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Dual-acting agents that possess reversing resistance and anticancer activities: Design, synthesis, MES-SA/Dx5 cell assay, and SAR of Benzyl 1,2,3,5,11,11a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′,4′:1,2] pyridin[3,4-*b*]indol-2-substitutedacetates

Jiawang Liu,[a] Guohui Cui,[a] Ming Zhao,[a,*] Chunying Cui,[a] Jingfang Ju[b,*] and Shiqi Peng[a,*]

[a]*College of Pharmaceutical Sciences, Capital Medical University, Beijing 100069, PR China*
[b]*Mitchell Cancer Institute, University of South Alabama, 307 N. University Boulevard, Mobile AL 36688-0002, USA*

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Abstract—Based on the structural analysis of fumitremorgin C (FTC), imidazoline and β-carboline amino acid benzylester, 14 novel 2-substitutedtetracyclic derivatives of tetrahydrocarboline **4a**–**n** were prepared. We demonstrated that the exposure of MES-SA/Dx5 cells to some of **4a**–**n** resulted in significant reduction of resistance of the cells against doxorubicin. This reduced resistance was accompanied by lowering of IC_{50} value to doxorubicin from 1.55 ± 0.26 μmol/L to 0.33 ± 0.05 μmol/L for 2-(2-butyl)-derivative **4c**, to 1.03 ± 0.22 μmol/L for 2-methyl-derivative **4d**, to 0.46 ± 0.04 μmol/L for 2-benzyl-derivative **4f**, to 0.98 ± 0.25 μmol/L for 2-indole-3-yl-methyl-derivative **4h**, to 0.36 ± 0.03 μmol/L for 2-benzyloxycarbonylmethyl-derivative **4i**, to 0.77 ± 0.08 μmol/L for 2-benzyloxycarbonylethyl-derivative **4j**, and to 0.77 ± 0.08 μmol/L for 2-benzyloxycarbonylamino-*n*-butyl-derivative **4l**. Proliferation assays of **4a**–**n** indicated **4c**,**f**,**i**,**j** were able to inhibit the proliferation of doxorubicin resistant MES-SA/Dx5 cells. The SAR analysis revealed that the benzylester form and the tetracyclic structure of **4a**–**n** were critical for both sensitizing doxorubicin and the cellular anti-proliferative effect.

1. Introduction

Chemoresistance is one of the major obstacles in cancer chemotherapy due to intrinsic or acquired drug resistance. A broad-spectrum resistance to structurally and mechanistically diverse antitumor agents is known as multidrug resistance (MDR).[1,2] Diverse mechanisms are involved in MDR, including drug inactivation, amount reduction of intracellular drugs, drugs inducing block of apoptotic pathway, etc. Among these, overexpression of members of ATP-binding cassette (ABC) transporter family proteins is generally known as a predominant cause of MDR.[3] As plasma membrane proteins, they can actively extrude a wide variety of structurally diverse anticancer agents, thereby reducing intracellular drug amount.[4] P-glycoprotein (Pgp) is one of the most extensively studied ABC transporters.[5] Currently, a number of substances such as vinca alkaloids, anthracyclines, linear peptides, cyclic peptides, ethidium bromide, rhodamine 123, calcein/AM, and Fluo-3/AM are known to be transported by Pgp.[6–9] As Pgp transported compounds, vinca alkaloids and anthracyclines can be effluxed through the cellular membrane, thus limiting their anti-proliferative effects.[10,11] Recently, it has become apparent that Pgp is potentially an important mediator of MDR.[12] Based on this fact, Pgp inhibitors could be designed to reverse MDR.

Fumitremorgin C (FTC), a member of a group of indole alkaloids, is a selective inhibitor of the breast cancer resistance protein (BCRP/ABCG2).[13–15] However, this natural product of fungal origin also has tremor-inducing side effects and can trigger cell cycle arrest at the G2/M transition.[16,17]

Keywords: MES-SA/Dx5; Reversing resistance; Anti-proliferation; SAR; Benzyl 1,2,3,5,11,11a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′,4′:1,2]pyridin[3,4-*b*]indol-2-substituted-acetates.
* Corresponding authors. Tel.: +1 251 460 7393; fax: +1 251 460 6994 (J.J.); tel.: +86 10 8391 1535; fax: +86 10 8391 1535 (M.Z.); tel.: +86 10 8391 1528; fax: +86 10 8391 1528 (S.P.); e-mail addresses: jju@usouthal.edu; sqpeng@mail.bjmu.edu.cn

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A series of investigations demonstrated that imidazoline is an important pharmacophore.[18–20] Studies related to anticancer indole alkaloids found that the benzylesters of β-carboline amino acid conjugates exhibited higher anticancer activity than their parental acid.[21] To improve the specificity and selectivity of FTC while eliminating the potential side effects, we analyzed the structural features of FTC, imidazoline and β-carboline amino acid benzylester conjugates, and established a structural connection (Fig. 1), from which benzyl 1,2,3,5,11,11a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′,4′:1,2]-pyridin[3,4-*b*]indol-2-substitutedacetate was considered to be the common structural feature of FTC, imidazoline, and β-carboline amino acid benzylester conjugates. In this study, we report the synthesis and characterization of their potency in reversing doxorubicin resistance using doxorubicin resistant MES-SA/Dx5 cells as a model system.

2. Results and discussion

2.1. Evaluation of chemosensitizing activities of 4a–n in doxorubicin resistant MES-SA/Dx5 cells

2.1.1. Chemistry. As depicted in Scheme 1, using H_2SO_4 as the catalyst, 3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**1**) was synthesized via the Pictet–Spengler condensation of L-tryptophane and formaldehyde with 95% yield. Coupling **1** and Boc_2O generated 3*S*-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**2**) in 76% yield. The amidation of **2** and L-amino acid benzylesters gave 3*S*-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carbonyl-L-amino acid benzylesters **3a**–**n** in 85–96% yields. After removal of Boc group from **3a**–**n**, the products were treated in situ with acetone and triethylamine to provide benzyl 1,2,3,5,11,11a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′,4′:1,2]pyridin[3,4-*b*]indol-2-substitutedacetates. The yields of this one-pot-two-step synthesis ranged from 45% to 60%. The data indicates that our synthetic route can provide all intermediates and final products with good yields.

2.1.2. Effect of doxorubicin on the growth of MES-SA and MES-SA/Dx5 cells. To determine the effect of doxorubicin on the growth of both doxorubicin sensitive MES-SA cells and doxorubicin resistant MES-SA/Dx5 cells, both of which achieve their relative levels of resistance via the high expression of P-gp, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide assays (MTT assay) were performed according to manufacturer's standard procedure. It was found that when the cells were exposed to a doxorubicin solution of concentrations ranging from 0.04 μM to 10.00 μM, survival differed between both cell groups. For MES-SA cells, the IC_{50} of doxorubicin was 0.347 μM. For MES-SA/Dx5 cells, the IC_{50} of doxorubicin was 1.55 μM. These data imply that, in the presence of doxorubicin, both doxorubicin sensitive MES-SA cells and doxorubicin resistant MES-SA/Dx5 cells grew reasonably. Thus, both MES-SA and MES-SA/Dx5 cells may be effectively used for the evaluation of reversing resistance agents. On the other hand, from these IC_{50} values, a resistance index of 4.47 (1.55 μM/0.347 μM) was obtained.

2.1.3. Effect of 4a–n on the resistance of MES-SA/Dx5 cells against doxorubicin. To determine the effect of **4a**–**n** on the resistance of MES-SA/Dx5 cells against doxorubicin, MTT assays were performed according to a standard procedure. It was found that when the cells were exposed to a doxorubicin solution of concentrations ranging from 0.04 μM to 10.00 μM and a 1.00 μM solution of **4a**–**n**, the resistance of MES-SA/Dx5 cells to doxorubicin was decreased in varying degrees. The IC_{50} values corresponding to compounds **4a**–**n** are summarized in Table 1 and are directly compared by Figure 2. As the representative description, the survival of MES-SA/Dx5 cells exposed to doxorubicin solution with a series of concentrations and the 1 μM solution of **4f, 4i** and **4c** are summarized in Figure 3.

The data demonstrate that in the 14 novel compounds, **4c**–**f**,**h**–**l** may significantly decrease the resistance of MES-SA/Dx5 cells to doxorubicin, and **4a,b,g,m,n** are inactive. Since the decrease of the IC_{50} of doxorubicin

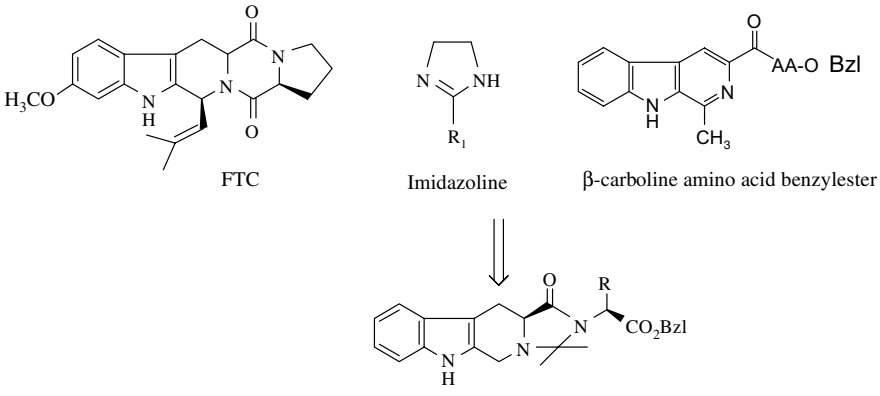
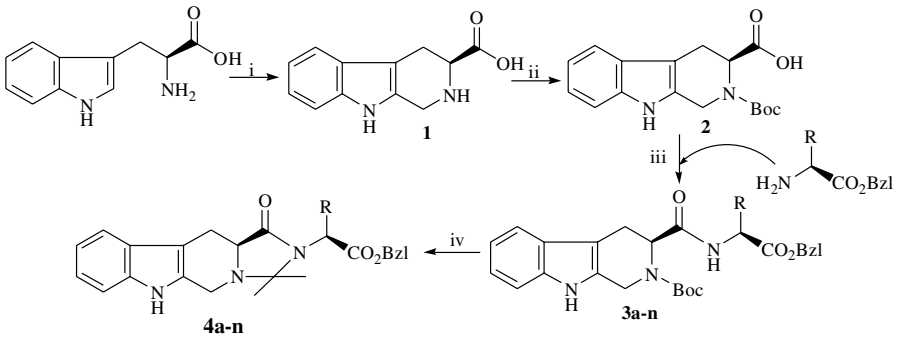


Figure 1. The structural connection for FTC, imidazoline, β-carboline amino acid benzylester and benzyl 1,2,3,5,11,11a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′,4′:1,2]pyridin[3,4-*b*]indol-2-substitutedacetate. Wherein R represents the side chains of L-amino acids, $R_1 = CH_2CH_2Ph$, NHPh or Ph.



Scheme 1. Synthetic route of benzyl 1,2,3,5,11,11a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′,4′:1,2]pyridin[3,4-*b*]indol-2-substitutedacetates. (i) HCHO and dilute H_2SO_4; (ii) $(Boc)_2O$, DMF; (iii) DCC, HOBt, NMM; (iv) HCl in EtOAc(4N), MeOH, acetone, Et_3N. In **3a** and **4a** R = $CH(CH_3)CH_2CH_3$; **3b** and **4b** R = $CH(CH_3)_2$; **3c** and **4c** R = $CH_2CH(CH_3)_2$; **3d** and **4d** R = CH_3; **3e** and **4e** R = H; **3f** and **4f** R = $CH_2C_6H_5$; **3g** and **4g** R = $CH_2C_6H_4$-OH-*p*; **3h** and **4h** R = Indole-3-yl-CH_2; **3i** and **4i** R = CH_2CO_2Bzl; **3j** and **4j** R = $CH_2CH_2CO_2Bzl$; **3k** and **4k** R = CH_2OBzl; **3l** and **4l** R = $CH_2CH_2CH_2CH_2NHCBz$; **3m** and **4m** R_1 = CH_2CONH_2; **3n** and **4n** R = $CH_2CH_2CONH_2$.

Table 1. $^{Dx}IC_{50}$ values and resistance index values of MES-SA/Dx5 in the presence of **4a**–**n**[a]

Compound	2-Substituent	$^{Dx}IC_{50}$ (μM)	R.I.	Compound	2-Substituent	$^{Dx}IC_{50}$ (μM)	R.I.
4a	$CH(CH_3)CH_2CH_3$	1.53 ± 0.32	4.41	**4h**	Indole-3-yl-CH_2	0.98 ± 0.25[c]	2.82
4b	$CH(CH_3)_2$	1.61 ± 0.42	4.64	**4i**	CH_2CO_2Bzl	0.36 ± 0.03[b]	1.04
4c	$CH_2CH(CH_3)_2$	0.33 ± 0.05[b]	0.95	**4j**	$CH_2CH_2CO_2Bzl$	0.77 ± 0.08[b]	2.22
4d	CH_3	1.03 ± 0.22[c]	2.97	**4k**	CH_2OBzl	1.27 ± 0.20[c]	3.66
4e	H	1.35 ± 0.19[c]	3.89	**4l**	$CH_2(CH_2)_3NHCBz$	0.94 ± 0.30[c]	2.71
4f	$CH_2C_6H_5$	0.46 ± 0.04[b]	1.33	**4m**	CH_2CONH_2	1.56 ± 0.30	4.50
4g	$CH_2C_6H_4$-OH-*p*	1.55 ± 0.33	4.47	**4n**	$CH_2CH_2CONH_2$	1.50 ± 0.23	4.32

[a] R.I. represents resistance index of MES-SA/Dx5 to doxorubicin; R.I. of doxorubicin alone = 4.47; $^{Dx}IC_{50}$ of doxorubicin alone = 1.55 ± 0.26 μM; $n = 6$.

[b] Compare to doxorubicin alone, $P < 0.01$.

[c] Compare to doxorubicin alone, $P < 0.05$.

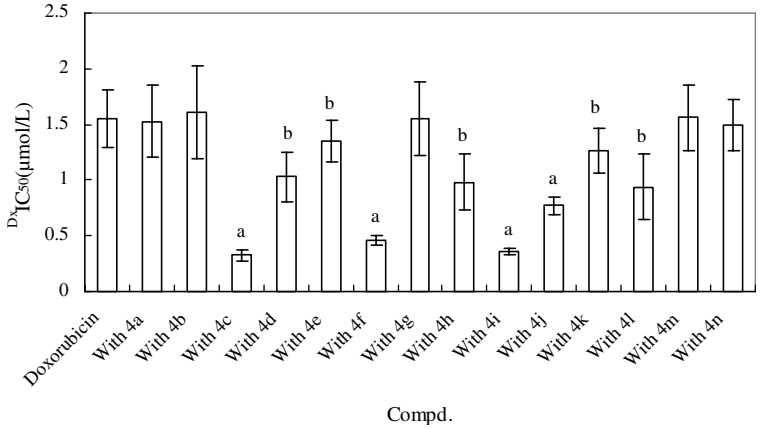


Figure 2. The $^{Dx}IC_{50}$ values of doxorubicin for MES-SA/Dx5 cells in a 1.00 μM solution of **4a**–**n**. (a) Compare to doxorubicin alone, $P < 0.01$; (b) Compare to doxorubicin alone, $P < 0.05$; $n = 6$.

against MES-SA/Dx5 cells may involve contributions from the inhibition of the resistance of MES-SA/Dx5 cells to doxorubicin and from the possible cytotoxic actions of **4c**–**f**,**h**–**l**, to strip their cytotoxic contribution should be necessary.

