

## Quantitative structure – activity relationships (QSAR) in flavonoid compound class

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### Abstract

The paper presents a quantitative structure-activity relationship (QSAR) study on the cytochrome P450 inhibitory activity of a series of 11 natural flavonoids. The molecular modeling, structural descriptor calculations, and mathematical models evaluation for these flavonoids were performed by using HyperChem package. Statistically relevant mono- and biparametrial models by using the number of donor atoms of H-bonds, percent of polar molecular surface, and Lipinski octanol-water partition coefficient were obtained (correlation coefficient > 0.8).

**Keywords:** cytochrome P450, flavonoids, quantitative structure-activity relationship (QSAR), molecular modeling, mathematical model

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### 1. Introduction

Cytochrome P450 enzymes (abbreviated as CYP, P450, or CYP450) represent a coupled enzyme system composed of the heme- containing cytochrome P450 and the nicotinamide, adenine dinucleotide phosphate (H) (NADPH)- containing cytochrome P450 reductase [1,2]. Cytochrome P450 catalyzes mono-oxidation reactions. The name cytochrome P450 is a generic term applied to a group of hemoproteins defined by a unique spectral property observed when reduced cytochrome P450 (containing  $\text{Fe}^{2+}$ ) is treated with carbon monoxide [1]. The complex formed has a maximum absorption at 450 nm, which results from the presence of an axial thiolate ligand to the heme iron. This spectral characteristic is present only when the protein is intact and catalytically functional. In vertebrates, the highest concentrations of cytochrome P450 are found in the liver; however, cytochrome P450 enzymes are also present in the lung, kidney, testes, skin, and gastrointestinal tract [1,2]. Cytochromes P450 may catalyze the hydroxylation of carbon-hydrogen bonds to transform hydrocarbons to the

corresponding alcohols. In larger aliphatic chains, the ( $\omega$ -1) position is often a favored point of attack. Oxidative *N*-, *O*- or *S*-dealkylations and oxidative dehalogenations are similar in mechanism to aliphatic hydroxylation, but give different end products due to secondary reactions of the intermediate products formed. Olefins are also oxidized by cytochrome P450, and with some substrates, epoxides are formed as products [1]. A great number of enzymes, like hydrolases, oxidoreductases, DNA synthetases, RNA polymerases, phosphatases, protein-phosphokinases, oxygenases, aminoacid-oxidases, as well as cytochrome P450 are inhibited by bioflavonoids, and thus these natural compounds could regulate the actions of above mentioned enzymes [3,4]. This study try to find the main structural parameters of flavonoids which are implied in the inhibitory action against cytochrome P450 and to establish statistically significant mathematical models for this biological activity, and furthermore to predict new flavonoid-like molecules with given activity.

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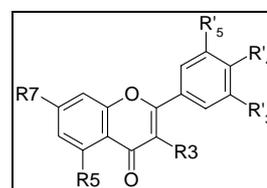
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## 2. Materials and Method

**Structures and biological activities.** A series of 11 natural compounds from flavonoid class with inhibition activity against Cytochrome P450 (monooxygenase) was selected from the Wombat database [5]. Structural variation appear especially on the benzen moiety from the benzopyrane structure of flavonoids and also on the 2-phenyl moiety (Table 1). The biological activity ( $A_{exp}$ ) was considered the negative logarithm of the 50% inhibitory concentration (mole/l, M) against cytochrome P450:  $A_{exp} = \log(1/IC_{50})$ .

**Molecular modeling and conformational analysis.** Molecular modeling of the selected flavonoids was performed by using the HyperChem 5.1 package (MM+ program) [6]. The RMS gradient was 0.01 kcal/mole and for structural optimization the conjugated gradient Polak-Ribiere algorithm was used. Conformational analysis of the flavonoids was realized with

the Conformational Search program from the HyperChem package, but the number of flexible torsion angles was low due to the advanced rigidity of flavonoid skeleton. The following condition were used: RMS gradient of 0.01 kcal/mole, range for acyclic torsion variation of  $\pm 60^\circ - \pm 180^\circ$ , maximum acceptance criterion of 4 kcal/mole above best, maximum iterations or optimizations of 250. Conformations were considered duplicate if the energy was within 0.01 kcal/mole, the distance between the corresponding atoms were lower than 0.5Å or the difference between the corresponding torsion angles was lower than  $15^\circ$ .



