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Synthesis and cytotoxic activities of β-carboline amino acid ester conjugates

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Abstract—β-Carboline represents a class of compounds with potent anti-tumor activity by intercalating with DNA. To further enhance the cytotoxic potency and bioavailability of β-carboline, a series of novel β-carboline amino acid ester conjugates were designed and synthesized, and the cytotoxic activities of these compounds were tested using a panel of human tumor cell lines. In addition, the membrane permeability of these compounds was evaluated in vitro using a Caco-2 cell monolayer model. The β-carboline amino acid ester conjugates demonstrated improved cytotoxic activity compared to the parental β-carbolines. In particular, the Lys/Arg conjugates were the most potent analogs with an IC_{50} value of 4 and 1 μM against human cervical carcinoma cells. The low interaction energy of Arg conjugate based on molecular modeling may contribute to its enhanced cytotoxicity. Taken together, this study provided new insights into structure–activity relationships in the β-carboline amino acid ester conjugates and identified the β-carboline Lys/Arg conjugates as promising lead compounds for further in vivo biological and molecular evaluation.
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1. Introduction

The discovery of new compounds with potent anti-tumor activity is one of the most important goals in medicinal chemistry. One interesting group of chemotherapeutic agents used in cancer therapy comprises molecules, such as cisplatin and CPT-11, target directly to DNA.[1] During the past decades, numerous studies have demonstrated DNA recognizing molecules that act as anticancer agents, including groove binders, alkylating and intercalator compounds. Among these, DNA intercalators represent one of the most important classes of anticancer drugs in clinical oncology, and a few representatives (anthracyclines, acridines, and anthraquinones) are routinely used in the clinic for the treatment of cancers.[1] DNA intercalating agents are applied to those compounds having a planar polycyclic aromatic residue capable of stacking between the DNA pairs at the intercalation sites.[2,3] Intercalation causes conformational changes in the double helix, accordingly, DNA replication, transcription, and repair are altered.[4] As a consequence, they interfere with these functions resulting in killing of fast growing cells to create a therapeutic window.[5] Therefore, potential applications of DNA intercalators can be directed toward anticancer drugs.[6–8]

Recently, a number of compounds that intercalate with DNA have been synthesized and tested for their antitumor activities.[9–16] For example, some β-carboline alkaloids such as harmine and its derivatives were highly cytotoxic against human tumor cell lines.[17–23] The further mechanistic studies indicated that the β-carbolines could intercalate into the DNA helix and inhibit DNA topoisomerase I and II to cause DNA damage.[24–28] The studies implicated a direct correlation between DNA intercalating ability and cytotoxicity.[24–28]

As part of our ongoing efforts to discover natural products with potent anti-tumor activity,[29,30] we recently

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designed and synthesized a series of β-carboline amino acid ester conjugates. Amino acids are attractive substrates because they are not only the fundamental building blocks of biological systems and many natural products, they also possess structurally diverse side chains. It is well known that many amino acids with functional side chains are capable of making base-specific contacts with more than one type of DNA bases.[31] With this in mind, we introduced a series of amino acids containing either nonpolar, acidic, basic, or aromatic sides, various functionalities with absolute stereochemistry into the β-carbolines' core. We postulated that the conjugation of amino acids with the β-carbolines could modulate the DNA intercalating capability and consequently improve their biological activity through the possible synergetic interactions between DNA with the structurally diverse amino acid side chains and β-carbolines. It was anticipated that the introduction of amino acid side chains would provide extra structural contribution to enhance β-carbolines' interaction capability with DNA.

Another reason for the design of amino acid conjugates of β-carbolines was to improve their bioavailability. Anticancer drug efficiency is often limited by poor bioavailability. In general, the bioavailability of the antitumor agents not only affects the absorption and transportation of the antitumor agents, but also affects the cytotoxic potencies of the antitumor agents. Thus, an attractive approach to improve and optimize the pharmacologic profiles of antitumor agents is to increase their oral bioavailability. Previous studies on the pharmacokinetic disposition revealed that the oral bioavailability of β-carbolines was relatively low (<20%).[32] Considering amino acid conjugates might target the gastrointestinal transporters involved in the absorption of amino acids and small peptides therefore result in improved oral bioavailability,[33–36] amino acids were engineered with β-carbolines to improve the bioavailability and biological activity. In this regard, we expect that amino acid conjugate approach would be a useful strategy for improving the intestinal absorption of drug with low oral bioavailability.[33–38]

In this study, a series of novel β-carboline derivatives were synthesized, and their cytotoxic activities were tested against a panel of human tumor cell lines. In addition, the structure–activity relationship studies of substitution preferences for enhanced cytotoxic activity have been elucidated. Furthermore, the membrane permeability of the derivatives was evaluated using a Caco-2 cell monolayer experiment.

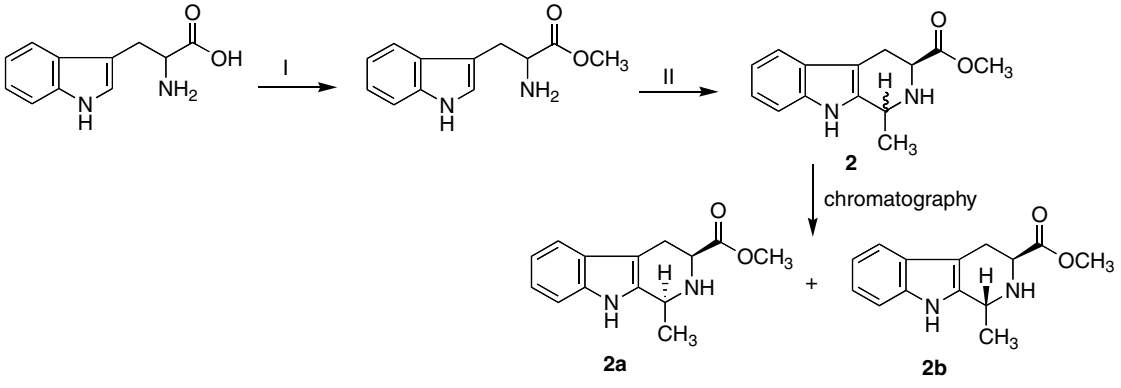
2. Results and discussion

2.1. Synthesis of β-carboline derivatives

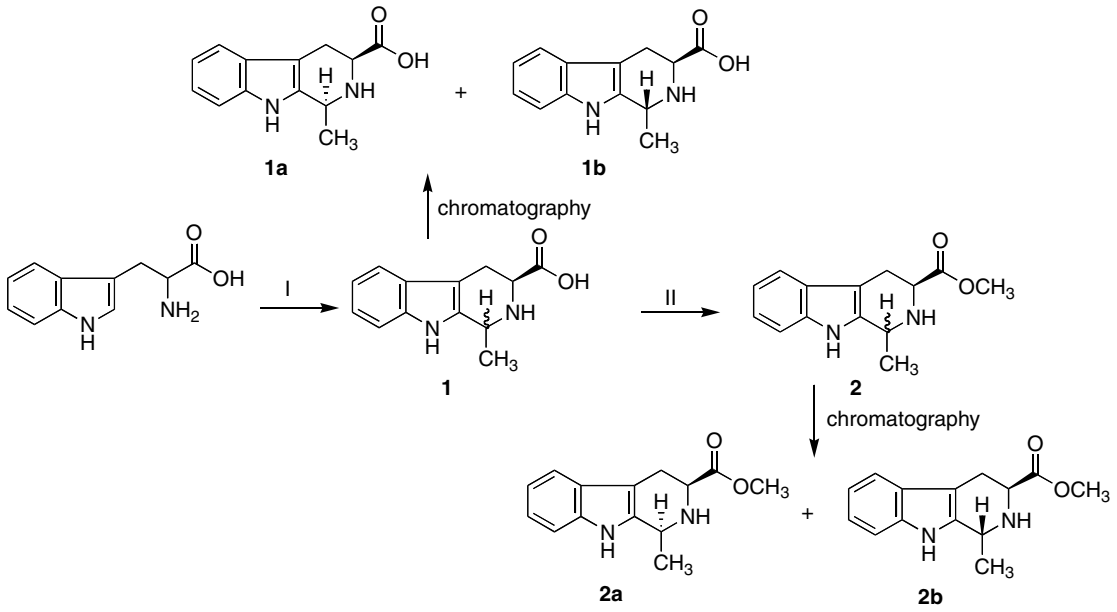
The synthesis of β-carboline derivatives was accomplished by adopting the Pictet–Spengler cyclization, which involves the acid-catalyzed cyclocondensation of β-arylethylamine with an aldehyde or ketone. The synthetic route is outlined in Schemes 1–3. Starting from the optically active L-tryptophane, after esterification of the carboxylic moiety with $MeOH/SOCl_2$, the resulting L-tryptophane methyl ester was then subjected to Pictet–Spengler cyclization with aldehyde to yield methyl 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate **2** in 47% yield. The cyclization of the chiral substrate was carried out using sulfuric acid as catalyst, 2:1 *trans*/*cis* (**2a**/**2b**) diastereomers of tetrahydro-β-carboline derivatives were isolated by chromatography.

To control the chirality in the Pictet–Spengler reaction for the enantiospecific synthesis of indole alkaloids,[30a,b] the effect of substitution on the stereospecificity was investigated. When L-tryptophane was directly employed as the substrate, the Pictet–Spengler reaction provided *trans*/*cis* diasetereomers **1a**/**1b** in a ratio of 16:1 (85% yield). After the esterification, *trans* diastereomer of the carboxylate methyl ester **2a** was generated as the predominant product (*trans*/*cis* 16:1). The increase in stereoselectivity in favor of the *trans* diastereomer was notable, which was probably due to the influence of the substituents on the stereospecificity.

