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Synthesis and biological evaluation of Matijing-Su derivatives as potent anti-HBV agents

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ABSTRACT

A series of Matijing-Su (MTS, *N*-(*N*-benzoyl-L-phenylalanyl)-*O*-acetyl-L-phenylalanol) derivatives were synthesized and evaluated for their anti-hepatitis B virus (HBV) activity in 2.2.15 cells. The IC_{50} of compounds **14a** (0.71 μM), **13c** (2.85 μM), **13b** (4.37 μM), etc. and the selective index of **13g** (161.01), **13c** (90.45), **13a** (85.09) etc. of the inhibition on the replication of HBV DNA were better than those of the positive control lamivudine (IC_{50}: 82.42 μM, SI: 41.59). Compounds **13o**, **13p**, and **16a** also exhibited significant anti-HBV activity.

1. Introduction

Despite the presence of an effective vaccine, hepatitis B virus (HBV) infection remains a global public health problem with over 400 million people chronically infected worldwide.[1,2] Approximately, 80% of carriers have different levels of hepatocyte destruction, which may develop into liver cirrhosis and hepatocellular carcinoma.[3] HBV causes acute and chronic infections of the liver and is responsible for 1.2 million deaths annually.[4,5] Nowadays, at least two different treatment options, including interferon and nucleoside analogs, such as lamivudine, adefovir dipivoxil, and entecavir are considered as antiviral therapy for chronic hepatitis B infection.[6] Although various treatment options exist for chronic HBV infection, none is entirely satisfactory.[7–11] The development of new anti-HBV agents is focused on discovering diverse compounds with either novel chemical structures or novel anti-HBV targets and mechanisms.[12,13]

Matijing-Su (MTS, *N*-(*N*-benzoyl-L-phenylalanyl)-*O*-acetyl-L-phenylalanol), a dipeptide derivative, was isolated from a Chinese ethnic drug Matijing (*Dichondra repens* Forst.) which has been widely used in the treatment of chronic liver diseases as folk medicine in China. The anti-HBV activity of MTS and its derivatives had been reported in our previous studies.[14,15] Some derivatives showed the significative inhibitions on the replication of HBV DNA and the SI and IC_{50} of them were better than those of the positive control lamivudine. These results greatly encouraged us to make further research on it for discovery of more potent anti-HBV derivatives. In this paper, the synthesis of new MTS derivatives and the evaluation of their anti-HBV activity were reported.

2. Results and discussion

2.1. Chemistry

The synthesis of the key synthetic intermediates was described in Scheme 1. 2-Chloroacetyl chloride was added to the solution of 4-amino-benzoic acid in 1.0 M NaOH to produce compound **2**.[16] Ac_2O or $(EtCO)_2O$ was treated with the solution of 4-amino-benzoic acid in pyridine to obtain compound **3** or **4**. *N*-Bromosuccinimide (NBS) and azobisisobutyronitrile (AIBN) were added to the solution of methyl 4-methylbenzoate in dry CCl_4 to give compound **6**,[17] which was dropped into the mixture of amine, K_2CO_3, and KI in ethanol. The product was salified with HCl to produce compound **7**. The solution of L-phenylalanine in H_2SO_4 was reacted with a mixed acid of concentrated HNO_3 and concentrated H_2SO_4 to obtain compound **9**,[18] which was treated with $SOCl_2$ to afford compound **10**.

The procedure for synthesis of the target compounds was illustrated in Scheme 2. The substituted L-phenylalanine methyl ester

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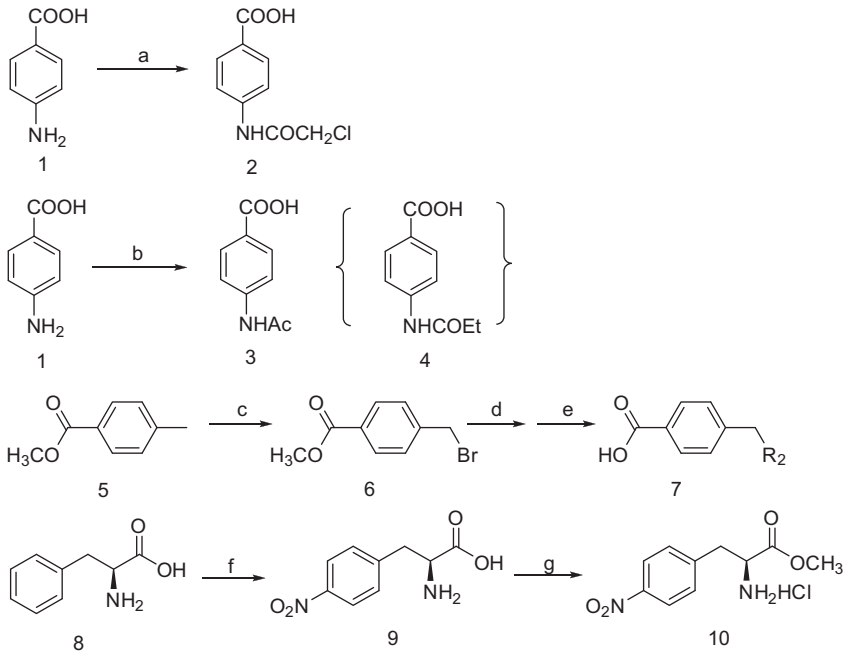
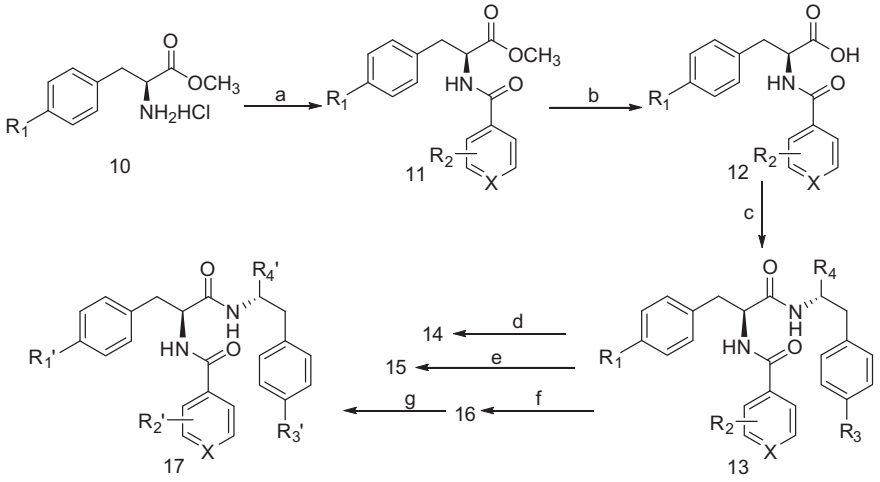


Scheme 1. Synthesis of the key intermediates. Reagents and conditions: (a) $ClCH_2COCl$, 1.0 M NaOH, −5 °C, 2 h, then stirred overnight, rt; (b) $Ac_2O/(EtCO)_2O$, pyr., rt; (c) NBS, AIBN, CCl_4, reflux for 12 h; (d) amine, K_2CO_3, KI, EtOH, rt, 24 h; (e) 1.0 M HCl, reflux for 8 h; (f) HNO_3/H_2SO_4 (V:V = 1.4:1.1), 10 °C, 2.5 h; (g) MeOH, thionyl chloride ($SOCl_2$), rt, 2.5 h, then reflux for 30 min.



Scheme 2. Synthesis of the target compounds. Reagents and conditions: (a) substituted benzoic acid or isonicotinic acid, CH_2Cl_2, IBCF, NMM, 0 °C; (b) NaOH, DMF, rt; (c) substituted L-phenylalanine methyl ester hydrochloride or substituted L-phenylalanol, CH_2Cl_2, IBCF, NMM, 0 °C; (d) $SOCl_2$, 1,4-dioxane, 70 °C; (e) Ac_2O, pyr, rt; (f) K_2CO_3, DMF, rt; (g) NaOH/EtOH–$CHCl_3$, rt.

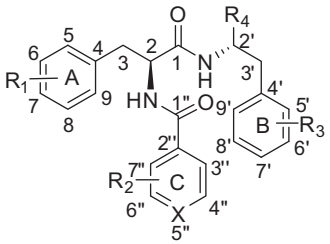
was treated with substituted benzoic acid or isonicotinic acid to give compound **11**,[19] which was then hydrolyzed in the 1 M solution of sodium hydroxide to give compound **12**. Compound **12** was reacted with substituted L-phenylalanine methyl ester hydrochloride or L-phenylalanol to afford compound **13**, which was followed by treatment with $SOCl_2$ in 1,4-dioxane to provide compound **14**. Compound **13** was reacted with Ac_2O in pyridine at room temperature to provide compound **15**. Compound **13** was treated with chlorides or iodides to obtain compound **16**. The target compound **17** was synthesized by hydrolysis of compounds **16** with NaOH in EtOH—$CHCl_3$. Thus, the structures of the target compounds are listed in Table 1.

2.2. Biological activity test

The target compounds **13a–13p**, **14a**, **15a**, **15b**, **16a–16g**, and **17a–17c** were tested for their abilities to inhibit the replication of HBV DNA and the production of HBsAg and HBeAg in HBV-infected 2.2.15 cells. The bioactivity of each compound was evaluated by the combination of its IC_{50} values and SI. The results of the inhibition on the replication of HBV DNA were summarized in Table 2.

