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Development of accurate binding affinity predictions of novel renin inhibitors through molecular docking studies

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ABSTRACT

In this study, an attempt was made to explore a possible correlation between different docking scoring functions (Glide InducedFit docking score and GOLD's GoldScore and ChemScore) and binding energy values of a set of renin inhibitors, using linear regression model. The renin inhibitors under study are characterized by known bound to the receptor crystal structures possessing a great variety of pharmacophore groups and a wide range of IC_{50} values. Linear regression models were derived to relate the docking scoring function and pIC_{50} values of renin inhibitors under study. The developed derived models are seeking to be helpful for the rational design of new, more potent renin inhibitors.

1. Introduction

The renin–angiotensin–aldosterone system (RAAS) plays an important role in the regulation of blood pressure (hypertension) [1]. Drugs available for the treatment of hypertension include diuretics, β-blockers, aldosterone receptor antagonists, angiotensin converting enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARBs). Nonetheless, hypertension is poorly controlled in many patients and the drugs prescribed may produce significant side effects [2].

Renin has been recognized as a desirable target for antihypertensive drugs for almost four decades [3]. Renin is a 335-amino acid, glycosylated aspartic protease and is a member of pepsin-like family [4,5]. The active site of the renin is a deep cleft between the N- and the C-terminal domains to which the inhibitors bind in an extended conformation [6,7] (Fig. 1). During the past few decades the renin inhibitors were based on peptidic or peptidomimetic scaffold which confers low stability and poor oral bioavailability in human [8]. Molecular modeling and determination of the X-ray crystallographic structure of the active site of renin have led to the identification of new renin inhibitors [9,10]. The first representative of this class of non-peptide drugs is aliskiren, an orally active, renin inhibitor with a very high binding affinity for renin [11–14]. Aliskiren received approval but still it is a complicated molecule with many steps to be synthesized. A drug simpler in structure and with even higher bioavailability is desirable in the drug market.

Computational (*in silico*) methods are widely used as an aiding tool for the design of novel enzyme inhibitors. The docking protocols can be described as a two-part procedure: a search strategy and a subsequent scoring function [15–17]. Docking programs mostly keep receptors' amino acids rigid. This may decrease the chance of the correct location of ligand binding at the active site. Recently some improvements have been implemented with two well-known molecular docking programs GOLD and Glide/Induced Fit Docking (IFD) to allow full flexibility to the side chains of amino acids at active site or to take into account the crystal water molecules at the docking studies are now possible. Thus, in our study we used these docking tools in order to seek new insights into the relationship between scoring function and the renin inhibitory potency. The data that were used for this study comprises representative sample of renin inhibitors with the following characteristics: known bound to the receptor crystal structures; diverse variety of pharmacophore groups and a wide range of IC_{50} values from 0.4 to 6560 nM.

The main aim of the docking tools is to map the drug/receptor interactions in order to assist to rational drug design as well as to

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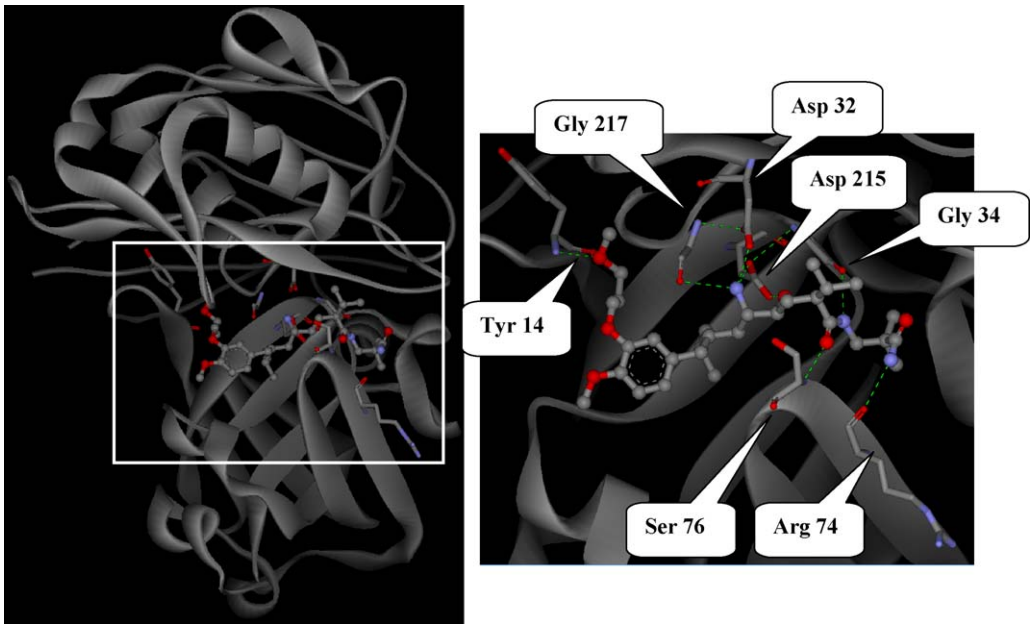


Fig. 1. Binding mode of aliskiren as produced from crystallographic data. The protein backbone is shown in ribbons. Residues of the binding site are displayed as grey sticks and aliskiren as ball and sticks. The right panel shows a zoom in the active site and the formed H-bonds with aliskiren. Molecular graphics are generated using DS Visualizer.

give an idea about the estimation of binding affinities of novel drugs before the synthesis and biological measurement steps. Thus, we found it interesting to compare the docking score functions and their experimental values for the set of renin inhibitors. As the searching algorithms and scoring functions are helpful to predict the biological activity of the ligand, our aim is to investigate which of the two is more suitable to explain the biological results. Such a correlation between any of the factors associated with the docking and the biological activity would help the rational design of novel renin inhibitors.

