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Research Article

### DESIGN, SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL EVALUATION OF TRISUBSTITUTED 4,5- DIHYDROPYRAZOLES DERIVATIVES

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**Abstract:**

**Objective:** The objective of the paper is to design, synthesis and characterization of trisubstituted 4,5-dihydropyrazoles and evaluated for their antibacterial activity.

**Material and methods:** The derivatives have been synthesized by the two steps process, in first step 1-(4-aminophenyl)ethan-1-one (0.002M) (1) react with 4-substituted benzaldehyde (2) to form (E)-1-(4-substituted phenyl)-3-(p-substituted)prop-2-en-1-one [3]. The final compound (4), 1-(4-substitutedphenyl)-3-(4-substitutedphenyl)-5-(p-substituted)-4,5-dihydro-1H-pyrazole was synthesized by the reaction of compound 3 with different (4-fluorophenyl) hydrazine and (4-chlorophenyl) hydrazine. Total Sixteen compounds have been synthesized and characterized by physicochemical and spectral analysis. The final compounds (TDHP-1 to TDHP-16) have been evaluated for antibacterial activity by disk diffusion method.

**Result and Discussion:** The synthesized compounds have characterized by the IR, <sup>1</sup>HNMR and mass spectral analysis. The IR spectrum of the compounds has shown the characteristics peak (cm<sup>-1</sup>) at 3012 (C-H), 2912 (C-H), 1675 (C=C), 1595 (C-C), 1462 (C=N), 1308 (C-H), 1512 (N=O), 1334 (N-O), 745 (C-Cl), 1018 (C-Br), 1102 (C-F). The <sup>1</sup>HNMR spectra of synthesized compounds depicted the peak at δ7.23-8.62 (m, 4H, CH=CH-CH=CH), 7.03-8.80 (m, 3H, Ar-H), 3.08, 2.47 (d, 2H, CH<sub>2</sub>), 3.42 (d, 1H, C-5, CH), 11.431 (s, 1H, NH). The Data of antibacterial activity against the gram-positive bacterial strains (*Bacillus subtilis* and *Staphylococcus aureus*) suggested that among substituted pyrazoline derivatives (TDHP-1 to TDHP-16), compound TDHP-2, TDHP-3, TDHP-4, TDHP-5, TDHP-6, TDHP-7, TDHP-8 have shown best activity against gram positive bacteria and The Data of antibacterial activity against the gram-negative bacterial strains (*Salmonella typhimurium* and *Pseudomonas aeruginosa*) suggested that among substituted pyrazoline derivatives (TDHP-1 to TDHP-16), compound TDHP-1, TDHP-2, TDHP-3, TDHP-4, TDHP-5 TDHP-6, TDHP-7, TDHP-8 have shown best activity against gram negative bacteria.

**Conclusion:** In general, trisubstituted 4,5-dihydropyrazoles derivatives having amine termination has shown the better anti-microbial activity as compared to standard drug. There is a need in drug design strategy to achieve more anti-microbial potency in different bacterial strain on novel future synthetic heterocyclic compounds.

**Keywords:** Antimicrobial activity, disk diffusion method, antibacterial, pyrazole, gram positive

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**INTRODUCTION:**

The pharmaceutical chemistry or the medicinal chemistry is at the intersection of chemistry and pharmacology involving the designing, synthesizing, identifying and developing of new chemical entities suitable for therapeutic use.[1] In the early stages of the medicinal chemistry scientists isolated medicinal agents found in plants. Now, scientists in this field are creating new synthetic compounds as drugs.[2] Pharmaceutical chemistry is almost always geared toward drug discovery and development. Heterocyclic compounds are the most complex branches of organic chemistry that have been studied extensively and attracted attention of medicinal and pharmacological activities.[3] The chemistry of heterocyclic compounds is interesting because many natural products and drugs belong to this group. Heterocyclic compounds are cyclic organic substances which contain in the ring system at least one atom other than carbon. Many alkaloids, vitamins, antibiotics, synthetic medicines and dyestuffs are heterocyclic derivatives. [4]

The  $\alpha,\beta$ -Unsaturated ketone-chalcone is an important class of naturally occurring flavonoid compounds[5]. Flavonoids and chalcones are natural antioxidants present in plants and preventing oxidative damages of the cell. [6] They exhibit a wide spectrum of biological activities such as anticancer, anti-inflammatory and antimicrobial activities. Chalcones are intermediates for the synthesis of a large number of bioactive molecules, such as pyrazolines and pyrazole derivatives. [7] The high medicinal significance of this scaffold has attracted considerable attention from many researchers and encouraged the design and synthesis of numerous Pyrazole containing compounds with diverse pharmacological activities, such as antibacterial,[8] antifungal, antiprotozoal, antiviral, anticancer, anti-inflammatory, antioxidant, analgesic, anticonvulsant, antidiabetic, and antihypertensive activities[9].

