 mmol/g) was purchased from BeadTech Inc., Korea. All library compounds were characterised by HPLC-MS (Agilent-1200 series) with a DAD detector and a single quadrupole mass spectrometer (6130 series) with an ESI probe. Unless indicated the analytical method: eluents: A: H_2O (0.1% HCOOH), B: ACN (0.1% HCOOH), gradient from 5 to 95% B in 7 min; C18(2) Luna column (4.6 x 50 mm^2, 5um particle size). ^1H NMR spectra were recorded on a Bruker Avance 500 NMR spectrometer. Spectroscopic measurements were done in BioTek microplate reader or SpectraMax M2 spectrophotometer (Molecular Devices). All the spectroscopic measurements were done in DMSO solutions and coumarin 1 (quantum yield=0.59) was used as a reference for quantum yield calculations. Hoechst 33342 (1:5000, H3570) and Vybrant®DyeCycle Ruby (5 μM, v10273) were from Life Technologies (Carlsbad, CA, USA). The human retinal pigment epithelial cell line (RPE1) was obtained from ATCC. The cells were maintained in DMEM high glucose supplemented with 8% FBS/1% PS at 37°C. Jurkat clone E6-1 was kindly provided by Singapore Immunology Network. Jurkat cells were maintained in DMEM high glucose supplemented with 10% FBS with 1% PS.

Synthetic procedure for AX library compounds and intermediates

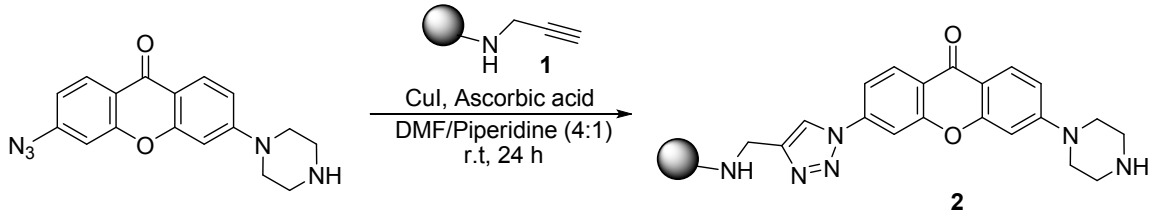
Loading of propargylamine to solid support (1)



Chlorotrityl resin **1**

DIEA (6.45 g, 50 mmol) was added to a solution of propargylamine hydrochloride (910 mg, 10 mmol) in DMF. Then the chlorotrityl resin (1 g, 1.37 mmol/g) was added to this solution and was shaken for 26 hours. The resin was washed then with DMF, MeOH and DCM (5 times each). After washing the resin was quenched with 40% MeOH in DMF for 1 hour. Finally, the resin was washed with DMF, MeOH and DCM (5 times each) and dried under high vacuum.

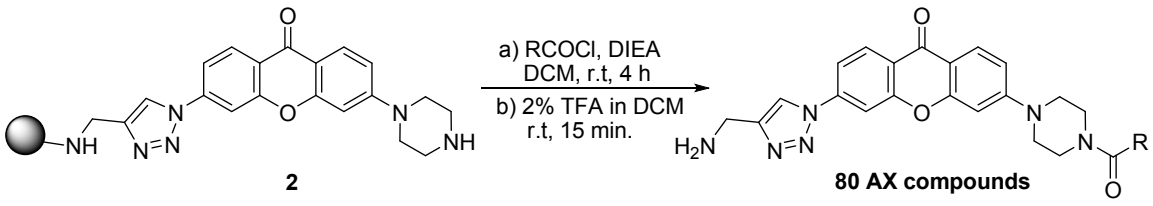
Synthesis of intermediate (2)



2

The resin **1** (778 mg, 0.788 mmol, 1 eq) was suspended to a solution of xanthone azide[1] (750 mg, 2.33 mmol, 3 eq) in DMF/piperidine (4:1). The copper iodide (741 mg, 3.89 mmol, 5 eq) and ascorbic acid (684 mg, 3.89 mmol, 5 eq) were then dissolved in the same solution and added to the resin. The reaction mixture was then shaken for 24 hours at room temperature. After the reaction, the resin was washed with, 1% Diethylthiocarbamate and 1% DIEA in DMF, 10 % water in DMF, MeOH and DCM (5 times each). Lastly, the resin was dried under vacuum. LCMS (ESI): calc for $C_{20}H_{20}N_6O_2$ (M+H) 377.1; found: 377.2

