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DESIGN, SYNTHESIS AND *IN-VITRO* EVALUATION FOR ANTI-TUBERCULAR BY MABA METHOD FOR THE NOVEL DERIVATIVES OF AZETIDIN-2-ONE CONTAIN A POTENT PHENYL BENZOTHIAZOLE

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ABSTRACT

A Novel series of 2-Azetidinone heterocyclic derivatives containing a potent bioactive phenyl benzothiazole are designed by ***In-Silico*** tool using Mcule software and studied for their physicochemical and anti-tubercular properties by targeting on two of the protein structure of mycobacterium organism Inositol-3- phosphate synthetase (**PBI-1gro)** & Pantothenate synthetase (**PBI-1n2g**). Selected a good glide scoring compounds structure is synthesized by web lab method using some modified procedure. A series of six 2-Azetidine contain phenyl benzothiazole and its derivatives are prepared by cyclo-condensation process of prepared Schiff's base of phenyl benzothiazole with chloroacetic acid in rectified spirit using TEA (tri ethyl amine) as base catalyst by conventional way. The obtain compounds are purified, characterized by physical and analytical and evaluated for ***In-vitro*** Anti-tubercular activity by standard MABA (micro plate alamar blue assay) method. From the data it shows the compound PBA-4,5 & 6 exhibit good glide score of **-9.5, -9.6 & -9.8** and good probability of physico chemical property. All compound structure are confirmed from I.R, H-NMR and EI-MS data, all compounds exhibit good anti-tubercular activity but compound **PBA- 3** show potent and compound **PBA-4** show significant activity of MIC value **1.6 µg/ml** and **3.12 µg/ml** concentration against a selected standard Anti-TB drugs.

INTRODUCTION

Persuading the research activity in the field of drug design & development provides new and improved chemical entities for the successful treat in clinical practice. It is an area bridge between synthetic chemistry and molecular biology for design and develops active entities. The synthesized compounds are introduced as magic moiety in the late 19th century and their far-flung to 21th century. The relationship between chemical entity & its binding nature with the proteins gives a structure based relationship, which enhances the mode of action, are termed as lead compounds. The role of researcher is to Identify and develop those leads which can over come from cost and treatment effectiveness.[1,2]. Docking is a tool used to predict the structure of the molecule obtained from various constituents. And also predicts its property like permeability, bioavailability, partition coefficient, polar surface area, structural confirmations, toxicity, ADME, its interaction with macromolecules like proteins, carbohydrates and lipids in body. In endeavor to develop the structural and functional genomics which ensues to identify new proteins which can identified for possible drug targets. This arouses to invent a rapid and

accurate result by computer based over wet-lab high-throughput screens for drug discovery. The most approached method using now are computational screening methods known as molecular docking. This will predict the chemical structure of the ligand and its plausible binding affinity with protein. The molecular docking for the synthesized compounds are performed by using Mcule online soft ware follows docking vina 3.0. Azetidinone is a β-lactam tetra cyclic nitrogen and carbonyl group on ring substituted with some potent heterocyclic ring like benzothiazole or phenyl benzothiazole appraise some new products that possess interesting biological activities. Already It has been reported that azetidin-2-one its derivatives posses antimicrobial, antifungal, some enzymes inhibitions like human tryptase, chymase, thrombin, leukocyte elastase, human cytomegalovirus protease and serine protease enzyme activities, it also shows anti-TB, anti-inflammatory, anti-tumor, anti-HIV, anti-parkinsonism, anti-diabetic and vasopressin V1a antagonist activity.[3,4] To exhibit the potency of activity we contrive to synthesis a novel derivative of 2-azetidinone contain a potent phenyl benzothiazole by performing in docking studies and in wet lab it is synthesized and evaluated for *In-vitro* anti-tubercular activity by MABA method for to exploring its nature of cognizance.

MATERIALS AND METHODS:

The software tool Mcule is an online available of version 3 Docking vina, the physic chemical parameters and 3D docking score are determined from it. The chemicals employed in the titled work were purchased from Hi-media, Merck, Otto and SD fine chemicals of high grade. The melting point for the synthesized compounds were determined by open capillary method which are incorrect, all the synthesized compounds are characterized and identified by FT-IR by KBr method using SHIMADZU IR-Spirit FTIR spectrophotometer. Few compounds are characterized by ^{1}H-NMR by Bruker-AVN III 500 MHz using TMS as internal standard in DMSO-d6 solvent and Mass by EI-MS for confirmation studies. All Six Azetidin-2-one derivatives are proposed to evaluate *In-vitro* anti-tubercular activity by MIC using MABA method. The result shows compound PBA-3 which contain 2-Azetidinone ring with 1-N phenyl benzothiazole and 4th aryl group show 1.6 µg/ml and PBA-4 have 2-Azetidinone ring with 1-N phenyl benzothiazole and 4-OH, 3-OCH_3 aryl on 4th position show 3.12 µg/ml compared to standard.

