

COMPUTATIONAL DETERMINATION OF POTENTIAL CK2 INHIBITORS

by

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Submitted to Graduate School of Natural and Applied Sciences
in Partial Fulfillment of the Requirements
for the Degree of Master of Science in
Chemical Engineering

Yeditepe University

2022

COMPUTATIONAL DETERMINATION OF POTENTIAL CK2 INHIBITORS

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ACKNOWLEDGEMENTS

I would like to thank my dear MSc supervisor Prof. Dr. Nihan ÇELEBİ ÖLÇÜM for her continuous support and motivation during my study. In addition, taking part in this research, provided me with invaluable experiences and the courage to work harder for further achievements.

To my dear teacher Prof. Dr. Tuğba DAVRAN CANDAN deeply thanks for supporting me with the balance of work and school life. I was an intern and then became an executive employee at my dream job with her support.

I really would like to thank Nermin ŞEN, Yeşim ÇAMLISOY, Sezen ALSANCAK, Hande ABEŞ for helping me and answering my questions at any time, day, and place.

Also, I sincerely thank my dear friend Emre Tankut SARI for help, support, and endless cheerfulness.

Finally, I would like to special thanks to my family Alper KOLUKISA for his support. I'd like to express my heartfelt gratitude to him for his steady support and patience throughout all of my processes.

ABSTRACT

COMPUTATIONAL DETERMINATION OF POTENTIAL CK2 INHIBITORS

Protein kinase CK2 (casein kinase II) has been an important physiopathological target for the development of chemical inhibitors in recent years due to its physiological role in supporting the survival of the cell and its irregularity in many cancer cells. CX-4945, also known as Silmitasertib, (5-(3chlorophenylamino) benzo[c][2,6] naphthyridine-8-carboxylic acid), is so far the only drug candidate targeting CK2 that is in phase 2 human clinical trials. Therefore, there is significant interest in developing efficient CK2 inhibitors for cancer treatment. Although thienobenzocarbazoles, bioisosteres of pyridocarbazoles that display very high biological potency, have great potential for CK2 inhibition, their activities have not been explored computationally, nor experimentally. In this thesis, thienobenzocarbazoles were computationally explored as potent CK2 inhibitors. The binding patterns and affinities of thienobenzocarbazole derivatives to CK2 were investigated using docking studies and molecular dynamics simulations. With the aim of improving the binding affinities of the 33 parent thienobenzocarbazoles that were found to have relatively lower docking scores compared to CX-4945, selected compounds were further structurally modified using computational derivatization tools generating a new library of thienobenzocarbazole derivatives. Docking of these new derivatives to CK2 active site identified inhibitor candidates with improved affinities. Potential interactions between CK2 and selected inhibitor candidates were then investigated using molecular dynamics simulations in a dynamic and solvated environment. Our results suggest that thienobenzocarbazole derivative “K93” can be a promising candidate for additional development targeting CK2 inhibition.

ÖZET

CK2 (KASEIN KINASE II) POTANSİYEL İNHİBİTÖRLERİNİN HESAPSAL OLARAK BELİRLENMESİ

Protein kinaz CK2 (kazein kinaz II), hücrenin hayatta kalmasını desteklemedeki fizyolojik rolü ve birçok kanser hücresindeki düzensizliği nedeniyle son yıllarda kimyasal inhibitörlerin gelişimi için önemli bir fizyopatolojik hedef olmuştur. Klinik çalışmalarında bir CK2 inhibitörü olan Silmitasertib olarak da bilinen (5-(3chlorophenylamino) benzo[c][2,6] naphthyridine-8-carboxylic acid) CX-4945'e benzer etkili ilaç adayları geliştirmek için çok çaba sarf edilmiş olup klinik çalışmalarda faz 2 aşamasına geçirilen tek potansiyel CK2 inhibitörüdür. Tiyenobenzokarbazoller, piridokarbazollerin biyoizosteri nedeniyle çok yüksek biyolojik potensiyel sergilemelerine ve CK2 inhibitörleri olarak büyük potansiyele sahip olmalarına rağmen, aktiviteleri hesaplamalı veya deneysel olarak araştırılmamıştır. Bu çalışmada, tiyenobenzokarbazoller, CX-4945 ile yapısal benzerlik gösterdikleri için CK2 inhibitörleri olarak hesaplamalı araştırılmıştır. Tiyenobenzokarbazol türevlerinin CK2'ye bağlanma modelleri, hesaplama yöntemleri kullanılarak incelenmiştir. Çalışmanın ilk aşamasında, CX-4945'e kıyasla nispeten daha düşük docking skorlarına sahip olduğu bulunan 33 AK molekülünün bağlanma afinitelerini geliştirmek amacıyla seçilen bileşikler, hesaplamalı türevlendirme araçları kullanılarak yapısal olarak modifiye edildi. Bu yeni türevler CK2 aktif bölgesine kenetlenerek, geliştirilmiş afinitelere sahip inhibitör adayları tanımlandı. İkinci adımda, CK2 ve seçilen inhibitör adayları arasındaki potansiyel etkileşimler, dinamik ve çözülmüş bir ortamda moleküler dinamik simülasyonları kullanılarak araştırıldı. Sonuçlarımıza göre, tiyenobenzokarbazol türevi olan K93'ün, CK2 inhibitörü adayı olabileceği gözlemlenmiştir.

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LIST OF SYMBOLS/ABBREVIATIONS

ARG	Arginine
QSAR	Quantitative structure activity relationship
CYS	Cysteine
CTMD	Constant temperature molecular dynamics
GAFF	Generalized amber force field
HIS	Histidine
LYS	Lysine
LEU	Leucine
MD	Molecular dynamics
MET	Methionine
MOE	Molecular operating environment
ns	nanosecond
PDB	Protein data bank
PDB ID	Protein data bank identifier
ps	Picosecond
RMSD	Root mean square deviation
SABER	Selection of active/binding sites for enzyme redesign
SER	Serine
VAL	Valine
Å	Angstrom
K_b	Force constant for strength of bond
K_θ	Force constant for bond angle
l	Covalent bond length
l_0	Ideal covalent bond length
θ	Bond angle
θ_0	Ideal bond angle
V_n	Torsion angle potential function constant
γ	Phase angle for torsional angle parameter
n	Multiplicity
q_i	Partial atomic charge of the atom i

q_j	Partial atomic charge of the atom j
ϵ_0	Lennard-Jones well depth
ω	Dihedral angle
R_{ij}	Distance between the atoms i and j, respectively
A_{ij}	Non-bonded force field parameter for atomic distances
B_{ij}	Non-bonded force field parameter for atomic distances
N	Number of atoms
F_i	Force action on an atom i
m_i	Mass of an atom i
a_i	Acceleration of an atom i
v_i	Velocity of an atom i
x_i	Position of an atom i
t	time
E	Potential energy
∇	Nabla
δ	delta
$\langle A \rangle$	Average
M	Time steps in simulation
P	Pressure
T	Temperature
N	Amount of substance

1. INTRODUCTION

CK2 (casein kinase II) is an enzyme naturally found in human body and is a significant potential target for cancer treatment [1]. CK2 is a fundamentally active protein kinase that phosphorylates several substrates. It is expressed in all cells, however, its level is more in cancer cells, where its anti-apoptotic functions are present [2]. Despite the fact that CK2 is a constitutively active kinase, it contributes to the potentiation of the tumor phenotype by generating favorable conditions for carcinogenesis anytime its activity exceeds a crucial level. In other words, it appears that cells with increased CK2 are positively selected by the tumor due to a number of characteristics, including the ability to evade apoptosis, poor druggability, and a particular ability to survive. This makes CK2 an appealing target in a variety of neoplastic disorders, because its pharmacological down-regulation could eliminate a number of generic devices used to promote tumor progression, even if the molecular change underlying the disease's genesis is not found in CK2 [3]. CK2 is comprised of two subunits, a catalytic subunit, CK2 α , and a regulatory subunit, CK2 β . The alpha subunits shown in Figure 1.1 is responsible for its catalytic activity while the beta subunits undergo auto phosphorylation. The relationship of protein kinase CK2 with many diseases, primarily cancer, due to its dual function that promotes cell growth and prevents apoptosis, makes this protein an important therapeutic target.

Although CK2 has emerged as an important target for cancer treatment, there is only one drug candidate that has succeeded to pass to clinical trials: CX-4945. Therefore, much effort has been devoted to develop efficient drug candidates similar to CX-4945, also known as Silmitasertib, (5-(3chlorophenylamino) benzo[c][2,6] naphthyridine-8-carboxylic acid), a CK2 inhibitor in human clinical trials. Figure 1.2. shows the crystal structure of CK2 complexed with CX-4945. Both computational and experimental studies on the development of CK2 inhibitors have increased significantly in recent years.

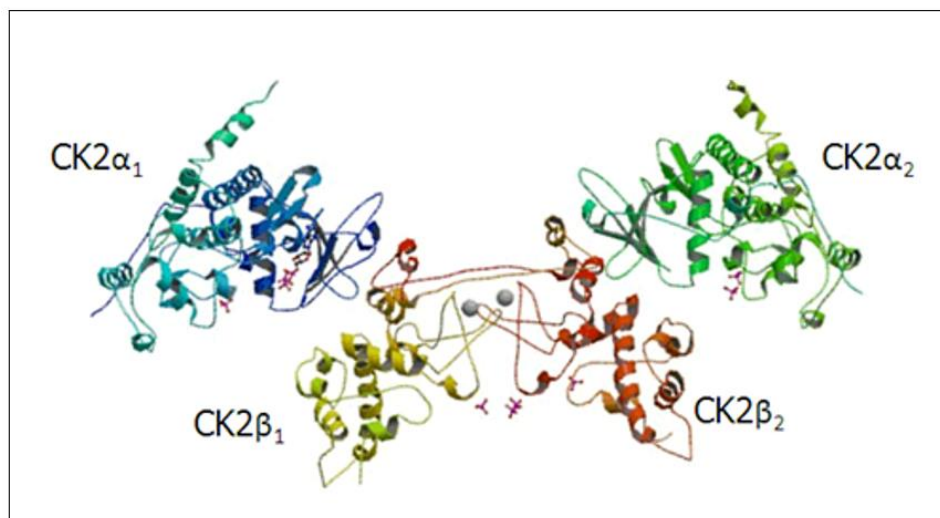


Figure 1.1. The structure of protein kinase CK2 [4]

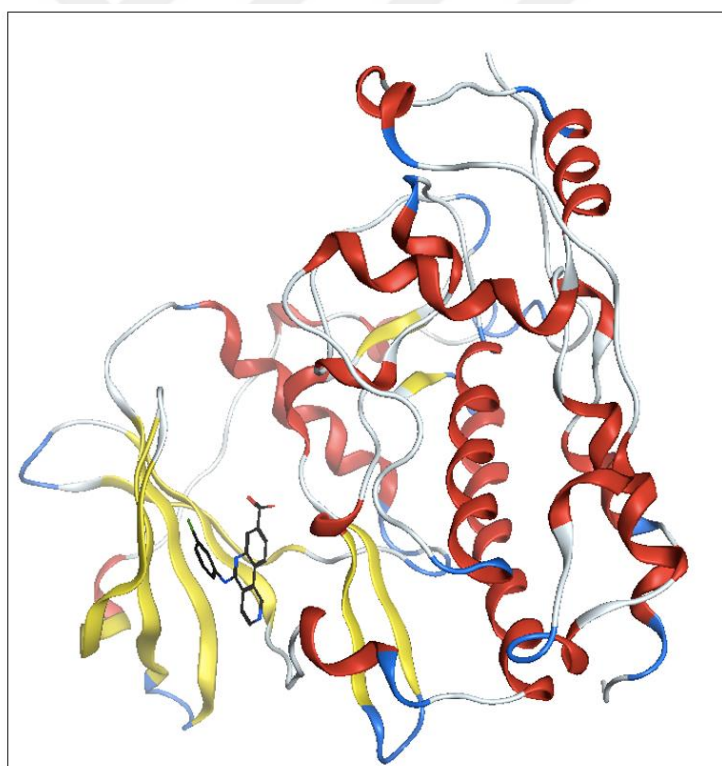
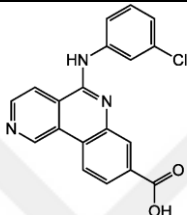
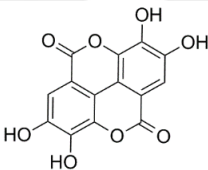
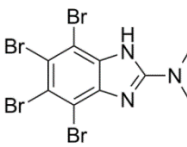
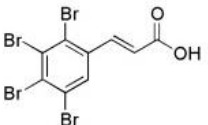
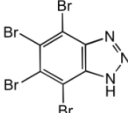
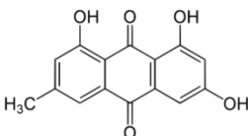


Figure 1.2. Crystal structure of human protein kinase CK2 alpha subunit in complex with the inhibitor CX-4945

Until today, many active CK2 inhibitors from different chemical classes have been developed [5]. The most active and known inhibitors are presented in Table 1.1, where IC_{50} is the half maximal inhibitory concentration, and K_i is the inhibitor constant. IC_{50} is a

measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function. K_i is an indication of how potent an inhibitor is; it is the concentration required to produce half-maximum inhibition [5]. Except for CX-4945, which is in a phase 2 clinical trial for cancer, none of these drugs candidates were able to pass to the clinical testing phase [7].

Table 1. 1. Potential protein kinase CK2 inhibitors

Common Name	Structure	IUPAC Name	IC ₅₀ (μM)	K _i (μM)
CX-4945		5-(3-chlorophenylamino)benzo[c][2,6]naphthyridine-8-carboxylic acid	0.001 ^[7]	0.00038 ^[5]
Ellagic Acid		2,3,7,8-tetrahydroxy-[2]benzopyrano[5,4,3-cde][2]benzopyran-5,10-dione	0.04 ^[7]	0.02 ^[3]
DMAT		2-dimethylamino-4,5,6,7-tetrabromo-1H-benzimidazole	0.14 ^[7,8]	0.040 ^[9]
XTBCA		(2E)-3-(2,3,4,5-tetrabromophenyl)-2-propenoic acid	0.11 ^[8]	0.077 ^[3]
TBB		4,5,6,7-tetrabromo-2-azabenzimidazole	0.9 ^[10]	0.40 ^[3]
Emodin		3-methyl-1,3,8-trihydroxyanthraquinone	2 ^[7]	1.85 ^[3]

Emodin and ellagic acid are inhibitors derived from natural resources [7]. Emodin (1,3,8-trihydroxy-6-methyl-antraquinone) is one of the natural substances that has been shown to inhibit a number of protein kinases, including CK2. However, it is not very selective for CK2 and is not very effective (IC₅₀ around 2 μ M) also. It is suggested that proper derivatizations could improve the emodin scaffold's potency and selectivity, resulting in molecules with IC₅₀ values in the submicromolar range [3].

CK2 inhibitors developed from the "ancestor" inhibitor DRB by eliminating the sugar moiety and substituting the chlorines with bulkier bromine atoms are currently the most extensively studied. This gave rise to the tetrabromobenzimidazole (TBI or TBBz) scaffold and its congener tetrabromobenzotriazole (TBB), both of which were shown to be more powerful than DRB. TBB was found to be very selective when tested against a 33-kinase panel, and it has also been utilized to treat mice *in vivo*, where it was well tolerated for several weeks [3].

Simultaneously, efforts were made to increase TBI's effectiveness by derivatizing its imidazole ring properly. With K_i values in the submicromolar range, a panel of highly selective, powerful, and cell-permeable inhibitors of protein kinase CK2 from the groups of condensed polyphenolic compounds, tetrabromobenzimidazole/triazole derivatives, and indoloquinazolines has been discovered. TBB and TBB derivatives (including DMAT) block another protein kinase, DYRK1a, with efficiency comparable to CK2, as indicated by the addition of other enzymes to the panel of protein kinases used to profile the specificity of inhibitors [9]. The dimethylamino derivative of TBI, DMAT, was the most successful molecule, much more potent than TBB when tested on cell cultures [3].

The information gathered from two recently solved crystal structures of CK2 α in contact with emodin and with 4,5,6,7-tetrabromo-2-azaben-zimidazole (TBB) is valuable for understanding the mode of activity of CK2 inhibitors. The size and form of the hydrophobic pocket in CK2 almost perfectly accommodates the TBB molecule, whereas in the other kinases, it is often larger and fails to entrap TBB, which can readily enter and exit the cavity [10].

Computational studies recently been conducted in the hopes of discovering CK2 inhibitors described a set of indeno[1,2-b]indoles as CK2 inhibitors, together with their *in vitro*

activities, and further presented a detailed description of a QSAR (Quantitative structure activity relationship) study built from these indeno[1,2-b]indoles [11]. This model was used to predict the activity of 25 compounds with naphtho[2,3-b]furan-4,9-dione derivatives, which were previously predicted as CK2 inhibitors via a molecular modeling approach [11]. The obtained QSAR model was exploited to predict the activity of a group of naphtho[2,3-b]furan-4,9-diones that have never been tested as CK2 inhibitors before. The results suggested that a naphtho[2,3-b]furan-4,9-dione backbone is an appropriate skeleton for the development of novel inhibitors for this kinase target [11].

As new potential CK2 inhibitors 298 compounds were selected for biochemical testing based on the results of virtual screening of a small organic compounds library consisting of 150,000 molecules using the Autodock program. *In vitro* experiments showed that 18 compounds have inhibitory activity against CK2 with IC₅₀ in the range of 1.4 to 20 μ M. The active compounds belonged to 15 chemical classes. The physicochemical properties of the studied inhibitors are in accordance with the Lipinski's rules, which makes the compounds suitable for further research and development of potential drugs. Additionally, parameter LE (ligand efficiency) was used to estimate the contribution of each atom to the compound activity.

CX-4945, a tricyclic quinoline compound is the first representative of CK2 inhibitors in human clinical trials. Molecular docking, molecular dynamics simulations and energy analysis were employed to explore the pivotal roles of the hinge region sub-pocket in the binding of tricyclic quinolone analogues with CK2. The loss of H-bonds between 2,6-naphthyridine substituent compounds and the hinge region induce the reorientation of the compound. This study presents a summary of known polar pharmacophore groups of ATP-competitive inhibitors [13].

It has been shown very recently that oxazinocarbazole derivatives studied with different biological test methods for CK2 activity show strong inhibition activity against CK2 and cause death of IPC-81 leukemia cells. At concentrations well below 80 μ M, the oxazinocarbazole derivatives were found to be capable of inducing leukemia cell death.

It has been determined that pyridocarbazoles, the bioisosters of thienobenzocarbazoles show antitumoral effects *in vitro* and *in vivo* as new ATP-competitive protein kinase CK2 inhibitors [16].

Carbazole structures have been used in the treatment of many diseases for many years, however, there is no information available on thienobenzocarbazoles, which are carbazole derivatives that can be isolated from plants as natural products [14]. In spite of the fact that synthetically different isomers of thienobenzocarbazoles are synthesized, there are few studies on their biological properties that have identified their antibacterial activities [15]. Therefore, what activities thienobenzocarbazoles have and which new features they will demonstrate is a topic waiting to be investigated. Thienobenzocarbazole derivatives, being bioisosters of pyridocarbazoles and displaying high structural similarity with CX-4945, have great potential in inhibiting CK2, yet, there are no studies exploring their activities towards CK2 inhibition.

In this thesis, new thienobenzocarbazole derivatives were generated from promising inhibitor candidates among the 33 parent molecules based on their docking scores to protein kinase CK2. New inhibitors with a better score were proposed by docking these new derivatives. As a last step, the potential interactions between CK2 and the selected inhibitor candidates were investigated using molecular dynamics simulations in a dynamic and solvated environment.

The next section of this thesis summarizes the computational methods that have been used followed by the docking and molecular dynamics simulation results of different thienobenzocarbazole derivatives. Finally, a conclusion for the project is given with the potential future work of the project.

2. METHODOLOGY

2.1 COMPUTATIONAL APPROACH

33 parent AK molecules, which are derivatives of thienobenzocarbazole, were docked to the active site of CK2 and affinity scores were determined by Autodock. 2 AK molecules with promising affinity score were selected, AK9 and AK10, which were derivatized using MOE. The newly obtained AK derivatives were again docked to the active region of CK2 and the affinity values obtained were evaluated. Selected new AK derivatives and the parent compounds used in the derivatization were evaluated with MD simulations. The interactions with the active site were further investigated and compared with CX-4945.

2.2 METHODS

2.2.1. Docking

Docking is the modeling of the interaction between the active region of a receptor and the ligand by computer simulation and is one of the most important tools used in structure based drug design. The docking technique involves locating the optimal binding geometry and energy by placing it in various orientations and conformations to the binding region of the ligand. Molecular docking is an estimate of conformational complementarity between ligand and receptor molecules to predict the tentative binding parameters of the ligand-receptor complex beforehand. The docking process is designed to examine the ligand conformations and to sort the selected conformations according to the scoring functions [18]. Docking simulations estimate optimized docking compatibility based on the total energy of the system. For the application of molecular docking, the methodology requires preparation of database and ligand for searching the appropriate target [19].

Molecular docking has brought together revolutionary developments, with the advances in other fields such as computer science, physics and statistics. However, the basic idea is the "Lock and Key Hypothesis" in the context of enzyme-substrate geometric interaction as proposed by Emil Fischer in 1894 [18].

During the developmental stages of molecular biology, various modifications have been recommended to figure out the incoming involution of the docking. In 1958, Koshland introduced the idea of “induced fit” instead of the “Lock and Key Hypothesis” (Figure 2.1). He suggested that conformational changes in the active region of the receptor are induced after binding of the substrate [20].

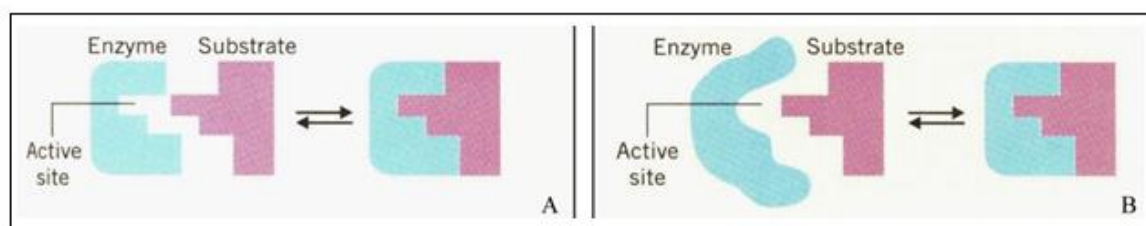


Figure 2.1. (a) The lock and key theory of enzyme-substrate interaction. (b) The induced fit theory of enzyme-substrate interaction [21]

Before late 1970s, the study of molecular docking was more of a molecular interaction phenomenon between molecules. The idea of docking as we understand today originated from works in the late 1970s. In the context of computer-aided drug design, the emergence of molecular docking is closely related to the development of various methodologies such as target recognition, virtual screening or high-throughput screening, molecular dynamics simulation, scoring function, and search algorithms. It integrates many entities, such as drug discovery databases, ligand libraries, molecular docking tools, and various evaluation tools [18].

The docking procedure can be divided into 3 stages:

- Ligand and macromolecule preparation: This is based on force fields that allow for surface representation and possible ligand sites in cavities.
- Determining the docking type: Rigid or Flexible,
- Determining the ligand conformation search strategy: Stochastic or Systematic [22].

During ligand and protein preparation part, before performing any computations, the system must be thoroughly picked and set up. The initial step is to obtain a protein structure, preferably one that has a bound ligand. Three-dimensional structures with excellent

resolution, as well as structures co-crystallized with high affinity ligands or natural substrates, are highly suggested [22].

