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Original article

QSAR modeling of the interaction of flavonoids with GABA(A) receptor

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Abstract

Experimentally assigned values to binding affinity constants of flavonoid ligands towards the benzodiazepine site of the GABA(A) receptor complex were compiled from several publications, and enabled to perform a predictive analysis based on Quantitative Structure–Activity Relationships (QSAR). The best linear model established on 78 molecular structures incorporated four molecular descriptors, selected from more than a thousand of geometrical, topological, quantum-mechanical and electronic types of descriptors and calculated by Dragon software. An application of this QSAR equation was performed by estimating the binding affinities for some newly synthesized flavonoids displaying 2-,7-substitutions in the benzopyrane backbone which still do not have experimentally measured potencies.

Keywords: QSAR; Dragon molecular descriptors; Replacement method; Flavone derivative; Benzodiazepine receptor; GABA(A); Flunitrazepam

1. Introduction

Nowadays, different brain states of mammals such as anxiety, sedation, convulsion, myorelaxation, hypnotic or amnestic are regulated through a large series of psychoactive drugs. A widely prescribed anxiolytic is the synthetic benzodiazepine diazepam, while other therapeutic agents involve barbiturates, neurosteroids, some anti-convulsionants and general anesthetics [1]. Most of these compounds share a structural similarity to the benzodiazepine (BDZ) nucleus. Although BZDs are considered the most safe psychotropic drugs available today for clinical use, they still have a series of unwanted side-effects, such as sedative and myorelaxant actions, ethanol and barbiturate potentiation, recorded amnesia, ataxia and the potential for drug abuse and tolerance [2], and this has stimulated research into alternatives to conventional BZDs.

It is known that the overall balance between neuronal excitation and inhibition in the central nervous system (CNS) is due to the affinity of such type of ligands to the benzodiazepine binding site (BZD-bs) of the γ-aminobutyric acid type A (GABA(A)) receptor complex [3]. GABA(A) receptors are transmembrane hetero-oligomeric proteins which are expressed in the CNS as a pentameric assembly derived from the combination of various subunits, identified as α1–α6, β1–β3 (plus β4 in chick brain), δ, ε, π and ρ1–ρ3 [4]. The BZD binding site in the brain was identified and described by radio-ligand receptor binding assays, employing [^{3}H]BZDs as ligands, and it was found to be located at the interface of the α and γ subunits in the receptor [5,6]. In addition, GABA(A) are ligand-gated chloride ion channel complexes, with BZD exerting their pharmacological effect by potentiating GABA-induced Cl^- currents, which results in membrane hyper-polarization and thus in a reduction of neuronal excitability [7,8].

During last years, the screening of traditional medicinal herbs has proven invaluable to drug development and

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discovery [3]. More than 4000 chemically unique flavonoids (phenyl-benzopyranes) have been isolated from vascular plants and many of them are used as tranquilizers in folkloric medicine. Such kind of compounds are important constituents of the human diet, being derived largely from fruits, vegetables, nuts, seeds, stems and flowers, and thus constitute one of the most important classes of metabolites. Chrysin (5,7-dihydroxyflavone) and Apigenin (5,7,4′-trihydroxyflavone) are among the first compounds from the natural flavone family that demonstrated to possess a potent *in vivo* anxiolitic activity [9], and do not involve unwanted size effects. This anxioselective property of some flavones arises as a consequence of the absence of a simultaneous myorelaxant, amnestic, or sedative effect [10]. Despite their selectivity, flavonoid compounds often exhibit only moderate affinities *in vitro* [11–13].

Several research groups have been able to generate synthetic flavone derivatives with higher affinities for the GABA(A) receptor, by means of the synthesis of small organic molecules libraries prepared by combinatorial chemistry, performed on solid or solution phases, and assisted with the molecular modeling of the flavonoid binding to the BZD-bs pharmacophore [13–18]. In addition, although a large number of qualitative Structure–Activity relationships (SAR) have been reported previously for analyzing this interaction [19–21], only few Quantitative Structure–Activity Relationships (QSAR) were developed [16,22–26]. It has to be mentioned, however, that most of these QSAR involved only a few flavone derivatives during the training stage of the model, thus resulting in an incomplete description of the chemical universe.

In the present study, we establish a QSAR model for the inhibition of GABA(A) receptor that could serve as a guide for the rational design of further potent and selective inhibitors having the flavone backbone. For this purpose, it was necessary to search in the literature for reported experimental affinities of flavonoids towards BZD-bs, in order to surmount the aforementioned limitation related to the number of data employed during model design [16,22–26]. We resort to the widely applied Replacement Method (RM) approach for performing the optimal variable subset selection [27–30], and explore a great number of structural molecular descriptors including definitions of all classes. Our main interest is to apply the so-derived QSAR model for estimating the binding affinities of some new 2-,7-substituted benzopyranes [31], which still do not have experimentally measured potencies. Up to now, few attempts have been carried out to synthesize flavonoids with substitutions of such type. It has to be mentioned here that few biological characterization for this sort of newly synthesized molecules is available, and in this way we expect to provide more knowledge on the underlying phenomena.

2. Methods

2.1. Data set

The experimental binding affinity constants (K_i [μM]) of flavonoid ligands for the benzodiazepine site of the GABA(A) receptor complex were obtained from displacement curves, estimating the ability of the of natural/synthetic flavonoid for displacing the radio-ligand [^{3}H]Flunitrazepam in washed crude synaptosomal membranes from rat cerebral cortex [11–13,16,25,32]. Since the potency values cover a wide range, from low nanomolar to high micromolar, these data are converted into logarithm units ($\log_{10} K_i$) for modeling purposes and are presented in Table 1.

