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Synthesis and Characterization of trisubstituted 4,5-dihydropyrazoles and evaluated for their antimicrobial activity

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Abstract:
Pharmaceutical chemistry is predominantly focused on drug discovery and development. Among the vast array of bioactive compounds, α,β-unsaturated ketones, particularly chalcones, represent an essential class of naturally occurring flavonoids with diverse pharmacological properties. In this study, different 4-substituted benzaldehydes, specifically (4-fluorophenyl) hydrazine and (4-chlorophenyl) hydrazine, were utilized to synthesize a series of novel trisubstituted 4,5-dihydropyrazole derivatives. The synthesized compounds were structurally characterized using standard spectroscopic techniques, confirming the successful formation of the targeted heterocyclic frameworks. The antimicrobial activity of these new derivatives was evaluated against various bacterial strains. Results indicated that the compounds exhibited a spectrum of antimicrobial efficacy ranging from mild to significant, with the trisubstituted 4,5-dihydropyrazole derivatives bearing amine terminations displaying superior antimicrobial activity when compared to standard drugs. This suggests their potential as potent antimicrobial agents. The findings emphasize the need for continued optimization in drug design strategies to enhance antimicrobial potency across a broader range of bacterial strains. Additionally, future work should involve testing these novel compounds in diverse biological assays to explore their therapeutic potential in other areas, such as anti-inflammatory and anticancer activities, particularly against carcinoma cell lines. This study underscores the importance of synthetic heterocyclic compounds in the development of novel therapeutics.

Keywords: Pharmaceutical Chemistry, Drug Discovery, A,B-Unsaturated Ketone, Chalcones, Trisubstituted 4,5-Dihydropyrazoles, Antimicrobial Activity, Heterocyclic Compounds, Synthetic Chemistry, Antibacterial Agents, Anti-Inflammatory, Anticancer

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 α,β-unsaturated ketone-chalcone is an important class of naturally occurring flavonoid compounds[5],Flavonoids and chalcones are natural antioxidants present in plants and preventing oxidativedamages of the cell.[6] Chalcones are intermediates for the synthesis of a large number of bioactive molecules, such aspyrazolines andpyrazole 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]

Furthermore, thiazole compounds exhibiting a promising biological potential for the treatment of Alzheimer's disease and metabolic syndrome have been reported in the scientific literature. Nowadays, research in the field of antimicrobial drug design is focused on the discovery of novel targets and chemical entities that possess antibacterial activity in order to overcome the rapid development of drug resistance.[10] The few antibiotics that have been recently approved are structurally related to older drugs, being susceptible to the same mechanisms of resistance.

Experimental

4.1 Materials 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

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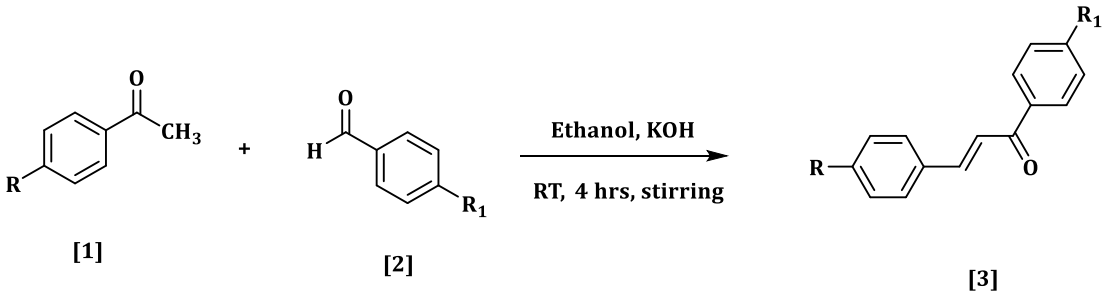
used for the reactions were distilled before use.The melting points of the synthesized compounds were determined in open glass capillaries. IR spectra were recorded on Bruker-alpha FTIR spectrometer. Elemental analysis was performed and found values were within 0.4% of theoretical values. 1HNMR spectra were recorded at 400 MHz, Mass Spectra were recorded using Mass Spectrometers Jeol FSX-112 (FAB) by ESI.

4.2 Synthesis scheme
Synthetic strategy planning for synthesis

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



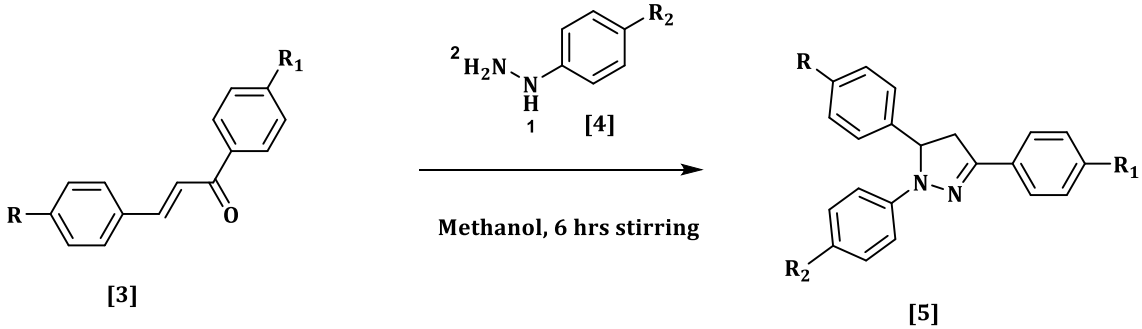
[1] [2] [3]

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.[63] The reaction mixture was poured into ice-cold water, solidformed**(3)** was filtered, washed several times with the ice-cold water and dried at room temperature.Percentage yield was found to be 85.20%.

