Electronic and Structural Insights of BCR-ABL Inhibitors Under LMC Treatment Perspective

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Abstract Protein-Tyrosine Kinases (PTK) are responsible for the protein–protein transfer of the phosphate present in ATP molecule. This activity is essential and allowed due to its activation through phosphorylation. However, when an inadequate activation occurs, then triggers neoplasms, such as Chronic Myeloid Leukemia (CML). The treatment of these diseases in their chronic phase occurs through tyrosine kinase inhibitors (TKIs). Examples of TKIs are imatinib, dasatinib, gefitinib, erlotinib, nilotinib, afatinib, sorafenib and ponatinib, which act specifically on the protein target. TKIs are a major scientific development for the treatment of BCR-ABL related cancers. However, the numerous possible mutations of the active site of the protein prevent the action of these inhibitors and consequently induces the spread of the disease. Therefore, the computational study of the electronic structure and the molecular docking of these drugs are essential to understand the conformations of the protein active site. This study also leads to effectively advance in the treatment involving TKIs. For a good direction of these studies, it is necessary to know their electronic properties and the orientation of their respective ligands in the active region of the enzyme. Therefore, this work aims to shed light on the development of new potential drugs centered in protein mutations, against neoplasms related to BCR-ABL.

Keywords Tyrosine kinases · Tyrosine kinase inhibitors · Electronic properties · Molecular docking · DFT

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1   Introduction

## 1.1   *Chronic Myeloid Leukemia and Tyrosine Kinase Inhibitors*

The class of protein-Tyrosine Kinase (PTK) is responsible for a range of intra- and extracellular processes, such as metabolic regulation, signaling, cell adhesion, cell division and differentiation, as well as apoptosis. PTKs are responsible for transferring the phosphate present in adenosine triphosphate (ATP) [1–3]. This activity is essential and allowed due to its activation through phosphorylation. However an inadequate activation can triggers neoplasms, such as Chronic Myeloid Leukemia (CML) [4–7]. The treatment of these diseases in the chronic phase occurs through tyrosine kinase inhibitors (TKIs), such as imatinib, gefitinib, erlotinib, nilotinib and afatinib, which acts specifically on the protein target. However, the numerous possible mutations of the protein active site prevent the action of these inhibitors, which consequently induces the spread of the disease. Therefore, the computational study of the electronic structure, as well as the study through molecular docking of these drugs plays a key role to understand the conformations of the protein active site and how to effectively advance in the treatment involving TKIs [8].

CML is a clonal chronic myeloproliferative neoplasm, characterized by the reciprocal translocation between chromosomes 9 and 22-t(9;22) (q34;q11), giving rise to the Philadelphia chromosome (Ph). In the Ph chromosome—disease indicator—the gene from the BCR-ABL1 fusion is found, a determinant factor for CML and responsible for the synthesis of the oncoprotein tyrosine kinase BCR-ABL intrinsic to the pathology[6]. In this context, the BCR-ABL protein is present in all patients with CML, and its hyperactivity triggers the release of cell proliferation effectors and apoptosis inhibitors, and its activity is responsible for the initial oncogenesis of CML [9].

Until the advent of imatinib mesylate TKI, in the 2000s, the treatment options that allowed an improvement in the survival rate and quality of life were limited to scarce drugs, such as bisulfan. However, these drugs did not have the desired efficacy and safety for the patient [10, 11]. Thus, the target therapy is through TKIs, which are the first-line for the treatment of cancers related to BCR-ABL and their respective mutations. According to Cortez et al. [12] the biggest challenge for the treatment of CML is primary or acquired drug resistance through tyrosine kinase mutations in the ATP binding region.

In the present study, the inhibitors afatinib, axitinib, dasatinib, gefitinib, erlotinib, imatinib, lapatinib, nilotinib, pazopanib, ponatinib, sorafenib e and tozasertib were analyzed (see Fig. 1). They act competitively with ATP, natural substrate of TK, and binds to the intracellular domain of the TK portion of the EGFR. Then, suppressing its autophosphorylation and consequent cascade signaling of the BCR-ABL signal transduction pathway [13].