The molecular structure for flavone is shown in Fig. 1. As can be appreciated from Table 1, the flavone derivatives considered in the present analysis appear substituted in positions 5, 6, 7, 8, 2′, 3′, 4′, and 6′ by different electron-donating and electron-withdrawing groups, such as F, Cl, Br, I, CH_3O, NO_2, CH_3, and OH. We partitioned the complete data set into a training set of 70 flavone derivatives and employed the rest of the molecules (molecules 71–78 from Table 1) as a way to assess if these data were correctly predicted by the best QSAR finally derived. It is mentioned that this test set of molecules (denoted as *val*) is considered ''unknown'' and was not employed during the training stage of the relationship. The validation flavonoids were chosen by hand from the complete set of compounds in such a way to share, in the most possible manner, similar structural characteristics with the training series. This is not complicated to carry out in practice since the present data set is quite homogeneous.

2.2. Molecular descriptors

The structures of the compounds are firstly pre-optimized with the Molecular Mechanics Force Field (MM+) procedure included in the Hyperchem 6.03 package [33], and the resulting geometries are further refined by means of the semiempirical method PM3 (Parametric Method-3) using the Polak–Ribiere algorithm and a gradient norm limit of 0.01 kcal Å^{-1}. We computed the molecular descriptors using the software Dragon 5.0 [34], including parameters of all types such as Constitutional, Topological, Geometrical, Charge, GETAWAY (Geometry, Topology and Atoms-Weighted AssemblY), WHIM (Weighted Holistic Invariant Molecular descriptors), 3D-MoRSE (3D-Molecular Representation of Structure based on Electron diffraction), Molecular Walk Counts, BCUT descriptors, 2D Autocorrelations, Aromaticity Indices, Randic Molecular Profiles, Radial Distribution Functions, Functional Groups, Atom-Centered Fragments, Empirical and Properties [35]. Finally, five quantum-chemical descriptors not provided by the program Dragon were added to the pool: molecular dipole moments, total energies, homo–lumo energies, and homo–lumo gap ($\Delta_{\text{homo–lumo}}$). The total pool of explored descriptors consisted of $D = 1176$ variables.

2.3. Model search

In our calculations we employ the computer system Matlab 5.0 [36]. It is our purpose to search the set **D**, containing D descriptors, for an optimal subset **d** of $d \ll D$ ones with minimum standard deviation S, by means of the Multivariable Linear Regression (MLR) technique:

Table 1
Experimental and predicted (Eq. (3) $\log_{10} K_i$ ([μM])

No.	Compound	Exp.	Pred.	Pred. *loo*
Training set				
1	6-Fluoro-3′-methoxyflavone	0.398	0.255	0.248
2	6-Bromo-3′-methoxyflavone	−0.215	−0.452	−0.461
3	6-Chloro-4′-methoxyflavone	0.097	−0.467	−0.484
4	6-Bromo-4′-methoxyflavone	0.322	−0.264	−0.277
5	6-Chloro-2′-fluoroflavone	−0.380	−0.070	−0.059
6	6-Bromo-2′-fluoroflavone	−0.424	−0.377	−0.375
7	6,3′-Difluoroflavone	−0.036	−0.167	−0.175
8	6-Chloro-3′-fluoroflavone	−0.932	−0.450	−0.430
9	6-Bromo-3′-fluoroflavone	−1.377	−0.882	−0.858
10	4′-Fluoroflavone	0.556	0.006	−0.047
11	6,4′-Difluoroflavone	0.398	−0.496	−0.526
12	6-Chloro-4′-fluoroflavone	−0.742	−1.168	−1.181
13	3′-Chloroflavone	−0.212	0.000	0.010
14	6,3′-Dichloroflavone	−1.638	−0.950	−0.922
15	3′-Bromoflavone	−0.384	−0.299	−0.294
16	6-Fluoro-3′-bromoflavone	−0.627	−0.754	−0.762
17	6-Chloro-3′-bromoflavone	−1.638	−1.192	−1.169
18	6,3′-Dibromoflavone	−1.721	−1.812	−1.818
19	6-Bromo-4′-nitroflavone	−0.699	−0.435	−0.426
20	6-Bromoflavone	−1.155	−1.073	−1.062
21	6-Chloroflavone	−0.785	−0.334	−0.302
22	6-Nitroflavone	−0.561	−0.642	−0.648
23	6-Methoxyflavone	−0.066	0.360	0.434
24	6-Fluoroflavone	0.653	0.138	0.108
25	6-Bromo-3′-nitroflavone	−3.000	−2.550	−2.438
26	6-Methyl-3′-nitroflavone	−2.252	−1.793	−1.744
27	6-Chloro-3′-nitroflavone	−2.097	−1.959	−1.946
28	6-3′-Dinitroflavone	−1.585	−1.524	−1.521
29	6-Fluoro-3′-nitroflavone	−0.745	−0.946	−0.959
30	3′-Nitroflavone	−0.545	−0.484	−0.481
31	6-Methyl-3′-bromoflavone	−1.886	−1.195	−1.148
32	6-Nitro-3′-bromoflavone	−1.602	−1.340	−1.330
33	6-Hydroxy-3′-bromoflavone	0.000	−1.306	−1.360
34	6-Methoxy-3′-bromoflavone	0.000	0.734	0.861
35	6-3′-Dimethylflavone	−0.682	−0.709	−0.711
36	3′-Methylflavone	1.000	1.251	1.269
37	5,2′-Dihydroxy-6,7,8,6′-tetramethoxyflavone	−0.444	−0.343	−0.284
38	5,7,2′-Trihydroxy-6,8-dimethoxyflavone	−2.215	−1.094	−1.013
39	2′-Hydroxy-β-napthoflavone	−1.569	−0.732	−0.706
40	6,2′-Dihydroxyflavone	−1.469	−0.908	−0.893
41	5,7,2′-Trihydroxy-6-methoxyflavone	−1.420	−0.286	−0.248
42	5,7,2′-Trihydroxyflavone	−1.125	−0.111	−0.075
43	2′-Hydroxyflavone	−0.678	−0.082	−0.065
44	5,7-Dihydroxy-6,8-dimethoxyflavone	−0.699	−1.138	−1.155
45	7,2′-Dihydroxyflavone	−0.252	−0.803	−0.835
46	5,7-Dihydroxy-6-methoxyflavone	−0.051	0.092	0.099
47	5,7-Dihydroxy-8-methoxyflavone	0.182	0.381	0.392
48	6-Hydroxyflavone	0.422	−0.351	−0.403
49	7-Hydroxyflavone	0.623	0.883	0.912
50	5,6,7-Trihydroxyflavone	0.747	0.154	0.092
51	6-Hydroxy-2′-methoxyflavone	0.976	1.220	1.237
52	2′-Methoxyflavone	1.508	1.070	1.025
53	2′-Amino-6-methoxyflavone	0.544	0.199	0.161
54	Flavone	0.000	−0.845	−0.916
55	5,7-Dihydroxyflavone	0.477	0.792	0.821
56	5,3′,4′-Trihydroxy-6,7-dimethoxyflavone	2.301	1.459	1.338
57	5,4′-Dihydroxy-6,7-dimethoxyflavone	1.362	0.548	0.423
58	5,7,4′-Trihydroxy-6-methoxyflavone	0.000	0.075	0.081
59	5,7-Dihydroxy-2′-chloroflavone	0.903	0.322	0.302
60	5,7-Dihydroxy-2′-fluoroflavone	0.903	0.043	0.013
61	5,7-Dihydroxy-6,8-dibromoflavone	−0.155	0.009	0.031
62	5,7,4′-Trihydroxyflavone	0.602	0.587	0.584

