Articles

Effects of Dimaine, Diacid and Dintitro Derivatives on the Inhibition of Adenosine Deaminase; Experimental, Molecular Docking and QSAR Studies

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Effects of some diacid, diamine and dinitro aromatic compounds on the structure and activity of adenosine deaminase (ADA) were investigated by UV-Vis spectrophotometry in 50 mM phosphate buffer at pH = 7.5 and 27 °C and molecular docking studies. The results showed that all tested ligands are showing inhibition: five ligands are uncompetitive and other two ligands are mixed of competitive and noncompetetive inhibitors with majority of competitive behavior. For the later case analysis was done based on competitive inhibition. Diacids have larger size and higher inhibition constant (K_i) relative to others. A logical correlation between calculated free energy of binding and experimental values was obtained for un-competitive. Experimental and calculated data showed that competitive inhibitors are distributed near the active site of enzyme and form several cluster of ranks, whereas uncompetitive inhibitors bind to the enzyme-substrate complex and distributed far from the active site. Results of structure-activity relationship showed that, larger, more hydrophobe, less spherical and more aromatic ligands have higher inhibition constants.

Key Words: Binding constant, QSAR, Enzyme inhibition, 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] The enzyme is present virtually in all human tissues, but the highest level is found in the lymphoid system such as lymph nodes, spleen, and thymus.[2-4]

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.[5] Adenosine has come to be considered as an important factor in the attenuation of inflammation,[6-7] since it has been reported that the concentration of adenosine is increased in inflammatory lesions.[8-11] 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.

Effect of inosine,[12] caffeine,[13] theophyline,[14] acetaminophen,[15] theobromine,[16] as inhibitors on ADA activity has been studied by spectroscopy and calorimetry. The enthalpy, equilibrium and inhibition constants for binding were obtained.[17,18]

On the other hand, performing QSAR analysis for several series of drugs, macromolecules enzyme and biological active compounds is now well appreciated. 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.[19] The kinetic parameters for adenosine deaminase were determined as well as the QSAR of these derivatives was studied. QSAR analysis revealed that the binding affinity depends on the molecular volume, dipole moment, electric charge, and the highest positive charge.[19]

We previously investigated the effect of aspirin and diclofenac as non-steroid anti-inflammatory drugs (NSAIDs)[20] on ADA using spectrophotometry and isothermal titration calorimetry. QSAR studies show that the large, polar, planar, and aromatic nucleoside and small, aromatic and polar non-nucleoside molecules have lower inhibition constant.[20]

We have investigated effect of salts; solvents; and ionic surfactants on the structure and activity of adenosine deaminase (ADA) by UV-Vis spectrophotometery, circular dichroism and molecular dynamics (MD).[21] Relative activity, experimental and computational helix content, total accessible surface area (ASA) and exposed charged surface area were obtained. It was shown that increasing the surface area and decreasing helix is associated to decreasing the activity.

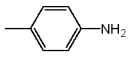
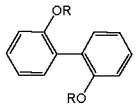
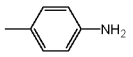
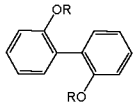
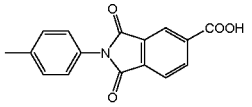
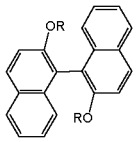
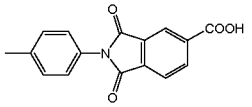
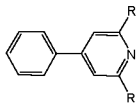
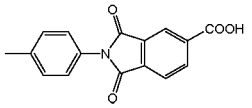
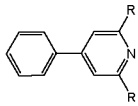
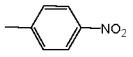
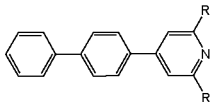
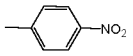
The aim of this work was to study the effects of seven diacid, diamine and dinitro aromatic compounds on the activity of adenosine deaminase by UV-Vis spectrophotometry molecular docking and QSAR methods in order to more understanding the structure-activity relationship.

Experimental

Materials. Adenosine deaminase (type IV, from calf intestinal mucosa), were obtained from Roche.

Wholly aromatic ether-diamine (DA1) and wholly aromatic

Scheme 1. Chemical structure and name of studied aromatic compounds in this work

Name	Structure	R
DA1	OR_2	
DA2		
DAC1		
DAC2		
DAC3		
DN1		
DN2		

DA1	4,4′-diaminodiphenyl ether
DA2	2,2′-(4-aminophenoxy)biphenyl
DAC1	2,2′-bis(4-trimellitimidophenoxy)biphenyl
DAC2	2,2′-bis(4-trimellitimidophenoxy)-1,1′-binaphthyl
DAC3	4-phenyl-2,6-bis(4-trimellitimidophenyl)pyridine
DN1	4-phenyl-2,6-bis(4-nitrophenyl)pyridine
DN2	4-*p*-biphenyl-2,6-bis(4-nitrophenyl)pyridine

diether-diamine (DA2) were obtained from Merck and distilled in vacuum. Biphenyl-based diamine diacids (DAC1 and DAC2) synthesized same as literature.[22] Imide containing aromatic diacid (DAC3) pyridine-based fully aromatic dinitro compounds (DN1, DN2) were synthesized as literature.[23] The name, abbreviation and the structure of cited ligands were shown in Scheme 1. All of the other reagents were obtained from Merck chemical company.