**Figure 1.** The basis structure of flavonoids used for analysis

**Table 1.** Flavonoid structures used molecular modeling and QSAR analysis

No	Structure	Name	$A_{exp}$	No	Structure	Name	$A_{exp}$
1		chrysin	6.6990	7		morin	5.0241
2		apigenin	5.8697	8		kaempferol	4.1343
3		luteolin	4.8729	9		myricetin	3.7328
4		galangin	5.5143	10		avicularin	3.4237
5		quercetin	3.7721	11		quercitrin	3.6498
6		fisetin	3.6253				

**Structural parameters.** Among the constitutional, topological, geometrical, and molecular property descriptors, the following structural parameters, calculated according to [7] or by using *in house* programs, seems to have importance on the cytochrome P450 inhibition: octanol-water partition coefficient calculated with the AlogPS and ClogP programs (*estLogKow* and *LpkCLogP*, respectively) or according to Moriguchi (*MLogP*), water solubility calculated with the same ALogPS program (*estLogWsol*), the percent of polar or non-polar molecular surface (*SfaPercPol* and *SfaPercNonpol*, respectively), the mean atomic Sanderson electronegativity (*Me*), the mean information index on atomic composition (*AAC*), the average connectivity index chi-4 (*X4A*), the topological charge index of order 4 (*GGI4*), the mean topological charge index of order 2 (*JGI2*), the Moran autocorrelation - lag 4 / weighted by atomic Sanderson electronegativities (*MATS4e*), the total number of donor atoms for H-bonds (with N and O) (*nHdon*), and also the hydrophilic factor (*Hy*).

**QSAR analysis.** For the quantitative structure – activity relationships (QSAR)

analysis in the flavonoid compounds class with inhibition activity against cytochrome P450 the following mono- and bilinear mathematical models were used:

$$\log(1/IC_{50})_i = a_0 + \sum_j b_{ij} \cdot P_{ij}$$

where  $P_{ij}$  represents the  $j$  parameter of the structure  $i$ ,  $a_0$  and  $b_{ij}$  are coefficients of the model.

### 3. Results and Discussion

Molecular modeling and conformational analysis of the flavonoid structures with inhibitory activity against cytochrome P450 indicate that the number of stable conformations was lower, especially for the structures with a lower flexible bonds. Excepting the flavonoside compounds (avicularin and quercitrin), which have saccharide moieties in the 3-position of the 2-phenyl-4H-benzo[e]piran-4-one skeleton, all the remain nine compounds have maximum two stable conformations (due to the presence of the flexible bond from the 2-position of the base structure). The specified descriptors were calculated only for the most stable conformation and the intercorrelational matrix is presented in Table 2.

**Table 2.** Intercorrelational matrix for the calculated descriptors in the case of flavonoid compound class

	Me	AAC	X4A	GGI4	JGI2	MATS4e	nHdon	Hy	MLogP	estLogKow	LpkCLogP	estLogWsol	SfaPercPol	SfaPercNonpol	log(1/IC50)
Me	1.00	0.89	-0.76	0.68	0.88	-0.75	0.75	0.78	-0.79	-0.75	-0.76	0.65	0.9	-0.9	-0.85
AAC		1.00	-0.89	0.89	0.89	-0.76	0.95	0.96	-0.97	-0.90	-0.94	0.82	1.0	-1.0	-0.89
X4A			1.00	-0.89	-0.89	0.87	-0.87	-0.87	0.88	0.95	0.93	-0.93	-0.8	0.8	0.93
GGI4				1.00	0.79	-0.70	0.96	0.95	-0.94	-0.89	-0.93	0.89	0.8	-0.8	-0.81
JGI2					1.00	-0.94	0.81	0.82	-0.82	-0.78	-0.87	0.74	0.9	-0.9	-0.95
MATS4e						1.00	-0.71	-0.71	0.72	0.69	0.81	-0.67	-0.7	0.7	0.94
nHdon							1.00	1.00	-1.00	-0.87	-0.96	0.82	0.8	-0.8	-0.82
Hy								1.00	-1.00	-0.88	-0.96	0.81	0.9	-0.9	-0.83
MLogP									1.00	0.89	0.97	-0.82	-0.9	0.9	0.84
estLogKow										1.00	0.89	-0.97	-0.8	0.8	0.84
LpkCLogP											1.00	-0.85	-0.8	0.8	0.87
estLogWsol												1.00	0.7	-0.7	-0.79
SfaPercPol													1.00	-1.0	-0.83
SfaPercNonpol														1.00	0.83
log(1/IC50)															1.00

For the QSAR analysis mono- and multiparametrial mathematical models were obtained for the cytochrome P450 inhibition activity of natural flavonoids. For the multiparametrial equations the descriptor used did not intercorrelate.

Thus, the best monoparametrial mathematical models were obtained in the case of the mean atomic Sanderson electronegativity ( $Me$ ), with a correlation coefficient of 0.85 (Eq. 1), for the mean information index on atomic composition ( $AAC$ ), or for the average connectivity and topological charge index ( $X4A$ ,  $GGI4$ ). Very good results for monoparametrial QSARs were obtained by using the mean topological charge index of order 2 ( $JGI2$ ), with a correlation coefficient of 0.95 (Eq. 2). Even these parameters have reduced physical significance, it can be observed that the biological activity increase with the decrease of the parameter values; these parameters finally describe the hydrophylic-hydrophobic properties or polarizabilities of the molecules.

$$\log\left(\frac{1}{IC_{50}}\right)_i = 128.7(\pm 25.5) - 118.5(\pm 24.4) \cdot (Me)_i \quad (1)$$

