MOLECULAR MODELING

Computer-assisted simulated docking experiments were carried out using Molecular Operating Environment (MOE Dock 2008) software, Chemical Computing Group, Montreal, Canada.

Antimicrobial docking study

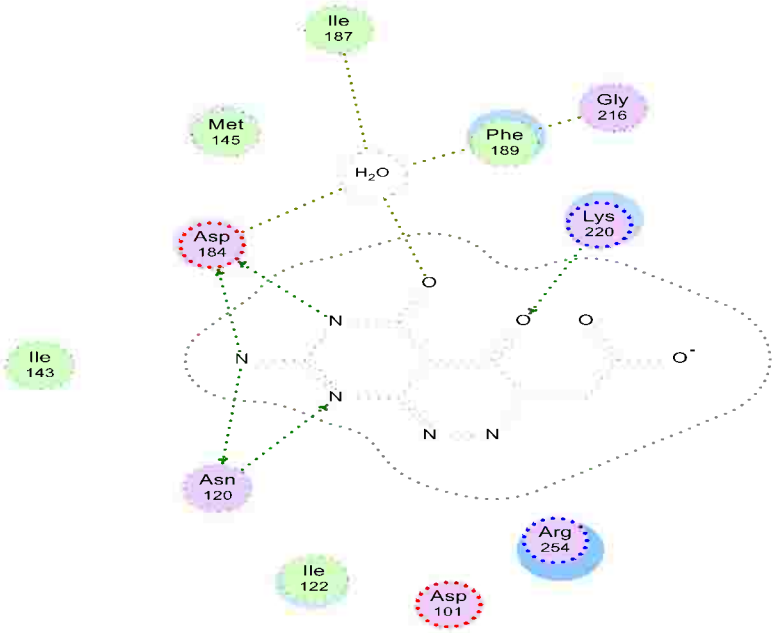
Dihydropteroate synthase (DHPS) is the validated drug target for sulfonamides antimicrobial therapy. However, due to widespread drug resistance and poor tolerance, the use of the sulfonamide antibiotics is now limited. The pterin binding pocket in DHPS has a high degree of conservation and is distinct from the sulfonamide binding site, and therefore represents an attractive alternative target for the design of novel antibacterial agents. DHPP binds in the pterin binding pocket, deep within the DHPS β-barrel where there is a high degree of conservation and in which sulfa drug resistance mutations have never been observed. The pterin binding site is therefore a very attractive alternative target for the design and development of novel antimicrobial agents, and we have been pursuing this goal. [110]

Experimental:

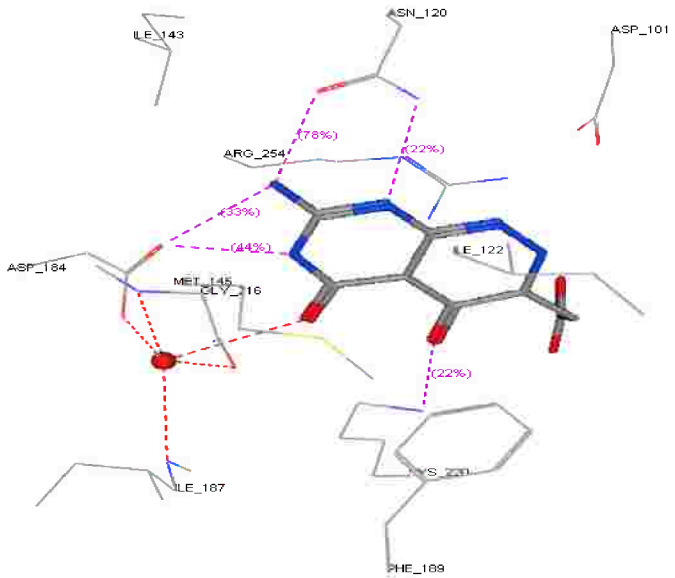
Co-crystal structures of *B. anthracis* DHPS with (7-Amino-4,5-dioxo-1,4,5,6-tetrahydro-pyrimido[4,5-*c*]pyridazin-3-yl)-acetic acid (PDB ID code: **4DAI**) was downloaded from the protein data bank. The ligand molecules were constructed using the builder module and were energy minimized using Force Field MMFF94X. The active site of DHPS was generated using the MOE-Alpha site finder, and then ligands were docked within this active site using the MOE Dock. MOE was also used to calculate the best score between the ligands and the active site interactions. Ten conformers of the ligand were retained with the highest and best score by default which shows the best ligand active site interactions.

