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Natural Coumarins: QSTR Approaches Regarding Their Genotoxicity

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Abstract: Coumarins are a group of phytochemicals with multiple applications in different fields, such as food and medicine. Many of their benefits are based on the different activities that they display, within which stand antioxidant properties. However, some conflicting evidences suggest the need to clarify or estimate the safety aspects and genotoxicity of this group of compounds. In this sense it has been shown in previous studies that some of them have presented pro-oxidant activity *in vitro* and clastogenic activity *in silico*. Therefore, in this paper chemical structures of coumarins that come from several natural sources were studied. These coumarins belong to the chemical subclasses: simple coumarins, furocoumarins, dihydrofurocoumarins, pyranocoumarins, phenylcoumarins and biscoumarins. Thepre-selected database was formed taking into account topological-structural information, using molecular descriptors from the TOPSMODE approach. A virtual screening, that used a structure-clastogenic activity model and linear discriminant analysis (LDA) technique, was also performed. Several natural coumarins showed clastogenic activity *in silico*. For this family, the QSTR associated the probability of being active to the presence of hydroxy and methoxy groups in the molecules. It is of positive contribution of the fragment that forms the *bay region* of the pyranocoumarinic system. These *in silico* results may contribute to the safe design of new foods, nutraceuticals or drugs. It may also be important in the prevention of cancer, in which pathology these substances show pro-oxidant activity.

Keywords: Coumarins, clastogenic activity, pro-oxidant activity, TOPSMODE approach.

1. Introduction

Nutrition, which was once intended to meet the nutrient needs, it is today directed to a research toward preventing and treating chronic diseases [1]. Constitutes an alternative seeking nutritional bioactive components other than medicinal purposes, which is a challenge to the biomedical sciences [2]. It is in this context that the concept of functional foods emerged [3]. There are, several bioactive compounds that confer functionality to food

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and are part of the daily diet [4]. Therefore numerous studies direct their efforts to identify these components and evaluate their isolated health benefits or as part of dietary regimens.

Within this huge range of compounds there are included the phenolic compounds, many of which have been recognized as *in vitro* antioxidants [5-8]. This activity has been linked to the possible prevention of diseases such as cardiovascular, cancer, neurodegenerative, etc [9, 10]. However, many of these compounds have been presented pro-oxidant activity [11-15] and even *in vitro*, *in vivo* and *in silico* clastogenic activity [16-20]. Examples of this are some phenolic acids present in many food sources of plant origin, which have shown dual behaviour [17, 19]. These considerations demonstrate the importance of continuing research on the safety associated with this family of compounds respects. The pro-oxidant activity causes the formation of reactive oxygen species and inhibition of antioxidants systems [21]. This can generateoxidative damageto cells and tissues [22, 23] and biomolecules such asproteins, DNA and lipids [21, 24]. It is added thefact that it is recognized that the development of many chronic diseases may be due to oxygen reactive species (ROS) [25-27], where the antioxidants and pro-oxidants levels balance is not achieved and, the result is a pathological process. The pro-oxidants catalyse, then, oxidative reactions to these biomolecules, which may lead to cellular dysfunction that ends with cell death [21]. Some pro-oxidant compounds presented clastogenic activity. Clastogenic processes are considered the endpoint of oxidative damage to DNA, in conjunction with mutations [28].

Another group of phenolic type compounds are the coumarins (benzo-α-pyrones), which have been less investigated. There are few studies evaluating the genetic toxicity of natural and synthetic coumarin derivatives in the literature [29]. Coumarins are a family of phenolic compounds that represent different constituents of the non-energetic part of the human diet [30]. The simplicity and versatility of the coumarin scaffold make it an interesting starting-point for a wide range of applications [31-33]. Coumarin derivative molecules (or coumarin metabolites) are naturally generated by the metabolism of coumarin in the cell. These derivatives have been described for their numerous therapeutic applications, such as: antitumor and anti-HIV agents and central nervous system stimulants, anti-inflammatory and antibacterial agents, anti-coagulants, among others [34]. Their structural variability and similarity to other phenolic compounds, suggesting the need to identify structural alerts associated with genotoxicity. Some background has been *in silico* studied and showed clastogenic activity in some of them [20]. This leads to the hypothesis that some natural coumarins might also have clastogenic activity based on *in silico* studies and reports. For these reasons, the objective of this study is to conduct a virtual screening based on the TOPSMODE approach, considering an external database of natural coumarins present in edible and medicinal plants.

