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Research Article

AN *IN SILICO* APPRAISAL OF FEW BIOACTIVE COMPOUNDS AGAINST KAS-A FOR ANTITUBERCULAR DRUG EFFICACY

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ABSTRACT

β-ketoacyl [acyl carrier protein] synthase A is a key enzyme in the mycobacterial cell wall. The inhibitors for this enzyme can block bacterial cell wall synthesis by blocking Fatty Acid Synthase-II enzyme and thereby stopping mycolic acid synthesis, hence kills the bacteria. Seven ligands were found out after checking their toxicity properties and were docked against the drug target. The result showed highest score for ligand 7 and lowest for ligand 1 i.e., -14.71 and -2.88 respectively. Again ligand L4 and L5 showed moderate score of -14.33 and -13.89 respectively. QSAR model was prepared for eight thiolactomycin compounds and the plot was generated. That was employed to predict the activity of the selected ligands. The prediction showed that ligand L4 and L5 have lower IC50 value of 2.81 μM and 1.62 μM. So these can be potent drugs for the treatment of tuberculosis. The statistical analysis revealed highly significant correlation amongst the data.

Keywords: beta-ketoacyl [acyl carrier protein] synthase A; KasA; ADME/Tox; FlexX; Meromycolic acid; Mycolic acid; tuberculosis.

INTRODUCTION

Tuberculosis now-a-days is one of the major reasons of death all across the world. The responsible microbe for this dreaded disease is none but a bacterium, *Mycobacterium tuberculosis*, which has an unusual cell wall composition for its survival. The cell wall component has mycolic acid which is synthesized due to the Fatty acid synthase-II enzyme (FAS-II). This prevents binding of broad range of drug molecule due to presence of a precursor of mycolic acid, the Meromycolic acid. This facilitates the bacterium with Pathogenicity, survival and multi drug resistant functionality. Every year huge population is being chomped by *tuberculosis* at a rate of about 2-3 million annually (Sullivan et al.). The FAS-II enzyme has a core part of β-ketoacyl ACP synthase A (Kas-A), which must be blocked to ensure the blockage of FAS-II and hence the mycolic acid leading to the extinction of the infection. The procedure of procuring drugs for this bacterium has become a hard-hitting job due to the protruding multidrug resistant (MDR) variety. So, the need for a drug against the Kas-A is a must to cut short mycolic acid, hence the pathogen (Al-Balas *et al.,* 2009, Oishi *et al.,* 1982; Slayden *et al.,* 1996; Choi *et al.,* 2000; Kremer *et al.,* 2002).

MATERIALS AND METHODS

Data collection

Drug target is a necessity for docking and QSAR analysis. Drug target i.e., β-ketoacyl ACP synthase-A (Kas-A) of *Mycobacterium tuberculosis* was retrieved from Protein DataBank (PDB) in ".PDB" format. The PDB ID of the target is 2WGE. It has got 416 amino acid residues having 21 α helices and 22 β strands. Seven ligands i.e., **L1** i.e., N-(bicyclo[2.2.1]hept-2-en-7-yl) acetamide (CID 537495), **L2** i.e., 9-hydroxychrysene-1,2-diol-3,4-oxide (CID 3036469), **L3** i.e., (*S*)-2-amino-3-(1H-imidazol-5-yl) propanoic acid (CID 6274), **L4** i.e., 6,9-dimethyl-1a,2,3,9c-tetrahydrophenanthro[3,4-b]oxirene-2,3-diol (CID 129583), L5 i.e., (1aS,9bS)-1a,1b,2,3,4,5,5a,9b-octahydrophenanthro[9,10-b] oxirene (CID 147280), L6 i.e., 5,8-dimethyl-6-oxo-7-oxabicyclo[3.2.1]oct-3-ene-8-carboxylic acid (CID 572118), L7 i.e., 9-methoxy-9-phenyl-3-(2-phenylethyl)-3-azabicyclo[3.3.1] nonane (CID 3040764) were taken from PubChem by their property to attack cell wall proteins and were saved in ".sdf" format. Eight Thiolactomycin (TLM) derivatives D1 i.e., 4,5-dichloro-3H-1,2-dithiole-3-thione, D2 i.e., 4-chloro-5-methyl-3H-1,2-dithiole-3-thione, D3 i.e., 4,5-dimethyl-3H-1,2-dithiole-3-thione, D4 i.e., 4-phenyl-5-(trifluoromethyl)-3H-1,2-dithiole-3-thione, D5 i.e., 4-phenyl-5-(trifluoromethyl)-3H-1,2-dithiol-3-one, D6 i.e., 4-(4-bromophenyl)-5-(trifluoromethyl)-3H-1,2-dithiole-3-thione, D7 i.e., 4-(4-methoxyphenyl) -5-(trifluoromethyl)-3H-1,2-dithiol-3-one, D8 i.e., 4-(4-methoxyphenyl)-5-(trifluoromethyl)-3H-1,2-dithiole-3-thione with IC50 values 26.0, 22.4, 50.5, 35.2, 102.0, 15.6, 100.0, 100.0 respectively were taken from literature (He *et al.,* 2004). All these compounds followed rule of five (Lipinski *et al.,* 2001).

