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Synthesis and anti-hepatitis B virus activities of Matijing-Su derivatives

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ABSTRACT

A series of derivatives of Matijing-Su (MTS, *N*-(*N*-benzoyl-L-phenylalanyl)-*O*-acetyl-L-phenylalanol) was synthesized and evaluated for their anti-hepatitis B virus (HBV) activities in 2.2.15 cells. The IC_{50} of compounds **9c** (1.40 μM), **9g** (2.33 μM) and **9n** (2.36 μM), etc. and the selective index of **9n** (45.93) of the inhibition on the replication of HBV DNA were higher than those of the positive control lamivudine [41.59, (IC_{50}: 82.42 μM)]. Compounds **11d**, **12a** and **12e** also exhibited significant anti-HBV activities.

1. Introduction

Hepatitis B virus (HBV)-infected hepatitis is the most common infectious disease all over the world. More than 400 million people worldwide are infected by HBV.[1] Previous research results showed that HBV played a major role in occurrence and development of liver fibrosis[2,3] and hepatocelluar carcinoma.[4–6] Important advances in the anti-HBV agents have been made with the introduction of non(low)-toxic, efficacious nucleotide analogues that can interfere directly with HBV genomic DNA synthesis. Lamivudine (LMV), adefovir (ADV) and entecavir (ETV) are well-tolerated and may lead to viral suppression in the majority of treated patients while the therapy is maintained.[7–10] Interferon (IFN) alpha has both immunostimulatory and direct antiviral properties. Although various treatment options exist for chronic HBV infection, none is entirely satisfactory. The clinical application of these agents, particularly LMV, is limited by the frequent development of resistance, occurring at the rates of up to 15–30% per year.[11,12] For ADV, the development of resistance is slower, however, 28% of treated patients develop genotypic resistance by year 5.[13] Interferon (IFN) alpha is effective in only one-third of patients[14] and the treatment is greatly hampered by significant adverse effects.[15]

On the other hand, the natural products, with their enormous structural diversity, provide a large opportunity for screening anti-HBV agents possessing novel basic structural backbones and mechanisms of action. 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 in China. The anti-HBV activities of MTS were found in our previous studies.[16] Since the anti-HBV activities of the compounds with a dipeptide framework still have not been found in the literatures and patents before, these study results of MTS greatly encouraged us to make further research on it for discovery of more potent anti-HBV derivatives. In this paper, we report some results of synthesis of the new derivatives of MTS and evaluation of their anti-HBV activities.

2. Results

2.1. Synthetic approach

The synthesis of the key synthetic intermediates was described in Scheme 1.

The procedure for synthesis of the target compounds was illustrated in Scheme 2. The substituted benzoic acid was treated with thionyl chloride to give the substituted benzoyl chloride (**7**), which was then reacted with the substituted L-phenylalanine in a 2 M NaOH solution to give compound **8**. Compound **8** was reacted with isobutylchloroform (IBCF) and substituted L-phenylalanine methyl ester hydrochloride or substituted L-phenylalanol in the presence of *N*-methylmorpholine (NMM) to afford compound **9**. For the one route, hydrogenation of nitro group of compound **9** give compound **10,** which was followed by treatment with Ac_2O in pyridine at room temperature to provide compound **11.** At this stage, the target compound **12** was synthesized by hydrolysis of compounds **9** or **11** with NaOH in DMF/EtOH. Thus, the structures of the synthesized target compounds are listed in Table 1.

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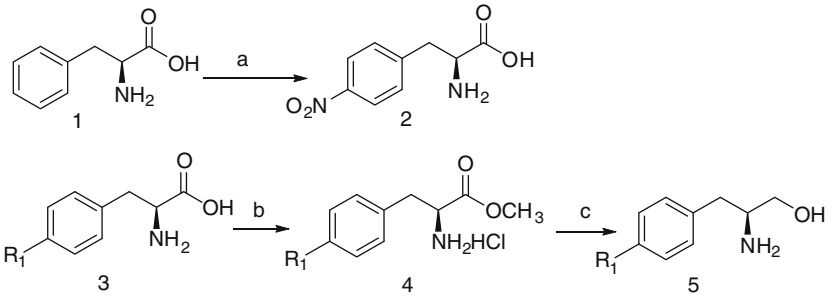
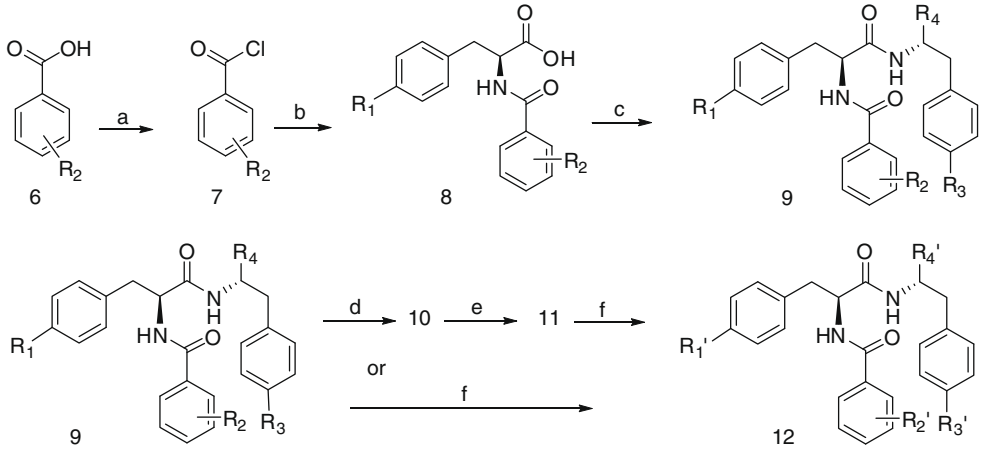


Scheme 1. Synthesis of the key intermediates. Reagents and conditions: (a) HNO_3/H_2SO_4 (1.4: 1.1), rt.; (b) MeOH, thionyl chloride ($SOCl_2$), rt, 2.5 h, then reflux for 30 min; (c) $NaBH_4/H_2O$.



Scheme 2. Synthesis of the target compounds. Reagents and conditions: (a) $SOCl_2$, reflux 30 min; (b) substituted L-phenylalanine, 2 M NaOH (aq), −5 °C to rt, 3 h; (c) substituted L-phenylalanine methyl ester hydrochloride or substituted L-phenylalanol, CH_2Cl_2, IBCF, NMM, −5 °C; (d) MeOH, H_2, Pd/C (10%), rt, 3 h; (e) Ac_2O, pyr, rt.; (f) NaOH, DMF/EtOH, rt.

2.2. Biological activity test

Hep G2.2.15 cell contained multiple copies of the HBV genome, which were stably integrated into the host cell genome and was widely used as a useful 'in vitro' model for evaluation of novel anti-HBV drugs. So, in the experiment, the Hep G2.2.15 cell line as in vitro cellular model was chosen. The results indicated that the derivatives of MTS could be potent HBV inhibitors (there were four derivatives which $IC_{50} < 4$ μM) with high selectivity indices (SI > 20).

The target compounds **9a–9r**, **11b–11d**, **12a–12g** were evaluated for their cytotoxicities and anti-HBV activities, namely the ability to inhibit the replication of HBV DNA and the production of HBsAg and HBeAg in HBV-infected 2.2.15 cells. The results of the inhibition for the replication of HBV DNA were summarized in Table 2.

As shown in Table 2, compound **9c** showed the most potence in vitro anti-HBV activity within all of the tested compounds. Its IC_{50} was 1.40 μM, which was about 59 times higher than that of the control lamivudine (82.42 μM). Compounds **9g**, **9n** and **12a** also exhibited significant inhibitory efficacy on the replication of HBV DNA (IC_{50}: 2.33, 2.36, 3.94 μM).

However, the inhibition for the production of HBsAg and HBeAg of the tested compounds did not be found in the examination.

3. Conclusion

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

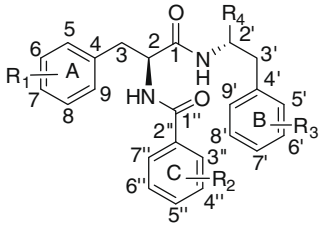
(a) In general, a series of derivatives of MTS could be potent HBV inhibitors (there were four derivatives which $IC_{50} < 4$ μM) with high selectivity indices (SI > 20), such as the IC_{50} of compounds **9c** (1.40 μM), **9g** (2.33 μM) and 9n (2.36 μM) and the selective index of **9n** [45.93] of the inhibition on the replication of HBV DNA were higher than those of the positive control lamivudine [41.59, (IC_{50}: 82.42 μM)].
(b) The substitution with deferent groups on the ring A, B and C of the derivatives of MTS could change the anti-HBV activities and cytotoxicities. The 5″-halide substituted on the benzoyl ring C was an important feature in conferring relatively potent inhibitory activity; but the 3″-halide substitution on the ring C might weaken the inhibitory activity.
(c) Both hydroxymethyl and methyl carboxylate substituted on the 2′-position derivatives might be the pharmacophores from the evaluation of their cytotoxicities and anti-HBV activities with potent HBV inhabitation.

