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Original Research

Comprehensive Analysis of Homologous Proteins for Specific Drug Design

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Abstract

A drug is a chemical substance used in the diagnosis, treatment or prevention of disease or as a component of a medication, should be specific and freedom from side affect. Many issues should be addressed while designing a new drug or improving existing compound. The increase in the interdisciplinary nature of science gives bioinformatics, systems and computational biology, which helps in reducing research and development costs, minimize drug failures by predicting drug efficacy and toxicity. One of the most important pathogenic bacterium is *Aeromonas* species which causes tissue damage, acute gastroenteritis and neonatal septicemia. Bacterial proteins are the ultimate target to inhibit their growth and these are the executors of cellular function. In related to this we selected four such different proteins Flavohemo protein, Guanylate kinase, Topoisomerase and Oligopeptidase found to be present in both humans and *Aeromonas* to study the effects of antibiotics through *in silico* approaches. An attempt has been made to classify the inhibitors as host protein inhibitors or guest protein inhibitors. Finally we conclude that the molecule AgkI5 (2-morpholin-4-yl-thianthren-1-ylpyron-4-one) shown good inhibition with minimum binding energy -9.30, docking energy -10.03, inhibition constant 1.53e-007 and RMS 0.0 against *Aeromonas* Guanylate kinase [*Aeromonas*: Modelled] when compared to human Guanylate kinase [PDB ID: 1KJD]. So AgkI5 was predicted as a good antibiotic against *Aeromonas* Species.

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INTRODUCTION

A drug, is any substance that, when absorbed into the body of a living organism, alters normal bodily function. In the field of pharmacology, a drug can be defined as a chemical substance used in the treatment, cure or prevention of disease otherwise used to enhance physical or mental well-being. Drugs must be not only effective but safe; side-effects can range from minor to dangerous.

Humans have been Figurehting against bacterial pathogens for many decades. Since, then humans have been utilizing various chemical substances with antibacterial or bacteriostatic properties. In the past 50 years, borrowing anti-bacterials from other bacteria and fungi even produced an impression of success in this battle (Galperin *et al.,* 1999).

The bactericidal antibiotic killing mechanisms are currently attributed to the class-specific drug-target interactions. Current antimicrobial therapies, which cover a wide array of targets, fall into two general categories: bactericidal drugs-which kill bacteria with an efficiency of >99.9% and bacteriostatic drugs- which merely inhibit growth (Michael *et al.,* 2007).

Bacteria belonging to *Aeromonas* species have been identified as common enteric pathogens from several countries. They cause acute gastroenteritis of both adults and children's, ranging from watery to blood diarrhoea of either short or prolonged (over 2 weeks) duration. In mammals, *Aeromonas* species causes neonatal septicemia (Bharath and Manjunatha, 2013). They occur widely in the environment, especially in water. They are found in both raw and chlorinated water supplies (Kudinha *et al.,* 2004).

Aeromonas hydrophila, *Aeromonas caviae*, and *Aeromonas sobria* are all considered to be "opportunistic pathogens," meaning they only infect hosts with weakened immune responses. Because of *Aeromonas hydrophila's* structure, it is very toxic to many organisms. When it enters the body of its victim, it travels through the bloodstream to the first available organ. It produces Aerolysin Cytotoxic Enterotoxin (ACT), a toxin that can cause tissue damage (Chopra *et al.,* 2000). Though *Aeromonas hydrophila* is considered a pathogenic bacterium, scientists not been able to prove that it is the actual cause of some of the diseases it is associated with. It is believed that this bacterium aids in the infection of diseases, but do not cause the diseases themselves.

Foods have been implicated in the transmission of *Aeromonas* species. Motile *Aeromonas* have been

isolated from fresh foods of animal origin. The microorganism has the potential to be a food borne pathogens. The disease spectrum associated with these microorganisms includes gastroenteritis, Septicemia, aquatic wound infections (Kudinha *et al.,* 2004).

Proteins are the ultimate executors of cellular function, and thus are directly responsible for a biological phenotype (Anderson *et al.,* 2002). Proteomics is the study of the expression, modification and activity of proteins in order to better understand a biological system. Diseased (or drug treated lysates) are identically processed and comparative analysis performed to evaluate protein expression between the two samples (Aebersold *et al.,* 2003). So in the present study, we selected four different proteins present in both *Aeromonas* and human system they are Guanylate Kinase protein, Oligopeptidase protein, Flavohemoprotein and Topoisomerase protein by functional analysis we will come to know that whether the proteins are orthologs or not using molecular docking studies as the force field is based on the concept of residue- residue contact energies. Reduced structures can be translated to atomic resolution, and further evaluated (Andrzej *et al.,* 2003).

MATERIALS AND METHODS

Sequence, structure and domain analysis was performed to predict the efficacy of inhibitor molecules against proteins. Protein sets were downloaded by Integr8 database and protein families were studied in Interpro, and then BLAST was performed for protein sequences against PDB to verify about the availability of structural information. BLAST and Genius clustalW tools were employed for sequence analysis. Structure analysis was performed using PDB database and protein homology modeling was achieved in Swiss modeler and validated through ADIT validation server by plotting Ramachandran plot for some proteins whose structural information was not available in PDB, Topmatch server was used for protein superimposition. Domain analysis was performed for all the proteins using Prosite server. Molecular docking studies were done using AutoDock v3.0 for known inhibitors downloaded from PubChem against selected proteins. An attempt has been made to classify the inhibitors as human protein inhibitors or *Aeromonas* protein inhibitors.

RESULTS AND DISCUSSION

From the integr8, we have downloaded the proteomic sets of human and bacteria. Interpro IDs From that proteome set were taken and submitted for Interpro, which shows the protein family, out of them we have selected four families and cross referenced with Pfam database for confirmation and selected four below showed in Table 1. The protein sequences for Guanylate kinase, Oligopeptidase, Flavohemoprotein and DNA Topoisomerase with a Uniprot ID's A0KEC4, A0KEG4, A0KG18 and A5UCC4 respectively in FASTA format were collected and subjected for sequence analysis.

Table 1: The Pfam IDs for four proteins

Protein	Pfam ID
Guanylate kinase	PF00625
Oligopeptidase A	PF01432
Flavohemoprotein	PF00175
Topoisomerase	PF00204

Sequence Analysis

psi-BLAST was performed for Flavohemoprotein, Guanylate kinase, Topoisomerase and Oligopeptidase protein sequences in FASTA format against non-redundant database, and the sequences from different species from Human to bacteria were selected from the obtained hits (Stephen *et al*., 1997). The Multiple Sequence Alignment was performed for the selected sequences using T-coffee to analyze the conserved regions (Figure 1-4).

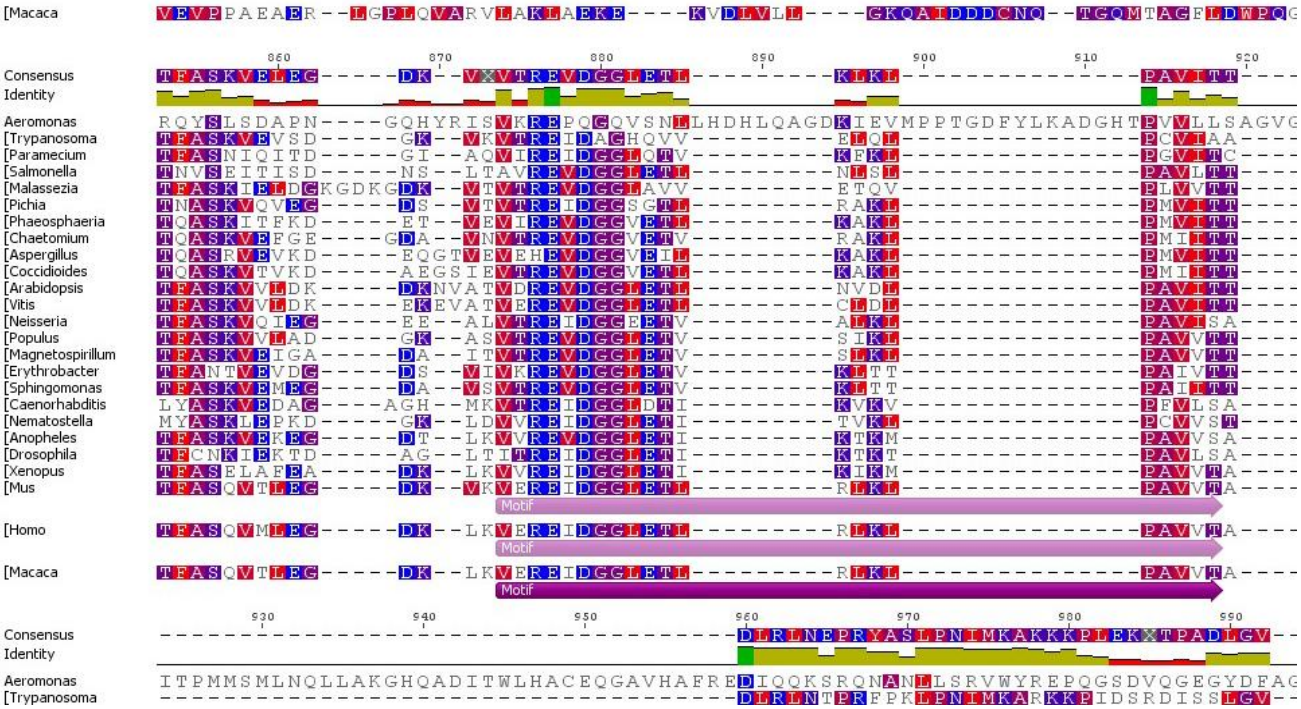


Figure 1: Flavohemoprotein.

