IJPSR (2016), Vol. 7, Issue 7 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 04 February, 2016; received in revised form, 28 March, 2016; accepted, 13 April, 2016; published 01 July, 2016

DESIGN, SYNTHESIS AND EVALUATION OF ANTITUBERCULAR ACTIVITY OF AMINO AZETIDINONES FROM ISONIAZID

Beena Thomas [*1] and Jyoti Harindran[2]

Department of Pharmaceutical Sciences [1], Regional Institute of Medical Science and Research, Mahatma Gandhi University, Kottayam, Kerala, India.
Department of Pharmaceutical Sciences [2], Cheruvandoor campus, Mahatma Gandhi University, Kottayam, Kerala, India

Keywords:

Isoniazid (INH), 2-azetidinone, amino azetidinone, 4-amino 1, 2, 4-triazole, Alamar blue assay, Chang liver cell, *In vitro* hepatotoxicity

Correspondence to Author:
Beena Thomas

Senior Lecturer, Department of Pharmaceutical Sciences, RIMSR, Rubber Board P.O., Kottayam, Kerala - 686009, India.

E mail: beenakku@gmail.com

ABSTRACT: Azetidin-2-one analogues are reported to exhibit various pharmacological activities like cholesterol absorption inhibitory activity, human tryptase, thrombin and chymase inhibitory activity, vasopressin V1a antagonist activity, antidiabetic, anti-inflammatory, antiparkinsonian and anti-HIV activity in addition to antimicrobial.[1-6] In the present study, Isoniazid (INH), the established antitubercular drug was selected as the lead for the design and development of antitubercular agents with minimal toxic effects. A novel series of amino azetidinones were designed from corresponding azetidin-2-ones using various *in silico* methods. Docking studies were performed at *Mtb* enoyl acp reductase (4DRE) and the derivatives exhibited best docking scores were prepared from corresponding azetidin-2-ones by treating with various molecules containing amino groups in the presence of TEA. Azetidin-2-ones in turn were obtained from a series of INH Schiff bases by reaction with chloro acetyl chloride. Structures of the newly synthesized compounds were assigned on the basis of elemental analysis, IR, ^{1}H NMR, ^{13}CNMR and mass spectral studies. The newly synthesized compounds were screened for their *in vitro* antitubercular activity using Alamar blue assay method and the hepatotoxicity was determined by MTT assay method using chang liver cells. AAZ1V, the amino azetidinone obtained from N-[3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl] pyridine-4-carboxamide (AZ1V) by combining with 4- amino 1, 2, 4-triazole produced significant antitubercular activity. The percentage viability produced by AAZ1V against Chang liver cells for hepatotoxicity was better than the percentage viability produced by INH.

INTRODUCTION: The synthesis of heterocyclic compounds has always drawn the attention of chemists over the years mainly because of their biological properties. Compounds classified as heterocyclic probably constitute the largest and most varied family of organic compounds. [7]

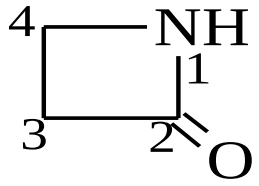
QUICK RESPONSE CODE

DOI:
10.13040/IJPSR.0975-8232.7(7).2795-04

Article can be accessed online on:
www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (7).2795-04

One such heterocyclic, 2-azetidinone, the 2-carbonyl derivatives of the 4-membered heterocyclic compounds containing nitrogen is aimed to evaluate new products that possess interesting biological activities.



Drug discovery based on the existing lead molecules by modification of functional groups is a common strategy. A number of already existing

drugs have been structurally modified for improving the activity, to reduce the side effects or in some cases to make the compound devoid of any unwanted effect. Moreover, introducing two or more established rings in a single molecule for a combined effect is also tried. [8]

Isoniazid (Isonicotinic acid hydrazide) is the most commonly used drug for active infection and prophylaxis since its introduction for treatment of TB in 1952. Isoniazid is a prodrug that is activated by Kat G, the mycobacterial catalase-peroxidase. The activated form of isoniazid forms a covalent complex with an acyl carrier protein (AcpM) and Kas A, a beta-ketoacyl carrier protein synthetase, which blocks mycolic acid synthesis. [9] Enzymatic acetylation of INH in human beings greatly reduces its therapeutic activity resulting in under dosing, decreased bioavailability and acquired INH resistance. Chemical modification of INH with a functional group that blocks acetylation, while maintaining strong antimycobacterial action, may improve clinical outcomes and help to reduce the rise of INH resistance.

Hepatotoxicity refers to liver dysfunction or liver damage that is associated with an overload of drugs or xenobiotics.[10] The three key antituberculosis drugs, isoniazid, pyrazinamide and rifampicin are potentially hepatotoxic. A Meta analysis has shown an incidence rate of liver toxicity of 2.6% with isoniazid. With the changing demographics and clinical characteristics of tuberculosis patients in many parts of the world, hepatotoxicity is of increasing concern in the treatment of this disease.

Tuberculosis (TB) is a chronic disease caused by *Mycobacterium tuberculosis* which has been threatening the man kind since ages. TB remains a major global health problem, responsible for ill health among millions of people each year. According to the latest estimates included in the WHO report 2015, there were 9.6 million incidental TB cases in 2014. [11]

In the present work, modification of INH by introducing 2-azetidinone ring followed by introduction of various rings by aminoalkylation and thus blocking acetylation to develop a better antitubercular drug with minimal hepatotoxic effect is aimed.

