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Synthesis of Novel and Potential Antimicrobial, Antioxidant and Anticancer Chalcones and Dihydropyrazoles Bearing Isoxazole Scaffold †

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Abstract: A series of isoxazole based (*E*)-1-(isoxazole-5-yl)-3-(substituted phenyl)-prop-2-en-1-ones (chalcones, **3a-3o**) and 3-(isoxazol-5-yl)-5-(substituted phenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide (dihydropyrazoles, **4a-4o**) were synthesized, characterized and evaluated for their antimicrobial, antioxidant and anticancer properties. Chalcones exhibited excellent antibacterial and antioxidant activities whereas the dihydropyrazoles shown superior antifungal and anticancer activities. The compound **3l** containing 3,4,5-trimethoxy phenyl ring showed the potent antibacterial activity (MIC = 1 µg/mL) as well as the antioxidant activity (IC_{50} = 5 µg/mL) whereas the dihydropyrazole, **4o** (MIC = 0.5 µg/mL) bearing the 2-chloro-3,4-dimethoxyphenyl was the potent antifungal compound identified. The dihydropyrazoles **4n** and **4h** possessing 2-fluoro-3,4-dimethoxyphenyl and 3,4-dimethoxyphenyl substituents exhibited potent anticancer activity against prostate cancer cell line (DU-145) with MIC 2 and 4 µg/mL respectively. The structure activity relationships had shown that there is a marked influence of both electron withdrawing halogens and electron releasing methoxyl groups on the above biological activities. All the compounds were evaluated for toxicity on normal human cell lines (LO2) and found to be non-toxic. These studies could help to synthesize, explore and identify new isoxazole containing leads for antimicrobial, antioxidant and anticancer properties.

Keywords: isoxazole; chalcones; dihydropyrazole; antibacterial activity; antifungal activity; anticancer activity; structure activity relationships

1. Introduction

Heterocyclic compounds constitute the most important class of organic compounds present in nature or synthesized in the laboratory. These compounds enjoy an array of biological activities and are employed in the treatment of a variety of ailments. Literature survey revealed that chalcones and isoxazoles and dihydropyrazoles possess a broad spectrum of biological activities like antimicrobial, anticancer, antimalarial, antidepressant, antihistaminic, antitubercular and anti-inflammatory [1–31].

Chalcones are a type of open chain flavonoids bearing two aryl rings connected through a three carbon spacer, the propenone linkage. The reactive α, β-unsaturated propenone fragment is not only responsible for the bioactivities but is also useful for the conversion of chalcones to different classes of heterocyclic compounds. There is a difference in the intensity of the bioactivities shown by chalcones due to variation in the nature and type of aryl rings. It was observed with many chalcones that the compounds containing heteroaromatic rings in their aryl portion showed excellent biological profiles.

Isoxazole is a five membered heterocyclic ring present in the drugs used in the therapy including valdecoxib (anti-inflammatory), sulfisoxazole and sulfamethoxazole (antibacterials), drazoxolon (antifungal), cloxacillin and dicloxacillin (*β*-lactam antibiotics) and leflunomide (immunosuppressive disease-modifying antirheumatic drug (DMARD)). The completely reduced form of isoxazole i.e., isoxazolidine scaffold is found in the antitubercular antibiotic cycloserine. Dihydropyrazole is another interesting heterocyclic compound with two nitrogen atoms present in the adjacent positions of a five membered ring. This ring can be conveniently synthesized from *α*, *β*-unsaturated carbonyl compounds by reacting with different kinds of hydrazines. The dihydropyrazole scaffold constitutes the part of drugs including diuretic-Muzolimine; analgesic-Antipyrine, Aminopyrine, Ramifenazone and Dipyrone; anti-obesity cannabinoid receptor type 1 (CB_1) antagonist-Ibipinabant (Figure 1).

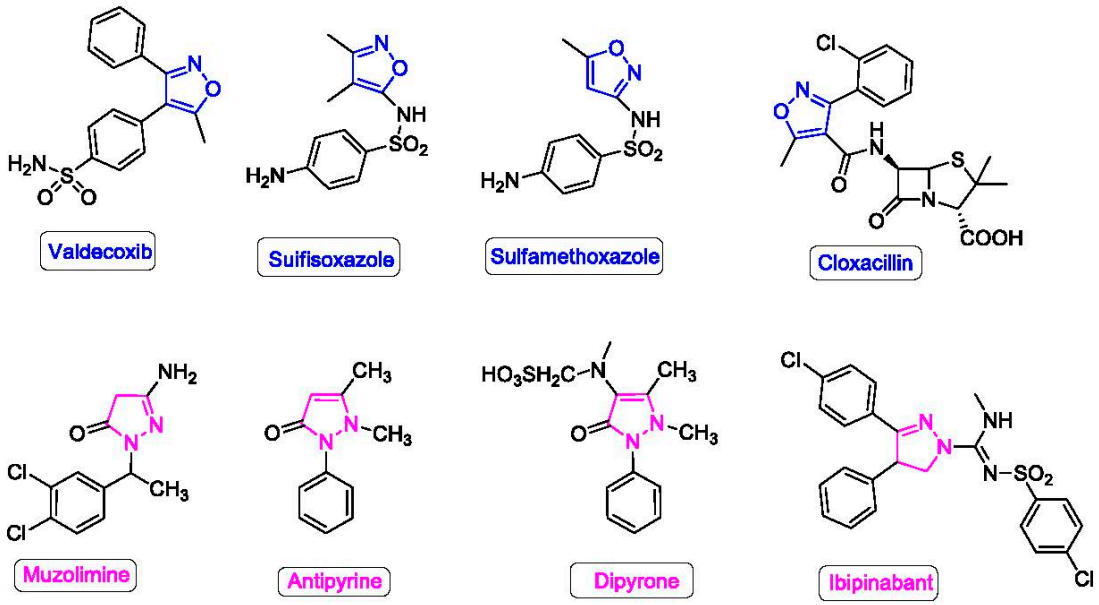

Figure 1. Structures of some clinically useful drugs containing isoxazole and dihydropyrazole rings.

In view of the above facts, we planned to prepare isoxazole containing chalcones and their dihydropyrazole derivatives to study the effect of these privileged heterocyclic compounds for their antibacterial, antifungal and anticancer activities.

