Supporting Information

Introduction of the α-ketoamide structure: En route to develop hydrogen peroxide responsive prodrugs

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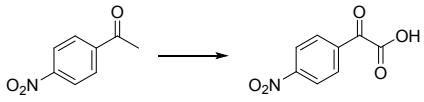
General Chemistry Experimental Information

All reagents and solvents were received from commercial suppliers and used without further purification. When necessary, solvents were dried using standard protocols. TLC was performed on silica gel plates and visualized under UV light (254 nm). Flash chromatography was performed using chromatography grade silica gel (200–300 mesh) purchased from Qingdao Haiyang Chemical Co., Ltd. Melting points were determined on a Mel-TEMP II melting point apparatus and were uncorrected. 1H NMR and 13C NMR spectrum were recorded with a JEOL 400 MHz spectrometer at 300 K. Mass spectrum were obtained on an Agilent Q-TOF 6540 spectrometer. High resolution mass spectra were obtained on Agilent LC/MSD TOF.

Synthesis and characterizations of target compounds

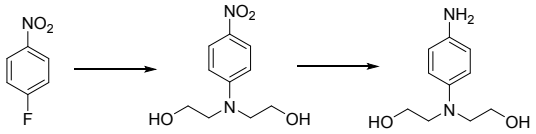
2-(4-Nitrophenyl)-2-oxoacetic acid (1)



1

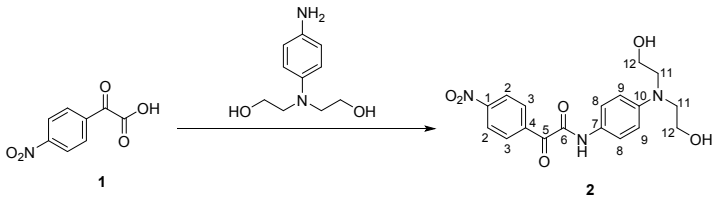
1-(4-Nitrophenyl)ethan-1-one (1 g, 6.06 mmol) was dissolved in pyridine (10 mL) and SeO_2 (1008 mg, 9.08 mmol) was added. The reaction mixture was heated in an oil bath at 90 °C under nitrogen for 5 h. The reaction was monitored by TLC. Upon completion, the reaction mixture was filtered, and the residue was washed with ethyl acetate (EA) (3 × 20 mL). The combined filtrate was treated with 1 M HCl (40 mL), the organic layer was separated, and the aqueous layer was re-extracted with EA (3 × 20 mL). The organic layers were combined and treated with 1 M NaOH (50 mL), and the aqueous layer was separated. The aqueous layer was acidified with 1 M HCl to pH 1.5. The mixture was extracted with EA (3 × 50 mL). The combined organic layers were dried over Na_2SO_4 and concentrated. The crude products were further purified by column chromatography using petroleum ether (PE)/EA (9:1 to 7:3) to afford **1** as a yellow solid in 35% yield.

***N*-4-Aminophenyl diethanolamine**



This intermediate was prepared using reported methods.[1, 2] Diethanolamine (2.73 mL, 28.48 mmol) was added to 4-nitrofluorobenzene (1 mL, 9.38 mmol) in 20 mL DMSO, and the solution was heated at 140 °C for 3.5 h. Upon completion of the reaction as monitored by TLC, the reaction mixture was cooled to room temperature (r.t.) and extracted with EA (3 × 50 mL). The organic layers were combined, dried over Na_2SO_4, and concentrated to afford *N*-4-nitrophenyl-diethanolamine in 55% yield. The nitro intermediate was then dissolved in 24 mL of dichloromethene (DCM)/MeOH (v/v, 1/2), and 10% palladium on carbon (0.5 g) was added. The reaction mixture was stirred under a hydrogen atmosphere for 4 h, filtered through Celite, and concentrated to give *N*-4-aminophenyl diethanolamine as a brown solid in 80% yield.

***N*-(4-(Bis(2-hydroxyethyl)amino)phenyl)-2-(4-nitrophenyl)-2-oxoacetamide (2)**



1 **2**

Compound **1** (780 mg, 3.98 mmol) was dissolved in DMF, and *N*-4-aminophenyl diethanolamine (775 mg, 3.98 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) (1145 mg, 5.96 mmol), and *N*,*N*-diisopropylethylamine (DIEA) (986 μL, 5.96 mmol) were added. The reaction mixture was stirred at r.t. for 1.5 h and monitored by TLC. Upon completion, the reaction mixture was poured into water and extracted with EA (3 × 50 mL). The organic layers were combined, dried over Na_2SO_4, concentrated and purified by column chromatography to give **2** as a blackish brown solid in 42% yield.

m.p.158–160 °C.

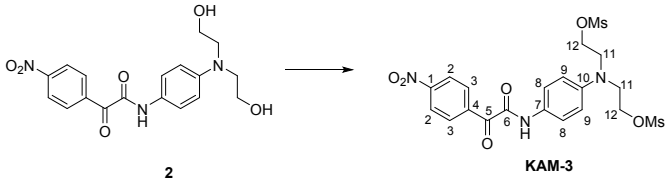
^{1}H NMR (400 MHz, d^6-DMSO) δ 10.71 (s, 1H, NH), 8.42–8.24 (d, 4H, H_2, H_3), 7.54 (d, J = 8.3 Hz, 2H, H_8), 6.67 (d, J = 8.1 Hz, 2H, H_9), 4.76 (s, 2H, 2 × OH), 3.52 (brs, 4H, H_{11} × 2), 3.40 (brs, 4H, H_{12} × 2).

^{13}C NMR (100 MHz, d^6-DMSO) δ 188.05 (C_5), 160.66 (C_6), 150.45 (C_1), 145.40 (C_{10}), 137.96 (C_4), 131.58 ($C_3 \times 2$), 125.96 (C_7), 123.94 ($C_2 \times 2$), 121.81 ($C_8 \times 2$), 111.22 ($C_9 \times 2$), 58.20 ($C_{11} \times 2$), 53.36 ($C_{12} \times 2$)

ESI-MS: 374.1 $[M + H]^+$.

HRMS: $[M + H]^+$ calcd. $[C_{18}H_{19}N_3O_6]^+$ 374.1352; found 374.1347.

KAM-3



To a stirred solution of **2** (200 mg, 0.536 mmol) and triethylamine (TEA) (260 µL, 1.876 mmol) in anhydrous DCM (5.0 mL) was added methanesulfonyl chloride (MsCl) (166 µL, 2.144 mmol) and DMAP (13 mg, 0.1072 mmol). The mixture was stirred at reflux for 3.5 h. Reaction was quenched with water, extracted with EA (3 × 30 mL), dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by flash chromatography (PE/EA v/v, 1:1) to afford **KAM-3** as a blackish brown solid in 70% yield.

m. p. 50–51 °C.

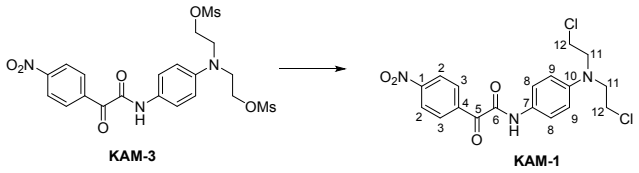
^{1}H NMR (400 MHz, d^6-DMSO) δ 10.78 (s, 1H, NH), 8.40 (d, J = 8.5 Hz, 2H, H_2), 8.31 (d, J = 8.5 Hz, 2H, H_3), 7.64 (d, J = 8.5 Hz, 2H, H_8), 6.84 (d, J = 8.7 Hz, 2H, H_9), 4.32 (t, J = 5.7 Hz, 4H, $H_{11} \times 2$), 3.75 (t, J = 5.9 Hz, 4H, $H_{12} \times 2$), 3.17(s, 6H, $CH_3 \times 2$).

^{13}C NMR (100 MHz, d^6-DMSO) δ 187.89 (C_5), 160.81 (C_6), 150.42 (C_1), 143.98 (C_{10}), 137.83 (C_4), 131.49 ($C_3 \times 2$), 127.50 (C_7), 123.84 ($C_2 \times 2$), 121.80 ($C_8 \times 2$), 112.31 ($C_9 \times 2$), 67.22 ($C_{12} \times 2$), 49.49 ($C_{11} \times 2$), 36.67 ($CH_3 \times 2$).

ESI-MS: 530.0 $[M + H]^+$, 552.1 $[M + Na]^+$.

HRMS: $[M + H]^+$ calcd. $[C_{20}H_{24}N_3O_{10}S_2]^+$ 530.0903; found 530.0898; $[M + Na]^+$ calcd. $[C_{20}H_{23}N_3O_{10}S_2Na]^+$ 552.0723; found 552.0721.

KAM-1



A mixture of **KAM-3** (100 mg, 0.189 mmol) and LiCl (40.07 mg, 0.945 mmol) in DMF (3 mL) was stirred at 60 °C for 2 h. Upon completion, the reaction mixture was extracted with DCM (3 × 30 mL), washed with brine, water, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography to afford **KAM-1** (PE/EA v/v, 9:1) as a blackish brown solid in 65% yield.

m.p.141–143 °C.

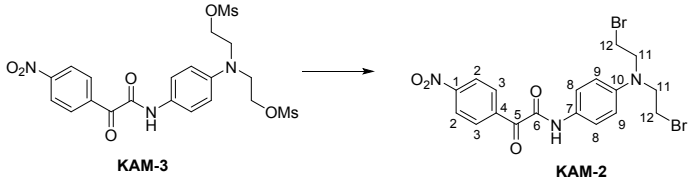
^{1}H NMR (400 MHz, Chloroform-d) δ 8.83(s, 1H, NH), 8.64–8.57 (m, 2H, H_2), 8.38–8.30 (m, 2H, H_3), 7.62–7.55 (m, 2H, H_8), 6.76–6.67 (m, 2H, H_9), 3.80–3.71 (m, 4H, $H_{11} \times 2$), 3.68–3.62 (m, 4H, $H_{12} \times 2$).

^{13}C NMR (100 MHz, Chloroform-d) δ 186.27 (C_5), 157.38 (C_6), 150.84 (C_1), 144.06 (C_{10}), 137.87 (C_4), 132.57 ($C_3 \times 2$), 126.70 (C_7), 123.50 ($C_2 \times 2$), 122.01 ($C_8 \times 2$), 112.33 ($C_9 \times 2$), 53.50 ($C_{12} \times 2$), 40.36 ($C_{11} \times 2$).

ESI-MS: 409.1 $[M + H]^+$.

HRMS (m/z): $[M + H]^+$ calcd. $[C_{18}H_{18}Cl_2N_3O_4]^+$ 410.0674, found 410.0666.

KAM-2



A mixture of **KAM-3** (100 mg, 0.189 mmol) and NaBr (97 mg, 0.945 mmol) in DMF (3 mL) was stirred at 60 °C for 2 h. Upon completion, the reaction mixture was extracted with DCM (3 × 30 mL), washed with brine, water, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography to afford **KAM-2** (PE/EA v/v, 9:1) as a blackish brown solid in 67% yield.

m.p.95–96 °C.

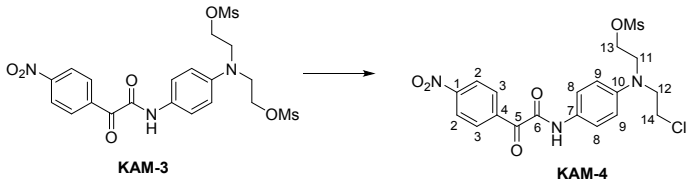
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.83(s, 1H, NH), 8.63–8.58 (m, 2H, H_2), 8.40–8.26 (m, 2H, H_3), 7.63–7.56 (m, 2H, H_8), 6.74–6.68 (m, 2H, H_9), 3.80 (t, J = 7.5 Hz, 4H, H_{11} × 2), 3.52–3.41 (m, 4H, H_{12} × 2).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 186.40 (C_5), 157.57 (C_6), 151.03 (C_1), 143.94 (C_{10}), 138.03 (C_4), 132.71 (C_3 × 2), 127.02 (C_7), 123.63 (C_2 × 2), 122.22 (C_8 × 2), 112.55 (C_9 × 2), 53.50 (C_{11} × 2), 28.31 (C_{12} × 2).

ESI-MS: 499.0 $[M + H]^+$.

HRMS (m/z): $[M + H]^+$ calcd. $[C_{18}H_{18}Br_2N_3O_4]^+$ 499.9644, found 499.9638.

KAM-4



A mixture of **KAM-3** (100 mg, 0.189 mmol) and LiCl (9 mg, 0.189 mmol) in acetonitrile (3 mL) was stirred at 60 °C for 10 h. After removal of the solvent, the residue was purified by column chromatography to afford **KAM-4** (PE/EA v/v, 6:4) as a blackish brown solid in 23% yield.

m.p. 83–85 °C.

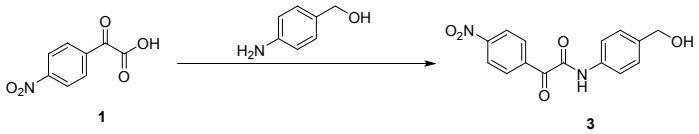
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.84(s, 1H, NH), 8.64–8.55 (m, 2H, H_2), 8.37–8.29 (m, 2H, H_3), 7.63–7.57 (m, 2H, H_8), 6.78–6.69 (m, 2H, H_9), 4.37 (t, J = 5.9 Hz, 2H, H_{11}), 3.83–3.72 (m, 4H, H_{12}, H_{14}), 3.69-3.62 (m, 2H, H_{13}), 2.99 (s, 3H, CH_3).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 186.25 (C_5), 157.47 (C_6), 150.90 (C_1), 144.18 (C_{10}), 137.88 (C_4), 132.57 (C_3 × 2), 127.05 (C_7), 123.50 (C_2 × 2), 122.04 (C_8 × 2), 112.78 (C_9 × 2), 66.15 (C_{13}), 53.64 (C_{11}), 50.71 (C_{14}), 40.44 (C_{12}), 37.59 (CH_3).

ESI-MS: 468.3 $[M – H]^-$.

HRMS (m/z): $[M + H]^+$ calcd. $[C_{19}H_{21}ClN_3O_7S]^+$ 470.0789, found 470.0783.

***N*-(4-Hydroxymetheyl)phenyl)-2-(4-nitrophenyl)-2-oxoacetamide (3)**

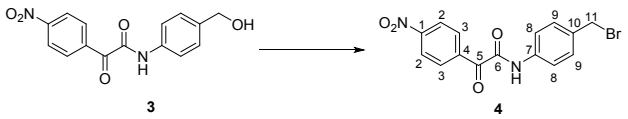


(4-Aminophenyl)methanol (633 mg, 5.13 mmol) was added to a solution of **1** (1 g, 5.13 mmol) in DMF (10 mL). EDCI (7.7 mmol, 1475 mg) and DIEA (7.7 mmol, 1272 μL) were added in turn. The reaction mixture was stirred at r.t. for 1.5 h and monitored by TLC. Upon completion, the reaction mixture was extracted with EA (3 × 50 mL), dried over Na_2SO_4, filtered, and concentrated to give **3** in 33% yield, which was used directly in the following reaction without purification.

ESI-MS: 299.3 $[M – H]^-$.

HRMS (m/z): $[M + H]^+$ calcd. $[C_{15}H_{13}ClN_2O_5]^+$ 301.0824, found 310.0817.

***N*-(4-(Bromomethyl)phenyl)-2-(4-nitrophenyl)-2-oxoacetamide (4)**



Compound **3** (300 mg, 1 mmol) was dissolved in DCM (10 mL) at 0 °C under nitrogen. PPh_3 (394 mg, 1.5 mmol) and CBr_4 (498 mg, 1.5 mmol) were added in turn, and the reaction mixture was allowed to warm to r.t. and stirred for 2 h. The mixture was concentrated under reduced pressure, and the residue was further purified by column chromatography (PE/EA v/v, 9:1) to obtain **4** as a yellow solid in 50% yield.

m.p. 133–135 °C.

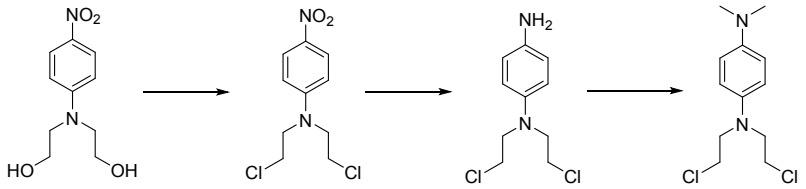
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.98 (s, 1H, NH), 8.66–8.54 (m, 2H, H_2), 8.41–8.29 (m, 2H, H_3), 7.74–7.63 (m, 2H, H_8), 7.50–7.39 (m, 2H, H_9), 4.51 (s, 2H, H_{11}).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 185.94 (C_5), 157.80 (C_6), 151.10 (C_1), 137.64 (C_4), 136.30 (C_7), 135.35 (C_{10}), 132.76 (C_3 × 2), 130.29 (C_9 × 2), 123.71 (C_2 × 2), 120.33 (C_8 × 2), 32.98 (C_{11}).

ESI-MS: 361.1 $[M – H]^-$.

HRMS (m/z): $[M – H]^+$ calcd. $[C_{15}H_{10}BrN_2O_4]^-$ 360.9829, found 360.9833.

***N*-(4-*N*',*N*'-Dimethylaminophenyl)-bis(2-chloroethyl)amine**

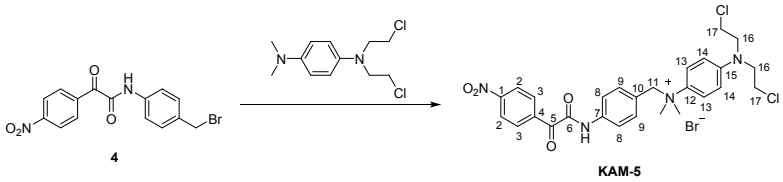


These intermediates were prepared using the methods described before.[1, 3] *N*-4-Nitrophenyl diethanolamine (1.6 g, 7.1 mmol) was suspended in DCM (16 mL) and pyridine (1 mL) was added at 0 °C. Thionyl chloride (1.16 mL, 16 mmol) was then added slowly and the mixture was heated at reflux for 1 h. After cooling to r.t., the mixture was extracted with DCM (3 × 50 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated to give *N, N*-bis(2-chloroethyl)-4-nitroaniline in 85% yield.

N,N-Bis(2-chloroethyl)-4-nitroaniline (300 mg, 1.14 mmol) was dissolved in 12 mL DCM/MeOH (v/v, 1/2), and 10% palladium on carbon (100 mg) was added. The reaction mixture was stirred under a hydrogen atmosphere for 4 h, filtered through Celite, and concentrated to give *N, N*-bis(2-chloroethyl)benzene-1,4-diamine in 74% yield.

The above phenylamine intermediate (220 mg, 1.12 mmol) and $NaBH_4$ (123 mg, 2.81 mmol) was added to a mixture of formalin (300 μL, 2.81 mmol) and concentrated H_2SO_4 (0.264 mL) in THF (6 mL). The reaction mixture was stirred at r.t. for 1.5 h and quenched with 1M NaOH solution, extracted with DCM (3 × 20 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography (PE/EA v/v, 9:1) to give the title compound in 75% yield.

KAM-5



Compound **4** (70 mg, 0.193 mmol) and *N*-(4-*N*',*N*'-Dimethylaminophenyl)-bis(2-chloroethyl)amine (50 mg, 0.193 mmol) were dissolved in MeCN (3 mL) and stirred at r.t. overnight. The solvent was concentrated and the residue was purified by column chromatography using (DCM/MeOH v/v, 95:5) to afford **KAM-5** as a yellow solid in 43% yield.

m.p. 82–84 °C.

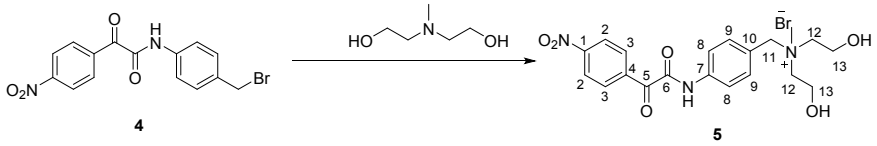
^{1}H NMR (400 MHz, *d*^{6}-DMSO) δ 11.16 (s, 1H, NH), 8.45–8.38 (m, 2H, H_2), 8.34–8.26 (m, 2H, H_3), 7.75 (d, *J* = 8.3 Hz, 2H, H_8), 7.60 (d, *J* = 9.1 Hz, 2H, H_9), 7.11 (d, *J* = 8.3 Hz, 2H, H_{13}), 6.89 (d, *J* = 9.3 Hz, 2H, H_{14}), 4.94 (s, 2H, H_{11}), 3.85–3.74 (m, 8H, H_{16}, H_{17}), 3.50 (s, 6H, CH_3 × 2).