As shown in Table 2, some MTS derivatives could be potent HBV inhibitors with high selectivity indices. However, the inhibition on the production of HBsAg and HBeAg for most of the tested compounds did not be found in this test, except for compound

Table 1
Structures of the target compounds**13a–13p**, **14a**, **15a**, **15b**, **16a–16g**, and **17a–17c**



No.	R_1	R_2	R_3	R_4	X	Compound
1	H	H	7-OCH_2CH_3	$COOCH_3$	C	**16a**
2	H	H	7-$O(CH_2)_3CH_3$	$COOCH_3$	C	**16b**
3	H	5-NHAc	7-OH	$COOCH_3$	C	**13a**
4	H	5-NHCOEt	7-NO_2	$COOCH_3$	C	**13b**
5	H	5-NHCOEt	H	$COOCH_3$	C	**13c**
6	H	5-$O(CH_2)_2N(CH_3)_2$	H	$COOCH_3$	C	**16c**
7	H	5-$NHCOCH_2Cl$	H	$COOCH_3$	C	**13d**
8	H	5-$NHCOCH_2N(CH_3)_2$	H	$COOCH_3$	C	**16d**
9	H	5-Pyrrolidinylacetamido	H	$COOCH_3$	C	**16e**
10	H	H	H	$COOCH_3$	N	**13e**
11	H	H	7-NO_2	$COOCH_3$	N	**13f**
12	H	H	7-OH	$COOCH_3$	N	**13g**
13	7-OH	H	H	$COOCH_3$	N	**13h**
14	7-OH	H	7-NO_2	$COOCH_3$	N	**13i**
15	H	H	H	CH_2OH	N	**13j**
16	7-OH	H	H	CH_2OH	N	**13k**
17	7-OH	5-NHAc	H	CH_2OH	C	**13l**
18	H	5-NHAc	H	CH_2Cl	C	**14a**
19	H	7-OH	H	CH_2OH	C	**13m**
20	H	7-$NHCOCH_2N(CH_3)_2$	H	CH_2OH	C	**16f**
21	H	5-$CH_2N(CH_3)_2$	H	CH_2OH	C	**13n**
22	H	5-$O(CH_2)_2N(CH_3)_2$	H	CH_2OH	C	**16g**
23	H	5-Pyrrolidin-1-ylmethyl	H	CH_2OH	C	**13o**
24	H	5-Morpholinomethyl	H	CH_2OH	C	**13p**
25	H	5-Morpholinomethyl	H	CH_2OAc	C	**15a**
26	H	5-$CH_2N(CH_3)_2$	H	CH_2OAc	C	**15b**
27	H	H	7-OCH_3	COONa	C	**17a**
28	H	H	7-OCH_2CH_3	COONa	C	**17b**
29	H	H	7-$O(CH_2)_3CH_3$	COONa	C	**17c**

17c, which exhibited significant inhibitory for the inhibition on the production of HBsAg in 2.2.15 cells.

The IC_{50} of compound **14a** was 0.71 μM, which was about 116 times higher than that of the control lamivudine (IC_{50}: 82.42 μM). Compound **14a**, with a SI of 42.26, showed the most potent in vitro anti-HBV activity within all of the tested compounds. Compounds **13c** (IC_{50}: 2.85 μM, SI: 90.45), **13b** (IC_{50}: 4.37 μM, SI: 33.53), **13g** (IC_{50}: 5.39 μM, SI: 161.01), **13a** (IC_{50}: 9.06 μM, SI: 85.09), **16a** (IC_{50}: 13.95 μM, SI: 58.61), etc. also exhibited significant inhibitory on the replication of HBV DNA in 2.2.15 cells.

3. Conclusions

A series of novel MTS derivatives were synthesized and their anti-HBV activities in vitro were examined with lamivudine as reference control. According to the above experiment results, conclusions could be made as:

a. In general, a series of MTS derivatives exhibited significant anti-HBV activity. The IC_{50} of the inhibition on the replication of HBV DNA of the thirteen MTS derivatives were lower and the SI of the five MTS derivatives were higher than those of the positive control lamivudine (IC_{50}: 82.42 μM, SI: 41.59). Some MTS derivatives could be potent HBV inhibitors, such as the compounds **14a** (IC_{50}: 0.71 μM, SI: 42.26), **13c** (IC_{50}: 2.85 μM, SI: 90.45), **13g** (IC_{50}: 5.39 μM, SI: 161.01), and **13a** (IC_{50}: 9.06 μM, SI: 85.09).
b. The substituting groups on ring A, B, and C of the MTS derivatives could affect their anti-HBV activities. The 5″-acetamido or 5″-propionamido substituted on the benzene ring C and no substituent on the benzene ring A was an important feature in conferring relatively potent inhibitory activity. When ring C was pyridine and the 7″-hydroxyl was substituted on ring B, the inhibitory activities of the derivatives were obviously strengthen. When ring C was pyridine and the 7″-nitro were substituted on ring B, the inhibitory activities of the derivatives were obviously weaken. Ring C of the derivatives might be one of the pharmacophores. Most of the MTS derivatives substituted with pyridine or amines on ring C exhibited more potent inhibitory activity.
c. The methyl carboxylate and hydroxymethyl substitution at 2′-position were potential pharmacophores for anti-HBV activities. Whereas both CH_2OAc and COONa substitution at 2′-position, the inhibitory activities of the derivatives were obviously weaken.

4. Experimental

4.1. Chemistry

Melting points were measured with a model XT-4 apparatus and are uncorrected. ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Varian Unit INOVA-400 spectrometer in

Table 2
The results of the inhibition for the replication of HBV DNA of compounds **13a–13p**, **14a**, **15a**, **15b**, **16a–16g**, and **17a–17c**

No.	TC_{50}[a] (μM)	DNA replication		Compounds
		IC_{50}[b] (μM)	SI[c]	
1	817.70	13.95	58.61	**16a**
2	1148.73	174.09	6.60	**16b**
3	770.59	9.06	85.09	**13a**
4	146.61	4.37	33.53	**13b**
5	257.87	2.85	90.45	**13c**
6	497.10	104.86	4.74	**16c**
7	223.26	–[d]	–	**13d**
8	110.51	–	–	**16d**
9	106.33	–	–	**16e**
10	1057.08	–	–	**13e**
11	812.44	–	–	**13f**
12	867.14	5.39	161.01	**13g**
13	219.23	–	–	**13h**
14	237.82	–	–	**13i**
15	213.70	28.92	7.39	**13j**
16	612.15	–	–	**13k**
17	748.87	–	–	**13l**
18	30.13	0.71	42.26	**14a**
19	1055.00	57.83	3.69	**13m**
20	73.70	–	–	**16f**
21	187.63	15.84	11.84	**13n**
22	327.30	27.59	11.86	**15g**
23	128.39	14.09	9.11	**13o**
24	156.53	13.91	11.25	**13p**
25	608.96	–	–	**15a**
26	507.70	–	–	**15b**
27	184.06	20.47	8.99	**17a**
28	1010.07	–	–	**17b**
29	979.32	64.56	15.20	**17c**
MTS	120.18	11.16	10.78	MTS
3TC	3427.82	82.42	41.59	Lamivudine[e]

[a] TC_{50}: 50% toxic concentration in HepG2.2.15 cells.
[b] IC_{50}: 50% inhibitory concentration.
[c] SI (Selectivity index) = TC_{50}/IC_{50}.
[d] Means no antiviral activity at a concentration lower than its TC_{50}.
[e] Lamivudine: an antiviral agent used as positive control.

$CDCl_3$ or DMSO-d_6 with Me_4Si (TMS) as an internal standard. Mass spectra (MS) were run on a Agilent MS-5973 spectrometer.

4.1.1. Synthesis of some intermediates

4.1.1.1. 4-(2-Chloroacetamido)-benzoic acid (2). 2-Chloroacetyl chloride (12 mL, 150.0 mmol) was added to the solution of 4-amino-benzoic acid (**1**) (13.70 g, 100.0 mmol) in 1.0 M NaOH (220 mL, 220.0 mmol) cooled in an ice-salt bath. The reaction mixture was stirred at −5 °C in ice-salt bath for 2 h, then stirred overnight at room temperature. The reaction was acidified to pH 1–2 with concentrated hydrochloric acid. The resulting precipitate was collected by filtration, washed with water (80 mL) and dried to give 4-(2-chloroacetamido)-benzoic acid (**2**) as a white powder (15.58 g, 73%).

4.1.1.2. 4-Acetamido-benzoic acid (3). Ac_2O (5.7 mL, 60.3 mmol) was added to the solution of 4-amino-benzoic acid (**1**) (6.86 g, 50.0 mmol) in pyridine (25 mL). The reaction was stirred at room temperature for 5 h. The solvent was removed in vacuo and the residue dispersed in water (100 mL) and acidified to pH 2–3 with concentrated hydrochloric acid. The resulting precipitate was collected by filtration, washed with water (30 mL) and dried to give 4-acetamido-benzoic acid (**3**) as a pale yellow powder (7.80 g, 99%).

4.1.1.3. 4-Propionamido-benzoic acid (4). It was prepared with a similar procedure to that described for the synthesis of compound **3**.

4.1.1.4. 4-Bromomethylbenzoate (6). NBS (6.57 g, 36.9 mmol) and AIBN (12 mg) were added to the solution of methyl 4-methylbenzoate (4.51 g, 30.0 mmol) in dry CCl_4 (60 mL). The reaction was refluxed for 12 h under an Ar atmosphere. The solid was filtered off and the filtrate was evaporated in vacuo to give methyl 4-bromomethylbenzoate (**6**) as milk white oil (6.58 g, 96%).

4.1.1.5. 4-Dimethylaminomethyl-benzoic acid hydrochloride (7). 4-Bromomethylbenzoate (**6**) (11.50 g, 50.2 mmol) was dropped to the mixture of amine (75.0 mmol), K_2CO_3 (17.25 g, 125.0 mmol), and KI (1.66 g, 10.0 mmol) in absolute ethanol (50 mL). The reaction was stirred overnight under an Argon atmosphere. The product was purified by extraction and hydrolyzed with 1.0 M HCl to give 4-dimethylaminomethyl-benzoic acid hydrochloride (**7**) as a pale white powder.

4.1.1.6. 4-Nitro-L-phenylalanine (9). To the solution of L-phenylalanine (**8**) (8.26 g, 50.0 mmol) in H_2SO_4 (85%, 25 mL) was added dropwise the mixed acid of concentrated HNO_3 7 mL and concentrated H_2SO_4 5.5 mL (V/V = 1.4/1.1) under stirring, which was pre-prepared and cooled down to 10 °C. The reaction mixture was stirred at 10 °C for 2.5 h. The reaction solution was adjusted to pH 5–6 with ammonia solution and the resulting precipitate was collected by filtration and dried to give 4-nitro-L-phenylalanine (**9**) as a pale yellow powder (9.86 g, 94%).

4.1.1.7. 4-Nitro-L-phenylalanine methyl ester hydrochloride (10). $SOCl_2$ (4.75 mL, 66.0 mmol) was added to methanol (45 mL), which was cooled in an ice-salt bath. The mixture was warmed up to room temperature. 4-Nitro-L-phenylalanine (**9**) (10.51 g, 50.0 mmol) was added and the mixture was stirred for 2.5 h at room temperature, then refluxed for 60 min. The solvent was removed and the residue was recrystallized from methanol/

ether to afford L-phenylalanine methyl ester hydrochloride (**10**) as colorless needles (11.56 g, 88%).

