# Electronic Supplementary Information

## A highly selective fluorogenic substrate for imaging intracellular glutathione S-transferase P1: development, cellular imaging, and applicabilty to epigenetic studies

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# 1. General methods

**Materials.**

Dulbecco's modified Eagle's medium (DMEM), Roswell Park Memorial Institute 1640 medium (RPMI1640), and phosphate-buffered saline (PBS) were purchased from Nissui Pharmaceutical (Tokyo Japan). Hanks' Balanced Salt Solution (HBSS) was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). McCoy's 5A (modified) medium, 100 ×penicillin/streptomycin/glutamine, trypsin-EDTA (0.25%), Lipofectamine™ *RNAiMAX* transfection reagent, Hoechst 33258 pentahydrate (10 mg/ml in water), SuperSignal West Pico Chemiluminescent Substrate, and ProLong® Gold Antifade Reagent were obtained from Thermo Fisher Scientific (Waltham, MA, USA). Fetal bovine serum (FBS) was obtained from Sigma-Aldrich (St. Louis, MO, USA). FuGENE6 transfection reagent was obtained from Roche Applied Science (Penzberg, Germany). *GSTP1* siRNA (siRNA ID: SASI_Hs01_00089970) and MISSION siRNA Universal Negative Control (siRNA ID: SIC-001) were obtained from Sigma-Aldrich. Protease inhibitor cocktails were obtained from Sigma-Aldrich (Cat. No. P8340) and Nacalai Tesque (Cat. No. 25955-24) (Tokyo, Japan). pIRES2 DsRed-Express2 was purchased from Clontech Laboratory, Inc., (Mountain View, CA). Antibodies against GSTP1 (Code No. 312) and β-actin (Code No. 053) were obtained from MBL (Nagoya, Japan). The monoclonal anti-FLAG M2 mouse antibody (F3165) was purchased from Sigma-Aldrich. Anti-rabbit goat IgG conjugated with horseradish peroxidase (W4011) was obtained from Promega (Fitchburg, WI, USA). Alexa647-conjugated Goat Anti-Mouse IgG H&L (Alexa Fluor® 647) (Cat. No. ab150115) was obtained from Abcam, Inc. (Cambridge, UK). General chemicals for organic synthesis and analysis were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), Tokyo Chemical Industries Co., Ltd. (Tokyo, Japan), Kanto Chemical Co., Inc. (Tokyo, Japan), and Sigma-Aldrich Chemical Co., Ltd. (St. Louis, MO, USA), and used without further purification.

**Identification of synthesized compounds.** NMR spectra of synthesized compounds were recorded on a Bruker DRX-400 spectrometer at 400 MHz for $^{1}$H-NMR and at 100 MHz for $^{13}$C-NMR. Chemical shifts (δ values) in $^{1}$H- and $^{13}$C-NMR were calibrated to the residual solvent resonance at 2.05 and 29.84 ppm for acetone-$d_6$, and 2.5 and 39.52 ppm for DMSO-$d_6$. Mass spectra were obtained using a Micromass LCT spectrometer (Waters, Milford, MA, USA) in ESI positive mode.

**Semi-preparative High-performance liquid chromatography (HPLC).** Semi-preparative HPLC was performed on a Mightysil RP-18 GP column (250 mm × 20 mm (5 μm); Kanto Chemical, Tokyo, Japan) at a flow rate of 9 ml/min using an

HPLC system composed of a pump (PU-980, JASCO) and a detector (UV-975, JASCO). Data were acquired by Clarity Lite software (DataApex, Prague, Czech Republic). Eluent: A:B = 90:10 → 10:90 (20 min), A: 10 mM ammonium acetate, B: acetonitrile. Subsequently, a fraction containing the target compound was analysed by reverse-phase HPLC on an Intertsustain C18 column (250 mm × 4.6 mm; GL Sciences, Inc.) at a flow rate of 0.2 ml/min using an HPLC system composed of two pumps (LC-20AD, Shimadzu) and a detector (SPD-M20A and RF-20Axs for absorbance and fluorescence, respectively) operated by Clarity software through a system controller (CVM-20A, Shimadzu). Eluent: A: B = 90:10 → 5:95 (20 min), A: 10 mM ammonium acetate, B: acetonitrile.

**HPLC and LC-MS analysis.** HPLC analysis was performed on an Intertsustain C18 column (250 mm × 4.6 mm; GL Sciences, Inc.) at a flow rate of 1.0 ml/min using an HPLC system composed of a pump (LC-20AD, Shimadzu) and a detector (SPD-M20A and RF-20Axs for absorbance and fluorescence, respectively). Eluent: A: B = 90: 10 → 5: 95 (20 min) A: 10 mM ammonium acetate, B: acetonitrile. LC-MS analysis was performed on an Intertsustain C18 column (150 mm × 3.0 mm; GL Sciences, Inc.) at a flow rate of 0.2 ml/min using LC-MS (Shimadzu Quadrupole LC-MS). Eluent: A: B = 90: 10 → 5: 95 (20 min) A: 10 mM ammonium acetate, B: acetonitrile. LabSolutions software was used for operation of the system and data analysis.

**Measurement of absorbance and fluorescence spectra.**

UV-visible spectra were obtained on a V-550 UV/VIS spectrophotometer (JASCO Corp., Tokyo, Japan). Fluorescence photometric studies were performed on an RF-5300PC spectrofluorophotometer (Shimadzu Corp., Kyoto, Japan), SH-9000 microplate reader (Corona Electric, Ibaraki, Japan) or Ensight™ multimode plate reader (ParkinElmer, Tokyo, Japan).

**Construction of expression vectors for mammalian cells.**

Expression plasmids for 3xFLAG-GSTs in mammalian cells were prepared as described elsewhere (Fujikawa et al, Talanta in press).

**Expression and purification of recombinant human GSTs.**

The expression plasmids for human GSTs, pCOLD-III/GSTA1, GSTM1, and GSTP1, were each introduced into *Escherichia coli* BL21(DE3)pLysS competent cells. Individual colonies were inoculated into 5-ml aliquots of LB supplemented with ampicillin (Amp) and chloramphenicol (CP) (50 and 34 μg/ml, respectively) and incubated overnight at 37°C with shaking. Each overnight culture was seeded in 200 ml fresh LB-Amp/CP, then incubated at 37°C with shaking. When the $OD_{600}$ reached 0.5, the culture solution was cooled at 15°C for 30 min, then isopropyl thio-β-D-galactoside (IPTG) (0.1 mM final concentration) was added to induce expression of the recombinant protein. After agitation at 15°C for 24 h, the cells were collected by centrifugation and suspended in 100 mM sodium phosphate (pH 7.0) containing 300 mM NaCl. The cell suspension was sonicated on ice (10-s sonication pulses alternating with 20-s intervals for 10 min) and clarified by centrifugation at 4°C for 30 min. The supernatant was loaded on an affinity column of 2 ml Glutathione Sepharose™ 4 Fast flow (GE Healthcare, Piscataway, NJ, USA), then washed three times with 5 ml of 20 mM potassium phosphate (pH 7.0) containing 3 mM EDTA and 3 mM β-mercaptoethanol (β-ME). Recombinant proteins were eluted with 4 ml of 50 mM Tris-HCl (pH 7.0) containing 400 mM NaCl and 10 mM GSH. The eluate was dialyzed against 20 mM potassium phosphate buffer (pH 7.0) containing 3 mM EDTA and 3 mM β-ME at 4°C overnight, then against 20 mM potassium phosphate buffer (pH 7.0) containing 3 mM EDTA, 3 mM β-ME, and 20% (v/v) glycerol at 4°C for 5 h. The dialyzed enzyme solution was frozen and held at -80°C until use. Protein concentration was determined using the standard Bradford assay with bovine serum albumin (BSA) as a standard. Activities of purified recombinant GSTs were confirmed with the standard CDNB assay.

**Determination of quantum efficiency.**

The quantum efficiencies (QEs) of fluorogenic substrates and reaction products were measured as previously described[1] with fluorescein in 0.1 M NaOH (QE = 0.85) as a standard.

**Determination of enzymatic activity.**

Except as noted, assays were performed in 100 mM sodium phosphate buffer (pH 7.4, 0.1% DMSO as a cosolvent) containing a compound and GSH in the presence or absence of GST. The activity was determined fluorophotometrically (excitation and emission wavelengths: 490 nm and 510 nm). In all experiments, the non-enzymatic background reaction rate was subtracted prior to calculation of the rate of product formation, which was estimated as previously described[32].

**Determination of enzyme kinetic parameters.**

**Specific activity:** Assays were performed in 100 mM sodium phosphate buffer (pH 7.4, 0.1% DMSO as a cosolvent) containing 1 μM **Ps-TG**, 1 mM GSH, and various concentrations of GST. For **Ps-TG** assays, 0.1-0.8 μg/ml GSTP1-1 and 0.8-3.7 μg/ml GSTM1-1 were used. Specific activity was determined from the slope of the linear relationship between the initial velocity and the GST concentration.

**Apparent specificity constant (app. $k_{cat}/K_M$):** These assays were performed in 100 mM sodium phosphate buffer (pH 7.4, 0.1% DMSO as a cosolvent) containing 0.03-1.0 μM **Ps-TG**, 1 mM GSH, and GST at concentrations as follows: 0.2 μg/ml GSTP1-1 and 1.5 μg/ml GSTM1. The apparent $k_{cat}/K_M$ was determined based on the Michaelis-Menten equation using probe concentrations well below the $K_M$ ([S] << $K_M$). $k_{cat}$, $K_M$, and [S] represent catalytic constant, Michaelis constant, and substrate concentration, respectively.

**Cell culture and transfection.** MCF7 and HT-1080 cells were cultured in DMEM. HCT116 and HT29 cells were cultured in McCoy's 5A (modified) medium. HuCCT1, DU145, and LNCaP cells were cultured in RPMI1640 medium. All the media were supplemented with penicillin/streptomycin/glutamine and 10% (v/v) FBS. Cell culture was performed in a humidified incubator under 5% $CO_2$ in air. Transfection of plasmids and small interfering RNAs (siRNAs) was carried out with FuGENE6® Transfection Reagent or Lipofectamine™ *RNAiMAX* Transfection Reagent (respectively) according to the respective manufacturer's instructions.

**Immunoblotting.** Aliquots of each cell lysate containing equal quantities of protein were separated by SDS-PAGE (12.5% polyacrylamide), then transferred onto an Immobilon-P polyvinylidene difluoride membrane (0.45-μm pore size, Millipore). The membrane was blocked with 3% skim milk in Tris-buffered saline containing 0.1% Tween 20 (TBS-T) by overnight incubation. The blocked membrane was incubated with anti-GSTP1 (1:1000 dilution) or anti-β-actin (1:4000 dilution) rabbit IgG in TBS-T containing 5% BSA and 0.2% sodium azide overnight at 4°C followed by washing three times with TBS-T. The secondary antibody was anti-rabbit goat IgG conjugated with horseradish peroxidase (1:2500 dilution). SuperSignal West Pico Chemiluminescent Substrate (Thermo Fisher Scientific) was used for detection.

**Immunofluorescence staining.** Cells were fixed with 4% paraformaldehyde in PBS for 15 min at room temperature. After permeabilisation in PBS containing 0.1% Triton X-100, cells were incubated in PBS containing 1% BSA, 0.1% Triton X-100 and 0.1% sodium azide for 60 min, followed by incubation with monoclonal anti-FLAG M2 mouse antibody (1:500 dilution) for overnight at 4°C. Then, cells were incubated with secondary antibody (goat anti-mouse IgG H&L conjugated with Alexa Fluor® 647, 1:1000 dilution) for 1 h at 4°C, followed by mounting with ProLong® Gold Antifade Reagent. Slides were maintained at 4°C pending microscopic evaluation.

**Probe incubation.** MCF7 cells transfected with a plasmid encoding 3×FLAG-GST were seeded on 35-mm glass-bottom dishes or 8-well chambers one day before transfection. Prior to the imaging experiments, cells were rinsed twice with PBS, then incubated in HBSS (+) containing 2.5 μM fluorogenic substrates (0.1% DMSO as a cosolvent) for 5 min at 37°C. Cells were washed twice with PBS; 1 ml HBSS (+) was added, and the cells were subjected to microscopic imaging. Cancer cells seeded one day before transfection were washed twice with PBS and loaded with 10 μg/ml Hoechst 33258 in HBSS (+) for 20 min, and then incubated with 2.5 μM **Ps-TAc** in HBSS (+) at room temperature. During the incubation, fluorescence and bright-field images were captured every 2 min for 20 min.

**Fluorescence imaging.** Live cell imaging of probe-loaded cells was performed using a FV10i confocal microscope with the FLUOVIEW software and an oil immersion objective lens UPLSAPO60XO 60× (NA 1.35). Fluorescence images of the probe and DsRed Express2 were taken via the FITC (excitation at 473 nm) and the DsRed (excitation at 559 nm) channels, respectively. Immunofluorescence imaging was performed using a FV1000 confocal microscope with the FLUOVIEW software and an oil immersion objective lens UPLSAPO60XO 60× (NA 1.42). Fluorescence images of DAPI, DsRed Express2, and Alexa647 were taken via the corresponding channels with excitation at 473 nm, 559 nm, and 635 nm, respectively.

**Data analysis and statistics.** Sixteen-bit images were obtained and then analysed using ImageJ/Fiji. DsRed-expressing cells were distinguished from non-expressing cells using high-contrast fluorescence images in the DsRed channel. The DsRed-expressing cells were defined as the cells exhibiting fluorescence above the level of transfected MCF7 cells. The number of the cells shown in the figure legends were collected and subjected to data analysis. Data are represented using box plots

with whiskers or dot plots; plots were generated using Kaleida Graph 4 (Synergy Software) (Reading, PA). In box plots, the medians are plotted as the central line of the box and the edges of the box indicate the upper (75%) and lower (25%) quartiles; the edges of the higher and lower whiskers indicate the maximum and the minimum fluorescence intensities, respectively. Outliers are represented as points whose values are greater than upper quartiles + 1.5 x IQD or less than lower quartiles - 1.5 x IQD, where the IQD (interquartile distance) is defined as the distance between the upper and lower quartiles. In dot plots, single-cell data are plotted and the median values are indicated by the central line. Statistical tests for the comparison of green fluorescence intensities from multiple groups were performed using two-tailed one-way analysis of variance (ANOVA) followed by post-hoc Tukey test. P values of less than 0.05 were defined as statistically significant.

