https://doi.org/10.48047/AFJBS.6.Si3.2024.1914-1934

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Molecular Modelling Studies, Synthesis, Structural Activity Relationship and Biological Evaluation of Benzothiazinone Derivatives as Potent Anti-tubercular and Anti-microbial Agents

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Article Info

Volume 6, Issue Si3, 2024

Received: 19 April 2024

Accepted: 28 May 2024

doi: 10.48047/AFJBS.6.Si3.2024.1914-1934

Abstract

Tuberculosis has proved harmful to the entire history of mankind from past several decades. Among the strategies for the development of new anti-tubercular lead compounds, Benzothiazinone (BTZ) were studied, with highly selective mechanism of action on DprE1 (Decaprenyl phosphoryl-b-D-ribose 2-epimerase) flavoenzyme. DprE1 is a vital enzyme for cell wall synthesis, plays a crucial role in the formation of D-arabinofuranose, a component of lipo-arabinomannan and arabinogalactam. Formation of covalent and non-covalent bond by the interacting ligand with the enzyme causes loss of its catalytic activity which ultimately leads to death of the mycobacterium

The Benzothiazinone derivatives kill the mycobacterium by inhibiting the essential flavoenzyme DprE1. This study involves designing of a series of 30 Benzothiazinone derivatives that binds non covalently to DprE1. These derivatives were subjected to energy minimization and molecular docking studies with Schrodinger software, from which the binding free energy calculations showed that the suggested compounds had better binding affinity with DprE1. Ten compounds

were selected for synthesis on the basis of docking score. The synthesized compounds were characterized and evaluated for their antimicrobial and anti-tubercular activity against different strains. Out of 7 evaluated compounds 3 compounds exhibited potent activity against microbial strain (*Staphylococcus aureus and Escherichia coli*) and 4 compounds exhibited potent activity against tubercular strains (H37RV).

BTZ4 and BTZ7 and BTZ30 has shown good activity against anti-microbial strain and BTZ8, BTZ9, BTZ17 and BTZ28 has shown good activity against anti-tubercular strain. On the basis of docking results, we concluded that the selected new series of Benzothiazinone hybrid derivatives BTZ4, BTZ7, BTZ8, BTZ9, BTZ17, BTZ28, BTZ30 act on the Enzyme DprE1, against tuberculosis binding protein with PDB ID: 4NCR, as they may have better binding sites and better energy values, when compared with the standard drugs Ampicillin, Isoniazid and Rifampicin. So these derivatives can be taken as best hit molecule and it could be useful for development of more new antimicrobial and anti-tubercular agents, blocking the mycobacterial cell wall formation.

Keywords: Tuberculosis, DprE1 enzyme, Docking analysis, Benzothiazinone, Anti-microbial activity, Anti-tubercular activity.

1. Introduction

Tuberculosis

Tuberculosis (TB) is an airborne contagious disease, caused by Mycobacterium bacilli or Mycobacterium tuberculosis (Mtb) that was discovered and isolated by Robert Koch in 1882. It is a rod-shaped bacillus of 1-4 µm length and 0.3-0.6 µm width. It generally attacks the lungs and is known as pulmonary tuberculosis. It is transmitted from person to person through droplets from cough or sneeze. It can also affect other organs, such as skeleton, soft tissue, lymph nodes and brain leading to extra pulmonary TB or it can disseminate through the blood vessels and affect multiple organs. It is the second reason for death on the planet from an infectious disease after human immunodeficiency infection (HIV).

They can be arranged into several major groups according to their treatment and diagnosis; alongside the previously mentioned Mycobacterium tuberculosis, additionally *M. bovis*, *M. aficanum, M. bovis*, *M. caprae, M. canetti and M. microti* have a place with the Mycobacterium tuberculosis complex (MTBC), and may cause TB diseases in humans and animals.

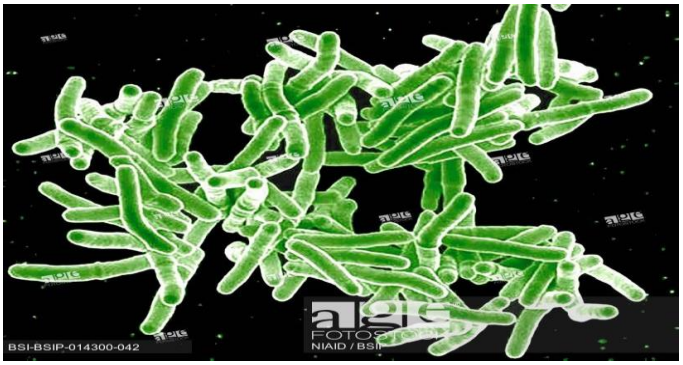


Figure 1: Scanning electron micrograph of Mycobacterium tuberculosis bacteria.

BTZ (Benzothiazinone) - A new class of anti-TB compounds having 1,3-benzothiazin-4-ones core (BTZs) has been recently disclosed. BTZ are the most promising, as it proved to kill *Mycobacterium tuberculosis in vitro, ex vivo*, and in a mouse models of TB infection and also responsible for blocking the synthesis of D-Arabinofuranose, a component of arabinogalactan and arabinomannan, with a mechanism of action highly selective in the cell wall of mycobacteria.

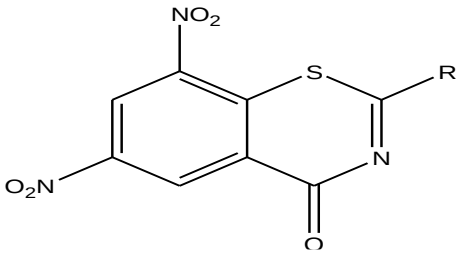
Benzothiazinones (BTZs), covalently binds to an active site with cysteine residue (Cys387 in *M. tuberculosis*, Cys394 in *Mycobacterium smegmatis*) in the essential enzyme DprE1, which catalyze biosynthesis of arabinose. Thus this enzyme losses its catalytic activity which ultimately leads to the death of mycobacteria. Thereby causing quantitative and irreversible inactivation.

Benzothiazinone inhibit the conversion of decaprenylphosphoryl-β-D-ribose(DPR) to decaprenylphosphoryl-β-D-arabinofuranose (DPA), a precursor of mycobacterial cell wall arabinan. This two-step epimerization reaction is catalyzed by the joint or successive action of the FAD-containing decaprenylphosphoryl-β-D-ribose 2'-epimerase (DprE1 or, Rv3790) and the NADH-dependent reductase DprE2 (Rv3791). BTZ inhibits the DprE1, thus provoking cell lysis and bacterial death.

