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ScienceDirect
European Journal of Medicinal Chemistry 43 (2008) 114–121
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Original article

QSAR study on a novel series of 8-azaadenine analogues proposed as A_1 adenosine receptor antagonists

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Received 25 September 2006; accepted 13 February 2007
Available online 1 March 2007

Abstract

8-Azaadenines have been recently proposed as a novel promising class of adenosine A_1 receptor antagonists. A QSAR study on 45 derivatives, synthesized in our laboratory as antagonists for A_1 receptor, is described here. The use of the CODESSA program allowed obtaining a quite simple equation capable of correlating the structural features of these ligands to their activity toward A_1 receptor. The model was investigated for reliability and stability by using statistical analysis criteria stricter than usual. Particular care was put in defining the chemical space where the model gave reliable predictions. The model allowed the identification of relevant structural features required for the interaction with the A_1 receptor, enabling the prediction of activity of molecules belonging to focused virtual libraries.

keywords: QSAR; A_1 adenosine receptor; CODESSA; 8-azaadenine

1. Introduction

Adenosine receptors (ARs) are members of the super-family comprising 7-transmembrane domain G-protein-coupled receptor. Structural, biochemical and pharmacological analyses of the AR genes and protein have led to the discovery of four distinct AR subtypes (A1, A2a, A2b, A3).

The different receptor subtypes can be distinguished on the basis of their agonist and antagonist specificities. The subtypes differ in their influences on cell metabolism and their tissue distribution [1]. The activation of the A_1 receptors inhibits adenylcyclase, decreases the concentration of intracellular cAMP and induces opening of potassium channels, which indirectly reduces calcium penetration into the cell. The stimulation of the A_2 receptors has an opposite effect, as it activates adenylcyclase. The effects of A_3 receptor stimulation are not well characterized yet; however, it is known that their stimulation induces mast cell degranulation and release of various pro-inflammatory transmitters [2,3].

Over the years, much attention has been focused on the development of antagonists for the adenosine receptors. The methylxanthines constitute the prototypical group of antagonists and modifications to the molecule resulted in a huge selection of derivatives some of which show distinct subtype selectivity. More recently, other structures including triazoloquinazolines, triazolotriazines, dihydropyridines and adenine derivatives served as the basis for a variety of non-xanthine antagonists [4].

In these last years many 8-azaadenine analogues were proposed by us as a novel promising class of A_1 receptor antagonists. A great number of A_1 adenosine receptor ligands have been synthesized, in which the substituent groups at the 3, 6 and 9 positions of the 8-azaadenine nucleus were changed.

Abbreviations: QSAR, quantitative structure–activity relationships; TR, training set; TS, test set; cAMP, cyclic adenosine monophosphate.

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doi:10.1016/j.ejmech.2007.02.009

Some of these compounds were shown to be very effective, as their affinity (K_i values) were found in the range of nanomolar units [5–12]. In order to obtain molecules characterized by high affinity toward the A_1 receptors, it turned out that the best substituent at the 2 position is the phenyl group, the ones at the 6 position are alkyl or cycloalkyl groups and one at the 9 position is a benzyl group, which can be *ortho* substituted. In this paper, we describe a quantitative structure–activity relationship (QSAR) study that was carried out on 45 8-azaadenine derivates synthesized in our laboratory as antagonists for adenosine A_1 receptor. Synthesis and biological assays are described in a paper already submitted for publication [13].

2. Results

2.1. CODESSA calculations

The heuristic correlation [14] performed by using CODESSA program [15] on the whole dataset (Table 1) provided several optimal equations based on different numbers of descriptors, which range from 1 to 9. The maximum number of descriptors to be used was set to 9, so that the ratio between the number of descriptor exploited and the available known molecules was about 1:5, as suggested when multiple linear regression (MLR) is used for the search of correlations. MLR calculates QSAR equations by performing standard multivariable regression calculations with multiple variables in a single equation. When using multiple linear regression, it is assumed that the variables belong to an orthogonal set, which is difficult to achieve in practice; nevertheless a poor correlation between variables is the condition ensuring the achievement of powerful predictive models. In this perspective, the number of independent variables initially considered should not be higher than one-fifth the number of known compounds in the training sets [16]. A higher ratio often leads to over-correlated equations, that in turn gives rise to poorly reliable predictions.

