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Synthesis and Biological Evaluation of a Novel Class of β-carboline Derivatives

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ABSTRACT

In this study, several novel β-carboline derivatives, 1-(4-hydroxy-3-methoxyphenyl)-β-carboline-3-carboxyl)-Trp-Trp-AA-OBzl compounds were designed and synthesized as potential anticancer agents. Their *in vitro* cytotoxic activities were evaluated using methylthiazoltetrazolium (MTT) assay. The *in vivo* anti-tumor activity of the newly synthesized β-carboline derivatives was determined in a S180 bearing mouse model and some of the compounds demonstrated tumor growth inhibition similar to the positive control, doxorubicin. The intercalation of the β-carboline derivatives synthesized with calf thymus (CT) DNA was also studied.

Keywords: Amino acid; β-carboline; Peptide; Anti-tumor; Cytotoxicity; CT DNA.

Introduction

Cancer has long been one of the serious diseases threatening human health and continues to be a major health problem worldwide. Therefore, discovering new compounds with potent anticancer activity is one important goal. Among current anticancer chemotherapeutic agents DNA-recognizing molecules, including intercalating agents, alkylating compounds and groove binders, are especially interesting. DNA intercalating agents are important in clinical oncology, and several representative compounds (anthracyclines, acridines, and anthraquinones) are routinely used.[1] Intercalation results in conformational alters of the double helix, and then changes the processes of DNA replication, transcription and repair. Thus the discovery of new DNA intercalating agents is considered as a promising approach toward anticancer drugs.

Polycyclic indolic compounds have attracted a great deal of interest amongst synthetic chemists over the years, largely because of the diverse biological activities demonstrated by this type of compounds.[2] Given their rigid structures, polycyclic indolic compounds are expected to show substantial selectivity in their interactions with enzymes or receptors. However, compounds bearing this ring system have displayed a diverse range of biological properties including antimalarial, anti-tumor, anti-HIV, anticonvulsive, hypnotic and anxiolytic, antiviral, and antimicrobial activities as well as inhibition of topoisomerase-II and cGMP-dependent processes.[3-8] In addition, antiplasmodial activity has also been reported.[9] The structure of β-carboline seems to be essential for many naturally occurring biologically important indole alkaloids and represents an important lead structure for the development of novel pharmacological agents.[10-17] Some β-carboline alkaloids such as harmine and its derivatives, are highly cytotoxic against human tumor cell lines.[18–23] In studies with β-carboline, DNA intercalation and cytotoxicity showed a correlation.[24-27] Thus, β-carbolines act through intercalation to inhibit DNA topoisomerases I and II and cause DNA damage.

One of the commonly used approaches in the search for new bioactive agents from natural products is chemical modifications of naturally occurring lead compounds. The β-carboline framework is used as an important pharmacophore for the design of novel anti-tumor agents in this study. Previous studies on a variety of synthetic β-carboline derivatives have demonstrated the influence of molecular planarity and substitutions at Positions 1, 3 and 9 of the β-carboline skeleton on anti-tumor activities.[28] It has also been shown that substitutions especially at Positions 1 and 3 could significantly reduce the toxicity of β-carboline derivatives. Some studies demonstrated that β-carboline derivatives containing a substituted phenyl group at Position 1 possess anti-tumor activities.[29] Several compounds having 1-(4-hydroxy-3-methoxyphenyl)-β-carbolines bearing the 2-substituted-1,3,4-oxadiazol-5-yl and 5-substituted-1,2,4-triazol-3-yl at C-3 were reported to possess antitumoral activities.[30]

It is well proved that Trp-Trp is biologically important either as a dipeptide or as a fragment of some peptides. Trp-Trp-OBz was used as a lead, and twenty tripeptide benzyl esters, Trp-Trp-AA-OBz , were synthesized as DNA intercalators.[31] In view of the importance associated with the above cited moieties in their potent anti-tumor activity, it was thought of considerable interest to employ derivatives of Trp-Trp-AA-OBz for the generation of new 1-(4-hydroxy-3-methoxyphenyl)-3-carboxyl β-carbolines which are represented in the Table 1. In addition, the use of tripeptide in β-carboline derivatives designed was based upon the concept that many amino acids with functional side chains are capable of making base-specific contacts with more than one type of DNA bases,[32] a series of amino acids, containing either nonpolar, acidic, basic, or aromatic sides, differently functionalities with absolute stereochemistry, were introduced into the β-carboline core. Moreover, amino acid conjugates might target the gastrointestinal transporters involved in the absorption of amino acids and small peptides resulting in improved oral bioavailability.[33-34] The various side chains of different amino acids allow

the addition of amino acids to β-carboline to manipulate the pharmacokinetics profiles of the compounds. In addition, various amino acids can be introduced to enhance solubility. Therefore, in order to search for better DNA intercalating agents, β-carboline was used as the basic system and a series of novel β-carboline derivatives bearing a 4-hydroxy-3-methoxyphenyl group at Position 1 and Trp-Trp-AA-OBzl at Position 3 were designed and synthesized and evaluated for their anti-tumor activity in the present study.

Results and discussion

Chemistry

As shown in Scheme 2, the syntheses of β-carboline derivatives were carried out using a multi-step synthetic route. Firstly, 1-(4-hydroxy-3-methoxyphenyl)-tetrahydro-β-carboline-3-carboxylic acid (**3**) was synthesized via the Pictet-Spengler reaction of tryptophan with vanillin in acetic acid. **3** obtained were composed of two isomers with 1S,3S-*cis* and 1R,3S-*trans* configurations.[13,30,35] Then esterification of **3** with methanol in the presence of $SOCl_2$ yielded methyl 1-(4-hydroxy-3-methoxyphenyl)-tetrahydro-β-carboline-3-carboxylate (**4**). Secondly, the intermediate methyl 1-(4-hydroxy-3-methoxyphenyl)-β-carboline-3-carboxylate (**5**) was prepared by the reaction of **4** with Selenium dioxide in acetic acid.[13,36] Subsequently, removal of the methyl group with NaOH resulted in 1-(4-hydroxy-3-methoxyphenyl)-β-carboline-3-carboxylic acid (**6**). Finally, tripeptide benzyl esters, NH_2-Trp-Trp-AA-OBzl, from Scheme **1** were introduced into **6** by the DCC/HOBt/*N*-methylmorpholine (NMM) procedure to provide the respective β-carboline derivatives, 1-(4-hydroxy-3-methoxyphenyl)-β-carboline-3-carboxyl)-Trp-Trp-AA-OBzl (**7-21**), as shown in Scheme **2** (chemical yields, 54–79%). The chemical structures of all 19 (**3-21**) β-carboline derivatives synthesized in this study are provided in Table **1** and they were confirmed by ^{1}HNMR, ^{13}CNMR, IR, and HR-ESIMS results. The spectroscopic data are given in the "Experimental" Section. The ^{1}HNMR

spectra of β-carboline derivatives **7-21** showed signals at δ 8.74-8.68 for one proton corresponding to H-4 of the pyridine ring in the β-carboline skeleton. The two pairs of 1H double doublet at δ 4.96-4.80 and 4.78-4.49 are in agreement with α-methine proton of tryptophan. Signals at 4.96-4.80, and 4.78-4.49 in the COSY spectrum indicated methylenes adjacent to methines. ^{1}HNMR spectroscopic signals at δ 4.80-3.95 are in agreement with α-methine proton of the AA in NH_2-Trp-Trp-AA-OBzl.

In vitro cytotoxicity

The *in vitro* cytotoxicity of the β-carboline derivatives synthesized above was evaluated in human colon cancer cells (HT-29), human lung adenocarcinoma cells (A549), chronic myeloid leukemia cells (K562) and human immature granulocyte leukemia cells (HL-60) using MTT assay.[22] Doxorubicin (adriamycin, ADM) was used as positive control. In brief, cells were exposed to **7-21** of concentrations ranging from 1 μM to 400 μM for 48 h and cell survival was then determined. IC_{50} is the concentration at which 50% of cells were killed. As shown in Table **2**, the IC_{50} values of ADM in K562, HT-29, HL60 and A549 cells were found to be 1.3, 8.1, 1.7 and 3.5 μM, respectively. **7-21** exhibited varied activities in the four cell lines and they were found most effective in K562 cells and least effective in HT-29 cells. In K562 cells, all compounds demonstrated IC_{50} values of less than 90 μM**,** and **9, 12, 15, 18, 19** and **21** were found to be most effective with IC_{50} values of lower than 20 μM. The IC_{50} of **12** (IC_{50}, 12.0 μM) is almost equipotent to that of **21** (IC_{50}, 14.3 μM) suggesting that the methyl group at the β or γ position of an amino acid residue does not cause changes in the cytotoxicity. In HL60 cells, all compounds showed IC_{50} values of less than 200 μM except for **16,** and **20** was found to be most effective with an IC_{50} value of 16.0 μM. In A549 cells, all but **13** demonstrated IC_{50} values of less than 200 μM and **8** was found to be the most potent with an IC_{50} value of 52.0 μM. Compared to **9** (IC_{50}, 56.4 μM), **19** (IC_{50}, 102.0 μM) exhibited a lower cytotoxicity in A549 cells suggesting that the

introduction of the additional methyl group in the Ser at the β position reduced the cytotoxicity. The results seem to suggest that the β-carboline derivatives synthesized exhibited the moderate cytotoxicity activity.

In vivo anti-tumor activity

The *in vivo* anti-tumor activity of fifteen β-carboline derivatives synthesized (**7-21**) was evaluated in mice bearing S180.[37] The mice were given a daily i.p injection of 0.1 μmol/kg of **7-21** in 0.2 mL of normal saline (NS) for seven consecutive days with NS as the negative control. ADM at 0.1 μmol/kg (0.2 mL) dissolved in 0.9% saline was used as positive control. The tumor weights were found to be 0.84 g to 0.45 g compared to 1.24 g in the negative control group. The tumor inhibition % in describing the anti-tumor effects was determined as:

$$\text{Tumor Inhibition \%} = (C–T)/C\times100$$

T: average tumor weight of treated group

C: average tumor weight of negative control group

The tumor inhibition % of the compounds tested were found to range from 31.9% (**11**) to 63.4% (**14**) compared to 61.1% for 0.1 μmol/kg of ADM as shown in Table **3**. The results suggested that the amino acid introduced in the Trp-Trp-AA-OBzl of the β-carboline derivatives synthesized affected their *in vivo* anti-tumor activities. **14** and **19** showed the highest tumor inhibition (63.4% and 62.9%, respectively) in mice bearing S180. Compared to **12** (52.4%)**, 11** and **21** exhibited a lower activity (31.9% and 33.0%) suggesting that the isopropyl group at the α or β position of an amino acid residue is likely responsible for the reduced activity observed. The *in vivo* results suggest that subtle changes in the size and shape of the alkyl side chain of neutral aliphatic amino acids of the β-carboline derivatives could cause changes in the *in vivo* anti-tumor activities, which may imply that the spatial orientation

and characteristics of the pendant group is important in DNA recognition and binding.[38] It was also noticed that the *in vitro* efficacy did not match the *in vivo* efficacy. It has been well known that the *in vitro* efficacy does not match with the *in vivo* efficacy is not a rare phenomena. Perhaps the absorption, delivery, distribution and metabolism are partly responsible for the mentioned phenomena of **7-21**.

