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SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL EVALUATION OF HYDRAZIDE BASED 1,3-BENZOXAZOLE DERIVATIVES AS ANTITUBERCULAR AGENTS

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ABSTRACT

The purpose of present research work to synthesize hydrazide based Benzoxazole derivatives, the hydrazide group was achieved by treatment of corresponding Ester with hydrazine hydrate in methanol at room temperature. Benzoxazole have extraordinary potential in biological activity. Benzoxazole is prepared by microwave assisted reaction and hydrazide compound react with substituted benzoic acid and other reactants to give desired products. The substituted benzoic acid compounds get cyclized to give (1,3)-cyclazolo- (4,5-pthalazine-5)6H)-one compounds. The synthesized compounds were characterized by spectroscopy techniques like IR, MASS and evaluate their physical constant and purity by TLC. The target compounds were in-vitro evaluated against M. tuberculosis using H37Rv strain. The activity screened by MABA using pyrazinamide as standard and DMSO as control. The majority of compounds shows their potential with MIC range 3.25ug/ml. All compounds shows prominant activity except two compounds.

Keywords: Anti-tubercular, Benzoxazole, MABA, Hydrazide, H37Rv

INTRODUCTION

Tuberculosis (TB) is one of the common, contagious, and fatal disease. Tuberculosis is public health threats due to develop resistance to drugs **[1]**. It is potentially serious infectious bacterial disease which mainly affects the lungs. The bacteria primarily attack on lungs and also other organs of body. TB is air-bonding disease

and spread through the cough, sneeze and person with the lungs infection. tuberculosis bacteria is slowly grow intracellular organism and having lipid rich cell wall, various drug develop resistant over TB and hence more critical forms of tuberculosis identified in patients first line drugs develops resistance over the tuberculosis bacteria Therefore, there is an urgentneed to developnew molecule with better activity and having low risk. Benzoxazole ring found to also useful for antibacterial, anti-inflammatory, anti-viral, antifungal, anticancer etc. synthesizing of new molecule through to expand anti-bacterial spectrum of existing drugs. Designing of new molecular structure which not used before. Synthesize new molecule or modifying existing drug which improve TB treatment. Another one requires extraordinary molecular diversity and chemical modification, this approach is easy and accessible through newly developed computational technique **[2]**. Another one method is molecular hybridization, where different bioactive pharmacophore is fused of combine to produce new molecule with better efficacy.

Mycobacterium tuberculosis:-

M. tuberculosis is growing in doubling time 12-24 hrs under desired conditions. The cell wall structure contain lipid bilayer, the inner portion is mycolic acid and glycolipid and outer part is waxy components. Peptidoglycan covalently linked with arabinogalactan and lipoarabinomanan and form periplasmic space between two membrane **[2]**. the person with infection, if coughed or sneeze that's small in size droplet nuclei which is suspended in air long time through this infectionn is initiated by inhalation, due to their small size it penetrate into terminal alveoli **[3]**. Mycobacterial adhesin HBHA and PE_PGRS proteins is main component in interaction between bacilli and host cells **[2]**. All the strains of Mycobacteria produces the α-alkyl, β-hydroxy fatty acids featured by higher molecular weight(60-90 Carbon atoms chain). Mycolic acid, 2-alkyl, 3-hydroxy long chain fatty acids are the main features of the *M. tuberculosis* cell wall **[4-9]**. *M. tuberculosis* and *M. bovis* produce three types mycolic acid: alpha mycolate, methoxymycolate, ketomycolate Chemically mycolic acid shows the presence of α-mycolic acid that contains the unsaturation as well as the cyclopropane rings. The mycolic acid of cell wall defence bacillus against oxidative stress **[5]**.

MATERIALS AND METHODS

All the reagents and solvents used were synthetic grade. The melting point of compounds were determined by capillary method.purity of compounds were checked by TLC using mobile phase methanol: acetonitrile:ethyl acetate (4:4:4). The

structure of compounds elucided by ATR-IR from government college of pharmacy karad, and MASS (+mode) spectroscopy from diya lab mumbai and solapur university, biological activity was evaluate from Maratha mandal research center belgaum.