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.[24] This method uses the change in the absorbance coefficient of adenosine (ε = 8400 M^{-1} cm^{-1}) on conversion to inosine by the catalytic activity of the enzyme. The adenosine concentration range was between 0.25 ~ 2.5 times that of the K_m. The concentration of enzyme in the assay mixture (50 mM sodium phosphate buffer, pH 7.5) was 0.83 nM with a final volume of 2 mL. Activities were measured using at least five different concentrations of adenosine and the assays were performed at least in triplicate. Care was taken to use experimental conditions where the enzyme reaction was linear during the first minutes of the reaction. It means that the linear range of v versus [S] substrate concentration curve was taken for data handling. This usually was carried out in the lower concentration range of substrate.

Structure-activity relationship. Structure-activity 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. In the first step, the molecular structure of different seven ligand 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, descriptors were generated using the Hyperchem-7.0 and Dragon-3.0 programs.[25,26] Dragon can calculate 1497 descriptors in 18 classes such as topological, geometrical, empirical, constitutional, charge and so on. We select more biosence and interpretable descriptors. 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). 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 descriptor that has same properties as original descriptors. For example size factor itself has property similar to molecular weight, surface area, number of atoms and number of functional group and so on.

Ligand docking. Version 3.0.5 of AutoDock[27] was used for the docking studies. ADA information was extracted from the protein databank (PDB) files of their X-ray crystal structures (1VFL) or optimized structure. Ligand PDB files were imported into Autodock tools (ADT), polar hydrogens were added, and Gasteiger charges were computed; the rigid root and the rotatable bonds were defined by the AutoTors tool of ADT. The PDB file was imported into ADT, all water molecules were removed, Kollman charges and solvation parameters were added. Grid maps of 126 × 126 × 126 points with a grid-point spacing of 0.375 Å were generated using the Auto Grid tool of ADT. The 250 genetic algoritlun (GA) runs were performed with the following parameters: population size of 50, maximum number of 2.5×10^5 energy evaluations, maximum number of 27,000 generations, an elitism of 1, a mutation rate of 0.02, and a crossover rate of 0.8. The resulting conformations were clustered using a root-mean-square deviation (rmsd) of 0.5 Å and the clusters were ranked in order of increasing binding energy of the lowest binding energy conformation in each cluster.

Results and Discussion

Enzyme kinetics. Scheme 1 shows the structure and nomenclature of seven compounds in three groups studied in this work. Dimaine (DA1 and DA2), diacid (DAC1, DAC2 and DAC3) and dinitro (DN1 and DN2) compounds have two amine (NH_2), two acid (COOH) and two nitro (NO_2) functional groups, respectively.

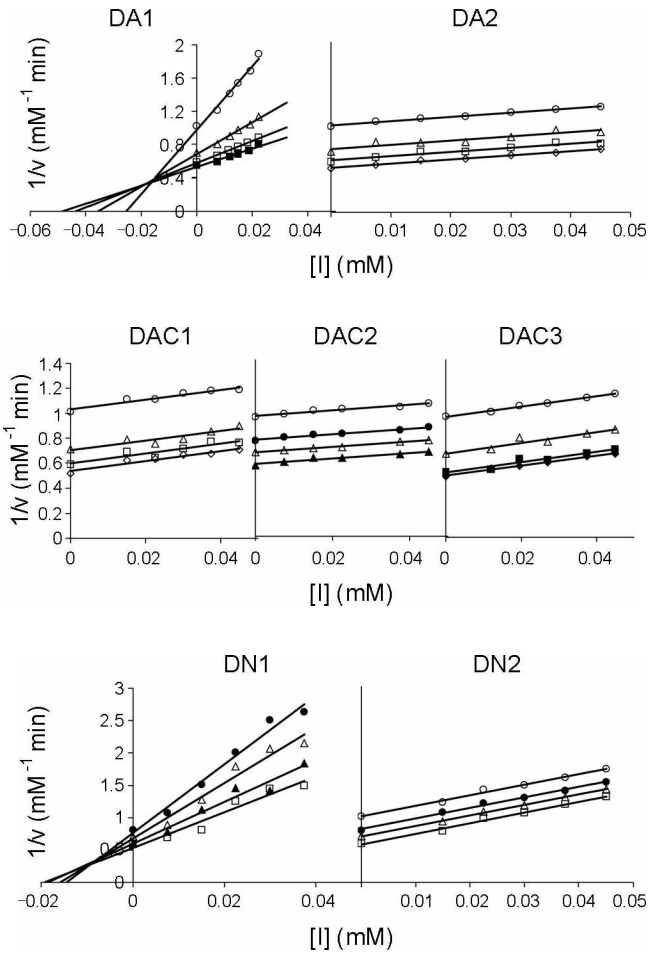


Figure 1. Dickson plot ($1/v$ against inhibitor concentration, [I]) for ADA in the absence (×) and presence of 0.015 (▫), 0.0225 (●) 0.030 (△) 0.0375 (▲), 0.0450 (⊏), 0.0525 (■) 0.060 (▯), 0.0675 (◆) mM of adenosine.