14, the most potent compound**,** was selected to further explore the effect of dose on the *in vivo* anti-tumor activity. As shown in Table **4**, at 0.001, 0.01, and 0.1 μmol/kg, **14** exhibited tumor inhibitions of 32.7, 44.3 and 63.4%, respectively.

Toxicity of β-carboline derivatives on mice

During chemotherapy, an increase body weight is an important parameter of health. To evaluate the preliminary toxicity of **7-21** during the administration the body weights of the mice were measured. The data are listed in Table **5** as increased body weight from start of treatment. **7–18**, **20** showed weight gain similar to that of the vehicle-treat group. Only two compounds (**19, 21**) showed significantly lower increases in body weight.

It is reported harmine and its derivatives had significant antitumor activities in mice bearing both Lewis lung cancer and Sarcoma 180, while they exhibited remarkable neurotoxic effects.[39] All the tested compounds caused neither obvious neurotoxic reaction including tremor, twitch, jumping, tetanus and supination nor death at tested dosage, except for partial contortion at injection point. The necropsy findings in surviving animals at the end of experimental period (7 days) revealed no apparent changes in any organs. The most potent compound was examined for the LD50 and neurotoxicity in mice model. The results indicate that even the dose of **14** was up to 500 mg/kg the mice neither exhibited neurotoxic behavior nor occurred death. Necropsy findings in **14** receiving mice on the 7th day revealed that the administration leaded no apparent changes in any organs. These suggest that **14** is comparatively

non-toxic and its LD50 values should be more than 500 mg/kg.

Intercalation of β-carboline derivatives toward CT DNA

There is compelling evidence that cellular DNA is the common target of many anti-tumor agents and the interaction of anti-tumor agents with DNA has been widely studied to understand the mechanisms of anti-tumor agents. Since two structural elements β-carboline and Trp-Trp of the β-carboline derivatives synthesized, each has a planar polycyclic aromatic pharmacophore capable of stacking between DNA pairs at the intercalation sites,[40-41] the intercalation of the β-carboline derivatives synthesized in this study with calf thymus (CT) DNA was studied using **14**. There are a number of techniques used to study the interaction of small molecules with DNA. UV absorption and fluorescence spectroscopy are well known as simple but sensitive methods. The UV and fluorescence spectra and viscosity of **14** in the presence of CT DNA were obtained and compared with those in the absence of CT DNA.

The UV spectrum of **14** in PBS (pH 7.4, 2 μM) was recorded on a Shimadzu 2550 spectrophotometer from 220 to 350 nm. Two (2) mL of **14** were then titrated with 10 μL of CT DNA in PBS (pH 7.4) solutions of 0, 1.0, 2.0, 4.0, 8.0, 16.0, and 32.0 μM, respectively. The corresponding UV spectra of the mixtures were also determined. As shown in Figure **1**, the absorption of **14** gradually decreased from 0.644 to 0.522 with the increase of CT DNA concentration and a hypochromic effect (18.9%) was induced. Most investigators believed that the small molecule binding to the base pairs can cause hypochromism. The classical intercalative binding has been characterized by large changes in the absorbance (hypochromism ≤ 35%).[42-44] This could be considered to be a direct evidence for the intercalation of **14** with CT DNA.

To further confirm the intercalation of **14** with CT DNA, the fluorescence spectrum of **14** in PBS (pH

7.4, 2 μM) was obtained and compared with that in the presence of CT DNA in PBS (final concentrations: 0, 1.0, 2.0, 4.0, 8.0, 16.0 and 32.0 μM, respectively, pH 7.4) on a Shimadzu RF-5310PC spectrofluorometer (emission wave-length 220-400 nm and excitation wavelength 310 nm).[45] It was found that the fluorescence intensity of **14** gradually decreased with the increase of CT DNA concentration as shown in Figure **2**. When the concentration of CT DNA was increased to 32.0 μM the fluorescence intensity of **14** was decreased by 42.2% as shown in Figure **2**. Fluorescence quenching is a very common phenomenon. It refers to the process of the fluorescence intensity samples decreasing. Many of molecular interactions can result in fluorescence quenching.[46] Fluorescence quenching is a result of intercalation of **14** with CT DNA.

The most convincing evidence for DNA intercalation is generally provided by viscosity measurements.[47-48] Insertion of ligands between adjacent nucleobase pairs leads to lengthening and stiffening of the double helix, i.e. to structural changes that are reflected in an increase in DNA viscosity while a non-classical intercalation or a groove mode would reduce the DNA viscosity.[48-49] To further clarify the interaction between the β-carboline derivatives synthesized and CT DNA, viscosity measurements were carried out. The effect of test compound on the viscosity of CT DNA solution is shown in Figure **3**. It is clear that as the concentration of **14** in the mixture of **14** and CT DNA increased the viscosity of the preparation increased, which suggests that the binding resulted in the lengthening of the DNA double helix. This is in consistent with the intercalation of **14** toward CT DNA.

Conclusions

In summary, novel β-carboline derivatives, 1-(4-hydroxy-3-methoxyphenyl)-β-carboline-3-carboxyl)-Trp-Trp-AA-OBzl **7–21** were prepared in acceptable yields using the five-step route described above.

In vitro cytotoxicity assays against four human carcinoma cell lines (HT-29, A549, K562 and HL-60) explored the cell selective anti-proliferation for individual compounds. The *in vivo* anti-tumor activity assays with S180 mice revealed **14** and **19** having the highest efficacy. The tumor inhibition % of **14** and **19** at 0.1μmol/kg (63.4% and 62.9%, respectively) is equipotent to that (61.1%) of the positive control doxorubicin ADM at 0.1μmol/kg. The UV and fluorescence spectra, as well as the relative viscosity measurements of **14** with or without CT DNA suggest that the binding of the β-carboline derivatives synthesized to DNA was intercalative.

Experimental

Syntheses

General Unless otherwise stated, all reactions were under a nitrogen atmosphere (1 bar). All reagents used were purchased from Sigma Chemical Co (USA). Optical rotations were determined with a Schmidt+Haensch Polartromic D instrument (Germany). IR spectra were recorded with an Avatar 330, Nicolet, USA spectrometer. ^{1}H and ^{13}C NMR spectra were recorded at 300 MHz on a VXR-300 instrument or at 500 MHz on a Bruker Am-500 instrument in $CDCl_3$ or in DMSO-*d*6 with tetramethylsilane as internal standard and chemical shifts are expressed in ppm (δ). Chromatography was carried out using Qingdao silica gel H (Qingdao of China). TLC analysis was carried out on silica gel F_{254}. The purities (> 95%) of the intermediates and the products were confirmed by TLC (Merck silica gel plates of type 60 F_{254}, 0.25 mm layer thickness, Germany) and HPLC (Waters, C_{18} column 4.6×150 mm, USA). MS was acquired on a Quattro Micro ZQ2000, Waters, USA instrument, m/z values are reported. High-resolution mass spectra were recorded with microTOF-Q mass spectrometer.

Methyl 1-(4-hydroxy-3-methoxyphenyl)-9H-pyrido[3,4-b]indole-3-carboxylate To a cooled, rapidly stirring solution of **4** (1 g, 2.57 mmol) in acetic acid (100 mL) was added SeO_2 (2.86 g**,** 25.8 mmol),

and the resulting mixture was refluxed for 1 h. The pH of the reaction mixture was adjusted to 9 with ammonia and kept on ice. The precipitate was collected and purified by silica gel column chromatography (40:1 CH_2Cl_2-MeOH) to provide **5** as brown powder (500 mg, 1.44 mmol, 56% yield). $[\alpha]_D^{25}$ -23.8 (c 0.1 in MeOH); IR (cm^{-1}, KBr, neat): 3055, 1716, 740; ^{1}HNMR (300 MHz, DMSO-d_6): δ 11.58 (1H, s, OH), 8.85 (1H, s, Ar-H), 8.72 (1H, s, Ar-H), 8.40 (1H, d, J= 8.1 Hz, Ar-H), 7.70 (1H, d, J= 8.4 Hz, Ar-H), 7.60 (1H, dt, J_1= 8.1 Hz, J_2= 0.9 Hz, Ar-H), 7.46 (1H, d, J= 1.8 Hz, Ar-H), 7.44 (1H, dd, J_1= 8.1 Hz, J_2= 1.8 Hz, Ar-H), 7.33 (1H, t, J= 7.2 Hz, Ar-H), 7.04 (1H, d, J= 8.1 Hz, Ar-H), 3.93 (3H, s, OCH_3), 3.91 (3H, s, OCH_3); ^{13}CNMR(125MHz, DMSO-d_6) δ 166.6, 148.2, 148.1, 143.1, 141.8, 136.9, 134.9, 129.2, 129.1, 128.9, 122.4, 122.0, 121.7, 120.8, 116.5, 116.1, 113.2, 112.9, 56.1, 52.5; ESIMS *m/z* 349 (M+1); HRMS calcd for: $C_{20}H_{15}N_2O_4$ - 1), *m/z* (347.1037); found, *m/z* (347.1003).

1-(4-Hydroxy-3-methoxyphenyl)-9H-pyrido[3,4-b]indole-3-carboxylic acid The solution of **5** (500 mg**,** 1.44 mmol) in NaOH (6 M) in tetrahydrofuran (THF, 20 mL) was stirred at 0°C until all the starting material was consumed as indicated by TLC (24 h). The pH of the reaction mixture was adjusted to 2 with hydrogen chloride (2 M) and the mixture was subject to evaporation to remove solvent. The residue was washed with saturated NaCl to provide **6** as green powder (400 mg, 1.20 mmol, 83% yield). ^{1}HNMR (300 MHz, DMSO-d_6): δ 11.43 (1H, s, OH), 8.40 (1H, d, J= 5.1 Hz, Ar-H), 8.24 (1H, d, J= 8.1 Hz, Ar-H), 8.04 (1H, d, J= 5.1 Hz, Ar-H), 7.65 (1H, d, J= 8.1 Hz, Ar-H), 7.56-7.46 (2H, m, Ar-H), 7.25 (1H, t, J= 7.2 Hz, Ar-H), 7.01 (1H, d, J= 7.2 Hz, Ar-H), 3.91 (3H, s, OCH_3); ^{13}CNMR(75MHz, DMSO-d_6) δ 171.6, 147.9, 147.5, 136.9, 136.7, 131.5, 131.4, 126.6, 122.3, 121.8, 119.1, 118.4, 115.5, 114.2, 111.7, 107.8, 107.7, 56.1.

