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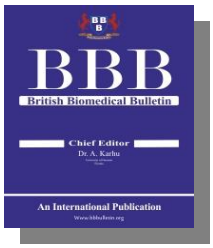

Original

Relationship Between Thermodynamic Parameters and Genotoxicity of Bioactive Phenolic Compounds

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A B S T R A C T

Background: The effects in health of food containing phenolic compounds require currently studies related to safety. It has been established that these compounds may exhibit dual activity (antioxidant/pro-oxidant effects).
Aims: The current work is focused on an *in silico* study to determine the relationships between different thermodynamic parameters and genotoxicity (GT) of phenolic compounds, such as flavonoids, cinnamic acids and coumarins.
Materials and Methods: It was modeled the influence of local and global parameters that characterize the structure (hydrophobic, steric, electronic, and logP properties) in the clastogenic capacity (chromosome aberration generated by DNA damage, due to its pro-oxidant activity). ChemDraw, MODESLAB and STATISTIC software were used. QSPR models were obtained using molecular descriptors based on the TOPSMODE approach.
Results: Properties thatcan influence the genetic damage caused by the studied pro-oxidant compounds, expressed through different Multivariate Linear Regression (MLR) statistical models, were identified. It was shown that steric (Sterimol, L) and hydrophobic (π) properties presented greater influence than the electronic properties (Hammett constant, σ *).
Conclusion: A decrease in the logP is associated with increased DNA damage by clastogenicity. From the local modulated properties it was shown that steric and hydrophobic properties influenced the activity.Hansch hydrophobicity constant was considered the one that most influenced the activity. The substituent that most affected the activity was the methoxy group. The results allow us to establish structure-toxicity relationships, important for design strategies to obtain nutraceuticals, functional food and novel drugs with the described phenolic compounds on their composition.

Introduction

Phenolic compounds derived from the secondary metabolism of plants, and can be found in various alimentary sources[1,2]. Among them, flavonoids and cinnamic acids are the most abundant in plant foods[3-8].Coumarin derivatives are describedas constituents of several classes of foods, such as cinnamon, ginger bread, green tea, chicory and fruits (bilberry and cloudberry)[9].Some essential oils, as cassia leaf oil, cinnamon bark oil and lavender oil have high levels of them.[10]. Therefore, flavonoids, in particular coumarins, and phenolic acids are of great interest due to their biological properties. Their physiological, bacteriostatic and anti-tumour activities make these families attractive for further backbone derivatization and screening as novel therapeutic agents[2, 10-15]. The coumarin derivatives have inhibitors as cellular proliferation potential in various carcinoma cell lines[16-18].

Recently the presence of these compounds in diet is being widely studied because of their different benefits to human health, such as antioxidant activity[19-22]. However, it has also been evidenced that many of these substances have pro-oxidant activity in various experimental systems, under certain conditions, such as high dosage or the presence of metal ions[23-26]. Studies about this duality have been carried out[27-37]. Such results lead to the necessity of continued studies to evaluate the phenolic compounds from the point of view of their possible "adverse effect" (according to the purpose of use), which is of particular attention during the design and development of new types of food (additives, functional foods andnutraceuticals), and even drugs.

It is known that some of these phenolic substances can be genetically active, and therefore capable of interacting with DNA[38]. These effects could be related to injuries that can take years to manifest. Also, there is a proved relationship between exposure to genotoxic substances (either occupational, accidental or due lifestyles) and increased risk of cancer[39]. The potential exposure to genotoxic agents, both physical and chemical, can produce chromosomal aberrations, being these agents called clastogens[40, 41]. DNA damage is an indicator of exposure to these agents, and it is commonly measured by the breakage of single or double chains[42, 43]. It can be easily observed by structural changes in the metaphase of the cell cycle, which are caused by breakage (clastogenic processes) of unrepaired or poorly repaired DNA chains[44]. This process is considered the endpoint of oxidative damage to DNA, in conjunction with mutations [38]. The DNA damage, chromosome aberrations and disorder in metabolic functioning are important in the initiation of the carcinogenetic process, through generation of reactive oxygen species[45]. There are reports of phenolic substances with clastogenicactivity[46-49]. It has also been predicted by *in silico* studies that some substances reported to have pro-oxidant activity can be clastogenic[50, 51].