Fig. 1 shows Dickson plot ($1/v$ *vs.* concentration of inhibitor, [I]) in different fixed concentration of adenosine and organic ligands at 50 mM phosphate buffer, pH 7.5 and 27 °C. Michaels constant (K_m) and maximum velocity (v_{max}) are 26.8 μM and 2.73 mM min^{-1}, respectively. Fig. 1 also shows that two ligands, DA1 and DN1, are mixed of competitive and non competitive inhibitors. They bind to the active site of enzyme, compete with substrate.[28] The inhibition constant can be obtained from slope of $1/v$ *vs.* [I] according to equation (1).

$$\frac{1}{v} = \frac{1}{v_{max}K_I}[I] + \frac{1}{v_{max}}\left(1 + \frac{K_m}{[S]}\right) \quad (1)$$

DA1 and DN1 have smaller size and more favorable binding to active site than others do. In addition, based on Fig. 1, the other five ligands are uncompetitive inhibitors. Uncompetitive inhibitors bind to the enzyme-substrate (ES) complex. The inhibition constant (K_I) for uncompetitive inhibitors can be obtained from slope of Dickson plot ($1/v$ *vs.* [I]) by equation (2):

$$\frac{1}{v} = \frac{K_m}{v_{max}K_I[S]}[I] + \frac{1}{v_{max}}\left(1 + \frac{K_m}{[S]}\right) \quad (2)$$

The values of K_I for DA1, DA2, DAC1, DAC2, DAC3, DN1 and DN2 are 15.24, 78.70, 97.05, 131.83, 79.61, 7.30 and 22.23 μM, respectively. DAC ligands have higher K_I relative to others.

Molecular docking. In order to clarifying differences between kinetic data for DA1, DN1 and other ligands, we used docking results. In the first step, structure of ADA was optimized up to 6 000 ps in water by GROMACS molecular dynamics software. Secondly, docking of seven ligands to optimized ADA was studied by Autodock 3.0.5. The number of runs was 250 and output of docking was sorted based on binding or docking free energy (ΔG_b). Most negative ΔG_b located in the first of data series. Table 1 listed the most ten negative ΔG_b. Relative ΔG_b for ligands are DAC3 < DN2 < DA1 < DAC1 < DA2 < DN1 < DAC2. Fig. 2 illustrated distribution of docking sites for the first 10 ranks of docking belong to seven ligands to ADA.

Table 1. Free energy of binding and number of runs in a cluster for the first ten ranks of ligand binding to optimized ADA

Rank	ΔG_b/ kcal/mol							Number of run in a cluster						
	DA1	DA2	DAC1	DAC2	DAC3	DN1	DN2	DA1	DA2	DAC1	DAC2	DAC3	DN1	DN2
1	-7.70	-9.92	-9.27	-6.12	-11.84	-10.68	-11.35	6	2	1	1	1	2	1
2	-7.01	-9.67	-8.10	-6.06	-11.66	-10.49	-11.31	12	1	1	1	1	2	1
3	-7.12	-9.53	-7.95	-6.05	-11.33	-10.51	-11.06	6	1	1	1	1	1	1
4	-7.06	-9.51	-7.93	-5.94	-11.12	-10.39	-10.26	2	1	1	1	1	1	1
5	-7.00	-9.44	-7.75	-5.57	-11.11	-9.89	-10.1	2	1	1	1	1	2	1
6	-6.88	-8.75	-7.71	-5.49	-11.05	-9.73	-10.05	5	3	1	1	1	8	1
7	-6.93	-9.40	-7.67	-5.49	-11.01	-9.80	-9.79	2	1	1	1	1	1	5
8	-6.97	-9.30	-7.54	-5.47	-10.97	-9.72	-9.77	1	1	1	1	1	1	6
9	-6.77	-9.24	-7.51	-5.30	-10.84	-9.60	-9.96	1	1	1	1	1	1	1
10	-6.75	-9.17	-7.36	-5.28	-10.81	-9.35	-9.75	1	1	1	1	1	3	2