2. Results and discussion

2.1. Evaluation of the docking procedure

Docking protocols are widely used in order to predict the binding affinities for a number of ligands. Our aim was to examine the possibility of an existing relationship between the inhibitory affinity of the renin inhibitors under study and the docking score. More specifically, the 14 renin inhibitors that were used for this study and obtained from the Protein Data Bank (PDB) were docked into the renin active site using three different scoring functions the GoldScore, ChemScore and Glide/IFD. Table 1 lists all the structures used for our study and their IC_{50} values [18,19].

2.1.1. GOLD docking

The most straightforward method of evaluating the accuracy of docking procedure is to determine how closely the binding conformation is predicted by the scoring functions of the docking program. Among the 20 poses generated using GOLD program for each compound we selected the best-docked based on two criteria: (i) ligand binding position; (ii) fitness function scores comparison. The parameter used for identifying the best ligand binding position was the root-mean-square distance (RMSD) value. A successful prediction is considered if the RMSD value from the comparison of docked binding and crystallographic model is below 2.0 Å. The RMSD values are listed in Table 2. In all the studied compounds we reliably reproduced the X-ray binding mode, as the RMSD values for both protocols were <2 Å. This is a successful result compared to the expected 70–80% success rate that is obtained for large test sets [20,21]. In general, it can be stated that the results confirm the accuracy of both scoring functions of GOLD software in predicting the correct binding conformation for the renin inhibitors, although GoldScore showed a slightly better predicting ability as the rate of inhibitors with RMSD < 1 is 79% while the corresponding rate for ChemScore is 71%. These results make clear the superiority of GOLD among other docking programs that usually fail to produce a well-docked complex as the top-ranked pose for highly flexible ligands with more than 10 rotatable bonds [22].

2.2. Comparison of GOLD scoring functions

The second step in our study was to compare the two different functions of GOLD software. For the scoring of studied compounds we used the following modified versions of GoldScore and ChemScore functions given in Eqs. (1) and (2), respectively [23]. The values of intramolecular terms have been subtracted because as it has been reported that these terms have arbitrary reference states [24]:

$$\text{GoldScore} = \text{GoldScore Fitness} - S_{\text{hb\_int}} + S_{\text{vdw\_int}} \quad (1)$$

$$\text{ChemScore} = \text{ChemScore Fitness} - E_{\text{int}} \quad (2)$$

The produced by the program values of the factors that contribute to each score are listed in Table 3 while in Figs. 2 and 3 are depicted the scores versus the pIC_{50} values of the tested com-

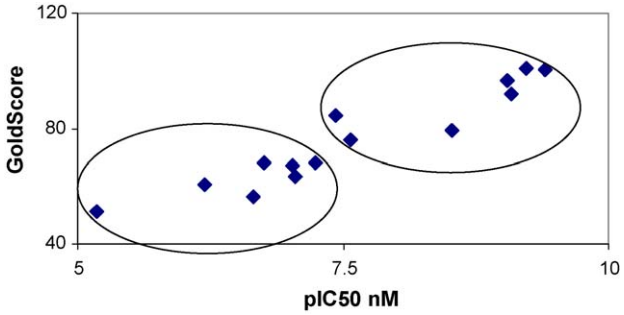


Fig. 2. Calculated GoldScore versus experimental pIC_{50} values for the 14 studied renin inhibitors.

Table 1
Structures, inhibitory activities and pdb codes of 14 renin inhibitors.

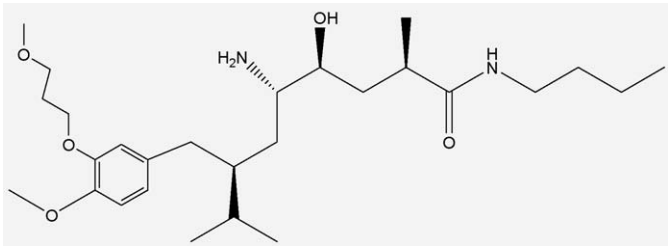
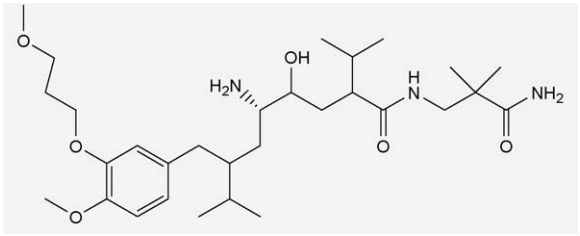
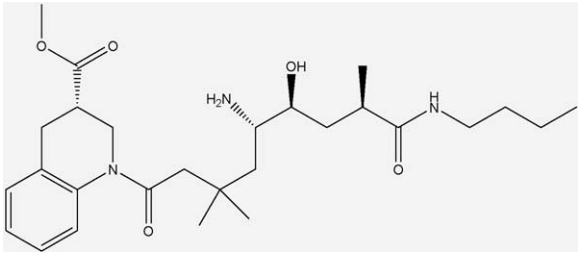
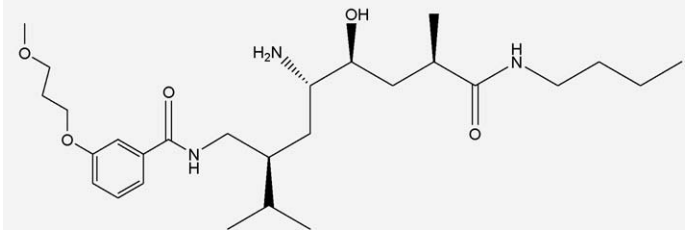
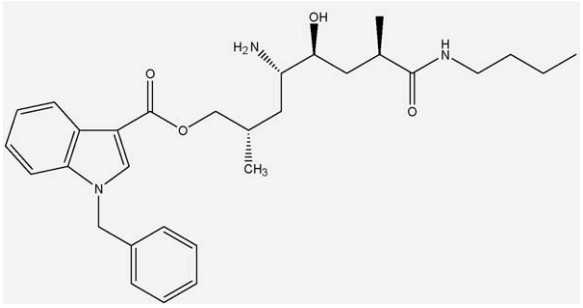
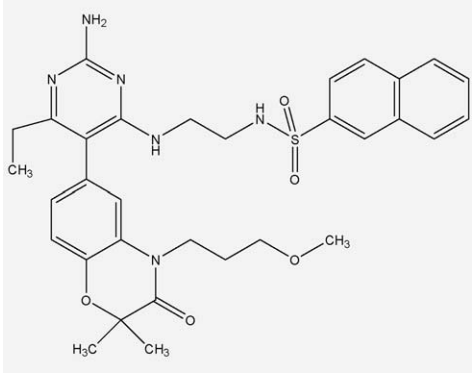
ID	Structure	IC_{50} (nM)	pIC_{50}	pdb code
1		0.4	9.40	2v10
2		0.6	9.22	2v0z
3		0.8	9.09	2v16
4		0.9	9.05	2v12
5		3	8.52	2v11
6		27	7.57	2il2

