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Original Article

Rationalization of Physicochemical and Structural Requirement of Some Substituted 5-(Biphenyl-4-ylmethyl)Pyrazole as Angiotensin II Receptor Antagonist: A QSAR Approach

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Abstract

A series of angiotensin II (A II) receptor antagonist of some substituted 5-(biphenyl-4-ylmethyl) pyrazole were subjected to QSAR analysis using Hansch and Fujita-Ban model, by using combination of thermodynamic, electronic, spatial descriptor and presence or absence of substituent respectively. Several QSAR model were obtained using stepwise regression analysis. Two models from both the method were selected on the basis of the statistical value that shows good significance with AII antagonistic activity. The best QSAR models further validated by leave one out cross validation method. The studies have help to ascertain the role of different substituent in explaining the observed antagonistic activity of this analogue. From Fujita-Ban model, it is predicted that butane and propane at position 1, COOH at position 4 are essential for activity. Group like CH_2CF_3 at position 1 and COOH in place of tetrazole at R_3 position contribute negative to the biological activity. In Hansch model it is predicted that molar refractivity at the 1 and 3 position shows positive contribution to the biological activity. Field effect at position 4 also shows positives contribution to the biological activity. Hydrogen donar at position R^3 and field effect at position 1 contributes negatively to the biological activity.

Keywords: Angiotensin II receptor antagonist; AT1 receptor; Fujita-Ban analysis; Hansch analysis; QSAR; Renin angiotensin system.
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1. Introduction

The vasoactive hormone angiotensin II (AII) produced by the rennin-angiotensin system (RAS) is a potent regulator of blood pressure, homeostasis, fluid volume and electrolyte balance in mammals [1]. The clinical success achieved by angiotensin converting enzyme (ACE) inhibitors in the treatment of the hypertension and congestive heart failure has made the RAS a major focus for the discovery of novel hypertensive agents. However, ACE also has kinase activity, and this lack of specificity has been implicated in the occasional side effect of ACE inhibitors such as dry cough and angiodema [2]. With the development of A II receptor antagonist, a

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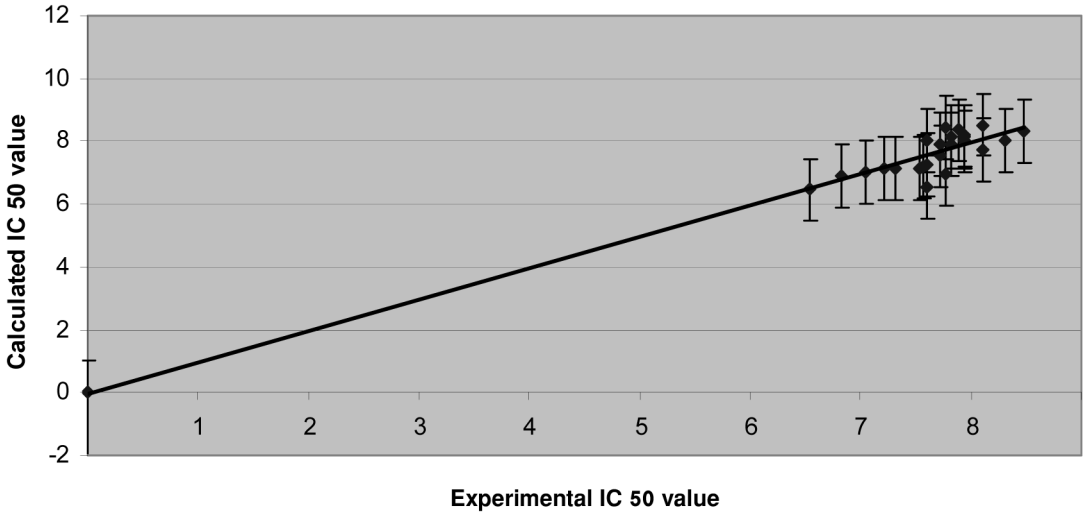


Figure 1. A plot of observed *vs.* calculated angiotensin II antagonistic activity with residual presentation using Fuzita- ban QSAR model.

specific attempt to inhibit the activity of RAS has become the main pharmacological approach.