Synthesis of AX compounds



80 mg (0.056 mmol, 1 eq) of resin **2**, was suspended in DIEA containing (72.24 mg, 0.56 mmol, 10 eq) DCM solution. After this, 10 equivalent of acid chloride was added to the solution and shake it for 4 hours at room temperature. After 4 hours, the resin was washed with DMF, MeOH and DCM (5 times each). Next the resin was cleaved with 2% TFA in DCM. Then the solution containing the product was evaporated and the excess TFA was removed by a simple work up, using 2 N NaOH and DCM. Finally, the excess unreacted proparzyl amine was removed from the DCM layer using short silica gel filtration.

Table S1. **HPLC-MS characterization and photophysical property of AX library**

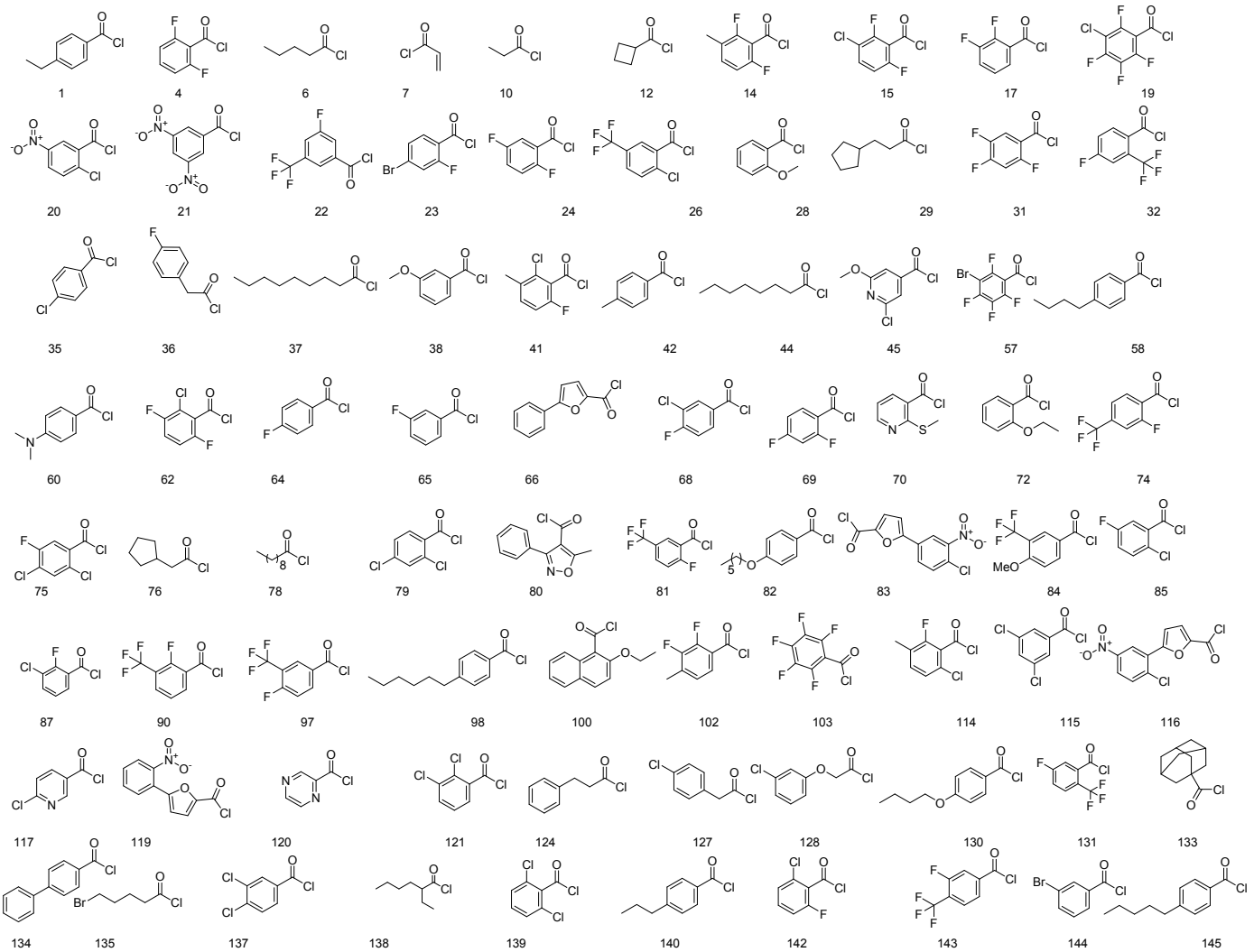
compound	M^+(calc.)	$M+H^+$ (exp.)	λ_{abs}(nm)	λ_{em}(nm)	ϕ	% yield	Purity
AX 1	508.2	509.0	369	500	0.34	53	91
AX 4	516.2	516.9	366	494	0.45	50	90
AX 6	460.2	461.2	370	502	0.3	61	92
AX 7	430.2	431.1	368	500	0.29	22	89
AX 10	432.2	433.0	369	503	0.29	31	92
AX 12	458.2	459.2	370	501	0.34	57	93
AX 14	530.2	531.0	366	494	0.54	53	90
AX 15	550.1	551.2	366	492	0.59	51	91
AX 17	516.2	517.1	368	495	0.42	44	92
AX 19	586.1	587.0	366	490	0.41	42	94
AX 20	559.1	560.1	368	491	0.01	23	95
AX 21	559.1	571.1	366	495	0.01	15	90
AX 22	566.2	567.1	368	499	0.15	20	94
AX 23	576.1	577.1	368	496	0.39	26	91
AX 24	516.2	517.2	367	494	0.37	18	96
AX 26	582.1	583.2	368	496	0.37	20	93
AX 28	510.2	511.2	369	498	0.34	27	92
AX 29	500.3	501.2	370	503	0.32	31	90
AX 31	534.2	535.2	367	491	0.34	19	95
AX 32	566.2	567.2	367	493	0.35	16	87
AX 35	514.2	515.2	368	500	0.29	18	89
AX 36	512.2	513.2	368	500	0.27	22	90
AX 37	516.3	517.3	369	505	0.3	48	93
AX 38	510.2	511.2	369	500	0.26	55	96
AX 41	546.2	547.2	367	493	0.39	68	85
AX 42	494.2	495.0	369	499	0.38	84	90
AX 44	502.3	503.2	369	503	0.31	26	90
AX 45	545.2	546.2	368	495	0.45	19	91
AX 57	630.1	632.3	366	493	0.29	47	92
AX 58	536.3	537.3	369	500	0.32	45	94
AX 60	523.2	524.3	365	504	0.04	18	91
AX 62	550.1	550.9	366	493	0.41	20	92
AX 64	498.2	498.9	369	500	0.3	15	91
AX 65	498.2	489.9	368	497	0.36	78	98
AX 66 (**CDb12**)	546.2	547.2	365	500	0.37	68	98

