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Synthesis and biological activity of (+)-hedychilactone A and its analogs from (+)-sclareolide

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Abstract—Natural product hedychilactone A (**3**) has been synthesized from (+)-sclareolide by an efficient route. Two of the synthetic intermediates, **10** and **12**, have shown strong growth inhibition effects against five cancer cell lines, human umbilical vein endothelial cell (HUVEC) and nitric oxide (NO) production. In particular, compound **15** showed selective inhibition activity against HUVEC growth without any cytotoxicity among tested cancer cell lines.

Zingiberaceae plant *Hedychium coronarium* is widely used in India, China, and Brazil as a traditional medicinal plant for the treatment of inflammation and rheumatism. In addition, the natural products extracted from *H. coronarium* have attracted much attention due to their promising biological activities, such as antitumor and antifungal activities.[1]

In the process of synthesizing these natural products, we have previously discovered that coronarin A (**1**), isolated from *H. coronarium*, 7-*epi*-coronarin A (**2**), and their synthetic intermediates have a moderate inhibitory activity against cancer cell lines.[2] In particular, we believe that these intermediates will be also the inhibitors against tumor angiogenesis based on the screening of human umbilical vein endothelial cell (HUVEC) proliferation and growth factor stimulated tube formation on Matrigel.[3]

Hedychilactone A (**3**) and its related natural products are also isolated from the rhizome of *H. coronarium*, which show inhibitory activities on nitric oxide (NO) production in LPS-activated mouse peritoneal macrophages and vascular permeability induced by acetic acid in mice.[4] In addition, these compounds have an inhibitory effect against the release of β-hexosaminidase induced by dinitrophenylated bovine serum albumin (DNP-BSA) from RBL-2H3 cells sensitized with anti-DNP IgE,[5] and inflammation[6] (Fig. 1).

Interestingly, hedychilactone A (**3**) had been already reported under a different name, pacovatinins A, which was isolated from the seeds of *Renealmia exaltata*, a medicinal plant, with stomachic and vermifugal effects.[7]

In this report, we describe an efficient synthesis of hedychilactone A (**3**) and the investigation on the biological activity of synthetic intermediates, including cytotoxicity against cancer cell lines, antiangiogenesis activity

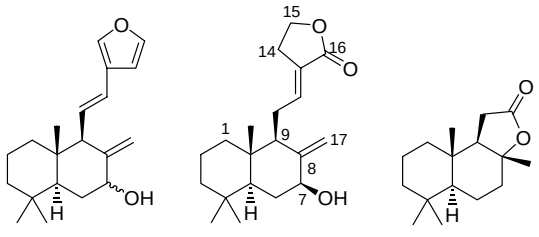

Figure 1.

