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ARTICLE

Synthesis of Pyrazolines and Pyrazoles Incorporated with Pyran-2-one Moiety and their Antimicrobial Evaluation

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ABSTRACT

In the present paper, we report herein synthesis of 5-aryl-3-(4-chloro-6-methyl-2*H*-pyran-2-on-3-yl)-1-phenylpyrazoles. All the compounds were characterized by spectroscopic methods such as IR, NMR and analytic methods and evaluated for their antibacterial and antifungal activities. It is found that some of these compounds are potent antimicrobial agents.

KEYWORDS

Dehydroacetic acid, Pyran-2-one, Hypervalent iodine, Pyrazoline, Pyrazole.

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INTRODUCTION

Heterocyclic compounds have gained much importance in medicinal chemistry due to its presence in large number of pharmacologically active moieties. Among the five membered heterocycles containing two heteroatom in its ring structure, pyrazole is one of the most important one. Pyrazoline is dihydropyrazole, a five membered heterocyclic compound containing two nitrogen atoms in adjacent positions and possessing only one endocyclic double bond.

Pyrazolines are very much promising when the biological activities of pyrazolines are taken into consideration. Pyrazolines are known to possess antitubercular [1], anti HIV [2], antiviral [3], antimicrobial [4], cerebroprotective [5], molluscicidal [6], antifungal [7], antiinflammatory [8], analgesic [9], anticonvulsant [10], anticancer [11] and antioxidant [12] properties, *etc*. One of the important applications of pyrazoline is the use of pyrazolines as a fluorescent brightening agent [13]. Pyrazolines are also acting as holes transporting material in organic electroluminescent device (OELD).

Furthermore, the oxidative aromatization of 1,3,5-trisubstituted-2-pyrazolines to pyrazoles is of great biological importance due to diverse biological properties of pyrazoles such as analgesic, anti-inflammatory, antipyretic, antiarrhythmic, muscle relaxant, psychoanaleptic, antidiabetic and antibacterial activities [14,15]. This discussion prompted us to synthesize some new pyrazole derivatives as an urgent need, which can possess biological and medicinal importance.

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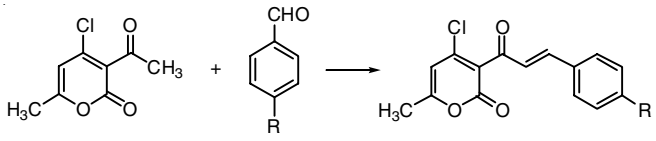
The present study is concerned with the synthesis of 4-chloro-3-cinnamoyl-6-methyl-2*H*-pyran-2-one and 5-aryl-3-(4-chloro-6-methyl-2*H*-pyran-2-on-3-yl)-1-phenylpyrazolines and the oxidation of pyrazoline to corresponding pyrazoles.

EXPERIMENTAL

Melting points were taken on slides in an electrical apparatus Labindia visual melting range apparatus and are uncorrected. The infrared spectra were recorded on a Perkin-Elmer 1800 FT-IR spectrophotometer. The ^{1}H NMR spectra were recorded in $CDCl_3$ on a Bruker Nuclear Magnetic Resonance (NMR) spectrophotometer at 300 MHz using tetramethylsilane (TMS) as an internal standard. Chemical shifts are expressed in ppm units (δ) downfield from TMS. The purity of compounds was checked by elemental analyses performed on Perkin-Elmer 2400 instrument. Mass spectra were recorded on 2500 ev (ESI source) using a water's Q-TOF micro instrument. All reagents were purchased from commercial sources and were used without purification.

4-Chloro-3-cinnamoyl-6-methyl-2*H*-pyran-2-one (1a): A solution of 4-chloro-dehydroacetic acid (2 mmol), 10 drops of piperidine and 2 mmol of aryl aldehyde in 25 mL of chloroform was refluxed for 8-10 h. The solution was concentrated over steam bath. Crystals of the product **1**, which separated on slow evaporation of the remaining chloroform were collected and recrystallized from ethanol (**Scheme-I**).



4-Chloro-DHA Aryl aldehyde **1**

Scheme-I

4-Chloro-3-cinnamoyl-6-methyl-2*H*-pyran-2-one (1a): m.p.: 130-132 °C; Yield: 78 %; IR (KBr, ν_{max}): 1725 cm^{-1} (C=O), 1636 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$, 300 MHz, δ): 2.39 (s, 3H, CH_3), 5.95 (s, 1H), 7.48-7.6 (m, 5H, Ar-H), 7.87 (d, 1H, *J* = 15.9 Hz, CH), 8.26 (d, 1H, *J* = 15.9 Hz, CH). Analysis, calculated for $C_{15}H_{11}O_3Cl$: C, 65.58; H, 4.04. Found: C, 65.50; H, 4.01.