Table 1 (*Continued*)

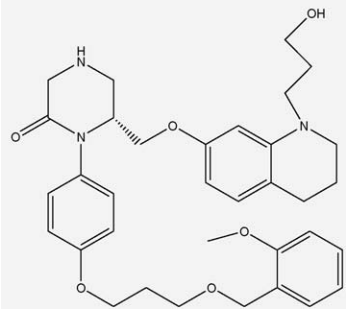
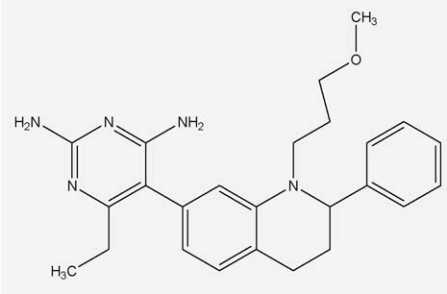
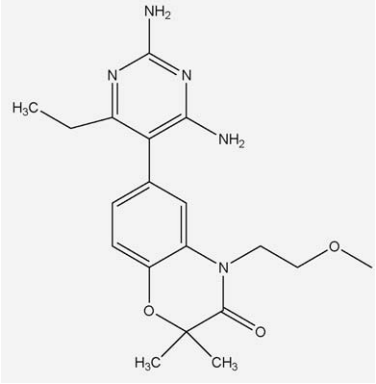
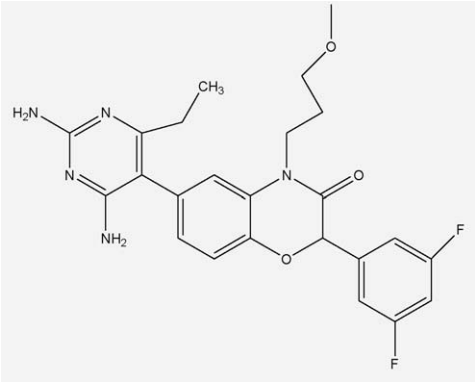
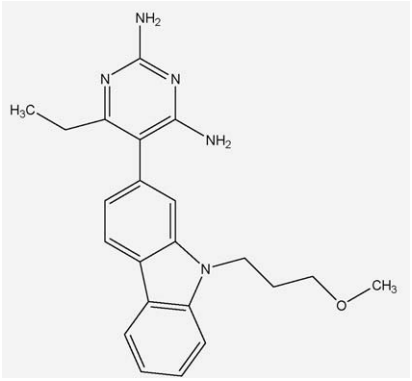
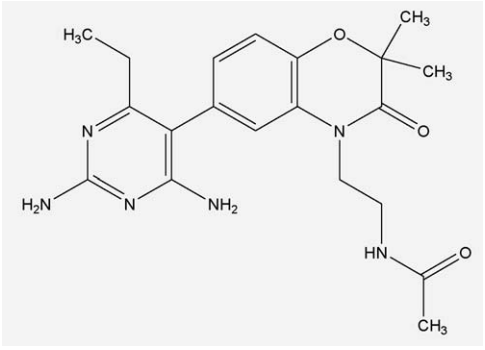
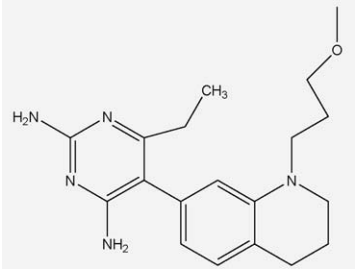
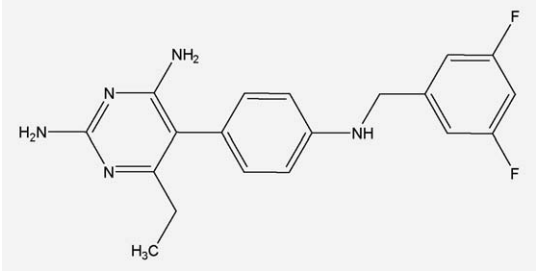
ID	Structure	IC_{50} (nM)	pIC_{50}	pdb code
7		37	7.43	2fs4
8		58	7.24	2iku
9		90	7.05	2g1y
10		95	7.02	2g1s

Table 1 (*Continued*)

ID	Structure	IC_{50} (nM)	pIC_{50}	pdb code
11		173	6.76	2g10
12		222	6.65	2g1r
13		650	6.19	2g21
14		6560	5.18	2iko

pounds. It can be observed that the GoldScore is correlated well with the reported pIC_{50} values. It is clearly visible that compounds with high pIC_{50} values correspond to high GoldScores. More specifically, compounds with IC_{50} value higher than 7.5 have a GoldScore superior to 80.00, while compounds with lower IC_{50} values are characterized by a lower GoldScore. When the docked molecules were evaluated with the ChemScore no such correlation was observed. For ChemScore the scatter is random as the most active compounds with $pIC_{50} < 7.5$ are observed with both high and low ChemScores. It appears that the GoldScore is better than ChemScore fitness function to explain the biological data and provides a qualitative agreement with the reported IC_{50} values of renin inhibitors. The above results highlight the superior scoring reliability of GoldScore.