MATERIALS AND METHODS:

Materials:

In silico molecular modeling studies were carried out on various softwares like Schrodinger suite Maestro v 9.3, ACD/Chem Sketch Free version 12.0 and Molinspiration. The chemicals were of AR and LR grade and were obtained from Merck, Hi-Media, Nice and Sigma-Aldrich. All the chemicals were dried and purified wherever necessary.

The melting points of the synthesized compounds were determined by Thiele melting point apparatus (open capillary tube method) and all the compounds gave sharp melting points and were uncorrected. The synthesized compounds were purified by recrystallization and purity of the compounds was ascertained by single spot on thin layer chromatography. The IR spectra of the synthesized compounds were recorded on IR Spectrometer PerkinElmer, Model: SPECTRUM 400.The NMR Spectra of the characteristic compound was recorded by NMR 400 MHZ Spectrometer Bruker –USA. The mass spectrum was recorded by Xevo GC Q-ToF –Waters-USA.

Antitubercular screening of the synthesized compounds was determined by alamar blue assay method using *M. tuberculosis* H_{37}Rv strain. *In vitro* hepatotoxicity was done using Chang liver cells by MTT assay method.

In silico design:

The protein structure of *Mtb* Enoyl-ACP reductase was obtained from PDB using their specific PDB ID (4DRE) was prepared using the protein preparation wizard in the Schrodinger software graphical user interface Maestro v9.3. Amino azetidin-2-ones derivatives of INH were selected as ligands and their structures were drawn using the workspace of Maestro and were converted to 3D form for the docking studies. The collected ligands were prepared for docking. Then the prepared ligands were docked into the generated grid in the prepared protein. The best docked pose with lowest Glide score value was recorded for each ligand. Extra precision (XP) was performed using the module Induced Fit Docking of Schrödinger-Maestro v9.3 (2012). Glide scores of designed analogues of azetidinones and aminoazetidinone of

INH are given in (**Table 3** and **4).** Best derivatives with good docking scores were selected and their ADME properties were checked using QIKPROP tool available in Schrodinger under Maestro (**Table 5**). [12] The Lipinski's rule of five (Table1) and drug likeness analysis (**Table 2**) of selected derivatives was also calculated.

Procedure for synthesis:

The strategy used in the synthesis of the title compounds is outlined in Scheme (**Fig. 1**).

Synthesis of Schiff base (SB1-SBX):

To a solution of isoniazid (0.01mol) in ethanol, substituted aromatic aldehyde (0.01mol) in ethanol was added slowly with constant stirring.Then catalytic amount of glacial acetic acid was added to it and refluxed for 5-6 h. The resulting reaction mixture after cooling was poured into ice cold water. The Schiff base obtained was filtered, dried and recrystallised from ethanol. Yield and melting points were determined.The procedure was repeated with various aldehydes to obtain compounds SB1-SBX. [13]

Synthesis of azetidinones (AZ1-AZX):

A mixture of Schiff base (0.01mol) and triethylamine (0.01mol) in dry 1, 4-dioxan (10 ml) was stirred well at 0--5°C temperature. To this mixture chloroacetyl chloride (0.01mol) was added drop wise for half an hour. The mixture was then shaken by a mechanical shaker at room temperature for additional 5 hours and then refluxed for 8-12h. The mixture was concentrated, cooled, poured into ice cold water. The product obtained was filtered, washed with cold water and then dried. The product was recrystallized from ethanol. This was repeated with remaining Schiff bases to obtain AZ1 to AZX. [14-15]

Synthesis of aminoazetidinone (AAZ1to AAZX):

Azetidinone from 4-chloro benzaldehyde (AZ1V, 0.01mol) and 4-amino-1, 2, 4-triazole (0.01mol) were separately dissolved in 1, 4- dioxan and mixed in a Round Bottom Flask (RBF). Then triethylamine (0.01mol) was added and the reaction mixture was refluxed for 4-6h. The reaction was monitored by TLC. The reaction mixture was then dumped in ice cold water and the precipitate was collected by suction and dried. The solid was recrystallised from rectified spirit. This was repeated with the same azetidinone and using various primary amines to obtain AAZ1-AAZX. [16]

Antitubercular screening:

Selected azetidinone and amino azetidinone analogues which exhibited best docking scores from each class were screened for antitubercular activity using Middlebrook 7H9 medium by microplate alamar blue assay (MABA) method. *Mycobacterium tuberculosis* H37Rv strain was used as the test organism. Each INH derivative was tested in a final concentration of 0.8µg/ml to 100µg/ml and was compared with the parent drug INH in the same concentration range (**Table 7**). [17-19]

***In vitro* hepatotoxicity studies:**

The effect of modifications in the hepatotoxicity of INH was evaluated by *in vitro* hepatotoxic screening using one representative analogue from each group of derivatives and was compared with that of the parent drug INH. Chang liver cells were used and 3 different concentrations of INH and its analogues SB1V, AZ1V & AAZ1V at the concentrations of 10µg/ml, 50µg/ml and100µg/ml were tested. The % difference in viability was measured using standard MTT assay method after 24hrs. Optical density was read at 540nm using DMSO as blank and percentage viability was calculated (Graph 1). [20-22]