2.1.4. Effect of 4a–n themselves on MES-SA/Dx5 cell proliferation. To strip the possible contribution of cytotoxicity of the 1.00 μM solution of **4a**–**n** to the decreasing resistance of MES-SA/Dx5 cells to doxorubicin, the effect of **4a**–**n** on the growth of MES-SA/Dx5 cells was also determined by MTT assays according to a standard procedure. It was found that when the cells were exposed to a 1.00 μM solution of **4a**–**n**, the growth of MES-SA/Dx5 cells was inhibited in varying degrees. The inhibition rates are summarized in Table 2 and Figure 4. The data indicate that the 1.00 μM solution of **4c,f,i,j** do exhibit low inhibition to the growth of

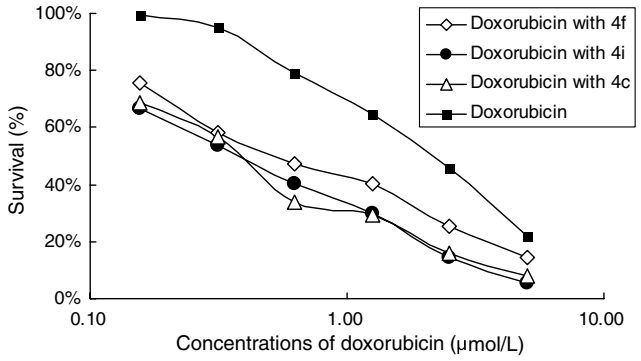


Figure 3. Survival of MES-SA/Dx5 cells exposed to doxorubicin solution with a series of concentrations and the 1 μM solution of **4f,i,c**.

MES-SA/Dx5 cells. Therefore, the reversing resistance of **4c,f,i,j** includes some contribution of their cytotoxicity against MES-SA/Dx5 cells. Accordingly, **4a**–**n** may be classified as the dual-acting agents of reversing resistance and cytotoxicity (**4c,f,i,j**), simply reversing resistance agents (**4d,e,h,k,i**), and carcinoma inactive agents (**4a,b,g,m,n**).

2.2. Effect of skeleton modification of 4a–n as reversing agents

To find a possible strategy for structural variation, the limited skeleton modifications of **4a**–**n** were performed. In these limited skeleton modifications, in order to make the results representative, three types of compounds were selected as model compounds. Accordingly, the dual-acting compounds of reversing resistance and cytotoxicity **4c,i** and the simply reversing resistance compound **4h** were used for the following modification.

2.2.1. Bioassay gave preference to 3-benzylester over 3-carboxylic acid and 3-methylester. When **4a**–**n** are considered as 2,2-dimethyl-4-oxoimidazo-[3′,5′:2,3]-β-carboline-3-carboxylic acid benzylesters, the 3-position conversion should be the most simple modification with the immediate analogs being carboxylic acids and methylesters. In order to confirm the importance of the benzylesters, **4i** was selected as a model compound and compared with its acid analogue **4″i** and methylester analogue **4′i**. Based on the synthetic route represented by Scheme 2, **4′i** and **4″i** were prepared. The coupling reaction of *N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid and HCl·L-Asp-$(OMe)_2$ generated *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carbonyl)-L-aspartic acid dimethylester (**3′i**) in 90% yield. Under the same conditions, the one-pot-two-step synthesis provided dimenthyl 1,2,3,5,11,11a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′,4′:1,2]pyridin[3,4-*b*]indol-2-succinate (**4′i**) in 56% yield. The saponification of **4′i** provided 1,2,3,5,11,11a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′,4′:1,2]pyridin[3,4-*b*]indol-2-succinic acid (**4″i**) in 90% yield.

Using the same assay as that used for **4a**–**n**, the $^{Dx}IC_{50}$ of both **4′i** and **4″i** were determined as 1.55 μmol/L and the resistance index values were determined as 4.47. In the anti-proliferation assay, both **4′i** and **4″i** exhibited no activity. Thus neither reversing action nor anti-proliferation action was observed for **4′i** and **4″i**.

Table 2. Effect of **4a**–**n** on the growth of MES-SA/Dx5[a]

Compound	2-Substituent	% Inhibition	Compound	2-Substituent	% Inhibition
4a	$CH(CH_3)CH_2CH_3$	0	**4h**	Indole-3-yl-CH_2	0
4b	$CH(CH_3)_2$	0	**4i**	CH_2CO_2Bzl	31.2 ± 4.6
4c	$CH_2CH(CH_3)_2$	38.9 ± 6.2	**4j**	$CH_2CH_2CO_2Bzl$	13.4 ± 3.2
4d	CH_3	0	**4k**	CH_2OBzl	0
4e	H	0	**4l**	$CH_2(CH_2)_3NHCBz$	0
4f	$CH_2C_6H_5$	31.2 ± 3.8	**4m**	CH_2CONH_2	0
4g	$CH_2C_6H_4$-OH-*p*	0	**4n**	$CH_2CH_2CONH_2$	0

[a] Concentration of **4a**–**n** = 1.00 μM; $n = 6$.

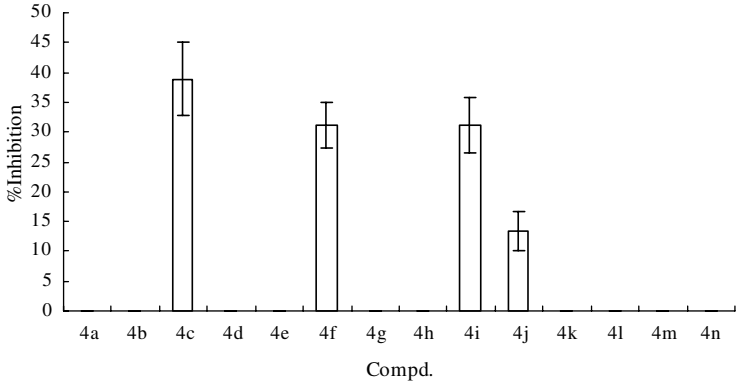
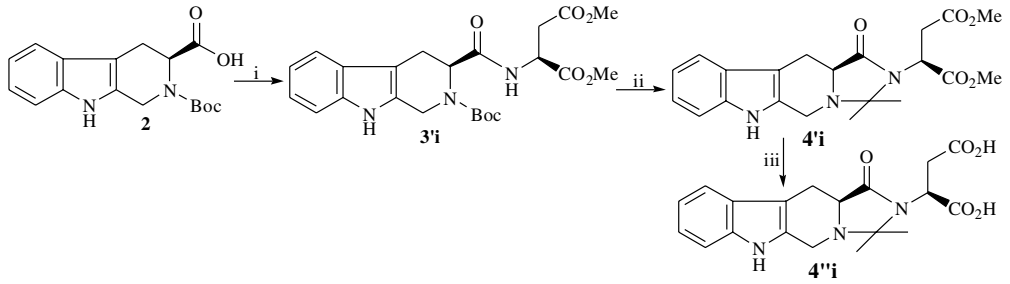


Figure 4. Inhibition effect of **4a**–**n** on MES-SA/Dx5 cell proliferation, $n = 6$.



Scheme 2. Synthetic route of 1,2,3,5,11,11a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′,4′:1,2]pyridin[3,4-*b*]indol-2-succinic acid. (i) DCC, HOBt, NMM and L-Asp(OMe)$_2$; (ii) HCl in EtOAc(4N), MeOH, acetone, Et_3N; (iii) Aqueous solution of NaOH (2N).

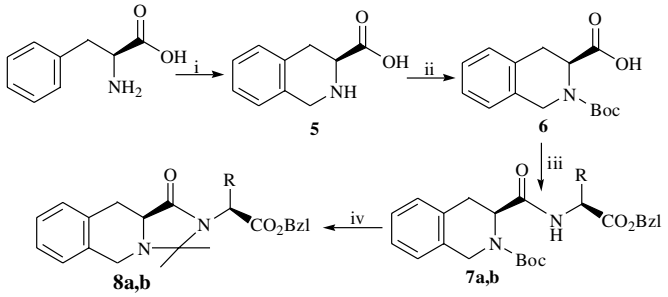
These observations imply that benzylester is a critical form for **4a**–**n** to exert reversing and anti-proliferation actions.

2.2.2. Effect of removing the pyrrole ring from 4a–n on the reversing and anti-proliferation actions. An immediate skeleton simplification should be removing the pyrrole ring from **4a**–**n**. To evaluate the benefit of the remaining indole alkaloid structure, **4c,i** were selected as model compounds, their pyrrole ring was abridged, and benzyl 1,2,3,5,10,10a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′,4′:1,2]-isoquinolin-2-substitutedacetates (**8a,b**) were prepared according to the synthetic route represented by Scheme 3. Using H_2SO_4 as the catalyst, 3*S*-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (**5**) was synthesized via the Pictet–Spengler condensation using L-phenylalnine and formaldehyde with 95% yield. Coupling **5** and Boc_2O offered 3*S*-*N*-Boc-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (**6**) in 80% yield. The amidation of **6** and L-Leu(OBzl) or L-Asp(OBzl)$_2$ gave **7a** and **7b** in 95% and 92% yield, respectively. After removal of Boc from **7a** and **7b**, the products were treated in situ with acetone and triethylamine to provide **8a** and **8b**, respectively. The yield of this one-pot-two-step synthesis was 60% and 58%. The data indicate that the synthetic route of Scheme 3 is appropriate not only for preparing the known compounds **5** and **6**, but also for preparing the new compounds **7a**, **7b**, **8a**, and **8b**.

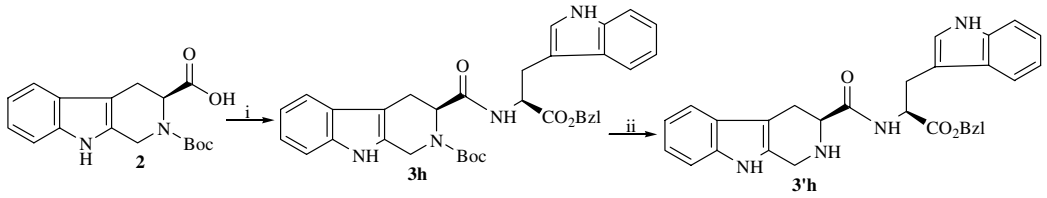
Using the same assay as that used for **4a**–**n**, the $^{Dx}IC_{50}$ of doxorubicin for MES-SA/Dx5 resistant cells in the presence of both **8a** and **8b** was determined to be 1.55 μmol/L and the resistance index was calculated to be 4.47. In the anti-proliferation assay, both **8a** and **8b** also exhibited no activity. Thus neither reversing action nor anti-proliferation action was observed for **8a** and **8b**. These observations imply that the pyrrole ring in **4a**–**n** is critical for them to exert both reversing and anti-proliferation actions.

2.2.3. Effect of removing the 4-oxoimidazol ring from 4a–n on the reversing and anti-proliferation actions. A direct skeleton simplification should be removing the 4-oxoimidazol ring from **4a**–**n**. To examine the benefit of the remaining 4-oxoimidazol ring, **4h** was selected as a model compound, its 4-oxoimidazol ring was abridged, and benzyl 3-(1*H*-indol-3-yl)-2-[*N*-(3*S*)-1,2,3,4-tetrahydro-β-carbonyl)amino]propanoate (**3′h**) was prepared according to the synthetic route represented by Scheme 4. Coupling **2** and L-Trp-OBzl offered 3*S*-*N*-Boc-1,2,3,4-tetrahydrocarboline-3-carbonyl-tryptophan benzylester (**3h**) in 90% yield. After removal of Boc from **3h**, the product was treated with triethylamine to provide **3′h** in 80% yield. The data indicate that using the synthetic route represented by Scheme 4, **3h** and **3′h** may be prepared in acceptable yields.

Using the same assay as that used for **4a**–**n**, the $^{Dx}IC_{50}$ of doxorubicin for MES-SA/Dx5 resistant cells in the presence of **3′h** was determined to be 1.04 μmol/L and the resistance index was calculated to be 2.98. However, in the anti-proliferation assay, **3′h** exhibited no activity. These observations imply that the oximidazole ring in **4a**–**n** is important for it to exert reversing action and is critical for it to exert anti-proliferation actions.



Scheme 3. Synthetic route of dibenzyl 1,2,3,5,10,10a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′,4′:1,2]isoquinolin-2-succinate and benzyl 1,2,3,5,10,10a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′,4′:1,2]isoquinolin-2-*iso*butylacetate. (i) Dilute H_2SO_4, HCHO; (ii) $(Boc)_2O$, DMF; (iii) DCC, HOBt, NMM, L-Leu-OBzl or L-Asp(OBzl)$_2$; (iv) HCl in EtOAc(4N), MeOH, acetone, Et_3N. In **7a** and **8a** R = CH_2CO_2Bzl; **7b** and **8b** R = $CH_2CH(CH_3)_2$.



Scheme 4. Synthetic route of benzyl 3-(1*H*-indol-3-yl)-2-[*N*-(3*S*)-1,2,3,4-tetrahydro-β-carbonyl)amino]propanoate. (i) DCC, HOBt, NMM, L-Trp-OBzl; (ii) HCl in EtOAc (4N).

2.2.4. Effect of removing the two rings from 4a–n on the reversing and anti-proliferation actions. Another direct skeleton simplification should be removing the two rings from **4a**–**n**. To assess the benefit of the remaining tetracyclic structure, **4h** was selected as model compound, its two rings were removed, and L-Trp-L-Trp-OBzl (**4″h**) was prepared according to the synthetic route represented by Scheme 5. Coupling Boc-L-Trp-OH and L-Trp-OBzl offered Boc-L-Trp-L-Trp-OBzl (**4′h**) in 92% yield. After removal of Boc, **4′h** was converted into **4″h** in 85% yield. The data indicate that the synthetic route of Scheme 5 is appropriate for preparing **4′h** and **4″h**.

Using the same assay as that used for **4a**–**n**, the $^{Dx}IC_{50}$ of doxorubicin for MES-SA/Dx5 resistant cells in the presence of **4″h** was determined to be 1.55 μmol/L and the resistance index value was calculated to be 4.47. In the anti-proliferation assay, **4″h** exhibited no activity. Thus neither reversing action nor anti-proliferation action was observed for **4″h**. These observations imply that the remaining tetracyclic structure for **4a**–**n** is critical for it to exert reversing and anti-proliferation actions.

2.3. Effect of 2-substituents on the activity of 4a–n

In Section 2.1.4, it was pointed out that the novel reversing agents **4a–n** may be classified as the dual-acting agents of reversing resistance and cytotoxicity (**4c,f,i,j**), the simply reversing resistance agents (**4d,e,h,k,l**), and the carcinoma inactive agents (**4a,b,g,m,n**). As the 2-substituted-tetracyclic derivatives of tetrahydrocarboline, the structural differences between them are characterized by their 2-substituents. Analyzing the association of the mentioned activity-based classification and the 2-substituents, it is likely that a polar 2-substitution leads to a carcinoma inactive structure, 2-benzylester substitution leads to dual-acting agents of reversing resistance and cytotoxicity, and 2-aromaticnonester substitution leads to simply reversing resistance agents. It should be emphasized that these are only very preliminary analyses and there are some obvious exceptions. In addition, a detailed SAR remains to be elucidated.

