Electronic Supplementary Information

(3-(Trifluoromethyl)-3*H*-diazirin-3-yl)coumarin as a carbene-generating photocross-linker with masked fluorogenic beacon

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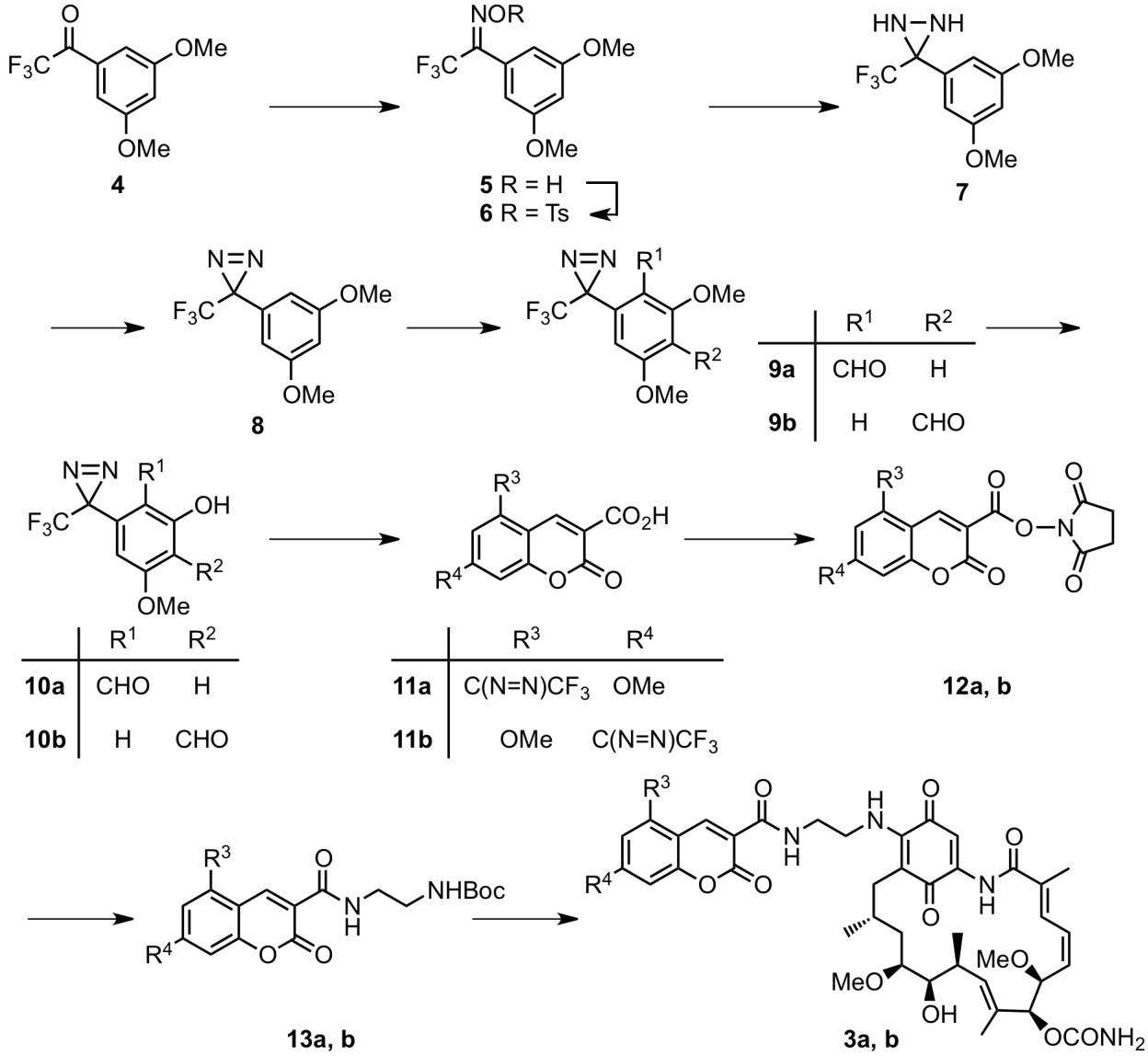
General

Melting points were measured on a Yanaco MP-S3 micro melting point apparatus, and uncorrected. All chemicals were of analytical grade and were used without further purification. Kieselgel 60 (70-230 mesh, Merck) was used for column chromatography. ^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded on a JEOL ECX400P spectrometer (400 MHz) and a Varian UNITYplus500 spectrometer (500 MHz), with chemical shifts (δ) reported in ppm relative to internal standards (Me_4Si for ^{1}H and ^{13}C, $CFCl_3$ for ^{19}F) and coupling constants (*J*) reported in Hz. Mass spectra and high-resolution mass spectra (HRMS) were recorded by electron impact ionization (EI) on a JEOL JMS-GCmate II or electrospray ionization (ESI) on a Thermo LTQ Orbitrap XL ETD. UV/Vis spectra were obtained by a Shimadzu UV-1800 spectrometer, and fluorescent spectra were measured on a JASCO FP-6500 spectrometer. Photoreactions performed by REX-250 high-pressure mercury lamp (Asahi Spectra). Fluorescence and chemiluminescence detection performed by Chemi-Print CX-EpiUV system (Relyon).

Preparation of the probe **3**



*2,2,2-Trifluoro-1-(3,5-dimethoxyphenyl)ethanone (**4**)*

A dry THF solution (70 mL) of 1-Chloro-3,5-dimethoxyphenylbenzene (34.5 g, 0.2 mol), magnesium (5.35 g, 0.22 mol) and 1,2-dibromoethane (376 mg, 2 mmol) was refluxed overnight in a three necked flask under argon. After cooled to -20 °C, a dry THF solution (70 mL) of 2,2,2-trifluoro-1-)piperidin-1-yl)ethanone (36.2 g, 0.2 mol) was added dropwise over a period of 30 minutes. The reaction mixture was then stirred at room temperature for 2 h followed by addition of sat. ammonium chloride aqueous solution (70 mL) and 1 M HCl (70 mL) at -20 °C. The products were extracted with ether three times. The combined organic solution was washed with 1 M HCl, brine, and then dried over $MgSO_4$. The crude residue was distilled under vacuum to give pale yellow oil (35.6 g, 76%), bp_1 77-79 °C. ^{1}H NMR ($CDCl_3$): δ 6.70 (d, *J* = 2.1 Hz, 2H), 6.46 (t, *J* = 2.1 Hz, 1H), 3.84 ppm (s, 6H); IR (liquid film): ν 1715 cm^{-1}; Anal: calcd for $C_{10}H_9F_3O_3$ C:51.29, H:3.89, found C:51.21, H:4.03; MS *m/z* (EI) 234 (M^+).