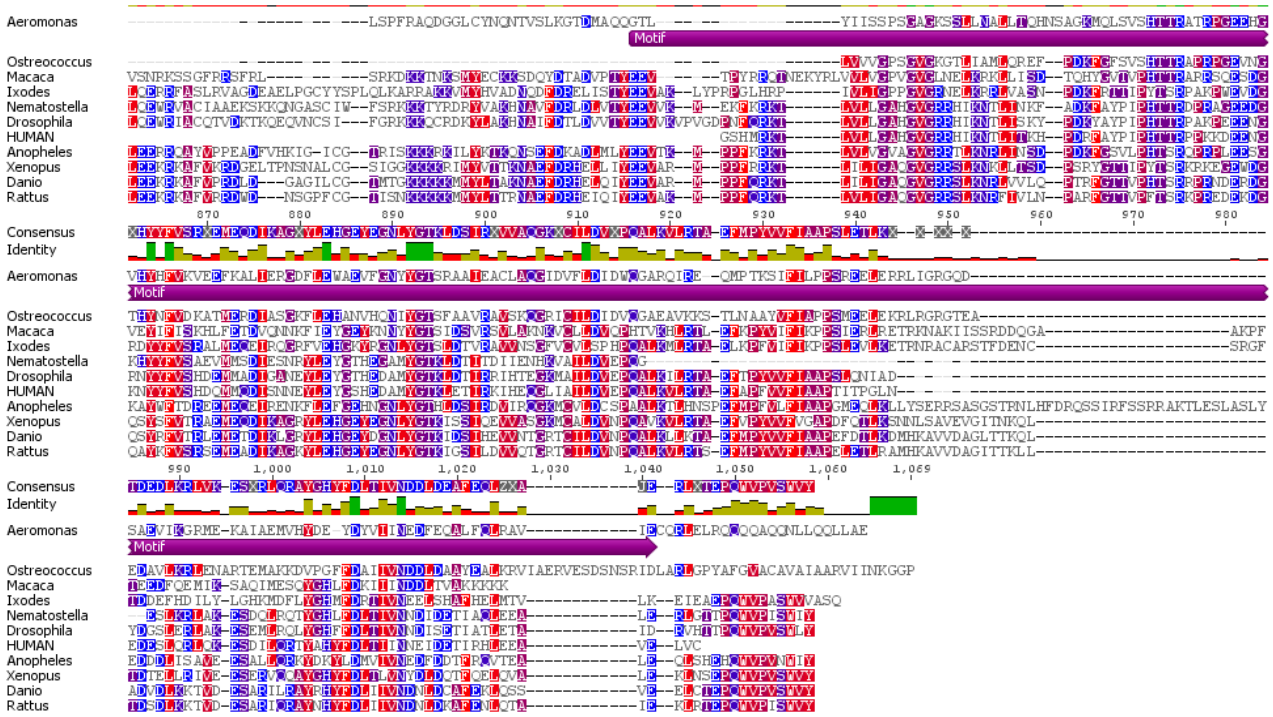


Figure 2: Guanylate kinase.

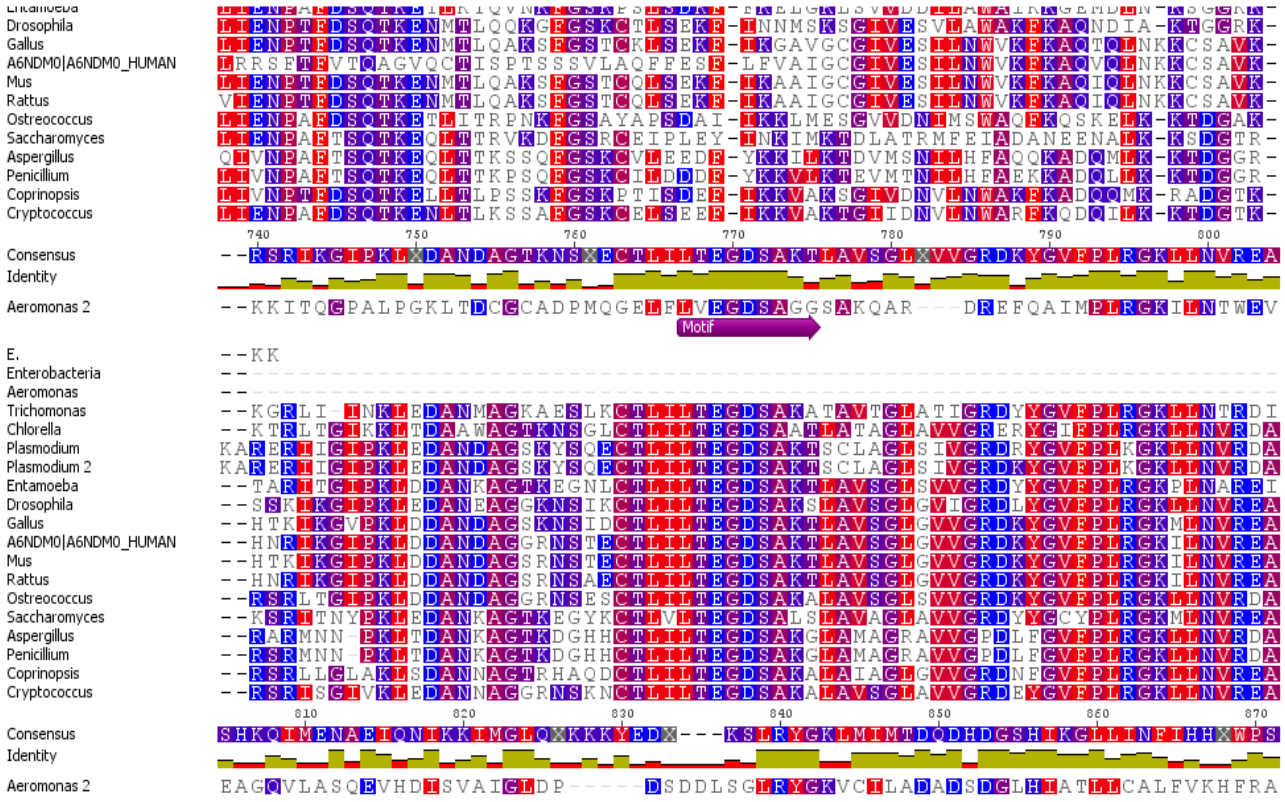


Figure 3: Topoisomerase.

The BLAST against Non-Redundant Database was performed for the FASTA sequences, numbers of hits for same protein were obtained, we have selected these sequences from different species and the FASTA sequences were downloaded. For downloaded sequences we performed Multiple Sequence Alignment using Genius Pro. (Figure 1-4). Here conserved regions among *Aeromonas*, Human and other species were analyzed, by this MSA we can say that the proteins both in Human and Bacteria performs same functions due to the conservation of the particular amino acid residues in their motifs.

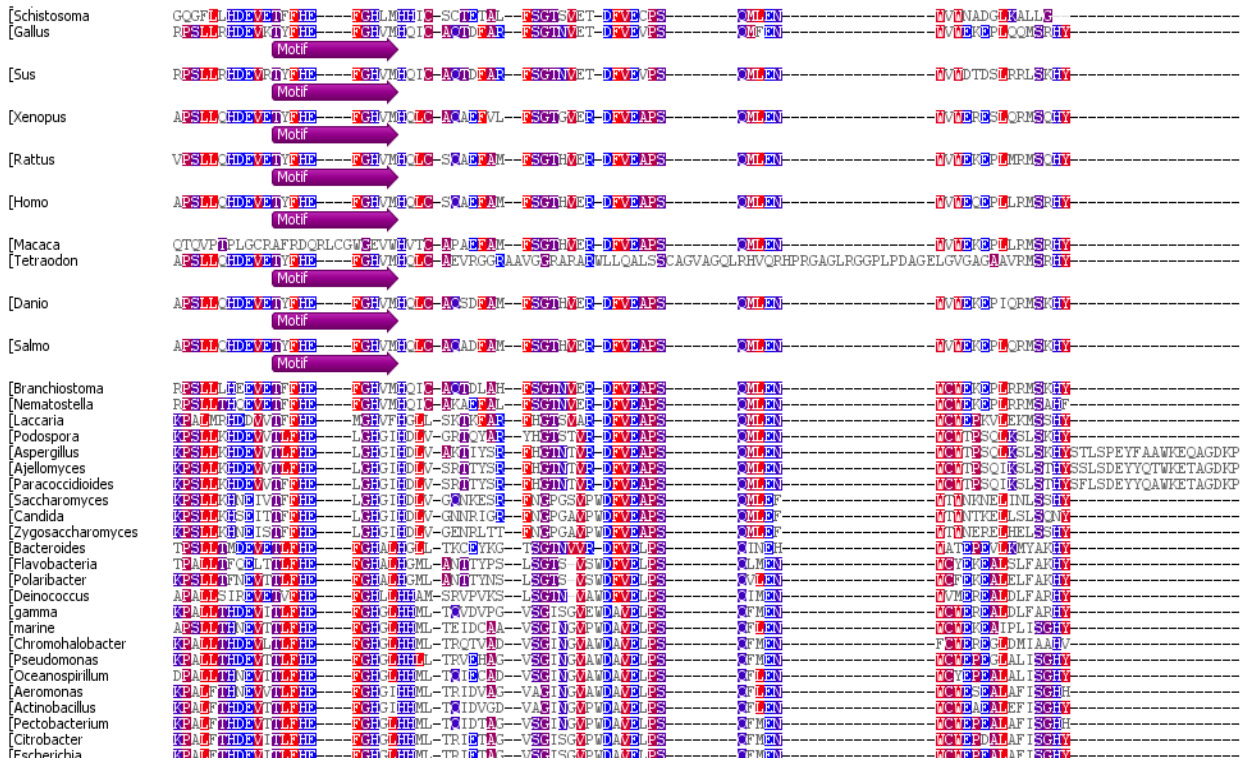


Figure 4: Oligopeptidase.

