Research Article

In silico Physicochemical Bioactivities and Toxicities Prediction of 3-chloro-6-arylpyridazines and 6-aryl-4,5-dihydropyridazine-3(2*H*)-thiones having Anti-tubercular Activity

Mohammad Asif[1*], Mrityunjoy Acharya[1], Lakshmayya[1] and Anita Singh[2]

[1]Department of Pharmacy, GRD (P.G) Institute of Management and Technology, 214-Rajpur Road, Dehradun, (U.K), India.
[2]Department of Pharmacy, Kumaun University, Bheemtal, Nainital, (U.K), India.

ABSTRACT

Purpose: In the present study, antitubercular activities and *in silico* physicochemical toxicities and bioactivity profile of some 3-chloro-6-arylpyridazines **(3a-d)** and 6-aryl-4,5-dihydropyridazine-3(2H)-thiones **(4a-d)** are studied. **Approach:** The compounds **(3a-d)** and **(4a-d)** were evaluated as antitubercular agents against *Mycobacterium tuberculosis* H37Rv by screening through *in vitro* Microplate Alamar Blue Assay (MABA) method. **Findings:** *In silico* physicochemical parameter revealed that the entire compounds follow Lipinski's rule-of-5 to become a "drug like" molecule. ADME (absorption, distribution, metabolism and excretions) profile prediction has shown that all the compounds can be absorbed through human intestine (HIA^+), Caco-2 cell ($Caco\text{-}2^+$) and can cross blood brain barrier (BBB^+), they all are non-substrate and non-inhibitor of p-glycoprotein. Compounds **4a-d** are inhibitor of human microsomal enzyme like CYP 450 1A2, CYP 450 2C19 and CYP 450 3A4. **Research limitations/implications:** Compounds **4a-4d** are better ligand for enzyme inhibition than **3a-d** compounds. The MIC of compounds **4a-d** and **3a** is 6.25 µg/ml. They are potent than compound **3b-d** with MIC 12.5 µg/ml. **Originality:** Toxicity prediction indicated that compounds **3a-3d** and **4a-d** are non-carcinogenic and non-mutagenic. Bioactivity prediction for compounds **3a-3d** and **4a-d** indicated better ligand as enzyme inhibitor in comparison to standard.

Key words: ADME, Anti-tubercular, *In silico*, Pyridazine, Physicochemical parameters.

Received Date : 10/01/2015
Revised Date : 13/06/2015
Accepted Date : 15/06/2015

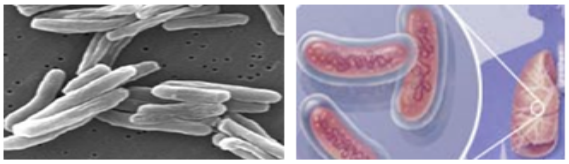
DOI: 10.5530/rjps.2015.2.7

Address for correspondence
Mr. Mohammad Asif,
Department of Pharmacy, GRD (P.G) Institute of Management and Technology, 214-Rajpur Road, Dehradun (U.K), 248009, India.
Phone No: +91-9897088910
E-mail: aasif321@gmail.com