Table 2. Symbol, definition and classification of used 43 descriptors

No	symbol	Definition	class
1	MW	molecular weight	constitutional descriptors
2	AMW	average molecular weight	constitutional descriptors
3	Sv	sum of atomic van der Waals volumes(scaled on Carbon atom)	constitutional descriptors
4	Se	sum of atomic Sanderson electronegativities (scaled on Carbon atom)	constitutional descriptors
5	Sp	sum of atomic polarizabilities (scaled on Carbon atom)	constitutional descriptors
6	Ss	sum of Kier-Hall electrotopological states	constitutional descriptors
7	Mv	mean atomic van der Waals volume (scaled on Carbon atom)	constitutional descriptors
8	Me	mean atomic Sanderson electronegativity (scaled on Carbon atom)	constitutional descriptors
9	Mp	mean atomic polarizability (scaled on Carbon atom)	constitutional descriptors
10	Ms	mean electrotopological state	constitutional descriptors
11	nAT	number of atoms	constitutional descriptors
12	nSK	number of non-H atoms	constitutional descriptors
13	nBT	number of bonds	constitutional descriptors
14	nBO	number of non-H bonds	constitutional descriptors
15	nBM	number of multiple bonds	constitutional descriptors
16	SCBO	sum of conventional bond orders (H-depleted)	constitutional descriptors
17	nCIC	number of rings	constitutional descriptors
18	nCIR	number of circuits	constitutional descriptors
19	RBN	number of rotatable bonds	constitutional descriptors
20	RBF	rotatable bond fraction	constitutional descriptors
21	nDB	number of double bonds	constitutional descriptors
22	nAB	number of aromatic bonds	constitutional descriptors
23	nH	number of Hydrogen atoms	constitutional descriptors
24	nC	number of Carbon atoms	constitutional descriptors
25	nO	number of Oxygen atoms	constitutional descriptors
26	nR06	number of 6-membered rings	constitutional descriptors
27	nBnz	number of benzene-like rings	constitutional descriptors
28	Ui	unsaturation index	empirical descriptors
29	Hy	hydrophilic factor	empirical descriptors
30	MR	Ghose-Crippen molar refractivity	properties
31	PSA	fragment-based polar surface area	properties
32	MLOGP	Moriguchi octanol-water partition coeff. (logP)	properties
33	HE	hydration energy	properties[a]
34	AROM	aromaticity (trial)	aromaticity indices
35	HOMT	HOMA total (trial)	aromaticity indices
36	SPH	Spherosity	geometrical descriptors
37	ASP	Asphericity	geometrical descriptors
38	FDI	folding degree index	geometrical descriptors
39	L/Bw	Length-to-breadth ratio by WHIM	geometrical descriptors
40	Mux	Dipole moment in x-direction	electrotopological[a]
41	Muy	Dipole moment in y-direction	electrotopological[a]
42	Muz	Dipole moment in z-direction	electrotopological[a]
43	Mutot	total dipole moment	electrotopological[a]

[a]Calculated by Hyperchem.

Structure-activity relationship. The 43 molecular properties (descriptors) were calculated using Dragon-3.0 and Hyperchem-7.0 softwares. Table 2 includes name and definition of these descriptors which classified as constitutional (1-27), empirical (28-29), properties (30-33), aromaticity (34-35), geometrical (36-39) and electrotopology (40-43).

The experimental log K_I and calculated values of descriptors were listed in Table 3. Linear correlation (R) between the log K_I and these calculated parameters (descriptors), for total ($R7$) and uncompetitive ($R5$) inhibitors were reported in the last two rows of this Table. Correlation between size and inhibition shows that larger size inhibitors have higher inhibition constant. Thus diacids have larger size and higher inhibition constant. Among diacids, DAC3 has smaller size and lower K_I relative to others based on Table 3. Trend of size for diacids is DAC3 < DAC1 < DAC2 and trend of log K_I is also DAC3 < DAC1 < DAC2.

In order to clarifying correlation between log K_I and descriptors, Fig. 3 was plotted. Fig. 3a shows majority of size dependent desciptors have positive correlation with log K_I, while some of them such as Hydration energy (HE) spherocity (SPH), folding degree index and dipole moment have negative correlation. Fig. 3b shows difference between descriptor values of a compe-

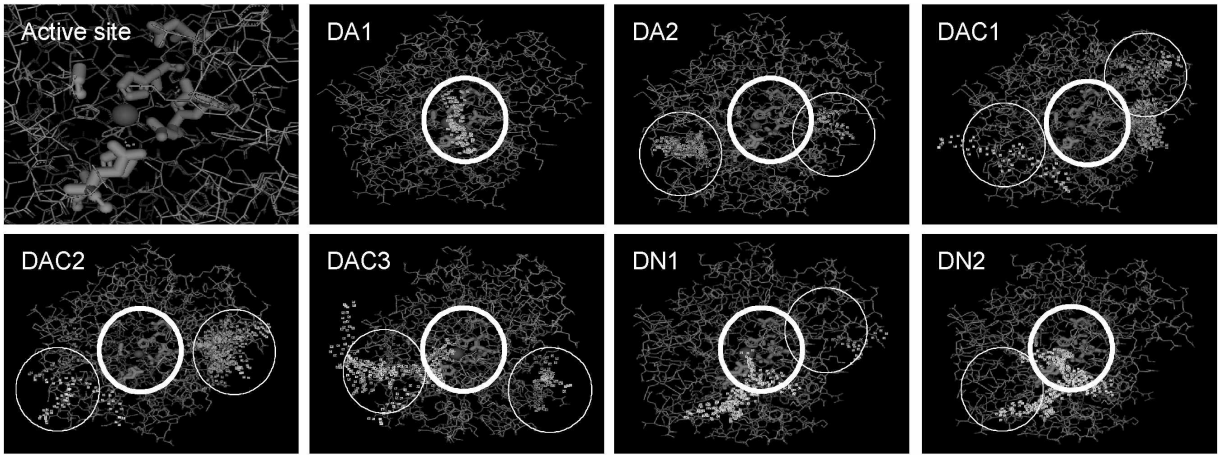


Figure 2. Snapshot of ligand distribution on the different sites of ADA extracted by Autodock 3.0.3. ADA optimized after 6000 ps by GROMACS 3.3 molecular dynamics. Yellow, green and pink color, represent the ligand molecules, active site amino acid and Zn atom, respectively.