As a first step, only models, for which the conditions $R^2(\mathrm{TR}) > 0.6$ and $q^2 > 0.5$ were satisfied, were considered for further selection. A subsequent validation step performed on the three TR–TS pairs described in Section 4.3 allowed us to discard several models from the initial ones. Finally, among these last rigorously validated models, the simplest was selected, as the most suitable model developed for predictive purposes. It consists of the five-parameter equation presented in Table 2. Calculated and experimental pK_i values for the 45 molecules are reported in Table 3 and in Fig. 1(a). In Table 3 the percentage error, [(calculated pK_i − experimental pK_i)/experimental pK_i] × 100%, is also reported.

The statistical parameters referring to the validation of the five-descriptors' model calculated for the three TR–TS pairs are reported in Table 4. In Fig. 1(b–d) calculated versus experimental pK_i values for the three pairs TR–TS are reported.

The five molecular descriptors (two topological, two electrostatic, one constitutional) involved in the selected model are: the average complementary information content (topological) [17,18] that could be considered an index of heterogeneity of a molecule; the Kier&Hall index (topological) [19] which is related to coordination numbers of atoms and to atomic connectivity; the relative number of Cl atoms (constitutional); the polarity parameter/square distance (electrostatic) [20,21] which represents the polarity parameter over the square of the distance between atoms bearing minimum and maximum partial charges; the HDCA-1/TMSA (electrostatic) is the fractional hydrogen donor charged surface area and represents the surface area multiplied by the corresponding partial charge of the compound that may act as hydrogen donor when interacting with its chemical environment [22].

Even though associating simple molecular features to most of the molecular descriptors considered in CODESSA is rather difficult, the analysis of the descriptor-based QSAR equation can give some interesting suggestions.

In particular it can be highlighted that

(i) the −Cl substituent on the phenyl group at position 9 of the adenine nucleus drastically decrease the activity of the compound.
(ii) the presence in the molecule of too many groups able to act as hydrogen bond donors in comparison with the total molecular surface area seem to decrease the activity.
(iii) the presence of polarized bonds (especially if short, as in the case of $-CF_3$ groups) seems to increase the activity of the compounds.

2.2. Chemical domain of model validity

The validity of QSAR model strictly depends upon the statistical parameters obtained from the training and the test sets, as previously highlighted, but it also depends upon the chemical domain where predictions are being carried out. Generally, QSAR models developed by exploiting very large databases, also characterized by high molecular diversity, show good predictive power over a structurally broad chemical domain. Their development yet requires that huge database of diverse chemical structures with known biological properties are available, which is quite difficult to be achieved in practice. Developing valuable QSAR models by exploiting datasets of limited size turns out to be much more feasible, on the basis of what the scientific literature makes available. More focused models may be successfully exploited provided that the virtual library, where predictions are being made, contains molecules belonging to a chemical space well defined by the training set. Hence, although models developed using a limited number of initial molecules should be handled cautiously, they may give rise to valuable predictions. Taking into account the above considerations, the domain of applicability of the models developed and described in this paper was carefully checked out, in order to ascertain which type of molecular structures could be successfully predicted by them. That is strictly related to the complex and, in some way still unsolved, problem of reliably measuring the chemical diversity/chemical similarity.