% viability = (OD of Test/ OD of Control) X 100

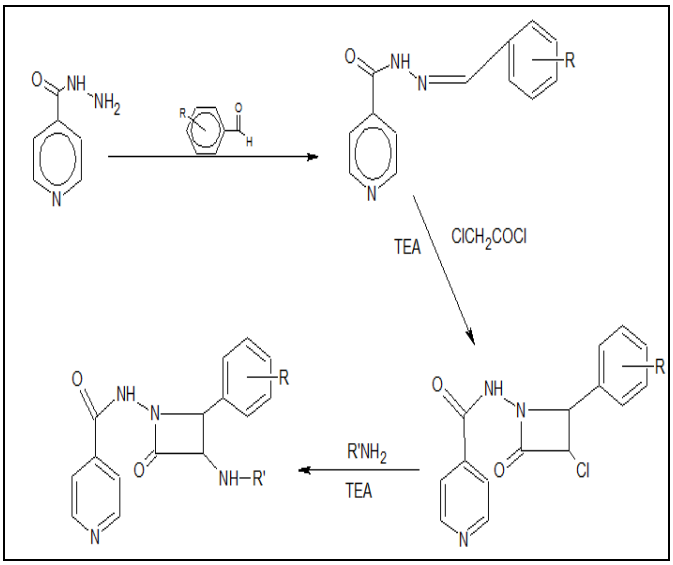


FIG.1: SCHEME

RESULTS AND DISCUSSION:

In silico Design:

TABLE1: ANALYSIS OF LIPINSKI'S RULE OF FIVE OF SELECTED AMINO AZETIDINONE ANALOGUES

Compound	miLogP	Mol. wt	NHDon	nHAcc	Nrotb	Lipinski's rule alert index
AAZ1	0.049	330.775	2	6	4	0
AAZ11	-0.448	436.859	3	9	6	0
AAZ111	0.841	435.871	3	8	6	0
AAZ1V	-0.83	383.799	2	9	5	0
AAZV	3.091	427.291	2	6	5	0
AAZV1	2.862	406.873	2	6	5	0
AAZV11	2.47	422.872	2	7	6	0
AAZV111	1.934	408.845	3	7	5	0
AAZ1X	2.324	436.855	3	6	8	0
AAZX	1.107	471.926	4	9	6	0

Drug likeness analysis:

TABLE 2: DRUG LIKENESS ANALYSIS OF SELECTED AMINO AZETIDINONE ANALOGUES

Compound	GPCR Ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
AAZ1	0.08	-0.24	0.00	-0.48	0.04	-0.14
AAZ11	-0.06	-0.29	-0.14	-0.45	-0.05	-0.17
AAZ111	-0.06	-0.29	-0.14	-0.46	-0.05	-0.18
AAZ1V	-0.20	-0.58	-0.17	-0.65	-0.31	-0.20
AAZV	-0.03	-0.27	-0.05	-0.34	-0.06	-0.19
AAZV1	-0.06	-0.33	-0.09	-0.37	-0.12	-0.24
AAZV11	-0.06	-0.33	-0.08	-0.35	-0.12	-0.23
AAZV111	0.01	-0.24	-0.02	-0.23	-0.06	-0.14
AAZ1X	-0.01	-0.26	-0.08	-0.21	-0.04	-0.13
AAZX	-0.10	-0.33	-0.07	-0.50	0.07	-0.07

DOCKING RESULTS:

Docking Scores of Azetidinones: (AZ1-AZXV)

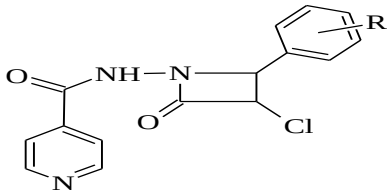


TABLE 3: GLIDE SCORES OF DESIGNED AZETIDINONE DERIVATIVES

Compound	-R	Glide Score
AZ1	--H	-8.08266
AZ11	4-$N(CH_3)_2$	-7.4164
AZ111	4-OCH_3	-7.82503
AZ1V	4-Cl	**-8.08748**
AZV	3-Cl	-7.96803
AZV1	2-Cl	-7.98143
AZV11	3-NO_2	-7.0308
AZV111	2-NO_2	-7.69068
AZ1X	4-NO_2	-6.29484
AZX	4-F	-8.08315
AZX1	4-Br	-7.86692
AZX11	4-I	-7.30216
AZX111	2,6-Dichloro	-6.24891
AZX1V	4-Cl,3-NO_2	-6.46219
AZXV	3,4-Dichloro	-5.88594
INH	-	-6.7018

Docking studies performed with the INH azetidinones showed that the one containing 4-chloro benzaldehyde (AZ1V) produced the best binding and was identified as the lead molecule in the azetidinone series. Hence to improve the binding interaction lead optimization was performed by exploring the azetidinone ring by incorporating other ring systems and by converting into amino azetidinones by combining with various primary and secondary amines. Some of the amino azetidinones showed tremendous binding affinity than the lead molecule itself.