Therefore, studies taking into account the wild and mutated forms of the BCR-ABL T315I protein were carried out together with molecular docking studies and
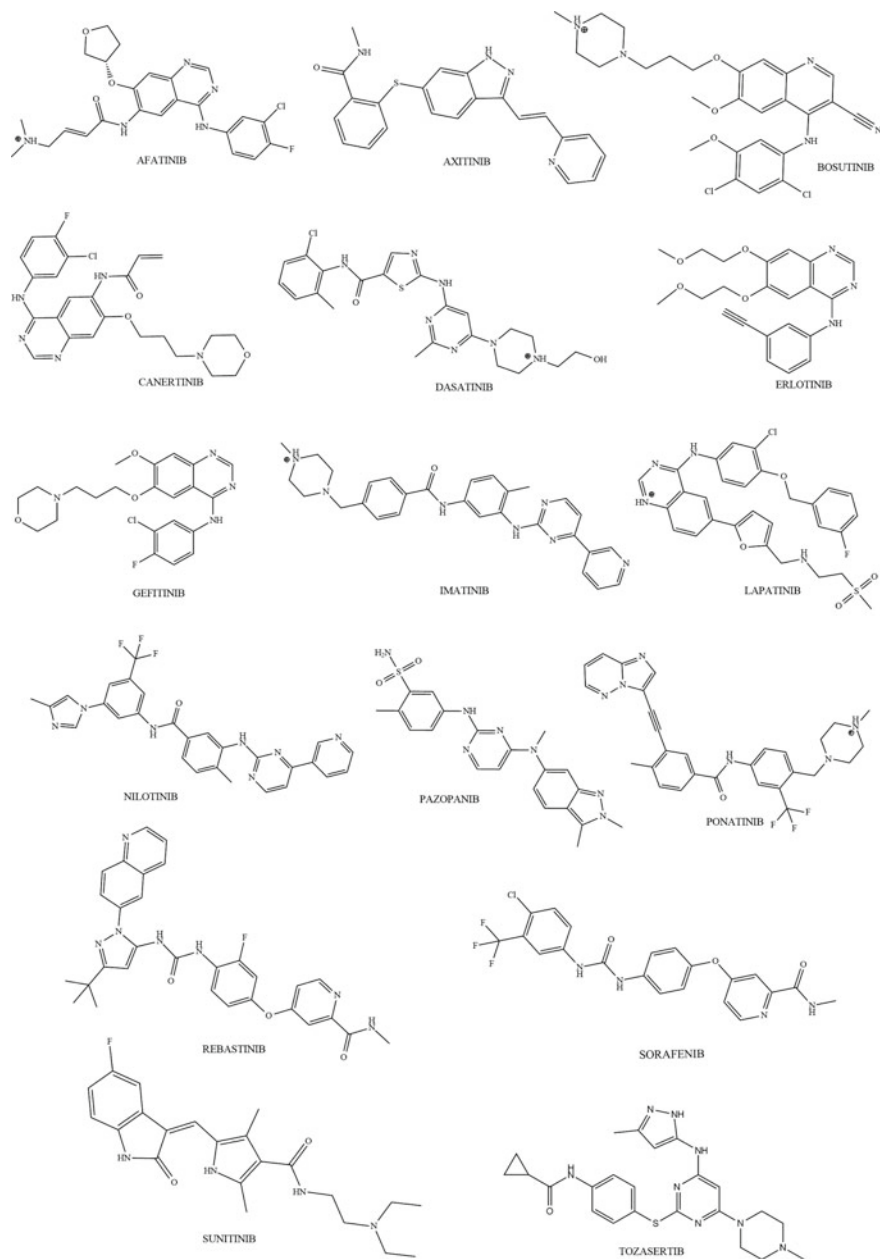
Fig. 1 2D molecular structures of studied TKIs

computational calculations, in order to characterize the inhibitor molecular structure. Therefore, to analyze their most primordial residues that present an effective interaction with the inhibitor and the active site of the protein. We have analyzed the positions presented by each of TKIs within the enzyme and the conformation of their orbitals. The aim is to understand the interaction between them, as well as to indicate which inhibitor is more effective in the BCR-ABL inhibition process.

1.2   BCR-ABL Protein

The BCR-ABL translocation, known as the Philadelphia Chromosome, results from the fusion of part of the ABL oncogene, located on chromosome 9, with the BCR gene, located on chromosome 22 [14], as well as having a tyrosine kinase activity, thus stimulating cell growth and proliferation [8]. It is activated through signal transduction cascades that start with the addition of a phosphate group coming from ATP (phosphorylation), which binds to the active site of the protein [8].

In this context, the substrate pocket is located in a deep crack where there is located an important residue named "gatekeeper", that of threonine 315 (Thr315) shown in Fig. 2, which is also involved in binding to ATP, thus being essential to stabilize the active conformation of BCR-ABL [15]. For this reason, the threonine residue is a determinant target for the interaction with the inhibitor. The T315I mutation confers resistance and decreases the molecular interaction to the first and second TKIs generation due to a replacement of the threonine residue by one of isoleucine [15]. Given the extreme importance of the Thr315 residue, in both cases of substrate and inhibitor binding, several studies and research interests were attracted, as it is believed that this region is a great potential pharmaceutical target [15].

Another important region of the protein is the so-called "A-loop" or activation loop (Fig. 3) which plays a central role in the activation of BCR-ABL, and is responsible for indicating the critical role in modulating the kinase activity, as contains a conserved catalytic site, called "DFG-out", formed by residues Asp381, Phe382, and Gly383 and their respective interactions [15]. In some cases, the "DFG-out" can undergo an inversion in relation to the active conformation of the protein, so this generates a resistance to inhibitors [16].

