MICROWAVE-INDUCED, EFFICIENT, CONVENIENT AND RAPID SYNTHESIS OF SUBSTITUTED 2-PYRAZOLINES AS POTENTIALLY ANTIMICROBIAL AGENT

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Microwave-induced an efficient, rapid and enviromentally begin condensation of substituted 2'-hydroxychalcones with hydrazine hydrate in 2-methoxyethanol afford 2-pyrazolines **2a-h** in high 88-95 % yields. The structure of newly synthesized compounds established on the basis of spectroscopic technique and laboratory chemical test. Further these compounds were screened for antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus, Aspergillus flavus* and *Candida albicans*. The most of the compounds shows good to better inhibitory activity.

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Introduction

The use of the hazardus reagent, solvent and high loading catalyst in synthetic organic chemistry leads to impact on environment. Due to this varied nature of the chemicals, chemist requiresvarious greener pathways in our quest towards attaining sustainability.[1] Microwave (MW) irradiation is one of them rout has been gaining increased popularity as an alternative heat source in green chemistry towards development of organic synthesis.[2] This technique has been applied to a variety of reactions resulting in reduction of reaction time, higher yield, greater selectivity, cleaner reaction products, and to reduce tadious job for isolation of product.[3] It also provides an opportunity to work with open vessels, thus avoiding the risk of high pressure and hazards of inflammable solvents.[4]

Among a wide range of biologically active heterocycles, a significant amount of research activity has been directed toward the study of pyrazolines. A number of pyrazoline derivatives have been found to exhibit physiological and pharmacological properties such as antiinnammatory,[5-6] antibacteria1,[7] antineoplastic,[8] antiallergic,[9] analgesic,[10] and hypoglycemic[11] activities. Classical route for the preparation of these compounds involesthe condensation of α,β-unsaturated carbonyl compounds with hydrazines.[12] The various modified methods have been reported for synthesis of 2-pyrazolines using different catalysts such as $KHSO_4H_2O/SiO_2$,[13] porous calcium hydroxyapatite,[14] chlorahine-T,[15] mercuric acetate,[16] $Bi(NO_3)_3{\cdot}5H_2O$,[17] Zn,[18] $H_3PW_{12}O_{40}$,[19] and Lewis acid/Lewis bases.[20] Many of these procedure invole combinations of solvents with various catalyst and long reaction time make these methods environmentally hazardous, economical expensive. Chemist require to improve these methods towards organic synthesis and reaction, increasing attention is being focused on green chemistry using environmentally benign reagents and conditions, particularly non-conventional heating. In view of these observations, it was thought worthwhile to synthesize 2-pyrazoline derivatives from α,β-unsaturated carbonyl compounds in 2-methoxyethanol by using nonconventional tool.

Methods and Materials

Instrumentation

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 MHz instrument in DMSO-d_6 as solvent and TMS as an internal standard. The mass spectra were recorded on Shimadzu GC/MS spectrometer. Elemental analyses were performed on a Perkin-Elmer 240 CHN elemental analyzer. A multimode microwave oven (2450 MHz, 300 W, Brand LG, India) were used for performing reaction. Reaction progress monitored on TLC using using hexane/ethyl acetate/petrolium ether combination as the mobile phase.

Typical procedure for synthesis of 2-pyrazolines

A mixture of 3-(3-bromo-4,5-dimethoxyphenyl)-1-(4-bromo-1-hydroxy-naphthalen-2-yl)propenone (10 mmol) and $NH_2NH_2{\cdot}H_2O$ (50 mmol) dissolved in 2-methoxyethanol (5 ml) in round bottom flask. To this reaction solution 0.001 mmol of glacial acetic acid was added. The resulting reaction mixtures were irradiated in microwave oven for 2-3 minutes with short interval of 20 seconds to avoid the excessive evaporation of solvent. Progress of reaction was monitored on TLC (mixture of hexane, ethyl acetate, petroleum ether). The separated solid was filtered and recrystallized from ethyl alcohol to give **2c**. Physical data of synthesized compounds **2a-h** are given in Table **1**.

