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Full Length Research Paper

Comparative study of inhibition of drug potencies of c-Abl human kinase inhibitors: A computational and molecular docking study

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Structural studies suggest that the c-Abl protein kinase domain exists in two conformations; an active and an inactive form. There are many inhibitors which bind this tyrosine kinase in both forms. Many of these kinase inhibitors are in clinical trials too. The inhibition potency of these inhibitors is a common topic of discussion. In the present study we have taken a library of eight different inhibitors and docked those using GLIDE. After GLIDE docking we have also calculated induced fit results. The validity of the docking scores was compared to the post-docking score calculated by the Molecular mechanics - gernalised Boltzman/ surface area (MM-GB/SA) approach. During this process, Imatinib and Nilotinib showed very similar scores and binding energy. A comparative study of all eight inhibitors suggest that Imatinib and Nilotinib have the best binding scores and hence, they can be considered as the best drugs relative to PHA, VX6, PD3, PD5, P17 and Dasatinib. Our findings provide further rationale for considering kinase conformation in the design of kinase inhibitors.

Key words: Docking, scoring, binding affinity.

INTRODUCTION

The development of protein crystallography over a last few decades has revolutionized advances in drug discovery. This technique enables us to visualize the precise positions of the individual atoms of a molecule which causes disease, thus revealing the mechanism of disease, which then assists us in drug discovery (Blundell et al., 2002). Comparative crystallographic studies of protein kinases have uncovered the secrets of irregularities in cell growth. A protein kinase is an enzyme that modifies other proteins by chemically adding phosphate groups to them. This phosphorylation usually results in a functional change of the target protein by changing enzyme activity (Goodshell, 2005). Thus, kinases cause changes in a cell's metabolism. Hence, deregulated protein kinase activities, occurring via mutation, can cause diseases like cancer due to uncontrolled changes in a cell. A protein kinase inhibitor is an enzyme that blocks the activity of these kinases by fitting into a binding site. In theory these inhibitors can be used to treat the irregularities and uncontrolled changes in cellular function that occur. During the last few decades there have been many groups who have investigated the conformation and molecular structure of many kinases. These studies reveal that among more than 500 different protein kinases, c-Abl kinase is a type of kinase which transmits messages to the cell for adding to their neighboring cells, to grow or to move to a new location. Since this kinase plays an important role in the structure and the function of organisms, its signaling should be carefully regulated. If it is not, then the total balance of the cell is destroyed resulting in diseases like chronic myelogenous leukemia (CML) (Deininger et al., 2000).

Tyrosine kinases are structurally separated into two domains, an N-terminal domain and a C-terminal domain. The N-terminal domain is made of largely of β sheets while the C-terminal domain is largely comprised of α helices. The N-terminal domain also contains an α helix, which is often known as the αC helix. The binding site for ATP is situated between these two domains. Between these two domains there is an activation loop (245 - 254) which contains a tyrosine residue, which after

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phosphorylation, electrostatically interacts with its neighboring arginine residue producing the open conformation of the kinase. This open loop conformation is suitable for peptide substrates binding. The α helical loop also plays an important role in the kinase's mechanism. This helix presents a glutamate residue that forms a salt bridge with a lysine residue from the N lobes and this pairing coordinates the phosphate groups of ATP. In the inactive form of the kinase, it projects outward preventing the formation of the salt bridge (Sicheri et al., 1997). The N lobes of the kinase also contain a flexible glycine residue, which plays an important role in kinase activity (Nagar, 2007).