(*continued on next page*)

Table 1 (*continued*)

No.	Compound	Exp.	Pred.	Pred. *loo*
63	3,5,7,4′-Tetrahydroxyflavone	1.969	1.479	1.423
64	5-Hydroxy-7-methoxyflavone	1.699	2.513	2.704
65	5,7-Dihydroxy-6,8-diiodoflavone	0.000	0.544	0.589
66	6-Fluoro,3′-hydroxyflavone	0.400	−0.535	−0.597
67	6-Chloro,3′-hydroxyflavone	−0.070	−0.805	−0.841
68	6-Bromo,3′-hydroxyflavone	−0.220	−1.226	−1.287
69	6-Bromo-2′-nitroflavone	−0.680	0.096	0.135
70	6-Nitro-4′-bromoflavone	−1.600	−1.445	−1.434
Test set				
71	3′-Methoxyflavone	0.380	−0.016	–
72	6-Chloro-3′-methoxyflavone	−0.072	−0.027	–
73	3′-Fluoroflavone	0.550	0.641	–
74	6-Bromo-4′-fluoroflavone	−0.939	−0.787	–
75	6-Fluoro-3′-chloroflavone	−0.701	−0.589	–
76	6-Bromo-3′-chloroflavone	−1.770	−1.571	–
77	6-Methylflavone	−0.903	−0.574	–
78	6-Bromo-3′-methylflavone	−0.812	−0.444	–
Estimation set				
79	7-Methoxyflavone	–	1.596	–
80	7-Chloroflavone	–	0.773	–
81	7-Bromoflavone	–	0.325	–
82	2-(2-Furanyl)-4*H*-1-benzopyran-4-one	–	−0.431	–
83	2-(β-Naphtyl)-4*H*-1-benzopyran-4-one	–	−2.678	–
84	2-(α-Naphtyl)-4*H*-1-benzopyran-4-one	–	−0.317	–
85	7-Bromo-2-(β-naphtyl)-4*H*-1-benzopyran-4-one	–	−1.741	–
86	7-Chloro-2-(α-naphtyl)-4*H*-1-benzopyran-4-one	–	0.408	–
87	7-Methyl-2-(α-naphtyl)-4*H*-1-benzopyran-4-one	–	0.379	–
88	7-Bromo-2-(α-naphtyl)-4*H*-1-benzopyran-4-one	–	0.202	–
89	7-Methoxy-2-(β-naphtyl)-4*H*-1-benzopyran-4-one	–	−3.990	–
90	7-Methoxy-2-(α-naphtyl)-4*H*-1-benzopyran-4-one	–	0.063	–
91	7-Chloro-2-(β-naphtyl)-4*H*-1-benzopyran-4-one	–	−1.657	–
92	7-Chloro-2-(2-furanyl)-4*H*-1-benzopyran-4-one	–	0.201	–
93	7-Fluoro-2-(α-naphtyl)-4*H*-1-benzopyran-4-one	–	0.663	–
94	7-Methyl-2-(β-naphtyl)-4*H*-1-benzopyran-4-one	–	−2.599	–

$$S = \frac{1}{(N-d-1)} \sum_{i=1}^{N} res_i \qquad (1)$$

where N is the number of molecules in the training set, and res_i the residual for molecule i, the difference between the experimental property ($\mathbf{p}$) and predicted property ($\mathbf{p}_{\text{pred}}$). More precisely, we want to obtain the global minimum of $S(\mathbf{d})$ where $\mathbf{d}$ is a point in a space of $D!/[d!(D-d)!]$ ones. A full search (FS) of optimal variables is impractical because it requires $D!/[d!(D-d)!]$ linear regressions. Some time ago we proposed the Replacement Method (RM) [27–30] that produces linear regression QSPR–QSAR models that are quite close to the FS ones with much less computational work. This technique approaches the minimum of S by judiciously taking into account the relative errors of the coefficients of the least-squares model given by a set of d descriptors $\mathbf{d} = \{X_1, X_2, \ldots X_d\}$. The RM gives models with better statistical parameters than the Forward Stepwise Regression procedure [37] and similar ones to the more elaborated Genetic Algorithms (GA) [38]. We believe that RM has a better behavior over GA since it takes into account the relative errors of the regression coefficients, and also because the replacement of the descriptor in the model is not random, in contrast to GA. In addition, the practical use of GA normally involves a problem that is not easy to solve, related to the adjustment of some parameters of mutation and crossover probability.

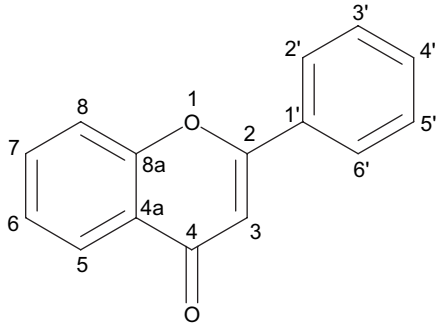

Fig. 1. Molecular structure of flavone.

