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Short communication

Caffeoylglycolic and caffeoylamino acid derivatives, halfmers of L-chicoric acid, as new HIV-1 integrase inhibitors

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Abstract

Human immunodeficiency virus (HIV) integrase (IN) catalyzes the integration of HIV DNA copy into the host cell DNA. L-Chicoric acid (**1**) has been found to be one of the most potent HIV-1 integrase inhibitor. Caffeoylglycolic and caffeoylamino acid derivatives' halfmeric structures of L-chicoric acid **2** were synthesized for the purpose of simplifying the structure of L-chicoric acid. Among synthesized, compounds **2c** and **3f** showed HIV-1 IN inhibitory activities with IC_{50} values of 10.5 and 12.0 μM, respectively, comparable to that of parent compound L-chicoric acid ($IC_{50} = 15.7$ μM).

Keywords: Human immunodeficiency virus; Integrase inhibitor; L-Chicoric acid; Caffeoylglycolic acid; Caffeoylamino acid

1. Introduction

The alarming spread of the acquired immune deficiency syndrome (AIDS) epidemic has stimulated the discovery of therapeutic agents to inhibit the replication of the causative virus, human immunodeficiency virus (HIV-1). Advanced understanding of the viral cell cycle has made it possible to define targets to interrupt the life cycle of the virus. One such target is viral integrase (IN), which is responsible for integration of proviral DNA into host cell DNA. This integration is essential for the production of progeny virus, therefore, therapeutic agents that can inhibit this process should be effective anti-HIV agents [1,2]. Over the past few years, extensive efforts have resulted in a large number of HIV-1 IN inhibitors [3–6]. Among them, L-chicoric acid (**1**) is one of the most potent HIV-1 IN inhibitors and has moderate anti-HIV activity (Fig. 1). Accordingly, the development of synthetic routes to **1** has been of interest to organic and medicinal chemists. Unfortunately, the procedures still suffer from low yields, lack of reproducibility or difficulty in the control of stereogenic centers [3,7–9]. On the other hand, several analogues of **1** have been synthesized to improve the anti-HIV effect of **1**. In general, the reported analogues of **1** were dimeric forms of caffeic acid, which are linked by aliphatic, alicyclic, or aromatic spacers [10]. Very recently, galloyl-substituted derivatives with γ-aminoalanine as a linker have been found to have improved anti-HIV activities [11]. We also reported several types of caffeoyl-based HIV-1 IN inhibitors that have glucose or five-membered heterocyclic ring or catechol-substituted L-chicoric acid analogues as alternative scaffolds [12–14].

Simplification of the complex structures of natural products is a promising strategy in the development of new leads for medicinal applications [15]. This strategy could also provide easier synthetic procedures to the target compounds since the number of stereogenic centers on the parent compounds

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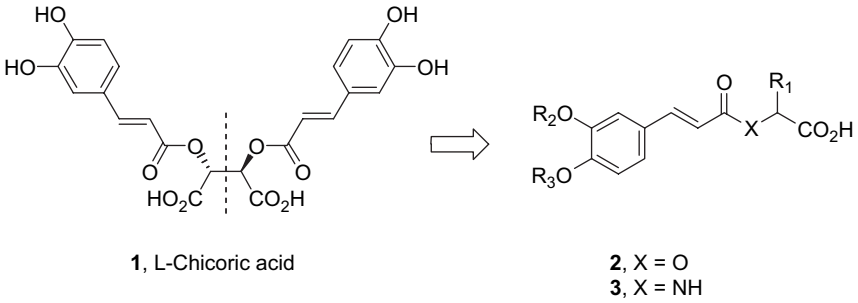


1, L-Chicoric acid

2, X = O
3, X = NH

Fig. 1. Design of new HIV-1 IN inhibitors from L-chicoric acid.

will be diminished. In this regard, we designed caffeoylglycolic acid derivatives **2**, halfmeric structures of **1**, by dividing the structure of **1** into half to produce new HIV-1 IN inhibitors. In addition, we also designed caffeoylamino acid derivatives **3** to examine the effect of amide on HIV-1 IN inhibitory activity. In this series of compounds **3**, more detailed SAR studies would be possible since a variety of chiral α-amino acids are readily available.