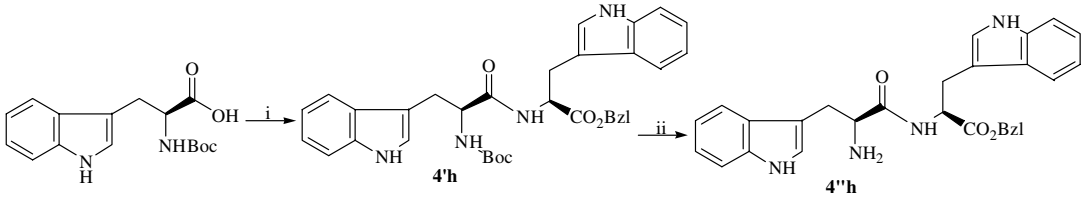
3. Conclusion

In conclusion, in this study, nine of the fourteen novel benzyl 1,2,3,5,11,11a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′,4′:1,2]pyridin[3,4-*b*]indol-2-substitutedacetates can increase the sensitivity of MES-SA/Dx5 cells to doxorubicin, suggesting that the structural combination of FTC, imidazoline and β-carboline-amino-acid benzylester may result in a desirable lead compound for finding reversing resistance agents. Relatively high reversing activities of our compounds demonstrate that the novel structure does provide a selection for the development of chemosensitizing agents. Furthermore, the revealed independent anti-proliferative action of some of these reversing agents from the MES-SA/Dx5 cell proliferation assay demonstrates that the novel reversing agents **4a**–**n** may be classified as the dual-acting agents of reversing resistance and cytotoxicity, the simply reversing resistance agents and the carcinoma inactive agents. The novel structure does have reversing and anti-proliferative dual-action. The association of the classification and the 2-substituents suggests that a polar 2-substitution leads to carcinoma inactive structure, 2-benzylester substitution leads to dual-acting agents of reversing resistance and cytotoxicity, and 2-aromaticnonester substitution leads to simply reversing resistance agents. A detailed SAR remains to be elucidated.

4. Experimental

4.1. Synthesis

General. The protected amino acids with L-configuration were purchased from Sigma Chemical Co. All of the coupling and deprotective reactions were carried out under anhydrous conditions. Chromatography was



Scheme 5. Synthetic route of L-tryptophyl-L-tryptophan benzylester. (i) DCC, HOBt, NMM, L-Trp-OBzl; (ii) HCl in EtOAc(4N).

performed on Qingdao silica gel H. The purities of the intermediates and the products were confirmed by TLC (Merck silica gel plates 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 with $p < 0.05$ as significant cut-off.

4.1.1. 3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (1). To a mixture of 5.0 g (24.5 mmol) of L-tryptophane, 25 ml of H_2SO_4 (1 mol/L), and 80 ml of water, 8 ml of formaldehyde (36–38%) was added. The reaction mixture was stirred at room temperature for 2 h and adjusted to pH 6–7 with concentrated ammonia liquor. The mixture obtained was kept at 0 °C for 12 h and the formed precipitates were collected by filtration. After recrystallization, 3.97 g (75%) of the title compound was obtained as a colorless powder. Mp 280–282 °C; ESI/MS (*m*/*z*) 217 $[M+H]^+$; IR (KBr): 3450, 3200, 3000, 2950, 2850, 1700, 1601, 1452, 1070, 900 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): $\delta = 10.99$ (s, 1H), 9.89 (s, 1H), 7.30 (t, $J = 7.5$ Hz, 1H), 7.22 (t, $J = 8.0$ Hz, 1H), 7.01 (d, $J = 8.0$ Hz, 1H), 6.81 (d, $J = 7.5$ Hz, 1H), 4.01 (t, $J = 4.8$ Hz, 1H), 3.75 (dd, $J = 10.5$ Hz, $J = 5.0$ Hz, 1H), 3.64 (dd, $J = 10.5$ Hz, $J = 2.4$ Hz, 1H), 2.91 (d, $J = 10.5$ Hz, 2H), 2.86 (s, 1H). Anal. Calcd for $C_{12}H_{12}N_2O_2$ C 66.65, H, 5.59, N 12.96. Found C 66.45, H 5.72, N 12.79.

4.1.2. *N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (2). The suspension of 1.1 g (5.0 mmol) of **1** in 15 ml of DMF and 1.4 ml of triethylamine was vigorously stirred at room temperature, to which 1.1 g (7.7 mmol) of Boc-N_3 was added in 30 min. The reaction mixture was stirred at room temperature for 24 h and at 40 °C for 80 h. To the reaction mixture, 5 ml of citrate in water (20%) was added and the solution was extracted with ethyl acetate (3×330 ml). The separated ethyl acetate layer was dried with anhydrous $MgSO_4$. After removal of $MgSO_4$ by filtration the filtrate was evaporated to dryness. The residue obtained was crystallized in $CHCl_3$ to give 1.20 g (76%) of the title compound. Mp 165–170 °C; ESI/MS (*m*/*z*) 317 $[M+H]^+$; IR (KBr): 3452, 3205, 3001, 2952, 2848, 1705, 1645, 1600, 1450, 1072, 901 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): $\delta = 10.87$ (s, 1H), 9.86 (s, 1H), 7.32 (t, $J = 7.6$ Hz, 1H), 7.21 (t, $J = 7.9$ Hz, 1H), 7.00 (d, $J = 7.9$ Hz, 1H), 6.84 (t, $J = 7.6$ Hz, 1H), 4.84 (t, $J = 5.0$ Hz, 1H), 4.20 (dd, $J = 10.2$ Hz, $J = 4.8$ Hz, 1H), 3.98 (dd, $J = 10.2$ Hz, $J = 3.2$ Hz, 1H), 2.93 (d, $J = 10.2$ Hz, 2H), 1.46 (s, 9H). Anal. Calcd for $C_{17}H_{20}N_2O_4$ C 64.54, H, 6.37, N 8.86. Found C 64.41, H 6.25, N 8.74.

General procedure for the preparation of *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carbonyl)-L-amino acid benzylesters (3a–n). At 0 °C to the solution of 2.0 g (6.33 mmol) *N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid in 30 ml of anhydrous THF, 1.2 g (8.9 mmol) of HOBt was added. After 10 min, 1.75 g (8.5 mmol) of DCC was then added. The suspension of 6.96 mmol of HCl·L-AA-OBzl in 3 ml of anhydrous THF was adjusted to pH 8–9 with *N*-methyl morpholine and stirred at room temperature for another 20 min. This suspension was then added to the solution of *N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid and the reaction mixture was stirred at 0 °C for 2 h and at room temperature for 16 h. On evaporation the residue was dissolved in 30 ml of ethyl acetate. The solution was 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 the title compound was obtained as powder.

4.1.3. *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-L-isoleucine benzylester (3a). Yield: 90%. Mp 134–136 °C; ESI/MS (*m*/*z*) 520 $[M+H]^+$. IR (KBr): 3445, 3339, 3210, 3010, 2955, 2840, 1758, 1732, 1645, 1600, 1452, 1390, 1371, 1064, 903 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): $\delta = 9.96$ (s, 1H), 8.00 (s, 1H), 7.27 (t, $J = 7.1$ Hz, 1H), 7.22 (t, $J = 7.0$ Hz, 2H), 7.20 (t, $J = 7.1$ Hz, 1H), 7.00 (d, $J = 7.1$ Hz, 1H), 7.13 (d, $J = 7.0$ Hz, 2H), 7.10 (t, $J = 7.0$ Hz, 1H), 6.88 (d, $J = 7.1$ Hz, 1H), 5.35 (s, 2H), 4.95 (t, $J = 5.0$ Hz, 1H), 4.42 (t, $J = 5.0$ Hz, 1H), 4.25 (dd, $J = 10.2$ Hz, $J = 4.0$ Hz, 1H), 4.05 (dd, $J = 10.2$ Hz, $J = 3.5$ Hz, 1H), 2.94 (d, $J = 6.0$ Hz, 2H), 2.91 (m, $J = 5.1$ Hz, 1H), 1.47 (s, 9H), 1.30 (m, $J = 5.2$ Hz, 2H), 1.05 (d, $J = 5.2$ Hz, 3H), 0.95 (t, $J = 5.2$ Hz, 3H). $[\alpha]_D^{20}$ −36 ° (*c* 0.37, $CHCl_3$–CH_3OH, 1:1, v/v); Anal. Calcd for $C_{30}H_{37}N_3O_5$ C 69.34, H 7.18, N 8.09. Found C 69.50, H 7.29, N 8.24.

4.1.4. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carbonyl)-L-valine benzylester (3b). Yield: 94%. Mp 169–171 °C; ESI/MS (*m*/*z*) 506 $[M+H]^+$. IR (KBr): 3441, 3336, 3205, 3004, 2953, 2843, 1757, 1732, 1645, 1600, 1453, 1390, 1372, 1064, 900 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): $\delta = 10.00$ (s, 1H), 7.98 (s, 1H), 7.27 (t, $J = 7.4$ Hz, 1H), 7.23 (t, $J = 7.0$ Hz, 2H), 7.20 (t, $J = 7.4$ Hz, 1H), 7.02 (d, $J = 7.4$ Hz, 1H), 7.13 (d, $J = 7.0$ Hz, 2H), 7.10 (t, $J = 7.0$ Hz, 1H), 6.87 (d, $J = 7.4$ Hz, 1H), 5.34 (s, 2H), 4.86 (t, $J = 5.2$ Hz, 1H), 4.40 (d, $J = 5.1$ Hz, 1H), 4.24 (dd, $J = 10.0$ Hz, $J = 4.3$ Hz, 1H), 4.05 (dd, $J = 10.0$ Hz, $J = 3.5$ Hz, 1H), 3.12 (m, $J = 5.2$ Hz, 1H), 2.93 (d, $J = 6.4$ Hz, 2H), 1.49 (s, 9H), 1.03 (d, $J = 5.1$ Hz, 6H). Anal. Calcd for $C_{29}H_{35}N_3O_5$ C 68.89, H 6.98, N 8.31. Found C 68.74, H 7.08, N 8.48.

4.1.5. *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-L-leucine benzylester (3c). Yield: 92%. Mp 140–142 °C; ESI/MS (*m*/*z*) 520 $[M+H]^+$. IR (KBr): 3440, 3207, 3005, 2951, 2842, 1735, 1644, 1603, 1452, 1393, 1370, 1060, 904 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): $\delta = 9.95$ (s, 1H), 8.06 (s, 1H), 7.27 (t, $J = 7.3$ Hz, 1H), 7.24 (t, $J = 7.1$ Hz, 2H), 7.21 (t, $J = 7.3$ Hz, 1H), 7.15 (d, $J = 7.1$ Hz, 2H), 7.11 (t, $J = 7.1$ Hz, 1H), 7.06 (d, $J = 7.3$ Hz, 1H), 6.82 (d,

J = 7.3 Hz, 1H), 5.33 (s, 2H), 4.93 (t, J = 5.2 Hz, 1H), 4.41 (t, J = 5.2 Hz, 1H), 4.26 (dd, J = 10.0 Hz, J = 4.5 Hz, 1H), 4.09 (dd, J = 10.0 Hz, J = 3.8 Hz, 1H), 2.91 (d, J = 6.2 Hz, 2H), 2.83 (d, J = 5.1 Hz, 2H), 1.53 (s, 9H), 1.34 (m, J = 5.1 Hz, 1H), 1.07 (d, J = 5.3 Hz, 6H). $[\alpha]_D^{20}$ −33 ° (c 0.31, $CHCl_3$–CH_3OH, 1:1, v/v); Anal. Calcd for $C_{30}H_{37}N_3O_5$ C 69.34, H 7.18, N 8.09. Found C 69.25, H 7.08, N 8.26.

4.1.6. *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-L-alanine bensylester (3d). Yield: 95%. Mp 110–112 °C; ESI/MS (m/z) 478 $[M+H]^+$. IR (KBr): 3447, 3342, 3001, 2945, 2842, 1761, 1733, 1602, 1455, 1391, 1373, 1062, 899 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): δ = 9.93 (s, 1H), 8.04 (s, 1H), 7.30 (t, J = 7.3 Hz, 1H), 7.24 (t, J = 7.3 Hz, 1H), 7.22 (t, J = 7.2 Hz, 2H), 7.14 (d, J = 7.2 Hz, 2H), 7.11 (t, J = 7.2 Hz, 1H), 6.95 (d, J = 7.3 Hz, 1H), 6.83 (d, J = 7.3 Hz, 1H), 5.35 (s, 2H), 4.86 (d, J = 5.4 Hz, 1H), 4.56 (m, J = 5.2 Hz, 1H), 4.22 (dd, J = 10.1 Hz, J = 4.5 Hz, 1H), 4.15 (dd, J = 10.0 Hz, J = 3.7 Hz, 1H), 2.92 (d, J = 10.0 Hz, 2H), 1.57 (d, J = 5.0 Hz, 3H), 1.46 (s, 9H). $[\alpha]_D^{20}$ −100 ° (c 0.38, $CHCl_3$–CH_3OH, 1:1, v/v); Anal. Calcd for $C_{27}H_{31}N_3O_5$ C 67.91, H, 6.54, N 8.80. Found C 67.72, H 6.40, N 8.90.

4.1.7. *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-L-glycine benzylester (3e). Yield: 95%. Mp 139–141 °C; ESI/MS (m/z) 464 $[M+H]^+$. IR (KBr): 3444, 3336, 3004, 2940, 2845, 1761, 1730, 1602, 1455, 1392, 1375, 1060, 903 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): δ = 9.96 (s, 1H), 8.05 (s, 1H), 7.27 (t, J = 7.4 Hz, 1H), 7.23 (t, J = 7.2 Hz, 2H), 7.18 (t, J = 7.4 Hz, 1H), 7.15 (d, J = 7.2 Hz, 2H), 7.11 (t, J = 7.2 Hz, 1H), 6.93 (d, J = 7.4 Hz, 1H), 6.85 (d, J = 7.4 Hz, 1H), 5.36 (s, 2H), 4.86 (d, J = 5.2 Hz, 1H), 4.25 (dd, J = 10.0 Hz, J = 4.3 Hz, 1H), 4.17 (dd, J = 10.0 Hz, J = 3.4 Hz, 1H), 4.15 (s, 2H), 2.93 (d, J = 10.0 Hz, 2H), 1.48 (s, 9H). $[\alpha]_D^{20}$ −84 ° (c 0.36, $CHCl_3$–CH_3OH, 1:1, v/v); Anal. Calcd for $C_{26}H_{29}N_3O_5$ C 67.37, H, 6.31, N 9.07. Found C 67.25, H 6.18, N 9.19.

