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Research Article

DESIGN, SYNTHESIS, QUANTUM MECHANICAL CALCULATION AND *INSILICO* SCREENING OF BENZOYLATED ISONIAZID HYDRAZONE DERIVATIVES AS MYCOBACTERIUM TUBERCULOSIS ENOYL REDUCTASE INHIBITORS

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ABSTRACT

A series of novel isonicotinic acid hydrazide derivatives were synthesized and benzoylated. The compounds were purified and characterized by IR, NMR, Mass spectral studies. Drug likeliness assessment conferred all compounds obeying rule of thumb. Binding energy calculation revealed compounds having electron donating groups having high HOMO values (ISB9, ISB10, BI9, and BI10). Structure based drug design was performed for 48 structures having various heterocyclic moieties, INH, and 10 compounds which are reported in literature as active Inh A inhibitors.

Docking and scoring study unravels that our compounds were having 1-5 H-bonding interactions with Tyr158, Ile95, Ile194, Pro 193 H2O 856, 502, 563, and 552. Hydrophobic interactions of compounds were found to be with Ala157, Gly96, Ile215, Leu218, Met103, Tyr158, Phe97, Pro156, Ser123 and lys165. All the compounds have good docking score compared to INH. In vitro anti oxidant activity by nitric oxide scavenging assay was also performed and the results inferred compounds having good docking score are having good activity. The compounds having electron donating groups are having good activity.

Keywords: InhA, Hydrazide derivatives, enoyl reductase inhibitors, Docking, Benzoylation.

INTRODUCTION

Tuberculosis is an acute but more frequently a chronic communicable disease spread through the air caused by several species of mycobacteria collectively termed the tubercle bacilli called Mycobacterium tuberculosis. Not only has the unfortunate synergy between TB and HIV increased the already high human life too [1], but also the emergence of multidrug resistant strains [2,3], which are both difficult and very costly to treat, poses an additional public health hazard and further roadblock in effective control of development of antimycobacterial agents there remains as much need for new drug discovery.

A chemical modification of an existing TB drug must show strong mycobacterial activity in vitro and in vivo with low toxicity and good bioavailability. Many INH derivatives have been described in the literature [4-6], some of them with improved MIC values against *M. tb*. A few of these derivatives are mentioned by computer automated structure evaluation and in vitro testing. 136 INH –derivatives were analysed. Most of the INH-derivatives are N-monosubstituted, one type constituted of alkyl substituted derivatives whereas the others included N-hydroxyallylsubstituted derivatives. Some of these drugs had minimum inhibitory concentration values better than INH.

It has been shown that the para-substituted isonicotinic benzylidene hydrazines retained a degree of activity against *M.tb* and were less toxic than the INH alone. An example of an N-monosubstituted drug is N-isonicotinyl-N-

(salicylidene) hydrazine which retained activity against *M.tb.* another derivative was a lipophilic Schiff base, N2-cyclohexylidenyl isonicotinic acid[6] hydrazide that was shown to block acetylation of INH. Literature review concluded that in order to improve the activity of INH, derivatisation should be directed towards increasing the lipophilicity of the compound, thereby slowing and prolonging the release of INH.

2.1 GENERAL

All the chemicals and reagents were purchased from Merck, Sd fine chem. Ltd, Himedia, SRL. All the solvents and starting materials were purified by standard methods. Melting points were determined in DBK programmed melting point apparatus and expressed in ^{0}C. Reactions were monitored by TLC using aluminium backed plates coated with silica gel 60 (MERCK). The chromatograms were visualized under UV light (254 nm) and stained with iodine. The IR spectra were recorded on schimadzu FT-IR affinity spectrophotometer using DRS-8000 and expressed in cm^{-1}. 1H NMR spectra was recorded on a Bruker Ac-80 MHz, Avance 400 MHz NMR spectrophotometer. The chemical shifts were reported as parts per million (δ ppm), using tetramethyl silane as internal standard. The solvents used for NMR are DMSO and $CDCl_3$. Mass spectrum was recorded on Apex mass spectrum system. The spectral characterization was done by referring to the basic principles provided in text books. Quantum mechanical calculations for the synthesized compound were done on Argus lab 4.0.1. Druglikeliness was performed in molinspiration of cheminformatics and ALOGPS 2.1. Docking was performed using Schrodinger 2010 (maestro 9.1) on Dell Precision T-1500 workstation (Intel(R) Core(TM) i7 CPU 860 @ 2.80 GHz 2.79 GHz; 12.0 GB Ram, 1 TB Hard disk).The In vitro Antioxidant activity was determined using Schimadzu UV-Visible spectrophotometer.

2.2 SYNTHESIS OF COMPOUNDS

2.2.1 General procedure for synthesis of Isonicotinic acid hydrazide (INH):

The mixture of isonicotinic acid (1.23 g, 0.01mol) and ethanol (10 mL) was refluxed with sulfuric acid (2 mL) at 60-70 0C for 3 h. Then the reaction mixture was added to 200 mL of ice cold water and excess of acid was neutralized by a saturated solution of sodium bicarbonate[7]. The crude ester was extracted with ether. The ether layer was separated and ester of isonicotinic acid was obtained on evaporation of ether layer. The resulting product (1.6 mL, 0.01mol) was dissolved in 10 mL of ethanol and hydrazine hydrate (0.583 mL, 0.012mol) then the reaction mixture was refluxed for 2 hrs, cooled to room temperature and the precipitated solid was recrystallized from ethanol.

