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Total synthesis and antiangiogenic activity of cyclopentane analogues of fumagillol

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Abstract—Novel cyclopentane analogues of fumagillol were synthesized and their endothelial cell proliferation inhibitory activities were evaluated. The cyclopentane-fumagillol derivatives were synthesized from (−)-2,3-*O*-isopropylidene-D-erythronolactone via stereoselective glycolate Claisen rearrangement and intramolecular ester enolate alkylation as key steps.

Angiogenesis is the phenomenon of generating a new capillary vessel, which is one of the normal physiological functions and one of the pathological functions caused by various diseases as well. It has deep connection with growth and transfer of solid cancer, rheumatic arthritis, diabetic retinopathy, psoriasis, etc.[1] Since the Folkman research group suggested a novel concept of treating solid cancer by inhibiting angiogenesis in 1971,[2] clinical importance of therapeutic agents controlling angiogenesis has been emphasized, and much endeavor on angiogenesis seems to have been bestowed on it.

Fumagillin (**1**), a natural product isolated from *Aspergillus fumigatus*,[3] was found by the Folkman group in 1990[4] to strongly inhibit endothelial cell proliferation. Many semi-synthetic analogues have been synthesized from fumagillol (**2**), the hydrolysis product of fumagillin.[4] Among these analogues, TNP-470 (**3**)[4a] and CKD-732 (**4**)[4c,d] are currently employed in clinical trials for the treatment of a variety of cancers (Fig. 1). The mechanism of action of these agents, including fumagillin, is proposed to inhibit angiogenesis by selective inhibition of methionine aminopeptidase type 2 (MetAP-2) through covalent bonding to the His231 residue of MetAP-2 by opening the spiro-epoxide.[5]

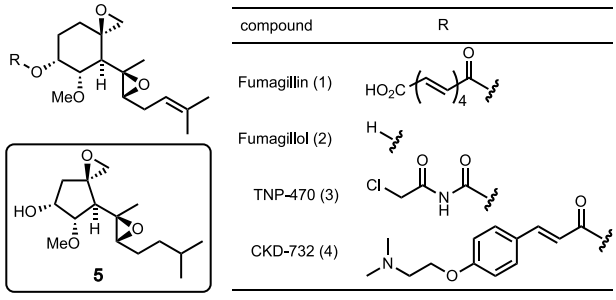

Figure 1.

We have reported the first asymmetric total synthesis of fumagillol, featuring the chelation-controlled glycolate Claisen rearrangement and the intramolecular ester enolate alkylation as the key steps.[6,7] As an ongoing project to expand structural diversity, the cyclopentane ring shown in **5** was introduced, instead of the naturally occurring cyclohexane skeleton of fumagillol (Fig. 1). We anticipated that this approach could lead to novel angiogenesis inhibitors since cyclopentane analogue **5** is not available from fermentation and it possesses a high level of homology to the fumagillol structure. It has a spiro-epoxide and an isoamyl side chain, which are proposed to be essential for activity and for

