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Synthesis And Antimicrobial Screening of Novel Chalcone and Pyrazoline Molecules Bearing 4-(difluoromethoxy)-3-hydroxybenzaldehyde Nucleus.

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ABSTRACT

A series of novel Chalcones **2a-i** and Pyrazolines **3a-i** have been synthesized and evaluated their antibacterial and antifungal activity. The Pyrazolines **3a-i** have been synthesized by reaction of various Chalcones **2a-i** with Isoniazide in presence of glacial acetic acid. The Chalcones of **2a-i** were prepared by the reaction of 4-(difluoromethoxy)-3-hydroxybenzaldehyde with substitued acetophenone in aqueous sodium hydroxide using ethanol as a solvent. The structures of the newly synthesized compounds were identify on the basis of ^{1}H-NMR, Mass spectra, IR and elemental analysis data. All the newly synthesized compounds were screened for their antibacterial activity against (Gram-positive bacteria) *S. aureus*, *M. luteus,* (Gram-negative bacteria) *E. coli, S. thphi* and antifungal activity against *Candida albicans.*

Keywords: Chalcones, Pyrazolines, isoniazide, Antimicrobial Screening.

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INTRODUCTION

The rising prevalence of multi-drug resistant bacteria continues to provide encourage for the research and discovery of novel antimicrobial agents active against these pathogens. Heterocycles are universal structures in pharmaceutical compounds [1]. Chalcone and Pyrazoline moieties are an important class of heterocycles widely used as key building blocks for pharmaceutical agents. Chalcones (1,3-diphenyl-propene-1-one) belonging to the flavonoid family, are natural and synthetic products that have been reviewed for their wide range of biological activities. It exhibits a wide spectrum of pharmacophore activities, as it can act as antibacterial [2], antitumor [3], anti-inflammatory [4], antioxidant agents [5] and PTP1B inhibitors[6].

Available data suggest that N-containing heterocyclic compounds such as pyrazoline synthesized from chalcones possesses good pharmacological activities and are present in a number of pharmacologically active molecules such as phenazone, amidopyrene, methampyrone (analgesic and antipyretic), azolid/ tandearil (anti-inflammatory), indoxacarb (insecticidal), anturane (uricosuric), etc. Considerable interest has been focused on the pyrazoline structure. The discovery of this class of drugs provides an outstanding case history of modern drug development and also points out the unpredictability of pharmacological activity from structural modification of a prototype drug molecule. It is having a variety of medicinal applications. Pyrazoline derivatives were found to have potential activities such as tranquillizing [7], muscle relaxant [8], psychoanaleptic [9], anticonvulsant[10], antihypotensive [11], and antidepressant[12] activities. These type of derivatives were also found to exhibit cytotoxic activity, inhibitory activity of platelet aggregation [13], herbicidal [14] activity and cannabinoid [15] CB1-receptor modulators.

In view of getting to synthesis of Chalcones **2a-i** and Pyrazolines **3a-i** were screened for their antimicrobial activity.

EXPERIMENTAL

All the melting points were determined on electro-thermal apparatus using open capillaries and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5mm thickness, and spots were located by Iodine and UV (254nm). The IR spectra were recorded on a Shimadzu FT-IR-8400 instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GCMS-QP2010 model using Direct Injection Probe technique. ^{1}H-NMR was determined in DMSO-d6 solution on a Bruker AC 400MHz spectrometer using TMS as internal standard and coupling constants (J) are expressed in Hertz (Hz). Elemental analysis of the all the synthesized compounds were carried out on Elementar Vario EL III Carlo Erba 1108 model, and the results are in agreements with the structures assigned. All the reagents were purchased from Rankem (New Delhi, India) and Sigma-Aldrich (New Delhi, India) and are used without further purification.

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General procedure for synthesis of (E)-3-(4-(difluoromethoxy)-3-hydroxyphenyl)-1-arylprop-2-en-1-one (2a-i)

To a solution of 4-(difluoromethoxy)-3-hydroxybenzaldehyde (0.01 mol) in ethanol was added substituded Acetophenone (0.01 mol) followed by catalytic amount of 40% aqueous NaOH solution and the reaction mixture was stirred for 5-6 hrs at room temperature. Completion of reaction checked by TLC. The reaction mixture was poured into crushed ice, filtere , dried. The product was crystallized in ethanol. The spectral analysis of compounds **2a-i** are as under.

