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Article

Inhibition of Farnesyl Protein Transferase by Ortho-substituted Cinnamaldehyde Derivatives

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Various cinnamaldehyde derivatives were synthesized and their inhibition activity (pI_{50}) of farnesyl protein transferase (FPTase) was measured to examine the structure-activity relationships (SAR) on the basis that FPTase was inhibited by ortho-hydroxycinnamaldehyde derived from extracts of the bark of *Cinnamomum cassia* Blume. The ortho-substituents on the phenyl backbone of cinnamaldehyde showed higher activity than those with meta- and para-substituents, and the side chain required unsaturated aldehyde. In particular, 2-chlorocinnamaldehyde, 5 showed the highest inhibition activity on the FPTase among them and its inhibition activity (pI_{50}) value was 4.45.

Key words : *farnesyl protein transferase inhibition, O-cinnamaldehydes, structure-activity relationships.*

Cancer, which is abnormally controlled cell division, results in irregular cell growth and proliferation through oncogene mediation. In the normal cell, cell division is specifically regulated by catalytic cell growth factors and epidermal growth factors (EGF).[1-3]

In the tumor cell, at least one of these factors has been activated or inactivated by carcinogens.[4-6] Intracellular signalling is mediated to the nucleus via protein-protein interactions and such interactions are related to control cell division.[7-10] Potentially, if this signalling could be blocked, the abnormal cell divisions of cancer cells could be inhibited. FPTase catalyzes the farnesylation of Ras protein on the cysteine residue near the C-terminus. This is critical for triggering the *ras* oncogene toward tumor formation.[11-13]

It has been found that the 2-hydroxycinnamaldehyde, isolated from the stem bark of *Cinnamomum cassia* Blume, shows inhibitory activity of FPTase.[14] Cinnamaldehyde derivatives were synthesized and SAR[15] between the inhibition activity on FPTase and the structure with changing of phenyl backbone and/or side chain are described.

Materials and Methods

Syntheses of substrates. All compounds including various substituents of cinnamaldehydes and cinnamic esters were synthesized according to the references.[19-20] The synthetic products have been confirmed with the instrumental ananysis including UV, FT-IR, NMR and MS.[19-20] The high-resolution mass spectra were measured on a VG ZAB-7070 by the University California at Riverside Mass Spectrometry Facility using the chemical ionization (CI) mode with NH_3.

Bioassay. The FPTase inhibition assay was conducted by using the Scintillation Proximity Asssay (SPA) kit which was purchased from Amersham. The enzyme used was a partially purified fraction of farnesyl protein transferase which was isolated from Sprague-Dowley rats brain cytosol.[18] In the FPTase-SPA, the transfer of the [^{3}H] farnesyl group of farnesyl pyrophosphate to the cysteine of biotin-YRASNRSCAIM was measured in the presence of FPTase which was isolated from bovine brain. An assay method of FPTase inhibition was followed.[16-18] After incubation at 37°C for 50 min, the reaction mixture of biotinylated peptide (biotin-YRASNRSCAIM), enzyme and farnesylpyrophosphate labelled with tritium (^{3}H-FPP) in the presence of inhibitor was stopped by an addition of stop buffer and bead solution (Streptavidin-agarose). After gentle vortexing, the mixture was then filtered and washed with washing buffer. The precipitated material (bound streptavidin-agarose to farnesylated peptide [^{3}H]) was finally counted on a liquid scintillation counter.

Results and Discussion

2-Methoxy cinnamaldehyde isolated from the bark of

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Abbreviations: FPTase, farnesyl protein transferase; SAR, structure-activity relationships.

Cinnamomum cassia Blume has been reported to have antifungal activity.[21] Its functional group, methoxy, was different from the hydroxy form isolated from the bark. Thus, there was an anticipation that its analogues would also show antifungal activity.