Protein kinases have been the focus of many targeted treatments. Tyrosine kinases are enzymes responsible for activating many proteins through signal transduction cascades. Proteins are activated by adding a phosphate group to the protein (phosphorylation):

$$\text{ATP + Protein - OH} \rightarrow \text{ADP + Protein - phosphorylated} + H^+.$$
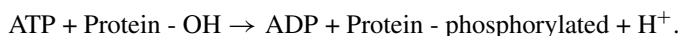
Tyrosine kinase inhibitors can compete with ATP, the phosphorylating entity, the substrate, or both, or they can act allosterically, binding to a site outside the active site, affecting its activity with conformational change. Aiming at the tyrosine kinases that regulate cell growth and proliferation, there are highly effective inhibitors, such
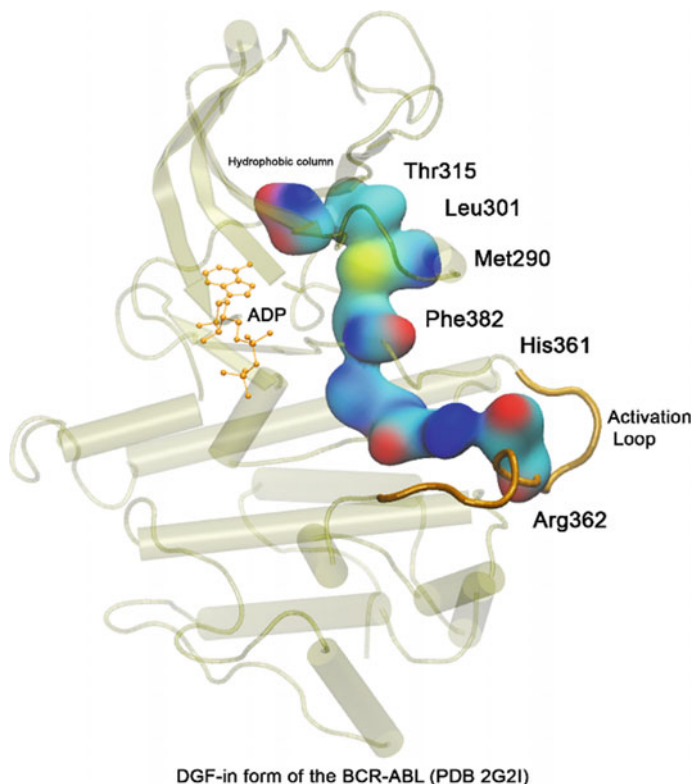
Fig. 2 Inactive conformation for the ABL domain of PTK, showing the Thr315 gatekeeper residue

as imatinib mesylate, (Gleevec, STI 571), and Nilotinib, (Tasigna, AMN107), for the treatment of CML and gastrointestinal stromal tumors (GIST) [8].

The resistance presented to imatinib inhibitor by some patients leads to the first-generation inhibitors. Subsequent inhibitors nilotinib, bosutinib and dasatinib, are also known as second generation were created and took the lead in inhibiting BCR-ABL as well as other TK mutations such as T315I.

In this sense, the objective of this work is to analyze the characteristics, such as chemical groups, volume and intermolecular forces of the first-generation optimized inhibitors as well as the second-generation ones to compare them with the other inhibitors and ligands presented in this work. Molecular docking methodology was used to qualitatively analyze the interactions between protein-inhibitor of BCR-ABL protein in the wild and mutated state.
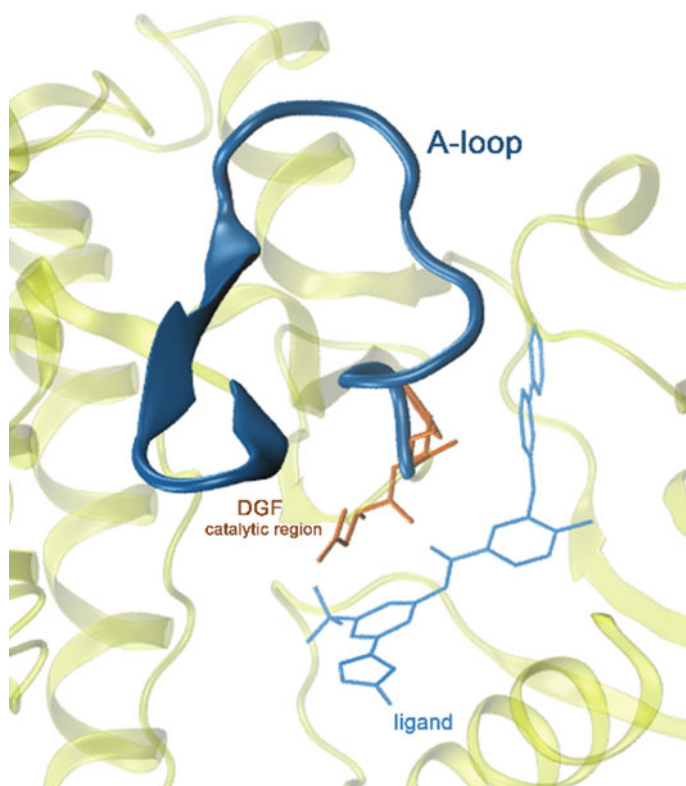
Fig. 3 BCR-ABL conformation for the A-loop, and the DFG catalytic site with nilotinib molecule complex in light blue

