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3D QSAR Analysis of 6-Isoxazolinyl and Isoxazolidinyl Substituted Quinazolinones as Angiotensin-II Receptor Antagonists

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Semi-emperical AM1 calculation was performed on 6-isoxazolinyl and isoxazolidinyl substituted quinazolinones as A-II receptor antagonists. The best QSAR equation obtained from 19 analogs of training set molecules revealed statically significant equation with following stastics; coefficient of determination (r^2) = 0.645525, cross validated value (q^2) = 0.894626, standard error of estimate (s) = 0.117655, fisher's F-value = 3.434560. Further, robustness and predictivity of the model was assessed externally by r^2 predicted value (0.114659). This analysis revealed that steric, thermodynamic and electronic interactions are responsible for A-II receptor antagonistic activity. Based on QSAR results new analogues were designed and their IC_{50} values were determined theoretically.

Key Words: Quantitative structure activity relationship, Angiotensin-II, Quinazolinones.

INTRODUCTION

The discovery by DuPont[1] of Losartan, a potent, specific, orally active, non-peptide A II receptor antagonist for the treatment of hypertension and congestive heart failure has led to an explosion of over 250 papers and patent application by various pharmaceutical companies in this field[2]. A-II receptor antagonists have been expected to lack adverse effects[3-6] observed with the use of ACE inhibitors. Dry cough, angioedema, aplastic anemia, conjunctivitis, headache, parenthesis and sinus tachycardia are associated side effects of ACE inhibitors[7] that are not associated with AII receptor antagonists. The potential ability of non-peptide A II antagonists to surmount these problems has made them exceedingly attractive targets for drug development. Since the last decades, several A II receptor antagonists have

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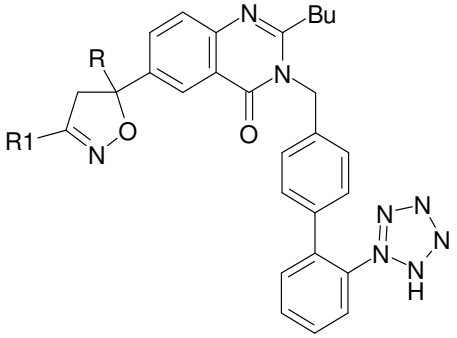
been developed and plethora of molecular modeling studies including QSAR investigations have been reported worldwide. Levin *et al.*[8] introduced 6-isoxazolinyl and isoxazolidinyl substituted quinazolinones lead structure as A II receptor antagonists. Inspite of unprecedented rate of progress in this therapeutic area, no molecular modeling-QSAR studies have been reported for these lead structures. In this paper, an effective QSAR analysis for 6-isoxazolinyl and isoxazolidinyl substituted quinazolinones series of molecules have been performed.

EXPERIMENTAL

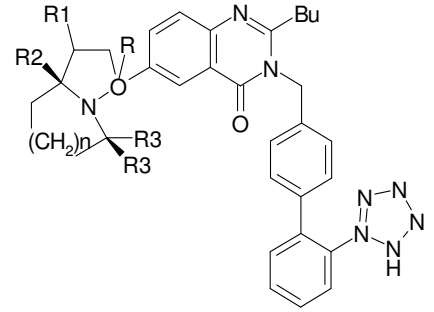
The structure and IC_{50} value of isoxazolines and bicyclic isoxazolidines are shown in Tables 1 and 2. A-II receptor antagonistic activity data (IC_{50} value in μg/mL) were converted to negative logarithm (pIC_{50}) to get the linear relationship. The sketched 2D structures were converted to 3D structures and were subjected to energy minimization using semi-emperical quantum mechanics module implemented on molecular orbital package (MOPAC) version, fixing maximum iteration limit to 1000, root mean square (RMS) gradient to 0.001 kcal/mol and applying the theory of AM1 Hamiltonian using closed shell restricted wave function. In order to describe the observed activity in a better way, thermodynamic, electronic and steric parameters were calculated for the energy minimized and geometrically optimized structures[9] from MM2 server.

