Journal of Advanced Zoology

ISSN: 0253-7214

*Volume **44** Special Issue-**2 Year 2023** Page **4634:4643***

DESIGN NOVEL SYNTHETIC ROOT OF BENZOXAZOLE DERIVATIVES AS *IN VITRO* ACTIVITIES: A RESEARCH ARTICLE

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Article History

Received: 08 Aug 2023

Revised: 29 Oct 2023

Accepted: 02 Nov 2023

ABSTRACT

Being a hetero- cyclic moiety, benzoxazole finds application in much medicinal research as an initial reactant. Benzoxazole derivatives have unique significance in the field of medical research due to their incredible biological potential. During recent year Benzoxazole derivative showed interesting development in medicinal research. Many Novel benzoxazole derivatives (G_3-G_6) were formed by using 4- methyl 2-amiono phenol reagent with methanol, potassium hydroxide, carbon di- sulfide. We observed the process employing thin-layer chromatography and interpreted spectral analysis via NMR, LC-MS, IR Spectrometry and antibacterial activity done by MIC method with *Escherichia Coli* a gram negative bacteria, *Staphylococcus aureus* a gram positive bacteria and anti-tubercular activities done through alamar blue dye technique(ABD) using *Mycobacterium Tuberculosis(MT)* bacteria . Some tested derivatives showed potent activity.

KEY WORD: Benzoxazole, 2-mercepto benzoxazole, antibacterial activities, Antitubercular activities and Serial dilution technique.

INTRODUCTION:

Benzoxazole is organic compound which show aromaticity. It contains chemical formula C_7H_5ON, even though Benzoxazole itself is of tiny product values, mostly novel derivative of Benzoxazole are commercially beneficial. Being a heterocyclic compounds, Benzoxazole find make use of do research as an initial substances used for the preparation of bulk, generally bio-active structure. It has aromaticity due to makes it comparatively secure, even though as a hetero cycle, it has many reactive sites which permit for functionalization [5, 15]. Modification on the Benzoxazole moiety has resulted in a many numbers of compound have assorted biological actions. The preparations, structure along with pharmacological activity of Benzoxazole derivatives focused for study significance in the area of medicines, because of probable activity exhibit through them. The moiety of Benzoxazole is escalating their Pharmaceutical significance and has been studied recurrently in favor of the investigation of their Biological assist in speckled Biological state of affairs [13, 14, 11]. The Pharmacological profile of this new generation of Benzoxazole represents a lot improvement with regard toward old one compound. Look into the therapeutic valuable of Benzoxazole nuclus, it is meaningful to prepare definite novel compounds of Benzoxazole furthermore monitor them for their pharmacological actions[4, 9]. For the period of modern year, there has been a little motivating development in the pharmacological actions of Benzoxazole compounds. These derivatives have unique importance in the field of medical Chemistry caused by their incredible biological potentials. Many Benzoxazole compounds were widely study for their anti-bacterial and anti tubercular activity[8,16,17]. Moiety of benzoxazole was showed various biological activity like, anticancer[2] anti- bacterial[1,3], anti-tubercular, analgesic, anti-oxidant ,anti-hypertensive, Immunomodulatory, anti-inflammatory[7,10,12] etc.

MATERIAL AND METHODOLOGY:

Material:

Chemical & solvent were of analytical grade that has been used in my research work and collected from TCI, Tokyo, Japan and CDH, Delhi, India. Chemicals were o-phenylenediamine, chloro acetic acid, 4-methyl -2- amino phenol, Methanol, carbon disulfide, silica gel G and substituted benzaldehyde.

Methods:

Synthetic process for the preparation of 2-(Chloro methyl)-benzimidazole (Compound B) [16]

Take 3.75 gm or 40 mmol of o-phenylenediamine in Beaker, add 3.75 gm or 35 mmol Chloro acetic acid mix well and add 30 ml of 5N hydrochloric acid in mixture, now poured the solution in a RBF and refluxed the solution in an oil bath for 6 hr. At room temperature solution was cooled and maintained the PH of the solution to 7 through ammonia solution. Yellow solid was obtained after filtration, washed residue by using water, with ethanol product was recrystallized and dried to furnish yellow solid product (Yield- 77%). M.P- 150-152 ^{0} C.

