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INHIBITORS FOR SECA1 OF *MYCOBACTERIUM TUBERCULOSIS* H37RV

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ABSTRACT

Background: *Mycobacterium tuberculosis* causes tuberculosis (TB), an infectious disease, needs an urgent intervention due to emergence of highly drug resistant strains. **Aim**: The study aimed to find out new inhibitors for strategic target (Translocase protein, SecA1, with pdb ID 1nkt), through building QSAR models using different descriptors of the available inhibitors of SecA protein. **Materials and Methods:** About 50 molecules that inhibit SecA in different microorganisms were collected from different sources, descriptors of these molecules were calculated and used to build QSAR regression models using Multiple Linear Regression (MLR). **Results:** Built model was satisfied most statistical requirements, used to estimate the IC50 of different molecules included in Zinc database, the virtual screening revealed about 104 molecules or ligands under strict restrictions. Docking studies showed that most obtained molecules docked strongly in the target protein (1nkt). The binding affinities (-5.9 to -9.2 kcal/mol), using RMSD value of zero. Further filtration and testing resulted in five molecules which can be used and applied as inhibitor to this protein.

KEYWORDS: *Mycobacterium tuberculosis*, SecA1 inhibitors, QSAR, Drug discovery.

INTRODUCTION

Tuberculosis (TB) is a chronic infectious disease, caused mainly by *Mycobacterium tuberculosis* (*M. tuberculosis*), it has been reported in most countries, WHO (World Health Organization) estimates that about a third world's population is infected with *M. tuberculosis*.[1] A serious problem to fight TB is the emergence of resistance at different levels (MDR, XDR, and XXDR-TB /TDR)[2], the latter resist all the drugs in use and recorded in Iraq among the immigrants[3]. Therefore, there is an emergency for developing new anti-mycobacterial agents with unique mechanism of action. So, it is important to put a strategy for target selection[4], Among these strategies, the target should play an indispensable function in bacterial survival without any existing alternative pathway for its mitigation and compensation, must not have closely related human homologs, and contributes vitally to bacterial virulence and pathogenicity.[5]

SecA 1 protein could represent a good target for *M. tuberculosis*, this protein is an integral membrane protein upon activation, it is an ATPase critical member of Sec family which responsible for translocation of membrane and secreted polypeptides/proteins in this bacteria, it provides energy for Sec-dependent protein translocation.[2,6]

On the other hand, advanced computer science has found broad applications in drug discovery and design.[1,7] Computer –aided drug design (CADD) has become an integral part of drug design and discovery processes in both academia and pharma companies. Elucidation of Quantitative structure-activity relationship (QSAR) is one of the main approach of CADD.[1] QSAR is a mathematical form represented by:

Activity = f (physicochemical properties and/or structural properties).[8]

2-D QSAR has been used to correlate and predict the activity of compounds[9], when the physicochemical properties or structural properties are expressed by numbers, so mathematical relationship or quantitative structure –activity relationship can be formed between them.[8] QSAR models are regression models to relate a set of predictor variables (Descriptors) to the potency of response (Activity), this relation can be used to evaluate properties of new chemical entities.[8] The aim of this study was to find inhibitors of SecA1 (Rv3240c) of *M. tuberculosis*.

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 SecA. Other source for inhibitors were used as well.[10]
pdb database: https://www.rcsb.org/
Used to find out the pdb structure of SecA1 protein of *M. tuberculosis*.
Uniprot database : https://www.uniprot.org/
To find out some information about the target (SecA1).
Zinc database: http://zinc.docking.org/
Used to download different chemical format, and information about compounds.

SOFTWARE

Marvin Sketch: https://chemaxon.com/products/marvin
Used for chemical format manipulation, and finding some molecule descriptors.

Molinspiration: https://www.molinspiration.com/cgi-bin/properties?textMode=1
Used for finding some molecules descriptors.

Online SMILES Translator and Structure File Generator: https://cactus.nci.nih.gov/translate/
Used to get SMILES format of some molecules.

OSRA Optical Structure Recognition: https://cactus.nci.nih.gov/cgi-bin/osra/index.cgi
Used to get SMILES format of some molecules from image.

Swiss ADME: http://www.swissadme.ch/
Used for finding characters of molecules.
Swiss Similarity: http://www.swisssimilarity.ch/

NCBI/BLASTp
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.

PyRx software v.8: https://pyrx.sourceforge.io/Used for docking.[11]

PyMOL software: https://pymol.org/2/
Used for docking vitalization.
Discovery Studio v2.5: Used for docking vitalization.

LigandScout software v 4.3: https://en.freedownloadmanager.org/Windows-PC/LigandScout.html
Used for building a pharmacophore.

OriginPro2016:
https://www.originlab.com/demodownload.aspx
Used for graphing and calculation of some results.

ZincPharmer
http://zincpharmer.csb.pitt.edu/pharmer.html
Used for virtual screening.

Model validation: The robustness, applicability and stability of the generated QSAR model have been established by internal and external validation.

