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Molecular Design of Isoniazid Analogues with Enhanced Therapeutic Potential as Antitubercular Agents: In Silico KNN-MFA, Pharmacophore and Virtual Screening

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Abstract

Over four decades of time, Isoniazid has been a popular drug but its glory has faded away with time though it is too strong scaffold to render it be of no use. In the following research work different computational strategies have been applied on isoniazid derivatives to re-evaluate its efficiency as anti tubercular scaffold by putting versatile substituent over the basic drug moiety with consideration of the reported data. Each data has defined specific substitution pattern on the scaffold to achieve higher activity. The following experiment accounts for 2D-QSAR, 3D-QSAR, docking analysis, pharmacophore mapping and further precise designing of the data set with the promising active moiety and concludes its chemistry to define its suitability in future. 2DQSAR has shows good reliability with r2=0.8031, q2_LOO=0.6757 and external pred_r2=0.6202, along with 3D QSAR has good predictability, q2 (r2cv)=0.8611, pred_r2=0.7691. Also, ligand based pharmacophore mapping for estimating atomic contribution of the molecular arrangement indicates that hydrogen accepter, donor and aromatic ring parameters are important for designing aspect. Finally, new chemical entities are designed taking considerations of all results and their activity is predicted with known data set. The predicted activity of the designed compounds exposed the re-utilization of the scaffold to be anti-TB active.

Keywords: QSAR, Docking, Pharmacophore mapping, Molecular designing, Anti-Tb activity, Validation.

Introduction

Global statistical data of the tuberculosis has affected population graph and multiplicity of the types and symptoms of TB with each year, as the medication and maintenance therapy is getting more vulnerable to the microorganism. In 2017 TB report,4.1% of new cases and 19% cases of multidrug resistance or rifampicin resistant tuberculosis have depicted alarming condition. Most of the first line drugs are getting resistant with time rendering a complex situation. In this context, Isoniazid has been the first line drug for decades without lapse. For its activation through KatG gene to generate nicotinic acid which produces bulky adduct after the reacting with one NAD molecule This activation procedure has now been the cause of its limitation and resistant development. So the new derivates must have the ability to bypass the process of activation to develop potent drug candidate. This might be achieved by introducing the bulkier group to the moiety, so that it can directly interact with the active site of the enoyl ACP reductase enzyme of *M. tuberculosis* [1].

In the present study, our effort is to re-evaluate the efficiency of isoniazid as antitubercular agent with the help of different computational strategies (**Figure 1**).

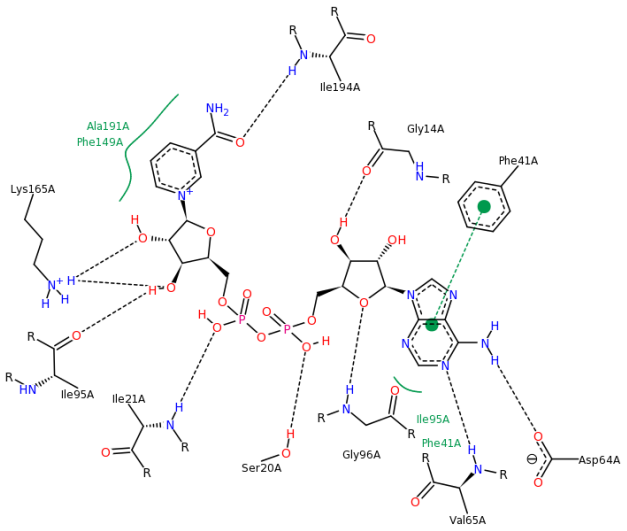

Figure 1: Interaction details of the Isoniazid-NAD adduct (co-crystal_1ENY) [Reported in RCSB-PDB].

Material and Methods

QSAR study using Vlife MDS

A set of 30Isoniazid derivatives reported by agar dilution method was subjected to the QSAR analysis for their antitubercular activity. All QSAR studies were performed in V-Life MDS software Version 4.4. The inhibitory activity data were reported as MIC50 and converted to pMIC50 by taking negative log of MIC50 to reduce the skewness of data and activity range was taken in log 2.5 fold scatter. (**Table 1**) shows the structure of the compounds along with their biological activity.