AX 68	532.1	533.2	369	498	0.49	70	98
AX 69	516.2	517.2	368	496	0.38	71	98
AX 70	527.2	528.2	368	497	0.41	40	97
AX 72	524.2	525.0	369	500	0.47	67	96
AX 74	566.2	567.2	367	494	0.29	67	97
AX 75	566.1	567.1	367	496	0.32	66	95
AX 76	486.2	487.1	369	500	0.27	80	97
AX 78	530.3	531.4	369	503	0.29	70	97
AX 79	548.1	549.0	368	496	0.41	71	96
AX 80	561.2	562.2	367	496	0.41	68	91
AX 81	566.2	567.2	368	497	0.42	73	93
AX 82	580.3	581.4	369	501	0.37	71	92
AX 83	625.1	626.1	366	496	0.07	33	90
AX 84	578.2	579.0	369	499	0.3	42	91
AX 85	532.1	533.2	368	497	0.36	52	92
AX 87	532.1	533.2	368	495	0.32	52	92
AX 90	566.2	567.1	368	494	0.32	53	90
AX 97	566.2	567.2	369	500	0.34	53	98
AX 98	564.3	565.3	369	500	0.33	73	98
AX 100	574.2	575.3	368	497	0.42	65	95
AX 102	530.2	531.1	368	496	0.43	74	94
AX 103	570.1	571.1	365	492	0.51	65	96
AX 114	546.2	547.2	367	494	0.38	80	96
AX 115	548.1	549.0	369	496	0.31	78	93
AX 116	625.1	626.0	366	496	0.03	59	94
AX 117	515.1	515.9	369	498	0.34	47	96
AX 119	591.2	592.2	365	502	0.02	65	97
AX 120	482.2	483.1	368	497	0.26	36	97
AX 121	548.1	549.2	368	493	0.38	49	94
AX 124	508.2	509.2	370	500	0.31	59	97
AX 127	528.2	529.2	369	501	0.29	56	90
AX 128	544.2	545.2	370	499	0.29	29	91
AX 130	552.2	553.2	369	500	0.29	45	92
AX 131	566.2	567.1	368	494	0.33	51	93
AX 133	538.3	539.3	369	505	0.22	55	93
AX 134	556.2	557.3	368	499	0.29	27	92
AX 135	538.1	539.2	366	496	0.21	22	94
AX 137	548.1	549.1	369	499	0.38	47	93