A quite simple criterion is considered here in regard. The validity chemical space is approximately defined by the range (max and min) of the values taken by each one of the

Table 1
Structure and name of the 45 dataset molecules

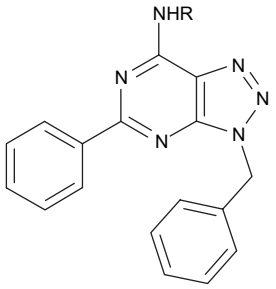
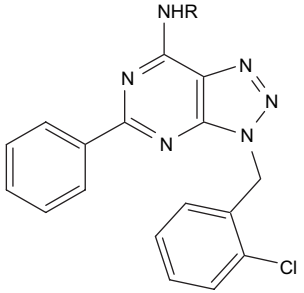
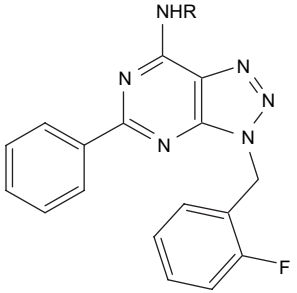
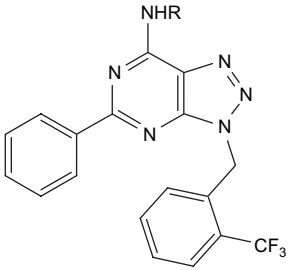
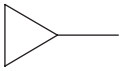
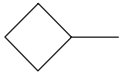
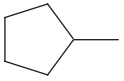
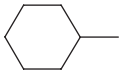
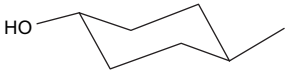
R				
$-CH_3$	A1	B1	C1	D1
$-C_2H_5$	A2	B2	C2	–
$-nC_3H_7$	A3	B3	C3	–
$-nC_4H_9$	A4	B4	C4	D4
$-C_6H_{13}$	A5	B5	C5	–
	A6	B6	C6	D6
	A7	B7	–	–
	A8	B8	C8	D8
	A9	B9	C9	D9
$-CH_2CH_2OH$	A10	B10	C10	–
$-CH_2CHOHCH_3$	A11	B11	C11	–
$-CH_2CH_2CH_2OH$	A12	B12	C12	D12
	A13	B13	C13	–
	A14	–	–	–

Table 2
The best five-parameter correlation for the full set of 45 compounds

No.	X	$\pm\Delta X$	t-Test	Descriptor
0	7.152e − 01	9.939e − 01	0.720	Intercept
1	−3.730e + 01	6.471e + 00	−5.764	Relative number of Cl atoms
2	5.118e − 01	9.590e − 02	5.337	Kier&Hall index (order 2)
3	−8.210e − 01	4.200e − 01	−1.954	Average complementary information content (order 1)
4	2.415e + 00	7.215e − 01	3.348	Polarity parameter/square distance
5	−1.324e + 02	5.467e + 01	−2.422	HA dependent HDCA-1/TMSA [Zefirov's PC]

descriptors involved in the equations defining the models themselves. In Table 5 the ranges for the five descriptors involved in the model are reported.

The developed models could be considered as good tools for a virtual library screening when the descriptor values, calculated for the molecules belonging to such a virtual library, fall into the above mentioned ranges of values calculated in the whole dataset, exploited in building the models themselves.

In order to further support the goodness of the predictive model presented here, the pK_i values of some molecules belonging to a further series of 8-azaadenine derivatives synthesized in our laboratory were predicted. These last compounds are only slightly different from the molecules of the initial dataset, as they bear an amidic group at position 6 of the adenine nucleus. At first, each molecule was checked out with regard to the chemical space defined by the model. Only molecules for which the values of the five descriptors fall in the specific range described by the model itself were considered in the prediction (Table 6).

On the basis of the above criterion only three molecules of the new series resulted to be predictable with the current model. The calculated pK_i for these molecules are reported in Table 6 together with their experimental pK_i and the percentage error.

The comparison between the experimental versus calculated pK_i values highlights the quite good predictive ability of the model presented here.

3. Conclusions

The development of QSAR models supplies a very helpful tool in drug discovery. Understanding the relationships between the structural features of a series of compounds and their biological activity allows optimizing features responsible for the expected interaction. That presents significant relevance, since QSAR models allow to quantitatively predict the biological property of newly designed compounds before their synthesis. It allows us removing undesirable molecules at early stages of their development, thus preventing waste of resources.