Discussion:

Concerning the 4DAI X-ray complex, the co-crystallized ligand displayed hydrogen bonding between amino, N6 and Asp 184, also between amino, N8 and Asn 120 and between 4-oxo and Lys 220.



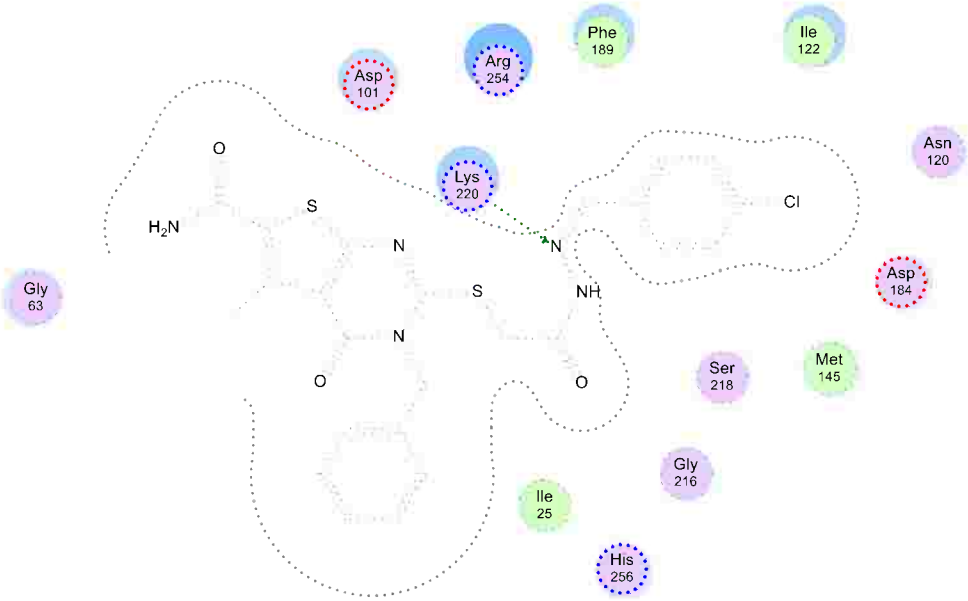
2D ligand- enzyme active site interactions



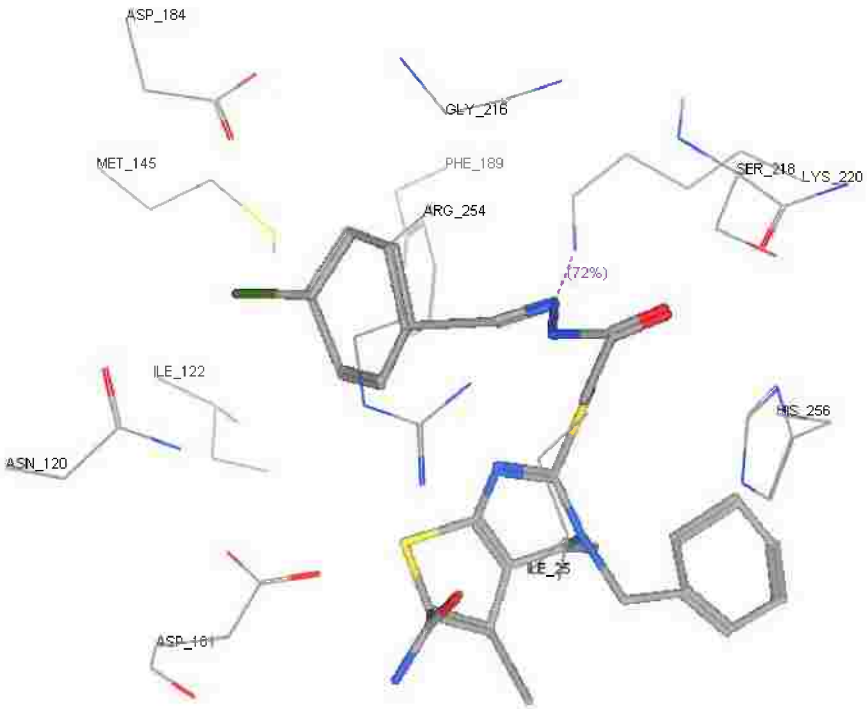
3D ligand-enzyme active site interactions