2.2.2 General procedure for the synthesis of isonicotinohydrazide Schiff bases (ISB1-ISB12)

To the reaction mixture of isonicotinicacid hydrazide (1.37 g, 0.01 mol) and appropriate ketone (0.01 mol), ethanol 30 mL and drops of glacial acetic acid was added. The reaction mixture was refluxed for about 0.5-8 h. It was then cooled at room temperature to obtain precipitate[8-10]. The precipitate obtained was filtered washed with water and recrystallized from ethanol.

2.2.3 General procedure for the synthesis of N1-benzoylated isonicotinohydrazide Schiff bases (BI5, BI7)

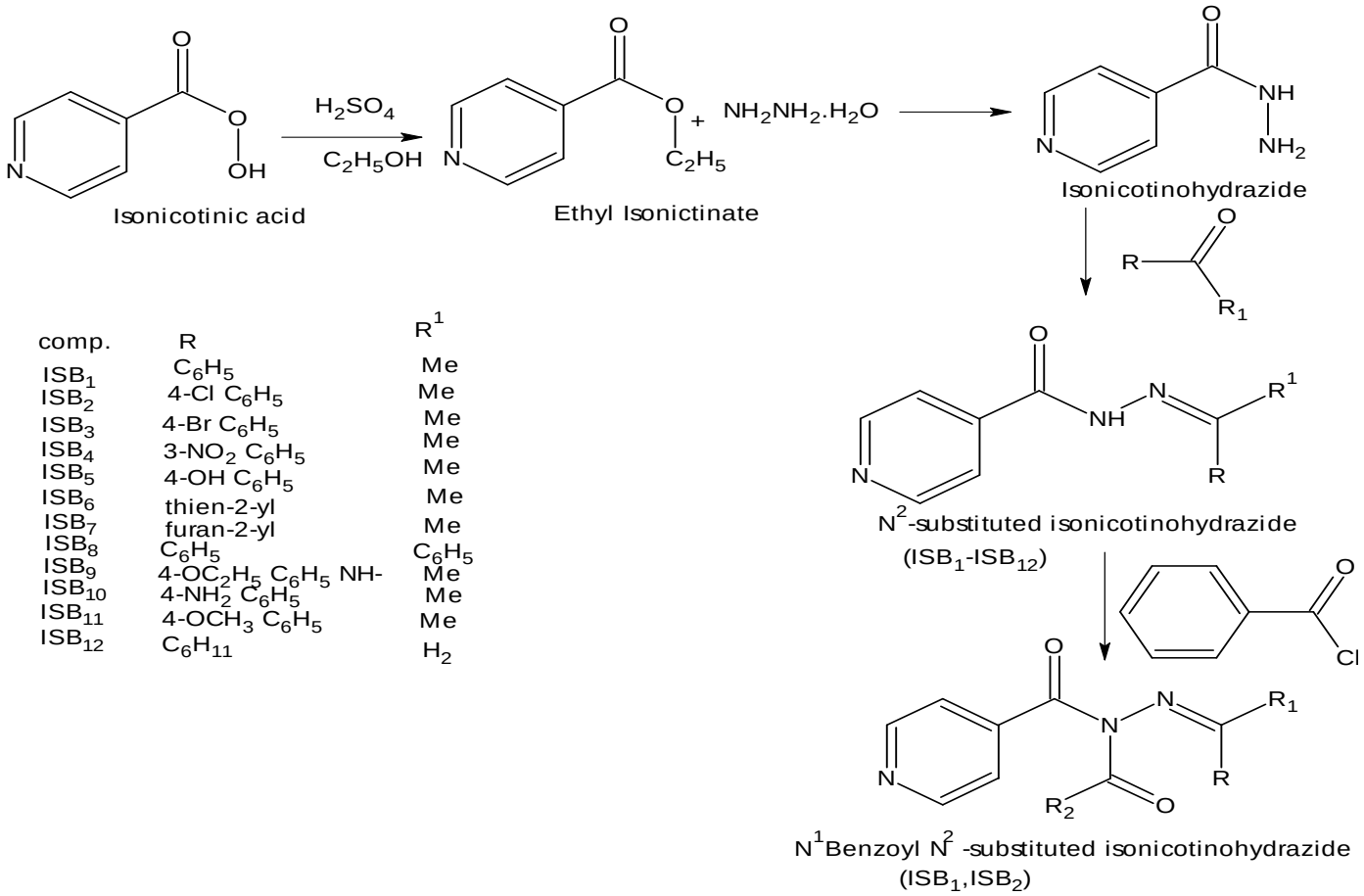
The Schiff base ISB5 and ISB7 (0.01 mol) was dissolved in 10 mL of dichloromethane and 5 mL of ethanol and the reaction mixture was taken in a flat bottomed flask, to which an equimolar amount of benzoyl chloride (1.16 mL, 0.01 mol) was added slowly with stirring for 0.5 h and refluxed at 60-70 ^{0}C for 2 h. The crude products were separated out by evaporating the solvents. The compounds BI5, BI7 were washed thoroughly with water and dried. Ethanol: water (7:3) was used as a solvent for recrystallization[11].

