

In silico studies of isatinyl-2-aminobenzoylhydrazone transition metal complexes against cyclin-dependent kinase 6 (CDK6)

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Abstract: Cyclin-dependent kinase 6 (CDK6) is an important member of protein kinases, involving in many cellular pathways especially cell cycle progression. Thus, CDK6 is a promising target in cancer therapy. This report aims to predict inhibition of CDK6 by some complex compounds by using molecular docking and pharmacological properties analysis. Those compounds are isatinyl-2-aminobenzoylhydrazone (ISABH) and cobalt (II), nickel (II), copper (II), and zinc (II) transition metal complexes. The molecular docking against CDK6 (PDB code: 3NUP) revealed that ISABH/ISABH-transition metal complexes established ligand-protein interaction as expressed by negative binding affinity values. Drug-likeness by SwissADME indicated that ISABH and Ni-ISABH met the Lipinski's rule of five. Both compounds also showed reasonable pharmacological criteria by admetSAR.

Keywords: Cyclin-dependent kinase, CDK6 inhibitors, drug-likeness, ADMET, isatinyl-2-aminobenzoylhydrazone

Introduction

Kinases are proteins that involved in the cellular pathways such as signal transduction and cell cycle progression mainly through phosphorylation [1]. Cyclin-dependent kinases (CDKs) are a member of the CMGC family of serine/threonine kinases and interact with cyclins to activate their catalytic action in cell cycle [2,3]. CDKs control the order of cell cycle development with specific cyclins and CKIs [4]. CDK6 gene is located on chromosome 7 in humans extends 231,706 base pairs and involves in cell cycle progression by encoding a 326 amino acids protein [5]. The activity of this kinase is controlled by the D-type cyclins regulatory subunits and CDK inhibitors i.e INK4 family. CDK6, as well as CDK4, has been shown to phosphorylate, and thus regulate the activity of retinoblastoma protein (Rb), a tumor suppressor protein [6]. The pathway of cyclin D-CDK4/6-INK4-RB has been observed in several cancer types due to uncontrolled cell cycle/growth [7]. Thus, these kinases are being used as a potential target of cancer therapeutic research and development. Palbociclib, a nitrogen-containing heterocyclic compound of CDK4/CDK6 targeted therapy, is approved for the treatment of breast cancer [8].

Isatin (1H-indole-2,3-dione) is a diketo (C2 and C3) heterocyclic compound derived from indole with transition metal ions binding capacity [9]. Isatin is also known for its flexible structure and diverse biological activities i.e. antiinflammation, antibacterial, anti-HIV, and anticancer CDK inhibitors [10]. Several isatin derivatives affected the proliferation of human breast cancer MCF-7 and/or MDA-MB-231 cell lines and displayed as efficient and potent inhibitors for CDK2, CDK4, CDK9 [11,12]. Hydrazones with the azometin group, -CH=N-NH-, are also exhibited various bioactivities including antimicrobial, anticonvulsant, analgesic, antiinflammatory, antiplatelet, antituberculosis, and antitumor. The carbon-nitrogen double bond plays an important role in the design and development of drugs [13]. Various transition metals of isatin-hydrazone complexes have been developed and further investigated in order to study their anticancer properties [14]. The present study reports on an attempt to develop CDK6 inhibitors based on the chemistry of isatin-hydrazone.

Methods

Protein preparation

The CDK6 (PDB ID: 3NUP) crystal structure with a resolution of 2.60 Å was retrieved from protein data

bank (<https://www.rscb.org>) [15]. The protein target preparation and optimization were executed with PyMOL by removing water molecules and 3NU co-crystalline ligand followed by the addition of hydrogen atoms. The 3D protein structure was then stored in pdb format and converted into dockable pdbqt format for docking.

Ligand preparation

The two-dimensional (2D) structure of isatinyl-2-aminobenzoylhydrazone (ISABH) and its transition metals Co (II), Ni (II), Cu (II), and Zn (II) complexes (Figure 1) were constructed using MarvinSketch and converted into a three-dimensional (3D) structure followed by protonation at a pH of 7.4 [16]. The geometry optimization was carried out using MMFF94 and the optimized structures were further saved into pdbqt format getting ready for docking process.

