

INHIBITORS FOR LEPB OF *MYCOBACTERIUM TUBERCULOSIS* H37RVZahra M. Al-Khafaji\*<sup>1</sup>, Marium B. Mahmood<sup>2</sup> and Aaisha B. Mahmood<sup>3</sup><sup>1</sup>Institute of Genetic Engineering and Biotechnology for Postgraduate Studies / University of Baghdad – Iraq.<sup>2</sup>University of Baghdad / Financial Affairs Dept./ Computer Science.<sup>3</sup>Ministry of Agriculture, Veterinary Directorate, Baghdad Veterinary Hospital, Al-Dora Hospital, Iraq.

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## ABSTRACT

Tuberculosis caused by *Mycobacterium tuberculosis* is under an urgent need for developing new drug with new target and action, as most available drugs are ineffective due to development of drug resistance at different levels. QSAR model was built and validated to find suitable ligands for inhibition of strategic target in *M. tuberculosis*. Fifteen molecules were obtained, these are non-mutagenic, non-carcinogenic, non-teratogenic and satisfied the ADME requirements. They docked strongly to the candidate target LepB (Rv2903c)/Spase I, a membrane bound protein. Binding affinities were (-6 to -7.8) kcal/mol, those molecules can be synthesized easily as the synthetic accessibility not exceed 5.5 out of 10.

**KEYWORDS:** Tuberculosis, Drug Targets, QSAR models, LepB, Spase I.

## INTRODUCTION

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis*. It represents a global problem. TB is treated with a complex treatment regimen with different classes of drugs targeting diverse cellular components.<sup>[1]</sup> The TB problem is complicated by rapid emergence of drug-resistant strains at different level and this renders most of currently available drugs ineffective over the time.<sup>[2]</sup> New classes of molecules/drugs with different mechanisms of action are much needed, especially to retain potency against multidrug, extensively and pan or totally drug resistant strains, to be effective against the causative agent and shortened treatment duration.<sup>[1,3]</sup> Some characters of target should be available for developing new drugs, among them, essentiality and the target should be novel and has no human homologue.<sup>[4]</sup> Bacterial signal peptidases represent good targets as they are essential for proprotein export.<sup>[5]</sup>

Type I signal peptidase (LepB) has most criteria required for drug target. It is a membrane protein, perform the cleavage of N-terminal leader sequences from secreted protein precursors, has no human homologue, has one copy of gene in *M. tuberculosis* genome (Rv2903c, and Uniprot ID P9WKA1). This signal peptidase I or Spase I has EC code EC3.4.21.89, act after translocation of extracellular protein and cleaves the signal peptide and release the mature protein outside the cell membrane. It was identified as a high-confidence drug target.<sup>[2,4,6,7]</sup>

Different approaches are used to discover/design new drugs at early steps. Among these Quantitative Structure-Activity Relationship (QSAR), which is one of the main approach on CADD.<sup>[8,9,10,11]</sup> QSAR models are regression models to relate a set of predictor variables (Descriptors) to the potency of the response variable (Biological activity). QSAR modeling alongside with molecular docking were used to predict the activities of various inhibitor compounds.<sup>[3,12,13]</sup> The relation represented by:

**Activity (Response) = f (physicochemical properties /or structural properties)**

When entirely the components are numerical a mathematical equation can be developed. The aim of this study is to develop a QSAR model for estimation of inhibitors for *M. tuberculosis* Spase I (LepB) and validate the built model and using it for finding more efficient compounds and docking them in the target candidate protein.

## MATERIALS AND METHODS

Different databases and software were used, for different purposes.

**Databases****Binding DB:** <https://www.bindingdb.org/bind/index.jsp>

Used to find out the inhibitors of LepB. Other source for inhibitors were used as well.

**Uniprot database:** <https://www.uniprot.org/>

To find out some information about the target (LepB)

**Zinc database:** <http://zinc.docking.org/>

Used to download different chemical format, and information about compounds.

#### Software

**Molinspiration:** <https://www.molinspiration.com/cgi-bin/properties?textMode=1>

Used for finding some molecules descriptors.

**SwissADME:** <http://www.swissadme.ch/>.<sup>[14]</sup>

Used for finding characters of molecules.

**SwissSimilarity:** <http://www.swissimilarity.ch/> <sup>[15]</sup>

#### NCBI/BLASTp

[https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&PAGE\\_TYPE=BlastSearch&LINK\\_LOC=blasthome](https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome)

Used to find out the similar protein to the target.

**T.E.S.T. software:** <https://www.epa.gov/chemical-research/toxicity-estimation-software-tool-test>. To find out the safety of molecules.<sup>[16]</sup>

**PyRx software v.8:** <https://pyrx.sourceforge.io/>. Used for docking.

**PyMOL software:** <https://pymol.org/2/>. Used for docking vitalization.

**Discovery Studio v2.5:** Used for docking vitalization and visualization of Ramachandran plot.

#### OriginPro2016:

<https://www.originlab.com/demodownload.aspx>. Used for graphing and calculation of some results.

