Inhibitory effects of flavonoids on human immunodeficiency virus type-1 integrase

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(Received September 17, 2001. Accepted November 27, 2001.)

Abstract

One hundred and eighty-three flavonoids were screened for their inhibitory effects on HIV-1 integrase (IN) using a multiplate integration assay (MIA). Of the tested flavonoids, 6-hydroxyluteolin, scutellarein, pedalitin, scutellarin, baicalein dimer, hypolaetin, 7-*O*-benzyl-6-hydroxyluteolin and baicalein showed appreciable inhibition with IC_{50} values of 0.4, 0.6, 1.3, 1.7, 2.0, 2.1, 3.0 and 3.6 μM, respectively. The potent inhibition was observed with flavonoids having at least one pair of vicinal hydroxyl groups and the activity was highly dependent on the number of vicinal hydroxyl groups.

On the other hand, the inhibitory activity tended to be decreased by replacing a hydroxyl group with one of methoxyl, acetoxyl, isopropoxyl, isopentenyl, benzyloxyl, glucuronyl and glycosyl groups. No flavanones, flavanonols and chalcones examined in this experiment showed any significant inhibitory activity.

Key words HIV-1, integrase, inhibitory effect, flavonoid.

Abbreviations HIV-1, human immunodeficiency virus type 1; IN, integrase; RT, reverse transcriptase; PR, protease; LTR, long terminal repeat; DMSO, dimethyl sulfoxide; DNA, deoxyribonucleic acid; MIA, multiplate integration assay; PBS, phosphate-buffered saline; MOPS, 3-(*N*-morpholino)propane sulfonic acid; DTT, dithiothreitol; AP, alkaline phosphatase; EDTA 2Na, ethylenediaminetetraacetate disodium salt; NP-40, nonidet-P40; IC_{50}, 50% inhibitory concentration; 5-CITEP; 1-(5-chloroindol-3-yl)-3-hydroxy-3-(*2H*-tetrazol-5-yl)propenone.

Introduction

HIV-1 infection remains a world-wide health concern with limited kinds of clinical drugs, in which some compounds have not been recommended for use for a long period of time due to the incidence of viral mutation followed by drug-resistance. Three HIV-1 enzymes are essential to the life cycle of the virus. HIV-1 reverse transcriptase (RT) is crucial for viral replication. HIV-1 protease (PR) processes viral proteins into functional enzymes and structural proteins, thereby facilitating maturation and infectivity of virion particles. HIV-1 integrase (IN) mediates the integration of the double strand DNA transcript of an HIV-1 RNA genome into the host genome.[1] HIV-1 IN presents an attractive possibility as an antiviral target because host cells do not make or require such enzymes. Recent evidence shows that HIV replication in T-lymphoid cells requires an integrase function.[2,3]

Flavonoids are one of the main constituents in plants, and show many biological activities such as antifungal, antibacterial,[4] anti-inflammatory,[5] antioxidant,[6,7] anticancer[8] and antiviral activities.[9-11] Effects of flavonoids on RT were investigated by Kusumoto *et al.*;[12] of their tested compounds, 6-hydroxyluteolin, pedalitin, 6-hydroxykaempferol and quercetagetin were the most potent inhibitors with IC_{50} values of less than 10

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μM. Flavonoids were also reported to show inhibitory effects on HIV-1 PR.[13] Recently, a new flavonol galloylglycoside from *Acer okamotoanum* was reported to exhibit HIV-1 IN inhibitory activity with an IC_{50} value of 18.1 μg/ml.[14] Since some flavonoids have been shown to have inhibitory action against various enzymes, it is of interest to investigate their inhibitory activity against HIV-1 IN. In the present paper, we report the inhibitory effects of some flavonoids obtained from natural sources, purchased and prepared by chemical synthesis on HIV-1 IN and their structure-activity relationships .

Materials and Methods

Chemicals and enzyme: The sources of flavonoids used in this experiment are described in Tables I and II.[15-59] All flavonoids were dissolved in 50% DMSO prior to the investigation of HIV-1 IN inhibitory activity. HIV-1 IN protein was expressed in *Escherichia coli* and the purified enzyme was stored at -80℃ before use. Oligonucleotides of long terminal repeat (LTR) donor DNA and substrate DNA were purchased from Japan Bioservice Co. (Asaka, Japan) and stored at -25℃ before use. The sequences of biotinylated LTR donor DNA and its unlabelled complement were 5'-biotin-ACCCTTTT-AGTCAGTGTGGAAAATCTCTAGCAGT-3' and 3'-GAAAATCAGTCACACCTTTTAGAGATCGTCA-5', respectively while those of digoxigenin-labelled target DNA were 5'-TGACCAAGGGCTAATTCACT-digoxigenin and 3'-digoxigenin-ACTGGTTCCCGATTAAGT-GA-5', respectively.

