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Synthesis and anti-angiogenesis activity of coumarin derivatives

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Abstract—A series of 7-diethylaminocoumarin compounds were synthesized and the cytotoxicities were tested against human umbilical vein endothelial cell (HUVEC) and some cancer cells. We found that the introduction of cyano groups at the 4-position will promote the bioactivity. In particular, compounds **9** and **10** strongly inhibited the proliferation of various cancer cell lines, and **12** and **15** showed a high selectivity for HUVEC. Therefore, these coumarin molecules can be utilized as lead compounds to develop potential nontoxic angiogenesis inhibitors and small molecular ligands to target HUVEC.

Angiogenesis, the formation of new blood vessels from pre-existing host vasculatures stimulated by biochemical stimulators,[1] is involved in wound healing,[2] embryonic development,[3] and the female reproductive cycle,[4] which is under elaborate regulations in normal vascular system. However, malignant angiogenesis plays a critical role in several fatal diseases including cancer, vascular insufficiency, diabetic retinopathy, and rheumatoid arthritis by a normal delivering mechanism of oxygen and nutrients to cell and tissue.[5] Because tumor angiogenesis caused by angiogenic inducers is the most critical factor in the growth of solid tumors,[6] as well as their invasion and metastasis,[7] early control of angiogenesis may be a promising therapeutic strategy for the related diseases.[8]

On the other hand, detection and imaging of specific marker for each disease at the initial stage are the emerging research areas in medicinal chemistry. For example, various fluorescent probes are useful in detecting the reactive oxygen species such as super oxide radical, hydrogen peroxide, singlet oxygen, and hydroxyl radical, which are involved in several biological malfunctions.[9] The use of fluorescence microscopy to detect early cancer cells and to discriminate between normal and neoplastic lesions has recently attracted considerable attention due to its simplicity and efficiency.[10] In particular, fluorescent probes for detection and imaging of angiogenesis are important and efficient to clinical treatment against cancer because tumor angiogenesis occurs drastically in the initial stage of the growing and metastasis of cancer cell.[11] Furthermore, if fluorescent molecules have the antiangiogenic activity, we can achieve both the imaging and the inhibition against angiogenesis at the same time.

Coumarins are one of the most important classes of fluorescent molecules, and they are found to possess versatile biological activities.[12] In addition, 6,7-dimethylcoumarin-based novel angiogenesis inhibitors were reported recently.[13] As an extension of our study in the combinatorial synthesis of coumarin derivatives for bioimaging,[14,15] we started to systemically screen the antiangiogenic activity of coumarins. In this communication, we report the synthesis of a series of 7-dialkylamino-3,4-substituted coumarin derivatives and the cell proliferation inhibitory activity test of these compounds against cell lines such as U87 MG, B16 BL6, HeLa, DLD-1, SiHa, NIH 3T3, and HUVEC.[16] In particular, by comparison of growth inhibition activity against HUVEC and other cell lines,[17] we will prove that the chemical library produced from a coumarin scaffold can produce novel angiogenesis inhibitors with high selectivity over tumor cells, and it is

