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Anti-Inflammatory activity of novel series of isoniazid derivatives

Beena Thomas, Jyoti Harindran and KS Devaky

Abstract
Two novel series of derivatives of isoniazid, the well-established antitubercular drug were designed with the aim of developing an antitubercular agent with better anti-inflammatory activity. Schiff bases of isoniazid were prepared by the treatment of isoniazid with various aldehydes. These Schiff bases on treatment with chloroacetyl chloride produced azetidinones, which on further treatment with different amines resulted in the first series of compounds, the amino azetidinones. The second series of derivatives were obtained by the conversion of isoniazid Schiff bases to thiazolidinones by treatment with thioglycolic acid followed by conversion to corresponding Mannich bases by treatment of the thiazolidinones with various secondary amines. Both series of derivatives were designed using *in silico* studies. Docking studies for anti-inflammatory activity were performed using cyclooxygenase-2(PDB ID: ICX2). Structures of the newly synthesized compounds were assigned on the basis of elemental analysis and various spectroscopic techniques like IR, ^{1}H NMR, ^{13}CNMR and mass analysis. The derivatives with best docking scores from each group were screened for anti-inflammatory activity by carrageenan induced paw edema method. The study performed using the compounds *N*-[2-(4-chlorophenyl)-4-oxo-1,3-thiazolidin-3-yl]pyridine-4-carboxamide (TZ1V), *N*-{2-(4-chlorophenyl)-5-[(dimethylamino)methyl]-4-oxo-1,3-thiazolidin-3-yl}pyridine-4-carboxamide (MB1V-2) and *N*-[2-(4-chlorophenyl)-3-(methylamino)-4-oxoazetidin-1-yl]pyridine-4-carboxamide (AAZ1) as representatives from each group were found to be significantly reducing edema formation at a dose level of 100 mg/kg.

Keywords: Isoniazid (INH), 2-azetidinone, aminoazetidinone, 4-thiazolidinone, mannich base, carrageenan, cyclooxygenase-2

1. Introduction
Heterocycles form by far the largest of classical divisions of organic chemistry and are of immense importance biologically and industrially. The structural feature inherent to heterocycles, which is of great advantage in drug industry, is their ability to manifest substituents around a core scaffold in defined three dimensional representations.[1] Literature survey shows that a number of heterocyclic compounds having condensed ring systems possess various types of physiological activities. Synthetic heterocycles have widespread therapeutic uses such as antibacterial, antifungal, trypanocidal, anti-HIV, antileishmanial, genotoxic, antitubercular, antimalarial, herbicidal, analgesic, anti-inflammatory, muscle relaxant, anticonvulsant, anticancer, lipid peroxidation inhibition, hypnotic, antidepressant, antitumoral, anthelmintic, insecticidal etc. [2-8].

Monocyclic β-lactams (2-azetidinones) are an important class of four-membered nitrogen heterocycles because of their use in the synthesis of biologically active classical or non-classical β-lactam antibiotics. Analogues of 2-azetidinones possess a wide range of biological activities like antimicrobial, anticancer, antiviral, antitubercular etc. β- Lactam is widely used in organic chemistry not only for the design and preparation of biologically active compounds but also as versatile building blocks for the synthesis of other types of nitrogen-containing compounds with potential biological properties [9-10]

4-Thiazolidinones are derivatives of thiazolidine with a carbonyl group at the fourth position and is another important pharmacophore present in a large number of compounds and its derivatives are reported with diverse biological activities such as antibacterial, antifungal, anticancer, anti-inflammatory, anticonvulsant, analgesic etc [11-14]

Mannich reaction plays a vital role in the construction of variety of organic molecules. In several instances, the Mannich derivatives exhibit better activity than the corresponding parent analogues. Modification of thiazolidinones by replacing the active hydrogen atom at C-5 by an amino alkyl group via Mannich reaction to improve biological activity is also reported [15]

Tuberculosis (TB) is a chronic disease caused by *Mycobacterium tuberculosis* (Mtb) which

has been threatening the man kind since ages. TB remains as a major global health problem, responsible for ill health among millions of people each year. Infection with Mtb is accompanied by an intense local inflammatory response which may be critical to the pathogenesis of TB [16] TB experts at Johns Hopkins conducted a 4-year series of experiments in mice and prove that anti-inflammatory drugs are effective in treating TB. The role of anti-inflammatory drugs as adjunct to the antitubercular treatment was reviewed by Majeed *et al* and they established the fact that, in tuberculosis therapy, killing of only pathogen is not mandatory, but the immunological imbalance created due to infection needs to be addressed [17]

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 [18].

In the present work, modification of INH by introducing 2—azetidinone and 4-thiazolidinone rings followed by introduction of various rings like triazole into both series of compounds by aminodehalogenation and Mannich reaction respectively to obtain an antitubercular drug with anti-inflammatory activity is aimed.

