Insilico Simulation Studies for Tuberculosis Drug Discovery and its Response

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Abstract
From high treatment and with such precaution measures TB (Tuberculosis) came with leading cause of deaths in the world. For Tuberculosis it increasing drug resistance for different kind of TB like Multidrug-resistant TB and Extensively drug-resistant TB, increasing relapse and in efficiency of drug for TB pathogen.it take longer duration, it affect on human body with infection from HIV and other autoimmune disorder have pushed it to a lethal level and for this we need more effective drugs. With the help of CADD technology we can findout more viable drug molecules for the better treatment of Mycobacterium tuberculosis .Insilico drug design is a more efficient ,time saving and cost effective process. With the help of inslico drug design we can identify/Generate lead molecules that can inhibit the activity of TB. In inslico process we took 4GZR protein which is Crystal structure of the Mycobacterium tuberculosis and lead(ligand) with the support of computer aided drug design (CADD) pipeline and bioinformatics software tools.

Key words: Tuberculosis, Insilico Drug Design, Computer Aided Drug Design (CADD), Bioinformatics Tool and Technology.

INTRODUCTION

Tuberculosis

TB (Tuberculosis) is a contagious disease which is trigger by the bacterium Mycobacterium tuberculosis (1). Though caused by a single pathogen.it is a major source of TB in worldwide. It's developed when a person with active TB disease in the lungs with coughs or sneezes and someone suck in the exhaust the droplets, which carry TB bacteria (2). Aerosol travel to larynx and reaches the bronchiole and to the parts of the alveoli and affects the respiratory system. According to the universal Tuberculosis control report, world health organization in 2016, almost 10.4 million new cases came out and registered for Tuberculosis throughout the world in 2015 (3). TB have many indication before infections , it will damage bone, the part of central nervous system, and many other organelles, but in beginning a pulmonary disease that initiated due to deposition of Mycobacterium tuberculosis, it holds in aerosol droplets over lung alveolar surfaces. From this place, the progression of the disease can find out many conclusions, and further it goes for determined more by the reactions of the host immune system. The advantages of this reactions is concerned by intrinsic points such as the genetics of the immune system, *e.g.*, damages may be occur for the host in their immune system and physiological and nutritional state.

Types Of Tuberculosis

There are two types of TB (i) latent and (ii) active. Through air the bacterium enters in to the body. The bacterium present does not affect the human body due to immune responses of the macrophage cells and it is harmless(2). This stage is latent TB . Nearly 10 % of the whole world is affected by latent TB . Active TB is when the bacterium affect the body. Latent TB may convert anytime with in Active TB. The mycobacterium tuberculosis surpasses host immune response, it becomes Active. Active TB can cause severe disease and death. Immune-suppressed people have more chances of acquiring active tuberculosis as the body has no ability to fight against the disease(4) . Hence TB is commonly seen as a co-infection with AIDS, Diabetes and other auto immune disorders.

insilico drug designing

In Silico Drug Design is Techniques and Methodologies that specify the appliance of **computational tools** which will be make use of this approaches. The covers of book theoretical background and methodologies of chemo bioinformatics techniques and network modeling and discusses the numerous applied strategies to systematically retrieve, integrate and analyze datasets from diverse sources. It is now the widely used technique in pharmaceutical industries and academics for aiding in drug discovery. Many drugs are now identified using computational methods. It is used before the drug discovery. It screens a number of compounds in a smaller time. It retained the same level of lead to hit discovery as wet lab(12) .it is gaining, popularity and is implemented in drug discovery as there are advances in software and hardware computational power . Availability of target and molecule structures graphically, databases on the required data makes In silico drug screening a larger possibility. The drug development and discovery process approx. take 7-12 years. The methods used were **High Throughput Screening (HTS)** and combinatorial library screening(13). This technique utilizes a large library of compounds that are tested against the pathogen cells or infected cells and the molecules which express the desired biological response are chosen as hits. But screening identifies a very low no. of hit to lead compounds. This conventional screening also requires minimal compound design knowledge and prior knowledge about it and high technologies are required for screening large compounds (32). Identifications of the hit can only take 2-3 years. It nearly consumes all the time and resources required. In case of Tuberculosis Mycobacterium tuberculosis is highly pathogenic and it has to be used in a highly protected manner. Also the pathogen takes time to replicate. It takes months to develop a colony. There are high chances of spread of the disease. The mode of action of the hit molecule can't be identified. These constraints are reduced in silico designing. In Silico screening requires minimal numbers of compounds to find the hit molecules (14). There is a high numbers of hit to lead identified. These hits are highly target specific and the modes of action are easily identified. The time-frame required for the insilico screening is fourfold lesser compared to the traditional methods. This also reduces the workload and price requirements. It requires less preparation time. While preparing for HTS assay, the In silico drug designing can be completed. Hence In silico method is a better and unidirectional option for drug design.

MATERIAL AND METHOD

METHODS

Preparation of Ligand Molecules

50 chemical compounds of anti-tuberculosis properties (Levofloxacin, Ethionamide, Aspirin) were retrieved from the database, this step is used for the retrieval of optimised Ligand molecules in Sdf format. After downloading this structure we went for energy minimization of ligand using chimera software where we edited the structure and minimize the structure and save it in to Pdb (Protein Data Bank) format.

Preparation of Protein Structure

Protein Optimization - To use the protein structures for performing the Molecular Docking with the suitable ligands for tuberculosis drug discovery we will retrieve the protein structure from Pdb. The crystall structure of 4gzr protein in complex with its substrate bring back from PDB database The ligands of the protein were removed. Ligand and protein were present in the active sites, so if ligands were removed the active site pocket remains empty and new molecules can be docked into it.

Protein Energy Minimization - After performing optimization process it become necessary to minimize the Energy of the protein in concern to stabilize the protein for performing the docking process. With the help of Spdbv software we go for energy minimization of protein (4gzr) and after energy minimization process our protein is also ready for docking.

Protein-Ligand Docking

In the molecular modelling, Docking is a method where we can check out the selected orientation of 1st molecule to 2nd when that combines to each other to form a stable complex and these predictions find best binding affinity with the help of their scoring functions. A protein-ligand docking study was performed using Autodock 4.0.

Hydrogen Bond analysis

The coordination of the docked protein along with the ligand will visualized using software within 6.5 Á region to discover the H-bond with active site residues (Ala 26 A) of our proteins.

Prediction of Toxicity

The molecules which have shown Hydrogen-Bond with the active site of the selected protein they were checked for toxicity analysis with Lipinski's rule of 5 and also we identified drug likeness of the molecule just to identify the drugability of the molecule.

As we all know **ADMET** analysis is very important process of any drug discovery process it helps us in identifying that how a drug can be **Administered, Distributed, Metabolised, Excreted** out without causing any **Toxicity**. Lipinski's rule of 5 is a major component of identifying ADMET for any molecule.

Molecular Dynamics and Simulation

molecular dynamics simulations is the One of the principal tools in the theoretical study of biological molecules. With the help of computational methods we can calculate the timing of simulations. MD simulations provides basic information on their fluctuations and conformational changes of proteins and nucleic acids of drug molecules and this methods are used for analysis of their structure ,and thermodynamics of the complexes.

RESULTS

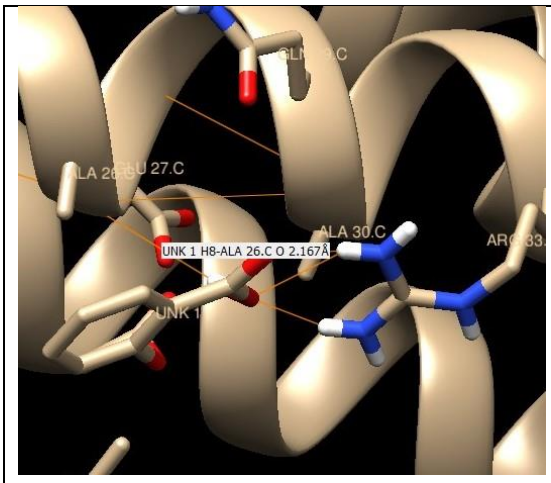
Minimum Binding Energy Table

After we performed molecular docking for all 50 molecules we have noted the minimum binding energy of the interaction along with the information about in which run out of 10 runs we have got best binding energy. So we have noticed that the minimum binding energy ranges between -3.52 to -9.46 hence it can be confirmed as positive result and can be continued for preparing the docking complex and visualization.

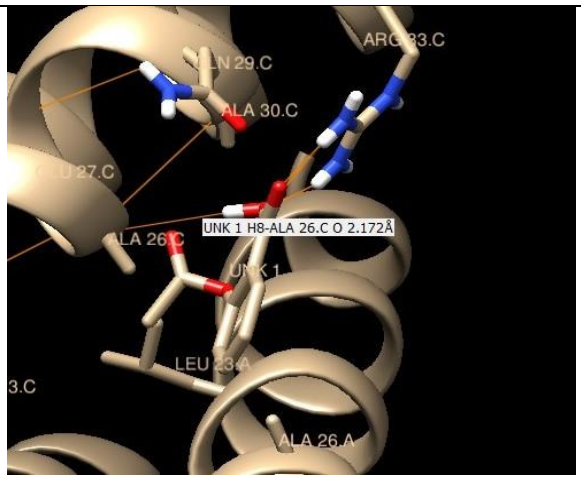
Molecules	Minimum Binding Energy	Run
3	-4.29	9
9	-4.26	8
10	-3.82	9
14	-5.13	6
25	-4.15	8
34	-6.9	5
47	-5.14	2
48	-5.06	2

H-Bond Interaction With Active Site Residues (Ala 26 A) Of Proteins

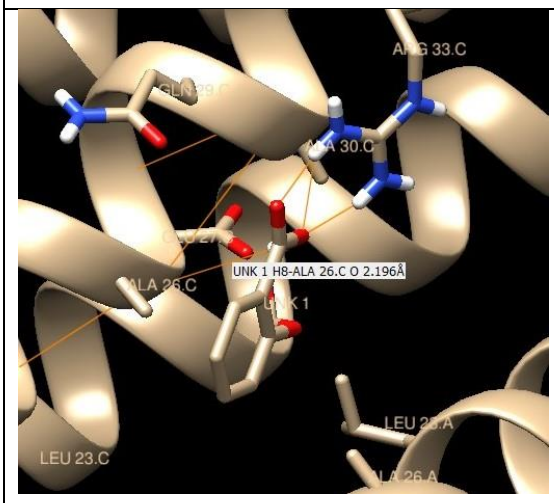
Once we prepared the docking complex (Drug molecule) we have performed visualization of these docking complexes to check whether the interaction has taken place between our specified Active site and the ligand molecule. After visualising our molecules (all 50) we found Hydrogen bond interaction for Molecule – 3, 9, 10, 14, 25, 34, 47 & 48.