Molecular docking

Docking was performed at MacBook Pro 2018 running on Intel® Core i5 quad-core 2.3 GHz, 8GB RAM with Intel Iris Plus Graphics 655 graphics card and MacOS Mojave 10.14.5 operating system. Flexible docking was accomplished using *AutoDock Vina* [17] in *PyRx* [18] with a grid size of X 16 Å, Y 16 Å, Z 16 Å and X, Y, Z dimensions of 38,9 Å, 27,3 Å, -12,3 Å resulted from the 3NU co-crystal binding location. Docking parameters and scoring function were set as default. The metal ions of the ISABH's complexes were automatically recognized by *Autodock Vina* as prepared in pdbqt format for ligands. Docking procedure was evaluated by redocking method based on $\text{RMSD} < 2 \text{ \AA}$ and interactions [19]. The binding

modes and receptor-ligand interaction were analyzed by using *PyMOL*.

Drug likeness and ADMET prediction

Drug-likeness analysis was assessed according to Lipinski's rule of five using SwissADME online web tool [20]. The pharmacological properties prediction based on absorption, distribution, metabolism, excretion, and toxicity (ADMET) was generated by using admetSAR [21].

Results

Molecular docking

3NU co-crystal was successfully re-docked back to the binding site of the 3NU-3NUP complex in order to validate the docking process. The alignment of the docked 3NU best pose to the 3NU co-crystallized showed a root-mean-squared deviation (RMSD) value of 1.083 Å (Figure 2a). The docked 3NU also indicated the same interactions compared to 3NU co-crystal in 3NUP complex (Figure 2b).

The cross-docking of ISABH and its complexes with metal ions of cobalt (II), nickel (II), copper (II), and zinc (II) on CDK6 protein (PDB ID: 3NUP) produced binding affinity of -9.4; -8.5; -6.6; -6.5; -5.4 kcal/mol for Ni-ISABH, ISABH, Co-ISABH, Cu-ISABH, and Zn-ISABH, respectively. Visualization of docking results using PyMOL was then carried out to determine the interactions and bind modes of ligands with 3NUP protein. Ni-ISABH interacted with Glu 99 and Asp 104 residues through hydrogen bonding (Figure 3). Co-ISABH, Cu-ISABH, and Zn-ISABH showed the same interaction with Asp 104 residue by hydrogen bonding. The interaction with Val 101 residue was demonstrated by ISABH as found in the 3NU co-crystals (Figure 4).