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 performed on a Varian Unit INOVA-400 spectrometers in $CDCl_3$ or DMSO-d_6 with Me_4Si (TMS) as an internal standard. Mass spectra (MS) were recorded on a Agilent MS-5973 spectrometer.

Table 1
Structures of the synthesized target compounds **9a–9r**, **11b–11d**, **12a–12g**



No	R_1	R_2	R_3	R_4	Compound
1	H	H	H	Methyl carboxylate	**9a**
2	H	H	7′-Hydroxy	Methyl carboxylate	**9d**
3	H	H	7′-Nitro	Methyl carboxylate	**9o**
4	H	H	7′-Acetamido	Methyl carboxylate	**11c**
5	H	5″-Fluoro	H	Methyl carboxylate	**9c**
6	H	3″-Chloro	H	Methyl carboxylate	**9j**
7	H	4″-Methyl	H	Methyl carboxylate	**9i**
8	7-Nitro	H	H	Methyl carboxylate	**9l**
9	7-Acetamido	H	H	Methyl carboxylate	**11b**
10	H	3″-Chloro	7′-Hydroxy	Methyl carboxylate	**9k**
11	7-Nitro	H	7′-Hydroxy	Methyl carboxylate	**9n**
12	H	5″-Methyl	7′-Hydroxy	Methyl carboxylate	**9q**
13	7-Nitro	5″-Methyl	7′-Hydroxy	Methyl carboxylate	**9r**
14	7-Acetamido	5″-Methyl	7′-Acetoxy	Methyl carboxylate	**11d**
15	H	H	H	Hydroxymethyl	**9b**
16	H	H	7′-Nitro	Hydroxymethyl	**9p**
17	H	H	7′-Acetamido	Hydroxymethyl	**12b**
18	H	3″-Chloro	H	Hydroxymethyl	**9h**
19	H	5″-Chloro	H	Hydroxymethyl	**9g**
20	H	4″-Methyl	H	Hydroxymethyl	**9e**
21	H	5″-Methyl	H	Hydroxymethyl	**9m**
22	7-Nitro	H	H	Hydroxymethyl	**9f**
23	7-Acetamido	H	H	Hydroxymethyl	**12a**
24	H	H	H	Carboxy	**12e**
25	H	H	7′-Hydroxy	Carboxy	**12f**
26	H	H	7′-Acetamido	Carboxy	**12c**
27	H	H	7′-Propionamido	Carboxy	**12d**
28	7-Nitro	H	H	Carboxy	**12g**

4.1.1. Synthesis of some intermediates[17,18]

4.1.1.1. 4-Nitro-L-phenylalanine (2). To a solution of L-phenylalanine (16.5 g, 100 mmol) in H_2SO_4 (85%, 50 mL) was added dropwise a mixed acid of concentrated HNO_3 and concentrated H_2SO_4 (V/V = 1.4/1.1) under stirring, which was pre-prepared and cooled down to room temperature. The reaction mixture was stirred for 5 h at room temperature. The pH of the reaction solution was then adjusted to 2–3 with 40% NaOH and the resulting precipitate was collected by filtration and dried to give 4-nitro-L-phenylalanine (**2**) as a pale yellow powder (20 g, 95.2%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.18 (2H, d, J = 8.4 Hz, H-6, 8), 7.55 (2H, d, J = 8.0 Hz, H-5, 9), 4.01 (1H, m, H-3), 3.5(br, NH_2), 3.27-3.12, (2H, m, H-3). EI-MS m/z: 194 [M−H_2O]$^+$, 184, 177, 138, 124, 110, 79 (100), 67, 52, 42.

4.1.1.2. L-Phenylalanine methyl ester hydrochloride (4a). $SOCl_2$ (9.5 mL, 132 mmol) was added to 90 mL of MeOH, which was cooled in an ice-salt-bath. The mixture was warmed up to room temperature. L-Phenylalanine (16.5 g, 100 mmol) was then added and the mixture was stirred for 2.5 h at room temperature, then refluxed for 30 min. The solvent was removed and the residue was recrystallized from methanol/ether to afford L-phenylalanine methyl ester hydrochloride (**4a**) as colorless needles (18.67 g, 86.8%). EI-MS m/z: 179 [M−HCl]$^+$, 120, 103, 91, 88 (100), 77, 65.

4.1.1.3. 4-Nitro-L-phenylalanine methyl ester hydrochloride (4b). It was prepared with a similar procedure to that described for the synthesis of compound **4a**. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.7 (3H, br, –NH_2·HCl), 8.19 (2H, d, J = 8.4 Hz, H-6, 8), 7.55 (2H, d, J = 8.4 Hz, H-5, 9), 4.37 (1H, m, H-2), 3.67 (3H, s, OMe), 3.35–3.22 (4H, m, H-3). EI-MS m/z: 223 [M−HCl−1]$^+$, 165, 119, 88 (100), 78, 60, 43.

4.1.1.4. 4-Nitro-L-phenylalanol (5a). Compound **4b** (2.4 g, 9.2 mmol) was dissolved in water (15 mL), then the solution was slowly added to a stirred solution of $NaBH_4$ (1.13 g, 30 mmol in 15 mL water) in an ice-salt-bath. The resulting mixture was warmed up to room temperature over 2 h and kept overnight at room temperature. The reaction mixture was extracted with EtOAc (3 × 50 mL). The combined organic phase was successively washed with saturated aqueous $NaHCO_3$ (20 mL), brine (20 mL) and dried over Na_2SO_4. The solvent was evaporated in vacuo to afford 4-nitro-L-phenylalanol **(5a)** as a yellow powder (1.23 g, 68.2%). EI-MS m/z: 196 (M$^+$), 178, 165, 88 (100), 78, 60.

4.1.2. Synthesis of the target compounds

The synthetic procedures used in the preparation of target compounds are illustrated in Scheme 2. Substituted benzoic acid was reacted with thionyl chloride to give substituted benzoyl chloride (**7**), then compound **7** was treated with substituted L-phenylalanine in 2 M NaOH solution to give compound **8**, 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 **9**.

4.1.2.1. General procedure for the synthesis of compounds 9a–9r. Benzoic acid or substituted benzoic acid (20 mmol) was

Table 2
The results of the inhibition for the replication of HBV DNA of compounds **9a–9r**, **11b–11d**, **12a–12g**

No.	TC_{50} (μM)	DNA replication		Compound
		IC_{50} (μM)	SI	
1	2557.75	—	—	**9a**
2	1009.34	115.97	8.70	**9d**
3	96.09	—	—	**9o**
4	530.65	—	—	**11c**
5	39.71	1.40	28.27	**9c**
6	620.95	—	—	**9j**
7	540.79	—	—	**9i**
8	816.05	—	—	**9l**
9	925.54	122.01	7.59	**11b**
10	1039.72	—	—	**9k**
11	108.69	2.36	45.93	**9n**
12	1010.27	—	—	**9q**
13	89.57	—	—	**9r**
14	693.41	23.45	29.57	**11d**
15	574.21	—	—	**9b**
16	116.96	—	—	**9p**
17	164.22	—	—	**12b**
18	381.55	—	—	**9h**
19	65.78	2.33	28.29	**9g**
20	184.97	60.48	3.06	**9e**
21	1863.29	—	—	**9m**
22	119.37	—	—	**9f**
23	93.82	3.94	23.82	**12a**
24	313.47	25.35	12.36	**12e**
27	1151.20	—	—	**12d**
28	840.80	54.17	15.52	**12g**
MTS	120.18	11.16	10.78	**MTS**
3TC	3427.82	82.42	41.59	Lamivudine

dissolved in $SOCl_2$ (7.2 mL, 100 mL). The reaction was refluxed for 5 h and evaporated in vacuo to give the intermediate of benzoyl chloride or substituted benzoyl chloride **7**. Then, compound **7** (10.0 mmol) was added, without further purification, to a solution of L-phenylalanine or substituted L-phenylalanine (10.0 mmol) in 2 M NaOH (11 mL) in ice-salt-bath. The mixture was allowed to warm up to room temperature within 2 h. The resulting mixture was acidified to pH 5–6 with hydrochloric acid (1.8 mL, 21.6 mmol) and the resulting precipitate was collected by filtration and dried to give compound **8**. IBCF (1.07 mmol) was added dropwise to a mixture of compound **8** (1.0 mmol), substituted L-phenylalanine methyl ester hydrochloride or substituted L-phenylalanol (1.05 mmol) and NMM (23.0 mmol) in (50 mL) at 0 °C within 60–70 min. The mixture was stirred for 30 min and the bulk of CH_2Cl_2 was removed in vacuo. The residue was dissolved in EtOAc and washed sequentially with water, dilute HCl (40 mL), saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4 and evaporated in vacuo. The residue was recrystallized from ethyl acetate or appropriate solvents to afford target compound **9.**

4.1.2.2. *N*-(*N*-Benzoyl-L-phenylalanyl)-L-phenylalanine methyl ester (9a). Colorless needl crystal, yield 65.0%, mp 206.0–210.0 °C (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.68 (2H, d, J = 7.6 Hz, H-3″, 7″), 7.51 (1H, m, H-5″), 7.42 (2H, t, H-4″, 6″), 7.31–7.13 (8H, m, H-5, 9, 6, 8, 5′, 9′, 6′, 8′), 6.97 (2H, m, H-7, 7′), 6.76 (1H, d, J = 7.4 Hz, NHCO), 6.37 (1H, d, J = 7.6 Hz, NHCO), 4.85 (1H, m, H-2), 4.77 (1H, m, H-2′), 3.70 (3H, s, OMe), 3.23–2.94 (4H, m, H-3, 3′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 171.3 (C-1), 170.4 (C-1′), 167.0 (C-1″), 136.4 (C-4), 135.5 (C-4′), 133.6 (C-2″), 131.8 (C-5″), 129.4 (C-6, 8), 129.1 (C-6′, 8′), 128.7 (C-4″, 6″), 128.6 (C-5, 9), 128.5 (C-5′, 9′), 127.1 (C-3″, 7″), 127.0 (C-7, 7′), 54.5 (C-2), 53.4 (C-2′), 52.3 (C–COMe), 38.0 (C-3), 37.8 (C-3′). EI-MS *m/z*: 444 (M^+), 384, 353, 311, 269, 252, 224, 172, 131, 105 (100), 91, 77, 60. Anal. Calcd for $C_{26}H_{26}N_2O_4$: C, 72.54; H, 6.09; N, 6.51. Found: C, 72.63; H, 5.95; N, 6.36.

