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An Improved Synthesis And Biological Evaluation Of Some New 4,5-dihydro-pyrazole-1-Carbaldehyde Derivatives

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ABSTRACT
An improved condensation of substituted chalcones **1a-g** with hydrazine hydrate and formic acid in 2-methoxyethanol to afford new series of 4,5-dihydro-pyrazole-1-carbaldehyde derivatives **2a-g**. Clean reaction conditions, simple workup procedure and short reaction time giving high yields of product are notable advantages of method. All newly synthesized compounds characterized by chemical test, spectral and elemental analysis. Further, all newly synthesized compounds were screened for their antimicrobial activity. Most of these title compounds exhibited potent activity.

Keywords: Synthesis, substituted chalcones, 2-methoxyethanol, 4,5-dihydro-1-carbaldehyde pyrazolines, antimicrobial activity

1. INTRODUCTION
Numerous pyrazolines type compounds have found to exhibit bioactivities[1,2] which has excellent role in many pharmaceutical, agrochemical and dystuff research[3,4]. Pyrazolines derivatives with a phenyl group at 5-position possess good flim-forming properties, exhibit excellent characteristics of blue photoluminescence and electroluminescence[5]. Moreover pyrazolines are extensively useful synthons in organic chemistry and also important in the development of theory in heterocyclic chemistry. These compounds are also well known for their pronounced biological activities including antimicrobial[5-7], antitumor[8], immunosuppressive[9], anti-inflammatory[10], anticancer[11], antidiabetic[12], antiamoebic[13], anticonvulsant[14], antidepressant[15], anesthetic[16], estrogen receptor agonists[17] and cyclooxygenase inhibitor[18] properties.

Synthesis of bicyclic pyrazoline derivatives were reported by condensation of 2,6-diarylidenecyclohexanones with hydrazine hydrate[19]. Acetone and acetophenones on base catalyzed condensation with substituted aldehyde affords α,β-unsaturated carbonyl compound which on treatment with hydrazine hydrate and formic acid yielded a 2-pyrazoline[20]. In view of these observations; it was thought worthwhile to synthesize some new different substituted pyrazoline derivatives by reacting chalcones with hydrazine hydrate and formic acid in 2-methoxyethanol as an alternative reaction solvent and to evaluate them for antimicrobial activity against *Bacillus subtilis (Bs), Staphylococcus aureus (Sa), Candida albicans (Ca) and Aspergillus flavus (Af).*

2. RESULTS AND DISCUSSION
In continuation of our work on different methodology in organic synthesis[21] and studies on some new bioactive compounds and their heterocycles[22], herein we report synthesis of some new series of 4,5-dihydro-pyrazole-1-carbaldehyde derivatives by condensation of chalcones with hydrazine hydrate and formic acid in 2-methoxyethanol as an alternative reaction solvent.

The starting chalcones **1a-g** were prepared by classical aldol condensation involving base-catalyzed condensation of the desired carbonyl compounds followed by dehydration forming α,β-unsaturated carbonyl compounds. Synthesis of 4,5-dihydro-pyrazole-1-carbaldehyde were attempted by reacting α,β-unsaturated carbonyl compounds (chalcones) with hydrazine hydrate and formic acid in presence of 2-methoxyethanol as solvent (Scheme-1). Recently the formation of 2-pyrazoline was reported by the reaction of chalcones with hydrazine hydrate take place in various conditions using ethanol[23], acetic acid[24], formic acid[20], or pyridine[25] as solvent. However, many of these reported procedures have one or more disadvantages such as use of expensive catalyst, low selectivity, harsh reaction conditions, low yield, relatively long reaction time and environmental concern. After some preliminary observation we found that 2-methoxyethanol as an efficient reaction medium in terms of clean reaction conditions, not expensive, short reaction time giving high yields of desired product. In view of these results, we turned our attention towards variety substituted chalcones. In all cases, reaction proceeds efficiently in high yields using 2-methoxyethanol (Table-3). The formation of products were assumed to proceed through the Micheal-type addition of hydrazine to activated double bond followed by intramolecular cyclization with the elimination of water molecule[26].