These regulatory elements imply that disruption of any one of them can result in changes in the activity of this protein kinase. This presents an important concept for designing inhibitors which bind to the ATP cleft (Al-Obeidi et al., 2000; Atwal et al., 2004). Fortunately many inhibitors have been developed as targets of this cleft (Zhang et al 2009). Imatinib is a type of ATP competitive inhibitor, which binds to the cleft between the N- and C-terminal domains of the kinase. It has been approved for use in patients affected with CML. The crystallographic structure of c-Abl protein kinase bound to Imatinib (Cowan-Jacob et al., 2007) revealed that after binding of Imatinib, the activation loop is folded inward (Nagar et al., 2003; Nagar, 2007). This inverted conformation prevents the aspartate residue from ligating with magnesium. Due to its strong binding behavior, Imatinib has been used in clinical trials. Besides Imatinib, Nilotinib is also a highly potent, very selective and active inhibitor against these types of kinases (Rix et al., 2007; Redaelli et al., 2008). Due to its increased inhibition, it was also used in clinical trials. A related inhibitor, Dasatinib, has also been studied; it has been found to be more effective for the Src/Abl kinase inhibition with potent anti proliferative activity against hematological malignancies harboring activated BCR-ABL (Tokaraski et al., 2006). A study shows that Dasatinib blocks the migration and the invasion of human cells without affecting proliferation and survival (Buettener et al., 2008). Another study shows that PHA 739358 also plays an important role as an Abl kinase inhibitor and binds the pan aurora as well as Abl kinase (Carpinelli et al., 2007). The role of the aurora kinase inhibitor, VX680 (Weisberg et al., 2006), as well its complex structure, have also been studied. It has been identified as a potent and selective small molecule inhibitor, which suppresses tumor growth (Young et al., 2006). By applying similar strategies, other chemo type inhibitors have been developed and their crystallographic studies have been done. These inhibitors have been predicted for therapeutic use by binding to the tyrosine kinases. The chemical structures of these inhibitors are shown in Figure 1.

In the present work we have investigated the c-Abl human kinase receptor from the 2hyy complex (Cowan-Jacob et al., 2007) and the above inhibitors, that is, Imatinib, Nilotinib, Dasatinib, PHA, PD3, PD5, P17, VX680 from different complexes. These drugs have already predicted for kinase inhibition. Computational docking of the inhibitors into kinases gives an accurate insight of the behavior of this molecule. We have calculated docking scores for each drug and each target complex separately, which theoretically explains binding of these inhibitors with the c-Abl human tyrosine kinases.

METHODOLOGY

Simulation was performed using SCHRODINGER Inc software package (Schrodinger Inc, New York). The initial structure was taken from the protein data bank (pdb id 2HYY, www.pdb.org), which was used as the starting coordinates for the simulation studies.

Structure preparation

The coordinates for all proteins were obtained from RCSB protein data bank (PDB code 2hyy). The imported structure was a tetramer in which all the units contain same binding site and ligand, so we have considered only one unit of the complex with a single ligand for our studies. The complex was prepared by protein preparation wizard software, where hydrogens were added automatically and refinement of the structure was also done. Since water molecules in the crystallographic complex were not critical to the functioning of the protein-ligand interaction, all of the crystallographic waters were deleted and the bond orders were re-assigned. Then the systems were minimized to a RMSD (Root Mean Square Deviation) 0.30 angstrom. The complex structure contains some missing residues, which were added through the prime application of the Schrödinger Suite.

Ligand preparation

Ligands were obtained from complex structures and we prepared them using the Ligprep module of the Maestro software. The crystallographic ligands did not have correct bond orders so we modified the bond orders manually according to their pdb data. Each ligand was subjected to a full minimization in the gas phase with the OPLS (Optimized Potential for liquid simulations) force field (Jorgenson et al., 1996) to eliminate the bond length and bond angles biased from the crystal structure. Under any physiological condition, a molecule can exist in a variety of protonation (ionization) states, so here we have produced multiple structures for each ligand with different combinations of ionized states based on the ionizable group present. This operation was done by help of ionizer in Ligprep. Since most proteins function in the pH range of 5 - 9, we have restricted the ionization to this range.

Since the crystal structure contains only one ligand structure but there is a chance that one of the tautomeric forms interacts more strongly with the binding site relative to the other forms, we have taken into account this concept and generated all of the other possible tautomeric states of one inhibitor.