Table 3. The values of experimental log K_I and calculated descriptors for studied ligands

Ligand	Log K_I	mass	AMW	Sv	Se	Sp	Ss	Mv	Me	Mp	Ms	nAT	nSK	nBT
DA_1	1.18	200.2	7.42	17.49	27.0	18.2	34.17	0.65	1.00	0.68	2.28	27	15	28
DA_2	1.90	368.4	7.68	32.39	47.9	33.7	60.33	0.67	1.00	0.70	2.15	48	28	51
DAC_1	1.99	716.7	9.19	55.68	80.3	56.9	142.3	0.71	1.03	0.73	2.64	78	54	85
DAC_2	2.12	816.8	9.08	64.88	92.1	66.4	157.0	0.72	1.02	0.74	2.53	90	62	99
DAC_3	1.90	685.7	9.14	54.05	76.8	55.2	136.3	0.72	1.02	0.74	2.62	75	52	82
DN_1	0.86	397.4	8.83	31.62	46.0	32.4	78.33	0.70	1.02	0.72	2.61	45	30	48
DN_2	1.35	473.5	8.61	38.81	55.8	39.9	89.67	0.71	1.01	0.73	2.49	55	36	59
	R7	0.74	0.33	0.76	0.77	0.77	0.69	0.39	0.29	0.44	0.03	0.78	0.74	0.78
	R5	0.60	0.25	0.62	0.64	0.63	0.55	0.05	0.41	0.09	0.09	0.65	0.60	0.65

Table 3. continued

nBO	nBM	SCBO	nCIC	nCIR	RBN	RBF	nDB	nAB	nH	Nc	nO	nR06	nBnz	Ui	Hy
16	12	22	2	2	4	0.14	0	12	12	12	1	2	2	3.70	2.17
31	24	43	4	4	6	0.12	0	24	20	24	2	4	4	4.64	1.56
61	42	85	8	10	8	0.09	6	36	24	42	10	6	6	5.43	-0.80
71	52	100	10	14	8	0.08	6	46	28	50	10	8	8	5.73	-0.80
59	42	83	8	10	4	0.05	6	36	23	41	8	6	5	5.43	-0.80
33	28	47	4	4	2	0.04	1	27	15	23	4	4	3	4.86	-0.80
40	34	58	5	5	2	0.03	2	32	19	29	4	5	4	5.13	-0.80
0.74	0.67	0.72	0.77	0.77	0.84	0.20	0.69	0.64	0.88	0.77	0.67	0.72	0.82	0.61	-0.20
0.60	0.47	0.57	0.64	0.68	0.89	0.65	0.52	0.44	0.80	0.63	0.59	0.54	0.71	0.40	0.10

Table 3. continued

MR	PSA	MLOGP	HE	HOMT	AROM	SPH	ASP	FDI	L/Bw	mux	muy	muz	mutot
61.7	9.2	2.21	-13.7	11.70	1.00	0.89	0.63	0.97	8.7	1.24	0.29	-1.49	1.96
113.1	18.5	3.81	-14.0	23.47	1.00	0.51	0.25	0.91	3.5	0.39	-0.25	-0.97	1.07
186.3	129.7	3.24	-19.3	34.52	0.99	0.88	0.56	0.96	5.8	-1.40	-3.82	-2.44	4.74
219.2	129.7	4.62	-18.8	38.93	0.95	0.78	0.48	0.93	5.1	-1.68	-4.11	-3.69	5.77
181.2	124.1	3.79	-17.7	34.57	0.98	0.97	0.47	1.00	3.5	2.65	-1.53	-0.91	3.19
113.4	93.5	4.76	-14.8	26.00	0.61	1.00	0.29	1.00	1.6	0.18	8.01	0.00	8.01
138.7	105.9	5.75	-15.7	31.03	0.76	1.00	0.29	1.00	1.6	0.19	8.48	0.00	8.48
0.74	0.35	-0.11	-0.77	0.62	0.73	-0.51	0.21	-0.60	0.19	-0.29	-0.83	-0.70	-0.39
0.59	0.11	-0.73	-0.62	0.35	0.89	-0.43	0.60	-0.60	0.89	-0.29	-0.99	-0.82	-0.57

