Supplementary Information

A class of novel AA-Trp-Trp-OBzl: Synthesis, *in vitro* anti-proliferation, *in vivo* anti-tumor action, and intercalation mechanism

Liu Liu, Lei Wei, Yifan Yang, Ming Zhao,* Xiaoyi Zhang, Meiqing Zheng, Yuji Wang, Li Li, Shiqi Peng*

College of Pharmaceutical Sciences, Capital Medical University, Beijing 100069, PR China

*To whom correspondence should be addressed. SP: College of Pharmaceutical Sciences, Capital University of Medical Sciences, Beijing 100069, P.R. China. Tel: 86-10-8391-1528, Fax: 86-10-8391-1528, E-mail: sqpeng@mail.bjmu.edu.cn; MZ: Tel.: +86-10-8280-2482; fax: +86-10- 8280-2482

Experimental

1. Chemistry

All chemicals were purchased from commercial suppliers and were purified when necessary. Protected amino acids with *L*-configuration were purchased from sigma chemical Co. Chromatography was performed on Qingdao silica gel H. The purities (>97%) of the intermediates and the products were measured by TLC analysis (Merck silica gel plates of type 60 F_{254}, 0.25 mm layer thickness) and HPLC analysis (waters, C_{18} column, 4.6 × 150 mm, column temperature was 22 °C, mobile phase was water/methanol). Melting points were determined in capillary tubes on an electrothermal SM/XMP apparatus and without correction. UV spectra were measured on Shimadzu UV 2550. ESI-MS was determined by Micromass Quattro micro TM API, Waters Co. ^{1}HNMR (500 Hz) and ^{13}C NMR (125 Hz) spectra were acquired on a Bruker AC 300 spectrometer in $CDCl_3$ or in DMSO-d_6 with TMS as internal standard, and chemical shifts are expressed in ppm. Optical rotations were determined with a Jasco P-1020 Polarimeter. Statistical analysis of all the biological data was carried out by use of ANOVA test, $p<0.05$ is considered significant.

Boc-Trp-Trp-OBzl (1)

At 0 ºC, 1.87 g (6.6 mmol) of 1-hydroxybenzotriazole (HOBt) and 1.48 g (7.2 mmol) of dicyclohexylcarbodiimide (DCC) were added to a solution of 2.84 g (7.2 mmol) of Boc-Trp and 2.17 g (6.6 mmol) of HCl·Trp-OBzl in 40 ml of anhydrous THF. At 0 ºC the reaction mixture was adjusted pH 8 with 1.44 ml (6.6 mmol) of

NMM and then stirred for 24 h. The precipitated DCU was removed by filtration. The filtrate was evaporated under reduced pressure, and the residue was dissolved in 80 ml of EtOAc. The solution was washed successively with saturated aqueous $NaHCO_3$, aqueous $KHSO_4$ (5%) and saturated aqueous NaCl. The organic phase was dried over anhydrous Na_2SO_4. After filtration and evaporation under reduced pressure, the residue was purified by column chromatography eluting with $CHCl_3$:CH_3OH (30:1) to provide 3.68 g (96%) of the title compound as colorless powder. Mp 189 - 191 ºC, ESI/MS (m/e) 581 $[M + H]^+$.

Trp-Trp-OBzl (2)

To 2.0 g (3.44 mmol) of Boc-Trp-Trp-OBzl 16 ml of 4 M solution of hydrochloride in ethyl acetate was added. The reaction mixture was stirred at room temperature for 60 min and TLC (chloroform/methanol, 5:1) indicated the disappearance of Boc-Ala-Trp-Trp-OBzl. The reaction mixture was evaporated under reduced pressure, the residue was dissolved in 40 ml of ethyl acetate, the solution was again evaporated under reduced pressure and the residue was washed with anhydrous ether to provide 1.74 g (98%) of the title compound as colorless powder. Mp. 197 - 199 ºC; ESI/MS (m/e) 481 $[M + H]^+$.

Boc-Ala-Trp-Trp-OBzl (3a)

Using the same procedure as described for Boc-Trp-Trp-OBzl from 517 mg (1.0 mmol) of Trp-Trp-OBzl and 208 mg (1.1 mmol) of Boc-Ala 641 mg (98%) of the title compound was obtained as as colorless powder. Mp 202 - 204 ºC, ESI/MS(m/e) 652

$[M + H]^+$.

Boc-Gly-Trp-Trp-OBzl (3b)

Using the same procedure as described for Boc-Trp-Trp-OBzl from 517 mg (1.0 mmol) of Trp-Trp-OBzl and 193 mg (1.1 mmol) of Boc-Gly 414 mg (65%) of the title compound was obtained as as colorless powder. Mp 206 - 208 ºC, ESI/MS(m/e) 638 $[M + H]^+$.

Boc-Phe-Trp-Trp-OBzl (3c)

Using the same procedure as described for Boc-Trp-Trp-OBzl from 517 mg (1.0 mmol) of Trp-Trp-OBzl and 292 mg (1.1 mmol) of Boc-Phe 682 mg (94%) of the title compound was obtained as as colorless powder. Mp 200 - 202 ºC, ESI/MS(m/e) 728 $[M + H]^+$.

Boc-Leu-Trp-Trp-OBzl (3d)

Using the same procedure as described for Boc-Trp-Trp-OBzl from 517 mg (1.0 mmol) of Trp-Trp-OBzl and 254 mg (1.1 mmol) of Boc-Leu 675 mg (97%) of the title compound was obtained as as colorless powder. Mp 206 - 208 ºC, ESI/MS(m/e) 694 $[M + H]^+$.

Boc-Ile-Trp-Trp-OBzl (3e)

Using the same procedure as described for Boc-Trp-Trp-OBzl from 517 mg (1.0 mmol) of Trp-Trp-OBzl and 254 mg (1.1 mmol) of Boc-Ile 687 mg (99%) of the title compound was obtained as as colorless powder. Mp 212 - 214 ºC, ESI/MS(m/e) 694

$[M + H]^+$.

Boc-Val-Trp-Trp-OBzl (3f)

Using the same procedure as described for Boc-Trp-Trp-OBzl from 517 mg (1.0 mmol) of Trp-Trp-OBzl and 239 mg (1.1 mmol) of Boc-Val 642 mg (95%) of the title compound was obtained as as colorless powder. Mp 210 - 212 ºC, ESI/MS(m/e) 680 $[M + H]^+$.

Boc-Ser-Trp-Trp-OBzl (3g)

Using the same procedure as described for Boc-Trp-Trp-OBzl from 517 mg (1.0 mmol) of Trp-Trp-OBzl and 226 mg (1.1 mmol) of Boc-Ser 563 mg (84%) of the title compound was obtained as as colorless powder. Mp 195 - 197 ºC, ESI/MS(m/e) 668 $[M + H]^+$.

Boc-Thr-Trp-Trp-OBzl (3h)

Using the same procedure as described for Boc-Trp-Trp-OBzl from 517 mg (1.0 mmol) of Trp-Trp-OBzl and 241 mg (1.1 mmol) of Boc-Thr 580 mg (85%) of the title compound was obtained as as colorless powder. Mp 190 - 192 ºC, ESI/MS(m/e) 682 $[M + H]^+$.