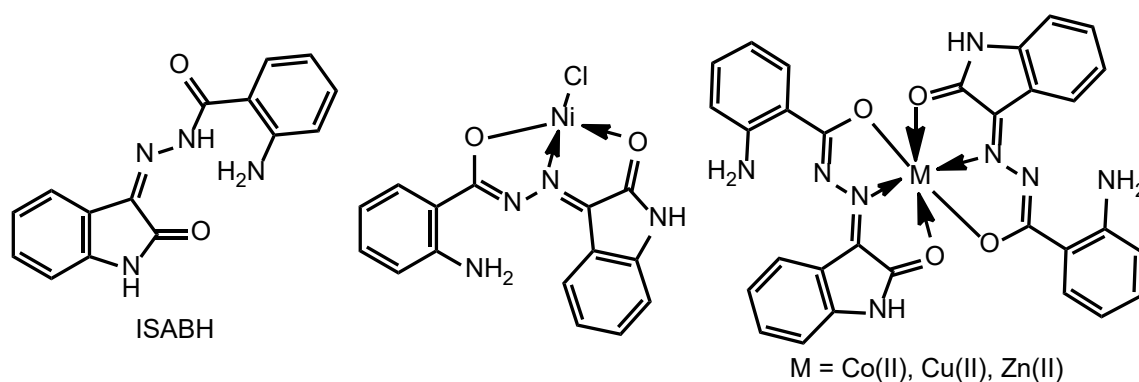
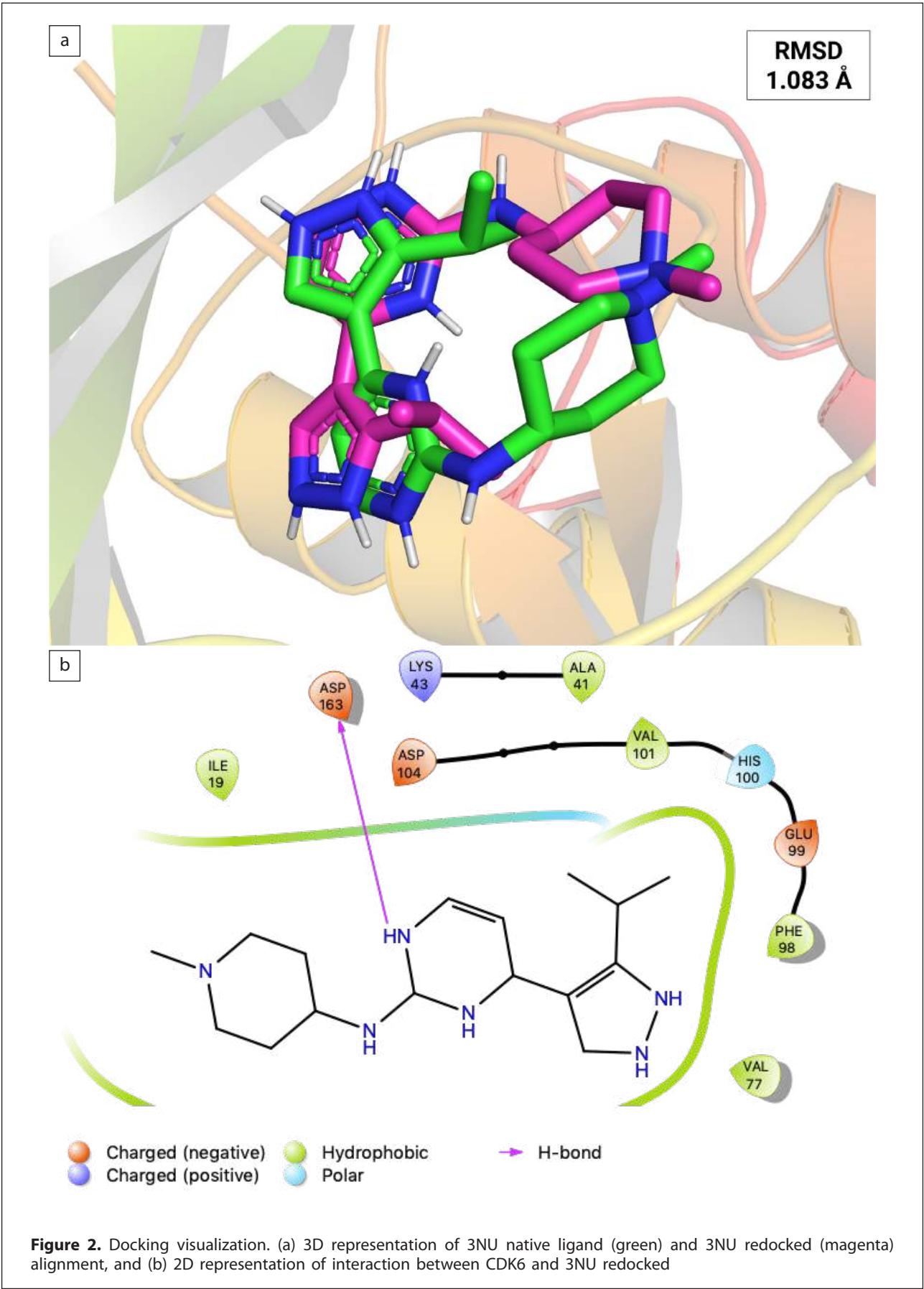


Figure 1. 2D structure of ISABH and Co (II), Ni (II), Cu (II), and Zn (II) transition metals complexes