2. Materials and Methods

2.1. Chemicals and Instruments

The chemicals, solvents and reagents were purchased from commercial sources and were used without further purification. Thin Layer Chromatography (TLC) was carried on precoated silica gel 60 F_{254} plates to monitor the reactions as well as to check the purity of the final products. The spots were visualized by UV lamp (254 nm). Chalcones were purified by recrystallization whereas the dihydropyrazoles by column chromatography employing silica gel (200 to 300 mesh) as stationary phase. Melting points were determined on Boitus melting point apparatus in open capillary tubes and were uncorrected. FT-IR spectra were scanned as KBR discs on Bruker Vertex 80v spectrometer whereas the ^1H and ^{13}C NMR spectrum were recorded Bruker AMX 400 MHz NMR spectrophotometer by dissolving the compounds in $CDCl_3$. TMS was used as internal standard for the NMR experiments and the chemical shift values were articulated in parts per million (ppm) relative to TMS. Mass spectra (MS) were obtained on Agilent LC-MS spectrometer by positive electron spray ionization method. All the spectral data was obtained from the facilities available with University College of Pharmaceutical Sciences, Andhra University, India. Elemental analyses was performed on Carlo Erba 1108 elemental analyzer

2.2. Synthesis

General Procedure for the Synthesis of Chalcones (**3a-3o**) and Dihydropyrazoles (**4a-4o**)

1 mmol of 1-(isoxazole-5-yl)ethanone and 1 mmol of appropriate aromatic aldehyde were dissolved in 7.5 mL of ethanol. To this solution, 10% aq.KOH (7.5 mL) was added and the reaction mixture was left for 24 h at room temperature. The reaction mixture after the completion of the reaction was charged into crushed ice which resulted in the separation of precipitate of the compound which was further filtered and washed thoroughly was distilled water and dried. The crude precipitate was purified with chloroform by recrystallization techniques. 1 mmol of (*E*)-1-(isoxazole-5-yl)-3-(substituted aryl)prop-2-en-1-one derivatives were dissolved in 7.5 mL of ethanol. Catalytic amount of pyridine and 1.5 mmol of semicarbazide was added to the above solution and allowed for reflux for 6–8 h. The crude product obtained was poured into crushed ice which resulted in the formation of the impure precipitate [9]. This was further charged into column and the pure compound was isolated (Scheme 1).

(E)-1-(isoxazole-5-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (**3a**): Yield 89%; m.p. 186–188 °C; IR (KBr, cm^{-1}): 1652 (C=O), 1601 (C=C of Ar), 1511 (CH=CH), 1611 (C=N), 1128 (-OCH_3), 3058 (Ar C-H); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 3.74 (3H, s, Ar-OCH_3), 7.22 (1H, d, *J* = 17 Hz, -CO-CH=), 7.73 (1H, d, *J* =17 Hz, =CH-Ar), 6.01–8.22 (6H, Ar-H); Anal. Calcd for: $C_{13}H_{11}NO_3$: C, 68.11; H, 4.84; N, 6.11; Found: C, 68.21; H, 4.86; N, 6.26.

(E)-1-(isoxazole-5-yl)-3-(3-methoxyphenyl)prop-2-en-1-one (**3b**): Yield 81%; m.p. 166–168 °C; IR (KBr, cm^{-1}): 1648 (C=O), 1600 (C=C of Ar), 1515 (CH=CH), 1618 (C=N), 1121 (–OCH_3), 3001 (Ar C-H); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 3.80 (3H, s, Ar-OCH_3), 7.18 (1H, d, *J* = 17 Hz, -CO-CH=), 7.68 (1H, d, *J* =17 Hz, =CH-Ar), 6.10–8.15 (6H, Ar-H); Anal. Calcd for: $C_{13}H_{11}NO_3$: C, 68.11; H, 4.84; N, 6.11; Found: C, 68.21; H, 4.86; N, 6.26.

(E)-1-(isoxazole-5-yl)-3-(2-methoxyphenyl)prop-2-en-1-one (**3c**): Yield 86%; m.p. 177–179 °C; IR (KBr, cm^{-1}): 1655 (C=O), 1606 (C=C of Ar), 1512 (CH=CH), 1609 (C=N), 1124 (–OCH_3), 3021 (Ar C-H); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 3.68 (3H, s, Ar-OCH_3), 7.23 (1H, d, *J* = 17 Hz, –CO-CH=), 7.70 (1H, d, *J* =17 Hz, =CH-Ar), 6.02–8.19 (6H, Ar-H); Anal. Calcd for: $C_{13}H_{11}NO_3$: C, 68.11; H, 4.84; N, 6.11; Found: C, 68.21; H, 4.86; N, 6.26.

(E)-1-(isoxazole -5-yl)-3-(2,3-dimethoxyphenyl)prop-2-en-1-one (**3d**): Yield 74%; m.p. 201–203 °C; IR (KBr, cm^{-1}): 1650 (C=O), 1608 (C=C of Ar), 1515 (CH=CH), 1611 (C=N), 1128 (-OCH_3), 3068 (Ar C-H); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 3.68 (3H, s, Ar-OCH_3), 3.70 (3H, s, Ar-OCH_3), 7.25 (1H, d, *J* = 17 Hz, -CO-CH=), 7.72 (1H, d, *J* =17 Hz, =CH-Ar), 6.02–8.19 (5H, Ar-H); Anal. Calcd for: $C_{14}H_{13}NO_4$: C, 64.86; H, 5.05; N, 5.40; Found: C, 64.95; H, 5.22; N, 5.49.

(E)-1-(isoxazole-5-yl)-3-(2,4-dimethoxyphenyl)prop-2-en-1-one (**3e**): Yield 88%; m.p. 146–148 °C; IR (KBr, cm^{-1}): 1652 (C=O), 1610 (C=C of Ar), 1504 (CH=CH), 1622 (C=N), 1135 (-OCH_3), 2966 (Ar C-H); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 3.54 (3H, s, Ar-OCH_3), 3.75 (3H, s, Ar-OCH_3), 7.29 (1H, d, *J* = 17 Hz, -CO-CH=), 7.66 (1H, d, *J* =17 Hz, =CH-Ar), 6.05–8.14 (5H, Ar-H); Anal. Calcd for: $C_{14}H_{13}NO_4$: C, 64.86; H, 5.05; N, 5.40; Found: C, 64.95; H, 5.22; N, 5.49.

