Enantiomeric Diketopiperazines: Getting Insight of the Impact of the Configuration to the Conformation, Nanoimage, u-PA Inhibition and Anti-metastatic Activity

Feng Wang,[a] Shan Li,[a] Yuji Wang,[a] Haimei Zhu,[a] Xiaoyi Zhang,[a] Ming Zhao,[a,b,*] Jianhui Wu,[a] and Shiqi Peng [a,*]

† Electronic Supplementary Information

1. General

The protected amino acids and the chemicals used were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and were purified, when necessary. Column chromatography was performed using silica gel (200-300 mesh, Qingdao Haiyang Chemical Co., Qingdao, P. R. China). Purity of intermediates (>95%) and products (>98%) were determined by TLC analysis (Qingdao silica gel plates of GF254) and HPLC analysis (CHIRALPAK AH-H column, 4.6 × 250 mm, Daicel Chemical IND., LTD.). ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Varian INOVA-300 MHz spectrometer with DMSO-*d6* as the solvent and tetramethylsilane as internal standard. ^{1}H NMR (800 MHz), ^{13}C NMR (200 MHz) and the ROESY 2D NMR spectra were recorded on a Bruker Avance II 800 MHz spectrometer with DMSO-*d6* as the solvent and tetramethylsilane as internal standard. ESI/MS spectra were recorded on ZQ 2000 (Waters, US) and solariX FT-ICR mass spectrometer (Bruker Daltonik) with an ESI/MALDI dual ion source and 9.4 T superconductive magnet.

2. Synthesis of four diastereomers SS, SR, RR and RS of AIPZ

2.1 Preparation of AIPZ

2.1.1 Preparing Boc-L-Lys(Z)-L-Trp-OBzl (1SS)

To a solution of 1.47 g (5.0 mmol) of L-Trp-OBzl, 1 mL (9.0 mmol) of N-methylmorpholine, 1.133 g (5.5 mmol) of DCC and 0.675 g (5.0 mmol) of HOBt in 20 mL of anhydrous THF 1.9 g (5.0 mmol) of Boc-L-Lys(Z) was added at 0 °C. After stirring at the same temperature for 1 h, the reaction mixture was stirred at room temperature for 5 h and TLC (chloroform/methanol, 30:1) indicated the complete disappearance of L-Trp-OBzl. The reaction mixture was filtered and the filtrate was evaporated under vacuum. The residue was dissolved in 150 mL of ethyl acetate and washed successively with 5% aqueous sodium bicarbonate, 5% aqueous citric acid, and saturated aqueous sodium chloride. The ethyl acetate phase was dried over anhydrous sodium sulfate. After filtration and evaporation under vacuum the residue was purified on silica gel column (Petroleum ether/ethyl acetate, 10:1-1:1) to provide 3.037 g (93%) of the title compound as colorless powder. ESI-MS (m/e): 657 $[M + H]^{+}$.

2.1.2 Preparing L-Lys(Z)-L-Trp-OBzl (2SS)

A solution of 2.62 g (4 mmol) of Boc-L-Lys(Z)-L-Trp-OBzl (**1**SS) in 26 mL of ethyl acetate containing hydrogen chloride (4 M) was stirred at 0 °C for 4 h. The reaction mixture was concentrated under vacuum. The residue was dissolved in ethyl acetate and the solution was concentrated under vacuum, which was repeated for 4 times to throughly remove hydrogen chloride. The residue was triturated with ether to provide 2.253 g (95%) of the title compound as yellow powder. ESI-MS (m/e): 557 $[M + H]^{+}$.

2.1.3 Preparing (3*S*,6*S*)-3-(4-Benzyloxycarbonylaminobutyl)-6-(1H-indole-3-ylmethyl)piperazine-2,5

-dione (3SS)

The solution of 2.0 g of L-Lys(Z)-L-Trp-OBzl (**2**SS) in 150 mL of ethyl acetate was washed with 5% aqueous sodium bicarbonate. The ethyl acetate phase was dried over anhydrous sodium sulfate. After filtration the solution was stirred at room temperature over night. Evaporated under vacuum and the residue was dissolved in 10 mL of methanol. The solution was filtered and precipitates were collected to provide 0.725 g (45%) of the title compound as colorless powder. ESI-MS (m/e): 449 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-*d6*): δ/ppm = 10.846 (s, 1 H), 8.017 (d, *J* = 1.5 Hz, 1 H), 7.911 (d, *J* = 1.8 Hz, 1 H), 7.574 (d, *J* = 7.8 Hz, 1 H), 7.341 (m, 6 H), 7.044 (m, 3 H), 6.920 (t, *J* = 7.2 Hz, 1 H), 5.000 (s, 2 H), 4.108 (s, 1 H), 3.505 (s, 1 H), 3.249 (dd, *J* = 4.2 Hz, *J* = 14.4 Hz, 1 H), 3.011 (dd, *J* = 4.8 Hz, *J* = 14.4 Hz, 1 H), 2.719 (q, *J* = 7.2 Hz, 2 H), 0.981 (m, 3 H), 0.513 (m, 3 H). ^{13}C NMR (75 MHz, DMSO-*d6*): δ/ppm = 167.54, 167.39, 156.42, 137.76, 136.36, 128.80, 128.29, 128.20, 125.04, 121.16, 119.43, 118.73, 111.59, 109.07, 65.55, 55.85, 54.32, 33.54, 29.41, 29.35, 21.26.

2.1.4 Preparing (3*S*,6*S*)-3-(4-aminobutyl)-6-(1H-indole-3-ylmethyl)-piperazine-2,5-dione (SS)

To the solution of 0.448 g (1.0 mmol) of (3S,6S)-3-(4-benzyloxycarbonylaminobutyl)-6-(1H-indole-3-ylmethyl)-piperazine-2,5-dione (**3**SS) in 10 mL methanol, 140 mg Pd/C was added. Replacing the air of the reaction bulb with H_2 and stirred at room temperature for 48 h. The Pd/C was removed by filtration. After evaporation under vacuum 0.260 g (83%) of the title compound was obtained as colorless powder. FT-MS (m/e): 315.1809 $[M+H]^+$. $[\alpha]_D^{20}$ = -10.00 (c = 0.31, CH_3OH). ^{1}H NMR (800 MHz, DMSO-d_6): δ/ppm = 10.979 (s, 1 H), 8.091 (s, 1 H), 7.933 (s, 1 H), 7.590 (d, *J* = 8.0 Hz, 1 H), 7.330 (d, *J* = 8.0 Hz, 1 H), 7.030 (dd, *J* = 15.2 Hz, *J* = 8.0 Hz, 2 H), 6.935 (t, *J* = 7.2 Hz, 1 H), 4.127 (d, *J* = 4.0 Hz, 1 H), 3.528 (d, *J* = 5.6 Hz, 1 H), 3.266 (dd, *J* = 4.0 Hz, *J* = 14.8 Hz, 1 H), 3.009 (dd, *J* = 4.0 Hz, *J* = 14.8 Hz, 1 H), 2.369 (m, 2 H), 1.073 (m, 2 H), 0.955 (m, 1 H), 0.597 (m, 2 H), 0.573 (m, 1 H). ^{13}C NMR (200 MHz, DMSO-*d6*): δ/ppm = 167.49, 167.25, 136.35, 128.37, 125.11, 121.11, 119.49, 118.73, 111.63, 109.04, 55.88, 54.19, 33.24, 29.22, 28.75, 20.86.

2.1.5 Preparing Boc-D-Lys(Z)-L-Trp-OBzl (1RS)

To a solution of 1.47 g (5.0 mmol) of L-Trp-OBzl, 1 mL (9.0 mmol) of N-methylmorpholine, 1.133 g (5.5 mmol) of DCC and 0.675 g (5.0 mmol) of HOBt in 20 mL of anhydrous THF 1.9 g (5.0 mmol) of Boc-D-Lys(Z) was added at 0 °C. After stirring at the same temperature for 1 h, the reaction mixture was stirred at room temperature for 5 h and TLC (chloroform/methanol, 30:1) indicated the complete disappearance of L-Trp-OBzl. The reaction mixture was filtered and the filtrate was evaporated under vacuum. The residue was dissolved in 150 mL of ethyl acetate and washed successively with 5% aqueous sodium bicarbonate, 5% aqueous citric acid, and saturated aqueous sodium chloride. The ethyl acetate phase was dried over anhydrous sodium sulfate. After filtration and evaporation under vacuum the residue was purified on silica gel column (Petroleum ether/ethyl acetate, 10:1-1:1) to provide 2.825 g (86%) of the title compound as colorless powder. ESI-MS (m/e): 657 $[M + H]^+$.