Figure 1: Co-crystallized ligand in the binding site of DHPS (PDB ID: 4DAI)

In compound **10b** strong hydrogen bonding is observed between imine nitrogen of the hydrazone and Lys 220 of score 72%.



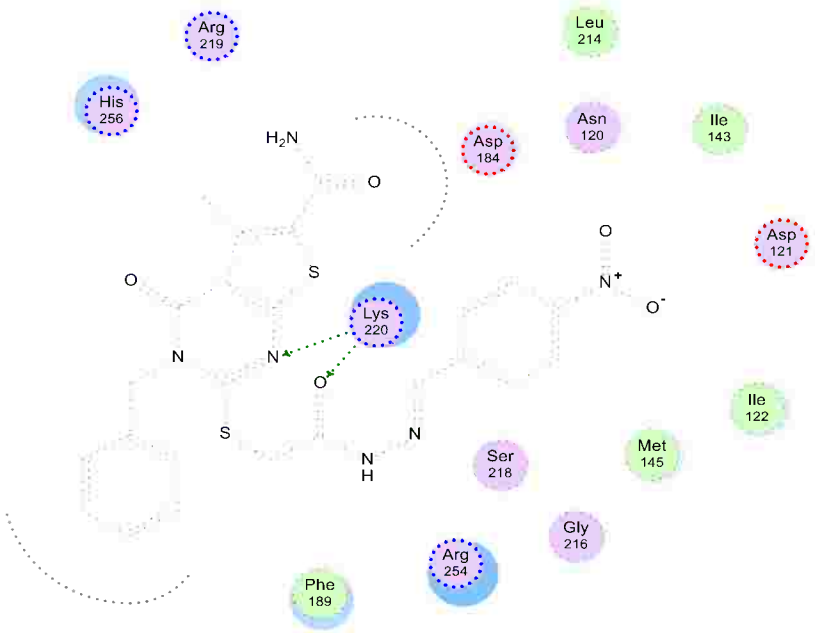
2D ligand (10b)- enzyme active site interactions



