International Journal of Innovative Research in Computer Science & Technology (IJIRCST)
ISSN: 2347-5552, Volume-5, Issue-6, November-2017
DOI:10.21276/ijircst.2017.5.6.1

A Systematic Bioinformatics Approach for Multiple Virtual Screening for Finding Novel MDR-TB Antagonists: Structure Based Screens & Toxicity Prediction Analyzing Poly Pharmacological Affects

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***Abstract*— Multidrug-resistant tuberculosis has been resistant over more than two primary drugs (isoniazid and rifampin) resulted due to misuse and mismanagement which leads to administration of improper treatment regimens and failure to ensure that patients complete the whole course of treatment. Screening the library of MDR-TB antagonists against the bacterial strains can improve the pharmacological optimization compared over the primary drugs.**

In the present study, virtual screening has been introduced using surflex dock targeting mycobacterial metabolite (DNA Gyrase) virtually screened against chemical library of Rifampin and Isoniazid. Consequently, HtrA2 has been virtually screened against chemical library of ciprofloxacin, ofloxacin and moxifloxacin according to their bio affinity and analysed polypharmacological affects.

Derivate of Ofloxacin (ZINCID_39383034) demonstrate better interactions with HtrA2 (PDBID_2PZD) and so on derivate of Rifampin (ZINC_ID85907485) demonstrate better interactions with DNA Gyrase (PDBID_3IFZ) following Lipinski's rule of five and without any mutagenic, toxic or carcinogenic effects.

Comparatively, analysing the binding patterns of derivates contrary to their primary drugs, inferred that docking score of the derivates have been significant than the primary drug and toxic level has been reduced in contrary to their primary drugs which has proved hints for the future design of new derivatives with higher potency and specificity.

***Index Terms*— Drug resistant, tumorigenic properties, pharmacological optimization, poly pharmacological affects.**

Manuscript Received October 20, 2017

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I. INTRODUCTION

Tuberculosis (TB) remains a significant global health problem, responsible for an estimated 1.7 million deaths per year worldwide. Resistance to anti-tuberculosis drugs is an important threat to tuberculosis control (1). The risk of treatment failure and death with standard short-course chemotherapy is highest with resistance to both isoniazid and rifampicin. Drug-resistant tuberculosis is "human made": The emergence of multidrug resistant tuberculosis i.e. involving resistance to at least isoniazid and rifampin could threaten the control of tuberculosis globally (2). Multi Drug Resistant TB (MDR-TB) is caused by bacteria that are resistant to the most effective anti-TB drugs (Isoniazid and Rifampicin) with or without resistance to other drugs (3, 4). Multidrug-resistant tuberculosis (MDR-TB) is a severe and feared problem that is difficult to control and has shown a tendency to increase worldwide (5). It results from treatment with inadequate drugs or drug regimens, improper case management, and preventable transmission. Its presence generally reflects weak tuberculosis control in the past or present. (6) The emergence and spread of multidrug-resistant tuberculosis (MDR-TB) will threaten global TB control. The treatment of patients with MDR-TB is prolonged, expensive and often unsuccessful (7). Many experts assert that standard TB control prevents the emergence of MDR-TB in a cost-effective way (8, 9). Multidrug-resistant tuberculosis (MDR-TB) is tuberculosis due to organisms which show high-level resistance to both isoniazid and rifampicin, with or without resistance to other anti-TB drugs (10). The molecular basis of resistance to isoniazid and rifampicin and some other drugs is now largely understood. Resistance to isoniazid is due to mutations at one of two main sites, in either the *katG* or *inhA* genes. Resistance to rifampicin is nearly always due to point mutations in the *rpo* gene in the beta subunit of DNA-dependent RNA polymerase (11 to 13). Antibiotic resistance has become a major hurdle to overcome bacterial diseases and thus there is always a need to find new drug targets or inhibitors or both (14). At present very few drugs are available in the market for treatment of M. tuberculosis infection as evolution of drug-resistant strains have resulted in little efficacy and some of them have shown undesired side-effects in host (15).

Most well-known bacterial drug target DNA Gyrase (tetramer A2B2 protein) has been encoded by gyrB-gyrA, which has been used to catalyse negative supercoiling of DNA and essential for efficient DNA replication, transcription and recombination (16). The subunit A (90 to 100 kDa, depending on the bacterial species) carries the breakage-reunion active site, whereas the subunit B promotes ATP hydrolysis, needed for energy transduction. It appears that DNA Gyrase is the sole topoisomerase drug target in Mycobacterium tuberculosis. The absence of a homologue in eukaryotic cells makes Mtb-DNA Gyrase an attractive target for small molecule inhibitors with the potential to have broad antibacterial activity (17, 18).

