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Design, Synthesis, Characterization and Biological Evaluation of New Heterocyclic Derivatives As Anti-Tubercular Agents

Ayyadurai Jerad Suresh[1]*, Geetha Lakshmi[1], Sundaralingam Saranya[1], Parakkot Ramakrishnan Surya[1]
1. College of Pharmacy, Madras Medical College, Chennai

ABSTRACT

Oxadiazole, a heterocyclic nucleus has attracted a wide attention of the chemist in search for the new therapeutic molecules. Some of the recent studies show that oxadiazole are reported to possess an anti-tubercular, anti-epileptic, analgesic, hypnotic, and sedative activity.[1] Catechol is a chemical, but a catechol may also be used as the name of a substance, where it represents a 1, 2-dihydroxy benzene group[2]. In the present study 1, 3, 4-oxadiazole and phenyl amino benzene 1,2 -diol derivatives were docked against methoxymycolic acid synthase-2 and synthesized using reflux condensation reaction. The newly synthesized compounds were characterized by elemental and spectral methods. Compounds RSP1, RSP3 and RSP4 are novel compounds. Anti-tubercular activities of these compounds were carried out using Alamar blue assay method and these compounds exhibited good activity. Compound with phenyl amino benzene 1, 2 diol showed good activity and oxadiazole showed moderate activity compared with standard drugs. RSP1, RSP3, and RSP4 shows good activity at the range of 0.8µg -50ng.RSP6 and RSP7 shows activity at 12.5µg. A further refinement to the structure of the synthesized compounds is expected to yield new outlook to the development of promising molecules against Mycobacterium tuberculosis.

Keywords: Oxadiazole, anti-tubercular, catechol, phenyl amino benzene1, 2-diol, analgesic.

*Corresponding Author Email: ajsuresh2001@yahoo.co.uk
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INTRODUCTION

Tuberculosis (TB) is a contagious disease caused by Bacteria (Mycobacterium tuberculosis), like the common cold, it spread through the air. [3]Tuberculosis is curable and preventable disease.[4] The recent rise in TB cases and especially the increase of drug resistant mycobacteria indicate an urgent need to develop new anti-TB drugs.[5] So, It is important to achieve a shortened therapy schedule to encourage patient compliance and to slow down the development of drug resistance in mycobacteria.[6]

Mycolic acids, a homologous series of C60-C90 long-chain alpha-alkyl- and beta-hydroxy fatty acids, represent essential components of the mycobacterial cell wall. They are important for mycobacterial growth, survival, and pathogenicity.[7] MmA2 is required for introduction of the distal cyclopropane ring in the formation of meroacids. Novel inhibitors of this enzyme could potentially be used as therapeutic agents. It will inhibit the cell wall synthesis of mycobacterium tubercular bacilli (i.e. distal cyclopropane modification of mycolic acid).[8]

Oxadiazole is a heterocyclic nucleus derived from furan by replacement of two methane (-CH=) group by two pyridine type nitrogen (-N=).There are four possible isomers of oxadiazole depending on the position of nitrogen atom in the ring.[9] Out of its four isomers 1, 3, 4-oxadiazole is widely exploited for various applications. Figure 1

Catechol is a chemical, but a catechol may also be used as the name of a substance, where it represents a 1, 2-dihydroxy benzene group. In the human body, the most abundant catecholamines are epinephrine, norepinephrine (nor-adrenaline), adrenaline, and dopamine.[10] Figure 2