Molecules 3



Molecules 9



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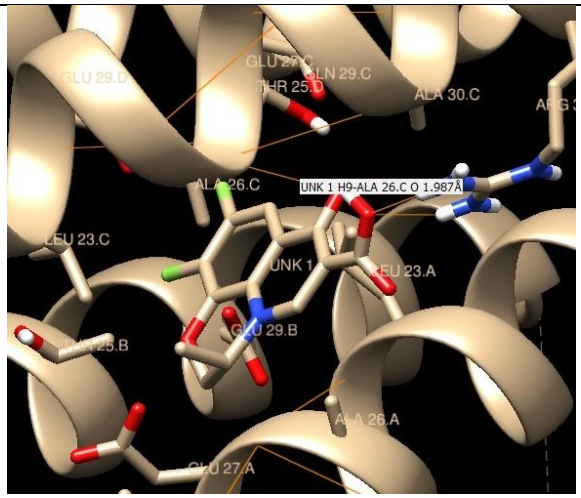
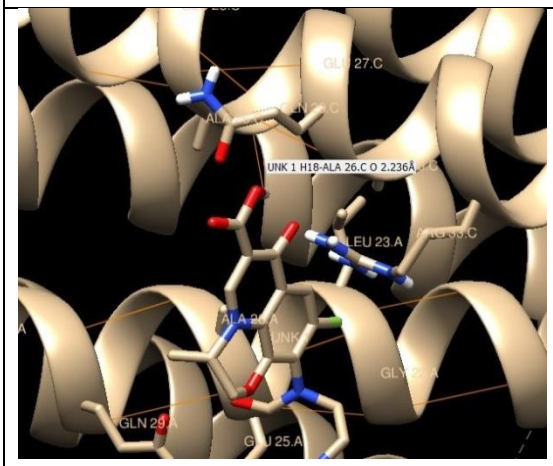
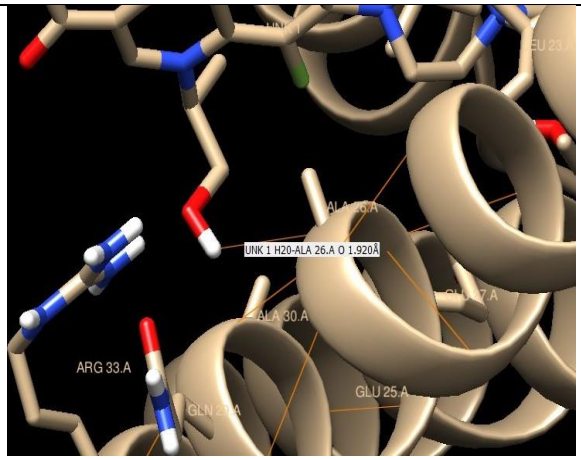


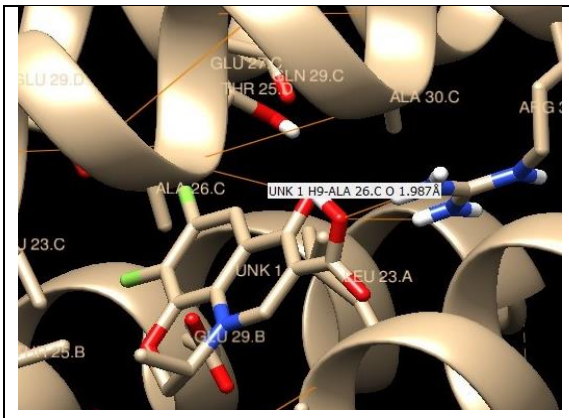
Molecules 14



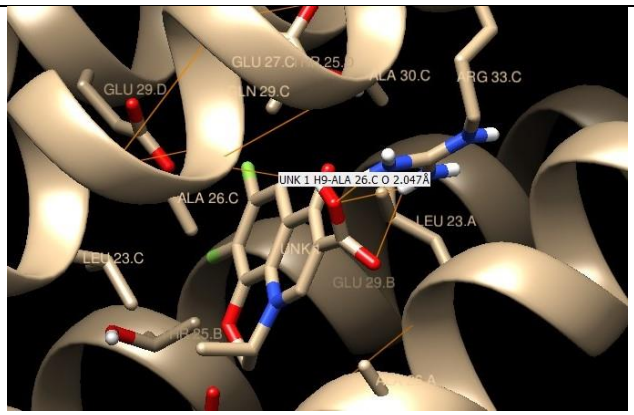
Molecules 25



Molecules 34



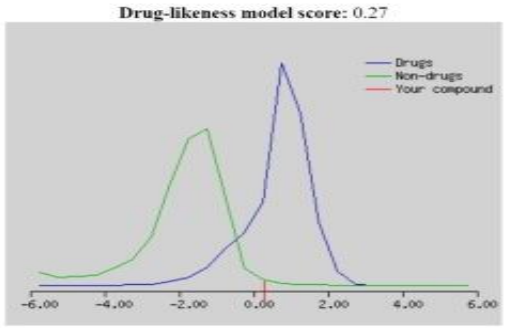
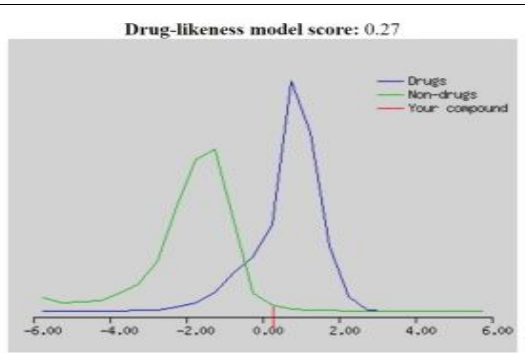
Molecules 47

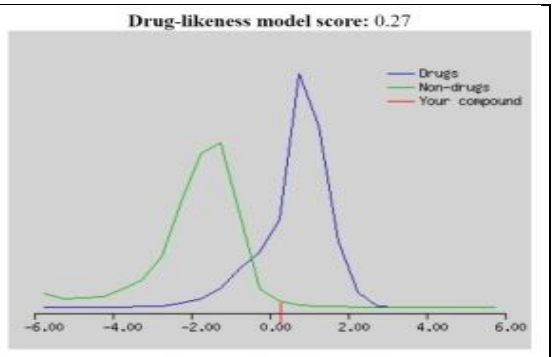
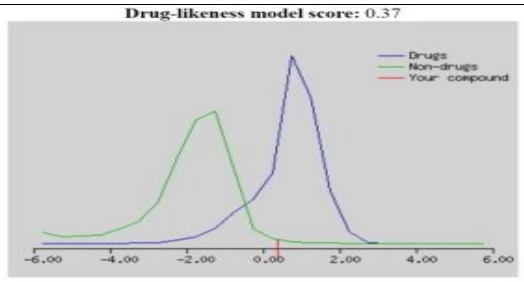
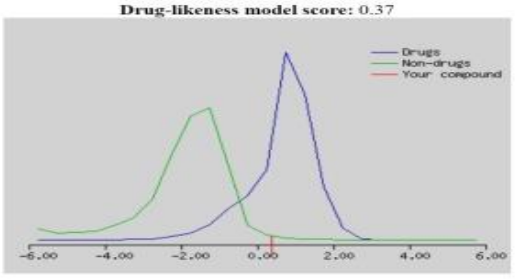
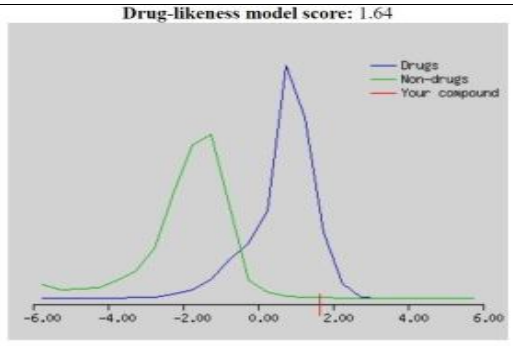
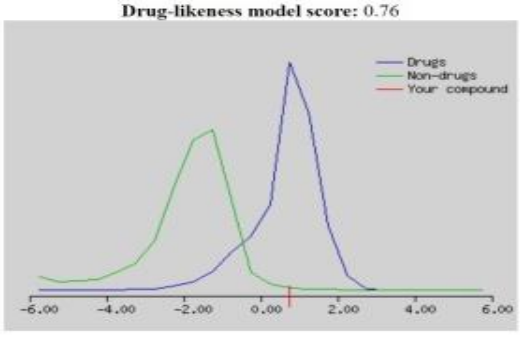


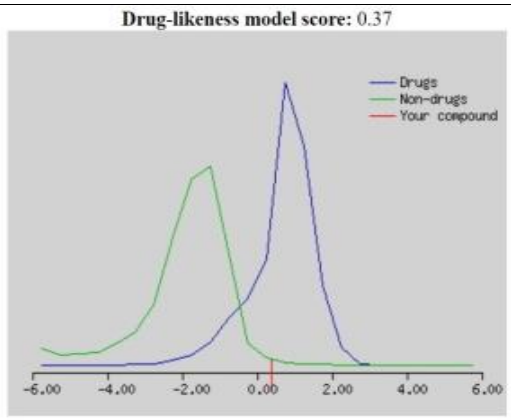
Molecules 48

Drug Likeness Of Selected Molecules

The molecules for which we found Hydrogen bond interconnection between the define Active site and ligand molecule (aspirin, ethionamide, levofloxacin), we have performed Lipinski's rule of 5 to check whether these molecules have any violations or not. Also we have checked the drug likeliness score to identify whether the molecule is a good drug or not. Hence we found No violations for Lipinski's rule of 5 from any of the 8 selected molecules. Now these molecules can be further analysed for stability with Molecular Dynamics Simulation.

