Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Supporting Information

Effect of 4'-substituted phenylaniline moiety of sansalvamide A peptide over antitumor activity

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Experimental Section

Chemistry

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance II 500 spectrometer or Varian Inova-500 spectrometer in $CDCl_3$ using TMS as an internal reference except otherwise stated, chemical shifts (δ) were reported downfiled from tetramethylsilane (Me_4Si) in parts per million (ppm), and *J* values were given in Hz. HRMS data were measured on a Micromass Autospec-Ultima ETOF spectrometer or a APEX II FT-ICRMS spectrometer (Bruker Daltonics, Inc.) with either of EI, or ESI ionisation methods.

All anhydrous solvents were freshly purified by standard techniques just before use. Reactions were carried out in an argon atmosphere when necessary, and monitored by thin layer chromatography (TLC) on silica gel plate (GF254). Organic extracts were washed with brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure on a rotary evaporator. Purification of products was performed by Flash Column Chromatography (FCC) on silica gel (200~300 mesh) purchased from Qing Dao Marine Chemical Co. (Qingdao, China), using petroleum ether (PE) and ethyl acetate (Et) mixtures as eluent except otherwise stated.

Methylation of carboxyl group was conducted in dry MeOH with thionyl chloride at 0°C to give the crude of the crude methyl ester hydrochloride according to the known method. [1]

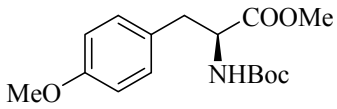
General procedure for synthesis of peptides

All of amide bond formation reactions were carried out under argon with freshly purified solvent, using methylene chloride/*N, N*-dimethylformamide (V/V = 25/2) for dipeptide, tripeptide, pentapeptide couplings, and methylene chloride/tetrahydrofuran/*N, N*-dimethylformamide (V/V = 4/4/1) for macrocyclization. The amine (1.0 equiv) and acid (1.5 ~ 2.0 equiv) were weighed into a dry flask along with HBTU (1.2 equiv), DIPEA (7.0 equiv) was used to maintain pH at 8 ~ 9. The mixture solvent was added for a 0.1 M solution. The solution was stirred at room temperature and monitored by TLC. Reactions were run for 12 ~ 24 hours before working up by concentration under vacuum. Added some water and extrected with ethyl acetate (or methylene dichloride), then washed with 5% pottssium carbonate, 2% hydrochloride, water and brine, respectively. After back extraction of aqueous layers with ethyl acetate (or methylene chloride), organic layers were combined, dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Flash column chromatography using 0 ~ 100% ethyl acetate-petroleum (or methylene chloride-methanol) as eluant gave the desired peptide.

General procedure for Macrocyclization (*in situ*)

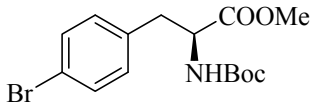
Under argon atmosphere double deprotected linear peptide (0.3 ~ 0.5mmol) (free acid and free amine) was added into a dry flask along with a solution of DCM/THF/DMF (described above), which would give a 0.001 ~ 0.005 M solution when adding the deprotected peptide. Coupling

agents were initially added: PyBOP (3.0 equiv), HATU (3.0 equiv), and including a minimum sodium chloride. DIPEA (3.0 ~ 5.0 equiv) was then added to ensure the pH was kept at 8 ~ 9. The solution was stirred at room temperature for about 3 ~ 4 days. Upon completion, the reaction was concentrated *in vacuo* and the residue was added some water, then extracted with ethyl acetate (or methylene chloride), washed with 5% potassium carbonate, 2% hydrochloride, water, and brine, respectively. After back extraction of aqueous layers with ethyl acetate (or methylene chloride), organic layers were combined, dried over with magnesium sulfate, filtered and concentrated. The macrocycle was purified by flash column chromatography on silica gel with ethyl acetate-petroleum (or methylene chloride-methanol) solvent as eluant. Finally, the desired cyclic peptide produced.



