Supporting Information

Developing visible fluorogenic 'click-on' dyes for cellular imaging

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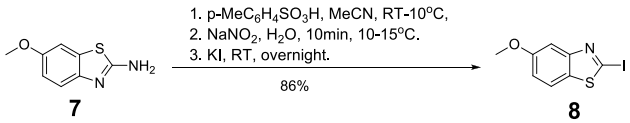
Materials and Reagents. Chemicals used in the syntheses were purchased from Aldrich or TCI without further purification. NMR spectra were recorded with a Bruker Avance Dpx 300 spectrometer and the δ values are in ppm vs $SiMe_4$ (0 ppm, 300MHz). Mass spectra were recorded using LCQ-Fleet mass spectrometer. UV spectra were recorded with a Varian 50 Bio UV-visible spectrometer; Fluorescence emission and excitation spectra were recorded using a FluoroMax-4 Spectrofluorometer and Spectra Max-M2 plate reader. HPLC was done by Varian 920-LC liquid chromatograph. Thin-layer chromatography (TLC) was performed on precoated silica gel 60F-254 glass plates.

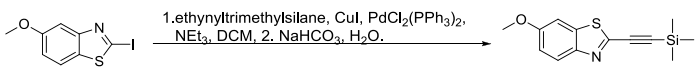
2-iodo-5-methoxybenzo[d]thiazole (8)[1, 2]



7 8

A solution of $NaNO_2$ (3.5 g, 50 mmol) and KI (10.5 g, 65 mmol) in H_2O was added dropwise to a mixture of 6-methoxybenzo[d]thiazol-2-amine (7) (4.5 g, 25 mmol), p-TsOH (15 g) in acetonitrile (100 ml) at 0°C around 30 min. The reaction was run overnight at RT, and then was quenched by 350 ml of water. The pH was adjusted to 8-9 with $NaHCO_3$. The precipitate was collected by filtration, and washed with water. The filter cake was dissolved in DCM, dried over Na_2SO_4, filtered through a silica gel column. The filtrate DCM solution was concentrated, and the crystallized pale brown solid (4.39 g) was collected by filtration. The remaining filtrate was further dried to give brown solid (1.87 g). The combined solid gave 6.25 g of compound **8** with 86% yield. TLC: Rf: 0.386 (hexane/ethyl acetate=6/1). MS (ESI) m/z 292 (M^+ +H); ^{1}HNMR ($CDCl_3$, 300MHz) δ 7.92 (1H, d, J=9Hz), 7.31 (1H, d, J=2.4Hz), 7.06 (1H, dd, J_1=9Hz, J_2=2.7Hz), 3.89 (3H, s).

6-methoxy-2-((trimethylsilyl)ethynyl)benzo[d]thiazole (9)[3, 4]

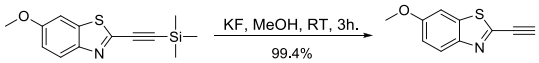


8 9

To a solution of 2-iodo-5-methoxybenzo[d]thiazole (8) (1.86 g, 6.39 mmol) and triethylamine (4 ml) in DCM (8 ml) was added ethynyltrimethylsilane (1.25 g, 12.78 mmol), CuI (26 mg), and $PdCl_2(PPh_3)_2$ (90

mg), and refluxed at 65 ^{o}C for 1h. The reaction solution was filtered through a short silica gel column, eluted with hexane/ethyl acetate=1/1. Solvent was removed by reduced pressure and the residue was purified by silica gel column using hexane/ethyl acetate=6/1 as eluent to give 1.14g yellow oil, 68.3% yield. TLC: Rf: 0.63 (hexane/ethyl acetate=6/1). MS (ESI) m/z 262 (M^+ + H); ^{1}HNMR ($CDCl_3$, 300MHz) δ 7.93 (1H, d, J=9Hz), 7.30 (1H, d, J=2.4Hz), 7.13 (1H, dd, J_1=9Hz, J_2=2.4Hz), 3.90 (3H, s), 0.32 (9H, s).

