Electronic Supplementary Information (ESI)

for

A Phenacrylate Scaffold for Tunable Thiol Activation and Release

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General

All reactions were conducted under a nitrogen atmosphere. All the chemicals were purchased from commercial sources and used as received unless stated otherwise. Solvents for reactions and petroleum ether and ethyl acetate (EtOAc) for chromatography were used as received. Column chromatography was performed on Rankem silica gel (60–120 mesh). $^1$H and $^{13}$C spectra were recorded on a JEOL 400 MHz (or 100 MHz for $^{13}$C) or a Bruker 500 MHz spectrometer using either residual solvent signals as an internal standard (CHCl$_3$ $\delta$$_H$, 7.26 ppm, $\delta$$_C$ 77.36 ppm). Chemical shifts (δ) are reported in ppm and coupling constants (J) in Hz. The following abbreviations are used: m (multiplet), s (singlet), d (doublet), t (triplet), dd (doublet of doublet). High-resolution mass spectra (HRMS) were obtained from HRMS-ESI-Q-Time of Flight LC/MS. FT-IR spectra were recorded using NICOLET 6700 FT-IR spectrometer as KBr disc. High performance liquid chromatography (HPLC) was performed on an Agilent model with Zorbax SB C-18 reversed phase column (250 mm $\times$ 4.6 mm, 5 $\mu$m). Fluorescence measurements were carried out using a SPEX Fluorolog HORIBA JOBIN VYON fluorescence spectrophotometer.

Experimental section:

Compounds 1a-1f,$^{23}$2a-2f,$^{21}$3g$^{26-27}$ and 11$^{28}$ were synthesized using reported procedures.

General procedure for synthesis of 2a-2f. To a solution of 1a-1f (1 equiv) in dry dichloromethane (DCM), phosphorous tribromide (PBr$_3$) was added drop by drop at 0 °C under N$_2$ atmosphere. The mixture was stirred at room temperature for 2 to 4 h. After complete consumption of the starting material (monitored by TLC), the reaction was quenched using water, extracted with multiple portions of chloroform (5 $\times$ 10 mL) and collected organic layer was dried over sodium sulfate (10 g). The solvent was separated and evaporated under reduced pressure to obtain the crude material. This was purified using silica gel column chromatography (60-120 mesh) using ethyl acetate (10 - 20%): hexane as an eluant system.
General procedure for synthesis of 4a-9a, 8b-8g. To a solution of 2a-2f in dry acetonitrile (ACN), cesium carbonate (Cs$_2$CO$_3$) and 3a-3g was added under N$_2$ atmosphere. The reaction mixture was stirred at room temperature for 2 to 4 h. After complete consumption of the starting material (monitored by TLC), reaction was quenched using water, extracted with multiple portions of chloroform (5 × 10 mL) and collected organic layer was dried over sodium sulfate (10 g) before evaporating the solvents under reduced pressure to obtain the crude material. This was purified using silica gel column chromatography (60-120 mesh) using ethyl acetate (5 - 15%): hexane as an eluant system.

(Z)-methyl 3-(4-cyanophenyl)-2-((phenylthio)methyl)acrylate (3h). To a solution of 2c (150 mg, 0.5354 mmol) in dry acetonitrile (5 mL) cesium carbonate (0.8032 mmol) and thiophenol (0.8032 mmol) were added under N$_2$ atmosphere. The reaction mixture was stirred at room temperature for 4.5 h, and complete consumption of the starting material was confirmed by TLC. The reaction mixture was diluted with water (100 mL), the aqueous layer was extracted with chloroform (5 × 10 mL), and the combined organic layer was dried over Na$_2$SO$_4$. The volatiles were evaporated under reduced pressure to obtain the crude product. The crude product was purified by silica gel column chromatography (60 – 120 mesh) using ethyl acetate hexane (2-10%) as an eluant system to obtain 3h as a colorless solid (161 mg, 98%): mp 54 – 56 °C; FT-IR (v$_{max}$, cm$^{-1}$): 2212, 1707, 1601, 1430, 1272, 1181, 1077; $^1$H NMR (400 MHz, CDCl$_3$): δ 7.65 (s, 1H), 7.56 – 7.59 (m, 2H), 7.31 – 7.35 (m, 4H), 7.20 – 7.24 (m, 3H), 3.92 (s, 2H), 3.81 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 167.0, 139.3, 138.7, 134.8, 132.3, 132.0, 131.1, 129.7, 129.0, 127.5, 118.5, 112.2, 52.6, 32.4; HRMS (ESI) for [C$_{18}$H$_{15}$NO$_2$S+H]$^+$: calcd., 310.0902. Found: 310.0902.