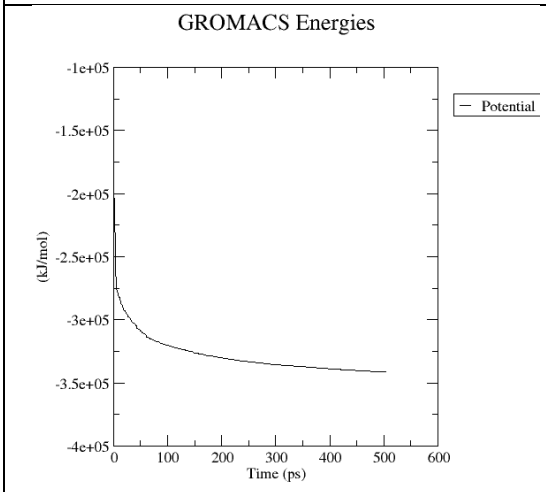
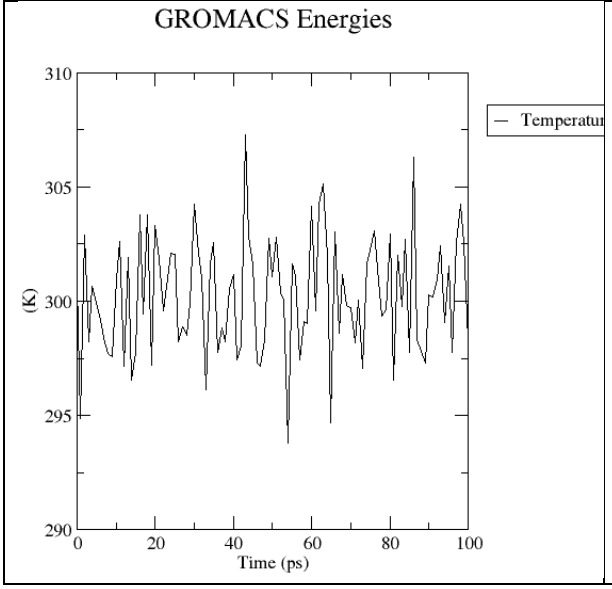
Molecules	Mol.wt(< 500 D)	Hba(<10)	Hbd(<5)	Log P(<5)	Drug likelines s score	screenshot
3	180.04	4	1	1.22	0.27	
9	180.04	4	1	1.22	0.27	

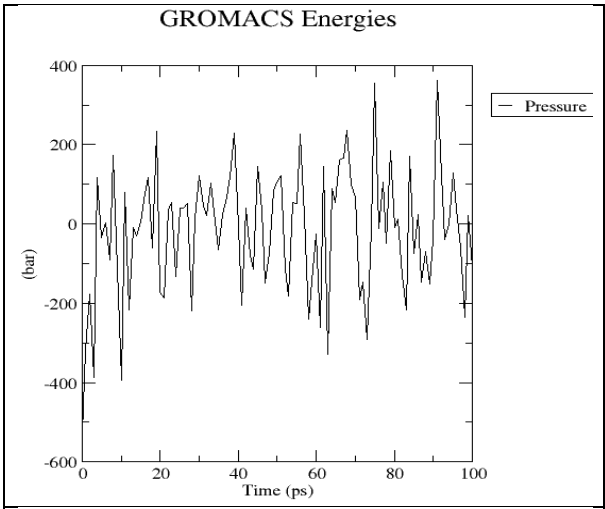
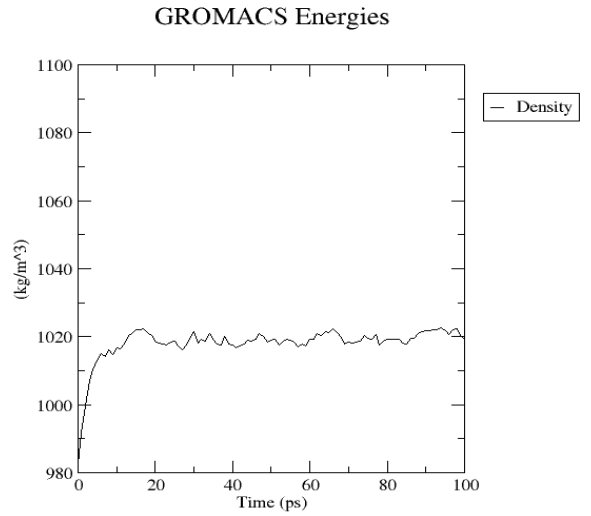
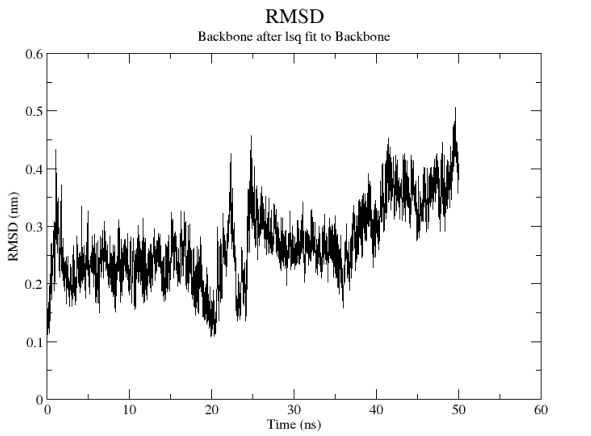
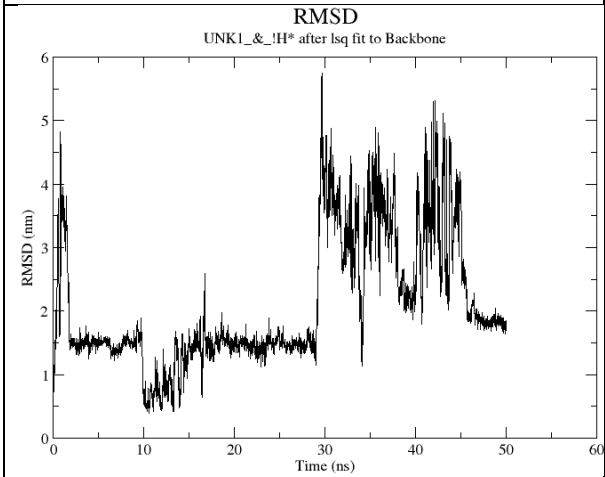
10	180.04	4	1	1.22	0.27	
14	281.05	4	1	0.77	0.37	
25	391.12	6	1	-0.98	0.77	
34	381.15	5	2	-0.10	1.64	
47	279.05	5	2	0.34	0.76	

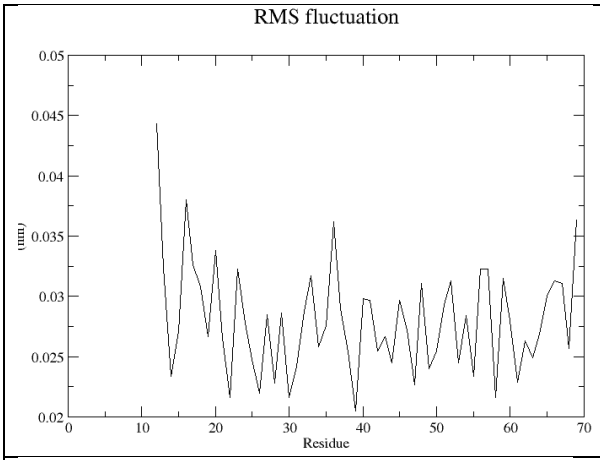
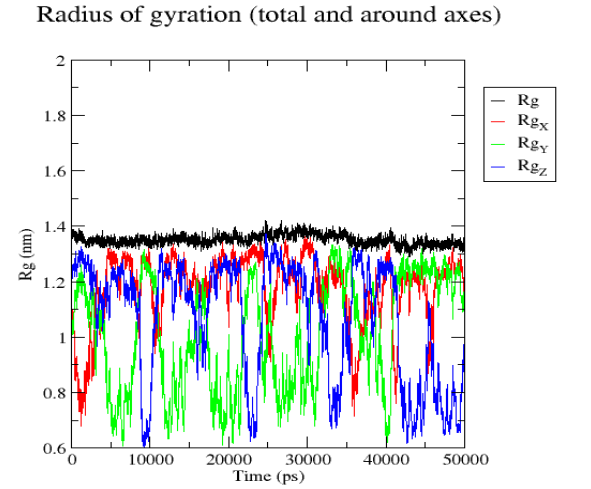
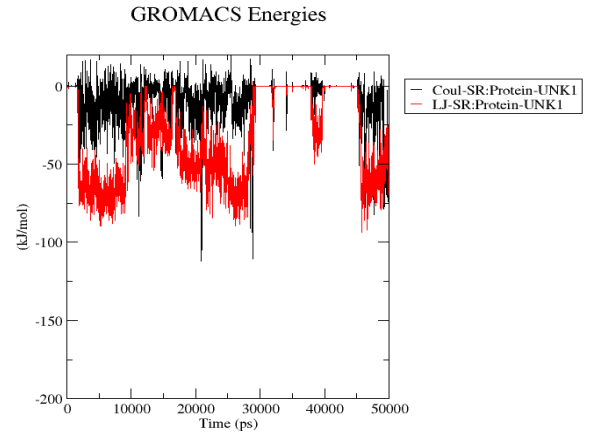
48	281.05	4	1	0.77	0.37	