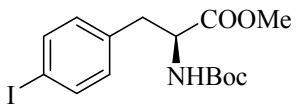
(s)-Methyl *N*-(*tert*-butyloxycarbonyl)-4-methoxyphenylalaninate

$[\alpha]^{20}$ +53.75° (*c* 1.2g/100ml, $CHCl_3$) (Ref.[2] $[\alpha]_D^{25}$ –58.4 (*c* 1.0, $CHCl_3$) , ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.04 ~ 7.03 (d, *J* = 8.5 Hz, 2 H), 6.84 ~ 6.82 (d, *J* = 9.5 Hz, 2 H), 4.96 ~ 4.95 (br s, 1H), 4.55 ~ 4.53 (m, 1H), 3.78 (s, 3 H), 3.71 (s, 3 H), 3.10 ~ 2.98 (m, 2 H), 1.42 (s, 9 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_c 172.34, 158.56, 155.03, 130.18(2), 127.86, 113.88(2), 79.75, 55.10, 54.47, 52.05, 37.34, 28.20(3);



(s)-Methyl *N*-(*tert*-butoxycarbonyl)-4-bromophenylalaninate

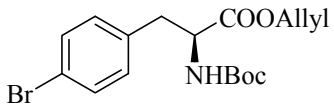
$[\alpha]^{20}$ +45.11° (*c* 1.35g/100ml, $CHCl_3$) (Ref.[3] $[\alpha]_D^{21}$+29.6° (*c* 1.35g/100ml, $CHCl_3$) , ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.42 ~ 7.41 (d, *J* = 8.0 Hz, 2 H), 7.01 ~ 6.99 (d, *J* = 8.0 Hz, 2 H), 4.98 ~ 4.97 (br d, *J* = 6.5 Hz, 1 H), 4.57 ~ 4.56 (br d, *J* = 6.0 Hz, 1 H), 3.72 (s, 3 H), 3.11 ~ 3.07 (dd, *J* = 13.5 Hz, *J* = 5.5 Hz, 1 H), 3.01 ~ 2.97 (dd, *J* = 13.5 Hz, *J* = 5.5 Hz, 1 H), 1.42 (s, 9 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_c 171.96, 154.91, 135.00, 131.53(2), 130.94(2), 120.95, 79.99, 54.13, 52.22, 37.74, 28.19(3).



(s)-Methyl *N*-(*tert*-butoxycarbonyl)-4-iodophenylalaninate

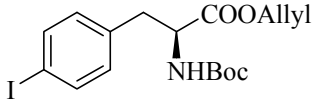
$[\alpha]^{20}$ +4.80° (c 1.0, CH_3OH), ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.62 ~ 7.61 (d, *J* = 8.0 Hz, 2 H), 6.88 ~ 6.87 (d, *J* = 8.5 Hz, 2 H), 4.98 ~ 4.96 (br d, *J* = 6.5 Hz, 1 H), 4.57 ~ 4.56 (br d, *J* = 6.5 Hz,

1 H), 3.72 (s, 3 H), 3.09 ~ 3.05 (dd, *J* = 13.5 Hz, *J* = 6.0 Hz, 1 H), 3.00 ~ 2.96 (dd, *J* = 13.5 Hz, *J* = 6.0 Hz, 1 H), 1.42 (s, 9 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_c 172.04, 155.00, 137.59(2), 135.76, 131.33(2), 92.51, 80.06, 54.20, 52.32, 37.91, 28.29(3); HRMS-ESI m/z $[M+H]^+$ calcd for $C_{15}H_{21}INO_4$: 406.0501, found 406.0506.