Multiplate integration assay (MIA) procedure: [60] First, a 96 well plate was coated with 50 μl of a streptavidin solution containing 40 μg/ml streptavidin, 90 mM Na_2CO_3 and 10 mM KCl. Fifty microliters of a biotinylated LTR donor DNA solution containing 10 mM

Table I. Inhibitory effects of flavonoids on HIV-1 integrase

Compound	Substituents of Flavonoids										IC_{50} (μM)	Source (Ref. No.)
	3	5	6	7	8	2'	3'	4'	5'	6'		
1. Chrysin		OH		OH							>100	15
2.		OH		OGent							>100	16
3. Chrysin 7-*O*-glucuronide		OH		OGlcUA							>100	16
4.		OPr		OPr							>100	17, 18
5.		OH		OBn							>100	17
6. Apigenin		OH		OH				OH			>100	15
7. Rhoiforin		OH		OGlc^{2}-^{1}Rha				OH			>100	19
8.		OH		OPr				OPr			>100	17, 18
9.		OAc		OAc				OAc			>100	17
10.		OH		OMe				OMe			>100	17
11.		OMe		OMe				OMe			>100	17
12. Luteolin		OH		OH			OH	OH			28	20
13.		OH		OH			OPr	OPr			>100	17
14.		OBn		OBn							>100	17
15.		OBn		OBn				OPr			>100	17, 18
16. Cosmosiin acetate		OAc		OGlc-Ac$_4$				OAc			>100	21
17. Acacetin		OH		OH				OMe			28	22
18.		OMe		OMe			OMe	OH			>100	Unp.
19. Luteolin tetramethyl-ether		OMe		OMe			OMe	OMe			>100	Unp.
20.		OBn		OBn			OPr	OPr			>100	17
21.		OH		OH			OMe	OH	OMe		>100	23
22.		OMe		OMe			OMe	OMe	OMe		>100	22
23. Baicalein		OH	OH	OH							3.6	24
24. Baicalein-7-*O*-Glc		OH	OH	OGlc							28	25
25.		OH	OH	OGlc^{6}-CHO							18	17
26. Oroxin B		OH	OH	OGent							>100	16
27. Baicalin		OH	OH	OGlcUA							5.9	26
28. Baicalein 6-*O*-Glc		OH	OGlc	OH							22	27
29.		OAc	OAc	OAc							15	17
30. Oroxylin A		OH	OMe	OH							>100	24
31.		OH	OMe	OGlcUA-ME							>100	26
32.		OMe	OMe	OMe							>100	17
33.		OH	OMe	OAc							>100	17
34. Scutellarein		OH	OH	OH				OH			0.63	28
35. Scutellarin		OH	OH	OGlcUA				OH			1.7	28