4.1.2. Synthesis of the target compounds

The synthetic procedures used in the preparation of target compounds are illustrated in Scheme 2. L-Tyrosine methyl ester hydrochloride or L-phenylalanine methyl ester hydrochloride was reacted with substituted benzoic acid or isonicotinic acid and IBCF in the presence of NMM to give compound **11**, then hydrolyzed in 2 M NaOH solution to give compound **12**, which was reacted with IBCF and substituted L-phenylalanine methyl ester hydrochloride or substituted L-phenylalanol in the presence of NMM to afford target compound **13**.

4.1.2.1. General procedure for the synthesis of compounds 13a–13p. IBCF (1.1 mmol) was added dropwise to the mixture of substituted benzoic acid or isonicotinic acid (1.0 mmol), L-tyrosine methyl ester hydrochloride or L-phenylalanine methyl ester hydrochloride (1.1 mmol) and NMM (2.3 mmol) in CH_2Cl_2 (50 mL) at 0 °C within 60 min. The mixture was stirred for 30 min and the bulk of CH_2Cl_2 was removed in vacuo. The residue was dissolved in ethyl acetate and washed sequentially with water, 5% HCl, saturated $NaHCO_3$ solution and brine, dried with Na_2SO_4. Removal of the solvent gave a residue which was recrystallized from ethyl acetate to afford target compound **11**. 2 M NaOH (1 mL) was added to the solution of compound **11** (1.0 mmol) in DMF (5 mL). After stirring at room temperature for 2 h, the mixture was acidified to pH 6–7 with concentrated hydrochloric acid, and partitioned between ethyl acetate and water. The organic phase was separated and washed with brine, dried over Na_2SO_4 and evaporated in vacuo to give the compound **12**. Compound **13** was prepared with a similar procedure to that described for the synthesis of compound **11**.

4.1.2.2. *N*-(*N*-4-acetamido-benzoyl-L-phenylalanyl)-L-tyrosine methyl ester (13a). White needle crystal, yield 63%, mp 216–217 °C (EtOAc), ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.23 (1H, s, Ar-NHCO), 9.26 (1H, s, Ar-OH), 8.47–8.43 (2H, m, NHCO × 2), 7.73 (2H, d, J = 8.4 Hz, H-3″, 7″), 7.62 (2H, d, J = 8.4 Hz, H-4″, 6″), 7.32 (2H, d, J = 7.2 Hz, H-5, 9), 7.23 (2H, t, H-6, 8), 7.14 (1H, t, H-7), 6.99 (2H, d, J = 8.4 Hz, H-5′, 9′), 6.62 (2H, d, J = 8.0 Hz, H-6′, 8′), 4.71 (1H, m, H-2), 4.40 (1H, m, H-2′), 3.56 (3H, s, OCH_3), 3.07–2.86 (4H, m, H-3, 3′), 2.05 (3H, CH_3CO); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 172.0, 171.7, 168.7 (Ar-NHCO), 165.7 (C-1″), 156.1 (C-7′), 142.0 (C-5″), 138.4 (C-4), 130.1 (C-5′, 9′), 129.2 (C-6, 8), 128.3 (C-3″, 7″), 128.2 (C-4′), 128.0 (C-5, 9), 127.0 (C-2″), 126.2 (C-7), 117.9 (C-4″, 6″), 115.1 (C-6′, 8′), 54.5 (C-2), 54.1 (C-2′), 51.8 (OCH_3), 36.9 (C-3), 35.9 (C-3′), 24.1 (CH_3CO); EI-MS m/z: 503 (M^+), 326, 309, 281, 178, 162 (100), 120; TOFESMS: calcd for $C_{28}H_{29}N_3O_6Na$ [M+Na]$^+$ 526.1954, found 526.1959.

4.1.2.3. *N*-(*N*-4-Propionamido-benzoyl-L-phenylalanyl)-4-nitro-L-phenylalanine methyl ester (13b). Pale yellow powder, yield 59%, ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.14 (1H, s, Ar-NHCO), 8.61 (1H, d, J = 7.2 Hz, NHCO), 8.42 (1H, d, J = 8.4 Hz, NHCO), 9.05 (2H, d, J = 8.0 Hz, H-6′, 8′), 7.72 (2H, d, J = 7.6 Hz, H-3″, 7″), 7.63 (2H, d, J = 8.4 Hz, H-5′, 9′), 7.51 (2H, d, J = 8.0 Hz, H-4″, 6″), 7.31 (2H, d, J = 7.2 Hz, H-5, 9), 7.23 (2H, t, H-6, 8), 7.14 (1H, t, J = 7.2 Hz, H-7), 4.68–4.59 (2H, m, H-2, 2′), 3.61 (3H, s, OCH_3), 3.25–2.90 (4H, m, H-3, 3′), 2.34 (2H, q, J = 7.6 Hz, $COCH_2CH_3$), 1.07 (3H, t, J = 7.6 Hz, $COCH_2CH_3$); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 172.4 (Ar-NHCO), 171.8 (C-1), 171.4 (C-1′), 165.7 (C-1″), 146.3 (C-7′), 145.5 (C-4′), 142.2 (C-5″), 138.3 (C-4), 130.6 (C-5′, 9′), 129.2 (C-6, 8), 128.3 (C-3″, 7″), 128.1 (C-5, 9), 128.0 (C-2″), 126.3 (C-7), 123.2 (C-6′, 8′), 117.9 (C-4″, 6″), 54.5 (C-2), 52.9 (C-2′), 52.1 (OCH_3), 36.8 (C-3), 36.2 (C-3′), 29.6 ($COCH_2CH_3$), 9.6 ($COCH_2CH_3$); EI-MS m/z: 546 (M^+), 354, 220, 192, 176 (100), 120, 91; TOFESMS: calcd for $C_{29}H_{30}N_4O_7Na$ [M+Na]$^+$ 569.2012, found 569.2018.

4.1.2.4. *N*-(*N*-4-Propionamido-benzoyl-L-phenylalanyl)-L-phenylalanine methyl ester (13c). White crystal, yield 62%, mp 225–227 °C (EtOAc), ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.09 (1H, s, Ar-NHCO), 8.52 (1H, d, J = 7.6 Hz, NHCO), 8.40 (1H, d, J = 8.4 Hz, NHCO), 7.74 (2H, d, J = 8.4 Hz, H-3″, 7″), 7.64 (2H, d, J = 8.8 Hz, H-4″, 6″), 7.34–7.13 (10H, m, H-5, 9, 5′, 9′), 4.73 (1H, m, H-2), 4.52 (1H, m, H-2′), 3.59 (3H, s, OCH_3), 3.08–2.91 (4H, m, H-3, 3′), 2.35 (2H, q, J = 7.2 Hz, $COCH_2CH_3$), 1.08 (3H, t, J = 7.6 Hz, $COCH_2CH_3$); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 172.4, 171.82, 171.78, 165.7 (C-1″), 142.1 (C-5″), 138.4 (C-4), 137.0 (C-4′), 129.2 (×2), 129.1 (×2), 128.3 (×4, C-3″, 7″, 5′, 9′), 128.1 (C-2″), 128.0 (C-5, 9), 126.6 (C-7), 126.2 (C-7′), 118.0 (C-4″, 6″), 54.4 (C-2), 53.7 (C-2′), 51.9 (OCH_3), 36.9 (C-3), 36.6 (C-3′), 29.6 ($COCH_2CH_3$), 9.5 ($COCH_2CH_3$); EI-MS m/z: 501 (M^+), 310, 295, 192, 176 (100), 120, 91; TOFESMS: calcd for $C_{29}H_{31}N_3O_5Na$ [M+Na]$^+$ 524.2161, found 524.2166.

4.1.2.5. *N*-(*N*- 4-Chloroacetamido-benzoyl-L-phenylalanyl)-L-phenylalanine methyl ester (13d). White powder, yield 55%, ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.64 (1H, s, Ar-NHCO), 8.57 (1H, d, J = 7.6 Hz, NHCO), 8.50 (1H, d, J = 8.4 Hz, NHCO), 7.78 (2H, d, J = 8.4 Hz, H-3″, 7″), 7.65 (2H, d, J = 8.8 Hz, H-4″, 6″), 7.34–7.14 (10H, m, H-5, 9, 5′, 9′), 4.74 (1H, m, H-2), 4.51 (1H, m, H-2′), 4.31 (2H, s, $NHCOCH_2Cl$), 3.59 (3H, s, OCH_3), 3.09–2.92 (4H, m, H-3, 3′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 171.9, 171.8, 165.6 (C-1″), 165.0 (Ar-NHCO), 141.2 (C-5″), 138.4 (C-4), 137.1 (C-4′), 129.2 (C-6, 8), 129.1 (C-6′, 8′), 129.0 (C-2″), 128.4 (C-3″, 7″), 128.3 (C-5′, 9′), 128.1 (C-5, 9), 126.6 (C-7′), 126.2 (C-7), 118.4 (C-4″, 6″), 54.5 (C-2), 53.8 (C-2′), 51.9 (OCH_3), 43.6 ($COCH_2Cl$), 37.0 (C-3), 36.6 (C-3′). EI-MS m/z: 521 (M^+), 343, 310, 196 (100), 120, 91; TOFESMS: calcd for $C_{28}H_{28}N_3O_5NaCl$ [M+Na]$^+$ 544.1615, found 544.1621.

