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Research Article

Molecular Docking Studies of Arylsubstituted Imidazoles on Oncogenic Protein Bcr-Abl Tyrosine Kinase

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Abstract

The treatment of chronic myeloid leukemia (CML) should be considered one of the medical successes of the last 30 years. CML is a myeloproliferative disorder that seriously compromises the health and life style of the patient who suffers. If not detected in time, the evolution of it can lead to a fatal end. The Philadelphia Chromosome (CrPh) is present in approximately 95% of patients with CML, constituting a diagnostic marker of the disease. It is formed by the Bcr-Abl oncogene. In the present work, molecular docking analysis was carried out to study the effect of twelve aryl substituted imidazoles on oncogene proteins Abl kinase (3CS9) and T315I mutation (2V7A). Molecular docking experiments were performed using Autodock 4.2 and the crystalline structure of the proteins was retrieved from Protein Data Bank. According to the results obtained, imidazoles 5a, 5d and 5j having minimum binding energy and have good affinity toward the active pocket of Abl kinase, whereas 5e, 5g and 5i by T315I mutation. In addition, these imidazoles were found to have good affinity than the co-crystallized ligands and known drugs studied.

Keywords: Chronic Myeloid Leukemia (CML), Molecular docking, aryl-substituted imidazoles, protein Bcr-Abl

Introduction

Chronic myeloid leukemia (CML) is a chronic myeloproliferative syndrome of a clonal nature, originated in the mother cell, which results in an excessive number of myeloid cells in all stages of maturation. It was the first malignant disease in which an acquired genetic abnormality was demonstrated and is currently the best studied molecular model of leukemia [1, 2]. It is a clonal myeloproliferative disease, which originates in hematopoietic stem cells (THC) and which is characterized by the presence of the Philadelphia chromosome and its oncogenic product BCR-ABL which is a type of protein known as tyrosine kinase. This protein has an increased and constitutive tyrosine kinase activity, essential for malignant transformation, which alters cellular properties such as adhesion, proliferation and apoptosis [3]. Its cause is unknown. It may appear after exposure to ionizing radiation or certain chemical agents, and the idea that its origin may be multifactorial, was raised more than 20 years ago. It is thought that some acquired molecular abnormality may precede the translocation t (9, 22). Also important is the hypothesis that the generation of the Bcr-Abl fusion gene in the pluripotent cell under conditions of reduced immunological survival is sufficient to initiate the expansion of the clone that modulates the behavior of the disease [4].

Although CML is one of the best known conditions, it is one of the most complex pathologies. Thanks to the numerous studies developed against this disease, a wide range of medications have been developed for its treatment and allow a better quality of life in patients, however, there are those who are refractory to this treatment and the adverse effects that these are still unknown drugs could cause when they are used for long periods of time [5]. This raises the need to look for new drugs that have a better action as inhibitors of tyrosine kinase by targeting the BCR-ABL protein, which is the mechanism of conventional treatment of CML, and that are as harmless as possible to the human organism. For the development of a new drug it is important to know a series of parameters that allow us to see how effective the drug is for the purpose in which we want to use it [6]. These parameters are obtained through a molecular docking study, which is a method to predict the preferred orientation of a molecule when it is coupled to a second to form a stable complex. The main objective of molecular docking is to accurately use ADT. Grid box centered at the active site of Abl kinase and T315I Abl mutant was selected (Table 1).

Grid box parameters selected for the target enzymesredict the affinity of a ligand confined to a receptor binding site and correctly estimate the strength of the binding [7]. Thus, it could be determined to what extent a ligand (possible inhibitory substance) could inhibit a protein such as tyrosine kinase. Taking into account the wide spectrum of activity of the substituted imidazoles, we reported several arylsusbituted imidazoles, some of which have been reported as potential anti-tuberculosis through molecular docking studies [8], so we have done this molecular docking study with oncogenic protein Abl-Bcr to predict their potential behavior as anticancer drugs.

Materials and Methods

Computational resource

Molecular modelling was performed using the High-performance computing capabilities of the Cluster of Chemistry Department of Exact and Natural Science Faculty, University of Oriente running the Linux operating system Debian 8.0 distribution.