4-Chloro-3-(4-methylcinnamoyl)-6-methyl-2*H*-pyran-2-one (1b): m.p.: 115-117 °C; Yield: 81 %; IR (KBr, ν_{max}): 1730 cm^{-1} (C=O), 1630 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$, 300 MHz, δ): 2.35, 2.46 (s, 6H, CH_3), 5.96 (s, 1H), 7.25 (d, 2H, *J* = 7.8 Hz, Ar-H), 7.59 (d, 2H, *J* = 7.8 Hz, Ar-H), 7.99 (d, 1H, *J* = 15.6 Hz, CH), 8.26 (d, 1H, *J* = 15.6 Hz, CH); Analysis, calculated for $C_{16}H_{13}O_3Cl$: C, 66.56; H, 4.54. Found: C, 66.50; H, 4.45.

4-Chloro-3-(4-fluorocinnamoyl)-6-methyl-2*H*-pyran-2-one (1c): m.p.: 175-177 °C; Yield: 77 %; IR (KBr, ν_{max}): 1728 cm^{-1} (C=O), 1632 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$, 300 MHz, δ): 2.42 (s, 3H, CH_3), 6.09 (s, 1H), 7.23 (d, 2H, *J* = 7.8 Hz, Ar-H), 7.60 (d, 2H, *J* = 7.5 Hz, Ar-H), 7.95 (d, 1H, *J* = 15.6 Hz, CH), 8.32 (d, 1H, *J* = 15.6 Hz, CH); Analysis, calculated for $C_{15}H_{10}O_3ClF$: C, 61.55; H, 3.44. Found: C, 61.49; H, 3.40.

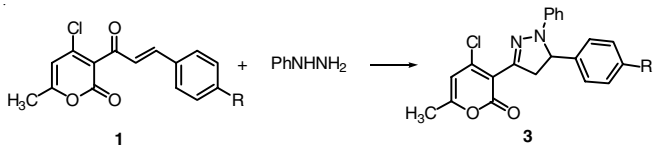
4-Chloro-3-(4-chlorocinnamoyl)-6-methyl-2*H*-pyran-2-one (1d): m.p.: 192-193 °C; Yield: 78 %; IR (KBr, ν_{max}): 1726 cm^{-1} (C=O), 1630 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$, 300 MHz, δ): 2.40 (s, 3H, CH_3), 5.96 (s, 1H), 7.25 (d, 2H, *J* = 7.5 Hz, Ar-H), 7.59 (d, 2H, *J* = 7.5 Hz, Ar-H), 7.94 (d, 1H, *J* = 15.6 Hz, CH), 8.26 (d, 1H, *J* = 15.6 Hz, CH); Analysis, calculated for $C_{15}H_{10}O_3Cl_2$: C, 58.28; H, 3.26. Found: C, 58.21; H, 3.22.

4-Chloro-3-(4-bromocinnamoyl)-6-methyl-2*H*-pyran-2-one (1e): m.p.: 183-186 °C; Yield: 80 %; IR (KBr, ν_{max}): 1725 cm^{-1} (C=O), 1631 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$, 300 MHz, δ): 2.40 (s, 3H, CH_3), 5.97 (s, 1H), 7.23 (d, 2H, *J* = 7.8 Hz, Ar-H), 7.59 (d, 2H, *J* = 7.8 Hz, Ar-H), 7.94 (d, 1H, *J* = 15.6 Hz, CH), 8.26 (d, 1H, *J* = 15.6 Hz, CH); Analysis, calculated for $C_{15}H_{10}O_3BrCl$: C, 50.95; H, 2.85. Found: C, 50.79; H, 2.82.

4-Chloro-3-(4-nitrocinnamoyl)-6-methyl-2*H*-pyran-2-one (1f): m.p.: 204-205 °C; Yield: 78 %; IR (KBr, ν_{max}): 1732 cm^{-1} (C=O), 1628 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$, 300 MHz, δ): 2.39 (s, 3H, CH_3), 6.02 (s, 1H), 7.82 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.89 (d, 2H, *J* = 8.4 Hz, Ar-H), 8.02 (d, 1H, *J* = 15.6 Hz, CH), 8.29 (d, 1H, *J* = 15.6 Hz, CH); Analysis, calculated for $C_{15}H_{10}NO_5Cl$: C, 56.35; H, 3.15. Found: C, 56.29; H, 3.13.

4-Chloro-3-(4-methoxycinnamoyl)-6-methyl-2*H*-pyran-2-one (1g): m.p.: 167-168 °C; Yield: 80 %; IR (KBr, ν_{max}): 1728 cm^{-1} (C=O str.), 1631 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$, 300 MHz, δ): 2.33 (s, 3H, CH_3), 3.88 (s, 3H, OCH_3), 5.96 (s, 1H), 6.96 (d, 2H, *J* = 8.7 Hz, Ar-H), 7.66 (d, 2H, *J* = 8.7 Hz, Ar-H), 8.01 (d, 1H, *J* = 15.9 Hz, CH), 8.20 (d, 1H, *J* = 15.9 Hz, CH); Analysis, calculated for $C_{16}H_{13}O_4Cl$: C, 63.06; H, 4.30. Found: C, 63.03; H, 4.27.