Autodock and SwissDock, use an in-house force field, whereas MOE and LeDock use AMBER and CHARMM force fields, respectively. It's worth noting that different programs' protein preparation modules generate slightly different output files, which has a direct impact on the docking findings. As a result, using the same preparatory technique for all docking calculations is strongly suggested in order to compare docking findings [22].

In most cases, the ligand and protein can be processed and prepared independently. Similar considerations apply to ligand preparation, with the first stage typically including its extraction from the protein structure. In virtual screening, ligands could come from places other than the PDB (e.g. Public repositories like PubChem, organic synthesis or virtual compounds). In such circumstances, the technique varies, but it usually entails either constructing the molecule from its Simplified Molecular Input Line Entry (SMILES) format or drawing the molecule and saving it as an SDF/ MOL file that serves as input. Numerous optimizations may be required for ligand preparation, including *ab initio* or semiempirical geometry and/or charge assignment, energy minimization, and conformational/tautomer search [22].

It is also worth noting that the protein can be prepared in the same way (using semi-empirical calculations). Due to lengthy computation periods, this may appear impracticable at first. Complex optimization and the use of detailed parameters, on the other hand, have been found to greatly improve outcomes and interaction modeling [22].

The type of docking must be chosen after the protein and ligand have been prepared. Residues are then picked as additional terms for energy computation in the case of flexible docking. This can be done either manually (Autodock, Vina) or automatically (Autodock, Vina) (ICM, MOE). This usually signifies that the potential has been "softened" to allow for residue flexibility and ligand interaction. In a nutshell, softening the interaction potential entails changing the parameters of the interaction description. The Lennard-Jones potential is frequently used since it is a conservative description for steric conflicts and does not sample small alterations that can occur in a protein pocket [22].

A Rigid Body Docking module should first be built before conformational ensembles can be used for Virtual Screening. Rigid Docking is a rapid method for applying small or rigid molecules, molecular fragments, or conformational ensembles to small or rigid molecules or molecular fragments. Semi-flexible Docking, which includes rigid targets but flexible ligands, can be added to the overall strategy. Full-flexible docking for proteins based on a conformation ensemble can be done using either conformation analysis on macromolecules or experimental data [23].

Internal distances must be fixed while minimizing a function under the rigid body constraint. Moving a point mass in a conservative force field along the negative gradient will doubtlessly minimize the field function. The same holds for a rigid body's center of mass.

The conformation and positioning of the ligand must be determined by the docking algorithm chosen. This includes conducting a conformational search and selecting the best solution based on the scoring function. Such a search could be systematic or random [22].

A wide sampling of conformations and a mix of structural factors are used in systematic search. This method, on the other hand, requires more resources and takes substantially longer to develop and analyze the conformers individually. A real systematic search may also result in a combinatorial explosion. To avoid this issue, a systematic search is carried out by constructing the ligand from various fragments, selecting one as the anchor and sequentially adding combinations of the remaining fragments [22].

The randomness of stochastic search is achieved using one of two methods: Monte Carlo (MC) or genetic algorithms (GA). Based on bond rotations as degrees of freedom, each creates its own conformation. The score function is then used to pick and filter poses for the structures [22].

The above-mentioned conformational search could result in a vast number of structures. Of course, only a small portion of these could be biologically significant. Scoring functions are then utilized to differentiate between excellent and bad solutions by evaluating a wide range of features such as intermolecular interactions, desolvation, electrostatic, and entropic effects, among others. There are three types of scoring functions: force field-based, empirical, and knowledge-based.

Force Field-Based scoring function employs parameter contribution to create a "master function" Equation 2.1 is an example of a hypothetical force field-based scoring function that accounts for H-bond, electrostatics, and non-bonding interactions. As a result, entropic contributions to solvation cannot be accounted for. Furthermore, these methods typically necessitate lengthier computation durations and the use of distance cutoffs, reducing the precision of long-range effects.

$$E = W_{vdw} \sum_{i,j} \left(\frac{A_{ij}}{r_{ij}} - \frac{B_{ij}}{r_{ij}^6} \right) + W_{Hbond} \sum_{i,j} E(t) \left(\frac{A_{ij}}{r_{ij}} - \frac{B_{ij}}{r_{ij}^6} \right) + W_{elec} \sum_{i,j} \frac{q_i q_j}{\epsilon(r_{ij}) r_{ij}} \quad (2.1)$$

The reproduction of experimental values is a requirement of empirical scoring functions. They could be connected to Quantitative structure activity relationship (QSAR) models, which use linear regressions of descriptors to simulate protein-ligand interactions. PLANTS scoring, shown in Equation 2.2, entails determining form complementarity based on atom types (f_{PLP}), ligand clash potential (f_{clash}), ligand torsion potential (f_{tors}), and site contributions to enforce ligand interactions (c_{site}). For score shifting, the value -20 is introduced. However, it has been suggested that such energy characteristics (unlike their QSAR counterparts) may not be as robust. Furthermore, because they are derived from individual protein-ligand complexes and varied data in training sets, most empirical scorings have severe drawbacks. Nonetheless, empirical scoring functions are far faster to compute than their force field-based counterparts and produce accurate binding energy estimations [22].

$$E_{PLANTS} = f_{PLP} + f_{clash} + f_{tors} + c_{site} - 20 \quad (2.2)$$

Knowledge-based scoring functions are designed to replicate structures rather than energies. Pairwise potentials generated from known receptor-ligand complexes are used to build the structure. This is based on the inverse Boltzmann relation, which originates in statistical mechanics and uses mean force potentials rather than real ones. Consider Equation 2.3 as an example. The score calculated by Small Molecule Growth (SMoG) 2001 is displayed. The first term refers to the normalization of protein-ligand contact probability; F^{rotx} signifies the

contribution of "frozen" flexible bonds, which lower ligand conformational entropy; and N^{rotx} denotes the number of flexible bonds [22].

$$F_{\text{SMoG2001}} = \sum_r \sum_p \sum_l F(r, \delta_p, \delta_l) \Delta(r, p, l) + F^{rot1} + N^{rot1} + F^{rot2} + N^{rot2} \quad (2.3)$$

AutoDock Vina uses Equation 2.4 as scoring function [24].

$$c = \sum_{i < j} f_{t_i t_j}(r_{ij}) \quad (2.4)$$

where; the summation is over all pairs of atoms that can move relative to each other, usually excluding 1-4 interactions, i.e., atoms separated by 3 consecutive covalent bonds. Here, each atom i is assigned a type t_i , and a symmetric set of interaction functions $f_{t_i t_j}$ should be defined for the interatomic distance r_{ij} [25].

2.2.2. Derivatization

In this method, part of the molecule can be changed or a group can be added to achieve a desired interaction. In the inhibitor design, the fragments need to be extended or linked with a scaffold [26]. Computational methods to accelerate these processes are highly desirable [27]. MOE's Linker pharmacophore tool, automatically creates complex scaffold replacement queries. Resulting molecules can be refined and scored against any available receptor atoms, and also filtered using molecular properties, pharmacophore, QSAR or fingerprint similarity models. Additionally the synthetic plausibility of the proposed molecules is predicted and can be included as a filter.

Computational methods for scaffold replacement include MOE's Linker pharmacophore features part of a pharmacophore query. A simple modification of the "Add group to ligand"

type requires a single pair of such features to define the binding vector. The combination of two (or more) pairs of features, each defining the binding of one R group to the scaffold of the ligand, selects fragments that can bind to all specified R groups, replacing the scaffold of the input molecule (Figure 2.2). Similar molecular changes are required for scaffold replacement, fragment linkage, and fragment growth. Once the bonds between the scaffold and the R groups are selected, the bonds are cut and the endpoints are used to define bond vectors. In the case of fragment growth, a binding vector is usually identified, along with other pharmacophore features in the volume to be filled [27].

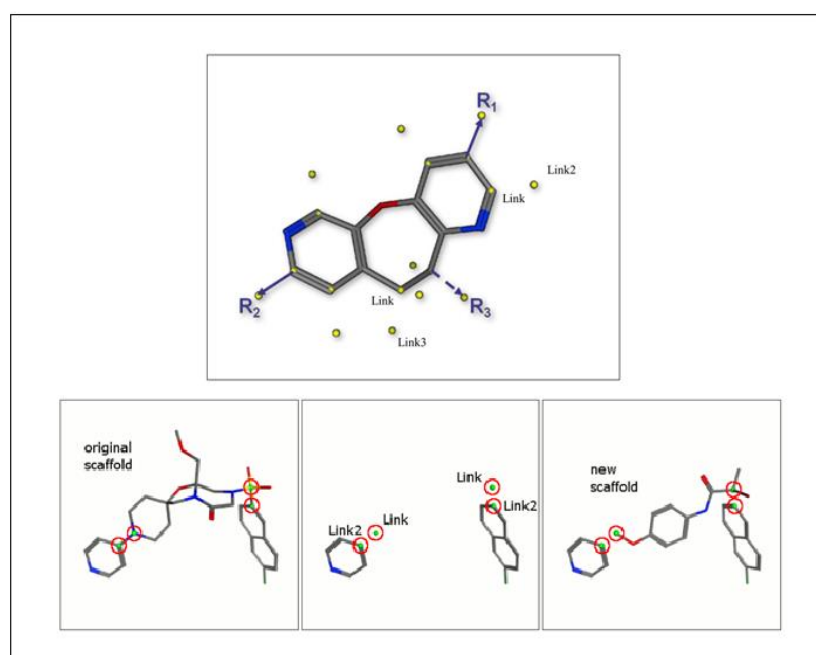


Figure 2.2. The combination of a Link feature with Link2 and/or Link3 features defines the bond vectors between scaffold and R-groups [27].

Derivatizations can be performed in the modes listed in Table 2.1.

Table 2.1. Derivatization types [26]

Mode	Description
Replace Scaffold (Select Scaffold)	Replace an internal portion of a molecule
Replace Scaffold (Select R Atoms)	Replace an internal portion of a molecule
Link Multiple Fragments	Find a scaffold that can link all specified fragments
Add Group to Ligand	Add a new fragment to a molecule
MedChem Transformations	Apply Medicinal Chemistry transformation rules to a molecule
BREED	Generate novel structures combining parts of existing aligned ligands
Combinatorial Builder	Enumerate a library of compounds in 3D

For the “Replace Scaffold”, portion of the input ligand can be selected for replacement using one of these modes:

1. “Select Scaffold”, replacing the selected atoms.
2. “Select R-group atoms”, where the linking atom of each retained R-group is selected.

An example scaffold replacement query is shown in Figure 2.3. The atoms highlighted in red were selected for replacement resulting in two bond vectors shown as green arrows, the R groups to be retained in the resulting molecules shown in black are selected [27].

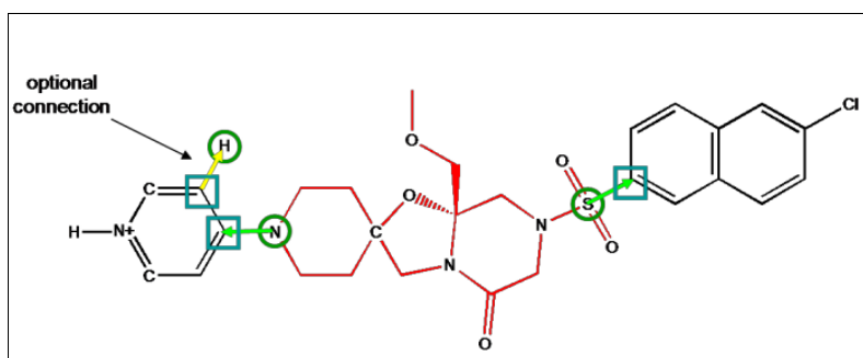


Figure 2.3. A scaffold replacement query showing two essential connections (green arrows) and a single optional connection (yellow arrow).

A single pharmacophore query is generated that places "Link2 Link3" (sp² or sp³) projected linker features on the bridgehead R group atoms (marked with blue squares). Link features are generated at a specified distance from the correct position for the heavy atom (green circles). Each pair is constrained to be formed by the same heavy atom to ensure that a bond can be formed. The bonds can be either essential or optional. Multiple queries are automatically generated containing both the essential compounds and all combinations of the optional compounds. Thus, a wide range of possible molecular modifications can be examined in a single search. Optional compounds are defined by the selection of hydrogen atoms used to orient the bond vector. If it is selected both the bonded heavy atom and the hydrogen atom, the compound is marked as essential. For example, when an optional compound is added to the pyridine ring Figure 2.3, two queries are generated: one with the two essential compounds and one with all three compounds. The molecules returned by the third query contain a ring fused to the pyridine R group.

Using the application in the second "replace scaffold" mode, "select R atoms", and the atoms in the blue squares, the R groups are defined. In this mode, a range of contiguous bonds where the connection can occur, so that multiple substitution points can be covered with a single search. For example, if it is also selected the sulfur atom, two searches will be generated, one for the N-C and S-C bonds and one for the N-C and N-S bonds.

Another method, "Add Group to Ligand" method is generally implemented to describe the process of adding a new fragment to a molecule already bound in the active site. The aim is to produce a new molecular entity with better binding properties and possibly greater selectivity than the original molecule.

This mode allows the user to expand a ligand in one or more sites. Compounds are indicated by the selection of hydrogen atoms, and if the heavy atom is also selected, the connection is essential. In Figure 2.4, there is one essential connection (green arrow) and two optional connections (yellow arrows). Four queries are created: one with only the essential connection, two queries with the essential connection and one of the optional connections, and one with all three connections. If only a single connection point is specified, this may result in a large number of hits. In this case, additional filters such as pharmacophore queries, QSAR, or fingerprint models can be applied to ensure a reasonable number of hits.

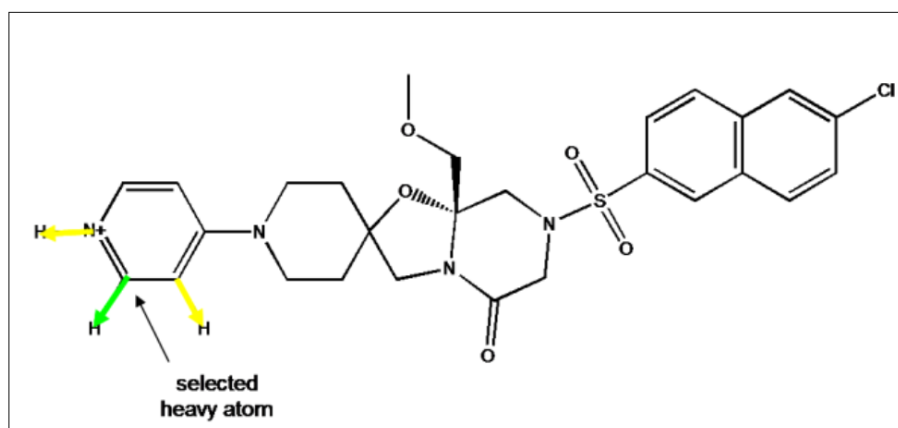


Figure 2.4. Add Group to Ligand mode, showing one essential connection (green arrow) and two optional connections (yellow arrows).

In “Link fragments” mode, two or more fragments can be joined with a linker. Links are indicated by marking hydrogen atoms, and at least one link is required for each fragment.

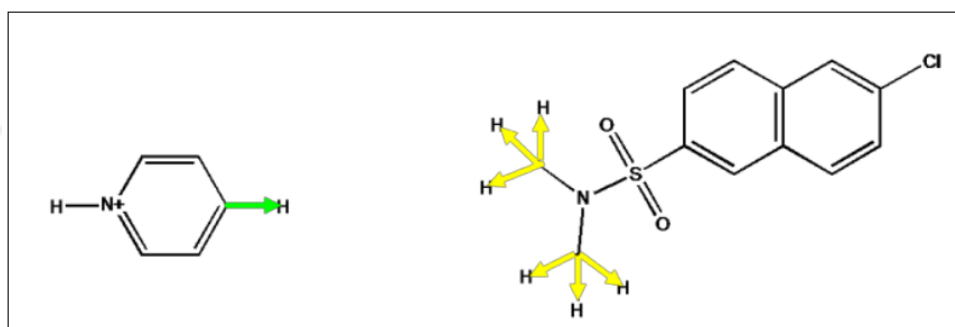


Figure 2.5. Linking multiple fragments with a combination of essential (green arrow) and optional (yellow arrows) exit vectors.

Figure 2.5 shows two fragments in which seven hydrogen atoms were selected as connecting points. The compound from pyridine on the left is considered essential because it is the only compound indicated for this fragment. The six compounds on the right are optional, although at least one of them must be present in each generated query. In this example, 56 queries are generated, all of which include the essential left compound and a combination of up to four optional compounds from the right fragment. (There are a total of four alternative connecting points.) The number of inquiries is decreased to eight when one of the optional connections is replaced with an essential connection: one with no optional connections, three with one, three with two, and one with three.

“Medchem Transformations” is a method for generating novel chemical structures by applying transformation rules to existing ligands. The transformations can change functional groups or either one or all sections of a ring while leaving the rest of the ligand unchanged. These transformations can be executed in a step-by-step process, resulting in cumulative alterations to the basic structures. Individual transformations result in small changes to the ligand's chemical structure. In the library, there are three sorts of transformations. Atom transformation, ring transformation, and ring creation. A match and replace algorithm is used to apply transformation rules [28].

- Every transformation rule's query is paired with the input molecule via a substructure search. Each of the resulting matches is taken into consideration for the next phase.
- The result's matched atoms are replaced.
- Transformations that result in heavy-atoms that are not linked are deleted. Once 3-D coordinates have been generated, duplicates are removed [28].

2.2.3. Molecular Dynamic Simulations (MD)

MD simulations create an interface between experiment and theory, using them to predict the dynamic properties of complex systems that cannot be calculated analytically as the equivalent of experiments [29].

Molecular Dynamics simulations are similar in many ways to real experiments. When we implement a real experiment, we prepare a sample of the material we would like to study with, connect this sample to the measuring device, and measure the property of the sample we are interested in at a given time interval. In molecular dynamics simulation, we follow the same steps. First, to prepare a sample, a model system containing N particles needs to be selected and Newton's equations of motion are solved for this system until the properties of the system no longer change with time. After equilibration, the actual measurement is performed [29].

The most important step in molecular modeling is to calculate the energy of an arrangement of atoms in three-dimensional space. There are several methods to perform these calculations. The most basic is the use of quantum mechanics, in which the Schroedinger equation is solved for the distribution of electrons and atoms in the system to derive a wave

function from which other properties can be derived. However, quantum mechanics has the major disadvantage that it is relatively time-consuming and is therefore rarely used for calculations involving large systems. Therefore, in molecular dynamics, the energy surface is explored by solving Newton's laws of motion for the system using a molecular mechanics force field model that ignores the electronic motions in the system and calculates the energy solely as a function of the positions of the atoms. The method uses a simple model of intramolecular and intermolecular interactions within a molecular system, dividing the energy into contributions from processes such as bond stretching, angular diffraction, rotation about individual bonds, and steric and electrostatic interactions between pairs of unbonded atoms [29].

The basic algorithm behind the MD calculations are as follows: First, divide time into discrete time steps no more than a few femtoseconds (10-15 s) apart. At each time step, calculate the forces acting on each atom using a forward molecular mechanics field. Assume that the force acting on each atom is constant during this time interval. Then move the atoms by updating their current positions and velocities to generate new positions and velocities in a short time interval using Newton's law of motion.

Newton's second law states that the force acting on a moving atom is equal to the mass of that atom times the acceleration of the atom.

$$F_i = m_i \times a_i \quad (2.1)$$

$$F_i = -m_i \frac{dv_i}{dt} = m_i \frac{d^2x_i}{dt^2} \quad (2.2)$$

Here v_i is the velocity of atom i and x_i is the position of atom i . Both are functions of time. The force is related to the acceleration and thus to the potential energy.

$$F_i = -\nabla_i E \quad (2.4)$$

For n atoms there are $3n$ position coordinates and $3n$ velocity coordinates, therefore the algebraic solution of the equations is impossible. The numerical solution is;

$$x_{i+1} = x_i + \delta_t v_i \quad (2.5)$$

$$v_{i+1} = v_i + \delta_t \frac{F(x_i)}{m} \quad (2.6)$$

Time-symmetric integration methods are used in the numerical solutions, such as Velocity Leapfrog Verlet.

$$x_{i+1} = x_i + \delta_t v_{i+1/2} \quad (2.7)$$

$$v_{i+1/2} = v_{i-1/2} + \delta_t \frac{F(x_i)}{m} \quad (2.8)$$

Among the most commonly used potential energy functions are the force fields AMBER, CHARMM, GROMOS, and OPLS/AMBER. The advancement of force fields continues to be an intense area of research that is important for both basic and applied research [22].

The simplest type of a force field (GAFF) that occurs in MD simulations is the following:

$$E = \sum_{\text{bonds}} K_b (l_i - l_{i,0})^2 + \sum_{\text{angles}} K_\theta (\theta_i - \theta_{i,0})^2 + \sum_{\text{torsions}} \frac{V_n}{2} (1 + \cos(n\omega - \gamma)) \\ + \sum_{i=1}^N \sum_{j>i}^N \left(\left[\left(\frac{A_{ij}}{R_{ij}^{12}} \right) - \left(\frac{B_{ij}}{R_{ij}^6} \right) \right] + \frac{q_i q_j}{\epsilon R_{ij}} \right) \quad (2.9)$$

The first term of equation 2.9 counts the harmonic potential, which represents the interaction between pairs of atoms when the atoms are separated by a covalent bond. This is the approximation of the energy of a bond as a function of displacement from the ideal bond length l_0 . The force constant, K_b , determines the strength of the bond. Both the ideal bond lengths and the force constants are specific to each pair of bonded atoms, i.e., they depend on the chemical nature of the atoms -the constituents. The second term of equation 2.9 is related to the change of bond angles θ from the ideal values θ_0 , which is also represented by a harmonic potential. The values of θ_0 , and K_θ depend on the chemical type of the atoms forming the angle. The third term represents the torsion angle potential function. The motion associated with this term is a rotation about the mean bond described by an dihedral angle and the symmetry coefficient $n=1,2,3$). This potential is assumed to be periodic and is often

expressed as a cosine function. The last term of equation 2.9 includes non-bond interactions such as Van der Waals and Coulombic interactions between non-bonded pairs of atoms [29].

Hydrogen bond analysis identifies the number and/or duration of hydrogen bonds in a system of interest. A hydrogen bond occurs when single hydrogen is shared between the heavy atom to which it is covalently bonded the "donor" and another heavy atom the "acceptor". This hydrogen bond which is actually a "weak interaction" usually exists with electronegative atoms such as fluorine (F), oxygen (O), or nitrogen (N) [30].

Nearly one hundred-year-old concepts about hydrogen bonding are brought to light to compare with today's views and theories [31]. Hydrogen bonds are among the most important non-bonded interactions in molecules. So far, several methods have been proposed to estimate the strength of hydrogen bonds [32].

In this study, inhibitor candidates that may be suitable for CK2 were investigated by calculating H-bond occupancy with MD simulations.

2.3. COMPUTATIONAL METHODOLOGY

2.3.1. Docking

The aim of docking in this project is to predict how well the ligand is settled in the protein of the crystalline structure of the human protein kinase CK2 alpha subunit (PDB ID: 3PE1). We assume that the receptor is rigid, that the lengths and angles of the covalent bonds are constant.

In the first phase of this project, the ways and interactions of thienocarbazole derivatives planned to be synthesized with protein kinase CK2 were studied by the docking method.

Preparation of macromolecule and ligand is required before starting the docking process. The crystal structure CK2 complexed with the inhibitor CX-4945 (PDB ID: 3PE1) was used to analyze the active site and implement molecular docking with thienobenzocarbazole derivatives.

