Supporting Information Available

"Development of synthetic Aminopeptidase N/CD13 inhibitors to overcome cancer metastasis and angiogenesis"

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Chemistry

The chemical materials were purchased from commercial suppliers and used without further purification. Solvents were dried over $MgSO_4$ or distilled prior to use and flash chromatography was performed using silica gel (60 Å, 200±300 mesh). Melting points are uncorrected. NMR spectra were recorded on a Bruker DRX-300 spectrometer. Chemical shifts are in parts per million (ppm). ESI-MS were determined on an API 4000 spectrometer. All tested compounds are >95% pure by elemental analysis (CHN or CHNS, and all values were within ±0.4%) performed on a Vario EI III Element Analyzer.

General procedure for the preparation of NH_2OK methanol solution. 28.1 g of KOH was dissolved in 70 mL of methanol and the precipitate was filtered out. Then the solution was added into the suspension of 23.5 g of hydroxylamine hydrochloride in 120 mL of methanol dropwise. The precipitate was filtered out and the filtrate was stored with seal at room temperature.

General procedure for the synthesis of 4a-4cc. One of the amines (**1a-1s,** 5 mmol, respectively) was added to a solution of triphosgene (2.22 g, 7.5 mmol) in dry toluene (80 mL) in room temperature. The reaction mixture was refluxed for 4 hours and then solvents removed under low pressure. The residue was dissolved in DCM (20 mL) and this solution was added to the mixture of L-Leucine methyl ester hydrochloride or the other four amino acid (L-Ile, L-Phe, L-PGy and L-Met) methyl ester hydrochlorides (15 mmol), respectively, and triethylamine (1.52 g, 15 mmol) in DCM (80 mL) under ice-bath. After stirring at room temperature for 1-2 hours, the reaction mixture was concentrated under vacuum and then ethyl acetate (20 mL) was added to the residue. The organic phase was washed with 1N HCl (10 mL) and saturated brine (10 mL) and dried over $MgSO_4$. After the solvent was removed under low pressure, the residue without purification was directly added to a solution of potassium hydroxylamine (8.37 g, 56 mmol) in methanol (20 mL). The reaction mixture was stirred at room temperature for 1-3 hours then methanol removed under low pressure. The residue was taken up with 1N HCl and extracted with ethyl acetate. The organic phase was washed with brine and dried with $MgSO_4$. After the solvent was removed under low pressure, the residue was separated by silica gel column chromatography to afford **4a-4cc**, respectively.

(*S*)-4-Methyl-2-[3-(3-phenyl-propyl)-ureido]-pentanoic acid hydroxyamide (4a). Yield 48%, mp = 159-160 °C; ^{1}H-NMR (600 MHz, DMSO-d_6): δ 10.66 (s, 1H), 8.78 (s, 1H), 7.30-7.14 (m, 5H), 6.03-5.95 (m, 2H), 4.09-4.01 (m, 1H), 3.00-2.94 (m, 2H), 2.57-2.55 (m, 2H). 1.68-1.51 (m, 3H), 0.88-0.84 (m, 6H); ^{13}C-NMR (300 MHz, DMSO-d_6): δ 166.9, 158.6, 141.8, 128.6, 128.2, 125.6, 64.8, 32.5, 31.8, 30.0, 18.6, 13.5; HRMS-ESI (+) calcd for $C_{16}H_{26}N_3O_3$, 308.1974; found, 308.1973 $[M+H]^+$. Elemental anal calcd for $C_{16}H_{25}N_3O_3$ C 62.52, H 8.20, N 13.67; found C 62.59, H 8.37, N 13.76.

(*S*)-2-[3-(4-Fluoro-benzyl)-ureido]-N-hydroxy-2-phenyl-acetamide (4b). Yield 43%, mp = 182-183 °C; ^{1}H-NMR (600 MHz, DMSO-d6): δ 11.00 (s, 1H), 8.98 (s, 1H), 7.37-7.11 (m, 9H), 6.91 (s, 1H), 6.70 (s,1H), 5.21-5.20 (m, 1H), 4.16-4.13 (m, 2H); ^{13}C-NMR (300 MHz, DMSO-d6): δ 167.3, 157.0, 140.1, 136.8, 128.8, 128.7, 128.2, 127.2, 126.5, 115.0,114.8, 54.3, 42.0; HRMS-ESI (+) calcd for $C_{16}H_{17}FN_3O_3$, 318.1254; found, 318.1257

[M+H]+. Elemental anal calcd for $C_{16}H_{16}FN_3O_3$ C 60.56, H 5.08, N 13.24; found C 60.58, H 5.15, N 13.20.

(*S*)-N-Hydroxy-4-methylsulfanyl-2-[3-(3-phenyl-propyl)-ureido]-butyramide (4c). Yield 47%, mp = 161-163 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 10.64 (s, 1H), 8.82 (s, 1H), 7.30-7.14 (m, 5H), 6.11-6.05 (m, 2H), 4.09-4.01 (m, 1H), 2.57-2.55 (m, 2H), 2.45-2.34 (m, 2H), 2.03 (s, 3H), 1.85-1.63 (m, 2H); 1.68-1.51 (m, 4H); ^{13}C-NMR (300 MHz, DMSO-d_6): δ 166.9, 159.0, 141.7, 128.6, 128.2, 125.7, 65.0, 55.1, 40.4, 32.4, 32.3, 29.2, 28.6, 18.6; HRMS-ESI (+) calcd for $C_{15}H_{24}N_3O_3S$, 326.1538; found, 326.1535 $[M+H]^+$. Elemental anal calcd for $C_{15}H_{23}N_3O_3S$ C 55.36, H 7.12, N 12.91, S 9.85; found C 55.41, H 7.27, N 12.88, S 9.81.