General synthetic procedure for the synthesis of 5-methyl-2-thio, benzoxazole (compound G_1)

A solution of 1.1 gm of 4-methyl -2-aminophenol in 15 ml of methanol was prepared in which a mixture of 3 ml of water and 0.7 gm of potassium hydroxide was added, further added 0.9 ml of carbon disulfide. At 65^0 C prepared solution was refluxed for 4 hr. TLC was used to monitor the reaction (chloroform: methanol, 4:1 Rf-0.50).The mixture were transferred in water after the completion of reaction. With concentrated hydrochloric acid, the mixture was now neutralized. After filtration, solid was obtained. Residue was cleaned with hexane, and Using ethanol, solid product was recrystallized, then dried to produce solid product (Yield, 86%). MP-160-163 ^{0} C.

General synthetic procedure for the synthesis of [(benzimidazole)-2-methyl thio] -5-methyl, benzoxazole (compound G_2)

A solution of 1.51 gm of 5-methyl-2-thio, benzoxazole (compound G_1) and 1.66 gm of 2-(Chloro methyl) benzo-imidazole (Compound B) in 30ml of tetra hydro furan, followed by 2 ml of triethylamine was missed for 6 hr at room temperature and TLC (Chloroform: Methanol: 4:1, Rf: 0.71) was used to monitor the reaction. Tetra hydro furan was separated by filtration and in residue 30 ml of ice cold water added with constant stirring. After filtration, Solid was obtained , washed solid through water and with ethanol, recrystallized, and dried to produce a solid product. (Yield, 78%). MP-165-168 ^{0} C.

General synthetic procedure for the synthesis of final derivative (G_3-G_6):

A mixture of 0.71 gm of [(benzimidazole)-2-methyl thio] - 5-methyl benzoxazole (compound G_2) and acetic acid, 5ml and added substituted aldehyde, 1ml. For 30 minutes, the solution was mixed at room temperature. TLC (Chloroform: Methanol/4:1) was used to observe the response. At room temperature, The solution were added to chill water & thoroughly mixed for 1/2 hr. Filtration was used to acquire the solid, which were then obtained, cleaned with water, ethanol was used for recrystallization and to produce a pure solid product after drying.

***In-vitro* antitubercular activity:**

The antituberculosis activity of derivatives were evaluated through *Mycobacterium* tuberculosis(MT). A thermally stable reagent is also used. During incubation, in order to reduce evaporation (loss) in the test wells, every outside perimeter well of a sterile 96-well plate received 200µl of sterile de-ionized water. The middle brook 7-H-9 broth, 100µl was added to the 96-well plates and derivatives were serially diluted right on the plate. Concentration of final drug examined ranged from 100µl/ml to 0.2µl/ml. The plates were covered and sealed with parafilm before being incubated for 120 hours at 37° C. Now the plate received 25 µl of a recently made mixture [1:1] of alamar blue reagent and 10% tween 80, which were then incubated for 1day. The well's pink colour indicated growth while the blue colour indicated no bacterial growth. The MIC was defined as the lowest drug concentration that prevented the transition from blue to pink. *Mycobacterium tuberculosis* (vaccine strain: H-37 RV strain),

A.T.C.C No: 27294, was the main strain utilized. Standard drugs value for the executed antitubercular test.