4.1.2.3. *N*-(*N*-Benzoyl-L-phenylalanyl)-L-phenylalanol (9b). Colorless needle crystal, yield 58.0%, mp 168.0–171.0 °C (MeOH), ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.72 (2H, d, J = 8.0 Hz, H-3″, 7″), 7.53 (1H, t, J = 7.2 Hz, H-5″), 7.44 (2H, t, H-4″, 6″), 7.35–7.07 (10H, m, H-5-9, 5′-9′), 6.80 (1H, d, J = 7.6 Hz, NHCO), 5.92 (1H, d, J = 7.6 Hz, NHCO), 4.77 (1H, m, H-2), 4.10 (1H, m, H-2′), 3.42 (2H, m, H-1′), 3.27 (1H, dd, J = 5.6, 13.6 Hz, H-3a), 3.04 (1H, dd, J = 9.2, 13.6 Hz, H-3b), 2.77 (1H, dd, J = 7.6, 13.6 Hz, H-3′a), 2.68 (1H, dd, J = 6.8, 13.6 Hz, H-3′b); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 170.9 (C-1), 166.1 (C-1″), 139.0 (C-4), 138.4 (C-4′), 134.1 (C-2″), 131.3 (C-5″), 129.2 (C-6, 8, 6′, 8′), 128.2 (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), 62.2 (C-1′), 54.8 (C-2), 52.5 (C-2′), 37.3 (C-3), 36.5 (C-3′). EI-MS *m/z*: 402 (M^+), 384, 311, 252, 224 (100), 92. Anal. Calcd for $C_{25}H_{26}N_2O_3$: C, 74.60; H, 6.51; N, 6.96. Found: C, 74.40; H, 6.90; N, 6.83.

4.1.2.4. *N*-(*N*-5″-Fluoro-benzoyl-L-phenylalanyl)-L-phenylalanine methyl ester (9c). Mp 194.0–196.0 °C (EtOAc), ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.69–7.72 (2H, m, H-3″, 7″), 7.07–7.28 (10H, m, H-5-9, 5′-9′), 6.96–6.98 (2H, m, H-4″, 6″), 6.80 (1H, d, J = 7.6 Hz, NH), 6.34 (1H, d, J = 7.6 Hz, NH), 4.76–4.84 (2H, m, H-2, 2′), 3.70 (3H, s, OCH_3), 2.98–3.18 (4H, m, H-3, 3′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 171.24 (s), 170.41 (s), 165.97 (2 × s), 136.28 (s), 135.44 (s), 129.47 (s), 129.38 (4 × d), 129.11 (2 × d), 128.69 (2 × d), 128.55 (2 × d), 127.11 (2 × d), 115.74 (d), 115.52 (d), 54.56 (d), 53.44 (d), 52.38 (q), 38.13 (t), 37.82 (t). EI-MS *m/z*: 448 (M^+), 416, 389, 357, 309, 286, 270, 242, 218, 180, 162, 147, 131, 123 (100), 120, 95, 91, 77, 41, 28, 15. Anal. Calcd for $C_{26}H_{25}FN_2O_4$: C, 69.63; H, 5.62; N, 6.25. Found: C, 69.43; H, 5.98; N, 6.02.

4.1.2.5. *N*-(*N*-Benzoyl-L-phenylalanyl)-L-tyrosine methyl ester (9d). White crystal, mp 184.0–186.0 °C (MeOH/EtOAc); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.18 (1H, s, Ar-OH), 8.52 (1H, d, J = 8.4 Hz, NHCO), 8.40 (1H, d, J = 7.6 Hz, NHCO), 7.77 (2H, d, J = 7.6 Hz, H-3″, 7″), 7.50 (1H, t, H-5″), 7.42 (2H, t, H-4″, 6″), 7.33 (2H, d, J = 7.2 Hz, H-5, 9), 7.24 (2H, t, H-6, 8), 7.16 (1H, t, H-7), 6.99 (2H, d, J = 8.4 Hz, H-5′, 9′), 6.62 (2H, d, J = 8.4 Hz, H-6′, 8′), 4.74 (1H, m, H-2), 4.45 (1H, m, H-2′), 3.60 (3H, s, OMe), 3.11–2.84 (4H, m, H-3, 3′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 171.8 (C-1), 171.5 (C-1′), 166.2 (C-1″), 156.0 (C-7′), 138.2 (C-4), 134.0 (C-2″), 131.1 (C-5″), 129.9 (C-5′, 9′), 129.1 (C-6, 8), 128.0 (×2), 127.9 (×2), 127.3 (C-3″, 7″), 126.8 (C-7), 126.1 (C-4′), 115.0 (C-6′, 8′), 54.4 (C-2), 53.9 (C-2′), 51.7 (C–OMe), 36.9 (C-3), 35.9 (C-3′). EI-MS *m/z*: 446 (M^+), 269, 252, 224, 178, 105 (100), 91, 77. Anal. Calcd for $C_{26}H_{26}N_2O_5$: C, 69.94; H, 5.87; N, 6.27. Found: C, 70.34; H, 5.93; N, 6.17.

4.1.2.6. *N*-(*N*-4″-Methyl-benzoyl-L-phenylalanyl)-L-phenylalanol (9e). White powder, mp 177.0–178.5 °C (MeOH); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.44 (1H, d, J = 8.4 Hz, NHCO), 7.89 (1H, d, J = 8.4 Hz, NHCO), 7.59–7.56 (2H, m, H-3″, 7″), 7.32–7.10 (12H, m, H-4″, 5″, H-5-9, 5′-9′), 4.83 (1H, t, CH_2OH), 4.67 (1H, m, H-2), 3.88 (1H, m, H-2′), 3.33–3.23 (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), 2.33 (3H, s, Ar-CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 171.0 (C-1), 166.2 (C-1″), 139.0 (C-4′), 138.5 (C-4), 137.5 (C-4″), 134.1 (C-2″), 131.9 (C-5″), 129.3 (×3, C-6′, 8′, 6″), 128.13 (×4, C-5′, 9′, C-6, 8), 128.07 (×2, C-5, 9), 128.0 (C-3″), 126.2 (C-7′), 126.0 (C-7), 124.6 (C-7″), 62.2 (C-1′), 54.8 (C-2), 52.5 (C-2′), 33.3, 36.5, 21.0 (ArCH_3). EI-MS *m/z*: 416 (M^+), 398, 386, 325, 283, 266, 238, 190, 131, 119 (100), 104, 91. Anal. Calcd for $C_{26}H_{28}N_2O_3$: C, 74.97; H, 6.78; N, 6.73. Found: C, 75.15; H, 6.87; N, 6.51.

4.1.2.7. *N*-(*N*-Benzoyl-7-nitro-L-phenylalanyl)-L-phenylalanol (9f). Pale yellow powder, mp 196.0–199.0 °C (MeOH); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.62 (1H, d, J = 8.4 Hz, NHCO), 8.12 (2H, d, J = 8.8 Hz, H-6, 8), 8.05 (1H, d, J = 8.8 Hz, NHCO), 7.78 (2H, d, J = 6.4 Hz, H-3″, 7″), 7.59 (2H, d, J = 8.4 Hz, H-5, 9), 7.51 (1H, t, H-

5″), 7.43 (2H, t, H-4″, 6″), 7.26–7.12 (5H, m, H-5′-9′), 4.85 (1H, t, OH), 4.76 (1H, m, H-2), 3.89 (1H, m, H-2′), 3.32–3.26 (2H, m, H-1′), 3.19–3.07 (2H, m, H-3), 2.92–2.81 (2H, m, H-3′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 170.5 (C-1), 166.1 (C-1″), 145.0 (C-7), 146.2 (C-4), 139.0 (C-4′), 133.8 (C-2″), 131.4 (C-5″), 130.5 (C-5, 9), 129.2 (C-6′, 8′), 128.2 (C-4″, 6″), 128.1 (C-5′, 9′), 127.4 (C-3″, 7″), 125.9 (C-7′), 123.2 (C-6, 8), 62.2 (C-1′), 54.3 (C-2′), 52.6 (C-2), 37.1 (C-3′), 34.9 (C-3). EI-MS *m/z*: 447 (M^+), 417, 356, 314, 297, 269, 134, 120, 105 (100), 91, 77, 60. Anal. Calcd for $C_{25}H_{25}N_3O_5$: C, 67.10; H, 5.63; N, 9.39. Found: C, 67.53; H, 5.89; N, 9.17.

