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Computational validation of 3-ammonio-3-(4-oxido-1H-imidazol-1-ium-5-yl) propane-1, 1-bis (olate) as a potent anti-tubercular drug against mt-MetAP

Abhishek Chowdhury[1]*, Shantanu Sen[1], Pradip Dey[1], Pankaj Chetia[1], Anupam Das Talukdar[2], Amitabha Bhattacharjee[3] & Manabendra Dutta Choudhury[1]

[1]Bioinformatics Centre (DBT-BIF), Assam University Silchar, Assam, India-788011; [2]Department of Life Science & Bioinformatics, Assam University Silchar, India-788011; [3]Department of Microbiology, Assam University Silchar, India-788011; Abhishek Chowdhury – Email: abhishek@bioinfoaus.ac.in; *Corresponding author

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Abstract:
The advent of Multi Drug Resistant (MDR) strain of *Mycobacterium tuberculosis* (TB) necessitated search for new drug targets for the bacterium. It is reported that 3.3% of all new tuberculosis cases had multidrug resistance (MDR-TB) in 2009 and each year, about 0.44 million MDR-TB cases are estimated to emerge and 0.15 million people with MDR-TB die. Keeping such an alarming situation under consideration we wanted to design suitable anti tubercular molecules for new target using computational tools. In the work Methionine aminopeptidase (MetAP) of *Mycobacterium tuberculosis* was considered as target and three non-toxic phenolic/ketonic compounds were considered as ligands. Docking was done with Flex X and AutoDock 4.2 separately. Ten proven inhibitors of MetAP were collected from literature with their IC50 and were correlated using EasyQSAR to generate QSAR model. Activity of ligands in question was predicted from QSAR. Pharmacophore for each docking was generated using Ligandscout 3.0. Toxicity of the ligands in question was predicted on Mobyle@rpbs portal and Actelion property explorer. Molecular docking with target showed that of all three ligands, 3-ammonio-3-(4-oxido-1H-imidazol-1-ium-5-yl) propane-1, 1-bis (olate) has highest affinity (-37.5096) and lowest IC50 (4.46 μM). We therefore, propose that -3-ammonio-3-(4-oxido-1H-imidazol-1-ium-5-yl) propane-1,1-bis(olate) as a potent MetAP inhibitor may be a new anti-tubercular drug particularly in the context of Multi Drug Resistant Tuberculosis (MDR-TB).

Keywords: Anti-tubercular drug, MetAP, 3-ammonio-3-(4-oxido-1H-imidazol-1-ium-5-yl) propane-1,1-bis(olate)

Background:
Search for new and stable drug target is an essential requirement of the day for treating *Mycobacterium tuberculosis* (TB) infection as the multidrug-resistant (MDR) TB strain is appearing at an alarming rate **[1]**. According to Times of India (13 Oct.2011) report, 3.3% of all new tuberculosis cases had multidrug resistance (MDR-TB) in 2009 and each year, about 0.44 million MDR-TB cases are estimated to emerge and 0.1 million people with MDR-TB, die. There are reports of more than 2.8 million cases of MDR pathotype worldwide and average death from TB reached to 9 million per annum **[2]**. The worldwide prevalence of monodrug-, multidrug- and extensively drug-resistant strains clearly indicates that the old targets in Mtb. are no longer effective. Well-validated targets with extensive biological characterization have proved to be more valuable for the development of new anti-tubercular drugs.

Methionine aminopeptidase (MetAP) is a pervasive enzyme occurring both in prokaryotic and eukaryotic systems and

carried out a significant co-translational modification of newly synthesized proteins. It is suggested that MetAP can be established as a prominent target for developing novel inhibitor of MDR-TB pathotype **[3].** MetAP removes terminal N-terminal methionine from nascent proteins and is required for post translational processing and targeting of the virulent protein to the host body. MetAP2 is found in all organisms and is especially important because of its crucial role in tissue repair and protein degradation **[4]**. Furthermore, MetAP2 is of particular interest because the enzyme plays a key role in angiogenesis, the growth of new blood vessels, which is necessary for the progression of diseases including solid tumour cancers and rheumatoid arthritis **[2]**.