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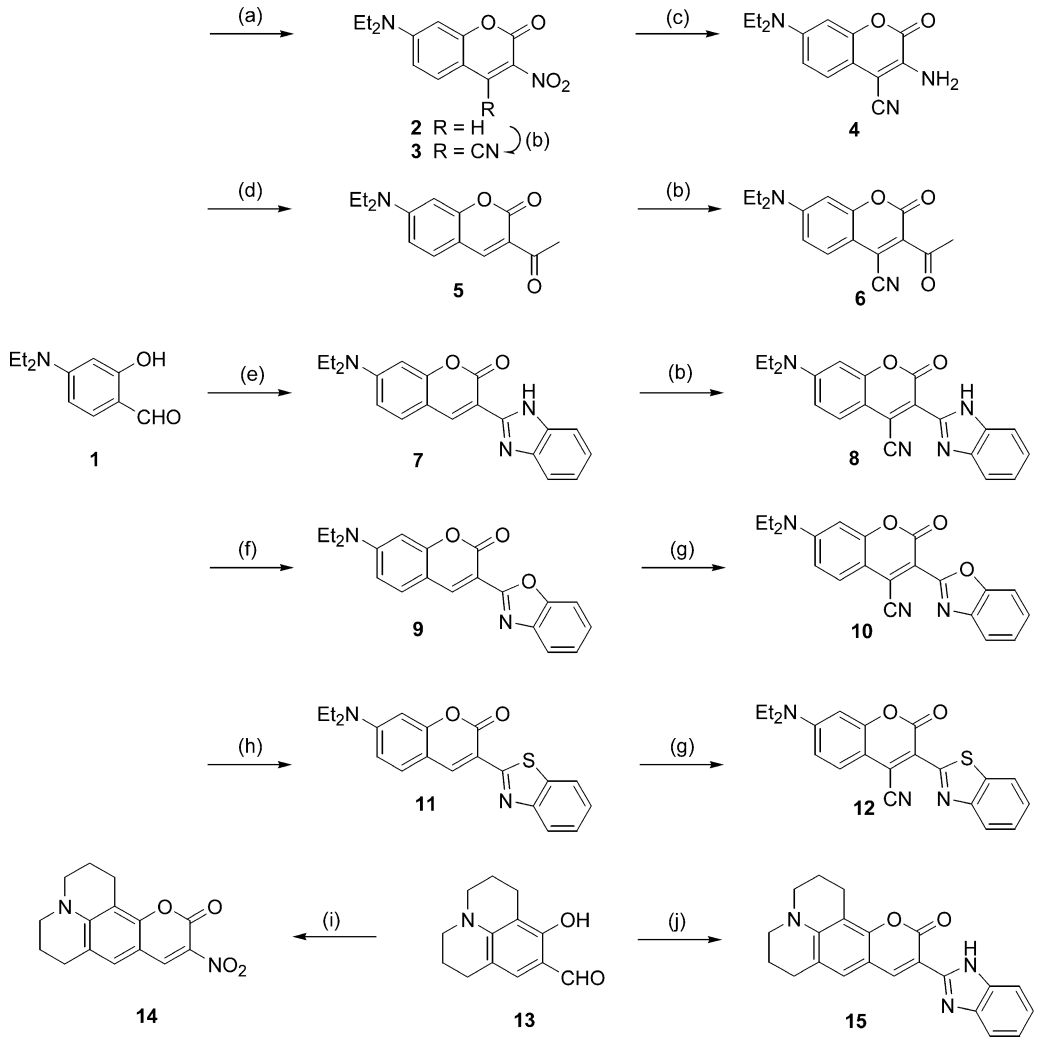

Scheme 1. Reagents and conditions: (a) ethyl nitroacetate (1 equiv), *n*-BuOH, morpholine (cat.), acetic acid (cat.), molecular sieve 3 Å, 0 °C, overnight, 79%; (b) NaCN (2 equiv), I_2 (1 equiv), DMSO, rt, for **4**: 83%, for **6**: 95%, for **8**: 57%; (c) $SnCl_2{\cdot}2H_2O$ (8 equiv), concd HCl, rt, 4 h, 67%; (d) ethyl acetoacetate (1 equiv), EtOH, morpholine (cat.), reflux, overnight, 30%; (e) i—2-cyanomethylbenzimidazole (1 equiv), MeOH, DMF, piperidine (cat.), rt, overnight, ii—17% HCl, rt, overnight, 70% for 2 steps; (f) (3-methoxypropyl)benzooxazol-2-yl-acetate (1 equiv), iso-propanol, reflux, 12 h, 33%; (g) NaCN (2 equiv), lead tetraacetate (1 equiv), DMSO, 40 °C, 2 h to rt, 10 h, for **10**: 88%, for **12**: 79%; (h) ethyl 2-(2-benzothiazolyl)acetate (1 equiv), EtOH, piperidine (cat.), 50 °C, 12 h, 82%; (i) Ethyl nitroacetate (1 equiv), *n*-BuOH, DMF, molecular sieve 3 Å, reflux, 12 h, 74%; (j) i—2-cyanomethylbenzimidazole (2 equiv), EtOH, piperidine (cat.), reflux, overnight, ii—concd HCl, reflux, 3 h, 70% for 2 steps.

potentially possible to inhibit and detect tumor angiogenesis simultaneously.