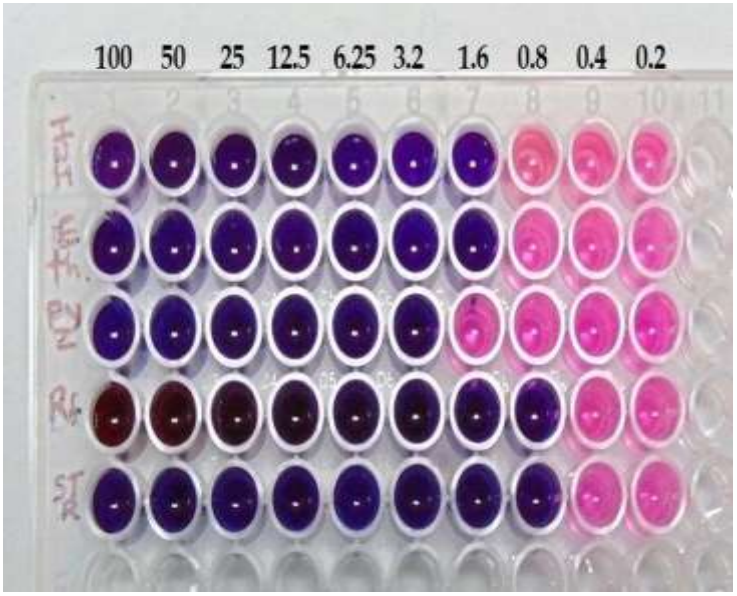


Figure: 1 Standard drug photograph of antitubercular activity

Antibacterial activity (MIC Test)

For MIC, each drug must be diluted nine times in thioglycollate broth; to the 380µl of Thioglycollate broth in the first tube and added 20µl of drug. The next 9 tubes each received 200 µl of Thioglycollate broth for dilution. 200µl was then transferred from initial tube to 1st tube that contained 200µl of Thioglycollate broth. This was regarded as a 10^{-1} dilution. 200 µl were transferred from the 10^{-1} diluted tube to the 2nd tube to generate the dilution of 10^{-2}. For each drug, the serialized dilution process was repeated up to a 10^{-9} dilution. 5 µl were collected from the maintained stock culture of the necessary organism and added to 2µl of Thioglycollate broth. Every serialized diluted tube received 200µl of the above culture solution. In an anaerobic jar at 37 ° C, the tubes were incubated. for 2-3 days, while being checked for turbidity. Standard values- *E.coli* - 2µg/ml, *S.Aureus* - 2µg/ml

RESULT AND DISCUSSION:

In this research work, all the benzoxazole derivates were prepared successfully according to the synthetic root (Figure-2). Physiochemical data of all prepared derivatives (G_3-G_6) shown in table-1 and confirmed by IR, NMR and LCMS.