During macromolecule and ligand preparation, in order to put the files in a compatible format with the docking program, protein and ligand files were converted to .pdbqt. Before converting to a pdpqt file, it is necessary to ensure that only polar hydrogens are present in both the ligand and protein files. This detail can be managed with the "polar only" option in MGL tools.

For the selecting the active site of the protein and docking calculations, water molecules and sulfates were removed from the structure of the protein 3PE1 in order to avoid any problems in the parameterization stage. This file has been saved as protein-ligand.pdb. In order to determine the grid box during the docking phase, CX-4945 was used as a reference. The xyz coordinates and the site of the gridbox, where the AK derivatives will be docked, as well as the input and output files of the docking process were included in a configuration file (Figure 2.6), which was feeded to Autodock Vina as an input (Figure 2.7) to generate the output involving the best 10 poses with the highest affinity score (Figure 2.8).

```
receptor = protein.pdbqt
ligand = k93.pdbqt

center_x = 22.71
center_y = -28.95
center_z = -14.59

size_x = 22
size_y = 24
size_z = 28

out = protein_k93.pdbqt
num_modes = 20
```

Figure 2.6. config.txt file

```
./vina --config config.txt
```

Figure 2.7. Docking calculation command in Autodock Vina

```

Detected 4 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 626716796
Performing search ...
0% 10 20 30 40 50 60 70 80 90 100%
|----|----|----|----|----|----|----|----|----|----|
*****
done.
Refining results ... done.

mode | affinity | dist from best mode
      | (kcal/mol) | rmsd l.b. | rmsd u.b.
-----+-----+-----+-----
1      -5.7      0.000      0.000
2      -5.5      3.405      5.389
3      -5.4      5.933      9.746
4      -5.4      2.301      4.007
5      -5.1      2.571      4.722
6      -5.1      3.724      8.535
7      -5.1      3.605      5.548
8      -5.0      8.760      9.944
9      -4.9      3.332      6.079
Writing output ... done.

```

Figure 2.8. Calculation results and affinity scores from AutoDock Vina output

2.3.2. Derivatization

In the first step, choosing an appropriate forcefield potential for the simulation is important. Amber10:EHT forcefield was chosen for modelling protein-ligand complexes.

The “QuickPrep” application prepares a PDB file for use in active site analysis and ligand modification. The first step was correcting any problems with the structure using the “Structure Preparation” tool, then adding hydrogens and assigning protonation states, which was performed by default using the “Protonate3D” application. Tethers were added to the receptor heavy atoms and distant atoms were fixed, and an energy minimization was performed.

Derivatization was performed in two different operating modes summarized in Table 2.2.

Table 2.2. Scaffold replacement modes

Mode	Description
MedChem Transformations	Apply Medicinal Chemistry transformation rules to a molecule
Add Group to Ligand	Add a new fragment to a molecule

2.3.3. Molecular Dynamic Simulations (MD)

In the MD phase, protein-ligand complexes for AK9, AK10 and their promising derivatives based on docking scores were evaluated.

MD simulations were performed using AMBER12 to evaluate the occupancy of the H-bond between the protein and the ligand in a dynamic and solvated environment [36]. The antechamber module of AMBER12 was used to generate the protein and derivatized ligand parameters using the general Amber forcefield (GAFF) [38], with partial charges set to fit the electrostatic potential computed at HF/6-31G(d) by RESP [33]. The Merz-Singh-Kollman scheme was used to calculate the charges using Gaussian 09 [35]. Protein and derivatized ligands were placed in a water box with solvent layer of at least 10 Å around the solute [39]. Initially, only the solvent molecules were relaxed, followed by an unrestrained minimization of all atoms. The systems were then heated in six 50 K, 50 ps steps too 300 K with a 1-fs time step at constant-volume periodic boundary conditions using a harmonic restraint of 10 kcal mol⁻¹ to the ligand. The temperature was controlled and equalized using the Langevin equilibration scheme. Each system was equilibrated for 2 ns with 2-fs time step at constant volume and another 2 ns at a constant pressure of 1 atm. A 20-ns production runs was performed using the isothermal-isobaric ensemble (NPT). Long-range electrostatic interactions were treated with the particle mesh-Ewald method. Trajectories were saved at every 100 ps, resulting in a total of 200 frames. The trajectories were analyzed using the ptraj module of AMBER12. MD populations was calculated as the percentage of the number of frames in the H-bond region with respect to the total number of frames in the simulation.

Instructions for MD parameterization details can be found in Appendix B.

3. RESULTS & DISCUSSION

Druglikeness attributes of the parent 33 thienobenzocarbazole derivatives (AK molecules) shown in Figure 3.1 were investigated with the basis of the Lipinski Rule of Five for comparison with CX-4945 [6]. In addition, total polar surface area (TPSA), aqueous solubility (logS), lipophilicity of a molecule (logD), octanol-water partition coefficient (logP), and toxicity values were found as well [6]. In an ideal drug, these properties satisfy the following criteria. For size, the molecular weight should be between 150 and 500 g/mol. For polarity, the TPSA should be between 20 and 130Å. For solubility, logS should not exceed 6. For octanol-water partition coefficient, logP should be in the range from -0.7 to $+6.0$. For flexibility, the molecule should not have more than 9 rotatable bonds [37]. Table 3.1 summarizes the ligand properties of the parent molecules compared to CX-4945. After screening all AK molecules that may show drug properties, all ligands were docked to the CK2 active site region centered around the location of CX-4945 in the crystal structure. The aim of finding the interactions and calculating the affinity scores was to determine how well CX-4945 and thienobenzocarbazole derivatives bind to the active site of CK2. AK9 and AK10 was found to have the best affinity scores [6].

Based on the docking of 33 AK molecules to CK2 active site (PDB ID: 3PE1) listed in Table 3.2, two of them, AK9 and AK10, were selected for further development based on their affinity scores, and considered for further structural modifications for improved binding.

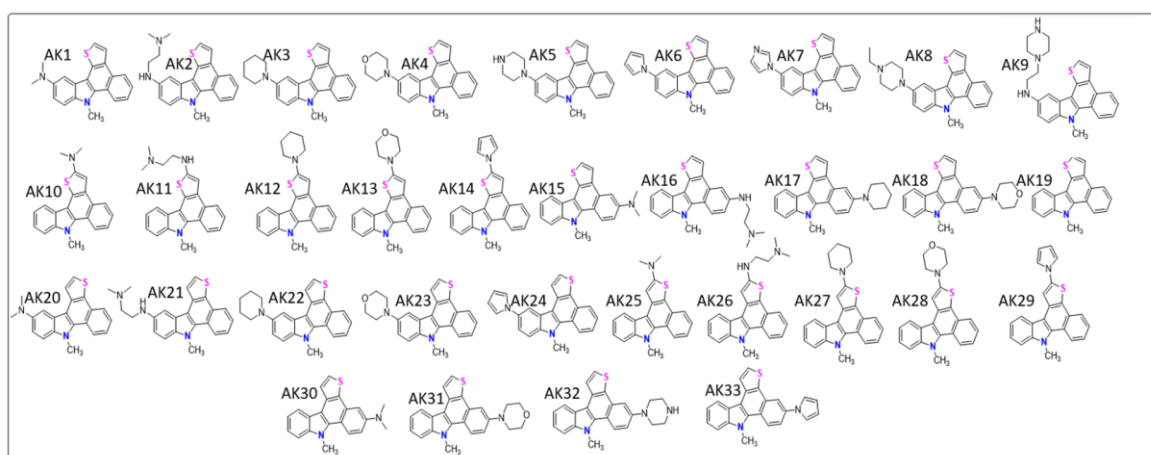


Figure 3.1. Thienobenzocarbazole derivatives (AK Molecules) investigated as CK2 inhibitors

Table 3.1. Lipinski's rule of five and toxicity results [6]

Molecule	Toxic	Weight (g/mol)	TPSA	HBD	HBA	logP	logD (pH=7)	logS
AK1	yes	330.45	8.17	0	0	5.71	5.71	-6.49
AK2	yes	373.52	20.20	1	1	5.51	3.13	-6.61
AK3	yes	370.52	8.17	0	0	5.94	5.94	-7.02
AK4	yes	372.49	17.40	0	1	5.20	5.20	-6.49
AK5	yes	371.51	20.20	1	1	5.08	3.48	-6.50
AK6	yes	352.46	9.86	0	0	6.89	6.89	-7.54
AK7	yes	353.45	22.75	0	1	6.02	5.93	-7.13
AK8	yes	399.56	11.41	0	1	5.61	4.00	-7.01
AK9	yes	414.58	32.23	2	2	4.85	2.49	-6.60
AK10	yes	330.45	8.17	0	0	5.73	5.73	-6.52
AK11	yes	373.52	20.20	1	1	5.51	3.20	-6.63
AK12	yes	370.52	8.17	0	0	5.97	5.97	-7.05
AK13	yes	371.48	17.40	0	1	2.84	2.84	-6.09
AK14	yes	352.46	9.86	0	0	7.04	7.04	-7.64
AK15	yes	330.45	8.17	0	0	5.74	5.74	-6.50
AK16	yes	373.52	20.20	1	1	5.53	3.21	-6.61
AK17	yes	370.52	8.17	0	0	5.98	5.98	-7.03
AK18	yes	372.49	17.40	0	1	5.24	5.24	-6.50
AK19	yes	287.39	4.93	0	0	5.66	5.66	-6.19
AK20	yes	330.45	8.17	0	0	5.69	5.69	-6.48
AK21	yes	373.52	20.20	1	1	5.50	3.11	-6.60
AK22	yes	370.52	8.17	0	0	5.92	5.92	-7.00
AK23	yes	372.49	17.40	0	0	5.15	5.18	-6.47
AK24	yes	352.46	9.86	0	0	6.88	6.88	-7.53
AK25	yes	330.45	8.17	0	0	5.73	5.73	-6.51
AK26	yes	373.52	20.20	1	1	5.50	3.21	-6.62
AK27	yes	370.52	8.17	0	0	5.97	5.97	-7.04
AK28	yes	371.48	17.40	0	1	2.85	2.85	-6.09
AK29	yes	352.46	9.86	0	0	7.03	7.03	-7.63
AK30	yes	330.45	8.17	0	0	5.73	5.73	-6.49
AK31	yes	372.49	17.40	0	1	5.23	5.23	-6.49
AK32	yes	371.51	20.20	1	1	5.10	3.62	-6.51
AK33	yes	352.46	9.86	0	0	6.90	6.90	-7.54
CX-4945	yes	349.78	75.11	2	4	4.42	4.10	-5.76

Table 3.2. Docking scores of 33 AK molecules [6]

Name	Affinity Score (kcal/mol)	Name	Affinity Score (kcal/mol)
AK1	-4.3	AK18	-4.6
AK2	-4.7	AK19	-4.4
AK3	-4.7	AK20	-3.9
AK4	-4.6	AK21	-4.5
AK5	-4.6	AK22	-4.5
AK6	-4.5	AK23	-4.7
AK7	-4.7	AK24	-4.4
AK8	-4.4	AK25	-4.3
AK9	-5.8	AK26	-4.3
AK10	-5.2	AK27	-4.6
AK11	-4.4	AK28	-4.4
AK12	-4.2	AK29	-4.4
AK13	-4.4	AK30	-4.2
AK14	-4.7	AK31	-4.5
AK15	-4.2	AK32	-4.4
AK16	-4.3	AK33	-4.6
AK17	-4.7	CX-4945	-10.3

Surface interactions of AK9 and AK10 were examined as a guide and interpreted in 3 different categories:

- 1- If the molecular surface of the ligand does not touch the molecular surface of the receptor, observed that little/no contact.
- 2- If the molecular surface of the ligand touches the molecular surface of the receptor observed as Favorable Contact.
- 3- If the molecular surface of the ligand penetrates the molecular surface of the receptor, observed that Steric Clash.

Figure 3.2 and 3.3 show AK9 and AK10 in the CK2 active side obtained from the docking results. In the zoomed image in the frame, the structure with the black mesh is the receptor, i.e., the surface of the protein structure, and the part with the white structure is the ligand surface of AK9 and AK10. The ligand touched the surface in the areas where there are H-bonds between the protein and ligand, indicating a favorable contact between the ligand

surface and the receptor surface. As shown in Figure 3.2 and Figure 3.3, there is a strong shape complementarity between the ligand surface and the receptor surface.

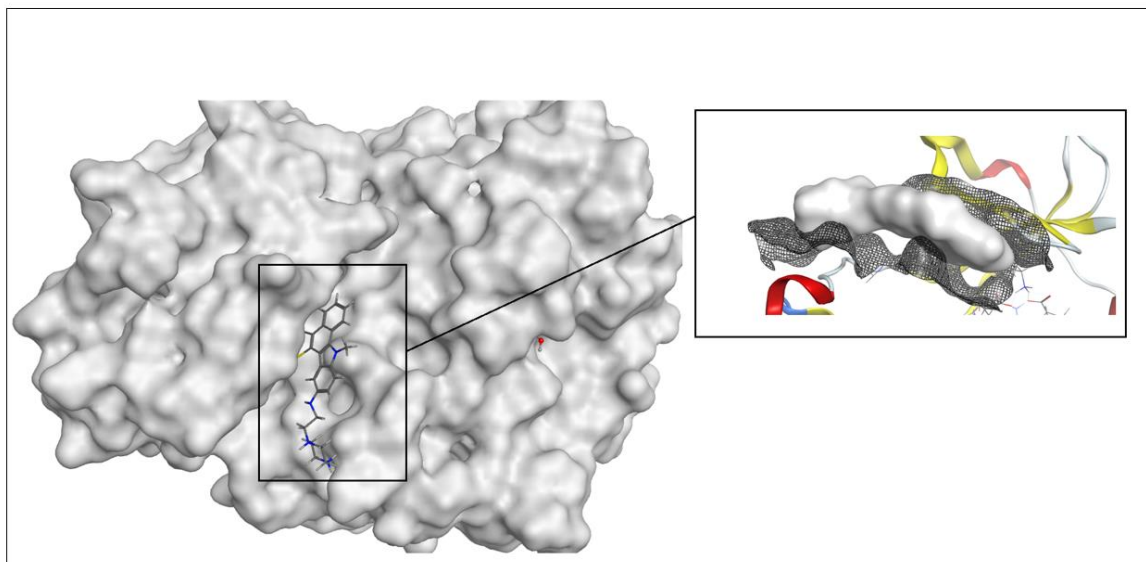


Figure 3.2. CK2 - AK9 protein & molecular surfaces

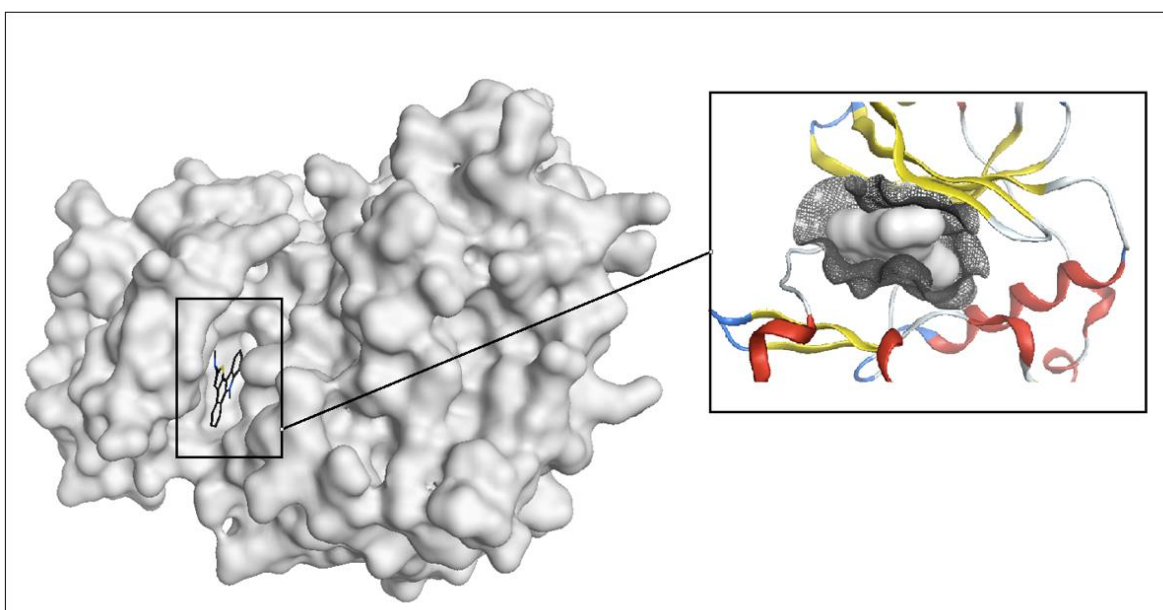


Figure 3.3. CK2 - AK10 protein & molecular surfaces

Besides shape complementarity, specific interactions between the ligand and protein involved in binding play an important role in obtaining acceptable affinities. Ligand

interaction diagram provides a means to visualize the active site in a concise and stylized manner, summarizing a large amount of spatial data. The layout of the diagram is optimised for the display of the current protein-ligand complex.

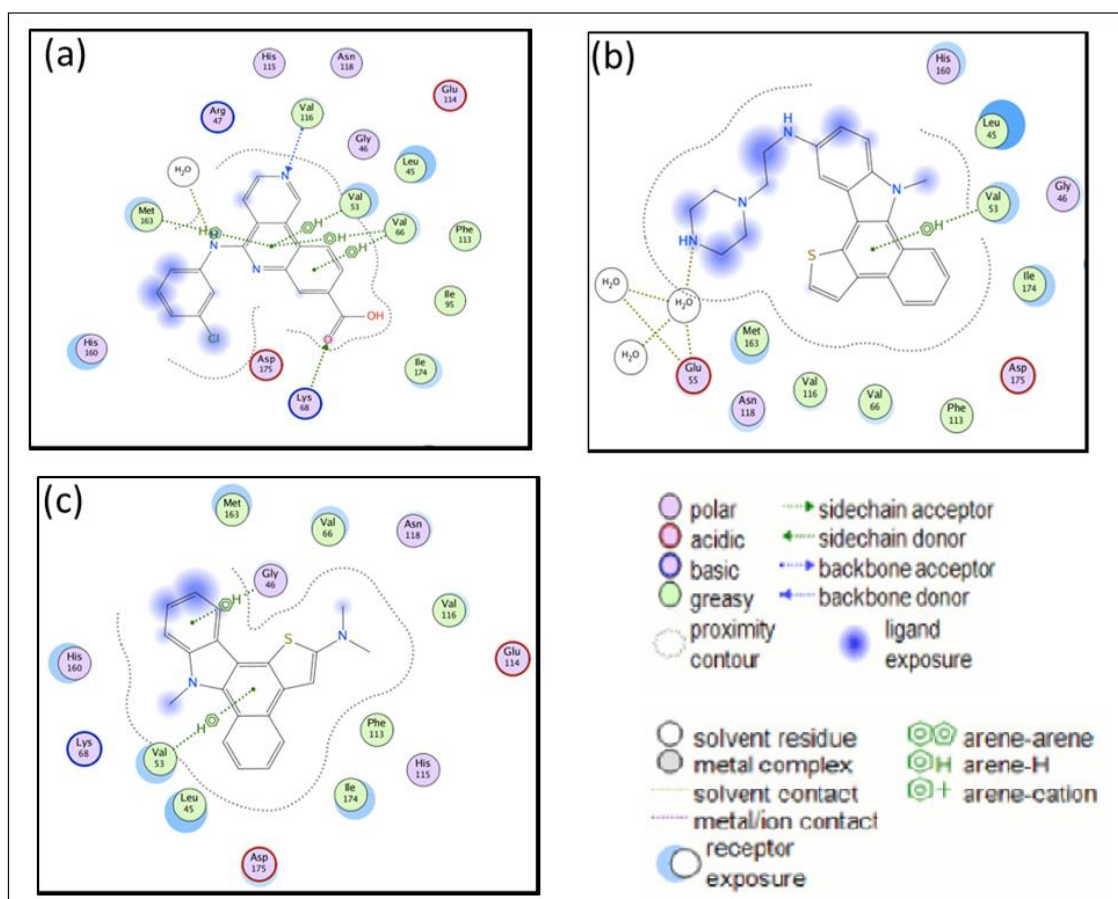


Figure 3.4. CK2 interactions of (a) CX-4945, (b) AK9 (c) AK10

Figure 3.4 shows the ligand interaction diagram of AK9, AK10 and CX-4945 as taken from the crystal structure as reference [6]. Even though AK9 and AK10 display better interactions among the 33 AK molecules also reflected in their affinity scores, the number of interactions is significantly less than that of CX-4945 [6].

Looking at Figure 3.4(a), there is a strong interaction between CX-4945 and the backbone NH of VAL116. There are three aromatic and C-H- π interactions between the ligand and the receptor. Salt bridge between the negatively charged carboxyl group of CX-4945 and

positively charged LYS68 is possibly one of the strongest interactions responsible for its higher affinity.

Looking at AK9 in Figure 3.4(b) it displays a C-H- π type interaction with VAL53 and some additional water interactions as can be expected by strong exposure of the ligand to the solvent. These interactions could result in the washing of the ligand out of the active site by the water molecules.

Looking at AK10 in Figure 3.4(c), same C-H- π interaction with VAL53 is observed as AK9, yet the ligand is now more buried in a hydrophobic pocket and less solvent exposed. AK10 has an additional C-H- π interaction with GLY46 compared AK9.

Figure 3.5 shows the three-dimensional layout of these interactions displayed in two-dimensional ligand interaction diagrams of CX-4945, AK9, and AK10.