2. Materials and Methods

For this study, three different *in silico* steps were defined. Figure 1 shows the *in silico* pathway for the elaboration of the virtual screening.

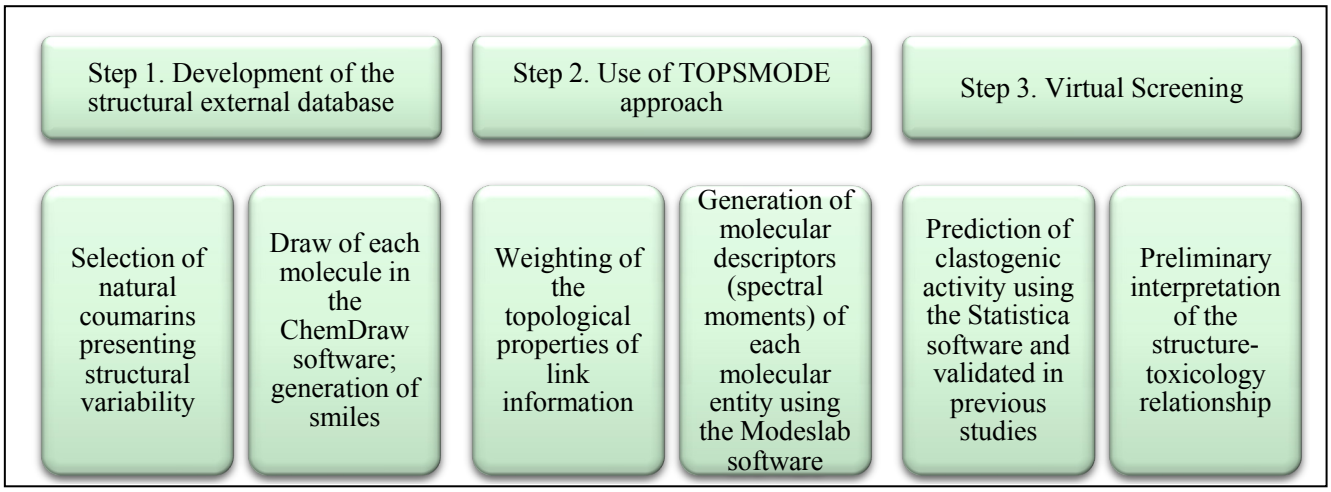


Fig 1. Different steps of a QSTR study taking into account the TOPSMODE approach.

To evaluate the studied coumarinic compounds, it was used an external database (DB1) that is represented in Table 1.

Table 1. Molecules used in the virtual screening (DB1).

Compounds	CAS[1]	SMILE[2]	ID in PubChem
Esculetin	895-61-4	C1=CC=C(C=C1)COC2=C(C=C3C=CC(=O)OC3=C2)O	1204535
Ammoresinol	643-57-2	CC(=CCCC(=CCCC(=CCC1=C(C2=C(C=C(C=C2)O)OC1=O)O)C)C)C	54712597
Ostruthin	148-83-4	CC(=CCCC(=CCC1=C(C=C2C(=C1)C=CC(=O)O2)O)C)C	5281420
Osthole	**484-12**-8	CC(=CCC1=C(C=CC2=C1OC(=O)C=C2)OC)C	10228
Novobiocin	303-81-1	CC1=C(C=CC2=C1OC(=O)C(=C2O)NC(=O)C3=CC(=C(C=C3)O)CC=C(C)C)OC4C(C(C(C(O4)(C)C)OC)OC(=O)N)O	54675769
Umbelliferone	5281426	C1=CC(=CC2=C1C=CC(=O)O2)O	93-35-6
Fraxidin	3083616	COC1=C(C(=C2C(=C1)C=CC(=O)O2)O)OC	525-21-3
Imperatorin	482-44-0	CC(=CCOC1=C2C(=CC3=C1OC=C3)C=CC(=O)O2)C	10212
Psoralen	66-97-7	C1=CC(=O)OC2=CC3=C(C=CO3)C=C21	6199
Bergapten	484-20-8	COC1=C2C=CC(=O)OC2=CC3=C1C=CO3	2355
Methoxsalen	298-81-7	COC1=C2C(=CC3=C1OC=C3)C=CC(=O)O2	4114
Marmesin	13849-08-6	CC(C)(C1CC2=C(O1)C=C3C(=C2)C=CC(=O)O3)O	334704
Rutaretin	13895-92-6	CC(C)(C1CC2=C(O1)C(=C3C(=C2)C=CC(=O)O3)O)O	44146779