In order to predict the clastogenic activity of phenolic compounds with reported pro-oxidant activity, QSAR methods have been previously employed, and they have been interpreted, in structural terms, structure-activity relationships[50-52]. This has allowed the identification of structural alerts associated with clastogenicity using the TOPSMODE approach[52]. It has been reported the influence of different substituents on polyphenolic congeneric structures, in a topological substructural level[52]. Research studies have allowed us to appreciate a

potential relationship between logP and polyphenols toxicity[37, 53].

The fundamentals of an extra-thermodynamic methodology [54, 55] can be formalized in a number of areas, including: (i) biological activity related to the structure of the drug, (ii) the structure of the drug entails certain overall properties such as hydrophobicity, net charge, solubility, etc., and certain local properties as distribution of hydrophobicity, charge and volume, in certain positions of the molecule, (iii) these global and local properties can be quantified through molecular or fragmental parameters, and (iv) there is always a function relating changes in biological activity with changes in the local and global properties, while this may not be simple nor obvious

Correlations may be established between the biological activity and a linear combination of indexes (parameters) representing the physical and chemical changes within a series of molecules. The parameters can be classified as: (i) molecular – regarding to the entire molecule, for example logP (partition coefficient), RM (molar refractivity), μD (dipolar moment), (ii) fragmental – related to the contribution of a fragment or substituent to the studied property, for example π (hydrophobic substituent constant), σ (Hammett constant), Es (Taft steric parameter), and (iii) other parameters that cannot be obtained experimentally, but can be obtained for example by molecular modelling[56], quantum-mechanical calculations (HOMO and LUMO energies) and molecular structure (connectivity index and molecular weight)[57].

In order to study the influence of the hydrophobic, steric and electronic properties, in the current work it was performed an *in silico* study to determine the relationship between different thermo-dynamic parameters and genotoxicity (GT) of phenolic compounds, including flavonoids, cinnamic acids and coumarins..

Materials and Methods

Characterization of the selected TOPSMODE model

It was used a model of clastogenic structure-activity relationship, validated and reported by Estrada *et al.*[58]. This model (Equation 1) explains the relationship between the chemical structure and clastogenicity, and coded topological information in each spectral moment (μ) bond weight (molecular descriptors): bond distance (SD), standard bond dipole moments (DM), hydrophobicity (H), polar surface area (PS), polarizability (Pol), molar refractivity (MR), van der Waals radii (vdW), and Gasteiger-Marsili charges (Ch). Global spectral moments were calculated by MODESLAB. This software package demonstrated to displayhigh predictive capacity for different pharmaceutical and toxic properties.

$$GT = 0.009\left[\Omega\left(\mu_1^{PS}\right)\right] - 1.5520\times10^{-4}\left[\Omega\left(\mu_5^{vdW}\right)\right] + 0.148\left[\Omega\left(\mu_4^{Ch}\right)\right] - 0.002\left[\Omega\left(\mu_2^{PS}\right)\right] + \\ + 2.6261\times10^{-4}\left[\Omega\left(\mu_3^{PS}\right)\right] - 3.8422\times10^{-5}\left[\Omega\left(\mu_4^{PS}\right)\right] + 1.1520\times10^{-4}\left[\Omega\left(\mu_4^{MR}\right)\right] + \\ + 1.2011\times10^{-6}\left[\Omega\left(\mu_5^{PS}\right)\right] - 9.8202\times10^{-5}\left[\Omega\left(\mu_5^{MR}\right)\right] - 3.8263\times10^{-5}\left[\Omega\left(\mu_8^{H}\right)\right] - \\ - 0.0626\left[\Omega\left(\mu_2^{Pol}\right)\right] + 1.6689\left[\Omega\left(\mu_1^{Pol}\right)\right] - 0.0078\left[\Omega\left(\mu_5^{Ch}\right)\right] + 0.1123\left[\Omega\left(\mu_3^{Ch}\right)\right] - 0.6517$$

……. (1)

The Ω is used to indicate that the corresponding variable in brackets was orthogonalized respecting to the rest of the variables included in the model. The classification model obtained is given below, together with the statistical parameters of the linear discriminate of the squared analysis, where λ is the Wilks' statistics, D2 is the Mahalanobis distance and F is the Fisher ratio (Wilks´- λ= 0.629; F(14.194)=8.148; D2=2.353; p<0.0000).

