

# Synthesis, Characterizations and Biological Screening of Tetrahydro-Quinazoline Analogues

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**Abstract** Quinazoline and their fused-ring systems are well known for their potential biological activity. In the present study a new Tetrahydro-quinazoline analogues (MB I-V) were synthesized. The newly synthesized compounds were characterized by IR, NMR and C, H, N, S analyses. All newly synthesized compounds were screened for their antibacterial (*Pseudomonas aurigenosa*, *Bacillus subtilis* and *Escherichia coli*) studies. The results revealed that all synthesized compounds have a significant biological activity against the tested microorganisms.

**Keywords** Tetrahydro-Quinazoline, Antibacterial Activity, Isophorone

## 1. Introduction

During the ancient era the isolation of various compounds was done by the process of extraction. But this process was time consuming as well as laborious. Moreover the yield was very low and the process of isolation required large amount of the starting material. Today the process of isolation has been replaced by the synthetic routes. A large number of compounds can be synthesized by using small amount of chemicals. More over the Synthetic routes take less amounts of time and can easily be carried out. Quinazoline derivatives hold a place of significant in today's world for their important application in chemical, clinical and biological spheres. Medicinally quinazoline has been used in various areas especially as an analgesic [1, 2], anti-oxidant [3-5], anti-cancer drugs [6-10], anti-inflammatory [11,12], anti-convulsant [13], anti-bacterial [14], anti-fungal [15] and anti-mycobacterial agents [16, 17]. It has also been found in the treatment of malaria [18, 19]. Considering the vast potential of quinazoline, it was thought appropriate to synthesize, characterize quinazoline analogues and investigate their biological activity. In this investigation, we have prepared tetrahydro quinazoline analogues and characterized them using spectral data. Biological screenings of these compounds were also reported also reported here.

## 2. Result and Discussion

### 2.1. Chemistry

Intermediates, 5,5,7-trimethyl-4-aryl-3,4,5,6-tetrahydro quinazoline -2-thiols (S I-V) synthesized from isophorone, different aromatic aldehydes and thiourea (Scheme Ia,b). Tetrahydroquinazoline analogues (MB I-V) synthesized from intermediates S I-V (Scheme IIa,b). The purity of the compounds was monitored by ascending thin layer chromatography (TLC) on silica gel-G coated on aluminum plates.

Developing solvents used in TLC were ethyl acetate: hexane (1:2) and the plates were viewed under UV light 254 nm and 265 nm respectively. Melting points were determined by the open capillary tubes equipped in electro thermal melting point apparatus. All the melting points are uncorrected. The yields of all the compounds reported are of crystallized form. The elemental analysis was studied by C, H, N, S analyzer on Perkin Elmer (U.S.A, 2400 Series II). Infrared (IR) spectra were recorded for the compounds on Perkin Elmer Spectrum GX using KBr pellet disc technique. The structure will further be elucidated by recording its mass spectra on Shimadzu LCMS 2010 eV. NMR was recorded in Torrent pharmaceuticals Ltd Research centre on Bruker Avance FT-NMR 400 MHz.

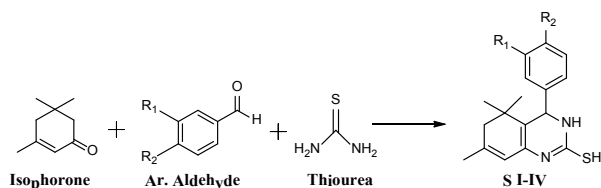
The M.P. of synthesized compounds were found between 258-300°C, while for the some analogues it was more than 300°C. The mol. wt. of the synthesized compound were in range of 271-427 g mole<sup>-1</sup>.

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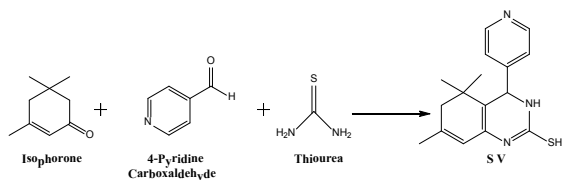
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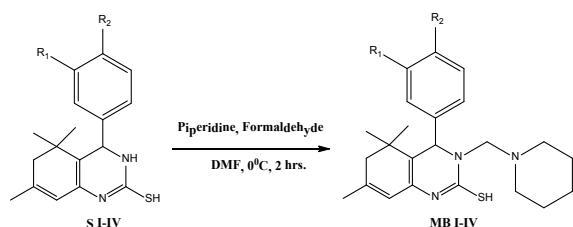