Pyrazole is an organic heterocyclic compound characterized by a 5-membered ring containing three carbon atoms and two adjacent nitrogen atoms with the formula  $C_3H_4N_2$ . It is a weak base with  $pK_b$ . One of nitrogen atoms is neutral in nature and the other is basic. Due to its planar conjugated ring structure, pyrazole is an aromatic molecule with six delocalized  $\pi$ - electrons. [10]. Among the various pyrazoline isomers, 2-pyrazolines appear to be the most frequently investigated compounds. The completely reduced form of pyrazole is pyrazolidine and the Oxo form of pyrazolines derivatives are pyrazolone.[11]

Several pyrazoles are commercially available as

pharmaceuticals. The pyrazole ring presents in a variety of drugs such as Lonazolac and Celecoxib which is the selective cyclooxygenase-2, (COX 2) inhibitor showing great promise as anti-inflammatory and analgesic agent.[12] Moreover, Celecoxib has less undesirable side effect than the other known anti-inflammatory agents. Currently, researchers have been attracted toward designing more potent pyrazole derivatives that have wide range of biological activities.[13] Pyrazoline and its substituted pyrazolone derivatives show biological activities such as antimicrobial, antitumor and anti-inflammatory properties. The versatility of new generation pyrazoline would represent a fruitful pharmacophore for further development of better medicinal agents. The synthesis of novel pyrazoline derivatives remains a main focus of modern drug discovery.[14]

Various chemists have designed several pyrazolines to examine their pharmacological influence. The compound of pyrazoline with benzene sulfonamide displayed remarkable anti-proliferative activity. A series of 1,3-thiazolone derivatives bearing pyrazoline moiety was synthesized and screened for their in vitro antitumor activity against human breast adenocarcinoma cell line (MCF-7).[15] It was found that five of the tested compounds exhibited good antitumor activity in comparison to the reference drug, doxorubicin. Some 3-(pyrid-2-yl)-pyrazolines were synthesized and the anti-proliferative activity in two cancer cell lines reported. Novel pyrazolines were synthesized by reacting propanone with phenyl hydrazine. These compounds possess moderate to good antimicrobial activity. Novel pyrazoline compounds were synthesized under ultrasonic irradiation and screened for their antimicrobial activity. Some of the compounds showed significant antimicrobial activity.[16]

The synthesis of a new series of acetyl pyrazoline derivatives via cyclization reaction of chalcones with hydrazine hydrate by conventional methods has been reported to undergo in excellent yields and in less reaction time when using ethanol and few drops of glacial acetic acid.[17] The objective of the paper was to design, synthesis, characterization and biological evaluation of new derivatives of trisubstituted 4,5-dihydropyrazoles and evaluate for antibacterial activity by agar disk-diffusion method.

**MATERIAL AND METHOD:**

The substituted 1-(4-aminophenyl)ethan-1-one, ethanol and potassium hydroxide was purchased from Sigma Aldrich, India. The different 4-substituted benzaldehyde, (4-fluorophenyl)hydrazine and (4-

chlorophenyl)hydrazine were purchased from sigma Aldrich. All the chemicals were purchased from Sigma Aldrich India. Commercial grade solvents used for the reactions were distilled before use. The melting points of the synthesized compounds were determined in open glass capillaries. IR spectra were recorded on Bruker-alpha FTIR spectrometer. Elemental analysis was performed and found values were within 0.4% of theoretical values. <sup>1</sup>HNMR spectra were recorded at 400 MHz, Mass Spectra

were recorded using Mass Spectrometers Jeol FSX-112 (FAB) by ESI.