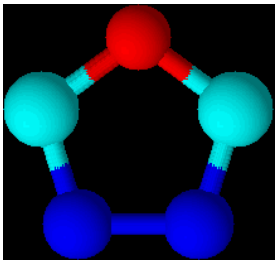


Figure.1: Image of oxadiazole nucleus

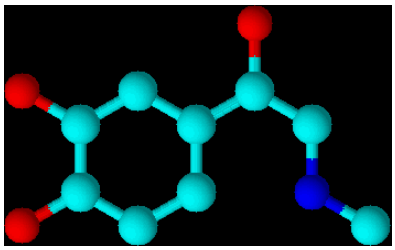


Figure.2: Image of catechol nucleus

MATERIALS AND METHOD

Docking

A process of design and discovery of new chemical entities using an automated docking program GLIDE (grid based ligand docking with energetics) maestro 9.0 Schrodinger suites, Auto Dock and Argus Lab. It searches molecules (ligands) having maximum favorable interactions with a receptor (target) usually a protein.[11] Docking is done by using ARGUS LAB Software. Argus lab 4.0 is distributed freely available for windows platforms by Planaria Software. It is an introductory molecular modeling package with academics [12]

Insilico screening of drug likeness

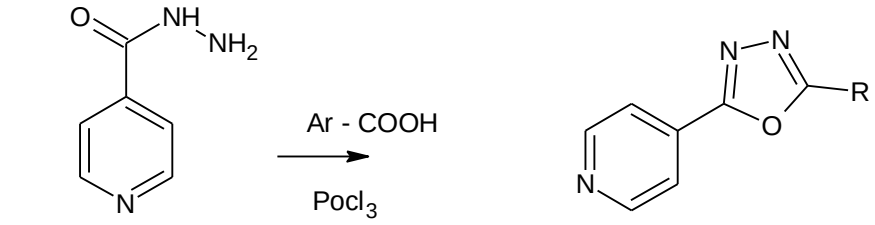
A drug to be pharmacologically active and exert the action it should possess pharmacokinetic properties like absorption, distribution, metabolism and excretion. In the field of drug research and development many drug failures occur due to unfavorable ADME properties. This has to be ruled out earlier in the process of drug discovery. Some computational methods have been evolved to investigate the most suitable drug molecules before synthesis. [13] "Lipinski's rule of five" it is also known as Pfizer's rule of five is rule to evaluate drug likeness. It is used to predict whether a molecule is likely to be orally bio-available or to evaluate drug likeness.[14]

Toxicity risk assessment

All the docked molecules are subjected to the toxicity risk assessment by using Osiris program, which is available online. Prediction results are color coded in which the red color shows high risks with undesired effects like mutagenicity or a poor intestinal absorption and green color indicates drug-conform behavior.[15]

Synthetic scheme 1: [16]

A mixture of isonicotinic acid hydrazide 2.6g (0.01mole) and various aromatic acids (0.01mole) in phosphorous oxychloride 5ml are refluxed for 5-6 hours. The contents are cooled and poured on to crushed ice. It is neutralized with sodium bi carbonate solution and the resulting solid is filtered and dried.



pyridine-4-carbohydrazide Oxadiazole derivatives

Aromatic acids: 4-methylamino benzoic acid3, 4-dichloro benzoic acid etc.

Scheme 2:

The compound was synthesized by two a step reaction.

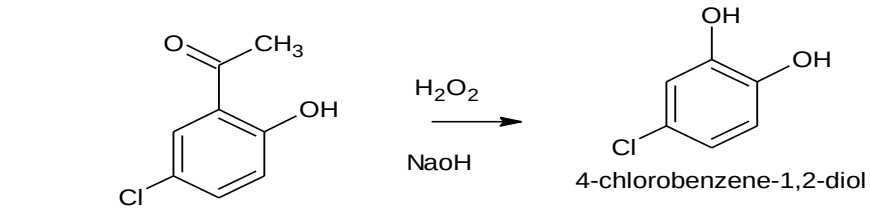
Step 1: dakin's reaction:[17]

5-chloro-2-hydroxy acetophenone (1mole) and sodium hydroxide (0.1mole) in presence of 3% hydrogen peroxide is heated at 45-50^{o}c .The solution is allowed to stand for 20 hrs. The completion of the reaction is monitored by TLC. After completion, few drops of acetic acid are added to neutralize excess alkali. The resulting solution is evaporated to dryness on water bath to get a crude product of 4-chloro catechol. It is recrystallized using ethanol to get pure product**.**