4.1.8. *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-L-phenylalanine benzylester (3f). Yield: 96%. Mp 144–146 °C; ESI/MS (m/z) 554 $[M+H]^+$. IR (KBr): 3442, 3350, 3202, 3009, 2944, 2842, 1758, 1734, 1642, 1601, 1455, 1390, 1371, 1065, 902 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): δ = 9.95 (s, 1H), 7.99 (s, 1H), 7.28 (t, J = 7.3 Hz, 1H), 7.25 (t, J = 7.6 Hz, 2H), 7.22 (t, J = 7.3 Hz, 2H),7.17 (t, J = 7.3 Hz, 1H), 7.15 (d, J = 7.4 Hz, 2H), 7.13 (d, J = 7.3 Hz, 2H), 7.11 (t, J = 7.3 Hz, 1H), 7.03 (t, J = 7.3 Hz, 1H), 6.98 (d, J = 7.6 Hz, 1H), 6.82 (d, J = 7.2 Hz, 1H), 5.34 (s, 2H), 4.95 (d, J = 5.2 Hz, 1H), 4.84 (t, J = 5.2 Hz, 1H), 4.25 (dd, J = 10.0 Hz, J = 4.1 Hz, 1H), 4.15 (dd, J = 10.0 Hz, J = 3.5 Hz, 1H), 3.19 (d, J = 5.2 Hz, 2H), 2.92 (d, J = 10.0 Hz, 2H), 1.49 (s, 9H). $[\alpha]_D^{20}$ −52 ° (c 0.38, $CHCl_3$–CH_3OH, 1:1, v/v); Anal. Calcd for $C_{33}H_{35}N_3O_5$C 71.59, H 6.37, N 7.59. Found C 71.74, H 6.22, N 7.77.

4.1.9. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carbonyl)-L-tyrosine benzylester (3g). Yield: 90%. Mp 155–157 °C; ESI/MS (m/z) 570 $[M+H]^+$; IR (KBr): 3444, 3200, 3006, 2950, 2842, 1730, 1647, 1600, 1456, 1395, 1370, 1060, 900 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): δ = 9.95 (s, 1H), 8.04 (s, 1H), 7.37 (t, J = 7.4 Hz, 1H), 7.23 (t, J = 7.6 Hz, 1H), 7.16 (d, J = 7.4 Hz, 2H), 7.14 (t, J = 7.1 Hz, 2H), 7.12 (d, J = 7.1 Hz, 2H), 7.09 (t, J = 7.1 Hz, 1H), 7.05 (d, J = 7.4 Hz, 1H), 6.93 (d, J = 7.6 Hz, 1H), 6.90 (d, J = 7.4 Hz, 2H), 5.40 (s, 2H), 4.99 (s, 1H), 4.91 (d, J = 5.5 Hz, 1H), 4.82 (t, J = 5.7 Hz, 1H), 4.26 (m, J = 5.3 Hz, 2 H), 3.17 (d, J = 5.3 Hz, 2H), 2.95 (d, J = 5.1 Hz, 2H), 1.47 (s, 9H). Anal. Calcd for $C_{33}H_{35}N_3O_6$ C 69.58, H 6.19, N 7.38. Found C 69.41, H 6.10, N 7.53.

4.1.10. *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-L-tryptophan benzylester (3h). Yield: 92%. Mp 136–138 °C; ESI/MS (m/z) 593 $[M+H]^+$. IR (KBr): 3444, 3337, 3208, 3005, 2945, 2835, 1758, 1736, 1645, 1602, 1449, 1390, 1370, 1067, 903 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): δ = 9.96 (s, 1H), 9.89 (s, 1H), 8.03 (s, 1 H), 7.29 (t, J = 7.3 Hz, 1H), 7.27 (t, J = 7.2 Hz, 1H), 7.22 (t, J = 7.0 Hz, 2H), 7.14 (d, J = 7.0 Hz, 2H), 7.12 (d, J = 7.5 Hz, 1H), 7.11 (t, J = 7.0 Hz, 1H), 7.10 (t, J = 7.5 Hz, 1H), 7.09 (d, J = 7.3 Hz, 1H), 7.07 (t, J = 7.5 Hz, 1H), 7.02 (d, J = 7.3 Hz, 1H), 6.97 (d, J = 7.2 Hz, 1H), 6.85 (s, 1H), 5.36 (s, 2H), 4.96 (d, J = 5.2 Hz, 1H), 4.73 (t, J = 5.1 Hz, 1H), 4.27 (d, J = 5.0 Hz, 2H), 3.17 (d, J = 5.2 Hz, 2H), 2.97 (d, J = 6.2 Hz, 2H), 1.47 (s, 9H). $[\alpha]_D^{20}$ −77 ° (c 0.36, $CHCl_3$–CH_3OH, 1:1, v/v); Anal. Calcd for $C_{35}H_{36}N_4O_5$ C 70.93, H 6.12, N 9.45. Found C 70.80, H 6.01, N 9.60.

4.1.11. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carbonyl)-L-aspartic acid dibenzylester (3i). Yield: 92%. Mp 132–134°C; ESI/MS (m/z) 612 $[M+H]^+$; IR (KBr): 3445, 3340, 3214, 3002, 2952, 2843, 1758, 1730, 1646, 1602, 1455, 1388, 1369, 1064, 901 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): δ = 10.01 (s, 1H), 8.02 (s, 1H), 7.30 (t, J = 7.2 Hz, 1H), 7.24 (t, J = 7.2 Hz, 1H), 7.22 (t, J = 7.0 Hz, 2H), 7.20 (t, J = 7.0 Hz, 2H), 7.15 (d, J = 7.0 Hz, 2H), 7.13 (d, J = 7.0 Hz, 2H), 7.11 (t, J = 7.0 Hz, 1H), 7.10 (t, J = 7.0 Hz, 1H), 7.02 (d, J = 7.2 Hz, 1H), 6.98 (d, J = 7.2 Hz, 1H), 5.36 (s, 2H), 5.34 (s, 2H), 4.94 (d, J = 5.2 Hz, 1H), 4.79 (t, J = 5.2 Hz, 1H), 4.27 (d, J = 5.2 Hz, 2H), 2.94 (d, J = 5.0 Hz, 2H), 2.87 (d, J = 5.2 Hz, 2H), 1.47 (s, 9H). Anal. Calcd for $C_{35}H_{37}N_3O_7$ C 68.72, H 6.10, N 6.87. Found C 68.54, H 6.19, N 7.00.

4.1.12. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carbonyl)-L-glutamic acid dibenzylester (3j). Yield: 90%. Mp 144–146°C; ESI/MS (m/z) 626 $[M+H]^+$; IR (KBr): 3445, 3204, 3007, 2948, 2826, 1735, 1640, 1600, 1452, 1391, 1375, 1062, 900 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): δ = 9.92 (s, 1H), 8.03 (s, 1H), 7.36 (t, J = 7.5 Hz, 1H), 7.27 (t, J = 7.5 Hz, 1H), 7.24 (t, J = 7.3 Hz, 2H), 7.22 (t, J = 7.4 Hz, 2H), 7.21 (d, J = 7.3 Hz, 2H), 7.19 (d, J = 7.4 Hz, 2H), 7.16 (t, J = 7.3 Hz, 1H), 7.14 (t, J = 7.4 Hz, 1H), 7.04 (d, J = 7.5 Hz, 1H), 6.86 (d, J = 7.5 Hz, 1H), 5.36 (s, 2H), 5.33 (s, 2H), 4.93 (d, J = 5.3 Hz, 1H), 4.46 (t, J = 5.5 Hz, 1H), 4.25 (d, J = 5.4 Hz, 2H), 2.95 (d, J = 5.2 Hz, 2 H), 2.25 (t, J = 5.5 Hz, 2H), 2.22 (t,

J = 5.6 Hz, 2H), 1.48 (s, 9H). Anal. Calcd for $C_{36}H_{39}N_3O_7$ C 69.10, H 6.28, N 6.72. Found C 69.27, H 6.18, N 6.89.

4.1.13. *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-*O*-benzyl-L-serine benzylester (3k). Yield: 93%. Mp 125–127 °C; ESI/MS (*m*/*z*) 494 $[M+H]^+$. IR (KBr): 3445, 3338, 3207, 3005, 2956, 2843, 1762, 1733, 1642, 1601, 1457, 1390, 1371, 1062, 903 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): δ = 9.97 (s, 1H), 7.99 (s, 1H), 7.27 (t, J = 7.2 Hz, 1H), 7.23 (t, J = 7.3 Hz, 1H), 7.21 (t, J = 7.0 Hz, 2H), 7.13 (d, J = 7.0 Hz, 2H), 7.10 (t, J = 7.0 Hz, 1H), 6.99 (d, J = 7.3 Hz, 1H), 6.85 (t, J = 7.2 Hz, 1H), 5.33 (s, 2H), 4.89 (d, J = 5.2 Hz, 1H), 4.50 (t, J = 5.0 Hz, 1H), 4.17 (d, J = 5.0 Hz, 2H), 4.15 (d, J = 5.2 Hz, 2H), 2.94 (d, J = 5.3 Hz, 1H), 2.90 (d, J = 5.3 Hz, 1H), 2.25 (s, 1H), 1.47 (s, 9H). $[\alpha]_D^{20}$ −51 ° (*c* 0.35, $CHCl_3$–CH_3OH, 1:1, v/v); Anal. Calcd for $C_{27}H_{31}N_3O_6$ C 65.71, H 6.33, N 8.51. Found C 65.58, H 6.39, N 8.70.

4.1.14. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carbonyl)-L-(Z)lycine benzylester (3l). Yield: 94%. Mp 152–154 °C; ESI/MS (*m*/*z*) 669 $[M+H]^+$; IR (KBr): 3445, 3002, 2943, 2845, 1732, 1604, 1457, 1062, 894 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): δ = 9.97 (s, 1H), 8.01 (s, 1 H), 7.98 (s, 1H), 7.28 (t, J = 7.6 Hz, 1H), 7.23 (t, J = 7.2 Hz, 1H), 7.21 (t, J = 7.1 Hz, 1H), 7.19 (t, J = 7.5 Hz, 1H), 7.16 (t, J = 7.2 Hz, 2H), 7.15 (d, J = 7.2 Hz, 2H), 7.13 (t, J = 7.2 Hz, 2H), 7.12 (t, J = 7.2 Hz, 2H), 6.94 (d, J = 7.6 Hz, 1H), 6.86 (d, J = 7.6 Hz, 1H), 5.37 (s, 2H), 5.35 (s, 2H), 4.91 (d, J = 5.5 Hz, 1H), 4.42 (t, J = 4.4 Hz, 1H), 4.21 (dd, J = 10.0 Hz, J = 4.5 Hz, 1H), 4.19 (dd, J = 10.0 Hz, J = 3.7 Hz, 1H), 2.96 (t, J = 4.4 Hz, 2H), 2.94 (d, J = 10.0 Hz, 2H), 1.92 (m, J = 4.4 Hz, 2H), 1.56 (m, J = 4.4 Hz, 2 H), 1.49 (s, 9H), 1.29 (m, J = 4.4 Hz, 2H). $[\alpha]_D^{20}$ −29 ° (*c* 0.35, $CHCl_3$–CH_3OH, 1:1, v/v); Anal. Calcd for $C_{38}H_{44}N_4O_7$ C 68.24, H, 6.63, N 8.38. Found C 68.09, H 6.51, N 8.54.

4.1.15. *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-L-asparagine benzylester (3m). Yield: 90%. Mp 142–144 °C; ESI/MS (*m*/*z*) 521 $[M+H]^+$. IR (KBr): 3443, 3207, 3004, 2932, 2833, 1735, 1630, 1604, 1451, 1392, 1375, 1064, 900 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): δ = 9.96 (s, 1H), 8.05 (s, 1H), 7.28 (t, J = 7.3 Hz, 1H), 7.23 (t, J = 7.4 Hz, 2H), 7.19 (t, J = 7.3 Hz, 1H), 7.17 (d, J = 7.4 Hz, 2H), 7.15 (t, J = 7.4 Hz, 1H), 7.06 (d, J = 7.3 Hz, 1H), 6.87 (d, J = 7.3 Hz, 1H), 6.05 (s, 2H), 5.33 (s, 2H), 4.97 (d, J = 5.3 Hz, 1H), 4.45 (t, J = 5.3 Hz, 1H), 4.24 (d, J = 5.3 Hz, 2H), 2.92 (d, J = 5.1 Hz, 2 H), 2.17 (t, J = 5.1 Hz, 2H), 1.47 (s, 9H). $[\alpha]_D^{20}$ −42 ° (*c* 0.30, $CHCl_3$–CH_3OH, 1:1, v/v); Anal. Calcd for $C_{28}H_{32}N_4O_6$ C 64.60, H 6.20, N 10.76. Found C 64.78, H 6.29, N 10.57.

4.1.16. *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-L-glutamine benzylester (3n). Yield: 87%. Mp 130–132 °C; ESI/MS (*m*/*z*) 535 $[M+H]^+$. IR (KBr): 3441, 3207, 3004, 2942, 2830, 1736, 1642, 1600, 1455, 1390, 1375, 1062, 903 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): δ = 9.95 (s, 1H), 8.04 (s, 1H), 7.27 (t, J = 7.3 Hz, 1H), 7.24 (t, J = 7.2 Hz, 2H), 7.18 (t, J = 7.3 Hz, 1H), 7.16 (d, J = 7.2 Hz, 2H), 7.13 (t, J = 7.2 Hz, 1H), 7.04 (d, J = 7.3 Hz, 1H), 6.85 (d, J = 7.3 Hz, 1H), 6.08 (s, 2H), 5.35 (s, 2H), 4.94 (d, J = 5.4 Hz, 1H), 4.43 (t, J = 5.4 Hz, 1H), 4.27 (d, J = 5.5 Hz, 2H), 2.90 (d, J = 5.2 Hz, 2H), 2.15 (t, J = 5.4 Hz, 2H), 2.16 (t, J = 5.4 Hz, 2H), 1.49 (s, 9H). $[\alpha]_D^{20}$ −50 ° (*c* 0.38, $CHCl_3$–CH_3OH, 1:1, v/v); Anal. Calcd for $C_{29}H_{34}N_4O_6$ C 65.15, H 6.41, N 10.48. Found C 65.32, H 6.52, N 10.31.

4.1.17. Benzyl 1,2,3,5,11,11a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′,4′:1,2]pyridin[3,4-*b*]indol-2-(2-butyl)-acetate (4a). At 0 °C to the solution of 2.0 g (3.85 mmol) of *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-L-isoleucine benzylester in 8 ml of ethyl acetate, 16 ml of hydrogen chloride/ethyl acetate (4N) was added dropwise. The reaction solution was stirred at 0 °C for 90 min and evaporated under reduced pressure. The residue obtained was dissolved in 60 ml of methanol and 20 ml of acetone. With triethylamine the reaction solution was adjusted to pH 9, at room temperature and in dark stirred for 240 h, and TLC ($CHCl_3$–MeOH, 10:1) indicated the complete disappearance of *N*-[(3*S*)-*N*-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-L-isoleucine benzylester. On evaporation the residue was dissolved in 200 ml of ethyl acetate. The solution was 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 the residue was dissolved in 5 ml of methanol to give 0.92 g (52%) of the title compound as yellowish crystals. Mp 192–193 °C; ESI-MS (*m*/*e*) 460 $[M+H]^+$; ^1H NMR (DMSO-d_6, 300 MHz) δ = 10.714 (s, 1H), 7.410 (m, 5H), 7.350 (d, J = 7.5 Hz, 1 H), 7.274 (d, J = 7.5 Hz, 1H), 7.023 (t, J = 7.5 Hz, 1H), 6.957 (t, J = 7.5 Hz, 1H), 5.183 (s, 2H), 4.356 (t, 1H), 3.970 (d, J = 16.5 Hz, 1 H), 3.919 (d, J = 16.5 Hz, 1H), 3.567 (dd, J = 10 Hz, J = 5 Hz, 1H), 2.869 (dd, J = 4 Hz, J = 15 Hz, 1H), 2.629 (t, 1H), 1.875 (m, 1H), 1.414 (m, 1H), 1.215 (m, 1H), 1.073 (s, 3H), 1.059 (s, 3H), 0.880 (d, J = 6.6 Hz, 3H), 0.833 (t, J = 6.6 Hz, 3H). ^{13}C NMR (DMSO-d_6, 300 MHz) δ = 173.39, 171.84, 136.34, 136.18, 134.19, 128.92, 128.62, 128.42, 127.52, 120.88, 118.74, 117.61, 111.33, 106.79, 78.65, 66.48, 56.50, 52.55, 42.14, 36.98, 25.29, 25.21, 24.54, 19.04, 15.92, 11.62; $[\alpha]_D^{20}$ −128.04 (*c* 0.25, acetone). Anal. Calcd for $C_{28}H_{33}N_3O_3$ C 73.18, H 7.24, N 9.14. Found C 73.33, H 7.37, N 9.01.

