Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 13 (2005) 5330–5337
Bioorganic & Medicinal Chemistry

QSAR study on thiazole and thiadiazole analogues as antagonists for the adenosine A_1 and A_3 receptors

Alice Borghini,[a] Daniele Pietra,[b] Patrick Domenichelli[a] and Anna Maria Bianucci[a,*]

[a]*Dipartimento di Scienze Farmaceutiche, Università di Pisa, Via Bonanno 6, 56126 Pisa, Italy*
[b]*Dipartimento di Psichiatria, Neurobiologia, Farmacologia e Biotecnologie, Università di Pisa, Via Bonanno 6, 56126 Pisa, Italy*

Received 3 March 2005; revised 18 May 2005; accepted 24 May 2005
Available online 28 June 2005

Abstract—Thiazole and thiadiazole analogues have been recently proposed as a novel promising class of adenosine A_1 and A_3 receptor antagonists. When appropriately modified, they show selectivity toward A_1 or A_3 receptors, which results in a variety of therapeutic potentialities of these ligands. In this work, we carried out a QSAR study on thiazole and thiadiazole analogues as antagonists for adenosine A_1 and A_3 receptors. To develop reliable models, we focused attention on any possible pitfalls of each step of QSAR process and approached each stage following accurate procedures. Application of datasets by using CODESSA software led to QSAR equations based on three and four descriptors for the adenosine A_1 and A_3 receptor ligands, respectively. The obtained models allowed us to understand the main structural features that strongly correlate with the target property.

1. Introduction

All adenosine receptors belong to the G-protein coupled receptor (GPCR) family. Classification of adenosine receptors is based on different sensitivities for agonist and antagonist compounds. So far, four subtypes of adenosine receptors have become known, namely A_1, A_{2A}, A_{2B}, and A_3.[1] Over the years, much attention has been focused on the development of ligands for the adenosine receptors, since they show a variety of therapeutic potentialities. The implications of adenosine receptors and their ligands in several potential therapeutic areas have been recently reviewed.[2] In particular, adenosine A_1 receptor antagonists appear to be involved in pain, kidney disorders, such as chronic renal failure, and metabolic disorders, such as obesity. Adenosine A_3 receptor antagonists appear to play a role in inflammatory pain, glaucoma, cerebral ischemia, and asthma.[2,3] Several classes of adenosine receptor antagonists have become known. In recent years, thiazole and thiadiazole analogues have been proposed as a novel promising class of A_1 and A_3 receptor antagonists. In fact, they can be appropriately modified to improve selectivity toward adenosine A_1[4,5] or A_3[6] receptors. In this paper, we describe a quantitative structure–activity relationship (QSAR) study that was carried out on thiazole and thiadiazole analogues as antagonists for adenosine A_1 and A_3 receptors.

2. Materials and methods

2.1. Statistical parameters

In the next section, statistical parameters used during the development and validation of QSAR models will be discussed, which are listed below. R^2 is the correlation coefficient, calculated for both the training (TR) and *test* (TS) *sets*; q^2 is the leave-one-out cross-validated R^2; F and s^2 are the F value and the standard deviation of the regression, respectively.

2.2. Dataset preparation

As recently suggested by Cronin and Shultz,[7] highly homogeneous biological data are required to develop a QSAR model with good predictive capability. High-quality biological data are ideally measured by the same protocol, within the same laboratory and by the same operator. We built our datasets by using data that were collected from the literature, but all data originated from the same author. An additional problem that any

Keywords: QSAR; Model validation; Adenosine receptors; Thiazole and thiadiazole analogues.
* Corresponding author. Tel.: +39 050 2219544; fax: +39 050 2219605; e-mail: bianucci@farm.unipi.it

0968-0896/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2005.05.041

analyst of structure–activity relationship may face lies in the need for knowing (or plausibly hypothesizing) the pharmacophoric conformations of the analyzed molecules. Ionization states and tautomeric equilibria are related to the pH of an experimental assay; it also adds to the difficulty in selecting proper conformations. Undertaking some assumptions is often mandatory, which will be supported after the model validation. In our case, we considered the amide tautomeric forms as the more probable ones. The initial molecular geometries were drawn on the basis of indications reported in the literature.[4,6] They were then optimized the protocol described below. Three compounds for each dataset (the most active compound, the less active one, and a molecule with an intermediate activity value) were submitted to Molecular Dynamics simulations (MD) to collect quite a large ensemble of structures: the conformations characterized by lower energy values subsequently underwent energy minimization, according to a commonly followed protocol. For each molecule, the best conformer was so selected as a more stable one. The superimposition of these structures in their optimal conformations showed a common 'template.' All the remaining molecules were then built on this conformation and subjected, in turn, to energy minimization, to fully relax them. Computational details are described below.