(*S*)-N-Hydroxy-2-phenyl-2-[3-(3-phenyl-propyl)-ureido]-acetamide (4d). Yield 52%, mp = 168-169 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 10.97 (s, 1H), 8.96 (s, 1H), 7.34-7.14 (m, 10H), 6.72 (d, 1H, J = 8.7 Hz), 6.28 (t, 1H, J = 5.7 Hz), 5.18 (d, 1H, J = 8.7 Hz), 2.99-2.94 (m, 2H), 2.58-2.52 (m, 2H), 1.68-1.59 (m, 2H); ^{13}C-NMR (300 MHz, DMSO-d_6): δ 167.3, 157.0, 141.7, 140.3, 128.2, 128.1, 127.2, 126.4, 125.7, 54.1, 40.4, 32.4, 31.7; HRMS-ESI (+) calcd for $C_{18}H_{22}N_3O_3$, 328.1661; found, 328.1660 $[M+H]^+$. Elemental anal calcd for $C_{18}H_{21}N_3O_3$ C 66.04, H 6.47, N 12.84; found C 66.09, H 6.47, N 12.86.

(*S*)-4-Methyl-2-[3-(4-phenyl-butyl)-ureido]-pentanoic acid hydroxyamide (4e). Yield 51%, mp = 170-171 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 10.63 (s, 1H), 8.76 (s, 1H), 7.29-7.14 (m, 5H), 5.93-5.90 (m, 2H), 4.08-4.00 (m, 1H), 3.00-2.96 (m, 2H), 2.58-2.49 (m, 2H), 1.58-1.48 (m, 3H), 1.40-1.24 (m, 4H), 0.89-0.82 (m, 6H); ^{13}C-NMR (300 MHz, DMSO-d_6): δ 169.5, 158.6, 142.2, 128.6, 128.2, 125.6, 65.0, 34.8,,30.0, 28.4, 18.6, 13.5; HRMS-ESI (+) calcd for $C_{17}H_{28}N_3O_3$, 322.2131; found, 322.2132 $[M+H]^+$. Elemental anal calcd for $C_{17}H_{27}N_3O_3$ C 63.53, H 8.47, N 13.07; found C 63.59, H 8.37, N 13.12.

(*S*)-3-Methyl-2-[3-(4-phenyl-butyl)-ureido]-pentanoic acid hydroxyamide (4f). Yield 61%, mp = 169-172 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 10.58 (s, 1H), 8.77 (s, 1H), 7.29-7.11 (m, 5H), 6.02-5.94 (m, 2H), 4.02-3.82 (m, 1H), 3.02-2.96 (m, 2H), 2.59-2.54 (m, 2H), 1.56-1.33 (m, 7H), 0.89-0.77 (m, 6H); ^{13}C-NMR (300 MHz, DMSO-d_6): δ 168.6, 157.5, 142.2, 128.2, 128.1, 125.6, 54.7, 38.7, 37.6, 34.8, 29.6, 28.3, 24.5, 15.2, 11.0; HRMS-ESI (+) calcd for $C_{17}H_{28}N_3O_3$, 322.2131; found, 322.2135 $[M+H]^+$. Elemental anal calcd for $C_{17}H_{27}N_3O_3$ C 63.53, H 8.47, N 13.07; found C 63.50, H 8.41, N 13.03.

(*S*)-N-Hydroxy-3-phenyl-2-[3-(4-phenyl-butyl)-ureido]-propionamide (4g). Yield 65%, mp = 173-175 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 10.62 (s, 1H), 8.80 (s, 1H), 7.29-7.14 (m, 10H), 6.08-6.01 (m, 2H), 4.36-4.22 (m, 1H), 2.96-2.52 (m, 6H), 1.56-1.46 (m, 2H), 1.37-1.20 (m, 2H); ^{13}C-NMR (300 MHz, DMSO-d_6): δ 168.6, 157.2, 142.2, 137.7, 129.2, 128.2, 128.1, 128.0, 126.1, 125.6, 52.0, 40.3, 38.8, 34.8, 29.6, 28.3; HRMS-ESI (+) calcd for $C_{20}H_{26}N_3O_3$, 356.1974; found, 356.2001 $[M+H]^+$. Elemental anal calcd for $C_{20}H_{25}N_3O_3$ C 67.58, H 7.09, N 11.82; found C 67.51, H 7.20, N 11.76.

(*S*)-N-Hydroxy-2-phenyl-2-[3-(4-phenyl-butyl)-ureido]-acetamide (4h). Yield 49%, mp = 177-178 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 10.96 (s, 1H), 8.95 (s, 1H), 7.40-7.13 (m, 10H), 6.68 (d, 1H, J = 8.7 Hz), 6.21 (t, 1H, J = 5.7 Hz), 3.07-2.96 (m, 2H), 2.58-2.53 (m, 2H), 1.58-1.44 (m, 2H), 1.40-1.30 (m, 2H); ^{13}C-NMR (300 MHz, DMSO-d_6): δ 167.3, 157.0, 142.1, 140.3, 128.3, 128.1, 128.0, 127.2, 126.4, 125.6, 54.1, 34.8, 29.6, 28.3; HRMS-ESI (+) calcd for $C_{19}H_{24}N_3O_3$, 342.1818; found, 342.1810 $[M+H]^+$. Elemental anal calcd for $C_{19}H_{23}N_3O_3$ C 66.84, H 6.79, N 12.31; found C 66.69, H 6.98, N 12.21.

(*S*)-2-(3-Benzyl-ureido)-N-hydroxy-2-phenyl-acetamide (4i). Yield 77%, mp = 198-200 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 10.98 (s, 1H), 8.97 (s, 1H), 7.38-7.21 (m, 10H), 6.87 (d, 1H, J = 8.7 Hz), 6.67 (t, 1H, J = 6.0 Hz), 5.21 (d, 1H, J = 8.7 Hz), 4.21-4.19 (m, 2H); ^{13}C-NMR (300 MHz, DMSO-d_6): δ 167.3, 157.0, 140.5, 140.2,

128.2, 128.2, 127.2, 126.9, 126.6, 126.4, 54.2, 42.8; HRMS-ESI (+) calcd for $C_{16}H_{18}N_3O_3$, 300.1348; found, 300.1346 $[M+H]^+$. Elemental anal calcd for $C_{16}H_{17}N_3O_3$ C 64.20, H 5.72, N 14.04; found C 64.59, H 5.57, N 14.16.