The serine protease HtrA2 belongs to the high-temperature requirement protein family, which contain a trypsin-like protease domain and a regulatory C-terminal PDZ domain (20). HtrA2 is imported into mitochondria by a mitochondrial targeting sequence and is then inserted into the inner mitochondrial membrane. A mature form is generated by proteolysis close to the trans membrane domain and resides within the inner mitochondrial space (21). HtrA2 are synthesized as precursor proteins with N-terminal mitochondrial localization signal peptides that are removed during maturation in the mitochondria to expose their N-terminal IBM. However, very little is known about the protease activity or substrates of Omi/HtrA2 after its release from the mitochondria during cell death (22). High temperature requirement A (HtrA2) is a mammalian serine protease that resides in the mitochondria of healthy cells, but apoptotic stimuli cause it to be released into the cytoplasm, where it binds the inhibitor of apoptotic proteins. This results in proteolytic degradation of these inhibitor proteins and in the following activation of the caspase cascade mechanism. HtrA2 is also involved in caspase-independent cell death through its serine protease activity exerted directly on specific targets. (23–25)

II. METHODOLOGY

A. Protein structure retrieval and active site prediction

The structure and sequence of HtrA2 (PDB ID: 2PZD) and DNA Gyrase (3IFZ) present in mycobacterium tuberculosis are retrieved from RCSB Protein Data Bank (http://www.rcsb.org) which have been selected for active site predictions through protomol generation and molecular docking of the protein models have been validated, optimized and purified by removing ligands and other hetro-atoms (water,ions,etc).

B. Substrate selection

The three dimensional chemical structures of Rifampin, Isoniazid and its derivatives along with commercially available anti MDR-TB drugs such as ciprofloxacin, moxifloxacin, and ofloxacin are retrieved from zinc database and Drug bank (http://www.drug bank.cal) (table-4).The optimization of ligand structures are carried out from Sybyl software .Further, the Rifampin, Isoniazid, Ciprofloxacin, Moxifloxacin, Ofloxacin and its 230 derivatives are evaluated to give their molecular properties using Osiris property explorer (27).

C. Molecular Docking and Screening

Derivatives of Rifampin and Isoniazid along with their reference ligands are docked into active site of DNA Gyrase and HtrA2 proteins using Sybyl Software. Screening has been carried out on the basis of subset preparation of primary drug and its derivates based on surflex Docking using Sybyl software. Fifty independent docking runs were performed for each ligand. Further protein ligand complex has been optimized and Hydrogen bond interaction between the derivate with the hydrophobic amino acid residual constituent of the target being analysed.

D. Statistical Analysis

The statistical analysis of the binding energy of the best molecules of Rifampin, Isoniazid , Ciprofloxacin, Moxifloxacin, Ofloxacin and its derivatives are being conducted using Sybyl software. The selected ten derivates form the reference primary drugs have shown the lowest binding energy against each of the protein models being selected for the analysis.

III. RESULTS AND DISCUSSION

Insilco approach for molecular docking has been proved to be a time effective and cost effective methodology to analyse the interaction profile of ligands with the target protein .The structural models of 3IFZ (DNA Gyrase) and 2PZD (HtrA2) from mycobacterium tuberculosis were selected for the binding interaction analysis against 230 derivates including primary molecules (Rifampin, Isoniazid, Moxifloxacin, ciprofloxacin and ofloxacin).The active site predictions carried out using active site predictor(table-11).Almost all the binding sites obtained, the site containing the catalytic serine residue has been found to be highly conserved in DNA Gyrase and HtrA2 which have been further taken into consideration for docking .Most of the active site residues were highly conserved, thus deciphering common organ and functionality. Rifampin, Isoniazid (FDA approved drugs) and its derivatives followed Lipinski's rule of five and are non-irritant, non-mutagenic, non-tumourgenic further analysis of the ligands have been based on their drug score which combines with druglikeness, logP, logS, molecular weight and toxicity risks associated with the ligand.. OSIRIS property explorer provides a cumulative score between 0and 1, which allow judging the overall potential of the compound to become a drug. It is observed that Isoniazid derivate (Zinc ID_331024) interacted with 3IFZ resulting to give -3158.512 Dscore which is least score in 3D chemical drug library of Isoniazid and rifampin derivate (Zinc ID_85907485) interacted with 3IFZ resulting to give -3501.96 Dscore which is least score in 3D chemical drug library of Rifampin , ciprofloxacin (Zinc ID_20220) interacted with 2PZD resulting to give -1162.52 D_score , moxifloxacin (Zinc ID_3826253) interacted with 2PZD resulting to give -1608.641.

