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Kinetic, thermodynamic and statistical studies on the inhibition of adenosine deaminase by aspirin and diclofenac

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Abstract

The kinetic and thermodynamic effects of aspirin and diclofenac on the activity of adenosine deaminase (ADA) were studied in 50 mM phosphate buffer pH = 7.5 at 27 and 37°C, using UV-Vis spectrophotometry and isothermal titration calorimetry (ITC). Aspirin exhibits competitive inhibition at 27 and 37°C and the inhibition constants are 42.8 and 96.8 μM respectively, using spectrophotometry. Diclofenac shows competitive behavior at 27°C and uncompetitive at 37°C with inhibition constants of 56.4 and 30.0 μM, at respectively. The binding constant and enthalpy of binding, at 27°C are 45 μM, − 64.5 kJ/mol and 61 μM, − 34.5 kJ/mol for aspirin and diclofenac. Thermodynamic data revealed that the binding process for these ADA inhibitors is enthalpy driven. QSAR studies by principal component analysis implemented in SPSS show that the large, polar, planar, and aromatic nucleoside and small, aromatic and polar non-nucleoside molecules have lower inhibition constants.

Keywords: *Adenosine deaminase inhibitors, quantitative structure-activity relationship, QSAR, aspirin, diclofenac, principal component analysis*

Introduction

Adenosine deaminase (E.C.3.5.4.4) is a monomeric protein (34.5 kDa), which catalyzes the deamination of adenosine and 2′-deoxyadenosine nucleosides to their respective inosine derivative nucleosides and ammonia with a rate enhancement of 2×10^{12} relative to the non-enzymatic reaction [1]. This catalysis requires a $Zn^{2}?^{+}$ cofactor [2].

The enzyme is present virtually in all human tissues, but the highest levels are found in the lymphoid system such as lymph nodes, spleen, and thymus [3–5]. Higher levels of ADA in the alimentary tract and decidual cells of the developing fetal-maternal interface put ADA among those enzymes performing unique roles related to the growth rate of cells, embryo implantation, and other undetermined functions [6,7]. ADA is widely distributed in the brain, and one important function of this enzyme is probably associated with regulation of the extracellular level of adenosine and 2′-deoxyadenosine in contact with cerebral blood vessels. The inhibition of adenosine deaminase in the brain would allow for an accumulation of adenosine, which would produce vasodilatation and increase in cerebral blood flow [8].

In recent years, adenosine has come to be considered as an important factor in the attenuation of inflammation [9,10] since it has been reported that the concentration of adenosine is increased in inflammatory lesions [11,12]. Furthermore, it is also believed that ecto-ADA has an extra enzymatic

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function via binding with CD26 on the surface of activated lymphocytes [13] and could perpetuate chronic inflammation by metabolizing adenosine released at inflamed sites that are toxic for lymphocytes [14]. Therefore, it is considered that an ADA inhibitor may prevent adenosine released specifically at inflamed sites from metabolism by ecto-ADA and would have great potential as an anti-inflammatory drug with few side effects.

A number of ADA inhibitors have been reported in the literature. However, almost all are purine nucleoside or alkyladenine analogues, for example, (+)-erythro-9- (2-hydroxy-3-nonyl) adenine ((+)-EHNA) (ground-state inhibitor)[15], pentostatin (transition-state inhibitor)[16], and various derivatives [17]. As a result, they have many problems, such as poor pharmacokinetics [18,19] and/or several toxicities [20,21]. Because of these problems, pentostatin is the only ADA inhibitor in clinical use; however, it is limited to the treatment of adult patients with hairy cell leukemia and is only available via intravenous administration [20,21]. ADA inhibitors with reduced toxicity and oral bioavailability would be expected to not only improve the treatment of leukemia but also have potential use as anti-inflammatory drugs.

Non-steroid anti-inflammatory drugs (NSAIDs) such as aspirin and diclofenac are major drugs against inflammation and pain. Aspirin uncouples oxidative phosphorylation leading to ATP catabolism, and pharmacologically relevant concentrations of sodium salicylate diminish intracellular ATP concentrations *in vitro*, thereby releasing micromolar amounts of adenosine, an autacoids with potent anti-inflammatory properties into extracellular fluids. Aspirin is an anti-inflammatory pain killer (NSAID), which is extensively used, worldwide, for pain relief, to reduce inflammation and pyrexin by affecting the prostaglandins, and to reduce the risk of heart attacks and strokes. It is widely used to prevent heart attacks and strokes [22].

Diclofenac is also a commonly used non-steroidal anti-inflammatory drug. It acts by reducing hormones that cause inflammation and pain and stiffness caused by many conditions, such as osteoarthritis, rheumatoid arthritis, abdominal cramps associated with menstruation, and *ankylosing spondylitis* [23].

The effect of inosine [24], caffeine [25], theophyline [26], acetaminophen [27] and theobromine [28] as inhibitors on ADA activity has been studied by calorimetery and spectroscopy. The enthalpy, equilibrium and inhibition constants for binding were obtained [29,30].

Understanding the interaction of ADA with its inhibitors and substrates at the molecular level will be important in the development of the next generation of pharmaceutical agents that acts as inhibitors or substrates. X-ray crystallography [31,32] and molecular dynamics [33] were analyzed at the molecular level for the ADA-inhibitor complex. Binding sites for some of inhibitors and the effect of their structure on the enzyme were investigated.

It is well known that, drug design is dependent on the structure of the drug, inter- and intra-molecular interactions between drug and receptor. X-ray crystallography, NMR and computational methods give the most information about the three-dimensional structure and molecular interactions but these methods are relatively expensive.

On the other hand, performing QSAR analysis for several series of drugs, enzyme and biological active compounds is now well appreciated. It is less expensive and a simple method relative to the above cited methods. Kinetic parameters were used for the QSAR analysis and as such, we found some theoretical descriptors which correlated the binding affinity of ADA towards several adenine nucleosides as inhibitors. The kinetic parameters of adenosine deaminase such as K_m and K_I were determined in the absence and presence of adenine derivatives as well as the QSAR of these derivatives was studied. QSAR analysis revealed that the binding affinity of the adenine nucleoside upon interaction with ADA depends on the molecular volume, dipole moment of the molecule, electric charge, and the highest positive charge for the related molecules [34].