(E)-3-(4-(difluoromethoxy)-3-hydroxyphenyl)-1-phenylprop-2-en-1-one (2a)

Yield 90% (light yellow solid); m.p 158-160 ^{O}C; IR (KBr, cm^{-1}): 2947, 1651, 1582, 1034, 972, 841; ^{1}H NMR (400 MHz, DMSO-d6) δ 6.63 – 6.68 (s, 1H), 6.83 – 6.90 (d, J = 7.4 Hz, 1H), 6.90 – 6.98 (d, J = 7.5, 1.9, 1.0 Hz, 1H), 7.19 – 7.24 (m, 1H), 7.36 – 7.62 (m, 4H), 7.84 – 7.97 (m, 3H) ; ES-MS :(m/z) 290.1 (M^{+}); Anal. Calcd. for $C_{16}H_{12}F_2O_3$: C:66.21%, H:4.17% Found: C: 67.05% , H: 4.59%.

(E)-3-(4-(difluoromethoxy)-3-hydroxyphenyl)-1-p-tolylprop-2-en-1-one (2b)

Yield 88% (yellow solid); m.p 140-143 ^{O}C; IR (KBr, cm^{-1}): 2961, 1645, 1578, 1028, 981, 837 ; ^{1}H NMR (400 MHz, DMSO-d6) δ 6.63 – 6.68 (s, 1H), 6.87 – 6.98 (m, 3H), 7.15 – 7.21 (d, 1H), 7.34 – 7.45 (m, 4H), 7.84 – 7.93 (d, J = 15.1 Hz, 1H), 7.93 – 8.01 (m, 3H) ; ES-MS :(m/z) 304.1(M^{+}); Anal. Calcd. For $C_{17}H_{14}F_2O_3$: C:67.10%, H:3.95% ; Found: C: 67.89%, H: 3.65%.

(E)-3-(4-(difluoromethoxy)-3-hydroxyphenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (2c)

Yield 95% (yellow solid); m.p 159-163 ^{O}C; IR (KBr, cm^{-1}): 2953, 1656, 1571, 1042, 965, 838; ^{1}H NMR (400 MHz, DMSO-d6) δ 3.76 – 3.81 (s, 3H), 6.63 – 6.68 (s, 1H), 6.83 – 6.90 (d, J = 7.5 Hz, 1H), 6.90 – 6.98 (m, 3H), 7.11 – 7.17 (d, 1H), 7.35 – 7.44 (d, J = 15.1 Hz, 1H), 7.82 – 7.91 (d, J = 15.1, 1.0 Hz, 1H), 7.93 – 8.01 (m, 2H) ; ES-MS :(m/z) 320.1 (M^{+}); Anal. Calcd. for $C_{17}H_{14}F_2O_4$: C: 63.75%, H: 4.41%; Found: C: 63.81%, H: 4.18%.

(E)-3-(4-(difluoromethoxy)-3-hydroxyphenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (2d)

Yield 88% (yellow solid); m.p 188-192 ^{O}C; IR (KBr, cm^{-1}): 3395, 3019, 2971, 1657, 1595, 1023, 991, 848; ^{1}H NMR (400 MHz, DMSO-d6) δ 6.63 – 6.68 (s, 1H), 6.83 – 6.98 (m, 4H), 7.11 – 7.17 (d, 1H), 7.34 – 7.43 (d, J = 15.1 Hz, 1H), 7.82 – 7.91 (m, 3H) ; ES-MS :(m/z) 306.1 (M^{+}); Anal. Calcd. for $C_{16}H_{12}F_2O_4$: C: 62.75%, H: 3.95% ; Found: C: 62.30%, H: 4.18%.

(E)-3-(4-(difluoromethoxy)-3-hydroxyphenyl)-1-(4-bromophenyl)prop-2-en-1-one (2e)

Yield 88% (yellow solid); m.p 160-163 ^{O}C; IR (KBr, cm^{-1}): 2983, 1659, 1586, 1021, 980, 845; ^{1}H NMR (400 MHz, DMSO-d6) δ 6.63 – 6.68 (s, 1H), 6.84 – 6.98 (m, 2H), 7.19 – 7.25 (d, 1H),

7.36 – 7.45 (d, J = 15.1 Hz, 1H), 7.71 – 7.79 (m, 2H), 7.84 – 7.94 (d, J = 15.0, 1H), 7.97 – 8.05 (m, 2H); ES-MS :(m/z) 368.0 (M^+) and 370.0 (M^{+2}); Anal. Calcd. for $C_{16}H_{11}BrF_2O_3$: C: 52.06%; H: 3.00% ; Found: C: 52.30%; H: 3.23%.