The Kubinyi function (FIT) [39,40] is a statistical parameter that is closely related to the Fisher ratio (F), but avoids the main disadvantage of the latter that is too sensitive to changes in small d values and poorly sensitive to changes in large

d values. The FIT(**d**) criterion has a low sensitivity to changes in small d values and a substantially increasing sensitivity for large d values. The greater the FIT value the better the linear equation. It is given by the following equation, where $R(d)$ is the correlation coefficient for a model with d descriptors.

$$\mathrm{FIT} = \frac{R(d)^2\left(N-d-1\right)}{\left(N+d^2\right)\left(1-R^2\right)} \tag{2}$$

In the present study, the optimal number of molecular descriptors (d_{opt}) to be included in the linear regression equation is deduced from the plot of FIT vs. d. As the Kubinyi function achieves a maximum value at d_{max}, it is possible to calculate d_{opt} in the following way:

1. calculate $d_1 = [d_{\mathrm{max}}/2]+1$, where $[x]$ denotes the integer part of x;
2. if the slope of FIT at d_1 is greater than at $d_1 + 1$, then $d_{\mathrm{opt}} = d_1$, otherwise, $d_{\mathrm{opt}} = d_1 + 1$.

Therefore, the d_{opt} value reflects a ''breaking point'' beyond which the FIT improvement can be considered negligible.

The theoretical validation practiced over each linear model is based on the Leave-More-Out Cross-Validation procedure (*l-n%-o*) [41], with $n\%$ representing the number of molecules removed from the training set. The number of cases for random data removal analyzed in every *l-n%-o* was of 5,000,000. The percentage $n\%$ depends simultaneously upon the number of compounds N (one cannot remove many molecules from the training set if a small sample is analyzed as the normality condition of the fitted data has to be obeyed) and their structural diversity (if the molecules are structurally very different, more compounds would have to be removed from the set for checking the predictive performance of the model). We chose the value of $n\% = 30\%$ (21 flavonoids) in Cross-Validation in order to properly validate the QSAR equations.

Table 2
Linear QSAR models established for the training set of $\log_{10} K_i$ ([μM]) ($N = 70$) (the best relationship found appears in bold)

Model	Descriptors involved	R	S	FIT
M1	*MATS8v*	0.591	0.861	0.514
M2	*BELe2*, *MATS8v*	0.708	0.759	0.909
M3	*R7u*$^+$, *MATS8v*, *BEHp6*	0.813	0.630	1.629
M4	***GATS8e***, ***IDDE***, ***RDF120e***, ***R7u***$^+$	**0.847**	**0.580**	**1.918**
M5	*R7u*$^+$, *GATS8e*, *BEHv5*, *RDF120v*, *IDDE*	0.871	0.541	2.107
M6	*MATS8v*, *BELe2*, *HATS7u*, *J*, *GATS8e*, *IDDE*	0.894	0.496	2.378
M7	*MATS8v*, *R4u*$^+$, *HATS7e*, *Mor22u*, *BEHp6*, *L1m*, *Mor22e*	0.930	0.410	3.342

3. Results and discussion

We established different predictive relationships capable to link the molecular structure of flavonoids with their inhibitory activities, by means of 1−10 variables linear regression models that were searched by selecting the most representative structural parameters appearing in the pool containing $D = 1176$ of them. The application of RM variable subset selection approach for the training set of 70 flavone derivatives resulted in the best QSAR models recorded in Table 2. It is of interest to mention here that the Cross-Validation technique was very useful for searching these linear models. Specific details of the molecular descriptors reported in this work are provided by Table 3. According to Fig. 2 (a), the FIT parameter improves with d up to a certain ''breaking point'', which according to the criterion mentioned in Section 2.3 corresponds to the value $d_{\mathrm{opt}} = 4$ descriptors. Therefore, we conclude that the best QSAR for modeling the interaction of flavonoids with GABA(A) receptor is the following:

$$\begin{aligned}\log_{10}K_i =& 3.222\left(\pm 1.1\right) + 2.064\left(\pm 0.3\right)\\ &\cdot GATS8e - 1.621\left(\pm 0.3\right)\cdot IDDE - 0.693\left(\pm 0.1\right)\\ &\cdot RDF120e + 48.951\left(\pm 7\right)\cdot R7u^+\end{aligned}$$

Table 3
Symbols for molecular descriptors involved in different models

Molecular descriptor	Type	Description
MATS8v	2D Autocorrelations	Moran autocorrelation − lag 8/weighted by atomic van der Waals volumes
BELe2	BCUT	Lowest eigenvalue no. 2 of Burden matrix/weighted by atomic Sanderson electronegativities
R7u$^+$	GETAWAY	*R* maximal autocorrelation of lag 7/unweighted
BEHp6	BCUT	Highest eigenvalue no. 6 of Burden matrix/weighted by atomic polarizabilities
GATS8e	2D Autocorrelations	Geary autocorrelation − lag 8/weighted by atomic Sanderson electronegativities
IDDE	Topological	Mean information content on the distance degree equality
RDF120e	Radial Distribution Function	Radial Distribution Function − 12.0/weighted by atomic Sanderson electronegativities
BEHv5	BCUT	Highest eigenvalue no. 5 of Burden matrix/weighted by atomic polarizabilities
RDF120v	Radial Distribution Function	Radial Distribution Function − 1.0/weighted by atomic van der Waals volumes
HATS7u	GETAWAY	Leverage-weighted autocorrelation of lag 7/unweighted
J	Topological	Balaban *J* index
R4u$^+$	GETAWAY	*R* maximal autocorrelation of lag 4/unweighted
HATS7e	GETAWAY	Leverage-weighted autocorrelation of lag 7/weighted by atomic Sanderson electronegativities
Mor22u	3D-MoRSE	3D-MoRSE-signal 22/unweighted
L1m	WHIM	1st component size directional WHIM index/weighted by atomic masses
Mor22e	3D-MoRSE	3D-MoRSE-signal 22/weighted by atomic Sanderson electronegativities