#### CarcinoPred-EL

<http://ccsipb.lnu.edu.cn/toxicity/CarcinoPred-EL/>. Used for estimation of compound carcinogenicity.<sup>[17]</sup>

#### Phyre2 server

<http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index> used for protein folding and estimation of pdb structure.

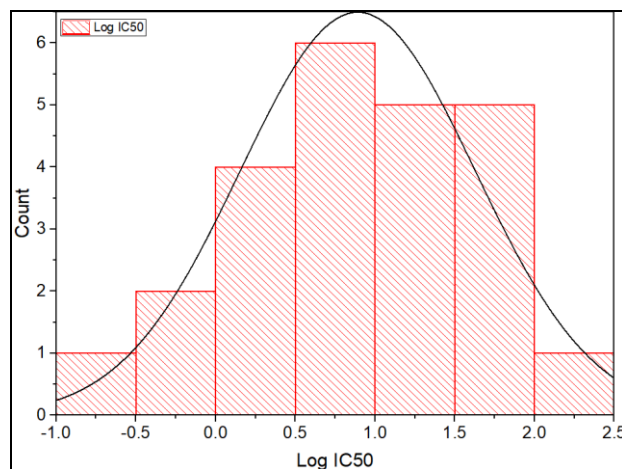
**Model validation:** The robustness, applicability and stability of the generated QSAR model have been established by validation. The model was externally validated by using compounds of Test set, and calculated the  $R^2$  pred.

**Docking:** This was done using PyRx package, after preparing the ligands (compounds), which were optimized to its lowest stable energy state.<sup>[18]</sup> The minimization was done until the energy change is less than (0.1) kcal/mol, the ligands were updated almost 200 times using PyRx software, and transformed into pdbqt format. The target macromolecule LepB was prepared to get pdbqt format, was docked after let the search space to

its maximum. The results recorded as binding affinity (kcal/mol) with RMSD value of zero.

## RESULTS

It has been found that variables in QSAR and QSPR follow some defined statistical distribution, most commonly the normal distribution as shown in Figure 1.



**Figure 1: Normal distribution of inhibitor activity (LogIC50).**

Descriptors used were, TPSA, MW and HB acceptor, they are correlated to biological activity as shown in Figure 2.