2. Materials and Methods

2.1 Materials

In silico molecular modeling studies were carried out on various softwares like Schrodinger suite Maestro v 9.3, ACD/ChemSketch 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.

Anti-inflammatory activity of the synthesized compounds was determined by carrageenan induced paw edema method.

Animals

Acute toxicity study

Mice- Female albino mice weighing around 20 to 25g- 18 no

Anti-inflammatory study

Wistar rats - Wistar albino rats weighing around 150 to 250g of either sex-30no.

2.2 Methods

2.2.1 *In silico* design

The 3-D structure of the protein cyclooxygenase-2 was obtained from PDB using their specific PDB ID (ICX2).The protein structure was prepared using the protein preparation wizard in the Schrodinger software graphical user interface Maestro v9.3. A set of derivatives of aminoazetidinones and Mannich bases of thiazolidinones 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 v 9.3 (2012) [19]. The Lipinski's rule of five and drug likeness analysis of selected derivatives were also calculated [20].

2.2.2 Scheme

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

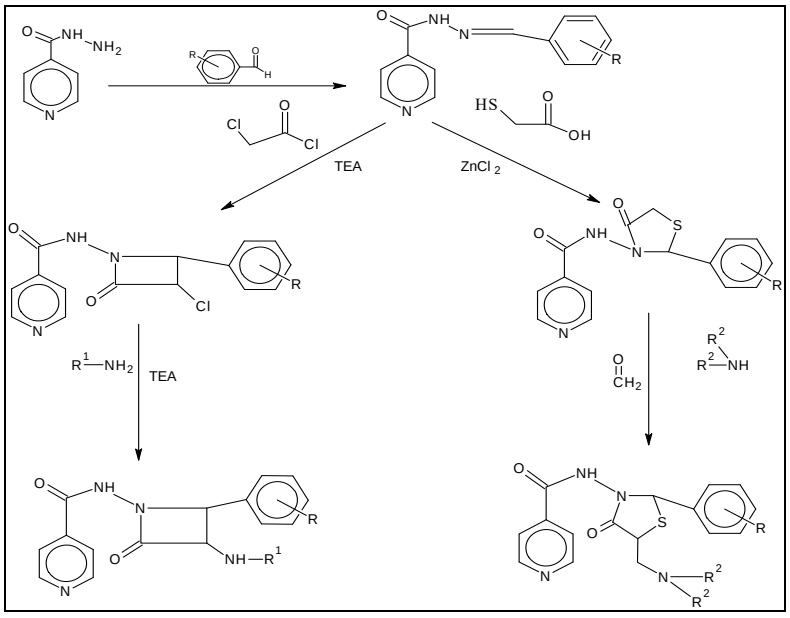


Fig 1: Scheme for the synthesis

Series 1: Aminoazetidinones

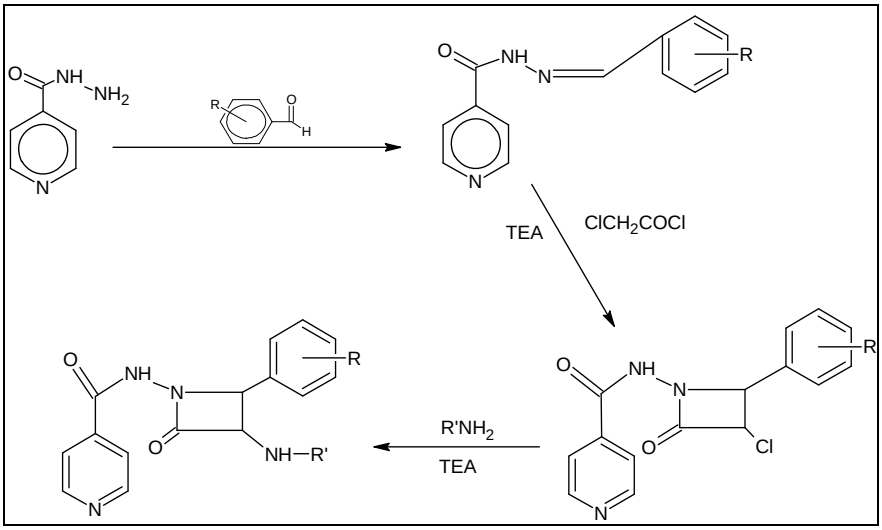


Fig 2: Scheme for the synthesis of aminoazetidinones

2.2.3 Procedure for synthesis

The strategy used in the synthesis of aminoazetidinones is outlined in (Fig.2).

2.2.3.1 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 [21].