Internal validation: was carried out using cross-validation leave-one out (LOO) procedure, the process was performed by removing one compound and creating and validating the model against the original model, this was done for all compounds of training set. Once complete, the mean is taken of all Q^2 values and reported.[12]

External 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.[8] 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 SecA1 (1nkt pdb ID) 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

In this study screening of potential anti-tubercular compounds was done through building QSAR model using multiple linear regression (MLR). The anti-tubercular activity of about 50 compounds were collected from databases and literatures and expressed as IC50 value, this subjected to data transformation using logarithmic value to base 10. This to ensure that are more uniformly distributed, in addition the normality test was done which indicates that the values within the normal distribution as shown in Figure 1.

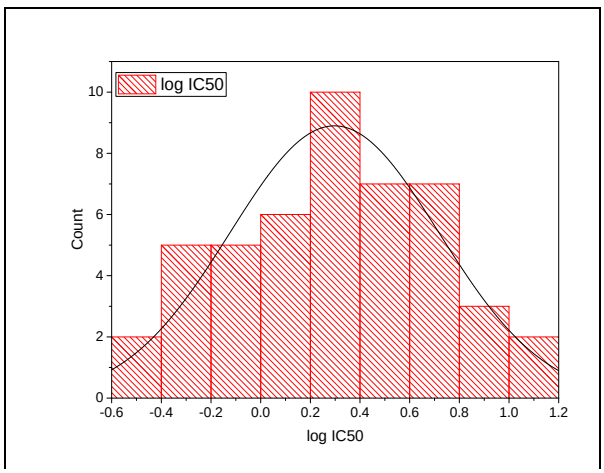


Figure 1: Normal distribution of SecA inhibitors (LogIC50) collected in this study.

Descriptor selection

Thousands of theoretical descriptors can be calculated[9], selection of descriptors is a critical step in the effective model development and requires significant efforts (so, primarily tens of models were developed, but fail upon validation and evaluation), therefore, descriptors were subjected to pretreatment. in this study, descriptors with correlation coefficient (r) > 0.7, one of them was used, and excluded descriptors having constant values. Among the descriptors used, constitutional descriptors such as Hbond donor and number of rotatable bonds. The electrostatic descriptors, polarization was used. In addition to LogP, which is considered to have main role in drug design.[13]

Model building

It is known that the majority of the cases, QSAR models have been used to modify previously discovered congeneric series of chemicals.[1] 2D QSAR models using MLR was carried out to correlate experimental response with physicochemical parameters, the data were divided into Training set and Test set (approximately Training set : Test set, 2:1), however, some molecules of Training set were excluded when there is an outliners and odd value relative to the majority of data. Large number of models were resulted, But high number of them were excluded upon validation. One model which considered as a good model was chosen for more study and validation in order to examine the internal stability and predictive ability:

Y= -6.28+ 0.33 LogP+ 0.26 HBdonor+ 0.17 Rotatable bonds+ 0.07 Polarizability

The statics of the model shown in Table 1.

Table 1: Statistics of generated QSAR model.

Validation Parameters	Name	Value
r	Correlation coefficient	0.982
R^2	Coefficient of determination	0.964
R^2_{adj}	Adjusted R2	0.939
Q^2cv	Cross validation coefficient	0.965
R^2_{pred}	Predicted coefficient	0.765
P(95%)	Confidence interval at 95% confidence level	<0.05
F-value	Significance of regression F-value	39.67
Tabulate F-value	Critical Significance of regression F-value (95%)	0.01
s	Standard error	0.09

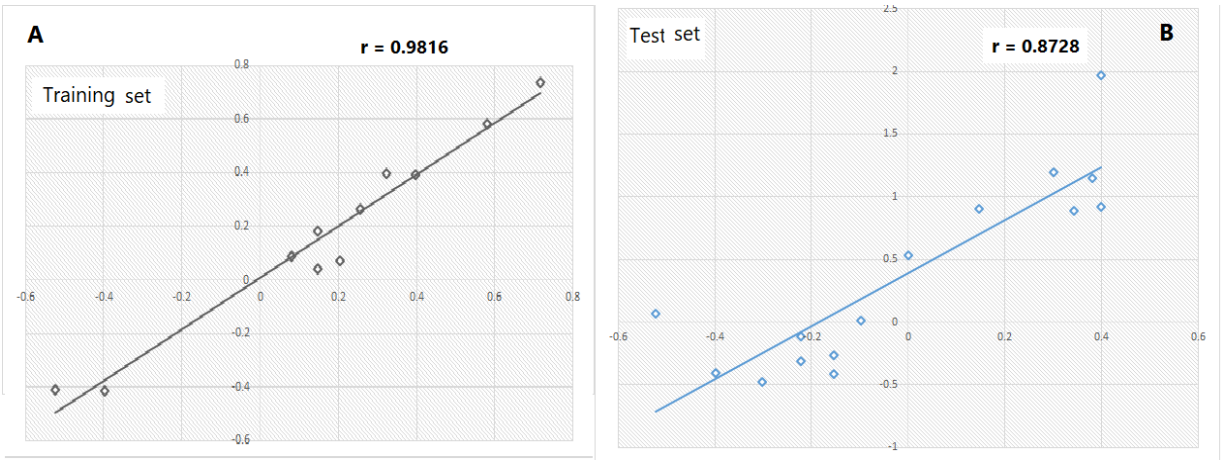


Figure 2: Correlation of Training set (A), Test set (B).

Figure 2A: Shows the results of Training set predictive values, and Figure 2B shows the results of Test set.

It seems that the model tended to be good for low values, i.e., high activity, as shown in Figure 3.

