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SYNTHESIS, CHARACTERIZATION OF BENZOXAZOLE DERIVATIVES FOR *IN-VITRO* ANTI-TUBERCULAR AND ANTI-BACTERIAL ACTIVITY - A RESEARCH ARTICLE

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Keywords:

Benz-oxazole derivatives, Biological activity, IR, NMR, LC-MS and Alamar blue assay

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ABSTRACT: Different-different modification of Benz-oxazole moiety gives various numbers of novel compounds which shows various biological activities. Some new derivatives (G_7-G_{10}) containing Benzoxazole moiety were prepared by the use of different methods and reagents. O- Phenylenediamine treated with Chloroacetic acid to form 2(chloro methyl) benzimidazole (compound B) and 4-methyl, 2- amino phenol with methyl alcohol and carbon di sulfide to form 2-thio, 5- methyl, benz-oxazole (compound G_1). Further Compound B treated with (compound G_1) to form [(benz-imidazole)-2-methyl thio]-5-methyl, benzoxazole (compound G_2). Finally compound G_2 treated with a substituted aldehyde to form benzoxazole derivatives (G_7-G_{10}). Compound spectral analysis was done by Infrared spectroscopy (IR), ^{1}H-Nuclear magnetic resonance (^{1}HNMR) & Liquid chromatography-mass spectroscopy (LCMS). The serial Dilution method (MIC test) was used for anti-bacterial activity, and Alamar blue assay test was used for Anti-tubercular activity. Compounds G_8 and G_9 showed potent anti-tubercular activity against *M. Tuberculosis,* and Compounds G_7, G_8 and G_{10} were showed good antimicrobial activity.

INTRODUCTION: Oxazole is a heterocyclic compound with five - Membered rings with oxygen & nitrogen atom in its structures. Benz-oxazole formed when the benzene ring is attached to oxazole rings [7, 9, 13]. Many of methods have been available meant for the preparation of the Benz - oxazole derivative. The best starting substance used for synthesizing Benz- oxazole hetero cyclic rings is Ortho-aminophenol because it has N & O into a structure capable of going into ring arrangement reactions by reactions [8, 10, 14].

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Due to the aromaticity of Benz-oxazole moiety, it has stability, although as a heterocyclic compound, it has many reactive sites [7]. Nowadays, tuberculosis is a major health issue with comprehensive types of indication caused by *Mycobacterium Tuberculosis*. Tuberculosis mainly affects the lungs organ. Recently data says that mycobacterium tuberculosis bacteria infect approximately 33% population. Nowadays, used therapy for this infectious bacteria takes more time.

Accordingly, it is required to prepare novel drugs with new modes of action other than a previously used drug, such as Isoniazid, Streptomycin, Pyrazinamide and Rifampicin [2-5]. Benz and oxazole ring containing new derivative has possessed various types of activities such as anticancer, antihyperglycaemic [13], antitumor, Immuno-modulatory, antifungal, anti-bacterial [1, 19],

analgesic, anti-tubercular [6, 12], anti inflammatory [11], antihypertensive and anticonvulsant activity. New prepared derivative (G_7-G10) was used for anti-tubercular and anti-bacterial activity.

MATERIAL AND METHODS:

Materials: All the solvents and chemical that has been used in research work were of analytical grade and collected from Central Drug House (P) Ltd. (Delhi, India) and Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan).

Synthesis:

Procedure for the preparation of 2-(chloro-methyl) – Benz-imidazole (Comp- B) [15]: Take 17.5 mmol Chloroacetic acids, add 20 mmol of 1, 2 phenylenediamines in conical flask, mix properly, and add 15 ml 5N, HCl in solution, poured the solution in RBF, refluxed the solution for 6 hr on oil bath. The mixture (solution) was cooled to room temperature pH maintained 7 by using an Ammonia solution. After filtration solid yellow product was obtained, the residue was rinsed with water, and the final product was recrystallized with ethanol and dried. (Yield 75%). M.P-151 - 153 °C.

Procedure for the preparation of 2-thio, 5-methyl, Benz-oxazole (comp G_1): 30 ml of methanol and solution of 2.2 gm of 2-amino, 4-methyl phenol pour in a beaker. Add a mixture of 1.4 gm of KOH and 6ml of water. Now 1.8 ml of carbon disulfide was added. The final mixture was refluxed for 4 hr at 65°C. The purification was monitored by TLC, (R_f - 0.51). When the reaction was completed than poured the mixture in normal water. Now pH is maintained to 7 by the use of HCl. After filtration, the solid was obtained, recrystallized the product with ethanol, and dried the final product (Yield 85%). M.P-161-162°C.