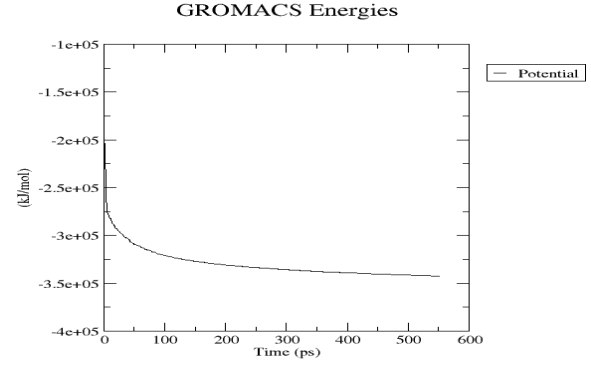
Molecular Dynamics Simulation

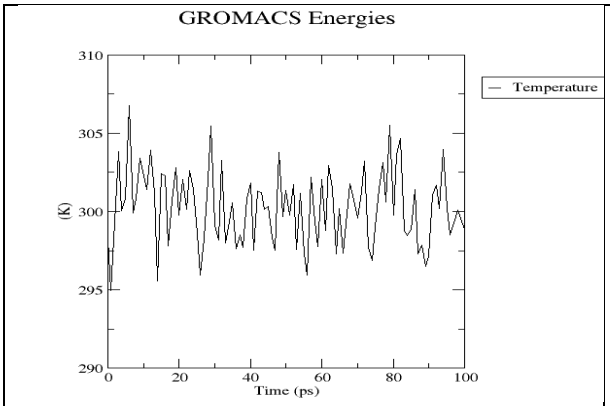
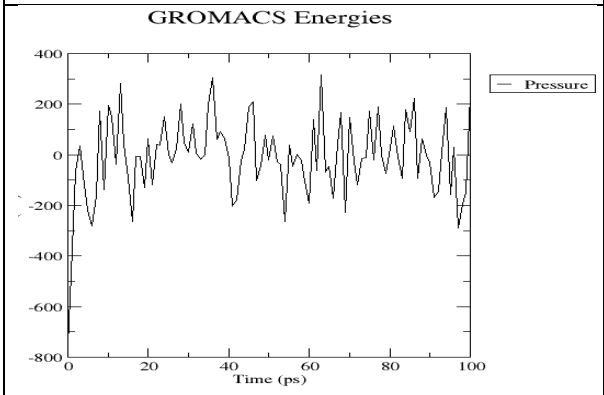
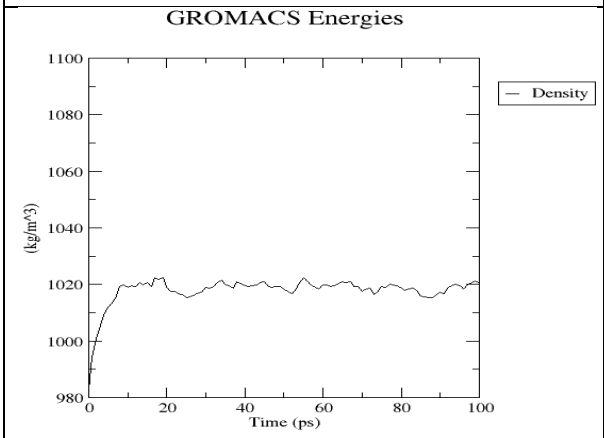
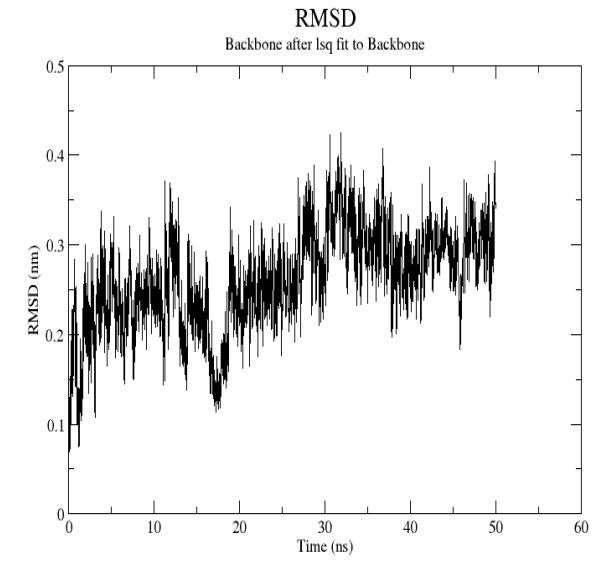
We performed MD Simulation for non toxic drug molecules to check the stability of these drug molecules. We have run Simulation for these molecules at 50 NS and then several graphs were generate to analyse the stability of the drug molecules. Mentioned below are the generated graphs along with their interpretations.

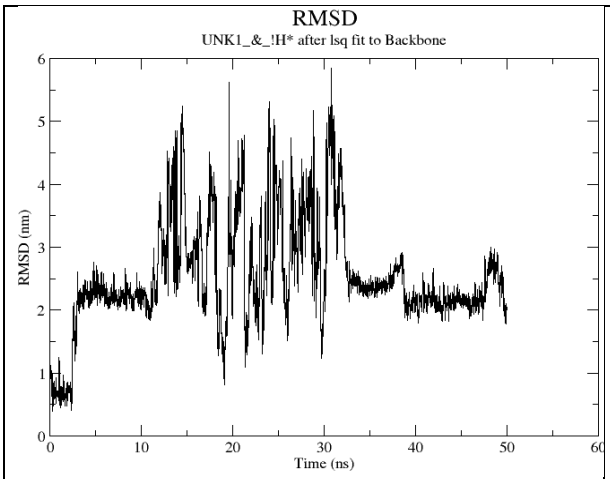
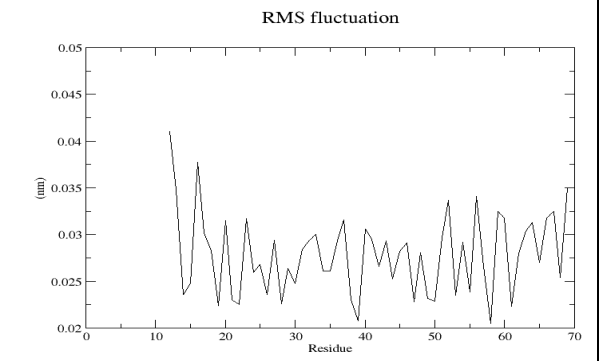
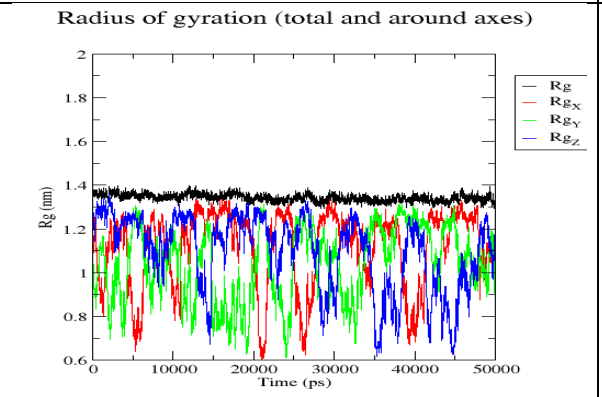
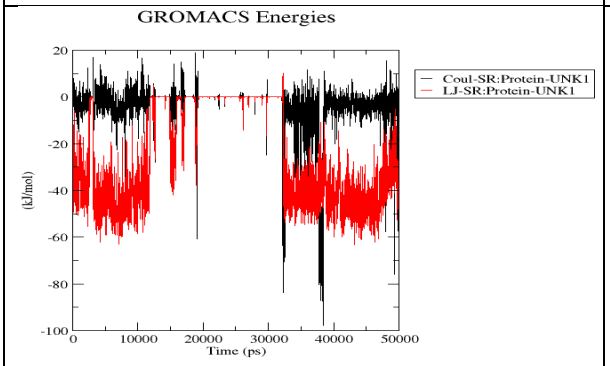
MOLECULE - 3	
Graph	**Interpretation**
	Potential energy graph confirm the energy minimisation of drug complex.
	It shows us the equilibrium range of drug complexes.

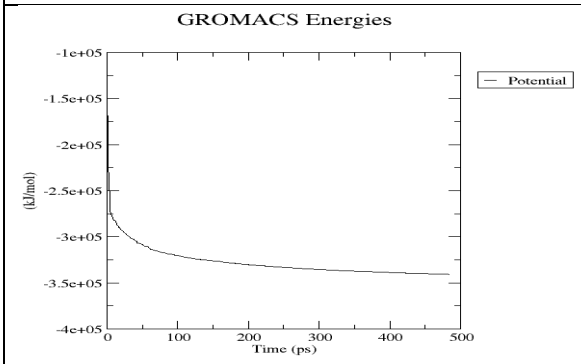
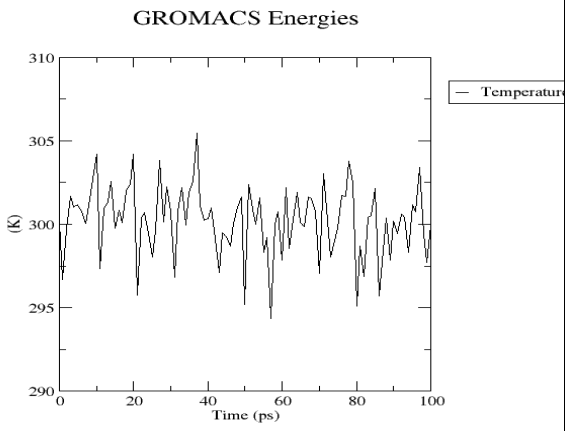
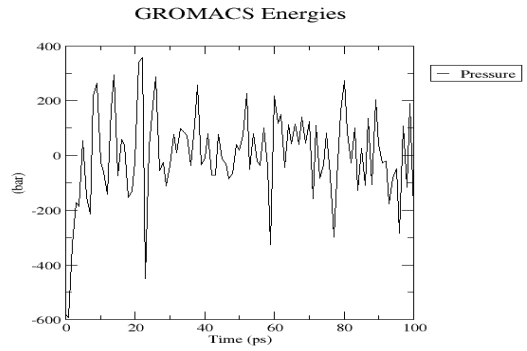
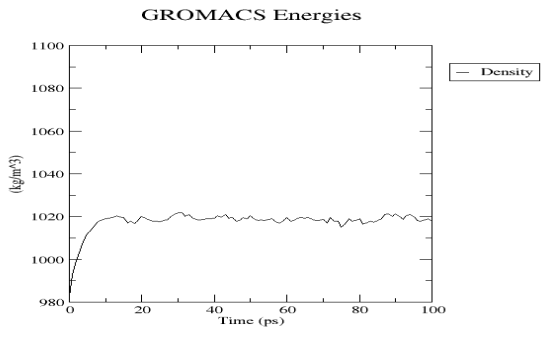
	Range is in between +200 to -200. If this comes as output for drug complex that means stability of drug is high
	The average value is 1000-1020 kg m-3. If this comes in between the ranges that means stability is good.
	RMSD value is less than of 3 that means stability of drug complex is good. RMSD (root mean square deviation) We go for 50 ns(Nano second)
	Depend upon the range and time of rmsd unk it shows its stability after 45 ns.

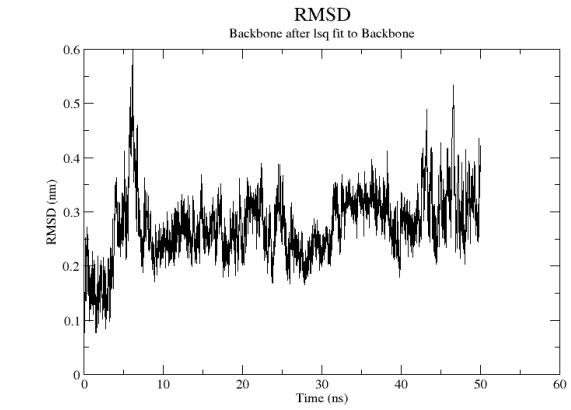
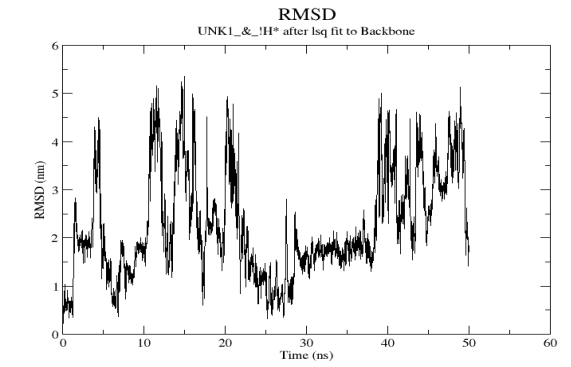
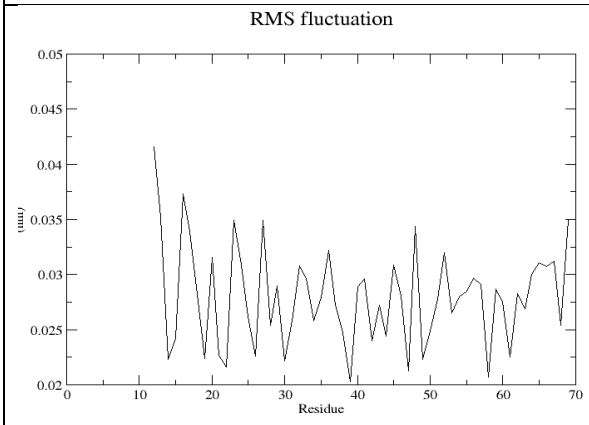
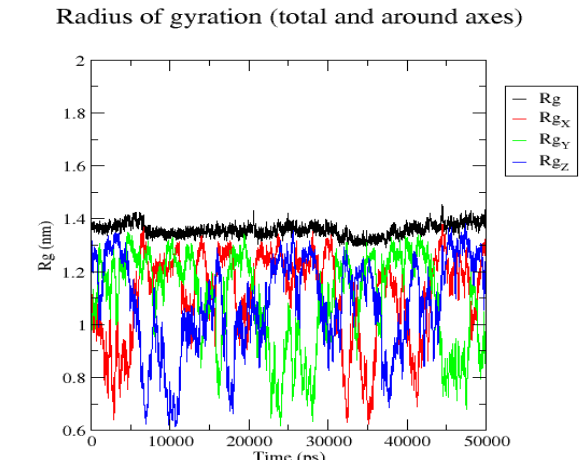
	RMSF(root mean square fluctuation) When the fluctuation appear with in certain range that means the molecule is stable.
	Radius of gyration determine the compactions of protein. Protein folding indicate the steady value of Rg.
	It shows Interaction energy of drug complex. Range of LJ is -99.1 ± 7.2 and CSR range is -20.5 ± 7.4 kJ mol-1. Stability of drug is depend upon the ranges In this graph we did not find stable complex of drug molecules.