Step 2: gold berg reaction: [18]

0.0850mole of substituted aromatic amines, 0.159 mole of 4-chloro catechol, 16.4g potassium carbonate and 0.3g copper bromide are added in 20ml of ethanol. The mixture is refluxed for 6-30 hours. The completion of the reaction is monitored by TLC. After completion, the mixture is cooled in ice bath and poured into the crushed ice. The resulting solution is neutralized by adding concentrated Hydrochloric Acid drop by drop. The precipitate that is obtained is filtered, dried and recrystallized using ethyl acetate.

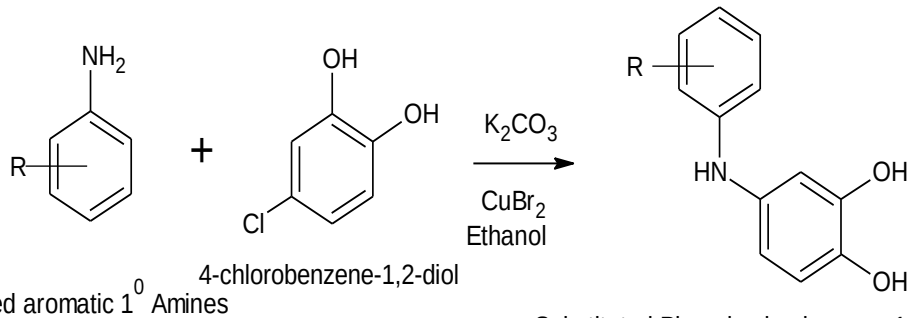
Step 1: Dakin's reaction



1-(5-chloro-2-hydroxyphenyl)ethanone

4-chlorobenzene-1,2-diol

Step 2: gold berg reaction



Substituted aromatic 1^{0} Amines

4-chlorobenzene-1,2-diol

Substituted Phenylamino benzene1,2 diol derivatives

Spectral analysis

4-[5-(3,4-dichlorophenyl)-1,3,4-oxadiazole-2-yl]pyridinole(RSP6) :IR- Using KBR pellet method. Ar C-H Str (3170.74 Cm^{-1}) ,Ar C=C St (1635.52 Cm^{-1}),C=N Str (2360.70 Cm^{-1}) C-O Str (1026.05Cm^{-1}), C-Clstr (856.33 Cm^{-1}).NMR:^{1}HNMR δ PPM (δ6.64-6.66 ppm,doublet,1H)(

7.17-7.40 ppm,multiplet,2H), (7.87-8.00 ppm, multiplet,3H),(8.77-8.852ppm, multiplet,1H) % PURITY:92%, MASS: 292.02(M^+), 294(M+2).Figure 3

N-Methyl-4-[5-(pyridine-4-yl)-1,3,4-oxadiazole-2-yl]aniline(RSP7):IR-N-HStr(3433.04Cm^{-1}), AliphaticC-HStr(2923.87Cm^{-1}),C=NStr(2360.70Cm^{-1}),Ar.C=CStr(1658.68Cm^{-1}).^{1}HNMR:(0.82-0.85ppm,triplet,1H),(1.22ppm,singlet,3H),(1.99ppm,singlet,1H),(7.26-8.84ppm,multiplet,6H),% purity:94.22%,mass: 252.17(M+).Figure 4