4.1.2.8. *N*-(*N*-5″-Chloro-benzoyl-L-phenylalanyl)-L-phenylalanol (9g). Mp 211.5–214.0 °C (MeOH), ^{1}H NMR (DMSO-d_6, 400 Mz) δ: 8.63 (1H, d, *J* = 8.4 Hz, NHCO), 7.93 (1H, d, *J* = 8.0 Hz, NHCO), 7.81 (2H, d, *J* = 8.0 Hz, H-3″, 7″), 7.51 (2H, d, *J* = 8.0 Hz, H-4″, 6″), 7.12–7.31 (10H, m, H-5-9, 5′-9′), 4.82 (1H, s, OH), 4.68 (H, m, H-2), 3.90 (1H, m, H-2′), 3.29–3.39 (2H, m, H-1′), 2.49–3.01 (4H, m, H-3, 3′), ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 170.9 (C-1), 165.1 (C-1″), 139.0 (C-4), 138.1 (C-4′), 136.2 (C-5″), 132.4 (C-2″), 129.4 (×2), 129.2 (×2), 128.3 (×2), 128.09 (×2), 128.07 (×2), 126.2, 125.9, 62.2 (C-1′), 54.9 (C-2), 52.5 (C-2′), 37.3 (C-3), 36.4 (C-3′). EI-MS *m/z*: 436 (M^+), 406, 345, 327, 303, 286, 258, 190, 139 (100), 120, 111, 104, 91, 73, 57, 43, 28. Anal. Calcd for $C_{25}H_{25}ClN_2O_3$: C, 68.72; H, 5.77; N, 6.41. Found: C, 69.21; H, 6.01; N, 6.08.

4.1.2.9. *N*-(*N*-3″-Chloro-benzoyl-L-phenylalanyl)-L-phenylalanol (9h). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.15–7.40 (14H, m, H-5-9, 5-9′, 4″-7″), 4.59–4.82 (1H, m, H-2), 4.07–4.10 (1H, m, H-2′), 3.41–3.42 (2H, m, 1′), 2.71–3.17 (4H, m, H-3, 3′), ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 172.81 (s), 169.53 (s), 139.61 (s), 138.42 (s), 137.02 (s), 132.27 (d), 131.96 (s), 130.92 (d), 130.45 (2 × d), 130.43 (2 × d), 130.01 (d), 129.46 (2 × d), 129.42 (2 × d), 127.96 (d), 127.80 (d), 127.33 (d), 63.73 (t), 56.55 (d), 54.14 (d), 38.93 (t), 37.87 (t). EI-MS *m/z*: 436 (M^+), 418, 406, 345, 327, 303, 286, 258, 190, 139 (100), 120, 104, 91, 73, 60, 43, 28, 18. Anal. Calcd for $C_{25}H_{25}ClN_2O_3$: C, 68.72; H, 5.77; N, 6.41. Found: C, 69.03; H, 5.80; N, 6.26.

4.1.2.10. *N*-(*N*-4″-Methyl-benzoyl-L-phenylalanyl)-L-phenylalanine methyl ester (9i). Mp: 190.0–192.0 °C (EtOAc), ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.51 (1H, s, H-3″), 7.46 (1H, d, *J* = 4.4 Hz, H-7″), 7.31–7.23 (6H, m, H-5″, 6″, H-6, 8, 6′, 8′), 7.16–7.11 (4H, m, H-5, 9, H-5′, 9′), 6.99–6.95 (2H, m, H-7, 7′), 6.68 (1H, d, *J* = 8.0 Hz, NHCO), 7.89 (1H, d, *J* = 7.4 Hz, NHCO), 4.80 (2H, m, H-2, 2′), 3.70 (3H, s, OCH_3), 3.23–2.93 (4H, m, H-3, 3′), 2.39 (3H, s, Ar-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 171.2 (C-1), 170.3 (C-1′), 167.2 (C-1″), 138.4 (C-4″), 136.4 (C-4), 135.5 (C-4′), 133.5 (C-2″), 132.6 (C-5″), 129.4 (×2), 129.1 (×2), 128.7 (×2), 128.5 (×2), 128.4 (C-6″), 127.7 (C-3″), 127.0 (×2, C-7, 7′), 124.0 (C-7″), 54.4 (C-2), 53.4 (C-2′), 52.4 (OCH_3), 38.0, 37.8. EI-MS *m/z*: 444 (M^+), 353, 309, 266, 238, 147, 119 (100), 104, 91. Anal. Calcd for $C_{27}H_{28}N_2O_4$: C, 72.95; H, 6.35; N, 6.30. Found: C, 73.16; H, 6.94; N, 6.14.

4.1.2.11. *N*-(*N*-3″-Chloro-benzoyl-L-phenylalanyl)-L-phenylalanine methyl ester (9j). Mp 152.5–153.5 °C (EtOAc), ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.51 (1H, d, *J* = 8.0 Hz, H-7″), 7.35–7.34 (2H, m, H-4″, 5″), 7.30–7.17 (9H, m, H-5, 6, 8, 9, 5′, 6′, 8′, 9′, 6″), 6.97–6.95 (2H, m, H-7, 7′), 6.75 (1H, d, *J* = 7.6 Hz, NHCO), 6.30 (1H, d, *J* = 7.6 Hz, NH), 4.85 (1H, m, H-2), 4.78 (1H, m, H-2), 3.67 (3H, s, OCH_3), 3.20–3.00 (4H, m, H-3, 3′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 171.2, 169.9, 166.1, 136.2, 135.5, 134.1, 131.6, 130.8, 130.3, 130.2, 129.4 (×2), 129.2 (×2), 128.7 (×2), 128.6 (×2), 127.14, 127.12, 127.0, 54.9, 53.4, 52.3, 37.9, 37.8. EI-MS *m/z*: 464 (M^+), 432, 373, 309, 302, 286, 258, 218, 180, 162, 147, 139 (100), 131, 120, 112, 102, 97, 91, 83, 73, 60, 44, 28. Anal. Calcd for $C_{26}H_{25}ClN_2O_4$: C, 67.17; H, 5.42; N, 6.03. Found: C, 66.89; H, 5.19; N, 6.22.

4.1.2.12. *N*-(*N*-3″-Chloro-benzoyl-L-phenylalanyl)-L-tyrosine methyl ester (9k). Mp 164.5–167.0 °C (EtOAc), ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.27 (1H, s, Ar-OH), 8.60 (1H, d, *J* = 8.4 Hz, NHCO), 8.40 (1H, d, *J* = 7.2 Hz, NHCO), 7.42–7.14 (9H, m, H-4″-7″, H-5-9), 7.03 (2H, d, *J* = 8.4 Hz, H-5′, 9′), 6.67 (2H, d, *J* = 8.4 Hz, H-6′, 8′), 4.75 (1H, m, H-2), 4.46 (1H, m, H-2′), 3.08–2.79 (4H, m, H-3, 3′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 171.9 (C-1), 171.1 (C-1′), 166.0 (C-1″), 156.1 (C-7′), 137.8 (C-4), 136.4 (C-5″), 130.8 (C-3″), 130.1 (C-5′, 9′), 130.0 (C-2″), 129.2 (4″), 129.2 (C-6, 8), 128.9 (7″), 128.0 (C-5, 9), 126.9 (C-6″, C-4′), 126.3 (C-7), 115.1 (C-6′, 8′), 54.0 (C-2), 51.8 (C-2′), 37.2, 36.0. EI-MS *m/z*: 480 (M^+), 303, 286, 258, 178, 139 (100), 107, 91, 77. Anal. Calcd for $C_{26}H_{25}ClN_2O_5$: C, 64.93; H, 5.24; N, 5.82. Found: C, 65.28; H, 5.66; N, 5.61.