2.2.3.2 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 8-12 h. 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 [22]

2.2.3.3 Synthesis of Aminoazetidinones (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. 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 [23]. (Table 9)

Series 2: Mannich Bases

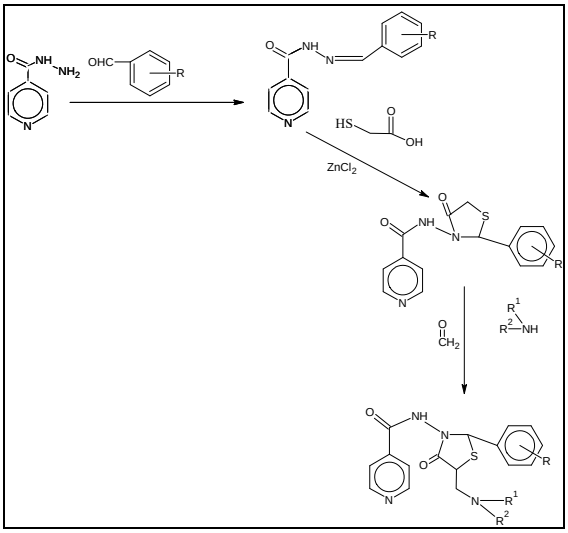


Fig 3: Scheme for the synthesis of Mannich bases

Procedure for synthesis
The strategy used in the synthesis of the Mannich bases is outlined in (Fig.3).

2.2.3.4 Synthesis of thiazolidinones (TZ1-TZX)
0.01mol of the Schiff base was dissolved in dry benzene.0.015mol of thioglycolic acid and a pinch of anhydrous zinc chloride were added. Dean Stark water separator was connected and refluxed for 36-48 h, during the course of the reaction the water was removed continuously. The completion of the reaction was monitored by TLC. The excess solvent was removed; the mixture was poured into cold water and neutralized with aqueous sodium bicarbonate solution. The precipitate obtained was filtered, washed repeatedly with water, dried and recrystallized from ethanol to obtain compoundsTZ1-TZX [24].

2.2.3.5 Synthesis of Mannich bases (MB1V-1to MB1V-10)
0.01mol of the synthesized thiazolidinone was dissolved in 15ml of absolute ethanol. To this 0.01mol of paraformaldehyde and 0.01 mol of appropriate amine were added. The mixture was refluxed for 6-12 h. Cooled, poured into cold water and the precipitate was recrystallized from ethanol to obtain compoundsMB1V-1to MB1V-10 [25]. (Table 10)

2.2.4 Anti-inflammatory Screening
2.2.4.1 Carrageenan Induced Paw Edema in Rats
TB disease activation is believed to arise as a result of lack of inflammatory homeostatic control either due to immunosuppression (decreased antimicrobial activity) or due to immunoactivation (excess inflammation). There are many reported studies indicating that suppression of excessive inflammatory process in the lung may have a beneficial antitubercular effect in tuberculosis sensitive animals. Hence selected analogues representing each group were screened for their anti-inflammatory activity by carrageenan induced paw edema method [26].
The carrageenan induced paw edema is a very useful method for the screening of anti-inflammatory agents. It has been used since 1962 and has been established as the most popular model for the evaluation of anti-inflammatory activity of synthesized compounds. Edema develops as carrageenan is injected in one paw (right) of the rat. Inflammation is determined by the degree of increased paw volume compared to the volume of the unaffected paw (left) which is measured by the plethysmograph over a time period [27].

Instruments Used: Plethysmograph, Oral feeding tube
Chemicals used: Carrageenan sodium, Sodium carboxymethylcellulose

2.2.4.2 Experimental procedure
The Institutional Animals Ethics Committee approved the use of animals for the study. Ethical Clearance number: (002/PHD/UCP/CVR/'13dtd14/5/13). Wistar albino rats weighed around 150 to 250g were used for this study. The initial right hind paw volume of the rats was measured using a Plethysmograph. They were divided into 5 groups consisting of 6 animals each.

Group 1: Served as positive control which received vehicle + 0.1ml carrageenan 1% sub plantar

Group 2: Standard group which has received diclofenac sodium (25 mg/kg) + 0.1ml carrageenan 1% sub plantar

Group 3: Test group which has received the test compound TZ1V (100 mg/kg) + 0.1ml carrageenan 1% sub plantar

Group 4: Test group which has received the test compound MB1V-2 (100 mg/kg) + 0.1ml carrageenan 1% sub plantar

Group 5: Test group which has received the test compound AAZ1 (100 mg/kg) +0.1ml carrageenan 1% sub plantar.
Prototype compounds selected for anti-inflammatory studies from each group based on the docking studies are TZ1V, MB1V-2 and AAZ1.The 3 different groups (Group-2, Group-3 & Group-4) received these test compounds (100 mg/kg) respectively. The stock solutions of each derivative were prepared in DMSO and depending upon the body weight, required volume was given. One group (Group-1) received control vehicle only. Diclofenac 25 mg/kg was used as the standard drug. After 30 min, the rats were challenged with sub plantar injection of 0.1 ml of 1% w/v solution of carrageenan into the right paw. The paw was marked with ink at the level of lateral malleolus. The paw volume was measured at 1, 2, 3 and 6 h after carrageenan injection using a Plethysmograph. The difference between initial and subsequent reading gave the actual edema volume. The anti-inflammatory activity in animals that received the test compounds (100 mg/kg) and diclofenac (25 mg/kg) was compared with that of vehicle control groups. The percentage inhibition of edema was calculated as follows:

Percentage inhibition of edema = $1 - Vt / Vc \times 100$

Where Vt is the inflammatory increase in paw volume in drug-treated rats,
Vc is the inflammatory increase in paw volume in control group of rats.
Percentage inhibition of edema is proportional to anti-inflammatory activity.
Results obtained were analysed to determine the significance of the difference between the control group and experimental animals treated with test drug for anti-inflammatory activity. All statistical calculations were carried out using Graph Pad Prism3.1statistical software. The data were subjected to statistical analysis using one-way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test.

3. Results and Discussion
3.1 *In Silico* Design
3.1.1 Analysis of Lipinski's rule of five
All the synthesized derivatives obey Lipinski's rule of five and the values are given in table 1 and 2.

Table 1: Analysis of Lipinski's rule of five of selected aminoazetidinones

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

Table 2: Analysis of Lipinski's rule of five of selected Mannich base analogues

Compound	miLogP	Mol. Wt	NHDon	nHAcc	Nrotb	Lipinski's rule alert index
MB1V-1	0.652	432.933	1	7	5	0
MB1V-2	0.806	390.896	1	6	5	0
MB1V-3	1.558	418.95	1	7	6	0
MB1V-4	-0.153	475.874	2	12	6	1
MB1V-5	2.395	508.047	1	7	6	1
MB1V-6	0.698	445.976	1	7	5	0
MB1V-7	1.209	416.934	1	6	5	0
MB1V-8	-0.149	444.9	1	8	5	0
MB1V-9	1.714	430.961	1	6	5	0
MB1V-10	1.955	444.988	1	6	5	0

3.1.2 Drug likeness analysis

Drug likeness analysis of the designed derivatives was compared with that of standard anti TB drugs and is given in table 3 and 4.

Table3: Drug likeness analysis of selected aminoazetidinone 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

Table4: Drug likeness analysis of selected Mannich base analogues

Compound	GPCR Ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
MB1V-1	-0.01	-0.31	-0.28	-0.49	-0.12	-0.15
MB1V-2	0.02	-0.29	-0.31	-0.55	-0.15	-0.15
MB1V-3	0.02	-0.29	-0.33	-0.50	-0.16	-0.15
MB1V-4	-0.24	-0.49	-0.50	-0.82	-0.34	-0.32
MB1V-5	0.04	-0.23	-0.23	-0.44	-0.14	-0.15
MB1V-6	0.06	-0.21	-0.23	-0.49	-0.11	-0.12
MB1V-7	0.08	-0.23	-0.28	-0.44	-0.04	-0.11
MB1V-8	0.00	-0.35	-0.43	-0.55	-0.16	-0.20
MB1V-9	0.07	-0.23	-0.30	-0.46	-0.09	-0.11
MB1V-10	0.04	-0.25	-0.35	-0.48	-0.09	-0.14
INH	-1.39	-1.45	-1.05	-2.33	-1.23	-0.66
Pyrazinamide	-1.97	-1.45	-1.71	-2.87	-1.84	-1.43

3.1.3 Docking Results

Docking scores of the designed derivatives azetidinones, aminoazetidinones, thiazolidinones and Mannich bases with the enzyme cyclooxygenase-2(PDB ID: ICX2) are given in table 5, table 6, table 7 and table 8 respectively.

3.1.3.1 Docking Scores of Azetidinones: (AZ1-AZXV)

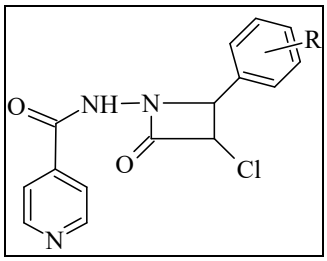


Table 5: Glide scores of designed azetidinone analogues with ICX2

Compound	Structure(-R)	Glide Score
AZ1	--H	-7.56413
AZ11	4-N(CH_3)$_2$	-7.86021
AZ111	4-OCH_3	-7.57127
AZ1V	4-Cl	**-7.88271**
AZV	3-Cl	-7.25512
AZV1	2-Cl	-6.50466
AZV11	3-NO_2	-7.87208
AZV111	2-NO_2	-7.18399
AZ1X	4-NO_2	-7.42974
AZX	4-F	-7.82687
AZX1	4-Br	-7.25155
AZX11	4-I	-7.05593
AZX111	2,6-Dichloro	-6.74102
AZX1V	4-Cl,3-NO_2	-7.0421
AZXV	3,4-Dichloro	-7.08305
INH	-	-5.86461
Diclofenac	-	-7.54597

Fifteen azetidinone derivatives of isoniazid were designed and docked with ICX2 for determining their binding affinity for anti-inflammatory activity. The glide scores indicate that almost all the azetidinone derivatives have more or less the same binding affinity which is comparable with diclofenac also. Azetidinone (AZ1V) obtained from N'-(4-chlorobenzylidene) pyridine-4-carbohydrazide (SB1V) has maximum binding affinity.