Sl No.	R	MIC50 (μM)	#NUM!
1	C_6H_4	9.9	-0.99564
2	2-CH_3-C_6H_4	9.56	-0.98046
3	3-CH_3-C_6H_4	9.56	-0.98046
4	4-CH_3-C_6H_4	9.56	-0.98046
5	2-OCH_3-C_6H_4	9.08	-0.95809
6	3-OCH_3-C_6H_4	9.08	-0.95809
7	4- OCH_3-C_6H_4	4.54	-0.65706
8	3,4,5- OCH_3-C_6H_4	7.76	-0.88986
9	4-OH-3- OCH_3-C_6H_4	8.7	-0.93952
10	2-OH-C_6H_4	18.98	-1.2783
11	3-OH-C_6H_4	9.49	-0.97727
12	4-OH-C_6H_4	18.9	-1.27646
13	4-$N(CH_3)_2$-C_6H_4	8.78	-0.94349
14	4-$CH(CH_3)_2$-C_6H_4	2.138	-0.33001
15	2-O-$CH_2C_6H_5$-C_6H_4	0.24	0.619789
16	3-O-$CH_2C_6H_5$-C_6H_4	0.24	0.619789
17	4-O-$CH_2C_6H_5$-C_6H_4	0.12	0.920819
18	2-NO_2-C_6H_4	2.12	-0.32634
19	3-NO_2-C_6H_4		0.251812
20	4-NO_2-C_6H_4	1.11	-0.04532
21	2-Cl -C_6H_4	1.15	-0.0607
22	4-Cl-C_6H_4	0.575	0.240332
23	2-Br-C_6H_4	0.25	0.60206
24	4-Br-C_6H_4	0.5	0.30103
25	2-F -C_6H_4	0.6	0.221849
26	3-F -C_6H_4	0.6	0.221849
27	4-F-C_6H_4	0.3	0.522879
28	2-CF_3-C_6H_4	0.52	0.283997
29	3-CF_3-C_6H_4	0.52	0.283997
30	4-CF_3-C_6H_4	0.13	0.886057

Table 1: Biological Activity Data And Substituent Present In The Compounds At Different Positions.

For 2D QSAR, all the compounds were subjected to energy minimization to get 3D structures, using Merck molecular force field (MMFF) followed by considering distance-dependent dielectric constant of 1.0 and convergence criterion of 0.001 kcal/mol. The QSAR work sheet was generated using biological activity as dependent variable and various 2D descriptors as independent variables. The data set was divided into training set and test set by considering the biological activity method along with 70% data selection method and SW PLS (Step wise forward-backward partial least square) method was incorporate for estimation [2].

In 3D QSAR, conformers were generated by Monte Carlo conformational search method and the conformers of least energy were selected for the alignment. All the compounds were aligned by template-based method, where a template was built by considering common substructures of the series. Usually, the lowest energy conformer of the most active compound is selected as a reference. In the present study, all the compounds were aligned against minimum energy conformation (RMSE value0.011189) by using isonicotinyl nucleus as template as shown in Fig2 and the alignment of molecules is shown in Fig 3.Several 3D QSAR models were generated using kNN-MFA (k nearest neighbour Molecular fragment arrangement) coupled with SW forward-backward variable selection method (SW kNN-MFA). These models were selected on the basis of the statistical parameters (**Figure 2**).

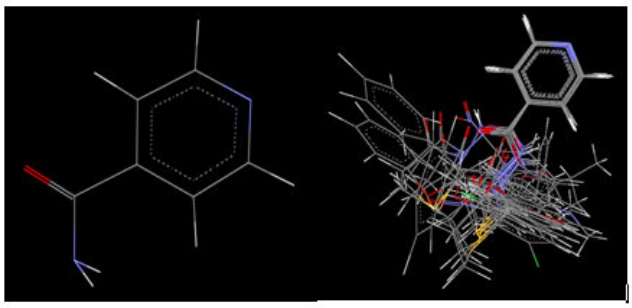

Figure 2: Template used for alignment of the compounds and Aligned molecular set over the template.

The pharmacophore estimation is carried out in Mol sign module of Vlife MDS 4.4. The series of anti-TB molecular set is first aligned on the basic common template structure of the series. A pharmacophore model is a set of three-dimensional features that are necessary for bioactive ligands. Thus, it makes logical sense to align molecules based on features that are responsible for its bioactivity rather only chemical features. The software was set to generate a minimum of five pharmacophoric features keeping the tolerance distance at 10 Å with maximum limit of 10 [3].

