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Synthesis of *trans*-caffeate analogues and their bioactivities against HIV-1 integrase and cancer cell lines

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ABSTRACT

Forty caffeate analogues were synthesized via a convenient method starting from vanillin with moderate to good yields. The testing of biological activity of these compounds against HIV-1 integrase indicates that four compounds: bornyl caffeate, bornyl 2-nitrocaffeate, 5-nitrocaffeic acid and 5-nitrocaffeic acid phenethyl ester (5-nitroCAPE) possess a good HIV integrase inhibitory activity, IC_{50} 19.9, 26.8, 25.0 and 13.5 μM, respectively. Twelve caffeate analogues were tested by MTT assay on growth of human hepatocellular carcinoma BEL-7404, human breast MCF-7 adenocarcinoma, human lung A549 adenocarcinoma and human gastric cancer BCG823 cell lines, respectively. And the best result is IC_{50} 5.5 μM for CAPE against BEL-7404.

Some naturally occurring caffeates are widely distributed in plant kingdom.[1] Most of them have bioactivities such as antibacterial,[2] antiviral, [3] anti-inflammatory,[4] antiatherosclerotic,[5] anti-HIV,[6] antitumor[7] and so on. Epidemiological studies indicate that a diet, rich in fruits and vegetables reduces cancer risk in humans, suggesting that certain dietary constituents may be effective in preventing cancer.[8] Especially, caffeic acid phenethyl ester (CAPE) has been identified as the major biologically active compounds.[9] Encouraged by the aforementioned information and as a part of our new drug discovery efforts, it was valuable to synthesize caffeate analogues and study the structure–activity relationship (SAR) on caffeate analogues.

Caffeate analogues have been synthesized previously by methods such as: acid-catalyzed esterification, alkylation of caffeic acid with halohydrocarbons, esterification via acyl chlorides, coupling reaction with DCC as coupling agent, transesterification and Wittig reaction.[10] Herein, we have developed a more convenient, one-pot method to prepare the caffeate analogues with moderate to good yields shown as Scheme 1. The synthesized forty compounds were summarized in Table 1. In addition, all caffeate analogues are *trans* (*E*) configuration confirmed by the ^1H NMR spectra in that the coupling constants of α-H and β-H on double bonds were 15.9–16.4 Hz.[11] Furthermore, it was confirmed caffeate analogues are *trans* (*E*) configuration by X-ray analysis of compound **III-26** (Fig. 1). [12]

The anti-HIV integrase activities of caffeates were evaluated by the Biotin-Avidin ELISA method. The results were summarized in Table 1. From Table 1, it has been found that compound **III-21**, **III-25**, **III-26** and **III-40** possess a good HIV-1 integrase inhibitory activity. In part of esterifiable site, aryl ring or multi-ring compound seemed to be required because of all alkyl esters being inactive; replacement of 3-hydroxyl groups with methyl ether, such as **III-25** to **III-22**, **III-26** to **III-23**, and **III-40** to **III-37**, resulted completely loss of potency whether adding a third group to the mother phenyl ring or not. So the presence of a catechol entity seems to be of importance.[6a]

Adding a third strong election-withdrawing group NO_2 on 3, 4-dihydroxyl pattern resulted in potential activity, such as compound **III-25**, **III-26** and **III-40**. These evidences would reveal that analogues with resonance electron-drawing group decrease election density to stabilize the corresponding conformers responsible for the higher activity.

Unfortunately, although the lead compound CAPE (**III-19**) was tested twice by the same ELISA method, it showed no activity against HIV integrase, which was not consistent with the results of the literature.[6a] These different results may be somewhat different from the Burke's method, and it need more work to identify.

