Using Peptidic Inhibitors to Systematically Probe the S1' Site of Caspases-3 and -7

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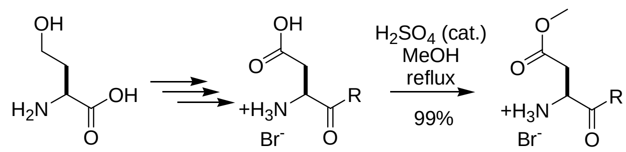
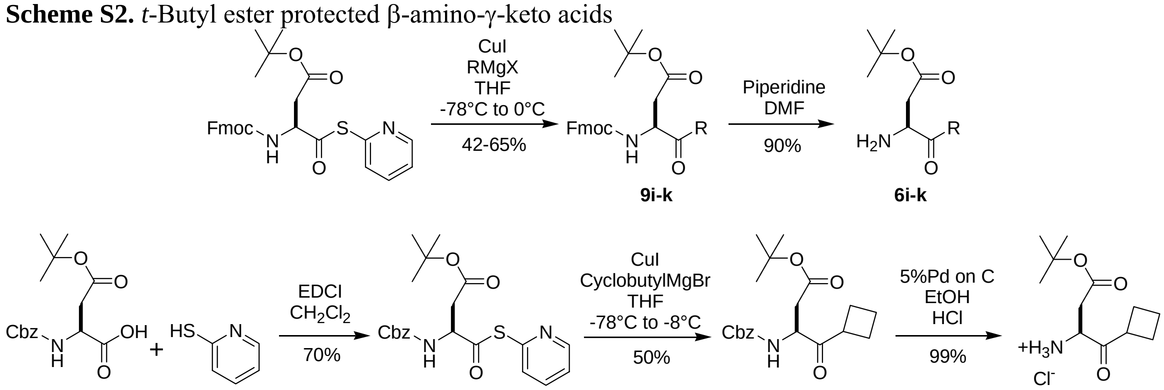
Scheme S1. Methyl ester protected β-amino-γ-keto acids



8a-h,m-o **6a-h,m-o**

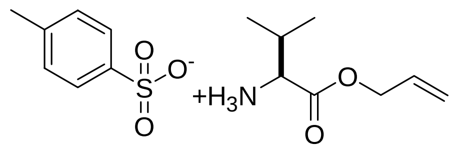
Scheme S2. *t*-Butyl ester protected β-amino-γ-keto acids



10 **11** **6l**

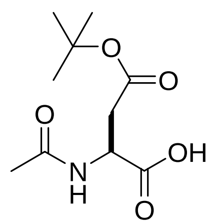
Experimental

General methods. All reactions requiring anhydrous conditions were conducted under a positive atmosphere of nitrogen or argon in oven-dried glassware. Standard syringe techniques were used for anhydrous addition of liquids. Unless otherwise noted, all starting materials, solvents, and reagents were acquired from commercial suppliers and used without further purification. Methlyene chloride was distilled over P_2O_5 under N_2 immediately prior to use. Methanol was distilled over Mg under N_2 atmosphere immediately prior to use. Tetrahydrofuran (THF) was distilled over $LiAlH_4$ under N_2 atmosphere immediately prior to use. Analytical thin-layer chromatography was performed using Merck silica gel plated with F254 indicator. Visualization of the plates was by UV and the indicated stain. Three solvent systems were used for TLC development and are indicated as System I-MeOH:EtOAc 1:9, System II-EtOAc:Hexanes 1:1, and System III-MeOH:EtOAc 1:1. Flash chromatography was performed using 230-400 mesh silica gel. ^{1}H NMR spectra were recorded on Varian Unity 500 (500MHz) and on Varian INOVA 500 (500MHz) spectrometers. ^{13}C spectra were recorded on a Varian Unity 500 (125MHz) spectrometer. Chemical shifts are reported in parts per million (ppm), and multiplicities and indicated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). Spectra were referenced to residual methanol (3.30 ppm ^{1}H and 49.0 ppm ^{13}C) or chloroform (7.26 ppm ^{1}H and 77.0 ^{13}C). IR spectra of non-water soluble compounds were obtained on a Perkin Elmer Spectra B spectrometer as thin films on NaCl plates. All mass spectra were obtained by the University of Illinois Mass Spectrometry Center and the data is reported as *m/e*.



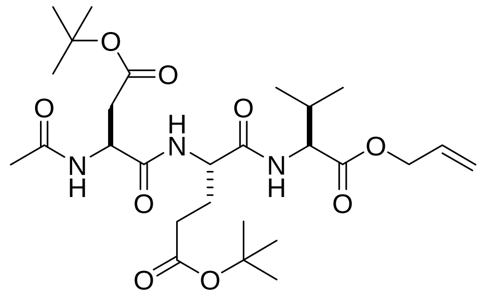
2

L-Valinylallyl ester *p*-toluenesulfonate (2). The allyl esterification of L-valine was carried out according to the procedure of Kunz.[1] Briefly, *p*-toluenesulfonic acid monohydrate (15.4 mmol) was added to L-valine (12.8 mmol) and 2-propenol (128.0 mmol) in benzene. The mixture was stirred at reflux with a Dean-Stark apparatus until the calculated amount of water had been collected. The benzene was then removed by rotary evaporation and the product was precipitated out of the mixture by petroleum ether and collected by vacuum filtration. The resulting solid was then taken up in a small amount of CH_2Cl_2 and repercipitated using petroleum ether. Product was obtained in 87.5% yield (11.2 mmol). Characterization data matched that previously reported.



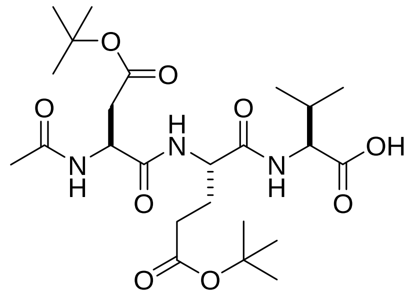
3

N^{α}-acetyl-β-*tert*-butylester-L-aspartic acid (3). To a slurry of β-*tert*-butylester-L-aspartic acid (7.93 mmol) in anhydrous THF at room temperature was added excess acetic anhydride. The solid dissolved slowly as the reaction mixture stirred. After 16 h, the pH was adjusted to 2 (as indicated by pH paper) using 1 M HCl. The solvent was then removed *in vacuo* leaving a white-yellow foam. This product was washed with EtOAc and collected via vacuum filtration. The white powder product was obtained in 95% yield. R_f 0.1 in System I, DPIP. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 4.73 (dd, *J* = 7.4 Hz, *J* = 5.4 Hz, 1H), 2.77 (dd, *J* = 16.2 Hz, *J* = 5.4 Hz, 1H), 2.66 (dd, *J* = 16.2 Hz, *J* = 7.4 Hz, 1H), 1.97 (s, 3H), 1.44 (s, 9H). ^{13}C NMR (125MHz, MeOH-d_4) δ_C 172.7, 171.8, 170.1, 81.3, 49.2, 37.3, 27.0, 21.2. HRMS (ESI) cacld for $C_{10}H_{18}NO_5$ 232.1185, found 323.1188.



4

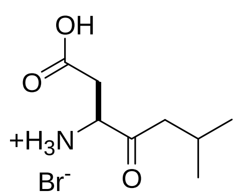
Ac-Asp(O*t*Bu)-Glu(O*t*Bu)-Val-allyl ester (4). To a solution of L-valinylallyl ester p-toluenesulfonate **2** (3.00 mmol, 1 eq), N^{α}-fmoc-γ-*tert*-butylester-L-glutamic acid (3.30 mmol, 1.1 eq), and PyBOP (3.30 mmol, 1.1 eq) in 17 mL of anhydrous CH_2Cl_2 at room temperature under N_2 was added DIEA (7.50 mmol, 2.5 eq). When the free amine had been consumed (as seen by TLC: System I, Ninhydrin), 7 mL of tris(aminoethyl)amine (TAEA) were added to the reaction mixture to deprotect the fluorenylmethylcarbamate and scavenge the produced dibenzofulvene. After 30 min, the reaction mixture was diluted to 50 mL with EtOAc and washed twice with brine, three times with pH 5.5 10% phosphate buffer, and once more with brine. The organic layer was dried over anhydrous $MgSO_4$ and then concentrated *in vacuo*. The resulting yellow oil was taken up in 17 mL of anhydrous CH_2Cl_2 with N^{α}-acetyl-β-*tert*-butylester-L-aspartic acid (3.30 mmol, 1.1 eq) and PyBOP (3.30 mmol, 1.1 eq) under N_2. DIEA (4.50 mmol, 1.5 eq) was then added. Upon complete consumption of the free amine (by TLC: System I, Ninhydrin), the reaction mixture was diluted to 50 mL with EtOAc and washed three times with pH 5.5 10% phosphate buffer and once with brine. After drying over anhydrous $MgSO_4$ and concentrating under reduced pressure, the product was obtained by flash silica gel chromatography (ethyl acetate/hexanes 3:1) as a white solid (46% yield over the three steps). R_f 0.6 in System I, DPIP. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 7.50 (dd, J = 24.1 Hz, J = 7.5 Hz, 1H), 7.17 (dd, J = 18.5 Hz, J = 8.6 Hz, 1H), 6.94 (t, J = 7.6 Hz, 1H), 5.87 (ddtd, J = 17.2 Hz, J = 10.4 Hz, J = 5.9 Hz, J = 1.9 Hz, 1H), 5.30 (ddt, J = 17.2 Hz, J = 2.4 Hz, J = 1.4 Hz, 1H), 5.21 (dq, J = 10.4 Hz, J = 1.2 Hz, 1H), 4.77 (m, 1H), 4.61 (ddq, J = 13.1 Hz, J = 5.8 Hz, J = 1.5 Hz, 1H), 4.56 (m, 1H), 4.46 (m, 2H), 2.82 (ddd, J = 24.1 Hz, J = 16.8 Hz, J = 4.9 Hz, 1H), 2.60 (ddd, J = 19.6 Hz, J = 16.8 Hz, J = 6.3 Hz, 1H), 2.35 (m, 2H), 2.18 (sp of d, J = 6.9 Hz, J = 5.0 Hz, 1H), 2.03 (m, 1H), 2.02 (d, J = 10.4 Hz, 3H), 1.91 (sp, J = 7.4 Hz, 1H), 1.41 (d, J = 2.4 Hz, 9H), 1.40 (s, 9H), 0.90 (dd, J = 6.9 Hz, J = 1.2 Hz, 3H), 0.88 (d, J = 7.0 Hz, 3H). IR 3294, 2976, 2252, 1728, 1632, 1528, 1368, 1150, 1049, 988, 955, 919, 850 cm^{-1}. MS *m/e* 556 ($M+H^+$); HRMS (ESI) calcd for $C_{27}H_{46}N_3O_9$ 556.3234, found 556.3262.



5

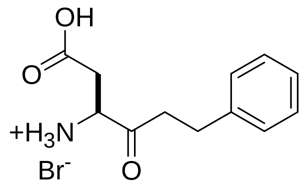
Ac-Asp(O*t*Bu)-Glu(O*t*Bu)-Val-OH (5). A solution of Ac-DEV-OAllyl **4** (1.26 mmol) in 30mL anhydrous THF with tetrakis(triphenylphosphine)palladium(0) (0.126 mmol) and morpholine (3.78 mmol) was stirred in the dark under an argon atmosphere at room temperature. After 20 min, concentrated HCl was slowly added until a pH of 3 was obtained (by pH paper). The solvent was removed under reduced pressure and the residue was immediately chromatographed over silica gel (methanol/ethyl acetate 1:3). The product was obtained as a yellow solid in 94% yield. R_f 0.6 in System I, DPIP. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 4.75 (m, 1H), 4.42 (ddd, *J* = 16.2 Hz, *J* = 9.6 Hz, *J* = 4.8 Hz, 1H), 4.15 (dd, *J* = 8.1 Hz, *J* = 5.4 Hz, 1H), 3.70 (t, *J* = 4.8 Hz, 2H), 3.05 (dt, *J* = 6.8 Hz, *J* = 1.3 Hz, 1H), 2.80 (ddd, *J* = 16.5 Hz, *J* = 6.1 Hz, *J* = 5.0 Hz, 1H), 2.64 (m, 1H), 2.50 (br s, 1H), 2.44-2.27 (m, 2H), 2.21-2.09 (m, 2H), 2.01 (d, *J* = 11.6 Hz, 3H), 1.96-1.87 (m, 1H), 1.45 (dd, *J* = 3.8 Hz, *J* = 0.5 Hz, 18H), 0.94 (d, *J* = 6.9 Hz, 3H), 0.92 (dd, *J* = 6.9 Hz, *J* = 2.1 Hz, 3H). IR 3293, 3068, 2977, 2934, 1731, 1651, 1575, 1420, 1393, 1368, 1257, 1157, 847 cm^{-1}. MS *m/e* 538 (M+Na$^+$); HRMS (ESI) calcd for $C_{24}H_{41}N_3O_9Na$ 538.2741, found 538.2734.

General Grignard reaction to produce β-amino-γ-keto acids (8a-h,m-o). All reactions were carried out as previously reported.[2] Compounds **8a-d, f, m-o** match the previously reported spectra.



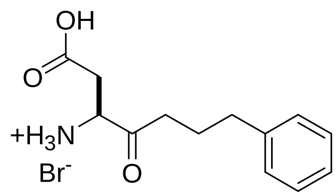
8e

1-Carboxymethyl-4-methyl-2-oxo-pentyl-ammonium bromide (8e). Overall yield for 3 steps: 11%. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 4.31 (dd, *J* = 6.5 Hz, *J* = 4.1 Hz, 1H), 3.11 (dd, *J* = 18.6 Hz, *J* = 3.9 Hz, 1H), 3.01 (dd, *J* = 18.1 Hz, *J* = 6.8 Hz, 1H), 2.51 (t, *J* = 7.0 Hz, 2H), 2.18 (m, 1H), 0.95 (d, *J* = 5.8 Hz, 3H), 0.94 (d, *J* 6.0 Hz, 3H). ^{13}C NMR (125MHz, MeOH-d_4) δ_C 203.676, 171.299, 55.743, 46.742, 33.059, 23.997, 21.479. HRMS (ESI) calcd for $C_8H_{16}NO_3$ 174.1130, found 174.1138.



8g

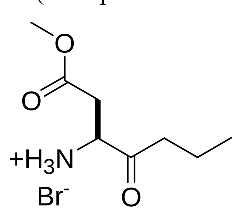
1-Carboxymethyl-2-oxo-4-phenyl-butyl-ammonium bromide (8g). Overall yield for 3 steps: 9%. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 7.24 (m, 4H), 7.16 (t, J = 7.0 Hz), 4.38 (br d, J = 25.4 Hz, 1H), 3.00 (m, 6H). ^{13}C NMR (125MHz, MeOH-d_4) δ_C 203.408, 171.359, 140.692, 128.358, 128.298, 126.092, 55.603, 39.980, 33.040, 28.943. HRMS (ESI) calcd for $C_{12}H_{16}NO_3$ 222.1130, found 222.1126.



8h

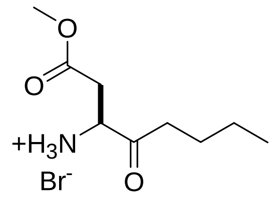
1-Carboxymethyl-2-oxo-5-phenyl-pentyl-ammonium bromide (8h). Overall yield for 3 steps: 6%. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 7.26 (m, 2H), 7.17 (m, 3H), 4.38 (ddd, J = 25.2 Hz, J = 6.4 Hz, J = 4.4 Hz, 1H), 3.09 (m, 2H), 2.64 (m, 4H). ^{13}C NMR (125MHz, MeOH-d_4) δ_C 204.017, 171.363, 141.730, 128.335, 128.302, 125.870, 52.020, 37.473, 34.542, 33.234, 24.890. HRMS (ESI) cacld for $C_{13}H_{18}NO_3$ 236.1287, found 236.1294.

General procedure for the methyl ester protection of β-amino-γ-keto acids (6a-h,m-o). A solution of the HBr salt of the aspartyl ketone (0.140 mmol) in anhydrous methanol with one drop of concentrated H_2SO_4 was stirred at reflux. When the reaction was complete as seen by TLC (System III, Ninhydrin), the solution was cooled to room temperature, and the solvent was removed under reduced pressure. The oily product was used without further purification (after product confirmation by NMR). Yields were all ~99%.



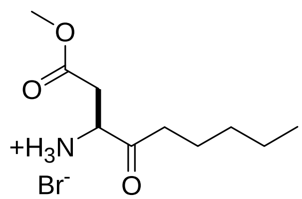
6a

1-Methoxycarbonylmethyl-2-oxo-pentyl-ammonium bromide (6a). R_f 0.5 in System III, Ninhydrin. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 4.38 (br s, 1H), 3.75 (s, 3H), 3.12 (m, 2H), 2.60 (t, J = 7.1 Hz, 2H), 1.64 (sx, J = 7.3 Hz, 2H), 0.94 (t, J = 7.3 Hz, 3H). ^{13}C NMR (125MHz, MeOH-d_4) δ_C 204.0, 170.3, 55.4, 54.0, 51.9, 39.9, 33.2, 16.4, 12.6.



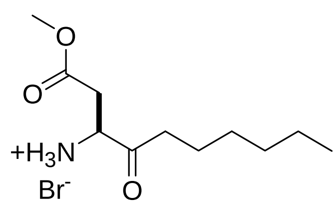
6b

1-Methoxycarbonylmethyl-2-oxo-hexyl-ammonium bromide (6b). R_f 0.5 in System III, Ninhydrin. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 4.36 (dd, J = 6.8 Hz, J = 4.0 Hz, 1H), 3.74 (s, 3H), 3.14 (dd, J = 18.1 Hz, J = 4.1 Hz, 1H), 3.06 (dd, J = 18.1 Hz, J = 7.0 Hz, 1H), 2.62 (td, J = 7.2 Hz, J = 3.0 Hz, 2H), 1.59 (quintet, J = 7.4 Hz, 2H), 1.34 (s of d, J = 7.4 Hz, J = 1.1 Hz, 2H), 0.92 (t, J = 7.3 Hz, 3H). ^{13}C NMR (125MHz, MeOH-d_4) δ_C 204.0, 170.3, 55.4, 54.0, 51.9, 37.7, 33.2, 25.1, 21.9, 13.0.



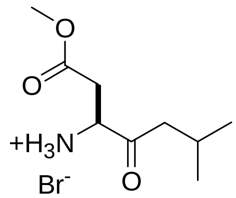
6c

1-Methoxycarbonylmethyl-2-oxo-heptyl-ammonium bromide (6c). R_f 0.6 in System III, Ninhydrin. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 4.37 (dd, J = 6.9 Hz, J = 4.3 Hz, 1H), 3.75 (s, 3H), 3.15 (dd, J = 18.1 Hz, J = 4.2 Hz, 1H), 3.07 (dd, J = 18.2 Hz, J = 7.1 Hz, 1H), 2.62 (td, J = 7.3 Hz, J = 2.5 Hz, 2H), 1.61 (pentet, J = 7.3 Hz, 2H), 1.31 (m, 4H), 0.91 (t, J = 7.0 Hz, 3H). ^{13}C NMR (125MHz, MeOH-d_4) δ_C 204.0, 170.3, 61.8, 55.4, 54.0, 51.9, 38.0, 33.2, 31.0, 22.7, 22.3, 13.0.



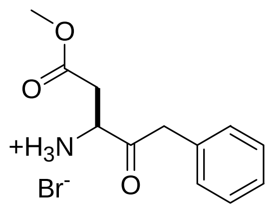
6d

1-Methoxycarbonylmethyl-2-oxo-octyl-ammonium bromide (6d). R_f 0.6 in System III, Ninhydrin. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 4.34 (br s, 1H), 3.72 (s, 3H), 3.12 (dd, J = 18.4 Hz, J = 4.3 Hz, 1H), 3.07 (dd, J = 18.2 Hz, J = 6.8 Hz, 1H), 2.60 (t, J = 7.1Hz, 2H), 1.57 (br pentet, J = 7.1 Hz, 2H), 1.28 (br m, 6H), 0.87 (t, J = 6.6 Hz, 3H). ^{13}C NMR (125MHz, MeOH-d_4) δ_C 204.0, 170.2, 55.4, 51.9, 38.0, 33.2, 31.5, 28.4, 22.9, 22.3, 13.1.



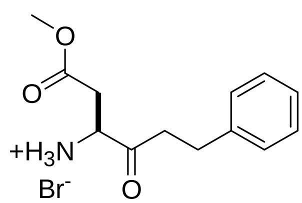
6e

1-Methoxycarbonylmethyl-4-methyl-2-oxo-pentyl-ammonium bromide (6e). R_f 0.5 in System III, Ninhydrin. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 4.33 (dd, J = 6.7 Hz, J = 4.0 Hz, 1H), 3.74 (s, 3H), 3.14 (dd, J = 18.2 Hz, J = 4.2 Hz, 1H), 3.07 (dd, J = 18.1 Hz, J = 6.9 Hz, 1H), 2.51 (ddd, J = 22.8 Hz, J = 17.6 Hz, J = 6.7 Hz, 2H), 2.17 (sp, J = 6.7 Hz, 1H), 0.94 (t, J = 6.5 Hz, 6H). ^{13}C NMR (125MHz, MeOH-d_4) δ_C 203.5, 170.2, 55.6, 46.7, 33.1, 24.0, 21.5.



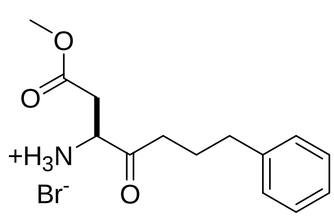
6f

1-Methoxycarbonylmethyl-2-oxo-3-phenyl-propyl-ammonium bromide (6f). R_f 0.6 in System III, Ninhydrin. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 7.28 (m, 5H), 4.51 (dd, J = 5.8 Hz, J = 4.1 Hz, 1H), 3.98 (s, 2H), 3.73 (s, 3H), 3.19 (m, 2H). ^{13}C NMR (125MHz, MeOH-d_4) δ_C 201.7, 170.2, 133.0, 129.7, 128.4, 127.1, 63.0, 56.5, 53.1, 46.0, 34.5.



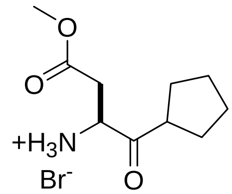
6g

1-Methoxycarbonylmethyl-2-oxo-4-phenyl-butyl-ammonium bromide (6g). R_f 0.6 in System III, Ninhydrin. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 7.24 (m, 4H), 7.17 (t, J = 7.1 Hz, 1H), 4.38 (m, 1H), 3.71 (s, 3H), 3.08 (dd, J = 18.2 Hz, J = 3.5 Hz, 1H), 3.02 (dd, J = 18.2 Hz, J = 6.9 Hz, 1H), 2.99-2.90 (m, 4H). ^{13}C NMR (125MHz, MeOH-d_4) δ_C 203.2, 170.2, 140.7, 128.4, 128.3, 126.1, 55.5, 51.9, 48.7, 39.9, 33.1, 28.9.



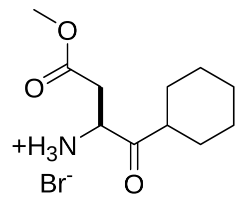
6h

1-Methoxycarbonylmethyl-2-oxo-5-phenyl-pentyl-ammonium bromide (6h). R_f 0.5 in System III, Ninhydrin. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 7.25 (t, J = 6.9 Hz, 2H), 7.16 (m, 3H), 4.35 (m, 1H), 3.72 (s, 3H), 3.31 (m, 2H), 3.09 (br m, 2H), 2.63 (br m, 4H). ^{13}C NMR (125MHz, MeOH-d_4) δ_C 203.9, 170.3, 141.8, 128.3, 128.2, 125.9, 55.4, 48.7, 37.3, 34.5, 33.2, 24.8.