Table 1. Synthesis of substitut ed 2-pyrazolines under microwave irradiation

Compund	R	R_1	R_2	R_3	Time, min.	Melting point,[a] ^{0}C	Yield,[b] %
2a	H	H	OMe	H	4	148-150	91
2b	H	OMe	OMe	Br	5-6	161-163	90
2c	Br	OMe	OMe	Br	2-3	177-179	92
2d	I	H	OMe	H	3	154	88
2e	H	H	F	H	4-5	130	93
2f	H	H	Cl	H	3-4	135-137	95
2g	I	OMe	OMe	Br	4	188	89
2h	H	OMe	OMe	OMe	5-6	190-192	88

[a]M.P. referred with solvent-free and other technique.[24-26] [b]Yield of isolated desired product.

2-[5-(4-Methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]naphthalen-1-ol. (2a)

UV/VIS (λ_{max}, nm): 413, 328. IR (KBr pellets): 1590 (C=N), 1474, 1542 (C=C), 1234 (C-N) cm^{-1}. ^{1}H NMR (300 MH$_Z$, DMSO-d$_6$) δ 12.45 (s, 1H, OH), 7.81-7.64 (m, 10H, Ar-H), 6.82 (s, 1H, NH), 3.26 (dd, *J* = 5.0, 17.4 H$_Z$, 1H, H$_A$), δ 3.64 (dd, *J* = 12.1, 17.5 H$_Z$, 1H, H$_B$), δ 4.82 (dd, *J* = 5.0, 12.1 H$_Z$, 1H, H$_X$), 3.77 (s, 3H, OCH$_3$). ^{13}CNMR (DMSO): 160.15 (C *of* Ar-OCH$_3$), 154.85 (C *of*Ar-OH), 152.29 (C *of* C=N), 138.28 (Ar-C), 137.92 (Ar-C), 136.26 (Ar-C), 135.88 (Ar-C), 134.74 (Ar-C), 128.64 (Ar-C), 128.49 (Ar-C), 127.75 (Ar-C), 127.70 (Ar-C), 126.93 (Ar-C), 126.80 (Ar-C), 124.19 (Ar-C), 122.38 (Ar-C), 117.26 (Ar-C), 56.74 (C *of* OCH$_3$), 52.10 (C *of* CH), 44.75 (C *of* CH$_2$). MS (EI, *m/z* (%): 318(M$^+$, 68 %). Anal.Cacld. for $C_{20}H_{18}O_2N_2$: C, 75.47; H, 5.66. found: C, 75.55; H, 5.59.

2-[5-(3-Bromo-4,5-dimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]naphthalen-1-ol. (2b)

UV/VIS (λ_{max}, nm): 409, 326. IR (KBr pellets):1585 (C=N), 1475, 1538 (C=C), 1228 (C-N) cm^{-1}. ^{1}H NMR (300 MH$_Z$, DMSO-d$_6$) δ 12.52 (s, 1H, OH), 7.76-7.39 (m, 8H, Ar-H),6.89 (s, 1H, NH), 3.31 (dd, *J* = 5.2, 17.6 H$_Z$, 1H, H$_A$), δ 3.67 (dd, *J* = 12.1, 17.6 H$_Z$, 1H, H$_B$), δ 4.86 (dd, *J* = 5.1, 12.1 H$_Z$, 1H, H$_X$), 3.83 (s, 3H, OCH$_3$), 3.76 (s, 3H, OCH$_3$).^{13}CNMR (DMSO): 154.65 (C *of*Ar-OH), 152.56 (C *of* C=N), 152.29 (C *of* Ar-OCH$_3$), 149.47 (C *of* Ar-OCH$_3$) 138.27 (Ar-C), 137.86 (Ar-C), 128.45 (Ar-C), 127.48 (Ar-C), 127.27 (Ar-C), 125.34 (Ar-C), 124.93 (Ar-C), 121.29 (Ar-C), 118.87 (Ar-C), 116.70 (Ar-C), 115.39 (Ar-C), 113.79 (Ar-C), 109.19 (C *of*Ar-Br), 57.84 (C *of* OCH$_3$), 56.45 (C *of* OCH$_3$), 53.28 (C *of* CH), 43.96 (C *of* CH$_2$).MS (EI, *m/z* (%): 427 (M$^+$, 42 %). Anal.Cacld. for $C_{21}H_{19}O_3N_2Br$: C, 59.01; H, 4.44; X (Br), 18.73. found: C, 59.16; H, 4.53; X (Br), 18.85.