5-Phenyl-3-(4-chloro-6-methyl-2*H*-pyran-2-on-3-yl)-1-phenylpyrazoline (3): A mixture of 3-cinnamoyl-4-chloro-6-methyl-2-pyrone (**1**, 2 mmol) and phenylhydrazine (2 mmol) in ethanol (25 mL) containing 6-8 drops of acetic acid was heated under reflux for 2 h. Half of ethanol was distilled off under reduced pressure. After cooling the reaction mixture, the product separated was filtered, washed with little alcohol and crystallized to afford compound **3** (**Scheme-II**).



1 **3**

Scheme-II

5-Phenyl-3-(4-chloro-6-methyl-2*H*-pyran-2-on-3-yl)-1-phenylpyrazoline (3a): m.p.: 168-170 °C; Yield: 83 %; IR (KBr, ν_{max}): 1723 cm^{-1} (C=O str.); ^{1}H NMR ($CDCl_3$, 300 MHz, δ): 2.4 (s, 3H, CH_3), 3.38-3.40 (dd, 1H, C_4-H_A), 4.13-4.16 (dd, 1H, C_4-H_B), 5.13-5.15 (dd, 1H, C_3-H_A), 6.06 (s, 1H, C_5'-H, DHA), 6.9-7.5 (m, 10H, Ar); Analysis, calculated for $C_{21}H_{17}N_2O_2Cl$: C, 69.14; H, 4.70; N, 7.68. Found: C, 69.05; H, 4.65; N, 7.63.

5-(4-Methylphenyl)-3-(4-chloro-6-methyl-2*H*-pyran-2-on-3-yl)-1-phenylpyrazoline (3b): m.p.: 189-190 °C; Yield: 80 %; IR (KBr, ν_{max}): 1725 cm^{-1} (C=O str.); ^{1}H NMR ($CDCl_3$, 300 MHz, δ): 2.30 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 3.41-3.43 (dd, 1H, C_4-H_A), 4.14-4.17 (dd, 1H, C_4-H_B), 5.11-5.12 (dd, 1H, C_3-H_A), 6.07 (s, 1H, C_5'-H, DHA), 7.0-7.5 (m, 9H, Ar);

Analysis, calculated for $C_{22}H_{19}N_2O_2Cl$: C, 69.75; H, 5.05; N, 7.39. Found: C, 69.71; H, 4.99; N, 7.33.

5-(4-Fluorophenyl)-3-(4-chloro-6-methyl-2*H*-pyran-2-on-3-yl)-1-phenylpyrazoline (3c): m.p.: 202-203 °C; Yield: 81 %; IR (KBr, ν_{max}): 1728 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$, 300 MHz, δ): 2.43 (s, 3H, CH_3), 3.37-3.38 (dd, 1H, C_4-H_A), 4.11-4.13 (dd, 1H, C_4-H_B), 5.14-5.15 (dd, 1H, C_3-H_A), 6.02 (s, 1H, C_5'-H, DHA), 7.3-7.7 (m, 9H, Ar); Analysis, calculated for $C_{21}H_{16}N_2O_2ClF$: C, 65.89; H, 4.21; N, 7.32. Found: C, 65.82; H, 4.15; N, 7.23.

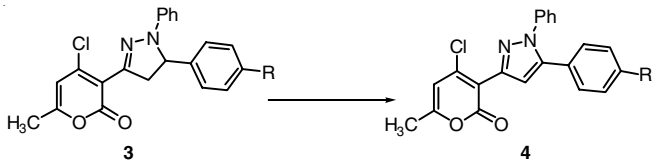
5-(4-Chlorophenyl)-3-(4-chloro-6-methyl-2*H*-pyran-2-on-3-yl)-1-phenylpyrazoline (3d): m.p.: 210-213 °C; Yield: 80 %; IR (KBr, ν_{max}): 1730 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$, 300 MHz, δ): 2.38 (s, 3H, CH_3), 3.40-3.41 (dd, 1H, C_4-H_A), 4.14-4.16 (dd, 1H, C_4-H_B), 5.13-5.15 (dd, 1H, C_3-H_A), 6.05 (s, 1H, C_5'-H, DHA), 7.2-7.7 (m, 9H, Ar); Analysis, calculated for $C_{21}H_{16}N_2O_2Cl_2$: C, 63.17; H, 4.04; N, 7.02. Found: C, 63.15; H, 4.01; N, 7.01.