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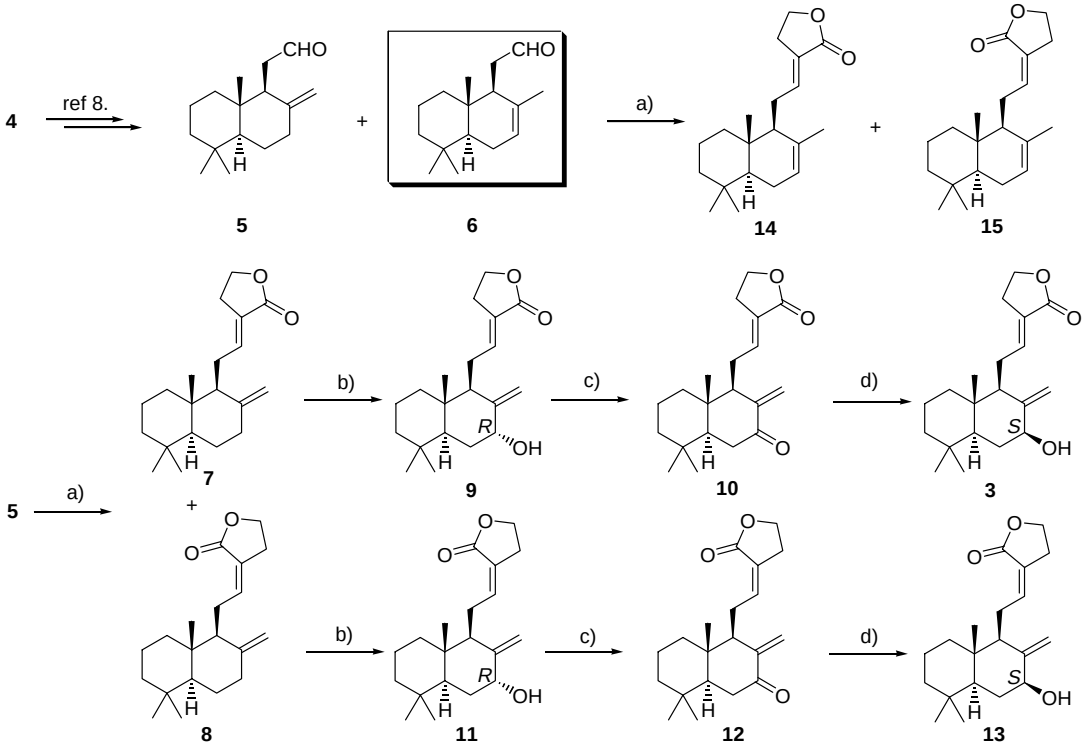
against HUVEC, and nitric oxide (NO) production inhibition activity.

As shown in Scheme 1, important synthetic intermediates (**5** and **6**) were synthesized from commercially available natural chiral building block, (+)-sclareolide (**4**), by a previously described method.[8] This protocol is a highly efficient for each *exo*- and *endo*-methylenedecaline system. For the introduction of α,β-unsaturated butyrolactone of natural target molecule (**3**), the olefination of **5** with diethylphosphono-2-butyrolactone ylide with NaH as base gave an isomeric mixture of major *E*-butyrolactone (**7**) and minor *Z*-butyrolactone (**8**) in a ratio of 3:1, respectively.[2] Each isomer was separated by flash column chromatography with a three solvent eluent system (chloroform/hexane/ethyl acetate = 10:1:1). We distinguished the stereochemistry by a comparison of the NOE effect between H-12 and H-14 in each isomer. Allylic hydroxylation of *E*-isomer (**7**) with SeO_2 and *t*-BuOOH in methylene chloride (MC) for 1 h gave the 7-α-hydroxy isomer (**9**) named as 7-*epi*-hedychilactone A as the only product in 76% yield, which result was very similar in the synthesis of the 7-α-hydroxylation of coronarin A.[3] 7-β-Hydroxyl group of the target compound (**3**) was obtained from 7-α-hydroxy isomer (**9**) by Swern oxidation and successive reduction of α,β-unsaturated ketone **10** with $NaBH_4$ in MeOH with a yield of 70% for two steps.[9]

In order to study the structure–activity relationship, we have also synthesized various isomers from synthetic intermediates. As shown in Scheme 1, it is possible to obtain the series of *Z*-isomers including 7-α-hydroxy **11**, α,β-unsaturated ketone **12**, and 7-β-hydroxy molecule **13** by the same procedures. In addition, two *endo*-methylene α,β-unsaturated butyrolactone analogs, **14** and **15**, were synthesized from *endo*-methylene intermediate **6** by same Horner–Wadsworth–Emmons reaction condition.[10]

As listed in Table 1, we have tried to test the three kinds of screening systems for the synthetic natural product, hedychilactone A (**3**), and its synthetic intermediates. Initially, we have tested the cytotoxicity against various cancer cell lines, including U87, U373, SiHa, sk-ov-3, and B16.[11] Unfortunately, hedychilactone A (**3**) and its stereoisomers (**9**, **11**, and **13**) have weak activities, which means the butyrolactone group did not affect the anticancer activity. Two *exo*-α, β-unsaturated butyrolactones (**7** and **8**) and two *endo*-molecules (**14** and **15**) have also shown low cytotoxicity. However, the compounds **10** and **12** with an α,β-unsaturated ketone skeleton showed strong inhibitions against various cancer cell lines, which was comparable to the anticancer drug, paclitaxel. In particular, the cytotoxicities of **10** and **12** against ovarian cancer are about 10 times more active than that of paclitaxel. We can conclude that the α,β-unsaturated ketone group acts as a Michael acceptor for the nucleophilic addition of cancer cells and plays an important role in enhancing the activity.