2.3 Drug likeliness

The Lipinski parameters were calculated by using online software Molinspiration of cheminformatics and ALOGPS 2.1. The structures of the molecules were drawn using java editor of the respective softwares and the drug likeliness parameters were calculated and tabulated in Table No 2.

2.4 Binding energy calculation

The structure of the compounds was drawn using Marwin sketch and hybridization is changed in Argus lab 4.0.1. The 3D structures of the compounds were geometry optimized using Austin model-1 (AM1) semi-empirical QM method. The Highest Occupied Molecular Orbital (HOMO) and Lowest Occupied Molecular Orbital (LUMO) energy values were estimated using Hamiltonian Parameterized method 3 (PM3) and closed shell Restricted Hartree – Fock - Single Consistent Field (RHF-SCF) methods[12,13]. HOMO and LUMO surfaces



comp.	R	R^1
ISB_1	C_6H_5	Me
ISB_2	4-Cl C_6H_5	Me
ISB_3	4-Br C_6H_5	Me
ISB_4	3-NO_2 C_6H_5	Me
ISB_5	4-OH C_6H_5	Me
ISB_6	thien-2-yl	Me
ISB_7	furan-2-yl	Me
ISB_8	C_6H_5	C_6H_5
ISB_9	4-OC_2H_5 C_6H_5 NH-	Me
ISB_{10}	4-NH_2 C_6H_5	Me
ISB_{11}	4-OCH_3 C_6H_5	Me
ISB_{12}	C_6H_{11}	H_2

Scheme of Synthesis

were visualized using a contour value of 0.05 in opaque mode using blue and red for positive and negative phase of the orbital in space. The estimated values of the energies of the tested compounds were given in the table no 3.

2.5 Protein-Ligand Interactions

2.5.2 Docking in Schrodinger maestro 9.1.

2.5.2.1 Protein preparation

The crystal structure of the Mycobacterium tuberculosis enoyl reductase (InhA) (PDB ID: 2H7M) has been downloaded from RCSB protein data bank. All bonds in the structure were assigned, including het groups (Het groups include ligands, metal ions, and cofactors) were added to all atoms in the structure, Selenomethionines were converted (MSE) to methionines (MET)[14-17], a Prime refinement was performed to place and optimize the missing side chains and missing loops. The water molecules 502, 552, 563, 856[14-19] which were found to be important for ligand protein interaction during preliminary docking studies were retained in the protein and was subjected to pre-process. Ionization states were generated at PH of 7±4 and the protein chain having lowest penalty was selected. The pre-processed protein was subjected to energy minimization using user defined OPLS_2005. The protein was then saved as *mae file.

2.5.2.2 Ligand preparation

Ligprep option was used to convert input 2D or 3D structures into corresponding low energy 3D structures. The ionization states for the ligands were generated at a PH 7±2 and Desalt was performed to remove any extra molecules or counter ions.

2.5.2.3 Receptor grid generation

Receptor grid generation was done with scaling factor 1 and partial cut off charge 0.25.A grid box of 20*20*20 A0 around the co-crystallized ligand was generated.

2.5.2.4 Ligand docking and scoring

GLIDE docking was done on XP extra precision mode with flexible docking. Docking simulations was performed in (Intel(R) Core(TM) i7 CPU 860. The compounds docked by XP were ranked based on affinity with the protein and were

studied in terms of glide score (G score), Lipophilic Evd W, H Bond, Rotational penalty.

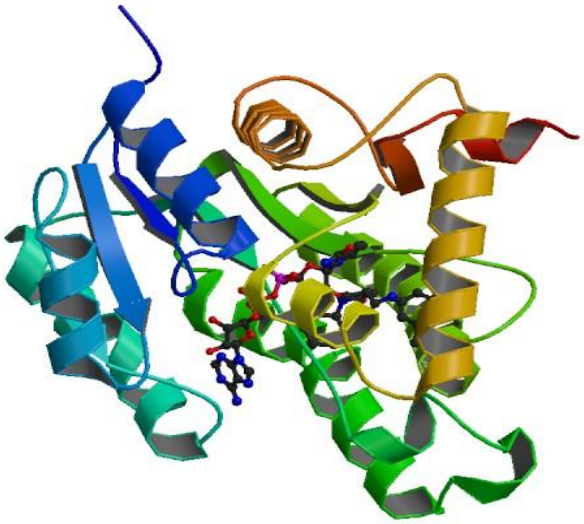


Figure: The image of protein (PDB ID-2H7M) downloaded from PDB

2.6 Pharmacological activity (Nitric oxide scavenging assay)

To 100 µM of test or standard compound dissolved in 1 mL of di methyl sulphoxide, 1mL of sodium nitroprusside (10 mM) in phosphate buffer was added and incubated at 37^{0}C for 150 min. Then to the reaction mixture 1mL of Griess reagent was added and the absorbance was measured at 546 nm[20,21]. The experiment was performed in duplicate and the average of both was taken. Then the scavenging ability was expressed as a percentage and was calculated using the following formula.

% Scavenging = [(Ac - As)]/Ac ×100

Where: As = the absorbance of the test sample

Ac = the absorbance of the control.