2.1.6 Preparing D-Lys(Z)-L-Trp-OBzl (2RS)

A solution of 2.62 g (4 mmol) of Boc-D-Lys(Z)-L-Trp-OBzl (**1**RS) in 26 mL of ethyl acetate containing hydrogen chloride (4 M) was stirred at 0 °C for 4 h. The reaction mixture was concentrated under vacuum. The residue was dissolved in ethyl acetate and the solution was concentrated under vacuum, which was repeated for 4 times to throughly remove hydrogen chloride. The residue was triturated with ether to

provide 2.253 g (95%) of the title compound as yellow powder. ESI-MS (m/e): 557 $[M + H]^+$.

2.1.7 Preparing (3*R*,6*S*)-3-(4-benzyloxycarbonylaminobutyl)-6-(1H-indole-3-ylmethyl)piperazine-2,5-dione (3RS)

At 80 °C to a solution of 1.832 g of D-Lys(Z)-L-Trp-OBzl (**2**RS) in 60 mL of ethyl acetate 2 mL TEA was added. After stirring at 80 °C for 24 h the reaction mixture was evaporated under vacuum and the residue was dissolved in 3 mL of methanol. The solution was filtered and precipitates were collected to provide 0.943 g (68%) of the title compound as colorless powders. ESI-MS (m/e): 449 $[M+H]^+$. $[\alpha]_D^{20}$ = 45.0 (c = 0.5, CH_3OH). ^1H NMR (300 MHz, DMSO-*d6*): δ/ppm = 10.89 (s, 1 H), 8.03 (s, 1 H), 7.85 (s, 1 H), 7.56 (d, *J* = 9.0 Hz, 1 H), 7.35 (m, 6 H), 7.19 (t, *J* = 6.0 Hz, 1 H), 7.05 (m, 2 H), 6.95 (t, *J* = 6.0 Hz, 1 H), 4.99 (s, 2 H), 4.07 (s, 1 H), 3.25 (dd, *J* = 3.0 Hz, *J* = 15.0 Hz, 1 H), 3.09 (dd, *J* = 3.0 Hz, *J* = 15.0 Hz, 1 H), 2.99 (s, 1 H), 2.91 (q, *J* = 6.0 Hz, 2 H), 1.48 (m, 2 H), 1.24 (m, 2 H), 1.16 (m, 2 H).

2.1.8 Preparing (3*R*,6*S*)-3-(4-aminobutyl)-6-(1H-indole-3-ylmethyl)-piperazine-2,5-dione (RS)

To the solution of 0.448 g (1.0 mmol) of (3R,6S)-3-(4-benzyloxycarbonylamino-butyl)-6-(1H-indole-3-ylmethyl)-piperazine-2,5-dione (**3**RS) in 10 mL methanol, 140 mg Pd/C was added. Replacing the air of the reaction bulb with H2 and stirred at room temperature for 48 h. The Pd/C was removed by filtration. After evaporation under vacuum 0.243 g (77%) of the title compound was obtained as colorless powder. FT-MS (m/e): 315.1814 $[M+H]^+$. Mp 189-190 ºC. IR(KBr): 3250, 2860, 2312, 1662, 1456, 1325, 1230, 1093, 1006, 736 cm^{-1}; $[\alpha]_D^{20}$ = 48.9 (c = 0.32, CH_3OH). ^1H NMR (800 MHz, DMSO-*d6*): δ/ppm = 10.92 (s, 1 H), 8.05 (s, 1 H), 7.88 (s, 1 H), 7.56 (d, *J* = 9.0 Hz, 1 H), 7.31 (d, *J* = 9.0 Hz, 1 H), 7.06 (s, 1H), 7.04 (t, *J* = 9 Hz, 1 H), 6.94 (t, *J* = 9 Hz, 1 H), 4.07 (s, 1 H), 3.26 (dd, *J* = 3 Hz, J = 12 Hz, 1 H), 3.02 (dd, *J* = 6.0 Hz, *J* = 15 Hz, 1 H), 2.84 (m, 2 H), 2.44 (m, 2 H), 1.19 (m, 2 H), 1.10 (m, 2 H). ^{13}C NMR (200 MHz, DMSO-*d6*): δ/ppm = 168.60, 168.09, 136.37, 128.05, 125.02, 121.31, 119.26, 118.85, 111.62, 108.86, 55.89, 53.59, 41.05, 31.94, 31.65, 29.39, 21.16.

2.1.9 Preparing Boc-D-Lys(Z)-D-Trp-OBzl (4RR)

To a solution of 1.47 g (5.0 mmol) of D-Trp-OBzl, 1 mL (9.0 mmol) of N-methylmorpholine, 1.133 g (5.5 mmol) of DCC and 0.675 g (5.0 mmol) of HOBt in 20 mL of anhydrous THF 1.9 g (5.0 mmol) of Boc-D-Lys(Z) was added at 0 °C. After stirring at the same temperature for 1 h, the reaction mixture was stirred at room temperature for 5 h and TLC (chloroform/methanol, 30:1) indicated the complete disappearance of D-Trp-OBzl. The reaction mixture was filtered and the filtrate was evaporated under vacuum. The residue was dissolved in 150 mL of ethyl acetate and washed successively with 5% aqueous sodium bicarbonate, 5% aqueous citric acid, and saturated aqueous sodium chloride. The ethyl acetate phase was dried over anhydrous sodium sulfate. After filtration and evaporation under vacuum the residue was purified on silica gel column (Petroleum ether/ethyl acetate, 10:1-1:1) to provide 2.923 g (89%) of the title compound as colorless powder. ESI-MS (m/e): 657 $[M + H]^+$.

2.1.10 Preparing D-Lys(Z)-D-Trp-OBzl (5RR)

A solution of 2.62 g (4 mmol) of Boc-D-Lys(Z)-D-Trp-OBzl (**4**RR) in 26 mL of ethyl acetate containing hydrogen chloride (4 M) was stirred at 0 °C for 4 h. The reaction mixture was concentrated under vacuum. The residue was dissolved in ethyl acetate and the solution was concentrated under vacuum, which was

repeated for 4 times to throughly remove hydrogen chloride. The residue was triturated with ether to provide 2.159 g (91%) of the title compound as yellow powder. ESI-MS (m/e): 557 $[M + H]^+$.

2.1.11 Preparing (3*R*,6*R*)-3-(4-benzyloxycarbonylaminobutyl)-6-(1H-indole-3-ylmethyl)-piperazine-2,5-dione (6RR)

The solution of 1.95 g (3.5 mmol) of D-Lys(Z)-D-Trp-OBzl (**5**RR) in 150 mL of ethyl acetate was washed with 5% aqueous sodium bicarbonate. The ethyl acetate phase was dried over anhydrous sodium sulfate. After filtration the solution was stirred at room temperature over night. Evaporated under vacuum and the residue was dissolved in 10 mL of methanol. The solution was filtered and precipitates were collected to provide 1.35 g (86%) of the title compound as colorless powder. ESI-MS (m/e): 449 $[M+H]^+$.