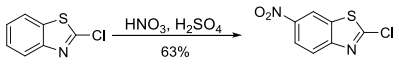
2-ethynyl-6-methoxybenzo[d]thiazole (10)



9 **10**

6-methoxy-2-((trimethylsilyl)ethynyl)benzo[d]thiazole (9) (1 g) in MeOH (30 ml) was reacted with KF (1 g) for 45 min at RT. Solvent was removed under reduced pressure, and the residue was purified by silica gel column using hexane/ethyl acetate=12/1 as eluent to give 0.72 g of pale yellow solid, yield 99.4%. TLC: Rf: 0.25 (hexane/ethyl acetate=6/1). MS (ESI) m/z 190 (M^+ +H); ^{1}HNMR ($CDCl_3$, 300MHz) δ 7.97 (1H, d, J=9Hz), 7.31 (1H, d, 2.4Hz), 7.15 (1H, dd, J_1=9Hz, J_2=2.4Hz), 3.91 (3H, s), 3.57 (1H, s).

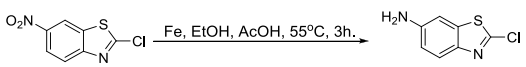
2-chloro-6-nitrobenzo[d]thiazole (12)[(5)]



11 **12**

2-Chlorobenzothiazole (11) (25.1 g, 147 mmol) and potassium nitrate (16.4 g, 162 mmol) were added into conc. H_2SO_4 (135 mL) on ice. The mixture was stirred at 0 ^{o}C for 30 min and then room temperature for 1 h. The resulting mixture was poured onto ice and the precipitate was collected by filtration. The residue was washed with water and ethanol to give off-white solid (20.0 g, 63%). The crude product was used for next step without further purification.

2-chlorobenzo[d]thiazol-6-amine (13)

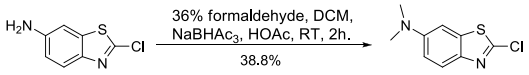


12 **13**

A suspension of Fe (10.00 g, 178.6 mmol) in ethanol (100 mL) and water (40 mL) was mixed with HCl (5 mL) at room temperature. The crude product from the previous step (5.00 g, 23.37 mmol) in ethanol (50

mL) was added to the suspension and refluxed at 90 ^{o}C for 3 hrs. The resulting mixture was extracted with chloroform (200 mL) and washed with water and brine. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography using ethyl acetate/hexane (5 to 30%) as eluent, giving yellow crystal solid 3.24 g (48% for two steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72 (d, J = 8.7 Hz, 1 H), 7.01 (d, J = 2.4 Hz, 1 H), 6.83 (dd, J = 6.0, 2.4 Hz, 1 H); ESI-MS (ES^+) m/z calcd for $C_7H_5ClN_2S$ 184.0, found 185.0 ($M + H^+$).

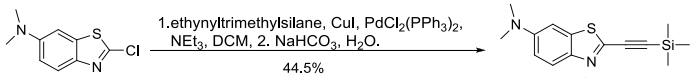
2-chloro-N,N-dimethylbenzo[d]thiazol-6-amine (14)



13 **14**

To a solution of 2-chlorobenzo[d]thiazol-6-amine (13) (920 mg, 5 mmol) in DCM (50 ml) was added 36% formaldehyde (5 ml) and $NaBHAc_3$ (3.25 g), and followed by HOAc (0.6 ml). The reaction solution was stirred overnight at RT. Extracted with DCM, washed with brine, and dried over Na_2SO_4. The solvent was removed, and the residue was purified by silica gel column using hexane/ethyl acetate=10/1 as eluent to give 411 mg white crystal **14** with 38.8% yield. TLC: Rf: 0.43 (hexane/ethyl acetate=5/1). MS (ESI) m/z 213 (M^+ +H); ^{1}HNMR ($CDCl_3$, 300MHz) δ 7.77 (1H, d, J=9Hz), 6.98 (1H, d, J=2.7Hz), 6.93 (1H, dd, J_1=9Hz, J_2=2.7Hz), 3.04 (6H, s).

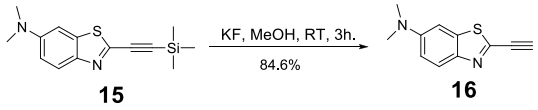
N,N-dimethyl-2-((trimethylsilyl)ethynyl)benzo[d]thiazol-6-amine (15)