(E)-methyl 3-phenyl-2-((p-tolyloxy)methyl)acrylate (4a). Starting from 2a (100 mg, 0.3919 mmol), compound 4a was isolated as a colorless oil (102 mg, 92%); FT-IR (v$_{max}$, cm$^{-1}$): 1715, 1508, 1441, 1215, 1114, 1015; $^1$H NMR (400 MHz, CDCl$_3$): δ 8.05 (s, 1H), 7.48 – 7.50 (m, 2H), 7.36 – 7.38 (m,
3H), 7.11 (d, J = 8.4 Hz, 2H), 6.88 (dd, J = 2.0, 6.6 Hz, 2H), 4.80 (s, 2H), 3.85 (s, 3H), 2.31 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 168.1, 156.7, 146.0, 134.8, 130.7, 130.3, 130.1, 129.9, 129.1, 127.7, 115.2, 63.2, 52.7, 20.9; HRMS (ESI) for [C$_{18}$H$_{18}$O$_3$ + Na$^+$]: calcd., 305.1154. Found: 305.1153.

(E)-methyl 2-((2-bromo-4-methylphenoxy)methyl)-3-phenylacrylate (5a). Starting from 2a (100 mg, 0.3919 mmol), compound 5a was isolated as a colorless oil (102 mg, 75%); FT-IR (ν$_{max}$, cm$^{-1}$): 1714, 1633, 1492, 1443, 1282, 1238, 1114; $^1$H NMR (400 MHz, CDCl$_3$): δ 8.07 (s, 1H), 7.56 – 7.58 (m, 2H), 7.38 – 7.41 (m, 4H), 7.03 – 7.06 (m, 1H), 6.92 (d, J = 8.3 Hz, 1H), 4.86 (s, 2H), 3.85 (s, 3H), 2.28 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 168.0, 153.3, 146.4, 134.7, 134.1, 132.6, 130.2, 130.0, 129.2, 129.0, 127.4, 115.2, 64.9, 52.7, 20.6; HRMS (ESI) for [C$_{18}$H$_{17}$BrO$_3$ + Na$^+$]: calcd., 383.0258. Found: 383.0251.

(E)-methyl 2-((3-methoxyphenoxy)methyl)-3-phenylacrylate (6a). Starting from 2a (100 mg, 0.3919 mmol), compound 6a was isolated as a colorless oil (110 mg, 95%); FT-IR (ν$_{max}$, cm$^{-1}$): 1715, 1595, 1484, 1446, 1283, 1235, 1197, 1152, 1112, 1036; $^1$H NMR (400 MHz, CDCl$_3$): δ 8.06 (s, 1H), 7.47 – 7.48 (m, 2H), 7.37 – 7.39 (m, 3H), 7.19 – 7.23 (m, 1H), 6.55 – 6.59 (m, 3H), 4.82 (s, 2H), 3.86 (s, 3H), 3.79 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 168.0, 161.2, 160.1, 146.1, 134.7, 134.1, 132.6, 130.2, 130.1, 130.0, 129.1, 127.5, 107.3, 107.2, 101.7, 63.1, 55.6, 52.7; HRMS (ESI) for [C$_{18}$H$_{18}$O$_4$ + Na$^+$]: calcd., 321.1103. Found: 321.1102.