3.1.3.2 Docking Scores of Aminoazetidinones: (AAZ1-AAZX)

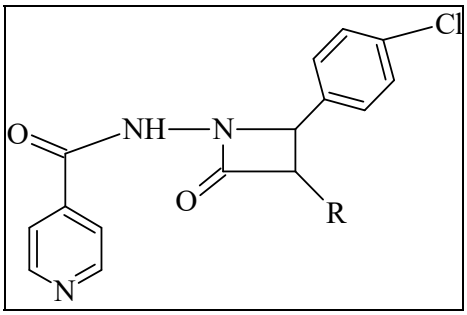
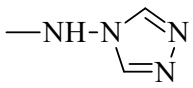
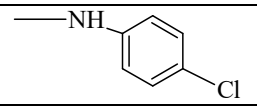
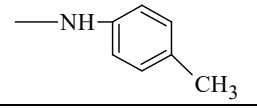
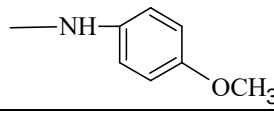
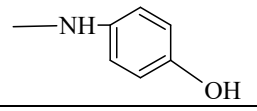
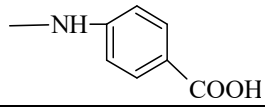
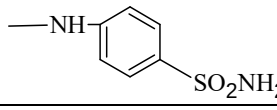


Table 6: Glide scores of designed aminoazetidinones with ICX2

Compound	Structure(-R)	Glide Score
AAZ1	-$NHCH_3$	**-8.88338**
AAZ11	-$NHNHCOC_5H_4N$	-7.88513
AAZ111	-$NHNHCOC_6H_5$	-4.57696
AAZ1V		-7.80132
AAZV		-7.80132
AAZV1		-6.57594
AAZV11		-8.43025
AAZV111		-5.68784
AAZ1X		-5.58441
AAZX		-7.70755

The ten aminoazetidinones designed from N-[3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl] pyridine-4-carboxamide (AZ1V) by combining with ten different amines were docked with cyclooxygenase-2(PDB: ID ICX2) to study their binding affinity. The aminoazetidinone (AAZ1) obtained by combining methyl amine exhibited best docking with a minimum glide score of -8.88338.

3.1.3.3 Docking Scores of Thiazolidinones: (TZ1-TZXV)

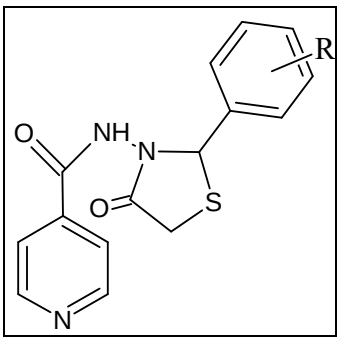


Table7: Glide scores of designed thiazolidinone analogues with ICX2

Compound	Structure(-R)	Glide Score
TZ1	-H	-7.50012
TZ11	4-N(CH_3)$_2$	-6.29314
TZ111	4-OCH_3	-7.68069
TZ1V	4-Cl	**-8.14832**
TZV	3-Cl	-7.02257
TZV1	2-Cl	-8.03723
TZV11	3-NO_2	-6.68682
TZV111	2-NO_2	-7.26086
TZ1X	4-NO_2	-7.06935
TZX	4-F	-8.14059
TZX1	4-Br	-7.98974
TZX11	4-I	-7.56435
TZX111	2,6-Dichloro	-6.80923
TZX1V	4-Cl,3-NO_2	-6.65547
TZXV	3,4-Dichloro	-8.05451
INH	-	-5.86461
Diclofenac	-	-7.76

For the second series of derivatives, fifteen thiazolidinones of isoniazid were selected corresponding to 15 Schiff base analogues and docked with ICX2. The glide scores indicate that most of them have good binding affinity with the protein which is better than the affinity of INH and comparable with that of diclofenac. The thiazolidinone (TZ1V) obtained from N'-(4-chlorobenzylidene) pyridine-4-carbohydrazide (SB1V) has maximum binding affinity (least glide score) with cyclooxygenase-2 and hence was selected for designing Mannich bases.