4.1.2.13. *N*-(*N*-Benzoyl-7-nitro-L-phenylalanyl)-L-phenylalanine methyl ester (9l). Mp 202.0–204.5 °C (MeOH), ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.09 (2H, d, *J* = 8.8 Hz, H-6, 8), 7.71 (2H, d, *J* = 7.6 Hz, H-3″, 7″), 7.54 (1H, t, H-5″), 7.43 (2H, t, H-4″, 6″), 7.38 (2H, d, *J* = 8.8 Hz, H-5, 9), 7.13 (3H, m, H-6′-8′), 6.99 (2H, m, H-5′, 9′), 6.93 (1H, br, NHCO), 6.56 (1H, br, NHCO), 4.94 (1H, m, H-2), 4.80 (1H, m, H-2′), 3.73 (3H, s, OCH_3), 3.29 (1H, dd, *J* = 7.2, 13.6 Hz, H-3′a), 3.22 (1H, dd, *J* = 5.6, 14.0 Hz, H-3′b), 3.09 (1H, dd, *J* = 5.6, 14.0 Hz, H-3a), 3.01 (1H, dd, *J* = 6.4, 14.0 Hz, H-3b); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 171.3 (C-1), 169.8 (C-1′), 167.1 (C-1″), 147.0 (C-7), 144.1 (C-4), 135.2 (C-4′), 133.1 (C-2″), 132.2 (C-5″), 130.3 (C-5, 9), 129.0 (C-6′, 8′), 128.7 (C-5′, 9′), 128.6 (C-4″, 6″), 127.2 (C-7′), 127.0 (C-3″, 7″), 123.7 (C-6, 8), 54.0 (C-2), 53.2 (C-2′), 52.5 (OCH_3), 38.0, 37.8. EI-MS *m/z*: 475 (M^+), 339, 297, 269, 162, 120, 105 (100), 91, 77. Anal. Calcd for $C_{26}H_{25}N_3O_6$: C, 65.67; H, 5.30; N, 8.84. Found: C, 65.87; H, 5.49; N, 8.58.

4.1.2.14. *N*-(*N*-5″-Methyl-benzoyl-L-phenylalanyl)-L-phenylalanol (9m). Mp 200.0–202.0 °C (EtOAc), ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.39 (1H, d, *J* = 8.8 Hz, NHCO), 7.86 (1H, d, *J* = 8.8 Hz, NHCO), 7.68 (2H, d, *J* = 8.0 Hz, H-3″, 7″), 7.29–7.11 (12H, m, H-4″, 6″, H-5′-9′, 5-9), 4.81 (1H, t, OH), 4.65 (1H, m, H-2), 3.88 (1H, m, H-2′), 3.33–3.24 (2H, m, H-1′), 3.03–2.90 (2H, m, H-3), 2.84 (1H, dd, *J* = 6.0, 13.6 Hz, H-3′a), 2.65 (1H, dd, *J* = 8.0, 13.6 Hz, H-3′b), 2.33 (3H, s, Ar-CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 171.0 (C-1), 166.0 (C-1″), 141.2 (C-5″), 139.0 (C-4′), 138.4 (C-4), 131.3 (C-2″), 129.2 (×4, C-6, 8, C-6′, 8′), 128.7 (C-4″, 6″), 128.1 (C-5′, 9′), 128.0 (C-5, 9), 127.5 (C-3″, 7″), 126.2 (C-7), 125.9 (C-7′), 62.2 (C-1′), 54.8 (C-2′), 52.5 (C-2), 37.3, 36.4, 21.0 (Ar-CH_3). EI-MS *m/z*: 432 (M^+), 414, 341, 297, 282, 254, 206, 147, 136, 119 (100), 107, 91, 77, 57. Anal. Calcd for $C_{26}H_{28}N_2O_3$: C, 74.97; H, 6.78; N, 6.73. Found: C, 74.93; H, 7.14; N, 6.56.

4.1.2.15. *N*-(*N*-Benzoyl-7-nitro-L-phenylalanyl)-L-tyrosine methyl ester (9n). Mp 200.0–202.0 °C (MeOH/EtOAc), ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.27 (1H, s, Ar-OH), 8.67 (1H, d, *J* = 8.4 Hz, NHCO), 8.57 (1H, d, *J* = 7.2 Hz, NHCO), 8.13 (2H, d, *J* = 8.4 Hz, H-6, 8), 7.76 (2H, d, *J* = 7.2 Hz, H-3″, 7″), 7.62 (2H, d, *J* = 8.4 Hz, H-5, 9), 7.51 (1H, t, H-5″), 7.43 (2H, t, H-4″, 6″), 7.00 (2H, d, *J* = 8.0 Hz, H-5′, 9′), 6.63 (2H, d, *J* = 8.0 Hz, H-6′, 8′), 4.83 (1H, m, H-2), 4.42 (1H, m, H-2′), 3.57 (3H, s, OMe), 3.22–2.84 (4H, m, H-3, 3′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 171.9 (C-1), 171.2 (C-1′), 166.3 (C-1″), 156.1 (C-7′), 146.8 (C-7), 146.2 (C-4), 133.8 (C-2″), 131.4 (C-5″), 130.6 (C-5, 9), 130.4 (C-4′), 130.1 (C-5′, 9′), 128.3 (C-4″, 6″), 127.4 (C-3″, 7″), 122.2 (C-6, 8), 115.1 (C-6′, 8′), 54.2 (C-2), 50.0 (C-2′), 51.9 (C-OMe), 36.8 (C-3), 35.9 (C-3′). EI-MS *m/z*: 491 (M^+), 385, 314, 297, 269, 178, 105 (100), 91, 77, 51. Anal. Calcd for $C_{26}H_{25}N_3O_7$: C, 63.54; H, 5.13; N, 8.55. Found: C, 63.88; H, 5.34; N, 8.19.

4.1.2.16. *N*-(*N*-Benzoyl-L-phenylalanyl)-7′-nitro-L-phenylalanine methyl ester (9o). Mp 220.0–221.5 °C (MeOH/EtOAc), ^{1}H NMR

(DMSO-d_6, 400 MHz) δ: 8.57 (1H, d, J = 8.0 Hz, NHCO), 8.52 (1H, d, J = 8.8 Hz, NHCO), 8.04 (2H, d, J = 8.4 Hz, H-6′, 8′), 7.73 (2H, d, J = 7.6 Hz, H-3″, 7″), 7.49 (3H, m, H-5″, H-5′, 9′), 7.41 (2H, t, 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, H-7), 4.70 (1H, m, H-2), 4.62 (1H, m, H-2′), 3.61 (3H, s, OCH_3), 3.25–2.90 (4H, m, H-3, 3′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 171.6 (C-1), 171.4 (C-1′), 166.2 (C-1″), 146.3 (C-7′), 145.5 (C-4′), 138.3 (C-4), 133.9 (C-2″), 131.4 (C-5″), 130.6 (C-5′, 9′), 129.2 (C-6, 8), 128.2 (×2), 128.1 (×2), 127.4 (C-3″, 7″), 126.3 (C-7′), 123.2 (C-6′, 8′), 54.5 (C-2), 52.9 (C-2′), 52.1 (OCH_3), 36.8, 36.2. EI-MS m/z: 475 (M^+), 354, 252, 224, 177, 131, 105 (100), 91, 77, 51. Anal. Calcd for $C_{26}H_{25}N_3O_6$: C, 65.67; H, 5.30; N, 8.84. Found: C, 65.36; H, 5.16; N, 9.03.

4.1.2.17. *N*-(*N*-Benzoyl-L-phenylalanyl)-7′-nitro-L-phenylalanol (9p). Mp 192.0–195.0 °C (MeOH/EtOAc), ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.46 (1H, d, J = 8.4 Hz, NHCO), 7.97 (2H, d, J = 8.0 Hz, H-6′, 8′), 7.92 (1H, d, J = 8.8 Hz, NHCO), 7.76 (2H, d, J = 8.0 Hz, H-3″, 7″), 7.52–7.40 (5H, m, H-4″-6″, H-5′, 9′), 7.29 (2H, d, J = 7.2 Hz, H-5, 9), 7.23 (2H, t, H-6, 8), 7.14 (1H, t, H-7), 4.89 (1H, t, OH), 4.63 (1H, m, H-2), 4.97 (1H, m, H-2′), 3.36–3.27 (2H, m, H-1′), 3.05–2.72 (4H, m, H-3, 3′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 171.0 (C-1), 166.1 (C-1″), 147.6 (C-7′), 145.8 (C-4′), 138.4 (C-4), 133.9 (C-2″), 131.4 (C-5″), 130.5 (C-5′, 9′), 129.2 (C-6, 8), 128.2 (×2), 128.0 (×2), 127.3 (C-3″, 7″), 126.2 (C-7′), 123.0 (C-6′, 8′), 62.6 (C-1′)54.8 (C-2), 51.9 (C-2′), 37.0, 36.4. EI-MS m/z: 447 (M^+), 429, 252, 224, 190, 131, 105 (100), 91, 77, 51. Anal. Calcd for $C_{25}H_{25}N_3O_5$: C, 67.10; H, 5.63; N, 9.39. Found: C, 67.51; H, 5.88; N, 9.02.