(E)-1-(isoxazole-5-yl)-3-(2,5-dimethoxyphenyl)prop-2-en-1-one (**3f**): Yield 76%; m.p. 140–142 °C; IR (KBr, cm^{-1}): 1656 (C=O), 1607 (C=C of Ar), 1501 (CH=CH), 1618 (C=N), 1131 (-OCH_3), 3064 (Ar C-H); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 3.64 (3H, s, Ar-OCH_3), 3.72 (3H, s, Ar-OCH_3), 7.21 (1H, d, *J* = 17 Hz, -CO-CH=), 7.71 (1H, d, *J* = 17 Hz, =CH-Ar), 6.04–8.22 (5H, Ar-H); Anal. Calcd for: $C_{14}H_{13}NO_4$: C, 64.86; H, 5.05; N, 5.40; Found: C, 64.95; H, 5.22; N, 5.49.

(E)-1-(isoxazole-5-yl)-3-(2,6-dimethoxyphenyl)prop-2-en-1-one (**3g**): Yield 80%; m.p. 139–141 °C; IR (KBr, cm^{-1}): 1649 (C=O), 1607 (C=C of Ar), 1508 (CH=CH), 1619 (C=N), 1133 (-OCH_3), 2995 (Ar C-H); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 3.58 (3H, s, Ar-OCH_3), 3.78 (3H, s, Ar-OCH_3), 7.20 (1H, d, *J* = 17 Hz, -CO-CH=), 7.73 (1H, d, *J* =17 Hz, =CH-Ar), 6.07–8.23 (5H, Ar-H); Anal. Calcd for: $C_{14}H_{13}NO_4$: C, 64.86; H, 5.05; N, 5.40; Found: C, 64.95; H, 5.22; N, 5.49.

(E)-1-(isoxazole-5-yl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (**3h**): Yield 75%; m.p. 181–183 °C; IR (KBr, cm^{-1}): 1649 (C=O), 1602 (C=C of Ar), 1505 (CH=CH), 1620 (C=N), 1129 (-OCH_3), 3028 (Ar C-H); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 3.55 (3H, s, Ar-OCH_3), 3.80 (3H, s, Ar-OCH_3), 7.18 (1H, d, *J* = 17 Hz, -CO-CH=), 7.67 (1H, d, *J* =17 Hz, =CH-Ar), 6.12–8.12 (5H, Ar-H); Anal. Calcd for: $C_{14}H_{13}NO_4$: C, 64.86; H, 5.05; N, 5.40; Found: C, 64.95; H, 5.22; N, 5.49.

(E)-1-(isoxazole-5-yl)-3-(3,5-dimethoxyphenyl)prop-2-en-1-one (**3i**): Yield 81%; m.p. 112–114 °C; IR (KBr, cm^{-1}): 1659 (C=O), 1610 (C=C of Ar), 1510 (CH=CH), 1621 (C=N), 1128 (-OCH_3), 3021 (Ar C-H); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 3.69 (3H, s, Ar-OCH_3), 3.84 (3H, s, Ar-OCH_3), 7.28 (1H, d, *J* = 17 Hz, -CO-CH=), 7.75 (1H, d, *J* =17 Hz, =CH-Ar), 6.11–8.21 (5H, Ar-H); Anal. Calcd for: $C_{14}H_{13}NO_4$: C, 64.86; H, 5.05; N, 5.40; Found: C, 64.95; H, 5.22; N, 5.49.

(E)-1-(isoxazole-5-yl)-3-(2,3,4-trimethoxyphenyl)prop-2-en-1-one (**3j**): Yield 74%; m.p. 169–171 °C; IR (KBr, cm^{-1}): 1655 (C=O), 1586 (C=C of Ar), 1511 (CH=CH), 1127 (-OCH_3); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 3.88 (3H, s, Ar-OCH_3), 3.92 (6H, s, 2x Ar-OCH_3), 7.25 (1H, d, *J* = 17 Hz, -CO-CH=), 7.55 (1H, d, *J* = 17 Hz, =CH-Ar), 6.21–8.11 (4H, Ar-H); Anal. Calcd for: $C_{15}H_{15}NO_2$: C, 62.28; H, 5.23; N, 4.84; Found: C, 62.55; H, 5.44; N, 5.85.

(E)-1-(isoxazole-5-yl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one (**3k**): Yield 76%; m.p. 154–156 °C; IR (KBr, cm^{-1}): 1652 (C=O), 1585 (C=C of Ar), 1498 (CH=CH), 1132 (-OCH_3); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 3.82 (3H, s, Ar-OCH_3), 3.90 (6H, s, 2x Ar-OCH_3), 7.21 (1H, d, *J* = 17 Hz, -CO-CH=), 7.52 (1H, d, *J* =17 Hz, =CH-Ar), 6.85–8.07 (4H, Ar-H); Anal. Calcd for: $C_{15}H_{15}NO_2$: C, 62.28; H, 5.23; N, 4.84; Found: C, 62.55; H, 5.44; N, 5.85.

(E)-1-(isoxazole-5-yl)-3-(2,4,6-trimethoxyphenyl)prop-2-en-1-one (**3l**): Yield 65%; m.p. 196–198 °C; IR (KBr, cm^{-1}): 1658 (C=O), 1578 (C=C of Ar), 1521 (CH=CH), 1133 (-OCH_3); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 3.85 (3H, s, Ar-OCH_3), 3.91 (6H, s, 2x Ar-OCH_3), 7.29 (1H, d, *J* = 17 Hz, -CO-CH=), 7.68 (1H, d, *J* =17 Hz, =CH-Ar), 6.25–8.28 (4H, Ar-H); Anal. Calcd for: $C_{15}H_{15}NO_5$: C, 62.28; H, 5.23; N, 4.84; Found: C, 62.55; H, 5.44; N, 5.85.

(E)-1-(isoxazole-5-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**3m**): Yield 88%; m.p. 222–224 °C; IR (KBr, cm^{-1}): 1661 (C=O), 1578 (C=C of Ar), 1522 (CH=CH), 1125 (-OCH_3), 3102 (Ar C-H); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 3.85 (3H, s, Ar-OCH_3), 3.86 (6H, s, 2x Ar-OCH_3), 7.19 (1H, d, *J* = 17 Hz, -CO-CH=), 7.66 (1H, d, *J* =17 Hz, =CH-Ar), 6.01–8.22 (4H, Ar-H); Anal. Calcd for: $C_{15}H_{15}NO_5$: C, 62.28; H, 5.23; N, 4.84; Found: C, 62.55; H, 5.44; N, 5.85.

