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A Simple QSAR Model for Trypsin Aminopeptidase Inhibitory Flavonoids

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A simple QSAR model of the trypsin aminopeptidase inhibitory flavonoids has been developed which enables prediction of the inhibitory potency of flavonoids as a function of their molecular properties. Based upon the results obtained, a possible mode of interaction between the flavonoid and its target receptor is proposed.

INTRODUCTION

Flavonoids, ubiquitously occurring and widely consumed secondary metabolites of plants,[1] are among the active components in vegetables and fruits that possess a diversity of therapeutic activities. Their pharmacological and pharmaceutical functions have been reviewed by Middleton and Kandaswami.[2] The effects exerted by flavonoids include antiinflammatory, antiallergic, antiviral, antimicrobial, antihypertensive, antimutagenic, antiulcerogenic and anticarcinogenic activities. Recently, antiHIV activity has also been reported.[3] Many flavonoids exhibit inhibitory activity against a variety of enzymes.[4] Thus, flavonoids have been attractive candidates for new drugs.[5] However, the molecular mechanisms underlying the pharmacological effects of flavonoids are insufficiently understood.

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In this paper, we present our attempt to quantitatively analyze the structure-activity relationships of flavonoids as trypsin aminopeptidase inhibitors. Quantitative structure-activity relationships (QSAR's) are traditionally developed by establishing a relationship between some measure of biological activity and multiple descriptors of chemical structure.[6] It is a powerful technique for the computer analysis of structure-activity relationships. The results are presented as an algebraic expression which provides useful information in a succinct manner. QSAR method has been proven to correctly forecast the potency of molecules before their synthesis. From the developed QSAR model, novel analogs of the existing flavonoid compounds can be proposed.

RESULTS AND DISCUSSION

The present study compares the inhibitory activity of flavonoids on trypsin protease involved in a variety of pathological processes. This enzyme has become a very useful target for the design of new therapeutic agents.[7] The structures of the flavonoid derivatives used in the analysis are shown in Figure 1.

In order to compare the inhibitory potency of the flavonoids considered, and to establish the relationships between structure and activity, the IC_{50} values were used as experimental biological activity. Inhibitory 50% concentrations (IC_{50}) are expressed as the concentrations in micromoles needed for 50% inhibition of the enzyme. A variety of the molecular descriptors is considered to clarify the effect of electronic, hydrophobic and steric properties on IC_{50}. The descriptor pool used contains the same descriptors as described in our earlier paper.[8] From the initial descriptor pool, the following descriptors were taken into account for the analysis: hydrophobicity term $\log P$, energy of the highest occupied molecular orbital E_{HOBO} (in β), and absolute hardness η (in $-\beta$). In addition, indicator variable I, which expresses the contribution of certain substituents on the magnitude of biological activity was used.

It is well known that hydrophobicity is a very important factor affecting the biological activity. As a standard parameter of hydrophobicity, the logarithm of the partition coefficient in the octanol-water system, $\log P$, is widely used.[9] The influence of $\log P$ on inhibition suggests that hydrophobic interactions are important in the inhibitory process. It is generally assumed that small molecules (MW < 500) penetrate membranes by hydrophobically facilitated passive diffusion. If specific interactions occur, being either electronic or steric interactions, the hydrophobicity parameter alone will not be sufficient and other parameters are needed. The energy level of HOMO has been previously reported as being one of the useful descriptors and is considered to describe the hydrogen-bond ability of a solute molecule.[10] The reactivity