Quantitative Structure-Property Relationship (QSPR) study

ChemDraw software was used for drawing each molecule, and for the calculation of SMILE codes. The multivariate linear regression (MLR), for the formation of the thermodynamic models, and linear discriminant analysis (LDA), for classification as active or inactive, were performed with the STATISTICA program version 4.13.For the development of the model associated with local properties (MLR), the percentage of GT of the studied compounds was used. In particular, the substituents presented in the compounds were studied, constituting GT the dependent variable. Overall properties were based on the properties of the substituents (local properties).

The analysis of local thermodynamic properties was taken into account for each of the corresponding thermodynamic models, considering as independent variables: (i) principal steric parameter, Sterimol (L), (ii) hydrophobic parameter, substituent constant (π) and (iii) electronic parameter, Hammett constant (σ^*). The values of these parameters were considered for each substituents presented in the tested structures. Each substituent value, for each parameter, was taken from bibliographic sources[59-61].

In the case of the model that relates a global property to the GT, constituted the independent variables: a) *dummy* variable corresponding to different kinds of phenolic compounds and b) lipophilicity data employing the values ofoctanol/water reported by references[37, 62].

Results and Discussion

External data set description

Table 1 contains a list of compounds used as external data set in the study. It has been established that these compounds may exhibit dual activity (antioxidant/pro-oxidant effects).Among them, it is presented the pro-oxidant reportfor all the studied cinnamic acids (compounds **7** and **8**), and most of the flavonoids (benzopyrones**1-4**), excepting morin and taxifolin.

Table 1. External data set description.

Several of the previously studied coumarinsshown in Table 2 presented clastogenic activity: compounds **9-11** and **13**, as well as compounds **2, 4, 5, 7** and **8**[33, 37, 51, 63-66].

A summary of the logP values used to prepare the model and the percentage of probability of being active (predicted by the statistic theoretical model MTE equation 1) are shown in Table 2.

Table 2. LogP values and prediction of GT activity of flavonoids, phenolic acids and coumarins.

The theoretical classification obtained through the ADL allows external validation of the model. 77.7% of good classification was obtained, since from nine compounds with clastogenic activity in experimental trials, seven of them agree with the theoretical prediction. As example, the prediction of caffeic acid corresponded to the description of Maistro *et al.*, which showed the clastogenic activity through the micronucleus test in drug-metabolizing rat hepatoma tissue cells (HTCs)[65]. This compound and gallic acid were reported by Stich et al[66] as clastogens, when tested in hamster ovary cells. The activity was enhanced by the addition of transition metals (Cu^{2+} and Mn^{2+})[66]. Clastogenic activity of caffeic acid was also previously reported by Hanham*et al*.[64].

A similar result was obtained by comparing the experimental and theoretical clastogenicactivityof kaemferol. Previous studies Gaspar *et al.* showed that this

compound was *in vitro* both pro-oxidant and clastogenic[63].

In this study it was introduced a new external data set corresponding to coumarins. The activity of compounds **9**, **10**, **11** and **13** was an important reason to confirm the prediction for this external data set. The coumarin and its derivative 7-methylcoumarin were considered as "false negatives". Their prediction contradicted what was experimentally observed, affecting the percentage of good classification.

The active compounds from this *in silico* study could cause DNA damage through clastogenicity. In the case of inactive compounds with pro-oxidant activity, it is possible to assume that the pro-oxidant activity and possible damage to DNA cannot be explained by means of the clastogenicity, but by other mechanisms that have been previously described, as cytotoxicity and mutagenicity[67].

In order to study the local hydrophobic, steric and electronic properties, new chemical structures (flavonoids and coumarins) were drawn.These new compoundswere subsequently checked for their existence. Table 3 shows a summary of these compounds, their substituents, and their GT probability values, which determined thermodynamic parameter values. Substituent's values for each thermodynamic parameter were taken from Kubinyi*et al.*[60].

Table 3. GT activity and substituents presented in the new flavonoids and coumarins.

QSPR study

Table 4 shows the QSPR models obtained for each parameter using molecular descriptors from TOPSMODE approach, and the corresponding statisticians. The results showed that three of the four obtained models account for more than 60 % correlation with the GT.

From the local modulated properties it was shown that steric (Sterimol, L) and hydrophobic (π) properties influence GT activity, but not the electronic parameter (Hammett constant, σ^*), as the MTE statisticians shown in Table 4.