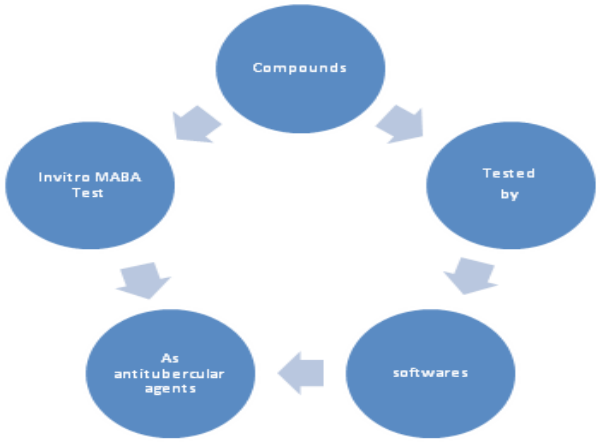
INTRODUCTION

Pyridazine is an important six member heterocyclic compound containing two nitrogen hetero atoms at 1 and 2 positions in the ring. Recently, there has been an increased interest in the pyridazine compounds because these compounds showed almost all types of pharmacological activities such as antimicrobial, antibacterial, antifungal, antiviral, analgesic, anti-inflammatory, antifeedant, antidiabetic, herbicidal, antiplatelet, anticancer, antitubercular, antidepressant, antithrombotic, diuretics, anti-HIV, antipyretics, cardio-tonic, antihypertensive, anticonvulsant, anti-asthamatic, antidepressant, anxiolytic and other anticipated activities. It is also used as chemical intermediates for drugs synthesis and agrochemicals.[1-3] Various pyridazinone and thiopyridazinones derivatives were synthesized and their anti-TB activity was tested.[4-6] Hence this feature of the ring system was tapped for the presence of any anti-TB activity. Drug resistance is a major public health problem that threatens progress made in TB care and control worldwide. Multidrug resistant tuberculosis (MDR-TB) is a form of TB that is resistant to two or more of the primary drugs like isoniazid (INH) and rifampin (RIF) used for the treatment of TB. The WHO estimated that

www.rjps.in



Mycobacterium tuberculosis



Tuberculosis is a contagious and often severe airborne disease caused by infection with *Mycobacterium tuberculosis*. TB typically affects the lungs, but it also can affect any other organ of the body. Anti-TB activities and *in silico* physicochemical toxicities and bioactivity profile of some 3-chloro-6-arylpyridazines and 6-aryl-4,5-dihydropyridazine-3(*2H*)-thiones were evaluated as using Med Chem Designer software, online software Molinspiration, ADMET SAR online tool and *in vitro* Microplate Alamar Blue Assay (MABA) method.

Graphical Abstract

there were about 310,000 cases of MDR-TB worldwide in 2011.[7] New drug synthesis is required to fight against MDR-TB strain. Majority of the synthesized drugs fails because of inadequate information about their (absorption, distribution, metabolism and excretions) (ADME), toxicity and bioactivity profile. In the present study we designed and synthesized some pyridazine **(3a-d** and **4a-d)** compounds on the basis of their physicochemical parameter ADME, toxicity and bioactivity profiles by the help of online *in silico* software Molinspiron and ADMET SAR.[8,9] All synthesized compounds were evaluated their anti-TB activity by using Microplate Alamar Blue (MABA) test.

MATERIALS AND METHOD

Structure designing, physico-chemical property and bioactivity prediction software

In present study structure was designed by Med Chem Designer software. Physicochemical property and bioactivity prediction study was done by help of online software Molinspiration.[8] ADME and toxicity was predicted by ADMET SAR online tool.[9]

In silico physico-chemical property prediction

Molinspiration provides a wide range of software tools by which we predict intestinal absorption property in compliance with Lipinski's rule-of-five. "Rule-of-five" is set of simple molecular descriptors used by Lipinski in formulating his "Rule-of-five".[7] The rule states, that most "drug-like" molecules have logP <=5, molecular weight <=500 daltons, number of hydrogen bond acceptors <=10, and number of hydrogen bond donors <=5. Molecules violating more than one of these rules may have problems with bioavailability. Physicochemical parameters play a vital role in generation and escalation of bioactivity of chemical entity. Molinspiration, web based software was used to obtain parameter such as Log P, TPSA, drug likeness. LogP (Octanol-water partition coefficient) is used in QSAR studies and rational drug design as a measure of molecular hydrophobicity.[8] Hydrophobicity affects drug absorption, bioavailability, hydrophobic drug-receptor interactions, metabolism of molecules, as well as their toxicity. LogP (octanol/water partition coefficient) is calculated by the methodology developed by Molinspiration as a sum of fragment based contributions and correction factors and used to predict the permeability of molecule across the cell membrane. Topological polar surface area (TPSA) is a very useful parameter for prediction of drug transport properties. Polar surface area is defined as a sum of surfaces of polar atoms (usually oxygens, nitrogens and attached hydrogens) in a molecule. This parameter has been shown to correlate very well with the human intestinal absorption, Caco-2 mono layers permeability, and blood-brain barrier penetration. Molecular Polar Surface Area (MPSA) is calculated based on the methodology published by Ertl *et al* (include a reference, instead)