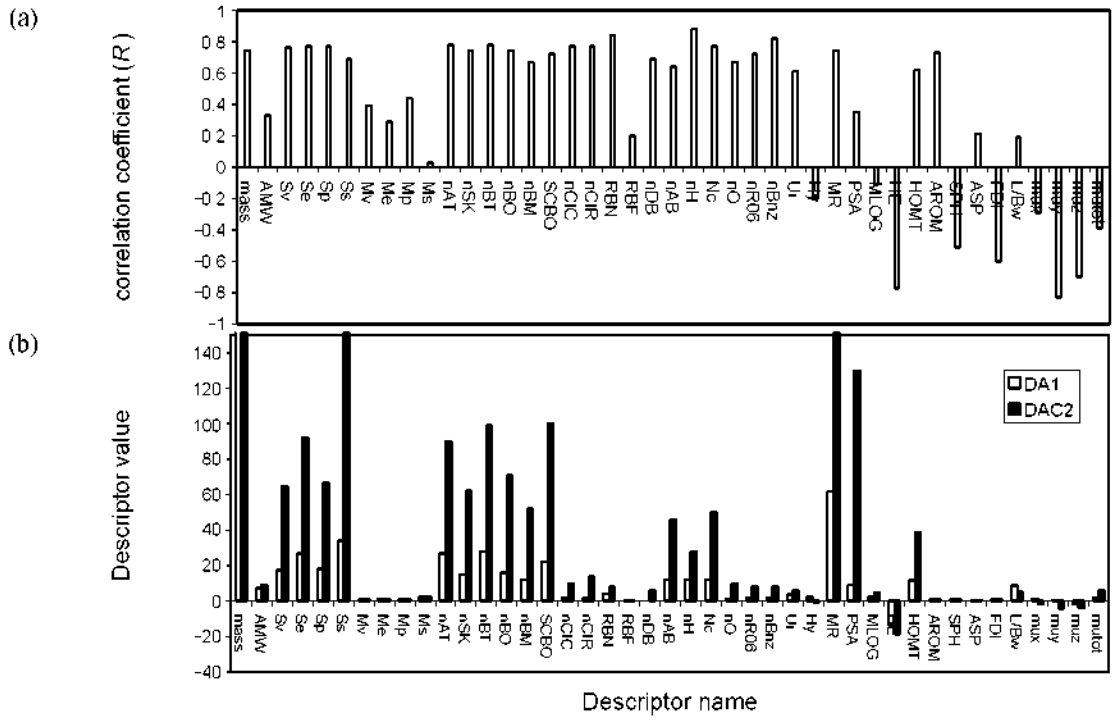


Figure 3. (a) correlation coefficient values between log K_I and descriptors (b) comparison between descriptor values of a competitive (DA1) and uncompetitive (DAC2) inhibitors. Scale of y axis for simplicity in comparison only was shown up to 150.

Table 4. Total variance explained by two principal components or PCs

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	Cumulative %	Total	% of Variance	Cumulative %
PC1	29.59	68.82	68.82	29.59	68.82	29.47	68.54	68.54
PC2	8.43	19.60	88.42	8.43	88.42	8.55	19.87	88.42

Extraction Method: Principal Component Analysis.

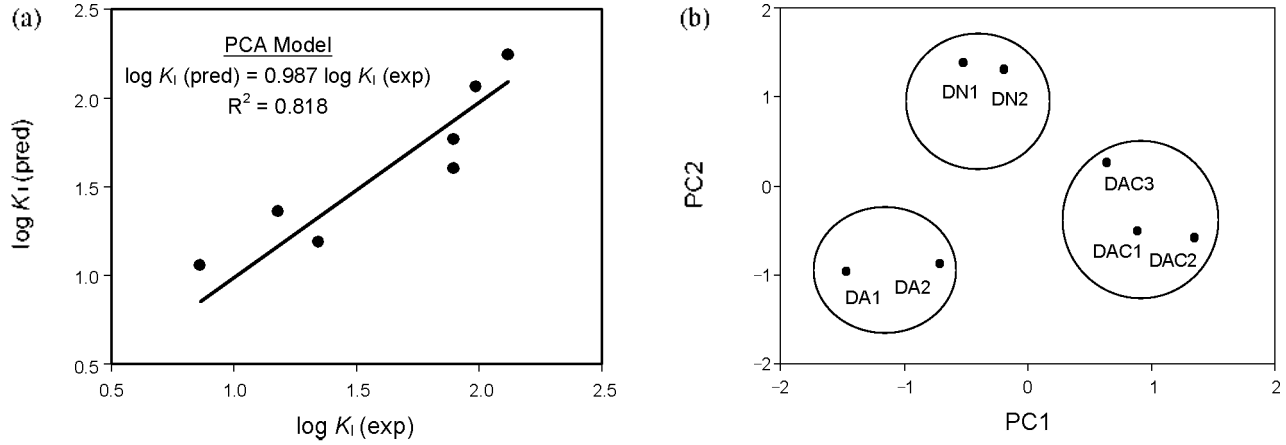


Figure 4. (a) Predicted log K_I by PCA methods against experimental log K_I (b) Plot of factor 2 or principal component 2 (PC2) against PC1.

titive (DA1) and uncompeteive (DAC2) inhibitors that shows DAC2 has larger size descriptors.

