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Preliminary Structure–Antiangiogenic Activity Relationships of 4-Senecioyloxymethyl-6,7-dimethoxycoumarin

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Abstract—Through a systematic modification of the novel angiogenesis inhibitor 4-senecioyloxymethyl-6,7-dimethoxycoumarin (**1**) we found that a 6,7-dimethoxy moiety is important for bioactivity of **1**. Replacement of the lactone functionality in coumarin **1** by an amide decreased its activity. By substitution of the senecioyl chain with various cinnamoyl groups we discovered **6d**, bearing a 4-methoxycinnamoyl instead of senecioyl side chain, with inhibitory activity in HUVEC tube formation assay enhanced by one order of magnitude compared to **1**. We have also synthesized compound **12**, an analogue of **6d**, with equipotency and improved water solubility.

Introduction

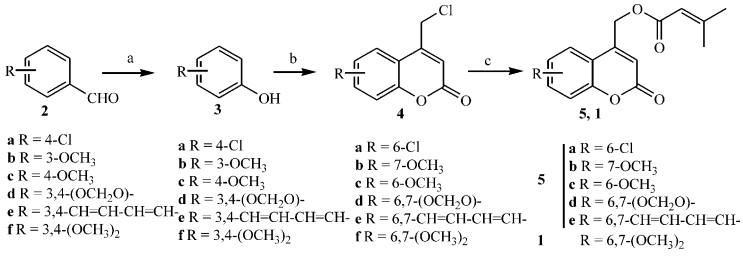
Coumarins constitute a class of compounds which are found widely in nature and possess diverse biological activities, for example, antimicrobial,[1–5] antifungal,[6–8] antiviral[9–14] including human immunodeficiency virus (HIV).[10–14] In cancer drug development arena, coumarin-type compounds have attracted increasing interests recently. Several studies reported a number of coumarins, natural[15–18] or synthetic,[19–26] with marked cytotoxic activities.

In our own works, we have previously reported the isolation of a novel metabolite, namely 4-senecioyloxymethyl-6,7-dimethoxycoumarin (**1**, Scheme 1), from the plant *Crinum latifolium*. This compound was shown to exhibit significant cytotoxicity against human umbilical venous endothelial cells (HUVEC) while manifesting a marginal effect on tumor cells.[27] Thus, **1** possesses a potential for further development as an antiangiogenic agent. As already widely known, angiogenesis inhibitors represent an important new class of anticancer agents.

From this perspective, we have initiated a study on the structure–activity relationship of this molecule. In this paper, the preliminary results from this study are presented and discussed.

Chemistry

Several series of simple analogues of **1** were synthesized as shown in Schemes 1–4. A series of 6- and/or 7- substituted 4-senecioyloxymethylcoumarins (**5**) were



Scheme 1. (a) $MeOH/H_2O_2$ (1:1), H_2SO_2 (cat.), rt, 48 h; (b) $ClCH_2COCH_2CO_2C_2H_5$, H_2SO_2 (excess), 0 °C, 2 h; (c) senecioic acid (excess), CH_3CN, rt, 30 min.

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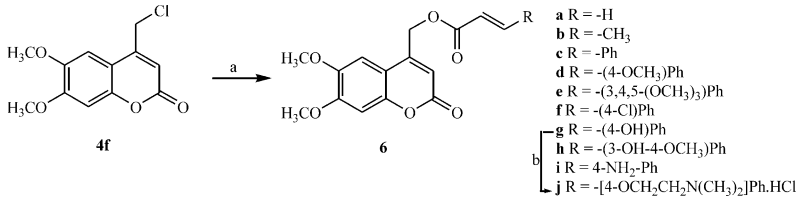
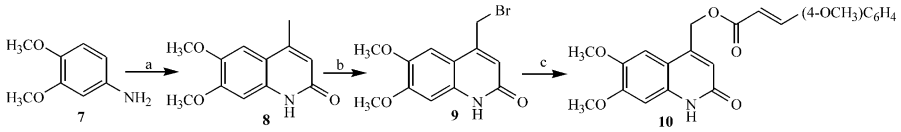
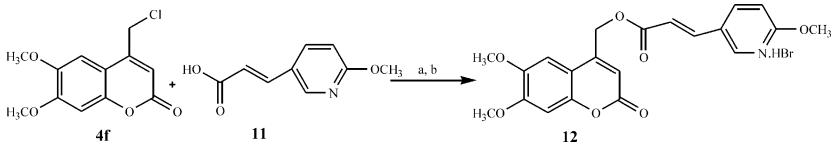


Scheme 2. (a) Carboxylic acid (excess), CH_3CN, TEA, rt, 30 min. (b) (i) NaH, NaI, DMF, rt, 3 h; (ii) 4 N HCl/dioxane, 1 h.