2 Methodology

2.1 PDB Structures and Molecular Docking

The wild and mutated forms of the BCR-ABL T315I protein were studied together with molecular docking studies and computational calculations. The aim is to investigate the molecular structure of inhibitors and to analyze their most primordial residues that present an effective interaction with the inhibitor and the active site of the protein. We have also analyzed the positions presented by TKIs within the enzyme and the conformation of their orbitals. In such a way, to understand the interaction between them, as well as to indicate descriptors for inhibitor effectiveness.

Although there are several mutations in the ABL-BCR protein, only the T315I mutation was selected due to its characteristic of resistance to imatinib. Ten three-dimensional structures of proteins were taken from the Protein Data Bank (PDB). The two proteins from *Homo sapiens* were selected, being 3QRK (UniProtKB:

P00519) and 3QRJ (UniProtKB: P00519), because they present minor alterations when compared to the literature. The catalytic sites (P1 and S1) of the structures were analyzed, keeping them intact. The ending residues presented in only one of the proteins were removed to obtain better similarity, and the marked difference between the two proteins was only the T315I mutation.

We carried out the study of the interaction of the BCR-ABL protein through docking, in its wild-type oncological conformation, using the structures deposited in the PDB database under the code 3QRK; and its oncological conformation with mutation, under the code 3QRJ(T315I). Two oncological conformations of BCR-ABL T315I were also studied: the wild type 3OXZ and the mutated type 3OY3. We compared the scores obtained, having the maximum DP-987 inhibitor reference, with the IC_{50} values, and the most likely conformations with the highest score values obtained. The docking study was carried out with the AutoDock4.2.6 [32] and VMD [33].

Inhibitors used for the treatment of CML, imatinib, ponatinib, nilotinib, bosutinib and dasatinib, were studied; and added inhibitors used in the treatment of other types of cancer: axitinib, erlotinib, gefitinib, lapatinib, pazopanib, sorafenib and tozasertib, to qualitatively and quantitatively assess the interactions of these inhibitors with their therapeutic target. The structures of the inhibitors were taken from DrugBank and PubChem (Table 1). The geometric structures of the ATP molecule (DrugBank ID: DB00171) were also obtained from the DrugBank database and from it the structure of the ADP ligand was generated with the removal of a phosphate group, the two molecules were optimized using the same computational protocol of the other inhibitors.

2.2 Electronic Structure

The TKI structures were geometry optimized at first using semiempirical PM6 method. Calculations were also performed using density functional theory (DFT) with the functionals B3LYP and ωB97XD with the 6-311+G(d,p) basis function using the Gaussian09 computational package [34]. The structural and electronic properties were studied, such as dipole, the SCF orbital energies of frontier orbitals, the highest occupied molecular orbital (HOMO) to the HOMO-−4, the lowest unoccupied molecular orbital (LUMO) to LUMO + 4, and the HOMO-LUMO gap. In addition to evaluating the volume and size of the molecules.

Table 1 Analyzed TKIs

| Inhibitor	Target protein	Drugbank ID	References
Afatinib	EGFR	DB08916	Keating [17]
Axitinib	VEGFR	DB06626	Roskoski [18]
Bosutinib	BCR-ABL, Src family	DB06616	Keller-von Amsberg and Schafhausen [19]
Canertinib	pan-erbB	DB05424	Arora and Scholar [20]
Dasatinib	BCR-ABL, Src family, c-KIT, PDGFR, Ephrins	DB01254	Müller et al. [21]
Erlotinib	EGFR	DB00530	Scheffler et al. [22]
Gefitinib	NSCLC	DB00317	Sordella et al. [23]
Imatinib	BCR-ABL, c-KIT, PDGFR	DB00619	Manley et al. [24]
Lapatinib	EGFR	DB01259	Arora and Scholar [20]
Nilotinib	BCR-ABL, c-KIT, PDGFR	DB04868	Fujita et al. [25]
Pazopanib	VEGFR, PDGFR, FGFR	DB06589	Sonpavde et al. [26]
Ponatinib	BCR-ABL, T315l, VEGFR, PDGFR, FGFR, EPH, KIT, RET, Tie-2 e FLT3	DB08901	Zhou et al. [27]
Rebastinib	Tie-2 tyrosine kinase receptor	DB13005	Janku et al. [28]
Sorafenib	RAF kinase, PDGF, VEGF receptor 2 & 3 kinases and c-KIT	DB00398	Keating and Santoro [29]
Sunitinib	RTK, GIST, PDGF-R, VEGFR, RET, CSF-1R, and FLT3	DB01268	Demetri et al. [30]
Tozasertib	BCR-ABL T315l	5494449	Ferreira and Andricopulo [31]