$n = 11$ ;  $r = 0.851$ ;  $s = 0.6$ ;  $F = 23.6$

$$\log\left(\frac{1}{IC_{50}}\right)_i = 13.2(\pm 0.96) - 58.7(\pm 6.5) \cdot (JGI2)_i \quad (2)$$

$n = 11$ ;  $r = 0.949$ ;  $s = 0.36$ ;  $F = 81.9$

Parameters with significative statistical importance as well as with physical evidence in the monoparametrial QSARs were the total number of donor atoms for H-bonds (with N and O) ( $nHdon$ ), with a correlation coefficient of 0.82 (Eq. 3), but also other parameters which more or less intercorrelate with the  $nHdon$ : octanol-water partition coefficient calculated with ClogP program ( $LpkCLogP$ ),  $r = 0.87$  (Eq. 4), and the percent of polar or non-polar molecular surface of the molecule ( $SfaPercPol$  and  $SfaPercNonpol$ ),  $r = 0.83$  (Eqs. 5 and 6).

$$\log\left(\frac{1}{IC_{50}}\right)_i = 7.08(\pm 0.61) - 0.55(\pm 0.13) \cdot (nHdon)_i \quad (3)$$

$n = 11$ ;  $r = 0.824$ ;  $s = 0.65$ ;  $F = 18.9$

$$\log\left(\frac{1}{IC_{50}}\right)_i = 3.21(\pm 0.31) + 0.82(\pm 0.15) \cdot (LpkCLogP)_i \quad (4)$$

$n = 11$ ;  $r = 0.870$ ;  $s = 0.57$ ;  $F = 28.0$

$$\log\left(\frac{1}{IC_{50}}\right)_i = 9.83(\pm 1.21) - 12.24(\pm 2.78) \cdot (SfaPercPol)_i \quad (5)$$

$n = 11$ ;  $r = 0.826$ ;  $s = 0.65$ ;  $F = 19.4$

$$\log\left(\frac{1}{IC_{50}}\right)_i = -2.41(\pm 1.60) + 12.24(\pm 2.78) \cdot (SfaPercNonpol)_i \quad (6)$$

$n = 11$ ;  $r = 0.826$ ;  $s = 0.65$ ;  $F = 19.4$

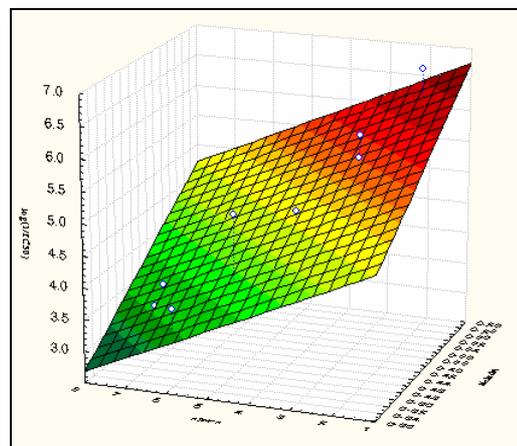
In this class of anti-cytochrome P450 flavonoid compounds multiparametrial QSARs were obtained. The relevant descriptors (which not intercorrelate) for the bilinear mathematical models were  $SfaPercPol$  with  $nHdon$  or  $LogWsol$ , the correlation coefficient in both cases being  $\sim 0.87$  (Eqs. 7 and 8). The quality of the biparametrial model in the case of  $SfaPercPol$  and  $nHdon$  is revealed by the graphical representation from Figure 2.

$$\log\left(\frac{1}{IC_{50}}\right)_i = 8.81(\pm 1.42) - 0.29(\pm 0.23) \cdot (nHdon)_i - 6.75(\pm 5.05) \cdot (SfaPercPol)_i \quad (7)$$

$n = 11$ ;  $r = 0.859$ ;  $s = 0.63$ ;  $F = 11.2$

$$\log\left(\frac{1}{IC_{50}}\right)_i = 4.81(\pm 3.32) - 0.23(\pm 0.77) \cdot (EstLogWsol)_i - 7.86(\pm 3.75) \cdot (SfaPercPol)_i \quad (8)$$

$n = 11$ ;  $r = 0.872$ ;  $s = 0.60$ ;  $F = 12.7$



**Figure 2.**  $\log(1/IC_{50})$  vs.  $nHdon$ ,  $SfaPercPol$  plot in the case of cytochrome P450 inhibitory flavonoids

#### 4. Conclusion

The study on the molecular modeling and quantitative structure – activity relationships (QSAR) for the case of natural flavonoids with inhibitory activity against cytochrome P450 revealed that the important structural parameters for this activity are the total number of donor atoms for H-bonds (with N and O), which are significant in the flavonoid-receptor site interaction (by means of hydrogen bonds), the octanol-water partition coefficient and the polar surface of the molecule; these two last parameters can act even in the transport process or in the hydrophobic and/or hydrophylic interaction between flavonoids and enzyme. Biparametrial mathematical models have lower statistical evidence. However, both mono- and biparametrial models could be used in order to design new structures (semi-synthetic compounds for example) with potent inhibitory activity against cytochrome P450.

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