4.1.18. Benzyl 1,2,3,5,11,11a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′,4′:1,2]pyridin[3,4-*b*]indol-2-*iso*-propyl-acetate (4b). With the same procedure as that used for the preparation of **4a** from 2.00 g (3.96 mmol) of *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carbonyl)-L-valine benzylester, 1.06 g (60%) of the title compound was obtained as colorless crystals. Mp 196–198 °C; ESI-MS (*m*/*e*) 446 $[M+H]^+$; ^1H NMR (DMSO-d_6, 300 MHz) δ/ppm = 10.725 (s, 1H), 7.425 (m, 5H), 7.353 (d, J = 7.5 Hz, 1H), 7.279 (d, J = 7.5 Hz, 1H), 7.032 (t, J = 7.5 Hz, 1 H), 6.963 (t, J = 7.5 Hz, 1H), 5.188 (s, 2H), 4.452 (t, 1H), 3.854 (d, J = 14 Hz, 1H), 3.701 (d,

J = 14 Hz, 1H), 3.536 (dd, J = 4.5 Hz, J = 11 Hz, 1H), 3.092 (dd, 1H, J = 14.4, J = 4.5), 2.951 (m, 1H), 2.726 (t, 1H), 1.452 (s, 3H), 1.325 (s, 3H), 1.121 (m, J = 6.6 Hz, 3H), 0.963 (m, J = 6.6 Hz, 3H); ^{13}C NMR (DMSO-d_6, 300 MHz): δ/ppm = 173.356, 171.856, 136.349, 136.124, 130.974, 128.896, 128.529, 127.965, 127.175, 121.682, 119.238, 118.022, 110.550, 108.235, 78.851, 66.366, 61.785, 57.148, 41.682, 33.936, 27.854, 23.902, 20.497, 19.830, 18.822; $[\alpha]_D^{20}$ −111.23 (c 0.25, acetone). Anal. Calcd for $C_{27}H_{31}N_3O_3$ C 72.78, H 7.01, N 9.43. Found C 72.64, H 7.12, N 9.59.

4.1.19. Benzyl 1,2,3,5,11,11a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′,4′:1,2]pyridin[3,4-*b*]indol-2-*iso*-butylacetate (4c). With the same procedure as that used for the preparation of **4a** from 2.00 g (3.85 mmol) of *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-L-leucine benzylester, 0.97 g (55%) of the title compound was obtained as colorless crystals. Mp 190–192 °C; ESI-MS (m/e) 460 [M+H]$^+$; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm = 10.834 (s, 1H), 7.445 (d, J = 7.2 Hz, 1H), 7.268 (d, J = 7.2 Hz, 1H), 7.064 (t, J = 7.2 Hz, 1H), 7.007 (t, J = 7.2 Hz, 1H), 5.186 (s, 2H), 4.438 (t, 1H), 4.023 (d, J = 14 Hz, 1 H), 3.859 (d, J = 14 Hz, 1H), 3.560 (dd, J = 4.2 Hz, J = 10.5 Hz, 1 H), 3.092 (dd, J = 4.2 Hz, J = 15 Hz, 1H), 2.734 (t, 1H), 2.226 (m, 2 H), 1.865 (m, 1H), 1.435 (s, 3H), 1.284 (s, 3H), 0.937 (d, 6H); ^{13}C NMR (DMSO-d_6, 300 MHz) δ/ppm = 171.648, 171.332, 136.266, 136.121, 131.124, 128.856, 128.334, 128.147, 127.156, 121.640, 119.545, 118.094, 110.762, 108.092, 78.478, 66.580, 57.852, 52.466, 41.656, 39.082, 25.218, 25.086, 23.633, 22.425, 22.412, 19.801; $[\alpha]_D^{20}$ − 103.78 (c 0.25, acetone). Anal. Calcd for $C_{28}H_{33}N_3O_3$ C 73.18, H 7.24, N 9.14. Found C 73.35, H 7.11, N 9.32.

4.1.20. Benzyl 1,2,3,5,11,11a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′,4′:1,2]pyridin[3,4-*b*]indol-2-methylacetate (4d). With the same procedure as that used for the preparation of **4a** from 2.00 g (4.19 mmol) of *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-L-alanine benzylester, 0.79 g (45%) of the title compound was obtained as colorless crystals. Mp 198–200 °C; ESI-MS (m/e) 418 [M+H]$^+$; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm = 10.722 (s, 1H), 7.486 (m, 5H), 7.329 (d, J = 7.5 Hz, 1H), 7.265 (d, J = 7.5 Hz, 1H), 7.014 (t, J = 7.5 Hz, 1 H), 6.959 (t, J = 7.5 Hz, 1H), 5.192 (s, 2H), 4.384 (m, 1H), 3.972 (d, J = 16.5 Hz, 1H), 3.756 (d, J = 16.5 Hz, 1H), 3.465 (dd, J = 4.3 Hz, J = 10.0 Hz, 1H), 3.063 (dd, J = 4.3 Hz, J = 15.0 Hz, 1H), 2.752 (t, 1 H), 1.491 (d, J = 4.8 Hz, 3H), 1.435 (s, 3H), 1.264 (s, 3 H).^{13}C NMR (DMSO-d_6, 300 MHz) δ/ppm = 173.545, 170.826, 136.714, 136.214, 131.442, 129.635, 128.564, 128.125, 127.722, 122.345, 120.115, 118.944, 111.245, 108.904, 70.870, 66.026, 60.134, 44.547, 42.109, 30.114, 29.115, 25.044, 14.117; $[\alpha]_D^{20}$ − 157.00 (c 0.25, methanol). Anal. Calcd for $C_{25}H_{27}N_3O_3$ C 71.92, H 6.52, N 10.06. Found C 71.78, H 6.40, N 9.90.

4.1.21. Benzyl 1,2,3,5,11,11a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′,4′:1,2]pyridin[3,4-*b*]indol-2-acetate (4e). With the same procedure as that used for the preparation of **4a** from 2.00 g (4.32 mmol) of *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-L-glycine benzylester, 0.87 g (50%) of the title compound was obtained as colorless crystals. Mp 203–205 °C; ESI-MS (m/e) 404 [M+H]$^+$; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm = 10.725 (s, 1H), 7.484 (m, 5H), 7.322 (d, J = 7.5 Hz, 1 H), 7.265 (d, J = 7.5 Hz, 1H), 7.012 (t, J = 7.5 Hz, 1H), 6.956 (t, J = 7.5 Hz, 1H), 5.183 (s, 2H), 4.335 (s, 2H), 3.962 (d, J = 16.5 Hz, 1 H), 3.756 (d, J = 16.5 Hz, 1H), 3.523 (dd, J = 4.5 Hz, J = 10.0 Hz, 1 H), 3.092 (dd, J = 4.1 Hz, J = 15 Hz, 1H), 2.744 (t, 1H), 1.438 (s, 3 H), 1.283 (s, 3H); ^{13}C NMR (DMSO-d_6, 300 MHz) δ/ppm = 172.168, 170.831, 136.651, 136.256, 131.224, 128.965, 128.689, 127.352, 124.333, 121.922, 120.400, 119.113, 111.363, 109.223, 71.152, 66.986, 61.144, 44.545, 42.854, 30.224, 29.543, 25.534; $[\alpha]_D^{20}$ − 81.98 (c 0.5, methanol). Anal. Calcd for $C_{24}H_{25}N_3O_3$ C 71.44, H 6.25, N 10.41. Found C 71.27, H 6.12, N 10.23.

4.1.22. Benzyl 1,2,3,5,11,11a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′,4′:1,2]pyridin[3,4-*b*]indol-2-benzylacetate (4f). With the same procedure as that used for the preparation of **4a** from 2.00 g (4.32 mmol) of *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-L-phenylalanine benzylester, 0.87 g (50%) of the title compound was obtained as colorless crystals. Mp 179–181 °C; ESI-MS (m/e) 494 [M+H]$^+$; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm = 10.912 (s, 1 H), 7.425–7.243 (m, 12H), 7.023 (t, J = 7.5 Hz, 1H), 6.956 (t, J = 7.5 Hz, 1H), 5.138 (s, 2H), 4.252 (t, 1H), 3.944 (d, J = 14 Hz, 1 H), 3.774 (d, J = 14 Hz, 1H), 3.552 (dd, J = 4.5 Hz, J = 10.5 Hz, 1 H,), 3.514 (d, J = 4.5 Hz, 2H), 2.924 (dd, 1H, J = 3.9, J = 14.7), 2.500 (t, 1H), 1.324 (s, 3H), 1.128 (s, 3H); ^{13}C NMR (DMSO-d_6, 300 MHz) δ/ppm = 171.124, 170.445, 138.247, 136.002, 135.654, 132.526, 132.544, 129.587, 128.123, 126.525, 119.458, 118.502, 112.263, 105.565, 77.665, 66.784, 56.856, 56.326, 41.658, 33.235, 24.987, 23.348, 22.888; $[\alpha]_D^{20}$ − 203.84 (c 0.25, acetone). Anal. Calcd for $C_{31}H_{31}N_3O_3$ C 75.43, H 6.33, N 8.51. Found C 75.29, H 6.45, N 8.68.

4.1.23. Benzyl 1,2,3,5,11,11a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′,4′:1,2]pyridin[3,4-*b*]indol-2-(*p*-hydroxylbenzyl)acetate (4g). With the same procedure as that used for the preparation of **4a** from 2.00 g (3.51 mmol) of *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-L-tyrosine benzylester, 0.84 g (47%) of the title compound was obtained as colorless crystals. Mp 182–184 °C; ESI-MS (m/e) 510 [M+H]$^+$; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm = 10.792 (s, 1 H), 7.405–7.017 (m, 9H), 6.934–6.600 (m, 4H), 5.638 (s, 1 H), 5.201 (s, 2H), 4.252 (t, 1H), 3.900 (d, J = 14.5 Hz, 1H), 3.844 (d, J = 14.5 Hz, 1H), 3.656 (dd, J = 3.8 Hz, J = 10.5 Hz, 1H), 3.237 (d, J = 4.8 Hz, 2H), 2.924 (dd, 1H, J = 3.8, J = 15), 2.635 (t, 1H), 1.423 (s, 3H), 1.245 (s, 3H); ^{13}C NMR (DMSO-d_6, 300 MHz) δ/ppm = 171.014, 170.532, 156.215, 136.034, 135.655, 132.652, 132.574, 130.710, 128.271, 127.856, 126.568, 120.532, 118.458, 117.812, 115.021, 110.907, 105.811, 78.028, 66.258, 56.806, 56.324, 41.256, 30.694, 24.856, 23.356, 17.994; $[\alpha]_D^{20}$ − 208.33 (c 0.25, methanol). Anal. Calcd for $C_{31}H_{31}N_3O_4$ C 73.06, H 6.13, N 8.25. Found C 73.21, H 6.24, N 8.11.

4.1.24. Benzyl 1,2,3,5,11,11a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′,4′:1,2]pyridin[3,4-*b*]indol-2-(indole-3-yl-methyl)acetate (4h). With the same procedure as that used for the preparation of **4a** from 2.00 g (3.38 mmol) of *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-L-tryptophan benzylester, 0.94 g (52%) of the title compound was obtained as colorless crystals. Mp 176–177 °C, ESI-MS (*m*/*e*) 533 $[M+H]^+$; ^1H NMR (DMSO-d_6, 300 MHz) δ/ppm = 10.796 (s, 1H), 10.635 (s, 1H), 7.567–7.247 (m, 9H), 7.022–7.010 (m, 4H), 6.782 (s, 1H), 5.143 (s, 2H), 4.261 (t, 1H), 3.954 (d, J = 14 Hz, 1H), 3.714 (d, J = 14 Hz, 1H), 3.514 (d, J = 4.5 Hz, 2H), 3.199 (dd, 1H, J = 3.9, J = 10.5), 2.911 (dd, 1H, J = 3.9, J = 14.7), 2.500 (t, 1H), 1.500 (s, 3H), 1.232 (s, 3H); ^{13}C NMR (DMSO-d_6, 300 MHz) δ/ppm = 170.865, 170.651, 136.034, 135.985, 135.562, 132.532, 132.383, 129.863, 128.965, 128.324, 127.595, 126.663, 124.385, 120.896, 120.525, 118.449, 118.012, 117.484, 111.426, 110.865, 110.554, 105.797, 77.9742, 66.218, 56.884, 56.660, 52.262, 41.253, 27.692, 24.444, 23.748, 23.365; $[\alpha]_D^{20}$ – 211.33 (*c* 0.25, acetone). Anal. Calcd for $C_{33}H_{32}N_4O_3$ C 74.41, H 6.06, N 10.52. Found C 74.57, H 6.17, N 10.43.

4.1.25. Dibenzyl 1,2,3,5,11,11a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′,4′:1,2]pyridin[3,4-*b*]indol-2-uccinate (4i). With the same procedure as that used for the preparation of **4a** from 2.00 g (3.63 mmol) of *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-L-aspartic acid dibenzylester, 1.10 g (55%) of the title compound was obtained as colorless crystals. Mp 187–189 °C; ESI-MS (*m*/*e*) 552 $[M+H]^+$; ^1H NMR (DMSO-d_6, 300 MHz) δ/ppm = 10.848 (s, 1H), 7.426–7.286 (m, 12H), 7.023 (t, J = 7.5 Hz, 1H), 7.015 (t, J = 7.5 Hz, 1H), 5.226 (s, 4H), 4.336 (t, 1H), 3.898 (d, J = 14 Hz, 1H), 3.758 (d, J = 14 Hz, 1H), 3.436 (dd, J = 4.2 Hz, J = 10.8 Hz, 1H), 3.257 (dd, J = 4.2 Hz, J = 15 Hz, 1H), 2.865 (dd, J = 4.5 Hz, J = 14.5, 1H), 2.796 (dd, J = 4.2 Hz, J = 14.5, 1H), 2.504 (t, 1H), 1.445 (s, 3H), 1.362 (s, 3H); ^{13}C NMR (DMSO-d_6, 300 MHz) δ/ppm = 171.056, 170.986, 169.859, 136.451, 135.923, 134.784, 132.424, 129.256, 128.455, 128.102, 121.632, 120.608, 118.463, 113.515, 110.914, 71.056, 66.635, 66.289, 56.556, 50.084, 41.262, 34.409, 24.595, 23.515, 23.298; $[\alpha]_D^{20}$ – 104.89 (*c* 0.25, acetone). Anal. Calcd for $C_{33}H_{33}N_3O_5$ C 71.85, H 6.03, N 7.62. Found C 71.69, H 6.14, N 7.46.