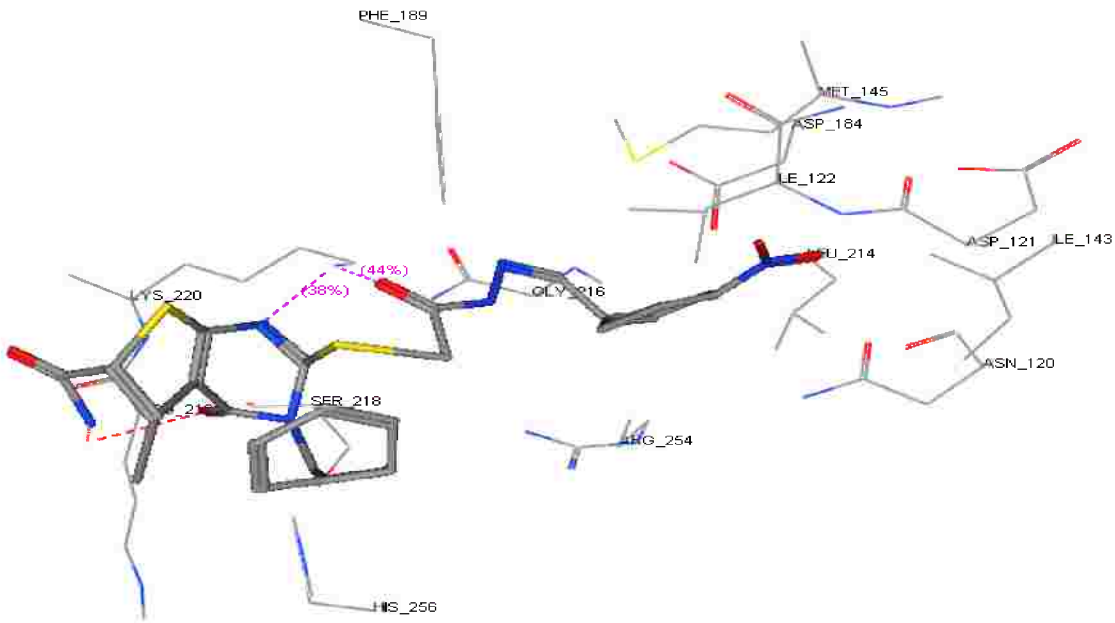
3D ligand-enzyme active site interactions

Figure 2: Compound 10b in the binding site of DHPS using MOE software

Molecular modeling studies of our target compound **10d** indicated that carbonyl oxygen of carbonyl methyl sulfanyl side chain and N1 of thienopyrimidinone ring contribute hydrogen bonding interaction with Lys 220 of score 44% and 38% respectively.



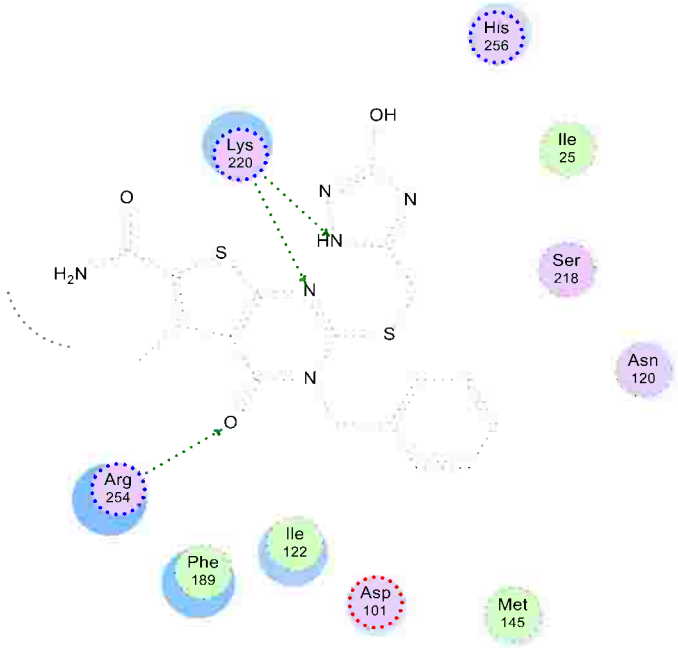
2D ligand (10d)- enzyme active site interactions