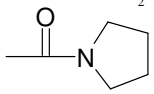
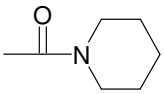
TABLE-1
in vitro BINDING OF ISOXAZOLINES



Compd.	R	R1	IC_{50}	pIC_{50}
1	H	P-CH_3-Ph	70	0.542
2	H	4-CH_3-furyl	180	0.4434
3	H	4-CH_3-thienyl	410	0.4171
4	H	CO_2Et	30	0.1694
5	H	CO_2H	500	0.4311
6	CH_3	CO_2Et	140	0.4659

TABLE-2
in vitro BINDING OF ISOXAZOLIDINES



Compd.	n	R	R1	R2	R3	IC_{50}	pIC_{50}
1	1	α-H	H	H	H	32	0.6644
2	1	α-H	H	H	Me	39	0.6285
3	2	α-H	H	H	H	38	0.6330
4	2	α-H	H	Me	H	65	0.5516
5	1	α-H	α,βCO_2Et	H	H	69	0.5440
6	1	α-Me	H	H	H	27	0.6988
7	1	β-Me	H	H	H	55	0.5746
8	1	α-Me	H	H	Me	65	0.5515
9	1	β-Me	H	H	Me	70	0.5420
10	2	α-Me	H	H	H	47	0.2232
11	2	β-Me	H	H	H	22	0.7440
12	1	α-Et	H	H	H	83	0.5023
13	1	β-Et	H	H	H	58	0.5670
14	1	α-Ph	H	H	H	56	0.572
15	1	α-CO_2Me	H	H	H	79	0.5269
16	1	α-CH_2OH	H	H	H	35	0.6476
17	1	α-CO_2H	H	H	H	46	0.6014
18	1	α-$CONMe_2$	H	H	H	57	0.5695
19	1		H	H	H	69	0.2645
20	1		H	H	H	61	0.6601

Twenty six derivatives selected for the study were divided into training set of 19 compounds and test set of 7 compounds by random selection method. Quantitative model building was accomplished through sequential multiple regression analysis using the method of least square in val_stat software. The stastical quality of the models was gauged by parameters like correlation coefficient (r) or coefficient of determination (r^2), standard

error of estimate (s), fisher's F-value. The significance of individual descriptor is gauged from its standard error. The correlation matrix among the various predictor variables was examined regularly in order to avoid simple colinearity problem. The parameters having intercorrelation above 0.5 and those are not significant at 99.9 % confidence interval were not considered whilst deriving QSAR models. To ascertain the predictivity of the model, internal validation using leave one out (LOO) cross-validation process, bootstrapping technique and randomization test were performed.

The external predictivity of the models was assessed using r^2_{pred}. The presence of outlier was confirmed by higher Z-score value.

RESULTS AND DISCUSSION

The A-II receptor antagonistic activity retained for study was obtained from the renin dependent aorta coarected rat model of hypertension[6]. In order to explore the nature of interactions of R, R1, R2 and R3 groups with A-II receptor, statistically significant QSAR models were developed. The best correlations obtained through multiple regression equations are discussed below.

Model 1: BA = [-3.75129 (± 3.01091)] + CC [0.169552(± 0.228287)] + MTI [0.0757497(± 0.0512852)] + SOD [-0.000447307 (± 0.000311253)] n = 19, r = 0.638123, r^2 = 0.407201, variance = 0.0138426, std = 0.117655, F =3 .43456.

CC = Cluster count, MTI = molecular topology index and SOD = sum of degree

Validation parameters: q^2 = 0.140065, Spress = 0.141706, S_{DEP} = 0.125909, r^2_{pred} = -0.0180909

Optimized model no. 1: BA = [-4.0947(± 2.03571)] + CC [0.178128 (± 0.153878)] + MTI [0.0837055(± 0.0347746)] +SOD [-0.000505642 (± 0.000211655)].

n = 18, r = 0.814447, r^2 = 0.663325, variance = 0.00619772, std = 0.0787256, F = 9.19436.