4.1.2.18. *N*-(*N*-5″-Methyl-benzoyl-L-phenylalanyl)-L-tyrosine methyl ester (9q). Mp 207.0–208.5 °C (EtOAc), ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.24 (1H, s, Ar-OH), 8.45 (2H, m, NHCO × 2), 7.68 (2H, d, J = 8.0 Hz, H-3″, 7″), 7.33 (2H, d, J = 7.2 Hz, H-5, 9), 7.22 (4H, m, H-4″, 6″, H-6, 8), 7.14 (1H, t, H-7), 7.00 (2H, d, J = 8.0 Hz, H-5′, 9′), 6.63 (2H, d, J = 8.0 Hz, H-6′, 8′), 4.73 (1H, m, H-2), 4.42 (1H, m, H-2′), 3.57 (3H, s, OCH_3), 3.08–2.87 (4H, m, H-3, 3′), 2.32 (3H, s, Ar-CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 172.0, 171.7, 166.1 (C-1″), 156.1 (C-7′), 141.2 (C-5″), 138.4 (C-4), 131.2 (C-2″), 130.1 (C-5′, 9′), 129.2 (C-6, 8), 128.7 (C-4″, 6″), 128.0 (C-5, 9), 127.4 (C-3″, 7″), 127.0 (C-4′), 126.2 (C-7), 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′), 21.0 (Ar-CH_3). EI-MS m/z: 460 (M^+), 283, 266, 238, 119 (100), 91. Anal. Calcd for $C_{27}H_{28}N_2O_5$: C, 70.42; H, 6.13; N, 6.08. Found: C, 70.43; H, 6.76; N, 5.89.

4.1.2.19. *N*-(*N*-5″-Methyl-benzoyl-7-nitro-L-phenylalanyl)-L-tyrosine methyl ester (9r). Mp 213.0–215.0 °C (MeOH/EtOAc), ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.31 (1H, s, Ar-OH), 8.61–8.57 (2H, m, NHCO × 2), 8.12 (2H, d, J = 8.8 Hz, H-6, 8), 7.67 (2H, d, J = 7.6 Hz, H-3″, 7″), 7.60 (2H, d, J = 8.8 Hz, H-5, 9), 7.23 (2H, d, J = 8.0 Hz, H-4″, 6″), 7.00 (2H, d, J = 8.4 Hz, H-5′, 9′), 6.63 (2H, d, J = 8.0 Hz, H-6′, 8′), 4.81 (1H, m, H-2), 4.41 (1H, m, H-2′), 3.56 (3H, s, OCH_3), 3.21–3.06 (2H, m, H-3′), 2.94–2.84 (2H, m, H-3), 2.32 (3H, s, Ar-CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 172.0 (C-1), 171.3 (C-1′), 166.2 (C-1″), 156.1 (C-7′), 146.9 (C-7), 146.2 (C-4), 141.3 (C-5″), 131.0 (C-2″), 130.6 (C-5, 9), 130.1 (C-5′, 9′), 128.8 (C-4″, 6″), 127.4 (C-3″, 7″), 126.9 (C-4′), 123.2 (C-6, 8), 115.1 (C-6′, 8′), 54.2, 53.9, 51.9 (OCH_3), 36.8, 35.9, 21.0 (Ar-CH_3). EI-MS m/z: 505 (M^+), 328, 311, 174, 119 (100), 106, 91, 78, 65. Anal. Calcd for $C_{27}H_{27}N_3O_7$: C, 64.15; H, 5.38; N, 8.31. Found: C, 63.96; H, 5.64; N, 8.06.

4.1.2.20. *N*-(*N*-Benzoyl-7-acetamido-L-phenylalanyl)-L-phenylalanol (12a). A solution of compound **9f** (1.788 g, 4 mmol) in MeOH (100 mL) was vigorously stirred overnight under an atmosphere of hydrogen in presence of Pd/C (20%, 21 mg) at room temperature. The catalyst was removed by filtration and the solvent was evaporated under reduced pressure to give the compound **10a**, *N-(N-benzoyl-7-amino-L-phenylalanyl)-L-phenylalanol* (1.876 g), as a white powder. Without further purification, the compound **10a** (469 mg, <1.0 mmol) was dissolved in pyridine (5 mL), then Ac_2O (2 mL) was added, After stirred at room temperature for 5 h, the reaction was quenched with EtOH (5 mL) and concentrated in vacuo. The residue was dissolved in EtOAc (250 mL) and washed sequentially with water, saturated $NaHCO_3$ solution, and brine. The solution was dried and concentrated, the residue was recrystallized from EtOAc to afford *N-(N-benzoyl-7-acetamido-L-phenylalanyl)-O-acetyl-L-phenylalanol* (**11a**) as white powder (300 mg, 60.0%). Mp 233.5–235.5 °C (EtOAc), ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.84 (1H, s, ArNHCO), 8.46 (1H, d, J = 8.4 Hz, NHCO), 7.89 (1H, d, J = 8.0 Hz, NHCO), 7.79 (2H, d, J = 7.6 Hz, H-3″, 7″), 7.52 (1H, t, H-5″), 7.47–7.42 (4H, m, H-4″, 6″, H-6, 8), 7.23–7.11 (7H, m, H-5′-9′, H-5, 9), 4.83 (1H, t, OH), 4.64 (1H, m, H-2), 3.90 (1H, m, H-2′), 3.34–3.25 (2H, m, H-1′), 3.00–2.64 (4H, m, H-3, 3′), 1.99 (3H, CH_3CO); ^{13}C-NMR (DMSO, 100 MHz) δ: 170.9 (C-1), 168.0 (ArNHCOCH_3), 166.0 (C-1″), 139.0 (C-7), 137.5 (C-4′), 134.1 (C-2″), 132.9 (C-4), 131.3 (C-5″), 129.4 (C-5, 9), 129.2 (C-6′, 8′), 128.2 (×2), 128.1 (×2), 127.4 (C-3″, 7″), 125.9 (C-7′), 118.6 (C-6, 8), 62.2 (C-1′), 54.9 (C-2), 52.5 (C-2′), 36.7 (C-3′), 36.4 (C-3), 24.0 (NHCOCH_3). EI-MS m/z: 501 (M^+), 380, 309, 281, 229, 203, 188, 148, 105 (100), 91, 77. Anal. Calcd for $C_{29}H_{31}N_3O_5$: C, 69.44; H, 6.23; N, 8.38. Found: C, 69.86; H, 6.45; N, 8.27. Then, 2.0 mL NaOH (5 M) was added to a solution of compound **11a** (1.003 g, 2.0 mmol) in dry DMF (5 mL) and EtOH (10 mL). After stirring at room temperature for overnight the reacted mixture was acidified to pH 2–3 with concentrated hydrochloric acid and partitioned between EtOAc (150 mL) and water (50 mL). The organic phase was combined and washed successively with dilute HCl (40 mL), brine (2 × 30 mL), dried and removed the solvent. The residue was recrystallized from EtOAc to afford target compound **12a** as white needles (680 mg, 74.0%). mp 233.5–235.5 °C (EtOAc), ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.84 (1H, s, ArNHCO), 8.46 (1H, d, J = 8.4 Hz, NHCO), 7.89 (1H, d, J = 8.0 Hz, NHCO), 7.79 (2H, d, J = 7.6 Hz, H-3″, 7″), 7.52 (1H, t, H-5″), 7.47–7.42 (4H, m, H-4″, 6″, H-6, 8), 7.23–7.11 (7H, m, H-5′-9′, H-5, 9), 4.83 (1H, t, OH), 4.64 (1H, m, H-2), 3.90 (1H, m, H-2′), 3.34–3.25 (2H, m, H-1′), 3.00–2.64 (4H, m, H-3, 3′), 1.99 (3H, CH_3CO); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 170.9 (C-1), 168.0 (ArNHCOCH_3), 166.0 (C-1″), 139.0 (C-7), 137.5 (C-4′), 134.1 (C-2″), 132.9 (C-4), 131.3 (C-5″), 129.4 (C-5, 9), 129.2 (C-6′, 8′), 128.2 (×2), 128.1 (×2), 127.4 (C-3″, 7″), 125.9 (C-7′), 118.6 (C-6, 8), 62.2 (C-1′), 54.9 (C-2), 52.5 (C-2′), 36.7 (C-3′), 36.4 (C-3), 24.0 (NHCOCH_3). EI-MS m/z: 459 (M^+), 441, 338, 309, 281, 229, 204, 188, 148, 105 (100), 91, 77. Anal. Calcd for $C_{27}H_{29}N_3O_4$: C, 70.57; H, 6.36; N, 9.14. Found: C, 70.08; H, 6.18; N, 8.68.

4.1.2.21. By a similar synthetic method described above for compound 12a (from compound 9f), the compounds of 12b (from 9p), 12c (from 9o), 12d (from 9o) was synthesized.

*4.1.2.21.1. **N**-(**N**-Benzoyl-L-phenylalanyl)-7′-acetamido-L-phenylalanol (**12b**).* Mp 235.5–238.0 °C (MeOH), ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.85 (1H, s, ArNHCO), 8.54 (1H, d, J = 8.0 Hz, NHCO), 7.95 (1H, d, J = 8.4 Hz, NHCO), 7.77 (2H, d, J = 7.6 Hz, H-3″, 7″), 7.50 (1H, t, H-5″), 7.44–7.40 (4H, m, H-4″-6″, 6′, 8′), 7.31 (2H, d, J = 7.6 Hz, H-5, 9), 7.23 (1H, t, H-6, 8), 7.15–7.12 (3H, m, H-7, 5′, 9′), 4.84 (1H, t, OH), 4.67 (1H, m, H-2), 3.86 (1H, m, H-2′), 3.34–3.27 (2H, m, H-1′), 3.05–2.92 (2H, m, H-3), 2.79 (1H, dd, J = 6.0, 13.2 Hz, H-3′a), 2.61 (1H, dd, J = 7.6, 13.2 Hz, H-3′b), 2.00 (3H, s, $COCH_3$); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 171.0 (C-1), 168.1 (ArNHCO), 166.2 (C-1″), 138.5 (C-4), 137.4 (C-7′), 134.1 (C-2″), 133.5 (C-4′), 131.3 (C-5″), 129.4 (C-5′, 9′), 129.2 (C-6, 8), 128.2 (C-4″, 6″), 128.1 (C-5, 9), 127.4 (C-3″, 7″), 126.2 (C-7′), 118.8 (C-6′, 8′), 62.1 (C-1′), 55.0 (C-2), 52.6 (C-2′), 37.4 (C-3), 35.8 (C-3′), 24.0 ($COCH_3$). EI-MS m/z: 459 (M^+), 441, 293, 252, 224, 191, 177, 149, 106, 91, 77, 60 (100), 43. Anal. Calcd for $C_{27}H_{29}N_3O_4$: C, 70.57; H, 6.36; N, 9.14. Found: C, 70.69; H, 6.58; N, 8.98.