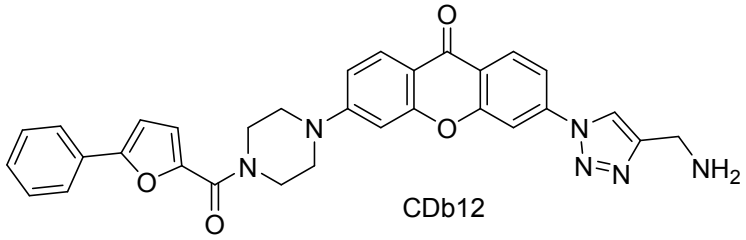
AX 138	502.3	503.3	368	501	0.31	58	94
AX 139	548.1	548.8	366	490	0.41	46	95
AX 140	522.2	523.1	369	500	0.4	50	94
AX 142	532.1	533.0	367	494	0.4	57	98
AX 143	566.2	567.1	368	497	0.31	53	90
AX 144	558.1	560.4	369	498	0.31	65	93
AX 145	550.3	551.3	369	499	0.35	64	98

HPLC analytical method: eluents: A: H_2O (0.1% HCOOH), B: ACN (0.1% HCOOH), gradient from 5 to 95%B in 7 min; C18(2) Luna column (4.6 x 50 mm^2, 5um particle size). Φ quantum yields are measured in DMSO solvent using Coumarin 1 as a standard (Φ=0.59). Purities were determined according to UV absorbance at 254 nm.

Chart 1. Acid Chloride building blocks used in AX Library synthesis (RCOCl in Scheme 1).



Characterization of CDb12



CDb12

^{1}H NMR (500MHz, DMSO): δ8.93 (s, 1H), 8.33 (d, *J* = 8.5 Hz, 1H), 8.11 (s, 1H), 8.04 (d, *J* = 9.0 Hz, 1H), 8.00 (d, *J* = 8.5 Hz, 1H), 7.83 (d, *J* = 7.7, 2H), 7.50 (t, *J* = 7.6 Hz, 2H), 7.40 (t, *J* = 7.2 Hz, 1H), 7.22 (d, *J* = 3.4 Hz, 1H), 7.18-7.12 (m, *2*H), 6.94 (s, 1H), 3.99 (s, 4H), 3.68 (s, 6H). ^{13}C NMR (125MHz, DMSO): δ29.4, 35.8, 46.5, 96.2, 99.1, 107.7, 108.6, 112.2, 115.6, 118.9, 121.4, 122.3, 124.5, 127.7, 128.5, 129.1, 129.5, 129.7, 140.7, 146.7, 154.8, 155.4, 156.5, 158.3, 158.6, 173.1 HPLC-MS : calc for $C_{31}H_{26}N_6O_4$ (M+H) 547.2; found: 547.2 Melting point: 287-289 ^{0}C

M-phase synchronization and imaging-based screening using ImageXpress Macro™ cellular imaging system

RPE1 cells were seeded in 96-well plates at 5000 cells per well. After tubulyzine B (10 μM) treatment for 24 h, the cells were stained by AX library compounds at 0.1 to 1 μM for 1 h. The live cell images were taken by well randomly using ImageXpress Macro™ cellular imaging system with 10X phase contrast objectives at various time points throughout the experiment. We have calculated the m-phase-mediated hit candidates through the fluorescence microscope, which shows brighter fluorescent signal than untreated group. All controls in subsequent experiments included 0.1% DMSO.