(E)-3-(4-(difluoromethoxy)-3-hydroxyphenyl)-1-(4-chlorophenyl)prop-2-en-1-one (2f)

Yield 76% (yellow solid); m.p 177-181 ^OC; IR (KBr, cm^{-1}): 2997, 1657, 1579, 1039, 981, 831; ^1H NMR (400 MHz, DMSO-d6) δ 6.63 – 6.68 (s, 1H), 6.85 – 6.98 (m, 2H), 7.20 – 7.25 (d, 1H), 7.37 – 7.46 (d, J = 15.1, 1H), 7.47 – 7.55 (m, 2H), 7.85 – 7.96 (m, 3H) ; ES-MS :(m/z) 324.0 (M^+) and 326.0 (M^{+2}); Anal. Calcd. for $C_{16}H_{11}ClF_2O_3$: C: 59.18%, H: 3.41% ; Found: C: 59.32%, H: 3.91% .

(E)-3-(4-(difluoromethoxy)-3-hydroxyphenyl)-1-(4-fluorophenyl)prop-2-en-1-one (2g)

Yield 85% (yellow solid); m.p 131-134 ^OC; IR (KBr, cm^{-1}): 2987, 1649, 1573, 1042, 962, 836; ^1H NMR (400 MHz, DMSO-d6) δ 6.63 – 6.68 (s, 1H), 6.85 – 6.98 (m, 2H), 7.19 – 7.34 (m, 3H), 7.37 – 7.46 (d, J = 15.1 Hz, 1H), 7.85 – 7.96 (m, 3H) ; ES-MS :(m/z) 308.1 (M^+); Anal. Calcd. for $C_{16}H_{10}F_3O_3$: C: 62.34%, H: 3.60% ; Found: C: 62.67%, H: 3.42% .

(E)-3-(4-(difluoromethoxy)-3-hydroxyphenyl)-1-(thiophen-2-yl)prop-2-en-1-one (2h)

Yield 89% (yellow solid) ; m.p 172 -174 ^OC; IR (KBr, cm^{-1}): 3002, 1648, 1565, 1021, 983, 839; ^1H NMR (400 MHz, DMSO-d6) δ 6.63 – 6.68 (s, 1H), 6.90 – 7.04 (m, 3H), 7.17 – 7.25 (m,2H), 7.67 – 7.76 (d, J = 15.1, 1H), 7.77 – 7.86 (d, 2H); ES-MS :(m/z) 296.0 (M^+); Anal. Calcd. for $C_{14}H_{10}F_2NO_3S$: C: 56.75%, H: 3.40% ; Found: C: 56.66%, H: 3.67%.

(E)-3-(4-(difluoromethoxy)-3-hydroxyphenyl)-1-(pyridin-3-yl)prop-2-en-1-one (2i)

Yield 77% (yellow solid); m.p 166-169 ^OC; IR (KBr, cm^{-1}): 3007, 1667, 1569, 1043, 980, 851; ^1H NMR (400 MHz, DMSO-d6) δ 6.44 – 6.54 (m, 1H), 6.63 – 6.68 (s, 1H), 6.78 – 6.86 (m, 1H), 6.90 – 6.98 (d, 1H), 7.10 – 7.16 (d, 1H), 7.46 – 7.54 (d, 1H), 7.64 – 7.73 (d, J = 15.1, 1H), 8.27 – 8.35 (d, 1H), 8.67 – 8.73 (d, 1H), 9.14 – 9.20 (d, 1H); ES-MS :(m/z) 291.1 (M^+); Anal. Calcd. for $C_{15}H_{11}F_2NO_3$: C: 61.86%, H: 3.81%, N: 4.81%; Found: C: 61.71%, H: 3.78%, N: 4.98%.

General procedure for synthesis of *5-(4-(difluoromethoxy)-3-hydroxyphenyl)-4,5-dihydro-3-arylpyrazol-1-yl)(pyridin-4-yl)methanone* (3a-i).

A mixture of Chalcone **2a-i** (1.0 mol) in acetic acid (5 ml) and isoniazide (1.2 mol). The reaction mixture was heated at 120 ^OC for 8 hrs. After completion of the reaction, the reaction mixture was poured into crushed and basified to pH 7.5 using sodium bicarbonate and extracted with ethyl acetate (2 x 20 ml). The organic layer was washed with brine, dry over sodium sulphate and evaporated under reduced pressure. The crude product was washed with diethyl ether to give pure product.