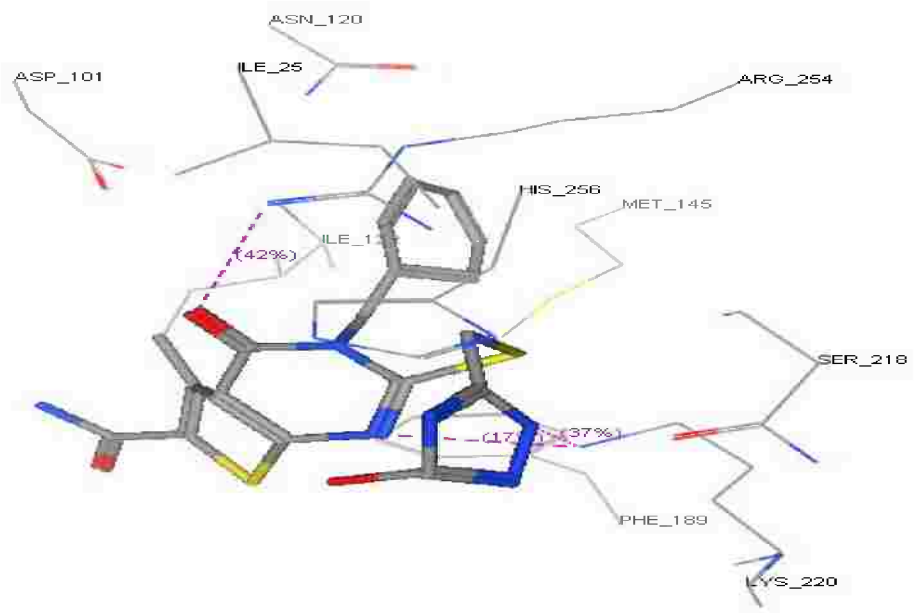
3D ligand-enzyme active site interactions

Figure 3: Compound 10d in the binding site of DHPS using MOE software

In compound **17a** hydrogen bonding was observed between triazolyl NH, N1 in theinopyrimidin-4-one and Lys 220 of score 37% and 17% respectively as well between carbonyl of thienopyrimidin-4-one and Arg 254 of score 42%.



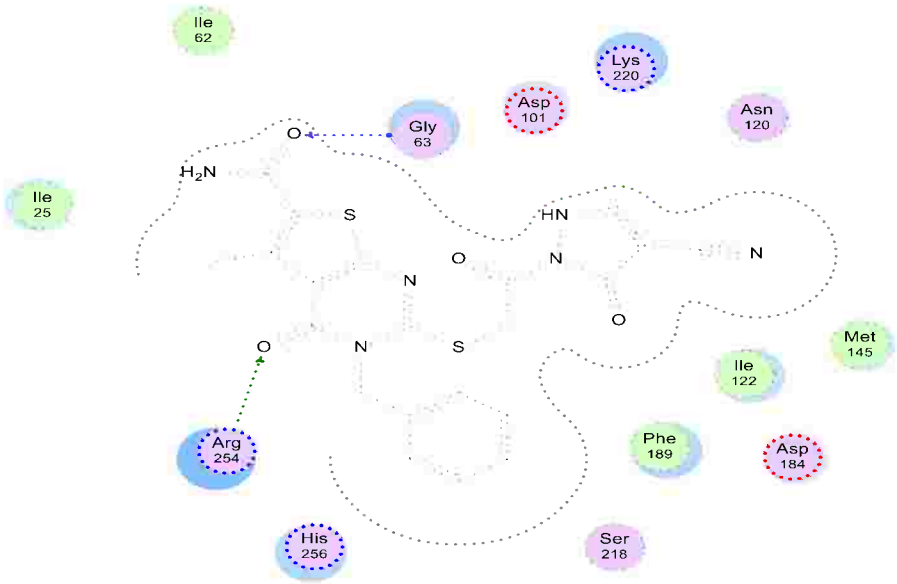
2D ligand (17a)- enzyme active site interactions