In this paper, QSAR models developed on a dataset of 45 8-azaadenosine derivatives proposed as antagonist of A_1 adenosine receptor is reported. The final model proposed by us was selected as the simplest (lower number of descriptors) among all the obtained models which were capable of satisfying the rigorous statistical criteria imposed for validation. It involves only five molecular descriptors.

From the analysis of the QSAR equation obtained, some features responsible for the behavior of these compounds toward bovine A_1 receptor emerged. In particular, it was highlighted that the −Cl substituent on the phenyl group at position 9 of the nucleus drastically decreases the activity of the compound.

The same effect is obtained when a molecule bears too many groups capable of acting as hydrogen bond donors in comparison with the total molecular surface area. This could suggest to keep number of −OH or aminic groups under a proper threshold.

On the other hand, the presence of polarized bonds (especially if short) seems to increase the activity of the compounds and this explains, for example, the increased antagonist behavior of our compounds belonging to the ''D'' series (D1, D4, D6, D8, D9, D12) which bear a $-CF_3$ substituent on the phenyl group at the position 9 of the nucleus.

Finally, it must be pointed out that the developed model was used as a predictive tool with highly satisfying results. As a future development the results presented here can be further improved by enlarging the initial dataset with other derivatives once they will in turn be designed, synthesized and biologically

Table 3
Experimental and calculated pK_i values for the dataset molecules provided by the best five-parameter correlation

Mol.	Exp.	Calc.	%Err.	Mol.	Exp.	Calc.	%Err.	Mol.	Exp.	Calc.	%Err.
A1	0.4522	1.0809	139.0314	B2	0.3344	0.8448	152.6316	C4	2.3665	2.0366	−13.9404
A2	1.041	1.2285	18.02113	B3	0.9957	1.1422	14.7133	C5	1.6021	2.2607	41.1148
A3	1.4318	1.4968	4.53974	B4	1.1024	1.2874	16.7906	C6	1.8239	2.1898	20.0614
A4	1.9586	1.6942	−13.5045	B5	1.5086	1.552	2.8702	C8	2.1739	2.6229	20.6541
A5	1.8794	1.7844	−5.0548	B6	1.9586	1.4079	−28.1170	C9	2.9393	2.5371	−13.6835
A6	1.9586	1.7471	−10.7985	B7	1.6198	1.8403	13.6128	C10	1.2676	1.8155	43.2234
A7	2.301	2.2083	−4.02868	B8	2.0458	1.6746	−18.1445	C11	2.3188	2.2184	−4.3298
A8	1.9586	1.9264	−1.64403	B9	1.4685	1.8873	28.5189	C12	2.4318	1.8573	−23.6245
A9	2.7959	2.0577	−26.4029	B10	0.9355	0.8452	−9.6633	C13	2.7328	2.8402	3.9300
A10	1.4202	1.3059	−8.04816	B11	1.4949	1.6495	10.3418	D1	1.8268	1.5665	−14.2544
A11	2.1249	2.0584	−3.12956	B12	1.4815	1.3031	−12.0418	D4	2.1871	2.1055	−3.7310
A12	1.6696	1.4632	−12.3563	B13	2.5086	2.2929	−8.5984	D6	2.1079	2.2295	5.7688
A13	2.5686	2.5784	0.377638	C1	1.6021	1.4966	−6.5851	D8	3.2218	2.5824	−19.8492
A14	1.9872	2.663	34.01268	C2	1.9586	1.8196	−7.0969	D9	2.1675	2.571	18.6159
B1	0.719	0.5025	−30.0974	C3	2.4948	1.9868	−20.3664	D12	0.8327	1.5176	82.2505