Docking study using GOLD

The set of 30 molecules were docked with two protein molecules reported to be important in the anti-TB activity and the crystalline structures of the respective proteins were taken from the RSCB PDB site, in order to estimate the ligand macromolecular interaction. The chemical nature of the macromolecule and ligands greatly influence the performance of docking routines. An evaluation criterion was based on the docking scores and interacting atoms. The docking study has been carried out in GOLD software Version 5.2 using ChemPLP-GA (ChemPLP genetic algorithm) scoring method and ChemPLP-GOLD-GA (ChemPLP-GOLD-gentic algorithm) rescoring method [8, 9]. The ligands were prepared using HyperCHEM 7 professional. The energy of all ligands wasminimized with MM+,

PM3 and abintio quantum mechanics. (**Figure 3**) shows minimized energy profile of all ligands.

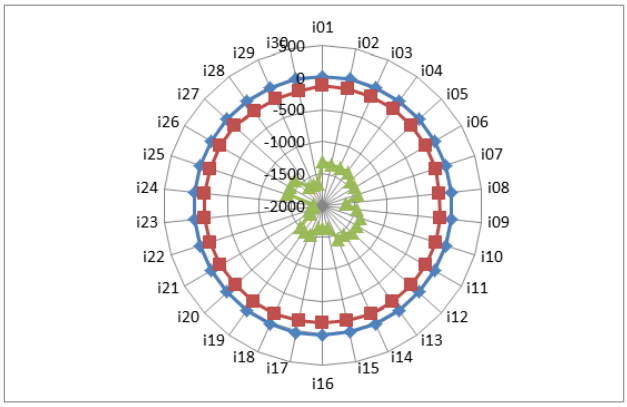

Figure 3: Graphical chart representation of the local energy minimization of the molecular set.

Designing of new molecule

Depending on the results of the above studies and taking consideration of the structural constituents of the series, the new molecular set has been designed with respect to the template moiety,using Chemdraw Ultra 8.0 and Hyperchem7 professional. The generated new molecular set has been first subjected to optimization process and then conformers were generated by Monte Carlo conformational search method. The conformers of least energy were selected for the alignment. All the compounds were aligned by template-based methodwith respect to the template and evaluated through the 3D QSAR model prediction which estimated probable activity of the molecular set using MDS Vlife 4.4 software suite [3].

Results and Discussion

2D QSAR Analysis

In this statistical assessment, training and test sets were generated by using 70% search method followed by SW-PLS treatment for the statistical estimation of the model. Compounds were divided in training and test set in such a way that biological activities of all compounds in test set lie within the maximum and minimum values of biological activities of training set compounds. 2D QSAR equations were selected by optimizing the statistical results developed with the variation of descriptors in these models. The frequency to use a particular typeof descriptor in the population of equations indicated the relevant contributions of the descriptors which are also within acceptability range. Among the several QSAR models, the best one regression equation is represented in Eq. 01:

pMIC50 = -1.56667(XlogP) + 0.293387(HydrogensCount)

-3.22126(chi3Cluster) + 1.97619 ……(Eq. 01)

The statistical data of the best 2D QSAR model has been given in table 2 and the correlation matrix has been reported in table 3. Among the prepared models the best model reported, explains 80.31% (r2 = 0.8031) of the total variance in the training set as well as it has internal (q2) and external (pred_r2) predicative ability of 67.57% and62.02% respectively. The low standard error of r2_se=0.29, q2_se=0.32 and pred_r2se = 0.23 demonstrates accuracy of the model. The F-test = 58.15% shows the statistical significance of the model which means that probability of failure of the model is 1 in 10,000. In addition randomization test shows confidence of 99.9% that the generated model is not random and hence it is selected as the QSAR model. Furthermore, the contribution plot the percentage of contributing descriptors. Three descriptors have contributed for the estimation of the model those are, XlogP, Hydrogens Count and chi3Cluster. Among them XlogP and chi3Cluster descriptors are negatively contributing with values 40% and 20% respectively on the other hand, HydrogensCount has positive contribution approx 40% to the estimated model [4].

XlogP: It signifies the hydrophobicity and steric hindrance of the molecular set under investigation. It is a theoretical estimation of partition coefficient of the molecule which directly relates to the lipophilicity of the molecule. It should be decreased according to the model estimation. But an optimum level must be balanced between hydrophobicity and steric hindrance factors to generate better active lead molecule.