as a sum of fragment based contributions in which O- and N-centered polar fragments are to be considered and calculated by surface areas that are occupied by oxygen, nitrogen atoms and by hydrogen atoms attached to them.[10,11] Molecular volume (MV) determines transport characteristics of molecules, such as intestinal absorption or blood-brain barrier penetration. Volume is therefore often used in QSAR studies to model molecular properties and biological activity. Method for calculation of MV developed at Molinspiration is based on group contributions. Number of Rotatable Bonds (nrotb) is a simple topological parameter that measures molecular flexibility. It has been shown to be a very good descriptor of oral bioavailability of drugs.[11,12] Rotatable bond is defined as any single non-ring bond, bounded to non-hydrogen atom. All the above mentioned results were compared with standard reference.

In silico ADME and Toxicity prediction

In silico ADME and toxicity prediction was accomplished by the help of ADMET SAR on line tool provides the latest and most comprehensive manually accumulated data for diverse chemicals associated with known Absorption, Distribution, Metabolism, Excretion and Toxicity profiles.[13,14]

In silico Bioactivity prediction

The drugs were also checked for the bioactivity by calculating the activity score for different molecular target like GPCR (G-protein coupled receptor) ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand. All the parameters were checked with the help of software Molinspiration drug-likeness score online.[8]

General Procedure for Synthesis

3-chloro-6-aryl-4,5-dihydropyridazines (3a-d) and 6-aryl-4,5-dihydropyridazine-3(2H)-thiones (4a-d)

All these compounds **(3a-3d)** and **(4a-4d)** were previously synthesized by following methods.[15-17] Compounds 3a-d were formed by reaction of compounds **2a-d** [6-phenyl-4,5-dihydropyridazin-3(2H)-one **(2a)**, 6-p-tolyl-4,5-dihydropyridazin-3(2H)-one **(2b)**, 6-(4-ethylphenyl)-4,5-dihydropyridazin-3(2H)-one **(2c)** and 6-(3,4-dimethylphenyl)-4,5-dihydropyridazin-3(2H)-one **(2d)**] with phosphorus oxychloride and compounds 4a-d were formed by reaction of compounds **2a-d** with phosphorus pentasulphide. Compounds **2a-d** has been synthesized from aroyl propionic acids **(1a-d)** [benzoyl propionic acid **(1a)**, 4-methyl benzoyl propionic acid **(1b)**, 4-ethyl benzoyl propionic acid **(1c)** and 3,4-dimethyl benzoyl propionic acid **(1d)**] by reaction with hydrazine hydrate. Compounds **(1a-d)** were formed from reaction of appropriate aromatic hydrocarbons, benzene, toluene, p-ethyl benzene, 3,4-dimethylbenzene with succinic anhydride.[15-17]

3-chloro-6-aryl-4,5-dihydropyridazine (3a-d)

A mixture of compound **2a** (0.01 mol) and phosphorous oxychloride (POCl3) (20 mL), was heated on a steam bath for 6 h. After heating, the mixture was poured on crushed ice and made alkaline by addition of sodium bicarbonate. Crude 3-chloropyridazine was collected by filtration.

3-chloro-6-phenyl-,4,5-dihydro pyridazine ***(3a)***

3-chloro-6-tolyl-4,5-dihydropyridazine ***(3b)***

3-chloro-6-p-ethylphenyl-4,5-dihydropyridazine ***(3c)***

3-chloro-6-(3,4-dimethyl)phenyl-4,5-dihydro pyridazine ***(3d)***

All the remaining acids were synthesized by analogous procedure with minor modification in temperature of reaction and use of nitrobenzene as a solvent.

6-Aryl-4,5-dihydropyridazine-3(2H)-thione (4a-d)

Compounds **(4a-d)** were prepared by refluxing 0.1 mole of **(2a-d)** dissolved in xylene, with phosphorus pentasulphide (0.1 mol) for 4 hours at a temperature of 150º. The contents were poured into a beaker and concentrated to a small volume. Yellow colored crystals were collected, crystallized from ethanol and dried at room temperature. The two groups of compounds were obtained as a result of condensation with hydrazine hydrate of aroyl propionic acid and substitution with sulphur.