The formation of chalcones **1a-g** was confirmed by IR spectra, absence of a band around 1710-1720 cm^{-1} due to the ketonic C=O stretch and the appearance of characteristic band near 1655 cm^{-1} and near 1610 cm^{-1} due to α,β-unsaturated carbonyl group and ν (C=C) respectively. In ^1H NMR spectrum of chalcones two doublet in range at δ 6.86 (H-α, J = 16.5 H_Z) and δ 7.32 (H-β, J = 16.5 H_Z) suggested the presence of olefin protons at α,β-position to the carbonyl group. The IR spectrum of newly synthesized 2-pyrazolines **2a-g** showed a strong band for carbonyl group near 1635 cm^{-1} and band at 1577 cm^{-1} due to C=N. In the ^1H NMR spectra, an ABX pattern was observed for H_A, H_B and H_X proton which appear as pair of doublets near δ 3.20, 3.72 and 5.42 ppm. Trans olefin proton appears as

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doublets near δ 6.85 and 7.14 ppm with J = 16 H_Z. The singlet of CHO appeared at δ 8.92 ppm which conforms the N-H of 2-pyrazoline replaced by N-CHO group. This also conforms on the basis of silver mirror test[27].

The results of antimicrobial screening data are given in Table **4**. In comparison with reference drugs, compounds **2e**, **2f** and **2g** showed effective activity against all tested microbes. Compounds **2f** and **2g** showed near to par activity against *Candida albicans*. Only compound **2f** showed potent activity against *Aspergillus flavus* than standard fluconazole fungicide. Moreover compound **2f** showed stronger activity against *Staphylococcus aureus* than reference streptomycin drug. The remaining compounds **2a-d** displayed moderate antimicrobial activity. On the other hand compound **2a** and **2b** are inactive against *Bacillus subtilis* and *Candida albicans*. Results show that presence of halogen with hydroxy and methoxy substituent in basic pyrazole nucleus exhibits potent antimicrobial activity against various pathogens.

3. CONCLUSION

In summary, we have developed simple and easy procedure for synthesis of some novel 4,5-dihydro-pyrazole-1-carbaldehyde derivative by condensation of chalcones with hydrazine hydrate and formic acid in 2-methoxyethanol as efficient and alternative reaction solvent. The advantages of present protocol are simplicity of operation, high yields of products and avoidance of expensive catalyst and usage of volatile organic solvent.

The preliminary *in vitro* antimicrobial screening of this series revealed that compounds **2e**, **2f** and **2g** showed potent activity when compared with standard drug. Therefore, the present study is useful drug in medicinal investigation against bacterial and fungal diseases.

4. EXPERIMENTAL

Melting points were determined in an open capillary tube and are uncorrected. IR spectra were recorded in KBr on a Perkin-Elmer spectrometer. ^{1}H NMR spectra were recorded on a Gemini 300-MH_Z instrument in DMSO as solvent and TMS as an internal standard. The mass spectra were recorded on EISHIMADZU-GC-MS spectrometer. Elemental analyses were performed on a Perkin-Elmer 240 CHN elemental analyzer.

4.1 *General Procedure for synthesis of chalcones* 1a-g

To a mixture of different substituted benzaldehyde **1** (0.02 mol) and acetone **2** (0.01 mol) in ethanol, 10 % aqueous sodium hydroxide (10 ml) was added drop by drop with constant stirring at 0-5 ^{0}C. After complete addition of NaOH solution, the reaction mixture left to stand in ice bath for 20 min. Then obtained yellow coloured solid was filtered washed with cold water and crystallized from ethanol to give the corresponding chalcones derivative. The physical data of synthesized chalcones are given in Table-1.

Table-1: Physical Data Of Synthesized Products (la-g)

Product	R	R1	R2	R3	Yeild(%)	M.P (^{O}C)	(Reported)
1a	OH	H	H	H	80	110-113	(112)[28]
1b	H	H	OCH_3	H	84	117-119	(118)[29]
1c	H	OCH_3	OH	H	82	124-126	(124)[30]
1d	H	OC_2H_5	OH	H	79	138-140	(139)[31]
1e	H	OCH_3	OH	Br	86	145-147	(145)[32]
1f	OH	Br	H	Br	83	157-159	----
1g	OH	I	H	I	88	132-135	----