HydrogensCount: This descriptor signifies number of hydrogen atoms in a compound. Number of hydrogen greatly affects the hydrogen bond within the system both inter and intra hydrogen bond orientation and directly affects the hydrophobicity of the molecular set.

chi3Cluster: This physicochemical descriptor signifies simple 3rd order cluster chi index in a compound. There aredifferent order of graph possible, among them third-order subgraph called a cluster, which involves four skeletal atoms in a trigonal relationship and this structural motif appears only in trifluoromethane and tetrafluoromethane. The negative coefficient describes that this type of substitution pattern will not favor the activity.

The kNN MFA QSAR method explores formally the active analogue approach where the activity of each compound is predicted as average activity of the most chemically similar compounds from that data set. Several 3D QSAR models were generated using SW kNN-MFA method. These models were selected on the basis of values of statistical parameters. The best SW kNN-MFA 3D QSAR model with 21 training compounds has a q2 (r2cv) value of 0.8611 and pred_r2 valueof 0.7691 was considered along with consideration of the acceptable range for the error factors used for further cross validationdescribed [5].

In 3D QSAR studies, 3D lattice generated around Isonicotinic pharmacophore were used to optimize the electrostatic and steric requirements of the active nucleus for desired anti-TB activity. The interactive lattice produced in the generated data points help for the designing of new compounds. The points generated in SW KNN MFA 3D QSAR model H_688 (0.3172 0.3920), E_266 (-0.0387 -0.0063), E_1537 (-0.0609 -0.0272) given in Fig. 6. The groups on the molecules near the lattice points need to have the interaction energy with the lattice point

within the given range to be active. The descriptors involved in 3d model are being explained below.

H_688 (0.3172 0.3920): positive range indicates that at lattice point 688 positive hydrophobic potential is favorable for increase in the activity of the molecular set and hence a hydrophobic substituent will be favorable in that region to get more potent congeners. Alkyl chain or phenyl ring as well as substituted benzene ring contribute to enhance the hydrophobicity increase which will be suitable for getting more potent molecular set.

E_266 (-0.0387 -0.0063): negative range indicates that at lattice point 266 negative electrostatic potential is favorable for increase in the activity and hence either a strong electronegative or less electropositive substituent will be the choice for substitution as a functional groups. It was found that the electropositive groups will be the desirable choice as substituent for the future designing approach to get a potent lead molecule.

E_1537 (-0.0609 -0.0272): negative range indicates that at lattice point 1537 negative electrostatic potential is favorable for increase in the activity and hence either a strong electronegative or less electropositive substituent will be the choice for substitution as a functional groups (Figure 4).

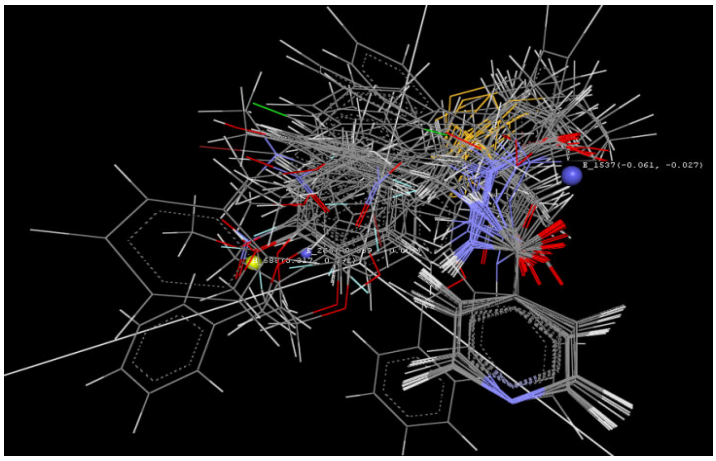

Figure 4: Relative positions of the local fields around aligned molecules of isoniazid analogues Validation of QSAR study:

2D QSAR model validation has been done by considering various statistical parameters. The q2_LOO value shows the robustness of the model developed which is in acceptable limits. Estimation of Z score as well as residual error isother validation parameters. Z score is crucial statistical parameter for validation. All the parameters, which support Z score functionalities, are in acceptable limits, which ensure the models reliability and acceptability that contributes directly to the validation of the prepared 2D QSAR model [6].

The actual vs. predicted and vice versa have been plotted to estimate the reliability of the model's predictability. The high value of cross validation parameter doesn't ensure reliable predictability of the model which is the key feature of a QSAR model. Here, R^2 of the two regression lines are equal and the slope values have differed by less than 0.1 which ensures the reliability of the model shown in the (**Figure 5**). The residual error of each compound is less than 0.01 which is within acceptable limit and confirms that the model has a predictability of less error chance and failure.