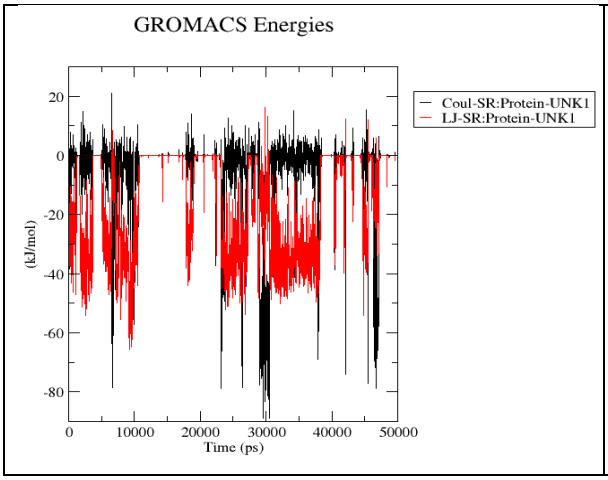
MOLECULE - 9	
Graph	**Interpretation**
	Potential energy graph confirm the energy minimisation of drug complex.

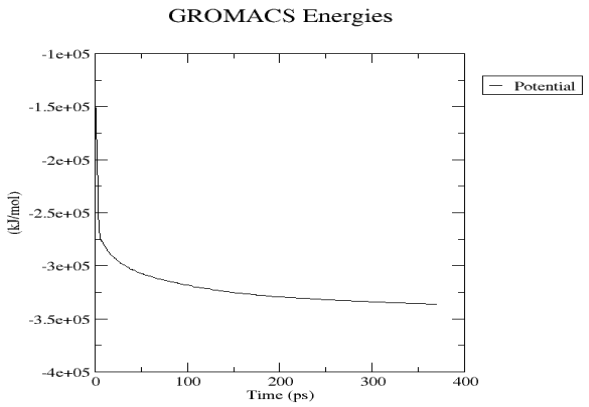
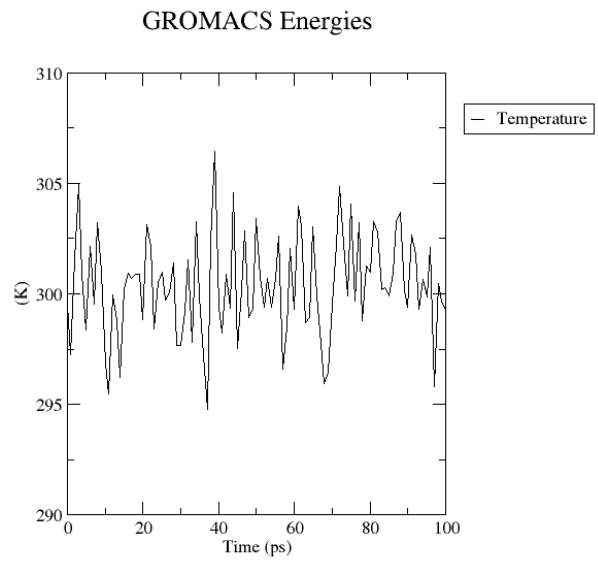
	It shows us the equilibrium range of drug complexes.
	Range is in between +200 to -200. If this comes as output for drug complex that means stability of drug is high
	The average value is 1000-1020 kg m-3. If this comes in between the ranges that means stability is good.
	RMSD value is less than of 3 that means stability of drug complex is good. RMSD (root mean square deviation) We go for 50 ns(Nano second)

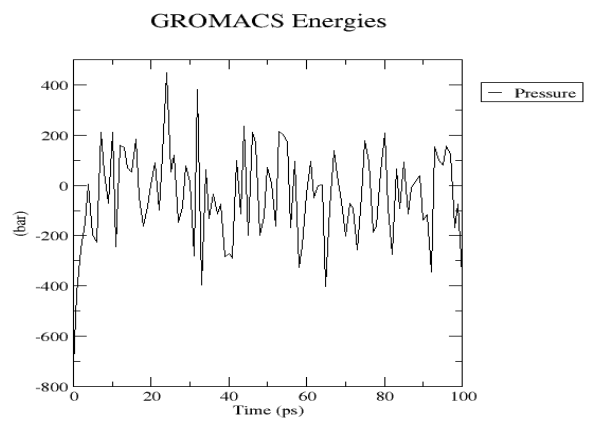
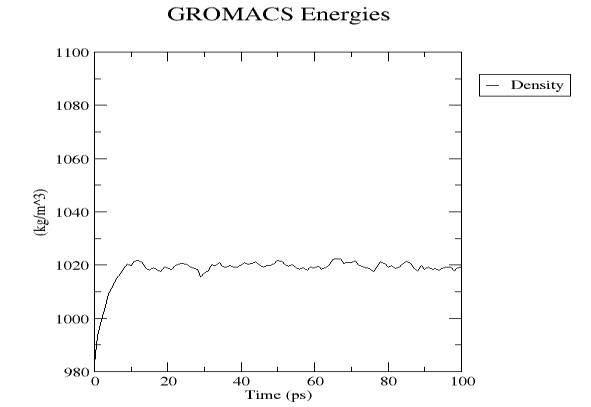
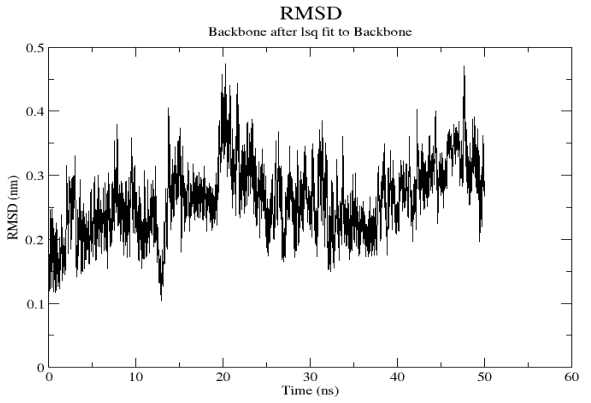
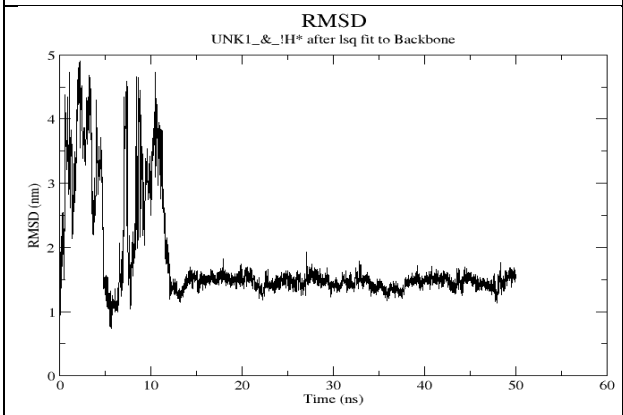
	Depend upon the range and time of rmsd unk it shows its stability after 45 ns.
	RMSF(root mean square fluctuation) When the fluctuation appear with in certain range that means the molecule is stable.
	Radius of gyration determine the compactions of protein. Protein folding indicate the steady value of Rg.
	It shows Interaction energy of drug complex. Range of LJ is -99.1 ± 7.2 and CSR range is -20.5 ± 7.4 kJ mol-1. Stability of drug is depend upon the ranges In this graph we did not find stable complex of drug molecules.

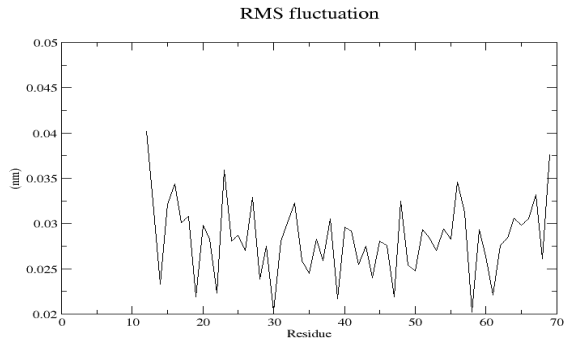
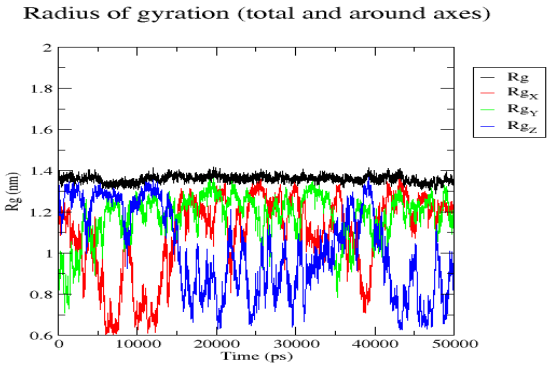
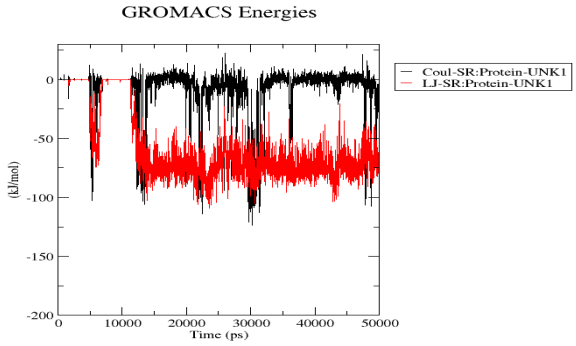
MOLECULE - 10	
Graph	**Interpretation**
	Potential energy graph confirm the energy minimisation of drug complex.
	It shows us the equilibrium range of drug complexes.
	Range is in between +200 to -200. If this comes as output for drug complex that means stability of drug is high
	The average value is 1000-1020 kg m-3. If this comes in between the ranges that means stability is good.