Ligand docking and scoring

Prepared ligand and receptor were used as the initial coordinates for docking purposes. We have used c-Abl tyrosine kinase as the target receptor. The principle ligand can be docked by two methods: (1) Assuming that the ligand is flexible and the receptor is rigid and (2) Assuming that the ligand is rigid and the receptor is flexible. So here we have used both strategies of ligand docking. In

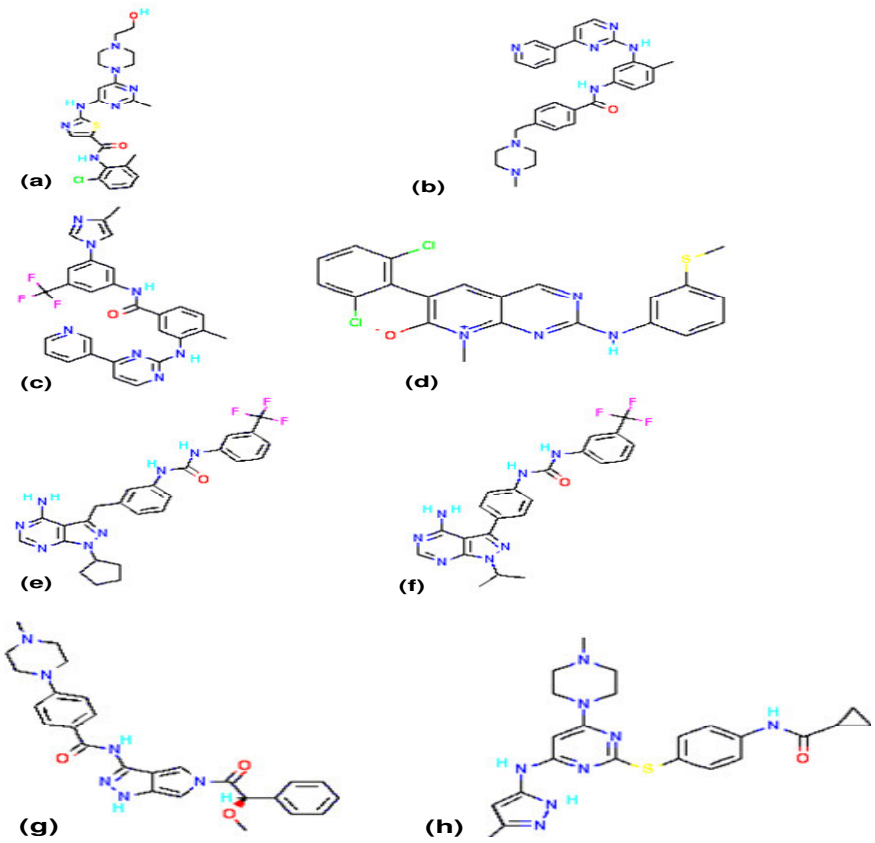

Figure 1. Chemical structure of different kinase inhibitors in their minimized positions. Non-carbon hydrogens are colored in green. The structures were generated using Chimera (a) Dasatinib, (b) Imatinib, (c) Nilotinib, (d) P17, (e) PD3, (f) PD5, (g) PHA and (h) VX-6.

both processes we have used GLIDE for docking (Friesner et al., 2004; Halgren et al., 2004).

The first stage for ligand docking was the receptor grid generation; for that purpose we have used the kinase protein structure complexed with Imatinib. During the grid generation, no Vander Waal radius sampling was done and the partial charge cutoff (Cho et al., 2007) has been taken as 0.25 and no constraints were applied (Sherman et al., 2006). The location of Imatinib was taken as binding site for docking of all of the other ligands. The ligand docking calculations were done in the standard precision mode of GLIDE. During the docking process, the receptor was treated as fixed while ligand was flexible. In the minimization of ligands, we have used a distance-dependent dielectric constant with a value of 2.0 and a conjugate gradient algorithm with small 100 steps. All of the inhibitors were passed through a scaling factor of 0.80 and partial charge cutoff of 0.15. After docking, we have performed post-docking minimization to improve the geometry of the poses. The post-docking minimization specifies a full force-field minimization of those poses which are considered for the final scoring. After docking, the results were used for binding energy calculations and docking scores (Bissantz et al., 2000). The MM-GBSA approach was used to predict the free energy of binding for a receptor and a set of ligands (Nu et al., 2006; Gohlke et al., 2000, 2002).