6-phenyl-4,5-dihydropyridazine-3(2H)-thione ***(4a)***

6-p-tolyl-4,5-dihydropyridazine-3(2H)-thione ***(4b)***

6-p-ethyl phenyl-4,5-dihydropyridazine-3(2H)-thione ***(4c)***

3,4-dimethyl-6-phenyl-4,5-dihydrothiopyridazine-3(2H)-one ***(4d)***

All the remaining acids were synthesized by analogous procedure with minor modification in temperature of reaction and use of nitrobenzene as a solvent.

Anti-TB activity by using Microplate Alamar Blue Dye (MABA) test

The anti-TB activities of synthetic compounds **(3a-d** and **4a-d)** were assessed against *M. tuberculosis* using microplate Alamar Blue Assay (MABA). Briefly, 200 µl of sterile de-ionized water was added to all outer perimeter wells of sterile 96 wells plate to minimize evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middle brook 7H9 broth and serial dilution of compounds was made directly on plate. The final drug concentrations **3a-d** and **4a-d** tested were 100 to 0.2 µg/ml. Plates were sealed with parafilm and incubated at 37º for five days.

Table 1: Physicochemical property of the compounds (3a-d and 4a-d)

Compound	LogP	TPSA	MW	nON	nOHNH	Nviolation	Nrotb	MV
PZA	-0.711	68.878	123.11	4	2	0	1	106.003
3a	2.75	24.728	192.64	2	0	0	1	166.862
3b	3.199	24.728	206.67	2	0	0	1	183.423
3c	3.665	24.728	220.70	2	0	0	2	200.225
3d	3.575	24.728	220.70	2	0	0	1	199.984
4a	1.511	24.391	190.27	2	1	0	1	170.33
4b	1.96	24.391	204.29	2	1	0	1	186.891
4c	2.426	24.391	218.32	2	1	0	2	203.692
4d	2.336	24.391	218.32	2	1	0	1	203.452

Log P=Octanol-water partition coefficient. TPSA=Topological polar surface area. MW=Molecular weight. nON=Number of hydrogen-bond acceptors. nOHNH=Number of hydrogen-bond donors. nviolation=Number of "Rule-of-five " violation. MV=Molecular volume.

Table 2: Bioactivity score of the compounds (3a-d and 4a-d)

Compounds	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
PZA	-1.97	-1.45	-1.71	-2.87	-1.84	-1.43
3a	-0.43	-0.41	-1.28	-0.91	-0.95	-0.55
3b	-0.41	-0.47	-1.19	-0.82	-0.93	-0.56
3c	-0.24	-0.27	-1.07	-0.58	-0.69	-0.37
3d	-0.29	-0.41	-1.03	-0.63	-0.79	-0.47
4a	-1.04	-0.46	-1.59	-1.04	-1.26	-0.39
4b	-0.98	-0.51	-1.48	-0.94	-1.22	-0.41
4c	-0.76	-0.31	-1.34	-0.70	-0.96	-0.23
4d	-0.82	-0.45	-1.29	-0.75	-1.06	-0.33

GPCR= G-protein coupled receptor

After this time, 25 µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.

RESULTS AND DISCUSSION

The results of physicochemical properties (Drug-like properties) of the compounds **(3a-d** and **4a-d)** are shown in the Table 1. Physicochemical parameters were compared with reference standards. Isoniazid (INH), Streptomycin (STR) and Pyrazinamide (PZA) were used as standard. Oral dosing is still most preferable route for drug administration and therefore to get optimum therapeutic efficacy, intestinal absorption of the drug is the first criteria to be considered. Lipinski's "rule-of-five"- identified some critical property maintained precisely the general requirement for absorption of drug molecule. LogP, TPSA, MW, nON, nOHNH, nviolation, nrotb, MV data revealed that all the synthesized compounds (**3a-d** and **4a-d**) possess "drugs like property" and complying "rule-of-five". Hence all compounds may exhibit optimum absorption and bioavailability. For accurate and effective prediction of intestinal absorption, several *in vitro* methods have been developed. Among them, the most popular cell-based model for intestinal permeability is the Caco-2 cell system.[10,11] Caco-2 cell permeability, human intestinal absorption and blood brain barrier penetration data further support optimum absorption and distribution possibility of the synthesized compounds. P-glycoprotein (P-gp) activity in the apical cell membrane may limit the bioavailability of the drug molecule.[18,19] Result revealed that all the compounds were neither substrate nor the inhibitor of p-gp Table 2. ADME and Toxicity predictions are presented in the Table 3-5. Cytochrome P450 (CYP) enzymes are important enzymes for drug metabolism. The CYP enzymes are a family of heme proteins involved in the metabolism of numerous pharmacologically active compounds and can cause drug-drug-interactions with co-administered drugs as well as unwanted adverse side effects.[20] *In silico* data indicate the entire designed compounds cannot metabolize (non substrate) by CYP 450 2C9, CYP 450 2D6, and CYP 450 3A4 enzymes. All designed drugs are inhibitor of CYP