General synthetic procedure for preparing 1-(4-Hydroxy-3-methoxyphenyl)-9H-pyrido[3,4-b]indole-

3-carboxyl)-Trp-Trp-AA-OBzl HOBt (1.2 eq.) and DCC (1.2 eq.) were added to a solution of **6** (1 eq.) in anhydrous THF at 0°C. The reaction mixture was stirred at 0°C for 30 min. The solution of NH_2-Trp-Trp-AA-OBzl (1.2 eq.) in anhydrous THF was added and the pH of the reaction mixture was adjusted to 9 with *N*-methylmorpholine. The reaction mixture obtained was kept at 0°C for 2 h followed by at room temperature for 24 h. DCU formed was removed by filtration. The filtrate was subject to evaporation under reduced pressure and the residue was dissolved in EtOAc (50 mL). The solution was washed successively with saturated $NaHCO_3$, 5% $KHSO_4$ and saturated NaCl and the organic phase was collected and dried using Na_2SO_4. Following filtration and evaporation under reduced pressure, purification of the residue by chromatography (100:1 CH_2Cl_2-MeOH) provided **7-21** as colorless powder in 54–79% yields.

1-(4-Hydroxy-3-methoxyphenyl)-9H-pyrido[3,4-b]indole-3-carboxyl)-Trp-Trp-Tyr-OBzl HOBt (162 mg, 1.20 mmol), DCC (247 mg, 1.20 mmol), **6** (334 mg, 1.00 mmol) in anhydrous THF (10 mL) and NH_2-Trp-Trp-Tyr-OBzl (815 mg, 1.20 mmol) in anhydrous THF (5 mL). Yield: 70%. mp 163-165 °C. $[\alpha]_D^{25}$ -10.7 (c 0.1 in MeOH); IR (cm^{-1}, KBr, neat): 3356, 1658, 1519, 740; ^{1}HNMR (300 MHz, DMSO-d_6): δ 11.76 (1H, s, OH), 10.77 (2H, brs, N-H), 9.40 (1H, s, N-H), 9.23 (1H, s, N-H), 8.70 (1H, s, Ar-H), 8.69 (1H, d, J= 5.4 Hz, N-H), 8.49 (1H, d, J= 7.2 Hz, N-H), 8.39 (1H, d, J= 9.9 Hz, N-H), 8.34 (1H, d, J= 7.8 Hz, Ar-H), 7.69 (1H, d, J= 9.1 Hz, Ar-H), 7.61-7.54 (4H, m, Ar-H), 7.41 (1H, dd, J_1= 9.1 Hz, J_2= 1.8 Hz, Ar-H), 7.36-7.23 (8H, m, Ar-H), 7.16-7.15 (2H, m, Ar-H), 7.14-7.02 (6H, m, Ar-H), 6.83 (1H, t, J= 7.5 Hz, Ar-H), 6.66 (2H, d, J= 8.4 Hz, Ar-H), 5.05 (2H, dd, J= 15.3 Hz, CH_2Ph), 4.84 (1H, dd, J_1= 12.6 Hz, J_2= 7.8 Hz, CH), 4.71 (1H, dd, J_1= 13.2 Hz, J_2= 8.4 Hz, CH), 4.50 (1H, dd, J_1= 14.4 Hz, J_2= 7.2 Hz, CH),3.85 (3H, s, OCH_3), 3.27-3.09 (3H, m, CH_2), 3.02-2.87 (3H, m, CH_2); ^{13}CNMR(75MHz, DMSO-d_6) δ 172.0, 171.7, 171.6, 164.8, 156.6, 148.2, 148.1, 141.9, 141.5, 139.4,

136.6, 136.5, 136.2, 134.5, 130.5, 129.9, 129.1, 128.8, 128.4, 128.3, 127.9, 127.8, 127.3, 124.2, 124.0, 122.3, 121.8, 121.7, 121.3, 120.6, 119.0, 118.9, 118.6, 116.2, 115.6, 113.2, 112.9, 112.7, 111.7, 110.5, 110.2, 66.4, 55.9, 55.4, 54.8, 53.9, 53.6, 36.6, 28.6, 28.3; ESIMS *m/z* 958 (M-1); HRMS calcd for: ($C_{57}H_{48}N_7O_8$ - 1), *m/z* (958.3569); found, *m/z* (958.3620).

1-(4-Hydroxy-3-methoxyphenyl)-9H-pyrido[3,4-b]indole-3-carboxyl)-Trp-Trp-Glu(OBzl)-OBzl

HOBt (162 mg, 1.20 mmol), DCC (247 mg, 1.20 mmol), **6** (334 mg, 1.00 mmol) in anhydrous THF (10 mL) and NH_2-Trp-Trp-Glu(OBzl)-OBzl (883 mg, 1.20 mmol) in anhydrous THF (5 mL). Yield: 70%. mp 139-141 ºC. $[\alpha]_D^{25}$ +58.3 (c 0.1 in MeOH); IR (cm^{-1}, KBr, neat): 3379, 1736, 1658, 1519,740; ^{1}HNMR (300 MHz, DMSO-d_6): δ 11.77 (1H, s, OH), 10.78 (2H, brs, N-H), 8.70 (1H, d, *J* = 8.1 Hz, N-H), 8.68 (1H, s, Ar-H), 8.71 (1H, d, *J* = 7.8 Hz, N-H), 8.44 (1H, d, *J* = 7.8 Hz, N-H), 8.35 (1H, d, *J* = 7.8 Hz, Ar-H), 7.69 (1H, d, *J* = 8.4 Hz, Ar-H), 7.60-7.53 (4H, m, Ar-H), 7.42-7.25 (14H, m, Ar-H), 7.17 (1H, d, *J* = 2.1 Hz, Ar-H), 7.15 (1H, d, *J* = 2.1 Hz, Ar-H), 7.07-6.84 (5H, m, Ar-H), 5.15 (2H, s, CH_2Ph), 5.04 (2H, s, CH_2Ph), 4.80 (1H, dd, J_1 = 12 Hz, J_2 = 7.5 Hz, CH), 4.68 (1H, dd, J_1 = 13.2 Hz, J_2 = 8.7 Hz, CH), 4.44 (1H, dd, J_1 = 13.2 Hz, J_2 = 8.4 Hz, CH), 3.83 (3H, s, OCH_3), 3.27-3.10 (3H, m, CH_2), 3.01 (1H, dd, J_1 = 15 Hz, J_2 = 9 Hz, CH_2), 2.54-2.37 (2H, m, CH_2), 2.14-1.94 (2H, m, CH_2); ^{13}CNMR(75MHz, DMSO-d_6) δ 172.5, 172.3, 171.8, 171.7, 164.9, 148.2, 148.0, 141.8, 141.4, 139.4, 136.6, 136.5, 136.4, 136.3, 134.3, 129.9, 129.1, 128.9, 128.8, 128.5, 128.4, 128.3, 127.9, 127.8, 124.2, 123.9, 122.4, 121.8, 121.7, 121.3, 120.6, 119.0, 118.8, 118.7, 116.2, 113.1, 112.8, 112.6, 111.7, 110.5, 110.2, 110.4, 110.2, 66.5, 65.9, 55.9, 54.0, 53.7, 51.9, 28.6, 28.0, 26.5; ESIMS *m/z* 1015 (M); HRMS calcd for: ($C_{60}H_{52}N_7O_9$ - 1), *m/z* (1014.3832); found, *m/z* (1014.3814).

1-(4-Hydroxy-3-methoxyphenyl)-9H-pyrido[3,4-b]indole-3-carboxyl)-Trp-Trp-Thr-OBzl HOBt (162 mg, 1.20 mmol), DCC (247 mg, 1.20 mmol), **6** (334 mg, 1.00 mmol) in anhydrous THF (10 mL) and

NH_2-Trp-Trp-Thr-OBzl (741 mg, 1.20 mmol) in anhydrous THF (5 mL). Yield: 75%. mp 159-161 ºC. $[\alpha]_D^{25}$ +46.4 (c 0.1 in MeOH); IR (cm^{-1}, KBr, neat): 3356, 1658, 1519, 740; ^{1}HNMR (300 MHz, DMSO-d_6): δ 11.82 (1H, s, OH), 10.83 (1H, brs, N-H), 9.50 (1H, brs, N-H), 8.75 (1H, d, *J*= 8.1 Hz, N-H), 8.73 (1H, s, Ar-H), 8.54 (1H, d, *J*= 8.1 Hz, N-H), 8.37 (1H, d, *J*= 7.8 Hz, Ar-H), 8.30 (1H, d, *J*= 8.1 Hz, N-H), 7.73 (1H, d, *J*= 8.1 Hz, Ar-H), 7.63-7.55 (4H, m, Ar-H), 7.46-7.30 (9H, m, Ar-H), 7.24 (1H, s, Ar-H), 7.19 (1H, s, Ar-H), 7.11-6.99 (4H, m, Ar-H), 6.87 (1H, t, *J*= 7.5 Hz, Ar-H), 5.19 (2H, s, CH_2Ph), 5.13 (2H, d, *J*= 6 Hz, N-H), 4.92-4.82 (2H, m, CH), 4.48 (1H, dd, J_1= 8.1 Hz, J_2= 3.3 Hz, CH), 4.26-4.21 (1H, m, CH), 3.86 (3H, s, OCH_3), 3.35-3.16 (3H, m, CH_2), 3.11-3.04 (1H, m, CH_2), 1.15 (3H, d, *J*= 6.3 Hz,CH_3); ^{13}CNMR(75MHz, DMSO-d_6) δ 172.6, 171.6, 170.9, 164.9, 148.2, 141.9, 141.8, 141.5, 141.4, 139.4, 136.6, 136.5, 136.4, 136.3, 134.5, 134.3, 129.9, 129.1, 128.9, 128.4, 128.2, 128.0, 127.9, 127.8, 124.3, 124.1, 123.9, 122.3, 121.8, 121.3, 120.6, 119.0, 118.9, 118.7, 116.2, 113.2, 112.8, 112.7, 111.7, 110.6, 110.2, 67.0, 66.9, 66.4, 58.7, 55.9, 53.8, 28.7, 28.2, 20.6; ESIMS *m/z* 897 (M), 896 (M-1); HRMS calcd for: ($C_{52}H_{46}N_7O_8$ - 1), *m/z* (896.3413); found, *m/z* (896.3452).