In vitro cytotoxicity of synthesized cinnamaldehyde analogues to human solid tumor cells such as A549, SK-OV-3, SK-MEL-2, XF498 and HCT15 were reported.[19] The results have revealed that cinnamic acids, cinnamates and cinnamyl alcohols did not show any cytotoxicity against the human tumor cells. Cinnamaldehydes and related compounds were not only resistant to A549 cell line (15 μg/ml) but also to HCT15. SK-MEL-2 cells were much sensitive to those synthesized compounds (ED_{50} values 0.63~8.1 μg/ml).[19]

From the basis on the findings, various cinnamaldehyde derivatives were synthesized and their inhibition activity of FPTase were measured, in order to examine SAR. Structure modification focused on the positions of the phenyl backbone and the side chain. The results of the assay of the FPTase inhibition using isolated 2-hydroxycinnamaldehyde and synthesized cinnamaldehydes were summarized in Tables 1 and 2. Inhibition activity was expressed as the pI_{50} value, the negative logarithm of the molar concentration for 50% inhibition (I_{50}) *in vitro* against FPTase. Styryl group-substituted compounds (Table 1) showed lower inhibition activity (pI_{50}=1.82~2.43) than ortho-substituted cinnamaldehyde derivatives (pI_{50}=3.03~4.45).

The quantitative SAR between the activity in the inhibition of FPTase and the physico-chemical parameters of the substituents on the phenyl backbone was attempted, but reasonable regression equation could not be obtained. In Table 1, cinnamic acids, cinnamate and some cinnamyl alcohols did not inhibit FPTase. The allylic aldehyde group was found to be active. However, the saturated compound moieties [3-(2-methoxyphenyl)propanal, 3-(2-hydroxyphenyl)propanal] did not inhibit FPTase. It demonstrated that the side chain was required to be activated with an aldehyde moiety. Another inhibitor isolated from the dried stem and leaves of *Artemisia sylvatica* Maximowicz, artecanin,[20] was found to have lower activity (pI_{50}=3.20) than substituted phenyl backbone derivatives (Table 2). But the pI_{50} value of artecanin showed higher than cinnamic acids, cinnamate and some cinnamyl alcohols.

Table 1. Inhibition activity values (pI^{50}) of FPTase by the some compounds.

Compounds	pI_{50}	mp (°C)
2-hydroxycinnamic acid	2.31	217[a]
2-hydroxycinnamic methyl ester	2.24	133~136
2-hydroxycinnamyl alcohol	2.43	83~88
3-hydroxycinnamyl alcohol	2.07	87~88
4-hydroxycinnamyl alcohol	2.32	124~126
2-chlorocinnamic acid	2.21	208~210
2-chlorocinnamyl alcohol	1.82	142~146
3-(2-methoxyphenyl)propanal	1.99	77[b]
3-(2-hydroxyphenyl)propanal	2.18	liquid
Artecaninc	3.20	248~251

[a]Purchased from Aldrich Co.
[b]Boiling point
[c]Isolated from dried stem and leaves of *Artemisia sylvatica* Maximowicz[20].

Table 2. Inhibition activity values (pI_{50}) of FPTase by the phenyl substituted cinnamaldehyde derivatives.

No.	Compounds	pI_{50}	mp (°C)
1	cinnamaldehyde	2.27	119~122[b]
2	3-hydroxycinnamaldehyde	2.77	118~120
3	4-hydroxycinnamaldehyde	2.47	136~138
4	2-hydroxycinnamaldehydea	3.34	130~133
5	2-chlorocinnamaldehyde	4.45	52~54
6	2-benzyloxycinnamaldehyde	3.68	53~55
7	2-(4-methylbenzyloxy)cinnamaldehyde	3.37	76~79
8	2-(4-methoxybenzyloxy)cinnamaldehyde	3.41	84~87
9	2-(4-chlorobenzyloxy)cinnamaldehyde	3.48	94~95
10	2-(4-nitrobenzyloxy)cinnamaldehyde	3.03	122~124
11	2-(4-bromobenzyloxy)cinnamaldehyde	3.43	92~95
12	2-(2-ethoxyphenyl)cinnamaldehyde	3.38	48~52
13	2-propoxycinnamaldehyde	3.44	37~39
14	2-(1-methylpropoxy)cinnamaldehyde	3.30	40~43
15	2-methoxycinnamaldehyde	3.24	47~50
16	2-farnesylcinnamaldehyde	2.85	liquid
17	2-benzoyloxycinnamaldehyde	3.19	78~80
18	2-acetoxycinnamaldehyde	3.26	78~80
19	2-methoxycarbonylcinnamaldehyde	3.44	38~41

[a]Isolated from stem bark of *Cinnammomum cassia* Blume.[19]
[b]Boiling point at 10 mmHg.