ISSN: 2347-5552, Volume-5, Issue-6, November-2017
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Table 1: Value/ Drug score of FDA approved MDR-TB antagonist drugs targeting against DNA Gyrase (3IFZ) and Htra2 (2PZD)

S.No.	Targeting against receptor	Drug name	Zinc ID	No. of poses	Drug Score
1.	DNA Gyrase **(3IFZ)**	Isoniazid	1590	5	3.5149
2.	DNA Gyrase **(3IFZ).**	Rifampin	94313219	5	7.4105
3.	HtrA2 **(2PZD)**	Moxifloxacin	3826253	5	3.9702
4.	HtrA2 **(2PZD)**	Ciprofloxacin	20220	5	5.3059
5.	HtrA2 **(2PZD)**	Ofloxacin	537891	5	2.7406

Dscore and the ofloxacin derivate (Zinc id_39383034) has the highest drug score of 0.9 and interacted with 2PZD resulting to give -1642.279 D_score , one of the other ofloxacin derivative (Zinc_Id 44830343) also has a comparable drug score of around0.85 (table no-5)which make them a better candidate for the analysis. Rifampin , Isoniazid and its derivatives are evaluated for their inhibitory potential against serine proteases based on their affinity and the interaction patterns. The computational study predicts the higher affinity of the antagonist ligand in terms of least docking score of ligand against the receptor. In the present investigation, Rifampin, Isoniazid and its derivatives molecules which are showing lowest docking score are considered as the best docked drugs and these derivates of each drug have a higher affinity for proteins (Table No.6,7,8,9,and 10). It is important to mention that docking score itself is not sufficient to predict ligand's potential to inhibit the protein .The stability and interaction between a ligand and the amino acid residues of a protein also play a crucial role for the same. Hydrogen bonds are one of the important parameters for interaction profile analysis. In this regard hydrogen bonds formed in protein ligand complex were illustrated (table no-6.1, 7.1, 8.1, 9.1 and 10.1).

Table 2: Classification of MDR-TB Antagonist drugs on the basis of their partial concentration, solubility, H-bond donor, and acceptor

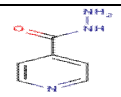
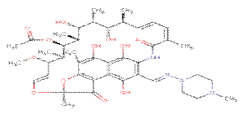
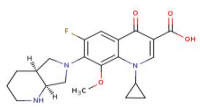
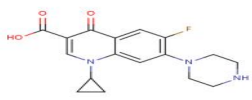
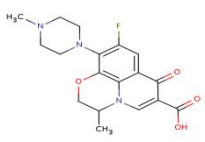
Drug name	Structure	Log p	Log s	pKa (at 20 °C)	water solubility	hydrogen acceptor count	hydrogen donor count
Isoniazid		-0.70	0.01	1.82	1.4E+005 mg/L (at 25 °C)	3	2
Rifampin		2.7	-4.3	1.7	1400 mg/L (at 25 °C)	14	6
Moxifloxacin		2.9	-3.4	5.69	1.68e-01g/l	7	2
Ciprofloxacin		0.28	-2.4	6.09	1.35e+00g/l	6	2
Ofloxacin		-0.39	-2.4	5.45	28.3mg/ml	7	1

Table 3: Osiris property explorer prediction: molecular properties of isoniazid[$], rifampin[#] ciprofloxacin[*], ofloxacin[&] and moxifloxacin[%] and their derivates