36.		OH	OH	OPr				OPr			14	17, 18
37.	Hispiculin	OH	OMe	OH				OH			>100	16
38.	Cirsimaritin	OH	OMe	OMe				OH			>100	29
39.	Cirsimarin	OH	OMe	OMe				OGlc			>100	29
40.	Embinin	OH	Glc^2	OMe				OMe			>100	30
41.	Isovitexin	OH	Glc	OH				OH			>100	31
42.	Swertisin	OH	Glc	OMe				OH			>100	32
43.		OAc	Glc-Ac_4	OMe				OAc			>100	33
44.		OH	Glc	OMe				OMe			>100	32
45.	6-Hydroxyluteolin	OH	OH	OH			OH	OH			0.37	33
46.	Eupatorin	OH	OMe	OH			OH	OMe			25	34
47.	Pedalitin	OH	OH	OMe			OH	OH			1.3	17
48.	Cirsiliol	OH	OMe	OMe			OH	OH			12	17
49.	Homuorientin	OH	Glc	OH			OH	OH			12	35
50.	Swertiajaponin	OH	Glc	OMe			OH	OH			>100	35
51.		OBn	OBn	OBn			OBn	OBn			>100	17
52.		OH	OMe	OPr			OH	OH			12	17
53.		OH	OH	OBn							>100	17
54.		OH	OH	OGlcUA-BE							17	25
55.		OBn	OBn	OBn							>100	17
56.		OH	OBn	OBn							>100	17
57.	Plantaginin	OH	OH	OGlc				OH			4.8	36
58.		OH	OH	OBn				OH			26	17
59.		OBn	OBn	OBn				OBn			>100	17
60.		OH	OH	OBn			OH	OH			3.0	17
61.	Hypolaetin	OH		OH	OH		OH	OH			2.1	37
62.		OMe		OH	OMe		OBn	OBn			>100	17
63.		OMe		OBn	OMe		OBn	OBn			>100	17
64.	Norwogonin	OH		OH	OH						17	38
65.		OH		OBn	OH						100	17
66.		=O		OBn	=O						>100	17
67.	Wogonin	OH		OH	OMe						>100	23
68.		OH		OGlcUA	OMe						>100	22
69.		OH		OMe	OMe						60	38
70.		OH		OBn	OBn						>100	17
71.	Isoscutellarein	OH		OH	OH			OH			20	15
72.		OH		OH	OGlcUA			OH			>100	15
73.		OH		OH	OH			OMe			17	17
74.		OH		OH	OMe			OH			>100	25
75.		OH		OMe	OH			OMe			>100	17
76.		OH		OPr	OH			OPr			>100	17
77.		OH		OPr	OMe			OPr			>100	17
78.		OH		OPr	OMe			OH			>100	17
79.		OH		OPr	OMe			OMe			75	17
80.	Vitexin	OH		OH	Glc			OH			>100	39
81.	Isoswertisin	OH		OMe	Glc			OH			>100	32
82.		OMe		OMe	Glc			OMe			>100	31
83.		OBn		OBn	OBn			OPr			>100	17
84.		OMe		OMe	Me			OMe			>100	17
85.		OH		OH	OMe	OMe					>100	40
86.		OH		OGlcUA	OMe	OMe					>100	41
87.	Skullcapflavone I	OH		OMe	OMe	OH					>100	40
88.	Scutevulin	OH		OH	OMe	OH				OMe	>100	26
89.	Rivularin	OH		OMe	OMe	OH				OMe	>100	41
90.		OMe		OMe	OMe	OMe				OMe	>100	42
91.		OH		OH	OMe	OH			OH	OMe	>100	22
92.		OH		OMe	OMe	OGlc					>100	43
93.		OH		OH	OBn		OPr	OPr			>100	17
94.		OH		OBn	OBn		OPr	OPr			40	17
95.		OH		OH	OH		OPr	OPr			4.5	17
96.		OH		OBn	OH		OPr	OPr			>100	17
97.		OH		OBn	OBn		OBn	OBn			50	17
98.		OH		OH	OBn						70	17
99.		OH		OMe	OH						>100	17
100.		OH		OBn	$OCH_2O(CH_2)_2OMe$						>100	Unp.
101.		OH		OH	Bn			OPr			>100	17
102.		OH		OH	OH			OPr			8	17
103.		OH		OBn	OH			OPr			>100	17
104.		OH		OBn	OBn			OPr			>100	17
105.		OH		OMe	OH			OMe			>100	Unp.
106.		OH		OPr	OH			OPr			80	17
107.	Skullcapflavone II	OH		OMe	OMe	OH					>100	44

