INTRODUCTION

SIRT1 enzyme activity has two primary roles as a target for therapy, namely SIRT1 inhibition in the catalytic region which is used in cancer treatment and SIRT1 activation in the allosteric region which is used in metabolic damage treatment, including diabetes [1,2] and anti-aging [3-5]. SIRT1 is a nicotinamide adenine dinucleotide (NAD+)-dependent deacetylase which catalyzes of deacetylating protein in lysine residues [6,7].

The structure of human sirtuin protein includes the SIRT1 isoform (UniProt accession code Q6EB6E, available online: NCBI, Research Collaboratory for Structural Bioinformatics). The SIRT1 sequence comprises 747 amino acids; amino acids 1-180 are located at the N-terminal region, amino acids 195-240 in the allosteric region (active regulator of SIRT1), amino acids 244-512 in the catalytic region, and amino acids 513-747 in the C-terminal region [8].

Nucleotide bonds including NAD+ are found in the amino acid regions 261-280, 345-348, 440-442, and 465-467; metal bonds are present at amino acids 371, 374, 395, and 398; the deacetylase area is at amino acids 244-498; and the activation area including sirtuin-activating compounds (STACs) are at amino acids 195-243 and Glu(E)230 [9,10]. Homologous sequences identical to SIRT1 and with allosteric regions obtained from the protein data bank (PDB) [6] include PDB IDs 4ZZI, 4ZZJ, 4ZZH [1,1], and 5BTR [12].

The structure of SIRT1 PDB ID 4ZZI comprises a ligand activator 4TQ (JSSS-1,3-dimethyl-N-[3-[1,3-oxazol-5-yl]phenyl]-6-[3-(trifluoromethyl)phenyl]-2,3-dihydropyrido[2,3-b]pyrazine-4(1H)-carboxamide) that interacts at the allosteric region, and ligand inhibitor 1NS 4-[(2-[methylsulfonyl] amino)ethyl] piperidin-1-yl) thieno(3,2-d)pyrimidine-6-carboxamide in the catalytic region. 4TQ activators demonstrate hydrophobic interactions (H) at amino acids Thr219, Leu215, Ile223, and hydrogen bond at Asn226. The 1NS inhibitor shows Hs at residues Phe297 and His363 and hydrogen bonds at Phe273, Ile347, and Asp348.

The structure of SIRT1 PDB ID 4ZZJ (www.rcsb.org/structure/4ZZJ) shows interactions between 4TQ at the allosteric region of the N-terminal region of the H of amino acids Thr219 and Ile223, hydrogen bonding at Asn226, and with CNA (carba-nicotinamide-adenine-dinucleotide) ligand at the catalytic region. The active conformation model of SIRT1 comprises a STAC complex with the Ac-p53 substrate, indicating the presence of amino acid hydrogen bond interactions at Glu230 and Arg496. The structure of the complex of SIRT1 with STAC compounds shows binding of STAC-1 to the STAC binding domain (SBD). Binding of STAC encourages SBD interaction with the central catalytic region to the substrate, increasing the activity of SIRT1 and stabilizing the deacetylase activity. Replacement of Glu (E) 230 with Lys residue disrupts enzymatic activity by reducing the electrostatic interactions between Glu230 in the SBD and Arg446 at the substrate-binding site [13].

The structure of SIRT1 PDB ID 4ZZH (www.rcsb.org/structure/4ZZH) shows Hs of STACs (4TO) ([4S]-N-[3-[1,3-oxazol-5-yl]phenyl]-7-[3- trifluoromethyl] phenyl)-3,4-dihydro-1,4-methanopyridine[2,3-b] [1,4] diazepine-5(2H)-carboxamide) with Leu 215, Thr219, and ILE223 and hydrogen interactions with Asn226 in the allosteric region.
The structure of SIRT1 PDB ID 5BTR (www.rcsb.org/structure/5btr) is the SIRT1 activator located in the allosteric region, a complex containing three resveratrol ligands (STL), and shows hydrogen bonding interactions at Gln222, Asn226, Lys444, Asp296, Asp292, and Glu230 and Hs at Gly415, Ile223, Gln294, Ile223, and Arg446. Intercation of 7-amino-4-methyl coumarin with 5BTR shows Hs at Phe412, Val414, His363, and Phe414 and hydrogen bond interactions at Arg446, Asn226, Gly415, Asp150, Glu416, and Val412 [12].