2. Results and discussion

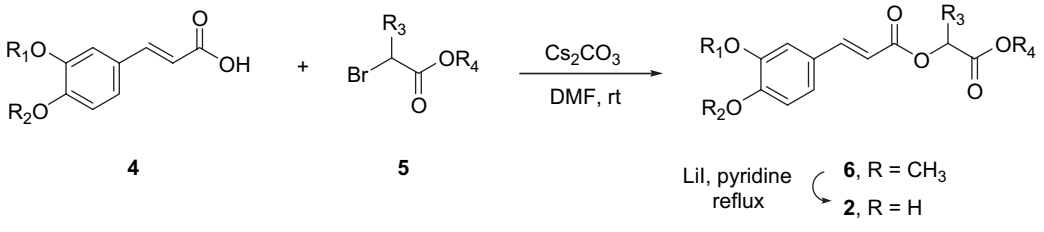
2.1. Chemistry

Caffeoylglycolic and caffeoylamino acid derivatives were synthesized from caffeic acid derivatives by alkylation with methyl esters of α-bromo carboxylic acids or EDC coupling with α-amino acids followed by demethylation of methyl ester as shown in Schemes 1 and 2. Cinnamic acid derivatives **4a–c** were treated with methyl α-bromo esters **5a** and **b** using cesium carbonate as a base to afford cinnamoylglycolic acid methyl esters **6a–d** [16]. Demethylation of **6a–d** was accomplished using the lithium iodide-promoted ester dealkylation method since demethylation in alkaline hydrolysis conditions can afford caffeic acid. Treatment of **6a–d** with lithium iodide in refluxing pyridine gave caffeoylglycolic acid derivatives **2a–d** [17].

Caffeoylamino acids **3a–f** were synthesized as shown in Scheme 2. The carbodiimide-based coupling reaction of caffeic acid with amino acid methyl ester **7** using dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP) in CH_2Cl_2/THF afforded caffeoylamino acid methyl esters **8a–f**. The methyl ester group was hydrolyzed cleanly by lithium hydroxide/water to give caffeoylamino acids **3a–f**.

2.2. Biological activity

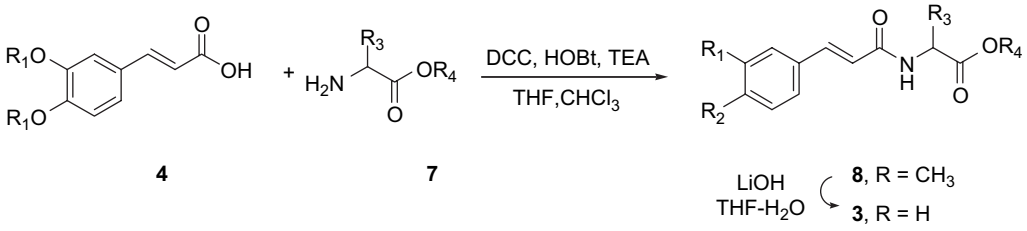
The resulting caffeoylglycolic and caffeoylamino acid derivatives **2–3** were assayed for HIV IN inhibition (Table 1) [13,18]. To compare inhibitory activities, L-chicoric acid (**1**) was prepared by a known procedure [9] and its activity data are included as a reference in the table. Although the catechol moiety has previously been found to be important for HIV-1 IN inhibitory activity in the di-caffeoyl series of compounds, 3,4-dimethoxy-, 4-hydroxy-3-methoxy- and 3,4-dihydroxy-cinnamic acid derivatives (**4a–c**) were prepared and assayed to examine the substituent effect on HIV-1 IN inhibitory activity. As expected, O-methylated compounds (**2a–b**) completely lacked inhibitory activity ($IC_{50} \gg 300$ μM), again indicating the importance of the catechol group for activity. Interestingly, the inhibitory activity of caffeoylglycolic acid (**2c**) ($IC_{50} = 10.5$ μM), the simplest compound, was comparable to that of L-chicoric acid ($IC_{50} = 15.7$ μM), even though it contained one less caffeoyl group than L-chicoric acid. On the other hand, in C-1 methyl-substituted caffeoylglycolic acid (**2d**) and caffeoylamino acid derivatives, the inhibitory activities were decreased about 10-fold from L-chicoric acid. However, 3,4-dihydroxybenzyl-substituted caffeoylamino acid (**3f**) also exhibited equipotent HIV-1 IN inhibitory activity to L-chicoric acid ($IC_{50} = 12.0$ μM), implicating the importance of the catechol group for activity. These data indicate that the inhibitory activity can be retained or even increased upon simplification of L-chicoric acid to halfmeric structures. However, more SAR studies are needed on this series of compounds, since compounds **2d** and **3f** did not show anti-HIV activity in cell culture assays with HIV-1_{IIIB} infected MT-4 cells at non-toxic concentrations (200 μM) in spite of their potent HIV-1 IN inhibitory activities [19]. On the other hand, L-chicoric acid exhibited



4 **5**

LiI, pyridine reflux → **6**, R = CH_3; **2**, R = H

Scheme 1.



Scheme 2.

anti-HIV activity with an EC_{50} value of 7.5 μM ($CC_{50} > 42.2$ μM, TI > 5.6).