All the initial geometries were built by using the Insight molecular modeling program (Accelrys Inc., San Diego, CA), then the molecules were solvated with a layer of 5 Å, and the pH was fixed to a physiological value of 7.4. The structures initially selected for accurate geometry optimization were first energy minimized to prepare them for MD simulations. All the energy minimizations were carried out as per a Molecular Mechanics (MM) approach implemented in the Discover program (Accelrys Inc., San Diego, CA), which also implements the MD simulations algorithm used in this work. The cff91 force field was selected and a distance-dependent dielectric function ($\varepsilon = 1r$) was used both for MM and MD calculations. Molecular Dynamics simulations were carried out on all the molecules selected for accurate geometry optimization. Each system was initially heated to 300 K, equilibration being reached after 5 ps simulation. Structures were collected after a 50 ps simulation. The collected structures showing lower energy values were then further relaxed by a second step of energy minimization that was carried out with 1000 steps of steepest descent, followed by conjugated gradient until potential energy rms <0.05 kcal/mol. All the calculations were run on Silicon Graphics (SGI) R10000 197 MHz.

All the affinity data about the thiazole and thiadiazole derivatives of interest were collected from the literature (see individual QSAR for respective references). The pK_i, arising from the logarithmic transformation of K_i values, obtained from radio-ligand binding data on adenosine A_1 and A_3 receptors, were, respectively, chosen as target property.

We split each dataset into a training set (TR) and a test set (TS) using a stratified random selection method. TS included at least five compounds, whose activities reflected the whole range of activities of the TR, to get the TS as much representative as possible of the whole dataset.[8]

2.3. Data analysis and QSAR equation development

All the minimized structures, as well as their pK_i values, were processed by the CODESSA program,[9] which automatically calculated a number of molecular descriptors for each molecule. In CODESSA, the molecular descriptors are grouped into different families: constitutional, topological, geometrical, and electrostatic types being included among them. Constitutional and topological descriptors require only 2D molecular structure information. Geometrical descriptors, instead, are based on 3D coordinates, and electrostatic ones reflect charge distribution of the molecules. At this level, plausible molecular conformations were required. The protocol we used to select the best descriptors and models is summarized below.

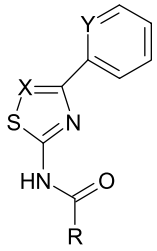
1. Each one of the TR sets (both for adenosine A_1 and A_3 receptors) was split into several couples of subclasses (X_1 and Y_1, X_2 and Y_2, …, X_n and Y_n) so that X as training sets and Y as test sets. In general, many criteria could be adopted for the generation of X and Y subclasses. In our cases, five couples of X/Y were generated, where every X contained 2/3 of the compounds belonging to the initial TR and every Y included 1/3 of the compounds chosen with a stratified random selection.
2. For each X subclass, correlation equations with 1, 2, …, ND_{max} descriptors (maximum number of descriptors) were calculated using the heuristic correlation, which is automatically accomplished by the program. The heuristic pre-selection method, accurately described in the CODESSA reference manual, is based on a stepwise procedure which, first of all, discards all descriptors with missing values or with a poor degree of variation between different structures. A further exclusion of descriptors highly inter-correlated is also carried out. At this level, the use of some control parameters for the heuristic pre-selection of descriptors is required to avoid over-correlation. A QSAR model is valid and stable when the ratio of the number of observations (compounds) and number of variables (descriptors) is at least 5:1.[7] Moreover, we set the control parameters a little lower than those taken by default by the program, to decrease further the acceptable inter-correlation allowed between descriptors. Maximum number of descriptors, $ND_{max} = 5$; one-parameter significance criteria, $R^2_{min} = 0.01$; high inter-correlation level, $r_{full} = 0.90$; significant intercorrelation level, $r_{sig} = 0.70$; one-parameter t test for significance, $t_1 = 0.1$; multi-parameter t test for significance, $t_2 = 3$, branching criteria, $NS = 3$. Based on the pre-selected descriptors, the program provides several basic correlation equations. The remaining descriptors are then added by the program to provide the best correlation equations.
3. Descriptors involved in the best models that resulted for each subclass X were applied to the other subclasses X by means of a multilinear regression (MLR) treatment, to identify all the diagnostic descriptors.