There are at least two distinct AII receptor subtype, designated as AT_1 and AT_2 [3]. The AT_1 receptor is G-protein coupled and mediates most of the known physiological effect of AII, including the maintenance of blood pressure [4]. The AT_2 receptor is thought to be involved in fetal growth and adult tissue repair and remodeling, especially in cardiovascular system [5]. Losartan, the most advanced nonpeptide AII antagonist, mediates its effect by blocking the AII AT_1 receptor subtype [6]. Due to our interest in various structural and new potential treatments for hypertensive disorders, we subjected a series of some substituted methyl pyrazole to QSAR analysis. QSAR is an important tool in drug designing technique [7, 8] to achieve different objective like diagnosis of mechanism of action of drug, quantitative prediction of biological activity of compound, classification of compound into various classes, optimization of lead compound and refinement of synthetic target. To achieve

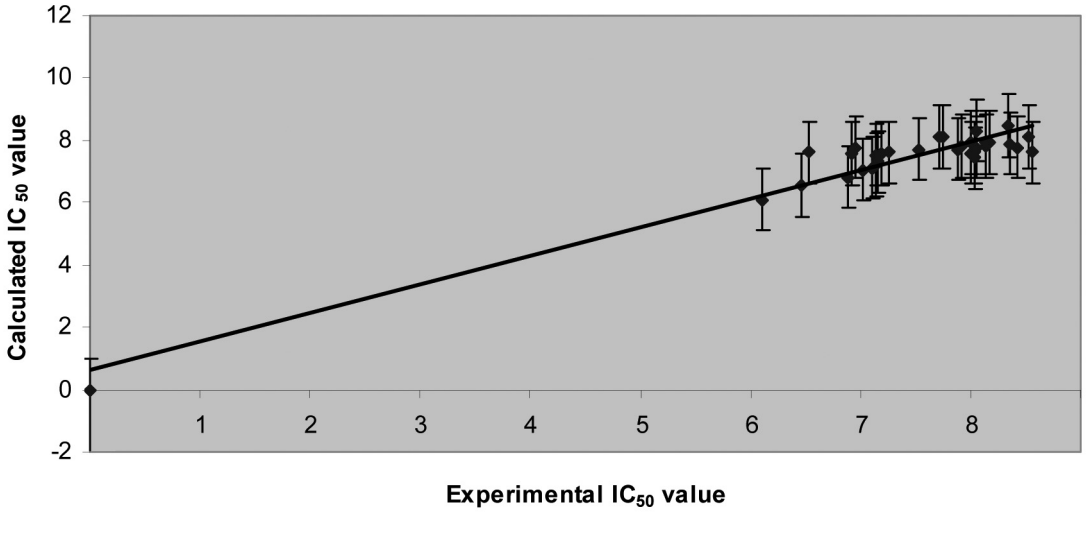
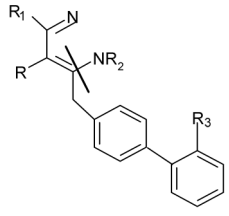


Figure 2. A plot of observed *vs.* calculated angiotensin II antagonistic activity with residual presentation using Hansch QSAR model.

Table 1. Analogs of 5-(biphenyl-4- ylmethyl) pyrazoles and their biological activity.



S. No.	R(4)	R_1(3)	R_2(1)	R_3	IC_{50}[a]	$LogIC_{50}$[b]
1	COOH	H	Bu	tetrazolyl	9.1	8.041
2	COOH	Me	Bu	tetrazolyl	2.2	8.567
3	COOH	Me	Pr	tetrazolyl	9.3	8.031
4	COOH	Me	Et	tetrazolyl	120	6.920
5	COOH	Me	CH_2CF_3	tetrazolyl	770	6.113
6	COOH	Me	Ph	tetrazolyl	78	7.102
7	COOH	Et	Bu	tetrazolyl	8.7	8.060
8	COOH	I-Pr	Bu	tetrazolyl	7.3	8.136
9	COOH	Pr	Bu	tetrazolyl	10	8
10	COOH	Ph	Bu	tetrazolyl	9	8.045
11	COOH	Et	Pr	tetrazolyl	3.7	8.431
12	COOH	I-Pr	Pr	tetrazolyl	6.7	8.173
13	COOH	C-Pr	Pr	tetrazolyl	4.4	8.356
14	COOH	Butyl	Pr	tetrazolyl	3	8.522
15	COOH	CH_2Ph	Pr	tetrazolyl	4.6	8.337
16	COOH	Me	Bu	tetrazolyl	290	6.537
17	COOH	Et	Bu	tetrazolyl	110	6.958
18	COOH	I-Pr	Pr	tetrazolyl	490	6.309
19	$COOCH_3$	Me	Bu	tetrazolyl	55	7.259
20	$COOCH_3$	t-Bu	Pr	tetrazolyl	19	7.721
21	$CONH_2$	Me	Bu	tetrazolyl	70	7.154
22	$CONH_2$	C-Pr	Pr	tetrazolyl	9.9	8.004
23	CH_2OH	Me	Bu	tetrazolyl	350	6.456
24	CHO	Me	Bu	tetrazolyl	74	7.130
25	$COCH_3$	Me	Bu	tetrazolyl	65	7.187
26	H	t-Bu	Pr	tetrazolyl	94	7.026
27	COOH	Me	Bu	SO_2NHPh	12	7.920
28	COOH	Me	Bu	$SO_2NHCOOBu$	13	7.886
29	COOH	Me	Bu	SO2NHCOOBu	30	7.522
30	COOH	C-Pr	Pr	SO_2NHPh	18	7.744
31	$CONH_2$	C-Pr	Pr	SO_2NHPh	7.4	8.130
32	COOH	I-Pr	Pr	COOH	130	6.886
33	COOH	I-Pr	Pr	$NHSO_2CF_3$	74	8.041