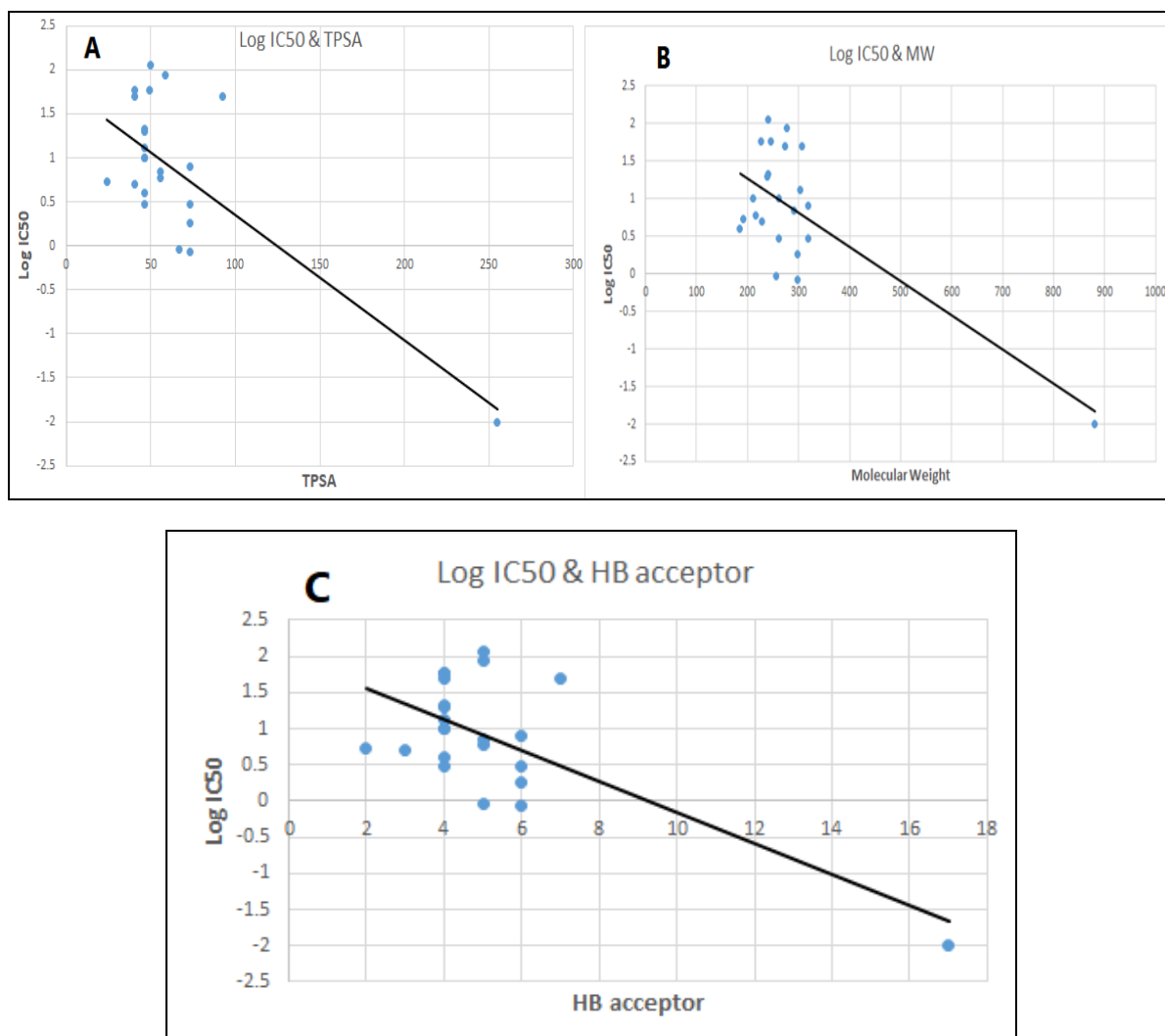


Figure 2: Correlation of activity (LogIC<sub>50</sub>) with A: TPSA, B: MW, C: HB acceptor.

These descriptors were used to build many QSAR models, one of them satisfied most criteria of validation was used.

Table 1 Shows the statistics of the selected model which was with highest R<sup>2</sup> (0.930318).

$$Y = -0.07699 * \text{TPSA} + 0.00548 * \text{MW} + 0.75442 * \text{HB Acceptor} + 0$$

Table 1: Statistics of built QSAR model.

Validation Parameters	Name	Value
r	Correlation coefficient	0.96453
R <sup>2</sup>	Coefficient of determination	0.930318
R <sup>2</sup> <sub>adj</sub>	Adjusted R <sup>2</sup>	0.803721
Q <sup>2</sup> <sub>cv</sub>	Cross validation coefficient	0.925157
R <sup>2</sup> <sub>pred</sub>	Predicted coefficient	0.5232
P(95%)	Confidence interval at 95% confidence level	<0.05
F-value	Significance of regression F-value	40.05246
Tabulate F-value	Critical Significance of regression F-value (95%)	3.64E-05
s	Standard error	0.278689

The regression model applied to Training set and Test set values, shown in Figure 3.

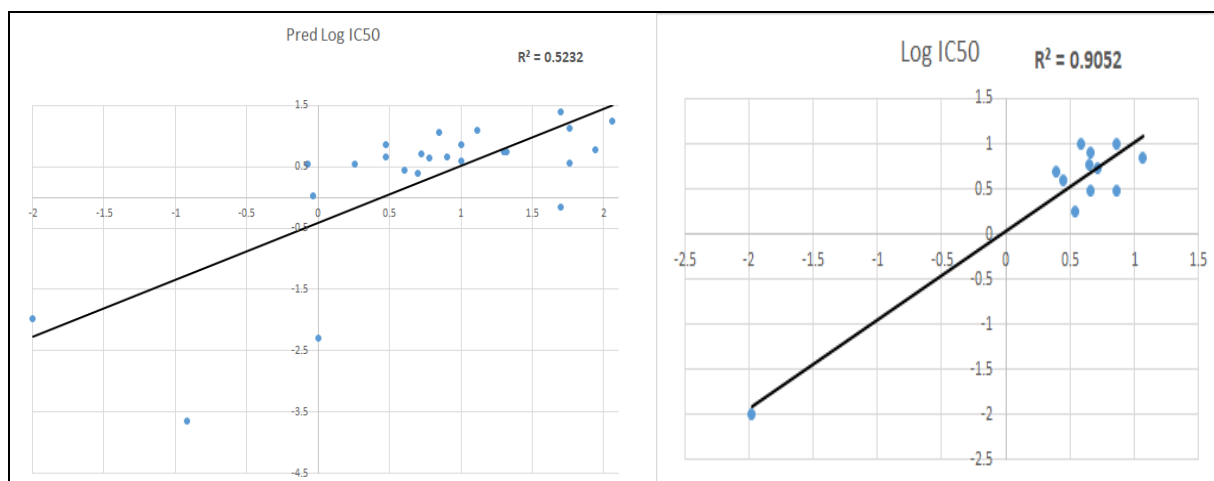


Figure 3: Regression of model, Left for real or observed values, Right for predicted values (Test set).