3. RESULTS AND DISCUSSION

3.1 Synthesis

The series of Schiff bases and their benzoylated derivatives were characterized by melting point, Rf values, IR, 1HNMR and mass spectroscopy[22]. All the relevant data regarding characterization of compounds is given Table No 1.

N1-(1-phenyl ethylidene) isonicotinohydrazide (ISB1)

IR bands (ν cm-1)3169.04 (NH amide), 3051.39 (Ar C-H str),1699.29 (C=O str), 1666.43- (C=N str),1544.98 (C=C str), 1452.40 (CH3 bend), 758.02 (Ar CH bend).

N1-(1-(4-chlorophenyl) ethylidene) isonicotinohydrazide (ISB2)

IR bands (ν cm-1)3188.33 (NH amide), 3034.03 (Ar C-H str),1678.07 (C=O str), 1653 (C=N str),1548.84 (C=C str), 1394.53 (C-N str),688.59 (C-Cl str), 752.24 (Ar CH bend).

N1-(1-(4-bromophenyl) ethylidene) isonicotinohydrazide (ISB3)

IR bands (ν cm-1)3184.4 (NH amide), 3034.03 (Ar C-H str),1672.28 (C=O str), 1616.35 (C=N str),1548.84 (C=C str), 1394.53 (C-N str).1H NMR (δ)11 (s,1H,NH), 8.77 (d,2H,ArH), 7.83-7.81 (d,2H,ArH), 7.67-7.61 (d,2H,ArH), 7.54-7.51 (d,2H,ArH), 2.37 (s,3H, CH3).Mass (m/z)318.1 (m/z), 319.2 (M+H+)

N1-(1-(3-nitrophenyl) ethylidene) isonicotinohydrazide (ISB4)

IR bands (ν cm-1)3184.90 (NH amide), 3034.03 (Ar C-H str),1672.28 (C=O str), 1643.35 (C=N str),1531.48 (C=C str), 1394.53 (C-N str),1492.90 (C-NO2 str), 800.46 (Ar CH bend).

N1-(1-(4-hydroxyphenyl)ethylidene)isonicotinohydrazide (ISB5)

IR bands (ν cm-1)3288.63 (NH amide, 3030.17 (Ar C-H str),1697.36 (C=O str), 1666.56 (C=N str),1535.34 (C=C str), 1392.61 (C-N str),1184.29 (C-O str),742.59 (OH bend). 1H NMR (δ)10.8 (s,1H,NH), 5.5(s,1H,OH), 8.75-8.71 (d,2H,ArH), 7.79-7.78 (d,2H,ArH), 7.61-7.59 (d,2H,ArH), 6.60-6.58 (d,2H,ArH), 2.26 (s,3H, CH3).

N1-(1-(thien-2-yl)ethylidene) isonicotino hydrazide(ISB6)

IR bands (ν cm-1)3174.83 (NH amide str), 3061.03 (Ar C-H str), 1666.5 (C=O str), 1597.06 (C=N str), 1546.91 (C=C str), 1155.36 (C-S str),1396.46 (C-N str), 833.25 (Ar CH bend).

N1-(1-(furan-2-yl)ethylidene) isonicotino hydrazide(ISB7)

IR bands (ν cm-1)3242.34 (NH amide), 3043.67 (Ar C-H str),1670.35 (C=O str), 1598.99 (C=N str),1535.34 (C=C str), 1153.43 (C-O str), 1454.33 (CH3 bend). 1H NMR (δ)11 (s,1H,NH), 8.76 (d,2H,ArH), 7.79-7.87 (d,2H,ArH), 7.7-7.65 (d,1H,ArH), 7 (t,1H,ArH),6.6-6.5 (d,1H,ArH), 2.31 (s,3H, CH3).Mass (m/z)230.2 (M+H+), 231.2 (M+2), 252.2(M+Na+).

N1-(Diphenyl methylene) isonicotino hydrazide(ISB8)

IR bands (ν cm-1) 3199.12 (NH amide), 3034.03 (Ar C-H str), 1697.36 (C=O str), 1666.5 C=N str), 1554.63.26 (C=C str).

N1-(1-(4-ethoxyphenylamino)ethylidene)isonicotino hydrazide (ISB9)

IR bands (ν cm-1)3427.51 (NH str) ,3199.12 (NH amide),3074.53 (Ar C-H str), 1653 (C=O str), 1606.7 (C=N str), 1510.26 (C=C str), 1409.96 (CH3 bend), 837.11 (Ar H bend).

N1-(1-(4-aminophenyl)ethylidene)isonicotinohydrazide (ISB10)

IR bands (ν cm-1)3503 (NH2 str, 3242.34 (NH amide),3045.60 (Ar C-H str), 1687.71 (C=O str),1643.36 (C=N str), 1357.53 (NH2 bend).

N1-(1-(4-methoxyphenyl)ethylidene)isonicotino hydrazide(ISB11)

IR bands (ν cm-1)3215.34 (NH amide), 3032.2 (Ar C-H str),1653.0 (C=O str), 1606.7 (C=N str), 1182.86 (C-O str).