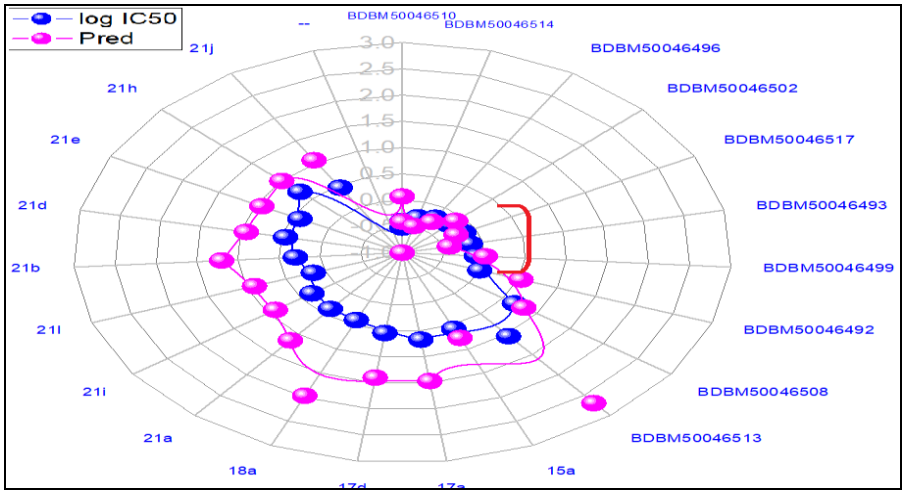


Figure 3: The observed Log IC50 and predicted Log IC50 obtained from model application.

The model was applied to all collected data (i.e. Training and Test sets), the results shown in Figure 4

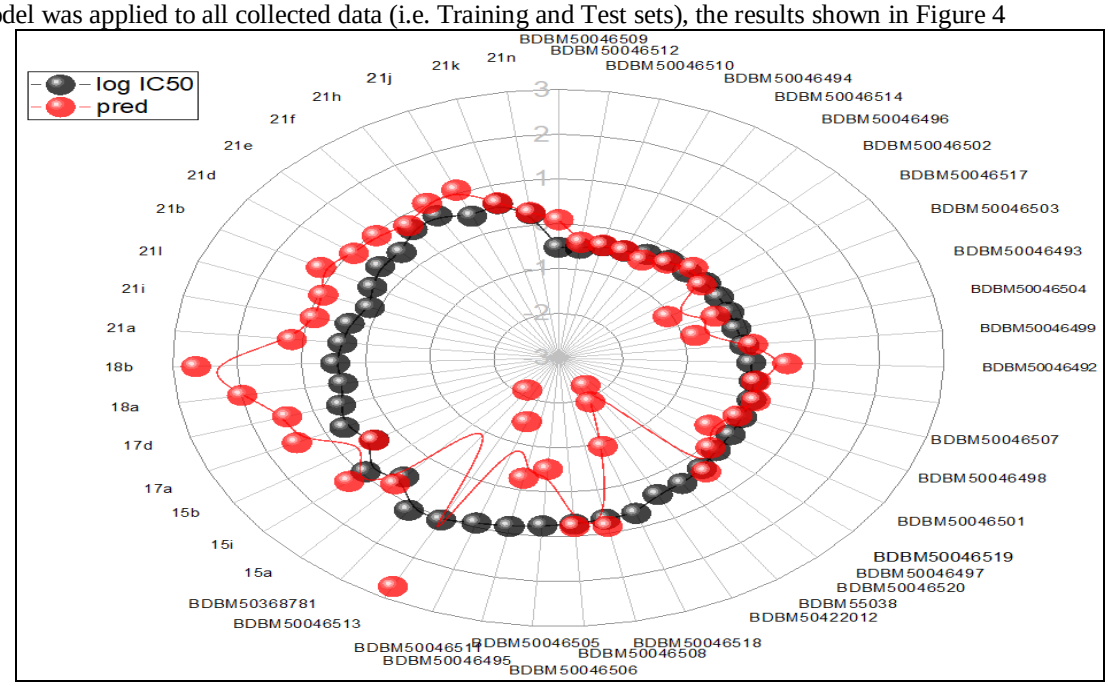


Figure 4: The estimation of predicted Log IC50 with the total observed Log IC50 upon application of QSAR generated model.