The predicted relation between observed values (LogIC50) for Training set and predicted (IC50) shown in Figure 4A The overall relation of observed and

predicted values for Training and Test set are shown in Figure 4B.

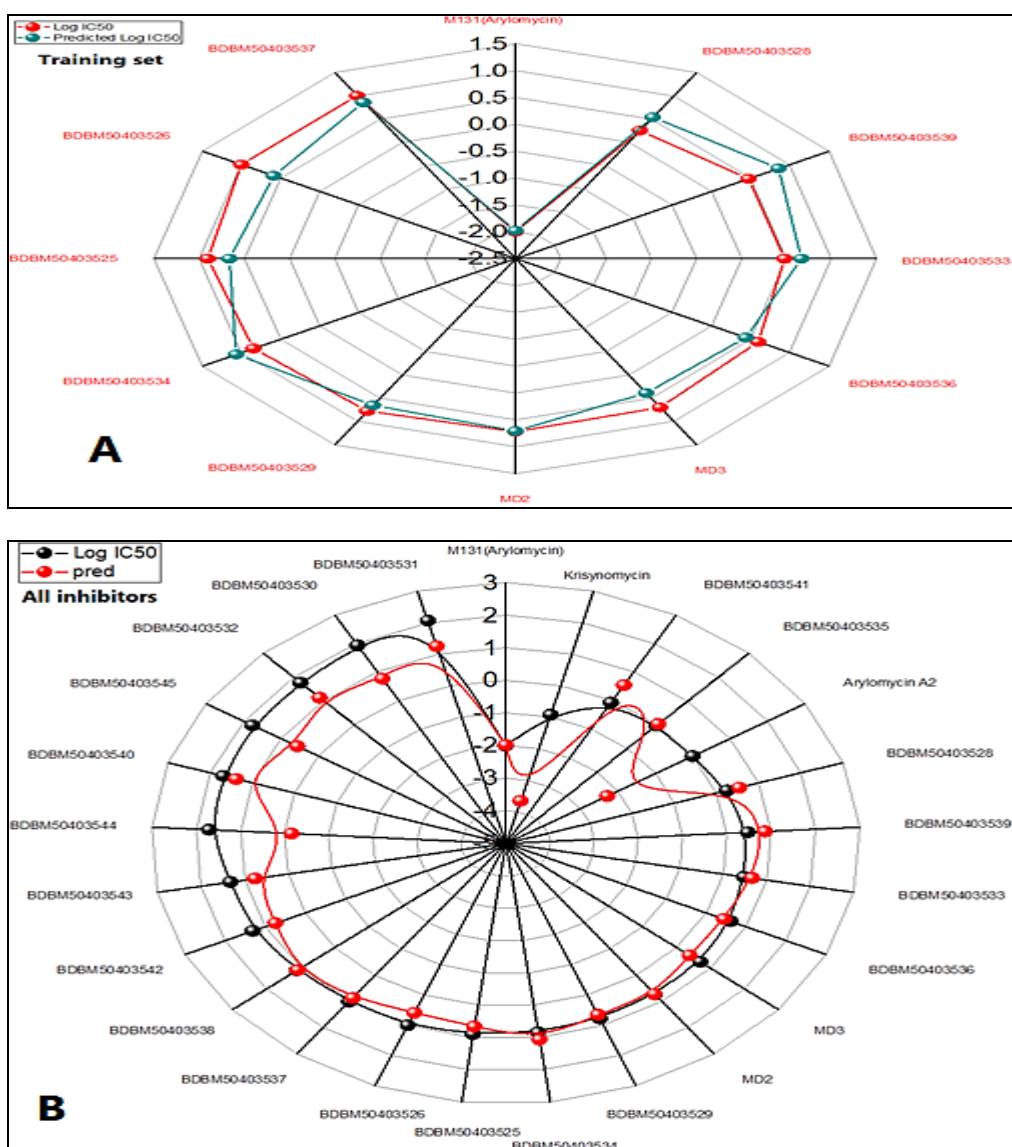


Figure 4: The values of observed and predicted values for Training set, of the all inhibitors (i.e., Training and Test set).

The selected target has no pdb structure, so it was modeled using Phyer2 server, the resulted 3D structure

was subjected to get Ramachandran plot using Discovery Studio v2.5, these results shown in Figure 5A and 5B.