4.2 *1,5-Bis-(3,5-dibromo-2-hydroxy-phenyl)-penta-1,4-dien-3-one.1f*

IR (KBr): 3236 (OH), 1656 (C=O), 1608 (C=C) cm^{-1}. ^{1}H NMR (300 MH_Z, DMSO) δ 6.86 (d, J = 16.5 H_Z, 2H, H_α), δ 7.31 (d, J = 16.5 H_Z, 2H, H_β), δ 12.21 (s, 2H, OH), δ 7.14-7.88 (m, 4H, Ar-H). MS m/z: 582 (M^+). Anal. Cacld for $C_{17}H_{10}O_3Br_4$: C, 35.05; H, 1.71; X (Br), 54.98. Found: C, 33.08; H, 1.75; X (Br), 55.02.

4.3 *1,5-Bis-(2-hydroxy-3,5-diiodo-phenyl)-penta-1,4-dien-3-one.1g*: IR (KBr)

3232 (OH), 1652 (C=O), 1610 (C=C) cm^{-1}. ^{1}H NMR (300 MH_Z, DMSO) δ 6.89 (d, J = 16.5 H_Z, 2H, H_α), δ 7.34 (d, J = 16.5 H_Z, 2H, H_β), δ 12.26 (s, 2H, OH), δ 7.14-7.82 (m, 4H, Ar-H). MS m/z: 770 (M^+). Anal. Cacld for $C_{17}H_{10}O_3I_4$: C, 26.49; H, 1.29; X (I), 65.97. C, 26.52; H, 1.31; X (I), 66.01.

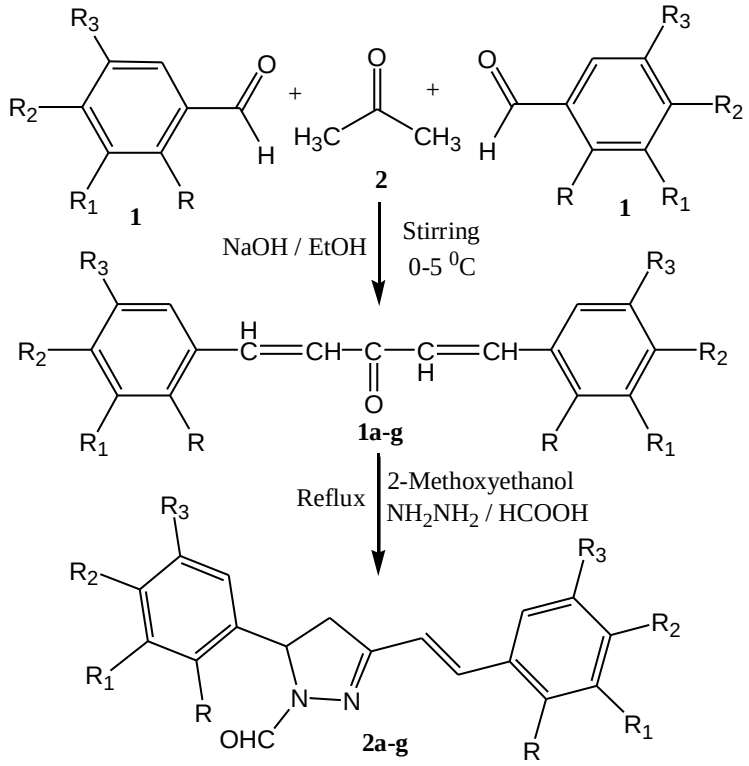
4.4 *Typical procedure for synthesis of 4,5-dihydro-pyrazole-1-carbaldehyde*. 2a-g

A mixture of **1a** (0.01 mol) hydrazine hydrate (0.02 mol) and formic acid (2ml) was dissolved in 2-methoxyethanol (10 ml). The reaction mixture was refluxed for period as shown in Table-2. The progress of reaction was monitored by TLC. After completion, reaction solution get cooled to room temperature and poured into crushed ice, obtained crude product was filtered washed with cold water and recrystallized from mixture of ethanol: dioxane to give the product **2a-g**. Similarly, other analogues of this were synthesized by using same procedure.