Table 3: Absorption prediction of compounds (3a-d and 4a-d)

Compound	Blood brain barrier (P)	Human intestinal absorption (P)	Caco-2 Cell permeability (P)	p-gp substrate (P)	p-gp inhibitor (P)
PZA	BBB^+ (0.9745)	HIA^+ (0.9813)	Caco-2^+ (0.7222)	NS (0.8219)	NI (0.9971)
3a	BBB^+ (0.9894)	HIA^+ (0.9950)	Caco-2^+ (0.6563)	NS (0.6760)	NI (0.7038)
3b	BBB^+ (0.9882)	HIA^+ (0.9971)	Caco-2^+ (0.6489)	NS (0.6000)	NI (0.6528)
3c	BBB^+ (0.9849)	HIA^+ (1.000)	Caco-2^+ (0.6148)	NS (0.5475)	NI (0.5070)
3d	BBB^+ (0.9855)	HIA^+ (0.9960)	Caco-2^+ (0.6713)	NS (0.5946)	NI (0.9653)
4a	BBB^+ (0.9805)	HIA^+ (0.8561)	Caco-2^+ (0.5138)	NS (0.7088)	NI (0.7472)
4b	BBB^+ (0.9755)	HIA^+ (0.9126)	Caco-2^+ (0.5090)	NS (0.6391)	NI (0.6857)
4c	BBB^+ (0.9690)	HIA^+ (0.9370)	Caco-2^+ (0.5000)	NS (0.5874)	NI (0.56672)
4d	BBB^+ (0.9606)	HIA^+ (0.8811)	Caco-2^+ (0.5220)	NS (0.6354)	NI (0.9908)

BBB^+ Indicate drug can cross blood brain barrier; HIA^+ Indicate drug can absorb through intestine; Caco-2^+ Indicate drug can cross Caco-2 cell; p-gp=p-glycoprotein; NS=Non-substrate; NI=Non inhibitor; P=Probability.

Table 4: Metabolism prediction of compounds (3a-d and 4a-d)

Compound	CYP 450 2C9 S (P)	CYP 450 2D6 S (P)	CYP 450 3A4 S (P)	CYP 450 1A2 I (P)	CYP 450 2C9 I (P)	CYP 450 2D6 I (P)	CYP 450 2C19 I (P)	CYP 450 3A4 I (P)
PZA	NS (0.8861)	NS (0.8760)	NS (0.7754)	NI (0.8791)	NI (0.9545)	NI (0.9731)	NI (0.9547)	NI (0.9697)
3a	NS (0.8402)	NS (0.7780)	NS (0.5882)	I (0.8599)	NI (0.6559)	NI (0.8160)	NI (0.5373)	NI (0.9678)
3b	NS (0.7868)	NS (0.7794)	NS (0.5412)	I (0.8108)	NI (0.6539)	NI (0.8158)	NI (0.5221)	NI (0.9553)
3c	NS (0.8054)	NS (0.7672)	NS (0.5729)	I (0.8428)	NI (0.5765)	NI (0.7388)	NI (0.6339)	NI (0.9665)
3d	NS (0.8069)	NS (0.7795)	NS (0.5366)	I (0.7144)	NI (0.5975)	NI (0.7568)	NI (0.6488)	NI (0.9415)
4a	NS (0.8299)	NS (0.7468)	NS (0.6880)	I (0.8033)	NI (0.6286)	NI (0.6357)	I (0.5658)	I (0.6391)
4b	NS (0.7738)	NS (0.7465)	NS (0.6511)	I (0.7845)	NI (0.6348)	NI (0.6554)	I (0.6103)	I (0.6768)
4c	NS (0.7929)	NS (0.7438)	NS (0.6828)	I (0.7736)	NI (0.5505)	NI (0.6121)	I (0.6677)	I (0.5947)
4d	NS (0.7961)	NS (0.7519)	NS (0.5783)	I (0.6901)	NI (0.6003)	NI (0.5838)	I (0.7031)	I (0.6665)