(E)-1-(isoxazole-5-yl)-3-(2-fluoro-3,4-dimethoxyphenyl)prop-2-en-1-one (**3n**): Yield 91%; m.p. 272–274 °C; IR (KBr, cm^{-1}): 1659 (C=O), 1580 (C=C of Ar), 1524 (CH=CH), 1121 (-OCH_3), 935 (C-F), 3102 (Ar C-H); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 3.64 (3H, s, Ar-OCH_3), 3.83 (3H, s, 2x Ar-OCH_3), 7.25 (1H, d, *J* = 17 Hz, -CO-CH=), 7.71 (1H, d, *J* =17 Hz, =CH-Ar), 6.51–8.25 (4H, Ar-H); Anal. Calcd for: $C_{14}H_{12}FNO_4$: C, 60.65; H, 4.36; N, 5.05; Found: C, 61.21; H, 5.01; N, 5.64.

(E)-1-(isoxazole-5-yl)-3-(2-chloro-4,6-dimethoxyphenyl)prop-2-en-1-one (**3o**): Yield 87%; m.p. 248-250 °C; IR (KBr, cm^{-1}): 1663 (C=O), 1576 (C=C of Ar), 1521 (CH=CH), 1119 (-OCH_3), 895 (C-F), 3110 (Ar C-H); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 3.56 (3H, s, Ar-OCH_3), 3.81 (3H, s, 2x Ar-OCH_3), 7.23 (1H, d, *J* = 17 Hz, -CO-CH=), 7.62 (1H, d, *J* =17 Hz, =CH-Ar), 6.45–8.20 (4H, Ar-H); Anal. Calcd for: $C_{14}H_{12}ClNO_4$: C, 57.25; H, 4.12; N, 4.77; Found: C, 58.01; H, 4.32; N, 4.91.

3-(isoxazol-5-yl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide (**4a**): Yield 56%; Molecular Weight: 286.29; m.p. 166-168 °C; IR (KBr, cm^{-1}): 1622 (C=N), 1677 (C=O), 3367 (−NH_2); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 3.13 (1H, H_A, dd, J_{AX} = 3.6 Hz, dd, J_{AB} = 16Hz), 3.87 (1H, H_B, dd, J_{AB} = 16 Hz, dd, J_{BX}= 12 Hz), 5.23 (1H, H_x, dd, J_{AX} = 3.6 Hz, dd, J_{BX} = 12 Hz), 10.44 (2H, s, NH_2, D_2O exchangeable), 3.70 (3H, s, Ar-OCH_3), 6.15–8.59 (6H, Ar-H); MS (*m*/*z*, %): 287.1 (M+1, 99.16); Anal. Calcd for: $C_{14}H_{14}N_4O_3$: C, 58.73; H, 4.93; N, 19.57; Found: C, 59.18; H, 4.99; N, 20.16.

3-(isoxazol-5-yl)-5-(3-methoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide (**4b**): Yield 60%; Molecular Weight: 286.29; m.p. 221–223 °C; IR (KBr, cm^{-1}): 634 (C=N), 1686 (C=O), 3391 (−NH_2); ^1H NMR (400

MHz, $CDCl_3$, ppm): δ 3.06 (1H, H_A, dd, J_{AX} = 3.6Hz, dd, J_{AB} = 16 Hz), 3.81 (1H, H_B, dd, J_{AB} = 16 Hz, dd, J_{BX}= 12 Hz), 5.19 (1H, H_x, dd, J_{AX} = 3.6 Hz, dd, J_{BX} = 12 Hz), 10.25 (2H, s, NH_2, D_2O exchangeable), 3.85 (3H, s, Ar-OCH_3), 6.15–8.22 (6H, Ar-H); MS (*m*/z, %): 287.1 (M+1, 99.22); Anal. Calcd for: $C_{14}H_{14}N_4O_3$: C, 58.73; H, 4.93; N, 19.57; Found: C, 59.18; H, 4.99; N, 20.16.

3-(isoxazol-5-yl)-5-(2-methoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide (**4c**): Yield 58%; Molecular Weight: 286.29; m.p. 256–258 °C; IR (KBr, cm^{-1}): 1619 (C=N), 1667 (C=O), 3375 (−NH_2); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 3.14 (1H, H_A, dd, J_{AX} = 3.6 Hz, dd, J_{AB} = 16 Hz), 3.82 (1H, H_B, dd, J_{AB} = 16 Hz, dd, J_{BX} = 12 Hz), 5.25 (1H, H_x, dd, J_{AX} = 3.6 Hz, dd, J_{BX} = 12 Hz), 10.41 (2H, s, NH_2, D_2O exchangeable), 3.72 (3H, s, Ar-OCH_3), 6.23–8.05 (6H, Ar-H); MS (*m*/z, %): 287.1 (M+1, 99.09); Anal. Calcd for: $C_{14}H_{14}N_4O_3$: C, 58.73; H, 4.93; N, 19.57; Found: C, 59.18; H, 4.99; N, 20.16.

3-(isoxazol-5-yl)-5-(2,3-dimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide (**4d**): Yield 52%; Molecular Weight: 316.31; m.p. 210–212 °C; IR (KBr, cm^{-1}): 1629 (C=N), 1669 (C=O), 3359 (−NH_2); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 3.18 (1H, H_A, dd, J_{AX} = 3.6 Hz, dd, J_{AB} = 16 Hz), 3.85 (1H, H_B, dd, J_{AB} = 16 Hz, dd, J_{BX} = 12 Hz), 5.19 (1H, H_x, dd, J_{AX} = 3.6 Hz, dd, J_{BX} = 12 Hz), 10.49 (2H, s, NH_2, D_2O exchangeable), 3.53 (3H, s, Ar-OCH_3), 3.68 (3H, s, Ar-OCH_3), 6.35–8.28 (5H, Ar-H); MS (*m*/z, %): 317.1 (M+1, 99.06); Anal. Calcd for: $C_{15}H_{16}N_4O_4$: C, 56.69; H, 5.10; N, 17.71; Found: C, 56.88; H, 5.31; N, 17.99.