(E)-methyl 2-((4-cyanophenoxy)methyl)-3-phenylacrylate (7a). Starting from 2a (100 mg, 0.3919 mmol), compound 7a was isolated as a colorless solid (105 mg, 92%): mp 115 – 117 °C; FT-IR (ν$_{max}$, cm$^{-1}$): 1709, 1607, 1523, 1438, 1349, 1259, 1171, 1106, 1023; $^1$H NMR (400 MHz, CDCl$_3$): δ 8.09 (s, 1H), 7.58 – 7.62 (m, 2H), 7.38 – 7.41 (m, 5H), 6.99 – 7.03 (m, 2H), 4.88 (s, 2H), 3.86 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 167.6, 162.0, 146.7, 134.4, 134.3, 130.2, 129.9, 129.2, 126.6, 119.5, 115.9, 104.7, 63.5, 52.8; HRMS (ESI) for [C$_{18}$H$_{15}$NO$_3$ + H$^+$]: calcd., 294.1130. Found: 294.1220.
(E)-methyl 2-((4-methyl-2-nitrophenoxy)methyl)-3-phenylacrylate (8a). Starting from 2a (150 mg, 0.5879 mmol), compound 8a was isolated as a colorless solid (187 mg, 97%): mp 106 – 108 °C; FT-IR (νmax, cm⁻¹): 1717, 1629, 1342, 1294, 1251, 1202, 1117; ¹H NMR (400 MHz, CDCl₃): δ 8.07 (s, 1H), 7.64 (d, J = 2.2 Hz, 1H), 7.52 – 7.54 (m, 2H), 7.39 – 7.42 (m, 3H), 7.30 – 7.32 (m, 1H), 7.11 (d, J = 8.5 Hz, 1H), 4.95 (s, 2H), 3.85 (s, 3H), 2.35 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 167.7, 150.2, 146.9, 140.7, 134.9, 134.5, 131.4, 129.1, 126.7, 125.9, 116.6, 65.4, 52.7, 20.6; HRMS (ESI) for [C₁₈H₁₇NO₅ + Na]⁺: calcd., 350.1004. Found: 350.1012.

(E)-methyl 2-((4-methyl-2-nitrophenoxy)methyl)-3-(4-nitrophenyl)acrylate (8b). Starting from 2b (150 mg, 0.4998 mmol), compound 8b was isolated as a colorless solid (184 mg, 99%): mp 148 – 150 °C; FT-IR (νmax, cm⁻¹): 1717, 1533, 1518, 1344, 1279, 1253, 1226, 1160, 1123; ¹H NMR (400 MHz, CDCl₃): δ 8.25 – 8.28 (m, 2H), 8.08 (s, 1H), 7.72 (d, J = 8.6 Hz, 2H), 7.65 (d, J = 2.1 Hz, 1H), 7.34 (dd, J = 2.2, 8.6 Hz, 1H), 7.13 (d, J = 8.6 Hz, 1H), 4.90 (s, 2H), 3.88 (s, 3H), 2.37 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 166.9, 149.8, 148.5, 144.0, 140.8, 135.1, 132.0, 130.8, 129.8, 126.0, 124.2, 116.6, 65.0, 53.1, 20.6; HRMS (ESI) for [C₁₈H₁₆N₂O₇ + Na]⁺: calcd., 395.0855. Found: 395.0838.

(E)-methyl 3-(4-cyanophenyl)-2-((4-methyl-2-nitrophenoxy)methyl)acrylate (8c). Starting from 2c (150 mg, 0.5354 mmol), compound 8c was isolated as a colorless solid (173 mg, 92%): mp 142 – 144 °C; FT-IR (νmax, cm⁻¹): 2226, 1719, 1641, 1532, 1504, 1430, 1348, 1279, 1254, 1228; ¹H NMR (400 MHz, CDCl₃): δ 8.03 (s, 1H), 7.70 (d, J = 8.3 Hz, 2H), 7.65 (d, J = 8.3 Hz, 3H), 7.33 (dd, J = 1.5, 8.5 Hz, 1H), 7.13 (d, J = 8.5 Hz, 1H), 4.88 (s, 2H), 3.86 (s, 3H), 2.36 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 167.0, 149.8, 144.5, 140.7, 138.9, 135.1, 132.8, 132.0, 130.5, 129.4, 126.0, 118.7, 116.6, 113.5, 65.1, 53.0, 20.6; HRMS (ESI) for [C₁₉H₁₆N₂O₅ + Na]⁺: calcd., 375.0957. Found: 375.0956.