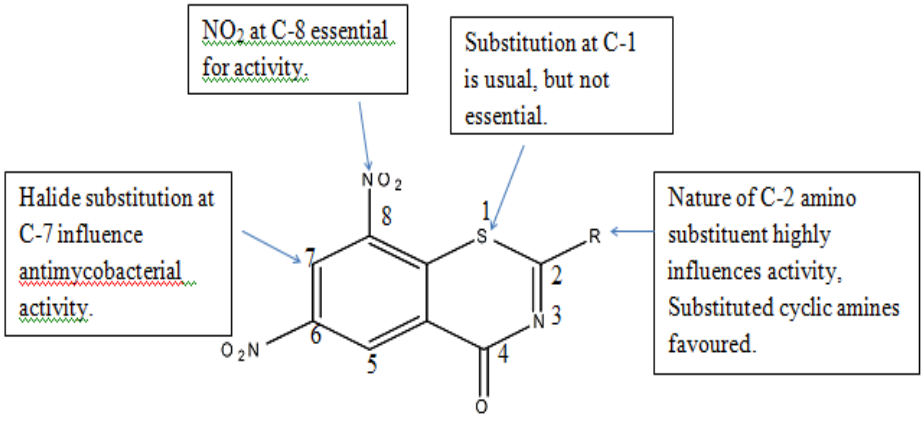
2. Materials And Method

2.1 Experimental

General Structure

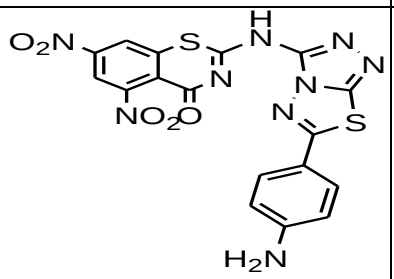
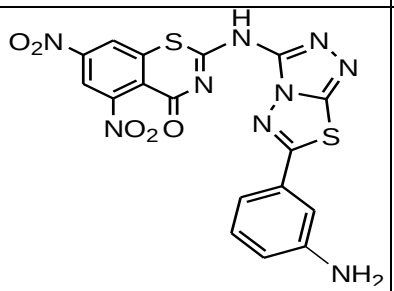
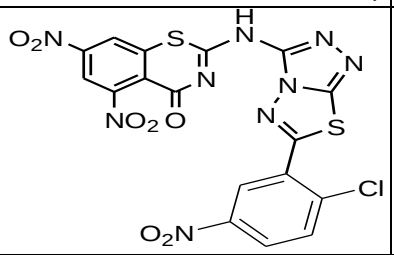
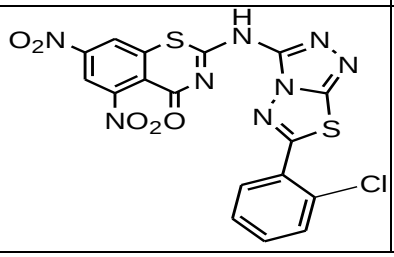


The present work addresses the synthesis of novel structural analogs of BTZ043. In particular, the chemical space at the arene moiety, introducing different substituents at positions 6 and 8 will be investigated. Furthermore, the influence of the position 2 substituent on BTZ activity will be investigated by introducing different cyclic amines as well as aryl and heteroaryl substituents.



Chemicals and reagents were purchased from Alfa Aesar and Chem Pura Enterprises Pvt. Ltd. All reactions were monitored by thin layer chromatography (TLC) on silica gel plates using DCM:Methanol (9:1) as eluent while UV lamp was used to visualize the spot. Melting points were determined by open capillary method and are uncorrected. IR spectra of the synthesized compounds was recorded on IR AFFINITY-1 1400 using KBr pellet technique. ^{1}H NMR spectra were recorded using JNM-ECX500FT NMR spectrometers using DMSO as solvent. Chemical shifts are expressed in δ ppm.

Table 1: Compound Code, Structure and IUPAC Name

Compound Code	Structure	IUPAC Name
BTZ4		2-[6-(4-Amino-phenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-ylamino]-5,7-dinitro-benzo[e][1,3]thiazin-4-one
BTZ7		2-[6-(3-Amino-phenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-ylamino]-5,7-dinitro-benzo[e][1,3]thiazin-4-one
BTZ8		2-[6-(2-Chloro-5-nitro-phenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-ylamino]-5,7-dinitro-benzo[e][1,3]thiazin-4-one
BTZ9		2-[6-(2-Chloro-phenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-ylamino]-5,7-dinitro-benzo[e][1,3]thiazin-4-one

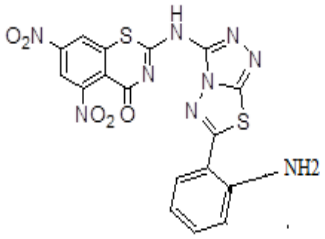
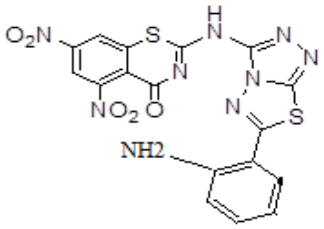
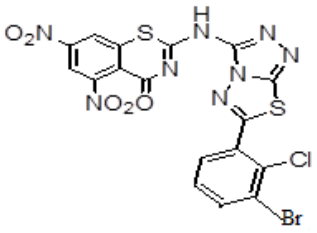
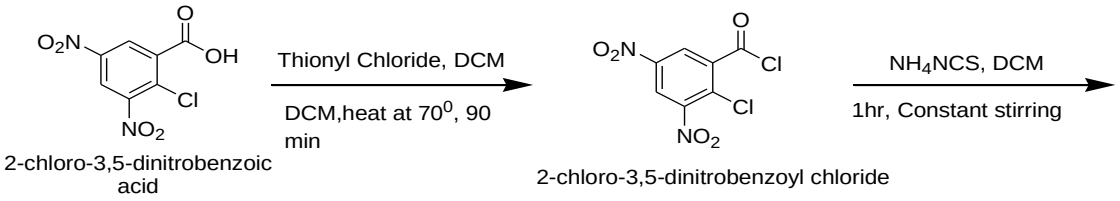
BTZ17		2-[6-(2-Amino-phenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-ylamino]-5,7-dinitro-benzo[e][1,3]thiazin-4-one
BTZ28		2-[6-(6-Amino-phenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-ylamino]-5,7-dinitro-benzo[e][1,3]thiazin-4-one
BTZ30		2-[6-(2-Chloro,3-bromo-phenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-ylamino]-5,7-dinitro-benzo[e][1,3]thiazin-4-one

Table 2: Docking Score, Glide emodel energy and RMSD of (BTZ1- BTZ30).

S.NO.	COMPOUND CODE	DOCKING SCORE	GLIDE EMODEL	RMSD
1	BTZ1	-8.893	-85.792	0.049
2	BTZ2	-6.528	-67.850	0.043
3	BTZ3	-7.063	-85.101	0.034
4	**BTZ4**	**-9.569**	**-99.185**	**0.042**
5	BTZ5	-6.928	-71.258	0.059
6	BTZ6	-8.282	-82.318	0.028
7	**BTZ7**	**-9.162**	**-84.914**	**0.038**
8	**BTZ8**	**-9.148**	**-92.617**	**0.042**
9	**BTZ9**	**-9.049**	**-95.031**	**0.005**
10	BTZ10	-5.782	-66.879	0.045
11	BTZ11	-6.246	-66.249	0.041
12	BTZ12	-5.685	-65.165	0.017
13	BTZ13	-6.755	-78.841	0.034
14	BTZ14	-6.300	-68.734	0.046
15	BTZ15	-5.146	-57.249	0.011
16	BTZ16	-5.345	-65.023	0.015

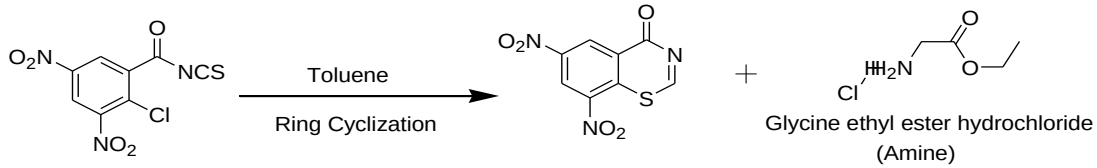
17	**BTZ17**	**-9.176**	**-94.111**	**0.023**
18	BTZ18	-4.582	-64.079	0.045
19	BTZ19	-5.158	-55.165	0.012
20	BTZ20	-6.400	-61.256	0.023
21	BTZ21	-6.755	-78.841	0.034
22	BTZ22	-6.300	-68.734	0.046
23	BTZ23	-5.582	-68.079	0.085
24	BTZ24	-5.345	-65.023	0.015
25	BTZ25	-5.245	-54.245	0.026
26	BTZ26	-6.356	-64.235	0.024
27	BTZ27	-4.782	-69.056	0.025
28	**BTZ28**	**-9.349**	**-91.031**	**0.008**
29	BTZ29	-4.154	-62.006	0.015
30	**BTZ30**	**-9.176**	**-94.111**	**0.023**
31	Delamanid	-5.041	35.783	0.012