Sample ID	R <sub>1</sub>	R <sub>2</sub>
S I	-OH	-OCH <sub>3</sub>
S II	-NO <sub>2</sub>	H
S III	H	H
S IV	-OCH <sub>3</sub>	-OCH <sub>3</sub>

Scheme Ia

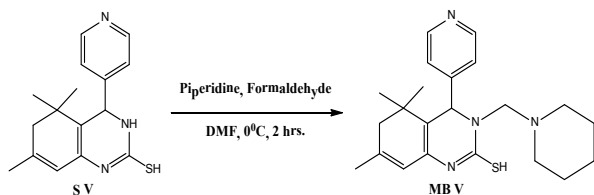


Scheme Ib



Sample ID (II)	R <sub>1</sub>	R <sub>2</sub>
MB I	-OH	-OCH <sub>3</sub>
MB II	-NO <sub>2</sub>	H
MB III	H	H
MB IV	-OCH <sub>3</sub>	-OCH <sub>3</sub>

Scheme IIa



Scheme IIb

The IR peaks obtained in study was correlated with the standard IR frequency of the vibration mode given by Nakamoto[20]. The IR spectra showed the presence of C=C stretching between 1580-1615 cm<sup>-1</sup>, C-C stretching between 1030-1350 cm<sup>-1</sup>, C-H stretching at ~3080 cm<sup>-1</sup>, C-O stretching (1° alcohol) at 1123 cm<sup>-1</sup>, C=N stretching between 1080-1360 cm<sup>-1</sup>, N-H stretching between 3380-3400 cm<sup>-1</sup>, C-N stretching between 1150-1210 cm<sup>-1</sup>, C-S stretching between 685-710 cm<sup>-1</sup>, -NO<sub>2</sub> stretching at ~1528 cm<sup>-1</sup>, -OH stretching at ~3426 cm<sup>-1</sup> for intermediates S I-V and final products MB I-V. In addition, <sup>1</sup>H NMR spectra of the synthesized compounds showed the

characteristic signals at ~ 6.1 δ due to SH group. Further the spectra of the intermediates (S I-V) showed a signal ~ 4.1 δ due to NH of pyrimidine ring and disappearance of these signals in synthesized compounds (MB I-V) established that attachment of piperidine moiety with pyrimidine NH group. The structures of synthesized compounds were supported by mass spectrum; it showed the molecular ion peaks in between range of 285 - 428 (M+1).

## 2.2. Antibacterial Activity

In present study the five compounds are screened for antibacterial activity against gram positive and gram negative bacteria. The result of antibacterial activities are comparatively studied (Table 1) and are recorded on the basis of the presence or absence of inhibition zone on media plate. The antibacterial activity against *Bacillus subtilis* was seen in all the compounds (MB I-V) used. The compound MB I showed high activity against *Bacillus subtilis* due to presence of OH group. All the compounds (MB I-V) did not show activity on *Pseudomonas aurigenosa* as well as the standard drug did not show any activity. The compound MB II showed high activity against *Escherichia coli*. This could be due to the presence of -NO<sub>2</sub> group at R<sub>1</sub> position of tetrahydroquinazoline derivatives.

Table 1. Zone of growth inhibition of synthesized compounds

Sample	Zone of Inhibition in mm		
	<i>Bacillus subtilis</i>	<i>Pseudomonas aurigenosa</i>	<i>Escherichia coli</i>
MB I	23	-	17
MB II	22	-	23
MB III	21	-	20
MB IV	18	-	21
MB V	16	-	15
Ciprofloxacin	46	-	20
DMSO	-	-	-

This activity of any compound is directly correlated with their zone of inhibition on the medium. More than 20 mm of inhibition zone is called high activity, More than 10 mm of inhibition is called good activity, inhibition zone between 6-9 mm moderate activity and least activity is assigned between 1-5 mm of inhibition zone[21].

## 3. Experimental

The raw materials used were of synthetic grade from Sigma Aldrich & Loba chemicals Ltd. Solvents used were distilled and dried. The TLC plates were from Merck. Melting points were determined by the open capillary tubes equipped reported are of crystallized form in electro thermal melting point apparatus. All the melting points are uncorrected. The elemental analysis was studied by C, H, N, S analyzer on Perkin Elmer (U.S.A, 2400 Series II). Infrared (IR) spectra were recorded for the compounds on Perkin Elmer Spectrum GX using KBr pellet disc technique. The structure will further be elucidated by recording its mass spectra on Shimadzu LCMS 2010 eV. NMR was recorded in

Torrent pharmaceuticals Ltd Research centre on Bruker Avance FT- NMR 400 MHz.