In line with that proposed research, we investigated the effect of aspirin and diclofenac on ADA using spectrophotometry and isothermal titration calorimetry. Thermodynamic parameters such as enthalpy, entropy, Gibbs free energy and the inhibition constant (K_I) were obtained. Using other data sets from literature, correlation between K_I for 20 inhibitors and 25 descriptors were investigated by multiple linear regression (MLR) and principal component analysis (PCA).

Materials and methods

Materials

Adenosine deaminase (type IV, from calf intestinal mucosa), were obtained from Roche. Aspirin and diclofenac from Sigma, adenosine and other related chemicals, of the highest grade, were obtained from Merck. The solutions were prepared in doubly distilled water.

Methods

Enzyme assay. Enzymatic activities were assayed by UV-Vis spectrophotometry with a GBC 916 spectrophotometer, by following the decrease in absorbance at 265 nm resulting from the conversion of adenosine to inosine based on the Kaplan method [35]. This method uses the change in the absorbance coefficient of adenosine ($\epsilon = 8400\ M^{-1}cm^{-1}$) on

conversion to inosine by the catalytic activity of the enzyme. The concentration of the enzyme in the assay mixture (50 mM sodium phosphate buffer, pH 7.5) was 0.94 nM with a final volume of 2 ml. Activities were measured using at least seven different concentrations of adenosine and the assays were performed at least in triplicate. The adenosine concentration range used was between 0.25–2.5 times that of the K_m. Care was taken to use experimental conditions where the enzyme reaction was linear during the first minutes of the reaction.

Isothermal titration calorimetry (ITC). Isothermal titration microcalorimetric experiments were performed with a 4-channel commercial microcalorimetric system, Thermal activity monitor 2277 (Thermometric, Sweden). The inhibitor solution (1.5 mM) was injected, using a Hamilton syringe, into a stirred calorimetric titration vessel, which contained 2 mL of enzyme solution. The injection of 50 μL inhibitor solution into the perfusion vessel was repeated 20 times. The heat of dilution of the drug solution was measured as described above except that the buffer solution was injected into the protein solution in the sample cell. The enthalpies of dilution for the drug and protein solutions were subtracted from the enthalpy of the ADA-aspirin interaction as previously reported [24–28]. The molecular weight of ADA was taken to be 34 500 [36].

Computational methods. A Data set was made for the literature selected compounds [24–33] and this work. QSAR analysis was performed on the cited compounds with regarding to the following steps 1) entry of the molecular structures into adequate software to perform the structural optimization, 2) generation of the molecular descriptors, 3) statistical analysis through the multiple linear regression (MLR) and principal component analysis, 4) defining of the model validation.

In the first step, the molecular structure of different nucleoside and non-nucleoside inhibitors was constructed and the three-dimensional structure optimized by the PM3 semi-empirical method in the Hyperchem–7.0 medium. In the second step, quantum mechanical descriptors were generated using the Hyperchem-7.0 and Dragon-3.0 programs [37,38]. Dragon calculates 1497 descriptors in 18 classes such as topological, geometrical, empirical, constitutional, charge and so on. The third step, was variable selection which was performed by the MLR method based on the construction of a linear mathematical model with regard to the observed inhibition constants. The final linear equation was formed by stepwise selection of terms. Some of descriptors may be correlated to each other. For the prevention of repeating descriptors and classifying them, we have used principal component analysis (PCA) [39]. PCA involves a mathematical procedure that transforms a number of (possibly) correlated variables into a (smaller) number of uncorrelated variables called principal components (PCs). Objectives of principal component analysis are to, 1) Discover or reduce the dimensionality of the data set. 2) Identify new meaningful underlying variables.

This method reduces the descriptors into a few factors. Each factor describes a property same as geometrical, topological, electronic and lipophilic properties. These factors can be considered as a new more overall descriptor that includes previous descriptors. We also performed MLR analysis on these factors and obtained a general equation between biological activity and common properties or factors.

In the fourth step, model validation was defined through randomly dividing the data-set into two groups, a training set and a prediction set was used for model generation and a prediction set for evaluation of the model. Generally, the good linear agreement between the predicted and experimental values can be used as an adequate parameter for testing the validity of the model. It should be noted that, in our statistical analysis the total number of selected compounds was 20. In the literature, there are many QSAR reports where a smaller set of compounds is utilized for statistical studies [40–42]. So, it can be deduced that here, an acceptable accuracy existed for the accomplished statistical work. In QSAR analysis it has been commonly accepted that the data set should contain five times as many compounds as the descriptors. The reason for this is that too few compounds relative to the number of descriptors will give a falsely high correlation. This aspect has also been considered in our QSAR study.

Results and discussion

Figure 1 shows a double reciprocal Lineweaver-Burk plots for ADA at different fixed concentrations of aspirin and diclofenac, in phosphate buffer, pH = 7.5 at 27°C and 37°C. The K_m values of the enzyme obtained were 39.0 and 61.8 μM at 27 and 37°C. The values of V_{max} are unchanged by aspirin at 27 and 37°C, and diclofenac at 27°C but the apparent Michaelis constant (K'_m or $K_{m,app}$) values are increased, confirming the competitive inhibition of these drugs on ADA at 27°C. On the other hand, at 37°C diclofenac shows uncompetitive inhibition because V_{max} has changed and K_m remains unchanged (Figure 1b). The values of K'_m at any fixed concentration of aspirin and diclofenac were obtained from Figure 1 and then plotted against concentrations of the inhibitor (in the inset of Figure, secondary plot) to obtain the inhibition constant (K_I). K_I values at 27 and 37°C were 42.8 and 96.8 μM for aspirin, and 56.4 and 30.0 μM, for diclofenac, respectively. The figures for aspirin