^{13}C NMR (100 MHz, *d*^{6}-DMSO) δ 187.21 (C_5), 161.66 (C_6), 150.54 (C_1), 147.22 (C_{15}), 139.19 (C_4), 137.45 (C_{12}), 133.53 (C_7), 133.28 (C_3 × 2), 131.63 (C_9 × 2), 124.45 (C_{10}), 123.92 (C_2 × 2), 122.64 (C_{13} × 2), 119.87 (C_8 × 2), 111.93 (C_{14} × 2), 71.44 (C_{11}), 52.62 (CH_3 × 2), 51.56 (C_{17} × 2), 41.10 (C_{16} × 2).

ESI-MS: 543.155 [M]$^+$.

HRMS (m/z): [M]$^+$ calcd. $[C_{27}H_{29}Cl_2N_2O_4]^+$ 543.1560, found 543.1565.

2-Hydroxy-*N*-(2-hydroxyethyl)-*N*-methyl-*N*-(4-(2-(4-nitrophenyl)-2-oxoacetamido)benzyl)ethan-1-aminium bromide (5)



Compound **4** (200 mg, 0.552 mmol) was dissolved in $CHCl_3$ (5 mL) and *N*-methyl-diethanolamine (220 μL, 1.822 mmol) was added. The reaction mixture was stirred at reflux for 3 h. Upon completion, the yellow precipitations appeared were filtered and washed with cold DCM to afford **5** as a yellow solid in 76% yield.

m.p. 185–186 °C.

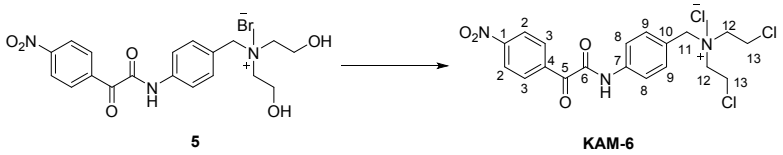
^{1}H NMR (400 MHz, *d*^{6}-DMSO) δ 11.25 (s, 1H, NH), 8.42 (d, *J* = 8.7 Hz, 2H, H_2), 8.34–8.28 (m, 2H, H_3), 7.91 (d, *J* = 8.3 Hz, 2H, H_8), 7.64 (d, *J* = 8.4 Hz, 2H, H_9), 5.34 (t, *J* = 4.9 Hz, 2H, OH × 2), 4.65 (s, 2H, H_{11}), 3.96–3.87 (m, 4H, H_{12} × 2), 3.58–3.28 (m, 4H, H_{13} × 2), 3.00 (s, 3H, CH_3).

^{13}C NMR (100 MHz, *d*^{6}-DMSO) δ 187.30 (C_5), 161.78 (C_6), 150.67 (C_1), 139.21 (C_4), 137.48 (C_7), 134.11 (C_3 × 2), 131.63 (C_9 × 2), 124.05 (C_{10}), 123.95 (C_2 × 2), 120.24 (C_8 × 2), 62.70 (C_{11}), 54.87 (C_{12} × 2, C_{13} × 2), 48.04 (CH_3).

ESI-MS: 402.2 [M]$^+$.

HRMS (m/z): [M]$^+$ calcd $[C_{27}H_{29}Cl_2N_2O_4]^+$ 402.1665, found 402.1660.

KAM-6



5 **KAM-6**

Compound **5** (100 mg, 0.208 mmol) was slowly added to $SOCl_2$ (3 mL) and the mixture was stirred at r.t. overnight. Upon completion, The solvent was evaporated and the residue was washed with cold DCM to afford **KAM-6** as a yellow solid in 62% yield.

m.p. 154–155 °C.

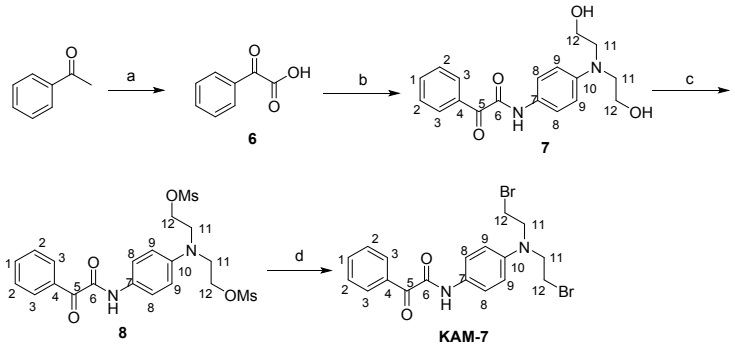
^{1}H NMR (400 MHz, d^6-DMSO) δ 11.35 (s, 1H, NH), 8.42 (d, J = 8.5 Hz, 2H, H_2), 8.31 (d, J = 8.6 Hz, 2H, H_3), 7.95 (d, J = 8.2 Hz, 2H, H_8), 7.62 (d, J = 8.3 Hz, 2H, H_9), 4.73 (s, 2H, H_{11}), 4.29–4.13 (m, 4H, H_{13}), 3.88–3.78 (m, 2H, H_{12}), 3.69 (m, 2H, H_{12}), 3.09 (s, 3H, CH_3).

^{13}C NMR (100 MHz, d^6-DMSO) δ 187.28 (C_5), 161.89 (C_6), 150.57 (C_1), 139.56 (C_4), 137.45 (C_7), 133.93 (C_3 × 2), 131.60 (C_9 × 2), 123.96 (C_2 × 2), 123.17 (C_{10}), 120.44 (C_8 × 2), 65.24 (C_{11}), 60.80 (C_{12} × 2), 47.26 (CH_3), 36.08 (C_{13} × 2).

ESI-MS: 438.1 $[M]^+$.

HRMS (m/z): $[M]^+$ calcd. $[C_{20}H_{22}Cl_2N_3O_4]^+$ 438.0982, found 438.0987.

Synthetic route of KAM-7



Reactants and conditions: a) SeO2, pyridine, 90 °C, N2; b) N-4-aminophenyl diethanolamine, EDCI, DIEA, DMF; c) MsCl, DMAP, TEA, DCM, reflux; d) NaBr (5 eq.), DMF, 60 °C.

2-Oxo-2-phenylacetic acid (6)

Using a similar procedure as described above for the synthesis of **1**, compound **6** was obtained from acetophenone as a pale yellow oil in 87% yield.

***N*-(4-(Bis(2-hydroxyethyl)amino)phenyl)-2-oxo-2-phenylacetamide (7)**

Using a similar procedure as described above for the synthesis of **2**, compound **7** was obtained from **6** and *N*-4-aminophenyl diethanolamine as a blackish brown solid in 83% yield.

m.p. 95–96 °C.

^{1}H NMR (400 MHz, d^6-Acetone) δ 9.68 (s, 1H, NH), 8.28–8.19 (m, 2H, H_3), 7.74–7.66 (m, 3H, H_1, H_2), 7.60–7.53 (m, 2H, H_8), 6.79–6.73 (m, 2H, H_9), 4.23 (t, J = 5.4 Hz, 2H, OH × 2), 3.76 (q, J = 5.6 Hz, 4H, H_{11} × 2), 3.57 (t, J = 5.7 Hz, 4H, H_{12} × 2).

^{13}C NMR (100 MHz, d^6-Acetone) δ 189.64 (C_5), 161.58 (C_6), 146.53 (C_{10}), 135.05 (C_1), 134.48 (C_4), 131.40 (C_2 × 2), 129.42 (C_3 × 2), 127.73 (C_7), 122.42 (C_8 × 2), 112.70 (C_9 × 2), 60.30 (C_{11} × 2), 55.26 (C_{12} × 2).

ESI-MS: 329.2 $[M + H]^+$, 351.1 $[M + Na]^+$.

ESI-MS: 329.2 $[M + H]^+$, 351.1 $[M + Na]^+$.

HRMS (m/z): $[M + H]^+$ calcd. $[C_{18}H_{21}N_2O_4]^+$ 329.1501, found 329.1497.

((4-(2-Oxo-2-phenylacetamido)phenyl)azanediyl)bis(ethane-2,1-diyl) dimethanesulfonate (8)

Using a similar procedure as described above for the synthesis of **KAM-3**, compound **8** was obtained from **7** as a blackish brown solid in 75% yield.

m.p. 96–98 °C.

^{1}H NMR (400 MHz, d^6-Acetone) δ 9.76 (s, 1H, NH), 8.28–8.19 (m, 2H, H_3), 7.78–7.70 (m, 3H, H_1, H_2), 7.61–7.55 (m, 2H, H_8), 6.95–6.89 (m, 2H, H_9), 4.44 (t, J = 5.8 Hz, 4H, H_{11} × 2), 3.88 (t, J = 5.9 H, 4H, H_{12} × 2), 3.10 (s, 6H, CH_3 × 2).

^{13}C NMR (100 MHz, d^6-Acetone) δ 189.59 (C_5), 162.06 (C_6), 145.37 (C_{10}), 135.17 (C_1), 134.46 (C_4), 131.46 (C_2 × 2), 129.51 (C_3 × 2), 129.28 (C_7), 122.66 (C_8 × 2), 113.54 (C_9 × 2), 68.04 (C_{11} × 2), 51.20 (C_{12} × 2), 37.17 (CH_3 × 2).

ESI-MS: 485.2 $[M + H]^+$, 507.1 $[M + Na]^+$.

HRMS (m/z): $[M + H]^+$ calcd. $[C_{20}H_{24}N_2O_8S_2]^+$ 485.1052, found 485.1046.

KAM-7

Using a similar procedure as described above for the synthesis of **KAM-2**, **KAM-7** was obtained from **8** as a blackish brown solid in 60% yield.

m.p. 87–89 °C.

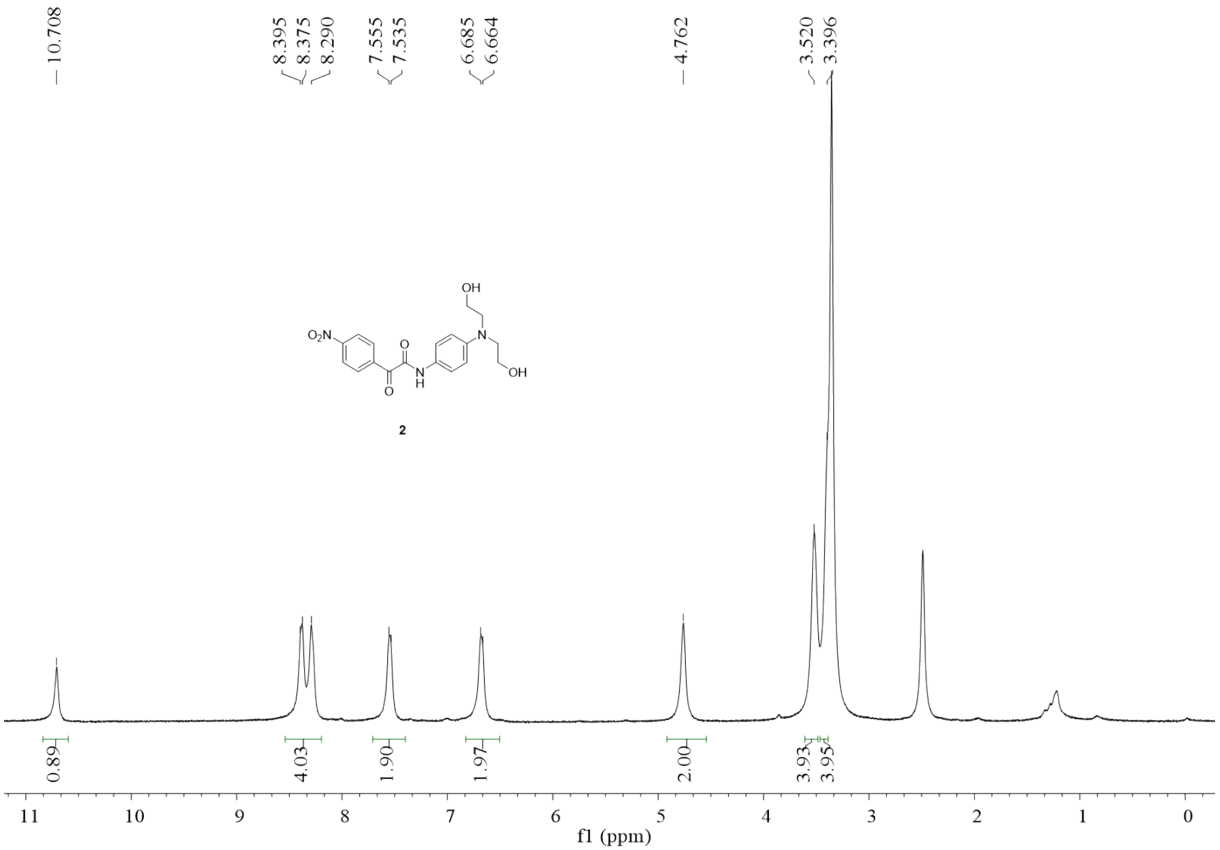
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.83 (s, 1H, NH), 8.40 (d, J = 7.8 Hz, 2H, H_3), 7.64 (t, J = 7.4 Hz, 1H, H_1), 7.58 (d, J = 8.6 Hz, 2H, H_2), 7.49 (t, J = 7.7 Hz, 2H, H_8), 6.70 (d, J = 8.6 Hz, 2H, H_9), 3.77 (t, J = 7.5 Hz, 4H, H_{11} × 2), 3.45 (t, J = 7.5 Hz, 4H, H_{12} × 2).

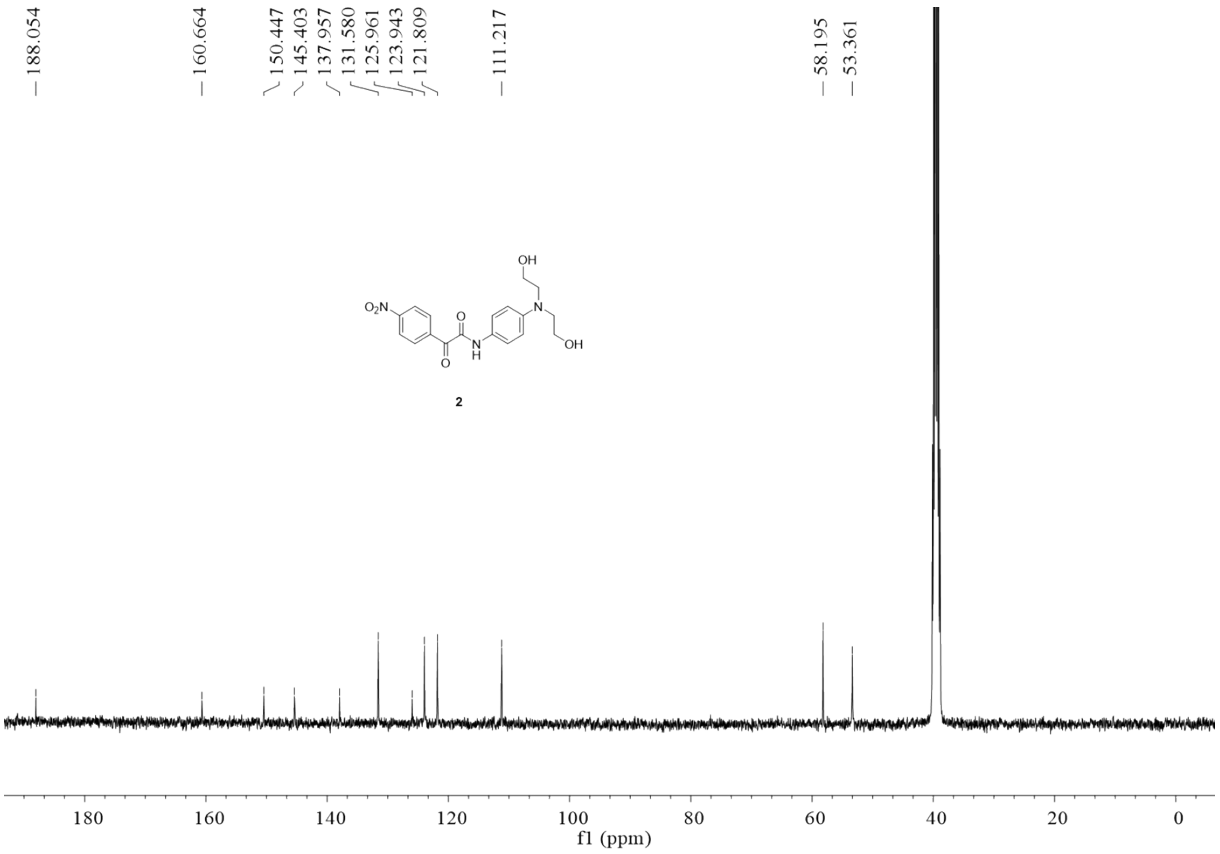
^{13}C NMR (100 MHz, Chloroform-*d*) δ 187.78 (C_5), 158.72 (C_6), 143.59 (C_{10}), 134.66 (C_1), 133.41 (C_4), 131.58 (C_2 × 2), 128.66 (C_3 × 2), 127.58 (C_7), 122.14 (C_8 × 2), 112.56 (C_9 × 2), 53.52 (C_{11}), 28.41 (C_{12}).

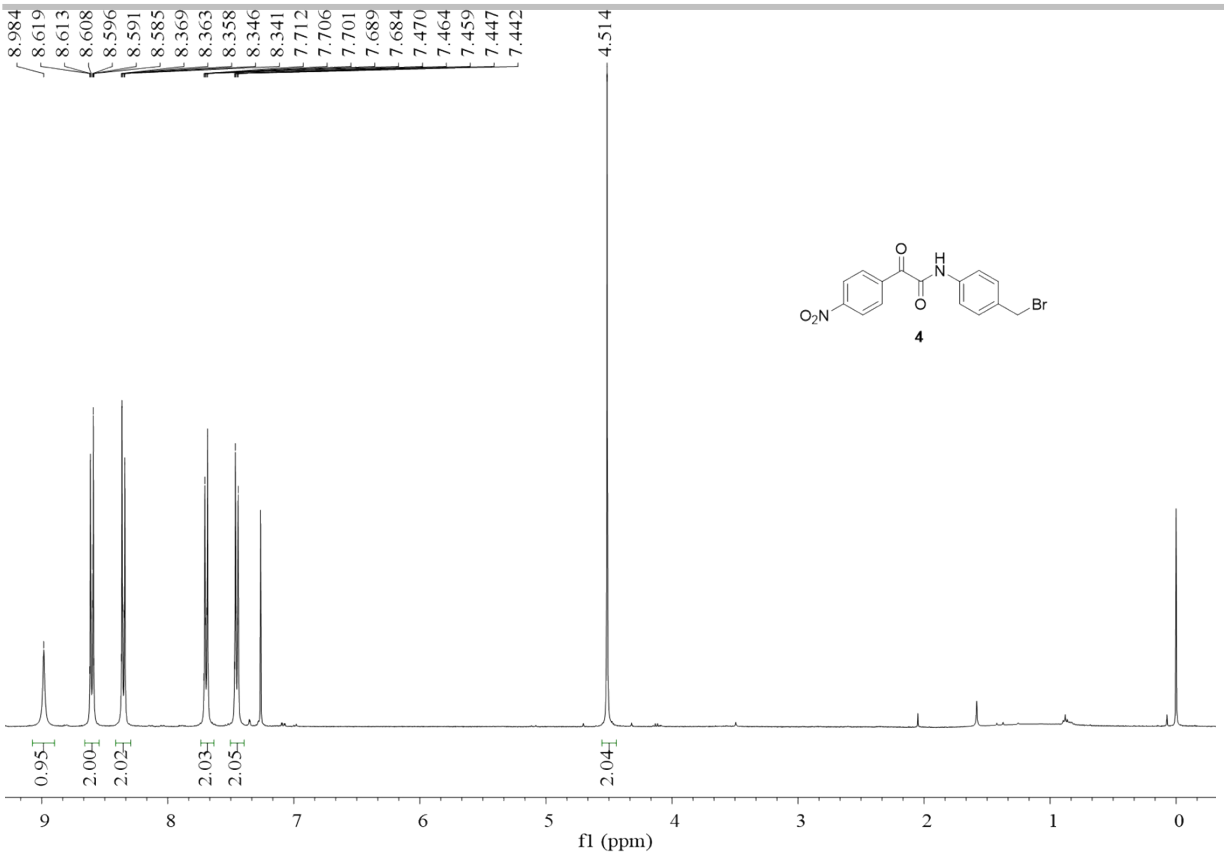
ESI-MS: 455.0 $[M + H]^+$.