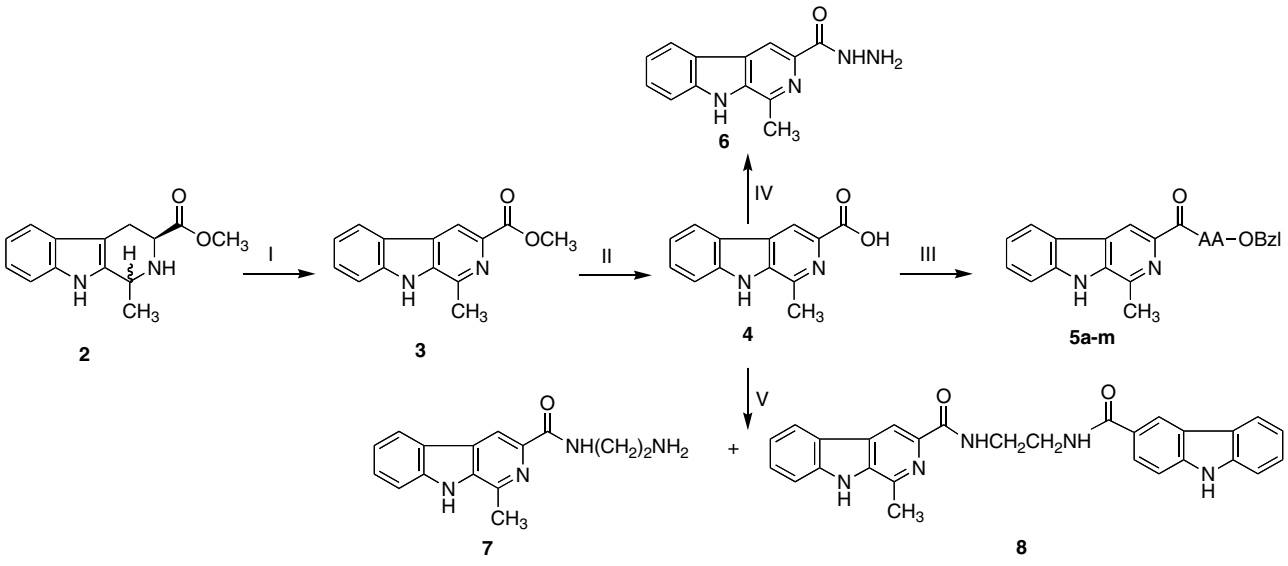
The conversion of the 1,2,3,4-tetrahydro-β-carboline derivative **2** to the corresponding β-carboline derivative **3** was carried out via a different oxidizing approach either by oxidation of the tetrahydro-β-carboline derivatives with sulfur in refluxing xylene, or with lead tetraacetate in glacial acetic acid, or with potassium



Scheme 1. Synthetic route of methyl-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate (**2a**,**b**). Reagents and conditions: (I) thionyl chloride and methanol; (II) sulfuric acid (cat) and aldehyde, rt.



Scheme 2. Alternative synthetic route of methyl-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate (**2a**,**b**). Reagents: (I) sulfuric acid and aldehyde; (II) thionyl chloride and methanol.



Scheme 3. Synthesis of β-carboline derivatives **5a**–**n**, **6**–**8**. Reagents and conditions: (I) potassium hypermanganate/DMF or sulfur flour/xylene or lead tetraacetate/HOAc; (II) sodium hydroxide (aq); (III) DCC/DMAP, HCl–Gly-OBzl for **5a**; HCl–Ala-OBzl for **5b**; HCl–Val-OBzl for **5c**; HCl–Phe-OBzl for **5d**; HCl–Thr-OBzl for **5e**; HCl–Tyr-OBzl for **5f**; HCl-Leu-OBzl for **5g**; HCl–Ile-OBzl for **5h**; HCl–Pro-OBzl for **5i**; HCl–Glu-OBzl for **5j**; HCl–Asp-OBzl for **5k**; HCl–Ser-OBzl for **5l**; HCl–Lys(Dde)-OBzl for **5m**; HCl–Arg-OBzl for **5n**. (IV) $NH_2NH_2H_2O$; (V) $NH_2CH_2CH_2NH_2$. In **5a**: AA = Gly; in **5b**: AA = Ala; in **5c**: AA = Val; in **5d**: AA = Phe; in **5e**: AA = Thr; in **5f**: AA = Tyr; in **5g**: AA = Leu; in **5h**: AA = Ile; in **5i**: AA = Pro; in **5j**: AA = Glu; in **5k**: AA = Asp; in **5l**: AA = Ser; in **5m**: AA = Lys; in **5n**: AA = Arg.

hypermanganate in DMF. Sulfur oxidation has the highest (65%) yield. Subsequently, compound **3** was hydrolyzed in the presence of sodium hydroxide to provide the key intermediate **4** (82% yield), 1-methyl-β-carboline-3-carboxylic acid, which was then subjected to the coupling reaction to generate a series of β-carboline amino acid ester conjugates **5a**–**n** (8–53% yield).

For the comparison with β-carboline Lys/Arg conjugate, the diamine chains were introduced to the β-carboline system. Refluxing of compound **3** with 85% hydrazine hydrate in methanol generated the hydrazide **6** with 38% yield. Similarly, the amidation of compound **3** and ethylenediamine yielded compound **7** (71% yield) and bis-β-carboline derivative **8** with 2% yield.

2.2. In vitro cytotoxicity screening of β-carboline derivatives

The cytotoxic potential of all synthesized β-carboline derivatives was evaluated against a panel of human tumor cell lines using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay to determine the drug concentration required to inhibit the growth of human cancer cells by 50% (IC_{50}) after 72 h.[39–41] The IC_{50} results are summarized in Table 1.

Our efforts to improve the cytotoxic activity of β-carboline derivatives resulted in the compounds **5a**–**n** and **6**–**8**. At the 3-position of β-carboline ring, we introduced the different amino acids to yield a series of β-carboline amino acid ester conjugates **5a**–**n**. These amino acids provide a variety of functional groups, which including the cationic residue (Lys, Arg), aromatic (Tyr, Trp, Phe), anionic (Glu, Asp), and neutral aliphatic (Ser, Thr, Gly, Ala, Leu, Val, Ile). Interestingly, as shown in Table 1, compared with the parent β-carbolines (**3**: IC_{50} = 121–167 μM; **4**: IC_{50} = 140–189 μM), introduction of amino acids to the β-carboline core showed enhanced cytotoxic activity (except for Tyr-, Pro conjugate). The incorporation of Gly showed moderate improvement in cytotoxicity activities against HeLa, MCF-7, and HepG-2 with IC_{50} values of 50, 48, and 45 μM, respectively. While other neutral aliphatic amino acid conjugates endowed with good cytotoxic activity against HeLa cell lines. (Thr: IC_{50} = 27 μM; Ala: IC_{50} = 19 μM; Leu: IC_{50} = 36 μM; Val: IC_{50} = 38 μM; Ile: IC_{50} = 27 μM). Although Ala and Val conjugates share a similar hydrophobic residue, they exhibited some difference in cytotoxic activity against the same cell lines (e.g., Ala and Val conjugates with IC_{50} values of 19 and 38 μM in HeLa cells). Similarly, when comparing Ser to Thr conjugate, it was also noticed that an extra carbon in the amino acid side chain caused a marked difference in the individual activity profile (Table 1). This observation implied the hydrophobic nature of the side chains might influence the potency of the amino acid ester conjugates. Both of the Leu and Ile conjugates displayed comparably effective cytotoxic activity against HeLa cell line (Leu: IC_{50} = 36 μM; Ile: IC_{50} = 27 μM). Glu conjugate showed improved activity against all the cell lines tested with IC_{50} values of 35, 46, and 50 μM. The Phe and Tyr appeared to be particularly interesting because these aromatic residues can intercalate with DNA.[31,42–44] However, the respective contribution from the Phe/Tyr residue to the cytotoxic activity was quite different. The Phe conjugate, as expected, indeed demonstrated better cytotoxic activity against HeLa, MCF-7, and HeG2 cell lines, with IC_{50} values of 12, 14, and 25 μM, respectively. Whereas in the case of Tyr conjugate, reduced cytotoxic activity regarding the tested cell lines was observed with IC_{50} higher than 100 μM. In the case of the Pro conjugate, least improved cytotoxic activity was observed with IC_{50} values of 85, 120, and 100 μM, respectively, against the cell lines tested.

Table 1. In vitro cytotoxic activities of β-carboline derivatives (IC_{50}, μM)[a,c]

Compound	HeLa[b]	MCF-7[b]	HepG2[b]
3	121	167	135
4	140	180	189
5a (Gly)	50	48	45
5b (Ala)	19	30	49
5c (Val)	38	54	44
5d (Phe)	12	14	25
5e (Thr)	27	23	53
5f (Tyr)	108	153	146
5g (Leu)	36	45	63
5h (Ile)	27	39	57
5i (Pro)	85	120	100
5j (Glu)	35	46	50
5k (Asp)	51	70	49
5l (Ser)	68	34	53
5m (Lys)	4	2	5
5n (Arg)	1	5	7
6	66	94	52
7	59	71	62
8	80	110	103

[a] Cytotoxicity as IC_{50} for each cell line is the concentration of compound, which reduced by 50% the optical density of treated cells with respect to untreated cells using the MTT assay.

[b] Cell lines include liver carcinoma (HepG-2), cervical carcinoma (HeLa), and human breast cancer (MCF-7).

[c] The data represent means ± SEM from at least three determinations.

A potent cytotoxicity activity was observed with the Lys/Arg conjugates. In the present study, regarding the activity against individual cell lines, Lys and Arg conjugates are the most active compounds toward the tumor cell lines tested. Lys and Arg conjugate showed the highest cytotoxic activity among all of the β-carboline amino acid ester conjugates toward HeLa cell line at IC_{50} values of 4 and 1 μM, respectively. Clearly, introducing Arg or Lys residue into the β-carboline dramatically enhanced its cytotoxic activity. It appears that the basic residues of Lys and Arg are crucial for DNA binding, and the positively charged amino acids might provide important structural contributions to the interaction with DNA. We speculate that the side chain of Arg/Lys was well suited to binding region on DNA because of its flexibility, its potential to form ionic bonds with the phosphate backbone and to donate multiple hydrogen bonds. Moreover, the guanidinium group of Arg can potentially interact with aromatic ring structures of DNA bases via its planar surface of shared π electrons.[31,42–44] In addition, the cationic amino acid side chain would not preclude from functioning as DNA minor groove directed component.[31,42–44] In this regard, it is conceivable to envision that β-carboline Arg conjugate acts as a complex of DNA intercalator and minor groove binder to initiate the amplification of DNA binding affinity and cytotoxic activity. The biological profile of β-carboline Arg conjugate was significantly improved by the incorporation of positively charged side chain of Arg. This observation was consistent with the previous suggestion that 'Arg is a generic DNA binding amino acid residue that creates or enhances DNA binding in a variety of contexts.'[45–47]

To further investigate the effect of substitutions on the cytotoxicity of β-carboline derivatives, we designed

and synthesized compounds **6**, **7**, and **8**. Regarding the individual cell lines, compound **6** showed moderate cytotoxicity with IC_{50} values of 66, 94, and 52 μM, respectively, and compound **7** exhibited slightly better activity than compound **6**, with IC_{50} values of 59, 71, and 62 μM. Furthermore, it was noticed the attachment of second bulk hydrophobic β-carboline appeared not beneficial to the cytotoxic activity. For example, compared with compound **7**, compound **8** has shown decreased cytotoxic activity with IC_{50} values of 80, 110, and 103 μM. The decreased cytotoxicity might be due to the steric effect.