Procedure for the preparation of [(benzimidazole)-2-methyl, thio] -5- methyl, benzoxazole (comp G_2): In RBF 0.82 gm of 2-(Chloro, methyl) benzoimidazole (Comp B) and a mixture of 0.75gm of 2-thio 5-methyl, benzoxazole (comp G_1) was added. Further, take 15 ml of THF, followed by TEA. The mixture (solution) was mixed at room temperature for six hours. Thin layer chromatography (TLC) was used to monitor the purification (Rf - 0.70). THF was removed by separating the funnel. 15ml chill water added in residue with regular stirring. After filtration solid was obtained. Recrystallized the product with ethanol, dried and get final product as solid (Yield 80%). M.P. – 162-164°C.

Procedure for the Preparation of Final Derivative (G_7-G_{10}): About 0.35 gm of [(benzimidazole)-2-methyl, thio] -5- methyl, benzoxazole (comp G_2) and 2.5 gm of acetic acid was mixed in a beaker. Now add 0.5 gm substituted aldehyde. The mixture was swirled for 0.5 hours at room temperature. Thin layer chromatography (TLC) was used to purify the reaction (9:1, chloroform: methanol ratio). The reaction solution was added to chilled water at room temperature & the mixture (solution) was properly mixed for 0.5 hrs. After filtration solid was obtained. Recrystallization was done by ethanol, dried the product and get final product a solid.

Anti-bacterial Activity: Gram-positive bacteria *S. aureus* & Gram-negative bacteria *E. coli* were used in a serial dilution experiment to test the anti-bacterial activity [18]. For MIC every drug has to 9 dilutions with thioglycollate broth, Take 20 µl of the drug in an initial tube and pour 380µl of thioglycollate broth. 200µl of thioglycollate was added individually to 9 tubes for dilution. 200 µl of the thioglycollate broth from the first tube was poured into the first tube.

The dilution was calculated at 10^{-1}. 200µl were transferred from the 10^{-1} diluted tube to the 2nd tube to create the 10^{-2} dilution. For each drug, the serial dilutions were carried out up to a 10^{-9} dilution. 5µl were collected from the regularly maintained stock culture of the necessary organisms and poured to 2 ml of thioglycollate broth. Every serially diluted tube received 200µl of the aforementioned culture solution. All tube was incubated for 2-3 days at 37°C in anaerobic jars while the turbidity was monitored [18].

Anti-tubercular Activity: *Mycobacterium tuberculosis*, vaccine strain- H37 RV strain (ATCC No. 27294) was tested for anti-tubercular activity by the Alamar blue assay method [16, 17]. This procedure employs a thermally stable reagent & non-toxic. In order to prevent the medium in the test well from evaporating too quickly throughout incubation, 200µl of sterile deionized water was

poured into every outer perimeter well of a sterile 96-well plate. The middle-brook 7H-9 broth was added to the ninety six-well plates in 100 µl, and subsequent serial dilutions of the derivatives were prepared right on the plate.

The final drug levels examined ranged from 100 to 0.2µg/ml. Plates were para-film enclosed and incubated at 37°C for 120 hours.

Afterward, the plate received 25µl of a freshly made solution [1:1] of alamar blue reagent and 10 % tween 80, which was then it incubated for 1 day. In the wells, pink color indicated bacterial growth while blue indicated no bacterial growth [16, 17].

RESULT AND DISCUSSION: In this research work, all the benzoxazole derivates were prepared successfully according to the synthetic root **Fig. 1**. Physiochemical data of all prepared derivatives (G_7-G_{10}) shown in **Table 1** The all newly prepared compound was confirmed by the interpretation of LCMS, NMR and IR data and Serial dilution was used to test for anti-bacterial activity. In contrast, alamar blue assay was used to test for anti-tubercular activity.