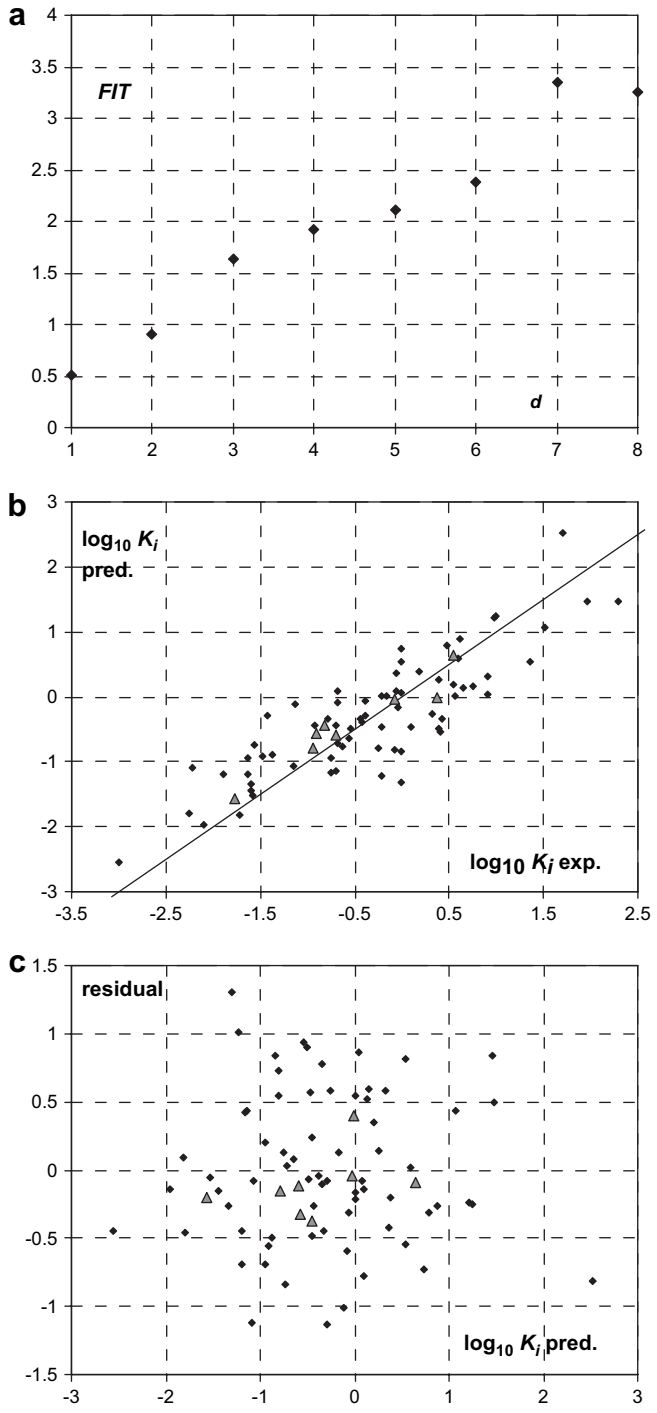

Fig. 2. (a) FIT parameter as a function of the number of descriptors for the training set. (b) Predicted (Eq. (3)) vs. experimental $\log_{10} K_i$ for the training (circles) and test (triangles) sets. (c) Dispersion plot of the residuals for the training and test sets according to Eq. (3).

$$N = 70,\ R = 0.847,\ S = 0.580,\ FIT = 1.919,\ p < 10^{-4} \qquad (3)$$

$$R_{loo} = 0.822,\ S_{loo} = 0.622,\ R_{l\text{-}30\%\text{-}o} = 0.700,\ S_{l\text{-}30\%\text{-}o} = 0.803$$

Here, the absolute errors of the regression coefficients are given in parentheses, p is the significance of the model, FIT the Kubinyi function, and *loo* and *l*-30%-*o* stand for the Leave-One-Out and Leave-More-Out Cross-Validation techniques, respectively.

With the purpose of demonstrating that Eq. (3) does not result from happenstance, a widely used approach to establish the model robustness is the so-called *y*-randomization [42]. It consists of scrambling the **p** experimental property in such a way that activities do not correspond to the respective compounds. After analyzing 5,000,000 cases of *y*-randomization, the smallest S value obtained using this procedure was 0.948, a poorer value when compared to the one found considering the true calibration ($S = 0.580$). In this way, the robustness of the model could be assessed, showing that the calibration was not a fortuitous correlation and therefore results in a Structure–Activity Relationship.

The plot of predicted vs. experimental $\log_{10} K_i$ shown in Fig. 2(b) suggests that the 78 flavone derivatives tend to follow a straight line trend. Table 1 also includes the predicted inhibitory potencies as obtained via Eq. (3) for the training and test set. The behavior of the residuals in terms of the predictions of Fig. 2(c) tends to show a normal distribution for both sets. This figure includes only one calibration outlier with a residual exceeding the value $2S = 1.160$: compound **33** (6-hydroxy-3′-bromoflavone, 1.306), while none of the training compounds exceeds the value 2.5 $S = 1.450$; the presence of this outlier can be attributed exclusively to be a pure consequence of the limited number of structural descriptors participating in Eq. (3). If compound **33** were omitted from the training data, the improvement of the statistical quality of the present model would not be significant, thus we decided to employ all data in the present modeling.

Considering the inter-correlation matrix in Table 4, it reveals that the descriptors of the linear model are not seriously inter-correlated ($R_{ij} < 0.4$), and this fact substantiates the presence of all the parameters in the equation. The predictive power of the linear model is satisfactory as revealed by its stability upon the inclusion or exclusion of compounds, as measured by the *loo* parameters $R_{loo} = 0.822$ and by *l*-*n*%-*o* $R_{l\text{-}30\%\text{-}o} = 0.700$. These results are in the range of a validated model: $R_{l\text{-}n\%\text{-}o}$ must be greater than 0.50, according to the literature [43]. The application of Eq. (3) to the eight flavonoids comprising the external test set, not considered during the model development, lead to appropriate predictions for the inhibitory potencies (see Table 1), i.e. $R_{val} = 0.960$, $S_{val} = 0.403$, thus verifying the predictive ability of the proposed relationship. For comparative purposes, Table 5 summarizes the results obtained with two other different QSARs based on MLR, for binding affinity prediction in terms of the number of calibration flavonoid employed, type of descriptors used to derive the model, number of parameters, and standard error.