Live cell imaging and flow cytometric analysis

To validate the distribution of **CDb12**, RPE1 cells were stained with **CDb12** at 1 μM for 1 h. Live cell imaging experiments were performed on a inverted Nikon's A1R+ confocal laser microscope systems with 562 nm, 672 nm, 405 nm lasers (Nikon Instruments Inc. Japan). Image processing and overlay analysis were performed using NIS Elements 3.10 software (Nikon Instruments Inc. Japan). To measure the flow cytometric analysis of **CDb12** and Vybrant®DyeCycle Ruby, RPE1 cells were stained

with **CDb12** at 5 μM for 1 h. The fluorescence intensity of samples were analyzed on a BD FACS Aria liu SORP Cell sorter with 405 nm (**CDb12**) and 633 nm (Vybrnt®DyeCycle Ruby)excitation filters (BD Biosciences, San Jose CA, USA). All controls in subsequent experiments included 0.1% DMSO. All images were assigned pseudo-color (**CDb12**, blue; scale bars, 10 μm; 100X oil). 2 channels were combined using an NIE software overlay protocol.

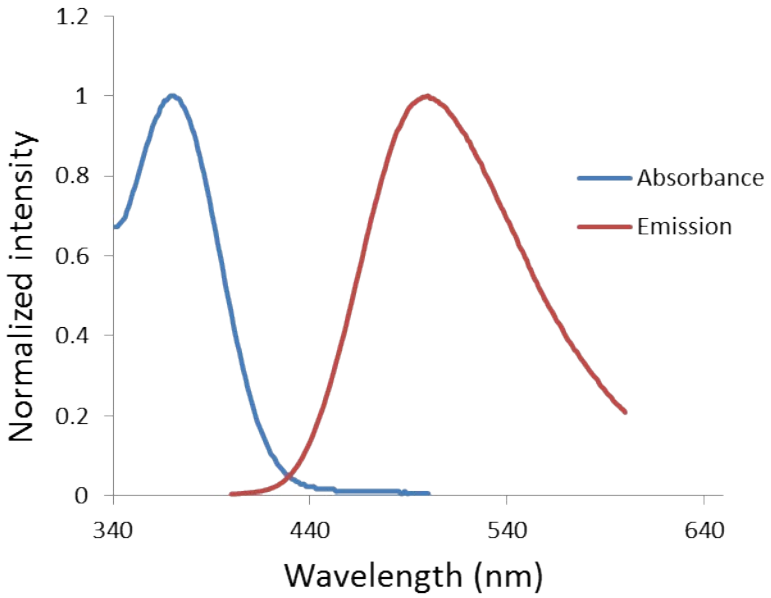


Fig. S1 Normalized absorbance and emission profiles of **CDb12** in DMSO.

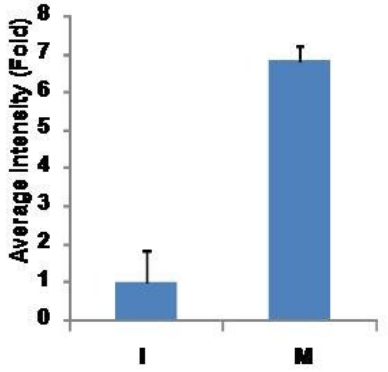


Fig. S2 Comparison between interphase (I) and mitosis (M) on fluorescent intensity after **CDb12** labeling.

Fluorescent intensity change of CDb12 against different concentrations of DNA and RNA

The DNA samples were dissolved in 20 mM HEPES buffer and then **CDb12** was added to the DNA sample so that the final concentration of the dye was 5 μM . Then the fluorescent spectra were recorded from 430 nm to 650 nm wavelength.