*2,2,2-Trifluoro-1-(3,5-dimethoxyphenyl)ethanone oxime (**5**)*

A solution of compound **4** (23.4 g, 0.1 mol) and hydroxylamine hydrochloride (7.64 g, 0.11 mol) in pyridine (100 mL) and EtOH (50 mL) was stirred at 60 °C overnight. After removal of the solvent, the residue was dissolved in ether followed by washing with water, 1 M HCl, brine, and then dried over $MgSO_4$. The solvent was evaporated to give colorless solid (24.2 g), mp 110-118 °C and was used for the following reaction without further purification. ^{1}H NMR ($CDCl_3$): δ 8.22 (s, 1H), 6.60 (d, *J* = 2.2 Hz, 2H), 6.57 (t, *J* = 2.2 Hz, 1H), 3.81 ppm (s, 6H); IR (Nujol): ν 3350 cm^{-1}; Anal: calcd for $C_{10}H_9F_3NO_3$ C:48.20, H:4.04, N:5.62, found C:48.23, H:4.44, N:5.66; MS *m/z* (EI) 249 (M^+).

*2,2,2-Trifluoro-1-(3,5-dimethoxyphenyl)ethanone O-p-toluenesulfonyloxime (**6**)*

To a CH_2Cl_2 solution (200 mL) of compound **5** (24.2 g, 0.1 mol), triethylamine (25.3 g, 0.25 mol) and dimethylaminopyridine (611 mg, 5 mmol) was slowly added toluenesulfonyl chloride (21.0 g, 0.11 mol) at 0 °C, and the solution was stirred at room temperature for 1 h. The reaction mixture was washed with water, brine, and then dried over $MgSO_4$. After removal of the solvent, the residue was recrystallized from EtOAc-hexane to give colorless prisms (36.5 g, 91% from compound **1**), mp 107-116 °C. ^{1}H NMR ($CDCl_3$): δ 7.88 (d, *J* = 8.0 Hz, 2H), 7.38 (d, *J* = 8.0 Hz, 2H), 6.57 (t, *J* = 2.1 Hz, 1H), 6.45 (d, *J* = 2.1 Hz, 2H), 3.80 (s, 6H), 2.48 ppm (s, 3H); IR (Nujol): ν 1590 cm^{-1}; Anal: calcd for $C_{17}H_{16}F_3NO_5S$ C:50.62, H:4.00, N:3.47, found C:50.37, H:3.85, N:3.34; MS *m/z* (EI) 403 (M^+).

*3-Trifluoromethyl-3-(3,5-dimethoxyphenyl)diaziridine (**7**)*

A CH_2Cl_2 solution (50 mL) of compound **6** (33 g, 82 mmol), liq. ammonia (ca. 10 mL) was tightly sealed in a glass pressure bottle at -78 °C, and was stirred at room temperature overnight. The reaction mixture was carefully removed pressure, and washed with water, brine, and then dried over $MgSO_4$. After removal of the solvent, the residue was recrystallized from hexane to give colorless prisms (20 g, quant.), mp 98-99 °C. ^{1}H NMR ($CDCl_3$): δ 6.75 (d, *J* = 1.9 Hz, 2H), 6.50 (t, *J* = 1.9 Hz, 1H), 3.80 (s, 6H), 2.75 (d, *J* = 9.2 Hz, 1H), 2.23 ppm (d, *J* = 9.2 Hz, 1H); IR (Nujol): ν 3250 cm^{-1}; Anal: calcd for $C_{10}H_{11}F_3N_2O_2$ C:48.39, H:4.47,

N:11.29, found C:48.68, H:4.45, N:11.49; MS *m/z* (EI) 248 (M^+).

*3-Trifluoromethyl-3-(3,5-dimethoxyphenyl)-3H-diazirine (**8**)*

To a suspension of compound **7** (14.9 g, 60 mmol), triethylamine (12.1 g, 0.12 mol) in MeOH (60 mL) was added iodine (16 g, 63 mmol) at room temperature and the mixture was stirred for additional 30 min. The reaction mixture was carefully removed pressure, and washed with water, brine, and then dried over $MgSO_4$. After removal of the solvent, the residue was dissolved in hexane. The solution was washed with 1 M HCl, 10% $Na_2S_2O_3$, brine, and dried over $MgSO_4$. The residue was purified by column chromatography on silica gel eluted with hexane-CH_2Cl_2 (2:1) to give pale yellow solid (14.5 g, 98%). ^{1}H NMR ($CDCl_3$): δ 6.46 (d, *J* = 2.4 Hz, 1H), 6.28 (s, 2H), 3.78 ppm (s, 6H); IR (liquid film): ν 1595 cm^{-1}; UV-vis (hexane) λ_{max} = 280 (ε 2400), 354 (ε 370), 373 nm (sh, ε 250); Anal: calcd for $C_{10}H_9F_3N_2O_2$ C:48.79, H:3.68, N:11.38, found C:48.85, H:4.13, N:11.38; MS *m/z* (EI) 246 (M^+), 218 (M^+-N_2).

2-(3-Trifluoromethyl)-3*H*-diazirin-3-yl)-4,6-dimethoxybenzaldehyde (**9a**)

To a CH_2Cl_2 solution (10 mL) of compound **8** (2.46 g, 10 mmol) was added $TiCl_4$ (2.85 g, 15 mmol) and dichloromethyl methyl ether (cancer suspect agent, 1.72 g, 15 mmol) at -20 °C under argon. The reaction mixture was stirred at -20 °C for 2 h and at room temperature for 1 h. Cold water was added to the mixture at 0 °C, and the solution was washed with water, sat. $NaHCO_3$, brine, and then dried over $MgSO_4$. After removal of the solvent, the residue was recrystallized from EtOAc-hexane to give pale yellow leaflets (1.09 g) as 2-(3-trifluoromethyl)-3*H*-diazirin-3-yl)-4,6-dimethoxybenzaldehyde (**9a**). The filtrate was purified by column chromatography on silica gel eluted with $CHCl_3$ to give compound **9a** (total 1.38 g, 50%, mp 150-154 °C (dec.)) and pale yellow solid as compound **9b** (1.30 g, 47%, mp 59-60 °C).

Compound **9a**: ^{1}H NMR ($CDCl_3$): δ 10.48 (s, 1H), 6.77 (d, *J* = 2.2 Hz, 1H), 6.55 (d, *J* = 2.2 Hz, 1H), 3.92 (s, 3H), 3.91 ppm (s, 3H); IR (Nujol): ν 1680 cm^{-1}; UV-vis (hexane) λ_{max} = 265 (ε 11600), 308 (sh, ε 8200), 315 nm (ε 8600); Anal: calcd for $C_{11}H_9F_3N_2O_5$ C:48.18, H:3.31, N:10.29, found C:47.96, H:3.07, N:10.55; MS *m/z* (EI) 274 (M^+), 246 (M^+-N_2).

