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Original Research Paper

THEORETICAL APPROACH FOR LIGAND BASED DRUG DESIGN OF LPXC: A KEY ENZYME OF LIPID A BIOSYNTHESIS

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ABSTRACT

Lipid A is an essential component of the outer membranes of the gram negative bacteria and genetic evidence has established that inhibition of its biosynthesis is lethal to gram-negative bacteria. It is seen that hydrophobicity is very important factor for the activity of LpxC inhibitors. Regression model developed in this work may be essential step for further development in designing and synthesis of good LpxC inhibitors.

Keywords: LpxC, Regression model, Docking, Hydrophobicity.

INTRODUCTION

Drug resistance of pathogens had necessitated the identification of novel targets for antibiotics and new libraries of antimicrobial compounds. An ideal antimicrobial compound should target biochemical pathways or molecules exclusive to the pathogen. Enzymes related to important biosynthetic pathway, which is both unique and essential to gram-negative bacteria, presents several good candidate enzymes for novel antimicrobial drugs. One such significant enzyme is LpxC, which catalyzes the first committed step in Lipid A biosynthesis. A novel lipid a biosynthesis inhibitors possessing antimicrobial efficacy was first reported by the researches of Merck Research Laboratories.[1] Lipopolysaccharide or LPS layer is an important structural unit of gram negative bacterial cell wall which is located in the outer leaflet of the outer membrane and consist four domain; the hydrophobic anchor lipid A, the inner core oligosaccharide, the outer core oligosaccharide and the O-antigen. Lipid A is an essential component of the outer membranes of the gram negative bacteria and genetic evidence has established that inhibition of its biosynthesis is lethal to gram-negative bacteria.[2,3] Thus making it an ideal target for antibiotics and antimicrobial inhibitors. The past two and a half decade has seen a tremendous emphasis on the advancement of novel antimicrobials against multi drug resistant GNB by aiming at the UDP-3-O-(R-3 hydroxymyristoyl)–N-acetylglucosamine deacetylase or LpxC which is the second enzyme in the Lipid A biosynthesis pathway and catalyzes the first committed step of Lipid A biosynthesis.[4-6] LpxC catalyzes the first committed step of lipid A biosynthesis which involves the deacetylation of uridine 5-diphosphate-3-O-[R-3-hydroxymyristoyl]-N-acetyl-glucosamine by UDP-3-O-[R-3 hydroxy myristoyl]-GlcNAC deacetylase (LpxC). It is an essential enzyme[7,8] with homologues in more or less 40 gram

negative species known till date. There is no mammalian homologue of LpxC. LpxC, though is a zinc dependent enzyme but is in fact a type of deacetylase which is dependent on zinc and has a distinct motif where zinc interacts with two histidine and one aspartic acid residues for proper helix packing.

From the structural point of view, utilizing X-ray crystallography and NMR data, the LpxC enzyme protein has been found to display a unique two domain 'β-α-α-β sandwich' fold (L1; 25 & 26) which belongs to the α+β class of folds (L3; 30). Both the LpxC domains I and II have a unique insert in the form of a small anti-parallel β-sheet for the former domain and a hydrophobic binding channel (L1; 24).

Broad screening has identified the lead inhibitor compounds L-573,655[1] as an inhibitor of this enzyme and also a series of carbohydroxamido-oxazolidine inhibitors. It was further observed that the aryl oxazoline hydroxamic acids were inhibitors of this enzyme and L-161,240 was a potent inhibitor of the LpxC from *Escherichia coli*.[9,10] Later Toni *et al.*[11] synthesized several focused small molecule libraries, each composed of a variable (Figure 1) aromatic ring, one heterocyclic /spacer moieties' and a hydroxamic acid and evaluated the LpxC inhibition of these compounds against purified *P. aeniginosa* enzyme. They gave efforts at expanding the original oxazoline molecule resulted in the first reported inhibitors of the *P. aeruiginosa* LpxC. For making the active compound the retained the hydroxamic acids for its well known chelating property, (in this case for Zn binding function) as the inhibition of metalloproteases by chelating to the metal in their active sites is well documented.[12] For the exploration of the contribution of heterocyclic and aromatic ring to overall activity of these compounds the two moieties were varied with different substituents. The most active heterocyclic ring appeared to be the oxazoline. Ring expansion (oxazines) reduced the activity and with a few exceptions ring opening (acyl series) or sulfur substitution (thiazolines) gave rise to considerable weaker inhibitors (see Table 1). The highest inhibitory potencies came from acyl oxazolines with 3, 4-disubstituted phenyl ringswith fluoro or trifluro methoxy in the para or meta position and a two to five atom hydrophobic group in the complementary position. These suggest that the electronic properties of the phenyl ring, the orientation of the oxygen lone pair and a certain optimal hydrophobicity are the chief determinants of good inhibitory potency. The m-trifluromethoxy function may be serving towards these functions. In the absence of OCF_3 or F-substituent, sub-micromolar potencies[13-15] were achieved by optimizing hydrophobic group at the para position. Another very important eight LpxC inhibitors are LPC009 series and CHIR09.[16] For the understanding of the molecular basis of the interaction of these inhibitors with LpxC, we applied molecular docking for evaluating binding and torsional energies. Further, we evaluated the HOMO, LUMO energies, and dipole moment to understand the properties of the inhibitors and to construct a correlation based phylogram. We also develop regression equations to correlate the electronic properties with activities of the compounds.

MATERIALS AND METHOD

A set of 70 compounds of carbohydroxamido-oxazolidine inhibitors and oxazoline hydroxamic acids has been selected from the work of Chen *et al.* and Toni *et al.*[11,17-19] The biological activity in IC50 in nM unit is collected from site Binding db[17-19] (www.bindingdb.org). The next step in developing a model is generation of the numerical descriptors of molecular structures. Carbohydroxamido-oxazolidine inhibitors were sketched using ACDLABS 10.0.[20] The molecular descriptors like HOMO, LUMO energy and Dipole moment were performed by DFT/B3LYP calculation using the software Gamess and the basis set 6-31G (d) was used.[21] The one lipophilic parameter LogP is calculated by Mervine LogP calculator.[22] LogP, the partiition coefficient is a ratio of un-ionised compound between the two solutions. It is also known as a measure of lipophilicity. It is widely used in medicinal chemistry to access drug lineness of a given molecule. It may be formulated as:

$$Log\ P_{\left(\frac{oct}{wat}\right)} = log\left(\frac{[Solute]_{octanol}}{[Solute]_{un-ionized\ water}}\right)$$

Another parameter Molar Volume (MV) which was calculated by ACDLABS 10.0. Crystal structure of the pseudomonas aeruginosa LpxC/LPC-009 complex (PDB code 3P3E)[23] is collected from Bindingdb (www.bindingdb.org) at a resolution of 1.28 Å.

RESULTS AND DISCUSSION

We have performed docking study with the ligand of the enzyme 3P3E (UDP-3-O-[3-Hydroxymyristoyl] N-Acetylglucosamine deacetylase) by using Autodock 4. The amino acid residues 62Met, 77Glu, 190Thr, 197Ile, 209Gly, 210Ser, 211Val, 237His, 241Asp, 264His and Zn atom lies within the 3 Å range from the binding site of 3P3E. It was also found that the residues 190Thr, 264His and Zn atom present within 2.5 Å from the binding site. All the amino acid residues within 3 Å range are initially taken as flexible residue and performed docking with compound 35. The lowest binding energy obtained for 190Thr (-5.32kcal/mol) which also lies within 2.5 Å from the binding site. Thus we consider 190Thr as the flexible residue in this docking Study.

Here we represent three docked images; compound 1, LPC009 and CHIR 09[16] with their nearest neighbours. Figure 2 represents the docked image of compound 1 which binds to the region of the enzyme involving 62Met, 158Ile, 159Asp, 160Phe, 191Phe, 192Gly, 193Phe 196Asp, 238Lys, 241Asp and 261Lys residues. It has two hydrogen bonds with 159Asp and 238Lys and also lies well inside the protein. Another important LpxC inhibitor LPC009 binds within the region of the enzyme involving 62Met, 143Lys, 158Ile, 159Asp, 160Phe, 191Phe, 192Phe, 196Asp, 197Ile, 206Ala and 261Lys. It forms hydrogen bond with 159Asp and 261Lys (Figure 3). Although the geometric structure of these two compounds are different but they bind in the same region of the enzyme. Another important inhibitor CHIR09 binds to the same region. The amino acid residues involving in this binding are 18Leu, 62Met, 158Ile, 159Asp, 160Phe, 156Phe, 191Phe, 197Ile, 206Ala, 214Ala, 238Lys, 262Ser, 261Lys and 263Gly (Figure 4). It forms four hydrogen bonds with the residues 159Asp, 238Lys, 261Lys and 262Ser which are also lies within 3 Å from the binding site. The amino acid residue 159Asp is common for all these inhibitors which form hydrogen bond. The energy of binding becomes -4.82 kcal/Mol for compound1, -5057 kcal/mol for Lpc 009 and -6.51kcal/mol for CHIR 09.