N1-cyclohexylidene isonicotinohydrazide (ISB12)

IR bands (ν cm-1)

3215.34 (NH str), 3032.10 (Ar C-H str), 2939.52, 2860.43 (CH3 str), 1690 (C=O str), 1641.42 (C=N str), 1531.48 (C=C str), 1450.47 (CH2 bend).1H NMR (δ) 10.9 (s, 1H,NH), 8.73-8.76 (d,2H,ArH), 7.74-7.73 (d,2H,ArH), 2.38 (s,2H,CH2), 2.34-2.31 (t,2H,CH2),1.68 (s,2H,CH2), 1.6 (s,4H, CH2).

N - benzoyl - N1- (1 - (4 - hydroxy phenyl) ethylidene) isonicotino hydrazide(BI5)

IR bands (ν cm-1)3082.25 (Ar C-H str), 1678.07 (C=O str),1606.70 (C=N str), 1531.48 (C=C str),1348.24 (OH bend). 1H NMR (δ) 5.42 (s,1H,OH), 7.5 -7.64 (m,5H,ArH), 7.84-7.86 (d,2H,ArH), 7.94-7.96 (d,2H,ArH), 8.29-8.31 (d,2H,ArH), 6.86-6.902 (d,2H,ArH) 2.33 (s,3H, CH3).Mass(m/z)401.2 (M+H+)

N – benzoyl - N1 - (1 - (furan – 2 - yl) ethylidene) isonicotino hydrazide (BI7)

IR bands (ν cm-1)2983.88 (Ar C-H str), 1703.14 (C=O str), 1693.50 (C=O str), 1529.55 (C=N str), 1510.26 (C=C str), 1456.26 (CH3 bend). 1H NMR (δ) 8.71-8.74 (d,2H,ArH), 7.98-7.99 (d,2H,ArH), 7.51-7.78(m,5H,ArH), 6.9 (t,1H,ArH),6.4-6.5 (d,2H,ArH), 2.26 (s,3H, CH3).

Drug likeliness characterization indicated all the compounds obeying Lipinski's rule of five and veber's rule of less than 10 rotatable bonds. TPSA of all compounds is also less than 120 A^0.

Table 1: Physical and analytical data of synthesized compounds (ISB1- ISB12).

Code	Physical state	R_f	m.r (^{0}C)	Yield (%)
ISB1	Cream colour crystals	0.65[a]	141-142	85
ISB2	White powder	0.69[a]	238-240	85.3
ISB3	White crystals	0.71[a]	249	85.7
ISB4	White crystals	0.65[a]	210-212	83
ISB5	Pale yellow crystals	0.56[a]	256	81
ISB6	Pale yellow powder	0.63[a]	179-180	73
ISB7	Light brown crystals	0.42[a]	183-184	75
ISB8	Light brown crystals	0.73[a]	197	58
ISB9	Brown crystals	0.6[a]	Above300	50
ISB10	Yellow crystals	0.4[a]	202-203	84.3
ISB11	White powder	0.67[a]	179-181	74
ISB12	White crystals	0.65[a]	153-155	63
BI5	Yellow solid	0.5[b]	245-247	55
BI7	Yellow solid	0.48[b]	198-200	49

Rf : retention factor ; m.r : melting point ; a = acetone : pet ether (9:1) ; b = toluene : ethyl acetate (6:4).

Table 2: Drug likeliness profile of synthesized compounds

Compd. code	M.wt	M.V	TPSA	CLogP	ALog P	HBA	HBD
ISB1	239.28	222.5	54.35	1.72	2.21±0.51	4	0
ISB2	273.7	236.04	54.35	2.401	2.83±0.51	4	0
ISB3	318.17	240.09	54.35	2.53	2.95±0.55	4	1
ISB4	284.3	245.8	100.17	1.68	1.99±0.41	7	1
ISB5	255.27	230.5	74.6	1.24	1.75±0.36	5	1
ISB6	245.3	213.2	54.3	1.622	1.86±0.57	4	1
ISB7	229.2	204.1	67.5	0.98	1.31±0.53	5	2
ISB8	301.34	277.35	54.3	2.94	3.4±0.66	4	1
ISB9	284.31	260.69	75.6	1.584	2.11±0.37	6	1
ISB10	254.29	233.79	80.3	0.8	1.38±0.37	5	1
ISB11	269.3	248.04	63.6	1.78	2.00±0.49	5	2
ISB12	217.2	207.7	54.35	1.67	2.07±0.80	4	3
BI5	359.38	321.29	82.86	2.51	2.92±0.55	5	1
BI7	333.3	294.84	75.7	2.26	2.54±0.65	6	0

M. wt : Molecular weight; M.V: Molar volume; TPSA : Topological polar surface area; HBA : Hydrogen bond acceptor; HBD : Hydrogen bond donors; No.v : Number of violations.