4-Bromo-2-[5-(3-bromo-4,5-dimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]naphthalen-1-ol. (2c)

UV/VIS (λ_{max}, nm): 411, 329. IR (KBr pellets):1594 (C=N), 1472, 1543 (C=C), 1231 (C-N) cm^{-1}. ^{1}H NMR (300 MH$_Z$, DMSO-d$_6$) δ 12.61 (s, 1H, OH), 7.89-7.46 (m, 7H, Ar-H),6.83 (s, 1H, NH), 3.26 (dd, *J* = 5.1, 17.5 H$_Z$, 1H, H$_A$), δ 3.65 (dd, *J* = 12.0, 17.5 H$_Z$, 1H, H$_B$), δ 4.88 (dd, *J* = 5.1, 12.1 H$_Z$, 1H, H$_X$), 3.86 (s, 3H, OCH$_3$), 3.78 (s, 3H, OCH$_3$).^{13}CNMR (DMSO): 155.32 (C *of*Ar-OH), 152.72 (C *of* C=N), 152.37 (C *of* Ar-OCH$_3$), 149.45 (C *of* Ar-OCH$_3$) 139.29 (Ar-C), 137.13 (Ar-C), 129.46 (Ar-C), 128.88 (Ar-C), 127.47 (Ar-C), 126.30 (Ar-C), 124.97 (Ar-C), 121.73 (Ar-C), 118.28 (Ar-C), 116.71 (Ar-C), 116.39 (Ar-C), 115.39 (C *of*Ar-Br), 109.22 (C *of*Ar-Br), 57.92 (C *of* OCH$_3$), 56.45 (C *of* OCH$_3$), 53.27 (C *of* CH), 43.98 (C *of* CH$_2$). MS (EI, *m/z* (%):506 (M$^+$, 78 %). Anal.Cacld. for $C_{21}H_{18}O_3N_2Br_2$: C, 49.80; H, 3.55, X (Br), 31.62. found: C, 49.74; H, 3.58; X (Br), 31.68.

4-Iodo-2-[5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl]naphthalen-1-ol (2d**)**

UV/VIS (λ_{max}, nm): 413, 331. IR (KBr pellets):1590 (C=N), 1479, 1544 (C=C), 1231 (C-N) cm^{-1}. ^{1}H NMR (300 MH$_Z$, DMSO-d$_6$) δ 12.56 (s, 1H, OH), 7.80-7.36 (m, 9H, Ar-H), 6.85 (s, 1H, NH), 3.28 (dd, *J* = 5.1, 17.5 H$_Z$, 1H, H$_A$), δ 3.66 (dd, *J* = 12.1, 17.5 H$_Z$, 1H, H$_B$), δ 4.87 (dd, *J* = 5.1, 12.1 H$_Z$, 1H, H$_X$), 3.74 (s, 3H, OCH$_3$).^{13}CNMR (DMSO): 159.97 (C *of* Ar-OCH$_3$), 155.13 (C *of*Ar-OH), 152.34 (C *of* C=N), 137.87 (Ar-C), 137.94 (Ar-C), 136.48 (Ar-C), 136.85 (Ar-C), 135.67 (Ar-C), 129.40 (Ar-C), 128.45 (Ar-C), 128.79 (Ar-C), 127.18 (Ar-C), 126.92 (Ar-C), 126.77 (Ar-C), 124.28 (Ar-C), 123.63 (Ar-C), 108.15 (C *of*Ar-I), 56.73 (C *of* OCH$_3$), 52.17 (C *of* CH), 44.62 (C *of* CH$_2$).MS (EI, *m/z* (%): 444 (M$^+$, 90 %). Anal.Cacld. for $C_{20}H_{17}O_2N_2I$: C, 54.05; H, 3.82; X (I), 28.60. found: C, 54.18; H, 3.79; X (I), 28.67.

2-[5-(4-Fluorophenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]-4-iodonaphthalen-1-ol. (2e)

UV/VIS (λ_{max}, nm): 410, 330. IR (KBr pellets): 1588 (C=N), 1468, 1542 (C=C), 1228 (C-N) cm^{-1}. ^{1}H NMR (300 MH$_Z$, DMSO-d$_6$) δ 12.48 (s, 1H, OH), 7.88-7.37 (m, 9H, Ar-H),6.87 (s, 1H, NH), 3.30 (dd, *J* = 5.2, 17.6 H$_Z$, 1H, H$_A$), δ 3.70 (dd, *J* = 12.1, 17.6 H$_Z$, 1H, H$_B$), δ 4.89 (dd, *J* = 5.2, 12.1 H$_Z$, 1H, H$_X$).^{13}CNMR (DMSO): 155.23 (C *of*Ar-OH), 152.48 (C *of* C=N), 137.18 (Ar-C), 137.82 (Ar-C), 136.25 (Ar-C), 136.96 (Ar-C), 135.27 (Ar-C), 129.31 (Ar-C), 129.57 (Ar-C), 128.62 (Ar-C), 127.19 (Ar-C), 127.91 (Ar-C), 126.73 (Ar-C), 125.57 (Ar-C),