1-(4-Hydroxy-3-methoxyphenyl)-9H-pyrido[3,4-b]indole-3-carboxyl)-Trp-Trp-Lys(N^{ω}-Z)-OBzl

HOBt (162 mg, 1.20 mmol), DCC (247 mg, 1.20 mmol), **6** (334 mg, 1.00 mmol) in anhydrous THF (10 mL) and NH_2-Trp-Trp-Lys(N^{ω}-Z)-OBzl (934 mg, 1.20 mmol) in anhydrous THF (5 mL). Yield: 79%. mp 124-126 ºC. $[\alpha]_D^{25}$ +52.8 (c 0.1 in MeOH); IR (cm^{-1}, KBr, neat): 3290, 1662, 1512, 744; ^{1}HNMR (300 MHz, DMSO-d_6): δ 11.80 (1H, s, OH), 10.80 (1H, d, *J*= 8.7 Hz, N-H), 8.71 (1H, d, *J*= 9.1 Hz, N-H), 8.69 (1H, s, Ar-H), 8.43 (1H, d, *J*= 7.2 Hz, N-H), 8.35 (1H, d, *J*= 7.8 Hz, Ar-H), 7.70 (1H, d, *J*= 8.1 Hz, Ar-H), 7.60-7.54 (4H, m, Ar-H), 7.42-7.28 (14H, m, Ar-H), 7.17 (2H, d, *J*= 5.4 Hz, Ar-H), 7.08-6.90 (4H, m, Ar-H), 6.84 (1H, t, *J*= 7.5 Hz, Ar-H), 5.14 (2H, s, CH_2Ph), 4.99 (2H, s, CH_2Ph), 4.82 (1H, m, CH), 4.72 (1H, m, CH), 4.33 (1H, t, *J*= 6.6 Hz, CH), 3.84 (3H, s, OCH_3), 3.24-3.13 (2H, m,

CH_2), 3.05-2.89 (4H, m, CH_2), 1.84-1.67 (2H, m, CH_2), 1.41-1.21 (4H, m, CH_2); ^{13}CNMR(75MHz, DMSO-d_6) δ 172.2, 172.1, 171.5, 164.9, 156.5, 148.2, 141.8, 141.5, 139.4, 137.7, 136.4, 136.3, 134.3, 129.9, 129.1, 128.9, 128.8, 128.5, 128.3, 127.9, 127.8, 124.0, 123.8, 122.3, 121.8, 121.3, 120.6, 118.9, 118.6, 116.2, 113.2, 112.8, 112.6, 111.6, 110.5, 110.1, 103.5, 66.4, 55.6, 63.4, 55.9, 53.9, 53.4, 52.7, 30.9, 29.5, 28.6, 28.2, 23.1; ESIMS *m/z* 1058 (M), 1057 (M-1); HRMS calcd for: ($C_{62}H_{57}N_8O_9$ - 1), *m/z* (1057.4254); found, *m/z* (1057.4296).

1-(4-Hydroxy-3-methoxyphenyl)-9H-pyrido[3,4-b]indole-3-carboxyl)-Trp-Trp-Val-OBzl HOBt (162 mg, 1.20 mmol), DCC (247 mg, 1.20 mmol), **6** (334 mg, 1.00 mmol) in anhydrous THF (10 mL) and NH_2-Trp-Trp-Val-OBzl (741 mg, 1.20 mmol) in anhydrous THF (5 mL). Yield: 68%. mp 136-138 ºC. $[\alpha]_D^{25}$ +14.8 (c 0.1 in MeOH); IR (cm^{-1}, KBr, neat): 3387, 1666, 1519, 748; ^{1}HNMR (300 MHz, DMSO-d_6): δ 11.78 (1H, s, OH), 10.85 (1H, brs, N-H), 10.81 (1H, brs, N-H), 8.71 (1H, d, *J*= 9.6 Hz, N-H), 8.69 (1H, s, Ar-H), 8.45 (1H, d, *J*= 8.1 Hz, N-H), 8.35 (1H, d, *J*= 7.8 Hz, Ar-H), 8.21 (1H, d, *J*= 8.1 Hz, N-H), 8.13 (1H, d, *J*= 7.8 Hz, N-H), 7.99 (1H, d, *J*= 8.1 Hz, N-H), 7.65 (1H, d, *J*= 8.4 Hz, Ar-H), 7.61-7.48 (3H, m, Ar-H), 7.48-7.27 (11H, m, Ar-H), 7.18-6.93 (5H, m, Ar-H), 6.82 (1H, t, *J*= 7.5 Hz, Ar-H), 5.15 (2H, s, CH_2Ph), 4.87-4.77 (1H, m, CH), 4.69-4.49 (1H, m, CH), 4.27 (1H, dd, J_1= 9 Hz, J_2= 6.3 Hz, CH), 3.38 (3H, s, OCH_3), 3.30-2.80 (4H, m, CH_2), 2.14-2.04 (1H, m, CH), 0.87 (3H, d, *J*= 8.7 Hz, CH_3), 0.84 (3H, d, *J*= 8.7 Hz, CH_3); ^{13}CNMR(75MHz, DMSO-d_6) δ 172.3, 171.6, 169.6, 164.7, 148.2, 141.8, 141.4, 139.4, 136.4, 134.3, 129.9, 129.1, 128.9, 128.5, 128.0, 127.8, 123.8, 122.3, 121.7, 121.3, 120.6, 118.9, 118.6, 116.1, 113.1, 112.8, 111.6, 110.4, 110.0, 66.4, 58.1, 55.9, 53.7, 53.4, 30.4, 27.9, 22.9, 19.4, 18.8; ESIMS *m/z* 895 (M), 894 (M-1); HRMS calcd for: ($C_{53}H_{48}N_7O_7$ - 1), *m/z* (894.3609); found, *m/z* (894.3591).

1-(4-Hydroxy-3-methoxyphenyl)-9H-pyrido[3,4-b]indole-3-carboxyl)-Trp-Trp-Ile-OBzl HOBt (162

mg, 1.20 mmol), DCC (247 mg, 1.20 mmol), **6** (334 mg, 1.00 mmol) in anhydrous THF (10 mL) and NH_2-Trp-Trp-Ile-OBzl (755 mg, 1.20 mmol) in anhydrous THF (5 mL). Yield: 64%. mp 144-146 ºC. $[\alpha]_D^{25}$ -14.1 (c 0.1 in MeOH); IR (cm^{-1}, KBr, neat): 3356, 1658, 1519, 748; ^{1}HNMR (500 MHz, DMSO-d_6): δ 11.77 (1H, s, OH), 10.80 (1H, d, J= 10.5 Hz, N-H), 10.79 (1H, d, J= 8.4 Hz, N-H), 9.43 (1H, s, N-H), 8.72 (1H, d, J= 7.8 Hz, N-H), 8.71 (1H, s, Ar-H), 8.42 (1H, d, J= 8.3 Hz, N-H), 8.35 (1H, d, J= 7.9 Hz, Ar-H), 8.30 (1H, d, J= 7.9 Hz, N-H), 7.69 (1H, d, J= 8.4 Hz, Ar-H), 7.63-7.51 (4H, m, Ar-H), 7.42-7.32 (9H, m, Ar-H), 7.21 (2H, d, J= 3.6 Hz, Ar-H), 7.03-6.96 (3H, m, Ar-H), 6.93 (1H, t, J = 7.3 Hz, Ar-H), 6.87 (1H, t, J= 7.5 Hz, Ar-H), 5.17 (2H, dd, J_1= 29.3 Hz, J_2= 13.2 Hz, CH_2Ph), 4.86-4.82 (1H, m, CH), 4.80-4.76 (1H, m, CH), 4.34 (1H, d, J= 14.1 Hz, CH), 3.87 (3H, s, OCH_3), 3.26 (1H, dd, J_1= 15 Hz, J_2= 7.2 Hz, CH_2), 3.17 (1H, dd, J_1= 15 Hz, J_2= 4.5 Hz, CH_2), 3.14 (1H, dd, J_1= 15 Hz, J_2= 4.5 Hz, CH_2), 3.02 (1H, dd, J_1= 15 Hz, J_2= 8.8 Hz, CH_2), 1.88-1.82 (1H, m, CH), 1.43-1.37 (1H, m, CH_2), 1.26-1.16 (1H, m, CH_2), 0.79 (3H, d, J= 2.75 Hz, CH_3), 0.77 (3H, d, J= 2.65 Hz, CH_3); ^{13}CNMR(125MHz, DMSO-d_6) δ 172.2, 171.6, 171.5, 164.8, 148.2, 148.0, 141.8, 141.4, 139.4, 136.4, 136.3, 136.2, 134.3, 129.9, 129.1, 128.9, 128.6, 128.5, 127.9, 127.8, 124.0, 123.9, 123.8, 122.3, 121.8, 121.7, 121.3, 120.6, 118.9, 118.8, 118.6, 116.1, 113.1, 112.8, 112.6, 111.6, 110.4, 110.1, 66.4, 57.1, 55.9, 53.7, 53.4, 36.7, 28.6, 28.1, 25.2, 15.8, 11.6; ESIMS *m/z* 908 (M-1); HRMS calcd for: ($C_{54}H_{50}N_7O_7$ - 1), *m/z* (908.3766); found, *m/z* (908.3782).

1-(4-Hydroxy-3-methoxyphenyl)-9H-pyrido[3,4-b]indole-3-carboxyl)-Trp-Trp-Ala-OBzl HOBt (150 mg, 1.11 mmol), DCC (228 mg, 1.11 mmol), **6** (308 mg, 0.92 mmol) in anhydrous THF (10 mL) and NH_2-Trp-Trp-Ala-OBzl (650 mg, 1.11 mmol) in anhydrous THF (5 mL). Yield: 69%. mp 149-151 ºC. $[\alpha]_D^{25}$ -8.9 (c 0.1 in MeOH); IR (cm^{-1}, KBr, neat): 3371, 1666, 1527, 748; ^{1}HNMR (300 MHz, DMSO-d_6): δ 11.78 (1H, s, OH), 10.81 (1H, brs, N-H), 10.78 (1H, brs, N-H), 8.71 (1H, d, J= 6.3 Hz,

N-H), 8.70 (1H, s, Ar-H), 8.57 (1H, d, *J*= 7.2 Hz, N-H), 8.44 (1H, d, *J*= 8.4 Hz, N-H), 8.37 (1H, d, *J*= 7.8 Hz, Ar-H), 7.69 (1H, d, *J*= 8.1 Hz, Ar-H), 7.61-7.55 (3H, m, Ar-H), 7.51 (1H, d, *J*= 1.5 Hz, Ar-H), 7.40-7.28 (9H, m, Ar-H), 7.17 (1H, s, Ar-H), 7.15 (1H, s, Ar-H), 7.05-6.96 (4H, m, Ar-H), 6.84 (1H, t, *J*= 7.5 Hz, Ar-H), 5.15 (2H, s, CH_2Ph), 4.83 (1H, dd, J_1= 12 Hz, J_2= 7.2 Hz, CH), 4.69 (1H, dd, J_1= 12 Hz, J_2= 4.8 Hz, CH), 4.47-4.38 (1H, m, CH),3.82 (3H, s, OCH_3), 3.29-3.11 (3H, m, CH_2), 2.99 (1H, dd, J_1= 15 Hz, J_2= 9.3 Hz, CH_2), 1.34 (3H, d, *J*= 7.2 Hz, CH_3); ^{13}CNMR(75MHz, DMSO-d_6) δ 172.8, 172.0, 171.4, 164.8, 148.1, 148.0, 141.8, 141.4, 139.4, 136.5, 136.3, 134.3, 129.9, 129.1, 128.9, 128.5, 128.2, 127.9, 127.8, 124.2, 124.1, 123.8, 122.4, 121.8, 121.7, 121.3, 120.6, 119.0, 118.9, 118.6, 116.1, 113.1, 112.6, 111.6, 110.5, 110.4, 110.1, 66.4, 55.9, 53.9, 53.8, 53.4, 48.3, 28.6, 28.3, 17.3; ESIMS *m/z* 867 (M); HRMS calcd for: ($C_{51}H_{44}N_7O_7$ - 1), *m/z* (866.3297); found, *m/z* (866.3036).

