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Synthesis and antitumor activity of feruloyl and caffeoyl derivatives

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This paper is dedicated to Prof. Wei-xiao Hu for his lifelong commitment to mentoring graduate students

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ABSTRACT

We developed two efficient protocols for the synthesis of feruloyl and caffeoyl derivatives from commercial vanillin and veratraldehyde. Pharmacological activities were assessed against a panel of human cancer cell lines in vitro. Most synthesized compounds demonstrated attractive cytotoxicity. Several new compounds demonstrated significant antiproliferative and cytotoxic activities against HeLa and Bewo tumor cell lines. In particular, 5-nitro caffeic adamantyl ester showed broad spectrum of tumor inhibition in 10 cell lines, and reduced tumor weight by 36.7% in vivo when administered at a dose of 40 mg kg^{-1}.

Naturally occurring feruloyl and caffeoyl derivatives are widely distributed throughout the plant kingdom.[1] Epidemiological studies demonstrate that a diet rich in fruit and vegetables, which contain abundant caffeoyl derivatives, can reduce cancer risk in humans. This suggests that certain dietary constituents may be effective in preventing cancer.[2] There is a large body of evidence demonstrating that feruloyl or caffeoyl derivatives, such as curcumin, caffeic acid, caffeic acid phenethyl ester, chlorogenic acid, L-cichoric acid, and rosmarinic acid possess a wide range of biological activities including anti-cancer,[3–7] anti-bacterial,[8] anti-oxidative,[9,10] and antiviral activities.[11] Although there is substantial evidence demonstrating the biological activities of feruloyl and caffeoyl compounds, little is known about the relationship between the structure and biological activity of these compounds.

In our previous work, SAR studies showed that four compounds inhibited against HIV-1integrase and some caffeates exhibited cytotoxicity with micromolar range. Moreover, compounds with nitro-substitutions, multiple cycloalkyl groups, or a combination of the two factors, showed superior inhibitory effects. These observations have led us to focus on caffeic analogues as a potential scaffold endowed with inhibitory effects on cancer.

Feruloyl and caffeoyl derivatives were prepared using two convenient methods as shown in Scheme 1. Ferulates and caffeates were prepared by Knoevenagel condensation of the substituted benzaldehydes[14–17] with mono-malonic esters in a one-pot manner.[18] Acetyl cinnamic acids were prepared by Perkin reaction, followed by the mixed carbonic anhydride method[19] to obtain feruloyl amides, and demethylated to obtain caffeoyl amides. Chlorogenic acid was separated and purified from honeysuckle using the HSCCC method (High Speed Counter-Current Chromatography).[12]

The ester condensation protocol can be very useful and applied to a wide range of cinnamic esters with a structure that is difficult to synthesize via other documented 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.[13] We have ever attempted to extended this protocol to prepare feruloyl and caffeoyl amides, but it was too difficult to separate pure amides by column chromatography, for the concentrated reaction solutions were very dark and gummy. Furthermore, yields of the desired amides were barely detectable due to excessive amounts of undetermined by-products. Fortunately, when we attempted