Table-2: Physical Data Of Newly Synthesized 4.5-dihydro-pyrazole-1-carbaldehyde derivatives (2a-g)

Product	R	R1	R2	R3	Time(h)	Yeild(%)	M.P (^{O}C)
2a	OH	H	H	H	3	78	129-131
2b	H	H	OCH_3	H	4	86	133-135
2c	H	OCH_3	OH	H	3.5	84	141-143
2d	H	OC_2H_5	OH	H	4	83	147-149
2e	H	OCH_3	OH	Br	3.5	80	154-156
2f	OH	Br	H	Br	3	88	169-171
2g	OH	I	H	I	4	91	158-160

The progress of reaction was monitored by TLC. After completion, reaction solution get cooled to room temperature and poured into crushed ice, obtained crude product was filtered washed with cold water and recrystallized from mixture of ethanol: dioxane to give the product **2a-g**. Similarly, other analogues of this were synthesized by using same procedure.



Scheme-1: Synthesis of 4,5-dihydro-pyrazole-1-carbaldehyde **2a-g**

4.5 *5-(2-Hydroxy-phenyl)-3-[2-(2-hydroxy-phenyl)-vinyl]-4,5-dihydro-pyrazole-1-carbaldehyde.2a*

IR (KBr): 3373 (OH), 1628 (C=O), 1578 (C=N) cm^{-1}. ^{1}H NMR (300 MH_Z, DMSO) δ 3.20 (dd, *J* = 5.0, 17.8 H_Z, 1H, H_A), δ 3.71 (dd, *J* = 12.0, 17.8 H_Z, 1H, H_B), δ 5.42 (dd, *J* = 5.1, 12.1 H_Z, 1H, H_X), δ 6.83 (d, *J* = 16.2 H_Z, 1H, H_α), δ 7.21 (d, *J* = 16.2 H_Z, 1H, H_β), δ 7.12-7.68 (m, 8H, Ar-H), δ 8.92 (s, 1H, CHO), 11.8 (s, 2H, *two* OH). ^{13}CNMR (DMSO) 162.48 (C=O), 156.19 (2C, *of two* Ar-OH), 144.72 (C=N), 138.24 (C_β, C=C *double bond*), 137.23 (C, Ar-C), 135.18 (C, Ar-C) 132.72 (2CH, *of two* Ar-C), 130.50 (2CH, *of two* Ar-C), 128.65 (2CH, *of two* Ar-C), 120.48 (2CH, *of two* Ar-C) 118.12 (C_α, C=C *double bond*), 50.57 (-CH), 39.44 (-CH_2). MS m/z 308 (M^+). Anal. Cacld for $C_{18}H_{16}O_3N_2$: C, 70.12; H, 5.19. Found: C, 70.16; H, 5.22.

4.6 *5-(4-Methoxy-phenyl)-3-[2-(4-methoxy-phenyl)-vinyl]-4,5-dihydro-pyrazole-1-carbaldehyde (2b)*

IR (KBr): 1626 (C=O), 1572 (C=N) cm^{-1}. ^{1}H NMR (300 MH_Z, DMSO) δ 3.82 (s, 6H, *two* OCH_3), δ 3.21 (dd, *J* = 5.1, 17.9 H_Z, 1H, H_A), δ 3.73 (dd, *J* = 12.2, 17.8 H_Z, 1H, H_B), δ 5.44 (dd, *J* = 5.1, 12.2 H_Z, 1H, H_X), δ 6.81 (d, *J* = 16.2 H_Z, 1H, H_α), δ 7.18 (d, *J* = 16.2 H_Z, 1H, H_β), δ 7.25-7.81 (m, 8H, Ar-H), δ 8.90 (s, 1H, CHO). ^{13}CNMR (DMSO) 165.58 (2C *of two para* Ar-ome), 163.22 (C=O), 144.88 (C=N), 138.26 (C_β of C=C *double bond*), 137.18 (C, Ar-C), 135.26 (C, Ar-C), 131.43 (4CH *of four* Ar-C), 118.14 (C_α of C=C *double bond*), 116.13 (4CH *of four* Ar-C), 58.42 (2C, *of two* OCH_3) 51.13 (-CH), 37.80 (-CH_2),. MS m/z: 336 (M^+). Anal. Cacl for $C_{20}H_{20}O_3N_2$: C, 71.42; H, 5.95. Found: C, 71.40; H, 5.92.