(*S*)-2-(3-Phenethyl-ureido)-N-hydroxy-2-phenyl-acetamide (4j). Yield 78%, mp = 178-180 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 10.98 (s, 1H), 8.96 (s, 1H), 7.34-7.17 (m, 10H), 6.81 (d, 1H, J = 9.0 Hz), 6.22 (t, 1H, J = 6.0 Hz), 5.18 (d, 1H, J = 9.0 Hz), 3.23-3.18 (m, 2H), 2.65 (t, 2H, J = 6.9 Hz); ^{13}C-NMR (300 MHz, DMSO-d_6): δ 167.3, 157.0, 140.3, 139.6, 128.6, 128.2, 128.1, 127.1, 126.4, 125.9, 54.2, 40.7, 36.0; HRMS-ESI (+) calcd for $C_{17}H_{20}N_3O_3$, 314.1505; found, 314.1498 $[M+H]^+$. Elemental anal calcd for $C_{17}H_{19}N_3O_3$ C 65.16, H 6.11, N 13.41; found C 65.19, H 6.21, N 13.46.

(*S*)-4-Methyl-2-(3-phenyl-ureido)-pentanoic acid hydroxyamide (4k). Yield 80%, mp = 166-168 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 10.77 (s, 1H), 8.85 (s, 1H), 8.54 (s, 1H), 7.36-7.34 (m, 2H), 7.24-7.18 (m, 2H), 6.91-6.86 (m, 1H), 6.33 (d, 1H, J = 9.0 Hz), 4.18-4.10 (m, 1H), 1.62-1.53 (m, 1H), 1.42-1.37 (m, 2H), 0.91-0.87 (m, 6H); ^{13}C-NMR (300 MHz, DMSO-d_6): δ 169.0, 154.5, 140.2, 128.6, 121.1, 117.4, 48.9, 42.4, 24.2, 22.7, 22.1; HRMS-ESI (+) calcd for $C_{13}H_{20}N_3O_3$, 266.1505; found, 266.1489 $[M+H]^+$. Elemental anal calcd for $C_{13}H_{19}N_3O_3$ C 58.85, H 7.22, N 15.84; found C 58.70, H 7.11, N 15.79.

(*S*)-2-(3-Furan-2-ylmethyl-ureido)-4-methyl-pentanoic acid hydroxyamide (4l). Yield 40%, mp = 137-139 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 10.67 (s, 1H), 8.79 (s, 1H), 8.54 (s, 1H), 7.56-7.55 (m, 1H), 6.38-6.31 (m, 4H), 4.18-4.16 (m, 2H), 4.12-4.03 (m, 1H), 1.57-1.46 (m, 1H), 1.35-1.30 (m, 2H), 0.88-0.84 (m, 6H); ^{13}C-NMR (300 MHz, DMSO-d_6): δ 169.3, 157.0, 153.4, 141.8, 110.3, 106.1, 49.0, 42.4, 36.2, 24.2, 22.7, 22.1; HRMS-ESI (+) calcd for $C_{12}H_{20}N_3O_4$, 270.1454; found, 270.1453 $[M+H]^+$. Elemental anal calcd for $C_{12}H_{19}N_3O_4$ C 53.52, H 7.11, N 15.60; found C 53.41, H 7.32, N 15.76.

(*S*)-4-Methyl-2-(3-naphthalen-1-yl-ureido)-pentanoic acid hydroxyamide (4m). Yield 70%, mp = 172-173 °C; $[\alpha]^{25}_D$ = +16.2 (*c* 0.5, MeOH); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 10.82 (s, 1H), 8.89 (s, 1H), 8.88 (s, 1H), 8.63-8.03 (m, 2H), 7.90-7.87 (m, 1H), 7.58-7.48 (m, 3H), 7.41 (t, 1H, J = 7.8 Hz), 6.93 (d, 1H, J = 9.0 Hz), 4.25-4.18 (m, 1H), 1.70-1.56 (m, 1H), 1.46 (t, 2H, J = 6.9 Hz), 0.94-0.90 (m, 6H); ^{13}C-NMR (300 MHz, DMSO-d_6): δ 169.0, 154.8, 135.0, 133.6, 128.4, 125.9, 125.7, 125.4, 125.1, 121.8, 121.1, 115.7, 49.0, 42.5, 24.3, 22.7, 22.2; HRMS-ESI (+) calcd for $C_{17}H_{22}N_3O_3$, 316.1661; found, 316.1646 $[M+H]^+$. Elemental anal calcd for $C_{17}H_{21}N_3O_3$ C 64.74, H 6.71, N 13.32; found C 64.71, H 6.68, N 13.26.

(*S*)-2-(3-Biphenyl-4-yl-ureido)-4-methyl-pentanoic acid hydroxyamide (4n). Yield 51%, mp = 169-170 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 10.79 (s, 1H), 8.87 (s, 1H), 8.66 (s, 1H), 7.76-7.40 (m, 8H), 7.90-7.87 (m, 1H), 7.58-7.48 (m, 3H), 7.41 (t, 1H, J = 7.8 Hz), 6.93 (d, 1H, J = 9.0 Hz), 4.25-4.18 (m, 1H), 1.70-1.56 (m, 1H), 1.46 (t, 2H, J = 6.9 Hz), 0.94-0.90 (m, 6H); ^{13}C-NMR (300 MHz, DMSO-d_6): δ 169.0, 154.8, 135.0, 133.6, 128.4, 125.9, 125.7, 125.4, 125.1, 121.8, 121.1, 115.7, 49.0, 42.5, 24.3, 22.7, 22.2; HRMS-ESI (+) calcd for $C_{19}H_{24}N_3O_3$, 342.1818; found, 342.1818 $[M+H]^+$. Elemental anal calcd for $C_{16}H_{25}N_3O_3$ C 66.84, H 6.79, N 12.31; found C 66.69, H 6.61, N 12.43.