HRMS (m/z): $[M]^+$ calcd. $[C_{18}H_{19}Br_2N_2O_2]^+$ 454.9793, found 454.9786.

^{1}H-NMR and ^{13}C-NMR spectra



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7.684
7.470
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7.459
7.447
7.442
4.514
O2N
O
H
N
O
Br
4
0.95
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2.03
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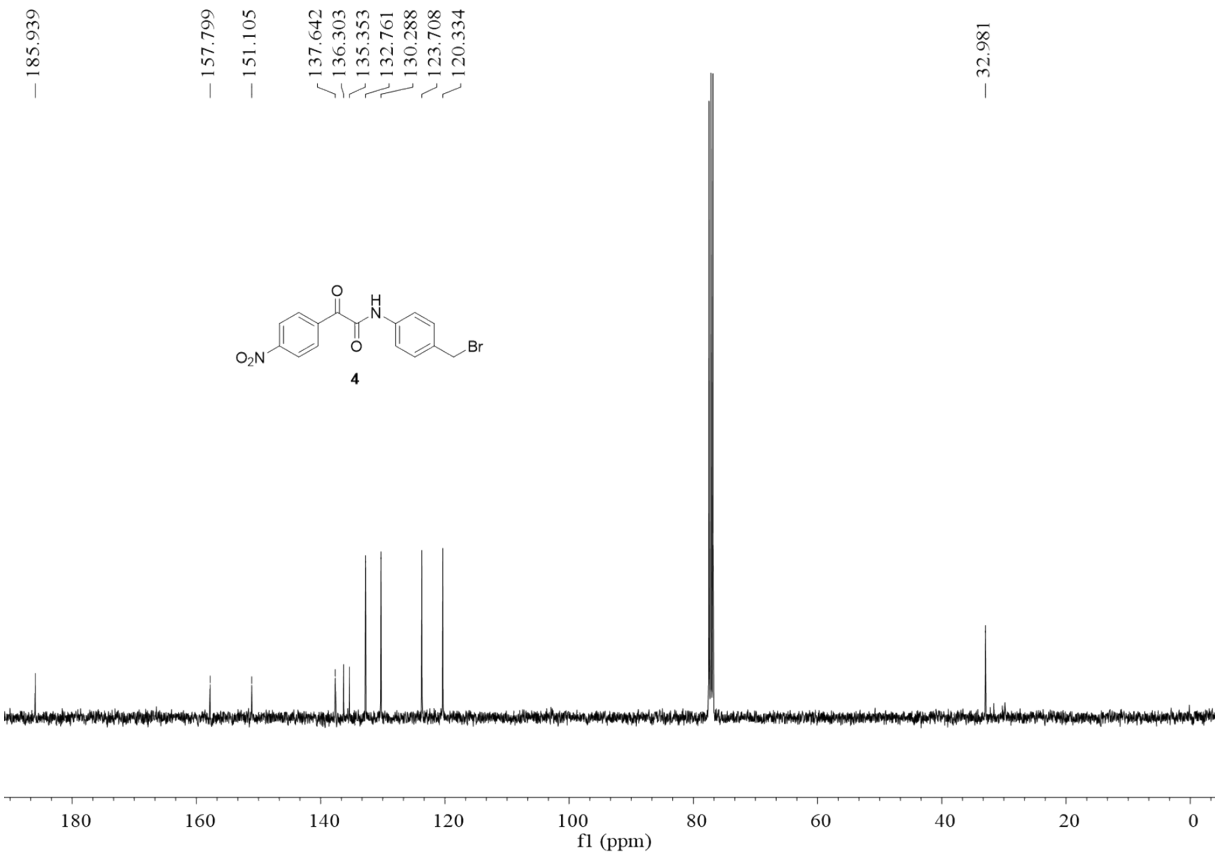
2.04
9
8
7
6
5
4
3
2
1
0
f1 (ppm)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N
O
Br
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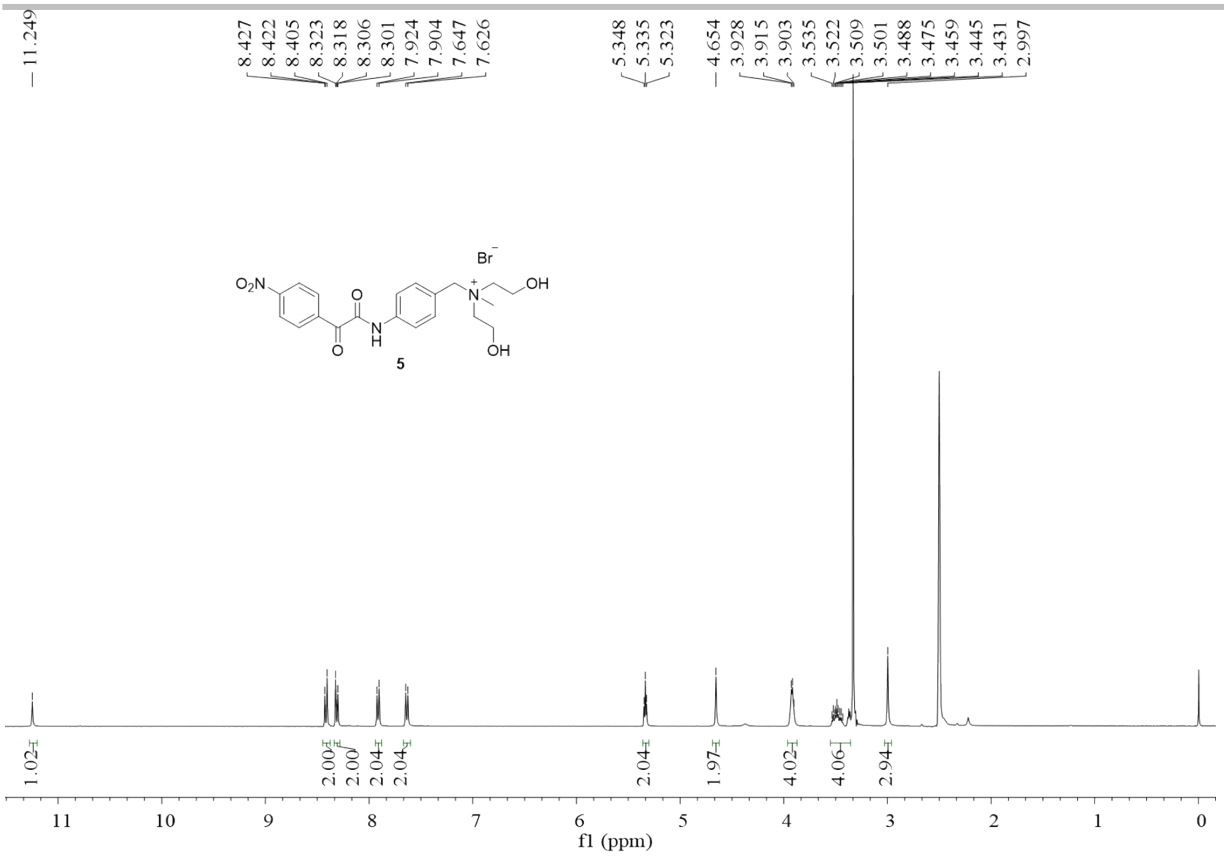
60
40
20
0
f1 (ppm)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8.301
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5.348
5.335
5.323
4.654
3.928
3.915
3.903
3.535
3.522
3.509
3.501
3.488
3.475
3.459
3.445
3.431
2.997
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OH
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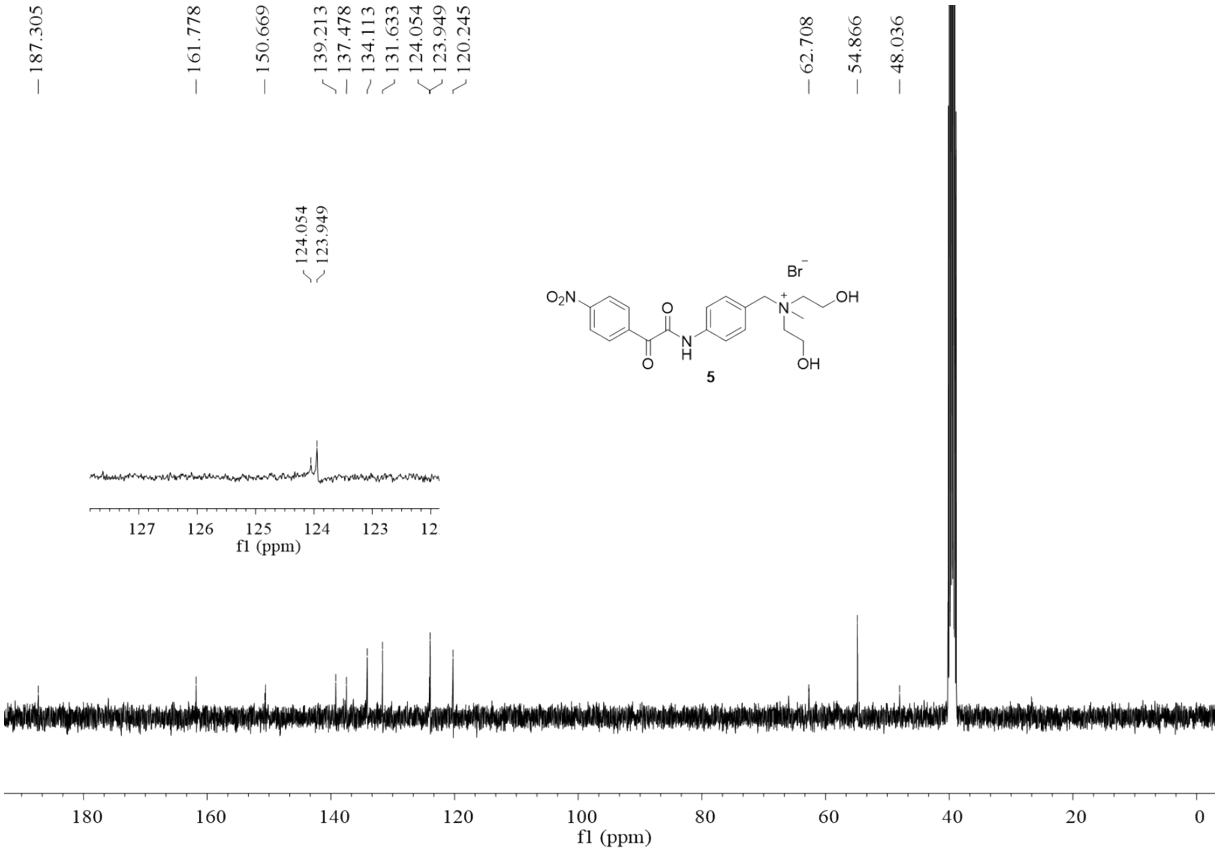
f1 (ppm)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O2N
OH
OH
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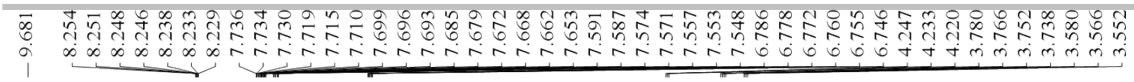
f1 (ppm)
f1 (ppm)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7.693
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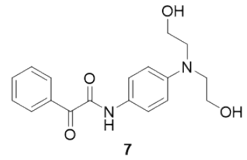
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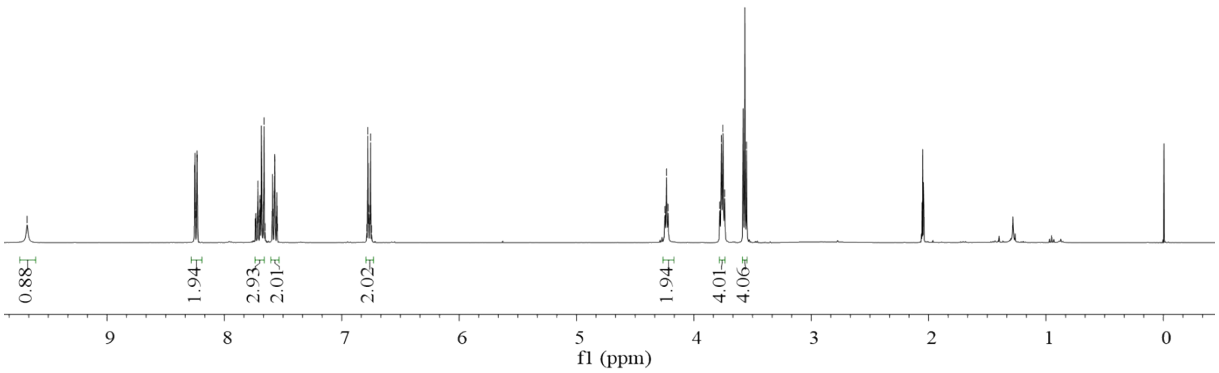
O
OH

7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4
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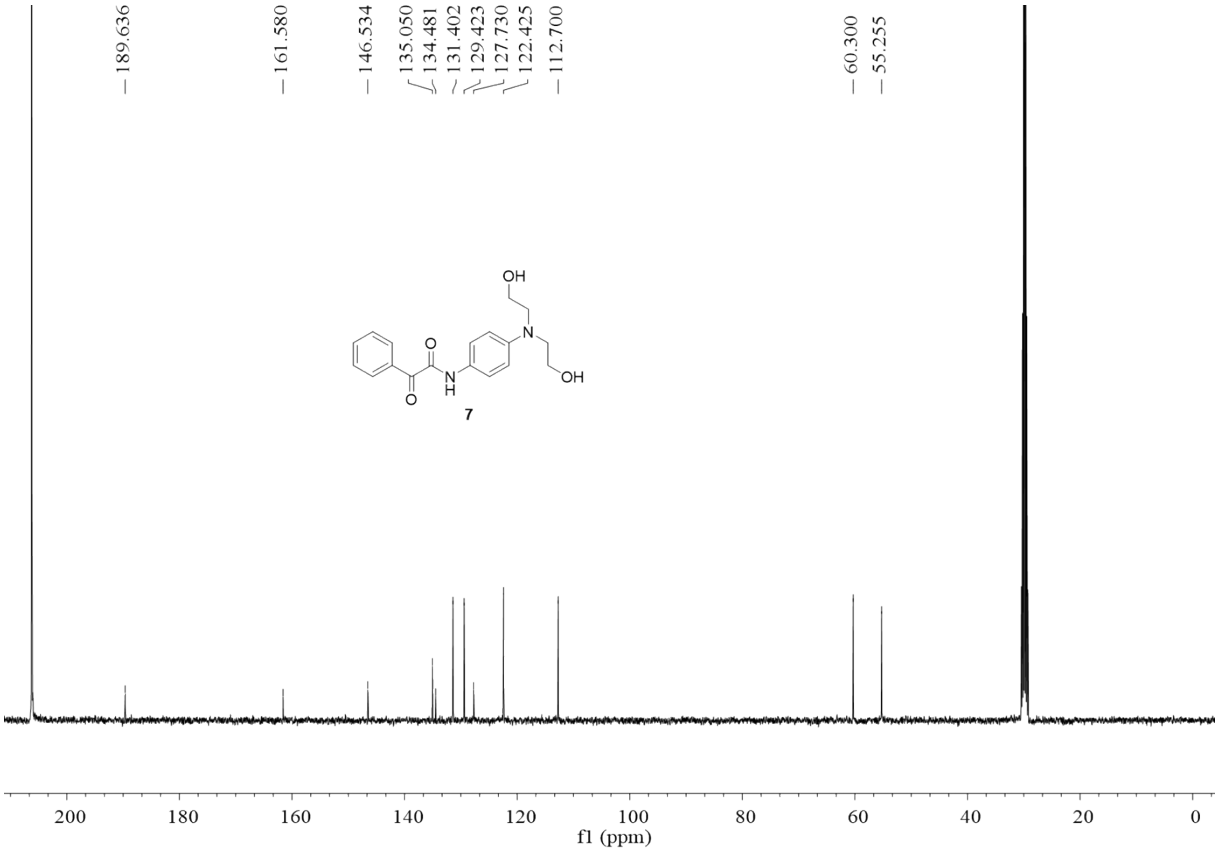
f1 (ppm)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O
OH
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60
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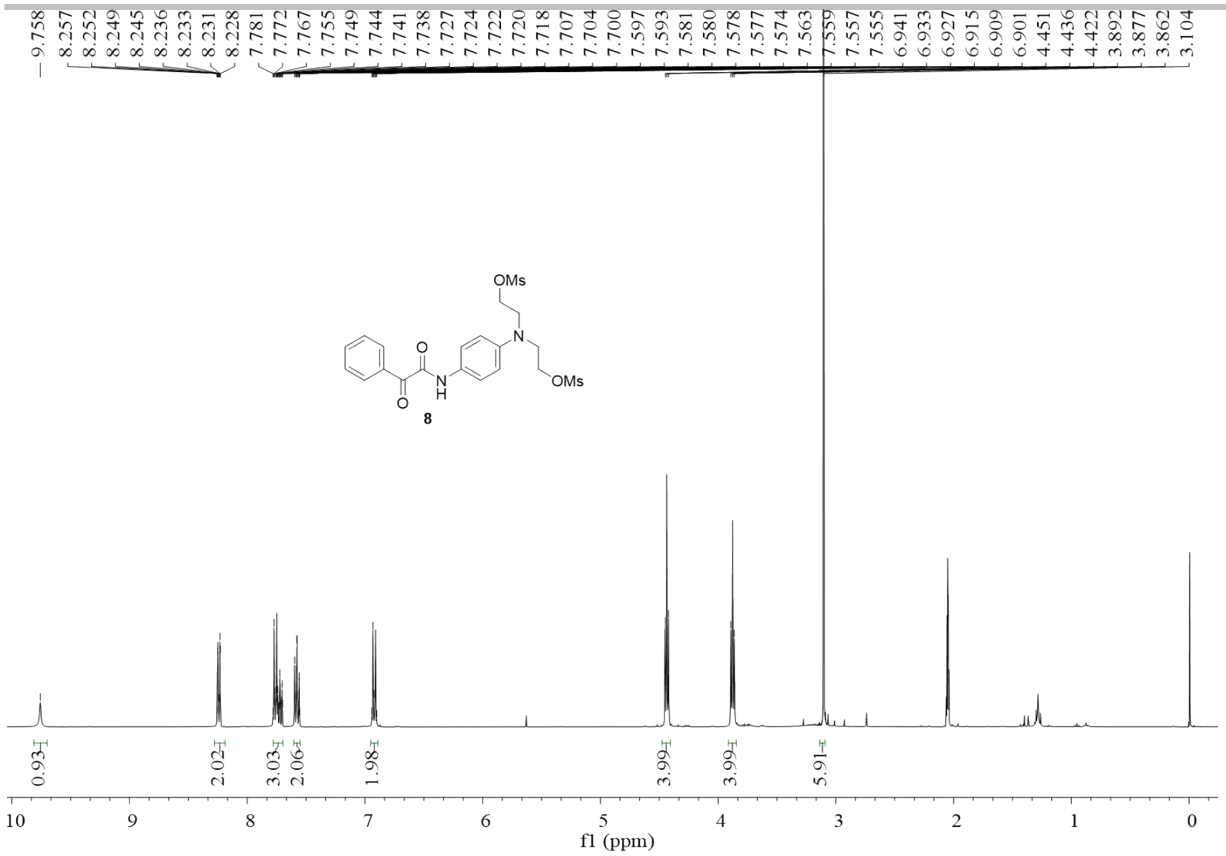
20
0
f1 (ppm)