### 3.1. General Procedure for Synthesis of 5, 5, 7-Trimethyl-4-Aryl-3, 4, 5, 6-Tetrahydroquinazoline-2-Thiois (S I-V)

Equivalent mixture of isophorone, an aromatic aldehydes and thiourea was refluxed in ethanol for 10 hours. Then the resulting reaction mixture was poured in ice bath. The obtain product (Scheme-Ia & Ib) was filtered, dried & recrystallized by 95% Ethanol[20].

5-(2-mercapto-5,5,7-trimethyl-3,4,5,6-tetrahydroquinazolin-4-yl)-2-methoxyphenol (S I): Yield: 85%; Colour: Brown; m.p.: >300°C; IR (KBr, cm<sup>-1</sup>): 1462 (Ar C=C), 1598 (Ar C-C), 3403 (N-H), 3292 (O-H), 1365 (CH<sub>3</sub>), 1638 (C=N), 1219 (C-S), 1274 (Ester C-O); <sup>1</sup>H NMR (DMSO): 1.25 δ (6H, s, CH<sub>3</sub>), 1.83 δ (3H, s, CH<sub>3</sub>), 2.19 δ (2H, s, CH<sub>2</sub>), 3.83 δ (3H, s, OCH<sub>3</sub>), 4.28 δ (1H, d, N-H), 4.60 δ (1H, d, CH), 5.35 δ (1H, s, OH), 5.7 δ (1H, s, CH), 6.3 δ (1H, s, S-H), 6.68 δ (1H, d, CH), 6.70 δ (1H, d, CH), 6.86 δ (1H, s, CH), DMSO 2.52 δ & 3.50 δ; MS, m/z: 317.34[M+1]. Anal.: Calc. for C, 65.42; H, 6.71; N, 8.48; S, 9.70. Found: C, 65.21; H, 6.50; N, 8.29; S, 9.35.

5,5,7-trimethyl-4-(3-nitrophenyl)-3,4,5,6-tetrahydroquinazoline-2-thiol (S II): Yield: 78%; Colour: Brown; m.p.: >300°C; IR (KBr, cm<sup>-1</sup>): 1664 (Ar C=C), 1468 (Ar C-C), 3383 (N-H), 3040 (Ar C-H), 1384 (CH<sub>3</sub>), 1619 (C=N), 1246 (C-S), 1528 (N-O); <sup>1</sup>H NMR (DMSO): 1.26 δ (6H, s, CH<sub>3</sub>), 1.82 δ (3H, s, CH<sub>3</sub>), 1.98 δ (2H, s, CH<sub>2</sub>), 4.12 δ (1H, d, N-H), 4.59 δ (1H, d, CH), 5.61 δ (1H, s, CH), 5.8 δ (1H, s, S-H), 7.62 δ (1H, d, CH), 7.59 δ (1H, d, CH), 8.07 δ (1H, d, CH), 8.12 δ (1H, s, CH), DMSO 2.48 δ & 3.47 δ; MS, m/z: 329.23[M+1]. Anal.: Calc. for C, 61.98; H, 5.81; N, 12.76; S, 9.73. Found: C, 61.75; H, 5.75; N, 12.50; S, 9.60.

5,5,7-trimethyl-4-phenyl-3,4,5,6-tetrahydroquinazoline-2-thiol (S III): Yield: 86%; Colour: Brown; m.p.: 258°C; IR (KBr, cm<sup>-1</sup>): IR (KBr, cm<sup>-1</sup>): 1663 (Ar C=C), 1452 (Ar C-C), 3438 (N-H), 2848 (Ar C-H), 1378 (CH<sub>3</sub>), 1617 (C=N) 1286 (C-S); <sup>1</sup>H NMR (DMSO): 1.24 δ (6H, s, CH<sub>3</sub>), 1.83 δ (3H, s, CH<sub>3</sub>), 2.2 δ (2H, s, CH<sub>2</sub>), 4.01 δ (1H, d, N-H), 4.62 δ (1H, d, CH), 5.68 δ (1H, s, CH), 5.9 δ (1H, s, S-H), 7.25 δ (2H, t, CH), 7.28 δ (1H, t, CH), 7.34 δ (2H, t, CH), DMSO 2.52 δ & 3.53 δ; MS, m/z: 284.10[M+1]. Anal.: Calc. for C, 71.79; H, 7.09; N, 9.85; S, 11.27. Found: C, 71.50; H, 6.95; N, 9.67 S, 11.01.