Mycobacterium tuberculosis possesses two types of MetAP i.e MetAP1b and MetAP 1c of which the later was found to be less virulent **[5]**. Interestingly none of the present tuberculosis therapy is targeting this enzyme as such MetAP 1b may be considered for designing new drug for MDR-TB. This enzyme belongs to the family dinuclearmetallo-hydrolases **[6-7]** and various cofactors like Ni (II) were found to empower the protein to act.

Although modification of target by the pathogen itself during infection process remains a question, in the present work we wanted to find out a suitable inhibitor of MetAP1b enzyme selecting few phenolic and ketonic compounds as ligands. Though there are a number of MetAP inhibitors known and available in NCBI PubChem compound database, no anti-tubercular drug from those known inhibitors could appear in the market till date. Therefore, our objective was to search out more suitable molecule(s) targeting MetAP1b with respect to higher binding potential, lower IC50 value etc than that of the known ones.

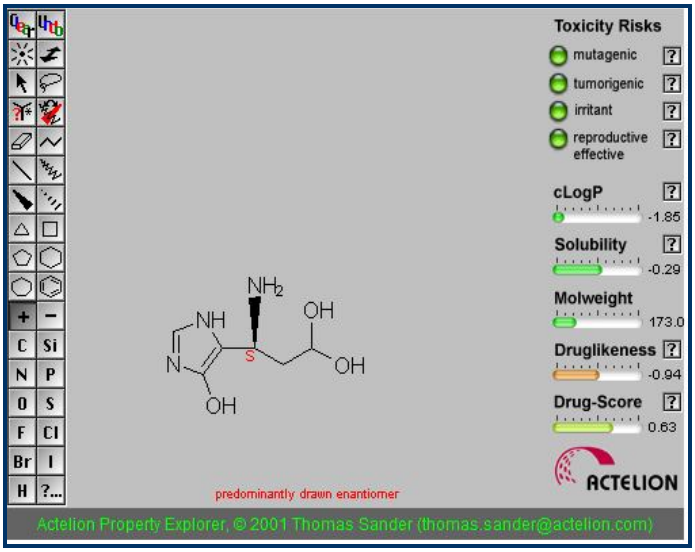

Figure 1: Actelion property Explorer view for drug likeliness of ligand1.

Methodology:

Data Collection

Drug target *i.e.* Methionine aminopeptidase (MetAP) of *Mycobacterium tuberculosis* (PDB ID -3PKA) was downloaded from Protein Data Bank (PDB) and saved in pdb format. The ligands were chosen with ketone and phenolic groups. Ligand1 had been generated using L-histidine based combinatorial library and saved in sdf format. Rest two ligands were retrieved from pubChem compound database in sdf format **Table 1 (see supplementary material).**

Ten proven inhibitors of mtMetAP1b were taken from literature with their respective IC50 value and were drawn using freeware ChemSketch and saved in mol format. Descriptors i.e., Molar Volume (MV), Index of Refraction (IR), Surface Tension (ST), Density (Den), Polarizability (Pol) and LogP were calculated for all the ligands using ChemSketch. IUPAC names of ligands and known inhibitors with their references are shown in **Table 1 (see supplementary material)**.

ADMETox screening

ADMETox screening was performed for the selected ligands using mobile@rpbs online portal and the results were recorded **Table 2 (see supplementary material).** The other drug likeliness properties were screened with Actelion property explorer and given in **(Figure 1).**

Molecular docking

Molecular docking was performed using FlexX with the three ligands in sdf format and Autodock 4.2 with the three ligands in mol2 format separately against target in pdb format. Results of Docking were recorded with binding energy, bonded residue, bond length and bond energy for FlexX in **Table 3 (see supplementary material)** and for Autodock 4.2 in **Table 4 (see supplementary material)**.