Table 4.1: Quantity of chemicals taken

S. N0.	Name of Chemicals	Mol. formula	Mol. Weight	Quant. (gm)
1.	1-(4-aminophenyl)ethan-1-one	C_8H_9NO	135.17	2.70
2.	1-(4-chlorophenyl)ethan-1-one	C_8H_7ClO	154.59	3.09
3.	4-chlorobenzaldehyde	C_7H_5ClO	140.57	2.81
4.	4-bromobenzaldehyde	C_7H_5BrO	185.02	3.70
5.	4-fluorobenzaldehyde	C_7H_5FO	124.11	2.48
6.	4-nitrobenzaldehyde	$C_7H_5NO_3$	151.12	3.02

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



[3] [4] [5]

Procedure:
The different substituted compounds 3 (0.01M) was dissolved in 10 ml methanolunderstirring,and take (4-fluorophenyl)hydrazine and (4-chlorophenyl)hydrazine(Compound 4, 0.02M) in beaker and added 10 ml of methanol and 2ml of concentrated sulfuricacid to dissolve the content. Both the solution was mixed in round bottom flask and stirring for 48*h* 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[64] (**Compound 5**). The compound coded as TDHP-1 to TDHP-12.

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Table 4.2: Quantity of chemicals taken

S. N0.	Name of Chemicals	Mol. Formula	Mol. Weight	Quant(gm)
1.	(E)-3-(4-aminophenyl)-1-(4-chlorophenyl)prop-2-en-1-one	$C_{15}H_{12}ClNO$	257.72	2.57
2.	(E)-3-(4-aminophenyl)-1-(4-bromophenyl)prop-2-en-1-one	$C_{15}H_{12}BrNO$	302.17	3.02
3.	(E)-3-(4-aminophenyl)-1-(4-fluorophenyl)prop-2-en-1-one	C15H12FNO	241.27	2.41
4.	(E)-3-(4-aminophenyl)-1-(4-nitrophenyl)prop-2-en-1-one	C15H12N2O3	268.27	2.68
5.	(E)-1,3-bis(4-chlorophenyl)prop-2-en-1-one	C15H10Cl2O	277.14	2.77
6.	(E)-1-(4-bromophenyl)-3-(4-chlorophenyl)prop-2-en-1-one	C15H10BrClO	321.60	3.21
7.	(E)-3-(4-chlorophenyl)-1-(4-fluorophenyl)prop-2-en-1-one	C15H10ClFO	260.69	2.60
8.	(E)-3-(4-chlorophenyl)-1-(4-nitrophenyl)prop-2-en-1-one	C15H10ClNO3	287.70	2.87
9.	(4-fluorophenyl)hydrazine	C6H7FN2	126.13	2.52
10.	(4-chlorophenyl)hydrazine	C6H7ClN2	142.59	2.85

4.3 Characterization of the synthesized compounds

4.3.1 List of Synthesized compounds: In this thesis, a total of sixteen substances were synthesized (Table 4.3) and purified using column chromatography. The IR, 1HNMR spectroscopy, mass, and elemental analyses were used to analyze the synthesized compounds.

Table 4.3: List of Final synthesized compounds

SN	Code	Chemical name
1.	TDHP-1	4-(3-(4-chlorophenyl)-1-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)aniline
2.	TDHP-2	4-(3-(4-bromophenyl)-1-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)aniline
3.	TDHP-3	4-(1,3-bis(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)aniline
4.	TDHP-4	4-(1-(4-fluorophenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-5-yl)aniline
5.	TDHP-5	4-(1,3-bis(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)aniline
6.	TDHP-6	4-(3-(4-bromophenyl)-1-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)aniline
7.	TDHP-7	4-(1-(4-chlorophenyl)-3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)aniline
8.	TDHP-8	4-(1-(4-chlorophenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-5-yl)aniline
9.	TDHP-9	3,5-bis(4-chlorophenyl)-1-(4-fluorophenyl)-4,5-dihydro-1H-pyrazole
10.	TDHP-10	3-(4-bromophenyl)-5-(4-chlorophenyl)-1-(4-fluorophenyl)-4,5-dihydro-1H-pyrazole
11.	TDHP-11	5-(4-chlorophenyl)-1,3-bis(4-fluorophenyl)-4,5-dihydro-1H-pyrazole
12.	TDHP-12	5-(4-chlorophenyl)-1-(4-fluorophenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole
13.	TDHP-13	1,3,5-tris(4-chlorophenyl)-4,5-dihydro-1H-pyrazole
14.	TDHP-14	3-(4-bromophenyl)-1,5-bis(4-chlorophenyl)-4,5-dihydro-1H-pyrazole
15.	TDHP-15	1,5-bis(4-chlorophenyl)-3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazole
16.	TDHP-16	1,5-bis(4-chlorophenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole

Qualitative analysis

(a) Melting point- Open capillary tubes were used to determine the melting point of the produced compounds, Electro Thermal Stuart SMP3 advanced melting point apparatus as stated in Table 4.4.

(b) Solubility- At room temperature (18-30°C), the solubility of the produced product was tested in various solvents. Table 4.5 shows the solubility of the produced chemical.

(c) Elements test (Lassaigne's test): Each compound's sodium fusion extract was produced and evaluated for nitrogen, sulphur, and halogens. All of the produced compounds tested positive for nitrogen, sulphur, and halogen [146].

(d) Bromine test for un-saturation: With continual shaking, dissolve the synthesised final chemical in a suitable solvent and add 4-5 drops of bromine water. The presence of a brown colour discharge indicates that the chemical was unsaturated and that a double bond was present. Wherever possible, the synthesized compounds were submitted to qualitative analyses for nitrogen, sulphur, and halogen. Elemental vario EL III Carlo-Erba 1108 was used for quantitative examination of nitrogen, oxygen, and sulphur.

(e) IR spectra were recorded on Bruker alpha-II software. FT-IR spectrometer apparatus used to provide detailed information about the structural changes in the region (4000–400 cm^{-1}).

(f) NMR spectra were recorded on C13 Advance Brucher DRX 400 MHz spectrometer. ^{1}H-NMR apparatus used to determine the proton spectra of compounds. The spectra recorded in deuterated solvent $CDCl_3$ in 400 MHz at 25 ºC or by DMSO-d6 solvent in 300MHz at 21ºC. Chemical shifts (δ) are given in parts per million (ppm) downfield relative to tetramethyl silane TMS.