- ***Reaction Scheme of Intermediate 1:***



2-chloro-3,5-dinitrobenzoic acid

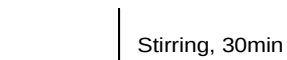
2-chloro-3,5-dinitrobenzoyl chloride

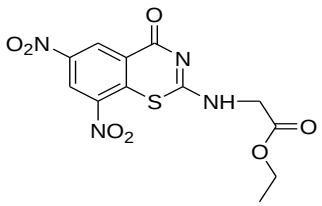


2-chloro-3,5-dinitrobenzoyl isothiocyanate

6,8-dinitro-4*H*-benzo[*e*][1,3]thiazin-4-one

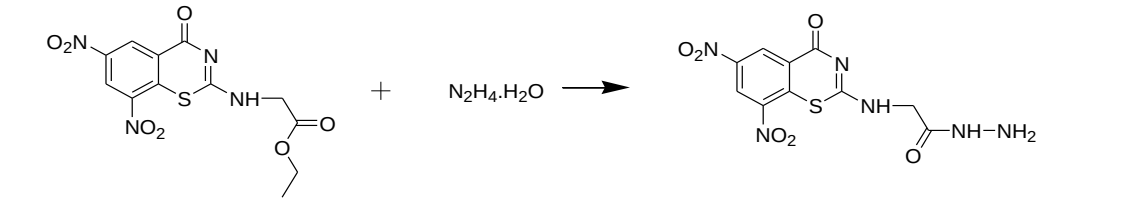
Glycine ethyl ester hydrochloride (Amine)





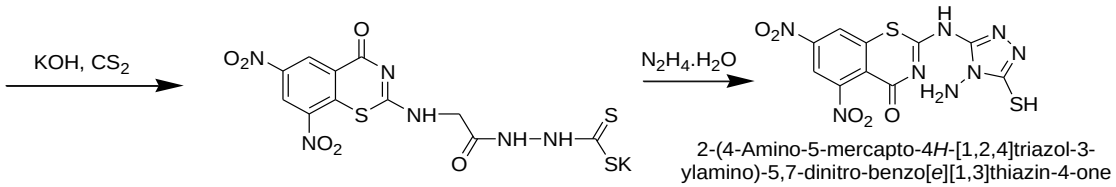
(6,8-Dinitro-4-oxo-4*H*-benzo[*e*][1,3]thiazin-2-ylamino)-acetic acid ethyl ester

- ***Reaction Scheme of Intermediate 2:***



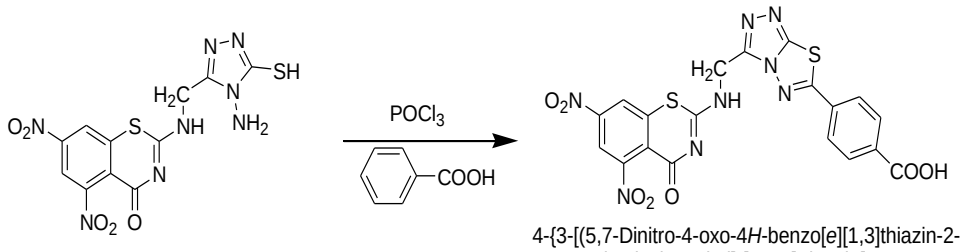
(6,8-Dinitro-4-oxo-4*H*-benzo[*e*][1,3]thiazin-2-ylamino)-acetic acid ethyl ester

(6,8-Dinitro-4-oxo-4*H*-benzo[*e*][1,3]thiazin-2-ylamino)-acetic acid hydrazide



2-(4-Amino-5-mercapto-4*H*-[1,2,4]triazol-3-ylamino)-5,7-dinitro-benzo[*e*][1,3]thiazin-4-one

- ***Reaction Scheme of Substitution:***



2-[(4-Amino-5-mercapto-4*H*-[1,2,4]triazol-3-ylmethyl)-amino]-5,7-dinitro-benzo[*e*][1,3]thiazin-4-one

4-{3-[(5,7-Dinitro-4-oxo-4*H*-benzo[*e*][1,3]thiazin-2-ylamino)-methyl]-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-6-yl}-benzoic acid

2.2 General Procedure for synthesis

2.2.1 Synthesis of (6,8-Dinitro-4-oxo-4H-benzo[e][1,3]thiazin-2-ylamino)-acetic acid ethyl ester (Intermediate 1)

To a RBF 2-Chloro-3,5-dinitro benzoic acid (2 g, 0.008 mole) in Dichloromethane (4-5 ml) with Thionyl chloride (0.58 ml, 0.004 mole) and catalyst DMF (2-3 ml) was added. The reaction mixture was heated at 70ºC for 90 min. Clear solution was obtained and all the acid was consumed, then Ammonium thiocyanate (0.6 g,0.001 mole) was added portion wise during 10 min time interval with constant stirring for further 1 hr. Then Glycine ethyl ester (1.1 g, 0.001 mole) was added and stirring was continued for next 30 min. The progress of reaction was monitored by TLC (DCM:Methanol 9:1) After completion of the reaction, the reaction mixture was mixed with saturated sodium bicarbonate solution and separated organic layer (DCM) was dried over anhydrous $MgSO_4$. DCM was removed under reduced pressure. The desired product obtained as brown solid; m.p: 330-335 ºC; yield: 92%.

2.2.2 Synthesis of 2-[(4-Amino-5-mercapto-4H-[1,2,4]triazol-3-ylmethyl)-amino]-5,7-dinitro-benzo[e][1,3]thiazin-4-one (Intermediate 2)

To a RBF, the intermediate ester (2 g, 0.0056 mole) was mixed with Hydrazine hydrate (0.28 ml, 0.0091 mole) in ethanol (2-3 ml) and stirring was done with reflux. To it, KOH (0.31 g, 0.0023 mole) dissolved in ethanol, and CS_2 (0.52 ml, 0.0091 mole) was added drop wise with stirring at

RT and maintained for 12 hr. The salt was formed, filtered and washed with ethanol 3 times. Then the intermediate was dissolved in hot water (2-3 ml) and Hydrazine hydrate (0.17 ml, 0.0068 mole) was added. This mixture was heated and refluxed for 3 hr. After completion of the reaction, the reaction mixture was poured into ice water and acidified with conc. HCl. The precipitate was filtered, washed with water. The desired product obtained as grey solid; m.p: 315-325 ºC; yield: 90%.

2.2.3 Synthesis of 2-[6-(4-Amino-phenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-ylamino]-5,7-dinitro-benzo[e][1,3]thiazin-4-one (BTZ4).

In a 100 ml RBF, a solution of Intermediate (100 mg, 0.0027 mole) was taken, added Phosphoryl Chloride (0.3 ml, 0.0082 mole) dropwise. To it added p-amino benzoic acid (0.2 g, 0.0047 mole) and the reaction mixture was stirred for 3 hrs reflux. After completion of reaction, the reaction mixture was poured into ice water and pH was adjusted to 8 with NaOH solution. The ppt was formed, filtered and washed 3 times with ethanol. The desired product obtained as cream color solid; m.p: 278-285 ºC; yield: 91%.