5-(4-Bromophenyl)-3-(4-chloro-6-methyl-2*H*-pyran-2-on-3-yl)-1-phenylpyrazoline (3e): m.p.: 159-162 °C; Yield: 81 %; IR (KBr, ν_{max}): 1725 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$, 300 MHz, δ): 2.35 (s, 3H, CH_3), 3.36-3.39 (dd, 1H, C_4-H_A), 4.10-4.13 (dd, 1H, C_4-H_B), 5.14-5.15 (dd, 1H, C_3-H_A), 6.01 (s, 1H, C_5'-H, DHA), 7.0-7.4 (m, 9H, Ar); Analysis, calculated for $C_{21}H_{16}N_2O_2BrCl$: C, 56.84; H, 3.63; N, 6.31. Found: C, 56.75; H, 3.59; N, 6.29.

5-(4-Nitrophenyl)-3-(4-chloro-6-methyl-2*H*-pyran-2-on-3-yl)-1-phenylpyrazoline (3f): m.p.: 218-219 °C; Yield: 78 %; IR (KBr, ν_{max}): 1735 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$, 300 MHz, δ): 2.42 (s, 3H, CH_3), 3.39-3.40 (dd, 1H, C_4-H_A), 4.16-4.17 (dd, 1H, C_4-H_B), 5.13-5.15 (dd, 1H, C_3-H_A), 6.08 (s, 1H, C_5'-H, DHA), 7.2-7.6 (m, 9H, Ar); Analysis, calculated for $C_{21}H_{16}N_3O_4Cl$: C, 61.54; H, 3.94; N, 10.25. Found: C, 61.51; H, 3.87; N, 10.21.

5-(4-Methoxyphenyl)-3-(4-chloro-6-methyl-2*H*-pyran-2-on-3-yl)-1-phenylpyrazoline (3g): m.p.: 158-159 °C; Yield: 82 %; IR (KBr, ν_{max}): 1723 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$, 300 MHz, δ): 2.31 (s, 3H, CH_3), 3.36-3.38 (dd, 1H, C_4-H_A), 3.80 (s,3H, OCH_3), 4.10-4.11 (dd, 1H, C_4-H_B), 5.12-5.14 (dd, 1H, C_3-H_A), 5.99 (s, 1H, C_5'-H, DHA), 6.8-7.3 (m, 9H, Ar); Analysis, calculated for $C_{22}H_{19}N_2O_3Cl$: C, 66.92; H, 4.85; N, 7.09. Found: C, 66.87; H, 4.80; N, 7.03.

5-Aryl-3-(4-chloro-6-methyl-2*H*-pyran-2-on-3-yl)-1-phenylpyrazoles: To a stirred solution of **3** (10 mmol) in dichloromethane (15 mL) was added iodobenzene diacetate (12 mmol) at room temperature. The reaction mixture was stirred for 5 h. Dichloromethane was distilled off on a steam bath and the residual gummy mass was triturated with pet-ether to remove iodobenzene and was recrystallized from alcohol to afford the title compound **4** in excellent yield (**Scheme-III**).



Scheme-III

5-Phenyl-3-(4-chloro-6-methyl-2*H*-pyran-2-on-3-yl)-1-phenylpyrazole (4a): m.p.: 130-132 °C; Yield: 85 %; IR (KBr, ν_{max}): 1728 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$, 300 MHz, δ): 2.40 (s, 3H, CH_3), 6.01 (s, 1H, C_5'-H, DHA), 7.2 (s, 1H, C_4-H), 7.1-7.6 (m, 10H, Ar); Analysis, calculated for $C_{21}H_{15}N_2O_2Cl$: C, 69.52; H, 4.17; N, 7.72. Found: C, 69.49; H, 4.11; N, 7.70.

5-(4-Methylphenyl)-3-(4-chloro-6-methyl-2*H*-pyran-2-on-3-yl)-1-phenylpyrazole (4b): m.p.: 121-122 °C; Yield: 83 %; IR (KBr, ν_{max}): 1726 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$, 300 MHz, δ): 2.32 (s, 3H, CH_3), 2.40 (s, 3H, CH_3) 6.03 (s, 1H, C_5'-H, DHA), 7.1 (s, 1H, C_4-H), 7.0-7.5 (m, 9H, Ar); Analysis, calculated for $C_{22}H_{17}N_2O_2Cl$: C, 70.12; H, 4.55; N, 7.43. Found: C, 70.09; H, 4.49; N, 7.40.

5-(4-Fluorophenyl)-3-(4-chloro-6-methyl-2*H*-pyran-2-on-3-yl)-1-phenylpyrazole (4c): m.p.: 155-157 °C; Yield: 80 %; IR (KBr, ν_{max}): 1730 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$, 300 MHz, δ): 2.42 (s, 3H, CH_3), 6.06 (s, 1H, C_5'-H, DHA), 7.3 (s, 1H, C_4-H), 7.1-7.6 (m, 9H, Ar); Analysis, calculated for $C_{21}H_{14}N_2O_2ClF$: C, 66.24; H, 3.71; N, 7.36. Found: C, 66.19; H, 3.68; N, 7.31.