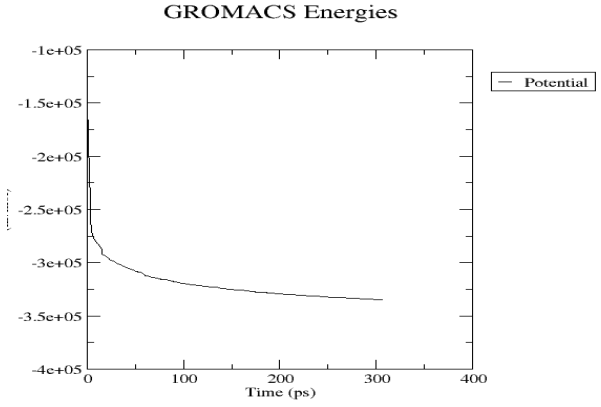
	RMSD value is less than of 3 that means stability of drug complex is good. RMSD (root mean square deviation) We go for 50 ns(Nano second)
	Depend upon the range and time of rmsd unk it shows its stability after 45 ns.
	RMSF(root mean square fluctuation) When the fluctuation appear with in certain range that means the molecule is stable.
	Radius of gyration determine the compactions of protein. Protein folding indicate the steady value of Rg.

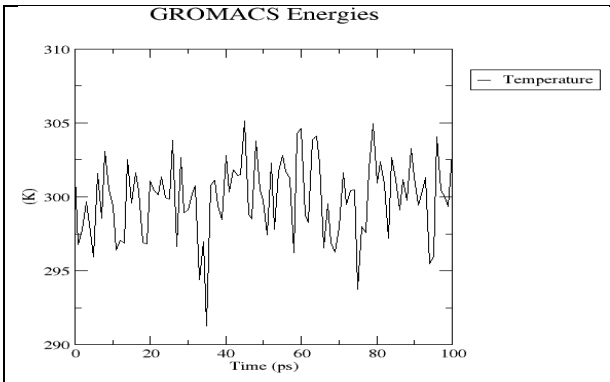
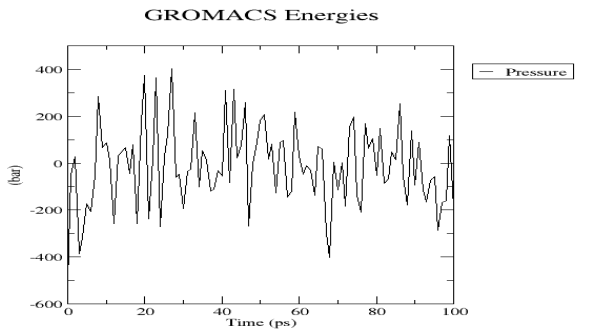
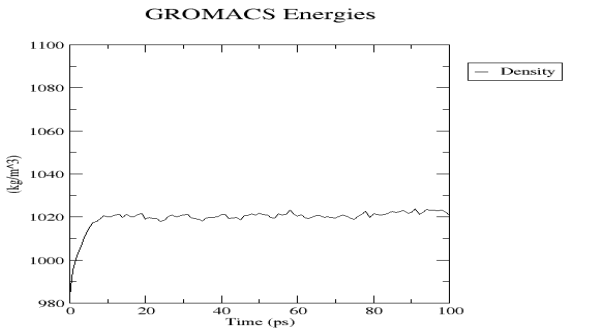
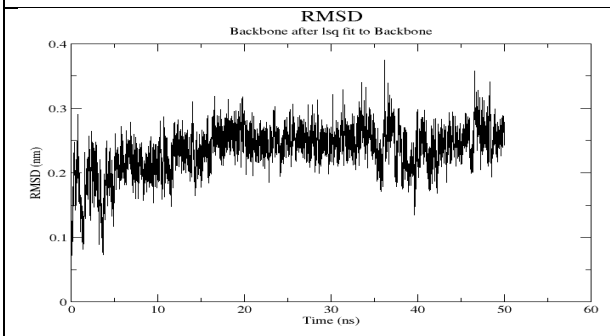
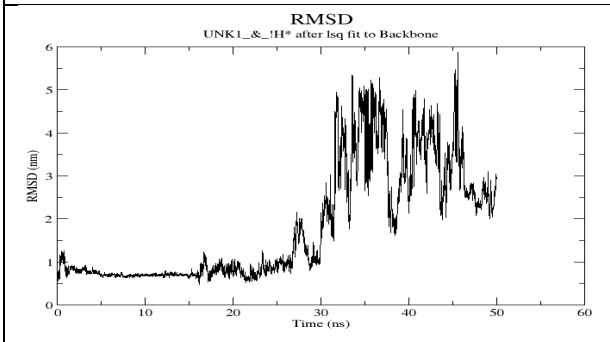
	It shows Interaction energy of drug complex. Range of LJ is -99.1 ± 7.2 and CSR range is -20.5 ± 7.4 kJ mol-1. Stability of drug is depend upon the ranges In this graph we did not find stable complex of drug molecules.

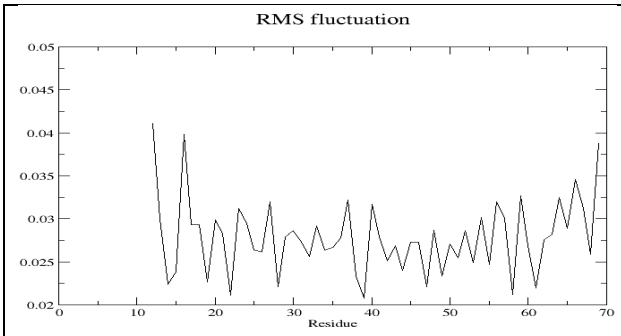
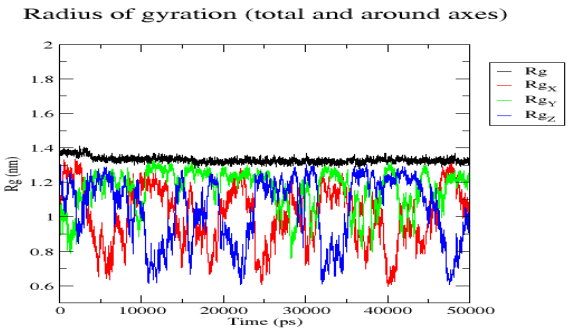
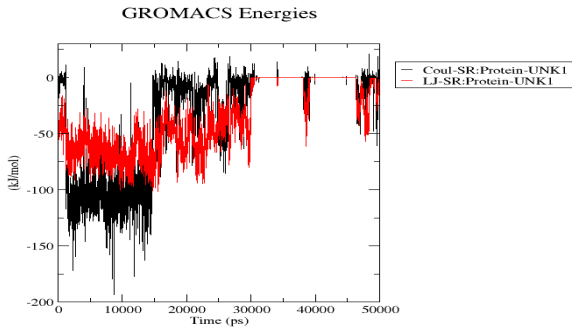
MOLECULE - 14	
Graph	**Interpretation**
	Potential energy graph confirm the energy minimisation of drug complex.
	It shows us the equilibrium range of drug complexes.

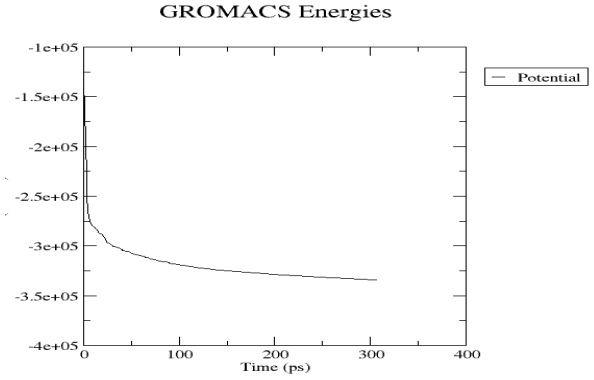
	Range is in between +200 to -200. If this comes as output for drug complex that means stability of drug is high
	The average value is 1000-1020 kg m-3. If this comes in between the ranges that means stability is good.
	RMSD value is less than of 3 that means stability of drug complex is good. RMSD (root mean square deviation) We go for 50 ns(Nano second)
	Depend upon the range and time of rmsd unk it shows its stability after 45 ns.