4.1.26. Dibenzyl 1,2,3,5,11,11a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′,4′:1,2]pyridin[3,4-*b*]indol-2-glutarate (4j). With the same procedure as that used for the preparation of **4a** from 2.64 g (4.23 mmol) of *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-L-glutamic acid dibenzylester, 1.08 g (45%) of the title compound was obtained as colorless crystals. Mp 182–184 °C; ESI-MS (*m*/*e*) 566 $[M+H]^+$; ^1H NMR (DMSO-d_6, 300 MHz) δ/ppm = 10.824 (s, 1 H), 7.436–7.269 (m, 12H), 7.045 (t, J = 7.5 Hz, 1H), 6.976 (t, J = 7.5 Hz, 1H), 5.166 (s, 4H), 4.252 (t, 1H), 3.944 (d, J = 14 Hz, 1 H), 3.774 (d, J = 14 Hz, 1H), 3.527 (dd, J = 4.5 Hz, J = 11 Hz, 1 H), 2.877 (dd, J = 3.5 Hz, J = 14.5 Hz, 1H), 2.445 (t, 1H), 2.613–2.413 (m, 3H), 2.316 (m, 1H), 1.375 (s, 3H), 1.314 (s, 3H). ^{13}C NMR (DMSO-d_6, 300 MHz) δ/ppm = 172.92, 171.34, 170.59, 136.59, 136.35, 133.09, 128.93, 128.42, 127.99, 127.17, 121.03, 118.29, 117.95, 111.41, 106.43, 78.45, 66.64, 66.10, 57.07, 53.05, 41.85, 31.13, 25.09, 24.96, 23.79, 19.93; $[\alpha]_D^{20}$ – 70.63 (*c* 0.25, acetone). Anal. Calcd for $C_{34}H_{35}N_3O_5$ C 72.19, H 6.24, N 7.43. Found C 72.04, H 6.09, N 7.25.

4.1.27. Benzyl 1,2,3,5,11,11a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′,4′:1,2]pyridin[3,4-*b*]indol-2-(benzyloxy-methyl)acetate (4k). With the same procedure as that used for the preparation of **4a** from 2.00 g (4.23 mmol) of *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-*O*-benzyl-L-serine benzylester, 1.08 g (54%) of the title compound was obtained as colorless crystals. Mp 186–187 °C; ESI-MS (*m*/*e*) 524 $[M+H]^+$; ^1H NMR (DMSO-d_6, 300 MHz) δ/ppm = 10.528 (s, 1H), 7.429–7.293 (m, 12H), 7.012 (t, J = 7.4 Hz, 1H), 6.978 (t, J = 7.4 Hz, 1H), 5.135 (s, 2H), 5.012 (s, 2H), 4.176 (t, J = 5.4 Hz, 1H), 4.094 (d, J = 13.5 Hz, 1H), 3.956 (d, J = 5.4 Hz, 2H), 3.814 (d, J = 13.5 Hz, 1H), 3.440 (dd, J = 4.2 Hz, J = 10.2 Hz, 1H), 2.879 (dd, J = 4.2 Hz, J = 15 Hz, 1 H), 2.453 (t, 1H), 1.379 (s, 3H), 1.310 (s, 3H); ^{13}C NMR (DMSO-d_6, 300 MHz) δ/ppm = 170.989, 169.454, 136.256, 135.856, 132.568, 128.685, 127.589, 127.326, 126.526, 120.585, 118.456, 117.521, 110.825, 105.868, 78.185, 66.528, 65.354, 58.386, 56.794, 56.481, 41.465, 24.502, 23.342, 19.267; $[\alpha]_D^{20}$ – 128.61 (*c* 0.25, acetone). Anal. Calcd for $C_{32}H_{33}N_3O_4$ C 73.40, H 6.35, N 8.02. Found C 73.25, H 6.46, N 8.20.

4.1.28. Benzyl 1,2,3,5,11,11a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′,4′:1,2]pyridin[3,4-*b*]indol-2-(benzyloxy-carbonylamino-*n*-butyl)acetate (4l). With the same procedure as that used for the preparation of **4a** from 2.007 g (3.00 mmol) of *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carbonyl)-L-(Z)lycine benzylester, 1.00 g (55%) of the title compound was obtained as colorless crystals. Mp 196–198 °C; ESI-MS (*m*/*e*) 609 $[M+H]^+$; ^1H NMR ($CDCl_3$, 300 MHz) δ/ppm = 8.863 (s, 1H), 8.017 (s, 1H), 7.320 (t, J = 7.5 Hz, 1 H), 7.297 (t, J = 7.5 Hz, 1H), 7.292 (t, J = 7.5 Hz, 1H), 7.282 (t, J = 7.5 Hz, 1H), 7.280 (d, J = 7.3 Hz, 2H), 7.277 (d, J = 7.3 Hz, 2 H), 7.274 (t, J = 7.3 Hz, 2H), 7.271 (t, J = 7.3 Hz, 2H), 7.007 (d, J = 7.5 Hz, 1H), 6.966 (d, J = 7.5 Hz, 1H), 5.395 (s, 2H), 5.363 (s, 2 H), 4.561 (t, J = 5.6 Hz, 1H), 4.055 (t, J = 4.6 Hz, 1H), 3.757 (dd, J = 4.2 Hz, J = 10.2 Hz, 1H), 3.490 (dd, 1H, J = 4.2 Hz, J = 10.2 Hz, 1H), 2.977 (d, J = 5.6 Hz, 2H), 2.870 (d, J = 4.6 Hz, 2H), 1.879 (m, J = 5.6 Hz, 2H), 1.568 (m, J = 5.6 Hz, 2H), 1.370 (s, 3H), 1.312 (s, 3H), 1.336 (m, J = 5.6 Hz, 2 H), ^{13}C NMR ($CDCl_3$, 300 MHz) δ/ppm = 173.625, 172.306, 161.080, 160.700, 141.363, 141.112, 135.881, 135.355, 132.437, 128.889, 128.726, 128.658, 128.522, 127.488, 127.450, 127.333, 127.302, 127.269, 127.247, 121.693, 120.714, 119.554, 112.530, 111.202, 72.294, 70.883, 69.589, 65.400, 55.282, 44.577, 42.491, 31.347, 31.274, 28.701, 28.670, 28.593, 24.810, 21.903; $[\alpha]_D^{20}$ – 135.82 (*c* 0.25, acetone). Anal. Calcd for $C_{38}H_{44}N_4O_7$ C 68.24, H 6.63, N 8.38. Found C 68.40, H 6.74, N 8.23.

4.1.29. Benzyl 1,2,3,5,11,11a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′,4′:1,2]pyridin[3,4-*b*]indol-2-(aminocarbonylmethyl)acetate (4m). With the same procedure as that used for the preparation of **4a** from 2.00 g (3.84 mmol) of *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-L-asparagine benzylester, 0.80 g (45%) of the title compound was obtained as colorless crystals. Mp 186–188 °C; ESI-MS (*m*/*e*) 461 $[M+H]^+$; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm = 10.429 (s, 1H), 7.524 (m, 5H), 7.427 (d, J = 7.4 Hz, 1H), 7.348 (d, J = 7.4 Hz, 1H), 7.010 (t, J = 7.4 Hz, 1H), 6.994 (t, J = 7.4 Hz, 1H), 6.217 (s, 2H), 5.204 (s, 2H), 4.253 (t, 1H), 3.936 (d, J = 14 Hz, 1H), 3.784 (d, J = 14 Hz, 1H), 3.277 (dd, J = 4.5 Hz, J = 10 Hz, 1H), 2.973 (d, J = 4.5 Hz, J = 15 Hz, 1H), 2.812 (d, J = 4.7 Hz, 2H), 2.458 (t, 1H), 1.452 (s, 3H), 1.396 (s, 3H); ^{13}C NMR (DMSO-d_6, 300 MHz) δ/ppm = 177.124, 172.984, 171.876, 136.256, 135.976, 134.732, 132.463, 129.356, 128.558, 127.846, 121.614, 120.622, 118.403, 113.594, 111.025, 71.108, 66.156, 56.587, 46.214, 45.589, 34.998, 24.626, 23.514, 23.296; $[\alpha]_D^{20}$ − 63.38 (*c* 0.25, methanol). Anal. Calcd for $C_{26}H_{28}N_4O_4$ C 67.81, H 6.13, N 12.17. Found C 67.64, H 6.02, N 12.29.

4.1.30. Benzyl 1,2,3,5,11,11a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′,4′:1,2]pyridin[3,4-*b*]indol-2-(aminocarbonylethyl)acetate (4n). With the same procedure as that used for the preparation of **4a** from 2.00 g (4.22 mmol) of *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-L-glutamine benzylester, 0.90 g (45%) of the title compound was obtained as colorless crystals. Mp 196–198 °C; ESI-MS (*m*/*e*) 475 $[M+H]^+$; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm = 10.836 (s, 1H), 7.458–7.241 (m, 7H), 7.042 (t, J = 7.5 Hz, 1H), 6.985 (t, J = 7.5 Hz, 1H), 6.242 (s, 2H), 5.201 (s, 2H), 4.248 (t, 1H), 3.965 (d, J = 14 Hz, 1H), 3.784 (d, J = 14 Hz, 1H), 3.534 (dd, J = 4.5 Hz, J = 11 Hz, 1H), 2.885 (dd, J = 4.5 Hz, J = 14.5 Hz, 1H), 2.472 (t, 1H), 2.625–2.416 (m, 3H), 2.323 (m, 1H), 1.387 (s, 3H), 1.336 (s, 3H); ^{13}C NMR (DMSO-d_6, 300 MHz) δ/ppm = 176.023, 172.987, 170.882, 136.487, 135.947, 134.776, 132.445, 129.635, 128.54, 128.124, 121.656, 120.627, 118.506, 113.747, 111.204, 71.156, 66.708, 56.551, 50.668, 44.881, 31.440, 25.884, 24.912, 23.516, 23.308; $[\alpha]_D^{20}$ − 69.20 (*c* 0.25, methanol). Anal. Calcd for $C_{27}H_{30}N_4O_4$ C 68.34, H 6.37, N 11.81. Found C 68.47, H 6.25, N 11.96.

4.1.31. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carbonyl)-L-aspartic acid dimethylester (3′i). At 0 °C to the solution of 2.0 g (6.33 mmol) *N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid in 30 ml of anhydrous THF, 1.2 g (8.9 mmol) of HOBt was added. After 10 min, 1.75 g (8.5 mmol) of DCC was then added. The suspension of 1364 mg (6.96 mmol) of HCl·L-Asp-$(OMe)_2$ in 3 ml of anhydrous THF was adjusted to pH 8–9 with *N*-methyl morpholine and stirred at room temperature for 20 min. This suspension was then added to the solution of *N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid and the reaction mixture was stirred at 0 °C for 2 h and at room temperature for 16 h. On evaporation the residue was dissolved in 30 ml of ethyl acetate. The solution was 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 2.9 g (90%) of the title compound was obtained as powder. Mp 158–160 °C; ESI/MS (*m*/*z*) 460 $[M+H]^+$; IR (KBr): 3441, 3210, 3004, 2955, 2841, 1732, 1643, 1604, 1453, 1390, 1371, 1061, 903 cm^{-1}; ^{1}H NMR (BHSC-500, DMSO-d_6): δ/ppm = 10.05 (s, 1H), 8.05 (s, 1H), 7.37 (t, J = 7.4 Hz, 1H), 7.25 (t, J = 7.4 Hz, 1H), 7.00 (d, J = 7.6 Hz, 1H), 6.95 (d, J = 7.4 Hz, 1H), 4.92 (d, J = 5.5 Hz, 1H), 4.77 (t, J = 5.5 Hz, 1H), 4.24 (d, J = 5.6 Hz, 2H), 3.62 (s, 3H), 3.58 (s, 3H), 2.91 (d, J = 5.2 Hz, 2H), 2.85 (d, J = 5.4 Hz, 2H), 1.49 (s, 9H). Anal. Calcd for $C_{23}H_{29}N_3O_7$ C 60.12, H 6.36, N 9.14. Found C 60.03, H 6.49, N 8.99.

4.1.32. Dimenthyl 1,2,3,5,11,11a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′,4′:1,2]pyridin [3,4-*b*]indol-2-succinate (4′i). With the same procedure as that used for the preparation of **4a** from 2.00 g (4.36 mmol) of *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-L-aspartic acid benzylester, 0.97 g (56%) of the title compound was obtained as colorless crystals. Mp 234–236 °C; ESI-MS (*m*/*e*) 400 $[M+H]^+$; ^{1}H NMR ($CDCl_3$, 300 MHz) δ/ppm = 8.782 (s, 1H), 7.411 (t, J = 7.5 Hz, 1H), 7.301 (t, J = 7.5 Hz, 1H), 7.007 (d, J = 7.5 Hz, 1H), 6.995 (d, J = 7.5 Hz, 1H), 4.439 (t, J = 4.5 Hz, 1H), 3.971 (t, J = 5.4 Hz, 1H), 3.648 (s, 3 H), 3.639 (s, 3H), 3.443 (dd, J = 4.2 Hz, J = 10.8 Hz, 1H), 3.274 (dd, J = 4.2 Hz, J = 10.8 Hz, 1H), 2.886 (d, J = 4.5 Hz, 2H), 2.796 (d, J = 4.2 Hz, 2H), 1.465 (s, 3H), 1.377 (s, 3H). ^{13}C NMR (DMSO-d_6, 300 MHz) δ/ppm = 171.020, 170.979, 169.866, 135.911, 134.762, 132.424, 121.631, 120.606, 118.463, 113.515, 110.914, 71.054, 56.511, 52.637, 51.747, 50.074, 41.223, 34.407, 24.583, 23.501, 23.280. Anal. Calcd for $C_{21}H_{25}N_3O_5$ C 63.14, H 6.31, N 10.52; Found: C 63.30, H 6.45, N 10.37.