[a]Concentration of 50 percent antihypertensive activity data against against A II receptor; [b]Negative logarithm of IC_{50} activity data.

this target, various QSAR model have been used such as Hansch, Free-Wilson and Fujita-Ban model [9]. To gain insight into the structural and molecular requirement influencing the AII antagonistic activity, we herein describe QSAR analysis of substituted pyrazole derivatives. A QSAR Model has been obtained for AII antagonistic activity. The relevance of the model for the design of novel derivatives should be assessed only in terms of predictivity, internal or external, but also in terms of their ability to provide a

Table 2. Substituent constants, calculated, and predicted value with residual and Z-score data analogs of 5-(biphenyl-4- ylmethyl) pyrazoles and their data used in Fuzita-ban analysis.

S. No.	CH_2CF_3(N1)	Bu2(N1)	Pr2(N1)	COOH	$COOH_3$ exp.	Calculated	$Cal._{Res.}$	Z-score
1	0	1	0	1	0	8.041	7.945	0.095	0.311
2	0	1	0	1	0	8.567	7.945	0.622	2.020
3	0	0	1	1	0	8.031	8.324	-0.293	-0.952
4	0	0	0	1	0	6.920	6.765	0.154	0.502
5	1	0	0	1	0	6.113	6.113	0	0.403
6	0	0	0	1	0	7.102	6.765	0.336	1.093
7	0	1	0	1	0	8.060	7.945	0.115	0.374
8	0	1	0	1	0	8.136	7.945	0.191	0.621
9	0	1	0	1	0	8.00	7.945	0.054	0.177
10	0	1	0	1	0	8.045	7.945	0.100	0.326
11	0	0	1	1	0	8.431	8.324	0.107	0.347
12	0	0	1	1	0	8.173	8.324	-0.150	-0.489
13	0	0	1	1	0	8.356	8.324	0.031	0.103
14	0	0	1	1	0	8.522	8.324	0.198	0.643
15	0	0	1	1	0	8.337	8.324	0.012	0.040
16	0	0	0	1	0	6.537	6.765	-0.228	-0.74
17	0	0	0	1	0	6.958	6.765	0.192	0.625
18	0	0	0	1	0	6.309	6.765	-0.456	-1.48
19	0	1	0	0	0	7.259	7.172	0.086	0.282
20	0	0	1	0	0	7.721	7.552	0.169	0.548
21	0	1	0	0	0	7.154	7.172	-0.017	-0.057
22	0	0	1	0	0	8.004	7.552	0.452	1.468
23	0	1	0	0	0	6.456	7.172	-0.767	-2.326
24	0	1	0	0	0	7.130	7.172	-0.041	-0.135
25	0	1	0	0	0	7.187	7.172	0.014	0.047
26	0	0	1	0	0	7.026	7.552	-0.525	-1.705
27	0	1	0	1	0	7.920	7.945	-0.024	-0.079
28	0	1	0	1	0	7.886	7.945	-0.059	-0.191
29	0	0	0	1	0	7.522	7.945	-0.422	-1.370
30	0	0	1	1	0	7.744	8.324	-0.580	-1.883
31	0	0	1	1	1	8.130	7.552	0.578	1.878
32	0	0	1	0	1	6.886	6.886	0	0.346

chemical and structural explanation of their binding interaction. Here, we propose general models from two methods for the antagonist and present minimal structural requirement for an AII antagonist. These results should serve as a guideline in design of more potent and selective AII antagonist.