Their probability shown upon application of Q-Q plot shown in Figure 5

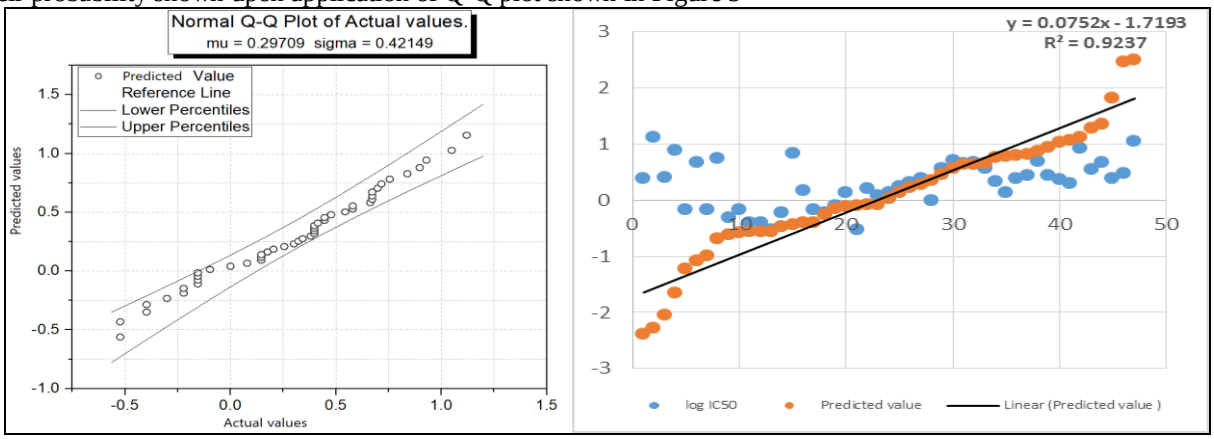


Figure 5: Q-Q plot for probability in comparison of distribution of real obsereved and predicted values of Log IC50.

Training set molecules were used for virtual screening of databases of SwissSimilarity using FP2 fingerprints and Electroshape for drugs under different entries, only one molecule was found under electroshape, but it was unsatisfactory. So pharmacophores were built using LigandScout software[14] and the best pharmacophore was used to screen Zinc database (22,723,923 compounds) using ZincPharmer Software[15], filtering and restriction of screening was performed by setting the RMSD value (0.05-0.2) and Rotatable bonds 4-6. The pharmacophore encounters the essential features, such as Aromatic, HBond donor, HBond Acceptor and Hydrophobic item as shown in Fig 6.

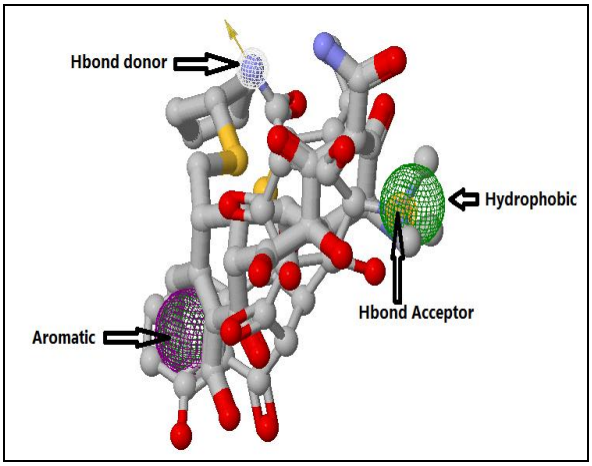


Figure 6: Built pharmacophore using Training set compounds.

About 104 molecules were obtained, duplication were excluded and molecules with more than RMSD values, only the lowest one were considered, 84 molecules were chosen and subjected for further studies, their activity were estimated using the model built in this study and shown in Table 2.

Table 2: IC50 values of Zinc database compounds screened in this study.

Molecule	Log IC50	IC50 µM
ZINC44921797	-3.0624	0.000866
ZINC95366186	-2.2271	0.005928
ZINC91784151	-2.099	0.007962
ZINC67820379	-2.0718	0.008476
ZINC67541660	-2.0011	0.009975
ZINC91782950	-1.9939	0.010141
ZINC67676669	-1.8489	0.014161
ZINC92914400	-1.7133	0.019351
ZINC12016213	-1.6436	0.02272
ZINC67446558	-1.6355	0.023147
ZINC71281691	-1.6183	0.024082
ZINC54257009	-1.5727	0.026749
ZINC40301277	-1.5442	0.028563
ZINC92920518	-1.5237	0.029943
ZINC64316375	-1.3919	0.04056
ZINC54257285	-1.3631	0.043341
ZINC13136296	-1.3024	0.049843
ZINC18331740	-1.2698	0.053728
ZINC54257285	-1.2695	0.053765
ZINC54257287	-1.2695	0.053765
ZINC75879678	-1.2628	0.054601
ZINC09608425	-1.2366	0.057996
ZINC54257032	-1.2041	0.062503
ZINC40153048	-1.1644	0.068486
ZINC12902120	-1.1554	0.06992
ZINC02962430	-1.1234	0.075266
ZINC27696987	-1.1068	0.078199
ZINC69663055	-1.0814	0.082909
ZINC93543399	-0.9994	0.100138
ZINC05092281	-0.9975	0.100577
ZINC01147917	-0.9454	0.113397
ZINC39947334	-0.901	0.125603