Protein Preparation

Abl kinase (PDB ID : 3CS9) and T315I Abl mutant (PDB ID : 2V7A) were retrieved from Protein Data Bank (http://www.rcsb.org/pdb/). Both proteins contain identical domain, for these reason we used chain A for each enzyme, 3D structure resulting of the proteins were saved as PDB file. UCSF Chimera molecular graphic system v.1.10.2 [9] and Autodock Tools 1.5.6 (ADT) [10] was used to generate the docking input files. Polar hydrogen, solvatation paramater and Kollman charges were added to the protein.

Hetero-atoms and drug molecules were removed and both macromolecules were saved in PDBQT file format using ADT. Grid and docking parameter files were prepared in ADT considering the ligands as rotables and macromolecules as rigid.

Identification of binding site residues

A protein is a very large molecule with respect to test ligands; for this reason, the method of molecular docking choose suggests that region

Table 1: Grid box parameters selected for the target enzymes

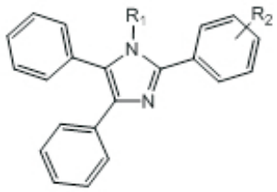
PDB ID (Resolution)	Enzyme	Coordinates of center of box (x,y,z)	Size (Points)	Spacing (Å)
3CS9 (2.21Å)	ABL kinase	27.437, 2.895, 51.243	60 x 60 x 60	0.375
2V7A (2.50 Å)	T315I Abl mutant	-41.534, -46.382, -10.483		

of the macromolecule where the active site is located; which it is known by analyzing the structures of each protein and its reference, by identifying the binding site of cases studied depending on the position of the co-crystallized within the protein. It should be noted that if this area is not should choose the calculation becomes more complicated and time requirement considerably increases, as well as be possible to obtain results far from reality. The binding site residues for Bcr-Abl were identified from the analysis of Human ABL kinase in complex with nilotinib – PDB ID:3CS9 [11] and T315I Abl mutant in complex with danusertib - PDB ID:2V7A[12].

Ligands preparation

The representation and optimization (MMFF94) of the arylsubstituted imidazoles (Table 2) were performed in the ChemOffice 2015, using the ChemDraw v.15.0 and Chem3D v.15.0 respectively. The pdb file was prepared using the software UCSF Chimera molecular graphic system v.1.10.2.Using the program ADT,as ligands are not peptides, Gasteiger charges were assigned and non-polar hydrogens were merged.

Table 2: Chemical structure of arylsubstituted imidazole



ID	R1	R2	ID	R1	R2
5a	C_6H_5-CH_2	p-$N(CH_3)_2$	5g	m-CH_3-C_6H_5	p-NO_2
5b	C_6H_5-CH_2	p-OH	5h	p-CH_3-C_6H_5	p-NO_2
5c	C_6H_5-CH_2	H	5i	p-SO_3H-C_6H_5	p-$N(CH3)_2$
5d	C_6H_5-CH_2	p-OCH_3	5j	H	p-$N(CH3)_2$
5e	m-CH_3-C_6H_5	m-NO_2	5k	Fu-CH_2	4-OH, 3-CH_3O
5f	o-CH_3-C_6H_5	p-NO_2	5n	Fu-CH_2	p-OH

Molecular docking

The molecular docking program AutoDock 4.2 [13] was employed to perform the docking experiment. AutoDock requires pre-calculated grid maps, one for each atom type, present in the ligand being docked. Autogrid was used to obtain pre calculated grid maps [14]. Lamarckian Genetic Algorithm was used to explore the best conformational space with a 50 docking runs for each ligand. The maximum numbers of generation were 27000 and evaluation 2500000 with a mutation and crossover rate 0.02 and 0.80 respectively.

Docking calculations can be validated redocking the ligands that were co-crystallized in the receptor structure, Nilotinib (Abl kinase) and Danusertib (T315I mutation) (Figure 1) and we included other ligands Dasatinib [15], Bosutinib [16] and Ponatinib [17] as inhibitors of Bcr-Abl protein (Figure 2).