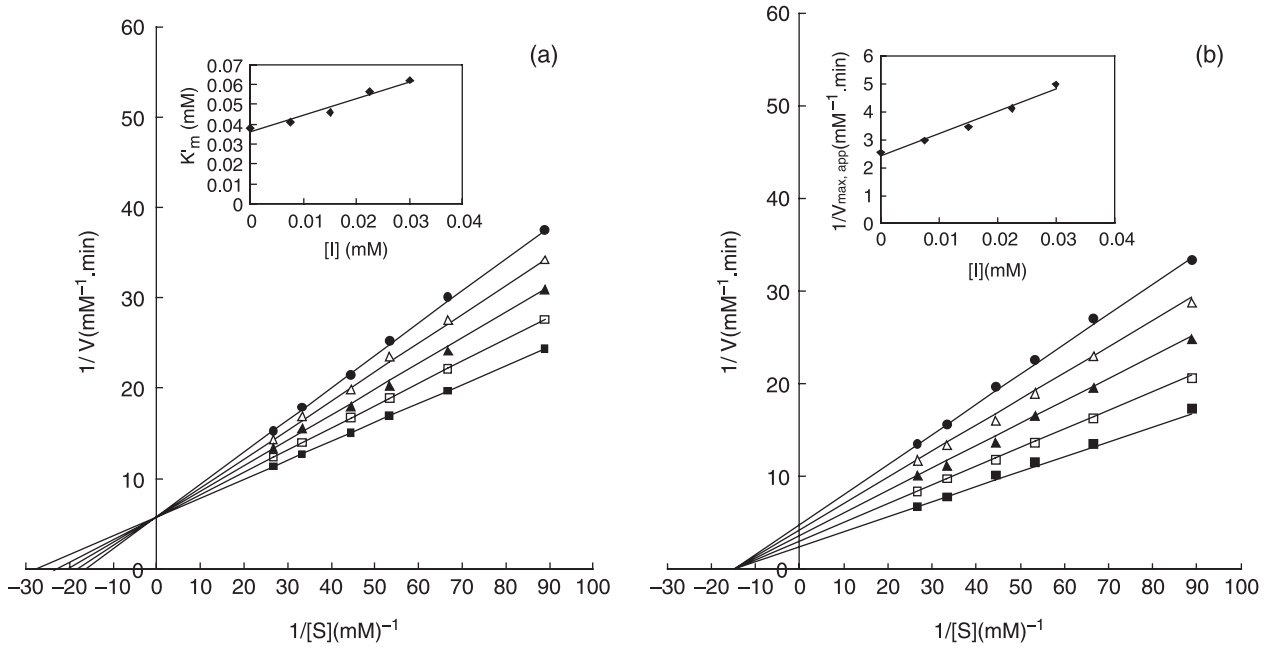


Figure 1. Double reciprocal Lineweaver–Burk plots for kinetics of 0.94 nM ADA at pH = 7.5 in the presence of 0.0 (■), 7.5? (□), 15.0 (▲), 22.5 (△) and 30.0 μM (() of (a) aspirin at 27°C and (b) diclofenac at 37°C. Inset of each figure (secondary plots): apparent Michaelis constant, K'_m ($K_{m,app}$) versus concentration of inhibitor, [I].

at 37°C and diclofenac at 27°C were similar to aspirin at 27°C (data not shown).

By titration of a solution containing an enzyme (E) with a solution of inhibitor (I), the equilibrium reaction moves toward an increasing concentration of the EI complex. The heat of the reaction depends on the concentration of the EI complex. Thus, the reaction under consideration can be written as follows [29,43]:

$$EI \rightleftharpoons E + I \quad K_I = [E][I]/[EI] \tag{1}$$

$$\Delta H^o = 1/A_i\{(B_i + K_I) - [(B_i + K_I)^2 - C_i]^{1/2}\} \tag{2}$$

Where

$$A_i = V_i/2q_i \tag{3}$$

$$B = [E]_{total} + [I]_{total}, \quad C = 4\,[E]_{total}[I]_{total} \tag{4}$$

where q_i is the sum of heat evolutions following the i-th titration step, V_i is the volume of the reaction solution and ΔH^o is the enthalpy of binding. A_i, B_i and C_i can be calculated in each injection; thus, Equation (2) contains two unknowns, K_I and ΔH^o. A series of reasonable values for K_I is inserted into Equation (2), corresponding values for ΔH^o are calculated and a graph of ΔH^o vs. K_I is constructed. Curves of all titration steps will intersect at one point, which represents the true value for ΔH^o and K_I.

The plots of ΔH^o vs. K_I, according to Equation (2), for the first 10 injections are shown in Figure 2.