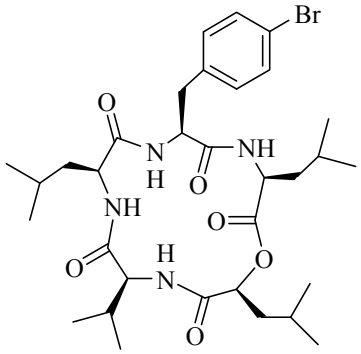
(s)-Allyl *N*-(*tert*-butoxycarbonyl)-4-bromophenylalaninate

$[\alpha]^{20}$ -4.20° (c 1.0, CH_3OH), ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.42 ~ 7.40 (d, *J* = 8.0 Hz, 2 H), 7.02 ~ 7.01 (d, *J* = 8.0 Hz, 2 H), 5.90 ~ 5.82 (m, 1 H), 5.32 ~ 5.25 (dd, *J* = 17.0 Hz, *J* = 10.5 Hz, *J* = 1.5 Hz, 2 H), 4.98 ~ 4.97 (br d, *J* = 7.0 Hz, 1 H), 4.61 ~ 4.58 (m, 3 H), 3.12 ~ 3.08 (dd, *J* = 13.5 Hz, *J* = 5.5 Hz, 1 H), 3.03 ~ 2.99 (dd, *J* = 13.5 Hz, *J* = 5.5Hz, 1 H), 1.42 (s, 9 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_c 171.22, 154.94, 135.01, 131.53(2), 131.32, 131.04(2), 120.96, 119.08, 79.98, 65.97, 54.21, 37.74, 28.21(3); HRMS-ESI m/z $[M+H]^+$ calcd for $C_{17}H_{23}BrNO_4$: 383.0732 and 385.0712, found 383.2892 and 385.0719.



(s)-Allyl *N*-(*tert*-butoxycarbonyl)-4-iodophenylalaninate

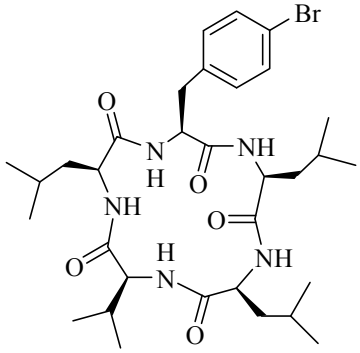
$[\alpha]^{20}$ -1.20° (c 1.0, CH_3OH), ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.62 ~ 7.60 (d, *J* = 8.0 Hz, 2 H), 6.90 ~ 6.88 (d, *J* = 8.5 Hz, 2 H), 5.90 ~ 5.82 (m, 1 H), 5.32 ~ 5.25 (ddd, *J* = 16.0 Hz, *J* = 9.5 Hz, *J* = 1.5 Hz, 2 H), 4.98 ~ 4.97 (br d, *J* = 7.5 Hz, 1 H), 4.61 ~ 4.57 (m, 3 H), 3.10 ~ 3.06 (dd, *J* = 13.5 Hz, *J* = 5.5 Hz, 1 H), 3.02 ~ 2.98 (dd, *J* = 13.5 Hz, *J* = 6.5Hz, 1 H), 1.42 (s, 9 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_c 171.28, 154.99, 137.58(2), 135.70, 131.41(3), 119.18, 92.50, 80.09, 66.06, 54.24, 37.92, 28.29(3); HRMS-ESI m/z $[M+H]^+$ calcd for $C_{17}H_{23}INO_4$: 432.0672, found 432.0668.



Cyclodepsipeptide 3:

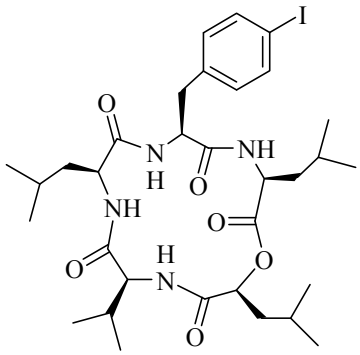
^{1}H NMR (500 MHz, $CDCl_3$): δ_H 8.23 ~ 8.21 (d, *J* = 7.5 Hz, 1 H), 7.45 (br, 1 H), 7.38 ~ 7.36 (d, *J* = 8.0 Hz, 2H), 7.13 ~ 7.11 (d, *J* = 8.0 Hz, 2 H), 6.98 (br, 1 H), 6.68 ~ 6.66 (d, *J* = 9.5 Hz, 1 H),