Scheme 3. (a) $CH_3COCH_2CO_2C_2H_5$, H_2SO_2 (excess), 0 °C, 2 h; (b) *N*-bromosuccinimide (1 mol equiv), benzene, reflux, 6 h; (c) 4-methoxycinnamic acid (excess), CH_3CN, TEA, rt, 30 min.



Scheme 4. (a) CH_3CN, TEA, rt, 30 min; (b) 40% HBr/AcOH.

obtained simply by reaction of senecioic acid with **4** which were reached from appropriate phenols **3** and ethyl 4-chloroacetoacetate by using Pechman condensation conditions, thus involving stirring of phenol and ethyl 4-chloroacetoacetate in neat sulfuric acid at room temperature for 12–16 h (Scheme 1). The phenols **3** were achieved by acid-catalyzed oxidation of the aldehydes **2** with hydrogen peroxide using a protocol developed by Matsumoto and co-workers.[28] This reaction proved to be superior to Bayer–Villiger reaction, as separation from by-products was much more convenient.

Analogues with the common structure **6** were obtained simply by reaction of various carboxylic acids, for example, acrylic acid, tigloic acid, substituted cinnamic acids, with 4-chloromethyl-6,7-dimethoxycoumarin (**4f**) synthesized above. Nucleophilic displacement of the chlorine in 2-(dimethylamino)ethyl chloride with **6g** led to **6j** (Scheme 2).

The quinoline analogue **10** of **6g** was explored through a sequence shown in Scheme 3. The Pechman condensation of 4-aminoveratrole (**7**) with ethyl acetoacetate afforded the intermediate **8** (6,7-dimethoxy-4-methylquinolin-2-one). When ethyl 4-chloroacetoacetate was used, a complex mixture of products was formed, probably due to the reaction of the amino group with the chloro moiety also occurring. The intermediate **8** was transferred into **9** in 67% yield by a reaction with *N*-bromosuccinimide in a molar ratio of 1:1 in anhydrous benzene at reflux temperature for 6 h. If excess *N*-bromosuccinimide was used, 4-dibromomethyl-6,7-dimethoxyquinolin-2-one was also obtained as a side product. The target compound **10** was furnished simply by a reaction of **9** with 4-methoxycinnamic acid as described above.

The final analogue **12** was synthesized by reacting **4f** with **11**, which was synthesized as described in the literature.[29] All compounds were confirmed straightforwardly by spectroscopic methods (MS, IR and ^1H NMR).[30]

Biological Results and Discussion

Attempts to discern structural features essential for the antiangiogenic activity of compound **1** were first directed on the substituent pattern on ring B of the coumarin skeleton. Accordingly, a series of compounds **5** were synthesized. The synthesized compounds **5a–e** and the parent compound **1** were examined for cytotoxicity in two tumor cell lines, namely B16 and HCT116.[31] It was revealed that all compounds assayed showed no significant cytotoxic activity in both cell lines tested (Table 1). To evaluate the antiangiogenic property of the compounds in this series, all compounds were subjected to in vitro HUVEC assay[31] at 3 μg/mL. The results summarized as inhibition percentages in Table 1 revealed that removal of a methoxy group at positions 6 and 7 was detrimental for antiangiogenic activity. Compound **5a** bearing a 6-chloro substituent was also inactive in the HUVEC assay model. Contraction of the 6,7-dimethoxy group to the 6,7-methylenedioxy substituent (**5d**) led to a decrease in bioactivity. Meanwhile, extension to one additional benzene moiety at 6,7-position brought about a similar result with compound **5e** being less potent than **1**. Though other substituents have not yet been examined, at this stage, it could be suggested that the 6,7-dimethoxy substituent is important for the antiangiogenic property of compound **1**. Therefore, it was maintained throughout the next phase of this work.