TABLE 1: PHYSIOCHEMICAL CHARACTERISTICS OF NEWLY PREPARED DERIVATIVES (G_7- G_{10})

Derivative Name	Chemical formula	IUPAC name	Molecular mass	Melting point ^{0}C	Rf value	Yield (In %)
G_7	$C_{23}H_{18}N_3OSF$	1-[(4'-Fluoro benzyl, benzimidazole)-2-methyl thio] 5-methyl benz-oxazole.	403	170-172	0.60	82
G_8	$C_{24}H_{21}N_3O_2S$	1-[(4'-Methoxy benzyl, benzimidazole-2-methyl thio] 5-methyl benz- oxazole.	415	188-190	0.65	85
G_9	$C_{23}H_{18}N_3OSBr$	1-[(3'-bromo benzyl, benzimidazole)-2-methyl thio] 5-methyl benz-oxazole.	464	215-216	0.58	78
G_{10}	$C_{23}H_{18}N_3OSCl$	1-[(3'-chloro benzyl, benzimidazole)-2-methyl thio] 5-methyl benz-oxazole.	419	205-207	0.83	85

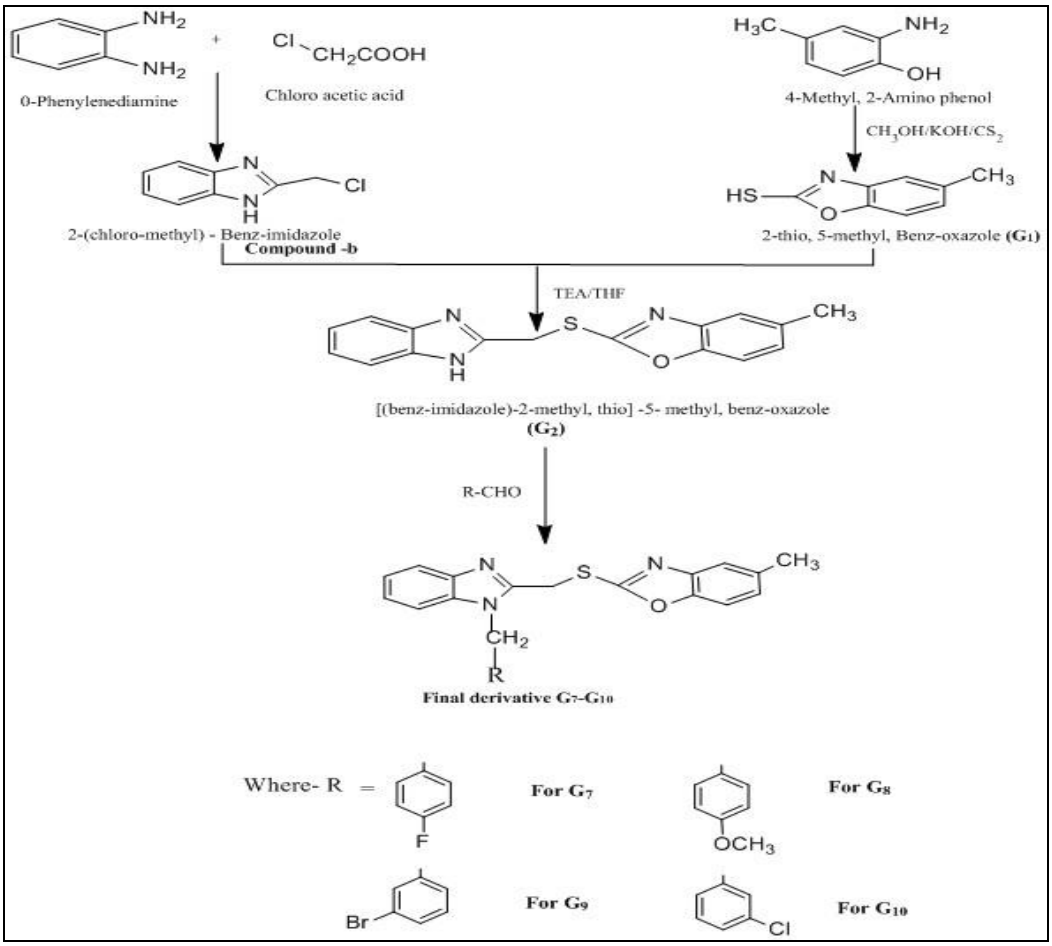

FIG. 1: SYNTHETIC ROOT FOR NEW BENZOXAZOLE DERIVATIVE

Interpretation of Intermediate 2-thio, 5-methyl, Benz-oxazole (comp G_1): ^{1}HNMR (400MHz, DMSO) δ- 2.98 (3-H, s, -CH_3),indicate the prescence of methyl group, δ-6.76-6.98 (2-H, m, Ar-H, δ-6.61 (1-H, dd, 1.8Hz, Ar-H), show the prescence of aromatic ring, 7.76 (0.5Hz), 7.98 (0.5Hz)), IR (KBr): 1694cm-1 (stretching C=N), 2884cm-1 (stretching Ar-H), 1523cm-1 (stretching Ar-CH3) and 738 cm-1 (stretching, C-S), Mass, (ESI-MS): m/z 166 (M+H).