4-(3,4-dimethoxyphenyl)-5,5,7-trimethyl-3,4,5,6-tetrahydroquinazoline-2-thiol (S IV): Yield: 91%; Colour: Orange; m.p.: 285°C; IR (KBr, cm<sup>-1</sup>): 1652 (Ar C=C), 1462 (Ar C-C), 3192 (N-H), 2955 (Ar C-H), 1381 (CH<sub>3</sub>), 1612 (C=N), 1249 (C-S); <sup>1</sup>H NMR (DMSO): 1.26 δ (6H, s, CH<sub>3</sub>), 1.82 δ (3H, s, CH<sub>3</sub>), 2.20 δ (2H, s, CH<sub>2</sub>), 3.88 δ (6H, s, OCH<sub>3</sub>), 4.11 δ (1H, d, N-H), 4.59 δ (1H, d, CH), 5.69 δ (1H, s, CH), 6.01 δ (1H, s, S-H), 6.76 δ (1H, d, CH), 6.67 δ (1H, d, CH), 6.73 δ (1H, s, CH), DMSO 2.5 δ & 3.51 δ; MS, m/z: 344.40[M+1]. Anal.: Calc. for C, 66.25; H, 7.02; N, 8.13; S, 9.31. Found: C, 65.99; H, 6.88; N, 8.04; S, 9.15.

5,5,7-trimethyl-4-(pyridin-4-yl)-3,4,5,6-tetrahydroquinazoline-2-thiol (S V): Yield: 77%; Colour: Yellow; m.p.: 278°C; IR (KBr, cm<sup>-1</sup>): 1667 (Ar C=C), 1467 (Ar C-C), 3406 (N-H), 2853 (Ar C-H), 1367 (CH<sub>3</sub>), 1599 (C=N), 1254 (C-S); <sup>1</sup>H NMR (DMSO): 1.26 δ (6H, s, CH<sub>3</sub>), 1.82 δ (3H, s, CH<sub>3</sub>), 2.22 δ (2H, s, CH<sub>2</sub>), 4.19 δ (1H, d, N-H), 4.61 δ (1H, d, CH), 5.72 δ (1H, s, CH), 6.05 δ (1H, s, S-H), 7.35 δ (2H, d, CH), 8.55 δ (2H, d, CH), DMSO 2.51 δ & 3.53 δ; MS, m/z: 285.15[M+1]. Anal.: Calc. for C, 67.33; H, 6.71; N, 14.72; S, 11.23. Found: C, 67.28; H, 6.56; N, 14.51; S, 11.02.

### 3.2. General Procedure for Synthesis of 4-Aryl-5, 5-Dimethyl-7-(2'-Piperidin-1'-yl-Ethyl)-2-Thiol-3,4, 5,6-Tetrahydroquinazolines (MB I-V)

A mixture of 0.01 moles appropriate intermediate (S I-V), Piperidine & formaldehyde in DMF were stirred for 2 hours in cooling conditions. Then the resulting reaction mixture was poured in ice bath. The obtain product (Scheme-IIa & IIb) was filtered, dried & recrystallized by 95% Ethanol.

5-(2-mercapto-5,5,7-trimethyl-3-(piperidin-1-ylmethyl)-3, 4, 5, 6 - tetrahydroquinazolin- 4- yl)-2-methoxyphenol (MB I): Yield: 82%; Colour: Brown; m.p.: 292°C; IR (KBr, cm<sup>-1</sup>): 1652 (Ar C=C), 1417 (Ar C-C), 2894 (Ar C-H), 1061 (C-N), 3426 (N-H), 3035 (O-H), 1219 (Ester C-O), 1314 (CH<sub>3</sub>), 1265 (C-S), 1600 (C=N); <sup>1</sup>H NMR (DMSO): 1.25 δ (6H, s, CH<sub>3</sub>), 1.28 δ (2H, m, CH<sub>2</sub>), 1.53 δ (4H, m, CH<sub>2</sub>), 1.83 δ (3H, s, CH<sub>3</sub>), 2.2 δ (2H, s, CH<sub>2</sub>), 2.45 δ (4H, m, CH<sub>2</sub>), 3.65 δ (2H, s, CH<sub>2</sub>), 3.84 δ (3H, s, OCH<sub>3</sub>), 4.59 δ (1H, d, CH), 5.33 δ (1H, s, OH), 5.70 δ (1H, s, CH), 6.32 δ (1H, s, S-H), 6.66 δ (1H, d, CH), 6.69 δ (1H, d, CH), 6.87 δ (1H, s, CH), DMSO 2.52 δ & 3.50 δ; MS, m/z: 427.24[M+1]. Anal.: Calc. for C, 67.41; H, 7.78; N, 9.83; S, 7.50. Found: C, 67.22; H, 7.52; N, 9.60; S, 7.25.