S.No.	Compound ZINC_ID	Molecular properties					
		cLogP	Molecular Weight	Drug likeness	Drug score	Tumorigenic/ Mutagenic	Reproductive effective
1	1590[$]	-1.02	137.6	-5.06	0.06	Yes/yes	Yes
2	1726073	-0.42	148.6	2.75	0.27	Yes/no	Yes
3	258332	-0.38	242.6	2.75	0.33	Yes/no	Yes
4	345613	-0.98	151.6	0.22	0.17	Yes/no	Yes
5	1664678	-1.4	181.6	3.17	0.35	Yes/no	Yes
6	5414781	0.9	177.6	5.62	0.34	Yes/no	Yes
7	16051610	0.38	135.6	0.68	0.65	No/no	No
8	3071307	-0.6	213.6	2.56	0.2	Yes/yes	Yes
9	1563455	-0.62	151.6	-5.86	0.18	Yes/no	Yes
10	94313219[#]	4.7	822.6	10.5	0.09	Yes/yes	No
11	85907267	4.71	778.6	5.71	0.22	No/no	No
12	77312918	3.86	695.6	6.59	0.28	No/no	No
13	85896170	3.3	821.6	8.77	0.27	No/no	No
14	79221574	3.88	722.6	7.46	0.17	No/no	No
15	20220[*]	-1.53	331.6	2.07	0.82	No/no	No
16	5929712	-0.26	375.6	2.35	0.74	No/no	No
17	0537891[&]	-0.34	361.6	5.77	0.87	No/no	No
18	13535560	-0.44	343.6	7.15	0.9	No/no	No
19	39383034	-0.44	343.6	7.15	0.9	No/no	No
20	44830343	-0.95	405.6	6.49	0.84	No/no	No
21	3826253[%]	-0.95	401.6	1.6	0.68	No/no	No

Table 4: Comparative docking score analysis of isoniazid[*] and its 10 derivatives with least docking score out of 50 derivatives against DNA Gyrase (3IFZ)

Serial no	Drug name	Total score	No. of poses	Hydro bond with AA
1	1590[*]	3.5149	5	-
2	1726073	0.5318	5	A/ Ser 118
3	1402787	0.7091	5	-
4	16051610	1.3087	5	A/ Gly 179
5	1664678	1.4029	5	-
6	258332	1.4048	5	-
7	3071307	1.4187	5	A/Ser104,A /Gly101
8	5414781	1.4738	5	A/Ser 104
9	34561360	1.6031	5	-
10	**331024**	**1.8084**	**5**	**A/ TYR 276**
11	1563455	1.8856	5	-

Table 4.1: Best docked drugs of isoniazid out of 10 least docking score drug against DNA Gyrase (3IFZ)

Serial no	Drug	Total score	Crash	Polar	D score	PMF
1	331024	2.05	-0.21	1.71	-3158.51	-4.120
2	5414781	1.89	-1.61	1.71	-260.834	15.196
3	3071307	1.64	-0.12	1.10	-228.503	10.292
4	1726073	1.52	-0.19	1.69	-158.019	22.892
5	16051610	1.51	-0.30	1.65	-175.820	-23.386
6	258332	0.08	-0.69	0.67	-277.287	-18.097

ISSN: 2347-5552, Volume-5, Issue-6, November-2017
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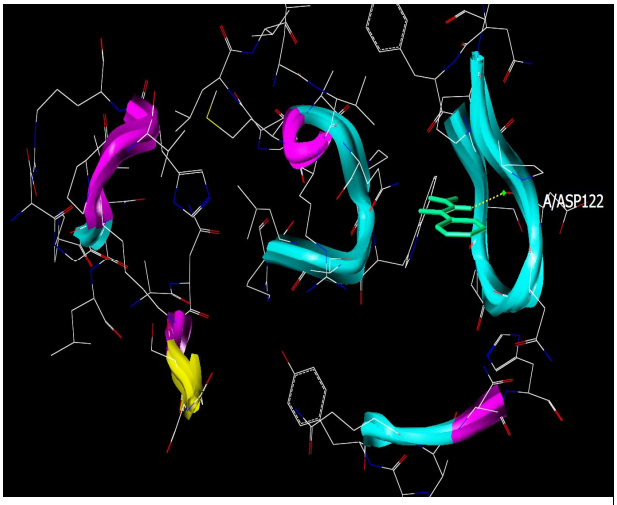

Figure 1: Docking interaction of Isoniazid (Zinc ID_1590) against DNA Gyrase (3IFZ) bonding with residue A/ASP122.

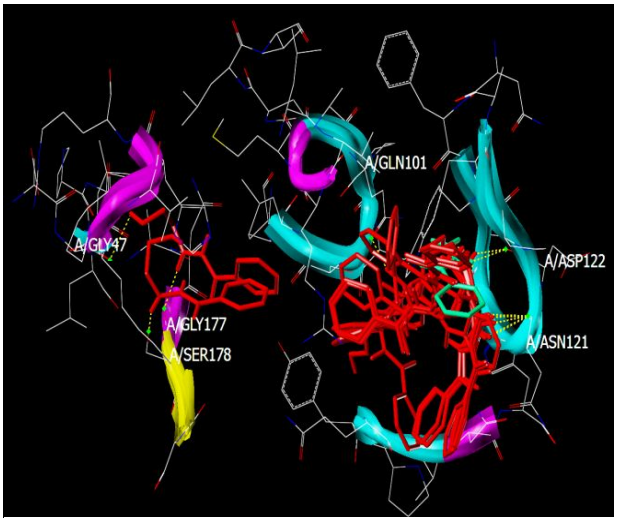

Figure 2: Multiple screening image of Isoniazid (Zinc ID_1590) against DNA Gyrase (3IFZ) bonding with residue A/ASP122, A/ASN121, A/GLN101, A/GLY47, and A/GLY177and A/SER178.

