Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 19, pp. 2473-2476, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)10008-7

SYNTHESIS AND BIOLOGICAL ACTIVITY OF CINNAMALDEHYDES AS ANGIOGENESIS INHIBITORS

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Abstract: A series of 2-hydroxycinnamaldehyde derivatives was synthesized for examing a structure-activity relationship for inhibition of angiogenesis. The anti-angiogenic effects of 2'-substituted cinnamaldehdes and related analogs were determined in a chick embryo chorioallantoic membrane assay system. © 1997 Elsevier Science Ltd.

Angiogenesis, the process of new blood vessel formation, is involved in a variety of pathological events such as diabetic retinopathy, rheumatoid arthritis and cancer.[1,2] Especially, neovascularization is critical for the growth and metastasis of solid tumors.[3] Recent works have shown that potent inhibitors of angiogenesis might be clinically useful as therapeutic agents for these diseases.[4]

In the course of a screening for new inhibitor of angiogenesis among herbal medicines, we isolated compounds **1a** and **1b** from the stem bark of *Cinnamomum cassia* Blume (Lauraceae)[4]. **1a, 1b** and related analogs were synthesized for examing a structure-activity relationship for inhibition of angiogenesis. This paper describes synthesis and antiangiogenic effects of 2'-substituted cinnamaldehydes and their analogs.

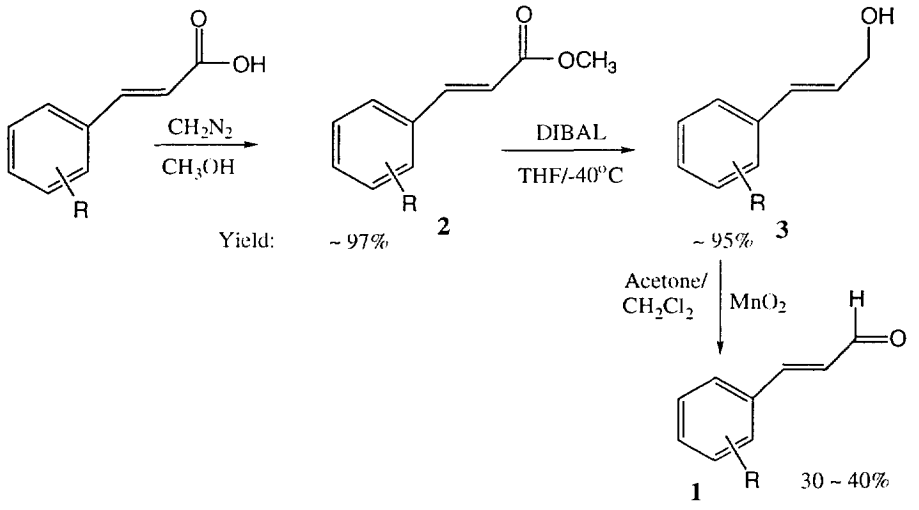
The antiangiogenic activity was examined in a chick embryo chorioallantoic membrane (CAM) assay system, according to a modification of the method described previously.[6] In brief, fertilized eggs were incubated at 37 °C for 3-4 days. A test sample(10 μL) dissolved in ethanol was placed on a thermanox coverslip and dried. The coverslip coated with a test sample was placed on the surface of 4.5-day-old CAM. After 5 or 6 days, 1 mL of intralipose (fat emulsion)

was injected into the chorioallantois and the antiangiogenic activity was determined by measuring an avascular zone in the CAM, followed by taking photographs of the treated CAMs.