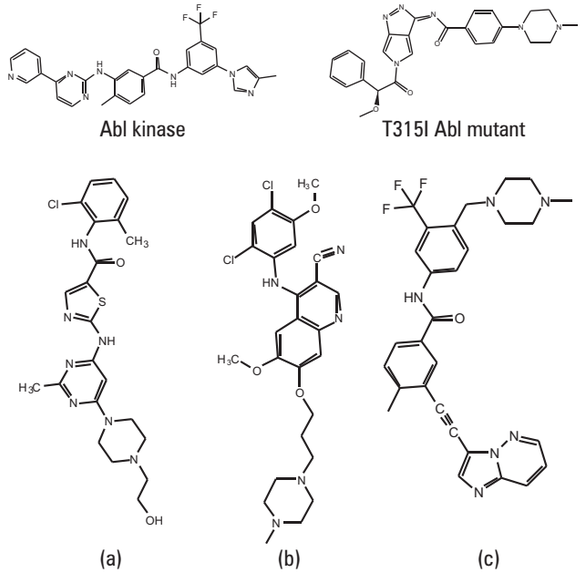


Figure 2: Structure of control inhibitors Dasatinib(a), Bosutinib (b) and Ponatinib (c)

Results and Discussion

The docked poses for each of the compounds were evaluated and the pose with the lowest binding free energy and the inhibition constant was thereby chosen (Table 3). These results reveal that 12 arylsubstituted imidazoles selected (Table 2) show a good affinity for the Abl protein kinase (3CS9) and Abl T315I (2V7A).

Table 3: Binding energy and inhibition constant observed between the aryl-substituted imidazoles and the target enzymes chosen

Ligands	ABL kinase (3CS9)		T315I Abl mutant (2V7A)	
	ΔG (kcal/mol)	Ki (nM)	ΔG (kcal/mol)	Ki (nM)
5a	-10.28	29.29	-8.29	842.39
5b	-9.72	74.7	-8.4	693.04
5c	-9.97	49.51	-8.49	599.33
5d	-10.11	39.08	-8.84	948.03
5e	-9.03	240.16	-9.68	79.59
5f	-8.48	611.12	-8.53	557.38
5g	-9.23	171.01	-9.18	187.81
5h	-9.33	145.83	-8.83	337.63
5i	-9.91	54.3	-10.12	38.28
5j	-10.6	16.96	-7.14	5870
5k	-9.7	77.26	-8.44	647.15
5n	-9.53	103.89	-8.13	11500

As observed, the ligands possess an excellent affinity towards the Abl kinase protein, highlighting the ligands 5a, 5d and 5j with values of -10.28, -10.11 and -10.6 kcal / mol and Ki of 29.29, 39.08 and 16.96 nM, respectively. However, in the case of the T315I mutation, the binding energy values are above -8 kcal/mol, where the ligands with the highest interaction nature are 5e, 5g and 5i, because of their high union energy values, which demonstrate greater affinity of interaction towards the T315I mutation. According to these results, we could generalize the high affinity that the ligands have towards the Abl kinase protein, not being so for the T315I mutation, which is consistent with the literature [18] and the high levels of resistance shown by the drugs towards this protein.

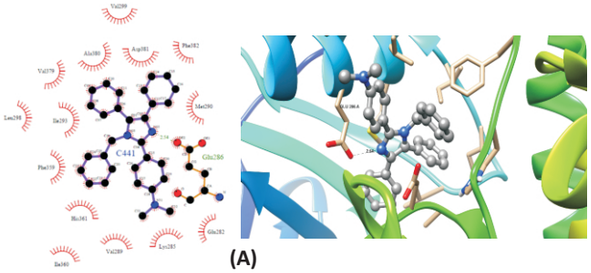
Abl kinase

The function of this protein is based on the formation of the Philadelphia chromosome by the union of the Abl and Bcr oncogenes, responsible for the phosphorylation and consequent proliferation of the oncogenic cells in the CML. Table 4 shows the nature of the interactions between the best imidazoles and the Abl kinase protein, we can observe a H-bond interactions between the imidazoles 5a, 5d and 5j with the amino acid residue Glu286 (present in the active site of the protein [11], which occurs through the pyridine nitrogen of imidazole for the case of ligands 5a and 5d (Figure 4A and B) with a distance of 2.54 and 2.52Å respectively, while for the case of ligand 5j (Figure 4C) this interaction occurs with pyrrole nitrogen with a distance of 2.82Å.