We have obtained binding energy of docking of 50 compounds (compound no. 1 to 50). The chemical structures of these 50 compounds are given in Table 1A-C. Binding energies range between -2.84 kcal/mol to -7.62 kcal/mol. Table 2 represents the obtained binding energies. The correlation coefficient between binding energy and Ic50 is 0.096 which is very poor. Thus, only binding energy cannot specify activity clearly and so obviously other factors must be incorporated to account for their activity. Now we introduce another parameter logP which is also known as liopholicity. The obtained LogP value with their activity is shown in the Table 3. The obtained values range between 0.10 and 3.83.

In the QSAR study, 70 potent LpxC inhibitors were taken. They were divided into two parts; training and test set. The 50 compounds which had already been taken for docking were taken as the training set and the remaining 20 compounds were taken as the test set. Chemical structures of these test compounds are presented in Table 4. Another eight (8) important LpxC inhibitors which have different chemical structure are also taken as test set is given in Table 5.

In our QSAR study, we have calculated other quantum chemical parameter like HOMO, LUMO dipole moment and molar volume. Foe scaling the data we have converted the Ic50 and molar volume into their natural logarithm. Table 6 represent the value of HOMO, LUMO, dipole moment, LogP, natural logarithm of molar volume and natural logarithm of Ic50. To understand the dependence of various inhibitors on their indices, we have calculated correlation between indices with LnIc50. The correlation matrix is given in Table 7. The correlation matrix shows that there is a good anti-correlation

between molar volume and activity (r=-0.8034) and moderate correlation with LogP (r=0.3294). Thus these two indices have a good effect in predicting activity. There are also good inter correlation between HOMO-LUMO (r=0.44) and between LUMO-dipole moment (r=-0.37). Thus it is likely that the hydrophobicity index (LogP) and molar volume correlate well with Ic50 values.

Based on correlation we have initially undertaken regression with LogP. This shows a correlation 0.45 and F-value 6.06. For molar volume with LogP yields r=0.48 and F=6.98, which is better than the previous one. In the third step we have used HOMO, LUMO and dipole moment. This regression gives very poor correlation (0.088) and F value (0.12). Thus this equation cannot be accepted statistically. Inclusion of LogP with third regression improves both correlation and F value (r=0.50, F=7.842). Lastly, we include molar volume with fourth regression we form a model which is better than the other four models which gives correlation 0.88 and F value 37.52. So this is the best model. Regression models are shown in Table 8. We have calculated the LnIc50 values of 50 training compounds and 20 test compounds by using last model (fifth regression equation) which is presented in Table 9. We have also drawn correlation graph of training and test compound given in Figure 5 and 6 respectively. These two graphs also show a good agreement between the predicted and the experimental activity for training as well as test set. To predict the activities of other eight LpxC inhibitors, we have calculated their indices (HOMO, LUMO, dipole moment, LogP, molar volume). By using last model the predicted activity are given in Table 11.

Based on correlations between indices and activity we have obtained a Cladogram using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) of 50 training compounds. The cladogram of training compounds is presented in Figure 7. From the cladogram it is found that compounds having similar molecular properties are in the same clade, and activities are also comparable with few exceptions which is attributed to the replacement of hydrophobic chain or fluoride atom by some other atom or group. As for example, it is found that compound 1 and compound 7 are in the same branch and these are very similar. The only difference between these two compounds is the hydrophobic side chain in benzene ring, the 3-methylbut-2-en-1-yloxy group is replaced by but-3-en-1-yloxy group and it it is observed that the hydrophobicity of the compound is increased which is clear by logP value and thus its activity is also increased.

Compounds 2, 3, 6, 10 and 46 are in the same clade. The activity of compound 10 is less than compound 2. Here the hydrophobic OCF_3 group is replaced by F atom. The activity of the compound 3 and compound 6 are nearly the same. The activity of compound 46 is much lower, here Cl atom is inserted in the benzene ring which is the main reason of reducing hydrophobicity and the activity. In another clade compounds 5, 11, 18 and 37 are present. Compound 11 and 37 are in a pair. Activity of 37 is much less than that of compound 11 due to replacement of OCF_3 group by F atom and n-propyl group. Activity of the compound 18 decreases with respect to that of compound number 5 due to the replacement of n-propyl group by three methoxy group. Compounds 19, 32, 35 and 40 are in the same clade. In this clade 19 and 40 are in the same pair. Activity of compound decreases due to replacement of furan ring by p-tplyl ring with oxazoline. Compounds 32 and 35 has small change in activity due to replacement CF_3 group by 1-butene.

In another cladogram (Figure 8), compounds LpxC and CHIR090 are found to be in a clade with compounds 42 and 32 whose molecular properties are the same but their activity is much higher. Compounds D, E, G, H are in same clade due their similar geometry and molecular property. Compound B and compound F are in different branch due to the different position of NH_2 in the benzene ring.

CONCLUSION

Our investigations suggest that the molecular properties along with the docking study show a way to assign inhibitors of LpxC and proper

designing and screening of inhibitors which is a very essential step for drug synthesis.

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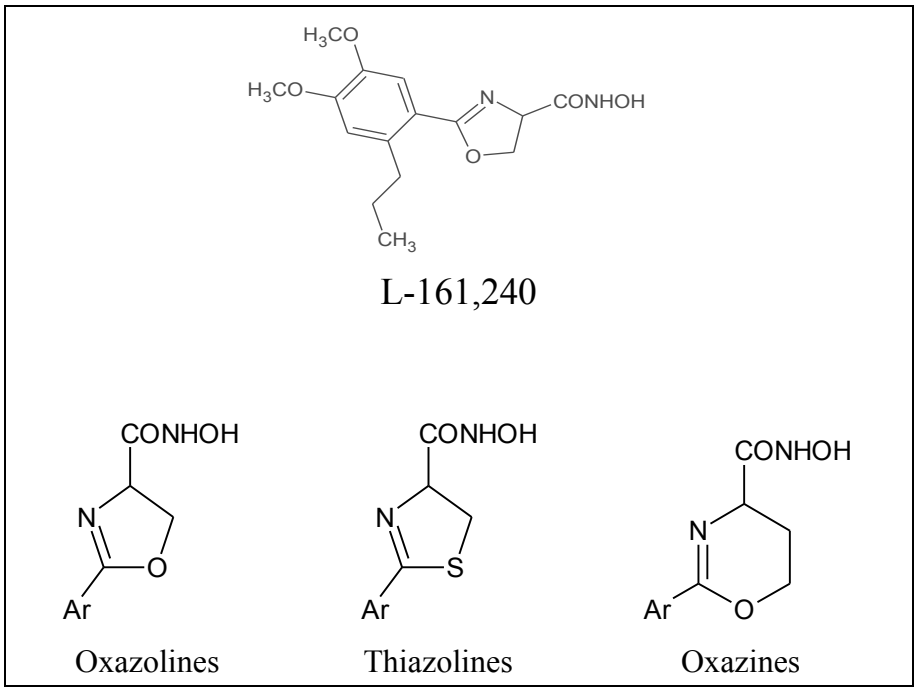


Figure 1: Series of related compounds by varying a set of aromatic rings.