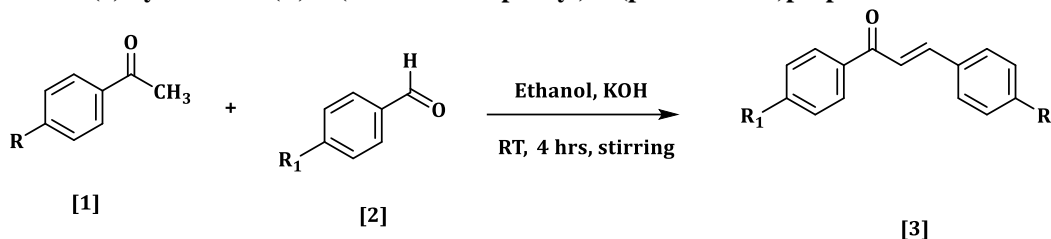
### Chemistry:

Synthetic strategy planning for synthesis

(a) Synthesis of (E)-1-(4-substituted phenyl)-3-(p-substituted)prop-2-en-1-one

(b) Synthesis of 1-(4-substitutedphenyl)-3-(4-substitutedphenyl)-5-(p-substituted)-4,5-dihydro-1H-pyrazole

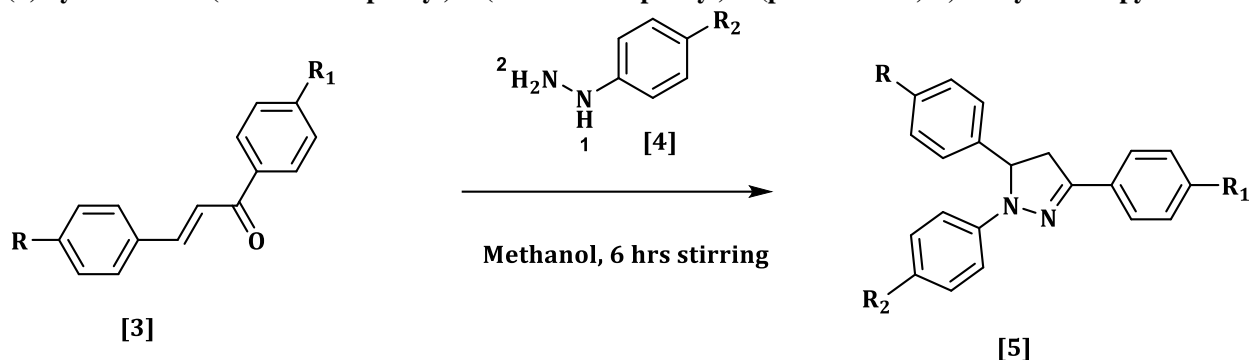
#### (a) Synthesis of (E)-1-(4-substituted phenyl)-3-(p-substituted)prop-2-en-1-one



**Procedure:** A 1-(4-aminophenyl)ethan-1-one (0.002M) (**1**) was added to an ethanolic solution (0.002M of KOH in 40 mL ethanol 96%) and stirred for 15 min, then (0.002M) of 4-substituted benzaldehyde (**2**) was added drop wise to the solution and stirred at room temperature for 48 hrs. The TLC method is utilized to monitor the reaction progress

using n-hexane: ethyl acetate as solvent system.[18] The reaction mixture was poured into ice-cold water, solid formed (**3**) was filtered, washed several times with the ice-cold water and dried at room temperature. Percentage yield was found to be 85.20%.

#### (b) Synthesis of 1-(4-substitutedphenyl)-3-(4-substitutedphenyl)-5-(p-substituted)-4,5-dihydro-1H-pyrazole



**Procedure:** The different substituted compounds **3** (0.01M) was dissolved in 10 ml methanol under stirring, and take (4-fluorophenyl)hydrazine and (4-chlorophenyl) hydrazine (Compound **4**, 0.02M) in beaker and added 10 ml of methanol and 2 ml of concentrated sulfuric acid to dissolve the content. Both the solution was mixed in round bottom flask and stirring for 48h at room temperature. The reaction was monitored by the TLC technique using N-hexane: methanol (7:2). The obtained solution was poured into crushed ice, then the solution was evaporated to obtain the pyrazoline product<sup>[19]</sup> (Compound **5**). The compound coded as TDHP-1 to TDHP-12.