4. All the correlation equations so obtained for every subclass X were used, in turn, to estimate pK_i values of the respective subclass Y.
5. All the best QSAR equations developed for all the couples X/Y and involving 1, 2, ..., ND_{max} descriptors were selected on the basis of the q^2 calculated from X and R^2 calculated from Y.
6. Descriptors included in the equations selected as the best ones were applied to the whole TR by means of a multilinear regression. This resulted in a set of correlation equations, based on 1, 2, ..., ND_{max} descriptors, which were used for the prediction of the TS.
7. Several QSAR models developed for the class of thiazole and thiadiazole analogues on both adenosine A_1 and A_3 receptors turned out to be validated according to the above-mentioned criteria. Each equation contained a different number of descriptors. In general, every additional descriptor improves the statistical quality of a model, besides also increasing its complexity. In this work, the selection of the best model was performed, by considering the variations in both q^2 and R^2 of the TS as the number of involved descriptors increased.[10] The point beyond which no significant improvements could be observed is so identified; the corresponding model is selected as the best one.

2.4. QSAR model validation

As reported by Golbraikh and Tropsha,[8] one of the most-used criteria for an internal estimate of the predictive ability of a QSAR model is q^2. Nevertheless, these authors have pointed out that a high value of q^2 is not

Table 1. The structural features of thiazole and thiadiazole analogues, their affinity values at the adenosine A_1 receptors, and their splitting into TR and TS



Compound	X	Y	R	pK_i	TR/TS
1 (LUF5433)	CH	CH	4-$OCH_3C_6H_4$	7.119186	TS
2 (LUF5417)	N	CH	4-$OCH_3C_6H_4$	7.49485	TR
3	CH	N	C_6H_5	5.769551	TR
4	CH	N	4-ClC_6H_4	6.69897	TR
5	CH	N	4-IC_6H_4	5.619789	TR
6	CH	N	4-$CH_3C_6H_4$	5.79588	TR
7	CH	N	4-$OCH_3C_6H_4$	5.49485	TS
8	CH	N	3,4-diClC_6H_3	5.79588	TS
9	CH	N	3-ClC_6H_4	5.769551	TR
10	CH	N	4-$NO_2C_6H_4$	5.823909	TR
11	CH	N	4-$OCH(CH_3)_2C_6H_4$	5.236572	TR
12	CH	N	Cyclopentyl	6.036212	TR
13	N	CH	C_6H_5	7.508638	TR
14	N	CH	4-ClC_6H_4	7.387216	TS
15	N	CH	4-$CH_3C_6H_4$	7.522879	TR
16	N	CH	4-OHC_6H_4	8.136677	TR
17	N	CH	4-$OCH_2CO_2HC_6H_4$	7	TR
18	N	CH	Cyclohexyl	5.853872	TR
19	N	CH	(*trans*) 4-OCH_3-cyclohexyl	7.49485	TR
20	N	CH	(*cis*) 4-OCH_3-cyclohexyl	6.958607	TR
21	N	CH	(*trans*) 4-OH-cyclohexyl	7.69897	TS
22	N	CH	(*cis*) 4-OH-cyclohexyl	7.376751	TR
23	N	CH	NHC_6H_5	6	TR
24	CH	N	NHC_6H_5	6.031517	TS
25	CH	CH	C_6H_5	7.408935	TR
26	CH	CH	3-ClC_6H_4	7.065502	TR
27	CH	CH	4-BrC_6H_4	7.481486	TS
28	CH	CH	4-ClC_6H_4	7.744727	TR
29	CH	CH	4-$NO_2C_6H_4$	7.657577	TR
30	CH	CH	4-$CH_3C_6H_4$	7.443697	TR
31	CH	CH	4-$C(CH_3)_3C_6H_4$	5.866461	TR
32	CH	CH	4-$CF_3C_6H_4$	6.782516	TR
33	CH	CH	3,4-diClC_6H_3	7.229148	TR
34	CH	CH	2,4-diClC_6H_3	7.236572	TR

Table 2. Comparison between experimental and calculated pK_i values at the adenosine A_1 receptor, and evaluation of the minimum, means, and max error for TR