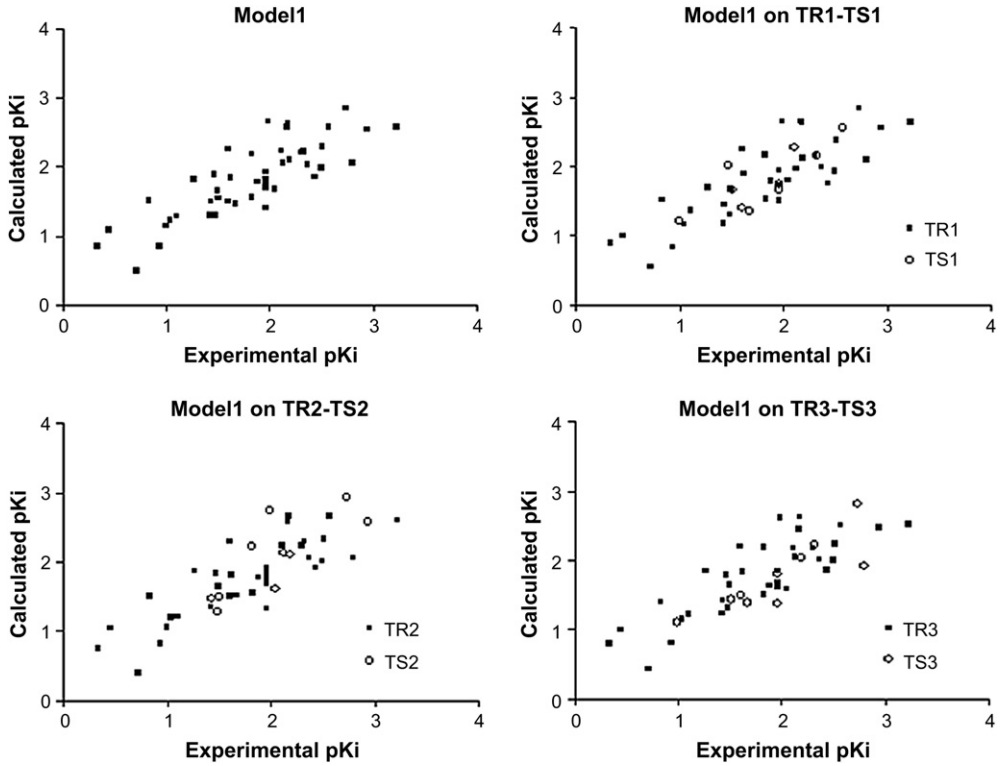


Fig. 1. Final model: (a) calculated and experimental pK_i values for the initial dataset provided by the five-parameter model. (b–d) Calculated and experimental pK_i values provided by the five-parameter model on the three TR–TS couples.

tested in our laboratory. This, consequently, will lead to an enlargement of the domain of validity of the model itself.

Drug discovery, hence, could be an interactive dynamic process in which preliminary experimental results, analyzed by computational tools, could suggest how to optimize an initial class of derivatives. In turn, the experimental results collected from the newly designed derivatives act as a feedback for the computational studies and at the same time they give an enlargement of the initial content of information. This lead to a continuous improvement of the knowledge about a specific topic.

4. Experimental protocols

4.1. Dataset collection and preliminary handling of all molecules

The overall dataset consists of 45 8-azaadenine derivates (Table 1) for which the K_i values toward bovine A_1 adenosine receptor were measured through radioligand binding assay. Synthesis and biological assays are described in Ref. [13] as mentioned above.

The molecular structures were initially built by using the InsightII program [23] and subsequently optimized by the molecular docking program DOCK5.2 [24] on the basis of the 3D structure of the receptor modeled by homology building techniques [13].