Therefore, the present study used an in silico study with molecular docking and dynamics simulations of the SIRT1 enzyme (PDB IDs 4ZZI, 4ZZJ, 4ZZH, and 5BTR) to determine the bonding interactions of SIRT1 required for ligand selectivity and selective identification of bond interactions and target bond interactions with biological function [14,15].

MATERIALS AND METHODS

Materials

The following materials were used: IntelXeon(R) central processing unit ES620 at 2.40 GHz × 16, processor 2.6 GHz Intel Core i7, random access memory 32 GB 1600 MHz DDR3, and Graphics Intel Iris 15.36 MB. The operating system used was Linux Ubuntu 12.04 LTS with an uninterrupted power supply.

Protein preparation

Preparation of macromolecular three-dimensional structure was done by downloading macromolecules SIRT1 (PDB IDs: 4ZZI, 4ZZJ, 4ZZH, and 5BTR) was obtained from the Research Collaboratory for Structural Bioinformatics PDB (http://www.rcsb.org/pdb) [16]. All macromolecules were clean-up from ligands, substrates, ions, and waters before minimization with Amber program [17].

Ligand preparation

The ligands used were the 4TO crystals of macromolecular SIRT1 (PDB IDs: 4ZZI, 4ZZH). Minimization of ligand used the AMBER program, and 1NS compound (SIRT1 inhibitor) was used as a negative control.

Molecular docking validation

Autodock4Zn was used to perform molecular docking in this study [18,19]. Validation of the autodock4Zn program measured the root mean square deviation (RMSD) value analyzed using the visual molecular dynamics (VMD) program and the initial crystal structure of PDB against the conformational crystal positioning after molecular redocking was done.

Molecular docking

Molecular docking was performed using AutoDock4Zn, with ligand–macromolecular grid binding sites by determining npts and gridcenter.

Analysis of the docking results in the form of affinity and RMSD binding values (for docking performance validation) and visualization of docking results to analyze ligand interaction with macromolecules used LigandScout (InteLigand, Austria) [20] and CHIMERA (UCSF, USA) respectively [21].

Molecular dynamics

The best compounds for molecular docking were selected for the molecular dynamics study, and the simulation was performed using Amber12 [17] for 50 ns.

General amber force field (GAFF) was used for ligand preparation [13]. Ligands and macromolecules topologies and coordinates were created in a vacuum and explicit waters environment. At this stage, the ligand structure was provided with an AM1–bond charge correction charge [14] using Antechamber software (UCSF USA) accessed via PuTTY. The file output was obtained in the form of *.mol2, and the antechamber result created a *.frcmod file.

Preparation of parameters and coordinates of macromolecule files containing Zn was performed online (http://mayoresearch.mayo.edu/mayo/research/camdl/zinc_protein.cfm).

Preparation of peptide minimization (substrate) used parameters and coordinates of peptide-containing (NArg His Lys Lys Leu Met CPh) macromolecular files [22]. Preparation for the formation of NAD+ used NAD+ as a cofactor with a positive charge. The creation of macromolecules with NAD+ required NAD+:lib, and NAD+-frcmod parameter files were obtained from Ross Walker, and the coordinates were altered using the coordinates of the NAD+ file associated with the macromolecules. Further complexes of ligand: Zn: NAD+:Macromolecules were used as topological and coordinate files with the addition of water molecules TIP3PBOX 12 Å, followed by minimization, heating, density, equilibration, and production.

Before continuing the dynamics simulation, verification of the system was balanced using the command “process_mdout. pl” to extract useful information from the output files: heat, out, density.out, and equil.out. A balanced system was seen from the temperature, density, and RMSD.

RESULTS AND DISCUSSION

The present study was performed by visualizing 4TO cocryystal interactions with SIRT1 macromolecules (SIRT1 PDB IDs 4ZZI, 4ZZJ, and 4ZZH) and one with three STLs (PDB ID 5BTR). Analysis of complex compound interactions (STACs) with SIRT1 activator receptors in the allosteric region was used to examine the bonding interactions that play an important role in ligand selectivity. Activator compounds act as sirtuin activators bound to the amino acid allosteric site at residues 183–243 and generally interact at the hydrophobic chains at Thr209, Pro211, Pro212, Leu215, Thr219, Ile223, and Ile227 at the shape of the (helix-turn-helix) amino acid and one hydrophilic interaction at Asn226 (Supplemental Fig. 1).