Experimental process:

Step 1: Synthesis of different azomethine of phenyl benzothiazole from 2-(4-amino phenyl) benzothiazole: An equate amount of 0.01M of 2-(4-amino phenyl) benzothiazole in 20 ml of ethanol (previously prepared from 2-aminothiophenol, procedure adopted from Oztekin Algul *etal*, *Molecules 13 (*2008*)*736-748)[8] taken into 100ml RB flask placed on magnetic stirrer, to this is treated with 0.01M of substituted aryl aldehyde previously dissolved in 10ml of ethanol in small portion with constant stirring to get homogenize mixture, finally the mixture is refluxed to a condenser on water bath and heated at 85^{0}C, initially after 30 min of condensation added few drops of GAA as catalytic reagent and condensation was continue for 4-6 hrs, at the same temperature with occasional shaking. Later the reaction mixture is cooled and poured into a beaker containing crushed ice and stirred rapidly to precipitate out. The precipitate is filtered by using vacuum filtration and dried at room temperature, and finally the product was re-crystallized in appropriate solvent, dried to get desired azomethine derivatives contain phenyl benzothiazole.[9,11,13]

Step-2: Synthesis of Azetidin-2-one heterocyclic compounds contains potent phenyl benzothiazole from above obtain azomethine derivatives: Respective obtained azomethine derivative of phenyl benzothiazole, are taken about 0.01M in to a clean conical flask with 30 ml of ethanol, to this a double strength of 0.02M of acetyl chloride and a small amount of tri ethyl amine (TEA) as catalyst was added. Keep this mixture on boiling water bath attached to reflex condenser and continued the heating at 60-70^{0}C for 3 hrs or more based on reaction process was confirmed by performing TLC on timely based, finally the mixture was cooled and neutralized it by cold water to precipitate the desired product 2-azetidinone.