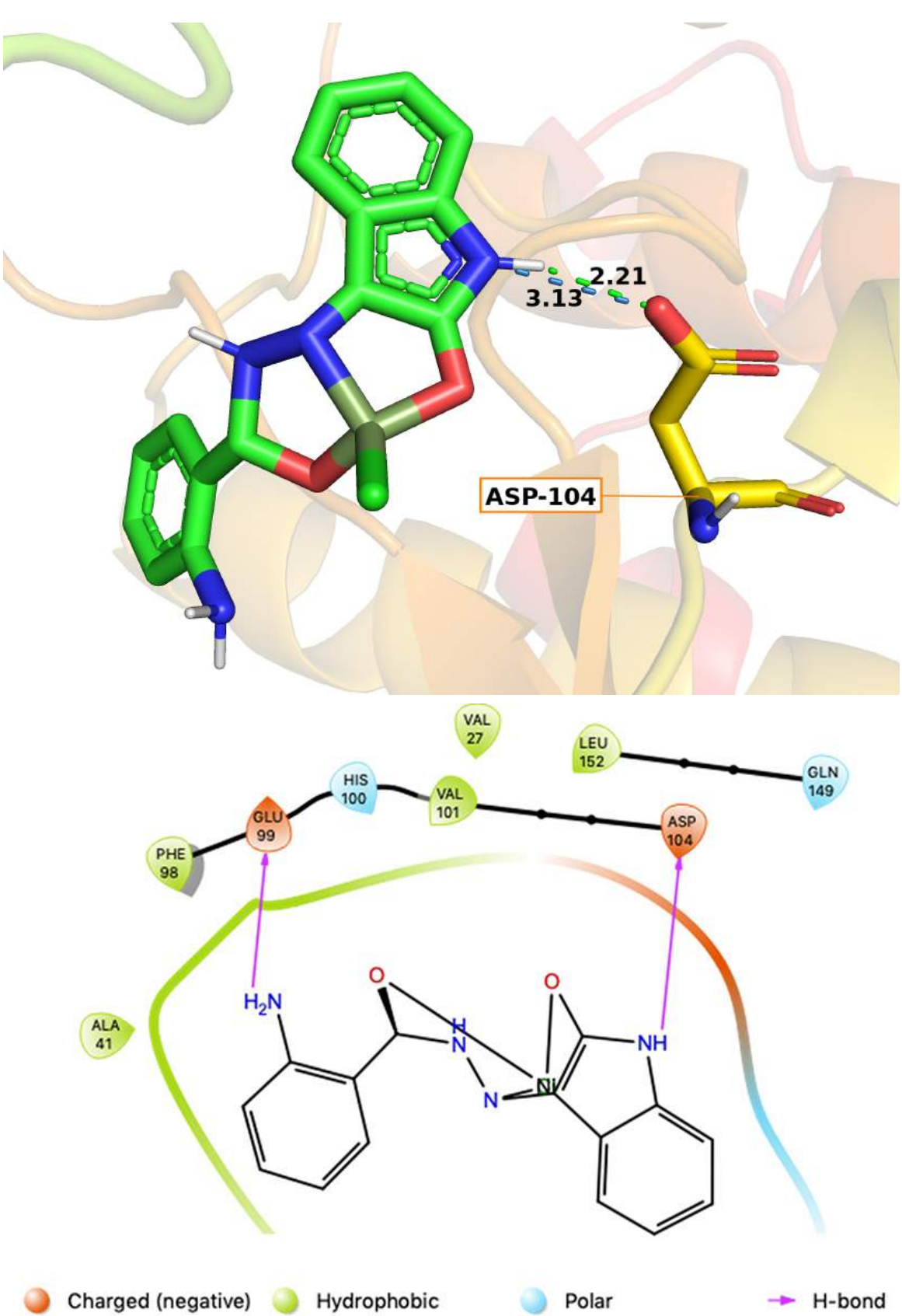


Figure 3. 3D and 2D representation of Ni-ISABH interaction with CDK6

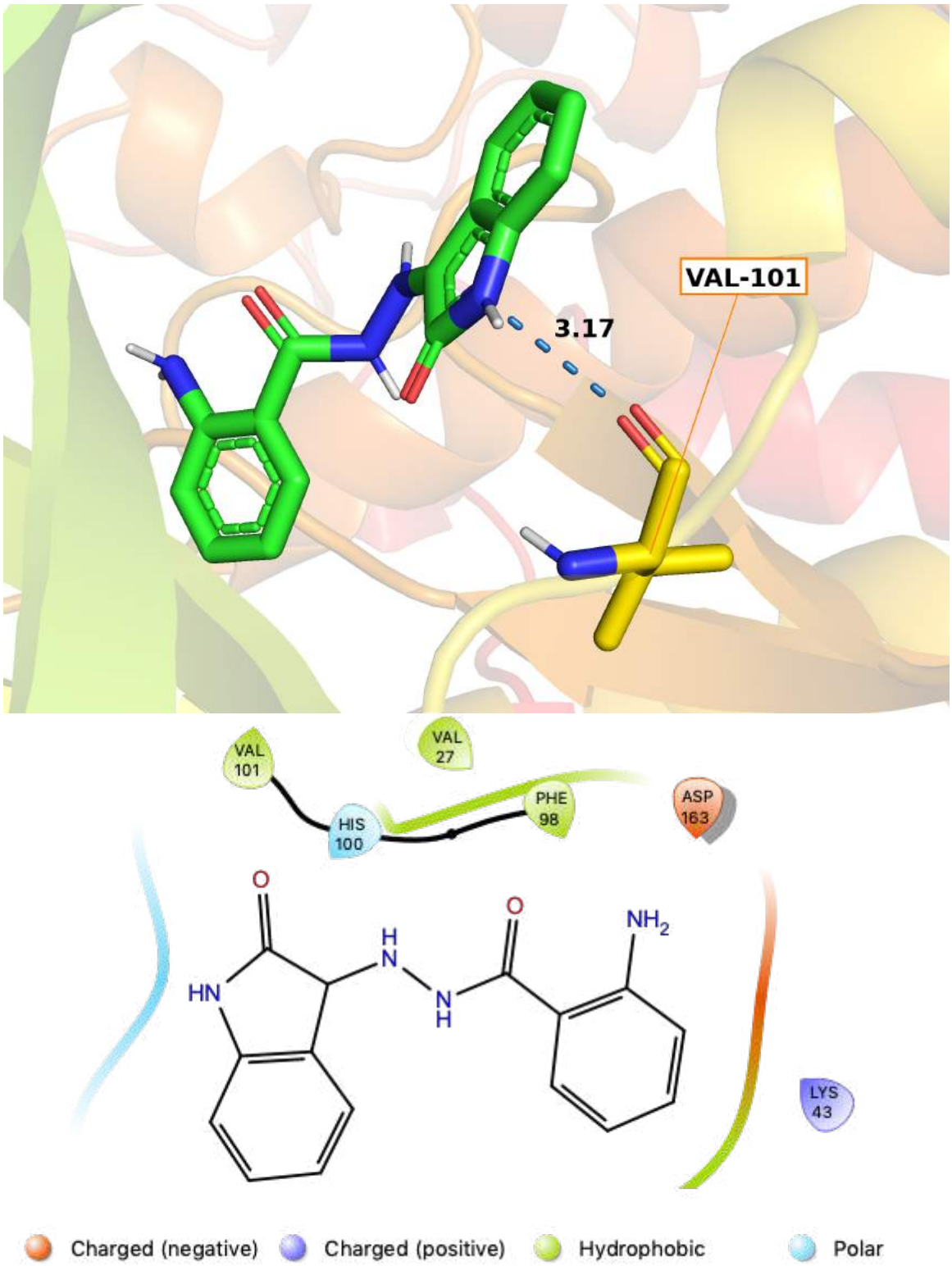


Figure 4. 3D and 2D representation of ISABH interaction with CDK6

ADMET profile

Table 1 summarizes the results of drug-likeness analysis using SwissADME. ISABH and its complexes with cobalt (II), nickel (II), copper (II), and zinc (II) showed log P of 1.44-2.48, hydrogen bond acceptors 6-12, hydrogen bond donors 4 -6, and molecular weights 280.28-623.93. The pharmacological properties prediction of ISABH and Ni-ISABH using admetSAR are shown in Table 2. ISABH and Ni-ISABH exhibited positive values for human intense absorption, human oral bioavailability, and blood brain barrier. Both were also non-inhibitory non-substrate for P-glycoprotein inhibition and substrate parameters and were non-carcinogenic. The acute oral toxicities of ISABH and Ni-ISABH were 2,057 and 2,810, while the plasma protein binding values were 0.677 and 0.807.

Discussion

The binding modes of protein-ligand CDK6 3NU co-crystal was reproduced by redocking method prior to docking study. This method was carried out by removing 3NU co-crystal from the binding site of 3NUP and inserting back to the same position [22]. The redocking procedure was successfully done and alignment of the re-docked 3NU to 3NU co-crystal gave RMSD value of 1.083 Å. Further, the docked 3NU ligand interacted with Val 101 through hydrogen bonding as in co-crystallized 3NU. These two parameters are enough in reproducing the interaction of 3NU and 3NUP crystal structure and indicate that it can be extended for the next assessment [23].