Table 3: HOMO, LUMO, GAP, Binding energies of synthesized compounds, test set and standards

S. No	Comp code	HOMO	LUMO	GAP	Binding energy (Kcal/mol)
1	INH	-9.65	-0.85	8.8	-7.09
2	ISB1	-9.051	-0.715	8.336	-10.03
3	ISB2	-8.79	-0.88	7.91	-10.56
4	ISB3	-9.21	-0.93	8.28	-9.86
5	ISB4	-9.27	-2.52	6.75	-8.76
6	ISB5	-8.63	-0.83	7.8	-10.07
7	ISB6	-8.55	-0.85	7.7	-9.99
8	ISB7	-8.48	-0.77	7.71	-7.86
9	ISB8	-8.38	-0.89	7.49	-9.34
10	ISB9	-8.19	-0.72	7.47	-9.13
11	ISB10	-8.16	-0.71	7.45	-9.69
12	ISB11	-8.73	-0.66	8.07	-8.66
13	ISB12	-9.15	-0.7	8.45	-8.51
14	BI5	-9.98	-1.79	8.19	-12.55
15	BI7	-9.38	-0.92	8.46	-12.185
16	A1	-8.65	-0.73	7.92	-9.192
17	A2	-8.77	-0.8	7.97	-8.55
18	A3	-8.57	-0.71	7.86	-8.06
19	A4	-8.6	-0.88	7.72	-7.28
20	A5	-8.66	-0.84	7.82	-10.34
21	BIH	-8.65	-0.73	7.92	-10.37

HOMO: energy of Highest occupied molecular orbital; LUMO: energy of lowest un occupied molecular orbital; GAP: energy difference between HOMO and LUMO.

Table 4: Schrodinger scoring parameters and in vitro minimum inhibitory concentration values reported in literature for test compounds and standards.

Compd. code	GScore	LipophilicEvdW	HBond	RotPenal	MIC MTB-H37Rv (μM * 10^{-3})
A1	-8.38	-4.18	-1.44	0.23	11
A2	-7.2	-4.13	-0.7	0.23	12
A3	-8.1	-3.01	-1.18	0.29	11
A4	-6.59	-3.52	0	0.24	20
A5	-6.92	-3.33	-0.29	0.39	52
A6	-7.46	-3.65	-0.52	0.31	12
BIH	-8.09	-4.02	-1.08	0.38	4.9
INH	-6.26	-2.35	-0.7	0	2.04
etambutol					15.31
rifampicin					9.4
ciprofloxacin					0.24

G Score: glide score; H-bond: hydrogen bonding term; RotPenal : Rotatable bond penalty; Lipophilic EvdW: Lipophilic term derived from hydrophobic grid potential.

Table 5: Scrodinger scoring parameters of synthesized compounds

Compd. code	GScore	LipophilicEvdW	HBond	RotPenal	Electro	Sitemap
ISB1	-7.26	-3.31	-0.34	0.26	-0.39	-0.61
ISB2	-6.87	-2.86	-1.13	0.21	-0.14	-0.35
ISB3	-6.9	-3.87	0	0.16	0.02	-0.31
ISB4	-5.98	-2.73	-1.06	0.29	-0.13	-0.35
ISB5	-7.16	-2.64	-1.89	0.35	-0.86	-0.12
ISB6	-6.45	-2.93	-0.28	0.25	-0.3	-0.64
ISB7	-6.66	-3	-0.34	0.28	-0.41	-0.62
ISB8	-6.54	-3.57	-0.51	0.35	-0.24	-0.26
ISB9	-7.86	-4.58	-1.1	0.44	-0.52	-0.1
ISB10	-6.67	-3.46	-0.32	0.35	-0.26	-0.26
ISB11	-6.43	-2.72	-0.56	0.32	-0.29	-0.53
ISB12	-7.48	-3.35	-1.17	0.3	-0.5	-0.17
BI5	-8.46	-4.02	-1.3	0.25	-0.71	0
BI7	-8.14	-4.3	-1.23	0.14	-0.52	-0.25

GScore: glide score; Hbond : hydrogen bonding term; RotPenal : Rotatable bond penalty; LipophilicEvdW: Lipophilic term derived from hydrophobic grid potential; Electro: Electrostatic rewards; Sitemap: SiteMap ligand/receptor non-H bonding polar/hydrophobic and hydrophobic/hydrophilic complementarity terms.

Table 6: Absorbances and %scavenging of synthesized compounds by nitric oxide method

S. No.	Compd. code	A_s (A^0)	% Scavenging
1	ISB1	1.349	55
2	ISB2	1.262	57.91
3	ISB3	1.731	42.25
4	ISB4	1.768	41.03
5	ISB5	1.216	59.46
6	ISB8	1.217	59.43
7	ISB9	1.07	64.3
8	ISB10	1.07	64.3
9	ISB11	1.175	60.82
10	ISB12	1.195	60.153
11	BI5	1.07	64.3
12	BIH	1.26	56.4
13	Ascorbic acid	0.990	66.8
14	INH	1.195	60.153

Ac : the absorbance of the control (2.999); As : the absorbance of the test sample.

Table 7: Hydrophilic and Hydrophobic interactions of some high scored compounds

S. No	Compd. Code	Interacting residues	
		Hydrophilic	**Hydrophobic**
1	ISb1	C=O- H_20 856(2.035).	Met 103, Ala157, Met199, Leu218.
2	ISB5	C=O-Tyr158 (1.963).	Ile21, Met 103, Met147, Ala191, Tyr 158.
3	ISB9	C=O-Tyr158 (2.216), NH- H_20 563(2.137).	Ile21, Met 103, Phe149, Tyr 158, LEU 218.
4	ISB12	C=O-Tyr158 (2.204), NH- H_20 563(2.066).	Ile21, Met 103, Phe149, Met199, Tyr 158.
5	BI5	C=O- H_20 552(2.607, 2.323), C=O-Tyr158 (1.689), phenolic OH-. H_20 856(2.049).	Ile21, Met 103, Phe149, Tyr 158, Ala 191, Ile 194.
6	BI7	C=O-Tyr158 (2.081), C=O- H_20 552(2.115)	Ile21, Met98, Met 103, Phe149, TYR 158.
7	INH	NH- H_20 563(2.083).	Met103, Tyr158.