In order to answer the question: "What is the predictive IC_{50} of a new renin inhibitor?" it must be determined if there is any correlation between the two variables GoldScore versus pIC_{50}. From the plot of Fig. 4, it appears that such a correlation exists between the two variables. The next step was to establish this correlation using a linear regression model. To quantify the extent of the correlation and examine if it is significant, a statistical analysis was applied.

The following linear regression model was found to be explanatory of the data:

$$\text{GoldScore} = -22.521 + 12.830\,pIC_{50} \quad (3)$$

where $pIC_{50} = -\log IC_{50}$.

Table 2
The RMSD values for the 14 tested inhibitors using both GoldScore and ChemScore.

Compound	Goldscore RMSD	ChemScore RMSD
1	0.65	0.56
2	0.62	1.21
3	0.43	0.67
4	1.31	0.84
5	0.77	0.83
6	0.23	0.83
7	0.95	1.06
8	0.53	0.43
9	1.98	1.02
10	0.34	0.55
11	1.53	1.10
12	0.84	0.73
13	0.62	0.26
14	0.45	0.86

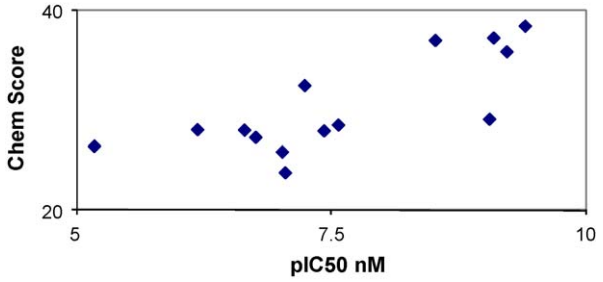


Fig. 3. Calculated ChemScore versus experimental pIC_{50} values for the 14 studied renin inhibitors.

The strength of the linear regression model can be given by calculation of the correlation coefficient and r^2. The correlation coefficient was calculated to be 0.94 meaning that the relationship between pIC_{50} values and GoldScore is pretty strong. Moreover, the very high r^2 value reveals that 88% of the variation in GoldScore can be explained by variation in pIC_{50}. The overall significance of the model was tested then. The very low p-value (Sig. = 0.000) indicated that the relationship between GoldScore and pIC_{50} is highly significant, and thus very unlikely to occur by chance alone. The obtained statistical results support the strong relationship that is evident in Fig. 4.

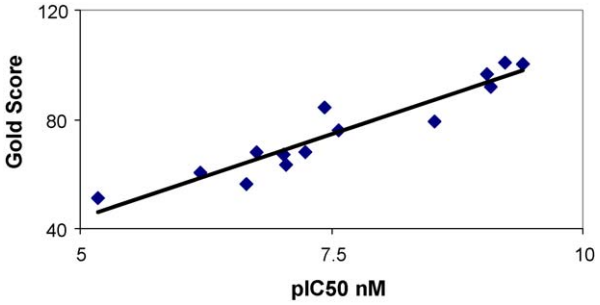


Fig. 4. The linear regression between GoldScore and pIC_{50}.

The above linear regression model was found to be promising in predicting the potency of new renin inhibitors; however, validation of this model was sought necessary. In order to validate the developed linear regression model, six renin inhibitors with known IC_{50} values were used as a test set. Their pIC_{50} values range between 7.04 and 9.10 and their biological activities are predicted by Eq. (3). The structure of the test set molecules (15–20) as well as the predicted and experimental values of them are presented in Table 4. The produced average deviation from the study is approximately 1.0 suggesting that the developed model gives a successful prediction for the pIC_{50} values.

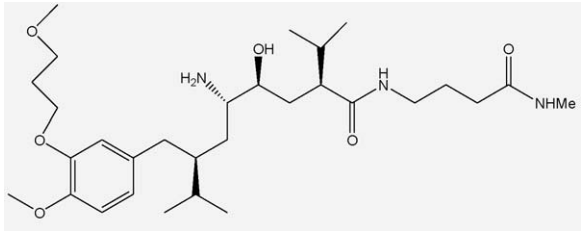
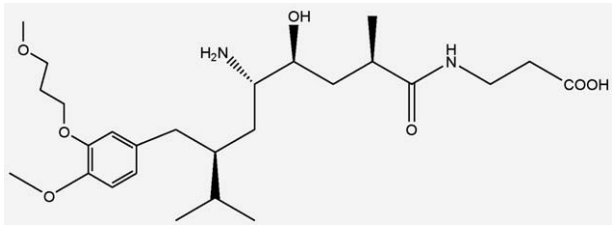
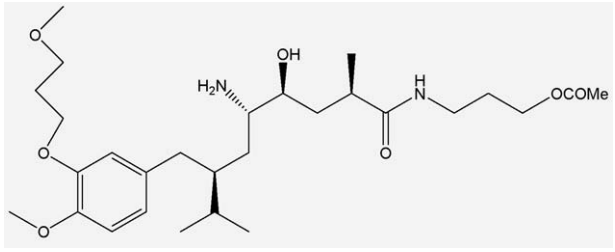
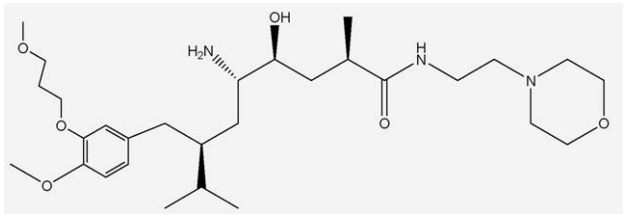
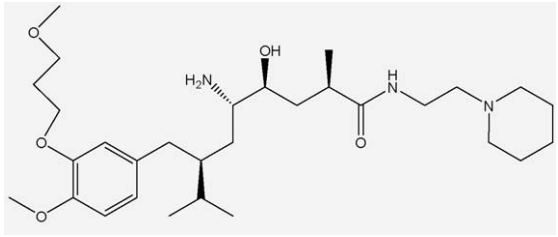
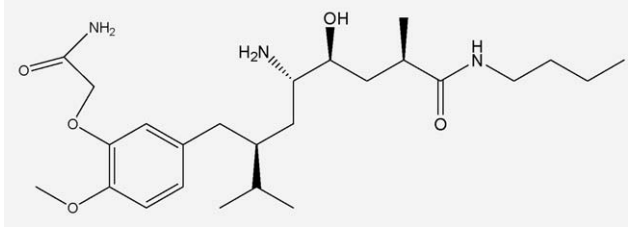
The used renin inhibitors belong to three different categories: (a) the 2,7-dialkyl-substituted 5(S)-amino-4(S)-hydroxy-8-phenyl-octanecarboxamides (**1**–**5**), (b) the 6-(2,4-diaminopyrimidinyl)-1,4-benzoxazin-3-ones (**6**, **8**–**14**) and (c) one ketopiperazine-based