Table 4: Interactions observed between the better imidazoles with the Abl kinase protein

Ligands	Residues making hydrophobic contacts	H-bond/Coordination Interactions
5a	Val299 Asp381 Phe382 Met290 Glu282 Lys285 Val289 Ile360 His361 Phe359 Leu298 Ile293 Val379 Ala380	Glu286[OH….$N_{pyridinic}$]
5d	Lys285 Glu282 Met290 Thr315 Phe382 Val379 Asp381 Leu298 Val299 Ala380 His361 Ile293 Ile360 Phe359 Val289	Glu286[OH….$N_{pyridinic}$]
5j	His361 Val289 Met290 Ala269 Thr315 Lys271 Val270 Val256 Phe382 Val299 Asp381 Ala380 Val379 Phe359 Leu298 Leu354	Glu286[OH….$N_{pyridinic}$]

In additionof these interaction by H-bond we can observe a lot of hydrophobic interactions (Table 5) found due to primarily to the presence a lot of bencenic rings in ligands studied. As shown the ligands 5a, 5d y 5j share hydrophobic interactions with the residues Phe382, Glu282, Thr315, Lys285, His361 and Phe359 all them involved in the active site of protein Abl kinase [11] (Figure 3).



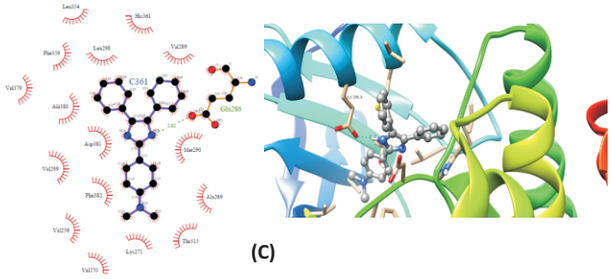


Figure 3: Ligand 5a (A), 5d (B) and 5j (C) as ball and stick at the binding pocket of Abl kinase receptor.

T315I Abl mutation

Protein T315I known as the mutation greater resistance to inhibitors tyrosine kinase, the main difference between her and protein Abl kinase lies in the change of aminoacids residues Thr315 by Ile315, weakening well interaction of inhibitors with the residues Thr315, main responsible for the process of inhibition of protein Bcr-Abl. As we saw above all ligands have better affinity taking into account the binding energy and inhibition constant to this protein. However, the ligands 5e, 5g and 5i showed a good affinity to this mutation, also to have H-bond interaction with the residues Lys271, Asp381, Gln252 and Gly249 all involved in the active site of the protein [12]. The hydrogen bond interaction involved the NO_2 group in ligand 5e (Figure 4A), the NO_2 group and pyridinic nitrogen in ligand 5g (Figure 4B) and SO_3H group in ligand 5i (Figure 4C).

We can observe as there are many hydrophobic interactions where exist some residues involved in the active site (Table 5) as Met318, Phe317, Asp381, Leu370 including the residue Ile315 responsible of the mutation in the protein.

Table 5: Interactions observed between the best imidazoles with the T315I protein

Ligands	Residues making hydrophobic contacts	H-bond/Coordination Interactions
5e	Ala269 Leu370 Thr319 Met318 Phe317 Gly321 Gly249 Asn322 Leu248 Gly250 Arg367 Val299 Asn368 Ala380 Ile315	Lys271[NH….O], Asp381[NH….O]
5g	Ile315 Leu370 Ala269 Gly321 Met318 Leu248 Val256 Gly251 Gly250 Asp381	Gly249[OH….N], Gln252[NH….O], Lys271[NH….O]
5i	Gly251 Val256 Ala269 Ile315 Leu370 Phe317 Met318 Leu248 Asn322 Arg367 Gly250	2Gln252[NH….O], Asp381[OH….O], Lys271[NH….O]