4.1.2.6. *N*-(*N*- 4-Pyridinyl-formyl-L-phenylalanyl)-L-phenylalanine methyl ester (13e). White needle crystal, yield 49%, mp 174–175 °C (EtOAc), ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.71 (2H, d, J = 6.0 Hz, H-4″, 6″), 7.52 (2H, d, J = 5.6 Hz, H-3″, 7″), 7.31–7.17 (8H, m, H-5, 6, 8, 9, 5′, 6′, 8′, 9′), 7.07 (1H, br, NHCO), 6.99–6.97 (2H, m, H-7, 7′), 6.30 (1H, br, NHCO), 4.86–4.75 (2H, m, H-2, 2′), 3.72 (3H, s, OCH_3), 3.20 (1H, dd, J = 6.0, 14.0 Hz, H-3a), 3.14–3.08 (2H, m, H-3b, 3′a), 2.97 (1H, dd, J = 7.6, 13.6 Hz, H-3′b); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 171.2 (C-1), 170.0 (C-1′), 165.0 (C-1″), 150.6 (C-4″, 6″), 140.7 (C-2″), 136.0, 135.3, 129.3 (C-6′, 8′), 129.0 (C-6, 8), 128.7 (C-5′, 9′), 128.6 (C-5, 9), 127.24, 127.19, 120.8 (C-3″, 7″), 54.6 (C-2), 53.5 (C-2′), 52.4 (OCH_3), 38.2 (C-3), 37.8 (C-3′); EI-MS m/z: 431 (M^+), 309, 269, 253, 225, 162, 147, 131, 106 (100), 78, 57, 43; TOFESMS: calcd for $C_{25}H_{25}N_3O_4Na$ [M+Na]$^+$ 454.1743, found 454.1748.

4.1.2.7. *N*-(*N*- 4-Pyridinyl-formyl-L-phenylalanyl)-4-nitro-L-phenylalanine methyl ester (13f). Pale yellow powder, yield 48%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.77 (2H, s, H-4″, 6″), 8.06 (2H, d, J = 8.0 Hz, H-6′, 8′), 7.54 (2H, s, H-3″, 7″), 7.29–7.19 (7H, m, H-5, 9, 5′, 9′), 6.86 (1H, br, NHCO), 6.29 (1H, br, NHCO), 4.79 (2H, m, H-2, 2′), 3.72 (3H, s, OCH_3), 3.21–3.08 (4H, m, H-3, 3′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 170.5 (C-1), 170.1 (C-1′), 165.2 (C-1″), 150.7 (C-4″, 6″), 147.1 (C-7′), 143.2 (C-4′), 140.4 (C-2″), 135.7 (C-4), 130.1 (C-5′, 9′), 129.2 (C-6, 8), 128.9 (C-5, 9), 124.4 (C-7), 123.7 (C-6′, 8′), 120.7 (C-3″, 7″), 54.8 (C-2′), 53.0 (C-2), 52.7 (OCH_3), 38.1 (C-3′), 37.1 (C-3); EI-MS m/z: 476 (M^+), 385, 354, 225, 131, 106 (100), 78, 57, 43; TOFESMS: calcd for $C_{25}H_{24}N_4O_6Na$ [M+Na]$^+$ 499.1594, found 499.1598.

4.1.2.8. *N*-(*N*- 4-Pyridinyl-formyl-L-phenylalanyl)-L-tyrosine methyl ester (13g). White needle crystal, yield 46%, mp 216–

217 °C (MeOH/EtOAc), ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.26 (1H, s, Ar-OH), 8.91 (1H, d, J = 8.8 Hz, NHCO), 8.70 (2H, d, J = 5.2 Hz, H-4″, 6″), 8.57 (1H, d, J = 7.6 Hz, NHCO), 7.66 (2H, d, J = 5.2 Hz, H-3″, 7″), 7.35 (2H, d, J = 7.6 Hz, H-5, 9), 7.25 (2H, t, H-6, 8), 7.16 (1H, t, H-7), 7.02 (2H, d, J = 8.0 Hz, H-5′, 9′), 6.64 (2H, d, J = 8.0 Hz, H-6′, 8′), 4.78 (1H, m, H-2), 4.44 (1H, m, H-2′), 3.59 (3H, s, OCH_3), 3.12–2.85 (4H, m, H-3, 3′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 172.0 (C-1), 171.3 (C-1′), 164.7 (C-1″), 156.1 (C-7′), 150.2 (C-4″, 6″), 140.9 (C-2″), 138.1 (C-4), 130.1 (C-5′, 9′), 129.2 (C-6, 8), 128.1 (C-5, 9), 127.0 (C-4′), 126.3 (C-7), 121.4 (C-3″, 7″), 115.1 (C-6′, 8′), 54.5 (C-2), 54.2 (C-2′), 51.9 (OCH_3), 37.0 (C-3), 35.8 (C-3′); EI-MS m/z: 447 (M^+), 270 (100), 253, 225, 178, 106, 78; TOFESMS: calcd for $C_{25}H_{25}N_3O_5Na$ $[M+Na]^+$ 470.1692, found 470.1696.

4.1.2.9. *N*-(*N*- 4-Pyridinyl-formyl-L-tyrosineyl)-L-phenylalanine methyl ester (13h). Pale yellow powder, yield 46%, ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.17 (1H, s, Ar-OH), 8.82 (1H, d, J = 8.8 Hz, NHCO), 8.70 (2H, d, J = 5.6 Hz, H-4″, 6″), 8.59 (1H, d, J = 7.6 Hz, NHCO), 7.67 (2H, d, J = 5.6 Hz, H-3″, 7″), 7.27–7.18 (5H, m, H-5′, 9′), 7.12 (2H, d, J = 8.4 Hz, H-5, 9), 6.63 (2H, d, J = 8.4 Hz, H-6, 8), 4.68 (1H, m, H-2), 4.50 (1H, m, H-2′), 3.59 (3H, s, OCH_3), 3.09–2.77 (4H, m, H-3, 3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 171.8 (C-1), 171.4 (C-1′), 164.6 (C-1″), 155.7 (C-7), 150.2 (C-4″, 6″), 141.0 (C-2″), 137.1 (C-4′), 130.1 (C-5, 9), 129.1 (C-6′, 8′), 128.3 (C-5′, 9′), 128.1 (C-4), 126.6 (C-7′), 121.4 (C-3″, 7″), 114.9 (C-6, 8), 54.9 (C-2), 53.8 (C-2′), 51.9 (OCH_3), 38.9, 36.5; EI-MS m/z: 447 (M^+), 325, 241, 180, 163, 147, 123, 106 (100), 91; TOFESMS: calcd for $C_{25}H_{25}N_3O_5Na$ $[M+Na]^+$ 470.1692, found 470.1696.

4.1.2.10. *N*-(*N*- 4-Pyridinyl-formyl-L-tyrosineyl)-4-nitro-L-phenylalanine methyl ester (13i). Pale yellow powder, yield 43%, ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.17 (1H, s, Ar-OH), 8.80 (1H, d, J = 8.4 Hz, NHCO), 8.68 (2H, d, J = 4.8 Hz, H-4″, 6″), 8.63 (1H, d, J = 8.0 Hz, NHCO), 8.06 (2H, d, J = 8.4 Hz, H-6′, 8′), 7.64 (2H, d, J = 5.6 Hz, H-3″, 7″), 7.50 (2H, d, J = 8.4 Hz, H-5′, 9′), 7.09 (2H, d, J = 8.4 Hz, H-5, 9), 6.62 (2H, d, J = 8.0 Hz, H-6, 8), 4.65–4.58 (2H, m, H-2, 2′), 3.61 (3H, s, OCH_3), 3.22 (1H, dd, J = 5.6, 13.6 Hz, H-3′a), 3.09 (1H, dd, J = 9.2, 13.2 Hz, H-3′b), 2.93 (1H, dd, J = 4.4, 14.0 Hz, H-3a), 2.79 (1H, m, H-3b); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 171.4 (×2, C-1, 1′), 164.5 (C-1″), 155.8 (C-7), 150.2 (C-4″, 6″), 146.3 (C-7′), 145.5 (C-4′), 140.9 (C-2″), 130.6 (C-5′, 9′), 130.1 (C-5, 9), 128.0 (C-4), 123.2 (C-6′, 8′), 121.3 (C-3″, 7″), 114.9 (C-6, 8), 54.9 (C-2), 52.9 (C-2′), 52.1 (OCH_3), 38.9, 36.2; EI-MS m/z: 492 (M^+), 370, 162, 123, 107 (100), 91, 78; TOFESMS: calcd for $C_{25}H_{24}N_4O_7Na$ $[M+Na]^+$ 515.1543, found 515.1548.

4.1.2.11. *N*-(*N*- 4-Pyridinyl-formyl-L-phenylalanyl)-L-phenylalanol (13j). Yellow needle crystal, yield 40%, mp 199–201 °C (MeOH/EtOAc), ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.83 (1H, d, J = 8.8 Hz, NHCO), 8.69 (2H, d, J = 6.4 Hz, H-4″, 6″), 7.96 (1H, d, J = 8.0 Hz, NHCO), 7.66 (2H, d, J = 6.0 Hz, H-3″, 7″), 7.31–7.11 (10H, m, H-5, 9, 5′, 9′), 4.81 (1H, t, J = 5.2 Hz, OH), 4.68 (1H, m, H-2), 3.89 (1H, m, H-2′), 3.35–3.25 (2H, m, H-1′), 3.04 (1H, dd, J = 4.4, 14.0 Hz, H-3a), 2.95–2.83 (2H, m, H-3b, 3′a), 2.65 (1H, dd, J = 7.6, 13.2 Hz, H-3′b); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 170.5 (C-1), 164.5 (C-1″), 150.1 (C-4″, 6″), 141.0 (C-2″), 139.0 (C-4′), 138.2 (C-4), 129.2 (×4, C-6, 8, 6′, 8′), 128.1 (×4, C-5, 9, 5′, 9′), 126.3 (C-7), 125.9 (C-7′), 121.4 (C-3″, 7″), 62.2 (C-1′), 54.8 (C-2), 52.5 (C-2′), 37.3 (C-3), 36.4 (C-3′); EI-MS m/z: 403 (M^+), 270, 253, 225, 190, 120, 106 (100), 91, 78; TOFESMS: calcd for $C_{24}H_{25}N_3O_3Na$ $[M+Na]^+$ 426.1794, found 426.1798.