3.1.3.4 Docking Scores of Mannich base analogues: (MB1V-1 toMB1V-10)

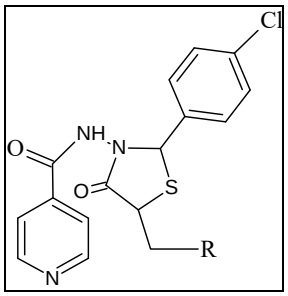
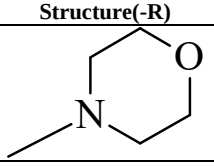
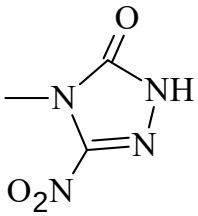
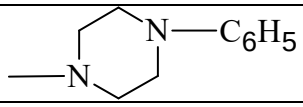
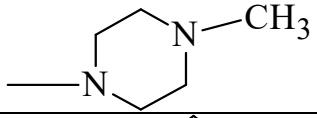
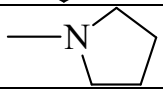
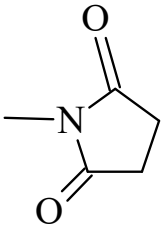
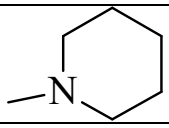
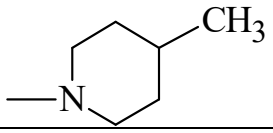


Table8: Glide scores of designed Mannich base analogues with ICX2

Compound	Structure(-R)	Glide Score
MB1V-1		-6.18292
MB1V-2	$-N(CH_3)_2$	-7.91145
MB1V-3	$-N(C_2H_5)_2$	-7.01298
MB1V-4		-
MB1V-5		-
MB1V-6		-6.06883
MB1V-7		-6.34616
MB1V-8		-5.52367
MB1V-9		-6.29372
MB1V-10		-5.7648

Mannich base analogues (MB1V-1 to MB1V-10) were designed from N-[2-(4-chlorophenyl)-4-oxo-1, 3-thiazolidin-3-yl] pyridine-4-carboxamide (TZ1V) by combining it with ten primary or secondary amines and the resulted Mannich bases were docked with cyclooxygenase-2 to study their binding affinity for anti-inflammatory activity. Among the eight derivatives got docked, the Mannich base (MB1V-2) containing dimethylamine exhibited best binding with a minimum glide score of -7.91145, followed by Mannich base (MB1V-3) with a docking score of -7.01298.

3.2 Physicochemical Properties of Synthesized Compounds

Table 9: Physicochemical Properties of aminoazetidinones

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-carboxamide	157	Dark brown	4.0:1.0
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

Table10: Physicochemical properties of Mannich bases

Compound	Molecular formula	IUPAC name	MP(˚C)	Physical state	TLC system (Toluene: methanol)
MB1V-1	$C_{20}H_{21}ClN_4O_3S$	*N*-[2-(4-chlorophenyl)-5-(morpholin-4-ylmethyl)-4-oxo-1,3-thiazolidin-3-yl]pyridine-4-carboxamide	148-150	Light yellow	4.2:0.8
MB1V-2	$C_{18}H_{19}ClN_4O_2S$	*N*-{2-(4-chlorophenyl)-5-[(dimethylamino)methyl]-4-oxo-1,3-thiazolidin-3-yl}pyridine-4-carboxamide	125-127	Yellow powder	4.1:0.9
MB1V-3	$C_{20}H_{23}ClN_4O_2S$	*N*-{2-(4-chlorophenyl)-5-[(diethylamino)methyl]-4-oxo-1,3-thiazolidin-3-yl}pyridine-4-carboxamide	160-161	Yellow powder	4.2:0.8
MB1V-4	$C_{18}H_{14}ClN_7O_5S$	*N*-{2-(4-chlorophenyl)-5-[(3-nitro-5-oxo-1,5-dihydro-4*H*-1,2,4-triazol-4-yl)methyl]-4-oxo-1,3-thiazolidin-3-yl}pyridine-4-carboxamide	178-180	Light yellow	4.5:0.5
MB1V-5	$C_{26}H_{26}ClN_5O_2S$	*N*-[2-(4-chlorophenyl)-4-oxo-5-(piperazin-4-yl phenyl)methyl]-1,3-thiazolidin-3-yl]pyridine-4-carboxamide	154-156	Creamy white	4.3:0.7
MB1V-6	$C_{21}H_{24}ClN_5O_2S$	*N*-[2-(4-chlorophenyl)-4-oxo-5-(piperazin-4-yl methyl)methyl]-1,3-thiazolidin-3-yl]pyridine-4-carboxamide	171	Yellow powder	4.2:0.8
MB1V-7	$C_{20}H_{21}ClN_4O_2S$	*N*-[2-(4-chlorophenyl)-4-oxo-5-(pyrrolidin-1-yl)methyl]-1,3-thiazolidin-3-yl]pyridine-4-carboxamide	165-166	Yellow powder	4.2:0.8
MB1V-8	$C_{20}H_{17}ClN_4O_4S$	*N*-{2-(4-chlorophenyl)-5-[(2,5-dioxopyrrolidin-1-yl)methyl]-4-oxo-1,3-thiazolidin-3-yl}pyridine-4-carboxamide	155-157	Brown powder	4.3:0.7
MB1V-9	$C_{21}H_{23}ClN_4O_2S$	*N*-[2-(4-chlorophenyl)-4-oxo-5-(piperidin-1-ylmethyl)-1,3-thiazolidin-3-yl]pyridine-4-carboxamide	183-185	Yellow crystalline	4.2:0.8
MB1V-10	$C_{22}H_{25}ClN_4O_2S$	*N*-[2-(4-chlorophenyl)-4-oxo-5-(4-methyl piperidin-1-ylmethyl)-1,3-thiazolidin-3-yl]pyridine-4-carboxamide	135-137	Brown powder	4.2:0.8