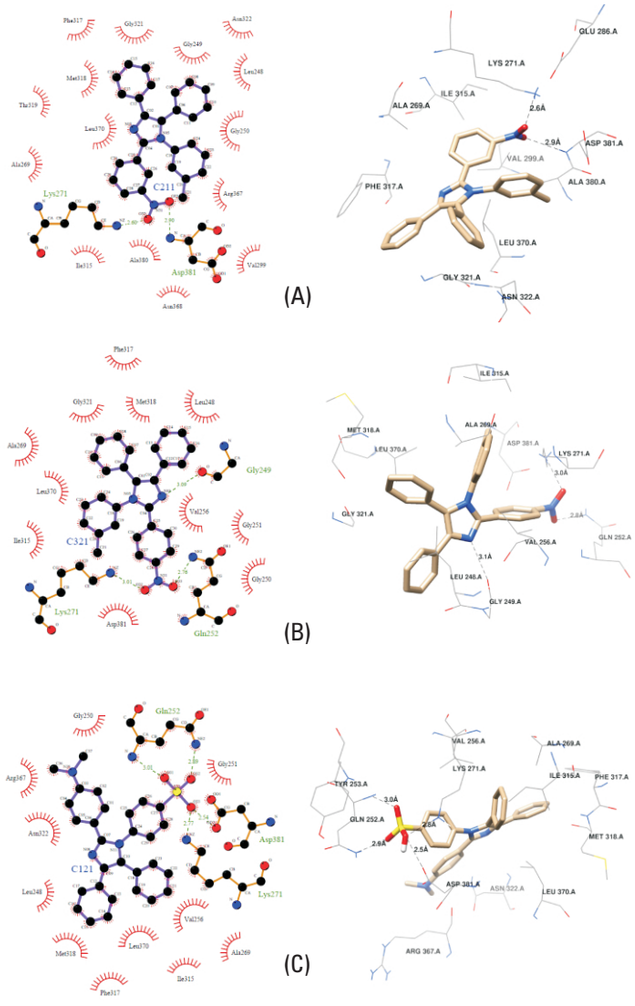


Figure 4: Ligand 5e (A), 5g (B) and 5i (C) as stick at the binding pocket of T315I Abl mutation receptor.

Control inhibitors

The control inhibitors were docked under the same docking conditions against the enzymes Abl kinase and T315I mutation. The docked poses for each inhibitorwere evaluated and the pose with the lowest binding free energy and the inhibition constant was thereby chosen. These results were compared with the better ligand for each protein analyzed.

Abl kinase

As we can see, the values of binding energy and inhibition constant of the imidazoles 5a, 5d and 5j reflect the great affinity that these have with the co-crystallized ligand (Nilotinib). As shown in Table 6, the values of ΔG and Ki obtained for the drugs Dasatinib and Ponatinib reflect a greater affinity with the Abl kinase protein, mainly due to the fact that these drugs are part of the third generation of tyrosine kinase inhibitor drugs (ITK), however, it is shown that these ligands have higher affinity than Bosutinib (-9.2 kcal / mol) which is 2nd generation.

Table 6: Comparison of predicted binding free energies and inhibition constant between the better ligands and control inhibitors for Abl kinase.

Ligand	ΔG (kcal/mol)	Ki (nM)
5a	-10,28	29,29
5d	-10,11	39,08
5j	-10,6	16,96
Nilotinib[a]	-9,23	170,9
Dasatinib	-11,31	5,16
Bosutinib	-9,2	180,22
Ponatinib	-13,91	0,064

We can observe (Figure 5) how the ligands (5a, 5d and 5j) have a conformation similar to the co-crystallizedNilotinib conformation after the coupling in the active site of the protein Abl kinase (PDB ID 3CS9), which could justify the high affinity of these ligands with the protein.

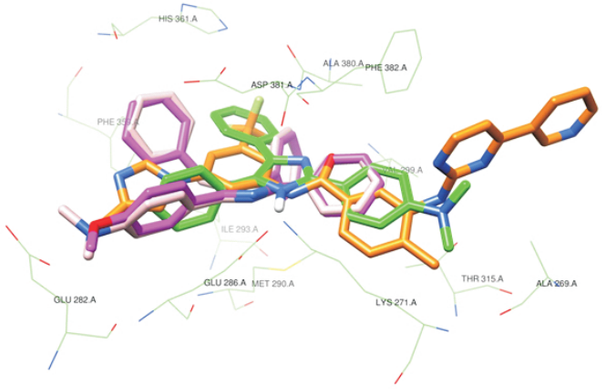


Figure 5: Superimposed conformations of ligands 5a (pink), 5d (magenta), 5j (green) and Nilotinib (orange) after the docking performed.

While the drugs Dasatinib and Ponatinib showed higher affinity than the ligands, mainly due to the structure of them that encompass a greater area of interaction within the protein and a greater number of both hydrophobic and hydrogen bonding interactions, stabilizing their conformational structure even more than the ligands (Figure 6).