2. Materials and methods

The AII antagonistic activity data of 5-(Biphenyl-4-ylmethyl) pyrazoles analogs (Figure 1) were taken from the reported work of Carmen Almansa *et al.* [10]. The antihypertensive activity data against AII receptor (IC_{50} in nm) was converted to negative logarithmic mole dose (-logIC_{50}) in order to reduce the skewness of the data set, for quantitative structure activity relationship analysis (Table 1).

Initially, series was subjected to Fujita-Ban analysis using regression technique in order to estimate the *de novo* contribution of substituents to the activity of the molecules. Further Hansch approach was carried out to established correlations between AII antagonistic activity and various substituents constants at position R_1, R_2, R_3, R_4 and R'of the molecule. Values of the substituents constants like hydrophobic (π), steric (Molar refractivity or MR), hydrogen acceptor (HA), hydrogen donor (HD) and electronic (field effect or F, resonance effect or R and

Table 3. Substituent constants, calculated, and predicted value with residual and Z-score data analogs of 5-(biphenyl-4- ylmethyl) pyrazoles and their data used in Hansch analysis.

S. No	F2(R_2)	F(4)	MR1(R_2)	MR3(R_1)	Hdon 3(R_3)	Exp.	Calculated	Cal.$_{Res.}$	Z-score
1	-0.06	0.33	1.03	18.33	0	8.041	7.437	0.603	1.385
2	-0.06	0.33	5.65	18.33	0	8.567	7.603	0.964	2.210
3	-0.06	0.33	5.65	18.33	0	8.031	7.603	0.427	0.041
4	-0.05	0.33	5.65	18.33	0	6.920	7.568	-0.647	-1.485
5	0.37	0.33	5.65	18.33	0	6.113	6.095	0.018	0.981
6	0.08	0.33	5.65	18.33	0	7.102	7.111	-0.208	-0.021
7	-0.06	0.33	10.30	18.33	0	8.060	7.771	0.289	0.663
8	-0.06	0.33	14.96	18.33	0	8.136	7.939	0.197	-0.615
9	-0.06	0.33	14.96	18.33	0	8	7.939	0.060	0.139
10	-0.06	0.33	25.36	18.33	0	8.045	8.313	-0.268	0.453
11	-0.06	0.33	10.30	18.33	0	8.431	7.771	0.660	1.510
12	-0.06	0.33	14.96	18.33	0	8.173	7.939	0.234	-0.331
13	-0.06	0.33	13.53	18.33	0	8.356	7.887	0.468	1.075
14	-0.06	0.33	19.61	18.33	0	8.522	8.106	0.416	0.954
15	-0.06	0.33	30.01	18.33	0	8.337	8.481	-0.144	0.538
16	-0.06	0.33	5.65	18.33	0	6.537	7.603	-1.065	-1.863
17	-0.06	0.33	10.30	18.33	0	6.958	7.771	-0.812	-2.444
19	-0.06	0.33	5.65	18.33	0	7.259	7.603	-0.343	-0.788
20	-0.06	0.33	19.62	18.33	0	7.721	8.107	-0.385	-0.884
21	-0.06	0.24	5.65	18.33	0	7.154	7.315	-0.160	-0.368
22	-0.06	0.24	13.53	18.33	0	8.004	7.599	0.404	0.386
23	-0.06	0	5.65	18.33	0	6.456	6.547	-0.091	-0.209
24	-0.06	0.31	5.65	18.33	0	7.130	7.539	-0.408	-0.937
25	-0.06	0.32	5.65	18.33	0	7.187	7.571	-0.384	-0.881
26	-0.06	0	19.62	18.33	0	7.026	7.050	-0.024	-0.055
27	-0.06	0.33	5.65	42.85	1	7.920	7.822	0.098	0.225
28	-0.06	0.33	5.65	40.03	1	7.886	7.717	0.168	0.928
29	-0.06	0.33	5.65	40.03	1	7.522	7.717	-0.194	-0.446
30	-0.06	0.33	13.53	42.85	1	7.744	8.106	-0.361	-0.198
31	-0.06	0.24	13.53	42.85	1	8.130	7.818	0.312	0.716
32	-0.06	0.33	14.96	6.93	1	6.886	6.823	0.063	0.144
33	-0.06	0.33	14.96	17.54	1	7.130	7.217	-0.086	-0.829

Hammett's constant or σ), taken from the reported work of Hansch *et al.* [11] were selected as independent variable and biological activity as dependant variable. Stepwise multiple regression analysis [12, 13] was performed to derive QSAR model and in addition to advance statistical validation procedure to select best QSAR model from high populated QSAR model by software Valstat [14]. Resulting QSAR model assessed through a number of statistics obtained in conjunction with such calculation: Correlation coefficient (r), standard deviation (s), F-test, Bootstrapping (r^2), Cross validation (Q^2), chance statistics (evaluated as the ratio of the equivalent regression equations to the total number of randomized sets; a chance value of 0.001 corresponds to 0.1% chance of fortuitous correlation), outliers (on the basis of Z-score value) and leave one out method (Loo) was employed for cross validation of the best equation.