108.			OH	OMe	OH	OMe						>100	45
109.			OH	OMe	OGlcUA	OMe						>100	45
110.			OH	Glc	OH	Ara						>100	46
111.			OH	OMe	OH	OMe	OH					>100	45
112.			OH	OMe	OMe	OMe		OMe	OMe			>100	22
113.		OH	OH		OGlc	2-Pt			OH			26	47
114.	Amurensin	OH	OH		OGlc	Pe			OH			15	47
115.		OAc	OAc		OGlc-Ac$_4$	Pe			OAc			18	47
116.	Panasenoside	OGal^2-^{1}Glc	OH		OH				OH			>100	48
117.	Kaempferol	OH	OH		OH				OH			40	Com (W)
118.	Quercetin	OH	OH		OH			OH	OH			15	Com (W)
119.	Quercimeritrin	OH	OH		OGlc			OH	OH			7	49
120.	Quercitrin	ORha	OH		OH			OH	OH			>100	Com
121.	Hyperin	OGal	OH		OH			OH	OH			>100	(T.K)
122.	Rutin	OGlc^6-^{1}Rha	OH		OH			OH	OH			>100	47
123.		OMe	OH		OMe			OMe	OMe			>100	Com (F)
124.	Datiscetin	OH	OH		OH		OH				OH	82	Com (F)
125.	Myricetin	OH	OH		OH			OH	OH	OH		5.2	50
126.	Myricitrin	ORha	OH		OH			OH	OH	OH		6.2	Com (F)
127.		OAc	OAc		OAc			OAc	OAc	OAc		13	Com (F)
128.	Anhydroicaritin	OH	OH		OH	Pe			OMe			50	17
129.	Nor-icariin	ORha	OH		OGlc	Pe			OH			>100	51
130.	Icariin	ORha	OH		OGlc	Pe			OMe			50	52
131.		H, H	OH		OH		OH					>100	52
132.	Dihydrobaicalein	H, H	OH	OH	OH							46	41
133.		H, H	OH	OMe	OMe							>100	24
134.	DihydrooroxylinA	H, H	OH	OMe	OH							>100	17
135.		H, H	OMe	OMe	OMe							>100	24
136.		H, H	OH	OH	OGlcUA-ME				OH			42	17
137.		H, H	OMe	Me	OMe		OMe	OMe	OMe	OMe		>100	15
138.		H, H	OH		OH	OMe			OH			>100	53
139.		H, H	OH		OH	OMe	OH					>100	20
140.		H, H	OMe		OH	OMe	OMe					>100	41
141.		H, H	OH		OMe	OMe	OH				OMe	>100	54
142.		H, H	OH		OMe	OMe	OGlcUA-ME				OMe	>100	54
143.	Naringin	H, H	OH		OH				OH			>100	41
144.	Nephellamuretin	H, OH	OH		OH	Pe			OH			>100	55
145.	Phellamurin	H, OH	OH		OGlc	Pe			OH			>100	47
146.		H, OH	OH		OGlc^6-Mem	Pe			OH			100	47
147.		H, OH	OH		OGlc	2-Pt			OH			>100	47
148.			OH	OMe	OMe	OH						>100	47
149.		H, OAc	OAc		OGlc-Ac$_4$	Pe			OAc			>100	22
150.	Astilbin	H,ORha	OH		OH			OH	OH			>100	47
151.		H, OH	OH		OH		OH				OH	>100	56
152.			OH		OH				OPr			32	51
153.	Luteolin 7-*O*-Glc		OH		OGlc			OH	OH			15	17
154.	Luteolin 5-*O*-Glc		OGlc		OH			OH	OH			40	39
155.			OGlc x Ac$_4$		OAc			OAc	OAc			11	57 57
156.			OH		OH		OH			OH		>100	58
157.			OH		OGlc-*p*-Cou				OH			12	59
158.	Saponarin		OH	Glc	OGlc				OH			>100	22

*Ara = Arabinose, Glc = Glucose, Gal = Galactose, Gent = Gentiobiose, Rha = Rhamnose, Ac = Acetyl, BE = Butyl-ester, Bn = Benzyl, Me = Methyl, ME = Methyl-ester, GlcUA = Glucuronic acid, Pe = Isopentenyl, Pt = Isopentanol, Pr = Isopropyl, *p*-Cou = para-Coumaroyl, Mem = Methylmalonyl, Unp. = Unpublished, Com (F) = Commercial reagent (Funakoshi), Com (W) = Commercial reagent (Wako), Com (T.K.) = Commercial reagent (Tokyo Kasei). Suramin was used as a positive control and its IC_{50} value was 2.4 μM under the same conditions.

Table II. Inhibitory effects of some flavonoids and related compounds on HIV-1 integrase

Compound	IC_{50} (μM)	Source (Ref. No.)
159	2	45
160	>100	17
161	>100	17
162	>100	54
163	>100	54
164	10	37
165	23	37
166	52	19
167	>100	37
168	>100	37
169	>100	37
170	>100	37
171	6.1	37
172	17	Unp.
173	>100	Unp.
174	17	Unp.
175	5.6	37
176	50	19
177	>100	19
178	>100	19
179	>100	19
180	>100	19
181	>100	19
182	>100	19
183	>100	19