2.1.12 Preparing (3*R*,6*R*)-3-(4-aminobutyl)-6-(1H-indole-3-ylmethyl)-piperazine-2,5-dione (RR)

To the solution of 0.732 g (1.5 mmol) of (3R,6R)-3-(4-benzyloxycarbonyl-aminobutyl)-6-(1H-indole-3-ylmethyl)-pipe- razine-2,5-dione (**5**RR) in 10 mL methanol, 140 mg Pd/C was added. Replacing the air of the reaction bulb with H_2 and stirred at room temperature for 48 h. The Pd/C was removed by filtration. After evaporation under vacuum 0.260 g (83%) of the title compound was obtained as colorless powder. FT-MS (m/e): 315.1809 $[M+H]^+$. $[\alpha]_D^{20}$ = 10.00 (c = 0.35, CH_3OH). ^1H NMR (800 MHz, DMSO-d_6): δ/ppm = 10.979 (s, 1 H), 8.091 (s, 1 H), 7.933 (s, 1 H), 7.590 (d, *J* = 8.0 Hz, 1 H), 7.330 (d, *J* = 8.0 Hz, 1 H), 7.030 (dd, *J* = 15.2 Hz, *J* = 8.0 Hz, 2 H), 6.935 (t, *J* = 7.2 Hz, 1 H), 4.127 (d, *J* = 4.0 Hz, 1 H), 3.528 (d, *J* = 5.6 Hz, 1 H), 3.266 (dd, *J* = 4.0 Hz, *J* = 14.8 Hz, 1 H), 3.009 (dd, *J* = 4.0 Hz, *J* = 14.8 Hz, 1 H), 2.369 (m, 2 H), 1.073 (m, 2 H), 0.955 (m, 1 H), 0.597 (m, 2 H), 0.573 (m, 1 H). ^{13}C NMR (200 MHz, DMSO-*d6*): δ/ppm = 167.49, 167.25, 136.35, 128.37, 125.11, 121.11, 119.49, 118.73, 111.63, 109.04, 55.88, 54.19, 33.24, 29.22, 28.75, 20.86.

2.1.13 Preparing Boc-L-Lys(Z)-D-Trp-OBzl (4SR)

To a solution of 1.47 g (5.0 mmol) of D-Trp-OBzl, 1 mL (9.0 mmol) of N-methylmorpholine, 1.133 g (5.5 mmol) of DCC and 0.675 g (5.0 mmol) of HOBt in 20 mL of anhydrous THF 1.9 g (5.0 mmol) of Boc-L-Lys(Z) was added at 0 °C. After stirring at the same temperature for 1 h, the reaction mixture was stirred at room temperature for 5 h and TLC (chloroform/methanol, 30:1) indicated the complete disappearance of D-Trp-OBzl. The reaction mixture was filtered and the filtrate was evaporated under vacuum. The residue was dissolved in 150 mL of ethyl acetate and washed successively with 5% aqueous sodium bicarbonate, 5% aqueous citric acid, and saturated aqueous sodium chloride. The ethyl acetate phase was dried over anhydrous sodium sulfate. After filtration and evaporation under vacuum the residue was purified on silica gel column (Petroleum ether/ethyl acetate, 10:1-1:1) to provide 2.989 g (91%) of the title compound as colorless powder. ESI-MS (m/e): 657 $[M + H]^+$.

2.1.14 Preparing L-Lys(Z)-D-Trp-OBzl (5SR)

A solution of 2.62 g (4 mmol) of Boc-L-Lys(Z)-D-Trp-OBzl (**4**SR) in 26 mL of ethyl acetate containing hydrogen chloride (4 M) was stirred at 0 °C for 4 h. The reaction mixture was concentrated under vacuum. The residue was dissolved in ethyl acetate and the solution was concentrated under vacuum, which was repeated for 4 times to throughly remove hydrogen chloride. The residue was triturated with ether to provide 2.04 g (86%) of the title compound as yellow powder. ESI-MS (m/e): 557 $[M + H]^+$.

2.1.15 Preparing (3*S*,6*R*)-3-(4-benzyloxycarbonylaminobutyl)-6-(1H-indole-3-ylmethyl)piperazine-

2,5-dione (6SR)

At 80 °C to a solution of 1.832 g of L-Lys(Z)-D-Trp-OBzl (**5**SR) in 60 mL of ethyl acetate 2 mL TEA was added. After stirring at 80 °C for 24 h the reaction mixture was evaporated under vacuum and the residue was dissolved in 3 mL of methanol. The solution was filtered and precipitates were collected to provide 0.902 g (65%) of the title compound as colorless powder. ESI-MS (m/e): 449 $[M+H]^+$. $[\alpha]_D^{20}$ = -45.0 (c = 0.5, CH_3OH). ^1H NMR (300 MHz, DMSO-*d6*): δ/ppm = 10.89 (s, 1 H), 8.03 (s, 1 H), 7.85 (s, 1 H), 7.56 (d, *J* = 9.0 Hz, 1 H), 7.35 (m, 6 H), 7.19 (t, *J* = 6.0 Hz, 1 H), 7.05 (m, 2 H), 6.95 (t, *J* = 6.0 Hz, 1 H), 4.99 (s, 2 H), 4.07 (s, 1 H), 3.25 (dd, *J* = 3.0 Hz, *J* = 15.0 Hz, 1 H), 3.09 (dd, *J* = 3.0 Hz, *J* = 15.0 Hz, 1 H), 2.99 (s, 1 H), 2.91 (q, *J* = 6.0 Hz, 2 H), 1.48 (m, 2 H), 1.24 (m, 2 H), 1.16 (m, 2 H).

2.1.16 Preparing (3*S*,6*R*)-3-(4-aminobutyl)-6-(1H-indole-3-ylmethyl)-piperazine-2,5-dione (SR)

To the solution of 0.448 g (1.0 mmol) of (3S,6S)-3-(4-benzyloxycarbonylaminobutyl)-6-(1H-indole-3-ylmethyl)-piperazine-2,5-dione (**6**SR) in 10 mL methanol, 116 mg Pd/C was added. Replacing the air of the reaction bulb with H_2 and stirred at room temperature for 48 h. The Pd/C was removed by filtration. After evaporation under vacuum 0.239 g (76%) of the title compound was obtained as colorless powder. FT-MS (m/e): 315.1814 $[M+H]^+$. Mp 183-185 ºC. IR(KBr): 3223, 2926, 2862, 1670, 1456, 1327, 1099, 742 cm^{-1}; $[\alpha]_D^{20}$ = -48.9 (c = 0.28, CH_3OH). ^1H NMR (800 MHz, DMSO-*d6*): δ/ppm = 10.92 (s, 1 H), 8.04 (s, 1 H), 7.88 (s, 1 H), 7.56 (d, *J* = 9.0 Hz, 1 H), 7.31 (d, *J* = 9.0 Hz, 1 H), 7.06 (s, 1H), 7.04 (t, *J* = 9 Hz, 1 H), 6.94 (t, *J* = 9 Hz, 1 H), 4.07 (s, 1 H), 3.26 (dd, *J* = 3 Hz, *J* = 12 Hz, 1 H), 3.02 (dd, *J* = 6.0 Hz, *J* = 15 Hz, 1 H), 2.98 (m, 2 H), 2.44 (m, 2 H), 1.21 (m, 2 H), 1.09 (m, 2 H). ^{13}C NMR (200 MHz, DMSO-d_6): δ/ppm = 168.00, 167.57, 135.79, 127.48, 124.43, 120.72, 118.68, 118.27, 111.03, 108.30, 55.28, 53.10, 32.62, 31.51, 28.79, 20.69.