(5-(4-(difluoromethoxy)-3-hydroxyphenyl)-4,5-dihydro-3-phenylpyrazol-1-yl)(pyridin-4-yl) methanone (3a)

Yield 78% (off white solid); m.p 171-173 ^{O}C; IR (KBr, cm^{-1}): 2939, 1602, 1458, 1011, 972, 856; ^{1}H NMR (400 MHz, DMSO-d6) δ 3.27 – 3.36 (dd, 1H), 3.53 – 3.63 (dd, 1H), 5.27 – 5.35 (dd, 1H), 6.63 – 6.68 (s, 1H), 6.73 – 6.84 (m, 2H), 6.85 – 6.92 (d, 1H), 7.34 – 7.44 (m, 2H), 7.48 – 7.58 (m, 1H), 7.63 – 7.71 (m, 2H), 7.76 – 7.83 (m, 2H), 8.75 – 8.81 (m, 2H).;ES-MS :(m/z) 409.1 (M^{+}); Anal. Calcd. for $C_{22}H_{17}F_2N_3O_3$: C: 64.54%, H: 4.19%, N: 10.26%; Found: C: 65.01%, H: 3.88, N: 10.39%.

(5-(4-(difluoromethoxy)-3-hydroxyphenyl)-4,5-dihydro-3-p-tolylpyrazol-1-yl)(pyridin-4-yl) methanone (3b)

Yield 77% (off white solid); m.p 165-167 ^{O}C; IR (KBr, cm^{-1}): 2946, 1586, 1465, 1014, 969, 859; ^{1}H NMR (400 MHz, DMSO-d6) δ 3.27 – 3.37 (dd, 1H), 3.53 – 3.63 (dd, 1H), 5.28 – 5.37 (dd, 1H), 6.63 – 6.68 (s, 1H), 6.75 – 6.93 (m, 4H), 7.17 – 7.25 (m, 2H), 7.44 – 7.52 (m, 2H), 7.77 – 7.83 (m, 2H), 8.75 – 8.81 (m, 2H).; ES-MS :(m/z) 423.1 (M^{+}); Anal. Calcd. for $C_{23}H_{19}F_2N_3O_3$: C: 65.24%, H: 4.52%, N: 9.92%; Found: C: 65.62%, H: 4.83%, N: 10.11%

(5-(4-(difluoromethoxy)-3-hydroxyphenyl)-4,5-dihydro-3-(4-methoxyphenyl)pyrazol-1-yl) (pyridin-4-yl) methanone (3c)

Yield 81% (off white solid); m.p 211-213 ^{O}C; IR (KBr, cm^{-1}): 2945, 1593, 1443, 1006, 984, 848; 1H NMR (400 MHz, DMSO-d6) δ 3.29 – 3.39 (dd, 1H), 3.51 – 3.61 (dd, 1H), 3.76 – 3.81 (s, 3H), 5.27 – 5.36 (dd, 1H), 6.63 – 6.68 (s, 1H), 6.78 – 6.99 (m, 5H), 7.58 – 7.66 (m, 2H), 7.79 – 7.85 (d, 2H), 8.75 – 8.81 (m, 2H);. ES-MS :(m/z) 439.1 (M+); Anal. Calcd. for $C_{23}H_{19}F_2N_3O_4$: C: 62.87%, H: 4.36%, N: 9.56%; Found: C: 63.02%, H: 4.89%, N: 9.87%

(5-(4-(difluoromethoxy)-3-hydroxyphenyl)-4,5-dihydro-3-(4-hydroxyphenyl)pyrazol-1yl) (pyridin-4-yl) methanone (3d)

Yield 76% (light yellow solid); m.p 194-196 ^{O}C, IR (KBr, cm^{-1}): 2986, 1593, 1445, 1027, 961, 833; 1H NMR (400 MHz, DMSO-d6) δ 3.32 – 3.42 (dd, 1H), 3.52 – 3.62 (dd, 1H), 5.30 – 5.39 (dd, 1H), 6.63 – 6.68 (s, 1H), 6.75 – 6.83 (m, 1H), 6.85 – 6.94 (m, 3H), 7.03 – 7.08 (d, 1H), 7.50 – 7.58 (m, 2H), 7.77 – 7.83 (d, 2H), 8.75 – 8.81 (m, 2H);ES-MS :(m/z) 425.1 (M^{+}); Anal. Calcd. for $C_{22}H_{17}F_2N_3O_4$: C: 62.12%, H: 4.03%, N: 9.88%; Found: C: 61.99%, H: 4.19%, N: 9.98%.