*4.1.2.21.2. N-(N-Benzoyl-L-phenylalanyl)-7′-acetamido-L-phenylalanine (**12c**).* ^{1}H NMR (DMSO, 400 MHz) δ: 12.80 (1H, br, COOH), 9.87 (1H, s, ArNHCO), 8.55 (1H, d, J = 8.4 Hz, NHCO), 8.32 (1H, d, J = 8.0 Hz, NHCO), 7.75 (2H, d, J = 7.2 Hz, H-3″, 7″), 7.51–7.13

(12H, m, H-4″-6″, H-5–9, H-5′, 9′, 6′, 8′), 4.73 (1H, m, H-2), 4.44 (1H, m, H-2′), 3.10–2.88 (4H, m, H-3, 3′), 2.02 (3H, s, $COCH_3$); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 172.9 (C-1′), 171.6 (C-1), 168.1 (ArNHC-OCH_3), 166.3 (C-1″), 138.5 (C-4), 137.9 (C-7′), 134.1 (C-2″), 131.8 (C-4′), 131.3 (C-5″), 129.4 (C-5′, 9′), 129.2 (C-6, 8), 128.2 (C-4″, 6″), 128.1 (C-5, 9), 127.4 (C-3″, 7″), 126.3 (C-7), 118.8 (C-6′, 8′), 54.6 (C-2), 53.7 (C-2′), 37.0 (C-3), 36.1 (C-3′), 24.0 ($COCH_3$). EI-MS *m/z*: 473 (M^+), 455, 429, 251, 225, 148, 105, 91 (100), 77, 65, 51. Anal. Calcd for $C_{27}H_{27}N_3O_5$: C, 68.48; H, 5.75; N, 8.87. Found: C, 68.19; H, 5.89; N, 8.92.

*4.1.2.21.3. N-(N-Benzoyl-L-phenylalanyl)-7′-propionamido-L-phenylalanine (**12d**).* ^1H NMR (DMSO-d_6, 400 MHz) δ: 12.78 (1H, br, COOH), 9.81 (1H, s, ArNHCO), 8.56 (1H, d, J = 8.4 Hz, NHCO), 8.33 (1H, d, J = 8.0 Hz, NHCO), 7.75 (2H, d, J = 7.2 Hz, H-3″, 7″), 7.50–7.48 (3H, m, H-5″, H-6′, 8′), 7.42 (2H, t, H-4″, 6″), 7.34 (2H, d, J = 7.2 Hz, H-5, 9), 7.23 (2H, t, H-6, 8), 7.17–7.12 (3H, m, H-7, H-5′, 9′), 4.73 (1H, m, H-2), 4.44 (1H, m, H-2′), 3.10–2.86 (4H, m, H-3, 3′), 2.28 (2H, q, J = 7.6 Hz, NHC-OCH_2CH_3), 1.06 (3H, t, J = 7.6 Hz, $NHCOCH_2CH_3$); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 172.9 (C-1′), 171.9, 171.6, 166.3 (C-1″), 138.5 (C-4), 138.0 (C-7′), 134.1 (C-2″), 131.7 (C-4′), 131.3 (C-5″), 129.4 (C-5′, 9′), 129.2 (C-6, 8), 128.2 (C-4″, 6″), 128.1 (C-5, 9), 127.5 (C-3″, 7″), 126.3 (C-7), 118.9 (C-6′, 8′), 54.7 (C-2), 53.7 (C-2′), 37.0, 36.1, 29.6 ($NHCOCH_2CH_3$), 9.8 ($NHCOCH_2CH_3$). EI-MS *m/z*: 487 (M^+), 382, 252, 163, 148, 105 (100), 91, 77, 65, 43. Anal. Calcd for $C_{28}H_{29}N_3O_5$: C, 68.98; H, 6.00; N, 8.62. Found: C, 68.63; H, 6.18; N, 8.35.

4.1.2.22. By a similar synthetic method described above for compound 11a (from compound 9f), the compounds of 11b (from 91), 11c (from 9o), 11d (from 9r) was synthesized.

*4.1.2.22.1. **N**-(**N**-Benzoyl-7-acetamido-L-phenylalanyl)-L-phenylalanine methyl ester (**11b**).* Mp 221.0–222.0 °C (EtOAc); ^1H NMR (DMSO-d_6, 400 MHz) δ: 9.84 (1H, s, ArNHCO), 8.52 (2H, m, NHCO × 2), 7.76 (2H, d, J = 7.6 Hz, H-3″, 7″), 7.51 (1H, t, H-5″), 7.46–7.42 (4H, m, H-4″, 6″, H-6, 8), 7.26–7.17 (7H, m, H-5′-9′, H-5, 9), 4.71 (1H, m, H-2), 4.51 (1H, m, H-2′), 3.59 (3H, s, OCH_3), 3.09–2.85 (4H, m, H-3, 3′), 1.99 (3H, CH_3CO); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 171.9, 171.7, 168.1 ($ArNHCOCH_3$), 166.2 (C-1″), 137.6 (C-7), 137.1 (C-4′), 134.0 (C-2″), 132.8 (C-4), 131.3 (C-5″), 129.4 (C-5, 9), 129.1 (C-6′, 8′), 128.3 (C-4″, 6″), 128.2 (C-5′, 9′), 127.4 (C-3″, 7″), 126.6 (C-7′), 118.6 (C-6, 8), 54.6 (C-2), 53.8 (C-2′), 51.9 (OCH_3), 36.6, 36.4, 24.0 ($COCH_3$). EI-MS *m/z*: 478 (M^+), 366, 281, 204, 188, 148, 105 (100), 91, 77. Anal. Calcd for $C_{28}H_{29}N_3O_5$: C, 68.98; H, 6.00; N, 8.62. Found: C, 68.84; H, 5.88; N, 8.43.

*4.1.2.22.2. N-(N-Benzoyl-L-phenylalanyl)-7′-acetamido-L-phenylalanine methyl ester (**11c**).* Mp 228.0–229.5 °C (EtOAc), ^1H NMR (DMSO-d_6, 400 MHz) δ: 9.95 (1H, s, ArNHCO), 8.60 (1H, d, J = 8.8 Hz, NHCO), 8.57 (1H, d, J = 7.6 Hz, NHCO), 7.77 (2H, d, J = 6.8 Hz, H-3″, 7″), 7.53–7.14 (12H, m, H-4″-6″, H-5–9, H-5′, 9′, 6′, 8′), 4.74 (1H, m, H-2), 4.47 (1H, m, H-2′), 3.59 (3H, s, OCH_3), 3.09–2.91 (4H, m, H-3, 3′), 2.02 (3H, s, $COCH_3$); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 171.9, 171.7, 168.2 ($ArNHCOCH_3$), 166.2 (C-1″), 138.4 (C-4), 138.0 (C-7′), 134.0 (C-2″), 131.4 (C-4′), 131.3 (C-5″), 129.4 (C-5′, 9′), 129.2 (C-6, 8), 128.2 (C-4″, 6″), 128.1 (C-5, 9), 127.4 (C-3″, 7″), 126.2 (C-7), 118.8 (C-6′, 8′), 54.6 (C-2), 53.9 (C-2′), 51.9 (OCH_3), 37.0, 36.1, 24.0 ($COCH_3$). EI-MS *m/z*: 487 (M^+), 396, 269, 252, 219, 177, 148, 105 (100), 91, 77, 43. Anal. Calcd for $C_{28}H_{29}N_3O_5$: C, 68.98; H, 6.00; N, 8.62. Found: C, 68.58; H, 6.19; N, 8.79.