Following scheme **(Figure 1)** represent the general synthetic pathway and the possible end product, where R1, R2, represent the substituent to be added **[9-17]**.

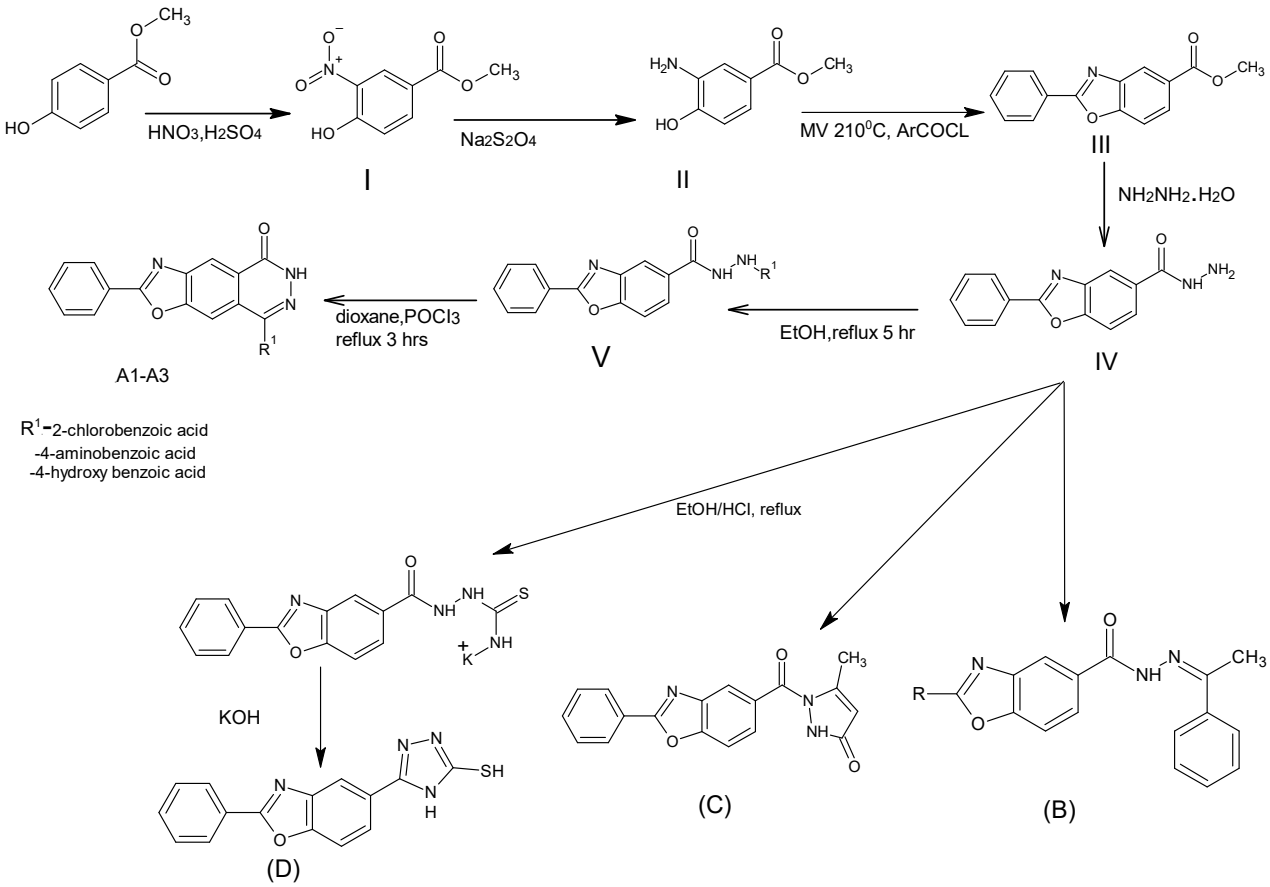

Figure 1: Synthetic scheme of the Reaction (1,2,3,4 and 5 represents intermediates and A1-A3, B, C, D represents the end products)