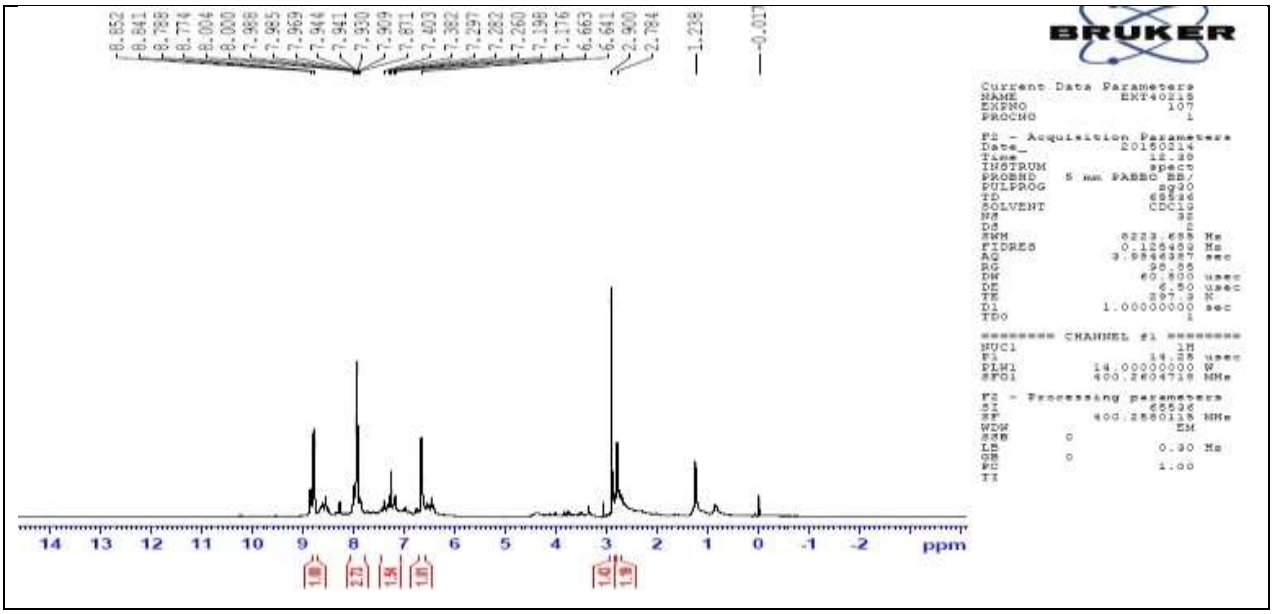


Figure 3: Image of 1H NMR spectrum of RSP64-[5-(3,4-dichlorophenyl)-1,3,4-oxadiazole-2-yl]pyridinole.