### Pharmacological Screening:

#### Antibacterial activity:

The sixteen compounds naming as TDHP-1 to TDHP16 was subjected to pharmacological screening for antibacterial activity by agar-based disk diffusion method. In antibacterial screening of compounds, both gram positive and gram-negative strains were utilized in that case of Gram-negative bacteria species *Pseudomonas aeruginosa* (ATCC/27853), *Salmonella typhimurium* (ATCC/25922). Gram-positive bacteria species *Bacillus subtilis* and *Staphylococcus aureus* (ATCC/25923). Disc diffusion method is applied for the determination of zone of inhibition.<sup>[20]</sup>

Gentamycin and Meropenem are used as antibiotic standards were used as standard drug for the antibacterial activity.<sup>[21]</sup> The antibacterial and antifungal activity of projected synthesized compounds was evaluated by dissolving the compound in DMSO at 50µg/ml concentration by disc diffusion method.<sup>[22]</sup> The inhibition of zones caused by the synthesized compounds and standard drug were examined and the diameter of zone of inhibition was observed and recorded.<sup>[23]</sup>

## RESULT AND DISCUSSION

### Chemistry:

IR, NMR, mass spectral, and elemental studies were used to characterize the target structures of the synthesized compounds. The 1-(4-aminophenyl)ethan-1-one (0.002M) (1) react with 4-substituted benzaldehyde (2) to form (E)-1-(4-substituted phenyl)-3-(p-substituted)prop-2-en-1-one [Compound 3]. The Compound 3 obtained as yellow crystal product having the melting point 115-117°C. The final compound (compound 4), 1-(4-substitutedphenyl)-3-(4-substitutedphenyl)-5-(p-substituted)-4,5-dihydro-1H-pyrazole was synthesized by the reaction of compound 3 with different 4-fluorophenyl hydrazine and (4-chlorophenyl) hydrazine. The synthesized compounds have characterized by the IR, <sup>1</sup>HNMR and mass spectral analysis. The IR spectrum of the compounds has shown the characteristics peak (cm<sup>-1</sup>) at 3012 (C-H), 2912 (C-H), 1675 (C=C), 1595 (C-C), 1462 (C=N), 1308 (C-H), 1512 (N=O), 1334 (N-O), 745 (C-Cl), 1018 (C-Br); 1102 (C-F). The <sup>1</sup>HNMR spectra of Synthesized compounds depicted the peak at δ7.23-8.62 (m, 4H, CH=CH-CH=CH), 7.03-8.80 (m, 3H, Ar-H), 3.08, 2.47 (d, 2H, CH<sub>2</sub>), 3.42 (d, 1H, C-5, CH), 11.431 (s, 1H, NH). Compound 1 (TDHP-1), mass spectrum has shown peak at m/z = 365.84, which matches the chemical formula C<sub>21</sub>H<sub>17</sub>ClFN<sub>3</sub>. All the other synthesized compound has also shown the molecular ion peak similar to their molecular formula and weight.

#### TDHP-1: 4-(3-(4-chlorophenyl)-1-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)aniline:

Chemical formula: C<sub>21</sub>H<sub>17</sub>ClFN<sub>3</sub>; Molecular weight: 365.84; IR (cm<sup>-1</sup>): 3066 (C-H), 3446 (N-H); 2901 (C-H), 1688 (C=C), 1597 (C-C), 1464 (C=N), 1301 (C-H), 740 (C-Cl); <sup>1</sup>HNMR (ppm): δ 8.40, 7.72, 7.51, 7.23 (m, 4H, CH=CH-CH=CH), 7.03, 7.53, 7.49, 7.40 (m, 4H, Ph-H), 3.02, 2.99 (d, 2H, C-4, CH<sub>2</sub>), 3.45 (d, 1H, C-5, CH), 2.05, 1.90, 1.87, 1.76 (m, 15H), 11.431 (s, 1H, NH); FAB Mass (m/z): 365.80.

#### TDHP-2: 4-(3-(4-bromophenyl)-1-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)aniline:

Chemical formula: C<sub>21</sub>H<sub>17</sub>BrFN<sub>3</sub>; Molecular weight: 410.29; IR (cm<sup>-1</sup>): 3012 (C-H), 2912 (C-H), 1675 (C=C), 1595 (C-C), 1462 (C=N), 1308 (C-H), 745 (C-Cl), 1018 (C-Br); 1102 (C-F); <sup>1</sup>HNMR (ppm): δ 8.40-7.23 (m, 4H, CH=CH-CH=CH), 7.03-7.53 (m, 4H, Ph-H), 2.99-3.02 (d, 2H), 3.45 (d, 1H), 11.431 (s, 1H, NH); FAB Mass (m/z): 410.25.