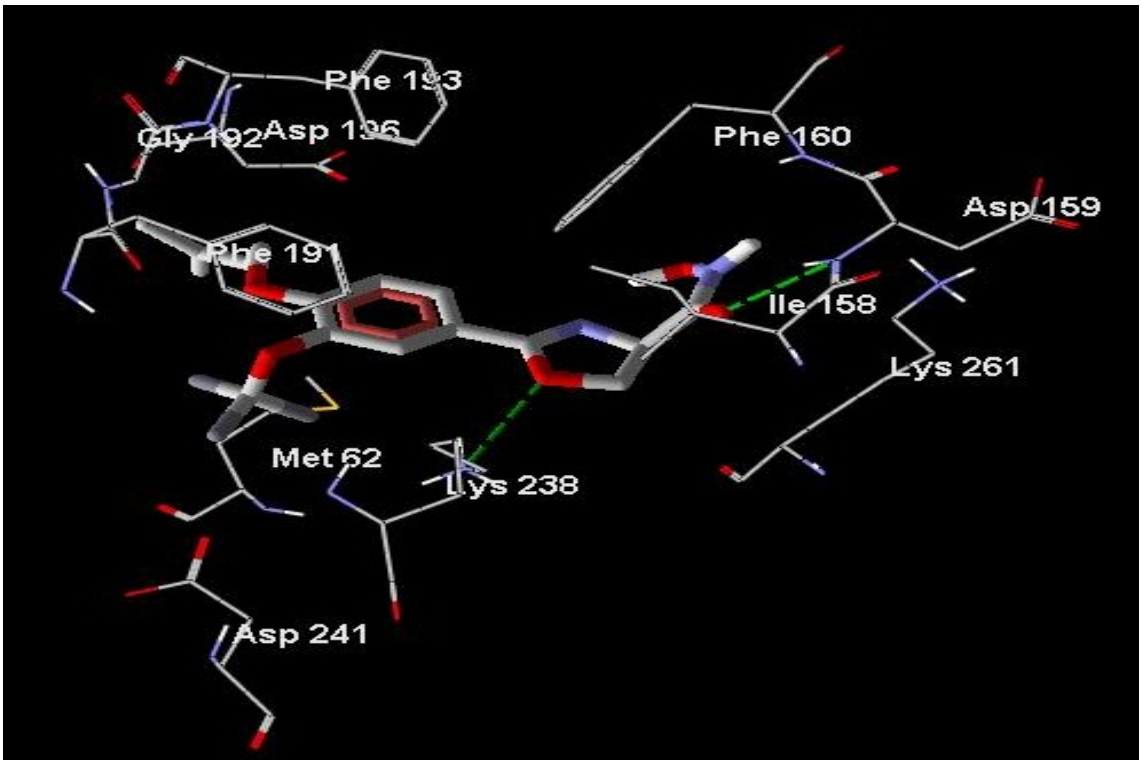


Figure 2: Docked image of compound 1 with 3P3E.

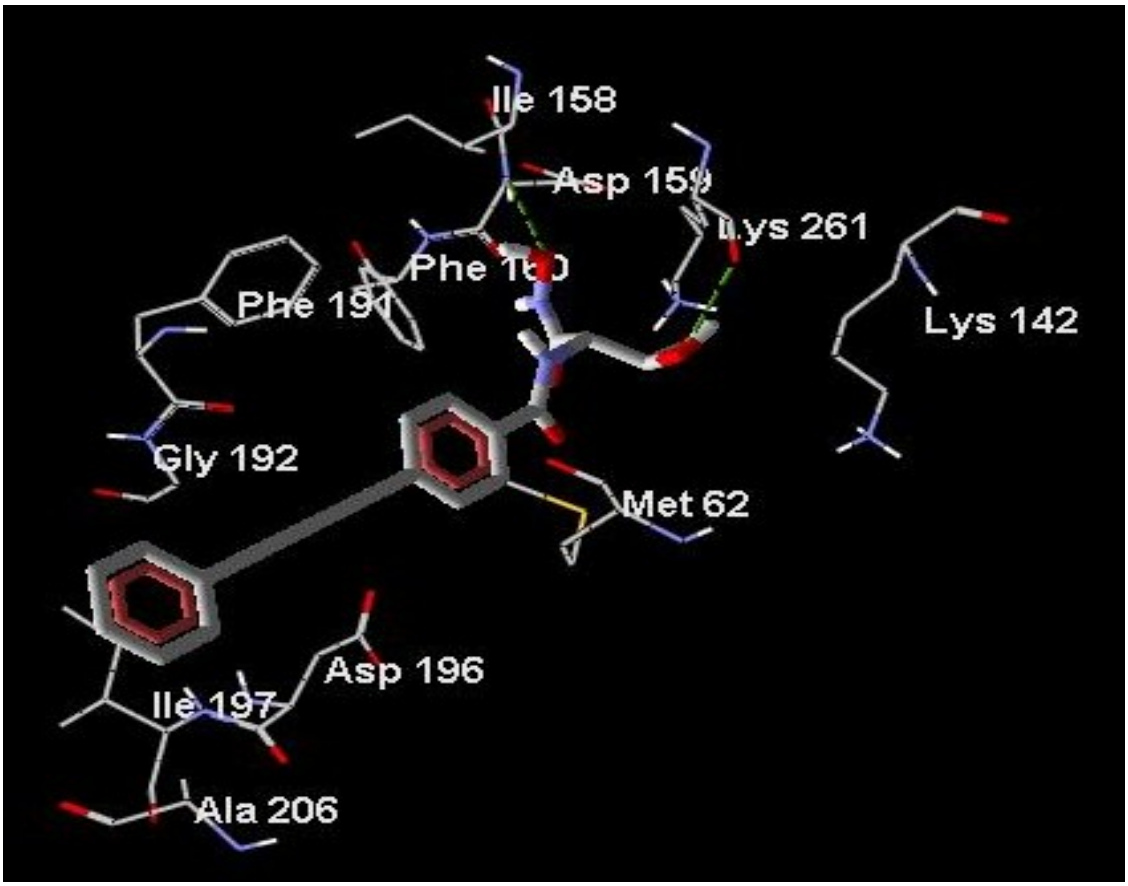


Figure 3: Docked image of compound 1 with LPC009.

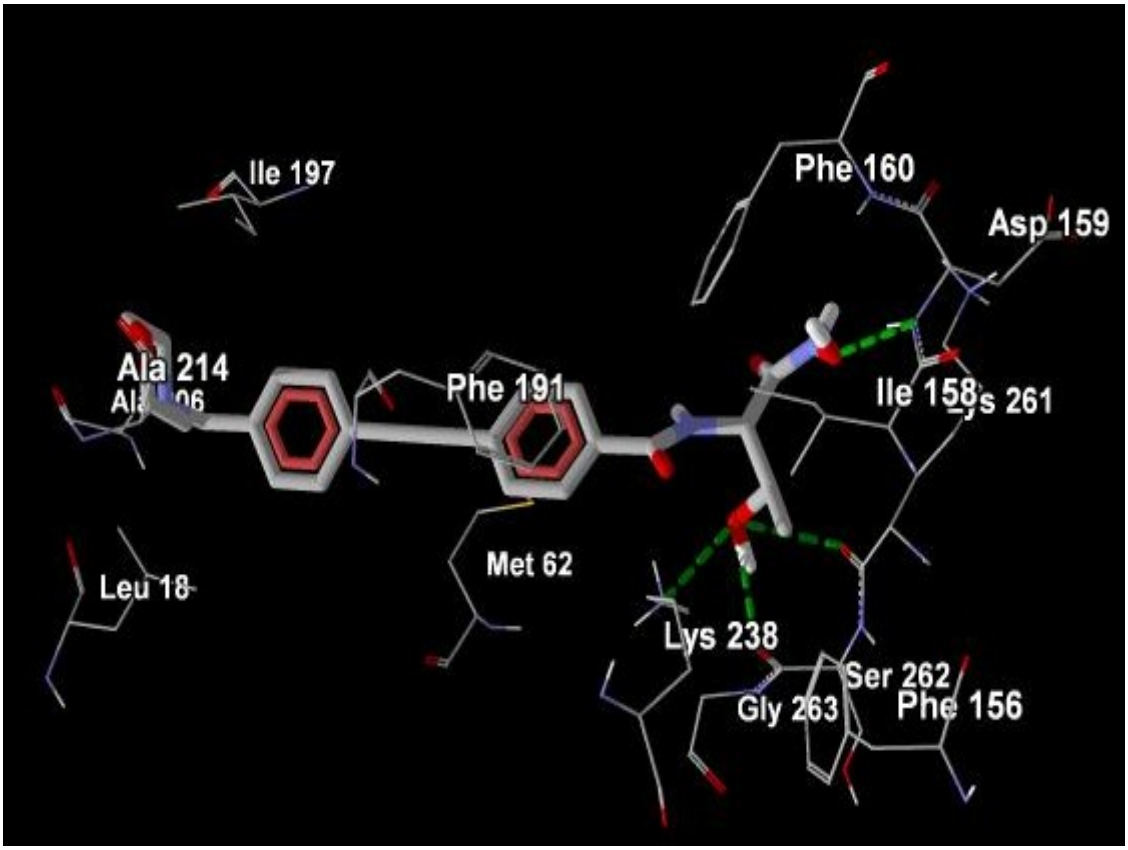


Figure 4: Docked image of compound 1 with CHIR09.