Validation parameters: q^2 = 0.379655, Spress = 0.106863, S_{DEP} = 0.0942442, r^2_{pred} = 0.0525294.

The derived model explains 66.33 % of variance in observed activity. The triparametric equation describes the A II antagonistic activity of 19 analogs as a function of their steric (CC, MTI and SOD) properties. Positive contribution of topology index and cluster count confirms the steric hindrance caused by increasing ring size at R, R1, R2 and R3 position. Compound 16 is outlier.

Model 2: BA = [1.67203(± 1.4962)] + Stretch-Bend Energy [-0.31285 (± 0.537512)] + Non-1, 4 VDW [0.0275453(± 0.030749)] + MR [-0.00309844 (± 0.00328904)]

n = 19, r = 0.482468, r^2 = 0.232775, variance = 0.0151855, std = 0.12323, F = 1.517

VDW = vander waals forces, MR = molar refractivity

Validation parameters: r^2_{bsp} = 0.382309, q^2 = -0.394611, Spress = 0.166142, S_{DEP} = 0.147621, r^2_{pred} = 0.328451

Optimized model no. 2a: BA = [1.63064(± 0.999191)] + Stretch-Bend Energy [0.0106527(± 0.391241)] + Non-1, 4 VDW [0.0254595 (± 0.0205552)] + MR [-0.00303471(± 0.00219626)]

n = 18, r = 0.733971, r^2 = 0.538713, variance = 0.00667605, std = 0.0817071, F = 5.44997

Optimized model no. 2b: BA = [1.2023(± 0.754451)] + Stretch-Bend Energy [-0.0486356(± 0.281785)] + Non-1,4 VDW [0.0264364 (± 0.0147098)] + MR [-0.00231117(± 0.00162297)]

n = 17, r = 0.803446, r^2 = 0.645525, variance = 0.00335484, std = 0.057921, F = 7.89131

Validation parameters: q^2 = 0.463763, Spress = 0.0712396, S_{DEP} = 0.0622972, r^2_{bsp} = 0.672155, r^2_{pred} = 0.114659.

The derived model explains 64.55 % of variance in observed activity. The satisfactory value of internal validation, cross-validated correlation coefficient (q^2), standard deviation of prediction (Spress), standard error of prediction (S_{DEP}), bootstrapping squared correlation coefficient (r^2_{bsp}) and chance correlation < 0.01 in the randomized biological activity test revealed that the results are not based on chance correlation. The models q^2 value > 0.4 supported the predictive ability and significance of the model. The triparametric equation describes the A II antagonistic activity of 19 analogs as a function of their thermodynamic (stretch-bend energy), electronic (non-1,4 vander Waals force) and steric (molar refractivity) parameters. Compound 16 and 25 were omitted stepwise upon deriving above model as outlier.

Although model-1 shows better r^2 value than 2, the predictive ability and robustness of model 2 is fairly good as compared to model 1 which is reflected from its better q^2 and low Spress and S_{DEP} value. Since all parameters are steric, the cross correlation among descriptors is more than 0.5, hence model 1 is utilized for designing new analogues.

Observed, predicted, calculated, Z score, residual value and descriptors of training and test set molecules are shown in Tables 3 and 4. The correlation between observed, predicted and calculated activity for training and test set molecules are given in Figs. 1-3 and 4-6.

Negative contribution of MR suggests that the quinazolinones moiety cannot tolerate too bulky and hydrophobic substituents at R1, R2 and R3 position. Better activity of bicyclic analogues (compounds **7-26**) demonstrates that there is significant amount of space available in the region of

TABLE-3
OBSERVED, PREDICTED, CALCULATED AND RESIDUAL VALUE OF TRAINING AND TEST SET MOLECULES (MODEL-1)