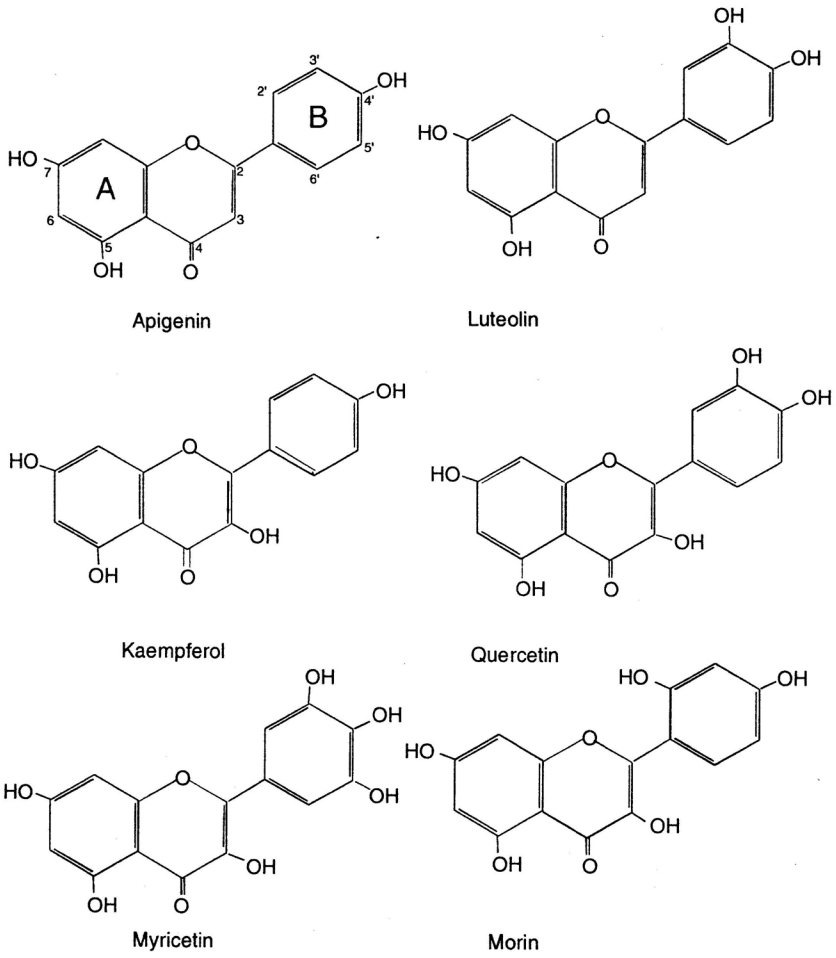

Figure 1. Structures of the studied flavonoid compounds.

of the electrons populating HOMO was also considered to be related to the biological activity. Absolute hardness η, represents half of the energy gap between HOMO and LUMO. It has been shown that absolute hardness is related to the polarizability of molecules.[11]

The structural requirements for a potent effect on the enzyme are much more complex than can be described solely by one molecular descriptor. From the many published structure-activity relationships of flavonoids as inhibitors of different enzymes, the following main features of the chemical structure influencing the activities can be stated. Generally, the planarity of the benzopyrone system, which implies the presence of the C-2, C-3 dou-

TABLE I

Molecular parameters, experimental and calculated activities of the studied flavonoids

Compounds	log P[a]	η[b]	E_{HOMO}[b]	I	IC_{50}	
					obsd.[c]	calcd.[d]
Apigenin	–0.920	0.4316	0.4159	0	141.5	139.5
Luteolin	–1.520	0.4166	0.3858	1	35.3	34.4
Kaempferol	–2.446	0.3915	0.3027	0	105.9	113.6
Quercitin	–2.966	0.3819	0.2835	1	7.1	12.0
Myricetin	–3.550	0.3729	0.2653	1	10.2	6.2
Morin	–2.637[e]	0.3784	0.2592	0	110.8	105.1

[a] log P values were taken from Ref. 14.
[b] Calculated using the HMO method.
[c] Experimental IC_{50} values were taken from Ref. 7.
[d] Calculated by Eq. 2.
[e] Estimated using Eq. 15 from Ref. 14.

ble bond, has been reported to be a requirement for inhibition.[12] Unsubstituted hydroxyl groups at positions 5 and 7, and the ketone group at position 4 confer a potent inhibitory effect.[13] Also, the presence of 3',4'-dihydroxyl substitution seems to be especially important for the activities. Hydroxyl groups may be involved in some H-bonding, which increases the affinity of the molecule to the active site of the enyzme. Indicator variable I was defined as the binary quantity, that is, 1 for the presence and 0 for the absence of vicinal OH groups in B ring. This variable also describes steric influences of the substituents in the phenyl moiety of the flavone core.

We examined the relationships between the chosen molecular descriptors of flavonoids and the biological activity expressed as IC_{50}. Table I lists the numerical values of favourable descriptors used in this study, experimental data for biological activity, and the calculated values of this activity using the obtained QSAR model.