The in vitro cytotoxicity experiment indicated a subtle change in the size, shape, and polarity of the substitution at position-3 of β-carboline derivatives can cause a large change in its biological profile. This observation may imply the spatial orientation and mobility of the pendant group is important in DNA recognition and binding.[48]

2.3. In vitro membrane permeation study

Oral delivery is a noninvasive method which is a preferred route for drug administration. To elicit their pharmacological and therapeutic effects, drug molecules first have to enter the systemic blood circulation, which requires the passage through the barrier membranes of the gastrointestinal tract. The prediction of drug-membrane permeability is important during the lead optimization stage of drug discovery. The experimental difficulty, high cost, and low-throughput involved in screening lead compounds in animals for oral drug absorption have led to the development of several in vitro prediction models. Caco-2 model has been widely used for the prediction of oral absorption in humans.[49–51] The membranes of Caco-2 cells have useful properties for correction with in vivo data and it is widely used for investigating transport, efflux, paracellular and transcellular diffusion, and metabolic activity during absorption.[52,53]

In our study, Caco-2 cell line monolayer was used as intestinal absorption model for screening β-carboline amino acid ester conjugates, and thus for evaluating the amino acid conjugation approach for enhanced drug absorption. Drug permeability is difficult to measure, thus it is simpler to estimate by the apparent permeability coefficient (P_{app}).[51]

P_{app} (A → B) is the permeability from the apical to the basolateral side (intestine to blood), and P_{app} (B → A) is the permeability from the basolateral to the apical side (blood to intestine).[51,54] The various amino acid conjugates across Caco-2 cell monolayers were valuated in triplicate in the apical to basolateral (A → B) or basolateral to apical directions (B → A).[49–51] It was initiated by adding the test solution to the apical or basolateral side of the monolayer. Investigation of the transport across the polarized Caco-2 cell monolayer in the basolateral to apical direction constitutes a means of evaluating the influence of P-gp and related efflux carriers which are located on the apical side of the monolayer.[51–54]

Table 2. The apparent permeability coefficients (P_{app}) of 4 and **5b**, **d**, **e**, **k**, **m**, **n**

Compound	$P_{app} \times 10^{-6}$ cm/s		
	A → B	B → A	(A → B)/ (B → A)
4	7.95	7.80	1.02
5b	10.25	6.34	1.62
5d	11.53	6.21	1.86
5e	11.19	6.32	1.77
5k	8.33	8.11	1.03
5m	12.03	6.10	1.97
5n	12.11	6.00	2.01

The standard deviations were generally less than 10% ($n = 4$); A → B: from apical side to basolateral side; B → A: from basolateral side to apical side.

The influence of efflux carriers on the permeability of the different AA ester conjugates was also examined by comparing the permeability ratio P_{ratio} of absorptive transport P_{app} (A → B) to the secretory one P_{app} (B → A).[49–54]

According to the previous study, the compound with permeability coefficients $P_{app} < 1 \times 10^{-6}$, $1\text{-}10 \times 10^{-6}$, and $>10 \times 10^{-6}$ cm/s is defined as poorly, moderately, and well absorbed, respectively.[51] From the permeability coefficient (P_{app}) data, as observed in Table 2, the compounds **5b**, **d**, **e**, **m**, **n**, that is, Ala-, Phe-, Thr-, Lys-, Arg-conjugate was identified as well-absorbed compound. By comparison of the apparent permeability coefficients P_{app} of the amino acid conjugates **5b**, **d**, **e**, **m**, **n**, with the parent compound **4**, the transepithelial transport of the amino acid conjugates across the Caco-2 cell monolayer in the absorptive (A → B) direction, and their respective P_{app} coefficients were higher than in the secretory (B → A) direction, which indicated the amino acid moiety in the β-carboline derivatives was favorable for the active transport across the biomembranes. In particular, the P_{app} (A → B) values of Lys- (**5m**) and Arg conjugate (**5n**) are higher than their corresponding P_{app} (B → A) values, and the permeability ratio of P_{app} (A → B)/P_{app} (B → A) is close to 2. These asymmetric permeation profiles implied AA conjugates might not be the substrates of the apically localized efflux P-gp carrier, which may limit their oral bioavailability by transporting the absorbed drug back into the intestinal lumen. However, additional experiments are required for evaluating the beneficial effect of amino acid conjugates on the transport.

2.4. Molecular modeling

Molecule modeling studies indicated that β-carboline amino acid ester conjugates might interact with DNA by intercalating, and consequently inhibit DNA synthesis. β-carboline Arg conjugate **5n** bearing guanidium group at the side chain had generally lower energy than the other conjugates. In particular, it was observed that β-carboline Arg conjugate slightly induced a conformational change in DNA suggesting that one key arginine residue or guanidine might contribute to trigger the minor conformational change in DNA. β-carboline Arg conjugate docking in DNA as an example is shown in

Figure 1. The modeling study of Arg conjugate indicated that the planar β-carboline aromatic ring system had been inserted into DNA such that the guanidine-terminated side chain was forced to lie in the minor groove of DNA. It seems that the relative positioning of side chains in the β-carboline amino acid ester conjugates may be an important factor in the DNA-drug recognition.

From the interaction energy data as shown in Table 3, it was noticed that, β-carboline Arg conjugate exhibited the lowest binding energy (**5n**: −40.9342 kcal/mol), which indicated that β-carboline Arg conjugate could form stable complex with DNA. It was noticed that the rigid proline ring seemed to induce the steric hindrance and therefore resulting in the relative higher binding energy in Pro conjugate (**5i**: −5.7609 kcal/mol). In addition, it was clearly seen that, Tyr and Phe conjugate exhibited similar binding energy (**5f**: −32.4253 kcal/mol; **5d**: −30.1275 kcal/mol). Likewise, it was observed in the case of Ser and Thr conjugate with similar binding energy (**5l**: −20.8982 kcal/mol; **5e**: −19.0324 kcal/mol). The reason for the differences in their biological activities might be explained by the differences in bioavailability such as drug uptake and different rate of metabolism.

From the molecular modeling analysis, we conclude that the size and shape of the side chain of these conjugates are crucial for high affinity and correct binding mode. However, other interaction factors also contribute to their distinct biological activities.

3. Conclusion

The in vitro cytotoxic activities of β-carboline derivatives demonstrated β-carboline nucleus was an important unit for the design and synthesis of new anti-tumor drugs; the cytotoxicity of β-carboline derivatives depended upon the presence and the nature of the substitutions, which were introduced into the β-carboline ring. The cytotoxic potencies of β-carboline derivatives were enhanced by the introduction of amino acids into 3-position of β-carboline ring. A cationic Lys/Arg residue was highly favorable for the enhanced cytotoxic activity compared to the uncharged amino acids. A minor structural alternation in the single amino acid chain of β-carboline amino acid ester conjugates can lead to marked multiple changes in membrane permeability, intercalating capability to the cytotoxicity, which implied that the improved biological profile might result from a synergic effect.

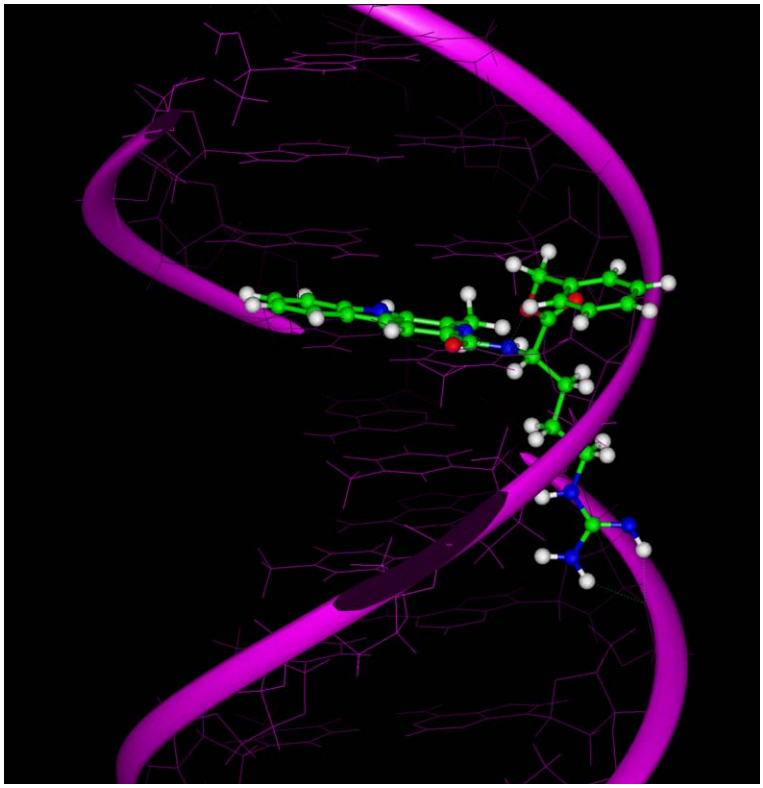


Figure 1. Model illustrating β-carboline-Arg conjugate (compound **5n**) docking in DNA.

Table 3. Computer simulation of the minimization binding energy β-carbolines amino acid conjugates and DNA

Compound	Phe (**5d**)	Thr (**5e**)	Tyr (**5f**)	Pro (**5i**)	Ser (**5l**)	Lys (**5m**)	Arg (**5n**)
E (kcal/mol)	−30.1275	−19.0324	−32.4253	−5.7609	−20.89822	−38.0016	−40.9342

Introducing the amino acid moiety onto β-carboline yielded a series of β-carboline amino acid ester conjugates representing one of the positive approaches to optimize the lead compound. Clearly, further in vitro and in vivo biological evaluation of β-carboline amino acid conjugates as potential antitumor agents is needed by application of mechanism-based bioassay systems, such as the inhibition of human topoisomerase I and II and molecular pathways that involved in cytotoxicity.

4. Experimental

4.1. General

The protected amino acids with L-configuration, used in this work, were purchased from Sigma Chemical. ^{1}H NMR (500 Hz) and ^{13}C NMR (75 Hz) spectra were acquired on a Bruker AC 300 spectrometer in $CDCl_3$ with TMS as internal standard, or in DMSO-d_6 and chemical shifts were expressed in ppm (δ). All of the coupling reactions were carried out under anhydrous conditions. Chromatography was performed on Qingdao silica gel H. The purities of the intermediates and the products were confirmed by TLC (Merck silica gel plastes of type 60 F_{254}, 0.25 mm layer thickness) and HPLC (Waters, C_{18} column 4.6 × 150 mm). The amino acid analysis was determined with a Hitachi 835-50 instrument. FAB-MS was determined by VG-ZAB-MS high resolution GC/MS/DS and HP ES-5989x. Optical rotations were determined with a Schmidt + Haensch Polartromic D instrument. The statistical analysis of all the biological data was carried out by use of ANOVA test, $p < 0.05$ is considered significant.

4.2. General procedure for preparation of amino acid benzylester hydrochloride

To the solution of 10.0 g polyphosphoric acid and 50 ml of benzyl alcohol, 0.02 mol amino acid was added. The reaction mixture was stirred at 92 °C for 8 h and then mixed with the solution of 20 ml of concentrated sulfuric acid in 200 ml of water. The mixture was treated with 20 ml of ether, and the aqueous phase was separated and then neutralized with aqueous solution of sodium carbonate adjusted to pH 10. The solution was extracted with ether (3× 70 ml) and the ether phase was dried with anhydride magnesium sulfate. After filtration, the filtrate was bubbled with hydrogen chloride to precipitate the hydrochloride of amino acid benzyl ester in 23–72% yield as colorless powder.