2.2.4 Synthesis of 2-[6-(3-Amino-phenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-ylamino]-5,7-dinitro-benzo[e][1,3]thiazin-4-one (BTZ7).

In a 100 ml RBF, a solution of Intermediate (140 mg, 0.0036 mole) was taken, added Phosphoryl Chloride (0.36 ml, 0.0099 mole) dropwise. To it added m-amino benzoic acid (0.23 g, 0.0082 mole) and the reaction mixture was stirred for 3 hrs reflux. After completion of reaction, the reaction mixture was poured into ice water and pH was adjusted to 8 with NaOH solution. The ppt was formed, filtered and washed 3 times with ethanol. The desired product obtained as light brown solid; m.p: 375-382 ºC; yield: 93%.

2.2.5 Synthesis of 2-[6-(2-Chloro-5-nitro-phenyl)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-3-ylamino]-5,7-dinitro-benzo[e][1,3]thiazin-4-one (BTZ8).

In a 100 ml RBF, a solution of Intermediate (130 mg, 0.0034 mole) was taken, added Phosphoryl Chloride (0.35 ml, 0.0097 mole) dropwise. To it added 2-Chloro,5-nitro benzoic acid (0.21 g, 0.0078 mole) and the reaction mixture was stirred for 3 hrs reflux. After completion of reaction, the reaction mixture was poured into ice water and pH was adjusted to 8 with NaOH solution. The ppt was formed, filtered and washed 3 times with ethanol. The desired product obtained as light brown solid; m.p: 266-274 ºC; yield: 92%.

2.2.6 Synthesis of 2-[6-(2-Chloro-phenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-ylamino]-5,7-dinitro-benzo[e][1,3]thiazin-4-one (BTZ9).

In a 100 ml RBF, a solution of Intermediate (200 mg, 0.0054 mole) was taken, added Phosphoryl Chloride (0.6 ml, 0.0164 mole) dropwise. To it added o-Chloro benzoic acid (0.4 g, 0.0096 mole) and the reaction mixture was stirred for 3 hrs reflux. After completion of reaction, the reaction mixture was poured into ice water and pH was adjusted to 8 with NaOH solution. The ppt was formed, filtered and washed 3 times with ethanol. The desired product obtained as brown solid; m.p: 392-395 ºC; yield: 95%.

2.2.7 Synthesis of 2-[6-(2-Amino-phenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-ylamino]-5,7-dinitro-benzo[e][1,3]thiazin-4-one(BTZ17).

In a 100 ml RBF, a solution of Intermediate (150 mg, 0.0038 mole) was taken, added Phosphoryl Chloride (0.37 ml, 0.0093 mole) dropwise. To it added o-amino benzoic acid (0.25 g, 0.0054 mole) and the reaction mixture was stirred for 3 hrs reflux. After completion of reaction, the reaction mixture was poured into ice water and pH was adjusted to 8 with NaOH solution. The ppt was formed, filtered and washed 3 times with ethanol. The desired product obtained as brown solid; m.p: 280-290ºC; yield: 93%.

2.2.8 Synthesis of 2-[6-(6-Amino-phenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-ylamino]-5,7-dinitro-benzo[e][1,3]thiazin-4-one(BTZ28).

In a 100 ml RBF, a solution of Intermediate (150 mg, 0.0038 mole) was taken, added Phosphoryl Chloride (0.37 ml, 0.0093 mole) dropwise. To it added 6-amino benzoic acid (0.25 g, 0.0054 mole) and the reaction mixture was stirred for 3 hrs reflux. After completion of reaction, the reaction mixture was poured into ice water and pH was adjusted to 8 with NaOH solution. The ppt was formed, filtered and washed 3 times with ethanol. The desired product obtained as light yellow solid; m.p: 255-268ºC; yield: 94%.

2.2.9 Synthesis of 2-[6-(2-Chloro,3-bromo-phenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-ylamino]-5,7-dinitro-benzo[e][1,3]thiazin-4-one(BTZ30).

In a 100 ml RBF, a solution of Intermediate (150 mg, 0.0038 mole) was taken, added Phosphoryl Chloride (0.37 ml, 0.0093 mole) drop wise. To it added 2-chloro, 3-bromo benzoic acid (0.25 g, 0.0054 mole) and the reaction mixture was stirred for 3 hrs reflux. After completion of reaction, the reaction mixture was poured into ice water and pH was adjusted to 8 with NaOH solution. The ppt was formed, filtered and washed 3 times with ethanol. The desired product obtained as yellow solid; m.p: 255-268ºC; yield: 92%.

2.3 Characterization of Synthesized Compounds

The identification and characterization of the prepared compounds were carried out by the following procedure to ascertain that the compounds were actually synthesized. Characterization was performed by the following methods:

2.3.1 Thin Layer Chromatography:

Thin Layer Chromatography is a method for analyzing mixtures by separating the compounds in the mixture. TLC can be used to determine the number of components in a mixture, the identity of compounds, and the purity of a compound. While observing the appearance of a product or the disappearance of a reactant, it can also be used to monitor the progress of a reaction.

Thin Layer Chromatography was carried out with silica gel plates (silica gel 60 F_{254}), and DCM:Methanol (9:1) or Chloroform:Ethanol (7:3) was used as mobile phase. R_f value of synthesized compounds are reported in Table 3. The differences in R_f values between starting compounds and the product were indicative of the transformation of starting compound to product and also purity of compounds.

Table 3: R_f value with solvent system of synthesized compounds.

Compound Code	Rf Value	Solvent System
BTZ1	0.366	Chloroform:Ethanol (7:3)
BTZ2	0.697	Chloroform:Ethanol (7:3)
BTZ3	0.538	Chloroform:Ethanol (7:3)
BTZ4	0.363	Ethyl acetate:Hexane(3:7)
BTZ5	0.346	Chloroform:Ethanol (7:3)
BTZ6	0.560	Chloroform:Ethanol (7:3)
BTZ7	0.625	Ethyl acetate:Hexane(3:7)
BTZ8	0.304	Chloroform:Ethanol (7:3)
BTZ9	0.608	Chloroform:Ethanol (7:3)
BTZ10	0.426	Ethyl acetate:Hexane(3:7)
BTZ11	0.527	Chloroform:Ethanol (7:3)
BTZ12	0.426	Chloroform:Ethanol (7:3)
BTZ13	0.459	Ethyl acetate:Hexane(3:7)
BTZ14	0.298	Chloroform:Ethanol (7:3)
BTZ15	0.611	Chloroform:Ethanol (7:3)
BTZ16	0.211	Chloroform:Ethanol (7:3)
BTZ17	0.689	Ethyl acetate:Hexane(3:7)
BTZ18	0.546	Chloroform:Ethanol (7:3)
BTZ19	0.247	Chloroform:Ethanol (7:3)
BTZ20	0.364	Chloroform:Ethanol (7:3)
BTZ21	0.542	Ethyl acetate:Hexane(3:7)
BTZ22	0.338	Ethyl acetate:Hexane(3:7)
BTZ23	0.521	Ethyl acetate:Hexane(3:7)
BTZ24	0.423	Chloroform:Ethanol (7:3)
BTZ25	0.659	Chloroform:Ethanol (7:3)
BTZ26	0.596	Ethyl acetate:Hexane(3:7)
BTZ27	0.457	Chloroform:Ethanol (7:3)
BTZ28	0.676	Ethyl acetate:Hexane(3:7)
BTZ29	0.359	Chloroform:Ethanol (7:3)
BTZ30	0.697	Ethyl acetate:Hexane(3:7)

2.3.2 Melting Point:

The melting point determination was done in melting point apparatus and are uncorrected. The melting points of all the synthesized compounds are reported in Table 4.