The antitumor activities in vitro for these compounds were evaluated by the MTT method for BEL-7404, MCF-7, A549 and BCG823 cell lines. The results are summarized in Table 2. All compounds except for **III-23** and **III-33** possessed potent activity. **III-19** and **III-37** possessed stronger BEL-7404 activities than positive control cisplatin. On the MCF-7 examination, **III-17** showed good anti-breast tumor potency. **III-39** showed good anti-lung cancer potency. But on the BCG832 examination, activities of all twelve compounds were not better than that of positive control. Comparing with the anti-HIV-1 integrase activities, replacement of the OH group with CH_3O group, such as **III**-40 to **III-37**, the antitumor activities did not lost. Adding NO_2 group as a third group, such as **III-19** to **III-**

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Table 1
Anti HIV-1 integrase activities of caffeate analogues (**III**)

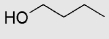
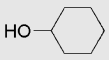
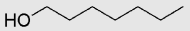
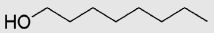
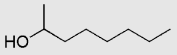
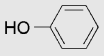
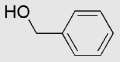
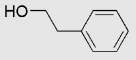
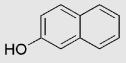
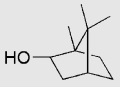
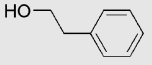
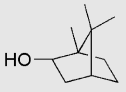
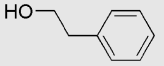
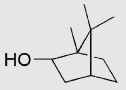
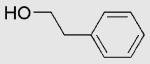
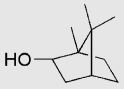
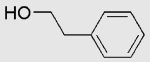
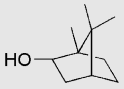
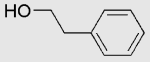
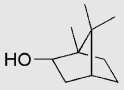
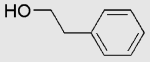
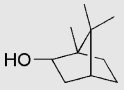
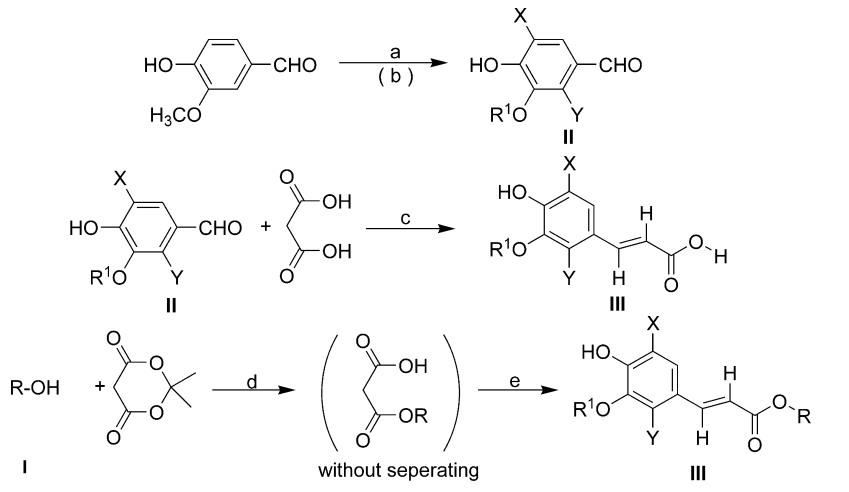
Entry	ROH (**I**)	R^1	X	Y	Inhibition of HIV-1 integrase IC_{50} (μM)
III-1	H	H	H	H	>100
III-2	HO—	H	H	H	>100
III-3		H	H	H	>100
III-4		H	H	H	>100
III-5		H	H	H	>100
III-6		H	H	H	>100
III-7		H	H	H	>100
III-8		H	H	H	>100
III-9		H	H	H	>100
III-10		H	H	H	>100
III-11		H	H	H	>100
III-12		H	H	H	>100
III-13		H	H	H	>100
III-14		H	H	H	>100
III-15		H	H	H	>100
III-16		H	H	H	>100
III-17		H	H	H	>100
III-18		H	H	H	>100
III-19		H	H	H	>100
III-20		H	H	H	>100
III-21		H	H	H	19.9
III-22	H	CH_3	NO_2	H	>100
III-23		CH_3	NO_2	H	>100
III-24		CH_3	NO_2	H	>100
III-25	H	H	NO_2	H	26.8
III-26		H	NO_2	H	25.0
III-27		H	NO^2	H	>100
III-28	H	CH_3	H	H	>100

Table 1 (*continued*)

Entry	ROH (**I**)	R^1	X	Y	Inhibition of HIV-1 integrase IC_{50} (μM)
III-29	H	CH_3	Cl	H	>100
III-30		CH_3	Cl	H	>100
III-31		CH_3	Cl	H	>100
III-32	H	CH_3	Br	H	>100
III-33		CH_3	Br	H	>100
III-34		CH_3	Br	H	>100
III-35	H	CH_3	H	NO_2	>100
III-36		CH_3	H	NO_2	>100
III-37		CH_3	H	NO_2	>100
III-38	H	H	H	NO_2	>100
III-39		H	H	NO_2	>100
III-40		H	H	NO_2	13.5



Scheme 1. The synthesis route of *trans*-caffeate analogues. (See above-mentioned references for further information).