Visualization of macromolecular interactions with SIRT1 PDB IDs 4ZZI, 4ZZJ, 4ZZH, and 5BTR showed differences in the position of the allosteric region receptors. Residues at Glu230–Asn241 showed a flexible helix shape. As Fig. 1 shows, the superimposition of SIRT1 PDB ID 4ZZI demonstrates a more closed form between the allosteric and catalytic regions, containing 4TO ligand crystals in the allosteric region and 1NS ligand crystals in the catalytic region. SIRT1 PDB ID 4ZZJ contains CNA and 4TO ligand crystals in the allosteric region, and the allosteric area form was catalytically more open than that of 4ZZL. PDB 4ZZH showed an allosteric and catalytic form that was more open and contained only 4TO ligand crystals in the allosteric region. PDB 5BTR contained resveratrol compounds in the allosteric region adjacent to the catalytic region.

Validation of molecular docking

In the present study, AutoDock4Zn was used to visualize molecular docking. Validation of this AutoDock program measured the RMSD value analyzed using the VMD program. The initial cocrystal PDB structure was analyzed against the conformational crystal positioning after molecular redocking restarted. The RMSD values of 4TO as a crystal were 0.81Å, 0.79Å, and 0.89Å for 4ZZI, 4ZZJ, and 4ZZH, respectively.

Molecular docking of macromolecular 4ZZI

The macromolecular receptor of 4ZZI has ligand crystals in the catalytic region as an activator. This ligand was 4TO ([4S]-[3-[(trifluoromethyl) phenyl]-7-[(3-oxazole-5-yl) phenyl]-3,4-dihydro-1,4-methanopyrido [2,3-b] [1,4] diazepine-5 [2H]-carboxamide) in the
The 3rd International Conference on Global Health (ICGH), Universitas Indonesia, Bali, Indonesia 239

Azminah et al.

Azminah et al.

Int J App Pharm, Vol 11, Special Issue 1, 2019

Int J App Pharm, Vol 11, Special Issue 1, 2019

allosteric region as an activator. The molecular docking results of the 4TO obtained bond energy values of $\Delta G = -6.86$ kcal/mol and a Ki value of 9.36 µM (predicted binding interaction).

Fig. 2 shows the 4TO ligand crystal compound interaction. 4TO bonded to the activating region of essential amino acids Ile223 and Ile227. The complex showed four hydrophobic features at amino acids namely Leu206 (HI), Thr219 (HI), Ile223 (HI), Ile227 (HI), Asn226 [hydrogen bond acceptor (HBA)], and Glu230. Docking of the 1NS compound (SIRT1 inhibitor) as a negative control on the allosteric region showed interactions at Thr219 (HBD) and Ile227 (HBD) and no HIs (Supplemental Fig. 2).

Molecular docking of macromolecular 4ZZJ

The AutodockZn program was used to analyze docking of the ligand crystal molecule and the active compound SIRT1 with 4ZZJ. The results obtained from the process showed a bond energy value of $\Delta G = -6.94$ kcal/mol, a Ki value of 8.24 µM (predicted binding interaction), and the complex interaction of ligand and receptor bonds.

Fig. 3 shows 4TO compound interaction. The complexes demonstrate four hydrophobic features at amino acids namely Leu206 (HI), Thr209, Leu215 (HI), Thr219 (HI), Ile223 (HI), and Ile227 (HI) and show acceptor hydrogen bonding interactions (HBA) at Asn226. Docking of the 1NS compound (SIRT1 inhibitor) as a negative control on the allosteric region showed interactions at Glu222 (HBD) and Thr219 (HBD) and no HIs (Supplemental Fig. 2).

Molecular docking of macromolecular 4ZZH

The AutodockZn program was used to generate molecular docking of the crystal ligand molecule and the active compound SIRT1 with 4ZZH. The results were obtained using a bond energy value of $\Delta G = -7.61$ kcal/mol and Ki = 5.6 µM (predicted binding interactions).