2.3.3 cLogP values:

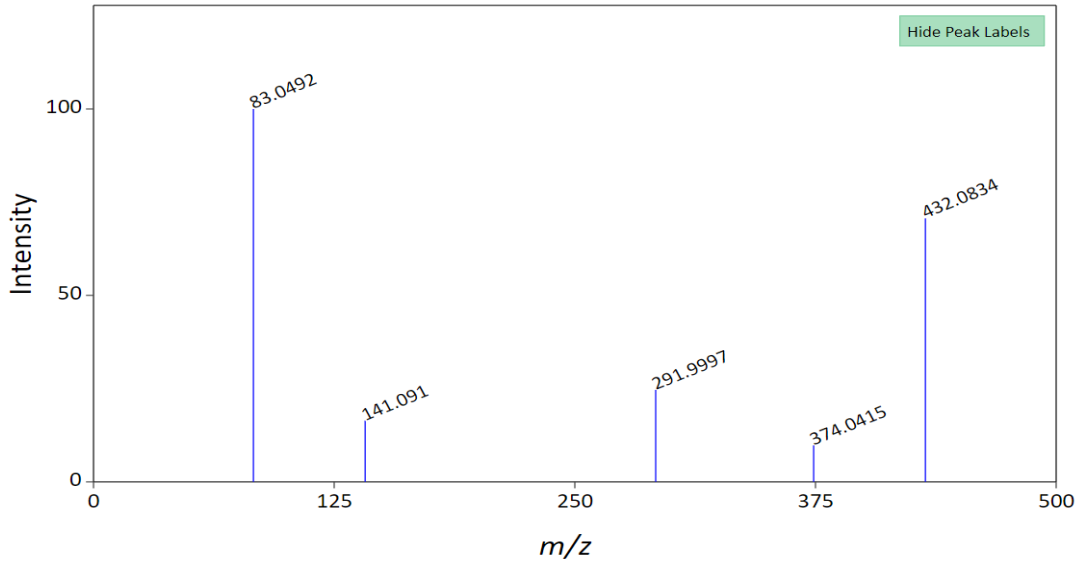
The cLogPValues, the indicative of hydrophobicity, were predicted for all the derivatives using CS ChemOffice- 2004 version 8.0. The cLogP values are reported in Table 4.

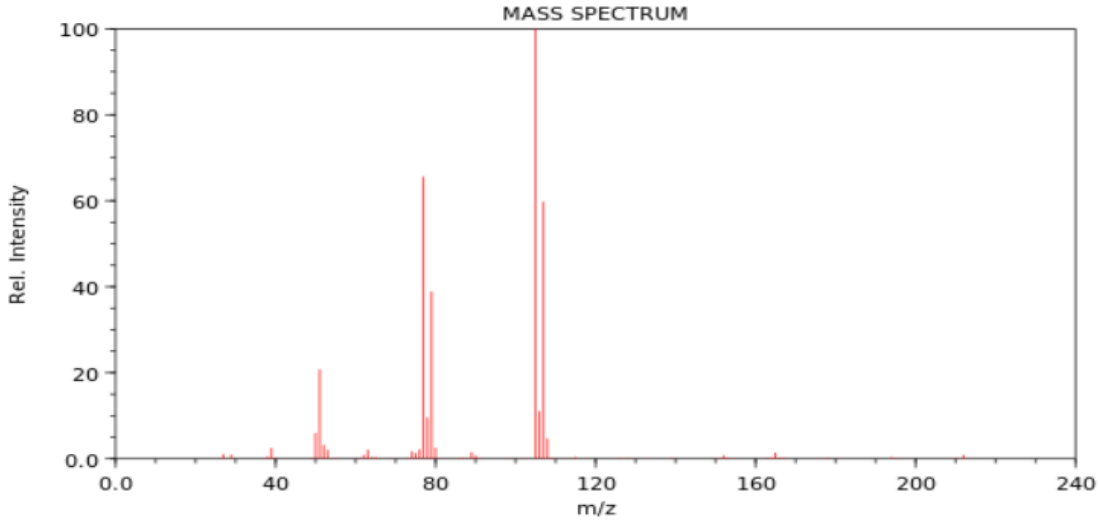
Table 4: Molecular Formula, Molecular Weight, cLogP and Melting Point.

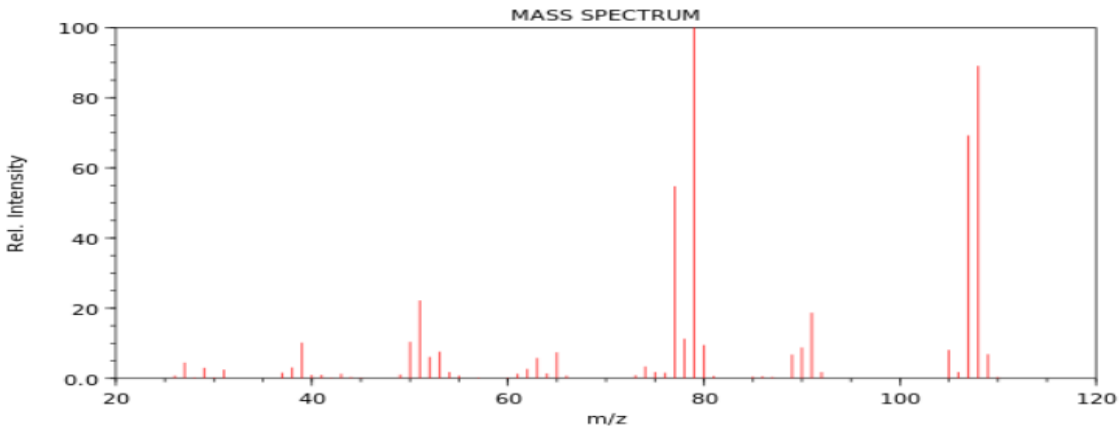
Compound Code	Molecular Weight	Molecular Formula	cLogP	Melting Point
BTZ1	512.44	$C_{18}H_8N_8O_7S_2$	2.599	280-285 ºC
BTZ2	537.32	$C_{17}H_6Cl_2N_8O_5S_2$	4.018	320-328 ºC
BTZ3	500.42	$C_{17}H_8N_8O_7S_2$	1.663	245-260 ºC
BTZ4	483.44	$C_{17}H_9N_9O_5S_2$	1.655	278-285 ºC
BTZ5	513.42	$C_{17}H_7N_9O_7S_2$	2.585	315-326 ºC
BTZ6	502.87	$C_{17}H_7ClN_8O_5S_2$	3.555	364-376 ºC
BTZ7	483.44	$C_{17}H_9N_9O_5S_2$	1.625	375-382 ºC
BTZ8	547.87	$C_{17}H_6ClN_9O_7S_2$	1.048	266-274 ºC
BTZ9	502.87	$C_{17}H_7ClN_8O_5S_2$	1.305	392-395 ºC
BTZ10	517.89	$C_{17}H_8ClN_9O_5S_2$	2.372	255-268ºC
BTZ11	547.89	$C_{17}H_7N_9O_7S_2$	2.348	275-289ºC
BTZ12	513.44	$C_{17}H_7ClN_8O_5S_2$	2.456	290-315ºC
BTZ13	479.32	$C_{17}H_9N_9O_5S_2$	4.123	300-320ºC
BTZ14	487.42	$C_{17}H_6ClN_9O_7S_2$	1.278	260-275ºC
BTZ15	427.44	$C_{17}H_7ClN_8O_5S_2$	1.478	280-295ºC
BTZ16	511.42	$C_{17}H_8ClN_9O_5S_2$	2.489	286-295ºC
BTZ17	508.87	$C_{17}H_6Cl_2N_8O_5S_2$	1.004	280-290ºC
BTZ18	412.44	$C_{17}H_8N_8O_7S_2$	4.235	245-260ºC
BTZ19	528.87	$C_{17}H_9N_9O_5S_2$	4.245	255-268ºC
BTZ20	502.87	$C_{17}H_7N_9O_7S_2$	3.247	275-280ºC
BTZ21	512.89	$C_{17}H_6ClN_9O_7S_2$	2.147	292-300ºC
BTZ22	510.44	$C_{17}H_7ClN_8O_5S_2$	3.058	285-295ºC
BTZ23	525.32	$C_{17}H_8ClN_9O_5S_2$	4.028	295-310ºC
BTZ24	550.42	$C_{17}H_7N_9O_7S_2$	3.789	265-280ºC
BTZ25	465.44	$C_{17}H_7ClN_8O_5S_2$	2.456	310-320ºC
BTZ26	486.42	$C_{17}H_6Cl_2N_8O_5S_2$	4.017	285-292ºC
BTZ27	524.87	$C_{17}H_8N_8O_7S_2$	4.587	265-280ºC
BTZ28	456.44	$C_{17}H_9N_9O_5S_2$	2.012	255-268ºC
BTZ29	478.87	$C_{17}H_7N_9O_7S_2$	2.945	285-295ºC
BTZ30	554.87	$C_{17}H_8ClN_9O_5S_2$	1.927	255-268ºC

