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QSAR and docking study of p-hydroxyphenylbenzohydrazide derivatives as ACE inhibiters- an antihypertensive agents

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Abstract: In the studies it was found that *p*-hydroxyphenylbenzohydrazide moiety has good biological activity in the form of antihypertensive agents. Therefore derivatives were selected for development of more active human ACE (angiotensin-I-converting enzyme) inhibiters. For QSAR study the substituted- thioxo- thiadiazol- *p*-hydroxyphenylbenzohydrazide derivatives were selected along with biological data to generate 3D-QSAR models. The molecular docking study was done by Glide-Schrodinger software into the angiotensin-I-converting enzyme with PDB code (2xy9). Compounds (4.a-4.n) were considered for docking study. The G-score of the standard ligand i.e. valsartan, in case of docking with 2xy9, was found as -6.11.The G-score of the compounds, (4.d), (4.i), (4.j) and (4.l) were also found as -5.98, -5.70, -5.87 and -5.70 for respectively. The number of H-bond interactions in the standard compound (valsartan) was compared with those of the designed compds. This has indicated the requirement of H-bond interaction for good antihypertensive activity.
Key words: angiotensin-I-converting enzyme**,** QSAR**,** docking, *p*-hydroxyphenylbenzohydrazide, valsartan.

1. Introduction:

One third of the world population is affected with cardiovascular diseases and the major part of it stems from hypertension. Hypertension is recognized as a major risk factor in a human patient with cerebral hemorrhage and heart and renal disease; therefore, diagnosis of hypertension is carried out by measuring blood pressure on a regular basis.

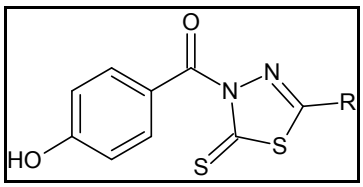
Human ACE (angiotensin-I-converting enzyme) has long been regarded as an excellent target for the treatment of hypertension and related cardiovascular diseases. To develop inhibitors with higher therapeutic efficacy and reduced side effects, efforts in this study have been directed towards the discovery of new compounds *p*-hydroxyphenylbenzohydrazide derivatives[1-3].

In studies it was found that this core moiety has good biological activity in the form of antihypertensive agents. Therefore derivatives were selected for development of more active human ACE (angiotensin-I-converting enzyme) inhibiters[4,5].

2. Experimental:

2.1 Data set:

For this study the substituted- thioxo- thiadiazol- *p*-hydroxyphenylbenzohydrazide derivatives were selected. Compounds reported along with biological data of *p*-Hydroxybenzohydrazide derivatives (a-n) was used to generate 3D-QSAR models in order to gain insights into the structural and molecular properties of these compounds; with the aid of the software program 3D-QSAR Pro (V-Life MDS) using various statistical regression methods [6,7].

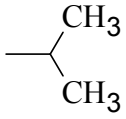
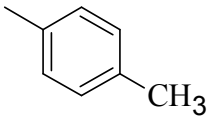
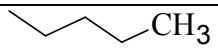
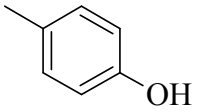
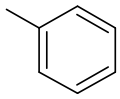
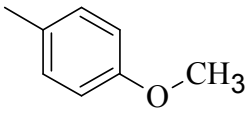
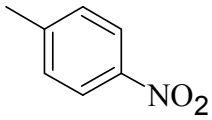
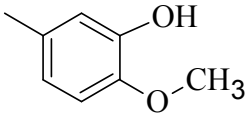
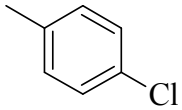
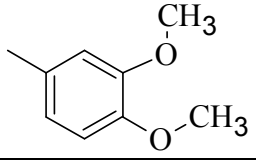
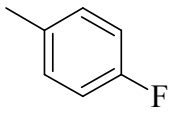
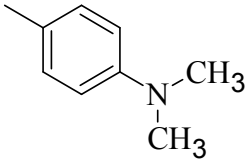
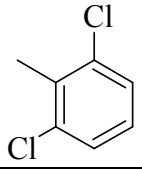
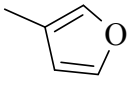