For the second process, we have assumed that the ligand was fixed while the receptor was flexible (that is, we have used an induced fit docking protocol in the first stage of this protocol). Softened potential docking was performed to generate 20 initial poses. The softened potential docking consisted of scaling the Vander Wall radii by a factor of 0.5; this process was done to give an extra cavity to ligand to be fit in the binding site (Moitessier et al., 2006). All of the docking calculations were performed using the standard Precision mode of Glide. For each of 20 poses from the initial softened potential docking step, a full cycle of protein refinement was performed. For refinement we have also used the OPLS_2005 parameter set and the surface Generalized Born implicit solvent model (Zhang et al., 2000). After convergence to a low energy solution, an additional minimization was performed to make all of the residues in the backbone and the side chains to be relaxed. The sum of molecular mechanics and solvation energy was calculated and those complexes having energy within the range of 30 kcal/ mol, were accepted for docking score.

RESULTS AND DISCUSSION

Virtual docking

We have applied the GLIDE docking method to seven inhibitors of tyrosine protein kinases to build a binding affinity model for the c-Abl human tyrosine kinase receptor that was then used to compute the free energy of binding for this kinase.

The ligand preparation procedure generated different

Table 1. Average Vander waals (vdw), electrostatic (coul) and Site energy (site) after GLIDE docking[a].

Inhibitors	E_{vdw}	E_{coul}	E_{site}	Docking score	Energy
Imatinib 1	-64.432	-11.216	-0.059	-11.953	-75.649
Imatinib 2	-64.165	-08.431	-0.063	-10.662	-72.596
Imatinib 3	-62.186	-16.276	-0.117	-12.302	-78.462
Imatinib 4	-61.354	-13.318	-0.099	-10.788	-74.673
Imatinib 5	-62.890	-18.320	-0.167	-13.136	-81.210
Imatinib 6	-60.489	-15.283	-0.163	-11.484	-75.773
Imatinib 7	-65.248	-21.593	-0.153	-13.400	-86.841
Imatinib 8	-59.592	-20.087	-0.244	-11.587	-79.680
Nilotinib 1	-67.135	-11.497	-0.121	-12.825	-78.633
Nilotinib 2	-66.141	-13.755	-0.136	-12.914	-79.897
Nilotinib 3	-65.086	-08.909	-0.108	-11.171	-73.995
Nilotinib 4	-64.384	-11.402	-0.133	-11.477	-75.786
Dasatinib	-57.271	-15.059	-0.174	-09.910	-72.276
PHA 1	-39.277	-07.407	-0.172	-04.622	-46.685
PHA 2	-46.271	-03.350	-0.032	-04.790	-49.622
PHA 3	-42.689	-12.486	-0.306	-05. 311	-59.195
PHA 4	-50.365	-08.830	-0.041	-07.629	-55.175
VX6 1	-36.666	-02.802	0.000	-05.391	-39.468
VX6 2	-34.512	-12.396	-0.302	-04.384	-46.908
PD17	-51.248	-04.064	0.000	-10.533	-55.314
PD3	-49.847	-06.670	-0.234	-06.780	-56.517
PD5	-49.545	-02.818	-0.040	-07.498	-52.363

[a] The energy data are written according to the structure generated by the docking program. The best docked poses are written in bold letter. All energies are given in Kcal/mol.