26 and **III-39**, **III-21** to **III-40**, the antitumor activities did not increase much. 5-Br (**III-23**) or 5-NO_2 (**III-33**) phenethyl ferulate was completely inactive. Comparing with all anti-cancer data, the best result is IC_{50} 5.5 μM for CAPE against BEL-7404. These results indicate that in our case there is no evidence for direct correlation between HIV-1 integrase and antitumor inhibition.

In summary, 40 caffeate analogues synthesized by the simple one-pot method with moderate to good yields, were tested by Biotin-Avi-

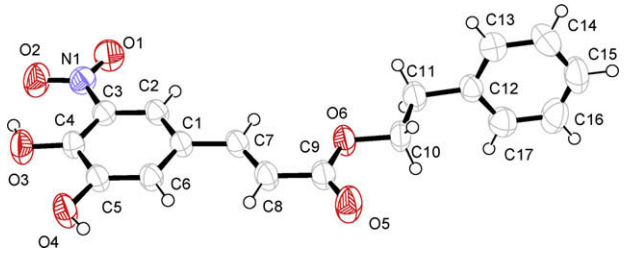


Figure 1. The crystal structure of 5-NO_2CAPE.

din ELISA method for the activities against HIV-1 integrase. It has been found there are four compounds possessing good activities. In part of esterifiable site, aryl or multi-ring group seems important for the activity; in the mother phenyl part, the catechol entity was important, and the replacement of 3-OH by CH_3O group will lost activity; the NO_2 group was assign to the mother phenyl ring could increase the activity. Some of compounds were tested by MTT method for antitutor activities. Most of them possessed antitumor activities. The best result is IC_{50} 5.5 μM for CAPE against BEL-740. Considering the structure–activity relationship, the ring substitutions and ester groups in the structure are probably important determinants for some potent biological activities which are worth to research further.

Acknowledgments

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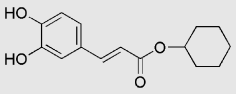
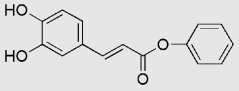
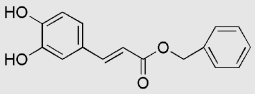
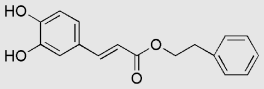
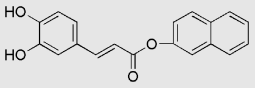
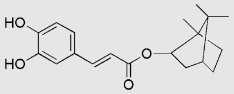
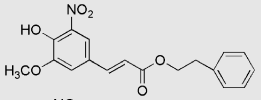
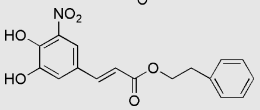
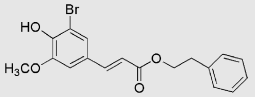
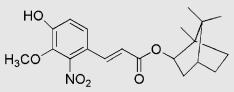
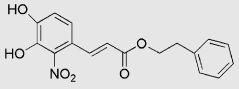
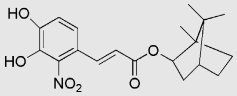
Table 2
Antitumor activities of some caffeate analogues (**III**)

Entry	Compound	IC_{50} (μM)			
		BEL-7404	MCF-7	A549	BCG832
III-12		15.5	30.3	89.3	97.6
III-17		44.3	5.9	75.9	56.56
III-18		17.4	14.1	27.3	48.1
III-19		5.5	26.7	83.6	44.6
III-20		58.1	12.8	50.0	59.8
III-21		28.2	>300	19.5	100.6
III-23		>300	>300	>300	>300
III-26		59.7	12.3	11.4	22.9
III-33		>300	>300	>300	>300
III-37		6.6	62.3	21.1	32.1
III-39		10.2	28.8	8.7	27.8
III-40		28.7	40.7	52.9	116.6
Control	Cisplatin	23.8	29.5	32.3	6.4

integrase and antitumor activities, respectively. We also acknowledge the financial support by the Science and Technology Department of Zhejiang Province (Grant No.2005C23022).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.10.046.