Interpretation of Intermediate [(benz-imidazole)-2-methyl, thio] -5- methyl, benz-oxazole (comp G_2): The following ^{1}HNMR (400MHz, DMSO) δ2.95 (3H, s,-CH_3), show the prescence of methyl group, 7.41-7.93 (H, m, Ar-H,) indicate the prescence of aromatic ring, 12.2 (1H, -NH-), 4.66 (2H,s, -CH_2-), indicate methylene group, 7.25(1H,1.2 Hz, Ar-H) , IR (KBr): 3015 cm-1 (stretching, Ar-H), 1695cm-1 (stretching C=N), 1434cm-1 (stretching Ar-CH3), 2301cm-1 (stretching N-H, Secondary Amine), and 734 cm-1 (stretching, C-S), Mass (ESI-MS): m/z 296(M+H).

Interpretation of New Derivative of Benzoxazole (comp-G_7): The ^{1}H NMR interpretation of G_7 compound, ^{1}HNMR (400MHz, DMSO) δ-2.24 (3H, s, -CH_3) indicates the presence of a methyl group, 4.10 (2H,s, -CH_2-), indicate the presence of methylene group 4.59 (2H,s,-CH_2-), δ-6.86-7.27 (11-H, m, Ar- H), indicate the presence of aromatic ring. IR interpretation, IR (KBr): 2992 cm-1 (stretching, Ar-H), 1694cm-1 (stretching C=N), 1438cm-1 (stretching Ar-CH3), 1146cm-1 (stretching C-F) & 737cm-1 (stretching C-S), LCMS interpretation, Mass (ESI-MS): m/z 404(M+H).

Interpretation of New Derivative of Benzoxazole (comp-G_8): ^{1}HNMR (400MHz, DMSO) δ- 2.23 (3H, s, -CH_3), indicate methyl group, 3.51 (3H, s, -OCH3) show prescence of Methoxy group δ-4.41 (2-H,s, -CH_2-), show methylene group δ-4.97 (2-H, s, -CH_2-) δ-7.19-7.63 (11H, m,Ar-H) indicate prescence of aromatic ring, IR (KBr): 3015cm-1 (stretching Ar-H), 2884cm-1 (stretching, Ar-OCH3), 1695cm-1 (stretching C=N), 1517cm-1 (stretching Ar-CH3), and 735 cm-1 (stretching, C-S), Mass (ESI-MS): m/z416(M+H).

Interpretation of New Derivative of Benzoxazole (comp-G_9): ^{1}HNMR (400-MHz, DMSO) δ-2.61 (3H, s, -CH_3), show prescence of methyl group 3.10 (2H, s, -CH_2-), δ-3.79 (2H, s,-CH_2-), indicate methylene group δ-6.86-7.27 (11H, m, Ar-H), indicate aromatic ring, IR (KBr): 2997 cm-1 (stretching, Ar-H), 1694cm-1 (stretching C=N), 1515cm-1 (stretching Ar-CH3), 792cm-1 (stretching C-S) & 737cm-1 (stretching C-Br), Mass (ESI-MS): m/z465(M+H).

Interpretation of New Derivative of Benzoxazole (comp-G10): ^{1}HNMR (400-MHz, DMSO) δ-2.08 (3-H, s, -CH_3), 3.90 (2-H, s, -CH_2-), 4.57 (2-H, s, -CH_2-), δ-6.65-7.16 (11H, m,Ar-H), IR (KBr): 2996 cm-1 (stretching, Ar-H), 1695cm-1 (stretching C=N), 1518cm-1 (stretching Ar-CH3), 792cm-1 (stretching C-Cl) & 737cm-1 (stretching C-S), Mass (ESI-MS): m/z420(M+H).

Anti-tubercular and Anti-bacterial Activity (G_7-G_{10}): By using the Alamar Blue assay Method, Newly synthesized derivative G_7 and G_{10} was showed anti-tubercular activity sensitive at 100 µg/ml and G_8, and G_9 was showed sensitivity at 100 and 50 µg/ml against the bacteria *Mycobacterium Tuberculosis* (H-37RV) **Table 2** and **Fig. 2** and against gram-negative bacteria like *E. coli*, G7, G8, and G10 shown anti-bacterial activity that was sensitive at 100 g/ml **Table 3** and G_7 and G_{10} was showed anti bacterial activity sensitive at 100 µg/ml doses against gram positive bacteria *S. aureus* **Table 4** by using serial dilution method (MIC).