QSAR and activity prediction

The QSAR study is normally performed to compare the efficacy of the compounds in question **[8]**. This was performed using ten known inhibitors of MetAP1b. IC50 values of those inhibitors were taken from literature and the correlation and regression were generated using freeware EasyQSAR. The regression equation was used to predict the activity of selected ligands and the F statistics was checked for the significance of the correlation and the equation. Multiple regression plot generated for QSAR model is shown in **(Figure 2).**

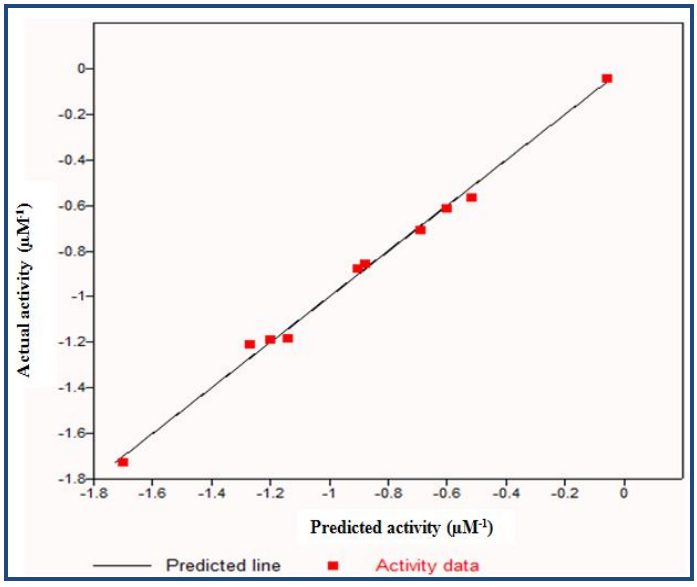

Figure 2: Multiple regression plot (Linear) of ten phenol compounds against MetAP of *M. tuberculosis*

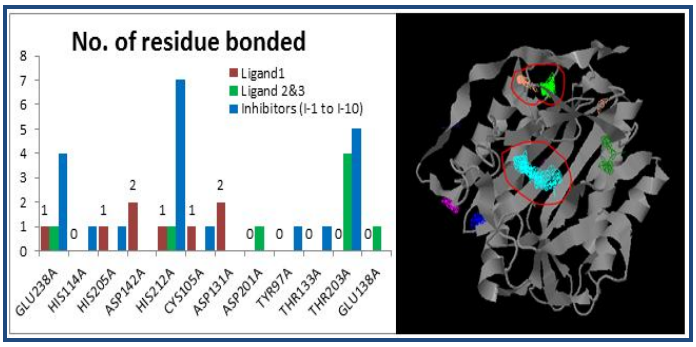

Figure 3: Residues of target docked by ligands (L-1 to L-3) and inhibitors (I-1 to I-10)

Results and Discussion:

PDB file showed that MetAP1b has 285 amino acid residues with 5 L helices and 23 M strands. The active site residues and their interactions with ligands were given in **(Figure 3)**. The three ligands in the present work proved their non-toxicity by passing ADME/Tox filter Table 2 and Actelion property explorer and the drug score was found to be 0.64 **(Figure 1).** Molecular docking with the target showed that Ligand1 has higher affinity (score-37.5096) and lowest IC50 i.e 4.46 EM **Table 3.**

QSAR model prepared showed R^2 (square of correlation co-efficient) is 99.50%. The F statistics value was found to be 99.62 which were much higher than F critical value 3.37 showing the significance of the regression equation. The equation generated was - *LogInvIC50= 61.36 - 0.093*MV- 42.16*IR - 0.014*ST + 4.70*DEN + 0.67*POL + 0.00052*LogP.*

During experimental process flexible docking of all three ligands was carried out in the active site of MetAP1b enzyme of *Mycobacterium tuberculosis* using FlexX tool. Fifty top ranking poses for each ligand were returned in the simulation; out of which one best pose for each ligand was selected on the basis of their re-rank score **Table 3**. For QSAR model Potencies *i.e.,* IC50 values against target for I-1 to I-10 were taken from literature **[9]**. Activities i.e., logarithm of inverse IC50s were correlated with six descriptors *viz.,* molar volume, index of refraction, surface tension, density, polarizability and logP. These descriptors showed significant correlation with the activities.