Domain Analysis

Domain analysis of these four proteins was done using PROSITE server. Here domains of *Aeromonas* protein are compared with the domains of human proteins. The domain hits obtained for Human guanylate kinase protein and *Aeromonas* Guanylate kinase sequences were observed with Guanylate Kinase_2 domain (Figure 5a and 5b) at 701th to 880th residue and 81th to 261th residue respectively, this indicates the domain shifting. Human guanylate kinase was observed with five more domains such as PROTEIN KINASE_OOM, two L27 domains, PDZ and 3H3 domains which were not seen on *Aeromonas* sequence (Laurent *et al*., 2002).

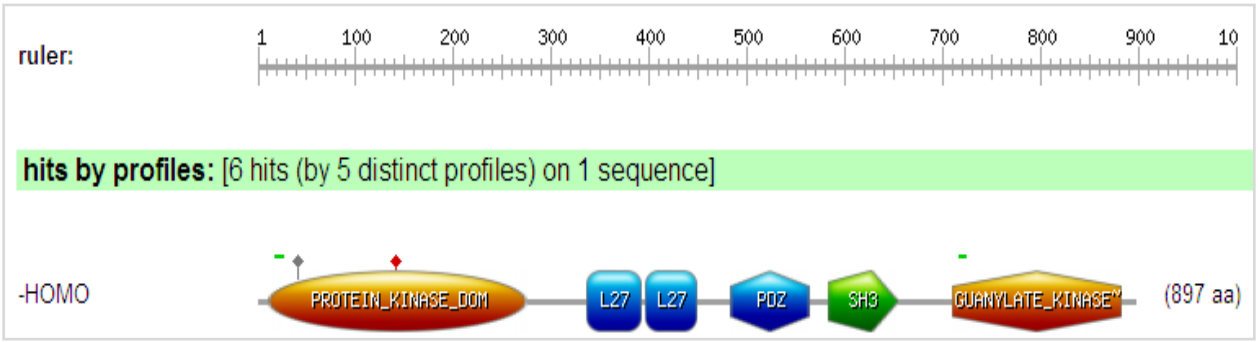


Figure 5a: Human Guanylate kinase.

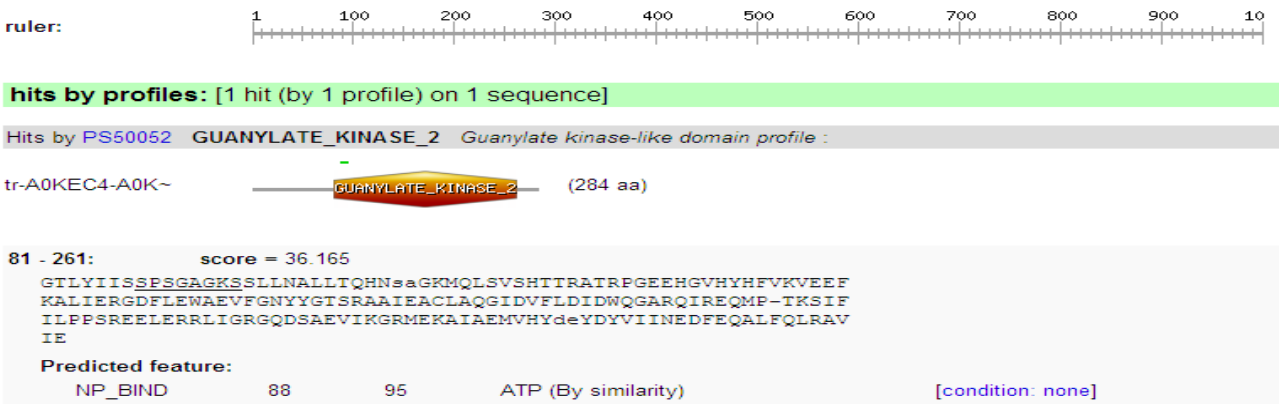


Figure 5b: *Aeromonas* Guanylate kinase

Similarly Oligopeptidase sequences were submitted and domain hits were obtained only for human sequence, no hits were found for bacterial sequence, and here we failed to observe the domain similarity between human and bacterial sequences. Neutral zinc metallopeptidases, zinc-binding region was observed on Human sequence as shown in Figure 6.

Domain analysis of Flavohemoprotein has received single hit corresponding the pattern for electron transfer flavoprotein beta-subunit on Human sequence and two hits by two distinct profiles namely Globin and FAD_FR on *Aeromonas* sequence as shown in Figure 7a and b.

In Topoisomerase domain analysis we observed the DNA topoisomerase II domain having eight amino acids length on both the sequences Figure: 8a and 8b, on Human sequence we observed the domain from 539th residue to 547th residue and on *Aeromonas* sequence the domain was from 416th residue to 424th residue. Here also we can observe the domain shifting as shown in Figure 8a and 8b.

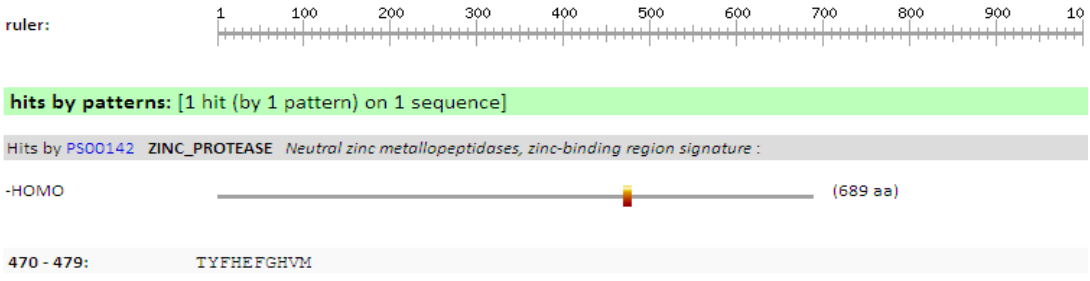


Figure 6: Human Oligopeptidase.

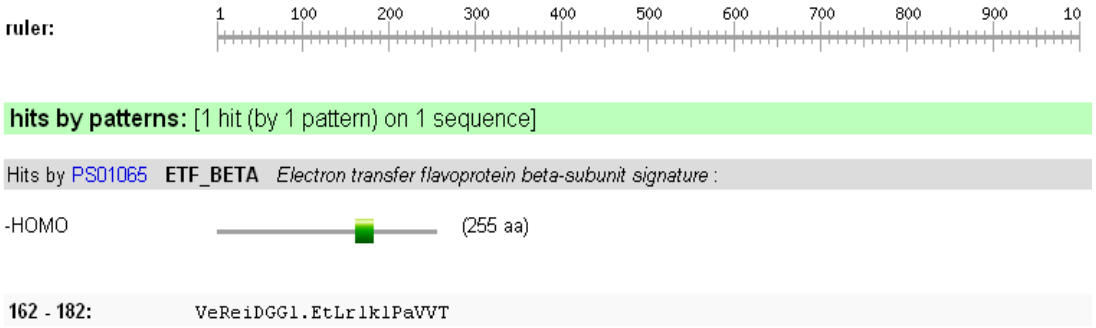


Figure 7a: Human Flavohemoprotein

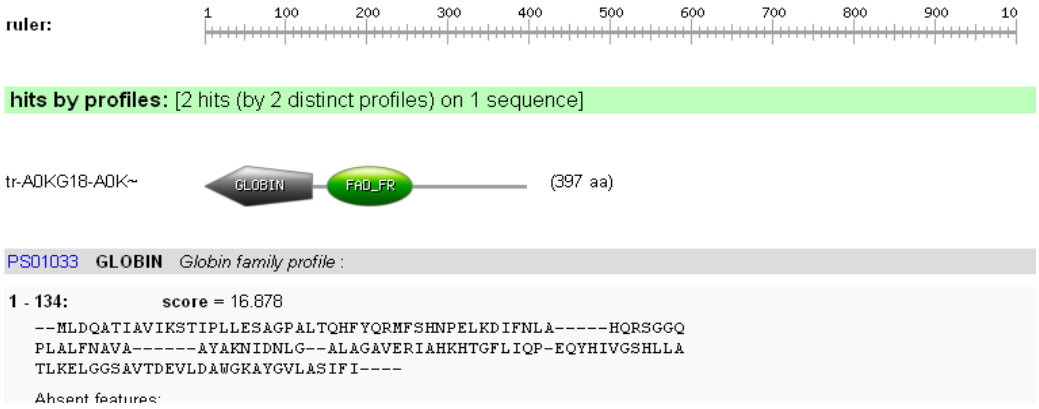


Figure 7b: *Aeromonas* Flavohemoprotein.