2.2 ^1H NMR, ^{13}C NMR and FT-MS

2.2.1 ^1H NMR and ^{13}C NMR spectra

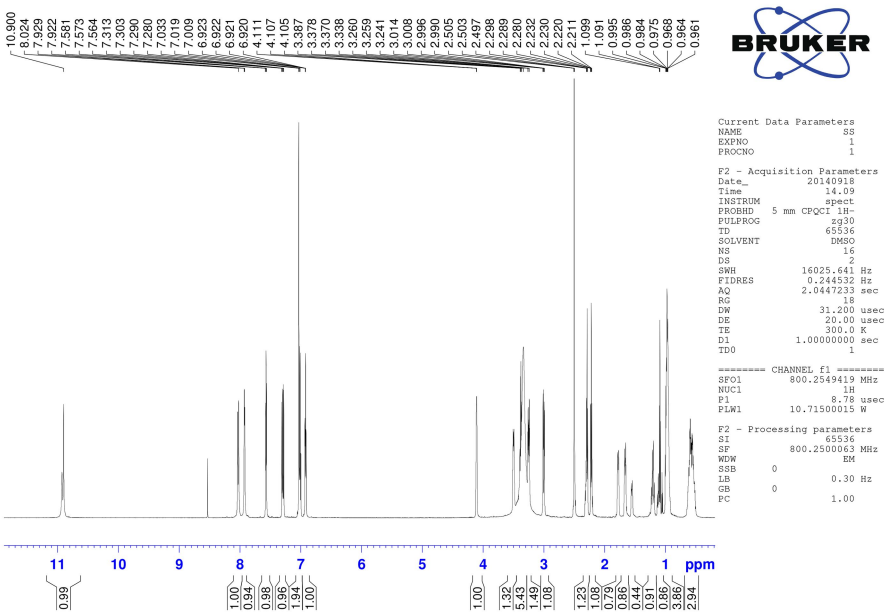

Figure S1. ^{1}H NMR spectrum of SS.

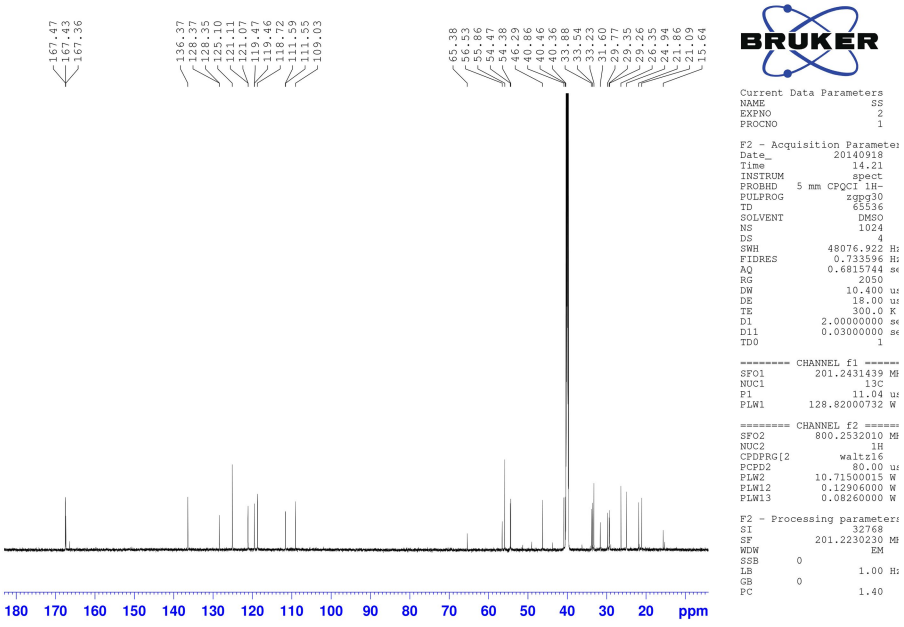

Figure S2. ^{13}C NMR spectrum of SS.

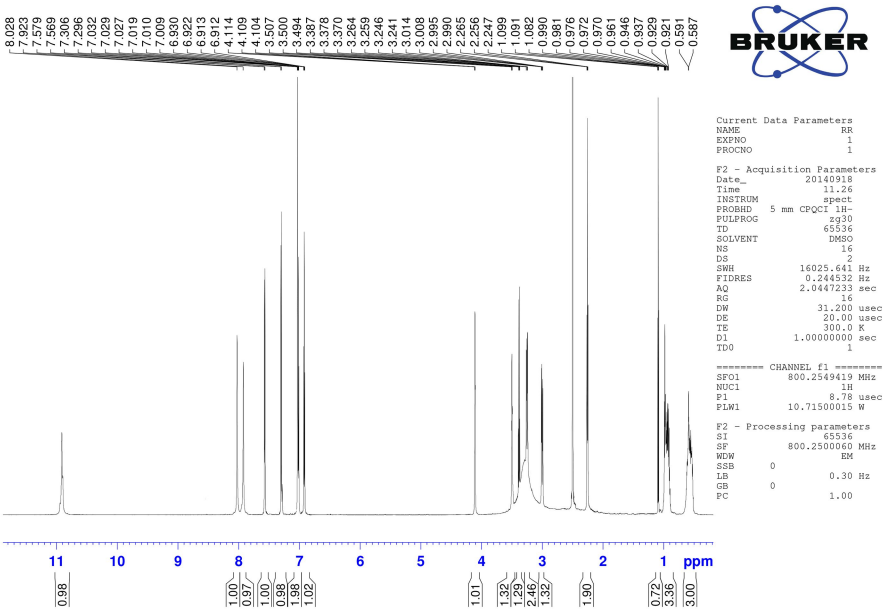

Figure S3. ^{1}H NMR spectrum of RR.

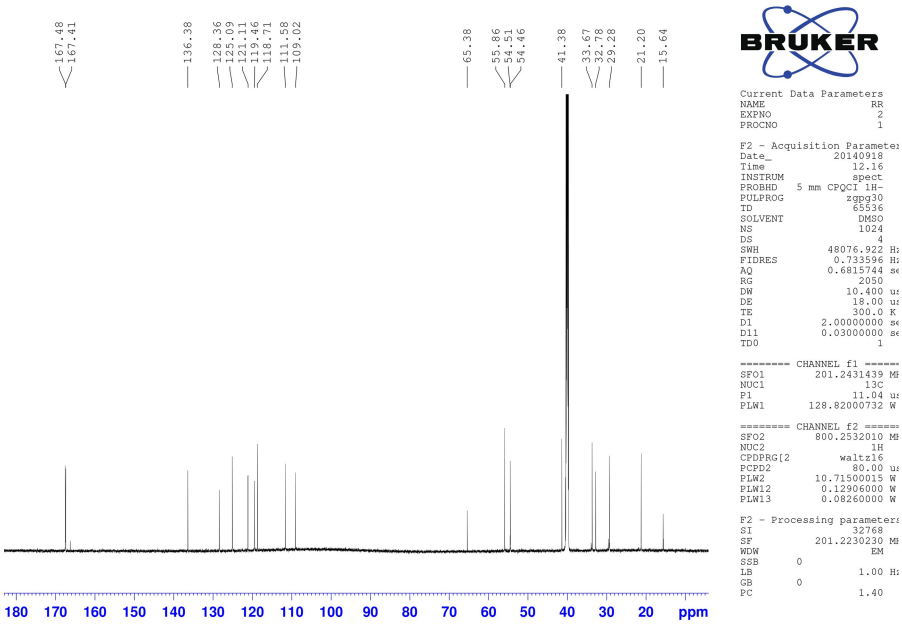

Figure S4. ^{13}C NMR spectrum of RR.

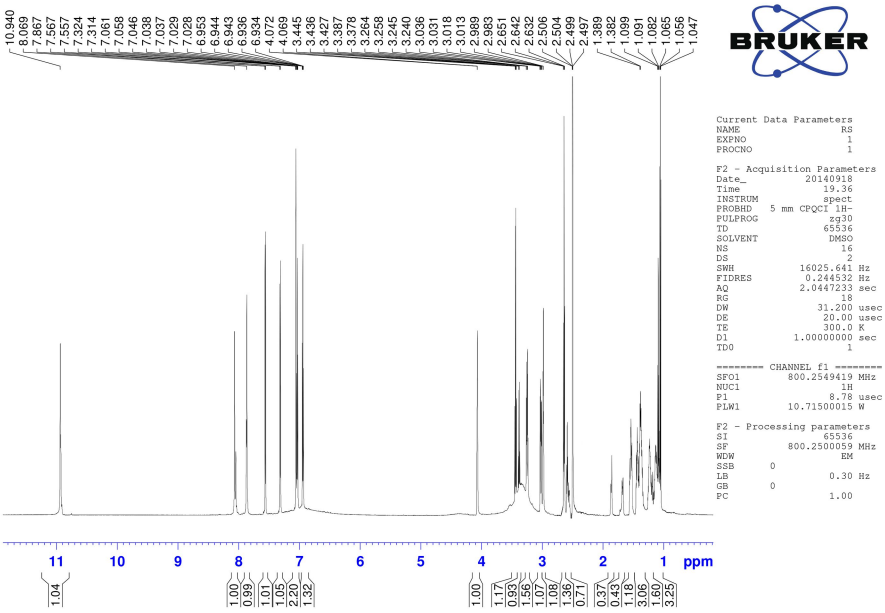

Figure S5. ^{1}H NMR spectrum of RS.