P=Probability; I=Inhibitor; NI=Non-inhibitor; S=Substrate; NS=Non-substrate.

450 1A2 and non inhibitor of CYP 450 2C9; CYP 450 2D6, CYP 450 2C19, CYP 450 3A4, but exceptionally compounds **4a-d** are inhibitor of CYP 450 2C9, CYP 450 2D6, CYP 450 2C19, CYP 450 3A4. The bacterial reverse mutation assay (Ames test) is a biological assay used to assess the mutagenic potential of chemical compounds. *In silico* AMES toxicity, carcinogenicity and rat LD50 data indicate all compounds were non mutagenic (non AMES toxic), non-carcinogenic and rat LD50 value was between the range of 2.5670-2.3567 mol/kg.[21] Bioactivity score was checked for different target like GPCR, Ion channel, Kinase, Nuclear receptor, Protease and Enzymes. Result indicate compound **3a** has better ion channel modulation property (Ion channel modulation >GPCR ligand >Enzyme inhibition >Nuclear receptor ligand >Protease inhibitor >Kinase inhibitor)

Table 5: Toxicity prediction of compounds (3a-d and 4a-d)

Compound	AMES Toxicity (Probability)	Carcinogens (Probability)	Rat acute toxicity (LD50, mol/kg)
PZA	Non AMES toxic (0.9133)	Non-carcinogen (0.9321)	1.8145
3a	Non AMES toxic (0.7230)	Non-carcinogens (0.7624)	2.5665
3b	Non AMES toxic(0.7127)	Non-carcinogen (0.7359)	2.5081
3c	Non AMES toxic (0.6855)	Non-carcinogens (0.6425)	2.5664
3d	Non AMES toxic (0.7533)	Non-carcinogen (0.7023)	2.3567
4a	Non AMES toxic (0.5211)	Non-carcinogens (0.8298)	2.5492
4b	Non AMES toxic (0.5322)	Non-carcinogen(0.8140)	2.5194
4c	Non AMES toxic (0.5677)	Non-carcinogens (0.7334)	2.5670
4d	Non AMES toxic (0.7880)	Non-carcinogen (0.7880)	2.5504

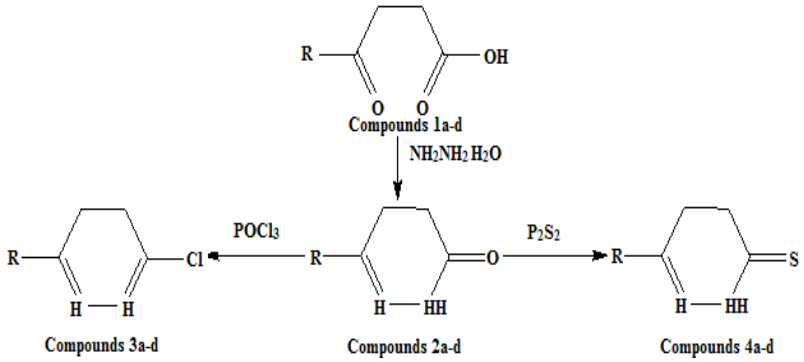


Figure 1: Protocol for the synthesis of pyridazine compounds

R=Phenyl, Methylphenyl, Ethylphenyl, 3,4-dimethylphenyl.

where as compound **3b-d** are better ligand for GPCR receptor than other targets (GPCR ligand >Ion channel modulation >Enzyme inhibition >Nuclear receptor ligand >Protease inhibitor >Kinase inhibitor). Compound **4a-d** are better ligand for enzyme inhibition as per *in silico* data prediction (Enzyme inhibition >Ion channel modulation >Nuclear receptor >GPCR ligand >Protease inhibitor >Kinase inhibitor). Furthermore data was compared with standard references and data indicate that all compounds **(3a-d** and **4a-d)** are better bioactivity score than reference standard PZA (Table 2).