Aegelinol	21860-31-1	CC1(C(CC2=C(O1)C=C3C(=C2)C=CC(=O)O3)O)C	1150962
Xanthyletin	553-19-5	CC1(C=CC2=C(O1)C=C3C(=C2)C=CC(=O)O3)C	65188
Inophyllum A	41135-07-3	CC1C(OC2=C(C1O)C3=C(C(=CC(=O)O3)C4=CC=CC=C4)C5=C2C=CC(O5)(C)C)C	455248
Inophyllum C	17312-30-0	CC1C(OC2=C(C1=O)C3=C(C(=CC(=O)O3)C4=CC=CC=C4)C5=C2C=CC(O5)(C)C)C	455252
Inophyllum G1	152135-65-4	CC1C(OC2=C(C1O)C3=C(C(=CC(=O)O3)C4=CC=CC=C4)C5=C2C6C(C6(C)C)O5)C	455254
Calanolide A	142632-32-4	CCCC1=CC(=O)OC2=C1C3=C(C=CC(O3)(C)C)C4=C2C(C(C(O4)C)C)O	64972
(+)-Dihydrocalanolide A	183904-53-2	CCCC1=CC(=O)OC2=C1C3=C(CCC(O3)(C)C)C4=C2C(C(C(O4)C)C)O	461796
Pseudocordatolide C	179461-48-4	CC1C(OC2=C(C1O)C3=C(C=CC(O3)(C)C)C4=C2C(=CC(=O)O4)C)C	467236
Isodispar B	98192-64-4	CC(C)CC(=O)C1=C(C=C(C2=C1OC(=O)C=C2C3=CC=CC=C3)O)O	6483316
Mammea AB	7058-70-0	CCCCCC1=CC(=O)OC2=C1C(=C(C(=C2CC=C(C)C)O)C(=O)C(C)CC)O	53325382
Dicoumarol	66-76-2	C1=CC=C2C(=C1)C(=C(C(=O)O2)CC3=C(C4=CC=CC=C4OC3=O)O)O	54676038
4-Methyldaphnetin	2107-77-9	CC1=CC(=O)OC2=C1C=CC(=C2O)O	-
Fraxetin	574-84-5	O=C1C=CC(C=C(OC)C(O)=C2O)=C2O1	-

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

TOPS-MODE approach represents a useful platform for the automatic generation of structural alerts [35]. It is based on the calculation of spectral moments of molecular bond matrices appropriately weighted taking into account the hydrophobic, electronic and steric molecular features. Spectral moments are the trace of the kth power of a matrix, i.e., the sum of all the main diagonal entries of such matrices [35]. These matrices represent the molecular skeleton without taking into account hydrogen atoms. Bond weights are placed as diagonal entries of such matrices and represent quantitative contributions to different physic-chemical properties.

Use of the TOPSMODE approach:

- Weighting of the topological properties of link information: 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*).
- Generation of molecular descriptors (spectral moments) of each molecular entity using the MODESLAB software and the theoretical statistic model (MTE) developed by [36]:

$$\begin{aligned} AC = {} & 0.0091\left[\Omega\left(\mu_1^{PS}\right)\right] - 1.5520\times10^{-4}\left[\Omega\left(\mu_5^{vdW}\right)\right] + 0.148\left[\Omega\left(\mu_4^{Ch}\right)\right] - 0.0021\left[\Omega\left(\mu_2^{PS}\right)\right] + \\ & + 2.6261\times10^{-4}\left[\Omega\left(\mu_3^{PS}\right)\right] - 3.8422\times10^{-5}\left[\Omega\left(\mu_4^{PS}\right)\right] + 1.1520\times10^{-4}\left[\Omega\left(\mu_4^{MR}\right)\right] + \\ & + 1.2011\times10^{-6}\left[\Omega\left(\mu_5^{PS}\right)\right] - 9.8202\times10^{-5}\left[\Omega\left(\mu_5^{MR}\right)\right] - 3.8263\times10^{-5}\left[\Omega\left(\mu_8^{H}\right)\right] - \\ & - 0.0626\left[\Omega\left(\mu_2^{Pol}\right)\right] + 1.6689\left[\Omega\left(\mu_1^{Pol}\right)\right] - 0.0078\left[\Omega\left(\mu_5^{Ch}\right)\right] + 0.1123\left[\Omega\left(\mu_3^{Ch}\right)\right] - 0.6517 \end{aligned} \quad (1)$$