#### TDHP-3: 4-(1,3-bis(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)aniline:

Chemical formula: C<sub>21</sub>H<sub>17</sub>F<sub>2</sub>N<sub>3</sub>; Molecular weight: 349.38; IR (cm<sup>-1</sup>): 3286 (C-H), 2899 (C-H), 3446 (N-H), 1737 (C=C), 1616 (C-C), 1447 (C=N), 1134(C-H); 1102 (C-F); <sup>1</sup>HNMR (ppm): δ7.187-8.621 (m, 4H, CH=CH-CH=CH), 7.55-8.373 (m, 3H), 2.470 (d, 2H, CH<sub>2</sub>), 3.304 (d, 1H, CH), 11.40 (s, 1H, NH); FAB Mass (m/z): 349.30.

#### TDHP-4: 4-(1-(4-fluorophenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-5-yl) aniline:

Chemical formula: C<sub>21</sub>H<sub>17</sub>FN<sub>4</sub>O<sub>2</sub>; Molecular weight: 376.39; IR (cm<sup>-1</sup>): 3286 (C-H), 2899 (C-H), 3446 (N-H), 1737 (C=C), 1616 (C-C), 1447(C=N), (1512 (N=O), 1334 (N-O), 1134 (C-H); 1102 (C-F); <sup>1</sup>HNMR (ppm): δ 7.18-8.62 (m, 4H, CH=CH-CH=CH), 7.50-8.35 (m, 3H, Ar-H), 2.470 (d, 2H), 3.304 (d, 1H), 11.431 (s, 1H, NH); FAB Mass (m/z): 376.14.

#### TDHP-5: 4-(1,3-bis(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)aniline:

Chemical formula: C<sub>21</sub>H<sub>17</sub>Cl<sub>2</sub>N<sub>3</sub>; Molecular weight: 382.29; IR (cm<sup>-1</sup>): 3062 (C-H), 2905 (C-H), 1688 (C=C), 3446 (N-H); 1597 (C-C), 1460 (C=N), 1301 (C-H), 740 (C-Cl), (3333, OH, C<sub>2</sub>H<sub>5</sub>OH); <sup>1</sup>HNMR (ppm): 7.20-8.38 (m, 4H, CH=CH-CH=CH), 7.02-7.50 (m, 4H), 2.95-3.00 (d, 2H), 3.42 (d, 1H), 11.431 (s, 1H, NH); FAB Mass (m/z): 382.08.

#### TDHP-6: 4-(3-(4-bromophenyl)-1-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)aniline:

Chemical formula: C<sub>21</sub>H<sub>17</sub>BrClN<sub>3</sub>; Molecular weight: 426.74; IR (cm<sup>-1</sup>): 3060 (C-H), 3446 (N-H); 2908 (C-H), 1688 (C=C) 1597 (C-C), 1464 (C=N), 1301(C-H), 740 (C-Cl), 1018 (C-Br); <sup>1</sup>HNMR (ppm): δ7.20-8.50 (m, 4H, CH=CH-CH=CH), 7.02-7.53 (m, 4H, Ph-H), 3.05, 2.97 (d, 2H, CH<sub>2</sub>), 3.45 (d, 1H, C-5, CH); 11.431 (s, 1H, NH); FAB Mass (m/z): 426.70.

#### TDHP-7: 4-(1-(4-chlorophenyl)-3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-5-

**yl)aniline:**

Chemical formula:  $C_{21}H_{17}ClFN_3$ ; Molecular weight: 365.84; IR ( $cm^{-1}$ ): 3066 (C-H), 2901 (C-H), 1688 (C=C), 1597 (C-C), 1464 (C=N), 1301 (C-H), 740 (C-Cl), 1102 (C-F), 1018 (C-Br);  $^1H$ NMR (ppm): 7.12-8.36 (m, 4H, CH=CH-CH=CH), 7.01-7.48 (m, 4H, Ph-H), 3.05, 2.95 (d, 2H, CH<sub>2</sub>), 3.54 (d, 1H, CH), 11.431 (s, 1H, NH); FAB Mass (m/z): 365.80.