4-(3-Trifluoromethyl)-3*H*-diazirin-3-yl)-2,6-dimethoxybenzaldehyde (**9b**): ^{1}H NMR ($CDCl_3$): δ 10.45 (s, 1H), 6.31 (s, 2H), 3.90 ppm (s, 6H); IR (Nujol): ν 1690 cm^{-1}; UV-vis (hexane) λ_{max} = 268 (ε 15400), 315 (ε 4300), 354 (ε 1140), 370 nm (sh, ε 770); Anal: calcd for $C_{11}H_9F_3N_2O_5$ C:48.18, H:3.31, N:10.29, found C:48.08, H:3.37, N:10.22; MS *m/z* (EI) 274 (M^+), 246 (M^+-N_2).

*6-Hydroxy-4-methoxy-2-(3-Trifluoromethyl)-3H-diazirin-3-yl)benzaldehyde (**10a**).*

To a CH_2Cl_2 solution (20 mL) of compound **9a** (1.37 g, 5 mmol) was added BBr_3 (1.25 g, 5 mmol) at -20 °C under argon. After stirring at 0 °C for 2 h, water was slowly added. The solution was washed with water and dried over $MgSO_4$. After removal of the solvent, the product was purified by chromatography on silica gel eluted with hexane-CH_2Cl_2 (1:1) to give colorless solid (1.29 g, 99%). The product was recrystallized from EtOAc-hexane to afford colorless prisms, mp 40-41 °C. ^{1}H NMR ($CDCl_3$): δ 12.37 (s, 1H), 10.55 (s, 1H), 6.79 (d, *J* = 2.4 Hz, 1H), 6.49 d, *J* = 2.4 Hz, 1H), 3.88 ppm (s, 3H); IR (Nujol): ν 1655 cm^{-1}; UV-vis (hexane)

λ_{max} = 235 (ε 9700), 274 (ε 12100), 322 nm (ε 8100); Anal: calcd for $C_{10}H_7F_3N_2O_5$ C:46.17, H:2.71, N:10.77, found C:46.30, H:2.62, N:10.56; MS *m/z* (EI) 260 (M^+), 232 (M^+-N_2).

*2-Hydroxy-6-methoxy-4-(3-trifluoromethyl)-3H-diazirin-3-yl)benzaldehyde (**10b**)*

Purification by chromatography on silica gel eluted with hexane-CH_2Cl_2 (2:1) gave pale yellow solid (1.25 g, 96%), mp 34-35 °C. δ_H ($CDCl_3$) 11.98 (1H, s), 10.31 (1H, s), 6.34 (1H, s), 6.04 (1H, s), 4.08 (3H, s); IR (Nujol): ν 1655 cm^{-1}; UV-vis (hexane) λ_{max} = 278 (ε 15900), 331 (sh, ε 3500), 345 (ε 4300), 361 nm (sh, ε 3300); Anal: calcd for $C_{10}H_7F_3N_2O_5$ C:46.17, H:2.71, N:10.77, found C:46.10, H:2.57, N:10.72; MS *m/z* (EI) 260 (M^+), 232 (M^+-N_2).

*Ethyl 7-methoxy-5-(3-Trifluoromethyl)-3H-diazirin-3-yl)coumarin-3-carboxylate (**1a**).*

A ethanol solution (5 mL) of compound **10a** (260 mg, 1 mmol), diethyl malonate (320 mg, 2 mmol), piperidine (119 mg, 1.4 mmol), acetic acid (54 mg, 0.9 mmol) was stirred at room temperature overnight. After removal of the solvent in vacuo, EtOAc was added to the residue followed by washing with H_2O, 1 M HCl, brine and being dried over $MgSO_4$. The product was purified by column chromatography on silica gel eluted with $CHCl_3$-acetone (50:1) to afford colorless solid (335 mg) in 94%, mp (dec) 129-131 °C. δ_H ($CDCl_3$) 9.08 (1H, s), 7.19 (1H, d, *J* 2.4), 6.90 (1H, d, *J* 2.4), 4.46 (2H, q, *J* 7.1), 3.93 (3H, s), 1.44 (3H, t, *J* 7.1); IR (Nujol): ν 1775, 1760 cm^{-1}; UV-vis (EtOH) λ_{max} = 253 (ε 5200), 263 (ε 4600), 347 (ε 20300), 354 (ε 18800), 370 nm (sh, ε 12900); Anal: calcd for $C_{10}H_7F_3N_2O_5$ C:50.57, H:3.11, N:7.86, found C:50.57, H:3.26, N:8.10; MS *m/z* (EI) 356 (M^+), 328 (M^+-N_2).

*Ethyl 5-methoxy-7-(3-Trifluoromethyl)-3H-diazirin-3-yl)coumarin-3-carboxylate (**1b**)*

Purification by chromatography on silica gel eluted with $CHCl_3$-acetone (50:1) to afford pale yellow solid (680 mg) in 95%, mp 94-95 °C. ^{1}H NMR ($CDCl_3$): δ 8.82 (s, 1H), 6.77 (s, 1H), 6.38 (s, 1H), 4.41 (q, *J* = 7.0 Hz, 2H), 3.98 (s, 3H), 1.40 ppm (t, *J* = 7.0 Hz, 3H); IR (Nujol): ν 1770 cm^{-1}; UV-vis (EtOH) λ_{max} = 246 (sh, ε 6100), 326 (ε 15400), 356 nm (sh, ε 10000); Anal: calcd for $C_{15}H_{11}F_3N_2O_5$ C:50.57, H:3.11, N:7.86, found C:50.28, H:2.88, N:8.05; MS *m/z* (EI) 356 (M^+), 328 (M^+-N_2).

*7-Methoxy-5-(3-trifluoromethyl-3H-diazirin-3-yl)coumarin-3-cabroxylic acid (**11a**)*

A solution of compound **10a** (520 mg, 2.0 mmol), meldrum's acid (317 mg, 2.2 mmol), diisopropylethylamine (646 mg, 5 mmol) in acetonitrile (2 mL) was stirred at room temperature for 1 h. After removal of the solvent, water was added to the residue and then acidified with conc. HCl. The product was extracted with EtOAc and washed with water and brine, then dried over $MgSO_4$. The solvent was evaporated, the product was purified by recrystallization from EtOAc-hexane to afford colorless needles (585 mg) in 89%, mp (dec) 122-124°C. ^{1}H NMR ($CDCl_3$): δ 9.46 (s, 1H), 7.33 (d, *J* = 2.4 Hz, 1H), 7.02 (d, *J* = 2.4 Hz, 1H), 3.99 ppm (s, 3H); IR (Nujol): ν 1785, 1675 cm^{-1}; UV-vis (EtOH) λ_{max} = 252 (ε 5550), 260 (ε 5300), 336 nm (ε 15600) ; MS *m/z* (EI) 328 (M^+), 300 (M^+-N_2).