Docking scores of amino azetidinones: (AAZ1-AAZX)

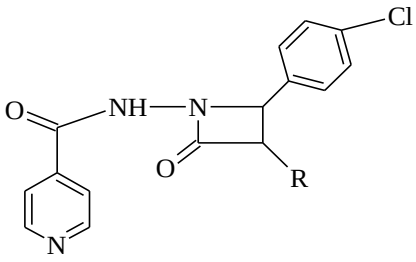
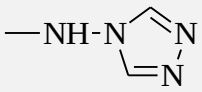
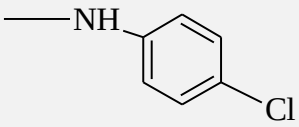
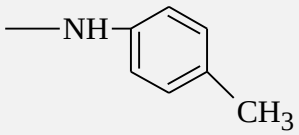
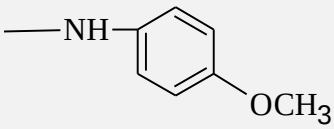
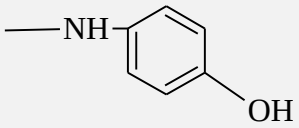
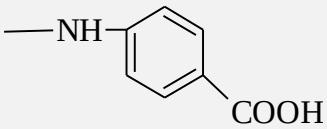
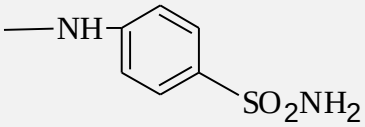


TABLE 4: GLIDE SCORES OF DESIGNED AMINO AZETIDINONE DERIVATIVES

Compound	-R	Glide Score
AAZ1	-$NHCH_3$	-6.12941
AAZ11	-$NHNHCOC_5H_4N$	-7.25174
AAZ111	-$NHNHCOC_6H_5$	**-7.47017**
AAZ1V		**-8.36693**
AAZV		-6.28839
AAZV1		**-7.38907**
AAZV11		-5.66394
AAZV111		-7.02691
AAZ1X		-6.99702
AAZX		-6.00561
INH	-	-6.7018

In the Amino azetidinone series AAZ1V, the derivative obtained by incorporating amino triazole into the azetidinone ring of AZ1V shows better docking interaction with energy of -8.367. Docking image (**Fig.2**) and ligand interaction (**Fig.3**) of AAZ1V with 4DRE are given below.