7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7.741
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3.104
OMs
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N
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O
OMs
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2.06
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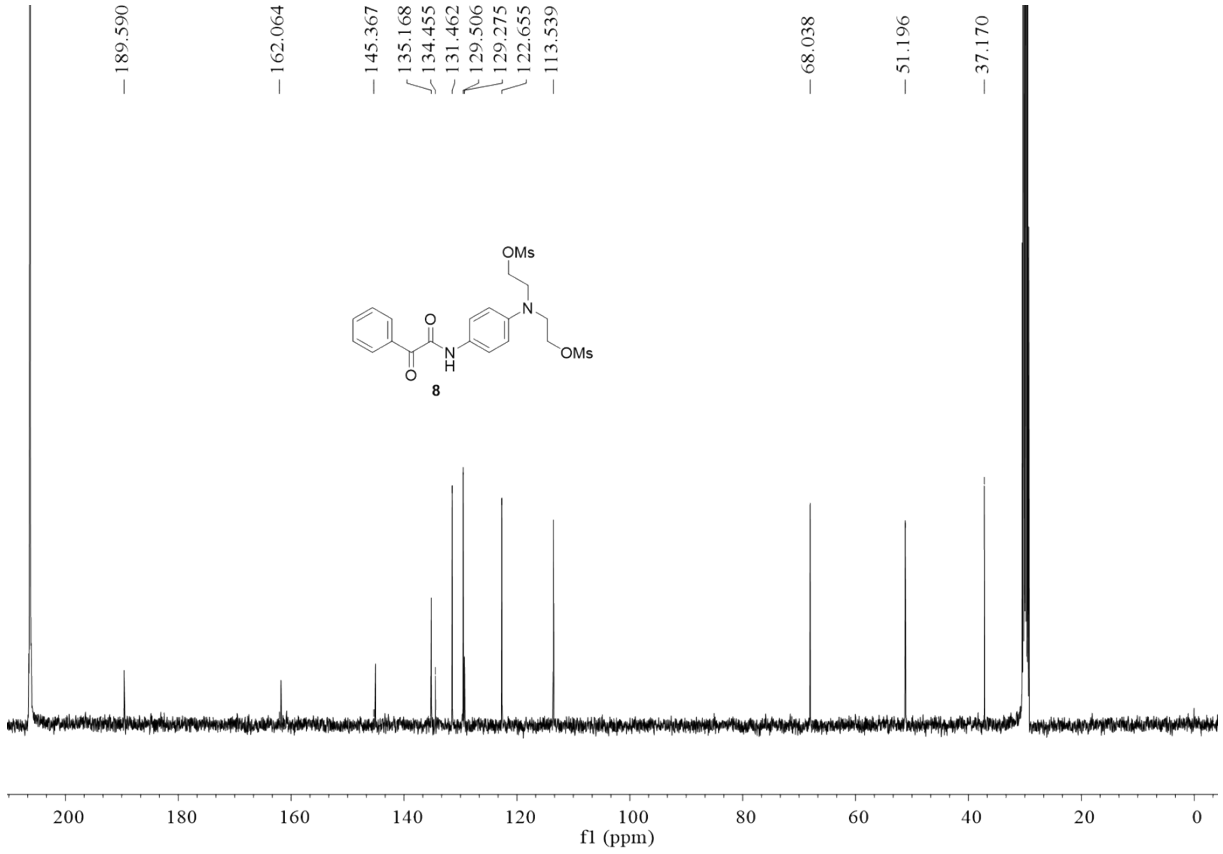
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37.170
OMs
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140
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100
80
60
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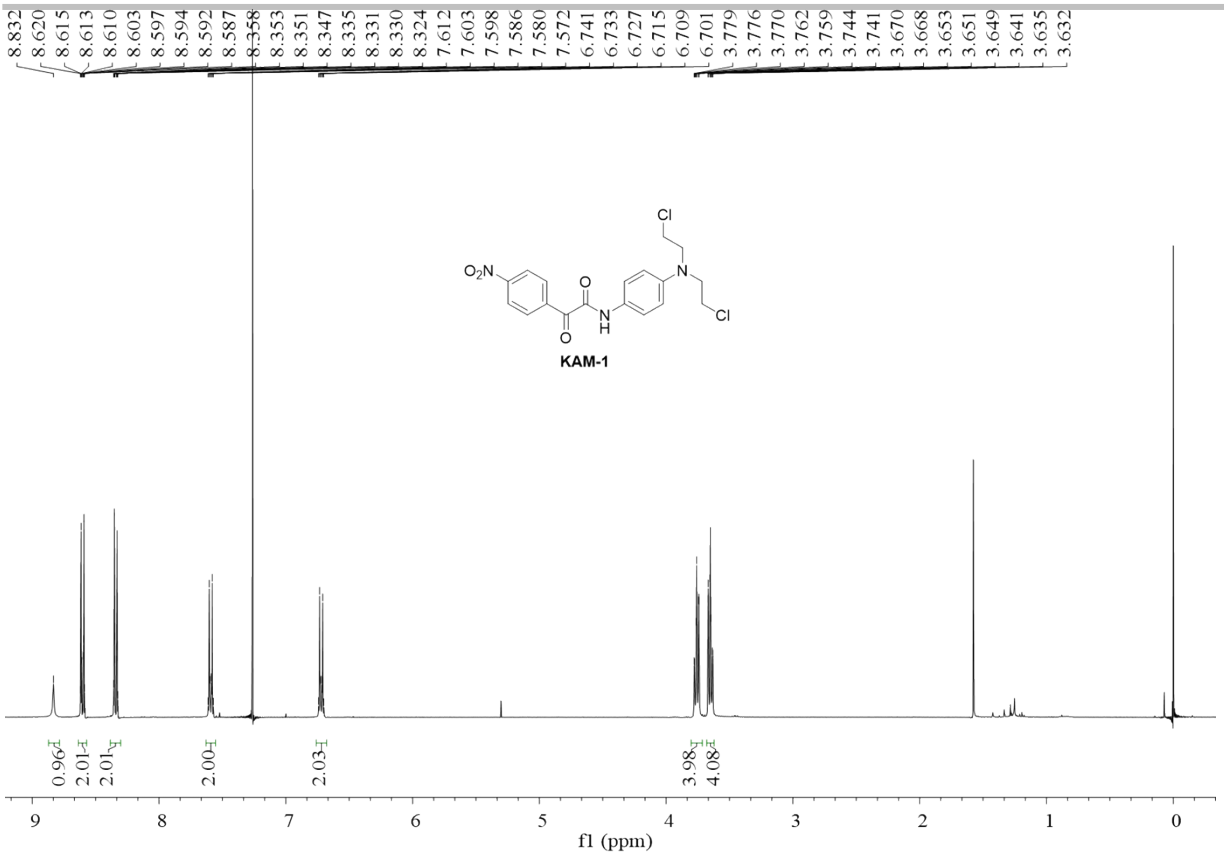
20
0
f1 (ppm)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8.347
8.335
8.331
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8.324
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7.598
7.586
7.580
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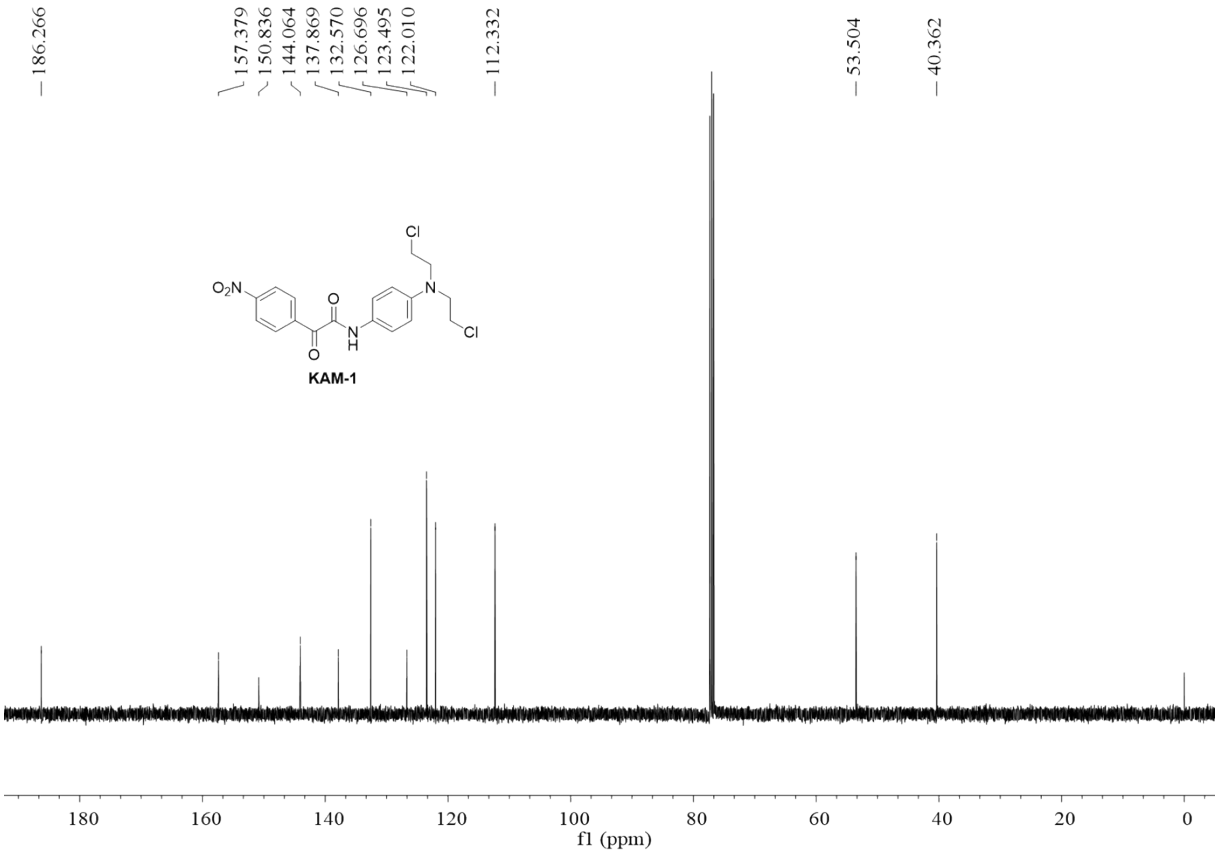
2.03
3.98
4.08
f1 (ppm)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O
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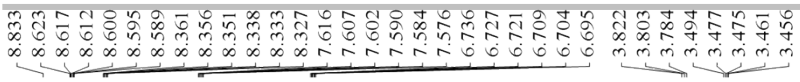
H
Cl
KAM-1
f1 (ppm)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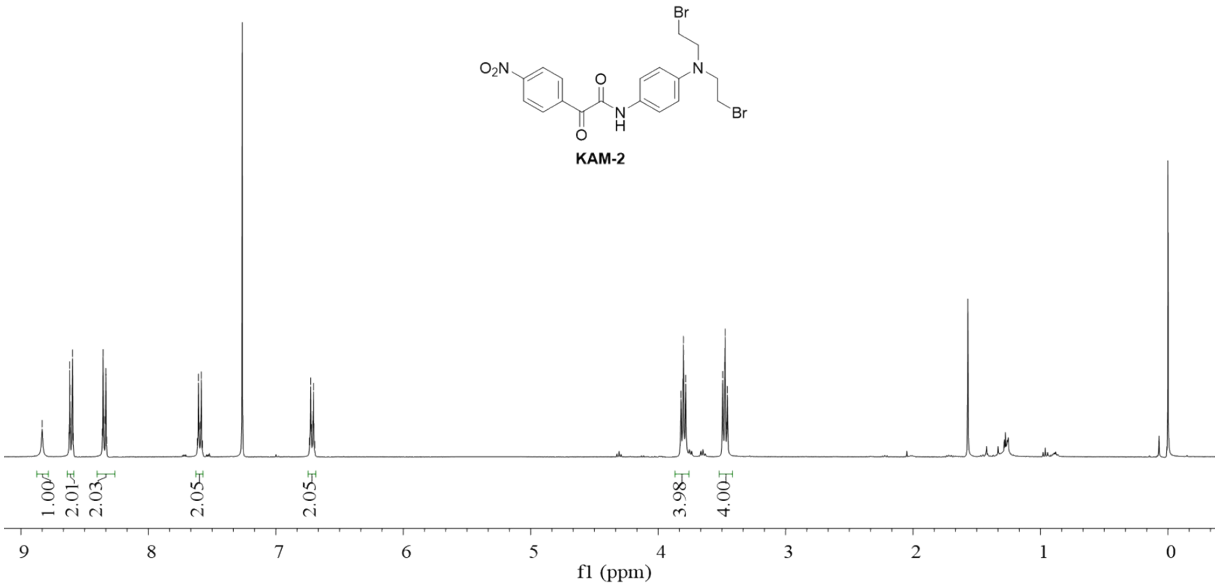
3.456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Br
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9
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7
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5
4
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1
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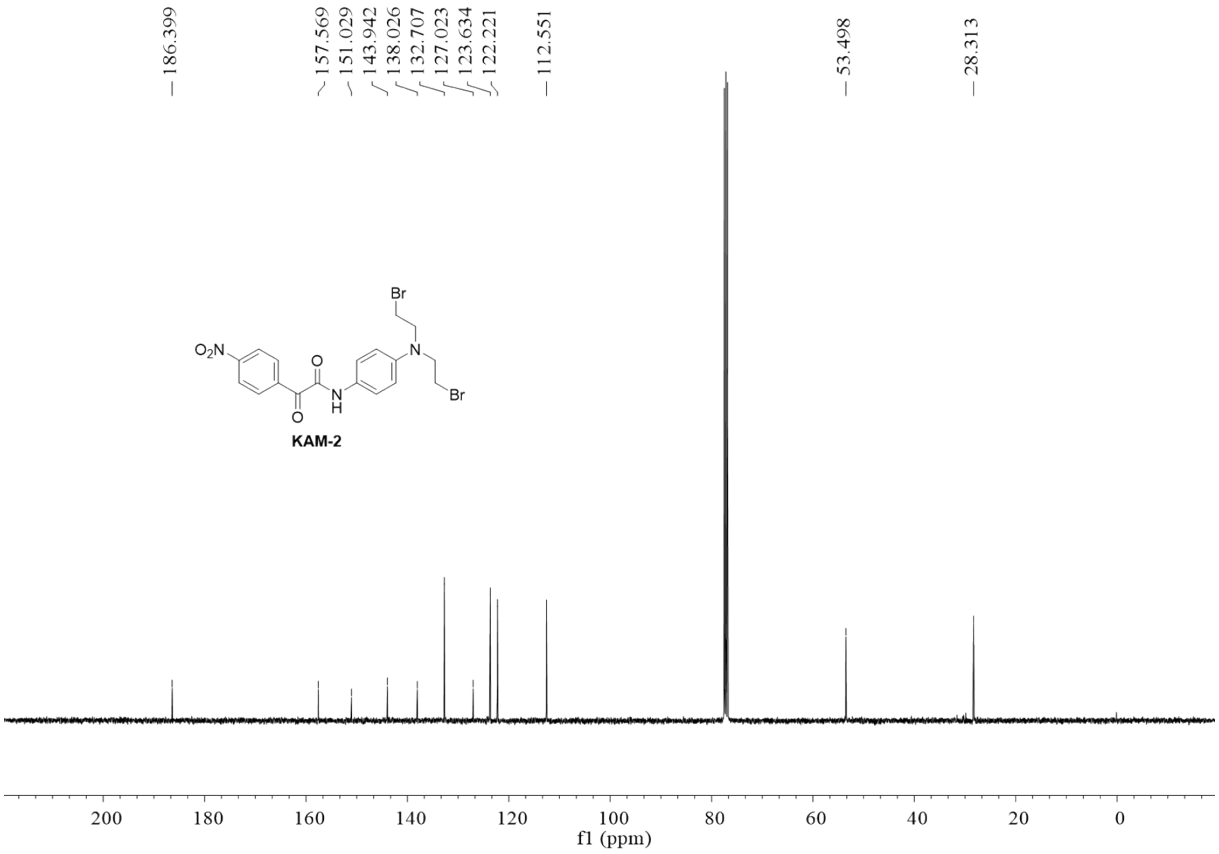
f1 (ppm)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20
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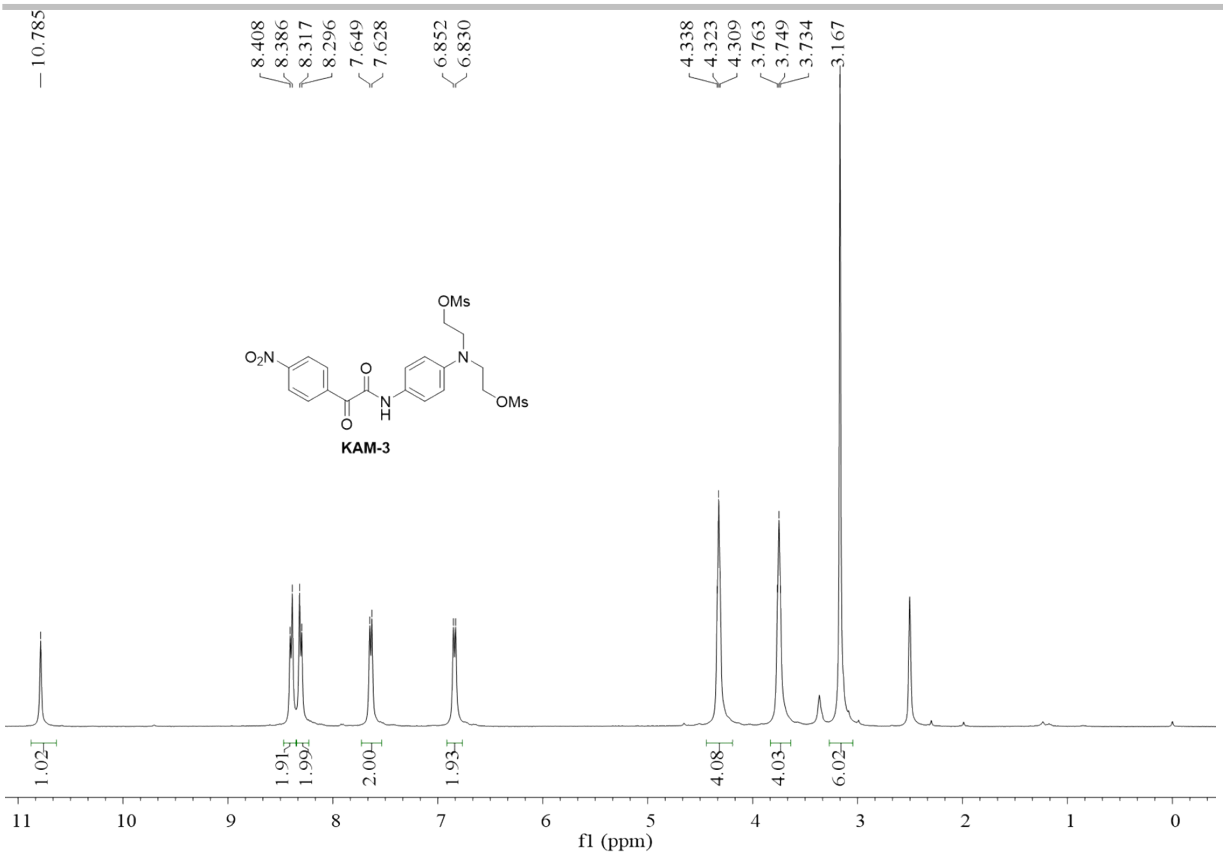
f1 (ppm)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3.749
3.734
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OMs
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N