ZINC91739677	-0.8362	0.145814
ZINC64640021	-0.8271	0.148902
ZINC92188174	-0.8062	0.156243
ZINC01123519	-0.7951	0.160288
ZINC01216814	-0.7786	0.166495
ZINC01107160	-0.7247	0.188495
ZINC69624123	-0.7152	0.192664
ZINC00709463	-0.7075	0.19611
ZINC40120437	-0.7047	0.197379
ZINC20886160	-0.6983	0.200309
ZINC01467860	-0.6907	0.203845
ZINC20884341	-0.6808	0.208545
ZINC92454920	-0.6729	0.212373
ZINC60531295	-0.6728	0.212422
ZINC92190345	-0.6702	0.213698
ZINC21218355	-0.5796	0.263269
ZINC07642788	-0.5779	0.264302
ZINC21223546	-0.5264	0.297577
ZINC00924951	-0.5048	0.312752
ZINC07638236	-0.502	0.314775
ZINC00913740	-0.5015	0.315137
ZINC03309431	-0.4411	0.36216
ZINC21260560	-0.3973	0.40059
ZINC40105000	-0.3905	0.406912
ZINC00681716	-0.3802	0.416677
ZINC18286111	-0.3476	0.449159
ZINC20882373	-0.3377	0.459515
ZINC20881658	-0.2731	0.533212
ZINC08945449	-0.2069	0.621012
ZINC16841888	-0.1638	0.685804
ZINC21251623	-0.1201	0.758403
ZINC08557885	-0.0873	0.8179
ZINC21230345	-0.0618	0.867361
ZINC01181909	-0.0298	0.933684
ZINC21251490	-0.0005	0.998849
ZINC21219139	0.0469	1.114038
ZINC21227741	0.0689	1.171925
ZINC72320019	0.0829	1.210319
ZINC20883326	0.1287	1.344931
ZINC21224948	0.1412	1.384204
ZINC21258299	0.2813	1.911173
ZINC21231176	0.3624	2.303563
ZINC09554158	0.4583	2.872764
ZINC21247498	0.5213	3.321238
ZINC12558203	0.6084	4.058822
ZINC04520832	0.7534	5.667611
ZINC20788240	0.8945	7.843321
ZINC12623860	1.1756	14.98304

All the obtained compounds (i.e., Zinc molecules) were prepared and docked using AutoDock Vina[11] incorporated in PyRx package, and vitalized using PyMOL and Discovery Studio software. These ligands were found to bind strongly to the binding site of the protein. Table 3 shows the binding affinities to the target protein.

Table 3: The binding affinities of Zinc database compounds to the target protein.

Molecule	Binding affinity (kcal/mol)
ZINC09554158	-9.2
ZINC20788240	-9.2
ZINC05092281	-9.1
ZINC21218355	-9.1
ZINC08557885	-9.1
ZINC21227741	-9.1
ZINC27696987	-8.9
ZINC00924951	-8.8
ZINC20882373	-8.8
ZINC67446558	-8.7
ZINC01216814	-8.6
ZINC21258299	-8.6
ZINC67676669	-8.5
ZINC39947334	-8.5
ZINC20886160	-8.5
ZINC01467860	-8.5
ZINC21223546	-8.5
ZINC21260560	-8.5
ZINC21247498	-8.5
ZINC18331740	-8.4
ZINC01123519	-8.4
ZINC01107160	-8.4
ZINC21230345	-8.4
ZINC20883326	-8.4
ZINC04520832	-8.4
ZINC40105000	-8.3
ZINC18286111	-8.3
ZINC21251490	-8.3
ZINC21219139	-8.3
ZINC40301277	-8.2
ZINC13136296	-8.2
ZINC01147917	-8.2
ZINC92190345	-8.2
ZINC16841888	-8.2
ZINC01181909	-8.2
ZINC20881658	-8.1
ZINC72320019	-8.1
ZINC21231176	-8.1
ZINC92920518	-8.0
ZINC20884341	-8.0
ZINC00681716	-8.0
ZINC21251623	-8.0
ZINC21224948	-8.0
ZINC00709463	-7.9
ZINC60531295	-7.9
ZINC00913740	-7.9
ZINC12558203	-7.9
ZINC54257287	-7.8
ZINC64640021	-7.6
ZINC12623860	-7.6
ZINC71281691	-7.5
ZINC40153048	-7.5
ZINC64316375	-7.3
ZINC69663055	-7.3
ZINC92188174	-7.3
ZINC08945449	-7.3
ZINC02962430	-7.2
ZINC91782950	-7.1
ZINC69624123	-7.1
ZINC07638236	-7.1
ZINC91784151	-7.0
ZINC12902120	-7.0
ZINC07642788	-7.0
ZINC54257009	-6.9
ZINC91739677	-6.9
ZINC67820379	-6.8
ZINC92454920	-6.8
ZINC03309431	-6.8
ZINC92914400	-6.7
ZINC75879678	-6.7
ZINC95366186	6.6
ZINC12016213	-6.5
ZINC44921797	-6.4
ZINC54257285	-6.4
ZINC67541660	-6.3
ZINC09608425	-6.3
ZINC93543399	-6.3
ZINC54257032	5.9

However, these molecules were subjected for further check, to estimate their developmental toxicity which is the only related activity to human in T.E.S.T. 2016 software[16] and mutagenicity.