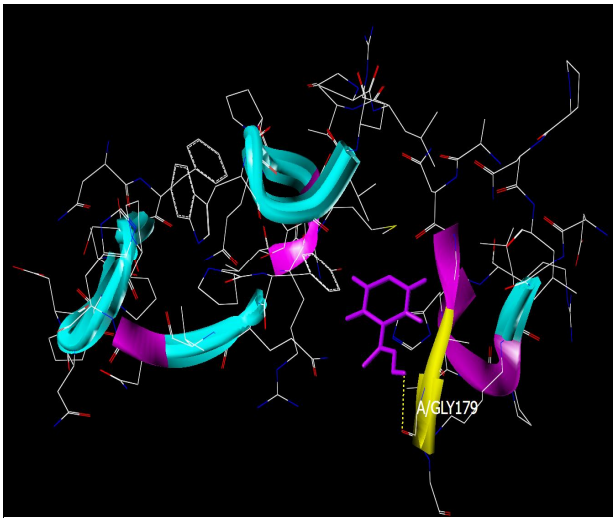

Figure 3: Docking interaction of Isoniazid derivate (Zinc ID_16051610) against DNA Gyrase (3IFZ) bonding with residue A/GLY179.

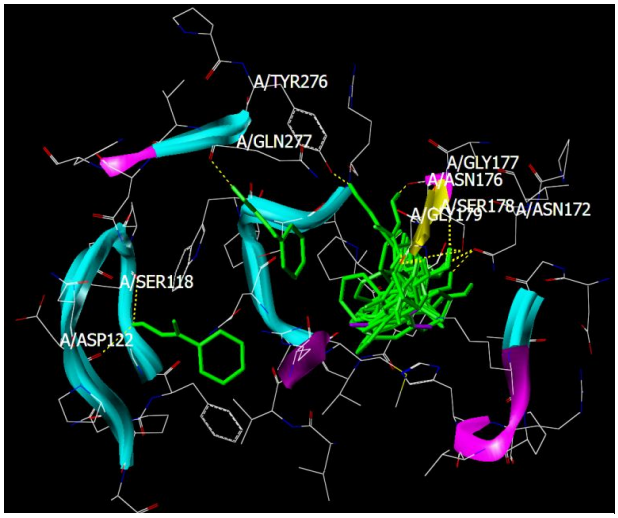

Figure 4: Multiple screening image of Isoniazid derivate (Zinc ID_16051610) against DNA Gyrase (3IFZ) bonding with residue A/TYR276, A/GLN277, A/SER118, A/ASP122, A/GLY177, A/ASN176, A/SER178, A/ASN172 and A/GLY179.

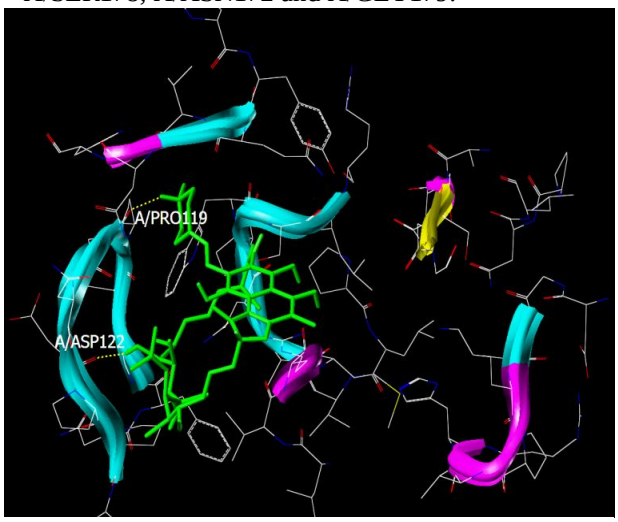

Figure 5: Docking interaction of Rifampin (Zinc ID_ 94313219) against DNA Gyrase (3IFZ) bonding with residue A/PRO119, A/ASP122.