Because coronarin A (**1**), the active ingredient of *H. coronarium*, and 7-*epi*-coronarin A (**2**) have shown its antiangiogenic activity based on the screening of HUVEC growth inhibition and tube formation on Matrigel,[3] we expected that hedychilactone A (**3**) and its isomers (**9**,



Scheme 1. Reagents and conditions: (a) diethoxyphosphonobutyrolactone, NaH, toluene, rt, for **7**, 51%; **8**, 17%; for **14**, 49%; **15**, 25%; (b) SeO_2, *t*-BuOOH, MC, rt, for **9**, 76%, for **11**, 82%; (c) oxalyl chloride, DMSO, MC, −78 °C to rt, for **10**, 76%, for **12**, 83%; (d) $NaBH_4$, MeOH, 0 °C to rt, for **3**, 92%, for **13**, 88%.

Table 1. Biological activity of hedychilactone A (**3**) and its analogs against cancer cell lines, HUVEC, and NO production

Compound	Cytotoxic activity against cancer cell lines[a] (IC_{50}, μg/mL)					(IC_{50}, μg/mL)	
	U87	U373	SiHa	sk-ov-3	B16	HUVEC growth inhibition[a]	NO production inhibition[a]
3	38.6	>50	>50	8.7	>50	12.6	11.0
4	>50	46.5	>50	6.8	12.4	7.4	7.2
5	4.1	7.0	14.5	3.1	4.0	2.9	>25
6	11.7	20.6	20.9	23.5	21.8	4.8	6.0
7	5.0	15.1	20.2	2.0	17.6	1.7	5.0
8	>50	40.4	>50	4.3	17.6	2.2	4.5
9	12.7	14.9	32.0	12.4	16.7	6.9	13.2
10	1.7	3.7	7.0	0.9	3.4	1.2	0.7
11	>50	>50	>50	10.1	>50	12.4	8.0
12	1.43	0.6	3.4	0.8	1.5	0.6	0.9
13	>50	15.7	31.7	5.3	>50	13.8	11.2
14	9.2	4.7	17.0	6.0	9.1	2.6	18.0
15	>50	>50	>50	>50	>50	4.3	>25
Paclitaxel	3.2	—[b]	1.3	8.7	<0.1	0.3	—[b]

[a] IC_{50} was calculated from the nonlinear regression by Graphpad Prism software.
[b] Not tested.

11, and **13**) could potentially have similar effect. Two compounds, **10** and **12**, demonstrated particularly strong inhibitory activity against the growth of HUVEC growth, which may result from the cytotoxicity as shown in the test against cancer cell lines.[11]

More interestingly, compound **15**, a regioisomer of **8**, showed very selective inhibition activity against HUVEC growth without any cytotoxicity toward all tested cancer cell lines. This compound and its potential derivatives can also be employed as targeting ligands for HUVEC, which can selectively deliver imaging agents such as fluorescent tags and isotopic probes to target angiogenesis.[12]

Finally, we have tested the inhibitory activity of NO production of hedychilactone A (**3**), its synthetic intermediates, and all structural isomers.[13] Most of the tested compounds effectively inhibited the production of NO in the screening test. In particular, compounds **10** and **12** also showed 10 times stronger activity than the natural product hedychilactone A (**3**).