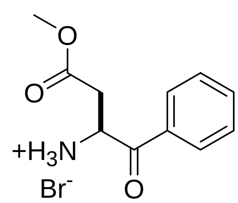
6m

2-Cyclopentyl-1-methoxycarbonylmethyl-2-oxo-ethyl-ammonium bromide (6m). R_f 0.5 in System III, Ninhydrin. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 4.47 (br s, 1H), 3.71 (s, 3H), 3.13 (m, 3H), 2.03-1.83 (m, 2H), 1.76 (br m, 2H), 1.63 (br m, 4H). ^{13}C NMR (125MHz, MeOH-d_4) δ_C 207.1, 170.3, 55.1, 54.2, 52.1, 33.1, 30.7, 28.5, 26.0, 25.9.



6n

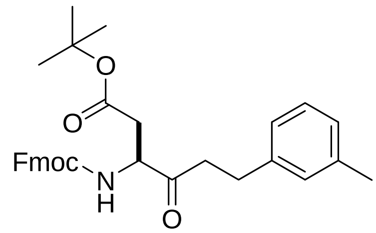
2-Cyclohexyl-1-methoxycarbonylmehtyl-2-oxo-ethyl-ammonium bromide (6n). R_f 0.5 in System III, Ninhydrin. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 4.50 (dd, J = 6.2 Hz, J = 3.3 Hz, 1H), 3.12 (dd, J = 18.1 Hz, J = 3.3 Hz, 1H), 3.02 (dd, J = 18.1 Hz, J = 6.2 Hz, 1H), 2.74 (tt, J = 11.4 Hz, J = 3.3 Hz, 1H), 1.96 (d, J = 12.4 Hz, 1H), 1.73 (m, 5H), 1.51-1.17 (m, 6H). ^{13}C NMR (125MHz, MeOH-d_4) δ_C 207.2, 171.4, 54.1, 46.2, 33.1, 29.5, 27.4, 25.6, 25.5, 24.8.



6o

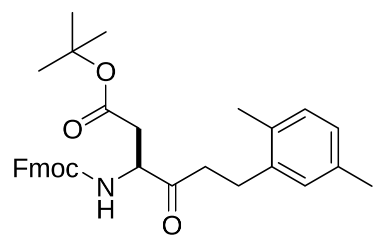
1-Methoxycarbonylmethyl-2-oxo-2-phenyl-ethyl-ammonium bromide (6o). R_f 0.6 in System III, Ninhydrin. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 8.00 (dd, J = 8.4 Hz, J = 1.2 Hz, 2H), 7.71 (tt, J = 7.5 Hz, J = 1.2 Hz, 1H), 7.58 (t, J = 7.8 Hz, 2H), 5.39 (dd, J = 7.8 Hz, J = 3.7 Hz, 1H), 3.69 (s, 3H), 3.09 (dd, J = 17.8 Hz, J = 3.8 Hz, 1H), 3.01 (dd, J = 17.8 Hz, J = 8.0 Hz, 1H). ^{13}C NMR (125MHz, MeOH-d_4) δ_C 194.4, 169.8, 134.7, 133.2, 129.2, 54.1, 52.4, 51.9, 48.7, 34.9.

General preparation of N^{β}-Fmoc-β-amino-γ-keto acid *tert*-butyl esters (9i-k). Reactions carried out according to the procedure by Vazquez and Albericio.[3]



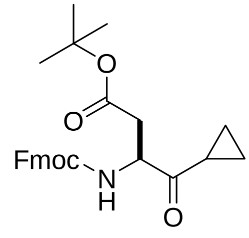
9i

3-(9H-Fluoren-9-ylmethoxycarbonylamino)-4-oxo-6-*m*-tolyl-hexanoic acid *tert*-butyl ester (9i). 35% yield. ^{1}H NMR (500MHz, $CDCl_3$) δ_H 7.80 (d, *J* = 10.8 Hz, 2H), 7.65 (m, 2H), 7.41 (t, *J* = 7.8 Hz, 2H), 7.35 (t, *J* = 7.8 Hz, 2H), 7.20 (q, *J* = 7.5 Hz, 1H), 7.05 (m, 2H), 5.90 (m, 1H), 4.65 (m, 1H), 4.40 (m, 4H), 4.26 (m, 1H), 2.90 (m, 4H), 2.35 (s, 3H), 1.45 (s, 9H). ^{13}C NMR (125MHz, MeOH-d_4) δ_C 207.4, 170.8, 156.3, 144.0, 143.9, 141.5, 140.9, 138.3, 130.4, 129.9, 129.4, 128.7, 128.7, 128.0, 127.7, 127.3, 127.2, 125.5, 125.4, 125.4, 125.3, 125.2, 120.4, 120.2, 82.2, 67.3, 56.8, 47.4, 41.0, 37.1, 29.6, 28.2, 21.6. IR 3392, 2974, 1734, 1715, 1609, 1507, 1448, 1394, 1368, 1340, 1212, 1152, 1044, 843, 760, 737 cm^{-1}. HRMS (ESI) calcd for $C_{32}H_{35}NO_5$ 514.2593, found 514.2601.



9j

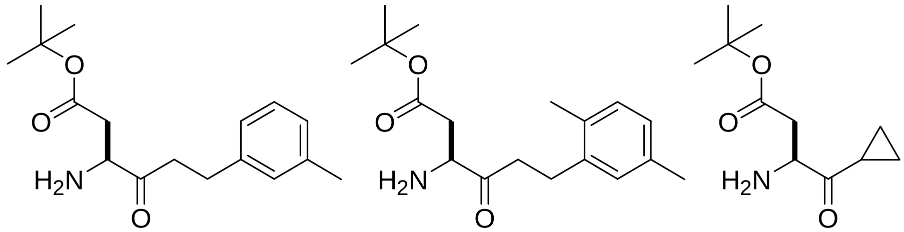
6-(2,5-Dimethylphenyl)-3-(9H-fluoren-9-ylmethoxycarbonylamino)-4-oxo-hexanoic acid *tert*-butyl ester (9j). 45% yield. ^{1}H NMR (500MHz, $CDCl_3$) δ_H 7.79 (m, 2H), 7.62 (m, 2H), 7.40 (m, 4H), 7.07 (d, *J* = 12.5 Hz, 1H), 6.98 (s, 2H), 5.99 (d, *J* = 12.0 Hz, 1H), 4.45 (m, 4H), 4.24 (t, *J* = 7.8 Hz, 1H), 2.86 (m, 5H), 2.30 (s, 3H), 2.27 (m, 3H), 1.44 (m, 9H). ^{13}C NMR (125MHz, MeOH-d_4) δ_C 207.5, 170.8, 156.3, 144.0, 141.6, 139.0, 135.8, 133.0, 130.6, 129.8, 128.0, 127.3, 125.4, 125.3, 120.3, 82.2, 67.5, 56.9, 47.3, 39.8, 37.2, 28.3, 27.1, 21.2, 19.0. HRMS (FAB) calcd for $C_{33}H_{37}NO_5$ 550.2569, found 550.2547.



9k

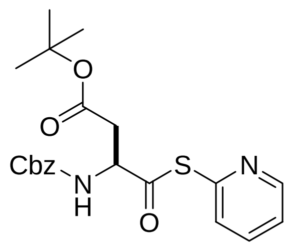
4-Cyclopropyl-3-(9H-fluoren-9-ylmethyoxycarbonylamino)-4-oxo-butyric acid *tert*-butyl ester (9k). 65% yield. ^{1}H NMR (500MHz, $CDCl_3$) δ_H 7.78 (d, *J* = 10.4 Hz, 2H), 7.60 (d, *J* = 11.8 Hz, 2H), 7.39 (t, *J* = 11.8 Hz, 2H), 7.29 (t, *J* = 10.4 Hz, 2H), 6.07 (d, *J* = 12.6 Hz, 1H), 4.71 (m, 1H), 4.49 (dd, *J* = 13.3 Hz, *J* = 10.3 Hz, 1H), 4.40 (dd, *J* = 12.8 Hz, *J* = 10.3 Hz, 1H), 4.25 (t, *J* = 13.0 Hz, 1H), 2.84 (qd, *J* = 16.3 Hz, *J* = 5.1 Hz, 2H), 2.08 (m, 1H), 1.44 (s, 9H), 1.09 (m, 2H), 0.95 (m, 2H). ^{13}C NMR (125MHz, MeOH-d_4) δ_C

207.4, 170.4, 156.3, 144.0, 141.6, 128.0, 127.9, 127.3, 125.4, 125.3, 120.3, 120.2, 81.9, 67.2, 57.2, 47.4, 37.1, 28.3, 17.9, 12.3, 12.0. IR 3365, 2977, 1732, 1504, 1450, 1367, 1214, 1151, 1042, 781, 758, 735 cm^{-1}. HRMS calcd for $C_{26}H_{30}NO_5$ 436.2124, found 436.2107.



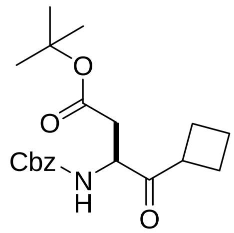
6i **6j** **6k**

Fmoc deprotection to give β-amino-γ-keto acid *tert*-butyl esters (6i-k). A mixture of 0.1 mmol of ketone, 0.03 mL of piperidine, and 0.3 mL of DMF was stirred for 20 minutes. At the end of the reaction, the crude reaction mixture was concentrated on rotary evaporator. The free-base residue was immediately used in the coupling reaction without purification or characterization in order to avoid polymerization by imine formation.



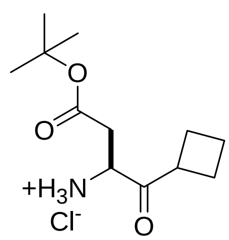
10

3-Benzyloxycarbonylamino-3-(pyridin-2-ylsulfanylcarbonyl)-propionic acid *tert*-butyl ester (10). To a clear solution of N^{α}-cbz-β-*tert*-butylester aspartic acid (1.0 mmol, 1 eq) and ethyl-3-(dimethylamino)propylcarbodiimide hydrochloride (1.0 mmol, 1 eq) in 10 mL dry CH_2Cl_2 was added 2-mercaptopyridine (1.1 mmol, 1.1 eq) and the reaction stirred at r.t. for 18 h. The reaction was then diluted with 10 mL of CH_2Cl_2 and washed with 0.1 M aq. HCl (2x10 mL), 10% aq. Na_2CO_3 (2x10 mL), and sat. brine (10 mL). The organic layer was then dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The yellow oil residue was then pushed through a silica pad using 3:1 EtOAc/Hex. Obtained a yellow foam product after concentration under reduced pressure (70% yield). R_f 0.6 in System I, DPIP. Used immediately in next step without further characterization due to instability in air.



11

3-Benzyloxycarbonylamino-4-cyclobutyl-4-oxo-butyric acid *tert*-butyl ester (11). To a dry Schlenk flask under Ar containing CuI (1.5 mmol, 3eq) in 3 mL of dry THF at –78°C was added Grignard reagent of cyclobutylbromide (3.0 mmol, 6eq). The reaction mixture was allowed to warm to –8°C and stir under Ar for 2 h. Then, thioester **10** (0.5 mmol, 1 eq) in 3 mL of dry THF was slowly added and the reaction stirred at –8°C under Ar for 10 min, after which the reaction was quenched by the addition of 20 mL of aq. sat. NH_4Cl. After warming to r.t. while stirring, the mixture was then extracted with Et_2O (2x20 mL), and the combined organic layers were washed with 10% aq. Na_2CO_3 (1x20 mL) and aq. sat. brine (1x15 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The desired product was purified by column chromatography (SiO_2, 9:1 Hex/EtOAc). A clear solid product that coated the flask walls was obtained in 50% yield. R_f 0.8 in System I, DPIP. ^1H NMR (500MHz, $CDCl_3$) δ_H 7.35 (m, 5H), 5.10 (m, 2H), 4.48 (dt, J = 8.9 Hz, J = 4.4 Hz, 1H), 3.50 (quintet, J = 8.5 Hz, 1H), 2.85 (dd, J = 16.8 Hz, J = 4.4 Hz, 1H), 2.68 (J = 16.8 Hz, J = 4.7 Hz, 1H), 2.30 (m, 2H), 2.1 (m, 2H), 1.77 (m, 2H), 1.38 (br s, 9H). ^{13}C NMR (125MHz, MeOH-d_4) δ_C 208.8, 170.6, 156.2, 136.5, 128.7, 128.7, 128.4, 128.3, 81.9, 74.7, 67.2, 55.3, 54.5, 42.9, 42.5, 42.3, 37.7, 36.9, 28.1, 25.1, 24.5, 23.7, 19.4, 19.0, 18.0. IR 3053, 2925, 2853, 2304, 1718, 1603, 1499, 1456, 1419, 1368, 1264, 1156, 895, 739, 705 cm^{-1}. HRMS (ESI) calcd for $C_{20}H_{28}NO_5$ 362.1967, found 362.1972.

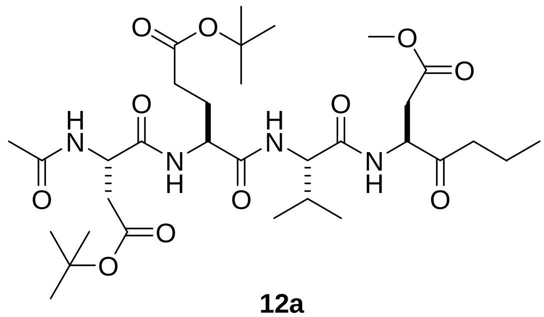


6l

3-Amino-4-cyclobutyl-4-oxo-butyric acid *tert*-butyl ester hydrochloride (6l). In a dry Schlenk flask, 5% palladium on carbon (6.4 mg, 0.003 mmol of Pd, 0.03 eq) was activated by evacuation and backfilling with H_2 5 times. The palladium was then quickly covered with 2 mL of absolute EtOH followed by the addition of **11** (0.111 mmol, 1 eq) and conc. aq. HCl (0.222 mmol, 2eq) in 1 mL of absolute EtOH. The reaction stirred at r.t. under a H_2 atmosphere until reaction was complete by TLC (System I, UV and Ninhydrin). The reaction was then removed from H_2 atmosphere, filtered through a celite plug, and concentrated to dryness under reduced pressure. The residue was then coevaporated once with toluene, and twice with CH_2Cl_2. The white solid product was obtained in 99% yield. R_f 0.3 in System I, Ninydrin. ^1H

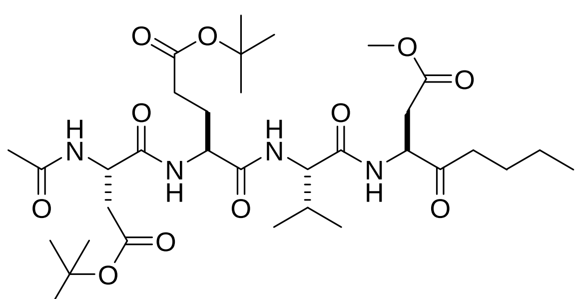
NMR (500MHz, MeOH-d_4) δ_H 4.30 (br s, 1H), 2.96 (m, 1H), 2.56 (m, 1H), 2.34-2.02 (m, 4H), 1.90-1.63 (m, 3H), 1.48 (m, 9H). ^{13}C NMR (125MHz, MeOH-d_4) δ_C 204.7, 169.1, 82.7, 54.2, 41.6, 40.5, 34.1, 27.1, 24.6, 17.6. HRMS (ESI) calcd for $C_{12}H_{22}NO_3$ 228.1600, found 228.1609.

General coupling procedure to give peptide-ketones 12a-o. To a solution of a β-amino-γ-keto acid ester **6a-o** (0.060 mmol, 1 eq), Ac-DEV-OH (**5**) (0.120 mmol, 2 eq), and PyBOP (0.126 mmol, 2.1 eq) in 0.8 mL of anhydrous CH_2Cl_2 was added DIEA (0.210 mmol, 3.5 eq) and the reaction mixture stirred under N_2 at room temperature until TLC (System I, Ninhydrin) showed consumption of the amine. The reaction mixture was then diluted to 15 mL with EtOAc and washed with pH 5.5 1 M phosphate buffer (3 x 5 mL) and brine (5 mL). The organic layer was then dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was separated by column chromatography (SiO_2, EtOAc) to give the desired product in the reported yield.



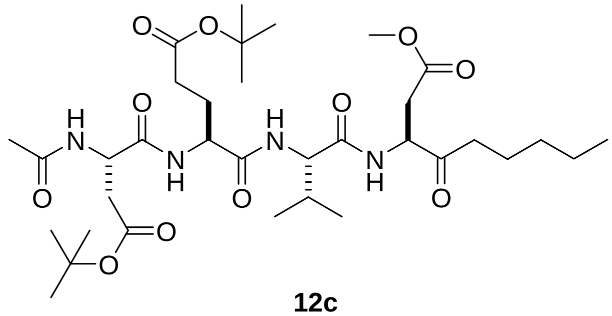
12a

Ac-Asp(O*t*Bu)-Glu(O*t*Bu)-Val-Asp(OMe)-propyl ketone (12a). 92% yield. R_f 0.6 in System I, DPIP. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 4.76-4.63 (m, 2H), 4.37 (m, 1H), 4.13 (td, J = 20.1 Hz, J = 7.4 Hz, 1H), 3.65 (d, J = 0.8 Hz, 3H), 2.90 (m, 1H), 2.78 (m, 1H), 2.72-2.43 (m, 4H), 2.33 (m, 2H), 2.10 (m, 2H), 1.99 (m, 3H), 1.90 (m, 1H), 1.57 (br sx, J = 7.5 Hz, 2H), 1.44 (m, 18H), 0.95 (m, 6H), 0.89 (tt, J = 7.4 Hz, J = 1.0 Hz, 3H). IR 3293, 2967, 1727, 1638, 1534, 1438, 1368, 1258, 1156, 847 cm^{-1}. MS *m/e* 671 (M+H$^+$); HRMS (ESI) calcd for $C_{32}H_{55}N_4O_{11}$ 671.3867, found 671.3878.



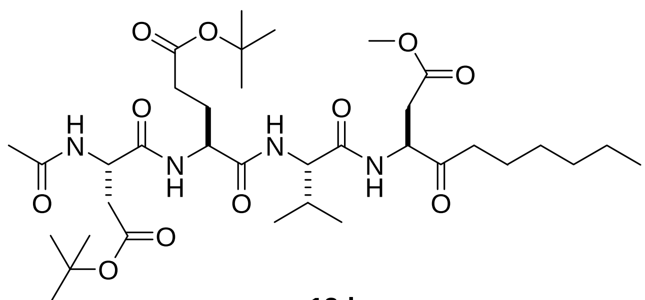
12b

Ac-Asp(O*t*Bu)-Glu(O*t*Bu)-Val-Asp(OMe)-butyl ketone (12b). 69% yield. R_f 0.6 in System I, DPIP. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 4.77-4.60 (m, 2H), 4.38 (m, 1H), 4.14 (m, 1H), 3.66 (s, 3H), 2.90 (m, 1H), 2.80 (m, 1H), 2.72-2.47 (m, 4H), 2.34 (m, 2H), 2.11 (m, 2H), 2.00 (m, 3H), 1.90 (m, 1H), 1.52 (m, 2H), 1.45 (m, 18H), 1.31 (sx, J = 7.5 Hz, 2H), 0.96 (m, 6H), 0.91 (t, J = 7.45 Hz, 3H). IR 3275, 3075, 2964, 2933, 1732, 1634, 1538, 1438, 1393, 1368, 1258, 1156 cm^{-1}. MS *m/e* 686 ($M+H^+$); HRMS (ESI) calcd for $C_{33}H_{57}N_4O_{11}$ 685.4023, found 685.4007.



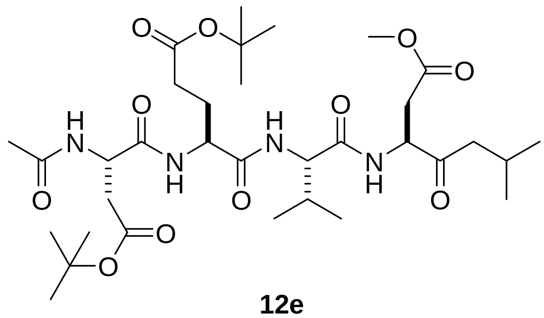
12c

Ac-Asp(O*t*Bu)-Glu(O*t*Bu)-Val-Asp(OMe)-pentyl ketone (12c). 83% yield. R_f 0.6 in System I, DPIP. ^1H NMR (500MHz, MeOH-d_4) δ_H 4.75-4.63 (m, 2H), 4.37 (m, 1H), 4.15 (td, *J* = 22.1 Hz, *J* = 7.0 Hz, 1H), 3.65 (s, 3H), 2.97-2.85 (m, 1H), 2.77 (m, 1H), 2.72-2.46 (m, 4H), 2.32 (m, 2H), 2.09 (m, 2H), 1.99 (m, 3H), 1.95-1.83 (m, 1H), 1.54 (m, 2H), 1.44 (m, 18H), 1.36-1.22 (m, 4H), 0.95 (m, 6H), 0.90 (t, *J* = 7.2 Hz, 3H). IR 3282, 3074, 2964, 2934, 2874, 2415, 1728, 1636, 1538, 1456, 1393, 1368, 1258, 1157, 1041, 1012, 957, 849 cm^{-1}. MS *m/e* 699 ($M+H^+$), 721 ($M+Na^+$); HRMS (ESI) calcd for $C_{34}H_{59}N_4O_{11}$ 699.4180, found 699.4199.



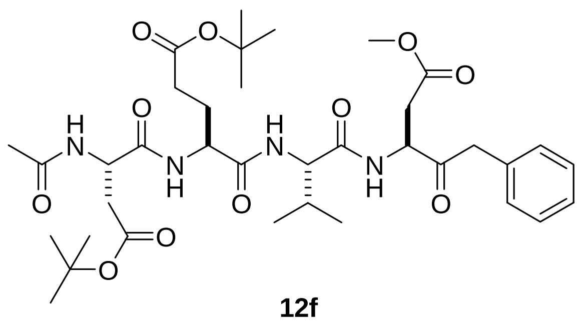
12d

Ac-Asp(O*t*Bu)-Glu(O*t*Bu)-Val-Asp(OMe)-hexyl ketone (12d). 55% yield. R_f 0.6 in System I, DPIP. ^1H NMR (500MHz, MeOH-d_4) δ_H 4.77-4.64 (m, 2H), 4.37 (dqd, *J* = 18.4 Hz, *J* = 9.2 Hz, *J* = 5.2 Hz, 1H), 4.15 (m, 1H), 3.66 (s, 3H), 2.92 (m, 1H), 2.78 (m, 1H), 2.74-2.46 (m, 4H), 2.34 (m, 2H), 2.10 (m, 2H), 2.00 (m, 3H), 1.90 (m, 1H), 1.54 (br m, 2H), 1.45 (m, 18H), 1.36-1.26 (m, 6H), 0.96 (m, 6H), 0.90 (t, *J* = 7.2 Hz, 3H). IR 3282, 3074, 2965, 2933, 2874, 2416, 1732, 1632, 1542, 1458, 1393, 1368, 1258, 1157, 1089, 1041, 957, 849 cm^{-1}. MS *m/e* 713 ($M+H^+$), 735 ($M+Na^+$); HRMS (ESI) calcd for $C_{35}H_{61}N_4O_{11}$ 713.4337, found 713.4327.