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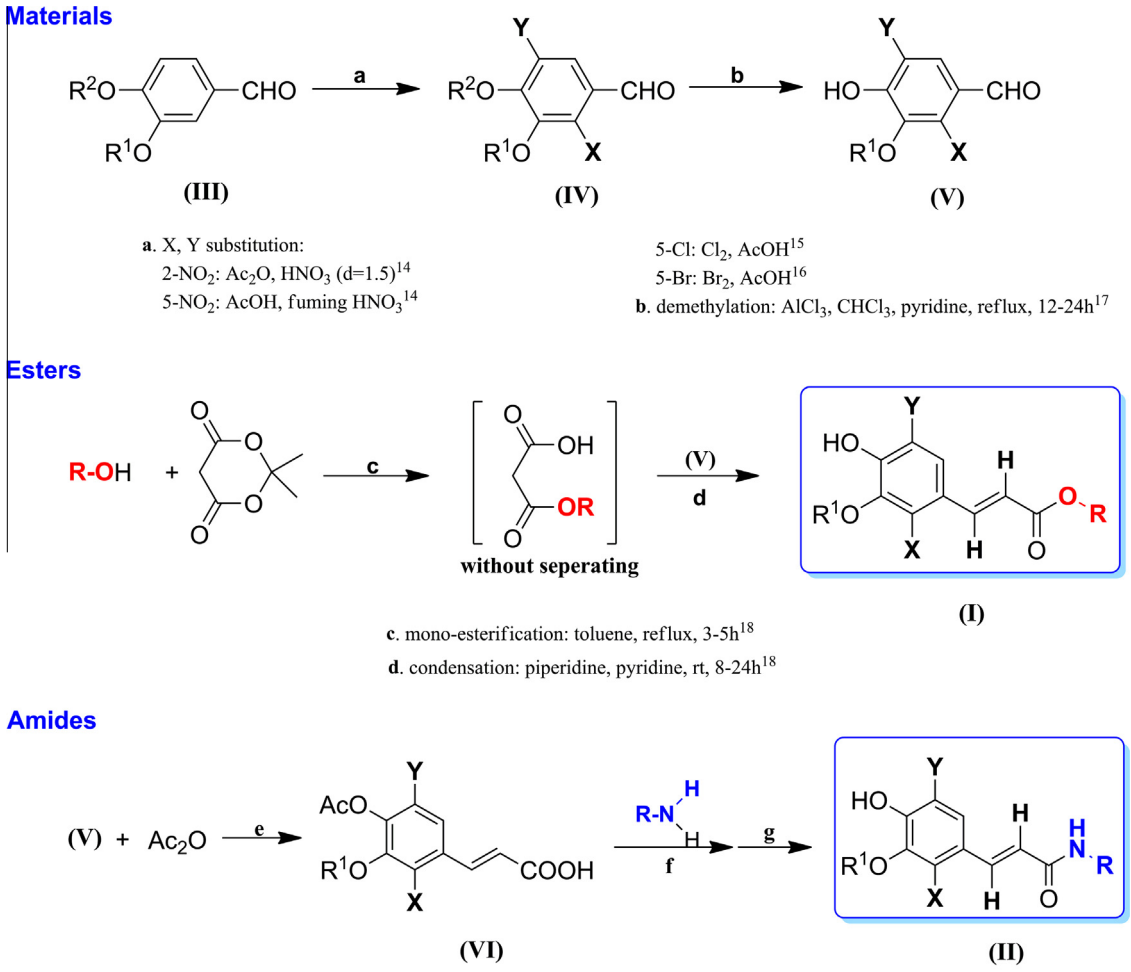
0960-894X/



Scheme 1. Synthesis of feruloyl and caffeoyl derivatives.

synthesis of 4-acetylvanillin, it was found that 4-acetylvanillin can easily form 4-acetyl feruloyl acid when catalyzed by sodium acetate at reflux temperature, followed by the mixed carbonic anhydride method to obtain feruloyl amides, and which can be demethylated to form caffeoyl amides. All synthesized esters and amides are summarized in Table 1, which shows the *trans* (*E*) configuration confirmed by the ^{1}H NMR spectra, a subset of the compounds were characterized by X-ray analysis.[20]

The antitumor activities of esters and amides against six human tumor cell lines including HeLa, Siha, Bewo, HL-60, SGC-7901 and HepG-2, were evaluated by MTT assay in vitro. Cisplatin and Doxorubicin were used as positive control in the MTT assay. The structures and anti-tumor activities are summarized in Table 1.

As shown in Table 1, most of feruloyl and caffeoyl compounds possessed potent anti-cancer activities. **I-24** and **I-30** showed good anti-Hela tumor potency. **I-30** showed potent Siha inhibition. In particular, nearly all compounds had significant cytotoxic effects against Bewo and HL-60 with small IC_{50} values. For the Bewo cell line, of the forty compounds screened, eighteen compounds showed good to excellent inhibitor activities, and IC_{50} values of **I-14** and **I-24** were less than 1 μM. Both compounds were at least 2–56 times more effective than the two positive controls (Cisplatin IC_{50} 1.07 μM, Doxorubicin IC_{50} 20.37 μM). Also there are 16 compounds that showed good inhibition on HL-60 cell line. **I-20, I-21** and **I-30** showed good SCG-7901 inhibitory activity. Compounds **I-15**, **I-21** and **I-30** showed potent HepG-2 inhibition. This observation suggests that the good to excellent inhibitory activities of the aforementioned compounds do not simply correlate with the size of the ester moiety or substitutions with electron-withdrawing group. The results of Bewo were not repeated with the other five tumor cell lines, so we can conclude that feruloyl and caffeoyl compounds possess higher selectivity for the Bewo cell line when compared to the other five cell lines.