3-(isoxazol-5-yl)-5-(2,4-dimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide (**4e**): Yield 65%; Molecular Weight: 316.31; m.p. 189–191 °C; IR (KBr, cm^{-1}): 1631 (C=N), 1682 (C=O), 3361 (−NH_2); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 3.10 (1H, H_A, dd, J_{AX} = 3.6 Hz, dd, J_{AB} = 16 Hz), 3.90 (1H, H_B, dd, J_{AB} = 16 Hz, dd, J_{BX} = 12 Hz), 5.22 (1H, H_x, dd, J_{AX} = 3.6 Hz, dd, J_{BX} = 12 Hz), 10.35 (2H, s, NH_2, D_2O exchangeable), 3.51 (3H, s, Ar-OCH_3), 3.72 (3H, s, Ar-OCH_3), 6.18–8.10 (5H, Ar-H); MS (*m*/z, %): 317.1 (M+1, 99.11); Anal. Calcd for: $C_{15}H_{16}N_4O_4$: C, 56.69; H, 5.10; N, 17.71; Found: C, 56.88; H, 5.31; N, 17.99.

3-(isoxazol-5-yl)-5-(2,5-dimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide (**4f**): Yield 59%; Molecular Weight: 316.31; m.p. 198–200 °C; IR (KBr, cm^{-1}): 1636 (C=N), 1675 (C=O), 3399 (-NH_2); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 3.15 (1H, H_A, dd, J_{AX} = 3.6 Hz, dd, J_{AB} = 16Hz), 3.85 (1H, H_B, dd, J_{AB} = 16 Hz, dd, J_{BX} = 12 Hz), 5.21 (1H, H_x, dd, J_{AX} = 3.6 Hz, dd, J_{BX} = 12 Hz), 10.18 (2H, s, NH_2, D_2O exchangeable), 3.66 (3H, s, Ar-OCH_3), 3.74 (3H, s, Ar-OCH_3), 6.09–8.18 (5H, Ar-H); MS (*m*/z, %): 317.1(M+1, 99.16); Anal. Calcd for: $C_{15}H_{16}N_4O_4$: C, 56.69; H, 5.10; N, 17.71; Found: C, 56.88; H, 5.31; N, 17.99.

3-(isoxazol-5-yl)-5-(2,6-dimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide (**4g**): Yield 45%; Molecular Weight: 316.31; m.p. 282–284 °C; IR (KBr, cm^{-1}): 1626 (C=N), 1671 (C=O), 3361 (−NH_2); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 3.13 (1H, H_A, dd, J_{AX} = 3.6 Hz, dd, J_{AB} = 16 Hz), 3.86 (1H, H_B, dd, J_{AB} = 16 Hz, dd, J_{BX} = 12 Hz), 5.29 (1H, H_x, dd, J_{AX} = 3.6 Hz, dd, J_{BX} = 12 Hz), 10.46 (2H, s, NH_2, D_2O exchangeable), 3.58 (3H, s, Ar-OCH_3), 3.78 (3H, s, Ar-OCH_3), 6.07–8.23 (5H, Ar-H); MS (*m*/z, %): 317.1 (M+1, 99.01); Anal. Calcd for: $C_{15}H_{16}N_4O_4$: C, 56.69; H, 5.10; N, 17.71; Found: C, 56.88; H, 5.31; N, 17.99.

3-(isoxazol-5-yl)-5-(3,4-dimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide (**4h**): Yield 55%; Molecular Weight: 316.31; m.p. 246–248 °C; IR (KBr, cm^{-1}): 1624 (C=N), 1678 (C=O), 3356 (−NH_2); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 3.20 (1H, H_A, dd, J_{AX} = 3.6 Hz, dd, J_{AB} = 16 Hz), 3.79 (1H, H_B, dd, J_{AB} = 16 Hz, dd, J_{BX} = 12 Hz), 5.30 (1H, H_x, dd, J_{AX} = 3.6 Hz, dd, J_{BX} = 12 Hz), 10.51 (2H, s, NH_2, D_2O exchangeable), 3.61 (3H, s, Ar-OCH_3), 3.83 (3H, s, Ar-OCH_3), 6.25–8.46 (5H, Ar-H); MS (*m*/z, %): 317.1 (M+1, 99.26); Anal. Calcd for: $C_{15}H_{16}N_4O_4$: C, 56.69; H, 5.10; N, 17.71; Found: C, 56.88; H, 5.31; N, 17.99.

3-(isoxazol-5-yl)-5-(3,5-dimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide (**4i**): Yield 48%; Molecular Weight: 316.31; m.p. 148-150 °C; IR (KBr, cm^{-1}): 1632 (C=N), 1679 (C=O), 3369 (−NH_2); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 3.21 (1H, H_A, dd, J_{AX} = 3.6 Hz, dd, J_{AB} = 16 Hz), 3.81 (1H, H_B, dd, J_{AB} = 16 Hz, dd, J_{BX} = 12 Hz), 5.33 (1H, H_x, dd, J_{AX} = 3.6 Hz, dd, J_{BX} = 12 Hz), 10.48 (2H, s, NH_2, D_2O

exchangeable), 3.51 (3H, s, Ar-OCH_3), 3.99 (3H, s, Ar-OCH_3), 6.33-8.61 (5H, Ar-H); MS (*m*/*z*, %): 317.1 (M+1, 99.08); Anal. Calcd for: $C_{15}H_{16}N_4O_4$: C, 56.69; H, 5.10; N, 17.71; Found: C, 56.88; H, 5.31; N, 17.99.

3-(isoxazol-5-yl)-5-(2,3,4-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide (**4j**): Yield 60%; Molecular Weight: 346.13; m.p. 262–264 °C; IR 1639 (C=N), 1662 (C=O), 3355 (−NH_2); ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 3.09 (1H, H_A, dd, J_{AX} = 3.6 Hz, dd, J_{AB} = 16 Hz), 3.82 (1H, H_B, dd, J_{AB} = 16 Hz, dd, J_{BX} = 12 Hz), 5.40 (1H, H_x, dd, J_{AX} = 3.6 Hz, dd, J_{BX} = 12 Hz), 10.39 (2H, s, NH_2, D_2O exchangeable), 3.65 (3H, s, Ar-OCH_3), 3.99 (6H, s, 2x Ar-OCH_3), 6.36–8.44 (4H, Ar-H); MS (*m*/*z*, %): 346.1 (M+1, 99.14); Anal. Calcd for: $C_{16}H_{18}N_4O_5$: C, 55.49; H, 5.24; N, 16.18; Found: C, 55.89; H, 5.65; N, 16.52.