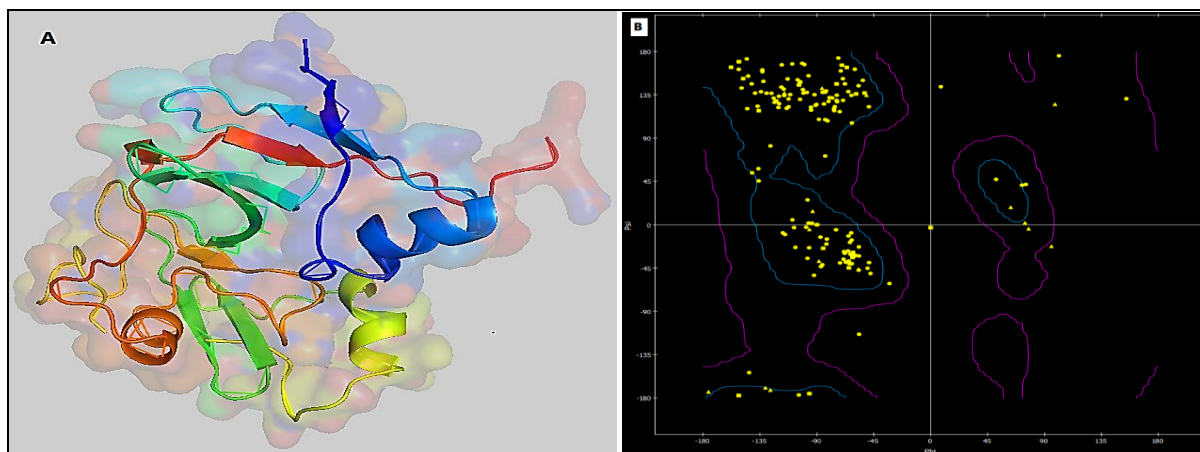


Figure 5: Modeled LepB (Rv2903c) protein using Phyer2 (A), Ramachandran plot (B).

For getting compounds outside the model, Training set molecules were used to find similar compounds included in SwissSimilarity server,<sup>[15]</sup> using Drug/FP2fingerprint and Zinc Drug-Like. Then top ten molecules for each Training set molecule were chosen for further study, this resulted in obtaining 108 compounds. Zinc compounds were filtered for mutagenicity and teratogenicity

(Developmental toxicity) using T.E.S.T (2016) software,<sup>[16]</sup> and estimation of carcinogenicity,<sup>[17]</sup> then subjected to ADME estimation to exclude compounds that are pg-substrate and molecules crossing the brain blood barrier (BBB) and other important characters using SwissADME,<sup>[14]</sup> these different steps of filtration resulted in 15 candidate molecules shown in Table 2.

Table 2: Characters of zinc candidate molecules.

Molecule	Log P	Solubility	GI absorption	BBB Permeant	Pgp Substrate	Lipinski #violations	Bioavailability Score	Pains #alerts	Leadlikeness #violations	Synthetic Accessibility
ZINC26892069	1.06	Soluble	High	No	No	0	0.55	0	1	5.5
ZINC26892076	1.04	Soluble	High	No	No	0	0.55	0	1	5.5
ZINC26892083	1.1	Soluble	High	No	No	0	0.55	0	1	5.5
ZINC26892088	0.98	Soluble	High	No	No	0	0.55	0	1	5.5
ZINC03781855	0.11	Very soluble	Low	No	No	0	0.11	0	0	5.07
ZINC03781858	0.13	Very soluble	Low	No	No	0	0.11	0	0	5.07
ZINC05751654	0.13	Very soluble	Low	No	No	0	0.11	0	0	5.07
ZINC05751655	0.13	Very soluble	Low	No	No	0	0.11	0	0	5.07
ZINC27196434	-0.13	Very soluble	High	No	No	0	0.56	0	0	4.01
ZINC26892092	-0.55	Very soluble	Low	No	No	0	0.11	0	0	4.55
ZINC03872559	0.23	Very soluble	High	No	No	0	0.56	0	0	4.75
ZINC03872560	0.25	Very soluble	High	No	No	0	0.56	0	0	4.75
ZINC03872561	0.21	Very soluble	High	No	No	0	0.56	0	0	4.75
ZINC03872562	0.24	Very soluble	High	No	No	0	0.56	0	0	4.75
ZINC03959242	0.2	Very soluble	High	No	No	0	0.56	0	0	4.75

QSAR model generated in this study was used to estimate the biological activity (LogIC<sub>50</sub> /IC<sub>50</sub>) inhibits LepB shown in Table 3