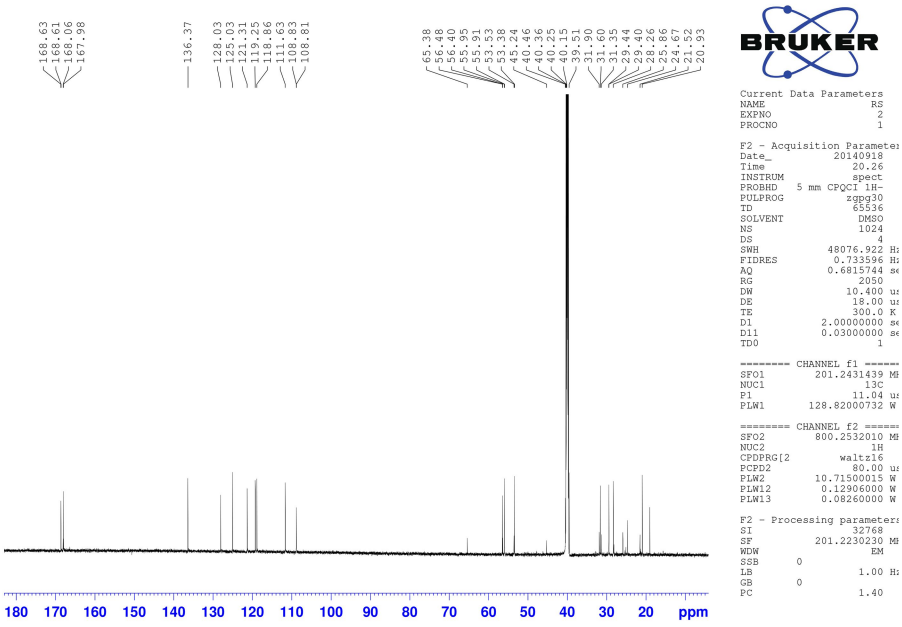

Figure S6. ^{13}C NMR spectrum of RS.

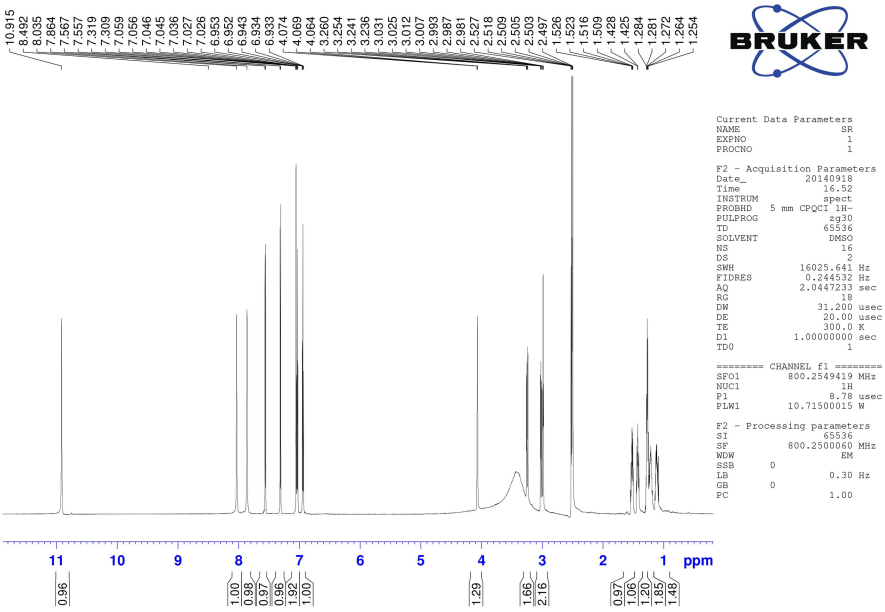

Figure S7. ^{1}H NMR spectrum of SR.

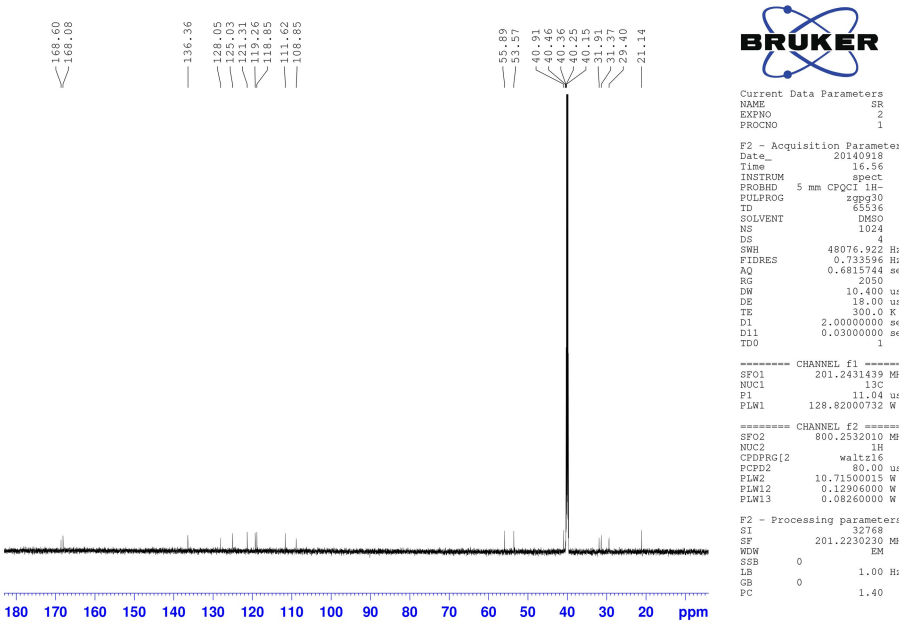

Figure S8. ^{13}C NMR spectrum of SR.

2.2.2 FT-MS spectra

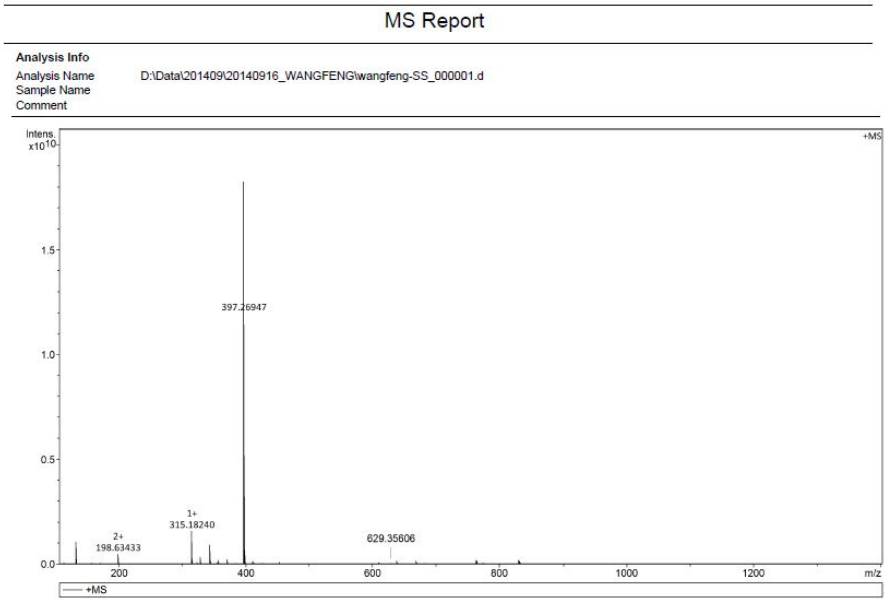

Figure S9. FT-MS spectrum of SS.