Synthetic procedure of compounds:

4-hydroxy-3-nitro-benzoic acid methyl ester (I)

In a 500 ml three necked round bottom flask with condenser, mechanical stirrer and thermometer, placed 0.065 mol (10 gm) p-hydroxy methyl benzoate and mixture of conc. nitric acid (6.5 ml) and conc. Sulphuric acid (6.5 ml) in dropping funnel, cool the flask in ice bath at 5-10 ºC and add continue upto 1 hour. Then poured the reaction mixture in crushed ice. Filter the residue and wash with cold water. Transfer solid in 500 ml beaker and slight stir with ice cold methanol to remove o-nitro isomer and other impurities. The mixture filter and washed with little

methanol and dried. The product is recrystallised by methanol.

3-amino-4-hydroxy-benzoic acid methl ester (II)

In 500 ml three necked flat bottom flask with reflux condencer and guard tube, compound I (10gm) was dissolved in boiling ethanol (40 % 150 ml) and only sodium dithionite was added to boiling ethanol solution until it becomes colourless. Then the solution was poured in crushed ice resulting colourless shiny product obtained, filtered, wash with cold water and dried. The product recrystalised by hot water.

2-phenyl benzoxazole-5-carboxylic acid methyl ester (III)

Benzoxazole is prepared via microwave assisted reaction.take 0.03 mol of intermediate II and 0.03 mol of benzoyl chloride in presence of dioxane at 210℃ for 15 min. After cooling the solid product obtained, it further recrystallized by methanol.

2-phenyl-benzoxazole-5-carboxylic acid hydrazide (IV)

A mixture of intermediate III (0.01 mol) and hydrazine hydrate (0.15 mol) in 25 ml methanol. Heated above mixture under reflux on water bath for 4-5 hours. Then the crushed ice was pour into the reaction mixture , product filtered and wash with cold water then dried. product recrystallized by methanol.

Synthesis of derivative (A1,A2,A3):

In this reaction intermediate IV taken 2 mmol (0.506 gm) and substituted benzoic acid 4mmol in ethanol. reflux the above mixture on water bath for 5 hours and cooled, the white product collected and dried (intermediate V).then again reflux above product in dioxane containing phosphorous oxychloride for 3 hours, then cooled and dilute with crushed ice the precipitate formed, product recrystallized by methanol.

A1-8-(2-chlorophenyl)-2-phenyl [1,3] oxazolo [4,5-*g*] phthalazin-5(6*H*)-one

A2-8-(4-aminophenyl)-2phenyl[1,3]oxazolo[4,5-*g*]phthalazin-5(6*H*)-one

A3-8-(4-hydroxyphenyl)-2 phenyl [1,3] oxazolo [4,5-*g*] phthalazin-5(6*H*)-one

Synthesis of derivative B:

(2-phenyl-*N*'-[(1*Z*)-1-phenylethylidene]-1,3- benzoxazole-5-carbohydrazide)

To a solution of 2 mmol (0.506 gm) of intermediate IV in 20 ml of 1,4-dioxane 2 mmol of acetophenone added. Stirred for one hour at RT and poured into crushed ice, the ppt. was collected, dried and recrystallized by ethyl acetate or methanol**.**

Synthesis of derivative C:

(5-methyl-1-(2-phenyl-1,3- benzoxazole-5-carbonyl)-1,2-dihydro-3*H*-pyrazol-3-one)

A mixture of 2mmol f intermediate IV and 0.0035 mol(0.455 gm) of ethylacetocetate and 1.0 cm^3 of actic acid was refluxed in 10 cm^3 ethanol for 5 hours. thethe mixture

cooled the precipitate formed was collected and driedand recrystallized by methanol.