4.3. (1*S*,3*S*)- and (1*R*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (1a, b)

To the mixture of 2.0 g (9.9 mmol) of L-tryptophane, 0.2 ml H_2SO_4 (1 mol/L), and 80 ml of water, 2 ml of aldehyde (40%) was added. The reaction mixture was stirred at room temperature for 6 h and adjusted to pH 6–7 with concentrated ammonia liquor. The mixture was kept at 0 °C for 12 h and the formed precipitate was collected by filtration to give 1.8 g (80%) of (1*S*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid as a colorless powder. The filtrate was concentrated to give 0.12 g (5%) of (1*R*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid as a colorless powder.

Compound **1a**: Mp 290–292 °C; ESI/MS: 231 $[M+H]^+$; IR (KBr): 3100–2400, 2960, 2900, 1700, 1620, 1590, 1500, 1450, 1379, 1070, 900 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 11.94 (s, 1H), 10.99 (s, 1H), 9.18 (s, 1H), 7.44 (d, J = 7.5 Hz, 1H), 7.33 (t, J = 8.0 Hz, 1H), 7.08 (t, J = 8.0 Hz, 1H), 6.99 (t, J = 7.5 Hz, 1H), 4.22 (q, J = 4.8 Hz, 1H), 3.69 (dd, J = 10.5 Hz, J = 5.0 Hz, 1H), 3.14 (dd, J = 10.5 Hz, J = 2.4 Hz, 1H), 2.83 (ddd, J = 10.5 Hz, J = 5.0 Hz, J = 2.4 Hz, 1H), 1.38 (d, J = 5.0 Hz, 3H). Anal. Calcd for $C_{13}H_{14}N_2O_2$: C, 67.81; H, 6.13; N, 12.17. Found: C, 67.65; H, 6.32; N, 11.99.

Compound **1b**: Mp 240–242 °C; ESI/MS: 231 $[M+H]^+$; IR (KBr): 3100–2400, 2960, 2900, 1700, 1620, 1590, 1500, 1450, 1379, 1070, 900 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 11.89 (s, 1H), 10.99 (s, 1H), 9.18 (s, 1H), 7.41 (d, J = 7.6 Hz, 1H), 7.30 (t, J = 8.1 Hz, 1H), 7.01 (t, J = 8.1 Hz, 1H), 6.97 (t, J = 7.6 Hz, 1H), 4.40 (q, J = 4.9 Hz, 1H), 3.67 (dd, J = 10.3 Hz, J = 5.1 Hz, 1H), 3.16 (dd, J = 10.3 Hz, J = 2.7 Hz, 1H), 2.53 (ddd, J = 10.3 Hz, J = 5.1 Hz, J = 2.6 Hz, 1H), 1.01 (d, J = 5.1 Hz, 3H). Anal. Calcd for $C_{13}H_{14}N_2O_2$: C, 67.81; H, 6.13; N, 12.17. Found: C, 67.70; H, 6.00; N, 12.01.

4.4. Methyl (1*S*,3*S*)- and (1*R*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate (2a,b)

(a) At 0 °C, to the solution of methanol (10 ml), 1 ml of thionyl chloride was added dropwise. The mixture was stirred at rt for 15 min, and then 2.0 g (8.7 mmol) of 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid was added in portion. The reaction mixture was stirred at rt for 16 h until TLC ($CHCl_3$/MeOH, 15:1) indicated the complete disappearance of 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid. The reaction mixture was neutralized with aqueous solution of sodium bicarbonate (10%) to pH 7.0. The formed precipitate was collected by filtration. After separation with silica gel chromatography (chloroform/acetone, 16:1), 1.88 g (88.5%) of methyl (1*S*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate and 125 mg (5.5%) of methyl (1*R*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate were obtained as a pale yellow powder.

Compound **2a**: Mp 75–76 °C; ESI/MS: 245 $[M+H]^+$; IR (KBr): 3400–3200, 2960, 2900, 2810, 1740, 1620, 1590, 1500, 1450, 1382, 1066, 897 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 10.04 (s, 1H), 7.42 (t, J = 6.4 Hz, 1H), 7.20 (t, J = 8.6 Hz, 1H), 7.11 (d, J = 7.5 Hz, 1H), 7.04 (d, J = 6.6 Hz, 1H), 4.28 (q, J = 6.6 Hz, 1H), 3.78 (s, 3H), 3.77 (m, J = 6.9 Hz, 1H), 3.08 (d, J = 11.2 Hz, 1H), 2.81 (t, J = 11.2 Hz, 1H), 2.25 (s, 1H), 1.42 (d, J = 6.0 Hz, 3H). Anal. Calcd for $C_{14}H_{16}N_2O_2$: C, 68.83; H, 6.60; N 11.47. Found: C, 68.67; H, 6.80; N, 11.64.

Compound **2b**: Mp 187–188 °C; ESI/MS: 245 $[M+H]^+$; IR (KBr): 3000, 2980, 2960, 2825, 1732, 1610, 1590,

1596, 1455, 1380, 1064, 899 cm^{-1}; ^1H NMR ($CDCl_3$): δ = 10.08 (s, 1H), 7.40 (t, J = 7.1 Hz, 1H), 7.28 (d, J = 8.4 Hz, 1H), 7.08 (t, J = 7.7 Hz, 1H), 7.00 (t, J = 6.5 Hz, 1H), 4.37 (q, J = 6.5 Hz, 1H), 3.98 (t, J = 4.9 Hz, 1H), 3.74 (s, 3H), 3.08 (q, J = 4.9 Hz, 1H), 2.95 (q, J = 6.6 Hz, 1H), 2.75 (s, 1H), 1.47 (d, J = 6.4 Hz, 3H). Anal. Calcd for $C_{14}H_{16}N_2O_2$: C, 68.83; H, 6.60; N, 11.47. Found: C, 68.99; H, 6.79; N, 11.31.

(b) To the mixture of 2.2 g (9.9 mmol) of L-tryptophane methyl ester and 0.1 ml H_2SO_4 in 80 ml of water, 2 ml of aldehyde (40%) was added. The reaction mixture was stirred at rt for 6 h and adjusted to pH 6–7 with concentrated ammonia liquor. The mixture was kept at 0 °C for 12 h and the formed precipitate was collected by filtration to give 1.18 g (47%) of methyl 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate. After separation with silica gel chromatography (chloroform/acetone, 16:1), 787 mg (31%) of methyl (1*S*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate and 394 (16%) of methyl (1*R*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate were obtained as a colorless powder.

4.5. Methyl-1-methyl-β-carboline-3-carboxylate (3)

(a) At 0 °C with stirring to the solution of 5.0 g (20.0 mmol) of (1*S*,3*S*)-methyl-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate in 50 ml DMF, 4.5 g (28.0 mmol) of potassium hypermanganate was added. The reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 14 h, and TLC (chloroform/methanol, 15:1) indicated the complete disappearance of methyl-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate. After evaporation, the residue was dissolved in 10 ml of methanol, filtered, and concentrated, 3.2 g (65%) of the title compound was obtained as yellow powder. Mp 243–244 °C; ESI/MS: 241 $[M+H]^+$; IR (KBr): 3310, 2954, 2922, 2901, 2811, 1742, 1600, 1581, 1566, 1450, 1380, 1066, 900 cm^{-1}; ^1H NMR ($CDCl_3$): δ = 9.98 (s, 1H), 7.41 (d, J = 5.6 Hz, 1H), 7.35 (d, J = 7.2 Hz, 1H), 7.15 (d, J = 8.2 Hz, 1H), 7.11 (t, J = 7.5 Hz, 1H), 6.97 (t, J = 6.4 Hz, 1H), 3.74 (s, 3H), 2.03 (s, 3H). Anal. Calcd for $C_{14}H_{12}N_2O_2$: C, 69.99; H, 5.03; N, 11.66. Found: C, 69.80; H, 5.20; N, 11.82.

(b) The suspension of 5.0 g (20.0 mmol) of methyl-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate, 1.4 g (20. mmol) sulfur flour, and 20 ml of anhydrous dimethyl benzene was refluxed for 96 h until TLC (chloroform/methanol, 15:1) indicated the complete disappearance of methyl 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate. On evaporation the residue was dissolved in 10 ml of methanol. After filtration and evaporation under reduced pressure, 3.2 g (65%) of the title compound was obtained as a yellow powder. Mp 242–243 °C; ESI/MS: 241 $[M+H]^+$; IR (KBr): 3310, 2954, 2922, 2901, 2811, 1742, 1600, 1581, 1566, 1450, 1380, 1066, 900 cm^{-1}; ^1H NMR (500, DMSO-d_6): δ = 9.98 (s, 1H), 7.41 (d, J = 5.6 Hz, 1H), 7.35 (d, J = 7.2 Hz, 1H), 7.15 (d, J = 8.2 Hz, 1H), 7.11 (t, J = 7.5 Hz, 1H), 6.97 (t, J = 6.4 Hz, 1H), 3.74 (s, 3H), 2.03 (s, 3H).

(c) At 0 °C, with stirring to the solution of 5.0 g (20.0 mmol) of methyl-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate in 10 ml of glacial acetic acid, 45.0 g (0.1 mol) of lead tetraacetate was added. After stirring at room temperature for 15 min to the reaction mixture, 10.0 g (0.1 mol) of oxalic acid was added. The reaction mixture was stirred at room temperature for 1 h and TLC (chloroform/methanol, 15:1) indicated the complete disappearance of methyl-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate. The formed precipitate was collected by filtration and then suspended in 150 ml of mixed solvent of water and chloroform (1:2). The suspension was neutralized with sodium bicarbonate and filtered. The filtered residue was washed with 20 ml of chloroform and the filtrate was extracted with chloroform (310 ml). The combined chloroform was dried with anhydrous sodium sulfate and filtered. The filtrate was evaporated to give 2.5 g (50%) of the title compound which was obtained as a yellow powder. Mp 242–243 °C; ESI/MS: 241 $[M+H]^+$; IR (KBr): 3310, 2954, 2922, 2901, 2811, 1742, 1600, 1581, 1566, 1450, 1380, 1066, 900 cm^{-1}; ^1H NMR (DMSO-d_6): δ = 9.98 (s, 1H), 7.41 (d, J = 5.6 Hz, 1H), 7.35 (d, J = 7.2 Hz, 1H), 7.15 (d, J = 8.2 Hz, 1H), 7.11 (t, J = 7.5 Hz, 1H), 6.97 (t, J = 6.4 Hz, 1H), 3.74 (s, 3H), 2.03 (s, 3H).

