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Natural Compounds with Bronchodilator Activity Selected by Molecular Topology[#]

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Abstract

Motivation. The main goal of the present work is selecting new bronchodilator lead compounds without an explicit knowledge of their mechanism of action. This is particularly interesting since asthma and other obstructive pulmonary diseases have become an increasingly concerning public health problem in many countries along the later years. The importance of the goal is reinforced by the fact that most of the drug design currently available methods need a previous knowledge about the mechanism of action involved.

Method. Molecular topology, a formalism based on describing the molecules as hydrogen–depleted graphs, as well as linear discriminant analysis, a statistical tool able to distinguish between two or more categories or objects, have been used to perform the task. The confirmation of the bronchodilator activity was performed by laboratory tests on Guinea pig trachea tissue.

Results. Several new bronchodilator–activity showing compounds have been selected, some of them significantly more potent than theophylline, the reference drug. Among the selected compounds, all of them derivatives of coumarine, flavonoids and antocianosides, stand out fisetin, an atoxic vegetal pigment which showed 88.9% relaxation on Guinea pig isolated trachea tissue at 0.1 mM with $pD_2 = 4.60$, and hesperetine, (hesperidine's aglycone), which produced a 87.4% relaxation effect at 0.1 mM with $pD_2 = 5.20$. As pattern drug it was used theophylline that relaxed up to 77% at 1 mM, having $pD_2 = 4.69$.

Conclusions. The results mentioned above confirms other previous results from our group, regarding the usefulness of molecular topology as a potent tool to discover new drugs, especially new leads, overcoming the need for a previous knowledge on the drugs mechanism of action.

Keywords. Molecular topology; bronchodilators; fisetin; hesperetin; QSAR; topological indices.

Abbreviations and notations

DF, discriminant function	LDA, lineal discriminant analysis
EC_{50}, effective concentration (50 %)	PD_2, negative log of EC_{50}
E_{max}, percent of relaxation	QSAR, quantitative structure–activity relationships

Dedicated on the occasion of the 70th birthday to Professor Alexandru T. Balaban.

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1 INTRODUCTION

The search for new bronchodilator compounds showing complementary anti–inflammatory activity for asthma, chronic obstructive pulmonary disease and other pulmonary inflammatory diseases has become one of the main targets of the health scientific community [1–3]. According to the estimations of World Health Organization (WHO), about 150 million people suffer from asthma and about 1.2 % dyes every year [4]. In Western Europe the number of cases has doubled during the last decade, and it is estimated that the asthma related deaths have increased in about 40 % in USA since 1982.

Currently, different methods are used to design new drugs; one of them being molecular topology, particularly molecular connectivity [5], which has demonstrated to be a useful formalism to find quantitative structure–activity relationships (QSAR). One of the most interesting advantages of molecular topology is the straightforward calculation of the topological descriptors: in this method each structure is assimilated as a hydrogen depleted graph where the atoms are represented by vertices and the bonds by edges; the connectivity between each atom to the others is included into the topological, either distance or adjacency, matrices.

Mathematical manipulation of such matrices provides different sets of numbers called topological descriptors [6–8], which characterize each molecule at different descriptive structural levels [9,10]. Whether well chosen, these indices can be used for the selection and design of new antivirals [11], sedatives [12], analgesics [13], betablockers [14], antifungals [15], antibacterials [16], citostatics[17], antihistaminics [18], many of which can be considered as lead drugs.

In a recent paper from this laboratory [19], it was proposed a topological–mathematical model able to identify and classify a given compound according to its bronchodilator activity. Among the selected compounds, it stands out acenocoumarol (a coumarinic derivative) and genistein (a flavonoid derivative). Acenocoumarol exhibited a good bronchodilator activity at 0.1 mM despite its low water solubility. These results encouraged us to follow up searching for new drugs in this field, following this model and applying it into a wide set of compounds such as coumarines, flavonoids, (natural substances essential to organism and widely distributed in plants, especially in some fruits as lemons, blackcurrants, buckwheat, and peppers, that show, among others, antiinfective, anti–inflammatory and antioxidant properties [20], as well as other compounds such as antocianosides, with similar actions and applications as the flavonoids.

2 MATERIALS AND METHODS

The search of new compounds showing bronchodilator activity was performed in the following steps.

2.1 Chemical Data

Molecular graphs can be represented by molecular matrices from which all the descriptors used in this work have been computed.