4.1.2.12. *N*-(*N*- 4-Pyridinyl-formyl-L-tyrosineyl)-L-phenylalanol (13k). Pale yellow powder, yield 43%, ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.17 (1H, s, Ar-OH), 8.79 (1H, d, J = 8.4 Hz, NHCO), 8.71 (2H, d, J = 5.6 Hz, H-4″, 6″), 7.95 (1H, d, J = 8.0 Hz, NHCO), 7.69 (2H, d, J = 5.6 Hz, H-3″, 7″), 7.23–7.09 (7H, m, H-5, 9, 5′, 9′), 6.63 (2H, d, J = 8.4 Hz, H-6, 8), 4.82 (1H, t, OH), 4.62 (1H, m, H-2), 3.90 (1H, m, H-2′), 3.37–3.27 (2H, m, H-1′), 2.96–2.79 (3H, m, H-3, 3′a), 2.66 (1H, dd, J = 8.0, 13.6 Hz, H-3′b); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 171.7 (C-1), 164.5 (C-1″), 155.7 (C-7), 150.2 (C-4″, 6″), 141.1 (C-2″), 139.0 (C-4′), 130.1 (C-5, 9), 129.2 (C-6′, 8′), 128.2 (C-4), 128.1 (C-5′, 9′), 125.9 (C-7′), 121.4 (C-3″, 7″), 114.9 (C-6, 8), 62.3 (C-1′), 55.3 (C-2), 52.5 (C-2′), 36.6, 36.4; EI-MS m/z: 419 (M^+), 297, 269, 241, 206, 162, 147, 123, 106 (100), 91, 78; TOFESMS: calcd for $C_{24}H_{25}N_3O_4Na$ $[M+Na]^+$ 442.1743, found 442.1747.

4.1.2.13. *N*-(*N*- 4-Acetamido-benzoyl-L-tyrosineyl)-L-phenylalanol (13l). Pale yellow powder, yield 54%, ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.17 (1H, s, Ar-NHCO), 9.14 (1H, s, Ar-OH), 8.28 (1H, d, J = 8.0 Hz, NHCO), 7.83 (1H, d, J = 8.4 Hz, NHCO), 7.76 (2H, d, J = 8.8 Hz, H-3″, 7″), 7.63 (2H, d, J = 8.4 Hz, H-4″, 6″), 7.22–7.07 (7H, m, H-5, 9, 5′, 9′), 6.61 (2H, d, J = 8.0 Hz, H-6, 8), 4.81 (1H, t, OH), 4.57 (1H, m, H-2), 3.88 (1H, m, H-2′), 3.34–3.25 (2H, m, H-1′), 2.91–2.50 (4H, m, H-3, 3′), 2.07 (3H, CH_3CO); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 171.2 (C-1), 168.7 (Ar-NHCO), 165.5 (C-1″), 155.7 (C-7), 142.0 (C-5″), 139.0 (C-4′), 130.1 (C-5, 9), 129.2 (6′, 8′), 128.4 (C-4, 2″), 128.3 (C-5′, 9′), 128.1 (C-3″, 7″), 125.9 (C-7′), 117.9 (C-4″, 6″), 114.8 (C-6, 8), 62.2 (C-1′), 55.1 (C-2), 52.4 (C-2′), 36.5, 36.4, 24.2 (CH_3CO); EI-MS m/z: 475 (M^+), 297, 206, 162 (100), 147, 120, 91; TOFESMS: calcd for $C_{27}H_{29}N_3O_5Na$ $[M+Na]^+$ 498.2005, found 498.2009.

4.1.2.14. *N*-(*N*- 4-Hydroxy-benzoyl-L-phenylalanyl)-L-phenylalanol (13m). White needle crystal, yield 61%, mp 223–225 °C (EtOAc), ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.98 (1H, s, Ar-OH), 8.22 (1H, d, J = 8.8 Hz, NHCO), 7.83 (1H, d, J = 8.4 Hz, NHCO), 7.66 (2H, d, J = 8.8 Hz, H-3″, 7″), 7.29–7.09 (10H, m, H-5, 9, 5′, 9′), 6.76 (2H, d, J = 8.4 Hz, H-4″, 6″), 4.80 (1H, t, J = 5.2 Hz, OH), 4.62 (1H, m, H-2), 3.87 (1H, m, H-2′), 3.34–3.22 (2H, m, H-1′), 3.02–2.92 (2H, m, H-3), 2.84 (1H, dd, J = 5.6, 13.6 Hz, H-3′a), 2.64 (1H, dd, J = 8.0, 13.6, Hz, H-3′b); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 171.2 (C-1), 165.8 (C-1″), 160.2 (C-5″), 139.0 (C-4′), 138.5 (C-4), 129.4 (C-3″, 7″), 129.23 (C-6′, 8′), 129.21 (C-6, 8), 128.09 (C-5′, 9′), 128.03 (C-5, 9), 126.2 (C-7), 125.9 (C-7′), 124.7 (C-2″), 114.7 (C-4″, 6″), 62.2 (C-1′), 54.7 (C-2), 52.5 (C-2′), 37.2 (C-3′), 36.5 (C-3); EI-MS m/z: 418 (M^+), 268, 240, 190, 121 (100), 91; TOFESMS: calcd for $C_{25}H_{26}N_2O_4Na$ $[M+Na]^+$ 441.1790, found 441.1794.

4.1.2.15. *N*-(*N*-(4-Dimethylamino-methyl-benzoyl)-L-phenylalanyl)-L-phenylalanol (13n). White needle crystal, yield 45%, mp 197–198 °C (EtOAc), ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.46 (1H, d, J = 8.8 Hz, NHCO), 7.87 (1H, d, J = 8.4 Hz, NHCO), 7.75 (2H, d, J = 8.0 Hz, H-3″, 7″), 7.34 (2H, d, J = 8.0 Hz, H-4″, 6″), 7.30–7.10 (10H, m, H-5′, 9′, 5, 9), 4.81 (1H, t, OH), 4.67 (1H, m, H-2), 3.87 (1H, m, H-2′), 3.43 (2H, s, Ar-CH_2N(CH_3)$_2$), 3.34–3.25 (2H, m, H-1′), 3.02–2.93 (2H, m, H-3), 2.84 (1H, dd, J = 5.6, 13.6 Hz, H-3′a), 2.65 (1H, dd, J = 8.4, 13.6 Hz, H-3′b), 1.14 (6H, s, Ar-CH_2N(CH_3)$_2$); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 171.0 (C-1), 165.9 (C-1″), 143.0 (C-5″), 139.0 (C-4′), 138.4 (C-4), 132.8 (C-2″), 129.2 (×4, C-6, 8, 6′, 8′), 128.5 (C-4″, 6″), 128.1 (C-5′, 9′), 128.0 (C-5, 9), 127.4 (C-3″, 7″), 126.2 (C-7), 125.9 (C-7′), 62.8 (Ar-CH_2N(CH_3)$_2$), 62.2 (C-1′), 54.8 (C-2), 52.5 (C-2′), 44.9 (Ar-CH_2N(CH_3)$_2$), 37.2, 36.4; EI-MS m/z: 459 (M^+), 416, 309, 281, 162 (100), 105, 91; TOFESMS: calcd for $C_{28}H_{33}N_3O_3Na$ $[M+Na]^+$ 482.2420, found 482.2425.

4.1.2.16. *N*-(*N*- (4-Pyrrolidin-1-ylmethyl-benzoyl)-L-phenylalanyl)-L-phenylalanol (13o). Yellow crystal, yield 43%, mp 164–165 °C (EtOAc), ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.44 (1H, d, J = 8.0 Hz, NHCO), 7.87 (1H, d, J = 8.4 Hz, NHCO), 7.74 (2H, d, J = 8.4 Hz, H-3″, 7″), 7.35 (2H, d, J = 8.0 Hz, H-4″, 6″), 7.31–7.09

(10H, m, H-5′, 9′, 5, 9), 4.81 (1H, t, OH), 4.67 (1H, m, H-2), 3.88 (1H, m, H-2′), 3.58 (2H, s, Ar-CH_2N), 3.34–3.24 (2H, m, H-1′), 3.04–2.91 (2H, m, H-3), 2.85 (1H, dd, J = 5.6, 13.6 Hz, H-3′a), 2.65 (1H, dd, J = 8.0, 13.6 Hz, H-3′b), 2.40 (4H, s, Ar-CH_2N(CH_2)$_2$), 1.67 (4H, s, N$CH_2CH_2CH_2CH_2$N); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 171.0 (C-1), 165.9 (C-1″), 143.0 (C-5″), 139.0 (C-4′), 138.4 (C-4), 132.6 (C-2″), 129.2 (×4, C-6, 8, 6′, 8′), 128.14 (C-4″, 6″), 128.08 (C-5′, 9′), 128.04 (C-5, 9), 127.4 (C-3″, 7″), 126.2 (C-7), 125.9 (C-7′), 62.2 (C-1′), 59.2 (Ar-CH_2N), 54.8 (C-2), 53.5 (Ar-CH_2N(CH_2)$_2$), 52.5 (C-2′), 37.2, 36.4, 23.1 (N$CH_2CH_2CH_2CH_2$N); EI-MS m/z: 416, 335, 307, 265, 188 (100), 152, 118, 91; TOFESMS: calcd for $C_{30}H_{36}N_3O_3$ [M+H]$^+$ 486.2757, found 486.2762.

4.1.2.17. *N*-(*N*- (4-Morpholinomethyl-benzoyl)-L-phenylalanyl)-L-phenylalanol (13p). White crystal, yield 46%, mp 183–185 °C (MeOH/EtOAc), ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.45 (1H, d, J = 8.4 Hz, NHCO), 7.87 (1H, d, J = 8.0 Hz, NHCO), 7.76 (2H, d, J = 7.6 Hz, H-3″, 7″), 7.37 (2H, d, J = 7.6 Hz, H-4″, 6″), 7.31–7.10 (10H, m, H-5′, 9′, 5, 9), 4.81 (1H, t, OH), 4.69 (1H, m, H-2), 3.89 (1H, m, H-2′), 3.57 (4H, s, CH_2OCH_2), 3.48 (2H, s, Ar-CH_2N), 3.34–3.25 (2H, m, H-1′), 3.05–2.92 (2H, m, H-3), 2.86 (1H, dd, J = 5.2, 13.6 Hz, H-3′a), 2.66 (1H, dd, J = 8.0, 13.6 Hz, H-3′b), 2.34 (4H, s, Ar-CH_2N(CH_2)$_2$); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 170.9 (C-1), 165.9 (C-1″), 141.4 (C-5″), 139.0 (C-4′), 138.4 (C-4), 132.8 (C-2″), 129.19 (×2), 129.17 (×2), 128.6 (C-4″, 6″), 128.06 (×2), 128.01 (×2), 127.4 (C-3″, 7″), 126.2 (C-7), 125.9 (C-7′), 66.2 (CH_2OCH_2), 62.2 (C-1′), 61.96 (Ar-CH_2N), 54.7 (C-2), 53.2 (Ar-CH_2N(CH_2)$_2$), 52.5 (C-2′), 37.2, 36.4; EI-MS m/z: 501 (M$^+$), 351, 323, 204 (100), 146, 118, 91; TOFESMS: calcd for $C_{30}H_{35}N_3O_4Na$ [M+Na]$^+$ 524.2525, found 524.2529.