3 Results and Discussion

### 3.1 Frontier Molecular Orbitals

In the present study, the inhibitors afatinib, axitinib, dasatinib, gefitinib, erlotinib, imatinib, lapatinib, nilotinib, pazopanib, ponatinib, sorafenib, and tozasertib were also evaluated (see Fig. 1), which act competitively with ATP, a natural substrate of TK. They also bind to the intracellular domain of the tyrosine portion EGFR kinase, suppressing its autophosphorylation and consequent cascade signaling of the BCR-ABL signal transduction pathway [13].

Figure 4 shows the profile energy of the frontier molecular orbitals (FMO) of the inhibitors obtained with semiempirical PM6 method, and B3LYP and ωB97XD functionals. The functionals and semiempirical methods presented almost the same behavior. However, some patterns can be observed. Imatinib, afatinib, dasatinib and ponatinib are in a separate group in both the HOMO − n and the LUMO + n series.
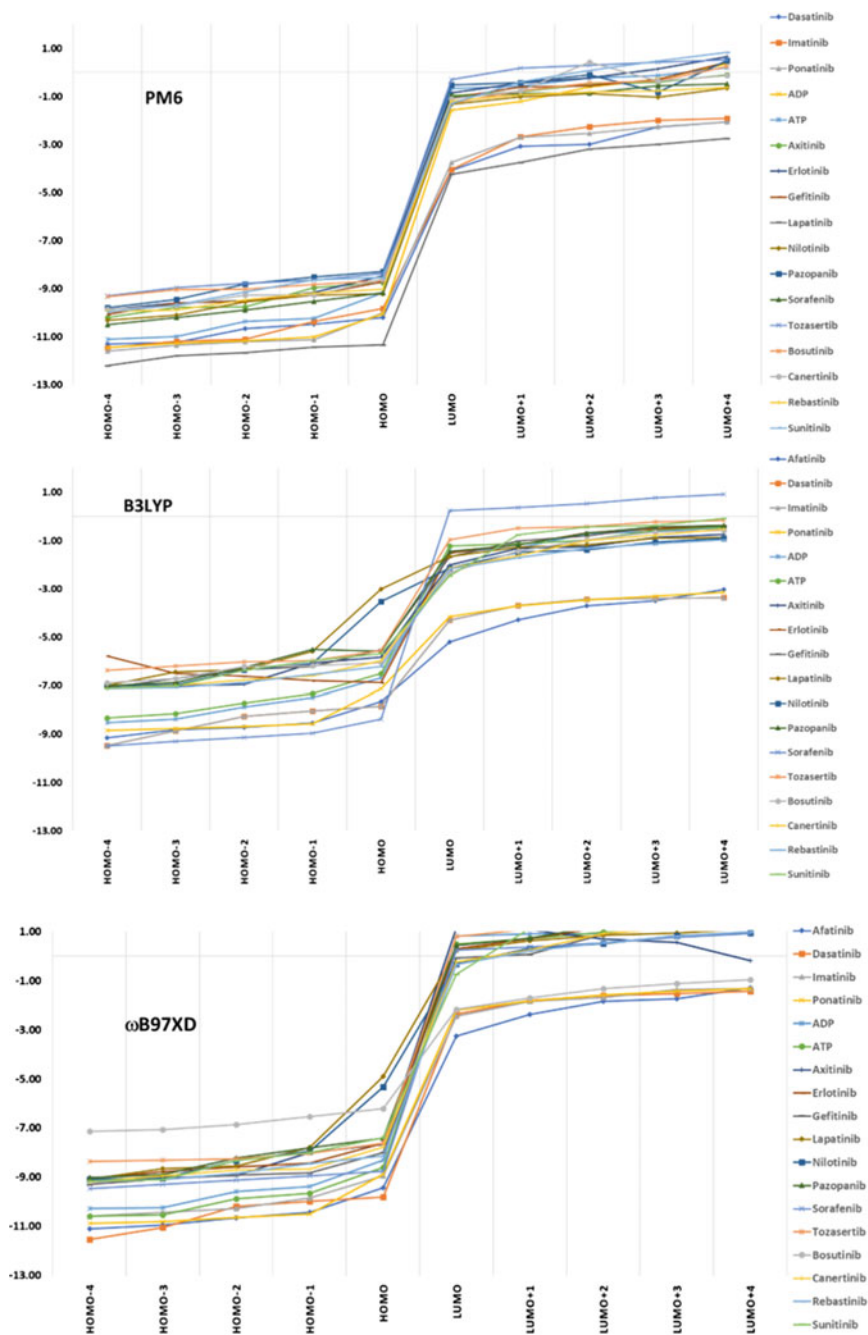
Fig. 4 Energy profile of the frontier molecular orbitals of TKIs of HOMO to HOMO − 4 and LUMO to LUMO + 4 series. Values are in eV. Calculations were performed with PM6 semiempirical method, and using hybrid functionals B3LYP and ωB97XD
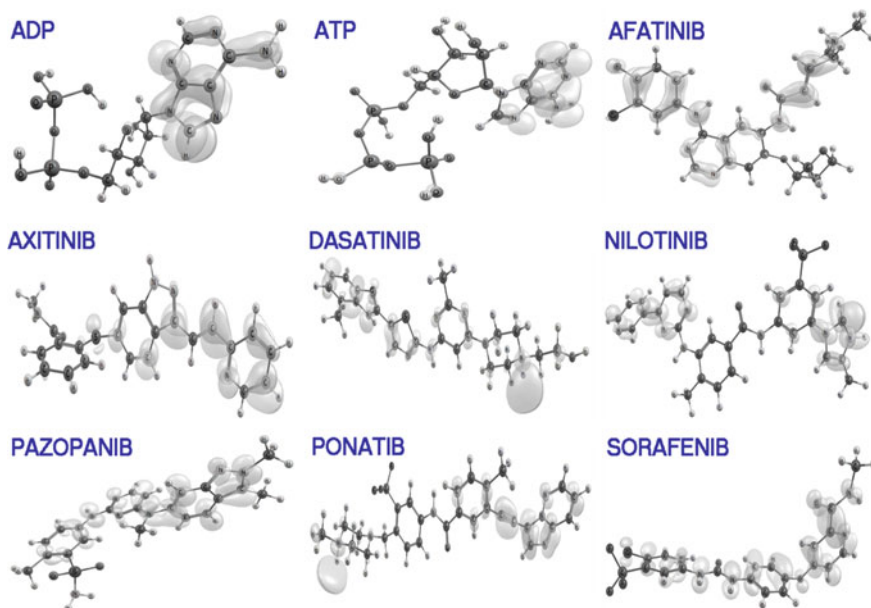
Fig. 5 The isosurface difference (LUMO-HOMO) distribution and localization for some TKIs studied. Calculations using B3LYP/6-311+G(d,p) level