2.1.1 Randić–Kier–Hall subgraph connectivity indices

As it is well–known the χ_i indices may be derived from the adjacency matrix and they are defined as:

$$ {}^{m}\chi_t = \sum_{j=1}^{Nm} {}^{m}S_j \tag{1}$$

where m is the subgraph order (the number of edges in the subgraph), Nm is the number of type t order m subgraphs within the whole graph, ${}^{m}S_j$ is a factor defined for each subgraph as:

$$ {}^{m}S_j = \prod_{i=1}^{m+1} \left(\delta_i\right)_j^{-1/2} \tag{2}$$

where j denotes the particular set of edges that constitutes the subgraph and δ_i is the degree of vertex i (the number of edges incident with vertex i).

Valence connectivity indices are defined similarly, substituting δ_i by δ_i^v:

$$ \delta_i^v = \frac{Z^v - h_i}{Z - Z^v - 1} \tag{3}$$

where Z is the atomic number of the atom i, Z^v is number of valence electrons, and h_i the number of H atoms attached to atom i.

2.1.2 Topological charge indices G_k and J_k

Topological charge indices have shown a good ability to evaluate the charge transfer between pairs of atoms and the global charge transfer, as demonstrated by the good correlation achieved with the dipole moment for a set of heterogeneous hydrocarbons [21].

For a given graph, they are defined as follows:

$$ G_k = \sum_{i=1}^{N-1} \sum_{j=i+1}^{N} \left|c_{ij}\right| \delta\left(k, d_{ij}\right) \tag{4}$$

$$ J_k = \frac{G_k}{\left(N-1\right)} \tag{5}$$

where N is the number of vertices in the chemical graph representing the molecular structure (the number of atoms different from hydrogen in the molecule), and c_{ij} is the charge term between the vertices i and j, defined as $c_{ij} = m_{ij} - m_{ji}$, with m_{ij} and m_{ji} being elements of the $N \times N$ matrix **M**, obtained as the product of two matrices: **M** = **A**·**Q**. Therefore,

$$m_{ij} = \sum_{h=1}^{N} a_{ih} q_{hj} \tag{6}$$

The matrix **A** is called the connectivity or adjacency matrix; its elements a_{ih} represent the bonds between the atoms corresponding to vertices *i* and *h* from the graph. The element a_{ih} takes the value 0 either if $i = h$ or if *i* is not bonded to *h*; it takes the value 1 if *i* is bonded to *h* by a simple bond, 1.5 if the bond is aromatic, 2 for double bonds, and 3 for triple bonds. The matrix **Q** is known as the Coulombian matrix; its element $q_{hj} = 0$ for $h = j$, otherwise $q_{hj} = 1/d_{hj}^2$, where d_{hj} is the number of bonds (the topological distance) between vertices *h* and *j*. In Eq. (4) δ represents the Kronecker delta symbol: $\delta(\alpha,\beta) = 1$ if $\alpha = \beta$, and $\delta(\alpha,\beta) = 0$ if $\alpha \neq \beta$, and d_{ij} is the topological distance between vertices *i* and *j* (the minimum number of bonds that separates the atoms *i* and *j*).

Therefore, G_k represents the overall sum of the absolute values of the c_{ij} charge terms for every pair of vertices *i* and *j* situated at a topological distance *k*. The valence topological charge indices G_k^V and J_k^V are calculated following the former procedure with a modified **A**′ matrix whose main diagonal values describe the electronegativity of the heteroatoms.

2.1.3 Other indices used in this work

Other descriptors used in this research are: *R*, the number of ramifications (the number of vertices bonded at least to three other ones), PR1, PR2, and PR3 representing the number of pairs of ramifications (separated by one, two and three edges, respectively).

2.2 Linear Discriminant Analysis

The objective of the linear discriminant analysis, LDA, which is considered as a heuristic algorithm able to distinguish between two or more categories or objects, is to find linear functions able to discriminate between the active and inactive compounds as for their different descriptor's values. Two large sets of compounds, the first with a proven pharmacological activity (in our case bronchodilator), and the second composed by inactive compounds, were considered for analysis. The discriminant ability is evaluated by the percentage of correct classifications into each group.

