INHIBITION OF AVIAN MYELOBLASTOSIS VIRUS REVERSE TRANSCRIPTASE BY FLAVONES AND ISOFLAVONES

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Reverse transcription is a pivotal step in the replication of retroviruses in which reverse transcriptase plays a crucial role. Therefore, inhibitors of reverse transcriptase are hopefully candidates for antiviral chemotherapeutics. We have undertaken a search for inhibitors of avian myeloblastosis virus (AMV) reverse transcriptase employing natural and synthetic compounds including known antibiotics and culture filtrates of soil actinomycetes[1]. In that screening, fisetin, a naturally occuring flavonoid compound, was found to be one of the most potent inhibitors. Fisetin had already been recognized as the inhibitor of mitochondrial ATPase[2] or phosphatidylinositol kinase[3]. Quercetin, another flavonoid compound closely related in structure to fisetin, shared many biological activities with the latter. Cyclic AMP-independent kinase[4], phosphorylase[5] and tyrosine protein kinase[5,6] were among the target enzymes of quercetin. Isoflavones such as genistein or daidzein were also studied extensively for their effects on various enzymes; catechol-*O*-methyltransferase[7], β-3,4-dihydroxyphenylalanine (DOPA) decarboxylase[8], β-galactosidase[9], phosphatidylinositol turnover[10], phosphatidylinositol kinase[3], protein kinase C[11] and tyrosine protein kinase[6,12,13] were adversely affected by isoflavones. On the basis of these findings, we are interested in the comparison of inhibitory activities of various flavones and isoflavones against AMV reverse transcriptase and the elucidation of structural requirement for potent inhibitors of this enzyme.

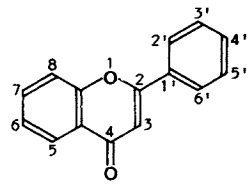
Genistein and myricetin were purchased from Funakoshi Pharmaceutical Co., Ltd. and Aldrich Chemical Co., Inc., respectively. Apigenin, quercetin and acacetin were products of Carl Roth GmbH Co. Fisetin, morin, 3-hydroxyflavone, 7,8-dihydroxyflavone, kaempferol, D-(+)-catechin and α-naphthoflavone were obtained from Tokyo Kasei Kogyo Co., Ltd. DN-23, 24 and 25 were kindly donated by Daiichi Pharmaceutical Co., Ltd. All other chemicals were commercial products of the analytical grade. AMV reverse transcriptase was assayed by the previous method[14].

As shown in Table 1, the inhibition of AMV reverse transcriptase was significant with quercetin, fisetin, myricetin or DN-24, whereas the effects of 7,8-dihydroxyflavone, baicalein and morin were moderate. Apigenin, acacetin, DN-23, DN-25, genistein, biochanin A, D-(+)-catechin and α-naphthoflavone were of no or poor activity.

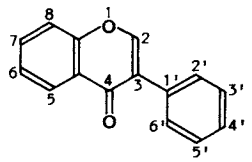
In all the flavones with a potent inhibitory activity against AMV reverse transcriptase, the hydroxy groups at 3, 3′ and 4′ positions exist in common. The hydroxy group at 4′ position, however, does not appear important as proven by the results for apigenin, DN-23 and DN-25. The hydroxy group at 3 position may contribute to some extent since morin shows a moderate inhibition with an ID_{50} of 11 μg/ml. 3-Hydroxyflavone was difficult to be solubilized in DMSO and a net effect of the 3-hydroxy group could not be elucidated. Flavones with the hydroxy group at 6 or 8 position (7,8-dihydroxyflavone and baicalein) are also moderate inhibitors. In contrast, the 5-, 7- and 4′-hydroxy groups are of no contribution to the inhibition of reverse transcriptase. The difference between the spectrum of inhibitors of reverse transcriptase and that of tyrosine protein kinase inhibitors was evident from the results obtained in this study and the previous findings of OGAWARA *et al.*[6]. According to OGAWARA *et al.*, the hydroxy group at 5 position of isoflavones is essential for inhibitors of tyrosine protein kinase and the hydroxy groups at 7 and 4′ positions are also necessary for full expression of the activity. As for the role of 5-hydroxy group, UMEZAWA *et al.*[8] suggested that chelation between the 5-hydroxy group and the carbonyl group at peri-position in isoflavones was required for the potent inhibition of DOPA decarboxylase and the same could be attributable to a structure-activity relationship for the inhibitors of tyrosine protein kinase. In the case of flavones, however, the 3-hydroxy group may be essential for the inhibition of tyrosine protein kinase, whereas the contribution of the 3′-hydroxy group is doubtful.