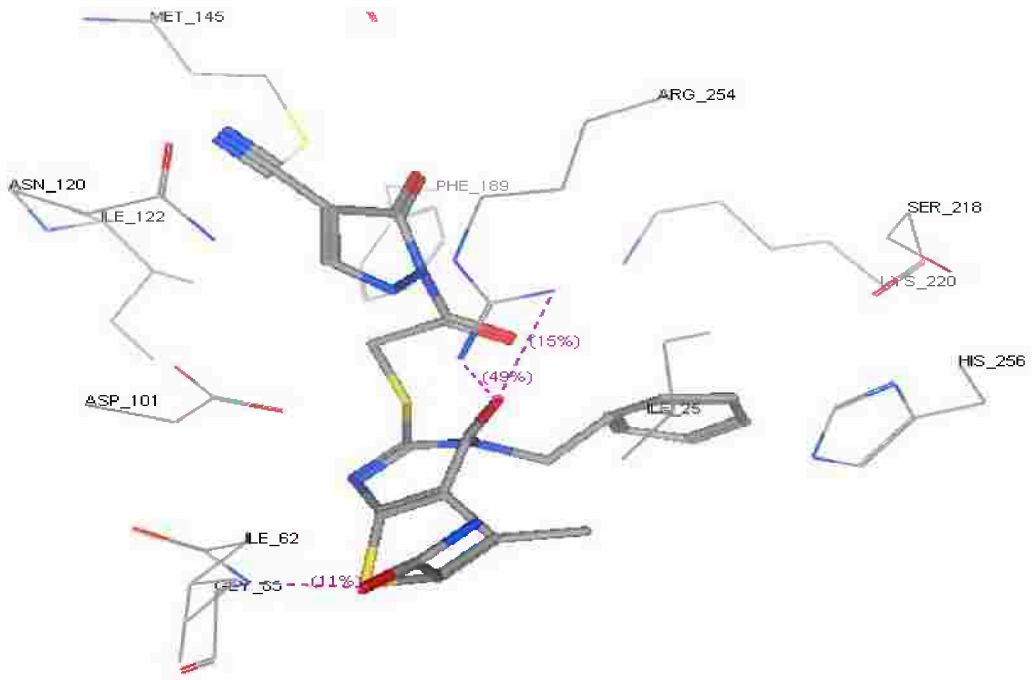
3D ligand-enzyme active site interactions

Figure 4: Compound 17a in the binding site of DHPS using MOE software

Whereas in compound **19** the interactions showed hydrogen bonding between carbonyl of theinopyrimidin-4-one and Arg 254 of score 49% and aslo between carbonyl of amide side chain at position 6 and Gly 63 of score 11.3%.



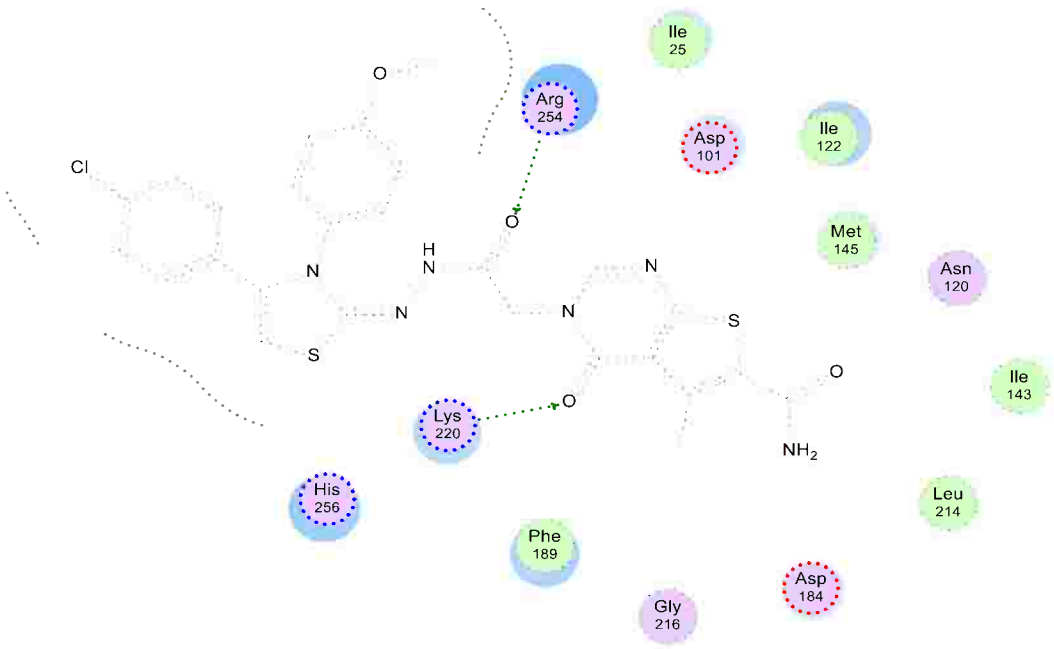
2D ligand (19)- enzyme active site interactions