ADME/Tox

ADME/Tox i.e Absorption, distribution, metabolism, excretion and toxicity were determined for the seven ligands (L1-L7) by mobyle@rpbs portal.

Docking

Docking was performed using the selected ligands (L1-L7) against β-keto acyl synthase-A of *M. tuberculosis* i.e., the drug target.

QSAR analysis

QSAR analysis was done by the eight TLM derivatives correlating their descriptors against the logarithm of inverse of IC50. The plot generated was used for prediction of activity of seven ligands, which were converted to IC50 (predicted) by taking antilogarithm of inverse of activity.

RESULTS AND DISCUSSION

The ADME/Tox properties of the ligands (L1-L7) were given in Table1. The docking was carried out with the beta-ketoacyl [acyl carrier protein] synthase (KasA) by seven drugs (L1-L7). The structures were given in Fig1. The Score, Match, Lipophilicity, Ambiguity, Rotation, Number of bonds, Bond energies and their Bond Lengths were found out. The results were given in Table2. The results showed that Ligand L7 and L1 showed higher and least score respectively i.e.,-14.716 and -2.888 whereas ligand L4 and L5 (in Fig3) showed comparative high score of 14.33 and 13.89 respectively. The active site residues CYS 171, MET 213, ARG 214, ALA 215, VAL 278, ALA 279, PRO 280, ALA 287, HIS 311, GLY 312, THR 313, ALA 314, THR 315, PRO 316, ILE 317, GLY 318, ASP 319, ALA 321, GLU 322, HIS 345 etc were found out using Q-site finder portal**.** In the present study most of the ligands docked with GLN, GLU, LYS, ALA, ILE, ARG residues which were common for the active site. This proved the capability of the ligands to bind the active site to block it. The QSAR properties for the eight drugs and seven ligands were given in Table3. In QSAR analysis eight drugs D1-D8 (in Fig2) were correlated against three descriptors *viz*., Index of refraction, surface tension and density to generate the following regression equation:

Log inverse IC50 (LOG 1/C) = -29.30+18.52*(Index of Refraction + (-0.89)*(Surface Tension) + 1.18*(Density).

The statistical analysis showed the Sum of squares due to Regression (SSR) =0.68, Sum of squared errors (SSE) =0.06, total sum of square (SST) =0.74, The R Square= 92.52%. F statistics value was found as 16.49 which is much higher than critical F i.e., 4.35, which shows high significance of the equation. The plot was given in graph1. By considering this equation the activity of the seven ligands were

found out and were converted by taking the antilogarithm of inverse of predicted activity to find the potency. Ligands L4 and L5 i.e., 6,9-dimethyl-1a,2,3,9c-tetrahydrophenanthro[3,4-b]oxirene-2,3-diol & (1aS,9bS)-1a,1b,2,3,4,5,5a,9b-octahydrophenanthro [9,10-b]oxirene showed low IC50 of 2.81 and 1.62 μM, which can be taken as potent drugs.

Table1: ADME/Tox properties of seven ligands and eight TLM derivatives

Parameters	MW	Drs	Ars	FB	RB	#R	RL	C	nC	C/nC	LogP	PSA
Parameter standards	150-500	0-6	0-12	0-15	0-50	0-7	0-12	<22	>2	0.1-1.0	(-2)-6	0-150
*L*1	151.1	1	2	1	10	2	5	9	2	0.22	0.70	29.11
*L*2	294.2	3	4	0	23	5	6	18	4	0.22	2.60	73.22
*L*3	190.1	1	5	3	8	1	5	6	6	1.00	0.36	85.22
*L*4	292.2	2	3	0	23	5	6	19	3	0.16	3.47	52.99
*L*5	292.2	2	3	0	23	5	6	19	3	0.16	3.47	52.99
*L*6	179.1	2	4	0	13	3	6	9	4	0.44	-0.66	65.88
*L*7	196.1	1	4	1	11	2	6	10	4	0.40	0.15	63.60
*D*1	203.1	0	0	0	6	1	5	3	5	1.66	1.53	82.69
*D*2	182.7	0	0	0	6	1	5	4	4	1.00	1.13	82.69
*D*3	162.3	0	0	0	6	1	5	5	3	0.60	0.73	82.69
*D*4	278.3	0	0	2	12	2	5	10	6	0.60	3.15	82.69
*D*5	262.2	0	1	2	12	2	6	10	6	0.60	2.90	67.67
*D*6	357.2	0	0	2	12	2	6	10	7	0.70	3.94	82.69
*D*7	292.2	0	2	3	12	2	6	11	7	0.63	2.81	76.90
*D*8	308.3	0	1	3	12	2	6	11	7	0.63	3.06	91.92