3. Experimental section

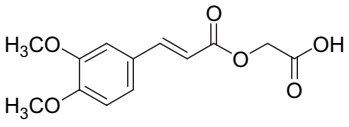
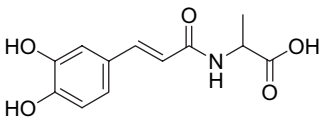
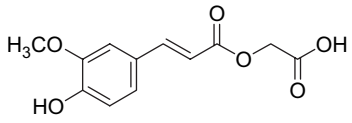
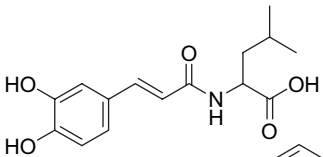
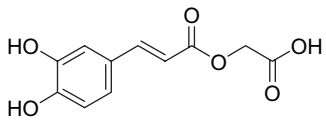
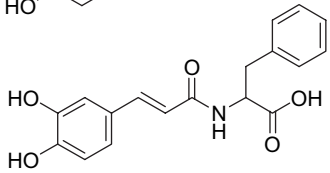
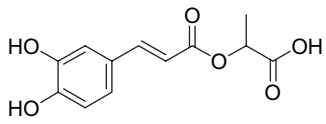
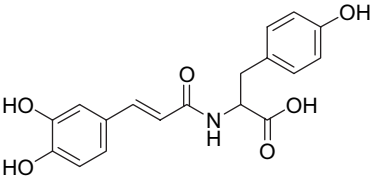
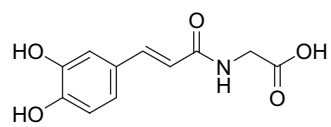
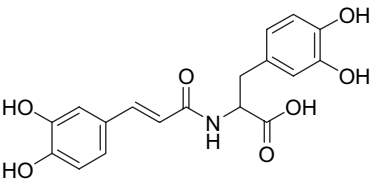
3.1. Chemistry

All reactions were carried out under nitrogen atmosphere. Flash column chromatography for purification was performed with Merck Kiesegel 60 Art 9385 (230–400 mesh). All solvents were purified according to standard procedures. ^1H and ^{13}C NMR spectra were recorded on a Gemini Varian-300 (300 and 75 MHz, respectively).

3.2. General procedure A for the synthesis of cinnamic acid methoxycarbonylmethyl esters *6a–d*

Cs_2CO_3 (1.56 g, 4.8 mmol) was added to solutions of cinnamic acid derivatives **4a–c** (4.8 mmol) in DMF (10 ml), and the reaction mixture was then stirred for 5 min at rt. Methyl bromoacetate **5** (4.8 mmol) was added dropwise to the mixture at 0 °C, which was then stirred at rt for 5 h. The reaction mixture was diluted with ethyl acetate and washed with water and saturated aqueous $NaHCO_3$ solution. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was purified by recrystallization or flash column chromatography to provide cinnamic acid methoxycarbonylmethyl esters **6a–d**.

Table 1
HIV-1 integrase inhibitory activities of catechol-substituted compounds (**2–3**)

Inhibitors	IC_{50} (μM)	Inhibitors	IC_{50} (μM)
2a 	>300	**3b** 	148.1
2b 	>300	**3c** 	129.6
2c 	10.5	**3d** 	95.0
2d 	128.1	**3e** 	129.0
3a 	171.6	**3f** 	12.0
L-Chicoric acid	15.7		

*3.2.1. 3-(3,4-Dimethoxy-phenyl)-acrylic acid methoxycarbonylmethyl ester (**6a**)*

Treatment of 3,4-dimethoxycinnamic acid (1.0 g, 4.8 mmol) with methyl bromoacetate (0.73 g, 4.8 mmol) according to general procedure A provided the desired product **6a** (0.85 g, 63%). ^{1}H NMR ($CDCl_3$) δ 7.72 (1H, d, $J = 15.9$ Hz), 7.13 (1H, dd, $J = 8.3$, 1.7 Hz), 7.07 (1H, d, $J = 1.7$ Hz), 6.88 (1H, d, $J = 8.3$ Hz), 6.41 (1H, d, $J = 15.9$ Hz), 4.76 (2H, s), 3.92 (6H, s), 3.80 (3H, s); ^{13}C NMR ($CDCl_3$) δ 168.4, 166.2, 151.3, 149.1, 146.2, 127.0, 122.9, 114.2, 111.0, 109.5, 60.5, 55.8, 55.7, 52.1.

*3.2.2. 3-(4-Hydroxy-3-methoxy-phenyl)-acrylic acid methoxycarbonylmethyl ester (**6b**)*

Treatment of 4-hydroxy-3-methoxycinnamic acid (1.0 g, 5.2 mmol) with methyl bromoacetate (0.80 g, 5.2 mmol) according to general procedure A provided the desired product **6b** (1.1 g, 79%). ^{1}H NMR (acetone-d_6) δ 7.70 (1H, d, $J = 15.9$ Hz), 7.42 (1H, s), 7.21 (1H, d, $J = 8.1$ Hz), 6.92 (1H, d, $J = 8.1$ Hz), 6.51 (1H, d, $J = 15.9$ Hz), 4.78 (2H, s), 3.96 (3H, s), 3.80 (3H, s); ^{13}C NMR (acetone-d_6) δ 169.1, 166.9, 150.3, 148.7, 147.0, 127.2, 124.3, 116.0, 114.5, 111.3, 61.0, 56.3, 52.2.