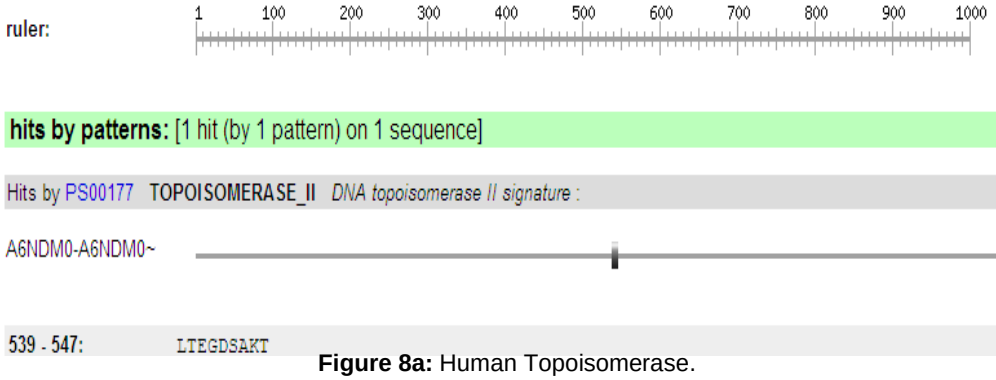


Figure 8a: Human Topoisomerase.

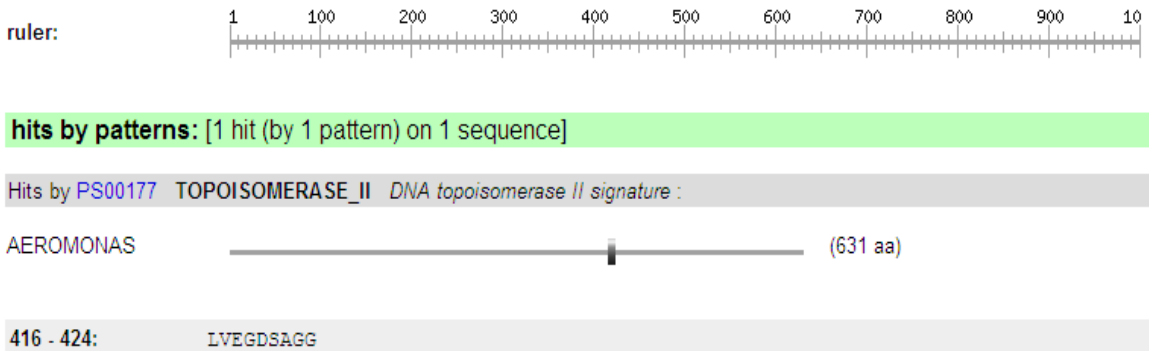


Figure 8b: *Aeromonas* Topoisomerase.

Structural Analysis

By domain analysis, we came to know the changes in domain composition that may lead to the structural differences. So we performed structural analysis for all the 4 proteins. For structural analysis we have collected the structures of proteins from PDB Table 1. We performed homology modeling using online modeling server Swiss model by taking *E. coli* structures as a template (Figure 9). The modeled structures were validated by ramachandran plot given by ADIT we can say that our models are good. The numbers of residues in favored region were 92.0%, 91.4% and 92.1% for Flavohemoprotein, Guanylate kinase and Topoisomerase respectively (Denis *et al*., 2002).

For structural similarity studies between Human and *Aeromonas* Protein structural superimposition was performed. The RMS values are 2.4, 3.3, 2.6 and 2.5 for Flavohemoprotein, Guanylate kinase, Topoisomerase and Oligopeptidase respectively as shown in Table 2. The modeled proteien structures were subjected for active pocket prediction, for PDB structures we referred ligplot provided by PDB only and for designed models castP server was used, the amino acids in active pockets are tabulated in Table 3. Even there is no complete structural similarity, domain similarity the proteins performing the same function in human and *Aeromonas* (Joe *et al*., 2006). This led us for species specific targeting and an attempt was made to illustrate the targeting results by performing molecular docking studies.

The protein structures of *Aeromonas* were superimposed against human protein structures using TOPMATCH server, and also RMS value is documented in Table 2 and we can observe the super imposition in the Figure 10. The active pockets on our PDB structures were identified by referring their ligplot and for designed models we used castP server.

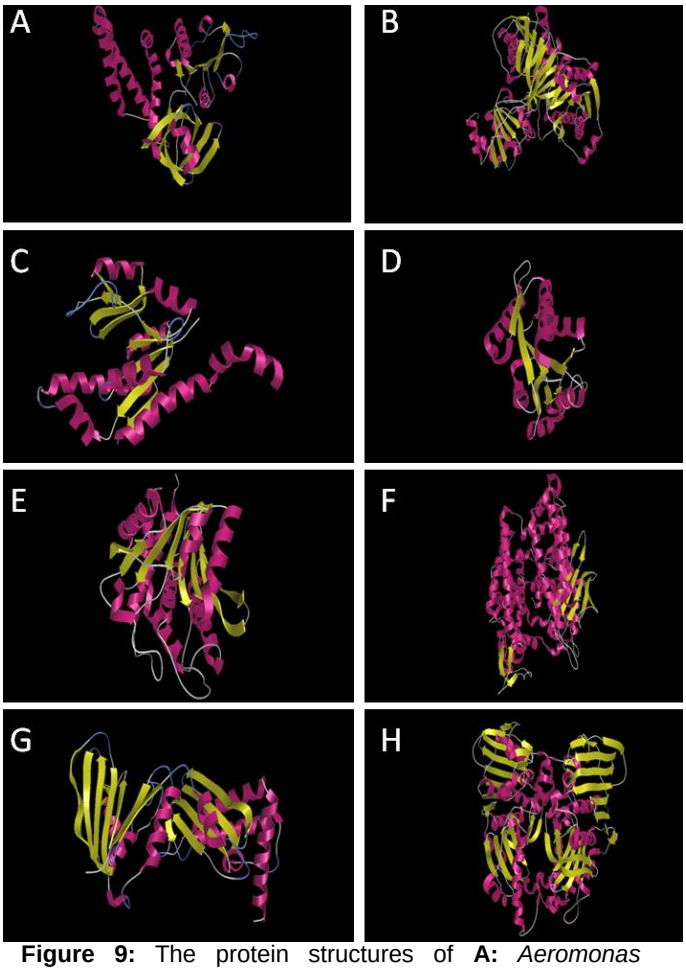


Figure 9: The protein structures of **A:** *Aeromonas* Flavohemoprotien, **B:** Human Flavohemoprotien, **C:** *Aeromonas* Guanylate kinase, **D:** Human Guanylate kinase, **E:** *Aeromonas* DNA Topoisomerase, **F:** Human DNA Topoisomerase, **G:** *Aeromonas* Oligopeptidase, **H:** Human DNA Oligopeptidase

Table 2: Structure superimposition using TopMatch.

Sl.No	Protein Name	Bacterial PDB ID	Human PDB ID	RMS Value
1.	Flavohemoprotein	Modeled	1EFV	2.4
2.	Guanylate kinase	Modeled	1KGD	3.3
3.	DNA Topoisomerase	Modeled	1ZXM	2.6
4.	Oligopeptidase	2DEA	1S4B	2.5

Table 3: Amino Acids in Active pocket.

N	Protein Name	PDB ID	Active Pocket	Active pocket amino acids
1.	*Aeromonas* Flavohemoprotein	Modeled	VAL-1, ALA-2, TYR-4, LYS-6, ASN-7, ALA-13, LEU-14, ALA-17, VAL-18, ARG-20, ILE-21, LYS-24, HIS-25, GLY-27, PHE-28, LEU-29, ILE-30, GLN-31, GLN-34, TYR-35, VAL-38, HIS-41, LEU-42, THR-45, LEU-46, LEU-49, TRP-62, ALA-65, TYR-66, LEU-69, ALA-70, PHE-73, SER-94, GLU-105, ILE-109, SER-111, TYR-130, SER-132, LYS-134, GLN-143, GLU-144, ILE-145, ARG-146, GLN-147, TYR-148, SER-149, ASP-152, ARG-160, SER-162, VAL-163, LYS-164, GLU-166, PRO-167, GLN-168, GLY-169, GLN-170, VAL-171, SER-172, VAL-187, MET-188, ALA-209, GLY-210, VAL-211, GLY-212, ILE-213, THR-214, PRO-215, MET-217, SER-218, HIS-236, ALA-237, CYS-238, GLU-239, GLN-240, ALA-242, VAL-243, HIS-244, ALA-245, PHE-246, ARG-247, TYR-266, ARG-267, THR-284, GLY-304, PRO-305, VAL-306, PHE-308, MET-309, GLN-310, LYS-313, GLN-314, ILE-317, ALA-323, TYR-328, GLU-329, VAL-330, PHE-331, GLY-332.	97
2.	Human Flavohemoprotein	1EFV	ASN-132, ALA-126, ASP-129, ASN39, CYS-42, GLY-123, ALA-9, THR-134, GLN-133, CYS-66	10
3.	*Aeromonas* Guanylate kinase	Modeled	SER-27, SER-28, PRO-29, SER-30, GLY-31, LYS-34, SER-35, LEU-38, ASN-39, LEU-42, HIS-45, SER-47, MET-51, GLN-52, LEU-53, SER-54, VAL-55, SER-56, HIS-57, ARG-60, ARG-63, PRO-64, VAL-70, HIS-71, TYR-72, HIS-73, GLU-91, ALA-93, VAL-95, PHE-96, ASN-98, TYR-100, GLY-101, THR-102, SER-103, ALA-106, ILE-107, CYS-110, ILE-115, VAL-117, LEU-119, ASP-120, ILE-121, ASP-122, GLY-125, ARG-151, LEU-152, ILE-153, GLY-154, ARG-155, GLY-156, GLN-157, ASP-158, ARG-166, LYS-169, ALA-170, GLU-173.	57
4.	Human Guanylate kinase	1KGD	GLU-802, GLY-812, TYR-811	3
5.	*Aeromonas* Topoisomerase	Modeled	ASP-2, GLN-3, SER-4, LEU-5, GLU-6, VAL-7, ILE-8, ASP-9, ASP-10, GLY-11, ARG-12, GLY-13, MET-14, PRO-15, HIS-19, GLY-26, LEU-29, ILE-30, ALA-36, GLY-37, GLY-38, LYS-39, PHE-40, LYS-43, ASN-44, TYR-45, PHE-47, SER-48, GLY-49, GLY-50, LEU-51, HIS-52, GLY-53, VLA-54, GLY-55, ILE-56, SER-57, VAL-58, VAL-59, LEU-62, SER-63, ARG-72, THR-103, ARG-104, VAL-105, ARG-106, PHE-107, PRO-109, PHE-114, ASP-115, SER-116, PRO-117, ARG-118, PHE-119, SER-120, VAL-121, SER-122, LYS-123, LEU-124, HIS-126, LEU-127, LEU-128, ALA-130, LYS-131, ALA-132, CYS-135, LEU-138, THR-139, ILE-140, LYS-141, PHE-142, LEU-143, ASP-144, LYS-145, ASN-146, THR-147, GLU-201, TYR-207, ASN-209, LEU-210, ILE-211, PRO-212, ALA-214, LYS-263, GLN-272, THR-273, LYS-274, GLU-275	87
6.	Human Topoisomerase	1S16	ARG-162, LYS-378, ASN-163, GLN-376, ASN-150, SER-148, ASN-91, SER-149, LYS-168, ALA-167, GLY-166, GLY-164, TYR-165, ASN-120.	14
7.	*Aeromonas* Oligopeptidase	2DEA	TYR-218.	1
8.	Human Oligopeptidase	1S4B	HIS-473, HIS-477, GLU-502.	3