Compound	Experimental pK_i	Calculated pK_i	Error
2 (LUF5417)	7.49485	7.4996	0.0047
3	5.769551	5.6833	−0.0862
4	6.69897	6.3246	−0.3743
5	5.619789	6.1965	0.5767
6	5.79588	5.9439	0.1481
9	5.769551	5.5798	−0.1897
10	5.823909	5.9993	0.1754
11	5.236572	5.5393	0.3028
12	6.036212	5.4739	−0.5623
13	7.508638	7.471	−0.0377
15	7.522879	7.3915	−0.1314
16	8.136677	8.2797	0.1431
17	7	7.1159	0.1159
18	5.853872	6.7945	0.9406
19	7.49485	6.7724	−0.7225
20	6.958607	6.8088	−0.1498
22	7.376751	7.148	−0.2288
23	6	5.9648	−0.0352
25	7.408935	7.5174	0.1084
26	7.065502	7.3067	0.2412
28	7.744727	7.5243	−0.2204
29	7.657577	7.1086	−0.5489
30	7.443697	7.3246	−0.1191
31	5.866461	6.5061	0.6396
32	6.782516	6.7828	0.0002
33	7.229148	7.3023	0.0732
34	7.236572	7.173	−0.0636

Minimum absolute error, 0.0002; mean absolute error, 0.2570; and maximum absolute error, 0.9406.

Table 3. Comparison between experimental and calculated pK_i values at the adenosine A_1 receptor and evaluation of the min, means, and max error for TS

Compound	Experimental pK_i	Calculated pK_i	Error
1 (LUF5433)	7.119186	6.8936	−0.2256
7	5.49485	5.9575	0.4626
8	5.79588	6.1499	0.3540
14	7.387216	7.9052	0.5180
21	7.69897	7.2719	−0.4270
24	6.031517	5.6649	−0.3666
27	7.481486	7.1797	−0.3018

Minimum absolute error, 0.2256; mean absolute error, 0.3749; and maximum absolute error, 0.5180.

sufficient for assessing a highly predictive power of the model. To accurately estimate the predictive ability of the model, further conditions over the validation parameters of the test set are required. We performed a statistical analysis for validating our models, as per to their suggestions. First of all, QSAR models were submitted to the so-called 'internal validation' (check the R^2 and q^2 over the TR). Only models with $q^2 > 0.5$ were selected for the so-called 'external validation' (check the R^2 and other parameters over the TS). We briefly analyzed such additive validation parameters. In the plot of experimental versus predicted activities, the obtained regression line ($y = ax + b$) is characterized by a correlation coefficient R^2. In an ideal QSAR model, the slope of the line (a) is 1, while the intercept with y axis (b) is 0 and R^2 (varying between 0 and 1) is 1. R^2 calculated on the TS must be $\geqslant 0.6$. When the regression line is forced to pass through the origin of axes (intercept set to 0), its slope (k) should be as close as possible to 1, and close to the slope of the actual regression line as well. $0.85 \leqslant k \leqslant 1.15$ is suggested to be in an acceptable range. Finally, the correlation coefficient R_0^2 of the regression line forced through the origin should be as close as possible to the value of R^2, so that $(R^2 - R_0^2)/R^2 < 0.1$. In conclusion, we adopted as validation criterion for our QSAR model the one stating that all the following conditions are simultaneously satisfied: $q^2 > 0.5$, $R^2 > 0.6$, $0.85 \leqslant k \leqslant 1.15$, and $(R^2 - R_0^2)/R^2 < 0.1$.

2.5. Chemical domain of model validity

The predictive ability of the model is usually tested by checking it over an external test set. But how chemically different can be the external set from the dataset which the model has been developed on? This point is still under discussion, and several authors face it in different ways, for example, by developing special algorithms,[11] or by a comparison of performances using prediction sets selected in different surroundings of a chemical space.[12] We assume, of course, that the chemical space where our models do have predictive ability is comprised in the same chemical class of thiazole and thiadiazole analogues examined. Moreover, we introduced a further restriction by analyzing the *descriptor space* defined by descriptors involved in the