(3-(4-bromophenyl)-5-(4-(difluoromethoxy)-3-hydroxyphenyl)-4,5-dihydropyrazol-1-yl)(pyridin-4-yl)methanone (3e)

Yield 76% (light yellow solid); m.p 194-196 ^{O}C, IR (KBr, cm^{-1}): 2986, 1593, 1445, 1027, 961, 833; ^{1}H NMR (400 MHz, DMSO-d6) δ 3.26 – 3.35 (dd, 1H), 3.53 – 3.62 (dd, 1H), 5.27 – 5.35 (dd, 1H), 6.63 – 6.68 (s, 1H), 6.71 – 6.77 (d, 1H), 6.77 – 6.91 (m, 2H), 7.41 – 7.49 (m, 2H), 7.54 – 7.62 (m, 2H), 7.76 – 7.82 (d, 2H), 8.75 – 8.81 (m, 2H).; ES-MS :(m/z) 487.0 (M^{+}) and 489.0 (M^{+2}) ;

Anal. Calcd. for $C_{22}H_{16}BrF_2N_3O_3$: C: 54.12%, H: 3.30%, N: 8.61%; Found: C: 54.35%, H: 3.61%, N: 8.49%.

(3-(4-chlorophenyl)-5-(4-(difluoromethoxy)-3-hydroxyphenyl)-4,5-dihydropyrazol-1-yl)(pyridin-4-yl)methanone (3f)

Yield 86% (off white solid); m.p 169-171 ^{O}C; IR (KBr, cm^{-1}): 2963, 1596, 1453, 1037, 969, 836; ^{1}H NMR (400 MHz, DMSO-d6) δ 3.27 – 3.36 (dd, 1H), 3.53 – 3.63 (dd, 1H), 5.28 – 5.36 (dd, 1H), 6.63 – 6.68 (s, 1H), 6.72 – 6.77 (d, 1H), 6.78 – 6.92 (m, 2H), 7.59 – 7.64 (s, 4H), 7.76 – 7.83 (m, 2H), 8.75 – 8.81 (m, 2H); ES-MS :(m/z) 443.1 (M^{+}) and 445.1 (M^{+2}); Anal. Calcd. for: $C_{22}H_{16}ClF_2N_3O_3$ C: 59.54%, H: 3.63%, N: 9.47%; Found: C: 59.96%, H: 3.56%, N: 9.45%.

(5-(4-(difluoromethoxy)-3-hydroxyphenyl)-3-(4-fluorophenyl)-4,5-dihydropyrazol-1-yl)(pyridin-4-yl) methanone (3g)

Yield 77% (light yellow solid); m.p 203-205 ^{O}C; IR (KBr, cm^{-1}): 2927, 1587, 1453, 1034, 957, 843; ^{1}H NMR (400 MHz, DMSO-d6) δ 3.27 – 3.36 (dd, 1H), 3.54 – 3.63 (dd, 1H), 5.28 – 5.37 (dd, 1H), 6.63 – 6.68 (s, 1H), 6.72 – 6.77 (d, 1H), 6.78 – 6.92 (m, 2H), 7.33 – 7.43 (m, 2H), 7.64 – 7.74 (m, 2H), 7.76 – 7.83 (d, 2H), 8.75 – 8.81 (m, 2H);ES-MS :(m/z) 427.1 (M^{+}) and 428.1 (M^{+1}); Anal. Calcd. for $C_{22}H_{16}F_3N_3O_3$: C: 61.83%, H: 3.77%, N: 8.61%; Found: C: 62.32%, H: 4.15%, N: 8.81%.

(5-(4-(difluoromethoxy)-3-hydroxyphenyl)-4,5-dihydro-3-(thiophen-2-yl)pyrazol-1-yl)(pyridin-4-yl) methanone (3h)

Yield 78% (off white solid); m.p 157-159 ^{O}C; IR (KBr, cm^{-1}): 2972, 1593, 1445, 1027, 961, 833; ^{1}H NMR (400 MHz, DMSO-d6) δ 3.45 – 3.54 (dd, 1H), 3.58 – 3.68 (dd, 1H), 5.22 – 5.30 (dd, 1H), 6.63 – 6.68 (s, 1H), 6.78 – 6.90 (m, 2H), 6.95 – 7.03 (d, 1H), 7.06 – 7.14 (m, 1H), 7.22 – 7.29 (d, 1H), 7.53 – 7.61 (d, 1H), 7.63 – 7.70 (m, 2H), 8.75 – 8.81 (m, 2H);ES-MS :(m/z) 520.3 (M^{+}); Anal. Calcd. for $C_{20}H_{15}F_2N_3O_3S$: C: 57.83%, H, 3.64%, N: 10.12%; Found: C: 57.89%, H: 4.03%, N: 10.36%.

