WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 14, 1202-1211. Research Article ISSN 2277– 7105

THE ADME/T PROFILES OF TB DRUGS – AN *IN SILICO* ANALYSIS

V.G. Shanmuga Priya[1]*

[1]Department of Biotechnology, KLE Dr. M. S. Sheshgiri College of Engineering and Technology, Belagavi, Karnataka, India.

Article Received on
18 Sept. 2017,
Revised on 08 October 2017,
Accepted on 29 October 2017
DOI: 10.20959/wjpr201714-10065

***Corresponding Author**
V.G. Shanmuga Priya
Department of Biotechnology, KLE Dr. M. S. Sheshgiri College of Engineering and Technology, Belagavi, Karnataka, India.

ABSTRACT

In an urge to identify potential drug candidates for the deadly tuberculosis disease, many *in silico* approaches are being carried out and numerous molecules are being identified. Their pharmacokinetic parameters need to be validated before taking them to the expensive experimental level. Comparison of their ADMET profile, with that of the profile of current existing TB drugs can provide a better analysis of the compounds. Hence in this study, 4 TB oral drugs Isoniazid, Pyrazinamide, Aminosalicylic Acid and Ethionamide, and 2 injectable TB drugs Streptomycin and Kanamycin, which belong to different lines of TB drug category were selected and their structures were retrieved from DrugBank database. With the structures, the ADME profiles of the drugs were calculated by the QikProp program of Schrodinger and their Toxicity profiles were known from admetSAR server. The ADME profile values of the oral drugs Isoniazid, Pyrazinamide, Aminosalicylic Acid and Ethionamide have very few deviations from the range of values calculated from 95% of known drugs and Streptomycin and Kanamycin being injectable antibiotics have wider deviations from the given range for comparatively many criteria. Regarding their toxicity profile, Isoniazid and Streptomycin are known to be mutagenic but are not carcinogenic. The other 4 drugs are both non-mutagenic and non-carcinogenic. These analysis carried out on known TB drugs widens the knowledge of evaluating new anti-TB drug candidates.

KEYWORDS: TB drugs, ADMET profile, DrugBank, QikProp program, admetSAR.

1. INTRODUCTION

The fight against Tuberculosis (TB), an infectious disease caused by the pathogen *Mycobacterium tuberculosis (Mtb)* is becoming intensive day by day. Though presently, there

are more than 20 drugs for the treatment of TB which are used in different combinations in different conditions (http://www.tbfacts.org/tb-drugs/) still the spread of the disease is not completely contained. Growing population, worsening environmental situations, patient negligence to adhere to the regime, spreading of drug resistant strains, alarming HIV co-infection, mainly the organisms tactics to survive, etc pose a challenge against ending the existence of this menace.[1]

Apart from experimental researches, many *in silico* works are being carried out in search of new anti-TB drugs. Both structure based and ligand based strategies carried out using bioinformatics tools and databases has identified many small molecules against new and existing targets in *Mtb*. Apart from their efficacy, these molecules are expected to posses drug-like properties. For this the pharmacokinetic parameters of the molecules need to be analyzed.

Pharmacokinetics study is done by analyzing ADME and toxicity profile of the molecules. ADME (Absorption, Distribution, Metabolism and Excretion) properties are a set of physico-chemical and biochemical properties of drug molecules that portrays what our body does to the drug. Toxicity on the other hand arises due to many factors like binding of the drug to other biomolecules, harmful functional groups present in the drug, inappropriate ADME etc. The effect of toxicity can vary from mild to adverse and can even be fatal. In the field of drug development, these data are used to prioritize lead series, guide structural modifications, select compounds for *in vivo* studies, and diagnose *in vivo* assay results.[2] *In silico* prediction of ADME/T properties prior to costly experimental procedures can eliminate unnecessary testing on compounds that will ultimately fail.

Instead of considering the generalized criteria for these studies, the properties of the existing drugs for a disease can be taken as a reference to consider the new molecules for the next stage of drug development against the same disease. Based on this idea, in this study, the ADME/T properties of randomly selected 2 first line oral drugs, 2 second line injectable drugs and 2 second line oral drugs, which are part of currently used medication for TB [3] are analyzed here.