Statisticians: Wilks′- λ= 0.629; F(14.194)=8.148; D^2=2.353; p<0.0000

AC indicates clastogenic activity. 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. μ_n are the spectral moments (molecular descriptors) and their exponents correspond to the bonds' properties mentioned before. 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, D^2 is the Mahalanobis distance and F is the Fisher ratio. The construction of this model by Estrada *et al.* (2006) was based in a dataset of 372 organic compounds, including known carcinogens, presented in the groups of drugs, food, agrochemicals, additives, medicinal products, cosmetics and household materials [36]. This model was internally and externally validated [20, 36, 37]. The linear discriminate analysis (LDA), implemented in Statistic software, has been used to generate the classification (active/inactive). A compound with probability of 50± 2.5 % was considered as not classified (NC).

The clastogenic model used in the virtual screening was externally validated from the percentage of good classification (expression indicating the correspondence between the theoretical and experimental prediction) to a series of external compounds. The percentage of good classification also indicates the robustness of the model to predictthe clastogenic activity. 6,7-Dihydroxycoumarinor esculetin (6,7-HC, CAS: 305-01-1) was deeply studied because it has been used in *in vitro* and *in vivo* clastogenicity studies [29] (Figure 2).

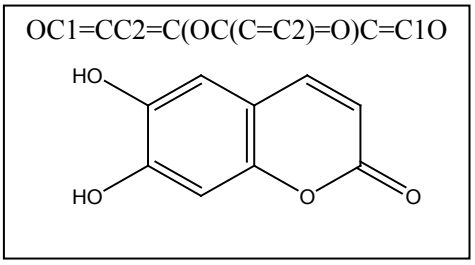


Fig. 2. Chemical structure and SMILE code of 6,7-HC.

3. Results and Discussion

1. Description of Natural Coumarins that Comprise the Databases

Compounds compiled in DB1 are presented in different families/species of DB2. In previous chemotaxonomic studies have been identified those plant families in which more genera and species with coumarins were reported and/or with greater structural diversity [38]. From this source and others, the database (DB2) of interest for future studies of coumarins, their medicinal or food uses and natural sources was designed (Table 2).

Table 2. Some plant families containing natural coumarins (DB2).

Family/specie (vernacular name)	Coumarin	Use*	Reference
Apiaceae			
Ammimajus (*Bishop's flower*)	Imperatorin, Bergapten, Marmesin	M	[39]
Angelica archangelica (Angelica)	Bergapten, imperatorin, osthol,umbelliferone	M, F	[40]
Apiumgraveolens (Celery)	bergapten, rutaretin, umbelliferone	M, F	[40, 41]
Coriandrumsativum (Coriander)	umbelliferone	M, F	[41]
Ferula assafoetida (Asafoetida)	umbelliferone	M	[40]
Foeniculumvulgare (Fennel)	bergapten, esculetin, umbelliferone, psoralen	M, F	[41]
Petroselinumcrispum (Parsley)	bergapten, imperatorin, psoralen	M, F	[40, 41]
Pimpinellaanisum (Aniseed)	umbelliferone, bergapten	M, F	[40, 41]
Asteraceae			
Arnica montana (Arnica)	umbelliferone	M, F	[40, 41]
Matricariarecutita (Chamomille)	umbelliferone	M, F	[40, 41]
Rutaceae			
C. limonum (Lemon Tree)	umbelliferone, bergapten	M, F	[41]
Zanthoxilumamericanum (Northern Prickly Ash)	xanthyletin	M	[40]
Fabaceae			
Glycyrrizaglabra (Liquorice)	umbelliferone	M, F	[40, 41]
Achanthaceae			
Justiciapectoralis (Tilo)	umbelliferone	M	[42]

Passifloraceae			
Passifloraincarnata (Passion Flower)	umbelliferone	M, F	[40, 41]
Caryophylacae			
Herniariaglabra (Rupture wort)	umbelliferone	M	[41]
Lamiaceae			
Salvia officinalis (Garden Sage)	esculetin	M	[41]
Clusiaceae			
C. brasiliense (*Guanandi, Ocuje*)	mammea A		[43]
C. cerasiferum	(-) calanolide B	M	[44]
Calophylluminophyllum (Borneo mahogany)	inophyllum A and P		[44]
Calophyllumlanigerum var. austrocoriaceum	(+)- calanolide A	M	[42]
C. teysmannii var. inophylloide	(-) calanolide B,	M	[42]
C. verticillatum	mammea A		[43]

Adapted from unpublished Work [30]; M: medicinal use; F: food use.