5-(4-Chlorophenyl)-3-(4-chloro-6-methyl-2*H*-pyran-2-on-3-yl)-1-phenylpyrazole (4d): m.p.: 198-202 °C; Yield: 82 %; IR (KBr, ν_{max}): 1723 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$, 300 MHz, δ): 2.45 (s, 3H, CH_3), 6.08 (s, 1H, C_5'-H, DHA), 7.3 (s, 1H, C_4-H), 7.1-7.5 (m, 9H, Ar); Analysis, calculated for $C_{21}H_{14}N_2O_2Cl_2$: C, 63.49; H, 3.55; N, 7.05. Found: C, 63.45; H, 3.52; N, 7.01.

5-(4-Bromophenyl)-3-(4-chloro-6-methyl-2*H*-pyran-2-on-3-yl)-1-phenylpyrazole (4e): m.p.: 105-109 °C; Yield: 81 %; IR (KBr, ν_{max}): 1723 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$, 300 MHz, δ): 2.42 (s, 3H, CH_3), 6.01 (s, 1H, C_5'-H, DHA), 7.3 (s, 1H, C_4-H), 7.0-7.4 (m, 9H, Ar); Analysis, calculated for $C_{21}H_{15}N_2O_2BrCl$: C, 57.10; H, 3.19; N, 6.34. Found: C, 57.09; H, 3.11; N, 6.29.

5-(4-Nitrophenyl)-3-(4-chloro-6-methyl-2*H*-pyran-2-on-3-yl)-1-phenylpyrazole (4f): m.p.: 140-141 °C; Yield: 81 %; IR (KBr, ν_{max}): 1732 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$, 300 MHz, δ): 2.44 (s, 3H, CH_3), 6.05 (s, 1H, C_5'-H, DHA), 7.3 (s, 1H, C_4-H), 7.1-7.7 (m, 9H, Ar); Analysis, calculated for $C_{21}H_{14}N_3O_4Cl$: C, 61.85; H, 3.46; N, 10.30. Found: C, 61.79; H, 3.43; N, 10.25.

5-(4-Methoxyphenyl)-3-(4-chloro-6-methyl-2*H*-pyran-2-on-3-yl)-1-phenylpyrazole (4g): m.p.: 127-129 °C; Yield: 82 %; IR (KBr, ν_{max}): 1720 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$, 300 MHz, δ): 2.39 (s, 3H, CH_3), 3.88 (s, 3H, OCH_3), 6.02 (s, 1H, C_5'-H, DHA), 7.1 (s, 1H, C_4-H), 7.0-7.5 (m, 9H, Ar); Analysis, calculated for $C_{22}H_{17}N_2O_3Cl$: C, 67.26; H, 4.36; N, 7.13. Found: C, 67.19; H, 4.31; N, 7.07.

Biological assay

Total six microorganism strains were selected on the basis of their clinical importance in causing disease in humans. Two Gram-positive bacteria namely, *Staphylococcus aureus* MTCC 96 and *Bacillus subtilis* MTCC 121; two Gram-negative bacteria namely, *Escherichia coli* MTCC 1652 and *Pseudomonas aeruginosa* MTCC 741 and two fungi, *Aspergillus niger* and *Aspergillus flavus*, the ear pathogens isolated from the patients of Kurukshetra [16], were used in the present study

for evaluation of antimicrobial activity of the chemically synthesized compounds.

Medium: All the cultures were procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India. The bacteria were sub cultured on nutrient agar and incubated aerobically at 37 °C.

***in vitro* Antibacterial assay:** The antibacterial activity of the compounds was evaluated by the agar well diffusion method. All the cultures were adjusted to 0.5 McFarland standard, which is visually comparable to microbial suspension of about 1.5×10^8 cfu/mL. Muller Hinton agar medium (20 mL) was poured into each petri plate and the agar plates were swabbed with 100 µL inocula of each test bacterium and kept for 15 min for adsorption. Using sterile cork borer of 8 mm diameter, wells were bored into the seeded agar plates and these were loaded with a 100 µL volume with concentration of 2 mg/mL of each compound reconstituted in the dimethylsulphoxide (DMSO). All the plates were incubated at 37 °C for 24 h. Antibacterial activity of each compound was evaluated by measuring the zone of growth inhibition against the test organisms with zone reader (Hi antibiotic zone scale). Dimethyl sulfoxide was used as a negative control whereas ciprofloxacin was used as a positive control. The experiments were performed in triplicates and the mean values of the diameter of inhibition zones [17,18].