Table 4
Correlation matrix for descriptors of Eq. (3) ($N = 70$)

	GATS8e	*IDDE*	*RDF120e*	*R7u*$^+$
GATS8e	1	0.162	0.018	0.203
IDDE		1	0.348	0.207
RDF120e			1	0.252
R7u$^+$				1

Table 5
Comparison of different MLR QSAR for flavonoid compounds

Number of molecules	Descriptors	Number of descriptors	S	Ref.
37	Lipophilic, steric and electronic	3	0.929	[25]
17	Constitutional and steric	3	0.576	[26]
70	Topological, GETAWAY, RDF, 2D Autocorrelation	4	0.580	Present study

RDF: Radial Distribution Function.

As can be appreciated, our MLR model outperforms the statistical quality of the previously reported ones and is built for a larger set of compounds.

The molecular descriptors appearing in the linear equation combine several 2D- and 3D-aspects of the molecular structure, and can be classified as follows: (i) a 2D Autocorrelation: *GATS8e*, Geary autocorrelation − lag 8/weighted by atomic Sanderson electronegativities; (ii) a Topological descriptor: *IDDE*, the mean information content on the distance degree equality; (iii) a Radial Distribution Function: *RDF120e*, Radial Distribution Function − 12.0/weighted by atomic Sanderson electronegativities; and finally, a GETAWAY descriptor: $R7u^+$, the R maximal autocorrelation of lag 7/unweighted. An important aspect that can be outlined from Eq. (3) is that it assumes that the distribution of atomic Sanderson electronegativities, an electronic effect, influences the variation of the affinity of the flavonoid ligands for the benzodiazepine site of the GABA(A) receptor complex. This is in line with the reported QSAR models for the interaction of flavonoid ligands with GABA(A) receptor [16,25].

Different structural variables introduced by Broto, Moreau, and Geary [44,45] correspond to 2D Autocorrelations between pairs of atoms in the molecule, and were defined in order to reflect the contribution of a considered atomic property to the experimental observations under investigation ($\log_{10} K_i$). The atomic properties that can be adopted to differentiate the nature of atoms are the mass (m), polarizability (p), electronegativity (e) or the volume (v). These indices can be readily calculated, i.e.: by summing products of atomic weights (employing atomic properties such as atomic polarizabilities, molecular volumes, etc.) of the terminal atoms of all the paths of a prescribed length. For the case of *GATS8e*, the path connecting a pair of atoms has length 8 and involves the atomic Sanderson electronegativities as weighting scheme to distinguish their nature.

Another descriptor that enters Eq. (3) is the topological variable *IDDE*, obtained from the Information Theory [46], and the descriptors derived therefrom measure the complexity of the molecule in terms of the diversity of elements that includes in its chemical structure, such as the type of atoms, bonds, cycles, etc. The Topological Distance matrix (**D**), introduced by Harary in the 1960s, accounts for the ''through bond'' interactions of atoms in molecules; descriptor *IDDE* characterizes the distribution of the topological distances in each chemical graph.

A Radial Distribution Function (RDF) [47] of an ensemble of atoms can be interpreted as the probability distribution of finding an atom in a spherical volume of certain radius, also incorporating different atomic properties in order to differentiate the contribution of atoms to $\log_{10} K_i$. For the case of *RDF120e*, the sphere radius is of 12.0 Å and the atomic Sanderson electronegativities are employed to distinguish their nature.

The GETAWAY (GEometry, Topology, and Atom-Weights AssemblY) type of descriptors [48] were designed with the main purpose of matching the 3D molecular geometry. These numerical variables are derived from the elements h_{ij} of the Molecular Influence matrix (**H**), obtained through the values of atomic cartesian coordinates. The diagonal elements of **H** (h_{ii}) are called leverages, and are considered to represent the influence of each atom in determining the whole shape of the molecule. For instance, the mantle atoms always have higher h_{ii} values than atoms near the molecule center, while each off-diagonal element h_{ij} represents the degree of accessibility of the j^{th} atom to interactions with the i^{th} atom. The Influence/Distance matrix (**R**) involves a combination of the elements of **H** matrix with those of the Geometric Matrix (**G**). Descriptor $R7u^+$ involved in Eq. (4) is of the R-GETAWAY type, and represents an **R** index of maximal contribution to the autocorrelation in lag 7 (topological distance).

The standardization of the regression coefficients of Eq. (3) [37], allows assigning a greater importance to the molecular descriptors that exhibit larger absolute standardized coefficients. This result is given as follows, with standardized coefficients shown between parentheses:

$$R7u^+\ (0.52) \rangle GATS8e\ (0.46) \rangle IDDE\ (0.39) \rangle RDF120e\ (0.35) \tag{4}$$

From this inequality it is deduced that both the GETAWAY descriptor $R7u^+$ and the 2D Autocorrelation *GATS8e* are the most relevant variables for the present set of flavonoids. As these four molecular descriptors take positive numerical values for all the compounds, and considering the sign of the regression coefficients, a compound would tend to exhibit relatively a higher binding affinity for the BZD-bs the lower the numerical values of $R7u^+$ and *GATS8e* descriptors, and the higher the values of *IDDE* and *RDF120e*. Of course, mixing effects among the four variables would also lead to a high estimated potency for the compounds. The accomplishment of the general tendency of descriptor's importance as given by Eq. (4) can be checked from the numerical values taken by the variables in Table 6 and by the predictions given in Table 1. The most relevant 3D descriptor $R7u^+$ is expected to have a high dependence on conformational changes, since it encodes information on pairs of atoms very far each other (lag of 7). For this reason, it is possible to argue that the affinity constants for the present set of flavone derivatives have high dependence on conformational changes.