Boc-Tyr-Trp-Trp-OBzl (3i)

Using the same procedure as described for Boc-Trp-Trp-OBzl from 517 mg (1.0 mmol) of Trp-Trp-OBzl and 309 mg (1.1 mmol) of Boc-Tyr 708 mg (95%) of the title

compound was obtained as as colorless powder. Mp 220 - 222 ºC, ESI/MS(m/e) 744 $[M + H]^+$.

Boc-Pro-Trp-Trp-OBzl (3j)

Using the same procedure as described for Boc-Trp-Trp-OBzl from 517 mg (1.0 mmol) of Trp-Trp-OBzl and 237 mg (1.1 mmol) of Boc-Pro 654 mg (97%) of the title compound was obtained as as colorless powder. Mp 182 - 184 ºC, ESI/MS(m/e) 678 $[M + H]^+$.

Boc-Met-Trp-Trp-OBzl (3k)

Using the same procedure as described for Boc-Trp-Trp-OBzl from 517 mg (1.0 mmol) of Trp-Trp-OBzl and 274 mg (1.1 mmol) of Boc-Met 692 mg (97%) of the title compound was obtained as as colorless powder. Mp 218 - 220 ºC, ESI/MS(m/e) 712 $[M + H]^+$.

Boc-Glu(OBzl)-Trp-Trp-OBzl (3l)

Using the same procedure as described for Boc-Trp-Trp-OBzl from 517 mg (1.0 mmol) of Trp-Trp-OBzl and 370 mg (1.1 mmol) of Boc-Glu(OBzl) 469 mg (59%) of the title compound was obtained as as colorless powder. Mp 202 - 204 ºC, ESI/MS (m/e) 800 $[M + H]^+$.

Boc-Asp(OBzl)-Trp-Trp-OBzl (3m)

Using the same procedure as described for Boc-Trp-Trp-OBzl from 517 mg (1.0 mmol) of Trp-Trp-OBzl and 355 mg (1.1 mmol) of Boc-Asp(OBzl) 473 mg (60%) of

the title compound was obtained as as colorless powder. Mp 208 - 210 ºC, ESI/MS (m/e) 786 $[M + H]^+$.

Boc-Cys(4-OMeBzl)-Trp-Trp-OBzl (3n)

Using the same procedure as described for Boc-Trp-Trp-OBzl from 517 mg (1.0 mmol) of Trp-Trp-OBzl and 375 mg (1.1 mmol) of Boc-Cys(4-OMeBzl) 801 mg (100%) of the title compound was obtained as as colorless powder. Mp 221 - 223 ºC, ESI/MS (m/e) 804 $[M + H]^+$.

Boc-Arg(NO_2)-Trp-Trp-OBzl (3o)

Using the same procedure as described for Boc-Trp-Trp-OBzl from 517 mg (1.0 mmol) of Trp-Trp-OBzl and 351 mg (1.1 mmol) of Boc-Arg(NO_2) 317 mg (41%) of the title compound was obtained as as colorless powder. Mp 207 - 209 ºC, ESI/MS (m/e) 782 $[M + H]^+$.

Boc-Lys(Z)-Trp-Trp-OBzl (3p)

Using the same procedure as described for Boc-Trp-Trp-OBzl from 517 mg (1.0 mmol) of Trp-Trp-OBzl and 418 mg (1.1 mmol) of Boc-Lys(Z) 829 mg (98%) of the title compound was obtained as as colorless powder. Mp 201 - 203 ºC, ESI/MS (m/e) 843 $[M + H]^+$.

Boc-Gln-Trp-Trp-OBzl (3q)

Using the same procedure as described for Boc-Trp-Trp-OBzl from 517 mg (1.0

mmol) of Trp-Trp-OBzl and 271 mg (1.1 mmol) of Boc-Gln 469 mg (66%) of the title compound was obtained as as colorless powder. Mp 195 - 197 ºC, ESI/MS (m/e) 709 $[M + H]^+$.

Boc-Asn-Trp-Trp-OBzl (3r)

Using the same procedure as described for Boc-Trp-Trp-OBzl from 517 mg (1.0 mmol) of Trp-Trp-OBzl and 255 mg (1.1 mmol) of Boc-Asn 633 mg (91%) of the title compound was obtained as as colorless powder. Mp 190 - 192 ºC, ESI/MS (m/e) 695 $[M + H]^+$.

Boc-His(Boc)-Trp-Trp-OBzl (3s)

Using the same procedure as described for Boc-Trp-Trp-OBzl from 517 mg (1.0 mmol) of Trp-Trp-OBzl and 391 mg (1.1 mmol) of Boc-His(Boc) 556 mg (68%) of the title compound was obtained as as colorless powder. Mp 189 - 191 ºC, ESI/MS (m/e) 818 $[M + H]^+$.

Boc-Trp-Trp-Trp-OBzl (3t)

Using the same procedure as described for Boc-Trp-Trp-OBzl from 517 mg (1.0 mmol) of Trp-Trp-OBzl and 334 mg (1.1 mmol) of Boc-Trp 664 mg (87%) of the title compound was obtained as as colorless powder. Mp 180 - 182 ºC, ESI/MS (m/e) 767 $[M + H]^+$.

Ala-Trp-Trp-OBzl (4a)

To 500 mg (0.78 mmol) of Boc-Ala-Trp-Trp-OBzl 7 ml of 4 M solution of

hydrochloride in ethyl acetate was added. The reaction mixture was stirred at room temperature for 60 min and TLC (chloroform/methanol, 5:1) indicated the disappearance of Boc-Ala-Trp-Trp-OBzl. The reaction mixture was evaporated under reduced pressure, the residue was dissolved in 40 ml of ethyl acetate, the solution was again evaporated under reduced pressure and the residue was washed with anhydrous ether to provide 449 mg (98%) of the title compound as colorless powder. Mp 205-207 ºC; ESI/MS (m/e) 552 $[M+H]^+$; $[\alpha]^{20}_D$ = 21.15 (*c* 1.05, CH_3OH); ^1H NMR (500 MHz, DMSO-d_6) δ/ppm = 10.89 (m, 2 H), 8.69 (d, *J* = 7.3 Hz, 1 H), 8.59 (d, *J* = 8.1 Hz, 1 H), 7.65 (d, *J* = 7.8 Hz, 1 H), 7.50 (d, *J* = 7.8 Hz, 1 H), 7.15 (m, 13 H), 5.03 (m, 2 H), 4.68 (m, 2 H), 3.77 (m, 1 H), 3.09 (m, 4 H), 2.01 (s, 2 H), 1.32 (m, 3 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ/ppm = 172.7, 142.3, 136.6, 130.1, 127.7, 127.5, 127.2, 122.9, 122.2, 120.1, 119.2, 119.0, 111.3, 111.1, 110.9, 68.6, 54.6, 49.8, 31.3, 30.8, 20.6. Anal. Calcd for $C_{32}H_{33}N_5O_4$: C, 69.67; H, 6.03; N, 12.70. Found: C, 69.48; H, 6.18; N, 12.92.