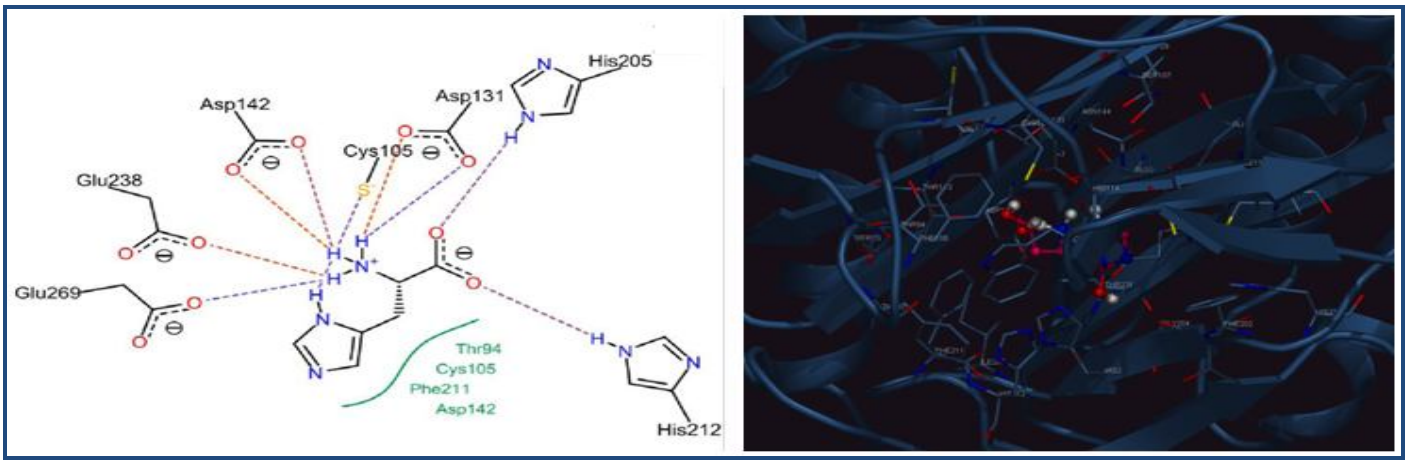

Figure 4: Docking poses of Ligand L1 against MetAp in FlexX and Autodock 4.2.

Analysis of result **Table 3** showed that of all three ligands, ligand L1 is the best option for MetAP1b inhibition as it has better docking scores -37.5096, with least IC50 value of 4.46μM. While choosing best ligands, the least score in docking was preferred as it indicates more stability in binding **[8].** Docking pattern of ligand L1 with the receptor is presented in **(Figure 4).** Potency of compounds in question has been calculated as inverse logarithm of IC50 for QSAR model.

In order to have more convincing result, all three ligands were again docked in the same active site of the same target using Autodock 4.2 **[10]** and again found that ligand1 is the best options out of the three, though docking scores in two different software varies **Figure 4 & Table 4** (**see supplementary material**) the results showed consistency in the hypothesis of ligand1 as the best option. Pharmacophores corresponding to each of the ligands were prepared using Ligandscout 3.2 software evaluation package **(Figure 5)** indicated that Ligand1 has more suitable bonding pattern with target in comparison to other ligands used. After choosing ligand1 as the best option on the basis of its docking score, IC50 value and bonding pattern, target fishing for Ligand1 was carried out using Pharmmapper tool. Very interestingly it is recorded that ligand1 has MetAP of human (1KQ0) as target at 205th rank with score 2.726 and fit 0.5453 (Linkhttp://59.78.96.61/pharmmapper/result.php?job_id=12031307 1321).