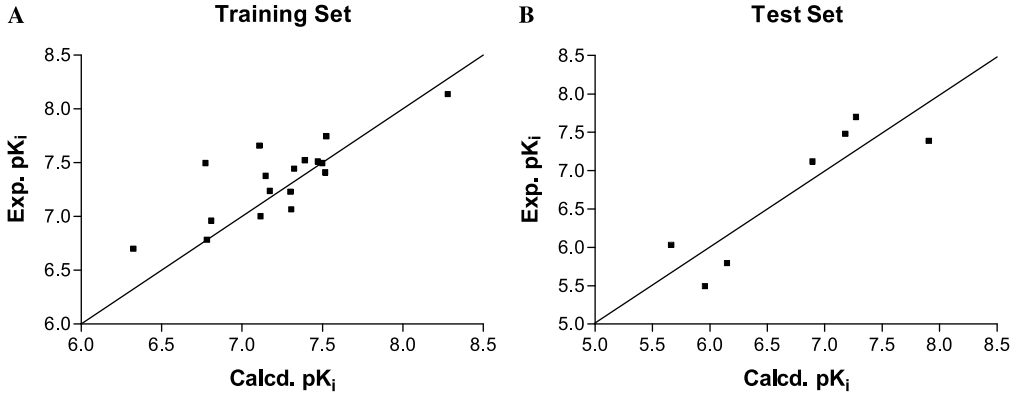


Figure 1. Experimental versus calculated pK_i values based on the three-descriptor correlation equation for the adenosine A_1 receptor, concerning TR (A) and TS (B).

Table 4. Minimum and maximum contribution coefficients of the three descriptors involved in the correlation equation for the adenosine A_1 receptor

	MPCN	ZXS/ZXR	PP/D^2
Min	−9.23E−02	5.60E−01	5.90E−03
Max	−8.14E−02	8.33E−01	2.69E−01

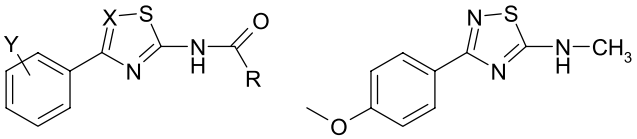
models. For each descriptor involved in every QSAR equation, the range between minimum and maximum value of the relevant contribution coefficient was considered. The intersection of all the ranges for each one of the involved descriptors gave the descriptor space of that equation. When those descriptors are calculated for the molecules whose biological properties have to be predicted, the contribution coefficients of all descriptors involved in the model must fall into the related range; if not, the corresponding compound has to be discarded as an outlier. Inside this domain of applicability, the model possesses an interpolation ability that is rigorously validated, while outside this domain it may have an extrapolation ability, whose goodness is not supported by statistics.

3. Results and discussion

3.1. QSAR study of adenosine A_1 receptor ligands

In this work, a dataset of 34 thiazole and thiadiazole analogues was analyzed. Their structures and affinity values at the rat adenosine A_1 receptor were collected from Refs. 4,5. The above dataset was split into a TR, including 27 compounds, and a TS, including seven compounds (Table 1). On the basis of the TR, several QSAR equations were built, each one containing a different number of descriptors. The model selected as the best one included the smallest number of descriptors together with the largest values of q^2 and R^2. Based on these criteria, the following equation was derived, based on three descriptors:

Table 5. The structural features of thiazole and thiadiazole analogues, their affinity values at the adenosine A_3 receptors, and their splitting into TR and TS



(2, 35-59, 61-65) **(60)**

Compound	X	Y	R	pK_i	TR/TS
2 (LUF5417)	N	H	4-$OCH_3C_6H_4$	7.086186	TR
35	CH	H	CH_3	7.737549	TR
36	CH	H	$(CH_3)_3CO$	5.29243	TR
37	CH	H	$NCCH_2$	6.69037	TR
38	CH	4-Cl	CH_3	7.293282	TR
39	CH	4-Cl	$C_6H_5CH_2$	7	TS
40	CH	4-CH_3O	CH_3	8.522879	TR
41	CH	3-CH_3O	CH_3	8.387216	TR
42	CH	2-CH_3O	CH_3	7.086186	TR
43	CH	4-CH_3O	CF_3	6.275724	TR
44	CH	4-CH_3O	CH_3CH_2	8.619789	TR
45	CH	4-CH_3O	$CH_3CH_2CH_2$	8.107905	TR
46	CH	4-CH_3O	$(CH_3)_2CH$	7.787812	TR
47	CH	4-CH_3O	$NCCH_2$	7.614394	TR
48	CH	4-CH_3O	$(CH_3)_3C$	7.496209	TR
49	CH	4-CH_3O	$(CH_3)_3O$	5.486782	TS
50	CH	4-CH_3O	C_6H_5	7.542118	TS
51	CH	4-CH_3O	$C_6H_5CH_2$	7.847712	TS
52	CH	4-CH_3O	$C_6H_5CH_2CH_2$	7.536107	TR
53	CH	4-CH_3O	*p*-$CH_3OC_6H_4CH_2$	5.935542	TR
54	CH	4-CH_3O	*p*-$CH_3OC_6H_4CH_2CH_2$	7.543634	TR
55	CH	4-CH_3O	$(C_6H_5)_2CH$	6.279014	TR
56	CH	4-CH_3O	$(C_6H_5)_2CHCH_2$	6.39794	TR
57	CH	4-CH_3O	2-Furan	7.501689	TR
58	CH	4-CH_3O	Thiophene-2-CH_2	7.490797	TS
59	CH	4-CH_3O	2-Thiophene	7.159267	TR
60				6.428291	TR
61	N	H	CH_3	8.638272	TS
62	N	H	$C_6H_5CH_2$	7.102373	TR
63	N	4-CH_3O	CH_3	9.102373	TR
64	N	4-CH_3O	$C_6H_5CH_2$	7.623423	TR
65	N	4-CH_3O	CH_3CH_2	8.946922	TR