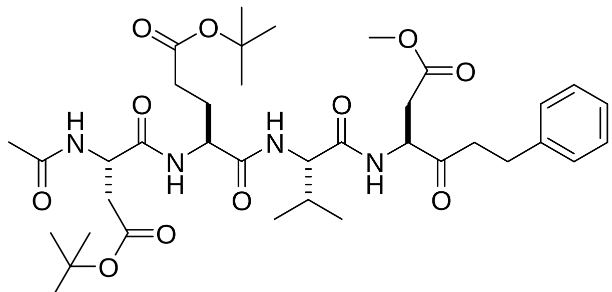
12e

Ac-Asp(O*t*Bu)-Glu(O*t*Bu)-Val-Asp(OMe)-isobutyl ketone (12e). 51% yield. R_f 0.6 in System I, DPIP. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 4.68 (m, 2H), 4.36 (m, 1H), 4.10 (m, 1H), 3.65 (s, 3H), 2.90 (m, 1H), 2.77 (m, 1H), 2.62 (m, 2H), 2.42 (m, 2H), 2.32 (m, 2H), 2.09 (m, 3H), 1.98 (s, 3H), 1.89 (m, 1H), 1.44 (s, 18H), 0.95 (m, 6H), 0.89 (m, 6H). IR 3650, 3406, 3298, 2967, 2934, 2875, 1725, 1648, 1534, 1458, 1370, 1257, 1157, 1088, 849 cm^{-1}. MS *m/e* 684 (M^+); HRMS (ESI) calcd for $C_{33}H_{57}N_4O_{11}$ 685.4023, found 685.2045.



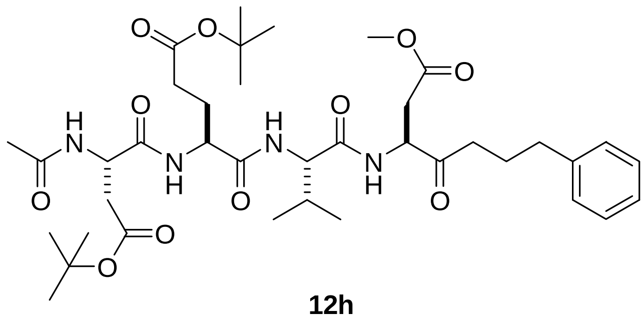
12f

Ac-Asp(O*t*Bu)-Glu(O*t*Bu)-Val-Asp(OMe)-benzyl ketone (12f). 65% yield. R_f 0.6 in System I, DPIP. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 7.28 (m, 2H), 7.24-7.16 (m, 3H), 4.67 (m, 2H), 4.40 (m, 1H), 4.14 (m, 1H), 3.86 (m, 2H), 3.63 (m, 3H), 2.92 (m, 1H), 2.79 (m, 1H), 2.75-2.53 (m, 2H), 2.34 (m, 2H), 2.10 (m, 2H), 1.98 (m, 3H), 1.90 (m, 1H), 1.44 (dd, *J* = 2.6 Hz, *J* = 1.9 Hz, 9H), 1.41 (m, 9H), 0.97 (m, 6H). IR 3275, 3068, 2975, 1730, 1634, 1534, 1368, 1258, 1155, 847 cm^{-1}. MS *m/e* 719 ($M+H^+$), 741 ($M+Na^+$); HRMS (ESI) calcd for $C_{36}H_{55}N_4O_{11}$ 719.3867, found 719.3896.



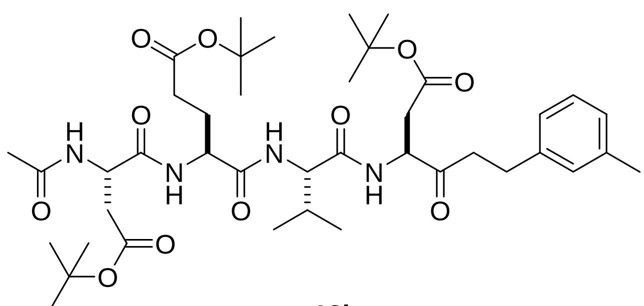
12g

Ac-Asp(O*t*Bu)-Glu(O*t*Bu)-Val-Asp(OMe)-2-phenylethyl ketone (12g). 93% yield. R_f 0.6 in System I, DPIP. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 7.24 (m, 2H), 7.19 (m, 2H), 7.14 (m, 1H), 4.76-4.62 (m, 2H), 4.35 (m, 1H), 4.13 (m, 1H), 3.65 (s, 3H), 3.05-2.56 (m, 9H), 2.31 (m, 2H), 2.09 (m, 2H), 1.99 (m, 3H), 1.86 (m, 1H), 1.45 (dd, *J* = 4.9 Hz, *J* = 1.9 Hz, 9H), 1.44 (t, *J* = 2.5 Hz, 9H), 0.94 (m, 6H). IR 3279, 3066, 2976, 2934, 1739, 1635, 1536, 1455, 1393, 1368, 1258, 1156, 1088, 849 cm^{-1}. MS *m/e* 733 ($M+H^+$), 755 ($M+Na^+$); HRMS (ESI) calcd for $C_{37}H_{56}N_4O_{11}Na$ 755.3843, found 755.3875.



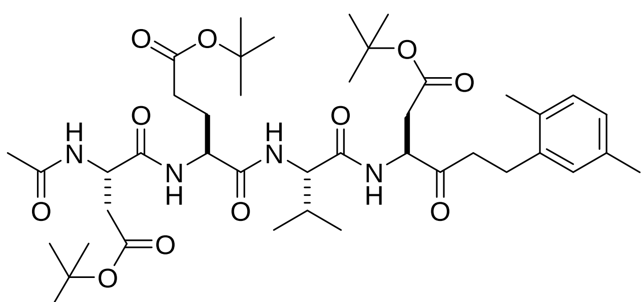
12h

Ac-Asp(O*t*Bu)-Glu(O*t*Bu)-Val-Asp(OMe)-3-phenylpropyl ketone (12h). 65% yield. R_f 0.6 in System I, DPIP. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 7.24 (t, *J* = 7.5 Hz, 2H), 7.17 (d, *J* = 7.1 Hz, 2H), 7.14 (t, *J* = 7.5 Hz, 1H), 4.67 (m, 2H), 4.37 (ddd, *J* = 19.2 Hz, *J* = 9.5 Hz, *J* = 5.0 Hz, 1H), 4.09 (m, 1H), 3.71 (s, 3H), 2.88 (m, 1H), 2.78 (m, 1H), 2.71-2.43 (m, 5H), 2.32 (m, 2H), 2.09 (m, 2H), 1.98 (dd, *J* = 4.3 Hz, *J* = 2.4 Hz, 3H), 1.91-1.81 (m, 3H), 1.44 (m, 9H), 1.42 (m, 9H), 0.94 (m, 6H). IR 3290, 3066, 2976, 2935, 1728, 1641, 1534, 1455, 1370, 1257, 1157, 1088, 849 cm^{-1}. MS *m/e* 747 ($M+H^+$), 769 ($M+Na^+$); HRMS (ESI) calcd for $C_{38}H_{59}N_4O_{11}$ 747.4180, found 747.4192.



12i

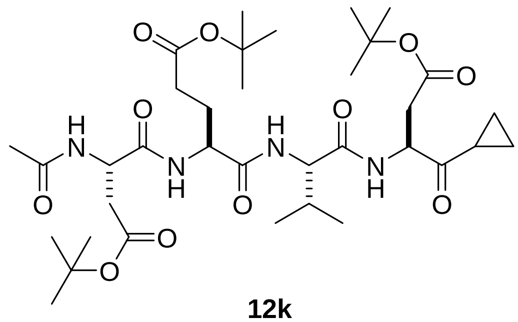
Ac-Asp(O*t*Bu)-Glu(O*t*Bu)-Val-Asp(O*t*Bu)-2-(3-methylphenyl)ethyl ketone (12i). 55% yield. R_f 0.6 in System I, DPIP. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 7.18 (t, *J* = 8.5 Hz, 1H), 7.05 (m, 3H), 4.73 (m, 2H), 4.42 (m, 1H), 4.28 (m, 2H), 4.20 (m, 1H), 2.88 (t, *J* = 8.3 Hz, 2H), 2.74 (m, 3H), 2.60 (m, 1H), 2.32 (br s, 4H), 2.08 (m, 2H), 2.00 (s, 3H), 1.90 (m, 1H), 1.44 (m, 27H), 0.98 (m, 6H). HRMS (ESI) cacld for $C_{41}H_{64}N_4O_{11}Na$ 811.4469, found 811.4490.



12j

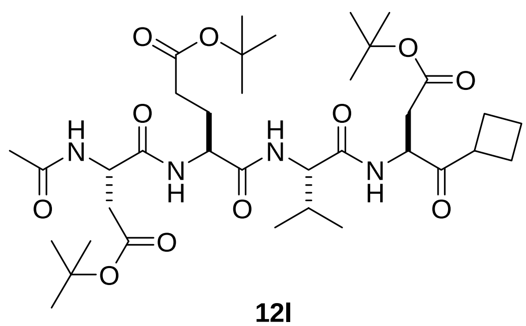
Ac-Asp(O*t*Bu)-Glu(O*t*Bu)-Val-Asp(O*t*Bu)-2-(2,5-dimethylphenyl)ethyl ketone (12j). 50% yield. R_f 0.6 in System I, DPIP. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 7.60 (m, 1H), 6.95 (m, 2H), 4.71 (m, 2H), 4.40

(m, 1H), 4.19 (m, 3H), 2.92 (t, *J* = 10.6 Hz, 1H), 2.75 (m, 3H), 2.60 (m, 2H), 2.27 (m, 6H), 1.44 (m, 27H), 0.98 (m, 6H). HRMS (ESI) cacld for $C_{42}H_{67}N_4O_{11}$ 803.4806, found 803.4832.



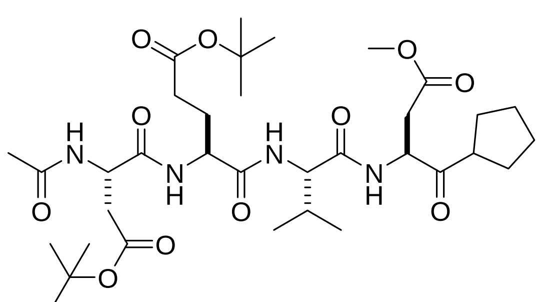
12k

Ac-Asp(O*t*Bu)-Glu(O*t*Bu)-Val-Asp(O*t*Bu)-cyclopropyl ketone (12k). 73% yield. R_f 0.6 in System I, DPIP. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 4.70 (m, 2H), 4.40 (m, 1H), 4.28 (m, 2H), 2.84-2.54 (m, 4H), 2.33 (m, 2H), 2.10 (m, 2H), 1.99 (m, 1H), 1.45 (s, 27H), 0.98 (m, 6H), 0.70 (m, 3H). IR 3289, 2976, 2931, 1732, 1687, 1655, 1640, 1537, 1455, 1393, 1367, 1257, 1154, 958, 847, 754 cm^{-1}. HRMS (ESI) cacld for $C_{35}H_{59}N_4O_{11}$ 711.4180, found 711.4179.



12l

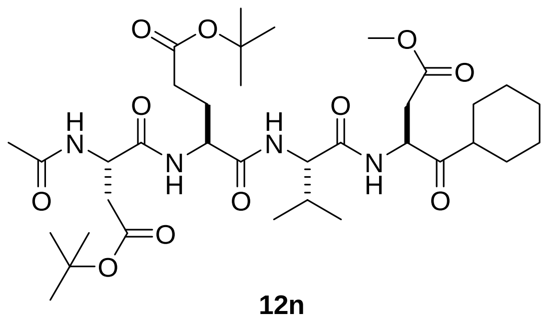
Ac-Asp(O*t*Bu)-Glu(O*t*Bu)-Val-Asp(O*t*Bu)-cyclobutyl ketone (12l). 70%yield. R_f 0.6 in System I, DPIP. ^{1}H NMR (500MHz, $CDCl_3$) δ_H 4.74 (m, 2H), 4.33 (m, 2H), 3.48 (sextet, *J* = 8.5 Hz, 1H), 2.83 (dt, *J* = 17.0 Hz, *J* = 5.2 Hz, 1H), 2.74 (dt, *J* = 15.7 Hz, *J* = 4.7 Hz, 2H), 2.65 (m, 1H), 2.45-2.32 (m, 2H), 2.32-2.16 (m, 3H), 2.16-2.00 (m, 6H), 1.95 (m, 2H), 1.78 (m, 1H), 1.42 (s, 9H), 1.40 (s, 9H), 1.38 (s, 9H), 0.92 (dd, *J* = 13.2 Hz, *J* = 6.7 Hz, 6H). IR (thin film) 3284, 2922, 2849, 1729, 1636, 1533, 1456, 1392, 1367, 1256, 1155, 847, 748 cm^{-1}. MS *m/e* 725 (M+H$^+$), 747 (M+Na$^+$); HRMS (ESI) calcd for $C_{36}H_{61}N_4O_{11}$ 725.4337, found 725.4358.



12m

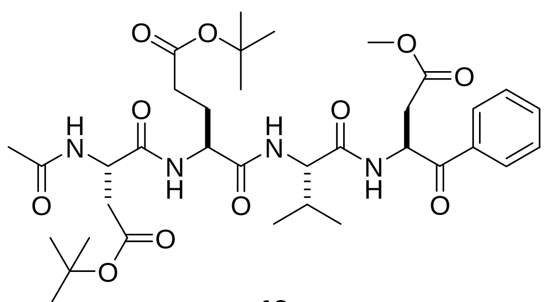
Ac-Asp(O*t*Bu)-Glu(O*t*Bu)-Val-Asp(OMe)-cyclopentyl ketone (12m). 51% yield. R_f 0.6 in System I, DPIP. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 4.83-4.63 (m, 2H), 4.37 (m, 1H), 4.11 (m, 1H), 3.65 (s, 3H), 3.16

(m, 1H), 2.91 (m, 1H), 2.78 (m, 1H), 2.70-2.55 (m, 2H), 2.32 (m, 2H), 2.09 (m, 2H), 1.99 (m, 4H), 1.89 (m, 2H), 1.67-1.53 (m, 4H), 1.44 (m, 18H), 0.95 (m, 6H). IR 3284, 3071, 2971, 2874, 1731, 1638, 1542, 1438, 1393, 1368, 1257, 1156, 1088, 847 cm^{-1}. MS *m/e* 698 ($M+H^+$), 720 ($M+Na^+$); HRMS (ESI) calcd for $C_{34}H_{57}N_4O_{11}$ 697.4024, found 697.4013.



12n

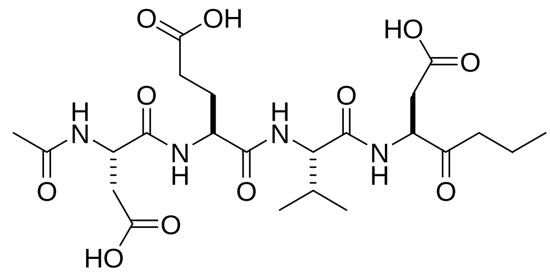
Ac-Asp(O*t*Bu)-Glu(O*t*Bu)-Val-Asp(OMe)-cyclohexyl ketone (12n). Isolated without the methyl ester from column in 41%. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 4.88-4.81 (m, 1H), 4.72-4.64 (m, 1H), 4.43 (m, 1H), 4.17 (m, 1H), 2.86-2.59 (m, 4H), 2.53 (m, 1H), 2.44-2.27 (m, 2H), 2.25-2.08 (m, 2H), 2.01 (m, 3H), 1.84 (br m, 2H), 1.75 (br m, 2H), 1.67 (br d, *J* = 12.5 Hz, 1H), 1.45 (m, 18H), 1.37-1.16 (m, 6H), 1.01-0.90 (m, 6H). IR 3423, 2975, 2361, 1726, 1648, 1543, 1448, 1368, 1259, 1156 cm^{-1}. MS *m/e* 698 ($M+H^+$), 719 ($M+Na^+$); HRMS (ESI) calcd for $C_{34}H_{57}N_4O_{11}$ 697.4024, found 697.4023.



12o

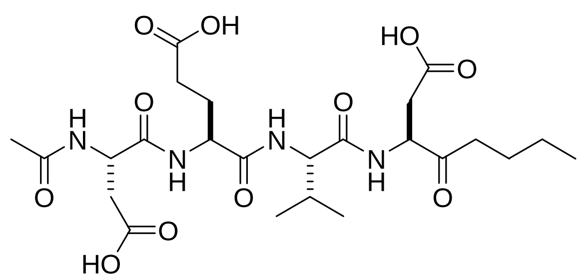
Ac-Asp(O*t*Bu)-Glu(O*t*Bu)-Val-Asp(OMe)-phenyl ketone (12o). 42% yield. R_f 0.6 in System I, DPIP. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 7.97 (m, 2H), 7.67-7.53 (m, 3H), 4.67 (m, 1H), 4.34 (td, *J* = 9.0 Hz, *J* = 5.0 Hz, 1H), 4.06 (ddd, *J* = 17.2 Hz, *J* = 6.86, *J* = 1.7 Hz, 1H), 3.65 (m, 3H), 3.04 (m, 1H), 2.82-2.55 (m, 4H), 2.29 (m, 2H), 2.08-1.92 (m, 4H), 1.83 (m, 1H), 1.44 (m, 18H), 0.79 (m, 6H). IR 3311, 3067, 2977, 2934, 1724, 1662, 1534, 1452, 1439, 1394, 1370, 1255, 1157, 848 cm^{-1}. MS *m/e* 705 ($M+H^+$), 727 ($M+Na^+$); HRMS (ESI) calcd for $C_{35}H_{53}N_4O_{11}$ 705.3711, found 705.3683; calcd for $C_{35}H_{52}N_4O_{11}Na$ 727.3530, found 727.3517.

General deprotection of peptide-ketones with methyl ester protecting group to give inhibitors 7a-h, m-o. To a flask containing the protected peptide-ketone (**12a-h,m-o)** (0.015 mmol, 1 eq) was added 2 mL of 1% (w/v) KOH in MeOH and the solution was stirred at room temperature for 1.5 hr. Then the reaction mixture was concentrated under reduced pressure. The residue was taken up in 15 mL using EtOAc and washed with 1 M HCl (4 mL), water (4 mL) and brine (4 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. To the resulting residue was added 1mL of trifluoroacetic acid and the solution stirred for 10 min. The solvent was removed under reduced pressure, and the residue washed with Et_2O (2 x 1 mL). The white solid products were then dried under high vacuum. Products obtained in the indicated yield.



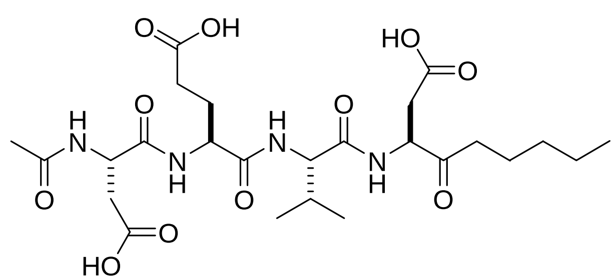
7a

Ac-Asp-Glu-Val-Asp-propyl ketone (7a). 36% yield. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 4.70 (m, 2H), 4.39 (m, 1H), 4.17 (m, 1H), 2.95-2.62 (m, 4H), 2.60-2.45 (m, 2H), 2.39 (t, J = 7.0 Hz, 2H), 2.10 (m, 2H), 1.99 (m, 3H), 1.92 (m, 1H), 1.56 (sextet, J = 7.3 Hz), 0.96 (m, 6H), 0.89 (t, J = 7.4 Hz, 3H). HRMS (ESI) calcd for $C_{23}H_{37}N_4O_{11}$ 545.2459, found 545.2448.



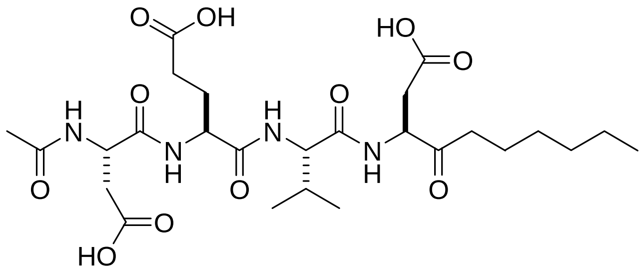
7b

Ac-Asp-Glu-Val-Asp-butyl ketone (7b). 97% yield. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 4.76-4.62 (m, 2H), 4.47-4.35 (m, 1H), 4.20-4.09 (m, 1H), 2.91-2.47 (m, 6H), 2.41 (m, 2H), 2.23-2.03 (m, 2H), 2.00 (m, 3H), 1.96-1.86 (m, 1H), 1.52 (m, 2H), 1.31 (m, 2H), 0.98 (m, 6H), 0.90 (t, J = 7.4 Hz, 3H). HRMS (ESI) calcd for $C_{24}H_{38}N_4O_{11}Na$ 581.2435, found 581.2449.



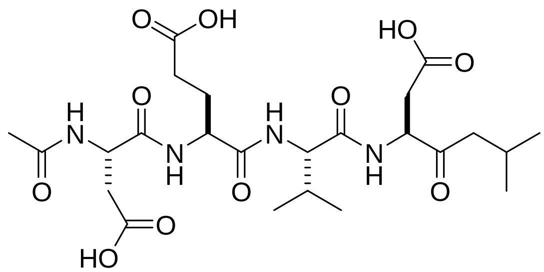
7c

Ac-Asp-Glu-Val-Asp-pentyl ketone (7c). 64% yield. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 4.70 (m, 2H), 4.37 (m, 1H), 4.16 (m, 1H), 2.90-2.62 (m, 4H), 2.61-2.48 (m, 2H), 2.40 (m, 2H), 2.10 (m, 2H), 1.98 (m, 3H), 1.92 (m, 1H), 1.54 (m, 2H), 1.28 (m, 4H), 0.97 (m, 6H), 0.89 (t, J = 7.0 Hz, 3H). HRMS (ESI) calcd for $C_{25}H_{41}N_4O_{11}$ 573.2772, found 573.2754.



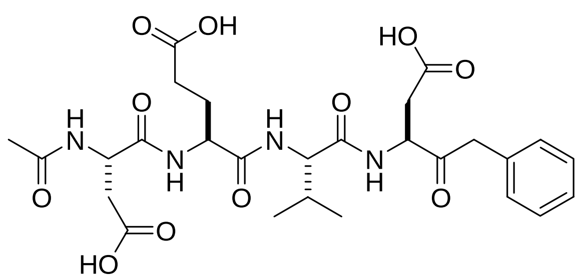
7d

Ac-Asp-Glu-Val-Asp-hexyl ketone (7d). 51% yield. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 4.74 (m, 1H), 4.64 (m, 1H), 4.38 (m, 1H), 4.20 (m, 1H), 2.90-2.64 (m, 4H), 2.55 (m, 2H), 2.40 (m, 2H), 2.10 (m, 2H), 1.99 (m, 3H), 1.92 (m, 1H), 1.53 (m, 2H), 1.29 (m, 6H), 0.97 (m, 6H), 0.89 (t, J = 6.9 Hz, 3H). HRMS (ESI) calcd for $C_{26}H_{43}N_4O_{11}$ 587.2928, found 587.2912.



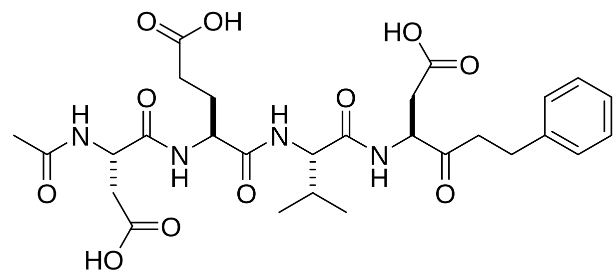
7e

Ac-Asp-Glu-Val-Asp-isobutyl ketone (7e). 52% yield. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 4.72 (m, 1H), 4.66 (m, 1H), 4.39 (m, 1H), 4.14 (m, 1H), 2.93-2.58 (m, 4H), 2.42 (m, 4H), 2.10 (m, 3H), 1.99 (m, 3H), 1.91 (m, 1H), 0.96 (m, 6H), 0.90 (d, J = 6.6 Hz, 6H). HRMS (ESI) calcd for $C_{24}H_{38}N_4O_{11}$ 559.2615, found 559.2612.