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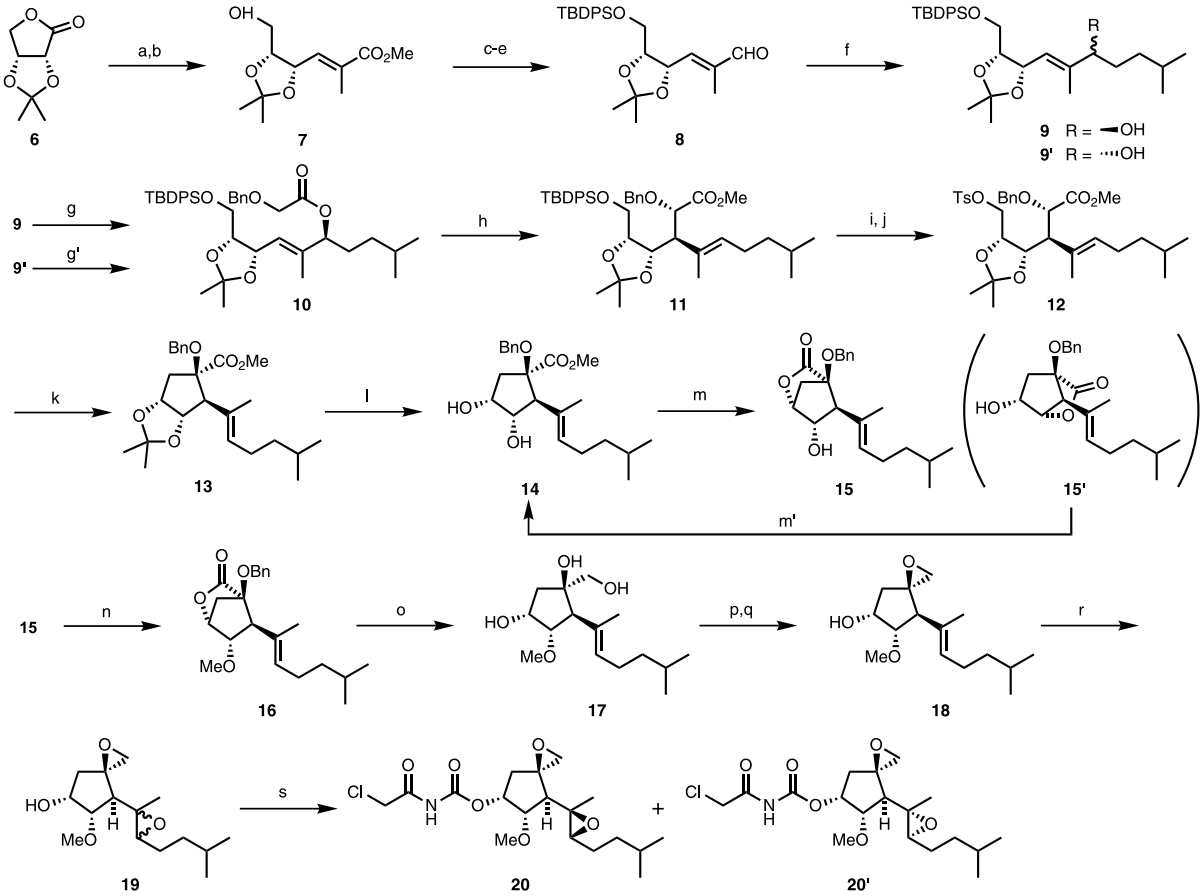
hydrophobic interaction with MetAP-2, respectively. Moreover, the stereochemistry of the substituents in the cyclopentane ring is identical to that of fumagillol.

Our synthesis, which is described in Scheme 1,[8] began with commercially available (−)-2,3-*O*-isopropylidene-D-erythronolactone **6**, which was converted to the corresponding lactol with DIBAL-H, followed by the Wittig reaction with (carbomethoxyethylidene)triphenyl-phosphorane to afford the α,β-unsaturated ester **7** in 87% yield for the two steps. After protection of the alcohol with the TBDPS group, the methyl ester was transformed to the aldehyde **8** by the sequence of reduction with DIBAL-H and MnO_2 oxidation (83% overall yield for the three steps). Addition of isoamylmagnesium bromide to the aldehyde **8** produced a 3:1 mixture of allylic alcohols **9** and **9′** where each compound could be isolated by silica column chromatography in 95% total yield. The absolute configuration of the newly generated stereocenter in **9** and **9′** was confirmed by spectroscopic analysis of the compounds **13** and **13′** (vide infra). The allylic glycolate ester **10** could be prepared using either DCC coupling from **9** or Mitsunobu reaction from **9′** in good yield. The γ,δ-unsaturated glycolate **11** was efficiently obtained as a single diastereomer by 1,3-chirality transfer through a chelation-controlled chair-like transition state of the Burke–Fujisawa–Kallmerten modification of the Ireland–Claisen rearrangement.[9,10] Intramolecular ester enolate alkylation of the tosylate **12**, which was converted from **11** by desilylation and tosylation, was performed with KHMDS at 0 °C in a short time to produce the desired cyclopentane-carboxylate **13** in 66% yield with a 10:1 mixture of inseparable diastereomers. The excellent diastereomeric excess implies that this cyclization may proceed through an allylic strain-controlled 'H-eclipsed' transition state.[10]

The absolute configuration of **13** was verified by chemical transformation and NMR analysis. An observation of the formation of the lactones (**15** or **15′**) in high yield in the case of treatment of the diol **14** with DBU is reliable evidence that expected stereo-chemistry of the C(*a*) position in **13** is correct. For the stereochemistry at the C(*b*) position, ^{1}H NMR studies were conducted.