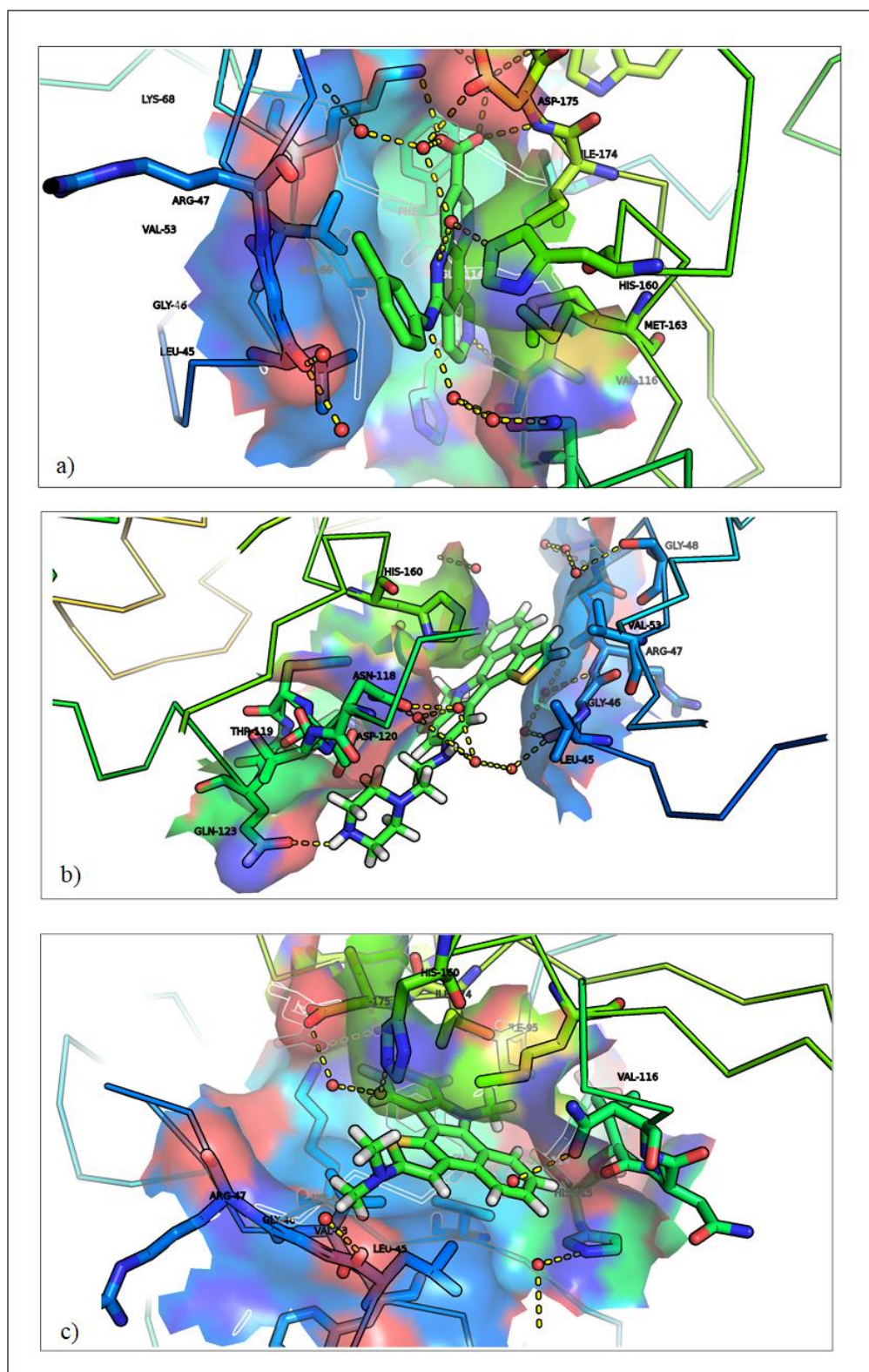


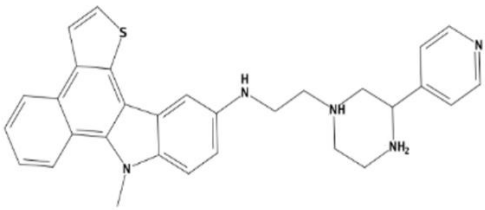
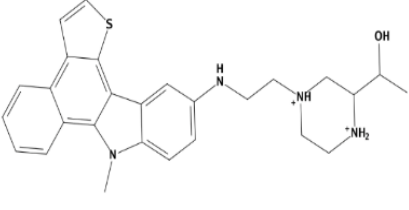
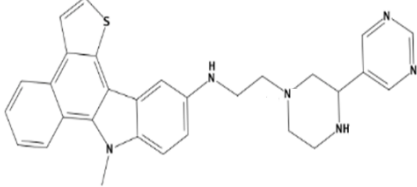
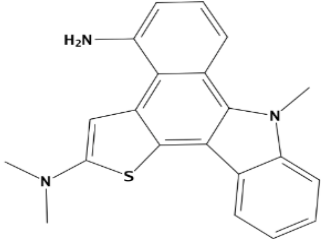
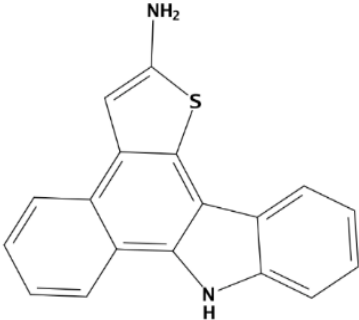
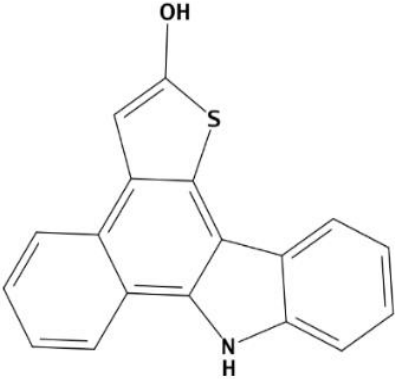
Figure 3.5. Interactions of (a) CX-4945 (b) AK9 (c) AK10 with CK2

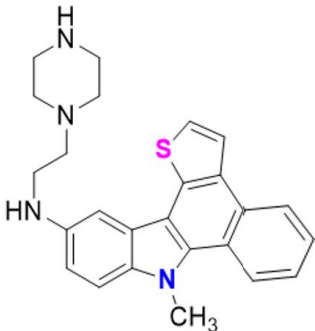
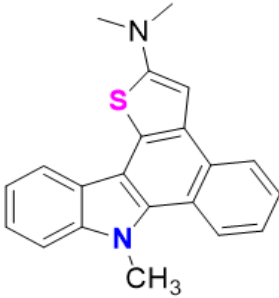
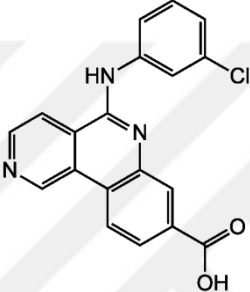
3.1 . DOCKING RESULTS

With the aim of improving the binding affinities of AK9 and AK10 to compete with CX-4945, AK9 and AK10 molecules were derivatized. A new derivative library was generated with the Scaffold Replacement and Medchem Transformations tools of MOE. A total of 119 derivatives were obtained. The library contained 72 derivates of AK9 and 47 derivatives of AK10.

Among these derivatives, structures with a smaller root-mean-square deviation (RMSD) value and a larger synthesisability score (rsynth) value were docked. Structures K91, K92 and K93 are derivatives of AK9 and structures K01, K06 and K07 are derivatives of AK10. All derivatives and docking results are given in Appendix Table A.2.

Table 3.3. Derivative molecules with best docking scores

	
K91 (-6.0 kcal/mol)	K92 (-5.6 kcal/mol)
	
K93 (-5.8 kcal/mol)	K01 (-5.4 kcal/mol)
	
K06 (-5.5 kcal/mol)	K07 (-5.4 kcal/mol)

	
AK 9 (-5.8 kcal/mol)	AK 10 (-5.2 kcal/mol)
	
CX-4945 (-10.3 kcal/mol)	

The docking results of the derivatives selected for evaluation using molecular dynamics simulations are listed in Table 3.3.

According to the docking results of derivatives of AK9, K91 gave the best docking score of -6.0 kcal/mol. K93 and AK9 have the same affinity scores (-5.8 kcal/mol), whereas K92 shows a lower affinity (-5.6 kcal/mol) compared to its parent compound AK9. As for AK10, derivatives K01, K06 and K07 show slightly improved binding affinities, -5.4 kcal/mol, -5.5 kcal/mol and -5.4 kcal/mol respectively. All of these derivatives, however have significantly lower docking docking scores compared to CX-4945 (-10.3 kcal/mol) presumably due to the lack of highly stabilizing carboxylate-lysine interaction.

3.2. MD RESULTS

Six thienobenzocarbazole derivatives K91, K92, K93, K01, K06 and K07 were evaluated with molecular dynamic simulations. AK9 and AK10 were also included for comparison.

Table 3.4. CK2 residues taken into account in MD analysis

Thienobenzocarbazole derivatives	Residues
K91	ARG41, LYS42, LEU43, GLY44, ARG45, GLY46, LYS47, TYR48, SER49, GLU50, VAL51, GLU53, VAL64, LYS66, LEU68, GLU79, LEU83, ILE93, PHE111, GLU112, HIE113, VAL114, ASN115, ASN116, THR117, ASP118, PHE119, LYS120, GLN121, HIE152, HIE158, ASN159, VAL160, MET161, ARG170, LEU171, ILE172, ASP173, TRP174, GLY175
K92	
K93	
AK9	
K01	
K06	
K07	
AK10	

Selected thienobenzocarbazole derivatives complexed with CK2 were immersed in a water box and subjected to 20 ns molecular dynamics simulation to monitor ligand-protein interactions in a dynamic solvated environment. For all of the derivatives subjected to MD simulations the ligand seems to fit well and stayed in the hydrophobic cavity of CK2. Therefore we turned our attention to polar contacts that can be discriminative in providing extra stabilization leading to higher affinities. Residues at a distance of 5 Å around the ligand were selected as active site residues (Table 3.4) and included in the analysis of the polar interactions which can be depicted from the H-bond occupancies of inter-residue contacts. H-bond occupancy can be defined as the percent ratio of frames having a H-bond distance less than 3.5 Å and H-bond angle greater than 120° over the total number of frames in the simulation. Key for the H-bond occupancy dumping scheme of the time series (0-20 ns) is given in Figure 3.6 and H-bond interactions along with their occupancies for each complex are summarized between Figure 3.7 and Figure 3.13.

Symbols		.	-	o	x	*	@
Occupancy (%)	0-5	5-20	20-40	40-60	60-80	80-95	95-100

Figure 3.6. H-bond occupancy intervals

The most stable H-bond interaction between the ligand and the protein is observed for K93. The occupancy of the H-bond between K93 and ASN-116 is 61% (Figure 3.7) as also represented by the cumulation of snapshots in the H-bond region defined by a distance cut-off 3.5 Å and an angle cut-off 120 ° in Figure 3.14 (a). Distance versus time graph for this interaction given in Figure 3.15 (a) shows that a strong H-bond formed at around 3.5 ns is disturbed at around 14 ns. A weak interaction between K93 and backbone -CO group of LEU43 (Figure 3.15 (b)) is found to have 16% occupancy (Figure 3.7 and 3.14 (b)). Figure 3.15 through Figure 3.20 show the strong complementarity between the aromatic portion of K93 structure and the hydrophobic active site pocket and the evolution of the polar contacts along the MD simulation. Derivative K91 display a similar interaction pattern to K93 but with somewhat lower occupancy for ASN116 (Figure 3.8, 39.5%) and a slightly higher occupancy for LEU43 (Figure 3.8, 27%). The H-bond occupancies of other derivatives as well as the parent compounds AK9, AK10 are found to be less than 10% (Figure 3.9 - Figure 3.13)

DONOR	ACCEPTOR	ACCEPTOR							
atom# :res@atom	atom# :res@atom	atom# :res@atom	atom# :res@atom	distance	angle	lifetime	maxocc		
1046 :6480	1937 :1118N	1936 :1118N	100.00	2.847 (0.099)	10.63 (8.62)	40.0 (80.0)	200		
838 :5180	498 :438H	697 :438H	100.00	2.847 (0.10)	20.46 (9.25)	40.0 (80.0)	200		
674 :4180	860 :538H	859 :538H	100.00	2.881 (0.11)	16.72 (8.85)	40.0 (80.0)	200		
2659 :15980	2882 :1728H	2881 :1728H	99.50	2.955 (0.15)	14.80 (8.15)	19.9 (48.9)	163		
722 :4480	824 :518H	823 :518H	99.50	2.942 (0.17)	16.28 (9.43)	19.9 (40.2)	112		
1055 :11180	1032 :648H	1031 :648H	98.50	3.030 (0.16)	15.76 (9.31)	9.9 (24.7)	50		
1342 :7980	1404 :838H	1403 :838H	98.50	3.028 (0.15)	16.41 (9.46)	9.9 (28.5)	120		
871 :5380E2	671 :418H21	670 :418H21	84.00	2.928 (0.20)	29.93 (13.80)	1.2 (4.1)	35		
838 :5180	717 :448H	716 :448H	80.50	3.226 (0.17)	21.27 (8.56)	1.0 (2.8)	19		
2899 :17280	2657 :15980D2	2655 :15980D2	80.00	3.127 (0.21)	22.20 (11.16)	1.0 (2.7)	17		
871 :5380E2	665 :418H2	664 :418H2	72.50	3.025 (0.24)	32.09 (13.61)	0.8 (2.8)	27		
2637 :1580D1	2630 :1580H	2629 :1580H	68.50	3.098 (0.16)	45.26 (7.72)	1.4 (3.7)	20		
870 :5380E1	665 :418H2	664 :418H2	63.50	2.990 (0.22)	30.31 (15.08)	0.8 (2.6)	20		
870 :5380E1	671 :418H21	670 :418H21	62.50	3.011 (0.21)	32.92 (15.23)	0.7 (2.2)	15		
1912 :11580D1	1919 :1168H	1918 :1168H	58.50	2.997 (0.19)	44.52 (8.16)	0.6 (2.0)	14		
873 :5380	652 :418H	651 :418H	48.50	3.314 (0.14)	10.63 (8.07)	0.4 (1.0)	7		
871 :5380E2	1883 :1138H2	1882 :1138H2	48.00	2.852 (0.15)	25.17 (12.48)	1.0 (3.1)	18		
5506 :3288H4	1928 :1168H21	1927 :1168H21	39.50	3.081 (0.17)	25.75 (13.97)	1.2 (3.7)	21		
2911 :17380	2937 :1758H	2936 :1758H	36.50	3.188 (0.19)	46.13 (8.87)	0.3 (0.9)	7		
1340 :7980E2	1082 :668H3	1079 :668H2	34.00	2.847 (0.15)	28.22 (14.34)	0.4 (1.0)	6		
1340 :7980E2	1080 :668H1	1079 :668H2	33.50	2.912 (0.17)	29.78 (12.97)	0.3 (0.8)	5		
1340 :7980E2	1081 :668H2	1079 :668H2	29.00	2.907 (0.17)	32.50 (11.31)	0.4 (0.9)	8		
1339 :7980E1	1081 :668H2	1079 :668H2	28.00	2.823 (0.13)	36.46 (12.56)	0.3 (0.8)	6		
1339 :7980E1	1080 :668H1	1079 :668H2	28.00	2.847 (0.13)	36.46 (12.56)	0.3 (0.8)	6		
715 :4380	5514 :3288H6	5513 :3288H2	27.00	3.081 (0.20)	40.79 (13.44)	0.3 (0.7)	4		
1339 :7980E1	1082 :668H3	1079 :668H2	25.50	2.849 (0.15)	40.18 (15.05)	0.3 (0.8)	5		
1340 :7980E2	2913 :1748H	2912 :1748H	25.00	3.248 (0.21)	42.65 (12.55)	0.3 (0.6)	3		
2909 :17380D2	1082 :668H3	1079 :668H2	24.50	2.757 (0.11)	25.21 (10.87)	0.3 (0.8)	5		
2909 :17380D2	1081 :668H2	1079 :668H2	24.50	2.784 (0.11)	22.52 (10.63)	0.4 (1.0)	6		
2909 :17380D2	2937 :1758H	2936 :1758H	23.50	3.190 (0.16)	27.02 (9.77)	0.3 (0.7)	6		
2909 :17380D2	1080 :668H1	1079 :668H2	21.50	2.796 (0.13)	22.36 (11.62)	0.4 (1.0)	8		
2911 :17380	2657 :15980D2	2655 :15980D2	20.00	3.206 (0.16)	49.63 (9.82)	0.3 (0.7)	4		
2908 :17380D1	1081 :668H2	1079 :668H2	11.00	2.785 (0.14)	26.71 (10.85)	0.3 (0.8)	4		
1903 :11480	1929 :1168H22	1927 :1168H22	11.00	3.064 (0.19)	21.54 (10.41)	0.7 (2.1)	10		
2908 :17380D1	1082 :668H3	1079 :668H2	10.50	2.787 (0.12)	25.28 (11.53)	0.2 (0.5)	2		
2908 :17380D1	1080 :668H1	1079 :668H2	9.00	2.838 (0.19)	27.40 (11.43)	0.3 (0.6)	2		
1945 :11780	1929 :1168H22	1927 :1168H22	9.00	3.033 (0.21)	32.82 (12.83)	0.7 (1.7)	7		
1926 :11680D1	1933 :1178H	1932 :1178H	8.50	2.915 (0.17)	41.25 (8.37)	0.4 (1.2)	7		
2908 :17380D1	2937 :1758H	2936 :1758H	8.00	3.259 (0.15)	30.44 (11.21)	0.2 (0.5)	2		
1878 :11380D1	1889 :1148H	1888 :1148H	7.00	3.016 (0.12)	42.97 (10.19)	0.2 (0.5)	2		

Figure 3.7. CK2-K91 H-bond occupancies from molecular dynamics simulations

DONOR		ACCEPTORH		ACCEPTOR		occupied		distance		angle		lifetime		maxocc	
atom#	res@atom	atom#	res@atom	atom#	res@atom	occupied	distance	angle	lifetime	maxocc					
2861	1708O	2677	1618H	2676	1618H	100.00	2.981 (0.14)	17.78 (9.64)	40.0 (80.0)	200					
1046	648O	1837	1118H	1836	1118H	100.00	2.838 (0.10)	17.10 (9.33)	40.0 (80.0)	200					
838	518O	698	438H	697	438H	100.00	2.815 (0.10)	21.24 (10.90)	40.0 (80.0)	200					
674	418O	860	538H	859	538H	100.00	2.875 (0.12)	18.08 (9.22)	40.0 (80.0)	200					
2659	1598O	2882	1728H	2881	1728H	99.50	2.934 (0.13)	14.43 (8.61)	19.9 (50.2)	168					
722	448O	824	518H	823	518H	99.50	2.954 (0.15)	17.45 (9.64)	13.2 (29.8)	98					
1342	798O	1404	838H	1403	838H	99.50	3.010 (0.16)	15.97 (9.45)	9.5 (37.2)	171					
2080	1718O	2923	1748H	2922	1748H	97.00	3.036 (0.18)	19.82 (9.31)	7.8 (17.4)	63					
2829	1618O	2839	1708H	2838	1708H	98.00	3.029 (0.15)	17.45 (9.64)	15.6 (6.6)	71					
1855	1118O	1032	648H	1031	648H	94.00	3.036 (0.18)	15.20 (8.09)	3.1 (10.1)	59					
1867	11208O1	2852	1708HE	2851	1708NE	84.50	3.002 (0.20)	23.65 (13.82)	1.2 (3.5)	20					
1868	11208O2	2858	1708HH21	2857	1708NH2	84.00	2.926 (0.19)	30.55 (12.78)	1.7 (5.0)	27					
838	518O	717	448H	716	448H	82.50	3.203 (0.16)	22.91 (10.43)	1.1 (3.2)	25					
871	538O2	671	418HH21	670	418NH2	81.50	2.950 (0.22)	28.85 (14.57)	1.3 (3.4)	17					
870	538O1	671	418HH21	670	418NH2	77.50	3.019 (0.22)	34.35 (13.42)	1.0 (3.0)	22					
1868	11208O2	2852	1708HE	2851	1708NE	75.50	3.067 (0.22)	34.76 (14.98)	0.8 (2.1)	15					
870	538O1	665	418HE	664	418NE	75.50	2.965 (0.23)	26.67 (13.63)	1.1 (3.2)	19					
2637	1588ND1	2630	1588H	2629	1588H	66.00	3.096 (0.17)	45.88 (7.36)	0.8 (2.0)	10					
871	538O2	1883	1138HE2	1882	1138NE2	64.00	2.846 (0.13)	23.14 (11.07)	1.8 (5.7)	37					
2069	1728O	2857	1708H	2856	1708H	62.50	3.102 (0.19)	23.02 (11.25)	0.7 (2.1)	19					
5505	3288N5	1929	1168HD22	1927	1168ND2	61.00	3.062 (0.15)	19.84 (11.20)	2.4 (8.1)	44					
1926	1168O1	1933	1178H	1932	1178H	55.50	3.020 (0.18)	44.63 (9.25)	0.7 (2.0)	16					
871	538O2	665	418HE	664	418NE	51.00	3.015 (0.24)	28.98 (13.51)	0.6 (2.2)	17					
873	538O	652	418H	651	418N	49.50	3.316 (0.13)	19.77 (8.98)	0.5 (1.4)	12					
2080	1718O	1556	938H	1555	938H	39.00	3.354 (0.10)	33.08 (11.39)	0.3 (0.8)	5	</				

Figure 3.9. CK2-K93 H-bond occupancies from molecular dynamics simulations

[illegible]

Figure 3.11. CK2-AK10 H-bond occupancies from molecular dynamics simulations

[illegible]

All H-bond occupancies from the MD simulations are given in Appendix B.1 and B.2.

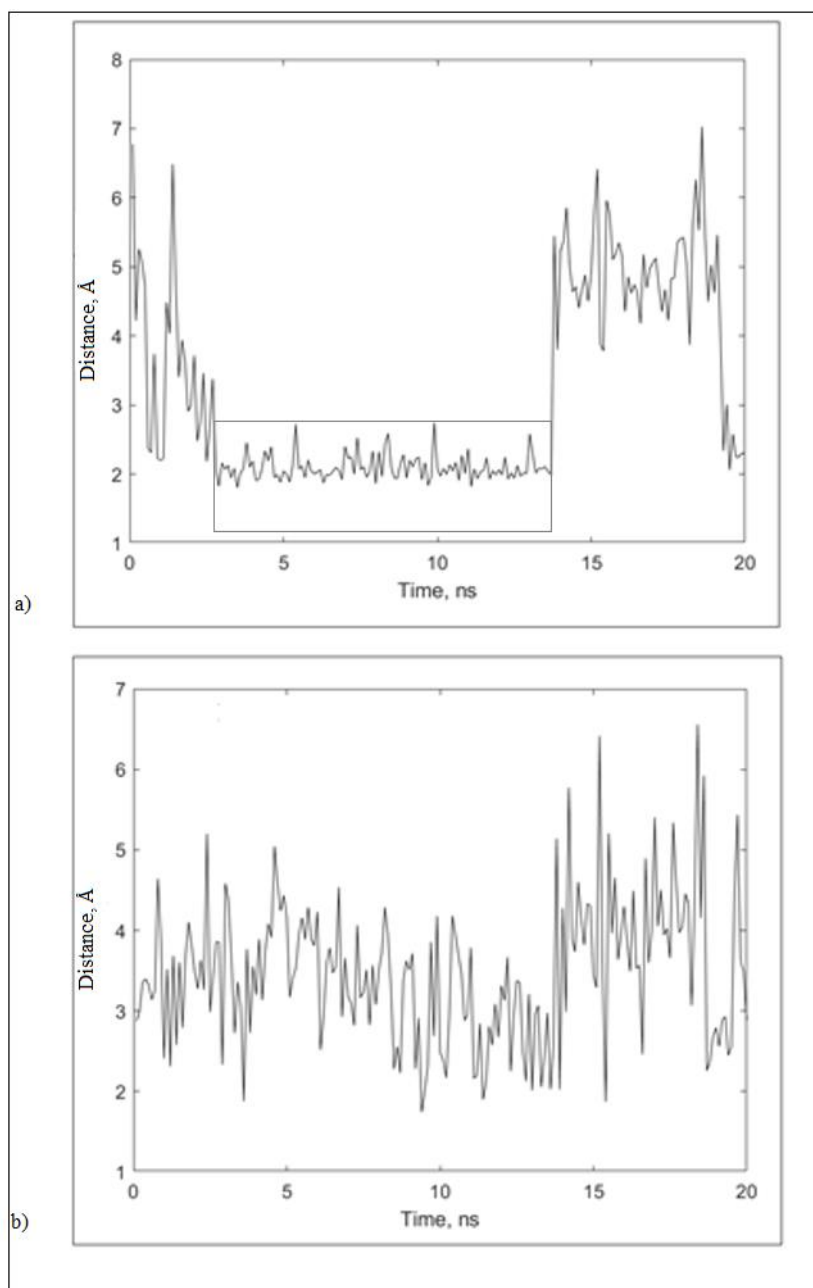


Figure 3.14. Distance vs. Time graphs of (a) K93 (328N5) - ASP (116HD22_116ND2) H-bond interaction (61% occupancy) (b) K93 (328H5_328N3) - LEU(43O) H-bond interaction - distance vs. time (16% occupancy)