Multiple regression analysis indicated that combinations of the electronic, steric and hydrophobicity descriptors were responsible for the variation in the enzyme inhibitory activity. The most predictive equations using two independent variables are shown below:

$$IC_{50} = 152.455(\pm 8.023) + 16.519(\pm 3.478) \log P - 90.672(\pm 6.120) I \quad (1)$$
$$n = 6 \quad r = 0.992 \quad s = 6.92 \quad F = 173.96$$

$$IC_{50} = -139.404(\pm 53.103) + 646.160(\pm 132.225) \eta - 95.360(\pm 5.666) I \quad (2)$$
$$n = 6 \quad r = 0.992 \quad s = 6.74 \quad F = 183.01$$

$$IC_{50} = 46.314(\pm 15.705) + 224.226(\pm 46.634)\, E_{HOMO} - 98.633(\pm 5.627)\, I \quad (3)$$
$$n = 6 \quad r = 0.992 \quad s = 6.84 \quad F = 177.78$$

In these equations, n represents the number of compounds, r the multiple correlation coefficient, s the standard deviation, F the ratio of regression and residual variances, and the figures in parentheses are the 95% confidence intervals. All the correlations are very good and highly significant based on their correlation coefficients and standard errors of estimate. In Figure 2, we give a plot of $(IC_{50})_{exp.}$ *vs.* $(IC_{50})_{calcd.}$ obtained from Eq. (2)

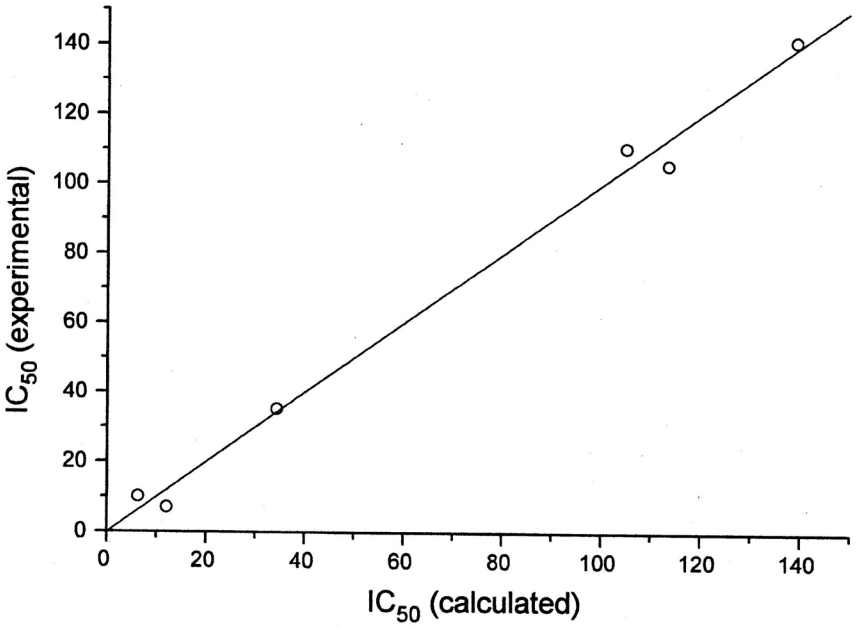

Figure 2. A plot of $(IC_{50})_{exp.}$ *vs.* $(IC_{50})_{calcd.}$.

The results obtained show that the interaction of the inhibitors with the binding niche is through electrostatic and hydrophobic forces. If flavonoids bind to the target by hydrogen bonds at the level of benzopyrone moiety as well as of the B ring, the presence of 3',4'-dihydroxylated positions does seem to be necessary. Thus, the proposed mechanism assumes that the vicinal hydroxyl groups maximize the hydrogen bonding interactions with the enzyme active site binding groove. Hydrophobicity term included in the models suggests that compounds able to inhibit enzymes must be hydrophobic enough to be able to gain access to their inhibitory site.

For synthetic chemists, the proposed QSAR models should be helpful in guiding them to improve of syntheses of new candidate compounds possessing stronger biological response. As a result, a new therapeutic agent could be designed.

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SAŽETAK

Jednostavni QSAR model inhibicije tripsin aminopeptidaze flavonoidima

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Razvijen je jednostavan QSAR model inhibicije tripsin aminopeptidaze flavonoidima, koji na temelju njihovih molekularnih osobina omogućuje predviđanje inihibicije. Na osnovi dobivenih rezultata predložen je mogući način interakcije flavonoida s receptorom.