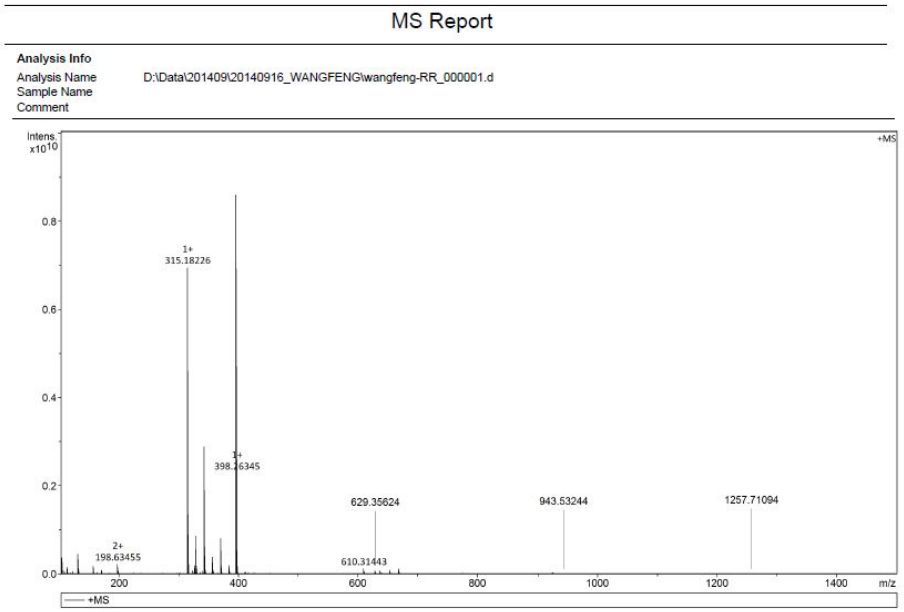

Figure S10. FT-MS spectrum of RR.

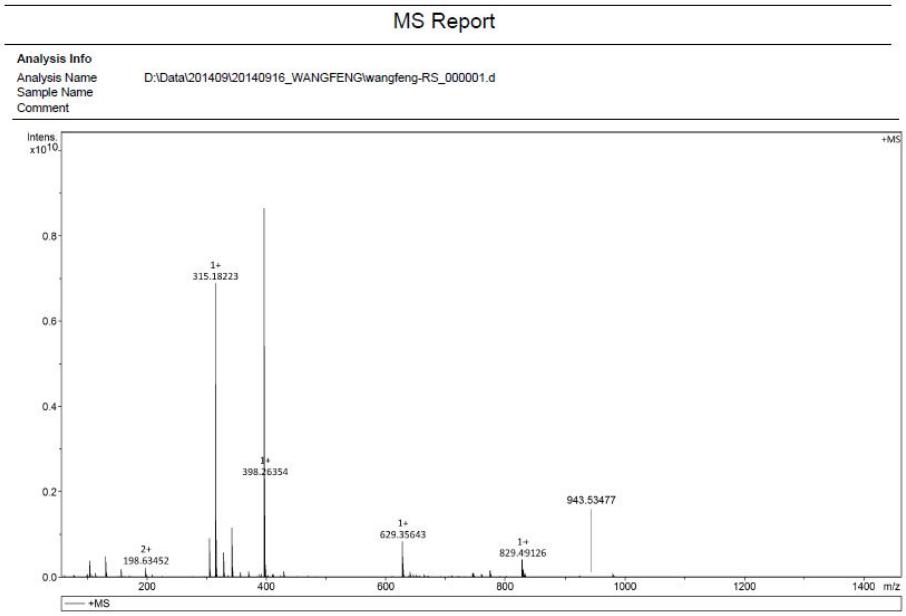

Figure S11. FT-MS spectrum of RS.

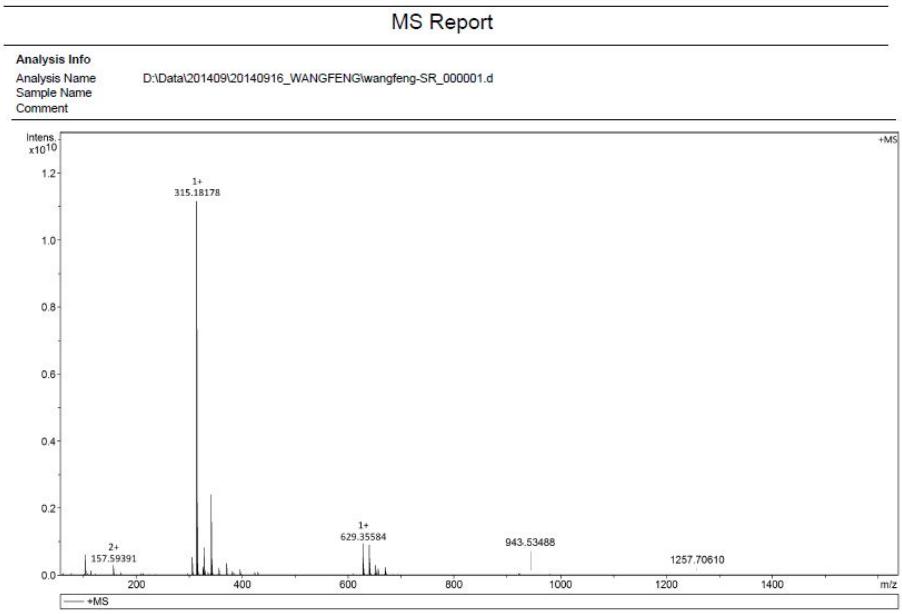

Figure S12. FT-MS spectrum of SR.

2.3 HPLC chromatogram

The purity of SS, RR, RS and SR were determined on HPLC with CHIRALPAK AH-H column (4.6 × 250 mm, Daicel Chemical IND., LTD.) and their chromatograms are shown in Figures S13 and S14. In brief, an Agilent 1100 Series HPLC system with a G1314A VWD Detector was used. The sample was separated on a CHIRALPAK AH-H column (250 mm × 4.6 mm, Daicel Chemical IND., LTD.). The column thermostat was maintained at 40 ºC. Onto the column 10 µL of RR, SS, RS or SR sample solution or 20 µL of man-made mixture of 1/o.5 of RR/SS sample solution or 20 µL of man-made mixture of 1/o.5 of SR/RS sample solution was injected for analysis. The mobile phase consisted of solvent A (chromatographically pure hexane) and solvent B (0.1% aqueous ammonia in chromatographically pure isopropanol, v/v). The flow rate was 1.2 mL/min. After each run, the column was washed with chromatographically pure methanol and equilibrated to initial conditions for 15 min. UV absorption spectra were recorded online. The VWD detector was set to a scanning wavelength of 278 nm.

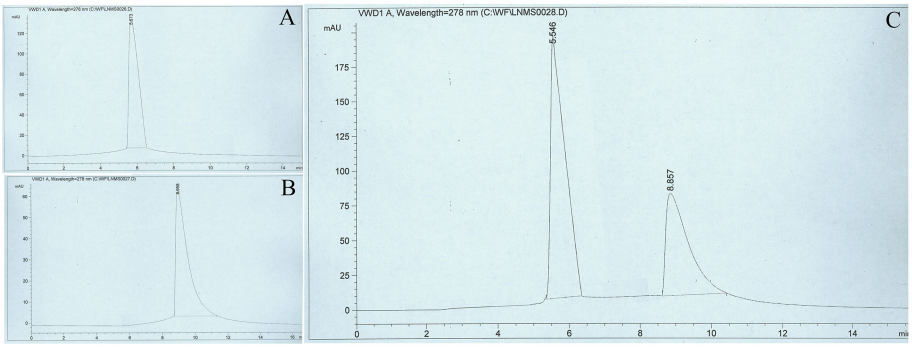

Figure S13. HPLC chromatogram of SS and RR, mobile phase is chromatographically pure hexane/isopropanol (containing 0.1% aqueous ammonia) = 70/30. A) HPLC chromatogram of the synthetic RR, purity 100%, retention time 5.673 min; B) HPLC chromatogram of the synthetic SS, purity 100%, retention time 8.958 min; C) HPLC chromatogram of a man-made mixture of 1/0.5 ratio of pure RR to pure SS, conforming the with CHIRALPAK AH-H column is capable of separating the enantiomeric RR and SS.