$$pK_i = 17.28(\pm 1.60) + 155.1(\pm 17.38)\text{MPCN} + 3.86(\pm 0.99)\text{ZXS/ZXR} + 2.12(\pm 1.11)\text{PP}/D^2.$$

Internal validation (over the *training set*) provided the following statistics: $R^2 = 0.8122$, $F = 33.15$, $s^2 = 0.1463$, $q^2 = 0.7418$. External validation (over the test set) provided the following statistics: $R^2 = 0.7856$, $k = 0.9996$, $R_0^2 = 0.7855$, $(R^2 - R_0^2)/R^2 = 0.0001273$. The differences between experimental and calculated biological data are reported for both TR (Table 2) and TS (Table 3). Regression lines obtained by plotting experimental versus predicted pK_i values and related statistical data for TR and TS are shown in Figure 1. The applicability domain of the QSAR model to the adenosine A_1 receptor ligands, considered as an intersection of the contribution coefficients of descriptors involved in the correlation equation, is reported in Table 4. The importance of each descriptor was evaluated by looking at the t test value. The first descriptor included in the equation, minimum partial charge on a N atom (MPCN) ($t = 8.9251$), is comprised, within the CODESSA background, of the electrostatic descriptors empirically calculated. It has a positive sign in the QSAR equation: an increasing contribution coefficient corresponds to an increase in pK_i. It reflects the importance of the second N atom on the central ring and accounts for an observed trend in affinity for the adenosine A_1 receptor: thiadiazoles > thiazoles > thiazolopyridines (Y = N). The presence of a substituent that is able to increase the partial charge, such as an electron-donor group at the position R, is so suggested. The second descriptor, ZX Shadow/ZX Rectangle (ZXS/ZXR) ($t = 3.9037$), is comprised, within the CODESSA background, among the so-called geometrical ones. It suggests that an aromatic group R, such as a phenyl ring, eventually bearing small substituents, should be preferred to aliphatic cycles. Its contribution coefficient is increased with the size of the substituent R, while the nature of the central ring does slightly affect it. Finally, the third descriptor, polarity parameter/square distance (PP/D^2) ($t = 1.9023$), also of electrostatic type, supports the importance of an electron-donor group substituent at position R. It is only affected by the nature of the R substituent; when R is an electron-donor group, this descriptor contributes with a higher coefficient. The results of this QSAR study are in agreement with the classical structure–activity relationships reported by IJzerman and co-workers.[4,5]

3.2. QSAR study of adenosine A_3 receptor ligands

Thirty-two thiazole and thiadiazole analogues and their affinity values at human adenosine A_3 receptor were collected from Ref. 6. The whole dataset was split into a TR made up of 26 compounds and a TS made up of 6 compounds (Table 5). Based on the TR, several QSAR equations were constructed, each one containing a different number of descriptors. The model including the smallest number of descriptors but leading to the largest increase in q^2 and R^2 was selected as the best one. The following equation was derived, based on four descriptors:

$$pK_i = -6.17(\pm 2.47) + 17296(\pm 260)\text{HACA-2/TMSA} + 22.73(\pm 4.44)\text{RNH} + 46.56(\pm 10.83)\text{MPCS} - 0.382(\pm 0.00935)\text{WNSA-3}$$