*3.2.3. 3-(3,4-Dihydroxy-phenyl)-acrylic acid methoxycarbonylmethyl ester (**6c**)*

Treatment of 3,4-dihydroxycinnamic acid (1.0 g, 5.6 mmol) with methyl bromoacetate (0.86 g, 5.6 mmol) according to general procedure A provided the desired product **6c** (0.85 g, 60%). ^{1}H NMR (DMSO-d_6) δ 9.69 (1H, br s), 9.20 (1H, br s), 7.56 (1H, d, $J = 15.9$ Hz), 7.10 (1H, s), 7.55 (1H, d, $J = 8.1$ Hz), 6.79 (1H, d, $J = 8.1$ Hz), 6.37 (1H, d, $J = 15.9$ Hz), 4.77 (2H, s), 3.70 (3H, s); ^{13}C NMR (acetone-d_6) δ 169.1, 166.8, 148.9, 146.9, 146.2, 127.6, 127.3, 122.8, 116.3, 115.3, 114.3, 61.0, 52.2.

*3.2.4. 3-(3,4-Dihydroxy-phenyl)-acrylic acid 1-methoxycarbonyl-ethyl ester (**6d**)*

Treatment of 3,4-dihydroxycinnamic acid (1.0 g, 5.6 mmol) with 2-bromopropionic acid methyl ester (0.94 g, 5.6 mmol) according to general procedure A provided the desired product **6d** (0.5 g, 30%). ^{1}H NMR (acetone-d_6) δ 8.56 (2H, br s), 7.53 (1H, d, $J = 15.9$ Hz), 7.13 (1H, d, $J = 1.9$ Hz), 7.01 (1H, dd, $J = 8.1$, 1.9 Hz), 6.82 (1H, d, $J = 8.1$ Hz), 6.27 (1H, d, $J = 15.9$ Hz), 5.06 (1H, q, $J = 7.2$ Hz), 3.64 (3H, s), 1.43 (3H, d, $J = 7.2$ Hz); ^{13}C NMR (acetone-d_6) δ 171.9, 166.8, 149.1, 146.8, 146.4, 117.2, 122.6, 116.3, 115.2, 114.4, 69.1, 52.3, 17.2.

*3.3. General procedure B for the syntheses of cinnamic acid carboxymethyl esters **2a–d***

Lithium iodide (9.68 mmol) was added to solutions of cinnamic acid methoxycarbonylmethyl esters **6a–d** (1.21 mmol) in pyridine (10 ml) under N_2 atmosphere, and the resulting solution was refluxed for 4.5 h. After cooling to rt, the reaction mixture was diluted with ethyl acetate and washed three times with water and 3 N HCl solution. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was purified by recrystallization or flash column chromatography to provide cinnamic acid carboxymethyl esters (**2a–d**).

*3.3.1. 3-(3,4-Dimethoxy-phenyl)-acrylic acid carboxymethyl ester (**2a**)*

General procedure B using **6a** (0.34 g, 1.2 mmol) afforded **2a** (0.2 g, 63%). ^{1}H NMR (acetone-d_6) δ 7.72 (1H, d, $J = 15.9$ Hz), 7.41 (1H, d, $J = 1.7$ Hz), 7.13 (1H, dd, $J = 8.3$, 1.7 Hz), 7.04 (1H, d, $J = 8.3$ Hz), 6.55 (1H, d, $J = 15.9$ Hz), 4.78 (2H, s), 3.94 (3H, s), 3.90 (3H, s); ^{13}C NMR (acetone-d_6) δ 173.4, 166.2, 145.9, 142.9, 133.6, 127.4, 123.3, 114.8, 111.7, 110.4, 60.3, 55.5.

*3.3.2. 3-(4-Hydroxy-3-methoxy-phenyl)-acrylic acid carboxymethyl ester (**2b**)*

General procedure B using **6b** (0.94 g, 3.7 mmol) afforded **2b** (0.4 g, 45%). ^{1}H NMR (acetone-d_6) δ 8.06 (1H, br s), 7.47 (1H, d, $J = 15.9$ Hz), 7.20 (1H, d, $J = 1.9$ Hz), 7.00 (1H, dd, $J = 8.1$, 1.9 Hz), 6.69 (1H, d, $J = 8.1$ Hz), 6.29 (1H, d, $J = 15.9$ Hz), 4.55 (2H, s), 3.74 (H, s); ^{13}C NMR (CD_3OD-d_6) δ 171.7, 168.4, 150.8, 149.4, 147.7, 127.6, 124.3, 116.5, 114.6, 111.7, 61.6, 56.4.

*3.3.3. 3-(3,4-Dihydroxy-phenyl)-acrylic acid carboxymethyl ester (**2c**)*

General procedure B using **6c** (0.50 g, 2.0 mmol) afforded **2c** (0.17 g, 35%). ^{1}H NMR (DMSO-d_6) δ 7.48 (1H, d, $J = 15.9$ Hz), 7.08 (1H, s), 6.97 (1H, d, $J = 8.1$ Hz), 6.77 (1H, d, $J = 8.1$ Hz), 6.28 (1H, d, $J = 15.9$ Hz), 4.63 (2H, s); ^{13}C NMR (DMSO-d_6 + CD_3OD) δ 173.7, 167.5, 149.4, 146.5, 145.7, 126.5, 122.2, 116.4, 114.8, 100.6, 63.5.