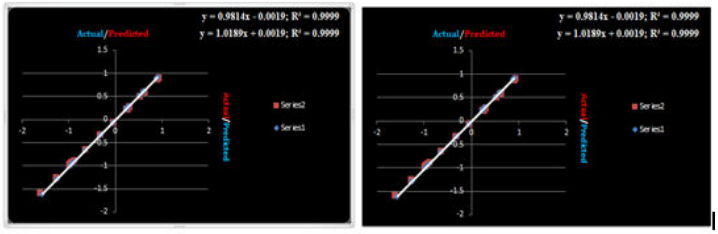

Figure 5: Plot of actual vs predicted values and reverse for model predictability estimation.

In accordance to, 2DQSAR result the partition coefficient i.e.XlogP andfour skeletal atoms in trigonal relationship i.e. chi3cluster are not productive for activity while the hydrogen count should be increased to an optimum level to get more active moiety.Therefore, the group substitution should be selective so that the new molecule will have a balanced featureof trigonal space orientation.In 3D QSAR study the hydrophobic potential should be increased and the electronic potential should be decreased at defined lattice point to produce more efficient molecules. These results are in accordance with the 2D QSAR results, which conclude that, an optimum balance of hydrophobicity and electronic potential distribution is required in the molecular systemto develop better moieties [7].

Pharmacophore modeling

Pharmacophore is a 3D description of the atomic contribution to the chemical features needed for activity, which include hydrogen bond donors and acceptors, aromatic groups, hydrophobic groups, positively ionisable and negatively ionisable groups. Here, a set of pharmacophore hypothesis was generated using Mol Sign.Each of the set contained the following features, hydrogen bond acceptors (blue colored cage), hydrogen bond donor (green colored cage) and aromatic group (yellow colored cage), shown in the which are the important features in regard of anti-TB activity.The ideal bond distance of the crucial points in the structure has been reported. (**Table 2**).

Sl no	1st Point	2nd Point	Distance (A)
1	Hydrogen acceptor1	Hydrogen donor	6.172
2	Hydrogen acceptor1	Hydrogen Acceptor 3	4.634
3	Hydrogen acceptor1	Aromatic ring	7.154
4	Hydrogen acceptor1	Hydrogen Acceptor 2	8.86
5	Hydrogen Acceptor 3	Hydrogen donor	5.03
6	Hydrogen Acceptor 3	Aromatic ring	3.229
7	Hydrogen Acceptor 3	Hydrogen Acceptor 2	4.395
8	Aromatic ring	Hydrogen donor	4.222

9	Aromatic ring	Hydrogen Acceptor 2	1.745
10	Hydrogen Acceptor 2	Hydrogen donor	5.251

Table 2: bond distance details of the pharmacophoric regional points.

The nitrogen atom and aromatic ring of the basic isonicotinyl ring, carbonyl group and sulfur atom of the cycloazathioxy hexane ring and the hydrogen bond donor substitution present at the aromatic ring positioned on the 2-cycloazathioxy hexane ring in the derivatives are the crucial features for the interaction. The overlapped phamacophore map of the molecular set also concluded the same chemical features shown by the reportedseries.

The recorded pharmacophoric features regarding activity are of significant value in contributing potent activity and interaction sites to the experimental moieties and each contributing factor has a relation with the results of 2D and 3DQSAR study.

So the outcomes of QSAR and pharmacophore model is inter related with each other providing support to the anti tubercular activity of the derivatives (**Figure 6**).

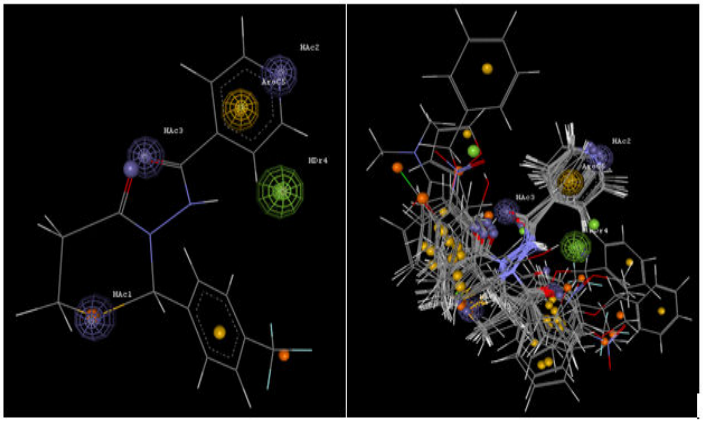

Figure 6: The principle pharmacophore model of the series and all molecules aligned on the basic pharmacophore.