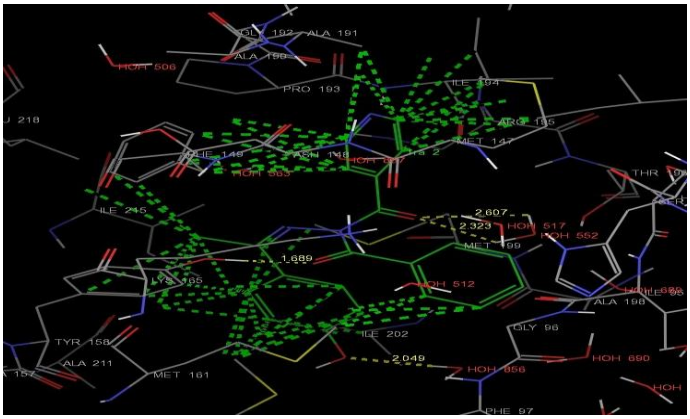


Figure 1: Hydrophilic and hydrophobic interaction of BI5

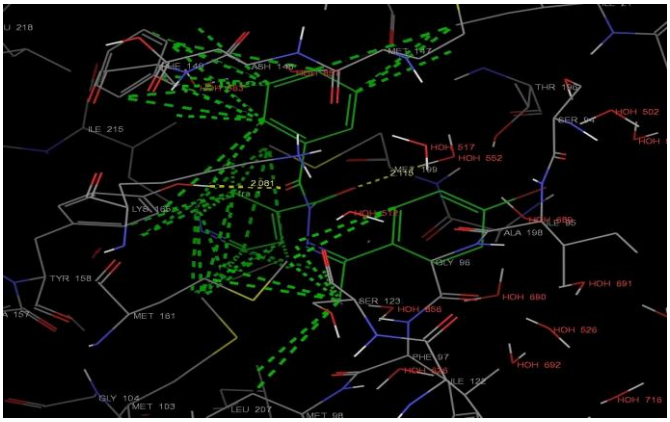


Figure 2: Hydrophobic interaction of BI7

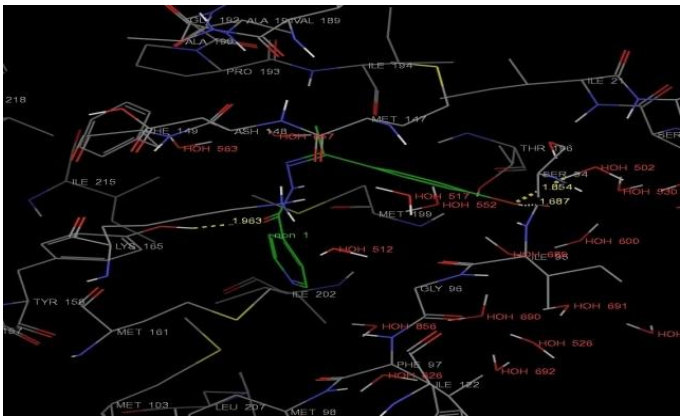


Figure 3: Hydrophilic interactions of ISB5

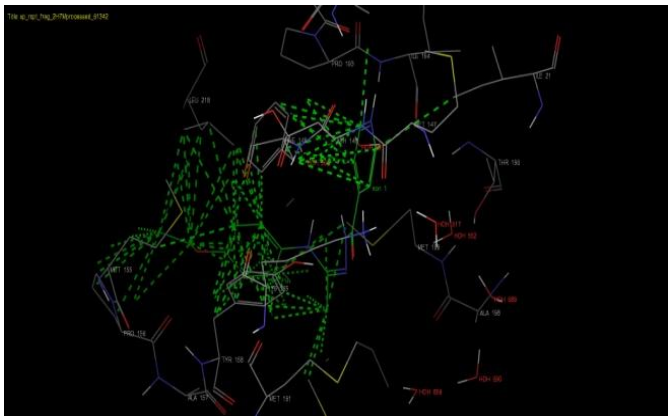


Figure 4: Hydrophobic interactions of ISB9

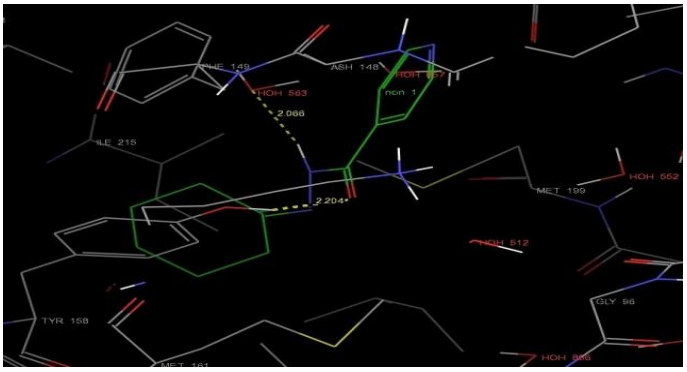


Figure 5: Hydrophilic interactions of ISB12

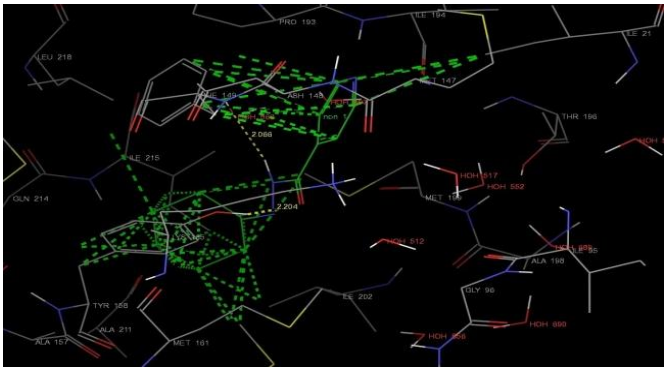


Figure 6: Hydrophobic interactions of ISB12

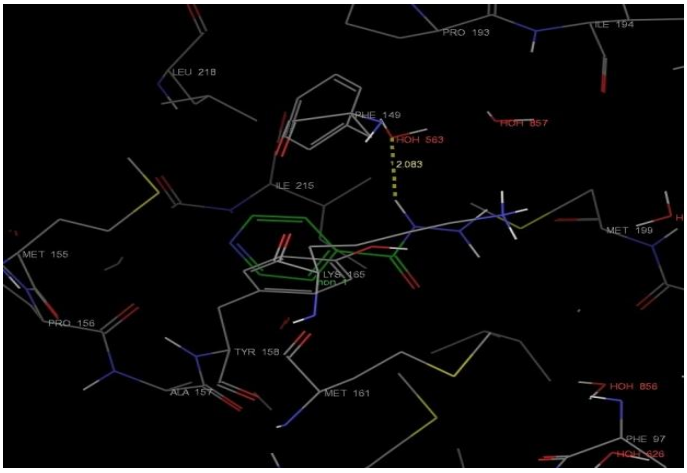


Figure 7: Hydrophilic interaction of INH

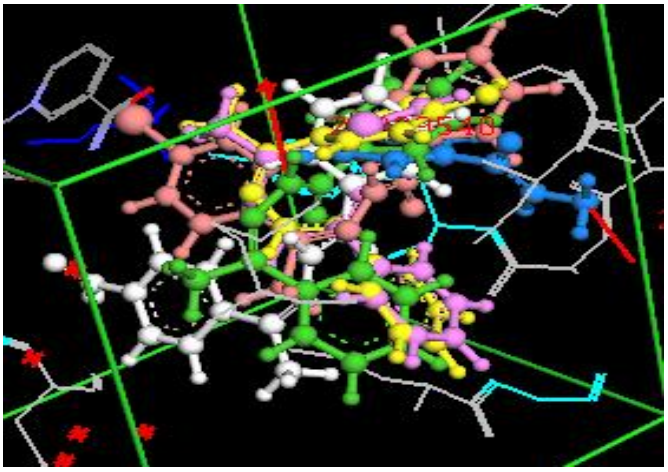


Figure 8: Stacking photo of INH (blue)

Binding energy studies

The quantum mechanical calculation indicated the greater homo values for electron donating substituents and large fall in lumo values for electron accepting substituents[23,24]. The lower GAP values indicated high binding capability which was in agreement in docking results.

Docking studies

All compounds have good binding capacity compared to standard drug INH (-6.26 Kcal/mol) except ISB4. Some of the compounds like BI8, BI9,ISB9, have score better than all test series compounds especially BIH (-8.09 Kcal / mol) which was reported in literature as equipotent active as INH and better activity than Ethambutol, Rifampicin and Ciprofloxacin.