Determination of minimum inhibitory concentration: Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation. MIC of the various compounds against bacterial strains was tested through a macrodilution tube method as recommended by NCCLS [19]. In this method, various test concentrations of the synthesized compounds were made from 256 to 0.5 µg/mL in sterile tubes no. 1-10. Sterile Muller Hinton Broth (100 µL) was poured in each sterile tube followed by addition of 200 µL test compound in tube 1. Two fold serial dilution was carried out from the tube 1 to the tube 10 and excess broth (100 µL) was discarded from the last tube no. 10. To each tube, 100 µL of standard inoculums (1.5×10^8 cfu/mL) was added. Ciprofloxacin was used as control. Turbidity was observed after incubating the inoculated tubes at 37 °C for 24 h.

***in vitro* Antifungal assay:** The antifungal activity of the compounds was evaluated by poisoned food technique. The moulds were grown on sabouraud dextrose sugar (SDA) at 25 °C for 7 days and used as inocula. About 15 mL of molten sabouraud dextrose sugar (45 °C) was poisoned by the addition of 100 µL volume of each compound having concentration of 4 mg/mL, reconstituted in the DMSO, poured into a sterile petri plate and allowed to solidify at room temperature. The solidified poisoned agar plates were inoculated at the centre with fungal plugs (8 mm diameter), obtained from the actively growing colony and incubated at 25 °C for 7 days. Dimethyl sulfoxide was used as negative control whereas fluconazole as positive control. The experiments were performed in triplicates. Diameter of fungal colonies was measured and expressed as percent mycelia inhibition determined by applying the formula given by Al-Burtamani *et al.* [20].

$$\text{Inhibition of myelial growth } (\%) = \frac{(dc - dt)}{dc} \times 100$$

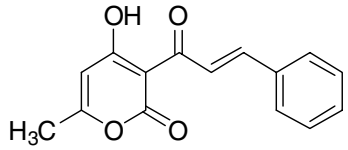
where, dc = average diameter of fungal colony in negative control plates, dt = average diameter of fungal colony in experimental plates.

***in vitro* Antibacterial activity:** All the synthesized compounds 5-aryl-3-(4-chloro-6-methyl-2*H*-pyran-2-on-3-yl)-1-phenylpyrazoles were screened for their *in vitro* antibacterial activity against two Gram-positive bacteria namely, *Staphylococcus aureus* and *Bacillus subtilis* and two Gram-negative bacteria namely, *Escherichia coli* and *Pseudomonas aeruginosa.* Standard antibiotics namely, ciprofloxacin, was used for comparison of antibacterial activity shown by compounds **4a-g**. The results were recorded for each tested compounds as average diameter of zone of inhibition of bacterial growth adjoining the well in millimetres. Minimum inhibitory concentration (MIC) measurements were performed using a modified agar well diffusion method. MIC of those compounds was determined which were showing activity in primary screening.

***in vitro* Antifungal activity:** All compounds tested for their *in vitro* antibacterial activity are also tested for their *in vitro* antifungal activity against two fungal strains, namely, *Aspergillus flavus* and *Aspergillus niger* through poisoned food method. Standard drug fluconazole was used for comparison with antifungal activity shown by the compounds **4a-g** and the results were recorded as percentage (%) of mycelial growth inhibition.

RESULTS AND DISCUSSION

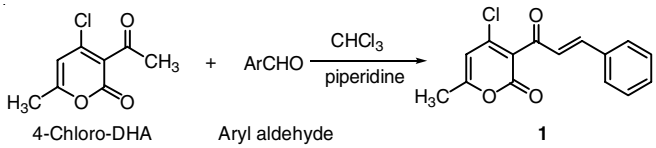
Pyrazoles and their derivatives are well recognized as an important class of heterocyclic compounds that have been found with extensive use in the pharmaceutical, material and agrochemical industries [21]. Compounds containing pyrazole moiety have exhibited diverse biological activities such as analgesic, antipyretic, anti-inflammatory, germicidal and antifungal [22,23]. Because of their diverse bioactivities, pyrazoles have received considerable attention of chemists. Efforts are continuously being made to evolve newer and facile procedures for the synthesis of this class of compounds. It is clear from the preceding description that a number of methods are available for the synthesis of pyrazole derivatives, one approach of Fischer and Knoevenagel which involves condensation reaction of α,β-unsaturated carbonyl compounds (chalcones) with hydrazine is most common approach. However, this strategy results in the formation of pyrazolines that need to be further oxidized to corresponding pyrazoles. Earlier, our research group have worked on chalcones of dehydroacetic acid (DHA) for the synthesis of pyrazoles [24].