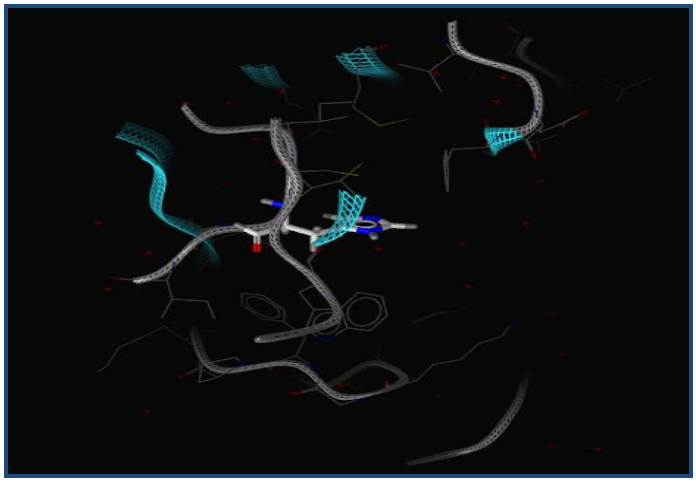

Figure 5: Ligandscout binding pattern of ligand1 with MetAp

Though cross validation of Flex X result with Autodock 4.2 confirms suitability of ligand1 for mtMetAP1b, result of target fishing opened up other possibilities for the ligand and as such Ligand1 was again docked with MetAP of human and E.coli. Results obtained from this docking **Table 5 (see supplementary material)** showed possibility of a wide range of spectrum of the Ligand1 as drug. As human MetAP is a target for colon cancer **[11]** and collagen-induced arthritis **[12]**, Ligand1 may be of use for those diseases and also for treatment of urinary tract infection with *E. coli* over and above its application as anti-tubercular drug. It is important to note that in drug development process compounds having a wide range of spectrum are always preferred and many such drugs with a diverse range of application are already in market. Example may be cited from Hydroxychloroquine which though known better for its anti-malarial efficacy is also used in the treatment of arthritis **[13]**. To have further convincing support, specific *in silico* test for mutagenecity and tumergenicity of Ligand1 was performed by using Actelion property explorer and found that Ligand1 is neither mutagenic nor tumergenic **(Figure 1)**.

Very interestingly it is observed that none of the known inhibitors of mtMetAP1b has comparable docking score and IC50 as that of ligand1 **(Table 3)**. In order to compare the bonding pattern of Ligand1 with that of known inhibitors, pharmacophore for each ligand was developed using ligandscout 3.0 and compared to that of ligand1. More over a massive pharmacophore combining all pharmacophores of known inhibitors was also developed and pharmacophore of Ligand1 is superimposed on it to see if Ligand1 has the same amino acid residue bonding with that of known inhibitors. It is observed that there are some common residues which are hit by both ligand1 and the known inhibitors (I-1to I-10). Ligand1 also had hit some amino acid residues that were not hit by the known inhibitors but these are the active site residues of the target as per record of Q-site portal. This also shows that ligand1 may have comparable efficacy to that of the aggregate efficacy of the ten inhibitors.

Analysis of pharmacophore thus indicates that having better docking score and better bonding pattern over all ligands and also over all known inhibitors of MetAP1b of *Mycobacterium tuberculosis*, Ligand1 may be the suitable option for developing anti-tubercular drug targeting this particular enzyme. As function of MetAP is to remove N terminal mathionine from nascent proteins which is required for post translational processing and targeting host body by the virulent protein **[4]**, inhibition of MetAP enzyme may make the pathogen non virulent.

Conclusion:

We therefore, propose that -3-ammonio-3-(4-oxido-1H-imidazol-1-ium-5-yl) propane-1,1-bis(olate) as a potent MetAP inhibitor and may be used in designing new anti-tubercular therapy particularly in the context of Multi Drug Resistant strains. However *in vivo* experimentation with both target and ligand is essential. Especially study on stability of MetAP1b as target is required as bacteria seldom have the ability to modify the target in acquiring drug resistance.

In the light of global struggle against emergence of MDR-TB and extensively drug resistant tuberculosis (XRD-TB), this novel compound may not only help to control the situation but also its wide spectrum of activity against other bacteria specially on *E coli* may develop potential second line drug to treat nosocomial infections.