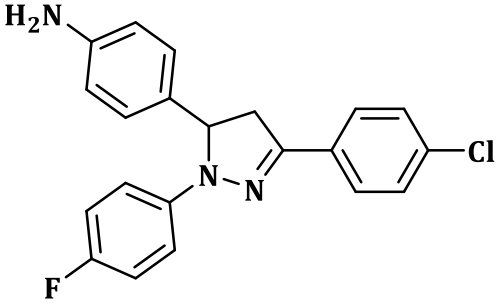
(g) Mass spectra were recorded on JeolSx 102/DA-6000 mass spectrometer using fast moving bombardment (FAB) technique.

(h) TLC (Thin-layer chromatography) plastic sheets silica gel, 20*20 cm, layer thickness 0.2 mm, was carried out on eluted with an ethyl acetate/n-hexane mixture, the spots were detected by UV light which monitored the progress of the reaction.

S. NO.	Code	Chemical formula	Mol. Weight	Percent Yield	Melting point
1.	TDHP-1	$C_{21}H_{17}ClFN_3$	365.84	88%	125-127°C
2.	TDHP-2	$C_{21}H_{17}BrFN_3$	410.29	76%	132-134°C
3.	TDHP-3	$C_{21}H_{17}F_2N_3$	349.38	82%	145-147°C
4.	TDHP-4	$C_{21}H_{17}FN_4O_2$	376.39	70%	114-116°C
5.	TDHP-5	$C_{21}H_{17}Cl_2N_3$	382.29	68%	122-124°C
6.	TDHP-6	$C_{21}H_{17}BrClN_3$	426.74	62%	112-114°C
7.	TDHP-7	$C_{21}H_{17}ClFN_3$	365.84	65%	150-152°C
8.	TDHP-8	$C_{21}H_{17}ClN_4O_2$	392.84	60%	138-140°C
9.	TDHP-9	$C_{21}H_{15}Cl_2FN_2$	385.26	69%	115-117°C
10.	TDHP-10	$C_{21}H_{15}BrClFN_2$	429.72	70%	162-164°C
11.	TDHP-11	$C_{21}H_{15}ClF_2N_2$	368.81	65%	193-195°C
12.	TDHP-12	$C_{21}H_{15}ClFN_3O_2$	395.82	78%	113-115°C
13.	TDHP-13	$C_{21}H_{15}Cl_3N_2$	401.72	62%	123-125°C
14.	TDHP-14	$C_{21}H_{15}BrCl_2N_2$	446.17	75%	117-119°C
15.	TDHP-15	$C_{21}H_{15}Cl_2FN_2$	385.26	68%	121-123°C
16.	TDHP-16	$C_{21}H_{15}Cl_2N_3O_2$	412.27	72%	127-129°C

4.5 CHARACTERIZATION OF THE SYNTHESIZED COMPOUNDS BY IR, NMR, MASS AND ELEMENTARY ANALYSIS

COMPOUND CODE: TDHP-1



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

Chemical Formula: $C_{21}H_{17}ClFN_3$**;** **Molecular Weight:** 365.84

Elemental analysis:

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Elements	C	N	F	Cl
Calculated	68.95	11.49	5.19	9.69
Found	68.92	11.45	5.20	9.66

IR (cm^{-1}):
3066 (C–H), 3446 (N-H); 2901(C–H), 1688 (C=C), 1597 (C-C), 1464 (C=N), 1301 (C-H), 740 (C-Cl)
1HNMR (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_2), 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

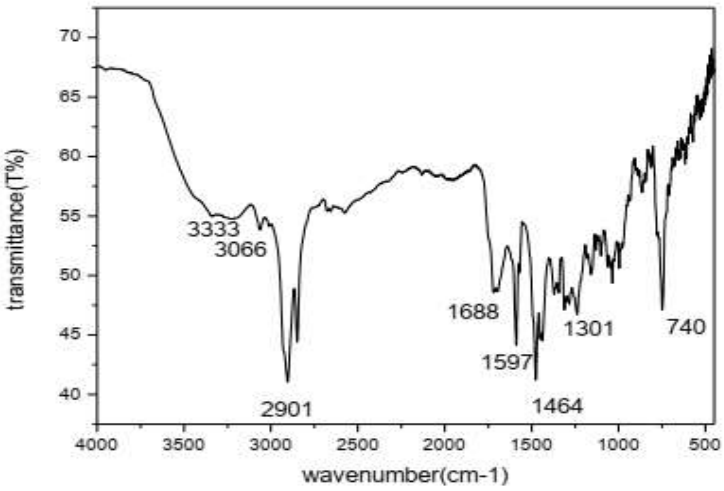


Fig. 1: FT-IR spectrum of compound TDHP-1

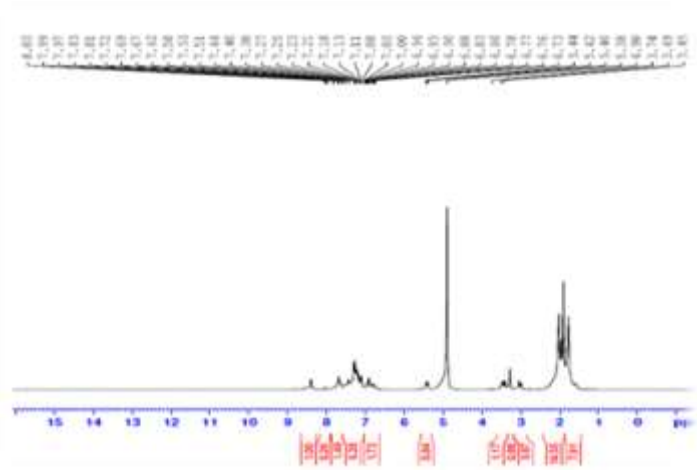
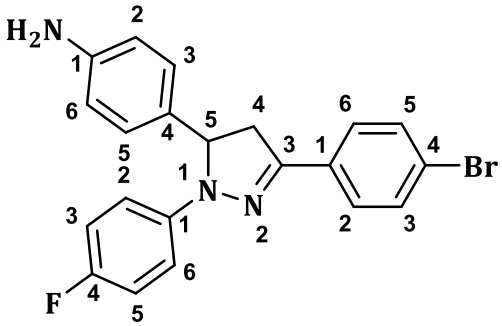