Tris-HCl (pH 8.0), 1mM NaCl and 40 fmol/μl of LTR donor DNA was added to each well, and the plate was gently shaken at room temperature for 30 min, then washed with phosphate-buffered saline (PBS) pH 7.3, 4 times. A mixture (45 μl) composed of 12 μl of IN buffer [containing 150 mM 3-(*N*-morpholino)propane sulfonic acid (MOPS) pH 7.2, 75 mM $MnCl_2$, 5 mM dithiothreitol (DTT), 25% glycerol and 500 μg/ml bovine serum albumin], 1 μl of 5 pmol/ μl digoxigenin-labelled target DNA and 32 μl of sterilized water was added into each well. Subsequently, 6 μl of a sample solution and 9 μl of 1/10 dilution of HIV-1 IN enzyme were added to the plate and incubated at 37°C for 80 min. The wells were washed with PBS 4 times and then 100 μl of 500 mU/ml alkaline phosphatase (AP) labelled anti-digoxigenin antibody were added and incubated at 37°C for 1 hr. The plate was washed again with washing buffer containing 0.05% Tween 20 in PBS 4 times and with PBS 4 times. Then, AP buffer (150 μl) containing 100 mM Tris-HCl (pH 9.5), 100 mM NaCl, 5 mM $MgCl_2$ and 10 mM *p*-nitrophenyl phosphate was added into each well and incubated at 37 °C for 1 hr. Finally, the visible absorbance of each well was determined with a microplate reader (BIO-RAD, model 3550 UV) at a wavelength of 405 nm. The positive control composed of the reaction mixture, 50% DMSO and HIV-1 IN enzyme, while the negative control was buffer-E containing 20 mM MOPS (pH 7.2), 400 mM potassium glutamate, 1 mM ethylenediaminetetraacetate disodium salt (EDTA 2Na), 0.1% Nonidet-P 40 (NP-40), 20% glycerol, 1 mM DTT and 4 M urea without HIV-1 IN enzyme.

Results and Discussion

The inhibitory effects of 183 flavonoids on HIV-1 IN were examined by an MIA (Fig. 1) as described in

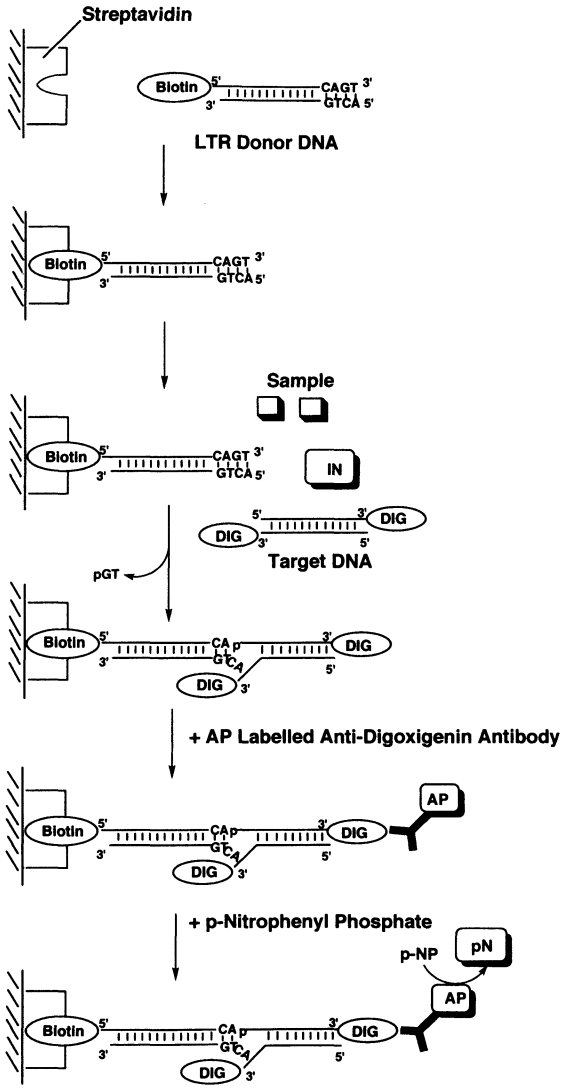
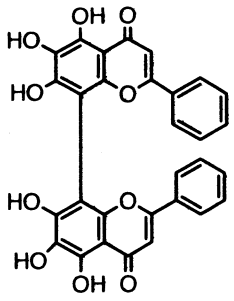
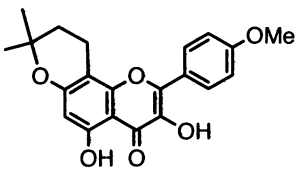


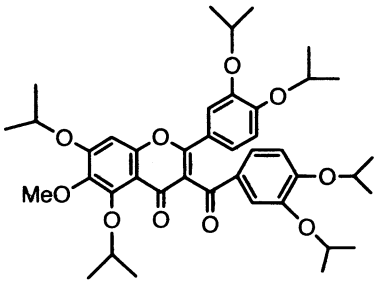
Fig. 1 Schematic illustration of multiplate integration assay procedures for the assay of inhibitory activity of flavonoids against HIV-1 integrase.