**Table 3: LogIC<sub>50</sub> and IC<sub>50</sub> of zinc candidate molecules calculated by the built model.**

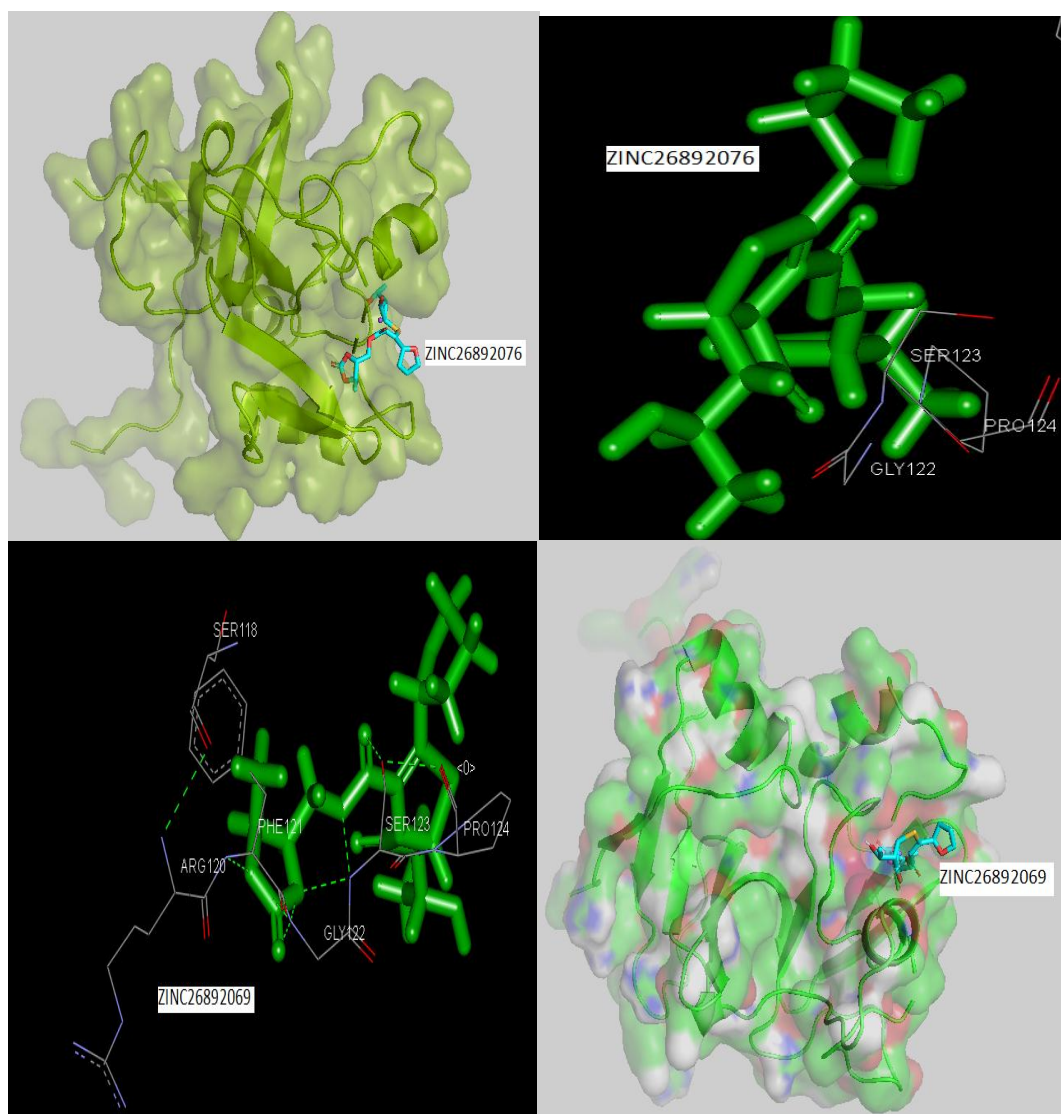
Molecule	logIC <sub>50</sub>	IC <sub>50</sub> $\mu$ M
ZINC03781855	-7.21812	6.05E-08
ZINC03781858	-7.21812	6.05E-08
ZINC03872559	-3.53913	0.000289
ZINC03872560	-3.53913	0.000289
ZINC03872561	-3.53913	0.000289
ZINC03872562	-3.53913	0.000289
ZINC03959242	-3.53913	0.000289
ZINC05751654	-7.21812	6.05E-08
ZINC05751655	-7.21812	6.05E-08
ZINC26892069	-2.31575	0.004833
ZINC26892076	-2.31575	0.004833
ZINC26892083	-2.31575	0.004833
ZINC26892088	-2.31575	0.004833
ZINC26892092	-6.0091	9.79E-07
ZINC27196434	-3.24404	0.00057

