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Syntheses of Certain 3-Aryl-2-propenoates and Evaluation of Their Cytotoxicity

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Abstract—A series of 3-aryl-2-propenoates including cinnamates, (*E*)-methyl/ethyl 3-[2-(1,4-dimethoxy-5,8-dione)naphthalenyl]-2-propenoates (**8ba**, **8bb**) and (*E*)-methyl/ethyl 3-[2-(1,4-dihydroxy-9,10-dione)anthracenyl]-2-propenoates (**9aa**, **9ab**) was synthesized and evaluated for antitumor cytotoxicity. It was found that the *ortho*- or *para*-dihydroxy funtionality on the aryl ring was essential for the cytotoxicity of cinnamates. Compounds **8ba**, **8bb** and **9aa**, **9ab** showed potent cytotoxicity against various tumor cell lines.

Introduction

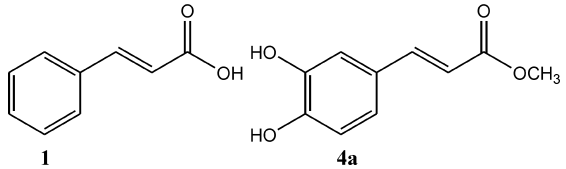
Cinnamic acid (**1**) and its derivatives are widely distributed in nature and constitute a class of compounds with a broad spectrum of activities such as antiinflammatory,[1] anticonvulsant,[2] antifungal,[3] analgesic[1] and antihypertensive.[4,5] They have been also reported to inhibit human immunodeficiency virus (HIV)[6] and a number of enzymes such as protein tyrosine kinases,[7] ras farnesyl transferase.[8] Several reports have unveiled the antiinvasive and antiangiogenic activity of this compound class.[9] However, the evaluation of cinnamates and synthesis of related derivatives, 3-aryl-2-propenoates as anticancer agents appear to be unexplored fields.

In our search for novel antitumor agents from natural sources, we isolated a phenyl propanoid, methyl 3,4-dihydroxycinnamate (methyl caffeate **4a**, Fig. 1), from the plant *Notopterygium incisum*. This compound showed a significant cytotoxicity against various cancer cell lines[10] and greatly inhibited the invasion of B16 melanoma cells.[11] Structurally, **4a** possesses an α,β-unsaturated carbonyl, which can be considered as a Michael acceptor, an active moiety often employed in the design of anticancer drugs.[12] In addition, a number of α,β-unsaturated ketones have demonstrated preferential activity toward thiol.[13] Alkylation with a cellular thiol such as glutathione (GSH) may also occur with cinnamates like **4a**, leading to adducts at the β-position. Hence, α,β-unsaturated carbonyl-containing compounds may be free from problems of mutagenicity and carcinogenicity that are associated with many alkylating agents used in cancer chemotherapy.[14] Taking into consideration this structural feature and the interesting bioactivity of the compound **4a**, the present investigation aims at preparing a number of **4a** analogues and related 3-aryl-2-propenoates in order to evaluate their cytotoxicity.

Results and Discussion

Chemistry

Caffeates (**4a**–**4c**, Table 1) were obtained by refluxing caffeic acid in excess alcohol used as solvent in the presence of a catalytic amount of HCl (Scheme 1). Other



1 **4a**

Figure 1.

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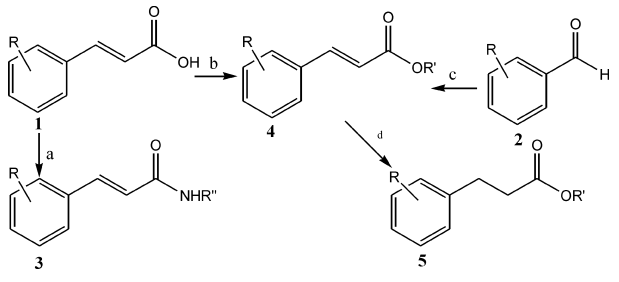
Table 1. Cytotoxicity of synthesized cinnamates and related compounds against some cancer cell lines[a]