training sets of the inhibitors whose scoring function is given in Table1. These different structures were found by using different orientations of the inhibitors and different positions of the hydrogens. The results of docking and scoring are given in Table 1. According to the table we see that among all the energy parameters the largest contribution for binding energy comes from Vander Waals interactions. The cavity energy term is very small, which indicates that there is a very low energy penalty when the ligand is buried in the cavity. The low rms deviation (0.30 Angstrom) indicates that the receptor has approximately the same structure as it does in the crystal structure. We then calculated docking scores for all other inhibitors. It is clear that among all of the 26 generated structures of the inhibitors, Imatinib and Nilotinib have very good docking scores. During the ligand preparation we have got eight different training sets of Imatinib. All of the training sets of Imatinib have different docking energies as well as docking scores. This observation shows that the inhibition of any ligand depends not only on the cavity site but also on the ionization and the tautomeric state of the inhibitors. Among all the eight training sets, Imatinib 7 had the best docking score, and the binding energy of this training set was also maximum. In best docked position, Imatinib forms five hydrogen bonds with THR-315, GLU-286, ASP-381, and ILE- 360 and MET-318 residues of the c-Abl kinase receptor (Figure 2a). The hydrogen binding with the ASP-381 plays an important role in kinase inhibition because the aspartate residue is the main constituent of the DFG loop of the tyrosine kinase receptor (Nagar, 2007). In the active kinase, the conformation of the aspartate residue of the DFG motif is oriented towards the bound ATP and is capable of ligating a critical magnesium ion bound to the phosphate group of ATP. After docking, Imatinib forms hydrogen bonds with the aspartate of the DFG loop, which negates its ability to bind to ATP. Thus, the activity of kinase may be reduced. Hence the mechanism of Imatinib inhibition has been clarified by computational docking. Nilotinib also has a very good docking score, which is slightly less than Imatinib. The docked structure shows that Nilotinib has approximately the same bound structure with the receptor (Figure 2b). Hence, we may conclude that the mechanism of inhibition of Nilotinib and Imatinib are similar. Nilotinib exhibits four different structures based on its different ionization and tautomeric states. When we compare the results of Table 1, it is obvious that the Vander Waals energy contribution for Nilotinib and Imatinib are approximately the same but there is a considerable difference in their columbic interactions.

This difference occurs due to the fluorine atoms present in the Nilotinib structure.