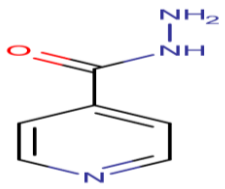
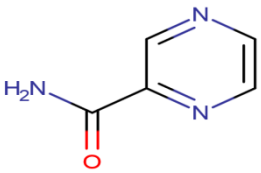
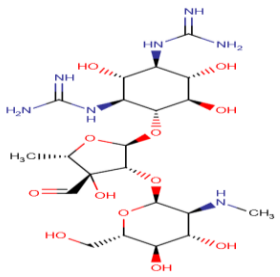
2. MATERIALS AND METHODS

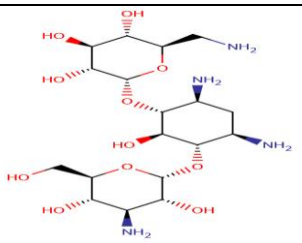
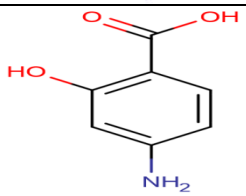
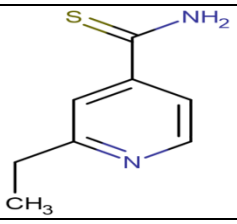
2.1 Structure and information retrieval

The DrugBank database is a cheminformatics resource that has a collection of drugs with details about their pharmacological activity, drug target, generic name and brand names, administration route, dosage etc, along with their structure. It has more than ten thousand drugs, which consists of small molecules, biotech (protein/peptide) drugs and nutraceuticals.[4](https://www.drugbank.ca/)

Current TB drugs, 2 first line oral drugs - Isoniazid and Pyrazinamide (Group 1), 2 second line injectable drugs - Streptomycin and Kanamycin (Group 2) and 2 second line oral drugs - Aminosalicylic Acid and Ethionamide (Group 4) were selected and their structures were retrieved from the database and also important informations about them were collected and tabulated in Table (1).

Table 1: Structure and details of 6 TB drugs.

TB drugs with DrugBank ID	Structure	Activity
DB00951 - Isoniazid		• Bacteriocidal & also bacteriostatic on slow growing bacteria • Specific for mycobacterium species • inhibits the synthesis of mycoloic acids, an essential component of the bacterial cell wall, by specifically inhibiting InhA, the enoyl reductase
DB00339 - Pyrazinamide		• highly specific agent and is both bacteriostatic & bactericidal • it interferes with fatty acid synthase FAS I and disables the ability to synthesize new fatty acids, required for growth and replication. • also binds to the ribosomal protein S1 (RpsA) and inhibit translation.
DB01082 - Streptomycin		• bactericidal • common antibiotic • binds to the bacterial 30S ribosomal subunit, causing misreading of t-RNA, leaving the bacterium unable to synthesize proteins vital to its growth.

DB01172 - Kanamycin		• being an aminoglycoside antibiotic is similar to kannamycin • common antibiotic
DB00233 - Aminosalicylic Acid		• for drug resistant tuberculosis • inhibits the onset of bacterial resistance to streptomycin and isoniazid. • inhibits folic acid synthesis and the synthesis of the cell wall component
DB00609 - Ethionamide		• for drug resistant tuberculosis • inhibits mycolic acid synthesis and acts in a similar fashion to isoniazid.

2.2 Calculating ADME profile

The ADME properties of the drug molecules were calculated using QikProp (v 4.0), which is an accurate, absorption, distribution, metabolism, and excretion (ADME) prediction program for ligands, from Maestro 9.8 of Schrodinger. It predicts pharmaceutically relevant properties of organic molecules and also provides the value ranges for the properties based on analysis of 95% known drugs, for comparison. The drug molecules were preprocessed using LigPrep program and provided as input to QikProp which was run in normal mode.
(QikProp, version 4, Schrödinger, LLC, New York, NY, 2014).

2.3 Calculating Toxicity profile

ADMET structure-activity relationship database (admetSAR) is an open source database that collects, curates, and manages available ADMET-associated properties data from the published literature for nearly one lakh unique compounds. It also provides an interface to query a specific chemical profile for compounds. As the ADME properties were calculated with QikProp, the 6 TB drugs were analyzed for their Toxicity profile using admetSAR. [5]
(http://www.admetexp.org.)