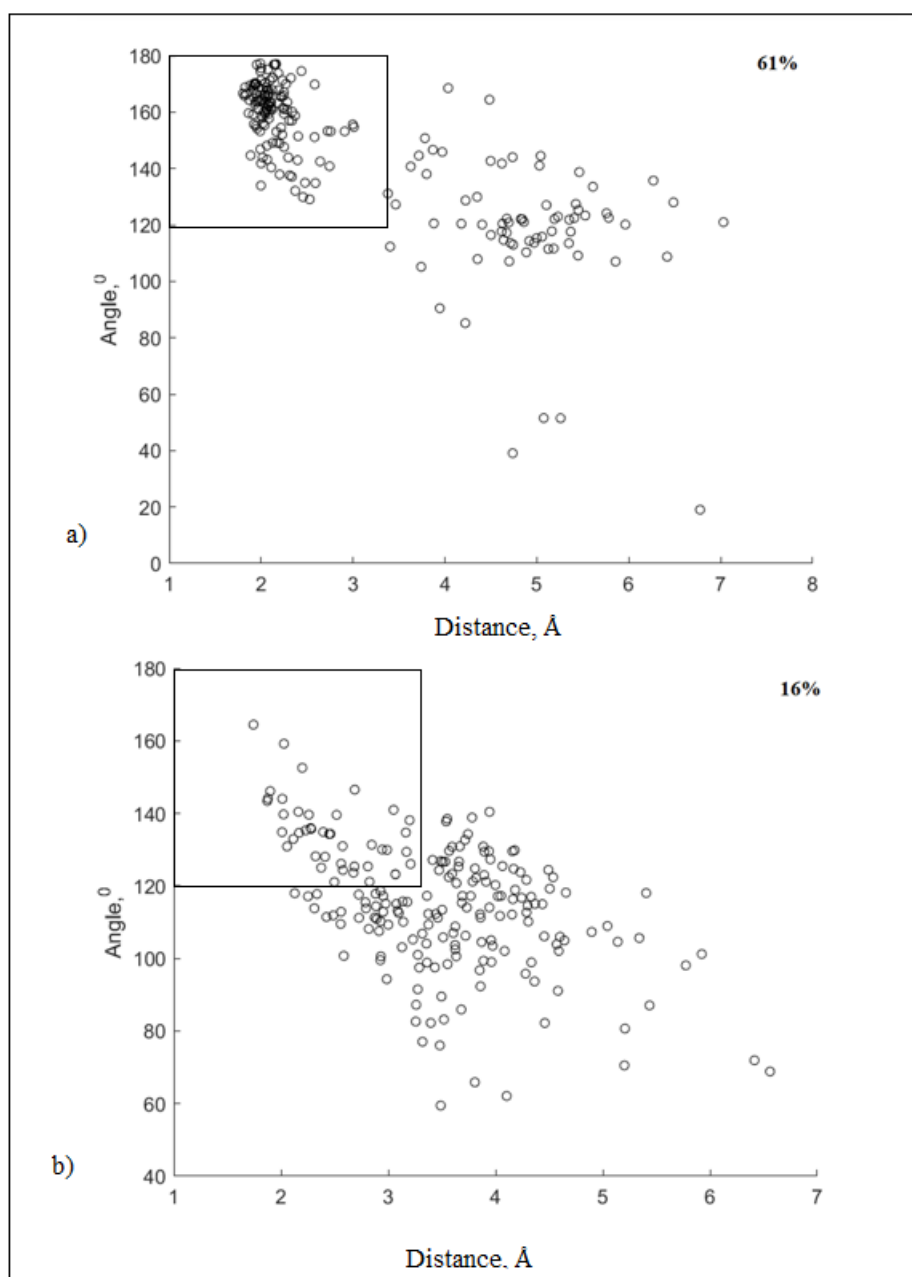


Figure 3.15. Angle vs. Distance graphs of (a) K93 (328N5) - ASP (116HD22_116ND2) H-bond interaction (61% occupancy) (b) K93 (328H5_328N3) - LEU(43O) H-bond interaction - angle vs. distance (16% occupancy)

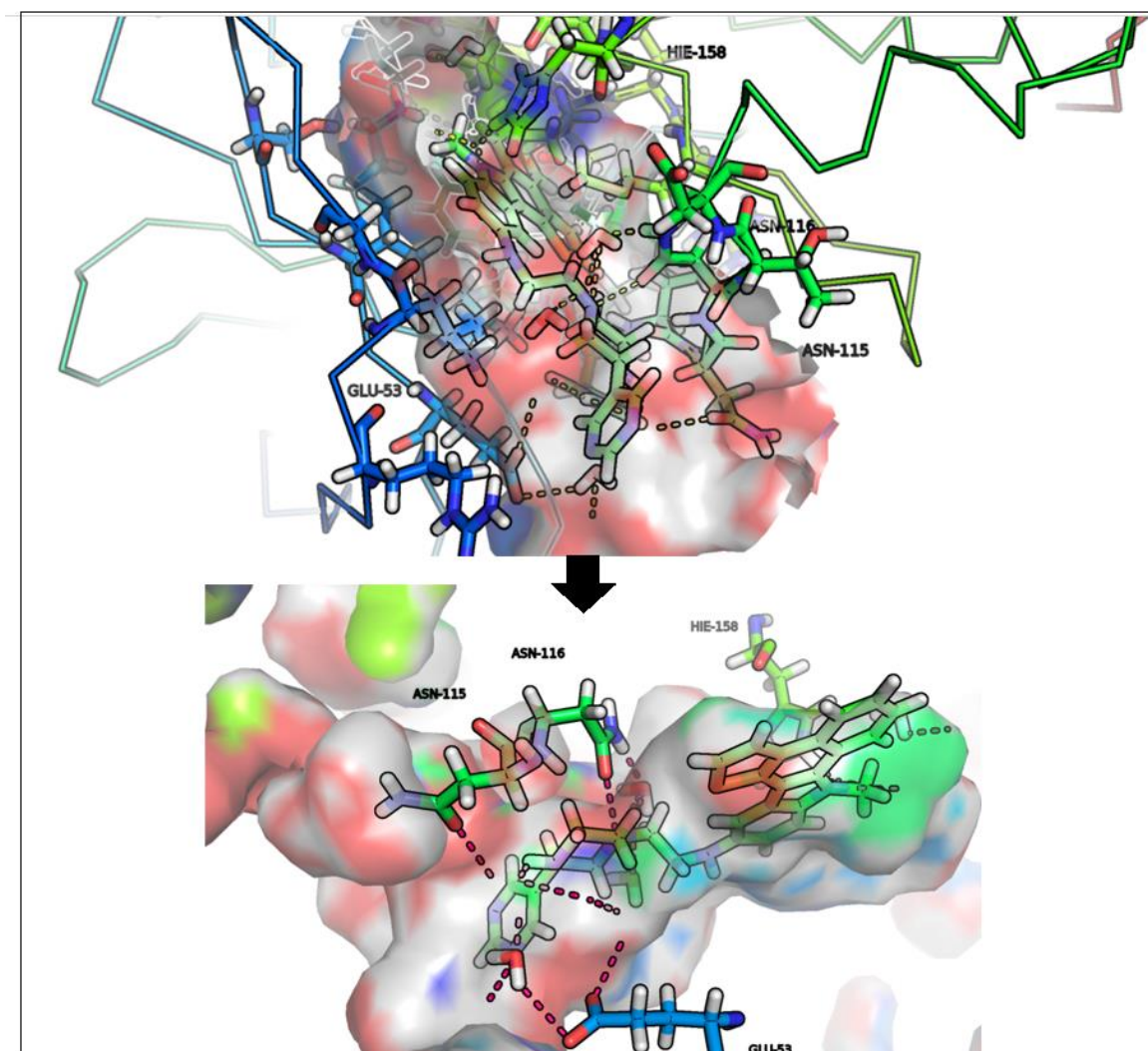


Figure 3.16. CK2-K93 polar contact with ASN116 before MD

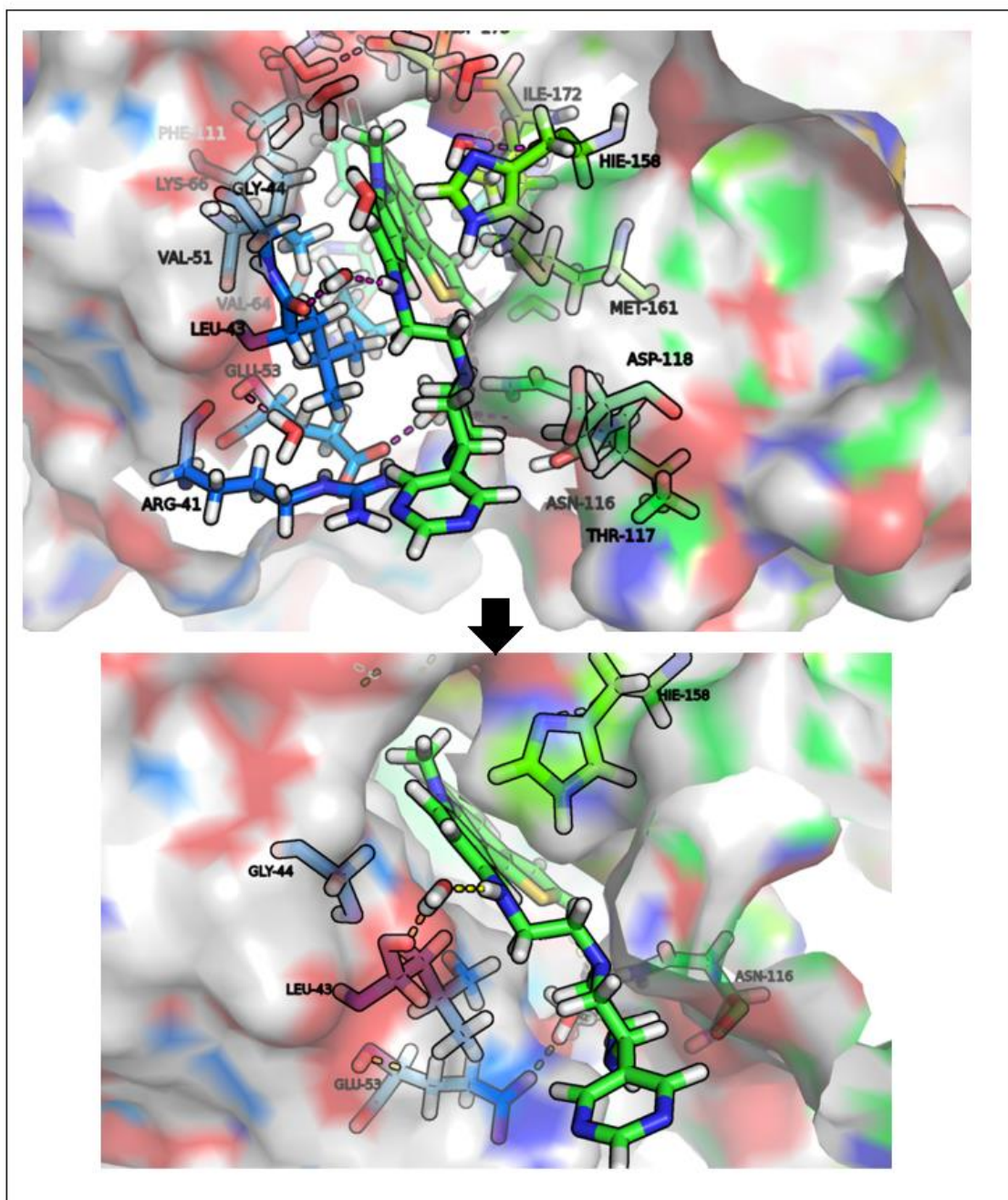


Figure 3.17. K93 interactions with CK2 active site amino acids after MD (1 ns)

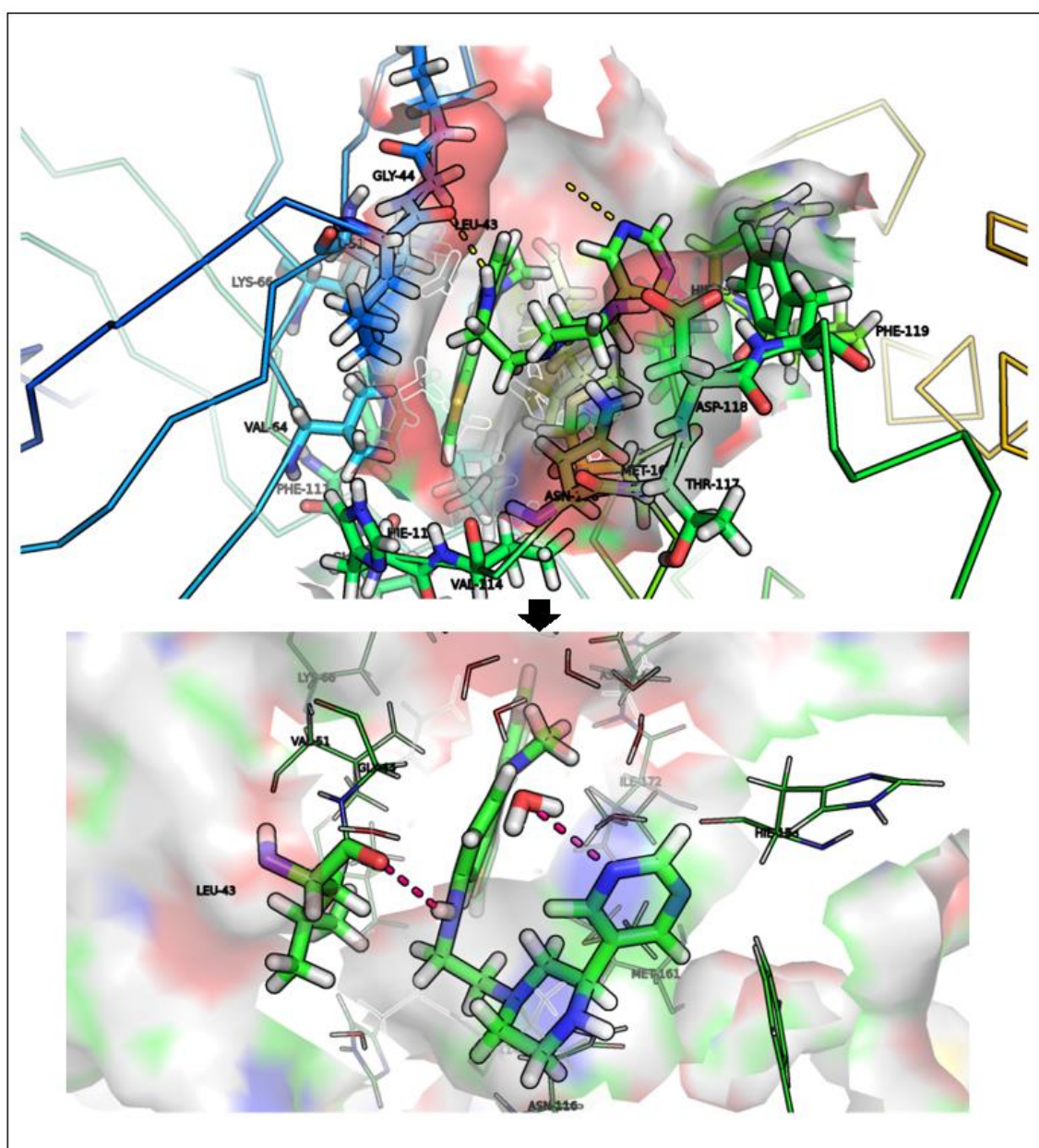


Figure 3.18. K93 interactions with CK2 active site amino acids after MD (7 ns)

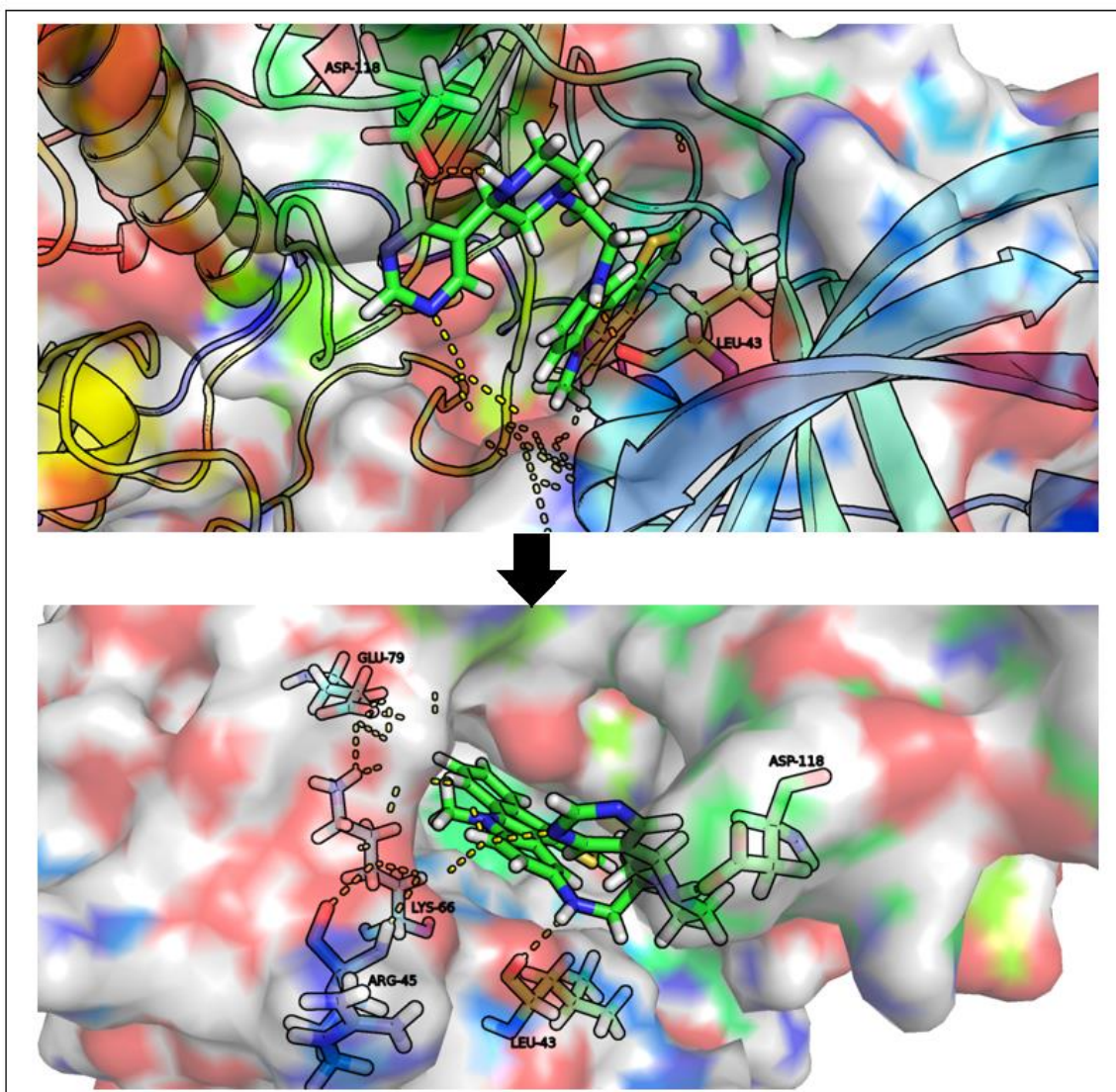


Figure 3.19. K93 interactions with CK2 active site amino acids after MD (12 ns)

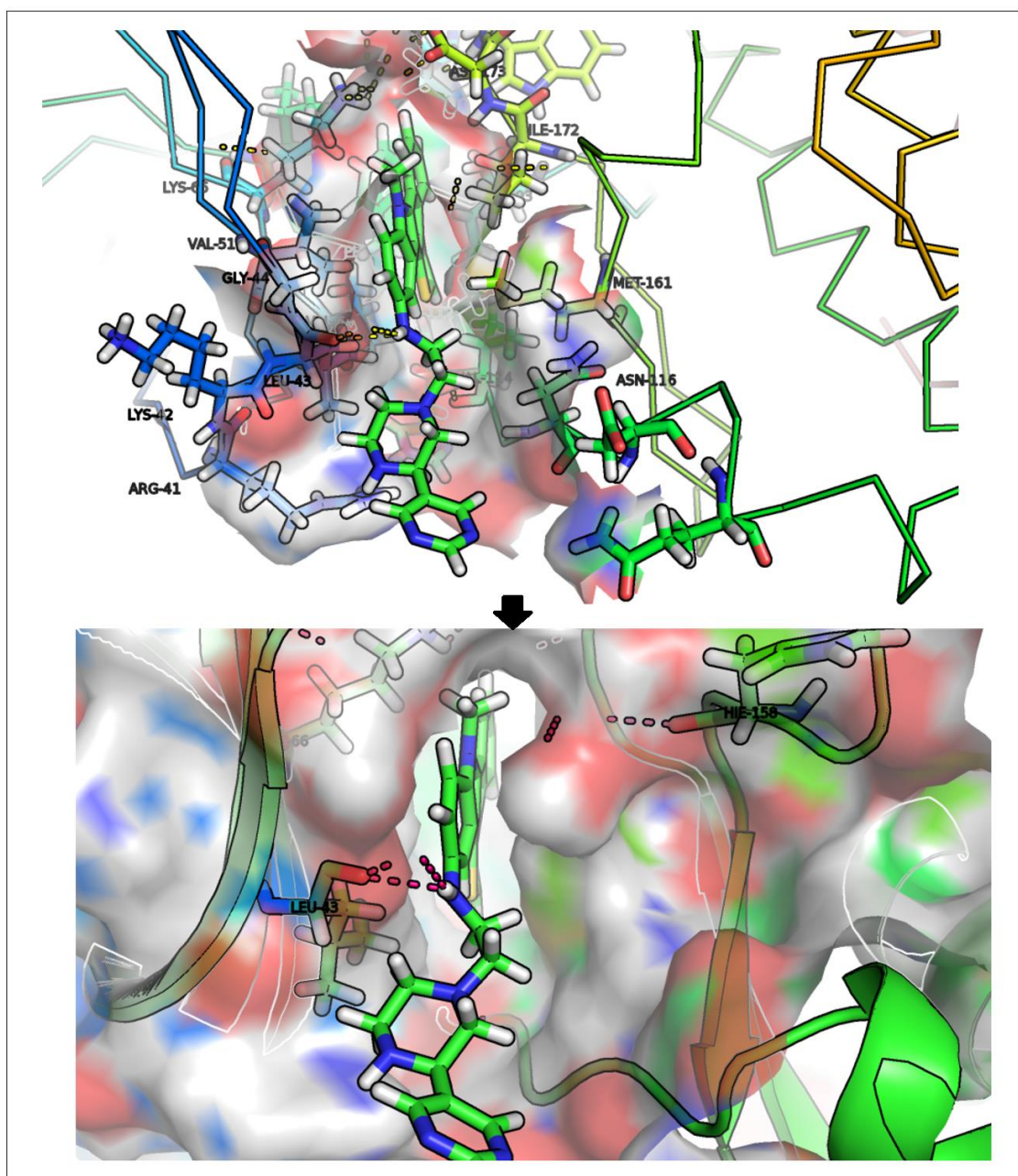


Figure 3.20. K93 interactions with CK2 active site amino acids after MD (20 ns)

4. CONCLUSION

In this thesis, thienobenzocarbazole derivatives were investigated as potential CK2 inhibitors using docking studies and molecular dynamics simulations. With the aim of improving the binding affinities of the 33 parent thienobenzocarbazoles that were found to have relatively lower docking scores compared to CX-4945, selected compounds were further structurally modified using computational derivatization tools generating a new library of thienobenzocarbazole derivatives. Docking and molecular dynamics evaluations of these new derivatives in a dynamic and solvated environment identified derivative “K93” as an alternative candidate for CK2 inhibition

Through our current results, we can confirm that a thienobenzocarbazole structure is an appropriate skeleton for the development of novel inhibitors for this kinase target. More compounds having this backbone with different functional groups should be designed with the aim of finding highly active CK2 inhibitors. After these structures are obtained in the experimental environment, the effects of the mentioned interactions on the human body should be investigated and this drug should be tested for further investigations.