124.88 (Ar-C),122.65 (Ar-C), 109.98 (C *of*Ar-I),52.26 (C *of* CH), 44.60 (C *of* CH_2).MS (EI, *m/z* (%): 432 (M^+, 38 %). Anal.Cacld. for $C_{19}H_{14}N_2OIF$: C, 52.77; H, 3.24; X (I); 29.39. found: C, 52.85; H, 3.27; X (I), 29.45.

2-[5-(4-Chloro-phenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]-4-iodo-naphthalen-1-ol. (2f)

UV/VIS (λ_{max}, nm): 409, 328. IR (KBr pellets):1590 (C=N), 1475, 1552 (C=C), 1232 (C-N) cm^{-1}. ^1H NMR (300 MH_Z, DMSO-d_6) δ 12.56 (s, 1H, OH), 7.92-7.39 (m, 9H, Ar-H), 6.90 (s, 1H, NH), 3.28 (dd, *J* = 5.1, 17.6 H_Z, 1H, H_A), δ 3.69 (dd, *J* = 12.1, 17.6 H_Z, 1H, H_B), δ 4.87 (dd, *J* = 5.1, 12.1 H_Z, 1H, H_X).^{13}CNMR (DMSO): 156.14 (C *of*Ar-OH), 152.67 (C *of* C=N), 138.41(Ar-C), 138.63 (Ar-C), 136.87 (Ar-C), 135.19 (Ar-C), 135.54 (Ar-C), 133.27 (Ar-C), 131.39 (Ar-C), 128.50 (Ar-C), 127.47 (Ar-C), 127.98 (Ar-C), 126.25 (Ar-C), 125.52 (Ar-C), 124.90 (Ar-C),122.69 (Ar-C) 109.83 (C *of*Ar-I),53.16 (C *of* CH), 44.65 (C *of* CH_2).MS (EI, *m/z* (%): 448 (M^+, 40 %). Anal.Cacld. for $C_{19}H_{14}N_2OICl$: C, 50.89; H, 3.12; X (I,Cl), 36.27. found: C, 50.82; H, 3.15; X (I,Cl), 36.30.

2-[5-(3-Bromo-4,5-dimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]-4-iodonaphthalen-1-ol. (2g)

UV/VIS (λ_{max}, nm): 410, 330. IR (KBr pellets):1592 (C=N), 1470, 1560 (C=C), 1234 (C-N) cm^{-1}.^{1}H NMR (300 MH_Z, DMSO-d_6) δ 12.48 (s, 1H, OH), 7.78-7.34 (m, 7H, Ar-H), 6.92 (s, 1H, NH) 3.32 (dd, *J* = 5.2, 17.5 H_Z, 1H, H_A), δ 3.70 (dd, *J* = 12.1, 17.5 H_Z, 1H, H_B), δ 4.88 (dd, *J* = 5.2, 12.1 H_Z, 1H, H_X), 3.88 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3).^{13}CNMR (DMSO): 154.88 (C *of*Ar-OH), 152.48 (C *of* C=N), 151.89 (C *of* Ar-OCH_3), 149.50 (C *of* Ar-OCH_3) 138.37 (Ar-C), 138.86 (Ar-C), 128.25 (Ar-C), 127.92 (Ar-C), 126.13 (Ar-C), 125.75 (Ar-C), 124.40 (Ar-C), 121.27 (Ar-C), 118.59 (Ar-C), 114.94 (Ar-C), 115.80 (Ar-C), 113.19 (Ar-C), 109.22 (C *of*Ar-Br), 57.66 (C *of* OCH_3), 56.40 (C *of* OCH_3), 53.29 (C *of* CH), 43.85 (C *of* CH_2).MS (EI, *m/z* (%): 553(M^+, 96 %). Anal.Cacld. for $C_{21}H_{18}O_3N_2IBr$: C, 45.56; H, 3.25; X(I, Br), 37.43. found: C, 45.64; H, 3.18; X (I,Br), 37.49.