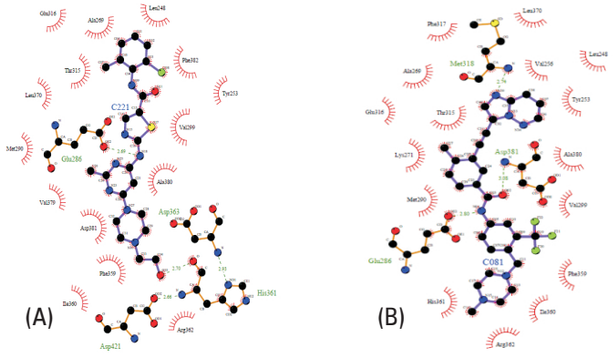


Figure 6: 2D protein-ligand interaction of the co-crystallizedDasatinib (A) and Ponatinib (B) with the Abl protein kinase using a Ligplot diagram.

However, the results for the drug Bosutinib were found to be similar to Nilotinib and lower than the best imidazoles analyzed for this protein (Table 6). This similarity between Bosutinib and the co-crystallized Nilotinib is evidenced in the superimposition conformational structures (Figure 7) which could justify the similar values of binding energy and inhibition constant obtained (Table 6).

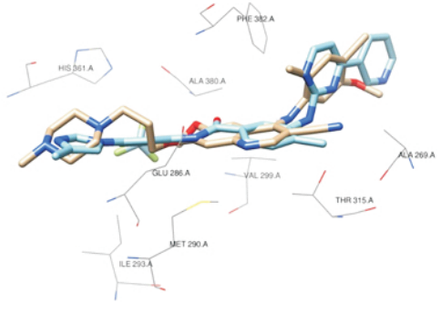


Figure 7: Superimposed conformations of the drug Bosutinib (tan) and the co-crystallized Nilotinib (blue) in the active site.

T315I Abl mutation

Within the more than 90 mutations presented by CML, the T315I mutation is the one that confers the highest resistance to Imatinib, Dasatinib and Nilotinib, and is associated with disease progression and poor prognosis. As shown in Table 7, the values of binding energy (ΔG) and inhibition constant of the imidazoles 5e and 5i are above the values obtained for the Imatinib co-crystallized, while the 5g has a similar affinity behavior. Whereas, when compared with drugs, both 5e and 5i are overcome by Ponatinib, which has been reported as the most powerful inhibitor of the T315I mutation according to the literature (Bendek 2015). We can observe how the superimposed conformations between the ligands 5e, 5g and 5i and the Imatinib co-crystallized show good conformational similarity (Figure 8), thus sustaining the values obtained.

Table 7: Comparison between imidazoles, inhibitors and drugs reported with the T315I protein.

Ligando	ΔG (kcal/mol)	Ki (nM)
5e	-9.68	79.59
5g	-9.18	187.81
5i	-10.12	38.28
Imatinib[a]	-9,2	180,22
Dasatinib	-9,82	63,7
Bosutinib	-9,68	80,63
Ponatinib	-10,63	16,18

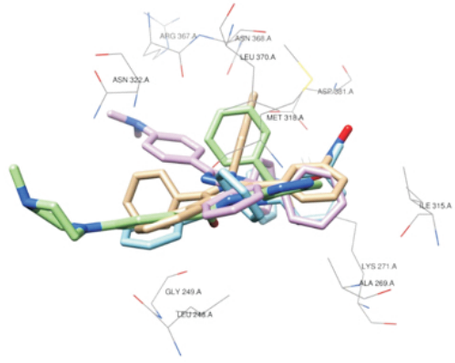


Figure 8: Superimposed conformations of the ligands 5e (brown), 5g (blue), 5i (violet) and Imatinib (green) after the coupling performed

According to the results obtained we corroborate what´s was proposed by Bendek [19] which raises the fact that the drugs Imatinib, Dasatinib, Nilotinib and Bosutinib have an effect against many mutations of the Bcr-Abl protein except against T315I, only having a marked effect on the Ponatinib.

Conclusion

The existence of good affinity of the arylsusbtituted imidazoles towards the oncogenic proteins Abl kinase and their mutation T315I was demonstrated using molecular docking studies. These in silico studies revealed that the imidazoles 5a, 5b and 5j for Abl kinase and 5e, 5g and 5i for T315I mutation have lesser binding energy and inhibition constant compared to co-crystallizedligands and standard drug Bosutinib, but these values for Ponatinib and Dasatinib were still

more low that imidazoles studied.The obtained results are useful to understand the structural features required to enhance the inhibitory activities and could prove to be useful for the design and development of future anticancer drugs.

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Conflicts of interest

The authors report no conflict of interest.

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