In conclusion, we have synthesized the natural product, hedychilactone A (**3**), and its structural isomers (**9**, **11**, and **13**) from (+)-sclareolide by efficient synthetic pathway. Based on the screening of inhibitory activity against five cancer cell lines, HUVEC, and NO production, we have discovered two molecules (**10** and **12**) have strong inhibition activity. In particular, unnatural *endo*-methylene α,β-unsaturated butyrolactone analog (**15**) shows a highly selective inhibitory activity against HUVEC growth, which can be potentially utilized as lead compounds to develop small molecular ligands to target HUVEC.

Acknowledgments

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9. Analytical data for **3**: white solid; mp 141–143 °C; $[\alpha]_D^{20}$ +12.7° (*c* 0.700, $CHCl_3$). Reported data from Ref. 4, $[\alpha]_D^{22}$ +12.3° (*c* 0.700, $CHCl_3$); for **13**: white solid; mp 147–148 °C; $[\alpha]_D^{20}$ +55.6° (*c* 0.055, $CHCl_3$); IR (KBr) ν_{max} 3271, 2924, 2862, 1742, 1667, 1441, 1378, 1207, 1168, 1079, 1020 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.15 (m, 1H, H-12), 5.18 (s, 1H, H-17α), 4.70 (s, 1H, H-17β), 4.31 (t, 2H, J = 7.3 Hz, H-15), 3.98 (m, 1H, H-7), 2.87 (m, 4H), 2.13–1.02 (m, 10H), 0.90 (s, 3H, H-18), 0.81 (s, 3H, H-19), 0.72 (s, 3H, H-20); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 150.1, 144.4, 123.2, 104.3, 73.7, 65.2, 55.3, 52.9, 41.8, 39.2, 38.7, 33.9, 33.4, 33.3, 28.9, 22.6, 21.5, 19.1, 14.3.
10. Analytical data for compound **9**: white solid; mp 127–128 °C; $[\alpha]_D^{20}$ −83.3° (*c* 0.02, $CHCl_3$); IR (KBr) ν_{max} 3401, 3080, 2926, 2867, 1754, 1674, 1440, 1386, 1289, 1216, 1032 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.68 (m, 1H, H-12), 5.03 (s, 1H, H-17α), 4.50 (s, 1H, H-17β), 4.37 (m, 3H, H-7, 15), 2.88 (m, 2H, H-14), 2.5–1.1 (m, 13H), 0.89 (s, 3H, H-18), 0.81 (s, 3H, H-19), 0.70 (s, 3H, H-20); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.1, 149.2, 141.5, 124.6, 109.9, 73.0, 65.2, 50.0, 47.0, 41.6, 39.3, 38.6, 33.0, 32.8, 30.3, 24.9, 24.7, 21.3, 19.0, 13.1. Compound **10**: white solid; mp 124–126 °C; $[\alpha]_D$ −16.7 (*c* 0.12, $CHCl_3$); IR (KBr) ν_{max} 2929, 2853, 1754, 1693, 1457, 1247, 1179,