3. Results and discussion

Fujita-Ban analysis gave significant tri-variant regression expression (Equation 1) which account for more than 84% variance in activity with *de novo* contribution of substituents to the activity of the molecules.

BA=[6.06(±0.24)]+(CH_2CF_3)2(N1)[-0.65(±0.43)]+Pr2 (N1)[1.43(±0.21)]+Bu2 (N1)[1.15(±0.21)]+COOH[0.70(±0.16)]+ COOH3[-1.31(±0.41)] (Equation 1)

n=33, r=0.84, r^2=0.718, variance=0.156, std=0.395, F=13.7911, FIT=118.88

The equation showed moderate corelation coefficient value 0.84 with one outlier. The fitness of model can be improved by removing outlier, hence remaining 32 compounds were considered for the QSAR analysis.

The optimized model showed equation have statistically significant corelation and significant for antagonistic activity.

BA=[5.99(±0.20)]+(CH_2CF_3)2(N1)[-0.65(±0.36)]+Pr2(N1)[1.5(±0.18)]+Bu2(N1) [1.17(±0.18)]+COOH[0.77(±0.14)]+$COOH_3$ [-1.43(±0.35)] (Equation 2)

n=32, r=0.895, r^2=0.801, variance=0.113, std=0.336, F=20.96, FIT=187.726

Fujita-Ban analysis (Table 2) of AII antagonistic data of 5-(Biphenyl-4-ylmethyl) pyrazole inferred that the 1st position of pyrazole ring is favorable for the butane, propane and the 4th position of ring is favorable for COOH group, respectively. Group like CH_2CF_3 at position 1and COOH in place of tetrazole at 2' position contribute negative to the biological activity. *De novo* contribution of groups also help in understanding of binding of molecule with AII receptor by means of possible hydrogen bond interaction in between COOH group at position 4 on the ring and polar positive charge region of AII receptor active site, second possible hydrophobic interaction of 1 position group of hydrocarbon and lipophillic pocket of AII receptor active site.

Hansch analysis 33 compounds was subjected to stepwise multiple linear regression analysis, in order to develop QSAR between antagonistic activity at A II as dependent variables and substituents constants as independent variables, several significant models were obtained. Amongst them best model No. 3 selected on the basis of statistical parameter as follows

BA=[5.54(±0.53)]+F2 [-3.35(±1.30)]+F [2.91(±1.21)]+MR_1[0.031(±0.015)]+MR_3 [0.036(±0.015)]+Hdon3[-0.60(±0.33)] (Equation 3)

n=33, r=0.66, r^2=0.43, variance=0.31, std=0.55, F=4.24, FIT=36.5

This equation showed moderate correlation coefficient value 0.66 with one outlier (Comp. No. 18). The fitness of the model can be improved by removing outlier. Hence remaining 32 compounds were considered for the QSAR analysis of AII antagonistic activity. The optimized model gave statistically significant correlation for antagonistic activity.

BA=[5.4(±0.46)]+F2[-3.50(±1.11)]+F [3.20(±1.04)]+MR_1[0.03(±0.013)]+MR_3 [0.037(±0.013)]+Hdon3[-0.69(±0.28)] (Equation 4)

n=32, r=0.747, r^2=0.558, variance=0.226, std=0.476, F=6.58, FIT=58.98

Only high correlation coefficient is not enough to select the equation as a model and hence various statistical approaches were used to confirm the robustness and practical applicability of equations. The equations 3 tested for presence of outliers one outlier was present suggested that although equation having good correlation coefficient but they are unable to explain the deviation of prediction of activity of a compound which involved in generation of expression. Equations 4 in randomize biological activity test showed probability of chance correlation were less than 0.1%.