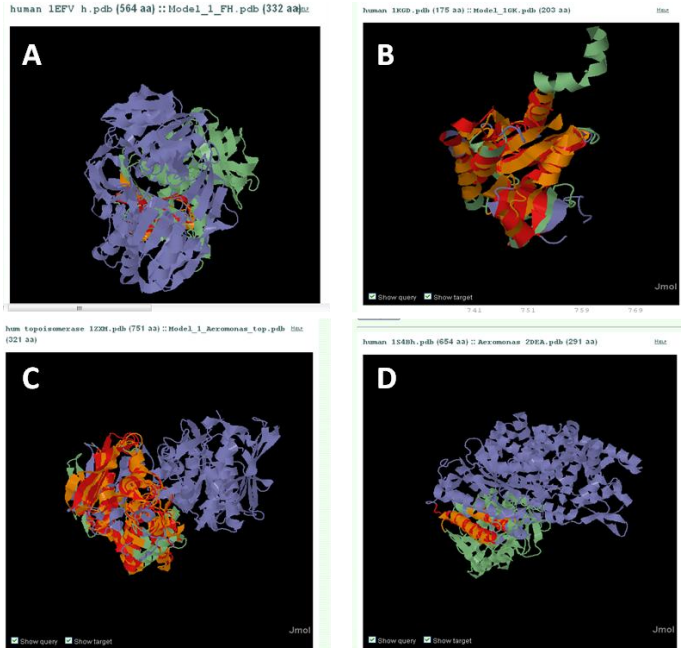


Figure 10: The super imposition protein structure from *Aeromonas* and Humans A: Flavohemoprotien, B: Guanylate kinase, C: DNA Topoisomerase, D: Oligopeptidase

Molecular Docking Studies

The inhibitors of all the four proteins were downloaded from pubchem data base, name, structure ID, structure and LogP of all the inhibitors subjected for docking are tabulated in Table 4, 5, 6 and 7 for Flavohemoprotein inhibitors, Guanylate kinase inhibitors, Topoisomerase inhibitors and Oligopeptidase inhibitors respectively (Rajesh *et al*., 2013). Flavohemoprotein inhibitors (4 and 8) and Oligopeptidase inhibitors (4 and 8) have not docked successfully.

Table 4: Flavohemoprotein Inhibitors

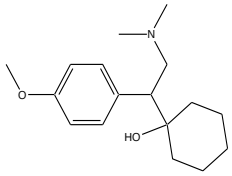
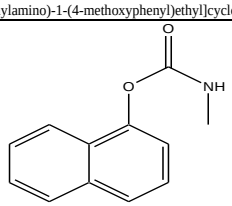
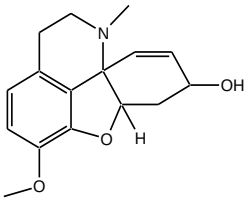
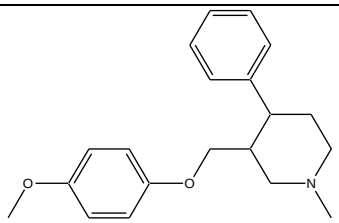
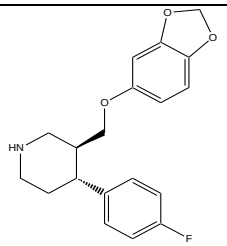
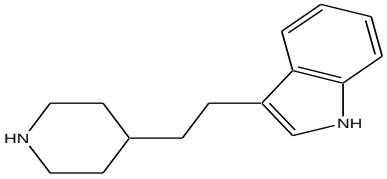
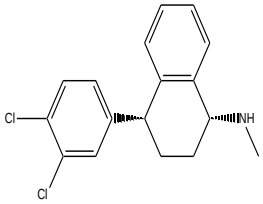
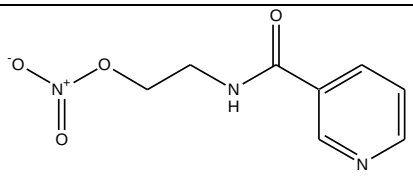
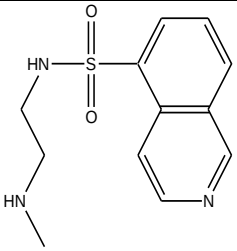
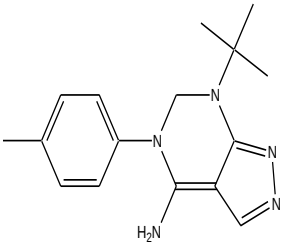
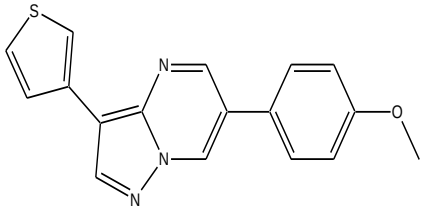
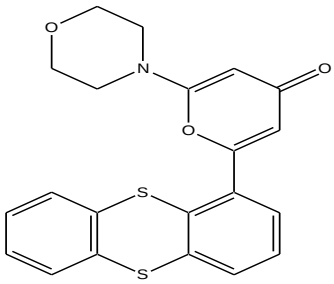
Mol. No	Name of Structure	Structure ID	Structure	Log P value
1	Venlafaxine	CID_5656	 1-[2-(dimethylamino)-1-(4-methoxyphenyl)ethyl]cyclohexan-1-ol	2.9
2	Carbaril	CID_6129	 naphthalen-1-yl N-methylcarbamate	2.4
3	Galanthamine	CID_9651		1.8
5	Femoxetine	CID_43103	 3-[(4-methoxyphenoxy)methyl]-1-methyl-4-phenylpiperidine	4.0
6	Paroxetine	CID_43815	 (3S,4R)-3-(1,3-benzodioxol-5-yloxymethyl)-4-(4-fluorophenyl)piperidine	3.5
7	Lipstene	CID_44668	 3-(2-piperidin-4-ylethyl)-1H-indole	3.3
9	Flesinoxan	CID_57347	 (1R,4R)-4-(3,4-dichlorophenyl)-N-methyl-1,2,3,4-tetrahydronaphthalen-1-amine	2.0

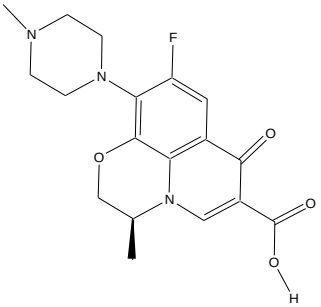
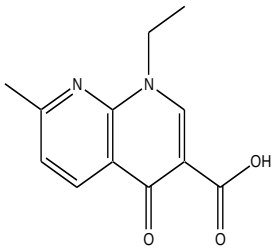
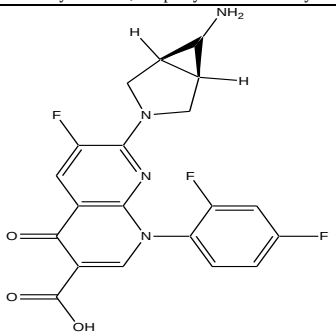
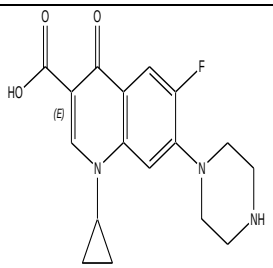
Table 5: Guanylate kinase inhibitor.