4.1.2.18. *N*-(*N*-4-Acetamido-benzoyl-L-phenylalanyl)-L-phenylalanol (14a). $SOCl_2$ (1.1 mmol) was added dropwise to the mixture of compound **13** (1.0 mmol) in 1,4-dioxane (10 mL), stirred at 70 °C for 1 h. The reaction solution was partitioned in ethyl acetate and water, washed sequentially with saturated $NaHCO_3$ solution, and brine. The solution was dried with Na_2SO_4 and concentrated, the residue was recrystallized from ethyl acetate to afford target compound **14a** as needle crystal. Yield 56%, mp 193–195 °C (EtOAc), ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.16 (1H, s, Ar-NHCO), 8.39 (1H, d, J = 8.4 Hz, NHCO), 8.26 (1H, d, J = 8.4 Hz, NHCO), 7.76 (2H, d, J = 8.4 Hz, H-3″, 7″), 7.62 (2H, d, J = 8.8 Hz, H-4″, 6″), 7.32–7.13 (10H, m, H-5, 9, 5′, 9′), 4.70 (1H, m, H-2), 4.17 (1H, m, H-2′), 3.64 (1H, dd, J = 4.8, 11.2 Hz, H-1′a), 3.52 (1H, dd, J = 7.0, 11.2 Hz, H-1′b), 3.05–2.78 (4H, m, H-3, 3′), 2.06 (3H, CH_3CO); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 171.4 (C-1), 168.7 (Ar-NHCO), 165.6 (C-1″), 142.0 (C-5″), 138.3 (C-4), 137.8 (C-4′), 129.2 (×4), 128.3 (×4), 128.2 (C-2″), 128.0 (C-3″, 7″), 126.4 (C-7), 126.2 (C-7′), 118.0 (C-4″, 6″), 54.7 (C-2), 51.5 (C-1′), 46.8 (C-2′), 37.3 (C-3), 37.0 (C-3′), 24.1 (CH_3CO); EI-MS m/z: 441 (100), 350, 279, 264, 162, 120, 91; TOFESMS: calcd for $C_{27}H_{28}N_3O_3ClNa$ [M+Na]$^+$ 500.1717, found 500.1722.

4.1.2.19. *N*-(*N*-(4-Morpholinomethyl-benzoyl)-L-phenylalanyl)-*O*- acetyl-L-phenylalanol (15a). Compound **13p** (502 mg, 1.0 mmol) was dissolved in pyridine (5 mL), then Ac_2O (1 mL) was added. After stirred at room temperature for 5 h, the reaction was quenched with ethanol (5 mL) and concentrated in vacuo. The residue was dissolved in ethyl acetate and washed sequentially with water, saturated $NaHCO_3$ solution, and brine. The solution was dried with Na_2SO_4 and concentrated, the residue was recrystallized from ethyl acetate to afford target compound **15a** as white powder (516 mg, 95%). Yield 44%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.68 (2H, d, J = 8.4 Hz, H-3″, 7″), 7.40 (2H, d, J = 8.4 Hz, H-4″, 6″), 7.30–7.05 (10H, m, H-5′, 9′, 5, 9), 6.84 (1H, d, J = 7.6 Hz, NHCO), 6.16 (1H, d, J = 8.4 Hz, NHCO), 4.80 (1H, m, H-2), 4.34 (1H, m, H-2′), 3.92 (1H, dd, J = 4.8, 11.2 Hz, H-1′a), 3.82 (1H, dd, J = 4.0, 11.2 Hz, H-1′b), 3.71 (4H, t, J = 4.4 Hz, CH_2OCH_2), 3.54 (2H, s, Ar-CH_2N), 3.22 (1H, dd, J = 6.0, 13.6 Hz, H-3a), 3.07 (1H, dd, J = 8.0, 13.6 Hz, H-3b), 2.78–2.73 (2H, m, H-3′), 2.44 (4H, s, Ar-CH_2N(CH_2)$_2$), 2.02 (3H, s, OCOCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 171.1, 170.6, 167.2 (C-1″), 142.5 (C-5″), 137.0, 136.9, 132.8 (C-2″), 129.6 (×2), 129.5 (×2), 129.4 (×2), 129.0 (×2), 128.8 (×2), 127.4 (C-3″, 7″), 127.0 (C-7, 7′), 67.2 (CH_2OCH_2), 64.8 (C-1′), 63.2 (Ar-CH_2N), 55.2 (C-2), 53.9 (Ar-CH_2N(CH_2)$_2$), 49.7 (C-2′), 38.8, 37.7, 21.1 (OCOCH_3); EI-MS m/z: 543 (M$^+$), 398, 351, 323, 220, 204 (100), 146, 105, 91; TOFESMS: calcd for $C_{32}H_{37}N_3O_5Na$ [M+Na]$^+$ 566.2631, found 566.2638.

4.1.2.20. *N*-(*N*-(4-Dimethylamino-methyl-benzoyl)-L-phenylalanyl)-*O*- acetyl-L-phenylalanol (15b). White needle crystal, yield 44%, mp 196–198 °C (EtOAc), ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.68 (2H, d, J = 8.4 Hz, H-3″, 7″), 7.40 (2H, d, J = 8.0 Hz, H-4″, 6″), 7.30–7.06 (10H, m, H-5′, 9′, 5, 9), 6.76 (1H, br, NHCO), 6.07 (1H, br, NHCO), 4.78 (1H, m, H-2), 4.35 (1H, m, H-2′), 3.92 (1H, dd, J = 4.8, 11.6 Hz, H-1′a), 3.81 (1H, dd, J = 4.0, 11.6 Hz, H-1′b), 3.49 (2H, s, Ar-CH_2N(CH_3)$_2$), 3.22 (1H, dd, J = 5.6, 13.6 Hz, H-3a), 3.06 (1H, dd, J = 8.4, 13.2 Hz, H-3b), 2.76–2.73 (2H, m, H-3′), 2.26 (6H, s, Ar-CH_2N(CH_3)$_2$), 2.03 (3H, s, COCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 170.8 (C-1), 170.2 (O$COCH_3$), 166.9 (C-1″), 143.0 (C-5″), 136.7, 136.6, 132.5 (C-2″), 129.3 (×4, C-6, 8, 6′, 8′), 129.1 (C-4″, 6″), 128.7 (C-5′, 9′), 128.5 (C-5, 9), 127.1 (C-3″, 7″), 126.7 (C-7, 7′), 64.5 (C-1′), 63.8 (Ar-CH_2N(CH_3)$_2$), 54.9 (C-2), 49.3 (C-2′), 45.3 (Ar-CH_2N(CH_3)$_2$), 38.4, 37.4, 20.8 (OCOCH_3); EI-MS m/z: 501 (M$^+$), 309, 281, 176, 162 (100), 91; TOFESMS: calcd for $C_{30}H_{35}N_3O_4Na$ [M+Na]$^+$ 524.2525, found 524.2529.

4.1.2.21. *N*-(*N*-Benzoyl-L-phenylalanyl)-*O*- ethyl-L-tyrosine methyl ester (16a). CH_3CH_2I (1.40 g, 9.0 mmol) was added to the mixture of compound 13 (1.34 g, 3.0 mmol) and K_2CO_3 (3.11 g, 22.5 mmol) in DMF (15 mL). After stirring at room temperature overnight. The reaction mixture was diluted with water and extracted with ethyl acetate. The organic layer was washed with brine, dried with Na_2SO_4 and evaporated in vacuo to give the compound **16a** as a white solid (1.29 g, 91%). Yield 56%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.69 (2H, d, J = 7.6 Hz, H-3″, 7″), 7.51 (1H, t, H-5″), 7.42 (2H, t, H-4″, 6″), 7.31–7.20 (5H, m, H-5, 9), 6.86 (2H, d, J = 8.4 Hz, H-5′, 9′), 6.76 (1H, br, NHCO), 6.66 (2H, d, J = 8.4 Hz, H-6′, 8′), 6.29 (1H, br, NHCO), 4.84 (1H, m, H-2), 4.72 (1H, m, H-2′), 3.90 (2H, m, Ar-OCH_2CH_3), 3.70 (3H, s, OMe), 3.21 (1H, dd, J = 6.0, 14.0 Hz, H-3′a), 3.12 (1H, dd, J = 7.6, 14.0 Hz, H-3′b), 3.02 (1H, dd, J = 5.6, 13.6 Hz, H-3a), 2.90 (1H, dd, J = 6.4, 13.6 Hz, H-3b), 1.36 (3H, t, Ar-OCH_2CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 171.7 (C-1), 170.6 (C-1′), 167.3 (C-1″), 158.3 (C-7′), 136.7 (C-4), 133.9 (C-2″), 132.2 (C-5″), 130.4 (C-5′, 9′), 129.8 (C-6, 8), 129.0 (×2), 128.9 (×2), 127.5 (C-4′), 127.4 (C-7), 127.3 (C-3″, 7″), 114.8 (C-6′, 8′), 63.5 (Ar-OCH_2CH_3), 54.8 (C-2), 53.8 (C-2′), 52.7 (COO*Me*), 38.4 (C-3), 37.3 (C-3′), 15.1 (Ar-OCH_2CH_3); EI-MS m/z: 474 (M$^+$), 224, 206 (100), 178, 135, 105, 91, 77; TOFESMS: calcd for $C_{28}H_{30}N_2O_5Na$ [M+Na]$^+$ 497.2052, found 497.2056.