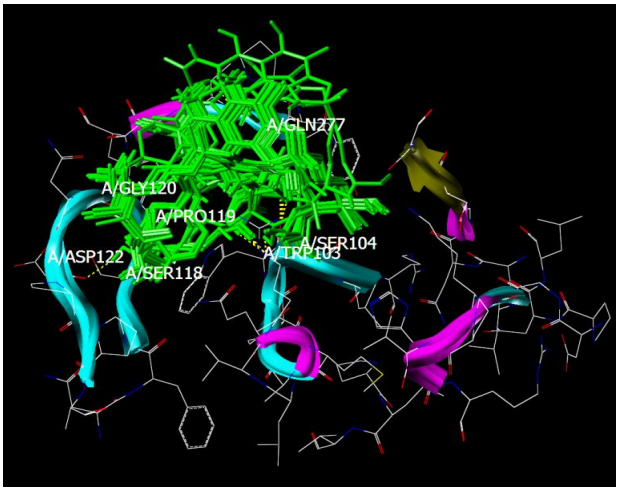

Figure 6: Multiple screening image of Rifampin (Zinc ID_94313219) against DNA Gyrase (3IFZ) bonding with residue A/GLN277, A/GLY120, A/PRO119, A/ASP122, A/SER118, A/SER104 and A/TRP103

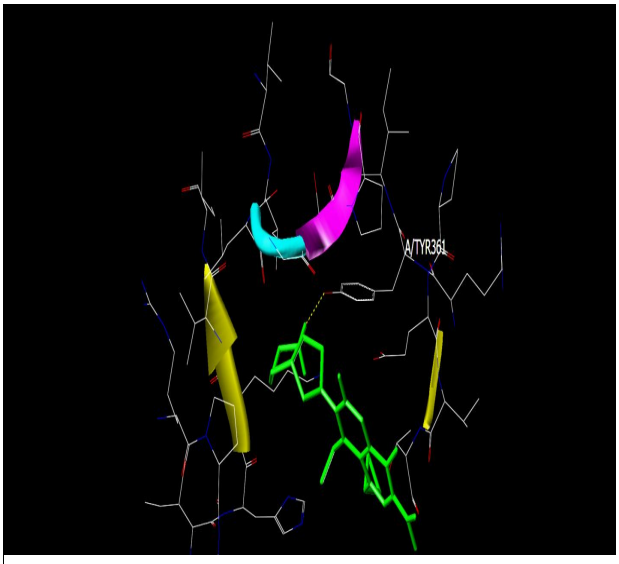

Figure 7: Docking interaction of moxifloxacin (Zinc ID 3826253) against HtrA2 (2PZD) bonding with residue A/TYR361.

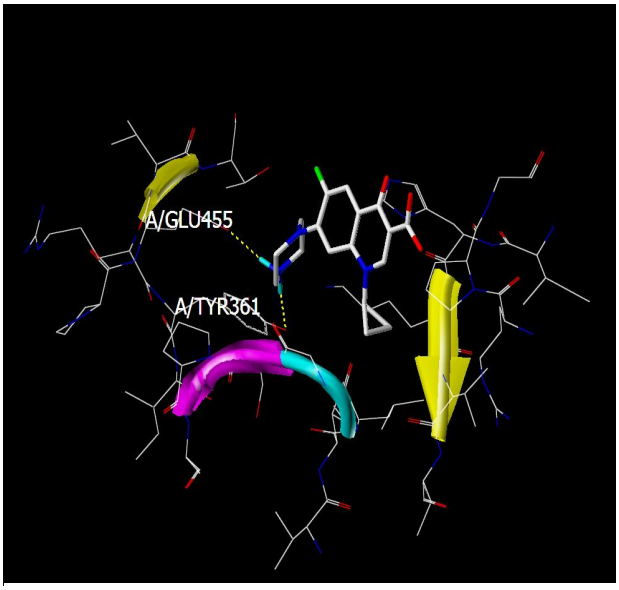

Figure 8: Docking interaction of ciprofloxacin (Zinc ID_ 20220) against HtrA2 (2PZD) bonding with residue A/GLU455 and A/TYR361.

