QSAR of Flavylium Salts as Inhibitors of Xanthine Oxidase

Dragan Amić,* Dušanka Davidović-Amić, and Drago Bešlo

Faculty of Agriculture, The Josip Juraj Strossmayer University, P.O. Box 117, HR-31001 Osijek, The Republic of Croatia

Bono Lučić and Nenad Trinajstić

The Rugjer Bošković Institute, P.O. Box 1016, HR-10001 Zagreb, The Republic of Croatia

Received January 5, 1998

A simple QSAR model of xanthine oxidase (XO) inhibitory flavylium salts, which enables prediction of the inhibitory potency of anthocyanidins as a function of their molecular properties, has been developed. The results obtained in the present work help to understand which of the several tautomeric anhydrobase species present in nearly neutral solution are mainly responsible for the inhibition of XO.

INTRODUCTION

Flavylium salts (anthocyanins, anthocyanidins, and related compounds) are a part of the very large and widespread group of plant pigments known collectively as flavonoids.[1] Many of the red and blue fruits, vegetables, and flowers owe their attractive coloration to the anthocyanins dissolved in the cell sap. Apparently harmless to health, anthocyanins have a considerable potential in the food industry as safe and effective food colorants.[2] There has also been current medicinal interest in anthocyanins as biologically active substances. Several reviews on the pharmacological and medicinal properties of flavylium salts have been published.[3−6] For example, anthocyanin extracts are effective in decreasing capillary permeability and fragility; they possess antiinflammatory, antioedematic, antioxidative, and antiulcer activities, show inhibitory activity of larval growth in insects, and exert pharmacological activities toward several enzymes.

Very recently, inhibitory activity of some flavylium salts on xanthine oxidase (XO) has been published.[7] XO is an unusual enzyme (containing iron and molybdenum) which oxidizes xanthine to uric acid.[8] Since accumulation of excess uric acid in the body results in the painful disease gout (caused by crystallization of uric acid in the joints), there has been considerable interest in designing XO inhibitors.

Species of Flavylium Salts Present at Physiological pH. Using QSAR for compounds having a wide variety of structures appears to be an extremely complicated problem. Tied to this is the question of the form of the compound which is the active species. At physiological pH, depending on the hydroxylation pattern, flavylium salts can exist in many different chemical entities, such as various neutral anhydrobases and their anionic forms in prototropic tautomeric equilibrium.[9] All the tautomers shown in Figure 1 can be present in neutral aqueous solution. In addition, carbinol pseudobases and chalkones resulting from the hydration reactions also exist.[9−11]

Recently, Rastelli *et al.*[12,13] have proposed a method for calculating the percentage composition of the various forms of anthocyanidins present in solution. These tautomers are likely to play different roles in pharmacological activity. Owing to the specificity of the enzyme−inhibitor interactions, one has to take into account that unfavored tautomeric forms could even become the preferred enzyme-bound forms. Thus, to establish a quantitative relationship between the chemical structure and biological activity we had to predict which of the species present in solution is/are mainly responsible for the exerted activity.

RESULTS AND DISCUSSION

To compare the inhibitory potency of the flavylium salts considered and to establish the relationships between structure and activity, the dissociation constant of the enzyme−inhibitor complex, K_{EI}, was used as experimental biological activity. The structures of the flavylium salts used in the analysis are shown in Figure 2.

The variety in molecular descriptors is considered to clarify the effect of electronic, hydrophobic, and steric properties on K_{EI}. We started with a descriptor pool containing the same descriptors as described in our recent paper.[14] In addition, Hansch's hydrophobocity parameter π, molar refractivity MR, and Hammett's electronic constants of substituents σ_p were used. At physiological pH, both the neutral and the anionic anhydrobase forms of flavylium salts are present and could be involved in the interaction with the enzyme. Therefore, descriptors were calculated for all possible tautomeric species. To determine the contribution of each tautomeric form to explaining the enzyme inhibitory activity, models were calculated for all combinations of one to six tautomeric forms. For example, compound **1** possesses six tautomeric forms (A_5, A_7, $A_{4'}$, $A_{54'}^-$, $A_{74'}^-$, and A_{57}^-), compound **2** has three tautomeric forms (A_7, $A_{4'}$, and $A_{74'}^-$), while compound **8** possesses only one tautomeric form ($A_{4'}$).