**TDHP-8: 4-(1-(4-chlorophenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-5-yl) aniline:**

Chemical formula:  $C_{21}H_{17}ClN_4O_2$ ; Molecular weight: 392.84; IR ( $cm^{-1}$ ): 3150 (C-H), 2902 (C-H), 3446 (N-H), 1650 (C=C), 1585 (C-C), 1465 (C=N), 1512 (N=O), 1334 (N-O), (1250, C-H bend), 740 (C-Cl);  $^1H$ NMR (ppm):  $\delta$  7.23-8.62 (m, 4H, CH=CH-CH=CH), 7.03-8.80 (m, 3H, Ar-H), 3.08, 2.47 (d, 2H, CH<sub>2</sub>), 3.42 (d, 1H, C-5, CH), 11.431 (s, 1H, NH); FAB Mass (m/z): 392.60.

**TDHP-9: 3,5-bis(4-chlorophenyl)-1-(4-fluorophenyl)-4,5-dihydro-1H-pyrazole:**

Chemical formula:  $C_{21}H_{15}Cl_2FN_2$ ; Molecular weight: 385.26; IR ( $cm^{-1}$ ): 3066 (C-H), 2901 (C-H), 1688 (C=C), 1597 (C-C), 1464 (C=N), 1301 (C-H), 740 (C-Cl), 1102 (C-F);  $^1H$ NMR (ppm):  $\delta$  7.20-8.45 (m, 4H, CH=CH-CH=CH), 7.03-7.53 (m, 4H, ph-H), 3.08, 2.82 (d, 2H, CH<sub>2</sub>), 3.45 (d, 1H, CH); FAB Mass (m/z): 385.20.

**TDHP-10: 3-(4-bromophenyl)-5-(4-chlorophenyl)-1-(4-fluorophenyl)-4,5-dihydro-1H-pyrazole:**

Chemical formula:  $C_{21}H_{15}BrClFN_2$ ; Molecular weight: 429.72; IR ( $cm^{-1}$ ): 3066 (C-H), 2901 (C-H), 1688 (C=C), 1597 (C-C), 1464 (C=N), 1301 (C-H), 740 (C-Cl), 1102 (C-F); 1018 (C-Br);  $^1H$ NMR (ppm):  $\delta$  7.20-8.45 (m, 4H, CH=CH-CH=CH), 7.03-7.53 (m, 4H, ph-H), 3.08, 2.82 (d, 2H, CH<sub>2</sub>), 3.45 (d, 1H, CH); FAB Mass (m/z): 429.70.

**TDHP-11: 5-(4-chlorophenyl)-1,3-bis(4-fluorophenyl)-4,5-dihydro-1H-pyrazole:**

Chemical formula:  $C_{21}H_{15}ClF_2N_2$ ; Molecular Weight: 368.81; IR ( $cm^{-1}$ ): 3066 (C-H), 2901 (C-H), 1688 (C=C), 1597 (C-C), 1464 (C=N), 1301 (C-H), 740 (C-Cl), 1102 (C-F);  $^1H$ NMR (ppm):  $\delta$  7.25-8.40 (m, 4H, CH=CH-CH=CH), 7.05-7.53 (m, 4H, Ph-H), 3.05, 2.95 (d, 2H, CH<sub>2</sub>), 3.54 (d, 1H, CH); FAB Mass (m/z): 368.20.

**TDHP-12: 5-(4-chlorophenyl)-1-(4-fluorophenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole:**

Chemical formula:  $C_{21}H_{15}ClFN_3O_2$ ; Molecular weight: 395.82; IR ( $cm^{-1}$ ): 3150 (C-H), 2902 (C-H), 3446 (N-H), 1650 (C=C), 1585 (C-C), 1465 (C=N), 1512 (N=O), 1334 (N-O), (1250, C-H bend), 740 (C-

Cl);  $^1H$ NMR (ppm):  $\delta$  7.23-8.62 (m, 4H, CH=CH-CH=CH), 7.03-8.80 (m, 3H, Ar-H), 3.08, 2.47 (d, 2H, CH<sub>2</sub>), 3.42 (d, 1H, C-5, CH), 11.431 (s, 1H, NH); FAB Mass (m/z): 395.80.

**TDHP-13: 1,3,5-tris(4-chlorophenyl)-4,5-dihydro-1H-pyrazole:**

Chemical formula:  $C_{21}H_{15}Cl_3N_2$ ; Molecular weight: 401.72; IR ( $cm^{-1}$ ): 3066 (C-H), 2901 (C-H), 1688 (C=C), 1597 (C-C), 1464 (C=N), 1301 (C-H), 740 (C-Cl), 1102 (C-F);  $^1H$ NMR (ppm):  $\delta$  7.20-8.45 (m, 4H, CH=CH-CH=CH), 7.03-7.53 (m, 4H, ph-H), 3.08, 2.82 (d, 2H, CH<sub>2</sub>), 3.45 (d, 1H, CH); FAB Mass (m/z): 401.20.