(a-n)

[(5-Substituted alkyl/aryl)-2-thioxo-1,3,4-thiadiazol-

3-yl]-4-hydroxyphenylbenzohydrazide

Table 1 Compounds (a-n) with biological activity (pIC_{50})

Compd.	R	pIC_{50}	Compd.	R	pIC_{50}
a		0.30	h		0.02
b		0.07	i		0.17
c		0.07	j		0.05
d		0.06	k		0.05
e		0.01	l		0.07
f		0.02	m		0.09
g		0.07	n		0.01

2.2 Molecular docking study [8,9]

All the docking calculations were performed using "standard precision (SP) and extra Precision" (XP) mode of Glide 8.5 program; Schrodinger LLC and the 2005 implementation of OPLS_2005 force field. The binding site, for which the various energy grids were calculated and stored, was defined in terms of two concentric cubes; the bounding box, which was contained the center of any acceptable ligand pose, and the enclosing box, which was contained all ligand atoms of an acceptable pose. Cubes with an edge length of 12 Å and centered at the midpoint of the longest atom-atom distance in the respective co-crystallized ligand was defined the bounding box in the protein. The large enclosing box was also defined in terms of the co-crystallized ligand: an edge length of 30 Å was used. Poses with an RMSD of less than 0.3 Å was used for optimization.

The scale factor for *van-der Waals* radii was applied to those atoms with absolute partial charges less than or equal to 0.15 (scale factor of 0.8) and 0.25 (scale factor of 1.0) electrons for ligand and protein, respectively. The maxkeep variable which got the maximum number of poses generated during the initial phase of the docking calculation were set to 32 and the kept best variable which got the number of poses per ligand that entered protocol included dielectric constant of 4.0 and 1000 steps of calculation, at most 100 poses per ligand were generated. The best docked structure was chosen using a glide score (G-score) function. The G-score was modified and extended version of the empirically based chemscore function. Another scoring function used by glide was emodel, which itself derived from a combination of G-score, H bond, *van- der Waals* and the strain energy of the ligand. Beside this the energy, contacts which included good, bad and ugly were also used for the evaluation of the docked complexes.

The molecules from QSAR studies were used for docking purpose. Fourteen molecules were selected (a-n) or docking via standard precision method. To obtain the precise results, these molecules were then subjected for extra precision method. Both the results were noted and compared. Extra precision method was showing good results in the form of Glide score, PhobEnHB, PhobEnPairHB, H Bond XP Lipophilic EvdW XP electro, and Glide pose number.

3. Results:

3.1 QSAR studies:

The biological activity data had selected and converted in the form of pIC_{50} value, table 1. Various 3D descriptors like steric, electrostatic and hydrophobic were calculated and used as independent variables. The dataset of 14 compounds was divided into training and test set by Random and manual selection method for MLR, PCR, PLS and kNN-MFA model.

The results were in terms of r^2, q^2 and predicted r^2 values. The QSAR models having significant values were selected for the design of compounds. The standard model was explained in Table 2.

Table 2 Comparative 3D QSAR results

Method	r^2	q^2	"F" test	Pred_r^2	Pred_r^2se
MLR	**0.9983**	**0.987**	**270.40**	**0.976**	**0.012**
PLS	0.995	0.834	522.10	0.124	0.063
PCR	0.973	0.821	423.6	0.921	0.042