H
N
OMs
O
KAM-3
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1.91
1.99
2.00
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6.02
11
10
9
8
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5
4
3
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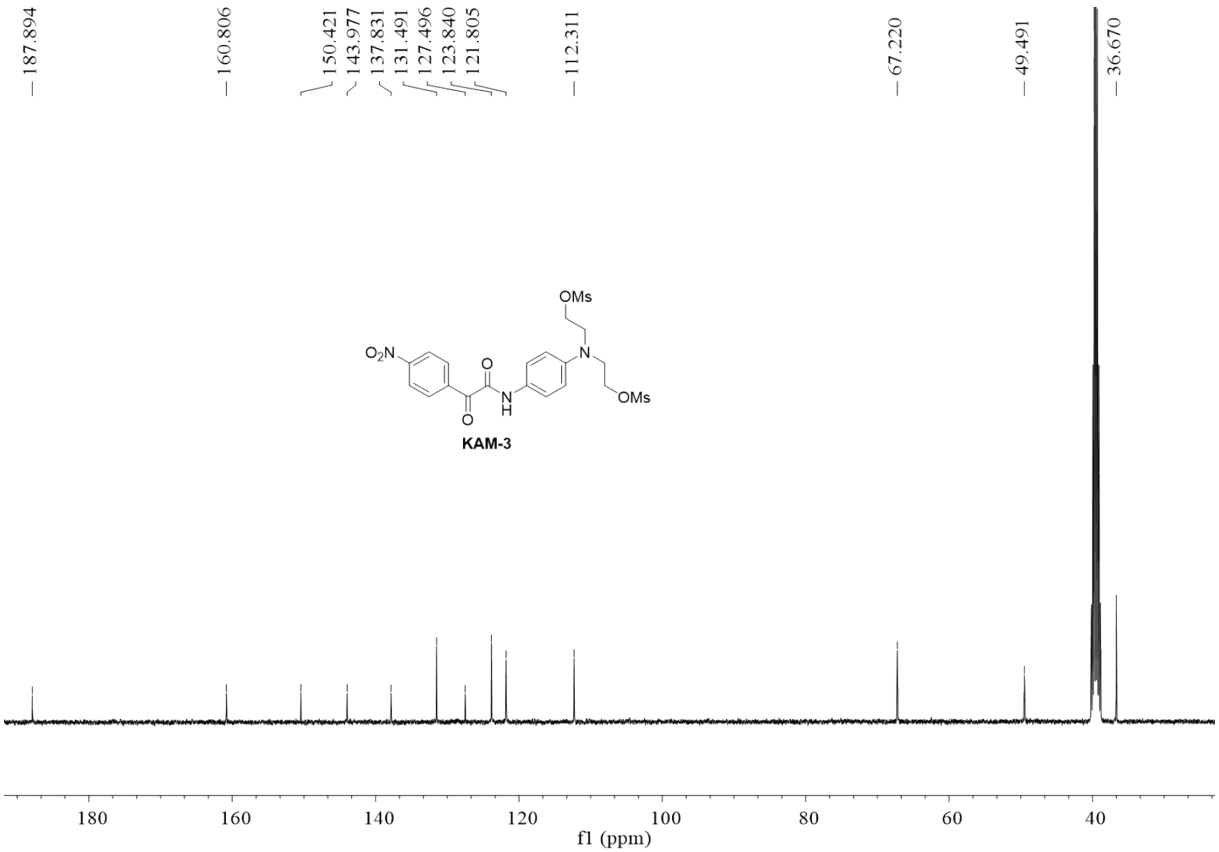
0
f1 (ppm)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KAM-3
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140
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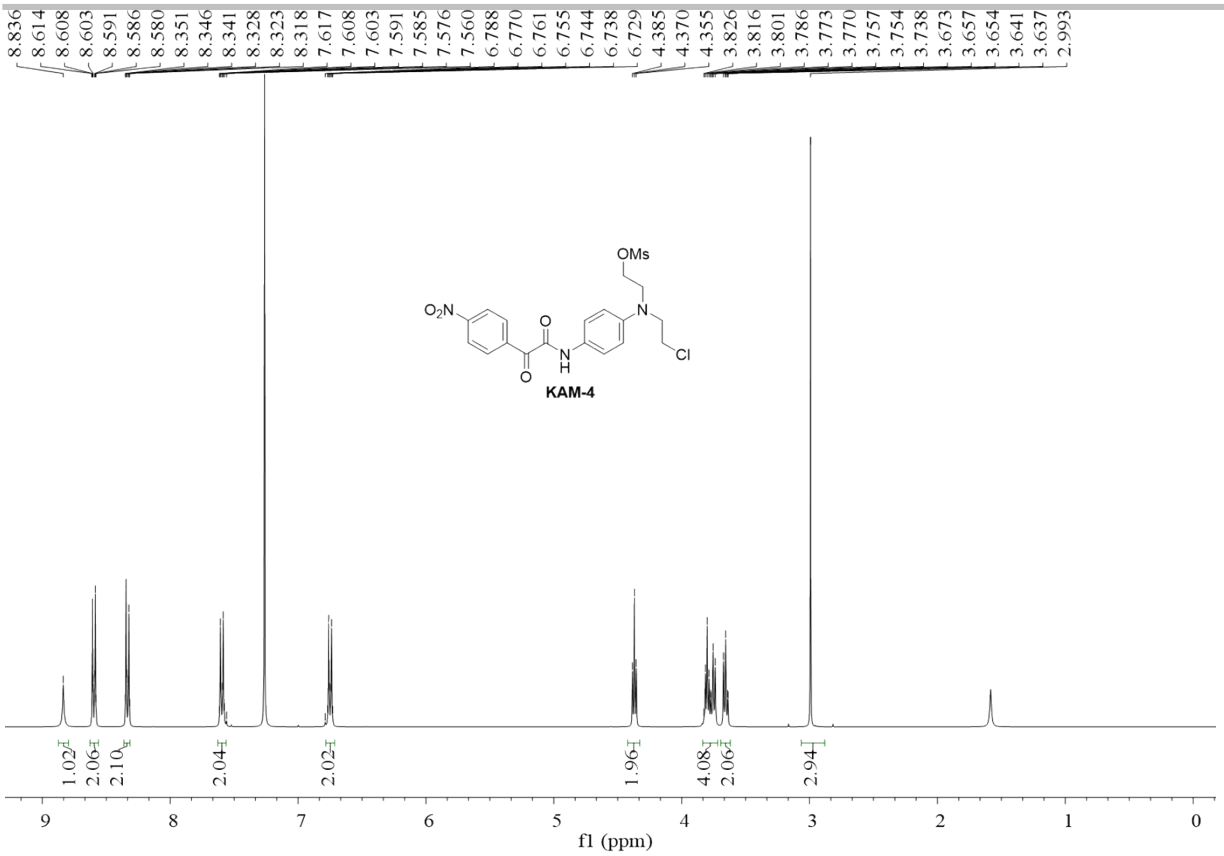
60
40
f1 (ppm)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4.370
4.355
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5
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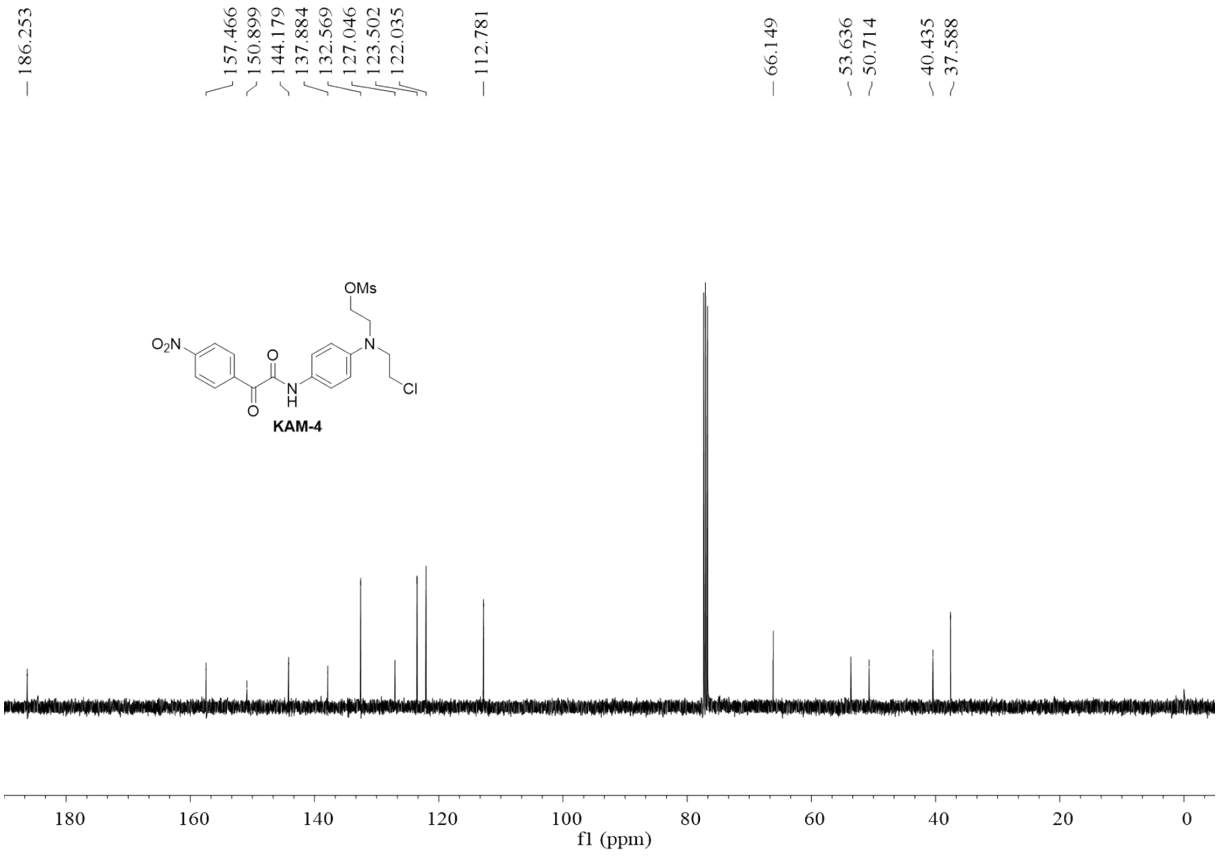
f1 (ppm)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123.502
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112.781
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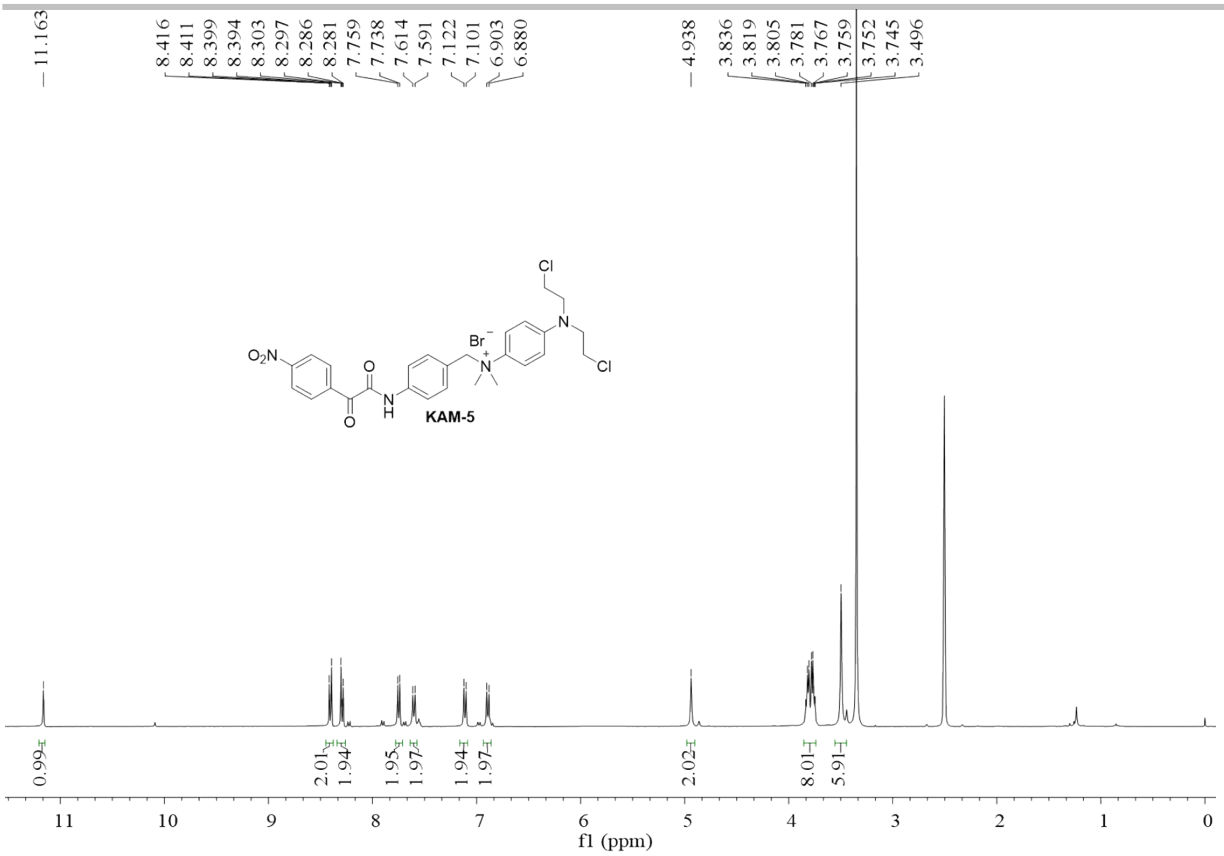
50.714
40.435
37.588
OMs
O2N
O
N
H
O
N
Cl
KAM-4
180
160
140
120
100
80
60
40
20
0
f1 (ppm)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7.122
7.101
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6.880
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3.836
3.819
3.805
3.781
3.767
3.759
3.752
3.745
3.496
KAM-5
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1.97
1.94
1.97
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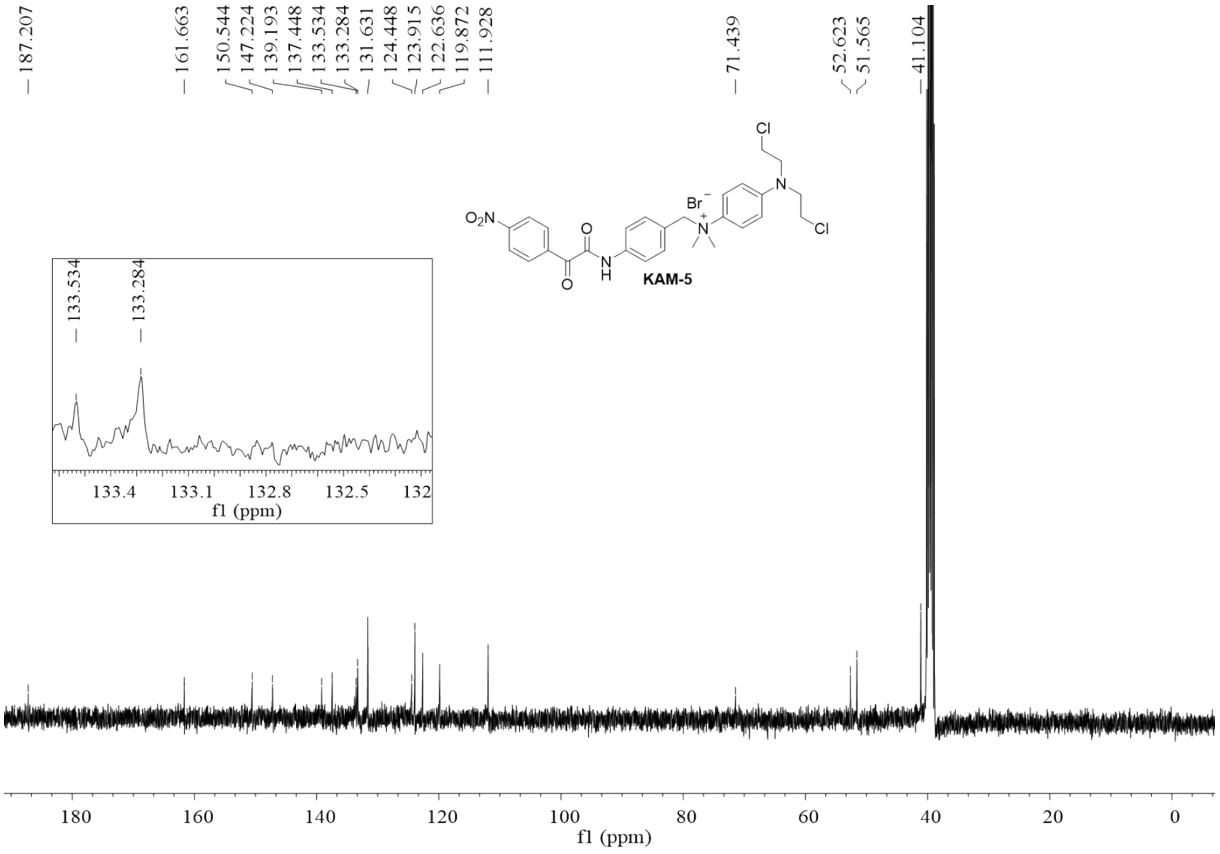
5.91
f1 (ppm)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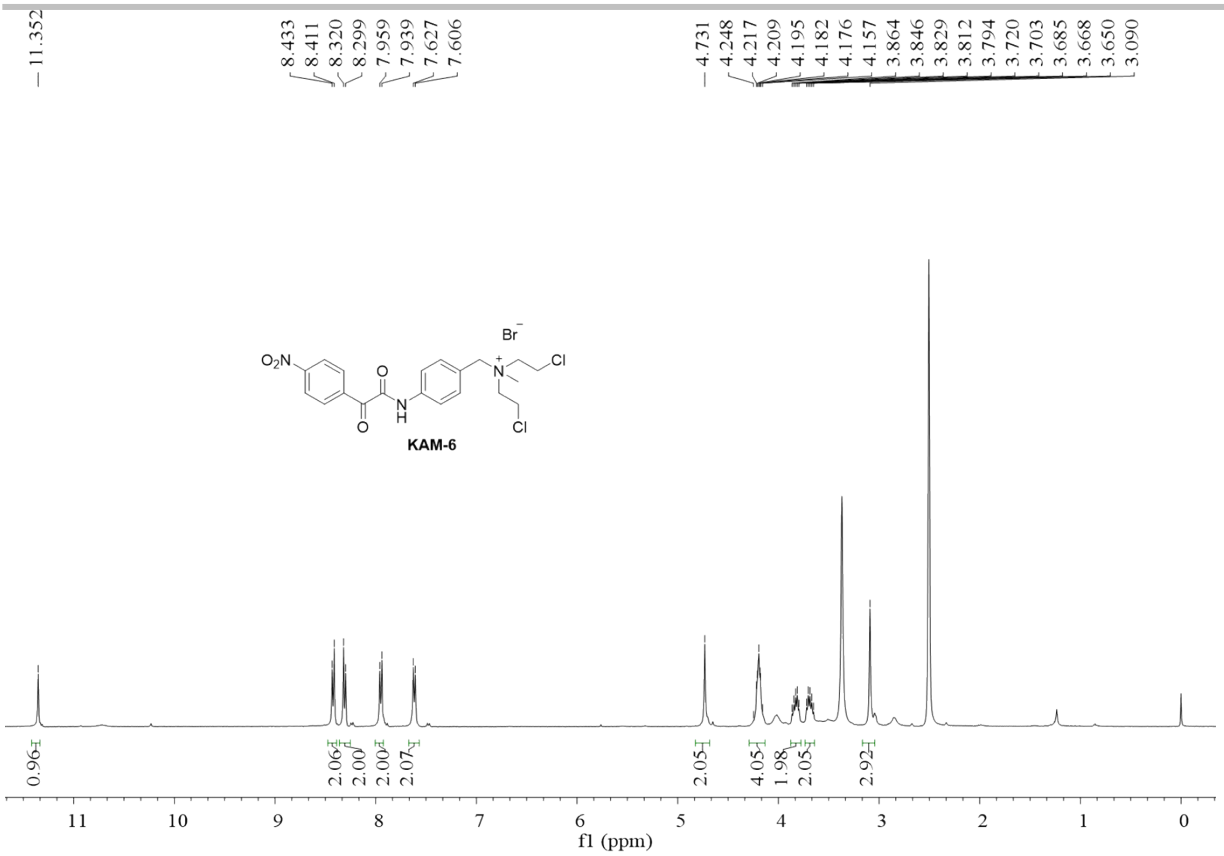
51.565
41.104
KAM-5
133.534
133.284
f1 (ppm)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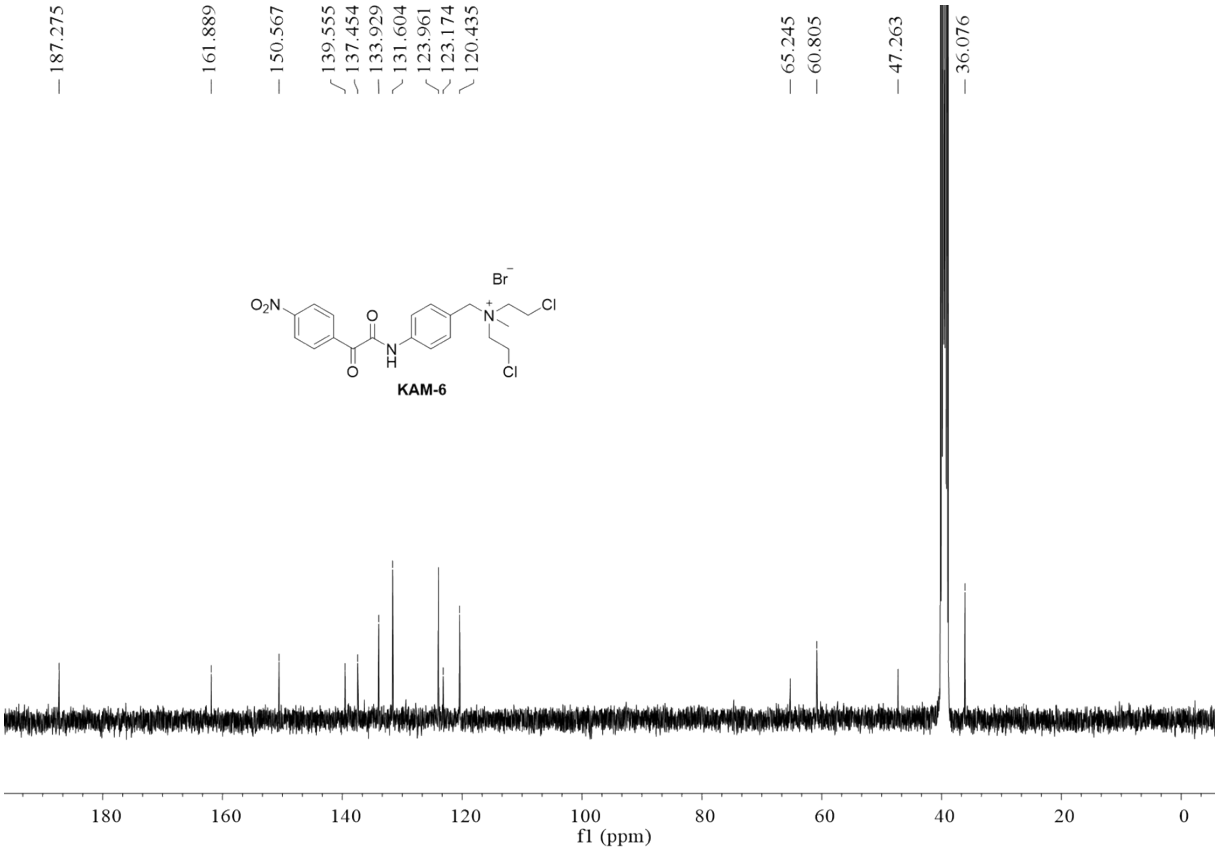
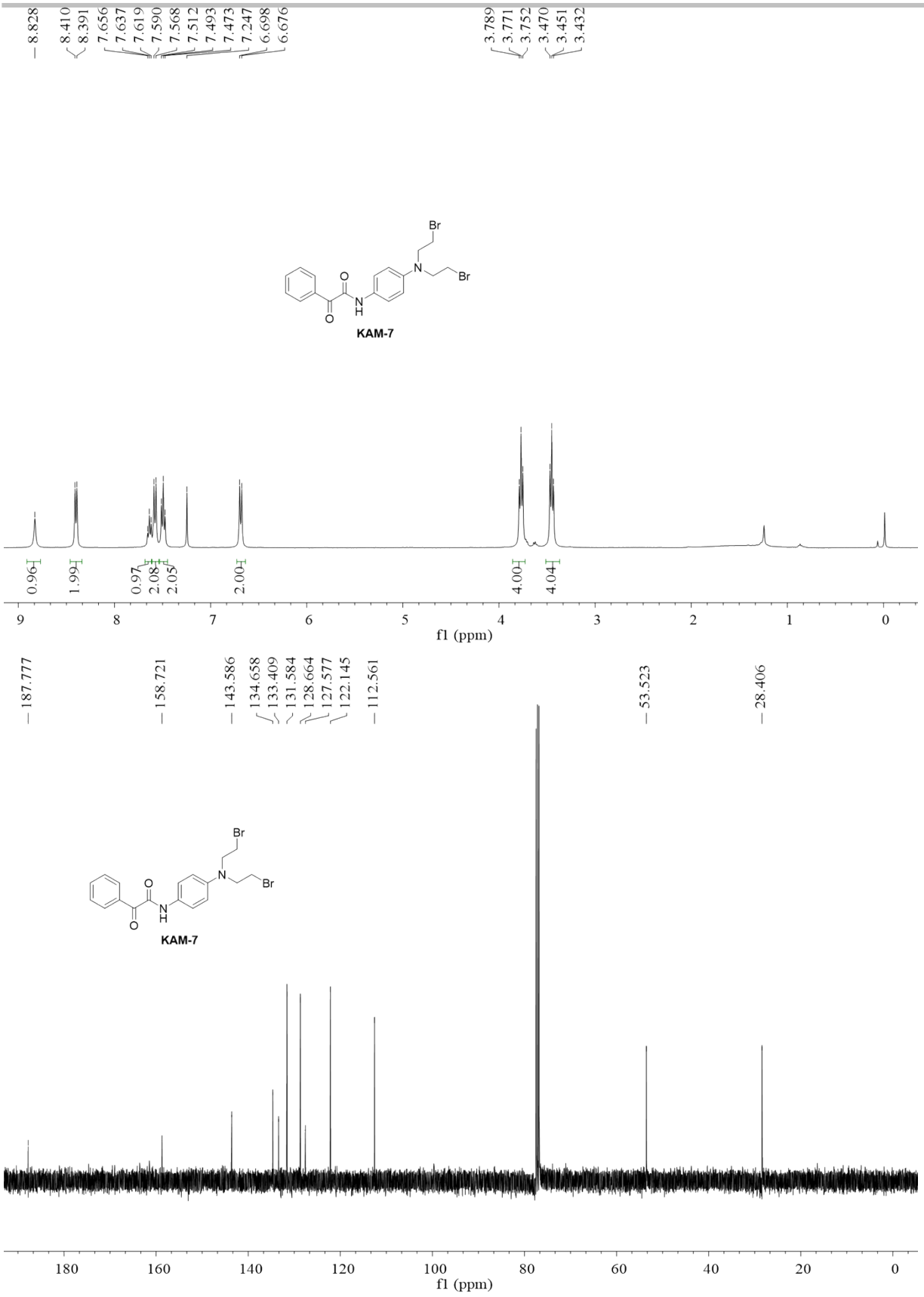
8.299
7.959
7.939
7.627
7.606
4.731
4.248
4.217
4.209
4.195
4.182
4.176
4.157
3.864
3.846
3.829
3.812
3.794
3.720
3.703
3.685
3.668
3.650
3.090
Br⁻
O2N
O
N
H
Cl
KAM-6
0.96
2.06
2.00
2.00
2.07
2.05
4.05
1.98
2.05
2.92
f1 (ppm)