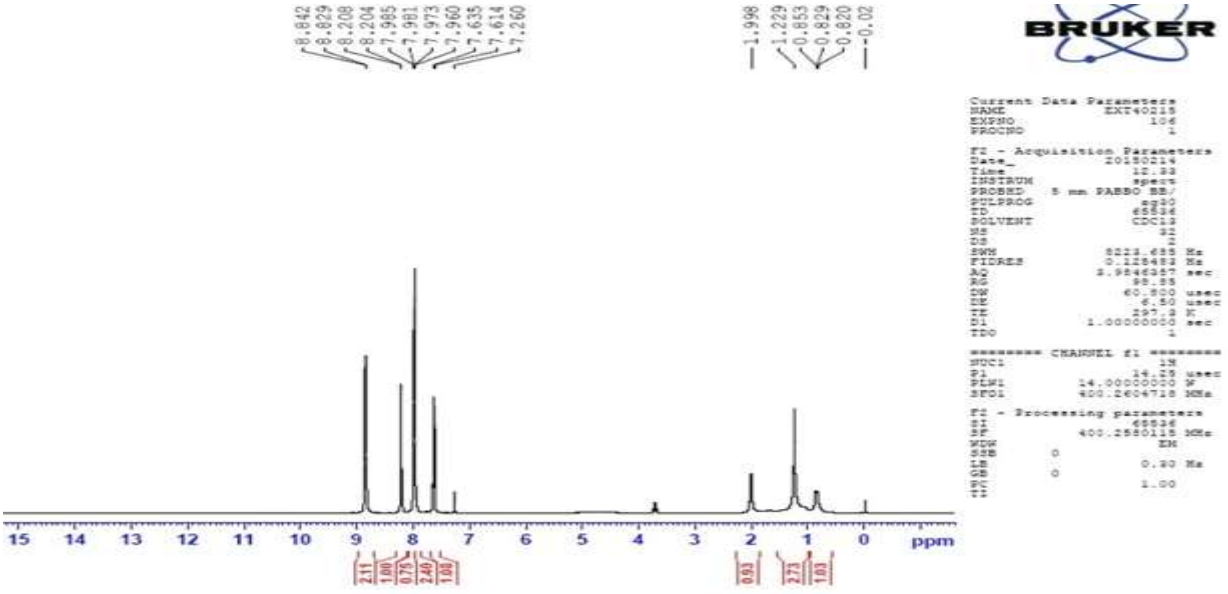


Figure 4: It shows 1HNMR spectrum of RSP7N-Methyl-4-[5-(pyridine-4-yl)-1,3,4-oxadiazole-2-yl]aniline.

4[(3,5-dichloropyridine-4-yl)amino]benzene -1,2 diol(RSP1): IR- N-H str(3448.87 Cm^{-1}),Ar C-H str(3116.74 Cm-1),Ar C=C str(1635 Cm-1),C=N str(2352.98 Cm-1),C-N str(1326.93 Cm-1).[1] H NMR:δ(1.25 ppm,singlet,1H),(4.98 ppm,singlet,1H).(8.20 ppm,singlet,1H),(1.9 ppm,singlet,1H).% purity:100%.

4-(pyridine-4-ylamino) benzene-1,2 diol(RSP3): : IR- N-H str(3379.04 Cm-1), O-H str (3471.61 Cm^{-1}),Ar C-H str(3193.88 Cm-1),Ar C=C str(1612.37 Cm-1),C=N str(2306.69 Cm-1),C-N str(1326.93 Cm-1).[1] H NMR:δ(5.24 ppm,singlet,1H),(6.53-6.55 ppm,doublet,2H).(6.99-7.01ppm,doublet,2H),(3.4 ppm,singlet,1H).(2.5ppm,singlet,1H),% purity:100%.

Anti-activity

The anti-mycobacterial activity of compounds were assessed against M.tuberculosis using microplate Alamar Blue assay (MABA).This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Briefly, 200µl of sterile deionized water was added to all outer perimeter wells Of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middlebrooks 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37ºC for five days. After this time, 25µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.

RESULTS AND DISCUSSION

In present research work, we have synthesized derivatives of oxadiazole4-[5-(3,4-dichlorophenyl)-1,3,4-oxadiazole-2-yl]pyridinole and catechol 4[(3,5-dichloropyridine-4-yl)amino]benzene -1,2 diol. The oxadiazole was obtained by cyclisation of hydrazide in isoniazid with different aromatic acid and 4-chloro catechol react with different amines to form phenylamino benzene -1,2 diol (catechol).The physicochemical properties of derivatives were calculated from computational tools and characterization of compounds were carried out by spectral methods. The final compounds were screened for anti-mycobacterial activity.

Chemistry

The structures of the final compounds were confirmed on the basis of spectral studies. All the newly synthesized compounds were characterized by IR,[1]H NMR, and MASS spectroscopic

data.[21]IR spectra of 4-[5-(3,4-dichlorophenyl)-1,3,4-oxadiazole-2-yl]pyridinole(RSP6) showed a strong absorption band at 2360 and 1026 Cm^{-1} for C=N and C-O stretching in oxadiazole ring. Absorption band at 3170 Cm^{-1} and 1635 Cm^{-1} for Aromatic C-H and C=C stretching in pyridine ring. C-Cl stretching at 856 Cm^{-1}.^{1}H NMR (δ7.8-8.0ppm,3H,phenylringprotons),(δ 6.4-6.6,1H,7.1-7.42H,8.7-8.8,1H,pyridine ring protons).The mass spectra of the compound RSP6 revealed the molecular ion peak at 292 m/z.M+2 at 294m/z (Cl substitution) corresponding to the molecular mass of the compound.