Some polyphenolic flavonoid-type substances presenting reported pro-oxidant activity have also proved to have *in vitro*clastogenic activity [45]. *In silico*previous studies, it seems to be a relationship between the clastogenic activity and pro-oxidant [46]. This suggests the fact that it is possible to estimate the pro-oxidant activity using Equation 1, since currently has not been able to get a QSAR model specifically for pro-oxidant activity. If these postulates are used, the active compounds, behind clastogenic activity, could present pro-oxidant activity, corroborating the relationship that was proposed in our unpublished works. From all the compounds represented in DB1, fraxetin and 4-methyldaphnetin were studied for their pro-oxidant activity.

2. Classification Model and Virtual Screening

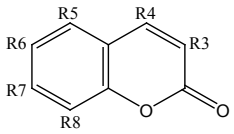
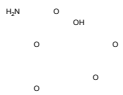
The prediction obtained for each of the analysed subclasses, are shown in Tables 3-9. The probability of belonging to the group of active compounds (G_2: 1) or possible genotoxic or inactive compounds (G_1: -1), was expressed in percentage of good probability.

2.1. QSTR of Simple Coumarins, Furocoumarins, Dihydrofurocoumarins

The results obtained for simple coumarins are shown in Table 3. It can be observed that the combination of hydroxy and methoxy groups seems to be related to the probability of being active (iefraxidin). Similar

chemoinformatics results were obtained for simple methoxylated coumarins, being in correspondence with the clastogenic activity exhibited *in vitro* [20]. Another group that appears to influence the activity is the amide group esterified with a glucoside, as in the case of novobiocin.

Table 3. Predictions made using TOPSMODE classification model to simple coumarins compounds.

Simple coumarins 	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**	**Class.**	**Prob. (%)**
Umbelliferone	H	H	H	H	OH	H	G_1:-1	70.3
Osthole	H	H	H	H	OCH_3		G_1:-1	60.9
Fraxetin	H	H	H	OCH_3	OH	OH	G_1:-1	52.0
Fraxidin	H	H	H	OCH_3	OCH_3	OH	G_2:1	94.6
4-Methyldaphnetin	H	CH_3	H	H	OH	OH	G_1:-1	55.0
Mammea AB	H	C_5H_{11}	OH		OH		G_1:-1	92.1
Ostruthin	H	H	H		OH	H	G_1:-1	94.2
Ammoresinol		OH	H	H	OH	H	G_1:-1	95.7
Esculetin	H	H	H	OH		H	G_1:-1	64.6
Novobiocin		OH	H	H		CH_3	G_2:1	65.6

The scaffold without substituents (coumarin) was predicted as not clastogenic in previous studies [20]. While in the present data, mammea AB presenting two (saturated and unsaturated) aliphatic radicals and a carbonyl group, is also an inactive molecule. It could be argued that as these types of radicals appear more often, increases the probability of being inactive, as in the case of ostruthin (94.2%). The presence of a group esterified with aromatic or aliphatic unsaturated chain, seems to be a structural feature for an inactive molecule, as in the case of esculetin.

Furocoumarins (ie psoralen) are inactive compounds (Table 4), but are activated when methoxy radical (ie bergapten, methoxsalen) are introduced. The analysis of this subclass corroborated the information noted above, that the molecule is inactivated when esterified with unsaturated aliphatic groups (ie imperatorin).

Table 4. Predictions made using TOPS-MODE classification model to furocoumarins.

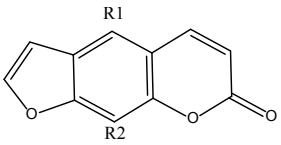
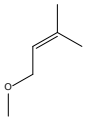
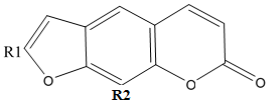
Furocoumarins 	**R1**	**R2**	**Class.**	**Prob. (%)**
Psoralen	H	H	G_1:-1	69.1
Imperatorin	H		G_1:-1	55.7
Bergapten	OCH_3	H	G_2:1	73.2
Methoxsalen	H	OCH_3	G_2:1	75.2

Table 5 shows the results obtained for the dihydrofurocoumarins. The two molecules considered in the study are inactive, considering the tert-butyl radical. The presence of the hydroxy radical in the rutaretin decreases the probability of toxicity.