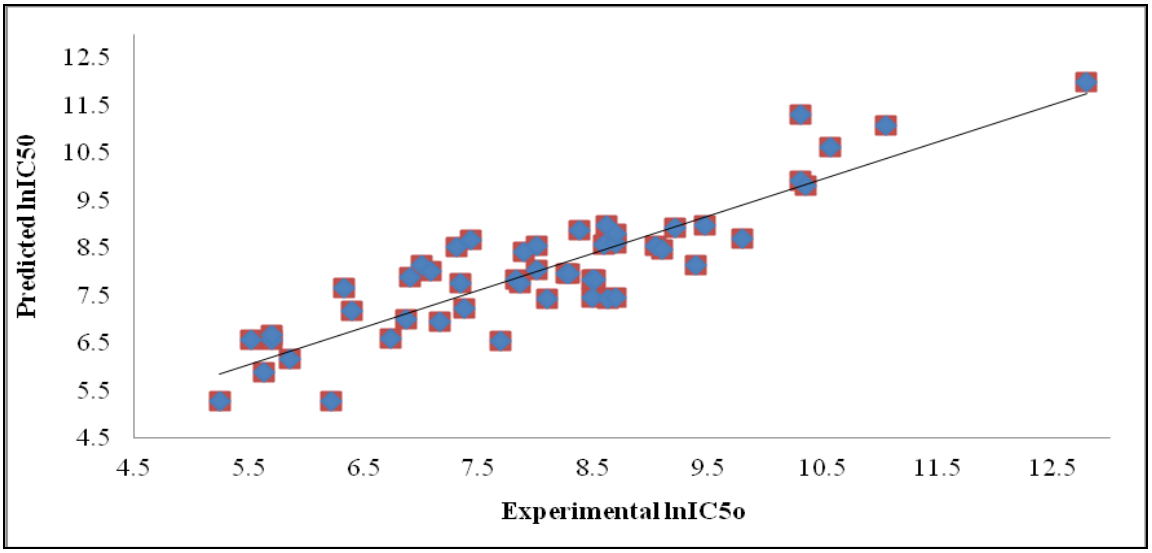


Figure 5: Correlation graph between experimental and predicted activity of 50 training compounds.

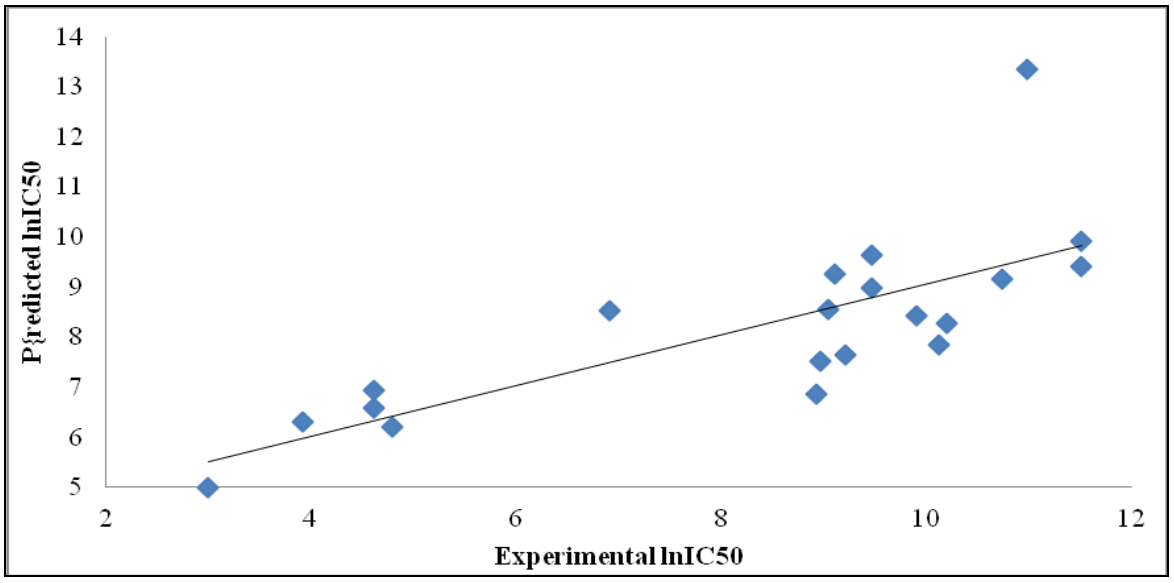


Figure 6: Correlation graph between experimental and predicted activity of 20 test compounds.

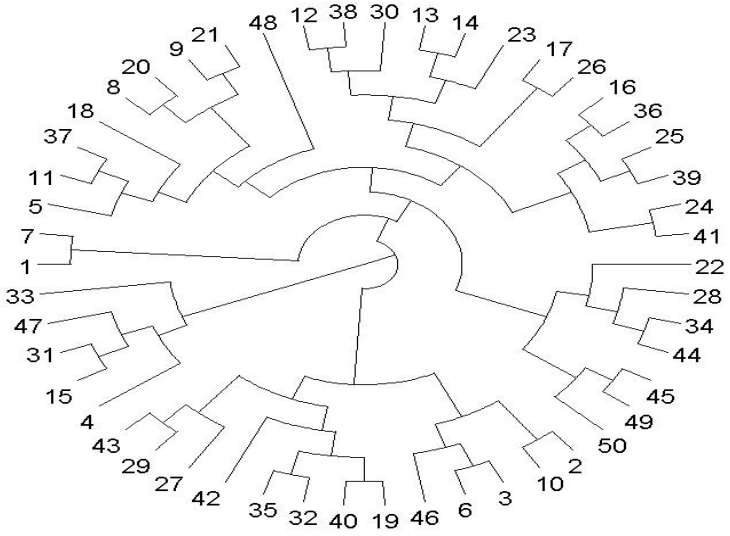


Figure 7: Cladogram using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) of 50 training compounds.

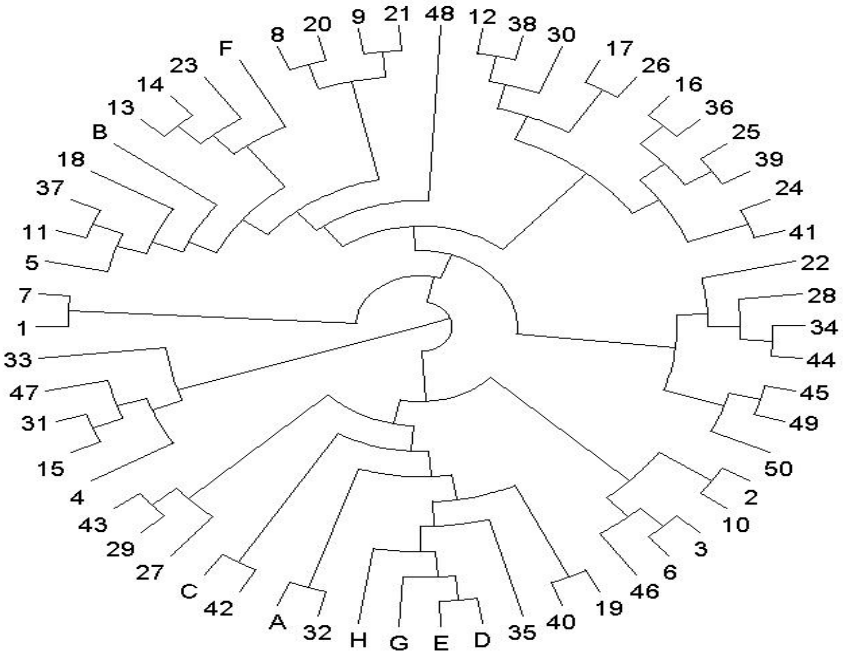
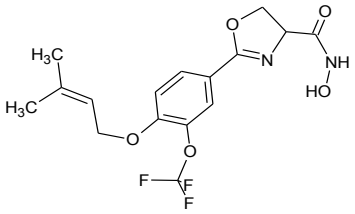
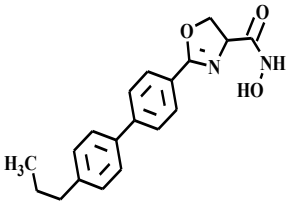
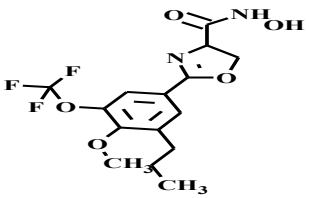
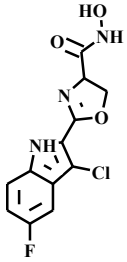
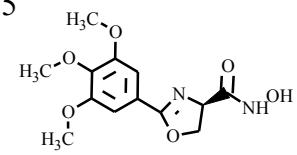
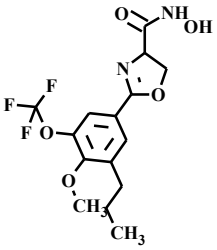
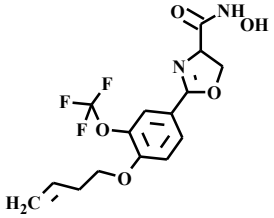
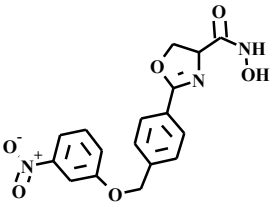
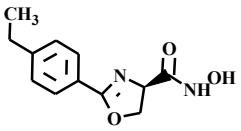
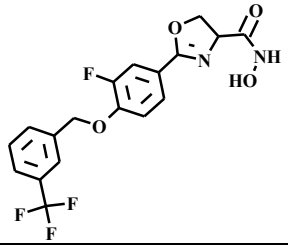
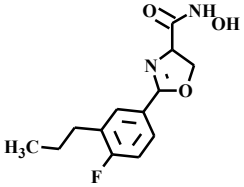
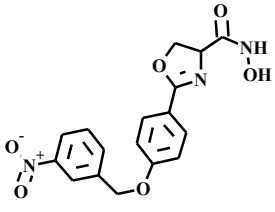