Fig. 2: 1HNMR spectrum of compound TDHP-1

COMPOUND CODE: TDHP-2



IUPAC NAME:4-(3-(4-bromophenyl)-1-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-5-yl) aniline

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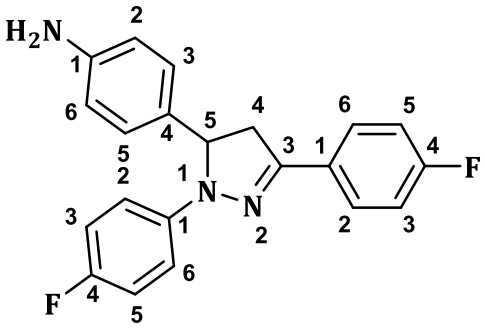
Chemical Formula: $C_{21}H_{17}BrFN_3$**;** **Molecular Weight:** 410.29

Elemental analysis:

Elements	C	N	F	Br
Calculated	61.48	10.24	4.63	19.47
Found	61.45	10.20	4.60	19.45

IR (cm^{-1}):
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)
1HNMR (ppm):
δ8.40-7.23 (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

COMPOUND CODE: TDHP-3



IUPAC NAME: 4-(1,3-bis(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)aniline
Chemical Formula: $C_{21}H_{17}F_2N_3$**;** **Molecular weight:** 349.38

Elemental analysis:

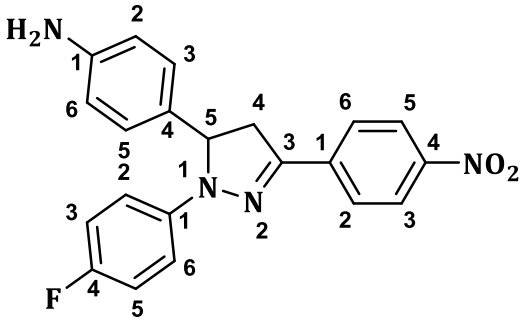
Elements	C	N	F
Calculated	72.19	12.03	10.88
Found	72.15	12.00	10.85

IR (cm^{-1}):
3286 (C–H), 2899 (C–H), 3446 (N-H), 1737 (C=C), 1616 (C-C), 1447 (C=N),1134(C-H); 1102 (C-F)

1HNMR (ppm):
δ 7.187-8.621(m, 4H, CH=CH-CH=CH), 7.55-8.373 (m, 3H), 2.470 (d, 2H, CH_2), 3.304 (d, 1H, CH), 11.40 (s, 1H, NH).

FAB Mass (m/z): 349.30

COMPOUND CODE: TDHP-4



IUPAC Name:4-(1-(4-fluorophenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-5-yl)aniline
Chemical Formula: $C_{21}H_{17}FN_4O_2$; **Molecular weight:** 376.39

Elemental analysis:

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Elements	C	N	F
Calculated	67.01	14.89	5.05
Found	67.00	14.85	5.02

IR (cm^{-1}):
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)

1HNMR (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

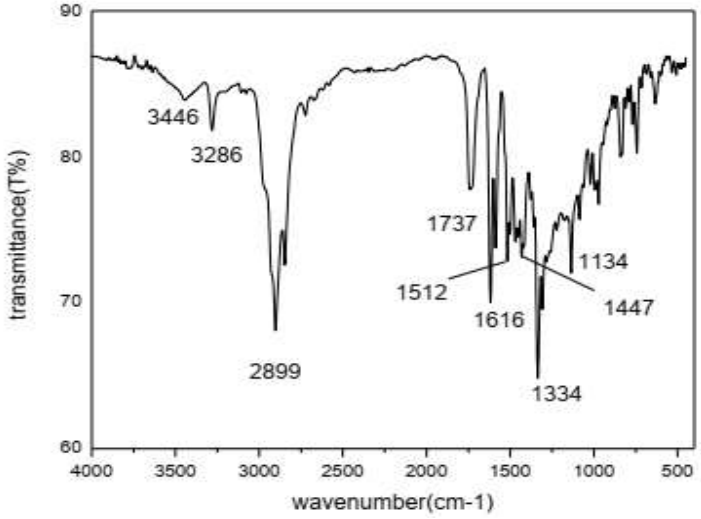


Fig. 3: FT-IR spectrum of compound TDHP-4

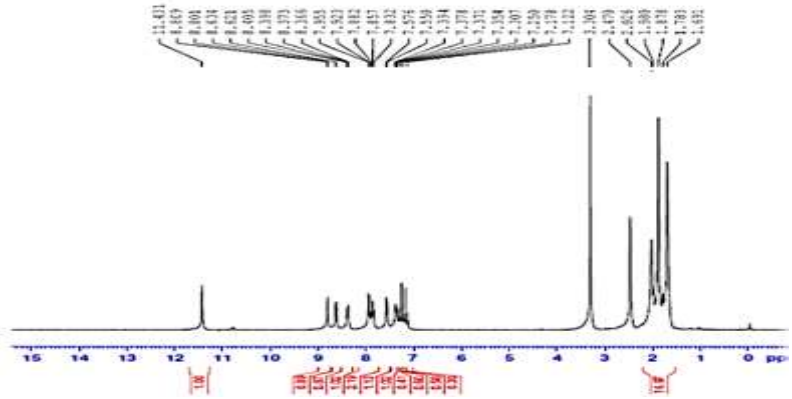
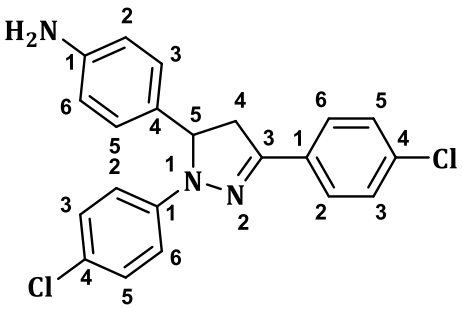