4.84 ~ 4.81 (dd, J = 9.0 Hz, J = 5.0 Hz, 1 H), 4.67 ~ 4.66 (d, J = 7.5 Hz, 1 H), 4.58 ~ 4.57 (d, J = 7.0 Hz, 1 H), 4.23 (t, J = 9.0 Hz, 1 H), 3.52 ~ 3.49 (t, J = 9.0 Hz, 1 H), 3.04 ~ 3.00 (dd, J = 13.5 Hz, J = 8.5 Hz, 1 H), 2.95 ~ 2.91 (dd, J = 13.0 Hz, J = 6.5 Hz, 1 H), 2.24 ~ 2.00 (m, 3 H), 1.89 ~ 1.86 (m, 1 H), 1.77 ~ 1.67 (m, 2 H), 1.50 ~ 1.40 (m, 2 H), 1.40 ~ 1.31 (m, 2 H), 0.99 ~ 0.91 (m, 15 H), 0.89 ~ 0.88 (d, J = 6.5 Hz, 3 H), 0.87 ~ 0.85 (d, J = 6.5 Hz, 3 H), 0.81 ~ 0.80 (d, J = 6.5 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$): δ_c 172.9, 172.1, 171.5, 171.4, 170.6, 135.9, 131.7(2), 131.0(2), 120.9, 75.1, 56.8, 55.1, 51.3, 40.3, 39.6, 38.9, 36.1, 30.4, 29.7, 24.9, 24.8, 24.7, 23.0, 22.8, 22.7, 22.0, 21.8, 21.6, 19.6, 18.4; HRMS-ESI m/z $[M+H]^+$ calcd for $C_{32}H_{50}BrN_4O_6$: 665.2914 and 667.2893, found 665.2899 and 667.2899.



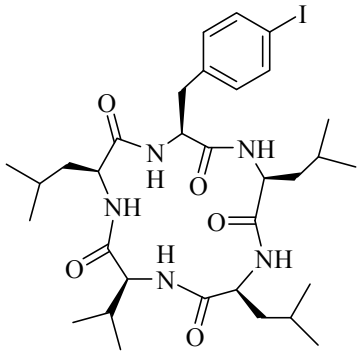
Cyclopentapeptide 4:[4]

^{1}H NMR (500 MHz, $CDCl_3$): δ_H 0.85 (d, J = 6.5Hz, 3 H), 0.89 (d, J = 7.0 Hz, 3 H), 0.93 (d, J = 6.5Hz, 3 H), 0.95 ~ 0.98 (m, 9 H), 1.01 ~ 1.4 (m, 6 H), 1.35 ~ 1.40 (m, 1 H), 1.47 ~ 1.50 (m, 2 H), 1.56 ~ 1.60 (m, 2 H), 1.70 ~ 1.72 (m, 2 H), 1.81 ~ 1.88 (m, 2 H) , 1.90 ~ 2.00 (m, 1 H), 2.36 (m, 1 H), 3.09 (dd, J = 13.0 Hz, 5.5 Hz, 1 H), 3.22 (t, J = 7.5 Hz, 1 H), 3.68 (br, 1 H), 4.06 (t, J = 7.5 Hz, 1 H), 4.32 (dd, J = 10.5 Hz, 6.0 Hz, 1 H), 4.44 (br, 1 H), 4.78 (dd, J = 9.0 Hz, 3.0 Hz, 1 H), 6.39 (d, J = 8.0 Hz, 1 H), 7.02 (d, J = 8.0 Hz, 1 H), 7.20 (d, J = 8.0 Hz, 2 H), 7.38 (d, J = 8.0 Hz, 2 H), 7.82 (br, 1 H), 8.15 (d, J = 8.0 Hz, 1 H); ^{13}C NMR (125 MHz, CD_3OD): δ_c 174.6, 174.2, 173.9, 173.3, 172.8, 135.7, 132.2(2), 130.9(2), 129.1, 62.8, 60.7, 58.3, 56.5, 52.7, 42.2, 41.8, 39.4, 38.0, 32.2, 26.8, 26.4, 26.3, 24.2, 23.8, 23.4, 23.1, 22.6, 22.4, 21.2, 19.4; HRMS-ESI m/z $[M+H]^+$ calcd for $C_{32}H_{51}BrN_5O_5$: 664.3074 and 666.3053, found 664.3061 and 666.3063.