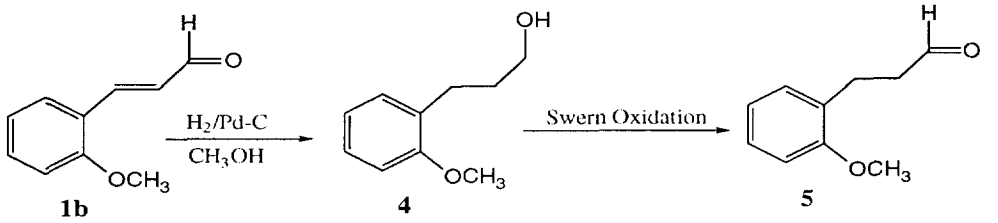
At 10 μg, 2'-hydroxycinnamaldehyde (**1a**, 91% inhibition) isolated from *Cinnamomum cassia* Blume strongly inhibited CAM angiogenesis in comparison with well known angiogenic inhibitors AGM-1470 (20% inhibition)[6,7] and genistein (68% inhibition) as summarized in Table 1.[8]

Based on finding obtained using the natural compound **1a**, the propenal and free phenolic hydroxyl groups were identified as targeting sites to study the structure-activity relationship. Therefore, we prepared compounds **1a-e** and **2~5** from 2'- or 3'-substituted cinnamic acids as shown in Schemes 1 and 2.[9]

Scheme 1



Scheme 2



Functionalization of the free phenolic hydroxyl group was accomplished using esterification and alkylation. The propenal was converted to saturated or unsaturated alcohol by reduction

with Pd-C/H_2 and DIBAL, respectively. The two derivatives, 2'-hydroxyl-(**1a**) and 2'-O-benzoyl-cinnamaldehyde (**1e**), appeared to be more potent angiogenic inhibitors in a CAM assay than genistein and AGM-1470 as well. **1a** and **1e** induced avascular zones in the CAM in a dose-dependent manner as shown in Table 2.

Table 1. Antiangiogenic activity of cinnamaldehydes and analogs in a CAM assay[a]

Compound	No.(%) of CAM avascular/total	Compound	No.(%) of CAM avascular/total
1a (2'-OH)	16/17(94)	**1b** (2'-OCH_3)	20/33(61)
1c (2'-$OC(O)CH_3$)	10/20(50)	**1d** (2'-$OC(O)C_2H_5$)	7/17(41)
1e (2'-OC(O)Ph)	15/17(91)	**1f** (2'-$C(O)OCH_3$)	14/30(47)
1g (3'-OH)	8/16(50)	**1h** (2'-OCH_2Ph)	14/30(47)
1i (2'-Acrylate)	12/22(54)	**1j** (2'-p-methylbenzoate)	6/17(35)
1k (2'-o-methylbenzoate)	6/17(35)	**1l** (2'-o-Methoxybenzoate)	5/21(24)
2 (2'-OH)	4/16(25)	**3** (2'-OH)	6/16(37)
4 (2'-OCH_3)	5/16(31)	**5** (2'-OCH_3)	10/20(50)

[a]A 10μg dose for each compound was used.

Table 2. Angiogenesis Inhibitory effects of **1a** and **1e** in different doses in a CAM assay

Dose (μg/egg, **1a**)	No.(%) of CAM avascular/total	Dose (μg/egg, **1e**)	No.(%) of CAM avascular/total
10	16/17 (94)	10	15/17 (91)
5	19/29 (65)	5	29/37 (78)
2	16/32 (50)	2	17/29 (59)
1	5/16 (31)	1	9/16 (56)
0.5	4/16 (25)	0.5	6/15 (40)

These results suggested that the aldehyde group of the side chain seems to play a critical role in the antiangiogenic activity of the compounds and that the double bond of propenal was no effect on the inhibitory activity of angiogenesis because of a similar activity of **1b** and **5**. New angiogenic inhibitors described here are easily sythesized from commercially available cinnamic acids at low prices in 3 to 5 chemical reaction steps and their activities in a CAM assay might provide valuable ideas for development of new lead compounds. 2'-Hydroxycinnamaldehyde

was found to inhibit farnesyl transferase,[5] which is one of key enzymes for triggering *ras* oncogene toward tumor formation. And it is also reported that the oncogenic *ras* stimulated tumor angiogenesis.[10] Therefore, the results are of interest in connection with angiogenesis and Ras signaling pathways.

Acknowledgements: This research was supported by the Star Project (Ministry of Science & Technology), Ministry of Agriculture, Forestry and Fisheries-Special Grants Research Program and Good Health R&D Project (Ministry of Health & W elfare) in Korea.

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(*Received in Japan* 9 *July* 1997; *accepted* 29 *August* 1997)