Table 3
Values of the factors that contribute to GoldScore and ChemScore.

#	GOLD Fitness	GoldScore	S_{hb_ext}	S_{vdw_ext}	S_{vdw_int}	S_{hb_int}
1	82.43	100.18	12.84	87.35	−12.58	−5.18
2	85.03	101.03	11.43	89.60	−11.70	−4.30
3	82.13	91.91	11.72	80.19	−4.44	−5.34
4	85.37	96.67	9.68	86.99	−6.18	−5.12
5	70.79	79.20	14.53	64.67	−3.41	−4.99
6	60.61	76.23	0.12	76.11	−1.15	−14.47
7	52.38	84.62	1.75	82.87	−4.36	−27.88
8	66.32	67.96	2.39	65.85	3.13	−4.28
9	59.12	59.55	1.3	57.11	0.11	−6.60
10	63.41	67.03	4.28	62.76	−1.02	−2.61
11	64.12	57.35	1.05	66.91	1.57	−5.42
12	53.20	56.15	8.42	47.72	0.37	−3.32
13	58.23	60.53	4.23	56.30	−0.71	−1.59
14	42.87	51.02	5.38	45.64	−1.78	−6.38

#	ChemScore	$\Delta G_{binding}$	S_{hbond}	S_{lipo}	E_{clash}	E_{int}	H_{rot}
1	38.44	−42.56	3.9	289.30	1.48	2.64	9.79
2	35.86	−42.67	4.62	299.07	1.55	5.26	13.24
3	37.25	−40.55	3.84	272.28	0.76	2.54	9.61
4	29.07	−36.98	3.07	271.48	3.83	4.08	10.51
5	37.01	−43.46	3.97	285.83	0.2	6.25	8.71
6	28.58	−41.47	2.81	278.87	7.35	5.53	6.01
7	27.91	−38.67	1.9	304.19	3.88	6.89	8.73
8	32.46	−37.82	2.67	238.22	1.8	3.56	4.46
9	23.72	−26.58	1.27	183.87	0.36	2.49	4.65
10	25.80	−27.47	1.34	195.28	1.24	0.43	5.34
11	27.25	−32.43	1.87	214.24	2.83	2.35	4.37
12	27.98	−30.81	2.37	175.46	2.81	0.02	3.11
13	28.02	−29.12	1.79	188.55	0.47	0.63	4.40
14	26.38	−30.28	1.9	190.13	0.41	3.49	3.78

Table 4
The structure of 6 renin inhibitors using as test set, their experimental and predicted activities and their GoldScores.

ID	Structure	Experimental pIC_{50}	Predicted pIC_{50}	GoldScore	Residual
15		9.10	9.97	80.01	0.9
16		8.52	9.29	73.39	0.8
17		8.22	8.92	69.85	0.7
18		7.96	9.20	72.66	1.2
19		7.66	8.62	66.89	1.0
20		7.04	8.57	66.37	1.53

renin inhibitor (**7**). Docking results of the binding orientation and interactions of representative renin inhibitors for each class with the active site of renin are shown in Fig. 5. More specifically, are shown the best docking conformations of molecules **2** (Aliskiren), **14** and **10** which represent the most active, the last active and the one with intermediate activity, of the series, respectively. All the molecules are placed inside the active site and demonstrate the following interactions. The –OH group of compound **2** forms H bond with both oxygens of Asp 32 (∼1.9 and 2.4 Å), while the distance of –OH from Asp 215 is approximately 4 Å. The $-NH_2$ group forms H bond with C=O of Gly 217 (∼1.8 Å) and the oxygen of Asp 32 (∼1.9 Å), while the methoxy group of the side chain has a 3.4 Å distance from NH of Tyr 14. The primary CONH forms H-bonds with NH of Ser 76 (∼1.7 Å) and has a 3 Å distance from C=O of Gly 34. The terminal NH_2 interacts with C=O of Arg 74 (∼2.7 Å). In compound **14** the $-NH_2$ group forms hydrogen bonds with C=O of Gly 34 (2.3

