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DOCKING STUDIES OF SOME NEW DERIVATIVES OF *P*-HYDROXYBENZOHYDRAZIDE AS AN ANTIHYPERTENSIVE AGENT

RITESH P. BHOLE*, KISHORE P. BHUSARI
Sharad Pawar College of Pharmacy, Wanadongri, Hingna Road, Nagpur, (MS), India-441110

Docking study was perform on prior synthesized and evaluated novel (4-hydroxyphenyl)-[(5-substituted-alkyl/aryl)-2-thioxo-1,3,4-thiadiazol-3-yl]methanone, (4.a-4.n) and N'-[-(3-substituted-4-oxo-1,3-thiazolidin-2-ylidene] 4-hydroxy benzohydrazide, (6.a-6.i) derivatives by the use of Schrödinger GLIDE program. Incorporating available biochemical and computational data to the model by correcting the conformation of a single residue lining the binding pocket resulted in significantly improved docking poses. These results support the applicability of GPCR modeling to the design of site-directed mutagenesis experiments and to drug discovery. The molecular modeling study allowed confirming the preferential binding mode of *p*-hydroxybenzohydrazide inside the active site.

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Keywords: Molecular Docking, GLIDE, Antihypertensive activity

1. Introduction

High blood pressure (BP) is one of the most potent risk factors for the first and recurrent stroke [1]. One third of world population is affected with cardiovascular diseases and the major part of it is occupied by hypertension [2-4]. Hypertension is recognized as a major risk factor in a human patient with cerebral hemorrhage, heart and renal disease, therefore, diagnosis of hypertension is carried out by measuring blood pressure on routine basis [5-7]. *p*-Hydroxy benzohydrazide moiety and its analogs seemed to be suitable parent compounds upon which variety of biological activities were reported such as antitumor[8,9], antianginal [10], antitubercular [11,12], antihypertensive[13], MAO enzyme inhibitor [14] , antibacterial[15] etc.

Discovering three-dimensional pharmacophores which can explain the activity of a series of ligands is one of the most significant contributions of computational chemistry to drug discovery [16]. Quantitative drug design embraces two major activities, the quantitative description of the structural differences among series of chemical compounds of biological interest, and the formulation of "QSAR" useful in the design of new and better therapeutic agents [17]. A QSAR is a mathematical relationship between a biological activity of a molecular system and its geometric and chemical characteristics.QSAR attempts to find consistent relationship between biological activity and molecular properties, so that these "rules" can be used to evaluate the activity of new compounds 3D models are more easily interpretable than 2D descriptor or fingerprint-based QSAR models, making it easier to suggest new compounds for synthesis.

In recent years, the actions of Ang II are mediated by angiotensin receptors, AT_1 and AT_2. These receptors are members of the G protein-coupled receptors family which are seven transmembrane helices, connected by interchanging extracellular and intracellular loops. Each G protein-coupled receptor couples to a specific G-protein which leads to activation of a special effectors system. AT_1 receptors are for instance primarily coupled through the $G_{q/11}$ group of G-proteins. Two more angiotensin receptors have been described, AT_3 and AT_4, but their role is still unknown. One of the G protein coupled receptor (GPCR) with crystallographic structural information available. [18–22] In this context, structural studies of other GPCR heavily relied on

mutagenesis experiments combined with sequence comparison and homology modeling. The first evidence of a general structure shared by the family of receptors that were later identified under the label of GPCR came during the nineties, with the report of a significant amino acid homology between rhodopsin and the AT1 receptor.[22] In subsequent years, it became clear that, just like rhodopsin, GPCR are formed by a single polypeptide chain that crosses the cell membrane seven times with seven R-helical transmembrane domains (Residue A) bundled together in a very similar manner Supporting the idea of a common folding of the sequence comparison revealed specific amino acid patterns characteristic of each TM and highly conserved in the great majority of Class GPCR.