In order to establish chemical library with various functionalities and structural diversity, we began with the synthesis of simple coumarin derivatives from 4-(*N*,*N*-diethylamino)salicylaldehyde (**1**) and 8-hydroxy-9-formyljulolidine (**13**) initially. As shown in Scheme 1, 3-amino-7-diethylaminocoumarin (**3**) was synthesized from reaction of **1** with ethyl nitroacetate in butanol and successive reduction of nitro group of **2** with $SnCl_2{\cdot}2H_2O$ in a concentrated HCl solution. The cyano group was introduced at the 4-position in the coumarin moiety by treating **3** with NaCN and iodine in DMSO regioselectively to afford 3-amino-4-cyano-7-diethylamino coumarin (**4**).[18] 3-Acetyl-7-diethylamino coumarin (**5**) synthesized from a similar condensation reaction of **4** and ethyl acetoacetate in ethanol was transformed to 4-cyanocoumarin (**6**) in 95% yield.

For structural diversity, commercially available yellow coumarin fluorescent dyes containing electron accepting heteroaromatic groups at the 3-position were synthesized by the known method.[19] This was accomplished through base-catalyzed condensation of **1** with 2-cyanomethylbenzimidazole to give a 3-(2′-benzimidazolyl)coumarin known as coumarin-7 via hydrolysis of iminolactone intermediate,[19] which was transformed to 4-cyanocoumarin derivative **8** by cyanation with NaCN.[20] 4-Cyano-3-(2′-benzoxazolyl)coumarin (**10**) and 4-cyano-3-(2′-benzothiazolyl)coumarin (**12**) were synthesized by a similar pathway and the reaction conditions are shown in Scheme 1.[21] Additionally, two benzoquinolizine coumarin structures **14** and **15** with similar electronic property but different structural features at 7-position were also synthesized.

As listed in Table 1, simple coumarin derivatives (**2**–**6**) did not show any cytotoxicity against both tested cancer

Table 1. Biological activity of fluorescenic coumarin derivatives against various cancer cell lines, and HUVEC

Compound	Cytotoxic activity against cancer cell lines[a] (IC_{50}, μM)						HUVEC growth inhibition[a] (IC_{50}, μM)
	U87	B16	HeLa	DLD-1	SiHa	NIH 3T3	
2	>70	>70	>70	>70	42.3	37.7	25.9
3	>70	>70	>70	>70	>70	>70	>70
4	>70	>70	>70	>70	>70	31.2	>70
5	>70	>70	>70	>70	>70	>70	>70
6	>70	>70	>70	>70	>70	17.9	>70
7	15.0	12.6	>70	>70	62.1	9.3	9.6
8	43.0	43.0	54.4	>70	>70	20.4	19.8
9	0.21	1.3	1.2	0.21	2.0	2.8	0.12
10	1.8	5.6	4.8	1.8	>70	4.8	3.0
11	>70	>70	>70	>70	>70	>70	>70
12	>70	>70	>70	>70	>70	>70	4.8
13	>70	>70	>70	>70	>70	>70	35.4
14	>70	>70	>70	>70	>70	>70	>70
15	33.4	8.9	11.6	35.2	14.6	41.4	3.3
Paclitaxel	0.08	0.08	0.47	0.09	3.5	1.29	0.06

[a] IC_{50} was calculated from the nonlinear regression by Graphpad Prism software.

cell lines and HUVEC, except that compound **2** showed weak inhibition activity against HUVEC. Because **2** has selective activity against HUVEC over other cancer cells, it could be interesting to perform further synthesis and screening of analog coumarin molecules for development of selective angiogenesis inhibitors.