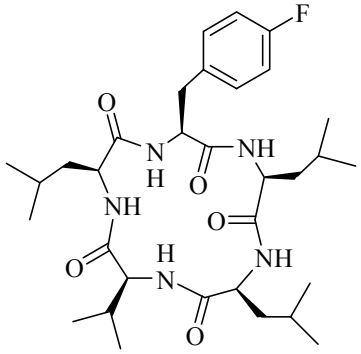
Cyclodepsipeptide 5:

^{1}H NMR (500 MHz, $CDCl_3$): δ_H 7.77 (br, 1 H), 7.64 ~ 7.62 (d, J = 9.0 Hz, 2 H), 7.40 (br, 1 H), 6.97 ~ 6.96 (d, J = 9.0 Hz, 2 H), 4.92 (br, 1 H), 4.57 ~ 4.56 (d, J = 6.5 Hz, 1 H), 4.41 (br, 1 H), 3.91 (br, 1 H), 3.75 (br, 1 H), 3.33 ~ 3.30 (t, J = 9.5 Hz, 1 H), 3.12 ~ 3.08 (dd, J = 14.0 Hz, J = 6.0 Hz, 1 H), 2.26 (br, 1 H), 2.01 (t, J = 6.0 Hz, 1 H), 1.77 ~ 1.64 (m, 5 H), 1.66 ~ 1.58 (m, 2 H), 1.53 ~ 1.45 (m, 1 H), 1.45 ~ 1.36 (m, 2 H), 0.99 ~ 0.86 (m, 24 H); ^{13}C NMR (125 MHz, $CDCl_3$): δ_c 172.9, 172.2, 171.4, 170.7, 169.8, 137.7(2), 136.5, 131.3(2), 129.2, 75.2, 56.7, 55.1, 51.3, 40.3, 39.5, 38.8, 36.2, 30.4, 29.7, 24.9, 24.8, 24.7, 23.0, 22.8, 22.7, 22.0, 21.8, 21.6, 19.6, 18.3; HRMS-ESI m/z $[M+H]^+$ calcd for $C_{32}H_{50}IN_4O_6$: 712.2697, found 713.2767.



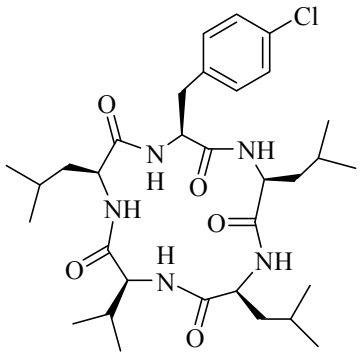
Cyclopentapeptide 6:[5]