3. RESULTS AND DISCUSSION

3.1 Analysis of ADME profile

Physically significant descriptors and pharmaceutically relevant properties of the 6 selected TB drugs were calculated using QikProp and are listed in the Table (2).

Table 2: ADME values for the selected TB drugs.

Molecular Descriptors or Properties	DB00951 Isoniazid	DB00339 Pyrazinamide	DB01082 Streptomycin	DB01172 Kanamycin	DB00233 Aminosalicylic Acid	DB00609 Ethionamide	Range or Recommended values
stars	2	2	**15**	**12**	1	1	**0-5**
amine	**3**	**3**	**2**	**2**	1	**2**	**0-1**
amidine	0	0	0	0	0	0	**0**
acid	0	0	0	0	0	0	**0-1**
amide	0	0	0	0	0	0	**0-1**
rotor	3	2	6	4	4	3	**0-15**
rtvFG	**5**	**3**	**32**	**27**	**5**	**5**	**0-2**
CNS	-1	-1	***-2***	***-2***	-1	2	**-2(inactive) +2(active)**
mol_MW	145.204	131.177	581.579	482.487	161.2	174.304	**130-725**
dipole	4.494	3.488	10.302	**15.386**	8.026	4.526	**1.0-12.5**
SASA	365.688	335.97	1254.3	**1107.382**	368.672	409.683	**300-1000**
FOSA	200.096	170.1	**790.68**	654.434	163.368	271.719	**0.0-750**
FISA	165.593	165.87	**463.62**	**452.948**	205.304	71.905	**7-330**
PISA	0	0	0	0	0	0	**0-450**
WPSA	0	0	0	0	0	66.059	**0-175**
volume	568.622	510.877	**2128.207**	1812.789	577.565	653.785	**500-2000**
donorHB	5	5	**11.5**	**7.333**	5	3.8	**0-6**
accptHB	4.7	5.7	**23.7**	18.933	6.1	3	**2-20**
dip^2/V	0.0355	0.0238	0.0498	0.130	0.111	0.0313	**0-0.13**
ACxDN^.5/SA	0.028	0.0379	**0.0640**	0.046	0.0369	0.0142	**0-0.05**
glob	0.907	0.9199	**0.637**	**0.649**	0.909	0.889	**0.75-0.95**
QPpolrz	14.676	13.007	**75.164**	63.825	14.392	18.084	**13-70**
QPlogPC16	5.802	5.294	**26.538**	**22.012**	6.199	6.081	**4.0-18.0**
QPlogPoct	14.553	14.406	**55.968**	**44.536**	16.42	12.855	**8.0-35**
QPlogPw	13.016	14.152	44.755	33.973	14.452	9.076	**4.0-45.0**
QPlogPo/w	**-2.236**	**-2.693**	**-3.232**	**-2.433**	-1.714	0.293	**-2-6.5**
QPlogS	**2**	**2**	-5.948	-5.881	0.171	0.329	**-6.5-0.5**
CIQPlogS	**2.511**	**2.6**	**2.079**	**1.573**	**0.729**	**0.882**	**-6.5-0.5**
QPlogHERG	**-5.204**	**-5.007**	**-8.843**	**-8.659**	-3.718	-4.777	**concern below -5**
QPPCaco	**4.133**	**4.108**	**0.025**	**0.031**	27.919	128.181	**<25poor- >500 great**
QPlogBB	-0.16	-0.067	**-6.596**	**-6.044**	-0.933	0.736	**-3-1.2**
QPPMDCK	**1.776**	**1.765**	**0.006**	**0.008**	**11.44**	151.231	**<25poor- >500 great**
QPlogKp	-8.409	-8.51	**-13.613**	**-13.608**	-6.982	-6.682	**-8to-1**
IP(eV)	8.229	7.984	**6.103**	**4.915**	**6.905**	**6.779**	**7.9 -10.5**
EA(eV)	-0.114	-0.154	**2.58**	**3.177**	0.751	0.758	**-0.9-1.7**
metab	1	3	6	4	4	3	**1-8**
QPlogKhsa	-0.793	-0.787	-1.144	-0.701	-0.826	-0.497	**-1.5-1.5**
HumanOral Absorption	2	2	1	1	2	2	**1-low,2-med,3-high**
PercentHuma	**24.884**	**22.157**	**0**	**0**	42.79	66.389	**>80 is high**