4.2. CODESSA calculations

The optimized conformers were analyzed by the CODESSA program [15] in order to calculate constitutional, topological, geometrical, and electrostatic descriptors. Constitutional descriptors are related to the number of atoms and bonds in each molecule. Topological descriptors include valence and non-valence molecular connectivity indices calculated from the hydrogen-suppressed formula of the molecule, encoding information about the size, composition, and the degree of branching of a molecule. Geometrical descriptors are calculated from 3D atomic coordinates of the molecule and comprise moments of inertia, shadow indices, molecular volumes, molecular surface areas, and gravitation indices. Electrostatic descriptors reflect characteristics of the charge distribution of

Table 4
Statistical parameters referred to the internal validation of the five-parameter model

	Overall dataset		Training set		Test set			
	R^2	q^2	R^2	q^2	R^2	R_0^2	k	Ratio
TR1–TS1	0.6812	0.5785	0.6747	0.5328	0.6530	0.6006	0.9802	0.08
TR2–TS2			0.6883	0.5581	0.6300	0.6199	1.013	0.02
TR3–TS3			0.6868	0.5575	0.7094	0.6906	0.8845	0.03

Table 5
Range of values of the five descriptor involved in the model

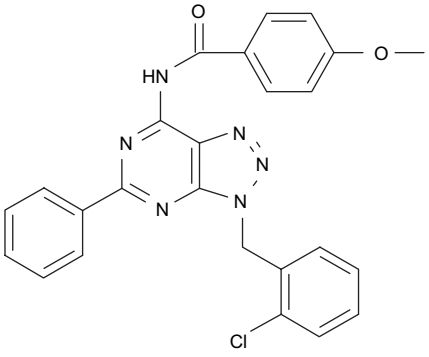
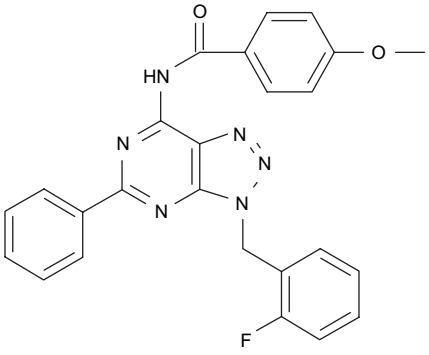
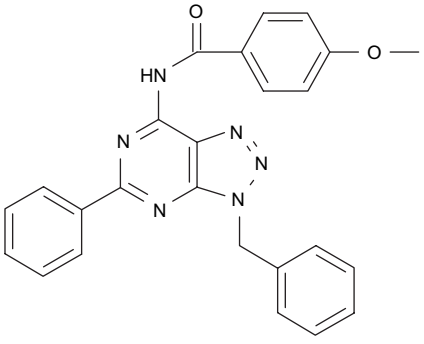
	Minimum values	Maximum values
Relative number of Cl atoms	0	0.025
Kier&Hall index (order 2)	5.4167	8.3295
Average complementary information content (order 1)	1.6356	2.5478
Polarity parameter/square distance	4.18e − 03	0.2785
HA dependent HDCA-1/TMSA [Zefirov's PC]	2.38e − 03	0.0103

the molecule. In the present work, about 200 descriptors were exploited.

For computational needs, the 'target' property values used in the QSAR analysis were obtained as a function of K_i [$\log(1/K_i) = pK_i$]. The CODESSA heuristic method [14] was applied to the whole dataset of 45 structures in order to accomplish a pre-selection step for the many available descriptors and to select the rough starting regression models. The advantages provided by the preliminary use of the heuristic method consist of a high speed (it gives correlations 2–5 times faster than other methods with comparable quality) even not requiring software restrictions on the size of the dataset. It can either quickly give a good estimate about the quality of correlation that may be expected from the dataset at hand, while giving different good regression models to be used as a starting point for further optimization. Besides, it enables us finding which descriptors cannot be calculated or when not plausible values are obtained, which descriptors are more or less significant (from the standpoint of a single-parameter correlation), and which descriptors are highly inter-correlated. This information turns out to help in optimizing the number of descriptors involved in the search for the best QSAR/QSPR model in the subsequent step of model development.