On the other hand, the spectrum of flavones inhibiting ATPase resembled to that of inhibitors of AMV reverse transcriptase. LANG and RACKER[2] studied the structure-activity relationship among flavones inhibitory against ATPase and proposed that the hydroxy groups at 3′ and perhaps 3 positions are important for the biological activity. Our results are in good agreement with those obtained by LANG and RACKER. However, we would like to propose

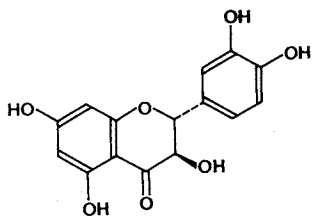
Table 1. Effect of flavones, isoflavones, D-(+)-catechin and α-naphthoflavone on AMV reverse transcriptase.



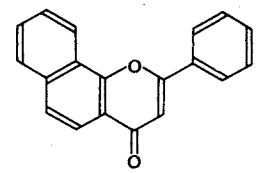
Compound	Position									IC_{50} (μg/ml)
	3	5	6	7	8	2′	3′	4′	5′	
7,8-Dihydroxyflavone	H	H	H	OH	OH	H	H	H	H	17
Chrysin	H	OH	H	OH	H	H	H	H	H	NT[a]
Apigenin	H	OH	H	OH	H	H	H	OH	H	>80
Acacetin	H	OH	H	OH	H	H	H	OCH_3	H	50
Baicalein	H	OH	OH	OH	H	H	H	H	H	9.5
DN-23	H	OH	OCH_3	OCH_3	H	H	H	OH	H	>80
DN-25	H	OH	OCH_3	OCH_3	H	OH	H	OH	OCH_3	>80
3-Hydroxyflavone	OH	H	H	H	H	H	H	H	H	NT[b]
Fisetin	OH	H	H	OH	H	H	OH	OH	H	1.6
Kaempferol	OH	OH	H	OH	H	H	H	OH	H	16
Quercetin	OH	OH	H	OH	H	H	OH	OH	H	2.8
Myricetin	OH	OH	H	OH	H	H	OH	OH	OH	3.0
Morin	OH	OH	H	OH	H	OH	H	OH	H	11
DN-24	OH	OH	OCH_3	OCH_3	H	H	OH	OH	H	1.3



Compound	Position			IC_{50} (μg/ml)
	5	7	4′	
Genistein	OH	OH	OH	>80
Biochanin A	OH	OH	OCH_3	>80



Compound	IC_{50} (μg/ml)
D-(+)-Catechin	>80



Compound	IC_{50} (μg/ml)
α-Naphthoflavone	>80

Each sample was dissolved in DMSO at a concentration of 5 mg/ml and then diluted with Tris-HCl buffer (less than 20 mM, pH 8.0).

[a] Insoluble in Tris-HCl buffer.

[b] Insoluble in DMSO.

NT: Not tested.

alternative explanation for the inhibition of AMV reverse transcriptase by flavones. There are two domains by which flavones are endowed with potency to inhibit reverse transcriptase. One of them is the 3-hydroxy group and the other is the structure consists of two or three hydroxy groups at adjacent positions on the aromatic ring (5,6,7-tri-, 7,8-di-, 2′,3′,4′-tri- or 3′,4′-dihydroxy structure). The compounds with either one of these two domains are classified as moderate inhibitors, *i.e.*, 7,8-dihydroxyflavone, kaempferol, morin and baicalein, and those with both of them (fisetin, quercetin, myricetin and DN-24) strongly inhibit reverse transcriptase, though we can not exclude the possibility that the existence of 3′-hydroxy group alone was enough to provide flavones with a potent inhibitory activity agansit AMV reverse transcriptase.