Synthesis of derivative D:

(5-(2-phenyl-1,3-benzoxazol-5-yl)-4*H*-1,2,4-triazole-3-thiol)

A mixture of 2 mmol (0.506 gm) of intermediate IV and 3.7 mmol of KSCN (0.358 gm) was refluxed in 25 cm^3 ethanol containing few drops of conc. HCI for 3 hrs.the precipitate was collected and dried, a mixture of above ppt. and 0.1 g of KOH was refluxed in 25 cm^3 of water for 3 hrs.the reaction mixture cooled and acidified with HCL and recrystallized by methanol.

RESULT AND DISCUSSION

Evaluation and characterization of compounds (Table 1-4).

Biological screening (Alamar Blue Assay For Estimating Anti-TB activity)

The biological activity of the molecules test against *M. tuberculosis* using microplate Alamar Blue Assay. MABA is harmless and used thermostable reagent which shows coreelation with BACTEC radiometric method. All 96 well filled with 200 µl sterile water. On the plate made 100 µl of middlebrook 7H9 and made dilutions of test solutions. The test samples tested at 100 to 0.2 µl conc. then incubate for 5 days at 37ºC. Then the plate added by 1:1 mixture of alamer blue dye and tween 80 and incubate for 24 hrs. The pink and blue colour in well was examined, the blue colour indicate the no bacterial growth while pink colour indicate growth. The lowest drug conce. Prevent colour change from blue to pink is MIC **[18-20]**.

The evaluation of all synthesized molecules A1, A2, A3, B, C, D for antitubercular activity against *M.tuberculosis* using strain H37RV was carried out through MABA assay. Pyrazinamide were screened as standard drug **[21-28] (Figure 4)**.

The standard used pyrazinamide MIC is 3.125 µg/ml and the solvent used DMSO as control. From all target compounds A1, B, C, D shows the good antitubercular activity, compound A1 show nearly similar activity to pyrazinamide **(Table 5)**.

Table 1: physicochemical data of intermediates (I-IV)

Intermediate	Mol. Formula	Mol. Wt.(gm)	Yield (%)	melting point	Rf value
1 (4-Hydroxy-3-nitro methyl benzoate)	C8H7NO5	197	77.93 %	67-69ºC	0.41
II (3-Amino 4-hydroxy methyl benzoate)	C8H9NO3	167	81.55 %	98-100 ºC	0.65
III (2-phenyl- benzoxazole-5-carboxylic acid methyl ester	C15H11NO3	253	63.40 %	134-136ºC	0.23
IV (2-phenyl- benzoxazole-5-carboxylic acid)	C14H11N3O2	253	85.77 %	122-126ºC	0.74

Table 2: End products physicochemical data ((TLC mobile phase- methanol: acetonitrile: ethyl acetate(4:4:4))

Comp. No.	Mol. far.	Mol.wt gm/mol	Yield (%)	melting point	Rf value
A1	C21H12N3O2CI	373.80	48.39 %	166-168 ºC	0.84
A2	C21H14N4O2	354.37	67.65 %	132-134 ºC	0.53
A3	C21H13N3O3	355.35	61.69 %	224-226 ºC	0.57
B	C22H17N3O3	355.40	67.74 %	198-202 ^{O}C	0.38
C	C18H12N3O3	319.34	66.98 %	312-314 ^{O}C	0.11
D	C15H10N4OS	294.32	92.34 %	326-328 ^{O}C	0.41

Table 3: Elemental analysis (found)

Comp.	Elements distribution					
	C	H	N	O	S	CI
A1	67.56	3.21	11.26	8.57	-	9.11
A2	71.18	3.95	15.81	9.03	-	-
A3	70.98	3.66	11.83	13.52	-	-
B	74.36	4.78	11.83	15.04	-	-
C	67.71	3.76	13.16	15.04	-	-
D	61.22	3.40	19.04	5.44	10.88	-

Table 4: Spectral data for synthesized compounds (MASS (+mode))