As usual, classical QSARs were calculated using stepwise multiple linear regression to fit the biological activity to molecular properties. This served to elucidate the relative significance of each independent variable in explaining the XO inhibitory activity as determined by regression analysis. Different models for predicting the inhibition of XO by a particular tautomeric form of anthocyanidins can be achieved by considering various combinations of molecular descrip-

S0095-2338(98)00002-X CCC: $15.00
Published on Web 08/20/1998

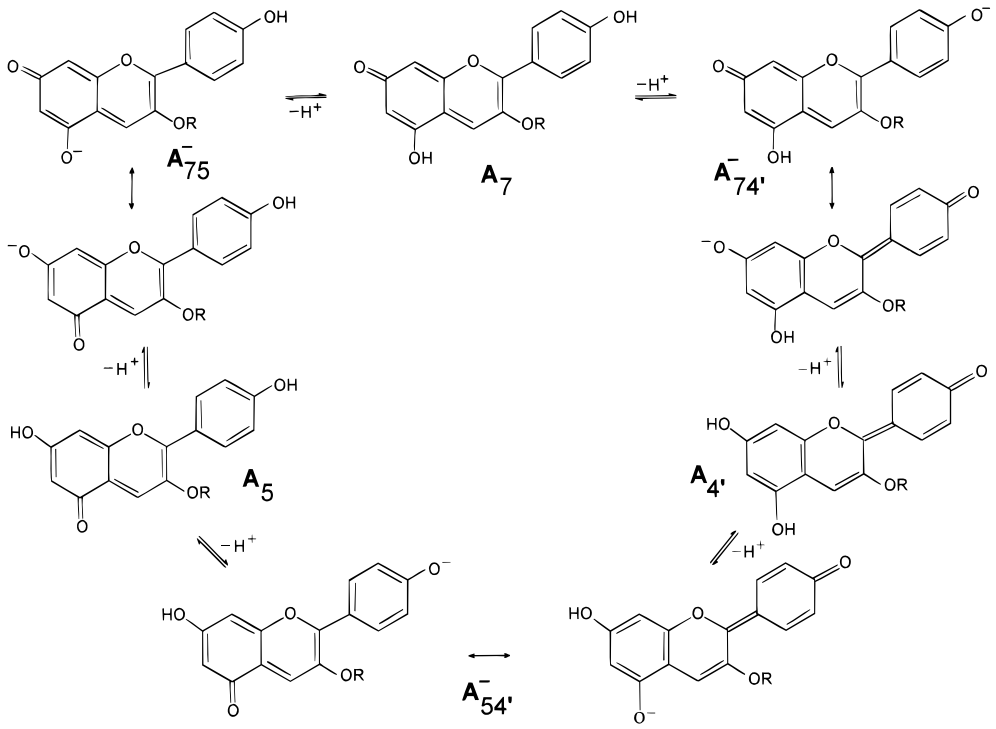
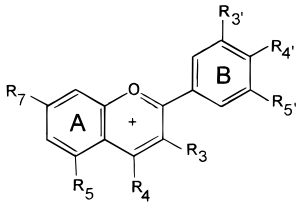


Figure 1. Prototropic equilibria of flavylium salts at physiological pH.



Substitution site

Flavylium salt	R_3	R_4	R_5	R_7	$R_{3'}$	$R_{4'}$	$R_{5'}$
1	OH	H	OH	OH	OH	OH	OH
2	OH	H	H	OH	OH	OH	OH
3	OH	H	OH	OH	OCH_3	OH	OCH_3
4	OH	H	OH	OH	OH	OH	H
5	OH	H	OH	OH	H	OH	H
6	H	H	OH	OH	H	OH	H
7	H	H	OH	OH	OH	OCH_3	H
8	H	H	H	OH	H	OCH_3	H
9	H	H	OCH_3	OH	H	OCH_3	H
10	H	H	OH	OCH_3	H	OCH_3	H
11	H	H	OCH_3	OCH_3	H	OH	H
12	H	H	OH	OH	H	OCH_3	H
13	H	CH_3	H	OH	H	OCH_3	H
14	H	H	OH	OCH_3	H	OH	H
15	H	H	OCH_3	OH	H	OH	H
16	H	H	H	OH	H	OH	H

Figure 2. Structures of studied flavylium salts.

tors. On the basis of the multiple regression analysis performed, tautomeric forms $A_{74'}^-$ and $A_{54'}^-$ seem to be most active. Table 1 lists the numerical values of descriptors for favorable tautomers and experimental data of biological activity.