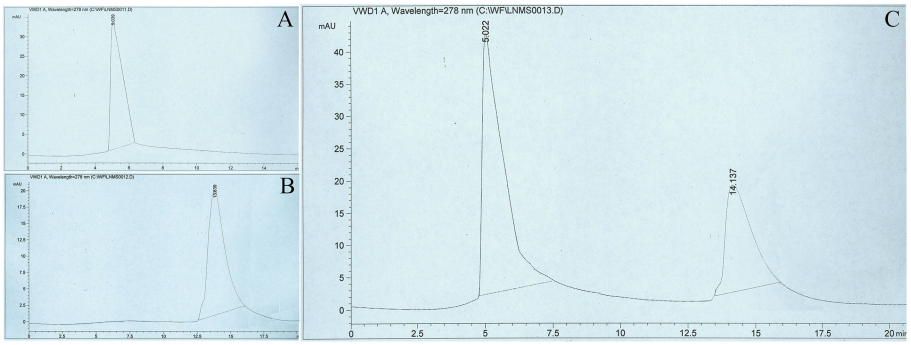

Figure S14. HPLC chromatogram of SR and RS, mobile phase is chromatographically pure hexane/isopropanol (containing 0.1% aqueous ammonia) = 65/35. A) HPLC chromatogram of the synthetic SR, purity 100%, retention time 5.039 min; B) HPLC chromatogram of the synthetic RS, purity 100%, retention time 13.839 min; C) HPLC chromatogram of a man-made mixture of 1/1 ratio of pure SR to pure RS, conforming the with CHIRALPAK AH-H column is capable of separating the enantiomeric SR and RS.

3. Measuring FT-MS spectra of 2 pairs of enantiomers of AIPZ

ESI mass spectra of four diastereomers, SS, SR, RR and RS of AIPZ in aqueous solution (10^{-6} nM) were measured on a solariX FT-ICR mass spectrometer (Bruker Daltonics, Billerica, MA, USA) consisted of an ESI/MALDI dual ion source and a 9.4 T superconductive magnet. The measurements were performed with the positive Maldi ion mode. The ion source was a Smart-beam-II laser (wavelength, 355 nm; focus setting, 'medium'; repetition rate, 1000 Hz). QCID mass was set to 2000 m/z, and the isolation window was 5 m/z. Data were collected by using solariXcontrol software. Spectral data were processed with Data Analysis software (Bruker Daltonics).

4. Measuring two-dimensional ROESY spectra of 2 pairs of enantiomers of AIPZ

One-dimensional ^{1}H NMR spectra of 10 mg of four diastereomers, SS, SR, RR and RS of AIPZ in 0.5 mL deuteron dimethyl sulfoxide (DMSO-d6) were measured on a Bruker 800MHz spectrometer. The probe temperature was regulated to 298 K. By using a simple pulse-acquire sequence zg30 the spectra were recorded. To ensure full relaxation of the ^{1}H resonances typical acquisition parameters consisted of 64 K points covering a sweep width of 16447 Hz, a pulse width (pw90) of 8.63 μs and a total repetition time of 24 s were used. Before Fourier Transformation the digital zero filling to 64 K and a 0.3 Hz exponential function were applied to the FID. The resonance at 2.5 ppm presented impurity (CD_2HSOCD_2H) in the residual solvents, and tetramethylsilane (TMS) was used as internal reference. Standard absorptive two-dimensional ^{1}H-^{1}H chemical shift correlation spectra (COSY) were tested with the same spectrometer. Each spectrum consisted of a matrix of 2 K (F2) by 0.5 K (F1) covering a sweep width of 9615.4 Hz. Before Fourier Transformation, the matrix was zero filled to 1 K by 1 K and the standard sinebell apodization functions were applied in both dimensions. Two-dimensional ROESY experiments were carried out in the phase-sensitive mode by using the same spectrometer. Each spectrum consisted of a matrix of 2 K (F2) by 1 K (F1) and covered a sweep width of 9615.4 Hz. Spectra were obtained using spin-lock mixing periods of 200 ms. Before Fourier Transformation, the matrix was zero filled to 1 K by 1 K and qsine apodization functions were applied in both dimensions.

5. Measuring TEM images of 2 pairs of enantiomers of AIPZ

Shape and size measurements of the nanospecies of four diastereomers, SS, SR, RR and RS of AIPZ were performed with transmission electron microscopy (TEM; JSM-6360 LV, JEOL, Tokyo, Japan). An aqueous solution of them (pH 6.7, 10^{-10} M) was dripped onto a formvar-coated copper grid, to which a drop of anhydrous ethanol was added to promote the removal of water. The grid was allowed to dry thoroughly in air and then was heated at 35 °C for 24 h. The copper grids were viewed under TEM. The shape and size distributions of the nanospecies were determined by counting >100 species in randomly selected regions on the copper grid. All of the determinations were carried out on triplicate grids and at 80 kV (the electron beam accelerating voltage). Images were recorded on an imaging plate (Gatan Bioscan Camera Model 1792, Pleasanton, CA, USA) with 20 eV energy windows at 6000 - 400,000× and were digitally enlarged.

6. Measuring SEM images of 2 pairs of enantiomers of AIPZ

The shape and size of the nanospecies of four diastereomers, SS, SR, RR and RS of AIPZ were measured by scanning electron microscopy (SEM, JEM-1230, JEOL, Tokyo, Japan) at 50 kV. The lyophilized powders were attached to a copper plate with double-sided tape (Euromedex, Strasbourg, France). The specimens were coated with 20 nm gold-palladium using a Joel JFC-1600 Auto Fine Coater. The coater was operated at 15 kV, 30 mA, and 200 mTorr (argon) for 60 s. The shape and size distributions of the nano-particles were measured by examining >100 particles in randomly selected regions on the SEM alloy. All measurements were performed on triplicate grids. Images were recorded on an imaging plate (Gatan Bioscan Camera Model1792) with 20 eV energy windows at 100 - 10,000 ×, and were digitally enlarged.

7. Energy-minimization of the conformations of 2 pairs of enantiomers of AIPZ

Four diastereomers, SS, SR, RR and RS of AIPZ were sketched in ChemDraw 10.0, converted to 3D conformation in Chem3D 10.0 and then energy minimized in Discovery Studio 3.5 with MMFF force field. Their energy-minimized conformations were utilized as the starting conformations for conformation generation. The energy- minimized conformations of the diastereomers, SS, SR, RR and RS of AIPZ were sampled in the whole conformational space via systematic search and BEST methods in Discovery Studio 3.5. Both of the systematic search and BEST methods were practiced with SMART minimizer using CHARMm force field. The energy threshold was set to 20kcal/mol at 300 K. The maximum minimization steps were set to 200 and the minimization RMS gradient was set to 0.1 Å. The maximum generated conformations were set to 255 with a RMSD cutoff 0.2 Å.

8. Molecular docking of 2 pairs of enantiomers of AIPZ

The 3D structures of four diastereomers, SS, SR, RR and RS of AIPZ and reference ligand, 4-iodobenzo[b]thiophene-2-carboxamidine, were built using a 3D-sketcher module and then energy minimized in Discovery Studio 3.5 with SMART minimizer using the CHARMm force field. The crystal structure of u-PA (PDB ID: 1C5X) was from the Protein Data Bank (PDB). The binding sphere (14.4 Å) of u-PA S1 pocket surrounding 4-iodobenzo[b] thiophene-2-carboxamidine-interacting domain was defined. The water molecules were removed, and hydrogen atoms were added under the CHARMm force field. Docking calculations were performed with LibDock module implemented in Accelrys Discovery studio 3.5, which is a high-throughput docking algorithm that positions catalyst generated ligand conformations in the protein active site based on polar and apolar interaction sites (hotspots).The results could be displayed by analyzing and scoring docked ligand poses. To find a top rank pose and measure the goodness of a docking study, LibDock Score was used as the criteria. The binding site “hotspots” were set to 100. The

conformation generation of the ligands was using the best method. The energy threshold was set to 20kcal/mol, the maximum minimization steps were set to 1000, the minimization RMS gradient was set to 0.001 Å, and the maximum generated conformations were set to 255 with a RMSD cutoff 1.0 Å.