This pattern was observed for all methods, with small variations, always grouping the imatinib, afatinib, dasatinib and ponatinib.

Figure 5 shows the energy difference between the isosurfaces of HOMO and LUMO orbitals. The distribution of the gap of TKIs molecules have different profile, which is the expected behavior in consonance with the kind of kinase protein where it acts more efficiently. The natural substrate ATP, and its product after the phosphorylation of the protein kinase (ADP) have similar distribution and location, in the adenosine group. The VEGFR and PDGFR inhibitors, axitinib, dasatinib, pazopanib, and sorafenib, presents their gap equally distributed throughout the fragments of their molecules. The BCR-ABL type II inhibitors imatinib, nilotinib and ponatinib show similar profile of distribution and location, in the opposite position. The EGFR inhibitor afatinib presented profile similar with ponatinib and sorafenib, both BCR-ABL inhibitors. This can indicate a new use of the afatinib as BCR-ABL inhibitor, or a new molecule derivates of this inhibitor.

3.2 Docking and the Electronic Structure Correlation

The protein has two essential sites for inhibition, named P1 site for the interaction site with ATP, and S1 site for the interaction site with protein phosphorylated by the

Fig. 6 Comparison of docking score values of wild protein 3QRK with mutated protein 3QRJ. Disclaimer for Dasatinib* referring to Dasatinib at S1 site. Pazopanib* referring to Pazopanib at P1 site. Ponatinib* referring to Ponatinib at S1 site, and Sorafenib* referring to Sorafenib at S1 site
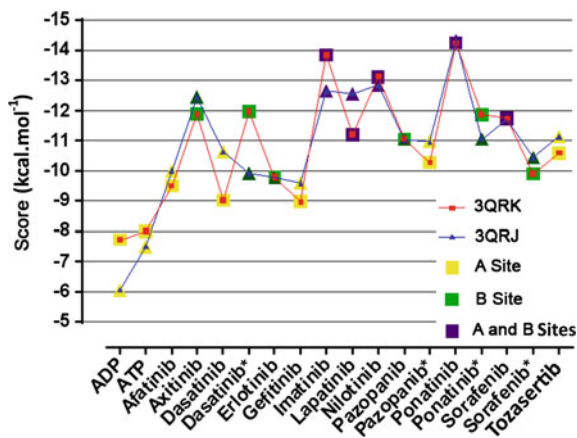
cascade process. Figure 6 illustrates docking score values of wild protein 3QRK and mutated protein 3QRJ. To identify whether the inhibitors were effective, we added the ATP and ADP molecules as a minimum score threshold. Thus, the inhibitors must have inhibition higher than the molecules that would be the natural substrates of the P1 site.