(*S*)-2-[3-(4-Methoxy-phenyl)-ureido]-4-methyl-pentanoic acid hydroxyamide (4o). Yield 62%, mp = 178-180 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 10.75 (s, 1H), 8.40 (s, 1H), 8.33 (s, 1H), 7.27-7.24 (m, 2H), 6.82-6.79 (m, 2H), 6.20 (d, 1H, J = 9.0 Hz), 4.16-4.08 (m, 1H), 3.68 (s, 3H), 1.62-1.51 (m, 1H), 1.38 (t, 2H, J = 6.9 Hz), 0.92-0.87 (m, 6H); ^{13}C-NMR (300 MHz, DMSO-d_6): δ 169.2, 154.7, 153.9, 133.4, 119.1, 113.9, 55.1, 48.8, 42.4, 24.2, 22.7, 22.1; HRMS-ESI (+) calcd for $C_{14}H_{22}N_3O_4$, 296.1610; found, 296.1592 $[M+H]^+$. Elemental anal calcd for $C_{14}H_{21}N_3O_4$ C 56.94, H 7.17, N 14.23; found C 56.70, H 6.94, N 13.99.

(*S*)-2-[3-(4-Fluoro-phenyl)-ureido]-4-methyl-pentanoic acid hydroxyamide (4p). Yield 68%, mp = 162-163 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 10.77 (s, 1H), 8.57 (s, 1H), 7.38-7.34 (m, 2H), 7.08-7.02 (m, 2H), 6.29 (d, 1H, J = 9.0 Hz), 4.17-4.09 (m, 1H), 1.62-1.51 (m, 1H), 1.40 (t, 2H, J = 7.2 Hz), 0.91-0.87 (m, 6H); ^{13}C-NMR (300 MHz, DMSO-d_6): δ 169.0, 158.4, 155.3, 154.5, 136.6, 119.0, 118.9, 115.2, 115.0, 48.9, 42.4, 24.2, 22.7, 22.1; HRMS-ESI (+) calcd for $C_{13}H_{19}FN_3O_3$, 284.1410; found, 284.1415 $[M+H]^+$. Elemental anal calcd for $C_{13}H_{18}N_3O_3$ C 55.11, H 6.40, N 14.83; found C 55.33, H 6.67, N 14.76.

(*S*)-4-Methyl-2-(3-p-tolyl-ureido)-pentanoic acid hydroxyamide (4q). Yield 72%, mp = 171-173 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 10.76 (s, 1H), 8.84 (s, 1H), 8.41 (s, 1H), 7.25-7.22 (m, 2H), 7.03-7.00 (m, 2H), 6.26 (d, 1H, J = 9.0 Hz), 4.16-4.08 (m, 1H), 2.21 (s, 2H), 1.64-1.51 (m, 1H), 1.39 (t, 2H, J = 7.2 Hz), 0.91-0.87 (m, 6H); ^{13}C-NMR (300 MHz, DMSO-d_6): δ 169.1, 154.5, 137.7, 129.8, 129.0, 117.5, 48.8, 42.4, 24.2, 22.7, 22.1, 20.2; HRMS-ESI (+) calcd for $C_{14}H_{22}N_3O_3$, 280.1661; found, 280.1666 $[M+H]^+$. Elemental anal calcd for $C_{12}H_{21}N_3O_3$ C 60.20, H 7.58, N 15.04; found C 60.52, H 7.23, N 15.21.

(*S*)-4-Methyl-2-(3-thiophen-2-ylmethyl-ureido)-pentanoic acid hydroxyamide (4r). Yield 46%, mp = 165-166 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 10.68 (s, 1H), 8.79 (s, 1H), 7.37-7.35 (m, 1H), 6.95-6.44 (m, 2H), 6.46 (t, 1H, J = 5.4 Hz), 6.13 (d, 1H, J = 9.0 Hz), 4.35 (d, 2H, J = 5.7 Hz), 4.13-4.05 (m, 1H), 1.58-1.47 (m, 1H), 1.34 (t, 2H, J = 6.9 Hz), 0.89-0.84 (m, 6H); ^{13}C-NMR (300 MHz, DMSO-d_6): δ 169.3, 157.0, 144.1, 126.6, 124.6, 124.6, 49.0, 42.4, 24.2, 22.7, 22.1; HRMS-ESI (+) calcd for $C_{12}H_{20}N_3O_3S$, 286.1225; found, 286.1220 $[M+H]^+$. Elemental anal calcd for $C_{12}H_{19}N_3O_3S$ C 50.51, H 6.71, N 14.73, S 11.24; found C 50.59, H 6.55, N 14.84, S 11.45.

(*S*)-2-[3-(2-Methoxy-benzyl)-ureido]-4-methyl-pentanoic acid hydroxyamide (4s). Yield 76%, mp = 156-158 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 10.66 (s, 1H), 8.78 (s, 1H), 7.25-7.12 (m, 2H), 6.97-6.86 (m, 2H), 6.26 (t, 1H, J = 6.0 Hz), 6.17 (d, 1H, J = 9.0 Hz), 4.14 (d, 2H, J = 8.7 Hz), 4.11-4.03 (m, 1H), 1.58-1.47 (m, 1H), 1.33 (t, 2H, J = 7.2 Hz), 0.89-0.84 (m, 6H); ^{13}C-NMR (300 MHz, DMSO-d_6): δ 169.4, 157.3, 156.6, 128.0, 127.9, 127.7, 120.0, 110.3, 55.2, 49.0, 42.5, 38.1, 24.1, 22.7, 22.2; HRMS-ESI (+) calcd for $C_{15}H_{24}N_3O_4$, 310.1767; found, 310.1774 $[M+H]^+$. Elemental anal calcd for $C_{15}H_{23}N_3O_4$ C 58.24, H 7.49, N 13.58; found C 58.29, H 7.34, N 13.35.