(E)-methyl 3-(4-bromophenyl)-2-((4-methyl-2-nitrophenoxy)methyl)acrylate (8d). Starting from 2d (150 mg, 0.449 mmol), compound 8d was isolated as a colorless solid (170 mg, 93%): mp 82 – 84
°C; FT-IR (v_{\text{max}}, \text{cm}^{-1}): 1706, 1530, 1356, 1222, 1207; ^1\text{H NMR (400 MHz, CDCl}_3): \delta 7.99 (s, 1H), 7.64 (d, J = 1.8 Hz, 1H), 7.53 – 7.56 (m, 2H), 7.42 (d, J = 8.4 Hz, 2H), 7.32 (dd, J = 2.0, 8.4 Hz, 1H), 7.13 (d, J = 8.5 Hz, 1H), 4.91 (s, 2H), 3.84 (s, 3H), 2.35 (s, 3H); ^13\text{C NMR (100 MHz, CDCl}_3): \delta 167.5, 150.0, 145.7, 140.6, 135.0, 133.3, 132.4, 131.6, 127.1, 126.6, 124.7, 116.6, 65.2, 52.8, 20.6; HRMS (ESI) for [C_{18}H_{16}BrNO_5 + Na]^+: calcd., 428.0110. Found: 428.0165; Elemental analysis for C_{18}H_{16}BrNO_5 calcd. C, 53.22; H, 3.97; N, 3.45. Found C, 53.40; H, 3.77; N, 3.23.

(E)-methyl 3-(4-fluorophenyl)-2-((4-methyl-2-nitrophenoxy)methyl)acrylate (8e). Starting from 2e (200 mg, 0.7323 mmol), compound 8e was isolated as a colorless solid (284 mg, 96%): mp 81 – 83 °C; FT-IR (v_{\text{max}}, \text{cm}^{-1}): 1699, 1599, 1533, 1505, 1441, 1342, 1257, 1227, 1159, 1116; ^1\text{H NMR (400 MHz, CDCl}_3): \delta 8.03 (s, 1H), 7.64 (d, J = 1.4 Hz, 1H), 7.54 – 7.58 (m, 2H), 7.31 (dd, J = 2.1, 8.5 Hz, 1H), 7.08 – 7.16 (m, 3H), 4.93 (s, 2H), 3.84 (s, 3H), 2.36 (s, 3H); ^13\text{C NMR (100 MHz, CDCl}_3): \delta 167.7, 165.2, 162.7, 150.1, 145.9, 140.7, 135.0, 132.4, 132.3, 131.6, 130.6, 126.4, 126.0, 116.6, 116.4, 116.2, 65.3, 52.8, 20.6; HRMS (ESI) for [C_{18}H_{16}FNO_5 + Na]^+: calcd., 368.0910. Found: 368.0882; Elemental analysis for C_{18}H_{16}FNO_5 calcd. C, 62.61; H, 4.67; N, 4.06. Found C, 62.72; H, 4.35; N, 3.87.

(E)-methyl 3-(4-methoxyphenyl)-2-((4-methyl-2-nitrophenoxy)methyl)acrylate (8f). Starting from 2f (150 mg, 0.526 mmol), compound 8f was isolated as a colorless solid (180 mg, 96%): mp 75 – 77 °C; FT-IR (v_{\text{max}}, \text{cm}^{-1}): 1709, 1607, 1523, 1438, 1349, 1259, 1171, 1106, 1023; ^1\text{H NMR (400 MHz, CDCl}_3): \delta 8.03 (s, 1H), 7.64 (d, J = 2.0 Hz, 1H), 7.53 – 7.56 (m, 2H), 7.31 (dd, J = 2.1, 8.5 Hz, 1H), 7.18 (d, J = 8.5 Hz, 1H), 6.92 – 6.95 (m, 2H), 4.99 (s, 2H), 3.83 (s, 6H), 2.35 (s, 3H); ^13\text{C NMR (100 MHz, CDCl}_3): \delta 168.1, 161.4, 150.2, 147.0, 140.6, 135.0, 132.3, 131.3, 127.1, 125.9, 124.1, 116.7, 114.6, 65.6, 55.7, 52.6, 20.6; HRMS (ESI) for [C_{19}H_{19}NO_6 + Na]^+: calcd., 380.1110. Found: 380.1179.