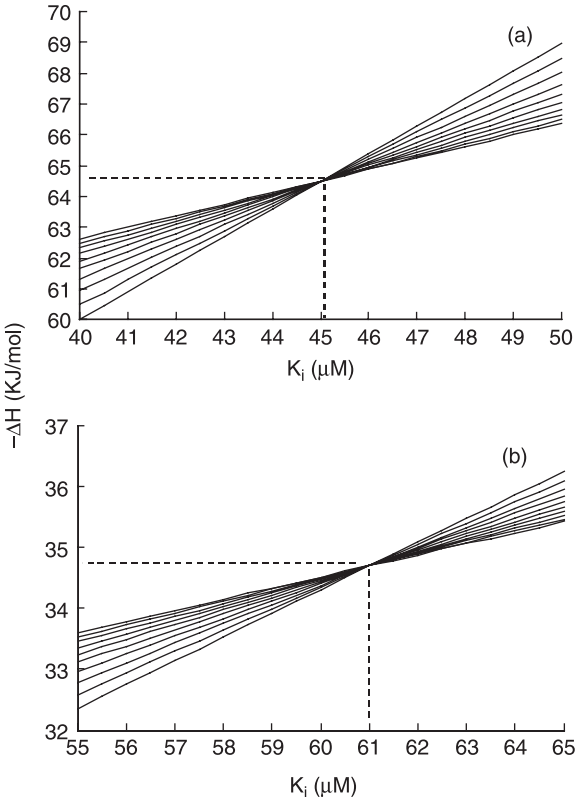
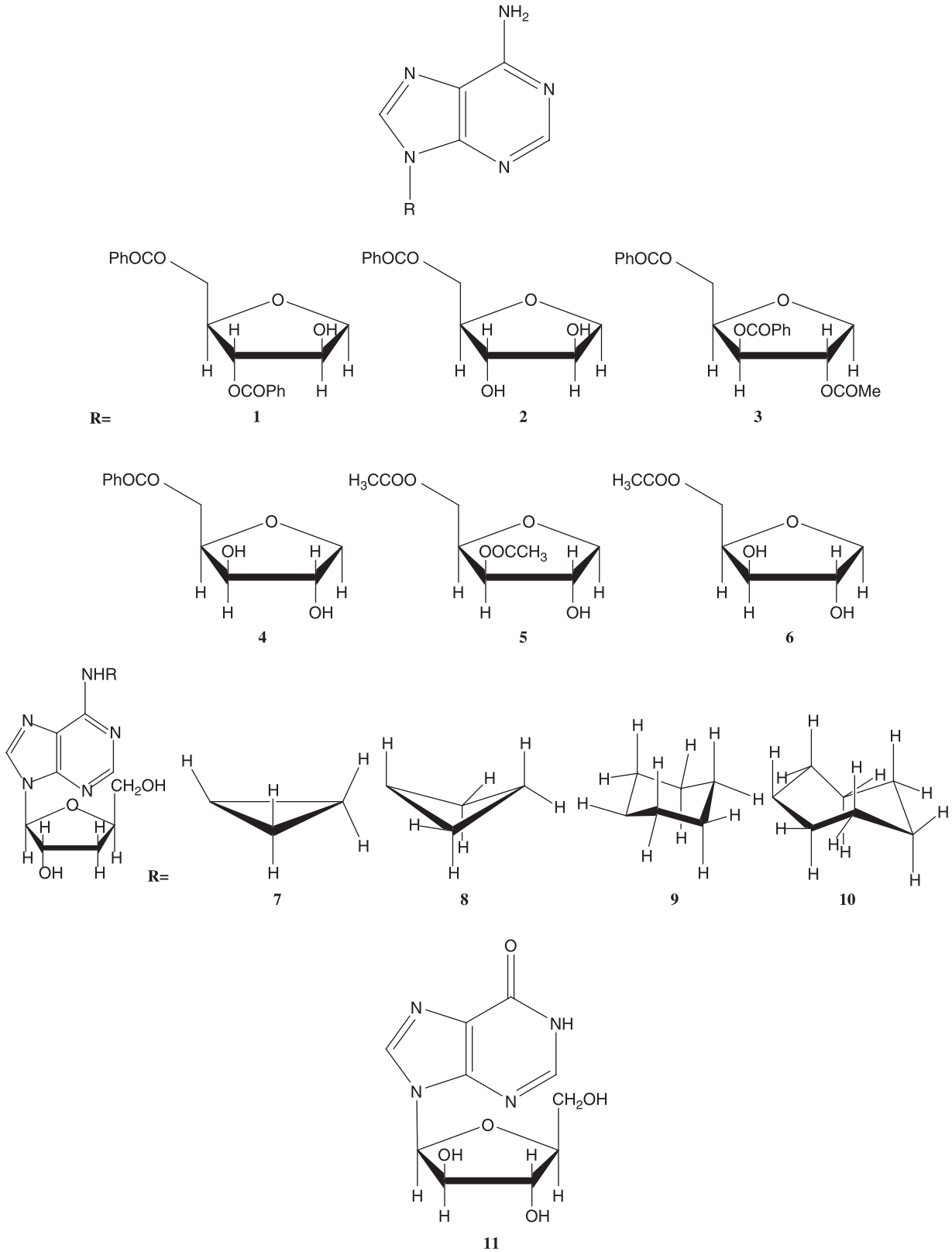
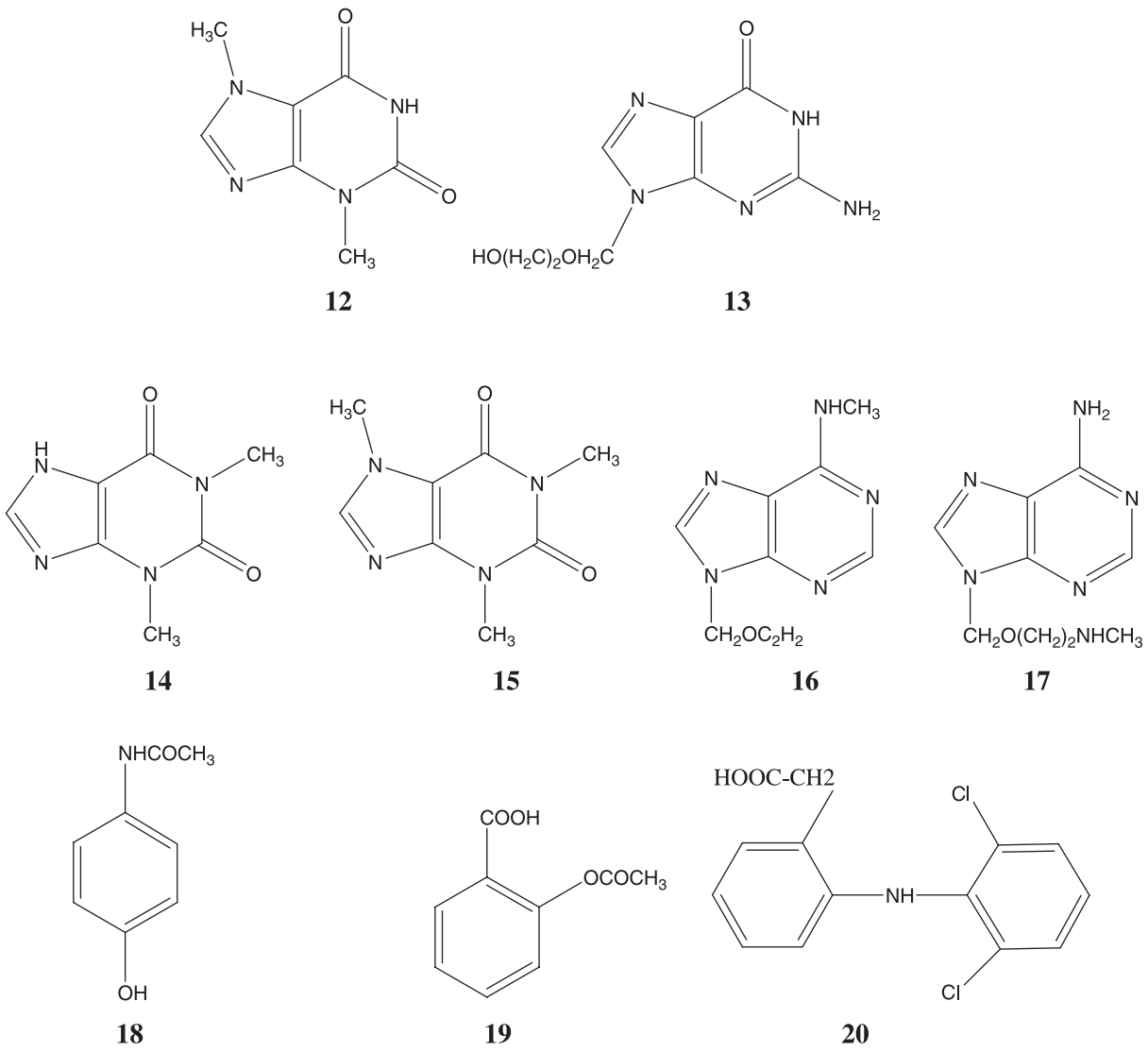