nOralAbsorption							**<25poor**
SAfluorine	0	0	0	0	0	0	**0-100**
SAamideO	0	0	0	0	0	0	**0-35**
PSA	83.666	82.065	**272.677**	**250.175**	94.046	40.891	**7-200**
NandO	4	4	**19**	15	4	2	**2-15**
RuleOfFive	0	0	3	2	0	0	**max 4**
RuleOfThree	1	1	2	2	0	0	**max 3**

Note: The values of the drug molecules which falls above or below the given range are highlighted in bold

Nearing 40 descriptors/ properties were calculated for the drug molecules and each property values were compared with the provided range values and discussed below:

Stars indicate the number of property or descriptor values of the test molecule that fall outside the range of values noted from 95% known drugs. Less the number of stars, the more druglikeness of the molecule. The Star value of Streptomycin and Kanamycin are far above the given range. The number of non-conjugated amine groups (**amine**) seems to be more than the range in all the drugs except Aminosalicylic Acid. Regarding the number of amidine and guanidine groups (**amidine**), carboxylic acid groups (**acid**), non-conjugated amide groups (**amide**) and non-hindered rotatable bonds (**rotor),** all the 6 molecules have values within the ranges indicated. The number of reactive functional groups which can lead to false positives in HTS assays and to decomposition, reactivity, or toxicity problems in vivo (**rtvFG)** are seen more in the injectable drugs Streptomycin and Kanamycin having 32 and 27 respectively. The other 4 drugs have nearly 5 groups which is more than the indicated range of max 2. Regarding their activity on central nervous system (**CNS**), drugs Streptomycin and Kanamycin are inactive and Ethionamide is predicted to be active, while the other 3 drugs are predicted to be less inactive. The molecular weight (**mol_MW**) of all 6 drugs are within the range and also the volume (**volume**) which is the total solvent-accessible volume in cubic angstroms calculated using a probe with 1.4 Å radius, of all the drugs except Streptomycin are within the range. Computed dipole moment (**dipole**) and the total solvent accessible surface area (**SASA**) of all the molecules are in the normal range except Kanamycin. Streptomycin has a higher hydrophobic component of the SASA (**FOSA)** and hydrophilic component of the SASA (**FISA)** than the indicated range. Also Kanamycin shows FISA value above the range. Carbon and attached hydrogen component of the SASA (**PISA)** and weakly polar component of the SASA (**WPSA**) are null in all the drugs except Ethionamide which has WPSA value within the indicated range.

Streptomycin has nearly 11 hydrogen bond donar groups (**donorHB**) and Kanamycin has nearly 7, while the indicated maximum range is only 6. Also only Streptomycin has more number of hydrogen acceptor groups (**accptHB)** than the indicated range of 2-20. The Square of the dipole moment divided by the molecular volume (**dip^2/V**) values of all the drugs are within the indicated range. The index of cohesive interaction in solids (**ACxDN^.5/SA**), which is related to number of hydrogen bond donars and acceptors, is known to be beyond the range for Streptomycin, while others have within the range.

The Globularity descriptor (**glob)** values are high in Streptomycin and Kanamycin compared to others. The predicted polarizability (**QPpolrz)** value is nearly 75 $Å^3$ for Streptomycin, while all the other drugs have their values within the indicated range of 13-70. The predicted hexadecane/gas partition coefficient (**QPlogPC16)** value is slightly higher than the indicated range for Streptomycin and Kanamycin, while the values for predicted octanol/gas partition coefficient (**QPlogPoct**) is very higher in Streptomycin and Kanamycin than the indicated range. The predicted water/gas partition coefficient (**QPlogPw)** values of all the drugs fall within the range given. The predicted octanol/water partition coefficient (**QPlogPo/w)** values for the drugs Isoniazid, Pyrazinamide, Streptomycin and Kanamycin are below the minimum range value given, while the values of the other 2 drugs fall within the range. The predicted aqueous solubility (**QPlogS)** value of drugs Isoniazid and Pyrazinamide is 2 which is higher than the maximum range of 0.5. The conformation-independent predicted aqueous solubility (**CIQPlogS)** values of all the drugs are higher than the indicated maximum value of 0.5. Here, the first 4 drugs Isoniazid, Pyrazinamide, Streptomycin and Kanamycin have a very higher value from 1.5-2.5. The predicted concentration of IC50 value for blockage of HERG K+ channels (**QPlogHERG)** is within the indicated range for 2 drugs Aminosalicylic Acid and Ethionamide and hence are considered safe, while the other drugs have concentration below -5 which needs to be considered while calculating the therapeutic dosage.