Fig. 4: 1HNMR spectrum of compound TDHP-4

COMPOUND CODE: TDHP-5



IUPAC name: 4-(1,3-bis(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)aniline
Chemical Formula: $C_{21}H_{17}Cl_2N_3$**;** **Molecular Weight: 382.29**
Elemental analysis:

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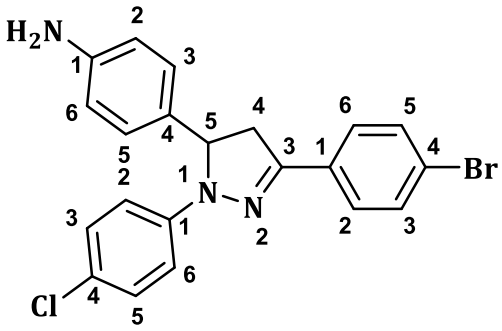
Elements	C	N	Cl
Calculated	65.98	10.99	18.55
Found	65.90	10.95	18.52

IR (cm^{-1}):
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_2H_5OH)

1HNMR (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
COMPOUND CODE: TDHP-6



IUPAC NAME: 4-(3-(4-bromophenyl)-1-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)aniline
Chemical Formula: $C_{21}H_{17}BrClN_3$**;** **Molecular weight:** 426.74

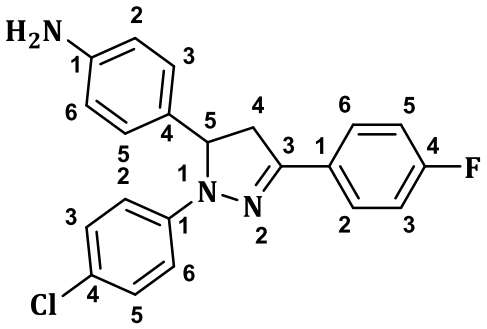
Elemental analysis:

Elements	C	N	Cl	Br
Calculated	59.11	9.85	8.31	18.72
Found	59.12	9.80	8.29	18.70

IR (cm^{-1}):
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);

1HNMR (ppm):
δ 7.20-8.50 (m, 4H, CH=CH-CH=CH), 7.02-7.53 (m, 4H, Ph-H), 3.05, 2.97 (d, 2H, CH_2), 3.45 (d, 1H, C-5, CH); 11.431 (s, 1H, NH)
FAB Mass (m/z): 426.70

COMPOUND CODE: TDHP-7



IUPAC NAME: 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

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Elemental analysis:

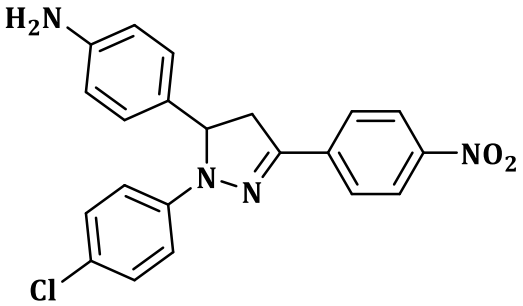
Elements	C	N	F	Cl
Calculated	68.95	11.49	5.19	9.69
Found	68.92	11.45	5.15	9.65

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)

1HNMR (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_2), 3.54 (d, 1H, CH), 11.431 (s, 1H, NH)

FAB Mass (m/z): 365.80

COMPOUND CODE: TDHP-8



IUPAC name: 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

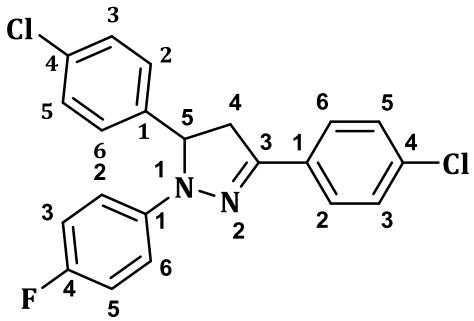
Elemental analysis:

Elements	C	N	Cl
Calculated	64.21	14.26	9.02
Found	64.18	14.25	9.05

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)

1HNMR (ppm):
δ 7.23-8.62 (m, 4H, CH=CH-CH=CH), 7.03-8.80 (m, 3H, Ar-H), 3.08, 2.47 (d, 2H, CH2), 3.42 (d, 1H, C-5, CH), 11.431 (s, 1H, NH).
FAB Mass (m/z):392.60

COMPOUND CODE: TDHP-9



IUPAC NAME: 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

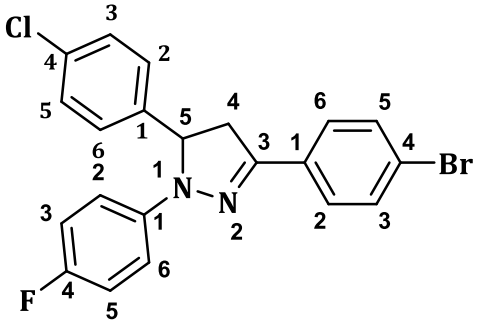
Elemental analysis:

Elements	C	N	F	Cl
Calculated	65.47	7.27	4.93	18.40
Found	65.45	7.25	4.90	18.35

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)
1HNMR (ppm):
δ 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_2), 3.45 (d, 1H, CH)

FAB Mass (m/z): 385.20

COMPOUND CODE: TDHP-10



IUPAC NAME: 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**

Elemental analysis:

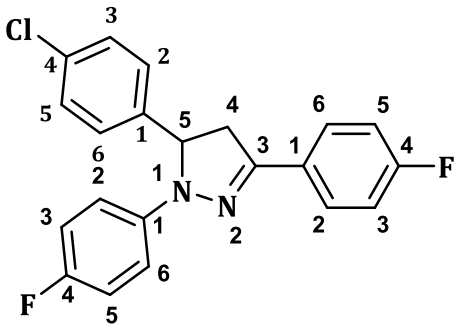
Elements	C	N	F	Br	Cl
Calculated	58.70	6.52	4.42	18.59	8.25
Found	58.65	6.50	4.40	18.57	8.20