Regarding the global property analysis (logP), which uses a *dummy* variable representing different groups belonging to the studied compounds, it was shown a correlation over 70 % between GT and lipophilicity. This means that there is a dependence of the studied activity to the hydrophobic character of these compounds. Previous studies agree that the lipophilicity is one of the characteristics that determine the toxicity of phenolic compounds[37, 53, 68]. The analytical expression of the model shown in Equation 5, allows theoretically explain that for the same subclass molecules with logP<0 (0<P<1), an increase in lipophilicity is associated with an increased probability of being clastogenic. However, for molecules with logP>0 (P>1), it would be the opposite. For experimental logP that ranged from 0.911-2.741 (Table 2), for the same subclass, a lower lipophilicity of phenolic compounds can influence in a greater probability for clastogenic activity, and DNA damage through this mechanism. The influence of this parameter (LogP) seems to behave the same way as to thegenotoxic and cytotoxic activities. This was also previously described by Sergediene *et al.*, who analyzed the influence of lipophilicity in the pro-oxidant nature of a group of polyphenols through QSPR study. This research group demonstrated experimentally that a increasing in the lipophilicity was directly related to increased IC_{50} in leukaemia cells [37]. From these results, it can be said that the cytotoxicity of pro-oxidant compounds increases with lipophilicity. It can be

suggested that the mechanisms by which both activities occur differ.

It would be useful to consider in future studies the possibility of analysing the nature of the molecular descriptors, which can be presented in very different ways: topology, topography and quantum chemical character of the descriptor; weighting properties of the graph; and weighting edges or vertices of the graph.

Table 4. RLM models related to the influence of different thermodynamic parameters on the GT of phenolic compounds

From a detailed analysis of local properties, Hansch hydrophobicity constant (π) was considered the one that most influencers of the activity (Table 5). As shown in Table 3, the substituent that affected the activity the most was the methoxy group (OCH_3), a strongly activating group. The thermodynamic parameter "hydrophobicity constant" was the responsible for describing the effects of the lipophilic substituents, which seems to have a better match with strongly activating groups (OH, OCH_3, NH_2, $N(CH_3)_2$). In order to demonstrate the relationship between lipophilic character and strength of the substituent, it was conducted a statistical analysis of RLM. This analysis was performed considering two compounds included in the analysed database – biochanin A and genistein– and two compounds that was necessary to generate (with NH_2 and $N(CH_3)_2$ substituents). The results can be seen in Table 5. It was found a high correlation between the GT and the hydrophobicity (and strength) of the substituent (R = 94.5 %).

Table 5. Results of RLM taking into account hydrophobicity and Hansch constants.

Substructural level analysis (substituents) indicated that the hydrophobic groups (OCH_3) have a Hansch constant value of -0.02[60]. Therefore OCH_3 groups contributed more favorably to the activity, assuming the positive value of the constant π in the equation 6. This may be because as bigger the substituent is, as higher is the possibility of hydrophobic interactions with the carbon chain of n-octanol. This indicates that the studied properties (global and local) may be correlated. Therefore, it is possible to explain them at substructural level. It is possible to study the influence of the substituent groups or fragments on the lipophilicity, resulting in the relationship between the reported pro-oxidant and clastogenic activity of the studied compounds.

Conclusions

QSPR models were obtained for each parameter using molecular descriptors based on the TOPSMODE approach. The results proved that there is a dependence of the studied activity to the hydrophobic character of these compounds. In addition, a decrease in the logP was associated with an increased DNA damage by clastogenic activity. Moreover, the study of the GT was dependent on the subclasses of phenolic compounds. From the local modulated properties it was shown that steric (Sterimol, L) and hydrophobic (π) properties influenced GT activity, while the electronic parameter (Hammett constant, σ *) did not.From a detailed analysis of local properties, Hanschhydrophobicity constant (π) was considered the one that most influenced the activity. The substituent that most affected the activity was the methoxy group (OCH_3). In addition, it was found a high correlation between the GT and the hydrophobicity (and strength) of the substituent. In summary, the results allowed

us to produce an analysis of the structure-toxicity relationship, which could be helpful for the design of new nutraceuticals, functional foods, and novel drugs with phenolic compounds on their structure.

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Conflict of interest

The authors declare no conflicts of interest.