1-(4-Hydroxy-3-methoxyphenyl)-9H-pyrido[3,4-b]indole-3-carboxyl)-Trp-Trp-Asp(OBzl)-OBzl

HOBt (162 mg, 1.20 mmol), DCC (247 mg, 1.20 mmol), **6** (334 mg, 1.00 mmol) in anhydrous THF (10 mL) and NH_2-Trp-Trp-Asp(OBzl)-OBzl (755 mg, 1.20 mmol) in anhydrous THF (5 mL). Yield: 67%. mp 136-138 ºC. $[\alpha]_D^{25}$ +24.1 (c 0.1 in MeOH); IR (cm^{-1}, KBr, neat): 3387, 1666, 1519, 748; ^{1}HNMR (300 MHz, DMSO-d_6): δ 11.78 (1H, s, OH), 10.80 (2H, brs, N-H), 8.71 (2H, d, *J*= 8.7 Hz, N-H), 8.70 (1H, s, Ar-H), 8.48 (1H, d, *J*= 8.1 Hz, N-H), 8.34 (1H, d, *J*= 7.8 Hz, Ar-H), 7.69 (1H, d, *J*= 8.1 Hz, Ar-H), 7.61-7.53 (4H, m, Ar-H), 7.46-7.27 (14H, m, Ar-H), 7.18 (2H, d, *J*= 1.8 Hz, Ar-H), 7.07-6.87 (5H, m, Ar-H), 5.11 (2H, s, CH_2Ph), 5.08 (2H, s, CH_2Ph), 4.89-4.80 (2H, m, CH), 4.80 (1H, dd, J_1= 13.5 Hz, J_2= 8.7 Hz, CH), 3.84 (3H, s, OCH_3), 3.29-3.12 (3H, m, CH_2), 3.04-2.89 (2H, m, CH_2), 2.78 (1H, dd, J_1= 16.8 Hz, J_2= 6.3 Hz, CH_2); ^{13}CNMR(75MHz, DMSO-d_6) δ 172.0, 171.7, 170.8, 170.3, 164.9, 148.2, 148.0, 141.8, 141.4, 139.4, 136.6, 136.5, 136.4, 136.3, 136.2, 136.1, 134.3, 129.9, 129.1, 128.9, 128.5, 128.4, 128.2, 127.9, 127.8, 124.2, 123.9, 123.8, 122.3, 121.8, 121.7, 121.3, 120.6, 119.0,

118.8, 118.6, 116.2, 113.1, 112.9, 112.7, 111.7, 110.4, 110.3, 110.2, 66.8, 66.4, 55.9, 53.9, 53.7, 49.1, 36.2, 28.7, 28.3; ESIMS *m/z* 1002 (M+1); HRMS calcd for: ($C_{59}H_{50}N_7O_9$ - 1), *m/z* (1000.3676); found, *m/z* (1000.3445).

1-(4-Hydroxy-3-methoxyphenyl)-9H-pyrido[3,4-b]indole-3-carboxyl)-Trp-Trp-Pro-OBzl HOBt (162 mg, 1.20 mmol), DCC (247 mg, 1.20 mmol), **6** (334 mg, 1.00 mmol) in anhydrous THF (10 mL) and NH_2-Trp-Trp-Pro-OBzl (736 mg, 1.20 mmol) in anhydrous THF (5 mL). Yield: 54%. mp 137-139 ºC. $[\alpha]_D^{25}$ -17.5 (c 0.1 in MeOH); IR (cm^{-1}, KBr, neat): 3425, 1635, 1519,1450,748; ^{1}HNMR (500 MHz, DMSO-d_6): δ 11.79 (1H, s, OH), 10.85 (1H, s, N-H), 8.78 (1H, d, *J*= 8.7 Hz, N-H), 8.74 (1H, s, Ar-H), 8.71 (1H, d, *J*= 8 Hz, N-H), 8.39 (1H, d, *J*= 7.5 Hz, Ar-H), 7.69 (1H, d, *J*= 7.5 Hz, Ar-H), 7.61-7.53 (4H, m, Ar-H), 7.44-7.28 (9H, m, Ar-H), 7.19 (1H, s, Ar-H), 7.12 (1H, s, Ar-H), 7.04-6.95 (4H, m, Ar-H), 6.83 (1H, t, *J*= 7 Hz, Ar-H), 5.15 (2H, dd, J_1 = 16.5 Hz, J_2 = 12.5 Hz, CH_2Ph), 4.87 (2H, m, CH), 4.45 (1H, m, CH), 3.81 (3H, s, OCH_3), 3.55 (1H, m, CH_2), 3.29-3.20 (3H, m, CH_2), 3.09-2.98 (2H, m, CH_2), 2.17 (1H, m, CH_2), 1.82 (3H, m, CH_2); ^{13}CNMR(125MHz, DMSO-d_6) δ 172.1, 171.2, 170.6, 164.6, 148.2, 141.8, 141.4, 136.5, 136.3, 134.3, 129.9, 129.1, 128.9, 128.8, 128.5, 128.3, 128.2, 128.1, 127.6, 124.1, 122.4, 121.7, 121.4, 121.2, 120.6, 118.9, 118.8, 118.6, 118.4, 116.1, 113.1, 112.8, 112.6, 111.8, 111.6, 110.1, 109.9, 66.3, 59.1, 55.8, 53.6, 51.5, 47.0, 29.1, 28.7, 27.6, 24.9; ESIMS *m/z* 893 (M); HRMS calcd for: ($C_{53}H_{46}N_7O_7$ - 1), *m/z* (892.2464); found, *m/z* (892.3256).

1-(4-Hydroxy-3-methoxyphenyl)-9H-pyrido[3,4-b]indole-3-carboxyl)-Trp-Trp-Gly-OBzl HOBt (178 mg, 1.32 mmol), DCC (272 mg, 1.32 mmol), **6** (400 mg, 1.20 mmol) in anhydrous THF (10 mL) and NH_2-Trp-Trp-Gly-OBzl (757 mg, 1.32 mmol) in anhydrous THF (5 mL). Yield: 61%. mp 140-142 ºC. $[\alpha]_D^{25}$ -37.8 (c 0.1 in MeOH); IR (cm^{-1}, KBr, neat): 3340, 1658, 1519, 748; ^{1}HNMR (300 MHz, DMSO-d_6): δ 11.76 (1H, s, OH), 10.78 (1H, d, *J*= 4.2 Hz, N-H), 8.72 (1H, d, *J*= 7.5 Hz, N-H), 8.71

(1H, s, Ar-H), 8.51-8.42 (2H, m, N-H), 8.37 (1H, d, *J*= 7.8 Hz, Ar-H), 7.69 (1H, d, *J*= 9.1 Hz, Ar-H), 7.60-7.49 (4H, m, Ar-H), 7.41-7.27 (9H, m, Ar-H), 7.20 (1H, d, *J*= 7.5 Hz, Ar-H), 7.06-6.93 (4H, m, Ar-H), 6.86 (1H, t, *J*= 7.5 Hz, Ar-H), 5.16 (2H, s, CH_2Ph), 4.86 (1H, dd, J_1= 12.3 Hz, J_2= 7.2 Hz, CH), 4.70 (1H, dd, J_1= 13.5 Hz, J_2= 8.4 Hz, CH), 3.95 (2H, dd, J_1= 10.5 Hz, J_2= 4.8 Hz, CH), 3.84 (3H, s, OCH_3), 3.26-3.14 (3H, m, CH_2), 3.02 (1H, dd, J_1= 14.7 Hz, J_2= 8.7 Hz,CH_2); ^{13}CNMR(75MHz, DMSO-d_6) δ 172.5, 171.6, 170.1, 164.8, 148.1, 141.8, 141.4, 139.4, 136.4, 134.3, 129.9, 129.1, 128.9, 128.5, 128.4, 127.9, 127.8, 124.1, 123.8, 122.3, 121.7, 121.3, 120.6, 119.0, 118.8, 118.7, 116.1, 113.1, 112.8, 111.6, 110.4, 110.1, 66.4, 65.4, 56.0, 55.9, 54.8, 53.9, 28.6, 28.3; ESIMS *m/z* 852 (M-1), 853 (M); HRMS calcd for: ($C_{50}H_{44}N_7O_7$ + 1), *m/z* (854.3297); found, *m/z* (854.3394).

1-(4-Hydroxy-3-methoxyphenyl)-9H-pyrido[3,4-b]indole-3-carboxyl)-Trp-Trp-Trp-OBzl HOBt (162 mg, 1.20 mmol), DCC (247 mg, 1.20 mmol), **6** (334 mg, 1.00 mmol) in anhydrous THF (10 mL) and NH_2-Trp-Trp-Trp-OBzl (843 mg, 1.20 mmol) in anhydrous THF (5 mL). Yield: 59%. mp 158-160 ºC. $[\alpha]_D^{25}$ +11.3 (c 0.1 in MeOH); IR (cm^{-1}, KBr, neat): 3356, 1658, 1519, 740; ^{1}HNMR (500 MHz, DMSO-d_6): δ 11.77 (1H, s, OH), 10.89 (1H, brs, N-H), 10.78 (2H, s, N-H), 9.42 (1H, s, N-H), 8.71 (1H, s, Ar-H), 8.69 (1H, d, *J*= 8 Hz, N-H), 8.60 (1H, d, *J*= 7 Hz, N-H), 8.43 (1H, d, *J*= 8 Hz, N-H), 8.33 (1H, d, *J*= 8 Hz, Ar-H), 7.69 (1H, d, *J*= 8 Hz, Ar-H), 7.61-7.51 (5H, m, Ar-H), 7.45-7.36 (2H, m, Ar-H), 7.31-7.28 (6H, m, Ar-H), 7.21 (5H, m, Ar-H), 7.17-7.06 (5H, m, Ar-H), 7.05-7.01 (1H, t, *J*= 7 Hz, Ar-H), 6.93 (1H, t, *J*= 7 Hz, Ar-H), 5.01 (2H, dd, J_1= 34 Hz, J_2= 13 Hz, CH_2Ph), 4.87-4.83 (1H, m, CH), 4.77-4.73 (1H, m, CH), 4.66 (1H, dd, J_1= 14 Hz, J_2= 7 Hz, CH), 3.85 (3H, s, OCH_3), 3.25-3.21 (2H, m, CH_2), 3.19-3.13 (3H, m, CH_2), 3.02 (1H, dd, J_1= 14.5 Hz, J_2= 9 Hz, CH_2); ^{13}CNMR(75MHz, DMSO-d_6) δ 172.2, 172.1, 171.6, 164.9, 148.2, 141.9, 141.5, 139.5, 136.6, 136.5, 136.4, 136.2, 134.5, 129.9, 129.1, 128.8, 128.4, 128.2, 127.9, 127.8, 127.6, 124.1, 123.9, 121.8, 121.5,

121.3, 118.9, 118.6, 116.1, 111.9, 111.7, 110.5, 110.3, 109.7, 66.4, 56.0, 54.0, 53.6, 28.6, 28.3,27.7; ESIMS *m/z* 981 (M-1), 982 (M); HRMS calcd for: ($C_{59}H_{51}N_8O_7$ + 1), *m/z* (983.3875); found, *m/z* (983.3944).