*3.3.4. 3-(3,4-Dihydroxy-phenyl)-acrylic acid 1-carboxy-ethyl ester (**2d**)*

General procedure B using **6d** (0.28 g, 1.1 mmol) afforded **2d** (0.13 g, 50%). ^{1}H NMR (DMSO-d_6) δ 7.50 (1H, d, $J = 15.9$ Hz), 7.01–7.06 (2H, m), 6.76 (1H, d, $J = 8.1$, 1.9 Hz), 6.31 (1H, d, $J = 15.9$ Hz), 4.98 (1H, q, $J = 7.1$ Hz), 1.43–1.45 (3H, d, $J = 7.1$ Hz); ^{13}C NMR (DMSO-d_6) δ 172.6, 166.4, 149.1, 146.4, 146.0, 125.8, 122.0, 116.2, 115.4, 113.7, 68.7, 17.3.

*3.4. General procedure C for the syntheses of N-methoxycarbonylmethyl cinnamamides **8a–f***

To solutions of cinnamic acids **4a–c** (4.8 mmol) in THF (12 ml) cooled to 0 °C were added successively α-amino acid methyl ester hydrochlorides **7** (4.8 mmol) and solutions of triethylamine (0.49 g, 4.8 mmol) in $CHCl_3$ (5 ml), 1-hydroxybenzotriazole (HOBt, 0.65 g, 4.8 mmol) in THF (10 ml), and dicyclohexylcarbodiimide (DCC, 1.09 g, 5.3 mmol) in $CHCl_3$ (10 ml). After stirring for 1 h at 0 °C, the mixture was warmed to rt and stirred overnight. After filtration of insoluble material (dicyclohexylurea) from the mixture, the filtrate was evaporated and dissolved in ethyl acetate. The ethyl acetate solution was washed with water, 10% citric acid aqueous solution, 10%

$NaHCO_3$ aqueous solution, and finally, with brine. The organic phase was dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was purified by recrystallization or flash column chromatography to provide *N*-methoxycarbonylmethyl cinnamamides **8a–f**.

*3.4.1. [3-(3,4-Dihydroxy-phenyl)-acryloylamino]-acetic acid methyl ester (**8a**)*

General procedure C using 3,4-dihydroxycinnamic acid (1.0 g, 5.6 mmol) and glycine methyl ester hydrochloride (0.70 g, 5.6 mmol) afforded **8a** (0.58 g, 42%). ^{1}H NMR (DMSO-d_6) δ 9.44 (1H, br s), 9.20 (1H, br s), 8.46 (1H, t, $J = 5.4$ Hz), 7.29 (1H, d, $J = 15.9$ Hz), 7.17 (1H, d, $J = 1.5$ Hz), 7.04 (1H, dd, $J = 8.1$, 1.5 Hz), 6.82 (1H, d, $J = 8.1$ Hz), 6.42 (1H, d, $J = 15.9$ Hz), 3.97 (2H, d, $J = 5.4$ Hz), 3.42 (3H, s); ^{13}C NMR (DMSO-d_6) δ 170.6, 166.0, 147.6, 145.6, 140.0, 126.2, 120.7, 117.6, 115.8, 113.9, 51.8, 40.8.

*3.4.2. 2-[3-(3,4-Dihydroxy-phenyl)-acryloylamino]-propionic acid methyl ester (**8b**)*

General procedure C using 3,4-dihydroxycinnamic acid (1.0 g, 5.6 mmol) and L-alanine methyl ester hydrochloride (0.78 g, 5.6 mmol) afforded **8b** (0.50 g, 34%). ^{1}H NMR (DMSO-d_6) δ 9.32 (2H, br s), 8.44 (1H, d, $J = 6.6$ Hz), 7.29 (1H, d, $J = 15.9$ Hz), 7.00 (1H, d, $J = 1.5$ Hz), 6.89 (1H, dd, $J = 8.1$, 1.5 Hz), 6.79 (1H, d, $J = 8.1$ Hz), 6.42 (1H, d, $J = 15.9$ Hz), 4.41 (1H, m), 3.67 (3H, s), 1.36 (3H, d, $J = 7.2$ Hz); ^{13}C NMR (DMSO-d_6) δ 173.4, 165.3, 147.6, 145.6, 140.0, 126.4, 120.6, 117.6, 115.8, 113.8, 51.9, 47.7, 17.1.

*3.4.3. 2-[3-(3,4-Dihydroxy-phenyl)-acryloylamino]-4-methyl-pentanoic acid methyl ester (**8c**)*

General procedure C using 3,4-dihydroxycinnamic acid (1.0 g, 5.6 mmol) and L-leucine methyl ester hydrochloride (1.02 g, 5.6 mmol) afforded **8c** (1.1 g, 66%). ^{1}H NMR ($CDCl_3$) δ 7.42 (1H, d, $J = 15.9$ Hz), 7.00 (1H, s), 6.94 (1H, d, $J = 7.5$ Hz), 6.75 (1H, d, $J = 7.5$ Hz), 6.26 (1H, d, $J = 15.9$ Hz), 4.77 (1H, q, $J = 5.1$ Hz), 3.75 (3H, s), 1.63–1.75 (3H, m), 0.96 (6H, d, $J = 5.1$ Hz); ^{13}C NMR ($CDCl_3$) δ 174.4, 167.7, 146.9, 144.3, 142.8, 127.0, 122.1, 116.3, 115.4, 114.2, 52.6, 51.3, 41.1, 24.9, 22.8, 21.7.