Data reduction or principal component analysis (PCA) was used to classifying the descriptors. The descriptors reduced to two factors or principal components (PCs). Table 4 shows that 88% of variation can be described by these two factors. Table 5 has listed two factors obtained by data reduction (PCA). Correlation between experimental log K_I and predicted log K_I by PCA was shown in Fig. 4a. Variation of PC1 *vs* PC2 also depicted in Fig. 4b. It is seen that functional groups properly was distingui

Table 5. Rotated component matrix[a] of reduced 43 descriptors into 2 factors

	Components	
Symbol	Size	Electrotopological
MW	0.99895	
AMW	0.86950	
Sv	0.99679	
Se	0.99653	
Sp	0.99605	
Ss	0.99465	
Mv	0.87638	
Me	0.79828	
Mp	0.89242	
Ms	0.66905	0.534
nAT	0.99506	
nSK	0.99884	
nBT	0.99526	
nBO	0.99852	
nBM	0.98741	
SCBO	0.99858	
nCIC	0.99279	
nCIR	0.97418	
RBN	0.58911	-0.754
RBF		-0.914
nDB	0.94786	
nAB	0.9675	
nH	0.94204	
nC	0.99575	
nO	0.97509	
nR06	0.97691	
nBnz	0.94692	
Ui	0.94832	
Hy	-0.76814	-0.629
MR	0.99618	
PSA	0.88353	
MLOGP		0.756
AROM	0.95491	
HOMT		-0.895
SPH		0.692
ASP		-0.556
FDI		0.779
L/Bw		-0.81
Mux		
Muy	-0.51188	0.832
Muz	-0.61049	0.683
Mutot		0.832
HE	-0.96838	

[a]Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization. Rotation converged in 3 iterations.

shed from each other by a circle.

Kinetic results showed diacids have higher inhibition constant relative to others. DA1 and DN1 are competitive and others ligands are uncompetitive. In addition, docking process was performed on the optimized ADA show that DA1 and DN1 mostly were distributed around active site (Fig. 2). Autodock could not distinguish between noncompetetive and uncompetitive inhibition. It only searches and finds binding sites as well as estimates binding free energy. On the other hand, kind of inhibition is related to position of binding. Since, it will be distinguished from experimental data. Autodock only support some experimental results specially difference between competitive and other two inhibitions i.e. noncompetitive and uncompetitive. Because, in the former case, inhibitor binds to the actives site, while in the last two inhibitors, ligand can be located in the other sites. Computational docking usually gives a new aspect or confirmation for some experimental results. In here by docking studies we support difference between competitive and uncompetitive inhibitors by difference between their binding sites. Thus, they are consistence with competitive inhibition behavior. They are belongs to different groups, diamine and dinitro compounds, but they have a common property, i.e. smaller size relative to other ligands so that they can easily enter to the active site and compete with substrate. Other ligands, specially, diacids have larger size and may could not enter to active site. Thus, they are distributed in the other sites. Larger molecules select different binding sites. Equations 1, 2 also show that, competitive ligands bind only to ADA and uncompetitive ones bind to ADA-substrate complex. It may be also related to higher degree of freedom due to higher number of torsion angle for larger molecules. On the other hand, the active site of ADA (His17, Asp19, Gly184, Glu217, His238, 247 Asp295 and Asp296) has relatively negative charge and also partial charge of oxygen on diacid and dinitro compounds are negative. Thus, the repulsion of charge on diacids and active site amino acids, prohibit the interaction of these ligands and active site. Therefore, diacids

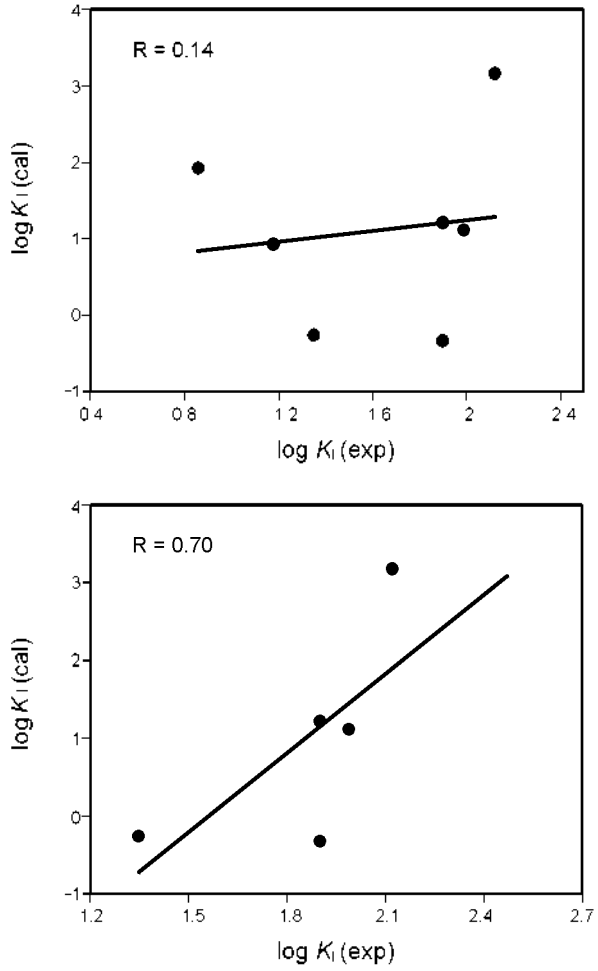


Figure 5. Calculated log K_I (cal) by Autodock against experimental log K_I (exp) for total ligands (up) and uncompetitive ligands (down).

could not bind to active site due to their size and charge.