(*S*)-2-[3-(3-Methoxy-benzyl)-ureido]-4-methyl-pentanoic acid hydroxyamide (4t). Yield 68%, mp = 161-162 °C; [α] $^{25}_{D}$ = +17.0 (*c* 0.5, MeOH); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 10.67 (s, 1H), 8.79 (s, 1H), 7.24-7.19 (m, 1H), 6.80-6.77 (m, 3H), 6.40 (t, 1H, J = 6.0 Hz), 6.12 (d, 1H, J = 8.7 Hz), 4.17 (d, 2H, J = 6.0 Hz), 4.13-4.05 (m, 1H), 1.61-1.48 (m, 1H), 1.34 (t, 2H, J = 6.9 Hz), 0.89-0.84 (m, 6H); ^{13}C-NMR (300 MHz, DMSO-d_6): δ 169.4, 159.3, 157.4, 142.4, 129.2, 119.0, 112.4, 112.0, 54.9, 49.1, 42.7, 42.5, 24.2, 22.8, 22.1; HRMS-ESI (+) calcd for $C_{15}H_{24}N_3O_4$, 310.1767; found, 310.1768 $[M+H]^+$. Elemental anal calcd for $C_{15}H_{23}N_3O_4$ C 58.24, H 7.49, N 13.58; found C 58.42, H 7.57, N 13.32.

(*S*)-2-[3-(3-Fluoro-benzyl)-ureido]-4-methyl-pentanoic acid hydroxyamide (4u). Yield 75%, mp = 167-168 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 10.66 (s, 1H), 8.79 (s, 1H), 7.38-7.31 (m, 1H), 7.08-7.01 (m, 3H), 6.47 (t, 1H, J = 6.0 Hz), 6.17 (d, 1H, J = 9.0 Hz), 4.21 (d, 2H, J = 6.0 Hz), 4.10-4.04 (m, 1H), 1.55-1.53 (m, 1H), 1.35 (t, 2H, J = 7.2 Hz), 0.89-0.84 (m, 6H); ^{13}C-NMR (300 MHz, DMSO-d_6): δ 169.5, 158.1, 157.3, 132.5, 128.2, 113.6, 55.0, 49.1, 42.4, 42.2, 24.2, 22.7, 22.1; HRMS-ESI (+) calcd for $C_{14}H_{21}FN_3O_4$, 298.1567; found, 298.1575 $[M+H]^+$. Elemental anal calcd for $C_{14}H_{20}FN_3O_4$ C 56.55, H 6.78, N 14.13; found C 56.59, H 6.75, N 13.96.

(*S*)-2-[3-(4-Methoxy-benzyl)-ureido]-4-methyl-pentanoic acid hydroxyamide (4v). Yield 67%, mp = 165-166 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 10.66 (s, 1H), 8.79 (s, 1H), 7.16-7.13 (m, 2H), 6.89-6.84 (m, 2H),

6.32 (t, 1H, J = 6.0 Hz), 6.07 (d, 1H, J = 8.7 Hz), 4.12-4.04 (m, 3H), 3.71 (s, 3H), 1.58-1.47 (m, 1H), 1.34 (t, 2H, J = 6.9 Hz), 0.89-0.84 (m, 6H); ^{13}C-NMR (300 MHz, DMSO-d_6): δ 169.4, 157.3, 144.0, 130.1, 122.8, 113.3, 113.1, 49.1, 42.4, 42.2, 24.2, 22.7, 22.1; HRMS-ESI (+) calcd for $C_{15}H_{24}N_3O_4$, 310.1767; found, 310.1760 $[M+H]^+$. Elemental anal calcd for $C_{15}H_{23}N_3O_4$ C 58.24, H 7.49, N 13.58; found C 58.56, H 7.19, N 13.42.

(*S*)-4-Methyl-2-[3-(4-methyl-benzyl)-ureido]-pentanoic acid hydroxyamide (4w). Yield 80%, mp = 165-166 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 10.66 (s, 1H), 8.78 (s, 1H), 7.13-7.11 (m, 4H), 6.34 (t, 1H, J = 6.0 Hz), 6.07 (d, 1H, J = 9.0 Hz), 4.15-4.04 (m, 3H), 2.26, (s, 3H), 1.58-1.50 (m, 1H), 1.34 (t, 2H, J = 7.5 Hz), 0.89-0.84 (m, 6H); ^{13}C-NMR (300 MHz, DMSO-d_6): δ 169.5, 157.3, 137.6, 135.6, 128.7, 126.9, 49.1, 42.5, 42.4, 24.2, 22.7, 22.1, 20.6; HRMS-ESI (+) calcd for $C_{15}H_{24}N_3O_3$, 294.1818; found, 294.1803 $[M+H]^+$. Elemental anal calcd for $C_{15}H_{23}N_3O_3$ C 61.41, H 7.90, N 14.32; found C 61.23, H 7.94, N 14.42.

(*S*)-2-[3-(4-Fluoro-benzyl)-ureido]-4-methyl-pentanoic acid hydroxyamide (4x). Yield 70%, mp = 155-156 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 10.66 (s, 1H), 8.78 (s, 1H), 7.28-7.23 (m, 2H), 7.16-7.10 (m, 2H), 6.42 (t, 1H, J = 6.3 Hz), 6.12 (d, 1H, J = 9.0 Hz), 4.18-4.04 (m, 3H), 1.58-1.49 (m, 1H), 1.34 (t, 2H, J = 7.2 Hz), 0.89-0.84 (m, 6H); ^{13}C-NMR (300 MHz, DMSO-d_6): δ 169.4, 157.3, 136.9, 128.8, 114.9, 49.1, 42.4, 42.0, 24.2, 22.7, 22.1; HRMS-ESI (+) calcd for $C_{14}H_{21}FN_3O_4$, 298.1567; found, 298.1559 $[M+H]^+$. Elemental anal calcd for $C_{14}H_{20}FN_3O_4$ C 56.55, H 6.78, N 14.13; found C 56.49, H 6.65, N 13.94.