Gly-Trp-Trp-OBzl (4b)

Using the same procedure as described for Ala-Trp-Trp-OBzl from 500 mg (0.79 mmol) of Boc-Gly-Trp-Trp-OBzl 446 mg (99%) of the title compound was obtained as as colorless powder. Mp 201-203 ºC; ESI/MS (m/e) 538 $[M+H]^+$; $[\alpha]^{20}_D$ = 26.06 (*c* 1.21, CH_3OH); ^1H NMR (500 MHz, DMSO-d_6) δ/ppm = 10.97 (s, 1 H), 10.89 (s, 1 H), 8.81 (d, *J* = 7.3 Hz, 1 H), 8.66 (d, *J* = 8.3 Hz, 1 H), 7.63 (d, *J* = 7.9 Hz, 1 H), 7.51 (d, *J* = 7.9 Hz, 1 H), 7.18 (m, 13 H), 5.06 (m, 2 H), 3.68 (m, 2 H), 3.15 (m, 6 H), 1.99 (s,

2 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ/ppm = 172.5, 142.0, 136.5, 130.1, 127.7, 127.5, 127.2, 122.9, 122.2, 120.1, 119.2, 119.0, 111.3, 111.1, 110.9, 68.3, 54.8, 49.7, 43.3, 31.5, 30.8. Anal. Calcd for $C_{31}H_{31}N_5O_4$: C, 69.26; H, 5.81; N, 13.30. Found: C, 69.05; H, 5.68; N, 13.09.

Phe-Trp-Trp-OBzl (4c)

Using the same procedure as described for Ala-Trp-Trp-OBzl from 500 mg (0.69 mmol) of Boc-Phe-Trp-Trp-OBzl 432 mg (98%) of the title compound was obtained as as colorless powder. Mp 199-201 ºC; ESI/MS (m/e) 628 $[M+H]^+$; $[\alpha]^{20}_D$ = 88.67 (*c* 1.32, CH_3OH); ^{1}H NMR (500 MHz, DMSO-d_6) δ/ppm = 10.96 (m, 2 H), 8.97 (d, *J* = 8.0 Hz, 1 H), 8.79 (d, *J* = 7.2 Hz, 1 H), 7.64 (d, *J* = 7.9 Hz, 1 H), 7.52 (d, *J* = 11.4 Hz, 1 H), 7.18 (m, 18 H), 5.03 (m, 2 H), 4.68 (m, 2 H), 3.41 (m, 1 H), 3.09 (m, 6 H), 2.00 (s, 2 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ/ppm = 173.1, 142.4, 139.5, 136.7, 130.1, 128.8, 127.7, 127.5, 127.2, 126.1, 122.9, 122.2, 120.1, 119.2, 119.0, 111.3, 111.1, 110.9, 68.7, 54.6, 53.7, 40.4, 31.2, 30.7. Anal. Calcd for $C_{38}H_{37}N_5O_4$: C, 72.71; H, 5.94; N, 11.16. Found: C, 72.50; H, 5.81; N, 11.38.

Leu-Trp-Trp-OBzl (4d)

Using the same procedure as described for Ala-Trp-Trp-OBzl from 500 mg (0.69 mmol) of Boc-Leu-Trp-Trp-OBzl 450 mg (99%) of the title compound was obtained as as colorless powder. Mp 222-224 ºC; ESI/MS (m/e) 594 $[M+H]^+$; $[\alpha]^{20}_D$ = 12.43 (*c* 1.05, CH_3OH); ^{1}H NMR (500 MHz, DMSO-d_6) δ/ppm = 10.91 (m, 2 H), 8.75 (d, *J* =

8.1 Hz, 1 H), 8.70 (d, *J* = 7.2 Hz, 1 H), 7.63 (d, *J* = 7.9 Hz, 1 H), 7.49 (d, *J* = 7.9 Hz, 1 H), 7.15 (m, 13 H), 6.83 (s, 2 H), 5.02 (m, 2 H), 4.63 (m, 2 H), 3.75 (m, 1 H), 3.15 (m, 4 H), 1.98 (s, 2H), 1.59 (m, 1 H), 0.89 (m, 6 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ/ppm = 172.9, 142.0, 136.7, 130.2, 127.7, 127.5, 127.2, 122.9, 122.2, 120.1, 119.2, 119.0, 111.3, 111.1, 110.9, 68.5, 54.8, 51.3, 43.9, 31.5, 30.7, 22.7, 22.2. Anal. Calcd for $C_{35}H_{39}N_5O_4$: C, 70.80; H, 6.62; N, 11.80. Found: C, 70.99; H, 6.78; N, 11.59.

Ile-Trp-Trp-OBzl (4e)

Using the same procedure as described for Ala-Trp-Trp-OBzl from 500 mg (0.72 mmol) of Boc-Ile-Trp-Trp-OBzl 450 mg (99%) of the title compound was obtained as as colorless powder. Mp 228-230 ºC; ESI/MS (m/e) 594 $[M+H]^+$; $[\alpha]^{20}_D$ = 35.28 (*c*= 1.15, CH_3OH); ^{1}H NMR (500 MHz, DMSO-d_6) δ/ppm = 10.97 (m, 2 H), 8.71 (d, *J* = 7.2 Hz, 1 H), 8.64 (d, *J* = 8.0 Hz, 1 H), 7.24 (m, 13 H), 6.81 (s, 2 H), 5.03 (m, 2 H), 4.67 (m, 2 H), 3.46 (m, 1 H), 3.11 (m, 4 H), 1.96 (s, 2 H), 1.25 (m, 1 H), 1.07 (m, 2 H), 0.82 (m, 6 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ/ppm = 172.8, 142.2, 136.7, 130.1, 127.7, 127.5, 127.2, 122.9, 122.2, 120.1, 119.2, 119.0, 111.3, 111.1, 110.9, 68.8, 57.3, 54.7, 39.5, 31.3, 30.6, 24.8, 14.6, 10.8. Anal. Calcd for $C_{35}H_{39}N_5O_4$: C, 70.80; H, 6.62; N, 11.80. Found: C, 70.61; H, 6.47; N, 11.58

Val-Trp-Trp-OBzl (4f)

Using the same procedure as described for Ala-Trp-Trp-OBzl from 500 mg (0.74 mmol) of Boc-Val-Trp-Trp-OBzl 440 mg (97%) of the title compound was obtained

as as colorless powder. Mp 200-202 ºC; ESI/MS (m/e) 580 $[M+H]^+$; $[\alpha]^{20}_D$ = 71.90 (*c* 1.31, CH_3OH); ^1H NMR (500 MHz, DMSO-d_6) δ/ppm = 10.89 (m, 2 H), 8.71 (d, *J* = 7.3 Hz, 1 H), 8.57 (d, *J* = 7.9 Hz, 1 H), 8.64 (d, *J* = 7.9 Hz, 1 H), 7.48 (d, *J* = 7.9 Hz, 1 H), 7.18 (m, 13 H), 5.04 (m, 2 H), 4.72 (dd, *J* = 5.4 Hz, *J* = 13.5 Hz, 1 H), 4.63 (dd, *J* = 7.1 Hz, *J* = 14.3 Hz, 1 H), 3.60 (m, 1 H), 3.15 (m, 4 H), 2.08 (m, 1 H), 1.88 (s, 2 H), 0.90 (m, 6 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ/ppm = 172.7, 142.1, 136.7, 130.1, 127.7, 127.5, 127.2, 122.9, 122.2, 120.1, 119.2, 119.0, 111.3, 111.1, 110.9, 68.5, 59.7, 54.7, 33.9, 31.3, 30.7, 17.3. Anal. Calcd for $C_{34}H_{37}N_5O_4$: C, 70.45; H, 6.34; N,12.08. Found: C, 70.66; H, 6.20; N, 11.86.