2. Material and methods

2.1. Molecular Docking Study

The series of compounds (4-hydroxyphenyl)-[5-substituted alkyl/aryl)-2-thioxo-1,3,4-thiadiazol-3-yl]methanone and *N*'-[(-3-substituted alkyl/aryl-4-oxo-1,3-thiazolidin-2-ylidene] (Table 1) were evaluated for antihypertensive activity by non-invasive tell cuff method and was then subjected for molecular docking study using Schrödinger GLIDE [23,26] module.

Table 1. Anti-hypertensive activity data at 10mg/kg dose.

Compd No.	Average blood pressure (mm Hg) at time (h)										
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
4.a	226±4	222±8	220±9	193±2	185±4	175±8	170±6	165±4	161±6	155±3	150±3
4.b	226±8	220±5	200±5	193±4	170±5	161±4	161±4	155±6	150±7	140±5	135±8
4.c	225±8	220±0	200±5	191±4	170±0	161±7	142±2	135±6	135±5	131±0	130±2
4.d	222±3	220±6	210±3	193±5	178±7	161±2	140±5	131±6	128±7	123±5	121±8
4.e	228±3	220±6	210±3	183±5	170±7	161±2	140±5	131±6	128±7	125±5	122±8
4.f	226±3	220±6	210±3	190±5	175±7	160±2	140±5	128±6	125±7	120±5	115±8
4.g	226±2	224±3	210±8	190±4	172±5	160±4	142±4	135±6	130±7	125±5	122±8
4.h	226±2	226±3	215±8	195±4	170±5	165±4	140±4	135±6	130±7	120±5	115±8
4.i	226±3	221±6	205±3	195±5	175±7	170±2	160±5	145±6	135±7	130±5	125±8
4.j	224±3	216±6	205±3	180±5	170±7	155±2	140±5	122±6	120±7	115±5	110±8
4.k	226±2	224±3	210±8	190±4	172±5	160±4	142±4	135±6	130±7	125±5	122±8
4.l	230±3	220±6	210±3	190±5	175±7	160±2	140±5	125±6	120±7	115±5	110±8
4.m	220±5	210±6	200±5	185±1	175±7	160±2	140±5	135±5	135±5	125±5	124±7
4.n	225±3	220±5	210±3	190±5	175±7	160±4	145±6	137±7	131±7	131±5	130±8
6.a	225±4	222±8	221±9	195±2	180±4	175±8	162±6	155±4	150±6	150±3	140±3
6.b	226±8	224±5	210±5	193±4	178±5	161±4	155±4	155±6	150±7	145±5	135±8
6.c	226±8	224±5	210±5	193±4	178±5	161±4	142±4	138±6	131±7	125±5	122±8
6.d	225±4	222±8	220±9	190±2	178±4	172±8	167±6	160±4	158±6	152±3	145±3
6.e	225±6	223±5	210±6	195±8	180±5	160±8	145±3	140±6	135±7	125±5	125±7
6.f	226±3	224±6	210±3	193±5	178±7	161±2	142±5	134±6	130±7	125±5	121±8
6.g	224±4	222±8	220±9	190±2	175±4	172±8	162±6	160±4	154±6	150±3	145±3
6.h	226±2	224±3	210±8	190±4	172±5	160±4	142±4	135±6	130±7	125±5	125±8
6.i	225±4	222±8	221±9	195±2	180±4	170±8	168±6	152±4	140±6	115±3	115±3
Control	225±2	224±1	225±1	224±3	224±4	224±1	225±1	224±4	225±2	224±3	225±2
Standard[1]	225±1	214±2	201±1	193±3	182±2	173±1	151±2	131±2	118±1	108±2	103±3

Standard[1]: Valsartan

3. Results and discussion

Molecular docking study was performed on series of compds (4.a-4.n) and (6.a-6.i) using glide module of Schrodinger. For each docking calculation a maximum of 100 poses were saved. The crystallographic coordinates with ACE inhibitor (3.2 resolution) were obtained from the Protein Databank[303] as entry *1R4L*. The reported crystal structure is a tetramer, having four asymmetric units (A, B, C, D) with the inhibitor bound to the A asymmetric unit. In the present study, asymmetric unit A was isolated and used further in the docking study, the predicted binding affinity of these compds was shown in Table-2.