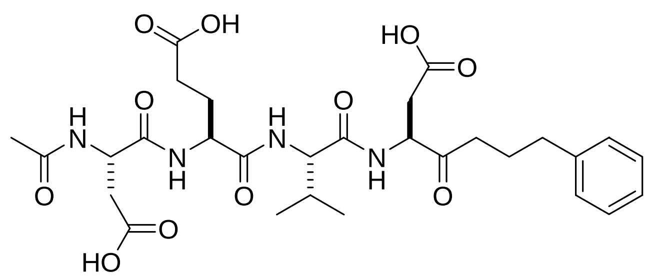
7f

Ac-Asp-Glu-Val-Asp-benzyl ketone (7f). 43% yield. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 7.29 (m, 2H), 7.20 (m, 3H), 4.69 (m, 1H), 4.40 (m, 1H), 4.17 (m, 1H), 3.86 (m, 2H), 2.94-2.62 (m, 4H), 2.41 (m, 2H), 2.30 (m, 1H), 2.12 (m, 2H), 1.98 (m, 3H), 1.92 (m, 1H), 0.98 (m, 6H). HRMS (ESI) calcd for $C_{27}H_{37}N_4O_{11}$ 593.2459, found 593.2446.



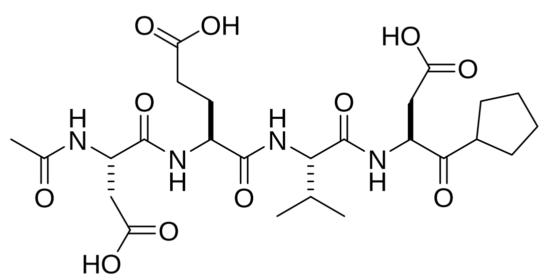
7g

Ac-Asp-Glu-Val-Asp-2-phenylethyl ketone (7g). 93% yield. [1]H NMR (500MHz, MeOH-d_4) δ_H 7.20 (m, 5H), 4.70 (m, 2H), 4.30 (m, 2H), 3.10-2.65 (m, 6H), 2.38 (m, 2H), 2.25 (m, 1H), 2.16 (br s, 2H), 2.08 (m, 1H), 1.98 (m, 3H), 1.90 (m, 1H), 0.98 (m, 6H). HRMS (ESI) calcd for $C_{28}H_{39}N_4O_{11}$ 607.2615, found 607.2610.



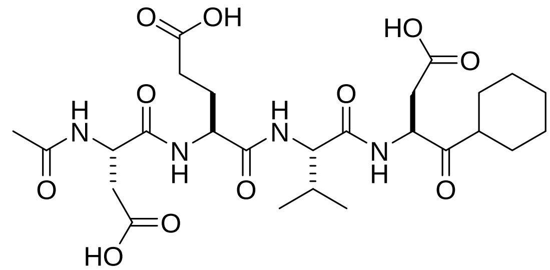
7h

Ac-Asp-Glu-Val-Asp-3-phenylpropyl ketone (7h). 65% yield. [1]H NMR (500MHz, MeOH-d_4) δ_H 7.25 (m, 2H), 7.17 (m, 3H), 4.75 (m, 1H), 4.69 (m, 1H), 4.49-4.20 (m, 2H), 2.90-2.57 (m, 5H), 2.40 (m, 2H), 2.20 (m, 3H), 2.02-1.81 (m, 7H), 0.98 (m, 6H). HRMS (ESI) calcd for $C_{29}H_{40}N_4O_{11}Na$ 643.2591, found 643.2547.



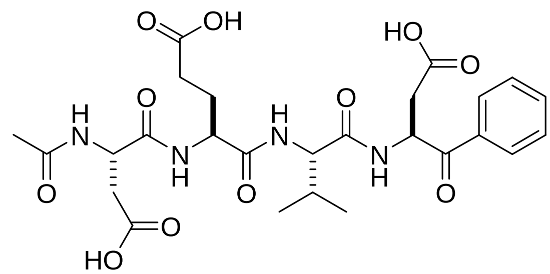
7m

Ac-Asp-Glu-Val-Asp-cyclopentyl ketone (7m). 36% yield. [1]H NMR (500MHz, MeOH-d_4) δ_H 4.76 (m, 1H), 4.38 (m, 1H), 4.27 (dd, J = 4.2 Hz, 1H), 4.17 (m, 1H), 3.25 (t, J = 3.2 Hz, 1H), 3.17 (m, 1H), 2.93-2.56 (m, 3H), 2.42 (m, 2H), 2.29 (m, 1H), 2.10 (m, 2H), 1.87 (m, 2H), 1.75 (m, 2H), 1.62 (m, 4H), 0.97 (m, 6H). HRMS (ESI) calcd for $C_{25}H_{39}N_4O_{11}$ 571.2615, found 571.2607.



7n

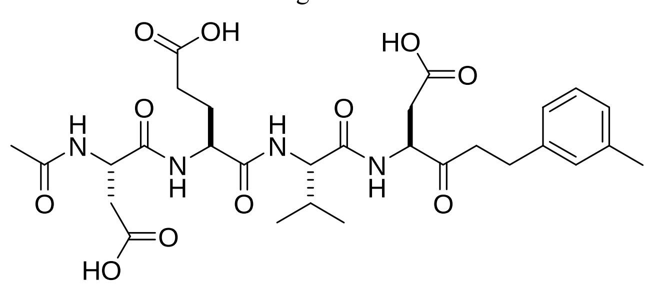
Ac-Asp-Glu-Val-Asp-cyclohexyl ketone (7n). 99% yield. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 4.67 (m, 1H), 4.38 (m, 1H), 4.16 (m, 1H), 3.70 (m, 1H), 2.80 (m, 4H), 2.56 (m, 1H), 2.40 (m, 2H), 2.16 (m, 2H), 2.00 (m, 4H), 1.92-1.64 (m, 6H), 1.30 (m, 6H), 0.98 (m, 6H). HRMS (ESI) calcd for $C_{26}H_{41}N_4O_{11}$ 585.2772, found 585.2770.



7o

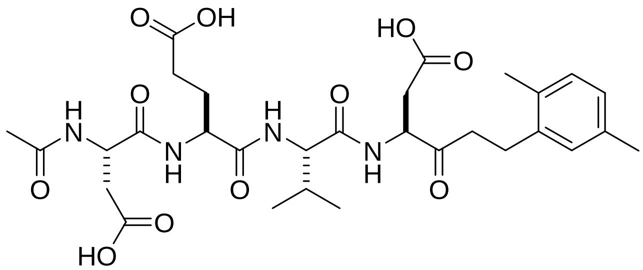
Ac-Asp-Glu-Val-Asp-phenyl ketone (7o). 55% yield. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 7.99 (m, 2H), 7.61 (m, 1H), 7.49 (m, 2H), 5.71 (m, 1H), 4.70 (m, 1H), 4.38-4.04 (m, 2H), 3.19 (m, 1H), 3.02 (m, 1H), 2.89-2.61 (m, 3H), 2.36 (m, 2H), 2.32-2.07 (m, 2H), 1.99 (m, 4H), 1.85 (m, 1H), 0.84 (m, 6H). HRMS (ESI) calcd for $C_{26}H_{35}N_4O_{11}$ 579.2302, found 579.2289.

General deprotection of peptide-ketones with *tert*-butyl ester protecting group to give inhibitors 7i-l. Protected peptide ketone (**12i-l**) (0.015 mmol, 1 eq) was stirred in 1 mL of trifluoroacetic acid at r.t. for 10 min. The solvent was then removed under reduced pressure and the residue was washed with Et_2O (2x1 mL). The white solid products were dried under high vacuum. Products obtained in the indicated yields.



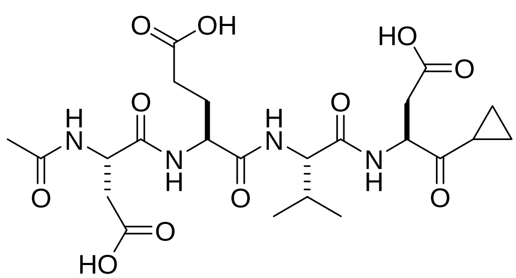
7i

Ac-Asp-Glu-Val-Asp-2-(3-methylphenyl)ethyl ketone (7i). 98% yield. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 7.05 (m, 4H), 4.71 (m, 2H), 4.42 (m, 1H), 4.30 (m, 2H), 4.20 (m, 1H), 2.94-2.68 (m, 6H), 2.42 (m, 2H), 2.30 (s, 3H), 2.15 (m, 2H), 1.99 (br s, 4H), 0.98 (br s, 6H). HRMS (ESI) calcd for $C_{29}H_{40}N_4O_{11}Na$ 643.2591, found 643.2579.



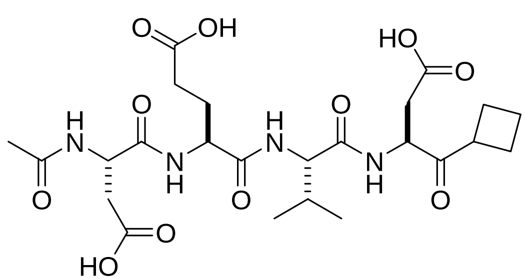
7j

Ac-Asp-Glu-Val-Asp-2-(2,5-dimethylphenyl)ethyl ketone (7j). 98% yield. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 795 (m, 1H), 6.90 (m, 2H), 4.70 (m, 2H), 4.45-4.00 (m, 4H), 2.95-2.55 (m, 6H), 2.40 (m, 2H), 2.25 (br s, 5H), 2.15 (br s, 2H), 2.00 (br s, 5H), 0.98 (br s, 6H). HRMS (ESI) calcd for $C_{30}H_{43}N_4O_{11}$ 635.2928, found 635.2930.



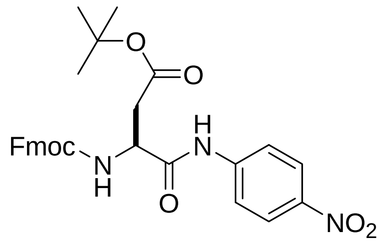
7k

Ac-Asp-Glu-Val-Asp-cyclopropyl ketone (7k). 98% yield. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 4.85 (m, 1H), 4.68 (m, 1H), 4.38 (m, 1H), 4.16 (m, 1H), 2.85 (m, 2H), 2.70 (m, 2H), 2.42 (m, 2H), 2.18 (m, 3H), 2.00 (m, 4H), 0.98 (m, 9H). HRMS (ESI) calcd for $C_{26}H_{35}N_4O_{11}$ 543.2303, found 543.2288.



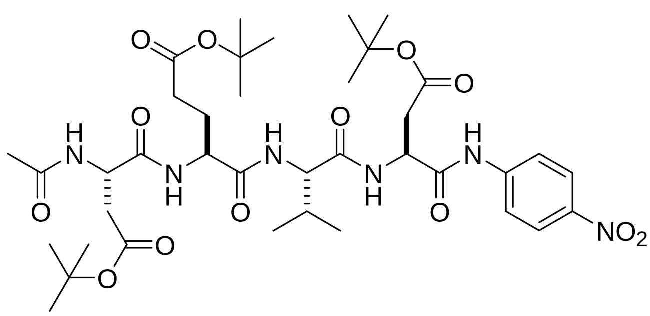
7l

Ac-Asp-Glu-Val-Asp-cyclopentyl ketone (7l). 82% yield. ^{1}H NMR (500MHz, MeOH-d_4) δ_H 4.68 (m, 2H), 4.42 (m, 1H), 4.17 (m, 1H), 3.53 (m, 1H), 2.88 (m, 2H), 2.70 (m, 2H), 2.42 (m, 2H), 2.32-2.03 (m, 4H), 2.00 (m, 5H), 1.80 (m, 1H), 0.96 (m, 6H). HRMS (ESI) calcd for $C_{24}H_{37}N_4O_{11}$ 557.2459, found 557.2455.



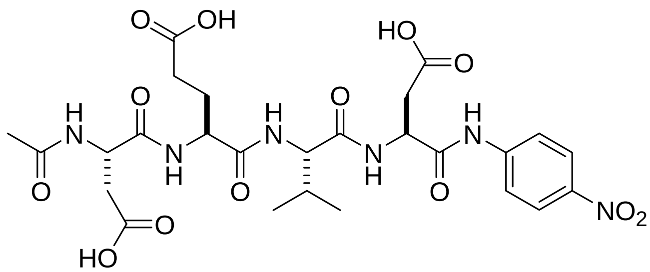
13

Fmoc-Asp(O*t*Bu)-*p*NA (13). To a solution of Fmoc-Asp(OtBu)-OH (1.0 mmol, 1 eq), and *p*-nitroanaline (1.0 mmol, 1 eq) in 3 mL of pyridine in the dark was added $POCl_3$ (1.1 mmol, 1.1 eq) and the reaction stirred at –15 °C for 10 min. Then the reaction was quenched by adding 10 mL of ice-water. When the ice had melted, the reaction mixture was extracted with EtOAc (3 x 15 mL). The combined organic layers were then washed with $NaHCO_3$ (15 mL) and brine (15 mL). This solution was then pushed through a pad of silica using EtOAc/hexanes (1:3). The solvent was removed under reduced pressure, and then the residue was coevaporated with toluene, EtOAc, and MeOH followed by drying *in vacuo*. 426.6 mg of a white-yellow solid were obtained (80% yield). R_f 0.7 in System I, DPIP. ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 9.11 (s, 1H), 8.17 (d, *J* = 9.2 Hz, 2H), 7.76 (d, *J* = 7.4 Hz, 2H), 7.66 (d, *J* = 9.2 Hz, 2H), 7.57 (d, *J* = 7.0 Hz, 2H), 7.34 (t, *J* = 7.3 Hz, 2H), 7.29 (br m, 2H), 6.14 (d, *J* = 7.3 Hz, 1H), 4.70 (br s, 1H), 4.49 (m, 2H), 4.21 (t, *J* = 6.8 Hz, 1H), 2.94 (dd, *J* = 16.6 Hz, *J* = 4.0 Hz, 1H), 2.72 (dd, *J* = 16.6 Hz, *J* = 6.6 Hz, 1H), 1.46 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 171.5, 169.4, 156.8, 143.8, 143.7, 143.6, 143.5, 141.5, 128.1, 127.3, 125.2, 125.1, 120.3, 119.5, 82.7, 67.7, 52.1, 47.2, 37.2, 28.2. IR 3307, 3066, 2979, 1718, 1695, 1684, 1652, 1616, 1597, 1559, 1512, 1451, 1410, 1368, 1341, 1302, 1252, 1153, 1112, 1048, 853, 739 cm^{-1}. HRMS (ESI) calcd for $C_{29}H_{30}N_3O_7$ 532.2084, found 532.2079.



14

Ac-Asp(O*t*Bu)-Glu(O*t*Bu)-Val-Asp(O*t*Bu)-*p*NA (14). To a solution of **13** (1.0 mmol, 1 eq) in 10 mL CH_2Cl_2 in the dark was added 3.5 mL of 4-aminomethylpiperidine (excess). After 20 min, the reaction was diluted to 40 mL using CH_2Cl_2, washed with brine (2x15 mL), pH 5.5 10% phosphate buffer (3x15 mL), and brine again (15 mL). The organic layer was dried over $MgSO_4$ and then concentrated under reduced pressure. [**Coupling**] To the residue was added 10 mL of CH_2Cl_2, N^{α}-fmoc-valine (1.1 mmol, 1.1 eq), and DIEA (1.5 mmol, 1.5 eq). Then PyBOP (1.1 mmol, 1.1 eq) was added and the reaction stirred at r.t. in the dark until all amine was consumed as seen by TLC (System I, Ninhydrin). [**Deprotection**] Then 4 mL of TAEA (excess) was added and the reaction stirred for 30 min, after which the solution was diluted with 20 mL of EtOAc and washed with sat. brine (1x15 mL), pH 5.5 10% phosphate buffer (3x15 mL), and again with sat. brine (1x15 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness. The **coupling** and **deprotection** process was repeated with appropriately protected amino acids to give the desired protected peptide, which was purified after the final washings by column chromatography (SiO_2, EtOAc). Obtained the product in 61% yield. R_f 0.7 in System I, DPIP. ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 8.17 (m, 2H), 7.97 (m, 2H), 4.96 (m, 1H), 4.62 (m, 1H), 4.44 (m, 1H), 3.86 (ddd, *J* = 16.5 Hz, *J* = 7.2 Hz, *J* = 5.8 Hz, 1H), 3.08 (m, 1H), 2.94-2.66 (m, 3H), 2.60-2.34 (m, 2H), 2.27 (m, 2H), 1.92 (s, 3H), 1.42 (m, 18H), 1.00 (m, 6H). IR 3283, 3085, 2978, 2934, 2255, 1731, 1633, 1598, 1514, 1458, 1412, 1393, 1368, 1342, 1302, 1257, 1156, 1113, 1041, 915, 855 cm^{-1}. HRMS (ESI) cacld for $C_{38}H_{59}N_6O_{13}$ 807.4140, found 807.4102.



15

Ac-Asp-Glu-Val-Asp-*p*NA (15). A solution of **14** (0.2 mmol) in 5mL of 95% trifluoroacetic acid/CH_2Cl_2 was stirred at room temperature in the dark for 10 min. The solution was then concentrated under reduced pressure, and the residue was washed twice with diethyl ether. A white solid was obtained from the ether by vacuum filtration. This was then lyophilized from water to give a white powder in 90% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 8.23 (m, 2H), 7.95 (dd, *J* = 9.2 Hz, *J* = 2.7 Hz, 2H), 4.61 (m, 1H), 4.50-4.30 (m, 1H), 4.06-3.91 (m, 1H), 3.03 (m, 1H), 2.92-2.66 (m, 3H), 2.49-2.36 (m, 2H), 2.16 (m, 2H), 2.00 (m, 3H), 1.97-1.88 (m, 1H), 0.99 (m, 6H). MS *m/e* 639 (M+H$^+$); HRMS (ESI) calcd for $C_{26}H_{35}N_6O_{13}$ 639.2262, found 639.2266.

Caspase inhibition assay. Caspase-3 and -7 were expressed and purified as previously reported[4] and the substrate **15** was used for all assays. In triplicate, fourteen concentrations of each inhibitor (ranging from 1 mM to 10 pM) were incubated with enzyme (diluted to give an activity of 0.85 pmol *p*NA/min) for 10 min after which the Ac-DEVD-*p*NA substrate was added to give a final concentration of 200µM (50µL total volume). A blank and zero inhibitor concentration (with vehicle control) were also included. The release of *p*-nitroaniline was monitored at 405 nm over 2 h in clear 384-well plates (Nunc) by a Molecular Devices SpectraMax Plus 384 plate reader. All experiments were carried out at room temperature in pH 7.4 50 mM HEPES, 100 mM NaCl, 0.10% CHAPS, 10 mM DTT, 0.1 mM EDTA, and 10% glycerol buffer with <1% DMSO per well. The relative enzyme activity was calculated from the slopes of the linear portions of the collected data and plotted versus inhibitor concentration. The data was fit to a logistic dose response curve by non-linear regression using TableCurve 2D (v.5.01) to give the IC_{50}, which was converted to apparent K_I using the following equation:

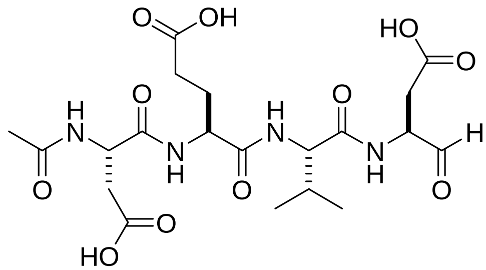
$$K_I = IC_{50}/(1+([\text{Substrate}]/K_M))$$

Where K_M was found for each 384-well plate by using the same enzyme dilution and a substrate range from 2 mM to 10 nM (data fit to the Michaelis-Menten equation).

Molecular modeling. Crystal structures of both caspase-3 and -7 with inhibitor **1** were obtained from the Protein Data Bank[5] (1PAU and 1F1J respectively). All energy minimizations were calculated in MOE[6] using the MMFF94x force field. Hydrogens were added to each crystal structure and allowed to minimize while the rest of the structure was fixed. Then, the entire protein/inhibitor structure was minimized. The appropriate P1' position was then built into the structure. The inhibitor and all residues of the enzyme within 9Å were then minimized while the rest of the protein was fixed. This last minimization was repeated with the added P1' position in different starting conformations, but always minimized to the same conformation shown. These minimized structures were then moved to Sybyl MOLCAD[7] for surface visualization (Connelly surface with a 1.4Å probe and a cavity depth mapped onto the surface).

References

(1) Waldmann, H.; Kunz, H. *Liebigs Ann. Chem.* **1983**, 1712-1725.
(2) Sharma, A. K.; Hergenrother, P. J. *Org Lett* **2003**, *5*, 2107-9.
(3) Vazquez, J.; Albericio, F. *Tetrahedron Letters* **2002**, *43*, 7499-7502.
(4) Stennicke, H. R.; Salvesen, G. S. *Methods* **1999**, *17*, 313-9.
(5) Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. *Nucleic Acids Res.* **2000**, *28*, 235-242.
(6) MOE version 2004, Chemical Computing Group, Montreal.
(7) SYBYL 7.0, Tripos Inc. St. Louis, Missouri.