When different electron-withdrawing groups such as NO_2, Cl and Br were present, the compounds showed more potent activity; it is obvious that a strong electron withdrawing group such as NO_2 contributes to increased activity, as can be seen with compounds **I-18**, **I-20, I-21**, **I-25, I-26, I-27, I-28**, **I-29** and **I-30**. These evidences would confirm that analogues with resonance election-drawing group decrease election density to stabilize the corresponding conformers responsible for the higher activity as well.[18] However, the activities decreased with caffeic adamantyl amides **II-1, II-2** and **II-3** when Cl or Br groups were introduced. The importance of the catechol entity was obviously greater for the activities of caffeoyl amides than for feruloyl amides. In addition, solubility problems were present with compounds containing adamantyl derivatives when used in the MTT assay, particularly in the case of feruloyl adamantyl amides.

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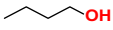
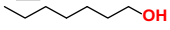
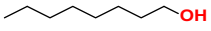
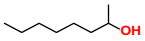
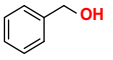
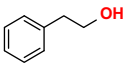
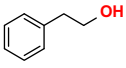
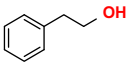
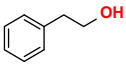
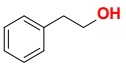
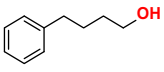
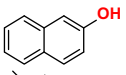
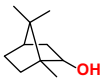
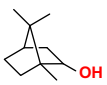
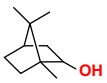
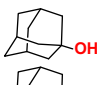
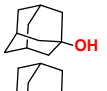
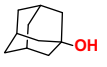
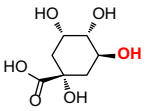
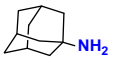
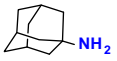
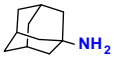
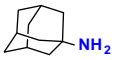
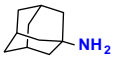
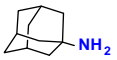
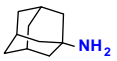
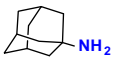
Table 1
Inhibitions of caffeoyl derivatives on tumor cells

Entry	Compound				Tumor cells (IC_{50}: μM)					
	X	Y	R_1	ROH/RNH_2	HeLa	Siha	Bewo	HL-60	SGC-7901	HepG-2
I-1	H	H	H		52.53	60.26	17.27	12.32	>100	81.55
I-2	H	H	H		23.27	34.09	6.20	8.94	>100	63.95
I-3	H	H	H		28.61	44.19	5.18	11.80	>100	>100
I-4	H	H	H		30.14	48.16	5.90	11.76	97.71	91.63
I-5	H	H	H		67.22	30.72	8.64	11.50	89.17	>100
I-6	H	H	H		19.17	39.08	8.99	12.34	>100	>100
I-7	H	H	H		40.80	40.97	5.42	7.54	>100	>100
I-8	H	H	H		13.77	33.77	3.18	12.46	>100	95.80
I-9	H	H	H		16.69	41.99	3.47	7.92	80.04	69.70
I-10	H	H	H		23.60	30.47	7.26	9.32	>100	38.05
I-11	H	H	H		23.86	51.53	2.75	8.32	95.54	45.54
I-12	H	H	H		21.51	35.75	2.77	4.46	33.99	44.71
I-13	H	H	H		34.94	45.93	13.12	8.29	55.96	54.29
I-14	H	H	H		38.22	49.94	0.41	5.10	17.43	24.08
I-15	H	H	H		>100	>100	>100	10.23	17.18	4.95
I-16	H	H	H		29.84	52.26	30.70	10.78	90.27	50.82
I-17	H	H	H		23.48	74.27	28.22	8.00	70.45	85.64
I-18	H	H	H		37.98	66.92	2.96	16.65	14.26	40.87
I-19	NO_2	H	H		10.91	17.17	4.38	18.23	ND	ND
I-20	H	Cl	H		ND	ND	ND	7.01	8.18	ND
I-21	H	Br	H		17.52	28.49	8.24	8.49	6.75	4.44
I-22	H	NO_2	H		ND	ND	ND	ND	ND	ND
I-23	H	H	H		31.57	88.71	5.80	11.25	40.09	48.43
I-24	H	H	H		8.04	39.51	0.36	3.40	44.80	14.77
I-25	H	H	H		20.60	73.74	35.86	3.35	12.03	31.37
I-26	NO_2	H	H		13.91	51.77	9.31	52.88	ND	ND
I-27	H	Cl	H		38.71	33.77	51.94	18.60	69.48	ND
I-28	H	Br	H		22.69	29.02	20.01	15.15	ND	ND
I-29	H	H	H		>100	>100	>100	8.15	29.68	29.97
I-30	H	NO_2	H		1.28	6.74	5.63	4.07	4.60	3.84
I-31	H	H	CH_3		59.94	>100	70.01	92.35	ND	ND