Figure 8: Cladogram using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) of 50 training and 8 other important inhibitors.

Table 1A: Chemical structures 50 training compounds (Compound 1 to 21)

1 	2 	3 
4 	5 	6 
7 	8 	9 
10 	11 	12 

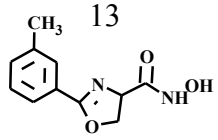
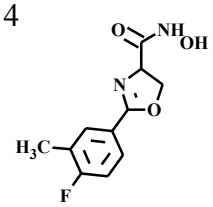
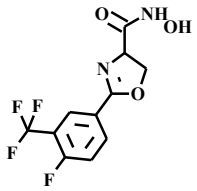
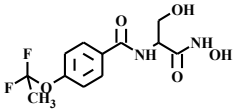
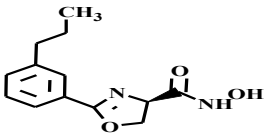
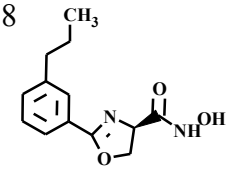
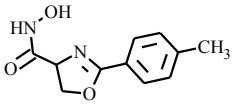
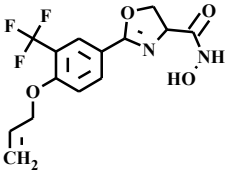
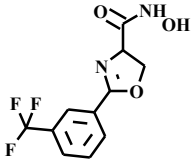
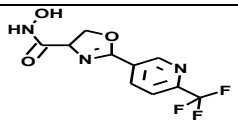
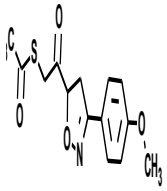
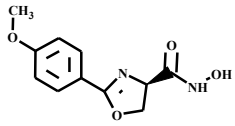
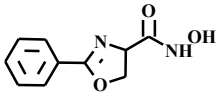
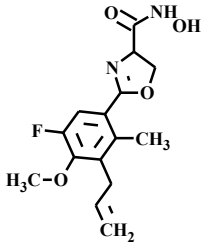
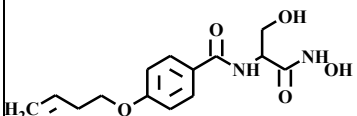
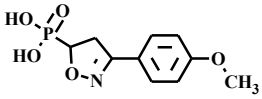
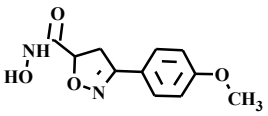
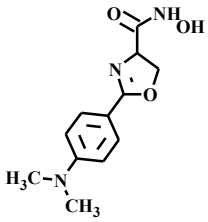
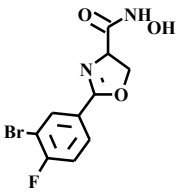
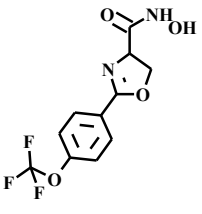
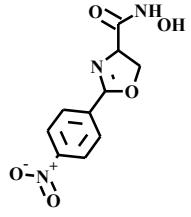
13 	14 	15 
16 	17 	18 
19 	20 	21 

Table 1B: Chemical structures 50 training compounds (Compound 22 to 42)

22 	23 	24 
25 	26 	27 
28 	29 	30 
31 	32 	33 

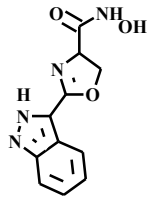
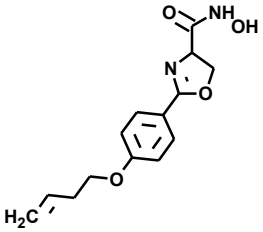
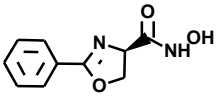
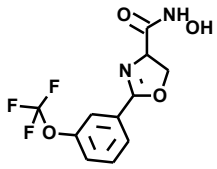
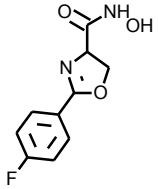
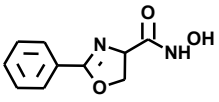
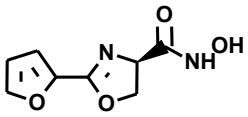
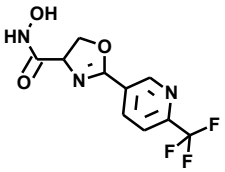
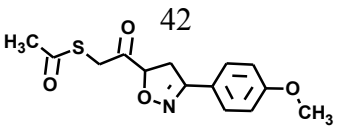
34 	35 	36 
37 	38 	39 
40 	41 	42 

Table 1C: Chemical structures 50 training compounds (Compound 43 to 50)

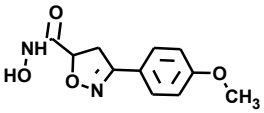
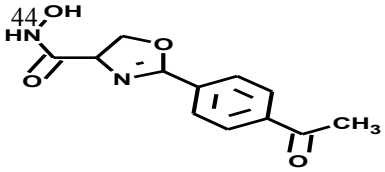
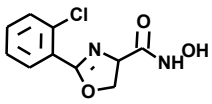
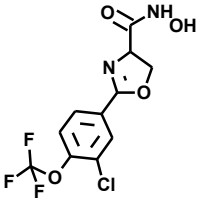
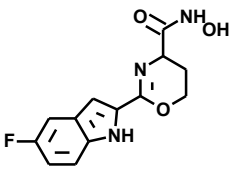
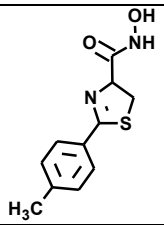
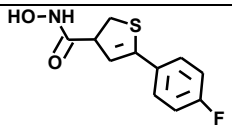
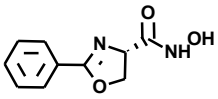
43 	44 	45 
46 	47 	48 
49 	50 	

Table 2: Binding energy of 50 training compounds with IC50 value

Molecule No.	IC50	Binding Energy	Molecule No.	IC50	Binding Energy
1	190.0000	-4.8200	26	3300.0000	-4.6100
2	250.0000	-4.9800	27	3900.0000	-4.2300
3	280.0000	-7.6200	28	4000.0000	-4.8600
4	300.0000	-5.5500	29	4370.0000	-4.8900
5	300.0000	-4.6500	30	4900.0000	-4.8800
6	350.0000	-3.9100	31	4900.0000	-4.6800
7	500.0000	-4.3900	32	5000.0000	-4.2000
8	560.0000	-4.1700	34	5400.0000	-5.6700
9	600.0000	-5.2900	33	5500.0000	-5.3200
10	840.0000	-4.2000	35	5600.0000	-4.8700
11	960.0000	-5.0200	36	6000.0000	-4.7300
12	1000.0000	-4.4900	37	6000.0000	-4.2100
13	1100.0000	-5.4300	38	6000.0000	-4.5500
14	1200.0000	-6.2200	39	8500.0000	-4.8900
15	1300.0000	-5.8200	40	9000.0000	-4.5500
16	1500.0000	-5.6400	41	10000.0000	-4.2300
17	1540.0000	-2.8400	42	12000.0000	-5.5800
18	1600.0000	-4.8200	43	13000.0000	-4.7600
19	1700.0000	-4.9000	44	18000.0000	-5.2500
20	2200.0000	-4.7400	45	30000.0000	-4.7400
21	2500.0000	-4.4300	46	30000.0000	-4.4000
22	2600.0000	-4.5600	47	31200.0000	-5.6200
23	2700.0000	-5.1500	48	38500.0000	-5.0500
24	3000.0000	-4.2400	49	62500.0000	-4.8500
25	3000.0000	-4.8100	50	360000.0000	-4.3500