O
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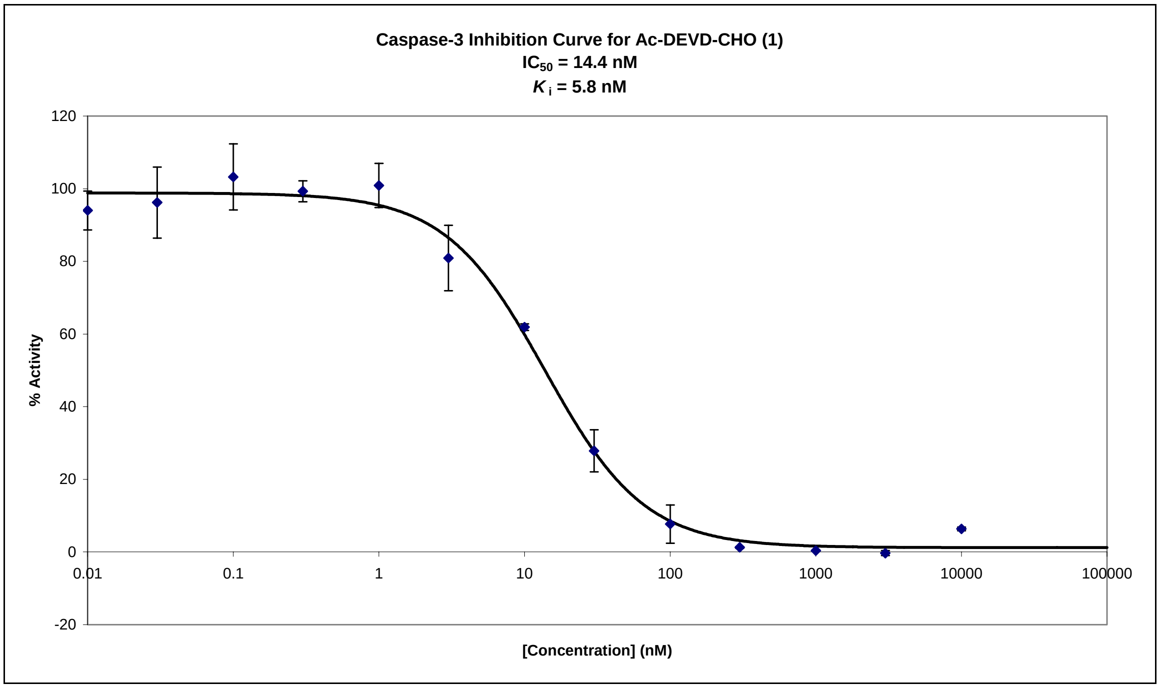
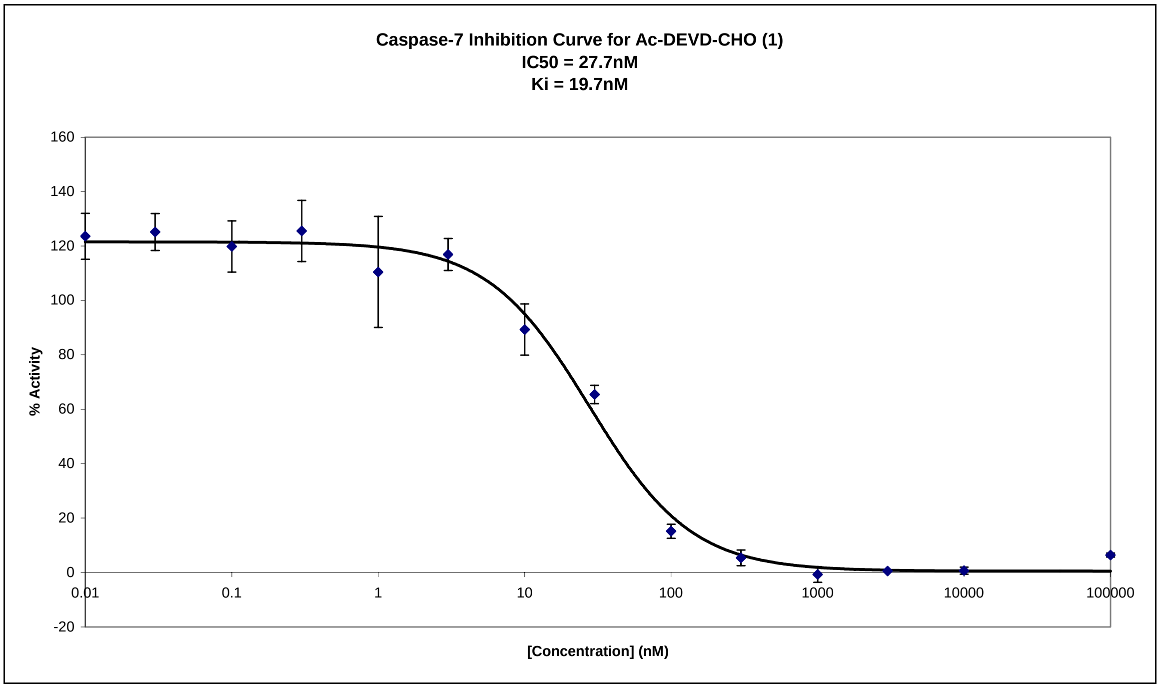
H
O
HO
O

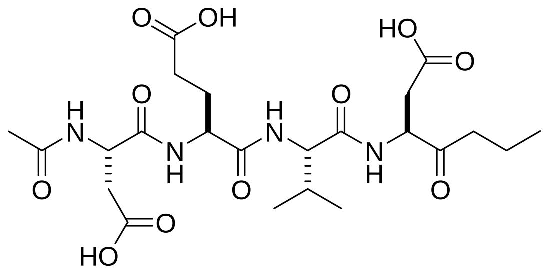
1



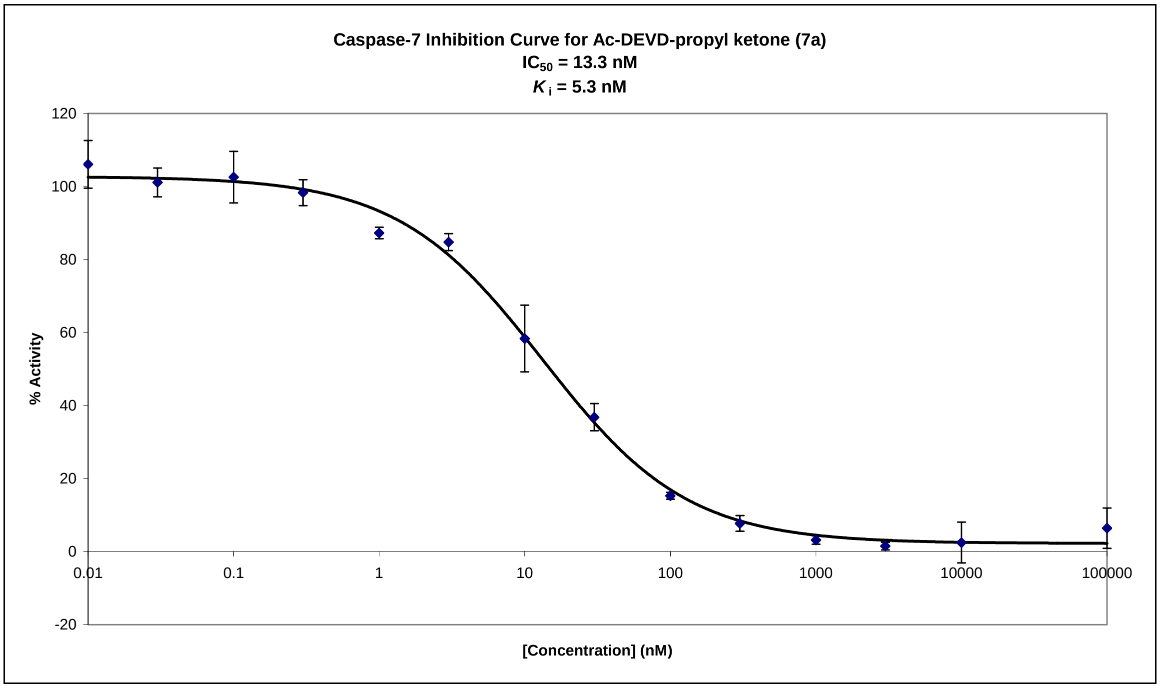
Caspase-3 Inhibition Curve for Ac-DEVD-CHO (1)
IC50 = 14.4 nM
K i = 5.8 nM
120
100
80
60
40
20
0
-20
% Activity
0.01
0.1
1
10
100
1000
10000
100000
[Concentration] (nM)

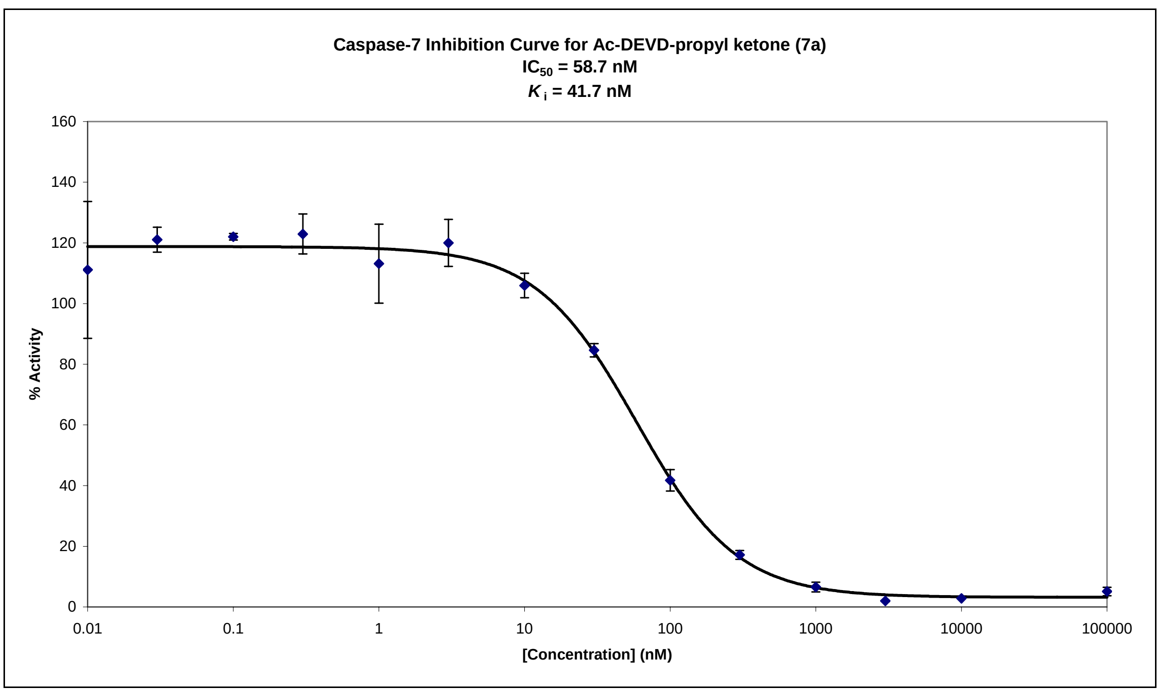


Caspase-7 Inhibition Curve for Ac-DEVD-CHO (1)
IC50 = 27.7nM
Ki = 19.7nM
160
140
120
100
80
60
40
20
0
-20
% Activity
0.01
0.1
1
10
100
1000
10000
100000
[Concentration] (nM)



7a


Caspase-7 Inhibition Curve for Ac-DEVD-propyl ketone (7a)
IC50 = 13.3 nM
K i = 5.3 nM
% Activity
[Concentration] (nM)
120
100
80
60
40
20
0
-20
0.01
0.1
1
10
100
1000
10000
100000


Caspase-7 Inhibition Curve for Ac-DEVD-propyl ketone (7a)
IC50 = 58.7 nM
K i = 41.7 nM
% Activity
[Concentration] (nM)
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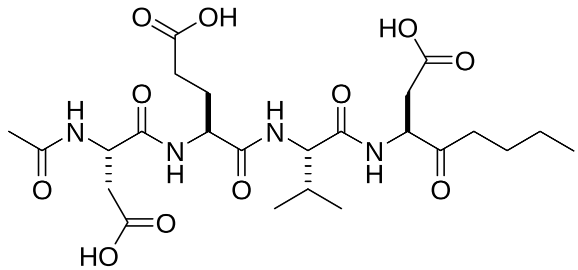
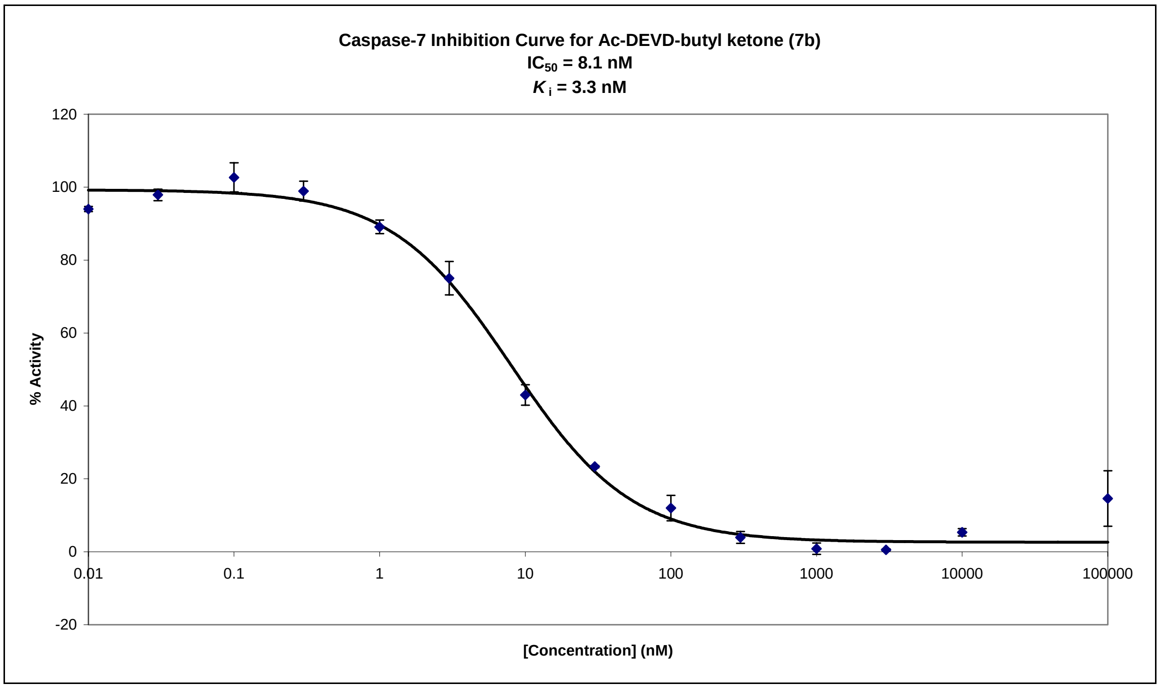
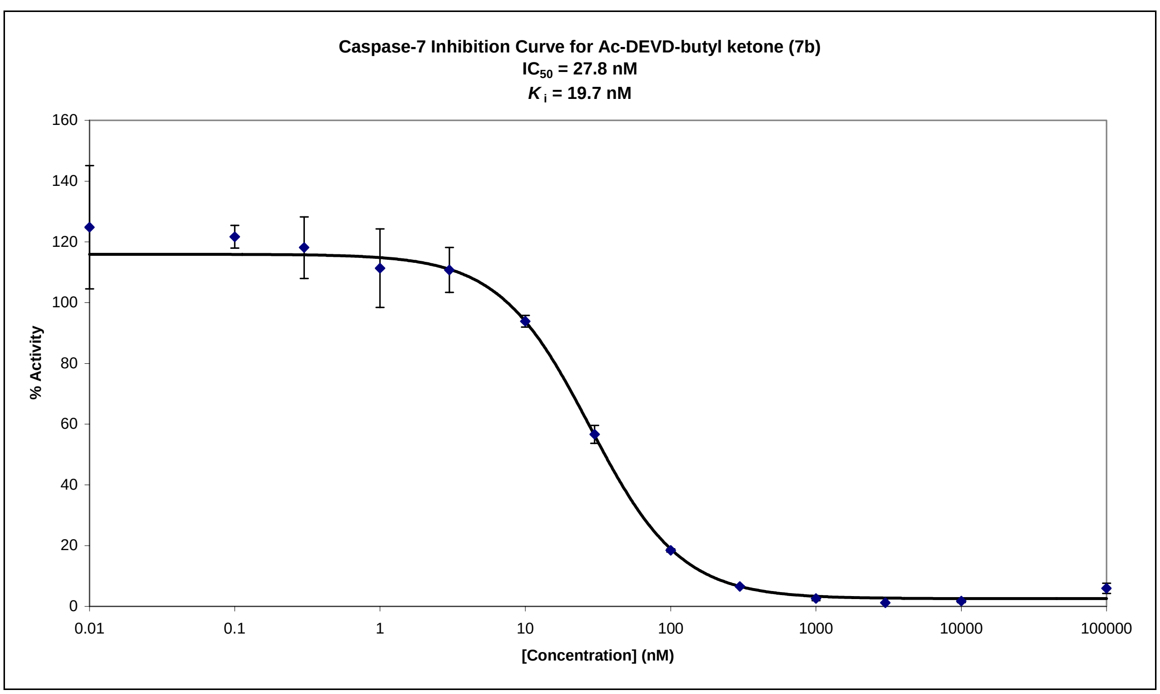
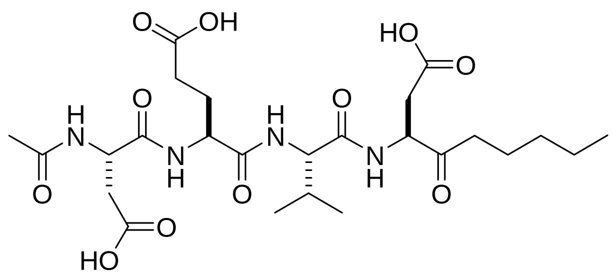
140
120
100
80
60
40
20
0
0.01
0.1
1
10
100
1000
10000
100000



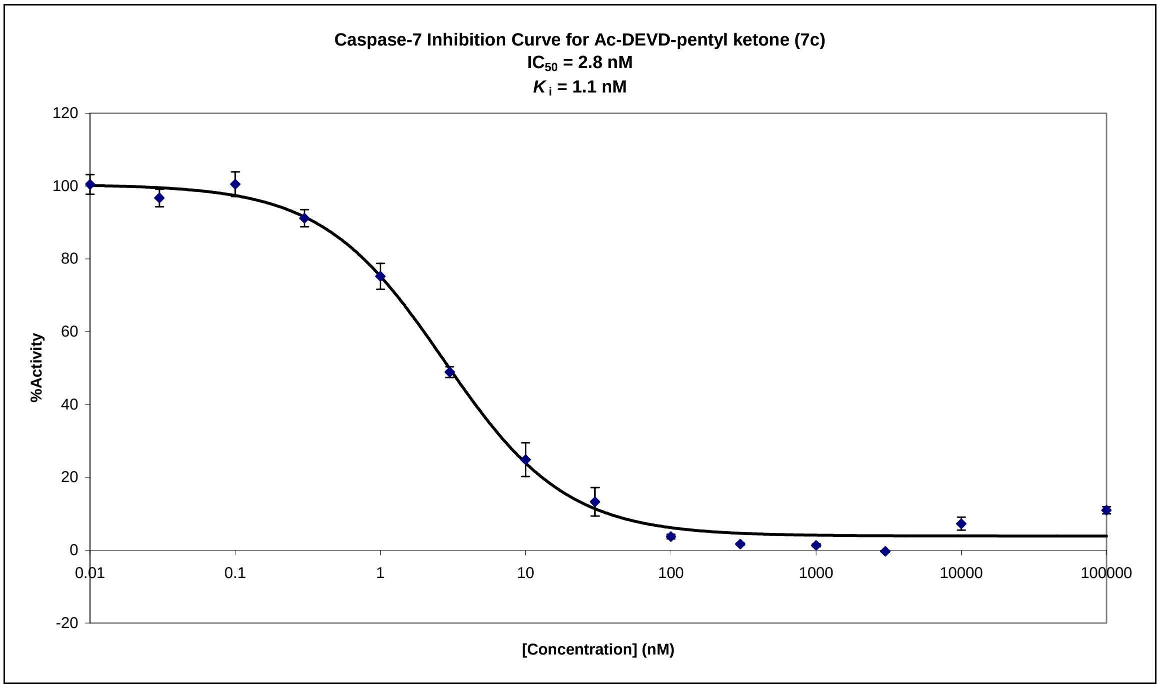
7b


Caspase-7 Inhibition Curve for Ac-DEVD-butyl ketone (7b)
IC_{50} = 8.1 nM
K_i = 3.3 nM
% Activity
[Concentration] (nM)


Caspase-7 Inhibition Curve for Ac-DEVD-butyl ketone (7b)
IC_{50} = 27.8 nM
K_i = 19.7 nM
% Activity
[Concentration] (nM)



7c



Caspase-7 Inhibition Curve for Ac-DEVD-pentyl ketone (7c)
IC50 = 2.8 nM
Ki = 1.1 nM
%Activity
[Concentration] (nM)
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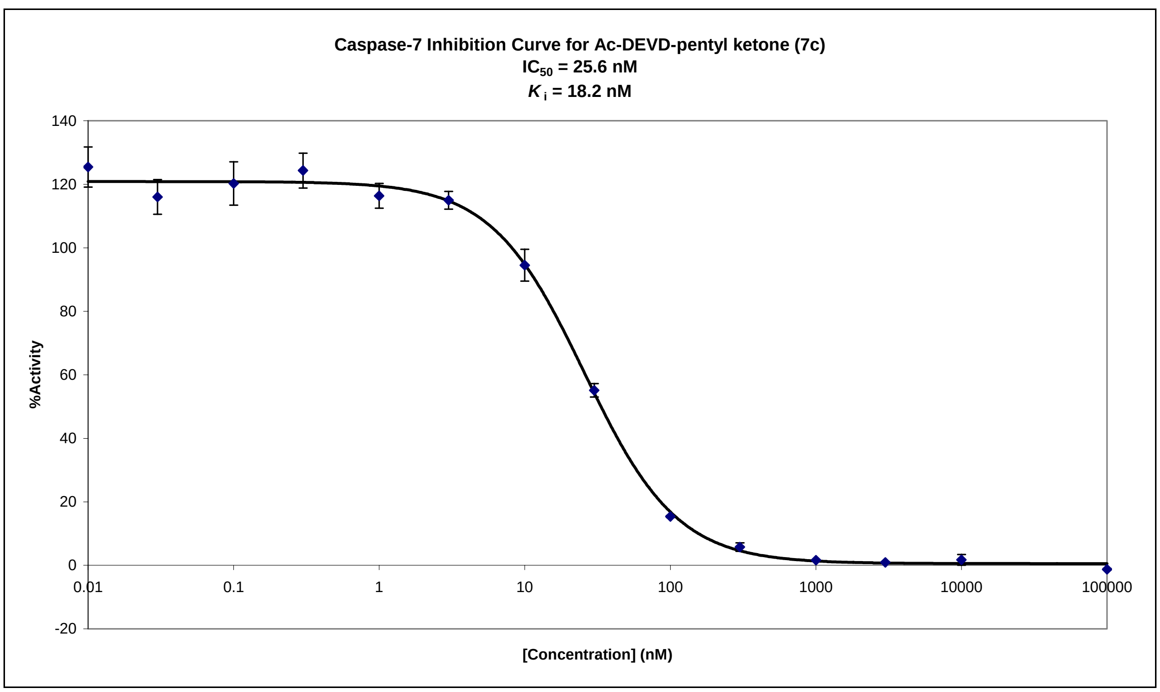
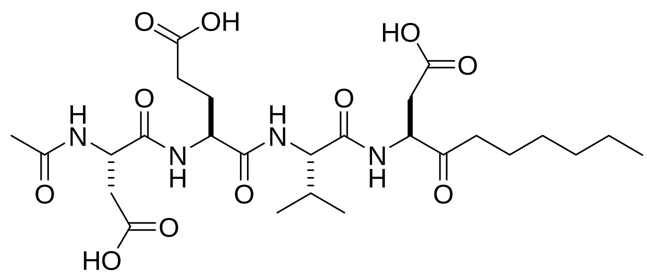
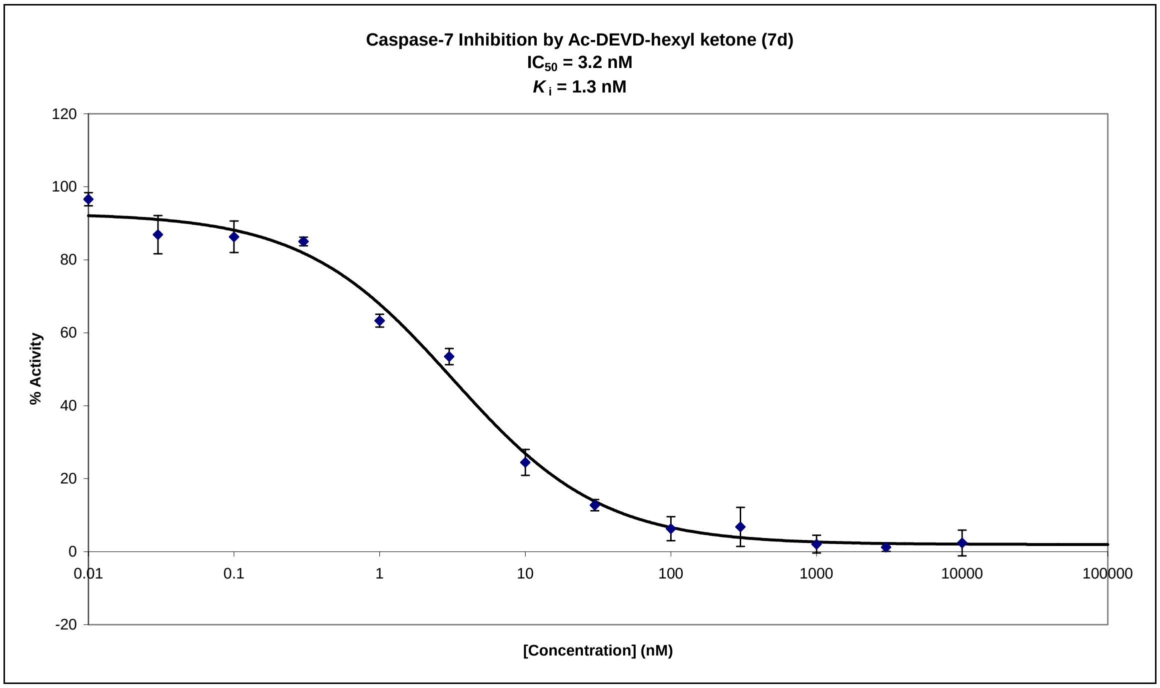
100
80
60
40
20
0
-20
0.01
0.1
1
10
100
1000
10000
100000