# 2. Supplementary Figures

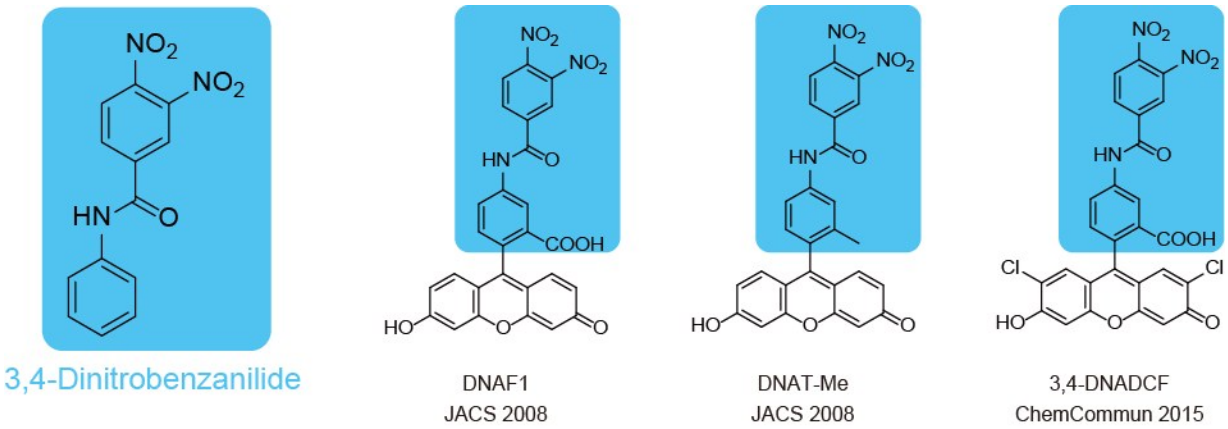



**Fig. S1 Previously described fluorogenic GST substrates based on 3,4-dinitrobenzanilide.**

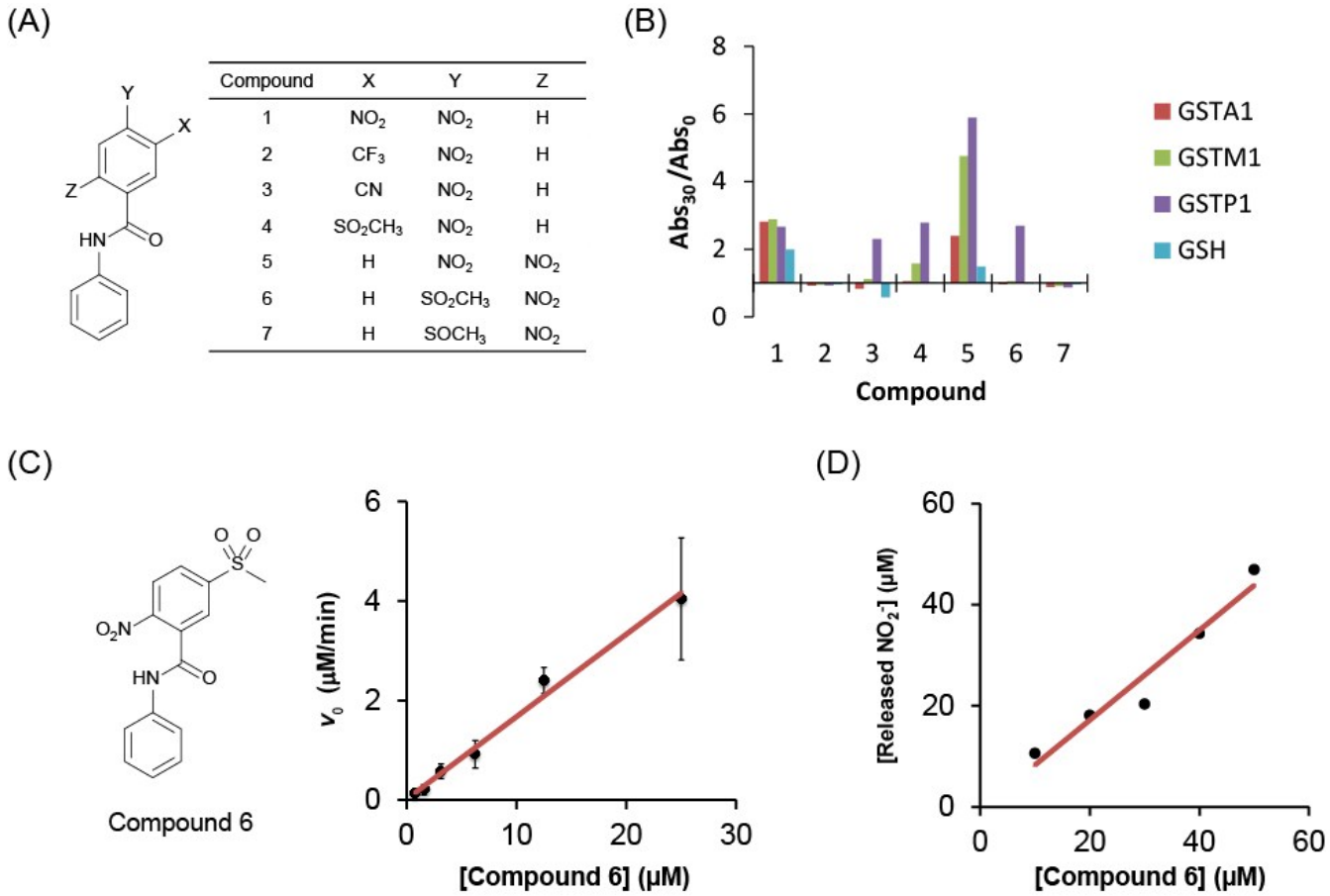



**Fig. S2 5-Mesyl-2-nitrobenzanilide is a GSTP1-selective substrate.**

(A) Molecular structure of the test compounds. (B) Absorbance ratio ($Abs_{30}/Abs_0$) at a specific wavelength (as described below) of the test compounds before and after incubation with GSH. Each compound (50 μM) was incubated with 1 mM GSH in the presence or absence of GSTP1-1 in 100 mM sodium phosphate buffer (pH 7.4) at 25°C for 30 min. The wavelengths used for analysis were as follows: **1**, 290 nm; **2**, 285 nm; **3**, 290 nm; **4**, 315 nm; **5**, 340 nm; **6**, 280 nm; **7**, 315 nm. (C) Linear relationship between the concentration of 5-mesyl-2-nitrobenzanilide (**6**) and the initial velocity ($v_0$) of the reaction. Reaction conditions: 100 mM sodium phosphate buffer (pH 7.4), 1 mM GSH, and 2 μg/ml GST at 25°C. Each point and error bars represent mean ± SEM (n = 3). (D) Quantification of the released nitrite ion from various concentrations of compound **6** after incubation with 1 mM GSH in the presence of GSTP1-1 at 25°C for 30 min.

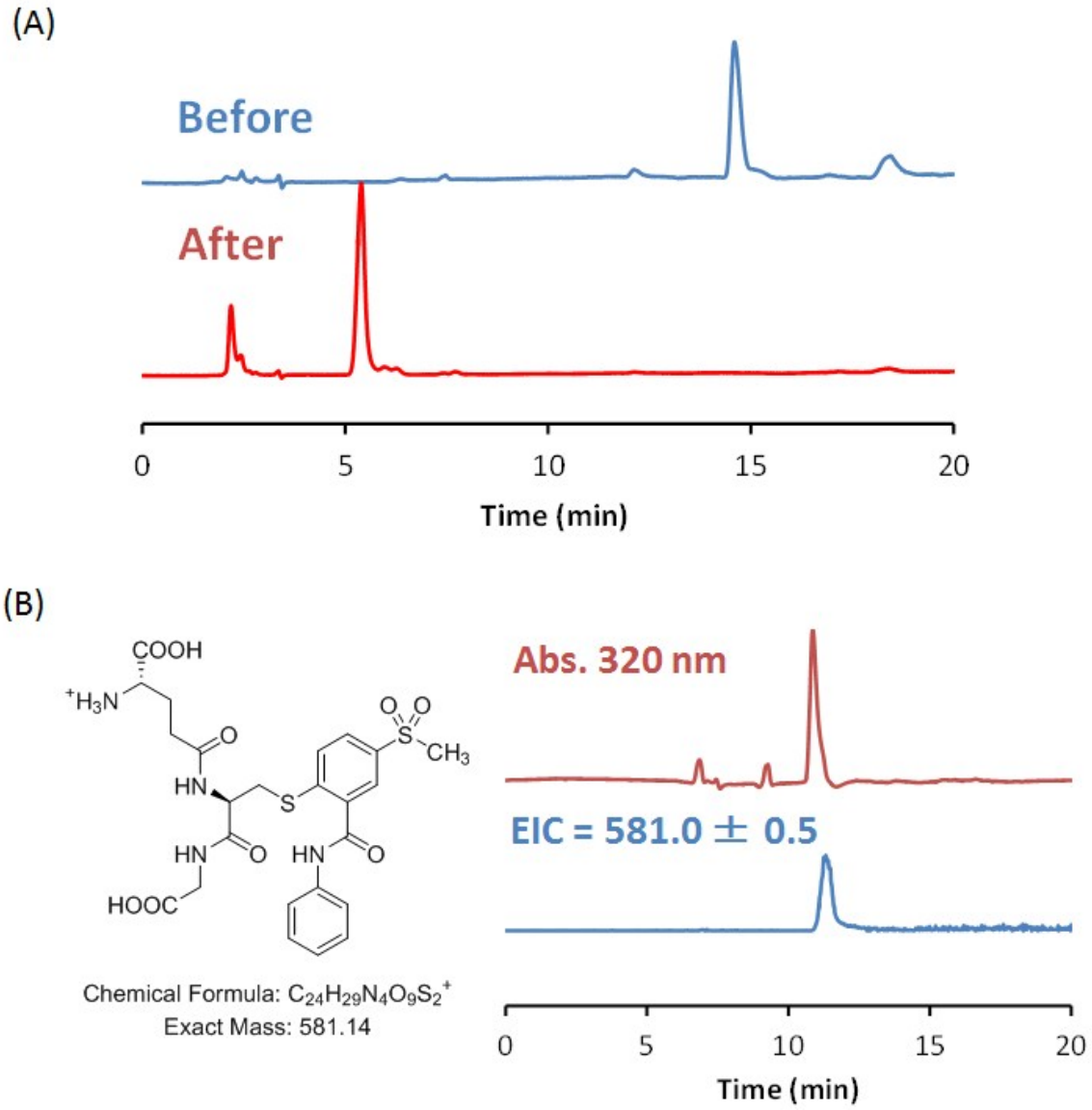



**Fig. S3 HPLC and LC-MS analysis of the conversion of 2-nitro-5-mesylbenzanilide by hGSTP1 catalysis.**

(A) HPLC analysis of 2-nitro-5-mesylbenzanilide (Before) and the reaction mixture after incubation with hGSTP1 (After). Absorbance at 280 nm was detected. (B) Confirmation of product formation by LC-MS. Left, molecular structure of the expected reaction product. Right, HPLC chromatogram (detected at 320 nm) and the extracted ion chromatogram (EIC) (*m/z* = 581.0 ± 0.5) are represented by red and blue lines, respectively. Eluents: A, 10 mM ammonium acetate (pH 6.5); B, acetonitrile. Gradient conditions; A:B = 90:10 (0 min) → 10:90 (20 min).

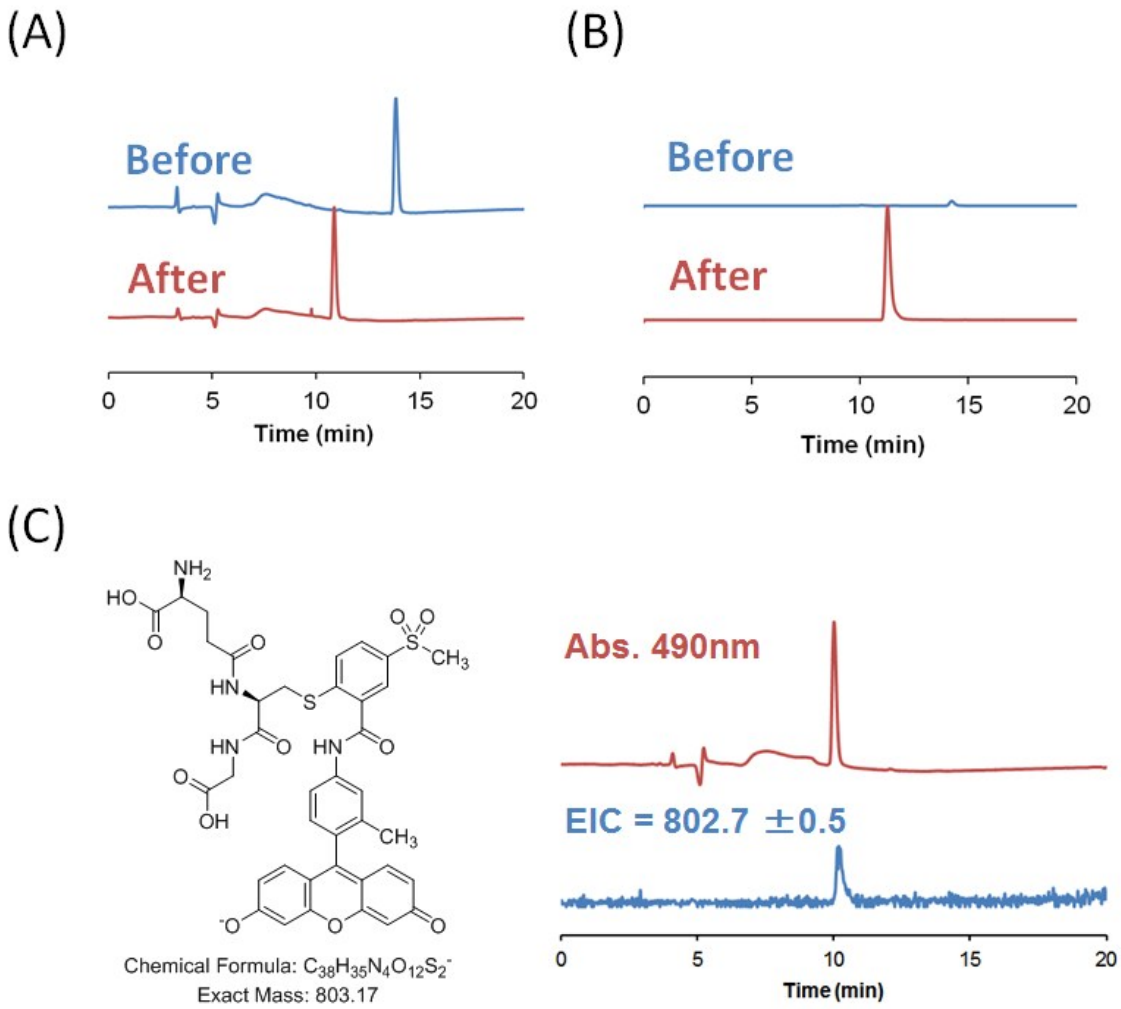



**Fig. S4 HPLC and LC-MS analysis of the reaction of Ps-TG by hGSTP1 catalysis.**

(A) HPLC chromatogram showing absorbance at 490 nm. (B) HPLC chromatogram showing fluorescence at 510 nm (ex. 490 nm). (C) LC-MS analysis of the product by GSTP1-catalyzed glutathionylation. Molecular structure of glutathionylated product (Left). Chromatogram detected by absorbance at 490 nm (upper right) and extracted ion chromatogram (EIC) of *m/z* at 802.7 ± 0.5 (lower right) are shown. Eluents: A, 10 mM ammonium acetate; B, acetonitrile. Gradient conditions: A:B = 90:10 (0 min) → 10:90 (20 min).