(E)-methyl 3-(4-cyanophenyl)-2-((4-(hydroxymethyl)-2-nitrophenoxy)methyl)acrylate (8g). Starting from 2c (500 mg, 1.785 mmol), compound 8g was isolated as a colorless solid (570 mg,
87%): mp 135 – 137 °C; FT-IR ($\nu_{\text{max}}$, cm$^{-1}$): 3466, 2229, 1702, 1622, 1575, 1529, 1447, 1355, 1243, 1003; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.05 (s, 1H), 7.85 (d, $J = 2.2$ Hz, 1H), 7.69 – 7.72 (m, 2H), 7.63 – 7.66 (m, 2H), 7.54 (dd, $J = 2.2$, 8.3 Hz, 1H), 7.22 (d, $J = 8.6$ Hz, 1H), 4.92 (s, 2H), 4.71 (s, 2H), 3.87 (s, 3H), 1.77 (s, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 166.9, 151.2, 144.7, 140.7, 138.8, 134.9, 132.8, 130.4, 129.1, 124.3, 118.6, 116.5, 113.5, 64.9, 63.9, 53.0; HRMS (ESI) for [C$_{19}$H$_{16}$N$_2$O$_6$ + Na]$^+$: calcd., 391.0906. Found: 391.0900.

(E)-methyl 2-((4-nitrophenoxy)methyl)-3-phenylacrylate (9a). Starting from 2a (100 mg, 0.3919 mmol), compound 9a was isolated as a colorless solid (115 mg, 94%): mp 100 – 102 °C; FT-IR ($\nu_{\text{max}}$, cm$^{-1}$): 1706, 1622, 1591, 1505, 1339, 1245, 1198, 1110; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.21 – 8.23 (m, 2H), 8.11 (s, 1H), 7.38 – 7.43 (m, 5H), 7.00 – 7.03 (m, 2H), 4.93 (s, 2H), 3.87 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 167.6, 163.8, 146.8, 142.1, 134.4, 130.3, 129.9, 129.2, 126.5, 126.3, 115.2, 63.9, 52.8; HRMS (ESI) for [C$_{17}$H$_{15}$NO$_5$ + H]$^+$: calcd., 314.1028. Found: 314.1107; Elemental analysis for C$_{17}$H$_{15}$NO$_5$: calcd. C, 65.17; H, 4.83; N, 4.47. Found C, 65.50; H, 4.55; N, 4.15.

Synthesis of 4-nitrophenyl (4-methoxyphenyl)carbamate. To a solution of anisidine (250 mg, 2.03 mmol) and 4-nitrophenylchloroformate (491 mg, 2.436 mmol) in dry tetrahydrofuran (5 mL), sodium bicarbonate (205 mg, 2.436 mmol) was added at 0 °C under N$_2$ atmosphere. The reaction mixture was slowly warm to room temperature for 2 h, and complete consumption of the starting material was confirmed by TLC. The reaction mixture was diluted with water (100 mL), the aqueous layer was extracted with ethyl acetate (5 x 10 mL), and the combined organic layer was dried over Na$_2$SO$_4$. The volatiles were evaporated under reduced pressure to obtain the crude product. The product was found to be unstable under chromatographic purification conditions, so used without purification.

Synthesis of (E)-methyl 3-(4-cyanophenyl)-2-((4-(((4-methoxyphenyl)carbamoyl)oxy)methyl)-2-nitrophenoxy)methyl)acrylate (9). To a solution of 8g (75 mg, 0.20 mmol) and 4-nitrophenyl (4-methoxyphenyl)carbamate (70.4 mg, 0.24 mmol) in dry tetrahydrofuran (3 mL), potassium carbonate (84.4 mg, 0.61 mmol) was added under N$_2$ atmosphere. The reaction mixture was stirred at room
temperature for 48 h, and complete consumption of the starting material was confirmed by TLC. The reaction mixture was diluted with water (75 mL), the aqueous layer was extracted with ethyl acetate (5 × 10 mL), and the combined organic layer was dried over Na₂SO₄. The volatiles were evaporated under reduced pressure to obtain the crude product. The crude product was purified by semi-preparative HPLC with reversed phase C-18 semi-preparative column. Eluant system containing a gradient of acetonitrile (ACN) 50 – 80% in milli-Q water with a flow rate of 2.5 mL/min for 20 min was used to obtain pure product as a colorless solid (24 mg, 22%): mp 108 – 110 °C; FT-IR (νmax, cm⁻¹): 2229, 1719, 1622, 1532, 1438, 1352, 1216, 1115, 1058, 1030; ¹H NMR (500 MHz, CDCl₃): δ 8.06 (s, 1H), 7.90 (s, 1H), 7.71 (d, J = 7.9 Hz, 2H), 7.64 (d, J = 7.9 Hz, 2H), 7.59 (d, J = 8.2 Hz, 1H), 7.26 – 7.28 (m, 2H), 7.22 (d, J = 8.5 Hz, 1H), 6.86 (d, J = 8.6 Hz, 2H), 6.62 (s, 1H), 5.17 (s, 2H), 4.93 (s, 2H), 3.87 (s, 3H), 3.79 (s, 3H); ¹³C NMR (125 MHz, CDCl₃): 166.5, 151.4, 144.4, 140.3, 138.4, 134.0, 132.4, 130.1, 130.0, 128.7, 125.4, 120.8, 118.2, 116.0, 114.3, 113.2, 65.1, 64.5, 55.5, 52.7; HRMS (ESI) for [C_{27}H_{23}N_{3}O_{8}+Na]^+: calcd., 540.1383. Found: 540.1385.