14 **15**

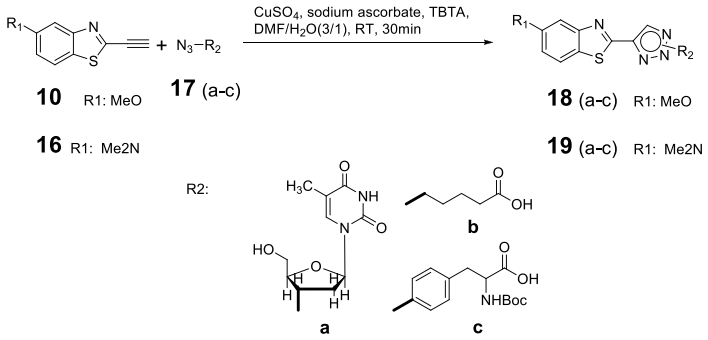
To a solution of 2-chloro-N,N-dimethylbenzo[d]thiazol-6-amine (14) (212 mg, 1.0 mmol) in DCM (2.2 ml) and NEt_3 (1.1 ml) was added ethynyltrimethylsilane (217 mg, 2 mmol), CuI (20 mg), and $PdCl_2(PPh_3)_2$ (20 mg) and refluxing at 65 ^{o}C for 3 h. The reaction solution was filtered through a short silica gel column, washed with hexane/ethyl acetate=1/1. The solvent was removed by reduced pressure, and the residue was purified by silica gel column using hexane/ethyl acetate=10/1 as eluent to give 122 mg yellow solid, 44.5% yield. TLC: Rf: 0.5 (hexane/ethyl acetate=5/1). MS (ESI) m/z 275 (M^+ +H); ^{1}HNMR ($CDCl_3$, 300 MHz) δ 7.87 (1H, d, J=9Hz), 7.02 (1H, d, J=2.7Hz), 6.98 (1H, dd, J_1=9Hz, J_2=2.7Hz), 3.06 (6H, s), 0.31 (9H, s).

2-ethynyl-N,N-dimethylbenzo[d]thiazol-6-amine (16)



To a solution of N,N-dimethyl-2-((trimethylsilyl)ethynyl)benzo[d]thiazol-6-amine (15) (40 mg) in MeOH (10 ml) was added KF (250 mg) and stirred 3 h at RT. Solvent was removed under reduced pressure, the residue was purified by silica gel column, eluted by hexane/ethyl acetate=5/1 to give 25 mg of yellow solid, yield 84.7%. TLC: Rf: 0.3 (hexane/ethyl acetate=5/1). MS (ESI) m/z 203 (M^+ +H); ^{1}HNMR ($CDCl_3$, 300 MHz) δ 7.88 (1H, d, J=8.7Hz), 7.02 (1H, dd, J_1=4.2Hz, J_2=2.7Hz), 6.98 (1H, d, J=2.4Hz), 3.53 (1H, s), 3.07 (6H, s).

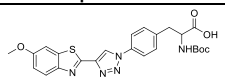
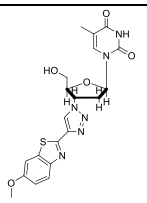
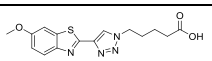
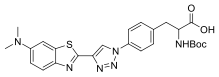
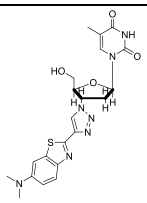
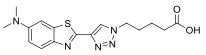
General 'click' reaction procedure (18, 19)



To a solution of AZT (**17a**) (14 mg, 0.052 mmol) in DMF (3 ml) and H_2O (1 ml) was added benzothiazole alkyne (**10**) (8 mg, 0.042 mmol), $CuSO_4$ (10 mg), sodium ascorbate (25 mg), and TBTA (2 mg), and stirred for 1 h at RT, followed by TLC and HPLC. No starting material **10** was detected after reaction (100% conversion), only the desired product **18a** was observed in HPLC. The reaction solution was extracted with EtOAc, washed with brine, and dried over Na_2SO_4. After removing solvent under reduced pressure, the residue was purified by silica gel column, eluted with 5% MeOH in EtOAc to give 11 mg white solid **18a** with 57% yield.

Compounds 18 (b, c) and **19 (a-c)** were prepared using the same procedure, the mole ratio of **10** or **16** and **7** were 1.1 to 1.5.

Table S-1 the Mass spectra and ^{1}HNMR data of the 'click on' products.