(*S*)-N-Hydroxy-2-[3-(4-methyl-benzyl)-ureido]-2-phenyl-acetamide (4y). Yield 69%, mp = 191-193 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11.00 (s, 1H), 8.99 (s, 1H), 7.35-7.10 (m, 9H), 6.87 (s, 1H), 6.63 (s, 1H), 5.21-5.20 (m, 1H), 4.16-4.13 (m, 2H), 2.26 (s, 3H); ^{13}C-NMR (300 MHz, DMSO-d_6): δ 167.3, 157.0, 140.2, 137.4, 135.6, 128.7, 128.2, 127.2, 126.9, 126.4, 54.2, 42.5, 20.6; HRMS-ESI (+) calcd for $C_{17}H_{20}N_3O_3$, 314.1505; found, 314.1501 $[M+H]^+$. Elemental anal calcd for $C_{17}H_{19}N_3O_3$ C 65.16, H 6.11, N 13.41; found C 65.13, H 6.19, N 13.38.

(*S*)-N-Hydroxy-2-[3-(4-methoxy-benzyl)-ureido]-2-phenyl-acetamide (4z). Yield 60%, mp = 189-190 °C; ^{1}H-NMR (600 MHz, DMSO-d_6): δ 11.00 (s, 1H), 8.98 (s, 1H), 7.34-6.84 (m, 10H), 6.61-6.59 (m, 1H), 5.21-5.20 (m, 1H), 4.16-4.10 (m, 2H), 3.70 (s, 3H); ^{13}C-NMR (300 MHz, DMSO-d_6): δ 167.3, 158.1, 157.0, 140.2, 132.4, 128.2, 128.1, 127.2, 126.5, 113.6, 55.0, 54.2,.42.2; HRMS-ESI (+) calcd for $C_{17}H_{20}N_3O_4$, 330.1454; found, 330.1459 $[M+H]^+$. Elemental anal calcd for $C_{17}H_{19}N_3O_4$ C 62.00, H 5.81, N 12.76; found C 61.97, H 5.95, N 12.73.

(*S*)-2-[3-(4-Fluoro-benzyl)-ureido]-N-hydroxy-2-phenyl-acetamide (4aa). Yield 72%, mp = 182-183 °C; ^{1}H-NMR (600 MHz, DMSO-d_6): δ 11.00 (s, 1H), 8.98 (s, 1H), 7.37-7.11 (m, 9H), 6.91 (s, 1H), 6.70 (s,1H), 5.21-5.20 (m, 1H), 4.16-4.13 (m, 2H); ^{13}C-NMR (300 MHz, DMSO-d_6): δ 167.3, 157.0, 140.1, 136.8, 128.8, 128.7, 128.2, 127.2, 126.5, 115.0,114.8, 54.3, 42.0; HRMS-ESI (+) calcd for $C_{16}H_{17}FN_3O_3$, 318.1254; found, 318.1257 $[M+H]^+$. Elemental anal calcd for $C_{16}H_{16}FN_3O_3$ C 60.56, H 5.08, N 13.24; found C 60.58, H 5.15, N 13.20.

(*S*)-N-Hydroxy-2-phenyl-2-(3-phenyl-ureido)-acetamide (4bb). Yield 65%, mp = 185-186 °C; ^{1}H-NMR (600 MHz, DMSO-d_6): δ 11.07 (s, 1H), 9.05 (s, 1H), 7.40-7.20 (m, 10H), 7.10 (s, 1H), 6.89 (s, 1H), 5.24-5.22 (m, 1H); ^{13}C-NMR (300 MHz, DMSO-d_6): δ 166.9, 154.1, 140.1, 139.8, 128.7, 128.3, 127.4, 126.5, 121.2, 117.4; HRMS-ESI (+) calcd for $C_{15}H_{16}N_3O_3$, 286.1192; found, 286.1190 $[M+H]^+$. Elemental anal calcd for $C_{15}H_{15}N_3O_3$ C 63.15, H 5.30, N 14.73; found C 63.16, H 5.49, N 14.69.

(*S*)-4-Methyl-2-(3-naphthalen-1-yl-methyl-ureido)-pentanoic acid hydroxyamide (4cc). Yield 52%, mp = 168-170 °C; $[\alpha]$ $^{25}_D$ = +11.0 (*c* 0.5, MeOH); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 10.68 (s, 1H), 8.79 (s, 1H),

8.78-7.82 (m, 3H), 7.56-7.41 (m, 4H), 6.45 (t, 1H, J = 5.7 Hz), 6.11 (d, 1H, J = 9.0 Hz), 4.74-4.60 (m, 2H), 4.17-4.10 (m, 1H), 1.60-1.51 (m, 1H), 1.37-1.32 (m, 2H), 0.89-0.87 (m, 6H); ^{13}C-NMR (300 MHz, DMSO-d_6): δ 169.4, 157.2, 135.9, 133.3, 130.8, 128.4, 127.4, 126.1, 125.7, 125.4, 125.0, 123.5, 49.1, 42.6, 2.2, 22.8, 22.1; HRMS-ESI (+) calcd for $C_{18}H_{24}N_3O_3$, 330.1818; found, 330.1820 $[M+H]^+$. Elemental anal calcd for $C_{18}H_{24}N_3O_3$ C 65.63, H 7.04, N 12.76; found C 65.66, H 7.12, N 12.80.