Multiple regression analysis indicated that combinations of the hydrophobicity descriptor π and indicator variable I were responsible for variation in the enzyme inhibitory activity. Descriptor π_{7O}- (π_{5OH}) represents π values for substituents in position C-7 (C-5) with preferably ionized 7-OH group; π_{5O}- (π_{7OH}) represents π values for substituents in position C-5 (C-7) with preferably ionized 5-OH group. The applied values of π for H, OCH_3, OH, and O^- substituent were 0, −0.02, −0.67, and −3.87, respectively.[15] Indicator variable I was defined as the binary quantity, that is, 1 for the presence and 0 for the absence of 5,7-diOH groups. This variable also describes steric influences of substituents in the benzopyrylium moiety of the flavylium pharmacophore. The following three term equations were developed:

$$K_{EI} = 313.064(\pm 16.186) + 75.857(\pm 4.460)\pi_{7O}\text{-} + 422.222(\pm 29.422)\pi_{5OH} + 264.859(\pm 21.366)I \quad (1)$$

$$n = 16, \quad r = 0.982, \quad s = 15.63, \quad F = 111.13, \quad Q = 0.0040$$

$$K_{EI} = 314.196(\pm 15.213) + 443.339(\pm 24.470)\pi_{7OH} + 71.594(\pm 4.671)\pi_{5O}\text{-} + 261.376(\pm 19.807)I \quad (2)$$

$$n = 16, \quad r = 0.985, \quad s = 14.68, \quad F = 126.47, \quad Q = 0.0046$$

In the above and subsequent equations, n represents the number of compounds, r the multiple correlation coefficient, s the standard deviation, and F the ratio of regression and residual variances. The quality factor[16] Q was defined as $Q = r/s^2$. The figures in parentheses are the 95% confidence interval. The generated QSAR models are statistically fairly good despite the diversity of the molecular structures of the compounds investigated.

The activity of anthocyanidins is very sensitive to changes in the structure of tautomeric forms: activity of ionized tautomers is quite different from that of neutral forms. It would appear that the primary requirement for high activity is the presence of ionized forms. Moreover, despite the fact that mutagenicity of anthocyanidins is a highly restricted event, tautomers of some synthetic flavylium salts having nonionic structure at neutral pH showed mutagenicity.[17] Thus, monohydroxyflavylium salts could be excluded from useful XO inhibitors.

Table 1. Favorable Descriptors[a] and Experimental Activities[b]

flavylium salt	$A_{7'}^{-}$		$A_{5'}^{-}$				K_{EI}
	π_{7O^-}	π_{5OH}	π_{7OH}	π_{5O^-}	I	Q_A	(μM)
1	−3.87	−0.67	−0.67	−3.87	1	6.106	0.07
2	−3.87	0	−0.67	0	0	6.028	0.15
3	−3.87	−0.67	−0.67	−3.87	1	6.106	1.34
4	−3.87	−0.67	−0.67	−3.87	1	6.104	0.53
5	−3.87	−0.67	−0.67	−3.87	1	6.102	1.64
6	−3.87	−0.67	−0.67	−3.87	1	6.106	3.10
7	−3.87	−0.67	−0.67	−3.87	1	6.105	0.99
8	−3.87	0	−0.67	0	0	6.022	5.18
9	−3.87	−0.02	−0.67	−0.02	0	6.093	18.89
10	−0.02	−0.67	−0.02	−3.87	0	6.101	50.68
11	−0.02	−0.02	−0.02	−0.02	0	6.037	304.00
12	−3.87	−0.67	−0.67	−3.87	1	6.104	2.60
13	−3.87	0	−0.67	0	0	6.078	41.85
14	−0.02	−0.67	−0.02	−3.87	0	6.047	5.74
15	−3.87	−0.02	−0.67	−0.02	0	6.095	32.37
16	−3.87	0	−0.67	0	0	6.024	1.65

[a] π values were taken from ref 15. Q_A values were calculated using the HMO method. [b] Experimental K_{EI} values were taken from ref 7.