Table 1. Cytotoxicity and antiangiogenic activity of synthesized compounds

Compd	R in **5** and **6**	Cytotoxicity[a] (IC_{50},[b] μg/mL)		Activity in the in vitro HUVEC assay	
		B16	HCT116	Concentration[c]	Inhibitory percentage[d]
1	6,7-$(OCH_3)_2$	>10	>10	3	86.5
5a	6-Cl	>10	>10	3	—[e]
5b	7-OCH_3	>10	>10	3	—
5c	6-OCH_3	>10	>10	3	—
5d	6,7-(OCH_2O)—	>10	>10	3	43.2
5e	6,7-CH=CH–CH=CH—	>10	>10	3	62.5
6a	–H	>10	>10	3	—
6b	$–CH_3$	>10	>10	3	—
6c	$–C_6H_5$	>10	>10	3	12.0
6d	–(4-OCH_3)C_6H_4	0.96	1.02	1 (toxic)	100.0
				0.3	81.7
	HCl			0.1	47.5
6e	–(3,4,5-OCH_3)$_3C_6H_2$	6.74	8.31	3	18.7
6f	–(4-Cl)C_6H_4	>10	>10	3	—
6g	–(4-OH)C_6H_4	4.15	5.21	3	19.7
6h	–(3-OH-4-OCH_3)C_6H_3	3.00	3.41	3 (toxic)	35.5
6i	–(4-NH_2)C_6H_4	>10	>10	3	—
6j	–[4-$O(CH_2)N(CH_3)_2$]$C_6H_4 \cdot HCl$	>10	>10	3	36.5
10		>10	>10	3	46.4
12		1.27	2.43	0.3	78.8
Suramin[f]		#[g]	#	30 μM	
Adriamycin		0.09	0.11		#

[a]Measured in tumor cells: B16, murine melanoma; HCT116, human colon.
[b]The concentration (g/mL) that produces a 50% reduction in cell growth.
[c]Non-cytotoxic concentration in both B16 and HCT116 cell lines, unless otherwise noted.
[d]Calculated as described in ref 31. The numbers represent the averaged results from triplicate experiments with deviation of less than 10%.
[e]Inactive (inhibition percentage was less than 10%).
[f]An established angiogenesis inhibitor.
[g]Not assayed.

The next phase of structural modifications carried out on **1** was focused on the senecioyl moiety. We initially replaced this moiety by acryloyl and tigloyl groups and found that the corresponding compounds **6a**,**b** were completely inactive. Compound **6c**, resulting from the replacement of the senecioyl chain by a cinnamoyl, was also found to be inactive. We continued our attempts with some substituted cinnamoyl chains and obtained compound **6d**, bearing a 4-methoxycinnamoyl side chain, with much enhanced bioactivity. This compound showed cytotoxicity against tumor cell lines (B16 and HCT116) with IC_{50} values of 0.96 and 1.02 μg/mL, respectively. Thus, the cytotoxicity of this compound was increased by more than one order of magnitude. In parallel, its inhibitory effects in HUVEC tube formation assays were also found to be enhanced by nearly 10 times; at 0.3 μg/mL, **6d** showed inhibitory percentage of 81.7%, almost comparable to that of **1** at 3 μg/mL. A higher concentration of this compound (1 μg/mL) completely inhibited the formation of tube network by HUVECs; however, this concentration was toxic in tumor cells. A lower concentration of **6d** (0.1 μg/mL) exhibited considerable activity with an inhibitory percentage of 47.5%.

Replacement of the 4-methoxy group in **6d** by either 4-Cl or 4-NH_2 substituents (**6e**,**h**) was detrimental for its bioactivity. Introduction of additional substituents into the 3- and 5-positions (**6f**,**i**) led to decreased activity. Thus, the 4-methoxy group seems to be the optimal substituent on the cinnamoyl side chain.

The quinoline **10** was found to be less potent than **1**. Thus, it appears that the lactone functionality in coumarin **1** is important for the binding of the molecule to receptors, probably through hydrogen bonding.