*5-Methoxy-7-(3-trifluoromethyl-3H-diazirin-3-yl)coumarin-3-cabroxylic acid (**11b**)*

Recrystallization from EtOAc-hexane afforded pale yellow needles (300 mg) in 91%, mp (dec) 137-139 °C.

δ_H ($CDCl_3$) 9.25 (1H, s), 6.87 (1H, s), 6.49 (1H, s), 4.02 (3H, s); IR (Nujol): ν 1750, 1675 cm^{-1}; UV-vis (EtOH) λ_{max} = 244 (sh, ε 4300), 314 nm (ε 10900); Anal: calcd for $C_{15}H_{11}F_3N_2O_5$ C:47.57, H:2.15, N:8.54, found C:47.59, H:1.97, N:8.79; MS *m/z* (EI) 328 (M^+), 300 (M^+-N_2).

7-Methoxy-5-(3-trifluoromethyl-3H-diazirin-3-yl)coumarin-3-carboxyl succimidyl ester ***(12a)***

A solution of compound **11a** (328 mg, 1.0 mmol), DCC (305 mg, 1.1 mmol), *N*-hydroxysuccinimide (127 mg, 1.1 mmol) in CH_2Cl_2 (5 mL) was stirred at room temperature for 1 h. After removal of the solvent, EtOAc and a drop of AcOH was added to the residue and the solvent was filtrated. The filtrate was washed with sat $NaHCO_3$, water and brine, and then dried over $MgSO_4$. The solvent was evaporated, the product was purified by recrystallization from EtOAc-hexane to afford colorless prisms (385 mg) in 91%, ^1H NMR ($CDCl_3$): δ 9.31 (s, 1H), 7.24 (d, *J* = 2.4 Hz, 1H), 6.92 (d, *J* = 2.4 Hz, 1H), 3.97 (s, 3H), 2.93 ppm (s, 4H); IR (Nujol): ν 1830, 1810, 1780, 1740 cm^{-1}; UV-vis (EtOH) λ_{max} = 252 (sh, ε 6000), 263 (sh, ε 4900), 360 nm (ε 22900); MS *m/z* (EI) 425 (M^+), 397 (M^+-N_2).

5-Methoxy-7-(3-trifluoromethyl-3H-diazirin-3-yl)coumarin-3-cabroxyl succimidyl ester ***(12b)***

Recrystallization from EtOAc-hexane afforded pale yellow needles (181 mg) in 85%, mp (dec) 140-142 °C. δ_H ($CDCl_3$) 9.09 (1H, s), 6.78 (1H, s), 6.41 (1H, s), 4.00 (3H, s), 2.91 (4H, br s); IR (Nujol): ν 1805, 1785, 1735 cm^{-1}; UV-vis (EtOH) λ_{max} = 246 (sh, ε 6500), 330 nm (ε 17000); Anal: calcd for $C_{15}H_{11}F_3N_2O_5$ C:48.01, H:2.37, N:9.88, found C:48.38, H:2.17, N:9.99; MS *m/z* (EI) 425 (M^+), 397 (M^+-N_2).

tert-Butyl (2-(5-metoxy-2-oxo-7-(3-(trifluoromethyl)-3H-diazirin-3-yl)-2H-chromene-3-carboxamido)ethyl) carbamate ***(13a)***

Compound **12a** (30.0 mg, 66 μmol), *t-butyl N-(2-aminoethyl)carbamate* (17.2 mg, 108 μM) and DIEPA (5.3 mg, 41 μM) were dissolved dry acetonitrile (0.6 mL) under argon. The reaction mixture was stirred overnight at room temperature. After removal of the solvent, the residue was partitioned between EtOAc and water. The organic phase was further washed with water (x 3) and brine, and dried over $MgSO_4$. The residue was purified by preparatory thin-layer chromatography (silica gel, EtOAc : hexane = 2 : 1) to give **13a** as yellow solid (23.1 mg, 73%). δ_H (400 MHz; $CDCl_3$) 9.46 (1H, s), 8.87 (1H, br s), 7.24 (1H, d, *J* 2.4), 6.95 (1H, d, *J* 2.4), 4.97 (1H, br s), 3.95 (3H, s), 3.55-3.68 (2H, m), 3.34-3.48 (2H, m), 1.45 (9H, s); HRMS (ESI+) *m/z* 941.2941 ($2M+H^+$) $C_{22}H_{27}F_3N_4O_6$ requires 941.2905.

tert-Butyl (2-(5-metoxy-2-oxo-7-(3-(trifluoromethyl)-3H-diazirin-3-yl)-2H-chromene-3-carboxamido)ethyl) carbamate ***(13b)***

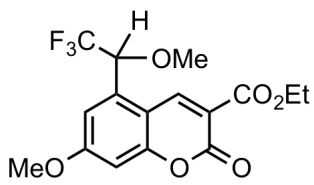
Purification by preparatory thin-layer chromatography (silica gel, EtOAc : hexane = 1 : 1) gave yellow solid (14.2 mg, 74%). δ_H (400 MHz; $CDCl_3$) 9.19 (1H, s), 8.87 (1H, br s), 6.81 (1H, s), 6.42 (1H, s), 4.97 (1H, br s), 3.98 (3H, s), 3.58 (2H, dt, *J* 5.8, 5.8), 3.43-3.33 (2H, m), 1.44 (9H, s); δ_C (101 MHz, $CDCl_3$) 162.0, 160.7, 157.8, 156.0, 155.1, 142.9, 136.1, 122.9, 120.2, 117.4, 110.4, 107.2, 103.1, 79.5, 56.4, 40.5, 40.1, 28.3; δ_F (376 MHz, $CDCl_3$) -64.44 (3F, s); HRMS (ESI+) *m/z* 471.1491 (M^+) $C_{22}H_{27}F_3N_4O_6$ requires 471.1491.