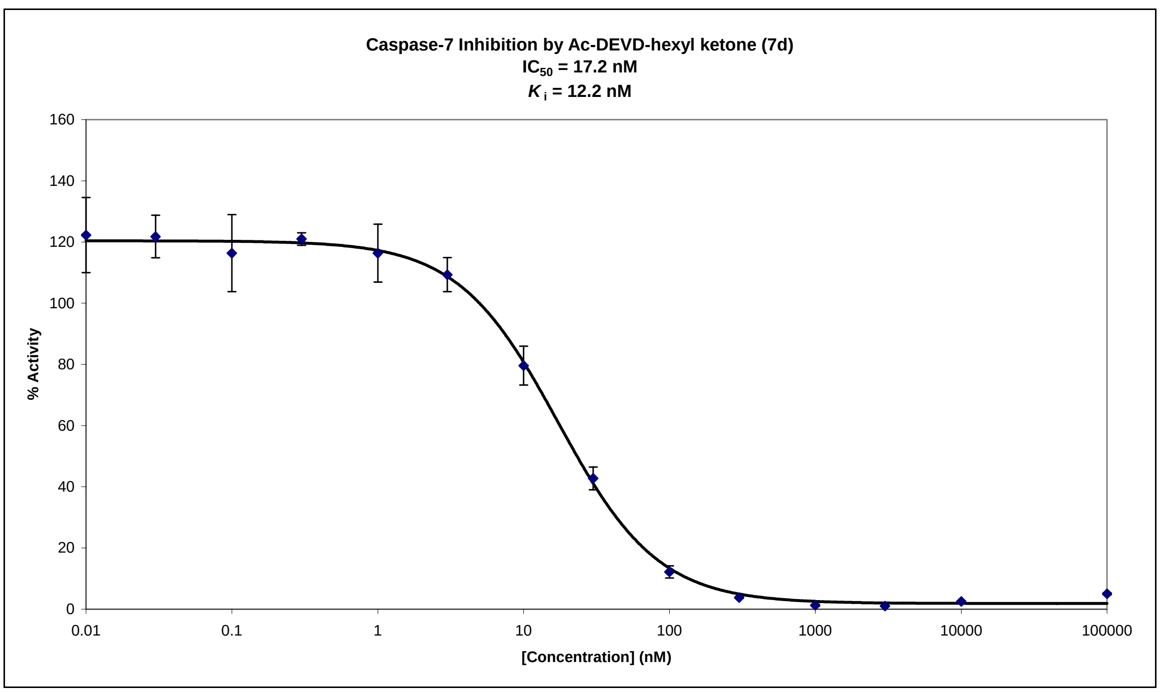
Caspase-7 Inhibition Curve for Ac-DEVD-pentyl ketone (7c)
IC50 = 25.6 nM
Ki = 18.2 nM
%Activity
[Concentration] (nM)
140
120
100
80
60
40
20
0
-20
0.01
0.1
1
10
100
1000
10000
100000



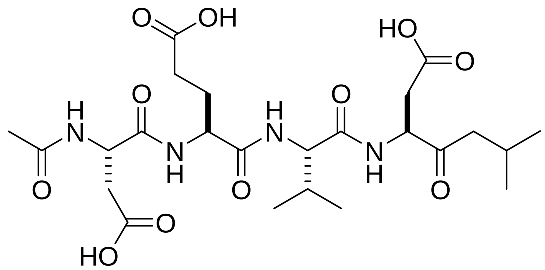
7d



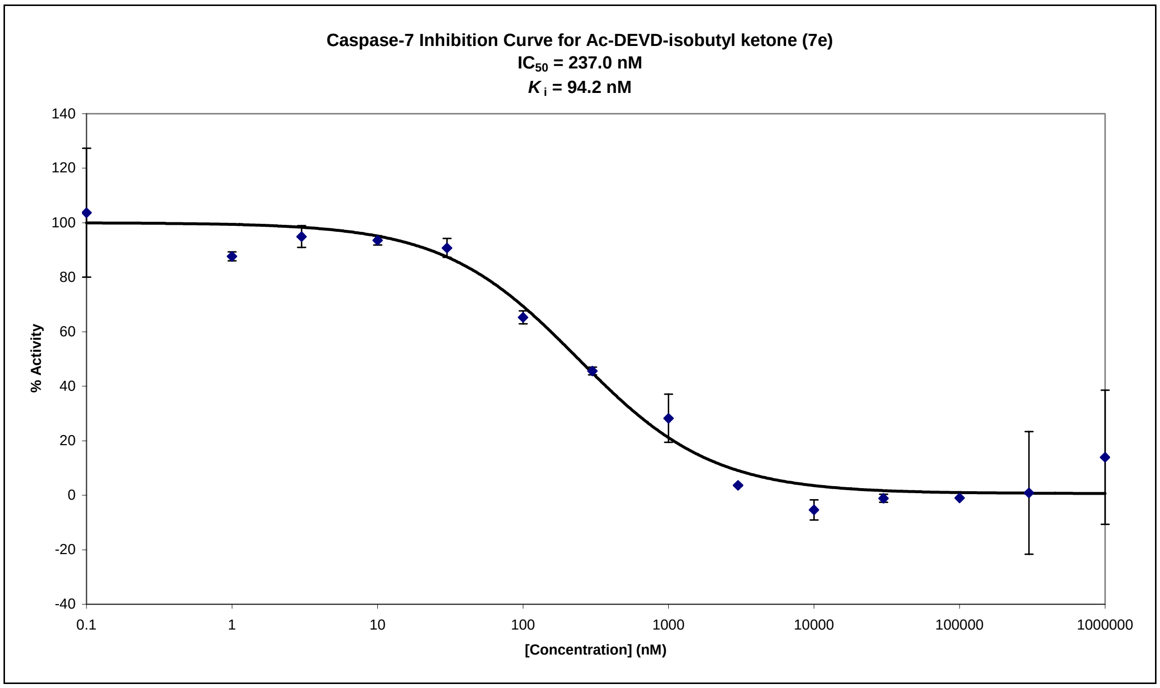
Caspase-7 Inhibition by Ac-DEVD-hexyl ketone (7d)
IC50 = 3.2 nM
K i = 1.3 nM
120
100
80
60
40
20
0
-20
% Activity
0.01
0.1
1
10
100
1000
10000
100000
[Concentration] (nM)

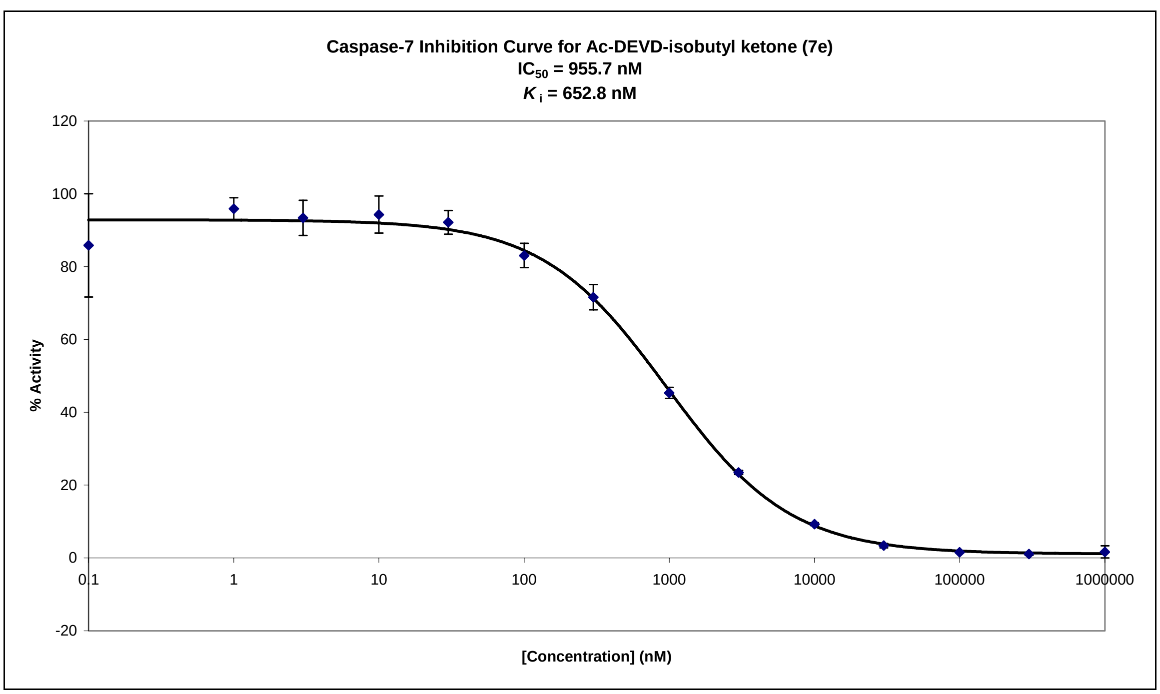


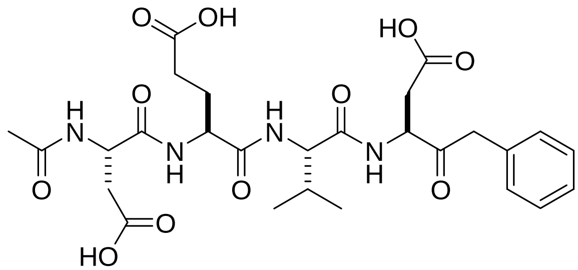
Caspase-7 Inhibition by Ac-DEVD-hexyl ketone (7d)
IC50 = 17.2 nM
K i = 12.2 nM
160
140
120
100
80
60
40
20
0
% Activity
0.01
0.1
1
10
100
1000
10000
100000
[Concentration] (nM)



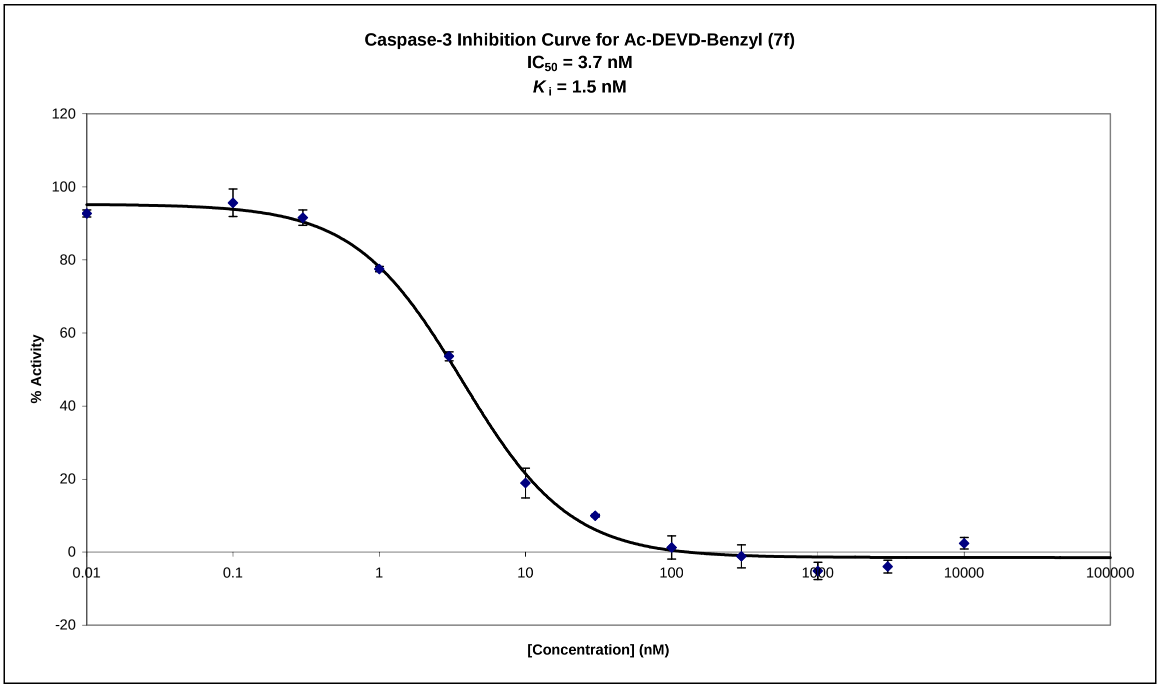
7e

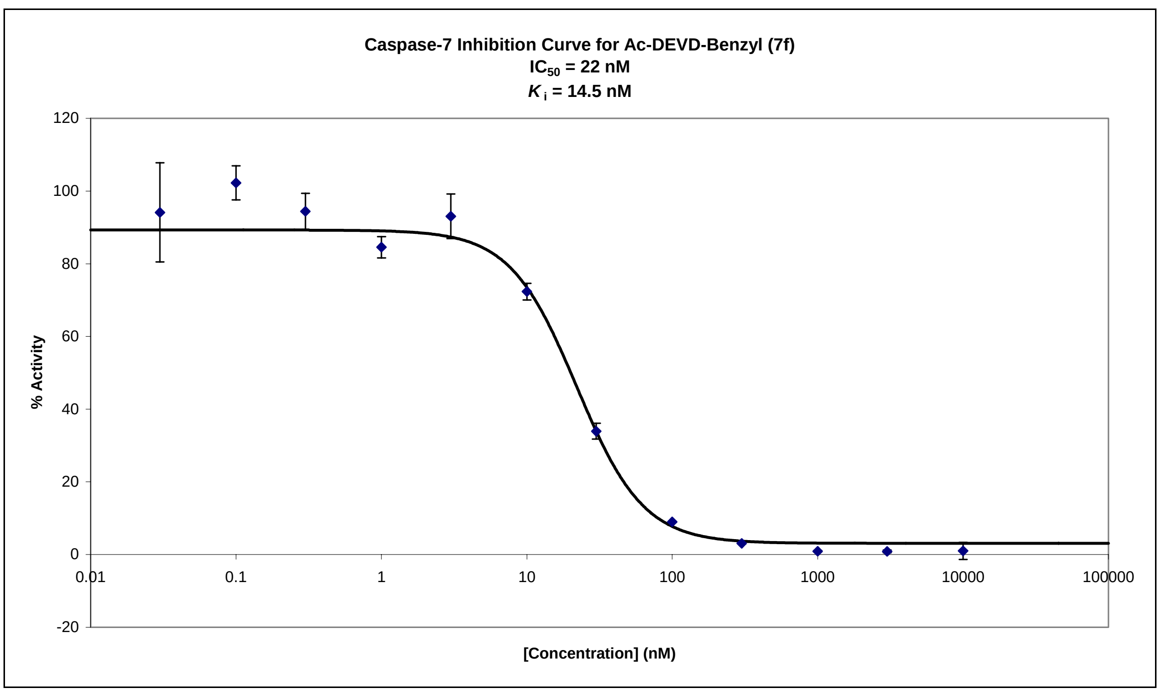

Caspase-7 Inhibition Curve for Ac-DEVD-isobutyl ketone (7e)
IC50 = 237.0 nM
K i = 94.2 nM
% Activity
[Concentration] (nM)


Caspase-7 Inhibition Curve for Ac-DEVD-isobutyl ketone (7e)
IC50 = 955.7 nM
K i = 652.8 nM
% Activity
[Concentration] (nM)



7f


Caspase-3 Inhibition Curve for Ac-DEVD-Benzyl (7f)
IC50 = 3.7 nM
K i = 1.5 nM
% Activity
[Concentration] (nM)
120
100
80
60
40
20
0
-20
0.01
0.1
1
10
100
1000
10000
100000


Caspase-7 Inhibition Curve for Ac-DEVD-Benzyl (7f)
IC50 = 22 nM
K i = 14.5 nM
% Activity
[Concentration] (nM)
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80
60
40
20
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-20
0.01
0.1
1
10
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10000
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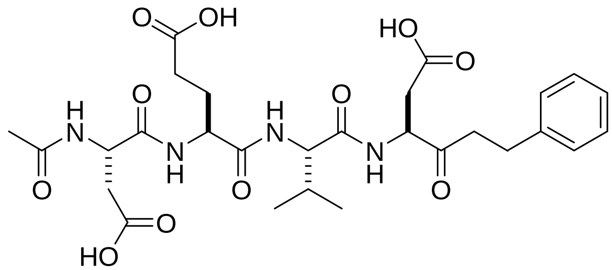
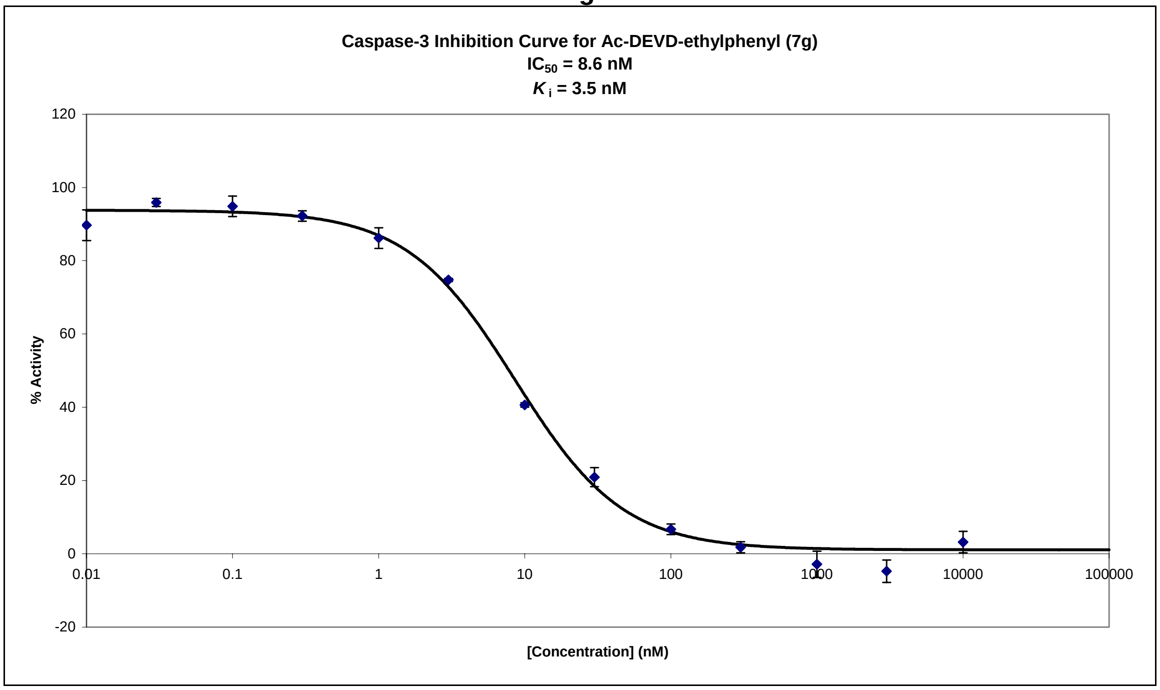


7g



Caspase-3 Inhibition Curve for Ac-DEVD-ethylphenyl (7g)
IC50 = 8.6 nM
K i = 3.5 nM
% Activity
[Concentration] (nM)
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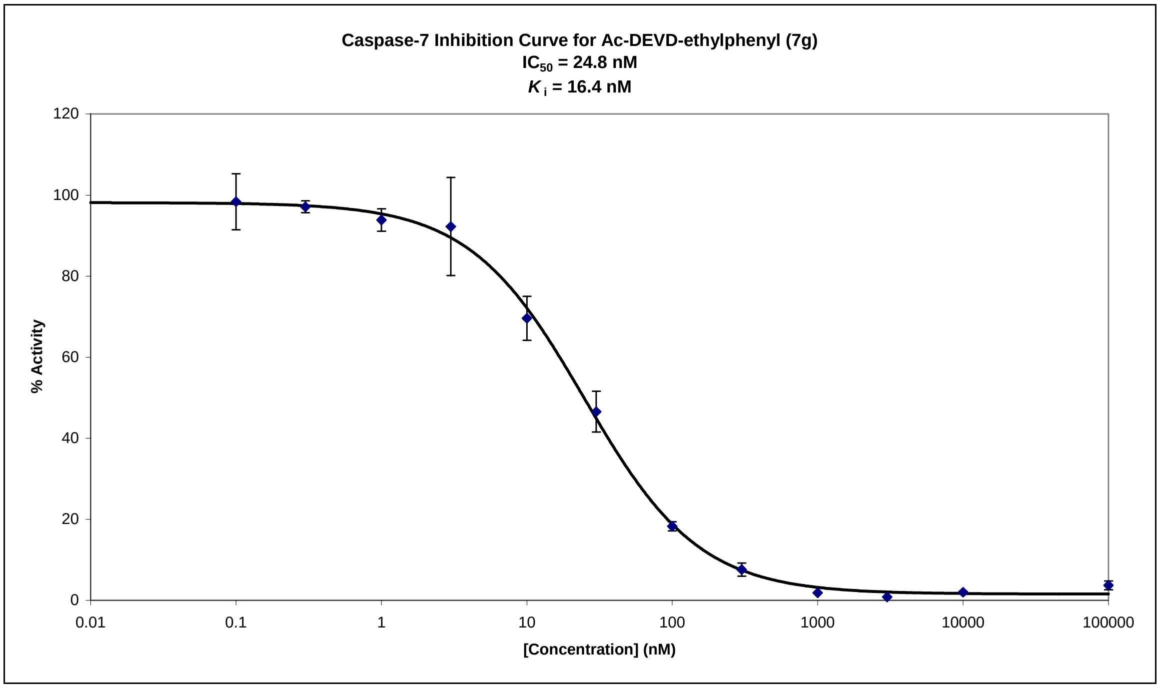
100
80
60
40
20
0
-20
0.01
0.1
1
10
100
1000
10000
100000