Scheme 1. Reagents and conditions: (a) DIBAL-H, CH_2Cl_2, −78 °C, 3 h, 92%; (b) $Ph_3P{=}C(Me)CO_2Me$, toluene, reflux, 1 h, 95%; (c) TBDPSCl, imidazole, DMF, rt, 3 h, 95%; (d) DIBAL-H, CH_2Cl_2, −78 °C, 4 h, 96%; (e) MnO_2, CH_2Cl_2, rt, 20 h, 91%; (f) isoamylMgBr, THF, −78 °C, 1 h, 70% of **9** and 25% of **9′**; (g) $BnOCH_2CO_2H$, DCC, DMAP, CH_2Cl_2, rt, 3 h, 77%; (g′) $BnOCH_2CO_2H$, PPh_3, DIAD, THF, 0 °C, 30 min then rt, 3 h, 82%; (h) (i) LiHMDS, TMSCl/Et_3N, THF, −78 °C, 1 h then rt, 10 h, (ii) CH_2N_2, Et_2O, 0 °C, 5 min, 70% (for 2 steps); (i) TBAF, THF, rt, 12 h, 80%; (j) TsCl, pyridine, $CHCl_3$, 4 °C, 20 h, 98%; (k) KHMDS, THF, 0 °C, 10 min, 66% (10:1 mixture of inseparable diastereomers); (l) PPTS, MeOH, reflux, 20 h, 98%; (m) DBU, CH_3CN, reflux, 6 h, 96% (**15**:**15′** = 1:1); (m′) NaOMe, MeOH, rt, 6 h, 95%; (n) Ag_2O, MeI, reflux, 4 h, 80%; (o) Li, *liq*. NH_3, −78 °C, 3 h, 62%; (p) TsCl, Et_3N, DMAP, CH_2Cl_2, rt, 18 h, 80%; (q) K_2CO_3, MeOH, rt, 2 h, 90%; (r) *m*-CPBA, $NaHCO_3$, CH_2Cl_2, 0 °C, 4 h, 94% (ca. 3:1 of inseparable diastereomeric mixture); (s) chloroacetyl isocyanate, Et_3N, DMAP, CH_2Cl_2, 0 °C, 71% (18% of **20** and 53% of **20′**).

Upon irradiation of the H(*b*) in **13**, nuclear Overhauser effect (NOE) was observed to be 1.4% for the H(*c*), while NOE in the diastereomer **13′** prepared from **9′** was 5.6%. These results nicely support the fact that H(*b*) and H(*c*) in **13** have a trans-relationship (Fig. 2).

Although several methods for the selective methylation of C(*c*)-alcohol in **14**, which was prepared from **13** by treatment with PPTS in methanol, with/without protection of the other hydroxyl group were attempted, we failed to obtain the desired mono-methoxy product in good yield. Lactonization turned out to be the best protection to give a 1:1 mixture of separable regioisomers (**15** and **15′**), with the undesired isomer **15′** being recycled. Then, methylation of the hydroxyl group in **15** was successfully performed with Ag_2O/MeI reaction system to give **16** in 80% yield. Removal of the benzyl-protecting group in **16** along with the concurrent reduction of the lactone was accomplished using excess lithium in liquid ammonia to produce the triol **17** in 62% yield. Selective mono-tosylation of the primary hydroxyl group in **17**, followed by treatment with mild base, afforded the spiro-epoxy compound **18** in 72% yield for the two steps. Epoxidation of the olefin in **18** with *m*-CPBA produced a 3:1 mixture of inseparable diastereomers **19** in 94% yield.

To measure the endothelial cell proliferation inhibitory activity of the novel cyclopentane analogue of fumagillol, chloroacetylcarbamoyl group, a side chain of TNP-470 (**3**), was introduced, and each diastereomer (**20** and **20′**) was isolated by silica column chromatography. Anti-proliferating activities were evaluated against calf pulmonary artery endothelial (CPAE) cells, lymphoma EL-4 cells, and murine leukemia P388. IC_{50} values were colorimetrically measured by SRB (CPAE cell) or MTT (EL-4, P388) methods. The biological data are shown in Table 1. The prepared novel cyclopentane analogues of fumagillol showed endothelial cell proliferation inhibitory activity at μM concentration. However, their activities turned out to be lower than those of fumagillin derivatives.