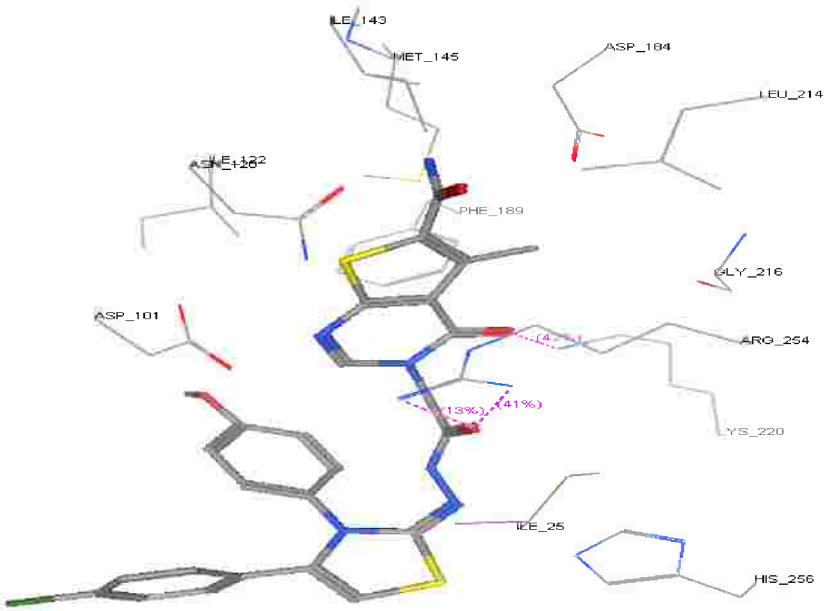
3D ligand-enzyme active site interactions

Figure 5: Compound 19 in the binding site of DHPS using MOE software

As for compound **23a** hydrogen bonding is shown between carbonyl of thienopyrimidin-4-one and Lys 220 of score 41.6% and also between carbonyl of hydrazino carbonyl methyl side chain and Arg 254 of score 41.4%



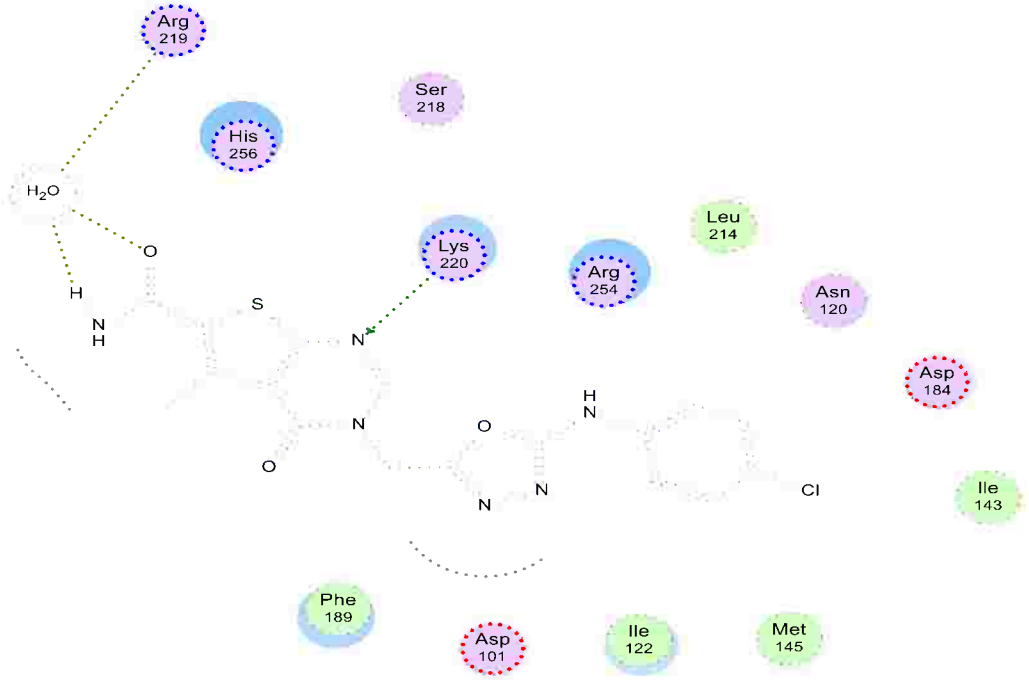
2D ligand (23a)- enzyme active site interactions