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12. Xia, C. N.; Hu W. X. Zhou W. *Acta Crystallogr., Sect. E.* **2007**, *63*, o4795. Crystal data of ***III-26***. $C_{17}H_{15}NO_6$, M = 329.30, Monoclinic, a = 8.584 (2), b = 5.373 (1), c = 33.904 (7) Å , β = 91.757(32)°, U = 1562.8 (3) Å^3, T = 296 (2) K, space group $P2_1/c$, Z = 4, D_c = 1.400 g/cm^3, μ(Mo-Ka) = 0.11 mm^{-1}, 9258 reflections measured, 3509 unique (R_{int} = 0.026) which were used in all calculations. Fine R_1 = 0.043, $\omega R(F^2)$ = 0.124 (all data). Full crystallographic details of ***III-26*** have been deposited at the Cambridge Crystallographic Data Center and allocated the deposition number CCDC 673003.
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17. *General procedure:*Preparation of caffeic acid3,4-Dihydroxybenzaldehyde (9.0 g, 65 mmol), malonic acid (7.3 g, 70 mmol) were added to a mixture of toluene (15 ml), pyridine (100 mmol) and aniline (0.7 ml). The solution was stirred at refluxing temperature 2 h. When the mixture was cooled to room temperature, yellow precipitation was filtrated, then washed with 50 ml 3 M HCl solution and 50ml water twice, respectively. The crude product was recrystallized from EtOH to give the light beige powder 5.2 g (44.2%). mp 197–200 °C; (Lit.211–213 dec.) [18]; IR ν_{max} (KBr/cm^{-1}): 3433, 3238, 1644, 1619, 1280, 1217; ^{1}H NMR (DMSO-d_6, σ): 12.02(1H, br s, COOH), 9.35(1H, br s, 2OH), 7.461(1H, d, J = 16.4 Hz, β-H), 7.03(1H, s, Ph-H), 7.02(1H, d, J = 7.6 Hz, Ph-H), 6.75(1H, d, J = 7.6 Hz, Ph-H),6.27(1H, d, J = 15.9 Hz, α-H).Preparation of Caffeic acid phenylethyl ester (CAPE)The Meldrum's acid (3.6 g, 25 mmol) was added into toluene (50 ml), and then added β-phenethanol (3.05 g, 25 mmol). The mixture was heated and refluxed for 4 h. When the mixture was cooled to room temperature, added 3,4-dihydroxybenzaldehyde (1.4 g, 10 mmol), pyridine (2.5 ml) and piperidine (0.25 ml). The stirring continued at room temperature 22 h, using TLC to trace the reaction until the reaction completely finished. The solvents were distilled out in vacuum; the residue was dissolved in diethyl ether (30 ml), washed with saturated solution of sodium bicarbonate 20 ml twice, then diluted hydrochloride 20 ml and distilled water 20 ml, respectively. The ether phase was dried by anhydrous $MgSO_4$ overnight. After removal of the drier, the solvent was distilled out to get yellow crude solid. The crude solid was recrystallized from a mixture of benzene and diethyl ether (8:2) to afford pale white needle crystal CAPE 2.56 g (90.1%). mp 126–128 °C (Lit. 128 °C)[6a]; IR ν_{max} (KBr/cm^{-1}): 3481, 3329, 1683, 1601, 1279, 1181; ^{1}H NMR (DMSO-d_6, σ): 9.59 (s, 1H, OH), 9.13 (s, 1H, OH), 7.45 (d, 1H, J = 15.9 Hz, β-H), 7.22–7.33 (m, 5H, 5Ph-H), 7.04 (d, 1H, J = 2.4Hz, Ph-H), 7.00 (dd,1H, J = 2.4, 8.0 Hz, Ph-H), 6.76 (d, 1H, J = 8.0 Hz, Ph-H), 6.23 (d, 1H, J = 15.9 Hz, α-H), 4.32 (t, 2H, J = 14.0 Hz, CH_2), 2.95 (t, 2H, J = 14.0 Hz, CH_2); IEMS (70 eV): 284 (3%, M$^+$), 180 (100), 163 (61), 134 (34), 105 (50), 104 (66), 89 (53), 77 (49), 51 (33).
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