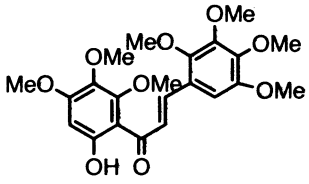
159 (Baicalein dimer)



160 (β-Anhydroicaritin)



161



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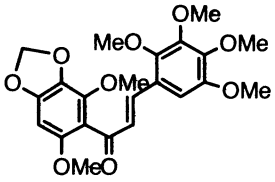

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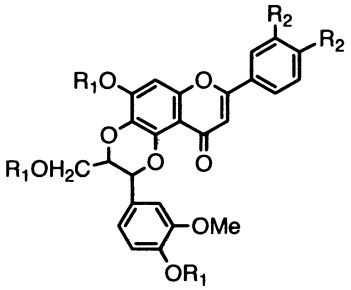
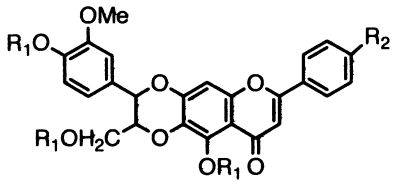
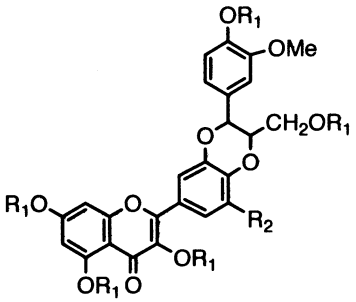


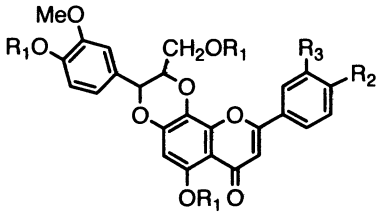
165 : $R_1 = R_2 = H$
166 : R_1 = Ac; $R_2 = H$
175 : $R_1 = H$; R_2 = OH
176 : R_1 = Ac; R_2 = OAc



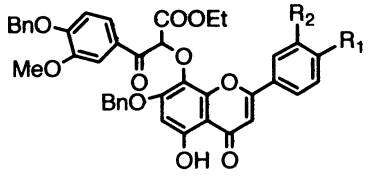
164 : $R_1 = R_2 = H$
167 : R_1 = Ac; R_2 = OAc



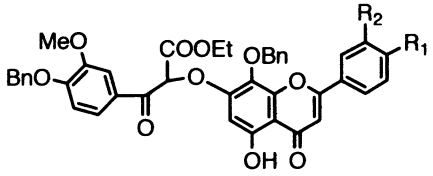
172 : $R_1 = R_2 = H$
173 : R_1 = Ac; $R_2 = H$
174 : $R_1 = H$; R_2 = OH



168 : $R_1 = R_2 = R_3 = H$
169 : R_1 = Ac; $R_2 = R_3 = H$
170 : $R_1 = R_3 = H$; R_2 = OH
171 : $R_1 = H$; $R_2 = R_3$ = OH
183 : $R_1 = R_3 = H$; R_2 = OPr



177 : $R_1 = R_2 = H$
178 : R_1 = OPr; $R_2 = H$
179 : $R_1 = R_2$ = OPr



180 : $R_1 = R_2 = H$
181 : R_1 = OPr; $R_2 = H$
182 : $R_1 = R_2$ = OPr

Fig. 2 Structures of some flavonoids and related compounds listed in Table II.