2.3.4 Mass Spectroscopy:

Mass spectra analysis was recorded using WATERS Q-TOF Micromass Spectrometer with an ESI source as m/z fragmentation pattern for molecular ion peak determination at Panjab University, Chandigarh.







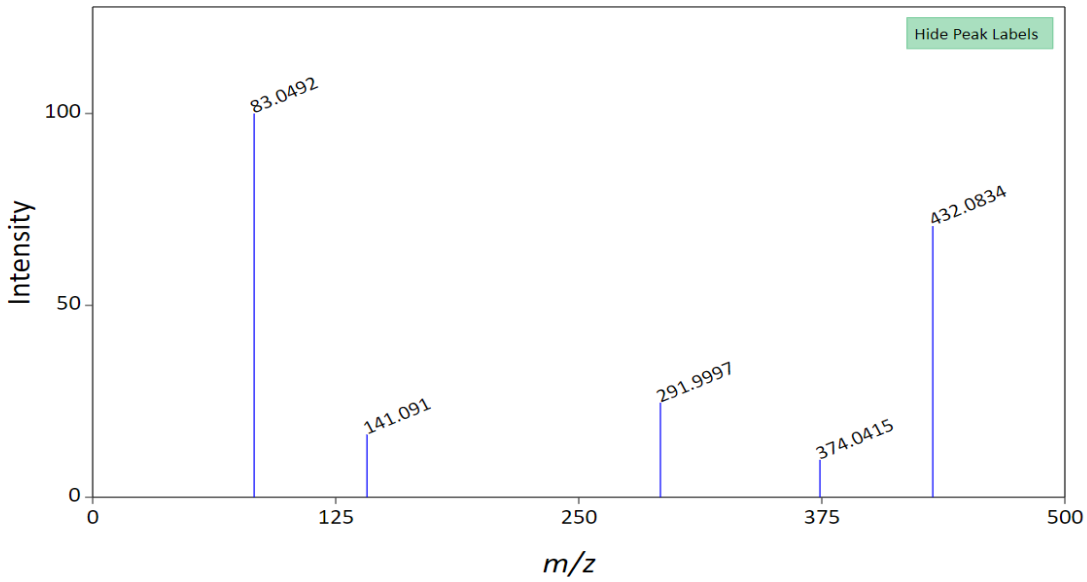


Figure 2: Mass Spectra of the compound (BTZ 4, BTZ 7, BTZ 28 & BTZ 30).

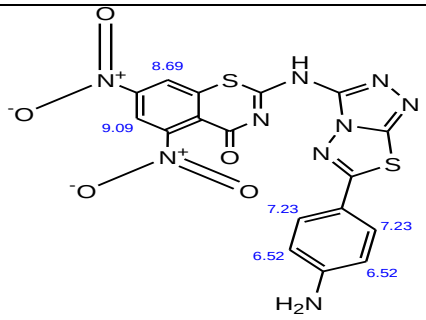
2.3.5 ^{1}H NMR Spectroscopy:

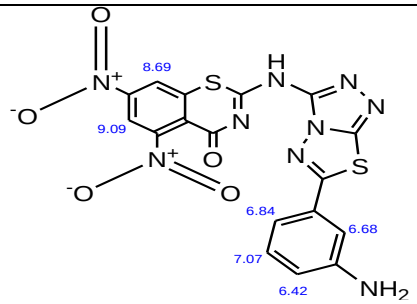
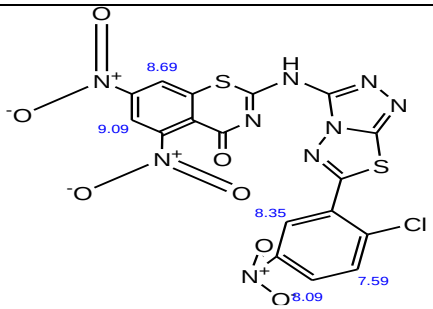
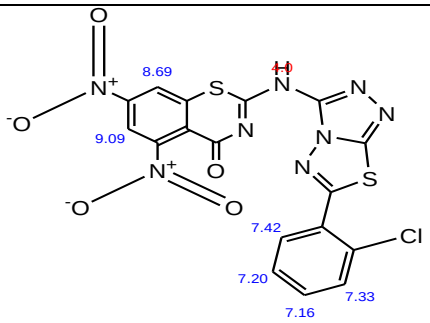
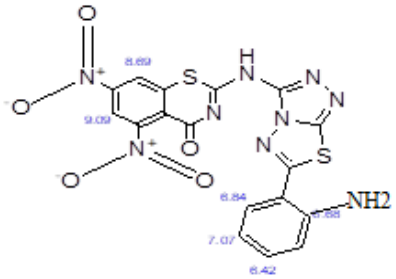
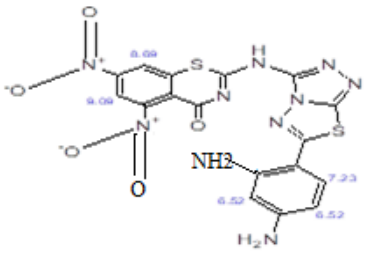
^{1}H NMR spectra were recorded using JNM-ECX500FTNMR spectrometers using DMSO as solvent at Dr. Harisingh Gour University, Sagar.^{1}H NMR data of compounds are summarized in Table 5.

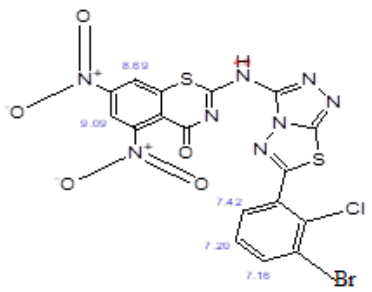
2.3.6 IR Spectroscopy:

The Infrared spectroscopy of all the synthesized compounds were recorded on IR AFFINITY-1 1400 using KBr pellet technique were carried out from Shreeji Analytical and Research Laboratory Pvt. Ltd, Indore and are expressed in cm^{-1}. The IR data of the compounds are summarized in Table 5.