*3.4.4. 2-[3-(3,4-Dihydroxy-phenyl)-acryloylamino]-3-phenyl-propionic acid methyl ester (**8d**)*

General procedure C using 3,4-dihydroxycinnamic acid (1.0 g, 5.6 mmol) and L-phenylalanine methyl ester hydrochloride (1.20 g, 5.6 mmol) afforded **8d** (1.5 g, 81%). ^{1}H NMR (acetone-d_6) δ 7.28 (1H, d, $J = 15.7$ Hz), 7.31–7.18 (5H, m), 6.94 (1H, d, $J = 1.8$ Hz), 6.83 (1H, dd, $J = 8.1$, 1.8 Hz), 6.74 (1H, d, $J = 8.1$ Hz), 6.38 (1H, d, $J = 15.7$ Hz), 4.59 (1H, q, $J = 5.6$ Hz), 3.57 (3H, s), 3.08 (1H, dd, $J = 13.7$, 5.5 Hz), 2.96 (1H, dd, $J = 13.7$, 9.2 Hz); ^{13}C NMR (acetone-d_6) δ 172.9, 166.4, 148.0, 146.2, 141.5, 138.0, 130.1, 129.2, 128.2, 127.5, 121.7, 118.8, 116.3, 114.9, 54.6, 52.2, 38.4.

*3.4.5. 2-[3-(3,4-Dihydroxy-phenyl)-acryloylamino]-3-(4-hydroxy-phenyl)-propionic acid methyl ester (**8e**)*

General procedure C using 3,4-dihydroxycinnamic acid (1.0 g, 5.6 mmol) and L-tyrosine methyl ester hydrochloride (1.30 g, 5.6 mmol) afforded **8e** (1.8 g, 89%). ^{1}H NMR (acetone-d_6) δ 7.44 (1H, d, $J = 15.9$ Hz), 7.10 (1H, d, $J = 2.2$ Hz), 7.09 (2H, d, $J = 8.1$ Hz), 6.87 (1H, dd, $J = 8.4$, 2.2 Hz), 6.87 (1H, d, $J = 8.4$ Hz), 6.79 (2H, d, $J = 8.1$ Hz), 6.39 (1H, d, $J = 15.7$ Hz), 4.83 (1H, m, $J = 5.6$ Hz), 3.70 (3H, s), 3.08 (1H, dd, $J = 13.8$, 5.6 Hz), 2.84 (1H, dd, $J = 13.8$, 9.0 Hz); ^{13}C NMR (DMSO-d_6) δ 173.1, 166.2, 156.7, 148.2, 146.3, 140.7, 130.7, 127.9, 126.9, 121.3, 118.3, 116.5, 115.8, 114.6, 54.8, 52.5, 36.9.

*3.4.6. 3-(3,4-Dihydroxy-phenyl)-2-[3-(3,4-dihydroxy-phenyl)-acryloylamino]-propionic acid methyl ester (**8f**)*

General procedure C using 3,4-dihydroxycinnamic acid (1.0 g, 5.6 mmol) and L-3,4-dihydroxyphenylalanine methyl ester (1.10 g, 5.6 mmol) afforded **8f** (1.2 g, 58%). ^{1}H NMR (DMSO-d_6) δ 8.37 (1H, d, $J = 7.5$ Hz), 7.25 (1H, d, $J = 15.7$ Hz), 6.97 (1H, d, $J = 1.8$ Hz), 6.87 (1H, d, $J = 8.2$, 1.8 Hz), 6.77 (1H, d, $J = 8.2$ Hz), 6.63–6.65 (2H, m, $J = 7.8$ Hz), 6.49 (1H, dd, $J = 8.0$, 1.9 Hz), 6.60 (1H, s), 6.43 (1H, d, $J = 15.7$ Hz), 4.47 (1H, m, $J = 5.2$ Hz), 3.60 (3H, s, $-OCH_3$), 2.88 (1H, dd, $J = 13.7$, 5.6 Hz), 2.81 (1H, dd, $J = 13.7$, 9.0 Hz); ^{13}C NMR (DMSO-d_6) δ 173.1, 166.2, 148.2, 146.3, 145.7, 144.7, 140.7, 128.6, 126.9, 126.9, 121.3, 120.5, 118.4, 117.1, 116.5, 116.1, 114.6, 54.9, 52.5, 37.1.

*3.5. General procedure D for the syntheses of compounds **3a–f***

Lithium hydroxide (5.40 mmol) was added to solutions of *N*-methoxycarbonylmethyl cinnamamides **8a–f** (1.08 mmol) in THF and water (1:1, 14 ml) at 0 °C. The mixture was stirred at rt for 40 min. The solution was diluted with water and acidified to pH 1 by addition of 3 N HCl. The mixture was extracted with ethyl acetate, and the organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was purified by recrystallization or flash column chromatography to provide *N*-carboxymethyl cinnamamides **3a–f**.