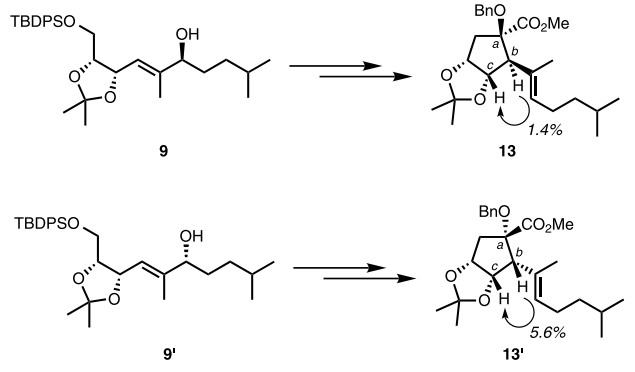


Figure 2.

Table 1. In vitro endothelial cell proliferation inhibitory activities against CPAE, EL-4, and P-388 cell lines

Compound	IC_{50} (μg/mL)		
	CPAE	EL-4	P-388
20	8.8×10^{-2}	3.0×10^{-1}	⩾10
20′	5.7×10^{-1}	1.1	⩾10
Fumagillin (1)	3.0×10^{-3}	1.6×10^{-3}	⩾10
TNP-470 (3)	2.0×10^{-4}	3.8×10^{-3}	⩾10

In summary, total synthesis of a novel cyclopentane analogue of fumagillol was carried out using glycolate Claisen rearrangement and intramolecular ester enolate alkylation as key steps. Studies on the structure–activity relationship of fumagillol analogues are underway.

Acknowledgments

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8. All new compounds exhibited satisfactory spectroscopic and analytical data, and the ratio of stereoisomers was

determined by the ^{1}H NMR analysis and/or isolation of individual isomers. Selected data for the key intermediates **11** and **13**: For **11** ^{1}H NMR (400 MHz, $CHCl_3$-*d*) δ 7.24–7.76 (m, 15H), 5.14 (t, 1H, *J* = 6.5 Hz), 4.48 (d, 1H, *J* = 11.3 Hz), 4.33 (d, 1H, *J* = 11.3 Hz), 4.21–4.26 (m, 2H), 4.00 (d, 1H, *J* = 10.2 Hz), 3.90 (dd, 1H, *J* = 10.8, 5.4 Hz), 3.82 (dd, 1H, *J* = 10.8, 5.4 Hz), 3.61 (s, 3H), 2.95 (dd, 1H, *J* = 0.2, 9.8 Hz), 1.88–1.92 (m, 2H), 1.58 (s, 3H), 1.42–1.51 (m, 1H), 1.37 (s, 3H), 1.29 (s, 3H), 1.08–1.11 (m, 2H), 1.06 (s, 9H), 0.85 (d, 6H, *J* = 6.6 Hz); ^{13}C NMR (100 MHz, $CHCl_3$-*d*) δ 172.1, 137.3, 136.2, 136.1, 134.4, 134.2, 131.4, 130.7, 129.7, 128.8, 128.5, 128.3, 127.9, 127.8, 125.9, 107.2, 81.5, 79.9, 73.2, 64.6, 51.8, 50.8, 41.4, 38.9, 27.9, 27.8, 27.4, 25.9, 25.7, 22.9, 22.8, 19.7, 13.5; EI-MS (*m*/*z*) 673 (M^++1). For **13** ^{1}H NMR (400 MHz, $CHCl_3$-*d*) δ 7.26–7.38 (m, 5H), 5.28 (t, 1H, *J* = 7.2 Hz), 4.85 (d, 1H, *J* = 7.1 Hz), 4.64–4.69 (m, 1H), 4.57 (d, 1H, *J* = 11.5 Hz), 4.34 (d, 1H, *J* = 11.5 Hz), 3.74 (s, 3H), 2.98 (d, 1H, *J* = 7.1 Hz), 2.64 (dd, 1H, *J* = 14.3, 5.3 Hz), 2.42 (dd, 1H, *J* = 14.3, 5.3 Hz), 2.02–2.04 (m, 2H), 1.72 (s, 3H), 1.47–1.54 (m, 1H), 1.32 (s, 3H), 1.26 (s, 3H), 1.19–1.21 (m, 2H), 0.87 (d, 6H, *J* = 6.6 Hz); ^{13}C NMR (100 MHz, $CHCl_3$-*d*) δ 172.3, 138.9, 131.1, 128.6, 127.9, 127.7, 113.7, 92.5, 82.5, 78.7, 68.1, 63.3, 60.8, 52.4, 39.1, 38.3, 28.0, 27.7, 26.3, 25.3, 23.0, 22.8, 21.4, 15.8, 14.6; EI-MS (*m*/*z*) 415 (M^+−1).

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10. Plausible transition states for the two transformations (**10** → **11** and **12** → **13**).