Ser-Trp-Trp-OBzl (4g)

Using the same procedure as described for Ala-Trp-Trp-OBzl from 500 mg (0.75 mmol) of Boc-Ser-Trp-Trp-OBzl 418 mg (92%) of the title compound was obtained as as colorless powder. Mp 218-220 ºC; ESI/MS (m/e) 568 $[M+H]^+$; $[\alpha]^{20}_D$ = 21.39 (*c* 1.11, CH_3OH); ^1H NMR (500 MHz, DMSO-d_6) δ/ppm = 10.97 (m, 2 H), 8.70 (m, 2 H), 7.28 (m, 15 H), 5.08 (m, 2 H), 4.63 (m, 2 H), 4.09 (m, 2 H), 3.45 (m, 1 H), 3.14 (m, 4 H), 2.07 (s, 2 H), 1.95 (s, 1 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ/ppm = 173.2, 142.3, 136.7, 130.1, 127.7, 127.5, 127.2, 122.9, 122.2, 120.1, 119.2, 119.0, 111.3, 111.1, 110.9, 68.5, 64.3, 56.4, 54.7, 31.3, 30.5. Anal. Calcd for $C_{32}H_{33}N_5O_5$: C, 67.71; H, 5.86; N, 12.34. Found: C, 67.50; H, 5.71; N, 12.11.

Thr-Trp-Trp-OBzl (4h)

Using the same procedure as described for Ala-Trp-Trp-OBzl from 500 mg (0.73 mmol) of Boc-Thr-Trp-Trp-OBzl 429 mg (95%) of the title compound was obtained as as colorless powder. Mp 195-197 ºC; ESI/MS (m/e) 582 $[M+H]^+$; $[\alpha]^{20}_D$ = 16.15 (*c* 1.13, CH_3OH); ^1H NMR (500 MHz, DMSO-d_6) δ/ppm = 10.95 (m, 2 H), 7.31 (m, 15 H), 6.78 (s, 2 H), 5.06 (m, 2 H), 4.65 (m, 2 H), 3.63 (m, 1 H), 3.39 (d, *J* = 8.2 Hz, 1 H), 3.13 (m, 4 H), 1.96 (s, 2 H), 1.15 (m, 3 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ/ppm = 172.8, 142.3, 136.7, 130.1, 127.7, 127.5, 127.2, 122.9, 122.2, 120.1, 119.2, 119.0, 111.3, 111.1, 110.9, 71.0, 68.8, 66.0, 54.6, 31.3, 30.5, 19.2. Anal. Calcd for $C_{33}H_{35}N_5O_5$: C, 68.14; H, 6.07; N, 12.04. Found: C, 68.33; H, 6.22; N, 12.25.

Tyr-Trp-Trp-OBzl (4i)

Using the same procedure as described for Ala-Trp-Trp-OBzl from 500 mg (0.67 mmol) of Boc-Tyr-Trp-Trp-OBzl 449 mg (98%) of the title compound was obtained as as colorless powder. Mp 207-209 ºC. ESI/MS (m/e) 644 $[M+H]^+$; $[\alpha]^{20}_D$ = 11.23 (*c* = 1.25, CH_3OH); ^1H NMR (500 MHz, DMSO-d_6) δ/ppm = 10.87 (m, 2 H), 8.76 (d, *J* = 7.3 Hz, 1 H), 8.73 (d, *J* = 8.2 Hz, 1 H), 7.65 (d, *J* = 8.0 Hz, 1 H), 7.52 (d, *J* = 7.9 Hz, 1 H), 7.05 (m, 17 H), 5.89 (s, 1 H), 5.01 (m, 2 H), 4.69 (m, 2 H), 3.89 (m, 1 H), 3.04 (m, 6 H), 1.96 (s, 2 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ/ppm = 172.9, 155.8, 142.5, 136.6, 132.2, 130.1, 129.0, 127.7, 127.5, 127.2, 122.9, 122.2, 120.1, 119.2, 119.0, 115.2, 111.3, 111.1, 110.9, 68.7, 54.8, 53.3, 40.6, 31.3, 30.7. Anal. Calcd for $C_{38}H_{37}N_5O_5$: C, 70.90; H, 5.79; N, 10.88. Found: C, 71.11; H, 5.93; N, 10.69.

Pro-Trp-Trp-OBzl (4j)

Using the same procedure as described for Ala-Trp-Trp-OBzl from 500 mg (0.74 mmol) of Boc-Pro-Trp-Trp-OBzl 451 mg (99%) of the title compound was obtained as as colorless powder. Mp 182-184 ºC. ESI/MS (m/e) 578 $[M+H]^+$; $[\alpha]^{20}_D$ = 27.30 (*c* 0.89, CH_3OH); ^1H NMR (500 MHz, DMSO-d_6) δ/ppm = 10.90 (s, 1 H), 10.86 (s, 1 H), 8.72 (m, 2 H), 7.64 (d, *J* = 7.9 Hz, 1 H), 7.51 (d, *J* = 7.9Hz, 1 H), 7.17(m, 13 H), 5.05 (m, 2 H), 4.65 (m, 2 H), 4.07 (m, 1 H), 3.17 (m, 6 H), 2.01 (m, 1 H), 1.78 (m, 4 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ/ppm = 172.8, 142.0, 136.6, 130.1, 127.7, 127.5, 127.2, 122.9, 122.2, 120.1, 119.2, 119.0, 111.3, 111.1, 110.9, 68.7, 60.6, 54.4, 45.6, 32.2, 31.2, 30.6, 24.8. Anal. Calcd for $C_{34}H_{35}N_5O_4$: C, 70.69; H, 6.11; N, 12.12. Found: C, 70.48; H, 6.26; N, 12.35.

Met-Trp-Trp-OBzl (4k)

Using the same procedure as described for Ala-Trp-Trp-OBzl from 500 mg (0.70 mmol) of Boc-Met-Trp-Trp-OBzl 448 mg (98%) of the title compound was obtained as as colorless powder. Mp 215-217 ºC. ESI/MS (m/e) 612 $[M+H]^+$; $[\alpha]^{20}_D$ = 27.56 (*c* 1.21, CH_3OH); ^1H NMR (500 MHz, DMSO-d_6) δ/ppm = 10.99 (m, 2 H), 8.78 (d, *J* = 7.3 Hz, 1 H), 8.73 (d, *J* = 7.9 Hz, 1 H), 7.74 (d, *J* = 7.8 Hz, 1 H), 7.65 (d, *J* = 7.8 Hz, 1 H), 7.18 (m, 13 H), 5.04 (m, 2 H), 4.69 (dd, *J* = 7.3 Hz, *J* = 14.2 Hz, 1 H), 4.61 (dd, *J* = 7.3 Hz, *J* = 14.2 Hz, 1 H), 3.19 (m, 5 H), 2.01 (m, 3 H), 1.98 (s, 2 H), 1.62 (m, 4 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ/ppm = 172.8, 142.3, 136.7, 130.1, 127.7, 127.5, 127.2, 122.9, 122.2, 120.1, 119.2, 119.0, 111.3, 111.1, 110.9, 68.8, 54.6, 53.1, 34.4,

31.3, 30.7, 29.5, 17.6. Anal. Calcd for $C_{34}H_{37}N_5O_4S$: C, 66.75; H, 6.10; N, 11.45. Found: C, 66.94; H, 6.25; N, 11.67.