^{1}H NMR (500 MHz, $CDCl_3$): δ_H 7.86 (d, J = 8.0 Hz, 1 H), 7.72 (br, 1 H), 7.58 (d, J = 8.0 Hz, 2 H), 7.32 (br, 1 H), 7.05 (d, J = 8.0 Hz, 2 H), 7.00 (d, J = 8.0 Hz, 1 H), 6.32 (d, J = 8.0 Hz, 1 H), 4.55 (br, 1 H), 4.41 (d, J = 6.5 Hz, 1 H), 4.29 (br, 1 H), 3.85 (d, J = 6.5 Hz, 1 H), 3.54 (br, 1 H), 3.23 (t, J = 9.0 Hz, 1 H), 3.02 (dd, J = 12.0 Hz, J = 6.0 Hz, 1 H), 2.20 ~ 2.28 (m. 2 H), 1.93 (t, J = 6.5 Hz, 1 H), 1.69 ~ 1.74 (m, 4 H), 1.57 ~ 1.63 (m, 2 H), 1.38 ~ 1.41 (m, 1 H), 1.09 (t, J = 6.5 Hz, 3 H), 0.94 ~ 0.99 (m, 12 H), 0.91 (t, J = 6.5 Hz, 3 H), 0.88 (t, J = 7.0 Hz, 3 H), 0.85 (t, J = 7.0 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$): δ_c 174.6, 174.2, 173.9, 173.3, 172.8, 136.8(2), 136.2, 131.7(2), 129.1, 62.2, 60.1, 58.4, 56.3, 52.7, 42.4, 41.8, 39.2, 37.8, 32.2, 27.1, 26.6, 26.2, 24.3, 23.6, 23.3, 23.0, 22.7, 22.5, 21.1, 19.2; HRMS-ESI m/z $[M+H]^+$ calcd for $C_{32}H_{51}IN_5O_5$: 712.2935, found 712.2954.



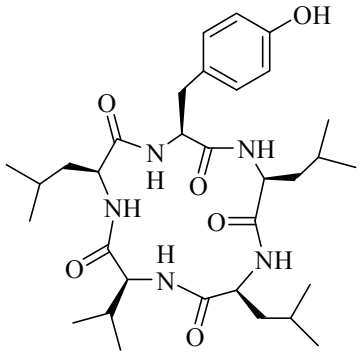
Cyclopentapeptide 7:

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 8.01 (br, 1 H), 7.52 (br, 1 H), 7.21 (br, 1 H), 7.15 (t, *J* = 8.0 Hz, 2 H), 6.95 (t, *J* = 8.0 Hz, 2 H), 6.66 (br, 1 H), 5.98 (br, 1 H), 4.79 (d, *J* = 7.2 Hz, 1 H), 4.43 (br, 1 H), 4.13 (br, 1 H), 3.80 (br, 1 H), 3.42 (br, 1 H), 3.12 (t, *J* = 7.6 Hz, 1 H), 2.95 (dd, *J* = 12.0 Hz, *J* = 6.0 Hz, 1 H), 2.45 ~ 2.51 (m, 1 H), 1.79 ~ 1.88 (m, 1 H), 1.70 ~ 1.76 (m, 2 H), 1.60 ~ 1.65 (m, 3 H), 1.49 ~ 1.54 (m, 2 H), 1.21 ~ 1.25 (m, 1 H), 1.02 ~ 1.08 (m, 3 H), 0.98 ~ 1.01 (m, 9 H), 0.93 ~ 0.96 (m, 6 H), 0.91 (d, *J* = 6.0 Hz, 3 H), 0.88 (t, *J* = 5.0 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$): δ_c 174.4, 173.9, 173.5, 172.8, 172.3, 159.7, 132.4, 126.8 (2), 114.1(2), 62.5, 61.1, 58.5, 56.3, 52.8, 42.5, 42.0, 39.9, 38.3, 32.2, 27.1, 26.7, 26.5, 24.1, 23.9, 23.6, 23.4, 22.9, 22.6, 22.1, 19.8; HRMS-ESI m/z $[M+H]^+$ calcd for $C_{32}H_{51}FN_5O_5$: 604.3874, found 604.3862.