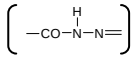
3.1. Binding mode of compounds (4.a-4.n)

A comparison of the different docking poses of compds (4.a-4.n) suggests that these compds adopt binding modes with the H-bonding network. To illustrate the binding mode of these compds, one of compd (4.j) was analyzed in more detail (being most active and showed more hydrogen bonding). Figure-1 shows a docked model of compd (4.j) into the active site of *1R4L* (ACE inhibitor).

The compd (4.j) having 4-methoxyphenyl substitution on thiadiazole ring and 4-hydroxyphenyl binds in a deep catalytic active site formed by the hinge region (residues 210–216) through three hydrogen bonds. The 4-hydroxyphenyl forms hydrogen bonding with Ala_{213} (O-H, d_1 = 2.07Å, θ =177.58) and 4-methoxyphenyl forms hydrogen bonding with Glu_{211} (– CH_3O-H, d_2 = 1.84 Å, θ=154.68) backbone. The oxygen in carbonyl group forms a hydrogen bond with the Lys_{162} side chain (O-H2N, d_3 = 1.88 Å, θ = 161.78) located in the upper lobe of the highly solvent-exposed phosphate binding site of *1R4L*. Further stabilization of the binding was mediated by the contact of the thioxo atom with the hydrophobic surface formed by Leu_{139}, Tyr_{212}, Pro_{214}, Leu_{215}, and Leu_{263} amino acid side chains. Being exposed to the solvent, this moiety offers a good handle for improving the pharmacokinetic profile through chemical modification. The 4-methoxyphenyl was found to interact with a hydrophobic surface formed by Val_{147}, Lys162, Lys_{141}, Ala_{273} and Leu_{164} residues found in the vicinity of a highly solvent-exposed phosphate binding site. On the basis of the docked geometry, it appears that compds (4.a-4.n) assume a v-shape conformation within the active site of *1R4L*.

3.2. Binding mode of compounds (6.a-6.i)

A comparison of different docking poses of compds (6.a-6.i) suggests that they bind to ACE in a same manner as described above. To illustrate the binding mode of this series of compds, compd (6.i) was selected for more detailed analysis (being most active in the Scheme and showed more hydrogen bonding). Figure-2 showed the docked model of compd (6.i) within the active site of *1R4L*. The compd (6.i) having linkage of p-hydroxyphenyl through hydrogen bonding with the backbone of Ala_{213} amino acid residue (–NH-O, d_1=2.08 Å, θ=149.28)



in the hinge region. The 2-methoxy function of 2,4-dimethoxyphenyl was found to be 3.17 A° away from the backbone carbonyl oxygen of Ala_{213}. The p-hydroxybenzohydrazide ring binds near the hinge region and forms hydrophobic contacts with Leu_{139}, Val_{147}, Ala_{160}, Leu_{194}, Leu_{210}, Tyr_{212}, Ala_{213} and Leu_{263}. The phenyl part of the benzohydrazide nucleus was found to bind to the inside of the selectivity pocket. It is worthwhile to note that 2,4-dimethoxyphenyl is located near the side chain of Arg_{137}.

The docking study of compds (4.a-4.n) and (6.a-6.n) gives hint that methoxyphenyl/ dimethoxyphenyl like substitution inhibits 1R4L which results in enhancement of the activity. *Also,* hydrophobic interaction of these compds with amino acids revels that, atleast two hydrogen bonds between compd and receptor are necessary for good inhibitory activity.