9. Conversion of plasminogen towards plasmin

SDS-PAGE is a general technique for detecting urokinase induced conversion of plasminogen (PLG) towards plasmin, and was carried out in a vertical slab gel unit DYY-6C. The separation gel (sodium dodecyl sulfate, GE healthcare, USA) was 10 cm in high, 1.5 mm in thickness, and had a total polyacrylamide (T) concentration of 12%, with a cross-linking component (C; bisacrylamide) of 4%, and contained 10% glycerol (GE healthcare, USA). A stacking gel of ~1.2 cm height with 4% T and 3% C was used. The solution of PLG (5 mL, 5 mg/mL, Sigma, USA) or UK (2.5 mL, 400 U/mL) or four diastereomers, SS, SR, RR and RS of AIPZ (1 mL, 20 mg/mL) or EACA (1 mL, 50 mg/mL) in Ultra-pure water was prepared. To 5 μL UK solution Ultra-pure water or AIPZ or EACA was added, the mixed solution was incubated at 37 ºC for 15 min. After which 5 μL PLG solution was added, the mixed solution was incubated at 37 ºC for the next 15 min. Then mixed with 5 μL loading buffer and at 100 ºC denaturalized for 5 min. The separations of these solutions were performed at a constant current of ~100 mA, starting at 90 V (increasing to ~120 V at the end of the run), and lasting for 2 h until the bromophenol blue (as a tracer, Sigma, USA) band had reached the bottom of the gel. Gels were stained in methanol/acetic acid/water (45:10:45) with coomassie brilliant blue R250 (0.1 %, Amresco, USA) for 0.5 h. The gels were kept in methanol/acetic acid/water (10:10:80) for ~12 h for destaining.

10. HCCLM3 cell migration assay

The *in vitro* invasion assay of AIPZ affects HCCLM3 cells was performed by following the method of literature[1,2]. In brief, HCCLM3 cells (2×10^5 cells/0.1 mL DMEM) were placed onto the upper compartment and incubated with NS or RGDS (20 μM) or four diastereomers, SS, SR, RR and RS of AIPZ (1, 10 μM). The plates were incubated at 37 ºC for 48 h in a humidified atmosphere with 95% air and 5% CO_2. The cells were then fixed with PBS containing 4% formaldehyde and stained with 2% crystal violet. The cells on the upper surface of the filter were removed by wiping with a cotton swab, and the cells in the lower surface of the filter which penetrated through the matrigel were counted under a light microscope at × 200 to take the count of the cells from 9 visual fields.

11. HCCLM3 cell invasion assay

The *in vitro* invasion assay of AIPZ affects HCCLM3 cells was performed by following the method of literature[1,2]. In brief, 24-well Trans well inserts with 8-μm porosity polycarbonate filters (Millipore, Billerica, MA, USA) were re-coated with 30 μg Englebreth-Holm-Swarm sarcoma tumor extract (EHS Matrigel Basement Membrane Matrix) at 25 ºC for 1 h to form a genuine reconstituted basement membrane. HCCLM3 cells (2×10^4 cells/0.1 mL DMEM) were placed onto the upper compartment and incubated with NS or RGDS (20 μM) or four diastereomers, SS, SR, RR and RS of AIPZ (1, 10 μM). The plates were incubated at 37 ºC for 48 h in a humidified atmosphere with 95% air and 5% CO_2. The cells were then fixed with PBS containing 4% formaldehyde and stained with 2% crystal violet. The cells on the upper surface of the filter were removed by wiping with a cotton swab, and the cells in the lower surface of the filter which penetrated through the matrigel were counted under a light microscope at × 200 to take the count of the cells from 9 visual fields.

12. *In vivo* anti-tumor assay

Male ICR mice were purchased from the Animal Center of Peking University. Work performed was based on a protocol reviewed and approved by the ethics committee of Capital Medical University. The committee assures that the welfare of the animals was maintained in accordance to the requirements of the Animal Welfare Act and in accordance to the NIH Guide for Care and Use of Laboratory Animals. Statistical analyses of all the biological data were carried out by use of ANOVA. P-values <0.05 were considered statistically significant. Male ICR mice were maintained at 21 °C with a natural day/night cycle in a conventional animal colony. Mice were 10 weeks old at the beginning of the assay. S180 ascites tumor cells were subcutaneously injected to form solid tumors. To initiate subcutaneous tumors, cells obtained in ascitic form from tumor-bearing mice were serially transplanted once per week. Subcutaneous tumors were implanted under the skin at the right armpit by injecting 0.2 mL NS containing 2 × 106 viable tumor cells. Twenty-four hours after implantation mice were randomly divided into treatment groups (15 per group) and treated with oral compound (5 μmol/kg) or intraperitoneal injection of doxorubicin (2 μmol/kg, positive control) or oral NS only (vehicle, negative control) every day for 10 days. Twenty-four hours after the last administration, mice were weighed, sacrificed by ether anesthesia, and dissected to immediately obtain and weigh the tumors.

13. *In vivo* anti-inflammation assay

Male ICR mice were purchased from the Animal Center of Peking University. Work performed was based on a protocol reviewed and approved by the ethics committee of Capital Medical University. The committee assures that the welfare of the animals was maintained in accordance to the requirements of the Animal Welfare Act and in accordance to the NIH Guide for Care and Use of Laboratory Animals. Statistical analyses of all the biological data were carried out by use of ANOVA. P-values <0.05 were considered statistically significant. Male ICR mice were maintained at 21 °C with a natural day/night cycle in a conventional animal colony. Mice were 10 weeks old at the beginning of the assay and were randomly divided into groups (10 per group) and orally treated with RR (5 μmol/kg) or aspirin (1.11 mmol/kg as a positive control) or NS (vehicle, blank control). Thirty minutes after dosage, 0.03 mL xylene was applied to both the anterior and posterior surfaces of the right ear. The left ear was used as a control. Two hours after xylene application, the mice were anesthetized with ether to sample the orbital blood, and sacrificed for removal of both ears. Several circular punches were taken (using a cork borer with a 7 mm diameter) and weighed. The increase in weight caused by the irritant was determined by subtracting the weight of the untreated left ear punch from the weight of the treated right ear punch.

14. *In vivo* anti-LLC growth and metastasis assay

Male C57BL/6 mice were purchased from the Animal Center of Peking University. Work performed was based on a protocol reviewed and approved by the ethics committee of Capital Medical University. The committee assures that the welfare of the animals was maintained in accordance to the requirements of the Animal Welfare Act and in accordance to the NIH Guide for Care and Use of Laboratory Animals. Statistical analyses of all the biological data were carried out by use of ANOVA. P-values <0.05 were considered statistically significant. Male C57BL/6 mice were maintained at 21 °C with a natural day/night cycle in a conventional animal colony. Mice were 10 weeks old at the beginning of the assay. LLC cells were subcutaneously injected to form solid tumors. To initiate subcutaneous tumors, cells obtained in homogenates of sarcoma form from tumor-bearing mice were serially transplanted once per 2 weeks. Subcutaneous tumors were implanted under the skin at the right armpit by injecting 0.2 mL NS containing 1×10^7 viable tumor cells. 10 days after implantation mice were randomly divided into treatment groups

(10 per group) and treated with oral compound (5 μmol/kg) or oral NS only (vehicle, negative control) every day for 11 days. Mice were weighed daily and the tumor volume was measured every 3 days. Twenty-four hours after the last administration, the tumor volume was measured and calculated with $L \times W \times W/2^3$, mice were weighed, sacrificed by ether anesthesia, and dissected to immediately obtain and weigh the tumors, the lungs were also removed and visually examined for the occurrence of tumor metastasis and the numbers of metastatic tumor nodules.

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