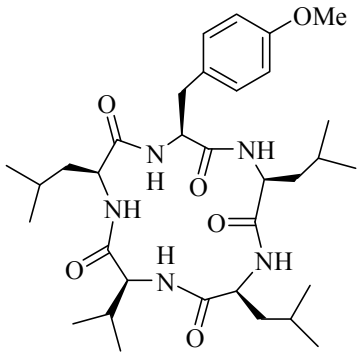
Cyclicpentapeptide 8:

^{1}H NMR (500 MHz, $CDCl_3$): δ_H 8.23 (br, 1 H), 7.81 (br, 1 H), 7.56 (br, 1 H), 7.21 (br, 1 H), 7.15 (t, *J* = 5.5 Hz, 2 H), 6.96 (t, *J* = 7.5 Hz, 2 H), 5.94 (d, *J* = 7.5 Hz, 1 H), 4.82 (dd, *J* = 15.0 Hz, *J* = 7.5 Hz, 1 H), 4.41 (br, 1 H), 4.13 (dd, *J* = 10.0 Hz, *J* = 7.0 Hz, 1 H), 3.63 (br, 1 H), 3.43 (t, *J* = 7.0 Hz, 1 H), 3.16 (t, *J* = 6.5 Hz, 1 H), 2.96 (d, *J* = 7.0 Hz, 1 H), 2.35 ~ 2.61 (m, 1 H), 1.79 ~ 1.92 (m, 1 H), 1.69 ~ 1.77 (m, 2 H), 1.60 ~ 1.66 (m, 3 H), 1.49 ~ 1.54 (m, 2 H), 1.26 ~ 1.30 (m, 1 H), 0.97 ~ 1.08 (m, 6 H), 0.91 ~ 0.95 (m, 12 H), 0.90 (t, *J* = 6.5 Hz, 3 H), 0.88 (d, *J* = 7.0 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$): δ_c 174.3, 173.7, 173.1, 172.7, 172.2, 138.5, 132.8, 130.9(2), 130.1(2), 62.4, 61.1, 58.1, 55.8, 52.2, 42.6, 42.1, 39.9, 38.8, 32.5, 27.3, 26.8, 26.6, 24.4, 23.7, 23.5, 23.3, 22.7, 22.3, 21.0, 18.9; HRMS-ESI m/z $[M+H]^+$ calcd for $C_{32}H_{51}ClN_5O_5$: 620.3579, found 620.3587.



Cyclicpentapeptide 9:

^{1}H NMR (500 MHz, DMSO-d_6): δ_H 8.54 (br, 1 H), 8.18 (d, J = 6.5 Hz, 1 H), 7.45 (br, 1 H), 7.16 (br, 1 H), 7.05 (d, J = 7.5 Hz, 2 H), 6.83 (t, J = 8.0 Hz, 2 H), 6.01 (br, 1 H), 4.75 (d, J = 7.5 Hz, 1 H), 4.38 (br, 1 H), 4.25 (t, J = 6.5 Hz, 1 H), 4.02 (d, J = 7.5 Hz, 1 H), 3.56 (br, 1 H), 3.26 (dd, J = 11.5 Hz, J = 7.0 Hz, 1 H), 2.98 (t, J = 7.5 Hz, 1 H), 2.43 ~ 2.49 (m, 1 H), 1.73 ~ 1.81 (m, 3 H), 1.52 ~ 1.60 (m, 3 H), 1.26 ~ 1.41 (m, 3 H), 1.03 ~ 1.22 (m, 6 H), 0.94 ~ 1.01 (m, 12 H), 0.90 (t, J = 6.5 Hz, 3 H), 0.87 (d, J = 7.0 Hz, 3 H); ^{13}C NMR (125 MHz, DMSO-d_6): δ_c 171.4, 171.1, 170.9, 170.7, 170.5, 161.8, 153.5, 130.6 (2), 115.1 (2), 61.1, 56.8, 55.1, 53.5, 53.3, 41.2, 40.8, 36.1, 29.7, 24.6, 24.4, 24.1, 22.7, 22.5, 22.3, 21.9, 21.7, 21.6, 19.1, 18.9; HRMS-ESI m/z $[M+Na]^+$ calcd for ($C_{32}H_{51}N_5O_6$+Na): 624.3737, found 624.3732.