*(4E, 6Z, 8S, 9S, 10E, 12S, 13R, 14S, 16R)-13-Hydroxy-8,14-dimethoxy-19-((2-(7-methoxy-2-oxo-5-(3-(trifluoromethyl)-3H-diazirin-3-yl)-2H-chromene-3-carboxamido)ethyl)amino)-4,10,12,16-tetramethyl-3, 20,22-trioxo (**3a**)*

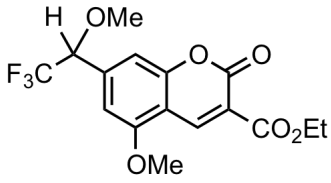
Compound **13a** (18.0 mg, 40 μmol) was stirred in 50% TFA-CH_2Cl_2 (0.40 mL) at room temperature for 1 h. After removal of the solvent, the product was used in the next reaction without further purification (15.7 mg). An acetonitrile solution (0.5 mL) of the product (15.7 mg), geldanamycin (7.0 mg, 12 μmol), and DIEPA (5.0 mg, 38 μmol) was stirred at room temperature overnight. The mixture was diluted with EtOAc (5 mL) and water (5 mL). The aqueous layer was extracted with EtOAc for three times. The combined organic layer was washed with brain, and was then dried over $MgSO_4$. After removal of the solvent, the residue was purified by preparatory thin-layer chromatography (silica gel, ether : MeOH = 20 : 1) to give **3a** as purple solid (6.0 mg, 18%). δ_H (500 MHz; $CDCl_3$) 9.49 (1H, s), 9.13 (1H, s), 9.02 (1H, t, *J* 5.6), 7.25 (1H, s), 6.92-6.98 (2H, m), 6.81 (1H, t, *J* 5.6), 6.58 (1H, t, *J* 11.3), 5.91 (1H, d, *J* 9.8), 5.86 (1H, t, *J* 10.5), 5.18 (1H, s), 4.78 (2H, br s), 4.31 (2H, d, *J* 9.8), 3.95 (3H, s), 3.72-3.94 (4H, m), 3.56-3.60 (1H, m), 3.43-3.47 (1H, m), 3.36 (3H, s), 3.27 (3H, s), 2.71-2.78 (1H, m), 2.68 (1H, d, *J* 12.8), 2.42 (1H, dd, *J* 10.7, 14.1), 2.02 (3H, s), 1.82-1.85 (1H, m), 1.80 (3H, d, *J* 0.9), 1.75 (1H, br s), 1.00 (6H, d, *J* 7.3); δ_F (376 MHz, $CDCl_3$) -67.97 (3F, s); HRMS (ESI+) *m/z* 921.3251 ($M+Na^+$) $C_{43}H_{49}F_3N_4NaO_{12}$ requires 921.3258.

*(4E, 6Z, 8S, 9S, 10E, 12S, 13R, 14S, 16R)-13-Hydroxy-8,14-dimethoxy-19-((2-(5-methoxy-2-oxo-7-(3-(trifluoromethyl)-3H-diazirin-3-yl)-2H-chromene-3-carboxamido)ethyl)amino)-4,10,12,16-tetramethyl-3, 20,22-trioxo (**3b**)*

Compound **13b** (11.5 mg, 24 μmol) was stirred in 50% TFA-CH_2Cl_2 (0.24 mL) at room temperature for 1 h. After removal of the solvent, the product was used in the next reaction without further purification (10.0 mg). A DMF solution (0.3 mL) of the product (10.0 mg), geldanamycin (2.8 mg, 5 μmol), and DIEPA (3.1 mg, 24 μmol) was stirred at room temperature overnight. The mixture was diluted with EtOAc (5 mL) and water (5 mL). The aqueous layer was extracted with EtOAc for three times. The combined organic layer was washed with brain, and was then dried over $MgSO_4$. After removal of the solvent, the residue was purified by preparatory thin-layer chromatography (silica gel, $CHCl_3$: EtOH = 20 : 1) to give **3b** as purple solid. δ_H (400 MHz; $CDCl_3$) 9.26 (1H, d, *J* 0.9), 9.13 (1H, s), 9.03 (1H, t, *J* 5.5), 7.26 (1H, s), 6.91-6.98 (2H, m), 6.81 (1H, d, *J* 0.9), 6.58 (1H, t, *J* 11.4), 6.43 (1H, s), 5.91 (1H, d, *J* 9.2), 5.86 (1H, t, *J* 10.5), 5.18 (1H, s), 4.31 (2H, d, *J* 9.2), 3.72-3.80 (3H, m), 3.80-3.88 (1H, m), 3.55-3.60 (1H, m), 3.44-3.48 (1H, m), 3.36 (3H, s), 3.27 (3H, s), 2.72-2.76 (1H, m), 2.68 (1H, d, *J* 12.8), 2.37-2.45 (1H, m), 2.05 (3H, s), 1.79-1.83 (1H, m), 1.80 (3H, d, *J* 0.9), 1.72 (1H, br s), 1.00 (3H, d, *J* 6.9), 0.98 (3H, d, *J* 6.9); δ_F (376 MHz, $CDCl_3$) -64.96 (3F, s); HRMS (ESI+) *m/z* 921.3248 ($M+Na^+$) $C_{43}H_{49}F_3N_4NaO_{12}$ requires 921.3258.



14a



14b

*Ethyl 5-methoxy-7-(1-trifluoromethyl-1-methoxymethyl)coumarin-3-cabroxylate (**14a**, methanol adduct)*

A methanol solution (40 mL) of compound **1a** (142 mg, 0.4 mmol) was irradiated by 100 W black light lamp at room temperature for 1 h. The product was purified by column chromatography on silica gel eluted with CH_2Cl_2-EtOAc (20 :1) to give a colorless solid (40 mg). δ_H ($CDCl_3$) 8.85 (1H, s), 7.06 (1H, d, *J* 2.5), 6.88 (1H, d, *J* 2.5), 4.93 (1H, q, *J* 6.4), 4.42 (2H, q, *J* 7.0), 3.93 (3H, s), 3.52 (3H, s), 1.41 (3H, t, *J* 7.0); HRMS (ESI+) *m/z* 383.0718 ($M+Na^+$) $C_{16}H_{15}F_3NaO_6$ requires 383.0718.

*Ethyl 7-methoxy-5-(1-trifluoromethyl-1s-methoxymethyl)coumarin-3-cabroxylate (**14b**)*

δ_H ($CDCl_3$) 8.87 (1H, s), 6.98 (1H, s), 6.83 (1H, s), 4.55 (1H, q, *J* 6.2), 4.42 (2H, q, *J* 7.0), 4.00 (3H, s), 3.51 (3H, s), 1.41 (3H, t, *J* 7.0); HRMS (ESI+) *m/z* 383.0718 ($M+Na^+$) $C_{16}H_{15}F_3NaO_6$ requires 383.0718.