In Table 2 showing pronounced activity of ortho-substituents, compound **6**, benzyloxy substituent, was proved to have high activity. Therefore, para-benzyloxy substituents on the phenyl ring were synthesized, but it was confirmed that the para-benzyloxy substituents did not show any appreciable activities. Electron donating groups displayed only slightly higher activity than electron withdrawing groups such as the nitro group. As a result, none substituted benzyloxy substituent, **6** showed the highest activity. However, the substituent modification of ortho- and meta-substituents (mono and di-substituents) on the benzyloxy group, respectively, was needed. Though a carbon on the side chain was extended in ethoxyphenyl substituent, **12**, the activity was not different from that of the para-benzyloxy substituents, **7~11**. Compounds **13** and **14** possessed longer aliphatic groups than the methoxy group and showed nearly the same results as that of **12**. Also, the activity changes were not conferred by benzoyl substituent, **17** and acetyl substituent, **18**, in which the carbonyl group was added for more convenient nucleophile attack. The methoxy and methylcarboxy groups did not show the changes either. Among them, 2-chloro substituent, **5** showed the highest inhibition activity and its inhibition activity (pI_{50}) value was 4.45.

The 2-farnesyl (or 3,7,11-trimethyl-2,6,11-dodecatrienyl) substituent (pI_{50}=2.85), **16** did not inhibit FPTase and

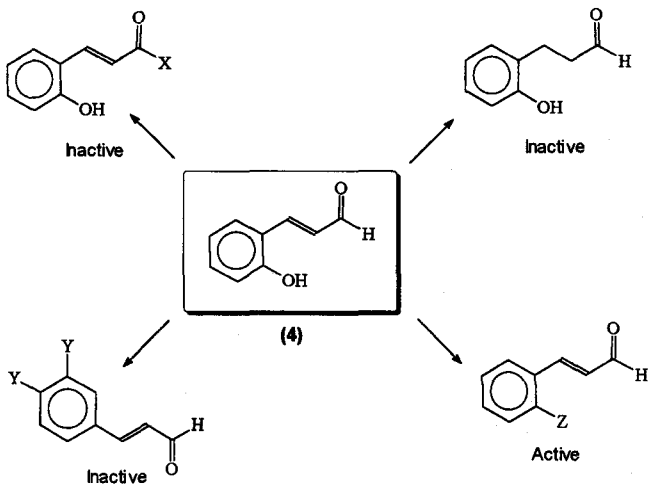

Fig. 1. SAR on the FPTase inhibition of cinnamaldehyde analogues. (X=OH, OCH_3, Y=OH or OCH_3, Z=various groups)

thus, conclusively it was not competitive to farnesyl pyrophosphate (FPP) in the reaction. The inhibition pattern of 2-hydroxycinnamaldehyde analogues was estimated such that the inhibitors interacted with one of the active sites of FPTase, though not to the sulfhydryl group of cysteine on the biotinylated-peptide. Also, it was not found to be a suitable environment to bind ^{3}H-FPP as one of the sites on the biotinylated-peptide in the SPA method.

The SAR between FPTase inhibition activity and modified structures from Table 1 and 2 was shown in Fig. 1. Analysis of the inhibition values from the assay results showed ortho-substituted cinnamaldehydes had higher activities than meta- and para-substituents. It was also necessary for substituent to be anchored to the α,β carbon-carbon double bond and carbonyl group in substrate for high FPTase inhibition activity. For further study of optimizing the main skeleton, the phenyl moiety should be changed to other heterocyclic compounds, and also ortho- and para-disubstituents should be synthesized.

Recently, according to the position of FPP substrate binding pocket and of nonapeptide residue from X-ray crystal structure of FPTase,[22] interaction between substrate and hydroxyl, and carboxyl group of Thr^7-Ser^6-Asp^5 in FPTase is hypothesized.[23]

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