4.7 *5-(4-Hydroxy-3-methoxy-phenyl)-3-[2-(4-hydroxy-3-methoxy-phenyl)-vinyl]-4,5-dihydro-pyrazole-1-carbaldehyde.2c*

IR (KBr): 3368 (OH), 1635 (C=O), 1577 (C=N) cm^{-1}. ^1H NMR (300 MH_Z, DMSO) δ 3.8 (s, 6H, *two* OCH_3), δ 3.23 (dd, *J* = 5.1, 17.9 H_Z, 1H, H_A), δ 3.71 (dd, *J* = 12.1, 17.8 H_Z, 1H, H_B), δ 5.42 (dd, *J* = 5.1, 12.2 H_Z, 1H, H_X), δ 6.82 (d, *J* = 16 H_Z, 1H, H_α), δ 7.14 (d, *J* = 16 H_Z, 1H, H_β), δ 7.28-7.76 (m, 6H, Ar-H), δ 8.93 (s, 1H, CHO), δ 10.73 (s, 2H, *two* OH). ^{13}CNMR (DMSO) 165.52 (2C *of two* Ar-ome), 162.25 (C=O), 156.21 (2C *of two* Ar-OH), 145.13 (C=N), 137.16 (C, Ar-C), 135.20 (C, Ar-C), 138.26 (C_β of C=C *double bond*), 121.87 (2CH, *of two* Ar-C), 118.38 (2CH, *of two* Ar-C), 118.13 (C_α of C=C *double bond*), 114.27 (2CH *of two* Ar-C), 58.45 (2C *of two* OCH_3), 50.41 (-CH), 38.37 ($-CH_2$). MS m/z: 368 (M^+). Anal. Cacl for $C_{20}H_{20}O_5N_2$: C, 65.21; H, 5.43. Found: C, 65.18; H, 5.40.

4.8 *5-(3-Ethoxy-4-hydroxy-phenyl)-3-[2-(3-ethoxy-4-hydroxy-phenyl)-vinyl]-4,5-dihydro-pyrazole-1-carbaldehyde.2d*

IR (KBr): 3372 (OH), 1628 (C=O), 1574 (C=N) cm^{-1}. ^1H NMR (300 MH_Z, DMSO) δ 1.38 (t, 6H, *two* CH_3), δ 3.89 (q, 4H, *two* OCH_2), δ 3.22 (dd, *J* = 5.2, 17.9 H_Z,1H, H_A), δ 3.76 (dd, *J* = 12.2, 17.9 H_Z, 1H, H_B), δ 5.48 (dd, *J* = 5.2, 12.2 H_Z, 1H, H_X), δ 6.79 (d, *J* = 16 H_Z, 1H, H_α), δ 7.12 (d, *J* = 16 H_Z, 1H, H_β), δ 7.22-7.79 (m, 6H, Ar-H), δ 8.92 (s, 1H, CHO), δ 10.68 (s, 2H, *two* OH). ^{13}CNMR (DMSO) 164.88 (2C, *of two* Ar-OEt), 162.78 (C=O), 156.23 (2C, *of two* Ar-OH), 145.22 (C=N), 137.18 (C, Ar-C), 135.16 (C, Ar-C), 138.24 (C_β of C=C *double bond*), 121.92 (2CH, *of two* Ar-C), 118.43 (2CH, *of two* Ar-C), 118.15 (C_α of C=C *double bond*), 114.32 (2CH, *of two* Ar-C) 51.17 (-CH), 38.33 ($-CH_2$, ring), 58.52 (2C *of two* OCH_2), 16 (2C, *of two* CH_3). MS m/z: 396 (M^+). Anal. Cacl for $C_{22}H_{24}O_5N_2$: C, 66.66; H, 5.05. Found: C, 66.68; H, 5.08.