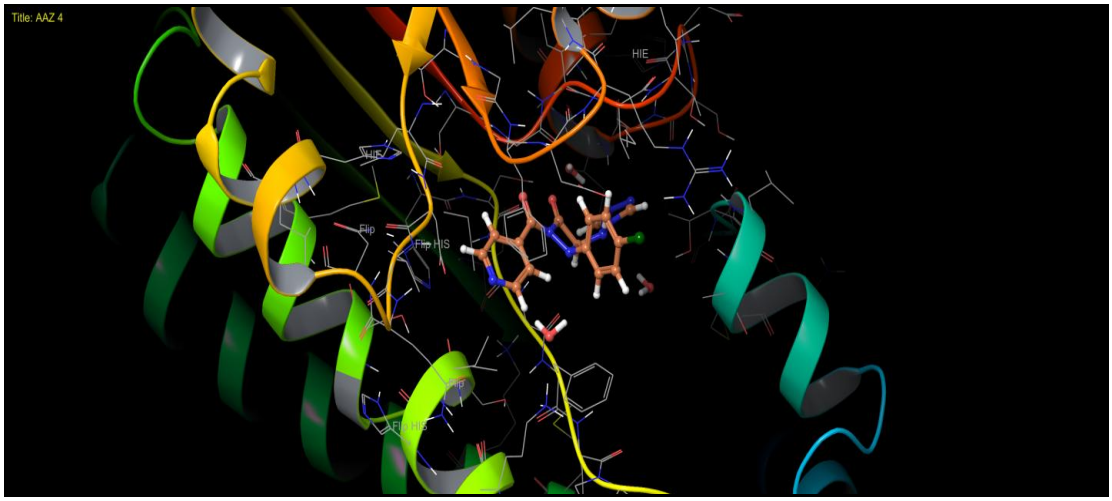


FIG.2: DOCKING IMAGE OF AAZ1V WITH 4DRE

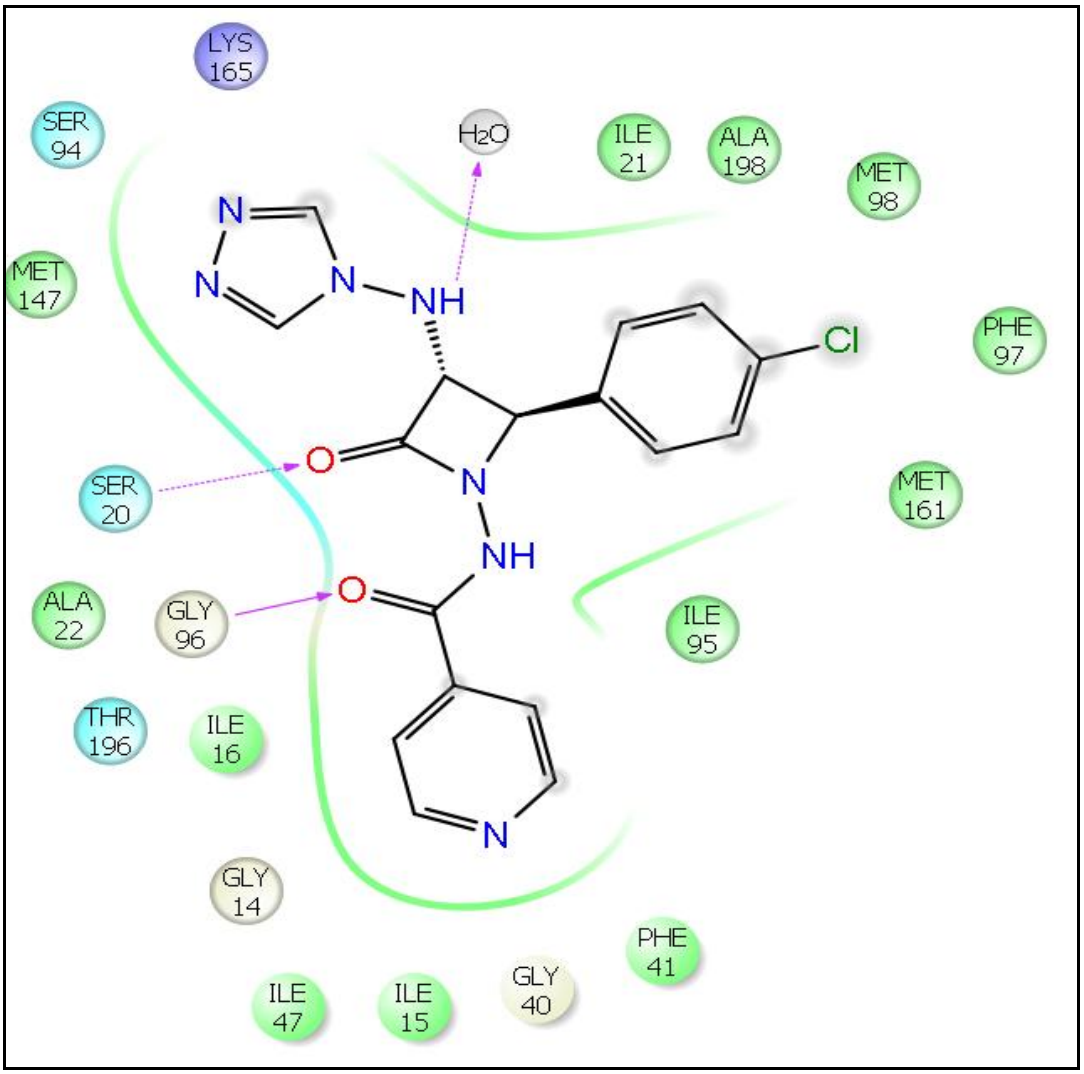


FIG.3: LIGAND INTERACTIONS OF AAZ1V WITH 4DRE

Hydrogen bonding of the –C=O group of hydrazide group with Gly96 is mainly seen in the ligand interaction diagram. Hydrogen bonding of the carbonyl group of azetidinone ring to the protein side chain Ser20 and also the same produced by – NH of amino triazole with water contribute to the better binding interaction. The $\pi - \pi$ stacking interaction of the pyridine ring is not seen with amino azetidinone derivatives.

ADME Property/Descriptors of Amino azetidinones (AAZ):

TABLE 5: PREDICTION OF ADME PROFILE OF SELECTED AMINO AZETIDINONE ANALOGUES

Compound	HOral Abs	% HOral Abs	QPlogKhsa	QPPCaco	QPlogBB	QPlogKp	QPlogHERG
AAZ1	1	77.548	0.028	130.096	-0.414	-4.719	-6.772
AAZ11	3	79.438	0.073	103.093	-1.836	-3.415	-7.044
AAZ111	1	88.723	0.426	165.601	-1.651	-2.871	-7.396
AAZ1V	3	74.77	-0.475	42.109	-1.014	-5.333	-7.148
AAZV	1	100	0.546	522.085	-0.733	-2.21	-6.684
AAZV1	1	100	0.635	881.743	-0.699	-1.681	-7.027
AAZV11	1	100	0.51	645.423	-0.932	-1.839	-7.128
AAZV111	3	88.779	0.425	183.869	-1.497	-2.839	-6.998
AAZ1X	1	70.307	0.246	14.778	-2.238	-3.797	-5.655
AAZX	2	66.014	-0.083	33.126	-2.566	-4.255	-7.31

TABLE 5: PREDICTION OF ADME PROFILE OF SELECTED AMINO AZETIDINONE ANALOGUES (CONTD)

Compound	QPlogS	Dipole	SASA	FOSA	FISA	#metab	QPPMDCK
AAZ1	-3.687	3.87	616.28	109.0	134.82	4	148.748
AAZ11	-5.689	7.698	734.077	33.816	209.076	5	104.748
AAZ111	-6.55	12.052	751.55	32.158	187.371	3	174.872
AAZ1V	-2.889	11.549	651.183	23.658	186.484	3	44.004
AAZV	-6.561	2.717	693.399	15.675	134.784	4	1483.103
AAZV1	-6.708	2.007	719.516	112.49	110.783	5	1062.943
AAZV11	-6.536	5.819	734.724	110.563	125.072	5	760.658
AAZV111	-6.123	2.62	712.582	13.915	182.578	5	195.781
AAZ1X	-6.746	1.591	773.321	19.122	235.144	4	16.324
AAZX	-5.757	12.386	771.511	11.364	261.07	4	31.388

Physico- Chemical Properties of Synthesized Compounds:

TABLE 6: PHYSICO- CHEMICAL PROPERTIES OF AMINO AZETIDINONES

Compound	Molecular formula	IUPAC name	MP(˚C)	Physical state	TLC system (Toluene:ethanol)
AAZ1	$C_{16}H_{15}ClN_4O_2$	*N*-[2-(4-chlorophenyl)-3-(methylamino)-4-oxoazetidin-1-yl]pyridine-4-carboxamide	145-147	Light brown	4.0:1.0
AAZ11	$C_{21}H_{17}ClN_6O_3$	*N*-{2-(4-chlorophenyl)-4-oxo-3-[2-(pyridin-4-ylcarbonyl)hydrazinyl]azetidin-1-yl}pyridine-4-carboxamide	150	Brown amorphous	4.0:1.0
AAZ111	$C_{22}H_{18}ClN_5O_3$	*N*-[3-(2-benzoylhydrazinyl)-2-(4-chlorophenyl)-4-oxoazetidin-1-yl]pyridine-4-carboxamide	155-156	Brown crystalline	4.0:1.0
AAZ1V	$C_{17}H_{14}ClN_7O_2$	*N*-[2-(4-chlorophenyl)-4-oxo-3-(4*H*-1,2,4-triazol-4-ylamino)azetidin-1-yl]pyridine-4-carboxamide	148-150	Brown powder	4.0:1.0
AAZV	$C_{21}H_{16}Cl_2N_4O_2$	*N*-{2-(4-chlorophenyl)-3-[(4-chlorophenyl)amino]-4-oxoazetidin-1-yl}pyridine-4-carboxamide	152	Yellow crystals	4.0:1.0
AAZV1	$C_{22}H_{19}ClN_4O_2$	*N*-{2-(4-chlorophenyl)-3-[(4-methylphenyl)amino]-4-oxoazetidin-1-yl}pyridine-4-carboxamide	150	Brown powder	4.0:1.0
AAZV11	$C_{22}H_{19}ClN_4O_3$	*N*-{2-(4-chlorophenyl)-3-[(4-methoxyphenyl)amino]-4-oxoazetidin-1-yl}pyridine-4-carboxamide	140-142	Brown powder	4.0:1.0
AAZV111	$C_{21}H_{17}ClN_4O_3$	*N*-{2-(4-chlorophenyl)-3-[(4-hydroxyphenyl)amino]-4-oxoazetidin-1-yl}pyridine-4-	157	Dark brown	4.0:1.0

		carboxamide			
AAZ1X	$C_{22}H_{17}ClN_4O_4$	4-({2-(4-chlorophenyl)-4-oxo-1-[(pyridin-4-ylcarbonyl)amino]azetidin-3-yl}amino)benzoic acid	138	Brown powder	4.0:1.0
AAZX	$C_{21}H_{18}ClN_5O_4S$	*N*-{2-(4-chlorophenyl)-4-oxo-3-[(4-sulfamoylphenyl)amino]azetidin-1-yl}pyridine-4-carboxamide	152-154	Brown powder	4.0:1.0

Characterization:

Representative Azetidinone: *N*-[3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl] pyridine-4-carboxamide **(AZ1V):**

Analytical data:

Calculated for $C_{15}H_{11}Cl_2N_3O_2$: C, 53.59; H, 3.30; N, 12.50:

Found: 53.61; H, 3.27; N, 12.51:

IR: 1677 (C=O), 1409 (C-N of ring), 3458 (N-H); ^{1}H NMR (500 MHz, MeOD) δ 8.3 (s,1H,-N-H), δ 8.74-8.76 (Double doublet, -CH-CH), δ 7.45-7.47 (m, 4H, pyridyl), δ7.8-7.9 (m,4H,Aromatic); ^{13}C NMR(100MHz, MeOD) δ123(pyridyl), δ 130-137 (δ Aromatic) 150 (-CH-CH carbon atoms of azetidinone ring), δ 210 (both –C=O). MASS (EI) m/z: Calcd for $C_{15}H_{11}Cl_2N_3O_2$ is 336; found: 337.

Representative amino azetidinone: *N*-[2-(4-chlorophenyl)-4-oxo-3-(4*H*-1, 2, 4-triazol-4-ylamino) azetidin-1-yl] pyridine-4-carboxamide **(AAZ1V):**

Analytical data:

Calculated for $C_{17}H_{14}ClN_7O_2$: C, 53.20; H, 3.68; N, 25.55:

Found: 53.22; H, 3.65; N, 25.57:

IR: 1632 (-NC=O), 1408 (C-N of ring), 3286 (N-H), 3087-3196 (C-H str); ^{1}H NMR (500 MHz, MeOD). δ 8.78 (s,2H, 2-CH of triazole), δ8.36(1H, -NH of ring), δ7.8(1H, -NH of triazole) δ 7.46-7.58 (m, 4H, pyridyl) δ9.078, 9.010(double doublet, -CH,-CH) δ7.86-7.93(m, 4H, aromatic); ^{13}C NMR(100MHz, MeOD) δ123--137(Aromatic & pyridyl), δ 142 (-2 equivalent –CH of triazole), δ150&151 (-CH-CH of azetidinone), δ 159 & 164 (both – C=O). MASS (EI) m/z: Calcd for $C_{17}H_{14}ClN_7O_2S$ is 383.8; found: 384.8