(E)-4-((3-(4-cyanophenyl)-2-(methoxycarbonyl)allyloxy)-3-nitrobenzyl 7-(dimethylamino)-2-oxo-2H-chromene-3-carboxylate (12). To a solution of 11 (32 mg, 0.135 mmol) in dry dichloromethane (DCM), oxalyl chloride (0.6 mL) was added and stirred for 1 h at 60 °C. Then, oxalyl chloride was removed under reduced pressure to afford the acid chloride of 7-dimethylamino coumarin-3-carboxylic acid as yellow solid. This crude solid used for the esterification reaction without further purification. To the solution of the acid chloride in dry DCM (5 mL), compound 8g (50 mg, 0.135 mmol) was added followed by triethylamine (28 μL, 0.203 mmol). The mixture was stirred at room temperature for 48 h and the solvent was removed under reduced pressure. The crude product was purified by column chromatography to give 12 as a yellow solid (4 mg, 5%): mp 209 – 211 °C; FT-IR (νmax, cm⁻¹): 2227, 1706, 1624, 1589, 1525, 1355, 1234, 1115; ¹H NMR (400 MHz, CDCl₃): δ 8.46 (s, 1H), 8.05 (s, 1H), 7.96 (d, J = 1.9 Hz, 1H), 7.69 – 7.71 (m, 3H), 7.62 – 7.65 (m, 2H), 7.38 (d, J = 8.9 Hz, 1H), 7.22 (d, J = 8.6 Hz, 1H), 6.64 (dd, J = 2.4, 8.9 Hz, 1H), 6.45 (d, J = 2.3 Hz, 1H), 5.32
(s, 2H), 4.92 (s, 2H), 3.86 (s, 3H), 3.12 (s, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 166.9, 164.2, 158.6, 158.4, 155.4, 151.6, 150.3, 144.8, 140.5, 138.8, 134.6, 132.8, 131.3, 130.4, 130.1, 129.0, 125.9, 118.6, 116.3, 113.5, 110.3, 108.7, 108.3, 97.4, 65.4, 64.8, 53.1, 40.6; HRMS (ESI) for [C$_{31}$H$_{25}$N$_3$O$_9$ +Na]$^+$: calcd., 606.1488. Found: 606.1490.
Stability studies with nucleophiles using HPLC:

**General procedure for glutathione-mediated decomposition of 4a-7a and 9a.** 10 mM stock solutions of compounds were prepared in acetonitrile. To a solution of 4a – 7a and 9a (10 mM, 30 µL, final concentration of 100 µM), 10 eq. of GSH (100 mM, 30 µL) and 1:1, v/v ACN (1.47 mL) and pH 7.4 phosphate buffer (1.47 mL, 25 mM) were added. The reaction mixture was stirred at 37 °C and after 4 h, an aliquot of the reaction mixture was filtered (0.22 µm filter) and injected (25 µL) in an Agilent high performance liquid chromatography (HPLC).

**Glutathione-mediated decomposition of 8a-8f and release of 3e.** Sample preparation was similar to what has been described earlier. The reaction was monitored until >90% of compound was consumed and the formation of 3e was monitored as well.