Compd.	Observed	Predicted	Calculated	Z-value	Residual
1	0.5420	0.457795	0.466627	0.67003000	0.0843
2	0.4434	0.449576	0.448118	-0.04194080	-0.0061
3	0.4171	0.459238	0.443043	-0.23062000	-0.0421
4#	0.1694	0.531858	-	-	-0.3624
5	0.4311	0.488389	0.468871	-0.33576300	-0.0572
6	0.4659	0.465885	0.465887	0.00011807	0.0001
7	0.6644	0.570356	0.589203	0.66846200	-0.0941
8#	0.6285	0.590275	-	-	0.0383
9	0.6330	0.581986	0.592720	0.35806600	0.0511
10#	0.5516	0.642686	-	-	-0.0910
11	0.5440	0.439522	0.467837	0.67704700	0.1045
12#	0.6988	0.630230	-	-	0.0686
13#	0.5746	0.625188	-	-	0.0505
14	0.5515	0.550717	0.550769	0.00649999	0.0008
15	0.5420	0.552907	0.552175	-0.09045220	-0.0109
16*	0.2232	0.657186	0.532699	-2.75128000	-0.4339
17	0.7440	0.567867	0.591378	1.35672000	0.1762
18	0.5023	0.621666	0.602158	-0.88768100	-0.1193
19#	0.5670	0.620024	-	-	-0.0530
20	0.5720	0.623784	0.601708	-0.26408700	-0.0517
21#	0.5269	0.560388	-	-	-0.0334
22	0.6476	0.593178	0.603004	0.39643100	0.0545
23	0.6014	0.495770	0.502338	0.88060500	0.1057
24	0.5695	0.520505	0.525774	0.38870400	0.0490
25*	0.2645	0.546855	0.481430	-1.92839000	-0.2167
26	0.6601	0.343673	0.433261	1.12753000	0.3165

#Test set molecules, *Outlier.

the receptor surrounding the C-6 substituents. Low activity of compound 25 and 26 showed that this region could tolerate bulky substituents but too bulky groups causes loss of activity. Bulkier and more hydrophobic substituents at C-1 and C-2 position hinder the interaction of compounds with receptor site. This finding indicates that the formation of hydrogen bond from the receptor to 1-position of quinazolinone moiety is important for receptor affinity. Thermodynamic parameter stretch-bend energy is negatively contributing while electronic parameter non-1,4 vander Waals force is positively contributing to biological activity. Non-1,4 vander Waals force has both a repulsive and attractive component. Positive contribution of this term indicates the dominating behaviour of attractive force, more the attractive force, more the legend-receptor interaction.

TABLE-4
OBSERVED, PREDICTED, CALCULATED AND RESIDUAL VALUE OF TRAINING AND TEST SET MOLECULES (MODEL-2)

Compd.	Observed	Predicted	Calculated	Z-value	Residual
1	0.5420	0.398325	0.459812	0.7652220	0.1437
2	0.4434	0.372768	0.387016	0.5249690	0.0707
3	0.4171	0.379414	0.387016	0.2800990	0.0916
4	0.1694	0.380491	0.293805	-1.1582900	-0.2110
5	0.4311	0.428494	0.429167	0.0179943	0.0027
6#	0.4659	0.277117	-	-	0.1888
7#	0.6644	0.598559	-	-	0.0659
8	0.6285	0.547501	0.557269	0.6632100	0.0810
9#	0.6330	0.556374	-	-	0.0767
10#	0.5516	0.529398	-	-	0.0223
11	0.5440	0.587495	0.571949	-0.2602210	-0.0434
12	0.6988	0.579978	0.600658	0.9137710	0.1189
13	0.5746	0.606148	0.600658	-0.2426150	-0.0315
14	0.5515	0.509893	0.512710	0.3611630	0.0417
15#	0.5420	0.512710	-	-	0.0293
16*	0.2232	0.586138	0.556236	-3.1007900	-0.3629
17	0.7440	0.539378	0.556236	1.7482100	0.2047
18	0.5023	0.593875	0.583969	-0.7603980	0.0915
19	0.5670	0.586028	0.583969	-0.1579970	-0.0190
20	0.5720	0.593861	0.585517	-0.1258510	-0.0218
21	0.5269	0.529368	0.529122	-0.0206912	-0.0024
22#	0.6476	0.583969	-	-	0.0637
23	0.6014	0.563190	0.566387	0.3259980	0.0383
24	0.5695	0.473499	0.490963	0.7312290	0.0961
25#	0.2645	0.525440	-	-	-0.2609
26	0.6601	0.663984	0.614339	-0.5050010	0.0038

#Test set molecules, *Outlier.