With the purpose of further analyzing the pharmacological properties of these flavonoid compounds, it is mentioned in the literature that the presence of electronegative groups in their structure is essential for activity [11,49]. It has been reported that halo, nitro, halo/nitro flavone derivatives with

Table 6
Numerical values for the best four descriptors participating in the linear QSAR

No.	Compound	*GATS8e*	*IDDE*	*RDF120e*	*R7u*$^+$
Training set					
1	6-Fluoro-3′-methoxyflavone	0.789	3.922	0.000	0.036
2	6-Bromo-3′-methoxyflavone	0.763	3.922	0.943	0.036
3	6-Chloro-4′-methoxyflavone	0.873	3.784	0.697	0.023
4	6-Bromo-4′-methoxyflavone	0.867	3.784	0.386	0.023
5	6-Chloro-2′-fluoroflavone	1.083	4.037	0.439	0.027
6	6-Bromo-2′-fluoroflavone	1.076	4.037	0.862	0.027
7	6,3′-Difluoroflavone	1.043	4.143	0.000	0.024
8	6-Chloro-3′-fluoroflavone	1.025	4.143	0.356	0.024
9	6-Bromo-3′-fluoroflavone	1.013	4.143	0.943	0.024
10	4′-Fluoroflavone	0.537	3.392	0.000	0.024
11	6,4′-Difluoroflavone	0.720	3.932	0.007	0.024
12	6-Chloro-4′-fluoroflavone	0.620	3.932	0.678	0.024
13	3′-Chloroflavone	0.699	3.572	0.000	0.023
14	6,3′-Dichloroflavone	0.782	4.143	0.283	0.023
15	3′-Bromoflavone	0.554	3.572	0.000	0.023
16	6-Fluoro-3′-bromoflavone	0.782	4.143	0.000	0.023
17	6-Chloro-3′-bromoflavone	0.665	4.143	0.283	0.023
18	6,3′-Dibromoflavone	0.609	4.143	1.011	0.023
19	6-Bromo-4′-nitroflavone	0.852	3.975	0.000	0.021
20	6-Bromoflavone	0.458	3.503	1.063	0.024
21	6-Chloroflavone	0.533	3.503	0.220	0.024
22	6-Nitroflavone	0.504	3.522	0.462	0.023
23	6-Methoxyflavone	0.525	3.827	0.555	0.054
24	6-Fluoroflavone	0.688	3.503	0.000	0.024
25	6-Bromo-3′-nitroflavone	0.731	4.107	2.100	0.017
26	6-Methyl-3′-nitroflavone	0.683	4.107	1.641	0.028
27	6-Chloro-3′-nitroflavone	0.754	4.107	1.315	0.017
28	6-3′-Dinitroflavone	0.705	4.089	0.513	0.016
29	6-Fluoro-3′-nitroflavone	0.803	4.107	0.000	0.017
30	3′-Nitroflavone	0.695	3.684	0.000	0.017
31	6-Methyl-3′-bromoflavone	0.542	4.143	0.345	0.029
32	6-Nitro-3′-bromoflavone	0.606	4.011	0.559	0.022
33	6-Hydroxy-3′-bromoflavone	0.698	4.143	0.546	0.023
34	6-Methoxy-3′-bromoflavone	0.622	3.922	0.012	0.053
35	6-3′-Dimethylflavone	1.067	4.143	0.925	0.025
36	3′-Methylflavone	1.068	3.572	0.000	0.033
37	5,2′-Dihydroxy-6,7,8,6′-tetramethoxyflavone	0.844	4.310	2.592	0.071
38	5,7,2′-Trihydroxy-6,8-dimethoxyflavone	0.732	4.252	0.157	0.024
39	2′-Hydroxy-β-napthoflavone	0.688	3.732	0.438	0.020
40	6,2′-Dihydroxyflavone	0.857	4.037	0.622	0.022
41	5,7,2′-Trihydroxy-6-methoxyflavone	0.793	4.005	0.033	0.028
42	5,7,2′-Trihydroxyflavone	0.828	3.684	0.000	0.019
43	2′-Hydroxyflavone	0.803	3.725	0.000	0.022
44	5,7-Dihydroxy-6,8-dimethoxyflavone	0.814	4.176	0.995	0.029
45	7,2′.Dihydroxyflavone	0.806	4.143	0.000	0.021
46	5,7-Dihydroxy-6-methoxyflavone	1.103	4.011	1.033	0.037
47	5,7-Dihydroxy-8-methoxyflavone	1.019	4.107	0.000	0.035
48	6-Hydroxyflavone	0.584	3.503	0.396	0.024
49	7-Hydroxyflavone	0.943	3.308	0.000	0.022
50	5,6,7-Trihydroxyflavone	1.219	3.722	0.694	0.019
51	6-Hydroxy-2′-methoxyflavone	1.109	3.922	0.126	0.044
52	2′-Methoxyflavone	1.168	4.143	0.000	0.044
53	2′-Amino-6-methoxyflavone	0.579	3.922	0.020	0.044
54	Flavone	0.394	3.735	0.000	0.024
55	5,7-Dihydroxyflavone	1.240	3.682	0.000	0.020
56	5,3′,4′-Trihydroxy-6,7-dimethoxyflavone	1.169	4.085	0.497	0.057
57	5,4′-Dihydroxy-6,7-dimethoxyflavone	1.082	4.263	1.206	0.058
58	5,7,4′-Trihydroxy-6-methoxyflavone	0.953	3.754	1.212	0.037
59	5,7-Dihydroxy-2′-chloroflavone	0.919	3.684	0.000	0.024
60	5,7-Dihydroxy-2′-fluoroflavone	0.855	3.684	0.000	0.021
61	5,7-Dihydroxy-6,8-dibromoflavone	1.359	3.975	1.011	0.023
62	5,7,4′-Trihydroxyflavone	1.031	3.309	0.546	0.020
63	3,5,7,4′-Tetrahydroxyflavone	1.272	3.939	0.622	0.050