4.6. 1-Methyl-β-carboline-3-carboxylic acid (4)

The solution of 5.0 g (20.8 mmol) of methyl 1-methyl-β-carboline-3-carboxylate in 120 ml of aqueous solution of sodium hydroxide (2.0 mmol/L) was stirred at 60 °C for 4 h and TLC (chloroform/methanol, 15:1) indicated the complete disappearance of methyl-1-methyl-β-carboline-3-carboxylate. The reaction mixture was neutralized with hydrochloric acid to pH 7.0. The formed precipitate was collected by filtration to give 3.86 g (82%) of the title compound as a yellow powder. Mp 292–293 °C; ESI/MS: 227 $[M+H]^+$; IR (KBr): 3230–2252, 2924, 2910, 2900, 1700, 1610, 1585, 1562, 1440, 1380, 1066, 900 cm^{-1}; ^1H NMR ($CDCl_3$): δ = 11.06 (s, 1H), 9.88 (s, 1H), 7.42 (d, J = 5.8 Hz, 1H), 7.39 (d, J = 7.1 Hz, 1H), 7.10 (d, J = 8.0 Hz, 1H), 7.10 (t, J = 7.6 Hz, 1H), 6.99 (t, J = 6.6 Hz, 1H), 2.10 (s, 3H). Anal. Calcd for $C_{13}H_{10}N_2O_2$: C, 69.02; H, 4.46; N, 12.38. Found: C, 69.20; H, 4.60; N, 12.22.

4.7. *N*-(1-Methyl-β-carboline-3-carbonyl)glycine benzyl ester (5a)

At 0 °C, to the solution of 364 mg (2.0 mmol) HCl–Gly-OBzl, 0.5 ml (4.5 mmol) of *N*-methylmorpholine, 454 mg (2.2 mmol) DCC, DMAP 23 mg (0.2 mmol), 5 ml of anhydrous THF, and 4 ml of anhydrous DMF, 452 mg (2.0 mmol) of 1-methyl-β-carboline-3-carboxylic acid was added. The reaction mixture was stirred at 0 °C for 1 h, at rt for another 16 h until TLC (chloroform/methanol, 15:1) indicated the complete disappearance of 1-methyl-β-carboline-3-carboxylic acid. The reaction mixture was filtered, concentrated, and

the residue was dissolved in 40 ml of ethyl acetate and washed successively with 5% sodium bicarbonate, 5% citric acid, and saturated sodium chloride, and the organic phase was separated and dried over anhydrous sodium sulfate. After filtration and evaporation under reduced pressure, 280 mg (37%) of the title compound was obtained as a colorless powder. Mp 156–158 °C; ESI/MS: 374 $[M+H]^+$. IR (KBr) 3341, 3028, 3009, 1751, 1690, 1601, 1582, 1501, 1460, 762, 701 cm^{-1}. ^1H NMR ($CDCl_3$) δ = 12.01 (s, 1H), 8.70 (s, 1H), 8.33 (s, 1H), 7.72 (d, J = 7.2 Hz, 1H), 7.48 (d, J = 7.5 Hz, 1H), 7.32 (d, J = 8.4 Hz, 2H), 7.29 (t, J = 7.2 Hz, 2H), 7.20 (t, J = 7.8 Hz, 1H), 7.12 (t, J = 7.2 Hz, 1H), 7.07 (t, J = 7.5 Hz, 1H), 5.28 (s, 2H), 4.01 (d, J = 5.4 Hz, 2H), 2.82 (s, 3H). Anal. Calcd for $C_{22}H_{19}N_3O_3$: C, 70.76; H, 5.13; N, 11.25. Found: C, 70.68; H, 5.22; N, 11.40.

4.8. *N*-(1-Methyl-β-carboline-3-carbonyl)-L-alanine benzyl ester (5b)

Using the similar procedure as that of **5a**, starting from 392 mg (2.0 mmol) of HCl–L-Ala-OBzl, 154 mg (20%) of the title compound was obtained as a colorless powder. Mp 158–160 °C; ESI/MS: 388 $[M+H]^+$. IR(KBr)3343, 3032, 3008, 1754, 1690, 1605, 1582, 1505, 1464, 762, 701 cm^{-1}. ^1H NMR ($CDCl_3$) δ = 12.02 (s, 1H), 8.70 (s, 1H), 8.43 (s, 1H), 8.04 (s, 1H), 7.59 (d, J = 7.5 Hz, 1H), 7.46 (d, J = 8.4 Hz, 1H), 7.41 (d, J = 8.2 Hz, 2H), 7.27 (t, J = 7.3 Hz, 2H), 7.22 (t, J = 7.7 Hz, 1H), 7.05 (t, J = 7.4 Hz, 1H), 5.29 (s, 2H), 4.91 (m, J = 6.4 Hz, 1H), 2.85 (s, 3H), 1.66 (d, J = 6.6 Hz, 3H). $[\alpha]_D^{20}$ 2.0 (c 0.5, EtOH/DMSO, 4:1), Anal. Calcd for $C_{23}H_{21}N_3O_3$: C, 71.30; H, 5.46; N, 10.85. Found: C, 71.49; H, 5.32; N, 10.66.

4.9. *N*-(1-Methyl-β-carboline-3-carbonyl)-L-valine benzyl ester (5c)

Using the similar procedure as that of **5a**, starting from 450 mg (2.0 mmol) of HCl–L-VaL-OBzl, 183 mg (22%) of the title compound was obtained as a colorless powder. Mp 162–164 °C; ESI/MS: 416 $[M+H]^+$; IR (KBr) 3338, 3025, 3009, 1751, 1690, 1602, 1581, 1504, 1460, 761, 698 cm^{-1}. ^1H NMR ($CDCl_3$) δ = 11.68 (s, 1H), 8.38 (s, 1H), 8.04 (s, 1H), 7.57 (d, J = 7.5 Hz, 1H), 7.56 (d, J = 7.2 Hz, 1H), 7.31 (t, J = 7.8 Hz, 2H), 7.29 (d, J = 7.6 Hz, 2H), 7.26 (d, J = 7.5 Hz, 1H), 7.15 (t, J = 7.5 Hz, 1H), 7.05 (t, J = 7.5 Hz, 1H), 5.29 (s, 2H), 4.91 (d, J = 7.2 Hz, 1H), 2.85 (s, 3H), 2.22 (m, J = 7.2 Hz, 1H), 1.66 (d, J = 6.6 Hz, 6H). $[\alpha]_D^{20}$ 5.0 (c 0.5, EtOH/DMSO, 4:1). Anal. Calcd for $C_{25}H_{25}N_3O_3$: C, 72.27; H, 6.06; N, 10.11. Found: C, 72.46; H, 6.22; N, 10.30.

4.10. *N*-(1-Methyl-β-carboline-3-carbonyl)-L-phenylalanine benzyl ester (5d)

Using the similar procedure as that of **5a**, starting from 546 mg (2.0 mmol) of HCl–L-Phe-OBzl, 490 mg (53%) of the title compound was obtained as a colorless powder. Mp 138–140 °C; ESI/MS: 464 $[M+H]^+$; IR (KBr) 3342, 3032, 3011, 1748, 1690, 1601, 1581, 1502, 1465, 761, 702 cm^{-1}. ^1H NMR ($CDCl_3$) δ = 12.02 (s, 1H), 8.70 (s, 1H), 8.24 (s, 1H), 7.70 (d, J = 7.2 Hz, 1H), 7.57 (d, J = 7.5 Hz, 1H), 7.16 (t, J = 7.2 Hz, 1H), 7.06 (t, J = 7.6 Hz, 1H), 7.32 (t, J = 7.6 Hz, 1H), 7.30 (t, J = 7.8 Hz, 1H), 7.29 (t, J = 7.5 Hz, 2H), 7.28 (t, J = 7.6 Hz, 2H), 7.27 (d, J = 7.6 Hz, 2H), 7.26 (d, J = 7.5 Hz, 2H), 5.26 (s, 2H), 5.14 (dd, J = 4.8 Hz, J = 3.5 Hz, 1H), 3.36 (d, J = 4.8 Hz, 2H), 2.72 (s, 3H). $[\alpha]_D^{20}$ 3.0 (c 0.5, EtOH/DMSO, 4:1). Anal. Calcd for $C_{29}H_{25}N_3O_3$: C, 75.14; H, 5.44; N, 9.07. Found: C, 75.01; H, 5.26; N, 9.20.

4.11. *N*-(1-Methyl-β-carboline-3-carbonyl)-L-threonine benzyl ester (5e)

Using the similar procedure as that of **5a**, starting from 454 mg (2.0 mmol) of HCl–L-Thr-OBzl, 126 mg (15%) of the title compound was obtained as a colorless powder. Mp 178–179 °C; ESI/MS: 418 $[M+H]^+$; IR (KBr) 3339, 3028, 3009, 1754, 1690, 1603, 1581, 1502, 1465, 761, 702 cm^{-1}. ^1H NMR ($CDCl_3$) δ = 12.02 (s, 1H), 8.79 (s, 1H), 8.22 (s, 1H), 7.73 (d, J = 7.8 Hz, 1H), 7.55 (t, J = 7.5 Hz, 1H), 7.45 (d, J = 7.2 Hz, 1H), 7.35 (t, J = 7.8 Hz, 1H), 7.33 (t, J = 7.8 Hz, 1H), 7.30 (t, J = 7.6 Hz, 2H), 7.27 (d, J = 7.5 Hz, 2H), 5.29 (s, 2H), 4.63 (m, J = 5.2 Hz, 1H), 4.52 (d, J = 5.2 Hz, 1H), 2.85 (s, 3H), 2.22 (s, 1H), 1.66 (d, J = 5.6 Hz, 3H). $[\alpha]_D^{20}$ 4.0 (c 0.5, EtOH/DMSO, 4:1). Anal. Calcd for $C_{24}H_{23}N_3O_4$: C, 69.05; H, 5.55; N, 10.07. Found: C, 68.88; H, 5.27; N, 10.21.

4.12. *N*-(1-Methyl-β-carboline-3-carbonyl)-L-tyrosine benzyl ester (5f)

Using the similar procedure as that of **5a**, starting from 578 mg (2.0 mmol) of HCl–L-Tyr-OBzl, 197 mg (20%) of the title compound was obtained as a colorless powder. Mp 136–137 °C; ESI/MS: 480 $[M+H]^+$; IR (KBr) 3342, 3031, 3012, 1754, 1693, 1605, 1582, 1501, 1460, 762, 701 cm^{-1}. ^1H NMR (DMSO-d_6) δ = 12.02 (s, 1H), 8.79 (s, 1H), 8.22 (s, 1H), 7.63 (d, J = 7.8,1H), 7.55 (d, J = 7.8 Hz, 1H), 7.35 (t, J = 7.5 Hz, 1H), 7.34 (t, J = 7.5 Hz, 2H), 7.32 (d, J = 7.8 Hz, 2H), 7.10 (t, J = 7.6 Hz, 1H), 7.07 (d, J = 7.5 Hz, 1H), 6.99 (d, J = 6.9 Hz, 2H), 6.61 (d, J = 6.8 Hz, 2H), 5.26 (s, 2H), 5.06 (s, 1H), 4.51 (t, J = 4.5 Hz, 1H), 3.14 (d, J = 4.5 Hz, 2H), 2.82 (s, 3H). $[\alpha]_D^{20}$ −25.0 (c 0.5, EtOH/DMSO, 4:1). Anal. Calcd for $C_{29}H_{25}N_3O_4$: C, 72.64; H, 5.25; N, 8.76. Found: C, 72.80; H, 5.44; N, 8.59.