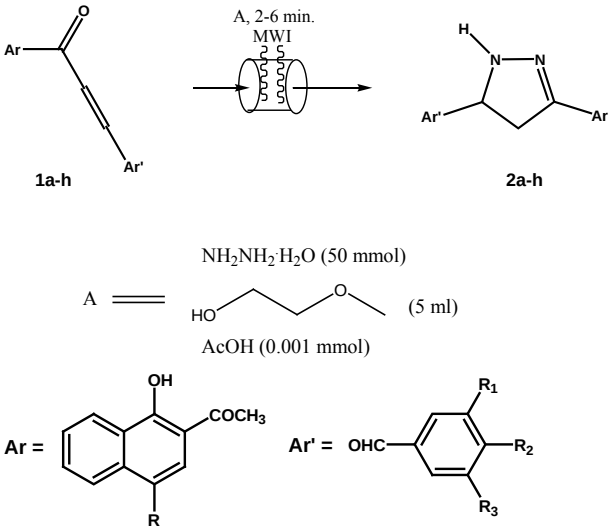
2-[5-(3,4,5-Trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]-naphthalen-1-ol. (2h)

UV/VIS (λ_{max}, nm): 413, 328. IR (KBr pellets): 1589 (C=N), 1472, 1542 (C=C), 1232 (C-N) cm^{-1}. ^1H NMR (300 MH_Z, DMSO-d_6) δ 12.40 (s, 1H, OH), 7.48-8.37 (m, 8H, Ar-H), 6.87 (s, 1H, NH), 3.28 (dd, *J* = 4.9, 17.5 H_Z, 1H, H_A), δ 3.72 (dd, *J* = 11.9, 17.5 H_Z, 1H, H_B), δ 4.89 (dd, *J* = 5.0, 11.9 H_Z, 1H, H_X), δ 3.86 (s, 3H, OCH_3), 3.72 (s, 6H, OCH_3). ^{13}CNMR (DMSO): 154.05 (C *of* Ar-OH), 153.28 (C *of* C=N), 148.67 (C *of* Ar-OCH_3), 148.42 (2C *of* Ar-OCH_3) 134.13 (Ar-C), 133.19 (Ar-C), 128.23 (Ar-C), 128.36 (Ar-C), 127.41 (Ar-C), 126.30 (Ar-C), 124.97 (Ar-C), 122.71 (Ar-C), 121.28 (Ar-C), 112.65 (Ar-C), 110.86 (Ar-C), 110.32 (Ar-C), 62.39 (3C *of* OCH_3), 56.36 (C *of* CH), 42.37 (C *of* CH_2). MS m/z: 378 (M^+, 80 %), 377, 361, 347, 287, 231, 211, 210, 181, 168, 140, 128, 115, 109, 95, 77, 65, 51, 40. Anal.Cacld. for $C_{22}H_{22}O_4N_2$: C, 69.84; H, 5.82. Found: C, 70.91; H, 5.96.

Results and discussion

Synthesis

In continuation of earlier research work on organic heterocyclic synthesis[21-23] and reported methods for compounds **2a-h** using various proceduure[24-26], we try to investigate herein a convenient route for synthesis of 2-pyrazolines using microwave irradiation. The reaction of 2'-hydroxychalcones with hydrazine in 2-methoxyethanol in presence of glacial acetic acid under MW irradiation gives 2-pyrazolines, Scheme-**1**.



Scheme 1. Synthesis of substituted 2-pyrazolines under microwave irradiation

The combination of 2-methoxyethanol with microwave technique found to be efficient process towards synthesis heterocyclic compounds [27,28]. Initially, we attempted the condensation of 3-(3-Bromo-4,5-dimethoxy-phenyl)-1-(4-bromo-1-hydroxy-naphthalen-2-yl)-propenone (10 mmol) with $NH_2NH_2{\cdot}H_2O$ (50 mmol) using 0.001 mmol of AcOH in 2-methoxyethanol as reaction solvent. The reaction went to completion within 2-3 minutes and corresponding product **2c** was obtained in 92% yield. In order to optimize the reaction conditions, we carried out the above reaction in different reaction medium such as ethanol, dichloromethane, dioxane, acetonitrile, DMF and 2-methoxyethanol, Table **2**. We found that use of 2-methoxyethanol as an efficient reaction medium in terms of clean reaction conditions, not expensive, higher yields of product and environmentally eco-friendly. As results observed from Table **2**, we pay our attention towards various substituted 2'-hydroxy chalcones. In all cases, reaction proceeded efficiently in high yields using 2-methoxyethanol.