Cyclicpentapeptide 10:

^{1}H NMR (DMSO, 500 MHz): δ_H 8.20 ~ 8.15 (m, 3 H), 8.07 ~ 8.05 (m, 2 H), 7.16 ~ 7.14 (d, J = 8.5 Hz, 2 H), 6.86 ~ 6.84 (d, J = 8.5 Hz, 2 H), 4.34 ~ 4.29 (dd, J = 11.5 Hz, J = 8.5 Hz, 1 H), 4.24 ~ 4.20 (m, 1 H), 4.20 ~ 4.15 (m, 1 H), 4.07 ~ 4.02 (m, 1 H), 3.82 ~ 3.78 (t, J = 9.0 Hz, 1 H), 3.73 (s, 3 H), 3.01 ~ 2.98 (m, 2 H), 2.91 (s, 1 H), 2.76 (s, 1 H), 2.16 ~ 2.11 (m, 1 H), 1.74 ~ 1.68 (m, 2 H), 1.58 ~ 1.49 (m, 6 H), 1.31 ~ 1.26 (m, 3 H), 0.94 (d, J = 6.0 Hz, 3 H), 0.93 ~ 0.87 (m, 13 H), 0.86 ~ 0.84 (d, J = 7.0 Hz, 6 H), 0.84 ~ 0.83 (t, J = 5.0 Hz, 3 H); ^{13}C NMR (125 MHz, DMSO): δ_c 171.38, 171.13, 171.01, 170.42, 170.36, 162.28, 157.88, 130.01(2), 129.10, 113.53(2), 60.86, 55.98, 54.93, 53.37, 53.19, 52.97, 40.65, 40.40, 35.91, 29.88, 24.56, 24.34, 24.27, 22.64, 22.48, 22.40, 21.75, 21.64, 21.60, 19.20, 18.71; HRMS-ESI m/z $[M+Na]^+$ calcd for ($C_{33}H_{53}N_5O_6$+Na): 638.3894, found 638.3879.

Evaluation of the antitumor activity

Cell Proliferation and Cytotoxicity Assays for MDA-MB-231, HT-29, HCT-15 and K562, A549 and HeLa Cells.

The ability of the compounds to inhibit cell proliferation or induce cytotoxicity was tested on six human cancer lines: MDA-MB-231 (breast cancer), HT-29 (colon cancer), HCT-15 (clorectal cancer), K562 (human erythroleukemia), A549 (adenocarcinoma cell) and HeLa. The procedures were adapted from Hanford *et al.*[6] Cells treated with the compounds were compared to DMSO

controls for their ability to proliferate as indicated by the incorporation of ^{3}H-thymidine into their DNA. Cells were cultured in 96-well plates at a concentration of 50 000 cells/well. All cells were grown in DMEM (dulbecco's modified eagle medium) supplemented with 10% heat-inactivated bovine serum and 100 μg/mL penicillin streptomycin and amphotericin B, and maintained at 37 °C in a humidified atmosphere of 95% O_2 and 5% CO_2. The compounds were dissolved in DMSO and diluted to a final concentration of 20 μg/mL (or lower as specified) and tested at the concentrations indicated in the manuscript. After the cells had been incubated with the compounds for 56 h, 1μ Ci ^{3}H-thymidine per well was added and the cells were cultured for an additional 16 h (for the cells to have a total of 72 h treatment), at which time the cells were harvested using a PHD cell harvester (Cambridge Technology Inc.). The samples were then counted in a scintillation counter for 5 min. Decreases in ^{3}H-thymidine incorporation, as compared to DMSO controls, are an indication that the cells are no longer progressing through the cell cycle or synthesizing DNA, as is shown in the studies presented.

Morphological changes using light microscopy. Cells were plated in 24-well plates at a concentration of 50 000 cells/well. After reaching 50% confluence, they were incubated in serum-free medium for 24 h, which was then replaced with fresh serum-free medium with or without treatment with the appropriate concentrations of cyclic peptide. After 24 h of treatment, the cells were viewed using light microscopy, and digital images were taken with a *Nikon Optem* CDC camera (Melville, NY) and acquired with ImagePro software (MediaCybernetics, Silver Spring MD).

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