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APPENDIX A

Table A.1. Docking Results for AK Molecules and CX-4945 [6]

Name	Mode	Affinity Score (kcal/mol)	Distance from the Best Mode rmsd l.b.	Distance from the Best Mode rmsd u.b.
AK-1	1	-5.7	0.000	0.000
	2	-5.5	5.881	8.641
	3	-5.2	5.990	8.372
	4	-5.1	5.757	8.905
	5	-4.5	10.672	12.335
	6	-4.5	11.041	12.871
	7	-4.4	10.694	11.638
	8	-4.3	11.236	13.471
	9	-4.3	10.575	11.630
AK-2	1	-5.7	0.000	0.000
	2	-5.4	2.356	4.029
	3	-5.1	3.702	5.690
	4	-5.1	3.714	8.831
	5	-5.1	3.224	5.948
	6	-5.0	2.724	4.981
	7	-5.0	8.765	10.013
	8	-4.7	2.492	8.300
	9	-4.7	8.853	13.128
AK-3	1	-5.6	0.000	0.000
	2	-5.5	3.516	6.095
	3	-5.4	2.000	3.793
	4	-5.2	3.012	8.450
	5	-5.1	3.640	6.026
	6	-5.1	3.767	8.394
	7	-5.1	3.229	6.256
	8	-5.0	8.655	9.933
	9	-4.7	9.225	13.383
AK-4	1	-5.6	0.000	0.000
	2	-5.5	4.456	8.372
	3	-4.9	12.000	14.896
	4	-4.9	11.817	14.487
	5	-4.7	11.567	13.995
	6	-4.7	3.285	7.456
	7	-4.7	12.525	16.761
	8	-4.6	10.950	15.083
	9	-4.6	10.928	12.713

Name	Mode	Affinity Score (kcal/mol)	Distance from the Best Mode rmsd l.b.	Distance from the Best Mode rmsd u.b.
AK-5	1	-5.7	0.000	0.000
	2	-5.2	11.431	14.519
	3	-5.1	3.186	8.287
	4	-5.0	11.572	14.817
	5	-4.8	11.256	13.932
	6	-4.7	1.851	3.462
	7	-4.7	13.849	18.070
	8	-4.7	10.958	12.852
	9	-4.6	11.082	12.675
AK-6	1	-5.1	0.000	0.000
	2	-4.8	11.743	14.557
	3	-4.7	10.373	13.375
	4	-4.7	11.592	14.394
	5	-4.7	10.812	12.684
	6	-4.6	10.856	12.450
	7	-4.6	11.201	13.397
	8	-4.5	11.439	14.791
	9	-4.5	8.960	12.390
AK-7	1	-5.7	0.000	0.000
	2	-5.5	2.972	7.574
	3	-5.0	11.230	13.082
	4	-4.8	1.818	3.488
	5	-4.8	21.987	23.973
	6	-4.7	11.683	14.113
	7	-4.7	11.492	14.589
	8	-4.7	3.534	8.022
	9	-4.7	10.859	13.025
AK-8	1	-5.4	0.000	0.000
	2	-5.0	8.489	12.924
	3	-5.0	6.215	13.570
	4	-4.9	1.814	3.392
	5	-4.9	8.001	12.172
	6	-4.7	8.086	11.278
	7	-4.6	3.925	6.200
	8	-4.5	8.740	12.869
	9	-4.4	8.614	13.063
	10	-4.4	8.614	13.063

Name	Mode	Affinity Score (kcal/mol)	Distance from the Best Mode rmsd l.b.	Distance from the Best Mode rmsd u.b.
AK-9	1	-6.4	0.000	0.000
	2	-6.2	4.244	7.184
	3	-6.2	4.964	6.911
	4	-5.9	3.067	4.759
	5	-5.9	4.986	10.398
	6	-5.8	5.412	10.263
	7	-5.8	2.082	3.733
	8	-5.7	6.195	9.196
	9	-5.4	8.083	9.486
AK-10	1	-7.4	0.000	0.000
	2	-5.5	6.214	10.108
	3	-5.2	5.913	9.826
	4	-4.7	10.918	11.818
	5	-4.7	10.871	12.884
	6	-4.5	10.901	12.919
	7	-4.5	17.158	18.658
AK-11	1	-6.7	0.000	0.000
	2	-5.4	3.301	10.466
	3	-5.1	2.480	9.891
	4	-5.1	15.299	18.155
	5	-4.9	6.011	9.775
	6	-4.8	5.979	9.763
	7	-4.8	9.370	11.013
	8	-4.6	10.935	13.169
	9	-4.4	10.935	12.855
AK-12	1	-5.6	0.000	0.000
	2	-5.5	1.045	1.322
	3	-5.1	2.100	6.163
	4	-5.1	6.717	11.173
	5	-5.0	10.827	13.876
	6	-4.9	8.027	10.614
	7	-4.8	6.739	11.802
	8	-4.6	9.544	12.980
	9	-4.2	7.950	10.873

Table A.1. Docking Results for AK Molecules and CX-4945 (cont'd)

Name	Mode	Affinity Score (kcal/mol)	Distance from the Best Mode rmsd l.b.	Distance from the Best Mode rmsd u.b.
AK-13	1	-5.4	0.000	0.000
	2	-5.1	9.348	11.845
	3	-5.0	9.631	12.603
	4	-5.0	7.961	12.017
	5	-4.8	3.038	6.018
	6	-4.7	10.448	13.797
	7	-4.5	9.868	13.205
	8	-4.5	11.697	15.171
	9	-4.4	9.883	13.674
AK-14	1	-6.7	0.000	0.000
	2	-5.7	6.002	9.943
	3	-5.7	6.356	9.939
	4	-5.5	5.841	9.792
	5	-5.1	5.978	9.072
	6	-5.0	10.687	11.632
	7	-4.8	10.785	13.218
	8	-4.7	10.498	12.062
	9	-4.7	10.708	13.166
AK-15	1	-4.5	0.000	0.000
	2	-4.4	2.781	5.165
	3	-4.3	2.560	6.570
	4	-4.3	10.221	12.772
	5	-4.3	2.020	6.724
	6	-4.2	1.502	6.458
	7	-4.2	1.937	3.720
	8	-4.2	2.579	5.601
	9	-4.2	2.259	7.321
AK-16	1	-5.7	0.000	0.000
	2	-5.6	2.277	4.374
	3	-5.3	3.616	8.683
	4	-4.8	1.419	2.071
	5	-4.7	8.885	10.358
	6	-4.6	10.023	14.28
	7	-4.4	9.665	13.414
	8	-4.4	19.090	21.490
	9	-4.3	5.490	10.762

Table A.1. Docking Results for AK Molecules and CX-4945 (cont'd)

Name	Mode	Affinity Score (kcal/mol)	Distance from the Best Mode rmsd l.b.	Distance from the Best Mode rmsd u.b.
AK-17	1	-5.5	0.000	0.000
	2	-5.3	3.595	8.704
	3	-5.3	1.976	8.072
	4	-5.2	1.786	3.548
	5	-4.9	8.786	12.487
	6	-4.9	7.552	13.342
	7	-4.8	6.611	12.366
	8	-4.8	11.961	16.204
	9	-4.7	13.221	17.780
AK-18	1	-5.5	0.000	0.000
	2	-5.2	1.759	3.573
	3	-5.2	12.625	16.242
	4	-4.8	9.008	12.606
	5	-4.8	12.340	16.739
	6	-4.7	8.015	13.419
	7	-4.7	6.858	12.206
	8	-4.7	10.856	14.687
	9	-4.6	8.595	12.351
AK-19	1	-6.6	0.000	0.000
	2	-5.6	6.718	9.014
	3	-5.4	7.294	10.804
	4	-5.0	6.788	9.305
	5	-4.7	18.804	19.643
	6	-4.5	18.520	19.543
	7	-4.5	18.657	19.564
	8	-4.4	11.252	12.798
	9	-4.4	11.293	12.130
AK-20	1	-5.3	0.000	0.000
	2	-4.9	5.766	8.880
	3	-4.8	1.487	6.101
	4	-4.5	10.914	14.267
	5	-4.5	11.512	16.276
	6	-4.3	10.875	13.962
	7	-4.1	3.644	7.171
	8	-4.0	6.134	8.073
	9	-3.9	5.253	7.709

Table A.1. Docking Results for AK Molecules and CX-4945 (cont'd)

Name	Mode	Affinity Score (kcal/mol)	Distance from the Best Mode rmsd l.b.	Distance from the Best Mode rmsd u.b.
AK-21	1	-5.5	0.000	0.000
	2	-5.5	2.987	5.631
	3	-5.4	2.929	5.484
	4	-5.4	3.833	8.160
	5	-5.2	2.057	3.761
	6	-4.8	1.767	3.393
	7	-4.6	9.881	11.756
	8	-4.6	1.912	3.461
	9	-4.5	10.136	14.198
AK-22	1	-5.0	0.000	0.000
	2	-4.9	6.577	8.333
	3	-4.9	7.957	11.757
	4	-4.7	7.118	10.662
	5	-4.6	8.696	14.464
	6	-4.6	6.903	9.920
	7	-4.5	12.748	16.611
	8	-4.5	8.607	13.746
	9	-4.5	6.657	9.621
AK-23	1	-5.7	0.000	0.000
	2	-5.5	4.114	8.270
	3	-5.2	3.772	7.775
	4	-5.1	4.646	8.333
	5	-5.0	12.255	14.578
	6	-4.9	3.430	7.614
	7	-4.8	10.526	12.622
	8	-4.8	10.151	12.737
	9	-4.7	10.123	14.218
AK-24	1	-5.1	0.000	0.000
	2	-4.8	10.393	15.149
	3	-4.7	10.094	14.158
	4	-4.7	6.923	11.711
	5	-4.7	9.146	11.723
	6	-4.6	9.940	11.605
	7	-4.5	9.039	11.515
	8	-4.4	7.898	11.929
	9	-4.4	2.290	5.322

Table A.1. Docking Results for AK Molecules and CX-4945 (cont'd)

Name	Mode	Affinity Score (kcal/mol)	Distance from the Best Mode rmsd l.b.	Distance from the Best Mode rmsd u.b.
AK-25	1	-6.8	0.000	0.000
	2	-5.5	5.796	9.586
	3	-5.5	5.816	9.063
	4	-5.3	5.452	9.409
	5	-4.6	10.777	12.804
	6	-4.6	10.955	12.937
	7	-4.5	10.932	12.238
	8	-4.4	10.362	13.005
	9	-4.3	11.357	13.570
AK-26	1	-5.5	0.000	0.000
	2	-5.2	2.843	5.590
	3	-5.2	1.879	6.056
	4	-5.1	1.941	6.716
	5	-4.9	2.074	3.009
	6	-4.9	1.118	1.603
	7	-4.8	2.332	5.580
	8	-4.6	1.928	5.028
	9	-4.3	10.787	15.416
AK-27	1	-6.1	0.000	0.000
	2	-5.9	6.041	9.305
	3	-5.7	5.414	9.599
	4	-5.6	5.847	8.791
	5	-5.0	5.301	9.343
	6	-4.9	10.758	11.978
	7	-4.9	10.637	13.399
	8	-4.8	10.290	11.546
	9	-4.6	10.808	12.147
AK-28	1	-5.8	0.000	0.000
	2	-5.4	3.340	6.524
	3	-5.0	11.045	13.581
	4	-4.9	10.899	14.666
	5	-4.9	9.107	12.249
	6	-4.8	9.197	12.566
	7	-4.7	6.402	9.364
	8	-4.5	10.508	13.846
	9	-4.4	3.235	6.714

Table A.1. Docking Results for AK Molecules and CX-4945 (cont'd)

Name	Mode	Affinity Score (kcal/mol)	Distance from the Best Mode rmsd l.b.	Distance from the Best Mode rmsd u.b.
AK-29	1	-7.0	0.000	0.000
	2	-5.8	6.122	9.386
	3	-4.9	10.645	13.187
	4	-4.8	10.853	12.067
	5	-4.7	10.597	11.776
	6	-4.7	12.455	14.568
	7	-4.6	5.205	10.525
	8	-4.6	10.864	13.284
	9	-4.4	4.908	10.484
AK-30	1	-5.0	0.000	0.000
	2	-4.5	11.437	13.259
	3	-4.4	11.970	15.877
	4	-4.4	2.396	7.101
	5	-4.3	11.169	13.524
	6	-4.2	9.445	12.819
	7	-4.2	11.482	14.402
	8	-4.2	10.394	13.28
	9	-4.2	12.396	15.108
AK-31	1	-5.4	0.000	0.000
	2	-5.3	1.681	3.354
	3	-5.2	12.338	15.933
	4	-5.0	4.699	8.463
	5	-4.8	9.125	12.726
	6	-4.7	8.641	13.689
	7	-4.6	10.700	14.665
	8	-4.5	8.072	9.471
	9	-4.5	8.281	11.171
AK-32	1	-5.4	0.000	0.000
	2	-5.0	3.764	7.914
	3	-4.9	9.067	12.922
	4	-4.9	9.370	14.469
	5	-4.7	7.198	12.965
	6	-4.6	7.224	12.375
	7	-4.6	8.269	11.033
	8	-4.5	8.486	11.168
	9	-4.4	9.248	13.664

Table A.1. Docking results for AK molecules and CX-4945 (cont'd)

Name	Mode	Affinity Score (kcal/mol)	Distance from the Best Mode rmsd l.b.	Distance from the Best Mode rmsd u.b.
AK-33	1	-5.8	0.000	0.000
	2	-5.7	3.657	7.891
	3	-5.7	3.434	8.224
	4	-5.2	2.865	8.026
	5	-4.8	9.876	14.114
	6	-4.8	2.458	8.037
	7	-4.7	12.062	15.902
	8	-4.6	11.916	16.434
	9	-4.6	11.743	14.470
CX-4945	1	-10.3	0.000	0.000

Table A.2. MOE medchem results for AK9

Rule	mseq	RMSD	rsynth
C[NOS]C_to_C(C=O)C.rxn	1	0.089015141	0.25806451
C[NOS]C_to_C(C=O)C.rxn	2	0.12612703	0.29032257
C[NOS]C_to_C(C=O)C.rxn	3	0.11159145	0.32258064
C[NOS]C_to_C(S=O)C.rxn	4	0.15824991	0.3548387
C[NOS]C_to_C(S=O)C.rxn	5	0.098220527	0.3548387
C[NOS]C_to_C(S=O)C.rxn	6	0.20005901	0.32258064
C[NOS]C_to_C(SO2)C.rxn	7	0.16427544	0.25
C[NOS]C_to_C(SO2)C.rxn	8	0.10973577	0.28125
C[NOS]C_to_C(SO2)C.rxn	9	0.18786822	0.34375
C[NO]C_to_CSC.rxn	10	0.14224824	0.30000001
C[NO]C_to_CSC.rxn	11	0.10291319	0.30000001
C[NO]C_to_CSC.rxn	12	0.12486414	0.30000001
C[NO]C_to_CSC.rxn	13	0.099382296	0.30000001
C[NS]C_to_COC.rxn	14	0.10051534	0.30000001
C[NS]C_to_COC.rxn	15	0.10595518	0.30000001
N14Subtraction.rxn	16	0.065926954	0.30000001
N14Subtraction.rxn	17	0.10228274	0.30000001
alkane_to_alkyne.rxn	18	0.22592047	0.33333334
amine34_to_alkane.rxn	19	0.25731942	0.26666668
amine_to_amide.rxn	20	0.24774575	0.25806451
amine_to_amide.rxn	21	0.11758096	0.22580644
amine_to_amide.rxn	22	0.22845218	0.22580644
amine_to_amide.rxn	23	0.1271929	0.22580644
aro5_N2.rxn	24	0.098125003	0.36666667
aro5_N3.rxn	25	0.10186297	0.33333334
aro5_N_13_switch.rxn	26	0.12928589	0.40000001
aro5_N_13_switch.rxn	27	0.085149996	0.56666666
aro5_[OS]_13_switch.rxn	28	0.11845361	0.30000001
aro5_[OS]_13_switch.rxn	29	0.10296609	0.30000001
aro5_[OS]_13_switch.rxn	30	0.12453032	0.30000001
benzene_to_pyridine.rxn	31	0.16128169	0.36666667
benzene_to_pyridine.rxn	32	0.13629614	0.36666667
benzene_to_pyridine.rxn	33	0.16114689	0.36666667
benzene_to_pyridine.rxn	34	0.098200209	0.33333334
benzene_to_pyridine.rxn	35	0.12406504	0.36666667
benzene_to_pyridine.rxn	36	0.098031186	0.33333334
benzene_to_pyridine.rxn	37	0.099303037	0.36666667
ethane_to_ethene.rxn	38	0.25576475	0.33333334
hom_[NOS]CH2.rxn	39	0.38130575	0.32258064
hom_[NOS]CH2.rxn	40	0.26930556	0.32258064

Table A.2. MOE medchem results for AK9 (cont'd)

Rule	mseq	RMSD	rsynth
hom_[NOS]CH2_inv.rxn	41	0.40567809	0.27586207
methylate_N.rxn	42	0.37584525	0.32258064
methylate_N.rxn	43	0.12799229	0.32258064
methylate_N_inv.rxn	44	0.12658535	0.27586207
phenyl_to_thiophene_2.rxn	45	0.30696923	0.37931034
phenyl_to_thiophene_2.rxn	46	0.37656465	0.37931034
phenyl_to_thiophene_2.rxn	47	0.3859486	0.34482759
phenyl_to_thiophene_2.rxn	48	0.15254328	0.31034482
phenyl_to_thiophene_2.rxn	49	0.18818417	0.31034482
phenyl_to_thiophene_2.rxn	50	0.19461817	0.31034482
phenyl_to_thiophene_2.rxn	51	0.17239015	0.31034482
phenyl_to_thiophene_2.rxn	52	0.1867127	0.31034482
phenyl_to_thiophene_2.rxn	53	0.18641655	0.31034482
phenyl_to_thiophene_2.rxn	54	0.15495977	0.31034482
phenyl_to_thiophene_2.rxn	55	0.1796298	0.31034482
phenyl_to_thiophene_2.rxn	56	0.18899497	0.31034482
phenyl_to_thiophene_3.rxn	57	0.46060622	0.31034482
phenyl_to_thiophene_3.rxn	58	0.43256196	0.31034482
phenyl_to_thiophene_3.rxn	59	0.43024451	0.31034482
phenyl_to_thiophene_3.rxn	60	0.41709274	0.37931034
phenyl_to_thiophene_3.rxn	61	0.35011962	0.37931034
phenyl_to_thiophene_3.rxn	62	0.34300652	0.37931034
phenyl_to_thiophene_4.rxn	63	0.46128571	0.44827586
phenyl_to_thiophene_4.rxn	64	0.42850834	0.44827586
phenyl_to_thiophene_4.rxn	65	0.43300071	0.41379312
piperidine_to_pyrrolidineN_1.rxn	66	0.2593202	0.30000001
piperidine_to_pyrrolidineN_1.rxn	67	0.27425674	0.30000001
piperidine_to_pyrrolidineN_2.rxn	68	0.26043952	0.32258064
piperidine_to_pyrrolidineN_2.rxn	69	0.40844122	0.32258064
thiophene[NOS]_to_phenyl_2.rxn	70	0.43807352	0.25806451
thiophene[NOS]_to_phenyl_2.rxn	71	0.45531124	0.25806451
thiophene[NOS]_to_phenyl_2.rxn	72	0.21770816	0.29032257

Table A.2. MOE medchem results for AK10

Rule	mseq	RMSD	rsynth
C[NOS]C_to_C(C=O)C.rxn	1	0.12272468	0.16
C[NOS]C_to_C(S=O)C.rxn	2	0.14985171	0.23999999
C[NOS]C_to_C(SO2)C.rxn	3	0.19070208	0.26923078
C[NS]C_to_COC.rxn	4	0.16493675	0.16666667
C[NS]C_to_COC.rxn	5	0.15786168	0.16666667
amine34_to_alkane.rxn	6	0.2667093	0.125
amine34_to_alkane.rxn	7	0.10896478	0.041666668
aro5_N3.rxn	8	0.13513854	0.20833333
aro5_N_13_switch.rxn	9	0.14971876	0.25
aro5_N_13_switch.rxn	10	0.093590833	0.45833334
aro5_[OS]_13_switch.rxn	11	0.11977259	0.16666667
aro5_[OS]_13_switch.rxn	12	0.10596975	0.16666667
aro5_[OS]_13_switch.rxn	13	0.2108991	0.16666667
benzene_to_pyridine.rxn	14	0.096144259	0.29166666
benzene_to_pyridine.rxn	15	0.15837929	0.20833333
benzene_to_pyridine.rxn	16	0.1360648	0.20833333
benzene_to_pyridine.rxn	17	0.16221657	0.29166666
benzene_to_pyridine.rxn	18	0.10793694	0.20833333
benzene_to_pyridine.rxn	19	0.14815225	0.25
benzene_to_pyridine.rxn	20	0.13880275	0.20833333
benzene_to_pyridine.rxn	21	0.1330941	0.25
hom_[NOS]CH2.rxn	22	0.3144471	0.2
hom_[NOS]CH2.rxn	23	0.2524263	0.2
methylate_N_inv.rxn	24	0.14649595	0.13043478
methylate_N_inv.rxn	25	0.11828435	0.13043478
phenyl_to_thiophene_2.rxn	26	0.161276	0.17391305
phenyl_to_thiophene_2.rxn	27	0.19137247	0.17391305
phenyl_to_thiophene_2.rxn	28	0.18737745	0.17391305
phenyl_to_thiophene_2.rxn	29	0.16669594	0.30434781
phenyl_to_thiophene_2.rxn	30	0.1941282	0.30434781
phenyl_to_thiophene_2.rxn	31	0.21835195	0.30434781
phenyl_to_thiophene_2.rxn	32	0.19902039	0.2173913
phenyl_to_thiophene_2.rxn	33	0.18709886	0.2173913
phenyl_to_thiophene_2.rxn	34	0.19143265	0.17391305
phenyl_to_thiophene_2.rxn	35	0.18495627	0.17391305
phenyl_to_thiophene_2.rxn	36	0.26645696	0.17391305
phenyl_to_thiophene_2.rxn	37	0.26379088	0.17391305
phenyl_to_thiophene_2.rxn	38	0.19958292	0.17391305

Table A.2. MOE medchem results for AK10 (cont'd)

phenyl_to_thiophene_2.rxn	39	0.23164757	0.17391305
phenyl_to_thiophene_2.rxn	40	0.23898919	0.17391305
phenyl_to_thiophene_2.rxn	41	0.21263742	0.17391305
phenyl_to_thiophene_2.rxn	42	0.23066537	0.17391305
phenyl_to_thiophene_2.rxn	43	0.23015402	0.17391305
thiophene[NOS]_to_phenyl_2.rxn	44	0.43999273	0.12
thiophene[NOS]_to_phenyl_2.rxn	45	0.45705095	0.12
thiophene[NOS]_to_phenyl_2.rxn	46	0.36524171	0.12
thiophene[NOS]_to_phenyl_3.rxn	47	0.43052778	0.12