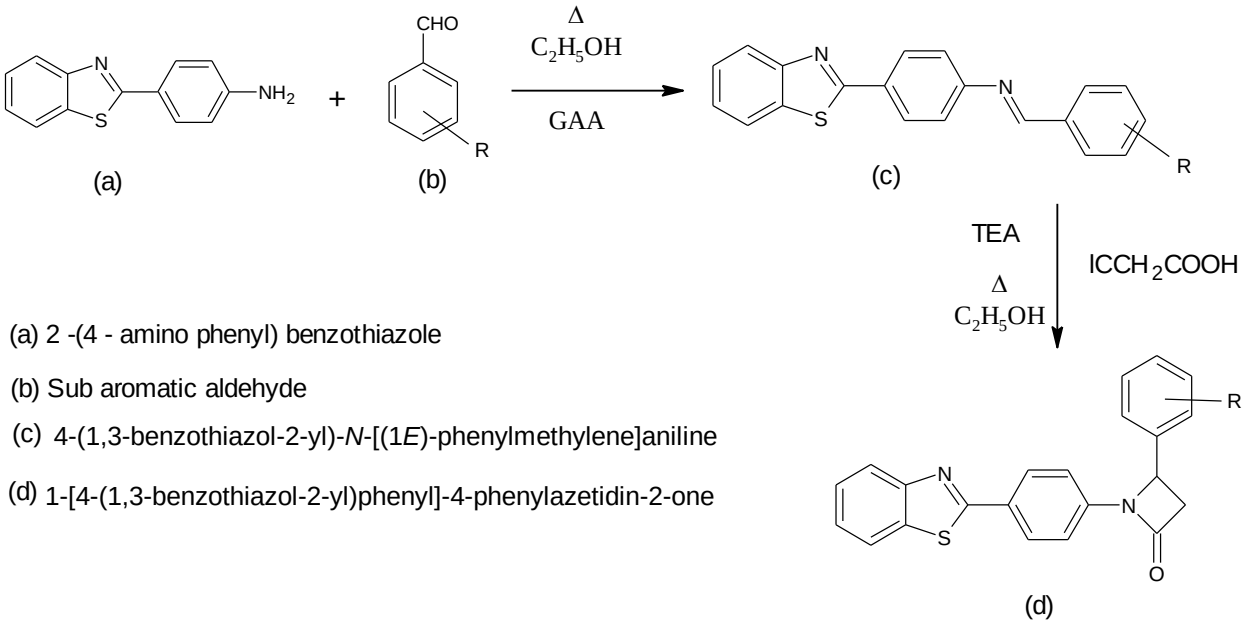


Fig 1: Scheme of Azetidin-2-one contain phenyl benzothiazole

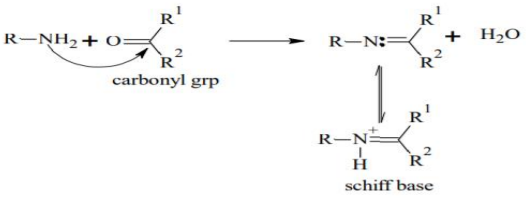


Fig 2: Mechanism of Imines formation

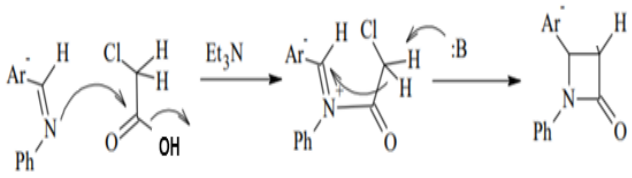


Fig 3: Mechanism of Azetidin-2-one formation from Imines

Spectroscopic characterization data of Azetidin-2-one containing phenyl benzothiazole and its derivatives:

APB-1: ***1-[4-(1,3-benzothiazol-2-yl) phenyl]-4-phenylazetidin-2-one.***

Mol form: $C_{22}H_{16}N_2SO$, **Mol wt(g):** 356, **IR υmax (cm^{-1}):** 1600(C=C); 1520(C-C); 1680(C=O); 1280(C-N); 1425 (C=N); 755(C-S). **^{1}H-NMR Spectra (δ value ppm in DMSO-d$_6$):** 2.20 (s, 1H –CH- of Azetidinone), 2.50 (s, 2H, -CH_2- Azetidinone), 7.35-8.48 (m, 13H, Aromatic, phenyl benzothiazole). **Mass (m/z)** (calculated- 356.44, observed – 357.82 (m+1).

APB-2: ***1-[4-(1,3-benzothiazol-2-yl)phenyl]-4-(4-hydroxy-3-methoxyphenyl)azetidin-2-one.***

Mol form: $C_{23}H_{18}N_2S\ O_3$, **Mol wt(g):** 403, **IR υmax (cm^{-1}):** 1610(C=C); 1525(C-C); 1690(C=O); 1270(C-N); 1425(C=N); 755(C-S); 1375(Ar-OH); 1130(Ar-OCH_3). **^{1}H-NMR Spectra (δ value ppm in DMSO-d$_6$):** 2.42 ((s, 1H –CH- of Azetidinone), 2.50 (s, 2H, -CH_2- Azetidinone), 3.42 (s, 3H, OCH_3), 10.23 (s, 1H, OH), 7.26-8.49 (m, 11H, Aromatic, phenyl benzothiazole). **Mass (m/z)** (calculated- 403.46, observed – 404.73 (m+1).

APB-3: ***1-[4-(1,3-benzothiazol-2-yl)phenyl]-4-(2-chlorophenyl)azetidin-2-one.***

Mol form: $C_{22}H_{15}N_2SOCl$, **Mol wt(g):** 391, **IR υmax (cm^{-1}):** 1600(C=C); 1575(C-C); 1680(C=O); 1275(C-N); 1425(C=N); 755(C-S); 760(Ar-Cl).

APB-4: ***1-[4-(1,3-benzothiazol-2-yl)phenyl]-4-(4-methoxyphenyl)azetidin-2-one.***

Mol form: $C_{23}H_{18}N_2SO_2$, **Mol wt(g):** 386, **IR υmax (cm^{-1}):** 1605(C=C); 1555(C-C); 1660(C=O); 1325(C-N) ; 1425(C=N); 755(C-S); 1170(Ar-OCH_3).