5,5,7-trimethyl-4-(3-nitrophenyl)-3-(piperidin-1-ylmethyl)-3,4,5,6-tetrahydroquinazoline-2-thiol (MB II): Yield: 87%; Colour: Brown; m.p.: >300°C; IR (KBr, cm<sup>-1</sup>): 1664 (Ar C=C), 1464 (Ar C-C), 2866 (Ar C-H), 1096 (C-N), 3404 (N-H), 1528 (N=O), 1382 (CH<sub>3</sub>), 1256 (C-S), 1620 (C=N); <sup>1</sup>H NMR (DMSO): 1.25 δ (6H, s, CH<sub>3</sub>), 1.29 δ (2H, m, CH<sub>2</sub>), 1.52 δ (4H, m, CH<sub>2</sub>), 1.82 δ (3H, s, CH<sub>3</sub>), 2.0 δ (2H, s, CH<sub>2</sub>), 2.45 δ (4H, m, CH<sub>2</sub>), 3.60 δ (2H, s, CH<sub>2</sub>), 4.62 δ (1H, d, CH), 5.64 δ (1H, s, CH), 5.84 δ (1H, s, S-H), 7.58 δ (1H, d, CH), 7.63 δ (1H, d, CH), 8.09 δ (1H, d, CH), 8.13 δ (1H, s, CH), DMSO 2.51 δ & 3.5 δ; MS, m/z: 426.21[M+1]. Anal.: Calc. for C, 64.76; H, 7.09; N, 13.13; S, 7.52. Found: C, 64.52; H, 6.89; N, 12.96; S, 7.36.

5,5,7-trimethyl-4-phenyl-3-(piperidin-1-ylmethyl)-3,4,5,6-tetrahydroquinazoline-2-thiol (MB III): Yield: 79%; Colour: Brown; m.p.: 277°C; IR (KBr, cm<sup>-1</sup>): 1660 (Ar C=C), 1423 (Ar C-C), 2885 (Ar C-H), 1077 (C-N), 3420 (N-H), 1370 (CH<sub>3</sub>), 1248 (C-S), 1596 (C=N); <sup>1</sup>H NMR (DMSO): 1.25 δ (6H, s, CH<sub>3</sub>), 1.27 δ (2H, m, CH<sub>2</sub>), 1.53 δ (4H, m, CH<sub>2</sub>), 1.82 δ (3H, s, CH<sub>3</sub>), 2.19 δ (2H, s, CH<sub>2</sub>), 2.44 δ (4H, m, CH<sub>2</sub>), 3.63 δ (2H, s, CH<sub>2</sub>), 4.61 δ (1H, d, CH), 5.7 δ (1H, s, CH), 5.97 δ (1H, s, S-H), 7.25 δ (1H, t, CH), 7.27 δ (2H, t, CH), 7.35 δ (2H, t, CH), DMSO 2.5 δ & 3.51 δ; MS, m/z: 381.22[M+1].

Anal.: Calc. for C, 72.40; H, 8.19; N, 11.01; S, 8.40. Found: C, 72.15; H, 8.09; N, 10.78; S, 8.19.