**TDHP-14: 3-(4-bromophenyl)-1,5-bis(4-chlorophenyl)-4,5-dihydro-1H-pyrazole:**

Chemical formula:  $C_{21}H_{15}BrCl_2N_2$ ; Molecular weight: 446.17; IR ( $cm^{-1}$ ): 3066 (C-H), 2901 (C-H), 1688 (C=C), 1597 (C-C), 1464 (C=N), 1301 (C-H), 740 (C-Cl);  $^1H$ NMR (ppm):  $\delta$  7.20-8.45 (m, 4H, CH=CH-CH=CH), 7.03-7.53 (m, 4H, ph-H), 3.08, 2.82 (d, 2H, CH<sub>2</sub>), 3.45 (d, 1H, CH); FAB Mass (m/z): 446.10.

**TDHP-15: 1,5-bis(4-chlorophenyl)-3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazole:**

Chemical formula:  $C_{21}H_{15}Cl_2FN_2$ ; Molecular weight: 385.26; IR ( $cm^{-1}$ ): 3066 (C-H), 2901 (C-H), 1688 (C=C), 1597 (C-C), 1464 (C=N), 1301 (C-H), 740 (C-Cl); 1102 (C-F);  $^1H$ NMR (ppm):  $\delta$  7.20-8.45 (m, 4H, CH=CH-CH=CH), 7.03-7.53 (m, 4H, ph-H), 3.08, 2.82 (d, 2H, CH<sub>2</sub>), 3.45 (d, 1H, CH); FAB Mass (m/z): 385.20.

**TDHP-16: 1,5-bis(4-chlorophenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole:**

Chemical formula:  $C_{21}H_{15}Cl_2N_3O_2$ ; Molecular weight: 412.27; IR ( $cm^{-1}$ ): 3150 (C-H), 2902 (C-H), 3446 (N-H), 1650 (C=C), 1585 (C-C), 1465 (C=N), 1512 (N=O), 1334 (N-O), (1250, C-H bend), 740 (C-Cl);  $^1H$ NMR (ppm):  $\delta$  7.23-8.62 (m, 4H, CH=CH-CH=CH), 7.03-8.80 (m, 3H, Ar-H), 3.08, 2.47 (d, 2H, CH<sub>2</sub>), 3.42 (d, 1H, C-5, CH), 11.431 (s, 1H, NH); FAB Mass (m/z): 412.20.

**Pharmacological evaluation:****Antibacterial activity:****Evaluation of Antibacterial Activity of synthesized compounds:**

The Zone of Inhibition results of the synthesized compounds against gram negative bacteria species *Pseudomonas aeruginosa*, and *Salmonella typhimurium*, gram positive bacteria species such as *Bacillus subtilis* and *Staphylococcus aureus*. The investigation of the antibacterial screening of the test

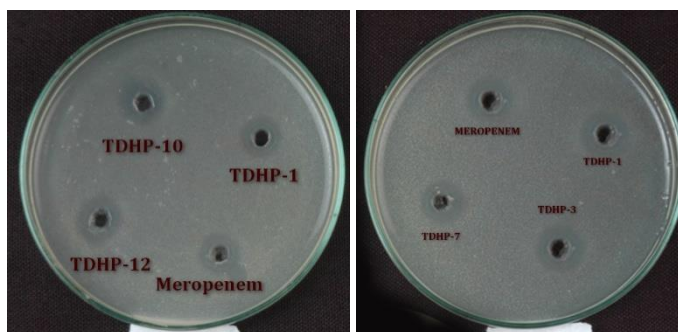
compound (TDHP-1 to TDHP-16) revealed that all these compounds exhibited different degrees of antibacterial activity in relation to the tested microbial species and showed moderate to weak antibacterial activity against all the organisms against

both of two types of bacterial strain. The results of MIC's determined reveal that the test compounds can act as a good anti-bacterial agent at higher concentrations, and no inhibition zone at lower concentration (Table 1).