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)

1HNMR (ppm):
δ 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_2), 3.45 (d, 1H, CH)

FAB Mass (m/z): 429.70

COMPOUND CODE: TDHP-11



IUPAC NAME: 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**
Elemental analysis:

Elements	C	N	Cl	F
Calculated	68.39	7.60	9.61	10.30
Found	68.35	7.56	9.58	10.30

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)

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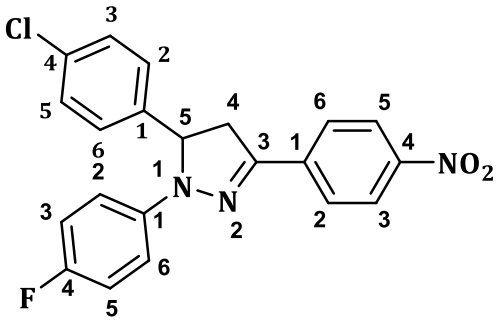
1HNMR (ppm):
δ 7.25-8.40 (m, 4H, CH=CH-CH=CH), 7.05-7.53 (m, 4H, Ph-H), 3.05, 2.95 (d, 2H, CH_2), 3.54 (d, 1H, CH),

FAB Mass (m/z): 368.20

COMPOUND CODE: TDHP-12

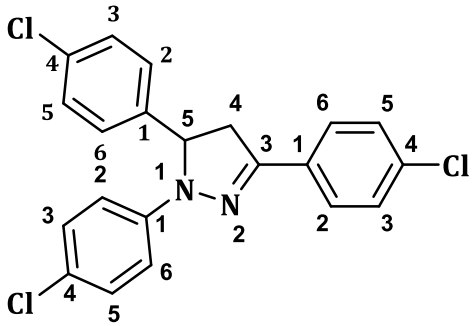


IUPAC NAME: 5-(4-chlorophenyl)-1-(4-fluorophenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole
Chemical Formula: $C_{21}H_{15}ClFN_3O_2$**;** **Molecular weight:** 395.82
Elemental analysis:

Elements	C	N	F	Cl
Calculated	63.72	10.62	4.80	8.96
Found	63.70	10.60	4.38	8.95

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)
1HNMR (ppm):
δ 7.23-8.62 (m, 4H, CH=CH-CH=CH), 7.03-8.80 (m, 3H, Ar-H), 3.08, 2.47 (d, 2H, CH2), 3.42 (d, 1H, C-5, CH), 11.431 (s, 1H, NH)
FAB Mass (m/z): 395.80

COMPOUND CODE: TDHP-13



IUPAC NAME: 1,3,5-tris(4-chlorophenyl)-4,5-dihydro-1H-pyrazole
Chemical Formula: $C_{21}H_{15}Cl_3N_2$**;** **Molecular weight:** 401.72
Elemental analysis:

Elements	C	N	Cl
Calculated	62.79	6.97	26.47
Found	62.75	6.95	26.45

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)
1HNMR (ppm):
δ 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_2), 3.45 (d, 1H, CH)

FAB Mass (m/z): 401.20

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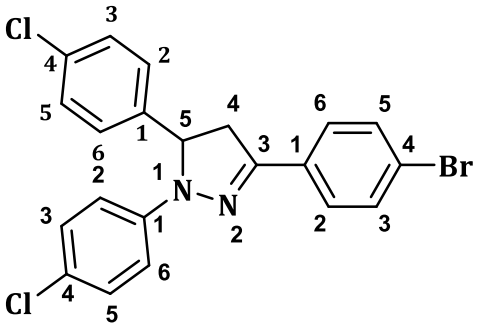
COMPOUND CODE: TDHP-14



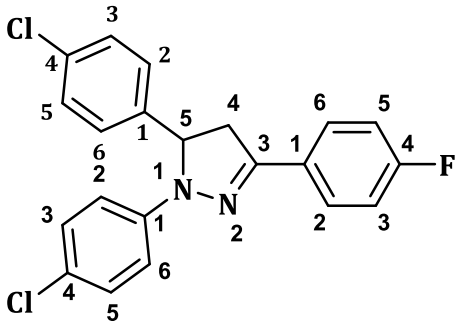
IUPAC NAME: 3-(4-bromophenyl)-1,5-bis(4-chlorophenyl)-4,5-dihydro-1H-pyrazole
Chemical Formula: $C_{21}H_{15}BrCl_2N_2$**;** **Molecular Weight: 446.17**

Elemental analysis:

Elements	C	N	Cl	Br
Calculated	56.53	6.28	15.89	17.91
Found	56.50	6.25	15.82	17.90

IR (cm^{-1}):
3066 (C–H), 2901 (C–H), 1688 (C=C), 1597 (C-C), 1464 (C=N), 1301 (C-H), 740 (C-Cl)
1HNMR (ppm):
δ 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_2), 3.45 (d, 1H, CH)
FAB Mass (m/z): 446.10

COMPOUND CODE: TDHP-15



IUPAC NAME: 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**
Elemental analysis:

Elements	C	N	F	Cl
Calculated	65.47	7.27	4.93	18.40
Found	65.45	7.25	4.90	18.35

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)
1HNMR (ppm):
δ 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_2), 3.45 (d, 1H, CH)

FAB Mass (m/z): 385.20

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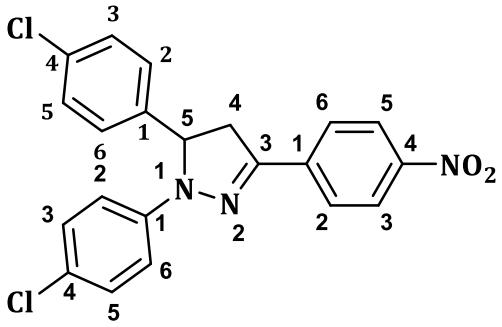
COMPOUND CODE: TDHP-16