*3.5.1. [3-(3,4-Dihydroxy-phenyl)-acryloylamino]-acetic acid (**3a**)*

General procedure D using **8a** (0.30 g, 1.20 mmol) afforded **3a** (0.09 g, 31%). ^{1}H NMR (DMSO-d_6) δ 8.33 (1H, t, $J = 6.0$ Hz), 7.28 (1H, d, $J = 15.9$ Hz), 6.98 (1H, d, $J = 1.5$ Hz), 6.88 (1H, dd, $J = 8.1$, 1.5 Hz), 6.77 (1H, d, $J = 8.1$ Hz), 6.43 (1H, d, $J = 15.9$ Hz), 3.88 (2H, d, $J = 6.0$ Hz); ^{13}C NMR (DMSO-d_6) δ 171.5, 165.8, 147.5, 145.6, 139.8, 126.3, 120.6, 117.9, 115.8, 113.8, 40.8.

*3.5.2. 2-[3-(3,4-Dihydroxy-phenyl)-acryloylamino]-propionic acid (**3b**)*

General procedure D using **8b** (0.35 g, 1.30 mmol) afforded **3b** (0.10 g, 30%). ^{1}H NMR (DMSO-d_6) δ 8.19 (1H, d,

$J = 6.9$ Hz), 7.44 (1H, d, $J = 15.9$ Hz), 7.05 (1H, d, $J = 1.8$ Hz), 6.94 (1H, dd, $J = 8.3$, 1.8 Hz), 6.80 (1H, d, $J = 8.3$ Hz), 6.47 (1H, d, $J = 15.9$ Hz), 4.54 (1H, m, $J = 6.9$ Hz), 1.48 (3H, d, $J = 7.2$ Hz); ^{13}C NMR (DMSO-d_6) δ 176.2, 168.9, 148.8, 146.7, 142.8, 128.2, 117.9, 116.4, 115.1, 49.5, 17.8.

3.5.3. 2-[3-(3,4-Dihydroxy-phenyl)-acryloylamino]-4-methyl-pentanoic acid (**3c**)

General procedure D using **8c** (0.50 g, 1.60 mmol) afforded **3c** (0.32 g, 67%). ^{1}H NMR (DMSO-d_6) δ 8.18 (1H, d, $J = 8.1$ Hz, −CON*H*CH−), 7.26 (1H, d, $J = 15.9$ Hz, −CH=C*H*−), 6.97 (1H, d, $J = 1.7$ Hz, phenyl-*H*2), 6.86 (1H, dd, $J = 8.3$, 1.7 Hz, phenyl-*H*6), 6.77 (1H, d, $J = 8.3$ Hz, phenyl-*H*5), 6.45 (1H, d, $J = 15.9$ Hz), 4.35 (1H, q, $J = 5.8$ Hz), 1.69−1.54 (3H, m), 0.90 (6H, d, $J = 4.7$ Hz); ^{13}C NMR (DMSO-d_6) δ 174.6, 165.4, 147.4, 145.6, 139.6, 126.4, 120.5, 118.1, 115.8, 113.9, 50.6, 24.4, 22.9, 21.4, 14.1.

3.5.4. 2-[3-(3,4-Dihydroxy-phenyl)-acryloylamino]-3-phenyl-propionic acid (**3d**)

General procedure D using **8d** (0.50 g, 1.47 mmol) afforded **3d** (0.32 g, 66%). ^{1}H NMR (DMSO-d_6) δ 8.30 (1H, d, $J = 8.1$ Hz), 7.32−7.20 (5H, m), 7.21 (1H, d, $J = 15.9$ Hz), 6.95 (1H, d, $J = 2.4$ Hz), 6.85 (1H, dd, $J = 8.1$, 2.4 Hz), 6.75 (1H, d, $J = 8.1$ Hz), 6.41 (1H, d, $J = 15.9$ Hz), 4.56 (1H, m), 3.13 (1H, dd, $J = 13.8$, 4.9 Hz), 2.94 (1H, dd, $J = 13.8$, 9.6 Hz). ^{13}C NMR (DMSO-d_6) δ 173.7, 165.8, 157.1, 147.9, 146.0, 140.5, 140.2, 138.2, 129.5, 128.7, 126.9, 126.7, 121.0, 118.4, 118.0, 116.2, 114.3, 54.1, 37.4.

3.5.5. 2-[3-(3,4-Dihydroxy-phenyl)-acryloylamino]-3-(4-hydroxy-phenyl)-propionic acid (**3e**)

General procedure D using **8d** (0.50 g, 1.40 mmol) afforded **3d** (0.16 g, 38%). ^{1}H NMR (DMSO-d_6) δ 8.13 (1H, d, $J = 8.0$ Hz), 7.18 (1H, d, $J = 15.7$ Hz), 7.02 (2H, d, $J = 8.3$ Hz), 6.94 (1H, s), 6.83 (1H, d, $J = 8.1$ Hz), 6.74 (1H, d, $J = 8.1$ Hz), 6.64 (2H, d, $J = 8.3$ Hz), 6.42 (1H, d, $J = 15.7$ Hz), 4.43 (1H, q, $J = 5.4$ Hz), 3.00 (1H, dd, $J = 13.8$, 4.9 Hz), 2.81 (1H, dd, $J = 13.8$, 9.2 Hz); ^{13}C NMR (DMSO-d_6) δ 176.6, 169.0, 157.2, 148.8, 146.7, 142.7, 131.3, 129.5, 128.3, 122.3, 118.1, 116.5, 116.2, 115.1, 56.5, 37.9.