LDA was performed with the BMDP 7M package [22]. The selection of the descriptors was based on the F–Snedecor parameter, and the classification criterion was the shortest Mahalanobis distance (distance of each case to the mean of all cases used in the regression equation). The 7M software chooses the variables used in computing the linear classification functions in a stepwise manner; at each step the variable that adds the most to the separation of the groups is entered into (or the variable that adds the least is removed from) the discriminant function. The quality of the discriminant function is evaluated by the Wilk's lambda parameter, λ, which is a multivariate analysis of variance statistic that tests the equality of group means for the variable(s) in the discriminant function.

2.3 Pharmacological Trials of Bronchodilator Activity

The viability of the topological–mathematical model used in this work to search and select new compounds with bronchodilator activity was confirmed by the adequate experimental bronchodilator tests. Guinea–pig isolated trachea was used in pharmacomechanical experiments to assess bronchodilatation [23,24]. Tracheal strips (3 mm of size) were opened by cutting longitudinally through the cartilage rings diametrically opposite the tracheal and were suspended in jacketed 10 mL tissue chambers containing physiological salt solution (Krebs–Henseleit solution). The tension changes were recorded with isometric transducers coupled to a multi–channel polygraph. The preparations were subjected to an initial imposed tension of 2 g. A cumulative log concentration–relaxation curve for each compound was obtained and results were expressed as percentage of the inhibition produced by theophylline (1 mM) added at the end of each experiment that was taken as 100% relaxation. The effective concentration 50% (EC_{50}), what is defined as the concentration leading to 50% of relaxation, was calculated by interpolation in the dose–response curves and expressed as pD_2, (negative log of EC_{50}). In this work, in order to avoid the solubility problems previously observed, tween (0.05 mL) was used as vehicle to prepare solutions of water–insoluble compounds, after checking the non–influence of the vehicle on relaxant activity.

3 RESULTS AND DISCUSSION

The topological–mathematical model used to find out new compounds with bronchodilator activity is comprised by two discriminant functions: the first one, namely DF1 was obtained by using a large data set of more than 300 compounds, representing bronchodilator drugs including xanthines, beta–adrenergic agonists, anti–cholinergics, leukotriene antagonists as well as other structurally heterogeneous set of drugs showing some extent of bronchodillator activity. Just to improve the DF1 discriminant efficiency, a second DF, DF2, was also put to work. That function was obtained with a smaller set of bronchodilator drugs, about 70, but including as many representative compounds as possible in order to consider drugs belonging to every family of bronchodilators. The discriminant functions chosen were [19]:

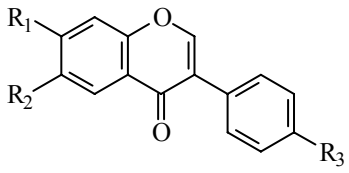
$$DF_1 = 3.07\ {}^{1}\chi^{V} - 3.58\ G_1 + 15.32\ J_2 + 55.50\ J_4 - 1.68\ PR1 + 0.879\ PR2 - 11.71$$
$$N = 739 \quad U\text{–statistics (Wilks}'\lambda) = 0.271 \quad Fs = 286.5 \tag{7}$$

$$DF_2 = 17.40\ ({}^{3}\chi_P - {}^{3}\chi_P{}^{V}) - 12.27\ ({}^{4}\chi_P - {}^{4}\chi_P{}^{V}) - 6.61$$
$$N = 192 \quad U\text{–statistics (Wilks}'\lambda) = 0.315 \quad Fs = 128.5 \tag{8}$$

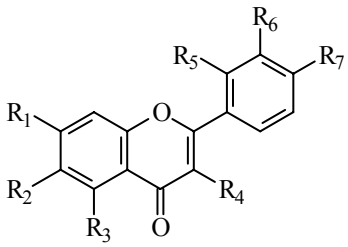
The topological descriptors used in these functions are the well known Kier and Hall's connectivity indices [25], ${}^{m}\chi_t$, as well as the more recently introduced charge indices [21], G_i and J_i, together with some *ad hoc* indices [26], namely PR1 and PR2. The charge indices evaluate the global charge transfer between pairs of atoms inside the molecule.