**Selectivity studies of compound 8b with amino acids.** Sample preparation and reaction were conducted as described in experimental section. Aqueous solutions of amino acids (10 eq.) were reacted with 8b, after 4 h compound's reactivity was monitored by HPLC analysis.

**Figure S1.** Selectivity studies of compound 8b with amino acids:
Table S1. Estimates of errors for the calculation of rate constants described in Table 3

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<td>8f</td>
<td>OMe</td>
<td>0.0274</td>
<td>0.9990</td>
<td>0.030</td>
<td>0.9954</td>
</tr>
</tbody>
</table>

Stability studies of 8c with thiophenol. 10 mM stock solution of 8c was prepared in acetonitrile. To a solution of 8c (10 mM, 20 μL, final concentration of 100 μM), 10 eq. of thiophenol (100 mM, 20 μL) and 1:1, v/v ACN (0.96 mL) and pH 7.4 phosphate buffer (1.0 mL, 25 mM) were added. The reaction mixture was stirred at 37 °C and after 1 h, an aliquot of the reaction mixture was filtered (0.22 μm filter) and injected (25 μL) in an Agilent high performance liquid chromatography (HPLC).

Figure S2. Stability studies of 8c with 10 equiv of thiophenol (PhSH) in ACN: pH 7.4 phosphate buffer (1:1 v/v) at 37 °C after 1 h was monitored using HPLC, the reaction mixture HPLC trace (8c + PhSH) was compared with HPLC traces of authentic PhSH and 3h.
Figure S3. Possible mechanisms for decomposition of phenacrylates.
Stability studies of 9 with GSH. 10 mM stock solution of 9 was prepared in acetonitrile. To a solution of 9 (10 mM, 10 μL, final concentration of 100 μM), 10 eq. of GSH (100 mM, 10 μL) and 1:1, v/v ACN (0.490 mL) and pH 7.4 phosphate buffer (0.490 mL, 25 mM) were added. The reaction mixture was stirred at 37 °C and after 1 h, an aliquot of the reaction mixture was filtered (0.22 μm filter) and injected (25 μL) in an Agilent high performance liquid chromatography (HPLC).

Figure S4: Mass spectra of reaction mixture of 9 with GSH in acetonitrile: buffer (1:1 v/v) obtained from MALDI (TOF-TOF) analysis
Thiol-mediated decomposition of 12 and release of 11. A stock solution of 12 was prepared in DMSO. The reaction mixture consisted of 12 (1 eq.), 2-mercaptoethanol (25 eq.) in methanol (80%) and pH 7.4 phosphate buffer (20%, 25 mM) and was stirred at 37 °C. Periodically, an aliquot was taken and diluted with (1:1 v/v) methanol and pH 7.4 phosphate buffer. The resulting solution was placed in a cuvette and excitation wavelength was 413 nm and fluorescence emission was recorded.

Figure S5. Calibration curve for compound 11:

Stability studies of 12 with esterase. A stock solution of 12 (10 mM) was prepared in DMSO. To a solution of 12 (10 mM, 30 μL, final concentration of 100 μM), esterase (1 Unit, 2 μL of 10 mg/mL in buffer), and pH 7.4 phosphate buffer (1.498mL, 25 mM) were added. The reaction mixture was stirred at 37 °C. After a time interval of 3 h an aliquot of the reaction mixture was filtered (0.22 μm filter) and injected (25 μL) in a high performance liquid chromatography (HPLC) and we found no significant formation of 11.
**Cell Viability Assay.** HeLa cells were purchased from National Centre for Cell Science, Pune (India). Cells were grown in DMEM supplemented with 10% heat inactivated fetal bovine serum (FBS), 100 IU/mL penicillin, 100 mg/mL streptomycin and 2 mM-glutamine. Cultures were maintained in a humidified atmosphere with 5% CO\textsubscript{2} at 37 °C. The cells were sub-cultured twice each week, seeding at a density of 10\textsuperscript{4} cells/mL. The cells were treated with 12 at 0.1 to 100 μM concentration. Control cells were supplemented with complete tissue culture medium containing DMSO (<0.1% final concentration). Cells were grown in 96-well plate by seeding 10\textsuperscript{4} cells/mL and incubated at 37 °C, 5% CO\textsubscript{2} for 24 h. A standard (3-(4,5- Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used for quantification of cell proliferation in response to treatment. This assay is based on the ability of metabolically active cells to reduce the tetrazolium salt MTT to water-soluble purple colored formazan compounds. The intensity of the formed dye, proportional to the number of metabolic active cells, was read at 570 nm using the Thermo scientific Varioscan microplate reader. Absorbance was recorded after 72 h incubation. (Concentration necessary for the inhibition of 50% of cell growth, IC\textsubscript{50} > 25 μM).