Table 3: Calculated value of logP of 50 training compounds with IC50 value

Molecule No.	IC50	LogP	Molecule No.	IC50	LogP
1	190.0000	3.32	26	3300.0000	0.32
2	250.0000	3.83	27	3900.0000	0.1
3	280.0000	3.74	28	4000.0000	0.66
4	300.0000	0.22	29	4370.0000	0.58
5	300.0000	1.93	30	4900.0000	0.8
6	350.0000	2.98	31	4900.0000	1.6
7	500.0000	3.36	32	5000.0000	2.12
8	560.0000	2.2	34	5400.0000	0.63
9	600.0000	1.65	33	5500.0000	0.29
10	840.0000	3.28	35	5600.0000	1.55
11	960.0000	2.23	36	6000.0000	0.69
12	1000.0000	1.03	37	6000.0000	2.12
13	1100.0000	1.2	38	6000.0000	0.83
14	1200.0000	1.34	39	8500.0000	0.69
15	1300.0000	1.71	40	9000.0000	1.44
16	1500.0000	0.69	41	10000.0000	0.73
17	1540.0000	0.26	42	12000.0000	1.79
18	1600.0000	2.09	43	13000.0000	0.58
19	1700.0000	1.2	44	18000.0000	0.25
20	2200.0000	2.14	45	30000.0000	1.29
21	2500.0000	1.57	46	30000.0000	2.72
22	2600.0000	1.2	47	31200.0000	0.91
23	2700.0000	1.46	48	38500.0000	1.73
24	3000.0000	0.53	49	62500.0000	1.36
25	3000.0000	0.69	50	360000.0000	0.69

Table 4: Chemical structures 20 test compounds

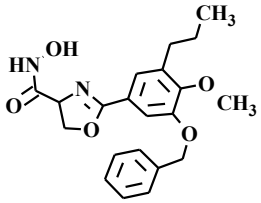
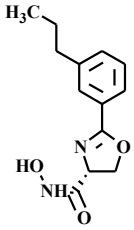
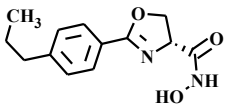
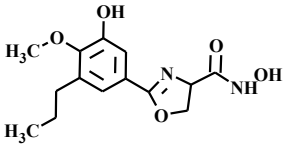
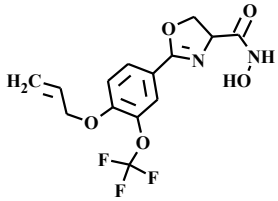
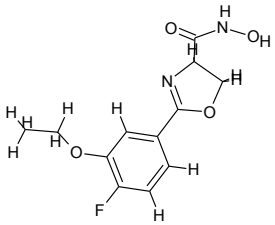
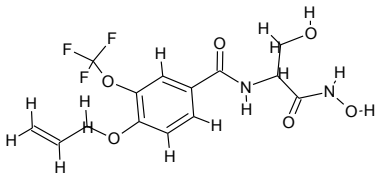
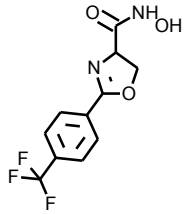
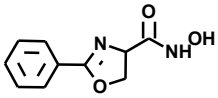
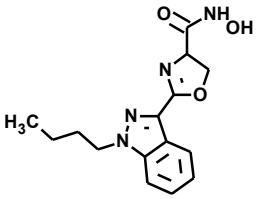
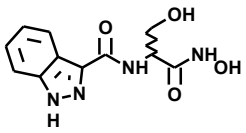
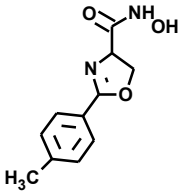
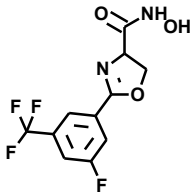
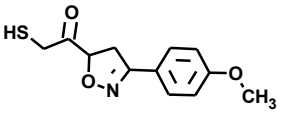
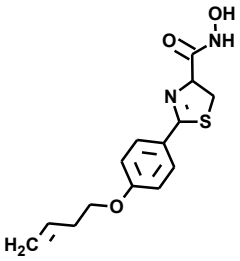
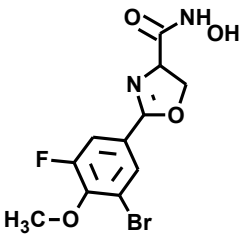
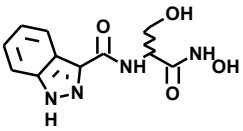
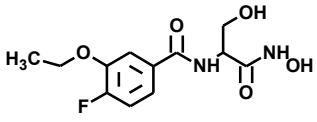
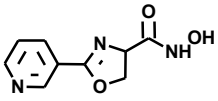
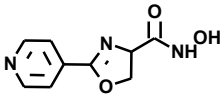
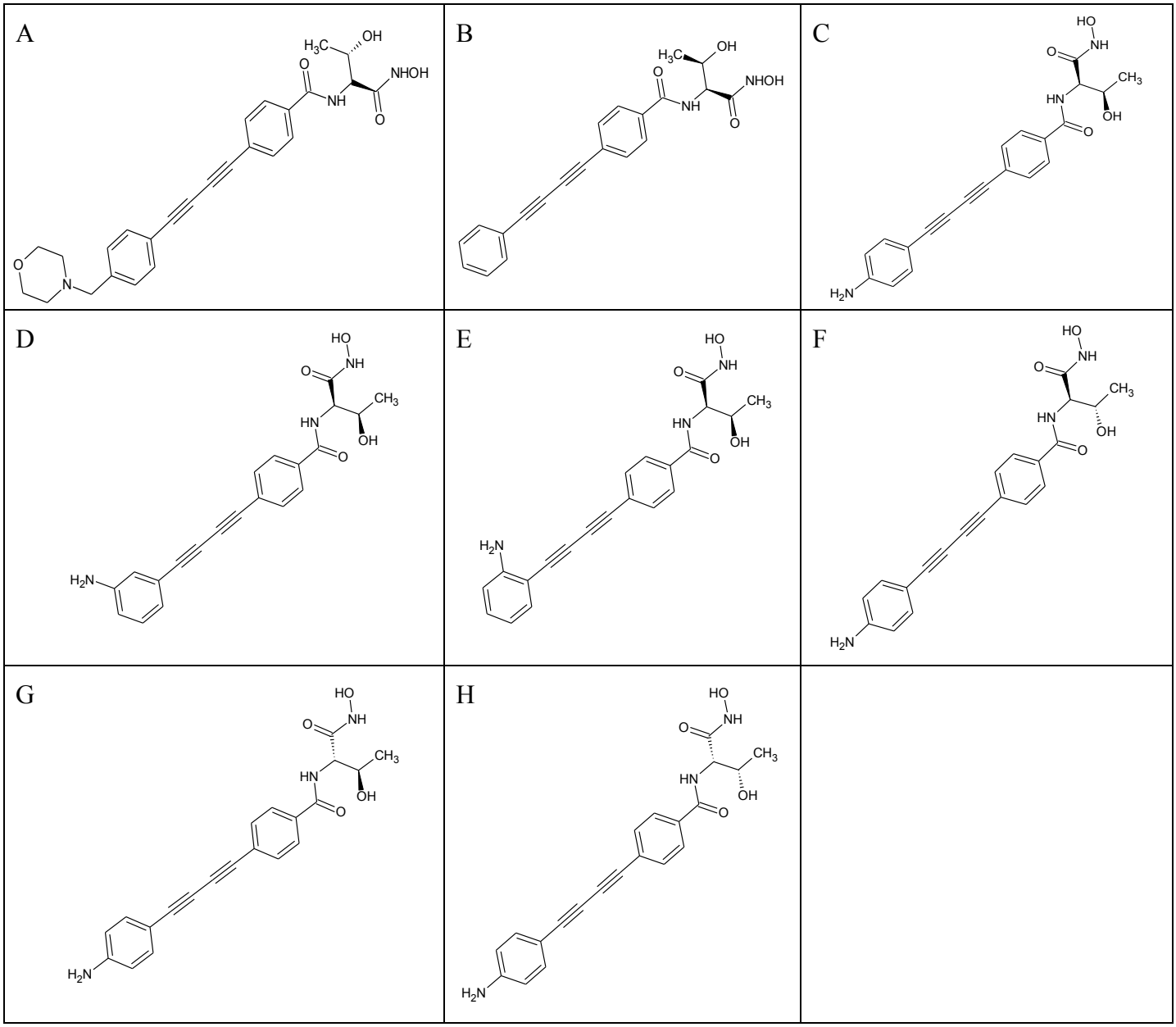
1 	2 	3 
4 	5 	6 
7 	8 	9 
10 	11 	12 
13 	14 	15 
16 	17 	18 
19 	20 	