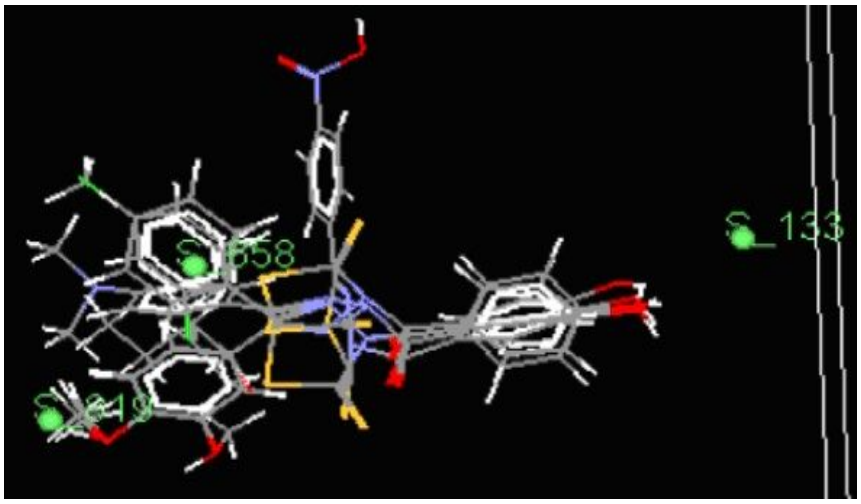


Figure 1 Distribution of points of QSAR method for antihypertensive activity

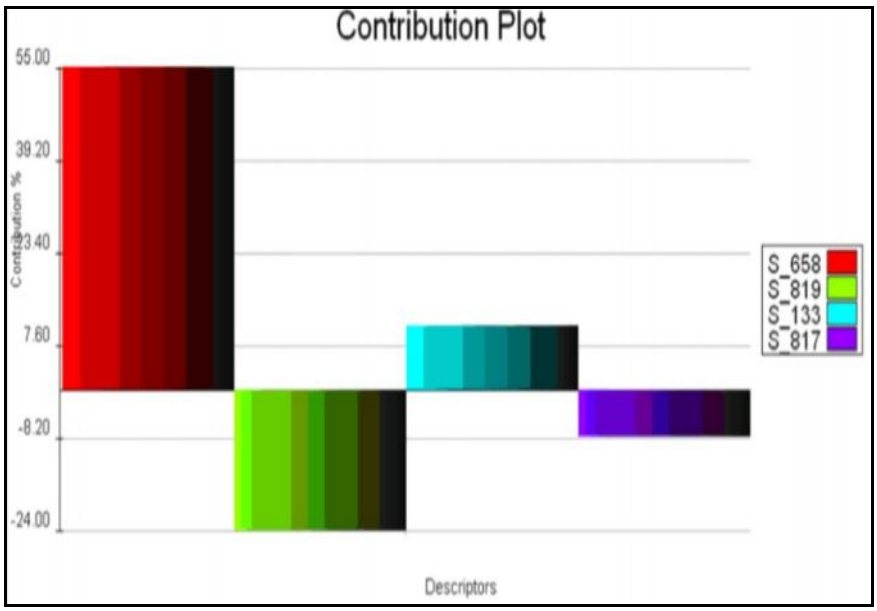


Figure 2 Contribution chart of QSAR method for antihypertensive activity

3.2 Molecular docking study

The following steps were undertaken for molecular docking studies.

The molecular docking tool, Glide (Schrodinger Inc. U.S.A.) software was used for docking studies of compounds (a-n) into the angiotensin-I-converting enzyme with PDB code (2xy9) [10].

3.2.1 Ligand preparation

The cocrystallized ligand was selected and by opening ligprep window ligand structures were taken. The force field parameter was selected as molecular mechanics force field (MMFF). The possible states of ligand generated were 32 and by keeping remaining data default ligand preparation was done[11].

3.2.2 Protein preparation

Protein preparation was done by selecting option of protein preparation wizard, from software. All hydrogens were added and keep as it as. As the protein selected was homodimer the unwanted chain from the protein was removed. Water molecules were removed from the protein and heterostates were generarted and

state having lowest penalty and highest probability was selected. After going to window Impref minimization all hydrogens and force field OPLS_2005 was selected.

3.2.3 Grid preparation

Grid generation was done with selection of rigid docking that is in this amino acids were not movable so scaling factor was applied upto not less than 0.7. By keeping remaining data unchanged grid was prepared [12].

3.2.4 Standard precision (SP) and extra precision (XP) mode.

Standard precision docking which is useful for most of docking it is having precision between extra precision (XP) and high throughput screening (HTVS). XP docking was used for refining molecules which were giving good results in SP docking. The compound which has shown good docking result in the form of good glide score. These molecules were again selected for docking via standard precision method[13-15].