This compound may also be considered for designing anticancer and anti-rheumatoid arthritis drug targeting MetAP enzyme of human.

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Supplementary material:

Table 1: IUPAC name of the ligands (L1-L3) and inhibitors (I-1 to I-10)

Compound	IUPAC Name	Reference
L-1	(S)-3-ammonio-3-(4-oxido-1H-imidazol-1-ium-5-yl)propane-1,1- bis(olate)	Designed from library
L-2	anti-10,11-Dihydroxy-8,9-epoxy-7-methyl-8,9,10,11-tetrahydrobenz(a)anthracene	CID 51372
L-3	1a,2,3,7b-tetrahydrooxireno[2,3-h]quinoline-2,3-diol	CID 154539
I-1	4,5,6,7-tetrachloro-2-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-1*H*-isoindole-1,3(2*H*)-dione	(comp 2) Olaleye *et.al*.
I-2	2-chloro-3-(piperidin-1-yl)naphthalene-1,4-dione	(comp 3) Olaleye *et.al*.
I-3	2,3-dichloronaphthalene-1,4-dione	(comp 4) Olaleye *et.al*.
I-4	2-chloro-3-(phenylamino)naphthalene-1,4-dione	(comp 16) Olaleye *et.al*.
I-5	2-chloro-3-[(3-methylphenyl)amino]naphthalene-1,4-dione	(comp 17) Olaleye *et.al*.
I-6	2-chloro-3-[(4-methoxyphenyl)amino]naphthalene-1,4-dione	(comp 18) Olaleye *et.al*.
I-7	2-chloro-3-[(3,5-dimethylphenyl)amino]naphthalene-1,4-dione	(comp 19) Olaleye *et.al*.
I-8	2,3-dibromonaphthalene-1,4-dione	(comp 20) Olaleye *et.al*.
I-9	2-(4-fluorophenoxy)-3-[2-(4-fluorophenyl)ethyl]naphthalene-1,4-dione	(comp 21) Olaleye *et.al*.
I-10	2-chloro-3-(4-fluorophenoxy)naphthalene-1,4-dione	(comp 22) Olaleye *et.al*.

Table 2: ADME/Tox properties of Ligands (L1 to L3) generated from Mobyle@rpbs portal

Parameters	MW	Drs	Ars	FB	RB	#R	RL	C	nC	C/nC	#Chrg	Chrg	LogP	PSA
Standards	200-600	0-6	0-12	0-15	0-50	0-7	0-12		>2	0.1-1.0	0-3	(-2)-2	(-2)-6	0-150
Ligand1	155.1	3	5	3	6	1	5	6	5	0.8333	0	0	-2.15	87.71
Ligand2	356.2	0	4	7	19	3	6	21	5	0.238095	0	0	3.79	32.78
Ligand3	292.2	2	3	0	23	5	6	19	3	0.157895	0	0	3.47	52.99

Table 3: Docking scores, bonded residues, bond energy, bond length and other descriptors of ligands and inhibitors