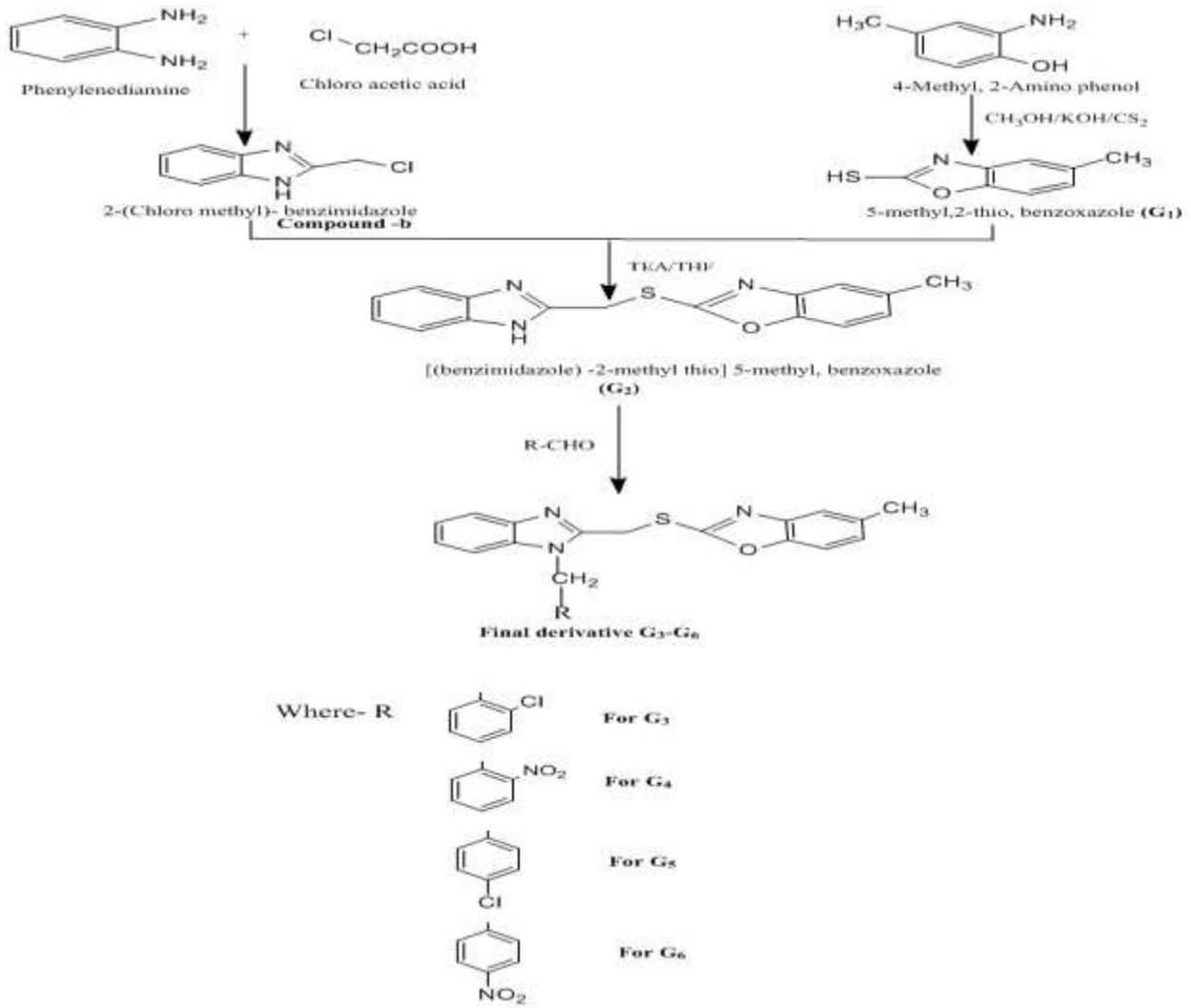
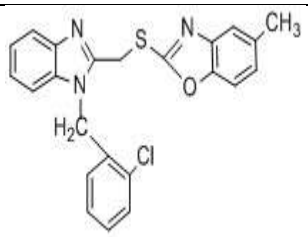
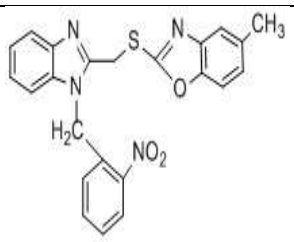
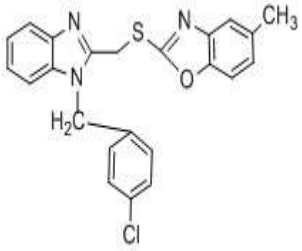
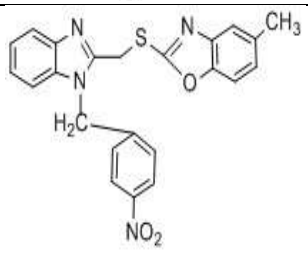


Figure-2: Scheme for noval Benzoxazole derivative (G_3-G_6)

Table: 1 Physiochemical properties of novel synthesized compounds (G_3-G_6):

Compound	Chemical formula	Chemical structure	Weight of Molecule	M. point ^{0}C	R_f value	% yield
G_3	$C_{23}H_{18}N_3OSCl$		419	178-180	0.62	85

G_4	$C_{23}H_{18}N_4O_3S$		430	238-240	0.69	83
G_5	$C_{23}H_{18}N_3OSCl$		419	215-216	0.76	78
G_6	$C_{23}H_{18}N_4O_3S$		430	190-191	0.53	74

Spectral analysis of Intermediate 5-methyl-2-thio, benzoxazole (G_1)

IR (KBr): 2884 cm-1 (stretching, Ar-H), 1694cm-1 (stretching C=N), 1523cm-1 (stretching, Ar-CH3) and 738 cm-1 (stretching, C-S), ^{1}HNMR (400 MHz,DMSO) δ-2.98 (3-H, s, -CH_3), δ -6.61 (1H, dd, 1.8Hz, Ar - H), δ - 6.76-6.98 (2H, m, Ar - H, δ - 7.76 (0.5Hz), 7.98 (0.5Hz)), Mass (ESI-MS):-m/z 166 (M+H).