4.1.33. 1,2,3,5,11,11a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′,4′:1,2]pyridin[3,4-*b*]indol-2-succinic acid (4″i). At 0 °C to the solution of 1.0 g(2.61 mmol)of dimenthyl 1,2,3,5,11,11a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′,4′:1,2]pyridin[3,4-*b*]indol-2-succinate, 10 ml of aqueous NaOH (2N) was added. The reaction mixture was stirred at 0 °C for 60 min and TLC ($CHCl_3$–MeOH, 10:1) indicated the complete disappearance of dimenthyl 1,2,3,5,11,11a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′, 4′:1,2] pyridin[3,4-*b*]indol-2-succinate. Using hydrochloric acid the reaction mixture was adjusted to pH 3 and evaporated under reduced pressure to remove methanol. The formed precipitates were collected by filtration, washed with distilled water and dried over anhydrous Na_2SO_4 to provide 0.42 g (90%) of the title compound as a colorless powder. ESI-MS (*m*/*e*) 372 $[M+H]^+$; ^{1}H NMR ($CDCl_3$, 300 MHz) δ/ppm = 10.708 (s, 1 H), 10.692 (s, 1H), 8.786 (s, 1H), 7.423 (t, J = 7.3 Hz, 1H), 7.315 (t, J = 7.3 Hz, 1H), 7.011 (d, J = 7.3 Hz, 1H), 7.004 (d, J = 7.3 Hz, 1 H), 4.462 (t, J = 4.7 Hz, 1H), 3.977 (t, J = 5.6 Hz, 1H), 3.450 (dd, J = 4.5 Hz, J = 10.2 Hz, 1H), 3.327 (dd, J = 4.5 Hz, J = 10.2 Hz, 1 H), 2.887 (d, J = 4.7 Hz, 2H), 2.799 (d, J = 4.5 Hz, 2H), 1.460 (s, 3 H), 1.382 (s, 3H). ^{13}C

NMR (DMSO-d_6, 300 MHz) δ/ppm = 176.853, 175.904, 172.217, 136.134, 134.777, 132.428, 122.313, 120.992, 119.400, 113.805, 111.030, 71.111, 57.000, 47.745, 41.568, 34.901, 25.136, 23.521, 23.342. Anal. Calcd for $C_{19}H_{21}N_3O_5$ C 61.45, H 5.70, N 11.31; Found: C 61.33, H 5.58, N 11.47.

4.1.34. *N*-[(3*S*)-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-L-tryptophan benzylester (3′h). At 0 °C to the solution of 2.0 g (3.38 mmol) of *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carboxyl]-L-tryptophan benzylester in 8 ml of ethyl acetate, 16 ml of hydrogen chloride/ethyl acetate (4N) was added dropwise. The reaction solution was stirred at 0 °C for 90 min and evaporated under reduced pressure. The residue obtained was dissolved in 60 ml of methanol. With triethylamine the reaction solution was adjusted to pH 9 and evaporated under reduced pressure. The residue was purified by chromatography (petroleum acetone, 2:1) to provide 1.33 g (80%) of the title compound as a colorless powder. Mp 206–208 °C; ESI-MS 493 $[M+H]^+$; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm = 10.662 (s, 1H), 10.575 (s, 1H), 8.013 (s, 1H), 7.582 (d, J = 7.8 Hz, 1H), 7.564 (d, J = 7.8 Hz, 1H), 7.385 (d, J = 7.8 Hz, 1H), 7.354 (d, J = 7.8 Hz, 1H), 7.325–7.176 (m, 5 H), 6.938 (s, 1H), 6.871–6.728 (m, 4H), 5.207 (s, 2H), 4.115 (t, 1 H), 3.781 (d, J = 14.5 Hz, 1H), 3.480 (d, J = 14.5 Hz, 1H), 3.199 (m, 2 H), 2.988 (dd, J = 3.5 Hz, J = 10 Hz, 1H), 2.729 (dd, J = 3.5 Hz, J = 12 Hz, 1H), 2.304 (t, 1H), 2.139 (s, 1H); ^{13}C NMR (DMSO-d_6, 300 MHz) δ/ppm = 170.855, 170.641, 136.350, 136.034, 135.985, 132.532, 132.383, 128.796, 128.563, 127.595, 127.324, 126.663, 124.389, 120.894, 120.556, 118.447, 118.002, 117.482, 111.400, 110.864, 110.551, 105.787, 66.536, 56.882, 56.654, 41.223, 27.690, 24.434; $[\alpha]_D^{20}$ − 89.57 (*c* 1, methanol); Anal. Calcd for $C_{30}H_{28}N_4O_3$ C 73.15, H 5.73, N 11.37; Found: C 73.32, H 5.83, N 11.20.

4.1.35. Boc-L-tryptophyl-L-tryptophan benzylester (4′h). At 0 °C to the solution of 2.0 g (6.58 mmol) L-Boc-Trp-OH in 30 ml of anhydrous THF, 1.2 g (8.9 mmol) of HOBt was added. After 10 min, 1.75 g (8.5 mmol) of DCC was then added. The suspension of 2.28 g (6.91 mmol) of HCl·L-Trp-OBzl in 3 ml of anhydrous THF was adjusted to pH 8-9 with *N*-methyl morpholine and stirred at room temperature for another 20 min. This suspension was then added to the solution of *N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid and the reaction mixture was stirred at 0 °C for 2 h and at room temperature for 16 h. On evaporation the residue was dissolved in 30 ml of ethyl acetate. The solution was 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 3.51 g (92%) of the title compound was obtained as a powder. ESI-MS 581 $[M+H]^+$; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm = 10.587 (s, 1H), 10.561 (s, 1H), 8.008 (s, 1H), 7.583 (d, J = 7.7 Hz, 2H), 7.372 (d, J = 7.7 Hz, 2H), 7.320–7.181 (m, 5H), 6.863 (s, 2H), 6.682–6.620 (m, 4H), 5.227 (s, 2H), 4.523 (t, J = 5.2 Hz, 1H), 4.025 (t, J = 4.7 Hz, 1H), 3.061–2.820 (m, 4H), 2.104 (s, 1H), 1.472 (s, 9H); ^{13}C NMR (DMSO-d_6, 300 MHz) δ/ppm = 175.361, 171.869, 157.604, 140.881, 136.353, 136.295, 131.548, 131.520, 128.791, 128.704, 128.566, 127.592, 127.404, 127.365, 122.833, 122.800, 120.890, 120.541, 120.526, 118.947, 118.764, 112.077, 112.052, 111.013, 110.868, 72.077, 70.543, 60.800, 57.853, 31.289, 30.700, 28.654, 28.437, 28.221; $[\alpha]_D^{20}$ − 70.40 (*c* 1, methanol); Anal. Calcd for $C_{34}H_{36}N_4O_5$ C 70.33, H 6.25, N 9.65; Found: C 70.75, H 6.00, N 9.61.

4.1.36. L-Tryptophyl-L-tryptophan benzylester (4″h). At 0 °C to the solution of 2.0 g (3.45 mmol) of Boc-L-Tryptophyl-L-tryptophan benzylester in 8 ml of ethyl acetate, 16 ml of hydrogen chloride/ethyl acetate (4N) was added dropwise. The reaction solution was stirred at 0 °C for 90 min and evaporated under reduced pressure. The residue obtained was dissolved in 60 ml of methanol. With triethylamine the reaction solution was adjusted to pH 9 and evaporated under reduced pressure. The residue was purified by chromatography (petroleum acetone, 2:1) to provide 1.41 g (85%) of the title compound as a colorless powder. Mp 196–198 °C; ESI-MS 481 $[M+H]^+$; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm = 10.652 (s, 1H), 10.568 (s, 1H), 8.010 (s, 1H), 7.587 (d, J = 7.8 Hz, 2H), 7.376 (d, J = 7.8 Hz, 2H), 7.326–7.184 (m, 5H), 6.869 (s, 2H), 6.686–6.623 (m, 4H), 5.221 (s, 2H), 4.526 (t, 1H), 4.025 (t, 1H), 3.065–2.824 (m, 4H), 2.108 (s, 1H); ^{13}C NMR (DMSO-d_6, 300 MHz) δ/ppm = 171.855, 171.641, 136.365, 136.121, 129.658, 128.796, 128.563, 127.595, 124.389, 120.894, 120.556, 118.447, 111.400, 110.864, 66.458, 55.856, 55.214, 34.356, 27.690; $[\alpha]_D^{20}$ − 75.68 (*c* 1, methanol); Anal. Calcd for $C_{29}H_{28}N_4O_3$ C 72.48, H 5.87, N 11.66; Found: C 72.65, H 6.01, N 11.47.

4.1.37. (3*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (5). To the solution of 1.65 g (10.0 mmol) of L-Phe-OH in 90 ml of concentrated hydrochloric acid, 35 ml of formaldehyde (36–38%) were added. The reaction mixture was stirred at 85 °C for 9 h and TLC ($CHCl_3$–CH_3OH, 10:1) indicated the complete disappearance of L-Phe-OH. The reaction was cooled to room temperature, filtered, and the collected powder was washed with water (3×330 ml) and acetone (3×30 ml) to provide 1.92 g (95%) of the title compound as a colorless powder. Mp. 302–303; ESI-MS (*m*/*e*) 178 $[M+H]^+$; ^{1}H NMR ($CDCl_3$-*d*) δ/ppm = 11.004 (s,1 H), 7.182 (t, J = 7.4 Hz, 1H), 7.089 (d, J = 7.4 Hz, 1H), 7.054 (d, J = 7.4 Hz, 1H), 7.002 (t, J = 7.4 Hz, 1H), 3.873 (dd, J = 5.8 Hz, J = 3.4 Hz, 1H), 3.806 (d, J = 5.0 Hz, 2H), 3.013 (d, J = 5.8 Hz, 1H), 2.782 (d, J = 5.5 Hz, 1H), 2.004 (s, 1H); ^{13}C NMR ($CDCl_3$-*d*) δ/ppm = 174.913, 136.207, 134.200, 128.411, 127.194, 126.004, 125.688, 57.601, 47.410, 29.406. Anal. Calcd for $C_{10}H_{11}NO_2$ C 67.78, H 6.26, N 7.90; Found: C 67.62, H 6.11, N 7.67.

4.1.38. (3*S*)-*N*-Boc-1,2,3,4-tetrahydroisoquinloline-3-carboxylic acid (6). The suspension of 4.0 g (18.7 mmol) of (3*S*)-1,2,3,4-tetrahydroisoquinloline-3-carboxylic acid and 5.2 g (23.9 mmol) of Boc-N_3 in 40 ml of DMF was vigorously stirred at room temperature and was ad-

justed to pH 10 by adding triethylamine. The reaction mixture was stirred at room temperature for 48 h and TLC ($CHCl_3$–CH_3OH, 10:1) indicated the complete disappearance of (3*S*)-1,2,3,4-tetrahydroisoquinloline-3-carboxylic acid. To the reaction mixture 5 ml of citrate in water (20%) was added and the solution was extracted with ethyl acetate (3×30 ml). The separated ethyl acetate layer was dried with anhydrous $MgSO_4$. After removal of $MgSO_4$ by filtration the filtrate was evaporated to dryness. The residue obtained was crystallized in $CHCl_3$ to give 4.14 g (80%) of the title compound. ESI/MS: 278 $[M+H]^+$; ^1H NMR ($CDCl_3$-*d*) δ/ppm = 11.012 (s,1 H), 7.185 (t, J = 7.4 Hz, 1H), 7.086 (d, J = 7.4 Hz, 1H), 7.058 (d, J = 7.4 Hz, 1H), 7.003 (t, J = 7.4 Hz, 1H), 4.824 (dd, J = 5.7 Hz, J = 3.5 Hz, 1H), 4.213 (d, J = 5.1 Hz, 2H), 3.024 (d, J = 5.7 Hz, 1H), 2.950 (d, J = 5.6 Hz, 1H), 1.467 (s, 9H); ^{13}C NMR ($CDCl_3$-*d*) δ/ppm = 175.305, 155.412, 136.894, 134.500, 128.711, 127.906, 126.403, 125.789, 70.600, 57.912, 47.705, 29.814, 28.620, 28.416, 28.214. Anal. Calcd for $C_{15}H_{19}NO_4$ C 64.97, H 6.91, N 5.05; Found: C 65.18, H 6.82, N 5.37.

4.1.39. (3*S*)-*N*-Boc-1,2,3,4-tetrahydroisoquinloline-3-carbonyl-L-aspartic acid dibenzylester(7a). At 0 °C to the solution of 2.0 g (7.19 mmol) of (3*S*)-*N*-Boc-1,2,3,4-tetrahydroisoquinloline-3-carboxylic acid in 30 ml of anhydrous THF, 1.2 g (8.9 mmol) of HOBt was added. After 10 min, 1.75 g (8.5 mmol) of DCC was then added. The suspension of 2.64 g (7.75 mmol) of HCl·L-Asp-$(OBzl)_2$ in 3 ml of anhydrous THF was adjusted to pH 8–9 with *N*-methyl morpholine and stirred at room temperature for another 20 min. This suspension was then added to the solution of *N*-Boc-3*S*-1,2,3,4-tetrahydro-isoquinloline-3-carboxylic acid. The reaction mixture was stirred at 0 °C for 2 h and at room temperature for 16 h. On evaporation the residue was dissolved in 30 ml of ethyl acetate. The solution was 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 3.91 g (95%) of the title compound was obtained as a powder. ESI-MS 573 $[M+H]^+$; ^1H NMR ($CDCl_3$-*d*) δ/ppm = 8.012 (s,1 H), 7.242 (t, J = 7.2 Hz, 1H), 7.221 (t, J = 7.0 Hz, 2H), 7.205 (t, J = 7.0 Hz, 2H), 7.203 (t, J = 7.2 Hz, 1H), 7.186 (t, J = 7.4 Hz, 1H), 7.157 (d, J = 7.0 Hz, 2H), 7.138 (d, J = 7.0 Hz, 2H), 7.083 (d, J = 7.4 Hz, 1H), 7.055 (d, J = 7.4 Hz, 1H), 7.001 (t, J = 7.4 Hz, 1 H), 5.228 (s, 2H), 5.224 (s, 2H), 5.073 (t, J = 5.7 Hz, 1H), 4.821 (dd, J = 5.6 Hz, J = 3.5 Hz, 1H), 4.210 (d, J = 5.2 Hz, 2H), 3.022 (d, J = 5.6 Hz, 1H), 2.953 (d, J = 5.7 Hz, 1H), 2.871 (d, J = 5.7 Hz, 2H), 1.464 (s, 9H). Anal. Calcd for $C_{33}H_{36}N_2O_7$ C 69.21, H 6.34, N 4.89; Found: C 69.06, H 6.22, N 5.05.

4.1.40. Dibenzyl 1,2,3,5,10,10a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′,4′:1,2]isoquinolin-2-succinate (8a). At 0 °C to the solution of 2.0 g (3.50 mmol) of (3*S*)-*N*-Boc-1,2,3,4-tetrahydroisoquinloline-3-carbonyl-L-aspartic acid dibenzylester in 8 ml of ethyl acetate, 16 ml of hydrogen chloride/ethyl acetate (4N) was added dropwise. The reaction solution was stirred at 0 °C for 90 min and evaporated under reduced pressure. The residue obtained was dissolved in 60 ml of methanol and 20 ml of acetone. With triethylamine the reaction solution was adjusted to pH 9, at room temperature and in dark stirred for 240 h, and TLC ($CHCl_3$–MeOH, 10:1) indicated the complete disappearance of N-(3*S*)--1,2,3,4-tetrahydroisoquinloline-3-carbonyl-L-aspartic acid dibenzylester. On evaporation the residue was dissolved in 200 ml of ethyl acetate. The solution was 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 the residue was dissolved in 5 ml of methanol to give 1.08 g (60%) of the title compound as yellowish crystals. Mp 234–236 °C; ESI-MS (*m*/*e*) 513 $[M+H]^+$; ^1H NMR (DMSO-d_6, 300 MHz) δ/ppm = 7.346–7.184 (m, 10H), 7.179 (m, 4H), 5.207 (s, 2H), 5.113 (s, 2H), 4.116 (t, 1H), 3.962 (d, J = 14 Hz, 1H), 3.748 (d, J = 14 Hz, 1H), 3.345 (dd, J = 4.0 Hz, J = 11 Hz, 1H), 3.257 (dd, J = 4.2 Hz, J = 15 Hz, 1H), 2.981 (dd, J = 3.5 Hz, J = 12 Hz, 1H), 2.796 (dd, J = 4.2 Hz, J = 14.5, 1H), 2.654 (t, 1H), 1.562 (s, 3H), 1.289 (s, 3H); ^{13}C NMR (DMSO-d_6, 300 MHz) δ/ppm = 171.058, 170.974, 169.868, 136.402, 135.158, 129.896, 128.765, 128.345, 128.285, 128.156, 127.174, 126.978, 126.594, 78.056, 66.638, 66.292, 56.558, 50.086, 41.265, 34.417, 24.602, 23.518, 23.288; $[\alpha]_D^{20}$ − 138.94 (*c* 0.25, acetone); Anal. Calcd for $C_{31}H_{32}N_2O_5$ C 72.64, H 6.29, N 5.47; Found: C 72.76, H 6.14, N 5.59.