Compound	ArCH=CHCOR/ArCH$_2$CH$_2$COR			Cytotoxicity (IC_{50})[b] (μg/mL)		
	E/*Z*	Ar	R	B16	HCT116	A431
1	*E*	3,4-(OH)$_2$–C$_6$H$_3$-	H	>30	>30	>30
3a	*E*	3,4-(OH)$_2$–C$_6$H$_3$-	NHOH	2.69	2.49	2.16
3b	*E*	3,4-(OH)$_2$–C$_6$H$_3$-	NHC$_6$H$_5$	4.23	4.26	5.00
4a	*E*	3,4-(OH)$_2$–C$_6$H$_3$-	OCH$_3$	2.96	3.01	1.79
4b	*E*	3,4-(OH)$_2$–C$_6$H$_3$-	OC$_2$H$_5$	1.75	1.99	1.81
4c	*E*	3,4-(OH)$_2$–C$_6$H$_3$-	O^{n}C$_3$H$_7$	2.46	2.12	1.89
4d	*E*	2,5-(OH)$_2$–C$_6$H$_3$-	OCH$_3$	0.12	0.17	0.15
4e	*Z*	2,5-(OH)$_2$–C$_6$H$_3$-	OCH$_3$	30.07	>30	>30
4f	*E*	2,5-(OH)$_2$–C$_6$H$_3$-	OC$_2$H$_5$	0.21	0.19	0.17
4g	*E*	3,5-(OH)$_2$–C$_6$H$_3$-	OCH$_3$	>30	>30	>30
4h	*E*	2,3-(OH)$_2$–C$_6$H$_3$-	OCH$_3$	4.02	4.15	4.06
4i	*E*	3-OH-4-OCH$_3$–C$_6$H$_3$-	OCH$_3$	25.88	27.49	>30
4j	*E*	2-OH-5-OCH$_3$–C$_6$H$_3$-	OCH$_3$	>30	>30	>30
4k	*E*	3,4-(OCH$_3$)$_2$–C$_6$H$_3$-	OCH$_3$	>30	>30	>30
4l	*E*	2,5-(OCH$_3$)$_2$–C$_6$H$_3$-	OCH$_3$	>30	>30	>30
4m	*E*	3,4-OCH$_2$O–C$_6$H$_3$-	OCH$_3$	>30	>30	>30
5	*E*	2,5-(OH)$_2$–C$_6$H$_3$-	OCH$_3$	4.43	4.59	5.00
8aa		(Refer to Scheme 2)		>30	>30	>30
8ab		(Refer to Scheme 2)		>30	>30	>30
8ba		(Refer to Scheme 2)		0.50	1.31	3.22
8bb		(Refer to Scheme 2)		0.57	1.32	2.29
9aa		(Refer to Scheme 2)		0.18	0.65	0.87
9ab		(Refer to Scheme 2)		0.32	0.71	0.91
9ba		(Refer to Scheme 2)		8.69	14.35	1.78
9bb		(Refer to Scheme 2)		7.16	9.03	0.78
		DHAQ[c]		>30	>30	>30
		Adriamycin		0.11	0.17	0.21

[a]B16, murine melanoma; HCT116, human colon cancer; A431, human epidermoid carcinoma.
[b]The sample concentration produces a 50% reduction in cell growth.
[c]DHAQ, 1,4-dihydroxy-9,10-anthraquinone, was synthesized as described previously.[15]

cinnamates (**4d**–**4m**) were synthesized in good yields by a Wittig reaction. In all cases, predominant *E*-isomers (>90% of the total products) were formed which were unambiguously characterized based on a coupling constant value of the two olefinic protons (around 16.5 Hz for *E*-isomer and 8 Hz for *Z*-isomer) and NOE techniques. Isolation of a *Z*-isomer attempted for methyl 2,5-dihydroxycinnamate resulted in 7% yield of **4e**. The two caffeamides **3a**, **3b** were synthesized through a sequence shown in Scheme 1: protection of the phenol groups with methoxycarbonyls, coupling of the protected caffeic acid with hydroxyamine or aniline and deprotection by sodium methoxide. Reduction of the double bond of the propenoate **4d** was carried out using hydrogen (1 atm) and Pd/C (10%) to afford the dihydrocinnamate **5** in a quantitative yield.