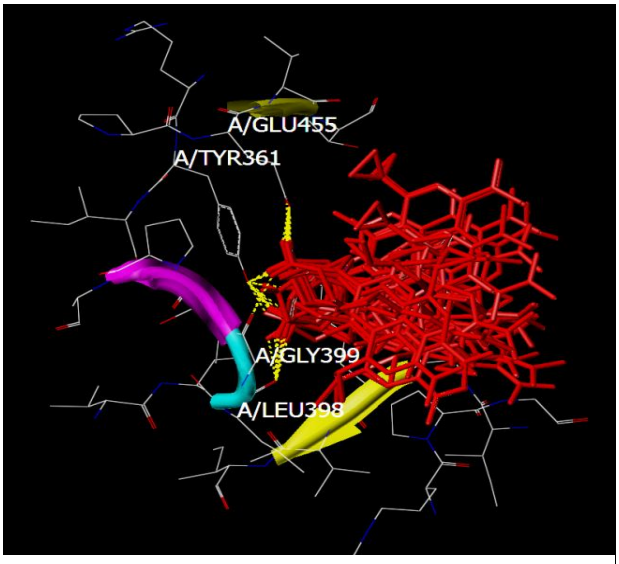

Figure 9: Multiple screening image of ciprofloxacin (Zinc ID_ 20220) against HtrA2 (2PZD) bonding with residue A/GLU455, A/TYR361, A/GLY399 and A/LEU398.

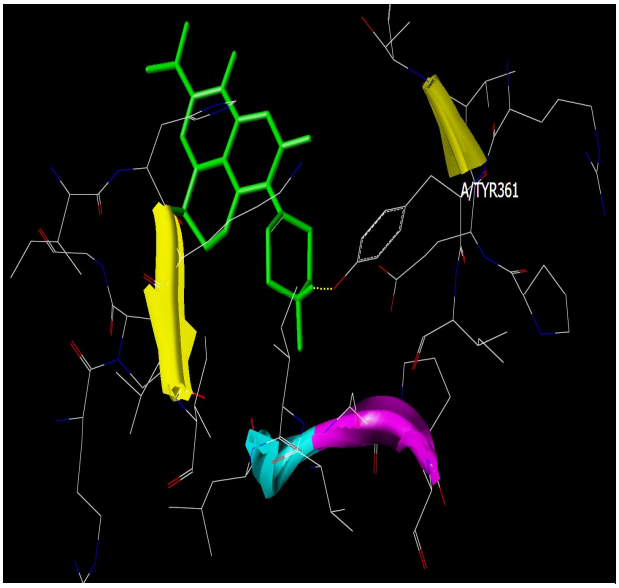

Figure 10: Docking interaction of oflaxacin (Zinc ID 537891) against HtrA2 (2PZD) bonding with residue A/TYR361.

Table 5: Comparative docking score analysis of Rifampin* and its 10 derivatives with least docking score out of 31derivatives against DNA Gyrase (3IFZ)

Serial no.	Drug name	Total score	Number of poses	Hydro bond with AA
1	94313219*	7.4105	5	-
2	95100869	-5.7439	5	-
3	85896181	-1.2636	5	-
4	85907485	0.7653	5	A/VAL278
5	79210126	1.1522	5	-
6	94313220	1.8382	5	A/GLN277
7	85907267	2.4754	5	-
8	85896170	2.6236	5	-
9	79221574	2.9294	5	A/GLY120
10	77312918	3.0864	5	-
11	49538620	3.2841	5	-

Table 5.1: Best docked drugs of Rifampin out of 10 least docking score drug against DNA Gyrase (3IFZ)**.**

Serial no.	Drug	Total score	Crash	Polar	D score	PMF
1	85907485	6.41	-1.48	2.45	-3501.961	-19.587
2	85907267	5.28	-1.42	0.66	-679.814	-4.890
3	94313220	4.27	-2.96	2.77	857.932	32.081
4	79221574	3.83	-1.12	1.02	-606.157	2.838
5	95100869	-10.54	-18.56	0.97	-2687.529	51.751
6	85896181	-27.40	-40.61	0.72	-1485.852	144.474

Table 6: Comparative docking score analysis of ciprofloxacin* and its 10 derivatives with least docking score out of 50 derivatives against HtrA2(2PZD).

Serial no	Drug name	Total score	Number of poses	Hydro bond with AA
1	20220*	5.3059	5	-
2	1089984	0.4892	5	-
3	70460737	1.2494	5	-
4	3886912	1.8838	5	-
5	65739406	2.0182	5	-
6	366933	2.2711	5	-
7	2540776	2.4487	5	-
8	5161651	2.7283	5	-
9	3887391	2.7652	5	-
10	26740199	2.9823	5	-
11	5929712	3.0542	5	-

Table 6.1: Best docked drugs of ciprofloxacin out of 10 least docking score drug against HtrA2

Serial no	Drug	Total score	Crash score	Polar	D score	PMF score
1	2540776	3.02	-0.46	0.00	-735.147	-5.237
2	3887391	2.42	-0.28	0.00	-849.478	-26.550
3	1089984	-0.02	-0.49	0.00	-2001.970	-1.494

Table 7: Comparative docking score analysis of ofloxacin* and its 10 derivatives with least docking score out of 50 derivatives against HtrA2(2PZD).