TABLE 2: MINIMUM INHIBITORY CONCENTRATION DATA OF ANTI-TUBERCULAR ACTIVITY AGAINT MYCOBACTERIUM TUBERCULOSIS (H37RV STRAIN)

S. no.	Sample	100µg /ml	50µg /ml	25µg /ml	12.5µg /ml	6.25µg /ml	3.12µg /ml	1.6 µg /ml	0.8 µg /ml
1	G_7	S	R	R	R	R	R	R	R
2	G_8	S	S	R	R	R	R	R	R
3	G_9	S	S	R	R	R	R	R	R
4	G_{10}	S	R	R	R	R	R	R	R

Where, S –Sensitive, R- Resistant, Bacteria- *M. tuberculosis.*

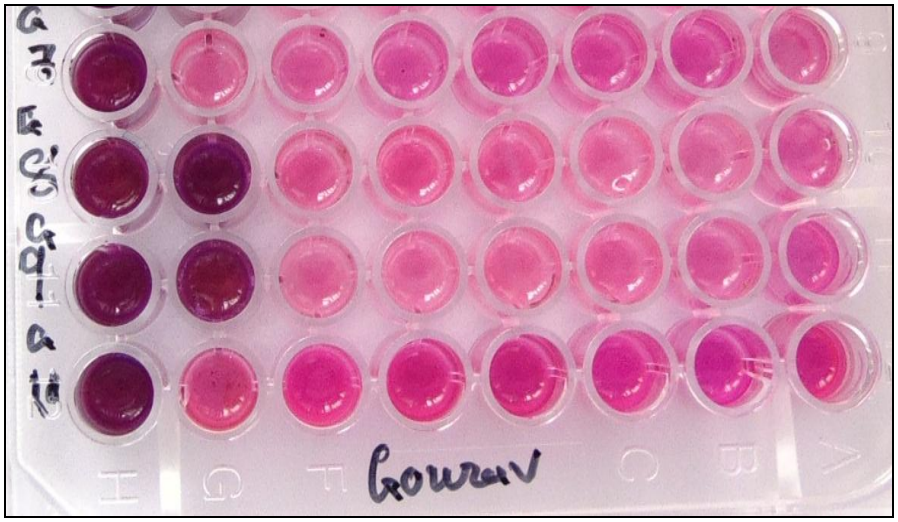

FIG. 2: RESULT OF ANTI-TUBERCULAR ACTIVITY (BACTERIA-*M. TUBERCULOSIS*), DARK BLUISH COLOR SHOWS SENSITIVITY, AND PINK COLOUR SHOWS RESISTIVITY

TABLE 3: MINIMUM INHIBITORY CONCENTRATION DATA OF ANTI-BACTERIAL ACTIVITY AGAINST GRAM-NEGATIVE BACTERIA *E. COLI*

S. no.	Sample	100	50	25	12.5	6.25	3.12	1.6	0.8	0.4	0.2 µg/ml
1	G_7	S	R	R	R	R	R	R	R	R	R
2	G_8	S	R	R	R	R	R	R	R	R	R
3	G_9	R	R	R	R	R	R	R	R	R	R
4	G_{10}	S	R	R	R	R	R	R	R	R	R

Where, R- Resistant and S- sensitive.

TABLE 4: MINIMUM INHIBITORY CONCENTRATION DATA OF ANTI-BACTERIAL ACTIVITY AGAINST GRAM-POSITIVE BACTERIA *S. AUREUS*

S. no.	Sample	100	50	25	12.5	6.25	3.12	1.6	0.8	0.4	0.2 µg/ml
1	G_7	S	S	R	R	R	R	R	R	R	R
2	G_8	R	R	R	R	R	R	R	R	R	R
3	G_9	R	R	R	R	R	R	R	R	R	R
4	G_{10}	S	R	R	R	R	R	R	R	R	R

Where, R- Resistant and S- sensitive.

CONCLUSION: All the novel synthesized benzoxazole derivatives (G_7-G_{10}) were confirmed by spectral analysis, *i.e.*, proton nuclear magnetic resonance, infra-red spectrometry, and liquid chromatography-mass spectrometry. Compounds G_8 & G_9 showed potent anti-tubercular activity at 100 and 50 µg/ml against the bacteria *Mycobacterium Tuberculosis* (H-37RV), and Compounds G_7, G_8 & G_{10} showed potent anti-bacterial activity at 100 µg/ml against *E. coli*. And Compound G_7 showed potent anti-bacterial activity at 100 & 50 µg/ml against *S. aureus*

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CONFLICTS OF INTEREST: No conflict of interest

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