(MW: Molecular weight, Drs: Donors, Ars : Acceptors, FB : flexible bonds, RB : Rigid Bonds, #R : Ring Number, RL: Ring Length, C: carbons, nC: non carbons, C/nC: ratio carbons/non-carbons, LogP : logP (octanol/ water), PSA: Polar surface area).

Table2: Docking results of seven drugs against β-keto acyl synthase-A.

Ligands	Score	Match	LIPO	AMBIG	Clash	ROT	NOB	Bonded Atom (Protein-Ligand)	BE (Kcal/Mol)	BL (Å)
Ligand L1	-2.888	-6.10	-3.3977	-2.3724	2.1818	1.4	1	H15-OE1 GLN93A	-4.70	2.01
Ligand L2	-9.131	-10.83	-5.9695	-3.5177	1.5871	4.2	3	H34-OE1 GLU96A	-3.20	1.82
								H36- O LYS 88A	-4.70	1.84
								H36-OE1GLN157A	-0.02	2.59
Ligand L3	-6.512	-9.79	-3.6682	-3.7135	2.4625	2.8	2	O7-HH21 ARG293A	-3.05	2.07
								O3-H ILE270A	-4.56	1.85
Ligand L4	-14.330	-13.21	-7.3402	-4.8146	2.8315	2.8	4	O21- HEARG293A	-1.82	1.90
								H38-OILE270A	-2.80	1.71
								022-HILE270A	-3.93	1.71
								O18- HH22ARG183A	-2.08	2.15
Ligand L5	-13.897	-15.73	-8.7693	-4.9039	7.3027	2.8	4	O21-HILE270A	-4.51	1.73
								H37-OILE270A	-1.16	2.17
								O22- HH21ARG183A	-4.70	1.83
								H38-OALA268A	-0.62	2.36
Ligand L6	-14.404	-15.00	-5.3160	-4.6941	2.4059	2.8	6	O7-HE ARG293A	-2.12	1.62
								O10- HH21 ARG183A	-4.70	1.86
								H21-O ILE270A	-1.90	1.67
								O11-H ILE270A	-0.38	1.35
								H20-O ALA268A	-2.77	1.90
								N12-HH21 ARG293A	-1.81	2.20
Ligand L7	-14.716	-17.08	-3.4621	-3.6620	2.6849	1.4	4	O11-HH21 ARG 293A	-5.23	2.06
								O10-HE ARG293A	-5.80	1.43
								O12-H ILE270A	-4.04	2.06
								O14-H ILE270A	-2.00	2.22

(LIPO: Lipophilicity, AMBIG: Ambiguity, ROT: Rotation, NOB: No. of Bonds, BE: Bond Energy, BL: Bond Length)

Table3: QSAR Descriptors of seven ligands and eight Thiolactomycins with their activity

Drug	Surface Tension (dyne/cm)	Index of refractivity	Density (g/cm^3)	Activity* (μM^{-1})	Ligands	Surface Tension (dyne/cm)	Index of refractivity	Density (g/cm^3)	Predicted (IC50)# (μM)
*D*1	82.9	1.786	1.85	-1.41497	*L*1	37.4	1.529	1.07	1023.29
*D*2	70.6	1.737	1.6	-1.35025	*L*2	42.1	1.557	1.146	660.69
*D*3	60.6	1.694	1.36	-1.70329	*L*3	47.4	1.588	1.42	245.47
*D*4	62.6	1.712	1.32	-1.54654	*L*4	67.1	1.786	1.429	2.81
*D*5	47.5	1.609	1.536	-2.00860	*L*5	70.1	1.81	1.48	1.62
*D*6	62.5	1.695	1.85	-1.19535	*L*6	76	1.703	1.581	398.10
*D*7	45.9	1.59	1.501	-2.00000	*L*7	50.2	1.542	1.314	4073.80
*D*8	54.9	1.639	1.53	-2.00000					

*** Calculated by taking the logarithm of inverted IC50 value;**
#calculated by taking antilogarithm of inverted activity predicted from QSAR model