'Click' products	Mass	^{1}HNMR
	496 (M^++H)	(CD_3OD) 9.09 (1H, s), 7.92 (1H, d, J=9.0Hz), 7.91 (2H, d, J=8.1Hz), 7.63 (1H, d, J=2.7Hz), 7.53 (2H, d, J=8.4Hz), 7.18 (1H, dd, J_1=9.0Hz, J_2=2.7Hz), 4.43 (1H, m), 3.93 (3H, s), 3.06 (1H,m), 1.4 (9H, s)
	457 (M^++H)	(CD_3OD) 8.72 (1H, s), 7.96 (1H, d, J=1.2Hz), 7.90 (1H, d, J=9.0Hz), 7.60 (1H, d, J=2.4Hz), 7.16 (1H, dd, J_1=9.0Hz, J_2=2.7Hz), 6.57 (1H, t, J=6.6Hz), 5.58 (1H,m), 4.48 (1H, m), 3.97 (1H, dd, J1= 12.3Hz, J_2= 3.3Hz), 3.92 (3H, s), 3.04 (1H, m), 2.84, (1H, m), 2.03 (1H, s), 1.94 (3H, s)
	333 (M^++H)	(CD_3OD) 8.59 (1H, s), 7.90 (1H, d, J=9.0Hz), 7.59 (1H, d, J=2.4Hz), 7.16 (1H, dd, J_1=9.0Hz, J_2=2.4Hz), 4.56 (2H, t, J=6.9Hz), 3.92 (3H, s), 2.03 (2H, m), 1.67 (2H, m)
	509 (M^++H)	(CD_3OD) 9.08 (1H, s), 7.95 (1H, d, J=9.0Hz), 7.92 (2H, d, J=8.4Hz), 7.61 (1H, d, J=2.4Hz), 7.53 (2H, d, J=8.4Hz), 7.38 (1H, s), 7.30 (1H, dd, J_1=9.0Hz, J_2=2.4Hz), 4.46 (1H, m), 3.17 (6H, s), 3.08 (2H,m), 1.41 (9H, s)
	470 (M^++H)	(CD_3OD) 8.68 (1H, s), 7.96 (1H, d, J=1.2Hz), 7.86 (1H, d, J=9.0Hz), 7.41 (1H, d, J=2.4Hz), 7.16 (1H, dd, J_1=9.0Hz, J_2=2.4Hz), 6.57 (1H, t, J=6.6Hz), 5.58 (1H,m), 4.48 (1H, m), 3.97 (1H, dd, J_1= 12.3Hz, J_2= 3.0Hz), 3.85(1H, dd, J_1= 12.3Hz, J_2= 3.0Hz), 3.10 (6H, s), 3.01 (1H, m), 2.83, (1H, m), 1.94 (3H, d, J=1.2Hz)
	346 (M^++H)	(D_2O+NaOD) 8.35 (1H, s) (very easy to be exchanged by D), 7.53 (1H, d, J=9.3Hz), 7.15 (1H, d, J=2.1Hz), 6.90 (1H, dd, J_1=9.3Hz, J_2=2.4Hz), 4.29 (2H, t, J=7.2Hz), 2.74 (6H, s), 2.08 (2H, t, J=7.5Hz), 1.79 (2H, m), 1.42 (2H, m).

pH effect of fluorescent intensity

The buffers of pH 3~10 were prepared by adding 0.1M citric acid or 0.1M NaOH solution to PBS Buffer. The pH 2 solution was prepared by adding 0.1M HCl solution. Compounds **10**, **18b, 16**, and **19b** (10μM) were dissolved in each pH and their fluorescence was measured by 316 nm or 375 nm excitation.

Cell culture

HeLa cells, human cervix adenocarcinoma epithelial cells, were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS),100 U/ml penicillin and 100 μg/ml streptomycin (Invitrogen). HeLa cells (2.5×10^4) were seeded with or without 2 μM AZT **17a** in a plastic petri dish with a bottom glass optical window (35 mm, MatTek) for 16 hrs at 37 ^{o}C. The cells were washed 3 times with medium before incubated with dye **16** (10 μM) and $CuSO_4$ (1 mM) for 3 hrs at 37 ^{o}C. After three washes with medium, reducing agent NaAsc (10 mM) in fresh medium was added to the cells and incubated for 30mins at 37 ^{o}C. The cell was fully washed by PBS and imaged by a fluorescence microscope using a conventional DAPI filter set. Exposure time was 125 ms. Fluorescence intensity of cells was quantified by manually outlined ROI (Region-of-interest) on 50 individual cells using an open source ImageJ program (v1.44, NIH). High resolution fluorescent images, 20X, were used for all quantitation.

References:

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