4-(3,4-dimethoxyphenyl)-5,5,7-trimethyl-3-(piperidin-1-ylmethyl)-3,4,5,6-tetrahydroquinazoline-2-thiol (MB IV): Yield: 65%; Colour: Orange; m.p.: >300°C; IR (KBr, cm<sup>-1</sup>): 1646 (Ar C=C), 1425 (Ar C-C), 2858 (Ar C-H), 1068 (C-N), 3443 (N-H), 1227 (Ester C-O), 1360 (CH<sub>3</sub>), 1258 (C-S), 1629 (C=N); <sup>1</sup>H NMR (DMSO): 1.28 δ (6H, s, CH<sub>3</sub>), 1.53 δ (2H, m, CH<sub>2</sub>), 1.59 δ (4H, m, CH<sub>2</sub>), 1.83 δ (3H, s, CH<sub>3</sub>), 2.21 δ (2H, s, CH<sub>2</sub>), 2.23 δ (4H, m, CH<sub>2</sub>), 3.61 δ (2H, s, CH<sub>2</sub>), 3.85 δ (6H, s, OCH<sub>3</sub>), 4.60 δ (1H, d, CH), 5.22 δ (1H, s, CH), 6.06 δ (1H, s, S-H), 6.67 δ (1H, d, CH), 7.73 δ (1H, d, CH), 7.76 δ (1H, s, CH), DMSO 2.50 δ & 3.52 δ; MS, m/z: 428.81[M+1]. Anal.: Calc. for C, 67.99; H, 7.99; N, 9.51; S, 7.26. Found: C, 67.65; H, 7.72; N, 9.26; S, 7.01.

5,5,7-trimethyl-3-(piperidin-1-ylmethyl)-4-(pyridin-4-yl)-3,4,5,6-tetrahydroquinazoline-2-thiol (MB V): Yield: 78%; Colour: Yellow; m.p.: 268°C; IR (KBr, cm<sup>-1</sup>): 1653 (Ar C=C), 1418 (Ar C-C), 2894 (Ar C-H), 1094 (C-N), 3441 (N-H), 1368 (CH<sub>3</sub>), 1265 (C-S), 1600 (C=N); <sup>1</sup>H NMR (DMSO): 1.25 δ (6H, s, CH<sub>3</sub>), 1.28 δ (2H, m, CH<sub>2</sub>), 1.53 δ (4H, m, CH<sub>2</sub>), 1.8 δ (3H, s, CH<sub>3</sub>), 2.21 δ (2H, s, CH<sub>2</sub>), 2.44 δ (4H, m, CH<sub>2</sub>), 3.62 δ (2H, s, CH<sub>2</sub>), 4.59 δ (1H, d, CH), 5.71 δ (1H, s, CH), 6.08 δ (1H, s, S-H), 7.36 δ (2H, d, CH), 8.54 δ (2H, d, CH), DMSO 2.52 δ & 3.51 δ; MS, m/z: 382.22[M+1]. Anal.: Calc. for C, 69.07; H, 7.90; N, 14.65; S, 8.38. Found: C, 68.92; H, 7.76; N, 14.49; S, 8.13.

### 3.3. Antibacterial Study

Culture of microorganisms: Bacterial samples used for primary screening of antibacterial activity for the compounds. Bacteria strains were supplied from ARIBAS, namely *Bacillus subtilis*, *Pseudomonas aurigenosa*, *Escherichia coli*. They were maintained by periodical transfer on fresh nutrient agar slant.

Preparation of media: The antibacterial activities were carried out by using Mueller-Hinton agar plate. The composition of the medium was Nutrient broth 13g & Agar-Agar powder 30 g. Both ingredients were completely dissolved in 1 L of distilled water & the pH of the medium was adjusted to 7.4 pH. The medium was sterilized in an autoclave at 121°C for 15 min. It was then cooled down to 45°C & 20 ml was poured in each sterilized Petri dish.

Preparation of inoculums: A fresh microbial seed was prepared separately by sub culturing in to nutrient broth medium and incubated at 37°C.

Antibacterial screening test: The antibacterial activity was performed after the checking of minimum inhibitory concentration (MIC) by taking (0.04g/mL) of sample using the cup method. Minimum inhibitory concentration is the lowest substance concentration at which no sign of bacterial growth was detectable microscopically. The test compounds were dissolved in DMSO to produce (0.04g/mL) for minimum inhibitory concentration (MIC). Different compounds on different microorganisms were placed in each cup. The plates were first placed in a refrigerator for 30

minutes and incubated at 37°C for 24 hours. The results were recorded by measuring the zone of inhibition in the plate and recorded for further calculation.

## 4. Conclusions

The antibacterial activity of the compound MB I to MB V was screened against the three different types of bacterial strains (*P. aurigenosa*, *E. coli*, *B. subtilis*). None of these compound was found to be effective against *P. aurigenosa*. However all these compounds are active against *B. subtilis* and *E. coli* compare to standard i.e. ciprofloxacin, the compound reported here are comparable against *E. coli*. However, all the compounds have very good activity against the bacterial strain, it is interesting to note that MB I and MB II are the most potent agents, have OH and NO<sub>2</sub> group attached to aromatic ring respectively.

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