Compound	Score	Boned Residues	BE	BL	MV	IR	ST	DEN	POL	LogP	IC50
L-1	-37.5096	H20-GLU238A	-6.88	1.91	131.3	1.587	65.2	1.302	17.51	-1.85	4.46*
		O9-HIS212A	-4.70	2.02							
		H19-ASP142A	-8.30	1.79							
		H19-ASP142A	-4.14	2.13							
		H13-CYS105A	-2.72	2.48							
		O10-HIS205A	-2.72	2.11							
		H21-ASP131A	-5.52	2.08							
		H21-ASP131A	-4.75	1.65							
L-2	-27.7303	O19-HIS212A	-3.59	2.12	204.5	1.786	67.1	1.429	34.24	3.79	8317.6*
		H52-GLU238A	-6.07	2.00							
		H52-GLU138A	-4.07	2.32							
		O2-THR203A	-3.61	1.59							
L-3	-27.1898	H40-ASP201A	-2.83	2.28	113.2	1.703	76	1.581	17.43	-0.62	549.5*
		O22-THR203A	-3.06	2.16							
		O23-THR203A	-3.24	1.90							
		H41-THR203A	-3.76	2.04							
I-1	-20.7690	HIS212A-O11	-4.28	1.56	252.1	1.753	86.9	1.88	40.86	5.47	4#
		GLN267A-O12	-4.70	1.91							
		THR203A-H27	-4.42	2.16							
I-2	-24.5957	GLU238A-H29	-4.68	3.24	204.8	1.62	55.5	1.34	28.83	6.16	8#
		HIS212A-O11	-3.98	1.80							
I-3	-15.8730	GLN267A-O12	-3.90	2.20	146.9	1.634	55.3	1.54	20.84	2.52	3.3#
		HIS212A-O11	-4.70	2.88							
I-4	-25.4427	GLN267A-O12	-4.70	1.98	202.9	1.67	59.7	1.39	30.17	6.18	50#
		THR203A-H27	-4.70	1.84							
		HIS212A-O11	-2.53	2.20							
I-5	-20.8518	HIS114A-O11	-4.29	1.93	218.6	1.65	57.5	1.36	32	4.38	18.6#
		TYR97A-H30	-1.23	1.75							
I-6	-26.2354	GLU238A-H29	-4.70	2.08	224.7	1.65	58.8	1.39	32.69	3.86	15.9#
		THR203A-O22	-4.17	1.86							
I-7	-16.2188	HIS212A-O12	-4.25	1.84	234.4	1.64	55.7	1.32	33.83	4.82	13.9#
		GLN267A-O11	-4.70	2.98							

I-8	-15.4025	THR203A-O11	-3.16	2.26	147.2	1.72	71.8	2.14	23.17	2.87	1.14#
I-9	-20.5658	THR203A-O11	-4.49	1.93	294.7	1.61	50.5	1.32	40.82	5.45	4.93#
		HIS212A-O12	-4.70	2.03							
		GLU238A-H19	-3.13	1.87							
I-10	-17.8363	HIS212A-O11	-4.18	1.65	206.0	1.64	55.9	1.46	29.45	3.95	7.58#
		HIS205A-O13	-1.63	2.44							
		CYS105A-H19	-0.28	2.73							

BE: Bond Energy, BL: Bond Length, IR: Index of Refraction, ST: Surface Tension, DEN: Density, POL: Polarizability
* Predicted IC50 from QSAR model; # IC50 taken from literature for QSAR model preparation

Table 4: Docking parameters of ligand1-3 against MetAP using Autodock 4.2

Ligand	Binding Energy	Inhibition Constant	Bond Energy	Electrostatic Energy	Interionic Energy	Frequency	Interact. Surface
L1	-8.67kcal / mol	4.39 mM	-4.05 kcal / mol	-6.91 kcal / mol	-10.96 kcal / mol	90%	468.638
L2	-7.62kcal / mol	2.62 μM	-7.89 kcal / mol	-0.25 kcal / mol	-8.13 kcal / mol	50%	656.083
L3	-4.46 kcal / mol	533.62 μM	-4.70 kcal / mol	-0.12 kcal / mol	-4.83 kcal / mol	60%	445.407

Table 5: Docking score of Ligand1 with human and *E.coli* MetAP

Compound	Target	Score	Boned Residues	Bond Energy	Bond Length
L1	Human MetAP	-36.3973	H25-ASP262A	-8.30	2.06
			H23-GLU364A	-7.18	1.92
			O10-HIS231A	-4.35	2.96
			H23-GLU364A	-4.39	1.92
			H24-ASP251A	-4.12	2.20
			O11-GLN457A	-3.52	1.72
			O10-HIS339A	-3.19	2.18
L1	*E. coli* MetAP	-13.5998	H23-MET300A	-8.30	1.50
			H14-CYS169A	-4.70	1.97
			O10-CYS169A	-3.72	2.18