Material and Methods. As shown in Tables I and II, 6-hydroxyluteolin (**45**), scutellarein (**34**), pedalitin (**47**), scutellarin (**35**), baicalein dimer (**159**) (see Fig. 2), hypolaetin (**61**), 7-*O*-benzyl-6-hydroxyluteolin (**60**) and baicalein (**23**) showed potent HIV-1 IN inhibitory activity with IC_{50} values of 0.4, 0.6, 1.3, 1.7, 2.0, 2.1, 3.0 and 3.6 μM, respectively. Most of these compounds contain hydroxyl substitutions at adjacent positions 5, 6 and 7 on the A-ring of flavonoids. Of these active compounds, 6-hydroxyluteolin (**45**) showed the most potent HIV-1 IN inhibitory effect. This compound contains two more vicinal hydroxyl groups at C-3' and C-4' on the B-ring. Therefore, it may imply that flavonoids with the higher number of vicinal hydroxyl groups tend to have more potent HIV-1 IN inhibitory activity compared to those with a lower number of them. This was also evident by comparison of anti-HIV-1 IN activity between quercetin (**118**, IC_{50} = 15 μM) and kaempferol (**117**, IC_{50} = 40 μM). Introduction of a hydroxyl group at C-3 seems to increase inhibitory activity as shown in the IC_{50} values of quercetin (**118**, IC_{50} = 15 μM) and luteolin (**12**, IC_{50} = 28 μM). 6-Hydroxyluteolin (**45**, IC_{50} = 0.37 μM) having three vicinal hydroxyl substituents at C-5, C-6 and C-7 on the A-ring was more potent inhibitor than myricetin (**125**, IC_{50} = 5.2 μM) having those at C-3', C-4', and C-5' on the B-ring.

Flavonoids, in which hydroxyl groups are replaced by other groups such as methoxyl, acetoxyl, isopropoxyl, isopentenyl, benzyloxyl, glucuronyl and glucosyl, were found to be lower in the inhibitory activity against HIV-1 IN than those with free hydroxyl groups, except for the case of luteolin 5-*O*-glucoside (**153**) and its hepta-acetate (**154**), where the latter compound showed more potent inhibitory activity (*cf.*, **153**, IC_{50} = 40 μM and **154**, IC_{50} = 11 μM). Of the *O*-glucuronyl and *O*-glucosyl compounds, baicalin (**27**, baicalein 7-*O*-glucuronide, IC_{50} = 5.9 μM) was more potent than baicalein 7-*O*-glucoside (**24**, IC_{50} = 28 μM).

Of the 5, 6, 7, 3', 4'-pentaoxygenated flavones, mono- and di-methylation of free hydroxyl groups on the A-ring resulted in appreciable reduction of inhibitory activity as shown in the case of pedalitin (**47**, 6-hydroxyluteolin 7-methyl ether, IC_{50} = 1.3 μM) and cirsiliol (**48**, 6-hydroxyluteolin 6,7-dimethyl ether, IC_{50} = 12 μM) *vs.* 6-hydroxyluteolin (**45**, IC_{50} = 0.37 μM). Furthermore, methylation of a hydroxyl group at C-4' on the B-ring also reduced inhibitory activity as evident from the comparison of the IC_{50} values between eupafolin (**46**, 6-hydroxyluteolin 6,7,4'-trimethyl ether, IC_{50} = 25 μM) and cirsiliol (**48**). Of the luteolin glucosides, luteolin 7-glucoside (**153**, IC_{50} = 15 μM) was more potent in the inhibition than luteolin 5-glucoside (**154**, IC_{50} = 40 μM). In most of the flavonoids examined, substitution of a hydroxyl group at C-5 by methoxyl and other groups resulted in abrupt loss of inhibitory activity except for some acetates (**29**, **115** and **127**). These findings also support the possible chelation of a metal cofactor with carbonyl and hydroxyl groups of granulatine[61] or salicylhydrazide at the catalytic site of IN.[62]

Flavanonols **144-147**, either substituted or unsubstituted at C-3 with a hydroxyl group, showed low inhibitory activity against HIV-1 IN with IC_{50} values of more than 100 μM. Flavanones **131-143** were also inactive except for **132** and **136** with low inhibitory activity (IC_{50} = 46 μM and IC_{50} = 42 μM, respectively), suggesting that the double bond at C-2 and C-3 in the flavonoids may be necessary to the inhibitory effects on HIV-1 IN. Saturation of the double bond leads to loss of planarity in the C-ring, which may prevent the interaction with the active site of IN and/or the intercalation to DNA. Chalcone derivatives **162-163** were also inactive against HIV-1 IN, their IC_{50} values being more than 100 μM. It is implied that the pyrone ring of flavonoids may be associated with potentiation of HIV-1 IN inhibitory activity by fixing two aromatic rings A and B. For flavolignans **164-176**, a hydroxymethyl group on the dioxane ring and adjacent hydroxyl groups at C-3' and C-4' on the B-ring seems to be required for the activity. These criteria could be observed from the inhibitory activity of **171** and **175**, their IC_{50} values being 6.1 and 5.6 μM, respectively.