(5-(4-(difluoromethoxy)-3-hydroxyphenyl)-4,5-dihydro-3-(pyridin-3-yl)pyrazol-1-yl)(pyridin-4-yl) methanone (3i)

Yield 76% (off white solid); m.p 147-149 ^{O}C; IR (KBr, cm^{-1}): 2962, 1593, 1445, 1027, 961, 833; 1H NMR (400 MHz, DMSO-d6) δ 3.31 – 3.41 (dd, 1H), 3.54 – 3.63 (dd, 1H), 5.26 – 5.34 (m, 1H), 6.63 – 6.68 (s, 1H), 6.75 – 6.86 (m, 2H), 6.92 – 6.98 (d, 1H), 7.53 – 7.61 (d, 1H), 7.73 – 7.79 (d, 2H), 7.96 – 8.03 (d, 1H), 8.71 – 8.81 (m, 4H); ES-MS :(m/z) 499.4 (M^{+}); Anal. Calcd. for $C_{21}H_{16}F_2N_4O_3$: C: 61.46%, H: 3.93%, N: 13.65%; Found: C: 61.81%, H: 3.97%, N: 13.93%.

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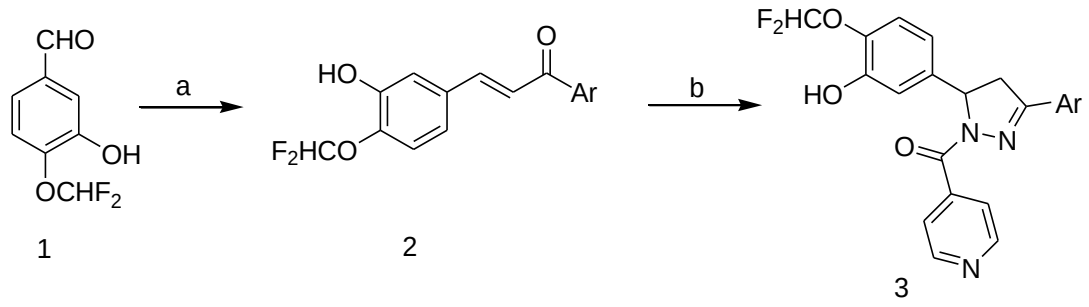
RESULTS AND DISCUSSION

Chemistry

The synthetic route adopted to obtain the Chalcone derivatives **2a-i** and Pyrazoline derivatives **3a-i** is shown in Scheme.



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Scheme 1: Reagents and conditions: (a) $ArCOCH_3$, 40% NaOH, Ethanol, RT, 6 hrs; (b) isoniazide , 120 ^{O}C, 8 hrs, gl. AcOH.

The Chalcones **2a-i** were prepared by conventional Claisen-Schmidt condensation of 4-(difluoromethoxy)-3-hydroxybenzaldehyde with substituted acetophenone using catalytic amount of 40% aq. NaOH in EtOH at room temperature. After recrystallization from ethanol all corresponding Chalcones were obtained in 68-90% yield. The pyrazoline derivatives **3a-i** were prepared from Chalcones **2a-i** by the reaction of isoniazide in acetic acid at 120 ^{O}C. The isolated product was washed with diethyl ether to get pyrazolines varied 66-72%. The structures of all newly synthesized compounds were assigned on the basis of spectral data such as IR, ^{1}H-NMR, Mass spectra and elemental analysis.

Spectral Analysis:

The structural assignment of the compounds **2a-i** and **3a-i** have been made on the basis of ^{1}H-NMR, Mass spectra , elemental analysis and IR spectral studies which were in full agreement with the proposed structures. The IR spectra of compounds **2a-i** showed a characteristic bands around 2947-3007 cm^{-1} due to C–H stretching vibrations. The strong bands observed between 1645-1670 cm^{-1} for carbonyl (C=O) stretching and 1565-1595 cm^{-1} for vinyl C=C stretching vibration. The □□□-unsaturated protons observed as a doublet with coupling constant (J) 15-16 Hz in the region δ 7.70-7.79 and δ 7.25-7.41. These observed coupling constant values indicate the presence of E-configuration in the Chalcone **2a-i**. The methyne group of -$OCHF_2$- observed as a singlet between δ 7.35-7.43. The remaining aromatic protons appeared at appropriate positions with appropriate multiplicity. The mass analysis of all the compounds **2a-i** showed M+H peak which support the formation of product. The compounds **3a-i** exhibited characteristic bands around 2927-2986 cm^{-1} due to C–H stretching vibrations, 1658-1682 cm^{-1} due to carbonyl stretching vibrations of acetyl group present in the compounds and 1587-1602 cm^{-1} due to C=N stretching vibrations of pyrazoline ring. ^{1}H-NMR of compounds