Table 5: ^{1}H NMR, and IR Prediction Values

Compound Code	Interpretation of NMR (ppm)	Interpretation of IR (cm^{1})
BTZ4		3350-3200 NH_2 Bending, 3000-2800 N-H Stretching, 2900-2800 C-H Stretching, 1690 C=O Stretching, 1600-1500 N-O Stretching.

BTZ7		3450-3300 C-H Stretching, 3400 N-H Stretching,3200-3100 NH_2 Bending, 2000-1500 C-H Bending, 1550-1500 N-O Stretching, 800 C-H Bending, 1650 C=O Stretching.
BTZ8		3500 N-H Stretching, 1690-1640 C=N Stretching, 1550-1500 N-O Stretching, 850-700 C-Cl Stretching, 800 C-H Bending 1290 C=S Stretching.
BTZ9		1600-1300 N-O Stretching, 1300-1200 C-N Stretching, 900-750 C-H Bending, 850-500 C-Cl Stretching, 1250 C=S 800 C-H Bending
BTZ17		3450-3300 NH_2 Bending, 1600-1300 N-O Stretching, 1300-1200 C-N Stretching, 900-750 C-H Bending, 850-500 C-Cl Stretching, 1250 C=S.
BTZ28		3200-3000 NH_2 Bending, 2900-2800 N-H Stretching, 2800-2700 C-H Stretching, 1600 C=O Stretching, 1500-1400 N-O Stretching

BTZ30		3500 N-H Stretching, 1690-1640 C=N Stretching, 1550-1500 N-O Stretching, 850-700 C-Cl Stretching, 800 C-H Bending 1290 C=S Stretching.

In the IR spectra, the relatively strong peaks at 3350-3200 cm^{-1} were attributed to the NH_2 bending vibrations and the weak single peaks at 1600-1500 cm^{-1} due to the N-O stretching vibrations for the compound BTZ4. However in BTZ8, the relatively strong peaks at 3500 cm^{-1} were attributed to the N-H stretching vibrations and the weak single peaks at 1550-1500 cm^{-1} due to the N-O stretching vibrations and C-Cl stretching peaks at 850-700 cm^{-1}

The ^{1}H NMR spectra of the benzothiazinone derivative (BTZ4) showed two characteristic proton signals at 8.69 and 9.09 ppm, which were attributed to the protons of N-O group and doublet proton signals at 7.23 and 6.52 ppm which may be due to C-H group. However in BTZ8, the characteristic proton signal at 8.09 and 7.59 ppm which may be due to N-O and C-Cl group respectively.

The mass spectra (m/z ratio, $M,^{+}$ & M^{+}+ 1 peaks) of the Benzothiazinone derivatives (BTZ 4, BTZ 7, BTZ 28 & BTZ 30) was found to be: FAB-MS (m/z):387[M^{+}], 388 [M^{+}+1], FAB-MS (m/z):405[M^{+}], 406[M^{+}+ 1], FAB-MS (m/z):403[M^{+}], 404[M^{+}+1], FAB-MS (m/z):448[M^{+}], 449[M^{+}+1].

2.4. Evaluation of Anti-microbial and Anti-tubercular Activity of Synthesized Compound

2.4.1 *Anti-microbial Activity*

The antimicrobial activity was performed on human pathogenic bacteria (*Staphylococcus aureus, Escherichia coli*). In assaying antibiotic activity, by Cup borer method, the test organism was grown on a suitable complete agar medium in Petri dishes. “Cups” cut out of the agar were filled with appropriate dilutions of a standard compound solution and of the test compound. After incubation the cups are found to be surrounded by circular “Zone of inhibition”. The Zone of inhibition was measured in terms of area in mm^2 as shown in Table 6 and compared with the area of whole quadrant to find out 50% inhibition compared with the standard drug Ampicillin.

Table 6: Zone of Inhibition of Synthesized Compound.

COMPOUND CODE	STAPHYLOCOCCUS AUREUS GRAM (+)	ESCHERICHIA COLI GRAM (-)
BTZ4	17mm	19mm
BTZ7	21mm	24mm
BTZ30	18mm	16mm
Ampicillin	24mm	22mm

2.4.2 ***Anti-tubercular activity***

2.4.2.1 ***Culture***

Tubercle bacilli are aerobes, grow slowly (generation time 14-15 hrs). optimum temperature 37^0C, pH 6.4-7.0. They grow only in specially enriched media containing egg, asparagine, potatoes, serum and meat extracts. Colonies appear in 2-6 weeks. *M. tuberculosis* grows more luxuriantly in culture (eugenic) than *M.bovis* which grows sparsely (dysgenic). The drug susceptibility test may be performed by either the direct or the indirect method. The direct drug susceptibility test is performed by using a subculture from a primary culture as the inoculum.

2.4.2.2 ***Determination of minimal inhibition concentrations by L.J Slope method.***

Materials and Method

1. All the synthesized drugs were used for anti-tubercle test procedures
2. All Necessary controls like:

- Drug control
- Vehicle control
- Agar control
- Organisms control
- Known antibacterial drugs control
- *M.tuberculosis* H37 RV cultures were tested against above mentioned known and unknown drugs.
- LJ was used as nutrient medium to grow and dilute the drug suspension for the test.
- Inoculum size for test strain was adjusted to 1mg/ml.
- Following common standard strain is used for screening of anti-tubercle activities: the strains were procured from institute of microbial technology, Chandigarh. *Mycobacterium tuberculosis* H37Rv (Acid Fast Bacilli) MTCC- 200 DMSO was as diluents/ Vehicle to get desired concentration of drugs to test upon Standard bacterial strains.

2.4.2.3 Methods used for Primary and Secondary Screening:

Each synthesized drug was diluted obtaining 2000 microgram/ml concentration, as a stock solution.

Primary screen: In primary screening 500 microgram/ml, 250 microgram/ml and 125 microgram/ml concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms.

Secondary screen: The drugs found active in primary screening were similarly diluted to obtain 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.250 µg/ml, 3.125 µg/ml and 1.5625 µg/ml concentrations.

Reading Result: The highest dilution showing at least 99 % inhibition is taken as MIC. The result of this is much affected by the size of the inoculum. The test mixture should contain 10^8organism/ml.

Table 7: IC_{50} Values of synthesized compound for anti-tubercular activity**.**

ANTITUBERCULOSIS ACTIVITY TABLE			
METHOD		L.J. MEDIUM (CONVENTIONAL METHOD)	
BACTERIA		H37RV	
STANDARD DRUG		ISONIAZID, RIFAMPICIN	
S.NO	**CODE NO**	**MIC µg/ml**	**REMARKS**
1	BTZ8	0.28	ISONIAZID = 0.39 µg/ml
2	BTZ9	0.37	RIFAMPICIN=0.48 µg/ml
3	BTZ17	0.31	
4	BTZ28	0.33	

3. Results and Discussion

3.1 Molecular docking

The main aim of the present study is to identify and optimize leads of benzothiazinone derivatives against DprE1 as new antitubercular agents. Thirty derivatives of benzothiazinone were designed. Glide score was obtained using GLIDE module (Grid based Ligand docking with Energetics, version Schrodinger 9.1, LLC, New York, 2010) at the CADD laboratory, S.G.S.I.T.S. Indore.

After preparation of ligand and protein, protein grid was generated by setting the grid box in which the ligands were expected to dock. Molecular docking of the designed compounds was performed to study the binding pattern of the structure with the protein. The docking studies were performed using protein (PDB ID; 4NCR)

Best docking scores of compounds were compared using docking score. The data shown in the given table revealed that all the designed compounds shows a prominent result as antitubercular and antimicrobial activity, but some compounds BTZ4, BTZ7, BTZ8, BTZ9, BTZ17, BTZ28, BTZ30 showed best Glide docking score as compared to other derivatives, which is compared with the Standard drugs (Ampicillin, Isoniazid and Rifampicin).