SCREENING OF ANTITUBERCULAR ACTIVITY

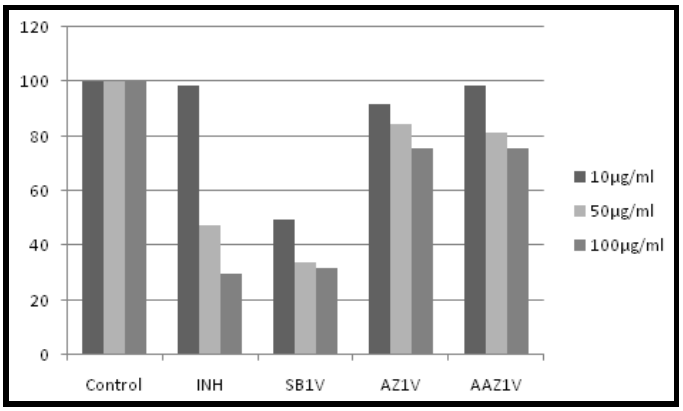
TABLE 7: ANTITUBERCULAR SCREENING RESULTS

Sl.No.	Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
1	INH(std)	S	S	S	S	S	S	R	R
2	SB1V	S	S	S	S	S	R	R	R
3	AZ1V	S	S	S	S	R	R	R	R
4	AAZ-1	S	S	S	S	S	R	R	R
5	AAZ-2	S	S	S	S	R	R	R	R
6	AAZ-3	S	S	S	S	S	S	R	R

S- Sensitive, **R**-resistant:

AAZ1- AAZ111; **AAZ2**- AAZV1; **AAZ3**- AAZ1V

Antitubercular activity of selected analogues of INH showed comparable activity with that of the parent drug INH. Schiff base (SB1V), azetidinone (AZ1V) and three amino azetidinones (AAZ111, AAZ1V and AAZV1) were screened for their sensitivity and compared with INH and with 3 other standard drugs. The Schiff base derivative SB1V was sensitive at 6. 25µg/ml while the azetidinone derivative AZ1V exhibited antitubercular activity at 12.5µg/ml. Among the three amino azetidinone analogues screened, AAZ1V, the one containing 4-amino 1, 2, 4-triazole exhibited comparable antitubercular activity (sensitive at 3.12µg/ml) with that of INH. AAZ111 with benzoyl hydrazine and AAZV1containing methyl aniline were effective at 6.25µg/ml and12.5µg/ml respectively.



GRAPH 1: BAR GRAPH SHOWING SAMPLE CONCENTRATION- % VIABILITY

The *in vitro* hepatotoxic potential of the derivatives determined by assessing the cytotoxicity using Chang liver cells by MTT assay method at the concentrations 10 µg/ml, 50µg/ml and 100µg/ml exhibited dose dependent toxicity.

The morphological analysis of the cells treated with derivatives like AZ and AAZ shows less membrane damage and cell death at low concentrations; only at higher concentrations they are showing some cells with membrane blebbing and detachment. The azetidinone (AZ1V) and amino azetidinone (AAZ1V) derivatives give comparatively better results. Even at concentrations 100µg/ml, the percentage viability is above 75% for both AZ1V and AAZ1V, which is very good in comparison with the percentage viability of INH at 50µg/ml and 100µg/ml.

CONCLUSION: The present work was focused on the rational approach in designing and development of derivatives of well known antitubercular drug isoniazid not only as a mode to improve its antitubercular activity but also to minimize other problems associated with INH therapy. A series of INH azetidin-2-ones and respective amino azetidinone derivatives were subjected to preliminary *in silico* designing and docking studies of the designed derivatives were performed using Schrodinger. Among the fifteen INH azetidinones docked at the active site of enoyl acp reductase (PDB ID: 4DRE) to study antitubercular effect, the one containing chlorine (from 4-Cl benzaldehyde) showed better docking score and hence *N*-[3-chloro-2-(4-chlorophenyl)-4-oxoazetidin – 1 - yl] pyridine-4-carboxamide (AZ1V) was used for the development of amino azeidinones.

Most of the derivatives have shown significant antitubercular activity when tested in Middlebrook7H9 medium by alamar blue assay method using *Mycobacterium tuberculosis* $H_{37}Rv$ strain and the results correlated well with the *in silico* studies performed. The amino azetidinone derivatives showed a promising increase in antitubercular activity in comparison with INH. Among the 3 amino azetidinones screened, the one with 4- amino 1, 2, 4- triazole ring (AAZ1V) showed sensitivity at a concentration of 3.12µg/ml which is equipotent as INH as far as antitubercular activity is considered.

The *in vitro* hepatotoxic potential determination by MTT assay exhibited dose dependent toxicity. The azetidinone (AZ1V) and amino azetidinone (AAZ1V) derivatives give comparatively better results. Even at concentrations 100µg/ml, the percentage viability is above 75% for both AZ1V and AAZ1V, which is very good in comparison with the percentage viability of INH at 50µg/ml and 100µg/ml. Therefore the present research work ascertains the findings of *in silico* studies. The antitubercular profile and the pharmacological studies conducted indicate the relevance of the work and these derivatives containing azetidinone and triazole moieties need further attention for molecular manipulation.

ACKNOWLEDGEMENTS: This work was financially supported by the AICTE under MODROB's scheme. The *in silico* studies and synthetic work were performed at DPS, Puthuppally, Kottayam. The authors express gratitude to all staffs of Maratha Mandal's NGH Institute of Dental Sciences & Research Centre, Belgaum for antitubercular studies conducted. The authors are thankful to IIRBS & School of Environmental science, Mahatma Gandhi University, Kottayam for carrying out the spectral analysis.

CONFLICT OF INTEREST: The authors do not have any conflict of interest.