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3a-i exhibited a singlet around 2.38-2.42 and 2.30-2.32 integrating for three protons. The protons of pyrazoline ring appeared as doublet of doublet between 3.27 – 3.57, 3.51-3.68 and 5.21-5.81 integration for one proton. The remaining aromatic protons appeared at appropriate positions and with appropriate multiplicity. The mass analysis of all the compounds **3a-i** showed M+H peak which support the formation of product.

Antibacterial and antifungal activity

Table 1: Antibacterial and antifungal activity of all the synthesized compounds 2a-i and 3a-i in MIC (µg/mL)

Id	Ar	Antibacterial activity			Antifungal activity	
		S.aureus	*M.luteus*	*E.coli*	*S.typhi*	*C.albicans*
2a	C_6H_5-	80	100	100	80	120
2b	4-CH_3-C_6H_4-	80	100	80	100	120
2c	4-OCH_3-C_6H_4-	80	80	80	100	100
2d	4-OH-C_6H_4-	60	80	80	60	80
2e	4-Br-C_6H_4-	80	60	60	100	100
2f	4-Cl-C_6H_4-	60	80	80	60	80
2g	4-F-C_6H_4-	40	60	40	60	80
2h	2-Thiophenyl	80	60	80	80	100
2i	3-Pyridinyl	80	60	80	60	80
3a	C_6H_5-	80	80	80	60	80
3b	4-CH_3-C_6H_4-	60	60	80	80	80
3c	4-OCH_3-C_6H_4-C6HG_4-	60	60	80	60	80
3d	4-OH-C_6H_4-	60	40	60	60	80
3e	4-Br-C_6H_4-	40	40	40	40	60
3f	4-Cl-C_6H_4-	60	40	40	60	80
3g	4-F-C_6H_4-	40	20	20	20	60
3h	2-Thiophenyl	40	40	60	40	60
3i	3-Pyridinyl	40	40	60	40	60
Ampiciline		10	10	20	10	-
Fluconazole		-	-	-	-	5

The newly synthesized compounds were screened for their antibacterial activity against Gram-positive (*S. aureus* ATCC 6538, *M. luteus* ATCC 9345), Gram negative (*E. coli* ATCC 4230, *S.thphi* ATCC 14028) bacteria, as described by the guidelines in NCCLS-approved standard document M7-A4, using the micro dilution broth procedure[16] Ampicillin trihydrate was used as the reference antibacterial agent. The antifungal activities of the newly synthesized chemical compounds were tested against yeast strain (*C. albicans* ATCC 14053) according to the guidelines in NCCLS-approved standard document M27-A2, using the micro dilution broth procedure[17]. Fluconazole was used as the reference antifungal agent. The solutions of test compounds and reference drug were prepared by dissolving in DMSO at a concentration of 2560 µg/mL. The 2-fold dilutions of the compounds and the reference drug were prepared (1280, 640, 320, 160, 80, 40, 20, 10, 5 µg/mL). Antibacterial activities of the newly synthesized

chemical compounds were performed in Mueller-Hinton broth medium at a pH of 7.2 with an inoculum of (1-2) × 10^3 cells/mL by the spectrophotometric method, and an aliquot of 100µL solution was added to each tube of serial dilution. The chemical compounds-broth medium serial tube dilutions inoculated with each bacterium were incubated on a rotary shaker at 37°C for 18 hr at 150 rpm. The minimum inhibitory concentration (MIC) of each chemical compound was recorded as the lowest concentration of each chemical compound in the tubes with no growth (i.e., no turbidity) of inoculated bacteria. Minimum inhibitory concentration (MIC, µg/mL) was measured and compared with control; the MIC values of the compound screened are given in Table 1.