Table 1. Structure of the 20 Compounds Selected by Molecular Topology Together with the Values of the Discriminant Function DF_1 and DF_2 and Activity Classification for Each Compound

Compound	Str.	R_1	R_2	R_3	R_4	R_5	R_6	R_7	DF_1 (DF_2)	Class
Genistein	I	H	H	OH					–0.75	+
Umbelliferone	IV	OH	H	H	H				1.10	+
Chrysin	II	OH	H	OH	H	H	H	H	1.89	+
Baicalein	II	OH	OH	OH	H	H	H	H	1.10	+
Apigenine	II	OH	H	OH	H	H	H	OH	–0.80	+
Acacetin	II	OH	H	OH	H	H	H	OCH_3	0.21	+
Morin	II	OH	H	OH	OH	OH	H	OH	–0.83	+
Fisetin	II	OH	H	H	OH	H	OH	OH	–2.40 (8.33)	+
Naringenin	III	OH	H	OH	H	H	H	OH	–0.80	+
Hesperetin	III	OH	H	OH	H	OH	OCH_3	H	–1.53 (1.03)	+
4–Methoxygenistein	I	H	H	OCH_3					0.27	+
Esculetin	IV	OH	OH	H	H				0.37	+
7–Mercapte–4–methylcoumarine	IV	SH	H	H	CH_3				2.96	+
7–Carboxi–methoxy–4–methylcoumarine	IV	OCH_2COH	H	H	CH_3				–1.50 (0.39)	+
Escopoletin (esculetin–6–methyl–ester)	IV	OH	OCH_3	H	H				1.14	+
4–Methyl–umbelliferyl–enantate	IV	$OCO(CH_2)_5CH_3$	H	H	CH_3				3.62	+
Coumarine 3–acid carboxilic	IV	H	H	H	H				0.22	+



Structure I



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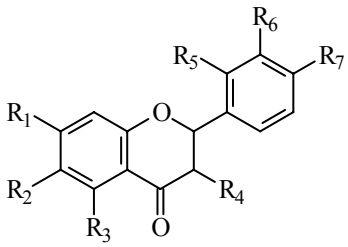

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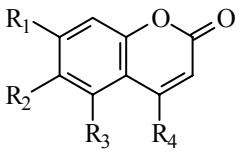
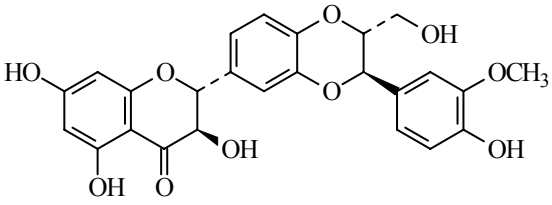
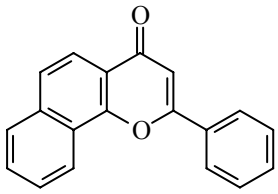


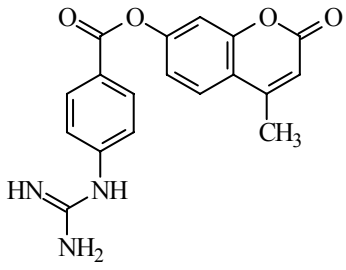
Structure IV



Silymarine
DF_1 (DF_2)= –4.5 (15.7)



α–Naphtoflavone
DF_1 = 4.82



4–Methyl–umbelliferyl
4–guanidine benzoate HCl
monohydrate
DF_1 (DF_2)= –4.8 (0.1)

Based on this model, a given compound is classified as a potential bronchodilator if either $DF_1 > -1$ and $DF_1 < 10$, or $DF_2 > 0$ and $DF_2 < 17$. Otherwise such a compound is classified as inactive [19]. Within these ranges stand most of the active compounds. After applying DF_2 and/or DF_1

functions to different structures related to coumarines, flavonoids and antocianosides from our working databases, twenty molecules were selected as theoretical active compounds. Table 1 shows the structure of each compound and the values of DF_1 and DF_2 as well as the classification for each candidate, while Table 2 illustrates the values of the descriptors selected by LDA.

Table 2. Topological Descriptors Used in the DF_1 and DF_2 Functions for the 20 Selected Compounds