187.275
161.889
150.567
139.555
137.454
133.929
131.604
123.961
123.174
120.435
65.245
60.805
47.263
36.076
Br⁻
O2N
O
N
H
Cl
KAM-6
f1 (ppm)


8.828
8.410
8.391
7.656
7.637
7.619
7.590
7.568
7.512
7.493
7.473
7.247
6.698
6.676
3.789
3.771
3.752
3.470
3.451
3.432
Br
N
Br
O
N
H
O
KAM-7
0.96
1.99
0.97
2.08
2.05
2.00
4.00
4.04
9
8
7
6
5
4
3
2
1
0
f1 (ppm)
187.777
158.721
143.586
134.658
133.409
131.584
128.664
127.577
122.145
112.561
53.523
28.406
Br
N
Br
O
N
H
O
KAM-7
180
160
140
120
100
80
60
40
20
0
f1 (ppm)

Analytical and biological experiments

HPLC analysis

HPLC analysis was performed on LC-10A HPLC system (C18 column, 4.6 mm × 200 mm, LC-10ATvp pumps, and SPD-10Avp UV detector).

The gradients of solvent A (CH_3CN, 0.1% TFA) and B (H_2O, 0.1% TFA) are listed below:

Time (min)	A (CH_3CN, 0.1% TFA) (%)		
	KAM-1–KAM-4, KAM-7	KAM-5, KAM-6	KAM-2 + H_2O_2
0–5	50	20	10
5–20	50–95	20–95	10-95
20–25	95	95	95

Cytotoxicity assays

The cytotoxicity of adhesive cells was determined using MTT assay. All the cell lines were purchased from American Tissue Culture Collection (ATCC, Rockville, MD, USA). Cells were planked in a 96 well plate with a concentration of 6×10^4 cells per well and cultured at 37 °C with 5% CO_2 for 24 h. Then cells were treated with tested compounds and incubated for an additional 72 h. MTT (5 mg/mL, 20 μL) was added and the plate was kept at 37 °C for another 4 h. Then the medium was carefully removed. Dimethyl sulfoxide (150 μL per well) was added and oscillated gently to make crystal dissolved. The absorbance at 490 nm was measured using a microplate reader.

The cytotoxicity of suspension cell HL-60 was determined using cell counting kit-8 (CCK-8) (MCE, NJ, USA) according to the manufacturer's protocol. HL-60 cells (1×10^4 per well) were seeded in 96-well plates and the plates were placed at 37 °C with 5% CO_2 for 12 h. Then cells were treated with tested compounds and incubated for an additional 72 h. CCK-8 (10 μL) was added and the plates were incubated at 37 °C with 5% CO_2. for another 4 h. The fluorescent signal was recorded at the wavelength of 450 nm on a microplate reader (Thermo Labsystems, Helsinki, Finland). In case of *N*-acetylcysteine (NAC) pretreatment, cells were treated with 20 mM NAC for 1 h at 37 °C, and the following procedures were the same as described above.

The percentage of cell death was calculated using the formula:

$$\text{Cell death\%} = 1 - \frac{\text{mean OD of drug treated cells} - \text{mean OD of medium}}{\text{mean OD of drug untreated cells} - \text{mean OD of medium}} \times 100\%$$

Selectivity of KAM-2 toward H_2O_2

The stock solutions of reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive sulfur species (RSS) were prepared as follow:

H_2O_2, *tert*-butylhydroperoxide (TBHP), and hypochlorite (NaOCl) were purchased from 30%, 70% and 13% commercial aqueous solutions, respectively.

H_2O_2 stock solution (100 mM) was prepared by adding 11 μL of 30% (w/v) H_2O_2 to 989 μL of H_2O.[4]

TBHP stock solution (100 mM) was prepared by adding 12 μL of 70% (w/v) TBHP to 998 μL H_2O.[4]

NaOCl stock solution (100 mM) was prepared by adding 22 μL of 13% (w/v) NaOCl to 978 μL H_2O.[4]

Hydroxyl radical (OH•) and *tert*-butoxy radical (^{t}BuO•) stock solutions (200 μM) were generated by reaction of 1 mM Fe^{2+} with 200 μM H_2O_2 or 200 μM TBHP respectively.[5]

Peroxynitrite solution ($ONOO^-$) was freshly prepared before use as reported.[4] A mixture of H_2O_2 (0.7 M, 1.5 mL), $NaNO_2$ solution (0.6 M, 3 mL) in an ice bath was acidified with HCl (0.6 M, 1.5 mL), and NaOH solution (1.5 M, 3 mL) was added within 1−2 s to make the solution alkaline. The final concentration of $ONOO^-$ was determined by measuring the absorbance at 302 nm ($\varepsilon = 1670\ M^{-1}\ cm^{-1}$). $[ONOO^-]$ (mM) = $Abs_{302\ nm}$ /1.67.

NO stock solution (100 mM) was freshly prepared before use by dissolving 29.8 mg sodium nitroprusside dihydrate in 1000 μL H_2O.

GSH (200 mM), Cys (200 mM) and Hcy (200 mM) stock solutions were prepared by adding 62.3 mg GSH, 12.1 mg Cys and 13.52 mg Hcy to 500 μL H_2O, respectivity.

KAM-2 (100 μM) and ROS (1 mM), RNS (1 mM), RSS (5 mM), or Fe^{2+} (100 mM) in PBS (pH 7.4, 10 mM)/MeCN (v/v, 80/20) solution was incubated at r.t., for 1 h, and the percentage of decomposed **KAM-2** was determined by RP-HPLC.

Annexin V-FITC/PI assay

HL-60 cells were planted at 5 × 10^5 per well in 6-well plate and incubated for 24 h. Cells were treated with different concentrations of **KAM-2** or MEC for 48 h, In case of NAC pretreatment, cells were treated with 20 mM NAC for 1h, and then incubated with 10 μM KAM-2 for 48 h. Cells were collected and washed twice with ice-cold PBS, resuspended in 300 μL 1× binding buffer. Annexin V-FITC (KGA105, Keygentec, China) (5 μL) was added into each sample and incubated for 15 min at r.t. in darkness. PI (KGA105, Keygentec, China) (5 μL) was added into cells and stained for 5 min at r.t. in darkness. At last, 200 μL 1× binding buffer was added, and cellular apoptosis was analysed by using flow cytometry with a BD FACScalibur (BD Bioscience, Heidelberg, Germany).

Cell cycle analysis

HL-60 cells were planted at 5 × 10^5 per well in 6-well plate and incubated for 24 h. Cells were treated with different concentrations of **KAM-2** or MEC for 48 h, In case of NAC pretreatment, cells were treated with 20 mM NAC for 1 h, and then incubated with 10 μM **KAM-2** for 48 h. Cells were washed in ice-cold PBS, and fixed in ice-cold 70% alcohol for at least 24 h. After that, cells were washed twice in ice-cold PBS, resuspended in 0.4 mL PBS and treated with 30 μL RNase A (1 mg/mL) (Thermo Scientific, MA, USA). Then 50 μL PI (500 ug/mL) (KGA105, Keygentec, Nanjing, China) was added into cells to stain the cellular DNA, and the staining process lasted 30 min at 4 °C in darkness. The DNA content of the stained cells was analyzed by flow cytometry with a BD FACScalibur (BD Bioscience, Heidelberg, Germany), and the cycle distribution was quantified.

Cell mitochondrial membrane potential assay

HL-60 cells were planted at 5 × 10^5 per well in 6-well plate and incubated for 24 h. Cells were treated with different concentrations of **KAM-2** or MEC for 48 h. In case of NAC pretreatment, cells were treated with 20 mM NAC for 1 h, and then incubated with 10 μM **KAM-2** for 48 h. The cells were stained with the lipophilic cationic dye JC-1 (KGA602, Keygentec, China) according to the manufacturer's instruction. The percentage of cells with healthy or collapsed mitochondrial membrane potentials was monitored by flow cytometry with a BD FACScalibur (BD Bioscience, Heidelberg, Germany).

Western blot Assay

HL-60 cells were planted at 5 × 10^5 per well in 6-well plate and incubated at 37 °C for 24 h. Then cells were treated with different concentration of **KAM-2** or 10 μM MEC for indicated time. In case of NAC pretreatment, cells were treated with 20 mM NAC for 1 h, and then incubated with 20 μM **KAM-2** for 48 h.

HL-60 cells were placed on ice for 10 min, followed by two rounds of centrifugation at 10000 rpm for 15 min and protein concentration was measured using a BCA (KGA902, Keygentec, China) protocol. Protein was stored at -70 °C until further use. SDS-PAGE (KGP113, Keygentec, China) was used to separate the proteins and then transferred to a nitrocellulose membrane. Membranes were blocked for 1 h in 5% milk in Tris-buffered saline. Membranes were incubated in antibodies, including anti-Caspase3 antibody (1:1000) (ab13847, Abcam, UK), anti-Cleaved Caspase3 antibody (1:1000) (ab2302, Abcam, UK), anti-Caspase9 antibody (1:1000) (ab32539, Abcam, UK), anti-Cleaved Caspase9 antibody (1:1000) (ab2324, Abcam, UK), anti-Bax antibody (1:5000) (ab32503, Abcam, UK), anti-Bcl-2 antibody (1:2000) (ab182858, Abcam, UK), anti-phosphorylated H_2AX antibody (γ-H_2AX; 1:1000) (ab81299, Abcam, UK), anti-BRCA1 antibody (1:1000) (ab191042, Abcam, UK), anti-RAD51 antibody (1:10000) (ab133534IK, Abcam, UK), anti-PARP1 antibody (1:1000) (ab191217, Abcam, UK), anti-Cleaved PARP1 antibody (1:1000) (ab32046, Abcam, UK) overnight at 4 °C. Following three washes in TBST (1×) (KGP103X, Keygentec, China), and then incubated with the secondary antibodies conjugated to horseradish peroxidase at r.t for 2 h. Membranes were washed three times in TBST and then incubated in an enhanced chemiluminescence (ECL) (KGP116, Keygentec, China) detection substrate for 5 min. Densitometry of the appropriate bands was performed using G: Box ChemiXR5 (Syngene, MD, USA).

Denaturing alkaline agarose gel electrophoresis

Linearization of Plasmid pBR322

pBR322 plasmid DNA (Thermo Scientific, MA, USA) was linearized by digestion with EcoR I (Thermo Scientific, MA, USA) according to the user instruction. Briefly, pBR322 plasmid DNA (4 μL) was incubated with FastDigest EcoRI (2 μL), EcoR I Buffer (2 μL) and nuclease-free water (12 μL) for 1 h at 37 °C. Following the restriction digest, the DNA was concentrated via ethanol precipitation and the resulting DNA pellet was resuspended in an appropriate volume of 10 mM Tris-HCl (pH 8.8), 0.1 mM Na_2EDTA to give a DNA stock concentration of no less than 100 ng/μL.

DNA cross-linking assay

DNA cross-linking reactions were carried out by treating linearized pBR322 plasmid DNA with various concentrations of test compounds (**KAM-2**, **KAM-7**) or MEC. H_2O_2 (2 μL) was mixed with **KAM-2** (2 μL), pBR322 (4 μL), and 50 mM phosphate buffer (2 μL, pH 7.2) to give a final volume of 10 μL. Stock solutions of **KAM-2**, **KAM-7**, MEC and H_2O_2 was added to a final concentration of 0-100 μM, 50 μM, 50 μM and 10 mM, respectively. The samples were incubated at 37 °C.

Urea/heat-denaturing agarose gel electrophoresis

The denaturing agarose buffer consisted of 0.5 mg/mL bromophenol blue, 8 M urea, 1 % (v/v) NP-40 and 1 mM Tris (pH 8) in DNase-free water. The mixture of denaturing agarose gel loading buffer (25 µL) and linearized pBR322 plasmid DNA (5 µL) was heat-denatured at 95 °C for 5 min and then immediately placed in an ice-water bath for 5 min. For urea/heat denaturing gel electrophoresis, 1.2% agarose gels containing 1 M urea were prepared. The electrophoresis buffer (1 × TAE) was supplemented with 1 M urea. The gel was run for 2–3 h at 40 V. After electrophoresis, the gel was washed at least three times with 1× TAE buffer (pH 8) and then stained with 0.5 mg/mL ethidium bromide (EB) for at least 30 min. The gel was visualised under UV light and photographed using Gel Doc™ XR+ System (BIO-RAD, CA, USA).

Comet assay

The comet assay was carried out as described.[6] HL-60 cells were planted at 5×10^5 per well in 6-well plate and incubated at 37 °C for 24 h. Then cells were treated with 20 µM **KAM-2** or 10 µM MEC containing EMEM for 48 h. In case of NAC pretreatment, cells were treated with 20 mM NAC for 1 h, and then incubated with 20 µM **KAM-2** for 48 h.