Table 2. Docking of compds with residue A of 1R4L with their binding energy[a]

Compd. No	Binding energy		Good VDW	Bad VDW	Ugly VDW
	G-Score[b]	E-Model[b]			
(4.a)	-10.23	-61.2	341	9	0
(4.b)	N.D	N.D	N.D	N.D	N.D
(4.c)	-10.24	-58.21	325	12	2
(4.d)	N.D	N.D	N.D	N.D	N.D
(4.e)	-10.27	-47.41	420	17	0
(4.f)	-8.86	-60.3	304	21	3
(4.g)	-8.82	-56.41	323	10	0
(4.h)	N.D	N.D	N.D	N.D	N.D
(4.i)	-7.86	-44.11	312	24	3
(4.j)	-7.45	-39.45	421	8	0
(4.k)	N.D	N.D	N.D	N.D	N.D
(4.l)	-6.94	-38.13	457	41	12
(4.m)	-4.13	-33.17	412	21	0
(4.n)	-3.31	-29.24	257	18	11
(6.a)	N.D	N.D	N.D	N.D	N.D
(6.b)	N.D	N.D	N.D	N.D	N.D
(6.c)	N.D	N.D	N.D	N.D	N.D
(6.d)	-3.62	-44.12	239	21	6
(6.e)	-3.28	-41.34	223	31	4
(6.f)	-3.25	-29.2	256	25	2
(6.g)	-3.17	23.13	225	19	1
(6.h)	-3.19	-34.26	219	5	2
(6.i)	-3.57	-11.28	267	3	0

[A]

.a more negative g-score (glide score) indicates a better fit in the binding site,[b].g-score and e-model were expressed as kcal/mol. N.d: values not determine.

4. Experimental

The docking studies were performed using Glide module (version 4.5, Schrödinger, LLC, NY)[268] which was installed on AMD athelon workstation. The crystallographic coordinates with ACE inhibitor (3.2 resolution) were obtained from the Protein Databank[269] as entry *1R4L*. The reported crystal structure is a tetramer, having four asymmetric units (A, B, C, D) with the inhibitor bound to the A asymmetric unit. In the present study, asymmetric unit A was isolated and used further in the docking study.

4.2. Steps undertaken in docking study

Step I. Data set for analysis

The antihypertensive activity of 23 compds i.e (4.a-4.n) and (6.a-6.i) were measured *in vitro* as IC_{50}. These inhibitors along with binding energy were shown in Table-29.

Step II Protein preparation

Protein preparation was performed by using protein preparation Wizard of Maestro software. The NMT protein structure *1R4L* was taken. The A chain was treated to add missing hydrogen, assign proper bond orders (i.e., breaking bonds to metal and correct the formal charge

on it and neighboring atoms), and to delete water compds that were more than 5Å from the heterogeneous groups. The H-bonds were optimized using sample orientations. All the polar hydrogens were displayed. Finally, the protein structure was minimized to the default Root Mean Square Deviation (RMSD) value of 0.30.

Step III Ligand preparation

Ligand preparation was accomplished on all the 23 compds using Ligprep module to clean the structure and generate tautomers as described in the PHASE CPH generation studies.

Step IV Receptor grid generation

Receptor was defined and the cocrystallized ligand was differentiated from the active site of receptor A chain. The atoms were scaled by vander Waals radii of 1.0 Å with the partial atomic charge less than 0.25 defaults. The active site was defined as an enclosing box at the centroid of the workspace ligand as selected in the receptor folder. The ligands similar in size to the workspace ligand were allowed to dock into the active site. No constraints either positional, H-bonding or hydrophobic were defined.

Step V Ligand docking

Ligand docking was performed using OPLSAA force field. The receptor grid defined in the receptor grid generation folder was selected for the docking of ligands prepared using Ligprep. Flexible docking was performed using the Extra Precision (XP) feature of Glide module. The van der Waals radii was scaled using a default scaling factor of 0.80 and default partial cutoff charge of 0.15 to decrease the penalties for close contacts. The core pattern comparison and similarity mode were not used since the aim was to study the binding of ligands to the active site. The constraints to defined ligand – receptor interactions were not set. The structure output format was set to poseviewer file so as to view the output of the resulting docking studies from pose-viewer. The XP-Glide predicted pose of active compds (4.j) and (6.i) in the Scheme are shown in Figure-1 and Figure-2.