Intermediate [(benzimidazole)-2-methyl thio] -5-methyl, benzoxazole (G_2) spectral analysis

IR (KBr): 3015cm-1 (stretching, Ar- H), 2301cm-1 (stretching, N-H, Secondary Amine), 1695cm-1 (stretching C=N), 1434cm-1 (stretching Ar-CH3), and 734 cm-1 (stretching, C-S), ^{1}H-NMR (400 M.Hz, DMSO) δ - 2.95 (3-H, s, -CH_3), δ-4.66 (2-H, s, CH_2-), δ-7.25(1H,1.2 Hz, Ar - H) δ - 7.41-7.93 (H, m, Ar - H,) δ - 12.2 (1-H, -NH-), Mass (ESI-MS): m/z 296(M+H).

Spectral analysis of final benzoxazole derivative i.e. 1-[(2'-Chloro benzyl, benzimidazole) -2-methyl thio]-5-methyl benzoxazole (G_3)

IR (KBr): 2990 cm-1 (stretching, Ar-H), 1694cm-1 (stretching C=N), 1435cm-1 (stretching, Ar-CH3), 792cm-1 (stretching, C- Cl) & 736cm-1 (stretching, C- S), ^{1}HNMR (400 M.Hz, DMSO) δ- 2.32 (3-H, s, -CH_3) δ - 4.18 (2-H, s, -CH_2-), δ-4.85(2-H, s, -CH_2-) δ - 6.94-7.35 (11H, m, Ar-H,), Mass (ESI-MS): m/z420(M+H)

Spectral analysis of final benzoxazole derivative i.e. 1-[(2'-Nitro benzyl, benzimidazole) -2-methyl thio]-5-methyl benzoxazole (G_4)

IR (KBr): 2909 cm-1 (stretching, Ar-H), 1695cm-1 (stretching C=N), 1507cm-1 (stretching, Ar-NO2), 1440cm-1 (stretching, Ar-CH3), 736 cm-1 (stretching, C-S), ^{1}HNMR (400 M.Hz, DMSO)

δ- 2.50 (3-H, s, -CH_3), δ-3.91 (2-H, s, -CH_2-), δ- 4.09(2-H, s, -CH_2-), δ- 6.19-7.24 (11H,m, Ar-H) , Mass (ESI-MS): m/z431(M+H)

Spectral analysis of final benzoxazole derivative i.e. 1-[(4'-Chloro benzyl, benzimidazole) -2-methyl thio]-5-methyl benzoxazole (G_5)

IR (KBr): 2843 cm-1 (stretching, Ar-H), 1694cm-1 (stretching C=N), 1441cm-1 (stretching Ar-CH3), 793cm-1 (stretching C-Cl) &738cm-1 (stretching C- S), ^{1}HNMR (400 M.Hz, DMSO) δ-2.51 (3-H, s, -CH_3), δ-3.46 (2-H, s, -CH_2-), δ- 4.38 (2- H, s, -CH_2-) δ - 7.13-7.68 (11H, m, Ar - H), Mass (ESI-MS): m/z420(M+H)

Spectral analysis of final benzoxazole derivative i.e. 1-[(4'-Nitro benzyl, benzimidazole) -2-methyl thio]-5-methyl benzoxazole (G_6)

IR (KBr): 2883 cm-1 (stretching, Ar-H), 1695cm-1 (stretching C=N), 1504cm-1 (stretching Ar-NO2), 1437cm-1 (stretching Ar-CH3), 737 cm-1 (stretching, C-S), ^{1}HNMR (400 M.Hz, DMSO) δ- 2.51 (3 - H, s, -CH_3), δ- 4.37 (2- H, s, -CH_2-) δ- 4.86 (2-H, s, -CH_2-), δ -7.13-8.18 (11H, m, Ar-H), Mass (ESI-MS): m/z431(M+H)

***In-Vitro,* Antitubercular activity:**

Antitubercular activity was completed through Alamar Blue dye test used *mycobacterium tuberculosis* bacteria. G_3- G_6 derivatives showed sensitivity at 100µg/ml, 50 µg/ml and showed resistivity against other (i.e.- 25µg/ml, 12.5µg/ml etc), (Figu-3 and table-2).