*3-Ethyloxycarbonyl-7-hydroxy-5-((3-trifluoromethyl)-3H-diazirin-3-yl)coumarin (**2**)*

A solution of 2-(3-trifluoromethyl)-3*H*-diazirin-3-yl)-4,6-dihydroxybenzaldehyde (708 mg, 3.0 mmol), diethy malonate (961 mg, 6 mmol), piperidine (415 μL, 4.2 mmol), and acetic acid (155 μL, 2.7 mmol) in ethanol (15 mL) was stirred at room temperature overnight. After removal of the solvent in vacuo, EtOAc was added to the residue followed by washing with H_2O, 1 M HCl, brine and dried over $MgSO_4$. The product was purified by column chromatography on silica gel eluted with EtOAc-hexane (1 :1) to afford yellow solid (351 mg) in 34%. δ_H (400 MHz; $CDCl_3$) 9.14 (1H, s), 9.13 (1H, s), 7.70 (1H, br s), 7.22 (2H, d, *J* 2.3), 7.12 (2H, d, *J* 2.3), 4.47 (2H, q, *J* 7.3), 1.45 (3H, t, *J* 7.3); δ_F (376 MHz, $CDCl_3$) -68.13 (3F, s); HRMS (ESI+) *m/z* 365.0363 ($M+Na^+$) $C_{14}H_9F_3N_2NaO_5$ requires 365.0361.

*Photoreaction of GA photoprobe **3a** with Hsp90.*

Probe **3a** was dissolved in DMSO (1 mM) and used to make an appropriate concentration in a reaction buffered solution. Each solution of probe **3a** (10 μM) and Hsp90 (2 μM) in a 20 mM Tris-HCl (MP Biomedicals ultra-pure grade, pH 7.5, 10 μL) solution containing 50 mM KCl, 5 mM $MgCl_2$ in the absence or presence of inhibitor at various concentrations was irradiated at 0 °C with 365 nm light (fwhm 10 nm) using REX-250 high-pressure mercury lamp for 15 seconds. The photoproducts were denature by incubation with SDS-sample buffer at room temperature for 1 hour, and separated by 10% SDS-PAGE (Bio-Rad TGX gel plate). The labeled band was detected by fluorescent method (420 nm through a bandpass filter, fwhm 10 nm, 320 nm for excitation through a bandpass filter, fwhm 10 nm).

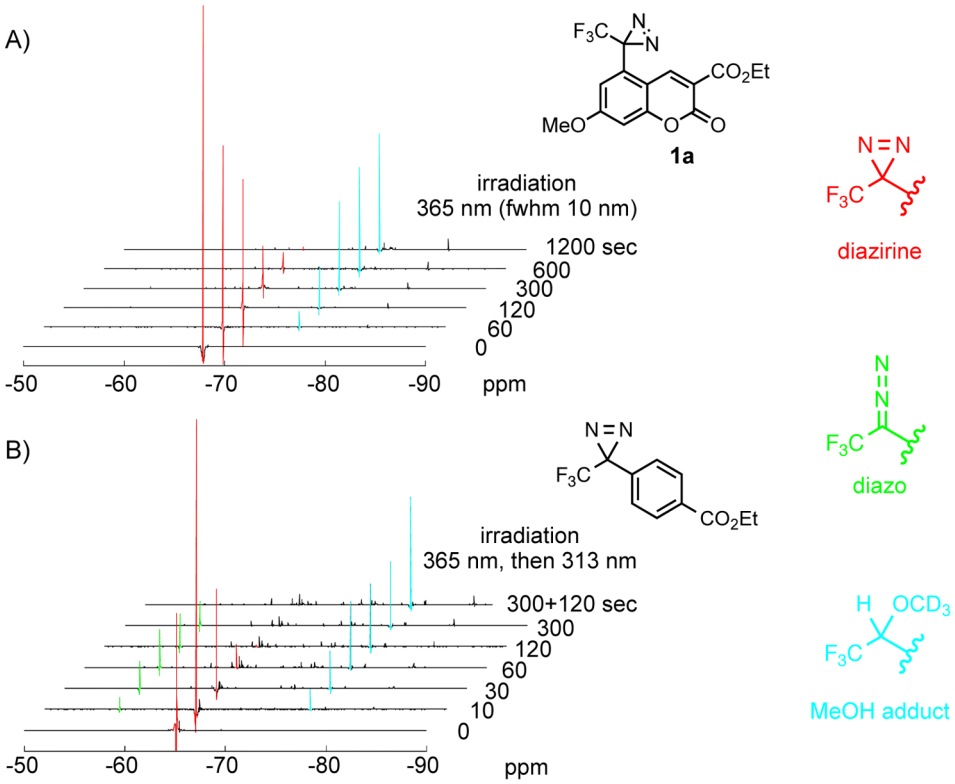


Fig. S1 ^{19}F NMR spectra showing the photolysis of compound **1a** (A) and ethyl 4-[3-trifluoromethyl)-3*H*-diazirin-3-yl]benzoate (B), respectively. The irradiation was performed in a CD_3OD solution of (20 mM) with 365 nm light through a bandpass filter at 0 °C over various periods of time. Panels A and B show the signals from CF_3 of diazirine compounds (red signals), the corresponding diazo compounds (green), and methanol adducts (blue). ^{19}F NMR signals from the CF_3 group of the photoproducts clearly distinguished the diazirine compound as a starting material observed at δ -67.9 and the methanol adduct at δ -82.2 ($CFCl_3$ as internal standard), respectively (panel A). The diazirine, diazo, and methanol adduct could be easily assigned by ^{19}F NMR since those signals are appeared separately at specific chemical shifts, as shown by a typical example for ethyl 4-[3-trifluoromethyl)-3*H*-diazirin-3-yl]benzoate in panel B. The spectra clearly show conversion among the three species; the peak δ -65.1, from the diazirine derivative, decreased with irradiation, the peak at δ -57.5, the diazo derivative, increased with irradiation at 365 nm and then disappeared with irradiation at 313 nm, and the peak at δ -76.4, from the methanol adduct, constantly increased. Hence, in panel A, the ^{19}F NMR spectra of compound **1a** do not show the signal from the corresponding diazo compound during photolysis.