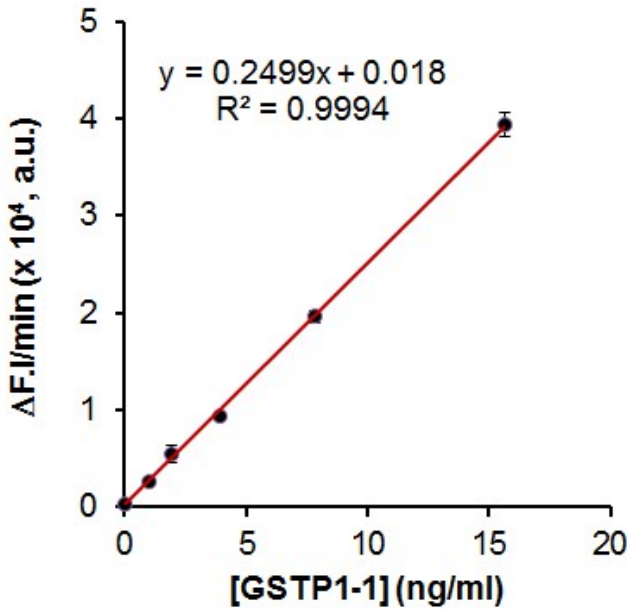



**Fig. S5. Relationship between initial rate and GSTP1-1 concentration.**

Ps-TG (1 μM) was incubated in 100 mM sodium phosphate buffer (with 0.1% DMSO as a cosolvent) containing 1 mM GSH and different concentrations of GSTP1-1. The enzyme concentration at the detection limit was calculated from $3SD_0$/slope, where $SD_0$ is the standard deviation of the initial rate in the absence of the enzyme. The detection limit was 0.115 ng/ml. Measurements were conducted in triplicate. The dots and error bars respectively represent the mean ± S.D.

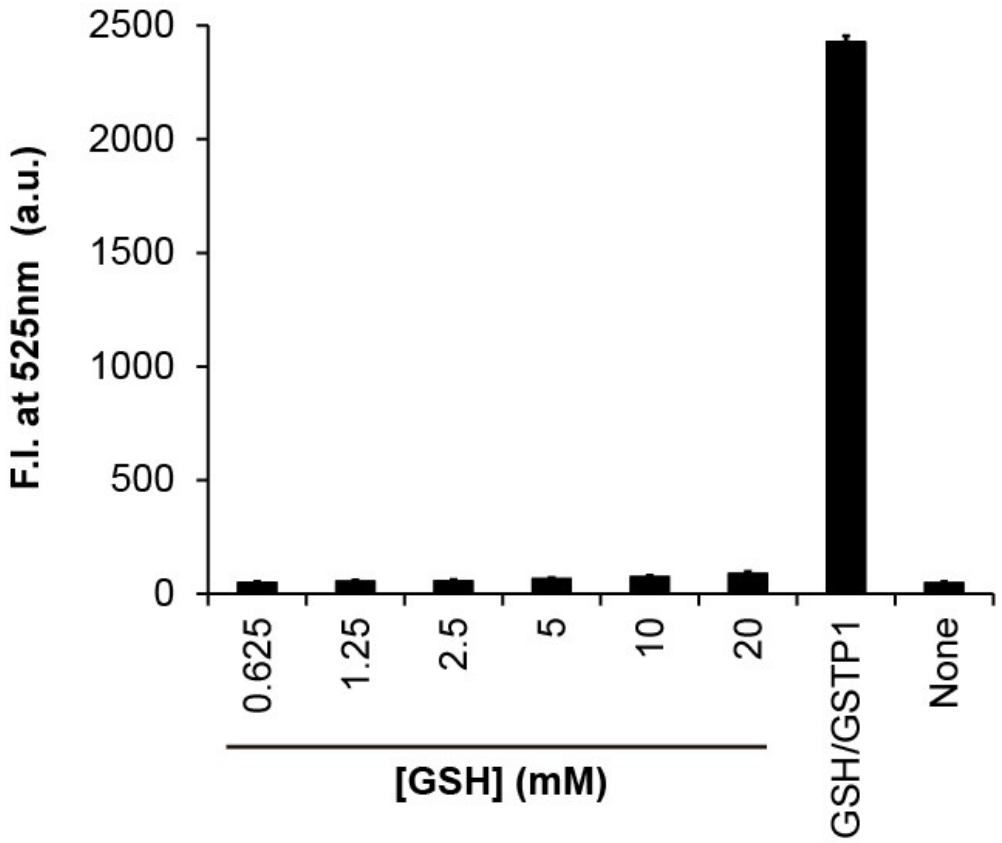



**Fig. S6 Non-enzymatic reactivity of Ps-TG with GSH.**

2 μM Ps-TG was incubated in sodium phosphate buffer (100 mM, pH 7.4) containing the indicated concentrations of GSH at room temperature for 30 min. 'GSH/GSTP1' represents Ps-TG that was incubated with 1 mM GSH in the presence of 5 μg/ml GSTP1. 'None' is a negative control containing only Ps-TG in sodium phosphate buffer (without GSH or GSTP1). Excitation and emission wavelengths were 490 nm and 510 nm, respectively.

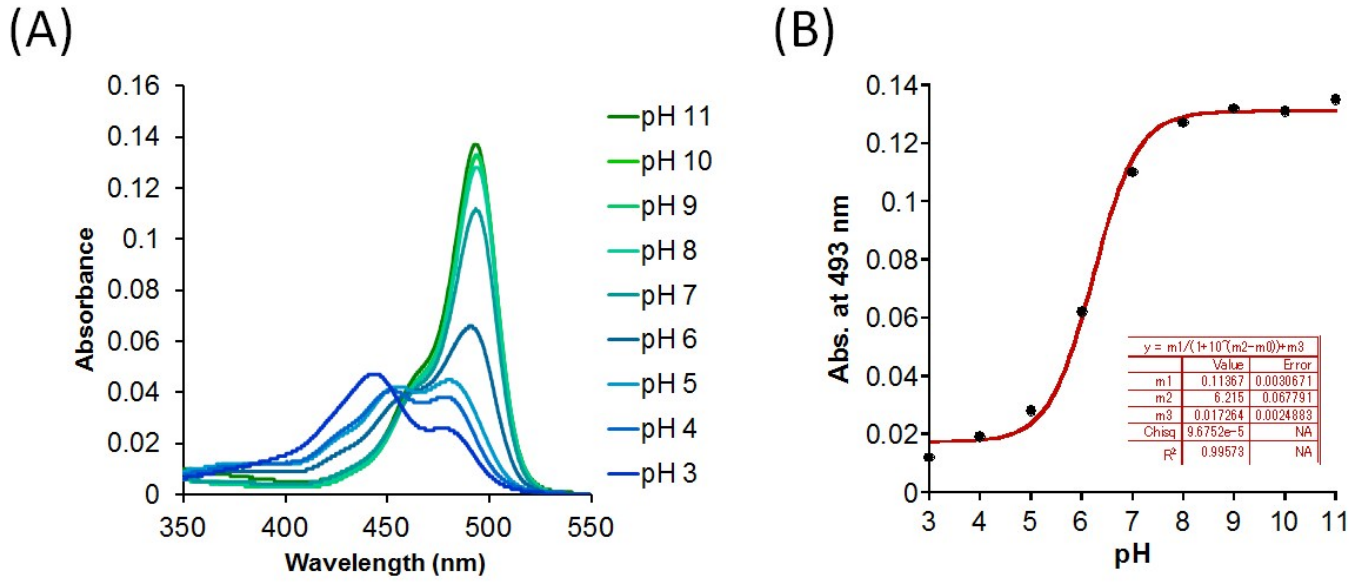



**Fig. S7. pH dependence of absorbance of 3,4-DNADCF.**

(A) Absorbance spectra of 2 μM Ps-TG in 100 mM sodium phosphate at various pH values. (B) pH dependence of absorbance at 493 nm. Data were fitted to the Henderson-Hasselbalch equation.

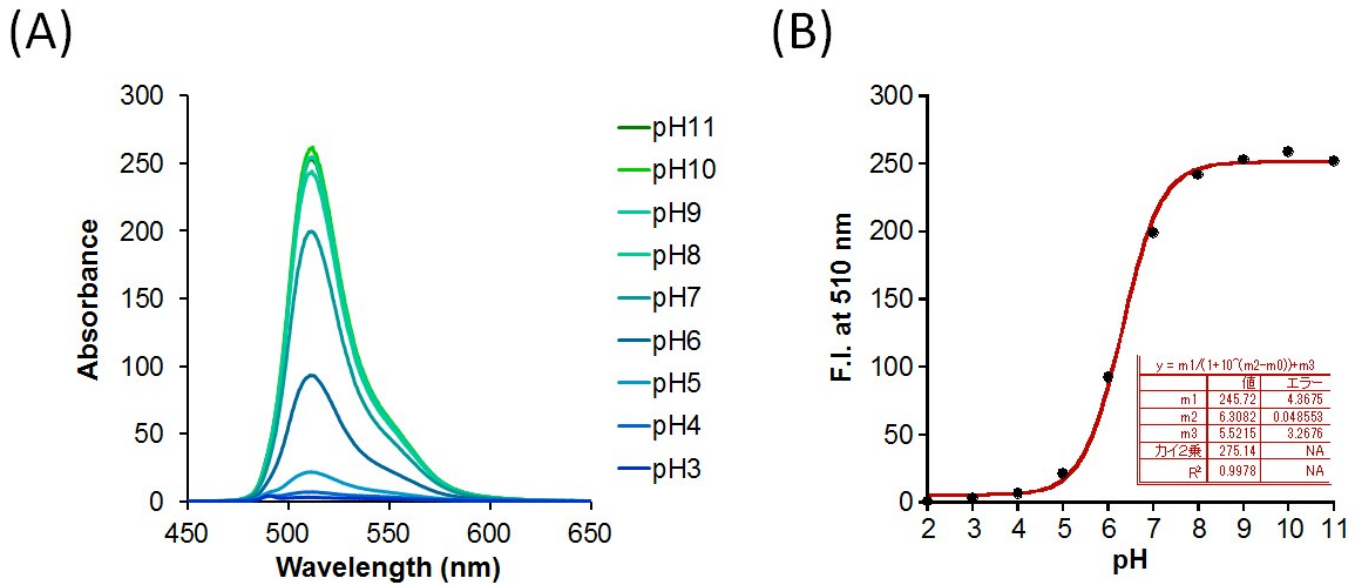



**Fig. S8. pH dependence of fluorescence after the reaction.**

(A) Fluorescence spectra after the reaction in 100 mM sodium phosphate at various pH values. Excitation was at 490 nm. (B) pH dependence of the fluorescence intensity at 510 nm. Data were fitted to the Henderson-Hasselbalch equation.

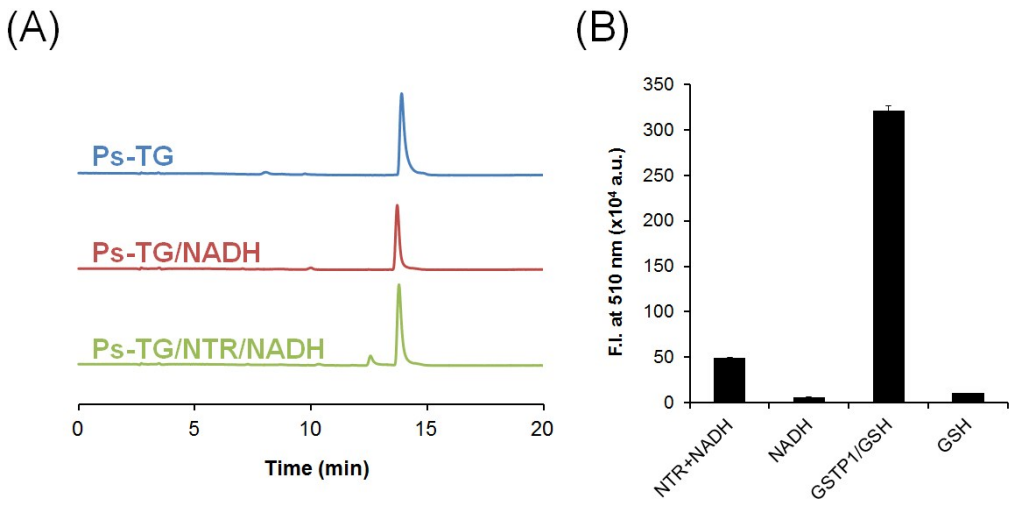



**Fig.S9. Sensitivity of reduction of Ps-TG catalyzed by nitroreductase.**

(A) HPLC chromatograms (detected at 490 nm) of Ps-TG and the reaction mixtures. Incubation was carried out in PBS containing 20 μM Ps-TG and 500 μM NADH in the absence or presence of nitroreductase (NTR, 1 μg/ml) at 25°C for 30 min. Eluents: A, 10 mM ammonium acetate; B, acetonitrile. Gradient conditions: A:B = 90:10 (0 min) → 50:95 (20 min). Absorbance at 490 nm. (B) The reaction was carried out in sodium phosphate buffer (100 mM, pH 7.4) containing 1 μM Ps-TG and 500 μM NADH in the absence or presence of NTR (1 μg/ml) at 30°C for 60 min. Ex/Em = 490 nm/510 nm. Data represent the mean ± S.D. of triplicate measurements.