Internal validation provided the following statistics: $R^2 = 0.6996$, $F = 12.23$, $s^2 = 0.3121$, $q^2 = 0.5478$. External validation provided the following statistics: $R^2 = 0.6755$, $k = 1.061$, $R_0^2 = 0.6719$, $(R^2 - R_0^2)/R^2 = 0.005329$. The differences between experimental and calculated biological data are reported for both TR (Table 6) and TS (Table 7). Regression lines obtained by plotting experimental versus predicted pK_i values and related statistical data for TR and TS are shown in Figure 2. The applicability domain of the QSAR model to the

Table 6. Comparison between experimental and calculated pK_i values at the adenosine A_3 receptor, and evaluation of the minimum, means, and max error for TR

Compound	Experimental pK_i	Calculated pK_i	Error
2 (LUF5417)	7.0862	7.3135	0.2273
35	7.7375	7.6463	−0.0913
36	5.2924	5.59	0.2976
37	6.6904	7.881	1.1906
38	7.2933	7.3082	0.0149
40	8.5229	7.8778	−0.6451
41	8.3872	7.8858	−0.5014
42	7.0862	7.4747	0.3886
43	6.2757	5.9299	−0.3459
44	8.6198	8.0885	−0.5313
45	8.1079	8.0029	−0.1050
46	7.7878	7.4277	−0.3601
47	7.6144	8.1726	0.5582
48	7.4962	7.6177	0.1215
52	7.5361	7.3555	−0.1807
53	5.9355	7.2413	1.3057
54	7.5436	7.8876	0.3439
55	6.279	6.1321	−0.1469
56	6.3979	6.5775	0.1795
57	7.5017	6.6302	−0.8715
59	7.1593	6.6667	−0.4926
60	6.4283	6.8145	0.3862
62	7.1024	6.9922	−0.1101
63	9.1024	8.781	−0.3214
64	7.6234	7.6632	0.0398
65	8.9469	8.5963	−0.3506

Minimum absolute error, 0.0149; mean absolute error, 0.3888; and maximum absolute error, 1.3057.

Table 7. Comparison between experimental and calculated pK_i values at the adenosine A_3 receptor and evaluation of the min, means, and max error for TS

Compound	Experimental pK_i	Calculated pK_i	Error
39	7	6.5455	−0.4545
49	5.4868	6.0622	0.5754
50	7.5421	6.8329	−0.7092
51	7.8477	6.9584	−0.8893
58	7.4908	6.4519	−1.0389
61	8.6383	8.5722	−0.0661

Minimum absolute error, 0.0661; mean absolute error, 0.6222; and maximum absolute error, 1.0389.

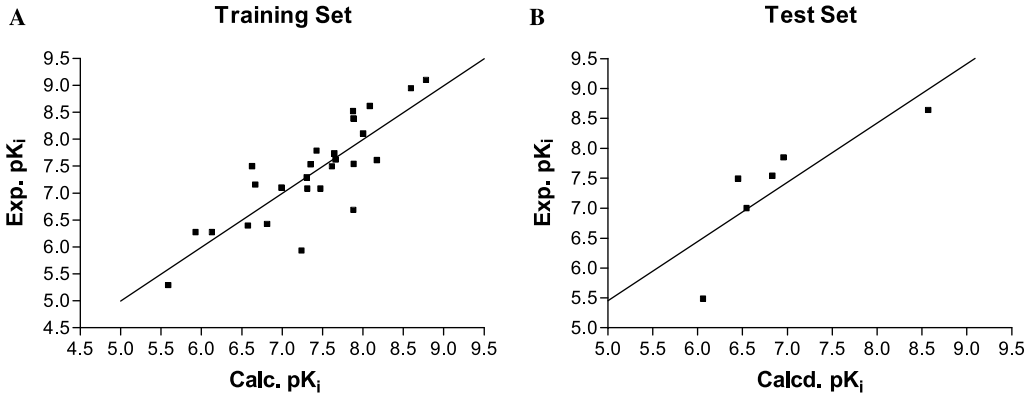


Figure 2. Experimental versus calculated pK_i values based on the four-descriptor correlation equation for the adenosine A_3 receptor, concerning TR (A) and TS (B).