1-(4-Hydroxy-3-methoxyphenyl)-9H-pyrido[3,4-b]indole-3-carboxyl)-Trp-Trp-Phe-OBzl HOBt (162 mg, 1.20 mmol), DCC (247 mg, 1.20 mmol), **6** (334 mg, 1.00 mmol) in anhydrous THF (10 mL) and NH_2-Trp-Trp-Phe-OBzl (815 mg, 1.20 mmol) in anhydrous THF (5 mL). Yield: 70%. mp 139-141 ºC. $[\alpha]_D^{25}$ +10.2 (c 0.1 in MeOH); IR (cm^{-1}, KBr, neat): 3387, 1666, 1519, 748; ^{1}HNMR (300 MHz, DMSO-d_6): δ 11.78 (1H, s, OH), 10.79 (2H, brs, N-H), 8.71 (1H, d, *J* = 6 Hz, N-H), 8.70 (1H, s, Ar-H), 8.59 (1H, d, *J* = 7.2 Hz, N-H), 8.44 (1H, d, *J* = 8.4 Hz, N-H), 8.34 (1H, d, *J* = 8.1 Hz, Ar-H), 7.69 (1H, d, *J* = 8.4 Hz, Ar-H), 7.61-7.53 (4H, m, Ar-H), 7.40 (1H, dd, J_1 = 8.4 Hz, J_2 = 2.1 Hz, Ar-H), 7.42-7.14 (15H, m, Ar-H), 7.07-7.02 (4H, m, Ar-H), 6.83 (1H, t, *J* = 7.5 Hz, Ar-H), 5.05 (2H, dd, *J* = 12.6 Hz, CH_2Ph), 4.84 (1H, dd, J_1 = 12.3 Hz, J_2 = 7.8 Hz, CH), 4.71 (1H, dd, J_1 = 13.2 Hz, J_2 = 8.4 Hz, CH), 4.59 (1H, dd, J_1 = 14.4 Hz, J_2 = 7.2 Hz, CH), 3.84 (3H, s, OCH_3), 3.37-2.94 (6H, m, CH_2); ^{13}CNMR(75MHz, DMSO-d_6) δ 172.1, 171.6, 164.8, 148.2, 148.0, 141.8, 141.4, 139.4, 137.3, 136.5, 136.4, 136.3, 136.2, 136.1, 134.3, 129.9, 129.6, 129.1, 128.8, 128.7, 128.5, 128.4, 127.9, 127.8, 127.0, 124.0, 122.3, 121.8, 121.7, 121.3, 120.6, 119.0, 118.9, 118.6, 116.1, 113.1, 112.8, 111.6, 110.4, 110.2, 66.5, 55.9, 54.4, 53.8, 53.6, 37.2, 28.7, 28.3; ESIMS *m/z* 942 (M-1), 944 (M+1); HRMS calcd for: ($C_{57}H_{50}N_7O_7$ + 1), *m/z* (944.3766); found, *m/z* (944.3890).

1-(4-Hydroxy-3-methoxyphenyl)-9H-pyrido[3,4-b]indole-3-carboxyl)-Trp-Trp-Ser-OBzl HOBt (162 mg, 1.20 mmol), DCC (247 mg, 1.20 mmol), **6** (334 mg, 1.00 mmol) in anhydrous THF (10 mL) and NH_2-Trp-Trp-Ser-OBzl (724 mg, 1.20 mmol) in anhydrous THF (5 mL). Yield: 65%. mp 166-168 ºC. $[\alpha]_D^{25}$ -21.4 (c 0.1 in MeOH); IR (cm^{-1}, KBr, neat): 3371, 1658, 1519,740; ^{1}HNMR (500 MHz,

DMSO-d_6): δ 11.80 (1H, s, OH), 10.86 (1H, d, J= 9.5 Hz, N-H), 9.48 (1H, brs, N-H), 8.72 (1H, d, J= 8.0 Hz, N-H), 8.71 (1H, s, Ar-H), 8.53 (1H, d, J= 7.5 Hz, N-H), 8.46 (1H, d, J= 9.0 Hz, N-H), 8.37 (1H, d, J= 9.0 Hz, Ar-H), 7.70 (1H, d, J= 9.0 Hz, Ar-H), 7.59 (3H, t, J= 9.0 Hz, Ar-H), 7.53 (1H, d, J = 1.5 Hz, Ar-H), 7.40 (3H, d, J= 7.0 Hz, Ar-H), 7.36 (2H, t, J= 7.5 Hz, Ar-H), 7.33-7.27 (4H, m, Ar-H), 7.20 (1H, s, Ar-H),7.16 (1H, s, Ar-H), 7.05 (1H, d, J= 9.0 Hz, Ar-H), 7.03-6.95 (2H, m, Ar-H), 6.93 (1H, t, J= 7.5 Hz, Ar-H), 6.84 (1H, t, J= 7.5 Hz, Ar-H), 5.19 (2H, s, CH_2Ph), 4.87-4.77 (1H, m, CH), 4.69-4.65 (1H, m, CH), 4.51 (1H, dd, J_1= 10 Hz, J_2= 5 Hz, CH), 3.84 (3H, s, OCH_3), 3.81-3.67 (2H, m, CH_2), 3.30-3.16 (1H, m, CH_2), 3.19-3.15 (2H, m, CH_2), 3.02 (1H, dd, J_1= 15 Hz, J_2= 9 Hz, CH_2); ^{13}CNMR(125MHz, DMSO-d_6) δ 172.2, 171.5, 170.8, 164.9, 148.2, 141.8, 139.4, 136.5, 136.4, 136.3, 134.3, 129.9, 129.1, 128.9, 128.8, 128.4, 128.1, 128.0, 127.9, 127.8, 124.0, 123.8, 122.3, 121.8, 121.7, 121.2, 120.6, 119.0, 118.9, 118.7, 116.2, 112.9, 112.6, 111.7, 111.6, 110.5, 110.4, 110.2, 110.1, 66.4, 61.7, 56.0, 55.3, 53.9, 53.5, 28.6, 28.3; ESIMS *m/z* 883 (M); HRMS calcd for: ($C_{51}H_{44}N_7O_8$ - 1), *m/z* (882.3257); found, *m/z* (882.3246).

1-(4-Hydroxy-3-methoxyphenyl)-9H-pyrido[3,4-b]indole-3-carboxyl)-Trp-Trp-Met-OBzl HOBt (162 mg, 1.20 mmol), DCC (247 mg, 1.20 mmol), **6** (334 mg, 1.00 mmol) in anhydrous THF (10 mL) and NH_2-Trp-Trp-Met-OBzl (777 mg, 1.20 mmol) in anhydrous THF (5 mL). Yield: 61%. mp 173-175 ºC. $[\alpha]_D^{25}$ -38.9 (c 0.1 in MeOH); IR (cm^{-1}, KBr, neat): 3356, 1658, 1519, 740; ^{1}HNMR (500 MHz, DMSO-d_6): δ 11.81 (1H, s, OH), 10.83 (1H, d, J= 12 Hz, N-H), 8.72 (1H, d, J= 7.5 Hz, N-H), 8.70 (1H, s, Ar-H), 8.50 (1H, d, J= 7.5 Hz, N-H), 8.47 (1H, d, J= 8 Hz, N-H), 8.36 (1H, d, J= 8 Hz, Ar-H), 7.70 (1H, d, J= 8 Hz, Ar-H), 7.61-7.54 (4H, m, Ar-H), 7.42-7.28 (9H, m, Ar-H), 7.19 (2H, d, J= 4 Hz, Ar-H), 7.08 (1H, d, J= 8.5 Hz, Ar-H), 7.03-7.01 (2H, m, Ar-H), 6.96 (1H, t, J= 7.5 Hz, Ar-H), 6.87 (1H, t, J= 7.5 Hz, Ar-H), 5.17 (2H, dd, J_1= 34 Hz, J_2= 13 Hz, CH_2Ph), 4.84-4.80 (1H, m, CH), 4.70

(1H, dd, J_1 = 9 Hz, J_2 = 5 Hz, CH), 4.53 (1H, dd, J_1 = 9 Hz, J_2 = 5 Hz, CH), 3.85 (3H, s, OCH_3), 3.25 (1H, dd, J_1 = 14.5 Hz, J_2 = 4 Hz, CH_2), 3.19-3.14 (2H, m, CH_2), 3.03 (1H, dd, J_1 = 15 Hz, J_2 = 9 Hz, CH_2), 2.50-2.46 (2H, m, CH_2), 2.05-1.96 (2H, m, CH_2); ^{13}CNMR(125MHz, DMSO-d_6) δ 172.2, 171.9, 171.6, 148.2, 148.1, 141.5, 139.3, 136.4, 136.3, 129.9, 129.1, 128.9, 128.6, 128.4, 127.9, 127.8, 124.0, 123.8, 122.3, 121.8, 121.7, 121.3, 120.6, 118.9, 118.8, 118.7, 116.2, 112.8, 112.7, 111.7, 110.5, 110.1, 66.6, 56.0, 53.9, 53.6, 51.7, 30.9, 29.9, 28.6, 28.0, 15.0; ESIMS *m/z* 927 (M); HRMS calcd for: ($C_{53}H_{48}N_7O_7S$ - 1), *m/z* (926.3341); found, *m/z* (926.3370).

1-(4-Hydroxy-3-methoxyphenyl)-9H-pyrido[3,4-b]indole-3-carboxyl)-Trp-Trp-Leu-OBzl HOBt (162 mg, 1.20 mmol), DCC (247 mg, 1.20 mmol), **6** (334 mg, 1.00 mmol) in anhydrous THF (10 mL) and NH_2-Trp-Trp-Leu-OBzl (755 mg, 1.20 mmol) in anhydrous THF (5 mL). Yield: 75%. mp 156-158 ºC. $[\alpha]_D^{25}$ +30.6 (c 0.1 in MeOH); IR (cm^{-1}, KBr, neat): 3371, 1666, 1519, 748; ^{1}HNMR (300 MHz, DMSO-d_6): δ 11.78 (1H, s, OH), 10.81 (1H, s, N-H), 10.79 (1H, s, N-H), 9.45 (1H, s, N-H), 8.70 (1H, d, J = 9.1 Hz, N-H), 8.68 (1H, s, Ar-H), 8.44 (2H, d, J = 7.8 Hz, N-H), 8.34 (1H, d, J = 9.1 Hz, Ar-H), 7.69 (1H, d, J = 8.4 Hz, Ar-H), 7.60-7.53 (4H, m, Ar-H), 7.46-7.26 (9H, m, Ar-H), 7.17 (2H, s, Ar-H), 7.07-6.95 (4H, m, Ar-H), 6.87 (1H, t, J = 7.5 Hz, Ar-H), 5.14 (2H, s, CH_2Ph), 4.82 (1H, dd, J_1 = 12.3 Hz, J_2 = 7.5 Hz, CH), 4.70 (1H, dd, J_1 = 13.2 Hz, J_2 = 8.4 Hz, CH), 4.42 (1H, dd, J_1 = 13.2 Hz, J_2 = 8.4 Hz, CH), 3.84 (3H, s, OCH_3), 3.24-3.12 (3H, m, CH_2), 3.15 (1H, dd, J_1 = 17.4 Hz, J_2 = 7.2 Hz, CH_2); ^{13}CNMR(75MHz, DMSO-d_6) δ 172.6, 172.2, 171.6, 164.9, 148.2, 148.0, 141.8, 141.4, 139.4, 136.6, 136.4, 136.3, 134.3, 129.9, 129.1, 128.9, 128.6, 128.3, 127.9, 127.8, 124.2, 123.9, 123.8, 122.3, 121.8, 121.7, 121.3, 120.6, 119.0, 118.9, 118.6, 116.1, 113.1, 112.8, 112.6, 111.7, 110.5, 110.1, 66.4, 55.9, 53.9, 53.6, 51.1, 40.77, 28.6, 28.1, 24.6, 23.2, 21.9; ESIMS *m/z* 909 (M); HRMS calcd for: ($C_{54}H_{52}N_7O_7$ + 1), *m/z* (910.3922); found, *m/z* (910.3965).