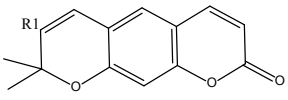
Table 5. Predictions made using TOPSMODE classification model to dihydrofurocoumarins.

Dihydrofurocoumarins 	**R1**	**R2**	**Class.**	**Prob. (%)**
Marmesin		H	G_1:-1	76.1
Rutaretin		OH	G_1:-1	59.9

2.2. QSTR of pyranocoumarins

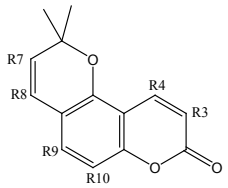
Table 6 shows the results of linear pyranocoumarins, which are predicted as inactive by the model (equation 1).

Table 6. Predictions made using TOPSMODE classification model to pyranocoumarins (linear type).

Pyranocoumarins (linear type) 	**R1**	**Class.**	**Prob. (%)**
Xanthyletin	H	G_1:-1	55.9
Aegelinol	OH	G_1:-1	63.9

In Table 7 it is shown the classification and probability of angular pyranocoumarins.

Table 7. Predictions made using TOPSMODE classification model to angular pyranocoumarins.

Pyranocoumarins (angular type) 	**R4**	**R7**	**R8**	**R9**	**R10**	**Class.**	**Prob. (%)**
Inophyllum A	C_6H_5	H	H	-OCH(CH_3)CH(CH_3)CH(OH)		G_2:1	68.6
Inophyllum C	C_6H_5	H	H	-OCH(CH_3)CH(CH_3)CO)-		G_2:1	68.1
Calanolide A	C_3H_7	H	H	-OCH(CH_3)CH(CH_3)CH(OH)		G_2:1	76.9
(+)-Dihydrocalanolide A	C_3H_7	H	H	-OCH(CH_3)CH(CH_3)CH(OH)		G_2:1	76.3
	R4	**R5**	**R6**	**R8**	**R9**	**R10**	
Inophyllum G1	C_6H_5	°		CH_3	CH_3	OH	G_2:1	51.6
	R4	**R5**		**R6**	**R9**	**R10**	
Pseudocordatolide C	CH_3	-OCH(CH_3)CH(CH_3)CH(OH)-			H	H	G_2:1	72.7

It can be observed that all the molecules are active and have the presence of a *bay region* in the pyranocoumarinic system (Figure 3b). Contributions fragments comprising this region were calculated according to equation 1, from the local spectral moments calculated using fragment contributions MODESLAB software module. Its basis isthat the spectral moments of the adjacency matrix of edges inwhich the TOPSMODE approach can be expressed as linearcombinations of the various structural fragments of the molecular graph.The bay region fragment has a positive contribution (0.892) to the activity (Figure 3). Similar *bay region* was designated as a structural alert of azafenantrene (Figure 3a) or polycyclic aromatic hydrocarbons [36], but with the difference in the presence of oxygen in the region. The contributions of the fragments that comprise it, are positive (Figure 3b) [36]. Saeki et al. (2003) observed that the BhQ is a potent ligand for the aryl hydrocarbon receptor (AhR) [47]. Meanwhile the AhR is a transcription factor that mediates ligand-activated cellular responses through dioxin and PAHs, causing the expression of gene disruption and toxicity [48].

It can then be argued for the analogy of the contributions in the *bay region*, that the fused ring system of active pyranocoumarins is a bioisoster of the azafenantrene or polycyclic aromatic hydrocarbons (PAHs). These bioisosteres could also be a transcription factor that mediates cellular responses causing toxicity. These assumptions should be considered in future work.

Within the structural features that are present in the compounds as inophyllum, it can be observed the permutation of a hydroxy group with a carbonyl one, in the C12 position. A slight decrease in toxicity (inophyllum C, 68.1%) compared to inophyllum A (68.6%), which could be explained by the presence of the carbonyl group (electron acceptor), is evident.