Table 8. Minimum and maximum contribution coefficients of the three descriptors involved in the correlation equation for the adenosine A_3receptor

	HACA-2/TMSA	RNH	MPCS	WNSA-3
Min	1.00E−03	3.10E−01	−4.17E−04	−1.04E+01
Max	3.49E−03	4.74E−01	−4.22E−02	−2.83E+00

adenosine A_3 receptor ligands is given in Table 8. The importance of each descriptor was evaluated by looking at the *t* test value. The first descriptor included in the equation is HACA-2/TMSA ($t = 6.6464$), where HACA-2 means *total charge weighted HACA*, HACA means hydrogen-acceptor charged surface area, and TMSA means total molecular surface area. This descriptor is not affected by any particular moiety in the molecule, it reflects, instead, the behavior of the whole molecule. It is an electrostatic descriptor, which emphasizes that the ligand should interact with the adenosine A_3 receptor by means of H-bonds, particularly it should be able to accept H-bonds from the receptor. The second descriptor, the constitutional descriptor relative number of H atoms (RNH) ($t = 5.1102$), suggests the importance of small alkyl groups at the position R, since they increase the contribution coefficient of this descriptor more than aromatic rings. It is only affected by the amide substituent since it shows the highest variability. The minimum partial charge on a S atom (MPCS) is the third descriptor ($t = 4.2999$) and belongs to the electrostatic family of descriptors. It is strongly affected by the nature of the central ring, and it is possible to establish a well-defined threshold value between thiazoles and thiadiazoles. The presence of a second N atom in the ring, near the S atom, probably affects its partial charge, because of its electronegativity. This descriptor suggests that thiadiazole should be preferred to thiazole derivatives. WNSA-3 is the fourth descriptor ($t = -3.3122$), where WNSA-3 means *total surface weighted PNSA* and PNSA means partial negative surface area. This electrostatic descriptor reflects both the negative charge and the total molecular surface properties. It is characterized by a negative sign in the QSAR equation and shows the importance of small molecule endowed with only a weak negative charge. The obtained results are in agreement with the structure-based studies on ligand–receptor interactions recently described by Jacobson and co-workers.[6]

4. Conclusions

We focused our attention on accurately affording each step that gives rise to the development of a QSAR model. Our aim was to increase the reliability of QSAR models for thiazole and thiadiazole derivatives under analysis, acting as antagonists for the adenosine A_1 and A_3 receptors. The analysis of our results shows that the obtained models are in agreement with previous structure–activity relationships and structure-based studies described in the literature. Moreover, the results appear to furnish useful suggestions for the design of new ligands acting as antagonists for the adenosine A_1 and/or A_3 receptors. More in detail, we expect that a thiadiazole derivative with an aromatic substituent may enhance the affinity for the adenosine A_1 receptor, especially when the aromatic ring brings an electron-donor group. On the contrary, a small thiadiazole analogue, able to accept hydrogen bonds and bearing a small, non-polar, alkyl substituent at the position R, should possess a high affinity for the adenosine A_3 receptor.

Acknowledgment

This work was supported by the Italian Ministry of Instruction, University and Research (MIUR).

References and notes

1. Fredholm, B. B.; IJzerman, A. P.; Jacobson, K. A.; Klotz, K.-N.; Linden, J. *Pharmacol. Rev.* **2001**, *53*, 527.
2. Fredholm, B. *Drug News Perspect.* **2003**, *16*, 283.
3. Müller, C. E. *Curr. Top. Med. Chem.* **2003**, *3*, 445.
4. van Muijlwijk-Koezen, J. E.; Timmerman, H.; Vollinga, R. C.; Frijtag von Drabbe Künzel, J.; de Groote, M.; Visser, S.; IJzerman, A. P. *J. Med. Chem.* **2001**, *44*, 739.

5. van Tilburg, E. W.; van der Klein, P. A. M.; de Groote, M.; Beukers, M. W.; IJzerman, A. P. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2017.
6. Jung, K.-Y.; Kim, S.-K.; Gao, Z.-G.; Gross, A. S.; Melman, N.; Jacobson, K. A.; Kim, Y.-C. *Bioorg. Med. Chem.* **2004**, *12*, 613.
7. Cronin, M. T. D.; Schultz, T. W. *THEOCHEM* **2003**, *622*, 39.
8. Golbraikh, A.; Tropsha, A. *J. Mol. Graphics Modell.* **2002**, *20*, 269.
9. http://www.codessa-pro.com.
10. Katritzky, A. R.; Fara, D. C.; Karelson, M. *Bioorg. Med. Chem.* **2004**, *12*, 3027.
11. Golbraikh, A.; Tropsha, A. *Mol. Divers.* **2000**, *5*, 231.
12. Szántai-Kis, C.; Kövesdi, I.; Kéri, G.; Örfi, L. *Mol. Divers.* **2003**, *7*, 37.