Genistein was isolated from subterranean clover (*Trifolium subterraneum* L.) as a main component showing oestrogenic activity[15] and, recently, from the culture filtrates of *Streptomyces roseolus*[7], *Aspergillus niger*[8], *Streptomyces xanthophaeus*[9] and *Pseudomonas* sp.[13] as the inhibitors of DOPA decarboxylase, catechol-*O*-methyltransferase, tyrosine protein kinase and β-galactosidase, respectively. Furthermore, orobol and daizein, isoflavones found in many plants, and psi-tectorigenin, a synthetic isoflavone, have been recovered from microbial culture filtrates as the inhibitors of certain enzymes[3,8~12]. None of them, however, satisfies the structural requirement for potent inhibitors of reverse transcriptase. Our unsuccessful screening of microbial culture filtrates for flavones or isoflavones inhibiting AMV reverse transcriptase could be accounted for, at least partly, by this fact.

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References

1) Take, Y.; Y. Inouye, S. Nakamura, H. S. Allaudeen & A. Kubo: Comparative studies of the inhibitory properties of antibiotics on human immunodeficiency virus and avian myeloblastosis virus reverse transcriptases and cellular DNA polymerases. J. Antibiotics 42: 107~115, 1989
2) Lang, D. R. & E. Racker: Effects of quercetin and F_1 inhibitor on mitochondrial ATPase and energy-linked reactions in submitochondrial particles. Biochim. Biophys. Acta 333: 180~186, 1974
3) Nishioka, H.; M. Imoto, T. Sawa, M. Hamada, H. Naganawa, T. Takeuchi & K. Umezawa: Screening of phosphatidylinositol kinase inhibitors from *Streptomyces*. J. Antibiotics 42: 823~825, 1989
4) Graziani, Y.; R. Chayoth, N. Karny, B. Feldman & J. Levy: Regulation of protein kinases activity by quercetin in Ehrlich ascites tumor cells. Biochim. Biophys. Acta 714: 415~421, 1981
5) Srivastava, A. K.: Inhibition of phosphorylase kinase, and tyrosine protein kinase activities by quercetin. Biochem. Biophys. Res. Commun. 131: 1~5, 1985
6) Ogawara, H.; T. Akiyama, S. Watanabe, N. Ito, M. Kobori & Y. Seoda: Inhibition of tyrosine protein kinase activity by synthetic isoflavones and flavones. J. Antibiotics 42: 340~343, 1989
7) Chimura, H.; T. Sawa, Y. Kumada, H. Naganawa, M. Matsuzaki, T. Takita, M. Hamada, T. Takeuchi & H. Umezawa: New isoflavones, inhibiting catechol-O-methyltransferase, produced by *Streptomyces*. J. Antibiotics 28: 619~626, 1975
8) Umezawa, H.; H. Tobe, N. Shibamoto, F. Nakamura, K. Nakamura, M. Matsuzaki & T. Takeuchi: Isolation of isoflavones inhibiting DOPA decarboxylase from fungi and Streptomyces. J. Antibiotics 28: 947~952, 1975
9) Hazato, T.; H. Naganawa, M. Kumagai, T. Aoyagi & H. Umezawa: β-Galactosidase-inhibiting new isoflavonoids produced by actinomycetes. J. Antibiotics 32: 217~222, 1979
10) Imoto, M.; T. Yamashita, T. Sawa, S. Kurasawa, H. Naganawa, T. Takeuchi, Z. Bai-quan & K. Umezawa: Inhibition of cellular phosphatidylinositol turnover by psi-tectorigenin. FEBS Lett. 230: 43~46, 1988
11) Osada, H.; J. Magae, C. Watanabe & K. Isono: Rapid screening method for inhibitors of protein kinase C. J. Antibiotics 41: 925~931, 1988
12) Umezawa, H.; M. Imoto, T. Sawa, K. Isshiki, N. Matsuda, T. Uchida, H. Inuma, M. Hamada & T. Takeuchi: Studies on a new epidermal growth factor-receptor kinase inhibitor, erbstatin, produced by MH435-hF3. J. Antibiotics 39: 170~173, 1986
13) Ogawara, H.; T. Akiyama, J. Ishida, S. Watanabe & K. Suzuki: A specific inhibitor for tyrosine protein kinase from *Pseudomonas*. J. Antibiotics 39: 606~608, 1986
14) Okada, H.; H. Mukai, Y. Inouye & S. Nakamura: Biological properties of streptonigrin derivatives. II. Inhibiton of reverse transcriptase activity. J. Antibiotics 39: 306~308, 1986
15) Bradbury, R. B. & D. E. White: The chemistry of subterranean clover. I. Isolation of formononetin and genistein. J. Chem. Soc. Chem. Commun. 1951: 3447~3449, 1951