4.13. *N*-(1-Methyl-β-carboline-3-carbonyl)-L-leucine benzyl ester (5g)

Using the similar procedure as that of **5a**, starting from 478 mg (2.0 mmol) of HCl–L-Leu-OBzl, 256 mg (30%) of the title compound was obtained as a colorless powder. Mp 183–185 °C; ESI/MS: 430 $[M+H]^+$; IR(KBr) 3338, 3027, 3012, 1749, 1689, 1601, 1582, 1502, 1461, 762, 701 cm^{-1}. ^1H NMR (DMSO-d_6) δ = 12.00 (s, 1H), 8.67 (s, 1H), 8.22 (s, 1H), 7.67 (d, J = 8.7 Hz, 1H), 7.54 (d, J = 7.8 Hz, 1H), 7.34 (d, J = 7.8 Hz, 2H), 7.32 (t, J = 7.5 Hz, 2H), 7.30 (t, J = 7.8 Hz, 1H), 7.13 (t, J = 7.6 Hz, 1H), 7.01 (t, J = 7.5 Hz, 1H), 5.26 (s, 2H), 4.60 (t, J = 5.1 Hz, 1H), 2.84 (s, 3H), 1.99 (m,

J = 5.1 Hz, 2H), 1.20(m, J = 5.0 Hz, 1H), 0.96 (d, J = 6.0 Hz, 6H). $[\alpha]_D^{20}$ 4.0 (c 0.5, EtOH/DMSO, 4:1). Anal. Calcd for $C_{26}H_{27}N_3O_3$: C, 72.71; H, 6.34; N, 9.78. Found: C, 72.90; H, 6.49; N, 9.59.

4.14. *N*-(1-Methyl-β-carboline-3-carbonyl)-L-isoleucine benzyl ester (5h)

Using the similar procedure as that of **5a**, starting from 478 mg (2.0 mmol) of HCl–L-Ileu-OBzl, 346 mg (40%) of the title compound was obtained as a colorless powder. Mp 182–183 °C; ESI/MS: 430 $[M+H]^+$; IR (KBr) 3341, 3032, 3011, 1751, 1690, 1602, 1581, 1502, 1461, 1380, 1371, 761, 701 cm^{-1}. ^1H NMR (DMSO-d_6) δ = 12.02 (s, 1H), 8.72 (s, 1H), 8.20 (s, 1H), 7.55 (d, J = 8.7 Hz, 1H), 7.34 (d, J = 7.8 Hz, 1H), 7.30 (d, J = 8.1 Hz, 1H), 7.28 (t, J = 7.5 Hz, 2H), 7.24 (t, J = 7.8 Hz, 1H), 7.12 (t, J = 7.5 Hz, 1H), 7.01 (t, J = 7.6 Hz, 1H), 5.26 (s, 2H), 4.51 (d, J = 5.1 Hz, 1H), 2.85 (s, 3H), 2.83 (m, J = 5.1 Hz, 1H), 1.29 (m, J = 5.1 Hz, 2H), 0.97 (d, J = 6.9 Hz, 3H), 0.93 (m, J = 6.9 Hz, 3H). $[\alpha]_D^{20}$ 3.0 (c 0.5, EtOH/DMSO, 4:1). Anal. Calcd for $C_{26}H_{27}N_3O_3$: C, 72.71; H, 6.34; N, 9.78. Found: C, 72.88; H, 6.51; N, 9.63.

4.15. *N*-(1-Methyl-β-carboline-3-carbonyl)-L-proline benzyl ester (5i)

Using the similar procedure as that of **5a**, starting from 446 mg (2.0 mmol) of HCl–L-Pro-OBzl, 134 mg (16%) of the title compound was obtained as a colorless powder. Mp 190–191 °C; ESI/MS: 414 $[M+H]^+$; IR (KBr) 3342, 3031, 3012, 1754, 1690, 1601, 1582, 1505, 1460, 761, 702 cm^{-1}. ^1H NMR (DMSO-d_6) δ = 11.95 (s, 1H), 8.45 (s, 1H), 7.72 (d, J = 7.2 Hz, 1H), 7.65 (d, J = 8.1 Hz, 1H), 7.31 (t, J = 7.8 Hz, 2H), 7.29 (d, J = 7.5 Hz, 2H), 7.07 (t, J = 7.5 Hz, 1H), 7.05 (d, J = 7.5 Hz, 1H), 5.28 (s, 2H), 4.47 (m, J = 6.6 Hz, 1H), 3.71 (t, J = 6.6 Hz, 2H), 2.81 (s, 3H), 2.27 (m, J = 6.6 Hz, 2H), 2.02 (m, J = 6.6 Hz, 2H). $[\alpha]_D^{20}$ 2.0 (c 0.5, EtOH/DMSO, 4:1). Anal. Calcd for $C_{25}H_{23}N_3O_3$: C, 72.62; H, 5.61; N, 10.16. Found: C, 72.44; H, 5.43; N, 9.95.

4.16. *N*-(1-Methyl-β-carboline-3-carbonyl)-L-glutamic acid benzyl ester (5j)

Using the similar procedure as that of **5a**, starting from 510 mg (2.0 mmol) of HCl–L-Glu-OBzl, 90 mg (8%) of the title compound was obtained as a colorless powder. Mp 211–213 °C; ESI/MS: 536 $[M+H]^+$; IR (KBr) 3340, 3029, 3008, 1752, 1692, 1603, 1584, 1502, 1461, 762, 701 cm^{-1}. ^1H NMR (DMSO-d_6) δ = 12.00 (s, 1H), 8.75 (s, 1H), 8.32 (s, 1H), 7.76 (d, J = 7.8 Hz, 1H), 7.60 (d, J = 7.8 Hz, 1H), 7.32 (t, J = 7.2 Hz, 2H), 7.31 (t, J = 7.2 Hz, 2H), 7.30 (t, J = 7.8 Hz, 2H), 7.29 (t, J = 7.8 Hz, 2H), 7.27 (t, J = 7.8 Hz, 1H), 7.25 (t, J = 7.8 Hz, 1H), 7.19 (t, J = 7.6 Hz, 1H), 7.02 (t, J = 7.6 Hz, 1H), 5.28 (s, 2H), 5.25 (s, 2H), 4.40 (m, J = 6.6 Hz, 1H), 2.79 (s, 3H), 1.90 (m, J = 6.4 Hz, 2H), 1.23 (t, J = 6.4 Hz, 2H). $[\alpha]_D^{20}$ 3.0 (c 0.5, EtOH/DMSO, 4:1). Anal. Calcd for $C_{32}H_{29}N_3O_5$: C, 71.76; H, 5.46; N, 7.85. Found: C, 71.90; H, 5.63; N, 7.68.

4.17. *N*-(1-Methyl-β-carboline-3-carbonyl)-L-aspartic acid benzyl ester (5k)

Using the similar procedure as that of **5a**, starting from 660 mg (2.0 mmol) of HCl–L-Asp-OBzl, 100 mg (10%) of the title compound was obtained as a colorless powder. Mp 202–204 °C; ESI/MS: 522 $[M+H]^+$; IR (KBr) 3342, 3027, 3009, 1754, 1690, 1601, 1582, 1501, 1463, 760, 704 cm^{-1}. ^1H NMR (DMSO-d_6) δ = 12.01 (s, 1H), 8.70 (s, 1H), 8.30 (s, 1H), 7.69 (d, J = 7.8 Hz, 1H), 7.52 (d, J = 7.8 Hz, 1H), 7.34 (t, J = 7.3 Hz, 2H), 7.32 (t, J = 7.3 Hz, 2H), 7.30 (t, J = 7.8 Hz, 2H), 7.28 (t, J = 7.8 Hz, 2H), 7.26 (t, J = 7.8 Hz, 1H), 7.24 (t, J = 7.8 Hz, 1H), 7.18 (t, J = 7.6 Hz, 1H), 7.01 (t, J = 7.6 Hz, 1H), 5.28 (s, 2H), 5.25 (s, 2H), 4.87 (m, J = 6.2 Hz, 1H), 3.20 (m, J = 6.2 Hz, 2H), 2.81 (s, 3H). $[\alpha]_D^{20}$ 4.0 (c = 0.5, EtOH/DMSO, 4:1). Anal. Calcd for $C_{31}H_{27}N_3O_5$: C, 71.39; H, 5.22; N, 8.06. Found: C, 71.22; H, 5.03; N, 8.23.

4.18. *N*-(1-Methyl-β-carboline-3-carbonyl)-L-serine benzyl ester(5l)

Using the similar procedure as that of **5a**, starting from 426 mg (2.0 mmol) of HCl–L-Ser-OBzl, 97 mg (12%) of the title compound was obtained as a colorless powder. Mp 158–160 °C; ESI/MS: 404 $[M+H]^+$; IR (KBr) 3342, 3027, 3011, 1754, 1691, 1602, 1581, 1503, 1462, 764, 703 cm^{-1}. ^1HNMR (DMSO-d_6) δ = 12.00 (s, 1H), 8.64 (s, 1H), 8.34 (s, 1H), 7.74 (d, J = 8.1 Hz, 1H), 7.52 (d, J = 7.8 Hz, 1H), 7.34 (d, J = 7.8 Hz, 2H), 7.30 (t, J = 7.5 Hz, 2H), 7.28 (t, J = 7.5 Hz, 1H), 7.16 (t, J = 7.6 Hz, 1H), 7.03 (t, J = 7.6 Hz, 1H), 5.27 (s, 2H), 4.72 (m, J = 5.6 Hz, 1H), 3.12 (m, J = 5.6 Hz, 2H), 2.83 (s, 3H), 2.05 (s, 1H). $[\alpha]_D^{20}$ 4.0 (c 0.5, EtOH/DMSO, 4:1). Anal. Calcd for $C_{23}H_{21}N_3O_4$: C, 68.47; H, 5.25; N, 10.42. Found: C, 68.62; H, 5.44; N, 10.23.

4.19. *N*-(1-Methyl-β-carboline-3-carbonyl)-L-lysine benzyl ester (5m)

Using the similar procedure as that of **5a**, starting from 744 mg (2.0 mmol) of HCl–Lys(Dde)-OBzl, the crude *N*-(1-methyl-β-carboline-3-carbonyl)-L-lysine (Dde) benzyl ester was directly subjected to 2% hydrazine in DMF (5 min) to remove Dde protecting group. After concentration, the residue was further purified by flash chromatography ($CHCl_3$/MeOH, 10:1) to give the title compound as a colorless powder 71 mg (8% yield). ESI/MS: 445 $[M+H]^+$; ^1H NMR (DMSO-d_6) δ = 11.80 (s, 1H), 8.78 (s, 1H), 8.28 (s, 1H), 7.70 (d, J = 8.0 Hz, 1H), 7.52 (d, J = 7.9 Hz, 1H), 7.34 (t, J = 7.0 Hz, 2H), 7.32 (t, J = 7.0 Hz, 2H), 7.30 (t, J = 7.6 Hz, 2H), 7.28 (t, J = 7.8 Hz, 2H), 7.26 (t, J = 7.8 Hz, 1H), 7.24 (t, J = 7.8 Hz, 1H), 7.18 (t, J = 7.6 Hz, 1H), 7.01 (t, J = 7.6 Hz, 1H), 5.27 (s, 2H), 4.43 (m, 1H), 2.65 (m, 2H), 2.55 (s, 3H), 1.89 (m, 2H), 1.55 (m, 2H), 1.30 (m, 2H). ^{13}C NMR (DMSO-d_6) δ = 171.5, 161.7, 152.8, 142.2, 140.6, 136.2, 135.4, 132.4, 129.0, 128.7, 127.1, 122.0, 120.1, 118.9, 113.4, 111.1, 105.9, 68.0, 52.8, 42.0, 32.5, 30.9, 21.0, 20.0.