4.9 *5-(3-Bromo-4-hydroxy-5-methoxy-phenyl)-3-[2-(3-bromo-4-hydroxy-5-methoxy-phenyl)-vinyl]-4,5-dihydro-pyrazole-1-carbaldehyde.2e*

IR (KBr): 3366 (OH), 1632 (C=O), 1576 (C=N) cm^{-1}. ^1H NMR (300 MH_Z, DMSO) δ 3.86 (s, 6H, *two* OCH_3), δ 3.24 (dd, *J* = 5.2, 17.9 H_Z, 1H, H_A), δ 3.71 (dd, *J* = 12.1, 17.8 H_Z, 1H, H_B), δ 5.47 (dd, *J* = 5.2, 12.1 H_Z, 1H, H_X), δ 6.82 (d, *J* = 16 H_Z, 1H, H_α), δ 7.14 (d, *J* = 16 H_Z, 1H, H_β), δ 7.26-7.81 (m, 4H, Ar-H), δ 8.95 (s, 1H, CHO), δ 10.64 (s, 2H, OH). ^{13}CNMR (DMSO) 165.58 (2C *of two* Ar-ome), 156.29 (2C *of two* Ar-OH), 162.88 (C=O), 145.51 (C=N), 138.35 (C_β *of* C=C *double bond*), 137.14 (C *of* Ar-C), 135.12 (C *of* Ar-C), 125.51 (2CH *of two* Ar-C), 118.24 (C_α *of* C=C *double bond*), 119.52 (2C *of two* Ar-Br), 113.78 (2CH *of two* Ar-C), 58.49 (2C *of two* -OCH_3), 51.25 (-CH), 38.26 ($-CH_2$). MS m/z: 526 (M^+). Anal. Cacl for $C_{20}H_{18}O_5N_2Br_2$: C, 45.62; H, 3.42; X (Br), 34.22. Found: C, 45.59; H, 3.40; X (Br), 34.18.

4.10 *5-(3,5-Dibromo-2-hydroxy-phenyl)-3-[2-(3,5-dibromo-2-hydroxy-phenyl)-vinyl]-4,5-dihydro-pyrazole-1-carbaldehyde.2f*

IR (KBr): 3371 (OH), 1630 (C=O), 1573 (C=N) cm^{-1}. ^1H NMR (300 MH_Z, DMSO) δ 3.23 (dd, *J* = 5.0, 17.8 H_Z, 1H, H_A), δ 3.70 (dd, *J* = 12.0, 17.8 H_Z, 1H, H_B), δ 5.46 (dd, *J* = 5.1, 12.1 H_Z, 1H, H_X), δ 6.85 (d, *J* = 16 H_Z, 1H, H_α), δ 7.12 (d, *J* = 16 H_Z, 1H, H_β), 7.25-7.83 (m, 4H, Ar-H), δ 8.92 (s, 1H, CHO), δ 12.0 (s, 2H, *two* OH). ^{13}CNMR (DMSO) 163.21 (C=O), 156.29 (2C *of two* Ar-OH), 144.97 (C=N), 138.28 (C_β *of* C=C *double bond*), 137.24 (C *of* Ar-C), 135.27 (C *of* Ar-C), 130.74 (2CH *of two* Ar-C), 129.61 (2CH *of two*Ar-C), 119.48 (2C *of two* Ar-Br), 118.18 (C_α *of* C=C *double bond*), 114.08 (2C *of two* Ar-Br), 51.32 (-CH), 37.88 ($-CH_2$). MS m/z: 624 (M^+). Anal. Cacl for $C_{18}H_{12}O_3N_2Br_4$: C, 34.61; H, 1.92; X (Br), 51.28. Found: C, 34.63; H, 1.95; X (Br), 51.30.

4.11 *5-(2-Hydroxy-3,5-diiodo-phenyl)-3-[2-(2-hydroxy-3,5-diiodo-phenyl)-vinyl]-4,5-dihydro-pyrazole-1-carbaldehyde*.**2g**