Chalcone of dehydroacetic acid

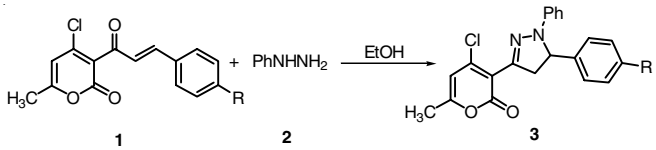
In the light of promising results obtained in that case and our ongoing interest in the chemistry of dehydroacetic acid and its derivatives, we decided to extend this synthetic approach with 4-chloro dehydroacetic acid (4-Cl DHA). In the present

investigation, 3-cinnamoyl derivative of 4-chloro dehydroacetic acid (**1**) have been taken as precursors for synthesis of pyrazoles. These were synthesized by condensation reaction of 4-chloro dehydroacetic acid with various aromatic aldehydes on refluxing in chloroform with few drops of piperidine (**Scheme-IV**).



Scheme-IV: Chalcone from DHA

The IR and ^{1}H NMR data of the compound was quite useful to arrive at its structure. In the ^{1}H NMR spectrum of chalcone, two doublets at 7.87 (H-α, *J* = 15.9 Hz) and 8.26 (H-β, *J* = 15.9 Hz) suggested the presence of olefinic protons at α,β-positions to carbonyl group. Accordingly, the pyrazoline derivatives (**3a-g**) were prepared by the reaction between equimolar quantity of chalcones **1** and phenylhydrazine in ethanol (**Scheme-V**) and characterized by spectral and analytical data. The methylene proton of pyrazoline ring appeared as two doublets one at 3.38-3.40 (C_4-H_A) and other at 4.13-4.16 (C_4-H_B). The appearance of these two doublets clearly revealed the magnetic non-equivalence of the two protons of CH_2 group adjacent to a chiral center.

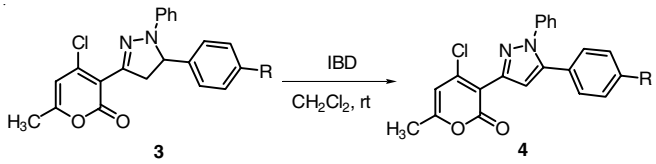


Scheme-V: Pyrazoline from DHA Chalcone

Our next attempt was to oxidize pyrazoline to pyrazoles. In this regard, a variety of oxidizing agents such as $Zr(NO_3)_4$ [25], Pd/C [26], Co(II) and oxygen [27], MnO_2 [28], lead tetraacetate, I_2-DMSO [29] have been reported. However, most of these reagents present several disadvantages including long reaction times, high temperature, unavailability of the reagents, toxicity because of the presence of certain toxic elements in these reagents, difficult work up and unsatisfactory yields of the products. To overcome these drawbacks, there is a need to search for new high-yielding, environmentally safe and cheaply available reagents for conversion of 2-pyrazolines to pyrazoles.

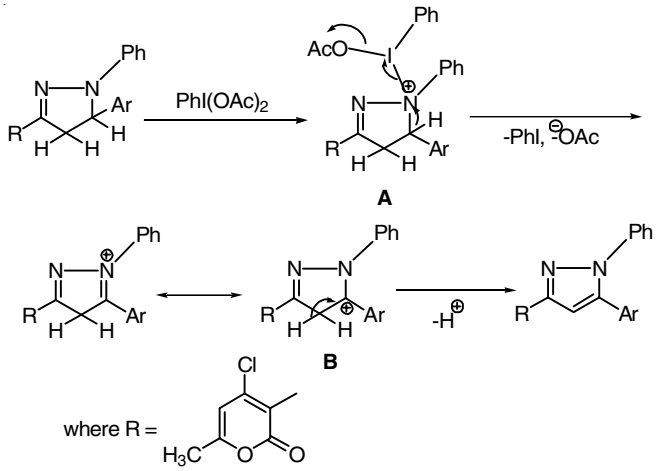
In continuation of ongoing research on oxidation of 1,3,5-trisubstituted 2-pyrazolines, we reported that iodobenzene diacetate works very smoothly for oxidative dehydrogenation of substituted pyrazoline derivatives in excellent yield. Therefore, we prepared iodobenzene diacetate to be used for oxidation of pyrazolines. Accordingly, we attempted the oxidation of 5-aryl-3-(4-chloro-6-methyl-2*H*-pyran-2-on-3-yl)-1-phenylpyrazolines (**3a-g**) using 1.1 eq of IBD in CH_2Cl_2. The reaction occurred at room temperature smoothly to afford the corresponding 5-aryl-3-(4-chloro-6-methyl-2*H*-pyran-2-on-3-yl)-1-phenylpyrazoles (**4a-g**) in good yields (**Scheme-VII**).

The confirmation of the occurrence of this transformation *i.e.*, formation of desired **4** was made by a careful comparison of ^{1}H NMR spectra. The signals around δ 3.38-3.40 (dd, 1H, C_4-H_a of pyrazoline), 4.13-4.16 (dd, 1H, C_4-H_b of pyrazoline), 5.13-5.15 (dd, 1H, C_3-H of pyrazoline) were missing from new spectra. Instead, a characteristic singlet at δ 7.2 due to C_4 of pyrazolyl proton appeared in the spectra.