3-(isoxazol-5-yl)-5-(2,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide (**4k**): Yield 51%; Molecular Weight: 346.13; m.p. 231–233 °C; IR (KBr, cm^{-1}): 1625 (C=N), 1672 (C=O), 3362 (−NH_2); ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 3.09 (1H, H_A, dd, J_{AX} = 3.6 Hz, dd, J_{AB} = 16 Hz), 3.75 (1H, H_B, dd, J_{AB} = 16 Hz, dd, J_{BX} = 12 Hz), 5.44 (1H, H_x, dd, J_{AX} = 3.6 Hz, dd, J_{BX} = 12 Hz), 10.40 (2H, s, NH_2, D_2O exchangeable), 3.80 (3H, s, Ar-OCH_3), 3.96 (6H, s, 2x Ar-OCH_3), 6.56–8.82 (4H, Ar-H); Anal. Calcd for: $C_{16}H_{18}N_4O_5$: C, 55.49; H, 5.24; N, 16.18; Found: C, 55.89; H, 5.65; N, 16.52.

3-(isoxazol-5-yl)-5-(2,4,6-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide (**4l**): Yield 41%; Molecular Weight: 346.13; m.p. 272–274 °C; IR (KBr, cm^{-1}): 1635 (C=N), 1681 (C=O), 3382 (−NH_2); ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 3.13 (1H, H_A, dd, J_{AX} = 3.6 Hz, dd, J_{AB} = 16 Hz), 3.85 (1H, H_B, dd, J_{AB} = 16 Hz, dd, J_{BX} = 12 Hz), 5.20 (1H, H_x, dd, J_{AX} = 3.6 Hz, dd, J_{BX} = 12 Hz), 10.32 (2H, s, NH_2, D_2O exchangeable), 3.79 (3H, s, Ar-OCH_3), 3.91 (6H, s, 2x Ar-OCH_3), 6.35–8.60 (4H, Ar-H); MS (*m*/*z*, %): 346.1 (M+1, 99.09); Anal. Calcd for: $C_{16}H_{18}N_4O_5$: C, 55.49; H, 5.24; N, 16.18; Found: C, 55.89; H, 5.65; N, 16.52.

3-(isoxazol-5-yl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide (**4m**): Yield 71%; Molecular Weight: 346.13; m.p. 291–293 °C; IR (KBr, cm^{-1}): 1628 (C=N), 1685 (C=O), 3369 (−NH_2); ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 3.11 (1H, H_A, dd, J_{AX} = 3.6 Hz, dd, J_{AB} = 16 Hz), 3.80 (1H, H_B, dd, J_{AB} = 16 Hz, dd, J_{BX} = 12 Hz), 5.15 (1H, H_x, dd, J_{AX} = 3.6 Hz, dd, J_{BX} = 12 Hz), 10.20 (2H, s, NH_2, D_2O exchangeable), 3.70 (3H, s, Ar-OCH_3), 3.95 (6H, s, 2x Ar-OCH_3), 6.11–8.33 (4H, Ar-H); MS (*m*/*z*, %):346.1 (M+1, 99.20); Anal. Calcd for: $C_{15}H_{15}NO_5$: C, 62.28; H, 5.23; N, 4.84; Found: C, 62.55; H, 5.44; N, 5.85.

3-(isoxazol-5-yl)-5-(2-fluoro-3,4-dimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide (**4n**): Yield 81%; Molecular Weight: 334.11; m.p. 302–304 °C; IR (KBr, cm^{-1}): 1630 (C=N), 1662 (C=O), 3360 (−NH_2); ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 3.06 (1H, H_A, dd, J_{AX} = 3.6 Hz, dd, J_{AB} = 16 Hz), 3.81 (1H, H_B, dd, J_{AB} = 16 Hz, dd, J_{BX} = 12 Hz), 5.31 (1H, H_x, dd, J_{AX} = 3.6 Hz, dd, J_{BX} = 12 Hz), 10.14 (2H, s, NH_2, D_2O exchangeable), 3.61 (3H, s, Ar-OCH_3), 3.89 (3H, s, 2x Ar-OCH_3), 6.22–8.41 (4H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 155.62 (C-3), 39.54 (C-4), 62.50 (C-5), 155.12 ($CONH_2$), 150.06 (C-3 of Isoxazolyl ring), 100.52 (C-4 of Isoxazolyl ring), 155.66 (C-5 of Isoxazolyl ring), 102.11 (C-1 of phenyl ring), 145.12 (C-2 of phenyl ring), 134.24 (C-3 of phenyl ring), 149.42 (C-4 of phenyl ring), 107.76 (C-5 of phenyl ring), 121.66 (C-6 of phenyl ring), 56.11 (Carbon of 2x-OCH_3). MS (*m*/*z*, %): 335.1 (M+1, 99.03); Anal. Calcd for: $C_{15}H_{15}FN_4O_4$: C, 53.89; H, 4.52; N, 16.76; Found: C, 54.21; H, 4.81; N, 16.98.

3-(isoxazol-5-yl)-5-(2-chloro-4,6-dimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide (**4o**): Yield 63%; Molecular Weight 350.76; m.p. 277–279 °C; IR (KBr, cm^{-1}): 1637 (C=N), 1675 (C=O), 3390 (−NH_2); ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 3.02 (1H, H_A, dd, J_{AX} = 3.6 Hz, dd, J_{AB} = 16 Hz), 3.79 (1H, H_B, dd, J_{AB} = 16 Hz, dd, J_{BX} = 12 Hz), 5.34 (1H, H_x, dd, J_{AX} = 3.6 Hz, dd, J_{BX} = 12 Hz), 10.49 (2H, s, NH_2, D_2O exchangeable), 3.45 (3H, s, Ar-OCH_3), 3.95 (3H, s, 2x Ar-OCH_3), 6.45-8.20 (4H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 155.92 (C-3), 38.91 (C-4), 57.20 (C-4), 155.26 ($CONH_2$), 150.11 (C-3 of Isoxazolyl ring), 100.21 (C-4 of Isoxazolyl ring), 158.76 (C-5 of Isoxazolyl ring), 120.76 (C-1 of phenyl ring), 134.25 (C-2 of phenyl ring), 107.22 (C-3 of phenyl ring), 161.08 (C-4 of phenyl ring), 98.35 (C-5 of phenyl ring), 158.28 (C-6 of phenyl ring), 55.8 (carbon of –OCH_3), 56.12 (carbon of –OCH_3). MS (*m*/*z*, %): 335.1 (M+1, 99.03); MS (*m*/*z*, %): 351.08 (M+1, 99.15); Anal. Calcd for: $C_{15}H_{15}ClN_4O_4$: C, 51.36; H, 4.31; N, 15.97; Found: C, 52.11; H, 4.65; N, 16.11.