Compounds	Spectral data
A1	m/z ratio, 373.9 IR,ν/cm^{-1}-3363.10(N-H),1712.91(C=O),1712.91(C=N),1209.32(C-N),1042.64(C-O)
A2	m/z ratio,355.4 IR,ν/cm^{-1} -3469.03(N-H 1^{0}stretch), 3175.74(2^{0} stretch),1601.00(C=O), 1601.00(C=N), 1175.54(C-N),1049.28(C-O)
A3	m/z ratio,356.1 IR,ν/cm^{-1}- 3186.56(N-H^{stretch}), 3312.49(O-H), 1586.27(C=N),1208.18(C-N), 1649.39(C=O), 1208.18(C-O)
B	m/z ratio, 356.2 IR,ν/cm^{-1}- 3204.98(N-H)1651.23(C=O),1622.21(C=N), 1051.15(C-N), 1544.60(C=C), 1102.44(C-O)
C	m/z ratio,319.3 IR,ν/cm^{-1}- 3183.37(N-H),1694.80(C=O), 1601.01(C=N), 1183.47(C-N), 1259.95(C-O)
D	m/z ratio,295.2 IR,ν/cm^{-1}- 3493.61(N-H), 2870.20(S-H), 1734.95(C=N),1092.96(C-N), 1092.969(C-O)

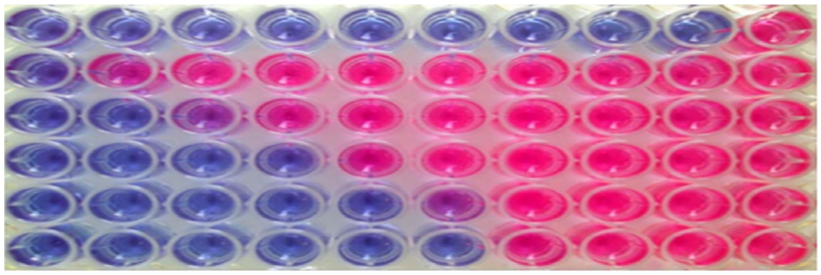

Figure 4: Screening of synthesized compounds.(blue colour is represent sensitive and pink colour represent resistant)

Table 5: MIC of Synthesized compounds. S= Sensitive And R= Resistant

Comp.	1000 µg/ml	500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml	31.2 µg/ml	15.6 µg/ml	7.8 µg/ml	3.9 µg/ml	1.95 µg/ml
A-1	S	S	S	S	S	S	S	S	S	R
A-2	S	R	R	R	R	R	R	R	R	R
A-3	S	S	R	R	R	R	R	R	R	R
B	S	S	S	S	R	R	R	R	R	R
C	S	S	S	S	S	R	R	R	R	R
D	S	S	S	S	S	S	R	R	R	R

DISCUSSION

All the above compounds synthesized from 2-phenyl benzoxazole -5 carboxylic acid hydrazide with the reactive carboxylic group, acetophenone analogs to forming cyclization followed by peptide bond **[17, 21]**. In the synthetic pathway, during synthesis of intermediate II i.e, reduction of nitro group into amino group done by sodium dithionite this reaction was optimized by using various reducing agents like Zn/HCL, stannous chloride, copper sulphate and sodium borohydride but there is no desired product obtained **[13, 14]**. The reaction of carboxylic group with intermediate II by conventionally not proceed the further hydrazine process, so we use the benzoyl chloride react at microwave to proceed the further steps **[23]**.

The synthesized compounds evaluated for anti-tubercular activity using strain H37 RV by microplate alamer blue assay method gives the all compounds be sensitive for activity with different concentration ,this activity compare by using pyrazinamide as standard and DMSO as control, there is all compounds shows less potent than standard.

CONCLUSION

The dissertation is concluded that the synthesized molecules are effective in inhibition of cell wall growth which play important role in mycolicbacterial cell wall growth. All compounds are showing the significant effect on target enzyme. Depending upon the biological activity we can conclude that the presence of functional groups like CI_2, OH, NH_2, CONH, increases the activity, this groups has been selected according to electron withdrawing groups and electron donating groups. Other than this there is again scope of various structure activity relationship and synthesized other such novel molecules. During the synthesis of derivatives the all methods should be optimized to obtained good yield and desired product purity.

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CONFLICT OF INTEREST

Author declare that no conflict of interest.

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