Anti-mycobacterial screening

All the compounds showed good and moderate activity against mycobacterium tuberculosis, the inhibition of growth of bacteria was measured in µg and ng. Out of the synthesized derivatives RSP1, RSP3, and RSP4 shows good activity at the range of 0.8µg -50ng.RSP6 and RSP7 shows activity at 12.5µg.Table:2, 3, 4.

Table 1: Compound Profile

S.l No	Mol Wt	Melting point	M. Formula	Solubility	Color	Yield	Molar refractivity
RSP6	292.12	69℃	$C_{15}H_7Cl_2N_3O$	Chloroform, Methanol	Light Brown	82.2%	71.80±0.3 cm3
RSP7	252.27	52℃	$C_{13}H_7Cl_2N_3O_2$	Chloroform, Ethanol	Dark Brown	85.2%	71.61± 0.3 cm3
RSP1	271.09	1310c	C11H8Cl2N2O2	Chloroform, ethyl acetate	White	82.25%	67.27 ± 0.3 cm3
RSP3	202.20	480c	C11H10N2O2	Chloroform, methanol	Dark brown	85.3%	57.48 ± 0.3 cm3
RSP4	280.11	1390c	C12H10BrN1O2	Chloroform, methanol	Light brown	80.21%	67.08 ± 0.3 cm3

Table.2: Anti-microbial activity of compounds compared with standard drugs.

S.NO	SAMPLE CODE	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	50 ng/ml
1	RSP6	S	S	S	S	R	R	R	R	R
2	RSP7	S	S	S	S	R	R	R	R	R
3	RSP1	S	S	S	S	S	S	S	S	S
4	RSP3	S	S	S	S	S	S	S	S	R
5	RSP4	S	S	S	S	S	S	S	S	R

Note:

S - Sensitive

R - Resistant

Strain used: **M. tuberculosis**(H37 RV strain)

Here are the **standard values** for the Anti-Tb test which was performed.