Serial no	Drug name	Total score	Number of poses	Hydro bond with AA
1	537891*	2.7406	5	-
2	44830343	0.6631	5	A/THR457
3	22065454	1.442	5	-
4	3426255	1.528	5	A/TYR361/B /HIS394
5	13535560	1.6617	5	-
6	22065452	1.7088	5	-
7	67665161	2.2698	5	-
8	65739900	2.4663	5	-
9	65735081	2.5841	5	-
10	**39383034**	**3.0366**	**5**	**A/LEU398**

Table 7.1: Best docked drugs of Ofloxacin out of 10 least docking score drug against HtrA2 (2PZD).

Serial no	Drug	Total score	Crash	Polar	D score	PMF
1	39383034	2.76	-0.22	1.66	-1642.279	-29.315
2	67665161	2.72	-0.92	0.00	-516.390	-12.880
3	65739900	2.62	-0.40	0.00	-268.527	-23.989
4	65735081	2.48	-0.39	0.00	-740.094	-4.452
5	22065454	1.97	-0.23	0.00	-183.217	-34.102
6	22065452	1.73	-0.31	0.00	-336.583	-13.586
7	03426255	1.69	-1.58	1.93	-1601.285	-85.279
8	44830343	0.88	-0.84	1.54	-402.976	-49.679

Table 8: Comparative docking scores analysis of moxifloxacin$ and its 10 derivatives with least docking score out of 50 derivatives against HtrA2 (2PZD).

Serial no.	Drug name	Total score	Number of poses	Hydro bond with AA
1	**3826253$**	**3.9702**	**5**	**A/TYR361**
2	65747896	2.4187	5	-
3	9210709	2.5336	5	-
4	8670498	2.6999	5	-
5	17527863	2.7958	5	-
6	9210710	3.0052	5	-
7	17297429	3.0162	5	-
8	30819403	3.1324	5	-
9	17297427	3.1373	5	-
10	65742956	3.4452	5	-
11	8670499	3.4760	5	-

Table 8.1: Best docked drugs of moxifloxacin out of 10 least docking score drug against HtrA2 (2PZD).

Serial no	Drug	Total score	Crash score	Polar score	D score	PMF
1	03826253	3.20	-0.48	0.12	-1608.641	-29.765
2	17297427	3.10	-0.36	0.00	-704.187	-17.679
3	08670498	2.98	-0.22	1.47	-503.868	-72.414

IV. CONCLUSION

Analyzing the binding patterns of derivates of rifampin ,isoniazid ,moxifloxacin ,ofloxacin and ciprofloxacin, it has been observed that Isoniazid derivate with Zinc ID_331024 has -3158.51 D_score and rifampin derivate with Zinc ID_85907485 has -3501.96 D_score, ciprofloxacin with Zinc ID_20220 has D_score -1162.52 but no hydrogen bond interactions were observed, moxifloxacin with Zinc ID_3826253 has -1608.64 D_score and the ofloxacin derivate with Zinc id_39383034 has the D_score of –1642.279 which is least D_score than other 3D chemical library targeting against Htra2(2PZD) and it has a drug_score of 0.9 compared with another Ofloxacin derivate Zinc Id 44830343 has comparable drug score of around0.85 which predict them as a better candidate for the analysis. The ofloxacin derivate Zinc ID_39383034 interacted with LEU398(Chain A of Htra2 receptor) and rifampin derivate with Zinc ID_85907485 interacted with

VAL278(Chain A of DNA gyrase receptor) has least docking score, validating least mutagenic and tumorigenic characteristic amongst all the 3-D chemical library including pro-drugs and hence it proves hints for the future design of new derivatives with higher potency and specificity.

V. ACKNOWLEDGEMENT

We wish to earnestly express our gratitude to BIFC, D.G.P.G.College, Kanpur funded by Department Of Biotechnology, India for providing us the financial assistance to conduct the research work. Also special thanks to Dr.P.P.Mathur , Professor & H.O.D, Department of Biochemistry and Molecular Biology, School of Life Sciences, Pondicherry University, Pondicherry, India for his technical support and useful discussions working with the SYBYL-X tool.

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