The 4TO compound showed interactions with four hydrophobic features of the amino acids namely Leu206 (HI), Leu215, Thr219 (HI), Ile223 (HI), and Ile227 (HI) and showed acceptor hydrogen bonding interactions (HBA) at Thr209 (HI) residues.

4TO compound interaction with macromolecules is shown in Fig. 4, showing interaction with four hydrophobic features at amino acids namely Leu206 (HI), Thr209, Leu215 (HI), Thr219 (HI), Ile223 (HI), and Ile227 (HI).

Fig. 1: Visualization superimposition crystal structure of protein data bank (4ZZI, 4ZZJ, 4ZZH, and 5BTR) shows the flexibility of allosteric region.

Fig. 2: (a) Molecular docking of 4TO:4ZZI at the allosteric region. (b) Visualization of 4TO:4ZZI complex interactions at Thr219, Ile223, Ile227, Asn226 (hydrogen bond acceptor), and Glu230 using the LigandScout program. (c) Visualization of the 4TO:4ZZI complex using a mesh surface image generated by CHIMERA (hydrophilic, blue; hydrophobic, orange).
Molecular docking of macromolecular 5BTR

Molecular docking of 4TO with 5BTR using the AutodockZn program was obtained using a bond energy value of $\Delta G = -13.1$ kcal/mol and $K_i = 2.51 \times 10^{-4} \mu$M (predicted binding interaction).

The 4TO ligand revealed bonds at the activating regions of essential amino acids Ile223 and Ile227. The complex showed hydrophobic features at amino acids namely Leu206 (HI), Thr219 (HI), Ile223 (HI), and Ile227 (HI) (Fig. 5). Docking of the 1NS compound (SIRT1 inhibitor) as a negative control on the allosteric region showed interactions at Leu206 (HBD) and no HIs (Supplemental Fig. 2).

Molecular dynamic simulation

Molecular dynamic simulation of 4TO compounds against 4ZZI, 4ZZJ, 4ZZH, and 5BTR receptors was performed for 50 ns using the Amber program. Analysis of the dynamics simulation result was carried out considering RMSD, RMSF, and hydrogen bonding conditions, and the binding energy was calculated using the MMGB/PBSA method.

RMSD

Conformational changes of the 4TO compound during the simulation were seen from the RMSD values. The RMSD curve for 50 ns showed a change in the stability of the 4TO complex dynamic simulation that corresponded to 4ZZI, 4ZZJ, 4ZZH, and 5BTR receptors. In Fig. 6, the 4TO:4ZZI, 4TO:4ZZJ, and 4TO:5BTR complexes showed an RMSD range of 2Å, whereas the 4TO:4ZZH complex had a RMSD range of 3Å.

RMSF

RMSF is the measure of the deviation between the atomic positions of each protein residue, i.e., the difference in fluctuations in the movement of each residue during the simulation is measured for 50 ns. Fig. 7 shows the RMSF value of the complex molecular dynamic simulation between the 4TO ligand (ligand crystal) and 4ZZI, 4ZZJ, 4ZZH, and 5BTR receptors. The 4ZZH receptor showed fluctuations of movement in the N-terminal domain of all RMSF compared with 4ZZI, 4ZZJ, and 5BTR receptors that did not show fluctuations, indicating that binding of the 4TO compound to the receptor is more stable.

Fig. 3: (a) Molecular docking of 4TO to 4ZZJ at the allosteric region. (b) Visualization of 4TO:4ZZJ complex interactions at Leu206 (hydrophobic interaction), Thr209, Leu215 (hydrophobic interaction), Thr219 (hydrophobic interaction), Ile223 (hydrophobic interaction), Ile227 (hydrophobic interaction), and Asn226 (hydrogen bond acceptor) using the LigandScout program. (c) Visualization of the 4TO:4ZZJ complex using a mesh surface image generated by CHIMERA (hydrophilic, blue; hydrophobic, orange)

Fig. 4: (a) Molecular docking of 4TO compound with protein data bank 4ZZH at the allosteric region. (b) Visualization of 4TO:4ZZH complex interactions at amino acids Leu206 (hydrophobic interaction), Thr209, Leu215 (hydrophobic interaction), Thr219 (hydrophobic interaction), Ile223 (hydrophobic interaction), and Ile227 (hydrophobic interaction) using the LigandScout program. (c) Visualization of 4TO:4ZZH complex using a mesh surface image generated by CHIMERA (hydrophilic, blue; hydrophobic, orange)