Glu(OBzl)-Trp-Trp-OBzl (4l)

Using the same procedure as described for Ala-Trp-Trp-OBzl from 500 mg (0.63 mmol) of Boc-Glu(OBzl)-Trp-Trp-OBzl 455 mg (99%) of the title compound was obtained as as colorless powder. Mp 214-216 ºC; ESI/MS (m/e) 700 $[M+H]^+$; $[\alpha]^{20}_D$ = 37.41 (*c* 1.12, CH_3OH); ^1H NMR (500 MHz, DMSO-d_6) δ/ppm = 10.92 (m, 2 H), 8.78 (d, *J* = 7.2 Hz, 1 H), 8.72 (d, *J* = 7.7 Hz, 1 H), 7.66 (d, *J* = 7.8 Hz, 1 H), 7.49 (d, *J* = 7.8 Hz, 1 H), 7.17 (m, 18 H), 5.02 (m, 4 H), 4.67 (m, 2 H), 3.81 (m, 1 H), 3.08 (m, 4 H), 2.56 (m, 2 H), 2.06 (m, 2 H), 1.96 (s, 2 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ/ppm = 173.2, 172.8, 142.2, 136.7, 130.1, 129.0, 127.7, 127.5, 127.2, 122.9, 122.2, 120.1, 119.2, 119.0, 111.3, 111.1, 110.9, 68.5, 54.7, 53.6, 31.3, 30.7, 29.1, 27.7. Anal. Calcd for $C_{41}H_{41}N_5O_6$: C, 70.37; H, 5.91; N, 10.01. Found: C, 70.15; H, 5.75; N, 10.23.

Asp(OBzl)-Trp-Trp-OBzl (4m)

Using the same procedure as described for Ala-Trp-Trp-OBzl from 500 mg (0.64 mmol) of Boc-Asp(OBzl)-Trp-Trp-OBzl 450 mg (98%) of the title compound was obtained as as colorless powder. Mp 210-212 ºC. ESI/MS (m/e) 686 $[M+H]^+$; $[\alpha]^{20}_D$ = 25.67 (*c* = 1.13, CH_3OH); ^1H NMR (500 MHz, DMSO-d_6) δ/ppm = 10.93 (m, 2 H), 8.76 (d, *J* = 7.9 Hz, 1 H), 7.73 (d, *J* = 7.3 Hz, 1 H), 7.31 (m, 20 H), 5.07 (m, 4 H),

4.64 (m, 2 H), 3.35 (m, 1 H), 3.15 (m, 4 H), 2.93 (m, 2 H), 1.98 (s, 2 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ/ppm = 173.4, 172.9, 142.2, 141.0, 136.7, 130.1, 129.2, 127.7, 127.5, 127.2, 122.9, 122.2, 120.1, 119.2, 119.0, 111.3, 111.1, 110.9, 68.7, 54.8, 49.6, 41.0, 31.2, 30.7. Anal. Calcd for $C_{40}H_{39}N_5O_6$: C, 70.06; H, 5.73; N, 10.21. Found: C, 70.27; H, 5.89; N, 10.00.

Cys(4-OMeBzl)-Trp-Trp-OBzl (4n)

Using the same procedure as described for Ala-Trp-Trp-OBzl from 500 mg (0.62 mmol) of Boc-Cys(4-OMeBzl)-Trp-Trp-OBzl 441 mg (96%) of the title compound was obtained as as colorless powder. Mp 219-221 ºC; ESI/MS (m/e) 704 $[M+H]^+$; $[\alpha]^{20}_D$ = 13.21 (*c* 1.20, CH_3OH); ^{1}H NMR (500 MHz, DMSO-d_6) δ/ppm = 10.93 (m, 2 H), 8.94 (d, *J* = 7.9 Hz, 1 H), 8.74 (d, *J* = 7.9 Hz, 1 H), 7.62 (d, *J* = 7.9 Hz, 1 H), 7.43 (d, *J* = 7.9 Hz, 1 H), 7.11 (m, 17 H), 4.98 (m, 2 H), 4.73 (dd, *J* = 7.5 Hz, *J* = 10.5 Hz, 1 H), 4.59 (dd, *J* = 7.5 Hz, *J* = 10.5 Hz, 1 H), 3.70 (m, 5 H), 3.39 (dd, *J* = 7.5 Hz, *J* = 10.5 Hz, 1 H), 2.99 (m, 6 H), 1.96 (s, 2 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ/ppm = 172.8, 159.2, 142.2, 136.7, 130.1, 129.8, 129.3, 127.7, 127.5, 127.2, 122.9, 122.2, 120.1, 119.2, 119.0, 114.0, 111.3, 111.1, 110.9, 68.5, 56.1, 54.7, 54.3, 38.4, 36.6, 31.3, 30.7. Anal. Calcd for $C_{40}H_{41}N_5O_5S$: C, 68.26; H, 5.87; N, 9.95. Found: C, 68.05; H, 5.71; N, 9.73.

Arg(NO_2)-Trp-Trp-OBzl (4o)

Using the same procedure as described for Ala-Trp-Trp-OBzl from 500 mg (0.64

mmol) of Boc-Arg(NO_2)-Trp-Trp-OBzl 445 mg (97%) of the title compound was obtained as as colorless powder. Mp 188-190 ºC. ESI/MS (m/e) 682 $[M+H]^+$; $[\alpha]^{20}_D$ = 19.08 (*c* 1.02, CH_3OH); ^1H NMR (500 MHz, DMSO-d_6) δ/ppm = 10.99 (m, 2 H), 8.52 (d, *J* = 7.5 Hz, 1 H), 8.37 (d, *J* = 7.4 Hz, 1 H), 7.33 (m, 15 H), 5.11 (m, 2 H), 4.19 (m, 1 H), 4.04 (m, 2 H), 3.14 (m, 4 H), 1.73 (m, 2 H), 2.02 (s, 2 H), 1.95 (m, 3 H), 1.58 (m, 4 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ/ppm = 172.7, 163.2, 142.1, 136.6, 130.1, 127.7, 127.5, 127.2, 122.9, 122.2, 120.1, 119.2, 119.0, 111.3, 111.1, 110.9, 68.8, 54.7, 53.6, 37.6, 31.9, 31.2, 30.7, 24.6. Anal. Calcd for $C_{35}H_{39}N_9O_6$: C, 61.66; H, 5.77; N, 18.49. Found: C, 61.45; H, 5.63; N, 18.72.

Lys(Z)-Trp-Trp-OBzl (4p)

Using the same procedure as described for Ala-Trp-Trp-OBzl from 500 mg (0.59 mmol) of Boc-Lys(Z)-Trp-Trp-OBzl 459 mg (99%) of the title compound was obtained as as colorless powder. Mp 193-195 ºC. ESI/MS (m/e) 743 $[M+H]^+$; $[\alpha]^{20}_D$ = 41.67 (*c* 1.05, CH_3OH); ^1H NMR (500 MHz, DMSO-d_6) δ/ppm = 10.98 (m, 2 H), 8.96 (d, *J* = 7.9 Hz, 1 H), 8.65 (d, *J* = 7.3 Hz, 1 H), 8.25 (d, *J* = 7.1 Hz, 1 H), 7.76 (d, *J* = 7.9 Hz, 1 H), 7.65 (d, *J* = 7.9 Hz, 1 H), 7.19 (m, 18 H), 5.00 (m, 4 H), 4.70 (m, 1 H), 4.35 (m, 1 H), 3.35 (m, 1 H), 3.05 (m, 6 H), 1.98 (s, 2 H), 1.49 (m, 6 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ/ppm = 172.6, 156.2, 142.0, 141.1, 136.7, 130.1, 129.3, 127.7, 127.5, 127.2, 122.9, 122.2, 120.1, 119.2, 119.0, 111.3, 111.1, 110.9, 68.4, 65.5, 54.7, 54.1, 42.0, 34.3, 31.3, 30.5, 29.5. Anal. Calcd for $C_{43}H_{46}N_6O_6$: C, 69.52; H, 6.24; N, 11.31. Found: C, 69.73; H, 6.38; N, 11.55.