Caspase-7 Inhibition Curve for Ac-DEVD-ethylphenyl (7g)
IC50 = 24.8 nM
K i = 16.4 nM
% Activity
[Concentration] (nM)
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60
40
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0
0.01
0.1
1
10
100
1000
10000
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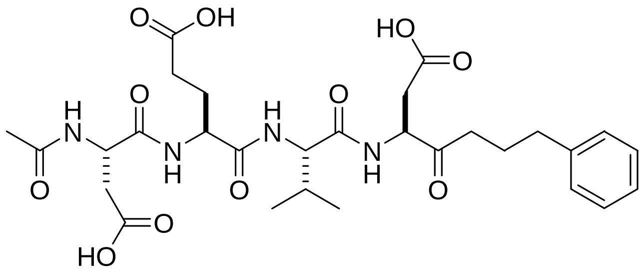
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HO
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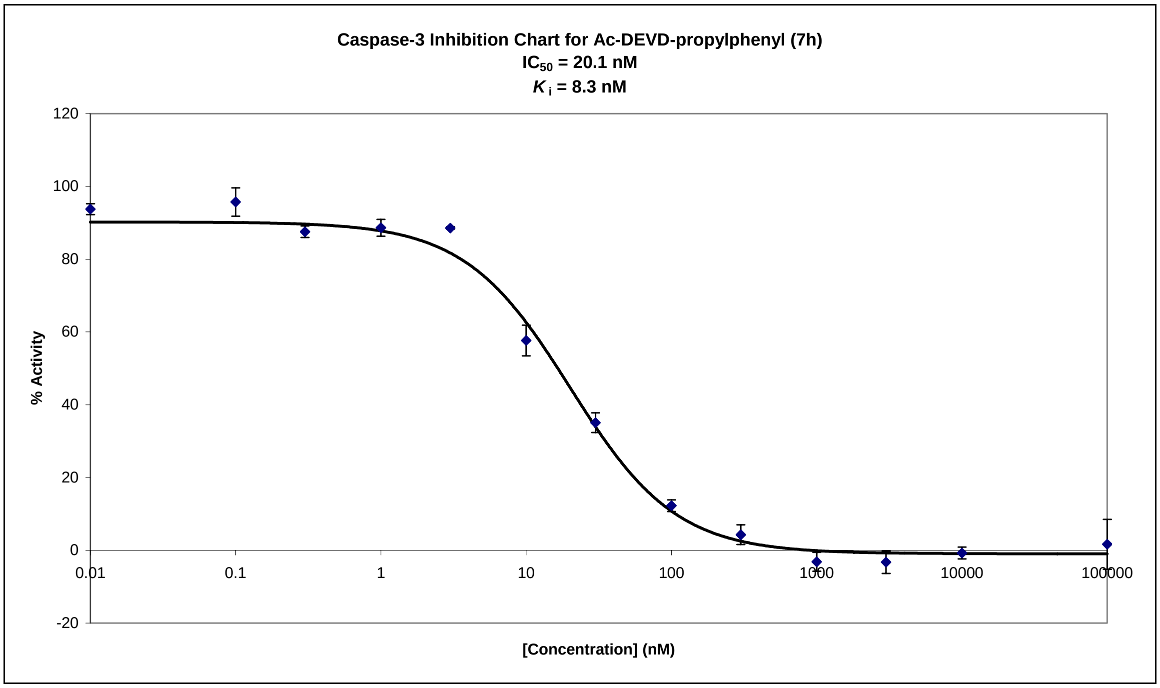
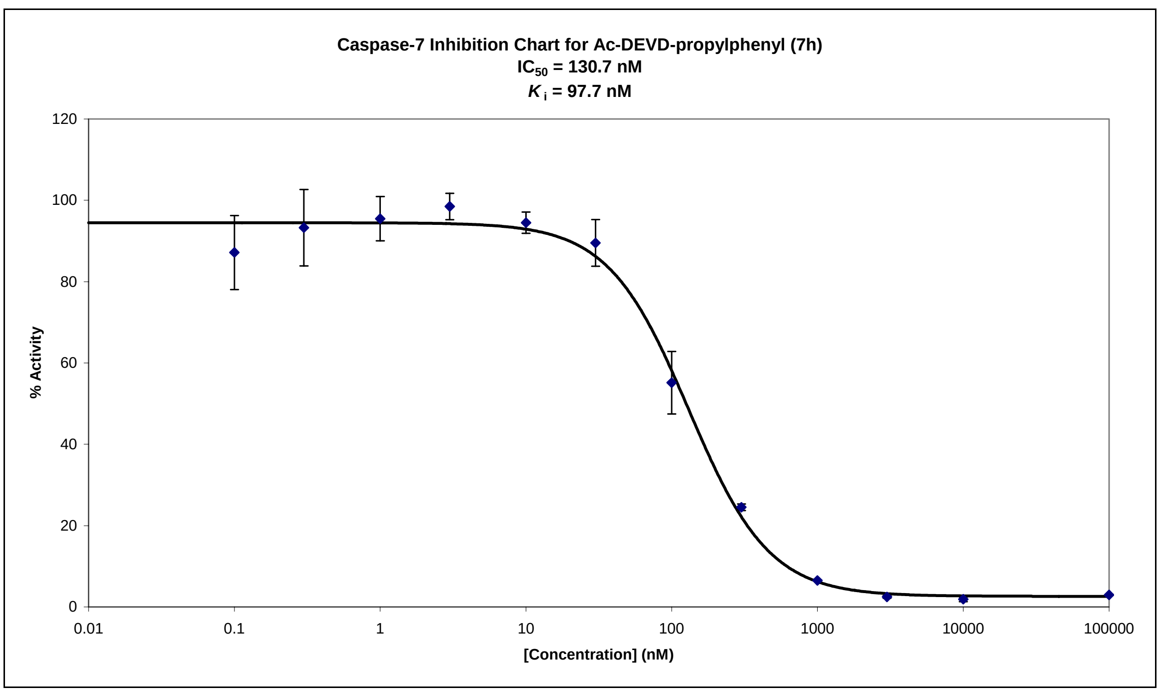
O
O

7h


Caspase-3 Inhibition Chart for Ac-DEVD-propylphenyl (7h)
IC50 = 20.1 nM
K i = 8.3 nM
% Activity
120
100
80
60
40
20
0
-20
0.01
0.1
1
10
100
1000
10000
100000
[Concentration] (nM)


Caspase-7 Inhibition Chart for Ac-DEVD-propylphenyl (7h)
IC50 = 130.7 nM
K i = 97.7 nM
% Activity
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100
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60
40
20
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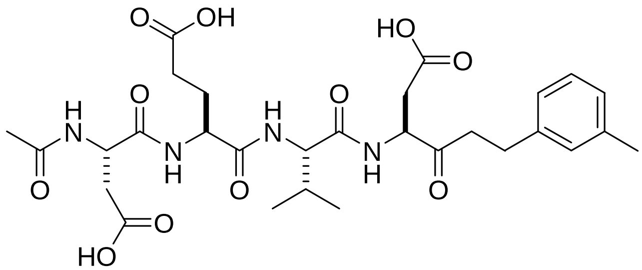
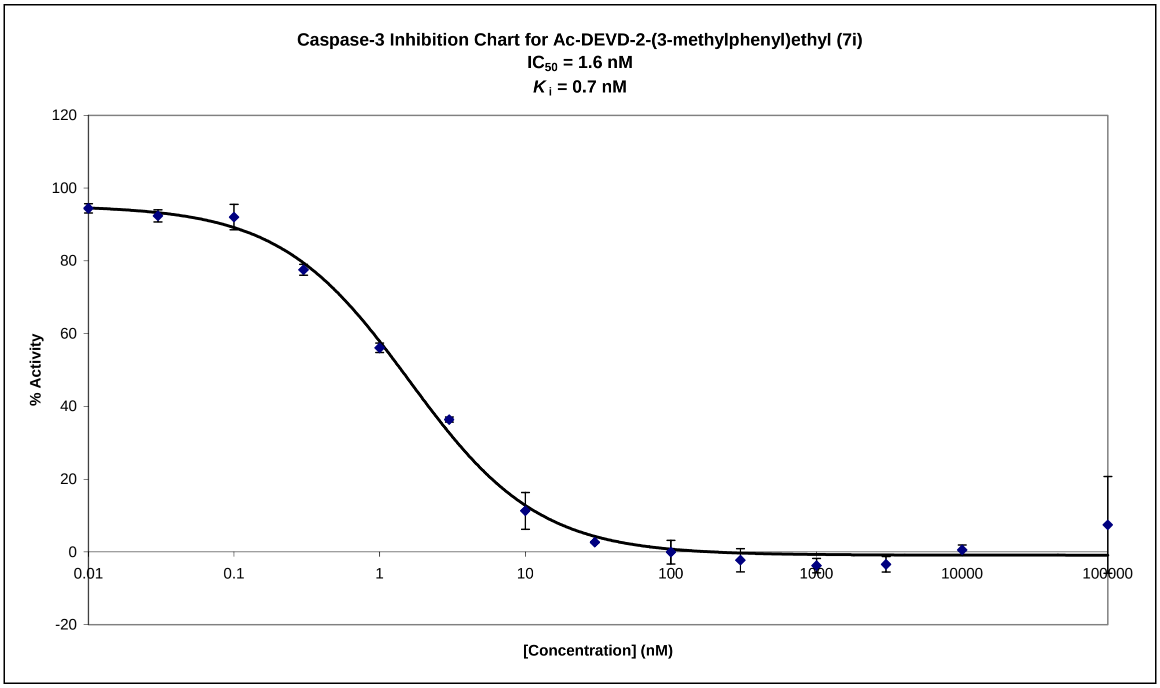
0.01
0.1
1
10
100
1000
10000
100000
[Concentration] (nM)



7i


Caspase-3 Inhibition Chart for Ac-DEVD-2-(3-methylphenyl)ethyl (7i)
IC50 = 1.6 nM
K i = 0.7 nM
% Activity
[Concentration] (nM)
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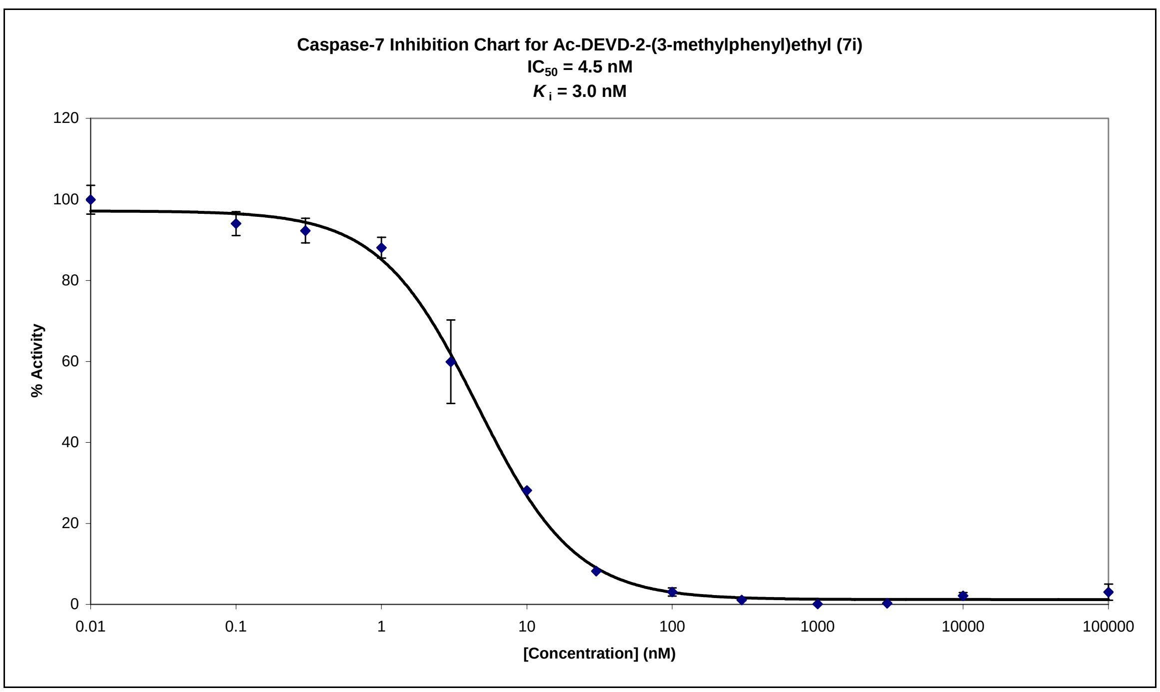
100
80
60
40
20
0
-20
0.01
0.1
1
10
100
1000
10000
100000


Caspase-7 Inhibition Chart for Ac-DEVD-2-(3-methylphenyl)ethyl (7i)
IC50 = 4.5 nM
K i = 3.0 nM
% Activity
[Concentration] (nM)
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10
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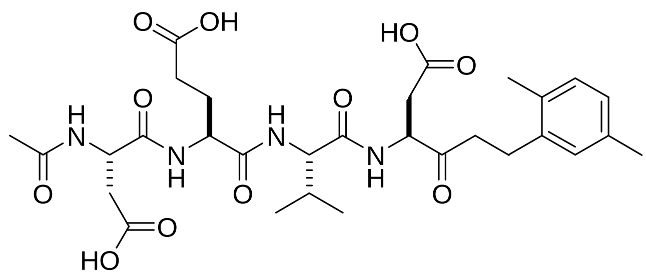
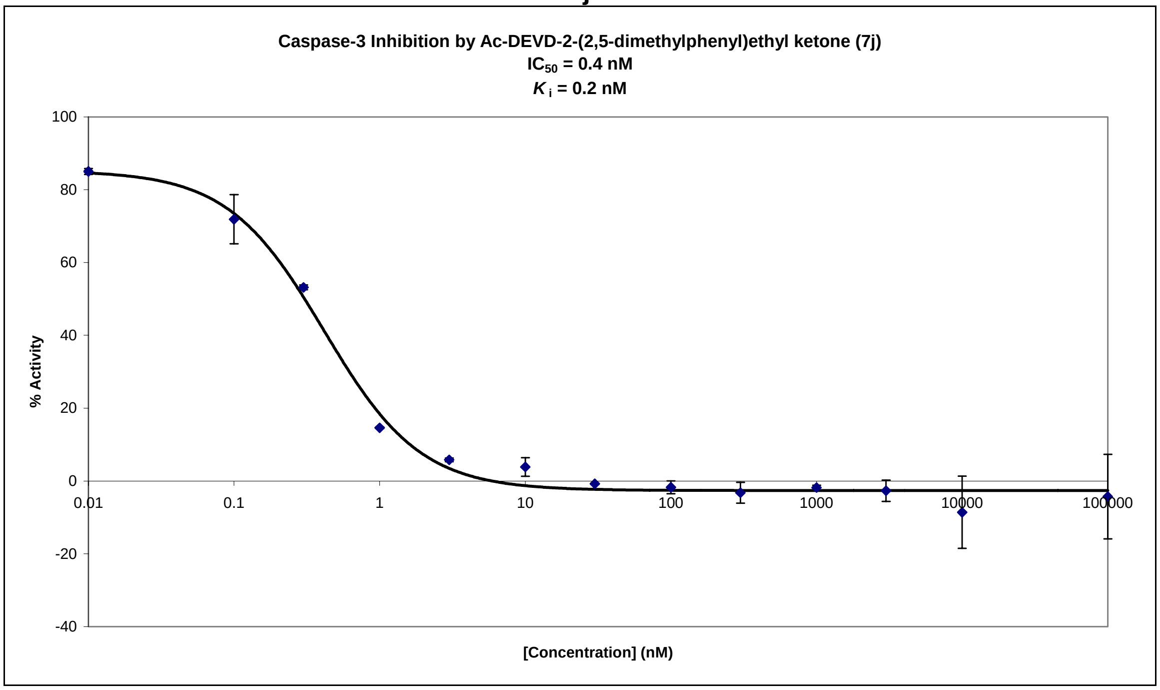
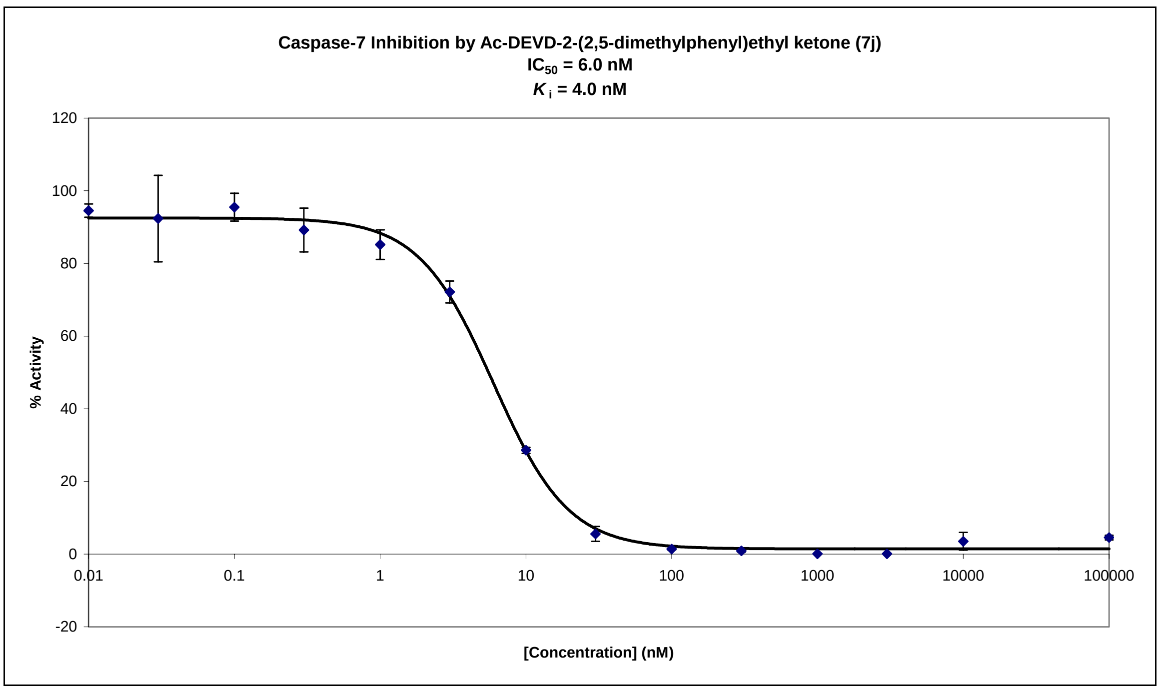
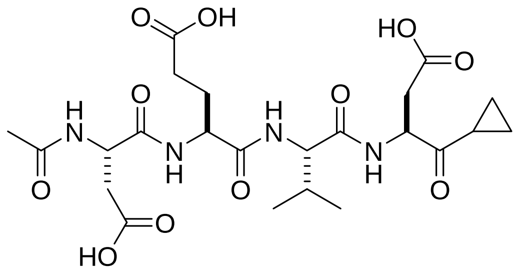
10000
100000



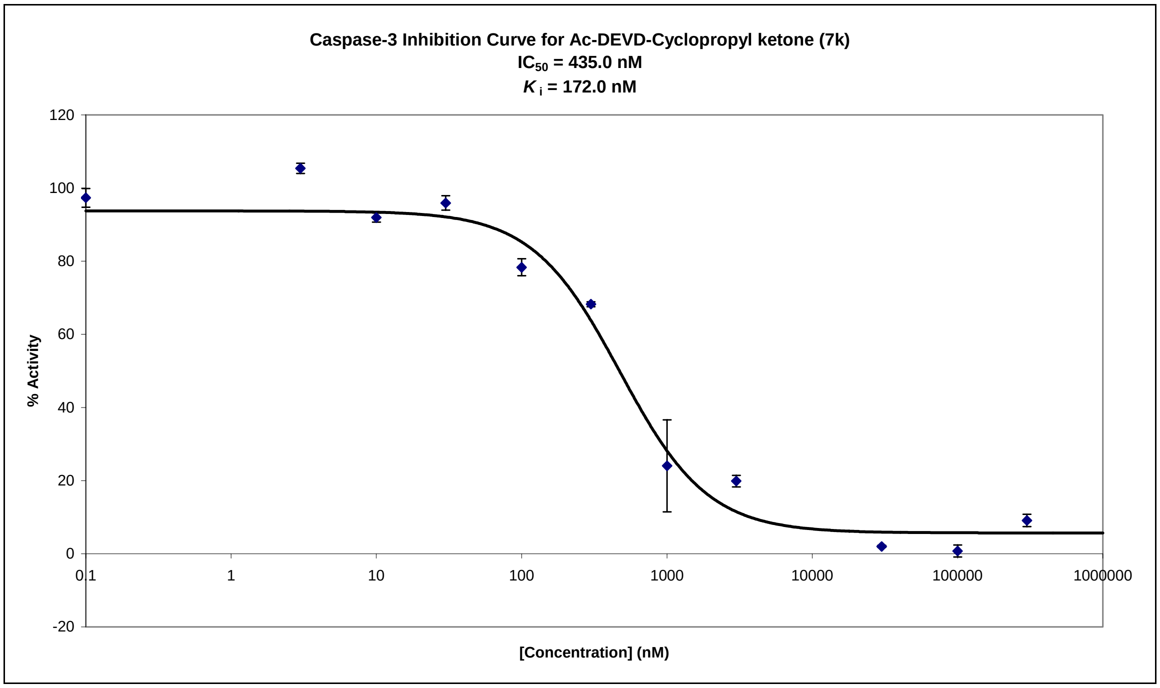
7j


Caspase-3 Inhibition by Ac-DEVD-2-(2,5-dimethylphenyl)ethyl ketone (7j)
IC50 = 0.4 nM
K i = 0.2 nM
% Activity
100
80
60
40
20
0
-20
-40
0.01
0.1
1
10
100
1000
10000
100000
[Concentration] (nM)

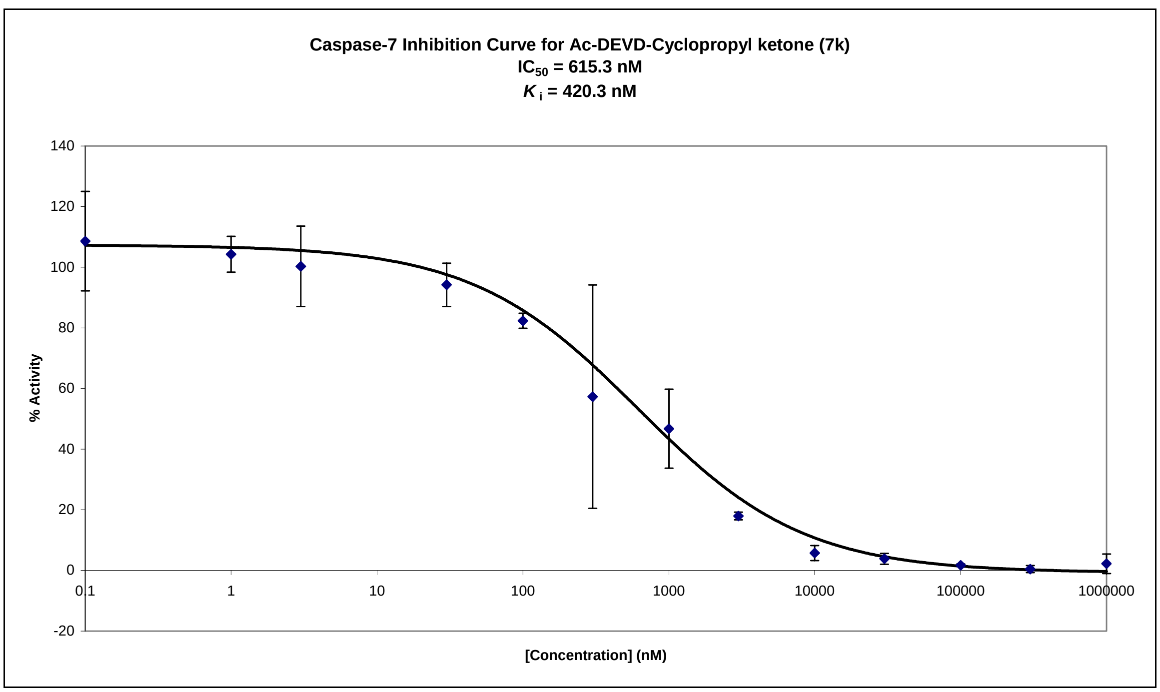

Caspase-7 Inhibition by Ac-DEVD-2-(2,5-dimethylphenyl)ethyl ketone (7j)
IC50 = 6.0 nM
K i = 4.0 nM
% Activity
120
100
80
60
40
20
0
-20
0.01
0.1
1
10
100
1000
10000
100000
[Concentration] (nM)