4.1.2.22. *N*-(*N*-Benzoyl-L-phenylalanyl)-*O*- butyl-L-tyrosine methyl ester (16b). White needle crystal, yield 59%, mp 177–178 °C, ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.69 (2H, d, J = 7.6 Hz, H-3″, 7″), 7.51 (1H, t, H-5″), 7.42 (2H, t, H-4″, 6″), 7.31–7.23 (5H, m, H-5, 9), 6.85 (2H, d, J = 8.4 Hz, H-5′, 9′), 6.76 (1H, br, NHCO), 6.66 (2H, d, J = 8.4 Hz, H-6′, 8′), 6.31 (1H, br, NHCO), 4.83 (1H, m, H-2), 4.72 (1H, m, H-2′), 3.82 (2H, m, Ar-O$CH_2CH_2CH_2CH_3$), 3.70 (3H, s, OMe), 3.21 (1H, dd, J = 5.6, 14.0 Hz, H-3′a), 3.12 (1H, dd, J = 7.2, 13.6 Hz, H-3′b), 3.01 (1H, dd, J = 5.6, 14.0 Hz, H-3a), 2.90 (1H, dd, J = 6.8, 14.0 Hz, H-3b), 1.70 (2H, m, Ar-$OCH_2CH_2CH_2CH_3$), 1.45 (2H, m, Ar-$OCH_2CH_2CH_2CH_3$), 0.96 (2H, t, J = 7.6 Hz, Ar-

$OCH_2CH_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 171.4 (C-1), 170.3 (C-1′), 167.0 (C-1″), 158.2 (C-7′), 136.4 (C-4), 133.6 (C-2″), 131.8 (C-5″), 130.1 (C-5′, 9′), 129.4 (C-6, 8), 128.7 (C-5, 9), 128.6 (C-4″, 6″), 127.04 (C-4′, C-7), 127.01 (C-3″, 7″), 114.5 (C-6′, 8′), 63.5 (Ar-$OCH_2CH_2CH_2CH_3$), 54.5 (C-2), 53.5 (C-2′), 52.3 (OMe), 38.1 (C-3), 36.9 (C-3′), 31.3 (Ar-$OCH_2CH_2CH_2CH_3$), 19.2 (Ar-$OCH_2CH_2CH_2CH_3$), 13.8 (Ar-$OCH_2CH_2CH_2CH_3$); EI-MS *m/z*: 502 (M^+), 234 (100), 178, 105; TOFESMS: calcd for $C_{30}H_{34}N_2O_5Na$ $[M+Na]^+$ 525.2365, found 525.2372.

4.1.2.23. *N*-(*N*-4-(2-(Dimethylamino)-ethoxy)-benzoyl-L-phenylalanyl)-L-phenylalanine methyl ester (16c). White needle crystal, yield 50%, mp 167–168 °C, ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.64 (2H, d, *J* = 8.8 Hz, H-3″, 7″), 7.30–6.95 (10H, m, H-5, 9, 5′, 9′), 6.92 (2H, d, *J* = 8.8 Hz, H-4″, 6″), 6.58 (1H, d, *J* = 7.6 Hz, NHCO), 6.35 (1H, d, *J* = 7.2 Hz, NHCO), 4.83–4.76 (2H, m, H-2, 2′), 4.10 (2H, t, *J* = 6.0 Hz, Ar-OCH_2), 3.70 (3H, s, OCH_3), 3.21–2.93 (4H, m, H-3, 3′), 2.75 (2H, t, *J* = 6.0 Hz, $CH_2N(CH_3)_2$), 2.35 (6H, s, $N(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 171.3 (C-1), 170.5 (C-1′), 166.6 (C-1″), 161.7 (C-5″), 136.4 (C-4), 135.5 (C-4′), 129.4 (C-3″, 7″), 129.1 (C-6, 8), 128.8 (C-6′, 8′), 128.7 (C-5′, 9′), 128.5 (C-5, 9), 127.0 (C-7, 7′), 125.9 (C-2″), 114.3 (C-4″, 6″), 66.1 (Ar-OCH_2), 58.1 ($CH_2N(CH_3)_2$), 54.4 (C-2), 53.4 (C-2′), 52.3 (OCH_3), 45.9 ($N(CH_3)_2$), 38.0, 37.8; EI-MS *m/z*: 517 (M^+), 192, 176 (100), 120, 91, 58 (100); TOFESMS: calcd for $C_{30}H_{36}N_3O_5$ $[M+H]^+$ 518.2655, found 518.2661.

4.1.2.24. *N*-(*N*- 4-(2-Dimethylamino-acetamido)-benzoyl-L-phenylalanyl)-L-phenylalanine methyl ester (16d). White powder, yield 49%, ^1H NMR (DMSO-d_6, 400 MHz) δ: 9.92 (1H, s, Ar-NHCO), 8.52 (1H, d, *J* = 7.6 Hz, NHCO), 8.41 (1H, d, *J* = 8.4 Hz, NHCO), 7.71 (4H, m, H-3″, 7″, 4″, 6″), 7.33–7.14 (10H, m, H-5, 9, 5′, 9′), 4.72 (1H, m, H-2), 4.50 (1H, m, H-2′), 3.57 (3H, s, OCH_3), 3.07 (2H, s, $NHCOCH_2N$), 3.06–2.89 (4H, m, H-3, 3′), 2.26 (6H, s, $N(CH_3)_2$); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 172.5, 172.4, 169.8 (Ar-NHCO), 165.3 (C-1″), 142.1 (C-5″), 139.0 (C-4), 137.7 (C-4′), 129.83 (C-6, 8), 129.78 (C-6′, 8′), 129.1 (C-2″), 128.9 (C-5′, 9′), 128.8 (C-3″, 7″), 128.7 (C-5, 9), 127.2 (C-7′), 126.9 (C-7), 119.1 (C-4″, 6″), 63.9 ($COCH_2N$), 55.1 (C-2), 54.4 (C-2′), 52.5 (OCH_3), 46.0 ($N(CH_3)_2$), 37.6 (C-3), 37.2 (C-3′); EI-MS *m/z*: 530 (M^+), 310, 205, 91, 58 (100); TOFESMS: calcd for $C_{30}H_{34}N_4O_5Na$ $[M+Na]^+$ 553.2427, found 553.2433.

4.1.2.25. *N*-(*N*- (4-Pyrrolidinylacetamido-benzoyl)-L-phenylalanyl)-L-phenylalanine methyl ester (16e). Pale yellow powder, yield 43%, ^1H NMR (DMSO-d_6, 400 MHz) δ: 9.93 (1H, s, Ar-NHCO), 8.53 (1H, d, *J* = 7.2 Hz, NHCO), 8.43 (1H, d, *J* = 8.8 Hz, NHCO), 7.75 (2H, d, *J* = 8.8 Hz, H-3″, 7″), 7.69 (2H, d, *J* = 8.8 Hz, H-4″, 6″), 7.34–7.15 (10H, m, H-5, 9, 5′, 9′), 4.74 (1H, m, H-2), 4.52 (1H, m, H-2′), 3.59 (3H, s, OCH_3), 3.26 (2H, $COCH_2N$), 3.07–2.94 (4H, m, H-3, 3′), 2.59 (4H, m, $COCH_2N(CH_2)_2$), 1.74 (4H, m, $NCH_2CH_2CH_2CH_2N$); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 171.8, 171.7, 169.2 (Ar-NHCO), 165.6 (C-1″), 141.4 (C-5″), 138.3 (C-4), 137.0 (C-4′), 129.2 (×2), 129.1 (×2), 128.5 (C-2″), 128.3 (×2), 128.2 (×2), 128.0 (×2), 126.6 (C-7′), 126.2 (C-7), 118.5 (C-4″, 6″), 59.5 ($COCH_2N$), 54.4 (C-2), 53.73 (C-2′), 53.69 ($COCH_2N(CH_2)_2$), 51.9 (OCH_3), 36.9 (C-3), 36.6 (C-3′), 23.5 ($NCH_2CH_2CH_2CH_2N$); EI-MS *m/z*: 556 (M^+), 231, 119, 84 (100); TOFESMS: calcd for $C_{32}H_{37}N_4O_5$ $[M+H]^+$ 557.2764, found 557.2770.

4.1.2.26. *N*-(*N*-4-(Dimethylamino)-acetamido-benzoyl-L-phenylalanyl)-L-phenylalanol (16f). Pale yellow crystal, yield 47%, mp 162–164 °C, ^1H NMR (DMSO-d_6, 400 MHz) δ: 9.94 (1H, s, Ar-NHCO), 8.39 (1H, d, *J* = 8.8 Hz, NHCO), 7.89 (1H, d, *J* = 8.4 Hz, NHCO), 7.77 (2H, d, *J* = 8.8 Hz, H-3″, 7″), 7.73 (2H, d, *J* = 8.8 Hz, H-4″, 6″), 7.31–7.11 (10H, m, H-5, 9, 5′, 9′), 4.82 (1H, t, OH), 4.67 (1H, m, H-2), 3.89 (1H, m, H-2′), 3.35–3.24 (2H, m, H-1′), 3.09 (2H, $COCH_2N$), 3.07–2.91 (2H, m, H-3), 2.86 (1H, dd, *J* = 5.6, 13.6 Hz, H-3′a), 2.66 (1H, dd, *J* = 7.6, 13.6 Hz, H-3′b), 2.28 (6H, s, $N(CH_3)_2$); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 171.0 (C-1), 169.1 (Ar-NHCO), 165.5 (C-1″), 141.4 (C-5″), 139.0 (C-4′), 138.5 (C-4), 129.2 (×4, C-6, 8, 6′, 8′), 128.6 (C-2″), 128.2 (C-3″, 7″), 128.1 (×2), 128.0 (×2), 126.2 (C-7), 125.9 (C-7′), 118.5 (C-4″, 6″), 63.3 ($COCH_2N$), 62.2 (C-1′), 54.8 (C-2), 52.5 (C-2′), 45.4 (×2, $N(CH_3)_2$), 37.3 (C-3), 36.4 (C-3′); EI-MS *m/z*: 502 (M^+), 474, 352, 205, 119, 91, 58 (100); TOFESMS: calcd for $C_{29}H_{35}N_4O_4$ $[M+H]^+$503.2658, found 503.2662.