The fully frosted microscope slides were covered with 100 µL 1% normal melting agarose (NMA) at 45 °C, immediately covered with a coverslip, and then kept at 4 °C for 4−8 min to solidify the agarose. Then the coverslips were removed and the cell suspension (400 cells per 100 µL PBS) was mixed with 1% low melting agarose (100 µL) at 37 °C. The coverslip was gently replaced and allowed to solidify on ice for 8 min. The coverslip was removed, and the slides were submersed in lysis solution (pH = 8.2−8.5, 2 M NaCl, 30 mM EDTA, 1 mM Tris base, 1% Triton-X 100 and 10% DMSO) for 1–3 h at 4 °C. The slides were then removed from the lysing solution and washed for 30 min. Subsequenctly, electrophoresis was performed at 300 mA, 25 V at 4 °C for 25 min. The slides were washed with neutral PBS (pH = 7.4) three times for 10 min. The slides were drained and stained with 1.0 µg/mL DAPI, and observed with a fluorescent microscope (Olympus, Tokyo, Japan).

EB-DNA fluorescence quenching assay

Fluorescence spectral method was carried out by adding various concentrations of **KAM-2** (0, 10, 25, 50 and 75 µM) into EB-DNA solution (2.5 µM EB and 50 µM linearized pBR322 plasmid DNA) with or without H_2O_2 (10 mM) in Tris-HCl/NaCl buffer (pH 7.2, 5 mM Tris-HCl, 50 mM NaCl). The samples were excited at 300 nm and emission was recorded at 600 nm.[7]

Immunofluorescence microscopy of γ-H_2AX

The immunofluorescence assay was carried out as described.[8] HL-60 cells were planted at 5×10^5 per well in 6-well plate and incubated for 24 h. Cells were treated with 10 µM **KAM-2** or MEC for 12 h. In case of NAC pretreatment, cells were treated with 20 mM NAC for 1 h, and then incubated with 10 µM **KAM-2** for 12 h. **KAM-2** supplemented media was removed, and cells were allowed to grow in **KAM-2** free media for another 0–12 h. Then cells were fixed in 4% paraformaldehyde for 15 min, washed with PBS, and permeabilized in 0.2% Triton X-100. After blocked with blocking serum for 1.5 h, samples were incubated with a mouse monoclonal anti-γ-H_2AX antibody (ab81299, Abcam, UK) (1:1000) for 2 h, followed with FITC-conjugated goat-anti-mouse secondary antibody (1:500) for 1 h. To stain the nuclei, DAPI (Thermo Scientific, MA, USA) was added to the cells and incubated for another 15 min. The cover slip was then removed from the plate and mounted on to a glass slide, and observed with a fluorescent microscope (Olympus, Tokyo, Japan).

Real-time PCR

HL-60 cells were planted at 5×10^5 per well in 6 per well plate and incubated for 24 h. Cells were then treated with 20 µM **KAM-2** for 24 or 48 h. In case of NAC pretreatment, cells were treated with 20 mM NAC for 1 h, and the following procedures were the same as described above.

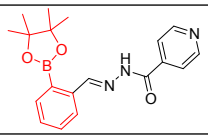
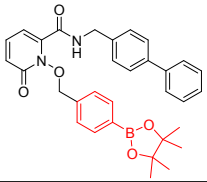
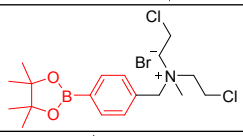
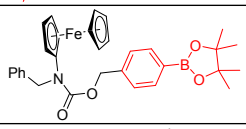
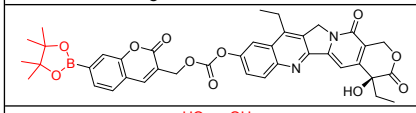
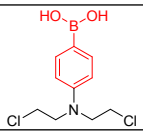
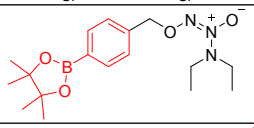
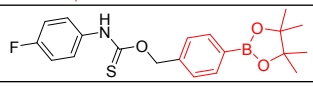
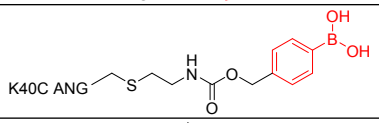
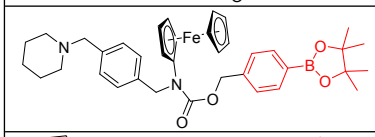
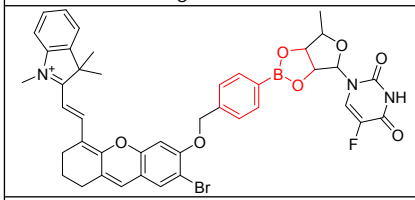
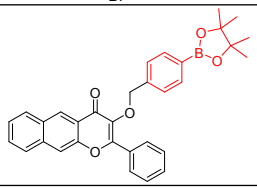
Total RNA was extracted using TRIzol (Thermo Scientific, MA, USA) and the concentration was determined using NanoDrop (λ = 260/280 nm (Nanodrop Technologies Inc, Wilmington, DE). Next, cDNA was synthesized from RNA with oligo (dT) as primer and AMV reverse transcriptase. The quantitative PCR amplification system was made up by mixing 20 µL of 2× Realltime PCR Master Mix (10 µL), 2 µL of forward and reverse primers, 1 µL cDNA template and 7 µL double-distilled H_2O.

The primer pairs are listed below. Samples were assayed in triplicate. The relative mRNA levels of Brca1, Parp and Rad51 were compared using the delta-delta cycle threshold (ΔΔCt) method. The GAPDH amplicon was used as internal control for normalization.

Genes	Foward primer	Reverse primer
Brca1	CCGTTGCTACCGAGTGTCTGTC	TGTTCCTGAGATGCCTTTGCCA
Parp1	GGCAGCAGCGACTCTCAGAT	TGTCCAGCAGGTTGTCAAGCAT
Rad51	GCCACCGCCCTTTACAGAACAG	GTCGCAGAAGCATCCGCAGAA

Supplementary Tables and Figures

Table S1 Selected H2O2-responsive prodrugs based on arylboronic acid/ester moiety.

Prodrug structure	Released drug	Bioactivity	Reference
	Salicylaldehyde isonicotinoyl hydrazine (SIH)	Cytoprotective	*J. Am. Chem. Soc.* 2006, **128**, 12424–12425
	MMP inhibitor	Reduce ischemia-reperfusion injury	*Angew. Chem. Int. Ed*. 2010, **49**, 6795–6797
	Nitrogen mustard	Anti-cancer	*J. Am. Chem. Soc*. 2011, **133**, 19278–19281
	Aminoferrocene	Anti-cancer	*J. Med. Chem*. 2012, **52**, 924-934
	Camptothecin derivative SN-38	Anti-metastatic cancer	*J. Am. Chem. Soc*. 2014, **136**, 13888-13894
	Nitrogen mustard	Anti-cancer	*J. Med. Chem*. 2014, **57**, 4498-4510
	Nitric oxide (NO)	NO donor	*Org. Lett.* 2014, **16**, 2610-2613
	Hydrogen sulfide (H_2S)	H_2S donor	*Angew. Chem. In.t Ed.*, 2016, **55**, 14638-14642
	Angiogenin (ANG)	Neuroprotective	*Angew. Chem. Int. Ed*. 2017, ***56***, 2619–2622
	Aminoferrocene	Anti-cancer	*Angew. Chem. Int. Ed*. 2017, **56**, 15545–15549
	5′-deoxy-5-fluorouridine (5-DFUR)	Anti-cancer	*Chem. Sci.* 2017, **8**, 7689–7695
	Carbon monoxide (CO)	CO donor	*Angew. Chem. Int. Ed*. 2018, **57**, 12415–12419

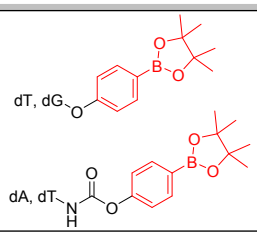
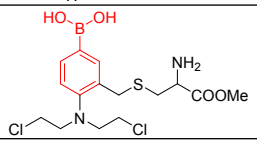
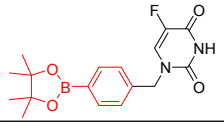
	Antisense oligonucleotides	Gene silencing	*Chem. Sci.* 2018, **9**, 1112–1118
	Nitrogen mustard	Anti-cancer	*J. Med. Chem*. 2018, **61**, 9132-9145
	5-Fluorouracil (5-FU)	Anti-cancer	*ACS Med. Chem. Lett.* 2019, **10**, 127-131.

Table S2 Calculated LopP values and Caco-2 permeability of KAM prodrugs.

	CLogP[a]	LogP[b]	Caco-2 permeability LogPapp, cm/s[c]
KAM-1	3.942	4.14	0.8777
KAM-2	4.222	4.62	0.8265
KAM-3	2.0936	1.86	0.6202
KAM-4	3.0178	3.01	0.6941
KAM-5	-0.014	1.93	0.7941
KAM-6	-1.003	1.54	0.8033

[a]Calculated by ChemDraw Professional 17.0, LopP = logarithm of the partition coefficient
[b]Calculated by http://vcclab.org/lab/alogps/
[c] Calculated by http://lmmd.ecust.edu.cn:8000/predict/

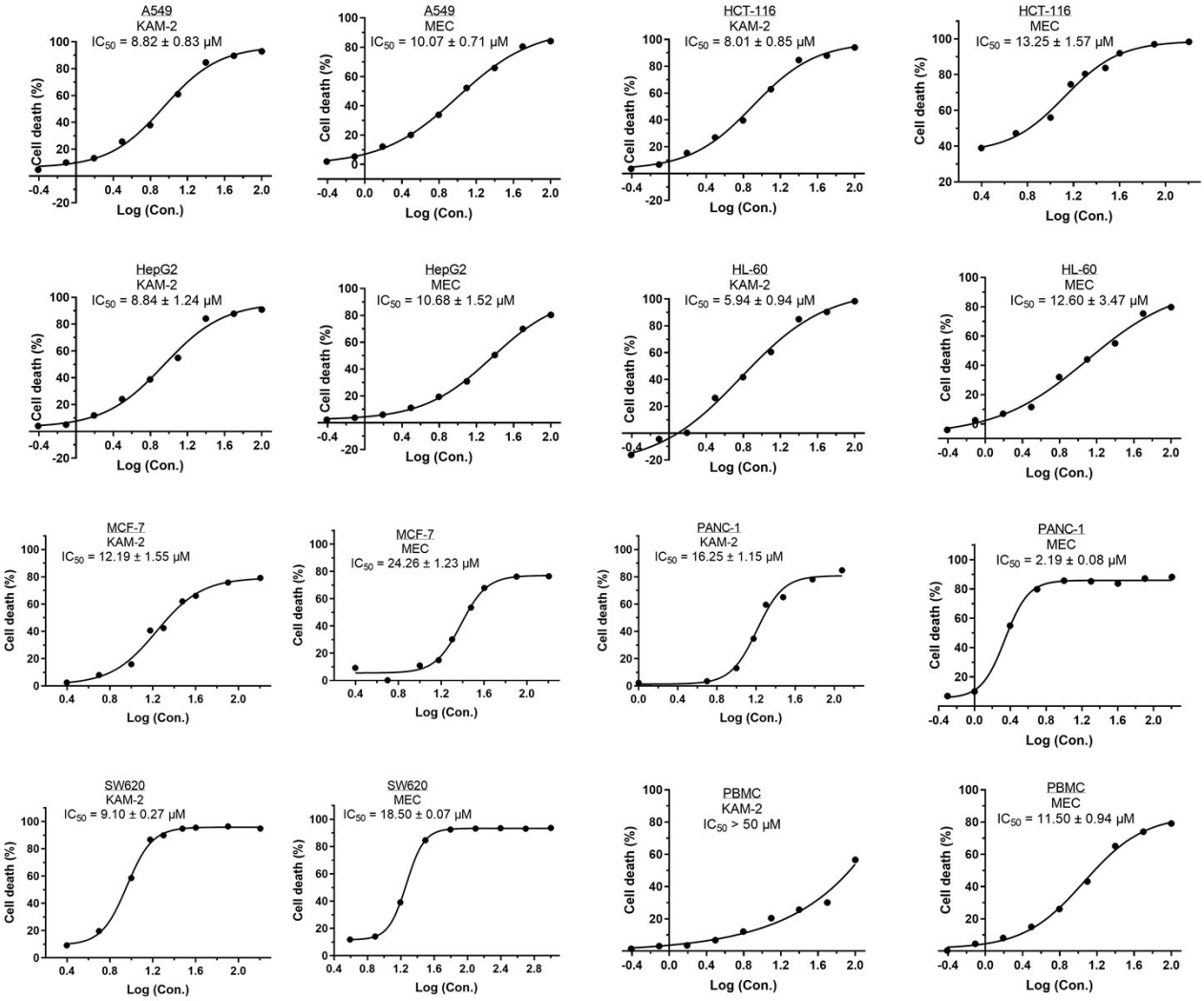


Fig. S1 Dose-response curves of **KAM-2** and MEC in indicated cell lines as determined by MTT or CCK-8 assays. IC_{50} values are expressed as mean ± SD (n = 3).

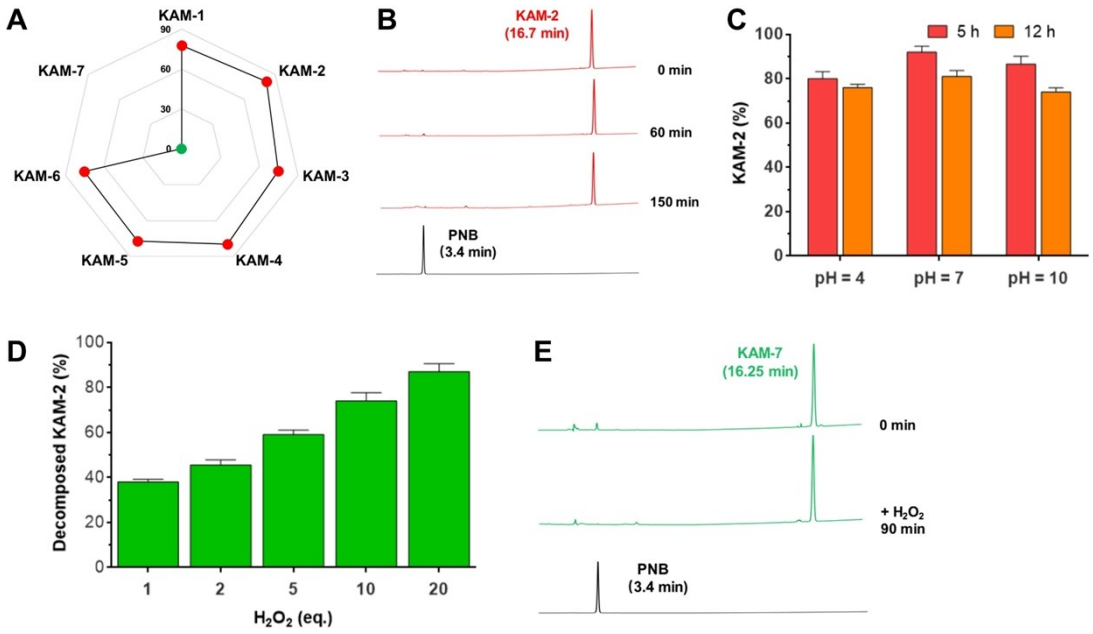


Fig. S2 Decomposition behaviours of KAM prodrugs with or without H_2O_2. (A) KAM prodrugs (100 µM) in PBS (pH 7.4, 10 mM)/MeCN (v/v, 80/20) solution were treated with 10 eq. H_2O_2 for 1 h at r.t., and the percentage of each decomposed prodrug was determined by RP-HPLC. (B) HPLC trace of **KAM-2** (100 µM) in PBS (pH 7.4, 10 mM)/MeCN (v/v, 80/20) solution at r.t. (C) Stability of **KAM-2** (100 µM) in PBS (pH = 4, 7 or 10,10 mM)/MeCN (v/v, 80/20) solution at r.t. The percentage of intact **KAM-2** at 5 h and 12 h was determined by HPLC. Mean ± SD (n = 3). (D) H_2O_2 equivalent-dependent decomposition of **KAM-2**. **KAM-2** (100 µM) in PBS (pH 7.4, 10 mM)/MeCN (v/v, 80/20) solution was treated with 1–20 eq. of H_2O_2 at r.t. for 1 h, and the percentage of decomposed **KAM-2** was determined by HPLC. Mean ± SD (n = 3). (E) HPLC trace of **KAM-7** (100 µM) in PBS (pH 7.4, 10 mM)/MeCN (v/v, 80/20) solution with 10 eq. H_2O_2 at r.t.

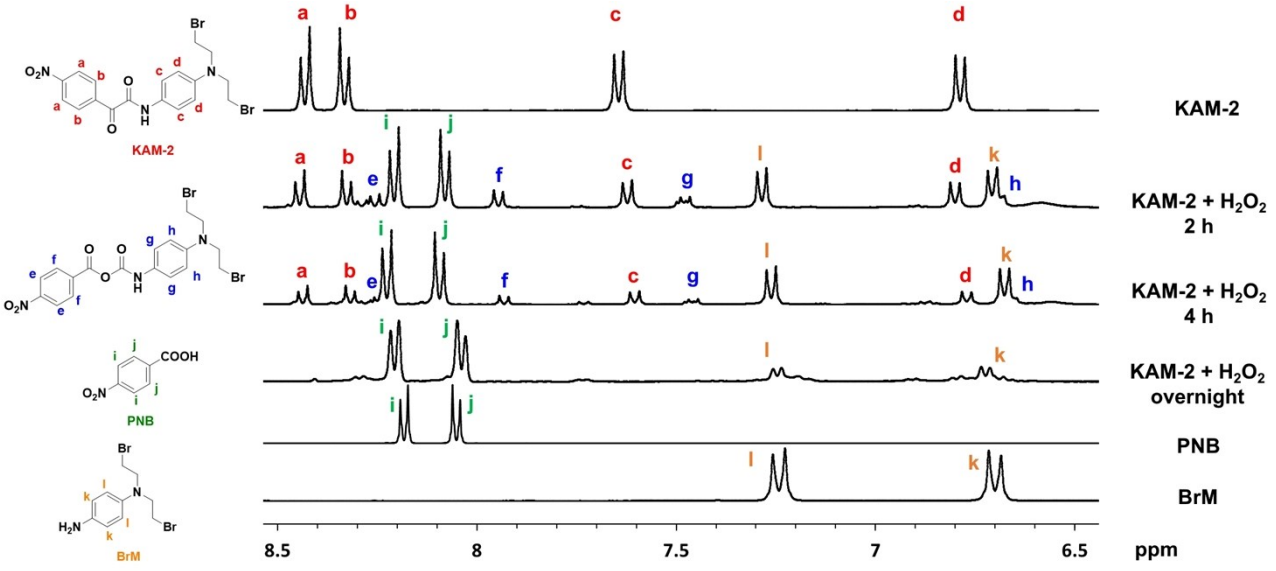


Fig. S3 ^{1}H NMR analysis of **KAM-2** (30 mM) degradation in a mixture of d^6-DMSO (550 µL) and D_2O (50 µL) in the presence of H_2O_2 (2 eq.) at r.t.

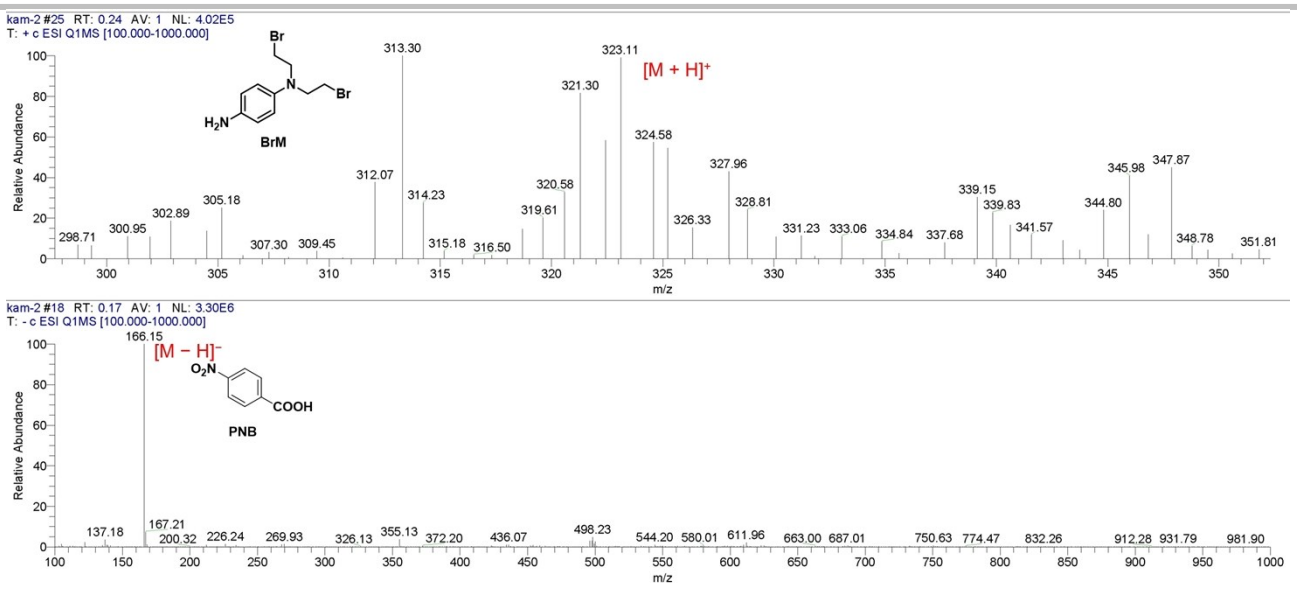


Fig. S4 ESI-MS spectrum of **KAM-2** (100 µM) after overnight H_2O_2 (10 eq.) treatment in PBS 7.4 (10 mM)/MeCN (v/v, 80/20).