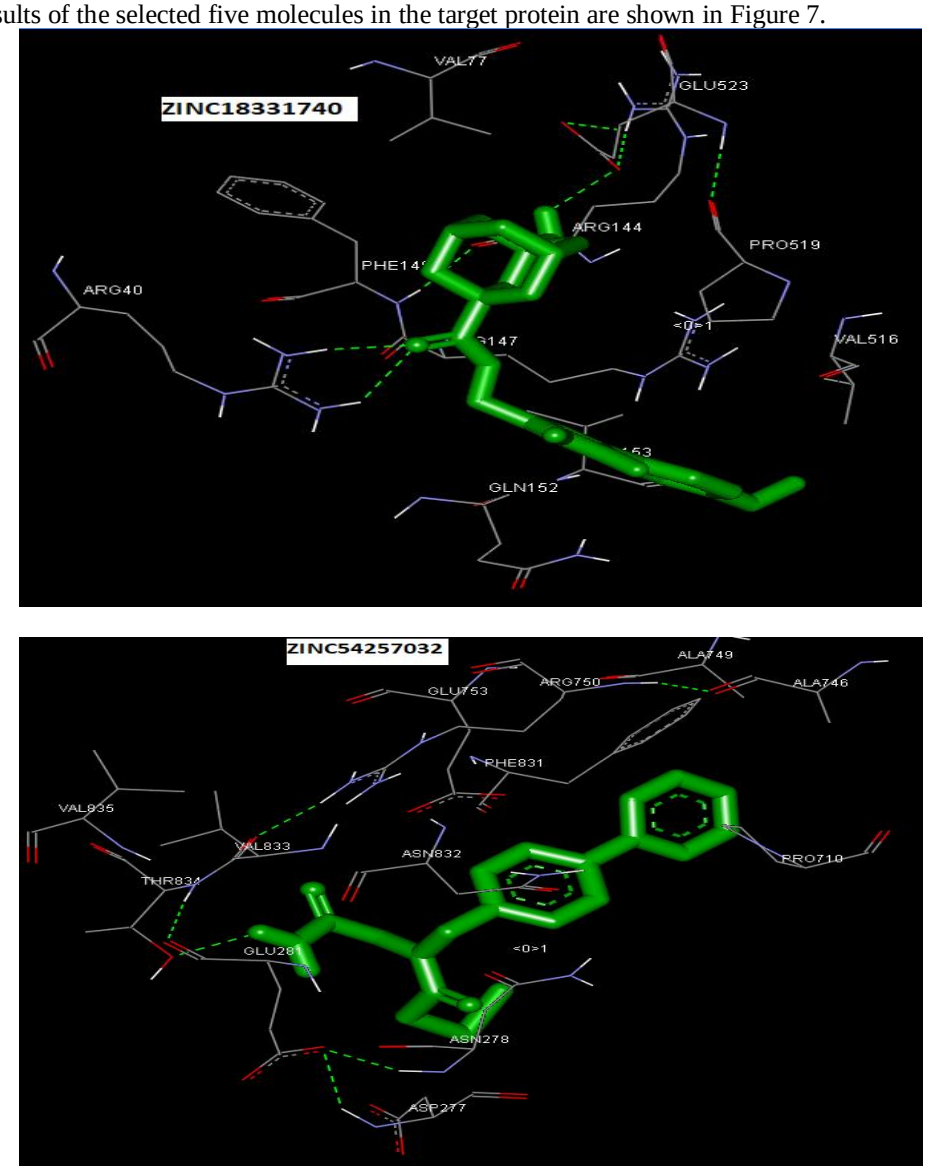
In addition, the compounds were used in Swiss ADME to check some characters qualified them as drugs, among these are GI absorption for orally used drugs, pg-substrate to avoid their efflux, their ability to cross brain blood barriers (BBB). In addition to find out the ability for synthesis (This measured as 1: very easy to synthesis, and 10 very difficult to synthesize). After these filtering, only 5 compounds passed these processes, shown in Table 4.

Table 4: Characters of non-mutagenic molecules and have no developmental toxicity of Zinc candidate compounds.

Molecule	Molecular weight	Solubility	GI absorption	P-gp substrate	BBB	Bioavailability Score	PAINS	Leadlikeness	Synthetic accessibility
ZINC07638236	390.49	Soluble	High	Yes	No	0.55	0 alert	2 violations: MW>350, Rotors>7	3.65
ZINC07642788	372.50	Soluble	High	Yes	No	0.55	0 alert	2 violation MW, RBs	3.63
ZINC12623860	515.82	Poorly soluble	Low	yes	No	0.17	0 alert	3 violations: MW>350, Rotors>7, XLOGP3>3.5	3.82
ZINC12902120	392.46	Soluble	low	No	yes	0.55	0 alert	2 violations: MW>350, Rotors>7	3.59
ZINC18331740	384.43	Soluble	High	No	No	0.55	0 alert	violation MW	5.59
ZINC27696987	341.41	Moderately soluble	High	yes	yes	0.55	0 alert	1 violation: XLOGP3>3.5	2.69
ZINC39947334	468.57	Moderately soluble	Low	Yes	No	0.55	0 alert	1 violation: MW>350	4.86
ZINC40105000	437.52	Moderately soluble	High	Yes	No	0.55	0 alert	2 violations: MW>350, Rotors>7	3.83
ZINC40153048	335.42	Soluble	High	Yes	No	0.55	0 alert	Yes	2.70
ZINC40301277	331.37	Moderately	High	Yes	yes	0.55	0 alert	Yes	2.67

		soluble							
ZINC54257032	328.43	Soluble	High	No	No	0.55	0 alert	1 violation: Rotors>7	2.31
ZINC67541660	333.84	Soluble	High	Yes	No	0.55	0 alert	Yes	3.13
ZINC67820379	313.42	Soluble	High	Yes	No	0.55	0 alert	Yes	3.12
ZINC69624123	385.44	Soluble	High	No	No	0.55	0 alert	2 violation MW, RBs	3.32
ZINC75879678	329.39	Soluble	High	No	No	0.55	0 alert	1 violation: Rotors>7	3.12
ZINC92454920	386.86	Moderately soluble	High	No	No	0.55	0 alert	2 violations: MW>350, XLOGP3>3.5	3.75
ZINC92920518	362.39	Soluble	High	Yes	No	0.55	0 alert	1 violation MW	4.07
ZINC95366186	382.50	Soluble	High	Yes	No	0.55	0 alert	1 violation: MW>350	3.62

The docking results of the selected five molecules in the target protein are shown in Figure 7.