The transition metal of Co (II), Ni (II), Cu (II) and Zn (II) complexes are well known for their interaction in the biological system as metalloprotein together with iron. Transition metal complexes also present as active substances in several inorganic-based pharmaceuticals for bioinorganic applications against various diseases, ranging from antibacterial to anticancer. In addition, these first-row transition metals are also have less toxic properties, affordable, and easily available [24]. The transition metals Co (II), Ni (II), Cu (II) and Zn (II) coordinates ISABH in a 3D conformation and enables them to optimally match on 3NUP. This ability mainly relies on the electron affinity which relates to metal-ligand polarization [25]. Cross-docking of ISABH, Co-ISABH, Cu-ISABH, Ni-ISABH, and Zn-ISABH was performed against CDK-6 protein (PDB ID: 3NUP). The binding affinity determined the strength of ligands and protein interaction indicates the more negative value the better binding strength and suggests the better molecular docking prediction [18]. Ni-ISABH displayed the lowest binding affinity among others with a value of -9.4 kcal/mol. The binding affinity of Co-ISABH, Cu-ISABH, Zn-ISABH was 6.6, 6.5, and 5.4 kcal/mol, respectively, and the order of binding affinity was Ni-ISABH > ISABH > Co-ISABH > Cu-ISABH > Zn-ISABH. The different binding affinity for each complexes relates to their structure (Figure 1), shielding effect of metal complexes, and metal-coordinating atom distances. As shown in Figure 1, metal ions of Co (II), Cu (II) and Zn (II) formed ML₂ type coordination with ISABH in octahedral fashion and is classified as sufficiently coordinated metal ion

Table 1. *In silico* drug likeness prediction using SwissADME

| Compounds | Molecular weight | log P | H-bond acceptor | H-bond donor | Lipinski's violation |
|-----------|------------------|-------|-----------------|--------------|----------------------|
| ISABH | 280.28 | 1.57 | 6 | 4 | 0 |
| Cu- ISABH | 622.09 | 2.47 | 12 | 6 | 1 |
| Co- ISABH | 617.48 | 2.47 | 12 | 6 | 1 |
| Ni- ISABH | 375.44 | 1.44 | 6 | 4 | 0 |
| Zn- ISABH | 623.93 | 2.48 | 12 | 6 | 1 |

Table 2. *In silico* ADMET properties of ISABH and Ni-ISABH

| Compounds | HIA | HOB | BBB | PPB | CYP Inhibitory/ substrate | Carcinogen-icity | Acute oral toxicity (kg/mol) |
|-----------|-----|-----|-----|-------|------------------------------|------------------|---------------------------------|
| ISABH | + | + | + | 0.677 | none | none | 2.057 |
| Ni-ISABH | + | + | + | 0.807 | none | none | 2.810 |

HIA: human intense absorption, HOB: human oral bioavailability, BBB: blood brain barrier, PPB: plasma protein binding

with optimum coordination number (OCN). This coordination type have negligible contributions to the binding affinities due to the large shielding effect of the metal complexes with OCNs. In contrast, Ni (II) with coordination number (CN) less than OCN produced ML type in tetrahedral mode and referred as hypo-coordinated metal ion, shows large contribution to binding affinities because of the small shielding effect. Figure 1 also shown that the distances of metal-coordinating atom in metal complexes increase with the increasing of CNs. This fact supports that the M-L strength in ML_n becomes weaker as n increases [26].

Interactions and binding modes of the best poses of ISABH, Co-ISABH, Cu-ISABH, Ni-ISABH, and Zn-ISABH with the CDK-6 protein were then visualized. Figure 3 indicates that Ni-ISABH interacted with Glu 99 and Asp 104 residues through hydrogen bonding, while Co-ISABH, Cu-ISABH, and Zn-ISABH connected with Asp 104 residue on CDK-6 protein. ISABH interacted with Val 101 residue as 3NU co-crystals on the 3NUP-3NU crystal structure (Figure 4).