Pyrazinamide- 3.125µg/ml

Streptomycin- 6.25µg/ml

Ciprofloxacin-3.125µg/ml

Table.3: sample drug photograph

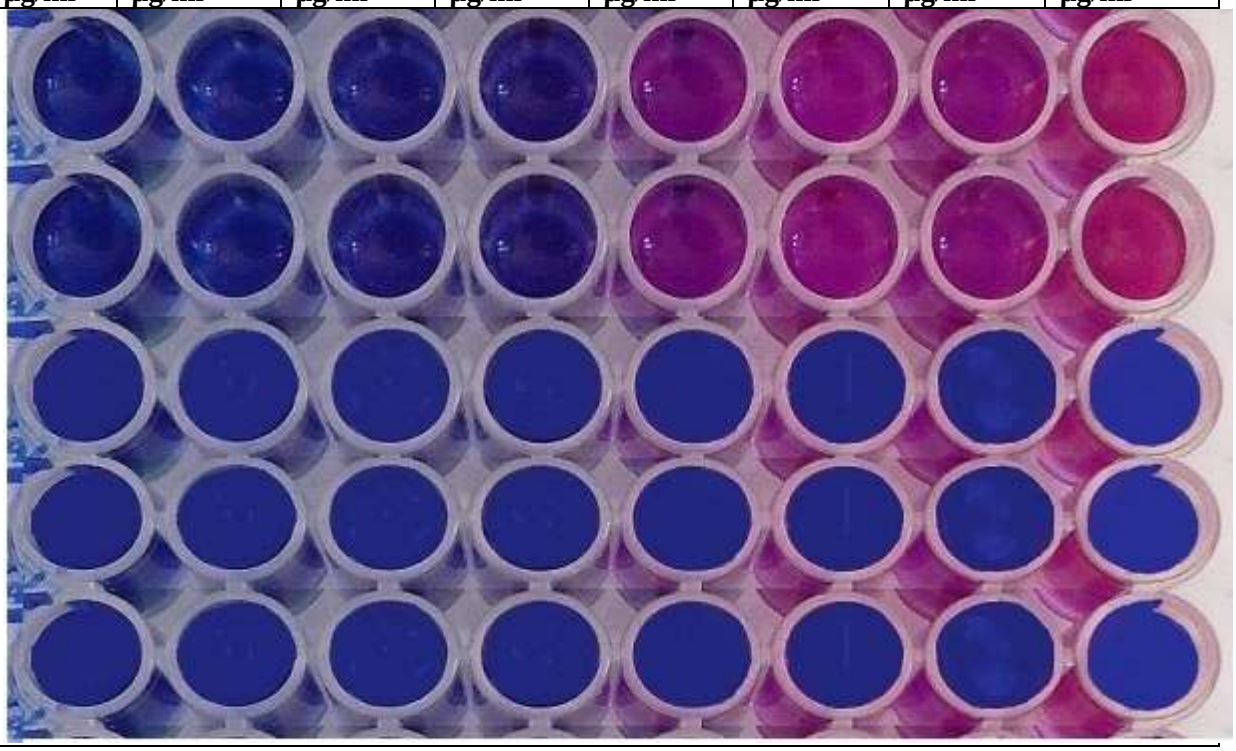
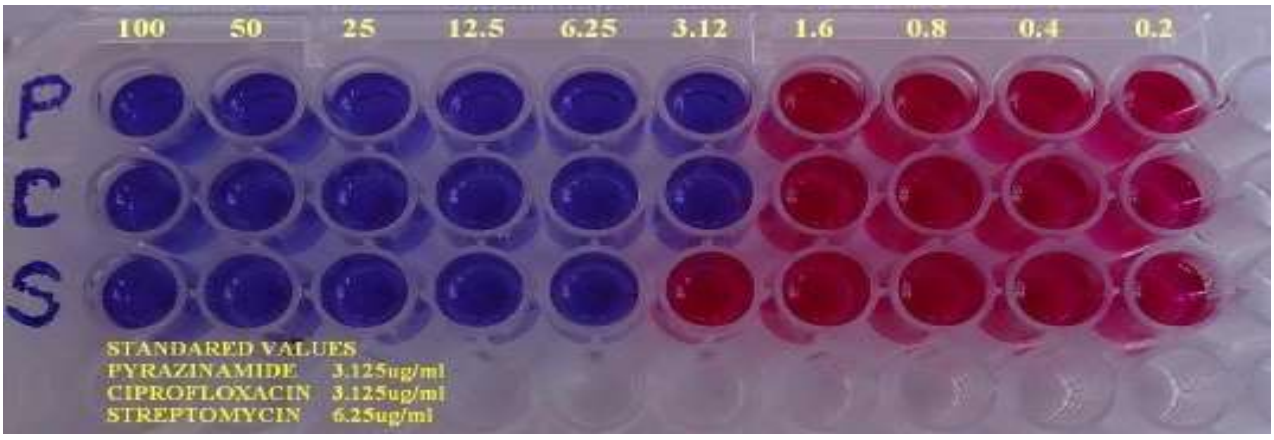
Sample Code	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
RSP6								
RSP7								
RSP1								
RSP3								
RSP4								

Table.4: standard drug photograph



CONCLUSION

Present work concludes that the successful synthesize and anti-tubercular activity of new 1,3,4-oxadizole and catechol derivatives. The anti-tubercular study revealed that all the compounds showed moderate to good activity against methoxymycolic acid synthase 2. Compound with phenyl amino benzene 1, 2 diol showed good activity and oxadiazole shows moderate activity compared with standard drugs. A further refinement to the structure of the synthesized

compounds is expected to yield a new outlook to the development of promising molecules against Mycobacterium tuberculosis.

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