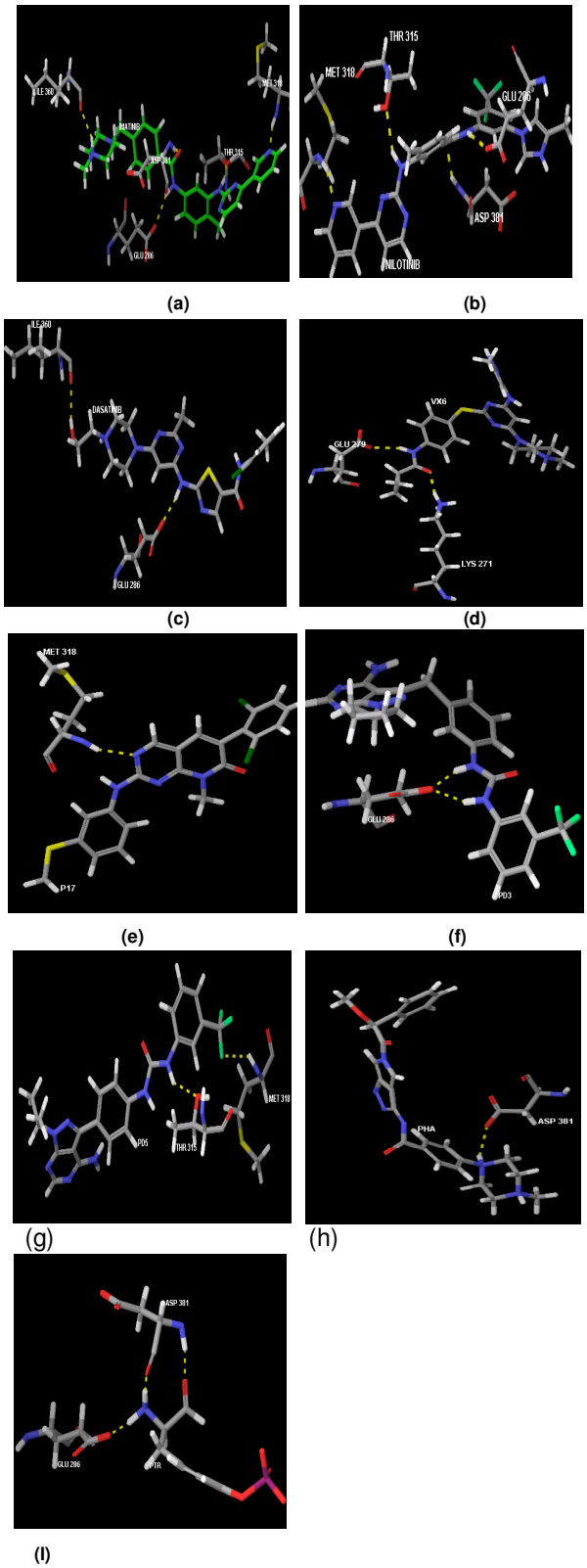

Figure 2. Structures of the different kinase inhibitors bound to the protein 2hyy. Only residues that undergo significant movement or are hydrogen bonded to the ligand are shown (a) binding of Imatinib, (b) binding of Nilotinib, (c) binding of Dasatinib, (d) binding of VX6, (e) binding of P17, (f) binding of PD3, (g) binding of PD5, and (h) binding of PHA.

Table 1 shows that, besides well known inhibitors like Imatinib and Nilotinib, there are also inhibition possibilities with Dasatinib, PHA, VX6, and PD5 and PD3. Interactions of these inhibitors with the c-Abl kinase receptor are shown in Figure 2c - I. The inhibition potential of these kinase inhibitors is in same sequence as written above. These inhibitors have significant docking scores and binding energies by MM-GB/SA approach. The binding energies of these inhibitors are shown in Table 2.

Induced fit results

In virtual docking (Taylor et al., 2002), the ligands are docked into binding site of the receptor where the receptor is held rigid and the ligand is free to move. But the c-Abl kinase receptor shows a critical hinging and on-off state, hence it may undergo side chain or backbone movement. These changes may allow alterations in the receptor so that it more closely conforms to the shape and binding modes of the ligands. Thus, we have also taken into account receptor flexibility (Broughton et al., 2000) by induced fit docking. In induced fit docking, we obtained 18 different poses for Imatinib, 19 different poses for Nilotinib, 19 for Dasatinib, 15 poses for VX680, 10 poses for PD3, 14 poses for PD5 and 14 different poses for P17. The results of the induced fit docking are given in Table 3, which displays the best docked poses. From the results of the induced fit docking, it is clear that there are some considerable changes in the docking scores and energies of the docked complexes. By comparing the results of flexible receptor docking and rigid receptor docking, we see that there is no noticeable change for Imatinib. In each case the docking score is approximately same. Structure analysis also shows no change in hydrogen bonding. The similarity in the results implies that Imatinib binds to receptor in an ideal manner and after the docking, it does not allow further flexibility in the binding site. But for other inhibitors, like Nilotinib, Dasatinib, PHA, PD3, PD5, P17, and VX6, there are noticeable changes in docking scores as well as in hydrogen bonding. These differences in the results may also be explained due to the strategy adopted in induced fit methods. The strategy in the induced fit method is to first dock the ligands into a rigid receptor using a softened energy function such that steric clashes, do not prevent at least one ligand pose from assuming a conformation close to the correct one. Then there is a sampling of the receptor degree of freedom and a minimization of the receptor-inhibitor complex for many different receptor poses and it is attempted to identify low free energy conformation of the each complex. A second round of the ligand docking is then performed on the refined protein structure, this time using a hard potential function to further sample ligand conformational space within the refined protein environment (Sherman et al., 2006).

Table 2. Binding energy[a] data for different inhibitors[b].