1030 cm^{-1}; ^1H NMR(300 MHz, $CDCl_3$) δ 6.70 (m, 1H, H-12), 5.90 (d, 1H, J = 1.3 Hz, H-17α), 5.03 (d, 1H, J = 1.3 Hz, H-17β), 4.40 (t, 2H, J = 7.3 Hz, H-15), 2.90 (m, 2H, H-14), 2.60 (m, 2H), 2.28 (m, 3H), 1.82–1.14 (m, 6H), 0.89 (s, 6H, H-18, H-19), 0.86 (s, 3H, H-20); ^{13}C NMR(75 MHz, $CDCl_3$) δ 202.2, 170.7, 147.5, 140.2, 125.3, 119.6, 73.0, 65.1, 54.7, 51.2, 41.4, 39.0, 38.2, 37.7, 33.4, 32.5, 26.6, 25.2, 20.8, 18.7, 13.9.Compound **11**: white solid; mp 135–136 °C; $[\alpha]_D^{20}$ −10.9° (c 0.10, $CHCl_3$); IR (KBr) ν_{max} 3401, 3075, 2927, 2862, 1746, 1663, 1457, 1377, 1170, 1088, 1026 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.13 (m, 1H, H-12), 5.03 (s, 1H, H-17α), 4.66 (s, 1H, H-17β), 4.39 (m, 1H, H-7), 4.30 (t, 2H, J = 7.3 Hz, H-15), 2.87 (m, 4H), 2.31 (m, 1H), 1.87–1.09 (m, 12H), 0.88 (s, 3H, H-18), 0.81 (s, H-19), 0.72 (s, H-20); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 149.2, 144.8, 123.0, 110.7, 73.7, 65.2, 51.2, 47.4, 41.9, 39.6, 38.6, 33.1, 32.9, 30.9, 28.9, 22.5, 21.4, 19.1, 13.2.Compound **12**: white solid; mp 129–130 °C; $[\alpha]_D$ +18.2 (c 0.11, $CHCl_3$); IR (KBr) ν_{max} 2990, 2923, 2843, 1741, 1692, 1441, 1388, 1224, 1023 cm^{-1}; ^1H NMR(300 MHz, $CDCl_3$) δ 6.12 (m, 1H, H-12), 5.80 (d, 1H, J = 1.7 Hz, C-17α), 5.11 (d, 1H, J = 1.8 Hz, H-17β), 4.32 (t, 2H, J = 7.3 Hz, H-15), 2.94 (m, 4H), 2.62 (m, 1H), 2.31 (m, 1H), 2.16 (m, 1H), 1.86 (m, 1H), 1.57(m, 4H), 1.19 (m, 1H), 0.88 (s, 9H, H-18,19,20); ^{13}C NMR(75 MHz, $CDCl_3$) δ 203.7, 170.1, 148.1, 143.2, 123.8, 118.9, 65.3, 55.9, 51.9, 41.5, 38.7, 38.6, 38.1, 33.5, 32.6, 28.8, 23.6, 20.7, 18.8, 13.9.Compound **14**: white solid; mp 88–89 °C; $[\alpha]_D^{20}$ −23.1° (c 0.13, $CHCl_3$); IR (KBr) ν_{max}2922, 2843, 1756, 1674, 1455, 1380, 1204, 1031 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.85 (m, 1H, H-12), 5.47 (t, 1H, J = 1.5 Hz), 4.38 (t, 1H, J = 7.3 Hz), 2.87 (m, 2H), 2.35 (m, 1H), 2.19–1.79 (m. 5H), 1.59 (s, 3H), 1.47 (m, 3H), 1.41–1.10 (m, 4H), 0.88 (s, 3H, H-18), 0.87 (s, 3H, H-19), 0.78 (s, 3H, H-20); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.2, 143.4, 133.3, 123.6, 123.5, 65.2, 54.3, 49.8, 41.9, 39.7, 36.5, 33.1, 32.8, 28.3, 25.2, 23.5, 22.2, 21.8, 18.7, 14.0. Compound **15**: white solid; mp 120–121 °C; $[\alpha]_D^{20}$ −75.0° (c 0.04, $CHCl_3$); IR (KBr) ν_{max} 2995, 2962, 2922, 2844, 1741, 1663, 1444, 1379, 1173, 1018 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.26 (m, 1H, H-12), 5.46 (br, 1H), 4.31 (t, 2H, J = 7.3 Hz), 3.04 (m, 1H), 2.90 (m, 2H), 2.66 (m, 1H), 1.90 (m, 4H), 1.60 (s, 3H), 1.5–1.3 (m. 3H), 1.17 (m, 2H), 0.88 (s, 3H, H-18), 0.86 (s, 3H, H-19), 0.73 (s, 3H, H-20); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.1, 146.6, 134.0, 123.4, 121.7, 65.2, 55.0, 50.1, 42.1, 39.7, 36.7, 33.2, 32.8, 29.0, 25.7, 23.6, 22.2, 21.9, 18.7, 14.2.

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