Docking score of inhibitors are suggestive of the binding capacity of inhibitors. The more negative their score, the greater the binding strength of interaction in a set of molecules. We chose substrates as minimum energy thresholds, from which is known that the inhibitor must score more negative than the natural substrate and to become effective for the wild 3QRK and mutant 3QRJ proteins (Table 2).

Mutant protein (3QRJ) has a mutation of the Thr315 residue by an isoleucine, and has a narrower catalytic pocket. Inhibitors showed greater difficulty for inhibition (interaction) of both sites of 3QRJ, especially at the P1 site. This is evidenced by the lower score value of the ATP molecule by the P1 site in the mutated protein than in the wild protein. Figure 7 depicts the correlation of score values with the gap and ΔE.

The ΔE (HOMO − (HOMO − 1)) shows a range of values between 0.05 and 1.44 eV for all inhibitors, and imatinib, afatinib and ponatinib presents the largest ΔE values. This trend is also followed by the gap values for imatinib, afatinib and ponatinib. Ponatinib has the largest score value for both proteins and presents the gap value close to 3.0 eV. Similar conclusions were observed for imatinib, ponatinib and ponatinib derivate molecules in the recent work of Pereira et. al [35]. In order of inhibition of mutated protein: ponatinib (-14.25 kcal mol^{-1}), nilotinib (-12.82 kcal mol^{-1}), imatinib (-12.65 kcal mol^{-1}), lapatinib (-12.56 kcal mol^{-1}), and axitinib (-12.46 kcal mol^{-1}).

The docking study demonstrated that for wild-type (3QRK) protein inhibition is favored due to a larger pocket volume [36]. The order of score for the wild protein is ponatinib (-14.25 kcal mol^{-1}), imatinib (-13.84 kcal mol^{-1}), nilotinib (-13.13 kcal mol^{-1}), and axitinib (-11.89 kcal mol^{-1}). This demonstrates that the

Table 2 Docking score of inhibitors, for both the wild 3QRK and mutant 3QRJ proteins BCR-ABL. The interaction site (P1 and S1), the gap energy, and the energy difference between HOMO and HOMO-1 using B3LYP/6-311+G(d,p) level

Ligand	3QRK/kcal.mol^{-1}	3QRJ/kcal.mol^{-1}	Site	Gap/eV	ΔE/eV
ADP	−7.73	−6.07	P1	5.24	0.80
ATP	−8.00	−7.50	P1	5.27	0.88
Afatinib	−9.50	−10.00	P1	2.43	0.82
Axitinib	−11.89	−12.46	S1	3.81	0.24
Dasatinib	−9.03	−10.63	P1	3.54	0.19
Erlotinib	−9.85	−9.79	S1	4.27	0.71
Gefitinib	−8.97	−9.60	P1	3.85	0.70
Imatinib	−13.84	−12.65	P1 and S1	2.70	0.77
Lapatinib	−11.20	−12.56	P1 and S1	3.65	0.29
Nilotinib	−13.13	−12.82	P1 and S1	3.87	0.05
Pazopanib	−11.06	−11.03	S1	4.14	0.32
Ponatinib	−14.25	−14.30	P1 and S1	2.98	1.44
Sorafenib	−11.75	−11.72	P1 and S1	4.80	0.52
Tozasertib	−10.59	−11.14	P1	4.58	0.42
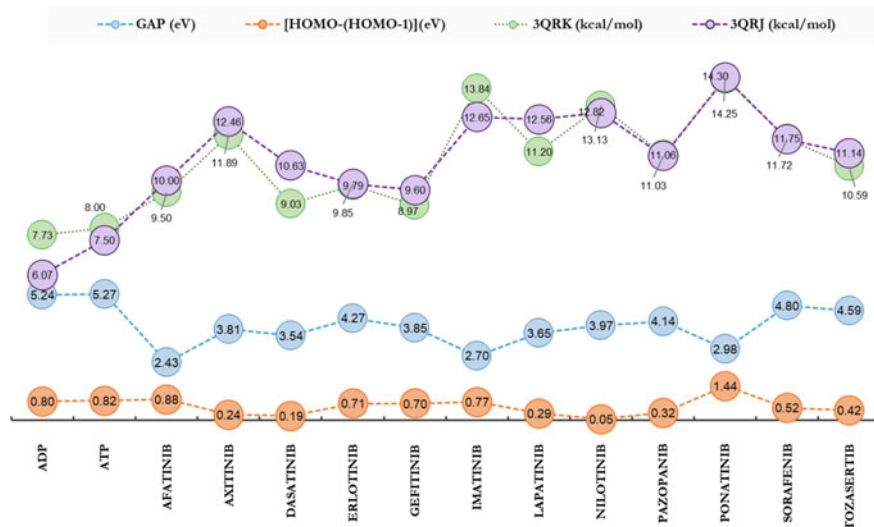
Fig. 7 Docking score for both the wild 3QRK and mutant 3QRJ proteins BCR-ABL compared to the gap energy, and the energy difference between HOMO and HOMO-1 at B3LYP/6-311+G(d,p) level