Table 6 (*continued*)

No.	Compound	*GATS8e*	*IDDE*	*RDF120e*	$R7u^+$
64	5-Hydroxy-7-methoxyflavone	1.423	3.422	0.011	0.039
65	5,7-Dihydroxy-6,8-diiodoflavone	1.280	3.975	0.074	0.024
66	6-Fluoro,3′-hydroxyflavone	0.983	4.143	0.000	0.019
67	6-Chloro,3′-hydroxyflavone	0.972	4.143	0.356	0.019
68	6-Bromo,3′-hydroxyflavone	0.965	4.143	0.943	0.019
69	6-Bromo-2′-nitroflavone	1.033	4.202	0.583	0.040
70	6-Nitro-4′-bromoflavone	0.417	3.821	0.662	0.023
Test set					
71	3′-Methoxyflavone	0.748	4.037	0.000	0.036
72	6-Chloro-3′-methoxyflavone	0.772	3.922	0.356	0.036
73	3′-Fluoroflavone	0.986	3.572	0.000	0.024
74	6-Bromo-4′-fluoroflavone	0.578	3.932	0.003	0.024
75	6-Fluoro-3′-chloroflavone	0.862	4.143	0.000	0.023
76	6-Bromo-3′-chloroflavone	0.743	4.143	1.063	0.023
77	6-Methylflavone	0.390	3.503	0.493	0.029
78	6-Bromo-3′-methylflavone	1.052	4.143	1.063	0.033
Estimation set					
79	7-Methoxyflavone	1.284	3.537	0.016	0.030
80	7-Chloroflavone	0.866	3.308	0.000	0.023
81	7-Bromoflavone	0.649	3.308	0.000	0.023
82	2-(2-Furanyl)-4*H*-1-benzopyran-4-one	0.359	3.375	0.000	0.022
83	2-(β-Naphtyl)-4*H*-1-benzopyran-4-one	0.843	4.297	2.456	0.021
84	2-(α-Naphtyl)-4*H*-1-benzopyran-4-one	0.898	4.202	0.000	0.029
85	7-Bromo-2-(β-naphtyl)-4*H*-1-benzopyran-4-one	0.975	3.936	2.271	0.020
86	7-Chloro-2-(α-naphtyl)-4*H*-1-benzopyran-4-one	1.064	3.936	0.000	0.028
87	7-Methyl-2-(α-naphtyl)-4*H*-1-benzopyran-4-one	0.813	3.936	0.000	0.038
88	7-Bromo-2-(α-naphtyl)-4*H*-1-benzopyran-4-one	0.988	3.936	0.000	0.027
89	7-Methoxy-2-(β-naphtyl)-4*H*-1-benzopyran-4-one	1.379	4.350	6.317	0.028
90	7-Methoxy-2-(α-naphtyl)-4*H*-1-benzopyran-4-one	1.363	4.437	0.004	0.025
91	7-Chloro-2-(β-naphtyl)-4*H*-1-benzopyran-4-one	1.070	3.936	2.433	0.020
92	7-Chloro-2-(2-furanyl)-4*H*-1-benzopyran-4-one	0.728	3.455	0.000	0.022
93	7-Fluoro-2-(α-naphtyl)-4*H*-1-benzopyran-4-one	1.211	3.936	0.000	0.027
94	7-Methyl-2-(β-naphtyl)-4*H*-1-benzopyran-4-one	0.769	3.936	4.380	0.041

substitutions preferentially in positions 6 and 3′ of the flavone nucleus show high affinity for the BZD-bs, and exhibit a wide spectrum of pharmacological profiles, ranging from full agonists to antagonists [13]. Further, it is known that 2′-hydroxyl group on flavonoid molecules is an important enhancing factor that is responsible for the interaction between the ligand and the receptor, resulting in a seven fold or higher affinity for the BZD-bs [32]. Our laboratory synthesized [31] the list of compounds that appear in Table 1 denoted as 'estimation set', consisting of benzopyrane compounds substituted in positions 2 and 7. As can be observed, these substitutions include some electronegative groups that are different than those reported previously, such as 2-furanyl, α-naphtyl, and β-naphtyl.

We applied the QSAR of Eq. (3) to estimate the affinity $\log_{10} K_i$ on our synthesized derivatives, and found some interesting results. The flavonoids involving the β-naphtyl group exhibit highly estimated binding affinities towards BZD-bs, such as 7-methoxy2-(β-naphtyl)-4*H*-1-benzopyran-4-one ($\log_{10} K_i = -3.990$), 7-methyl2-(β-naphtyl)-4*H*-1-benzopyran-4-one ($\log_{10} K_i = -2.599$), 2-(β-naphtyl)-4*H*-1-benzopyran-4-one ($\log_{10} K_i = -2.678$). These findings are in line with the structural requirements given by inequality of Eq. (4) and the data shown in Table 6, as these molecules tend to have relatively lower numerical values of $R7u^+$ and *GATS8e* descriptors, and higher numerical values of *IDDE* and *RDF120e*. On the contrary, 7-methoxyflavone is predicted to display a very low potency as 1.596, also due to the values adopted by the molecular descriptors as expressed in Eq. (4). It is logical to suspect that 7-methoxyflavone possess a low affinity, as the structurally similar 5-hydroxy-7-methoxyflavone has $\log_{10} K_i = 1.699$.

4. Conclusions

Because of their selective pharmacological profile and low intrinsic efficacy to BZRs, flavone derivatives represent an improved therapeutic tool in the treatment of anxiety and have been important leads for the development of potent and selective BZR ligands. In the present work, we established a predictive QSAR model for 78 flavonoids including only four molecular descriptors that encompass different 2D- and 3D-aspects of the chemical structure. An application of this QSAR was performed by estimating the binding affinities for some newly synthesized flavonoids displaying 2-,7-substitutions in the benzopyrane backbone, and whose experimental potency values still remain largely unknown. The main result achieved in our research was that the presence of the β-naphtyl group substituting the benzopyrane nucleus highly improve the

binding affinities towards the benzodiazepine binding site of the GABA(A) receptor complex.

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