General procedure for the synthesis of 7. To a mixture of L-Leucine methyl ester hydrochloride (2.76 g, 21 mmol) in saturated $NaHCO_3$ (80 mL) and DCM (80 mL) was added triphosgene (2.08 g, 7 mmol). The reaction mixture was vigorously stirred under ice-water bath for 15 min and the organic layer was collected. The water layer was extracted with DCM for three times and the organic phase was combined and dried with $MgSO_4$. After the solvent removed under vaccum, the residue was dissolved in DCM (20 mL). This solution was then added to the mixture of 3-Phenyl-propan-1-ol (2.86 g, 21 mmol) and triethylamine (2.12g, 21 mmol) in DCM (80 mL) under ice bath. The reaction mixture was stirred at room temperature for 30 min and then the solvent was removed under low pressure. The residue was taken up with ethyl acetate (40 mL) and washed with 1N HCl (10 mL) and brine (20 mL). The organic phase was dried with $MgSO_4$. After the solvent removed, the residue without purification was directly added to a solution of potassium hydroxylamine (8.37 g, 56 mmol) in methanol (20 mL). The reaction mixture was stirred at room temperature for 5 hours and then removed methanol under low pressure. The residue was taken up with 1N HCl and extracted with ethyl acetate. The organic phase was washed with brine and dried with $MgSO_4$. After the solvent removed under low pressure, the residue was separated by silica gel column chromatography to afford **7**. Yield 69%, mp = 137-139 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 10.48 (s, 1H), 8.18 (s, 1H), 7.26-7.06 (m, 5H), 4.08-4.00 (m, 1H), 3.78-3.72 (m, 2H), 3.00-2.94 (m, 2H), 2.57-2.55 (m, 2H), 1.68-1.51 (m, 3H), 0.88-0.84 (m, 6H); HRMS-ESI (+) calcd for $C_{16}H_{25}N_2O_4$, 309.1814; found, 309.1808 $[M+H]^+$. Elemental anal calcd for $C_{16}H_{24}N_2O_4$ C 62.32, H 7.84, N 9.08; found C 62.10, H 7.66, N 8.97.

General procedure for the synthesis of 9. To a mixture of L-Leucine methyl ester hydrochloride (2.76 g, 21 mmol) and triethylamine (2.12g, 21 mmol) in DCM (50 mL) was dropwise added phenylacetyl chloride (3.24 g, 21 mmol) in DCM (30 mL) under an ice-water bath for 30 min. After the addition was completed, the reaction mixture was allowed to be stirred at room temperature. 1 h later, DCM evaporated, the residue was taken up with ethyl acetate and washed with 1N HCl and brine, then dried with $MgSO_4$. After the solvent removed, the residue without purification was directly added to a solution of potassium hydroxylamine (8.37 g, 56 mmol) in methanol (20 mL). The reaction mixture was stirred at room temperature for 5 hours and then removed methanol under low pressure. The residue was taken up with 1N HCl and extracted with ethyl acetate. The organic phase was washed with brine and dried with $MgSO_4$. After the solvent removed under low pressure, the residue was separated by silica gel column chromatography to afford **9**. Yield 61%, mp = 136-137 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 10.69 (s, 1H), 8.81 (s, 1H), 8.23 (d, 1H, J = 8.4 Hz), 7.31-7.17 (m, 5H), 4.24-4.16 (m, 1H), 3.49-3.39 (m, 2H), 1.56-1.37 (m, 3H), 0.87-0.78 (m, 6H); HRMS-ESI (+) calcd for $C_{14}H_{21}N_2O_3$, 265.1552; found, 265.1553 $[M+H]^+$. Elemental anal calcd for $C_{14}H_{20}N_2O_3$ C 63.62, H 7.63, N 10.60; found C 63.57, H 7.72, N 10.82.

Biological Activity

Methods. Cell culture. ES-2 cells and B16BL6 cells were cultured in RPMI-1640 culture medium supplemented with 10% (v/v) fetal bovine serum (FBS).

Enzyme inhibitory activity of the compounds towards APN from porcine kidney (Microsomal, Sigma). IC_{50} values against APN were determined by using L-Leu-*p*-nitroanilide as substrate and Microsomal aminopeptidase from Porcine Kidney Microsomes (Sigma) as lymphilized powder 15-25 units/mg protein. In brief, the assay was performed in 96-well plates in 50 mM PBS, pH 7.2 as the assay buffer, at 37 °C. All solutions of inhibitors were prepared in the assay buffer with 0.5% DMSO in final concentration as the fluxing agent. All

inhibitors were pre-incubated with APN for 5 min at room temperature. The assay mixture, which contained 40 μL of the inhibitor solution (concentration dependent on the inhibitors), 100 μL of the enzyme solution (6 μg/mL in final concentration), 5 μL of the substrate solution and the assay buffer, was adjusted to 200μL, then incubated at 37°C for 30 min. The hydrolysis of the substrate was monitored by following the change in the absorbance measured at 405 nm with the UV-Vis spectrophotometer Pharmacia LKB, Biochrom 4060. The enzyme activity inhibitory rate was calculated from the photometric intensity readings, and the concentration of inhibiting 50% of the enzyme activity (IC_{50}) was determined through a regression analysis of the concentration/inhibitory rate data.

Enzyme inhibitory activity of the compounds towards APN from ES-2 cells or B16BL6 cells. IC_{50} values against APN were determined by using L-Leu-*p*-nitroanilide as substrate and ES-2 human ovarian clear cell carcinoma cells or B16BL6 mouse melanoma cells as the enzyme source. In brief, the assay was performed in 96-well plates in 10 mM PBS, pH 7.4 as the assay buffer, at 37°C. All solutions of inhibitors were prepared in the assay buffer with 0.2% DMSO in final concentration as the fluxing agent. Compounds were pre-incubated with ES-2 or B16BL6 cell suspension (1×10^5 cells per well) for 5 min at room temperature. The assay mixture, which contained 20 μL of the inhibitor solution (concentration dependent on the inhibitors), 70 μL of the ES-2 cell suspension, 10 μL of the substrate solution, or the assay buffer, was adjusted to 100μL, and then incubated at 37°C for 1 h. The hydrolysis of the substrate in the supernatant liquor after centrifugation was monitored by following the change in the absorbance measured at 405 nm with the UV-Vis spectrophotometer Pharmacia LKB, Biochrom 4060. The enzyme activity inhibitory rate was calculated from the photometric intensity readings, and the concentration of inhibiting 50% of the enzyme activity (IC_{50}) was determined through a regression analysis of the concentration/inhibitory rate data.