4.1.2.27. *N*-(*N*-4-(2-(Dimethylamino)-ethoxy)-benzoyl-L-phenylalanyl)-L-phenylalanol (16g). Pale yellow powder, yield 53%, ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.67 (2H, d, *J* = 8.8 Hz, H-3″, 7″), 7.31–7.07 (10H, m, H-5, 9, 5′, 9′), 6.92 (2H, d, *J* = 8.8 Hz, H-4″, 6″), 6.78 (1H, d, *J* = 7.6 Hz, NHCO), 6.28 (1H, d, *J* = 8.0 Hz, NHCO), 4.80 (1H, m, H-2), 4.11–4.07 (3H, m, Ar-OCH_2, H-2′), 3.42 (2H, m, H-1′), 3.23 (1H, dd, *J* = 6.4, 13.6 Hz, H-3a), 3.06 (1H, dd, *J* = 8.4, 13.6 Hz, H-3b), 2.80–2.66 (4H, m, H-3′, $CH_2N(CH_3)_2$), 2.34 (6H, s, $N(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 171.0 (C-1), 166.8 (C-1″), 161.8 (C-5″), 137.4 (C-4), 136.8 (C-4′), 129.3 (C-3″, 7″), 129.1 (C-6, 8), 128.9 (C-6′, 8′), 128.8 (C-5′, 9′), 128.5 (C-5, 9), 127.1 (C-7), 126.5 (C-7′), 125.8 (C-2″), 114.4 (C-4″, 6″), 66.2 (Ar-OCH_2), 63.4 (C-1′), 58.1 ($CH_2N(CH_3)_2$), 55.1 (C-2), 52.9 (C-2′), 45.9 ($N(CH_3)_2$), 38.6 (C-3′), 36.8 (C-3); EI-MS *m/z*: 489 (M^+), 418, 339, 192, 105, 91, 58 (100); TOFESMS: calcd for $C_{29}H_{36}N_3O_4$ $[M+H]^+$ 490.2706, found 490.2710.

4.1.2.28. Sodium *N*-(*N*-benzoyl-L-phenylalanyl)-*O*- methyl-L-tyrosinate (17a). 5 M NaOH (0.2 mL) was added to the solution of compound **16** (1.0 mmol) in EtOH–$CHCl_3$ (5 mL). After stirring at room temperature for 2 h, the reaction was evaporated in vacuo. The residue was recrystallised from EtOAc to afford the compound **17**. White needle crystal, yield 61%, mp 271–271 °C, ^1H NMR (DMSO-d_6, 400 MHz) δ: 8.81 (1H, d, *J* = 8.4 Hz, NHCO), 7.78 (2H, d, *J* = 7.2 Hz, H-3″, 7″), 7.48 (2H, m, H-5″, NHCO), 7.42 (2H, t, H-4″, 6″), 7.30 (2H, d, *J* = 8.4 Hz, H-5, 9), 7.22 (2H, t, H-6, 8), 7.13 (1H, t, H-7), 7.01 (2H, d, *J* = 8.4 Hz, H-5′, 9′), 6.57 (2H, d, *J* = 8.4 Hz, H-6′, 8′), 4.57 (1H, m, H-2), 3.95 (1H, m, H-2′), 3.59 (3H, s, Ar-OCH_3), 3.13–2.86 (4H, m, H-3, 3′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 172.7 (C-1′), 169.9 (C-1), 166.2 (C-1″), 157.3 (C-7′), 138.9 (C-4), 134.1 (C-2″), 131.3 (C-5″), 131.0 (C-4′), 130.7 (C-5′, 9′), 129.1 (C-6, 8), 128.2 (C-4″, 6″), 128.1 (C-5, 9), 127.4 (C-3″, 7″), 126.1 (C-7), 112.9 (C-6′, 8′), 55.6 (×2), 54.7 (Ar-OCH_3), 36.7, 36.3; EI-MS *m/z*: 446, 309, 246, 121 (100), 105, 91, 77; TOFESMS: calcd for $C_{26}H_{25}N_2O_5Na$ $[M+Na]^+$ 491.1559, found 491.1563.

4.1.2.29. *N*-(*N*-Benzoyl-L-phenylalanyl)-*O*- ethyl-L-tyrosine (17b). White needle crystal, yield 57%, mp 263–264 °C, ^1H NMR (DMSO-d_6, 400 MHz) δ: 12.85 (1H, br, COOH), 8.55 (1H, d, *J* = 8.4 Hz, NHCO), 8.24 (1H, d, *J* = 8.0 Hz, NHCO), 7.76 (2H, d, *J* = 7.6 Hz, H-3″, 7″), 7.52 (1H, t, H-5″), 7.43 (2H, t, H-4″, 6″), 7.33 (2H, d, *J* = 7.6 Hz, H-5, 9), 7.23 (2H, t, *J* = 7.6 Hz, H-6, 8), 7.15–7.10 (3H, m, *J* = 8.4 Hz, H-7, H-5′, 9′), 6.70 (2H, d, *J* = 8.4 Hz, H-6′, 8′), 4.73 (1H, m, H-2), 4.41 (1H, m, H-2′), 3.87 (2H, m, Ar-OCH_2CH_3), 3.10–2.84 (4H, m, H-3′, 3), 1.26 (3H, t, Ar-OCH_2CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 172.9 (C-1′), 171.4 (C-1), 166.2 (C-1″), 157.2 (C-7′), 138.4 (C-4), 134.0 (C-2″), 131.4 (C-5″), 130.2 (C-5′, 9′), 129.2 (C-6, 8), 129.0 (C-4′), 128.2 (C-4″, 6″), 128.1 (C-5, 9), 127.4 (C-3″, 7″), 126.2 (C-7), 114.1 (C-6′, 8′), 62.8 (Ar-OCH_2CH_3), 54.5 (C-2′), 53.8 (C-2), 36.8 (C-3), 35.9 (C-3′), 14.7 (Ar-OCH_2CH_3); EI-MS *m/z*: 460, 251, 207, 135, 121, 105, 91 (100), 77; TOFESMS: calcd for $C_{27}H_{27}N_2O_5Na_2$ $[M+Na]^+$ 505.1715, found 505.1720.

4.1.2.30. Sodium *N*-(*N*-benzoyl-L-phenylalanyl)-*O*- butyl-L-tyrosinate (17c). White needle crystal, yield 55%, mp 274–276 °C, ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.82 (1H, d, J = 8.4 Hz, NHCO), 7.78 (2H, d, J = 7.2 Hz, H-3″, 7″), 7.51–7.47 (2H, m, H-5″, NHCO), 7.41 (2H, t, H-4″, 6″), 7.30 (2H, d, J = 7.6 Hz, H-5, 9), 7.22 (2H, t, H-6, 8), 7.12 (1H, t, H-7), 6.99 (2H, d, J = 8.4 Hz, H-5′, 9′), 6.55 (2H, d, J = 8.0 Hz, H-6′, 8′), 4.57 (1H, m, H-2), 3.94 (1H, m, H-2′), 3.75 (2H, t, J = 6.4 Hz, Ar-O$CH_2CH_2CH_2CH_3$), 3.12–2.85 (4H, m, H-3, 3′), 1.61 (2H, m, Ar-OCH$_2$CH_2CH$_2$CH$_3$), 1.37 (2H, m, ArOCH$_2$CH$_2$CH_2-CH$_3$), 0.89 (2H, t, J = 7.6 Hz, Ar-OCH$_2$CH$_2$CH$_2$CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 172.5 (C-1′), 169.6 (C-1), 166.0 (C-1″), 156.6 (C-7′), 138.7 (C-4), 134.1 (C-2″), 131.0 (C-5″), 130.9 (C-4′), 130.5 (C-5′, 9′), 128.9 (C-6, 8), 127.93, 127.87, 127.3 (C-3″, 7″), 125.9 (C-7), 113.4 (C-6′, 8′), 66.8 (Ar-O$CH_2CH_2CH_2CH_3$), 55.4, 55.3, 36.6, 36.3, 30.7 (ArOCH$_2$CH_2CH$_2$CH$_3$), 18.6 (Ar-OCH$_2$CH$_2$-CH_2CH$_3$), 13.5 (Ar-OCH$_2$CH$_2$CH$_2$CH_3); EI-MS *m/z*: 488, 309, 163, 121, 105 (100), 91, 77; TOFESMS: calcd for $C_{29}H_{31}N_2O_5Na_2$ $[M+Na]^+$ 533.2028, found 533.2033.

4.2. Biological assay

4.2.1. In vitro anti-HBV assays

The anti-HBV activities in vitro included the ability to inhibit the production of HBsAg and HBeAg in 2.2.15 cells, and the replication of HBV DNA in HBV-infected 2.2.15 cells. Confluent cultures of 2.2.15 cells were maintained on 96-well flat-bottomed tissue culture plates in RPMI 1640 medium with 2% fetal bovine serum for the antiviral analyses. Cultures were treated with eight consecutive daily doses of the test compounds and lamivudine (produced by Glaxo & Welcome Co.). The Hep G2.2.15 cells were incubated in 24-well plates at a density of 1.0×10^5 cells/mL in 1 L MEM medium containing 10% FBS for 24 h. After attachment to plates, the supernatants in each well were replaced carefully with 1 mL of fresh DMEM containing different concentrations (50, 25, and 12.25 μg/mL) of compounds **13a–13p**, **14a**, **15a**, **15b**, **16a–16g**, and **17a–17c** and did four wells at each concentration. Cells grew in the presence of drugs for 9 days with changes of medium every 3 days. After 6 and 9 days, supernatant was collected and performed at 20 °C. The HBsAg and HBeAg in culture medium were simultaneously measured by EIA kits on 6 and 9 days. This test was done twice under the same condition. Untreated cells were used as the control. Medium was changed daily with fresh test compounds and positive control. The levels of HBV nucleic acid and the protein were measured 8 days after the first treatment. The HBsAg and HBeAg in the culture medium were evaluated by semiquantitative enzyme immunoassay (EIA) methods using commercial kits (Beijing North Institute of Biological Technology.) as previously described. The levels of intracellular HBV DNA were measured by quantitative Southern blot hybridization. The IC_{50} and selected index of the evaluated compounds and lamivudine were calculated by one-way repeated-measure ANOVA and *t*-test for comparisons between the groups, respectively.

4.2.2. Cytotoxicity assay

Cytotoxicity induced by the test compounds in cultures of 2.2.15 cells was also determined. The 2.2.15 cells were grown to confluence in 96-well flat-bottomed tissue culture plates and treated with test compound (in 0.2 mL culture medium/well) as described above. Untreated control cultures were maintained on each 96-well plate. The toxicity was determined by measuring neutral red dye uptake, as determined from the absorbance at 510 nm relative to untreated cells, at 24 hours following 9 days of treatment.

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