Mol. No	Name of Structure	Structure ID	Structure	Log P value
1	Nicorandil	CID_47528	 2-(pyridine-3-carbonylamino)ethyl nitrate	0.8
2	1yds; H-8 dihydrochloride	CID_3540	 N-[2-(methylamino)ethyl]isoquinoline-5-sulfonamide	0.6
3	PP1 cpd; tyrosine kinase inhibitor PP1	CID_10125426	 7-tert-butyl-5-(4-methylphenyl)-6H-pyrazolo[3,4-d]pyrimidin-4-amine	2.2
4	Pyrazolo[1,5-A]Pyrimidine 3g	CID_5329468	 6-(4-methoxyphenyl)-3-thiophen-3-ylpyrazolo[1,5-a]pyrimidine	3.2
5	Nchembio.63-Comp3	CID_5278396	 2-morpholin-4-yl-6-thianthren-1-ylpyran-4-one	3.9

In the Molecular docking study of Flavohemoprotein inhibitors against Human and *Aeromonas* Flavohemoprotein, the inhibitor HfhI9 showed minimum docking energy, binding energy, inhibition constant and 0.92 RMS value with Human Flavohemoprotein (Figure 11). The same molecule named AfhI9 showed minimum docking energy, binding energy, inhibition constant and 0.0 RMS value against *Aeromonas* Flavohemoprotein but less when compare with HfhI9. The AfhI7 shown good results than other *Aeromonas* protein inhibitors (Figure 12). This kind of inhibitors cannot use as an antibacterial drugs. The minimum binding energy, docking energy, Inhibition constant and RMS value are tabulated in Table 8 and 9.

Molecular docking study of Guanylate kinase inhibitors against Human and *Aeromonas* Guanylate kinase showed that the inhibitor HgkI5 showed minimum docking energy, binding energy, inhibition constant and 0.0 RMS value with Human Guanylate kinase protein (Figure 13). The same molecule named AgkI5 showed minimum docking energy, binding energy, inhibition constant and 0.0 RMS value against *Aeromonas* Guanylate kinase but less when compare with HgkI5. The AgkI5 inhibitor showed good results than HgkI5 (Figure.14). This kind of inhibitors can use as an antibacterial drugs. The minimum binding energy, docking energy, Inhibition constant and RMS value are tabulated in Table 10 and 11.

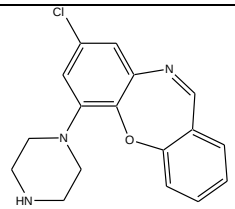
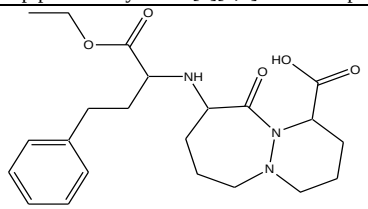
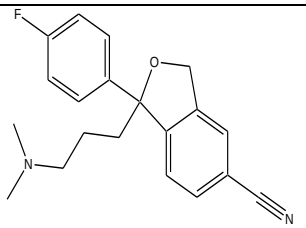
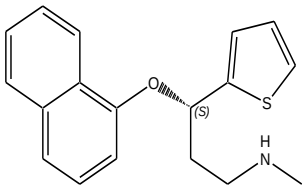
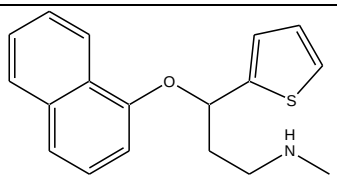
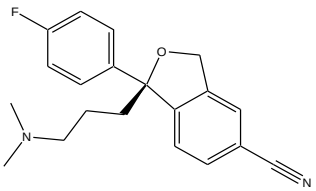
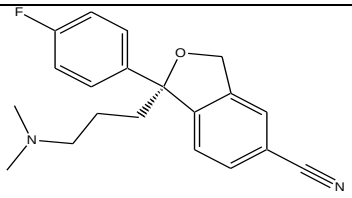
Table 6: Topoisomerase inhibitors

Mol. No	Name of Structure	Structure ID	Structure	Log P value
1	Levofloxacin	CID_149096	 8-Fluoro-3-methyl-9-(4-methyl-piperazin-1-yl)-6-oxo-2,3-dihydro-6*H*-1-oxa-3a-aza-phenalene-5-carboxylic acid	-0.4
2	Nalidixic acid	CID_4421	 1-ethyl-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid	1.4
3	Trovafloxacin	CID_62959	 7-[(1R,5S)-6-amino-3-azabicyclo[3.1.0]hexan-3-yl]-1-(2,4-difluorophenyl)-6-fluoro-4-oxo-1,8-naphthyridine-3-carboxylic acid	0.3
4	Ciprofloxacin	CID_2764	 1-cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid	-1.1

In the Molecular docking study of Topoisomerase inhibitors against Human and *Aeromonas* Topoisomerase, the inhibitor HtopI4 showed minimum docking energy, binding energy, inhibition constant and 0.0 RMS value with Human Topoisomerase (Figure 15). The same molecule named AtopI4 showed minimum docking energy, binding energy, inhibition constant and 0.0 RMS value against *Aeromonas* Topoisomerase but less when compare with HtopI4. The AtopI3 shown good results than other *Aeromonas* protein inhibitors (Figure 16). This kind of inhibitors cannot use as an antibacterial drugs. The minimum binding energy, docking energy, Inhibition constant and RMS value are tabulated in Table 12 and 13.

Eventually the molecular docking study of Oligopeptidase inhibitors against Human and *Aeromonas* Oligopeptidase was performed, here also the inhibitor HopI1 showed minimum docking energy, binding energy, inhibition constant and 0.0 RMS value with Human Oligopeptidase (Figure 17). The same molecule named AopI1 showed minimum docking energy, binding energy, inhibition constant and 0.0 RMS value against *Aeromonas* Oligopeptidase but less when compare with HopI1. The AopI1 shown good results than other *Aeromonas* protein inhibitors (Figure 18). This kind of inhibitors cannot use as an antibacterial drugs. The minimum binding energy, docking energy, Inhibition constant and RMS value are tabulated in Table 14 and 15.

Table 7: Oligopeptidase inhibitor.

Mol. No	Name of Structure	Structure ID	Structure	Log P value
1	Amoxapine	CID_2170	 8-chloro-6-piperazin-1-ylbenzo[b][1,5]benzoxazepine	2.6
2	Cilazapril	CID_2751	 9-[(1-ethoxy-1-oxo-4-phenylbutan-2-yl)amino]-10-oxo-1,2,3,4,6,7,8,9-octahydropyridazino[1,2-a]diazepine-1-carboxylic	0.6
3	Nitalapram	CID_2771	 1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-3H-2-benzofuran-5-carbonitrile	3.2
5	Cymbalta	CID_60835	 (3S)-N-methyl-3-naphthalen-1-yloxy-3-thiophen-2-ylpropan-1-amine	4.3
6	Duloxetine	CID_122252	 N-methyl-3-naphthalen-1-yloxy-3-thiophen-2-ylpropan-1-amine	4.3
7	Escitalopram	CID_146570	 (1S)-1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-3H-2-benzofuran-5-carbonitrile	3.2
9	Citalopram	CID_6101829	 (1R)-1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-3H-2-benzofuran-5-carbonitrile	3.2

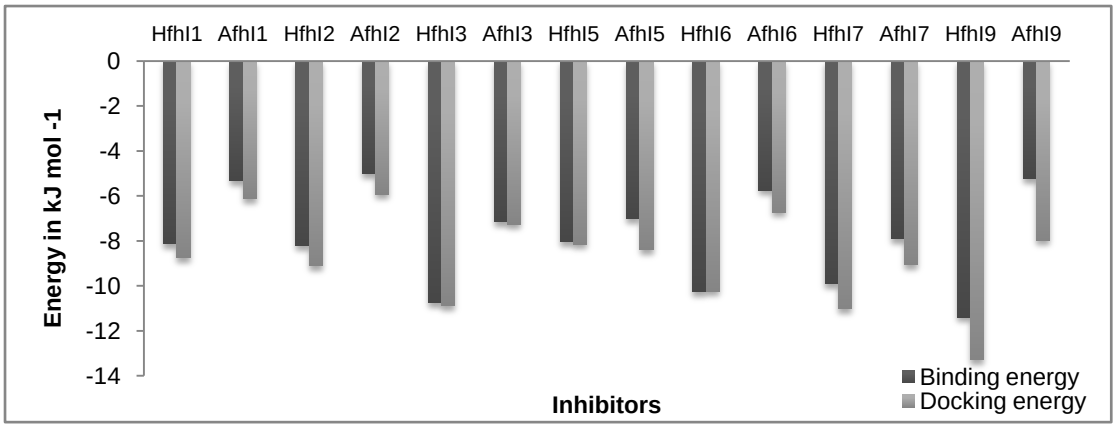


Figure 11: Graph showing binding energy and Docking energy of an inhibitors with both Human and *Aeromonas* Flavohemoprotein.

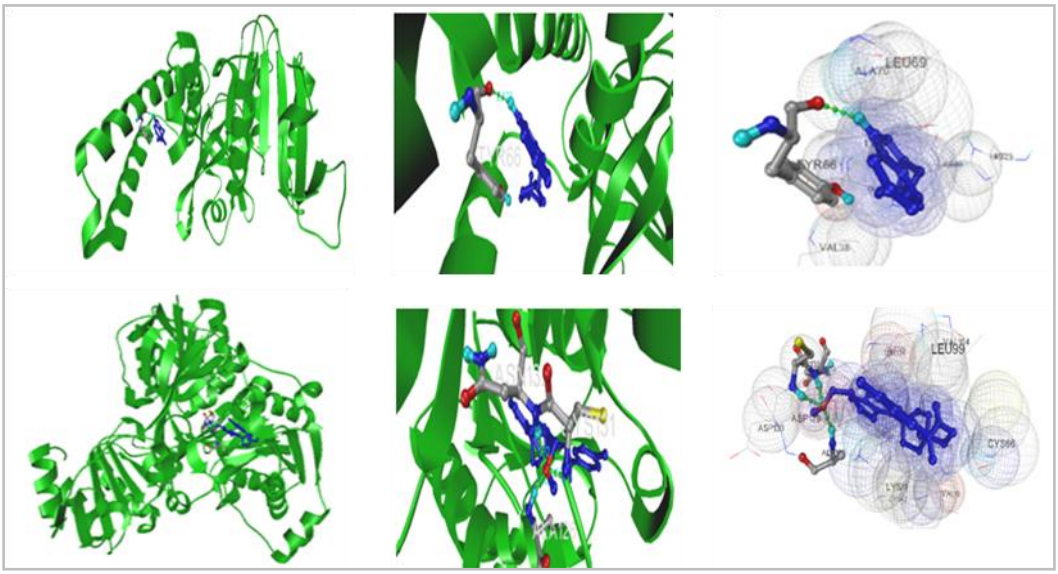


Figure 12: Docking of flavohemoprotein with the ligand flesinoxan, Top three corresponds to *Aeromonas* and Bottom three corresponds to human.