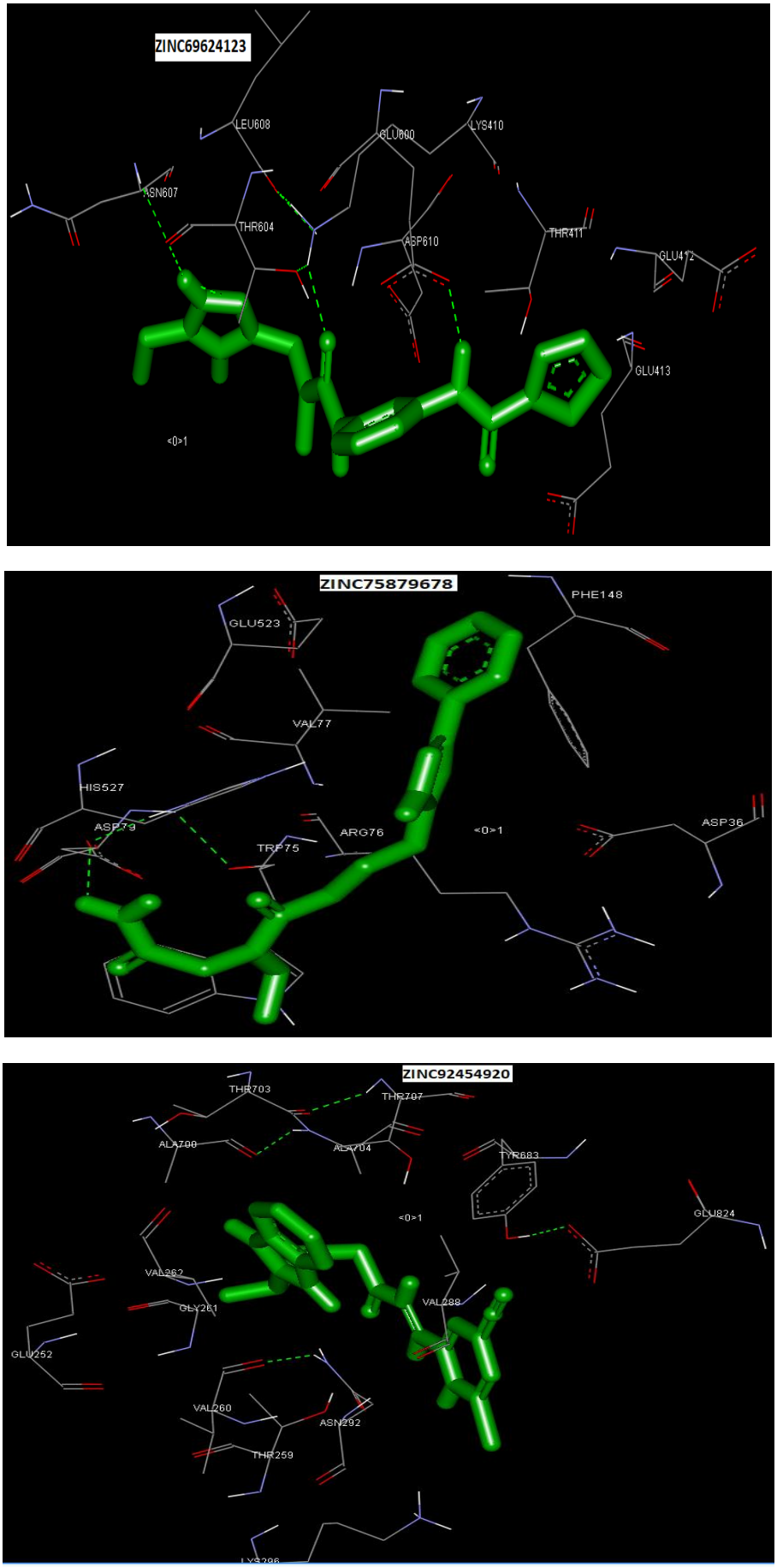


Figure 7: The docking of five candidate molecules in the target protein.

DISCUSSION

Quantitative structure–activity relationship has advantages over other computational techniques as it can be utilized for the prediction of physicochemical properties in the chemical, pharmaceutical and environmental fields. On the other side all the bacterial pathogens, the majority of *M. tuberculosis* virulence factors are extracytoplasmic proteins exported to the bacterial cell surface or secreted to the extracellular milieu[2] mainly via SecA1, this with pdb ID 1nkt and has high resolution 2.6A°.

The Adjusted R^2 was calculated, which is a modification of R^2, that adjusts for the number of explanatory terms in a model. Unlike R^2 in which the addition of descriptors to the developed QSAR model increases the value, the value of adjusted R^2 increases only if the new term improves the model more than what be expected by chance[1] and makes the model away from overfitting which developed upon increasing the number of descriptors.