APB-5: ***1-[4-(1,3-benzothiazol-2-yl)phenyl]-4-(4-chlorophenyl)azetidin-2-one.***

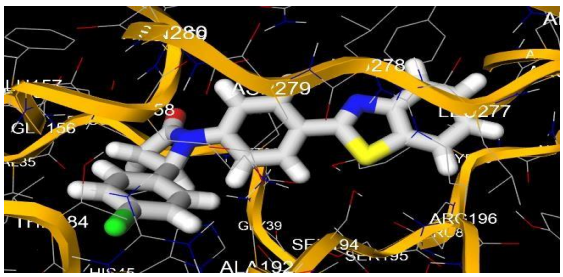
Mol form: $C_{22}H_{15}N_2SOCl$, **Mol wt(g):** 391, **IR υmax (cm^{-1}):** 1606(C=C); 1520(C-C); 1670 (C=O); 1260(C-N); 1425(C=N); 755(C-S). **^{1}H-NMR Spectra (δ value ppm in DMSO-d$_6$): 2.34 (s, 1H –**

CH- Azetidinone**), 2.50 (s, 2H, -CH2-** Azetidinone**), 7.16-8.49 (**m**, 12H,** Aromatic, phenyl benzothiazole**). Mass (m/z)** (calculated- 391.56, observed – 392.88 (m+1).
APB-6: ***1-[4-(1,3-benzothiazol-2-yl)phenyl]-4-(3,4-dimethoxyphenyl)azetidin-2-one.***
Mol form: $C_{24}H_{21}N_2SO_3$, **Mol wt(g):** 417, **IR υ_{max} (cm^{-1}):** 1600(C=C); 1510(C-C); 1670(C=O) 1320(C-N); 1425(C=N); 755(C-S); 1170(Ar-OCH_3).

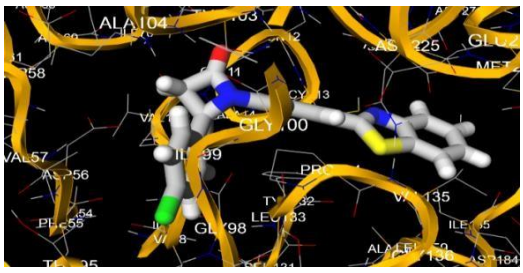
Table No 1
Physico chemical properties of Azetidin-2-one contain potent phenyl benzothiazole

Sl No	R-substituent	Melting Point – ^oC	R_f Values	clog P	H-BA	H-BD	Rotatable Bonds	Docking score (ΔG, kcal/mol)	
								1gro	1n2g
01	H	85	0.73*	3.506	3	1	3	-8.8	-8.6
02	3-OCH_3 , 4-OH	95	0.63*	3.225	5	1	4	-7.3	-9.3
03	2-Cl	75	0.69*	4.159	3	2	3	-8.4	-7.9
04	4-OCH_3	82	0.66**	3.514	4	1	4	-9.5	-9.3
05	4-Cl	92	0.57**	4.159	3	2	3	-9.3	-9.6
06	3,4-di OCH_3	90	0.52**	4.525	5	2	5	-9.5	-9.8

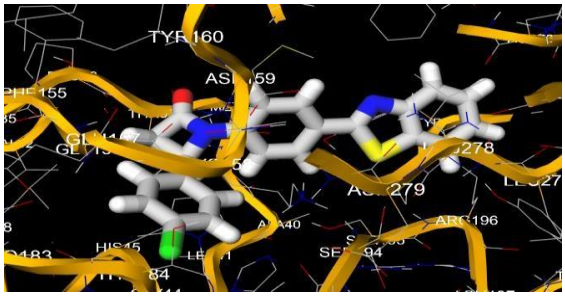
(Mobile phase: Cyclohexane : toluene (* 6:4 & **5:5), **PBI-** (**1gro) -** Inositol-3-phosphate synthetase & **(1n2g)** Pantothenate synthetase enzyme need for cell membrane formation of Mycobacterium tuberculosis organism)



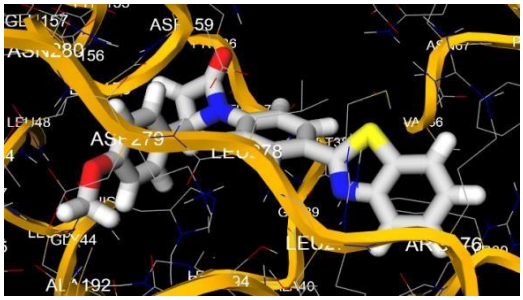
APB-4 on 1gro



APB-5 on 1n2g



APB-6 on 1gro



APB-6 on 1n2g

Figure 4: Docking view of compound APB-4,5and 6 on the enzyme surface of mycobacterium tuberculosis i.e Inositol-3-phosphate synthetase (**PBI-1gro**) & Pantothenate synthetase (**PBI-1n2g**) which is needed for integral protein synthesis on the cell wall formation of bacteria these are the good target site for ligands, compound APB-4 and 6 shows good interaction with the enzyme surface of glide score of -9.5 **ΔG, kcal/mol & compound APB-5 and 6 shows glide score of -9.6- -9.8 ΔG, kcal/mol.**