Compound	$^1\chi^v$	G_1	J_2	J_4	PR1	PR2	$\Delta^3\chi_p$ [a]	$\Delta^4\chi_p$ [a]
Genistein	5.830	5.750	0.614	0.117	5	7	1.057	1.085
Umbelliferone	3.485	3.750	0.667	0.095	1	2	0.608	0.512
Chrysin	5.702	5.000	0.580	0.102	4	7	0.904	1.032
Baicalein	5.849	5.250	0.643	0.123	6	8	1.431	1.105
Apigenine	5.837	6.250	0.632	0.113	4	7	1.088	1.124
Acacetin	6.225	6.250	0.606	0.116	4	7	1.176	1.182
Morin	6.123	7.250	0.720	0.164	7	11	1.682	1.599
Fisetin	5.983	6.500	0.689	0.136	6	7	1.624	1.086
Naringenin	5.837	6.250	0.632	0.113	4	7	1.088	1.124
Hesperetin	6.366	7.000	0.651	0.128	5	8	1.448	1.431
4–Methoxygenistein	6.218	5.750	0.589	0.120	5	7	1.145	1.142
Esculetin	3.625	4.500	0.741	0.116	2	3	0.955	0.625
7–Mercapte–4–methylcoumarine	4.180	4.500	0.722	0.121	2	4	0.415	0.500
7–Carboxi–methoxy–4–methylcoumarine	4.952	6.000	0.681	0.106	2	4	0.988	0.831
Escopoletin (esculetin–6–methyl–ester)	4.014	4.500	0.692	0.122	2	3	0.974	0.785
4–Methylumbelliferyl–enantate	7.351	6.000	0.528	0.092	2	5	0.882	0.899
Coumarine 3–acid carboxilic	3.945	3.750	0.684	0.093	3	3	0.728	0.633
Silymarine	10.703	10.500	0.503	0.156	10	14	3.331	2.903
α–Naphtoflavone	6.838	3.500	0.478	0.085	5	5	0.569	0.567
4–Methyl–umbelliferyl 4–guanidine benzoate HCl monohydrate	7.400	8.500	0.602	0.092	3	6	1.185	1.143

[a] $\Delta^3\chi_p = {}^3\chi_p - {}^3\chi_p{}^v$ and $\Delta^4\chi_p = {}^4\chi_p - {}^4\chi_p{}^v$

The compounds selected were: baicalein, lipoxidase and LTs biosynthesis inhibitor; genistein, which is a in vitro inhibitor of specific tyrosine protein kinase; 4–methoxygenistein; naringenin (from *Eucaliptus maculata*); umbelliferone, which shows antibacterial properties and it is an indicator as well; 7–mercapto–4–methylcoumarine (or 4–metil–7–thioumbelliferone); 4–methyl–umbelliferyl–enantate; chrysin; hesperetine, another flavonoid aglycone from hesperedine; 4–methyl–umbelliferyl–4–guanidine; morin, yellow strain from old wood of some trees (*Chlorophora tinctoria*), also used as luminescent indicator; esculetin, a U.V filter; escopoletin (esculetin–6–methyl–eter); fisetin, atoxic pigment from *Rhus cotinus* that inhibits cytotoxicity from aflatoxin; apigenine, inhibiting syntetase in human estrogens; α–naphtoflavone; silymarine, isolated from the *Silybum marianum* fruit and *Carduus marianum*, which is a flavolignane (flavonoid plus coniferilic alcohol) showing antihepatotoxic activity.

Later on, the bronchodilator effect was evaluated for the twenty compounds selected. Just as an example, the results for fisetin are represented on Table 3. Fourteen tests were carried out so that the percent of relaxation was carefully measured for each test at different concentrations.

From the mean relaxation values, Emax, the concentration–response curve (Emax(%) *vs* – log C)

is presented in Figure 1. The effective concentration 50% (EC_{50}) was calculated by interpolation and it was expressed as pD_2 (– log EC_{50}).

Table 3. Values of Percentage of Relaxation Obtained at Different Concentrations for Fisetin

Conc.	Test number														Emax(%)
(mol/L)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
10^{-9}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0 ± 0.0
10^{-8}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0 ± 0.0
10^{-7}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0 ± 0.0
10^{-6}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0 ± 0.0
10^{-5}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	26.7	15.6	12.4	44.3	0.0	44.1	30.2	12.4 ± 4.6
10^{-4}	97.6	91.2	92.3	96.8	90.0	90.9	89.3	93.3	75.0	85.1	94.3	95.1	79.4	74.4	88.9 ± 2.0
10^{-3} T	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100.0 ± 0.0

Table 4. Percentage of Relaxation and pD_2 (pD_2= – log EC_{50}) for the Selected Compounds