Predicted apparent Caco-2 cell permeability (**QPPCaco)** which is expressed in nm/sec is too low for the second line injectabe drugs Streptomycin and Kanamycin apparently. For the same criterion, the first line drugs Isoniazid and Pyrazinamide have a value which is less than the minimum range value indicating that they have poor gut-blood barrier permeability while the second line oral drugs have good gut-blood barrier permeability. The predicted brain/blood partition coefficient (**QPlogBB**) is very low for the injectable second line drugs Streptomycin and Kanamycin when compared to other drugs. Surprisingly, the predicted

apparent MDCK cell permeability which is said to be a mimic for the blood-brain barrier permeability (**QPPMDCK**) seems to be poorer for all the drugs except Ethionamide. Based on the predicted skin permeability **(QPlogKp)** values, it is known that injectable drugs Streptomycin and Kanamycin do not have skin permeability capacity, while the drugs Isoniazid and Pyrazinamide have better permeability, while the drugs Aminosalicylic Acid and Ethionamide have good permeability capacity.

Only the drugs Isoniazid and Pyrazinamide have PM3 calculated ionization potential (**IP(ev))** values within the range, while others have values below the minimum indicated range. The PM3 calculated electron affinity (**EA(eV))** value is higher than the maximum range value for injectable drugs Streptomycin and Kanamycin, while the other drugs have values within the range. For all the drug molecules, the number of likely metabolic reactions (**metab**) they participate and their binding ability to human serum albumin (**QPlogKhsa)** are within the indicated range of 1 to 8 and -1.5 to 1.5 respectively.

Predicted qualitative human oral absorption (**Human Oral Absorption)** value whose calculation includes knowledge-based set of rules, including checking for suitable values of Percent Human Oral Absorption, number of metabolites, number of rotatable bonds, logP, solubility and cell permeability, is low for injectable drugs, while for the other 4 oral drugs it is medium. Predicted human oral absorption percentage (**PercentHuman-OralAbsorption)** is null for the injectable drugs Streptomycin and Kanamycin, while the second line drugs Aminosalicylic Acid and Ethionamide have better oral absorption than the first line oral drugs Isoniazid and Pyrazinamide.

All the drugs have no solvent-accessible fluorine atoms (**SAFluorine**) and amide oxygen atoms (**SAamideO**). The van der Waals surface area of polar nitrogen and oxygen atoms (**PSA**) seems to be comparatively very high for Streptomycin and Kanamycin and is beyond the given range. The number of nitrogen and oxygen atoms (**NandO**) is comparatively high in drugs Streptomycin and Kanamycin and for streptomycin it is beyond the given range. All the 4 oral drugs Isoniazid, Pyrazinamide, Aminosalicylic Acid and Ethionamide has obeyed all the 4 laws of Lipinski's rule of five (**RuleOfFive**), while the injectable drugs Streptomycin and Kanamycin has 3 and 2 violations respectively. The drugs Aminosalicylic Acid and Ethionamide obey all 3 laws of Jorgensen's rule of three **(RuleOfThree),** while Isoniazid and Pyrazinamide show one violation and Streptomycin and Kanamycin violate 2 rules.

3.2 Analysis of Toxicity Profile

AdmetSAR calculated toxicity profiles of the 6 TB drugs are given in Table (3).

Table 3: Toxicity profiles of the drugs with their prediction probability values.