The high correlation coefficient (r = 0.98161487) indicates the susceptibility of used descriptors. The squared correlation coefficient R^2 (0.96356775) explains 96% variance in biological response of the tested compounds.

The statistical significance of the regression model can also be assessed by means of F-value[17], which represents the ratio between explained and unexplained variance for a given degree of freedom. The higher the F-value the greater the probability is that the equation (Model) is significant. The regression model equation is considered to be statistically significant if the observed F-value is greater than a tabulate value for the chosen level of significance (Typically, 95% level) and the corresponding degree of freedom of F.

Other item should be considered is standard error[17], for good model the standard error(s) should be low, this measures the dispersion of the observed values about the regression line, the smaller the value of standard error means higher reliability of the prediction.

The acceptable P-value normally <0.05, low enough P-value could be considered the best predictive descriptors with sufficient statistical confidence[18], this means that only 5% or lower probability that the decency found is obtained by chance correlation between the variables.[19]

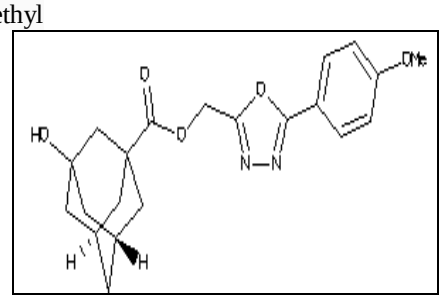
The contribution of descriptors in the built model can be deduced from the coefficients, and these are:
LogP> HBond donor > Rotatable bonds > Polarizability.

The results indicate that the molecules are very effective and most of them >10 µM, in that it is highly likely that different inhibitors may exhibit vary affinities for SecA. The suitability of SecA ATPase (SecA) as an ideal target for development of anti-tubercular agents have been increasingly recognized[10], as the binding of preproteins to SecA is an early step in protein export.[2] It is one of Sec machinery composed of SecA, SecD, SecE, SecF, SecG, SecY and YaiG. SecA becomes fully active as an ATPase and protein translocase. Therefore, SecA is essential for bacterial survival and pathogenicity[20], and the envision that inhibitors of SecA can be very useful as potential antimicrobial agents, especially because SecA has no human counterpart. In addition, SecA is a membrane protein in its translocation functional form, this more advantage in that the inhibitors can directly assess SecA without need to enter the cytoplasmic space. Thus drug permeation and intracellular concertation are less of an issue with these inhibitors. It is also expected that inhibition of SecA can affect the assembly of functional efflux pumps and can overcome the drug resistance.[4,5]

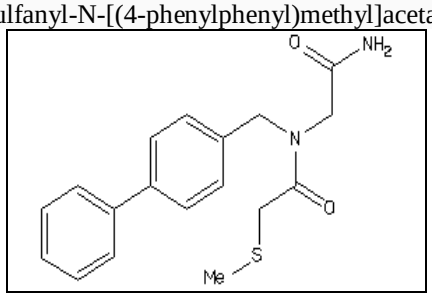
CONCLUSION

The generated QSAR model can be used to surveyed more molecules from other sources such as natural compound prior to experimental or wet lab studies.
The following represent the candidate molecules:

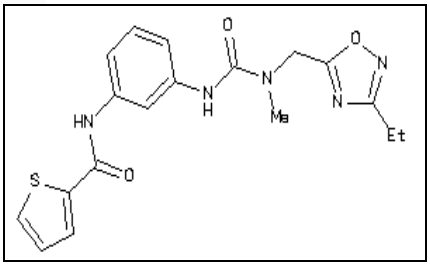
ZINC18331740: [5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl]methyl



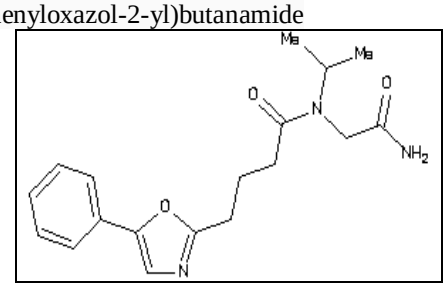
ZINC54257032: N-(2-amino-2-oxo-ethyl)-2-methylsulfanyl-N-[(4-phenylphenyl)methyl]acetamide



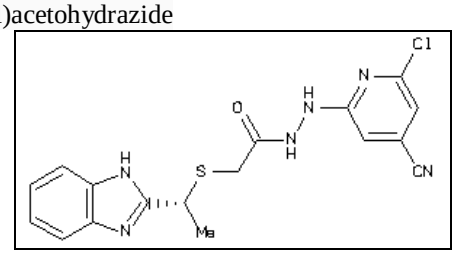
ZINC69624123: N-[3-[[(3-ethyl-1,2,4-oxadiazol-5-yl)methyl-methyl-carbamoyl]amino]phenyl]thiophene-2-carboxamide



ZINC75879678: N-(2-amino-2-oxo-ethyl)-N-isopropyl-4-(5-phenyloxazol-2-yl)butanamide



ZINC92454920: 2-[(1R)-1-(1H-benzimidazol-2-yl)ethyl]sulfanyl-N'-(6-chloro-4-cyano-2-pyridyl)acetohydrazide



Conflicts of interest

There is no conflicts of interest.

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