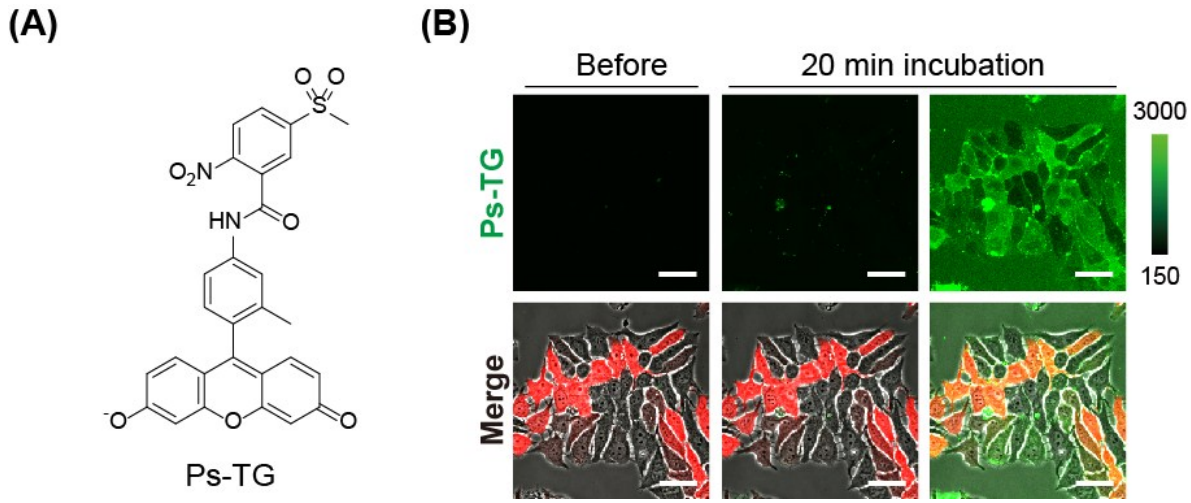



**Fig. S10 Fluorescence imaging of MCF7 cells expressing GSTP1 using Ps-TG.**

MCF7 cells transfected with pIRES2-DsRed Express2/3×FLAG-hGSTP1 were loaded with 2.5 μM Ps-TG (A) in HBSS for 20 min at room temperature and subjected to fluorescence imaging without washout of the probes. (B) Fluorescence and merged images with corresponding bright-field (BF) image before and after probe loading. In the images after 20 min incubation, the right panels show the same field as the left panels (sensitivity, 20%) with increased sensitivity (sensitivity, 50%). Images were taken with a ×60 objective. Bar: 40 μm.

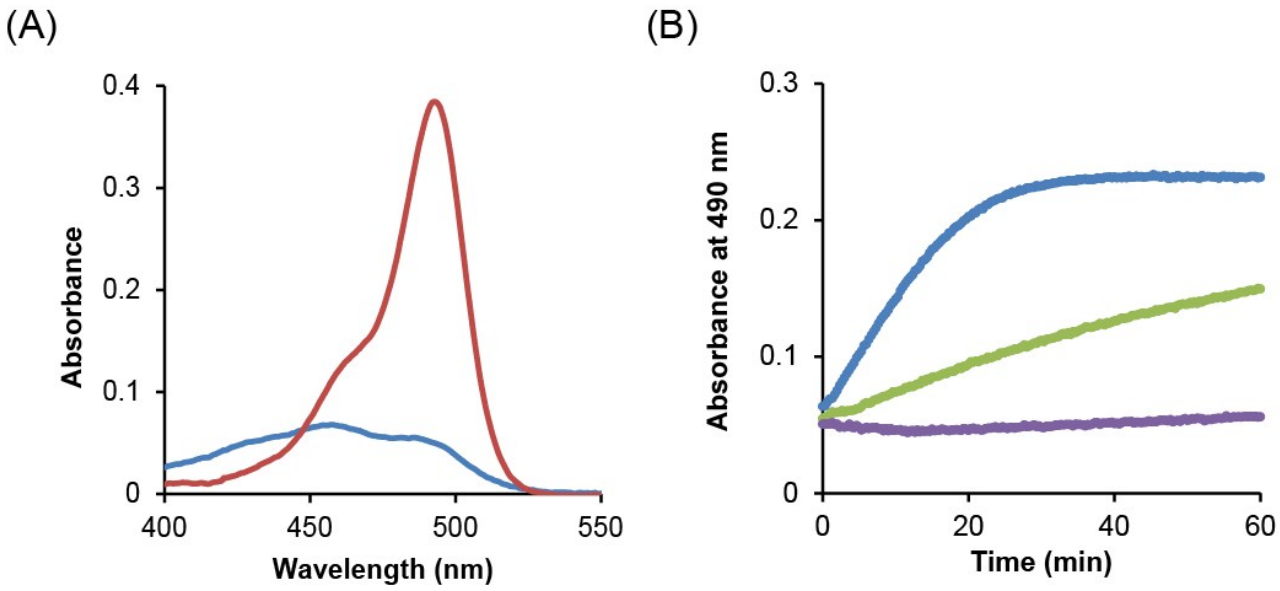
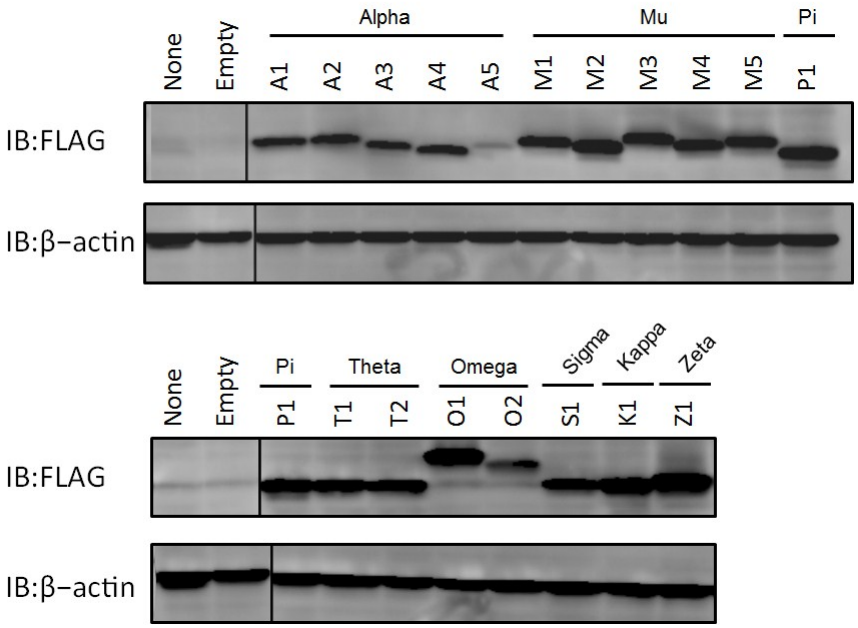



**Fig. S11 Conversion of Ps-TAc to Ps-TG due to esterase activity or spontaneous thiolysis by GSH.**

(A) Absorption spectra of 5 μM Ps-TAc untreated (blue line) and treated (red line) with porcine liver esterase (PLE). (B) Time course of absorbance at 490 nm. Ps-TAc was incubated with 10 μg/ml PLE (blue) or 1 mM GSH (green) in sodium phosphate buffer (100 mM, pH 7.4) at room temperature. Incubation of Ps-TAc without any additive is shown as a purple line.

(A)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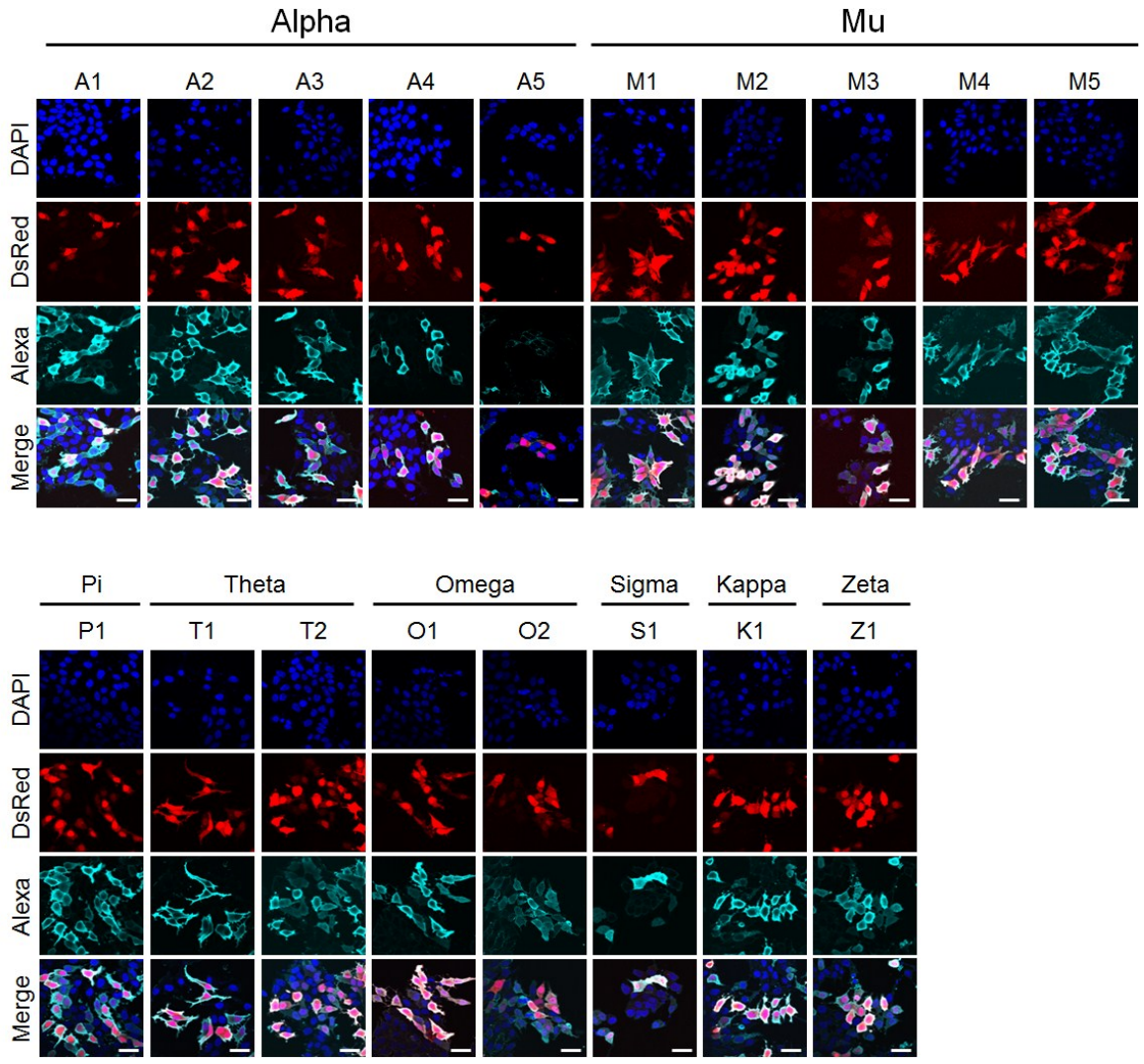
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**Fig. S12 Immunoblot and immunofluorescence analysis of 3×FLAG-GSTs in MCF7 cells transfected with pIRES2-DsRed Express2/3×FLAG-GST.**

(A) Immunoblot analysis of 3×FLAG-GSTs with anti-FLAG antibody. β-actin serves as loading control of cell lysate. (B) Immunofluorescence staining of exogenously expressed 3×FLAG-GSTs in fixed cells. Nuclei were stained with DAPI and transfected cells were identified by DsRed fluorescence. 3×FLAG-GSTs were detected with anti-FLAG mouse IgG and anti-mouse IgG-Alexa647 conjugate. Scale bar: 40 μm.