The extra precision docking was performed by using prepared ligands and preprocessed protein. The module glide was selected from the maestro and XP docking was performed which was indicated good results in the form of G- score, H- bond contacts ,lipophilic lvdW and electrostatic in (Table 3). The comparative analysis of the docking parameters was carried out with valsartan (standard) (Table 4). The molecules from QSAR studies were further used for docking purpose[16-18].

3.2.5 Viewing docking results

It was done using pose-viewer. G score, H- bond contacts ,lipophilic EvdW and electrostatic parameter used to visualized the results using default settings to analyze the binding modes of the ligands to receptor (Figure 4 to Figure 8)[19-22].

Table 3 XP docking of compds. (a-n)

Sr.no	Compound	GScore	H-Bond	Lipophilic EvdW	Electrostatic
1.	a	-3.40	-0.72	-2.29	-0.61
2.	b	-4.65	-0.86	-3.18	-0.19
3.	c	-5.38	-1.06	-3.84	-0.31
4.	d	-5.98	-1.03	-3.99	-0.36
5.	e	-4.73	-1.35	-3.20	-0.01
6.	f	-4.00	-0.94	-2.42	-0.61
7.	g	-5.38	-1.05	-3.85	-0.44
8.	h	-5.38	-0.93	-3.38	-0.27
9.	i	-5.70	-1.68	-2.70	-0.85
10.	j	-5.87	-1.61	-3.77	0.09
11.	k	-3.20	-1.07	-3.62	0.39
12.	l	-5.70	-1.68	-2.70	0.85
13.	m	-5.26	-0.92	-3.08	0.38
14	n	-3.13	-1.05	-3.41	0.22

Table 4 XP docking of Valsartan (as standard)

Sr. no	Comp	G - Score	Lipophilic EvdW	H-Bond	Electro static
1.	Valsartan	-6.11	-3.33	-2.27	-0.79
2.	Valsartan	-6.27	-4.56	-1.9	-0.93