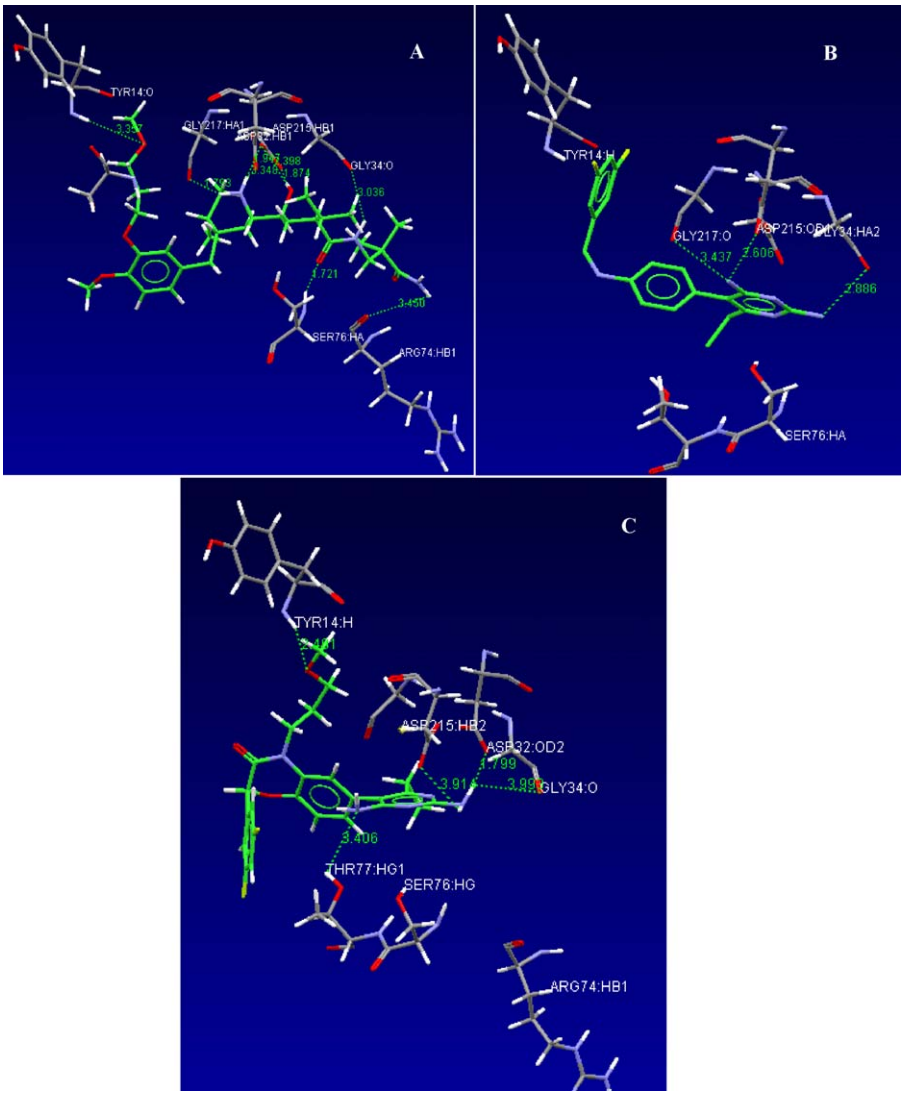


Fig. 5. GoldScore-based docking interactions of molecules **2** (A), **14** (B) and **10** (C) with active site residues.

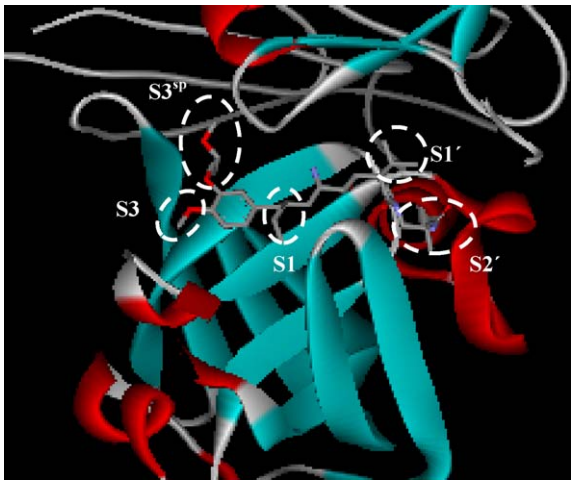


Fig. 6. Active site pockets that are occupied from compound **2** (Aliskiren).

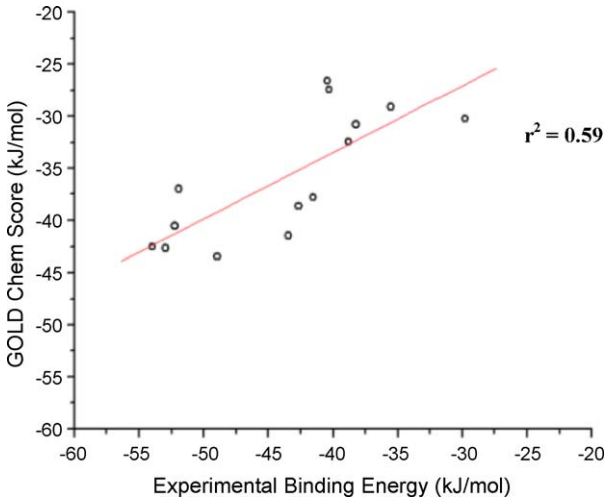


Fig. 7. Linear regression between the experimental binding energy values with GOLD ChemScore docking scores.