Fig. 5: (a) Molecular docking of compound 4TO:5BTR at the allosteric region. (b) Visualization of 4TO:5BTR complex interactions at Leu206, Thr209 (hydrogen bond acceptor), Leu215 (hydrophobic interaction), Thr219 (hydrophobic interaction), Ile223 (hydrophobic interaction), and Ile227 (hydrophobic interaction) using the LigandScout program. (c) Visualization of 4TO–5BTR complex using a mesh surface image generated by CHIMERA (hydrophilic, blue; hydrophobic, orange)
Fig. 6: Root mean square deviation and conformational changes of receptor structure at an interval of 10 ns (a). Zn:4ZZI, (b). peptide: Zn: NAD+:4ZZJ, (c). Zn:4ZZH, and (d). peptide: Zn:5BTR with 4TO at 300 K
The 3rd International Conference on Global Health (ICGH), Universitas Indonesia, Bali, Indonesia

Fig. 7: Root mean square fluctuation complex of 4TO ligand with 4ZZI, 4ZZJ, 4ZZH, and 5BTR macromolecules

Fig. 8: Root mean square fluctuation of the backbone atoms from 4ZZI, 4ZZJ, 4ZZH, and 5BTR complex with 4TO in the allosteric domain

Fig. 9: Snapshot dynamic simulation of 4TO interaction with 4ZZI and 4ZZH receptors, whereas Glu230 residue (allosteric region) with Arg446 (catalytic region) did not show hydrogen bonding

Hydrogen bond analysis
As an activator of SIRT1 PDB ID 4ZZJ, the 4TO ligand crystal shows the presence of hydrogen bond interactions at Arg234–Asp475 (NH–O) 2.83Å, Arg234–His473 (NH–O) 2.84Å, and Arg234–Val459 (NH–O) 3.01 Å [7]. The VMD program was used to perform hydrogen bond strength analysis (% occupancy) of 4TO with SIRT1 PDB IDs 4ZZI, 4ZZJ, 4ZZH, and 5BTR from molecular dynamic simulations for 50 ns. Based on the molecular dynamic simulations, 4TO in complex with 4ZZI and 4ZZH receptors did not show any hydrogen bond interactions between Glu230 (allosteric regions) and Arg446 (catalytic regions). Fig. 9 shows the binding of 4TO ligand to the allosteric region but not to the activator of SIRT1.

The results of the hydrogen bond strength (% occupancy) in the complex of the 4TO ligand with 4ZZJ receptor indicated the presence of important amino acid interactions, namely Glu230–Arg446, 51%; Arg234–Asp475, 65.1%; Arg234–His473, 22.5%; and Arg234–Val459, 11.7%. The hydrogen bond strength (% occupancy) in the complex of the 4TO ligand with the 5BTR receptor showed the presence of important amino acid interactions, namely Glu(E)230–Arg(R)446, 65.80%; Arg(R)234–Asp(D)475, 70.9%; Arg(R)234–His(H)473, 33.3%; and Arg234–Val459, 4.9% (Fig. 10). The results of this analysis indicate that the SIRT1 activator is showed by hydrogen bond interactions between Glu230 and Arg234 (allosteric region) with Arg446, Val459, His473, and Asp475 (catalytic region) which are close to the bound substrate region.

Free energy calculation (ΔG)
The result of the ΔG bond energy calculated from the molecular docking with AutoDock4Zn was then recalculated to determine the free bonding energy from the 4TO compound with SIRT1 PDB IDs 4ZZI, 4ZZJ, 4ZZH, and 5BTR using the MM-GBSA method, with simulation dynamics carried out for 50 ns. This was performed to ensure that the bond energy was more selective [23], and the energy value of the 4TO ligand bond with 4ZZJ receptor using the MMGBSA method was calculated, while MMPBSA measured bond energy on simulation dynamics for 50 ns.
The calculation of the compound complex of 4TO ligand crystal with peptide Zn: NAD+:4ZZJ found a value of MMGBSA (ΔG_{total}) = −31.4729 kcal/mol, ΔG_{total} = ΔG_{gas} + ΔG_{solv} = −51.2942 + 19.8212 = −31.479 kcal/mol.