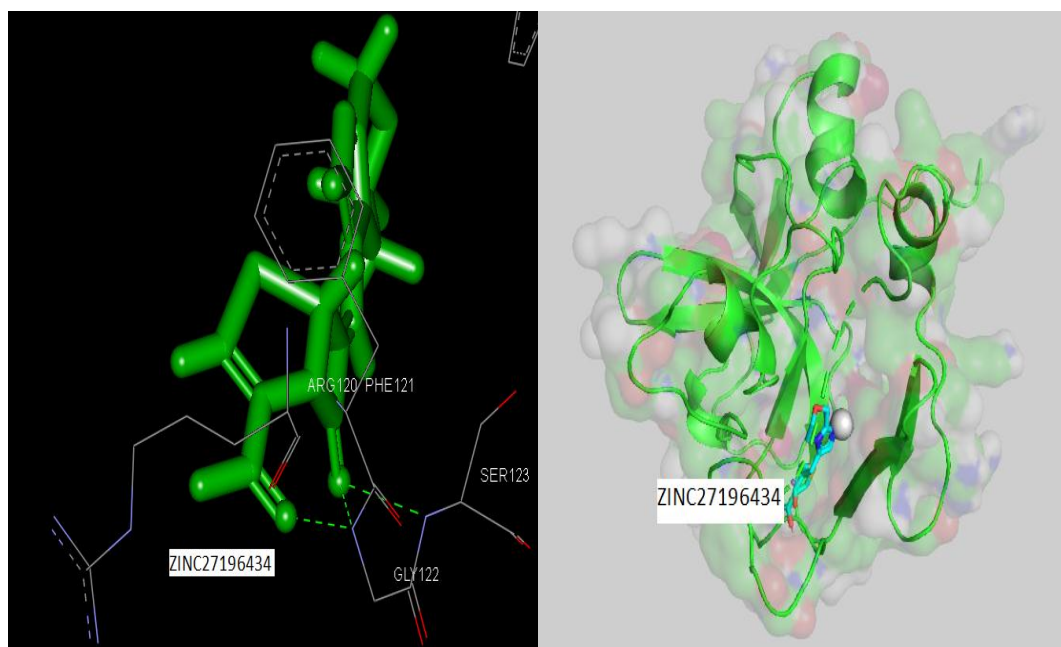
The results indicate that the candidate Zinc molecules are very active as it can be used at extremely low concentration.

**Table 4: Binding affinity of docked zinc candidate molecules.**

Compounds	Binding affinity (BA)Kcal/mol
ZINC26892076	-7.8
ZINC26892069	-7.6
ZINC27196434	-7.0
ZINC26892088	-6.8
zinc_3781855	-6.5
ZINC05751654	-6.5
ZINC26892083	-6.5
ZINC03872559	-6.4
ZINC03872560	-6.4
zinc_3781858	-6.3
ZINC03959242	-6.3
ZINC05751655	-6.2
ZINC26892092	-6.2
ZINC03872561	-6.0
ZINC03872562	-6.0

These ligands were found to bind strongly to the binding sites of target protein, the docking of top 4 ligands shown in Figure 6.





**Figure 6: Docking of top three zinc ligands in LepB protein.**

## DISCUSSION

The basis for mechanistic interpretation states that the properties of biological interactions of a chemical are inherent to its molecular structure.<sup>[19]</sup> The majority of QSAR modeling applications to design new anti-TB agents have been used to modify previously discovered congeneric group of chemicals.<sup>[12]</sup> In this study about 24 inhibitor molecules for LepB were collected from literatures and databases, represented by IC<sub>50</sub> values, these subjected to data transformation by taking the logarithm to base 10. In practice, for reasons of cost, time and animal welfare, these limited the number of inhibitors, so the obtained number or values were divided into Training set and Test set to develop QSAR model.

Descriptor selection is quite important step in developing QSAR model In this study constitutional descriptors such as molecular weight (MW) and HB acceptor were used, and topological polar surface area (TPSA) which defines the sum of surface polar atoms in a molecules.<sup>[20]</sup> was used as well, they are highly correlated to the biological activity (i.e., Log IC<sub>50</sub>) as shown in Figure 2.

### Model validation

The purpose of validation is to provide a statistically reliable model with selected descriptors as a consequence of cause-effect and not only of pure numerical relationship obtained by chance. Based on the high squared correlation coefficient ( $R^2$ ) and low standard error and high Fisher coefficient (F) and significant values of P of t-test, the above model was chosen. The difference of squared correlation ( $Q^2$ ) for internal validation and  $R^2$  not exceed 0.2-0.3 which is clear indication that the model not suffering from overfitting, in addition  $Q^2 > 0.5$  in all conditions.<sup>[21,22]</sup> The external validation, i.e., using Test set molecules to estimate the predictive ability of the model, which is a measure of

how the model can predict of new data that were not used in model building.<sup>[19]</sup> The regression relationship between the observed values and predicted values of Training set and Test set are shown in Figure 3A and Figure 3B. It is known that models with values of  $R^2_{pred}$  above stipulated value of 0.5 are considered to be well predictive.<sup>[23]</sup>