Pyridazinone compounds **(3a-d** and **4a-d)** are described in this study, and a reaction sequence for the preparation is outlined in Figure 1. The title compounds **3a-d** and **4a-d** were prepared by reacting appropriate aromatic hydrocarbons with succinic anhydride in presence lewis acid (anhydrous $AlCl_3$) by Friedal-Craft acylation to formed appropriate aroyl-propoinic acids **(1a-d)**. Compounds **(1a-d)** were reacted with hydrazine hydride in presence of sodium acetate and ethanolic solution (reaction time were varied from 4 to 6 h) to formed compounds **2a-d**. The synthesis of 6-arylpyridazinones **(2a-d)** reacted with phosphorus oxychloride to give 3-chloro-6-arylphenyl-pyridazines **(3a-d)**. The 6-aryl-4,5-dihydro pyridazines **(2a-d)** followed by substitution with sulphur by reaction with phosphorus pentasulphite to yield the compounds **(4a-d)**. All the final compounds were structurally confirmed on the basis of NMR and IR spectral data, (explanation of the procedure is not necessary, discuss only the results) and the final synthesized compounds were evaluated for anti-TB activity. All the compounds **3a-d** and **4a-d** were screened for anti-TB activity. The anti-TB activity was evaluated by the MABA test. Out of the ten compounds subjected to anti-TB screening by the MABA method and showed significant activity. The result indicate that MIC of compound **4a-d** and 3a is 6.25 μg/ml, potent than compound **3b-d** with MIC 12.5 μg/ml and results are represented in the Table 6.

Table 6: Anti-tubercular activity of synthetic compounds (3a-d and 4a-d)

Compounds (3a-d)	MIC (µg/ml)	Compounds (4a-d)	MIC (µg/ml)
3a	6.25	**4a**	6.25
3b	12.5	**4b**	6.25
3c	12.5	**4c**	6.25
3d	12.5	**4d**	6.25

Reference standard drug, pyrazinamide (PZA) MIC Value 3.12 µg/ml

CONCLUSION

From present study entire designed compounds fulfill the parameter to become a "drug like" molecule, having absorption property through human intestine (HIA^+), Caco-2 cell and can penetrate blood brain barrier (BBB^+) and non-inhibitor and non-substrate to p-gp. All compounds are inhibitor of CYP 450 1A2 and compound **4a-d** are inhibitor of CYP 450 2C9, CYP 450 2D6, CYP 450 2C19, and CYP 450 3A4. Compound **4a-d** can cause drug-drug interaction with co-administered drug metabolized by the enzymes. All the drugs were non mutagenic, non-carcinogenic and LD50 value more than PZA. Compound **4a-d** are better enzyme inhibition score and compound **3b-d** are better ligand for GPCR receptor except **3a**. From anti-tubercular activity study result indicate that compounds **4a-d** having sulfur group in their side chain are more potent anti-TB compound than compounds having chlorine group in their side chain (except compound **3a**). Results indicated that pyridazine compound with sulfur groups **(4a-d)** were more potent anti-TB agents than compounds containing chlorine (**3b-d**, except **3a**) group at their side chain. Pyridazines are noteworthy for their physiological and biological importance. Medicinal chemists are working on pyridazines due to their wide range of biological activities. A further extensive study is required to explore other biological potential of pyridazines.

ACKNOWLEDGMENTS

Authors are grateful to the Chairman, GRD (PG) IMT, Dehradun, India for performing synthesis and Department of Microbiology, Maratha Mandal's N.G.H. Institute of Dental Sciences and Research Centre, Belgaum, India for evaluating anti-TB activity of the compounds.