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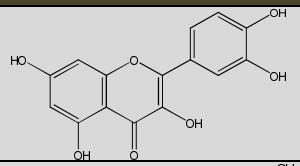
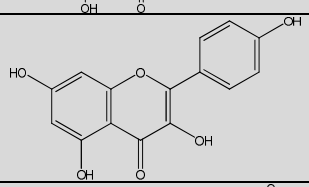
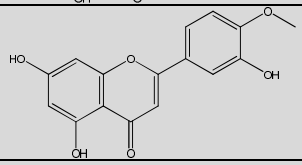
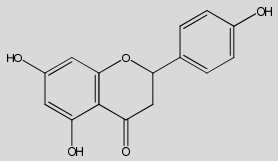
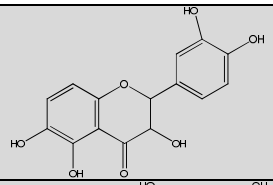
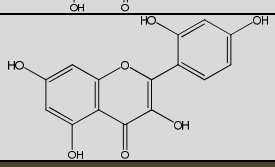
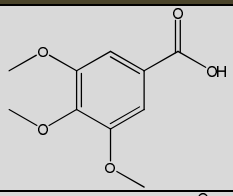
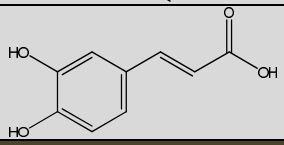
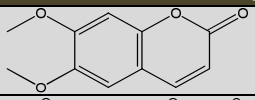
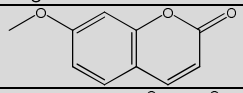
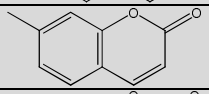
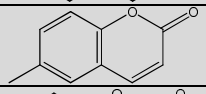
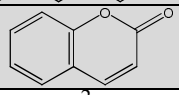
Table 1. External data set description

Code	Compounds	Structure	CAS number[1]	SMILE[2]
Benzo-γ-pyrones				
1	Quercetin		117-39-5	OC1=CC2=C(C(=C1)O)C(=O)C(=C(O2)C3=CC(=C(O)C=C3)O)O
2	Kaempferol		520-18-3	OC1=CC=C(C=C1)C2=C(O)C(=O)C3=C(O2)C=C(O)C=C3O
3	Hesperetin		520-33-2	COC1=CC=C(C=C1O)C2CC(=O)C3=C(O)C=C(O)C=C3O2
4	Naringenin		480-41-1	OC1=CC=C(C=C1)C2CC(=O)C3=C(O2)C=C(O)C=C3O

5	Taxifolin		480-18-2	OC(C1=O)C(OC2=C1C(O)=C(C=C2)O)C3=CC=C(C(O)=C3)O
6	Morin		480-16-0	OC1=C(C2=CC=C(O)C=C2O)OC3=C(C(O)=CC(O)=C3)C1=O
Cinnamic acids				
7	Gallic acid		118-41-2	COC1=C(OC)C(=CC(=C1)C(O)=O)OC
8	Caffeic acid		331-39-5	OC(=O)C=CC1=CC=C(O)C(=C1)O
Benzo-α-pyrones				
9	6,7-Dimethoxycoumarin		120-08-1	O=C1OC2=C(C=C(OC)C(OC)=C2)C=C1
10	7-Methoxycoumarin		531-59-9	O=C1OC2=C(C=CC(OC)=C2)C=C1
11	7-Methylcoumarin		2445-83-2	O=C1OC2=C(C=CC(C)=C2)C=C1
12	6-Methylcoumarin		92-48-8	O=C1OC2=C(C=C(C)C=C2)C=C1
13	Coumarin		91-64-5	O=C1OC2=C(C=CC=C2)C=C1

[1]Chemical Abstracts Service Number; [2] Simplified Molecular Input Line Entry System

Table 2. LogP values and prediction of GT activity of flavonoids, phenolic acids and coumarins