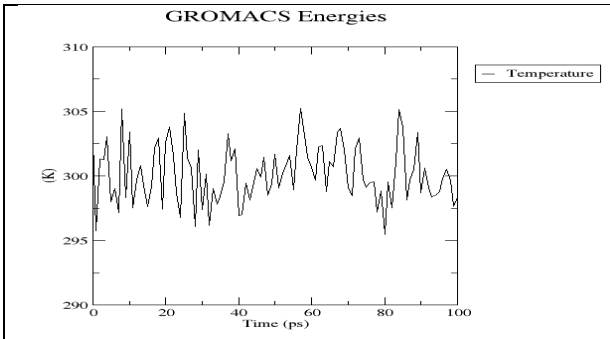
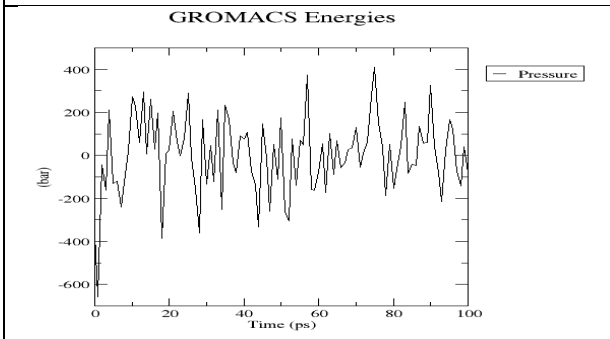
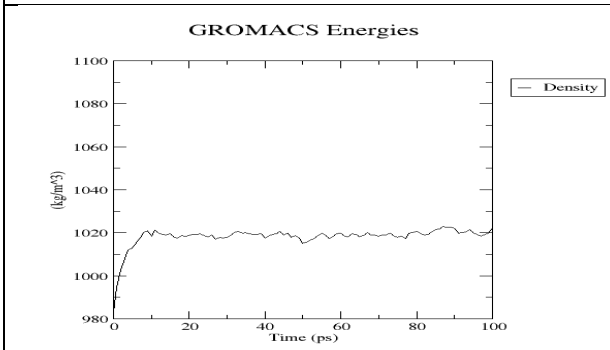
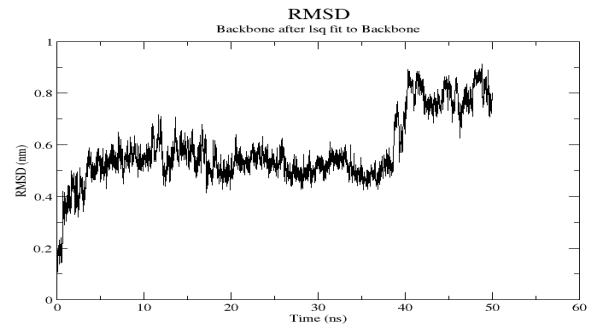
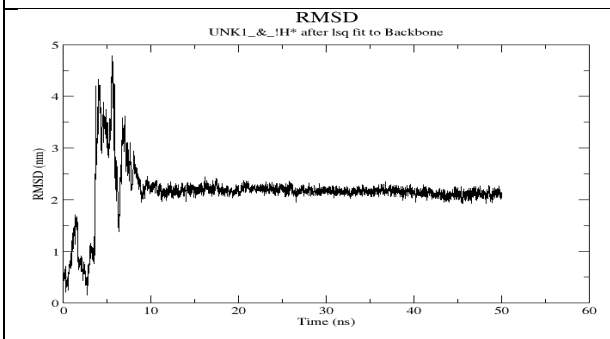
	RMSF(root mean square fluctuation) When the fluctuation appear with in certain range that means the molecule is stable.
	Radius of gyration determine the compactions of protein. Protein folding indicate the steady value of Rg.
	It shows Interaction energy of drug complex. Range of LJ is -99.1 ± 7.2 and CSR range is -20.5 ± 7.4 kJ mol-1. Stability of drug is depend upon the ranges In this graph we did not find stable complex of drug molecules.

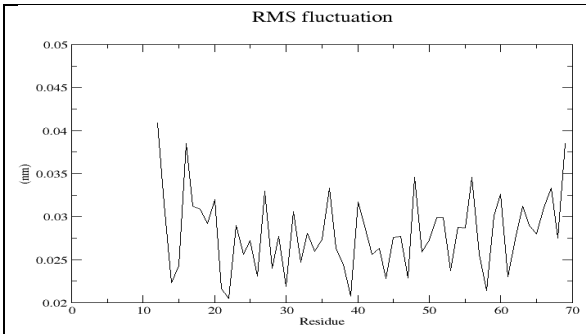
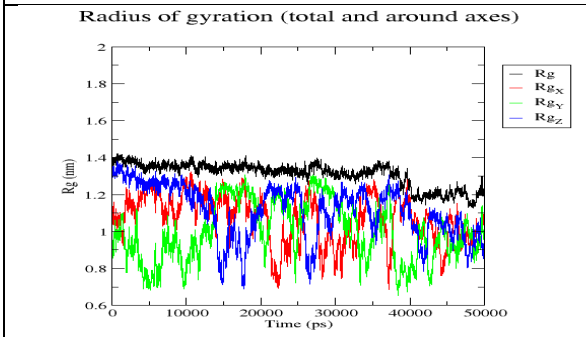
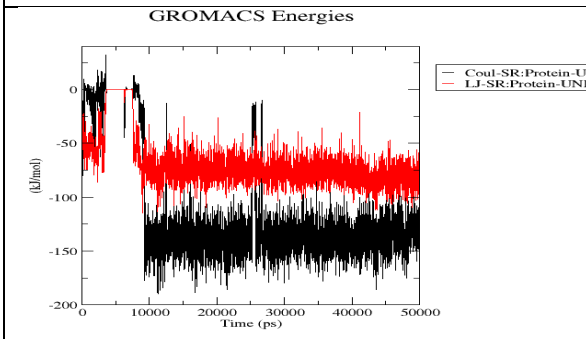
MOLECULE - 25	
Graph	**Interpretation**
	Potential energy graph confirm the energy minimisation of drug complex.

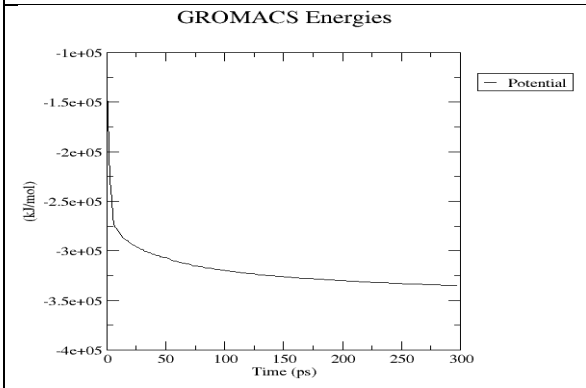
	It shows us the equilibrium range of drug complexes.
	Range is in between +200 to -200. If this comes as output for drug complex that means stability of drug is high
	The average value is 1000-1020 kg m-3. If this comes in between the ranges that means stability is good.
	RMSD value is less than of 3 that means stability of drug complex is good. RMSD (root mean square deviation) We go for 50 ns(Nano second)
	Depend upon the range and time of rmsd unk it shows its stability after 45 ns.

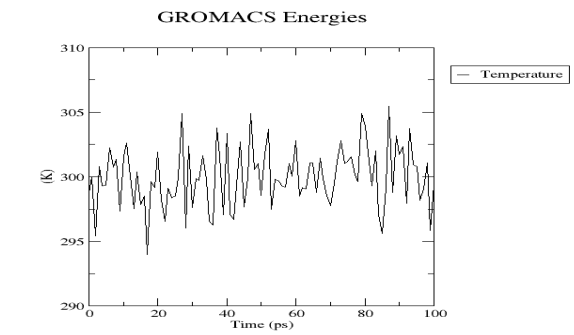
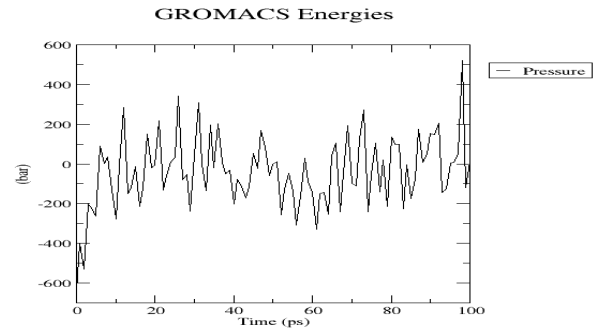
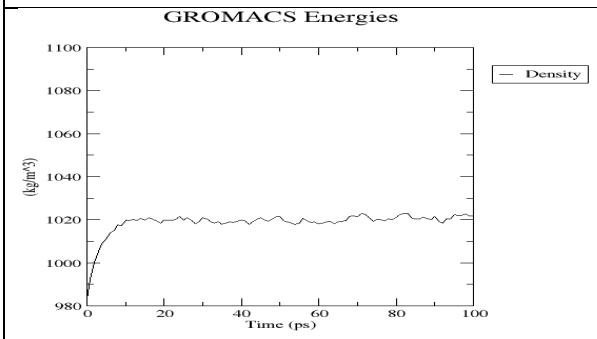
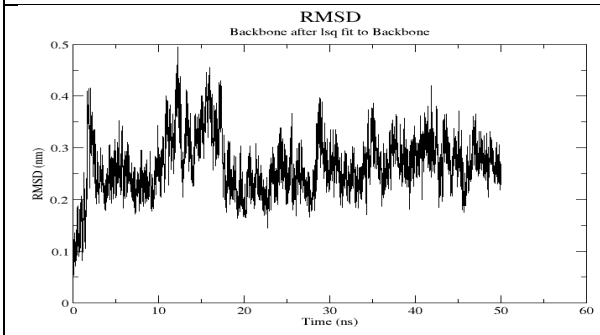
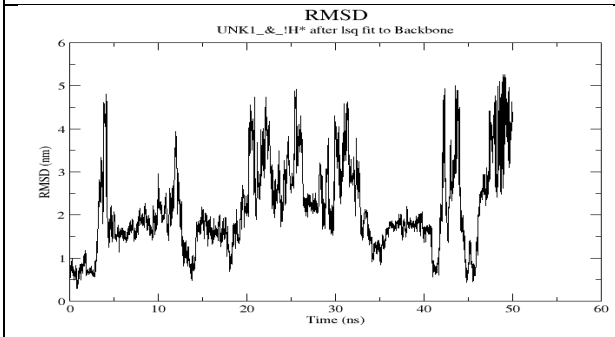
	RMSF(root mean square fluctuation) When the fluctuation appear with in certain range that means the molecule is stable.
	Radius of gyration determine the compactions of protein. Protein folding indicate the steady value of Rg.
	It shows Interaction energy of drug complex. Range of LJ is -99.1 ± 7.2 and CSR range is -20.5 ± 7.4 kJ mol-1. Stability of drug is depend upon the ranges In this graph we did not find stable complex of drug molecules.

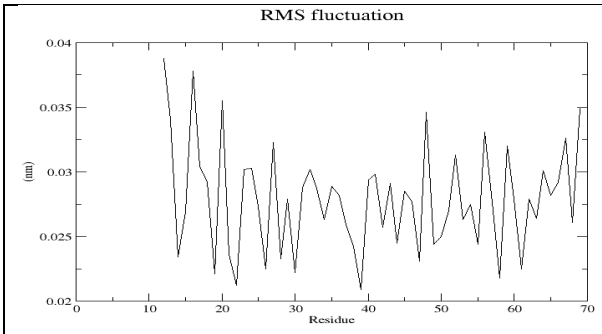
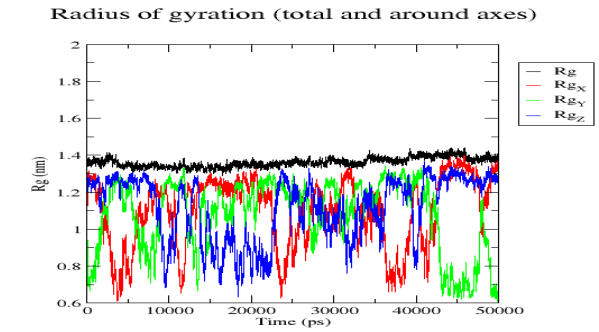
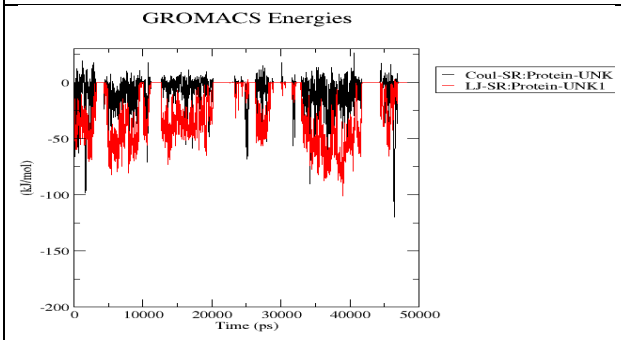
MOLECULE - 34	
Graph	**Interpretation**
	Potential energy graph confirm the energy minimisation of drug complex.