In vitro cytotoxicity

In vitro cytotoxicity of the compounds synthesized were determined in HT-29, A549, K562 and HL-60 cell lines using the MTT assay according to the method described by Al-Allaf and Rashan.[22] Cells were grown in DMEM or RPMI-1640 medium containing 10% (v/v) fetal bovine serum and 10,000 U/mL penicillin and 100 ug/mL streptomycin in a humidified incubator containing 5% CO_2 at 37 °C for 4 h. **7-21** of different concentrations (ranging from 1 to 400 μM) in DMSO were prepared. To each well of the 96-well plates, 25 μL of medium containing test compounds of different concentrations of **7-21** were added. The final concentration of DMSO in the growth medium was 1% (v/v). After 48 h, 25 μL (final concentration, 0.5 mg/mL) of MTT were added to each well, and plates were returned to incubator for an additional 4 h. Then culture medium was then removed. The resulting MTT–formazan product was dissolved in 100 μL DMSO, and the absorbance at 570 nm was determined. IC_{50} is defined as the concentration that results in a 50% inhibition of tumor cell growth.

Determination of in vivo anti-tumor activity

All animal experiments were conducted in accordance with China's National Guide for the Care and Use of Laboratory Animals. The experimental procedures were approved by the Committee on Animal Care and Usage, Capital Medical University, and all efforts were made to minimize animal suffering. This may be refined by selecting an injection site that will cause minimal pain and distress; using sharp needles of the narrowest possible gauge and catching mice by cupping in their home cage tunnel, instead of by the tail. This method of capture is less aversive and induces less anxiety. Male ICR mice (10-12 weeks old), purchased from Peking University Health Science Center, were maintained at 21˚C with a natural day/night cycle in a conventional animal colony. Food and water were supplied *ad libitum*. The breeding animals were maintained on solid floors with softwood shavings for bedding and

nesting. All animals were allowed one week to adapt to their environment before the treatment. S180 tumor cells passaged in mouse abdomen were harvested once a week. Mice were inoculated with the harvested S_{180} tumor cells (1×10^7 cells per mouse) by subcutaneous injection. Twenty-four hours after inoculation **7-21** dissolved in 0.9% saline were injected by intraperitoneal (i.p.) to mice (12 mice per group, 0.2 mL per mouse) at 0.1 μmol/kg for seven consecutive days. ADM at 0.1 μmol/kg (0.2 mL) dissolved in 0.9% saline was used as positive control and 0.2 mL of 0.9% saline was used as negative control. All the tested compounds did not cause obvious neurotoxic reaction including tremor, twitch, jumping, tetanus and supination at tested dosage. The weights of animals were recorded every day. Most of compounds showed weight gain similar to that of the vehicle-treat group. Only two compounds (**19, 21**) showed significantly lower increases in body weight. It is observed that only languishment was significant indicator of distress and the size of the tumor was the most significant predictor of the animal's condition. There were strong correlations between the incremental size of the tumor and further deterioration in the animal's condition. Twenty-four hours after the last administration, all mice were weighed, decapitated and dissected immediately to obtain the weight of the tumor. The surgical procedures were performed by personnel authorized by Beijing Association on Laboratory Animal Care.

The tumor inhibition % was calculated as: (C–T)/C×100

T: average tumor weight of treated group; C: average tumor weight of negative control group.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure and Scheme captions

Figure 1.

UV spectra of **14**(concentration 2 μM) in the absence and presence of CT DNA (pH = 7.4) of 2.0, 4.0, 6.0, 8.0, 10.0 and 32.0 μM, respectively.

Figure 2.

Fluorescence spectra of **14** (concentration 2 μM) in the absence and presence of CT DNA (pH = 7.4) of 1.0, 2.0, 4.0, 8.0, 16.0 and 32.0 μM, respectively.

Figure 3.

Effect of **14** on the relative viscosity of CT DNA (200 mM).

Scheme 1.

Preparation of NH_2-Trp-Trp-AA-OBzl. AA=Tyr, Glu, Thr, Lys, Val, Ile, Ala, Asp, Pro, Gly, Trp, Phe, Ser, Met or Leu. Reagents and conditions: (i) NMM, DCC, HOBt; (ii) 2M NaOH; (iii) NH_2-AA-OBzl, NMM, DCC, HOBt; (iv) hydrogen chloride in ethyl acetate (4 mol/L).

Scheme 2.

Synthesis of 1-(4-hydroxy-3-methoxyphenyl)-β-carboline-3-carboxyl)-Trp-Trp-AA-OBzl compounds [a]

[a] Compound 7 (AA= Tyr), 8 (AA= Glu), 9 (AA= Thr), 10 (AA= Lys), 11 (AA= Val), 12 (AA= Ile), 13 (AA= Ala), 14 (AA= Asp), 15 (AA= Pro), 16 (AA= Gly), 17 (AA= Trp), 18 (AA= Phe), 19 (AA= Ser), 20 (AA= Met), 21 (AA=Leu). Reagents and conditions: (i) AcOH; (ii) $SOCl_2$ and MeOH; (iii) SeO_2 and AcOH; (iv) 6M NaOH; (v) NH_2-Trp-Trp-AA-OBzl, NMM, DCC, HOBt.

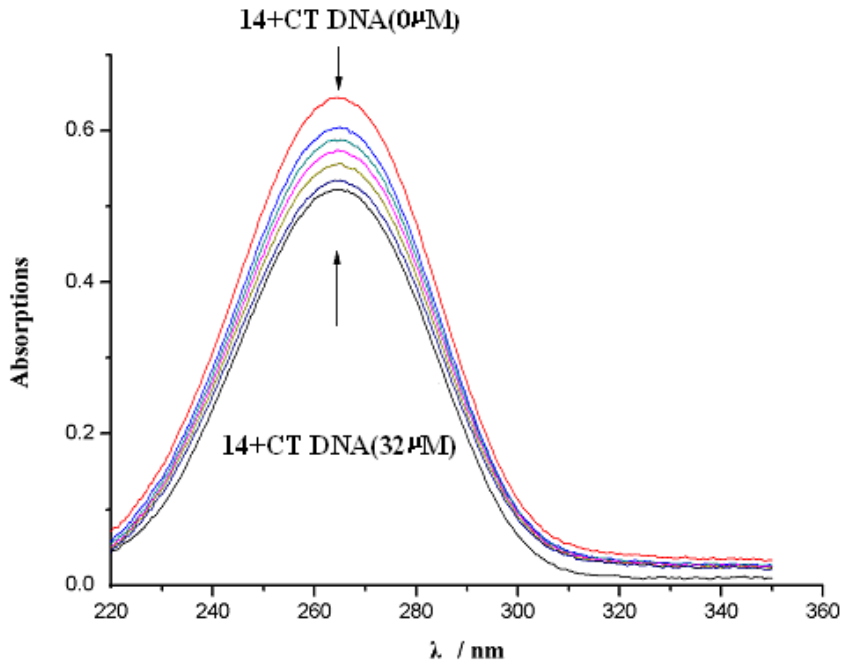


Figure 1.

UV spectra of **14**(concentration 2 μM) in the absence and presence of CT DNA (pH = 7.4) of 2.0, 4.0, 6.0, 8.0, 10.0 and 32.0 μM, respectively.

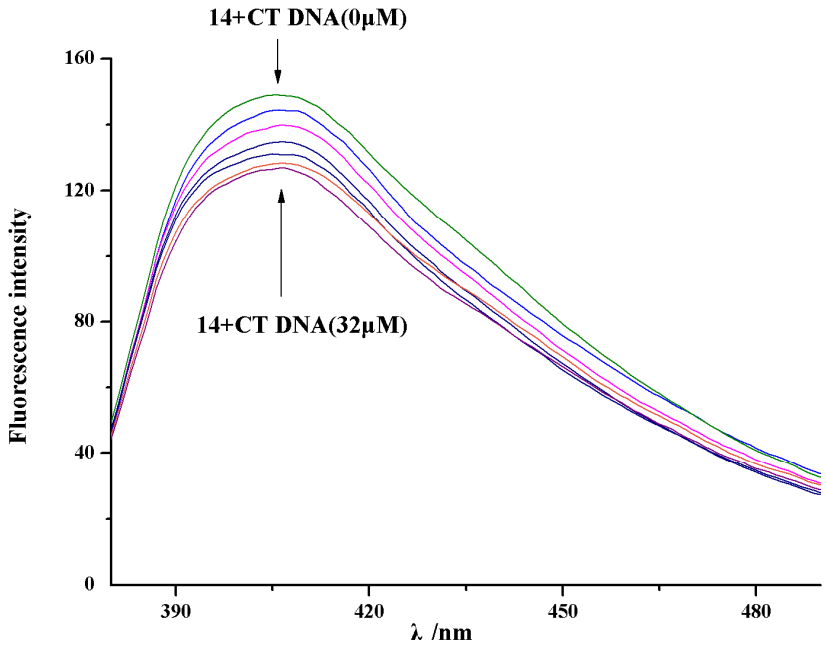


Figure 2.

Fluorescence spectra of **14** (concentration 2 μM) in the absence and presence of CT DNA (pH = 7.4) of 1.0, 2.0, 4.0, 8.0, 16.0 and 32.0 μM, respectively.

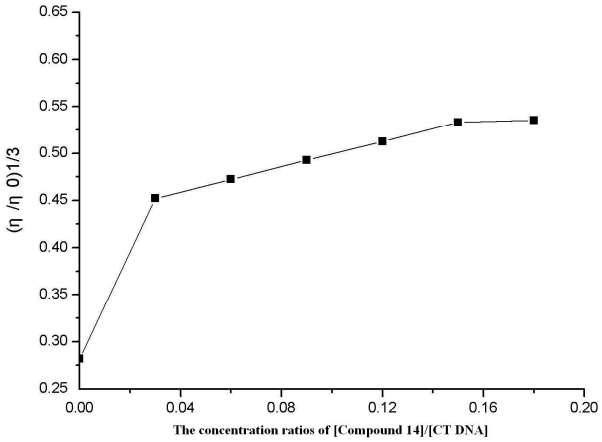
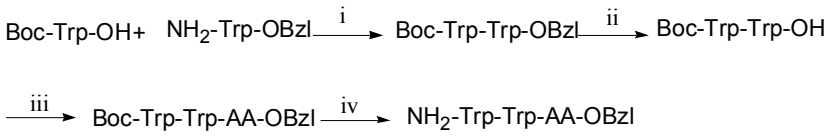


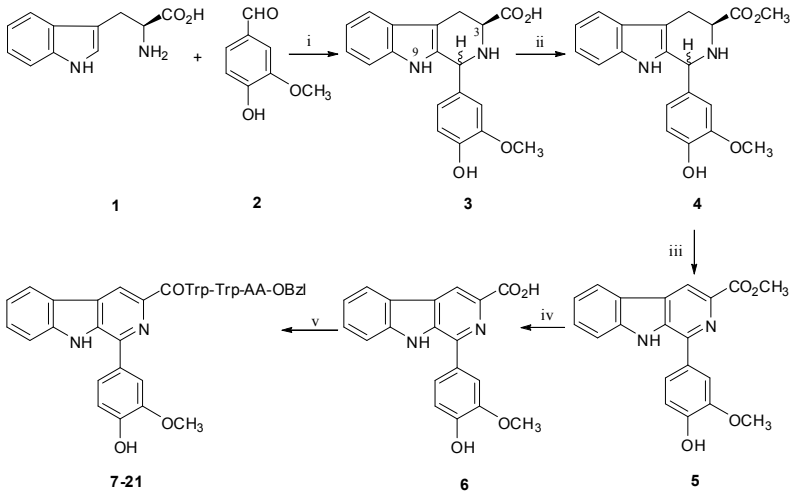
Figure 3.