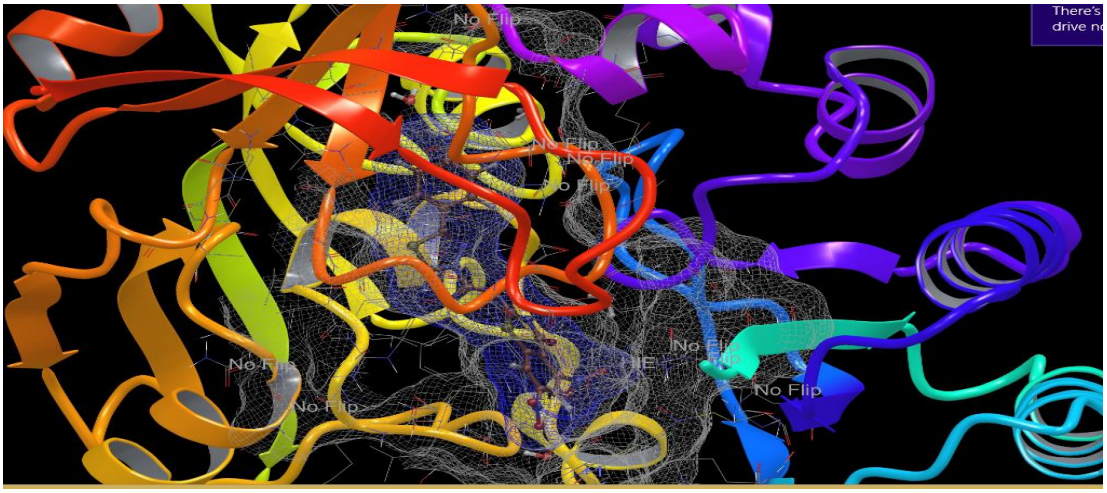


Figure 3: Best Binding Pose on Protein 4NCR of Compound (BTZ 4)

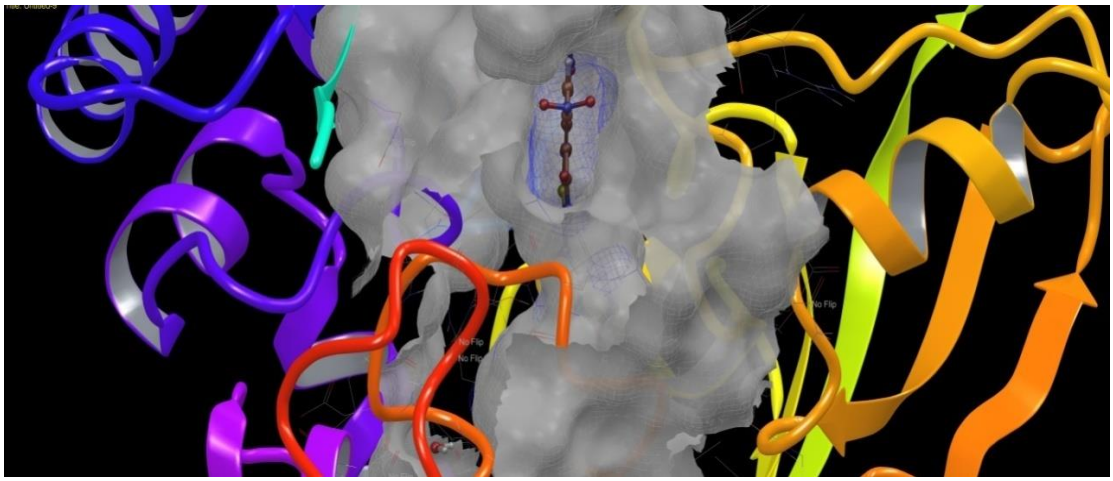


Figure 4: Best Binding Pose on Protein 4NCR of Compound (BTZ 7).

Structure based docking strategy was carried out using the poses predicted by Glide. All the ligands were docked within the binding site of the bacterial protein. Analysis of the docking result clarify that interaction of ligand with residue, is the crucial parameter for the inhibition of DprE1 (decaprenylphosphoryl- β-D-ribose 2'-epimerase) enzyme. Benzothiazinone derivatives form covalent or non-covalent binding with Cysteine C387 and Tyrosine Ty38c residue of the enzyme. In the molecular docking studies the compound BTZ 4 & BTZ 7 has shown good interaction with protein residue and glide properties.

3.2 Antimicrobial and Antitubercular activity

The antimicrobial activity was performed on human pathogenic bacteria (*Staphylococcus aureus, Escherichia coli*). In assaying antibiotic activity by Cup borer method, the test organism was grown on a suitable complete agar medium in Petri dishes. "Cups" cut out of the agar were filled with appropriate dilutions of a standard compound solution and of the test compound. After incubation the cups are found to be surrounded by circular "Zone of inhibition". The Zone of inhibition was measured in terms of area in mm^2 as shown in Table 6 and compared with the area of whole quadrant to find out 50% inhibition compared with the standard drug Ampicillin.

Out of 7 evaluated compounds 3 compounds exhibited potent activity against microbial strain (*Staphylococcus aureus and Escherichia coli*) and 4 compounds exhibited potent activity against tubercular strains (H37RV). BTZ4 and BTZ7 and BTZ30 has shown good activity against anti-microbial strain and BTZ8, BTZ9, BTZ17 and BTZ28 has shown good activity against anti-tubercular strain. The evaluation was carried out at New Alpha Micro Laboratory, Bhopal.

4. Conclusion

The main aim of the present study is to design various substituted benzothiazinone derivatives. On the basis of literature review, we envisaged that benzothiazinone derivatives have potentiated that non covalently react with the cysteine 387(C387) residue of DprE1 enzyme, which catalyze biosynthesis of arabinose. Formation of covalent and non-covalent bond by the interacting ligand with the enzyme causes loss of its catalytic activity which ultimately leads to the death of mycobacteria.

The Benzothiazinone derivatives kills the mycobacterium by inhibiting the essential flavoenzyme DprE1. This study involves designing of a series of 30 benzothiazinone derivatives that binds non-covalently to DprE1. These derivatives were subjected to energy minimization and molecular docking studies with Schrodinger software, from which the binding free energy calculations showed that the suggested compounds had better binding affinity with DprE1.

All the compounds were synthesized by conventional method. The synthesis was done under standard condition using AR grade reagents and the reaction progress was monitored by TLC. Structural conformation was done by IR spectroscopy, NMR and Mass spectroscopy. The synthesized compounds were evaluated for their anti-microbial and anti-tubercular activity against different strains. Out of 7 evaluated compounds 3 compounds exhibited potent activity against microbial strain (*Staphylococcus aureus and Escherichia coli*) and 4 compounds exhibited potent activity against tubercular strains (H37RV). BTZ4 and BTZ7 and BTZ30 has shown good activity against anti-microbial strain and BTZ8, BTZ9, BTZ17 and BTZ28 has shown good activity against anti-tubercular strain.

On the basis of docking results, we concluded that the selected new series of benzothiazinone hybrid derivatives BTZ4, BTZ7, BTZ8, BTZ9, BTZ17, BTZ28 and BTZ30 act on the Enzyme DprE1, against tuberculosis binding protein with PDB ID: 4NCR, as they may have better binding sites and better energy values, when compared with the standard drug Ampicillin, Isoniazid and Rifampicin. So these derivatives can be taken as best hit molecule and it could be useful for development of more new antimicrobial and antitubercular agents, blocking the mycobacterial cell wall formation.

5. References

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