In the next trial of synthesis, we introduced hydrophobic functional groups at 3-position for enhancing van der Waals interaction to the active site. Cytotoxicity of compounds **7** and **9** designed from this postulation was reasonably increased. In particular, when compared to the control drug, paclitaxel, compound **9** with oxazolidine substitution at the third position showed a higher inhibitory activity in submicromolar range, but, unfortunately, showed no selectivity for HUVEC over cancer cells. The most interesting results were obtained by 4-cyanation of **7**, **9**, and **11**, which led to 3-aromatic-4-cyanocoumarins showing some impacts on the inhibition activity or high selectivity, respectively. As shown in Table 1, although the cyanide compounds, **8** and **10** showed weaker inhibition activity upon cancer cells and HUVEC, 3-(2′-benzothiazole)-4-cyanocoumarin (**12**) gave a high selectivity as well as antiproliferation activity against HUVEC. Based on this synthetic trial, we could find a possibility to improve the potency and selectivity by adding a new functionality to mother scaffold. Therefore, it can potentially be a selective angiogenesis inhibitor from further elaboration.

Julolidine derivatives **14** and **15** synthesized from **13** also gave a similar result as former entries. Although **14** did not show any inhibition activity, **15** showed a moderate inhibition against cancer cells, and strong inhibition activity and selectivity against HUVEC.

In conclusion, from the preliminary biological activity screening by using the coumarin library synthesized from two staring materials **1** and **13**, we found that certain coumarin molecules showed growth inhibition activity against both cancer cells and HUVEC. Furthermore, we found that the introduction of benzothiazolyl and analogous groups at 3-position improved the overall inhibition activity and introducing a cyano group at the fourth position afforded the selectivity towards HUVEC. In particular, the inhibition activity of **9** against tested cancer cell lines was comparable to a positive control, paclitaxel, and compounds **12** and **15** showed a high selectivity for HUVEC, which can be potentially utilized as lead compounds to develop nontoxic angiogenesis inhibitors and small molecular ligands to target HUVEC. Moreover, it is potentially possible to inhibit and detect tumor angiogenesis at the same time from further screening in vivo.

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21. Selective analytical data for 4-cyanocoumarins. For **4**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40 (d, 1H), 6.63 (d, 1H), 6.50 (s, 1H), 3.48 (q, J = 7.2 Hz, 4H), 4.99 (s, 2H), 1.26 (t, J = 7.2 Hz, 6H); HRMS: calcd for $(M+H)^+$ $C_{14}H_{15}N_3O_2$ 258.1235, found 258.1242. For **6**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.79 (d, J = 9.0 Hz, 1H), 6.73 (dd, J = 9.3, 2.7 Hz, 1H), 6.47 (d, J = 2.4 Hz, 1H), 3.49 (q, J = 7.2 Hz, 4H), 4.99 (s, 2H), 1.27 (t, J = 7.2 Hz, 6H). For **8**: mp 265–267 °C; IR (KBr): 2210 (w), 1705 (s), 1620 (vs), 1528 (s), 1420 (m). ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.3 (s, 1H), 7.89 (m, 1H), 7.32–7.51 (m, 4H), 6.60 (d, J = 6.7 Hz, 1H), 6.5 (s, 1H), 3.48 (q, J = 7.4 Hz, 4H), 1.27 (t, J = 7.4 Hz, 6H). For **10**: δ 7.88 (dt, J = 6.9, 2.4 Hz, 1H), 7.77 (d, J = 9.0 Hz, 1H), 7.66 (dt, J = 6.9, 2.4 Hz, 1H), 7.43–7.40 (m, 2H), 6.75 (dd, J = 9.3, 2.4 Hz, 1H), 6.54 (d, J = 2.4 Hz, 1H), 3.51 (q, J = 7.2 Hz, 4H), 1.28 (t, J = 7.2 Hz, 6H). For **12**: mp 237–239 °C; IR (KBr): 2221 (m), 1712 (m), 1620 (m), 1558 (m), 1504 (s), 1420 (m). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.20 (d, J = 7.9 Hz, 1H), 7.87–7.98 (m, 2H), 7.34–7.55 (m, 2H), 6.78 (d, J = 6.8 Hz, 1H), 6.55 (s, 1H), 3.48 (q, J = 7.0 Hz, 4H), 1.27 (t, J = 7.0 Hz, 6H). For **14**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.60 (s, 1H), 6.99 (s, 1H), 3.23 (m, 4H), 2.64–2.70 (m, 4H), 1.84–1.89 (m, 4H).