Figure 2. ΔH^o versus K_I for first 10 injections, according to Equation (2) for (a) aspirin (b) diclofenac. The coordinates of intersection point of curves give true values for ΔH^o and K_I.

The intersection of the curves gives ΔH^o and K_I which are -64.5 kJ/mol, 45.0 μM, -34.5 kJ/mol and 61.0 μM, for aspirin and diclofenac, respectively.

Good conformity of the dissociation binding constant (K_I) obtained from thermodynamic and kinetic studies were observed. In addition, calorimetric measurements showed that the interaction





Scheme 1. Molecular structure of a) nucleoside and b) non-nucleoside inhibitors.

between ADA and the two drugs was an exothermic process.

The standard Gibbs free energy change of binding for aspirin and diclofenac to ADA can be calculated using the inverse of the K_I value as the association binding constants ($K = 1/K_I$). Molar Gibbs free energy (ΔG^{o}) and entropy (ΔS^{o}) of binding can be obtained by the following equations:

$$\Delta G^{o} = -\mathrm{RT}\ln K \tag{5}$$

$$\Delta S^{o} = (\Delta H^{o} - \Delta G^{o})/\mathrm{T} \tag{6}$$

The molar ΔG^{o} and ΔS^{o} of binding for aspirin were -25.0 kJ/mol and -131.7 J/(mol K) and for diclofenac were -24.2 kJ/mol and -32.0 J/(mol.K), respectively. Therefore, the binding process for these inhibitors on ADA is spontaneous enthalpy driven. The Binding constant of aspirin is higher than that of diclofenac.

To gain insight into the structure-activity relationship for the inhibitors, QSAR analysis has been performed through ascertaining the several suitable descriptors followed by defining of any linear correlation between the cited descriptors and the parameters of inhibition. Thus, a number of molecular descriptors have been primarily determined for a set of competitive inhibitors including nucleoside and non-nucleoside inhibitors. Fortunately, in the literature, [24–33] there are several inhibitors, where their kinetic parameters were reported during their interaction with ADA under environmental conditions that are similar those of our studies. In the previous work we reported only the QSAR of nucleoside inhibitors [34]. However, in the present study, we considered QSAR and factor analysis on nucleoside (Scheme 1a, compounds 1–11) and non-nucleoside inhibitors (Scheme 1b compounds 12–20).

The majority of these compounds had been experimentally studied previously. Only aspirin and

diclofenac (compounds 19, 20) were studied here by us. Table I shows the abbreviation and definition of the 25 descriptors.

The related values obtained from Hyperchem and Dragon were tabulated in Table II. These descriptors belong to different properties such as geometric, topologic, electronic, hydrophobic and aromatic properties. In the first step correlation between $\log K_I$ and these descriptors was investigated (final column of Table I); a negative sign shows a negative correlation.

Then the best descriptors were selected on the basis of the correlation obtained from stepwise selection in SPSS. Whenever the number of entered descriptors increases the correlation coefficient is enhanced. Thus, different models are observed on the basis of the number of entered descriptors. There are 20 compounds and so the number of descriptors is 1/5 number of compounds, i.e. 4 descriptors. Therefore, a model having 4 descriptors was selected. A Correlation between $\log K_I$ and selected descriptors was obtained for 20 compounds by MLR methods as follow:

$$\begin{aligned}\log K_I = &-0.224(\pm 0.033)(MLOGP)\\ &-0.285(\pm 0.022)(MUTOT)\\ &-0.0828(\pm 0.017)FDI\\ &+0.0719(\pm 0.021)MUY\\ &+3.475(\pm 0.141)\\ &R = 0.973 \quad S.E = 0.184\end{aligned} \tag{7}$$

As we see, the $\log K_I$ decreases with hydrophobicity (*MLOGP*), total dipole moment (*MUTOT*) and folding degree index (*FDI*) and increases with dipole moment in the Y-direction (*MUY*). On the other hand, aspirin has a lower value of *MUY* and *MLOGP* and a higher value of *MUTOT* relative to diclofenac. This means that aspirin is less hydrophobic and more polar relative to diclofenac.

Predicted values by this equation were tabulated in Table II [See column 3, $\log K_I$(pred1)]. Figure 3 shows a correlation between experimental and predicted data. The values for aspirin and diclofenac are encircled.