3.3 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 Aminoazetidinone: *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, -NHof 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

Representative Thiazolidinone: *N*-[2-(4-chlorophenyl)-4-oxo-1, 3thiazolidin-3-yl] pyridine-4-carboxamide (TZ1V):
Analytical data
Calculated for $C_{15}H_{12}ClN_3O_2S$: C, 53.97; H, 3.62; N, 12.59:
Found: 53.95; H, 3.64; N, 12.60:
IR: 1712, 1677 (C=O),1414 (-CH_2), 1493 (C-N of ring), 752 (C-Cl); ^{1}H NMR (500 MHz, MeOD) δ 6.0 (s,1H,-CONHN), δ 8.7 (s,1H, NCHS), δ 3.8(s, 2H, -CH_2 of ring), δ 7.3-7.7 (m, 8H, Aromatic & pyridyl);^{13}C NMR(100MHz, $CDCl_3$) δ122-151(Aromatic & pyridyl), δ 30 (-CH_2), δ 63 (-CH), δ 161&177(both –C=O). MASS (EI) m/z: Calcd for $C_{15}H_{12}ClN_3O_2S$ is 333; found: 334.

Representative Mannich base: *N*-{2-(4-chlorophenyl)-5-[(diethylamino) methyl]-4-oxo-1, 3-thiazolidin-3-yl} pyridine-4-carboxamide (MB1V-3):
Analytical data:
Calculated for $C_{20}H_{23}ClN_4O_2S$: C, 57.29; H, 4.89; N, 13.37:
Found: 57.38; H, 4.79; N, 13.30:
IR: 1710, 1676 (C=O), 1412 (-CH_2), 1491 (C-N of ring), 751 (C-Cl), 2898(C-H str); ^{1}H NMR (500 MHz, MeOD). δ 1.3 (t,6H,-CH_3), δ 3.3 (q,4H, CH_2 of ethyl), δ 3.8(d, 2H, -CH_2 of MB), δ6.0(1H, -NH) δ 7.4-7.5 (m, 4H, pyridyl) δ7.8-7.9(double doublet, -CH,-CH) δ8.6-8.7(m, 4H, aromatic) ; ^{13}C NMR(100MHz, $CDCl_3$) δ122-151(Aromatic & pyridyl), δ 30 (-CH_3), δ 63 (N-CH_2), δ122(-CH_2 of Mannich base) δ 151&164(thiazolidinone ring) δ 166&171(both –C=O) δ 141,142,150,123-137(Aromatic & pyridyl).MASS (EI) m/z: Calcd for $C_{20}H_{23}ClN_4O_2S$ is 419; found: 420.

3.4 Screening of Anti-inflammatory activity

The results obtained for the anti-inflammatory study with representative compound from each group (dose 100 mg/kg) by carrageenan induced paw edema method are shown as decrease in paw volume at different time intervals 1 h, 2 h, 3 h, 6 h and corresponding percentage inhibition (Table 11). The data were statistically analyzed by one way ANOVA followed by Dunnet's test and compared with toxic control.

Table 11: Effect of various analogues of INH on the carrageenan-induced rat hind paw edema

S. No.	Group	Decrease in paw volume(ml) at different time in hour				Percentage inhibition (%)			
		1h	2h	3h	6h	1h	2h	3h	6h
1	Toxic Control	0.525±0.04	0.525±0.04	0.525±0.04	0.35±0.02	-	-	-	-
2	Standard (Diclofenac)	0.200± 0.00**	0.150±0.00**	0.083±0.01**	0.008±0.01**	61.90	71.00	84.00	97.00
3	TZ1V	0.375±0.01**	0.275±0.01**	0.166±0.01**	0.108±0.01**	28.57	47.61	68.38	69.06
4	MB1V-2	0.291±0.00**	0.166±0.01**	0.101±0.00**	0.050±0.00**	23.00	68.38	80.65	85.71
5	AAZ1	0.291±0.00**	0.158±0.01**	0.091±0.01**	0.050±0.00**	23.00	69.85	82.55	85.71

Results are expressed as mean ± SEM for n=6 rats in each group. ***P*<0.01 against toxic control. Dose: Synthesized compounds-100 mg/kg. Diclofenac-25 mg/kg.