Scheme-VII: Oxidation of pyrazoline

It is clear from the results that the use of IBD not only gives a clean product, but also the reaction conditions are very mild and simple. A plausible mechanistic course for this transformation has been depicted below. The reaction may be initiated by the preferential electrophilic attack of IBD on sp^3 hybridized nitrogen resulting in the formation of intermediate **A**. Cleavage of the weak N-I bond in **A** is facilitated by the loss of C_5-H as a proton, giving rise to the resonance stabilized cation **B**. Finally, proton loss from **B** gives the product (**Scheme-VII**).



Scheme-VII: Mechanism of oxidation

***in vitro* Antibacterial activity:** From the results, it has been observed that in general, all the tested compounds possess moderate to good antibacterial activity against Gram-positive (*S. aureus* and *B. subtilis*) bacteria and Gram-negative (*E. coli* and *P. aeruginosa*) bacteria. On the basis of zone of inhibition against test bacterium, five compounds **4c, 4d, 4e, 4f** and **4g** showed excellent activity having maximum zone of inhibition > 18.0 mm as compared with standard drug ciprofloxacin which showed the zone of inhibition of 26.6 mm against *S. aureus*.

Similarly, on the basis of zone of inhibition three compounds **4c**, **4d** and **4g** were found to be most effective against *B. subtilis* showing maximum zone of inhibition >18.0 mm as compared to standard drug ciprofloxacin which showed the zone of inhibition of 24.0 mm against *B. subtilis* (Table-1). Out of these active compounds, three compounds **4c**, **4d**, **4g** are in common which showed activity against both the Gram-positive bacteria. However, in case of the Gram-negative bacteria some of the compounds showed moderate to good antibacterial activity against the test bacteria *E. coli* and *P. aeruginosa* as shown in Table-1.

TABLE-1
in vitro ANTIBACTERIAL ACTIVITY OF COMPOUNDS **4a-g** THROUGH AGAR WELL DIFFUSION METHOD

Compounds	Diameter of growth of inhibition zone (mm)[a]			
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*
4a	16.6	17.3	–	–
4b	15.0	15.6	–	–
4c	18.6	19.3	13.2	–
4d	18.6	19.3	14.1	–
4e	18.6	17.6	–	–
4f	19.0	17.0	13.9	13.2
4g	19.6	18.6	–	13.6
Ciprofloxacin	26.6	24.0	25.0	22.0

– No activity, [a]Values, including diameter of the well (8 mm), are means of three replicates.

In whole series, MIC of the synthesized compounds ranged between 32 and 256 µg/mL against the Gram-positive bacteria (Table-2). In case of *S. aureus*, compounds **4g** is the most potent member having MIC 32 µg/mL in comparison to standard drug having MIC of 5 µg/mL. Other compounds showing good activity are **4d** and **4f** having MIC of 64 µg/mL. In case of *B. subtilis*, most potent members having MIC 32 µg/mL are **4c** and **4g**. Other compound shows moderate activity having MIC ranged between 128 and 256 µg/mL. All other compounds showed reasonable activity against Gram-positive bacteria. In case of Gram-negative bacteria, MIC ranged between 128 and 256 µg/mL (Table-2).

TABLE-2
MINIMUM INHIBITORY CONCENTRATION (MIC) (µg/mL) OF COMPOUNDS **4a-g** BY USING MACRO DILUTION METHOD

Compounds	Minimum inhibitory concentration (µg/mL)			
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*
4a	128	64	-	-
4b	128	128	-	-
4c	128	32	-	128
4d	64	64	>256	-
4e	128	64	256	-
4f	64	128	-	256
4g	32	32	-	128
Ciprofloxacin	5	5	5	5

***in vitro* Antifungal activity:** Table-3 revealed that all the compounds showed variable antifungal activity against two pathogens. From the careful comparison of the results, it has been suggested that mainly the three compounds **4d**, **4f** and **4g** showed good antifungal activity with > 55 % inhibition of mycelial growth against both fungi in comparison with standard drug (*A. niger* 81.1 % and *A. flavus* 77.7 %). All other compounds showed fair activity against fungal pathogens.

TABLE-3
in vitro ANTIFUNGAL ACTIVITY OF COMPOUNDS **4a-g** THROUGH POISONED FOOD METHOD

Compounds	Mycelial growth inhibition (%)	
	Aspergillus flavus	*Aspergillius niger*
4a	52.5	56.6
4b	53.3	50.0
4c	50.0	51.1
4d	55.5	55.5
4e	53.3	54.4
4f	58.8	55.5
4g	58.8	56.6
Fluconazole	77.7	81.1

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