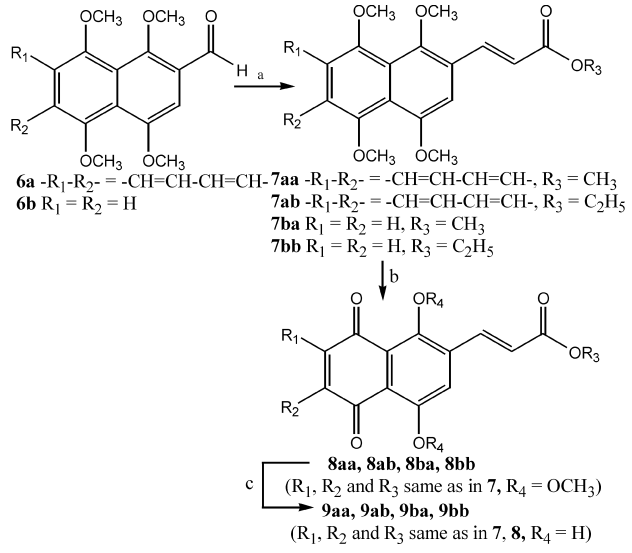
Scheme 1. (a) (i) CH_3OCOCl, TEA, THF; (ii) $R''NH_2$, DCC, HOBt, CH_3CN; (iii) CH_3ONa, CH_3OH; (b) $R'OH$, HCl, reflux, 3 h; (c) Ph_3PCH_2COOR', benzene, reflux, 12 h; (d) Pd–C (10% Pd), H_2, 1 atm, 30 min.

(*E*)-3-[2-(1,4-Dihydroxy-9,10-dione)anthracenyl]- and (*E*)-3-[2-(1,4-dihydroxy-5,8-dione)naphthalenyl]-2-propenoates were synthesized from 2-formyl-1,4,9,10-tetramethoxyanthracene[15] (**6a**) and 2-formyl-1,4,9,10-tetramethoxynaphthalene[16] (**6b**) as shown in Scheme 2. Oxidative demethylation of **7** with cerium diammonium nitrate afforded **8** in good yields (85–90%). Here, the electron-withdrawing effect of the α,β-unsaturated carbonyl side chain might be attributed to the occurrence of demethylation at 5,8-positions but not 1,4-positions of **7ba** and **7bb**. This has been fully explained previously.[17] The use of aluminum chloride in nitrobenzene as a solvent effected a further selective 1,4-demethylation while the ester moiety remained intact.[18]

Bioactivity

The synthesized compounds were assayed[19] against three cancer cell lines including murine melanoma (B16), human colon cancer (HCT116) and human epidermoid carcinoma (A431). From the IC_{50} values summarized in Table 1, one general observation for the cinnamates can be drawn: Compounds bearing *ortho*- or *para*-hydroquinone moieties (**4a**–**4d**, **4f**, **4h**, **5**) exhibited significant cytotoxic activity ($IC_{50} \leq 5$ μg/mL) while compounds with otherwise-substituted benzene rings were all inactive. For example, methylation of one hydroxy group (compounds **4i**, **4j**) or both (compounds **4k**, **4l**, and **4m**) abolished cytotoxicity. It has been reported that hydroquinones are easily autoxidized in