IUPAC NAME: 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**

Elemental analysis:

Elements	C	N	Cl
Calculated	61.18	10.19	17.20
Found	61.15	10.15	17.15

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)
1HNMR (ppm):
δ 7.23-8.62 (m, 4H, CH=CH-CH=CH), 7.03-8.80 (m, 3H, Ar-H), 3.08, 2.47 (d, 2H, CH2), 3.42 (d, 1H, C-5, CH), 11.431 (s, 1H, NH)
FAB Mass (m/z): 412.20

4 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 (**compound4**), **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 physicochemical properties of the synthesized compound (TDHP-1 to TDHP-16) was represented in Table 4.4.
The synthesized compounds have characterized by the IR, 1HNMRand mass spectral analysis. The IR spectrum of the compounds has shown the characteristics peak (cm^{-1}) 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 1HNMR spectra of Synthesized compounds depicted the peak of δ 7.23-8.62 (m, 4H, CH=CH-CH=CH), 7.03-8.80 (m, 3H, Ar-H), 3.08, 2.47 (d, 2H, CH2), 3.42 (d, 1H, C-5, CH), 11.431 (s, 1H, NH). Compound 1 (TDHP-1), mass spectrum have shown peak at m/z = 365.84, which matches the chemical formula $C_{21}H_{17}ClFN_3$. All the other synthesized compound has also shown the molecular ion peak similar to their molecular formula and weight.

5.0 PHARMACOLOGICAL EVALUATION

Antimicrobial Activity

Microorganisms require nutrition for all their metabolic activities. They draw the nutrition from theirsurrounding area. The growth of the microorganisms depends on the type of the nutrition theyutilize. Resistance of pathogenic bacteria to available antibiotics is quickly becoming a majorproblem in the community and hospital based healthcare settings. Antimicrobials are one of the veryimportant categories of drug. It is quite clear from its wide use that these categories of drugs are veryimportant from the medical point of view.[65] However, microbial resistance towards the drug creates avery serious problem because of development of resistance; many drugs are now useless which were very effective in the past. Moreover, the toxic effects produced by these antibiotics are also reducing their significance.[66]
There is a need for new antimicrobial agents for resisting microbial infections. Antimicrobial (antibacterial and antifungal) activity of the synthesized compounds was done by disc diffusion method.

Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antimicrobial that inhibit the visible growth of a microorganism after overnight incubation. MIC values can be determined by a number of standard test procedures. The most commonly employed methods are the broth dilution method and agar dilution methods. In agar dilution

method, the diluted compounds were inoculating into bacterial growth media, incubated and scored for growth. This procedure is a standard assay for antimicrobials.[67] MIC is important in diagnostic laboratories to confirm resistance of micro organisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism.[68] Clinically, the minimum inhibitory concentrations are used not only to determine the amount of antibiotic that the patient will receive but also the type of antibiotic used, which in turn lowers the opportunity for microbial resistance to specific antimicrobial agents.

Materials used in Microbiology Assays

Gram-negative bacteria species *Pseudomonas aeruginosa (ATCC/27853)*,*Salmonella typhimurium (ATCC/25922)*. Gram-positive bacteria species *Bacillus subtilis* and *Staphylococcus aureus (ATCC/25923)*. All microorganisms were obtained from Agricultural biological laboratory. (10µg) of gram-negative bacteria (*Gentamycin)* and (10µg) of gram-positive bacteria *(Meropenem)* are used as antibiotic standards, normal saline 0.9%, Mueller Hinton agar media (MHA).

Test Microorganisms

The synthesized compounds (TDHP-1 to TDHP-16) were tested for their antimicrobial activity against gram-negative bacteria species (*Psedomonas Aeruginosa* and *Salmonella typhimurium)*, gram-positive bacteria species (*Bacillus subtilis* and *Staphylococcus aureus)*.

Preparation of Stock Solution

Against gram-negative and gram-positive bacteria species, 50µg/ml drug concentration in DMSO were prepared and tested against standard drug concentration i.e. (10µg) of gram-negative bacteria (*Gentamycin)* and(10µg) of gram-positive bacteria *(Meropenem)* are used as antibiotic standards.[69]

Antibacterial Activity by Disc Diffusion Method

Gram-negative and gram-positive bacteria species were used as antibacterial test strains.The synthesized compounds (TDHP-1 to TDHP-16) were screened at the concentration (500µg/mL) in DMSO on the agar media for all bacterial strains. The antibiotic *Gentamycin* was used as standard drugs against gram negative bacteria. *Meropenem* was used as standard drugs against gram positive bacteria. The plate inoculated with bacteria was incubated for 24 *h* at 37ºC. After the period of incubation, the zone of inhibition produced by the test compounds was measured in *mm*. The screening tests were performed in triplicate and the results were taken as a mean of three determinations. **Figure 5.1** shows the evaluation of antibacterial activity by disc diffusion method.