4.20. *N*-(1-Methyl-β-carboline-3-carbonyl)-L-arginine benzyl ester (5n)

Using the similar procedure as that of **5a**, starting from 528 mg (2.0 mmol) of HCl–Arg–OBzl, 94 mg (10% yield) of the title compound purified by flash chromatography was obtained as a colorless powder. ESI/MS: 473 $[M+H]^+$; ^1H NMR (DMSO-d_6) δ = 11.78 (s, 1H), 8.78 (s, 1H), 8.27 (s, 1H), 8.0 (m, 1H), 7.90 (m, 1H), 7.70 (d, J = 8.0 Hz, 1H), 7.52 (d, J = 7.8 Hz, 1H), 7.35 (t, J = 7.1 Hz, 2H), 7.33 (t, J = 7.2 Hz, 2H), 7.30 (t, J = 7.6 Hz, 2H), 7.28 (t, J = 7.6 Hz, 2H), 7.26 (t, J = 7.6 Hz, 1H), 7.24 (t, J = 7.8 Hz, 1H), 7.18 (t, J = 7.6 Hz, 1H), 7.01 (t, J = 7.6 Hz, 1H), 5.30 (s, 2H), 4.42 (m, 1H), 2.66 (m, 2H), 1.89 (m, 2H), 1.55 (m, 2H). ^{13}C NMR (DMSO-d_6) δ = 171.6, 160.8, 158.0, 151.0, 141.0, 140.5, 135.4, 135.2, 131.4, 122.2, 120.1, 119.0, 129.0, 128.0, 127.0, 113.4, 111.0, 52.6, 37.5, 25.0, 28.0, 20.0.

4.21. 1-Methyl-β-carboline-3-carbonylhydrazide (6)

At 80 °C, to the solution of 2.5 ml (50.0 mmol) of hydrazine hydrate in 2 ml of methanol, a mixture of 480 mg (2.0 mmol) of methyl 1-methyl-β-carboline-3-carboxylate in 10 ml of chloroform and 2 ml of methanol was added dropwise. The reaction mixture was stirred at 80 °C for 5 h and TLC (chloroform/methanol, 15:1) indicated the complete disappearance of methyl 1-methyl-β-carboline-3-carboxylate. On evaporation the residue was purified by silica gel chromatography ($CHCl_3$/MeOH, 3:1) to provide 180 mg (38%) of the title compound as a yellow powder. Mp 294–295 °C; ESI/MS: 241 $[M+H]^+$. IR (KBr): 3340, 3335, 2924, 2910, 2900, 1620, 1600, 1585, 1562, 1440, 1380, 1066, 900 cm^{-1}; ^1H NMR (DMSO-d_6): δ = 11.90 (s, 1H), 8.63 (s, 1H), 8.34 (d, J = 7.8 Hz, 1H), 7.63 (d, J = 8.1 Hz, 1H), 7.56 (t, J = 7.5 Hz, 1H), 7.26 (t, J = 7.5 Hz, 1H), 4.60 (m, 1H), 2.80 (s, 3H). Anal. Calcd for $C_{13}H_{12}N_4O_2$: C, 64.99; H, 5.03; N, 23.32. Found: C, 65.18; H, 5.18; N, 23.16.

4.22. *N*-(1-Methyl-β-carboline-3-carbonyl)-*N*-(aminoethyl)-amine (7) and 1,2-di-(1-methyl-β-carboline-3-carbonyl)ethylenediamine (8)

At 88 °C, to 12 ml (180 mmol) of ethylenediamine, the mixture of 1.0 g (4.2 mmol) of methyl-1-methyl-β-carboline-3-carboxylate in 10 ml of methanol and 30 ml of chloroform was added dropwise. The reaction mixture was stirred at 88 °C for 5 h until TLC (chloroform/methanol, 15:1) indicated the complete disappearance of methyl-1-methyl-β-carboline-3-carboxylate. On evaporation the residue was separated by silica gel chromatography ($CHCl_3$/MeOH, 3:1) to afford 793 mg (71%) of *N*-(1-methyl-β-carboline-3-carbonyl)-*N*-(aminoethyl)-amine (**7**) and 20 mg (1.8%) of 1,2-di-(1-methyl-β-carboline-3-carbonyl)ethylenediamine (**8**) as a yellow powder.

Compound **7**: Mp 172–173 °C; ESI/MS: 269 $[M+H]^+$. IR (KBr) 3341, 3332, 3330, 3009, 1690, 1600, 1585, 1500, 1464, 762, 701 cm^{-1}. ^1H NMR (DMSO-d_6): δ = 8.85 (s, 1H), 8.46 (s, 1H), 7.99 (s, 1H), 7.73 (d, J = 7.5 Hz, 1H), 7.48 (d, J = 7.6 Hz, 1H), 7.12 (t, J = 7.6 Hz, 1H), 7.01 (t, J = 7.5 Hz, 1H), 3.28 (t, J = 4.5 Hz, 2H), 2.96 (t, J = 4.4 Hz, 2H), 2.82 (s, 3H), 2.78 (s, 2H). Anal. Calcd for $C_{15}H_{16}N_4O$: C, 67.15; H, 6.01; N, 20.88. Found: C, 67.33; H, 6.20; N, 21.06.

Compound **8**: Mp 210–211 °C; ESI/MS: 477 $[M+H]^+$. IR (KBr) 3345, 3334, 3331, 3010, 1692, 1603, 1586, 1501, 1460, 764, 702 cm^{-1}. ^1H NMR (DMSO-d_6) δ = 8.92 (s, 2H), 8.66 (s, 2H), 8.02 (s, 2H), 7.70 (d, J = 7.8 Hz, 2H), 7.45 (d, J = 8.1 Hz, 1H), 7.17 (t, J = 7.5 Hz, 2H), 7.07 (t, J = 7.5 Hz, 2H), 3.32 (t, J = 4.5 Hz, 2H), 3.02 (t, J = 4.4 Hz, 2H), 2.80 (s, 6H). Anal. Calcd for $C_{28}H_{24}N_6O_2$: C, 70.57; H, 5.08; N, 17.64. Found: C, 70.40; H, 5.27; N, 17.80.

4.23. In vitro cytotoxicity assay

In vitro cytotoxicity assays were carried out using 96-microtiter culture plate and MTT staining according to the previously reported procedures with a minor modification.[41] Cells (2×10^4 cells/well) were grown in RPMI-1640 medium containing 10% (v/v) fetal calf serum and 100 μg ml^{-1} penicillin and 100 μg ml^{-1} streptomycin. Cultures were propagated at 37 °C in a humidified atmosphere containing 5% CO_2. The synthetic compounds' stock solutions were prepared in DMSO. The tumor cell line panel consists of liver carcinoma (HepG2), cervical carcinoma (HeLa), and human breast cancer (MCF-7). The synthetic compounds in 2% DMSO were added to the cultures at day 1 after seeding. After 72 h, 20 μL MTT solution (5 μg/mL) per well was added to each culture medium and incubated for additional 4 h. The optical density was measured by the microplate reader after adding 100 μL of 10% SDS–0.01 N HCl solution to each well (n = 3).

4.24. Apparent permeability coefficient test

Caco-2 cells were obtained from the American Type Culture Collection, Rockville, MD, USA. The cells were cultivated on polycarbonate filters (transwell cell culture inserts, 12 mm in diameter, 3.0 μM in mean pore size) as described elsewhere.[51] Caco-2 cells grown on filter supports for 21 days were used for all transport tests, and the integrity of monolayers was routinely checked by measurements of transepithelial electrical resistance (approximately 700 Ω cm^2).

All absorption tests were performed in Hanks' balanced salt solution (HBSS). Compounds **5a**–**n** for evaluation were dissolved in HBSS to prepare drug solutions at a final concentration of 4×10^{-3} M. In apical to basolateral direction, transport was initiated by adding drug solutions (total AP volume, 0.5 ml) to the apical compartment of inserts held in transwells containing 1.5 ml HBSS (basolateral compartment). In basolateral to apical direction, transport was initiated by adding 1.5 ml of drug solution to basolateral compartment and adding 0.5 ml HBSS as receiving solution to apical side of the monolayers. The monolayers were incubated in air at 37 °C and 95% relative humidity. At 30, 60, 90

and 120 min, samples were withdrawn from the receiving side, and the concentrations of the samples were determined by HPLC analysis. The resistance of monolayers was checked at the end of each experiment. Apparent permeability coefficients (P_{app}) were calculated according to the following equation:

$$P_{app} = dQ/dt \cdot 1/(A \cdot C_0)$$

wherein dQ/dt is the initial permeability rate, C_0 is the initial concentration in the donor chamber, and A is the surface area of monolayer (1 cm^2).

4.25. Molecular modeling

Modeling study was performed using the Docking program Insight II from Accelrys (version 2000, Molecular Simulations, San Diego, CA) for structure visualization and analysis. The AMBER force field was used in all molecular mechanics (MM) calculations and minimization was performed using the steepest gradient algorithm with 1000 iteration steps or until convergence. All calculations were performed on a SGI IRIX6.5 workstation.