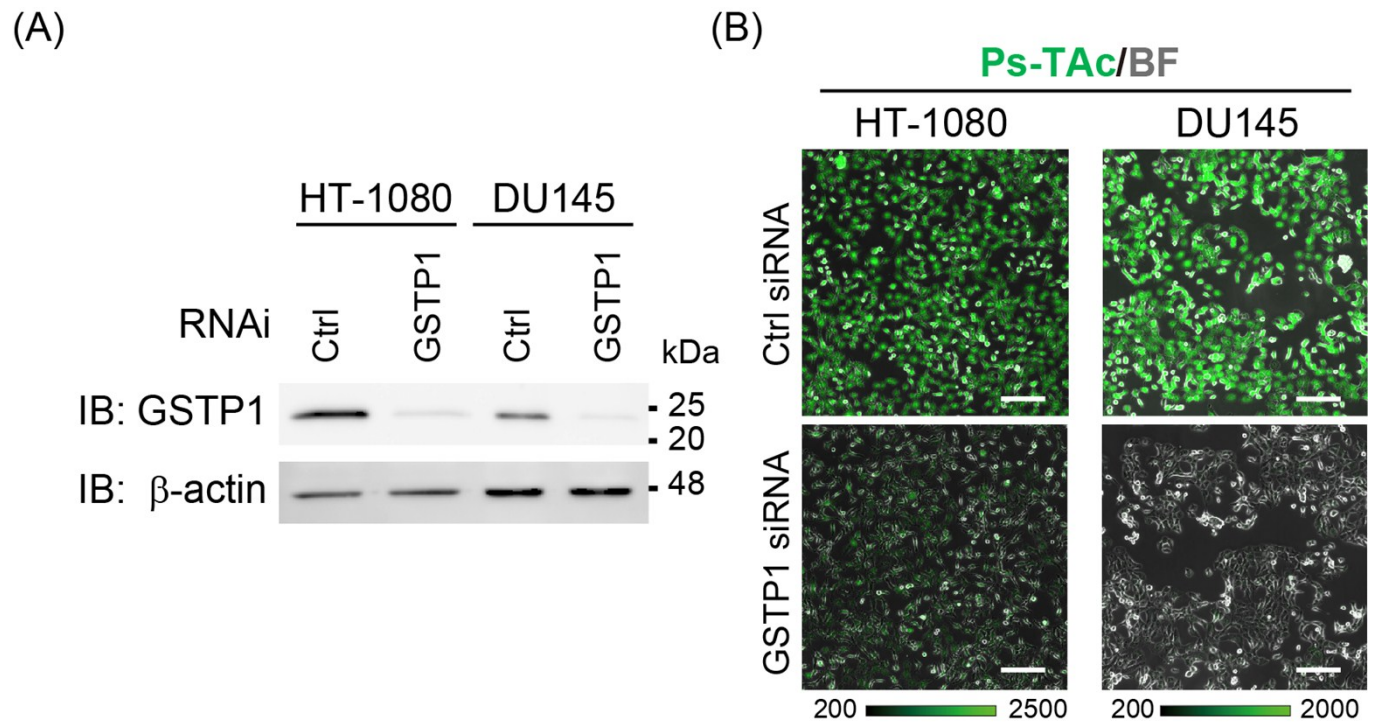



**Fig. S13 *GSTP1* RNAi abrogates fluorescence activation of Ps-TAc in HT-1080 and DU145 cells.**

(A) Immunoblot analysis of GSTP1 protein level. Cell lysates were prepared from each cell line subjected to control or *GSTP1* RNAi. β-actin serves as a loading control. (B) Merged fluorescence and bright-field images of the indicated cell lines subjected to control or *GSTP1* RNAi. Cells were loaded with 2.5 μM Ps-TAc for 15 min at room temperature. Images were taken with a ×10 objective. Bar: 200 μm.

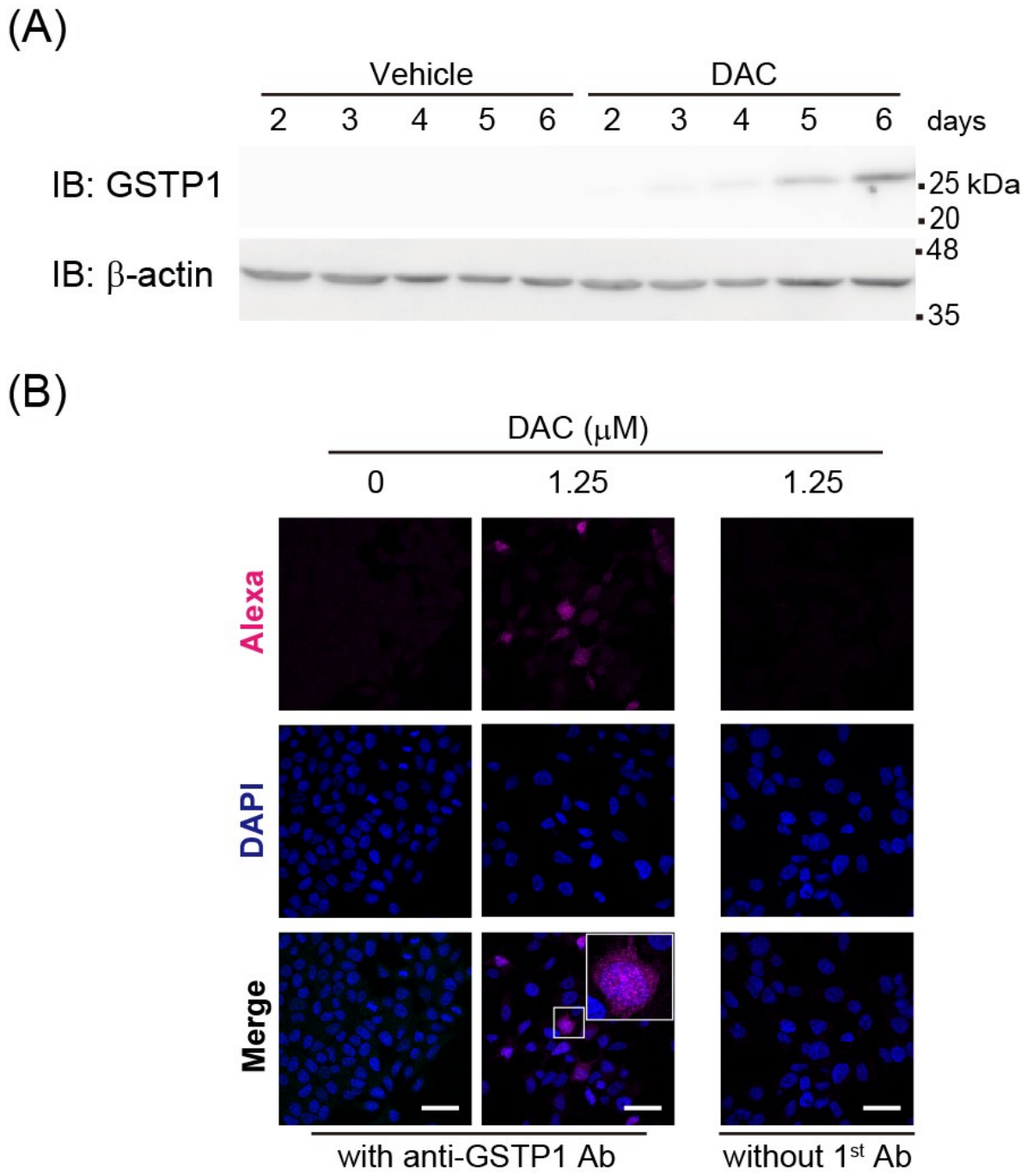



**Fig. S14 Immunoblot and immunofluorescence analysis of GSTP1 in MCF7 cells treated with DAC.**

(A) Immunoblot analysis of GSTP1 expression in MCF7 cells treated with DAC. β-actin was used as a loading control for lysate protein. (B) Confocal microscopy for GSTP1 and nuclei in MCF7 cells subjected to DAC treatment for six days. Nuclei were labeled with DAPI (blue), and GSTP1 was stained by indirect immunofluorescence with anti-GSTP1 mouse IgG and an Alexa647-conjugated secondary antibody (magenta), annotated here as "Alexa". "Without 1st Ab" serves as a negative control for immunostaining. Images were taken with a ×60 objective. Bar: 40 μm.

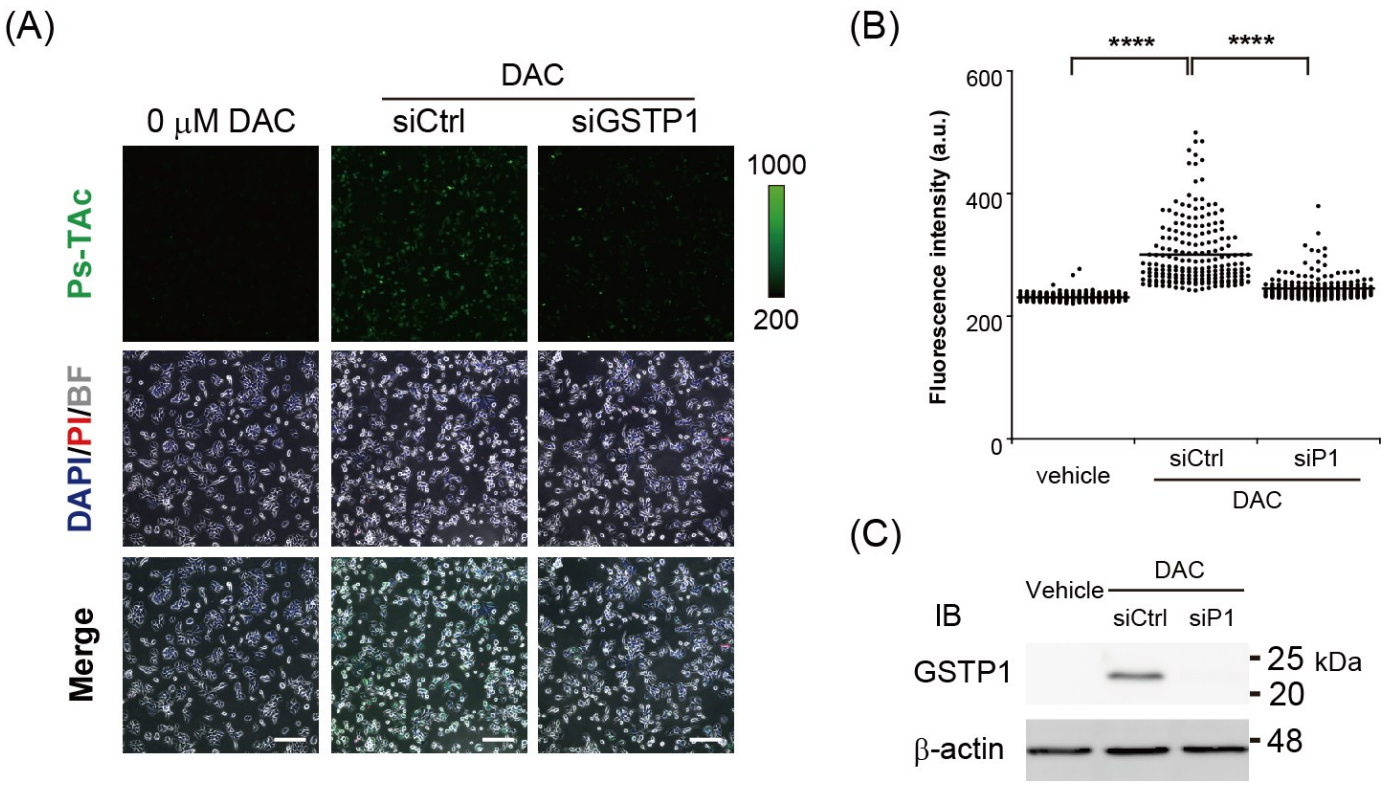



**Fig. S15 *GSTP1* RNAi abrogates fluorescence activation of Ps-TAc in MCF7 cells treated with DAC.**

(A) Representative fluorescence images alone and merged with bright-field (BF) images of MCF7 cells treated with 1.25 μM DAC for six days. Cells were transfected with control or GSTP1 siRNA on the fourth day of DAC treatment. MCF7 cells were incubated with 2.5 μM **Ps-TAc** and 10 μg/ml Hoechst 33258 in HBSS (0.1% DMSO as a cosolvent) at room temperature for 20 min. After washing out of the excess dye, fluorescence and BF images were acquired in HBSS containing 10 μg/ml PI. Imaging experiment was performed with a ×10 objective. Scale bars; 200 $\mu$m. Representative data from two independent experiments are shown. (B) Dot plot representation of green fluorescence intensity in MCF7 cells treated with DAC as shown in (A). Data from 200 cells each were represented by dot plots and statistically analysed. n = 200 cells, **** $p$ <0.0001 (two-tailed ANOVA with post-hoc Tukey) (C) Immunoblot analysis of GSTP1 expression in MCF7 cells treated with DAC with or without GSTP1 RNAi. β-actin was used as a loading control for lysate protein.

# 3. Supplementary Tables

**Table S1. Reduction potentials of chromogenic compounds.**

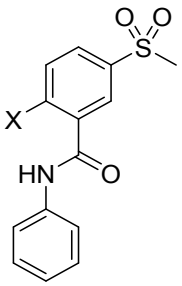
|  | Compound | X | Reduction potential (V vs. $Ag/Ag^+$) |
|---|---|---|---|
| | 5-Mesyl-2-nitrobenzanilide (**6**) | $NO_2$ | -1.15 |
| | 5-Mesyl-2-methylthiobenzanilide | $SCH_3$ | -2.46 |

**Table S2. Photochemical properties of Ps-TG before and after reaction with GSH.**

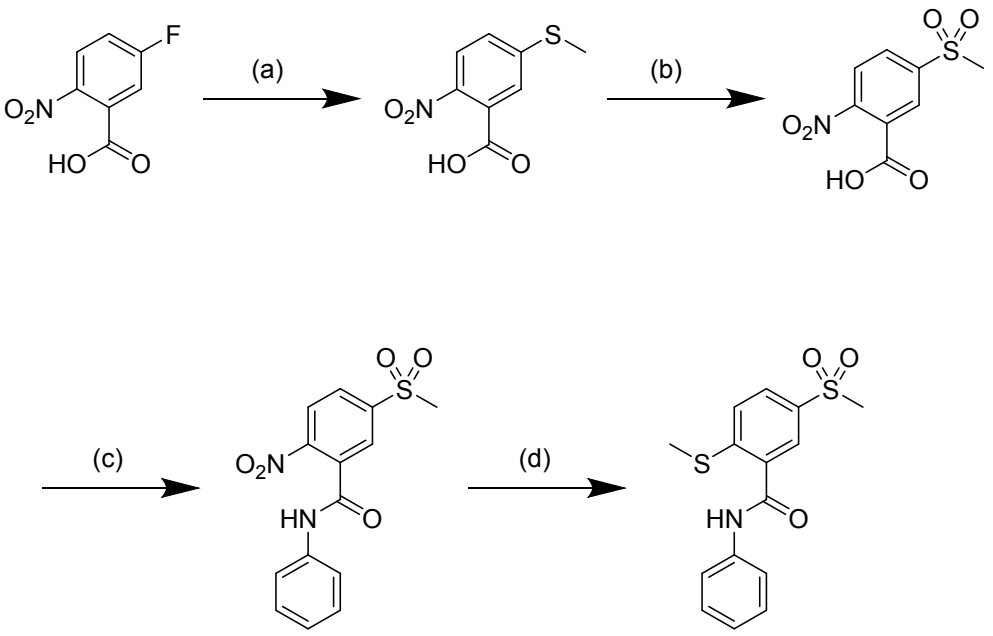
| | Reaction | Absorption maximum (nm) | Emission maximum (nm) | Fluorescence quantum efficiency |
|---|---|---|---|---|
| Ps-TG | Before | 493 | 510 | 0.008 |
| Ps-TG + GSTP1-1/GSH | After | 493 | 511 | 0.76 |