REFERENCES

1. Asif M. General study of pyridazine compounds against cyclooxygenase enzyme and their relation with analgesic, anti-inflammatory and anti-arthritic activities. Chron Young Sci. 2010; 1(3): 3-9.
2. Asif M, Singh A. Exploring potential, synthetic methods and general chemistry of pyridazine and pyridazinone: A brief introduction. Int. J Chem Tech Res. 2010; 2(2): 1112-28.
3. Asif M, Singh A, Siddiqui AA. Effect of pyridazine compounds on cardiovascular system. Med Chem Res. 2012; 21(11): 3336-46.
4. Asif M, Singh A, Lakshmayya. *In-vivo* anticonvulsant and *in vitro* Antitubercular activity of methyl indole derivative of some 6-aryl-4,5-dihydropyridazin-3(2H)-ones their expected anticonvulsant mechanisms. Iranian J Pharm Sci. 2013; 9(1): 67-80.
5. Asif M, Singh A, Lakshmayya. *In vivo* Anticonvulsant and *In vitro* Antimycobacterial activities of 6-aryl pyridazine-3(2H)-one derivatives. Am J Pharmacol Sci. 2014; 2(1): 1-6.
6. Asif M, Singh A, Lakshmayya, Husain A, Siddiqui AA. Anticonvulsant and antitubercular activities of 6-Phenyl/Biphenyl-4-yl-2-[2-(pyridin-2-ylamino)-ethyl]- and 6-(Biphenyl-4yl)-2-(2N-subtituted amin-1-yl)-ethyl derivatives of 4,5-dihyropyridazin-3(2H)-one. Lett Drug Design and Discov. 2013; 10(7): 651-60.
7. World Health Organization. Global tuberculosis control report; 2012.
8. Molinspiration cheminformatics. Nova ulica, SK-900 26 Slovensky Grob, Slovak Republic. [cited 2012 July 3], Available from: URL: http://www.molinspiration.com.
9. Cheng F, Li W, Zhou Y, Shen J, Wu Z, Liu G, *et al*. Admetsar: a comprehensive source and free tool for evaluating chemical ADMET properties. J Chem Inf Model. 2012; 52(11): 3099-105.
10. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Delivery Rev. 1997; 23: 4-25.
11. Ertl P, Rohde B, Selzer P. Fast Calculation of Molecular Polar Surface Area as a Sum of Fragment-Based Contributions and Its Application to the Prediction of Drug Transport Properties. J Med Chem. 2000; 43(20): 3714-7.
12. Veber DF, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD. Molecular Properties That Influence the Oral Bioavailability of Drug Candidates. J Med Chem. 2002; 45(12): 2615-23.
13. Cheng F, Li W, Zhou Y, Shen J, Wu Z, Liu G, *et al*. Admetsar: a comprehensive source and free tool for evaluating chemical ADMET properties. J. Chem. Inf. Model. 2012; 52(11): 3099-105.
14. Artursson P. Cell cultures as models for drug absorption across the intestinal mucosa. Crit Rev Ther Drug Carrier Syst. 1991; 8(4): 305-30.
15. Islam M, Siddiqui AA, Rajesh R, Bakht A, Goel S. Synthesis and antimicrobial activity of some novel oxadiazole derivatives. Acta Pol Pharm and Drug Res. 2008; 65(3): 353-62.
16. Banarjee PS, Sharma PK, Nema RK. Synthesis and Anticonvulsant Activity of Pyridazinone Derivatives Inter. J Chem Tech Res. 2009; 1(3): 522-5.
17. Husain A, Drabu S, Kumar N, Alam MM, Ahmad A. Synthesis and biological evaluation of some new pyridazinone derivatives. J. Enz Inhibit and Med Chem. 2011; 26(5): 742-8.
18. Tsuji A, Tamai I. Carrier-mediated intestinal transport of drugs. Pharm Res. 1996 Jul; 13(7): 963-77.
19. Lin JH, Lu AY. Role of pharmacokinetics and metabolism in drug discovery and development. Pharmacol Rev. 1997; 49(4): 403-49.
20. Laura M. *In vitro* and *in silico* methods to predict cytochrome P450 enzyme inhibition University of Eastern Finland, Faculty of Health Sciences, Publications of the University of Eastern Finland. Dissertations in Health Sciences 2012; 108: 5.
21. Modi S, Li J, Malcomber S, Moore C, Scott A, White A, *et al*. Integrated *in silico* approaches for the prediction of Ames test mutagenicity. J Comput Aided Mol Des. 2012; 26(9): 1017-33.