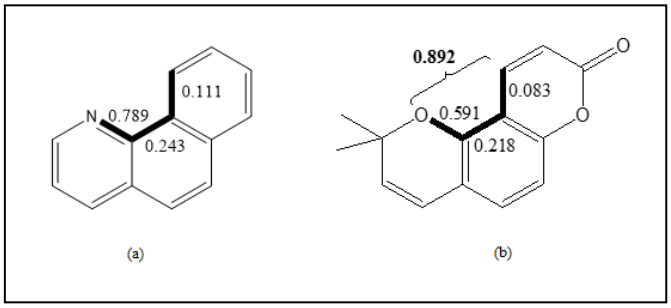


Fig.3. The *bay region* bond contributions.(a) Benzo[*h*]quinoline (BhQ), from Estrada *et al.*(2006); (b) Pyranocoumarins (angular type).

In the case of calanolides, an unsaturation between carbons C7:C8 is observed, for the case of calanolide A, while in the same position for the (+)-dihydrocalanolide A that position is saturated. This indicates that the toxicity seems to decrease with the saturation.

2.2.1. Inophyllum A, Inophyllum C and Inophyllum G1 Structures

The structures of inophyllum A, inophyllum C and inophyllum G1 are shown in Figure 4.

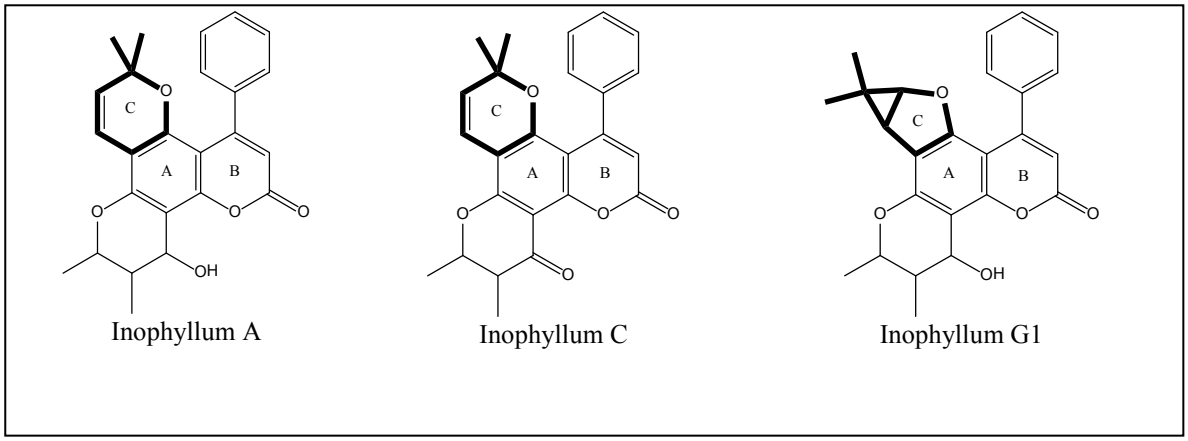


Fig. 4. Ring C fragment structures of inophyllum and pyranocoumarinic system.

As observed in Table 7, inophyllum G1 showed a lower toxicity probability value (51.6%). If its structure is compared to the rest of inophyllum compounds (Figure 4), it can be observed a structural difference (isomeric ratio) in the C ring of the pyranocoumarinic system. This characteristic could be the explanation for the decrease in the genotoxicity *in silico*.

2.3. QSTR of Phenylcoumarins and Biscoumarins

Table 8 shows the same regularity: carbonyl groups esterified with saturated aliphatic groups, and the presence of aromatic groups, inactivate the molecule (ie isodispar B). Meanwhile the biscoumarin studied (Table 9) was also predicted to be inactive (ie dicoumarol).

Table 8. Predictions made using TOPSMODE classification model to phenylcoumarins.

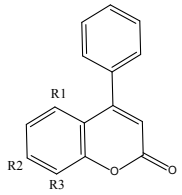
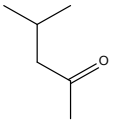
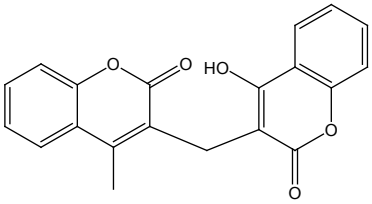
Phenylcoumarin 	**R1**	**R2**	**R3**	**Class.**	**Prob. (%)**
Isodispar B	OH	OH		G_1:-1	90.7

Table 9. Predictions made using TOPSMODE classification model to biscoumarins.