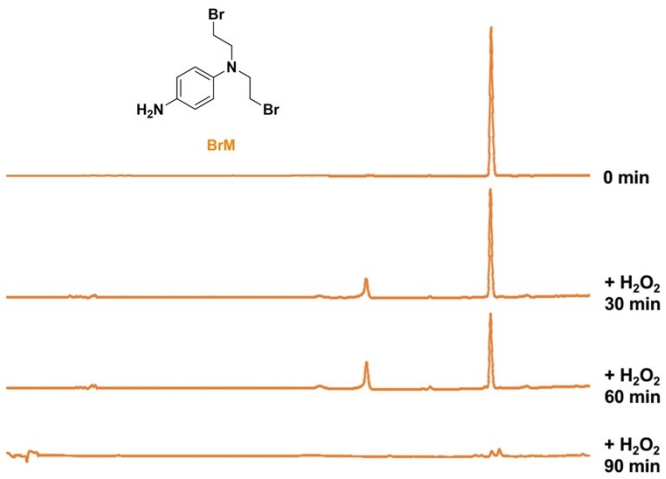


Fig. S5 HPLC trace (254 nm) of BrM (100 µM) in PBS (pH 7.4, 10 mM)/MeCN (v/v, 80/20) solution with 2 eq. H_2O_2 at r.t. The instability of BrM in the presence of H_2O_2 accounts for the shrinkage of BrM signal in HPLC during the **KAM-2** activation study.

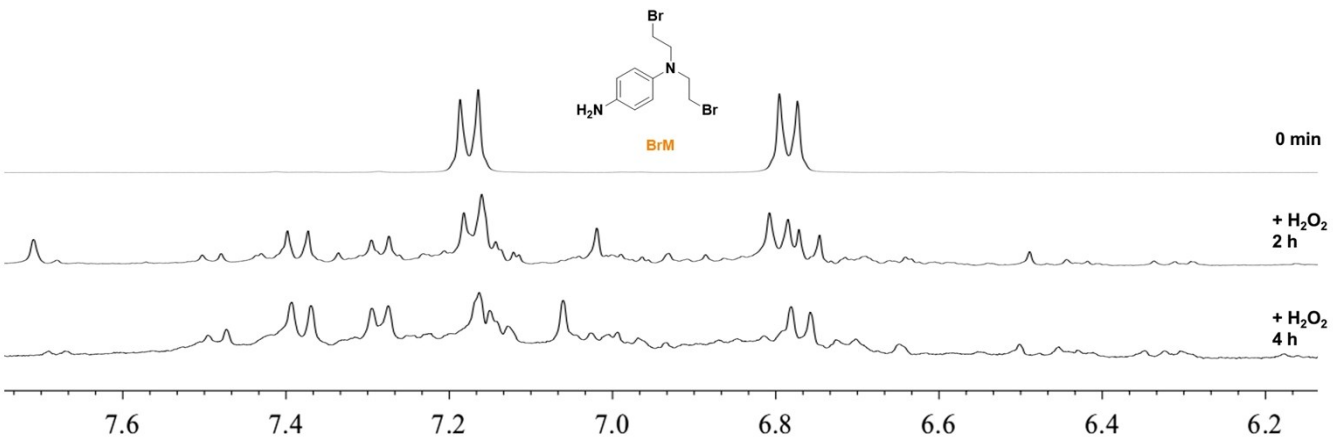


Fig. S6 ^{1}H NMR trace of BrM (30 mM) in a mixture of d^6-DMSO (550 µL) and D_2O (50 µL) in the presence of H_2O_2 (2 eq.) at r.t. The instability of BrM in the presence of H_2O_2 accounts for the shrinkage of BrM signal in NMR during the **KAM-2** activation study.

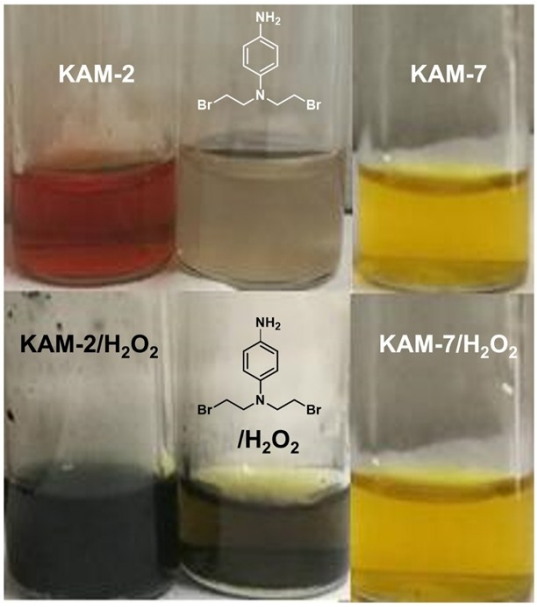


Fig. S7 Visible color change of **KAM-2**, **KAM-7** and *N*-4-aminophenyl-bis(2-bromoethyl)amine (5 mM) in PBS 7.4 (10 mM)/MeCN (v/v, 80/20) solutions 1 h after the addition of 10 eq. H_2O_2 at r.t.

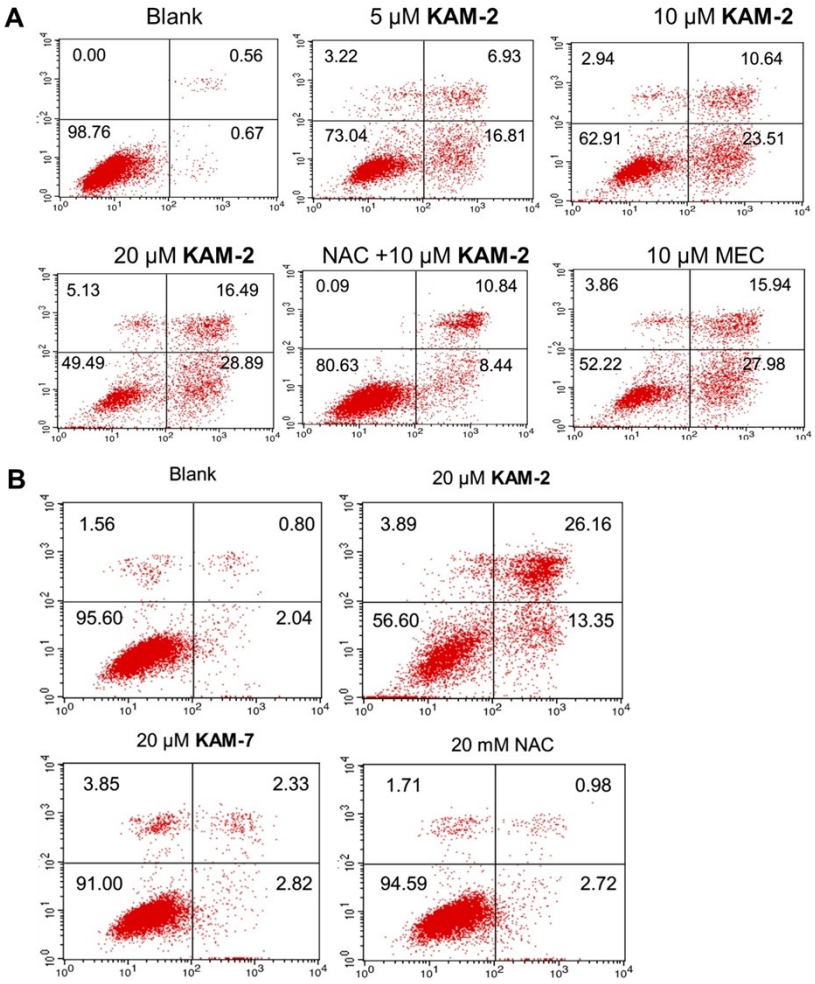


Fig. S8 Apoptotic effects of **KAM-2**, **KAM-7**, MEC, and NAC on HL-60 cells. HL-60 cells were treated with compounds at indicated concentrations for 48 h, or pre-incubated with ROS scavenger NAC (20 mM) for 1 h, followed by treatment with 10 μM **KAM-2** for 48 h. Apoptotic effects were measured by flow cytometry using annexin V-FITC/PI staining protocol. Percentage of cells in each quadrant (%) is labeled.

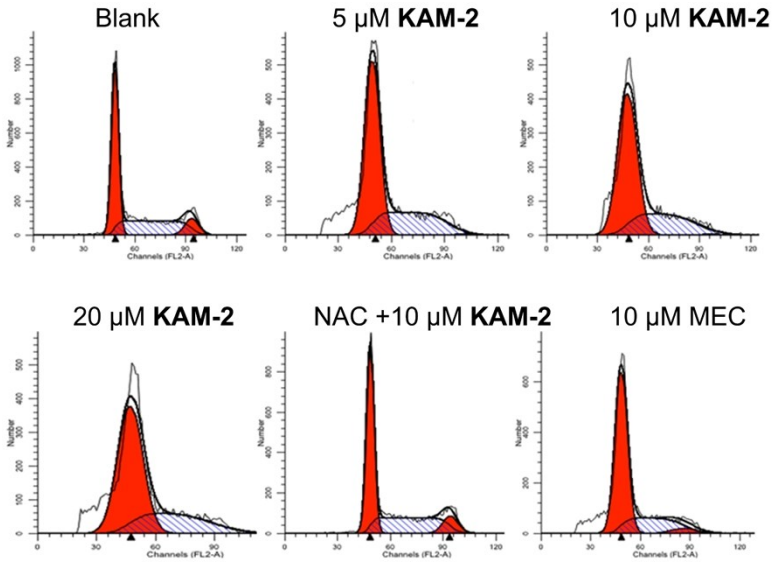


Fig. S9 Effects of **KAM-2** and MEC on cell cycle arrest. HL-60 cells were treated with compounds at indicated concentrations for 48 h, or pre-incubated with ROS scavenger NAC (20 mM) for 1 h, followed by treatment with 10 μM **KAM-2** for 48 h. The percentage of cells in each cell cycle phase was analyzed by flow cytometry.

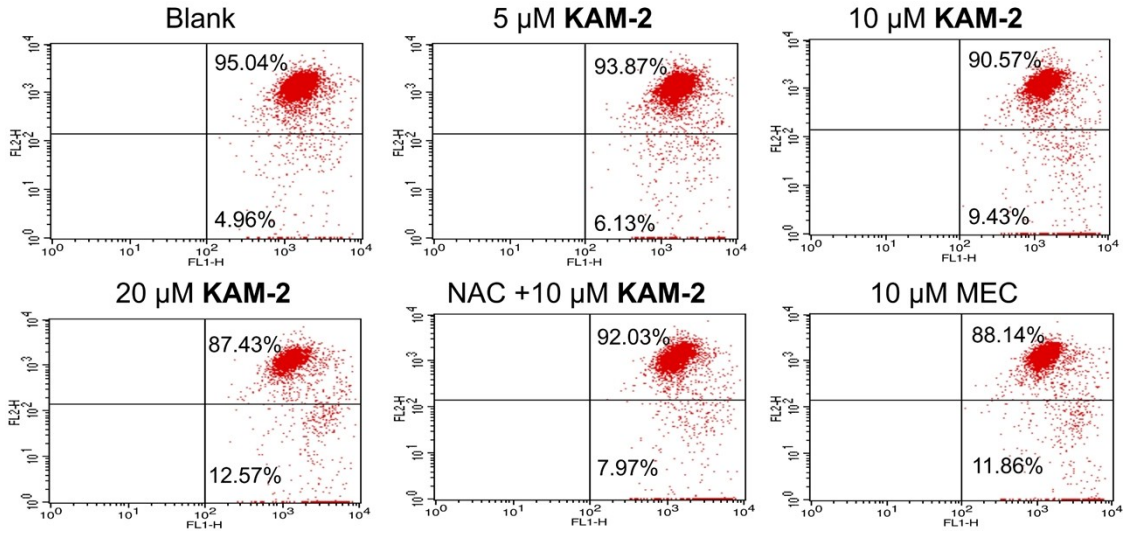


Fig. S10 Effect of **KAM-2** and MEC on mitochondrial membrane potentials (MMP) of HL-60 cells. HL-60 cells were treated with compounds at indicated concentrations for 48 h or pre-incubated with ROS scavenger NAC (20 mM) for 1 h, followed by treatment with 10 μM **KAM-2** for 48 h. MMP was measured by flow cytometry using JC-1 staining protocol.

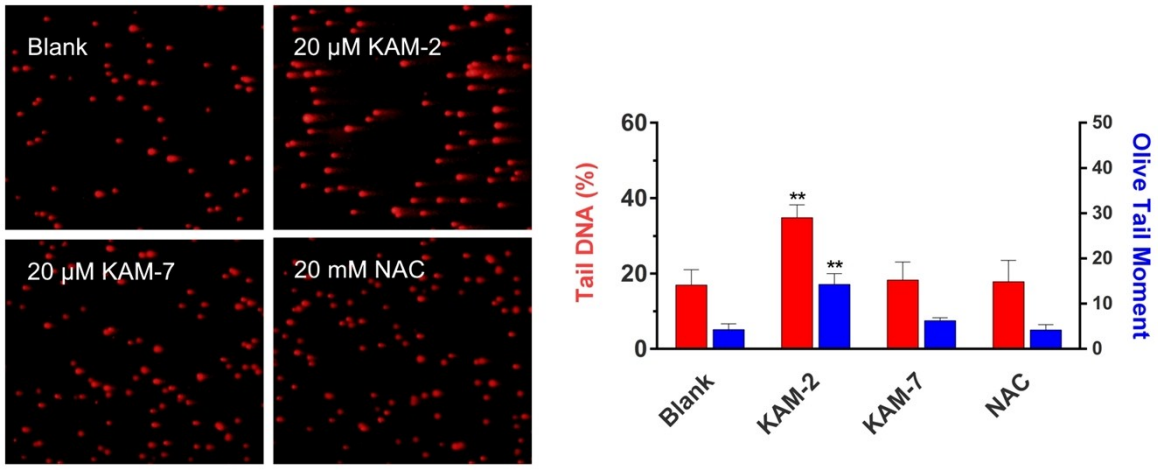


Fig. S11 Effects of **KAM-2**, **KAM-7**, and NAC on cell damage assessed by Comet assay. HL-60 cells were treated with 20 μM **KAM-2** or **KAM-7** for 48 h, or with 20 mM NAC for 1 h. Alkaline comet electrophoresis was performed. Quantitative analysis was conducted with the comet analysis software CASP. The tail DNA% and olive tail moment were employed to evaluate DNA damage. Mean ± SD, n = 3. $^{**}p < 0.01$ vs. blank.

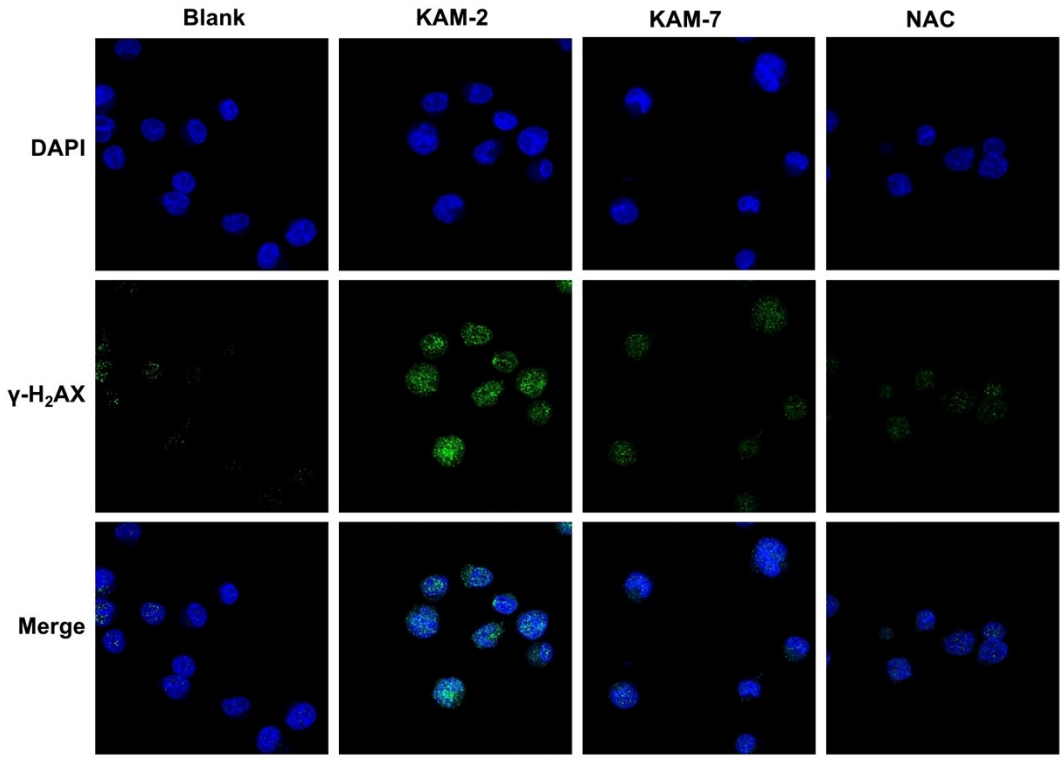


Fig. S12 Immunofluorescence of DNA cleavage protein γH_2AX. HL-60 cells were treated with **KAM-2** (10 μM) or KAM-7 (10 μM) for 12 h, or with NAC (20 mM) for 1 h.

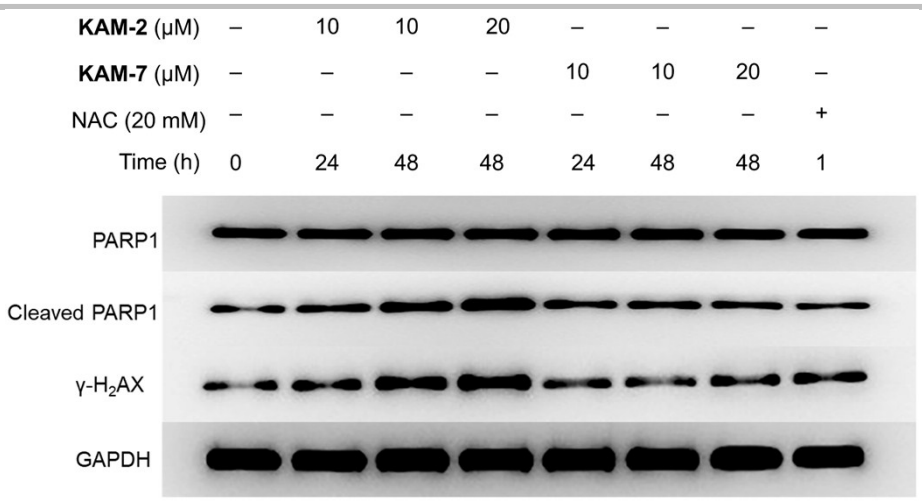


Fig. S13 Effects of **KAM-2**, **KAM-7** and **NAC** on the expression of PARP1, cleaved PARP1, and γ-H2AX by Western blot. HL-60 cells were treated with compounds at indicated concentrations for indicated time.

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