The double bonded oxygen atoms has a great effect on the molecular arrangement of the system hence it can affect the activity dramatically.

Replacement of this group will not be favorable. Apart from substitution of phenyl ring at R group, other ring systems such as cyclic rings, heterocylic ring, acyclic rings, spiro ring, bicyclic ring should be studied.

For effective activity, despite the reported substitution on the above mentioned benzene ring, fluorine, nitro or other functional group may be appliedto provide hydrophobicity with less steric hindrance (**Figure 7**).

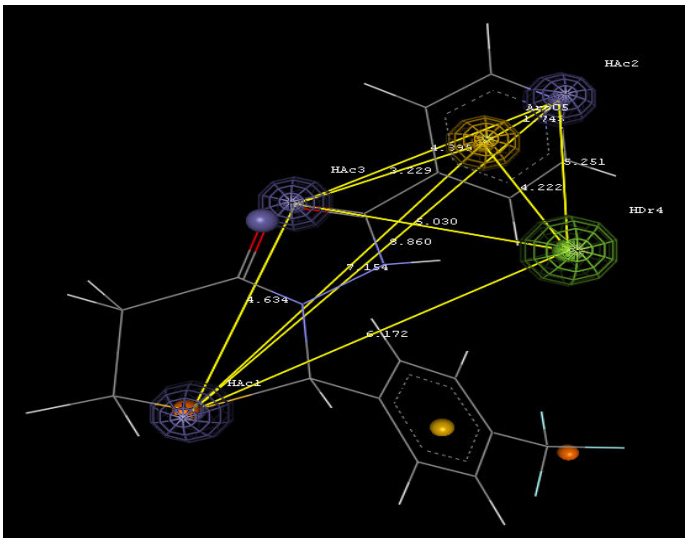

Figure 7: The distance of the main region of the considerable points in pharmacophore.

Docking study

In search of newer and potent anti-TB lead among the isoniazid analogues, structure based evaluation is being performed *via* docking analysis using GOLD Suite 5.2 software using ChemPLP-GA scoring method and ChemPLP-GOLD-GA rescoring method. Docking confirms theoretical aspect of binding affinity, orientation and potency through its scoring values and enlightens the activity of the molecules on a system under certain conditions applied. Here the docking study of the analogues has been done on two different proteins, KatG (PDB ID 2CCD) and enoyl ACP reductase enzyme(PDB ID 1ENY) along with the standard isoniazid molecule.Isoniazid first reacts to KatG protein and get oxidized and forms NAD adduct and binds to the enoyl-ACP reductase enzyme of *Mycobacterium tuberculosis* and shows anti TB effect. The binding patterns of top scored three molecules with enoyl-ACP reductase enzyme are represented. The docking score, H-bond, interacting water molecules and important interactions of these hits are discussed. The scoring and rescoring data of the docking study with 1ENY protein has been summarized. The KatG enzyme which is a dimeric catalytic enzyme has an important role in the oxidation procedure and the activation of isoniazid as isonicotinic acid. Here, the heme pocket made up of metaloporphyrin ring at both the chain A and B in the macro-molecule is embedded deep in the structure of the target. It is surrounded by a bed of water molecules forming a network like structure, better to be known as hydrogen bonded network which maintains it's potential [8].

In earlier wild strain of *M. tuberculosis* has been reported to show a channel of amino acid residue of 6Å at the narrowest part of the system, through which INH get its access to the heme pocket and the electro-potential effect of the hydrogen bonded system helps to carry out the oxidation process which is energy dependent [14]. In recent literature, the mutant [PDB ID: 2CCD] reported, has a different structure from these system and S315T is unable to form the oxyferrous intermediate which is needed to oxidize INH. Therefore, it has blocked the possible kinetic

process responsible for resistant to INH. It has been proposed that the extra methyl group introduced by Thr315 effectively restricts the accessibility to the heme by closing down the dimensions of the narrowest part of the channel from 6 Å in the wild type KatG to 4.7 A in the mutant. Besides, the extra methyl group has alter the hydrogen bond network and the electrostatic distribution surrounding the heme group, hence also affecting its redox potential and restricting the mode of action [9].