**Table S3. Kinetics parameters (mean ± SEM) of GSTs for Ps-TG in 100 mM sodium phosphate buffer (pH 7.4) at 25°C.**

| | Apparent $k_{cat}/K_M$ ($\times 10^4$ $M^{-1}s^{-1}$) [a] | | |
|---|---|---|---|
| | GSTA1-1 | GSTM1-1 | GSTP1-1 |
| Ps-TG | N.D.[b] | 0.94 ± 0.08 | 11.0 ± 0.3 |

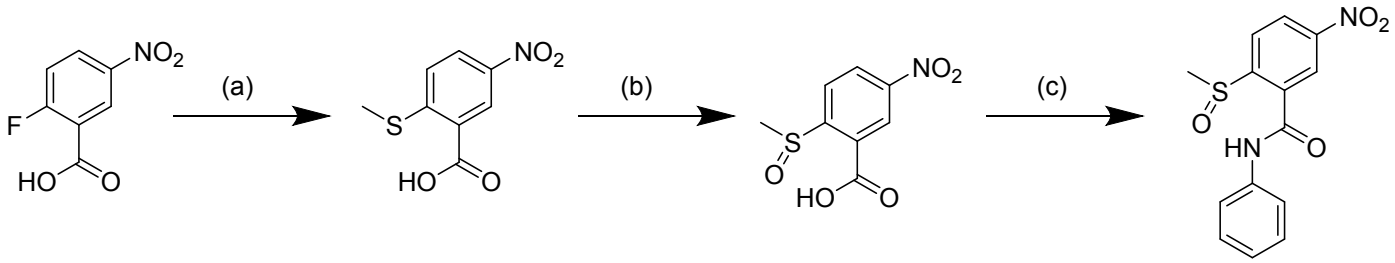
[a] Measured in sodium phosphate buffer (100 mM, pH 7.4) containing 1 mM GSH at 25°C. Apparent $k_{cat}/K_M$ value was directly determined using the Michaelis-Menten relationship at probe concentrations well below $K_M$ ([S] << $K_M$). [b] Not determined due to very low activity.

# 4. Organic Synthesis of Chemical Compounds



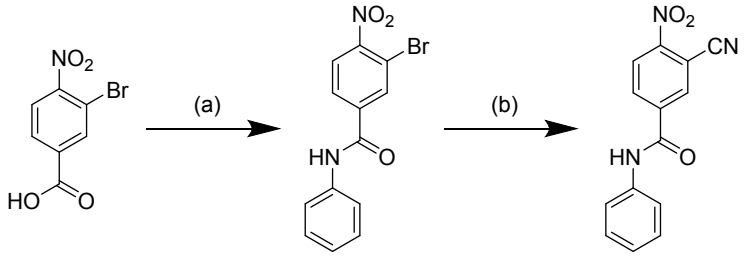

**Scheme S1. Synthesis of 5-(methylsulfonyl)-2-nitro-*N*-phenylbenzamide.**

(a) $NaSCH_3$, *i*-PrOH, r.t., yield 70%. (b) $H_2O_2$, $K_3PO_4$, $CH_3CN$, $H_2O$, r.t., yield 68%. (c) Aniline, EDC·HCl, $CH_3CN$, r.t., yield 58%. (d) $NaSCH_3$, DMSO, r.t., yield 73%.



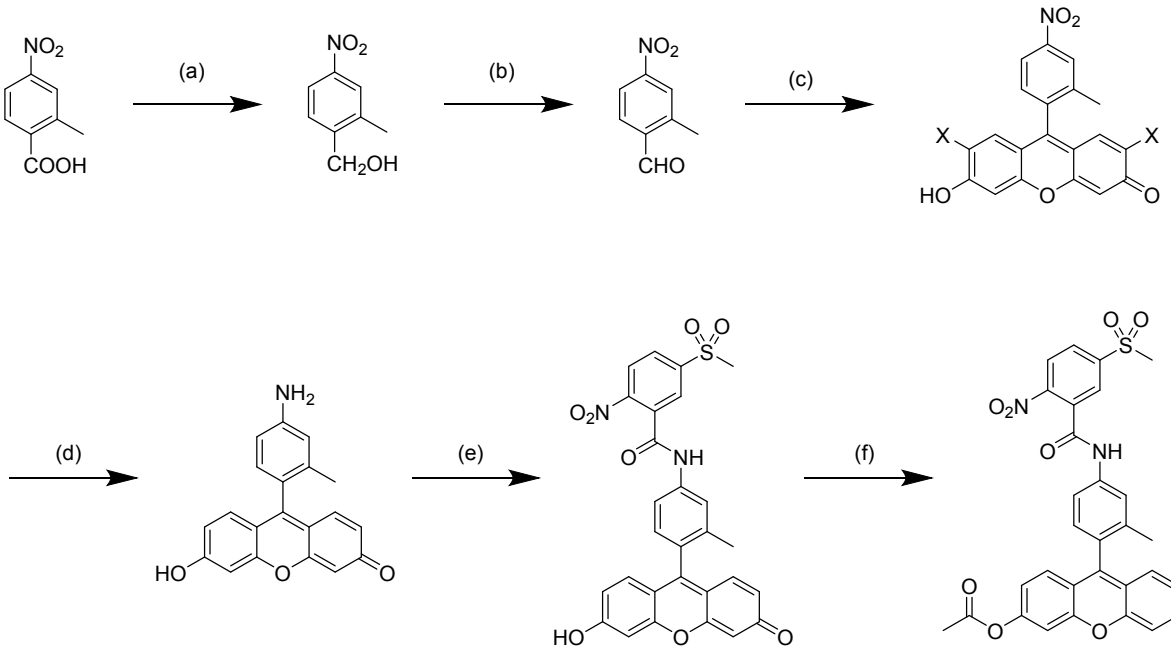

**Scheme S2. Synthesis of 2-(methylsulfinyl)-5-nitro-*N*-phenylbenzamide.**

(a) $NaSCH_3$, *i*-PrOH, r.t., yield 64%. (b) $H_2O_2$, $K_3PO_4$, $CH_3CN$, $H_2O$, r.t., yield 29%. (c) aniline, EDC·HCl, $CH_3CN$, r.t., yield 49%.



**Scheme S3. Synthesis of 3-cyano-4-nitro-*N*-phenylbenzamide.**

(a) (i) Thionyl chloride, DMF, reflux, (ii) aniline, $CH_2Cl_2$, r.t., yield 51%. (b) CuCN, NMP, 150°C, yield 77%.




**Scheme S4. Synthesis of Ps-TG and Ps-TAc.**

(a) $BH_3 \cdot S(CH_3)_2$, THF, 50°C, yield 81%. (b) Pyridinium chlorochromate (PCC), silica-gel, $CH_2Cl_2$, r.t., yield 91%. (c) Resorcinol, methanesulfonic acid, 90°C. (d) $NaSH \cdot nH_2O$, $Na_2S \cdot 9H_2O$, $H_2O$, reflux, yield 4%. (e) (i) 5-Mesyl-2-nitrobenzoic acid, EDC·HCl, $CH_3CN$. (ii) NaOH, $CH_3CN$, $H_2O$, r.t., yield 50%. (f) Acetyl chloride, DIEA, THF, r.t., yield 22%.

**Scheme S1**

**5-Methylthio-2-nitrobenzoic acid.** 5-Fluoro-2-nitrobenzoic acid (305 mg, 1.7 mmol) and sodium methane thiolate (620 mg, 8.8 mmol) were suspended into 10 ml isopropanol and stirred at room temperature for 30 min. The reaction was diluted with AcOEt and quenched with 1 N HCl, then partitioned between AcOEt and $H_2O$. The organic layer was washed twice with 1 N HCl and then with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was dissolved in hexane and precipitated by addition of AcOEt. The resulting precipitate was collected by filtration and dried under reduced pressure to afford the title compound as orange solid (246 mg, yield 70%).$^1$H-NMR (400 MHz, Acetone-$d_6$) δ 7.96 (1H, dd, $J$ = 7.5, 1.6 Hz), 7.58-7.56 (2H, m), 2.65 (3H, s). $^{13}$C-NMR (100 MHz, Acetone-$d_6$) δ 166.5, 148.5, 145.1, 130.0, 127.9, 125.9, 125.4, 14.7.

**5-Mesyl-2-nitrobenzoic acid.** To a mixture of 1 ml acetonitrile and 4 ml aqueous 30% $H_2O_2$ (4 ml, 39 mmol) containing 5-methylthio-2-nitrobenzoic acid (97 mg, 0.47 mmol), potassium phosphate was added until bubbling stopped. The reaction mixture was stirred for 20 min at ambient temperature. The solution was extracted with AcOEt, washed with 1 N HCl and then with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was dissolved in hexane and precipitated by addition of AcOEt. The resulting precipitate was collected by filtration and dried under reduced pressure to afford the title compound as off-white solid (79 mg, yield 68%). $^1$H-NMR (400 MHz, Acetone-$d_6$) δ 8.47 (1H, d, $J$ = 2.3 Hz), 8.38 (1H, dd, $J$ = 8.5, 2.1 Hz), 8.20 (1H, d, $J$ = 8.2 Hz), 3.32 (3H, s). $^{13}$C-NMR (100 MHz, Acetone-$d_6$) δ 164.3, 152.9, 145.5, 133.0, 130.7, 128.0, 125.9, 43.9. LRMS (EI$^+$): 246.

**5-Mesyl-2-nitrobenzanilide.** To 5 ml of acetonitrile containing 5-mesyl-2-nitrobenzoic acid (27 mg, 0.11 mmol) and EDC·HCl (50 mg, 0.26 mmol), aniline (15.2 mg, 0.17 mmol) was added at 4°C and stirred at room temperature for 15 min. The reaction mixture was diluted with AcOEt, washed three times with 0.1 N HCl, dried over anhydrous $MgSO_4$, filtered, and evaporated to dryness. The residue was dissolved in hexane and precipitated by addition of AcOEt. The resulting precipitate was collected by filtration and dried under reduced pressure to afford the title compound as slightly yellowish powder (21 mg, yield 58%).$^1$H-NMR (400 MHz, Acetone-$d_6$) δ 8.36-8.29 (3H, m), 7.74 (2H, d, $J$ = 8.2 Hz), 7.38 (2H, t, $J$ = 7.5 Hz), 7.16 (2H, t, $J$ = 7.3 Hz), 3.32 (3H, s) $^{13}$C-NMR (100 MHz, Acetone-$d_6$) δ 163.2, 151.1, 146.1, 139.5, 134.3, 130.9, 129.7, 129.1, 126.4, 125.3, 120.7, 43.8. HRMS (ESI-TOF) $m/z$ calcd for $C_{14}H_{12}N_2O_5SNa$ [M+Na] $^+$: 323.0367, found: 343.0367 (+0.0 mmu).

**5-Mesyl-2-methylthiobenzanilide.** To the suspension of 5-mesyl-2-nitrobenzanilide (46 mg, 0.14 mmol) in 5 ml DMSO, sodium methanethiolate (100 mg, 1.4 mmol) was added and stirred at ambient temperature for 10 min. The reaction mixture was diluted with AcOEt. The organic layer was washed twice with 1 N HCl, brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The material was further purified by silica-gel column chromatography (eluent: AcOEt/hexane = 1:1) to afford the title compound (34 mg, yield 73%). $^1$H-NMR (400 MHz, DMSO-$d_6$) δ 7.98 (1H, d, $J$ = 1.8 Hz), 7.95 (1H, dd, $J$ = 8.2, 1.8 Hz), 7.69 (2H, d, $J$ = 7.8 Hz), 7.62 (1H, d, $J$ = 8.7 Hz), 7.36 (2H, t, $J$ = 8.0 Hz), 7.12 (1H, t, $J$ = 7.3), 3.25 (3H, s), 2.52 (3H, s). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) δ 164.8, 145.5, 138.7, 136.1, 135.1, 128.8, 128.4, 125.9, 125.6, 124.0, 119.9, 43.6, 14.8. HRMS (ESI-TOF) $m/z$ calcd for $C_{15}H_{15}NO_3S_2Na$ $[M+Na]^+$: 344.0391, found: 344.0393 (+0.2 mmu).

**Scheme S2**

**2-(Methylthio)-5-nitrobenzoic acid.** 2-Fluoro-5-nitrobenzoic acid (235 mg, 1.3 mmol) and sodium methanethiolate (450 mg, 6.4 mmol) were suspended into 6 ml isopropanol and stirred at room temperature for 1 h. The reaction was quenched with 1 N HCl and diluted with AcOEt. The organic layer was washed twice with 1 N HCl, brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was dissolved in hexane and precipitated by addition of AcOEt. The resulting precipitate was collected by filtration and dried under reduced pressure to afford the title compound as slightly yellowish solid (173 mg, yield 64%). $^1$H-NMR (400 MHz, Acetone-$d_6$) δ 7.96 (1H, dd, $J$ = 7.5, 1.6 Hz), 7.58-7.56 (2H, m), 2.65 (3H, s). $^{13}$C-NMR (100 MHz, Acetone-$d_6$) δ 166.5, 148.5, 145.1, 130.0, 127.9, 125.9, 125.4, 14.7.

**2-(Methylsulfinyl)-5-nitrobenzoic acid.** To a suspension containing 2-methylthio-5-nitrobenzoic acid (97 mg, 0.47 mmol) in a mixture of 1 ml acetonitrile and aqueous 30% $H_2O_2$ solution (4 ml, 39 mmol), potassium phosphate was added until bubbling stopped; stirring at room temperature was continued for 30 min. The reaction mixture was diluted with AcOEt. The organic layer was washed twice with 1 N HCl, brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was dissolved in hexane and precipitated by addition of AcOEt. The resulting precipitate was collected by filtration and dried under reduced pressure to afford the title compound (33 mg, yield 29%). $^1$H-NMR (400 MHz, DMSO-$d_6$) δ 8.69 (1H, dd, $J$ = 8.5, 2.5 Hz), 8.65 (1H, d, $J$ = 2.7 Hz), 8.35 (1H, d, $J$ = 8.7 Hz), 2.82 (3H, s). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) δ 165.0, 157.5, 148.6, 129.1, 127.9, 125.8, 125.2, 43.7.