Gln-Trp-Trp-OBzl (4q)

Using the same procedure as described for Ala-Trp-Trp-OBzl from 500 mg (0.71 mmol) of Boc-Gln-Trp-Trp-OBzl 424 mg (93%) of the title compound was obtained as as colorless powder. Mp 188-190 ºC. ESI/MS (m/e) 609 $[M+H]^+$; $[\alpha]^{20}_D$ = 49.05 (*c* 1.02, CH_3OH); ^1H NMR (500 MHz, DMSO-d_6) δ/ppm = 10.87 (m, 2 H), 8.78 (d, *J* = 7.2 Hz, 1 H), 8.71 (d, *J* = 7.8 Hz, 1 H), 7.59 (m, 2 H), 7.17 (m, 13 H), 6.15 (s, 2 H), 5.02 (m, 2 H), 4.67 (m, 2 H), 3.88 (m, 1 H), 3.17 (m, 4 H), 2.27 (m, 2 H), 2.05 (s, 2 H), 1.96 (m, 2 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ/ppm = 174.1, 173.0, 142.3, 136.7, 130.1, 127.7, 127.5, 127.2, 122.9, 122.2, 120.1, 119.2, 119.0, 111.3, 111.1, 110.9, 68.8, 54.5, 53.7, 32.3, 31.3, 30.7, 30.1. Anal. Calcd for $C_{34}H_{36}N_6O_5$: C, 67.09; H, 5.96; N, 13.81. Found: C, 66.86; H, 5.81; N, 14.03.

Asn-Trp-Trp-OBzl (4r)

Using the same procedure as described for Ala-Trp-Trp-OBzl from 500 mg (0.72 mmol) of Boc-Asn-Trp-Trp-OBzl 431 mg (95%) of the title compound was obtained as as colorless powder. Mp 199-201 ºC. ESI/MS (m/e) 595 $[M+H]^+$; $[\alpha]^{20}_D$ = 11.43 (*c* 1.01, CH_3OH); ^1H NMR (500 MHz, DMSO-d_6) δ/ppm = 10.99 (m, 2 H), 8.73 (m, 2 H), 7.58 (m, 2 H), 7.19 (m, 13 H), 6.16 (s, 2 H), 5.04 (m, 2 H), 4.63 (m, 2 H), 2.96 (m, 7 H), 1.99 (s, 2 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ/ppm = 174.8, 172.7, 142.0, 136.7, 130.1, 127.7, 127.5, 127.2, 122.9, 122.2, 120.1, 119.2, 119.0, 111.3, 111.1, 110.9, 68.9, 54.4, 49.8, 40.0, 31.3, 30.2. Anal. Calcd for $C_{33}H_{34}N_6O_5$: C, 66.65; H,

5.76; N, 14.13. Found: C, 66.46; H, 5.62; N, 14.36.

His-Trp-Trp-OBzl (4s)

Using the same procedure as described for Ala-Trp-Trp-OBzl from 500 mg (0.61 mmol) of Boc-His(Boc)-Trp-Trp-OBzl 361 mg (90%) of the title compound was obtained as as colorless powder. Mp 211-213 ºC. ESI/MS (m/e) 618 $[M+H]^+$; $[\alpha]^{20}_D$ = 46.49 (*c* 0.89, CH_3OH); ^1H NMR (500 MHz, DMSO-d_6) δ/ppm = 11.02 (s, 1 H), 10.95 (s, 1 H), 9.03 (s, 1 H), 8.91 (m, 1 H), 8.44 (s, 3 H), 7.67 (d, *J* = 7.9 Hz, 1 H), 7.51 (d, *J* = 7.9 Hz, 1 H), 7.28 (m, 13 H), 5.04 (m, 2 H), 4.65 (m, 2 H), 3.21 (m, 6 H), 2.99 (m, 1 H), 2.03 (s, 2 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ/ppm = 173.0, 142.4, 136.7, 135.8, 133.6, 130.1, 127.7, 127.5, 127.2, 122.9, 122.2, 120.1, 119.8, 119.2, 119.0, 111.3, 111.1, 110.9, 68.6, 54.5, 53.7, 33.1, 31.3, 30.7. Anal. Calcd for $C_{35}H_{35}N_7O_4$: C, 68.06; H, 5.71; N, 15.87. Found: C, 67.87; H, 5.55; N, 15.65.

Trp-Trp-Trp-OBzl (4t)

Using the same procedure as described for Ala-Trp-Trp-OBzl from 500 mg (0.65 mmol) of Boc-Trp-Trp-Trp-OBzl 416 mg (91%) of the title compound was obtained as as colorless powder. Mp 212 - 214 ºC. ESI/MS (m/e) 667 $[M + H]^+$; $[\alpha]^{20}_D$ = 27.23 (*c* 1.11, CH_3OH); ^1H NMR (500 MHz, DMSO-d_6) δ/ppm = 11.10 (m, 3 H), 8.03 (m, 2 H), 7.39 (m, 20 H), 4.57 (m, 4 H), 3.59 (s, 1 H), 3.43 (m, 6 H), 2.05 (s, 2 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ/ppm = 172.8, 142.2, 136.7, 136.3, 130.1, 127.7, 127.5,

127.2, 122.9, 122.2, 120.1, 119.2, 119.0, 111.3, 111.1, 110.9, 68.5, 55.0, 54.7, 34.1, 31.3, 30.7. Anal. Calcd for $C_{40}H_{38}N_6O_4$: C, 72.05; H, 5.74; N, 12.60. Found: C, 71.84; H, 5.60; N, 12.82.

2. Pharmacology

2.1. *In vitro* anti-proliferation assay of 4a-t

Following a slightly modified procedure[13], the *in vitro* anti-proliferation assays were carried out with 96 microtiter plate cultures and MTT staining. $HepG_2$, S180, H22, K562 and B16 cells (final concentration, 1×10^4/ml growth medium) were grown in RPMI-1640 medium or DMEM medium containing10%(v/v) FCS, penicillin (100 μg/ml) and streptomycin (100 μg/ml) without (control) or with **4a-t** (final concentration, 0.01, 0.1, 1.0, 10.0, 100.0, 200.0, 300.0 400.0 and 500.0 μM). In a humidified atmosphere containing 5% CO_2 the cultures were propagated at 37 ºC for 24 h, after the first renew of the growth medium the cultures without or with **4a-t** were propagated for additional 48 h, the cultures with 25 μl of MTT solution (5 mg/ml) were propagated for another 4 h. On the removal of the growth medium the residue was dried in the air, dissolved in 100 μl of DMSO and the absorption values of light of the formed purple solution was recorded on Bio-rad 570 nm microplate reader (Biorad, USA). The inhibited rates were calculated according to I% = (C - T/C) %.