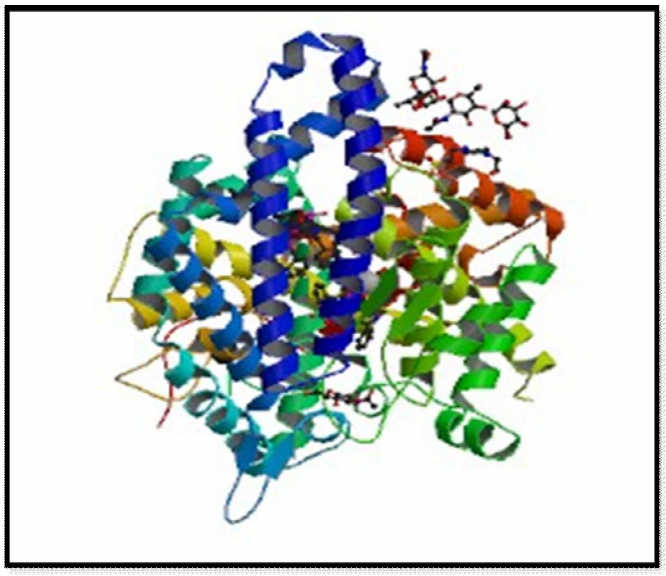


Figure 3 Structure of 2xy9 receptor

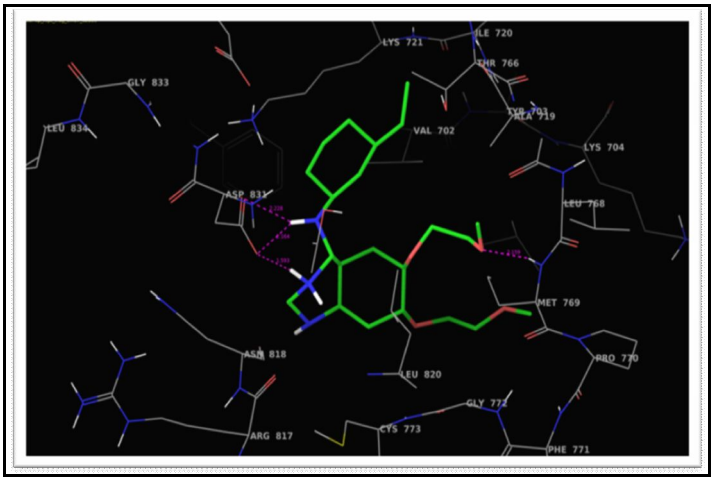


Figure 4 Grid selected for docking of compds. (4.a-4.m) with 2xy9 receptor

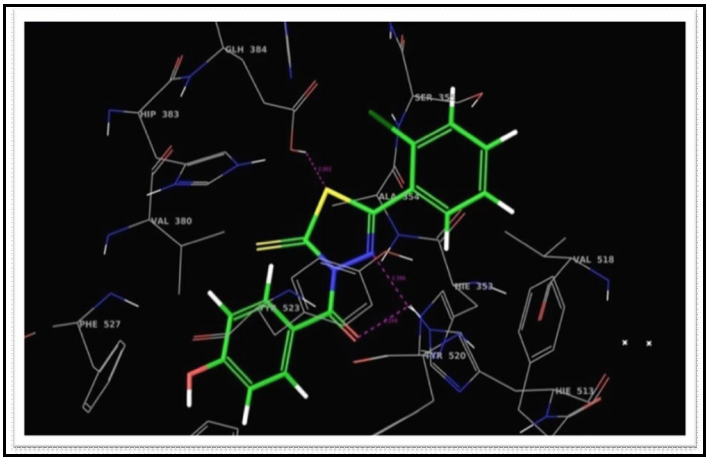


Figure 5 Compd. (d) docked in active site of 2xy9 receptor

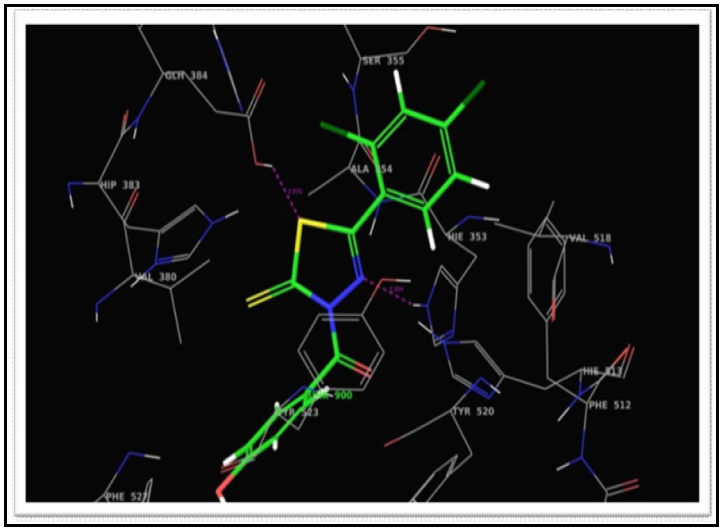


Figure 6 Compd. (i) docked in active site of 2xy9 receptor

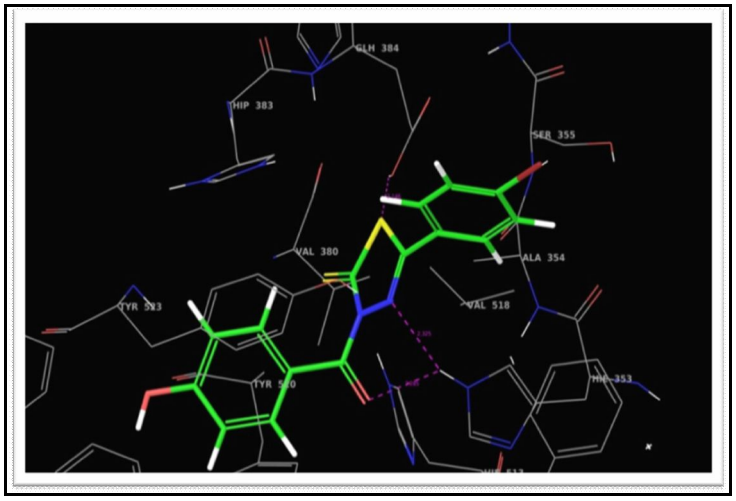


Figure 7 Compd. (j) docked in active site of 2xy9 receptor

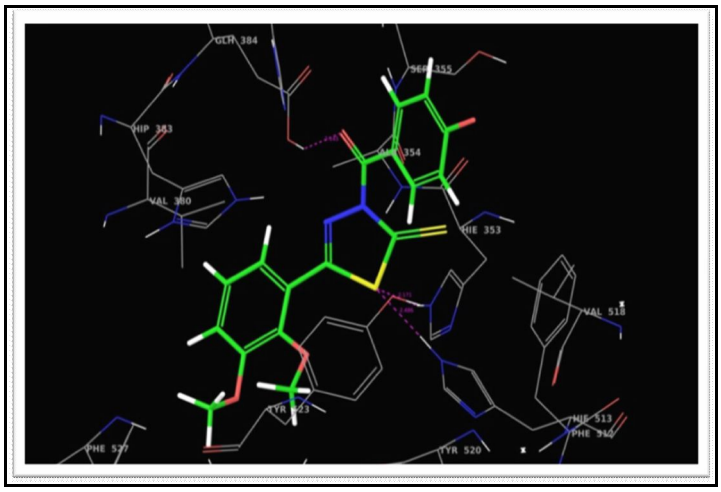


Figure 8 Compd. (l docked in active site of 2xy9 receptor

4. Discussion:

3D-QSAR study was performed on compds (a-.n) for the generation of 3D-QSAR models. The statistically significant 3D-QSAR models were generated using 14 compds with random and manual selection in to training set and test set**,** employing various statistical models like MLR, PCR, PLS and kNN-MFA model and evaluated on various points with 3D descriptors like steric, electrostatic and hydrophobic were calculated and used as independent variables.

The results were in terms of r^2, q^2 and predicted r^2 values. The QSAR models having significant values were selected for the design of compounds. The standard model was explained in Table 3. Among these methods, the MLR showed good results (Table 2) having r^2 0.9983, q^2 0.987 and standard error of estimate 0.012. This statistical data along with distribution points (Figure 1) and contribution chart (Figure 2) was used for design of new compounds. Using these results, newer compounds were designed and were subjected for the generic prediction. Steric points with positive coefficient S_819 (9.1762, 7.2460) at position R indicated that aromatic bulky groups were favourable on this site for increase in the activity.