IR (KBr): 3373 (OH), 1635 (C=O), 1573 (C=N) cm^{-1}. ^1H NMR (300 MH_Z, DMSO) δ 3.23 (dd, *J* = 5.1, 17.9 H_Z, 1H, H_A), δ 3.67 (dd, *J* = 12.1, 17.8 H_Z, 1H, H_B), δ 5.44 (dd, *J* = 5.1, 12.2 H_Z, 1H, H_X), δ 6.87 (d, *J* = 16 H_Z, 1H, H_α), δ 7.12 (d, *J* = 16 H_Z, 1H, H_β), 7.22-7.81 (m, 4H, Ar-H), δ 8.93 (s, 1H, CHO), δ 12.1 (s, 2H, *two* OH). ^{13}CNMR (DMSO) 163.27 (C=O), 156.24 (2C *of two* Ar-OH), 145.08 (C=N), 138.32 (C_β of C=C *double bond*), 137.26 (C *of* Ar-C), 135.22 (C *of* Ar-C), 130.57 (2CH *of two* Ar-C), 118.24 (C_α of C=C *double bond*) 112.36 (2CH, *of two* Ar-C), 88.17 (4C *of four* Ar-I), 51.37 (-CH), 37.96 ($-CH_2$). MS m/z: 812 (M^+). Anal. Cacl for $C_{18}H_{12}O_3N_2I_4$: C, 26.60; H, 1.47; X (I), 62.56. Found: C, 26.63; H, 1.49; X (I), 62.58

4.12 *Antimicrobial Assay*

The antibacterial activities of the synthesized compounds (**2a-g**) were determined by agar well diffusion method[33]. The compounds were evaluated for antibacterial activity against *Bacillus subtilis* [MTCC 2063] and *Staphylococcus aureus* [MTCC 2901]. The antifungal activity performed against *Aspergillus flavus* [MTCC 2501] and *Candida albicans* [MTCC 183] were procured from Institute of Microbial Technology (IMTech), Chandigarh, India. The antibiotic streptomycin (25µ/mL) and fluconazole used as reference drug for antibacterial and antifungal activity, respectively. Dimethyl sulphoxide (1%, DMSO) used a control without compound.

Table-3: Effect of solvent on synthesis of 5-(2-Hydroxy-3,5-diiodo-phenyl)-3-[2-(2-hydroxy-3,5-diiodo-phenyl)-vinyl]-4,5-dihydro-pyrazole-1-carbaldehyde : **2g**

Entry	Solvent	Time (h)	Yield (%)
1	Ethanol	14	70
2	Acetic acid	16	65
3	Formic acid	18	68
4	DMF	12	75
5	2-Methoxyethanol	04	91

Table-4: Antimicrobial screening of some new 4,5-dihydro-pyrozole-1-carbaldehyde

Entry	Zone of inhibition (mm)			
	Af	Ca	Bs	Sa
2a	12	15	--	13
2b	17	--	15	12
2c	21	19	18	17
2d	23	21	19	19
2e	26	24	28	26
2f	28	26	26	30
2g	26	26	28	26
Reference 1	26	28	NA	NA
Reference 2	NA	NA	30	28

Reference 1 = Fluconazole
Reference 2 = Streptomycin
NA= Not Applicable

The culture strains of bacteria were maintained on nutrient agar slant at 37 ± 0.5 ^{0}C for 24 h. The antibacterial activity was evaluated using nutrient agar plate seeded with 0.1 mL of respective bacterial culture strain suspension prepared in sterile saline (0.85%) of 10^5 CFU/mL dilutions. The wells of 6 mm diameter were filled with 0.1 mL of compound solution at fixed concentration 25 µ/mL separately for each bacterial strain. All plates were incubated at 37 ± 0.5 ^{0}C for 24 h. Zone of inhibition were noted in mm, Table-4.

For antifungal activity, all culture strains of fungi maintained on potato dextrose agar (PDA) slant at 27 ± 0.2 ^{0}C for 24-48 h, until sporulation. Spore of strains were transferred into 5 mL of sterile distilled water containing 1% Tween-80 (to suspend the spore properly). The spores were counted by haemocytometer (10^6 CFU/mL). Sterile PDA plate was prepared containing 2% agar; 0.1 mL of each fungal spore suspension was spread on each plate and incubated at 27± 0.2 ^{0}C for 12 h. After incubation well prepared using sterile cork borer and each agar well was filled with 0.1 mL of compound solution at fixed concentration 25 µ/mL. The plates were kept in refrigerator for 20 min for diffusion and then incubated at 27± 0.2 ^{0}C for 7 days except *Candida albicans*. After incubation, zone of inhibition of compounds were measured in mm along with standard, Table-4.

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