vivo to quinones[20] which are known to elicit cytotoxicity per se or through a cascade of a redox cycle.[21] Thus, the possible formation of quinonoid metabolites from *ortho*- or *para*-hydroquinone compounds (**4a**–**4d**, **4f**, **4h**, **5**) might be one of the mechanisms responsible for their cytotoxicity. Moreover, the quinonoid metabolites should be more electron-deficient at the β-position compared to the parent hydroquinones, thus more labile to cellular nucleophiles such as GSH. Compound **4g** bearing a 3,5-dihydroxy substituent, which is impossible for the formation of quinonoid metabolite, was found inactive. The 2,5-dihydroxy-substituted pattern seemed to be most favorable for the cytotoxic activity, followed by the 3,4- and 2,3-dihydroxy-substituted ones (compound **4d** was more potent than **4a** which was in turn more cytotoxic than **4h**). Compounds **4d** and **4f** were most potent among the cinnamates with IC_{50} values of less than 0.3 μg/mL in all three tested cell lines. Interestingly, it was found that the *E* configuration proved essential for the cytotoxicity; compound **4e**, a *Z*-isomer of cytotoxic **4f**, was inactive at the highest concentration assayed (30 μg/mL). Saturation of the double bond of the propene moiety (**5**) dramatically reduced the cytotoxicity. Thus, the α,β-unsaturated carbonyl moiety of cinnamates was demonstrated to be important for the cytotoxicity and appears to play an important role in the alkylation of cellular nucleophiles, preferentially via Michael addition.[14] Furthermore, the ester group was found to be necessary for the strong activity; replacement of the ester by amide or hydroxy groups substantially decreased or abolished cytotoxicity of the related compounds (**3a**, **3b** and **1** vs **4a**). Variation of the alkyl size did not affect the cytotoxicity significantly (**4a**–**4c**).



Scheme 2. (a) $Ph_3PCH_2COOR_3$, benzene, reflux, 12 h; (b) CAN, CH_3CN–H_2O; (c) $AlCl_3$, nitrobenzene.

The rationale behind the synthesis of compounds **9aa**, **9ab** was based on the consideration of the northeastern part in the structures of doxorubicin (Adriamycin®) and daunorubicin (Fig. 2), two drugs currently used in cancer chemotherapy. As shown in Table 1, compounds **9aa**, **9ab** were almost as cytotoxic as Adriamycin®. The two compounds **8aa**, **8ab**, bearing a 2-(1,4-dimethoxy-9,10-dione)anthracenyl moiety corresponding to the 2-(1,4-dihydroxy-9,10-dione)anthracenyl group in **9aa**, **9ab**, were found inactive, suggesting the essentiality of

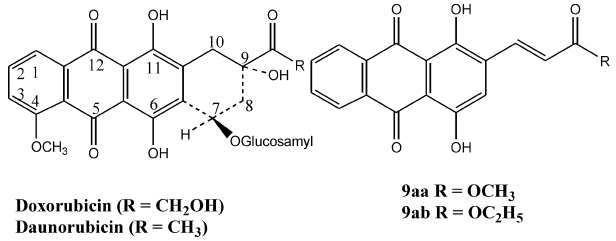


Figure 2.

Table 2. Cytotoxicity of representative compounds in a panel of cancer cell lines

Organ	Cell line	Cytotoxicity (IC_{50})[a] (μg/mL)						
		4d	**4f**	**8ba**	**8bb**	**9aa**	**9bb**	**ADR**[b]
Lung	NCI-H23	0.93	0.65	1.01	1.02	0.39	5.16	0.10
	A549	7.81	7.15	1.12	0.93	1.13	4.78	0.20
Colon	HCT 116	0.17	0.19	1.31	1.32	0.65	9.03	0.17
	HCT 15	0.27	0.26	0.97	0.68	0.66	1.58	0.53
	COLO 205	0.97	2.22	0.83	0.92	0.73	3.69	0.11
	HCC2998	5.22	5.43	3.21	0.78	0.95	4.59	0.17
	SW620	0.39	0.29	0.30	0.11	0.45	1.47	0.17
	HT 29	1.10	2.14	1.11	1.02	0.55	2.16	0.21
	DLD-1	0.68	0.77	0.99	1.15	0.89	3.28	0.32
Melanoma	LOX-IMVI	0.28	0.33	0.11	0.35	0.09	0.91	0.11
	UACC62	0.56	0.57	0.59	0.68	0.11	3.69	0.06
	B16	0.12	0.21	0.50	0.57	0.18	2.56	0.11
Breast	MDA-MB-231	0.82	0.53	0.98	1.14	0.74	3.69	0.24
	MCF7	0.37	0.67	1.21	0.96	0.46	4.69	0.13
Prostate	PC-3	0.42	0.74	0.49	0.64	2.72	5.67	0.30
CNS	SNB19	2.59	2.26	1.25	1.32	0.69	2.49	0.29
Renal	ACHN	0.73	0.82	0.61	0.58	0.42	3.19	0.14
	Mean	1.37	1.48	0.97	0.83	0.69	3.95	0.20