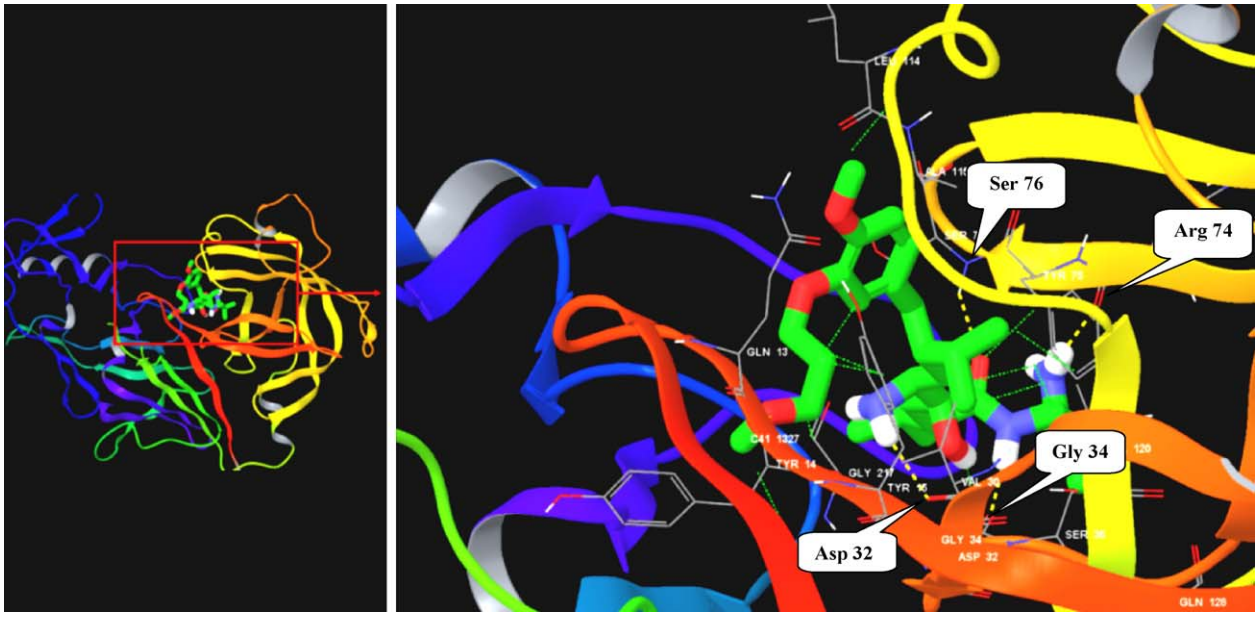


Fig. 8. (Right) Top binding pose derived from Glide/IFD for compound **2**. (Left) Close look to the binding interactions: yellow and green dashed bonds show H-bonds and close-van der Waals contacts, respectively.

and 2.6 Å) and has a distance of 3.4 Å from the oxygen of Asp 32 while the other $-NH_2$ group forms H-bonds with the oxygen of Asp 215 (~1.5 Å) and C=O of Gly 217 (~2.6 Å) and has a 4 Å distance from the –OH of Ser 76. In compound **10** the oxygen of the chain forms an H bond with the –NH group of amino acid Tyr 14. The $-NH_2$ group that is positioned between the two nitrogens of the pyrimidine ring forms an H bond with Asp 32 (~1.8 Å) and also has a distance of 4 Å from Asp 215 and 4 Å distance from C=O of Gly 34. The other $-NH_2$ group of the pyrimidine ring has a 3.4 Å distance from the –OH group of the amino acid Thr 77.

The binding to the catalytic aspartate residues is vital for all the protease inhibitors [25]. All the renin inhibitors in the above study demonstrate interactions with at least one of the aspartate residues of renin Asp 32 or Asp 215. This makes clear that any new renin inhibitor should interact with one of the aspartate aminoacids. On the other hand, the $S3^{sp}$ is unique for renin to provide additional potency and specificity against other aspartic proteases [18]. Compounds **2** and **10** that interact with Tyr 14, the amino acid of $S3^{sp}$ possess higher potency compared to compound **14** that does not interact with this sub pocket. Additionally, compound **2** occupies five of the active site pockets ($S3^{sp}$, S3, S1, S1′, S2′) while compounds **10** and **14** occupy four ($S3^{sp}$, S3, S1, S1′) and two pockets (S1, S1′), respectively. These additional interactions of compound **2** versus **14** and **10** justify its higher activity. The active site pockets that compound **2** occupy are depicted in Fig. 6.

Furthermore, the linear regression fit methods were used to correlate the experimental binding energy values with GOLD's ChemScore binding energy results (Fig. 7). Correlation coefficient r^2 was found as 0.59. Linear regression equation was derived as $y = -7.9094\ (\pm 6.80) + 0.63863\ (\pm 0.15)x$; where y is the ChemScore and x is the experimental binding energy.

Table 5
Glide/IFD docking results. Binding affinities were converted through free energy equation using $T = 300$ K.

Compounds	Exp. binding energies (kJ/mol)	Glide/IFD docking scores (kJ/mol)
1	−53.97	−48.53
2	−52.95	−43.22
3	−52.24	−49.87
4	−51.95	−42.13
5	−48.95	−35.82
6	−43.47	−48.74
7	−42.68	−35.80
8	−41.56	−37.78
9	−40.46	−42.38
10	−40.33	−39.37
11	−38.83	−39.46
12	−38.21	−40.00
13	−35.53	−36.53
14	−29.77	−38.03
15	−52.26	−45.73
16	−48.93	−42.05
17	−47.21	−47.32
18	−45.71	−45.43
19	−43.99	−44.85
20	−46.82	−46.50

2.2.1. Glide/IFD docking

Glide/IFD under Schrodinger molecular modeling package has been used for docking studies. Crystal structures were downloaded from the http://www.rcsb.org web site and renin inhibitors were

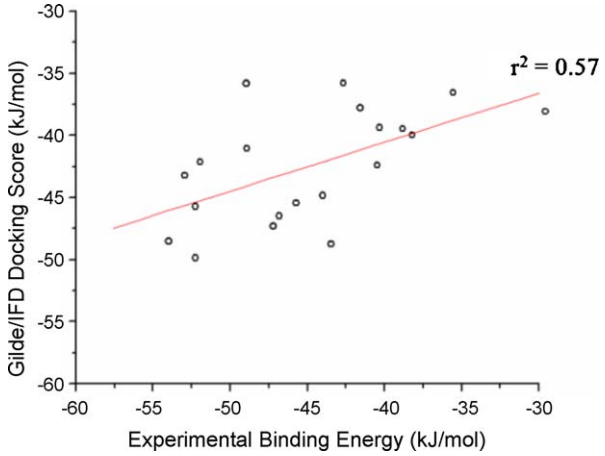


Fig. 9. Linear regression between the experimental binding energy values with Glide/IFD docking scores.

extracted from these coordinate files. These inhibitors were docked into the renin active site using Glide/IFD. Fig. 8 shows the best binding pose of **2** at the active site of the receptor which has similar binding pose with GOLD docking pose that has highest docking score. Table 5 lists the docking results of 20 inhibitors. For easy comparison of experimental and calculated values, binding affinities were converted to the binding energies. Linear regression fit methods were used to correlate the experimental binding energy values with Glide/IFD docking scores (Fig. 9). Correlation coefficient r^2 was found as 0.57. Linear regression equation was derived as $y = -24.78149\,(\pm 6.10) + 0.39395\,(\pm 0.13)\,x$; where y is the Glide/IFD score and x is the experimental binding energy.