Biscoumarin 	**Class.**	**Prob. (%)**
Dicoumarol	G_1:-1	60.1

3. Overview of QSTR Regarding Natural Coumarins from the DB1 and Virtual Screening Validation

From a scan for regularities between chemical subclasses, it can be observed that when the scaffold has minimal substitutions, these molecules are inactive, eiumbelliferone, psoralen and xanthyletin. The presence of an

electron-withdrawing group (carbonyl) and esterified oxygen, and saturated and unsaturated aliphatic and aromatic groups, are associated with inactivity of molecules (ieammoresinol, ostruthin, osthole and mammea AB).

Methoxy and hydroxy radicals seems to cause increased toxicity. This is related with the probability of clastogenicity, such in the cases of fraxidin, bergapten and methoxsalen. Similar results were obtained for other families of phenolic compounds in previous studies [37, 46]. The *bay region* present in pyranocoumarins (angular type) is also associated with genotoxicity.

There are few studies evaluating the genetic toxicity (clastogenic activity) of natural coumarin (described in the DB1) in the literature. However, genotoxic experimental studies performed with the 6,7-HC, showed *in vivo* antigenotoxic effects and that there were not clastogenic/aneugenic effects in bone marrow cells of mice (micronucleus test) [29]. The prediction proved to be similar to the experimental results obtained by Maistro*et al.* (2015), and it is also corroborated by preliminar genetic toxicity studied *in vitro*: Salmonella/microsome, comet and micronucleus assays. These results showed that this natural coumarin did not present mutagenic, genotoxic or clastogenic/aneugenic activities. In the present study, this compound was classified as inactive, with a probability of 56 %. The correspondence between prediction and experimental data allow to state that for this series of external data. Therefore, the model reached 100 % of goodclassification.6,7-HC, due to the presence of two hydroxyl groups on its benzene ring, seems to affect the formation and scavenging of ROS and influence free radical-mediated oxidative damage, being considered one of the most effective antioxidant in the family of coumarins [29]. Because of that, this compounds has a great potential to be used as an antioxidant, protecting against DNA damage, cancer and aging.

In addition, Paya*et al.* indicated that fraxetin and 4-methyldaphnetin showed *in vitro* pro-oxidant activity [49, 50]. The model did not explain these experimental results based on the hypothesis (relative clastogenicity-pro-oxidation) for the studied database, since these two molecules were considered inactive, although with very low percentage of probability (Table 3).

The most abundant compounds in the plant families of BD1 are inactive compounds (ie umbelliferone, imperatorin and esculetin, which are present in various species with food use). Of the most active compounds, the most abundant in natural sources is bergapten, which can be found in *Angelica archangelica, Apiumgraveolens, Foeniculumvulgare, Petroselinumcrispum, Pimpinellaanisum* and *C. limonum* (Table 2).

The structural features associated with *in silico* clastogenic activity that have been determined, can be considered in the formation of toxicological structural alerts associated with genotoxicity. This becomes important because the DNA damage, chromosome aberrations and consequently disorder in metabolic functioning, contributed to the initiation of the carcinogenetic process, through generation of ROS [51].

Another view of the phenomenon has been postulated in which is now recognized that the pro-oxidant action of bioactive natural phenols has a unique preference rather than their antioxidant action, since it can play an

important role in cancer prevention [52]. It was recently reported that dietary polyphenols could mobilize endogenous copper in humans, leading to oxidative DNA damage, which could be responsible for inducing anti-cancer properties [53].

4. Conclusion

Coumarins represent a diverse class of phytochemicals that are ubiquitous in the human diet and display several medicinal properties. *Apiaceae* family is a prominent food source of coumarins: carrots, celery, parsley, coriander, cumin, fennel and aniseed are present in the culinary practice around the world and in food industry. *Rutaceae* also proved to contain a great number of coumarins with nutritional and economic interest, standing out the citrus and some other like bael fruits. Besides, fruits and vegetables, olive oil, and beverages like coffee, wine, and black and green tea, are also important dietary sources of coumarins. Various natural coumarins showed clastogenic activity *in silico*. However, experimental studies are required to corroborate the information described in this chemoinformatic study. Generally, for this family, the QSTR associated the probability of being active to the presence of hydroxy and methoxy groups in the molecules. It is of particular significance the large number of active molecules from the subclass of pyranocoumarins (angular type), which has been linked to the positive contribution of the fragment that forms the *bay region* of the pyranocoumarinic system. These *in silico* results may contribute to the design of novel foods and drugs, contributing to its security. The genotoxicity of these compounds is of interest in the initiation of carcinogenic processes that occur through the generation of ROS. It may also be important in the prevention of cancer when these substances display pro-oxidant activity.

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