Table 5: Chemical structures eight other important compounds



A- CHIR-090, B- LPC-009, C- LPC-011, D- LPC-012, E- LPC-013, F- LPC-053, G- LPC-054, H- LPC-055

Table 6: Value of quantum chemical parameters and lnIc50 values of 50 training compounds

Compound	HOMO	LUMO	DM	LogP	LnMV	$LnIC_{50}$
1	-0.2278	-0.035	5.393	3.32	5.594	5.247
2	-0.226	-0.0559	2.0615	3.83	5.624	5.5215
3	-0.2215	-0.0514	4.3681	3.74	5.5699	5.6348
4	-0.2104	-0.0259	7.7559	0.22	5.3575	5.7038
5	-0.2144	-0.0224	4.5415	1.93	5.3762	5.7038
6	-0.2183	-0.0342	3.7861	2.98	5.5358	5.8579
7	-0.2273	-0.0396	6.198	3.36	5.5326	6.2146
8	-0.2298	-0.0979	5.1731	2.2	5.4926	6.3279
9	-0.2277	-0.0255	5.3136	1.65	5.184	6.3969
10	-0.2253	-0.0326	1.3686	3.28	5.6006	6.7334
11	-0.2211	-0.0314	4.5336	2.23	5.2852	6.8669

12	-0.2202	-0.0319	4.1254	1.03	5.2354	6.9078
13	-0.2297	-0.032	4.4138	1.2	5.0888	7.0031
14	-0.221	-0.0311	4.4488	1.34	5.1072	7.0901
15	-0.2353	-0.0432	6.9864	1.71	5.1935	7.1701
16	-0.2302	-0.0348	5.1075	0.69	4.9904	7.3132
17	-0.2404	-0.0265	3.9195	0.26	5.373	7.3395
18	-0.2273	-0.0332	4.2836	2.09	5.2704	7.3778
19	-0.2232	-0.0301	2.7781	1.2	5.0888	7.4384
20	-0.2226	-0.0367	4.9186	2.14	5.4429	7.6962
21	-0.2364	-0.0493	5.1143	1.57	5.1773	7.824
22	-0.2299	-0.0367	5.8871	1.2	5.0888	7.8633
23	-0.2344	-0.047	4.3565	1.46	5.0727	7.901
24	-0.2272	-0.0231	4.7203	0.53	5.1287	8.0064
25	-0.2297	-0.0373	5.2557	0.69	4.9904	8.0064
26	-0.2185	-0.0328	4.3377	0.32	5.4827	8.1017
27	-0.2324	-0.0201	2.4626	0.1	5.4498	8.2687
28	-0.2128	-0.0335	5.7459	0.66	5.1102	8.294
29	-0.2077	-0.0147	1.7752	0.58	5.1287	8.3825
30	-0.1871	-0.0157	3.7183	0.8	5.238	8.497
31	-0.2283	-0.043	6.5866	1.6	5.0907	8.497
32	-0.232	-0.0374	3.1969	2.12	5.2133	8.5172
33	-0.2389	-0.1019	9.2112	0.63	5.0265	8.5942
34	-0.2055	-0.0508	5.8372	0.29	4.9698	8.6125
35	-0.2223	-0.032	3.3778	1.55	5.3804	8.6305
36	-0.2295	-0.0358	4.9687	0.69	4.9904	8.6995
37	-0.2387	-0.041	4.5592	2.12	5.2133	8.6995
38	-0.2319	-0.0369	4.0428	0.83	5.01	8.6995
39	-0.2284	-0.038	5.2676	0.69	4.9904	9.0478
40	-0.2278	-0.0434	2.7545	1.44	5.216	9.105
41	-0.2476	-0.0631	4.6341	0.73	5.1114	9.2103
42	-0.2105	-0.0515	2.0269	1.79	5.4205	9.3927
43	-0.2044	-0.0189	1.7779	0.58	5.1287	9.4727
44	-0.2395	-0.0681	5.8421	0.25	5.1682	9.7981
45	-0.2286	-0.0431	5.3787	1.29	4.0307	10.309
46	-0.2435	-0.0489	3.1786	2.72	4.5326	10.309
47	-0.195	-0.0328	7.3135	0.91	4.3095	10.3482
48	-0.2227	-0.0399	4.5729	1.73	4.2528	10.5584
49	-0.2282	-0.0448	5.8062	1.36	4.0587	11.0429
50	-0.2301	-0.0379	5.5303	0.69	3.8501	12.7939

Table 7: Correlation matrix between chemical parameters and lnIc50 value of 50 training compounds

	HOMO	LUMO	DM	LogP	MV	LnIC50
HOMO	1	0.4395	-0.12	-0.108	0.0407	-0.0497
LUMO	0.4395	1	-0.3715	-0.1572	0.0293	-0.0851
DM	-0.12	-0.3715	1	-0.2257	-0.2896	0.0453
LogP	-0.108	-0.1572	-0.2257	1	0.3294	-0.453
MV	0.0407	0.0293	-0.2896	0.3294	1	-0.8034
LnIC50	-0.0497	-0.0851	0.0453	-0.453	-0.8034	1

Table 8: Regression models

Model	Equation	r	r2	F
1	Pred = 9.119750 + (-0.7323) LogP	0.45	0.2025	6.066
2	Pred = 5.609427 + (-0.3415) LogP + (-2.8535) MV			
3	Pred = -7.230433 + (2.1590) Ehomo + (-6.7943) Elumo + (0.0166) DM	0.088	0.077	0.12
4	Pred = 8.252387 + (-4.2761)Ehomo + (-20.0046)Elumo + (-0.1550) DM + (-0.8484) LogP	0.50	0.25	7.842
5	Pred = 25.763452 + (2.2033) E_{homo} + (-23.1379) E_{lumo} + (-0.3416) DM + (-0.4930) LogP + (-3.0945) LnMV	0.884	0.774	37.52

Where 50 numbers of compounds used, r is the correlation coefficient, r2 is the squared correlation coefficient, F is the Fisher ratio.

Table 9: Value of quantum chemical parameters and lnIc50 values of 20 test compounds and 8 important inhibitors

Compound	E_{homo}	E_{lumo}	DM	LogP	LnMV	$LnIC_{50}$
1	-0.2111	-0.0222	3.9985	3.5	5.7255	2.9957
2	-0.2273	-0.0329	4.3696	2.09	5.2704	3.912
3	-0.2266	-0.027	4.7498	2.09	5.2704	4.6052
4	-0.2137	-0.03	5.8219	1.63	5.3636	4.6052
5	-0.2322	-0.0343	4.6011	2.69	5.4706	4.7875
6	-0.2248	-0.1007	2.8909	2.2	5.4926	6.9078
7	-0.2263	-0.0273	3.6743	1.24	5.5475	8.9227
8	-0.233	-0.0488	6.041	1.57	5.1773	8.9619
9	-0.2284	-0.038	5.2676	0.69	4.9904	9.0478
10	-0.2048	-0.0318	2.6563	1.84	5.3641	9.2103
11	-0.2044	-0.0189	1.7779	0.58	5.1287	9.4727
12	-0.2275	-0.0323	3.6151	1.2	5.0888	9.9035
13	-0.2411	-0.0538	5.0253	1.71	5.1935	10.1266
14	-0.209	-0.0476	2.6521	1.95	5.2549	10.2036
15	-0.2187	-0.0214	4.1598	2.08	3.2229	10.9802
16	-0.2152	-0.0237	5.3936	-0.25	4.7983	9.105
17	-0.2183	-0.0376	3.3838	-1.05	5.134	9.4727
18	-0.2216	-0.0199	0.7662	-0.42	5.3414	10.7364
19	-0.2359	-0.0484	4.4699	-0.53	4.9097	11.5129
20	-0.2379	-0.0613	6.7848	-0.53	4.9097	11.5129