Table 2. Optimization of solvent effect on model reaction.[a]

Entry	Solvent	Time,min	Yield, %
1	Ethanol (10 ml)	12	78
2	Dichloromethane (10 ml)	18	63
3	Dioxane (15 ml)	15	68
4	Acetonitrile (15 ml)	20	75
5	DMF (10 ml)	16	60
6	2-Methoxyethanol (5 ml)	2-3	92

[a]Reaction of 3-(3-Bromo-4,5-dimethoxyphenyl)-1-(4-bromo-1-hydroxynaphthalen-2-yl)propenone (10 mmol) with $NH_2NH_2{\cdot}H_2O$ (50 mmol) using 0.001 mmol of AcOH under microwave irradiation.

The structure characterization of compounds **2a-h**confirmed on the basis of spectral technique, UV, IR, ^{1}H NMR, MS, ^{13}C-NMR and elemental analysis. In IR spectral data ofcondensed products, compounds **2a-h** display disappearance of band at 1625-1635 cm^{-1} due to C=O of 2'-hydroxychalcone and appearance of a band near 1588 cm^{-1}due to C=N formed. The N-H stretching band of pyrazoline nucleus appears near 3320 cm^{-1}.

The ^{1}H NMR spectra showing an ABX pattern were observed for H_A, H_B, and H_X proton which appear as pair of doublets near δ 3.28, 3.67, and 4.90 ppm with J_{AB}=17.6 Hz, J_{Ax} = 5.0 Hz, J_{AB}= 12.1 Hz. The chemical shift for aromatic protons was observed at δ 7.25-8.20 ppm and that of N-H pyrazolo at d 6.90 ppm.^{13}C NMR of five membered pyrazolo nucleous reveals the presence of peak near 44 and 53 which assign for methylene and methyne carbon. The peak near 161, 155 and 153 reveals the presence OCH_3, C-OH and C=N functionality in the basic structure of pyrazolines respectively.

Antimicrobial activity

The antibacterial activities of the synthesized compounds (**2a-h**) were determined by agar well diffusion method.[27] 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.

The culture strains of bacteria were maintained on nutrient agar slant at 37±0.5 °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 105 CFU/mL dilutions. The wells of 6 mm diameter were filled with 0.1 mL of compound solution at fixed concentration 25 μ mL^{-1} 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 **3**.

Table 3. Antmicrobial data of 2-pyrazolines.

Com-pound	Zone of inhibition in mm and minimum inhibitory concentration, μg mL^{-1}			
	A	**B**	**C**	**D**
2a	23 (50)	14 (<100)	14 (100)	16 (>100)
2b	21 (50)	17(<100)	12 (<200)	10 (<200)
2c	24 (25)	28 (15.5)	25 (50)	26 (50)
2d	20 (<100)	21(100)	19 (<100)	19 (<100)
2e	23 (50)	22 (<50)	21 (<100)	24 (<50)
2f	26 (12.5)	21 (100)	10 (200)	16 (100)
2g	28 (10.5)	30 (<10)	26 (<50)	29 (10)
2h	25 (12.5)	26 (50)	24 (50)	27 (12.5)
Fluco-nazole	26 (12.5)	28 (10)	--	--
Strepto-mycin	--	--	30 (25)	28 (10.5)

A: *B. subtilis* B: *S. aureus* C: *A. flavus* D: *C. albicans*

For antifungal activity, all culture strains of fungi maintained on potato dextrose agar (PDA) slant at 27 ± 0.2 ^{0}C for 24-48 hr, 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 (106 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^{-1}. 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-**3**.

The minimum inhibitory concentration values were determined by comparison to standard drugs at 10, 12.5, 25, 50, 100 and 200 μ mL^{-1}. A lower MIC's values indicate that less drug is required for inhibiting growth of the organism, therefore, drug with lower MIC scores more effective antimicrobial agent.

Conclusion

In summary we developed new methodology towards the synthesis of substituted 2'-pyrazolines from 2'-hydroxy chalcones using 2-methoxyethanol in presence of slightly acidic medium. The combination of microwave with 2-methoxyethanol found to be excellent and convenient reaction route in terms of simple reaction procedure, quick reaction time giving percent yield of product. The preliminary *in vitro* antimicrobial screening of this series revealed that compounds **2c**, **2g** and **2h** showed potent activity when compared with standard drug. Therefore, the present study is useful drug in medicinal investigation against bacterial and fungal diseases.

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