Fig. 11 shows examples of the calculation. Fig. 12 shows the overall results.

The bond energy of 4TO:peptide:Zn: NAD+:4ZZJ showed a range of bonding energy values as follows: MMGBSA ΔG = 4.7973 kcal/mol (−31.4729—−26.6756) and MMPBSA ΔG = 4.1432 kcal/mol (−32.6292—−28.486). The bond energy of 4TO:Zn:5BTR:Ac:peptide showed a range of energy values as follows: MMGBSA ΔG = 10.56 kcal/mol (−40.6255—−30.0653) and MMPBSA ΔG = 8.6762 kcal/mol (−34.6713—−25.9591). The results obtained from

**Fig. 10: Hydrogen bond strength (% occupancy) of important amino acid interactions in the complex of 4TO:4ZZJ and 4TO:5BTR**

**Fig. 11: Calculation of free bonding energy (MMGBSA) in 4TO: peptide:Zn: NAD+:4ZZJ complex. The calculation of the compound complex of 4TO ligand crystal with peptide: Zn:NAD+:4ZZJ found a value of MMGBSA (ΔG_{total}) = −31.4729 kcal/mol, ΔG_{total} = ΔG_{gas} + ΔG_{solv} = −51.2942 + 19.8212 = −31.479 kcal/mol.**
the four different receptors (4ZZI, 4ZZJ, 4ZZH, and 5BTR) showed that the strength of the 4TO bond to 5BTR was stronger than that of the other receptors because the receptor form was more closed. A small difference in MM-GB(PB)SA range values was seen in the interaction between 4TO:Zn:4ZZI.

CONCLUSIONS
The present study describes an in silico study of SIRT1 bond interaction using a simulation approach of molecular dynamics over 50 ns using the Amber program. Analysis of activator ligand (4TO) binding to the SIRT1 receptor (PDB IDs 4ZZI and 5BTR) showed selectivity of the ligand by marked hydrophobic bond features on Leu206, Ile223, Asn226, Ile227, and Glu230 of the 4ZZJ and 5BTR receptors.

Hydrogen bond interactions between Glu230 and Arg234 (allosteric regions) with Arg446, Val459, His473, and Asp475 (catalytic areas) ensured that they became close to the bounding substrate area. The bond energy values obtained for 4TO interacting with 4ZZJ using the MM-GB(PB)SA calculation using AMBER were as follows: MMGBSA $\Delta G = -31.4729$ $-$ $26.675$ and MMPBSA $\Delta G = -32.6292$ $-$ $28.486$. The bond energy values of the 4TO interaction with 5BTR were as follows: MMGBSA $\Delta G = -40.6255$ $-$ $30.0653$ and MMPBSA $\Delta G = -34.6713$ $-$ $25.9951$. These results are important for drug discovery and development as they give insight into target interaction of the bonds to the more selective SIRT1 activator [24-26].

ACKNOWLEDGMENTS
We thank to the Universitas Indonesia for the TADOK 2018 (Tugas Akhir Hibah Doktor) research grant.

CONFLICT OF INTEREST
There are no conflicts of interest to declare.

REFERENCES


**Supplemental Figure**

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Supplemental Fig. 1: (A) (i) Visualization of 4ZZI. (ii) The structure of the 4TO:4ZZI complex shown as a mesh surface image generated by CHIMERA (hydrophilic, blue; hydrophobic, orange). (iii) Three-dimensional image generated using LigandScout. (B) (i) Visualization of 4ZZJ. (ii) The structure of the 4TO:4ZZJ complex shown as a mesh surface image generated by CHIMERA (hydrophilic, blue; hydrophobic, orange). (iii) Three-dimensional image generated using LigandScout. (C) (i) Visualization of 4ZZH. (ii) The structure of the 4TO:4ZZH complex shown as a mesh surface image generated by CHIMERA (hydrophilic, blue; hydrophobic, orange). (iii) Three-dimensional image generated using LigandScout. (D) (i) Visualization of 5BTR. (ii) The structure of the resveratrol:4ZZJ complex shown as a mesh surface image generated by CHIMERA (hydrophilic, blue; hydrophobic, orange). (iii) Hydrogen bond interaction at the allosteric region.

Supplemental Fig. 2: Molecular docking of the 1NS compound (SIRT1 inhibitor) as a negative control in the allosteric site.