**2-(Methylsulfinyl)-5-nitrobenzanilide.** To 4 ml acetonitrile containing 2-methylsulfinyl-5-nitrobenzoic acid (16.9 mg, 0.069 mmol) and EDC·HCl (39.9 mg, 0.21 mmol), aniline (9.3 mg, 0.10 mmol) was added on ice. The reaction mixture was stirred at room temperature for 10 min and diluted with AcOEt. The organic layer was washed twice with 0.1 N HCl, brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was dissolved in hexane and precipitated by addition of AcOEt. Resulting precipitate was collected by filtration and dried under reduced pressure to afford the title compound (10.8 mg, yield 49%). $^1$H-NMR (400 MHz, DMSO-$d_6$) δ 10.8 (1H, s), 8.56 (1H, dd, $J$ = 8.2, 2.3 Hz), 8.53 (1H, d, $J$ = 1.8 Hz), 8.29 (1H, d, $J$ = 8.2 Hz), 7.72-7.63 (2H, m), 7.43-7.32 (2H, m), 7.18-7.11 (1H, m), 3.48 (3H, s). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) δ 163.9, 150.0, 143.0, 138.7, 138.5, 131.7, 128.8, 125.2, 124.3, 123.7, 120.1, 44.8. HRMS (ESI-TOF) $m/z$ calcd for $C_{14}H_{12}N_2O_4SNa$ $[M+Na]^+$: 327.0415, found: 327.0423(+0.8 mmu).

**Scheme S3.**

**3-Bromo-4-nitrobenzanilide.** 3-Bromo-4-nitrobenzoic acid (2.18 g, 8.9 mmol) and two drops of DMF were suspended in 15 ml thionyl chloride and refluxed for 3 h. Toluene was added to the mixture for azeotroping and excess thionyl chloride was evaporated. The residue was suspended in 10 ml $CH_2Cl_2$, then 5 ml $CH_2Cl_2$ containing aniline (1.25 g, 14 mmol) and triethylamine (2.8 g, 28 mmol) were added dropwise on ice and the mixture was stirred at room temperature for 1 h. After addition of 1 N HCl, the reaction mixture was diluted with $CH_2Cl_2$. The organic layer was washed three times with 1 N HCl, brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. Recrystallization from $CH_2Cl_2$/hexane gave the title compound as slightly yellowish solid (1.47 g, yield 51%). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.5 (1H, s), 8.42 (1H, d, $J$ = 1.8 Hz), 8.17 (1H, d, $J$ = 8.2 Hz), 8.12 (1H, dd, $J$ = 8.2, 1.8 Hz), 7.79-7.72 (2H, m), 7.43-7.33 (2H, m), 7.19-7.10 (1H, m) $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 162.5, 151.1, 139.3, 138.5, 133.6, 128.7, 128.6, 125.5, 124.3, 120.5, 113.0. HRMS (ESI-TOF) $m/z$ calcd for $C_{13}H_{10}BrN_2O_3$ $[M+H]^+$: 320.9875, found: 320.9865(-1.0 mmu).

**3-Cyano-4-nitrobenzanilide.** A solution of 10 ml *N*-methylpyrolidone (NMP) containing 3-bromo-4-nitrobenzanilide (805 mg, 2.5 mmol) and sodium cyanide (291 mg, 5.9 mmol) was heated and stirred at 150°C for 3.5 h. After cooling to room temperature, an excess amount of water was added, and the resulting precipitate was collected by filtration. The crude product was purified by silica-gel column chromatography (eluent: $CH_2Cl_2$) to give the title compound as slightly yellowish

solid (518 mg, 77%). $^{1}$H-NMR (400 MHz, DMSO-$d_6$) δ 10.66 (1H, s), 8.66 (1H, d, $J$ = 1.8 Hz), 8.52 (1H, d, $J$ = 8.8 Hz), 8.42 (1H, dd, $J$ = 8.8, 1.8 Hz), 7.80-7.72, (2H, m), 7.44-7.35 (2H, m), 7.20-7.10 (1H, m). $^{13}$C-NMR (100 MHz, Acetone-$d_6$) δ 162.1, 149.5, 140.0, 138.4, 134.6, 133.7, 128.8, 126.1, 124.4, 120.4, 115.3, 107.1. HRMS (ESI-TOF) $m/z$ calcd for $C_{14}H_{10}N_3O_3$ $[M+H]^+$: 268.0722, found: 268.0714 (-0.8 mmu).

**2,5-Dinitrobenzanilide.** To 1 ml acetonitrile containing 2,5-dinitrobenzoic acid (43 mg, 0.20 mmol) and EDC·HCl (81 mg, 0.42 mmol), aniline (23 mg, 0.25 mmol) was added on ice and the mixture was stirred at room temperature for 1 h. The reaction was quenched with 1 N HCl and the reaction mixture was diluted with AcOEt. The organic layer was washed three times with 1 N HCl, saturated $NaHCO_3$ aqueous solution, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was dissolved in hexane and precipitated by addition of AcOEt. Resulting precipitate was collected by filtration and dried under reduced pressure to afford the title compound as slightly yellowish solid (27 mg, yield 45%). $^{1}$H-NMR (400 MHz, DMSO-$d_6$) δ 10.89 (1H, s), 8.62 (1H, d, $J$ = 2.3 Hz), 8.55 (1H, m, $J$ = 8.7, 2.3 Hz), 8.36 (1H, d, $J$ = 9.1 Hz), 7.66-7.64 (2H, m), 7.40-7.36 (2H, m), 7.17-7.15 (1H, m). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) δ 161.7, 150.1, 149.3, 138.4, 133.2, 128.9, 126.3, 126.2, 124.6, 124.4, 119.9.

**4-Nitro-3-trifluoromethylbenzanilide.** 3-Trifluoro-4-nitrobenzoic acid (785 mg, 3.3 mmol) and two drops of DMF were suspended in thionyl chloride (3 ml) and the mixture was stirred at room temperature for 2 h. After cooling to ambient temperature, toluene was added to the reaction mixture for azeotroping and excess thionyl chloride was removed. The residue was suspended in $CH_2Cl_2$ (2 ml), then 5 ml $CH_2Cl_2$ containing aniline (307 mg, 3.3 mmol) and triethylamine (670 mg, 6.6 mmol) were added on ice and stirred at room temperature for 30 min. The reaction was quenched with 1 N HCl and the mixture was diluted with $CH_2Cl_2$. The organic layer was washed three times with 1 N HCl, brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. Recrystallization from AcOEt/hexane gave the title compound as slightly yellowish solid (613 mg, yield 59%). $^{1}$H-NMR (400 MHz, DMSO-$d_6$) δ 10.67 (1H, s), 8.50 (1H, m, 8.50-8.49), 8.46 (1H, dd, $J$ = 8.2, 1.8 Hz), 8.33 (1H, d, $J$ = 8.2 Hz), 7.79-7.71 (2H, m), 7.44-7.34 (2H, m), 7.20-7.11 (1H, m). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) δ 163.0, 149.2, 139.5, 139.0, 134.4, 129.3, 127.9, 126.3, 124.9, 123.8, 122.0, 121.7, 121.1. HRMS (ESI-TOF) $m/z$ calcd for $C_{14}H_{10}F_3N_2O_3$ $[M+H]^+$: 311.0644, found: 311.0636 (-0.8 mmu).

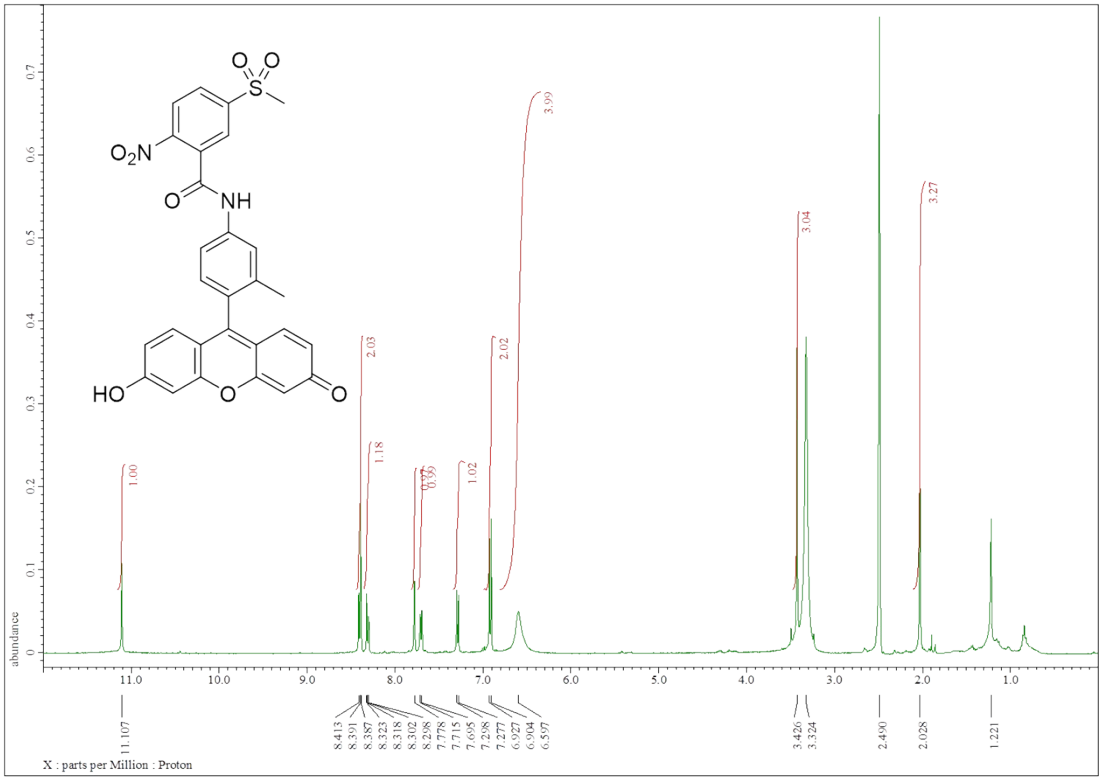
**Scheme S4.**

**Ps-TG.** A suspension of 2-methyl-4-amino-TokyoGreen (2-Me-4-$NH_2$-TG) (10.2 mg, 0.032 mmol, synthesized as previously described[3]), 5-mesyl-2-nitrobenzoic acid (15.6 mg, 0.064 mmol), and EDC·HCl (12.5 mg, 0.066 mmol) in 1 ml acetonitrile was stirred at room temperature for 1 h. The reaction mixture was diluted with AcOEt and the organic layer was washed three times with 1 N HCl, brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. To a stirred solution of the crude material in acetonitrile (2 ml), 1 N NaOH (0.2 ml) was added on ice and stirred at room temperature for 10 min. The reaction mixture was neutralized with 200 mM sodium phosphate (pH 7.4). The crude material was purified by semi-preparative HPLC (Eluent: A:B = 90:10 → 10:90 (20 min), A: 10 mM ammonium acetate, B: acetonitrile, flow rate: 9 ml/min). After evaporation of acetonitrile from the collected fraction under reduced pressure, the residue was acidified with 0.1 N HCl and diluted with AcOEt. The organic layer was washed three times with 0.1 N HCl, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo to afford the title compound (8.5 mg, 0.016 mmol, yield 50%). $^1$H-NMR (400 MHz, DMSO-$d_6$) δ 7.56 (2H, m), 7.48 (1H, dd, $J$ = 1.6, 8.8 Hz), 6.95 (1H, s), 6.86 (1H, m), 6.47 (1H, d, $J$ = 8.4 Hz), 6.08 (2H, d, $J$ = 9.2 Hz), 5.77 (4H, br), 2.59 (3H, s), 1.20 (3H, s). $^{13}$C-NMR (400 MHz, DMSO-$d_6$) δ 163.3, 159.1, 150.1, 145.2, 140.8, 137.2, 133.2, 130.6, 128.8, 127.6, 126.3, 121.8, 121.3, 117.8, 116.8, 103.1, 60.3, 21.6, 21.3, 14.6. HRMS (ESI-TOF) $m/z$ calcd for $C_{28}H_{21}N_2O_8S$ $[M+H]^+$: 545.1019, found: 545.1019 (+ 0.0 mmu).