First of all, all descriptors are checked out to ensure (a) that values of each descriptor are available for each molecular

Table 6
Experimental, calculated pK_i values and error provided by the best five-parameter correlation for the compounds belonging to a new series of 8-azaadenines

Compound	Structure	pK_{iexp}	pK_{icalc}	%Error
(5)		1.70	1.65	2.94
(6)		1.66	2.12	−27.71
(7)		2.49	1.95	21.69

structure and (b) that their values do change enough within the dataset so that their contribution to the correlation is expected to be significant. Descriptors for which values cannot be calculated for each compound in the dataset are discarded as well as descriptors showing low variance among the molecules of the analyzed dataset. Thereafter, all possible one-parameter regression models are checked out so that the poorly significant descriptors are removed. In the subsequent step, the pair-correlation matrix of descriptors is calculated and the descriptor pool is further reduced by elimination of highly correlated descriptors. All two-parameter regression models with significant descriptors are subsequently obtained and ranked by the regression correlation coefficient, R^2. A stepwise addition of further descriptors is performed to find the best multi-parameter regression models with optimal values of the statistical parameters related to the validation criterion adopted (highest values of R^2, the cross-validated R^2 (q^2), and the Fisher F-criterion value).

4.3. Validation of QSAR models

An important aspect of any QSAR study is the validation of the model obtained from the analysis step. Often, a high value of q^2 (i.e. the mean of the leave-one-out cross-validated R^2 values) has been considered as a proof of the high predictive ability of the model obtained by analyzing the whole available dataset. This assumption, generally incorrect, was confuted by Golbraikh and Tropsha [25] who highlighted that, when considering a TR fully disjoined from its TS, the threshold imposed for the statistical parameters referring to the TR may be lower than usual, provided that suitable thresholds are used for a number of statistical parameters, referring to the TS, higher than usual. According to that, in addition to q^2 (that must be >0.5), other significant statistical parameters were taken into account in building the predictive models of interest. The construction of the models was carried out through different steps and each step implied performing different experiments. Different subsets, taken from the whole database were exploited as training sets (TRs), as described in more detail later. The validation steps were than carried out on the relevant test sets (TSs) taking into account the above mentioned strict conditions, which had to be simultaneously satisfied. Such conditions are listed below:

TR: $R^2 > 0.6$, $q^2 > 0.5$,
TS: $R^2 > 0.6$, $0.85 < k < 1.15$, $(R^2 - R_0^2)/R^2 < 0.1$

where R^2 is the correlation coefficient of the regression line between the calculated versus experimental pK_i, R_0^2 is the correlation coefficient of the same regression line forced through the origin and k is the slope of this line. The values imposed for these criteria impose that the regression line correlating the x and y values (experimental versus predicted pK_is) should be as close as possible to the bisector of the axes as in the ideal QSAR model.

Going into more details, some preliminary experiments were carried out on the whole initial dataset, in order to find several starting molecular descriptors. The first treatment of the whole dataset implied calculating just R^2 and q^2 (from leave-one-out cross-validation). Subsequent experiments were carried out by splitting the parent dataset into three couples of training and test sets, referred as TR1–TS1, TR2–TS2, TR3–TS3, so that three independent models could be obtained, in order to ascertain their stability. The criterion followed in splitting the whole dataset into the above three TR*i*/TS*i* pairs consisted of ordering all the molecules according to the decreasing values of their target property. Several contiguous sub-groups were selected and molecules were randomly picked up from them. By this way the TR*i*/TS*i* pairs were filled up with different elements (molecular structures and relevant target property) so that maximum coverage of the available chemical space and maximum variability of the target property within each one of the sub-sets was ensured. For what concerns the composition of each TS, particular care was put in selecting molecules that could be comprised in the chemical space described by the corresponding TR. The chemical space, where each model is expected to be valid, was defined by considering the range between the minimum and maximum values of the descriptors found in the training equations.

For each training set (TR_i), the correlation equation was derived by using the same descriptors involved in the initial models obtained from the preliminary step where the entire dataset had been analyzed (whole model). Each one of the equations obtained was then used for predicting the pK_i values of compounds belonging to the corresponding test set (TS_i). The predictive power of each model was quantitatively assessed by verifying that the imposed validation criteria were simultaneously satisfied, when comparing calculated and experimental target property values.

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