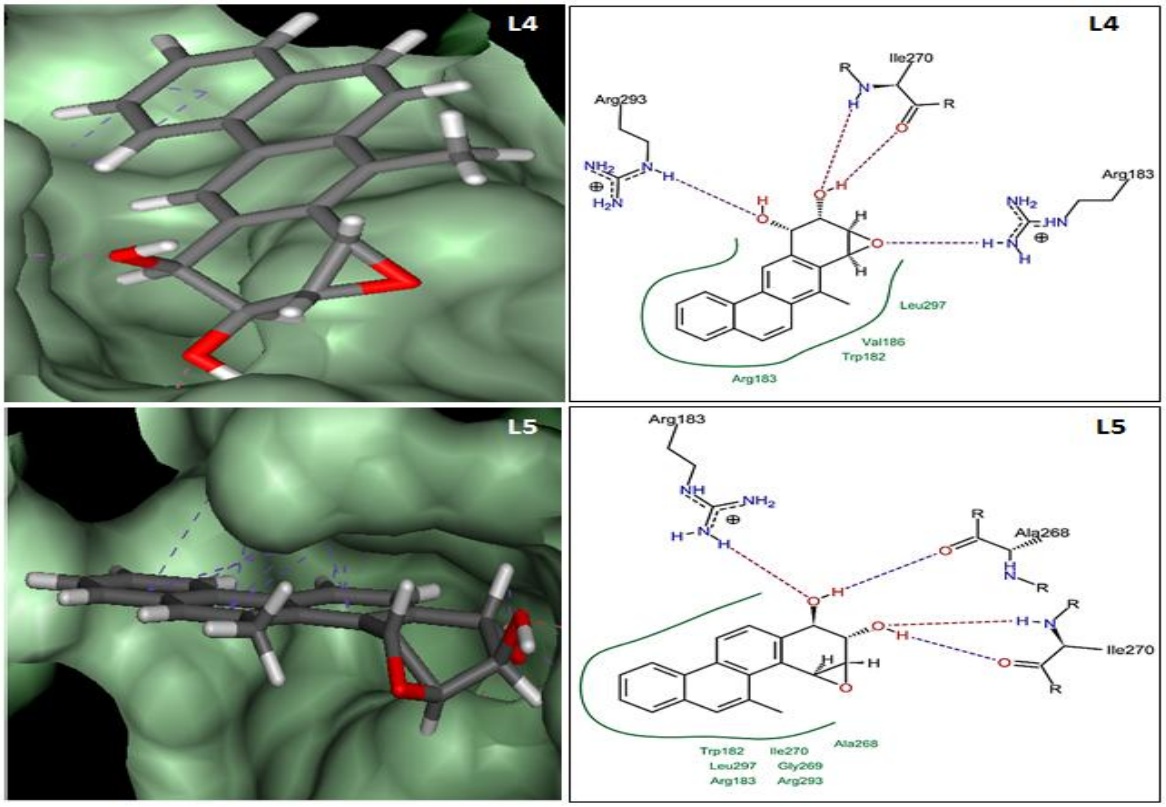
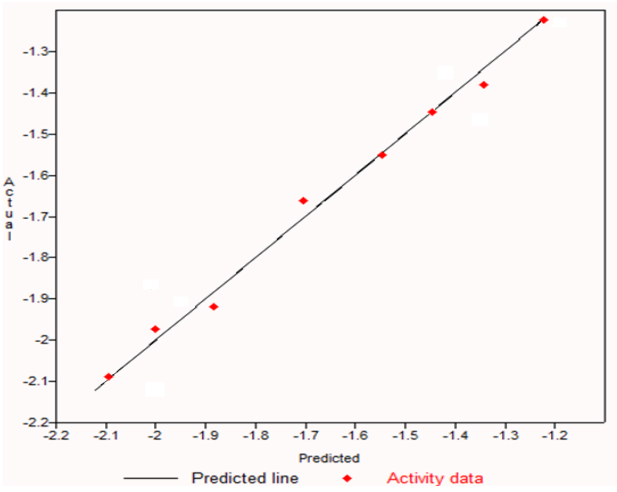


Fig1: Docking pattern (3d & 2D) of ligand L4 and L5 showing high potency against Kas-A



Graph1: Regression plot (linear) of thiolactomycin derivatives (D1-D8) against Kas-A

CONCLUSION

It was observed that beta-ketoacyl acyl carrier protein synthase when docked with the compounds, give good scores and the QSAR analysis also showed good result for ligand L4 and L5. The predicted potency of the seven compounds with unknown potency showed that two ligands had very low activity value which ensures the potentiality of the compounds as good anti-tubercular drugs.

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