Table 8: Docking results for *Aeromonas* Flavohemoprotein (modeled protein)

Molecule number	Binding energy	Docking energy	Inhibition Constant	RMS
AfhI1	-5.31	-6.13	0.000127	0.37
AfhI2	-5.01	-5.92	0.000213	0.91
AfhI3	-7.16	-7.26	5.62e-006	0.1
AfhI5	-7.01	-8.39	7.25e-006	0.0
AfhI6	-5.75	-6.72	6.12e-005	1.51
AfhI7	-7.9	-9.05	1.61e-006	0.0
AfhI9	-5.23	-7.97	0.000146	0.0

Table 9: Docking results for Human Flavohemoprotein (1EFV)

Molecule number	Binding energy	Docking energy	Inhibition Constant	RMS
HfhI1	-8.14	-8.74	1.07e-006	0.37
HfhI2	-8.21	-9.11	9.66e-007	0.0
HfhI3	-10.74	-10.88	1.34e-008	0.05
HfhI5	-8.04	-8.15	1.29e-006	0.0
HfhI6	-10.27	-10.24	2.95e-008	1.72
HfhI7	-9.9	-11.01	5.54e-008	0.73
HfhI9	-11.4	-13.28	4.42e-009	0.92

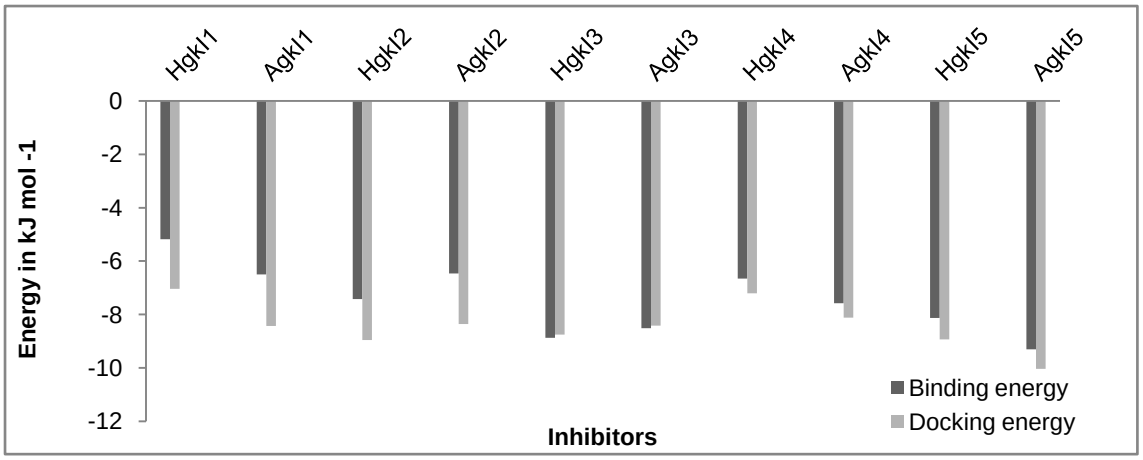


Figure 13: Graph showing binding energy and docking energy of an inhibitors with both Human and *Aeromonas* Guanylate kinase proteins.

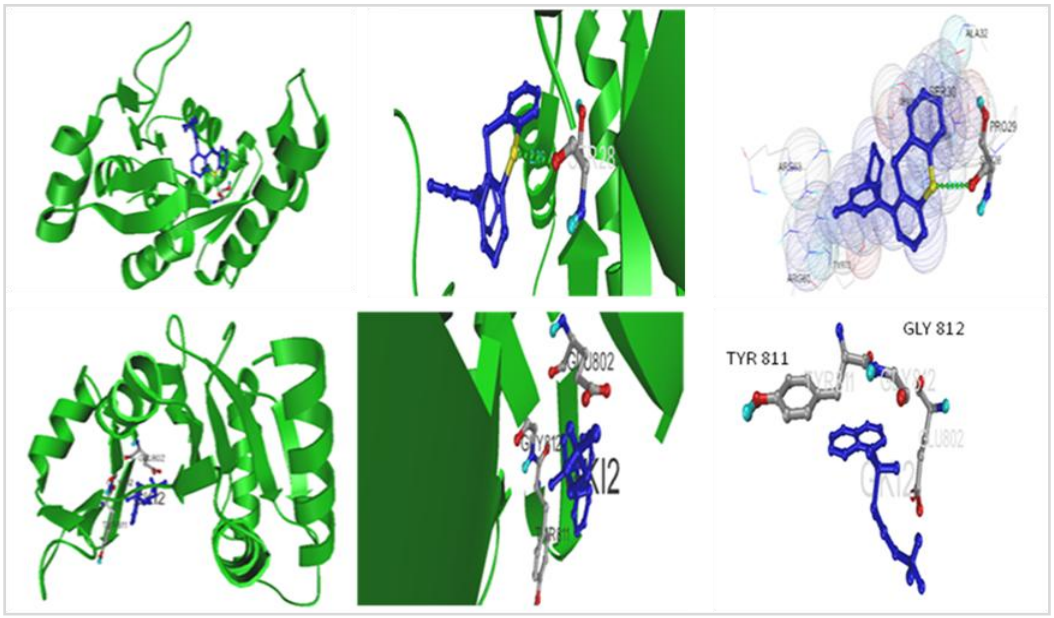


Figure 14: Docking of guanylate kinase with the ligand Nchembio.63-Comp3, Top three corresponds to *Aeromonas and* Bottom three corresponds to human Docking results.

Table 10: Docking results for *Aeromonas* Guanylate kinase (Modeled).

Molecule number	Binding energy	Docking energy	Inhibition Constant	RMS
AgkI1	-6.50	-8.43	1.71e-005	0.0
AgkI2	-6.46	-8.35	1.84e-005	0.0
AgkI3	-8.51	-8.42	5.78e-007	0.0
AgkI4	-7.58	-8.12	2.76e-006	0.0
AgkI5	-9.30	-10.03	1.53e-007	0.0

Table 11: Docking results for Human Guanylate kinase (1KGD).

Molecule number	Binding energy	Docking energy	Inhibition Constant	RMS
HgkI1	-5.18	-7.04	0.000159	0.0
HgkI2	-7.42	-8.95	3.65e-006	0.0
HgkI3	-8.87	-8.75	3.17e-007	0.0
HgkI4	-6.65	-7.21	1.33e-005	0.0
HgkI5	-8.13	-8.93	1.10e-006	0.0

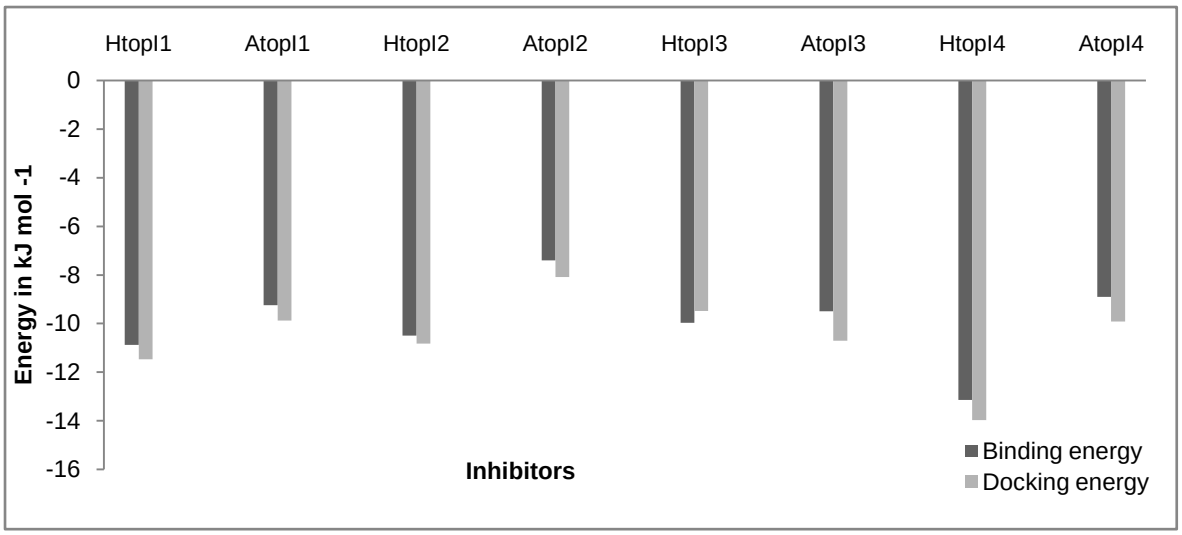


Figure 15: Graph showing Binding energy and Docking energy of an inhibitors with both Human and *Aeromonas* Topoisomerase proteins.