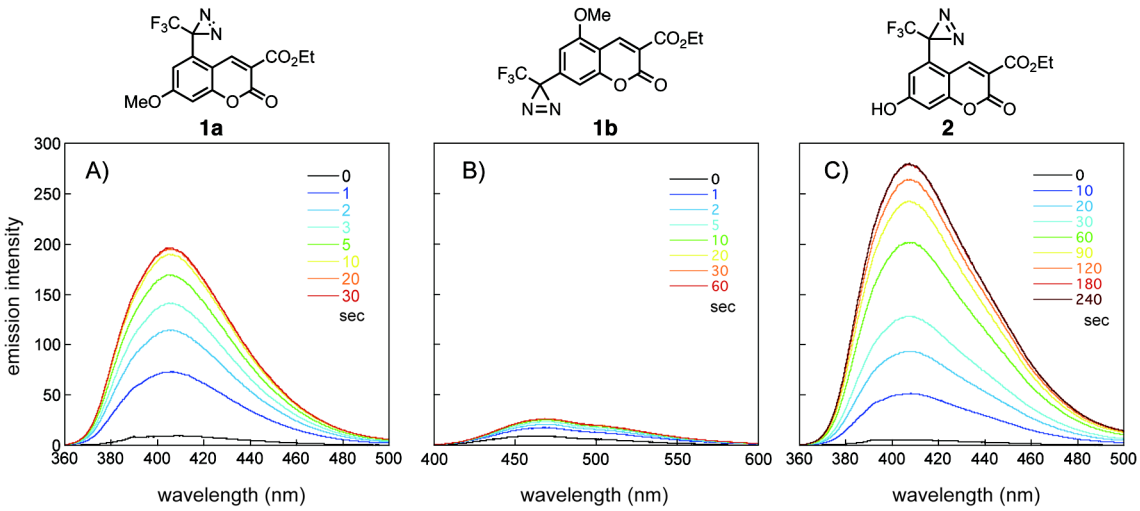
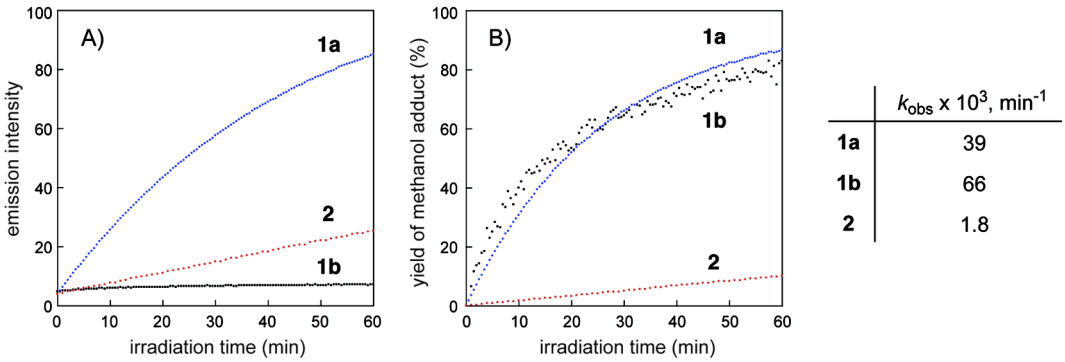


Fig. S2 Fluorescence of diazirinylcoumarin derivatives with irradiation under 365 nm light through a bandpass filter (fwhm = 10 nm), using a 250 W high pressure Hg lamp at 0 °C (panels A–C). Fluorescence spectra were measured at 20 °C for **1a**, **1b**, and **2** (1 μM in methanol) which were excited at 349, 329, and 351 nm, respectively.



	k_{obs} x 10^3, min^{-1}
1a	39
1b	66
2	1.8

Fig. S3 (A) Emission recovery of coumarin derivatives (1 μM in methanol for compound **1a** (blue), **1b** (black), and **2** (red): Emission intensities were measured at 406, 469, and 407 nm (λ_{ex} = 365 nm), respectively) with irradiation under 365 nm light using a spectrofluorometer at 20 °C. (B) Conversion yields (%) of the corresponding methanol adduct were determined from fluorescence intensities after additional irradiation under 365 nm light through a bandpass filter (fwhm = 10 nm) using a 250 W high pressure Hg lamp at 0 °C. Observed rate constants (x 10^{-3}) were calculated from the initial slope of panel B.

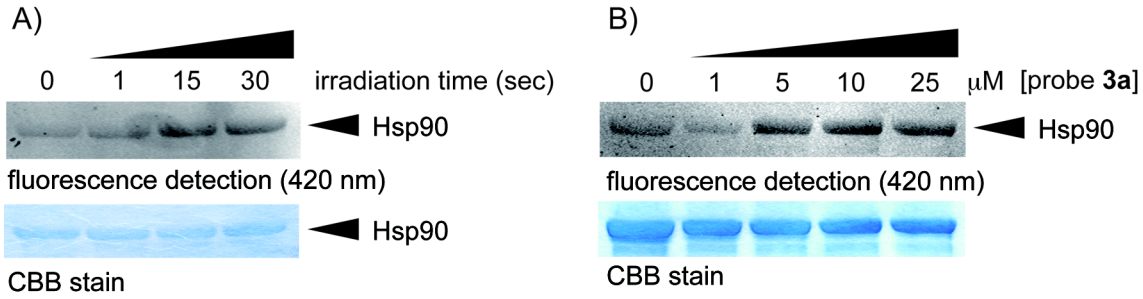


Fig. S4 (A) Photoaffinity labeling of Hsp90 (5 μM) was performed with GA photoprobe **3a** (50 μM) in a 10 μL buffered solution with irradiation under 365-nm light through a bandpass filter (fwhm 10 nm) using a 250 W high pressure Hg lamp at 0 °C. Fluorescence intensity of the labeled Hsp90 (indicated by arrow) was increased with the irradiation time; 0, 1, 15, 30 seconds, respectively. Lower panels show the results of Coomassie Brilliant Blue (CBB) staining of photoproducts. (B) Photoaffinity labeling of Hsp90 (5 μM) was performed with GA photoprobe **3b** at various concentrations. Fluorescence intensity of the labeled Hsp90 (indicated by arrow) was increased with the amount of probe; 0, 1, 5, 10, 25 μM, respectively.