Code	Compounds	Independent variables		Clastogenic Activity (GT)		
		Dummy variable	LogP	Experimental	Predicted	
					(Class.[4])	Percentage of *posterior probability*
1	Quercetin	*a*	2.741[1]	(-)	(G_2:1)	67.6
2	Kaempferol	*a*	2.691[1]	(*)	(G_2:1)	53.0
3	Hesperitin	*b*	2.301[1]	(-)	(G_2:1)	85.9
4	Naringenin	*b*	2.591[1]	(*)	(G_2:1)	52.6
5	Taxifolin	*b*	1.221[1]	(*)	(G_2:1)	71.3
6	Morin	*a*	1.971[1]	(-)	(G_2:1)	66.5
7	Gallic acid	*c*	0.911[1]	(*)	(G_2:1)	54.6
8	Caffeic acid	*c*	2.471[1]	(*)	(G_2:1)	54.2
9	6,7-Dimethoxycoumarin	*d*	1.940[2]	(*)	(G_2:1)	79.3
10	7-Methoxycoumarin	*d*	2.310[3]	(*)	(G_2:1)	60,4
11	7-Methylcoumarin	*d*	1.840[2]	(*)	(G_1:-1)	73.0
12	6-Methylcoumarin	*d*	1.854[2]	(-)	(G_1:-1)	73.4
13	Coumarin	*d*	1.394[2]	(*)	(G_1:-1)	62.7

[1]Reported by[37]
[2]Reported by http://www.thegoodscentscompany.com/[62]
[3] Calculated using Chemdraw program.
[4]The theoretical classification model "active" is represented as "G_2:1", and "inactive" as "G_1:-1
(*)experimentalclastogenicactivityreport; (-) non experimental clastogenicactivityreport

Table 3. GT activity and substituents presented in the new flavonoids and coumarins

Code	New designed molecules		Prediction (GT)
	SMILE	Substituent	Percentage of *posterior probability*
14	O=C2OC1=C(O)C=C(O)C=C1C=C2C3=CC=C(CC)C=C3	C_2H_5	65.4 (G_1:-1)
15	O=C2OC1=C(O)C=C(O)C=C1C=C2C3=CC=C(/C=C/C)C=C3	$CH=CHCH_3$	53.4 (G_2:1)
16	O=C2OC1=C(O)C=C(O)C=C1C=C2C3=CC=C(COC)C=C3	$COCH_3$	65.6 (G_2:1)
17	O=C2OC1=C(O)C=C(O)C=C1C=C2C3=CC=C(C(OC)=O)C=C3	$COOCH_3$	67.6 (G_2:1)
18	O=C2OC1=C(O)C=C(O)C=C1C=C2C3=CC=C(OC)C=C3	OCH_3	79.6 (G_2:1)
19	O=C2OC1=C(O)C=C(O)C=C1C=C2C3=CC=C(C#N)C=C3	CN	68,8 (G_2:1)
20	O=C2OC1=C(O)C=C(O)C=C1C=C2C3=CC=C([N+]([O-])=O)C=C3	NO_2	67.7 (G_2:1)
21	O=C2OC1=C(O)C=C(O)C=C1C=C2C3=CC=C(Br)C=C3	Br	64.6 (G_1:-1)

[1]The theoretical classification model "active" is represented as "G_2:1", and "inactive" as "G_1:-1".

Table 4. RLM models related to the influence of different thermodynamic parameters on the GT of phenolic compounds

Thermodynamic parameter	Model	Statisticians
Principal steric parameter, Sterimol (L)	$GT = -0{,}0008 \times L - 59.2481$ (2)	N = 12; R = 0.622; F (1,10) = 3.746; S_{CV} = 12.030;p< 0.082
Hydrophobic parameter, substituent constant (π)	$GT = -7.9742 \times \pi - 76.8216$ (3)	N = 9; R = 0.653; F (1,7) = 3.081; S_{CV} = 13.794; p< 0.123
Electronic parameter, Hammett constant (σ*)	$GT = 3.4782 \times \sigma * -86.3795$ (4)	N = 22; R = 0.207; F (1,20) = 0.897; S_{CV} = 12.010; p< 0.355
Global parameter, partition coefficient (log P)	$GT = 213.435 - 45.089 \log \mathrm{P} + 32.841\,\mathrm{dummy}$ (5)	N = 8; R = 0.721; F (2,5) = 2.7091; S_{CV} = 34.580; p< 0.159

Table 5. Results of RLM taking into account hydrophobicity and Hansch constants

Model	Statisticians
$GT = 113.131 \times \pi + 58.512$ **(6)**	$N = 4$; $R = 0.945$;$R^2 = 0.893$;F (1,2) = 16.648;S_{CV} = 30.846; p< 0.0551