Correlation between calculated data by docking and experimental inhibition constant (log K_I) for all inhibitors and uncompetitive inhibitors were obtained and has depicted in Fig. 4. This figure shows that correlation of uncompetitive inhibitor is better than all of inhibitors. This difference is due to difference in the nature of inhibition and binding sites in competitive and uncompetitive inhibitors (Fig. 1 and 2).

The experimental log K_I and calculated values of descriptors as well as correlation coefficient (R) between experimental log K_I and these calculated parameters (descriptors), for total ($R7$) and uncompetitive ($R5$) inhibitors were reported in the last two rows of this Table 4.

Correlation between log K_I and majority of descriptors is positive. Namely, values of log K_I increase by enhancing the descriptors values. Other descriptors, including unsaturation index (Ui), hydrophobicity (MLogP), hydration factor (Hy), sphero-city (SPH), folding degree index (FDI), dipole moment (mux, muy, muz and mutot), and Hydration energy (HE) have negative correlation.

In addition, the most of descriptors such as; constitutional descriptors (1-27) are size dependent, while another group (SPH, ASP, L/Bw and FDI) are shape or geometry dependent. Third group (MlogP, Hy, HE,) and fourth group (AROM and HOMT) are hydrophobicity and aromaticity dependent, respectively. Finally, some of them such as dipole moment depend on more than two properties. In here, dipole moment depends on the size and electrical properties (electrotopological).

Correlation between log K_I and size dependent descriptors is positive so that the larger molecules have more log K_I. Also, aspherocity (ASP) and length to breath ratio (L/Bw), (two geometrical or shape dependent descriptors), have positive correlation, and the other two shape dependent factors, spherocity (SPH) and folding degree index (FDI) have negative correlation. Thus, it seems that more spherical molecules and more compact, have lower inhibition constant.

The third group of descriptors related to hydrophobicity. Hydrophobicity factor (MlogP), hydration energy (HE) and hydration factor (Hy) have direct correlation with log K_I. More positive hydration energy corresponds to higher MlogP and Hy and less tendency for hydration.

Fourth group, are electronic, size and shape dependent dipole moment, which have negative correlation with log K_I. The most correlated descriptor which obtained from stepwise selection, is the number of hydrogen. This descriptor increases the log K_I.

Data reduction by principal components analysis was used for classification and reduction of descriptors to a few number of principal components (PCs). Table 4 listed only PC1 and PC2 that shows 88% of variation can be described by these two factors. Table 5 has listed two factors obtained by data reduction (PCA). The first factor is mostly related to size and the other is related to electrical and geometrical properties so that it is called as electrotopological descriptors which is common property between electronic and shape properties. For example hydration and shape are electronic and SPH, ASP, L/Bw, FDI, are shape descriptors, when two new reduced descriptors were studied by multiple linear regression the following equation was obtained:

$$\log K_I = 0.346\ (\textit{Size factor}) - 0.271\ (\textit{electrotopological factor}) + 1.6 \quad (3)$$

It is shown that the log K_I increases and decreases with size and electrotopological factors respectively. Thus, larger molecule has higher inhibition constant. We also reported previously that K_I for non-nucleosides inhibitors increases by size and shape.[20] Fig. 4a shows the correlation between predicted by equation (3) and experimental values of log K_I. Fig. 4b shows the variation of factor 2 (PC2) versus factor 1 (PC1). It indicates that each of three groups which have similar properties were surrounded by a circle. It means that, we can distinguish the similar groups only from descriptors without using any other techniques.

Conclusion

Effect of different organic aromatic ligands on the structure and activity of ADA was studied by experimental and computational methods. All of ligands showed inhibition behavior, some of them were mixed of competitive and non-competitive (one of diaamines, and one of dinitros) and the others (mostly diacids) were uncompetitive inhibitors. There is a logical correlation between experimental and calculated inhibition constant for uncompetitive inhibitors. Calculated binding energy and binding site of ligands showed that competitive inhibitors are relatively small ligands, and is distributed around active site of enzyme with higher cluster rank. The experimental results also showed that competitive inhibitors bind to the active site, whereas uncompetitive ligands are larger and have different binding site in accord to experimental results. Results of structure-property relationship showed that, larger, more hydrophobe, less spherical and more aromatic ligands have higher inhibition constant.

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