Enzyme Inhibitory Activity of the compounds towards MMP-2 (Recombinant, Sigma). IC_{50} values against MMP-2 in 96-well microtiter plates were determined by using succinylated gelatin as the substrate. In brief, the tested compounds and the enzyme were dissolved in sodium borate buffer (pH 8.5, 50 mM) and incubated at 37 °C for 10 min. The substrate was added and incubated at 37 °C for additional 60 min. The control and zero-setting groups were also carried out. Then 0.03% picrylsulfonic acid solution was added and incubated at room temperature for additional 20 min. The resulting solutions were measured under 450 nm with the UV-Vis spectrophotometer Pharmacia LKB, Biochrom 4060. The inhibitory rates were calculated from the photometric intensity readings, and the concentration of inhibiting 50% of the enzyme activity (IC_{50}) was determined through a regression analysis of the concentration/inhibitory rate data.

In Vitro Cytotoxity Assay. The in vitro cytotoxity of the tested compounds was assayed by determining the growth inhibition rates on certain concentrations by MTT method. In brief, the ES-2 or B16BL6 cells were plated in a 96-well plate (8000 cells per well) in RPMI-1640 culture medium with 10% FBS, and allowed to adhere and spread for 10 h. Then the culture medium was removed and certain concentrations (15 and 150 μM) of the compounds in RPMI-1640 culture medium with 1% FBS (with 0.2% DMSO as fluxing agent, also in the control the zero-setting wells) were added, and then the cells were cultured for 24 h at 37 °C in a CO_2 incubator. MTT solution (10 μL of 5 mg/mL) was added per well and the cells were cultured for additional 4 h. Then the medium were removed and the residue were dissolved in 100 μL DMSO per well. The optical density (OD) values were measured at 590 nm. The growth inhibitory rate was calculated as from the photometric intensity readings, and the concentration of inhibiting 50% of the enzyme activity (IC_{50}) was determined through a regression analysis of the concentration/inhibitory rate data.

Cell Migration Assay. The Thincert™ Chambers (Costar, Cambridge, MA) 150 μM of the tested compounds in 100 μL of RPMI-1640 culture medium with 1% FBS (with 0.1% DMSO in final concentration as the fluxing agents) were added to the upper chamber at the same time. ES-2 cells in 400 μL of RPMI-1640 culture medium

with 1% FBS (50000 cells per well) were added and allowed to migrate for 3 h at 37 °C in a CO_2 incubator. 6 h later, cells in the upper chamber were removed with a cotton swab. The migrated cells were fixed, stained with 0.1% crystal violet, and photographs were taken under a microscope. ES-2 migration was quantified by counting the number of cells in five random fields (× 100) per insert.

Anti-invasion Assay. The BD BioCoat™ Matrigel™ Invasion Chambers were rehydrated with 500 μL RPMI-1640 culture medium with 1% FBS in both of the upper and lower chambers for 2 h. After the medium was removed, 750 μL of RPMI-1640 culture medium with 10% FBS were added to the lower chambers. Various concentrations (5 and 50 μg/mL) of **4m**, **4q**, **4t** and **4zc** in 100 μL of RPMI-1640 culture medium with 1% FBS (with 0.1% DMSO in final concentration as the fluxing agents) were added to the upper chamber at the same time. Cells in 400 μL of RPMI-1640 culture medium with 1% FBS (100000 cells per well) were added and allowed to invade for 8 h at 37 °C in a CO_2 incubator. 8 h later, Matrigel and cells in the upper chamber were removed with a cotton swab. The remaining cells were fixed, stained with 0.1% crystal violet, and photographs were taken under a microscope. ES-2 invasion was quantified by counting the number of cells in five random fields (× 100) per insert.

HUVEC Anti-angiogenesis Assay. Before the HUVEC tubular structure formation assay, Matrigel (BD Bioscience, Bedford, MA) was polymerized in 24-well plates at 37 °C for 1 h. Then HUVEC was trypsinized and seeded onto Matrigel at a concentration of 10^5 cells per well and then incubated with various concentration of **4m**, **4q**, **4t** and **4zc** (25 μg/mL) for 8 h at 37 °C in a CO_2 incubator. Cellular morphology was taken under a microscope in five random fields per well.

Murine B16BL6 Melanoma Induced Angiogenesis. The effect of the compounds on tumor induced angiogenesis was accessed based on previously described methods[34] with minor modifications. The C57BL/6 mice aged 6 weeks (n = 8 mice per group) were inoculated intradermally with B16BL6 cells (5×10^5 cells/50 μL per mouse) at two sites on the back of mice on day 0. The treatment groups received 50 mg/kg/d or 100 mg/kg/d compounds intraperitioneally, and the control group received an equal volume of DMSO intraperitioneally, from day 0 to day 4. On day 5, animals were sacrificed and the skin was separated from the underlying tissues. Angiogenesis was quantified by counting the number of vessels oriented towards the tumor mass under a dissecting microscope[35]. The tumor volume was estimated using the following formula: tumor mass (mm^3) = long diameter × short diameter^2 × 1/2.

Murine B16BL6 Melanoma Experimental Lung Metastasis. C57BL/6 mice aged 6 weeks (n = 8 mice per group) were intravenously inoculated with B16BL6 melanoma cells (5×10^5 cells/100 μL per mouse) on day 0. The treatment groups received 100 mg/kg/d compounds intraperitioneally, and the control group received an equal volume of DMSO intraperitioneally, from day 0 to day 13. On day 14, animals were sacrificed and the lungs were fixed in Bouin's solution and counted for the number of metastatic nodules on the lung surface[31].

Statistical Analysis.

The statistical significance of differences between the groups was assessed by Student's *t* test. *P* values of <0.05 were considered as statistically significant.