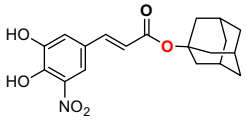
(*continued on next page*)

Table 1 (*continued*)

Entry	Compound				Tumor cells (IC_{50}: μM)					
	X	Y	R_1	ROH/RNH_2	HeLa	Siha	Bewo	HL-60	SGC-7901	HepG-2
I-32	H	H	H		ND	ND	ND	>100	>100	>100
II-1	H	H	H		10.16	41.28	1.09	8.18	29.78	30.06
II-2	H	Cl	H		50.03	18.93	68.36	35.65	ND	ND
II-3	H	Br	H		33.23	>100	60.85	>100	ND	ND
II-4	H	H	CH_3		>100	67.61	>100	>100	ND	ND
II-5	H	NO_2	CH_3		66.77	59.54	68.19	18.74	ND	ND
II-6	NO_2	H	CH_3		91.26	38.90	26.69	73.19	ND	ND
II-7	H	Cl	CH_3		98.42	34.82	44.49	67.87	ND	ND
II-8	H	Br	CH_3		>100	82.37	41.41	49.82	ND	ND
Control				Doxorubicin	0.33	2.04	1.07	0.42	ND	ND
				Cisplatin	0.97	13.97	20.37	10.40	ND	ND

ND—Not detective.

Table 2
Inhibition of **I-30** on four additional tumor cell lines (IC_{50}: μM)



Cell lines	BEL-7404	A549	BCG832	MCF-7	HL-60
IC_{50}	21.43	4.41	8.22	4.09	0.52
Control Doxorubicin	ND	ND	ND	ND	0.23
Cisplatin[18]	7.1	9.7	1.9	8.8	3.12

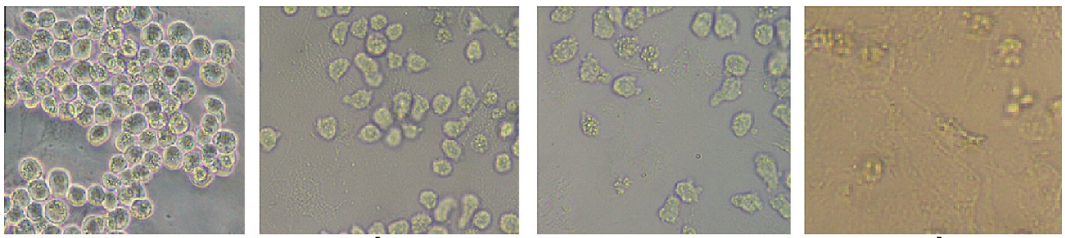


Figure 1. The influence of **I-30** on cell morphology of HL-60 (×400). (a) Control cells, (b) 2.5 μg/mL, (c) 5 μg/mL, and (d) 10 μg/mL.

As shown in Table 1, the compound **I-30,** 5-nitro caffeic adamantyl ester, had a broad-range of biological activities. **I-30** showed higher activities against all the tested cancer cell lines than most compounds. To verify the proposed broad-range activity of **I-30**, we tested four additional tumor cell lines for cytotoxicity alongside a repetition of HL-60. (Table 2)

Based on the in vitro anti-tumor data, **I-30** was chosen as a lead compound to test the morphology (Fig. 1) and flow cytometer analysis (Fig. 2) of the HL-60 cell line. The results showed that **I-30** significantly inhibited proliferation of HL-60 cells in a dose-dependent and time-dependent manner. Within certain dose ranges, the inhibition was time-effect relationship. Preliminary results from flow cytometric analyses revealed that compounds **I-30** significantly induced the level of apoptosis in HL-60 cells in vitro at low concentrations (Fig. 2).