REFERENCES:

1. Bhusari KP, Charbe NB, Mehere AP, Warokar AS, Kakde JW and Mishrab AP: Synthesis, cholesterol absorption inhibition and anti-bacterial activity of some novel 2-azetidinone derivatives. IJRPC. 2011; 1(4): 2231- 2781.

2. Bergman M, Morales H, Mellars L and Kosoglou T: 12th International Symposium on drugs affecting lipid Metabolism. Houston, TX, 1995; 7-10.
3. Slusarchyk WA, Bolton SA, Hartl KS and Huang MH: Synthesis of potent and highly selective inhibitors of human tryptase. Bioorg Med Chem Lett.2002; 12: 32-35.
4. Kayarmar R, Nagaraja GK, Naik P, Manjunatha H, Revanasiddappa BC and Arulmol T: Synthesis and characterization of novel imidazoquinoline based 2-azetidinones as potent antimicrobial and anticancer agents. Journal of Saudi Chemical Society. 2014. Article in press.
5. Guillon CD, Koppe A, Brownstein MJ and Chaney MO: Azetidinones as vasopressin V1a antagonists. Bioorg Med Chem. 2007; 15:2054.
6. Kendre BV, Landge MG and Bhusare SR: Microwave Assisted and Al2O3/K2CO3 Catalyzed Synthesis of Azetidin-2-One Derivatives Containing Aryl Sulfonate Moiety with Anti-Inflammatory and AntiMicrobial Activity. Open Journal of Medicinal Chemistry. 2012; 2(3): 98-104.
7. Hote SV and Bhoyar SP: Heterocyclic Compound –A Review. IOSR Journal of Applied Chemistry. 2014:43-46.
8. Kaminskyy D, Khyluk D, Vasylenko O, Zaprutko L and Lesyk R: A Facile Synthesis and Anticancer Activity Evaluation of Spiro [Thiazolidinone-Isatin] Conjugates. Sci Pharm. 2011; 79(4): 763–777.
9. Chambers HF and Deck DH: Antimycobacterial agents. In: Katzung BG, Masters SB, Trevor AJ, editors. Basic and Clinical Pharmacology. Tata McGraw-Hill, New Delhi, Edition 11, 2009: 823-830.
10. Singh A, Bhat TK andSharma OP: Clinical Biochemistry of Hepatotoxicity. J Clinic Toxicol 2011; S4:001.
11. WHO report2015.www.who.int/tb/data
12. Schrodinger suite user manual. Maestro 9.3 v 2012
13. Tomma JH, Khazaal MS and Al-Dujaili AH: Synthesis and characterization of novel Schiff bases containing pyrimidine unit. Arabian Journal of Chemistry. 2014; 7(1):157-163.
14. Chandra D, Mishra PS, Gupta SK and Ritu: Synthesis, Characterization and Antimicrobial Activity of 2-Azetidinone Derivatives. Wjjps. 2014; 3(12): 860-868.
15. Thomas B, George D and Harindran J: Novel 1, 2, 4 - Triazole Incorporated Azetidin-2-One Analogues as Anti-Tubercular Agents. Research and Reviews: Journal of Chemistry.2014; 3(4):52-59.
16. Ishwar BK, Sunil KM, Jainey PJ and Shastry CS: Antimicrobial studies of synthesized azetidinone derivatives from sulfamethoxazole moiety. J. Chem. Pharm. Res. 2011; 3(3):114-118.
17. Vanitha JD and Paramasivan CN. Evaluation of microplate Alamar blue assay for drug susceptibility testing of *Mycobacterium avium* complex isolates. Diagn Microbiol Infect Dis. 2004; 49(3): 179-82.
18. Lourenco MCS, DeSouza MVN, Pinheiro AC, Ferreira ML, Goncalves RB, Nogneira TCM et al. Evaluation of anti-Tubercular activity of nicotinic and isoniazid analogues. ARKIVOC. 2007; (xv): 181-191.
19. Franzblau SG, Witzig RS, McLaughlin JC, Torres P, Madico G, Hernandez A et al. Rapid, low-technology MIC determination with clinical *Mycobacterium tuberculosis* isolates by using the microplate Alamar Blue assay. J Clin Microbiol 1998; 36(2):362-6.
20. Singh M, Sasi P, Rai G, Gupta VH, Amarapurkar D and Wangikar PP: Studies on toxicity of antitubercular drugs namely isoniazid, rifampicin, and pyrazinamide in an in vitro model of HepG2 cell line. Med. Chem. Res. 2011; 20(9):1611-1615.
21. Shih T, Pai C, Yang P, Chang W, Wang N and Hu O: A Novel Mechanism Underlies the Hepatotoxicity of Pyrazinamide. Antimicrob. Agents Ch. 2013; 57(4): 1685-1690.
22. Mostafavi-Pour Z, Khademi F, Zal F, Sardarian AR and Amini F: *In vitro* Analysis of CsA-Induced Hepatotoxicity in HepG2 Cell Line: Oxidative Stress and α2 and β1 Integrin Subunits Expression. Hepat Mon. 2013; 13(8):e11447.

How to cite this article:
Thomas B and Harindran J: Design, Synthesis and Evaluation of Antitubercular Activity of Amino Azetidinones from Isoniazid. Int J Pharm Sci Res 2016; 7(7): 2795-04.doi: 10.13040/IJPSR.0975-8232.7(7).2795-04.