The correlation data (Table I) shows that there are some descriptors such as volume, surface area, and polarizability, refractivity, molecular weight, which have similar properties. They describe the size of molecule. Also geometrical descriptors such as *L/BW, RGYR, SPH, ASP* and *FDI* have similar properties. Therefore, it seems we can classify and reduce similar descriptors to a family or group.

So, in the next step, principal component analysis (PCA) was used for reduction and classification of descriptors. This method reduces the descriptors to a few factors or principal components (PCs). These factors include those descriptors which have similar effects and properties. In the current study, 25 descriptors were reduced into three factors which are tabulated in Table III.

Factor 1 includes volume, molecular weight, accessible and polar surface area, refractivity, polarizability, aromaticity, total dipole moment and the Z-direction dipole moment. The majority of them describe the size, thus we refer to them as the size

Table I. List of descriptors used in this work.

Descriptor	Definition	Correlation coefficient with $\log K_I$
UI	Unsaturation index	−0.775
HY	Hydrophilic factor	−0.089
ARR	Aromatic ratio	−0.401
MR	Ghose-Crippen molar refractivity	−0.907
PSA	Fragment-based polar surface area	−0.726
MLOGP	Moriguchi octanol-water partition coeff. (logP)	−0.608
HOMA	Harmonic Oscillator Model of Aromaticity index	−0.119
RCI	Jug RC index	−0.537
AROM	Aromaticity (trial)	−0.25
HOMT	HOMA total (trial)	−0.879
L/BW	Length-to-breadth ratio by WHIM	0.239
RGYR	Radius of gyration (mass weighted)	0.429
SPH	Spherosity	−0.04
ASP	Asphericity	−0.452
FDI	Folding degree index	−0.101
MUX	Dipole moment in X direction	0.332
MUY	Dipole moment in Y direction	0.519
MUZ	Dipole moment in Z direction	−0.838
MUTOT	Total dipole moment	−0.728
SA	Surface area	−0.854
VOL	Molecular volume	−0.874
HE	Hydration energy	0.036
POL	Polarizability	−0.89
MW	Molecular weight	−0.896

Table II. Values of descriptors was calculated by Hyperchem-7.0 and Dragon-3.0.

NO	LogK_I (Exp)	LogK_I (Pred1)	LogK_I (Pred2)	LogK_I (Pred3)	UI	HY	ARR	MR	PSA	MLOGP	HOMA	RCI	AROM	HOMT
1	0.50	0.54	0.38	0.34	4.64	0.88	0.56	117.8	113.39	1.20	0.89	1.42	0.95	19.5
2	1.11	1.36	1.31	1.43	4.17	1.81	0.53	88.51	87.09	−0.43	0.85	1.43	0.93	13.62
3	−0.10	0.12	0.14	0.07	4.70	0.23	0.52	127.0	139.69	1.64	0.89	1.43	0.95	19.48
4	1.56	1.50	1.29	1.43	4.17	1.81	0.53	88.51	87.09	−0.43	0.85	1.43	0.93	13.59
5	2.00	1.67	1.65	1.67	3.70	1.17	0.37	77.49	113.39	−1.21	0.77	1.42	0.89	7.66
6	2.15	2.07	1.91	2.15	3.59	2.06	0.42	68.34	87.09	−1.75	0.75	1.43	0.88	7.49
7	1.89	2.00	2.22	2.08	3.46	0.42	0.44	70.42	47.90	0.22	0.65	1.42	0.87	6.54
8	1.96	1.93	2.18	1.99	3.46	0.39	0.42	75.02	47.90	0.48	0.65	1.42	0.87	6.54
9	1.84	1.76	2.07	1.78	3.46	0.33	0.39	84.23	47.90	0.97	0.65	1.42	0.88	6.50
10	1.72	1.59	2.01	1.66	3.46	0.30	0.37	88.83	47.90	1.21	0.66	1.42	0.89	6.64
11	2.16	2.20	2.36	2.19	2.32	1.36	0.10	54.48	43.07	−2.15	0.83	1.40	0.9	1.66
12	2.49	2.42	2.44	2.44	2.32	0.03	0.14	42.59	41.79	−0.86	0.95	1.40	0.95	1.90
13	2.45	2.30	2.39	2.49	2.32	1.51	0.18	49.54	30.71	−1.17	0.89	1.40	0.91	2.66
14	2.30	2.65	2.49	2.43	2.32	0.03	0.14	42.59	56.41	−0.86	0.95	1.40	0.95	1.90
15	2.53	2.43	2.42	2.33	2.32	−0.56	0.13	47.49	45.03	−0.52	0.95	1.40	0.95	1.90
16	2.58	2.55	2.37	2.72	3.46	−0.04	0.59	58.18	60.79	−0.08	0.70	1.42	0.89	7.02
17	2.86	3.06	2.45	2.75	3.46	1.45	0.59	56.07	60.79	−1.14	0.70	1.42	0.89	7.02
18	2.10	2.04	2.11	1.98	3.00	−0.11	0.55	40.83	17.07	1.06	0.98	1.40	1.00	5.85
19	1.62	1.44	1.77	1.69	3.17	−0.67	0.46	43.95	43.37	1.7	0.98	1.4	1	5.89
20	1.75	1.77	1.50	1.83	3.81	−0.24	0.60	76.95	17.07	3.99	0.98	1.41	1	11.72