**Figure S6.** Cell viability assay
**Cell-based fluorescence assay.** HeLa cells were grown in DMEM supplemented with 10% heat inactivated fetal bovine serum (FBS), 100 IU/mL penicillin, 100 mg/mL streptomycin and 2 mM-glutamine. Cultures were maintained in a humidified atmosphere with 5% CO₂ at 37 °C. The cells were sub-cultured twice each week, seeding at a density of 10⁴ cells/mL. HeLa cells were incubated with a solution of 12 in DMEM medium (10 μM 0.2% DMSO, pH = 7.4) at 37 °C. In another vial, HeLa cells were pretreated for 30 min with a solution of 10 equiv of N-ethylmaleimide in DMEM (NEM, 100 μM, 0.2% DMSO, pH = 7.4), to this a solution of 12 in DMEM medium (10 μM) was added and incubated at 37 °C. After 2 h of incubation the fluorescence emission from the above cell suspensions were recorded using a Thermo Scientific Varioscan microplate reader by exciting at λ<sub>ex</sub> = 421 nm and emission at λ<sub>em</sub> = 460 nm.

**Figure S7.** HeLa cells were incubated in serum-free media in the presence of 12 and fluorescence was recorded after 2 h.

**Cell Imaging.** HeLa cells were grown in DMEM supplemented with 10% heat inactivated fetal bovine serum (FBS), 100 IU/mL penicillin, 100 mg/mL streptomycin and 2 mM-glutamine. Cultures were maintained in a humidified atmosphere with 5% CO₂ at 37 °C. Cells were sub-cultured twice each week, seeding at a density of 10⁴ cells/mL. Cells were grown in 30 mm dish by seeding 10⁴ cells/mL and were incubated at 37 °C, 5% CO₂ for 24 h. The cells were incubated with solution of 12 (10 μM in DMEM (0.2% DMSO, pH = 7.4) at 37 °C for 2 h. To avoid the background fluorescence from media, the cells were washed with PBS (1x, pH 7.4) before imaging. After washing with PBS (3 × 1 mL) the fluorescence images were recorded. The fluorescence images were recorded using an Olympus
Inverted IX81 equipped with Hamamatsu Orca R2 microscope by exciting at $\lambda_{\text{ex}} = 410$ nm (using DAPI filter).
Figure S8. HPLC traces of 4a-7a and 9a after 4 h of reaction with glutathione in acetonitrile: pH 7.4 phosphate buffer (1:1, v/v) at 37 °C
Figure S9. HPLC traces of 8a-8f and kinetics experiment with glutathione in acetonitrile: pH 7.4 phosphate buffer (1:1, v/v) at 37 °C.
HPLC traces for compounds purity:

- **4a**
- **5a**
- **6a**
- **7a**
- **9a**
NMR Spectra: $^1$H NMR of 3h

$^{13}$C NMR of 3h
$^1$H NMR of 4a

$^{13}$C NMR of 4a
\(^1\)H NMR of 5a

\[^{13}\]C NMR of 5a
$^1$H NMR of 6a

$^{13}$C NMR of 6a
$^1$H NMR of 7a

$^{13}$C NMR of 7a
$^1\text{H NMR of 8a}$

$^{13}\text{C NMR of 8a}$
$^1$H NMR of 8b

$^{13}$C NMR of 8b
$^{1}H$ NMR of $8c$

$^{13}C$ NMR of $8c$
$^1$H NMR of 8d

$^{13}$C NMR of 8d
$^{1}$H NMR of 8e

$^{13}$C NMR of 8e
$^1$H NMR of 8f

$^{13}$C NMR of 8f
$^1$H NMR of 8g

$^{13}$C NMR of 8g
$^1$H NMR of 9a

$^{13}$C NMR of 9a
$^1\text{H NMR of 9}$

$^{13}\text{C NMR of 9}$
$^1$H NMR of 12

$^{13}$C NMR of 12