Subsequently, Asp137 retains a hydrogen-bond with the main chain of Ile228 and developed an arrangement where it is positioned at a bottleneck of the access tunnel, and its position potentially controls the entrance of substrates. Therefore, the modification to the system has brought the justification of being less docking score for the molecular set used for docking to the mutant. Furthermore, the structure of the molecular set is far different and bulkier than isoniazid and the electro-potential and steric effect of the molecular set is also differs from the substituent present in INH. So the possibility of its getting access through the tunnel and to the heme pocket is very less. Hence the less docking score without any interaction between the target and the investigational molecular set predominantly confirms that the molecular set will not get access through the tunnel. So the molecular set has very less chances to undergo redox reaction process [10].

Validation of docking study

Validation of docking analysis was done using rescoring method for the blind docking and for the active site docking or direct docking. For validation, superimpose fitting method has been applied using Pymol 2.0.7. Graph of ChemPLP-GA scoring method vs ChemPLP-GOLD-GA rescoring method and vice versa of the total molecular set has been plotted to estimate the reliability of the docking method. The R^2 of the two regression lines are equal and the slope values have differed by less than 0.1 which ensures the reliability of the method. On the other side, the fitting has been done over 42 atoms of the 81 atoms constituted co-crystal system using the raw pdb file, which contributes over 50% of the atom fitted within 2A of acceptability range [11].

No interaction was observed with the KatGas well as possibility to reach the electro potential region of the macro molecule i.e.metaloporphyrin ring was very less. Moreover, the molecular set has shown better score and interaction with the active site of crystal structure and function of the isoniazid target of M. Tuberculosis (PDB ID 1ENY) over the reported co-crystal which is the NAD-isonicotinic acid adduct and isoniazid. So it is evident that from docking analysis compounds possess anti-TB activity. The bulk added to the basic isoniazid moiety has made the activation process by katG to be non essential which proves bulky substituent is the favourable for the molecular set to be active directly on the active site of the enoyl ACP reductase enzyme.

Designing of the new chemical moeity

For the development of better molecular set, the following major points of the QSAR results has been considered; the functional group substitution should be selective to produce a significant change in partition coefficient factor with increase in hydrogen bonding parameter. Finally, over all substitution pattern should preserve an optimum balance between hyrdophobicity and electronic potential distribution among the molecular system. Pharmacophore mapping accounts that, the nitrogen atom and aromatic ring of the basic Isonicotinylring, carbonyl group and sulfur atom of the cycloazathioxy hexane ring and the hydrogen bond donor substitution present at the aromatic ring positioned on the 2-cyclo azathioxy hexane ring are the crucial features for theprotein-ligand interaction. So considering this all points, a set of 1000 molecular system has been developed. The activity value has been predicted using the developed 3D QSAR model as molecule exists in 3 dimensional axis and among them only the good and efficient molecules having activity greater than the reported value, along with the predicted numerical activity in terms of negative log value.

The active compounds predicted from the developed 3D-model has all the quality ensured in the result of the pharmacophore and the QSAR results. The molecular structure was drawn in Chemdraw and subjected to optimization by MM +method then, PM3 and finally using DFT b3lyp/6-311g method. The designed molecule 01 are stable and real possible molecule. The IR spectrum of designed molecule is given respectively, which tells about the functional groups present in the system. This concludes the molecule is not an arbitrary outcome and imaginary. It can exist in real with a defined space and chemical features.

Conclusion

In this computational experiment we have followed the well defined protocol which manages to reach a notable conclusion that the isoniazid scaffold can be used to develop novel molecular set for potent anti tuberculosis activity with proper substitution pattern. According to 2DQSAR and 3DQSAR, group substitution should be selective so that it balances the hydrophobic character and electronic potential distribution required in the molecular system. Pharmacophore mapping reveals the important features required for protein ligand binding interaction and established that the molecular set is active for this contributing atomic arrangement. The docking results have concluded that the compounds with bulky substituents to the basic isoniazid, has made the activation process through katG to be non essential and hence, it can directly interacts to the active site of the enoyl ACP reductase enzyme with better activity. The highest PEC value of designed compounds predicted by 3D QSAR-models also explore them as most promising drug candidate. It can be expected, the likewise parent scaffold, the new generation of isoniazid derivatives can reduce the burden of the tuberculosis which includes the domain of all age groups of patients with the same efficiency for decades.

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