The methoxy group presence in ring A causes a decrease of activity. This could be ascribed to steric restrictions. It could be predicted that the preferred enzyme-bound forms are ionized tautomeric forms bearing negative charge on A-ring. Indeed, the anionic oxygen in A-ring of the flavylium pharmacophore has a very strong carbonyl character due to a substantial delocalization of the negative charge on the entire benzopyrylium core. This delocalization seems to be essential for the activity. The most active compounds have an extended π aromatic system which is capable of delocalizing the negative charge of the anionic tautomers. This delocalization may act to minimize the repulsive interaction between the ionic form of the anthocyanidins and the mitochondrial membrane. Generally, activity is improved by increasing the number of tautomeric forms of a particular anthocyanidin. For example, compound **1** is very active and possesses six tautomeric forms; on the contrary, compound **11** is much less active and has only one tautomeric form.

To examine the assumption that the anionic oxygen in A-ring has a considerable carbonyl character, models were generated using Q_A descriptor (the sum of charge densities at the A-ring carbons for anhydrobase form A_7). Addition of the Q_A term into eqs 3 and 4 yielded the corresponding correlations better than eqs 1 and 2.

$$K_{EI} = -3159.73(\pm 418.191) + 79.257(\pm 1.775)\pi_{7O^-} + 457.971(\pm 12.182)\pi_{5OH} + 260.917(\pm 8.290)I + 575.594(\pm 69.304)Q_A \quad (3)$$

$$n = 16, \quad r = 0.998, \quad s = 6.05, \quad F = 572.74, \quad Q = 0.0273$$

$$K_{EI} = -2710.81(\pm 519.313) + 458.901(\pm 12.924)\pi_{7OH} + 76.486(\pm 2.555)\pi_{5O^-} + 256.195(\pm 10.273)I + 501.177(\pm 86.029)Q_A \quad (4)$$

$$n = 16, \quad r = 0.996, \quad s = 7.59, \quad F = 363.68, \quad Q = 0.0173$$

A plot of $pK_{EI(exp)}$ vs $pK_{EI(calcd)}$ obtained from eq 3 can be seen from Figure 3.

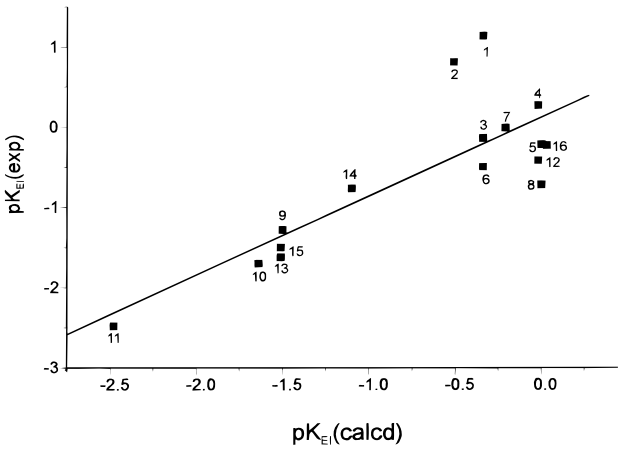


Figure 3. A plot of $pK_{EI(exp)}$ vs $pK_{EI(calcd)}$.

Based upon the QSAR results obtained, the following interactions at the molecular level are proposed:

1. The presence of the ionizable hydroxy group at positions 5 and/or 7 in A-ring seems to be indispensable for the activity. These groups are considered to be involved in either hydrogen bonding or electrostatic interaction with an amino acid residue in the close proximity of the active site of XO.

2. Lack of diversity in the contribution of B-ring substituents suggests the existence of a hydrophobic pocket or groove, which accommodates the phenyl moiety (B-ring), in the proximity of the active site.

Our results are consistent with those recently obtained by Rastelli et al.[18,19] They proposed a similar mechanism for the inhibition of XO by flavones. Two structurally related classes of flavonoids, flavylium salts and flavones, seem to achieve a high degree of similarity in acting as XO inhibitors.

In conclusion, we would like to stress that the proposed QSAR models should be helpful in guiding synthetic chemists to improve syntheses of new candidate compounds possessing an enhanced inhibitory activity on XO. As a result, a new therapeutic agent could be designed.

ACKNOWLEDGMENT

This work was supported in part by Grants 079301 (D.A., D.D.A., D.B.) and 00980605 (B.L., N.T.) awarded by the Ministry of Science and Technology of the Republic of Croatia.