2.2. *In vivo* anti-tumor assay of 4a-t

The assessments described here were performed based on a protocol reviewed and approved by the ethics committee of Capital Medical University. The committee

assures the welfare of the male ICR mice was maintained in accordance to the requirements of the animal welfare act and according to the guide for care and use of laboratory animals. The mice, purchased from Peking University Health Science Center, were 10 to 12 weeks old at the beginning of the experiments. S180 cells for the initiation of subcutaneous tumors were obtained from the ascitic form of the tumors in mice and serially transplanted once per week. Subcutaneous tumors were implanted by injecting 0.2 ml of NS containing 1×10^7 viable tumor cells under the skin on the right oxter of the mice. Twenty-four hours after the implantation the tumor-bearing mice were randomized into 22 experimental groups (12 per group). All the mice were given a daily i.p. injection of cytarabine (positive control, 8.9 μmol/kg/day in 0.2 ml of NS), or NS (negative control, 0.2 ml), or **4a-t** (8.9 μmol/kg/day in 0.2 ml of NS) for seven consecutive days. Twenty-four hours after the last i.p. injection, all mice were sacrificed by diethyl ether anesthesia, the tumors of the treated (T_W) and control (C_W) groups were separated and weighed to calculate the percentage of the tumor growth inhibition by using the following equation:

$$\text{Inhibition (\%)} = [1-(T_w/C_w)]\times 100$$

2.3. *In vivo* dose-dependent assay of 4r

Male ICR mice, purchased from Peking University Health Science Center, were 10 to 12 week old at the beginning of the experiments. The tumor used was S180 that forms solid tumors, when injected subcutaneously. S180 cells for initiation of the subcutaneous tumors were obtained from the ascitic form of the tumors in the mice, which were serially transplanted once per week. Subcutaneous tumors were implanted

by injecting 1×10^7 viable tumor cells in 0.2 ml of NS under the skin of the right oxter of the mice. Twenty-four hours after the implantation, the mice were randomized into 6 experimental groups (10 per group), and were given a daily i.p. injection of cytarabine (positive control, 8.9, 0.89 or 0.089 μmol/kg/day in 0.2 ml of NS), or NS (negative control, 0.2 ml), or **4r** (8.9, 0.89 or 0.089 μmol/kg/day in 0.2 ml of NS) for seven consecutive days. Twenty-four hours after the last i.p. injection, all mice were sacrificed by diethyl ether anesthesia, the tumors of the treated (T_W) and control (C_W) groups were separated and weighed to calculate the percentage of the tumor growth inhibition accordingly.

2.4. *In vivo* acute toxicity assay

Health male ICR mice of 24-week old were given a single i.p injection of 500 mg/kg of the anti-tumoral active **4c,e,f,g,i,k,l,n,o,q,r** in 0.2 mL of NS. The mice were monitored for 7 days to observe the neurotoxic behavior, such as tremor, twitch, jumping, tetanus, and supination, as well as the death. On the 7^{th} day the survival mice were sacrificed by diethyl ether anesthesia and dissected to immediately obtain the organs for examination.

3. Action mechanism

3.1. UV spectra of 4r without and with CT DNA

In the UV measurements (Figure 1) of **4r** alone (pH 7.4, final concentration 50 μM) and it plus CT DNA (pH 7.4, final concentration 0, 15, 30, 45, 60 and 75 μM) in PBS buffer were performed on a Shimadzu 2550 spectrophotometer over 220 nm to 400 nm. Figure 1 explores that CT DNA induces **4r** a hypochromic effect (7.52%)

and hypsochromic shift (9 nm).

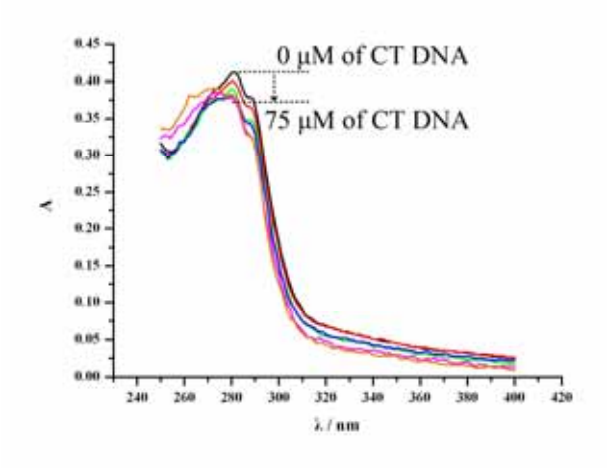

Figure 1 Hypochromic effect and hypsochromic shift occurred in the UV spectra of **4r** (PBS buffer, pH = 7.4, final concentration 50 μM) by adding CT DNA (PBS buffer, pH = 7.4, final concentration 0, 15, 30, 45, 60 and 75 μM)

3.2. CD of CT DNA without and with 4r

The CD spectra of CT DNA are characterized by the positive and negative bands, of which the former results from the base stacking and the latter results from the right-handed helicity. The change of the band intensity is usually the result of the intercalation of small molecules towards CT DNA. In CD experiments of CT DNA alone (pH 7.4, final concentration 2×10^{-4} μM) and it plus **4r** (pH 7.4, final concentration 0 M, 10^{-4} M and 5×10^{-4} M) in PBS buffer were incubated at 37 ˚C for 24 h, and determined the CD spectra according to a standard procedure. Figure 2 explains **4r** induced decrease of the intensity of both positive and negative bands, and reflects the intercalation of it towards CT DNA.

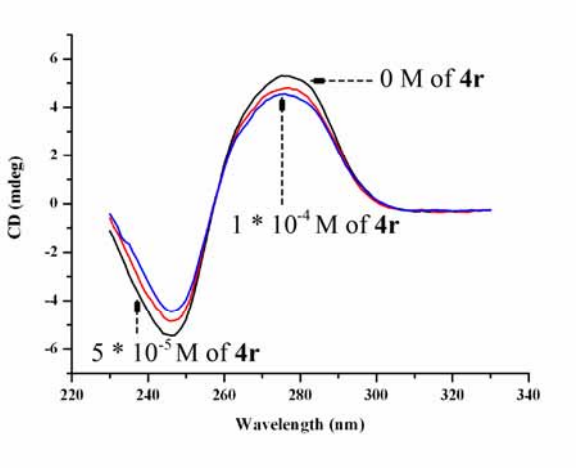

Figure 2 Effect of **4r** on the signals of the CD spectra of CT DNA (final concentration, 2×10^{-4} M).

3.3. UV spectra of 4t without and with CT DNA

In the UV measurements (Figure 3) of inactive **4t** alone (pH 7.4, final concentration 50 μM) and it plus CT DNA (pH 7.4, final concentration 0, 15, 30, 45, 60 and 75 μM) in PBS buffer were performed on a Shimadzu 2550 spectrophotometer over 220 nm to 400 nm. Figure 3 explores that CT DNA induces **4t** neither hypochromic effect nor hypsochromic shift.