Table A.3. AK9 & AK10 derivatives with docking scores

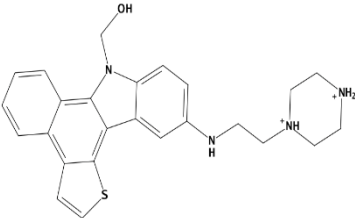
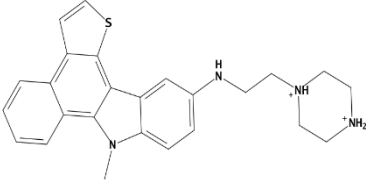
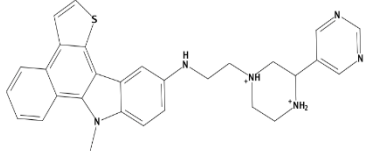
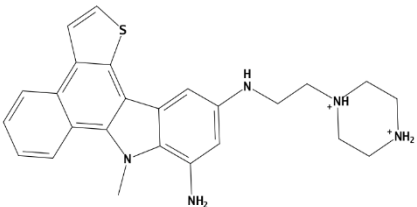
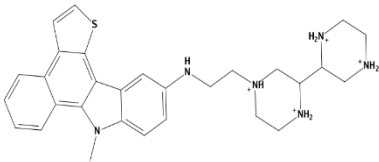
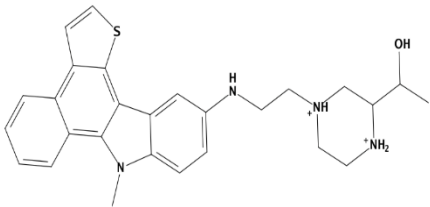
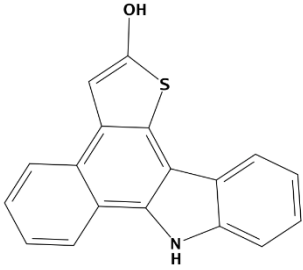
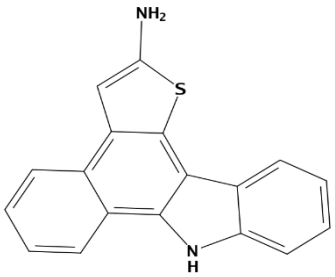
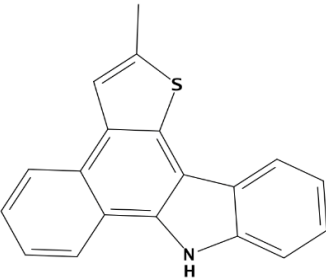
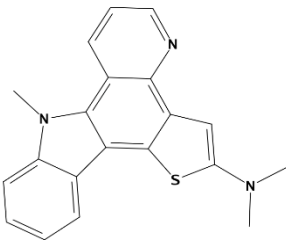
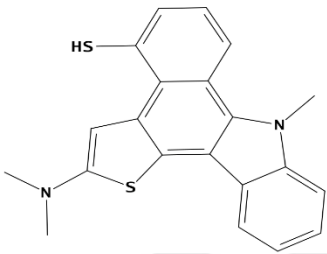
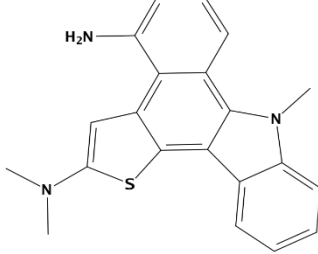
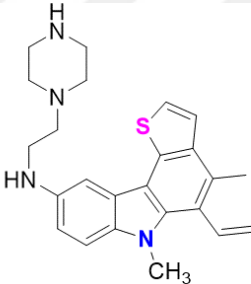
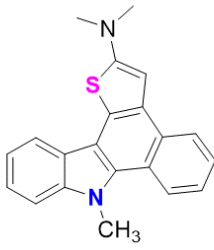
	
AK9MC3-22-1 (5.0 kcal/mol)	AK9MC3-22-2 (5.1 kcal/mol)
	
AK9-addnglig-202 (K91) (-5.8 kcal/mol)	AK9MC3-1 (5.2 kcal/mol)
	
AK9addnglig-104 (K93) (-6.0 kcal/mol)	AK9addnglig-65 (K92) (-5.6 kcal/mol)
	
AK10MC6-3 (K07) (-5.4 kcal/mol)	AK10MC6-2 (K06) (-5.5 kcal/mol)

Table A.3. AK9 & AK10 derivatives with docking scores (cont'd)

	
AK10MC6-1 (-5.0 kcal/mol)	AK10MC1-3 (-5.3 kcal/mol)
	
AK10MC3-4 (-5.0 kcal/mol)	AK10MC1-1 (K01) (-5.4 kcal/mol)
	
AK-9 (-5.8 kcal/mol)	AK-10 (M10) (-5.3 kcal/mol)

APPENDIX B

Table B.1 K93 Analysis results with active residues (20 nano-second)

atom#	res@atom	atom#2	res@atom3	atom#4	res@atom5	%occupied	distance	angle	lifetime	maxocc
5505	:328@N5	1929	:116@HD22	1927	:116@ND2	61.00	3.062 (0.15)	19.84 (11.20)	2.4 (8.1)	44
1926	:116@OD1	1933	:117@H	1932	:117@N	55.50	3.020 (0.18)	44.63 (9.25)	0.7 (2.0)	16
1912	:115@OD1	1919	:116@H	1918	:116@N	37.00	3.030 (0.16)	47.43 (7.42)	0.5 (1.4)	8
1903	:114@O	1919	:116@H	1918	:116@N	18.50	3.069 (0.18)	43.05 (7.44)	0.5 (1.1)	5
715	:43@O	5513	:328@H5	5512	:328@N3	16.00	3.086 (0.21)	44.47 (10.21)	0.3 (0.7)	4
1340	:79@OE2	1080	:66@HZ1	1079	:66@NZ	12.00	2.804 (0.14)	25.65 (12.01)	0.3 (0.6)	3
1340	:79@OE2	1082	:66@HZ3	1079	:66@NZ	11.50	2.812 (0.12)	28.73 (14.47)	0.3 (0.7)	3
1955	:118@OD2	5498	:328@H6	5497	:328@N6	11.00	3.097 (0.22)	23.81 (15.32)	0.4 (1.0)	6
1339	:79@OE1	1082	:66@HZ3	1079	:66@NZ	10.00	2.931 (0.21)	42.16 (13.54)	0.2 (0.6)	3
1339	:79@OE1	1080	:66@HZ1	1079	:66@NZ	9.50	2.942 (0.16)	36.82 (14.67)	0.3 (0.6)	2
1954	:118@OD1	5498	:328@H6	5497	:328@N6	9.00	2.954 (0.14)	22.82 (11.03)	0.4 (1.0)	4
2908	:173@OD1	1081	:66@HZ2	1079	:66@NZ	8.00	2.811 (0.13)	24.88 (8.75)	0.3 (0.7)	3
1340	:79@OE2	1081	:66@HZ2	1079	:66@NZ	7.50	2.823 (0.12)	24.41 (14.41)	0.3 (0.6)	3
1339	:79@OE1	1081	:66@HZ2	1079	:66@NZ	6.00	2.991 (0.23)	33.05 (16.53)	0.2 (0.5)	2
5505	:328@N5	1919	:116@H	1918	:116@N	5.50	3.308 (0.14)	53.57 (5.64)	0.3 (0.8)	4
2909	:173@OD2	1082	:66@HZ3	1079	:66@NZ	5.00	2.752 (0.10)	25.50 (10.36)	0.2 (0.5)	2
2908	:173@OD1	1082	:66@HZ3	1079	:66@NZ	5.00	2.791 (0.08)	25.73 (11.81)	0.3 (0.6)	2
2908	:173@OD1	1080	:66@HZ1	1079	:66@NZ	3.50	2.890 (0.16)	30.55 (9.75)	0.3 (0.6)	2
2909	:173@OD2	1080	:66@HZ1	1079	:66@NZ	3.00	2.732 (0.09)	19.46 (10.84)	0.2 (0.4)	1
1945	:117@O	1928	:116@HD21	1927	:116@ND2	3.00	3.054 (0.29)	54.88 (5.20)	0.2 (0.4)	1
2909	:173@OD2	1081	:66@HZ2	1079	:66@NZ	2.50	2.759 (0.09)	31.28 (10.95)	0.2 (0.4)	1
5488	:328@N2	669	:41@HH12	667	:41@NH1	1.00	3.362 (0.08)	42.86 (16.55)	0.2 (0.4)	1
5485	:328@N1	2641	:158@HE2	2640	:158@NE2	1.00	3.033 (0.13)	33.32 (0.96)	0.2 (0.4)	1
5485	:328@N1	672	:41@HH22	670	:41@NH2	1.00	3.054 (0.22)	53.79 (0.84)	0.2 (0.4)	1
5485	:328@N1	669	:41@HH12	667	:41@NH1	1.00	3.060 (0.12)	44.67 (8.86)	0.2 (0.4)	1
1926	:116@OD1	1919	:116@H	1918	:116@N	1.00	3.036 (0.03)	54.96 (0.69)	0.4 (0.8)	2
804	:49@OG	1082	:66@HZ3	1079	:66@NZ	1.00	2.952 (0.06)	54.39 (1.63)	0.2 (0.4)	1
5488	:328@N2	672	:41@HH22	670	:41@NH2	0.50	3.270 (0.00)	31.07 (0.00)	0.2 (0.4)	1
1945	:117@O	1929	:116@HD22	1927	:116@ND2	0.50	3.242 (0.00)	27.84 (0.00)	0.2 (0.4)	1
1931	:116@O	1943	:117@HG1	1942	:117@OG1	0.50	2.959 (0.00)	15.13 (0.00)	0.2 (0.4)	1
1903	:114@O	1929	:116@HD22	1927	:116@ND2	0.50	2.850 (0.00)	8.58 (0.00)	0.2 (0.4)	1

Table B.2 K91 Analysis results with active residues (20 nano-second)

atom#	res@atom	atom#2	res@atom3	atom#4	res@atom5	%occupied	distance	angle	lifetime	maxocc
1912	:115@OD1	1919	:116@H	1918	:116@N	58.50	2.997 (0.19)	44.92 (8.16)	0.6 (2.0)	14
5506	:328@N4	1928	:116@HD21	1927	:116@ND2	39.50	3.081 (0.17)	25.75 (13.97)	1.2 (3.7)	21
1340	:79@OE2	1082	:66@HZ3	1079	:66@NZ	34.00	2.847 (0.15)	28.22 (14.34)	0.4 (1.0)	6
1340	:79@OE2	1080	:66@HZ1	1079	:66@NZ	33.50	2.912 (0.17)	29.78 (12.97)	0.3 (0.8)	5
1340	:79@OE2	1081	:66@HZ2	1079	:66@NZ	29.00	2.907 (0.17)	32.50 (11.31)	0.4 (0.9)	8
1339	:79@OE1	1081	:66@HZ2	1079	:66@NZ	28.00	2.823 (0.13)	36.46 (12.56)	0.3 (0.8)	6
1339	:79@OE1	1080	:66@HZ1	1079	:66@NZ	28.00	2.842 (0.17)	38.69 (11.67)	0.3 (0.6)	5
715	:43@O	5514	:328@H6	5513	:328@N2	27.00	3.081 (0.20)	40.79 (13.44)	0.3 (0.7)	4
1339	:79@OE1	1082	:66@HZ3	1079	:66@NZ	25.50	2.849 (0.15)	40.18 (15.05)	0.3 (0.8)	5
2909	:173@OD2	1082	:66@HZ3	1079	:66@NZ	24.50	2.757 (0.11)	25.21 (10.87)	0.3 (0.8)	5
2909	:173@OD2	1081	:66@HZ2	1079	:66@NZ	24.50	2.784 (0.11)	22.92 (10.63)	0.4 (1.0)	6
2909	:173@OD2	1080	:66@HZ1	1079	:66@NZ	21.50	2.796 (0.13)	22.36 (11.62)	0.4 (1.0)	8
2908	:173@OD1	1081	:66@HZ2	1079	:66@NZ	11.00	2.785 (0.14)	26.71 (10.85)	0.3 (0.8)	4
1903	:114@O	1929	:116@HD22	1927	:116@ND2	11.00	3.064 (0.19)	21.54 (10.41)	0.7 (2.1)	10
2908	:173@OD1	1082	:66@HZ3	1079	:66@NZ	10.50	2.787 (0.12)	25.28 (11.53)	0.2 (0.5)	2
2908	:173@OD1	1080	:66@HZ1	1079	:66@NZ	9.00	2.839 (0.19)	27.40 (11.43)	0.3 (0.6)	2
1945	:117@O	1929	:116@HD22	1927	:116@ND2	9.00	3.033 (0.21)	32.82 (12.83)	0.7 (1.7)	7
1926	:116@OD1	1933	:117@H	1932	:117@N	8.50	2.915 (0.17)	41.25 (8.37)	0.4 (1.2)	7
5487	:328@N1	1947	:118@H	1946	:118@N	2.50	3.294 (0.13)	22.99 (11.79)	0.2 (0.5)	2
2686	:161@SD	1928	:116@HD21	1927	:116@ND2	2.00	3.343 (0.05)	39.72 (15.72)	0.2 (0.4)	1
1926	:116@OD1	1919	:116@H	1918	:116@N	1.50	2.850 (0.08)	53.25 (0.31)	0.2 (0.4)	1
2686	:161@SD	1929	:116@HD22	1927	:116@ND2	1.00	3.370 (0.13)	38.81 (17.68)	0.2 (0.4)	1
1931	:116@O	1943	:117@HG1	1942	:117@OG1	1.00	3.180 (0.22)	40.33 (13.89)	0.2 (0.4)	1
5506	:328@N4	1919	:116@H	1918	:116@N	0.50	3.075 (0.00)	17.65 (0.00)	0.2 (0.4)	1
5487	:328@N1	668	:41@HH11	667	:41@NH1	0.50	3.276 (0.00)	46.98 (0.00)	0.2 (0.4)	1
1931	:116@O	1929	:116@HD22	1927	:116@ND2	0.50	3.099 (0.00)	43.01 (0.00)	0.2 (0.4)	1

Table B.3 AK9 Analysis results with active residues (20 nano-second)

atom#	res@atom	atom#2	res@atom3	atom#4	res@atom5	%occupied	distance	angle	lifetime	maxocc
1340	79@OE2	1081	66@HZ2	1079	66@NZ	24.00	2.787 (0.14)	27.41 (11.86)	0.3 (0.8)	4
1340	79@OE2	1080	66@HZ1	1079	66@NZ	22.00	2.828 (0.16)	27.49 (13.77)	0.3 (0.8)	5
1340	79@OE2	1082	66@HZ3	1079	66@NZ	21.00	2.787 (0.09)	23.31 (13.25)	0.3 (0.8)	6
1339	79@OE1	1081	66@HZ2	1079	66@NZ	19.00	3.030 (0.23)	43.13 (10.94)	0.3 (0.7)	3
1339	79@OE1	1082	66@HZ3	1079	66@NZ	15.50	3.072 (0.24)	44.57 (11.45)	0.3 (0.6)	4
1903	114@O	1919	116@H	1918	116@N	15.00	2.995 (0.15)	43.31 (8.14)	1.2 (4.0)	20
1339	79@OE1	1080	66@HZ1	1079	66@NZ	13.00	2.914 (0.18)	42.55 (10.57)	0.3 (0.6)	3
1926	116@OD1	1933	117@H	1932	117@N	12.50	3.069 (0.16)	47.25 (8.80)	0.4 (0.9)	6
2908	173@OD1	1081	66@HZ2	1079	66@NZ	11.50	2.799 (0.11)	24.48 (11.89)	0.3 (0.6)	3
2908	173@OD1	1082	66@HZ3	1079	66@NZ	11.00	2.773 (0.12)	27.28 (10.72)	0.3 (0.7)	3
2908	173@OD1	1080	66@HZ1	1079	66@NZ	11.00	2.840 (0.15)	23.74 (13.47)	0.3 (0.8)	4
2909	173@OD2	1080	66@HZ1	1079	66@NZ	9.50	2.758 (0.07)	30.79 (10.97)	0.3 (0.7)	3
1945	117@O	1929	116@HD2	2 1927	116@ND2	8.50	3.077 (0.21)	30.20 (13.20)	0.3 (0.8)	4
1926	116@OD1	5505	328@H2	5504	328@N2	8.50	3.073 (0.23)	27.18 (10.68)	0.5 (1.4)	7
2909	173@OD2	1081	66@HZ2	1079	66@NZ	8.00	2.879 (0.18)	24.25 (12.52)	0.4 (1.0)	6
2909	173@OD2	1082	66@HZ3	1079	66@NZ	7.50	2.843 (0.14)	29.65 (12.00)	0.3 (0.6)	2
1926	116@OD1	1919	116@H	1918	116@N	5.50	3.026 (0.14)	55.48 (3.29)	0.2 (0.5)	2
1955	118@OD2	5490	328@H3	5489	328@N4	3.00	3.103 (0.29)	16.45 (11.79)	0.2 (0.5)	2
1954	118@OD1	5490	328@H3	5489	328@N4	2.50	2.956 (0.03)	15.60 (3.92)	0.2 (0.4)	1
804	49@OG	1081	66@HZ2	1079	66@NZ	2.00	2.950 (0.10)	33.00 (7.68)	0.4 (0.9)	3
2011	121@OE1	5490	328@H3	5489	328@N4	1.50	3.249 (0.25)	36.83 (13.89)	0.3 (0.6)	2
1931	116@O	5490	328@H3	5489	328@N4	0.50	2.928 (0.09)	20.97 (4.79)	0.6 (1.2)	3
1903	114@O	1929	116@HD2	2 1927	116@ND2	1.00	3.011 (0.04)	26.54 (8.67)	0.4 (0.8)	2
5497	328@N3	1947	118@H	1946	118@N	0.50	3.264 (0.00)	17.02 (0.00)	0.2 (0.4)	1
2686	161@SD	1929	116@HD2	2 1927	116@ND2	0.50	3.448 (0.00)	58.05 (0.00)	0.2 (0.4)	1
1945	117@O	1928	116@HD2	1 1927	116@ND2	0.50	2.709 (0.00)	51.42 (0.00)	0.2 (0.4)	1
1931	116@O	1929	116@HD2	2 1927	116@ND2	0.50	3.260 (0.00)	56.75 (0.00)	0.2 (0.4)	1
1926	116@OD1	5490	328@H3	5489	328@N4	0.50	3.129 (0.00)	52.83 (0.00)	0.2 (0.4)	1
804	49@OG	1082	66@HZ3	1079	66@NZ	0.50	3.199 (0.00)	28.38 (0.00)	0.2 (0.4)	1
804	49@OG	1080	66@HZ1	1079	66@NZ	0.50	2.885 (0.00)	44.31 (0.00)	0.2 (0.4)	1

Table B.4 K92 Analysis results with active residues (20 nano-second)

atom#	res@atom	atom#2	res@atom3	atom#4	res@atom5	%occupied	distance	angle	lifetime	maxoc c
5504	:328@N3	1929	116@HD22	1927	116@ND2	12.5	3.069 (0.13)	19.17 (10.47)	1.7 (4.5)	17
1955	:118@OD2	5489	328@H4	5488	328@O1	11.5	2.687 (0.18)	19.25 (9.10)	0.4 (1.2)	7
1954	:118@OD1	5489	328@H4	5488	328@O1	11.5	2.715 (0.18)	17.08 (11.73)	0.7 (1.7)	8
1954	:118@OD1	5489	328@H6	5496	328@N4	9.00	3.011 (0.17)	23.79 (8.99)	0.9 (2.1)	8
715	:43@O	5512	328@H5	5511	328@N1	6.00	3.026 (0.23)	38.85 (9.27)	0.3 (0.8)	3
1955	:118@OD2	5497	328@H6	5496	328@N4	5.00	2.842 (0.10)	23.21 (8.19)	0.7 (1.6)	5
1945	:117@O	5497	328@H6	5496	328@N4	2.00	3.191 (0.20)	44.74 (3.93)	0.4 (0.9)	3
2645	:158@O	2661	160@H	2660	160@N	1.5	3.211 (0.22)	48.87 (8.81)	0.2 (0.4)	1

Table B.5 AK10 (M10) Analysis results with active residues (20 nano-second)

atom#	res@atom	atom#2	res@atom3	atom#4	res@atom5	%occupied	distance	angle	lifetime	maxocc
1340	:79@OE2	1081	:66@HZ2	1079	:66@NZ	13.50	2.904 (0.20)	30.68 (14.55)	0.4 (0.9)	6
1339	:79@OE1	1081	:66@HZ2	1079	:66@NZ	13.00	2.860 (0.17)	30.30 (15.28)	0.4 (0.9)	4
1340	:79@OE2	1082	:66@HZ3	1079	:66@NZ	Ara.50	2.870 (0.21)	29.01 (16.17)	0.4 (0.9)	5
1339	:79@OE1	1080	:66@HZ1	1079	:66@NZ	11.00	2.785 (0.12)	28.52 (13.27)	0.5 (1.7)	11
2909	:173@OD2	1080	:66@HZ1	1079	:66@NZ	Eki.50	2.822 (0.16)	22.47 (12.72)	0.4 (1.0)	6
2909	:173@OD2	1082	:66@HZ3	1079	:66@NZ	9.00	2.767 (0.08)	22.81 (9.32)	0.5 (1.9)	11
1339	:79@OE1	1082	:66@HZ3	1079	:66@NZ	9.00	2.851 (0.15)	33.36 (14.93)	0.4 (1.1)	7
2909	:173@OD2	1081	:66@HZ2	1079	:66@NZ	Tem.50	2.797 (0.16)	24.87 (10.34)	0.4 (0.9)	5
1340	:79@OE2	1080	:66@HZ1	1079	:66@NZ	7.00	2.922 (0.23)	33.40 (15.57)	0.2 (0.5)	2
5487	:328@N2	2641	:158@HE2	2640	:158@NE2	Haz.50	3.161 (0.15)	34.06 (15.02)	0.4 (1.0)	5
2908	:173@OD1	1081	:66@HZ2	1079	:66@NZ	May.50	2.752 (0.12)	25.87 (11.68)	0.4 (1.4)	7
2908	:173@OD1	1082	:66@HZ3	1079	:66@NZ	Nis.50	2.854 (0.20)	35.61 (10.72)	0.3 (0.7)	3
2908	:173@OD1	1080	:66@HZ1	1079	:66@NZ	4.00	2.821 (0.09)	17.30 (9.65)	0.4 (1.0)	4
5487	:328@N2	1929	:116@HD22	1927	:116@ND2	0.50	3.435 (0.00)	11.36 (0.00)	0.2 (0.4)	1