REFERENCES AND NOTES

(1) Harborne, J. B. *Comparative Biochemistry of the Flavonoids*; Academic Press: London and New York, 1967.
(2) Markakis, P. Anthocyanins as Food Additives. In *Anthocyanins as Food Colors*; Markakis, P., Ed.; Academic Press: New York; 1982; pp 245−253.
(3) Harborne, J. B.; Grayer, R. J. The Anthocyanins. In *The Flavonoids: Advances in Research Since 1980*; Harborne, J. B., Ed.; Chapman and Hall: London; 1988; pp 1−20.
(4) Timberlake, C. F.; Henry, B. S. Anthocyanins as Natural Food Colorants. In *Plant Flavonoids in Biology and Medicine II: Biochemical, Cellular and Medicinal Properties*; Cody, V., Middleton, E., Jr., Harborne, J. B., Beretz, A., Eds.; Alan R. Liss: New York; 1988; pp 107−121.
(5) Mazza, G.; Miniati, E. *Anthocyanins in Fruits, Vegetables, and Grains*; CRC Press: Boca Raton, FL, 1993; pp 1−28.
(6) Tamura, H.; Yamagami, A. Antioxidative Activity of Monoacylated Anthocyanins Isolated from Muscant Bailey A Grape. *J. Agric. Food Chem.* **1994**, *42*, 1612−1615.

(7) Costantino, L.; Rastelli, G.; Albasini, A.; Anthocyanins as Inhibitors of Xanthine Oxidase. *Pharmazie* **1995**, *50*, 573−574.
(8) Elliott, W. H.; Elliott, D. C. *Biochemistry and Molecular Biology*; Oxford University Press: Oxford, 1997; p 230.
(9) Brouillard, R. Chemical Structure of Anthocyanins. In *Anthocyanins as Food Colors*; Markakis, P., Ed.; Academic Press: New York; 1982; pp 1−40.
(10) Jurd, L.; Geissman, T. A. Anthocyanins and Related Compounds. II. Structural Transformations of Some Anhydro Bases. *J. Org. Chem.* **1963**, *28*, 2394−2397.
(11) Baranac, J. M.; Amić, D. S. Structural Transformations of Apigeninidin-Type Flavylium Salts. *J. Agric. Food Chem.* **1990**, *38*, 2111−2115.
(12) Rastelli, G.; Costantino, L.; Albasini, A. Physico-Chemical Properties of Anthocyanidins. Part 1. Theoretical Evaluation of the Stability of the Neutral and Anionic Tautomeric Forms. *J. Mol. Struct. (THEOCHEM)* **1993**, *279*, 157−166.
(13) Costantino, L.; Rastelli, G.; Rossi, M. C.; Albasini, A. Quantitative Measurement of Proton Dissociation and Tautomeric Constants of Apigeninidin. *J. Chem. Soc., Perkin Trans. 2* **1995**, 227−234.
(14) Amić, D.; Davidović-Amić, D.; Bešlo, D.; Lučić, B.; Trinajstić, N. The Use of the Ordered Orthogonalized Multivariate Linear Regression in a Structure−Activity Study of Coumarin and Flavonoid Derivatives as Inhibitors of Aldose Reductase. *J. Chem. Inf. Comput. Sci.* **1997**, *37*, 581−586.
(15) Hansch, C.; Leo, A. *Exploring QSAR. Fundamentals and Applications in Chemistry and Biology*; American Chemical Society: Washington, DC, 1995.
(16) Pogliani, L. A Strategy for Molecular Modeling of a Physicochemical Property using a Linear Combination of Connectivity Indexes, *Croat. Chem. Acta* **1996**, *69*, 95−109.
(17) Sweeny, J. G.; Iacobucci, G. A.; Brusick, D.; Jagannath, D. R. Structure−Activity Relationships in the Mutagenicity of Quinone Methides of 7-Hydroxyflavylium Salts for *Salmonella typhimirium*. *Mutation Res.* **1981**, *82*, 275−283.
(18) Costantino, L.; Rastelli, G.; Albasini, A. A Rational Approach to the Design of Flavones as Xanthine Oxidase Inhibitors, *Eur. J. Med. Chem.* **1996**, *31*, 693−699.
(19) Rastelli, G.; Costantino, L.; Albasini, A. A Model of the Interaction of Substrates and Inhibitors with Xanthine Oxidase, *J. Am. Chem. Soc.* **1997**, *119*, 3007−3016.

CI980002R