3.5.6. 3-(3,4-Dihydroxy-phenyl)-2-[3-(3,4-dihydroxy-phenyl)-acryloylamino]-propionic acid (**3f**)

General procedure D using **8f** (0.50 g, 1.34 mmol) afforded **3f** (0.12 g, 25%). ^{1}H NMR (DMSO-d_6) δ 7.76 (1H, d, $J = 7.2$ Hz), 7.18 (1H, d, $J = 15.9$ Hz), 6.97 (1H, s), 6.83 (1H, d, $J = 7.8$ Hz), 6.74 (1H, d, $J = 7.8$ Hz), 6.65 (1H, s), 6.57 (1H, d, $J = 7.8$ Hz), 6.46 (1H, d, $J = 7.8$ Hz), 6.47 (1H, d, $J = 15.9$ Hz), 4.33 (1H, q, $J = 5.1$ Hz), 2.98 (1H, dd, $J = 13.5$, 4.2 Hz), 2.76 (1H, dd, $J = 13.5$, 7.8 Hz); ^{13}C NMR (DMSO-d_6) δ 175.7, 165.5, 148.1, 146.4, 145.5, 144.2, 139.5, 130.4, 127.1, 121.0, 120.7, 119.7, 117.5, 116.6, 115.9, 114.8, 56.2, 37.8.

3.6. HIV integrase assay [12,17]

Recombinant human immunodeficiency virus type 1 (HIV-1) integrase was expressed in *Escherichia coli* and purified in a one-step process using a nickel-chelated column. Aliquots of 0.5 mg/ml HIV-1 integrase stock solutions were stored at −70 °C until used.

3.6.1. Oligonucleotide substrates

Two complementary 20-mer oligonucleotides whose sequences resemble the end of U5-LTR were obtained from Korea Biotech, Inc.: K16 (U5-LTR, +strand), 5′-TGTGGAAAATC TCTAGCAGT-3′; K17 (U5-LTR, −strand), 5′-ACTGCTAGA GATTTTCCACA-3′. The oligonucleotides were purified in 20% polyacrylamide gels before use. To construct the duplex oligonucleotide substrate, 30 pmol of oligonucleotide K16 were labeled at the 5′-end using 250 μCi of [γ-^{32}P]-ATP (3000 Ci/mmol; 1 Ci = 37 GBq; Amersham) and 10 units of T4 polynucleotide kinase (T4 PNK, New England Biolabs) in 40 μl of reaction buffer (70 mM tris−HCl [pH 7.6], 10 mM $MgCl_2$, 5 mM dithiothreitol) at 37 °C for 15 min. The labeling reaction was supplemented with 10 mM EDTA and heated to 85 °C for 15 min to inactivate T4 PNK. After addition of 30 pmol of oligonucleotide K17, the reaction mixture was boiled for 3 min and cooled slowly. Labeled substrate was separated from unincorporated nucleotide on a Biospin 6 column (Bio-Rad).

3.6.2. HIV-1 integrase reaction

Standard endonucleolytic activity assays were performed in the presence of potential inhibitor with 0.1 pmol of duplex oligonucleotide substrate and 15 pmol of HIV-1 integrase in 15 mM tris−HCl (pH 7.4), 100 mM NaCl, 1 mM $MnCl_2$, 2 mM 2-mercaptoethanol, 2.5 mM CHAPS, 0.1 mM EDTA, 0.1 mM PMSF, 1% glycerol, and 10 mM imidazole in a total volume of 10 μl. Inhibitors or drugs were dissolved in 100% DMSO and added to the reaction for a final concentration of 5% DMSO. Reaction mixtures were incubated at 33 °C for 90 min and stopped by addition of 4 μl of 95% formamide, 20 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol FF. The reaction mixtures were heated to 90 °C for 3 min and electrophoresed on 20% denaturing polyacrylamide gels. Reaction products were visualized by autoradiography of wet gels. IC_{50} was calculated by scanning bands on Kodak-5 film (Image Master VDS, Pharmacia Biotech).

4. Conclusion

We synthesized and tested the HIV-1 IN inhibitory activities of caffeoylglycolic and caffeoylamino acid derivatives **2** and **3**. Compounds **2c** and **3f** showed equal or slightly increased HIV-1 IN inhibitory activity compared to parent compound L-chicoric acid. It is notable that while dimerization of a pharmacophore of bioactive compounds through a linker is relatively well-known strategy for obtaining more potent activity, compounds in this work retained activity even upon simplification into halfmeric structures.

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