**Table 1: Zone of Inhibition of the synthesized compounds against bacterial species in mm.**

Antibiotic	Gram Positive Bacteria		Gram Negative Bacteria	
	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Salmonella typhimurium</i>
Meropenem	30	28	-	-
Gentamycin	-	-	25	20
TDHP-1	22.7	23.4	22.5	19.2
TDHP-2	22.6	22.5	21.3	19.5
TDHP-3	28.5	21.4	21.5	16.3
TDHP-4	25.5	20.2	21.8	14.2
TDHP-5	29.5	23.5	21.4	18.3
TDHP-6	27.2	24.8	22.4	15.6
TDHP-7	23.5	22.5	22.9	14.2
TDHP-8	24.5	26.5	21.7	13.5
TDHP-9	12.5	12.5	12.5	13.8
TDHP-10	13.0	12.8	14.6	13.6
TDHP-11	18.0	13.6	14.2	11.5
TDHP-12	16.5	14.6	13.5	10.2
TDHP-13	12.3	15.6	13.4	9.2
TDHP-14	12.2	13.4	13.5	9.5
TDHP-15	13.5	12.3	13.2	8.9
TDHP-16	14.6	12.8	13.8	8.7

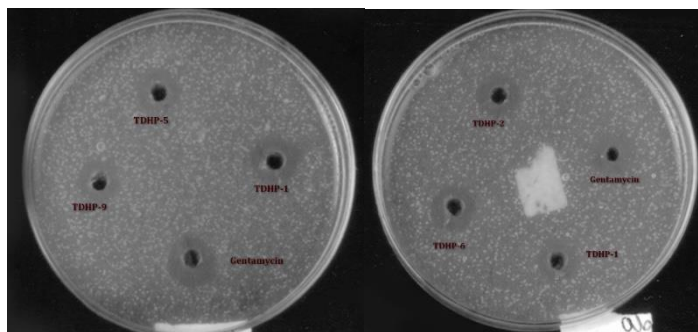
The Sixteen synthesized compounds (TDHP-1 to TDHP-16) were evaluated for the antibacterial screening and data obtained by the result stated that substituted pyrazoline derivatives has shown the mild to better activity against tested organism's strains. The screening carried out on both gram-positive and gram-negative bacteria separately. The pictorial representation of Zone of inhibition of compound against gram-positive bacteria was shown in Fig. 1.



**Fig. 1: The pictorial representation of Zone of inhibition of compound against gram-positive bacteria**

The Data of antibacterial activity against the gram-positive bacterial strains (*Bacillus subtilis* and *Staphylococcus aureus*) suggested that among substituted pyrazoline derivatives (TDHP-1 to TDHP-16), compound TDHP-9, TDHP-10, TDHP-13, TDHP-14 and TDHP-15 have shown mild activity while as compound TDHP-11, TDHP-12

TDHP-16 and TDHP-1 shown moderate activity and compound TDHP-2, TDHP-3, TDHP-4, TDHP-5, TDHP-6, TDHP-7, TDHP-8 have shown best activity against gram positive bacteria (Table 1). The pictorial representation of Zone of inhibition of compound against gram negative bacteria was shown in Fig. 2.



**Fig. 2: The pictorial representation of Zone of inhibition of compound against gram negative bacteria**

The Data of antibacterial activity against the gram-negative bacterial strains (*Salmonella typhimurium* and *Pseudomonas aeruginosa*) suggested that among substituted pyrazoline derivatives (TDHP-1 to TDHP-16), compound TDHP-9, TDHP-13, TDHP-14 TDHP-15 have shown mild activity while as compound TDHP-10, TDHP-11, TDHP-16 shown moderate activity and compound TDHP-1, TDHP-2, TDHP-3, TDHP-4, TDHP-5 TDHP-6, TDHP-7, TDHP-8 have shown best activity against gram negative bacteria.

### DISCUSSION:

The antibacterial activity of synthesized compounds (TDHP-1 to TDHP16) was screened against each gram-negative bacteria species *Pseudomonas aeruginosa*, and *Salmonella typhimurium*, gram positive bacteria species such as *Bacillus subtilis* and *Staphylococcus aureus*. by disc diffusion method using ciprofloxacin as standard drug. These compounds possessed the halogens on the aromatic ring and thus reveal the positive contribution of electron withdrawing groups to the antibacterial and antifungal activity. Presence of electronegative group (Cl, NO<sub>2</sub>) is required for the potent antibacterial activity. In general, trisubstituted 4,5-dihydropyrazoles derivatives having amine termination has shown the better anti-microbial activity as compared to standard drug.

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### Conflicts of Interest:

The author declares no conflicts of interest

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