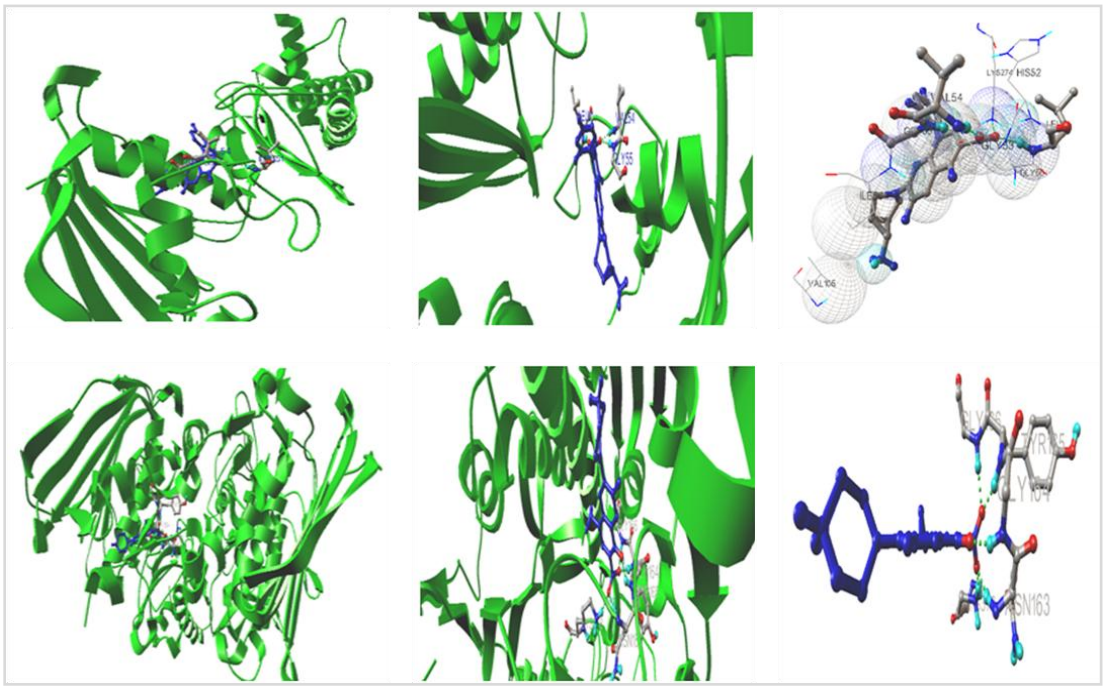


Figure 16: Docking of DNA Topoisomerase with the ligand Ciprofloxacin, Top three corresponds to *Aeromonas and* Bottom three corresponds to human.

Table 12: Docking results for *Aeromonas* Topoisomerase (Modeled)

Molecule number	Binding energy	Docking energy	Inhibition Constant	RMS
AtopI1	-9.24	-9.87	1.69e-007	0.0
AtopI2	-7.4	-8.08	3.73e-006	0.0
AtopI3	-9.49	-10.70	1.11e-007	0.84
AtopI4	-8.90	-9.91	2.98e-007	0.0

Table 13: Docking results for Human Topoisomerase (1S16)

Molecule number	Binding energy	Docking energy	Inhibition Constant	RMS
HtopI1	-10.88	-11.47	1.05e-008	0.05
HtopI2	-10.49	-10.83	2.05e-008	0.0
HtopI3	-9.97	-9.48	4.88e-008	0.72
HtopI4	-13.14	-13.97	2.35e-010	0.0

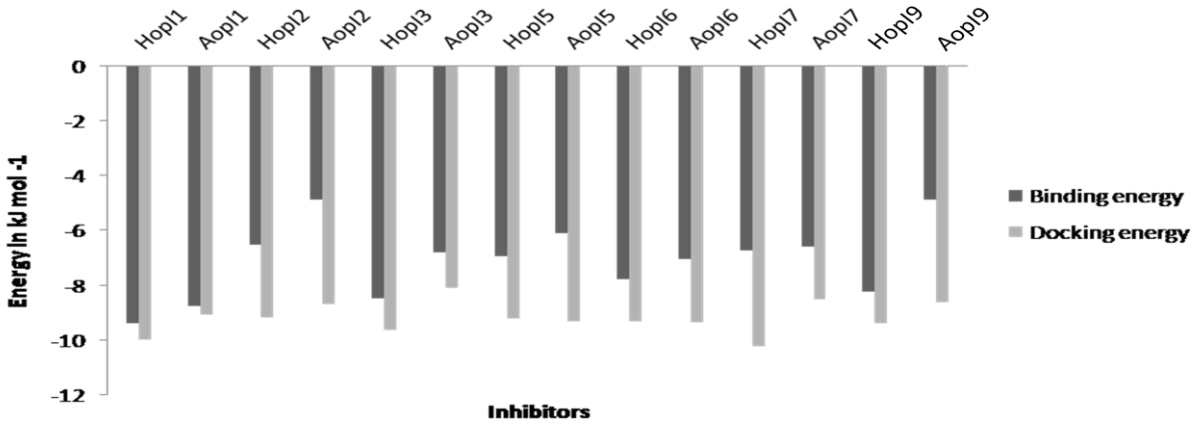


Figure 17: Graph showing Binding energy and docking energy of an inhibitors with both Human and *Aeromonas* Oligopeptidase proteins.

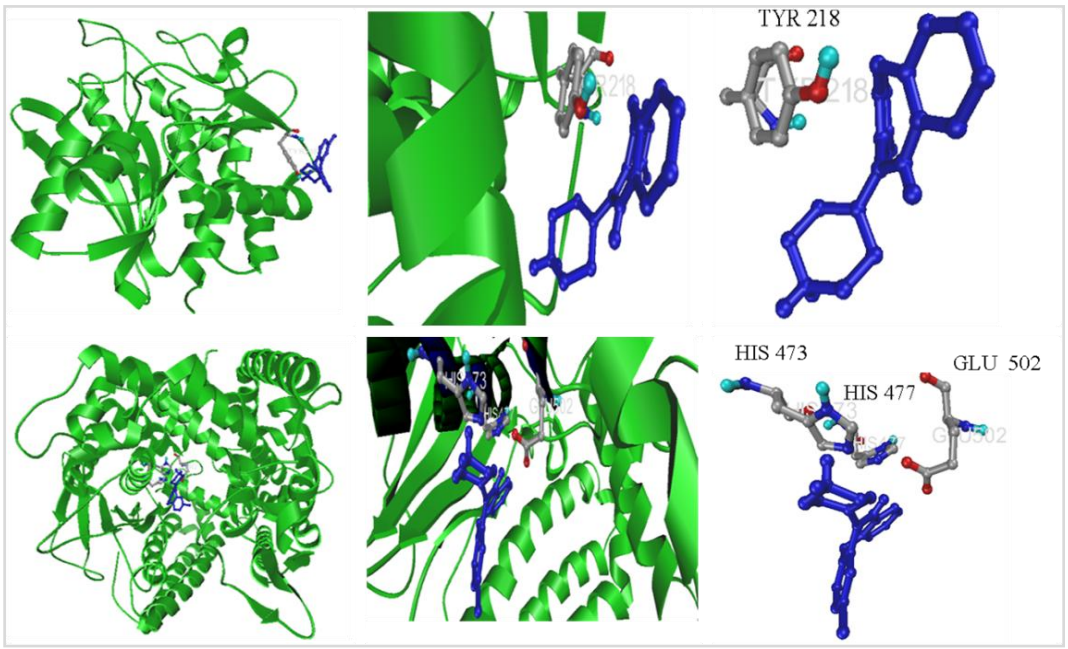


Figure 18: Docking of Oligopeptidase with the ligand Citalopram, Top three corresponds to *Aeromonas and* Bottom three corresponds to human.

Table 14: Docking results for *Aeromonas* Oligopeptidase (2DEA)

Molecule number	Binding energy	Docking energy	Inhibition Constant	RMS
AopI1	-8.74	-9.06	3.94e-007	0.0
AopI2	-4.88	-8.67	0.000266	0.0
AopI3	-6.77	-8.08	1.1e-005	0.0
AopI5	-6.08	-9.30	9.81e-006	0.0
AopI6	-7.04	-9.34	6.97e-006	0.0
AopI7	-6.56	-8.49	1.54e-005	0.0
AopI9	-4.87	-8.59	0.000267	0.0

Table 15: Docking results for Human Oligopeptidase (2DEA)

Molecule number	Binding energy	Docking energy	Inhibition Constant	RMS
HopI1	-9.39	-9.98	1.48e-007	0.0
HopI2	-6.50	-9.17	1.73e-005	0.0
HopI3	-8.45	-9.62	6.41e-007	0.0
HopI5	-6.91	-9.19	8.55e-006	0.0
HopI6	-7.78	-9.29	1.99e-006	0.0
HopI7	-6.71	-10.23	1.21e-005	0.0
HopI9	-8.23	-9.37	9.34e-007	0.0

CONCLUSIONS

By the sequence analysis, structural analysis, and functional analysis of the four proteins taken from the human and bacteria, we have performed the docking studies by taking the inhibitors for four proteins. These inhibitors showed good docking energy with human proteins than *Aeromonas hydrophila* proteins which mediates neonatal septicemia, gastroenteritis and aquatic wound infections in mammals. Thus, we conclude that the taken inhibitors cannot be used as antibiotics, but the AgkI5 inhibitor showed good results than HgkI5, this kind of inhibitors can be use as antibacterial drugs.

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