Tests an properties	Isoniazid	Pyrazinamide	Streptomycin	Kanamycin	Ethionamide	Aminosalicylic Acid
Ames test	AMES toxic (0.8557)	Non AMES toxic (0.9133)	AMES toxic (0.9107)	Non AMES toxic (0.7406)	Non AMES toxic (0.9132)	Non AMES toxic (0.9388)
Carcinogenicity	N-C (0.7514)	N-C (0.9321)	N-C (0.9528)	N-C (0.9488)	N-C (0.833)	N-C (0.8045)
Biodegradation	NRB (0.981)	NRB (0.9602)	NRB (0.9821)	NRB (0.849)	NRB (0.994)	Ready biodegradable (0.6246)
hERG inhibition (predictor I)	Weak inhibitor (0.9872)	Weak inhibitor (0.9939)	Weak inhibitor (0.9924)	Weak inhibitor (0.9772)	Weak inhibitor (0.9735)	Weak inhibitor (0.9689)

N-C : Non-carcinogens; NRB - Not ready biodegradable;

As seen from the above table, the toxicity properties of the compounds are predicted with high probability values by admetSAR. Isoniazid and Streptomycin are found to be toxic in Ames test, while the other molecules were non-Ames toxic. AMES toxicity property of the molecules indicates that they are mutagenic. But all the molecules were found to be non-carcinogens and this assures the safety of using these molecules in treatment. All the 6 drugs were found to be weak inhibitors of hERG subunit of potassium ion channel which is best known for its contribution to the electrical activity of the heart that coordinates the heart's beating.

4. CONCLUSION

As search for new lead and drug candidates for TB is done widely *in silico*, the valid molecules obtained need to be evaluated for drug likeness before carrying them to the next experimental step. ADME/T profile of current TB drugs can give a better understanding of the molecules being studied. The ADME and toxicity profile of 6 TB drugs Isoniazid, Pyrazinamide, Streptomycin, Kanamycin, Aminosalicylic Acid and Ethionamide calculated from QikProp program and admetSAR tool reveals that the second line oral drugs Aminosalicylic Acid and Ethionamide and the first line oral drugs Isoniazid and Pyrazinamide have an ADME profile similar to most of the known drugs. The values of many

criteria for the second line injectable drugs have wide deviations from the known range compared. This indicates that depending on the efficacy of the molecules they can be considered for treatment and based on the ADME profile their route of administration can be decided.

The toxicity profile of the drugs portrays that only Isoniazid and Streptomycin are mutagenic, and Aminosalicylic acid is readily biodegradable. But all the drugs were found to be non-carcinogenic and also assures cardiac safety. Thus the work carried out here reveals the main ADME/T criteria and the value ranges to be considered while validating the anti-tuberculosis drug candidates.

ACKNOWLEDGEMENT

I thank Dr. Priya Swaminathan, SRM University, Chennai, Tamil Nadu, India for her contributions in carrying out this work. I thank Dr. Kailas D.Sonawane, Shivaji University, Kolhapur, Maharashtra, India and Dr. Uday M. Muddapur, B.V.B CET, Hubballi, Karnataka, India for their valuable inputs in this work.

REFERENCES

1. Kumar A, Chettiar S, and Parish T. Current challenges in drug discovery for tuberculosis. Expert Opinion On Drug Discovery, 2015; *12*: 1-4.
2. Balani S, Miwa G, Gan L , Wu J, Lee F. Strategy of Utilizing In Vitro and In Vivo ADME Tools for Lead Optimization and Drug Candidate Selection. Curr. Top. Med. Chem., 2005; 5(11): 1033-8.
3. Ambrosio L, Centis R, Sotgiu G, Pontali E, Spanevello A, Migliori G. New anti-tuberculosis drugs and regimens: 2015 update. *ERJ Open Research*, 2015; 1(1): 00010-2015.
4. Wishart DS, Knox C, Guo AC, Cheng D, Shrivastava S, Tzur D, Gautam B, Hassanali M. DrugBank: a knowledgebase for drugs, drug actions and drug targets. Nucleic Acids Res, 2008; 36: D901-6.
5. Cheng F, Li W, Zhou Y, Shen J, Wu Z, Liu G, Lee P, Tang Y. admetSAR: A Comprehensive Source and Free Tool for Assessment of Chemical ADMET Properties. J. Chem. Inf. Model., 2012; 52: 3099-3105.