Lipinski's rule of five states that a compound with excellent drug-like properties has a molecular weight (MW) ≤ 500 Dalton, $\log P \leq 5$, number of hydrogen bond donors (HBD) ≤ 5 , and number of hydrogen bond acceptors (HBA) ≤ 10 [27]. The $\log P$ value which is the logarithm of the partition coefficient between *n*-octanol and water ($\log(c_{\text{octanol}}/c_{\text{water}})$) can be used to estimate the hydrophilicity of a compound. The high hydrophilicity correlates with low $\log P$ values and favorable absorption and permeation. Drug-likeness analysis (Table 1) showed that ISABH and the complexes of cobalt (II), nickel (II), copper (II), and zinc (II) have acceptable hydrophilicity indicated by low $\log P$ values (1.44-2.48). Cu-ISABH, Co-ISABH, and Zn-ISABH did not meet the requirement of hydrogen bond acceptors, hydrogen bond donors, and molecular weight parameters. Those three complexes have $MW \geq 600$ Dalton, $nHBA \geq 10$, and $nHBA \geq 5$. In contrast, ISABH and Ni-ISABH met all the criteria, namely $BM \leq 500$, $nHBA \leq 5$, and $nHBD \leq 5$. Based on these data, ISABH and Ni-ISABH have valuable drug-like properties.

The evaluation of absorption, distribution, metabolism, excretion, and toxicity was then performed to predict the pharmacological properties of ISABH and Ni-ISABH. The results (Table 2) indicated that both ISABH and Ni-ISABH have positive values for HIA and HOB indicating a decent absorption

in human body and tolerable absorption rates in human intestine. ISABH and Ni-ISABH also showed promising distribution expressed by positive blood brain barrier parameter and plasma protein binding (PPB) of 67.7% and 80.7%. An absolute distribution requires positive blood brain barrier and plasma protein binding distribution capabilities. The blood brain barrier parameter represents the central nervous system vascularization of blood vessels which strictly regulates the movement of ions, molecules, and cells between the blood and brain [28]. Furthermore, the large percentage of plasma protein binding illustrates the better distribution of compounds in the blood because of plasma protein binding value represents the degree of protein distribution in the blood [29]. The distribution prediction based on inhibition and substrate P-glycoprotein parameters is important because P-glycoprotein is one of the drug carriers that determines the absorption and excretion of various drugs. Table 2 informs that ISABH and Ni-ISABH were non-substrate and non-inhibitor to P-glycoprotein. Moreover, ISABH and Ni-ISABH were non-carcinogenic with fairly safe acute oral toxicity values of 2.057 and 2.810 kg/mol, respectively, which fall into the category III of acute oral toxicity.

To summarize, the docking study of ISABH, Co-ISABH, Cu-ISABH, Ni-ISABH, and Zn-ISABH have successfully been performed by redocking the 3NU co-crystal ligand on the active site of the CDK-6 protein followed by cross-docking. Ni-ISABH showed the best binding affinity value of -9.4 kcal/mol and interacted with Glu 99 and Asp 104 residues through hydrogen bonding. Ni-ISABH met the Lipinski rule of five and had acceptable drug-like and pharmacological properties.

Conclusion

The molecular docking study of ISABH, Co-ISABH, Cu-ISABH, Ni-ISABH, Zn-ISABH was carried out against the CDK-6 protein and resulted in a binding affinity value of -9.4 kcal/mol for Ni-ISABH lower than ISABH (-8.5 kcal/mol). Visualization of the docking results informed that Ni-ISABH interacted with CDK-6 with glutamic acid 99 and aspartic acid 104 residues via hydrogen bonding. The drug-likeness properties analysis showed that Ni-ISABH complied Lipinski rules of five with $BM \leq 500$, $\log P 1.44$, $nHBA \leq 5$, and $nHBD \leq 5$. Ni-ISABH displayed a valuable absorption, distribution,

metabolism, excretion and was non-carcinogenic with safe acute oral toxicity.

Acknowledgment

None.

Declaration of interest

The authors declare no conflict of interests in the journal publication of this article.

Author contribution

AF constructed the study design. YRN performed and analyzed the molecular docking and pharmacological properties studies. AF prepared the manuscript and revised the final version of the paper. YRN and AF have read the final manuscript and approved the submission.

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