It has been suggested that external validation might be the only way to estimate the predictive power of QSAR model and considered the most rigorous validation procedure, because the compounds in the external Test set do not affect the model development.<sup>[19]</sup> But this should be supported by high  $Q^2$  value, which can be regarded as necessary, although it is insufficient and needs more parameters for validation.<sup>[24,25,26,27]</sup>

Table 1 also shows the high correlation coefficient ( $r=0.96453$ ). The high squared correlation coefficient  $R^2$  (0.930318) explains that 93% variance in biological activity of tested compounds. To overcome the drawbacks and overfitting of  $R^2$ ,  $R^2_{adj}$  was calculated, which is a modified  $R^2$  that adjusts for the number of explanatory terms of the model,<sup>[23]</sup> as  $R^2_{adj}$  takes in consideration the number of degrees of freedom, and it decreases if the addition of new variable (descriptor) does not reduce the unexplained variance.<sup>[19]</sup>

For good model the standard error should be low, since it measures the dispersion of the observed values about the regression line.<sup>[19]</sup> Statistical significance of regression model can be assessed also by means of F values, which represents the ratio between explained and unexplained variance for a given number of degree of freedom, the higher the F value the greater the probability is that the model is significant,<sup>[19]</sup> when the values is greater than a tabulated value for chosen level of significance

(Typically 95%). And each regression coefficient should be significant at  $P < 0.05$  which is checked by t-test (i.e., P value,  $P < 0.05$ )<sup>[23]</sup>

Back to the used descriptors in model building, the most frequently used descriptor is LogP, because is a measure of hydrophobicity and reflects the ability of compounds to partition and accumulation in organisms. So if the chemical compound does not need partition, as it has special metabolism, or the chemical has a specific mode of action, or its target is easily assessable such as extracellular location, then the LogP is not reasonable descriptor.<sup>[19]</sup> The molecular size represented by MW was used since it indicated a good correlation with IC50 values (see Figure 2). While TPSA indicates the relation of chemical structure and their transport and affect the absorption in the intestine and oral bioavailability of oral drugs.<sup>[28]</sup> Figure 2 shows that TPSA correlated to LogIC50 values significantly. It has been shown that compounds with TPSA values about 60 Å<sup>2</sup> are generally regarded as poor-membrane permeable substances with predictively reduced CNS bioavailability.<sup>[29]</sup>

Since statics can never replace chemistry, non-statistical validation is required. So docking processes were performed. The molecules were prepared and optimized to its lowest stable energy state, the minimization was done until the energy change is less 0.1 kcal/mol, the molecules were updated almost 200 times in PyRx software. The prepared ligand/ molecules were docked with prepared target protein (all of them in pdbqt format) using AutoDock vina incorporated in PyRx software v.8.<sup>[18]</sup> Docking results evaluated by Binding Affinity at RMSD value of zero, Binding Affinity ranged (-6 to -7.8) kcal/mol as shown in Table 4.

In general, drug development for TB is very slowly, recently Linezolid was introduced to act against the XDR-TB,<sup>[2]</sup> but its target (16S rRNA) is eligible for mutant developing, as shown for *Staphylococcus haemolyticus* which developed resistance within 4-6 weeks under wet lab experiments.<sup>[30]</sup> So the most promising TB drug targets preferably have outside position.

LepB is considered as an ideal drug target for its essentiality and vulnerability for *M. tuberculosis*, the protein is druggable with more than one pocket for binding.<sup>[31]</sup> Vulnerability is a key feature for drug target, where the most ideal targets would be those that cause cell death or diverse effect to the active and dominant cell upon minimal inhibition.<sup>[2,4]</sup> And it would expected that corruption of this protein (LepB) with small molecules may be useful as this process could corrupt essential function for the cell (i.e., protein transport), especially for *M. tuberculosis* which has only one copy of gene coding this protein and cannot be compensated.<sup>[4]</sup>

On the other hand, the most effective class of Spase I inhibitor belongs to beta- lactam compounds. in

*Escherichia coli*, it was suggested that the unique Ser/Lys catalytic dyad active site allowing the development of highly specific inhibitors and is distinctly different from human Spases, suggesting the protein as a promising drug target. However, the number of Spase I inhibitors remain small.<sup>[4]</sup> and as shown in this study. For LepB of *M. tuberculosis* an inhibitor MD3 (beta aminoketone) was active exhibiting growth inhibition and bactericidal activity.<sup>[4]</sup>

In addition, detection of toxicity and other adverse effects such as carcinogenicity and teratogenicity remain a serious bottleneck in drug discovery, however, the compounds of this study were checked for these characters in Silico.

## CONCLUSION

The compounds introduced in this study might be promising as inhibitors and future drugs for essential target associated with membrane, and the permeation of molecules across the complex mycobacterial cell wall may be overcome by manipulation of used concentration especially the recorded IC50 inhibitory concentrations is extremely low.

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