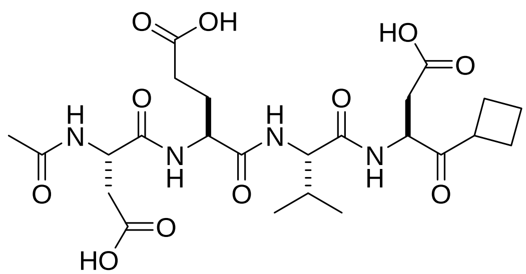
7k



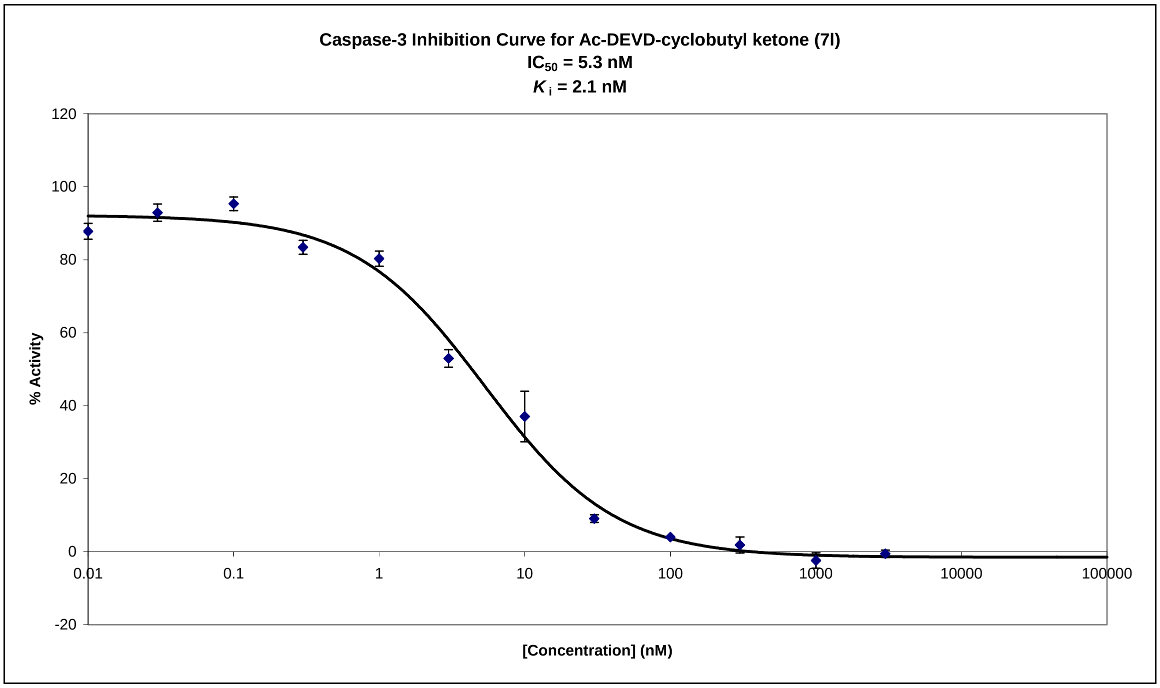
Caspase-3 Inhibition Curve for Ac-DEVD-Cyclopropyl ketone (7k)
IC50 = 435.0 nM
K i = 172.0 nM
% Activity
[Concentration] (nM)
120
100
80
60
40
20
0
-20
0.1
1
10
100
1000
10000
100000
1000000

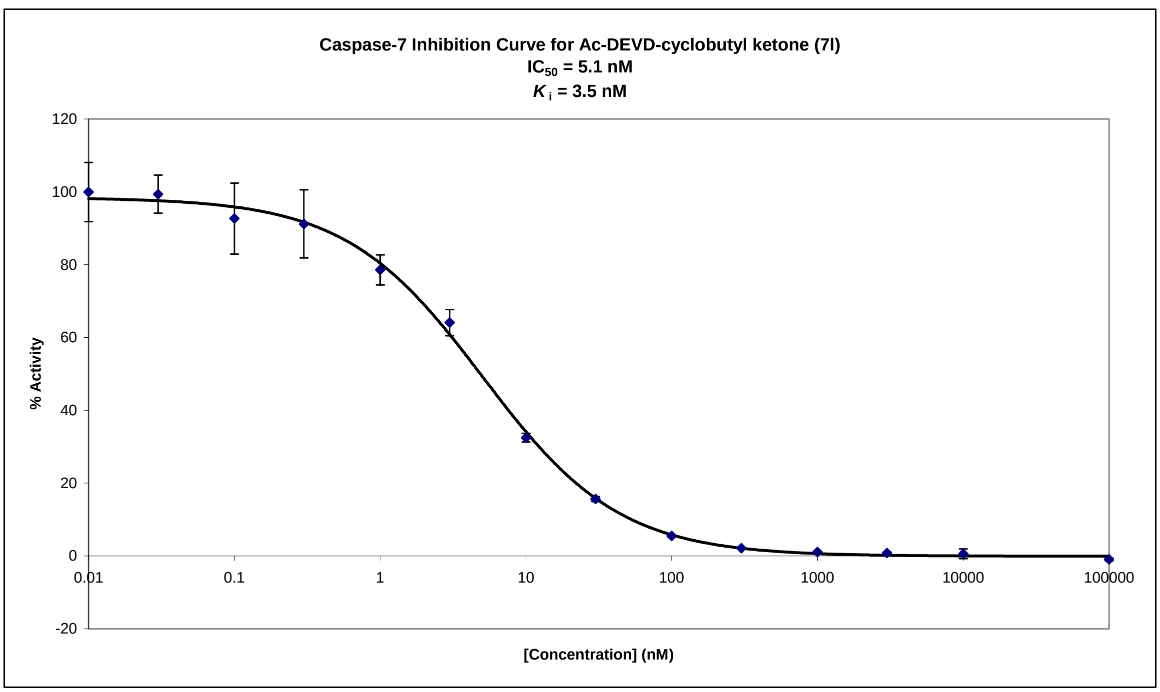


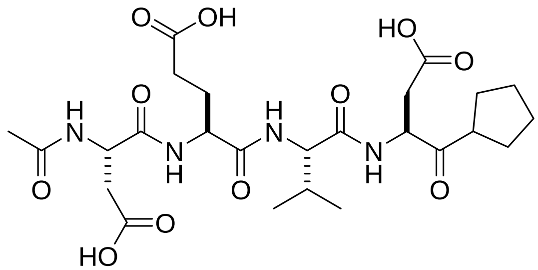
Caspase-7 Inhibition Curve for Ac-DEVD-Cyclopropyl ketone (7k)
IC50 = 615.3 nM
K i = 420.3 nM
% Activity
[Concentration] (nM)
140
120
100
80
60
40
20
0
-20
0.1
1
10
100
1000
10000
100000
1000000



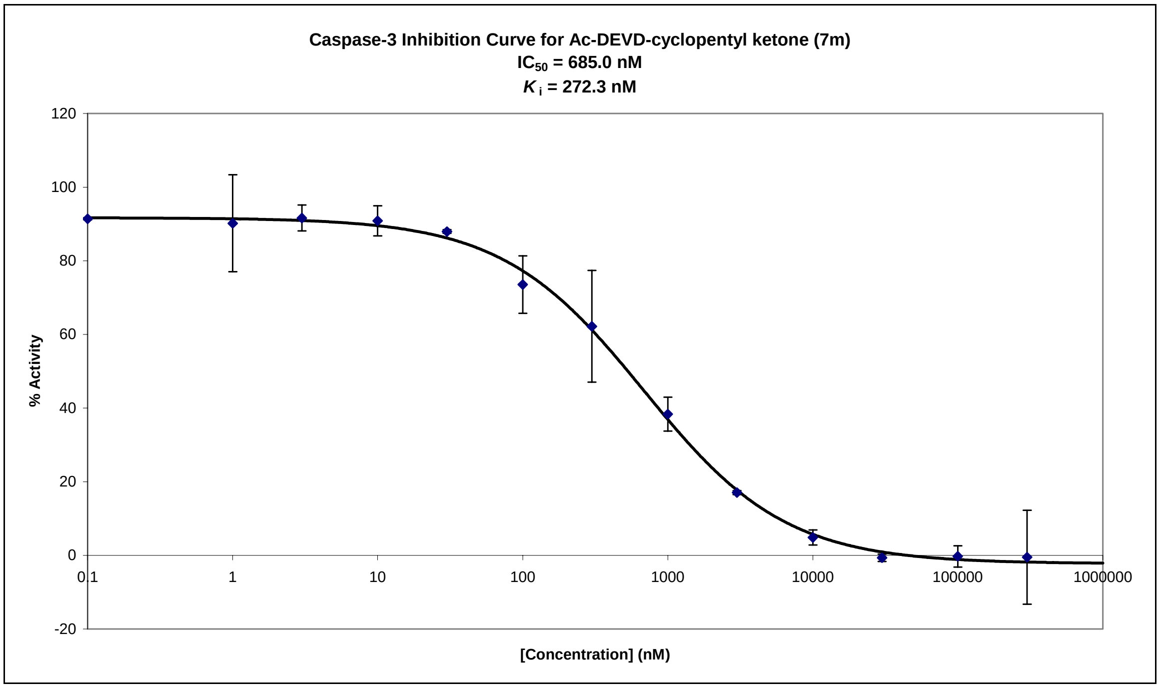
7l


Caspase-3 Inhibition Curve for Ac-DEVD-cyclobutyl ketone (7l)
IC_{50} = 5.3 nM
K_i = 2.1 nM
% Activity
[Concentration] (nM)
120
100
80
60
40
20
0
-20
0.01
0.1
1
10
100
1000
10000
100000

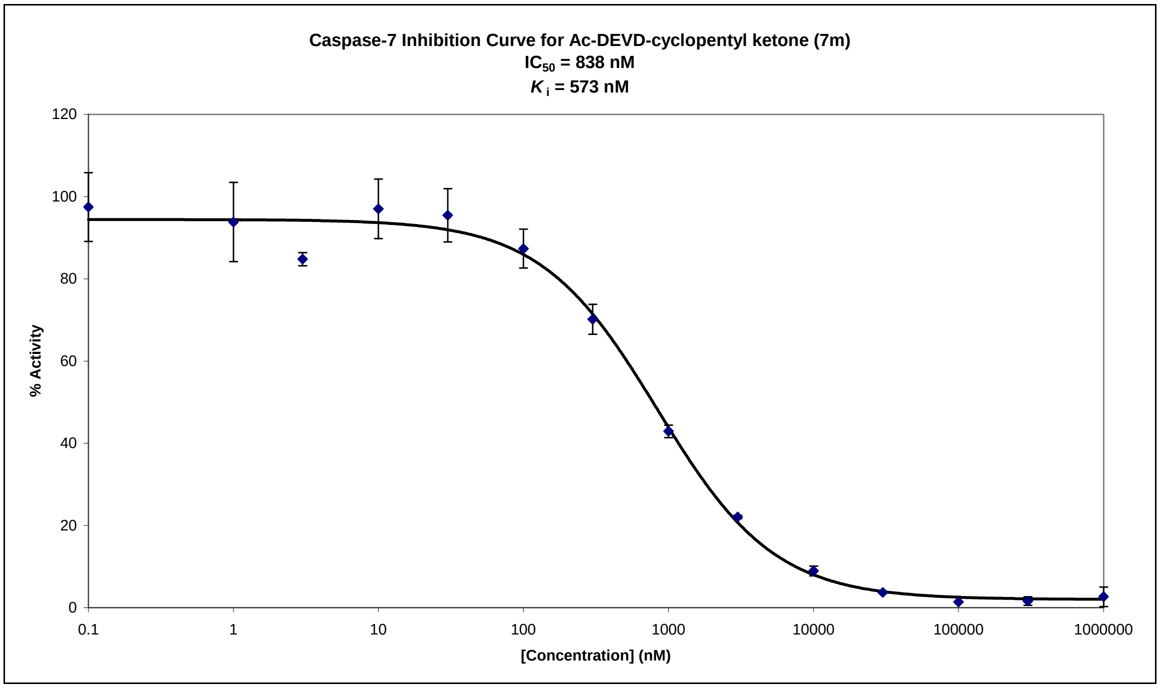

Caspase-7 Inhibition Curve for Ac-DEVD-cyclobutyl ketone (7l)
IC_{50} = 5.1 nM
K_i = 3.5 nM
% Activity
[Concentration] (nM)
120
100
80
60
40
20
0
-20
0.01
0.1
1
10
100
1000
10000
100000



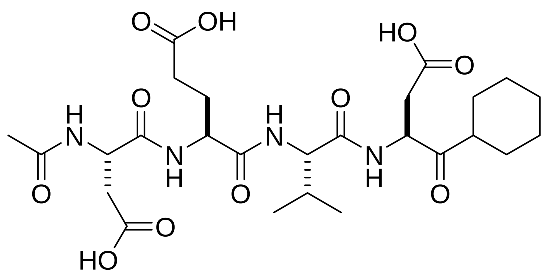
7m



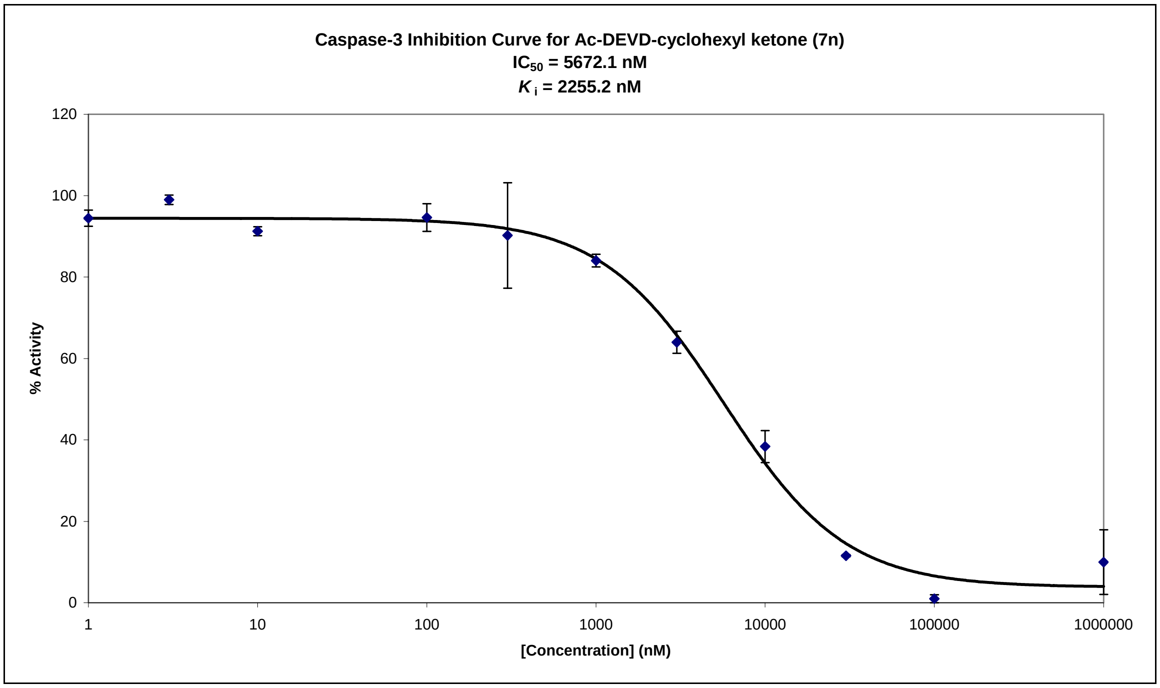
Caspase-3 Inhibition Curve for Ac-DEVD-cyclopentyl ketone (7m)
IC50 = 685.0 nM
K i = 272.3 nM
120
100
80
60
40
20
0
-20
% Activity
0.1
1
10
100
1000
10000
100000
1000000
[Concentration] (nM)



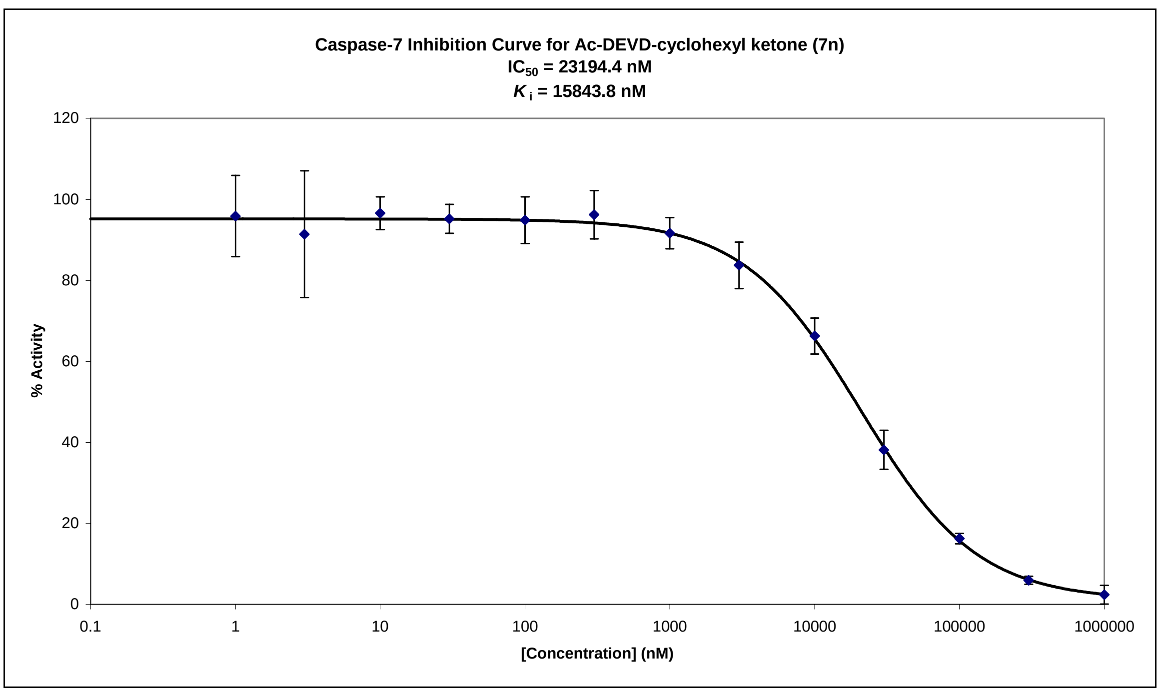
Caspase-7 Inhibition Curve for Ac-DEVD-cyclopentyl ketone (7m)
IC50 = 838 nM
K i = 573 nM
120
100
80
60
40
20
0
% Activity
0.1
1
10
100
1000
10000
100000
1000000
[Concentration] (nM)



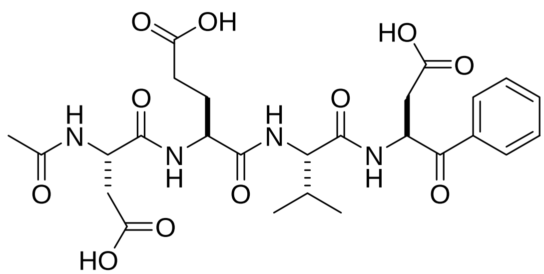
7n



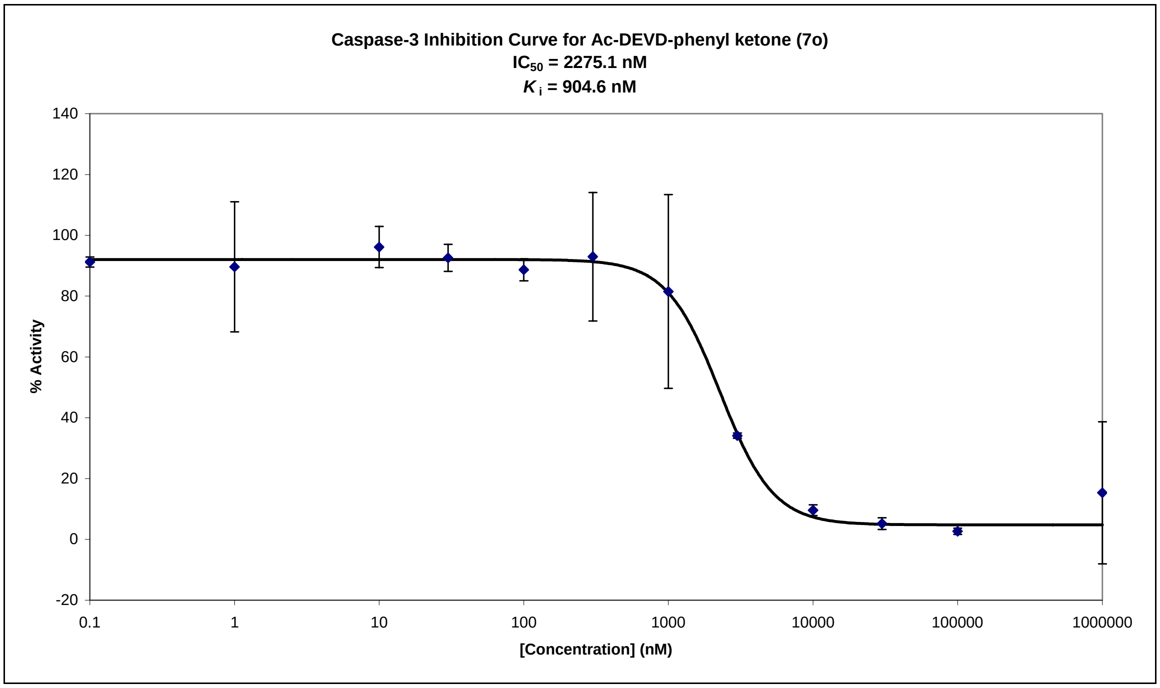
Caspase-3 Inhibition Curve for Ac-DEVD-cyclohexyl ketone (7n)
IC50 = 5672.1 nM
K i = 2255.2 nM
% Activity
0
20
40
60
80
100
120
1
10
100
1000
10000
100000
1000000
[Concentration] (nM)

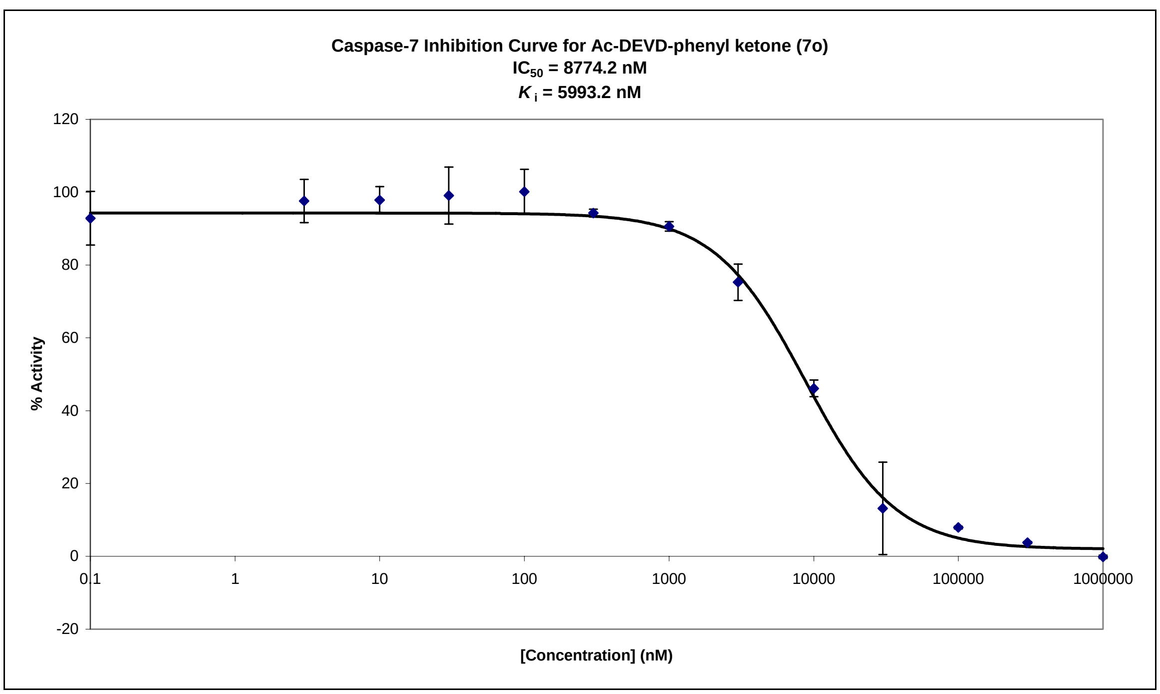


Caspase-7 Inhibition Curve for Ac-DEVD-cyclohexyl ketone (7n)
IC50 = 23194.4 nM
K i = 15843.8 nM
% Activity
0
20
40
60
80
100
120
0.1
1
10
100
1000
10000
100000
1000000
[Concentration] (nM)


O
OH
HO
O
O
H
N
N
H
O
H
N
O
N
H
O
O
O
HO

7o


Caspase-3 Inhibition Curve for Ac-DEVD-phenyl ketone (7o)
IC_{50} = 2275.1 nM
K_i = 904.6 nM
% Activity
140
120
100
80
60
40
20
0
-20
0.1
1
10
100
1000
10000
100000
1000000
[Concentration] (nM)


Caspase-7 Inhibition Curve for Ac-DEVD-phenyl ketone (7o)
IC_{50} = 8774.2 nM
K_i = 5993.2 nM
% Activity
120
100
80
60
40
20
0
-20
0.1
1
10
100
1000
10000
100000
1000000
[Concentration] (nM)