Ligand	dG(1)c	dG(2)	G_comp	G_lig	G_rec	Lstrain
VX 6 1	-05.07	-15.02	86218.83	-161.89	86385.80	09.95
VX6 2	-19.22	-22.75	86205.09	-161.49	86385.80	03.53
PTR2	71.49	61.03	86141.17	-316.12	86385.80	10.46
PTR1	76.23	73.83	86146.49	-315.54	86385.80	02.41
PHA3	-30.39	-38.60	86377.54	022.14	86385.80	08.20
PHA4	-33.22	-39.49	86374.10	021.53	86385.80	06.27
PHA2	-31.74	-38.22	86424.70	070.64	86385.80	06.48
PHA1	29.58	-37.23	86427.09	070.87	86385.80	07.65
PD5	-36.37	-41.49	86249.33	-100.10	86385.80	05.12
PD3	-34.96	-46.03	86270.14	-080.70	86385.80	11.07
Nilotinib2	-63.05	-73.84	86157.15	-165.60	86385.80	10.79
Nilotinib1	-67.79	-73.71	86178.41	-139.59	86385.80	05.91
Nilotinib4	-48.80	-60.16	86155.83	-181.16	86385.80	11.35
Nilotinib3	-54.13	-59.98	86176.73	-155.53	86385.80	05.84
Imatinib7	-67.37	-76.03	86134.53	-183.90	86385.80	08.66
Imatinib5	-70.65	-82.34	86183.64	-131.50	86385.80	11.69
Imatinib3	-73.02	-84.28	86186.79	-125.99	86385.80	11.27
Imatinib1	-46.62	-57.37	86262.92	-076.26	86385.80	10.75
Imatinib8	-47.96	-60.06	86138.57	-199.26	86385.80	12.10
Imatinib6	-55.91	-67.80	86182.36	-147.52	86385.80	11.89
Imatinib4	-57.00	-69.03	86186.68	-142.12	86385.80	12.03
Imatinib2	-31.52	-42.47	86261.67	-092.61	86385.80	10.96
Dasatinib	-51.83	-34.72	86158.09	-175.87	86385.80	10.

[a] All energy values are given in the Kcal/mol. [b] Binding energy data are not arranged in the best posed position. [c] dG1 and dG2 are binding energy in different condition of complex, which are calculated by using following formula. dG(1) = E_complex(minimized) - (E_ligand(minimized) + E_receptor). dG(2) = E_complex(minimized) - (E_ligand(from minimized complex) + E_receptor).

Table 3. Results of the induced fit docking[a].

Inhibitors	Docking energy	Docking score
Imatinib	-86.073	-13.313
Nilotinib	-86.630	-14.178
Dasatinib	-68.274	-11.220
PD5	-64.244	-09.951
PD3	-69.341	-10.866
VX6	-77.340	-13.066
PHA	-46.843	-05.311
P17	-65.392	-12.238
PTR	-44.743	-07.912

[a] docking energy is written only for the best docked poses. All energy values are given in Kcal/mol.

Finally, a composite scoring function is applied to rank the complexes, accounting for the receptor ligand interaction energy as well as strain and solvation energy. The validity of the induced fit method is already proved for flexible proteins as well as where little or no conformational changes in the receptor are required to properly dock the ligand.

Binding energy calculations

In the induced fit calculations, improvement in the ranking of the known ligand and better discrimination among the rest of compounds in the database was achieved by taking into account the ligand – receptor solvation energy (Jacobson et al., 2002, 2004). Hence in this methodology, the MM-GB/SA calculations have been performed also to enhance the docking scoring (Zhang et al., 2001). The MM-GB/SA results for docking all of the ligands are given in Table 2. Table 2 shows that the ligand strain energy is different for different inhibitors. The ligand strain energy is the difference of (1) the binding energy dG1 calculated from the difference of the energy of minimized complex and sum of the energies of the minimized ligand and receptor and (2) the binding energy dG2 calculated as difference of energy of the minimized complex to the sum of the energy of the ligand from minimized structure and energy of the receptor (Given as formula in the legend of Table 2).