At this stage, we have identified compound **6d** as the most potent one. However, this compound showed very poor solubility in aqueous systems which rendered the in vivo evaluation difficult. We further attempted to look for analogues with improved water solubility. Initially we synthesized compound **6j** with expectation that this compound might retain bioactivity of **6d** and in the meantime offer reasonable water solubility. Unfortunately, however, **6j** was shown to be much less active than **6d**, probably due the fact that the 4-substituent in **6j** is too bulky and not tolerable for bioactivity. Finally, we came up with the design of **12**. In this compound, the pyridinyl moiety should much resemble the phenyl moiety in **6d**. Thus, the two compounds are expected to be similar in conformation. Meanwhile, though we have not evaluated in detail, it is obvious that the water solubility of **12** in the hydrobromide salt form should be much enhanced compared to **6d**. Interestingly, compound **12** was found to be almost equipotent with **6d** in HUVEC assay, though it was slightly less active towards tumor cells.

In summary, through this study we have identified that the 6,7-dimethoxy moiety is important for bioactivity of compound **1**. Replacement of the lactone functionality in coumarin **1** by an amide decreased its activity. By substitution of the senecioyl chain with various cinnamoyl

groups we found compound **6d** with inhibitory activity in HUVEC tube formation assay enhanced by one order of magnitude. We have also synthesized compound **12**, an analogue of **6d**, with equipotency and expected to have much improved water solubility compared to **6d**. Further in vivo evaluations of **12** and more extended investigation on this new lead are underway in our lab.