4.1.41. (3*S*)-*N*-Boc-1,2,3,4-tetrahydroisoquinloline-3-carbonyl-L-leucine benzylester (7b). At 0 °C to the solution of 2.0 g (7.19 mmol) of (3*S*)-*N*-Boc-1,2,3,4-tetrahydroisoquinloline-3-carboxylic acid in 30 ml of anhydrous THF, 1.2 g (8.9 mmol) of HOBt was added. After 10 min, 1.75 g (8.5 mmol) of DCC were then added. The suspension of 1.94 g (7.55 mmol) of HCl·L-Leu-$(OBzl)_2$ in 3 ml of anhydrous THF was adjusted to pH 8-9 with *N*-methyl morpholine and stirred at room temperature for another 20 min. This suspension then was added to the solution of *N*-Boc-3*S*-1,2,3,4-tetrahydroisoquinloline-3-carboxylic acid and the reaction mixture was stirred at 0 °C for 2 h and at room temperature for 16 h. On evaporation the residue was dissolved in 30 ml of ethyl acetate. The solution was 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 3.18 g (92%) of the title compound was obtained as a powder. ESI-MS 481 $[M+H]^+$; ^1H NMR ($CDCl_3$-*d*) δ/ppm = 8.019 (s,1 H), 7.240 (t, J = 7.2 Hz, 1H), 7.220 (t, J = 7.2 Hz, 2H), 7.201 (t, J = 7.2 Hz, 2H), 7.088 (d, J = 7.4 Hz, 1H), 7.052 (d, J = 7.4 Hz, 1H), 7.004 (t, J = 7.4 Hz, 1 H), 5.229 (s, 2H), 4.825 (dd, J = 5.5 Hz, J = 3.6 Hz, 1H), 4.408 (t, J = 5.6 Hz, 1H), 4.213 (d, J = 5.3 Hz, 2H), 3.024 (d, J = 5.6 Hz, 1H), 2.955 (d, J = 5.7 Hz, 1H), 2.898 (m, J = 5.6 Hz, 2H), 1.466 (s, 9H), 1.287 (m, J = 5.3 Hz, 2H), 1.076 (t, J = 5.6 Hz, 3H), 0.899 (t, J = 5.6 Hz, 3H). Anal. Calcd for

$C_{28}H_{36}N_2O_5$ C 69.98, H 7.55, N 5.83; Found: C 70.21, H 7.39, N 5.99.

4.1.42. Benzyl 1,2,3,5,10,10a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′, 4′:1,2]isoquinolin-2-*iso*butylacetate (8b). At 0 °C to the solution of 2.0 g (4.17 mmol) of (3*S*)-*N*-Boc-1,2,3,4-tetrahydroisoquinloline-3-carbonyl-L-leucine benzylester in 8 ml of ethyl acetate, 16 ml of hydrogen chloride/ethyl acetate (4N) was added dropwise. The reaction solution was stirred at 0 °C for 90 min and evaporated under reduced pressure. The residue obtained was dissolved in 60 ml of methanol and 20 ml of acetone. With triethylamine the reaction solution was adjusted to pH 9, at room temperature and in dark stirred for 240 h, and TLC ($CHCl_3$–MeOH, 10:1) indicated the complete disappearance of N-(3*S*)--1,2,3,4-tetrahydroisoquinloline-3-carbonyl-L-leucine benzylester. On evaporation the residue was dissolved in 200 ml of ethyl acetate. The solution was 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 the residue was dissolved in 5 ml of methanol to give 1.02 g (58%) of the title compound as yellowish crystals. Mp 183–184 °C; ESI-MS (*m*/*e*) 421 $[M+H]^+$; ^1H NMR (DMSO-d_6, 300 MHz) δ/ppm = 7.326 (m, 5 H), 7.188 (m, 4H), 5.162 (s, 2H), 4.101 (t, 1H), 3.948 (d, J = 14 Hz, 1 H), 3.734 (d, J = 14 Hz, 1H), 3.330 (dd, J = 4.0 Hz, J = 11 Hz, 1 H), 2.924 (dd, J = 3.5 Hz, J = 12 Hz, 1H), 2.631 (t, 1H), 2.088 (m, 1 H), 1.923 (m, 1H), 1.691 (m, 1H), 1.551 (s, 3H), 1.270 (s, 3H), 0.944 (d, 6 H); ^{13}C NMR (DMSO-d_6, 300 MHz) δ/ppm = 171.180, 171.075, 136.398, 135.152, 129.885, 128.759, 128.334, 128.265, 128.147, 127.156, 126.968, 126.590, 78.775, 66.538, 56.403, 52.589, 43.814, 39.010, 25.152, 25.013, 23.673, 22.425, 22.298, 19.392; $[\alpha]_D^{20}$ − 86.24 (*c* 0.25, acetone); Anal. Calcd for $C_{26}H_{32}N_2O_3$ C 74.26, H 7.67, N 6.66; Found: C 74.41, H 7.52, N 6.82.

4.2. Determining doxorubicin sensitivity of MES-SA and MES-SA/Dx5

Doxorubicin sensitive MES-SA cells and doxorubicin resistant MES-SA/Dx5 cells (during the exponential phase of growth with final concentration at 1×10^5/mL in McCoy's 5 A medium containing 10% (v/v) fetal calf serum, penicillin (100 μg/mL), and streptomycin (100 μg/mL)) were seeded in 96-well plates (100 μL per well) coated with poly-L-lysine. The cultures were propagated at 37 °C in a humified atmosphere containing 5% CO_2 for 4 h. To the plates, 25 μl of the solution (in successive final concentrations: 10.00 μM, 5.00 μM, 2.50 μM, 1.25 μM, 0.63 μM, 0.31 μM, 0.16 μM, 0.08 μM, 0.04 μM) of doxorubicin in the growth medium was added and the cells were propagated for 48 h. For the control well, 25 μL of the growth medium was added and the cells were also propagated for 48 h. For the blank, which contains only the growth medium without cells, to the well, 25 μL of the growth medium was added and the cells were also propagated for 48 h. The growth medium was removed and the residue was dried in the air. The dried residues were dissolved in 10 μL of DMSO and the absorption values of light of the formed purple solutions were recorded on Bio-Rad 450 microplate reader (Bio-Rad, USA). The relative survival rates were calculated according to the following equation:

$$\text{Survival}\% = (D_{Dx} - D_{blank})/(D_{control} - D_{blank})$$

wherein D_{Dx} presents the light absorption values of cells + doxorubicin+growth medium, the $D_{control}$ presents the light absorption values of cells+growth medium, and D_{blank} presents the light absorption values of growth medium alone. To give the survival rates, the D_{Dx}, $D_{control}$, and D_{blank} values were substituted into the equation. The survival and concentrations of doxorubicin were plotted to define the IC_{50} of doxorubicin against both MES-SA cells and MES-SA/Dx5 cells. Accordingly, the resistance index value was given by (IC_{50} against MES-SA/Dx5)/(IC_{50} against MES-SA).

4.3. Determining the effect of 4a–n on the sensitivities of MES-SA/Dx5f to doxorubicin

Doxorubicin resistant MES-SA/Dx5 cells (during the exponential phase of growth with final concentration of 1×10^5/mL in McCoy's 5 A medium containing 10% (v/v) fetal calf serum, penicillin (100 μg/mL) and streptomycin (100 μg/mL)) were seeded in 96-well plates (100 μL per well) coated with poly-L-lysine. The cultures were propagated at 37 °C in a humified atmosphere containing 5% CO_2 for 4 h. To the plates, 25 μL of the solution (in successive final concentrations: 10.00 μM, 5.00 μM, 2.50 μM, 1.25 μM, 0.63 μM, 0.31 μM, 0.16 μM, 0.08 μM, 0.04 μM) of doxorubicin in the growth medium was added and the cells were propagated for 4 h. To the plates, 25 μL of the solution (final concentration, 1.00 μM) of **4a**–**n** in the growth medium were added and the cells were propagated for 48 h. To the control well, 25 μL of the growth medium was added and the cells were propagated for 48 h. To the blank well, which contains no cells, 25 μL of the growth medium was added and the cells were also propagated for 48 h. The growth medium was removed and the residue was dried in the air. The dried residues were dissolved in 10 μL of DMSO and the absorption values of light of the formed purple solutions were recorded on Bio-Rad 450 microplate reader (Bio-Rad, USA). The relative survival rates of MES-SA/Dx5 cells under the influence of **4a**–**n** were calculated according to the equation

$$\text{Survival}\% = (D_{Dx} - D_{blank})/(D_{control} - D_{blank}),$$

wherein D_{Dx} presents the light absorption values of cells+doxorubicin+growth medium+**4a**–**n**, the $D_{control}$ presents the light absorption values of cells + growth medium, and D_{blank} presents the light absorption values of growth medium alone. To give the survival rates, the D_{Dx}, $D_{control}$, and D_{blank} values were substituted into the equation. The survival and concentrations of doxorubicin were plotted to define the IC_{50} of doxorubicin against both MES-SA cells and MES-SA/Dx5 cells. Accordingly, the resistance index value was given by (IC_{50} of doxorubicin alone against MES-SA/Dx5)/ (IC_{50} of doxorubicin with **4a**–**n** against MES-SA/Dx5).

4.4. Determining the cytotoxicities of 4a–n for MES-SA/Dx5 cells

Doxorubicin resistant MES-SA/Dx5 cells (during the exponential phase of growth with final concentration at 1×10^5/mL in McCoy's 5 A medium containing 10% (v/v) fetal calf serum, penicillin (100 μg/mL) and streptomycin (100 μg/mL)) were seeded in 96-well plates (100 μL per well) coated with poly-L-lysine. The cultures were propagated at 37 °C in a humified atmosphere containing 5% CO_2 for 4 h. To the plates, 25 μL of the 1 μmol/L solution of **4a**–**n** in McCoy's 5 A medium was added and the cells were propagated for 48 h. To the control well, 25 μl of the growth medium was added and the cells were propagated for 48 h. To the blank well, which contains no cells, 25 μL of the growth medium was added and the cells were also propagated for 48 h. The growth medium was removed and the residue was dried in the air. The dried residues were dissolved in 10 μL of DMSO and the absorption values of light of the formed purple solutions were recorded on Bio-Rad 450 microplate reader (Bio-Rad, USA). The inhibition rates of **4a**–**n** to MES-SA/Dx5 cells were calculated according to the equation

$$\text{Inhibition}\% = 1 - (D_{\text{Drug}} - D_{\text{blank}})/(D_{\text{control}} - D_{\text{blank}}),$$

wherein D_{Drug} presents the light absorption values of cells + growth medium + **4a**–**n**, the D_{control} presents the light absorption values of cells + growth medium and D_{blank} presents the light absorption values of growth medium alone. To give the inhibition rates, the D_{Dx}, D_{control}, and D_{blank} values were substituted into the equation.

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References and notes

1. Gottesman, M. M.; Pastan, I. *Annu. Rev. Biochem.* **1993**, *62*, 385–427.
2. Breier, A.; Barančík, M.; Sulová, Z.; Uhrík, B. *Curr. Cancer Drug Targets* **2005**, *5*, 457–468.
3. Dietel, M. *Pathol. Res. Pract.* **1991**, *187*, 892–905.
4. Glavinas, H.; Krajcsi, P.; Cserepes, J.; Sarkadi, B. *Curr. Drug Delivery* **2004**, *1*, 27–42.
5. Sikic, B. I. *Oncology* **1999**, *13*, 183–187.
6. Wiese, M.; Pajeva, I. K. *Cur. Med. Chem.* **2001**, *8*, 685–713.
7. Kvačkajová-Kišucká, J.; Barančík, M.; Breier, A. *Gen. Physiol. Biophys.* **2001**, *20*, 215–237.
8. Weaver, J. L.; Pine, P. S.; Aszalos, A.; Schoenlein, P. V.; Currier, S. J.; Padmanabhan, R.; Gottesman, M. M. *Exp. Cell. Res.* **1991**, *196*, 323–329.
9. Orlicky, J.; Sulova, Z.; Dovinova, I.; Fiala, R.; Zahradníková, A., Jr.; Breier, A. *Gen. Physiol. Biophys.* **2004**, *23*, 357–366.
10. Lee, C. H. *Curr. Med. Chem. Anti-Cancer Agents.* **2004**, *4*, 43–52.
11. Abbott, B. L. *Clin. Adv. Hematol. Oncol.* **2006**, *4*, 63–72.
12. Mmbudkar, S. V.; Kimchi-Sarfaty, C.; Sauna, Z. E.; Gottesman, M. M. *Oncogene* **2003**, *22*, 7468–7485.
13. Rabindran, S. K.; He, H.; Singh, M.; Brown, E.; Collins, K. I.; Annable, T.; Greenberger, L. M. *Cancer Res.* **1998**, *58*, 5850–5858.
14. Hazlehurst, L. A.; Foley, N. E.; Gleason-Guzman, M. C.; Hacker, M. P.; Cress, A. E.; Greenberger, L. W.; De Jong, M. C.; Dalton, W. S. *Cancer Res.* **1999**, *59*, 1021–1028.
15. Rabindran, S. K.; Ross, D. D.; Doyle, L. A.; Yang, W.; Greenberger, L. M. *Cancer Res.* **2000**, *60*, 47–50.
16. Plate, R.; Hermkens, P. H. H.; Behm, H.; Ottenheijm, H. C. J. *J. Org. Chem.* **1987**, *52*, 560–564.
17. Cui, C.-B.; Kakeya, H.; Osada, H. *Tetrahedron* **1997**, *53*, 59.
18. Ozola, V.; Thorand, M.; Diekmann, M.; Qurishi, R.; Schumacher, B.; Jacobsonb, A. K.; Müller, E. C. *Bioorg. Med. Chem.* **2003**, *11*, 347–356.
19. Merriman, G. H.; Ma, L.; Shum, P.; McGarry, D.; Volz, F.; Sabol, J. S.; Gross, A.; Zhao, Z.; Rampe, D.; Wang, L.; Wirtz-Brugger, F.; Harris, B. A.; Macdonald, D. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 435–438.
20. Clark, R. D.; Jahangir, A.; Severance, D.; Salazar, R.; Chang, T.; Chang, D.; Jett, M. F.; Smithc, S.; Bley, K. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1053–1056.
21. Zhao, M.; Bi, L.; Wang, W.; Wang, C.; Baudy-Floc'h, M.; Ju, J.; Peng, S. *Bioorg. Med. Chem.* **2006**, *14*, 6998–7010.