The equation was has better correlation

coefficient (r=0.74), which accounts for more than 74.0% of the variance in the activity, the equation shows, that in multi-variant model, dependent variable can be predicted from a linear combination of the independent variables. The *p* value is less than 0.003 for each physiochemical parameters involved in model generation. The data showed overall internal statistical significance level better than 99.9% as it exceeded the tabulated $F_{(4, 27)\alpha\ 0.001}$=6.51 The model was further tested for outlier by Z-score method and one compound was found to be an outlier (Table 3) which suggested that the model is able to explain the structurally diverse analogs that is helpful in designing of more potent compounds using physiochemical parameters. Leave one out cross validation method was employed for prediction of the activity (Figure 1 & 2 and Table 3), cross-validated squared correlation co-efficient (Q^2=0.710), predictive residual sum of square (S_{PRESS}=0.426) and standard error of prediction (S_{DEP}=0.376) suggested good internal consistency as well as predictive ability of the biological activity with low S_{DEP}. Randomized biological activity test (Chance<0.001) revealed that the results were not based on chance correlation. In general, the model fulfills the statistical validation criteria in a significant echelon to achieve theoretical base for proposing more active compounds which is helpful for rationalizing the interaction between molecule and receptor. Molar refractivity at the 1 and 3 positions shows positive contribution to the biological activity. Field effect at position 4 also shows positives contribution to the biological activity. Hydrogen donor at position 2' and field effect at position 2 contributes negatively to the biological activity.

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Refrences

[1] Ferrario CM. The renin-angiotensin system: Importance in physiology. *J Cardiovasc Pharm* 1990; 15: S1-5.

[2] Cockcroft JR, Sciberras DG, Goldberg MR, Ritter MJ. Comparision of angiotensin converting enzyme inhibition with angiotensin II receptor antagonism in the human forearm. *J Cardiovasc Pharm* 1993; 22: 579-84.

[3] De Gaspro M, Jhussain A, Alexander W, Cattr KJ, Chiu AT, Drew M, Good Freiend T, Harding JW, Inagani T, Timmermans PBMWM. Proposed update of angiotensin receptor nomenclature. *hypertension* 1995; 25: 924-39.

[4] Timmermans PBMWM, Benfield P, Chiu AT, Herblin BF, Wong PC, Smith RD. *Am J Hypertension* 1992; 5: 2215-355.

[5] Nahmias C, Strosberg AD. The angiotensin AT2 receptor: Searching for signal transduction pathway and physiological function. *Trends Pharmacol Sci* 1995; 16: 223-5.

[6] Carini DJ, Dunica JV, Aldrich PE, Chiu AT, Johnson AL, Pieerce ME, Price WA, Santella JB, Wells GJ, Wexler RR, Wong PC, Yoo S, Timmermans, PBMWM. Nonpeptide angiotensin II receptor antagonist: The discovery of series of n-(biphenylmethyl) imidazoles as potent, orally-active antihypertensives. *J Med Chem* 1991; 34: 2525-7.

[7] Tute M.S. History and objectives of quantitative drug design. In: Hansch C, (editor). *Comprehensive medicinal chemistry*. Vol 2, 4. New York: Pergamon Press, 1990. p. 579.

[8] Kubinyi H. In: Wolff ME, (editor), *Berger's medicinal chemistry and drug discovery.* 5th ed. Vol. 1. New York: John Wiley and Sons Inc., 1995; p. 91.

[9] Sarathy KP, Girdhar R, Yadav MR. QSAR study by Fujita- Ban model of some substituted α, β-diaryl five membered hetrocycles as Cox-1/ Cox-2 inhibitors. *Indian Drugs* 2003; 40: 9-18.

[10] Almansa C, Gomez LA, Cavalcanti FL, de Arriba AF, Rafanell JG, Forn J. Synthesis and structure-activitry relationship of a new series of potent AT1 selective angiotensin II receptor antagonists: 5-(Biphenyl-4-ylmethyl) pyrazoles. *J Med Chem* 1997; 40: 547-58.

[11] Hansch C, Leo A. *Substituent constants for correlation analysis in chemistry and biology.* New York: John Wiley and Sons, 1979; p. 114.

[12] Draper NR, Smith H. *Applied regression analysis.* New York: John Wiley and Sons, 1996; p. 193.

[13] Chakarvarti SK, Ajmani S, Chaturvedi SC. SMRAILS-A lotus 1-2-3 spreadsheet for stepwise

multiple parameter regression analysis applied to QSAR analysis. *Indian J Pharm Sci* 1998; 60: 371-4.

[14] Gupta AK, Arokia BM, Khakhedikar SG. Valstat: Valiation program for quantitative structure acyivity relationship studies. *Indian J Pharm Sci* 2004; 66: 396-402.