Effect of **14** on the relative viscosity of CT DNA (200 mM).



Scheme 1.

Preparation of NH_2-Trp-Trp-AA-OBzl. AA=Tyr, Glu, Thr, Lys, Val, Ile, Ala, Asp, Pro, Gly, Trp, Phe, Ser, Met or Leu. Reagents and conditions: (i) NMM, DCC, HOBt; (ii) 2M NaOH; (iii) NH_2-AA-OBzl, NMM, DCC, HOBt; (iv) hydrogen chloride in ethyl acetate (4 mol/L).



Scheme 2.

Synthesis of 1-(4-hydroxy-3-methoxyphenyl)-β-carboline-3-carboxyl)-Trp-Trp-AA-OBzl compounds [a]

[a] Compound 7 (AA= Tyr), 8 (AA= Glu), 9 (AA= Thr), 10 (AA= Lys), 11 (AA= Val), 12 (AA= Ile), 13 (AA= Ala), 14 (AA= Asp), 15 (AA= Pro), 16 (AA= Gly), 17 (AA= Trp), 18 (AA= Phe), 19 (AA= Ser), 20 (AA= Met), 21 (AA=Leu). Reagents and conditions: (i) AcOH; (ii) $SOCl_2$ and MeOH; (iii) SeO_2 and AcOH; (iv) 6M NaOH; (v) NH_2-Trp-Trp-AA-OBzl, NMM, DCC, HOBt.

Table 1

Structures of β-carboline derivatives synthesized

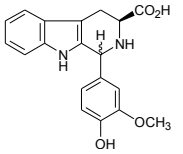
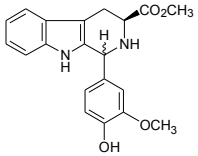
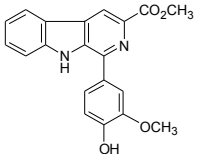
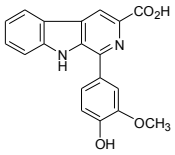
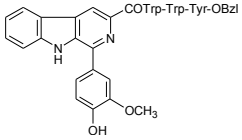
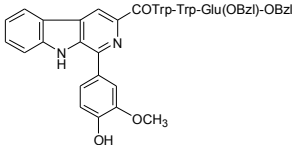
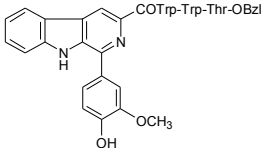
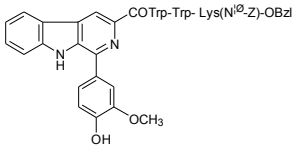
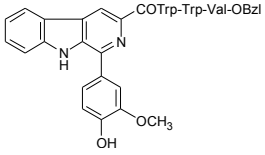
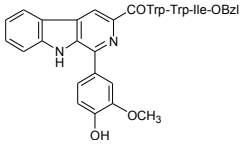
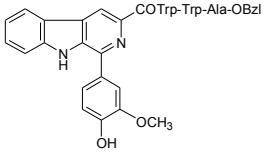
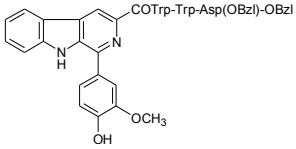
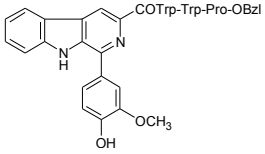
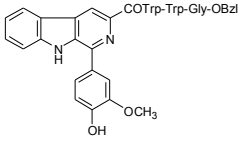
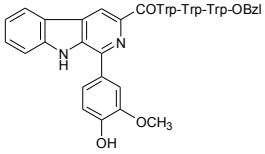
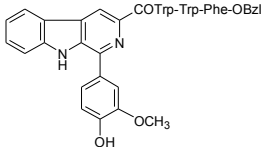
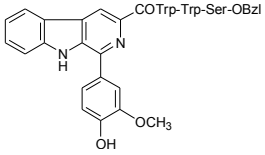
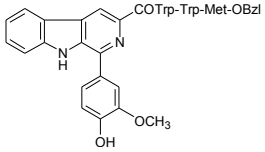
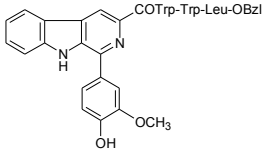
Compound No	Chemical structure	Compound No	Chemical structure
3		**4**	
5		**6**	
7		**8**	
9		**10**	
11		**12**	
13		**14**	
15		**16**	
17		**18**	
19		**20**	
21			

Table 2

IC_{50} values of **7-21** in K562, HT-29, HL-60 and A549 cell lines

Compounds	K562	HT-29	HL60	A549
7	34.0±3.1	>200	50.0±1.7	76.5±1.4
8	35.0±1.2	>200	28.5±0.9	52.0±1.4
9	17.3±3.5	>200	45.0±2.5	56.4±1.8
10	43.5±0.9	>200	39.0±0.9	113.1±1.5
11	43.0±1.2	>200	60.0±1.5	162.2±2.8
12	12.0±1.6	>200	84.0±3.1	140.0±1.1
13	25.1±0.9	>200	134.0±2.5	>200
14	30.2±1.1	>200	37.5±1.4	77.0±0.8
15	13.7±2.1	>200	51.0±2.4	98.0±1.7
16	86.3±1.9	>200	>200	175.0±0.8
17	36.4±1.5	>200	40.3±2.2	63.4±2.4
18	15.3±1.9	>200	33.1±1.9	79.0±1.5
19	18.6±2.6	>200	34.5±1.8	102.0±2.1
20	66.0±2.3	99.5±1.3	16.0±1.9	92.1±1.9
21	14.3±1.2	>200	68.0±2.5	114.0±0.7
ADM	1.3±0.1	8.1±0.6	1.7±0.4	3.5±0.6

Results shown are $\bar{x} \pm$SD μM; n = 6.

Table 3

Tumor inhibition % by **7-21** in S180 bearing mice

Compound	Dose (μmol/kg)	Tumor weight (g)	Tumor inhibition%
NS		1.24±1.30	
ADM	0.1	0.45±0.11	61.1±6.9
7	0.1	0.53±0.13[b]	57.3±10.4
8	0.1	0.68±0.18[c]	45.5±14.8
9	0.1	0.61±0.17[b]	51.1±13.8
10	0.1	0.66±0.13[c]	46.6±10.3
11	0.1	0.84±0.23[c]	31.9±18.6
12	0.1	0.59±0.14[b]	52.4±11.7
13	0.1	0.60±0.16[b]	51.9±13.0
14	0.1	0.45±0.11[b]	63.4±8.7
15	0.1	0.68±0.19[c]	45.1±15.6
16	0.1	0.62±0.16[b]	50.4±12.9
17	0.1	0.64±0.12[c]	48.1±9.5
18	0.1	0.73±0.16[c]	41.2±13.5
19	0.1	0.46±0.13[b]	62.9±10.7
20	0.1	0.62±0.12[b]	50.0±9.5
21	0.1	0.83±0.21[c]	33.0±16.9

ADM = Positive control, NS = Vehicle, n = 12, tumor weight is expressed by $\overline{X} \pm$ SD g.

[b]Compared to NS $p < 0.01$, to 0.1 μmol/kg ADM $p > 0.05$.

[c]Compared to NS $p < 0.01$, to 0.1 μmol/kg ADM $p < 0.05$.

Table 4

Tumor inhibition% by **14** at different doses in S180 bearing mice

Compound[a]	Dose (μmol/kg)	Tumor weight (g)	Tumor inhibition%
NS		1.24±1.30	
ADM	0.1	0.45±0.11	61.1±6.9
Compound 14	0.1	0.45±0.11[b]	63.4±8.7
Compound 14	0.01	0.69±0.14[c]	44.3±11.2
Compound 14	0.001	0.83±0.11[d]	32.7±8.5

[a]ADM = Positive control, NS = Vehicle, n = 12.

[b]Compared to NS and 0.01 μmol/kg of **14** $p < 0.01$.

[c]Compared to NS and 0.001 μmol/kg of **14** $p < 0.01$,

to 0.1 μmol/kg ADM $p < 0.01$.

[d]Compared to NS $p < 0.01$, to 0.1 μmol/kg ADM $p < 0.01$.

Table 5

Effect of **7-21** on the body weight increase of S180 mice

Compound[a]	Increased BW	Compound[a]	Increased BW
NS	6.03±2.75	**14**	5.90±3.04
ADM	6.64±2.54	**15**	8.78±2.32
7	6.16±3.65	**16**	7.23±2.99
8	5.64±3.16	**17**	6.11±1.69
9	6.76±2.78	**18**	7.07±2.92
10	6.96±1.68	**19**	4.61±2.98[b]
11	7.05±2.65	**20**	6.06±2.82
12	6.42±2.82	**21**	3.67±3.41[b]
13	5.93±3.07		

[a]Dose of ADM and **7-21**: 0.1 μmol/kg, NS = Vehicle, n = 12,

increased BW = increased body weight is expressed by $\overline{X} \pm$ SD g.

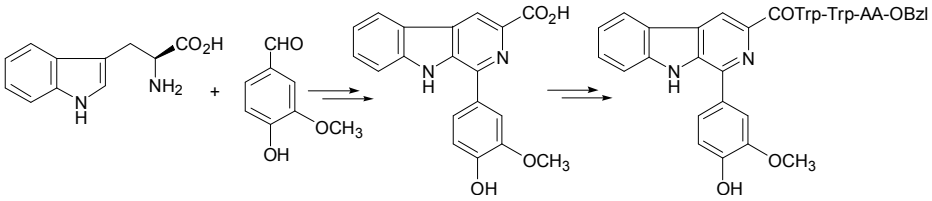
[b]Compared to NS $p < 0.05$

Graphic Abstract

Synthesis and biological evaluation of a novel class of β-carboline derivatives

Hao Chen, Pengchao Gao, Meng Zhang, Wei Liao, Jianwei Zhang*

β-carboline was modified with amino acids and several novel β-carboline analogues were obtained.



Wherein AA = amino acids.