2.3. Biological Activity Studies

2.3.1. Antibacterial and Antifungal Activities

The antibacterial and antifungal activity of the novel chalcones (**3a** to **3o**) and dihydropyrazoles (**4a** to **4o**) against the selected bacterial and fungal strains was assessed by following the procedure described in our previous paper [32].

2.3.2. Antioxidant Activity

DPPH assay: DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol. This free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to colorless ethanol solution. The use of the DPPH assay provides an easy and rapid way to evaluate antioxidants by spectrophotometry, so it can be useful to assess various products at a time. The purpose of this study was to evaluate the antioxidant activity of chalcones and pyrazolines using the DPPH free radical assay. The percentage of antioxidant activity (AA%) of all the compounds was performed by DPPH free radical assay. The measurement of the DPPH radical scavenging activity was performed according to methodology described by Brand-Williams et al. The samples were reacted with the stable DPPH radical in an ethanol solution. 0.1 mM solution of DPPH in methanol was prepared. Gallic acid was taken as a reference standard and different concentration of test samples (5–100 μg/mL) and standard (1.0 μg/mL, 2.5 μg/mL and 5.0 μg/mL) were prepared using methanol. 1 mL of 0.1 mM DPPH solution was added to 3 mL of all concentrations of test and standard separately. These mixtures were kept in dark for about 30 min and the absorbance was measured at 517 nm [33]. The capability to scavenge the DPPH radical was calculated using the formula.

$$\text{DPPH scavenged}(\%) = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} * 100$$

When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in colour (from deep violet to light yellow) were read [Absorbance (Abs)] at 517 nm after 100 min of reaction using a UV-VIS spectrophotometer.

2.3.3. Anticancer Activity

The *in vitro* anticancer activity of chalcones (**3a** to **3o**) and dihydropyrazoles (**4a** to **4o**) was evaluated by the Mosmann's MTT assay that was described elsewhere [34].

3. Results and Discussion

3.1. Chemistry

Chalcones were initially prepared by Claisen-Schmidt condensation reaction in the presence of aqueous potassium hydroxide as a catalyst and further theses compounds were treated with semicarbazide in catalytic amount of pyridine and obtained the target dihydropyrazoles. The FT-IR spectrum of chalcones shown characteristic bands around the wave numbers 1648–1661 cm^{-1} (-C=O) and 1498–1522 cm^{-1} (-C=C-) where as the dihydropyrazoles showed stretching absorption bands around 1619–1639 cm^{-1} (C=N), 1662–1686 (C=O) and 3355–3399 ($-NH_2$) respectively. The ^1H NMR spectrum displayed signals for –CO-CH= (α-H) around δ 6.7–7.4 ppm and δ 7.3–7.8 =CH-Ar (β-H) as doublets with coupling constant (J = 17 Hz) respectively which are typical for chalcones and the dihydropyrazoles shown their unique three peaks corresponding to the ABX system of dihydropyrazole in the regions of 3.02–3.21 ppm (1H, H_A, dd, J_{AX} = 3.6 Hz, dd, J_{AB} = 16 Hz), 3.75–3.90 ppm (1H, H_B, dd, J_{AB} = 16 Hz, dd, J_{BX} = 12 Hz) and 5.19–5.44 ppm (1H, H_x, dd, J_{AX} = 3.6Hz, dd, J_{BX} = 12 Hz) as well as a broad singlet at δ = 10.20–10.51 ppm for exchangeable $-NH_2$ respectively. Other protons exhibited additional resonance signals typically present in each compound in the ^1H NMR

spectrums of both chalcones and dihydropyrazoles respectively. In their mass spectrum the dihydropyrazoles showed the typical molecular ion peaks scanned in the positive mode confirming the molecular weight. Elemental analysis results were within ±0.4% of the calculated values and helped in determining the elemental composition of the compounds.

3.2. Biological Activity Studies

3.2.1. Antibacterial and Antifungal Activities

The target chalcones (**3a–3o**) and pyrazolines (**4a–4o**) were tested for their antimicrobial activities by serial tube dilution method against two types of bacterial and fungal strains (Tables 1 and 2). The bacterial strains used for the testing including gram positive *Staphylococcus aureus* (*Sa*) and gram negative *Pseudomonas aeruginosa* (*Pa*)where as the fungal strains employed include *Aspergillus niger* (*An*) and *Candida tropicalis* (*Ct*).

Table 1. Results of the antibacterial and antifungal activities of chalcones (3a-3o).

Entry	Compound	*Sa*	*Pa*	*An*	*Ct*
1	**3a**	4	8	8	8
2	**3b**	128	128	256	256
3	**3c**	4	4	8	16
4	**3d**	32	64	64	32
5	**3e**	2	4	8	16
6	**3f**	32	32	64	64
7	**3g**	4	4	4	4
8	**3h**	64	32	128	64
9	**3i**	256	256	256	512
10	**3j**	64	64	128	128
11	**3k**	16	16	32	32
12	**3l**	1	1	2	2
13	**3m**	64	128	128	256
14	**3n**	4	4	16	16
15	**3o**	2	2	4	4
16	**Ciprofloxacin**	2	2	**---**	**---**
17	**Fluconazole**	**---**	**---**	1	1

Chalcones exhibited excellent antibacterial potency where as the pyrazolines showed tremendous antifungal activity compared to the standard drugs ciprofloxacin and fluconazole respectively. This signifies that Chalcone link is essential for the antibacterial activity where as the pyrazoline scaffold for activity against the fungal strains. With both chalcones and pyrazolines, the nature and position of the substituents on the phenyl ring played a crucial role for the antimicrobial activity. Among the chalcones, **3l** containing 2,4,6-trimethoxyphenyl ring was the most potent even than ciprofloxacin with an MIC 1 μg/mL against tested bacteria. In contrast, its antifungal potency was at MIC 2 μg/mL which was less compared to fluconazole (1 μg/mL). **3o** containing 2-chloro-4,6-dimethoxyphenyl was next in potency to **3l** with respect to both the activities. Most of the chalcones demonstrated moderate antibacterial and antifungal activity with the MIC ranging from 4-64 μg/mL. Some of the compounds viz., **3b**, **3i**, **3j** and 3m showed poor activity with MIC ranging between 128–256 μg/mL. Structure activity relationship studies from the above results disclosed that the presence of more number of methoxyl and halogen substituents at ortho and para positions is critical for the antimicrobial activity and if the methoxyl groups are present in the meta position will reduce the antimicrobial potency.