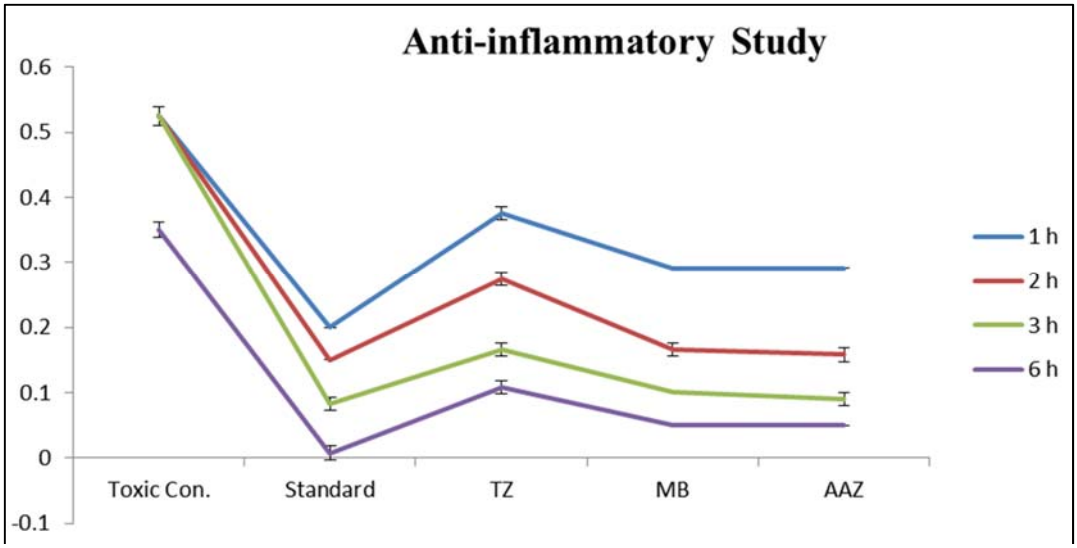


Fig 4: Decrease in paw volume produced by TZ1V, MB1V-2& AAZ1 on carrageenan induced paw edema model.

The study performed using the compounds TZ1V, MB1V-2 and AAZ1 as representatives from each group, with best docking scores were found to be significantly reducing edema formation at a dose level of 100 mg/kg. The thiazolidin-4-one derivative TZ1V of N'(4-chlorobenzylidene)pyridine-4-carbohydrazideshowed significant effect at the time intervals 1 h, 2 h, 3 h and 6 h with percentage inhibition 28.6%, 47.6%, 68.4% and 69.1% respectively.

The Mannich derivative MB1V-2 of TZ1V with dimethylamine exhibited percentage inhibition 23%, 68%, 80% and 85% at various time intervals which are considered to be very significant.

Significant anti-inflammatory activity was also shown by the methyl amino derivative of azetidin-2-one from INH (AAZ1) at various time intervals.

In comparison with standard diclofenac, all the representative compounds showed better percentage inhibition at higher time intervals.

4. Conclusion

Tuberculosis, the contagious disease caused by *Mycobacterium tuberculosis*, has been threatening the mankind since ages. The present work was focused on the rational approach in designing and development of derivatives of well-known antitubercular drug isoniazid in order to improve its anti-inflammatory effect.

A series of INH aminoazetidinones and Mannich base derivatives were subjected to preliminary *in silico* designing. Docking studies of the designed derivatives and prediction of their ADME properties were performed using QikProp prediction programme of Schrodinger.

Anti-inflammatory activity of the characteristic analogue (dose: 100 mg/kg) from each class of derivative was determined by carrageenan induced paw edema method in rat. Diclofenac (25 mg/kg) was the standard used and decrease in paw volume at various intervals 1 h, 2 h, 3 h and 6 h was determined and also corresponding percentage inhibition was calculated. In aminoazetidinone series, AAZ1, containing methylamine showed best docking score with cyclooxygenase-2(PDB ID: ICX2) enzyme and was used for anti-inflammatory screening. Among the designed Mannich bases, MB1V-2 obtained from TZ1V using dimethylamine as secondary amine, was selected considering its best docking score against ICX2 for anti-inflammatory screening.

The data statistically analyzed by one way ANOVA followed by Dunnet's test and compared with toxic control showed significance at $^{**}P<0.01$.This would be a supporting factor while the molecules are considered for antitubercular treatment as they could be beneficial to relieve TB associated granuloma formation.

5. Acknowledgements

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