Step-VI Viewing docking results

It was done using pose-viewer. The H-bonds and vander Waals contacts (good, bad and ugly) to the receptor were visualized using default settings to analyze the binding modes of the ligands to receptor.

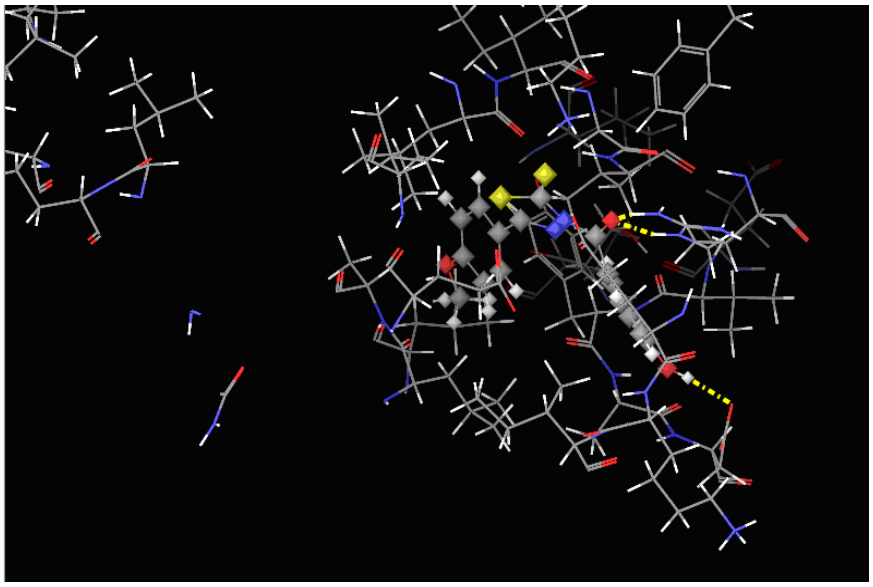


Fig. 1 XPGlide-predicted pose for compd (4.j) with active site

Note: Only the polar hydrogen bonds were shown in dotted yellow lines

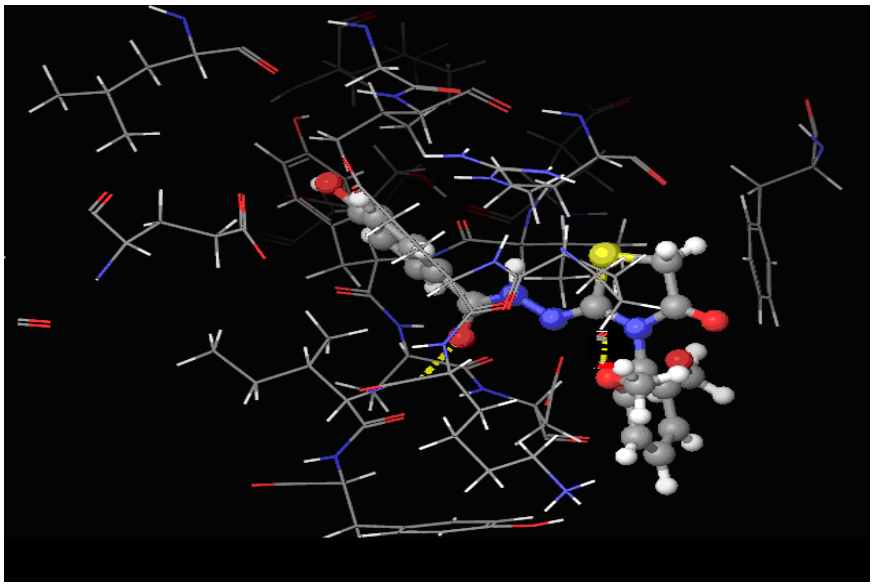


Fig. 2. XPGlide-predicted pose for compound (6.i) with active site

Note: only the polar hydrogens were shown in dotted yellow line.

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