Table II. *continued*

L/BW	RGYR	SPH	ASP	FDI	MUX	MUY	MUZ	MUTOT	SA	VOL	HE	POL	MW
0.91	0.25	0.93	0.81	1.60	0.571	−0.081	8.853	8.872	715	1245	−14.93	49.04	475
0.86	0.56	0.93	0.78	6.30	−1.222	0.243	5.854	5.985	572	972	−17.53	37.46	371
0.80	0.31	0.91	0.82	2.60	−2.868	−3.85	7.334	8.766	730	1315	−14.19	52.79	517
0.83	0.50	0.94	0.68	5.10	−0.837	−1.213	5.284	5.486	579	985	−17.00	37.00	371
0.76	0.39	0.95	0.89	3.70	−2.752	−0.305	5.486	6.145	556	934	−12.57	33.00	351
0.80	0.38	0.96	0.85	3.40	−0.588	−1.277	4.790	4.990	495	822	−15.16	29.60	309
0.73	0.47	0.95	0.83	6.20	0.253	−0.121	3.154	3.167	489	808	−9.50	30.04	274
0.74	0.49	0.94	0.91	6.60	0.12	0.310	3.171	3.188	508	851	−9.01	31.88	288
0.75	0.54	0.94	0.91	7.70	−0.014	0.637	3.106	3.171	550	928	−8.36	35.55	316
0.76	0.58	0.94	0.86	9.10	−0.035	0.613	3.101	3.161	569	969	−8.14	37.38	330
5.30	5.08	0.67	0.43	0.97	−3.17	6.744	−1.275	7.563	431	694	−18.24	23.81	268
1.70	3.24	0.94	0.30	1.00	4.194	1.194	−0.328	4.373	340	522	−41.60	17.04	180
4.30	5.03	0.80	0.46	0.99	−4.146	3.706	−1.330	5.675	431	658	−17.41	20.99	225
1.40	3.17	0.94	0.26	1.00	3.371	0.810	−0.110	3.469	339	522	−4.88	17.04	180
1.50	3.56	0.94	0.27	1.00	3.864	0.566	−0.068	3.906	364	573	−1.36	18.87	194
0.74	0.46	0.98	0.82	5.20	−0.16	2.328	0.472	2.381	449	705	−7.05	24.83	241
0.76	0.46	0.95	0.62	4.90	0.835	0.675	−0.059	1.076	443	696	−12.01	24.25	222
6.10	3.38	0.88	0.59	0.96	3.093	−1.622	0.315	3.506	327	492	−11.20	16.18	151
1.4	3.3	0.73	0.15	0.95	3.05	−3.041	−1.261	4.74	348	535	−8.95	17.38	180
2.2	5.12	0.78	0.25	0.95	−2.568	0.804	−0.658	2.771	461	767	−8.18	29.69	296

factor. In previous work [34] we also showed that volume decreases the logK_I while here we obtained several descriptors which show a size property.

Factor 2 includes *L/BW, RGYR, SPH, ASP* and *FDI* which belong to geometrical and *HOMA* and *AROM* which belong to aromaticity indices [39]. Aromaticity descriptors (*HOMA*, *HOMT, RCI, AROM, ARR*) distribute between three factors, because aromaticity depends on size, electronic properties and molecular shape. This factor is related to planarity or is two-dimensional such as aromaticity descriptors (*HOMA, AROM, L/BW*) or non-planarity three-dimensional (*RGYR, SPH, ASP* and *FDI*) descriptors. Therefore we refer to factor 2 as a shape factor.

Factor 3 includes *MLOGP, HY, MUX, MUY, ARR* and *HE*. *MLOGP*, *HY* and *HE* are related to hydrophobicity and electronic properties. Dipole moment in the X (*MUX*), Y (*MUY*) direction and *ARR* belong to electronic properties and describe hydrophobicity or electronic descriptors. We refer to this factor as the electronic factor. It is difficult to clearly define the boundary of these factors but each factor majority describes an overall property. Dipole moment depends on electronic charges and distance between them. Lengthy, polar molecule have a higher dipole moment. If we consider that the surface of the molecule locates in XY, then Z is perpendicular to the surface of the molecule. Increasing the *MUX* and

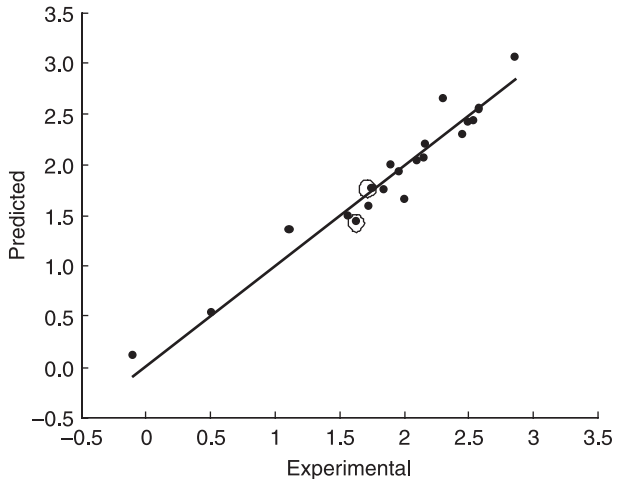


Figure 3. The linear correlation between the experimental values of logK_I and the related values of the prediction set by MLR analysis on descriptors.

MUY is related to enhancing the surface and planarity. On the other hand, more conjugated planar molecules have higher aromaticity. Increasing the *MUZ* reduces the two-dimensional shape (planarity) and increases the three-dimensional shape (spherosity or cylindrical). Table I, show that increasing the *MUTOT, MUZ, MUX*, bond conjugation and aromaticity decreases logK_I and so increases the binding constant. Thus lengthy, planar, aromatic and polar molecule has a lower logK_I and higher tendency to bind to ADA. These data are compatible with other reports where more aromatic and large inhibitors have a higher binding constant or lower inhibition constant [31–33]. Therefore, using these finding we can design a desirable drug for other proposals.

Table III. Rotated component matrix for studied descriptors.

	Components		
Descriptor	Size	Shape	Electronic
MW	0.965		
MR	0.944		
HOMT	0.942		
VOL	0.941		
POL	0.938		
SA	0.934		
MUZ	0.889		
UI	0.888		
PSA	0.831		
MUTOT	0.679	−0.525	
RCI	0.606		
HOMA		−0.914	
FDI		0.89	
AROM		−0.826	
RGYR	−0.547	−0.731	
ASP	0.525	0.721	
SPH		0.659	
LB/W		−0.597	
MLOGP			0.802
HY			−0.73
MUX			0.653
ARR			0.569
MUY			−0.54
HE			

On the other hand, we can obtain the dependence of logK_I on these factors by MLR. The resulting equation is as follow:

$$\log K_I = -0.641(Size) + 0.151(Shape) - 0.118(Electronic) + 1.874 \quad (8)$$

The equation shows that logK_I decreases with the size and electronic or hydrophobicity factor and increases with the shape factor. Calculated data are tabulated in Table II [logK_I(Pred2)]. The correlation coefficient, standard error and mean effect are tabulated in Table IV. The effect of each descriptor appears in the beta factor (mean effect).