A	-0.2118	-0.0573	3.0072	1.97	5.8348	6.5683
B	-0.2121	-0.0603	4.2492	2.25	5.5925	6.8246
C	-0.1865	-0.0462	1.7062	1.42	5.6036	7.7983
D	-0.1964	-0.0552	3.3445	1.42	5.6036	7.4250
E	-0.1955	-0.0642	3.3551	1.42	5.6036	7.6313
F	-0.1897	-0.0536	4.4708	1.42	5.6036	7.0181
G	-0.1889	-0.0533	3.3219	1.42	5.6036	7.4053
H	-0.1889	-0.0549	3.1764	1.42	5.6036	7.4921

Table 10: Experimental and predicted lnIC50 value of 50 training compounds by using model 5

Molecule No.	Experimental lnIC50	Predicted lnIC50	Molecule No.	Experimental lnIC50	Predicted lnIC50
1	5.247	5.281725	26	8.1017	7.435221
2	5.5215	6.563049	27	8.2687	7.961546
3	5.6348	5.892691	28	8.294	7.968016
4	5.7038	6.562491	29	8.3825	8.882845
5	5.7038	6.669836	30	8.497	7.840918
6	5.8579	6.180782	31	8.497	7.463415
7	6.2146	5.284556	32	8.517201	7.847866
8	6.3279	7.673752	34	8.594201	8.583195
9	6.3969	7.181313	33	8.612501	8.970076
10	6.7334	6.605734	35	8.630501	7.446417
11	6.8669	6.999714	36	8.699501	8.605862
12	6.9078	7.898412	37	8.699501	7.451038
13	7.0031	8.151122	38	8.699501	8.81264
14	7.0901	8.011552	39	9.0478	8.557085
15	7.1701	6.943703	40	9.105	8.473955
16	7.3132	8.533768	41	9.210301	8.91779
17	7.3395	7.753103	42	9.392701	8.142665
18	7.3778	7.22792	43	9.4727	8.986372
19	7.4384	8.680237	44	9.798101	8.699547
20	7.6962	6.54389	45	10.309	11.31069
21	7.824	7.841082	46	10.309	9.905492
22	7.8633	7.756149	47	10.3482	9.810062
23	7.901	8.429049	48	10.5584	10.6207
24	8.006401	8.052842	49	11.0429	11.08371
25	8.006401	8.542088	50	12.7939	11.98995

Table 11: Experimental and predicted lnIC50 value of 20 test compounds using model 5

Compound	Experimental lnIC50	Predicted lnIc50
1	2.9957	5.003048
2	3.912	7.1916
3	4.6052	6.926752
4	4.6052	6.596733
5	4.7875	6.218797
6	6.9078	8.529154
7	8.9227	6.863309
8	8.9619	7.520442
9	9.0478	8.557084
10	9.210301	7.634281
11	9.4727	8.986371
12	9.9035	8.435746
13	10.1266	7.846096
14	10.2036	8.275731
15	10.9802	13.35705
16	9.105	9.270128
17	9.4727	9.627038
18	10.7364	9.152008
19	11.5129	9.904873
20	11.5129	9.408176

REFERENCES

1. Chen, MH; Steiner, MG and De Laszlo, SE *et al.* (1999), "Carbohydroxamido-oxazolidines: antibacterial agents that target lipid A biosynthesis", ***Bioorg. Med. Chem. Lett***, Vol. 9 (3), 313-318.
2. Galloway, A and Raetz, CHR (1990), "A mutant of *Escherichia coli* defective in the first step of endotoxin biosynthesis", ***J. Biol. Chem***, Vol. 265(11), 6394-6402.
3. Kelly, TM; Stachula, SA; Raetz, CHR and Anderson, MS (1993), "The firA gene of *Escherichia coli* encodes UDP-3-O-(R-3-hydroxymyristoyl)-glucosamine N-acyltransferase. The third step of endotoxin biosynthesis", ***J. Biol. Chem***, Vol. 268 (26), 19866-19874.
4. Barb, AW and Zhou, P (2008), "Mechanism and inhibition of LpxC; an essential zinc-dependent deacetylase of bacterial lipid A biosynthesis", ***Curr Pharm Biotechnol***, Vol. 9(1), 9-15.
5. Raetz, CRH (1998), "Enzymes of lipid A biosynthesis; target for the design of new antibiotics", ***Prog. Clin. Biol. Res***, Vol. 397, 1-14.
6. Raetz, CRH; Reynolds, CM; Trent, MS and Bishop, RE (2007), "Lipid A modification systems in Gram-negative bacteria", ***Annu. Rev. Biochem*** Vol. 76, 295-329.
7. Young, K; Silver, LL and Bramhill, D *et al.* (1995), "The envA permeability/cell division gene of *Escherichia coli* encodes UDP-3-O-(R-3-hydroxymyristoyl)-N-acetylglucosamine deacetylase", ***J. Biol. Chem***, Vol. 270(51), 30384-30391.
8. Henrick, M; Gennadios, HA and Whittington, DA *et al.* (2005), "UDP-3-O-((R)-3-hydroxymyristoyl-N-acetylglucosamine deacetylase function through a general acid base catalyst pair mechanism", ***J. Biol. Chem***, Vol. 280 (17), 16969-16978.

9. Onishi, HR; Pelak, BA and Gerckens, LS, *et al.*(1996), "Antibacterial Agents That Inhibit Lipid A Biosynthesis", ***Science***, 274(5298), 980-2.
10. Chen, MH; Steiner, MG and de Laszlo, SE *et al.* (1999), "Carbohydroxamido-oxazolidines: antibacterial agents that target lipid A biosynthesis", ***Bioorg. Med. Chem. Lett***, Vol. 9(3), 313-318.
11. Kline, T; Andersen, NH and Harwood, EA *et al.* (2002), "Potent, Novel in Vitro Inhibitors of the *Pseudomonas aeruginosa* Deacetylase LpxC", ***J. Med. Chem***, Vol. 45(14), 3112-3129.
12. Rich, DH (1990), "***In: Comprehensive Medicinal Chemistry*** (Sammes, PG., Ed.)", Pergamon Press, Vol. 2, 391-441.
13. Otwinowski, Z and Minor, W (1997), "Processing of X-ray diffraction data collected in oscillation mode, In: Methods in Enzymology", ***Charles W. Carter, Jr., Ed. Academic Press***, Vol. 276, 307-326,
14. McCoy, AJ; Grosse-Kunstleve, RW; Adams, PD; Winn, MD; Storoni, LC and Read, RJ (2007), "Phaser crystallographic software", ***J. Appl. Cryst***, Vol. 40, 658-674.
15. Zwart, PH; Afonine, PV and Grosse-Kunstleve RW *et al.* (2008), "Automated structure solution with the PHENIX suite", ***Methods Mol. Biol***, Vol. 426, 419-435.
16. Liang, X; Lee, CJ and Chen, X *et al.* (2011) "Syntheses, structures and antibiotic activities of LpxC inhibitors based on the diacetylene scaffold", ***Bioorg. & Med. Chem***, Vol. 19(2), 852-860.
17. Chen, X; Liu, M and Gilson, MK (2002), "Binding DB: A Web-accessible molecular recognition database Comb", ***Chem. High Throughput Screen***, Vol. 4, 719–725.
18. Chen, X; Liu, M and Gilson, MK (2002), "The binding database: Data management and interface design", ***Bioinformatics***, Vol. 18: 130-139.
19. Chen, X; Liu, M and Gilson, MK (2002), "The binding database: Overview and user's guide", ***Biopolymers Nucleic Acid Sci***, Vol. 61, 127–141.
20. (2012), "***ACD/ChemSketch Freeware***", version 10.00, Advanced Chemistry Development, Inc., Toronto, ON, Canada, www.acdlabs.com.
21. Schmidt, MW; Baldridge, KK; Boatz, JA; Elbert, ST; Gordon, MS and Jensen, JH (1993), "GAMESS Version=24" Mar 2007 (R1) from lowa state University, ***J Comput. Chem***, Vol. 14, 1347-1363.
22. ChemAxon (2007), "***MarvinSketch 4.1.8. ChemAxon Kft***", Budapest, Hungary.
23. Lee, CJ; Liang, X and Chen, X *et al.* (2011), "Species- species and inhibitor dependent conformations of Lpxc; Implications for antibiotic design", ***Chem. Biol***, Vol. 18(1), 38-47.

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