References and Notes

1. Eid, A. I.; Ragab, F. A.; el-Ansary, S. L.; Gazayerly, S. M.; Mourad, F. E. *Arch. Pharm. (Weinheim)* **1994**, *327*, 211.
2. Hogberg, T.; Vora, M.; Drake, S. D.; Mitscher, L. A.; Chu, D. T. *Acta Chem. Scand. B* **1984**, *38*, 359.
3. el-Fattah, M. E. A. *Arch. Pharm. Res.* **1998**, *21*, 723.
4. Leolardo, B.; Philippo, C. H.; Elkhaili, F. J.; Silvio, M. L.; Giuseppe, L.; Fedele, M.; Henry, M.; Davide, P.; Daniela, S. *Il Farmaco* **1998**, *53*, 425.
5. Dejardin, J. V.; Stevens, U.; Jakubowski, B.; Lapiere, C. L. *Ann. Pharm. Fr.* **1983**, *41*, 437.
6. Nozawa, K.; Yamada, M.; Tsuda, Y.; Kawai, K.; Nakajima, S. *Chem. Pharm. Bull.* **1981**, *29*, 3486.
7. Nozawa, K.; Yamada, M.; Tsuda, Y.; Kawai, K.; Nakajima, S. *Chem. Pharm. Bull.* **1981**, *29*, 2491.
8. Sardari, S.; Mori, Y.; Horita, K.; Micetich, R. G.; Nishibe, S.; Daneshtalab, M. *Bioorg. Med. Chem.* **1999**, *7*, 1933.
9. Ishikawa, T.; Kotake, K.; Ishii, H. *Chem. Pharm. Bull.* **1995**, *43*, 1039.
10. Xie, L.; Takeuchi, Y.; Cosentino, L. M.; McPhail, A. T.; Lee, H. K. *J. Med. Chem.* **2001**, *44*, 664.
11. Yang, Z. Y.; Xia, Y.; Xia, P.; Brossi, A.; Cosentino, L. M.; Lee, H. K. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1003.
12. Xie, L.; Takeuchi, Y.; Cosentino, L. M.; Lee, H. K. *J. Med. Chem.* **1999**, *42*, 2662.
13. Huang, L.; Kashiwada, Y.; Cosentino, L. M.; Fan, S.; Chen, C. H.; McPhail, A. T.; Fujioka, T.; Mihashi, K.; Lee, H. K. *J. Med. Chem.* **1994**, *37*, 3947.
14. Flavin, M. T.; Rizzo, J. D.; Khilevich, A.; Kucherenko, A.; Sheikman, A. K.; Vilaychack, V.; Lin, L.; Chen, W.; Greenwood, E. M.; Pengsuparp, T.; Pezzoto, J. M.; Hughes, S. H.; Flavin, T. M.; Cibulski, M.; Boulanger, W. A.; Shone, R. L.; Xu, Z. Q. *J. Med. Chem.* **1996**, *39*, 1303.
15. Guh, J. M.; Yu, S. M.; Ko, F. N.; Wu, T. S.; Teng, C. M. *Eur. J. Pharmacol.* **1996**, *298*, 19.
16. Ahsan, M.; Zaman, T. A.; Hasan, C. M.; Ito, C.; Islam, S. K. N. *Fitotherapia* **2000**, *71*, 697.
17. Magiatis, P.; Melliou, E.; Skaltsounis, A. L.; Mitaku, S.; Leonce, S.; Renard, P.; Pierre, A.; Atassi, G. *J. Nat. Prod.* **1998**, *61*, 982.
18. Canedo, L. M.; Fernander Puentes, J. L.; Baz, J. P. *J. Antibiot.* **1997**, *50*, 175.
19. Egan, D.; James, P.; Cooke, D.; O'Kennedy, R. *Cancer Lett.* **1997**, *118*, 201.
20. Valenti, P.; Rampa, A.; Recanatini, M.; Bisi, A.; Belluti, F.; Da Re, P.; Carrara, M.; Cima, L. *Anticancer Drug Des.* **1997**, *12*, 443.
21. Valenti, P.; Rampa, A.; Recanatini, M.; Bisi, A.; Belluti, F.; Da Re, P.; Carrara, M.; Cima, L. *Anticancer Drug Des.* **1997**, *12*, 137.
22. Valenti, P.; Fabbi, G.; Rampa, A.; Bisi, A.; Gobbi, S.; Da Re, P.; Carrara, M.; Sgevano, A.; Cima, L. *Anticancer Drug Des.* **1996**, *11*, 243.
23. Manfredini, S.; Baraldi, P. G.; Bazzanini, R.; Guarneri, M.; Simoni, D.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1994**, *37*, 2401.
24. Baraldi, P. G.; Valenti, P.; Rampa, A.; Recanatini, M.; Bisi, A.; Belluti, F.; Da Re, P.; Carrara, M.; Cima, L. *J. Med. Chem.* **1992**, *35*, 1877.
25. Simoni, D.; Manfredini, S.; Tabrizi, M. A.; Bazzanini, R.; Baraldi, P. G.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1991**, *34*, 3172.
26. Baraldi, P. G.; Guarneri, M.; Manfredini, S.; Simoni, D.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1989**, *32*, 284.
27. Nam, N. H.; Kim, Y.; You, Y. J.; Hong, D. H.; Kim, H. M.; Ahn, B. Z. *Planta. Med.* Submitted for publication.
28. Matsumoto, M.; Kobayashi, H.; Hotta, Y. J. *J. Org. Chem.* **1984**, *49*, 4740.
29. Ashimiro, N.; Nagamura, S.; Kobayashi, E.; Gomi, K.; Saito, H. J. *Med. Chem.* **1999**, *42*, 669.
30. Illustrated data for compound **6d**: Yield 75%. IR (KBr, ν_{max} cm^{-1}) 1720, 1640; ^1H NMR (300 MHz, $CDCl_3$) 7.71 (1H, d, $J=15.43$ Hz), 7.42–7.25 (2H, m), 7.16–6.95 (2H, m), 6.80 (1H, s), 6.78 (1H, s), 6.39 (1H, d, $J=15.43$ Hz), 6.31 (1H, s), 5.24 (2H, s), 3.81 (3H, s), 3.78 (3H, s). HRMS: $[M]^+$ 396.1573 ($C_{22}H_{20}O_7$), calcd 396.1203.
31. (a) Cytotoxic and in vitro HUVEC tube formation assays were performed as described in our previous reports: Kim, Y.; Kim, S. B.; You, Y. J.; Ahn, B. Z. *Planta Med.* **2002**, *68*, 271. (b) Nam, N.H.; Kim, H. M.; Bae, K. H.; Ahn, B. Z. *Phytother. Res.* In press. The inhibition percentages were calculated as described therein.