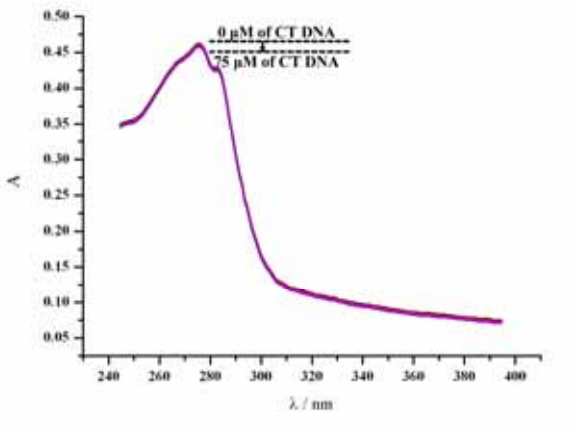

Figure 3 Hypochromic effect and hypsochromic shift did not occurr in the UV spectra of **4t** (PBS buffer, pH = 7.4, final concentration 50 μM) by adding CT DNA (PBS buffer, pH = 7.4, final

concentration 0, 15, 30, 45, 60 and 75 μM)

3.4. Fluorescence spectra of 4t without and with CT DNA

The fluorescence measurements of **4t** alone (pH 7.4, final concentration 50 μM) and it plus CT DNA (pH 7.4, final concentration 0, 10, 20, 30, 40, 50, 60 and 70 μM) in PBS buffer were performed on a Shimadzu RF-5310PC spectrofluorometer (emission wavelength, 359 nm and excitation wavelength, 277 nm). Figure 4 explains no fluorescence quenching phenomenon, i.e. CT DNA induces no concentration-dependent decrease of fluorescence intensity of **4t**.

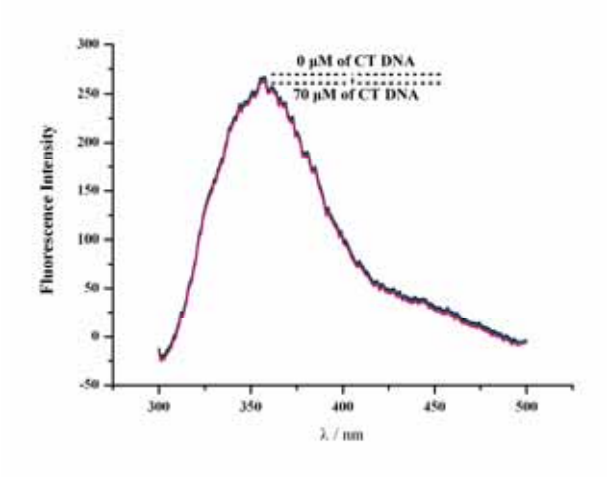

Figure 4 Fluorescence spectra explain no fluorescence quenching of **4t** (final concentration 50 μM, pH = 7.0, λ_{em} = 359 nm, λ_{ex} = 277 nm) by adding 10 μl of CT DNA (final concentration 0, 10, 20, 30, 40, 50, 60 and 70 μM) in PBS buffer.

3.5. CD of CT DNA without and with 4t

The CD spectra of CT DNA are characterized by the positive and negative bands, of which the former results from the base stacking and the latter results from the right-handed helicity. The change of the band intensity is usually the result of the intercalation of small molecules towards CT DNA. In CD experiments, of CT DNA

alone (pH 7.4, final concentration 2 × 10^{-4} μM) and it plus **4t** (pH 7.4, final concentration 0 M, 10^{-4} M and 5 ×10^{-4} M) in PBS buffer were incubated at 37 ˚C for 24 h, and determined the CD spectra according to a standard procedure. Figure 5 explains **4t** did not induce the decrease of the intensity of both positive and negative bands, and reflects the no intercalation of it towards CT DNA.

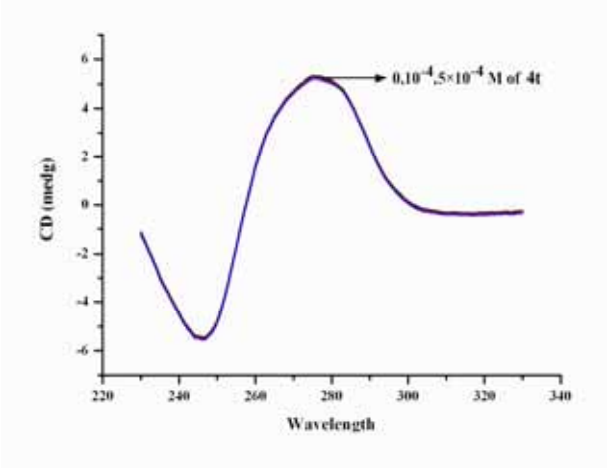

Figure 5 Effect of **4t** on the signals of the CD spectra of CT DNA (final concentration, 2 × 10^{-4} M).

3.6. Melting temperature of CT DNA without and with 4t

The melting temperature (Tm) of DNA is widely used to reflect the thermostability of the double helix. Once the temperature of DNA solution is high enough the double strands dissociate to single stand, and the temperature that induces the absorbance a 50% increase is termed as Tm. Here the temperature of the solution of CT DNA alone (100 μM) and it plus **4t** (10 μM) in PBS (pH 7.4) rose at a rate of 0.1. The melting curves of CT DNA alone and it plus **4r** were shown with Figure 6. The Tm of CT DNA plus **4t** is 2.8 ± 0.6 ºC higher than that of it alone indicates that the addition of **4t** does not increase the thermostability of the double helix, which should be attributed to no intercalation of **4t** toward CT DNA.

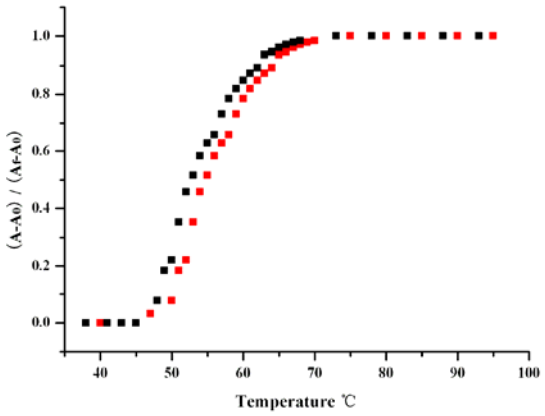

Figure 6 Thermal denaturation curves of CT-DNA in the absence and presence of **4t**. Tm measurements were performed in PBS at pH 7.4 with a **4t**/DNA ratio of 0.10.

3.7. Viscosity of CT DNA without and with 4t

In the intercalation, in order to accommodate and bind small molecule, DNA base pairs have to be pushed apart and consequently leads to viscosity increase. To define the intercalation here the relative viscosity of the sample solutions of [**4t**]/[CT DNA] in the ratios of 0 - 0.36 were measured. Figure 7 indicates that the relative viscosity of CT DNA is not increased with the increase of the concentration of **4t**, which is the result of no intercalation of **4t** towards CT DNA.

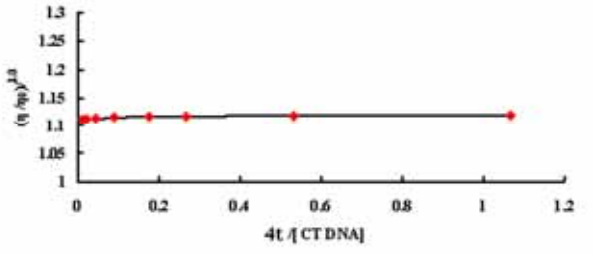

Figure 7 Effect of **4t** on the relative viscosity of CT DNA (200 μM)