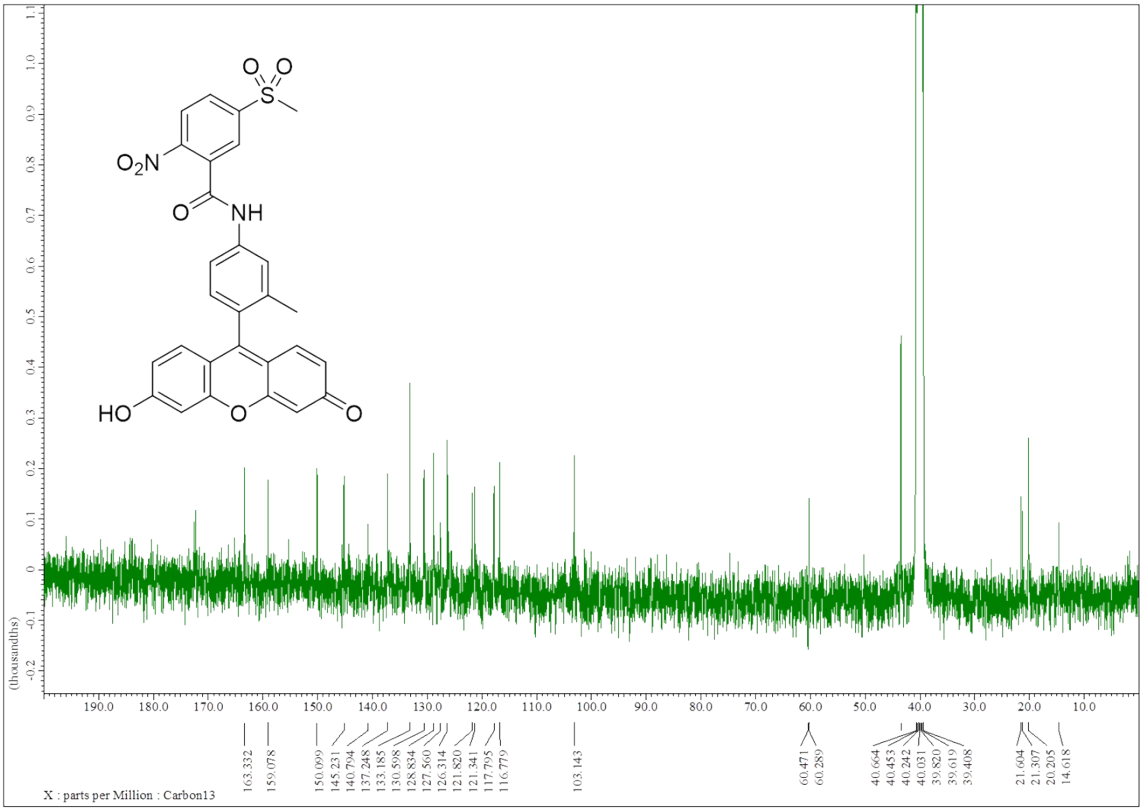
**Ps-TAc.** To a solution of Ps-TG (2.1 mg, 0.0039 mmol) and acetyl chloride (0.63 mg, 0.008 mmol) in 2 ml tetrahydrofuran, diisopropylethylamine (1.0 mg, 0.008 mmol) was added on ice. The reaction mixture was stirred at r.t. for 2 h and then partitioned between 1 N HCl and AcOEt. The organic layer was washed three times with 1 N HCl, brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by silica-gel column chromatography (AcOEt/ hexane = 2:1) to afford the title compound (0.5 mg, 0.00085 mmol, yield 22%). $^1$H-NMR (400 MHz, DMSO-$d_6$) δ 8.40 (1H, d, $J$ = 8.7 Hz), 8.39 (1H, d, $J$ = 1.8,), 8.31 (1H, dd), 7.80 (1H, s, $J$ = 8.2 Hz), 7.71 (2H, m), 7.50 (1H, d, $J$ = 1.8 Hz), 7.33 (1H, d, $J$ = 8.2 Hz), 7.12 (1H, d, $J$ = 4.1 Hz), 6.98 (1H, d, $J$ = 9.6 Hz), 6.49 (1H, dd, $J$ = 9.6, 1.8 Hz), 6.27 (1H, d, $J$ = 2.3 Hz), 3.42 (3H, s), 2.32 (3H, s), 2.06 (3H, s). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) δ 184.4, 168.7, 162.7, 158.1, 154.2, 152.4, 149.5, 147.5, 144.8, 139.6, 136.8, 132.9, 130.8, 130.5, 130.8, 130.1, 13.0, 129.1, 128.2, 127.6, 125.8, 121.2, 119.9, 119.2, 118.1, 117.5, 110.5, 105.2. HRMS (ESI-TOF) $m/z$ calcd for $C_{30}H_{23}N_2O_9S$ $[M+H]^+$: 587.1124, found: 587.1128 (+0.4 mmu).

1. Hirabayashi, K.; Hanaoka, K.; Takayanagi, T.; Toki, Y.; Egawa, T.; Kamiya, M.; Komatsu, T.; Ueno, T.; Terai, T.; Yoshida, K.; Uchiyama, M.; Nagano, T.; Urano, Y. *Anal. Chem.* **2015**, *87* (17), 9061.

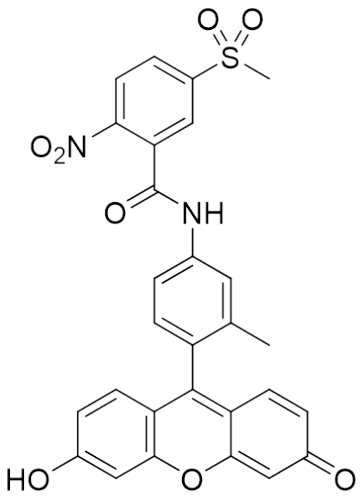
# 5. $^{1}$H and $^{13}$C NMR, and HRMS Spectra of Ps-TG and Ps-TAc




$^{1}$H-NMR (400 MHz, DMSO-$d_6$) of Ps-TG



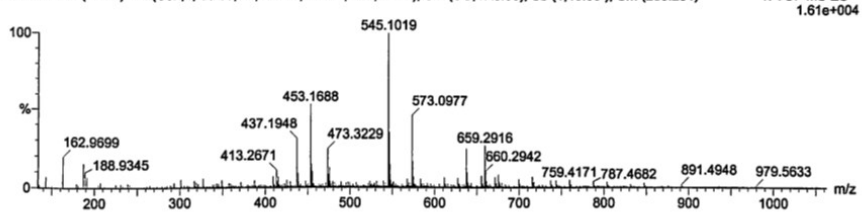

$^{13}$C-NMR (100 MHz, DMSO-$d_6$) of Ps-TG




**Elemental Composition Report** Page 1

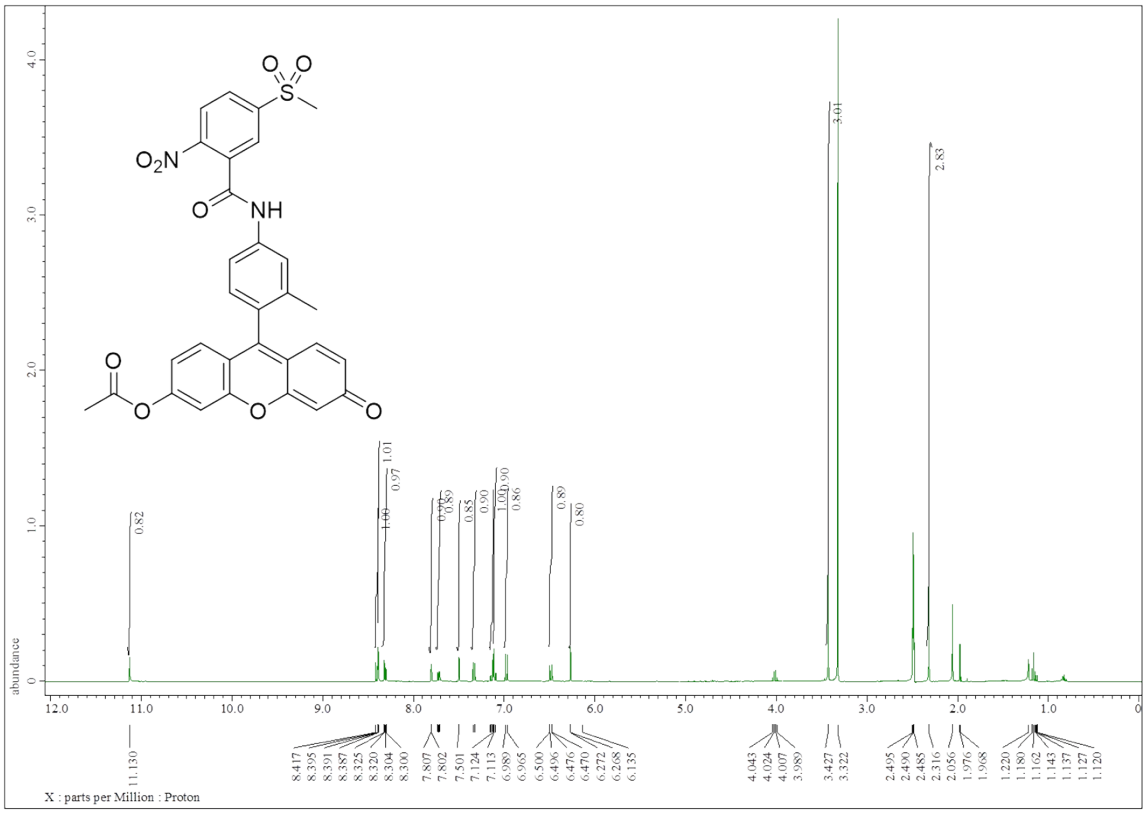
**Single Mass Analysis**
Tolerance = 20.0 PPM / DBE: min = -1.5, max = 300.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
5 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 1-300 H: 1-1000 N: 2-2 O: 8-8 S: 1-1
Ps-TG
M-9583 266 (3.157) AM (Cen,4, 80.00, Ar,8500.0,556.28,0.00,LS 10); Sm (SG, 2x3.00); Sb (1,40.00 ); Cm (260:294) 1: TOF MS ES+ 1.61e+004

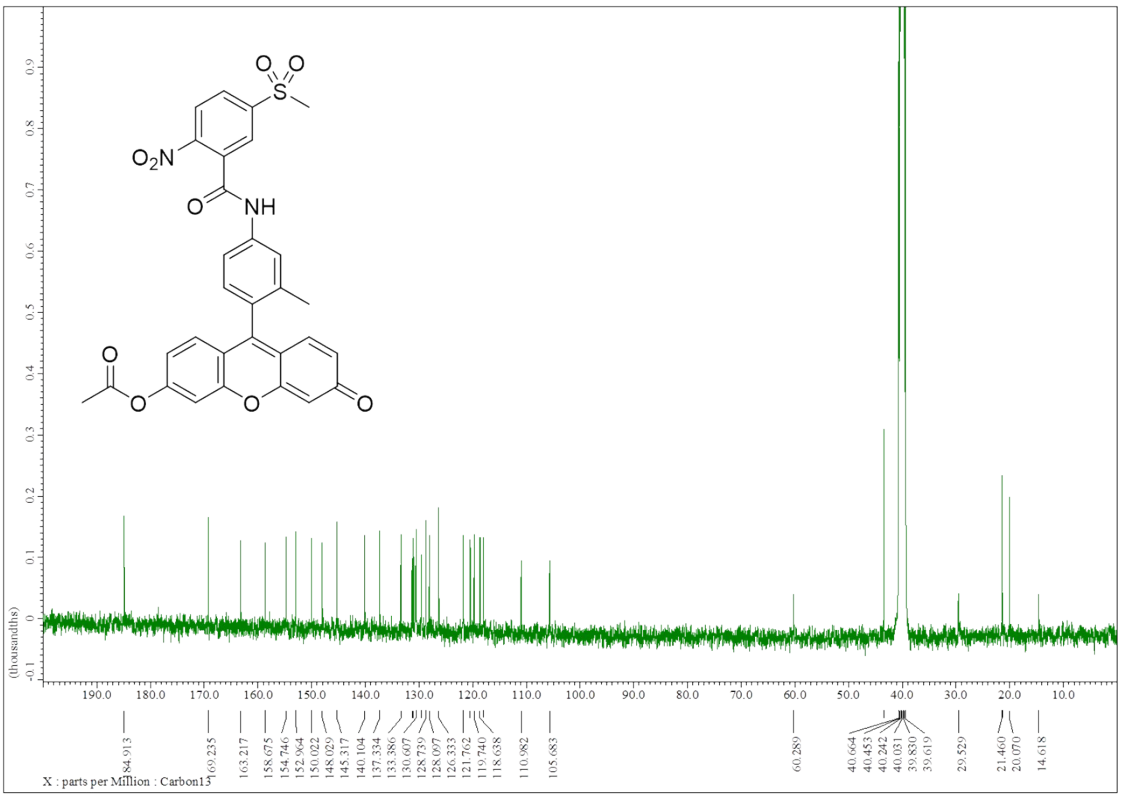



| | | | | | | |
|---|---|---|---|---|---|---|
| Minimum: | | | | -1.5 | | |
| Maximum: | | 100.0 | 20.0 | 300.0 | | |
| Mass | Calc. Mass | mDa | PPM | DBE | i-FIT | Formula |
| 545.1019 | 545.1019 | 0.0 | 0.0 | 19.5 | 61.9 | C28 H21 N2 O8 S |

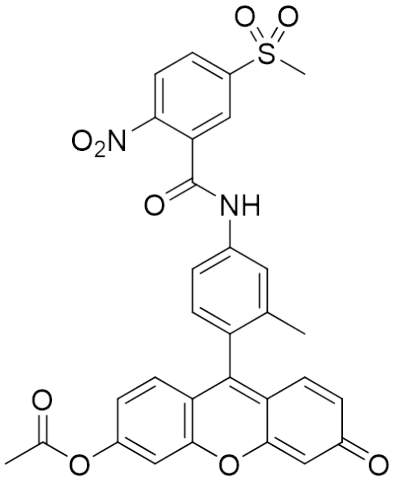
HRMS of Ps-TG




$^{1}$H-NMR (400 MHz, DMSO-$d_6$) of Ps-TAc



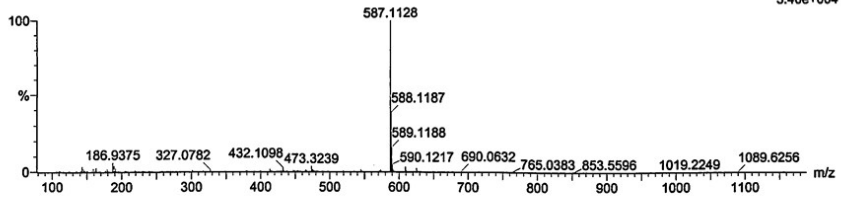

$^{13}$C-NMR (100 MHz, DMSO-$d_6$) of Ps-TAc




**Elemental Composition Report** **Page 1**

**Single Mass Analysis**
Tolerance = 20.0 PPM / DBE: min = -1.5, max = 300.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
5 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 1-300 H: 1-1000 N: 2-2 O: 9-9 S: 1-1
Ps-TAc
M-9588 306 (3.633) AM (Cen,4, 80.00, Ar,8500.0,556.28,0.00,LS 10); Sm (SG, 2x3.00); Sb (1,40.00 ); Cm (303:336) 1: TOF MS ES+ 3.40e+004




| | | | | | | |
|---|---|---|---|---|---|---|
| Minimum: | | | | -1.5 | | |
| Maximum: | | 100.0 | 20.0 | 300.0 | | |
| Mass | Calc. Mass | mDa | PPM | DBE | i-FIT | Formula |
| 587.1128 | 587.1124 | 0.4 | 0.7 | 20.5 | 137.0 | C30 H23 N2 O9 S |

HRMS of Ps-TAc