*4.1.2.22.3. N-(N-5″-Methyl-benzoyl-7-acetamido-L-phenylalanyl)-O-acetyl-L-tyrosine methyl ester (**11d**).* Mp 222.0–223.0 °C (MeOH/EtOAc), ^1H NMR (DMSO-d_6, 400 MHz) δ: 9.84 (1H, s, ArNHCO), 8.55 (1H, d, J = 7.2 Hz, NHCO), 8.42 (1H, d, J = 8.4 Hz, NHCO), 7.69 (2H, d, J = 8.0 Hz, H-3″, 7″), 7.44 (2H, d, J = 8.4 Hz, H-6, 8), 7.27–7.23 (6H, m, H-5, 9, 5′, 9′, 4″, 6″), 6.98 (2H, d, J = 8.4 Hz, H-6′, 8′), 4.71 (1H, m, H-2), 4.52 (1H, m, H-2′), 3.59 (3H, s, OCH_3), 3.07–2.86 (2H, m, H-3, 3), 2.34 (3H, s, Ar-CH_3), 2.24 (3H, s, $OCOCH_3$), 1.99 (3H, s, NHC-OCH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 171.4 (×2, C-1, 1′), 169.2 (ArOCO), 168.1 (ArNHCO), 166.0 (C-1″), 149.2 (C-7′), 141.2 (C-5″), 137.6 (C-7), 134.5 (C-4′), 132.8 (C-4), 131.2 (C-2″), 130.1 (C-5′, 9′), 129.4 (C-5, 9), 128.7 (C-4″, 6″), 127.4 (C-3″, 7″), 121.6 (C-6′, 8′), 118.7 (C-6, 8), 54.5 (C-2), 53.7 (C-2′), 51.9 (OCH_3), 36.5, 35.9, 24.0 ($OCOCH_3$), 21.0 ($ArCH_3$), 20.9 ($NHCOCH_3$). EI-MS *m/z*: 559 (M^+), 424, 382, 295, 203, 188, 119 (100), 91. Anal. Calcd for $C_{31}H_{33}N_3O_7$: C, 66.53; H, 5.94; N, 7.51. Found: C, 66.75; H, 6.09; N, 7.38.

4.1.2.23. *N*-(*N*-Benzoyl-L-phenylalanyl)-L-phenylalanine (12e). 5 M NaOH (1 mL) was added to a solution of compound **9a** (2.0 mmol) in dry DMF (5 mL) and EtOH (10 mL). After stirring at room temperature for overnight the mixture was acidified to pH 2–3 with concentrated hydrochloric acid, and partitioned between EtOAc (150 mL) and water (50 mL). The organic phase was separated and washed with brine (2 × 30 mL), dried and evaporated in vacuo to give the compound of (**12e**). As a white powder (750 mg, 90.1%). ^1H NMR (DMSO-d_6, 400 MHz) δ: 12.81 (1H, br, COOH), 8.53 (1H, d, J = 8.4 Hz, NHCO), 8.31 (1H, d, J = 8.0 Hz, NHCO), 7.74 (2H, d, J = 7.6 Hz, H-3″, 7″), 7.46 (1H, t, H-5″), 7.40 (2H, t, H-4″, 6″), 7.33–7.12 (10H, m, H-5–9, 5′-9′), 4.72 (1H, m, H-2), 4.46 (1H, m, H-2′), 3.10–2.90 (4H, m, H-3, 3′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 172.8 (C-1′), 171.5 (C-1), 166.2 (C-1″), 138.4 (C-4), 137.4 (C-4′), 134.0 (C-2″), 131.3 (C-5″), 129.2 (×4, C-6, 8, C-6′, 8′), 128.2 (×4, C-4″, 6″, C-5′, 9′), 128.1 (C-5, 9), 127.4 (C-3″, 7″), 126.5 (C-7′), 126.2 (C-7), 54.6 (C-2), 53.6 (C-2′), 36.9 (C-3), 36.7 (C-3′). EI-MS *m/z*: 416 (M^+), 398, 251, 120, 105 (100), 91, 77. Anal. Calcd for $C_{25}H_24N_2O_4$: C, 72.10; H, 5.81; N, 6.73. Found: C, 72.59; H, 6.16; N, 6.47.

4.1.2.24. By a similar synthetic method described above for compound 12e (from compound 9a), the compounds of 12f (from 9d), 12 (from 91) was synthesized.

*4.1.2.24.1. **N**-(**N**-Benzoyl-L-phenylalanyl)-L-tyrosine (**12f**).* ^1H NMR (DMSO-d_6, 400 MHz) δ: 12.75 (COOH), 9.22 (1H, s, Ar-OH), 8.55 (1H, d, J = 8.4 Hz, NHCO), 8.23 (1H, d, J = 7.6 Hz, NHCO), 7.76 (2H, d, J = 7.6 Hz, H-3″, 7″), 7.51 (1H, t, H-5″), 7.43 (2H, t, H-4″, 6″), 7.34 (2H, d, J = 7.2 Hz, H-5, 9), 7.24 (2H, t, H-6, 8), 7.15 (1H, t, H-7), 7.02 (2H, d, J = 8.4 Hz, H-5′, 9′), 6.61 (2H, d, J = 8.4 Hz, H-6′, 8′), 4.74 (1H, m, H-2′), 4.38 (1H, m, H-2), 3.11–2.82 (4H, m, H-3, 3′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 172.9 (C-1′), 171.5 (C-1), 166.3 (C-1″), 156.0 (C-7′), 138.4 (C-4), 134.0 (C-2″), 131.3 (C-5″), 130.1 (C-5′, 9′), 129.2 (C-6, 8), 128.2 (C-4″, 6″), 128.0 (C-5, 9), 127.4 (C-3″, 7″), 127.3 (C-4′), 126.2 (C-7), 115.0 (C-6′, 8′), 54.6 (C-2′), 53.9 (C-2), 36.9 (C-3), 35.9 (C-3′). EI-MS *m/z*: 432 (M^+), 414, 386, 269, 252, 224, 120, 105 (100), 91, 77. Anal. Calcd for $C_{25}H_{24}N_2O_5$: C, 69.43; H, 5.59; N, 6.48. Found: C, 69.13; H, 5.79; N, 6.18.

*4.1.2.24.2. N-(N-Benzoyl-7-nitro-L-phenylalanyl)-L-phenylalanine (**12g**).* ^1H NMR (DMSO-d_6, 400 MHz) δ: 12.85 (1H, COOH), 8.54 (1H, d, J = 8.8 Hz, NHCO), 8.42 (1H, d, J = 8.4 Hz, NHCO), 8.14 (2H, d, J = 8.8 Hz, H-6, 8), 7.77 (2H, d, J = 6.8 Hz, H-3″, 7″), 7.63 (2H, d, J = 8.4 Hz, H-5, 9), 7.52 (1H, t, H-5″), 7.45 (2H, t, H-4″, 6″), 7.26–7.18 (5H, m, H-5′-9′), 4.85 (1H, m, H-2), 4.49 (1H, m, H-2′), 3.24–2.94 (4H, m, H-3, 3′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 172.7 (C-1′), 171.0 (C-1), 166.3 (C-1″), 146.8 (C-7), 146.2 (C-4), 137.4 (C-4′), 133.8 (C-2″), 131.4 (C-5″), 130.5 (C-5, 9), 129.2 (C-6′, 8′), 128.2 (×4, C-4″, 6″, 5′, 9′), 127.4 (C-3″, 7″), 126.5 (C-7′), 123.2 (C-6, 8), 53.9 (C-2′), 53.6 (C-2), 36.8, 36.6. EI-MS *m/z*: 461 (M^+), 443, 296, 269, 148, 120, 105 (100), 91, 77, 57, 43. Anal. Calcd for $C_{25}H_{23}N_3O_6$: C, 65.07; H, 5.02; N, 9.11. Found: C, 64.67; H, 5.25; N, 8.96.

4.2. Biological assay

4.2.1. In vitro anti-HBV assays

The antiviral activities of compounds **9a**–**9r**, **12a**–**12** against HBV in 2.2.15 cells were evaluated .The anti-HBV activities

in vitro included the ability to inhibit the production of HBsAg and HBeAg, and the replication of HBV DNA in HBV-infected 2.2.15 cells. For the antiviral analyses, confluent cultures of 2.2.15 cells were maintained on 24-well flat-bottomed tissue culture plates in RPMI 1640 medium with 2% fetal bovine serum. Cultures were treated with eight consecutive daily doses of the test compounds and lamivudine (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 very carefully with 1 mL of fresh DMEM containing different concentrations (50 μg/mL, 25 μg/mL, 12.25 μg/mL) of compounds **9a**–**9r**, **12a**–**12g** and did 4 wells at each concentration. Cells grew in the presence of drugs for 9 d with changes of medium every 3 d. After 6 d and 9 d, Supernatant was collected and performed at 20 °C. The HBsAg and HBeAg in culture medium were simultaneously measured by EIA kits on days 6 and 9. This test was done twice under the same conditions. Untreated cells were used as the control. Medium was changed daily with fresh test compounds and positive control. HBV nucleic acid and protein levels were measured eight days after the first treatment. The HBsAg and HBeAg in the culture medium were evaluated by semiquantitative enzyme immunoassay (EIA) methods using commercial kits (HBsAg, HBeAg, Beijing North Institute of Biological Technology.) as previously described. Intracellular HBV DNA levels 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 groups, respectively.

4.2.2. Cytotoxicity assay

Cytotoxicity induced by the test compounds in cultures of 2.2.15 cells was also determined. Briefly, 2.2.15 cells were grown to confluence in 24-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 24-well plate. Toxicity was determined by measuring neutral red dye uptake, as determined from the absorbance at 510 nm relative to untreated cells, at 24 h following day 9 of treatment.

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