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References and notes

1. (a) Momekov, G.; Bakalova, A.; Karavanova, M. *Curr. Med. Chem.* **2005**, *12*, 2177–2191; (b) Hurley, L. H. *Nat. Rev. Cancer* **2002**, *2*, 188–200; (c) Demeunynck, M.; Bailly, C.; Wilson, W. D. *DNA and RNA Binders*; Wiley-VCH: New York, 2003.
2. (a) Lerman, L. S. *J. Mol. Biol.* **1961**, *3*, 18–30; (b) Lerman, L. S. *Proc. Natl. Acad. Sci. U.S.A.* **1963**, *49*, 94–102.
3. Martinez, R.; Chacon-Garcia, L. *Curr. Med. Chem.* **2005**, *12*, 127–151.
4. Wilson, W. D.; Jones, R. In *Intercalation Chemistry*; Whittingham, M. S., Jacobson, A. J., Eds.; Academic: New York, 1981, Chapter 14.
5. Denny, W. A.; Baguley, B. C.; Cain, B. F.; Waring, M. J. In *Mechanism of Action of Anticancer Drugs*; Neidle, S., Waring, M. J., Eds.; MacMillan: London, 1983.
6. Foye, W. O., Ed.; *Cancer Chemotherapeutic Agents*; American Chemical Society: Washington, DC, 1995.
7. Propst, C. L.; Perun, T. L., Eds.; *Nucleic Acid Targeted Drug Design*; Marcel Dekker: New York, 1992.
8. Neidle, S.; Waring, M. J., Eds.; *Molecular Aspects of Anti-Cancer Drug Action*; MacMillan: London, 1983.
9. Cholody, W. M.; Martelli, S.; Konopa, J. *J. Med. Chem.* **1990**, *33*, 2852–2856.
10. Capps, D. S.; Dunbar, J.; Kesten, S. R.; Shillis, J.; Werbel, L. M.; Plowman, J.; Ward, D. L. *J. Med. Chem.* **1992**, *35*, 4770–4778.
11. Cholody, W. M.; Martelli, S.; Konopa, J. *J. Med. Chem.* **1992**, *35*, 378–382.
12. Wanda, P.; Pognan, F.; Kaczmarek, L.; Boratynski, J. *J. Med. Chem.* **1994**, *37*, 3503–3510.
13. Gandolfi, C. A.; Beggiolin, G.; Menta, E.; Palumbo, M.; Sissi, C.; Spinelli, S.; Johnson, F. *J. Med. Chem.* **1995**, *38*, 526–536.
14. Bolognese, A.; Correale, G.; Manfra, M.; Lavecchia, A.; Mazzoni, O.; Novellino, E.; La Colla, P.; Sanna, G.; Loddo, R. *J. Med. Chem.* **2004**, *47*, 849–858.
15. Lynch, M. A.; Duval, O.; Sukhanova, A.; Devy, J.; Mackay, S. P.; Waigh, R. D.; Nabiev, I. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2643–2646.
16. Nakanishi, T.; Suzuki, M.; Mashiba, A.; Ishikawa, K.; Yokotsuka, T. *J. Org. Chem.* **1998**, *63*, 4235–4239.
17. Hayashi, K.; Nagao, M.; Sugimura, T. *Nucleic Acids Res.* **1977**, *4*, 3679–3685.
18. Meester, C. *Mutat. Res.* **1995**, *339*, 139–153.
19. Funayama, Y.; Nishio, K.; Wakabayashi, K.; Nagao, M.; Shimoi, K.; Ohira, T.; Hasegawa, S.; Saijo, N. *Mutat. Res.* **1996**, *349*, 183–191.
20. Ishida, J.; Wang, H. K.; Bastow, K. F.; Hu, C. Q.; Lee, K. H. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3319–3324.
21. Al-Allaf, T. A. K.; Rashan, L. J. *Eur. J. Med. Chem.* **1998**, *33*, 817–820.
22. Cao, R.; Peng, W.; Chen, H.; Ma, Y.; Liu, X.; Hou, X.; Guan, H.; Xu, A. *Biochem. Biophys. Res. Commun.* **2005**, *338*, 1557–1563.
23. Remsen, J. F.; Cerutti, P. A. *Biochem. Biophys. Res. Commun.* **1979**, *86*, 124–129.
24. Pognan, F.; Saucier, J. M.; Paoletti, C.; Kaczmarek, L.; Nantka-Namirski, P.; Mordarski, M.; Peczynska-Czoch, W. *Biochem. Pharm.* **1992**, *44*, 2149–2155.
25. Laronze, M.; Boisbrun, M.; Leonce, S.; Pfeiffer, B.; Renard, P.; Lozach, O.; Meijer, L.; Lansiaux, A.; Bailly, C.; Sapi, J.; Laronze, J. Y. *Bioorg. Med. Chem.* **2005**, *13*, 2263–2283.
26. Funayama, Y.; Nishio, K.; Wakabayashi, K.; Nagao, M.; Shimoi, K.; Ohira, T.; Hasegawa, S.; Saijo, N. *Mutat. Res.* **1996**, *349*, 183–191.
27. Beljanski, M.; Beljanski, M. S. *Exp. Cell Biol.* **1982**, *50*, 79–87.
28. (a) Peczynska-Czoch, W.; Pognan, F.; Kaczmarek, L.; Boratynski, J. *J. Med. Chem.* **1994**, *37*, 3503–3510; (b) Sobhani, A. M.; Ebrahimi, S. A.; Mahmoudian, M. *J. Pharm. Pharm. Sci.* **2002**, *5*, 19–23.
29. (a) Ju, J.; Pedersen-Lane, J.; Maley, F.; Chu, E. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 3769–3774; (b) Chu, E.; Ju, J.; Schmitz, J. C. *Antifolate Drugs Cancer Ther.* **1999**, 397–408; (c) Chu, E.; Copur, S. M.; Ju, J.; Chen, T.; Khleif, S.; Voeller, D. M.; Mizunuma, N.; Patel, M.; Maley, G. F.; Maley, F.; Allegra, C. J. *Mol. Cell. Biol.* **1999**, *19*, 1582–1594; (d) Ju, J.; Kane, S. E.; Lenz, H.; Danenberg, K. D.; Chu, E.; Danenberg, P. V. *Clin. Cancer Res.* **1998**, *4*, 2229–2236; (e) Liu, M.; Pelling, J. C.; Ju, J.; Chu, E.; Brash, D. E. *Cancer Res.* **1998**, *58*, 1723–1729.
30. (a) Bi, L.; Zhao, M.; Wang, C.; Peng, S.; Winterfeldt, E. *J. Org. Chem.* **2002**, *67*, 22–26; (b) Zhao, M.; Wang, C.; Guo, M.; Peng, S. *J. Prakt. Chem.* **1999**, *341*, 691–694; (c) Ding, W.; Zhang, J.; Yao, Z.; Lu, R.; Wu, D.; Li, G.; Shen, Z.; Sun, Y.; Lin, G.; Wang, C.; Zhao, M.; Peng, S. *Bioorg. Med. Chem.* **2004**, *12*, 4989–4994; (d) Wang, C.; Ding, W.; Zhao, M.; Lin, G.; Peng, S. *Prep. Biochem. Biotech.* **2003**, *33*, 189–195; (e) Aubin, S.; Martin, B.; Delcros, J.; Arlot-Bonnemains, Y.; Baudy-Floc'h, M. *J. Med. Chem.* **2005**, *48*, 330–334; (f) Bouget, K.; Aubin, S.; Delcros, J.; Arlot-Bonnemainsc, Y.; Baudy-Floc'h, M. *Bioorg. Med. Chem.* **2003**, *11*, 4881–4889.
31. (a) Hastings, C. A.; Barton, J. K. . *Biochemistry* **1999**, *38*, 10042–10051; (b) Pabo, C. O.; Sauer, R. T. *Annu. Rev. Biochem.* **1992**, *61*, 1053–1095; (c) Dias, N.; Goossens, J.-F.; Baldeyrou, B.; Lansiaux, A.; Colson, P.; Di Salvo, A.;

Bernal, J.; Turnbull, A.; Mincher, D. J.; Bailly, C. *Bioconjugate Chem.* **2005**, *16*, 949–958.
32. (a) Guan, Y.; Louis, E. D.; Zheng, W. *J. Toxicol. Environ. Health A.* **2001**, *64*, 645–660; (b) Fekkes, D.; Tuiten, A.; Bom, I.; Pepplinkuizen, L. *Life Sci.* **2001**, *69*, 2113–2121.
33. Ganapathy, M. E.; Huang, W.; Wang, H.; Ganapathy, V.; Leibach, F. H. *Biochem. Biophys. Res. Commun.* **1998**, *246*, 470.
34. Marma, M. S.; Kashemirov, B. A.; McKenna, C. E. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1787–1790.
35. Han, H.-K.; De Vrueh, R. L. A.; Rhie, J. K.; Covitz, K.-M. Y.; Smith, P. L.; Lee, C.-P.; Oh, D.-M.; Sadee, W.; Amidon, G. L. *Pharm. Res.* **1998**, *15*, 1154–1159.
36. Doring, F.; Will, J.; Amasheh, S.; Clauss, W.; Ahlbrecht, H.; Daniel, H. *J. Biol. Chem.* **1998**, *273*, 23211–23218.
37. Anand, B. S.; Katragadda, S.; Mitra, A. K. *J. Pharmacol. Exp. Ther.* **2004**, *311*, 659–667.
38. Marshall, N. J.; Andruszkiewicz, R.; Gupta, S.; Milewski, S.; Payne, J. W. *J. Antimicrob. Chemother.* **2003**, *51*, 821–831.
39. Alley, M. C.; Scudiero, D. A.; Monks, P. A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589–601.
40. Grever, M. R., Schepartz, S. A., Chabner, B. A.; The National Cancer Institute: Cancer Drug Discovery and Development Program, Seminars in Oncology, 1992, 19, 622–638.
41. (a) Al-Allaf, T. A. K.; Rashan, L. J. *Eur. J. Med. Chem.* **1998**, *33*, 817; (b) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55–63.
42. Luscombe, N. M.; Laskowski, R. A.; Thornton, J. M. *Nucleic Acids Res.* **2001**, *29*, 2860–2874.
43. Luscombe, N. M.; Thornton, J. M. *J. Mol. Biol.* **2002**, *320*, 991–1009.
44. Sathyapriya, R.; Vishveshwara, S. *Nucleic Acids Res.* **2004**, *32*, 4109–4118.
45. Marion, T. N.; Tillman, D. M.; Jou, N. T.; Hill, R. J. *Immunol. Rev.* **1992**, *128*, 123–149.
46. Radic, M. Z.; Weigert, M. *Annu. Rev. Immunol.* **1994**, *12*, 487–520.
47. Eilat, D.; Anderson, W. F. *Mol. Immunol.* **1994**, *27*, 203–210.
48. Madalengoitia, J. S.; Tepe, J. J.; Werbovetz, K. A.; Lehnert, E. K.; Macdonald, T. L. *Bioorg. Med. Chem.* **1997**, *5*, 1807–1815.
49. Artursson, P.; Karlsson, J. *Biochem. Biophys. Res. Commun.* **1991**, *175*, 880–885.
50. Stewart, B. H.; Chan, O. H. *J. Pharm. Sci.* **1998**, *87*, 1471–1478.
51. (a) Yee, S. *Pharm. Res.* **1997**, *14*, 763–766; (b) Kaldas, M. I.; Walle, U. K.; Walle, T. *J. Pharm. Pharmacol.* **2003**, *55*, 307–312.
52. Camenisch, G.; Alsenz, J.; Van de Waterbeemd, H.; Folkers, G. *Eur. J. Pharm. Sci.* **1998**, *6*, 313–319.
53. Camenisch, G.; Folkers, G.; Van de Waterbeemd, H. *Int. J. Pharm.* **1997**, *147*, 61–70.
54. Faassen, F.; Vogel, G.; Spanings, H.; Vromans, H. *Int. J. Pharm.* **2003**, *263*, 113–122.