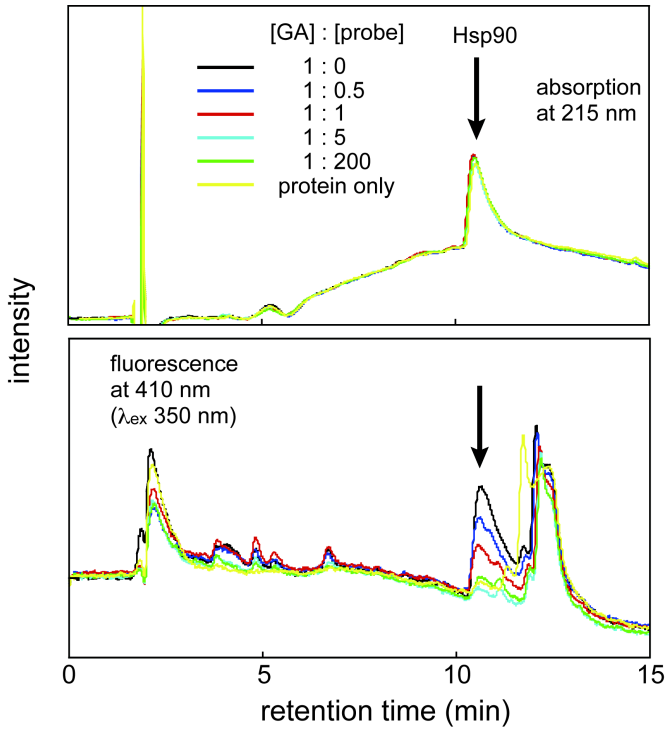


Fig. S5 Photoaffinity labeling of Hsp90 (5 μM) was performed with GA photoprobe **3a** (10 μM) in a 10 μL buffered solution in the absence or presence of GA at various concentrations (0.5, 1, 5, 200 μM), respectively, with irradiation under 365-nm light through a bandpass filter (fwhm 10 nm) using a 250 W high pressure Hg lamp at 0 °C. After ultrafiltration using Amicon Ultra-0.5 10K, the photoproducts were analyzed by reverse-phase HPLC on ODS (SHISEIDO Proteonavi C4, 5mm 2.0 mm x 250 mm) with a liner gradient of 20–100% solution B (90% acetonitrile-water containing 0.085% formic acid, solution A: 10% acetonitrile-water containing 0.1% formic acid) over 15 min at a flow rate of 0.4 mL/min (detection in the upper panel: absorption at 215 nm, the lower panel: emission at 410 nm excited at 350 nm).

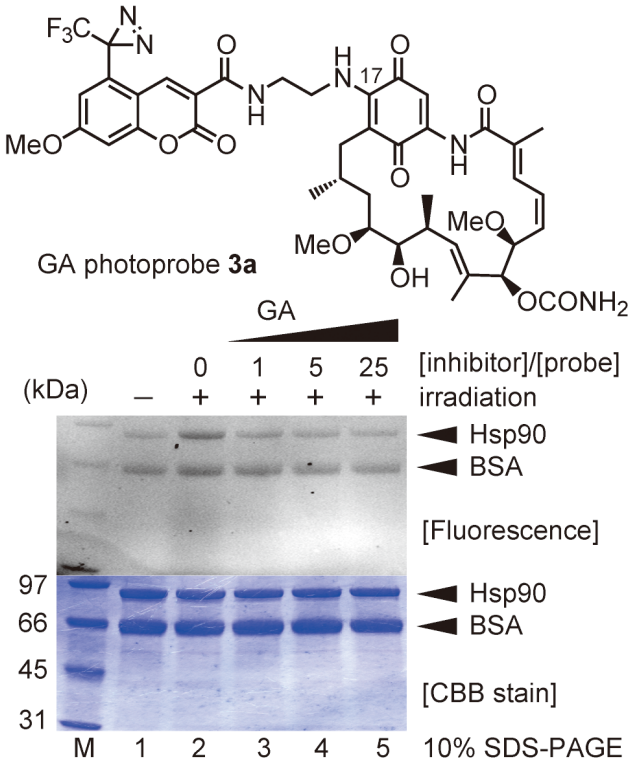


Fig. S6 (A) PAL of Hsp90 (5 μM) with photoprobe **3a** (10 μM) and the competitive inhibition assays in a solution (10 μL) including BSA (4 μg) in the absence (lanes 1,2) or presence of GA (lanes 3-5) at various concentrations (10, 50, 250 μM), respectively. Upper panel showed the result by fluorescence detection of the photoproducts (indicated by arrow). The bands were detected by a fluorescence method (λ_{ex} = 320 nm, λ_{em} = 420 nm, through bandpath filters (fwhm = 10 nm)). Lower panel shows the result of Coomassie Brilliant Blue (CBB) staining of photoproducts. Some emissions were detected from the protein bands and the intensity was depended on protein as shown in a lane of protein marker.

The emission from the band was not completely disappeared in a sample containing GA at high dose (lane 5). Weak emission was also observed from the protein band in a probe-free sample (lane 1). Other proteins showed similar emissions, and intensity was dependent on protein, as seen in the lane for protein marker. We used ultra-pure grade Tris (MP Biomedicals ultra-pure grade, pH 7.5) and Bio-Rad TGX gel plate as a low fluorescent gel plate for SDS-PAGE. Since bandpass filters for excitation and emission were used for detection, the intrinsic emissions from tryptophan and tyrosine residues of protein should not have affected the accuracy of the detection. Tris-HEPES SDS-PAGE made at pH 7.0 was tested either. It prevents the hydrolysis of polyacrylamide to acrylic acid which affects the migration of proteins. But the emission from proteins was similarly detected. From the data of HPLC (Fig. S5), it may be diffracted light, but the reason has not been clear yet.

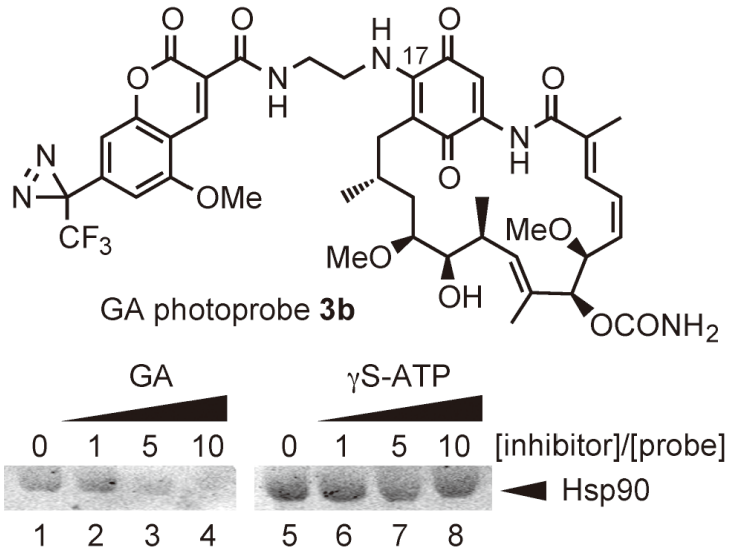


Fig. S7 PAL of Hsp90 (2 μM) with photoprobe **3b** (10 μM) and the competitive inhibition assays in the absence (lanes 1,5) or presence of GA (lanes 2-4) or γS-ATP (lanes 6-8) at various concentrations (10, 50, 100 μM), respectively.