inhibitors designed for the disease have high affinity for the protein and consequent inhibition. Otherwise, the inhibitor lapatinib is designed for breast cancer and has high inhibition for the wild protein [37], corroborating the result with the clinical treatment used of imatinib in breast cancer [38].

3.3 Comparison of the Docking at P1 and S1 Sites of the Wild and Mutated Proteins

The analysis demonstrates that the inhibitors showed greater inhibition in the wild protein than in the mutant protein, with one exception being ponatinib. On the other hand, ATP has greater interaction with the wild protein than the mutated one, demonstrating that the mutation has its characteristic due to greater interaction with the P1 site. This demonstrates that inhibitors with greater inhibitory activity in the region of the P1 site will present greater inhibition. Ponatinib inhibitor has the highest score value and, its characteristic of inhibiting both sites make it stand out compared to imatinib and nilotinib.

Our results for the wild protein show that classical inhibitors for leukemia have a greater number of interactions with P1 and S1 sites (Fig. 8). Despite not being designed for such protein, these results demonstrate that there is a characteristic correlation of the docking results. For the two proteins, ponatinib stand out with the highest score followed by nilotinib.

The inhibitor tozasertib is an exponent for its strong interaction that occurs with the Glu286 and His361 residues, components of the P1 site in the wild protein. In
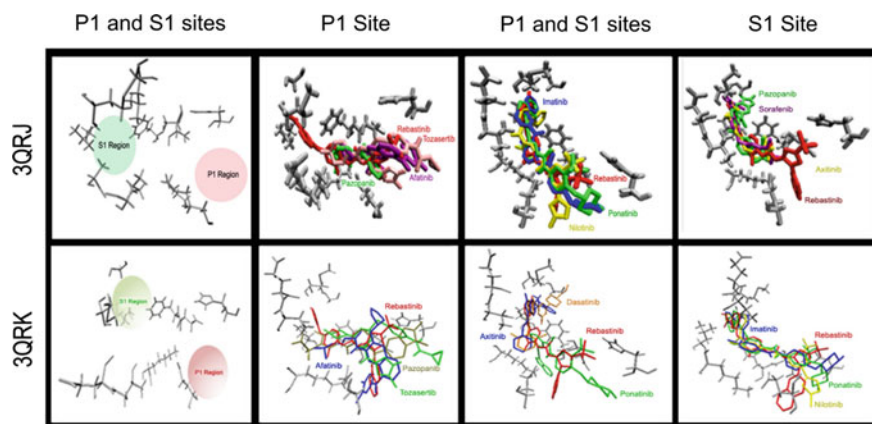
Fig. 8 Representation of docking from the inhibitor interaction with key amino acids. Column 1: representation of the pocket of P1 and S1 sites; Column 2 represents inhibitors with the highest score at the P1 site; Column 3 represents inhibitors with the highest score at P1 and S1 site; Column 4 represents inhibitors with the highest score at S1 site

the mutated protein, the inclusion of Ile 315 showed no difference for the reported bindings, see Fig. 8.

For S1 site, the best inhibitors are axitinib, dasatinib, ponatinib and pazopanib. The inhibitor axitinib has a high score in both proteins, demonstrating that the site present for protein interaction resulting from the inhibition cascade anchors the inhibitor. Such characteristics indicate a strong candidate as a basis for the design of new hybrid ligand or as a leader molecule in the proposition of new ligands for inhibiting the S1 site. It was observed that inhibitors that contain a sulfur atom in their molecular composition (axitinib, dasatinib, and pazopanib) have a strong interaction with the S1 site.

Final Remarks

The molecular docking study of a set of tyrosine kinase inhibitors were studied. The molecules include afatinib, axitinib, bosutinib, canertinib, dasatinib, erlotinib, gefitinib, imatinib, lapatinib, nilotinib, pazopanib, ponatinib, rebastinib, sorafenib, sunitinib, tozarsetib. They are divided into. The catalytic sites, P1 and S1, of the structures of 3QRK (UniProtKB: P00519) and 3QRJ (UniProtKB: P00519) were analyzed. Quantum chemistry descriptors were used, such as, frontier molecular orbital energies and distribution, and HOMO-LUMO and HOMO-(HOMO-1) energy differences were used to assess the binding affinity of those inhibitors. It is possible to devise some properties that help on the generation of new hybrid ligand with greater inhibition on both sites.

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