Compound	Relaxation (%) (0.1 mM)	pD_2 (– log EC_{50})	Number of Tests
Theophylline (reference drug)	77.0 ± 0.0	4.69	6
3–Coumarine carboxilic acid	0.0 ± 0.0		4
Genistein	73.7 ± 6.1	4.60	8
Naringenin	70.5 ± 10.1	4.60	8
4–Methoxygenistein	57.5 ± 4.9	4.65	9
Umbelliferone	75.4 ± 6.5	4.50	8
Esculetin	74.7 ± 6.8	5.35	9
Fisetin	88.9 ± 2.0	4.60	14
Hesperetin	87.4 ± 4.0	4.75	8
Chrysin	60.8 ± 5.6	4.70	13
Baicalein	65.4 ± 4.6	4.50	10
Apigenine	58.2 ± 2.4	4.60	7
7–Carboximethoxy–4–methylcoumarine	12.8 ± 3.2	6.90	5
Sylimarine	48.2 ± 3.5	4.50	8
Morin	58.2 ± 6.6	4.50	8
Acacetin	–38.4 ± 4.9		4
4–methyl–umbelliferylenantate	46.9 ± 5.6	4.65	17
Escopoletin	61.7 ± 2.1	4.80	8
α–Naphtoflavone	41.6 ± 4.7	4.95	10
7–Mercapte–4–methylcoumarine	55.1 ± 4.8	4.55	15
4–Methyl–umbelliferyl–4–guanidine benzoate HCl monohydrate	80.8 ± 1.5	4.45	9

The results obtained for all compounds are presented in Table 4. The second column shows the relaxation percentages at 0.1 mM, for each compound. Theophylline, used as reference drug, exhibits a relaxation of 77%. From the selected compounds, only two, namely 3–coumarine carboxilic acid and acacetin, resulted to be inactive, which implies about 90% of success for the model's predictive ability. Furthermore, 78% of the active compounds showed relaxation higher than 50%, namely fisetin, hesperetin and 4–methyl–umbelliferyl–4–guanidine benzoate, have values of 88.9, 87.4 and 80.8%, respectively, what is higher than theophylline–induced relaxation. Moreover, relaxation was observed at 0.01 mM for hesperetin and escopoletin. From relaxation curves, the EC_{50} values were obtained by extrapolation; the third column in Table 4 illustrates the potency values expressed as pD_2. Most of compounds show pD_2 values similar to that of

theophylline, standing out esculetin, with $pD_2 = 5.35$, which represents a fourfold lower EC_{50} value than theophylline.

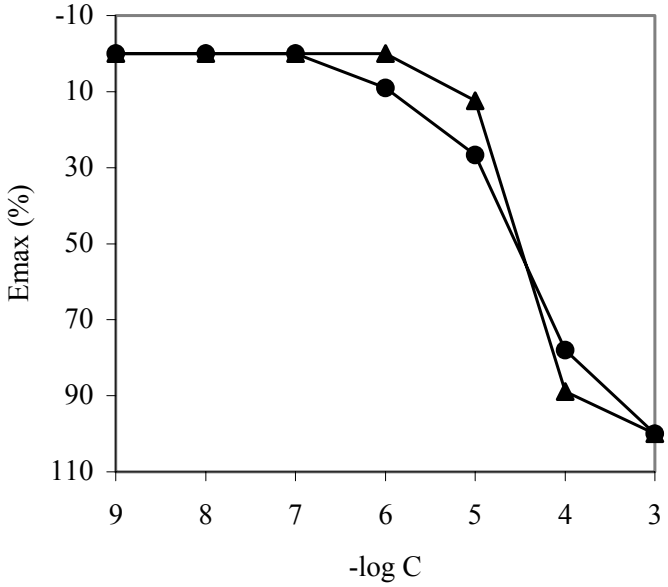


Figure 1. Dose-response curves for fisetin (triangle) and the reference drug teophylline (circle).

The pD_2 top value is that of 7–carboximethoxy–4–methylcoumarine ($pD_2 = 6.90$), that despite its low relaxant effect (12.8%), involves a significant bronchodilator activity, with initial relaxation level at lower concentrations than theophylline.

4 CONCLUSIONS

These results corroborate, once more, that molecular topology is an excellent tool for the selection of new bronchodilator compounds. Indeed, considering the low toxicity of most of the selected compounds it is to be expected that some of them will be the focus of therapeutic interest in the future.

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Supplementary Material

The files used in the LDA study: DF1–INPUT.txt, DF2–INPUT.txt, DF1–OUTPUT.txt and DF2–OUTPUT.txt.

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