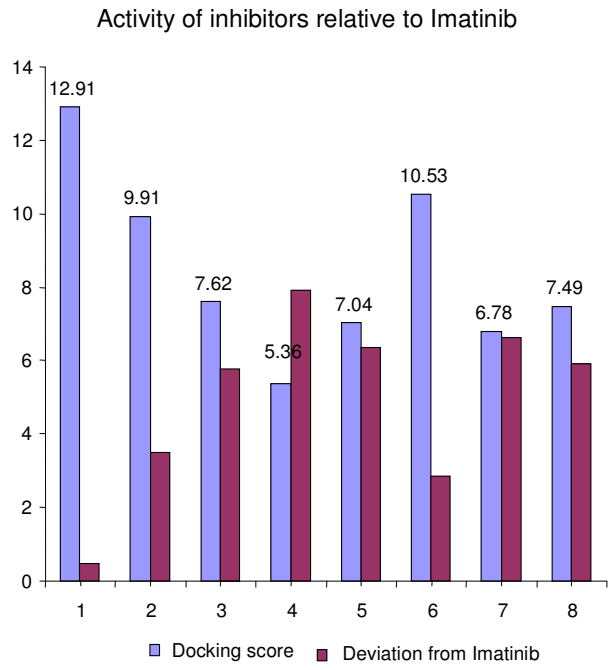

Figure 3. Comparative scoring of different inhibitors with respect to Imatinib. Here Imatinib is taken as the standard unit of kinase inhibition and the other ligands are compared to it. 1- Nilotinib, 2-dasatinib, 3-PHA, 4,-VX6, 5-PD17, 6- PD3, 7-PD5.

Comparing the results of dG1 and dG2, we see very considerable differences between these two free energies. In the first case when we minimized the ligand in the absence of receptor, the ligand adjusts its conformation independently. It does not show the exact position of ligand in the complex. But the minimization of the ligand into the complex represents ligand stabilization with respect to the receptor; hence there is a noticeable difference between the free energies in both cases. It is also obvious from the Table 2 that the free energy in the second case also gives better results than the first case. This shows that ligands in second case, bind the receptor more accurately than the first case. Hence the second case represents the better position of the ligand.

The first three training sets of Imatinib exhibit high free energy, whereas, the first two ligands of Nilotinib have more reliable free energies. Comparing these results with Table 1 we see that inhibitors which have good docking scores also have reliable free energies. Table 1 show that Imatinib, Nilotinib computationally behave as the best inhibitors because they have good docking scores and binding energies (Sotriffer et al., 2002; Taylor et al., 2002). Figure 3 shows a comparative graph of the other inhibitors with respect to Imatinib. It is clear from the graph that Nilotinib and Dasatinib are the best inhibitors compared to Imatinib. Also, comparing the results of Tables 1 and 2, it clear that by GLIDE docking methods we have approximately same free energy results for Nilotinib and Imatinib.

Conclusion

The glide score can be used as a semi-quantitative descriptor for the ability of ligands to bind to a specific conformation of the protein receptor. Generally speaking for low glide score good ligand affinity to the receptor may be expected. According to the glide score and MM-GB/SA binding energy, the results of the inhibition for the c-Abl human tyrosine kinase receptor may be arranged in the following manner: Imatinib> Nilotinib > P17 > Dasatinib > PHA > PD5 >PD3> > VX6. Conformational analyses of different docked complexes also show that residues ASP-381, THR-315, GLU-286, MET-318, ILE-360, LYS-281and GLU-279 play important role in this kinase's activity.

Docking studies performed by GLIDE has confirmed that above inhibitors fit into the binding pocket of the c-Abl kinase receptor. From the results we may observe that for successful docking, intermolecular hydrogen bonding and liphophilic interactions between the ligand and the receptor are very important. We can also explain why the glide score and MM-GB/SA results for Imatinib are higher than others. The main reason for this are penalties for close intra-ligand contacts. Using the results, we have described a structural-based model of inhibition of c-Abl tyrosine kinase. Docking results show that Imatinib, Nilotinib, Dasatinib, PHA, PD5, PD3, PD17, VX6 inhibitors may penetrate deeply into binding site. The results also suggest that Imatinib and Nilotinib bind with the tyrosine residue of the DFG loop and compete with ATP binding.

A comparison of the induced fit and virtual docking gives the role of protein flexibility. It is obvious from the results that a combined method of soft docking and side chain optimization gives better results. It is also clear that an average distribution of docking free energy ranging from 2 kcal/mol or more, is sufficient to mis-rank a potential drug candidate as a weak binder. However, by combining the MM-GB/SA and relaxed complex methods we are able to show the best ranked binding modes.

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