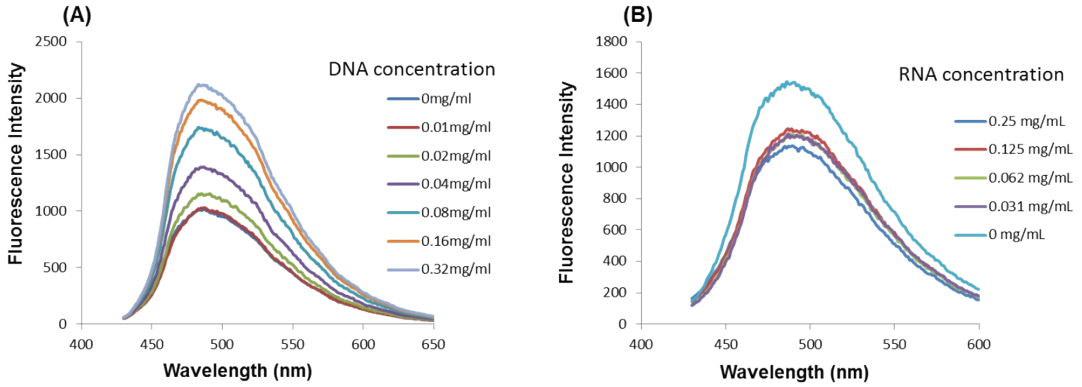


Fig. S3 Fluorescent intensity change of **CDb12** upon binding with DNA (A) and RNA (B).

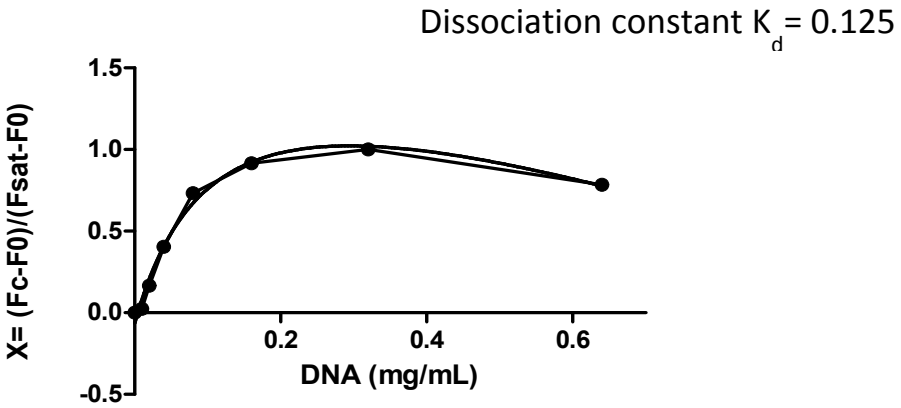


Fig. S4 *In vitro* Binding of **CDb12** with DNA

U2OS and RPE1 cells with CDb12 labeling does not affect viability and proliferation at concentrations below to 2 μM.

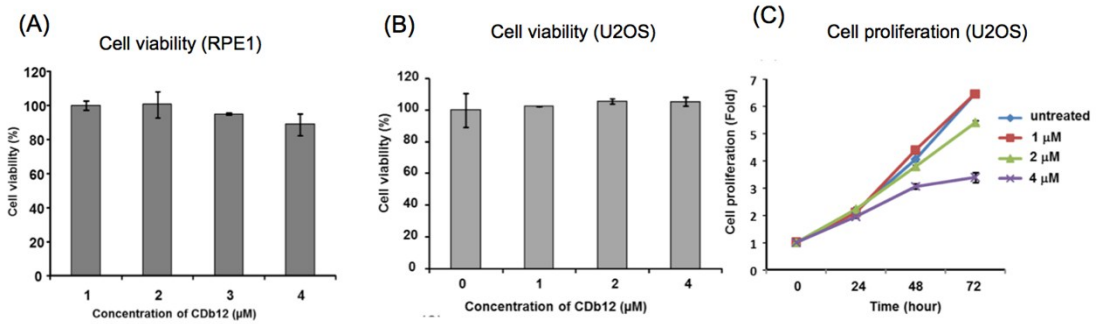


Fig. S5. (A) RPE1 cells and (B) U2OS cells were stained with the indicated doses of CDb12 for 24 hour. The cell viability was measured by the crystal violet staining method[2]. (C) After CDb12 labeling, the U2OS cell proliferation rate was measured by manual counting of cells under microscopy observation at indicated time points. All control cells were treated with 0.1% DMSO.

Reference

1. K. K. Ghosh, H. H. Ha, N. Y. Kang, Y. Chandran and Y. T. Chang, *Chem Commun*, 2011, **47**, 7448.
2. Y. M. Jeong, H. Li, S. Y. Kim, W. J. Park, H. Y. Yun, K. J. Baek, N. S. Kwon, J. H. Jeong, S. C. Myung and D. S. Kim, *Journal of photochemistry and photobiology. B, Biology*, 2011, **103**, 50-56.