The molecular docking study on these predicted compounds was done with angiotensin-I-converting enzyme, 2xy9 receptor which was showed good results in the form of docking score among which (a-n) compounds were subjected further docked using extra precision method which was resulted in docking score ranging from -5.98 to -3.20.

The docking studies were performed using standard precision mode of glide. The results of the docking studies were generated in the form of G-score. The more negative value of G-score indicated that the compound may be more potent and indicated the good binding potential of the compound. The G-score of the standard ligand i.e. valsartan, in case of docking with 2xy9, was found as -6.11.The G-score of the compounds, (d), (i), (j) and (l) were also found as -5.98, -5.70, -5.87 and -5.70 for respectively. Close analysis of these results suggested that compds were comparable with standard antihypertensive agent, is valsartan. Besides the G-score, other parameters like H-bond, lipophilic EvdW and Electrostatic.

The number of H-bond interactions in the standard compound, is valsartan was compared with those of the designed compds. In case of docking with 2xy9, the H-bond interactions of the standard compound, valsartan, was found as -2.27.

While those of (d), (i), (j) and (l) compds were found to be -1.03, -1.68, -1.61 and -1.68 respectively. This has indicated the requirement of additional functional groups which can form possible H-bond interaction with the 2xy9 receptor.

5. Conclusion:

3D-QSAR and molecular docking study of compounds (a- n), revealed that aromatic substitution on thiadiazole / thiazolidine moiety could be retain for better antihypertensive activity. Also, the activity may be increased by placing electron releasing and hydrophobic groups group/s on phenyl ring. Also, hydrophobic interaction of these compds with amino acids revels that at least two hydrogen bond required between, compd (ligand) and receptor required for good antihypertensive activity.

6. Acknowledgements:

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