[a]The sample concentration produces a 50% reduction in cell growth.
[b]ADR, Adriamycin.

the *para*-dihydroxy functionality for the strong cytotoxicity of **9aa** and **9ab**. Noteworthy however, a 1,4-dihydroxy-9,10-anthraquinone (DHAQ) was also inactive up to 30 μg/mL in all cell lines assayed. Thus, the incorporation of the propenoate moiety onto DHAQ conferred a strong cytotoxicity to the resulting compounds **9aa** and **9ab**. To the best of our knowledge, this practice has not been reported for the anthracyclines though hundreds of analogues of this class have been prepared in the past decades. In **9aa** and **9ab**, the propenoate side chain might play an important role in enhancing the oxidative formation of an anthracene-1,4,9,10-tetraone metabolite, which is then available for rereduction and hence undergoes a futile redox cycling, a known action mechanism of anthraquinones.[21] It is expected that the introduction of a double bond connecting C9–C10 in the structure of doxorubicin could produce a novel analogue with superior bioactivity without increasing complication of mutagenic and carcinogenic toxicity as explained in the preceding paragraph. This venture remains to be studied.

Encouraged by the interesting results obtained from **9aa** and **9ab**, we went on to synthesize two naphthoquinone derivatives **8ba** and **8bb**. Previously, Song et al.[16] reported several series of highly cytotoxic naphthoquinones against leukemic cell lines but none of them was active against solid tumor cells. Our result showed that naphthoquinones **8ba** and **8bb** were significantly cytotoxic against all three solid tumor cell lines with IC_{50} values as low as 0.5 μg/mL. No naphthoquinone derivatives exerting notable cytotoxicity against solid tumor cells have been documented at the time of writing. 1,4-Demethylation of **8ba** and **8bb** slightly enhanced their activity toward A431 but substantially decreased cytotoxicity against B16 and HCT116. This shift is still not fully understood though it has been noted for other naphthoquinones on leukemia cells previously.[16]

Further evaluation of the most active compounds was carried out in a panel of cancer cell lines and the results are shown in Table 2. The naphthoquinones **8ba** and **8bb** and anthraquinone **9aa** showed IC_{50} values of less than 1 μg/mL in most of the cell lines. It is noteworthy to mention that two cell lines A549 (lung carcinoma) and HCT116 (colon cancer) were rather resistant toward the two cinnamates **4d** and **4f**.

In conclusion, we have found potent cytotoxicities displayed by methyl and ethyl 2,5-dihydroxycinnamates against a variety of cancer cell lines. We also discovered simple analogues (*E*)-methyl/ethyl 3-[2-(1,4-dihydroxy-9,10-dione)anthracenyl]-2-propenoates with an almost comparable cytotoxicity to Adriamycin® and (*E*)-methyl/ethyl 3-[2-(1,4-dimethoxy-5,8-dione)naphthalenyl]-2-propenoates, the first naphthoquinone derivatives showing strong cytotoxicities against solid tumor cell lines. Evaluation against multi-drug resistance cells as well as in vivo experiments are underway.

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