3. Conclusion

The GoldScore function is considered as an initial criterion to estimate the binding affinity of a renin inhibitor. The results show significant correlation between GoldScore and pIC_{50} values. Linear regression analysis was also performed using Glide/IFD and ChemScore binding energy scores with experimental binding energy results. A satisfactory correlation ($r^2 > 0.5$) was observed. For both docking scores linear regression equations were derived. As discovery of novel renin inhibitors templates remain a clear need, derived equations can be used in predicting binding affinity values prior to the synthesis and *in vitro* biological measurements of novel compounds. The introduction of the model was based on docking studies demonstrating the power of this approach in predicting binding affinities of compounds.

A set of renin inhibitors with known crystal structures were used to construct these models. This developed methodology is not limited to renin inhibitors but it can be applied to any class of bioactive molecules. Additionally this methodology is of paramount importance when the experimental results are limited and no QSAR studies with compact models can be developed. A validation with *in vitro* experiments of novel renin inhibitors in futures studies will boost the predictability of this model.

4. Methods

The protein used in the docking studies was obtained from the protein data bank with the code 2v0z. All hydrogen atoms were added and the inhibitor and water molecules were removed. An active site of 10 Å around the docked inhibitor was created.

Fourteen renin inhibitors were taken from the reported literature [18,19] for the docking studies. The renin inhibitors under study have known crystal structure. The structure of these molecules, their IC_{50} values and the code of the pdb files that were extracted are provided in Table 1. All the crystal structures selected had a resolution better than 2 Å. The crystallographic bound renin inhibitors were removed from the binding site. Then, the structures were energy minimized using Steepest Descent, Conjugated Gradient and Powell algorithms with a convergence gradient value of 0.001 kcal/(mol Å) using SYBYL molecular modeling package.

Docking studies were performed using genetic optimization for ligand docking (GOLD) software that uses the genetic algorithm (GA) to explore the full range of ligand conformational flexibility with partial flexibility of the protein [20] as well as Glide/IFD docking programs which uses full flexibility to the docked ligands and active site residues.

4.1. GOLD

The maximum number of generic algorithm runs was set to 20 for each compound. The default generic algorithm parameters were selected (100 population size, 5 number of islands, 100,000 number of generic operations and 2 for the niche size). Default cutoff values of 2.5 Å (dH-X) for hydrogen bonds and 4.0 Å for van der Waals distance were employed. When the top three solutions attained RMSD values within 1.5 Å, GA docking was terminated.

The two scoring functions used, were the GoldScore fitness function and the ChemScore [21,24]. The GoldScore function is a molecular mechanics-like function with four terms:

$$\text{GoldScore Fitness} = S_{\text{hb\_ext}} + S_{\text{vdw\_ext}} + S_{\text{hb\_int}} + S_{\text{vdw\_int}} \quad (4)$$

where $S_{\text{hb\_ext}}$ is the protein–ligand hydrogen-bond score and $S_{\text{vdw\_ext}}$ is the protein–ligand van der Waals score. $S_{\text{hb\_int}}$ is the contribution to the Fitness due to intramolecular hydrogen bonds in the ligand; $S_{\text{vdw\_int}}$ is the contribution due to intramolecular strain in the ligand.

On the other hand, the ChemScore function estimates the free energy of binding of the ligand to a protein as follows:

$$\Delta G_{\text{binding}} = \Delta G_0 + \Delta G_{\text{hbond}} S_{\text{hbond}} + \Delta G_{\text{metal}} S_{\text{metal}} + \Delta G_{\text{lipo}} S_{\text{lipo}} + \Delta G_{\text{rot}} H_{\text{rot}} \quad (5)$$

where S_{hbond}, S_{metal}, and S_{lipo} are scores for hydrogen-bonding, acceptor-metal, and lipophilic interactions, respectively. H_{rot} is a score representing the loss of conformational entropy of the ligand upon binding to the protein.

The final ChemScore value is obtained by adding in a clash penalty and internal torsion terms, which militate against close contacts in docking and poor internal conformations. Covalent and constraint scores may also be included:

$$\text{ChemScore} = \Delta G_{\text{binding}} + E_{\text{clash}} + E_{\text{int}} \quad (6)$$

4.2. Glide/IFD

Geometry optimization calculations for ligands were performed with the Schrodinger's maestro module using Polak–Ribiere conjugate gradient (PRCG) minimization (0.0001 $kJÅ^{-1}\,mol^{-1}$, convergence criteria) [26,27]. Protonation states of ligands and residues were tested using LigPrep and Protein Preparation modules under Schrodinger package at neutral pH. The Glide-XP (extra precision) (v5.0) [27] combined with Induced Fit Docking (IFD) have been used for the docking calculations. IFD uses the Glide docking program to account the ligand flexibility and the refinement module and the Prime algorithm to account for flexibility of the receptor. Schrodinger's IFD protocol model uses the following steps (the description below is from the IFD user manual): (i) constrained minimization of the receptor with an RMSD cutoff of 0.18 Å; (ii) initial glide docking of each ligand using a soft potentials (0.5 van der Waals radii scaling of non-polar atoms of ligands and receptor using partial charge cutoff of 0.15); (iii) derived docking poses were refined using the Prime module of the Schrodinger. Residues within the 5.0 Å of ligand poses were minimized in order to form suitable conformations of poses at the active site of the receptor; (iv) Glide re-docking of each protein–ligand complex.

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