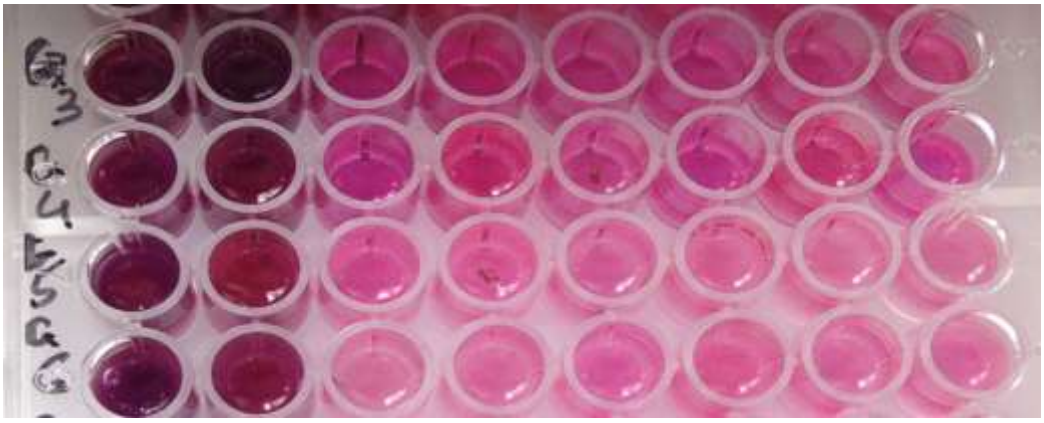


Figure: 3 Anti tubercular result against *Mycobacterium Tuberculosis* (Bluish colour shows sensitivity and pink colour shows resistivity)

Table: 2 anti tubercular result

S.No	compound	100	50	25	12.5	6.25	3.12	1.6	0.8µg/ml
01	G_3	S	S	R_t	R_t	R_t	R_t	R_t	R_t
02	G_4	S	S	R_t	R_t	R_t	R_t	R_t	R_t
03	G_5	S	S	R_t	R_t	R_t	R_t	R_t	R_t
04	G_6	S	S	R_t	R_t	R_t	R_t	R_t	R_t

Where: R_t - Resistant and S- Sensitive

Antibacterial activity:

Antibacterial activity was done by Minimum inhibitory concentration (MIC) method used *E.coli* and *S. Aureus bacteria.* G_3, G_5 and G_6 showed sensitivity at 100µg/ml and G_4 showed resistivity against *E.coli* (Table-3) and G_3 and G_6 showed sensitivity at 100µg/ml, 50µg/ml, G_5 was showed sensitivity at 100µg/ml and G_4 was showed resistivity against *S.Aureus* bacteria (Table-4).

Table: 3 Antibacterial activities results against *E.Coli* bacteria

S.No	Samples	100	50	25	12.5	6.25	3.12	1.6	0.8	0.4	0.2µg/ml
1	G_3	S	R_t	R_t	R_t	R_t	R_t	R_t	R_t	R_t	R_t
2	G_4	R_t	R_t	R_t	R_t	R_t	R_t	R_t	R_t	R_t	R_t
3	G_5	S	R_t	R_t	R_t	R_t	R_t	R_t	R_t	R_t	R_t
4	G_6	S	R_t	R_t	R_t	R_t	R_t	R_t	R_t	R_t	R_t

Where: S-Sensitive and R_t -Resistant

Table: 4 Antibacterial activities result against *S. Aureus* bacteria

S.No	Samples	100	50	25	12.5	6.25	3.12	1.6	0.8	0.4	0.2µg/ml
1	G_3	S	S	R_t	R_t	R_t	R_t	R_t	R_t	R_t	R_t
2	G_4	R_t	R_t	R_t	R_t	R_t	R_t	R_t	R_t	R_t	R_t
3	G_5	S	R_t	R_t	R_t	R_t	R_t	R_t	R_t	R_t	R_t
4	G_6	S	S	R_t	R_t	R_t	R_t	R_t	R_t	R_t	R_t

Where: S-Sensitive and R-Resistant

CONCLUSION:

All novels synthesized derivatives and intermediate confirmed by spectral data i.e. ^{1}H NMR, LC-MS & IR spectroscopy. Every prepared derivative further evaluated for anti tubercular & anti bacterial activity. It was observed that compound G_3, G_5 and G_6 showed potent antitubercular and antibacterial activity and compound G_4 showed the resistivity.

ACKNOWLEDGEMENT:

The Author is greatly Thankful to Mrs Swati Saini for his kind and unwavering motivation and support. This research is self sponsored and no funding Agencies were approached for this research.

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