Table-2 - *In-vitro* Anti-Tubercular MIC by MABA method 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
01.	PBA-1	S	S	S	S	S	R	R	R
02.	PBA-2	S	S	S	S	S	R	R	R
03.	PBA-3	S	S	S	S	S	S	S	R
04.	PBA-4	S	S	S	S	S	S	R	R
05.	PBA-5	S	S	S	S	S	R	R	R
06.	PBA-6	S	S	S	S	R	R	R	R
07.	Isoniazid	S	S	S	S	S	S	R	R
08.	Pyrazinamide	S	S	S	S	S	S	S	R
09.	Streptomycin	S	S	S	S	S	R	R	R
10.	Ciprofloaxin	S	S	S	S	S	S	R	R
11.	Rifampicin	S	S	S	S	S	S	S	R

NOTE: S-Sensitive, R-Resistant, Strain used: *M.tuberculosis* (H37Rv strain): ATCC No-27294

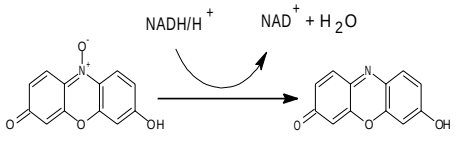
Contain phenyl benzothiazole and its derivatives. Dried it, re-purified it by DMF reagent and subjected for physico-chemical characterization studies.[5]

In-vitro Anti-Tubercular Activity by MABA method:

The Micro-plate Alamar blue assay (MABA) or Resazurin reduction assay is an florescence reduction assay of Alamar blue dye (chemically Resazurin) in microplate, it is highly sensitive and inexpensive method for assaying cell viability, toxicity or cellar growth of Mycobacterium species. The all synthesized Azetidin-2-one derivatives contain phenyl benzothiazole are evaluated for its anti-tubercular activity against *Mycobacterium tuberculosis* H37Rv strain by using a Middle brook 7H9 liquid broth media.[12]

Principle:

Resazurin (7-Hydroxy-3*H*-phenoxazin-3-one 10-oxide) is a weak fluorescent blue dye used as an oxidation-reduction indicator for cell viability estimation assay method. This Resazurin will irreversibly reduce to pink color Resorufin in the action produced by Mycobacterium tubercular bacteria. The color intensity is measured in fluorimeter.



oxidized state reduced state

Oxido-reductive process of Resazurin by NADH in *M.Tuberculosis*

Procedure: (J.C. Palomino and F. Portaels. Simple Procedure for Drug Susceptibility Testing of *Mycobacterium tuberculosis* Using a Commercial Colorimetric Assay, *Eur J Clin Microbiol Infect Dis* 18 (1999) 380–383).

CONCLUSION:

The novel series of six Azetidin-2-one derivatives contain potent phenyl benzothiazole are prepared by condensing 2-(4-amino phenyl) benzothiazole with substituted aryl aldehyde in ethanol medium under catalytic GAA to give azomethine derivatives of phenyl benzothiazole, and this further undergoes cyclo-condensation with chloroacetic acid in catalytic TEA in alcoholic medium to give titled compound of Azetidin-2-one its derivatives contain phenyl benzothiazole. All the synthesized compounds are re-crystallized, physical and spectral analysis are performed to identify the functional groups by FT-IR and characteristic proton signal from ^{1}H –NMR spectroscopy and EI-MS to determine the mass of the compounds. An *In-silico* analysis was done to predict the drug-likeness by follows Lipinski "rule-of-five were the compounds posses lipid solubility character whose clogP values are <5, hydrogen bond donor and acceptor within the limits and Molecular Docking was performed using Mcule docking software version 3.0 shows compound APB-4,5 & 6 shows good ligand-receptor interaction with selected Inositol-3-phosphate synthetase (**PBI-1gro**) & Pantothenate synthetase (**PBI-1n2g**) with glide score of average **-9.3 to -9.7**

ΔG, kcal/mol, this shows compounds have probable target interaction are identified. All the synthesized azetidin-2-one derivatives are evaluated for ***In-vitro*** anti-tubercular activity against ***Mycobacterium tuberculosis*** (H37Rv) strain by MABA method, compound code **APB-3** show potent activity at **1.6 µg/ml** concentration and compound **APB-4** shows significant activity at **3.12 µg/ml** concentration rest of the compounds shows moderate activity as compared to the selected standard anti-TB agents as shown in table no 2. Based on the obtain result some selected compound are further investigated for ***In-vivo*** activity in future aspect studies.

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