Various flavonoids have been tested for their inhibitory effects on RT.[12,63] The results indicated that the adjacent hydroxyl groups were important for the requirement of HIV-1 RT inhibitory activity, while flavanones, isoflavones and chalcones were inactive. These requirements were similar to those for HIV-1 IN inhibitory activity as shown in the present experiment. However, the structure-activity relationships of flavonoids on HIV-1 IN were somewhat different from those on HIV-1 PR. Baicalein (**23**) and quercetagetin, the potent HIV-1 RT and HIV-1 IN inhibitors were inactive against

HIV-1 PR with IC_{50} values of 480 and 1,000 μM, respectively.[13] This may imply that the mechanism of action of flavonoids on HIV-1 PR is different from those of HIV-1 RT and HIV-1 IN. On considering the structure of HIV-1 IN, its core domain requires a metal cation as a cofactor which is similar to that of HIV-1 RT. Therefore, it is suggested that the inhibition of flavonoids on HIV-1 RT and HIV-1 IN may be performed by binding or chelating with metal in this domain of these two enzymes HIV-1 IN and HIV-1 RT. In 1991, Kusumoto *et al.*[12] reported that 6-hydroxyluteolin (**45**) and myricetin (**125**) were non-competitive inhibitors against RT with respect to the substrate and the template-primer. The other consideration about the function of these enzymes is that both of HIV-1 IN and HIV-1 RT are DNA-binding enzymes, while that of HIV-1 PR is protein binding one. Therefore, it may have some hint to show the similarity of structure activity relationships of flavonoids with HIV-1 IN and HIV-1 RT.

Although inhibitory effects of flavonoids on HIV-1 IN were previously reported,[64] the assay was carried out with radioisotopic and gel-electrophoretic methods for determination of the inhibitory activity, and the number of flavonoids tested was not as many as described in the present study. However, the main conclusions of the structure-activity relationships of flavonoids on the inhibition of HIV-1 IN were similar to those obtained in our experiments; the inhibitory activity was enhanced in proportion to the number of adjacent hydroxyl groups, while the activity was reduced by substitution of a free hydroxyl group with methoxyl or glycosyl groups. It was also reported that stacking with DNA bases at the active site of enzyme could play an important role in the mechanism by which these flavonoids inhibit IN.[65] Another possible mechanism is that the flavonoids may directly interact at the active site of IN[66] and then interfere the binding of DNA with IN. For the latter proposed mechanism, the x-ray crystallography and 3-D computerization are now becoming useful techniques for determination of the binding properties between active compounds and IN.

Recently, Goldgur and his co-workers reported the structure of HIV-1 catalytic domain complexed with an inhibitor, 5-CITEP, 1-(5-chloroindol-3-yl)-3-hydroxy-3-(2*H*-tetrazol-5-yl)propenone using computergraphic (molecular docking) and x-ray crystallographic means.[67] These techniques are useful for determination of binding affinity of each active flavonoid with the active site of IN. In order to know the binding affinity between each active flavonoid and catalytic acidic residues of IN, some details should be further investigated. The IN/inhibitor complex of active flavonoids will usefully provide a platform for the design of new selective HIV-1 IN inhibitors.

Acknowledgment

Part of this study was financially supported by The Japan Health Science Foundation (Tokyo, Japan).

和文抄録

マルチプレートインテグレーション法を用いて183種のフラボノイド類のHIV-1インテグラーゼ阻害効果を検討した。これらのうち6-hydroxyluteolin, scutellarein, pedalitin, scutellarin, baicalein 二量体, hypolaetin, 7-*O*-benzyl-6-hydroxyluteolin および baicalein は強い阻害を示し，それらの50％阻害濃度はそれぞれ0.4, 0.6, 1.3, 1.7, 2.0, 2.1, 3.0および3.6 μMであった。フラボノイド類による阻害には少なくとも一対の隣接したヒドロキシル基が必要であった。また，隣接したヒドロキシル基の数が増加するに従い，HIV-1インテグラーゼ阻害活性も上昇した。他方，ヒドロキシル基がmethoxyl基, acetoxyl基，isopropoxyl 基，isopentenyl 基，benzyloxyl 基，glucuronyl基 及び glycosyl基に置換されると阻害活性は減少あるいは消失した。試験したフラバノン，フラバノール，カルコン類には顕著な阻害活性は認められなかった。

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