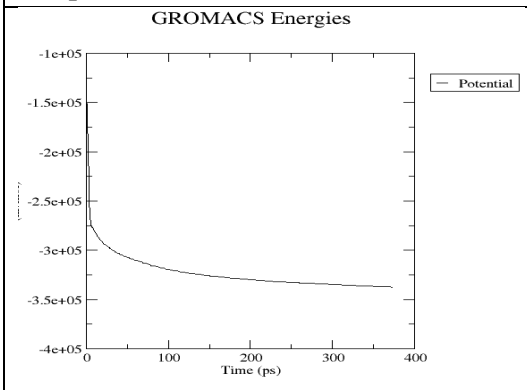
	It shows us the equilibrium range of drug complexes.
	Range is in between +200 to -200. If this comes as output for drug complex that means stability of drug is high
	The average value is 1000-1020 kg m-3. If this comes in between the ranges that means stability is good.
	RMSD value is less than of 3 that means stability of drug complex is good. RMSD (root mean square deviation) We go for 50 ns(Nano second)
	Depend upon the range and time of rmsd unk it shows its stability after 45 ns.

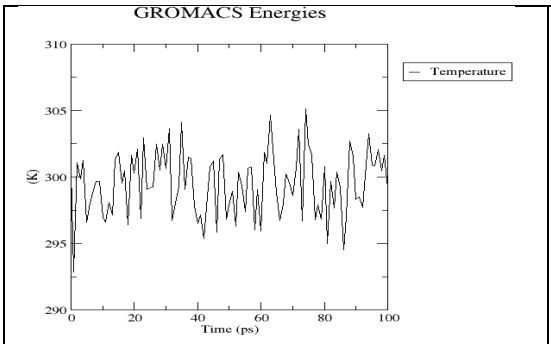
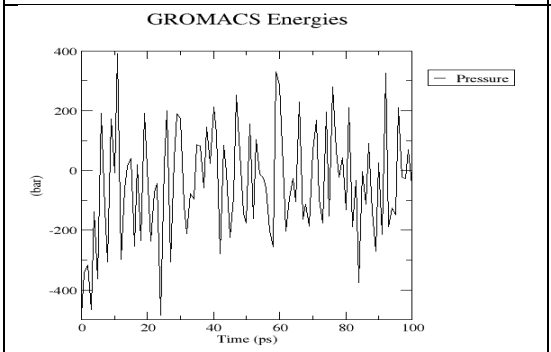
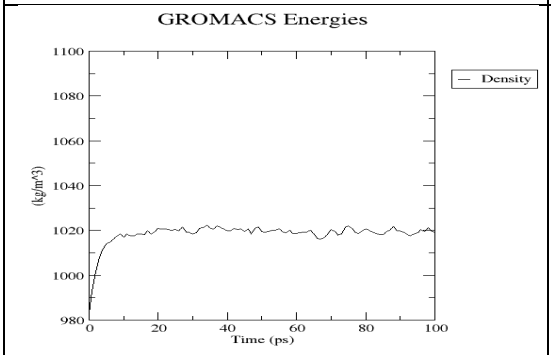
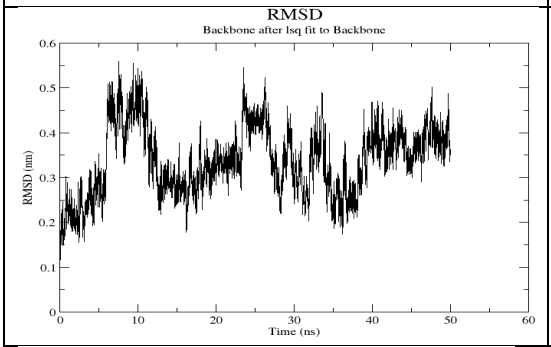
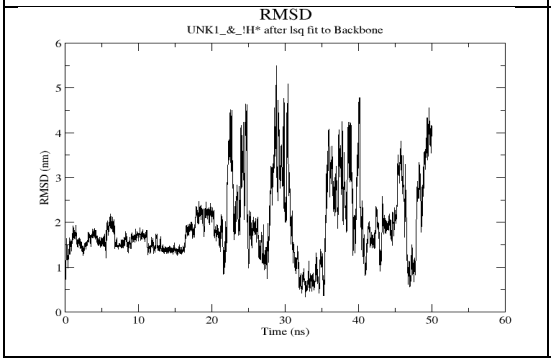
	RMSF(root mean square fluctuation) When the fluctuation appear with in certain range that means the molecule is stable.
	Radius of gyration determine the compactions of protein. Protein folding indicate the steady value of Rg.
	It shows Interaction energy of drug complex. Range of LJ is -99.1 ± 7.2 and CSR range is -20.5 ± 7.4 kJ mol-1. Stability of drug is depend upon the ranges In this graph we did not find stable complex of drug molecules.

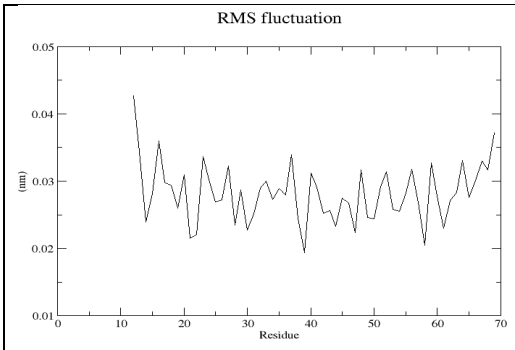
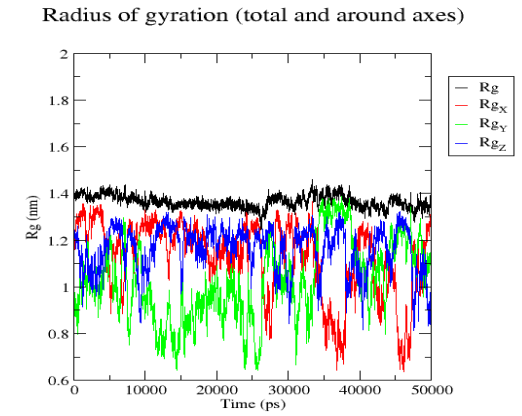
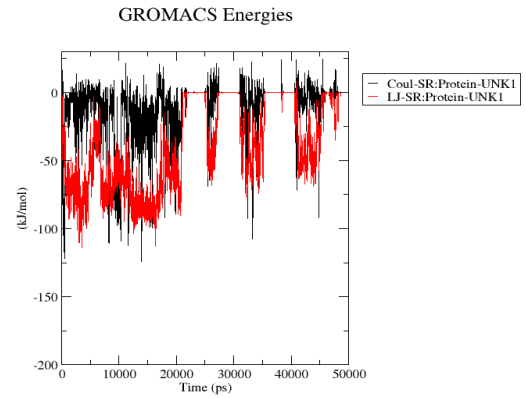
MOLECULE - 47	
Graph	**Interpretation**
	Potential energy graph confirm the energy minimisation of drug complex.

	It shows us the equilibrium range of drug complexes.
	Range is in between +200 to -200. If this comes as output for drug complex that means stability of drug is high
	The average value is 1000-1020 kg m-3. If this comes in between the ranges that means stability is good.
	RMSD value is less than of 3 that means stability of drug complex is good. RMSD (root mean square deviation) We go for 50 ns(Nano second)
	Depend upon the range and time of rmsd unk it shows its stability after 45 ns.

	RMSF(root mean square fluctuation) When the fluctuation appear with in certain range that means the molecule is stable.
	Radius of gyration determine the compactions of protein. Protein folding indicate the steady value of Rg.
	It shows Interaction energy of drug complex. Range of LJ is -99.1 ± 7.2 and CSR range is -20.5 ± 7.4 kJ mol-1. Stability of drug is depend upon the ranges In this graph we did not find stable complex of drug molecules.

MOLECULE - 48	
Graph	**Interpretation**
	Potential energy graph confirm the energy minimisation of drug complex.

	It shows us the equilibrium range of drug complexes.
	Range is in between +200 to -200. If this comes as output for drug complex that means stability of drug is high
	The average value is 1000-1020 kg m-3. If this comes in between the ranges that means stability is good.
	RMSD value is less than of 3 that means stability of drug complex is good. RMSD (root mean square deviation) We go for 50 ns(Nano second)
	Depend upon the range and time of rmsd unk it shows its stability after 45 ns.

	RMSF(root mean square fluctuation) When the fluctuation appear with in certain range that means the molecule is stable.
	Radius of gyration determine the compactions of protein. Protein folding indicate the steady value of Rg.
	It shows Interaction energy of drug complex. Range of LJ is -99.1 ± 7.2 and CSR range is -20.5 ± 7.4 kJ mol-1. Stability of drug is depend upon the ranges In this graph we did not find stable complex of drug molecules.

CONCLUSION

Now in worldwide all the countries are facing problems with Tuberculosis (TB) because they are multi drug resistant and extensively drug resistance from the other autoimmune disorder like HIV , diabetes etc. because of these type of problems we are facing a lot of things in our daily life . TB is most common disease in worldwide for this problems we need to develop a novel drug with high efficacy with short time periods. For the shorter time period we can go through inslico drug design method or computer aided drug design method. We can design the novel drug with the help of proteins and ligand molecules. We go for this inslico drug design methods to design a novel drug with the help of 4gzr protein which is crystal structure of mycobacterium tuberculosis and 50 chemical compounds of anti-tuberculosis properties (Levofloxacin, Ethionamide, Aspirin) and then go for energy minimization of protein and ligands. After that we go for docking process in which with the help of autodock 4.0 we made a docking complex/Drug complex. And after we go for Hydrogen Bond analysis and toxicity checkup of that complex we can go for Invitro study of this complex. Out of 50 molecules we got only 8 molecules that have H-bond interaction with active site residues (Ala 26 A) of our proteins. With the help of bioinformatics tool and techniques we find final drug molecules/Docking complex on the basis of their **active site** Alanine (26) and ligand molecules bonds. In the hydrogen bond analysis we found we selected three different type of chemicals **Aspirin, levofloxacin , and Ethionamide** for making drug molecules/ docking complex but we find Ethionamide is not working in this docking complex.

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