The hydrogen bonding interactions are formed by pyridyl nitrogen, keto groups, amide NH, and Hydrogen bond forming substituents at para-position of phenyl ring with Tyr 158, Ile 95, Ile194, Pro193 and water molecules 502, 552, 563, 856. All the compounds were buried in hydrophobic pocket created by mainly 13 amino acids Ala 157, Gly96, Gly104, Ile215, Leu218, Lys 165, Met103, Met 155, Met199, PHe97, Pro156, Ser123, and Tyr 158 as shown in figures. The data was tabulated in table no4 and table no5 and interacting residues of some high binding compounds is given in table no.7

Pharmacological activity

The in vitro anti oxidant activity by nitric oxide scavenging assay of the synthesized compounds depicted that the compounds having electron donating groups (OH, OCH3 at para-position observed to have better activity comparable to standards INH and ascorbic acid.

CONCLUSION

In conclusion the compounds isoniazid Schiff bases and their benzoylated derivatives were predicted in silico as good Inh A inhibitors .From literature review it was found that the compounds having anti oxidant effect like INH are first line tuberculosis drug so In-vitro anti oxidant activity was performed and results state that they are having good anti oxidant potential. The future scope of work includes synthesis of some more derivatives and lead molecules can be further tested for mycobacterial activity.

Disclosure of interest: The authors declare that they have no conflicts of interest concerning this article

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