The predicted value for aspirin is higher than that of diclofenac by this method. For improving it, we performed PCA analysis separately for the two categories. In addition the resulting equation from MLR of PCs for non-nucleosides is as follows:

$$\log K_I = 0.0852(Size) + 0.303(Shape) - 0.213(Electronic) + 2.298 \quad (9)$$

and for nucleosides is:

$$\log K_I = -0.704(Size) - 0.0598(Shape) - 0.04(Electronic) + 1.526 \quad (10)$$

The coefficients, their standard errors and mean effects for the three groups are tabulated in Table IV. The predicted values for non-nucleoside specially for aspirin and diclofenac, are shown in Table II [log K_I(Pred3)].

It is observed that all of the factors for nucleosides are negative but in non-nucleosides only the electronic factor is negative. The mean effects show that, the size in nucleosides and shape in non-nucleosides are more effective than the others. This finding related to correlation coefficient between logK_I and descriptors. Figure 4 shows the differences between the correlation coefficients of the two categories. It is interesting to note that the size and shape descriptors have a reverse correlation in the two groups (region A). It may be due to the differences in inhibitor structure, binding site and type of interaction between inhibitors and ADA. It can help us to prediction an inhibitor's behavior. We also found that diclofenac has a higher size factor and higher logK_I relative to aspirin which is compatible with non-nucleoside behavior.

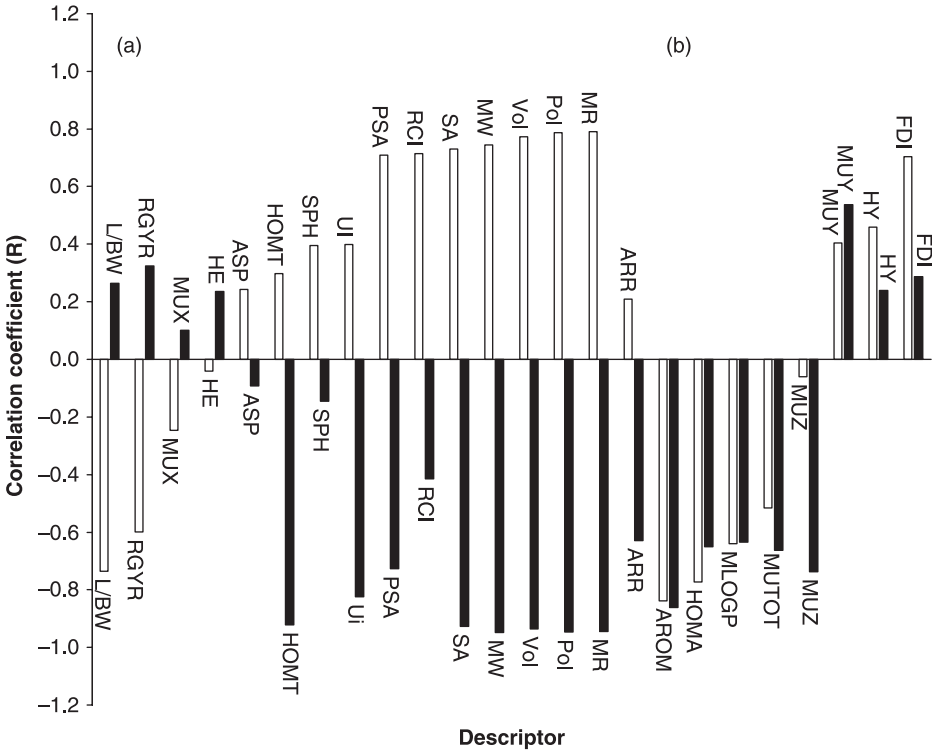


Figure 4. Correlation coefficients logK_I and descriptors for nucleoside (black) and non-nucleoside (white) bar line.

Table IV. Specification of the multiple linear regression model for factors.

	Total		Non-nucleosides		Nucleosides	
	Coefficient (error)	Mean effect	Coefficient (error)	Mean effect	Coefficient (error)	Mean effect
Constant	1.874 (± 0.057)		2.298 (± 0.058)		1.526 (± 0.066)	
Size factor (F1)	−0.641 (± 0.059)	−0.904	0.0852 (± 0.022)	0.211	−0.704 (± 0.069)	−0.964
Shape factor (F2)	0.151 (± 0.047)	0.213	0.303 (± 0.062)	0.750	−0.0598 (± 0.044)	−0.082
Electronic factor (F3)	−0.118 (± 0.023)	−0.166	−0.213 (± 0.016)	−0.526	−0.04 (± 0.01)	−0.055
	R = 0.94, F = 43, S.E = 0.25		R = 0.94, F = 12.7, S.E = 0.17		R = 0.969, F = 35, S.E = 0.21	

Thus we can gain an insight into structure-activity relationships and drug design with favorable properties by these methods, which are complementary methods for other studies.

Conclusion

Aspirin and diclofenac are anti-inflammatory drugs and inhibit the activity of ADA. They show different effects at different temperatures. The binding process for these ADA inhibitors is enthalpy driven. Diclofenac is a larger and more aromatic molecule compatible to its higher inhibition constant. Molecular structure and statistical analysis show that polar, aromatic, ribbon shape and planar molecules have higher tendency for binding to the enzyme and a lower inhibition constant. Analysis of the correlation between logK_I and factors of two categories shows that nucleosides have a negative correlation with size, shape and electronic parameters while non-nucleosides have a positive correlation with size and shape and negative with electronic. In nucleosides, size is more effective than shape, while in non-nucleosides it is the reverse. All the data show a proper prediction of logK_I for aspirin and diclofenac.

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