Based on our in vitro anti-tumor and acute toxicity data, we further evaluated the in vivo anti-tumor activity of **I-30** against S-180 tumor-bearing mice with CTX as positive control. The results showed that **I-30** can inhibit S-180 tumor by 36.7%, but its efficacy is lower than that of CTX, which inhibited tumors by 54.8%, although it showed efficiency anti-tumor activity in vitro. Given that the in vivo studies were performed on murine tumor cells, which are different from the in vitro tested cells, our results are still promising considering the possibility of selective inhibition. Further work needs to be done with human tumor cells.

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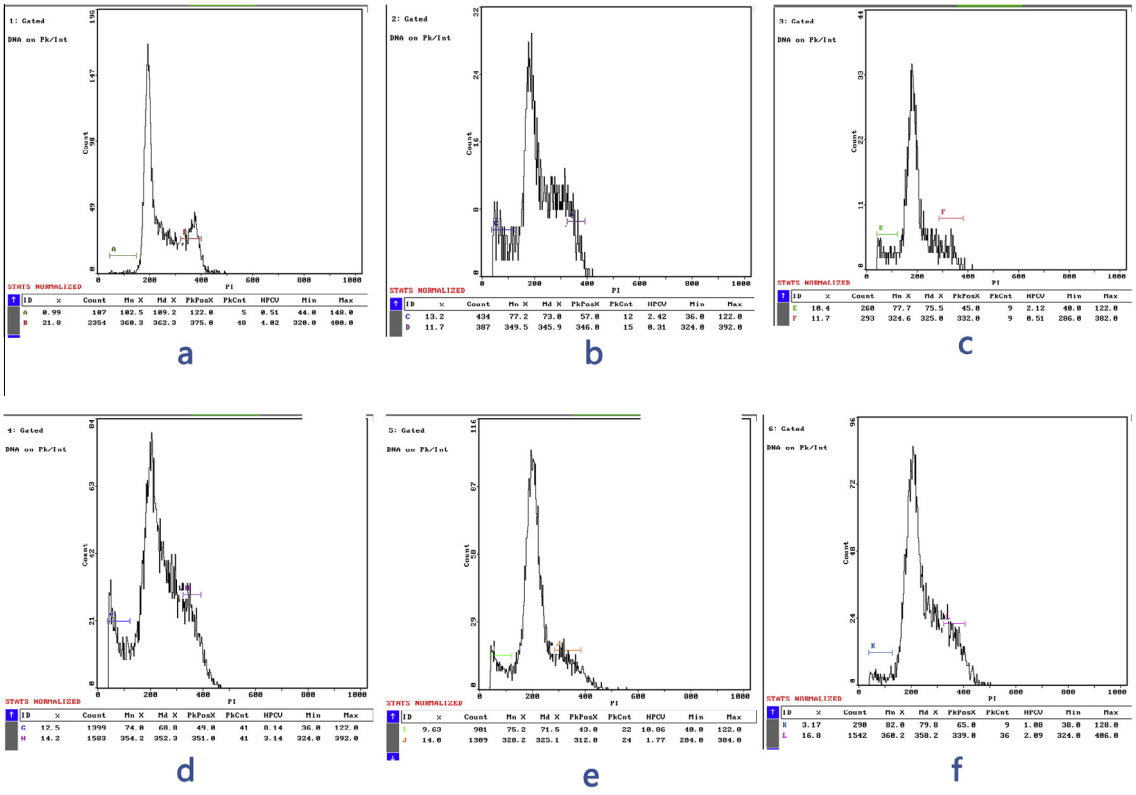

Figure 2. DNA cell cycle analysis in HL-60 cells with increasing doses of **I-30** (a) control cells, (b) 2 μg/mL, (c) 1 μg/mL, (d) 2 μg/mL (repeated), (e) 1 μg/mL (repeated), (f) 0.5 μg/mL.

In conclusion, a series of feruloyl and caffeoyl derivatives were synthesized and tested for in vitro anti-tumor activity against several cancer cell lines. The SAR study of these compounds led to the identification of a new caffeate, **I-30**, as a highly potent anti-cancer compound with broad range inhibitory activities. Further chemo-biological study of **I-30** with regards to its anti-tumor pathway and its enzymatic targets are ongoing in our laboratory, along with additional in vivo studies.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.08.024. These data include MOL files and InChiKeys of the most important compounds described in this article.

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