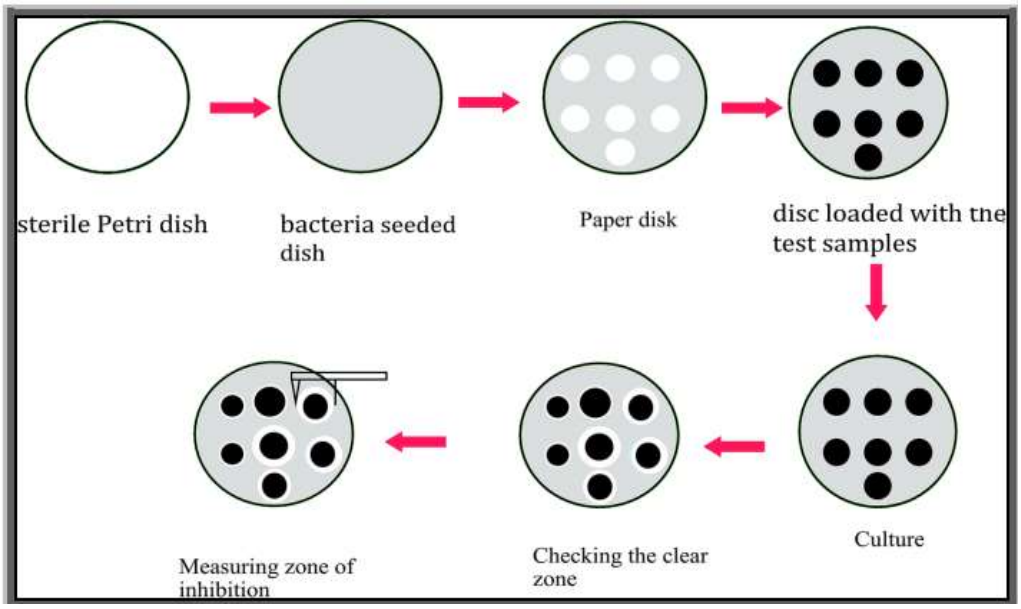


Fig 5.1: Schematic representation of Agar/ Disk diffusion method

The tested samples diffuse through the agar around its disks and inhibit germination of the microorganism by a characteristics zone of inhibition depending on the microorganism sensitivity to the test sample, then measuring the inhibition zones diameters in *mm*.

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RESULT AND DISCUSSION

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 5.1).

Table 5.1: Zone of Inhibition of the synthesized compounds against bacterial species in mm.

	Gram Positive Bacteria		**Gram Negative Bacteria**	
Antibiotic	***Bacillus subtilis***	***Staphylococcusaureus***	***Pseudomonas aeruginosa***	***Salmonella typhimurium***
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.

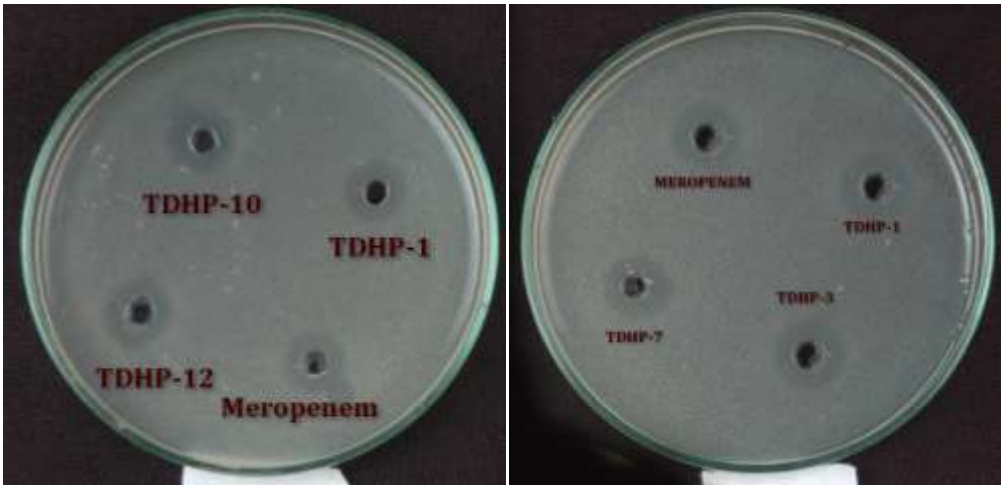

Fig. 5.1: The pictorial representation of Zone of inhibition of compound against gram-positive bacteria (*Micrococlus luteus* ATCC 9341)

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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 andTDHP-15 have shown mild activity while as compound TDHP-11,TDHP-12TDHP-16 andTDHP-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 5.1).

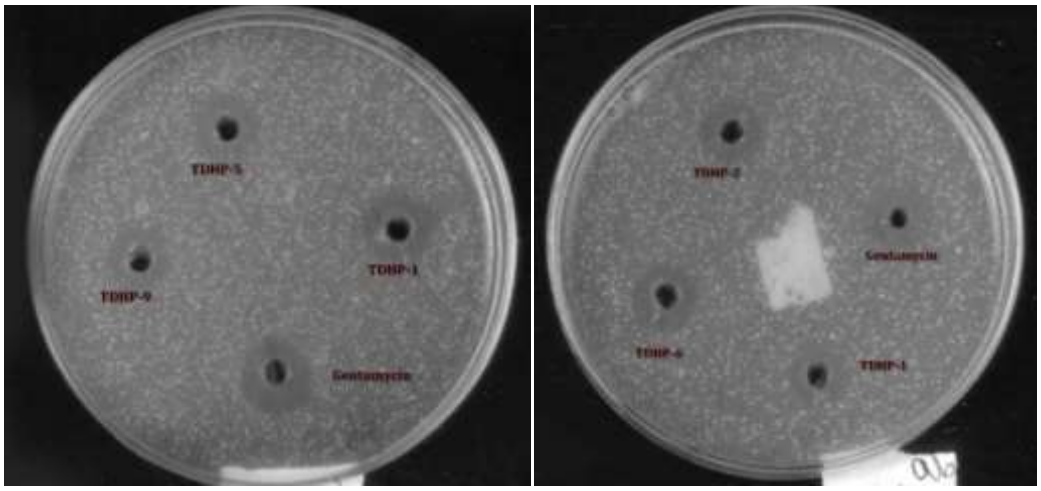

Fig. 5.2: The pictorial representation of Zone of inhibition of compound against gram negative bacteria

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-14TDHP-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-5TDHP-6, TDHP-7, TDHP-8 have shown best activity against gram negative bacteria.

Conclusions

The research work described the synthesis of new chemical entities of pyrazole derivatives. The synthesized compounds were characterized by standard spectroscopic techniques. The evaluation of the antimicrobial activity of all new compounds was carried out against bacterial strains and proven to have significant to mild to better activity. 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. Moreover, there is a need for other ways to test these compounds on various assays for additional therapeutic areas like inflammatory and carcinoma cell lines as well.

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