Table B.6 K07 Analysis results with active residues (20 nano-second)

atom#	res@atom	atom# 2	res@atom 3	Atom# 4	res@atom 5	%occupie d	distance	angle	lifetime	maxoc c
1819	:109@O	1064	:66@H	1063	:66@N	100.00	2.912 (0.12)	16.04 (7.40)	40.0(80.0)	200
1084	:66@O	1802	109@H	1801	109@N	97.50	2.967 (0.15)	19.56 (7.85)	13.0(40.2)	161
1340	:79@OE2	1082	:66@HZ3	1079	:66@NZ	34.00	2.876 (0.14)	27.93 (11.76)	0.3(0.8)	5
1340	:79@OE2	1081	:66@HZ2	1079	:66@NZ	33.00	2.893 (0.18)	28.44 (13.83)	0.3(0.8)	6
1340	:79@OE2	1080	:66@HZ1	1079	:66@NZ	29.50	2.885 (0.17)	26.87 (13.51)	0.4(0.8)	4
1339	:79@OE1	1081	:66@HZ2	1079	:66@NZ	29.50	2.857 (0.17)	37.72 (12.86)	0.3(0.8)	6
1339	:79@OE1	1082	:66@HZ3	1079	:66@NZ	28.00	2.851 (0.14)	42.43 (11.47)	0.3(0.7)	4
1339	:79@OE1	1080	:66@HZ1	1079	:66@NZ	23.50	2.892 (0.18)	40.38 (13.24)	0.3(0.7)	3
2909	:173@OD 2	1080	:66@HZ1	1079	:66@NZ	21.50	2.774 (0.13)	27.18 (13.67)	0.3(0.7)	4
2908	:173@OD 1	1082	:66@HZ3	1079	:66@NZ	18.00	2.795 (0.11)	26.39 (13.30)	0.4(0.9)	4
2909	:173@OD 2	1081	:66@HZ2	1079	:66@NZ	17.50	2.794 (0.11)	22.01 (10.56)	0.3(0.8)	4
2908	:173@OD 1	1081	:66@HZ2	1079	:66@NZ	13.50	2.796 (0.12)	28.10 (11.44)	0.3(0.6)	2
2909	:173@OD 2	1082	:66@HZ3	1079	:66@NZ	12.00	2.781 (0.11)	24.65 (11.96)	0.3(0.7)	3
2908	:173@OD 1	1080	:66@HZ1	1079	:66@NZ	Kas.50	2.823 (0.14)	23.04 (10.76)	0.3(0.6)	3
715	:43@O	5484	328@H1	5483	328@O1	Ağ.50	2.981 (0.17)	35.87 (14.03)	0.3(0.6)	2

Table B.7 Polar atom types

AK9 acceptor N2 H2	K93 donor N2	HIS donor NE2	LYN donor NZ
AK9 donor N3	K93 acceptor N3 H5	HIS acceptor ND1 HD1	SER donor OG
AK9 acceptor N4 H3	K93 donor N5	HIS acceptor HE2	SER acceptor OG HG
K92 acceptor N1 H5	K93 acceptor N6 H6	HID donor NE2	THR donor OG1
K92 donor N3	M10 donor N1	HID acceptor ND1 HD1	THR acceptor OG1 HG1
K92 acceptor N4 H6	M10 donor N2	HIE donor ND1	TYR donor OH
K92 acceptor O1 H4	M10 acceptor S1	HIE acceptor NE2 HE2	TYR acceptor OH HH
K01 acceptor N3 H14	GLU donor OE1	HIP acceptor ND1 HD1	ARG acceptor NH1 HH11
K01 acceptor N3 H2	GLU donor OE2	HIP acceptor NE2 HE2	ARG acceptor NH1 HH12
K01 donor N1	ASP donor OD1	GLN donor OE1	ARG acceptor NH2 HH21
K01 donor N2	ASP donor OD2	GLN acceptor NE2 HE21	ARG acceptor NH2 HH22
K01 donor S1	GLH donor OE1	GLN acceptor NE2 HE22	ARG acceptor NE HE
K07 acceptor O1 H1	GLH donor OE2	ASN donor OD1	TRP acceptor NE1 HE1
K07 acceptor N1 H11	GLH acceptor OE1 HE1	ASN acceptor ND2 HD21	CYS donor SG
K07 donor S1	GLH acceptor OE2 HE2	ASN acceptor ND2 HD22	CYS acceptor SG HG
K91 donor N1	ASH donor OD1	LYS acceptor NZ HZ1	CYC donor S
K91 acceptor N2 H6	ASH donor OD2	LYS acceptor NZ HZ2	CYC donor O
K91 donor N4	ASH acceptor OD1 HD1	LYS acceptor NZ HZ3	CYC acceptor N H
K91 acceptor N5 H7	ASH acceptor OD2 HD2	LYN acceptor NZ HZ2	CYM donor SG
K93 donor N1	HIS donor ND1	LYN acceptor NZ HZ3	MET donor SD

Derivatization Details

It has been compared the positions of AK9, AK10 molecules by derivatizing with Medchem and tested the different transformation data bases to see which one could get the most efficient results. The appropriate force field for modeling protein-ligand complexes is Amber10:EHT. When clicked on the force field screen, all bonded Vanderwall's electrostatic interactions and all restraints were taken into account. There should be a statement "The molecular system appears parameterized" so if the default force field is Amber10, there is no need to change it. First, randomly scanned without selecting any protein and opened only AK molecule with choosing the transformation as "\$ MOE / lib / medchem_rxn.mdb". The process was continued by leaving the descriptive filter blank.

Medchem in Cleft is the second type of conversion done. Here, it is prepared the input navigate to \$DESKTOP/(Tutorial)_MOE_ MedChem_Transformations_In-Cleft/ datafiles. Output data base is saved as "medchem.mdb". Proceeded by selecting the "GrowthRXN" database. Before preparing the complex, first, need to be ensured that no atoms were selected and clicked respectively QuickPrep and site view. Site view renders the molecular system according to a convention which is designed to provide feedback regarding which atoms belong to the receptor and which atoms are ligand atoms, to focus on the active sites of receptor-ligand complexes. Site view isolated the ligand in the active site by hiding distant receptor and solvent atoms. Distant backbone ribbons hidden. The receptor atoms rendered in line mode and ligands in stick mode. Non-polar hydrogens will be hidden and polar hydrogens shown.

Firstly based on the existing molecules, then ignored the side chains and scanned only on the scaffold of the molecule. It has been proceeded by leaving the descriptor filter blank in the same way.

Parameterization of the Ligand Flow

All ligands were converted to xyz file with pdb2xyz command and these .xyz files were converted to the correct input file format, .com, using the xyz2prep script. The .com file that was made was submitted as a Gaussian job. Gaussian input, .com file, generated from the ligand's xyz file. The .com files obtained were used for optimization. The job was sent with the script g09.tbag49. Files converted to .com were selected as the input file. The processor number was selected as 4. After seeing that the files output as opt-.out give normal termination, we use out2prep command for single point with the same basis set.

To extract partial charges from Gaussian output, the antechamber module was used. Where "i" input file, "fi" input file format, "gout" gaussian output file, "o" output, "fo" output file format, "c" charge, "resp" is the method of calculating charges. After preparing the prepin file of the ligand, it was used the parmcheck, which is the command to add improper dihedrals to the parameterization. The following command was used to convert the ligand to .pdb file.

This new parameterized ligand paired with the old ligand which was previously docked and placed in CK2 protein. The new ligand, parameterized and pair fitted, was saved in its new position. In the pair fitting process, only heavy hetero atoms such as Oxygen and Nitrogen were paired. In order to be saved as CK2-AK complex, the file was recorded by putting the "TER" expression between them in the text file, adding the protein first, then the new ligand and waters. The details to be checked on this file have been examined. Cysteines that are close to each other may have the possibility of reacting and forming a sulfur-sulfur bond. This situation in the structure was examined with the "select resn CYS" command. Then, for the protonation state of the histidines, a histidine close to the ligand was analyzed with "select resn HIS". It was investigated which hydrogen bonds were made. Bonded structures at a distance of 4 Å were observed. Except for the distant Asparagine and Lysine, there was no case in which had close contact. There was no close connection to histidine. Polar contacts around it were examined. It has also been observed that it is only a water molecule.

"tleap" was entered to generate input files that Amber could understand. The following command was used to save the ligand file and the "gaff" to write for parameters of the ligand

means the General Amber Forcefield. The force field required for the amino acids of the protein was entered as follows. Previously created parameter files for the ligand had been loaded. The charges of the enzyme were checked and neutralized. The axes were aligned. It was covered with water up to a minimum of 10 Å beyond the protein. The parm command was used to save the parameter, topology, and input xyz coordinate files.

Preparation of the Protein-Ligand Complex

The complex of CK2 and new ligands were aligned to the 3PE1 structure (Crystal structure of human protein kinase CK2 alpha subunit in complex with the inhibitor CX-4945) one by one on Pymol. In this way, observed that the new structure overlaps with 3PE1 and CX-4945 ligand. Water molecules were only present in the outer part of the protein. In order to take into account the water molecules close to the active site, it was included the water from the crystal structure.

Protein, ligand and water files were created separately. First, to save the protein, sequentially observed the structure of CK2 and ligand complexes. The whole structure was selected except ligand over the sequence representation. After selection, copied as a new object and removed the hydrogens. Secondly, it was selected only water and removed hydrogen in the same way. Finally, moved forward by choosing the only ligand in the structure. Since the docked ligands only contain polar hydrogens, this time it was removed all the hydrogens and added again. 3 different files (protein-ligand-water) were prepared for each different ligand. These 3 files were combined in text and added "TER" which means terminate after each ending chain.

ATOM	5464	CB	LYS	327	-2.599	15.979	-18.141	1.00	0.00
ATOM	5465	HB2	LYS	327	-2.713	16.502	-19.090	1.00	0.00
ATOM	5466	HB3	LYS	327	-1.959	15.108	-18.281	1.00	0.00
ATOM	5467	CG	LYS	327	-3.965	15.528	-17.643	1.00	0.00
ATOM	5468	HG2	LYS	327	-4.621	16.399	-17.619	1.00	0.00
ATOM	5469	HG3	LYS	327	-4.359	14.797	-18.350	1.00	0.00
ATOM	5470	CD	LYS	327	-3.895	14.908	-16.258	1.00	0.00
ATOM	5471	HD2	LYS	327	-3.107	14.155	-16.250	1.00	0.00
ATOM	5472	HD3	LYS	327	-3.659	15.688	-15.535	1.00	0.00
ATOM	5473	CE	LYS	327	-5.223	14.257	-15.887	1.00	0.00
ATOM	5474	HE2	LYS	327	-5.331	13.349	-16.480	1.00	0.00
ATOM	5475	HE3	LYS	327	-5.187	13.997	-14.829	1.00	0.00
ATOM	5476	NZ	LYS	327	-6.385	15.156	-16.138	1.00	0.00
ATOM	5477	HZ1	LYS	327	-6.419	15.397	-17.119	1.00	0.00
ATOM	5478	HZ2	LYS	327	-7.239	14.684	-15.878	1.00	0.00
ATOM	5479	HZ3	LYS	327	-6.285	15.998	-15.589	1.00	0.00
ATOM	5480	C	LYS	327	-0.597	17.454	-17.903	1.00	0.00
ATOM	5481	O	LYS	327	0.517	17.288	-17.405	1.00	0.00
ATOM	5482	OXT	LYS	327	-0.684	18.040	-18.979	1.00	0.00
TER									
ATOM	5483	C2	K93	328	10.718	16.705	9.506	1.00	0.00
ATOM	5484	H2	K93	328	9.661	16.790	9.320	1.00	0.00
ATOM	5485	N1	K93	328	11.222	17.539	10.401	1.00	0.00
ATOM	5486	C3	K93	328	12.516	17.440	10.622	1.00	0.00
ATOM	5487	H3	K93	328	12.934	18.113	11.349	1.00	0.00
ATOM	5488	N2	K93	328	13.352	16.603	10.039	1.00	0.00
ATOM	5489	C4	K93	328	12.833	15.777	9.150	1.00	0.00
ATOM	5490	H4	K93	328	13.511	15.100	8.662	1.00	0.00
ATOM	5491	C1	K93	328	11.482	15.770	8.829	1.00	0.00
ATOM	5492	C29	K93	328	10.878	14.805	7.833	1.00	0.00
ATOM	5493	C26	K93	328	10.659	13.423	8.457	1.00	0.00
ATOM	5494	H19	K93	328	11.612	13.078	8.869	1.00	0.00
ATOM	5495	H26	K93	328	9.958	13.511	9.280	1.00	0.00
ATOM	5496	H1	K93	328	9.895	15.180	7.542	1.00	0.00
ATOM	5497	N6	K93	328	11.753	14.652	6.681	1.00	0.00
ATOM	5498	H6	K93	328	11.932	15.546	6.268	1.00	0.00
ATOM	5499	C28	K93	328	11.214	13.738	5.688	1.00	0.00
ATOM	5500	H21	K93	328	11.924	13.655	4.874	1.00	0.00

CK2-ligand.pdb file

Minimization Details

The constraint existence is specified by the “ntr” parameter in the input file. If $ntr > 0$, a harmonic potential is used to constrain specified atoms in Cartesian space. If $ntr=0$, then there is no restraint [29]. As a result, the ntr values in the min1 and min2 steps were different. The option $imin=1$ was utilized during the minimization run. If a different simulation is run instead of minimization, $imin=0$. The non-bonded cutoff distance in 8 was specified using the $Cut=8$ parameterization. $Ntb=1$ denotes a volume that is constant. With a default value of 0, Igb is a parameter for the generalized Born or Poisson-Boltzmann implicit solvent models. $Ntc=1$ indicates that the SHAKE algorithm was not used to solve the system of non-linear constraint equations (the system of non-linear constraint equations is solved using the Gauss–Seidel technique, which approximates the solution of the linear system of equations using the Newton–Raphson method).

Heating Details

After the minimization phase, the minimized systems were heated in 6 steps from 0 to 300 K (50 K heating for each) to reach equilibrium. To execute the simulation effectively, a loose constraint of 10 was applied to the first residue after the protein residues, similar to the minimization step. The ligand was restrained in the heating steps for ligand-protein complexes by applying a constraint of 30 to it.

Figure 2.10 shows an example input file for the first heating simulation. Since it was not a minimization stage, there are additional parameters to minimization parameters provided in the heating input file, such as `imin=0`. The variable `irest=0` indicates that the simulation should not be restarted; instead, it should be executed as a new simulation. With this setting, any velocity in the input coordinate file was disregarded. `Ntx=1` implies only read the coordinates from the restart file, not the velocities. SHAKE eliminates the quickest motion, bond stretching, and allows for a bigger time step to be used as a result. A specific "three-point" approach is employed for water models.

As a result, `ntf` and `ntc` were set to 2 in order to use TIP3P. The initial temperature parameter is `Tempi`, and the end temperature value in the simulation step is `temp0`. The number of 3 implies temperature control with a Langevin thermostat, and `Ntt` is a temperature regulator. When `ntt = 3`, `gamma ln=1.0` is the collision frequency in ps1. The number of MD steps in the run was 50000, as indicated by `Nstlim=50000`. The duration length of each MD step in ps was `Dt=0.001`. Energy information was output in human-readable form to files "heatout" and "heatinfo" at every `ntpr` step, according to the `ntpr` parameter. The `ntwr` parameter indicates that the "restrt" file was written after each `ntwr` step. The coordinate format is `Ioutfm=1` [23].

Equilibrium Details

For equilibration runs, restart files generated after the last heating phase were used. Equilibrium was achieved in two stages. The first was performed by NVT (Canonical Ensemble), while the second was performed by NPT (Isobaric-Isothermal Ensemble). As a result, for NVT, a thermostat was employed, and for NPT, both a thermostat and a barostat were used. Figures 2.4.7 and 2.4.8 illustrate the input files for the first and second phases, respectively. As can be seen, the heating steps were controlled by the same thermostat, the

ntt=3, Langevin thermostat. The settings ntb=2, pres0=1.0, ntp=1, taup=2.0 were used for barostat. Constant pressure periodic boundary conditions were utilized with ntb=2. The reference pressure (in units of o) is Pres0=1.0.

Molecular Dynamic Simulations (MD)

In this project, MD simulations were performed using AMBER12 to evaluate the designed protein-ligand catalytic contacts in a dynamic environment.

Parameterization of the Ligand

Gaussian09 was used to parameterize the ligand as a single point geometry optimization. The computational model was the Hartree-Fock theory model (HF), which is the basis of electronic structure theory [33]. Basis set type 6-31G (d) was used to which common functions were added. Extra keywords were added to calculate SCF = tight Pop = MK iop = (6/33 = 2). Instructions for ligand parameterization can be found in Appendix B, under Parameterization of the Ligand Flow.

To extract partial charges from Gaussian output, the antechamber module was used. After preparing the prepin file of the ligand, it was used the parmcheck, which is the command to add improper dihedrals to the parameterization. The following command was used to convert the ligand to .pdb file.

```
antechamber -i sp-opt-ligand.out -fi gout -o ligand.prepin -fo prepi -c resp
parmchk -i ligand.prepin -f prepi -o ligand.frcmod
antechamber -i ligand.prepin -fi prepi -o ligand.pdb -fo pdb
```

Figure Obtaining necessary ligand files from Gaussian output

This new parameterized ligand paired with the old ligand which was previously docked and placed in CK2 protein. In the pair fitting process, only heavy hetero atoms such as Oxygen and Nitrogen were paired. Cysteines that are close together may react and form sulfur-sulfur bonds. Then, for the protonation state of histidine, "select resn HIS" was used to analyze the histidine near the ligand. Investigated which hydrogen bonds are created. Bonded structures

at a distance of 4 Å were observed. Except for the distant Asparagine and Lysine, there was no case in which had close contact. There was no close connection to histidine. Polar contacts around it were examined. It has also been observed that it is only a water molecule.

"tleap" was entered to generate input files that Amber could understand. The following command was used to save the ligand file and the "gaff" to write for parameters of the ligand means the General Amber Forcefield. The force field required for the amino acids of the protein was entered as follows. Previously created parameter files for the ligand had been loaded. The charges of the enzyme were checked and neutralized. The axes were aligned. It is covered with water, at least 10 Å beyond the protein.

```
source /truba/home/tbag27/amber12/dat/leap/cmd/leaprc.gaff
source /truba/home/tbag27/amber12/dat/leap/cmd/leaprc.ff99SB
loadamberprep ligand.prepin
loadamberparams ligand.frcmod
substrate-pdb = loadpdb ligand.pdb
enzyme = loadpdb CK2-ligand.pdb
check substrate-pdb
check enzyme
charge enzyme
addions enzyme Na+ 0 ( or Cl- 0)
CK2-ligand-TIP3P = copy enzyme
alignaxes CK2-ligand-TIP3P
solvatebox CK2-ligand-TIP3P TIP3PBOX 10
charge CK2-ligand-TIP3P
saveoff CK2-ligand-TIP3P CK2-AK-TIP3P.lib
saveamberparm CK2-ligand-TIP3P CK2-ligand-TIP3P.prmtop CK2-ligand-TIP3P.inpcrd
savepdb CK2-ligand-TIP3P CK2-ligand-TIP3P.pdb
quit
```

Figure Generating parameters for a protein with tleap

Minimization

The first category of simulation in MD is system minimization. The coordinate files utilized in simulations were inpcrd files. Minimizations were carried out in two stages. Each step was run for 20 nanoseconds. To run the process properly in the first minimization stage, a loose constraint was placed on an atom outside of the protein residues, however, no constraint was placed in the second minimization step. For the first minimization of the kinase-ligand complexes, a constraint with a high value of 150 was applied to the entire protein to freeze protein residues and only water molecules were released. In the second step,

water molecules, protein and ligand were minimized without applying any constraints in minimization. Figures 2.14 and 2.15 demonstrate an example input file for the minimization process.

```
minimization
&cntrl
  imin=1, maxcyc=20000, ncyc=15000,
  ntb=1, igb=0, ntc=1,
  cut=8,
  ntr=1, nmropt=0,
&end
Group input for restrained atoms
150
RES 1 328
END
END
```

Figure Minimization input file for the first step

```
minimization
&cntrl
  imin=1, maxcyc=20000, ncyc=20000,
  ntb=1, igb=0, ntc=1,
  cut=8,
  ntr=0, nmropt=0,
&end
```

Figure Minimization input file for the second step

Heating

After the minimization phase, the minimized systems were heated in 6 steps from 0 to 300 K (50 K heating for each) to reach equilibrium. To execute the simulation effectively, a loose constraint of 10 was applied to the first residue after the protein residues, similar to the minimization step. The ligand was restrained in the heating steps for ligand-protein complexes by applying a constraint of 30 to it.

Figure 2.16 shows an example input file for the first heating simulation.

```

heating of the system
&cntrl
  imin=0,
  irest=0,
  ntx=1,
  ntb=1,
  cut=8, ntc=2, ntf=2,
  tempi=0.0, temp0=50.0,
  ntt=3, gamma_ln=1.0,
  nstlim=50000, dt=0.001,
  ntpr=10000, ntwx=0, ntwr=10000,
  ioutfm=1,
  ntr=1, nmropt=0,
&end
Group input for restrained atoms
30
RES 328 328
END
END

```

Figure Example input file of the first heating simulation of a complex

Equilibrium

For equilibration runs, restart files generated after the last heating phase were used. Equilibrium was achieved in two stages. The first was performed by NVT (Canonical Ensemble), while the second was performed by NPT (Isobaric-Isothermal Ensemble). As a result, for NVT, a thermostat was employed, and for NPT, both a thermostat and a barostat were used. Figures 2.17 and 2.18 illustrate the input files for the first and second phases, respectively.

```

equilibration run (0 - 1 ns)
&cntrl
  imin=0,
  irest=1, ntx=7,
  ntb=1,
  cut=8, ntc=2, ntf=2,
  tempi=300.0, temp0=300.0,
  ntt=3, gamma_ln=1.0,
  nstlim=500000, dt=.002,
  ntpr=100000, ntwx=0, ntwr=100000,
  ioutfm=1,
  ntr=1,
&end
Group input for restrained atoms
10
RES 328 328
END
END

```

Example input file of the first equilibrium step of a complex

```

equilibration run (1 - 3 ns)
&cntrl
  imin=0,
  irest=1, ntx=7,
  ntb=2, pres0=1.0, ntp=1, taup=2.0,
  cut=8, ntc=2, ntf=2,
  tempi=300.0, temp0=300.0,
  ntt=3, gamma_ln=1.0,
  nstlim=1000000, dt=.002,
  ntp=100000, ntwx=0, ntwr=100000,
  ioutfm=1,
  ntr=1,
&end
Group input for restrained atoms
10
RES 328 328
END
END

```

Figure Example input file of the second equilibrium step of a complex

Production

The manufacturing run was the final phase in the MD simulations. There was only one sub-step in this phase, with a simulation time of 20 ns. In production runs, no constraints were applied to any residue. For the production runs, restart files generated after the final equilibration step were used. Figure 2.19 shows a sample input file for the md simulation.

```

production run (20 ns)
&cntrl
  imin=0,
  irest=1, ntx=7,
  ntb=2, pres0=1.0, ntp=1, taup=2.0,
  cut=8, ntc=2, ntf=2,
  tempi=300.0, temp0=300.0,
  ntt=3, gamma_ln=1.0,
  nstlim=1000000, dt=.002,
  ioutfm=1,
  ntp=100000, ntwx=50000, ntwr=100000,
  ntr=0,
&end

```

Figure Example input file of the production run of a complex

Analysis of the Derivative Molecules

PYMOL was used to open TIP3P files containing the ligand and determine the residues surrounding the 5 Å position of the ligand. For each ligand in their MD simulation work files, a new file called "analysis.input" was created, into which the number of residues calculated in the 5 Å diameter was inserted. The script "single trajectory analysis.local" was then run to obtain the MD simulation result files for analysis.

The script "single_trajectory_analysis.local" was entered and the queried numbers were entered as shown in the Figure 2.20.

The substrate residue number is the ligand sequence number. The number of the catalytic residue is also given as 66 because it forms a salt bridge with Lysine.

```
single_trajectory_analysis.local
subs residue : 328
catalytic residue: 66
how many mdcrd file : 1
distance cut off : 0.1
simulation time (ns) : 20
total data collection rate: 10
```

Figure MD Analysis Script

After the analyzes are completed, "DONE" appears on the screen. Among the files created, the file named HBOND.out was opened with the command "vi" and the occupancy of the hydrogen bonds was examined.