TABLE-5
CORRELATION MATRIX OF THE DESCRIPTORS USED IN DERIVED QSAR MODELS

Model 1			
	Stretch-B	Non-1,4	MR
Stretch-B	1.000000		
Non-1,4	0.446234	1.000000	
MR	0.125738	0.577144	1.000000
Model 2			
	CC	MTI	SOD
CC	1.000000		
MTI	0.567405	1.000000	
SOD	0.662792	0.963960	1.000000

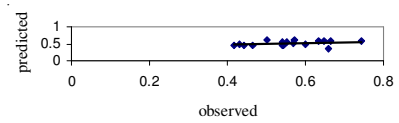


Fig. 1. Observed *vs.* predicted activity of training set molecules (model-1)

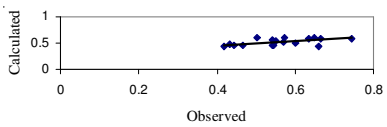


Fig. 2. Observed *vs.* calculated activity of training set molecules (model-1)

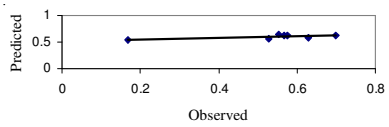


Fig. 3. Observed and predicted activity of test set molecules (model-1)

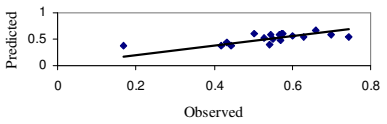


Fig. 4. Observed *vs.* predicted activity of training set molecules (model-2)

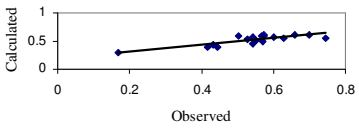


Fig. 5. Observed *vs.* calculated activity of training set molecules (model-2)

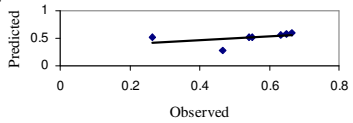
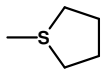
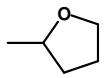
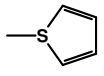
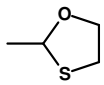


Fig. 6. Observed and predicted activity of test set molecules (model-2)

TABLE-6
STRUCTURE, CALCULATED DESCRIPTORS AND THEORETICALLY PREDICTED pIC_{50} VALUE OF DESIGNED COMPOUNDS

Compd.	n	R	R1	R2	R3	Descriptors			pIC_{50}
						Str.-Bend	Non 1,4 VEW	MR	
Des 1	2	α-COOH	H	H	H	0.3807	-8.1339	170.44	0.76614
Des 2	2	α-OH	H	H	H	0.5563	-10.9805	167.71	0.75930
Des 3	2		H	H	H	-1.6644	-15.1404	18.938	0.83910
Des 4	2		H	H	H	-6.1316	-10.7623	18.463	1.17340
Des 5	2	α-NO_2	H	H	H	0.4239	-11.3555	16.752	0.84287
Des 6	2		H	H	Me	-1.4957	-13.0307	19.366	1.53880
Des 7	2		H	H	Me	-6.1088	-12.1423	19.391	1.13320

On the basis of QSAR findings seven compounds were designed which are theoretically more potent than the reported series of compounds. The value of descriptors and calculated pIC_{50} value of all designed compounds were shown in Table-6. Compound **6** is most potent, which suggests that

sulfur containing heterocyclic ring is responsible for improved activity due to electron withdrawing property of S atom. Better potency of **D-6** as compared to **D-4** & **D-7** may be attributed to the direct attachment of S at C-6 position.

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