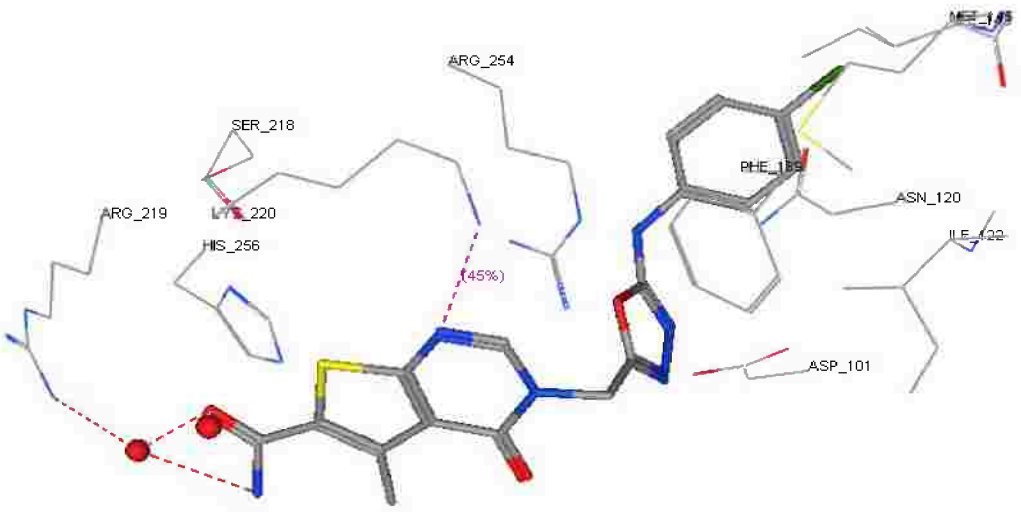
3D ligand-enzyme active site interactions

Figure 6: Compound 23a in the binding site of DHPS using MOE software

Also in compound **24a** hydrogen bonding is between N1 of theinopyrimidin-4-one and Lys 220 of score 45% and between amide side chain at position 6 of theinopyrimidin-4-one and water molecule that hydrogen bonds with Arg 219.



2D ligand (24a)- enzyme active site interactions



3D ligand-enzyme active site interactions

Figure 7: Compound 24a in the binding site of DHPS using MOE software

Conclusion:

In conclusion, modeling studies revealed that all the docked compounds **10b, 17a** and **24a** showed hydrogen bonding interactions of N1 of theinopyrimidin-4-one with the residue Lys 220 and also carbonyl of theinopyrimidin-4-one is important for activity as it exhibit hydrogen bonding with amino acid residue Arg 254 in compounds **17a** and **19** and Lys 220 in compound **23a** besides hydrophobic interactions between all the docked compounds and the enzyme active site. All of these could be attributed to their antimicrobial activity.

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