Table 2. Results of the antibacterial and antifungal activity of pyrazolines (4a-4o).

Entry	Compound	*Sa*	*Pa*	*An*	*Ct*
1	**4a**	16	16	4	4
2	**4b**	128	128	64	64
3	**4c**	16	16	8	8
4	**4d**	64	64	16	16
5	**4e**	8	8	4	8
6	**4f**	64	32	32	32
7	**4g**	32	16	2	2
8	**4h**	128	64	32	32
9	**4i**	256	256	64	64
10	**4j**	64	128	64	64
11	**4k**	32	16	16	16
12	**4l**	4	4	2	2
13	**4m**	64	256	16	16
14	**4n**	8	8	0.5	1
15	**4o**	8	8	0.5	0.5
16	**Ciprofloxacin**	2	2	**---**	**---**
17	**Fluconazole**	**---**	**---**	1	1

Pyrazolines **4n** and **4o** containing a blend of methoxyl and halogen substituents on the phenyl ring exhibit brilliant antifungal activity compared to fluconazole. **4o** was active at MIC 0.5 μg/mL against both the fungal strains whereas the compound **4n** displayed activity against *Aspergillus niger* at 0.5 μg/mL and 1 μg/mL on the *Candida albicans* strain. Compound **4l** and **4a** exhibited activity at 2 and 4 μg/mL respectively against the two fungal strains. The other compounds were active in the range of 16–64 μg/mL. The antibacterial potency of pyrazolines was less than chalcones. Among pyrazolines **4l** displayed the most potent antibacterial activity at MIC 4 μg/mL and **4e**, **4n** and **4o** were the subsequently potent with the MIC 8 μg/mL. Most of the compounds demonstrated activity at 16–64 μg/mL and some at 128 and 256 μg/mL. In summary, chalcones containing 2,4,6-trimethoxyphenyl moiety were potent antibacterial agents whereas the pyrazolines containing 2-halo-4,6-dimethoxyphenyl scaffold were potent antifungal agents

3.2.2. Antioxidant Activity

The antioxidant activity of all the compounds was done employing DPPH free radical assay and the results are average of three independent experiments (Table 3). Gallic acid was employed as the positive control. Both chalcones and pyrazolines have exhibited significant antioxidant potency and among these two the chalcones were more active. The structure activity relationships based on the above results activity. For instance, **3l** containing three methoxyl groups at positions 2,4 and 6 was the most potent of the thirty compounds and its activity was equal to that of the standard with an IC_{50} value 5 μg/mL. On the other hand **3n**, **3o**, **4n** and **4o** containing both halogen and methoxyl groups on the phenyl were least active. More is the number of methoxyl groups more pronounced was the antioxidant activity i.e., the compounds containing three methoxyl groups were first in potency followed by the compounds with two and one methoxyl groups respectively. This indicate that the substituents on the phenyl ring have a remarkable influence on the antioxidant

3.2.3. Anticancer Activity

All the thirty compounds were evaluated for their anticancer potency against prostate cancer cell line, **DU-145**, employing MTT assay. Pyrazoline derivatives exhibited superior activity than the chalcones (Table 4). The pyrazoline **4n** and **4h** was more potent compound with MIC 2 and 4 μg/mL respectively. The activity of these compounds was greater than the standard, Docetaxel (MIC = 5 μg/mL). The chalcone **3n** and pyrazoline **4m** exhibited equipotent activity as that of the standard.

The compounds **4h**, **3h**, **3m**, **4j** and **3j** were next in activity with MIC values 4, 6, 8, 9 and 10 μg/mL respectively. All the other compounds exhibited moderate anticancer activity with MIC ranging from 11–32 μg/mL. The SAR features indicate that heterocyclic pyrazoline ring is more essential than the propenone motif found in chalcones. The compounds were also tested against the human normal cells (**L02**) and found to be nontoxic to the normal cells.

Table 3. Comparison of the DPPH assay results of chalcones and pyrazolines (IC_{50} values in μg/mL).

Compound	IC_{50}	Compound	IC_{50}
3a	33 ± 1	**4a**	44 ± 1
3b	38 ± 2	**4b**	51 ± 2
3c	32 ± 2	**4c**	38 ± 2
3d	24 ± 1	**4d**	32 ± 1
3e	18 ± 2	**4e**	25 ± 2
3f	26 ± 2	**4f**	39 ± 2
3g	16 ± 1	**4g**	28 ± 1
3h	29 ± 1	**4h**	49 ± 1
3i	9 ± 1	**4i**	12 ± 1
3j	6 ± 1	**4j**	8 ± 1
3k	7 ± 2	**4k**	10 ± 2
3l	**5 ± 1**	**4l**	**6 ± 1**
3m	8 ± 2	**4m**	12 ± 2
3n	45 ± 2	**4n**	55 ± 2
3o	48 ± 1	**4o**	62 ± 1
Gallic acid		5 ± 1	

Table 4. Comparison of the MTT assay anticancer results of chalcones and pyrazolines. (IC50 values in μg/mL).

Entry	DU-145	Human Normal Cells (L02)	Entry	DU-145	Human Normal Cells (L02)
3a	32 ± 2	>40	**4a**	28 ± 1	>40
3b	20 ± 1	>40	**4b**	18 ± 2	>40
3c	36 ± 1	>40	**4c**	32 ± 2	>40
3d	22 ± 1	>40	**4d**	16 ± 2	>40
3e	29 ± 2	>40	**4e**	26 ± 2	>40
3f	38 ± 1	>40	**4f**	31 ± 2	>40
3g	33 ± 2	>40	**4g**	20 ± 2	>40
3h	6 ± 2	>40	**4h**	4 ± 2	>40
3i	12 ± 1	>40	**4i**	8 ± 2	>40
3j	10 ± 2	>40	**4j**	9 ± 1	>40
3k	26 ± 3	>40	**4k**	18 ± 2	>40
3l	30 ± 2	>40	**4l**	21 ± 2	>40
3m	8 ± 2	>40	**4m**	5 ± 2	>40
3n	5 ± 2	>40	**4n**	2 ± 1	>40
3o	16 ± 2	>40	**4o**	12 ± 2	>40
Docetaxel			5±1		

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