From the result of biological evaluation, it has been observed that the compounds exhibited interesting biological activity, however with a degree of variation. Most of the compounds tested were found to have moderate antibacterial and exhibit very low antifungal activity. From the Table 1, it can be observed that compounds 2d, 2e, 2g, 2h, 4i, 3d, 3e, 3g 3f, 3h and 3i showed moderate activity against *S. aureus* ATCC 6538, *M. luteus* ATCC 9345, *E.coli* ATCC 4230 and *S.thphi* ATCC 14028, while all the synthesized compounds lack antifungal activity against *C. albicans* ATCC 14053. It was observed that Pyrazoline showed improvement in activity compared to the corresponding Chalcone. The incorporation of heterocyclic moiety showed an improvement in antibacterial activity of corresponding Chalcone (2h & 2i) and pyrazoline (3h & 3i). However, similar improvement was not observed in case of antifungal activity.

CONCLUSION

In summary, we have synthesized a series of vanillin incorporated new Chalcone and Pyrazoline derivatives. All the newly synthesized compounds were confirmed with spectroscopic data like ^{1}H-NMR, Mass, IR Spectra, elemental analysis and evaluated antibacterial and antifungul activity. The antibacterial study shows that Chalcones (2d, 2e, 2h and 2i) have moderate activity with MICs between 60 and 100 µg/mL. Its pyrazoline derivatives (3d, 3e, 3f, 3g, 3h, 3i) showed improvement in activity with MICs between 20 and 80 µg/ mL. The Chalcones and Pyrazolines showed very low antifungal activity. The importance of such work lies in the possibility that the new compounds might be more efficacious drugs against bacteria, which could be helpful in designing more potent antibacterial agent for therapeutic use.

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REFERENCES

[1] (a) Eicher T, Hauptmann S. The Chemistry of Heterocycles, 2nd ed.; Wiley-VCH: Weinheim, 2003. (b) Suresh M, Jonnalagadda S B, Rao CV. Oriental. J. Chem. 2011;27:127.

[2] (a) Alcaraz LE, Blanco SE, Puig ON, Tomas F, Ferretti FH. J. Theor. Biol. 2000;205:231–240. (b) Baviskar BA, Baviskar B, Shiradkar MR, Deokate UA, Khadabadi SS. E-J. Chem. 2009;6: 196–200.
[3] (a) Echeverria C, Santibañez JF, Donoso-Tauda O, Escobar CA, Ramirez-Tagle R. Int. J. Mol. Sci. 2009;10:221–231. (b) Modzelewska A, Pettit C, Achanta G, Davidson NE, Huang P, Khan SR. Bioorg. Med. Chem. 2006;14:3491–3495. (c) Vogel S, Heilmann, J. J. Nat. Prod. 2008;71:1237–1241.
[4] (a) Babasaheb, P.B.; Sachin, A.P.; Rajesh, N.G. Bioorg. Med. Chem. Lett. 2010, 20, 730–733. (b) Vogel, S.; Barbic, M.; Jürgenliemk, G.; Heilmann, J. Eur. J. Med. Chem. 2010, 45, 2206–2213. (c) Tran, T. D.; Park, H.; Kim, H.P.; Ecker, G.F.; Thai, K.M. Bioorg. Med. Chem. Lett. 2009, 19, 1650–1653.
[5] (a) Beom-Tae K, Kwang-Joong O, Jae-Chul C, Ki-Jun H. Bull. Korean Chem. Soc. 2008;29: 1125–1130. (b) Doan TN, Tran TD. Pharmacol. Pharm. 2011;2:282–288. (c) Sivakumar, PM, Prabhakar PK, Doble M. Med. Chem. Res. 2011;20:482–492.
[6] (a) Chen RM, Hu LH, An TY, Li J, Shen Q. Bioorg. Med. Chem. Lett. 2002, 12, 3387. (b) Sun LP, Gao LX, Ma WP, Nan FJ, Li. J. Piao, H. R. Chem. Biol. Drug Des. 2012;80:584. (c) Liu Z, Lee W, Kim SN, Yoon, G.; Cheon, S.H. Bioorg. Med. Chem. Lett. 2011;21:3755.
[7] Polevoi lG, Nauchn TR and Konf Aspir Ordin. 1-yi(peruyi) Mosk Med Inst Moscow. 1964;159; Chem Abstr.1996;65:19147d.
[8] Batulin YM. Farmakol. Toksikol. 1968;31:533–536.
[9] Parmar SS, Pandey BR, Dwivedi C, Harbinson RD. J. Pharm. Sci. 1974;63:1152–1155.
[10] Soni N, Pande K. Pande, Kalsi R. Kalsi, Gupta TK, Parmar SS, Barthwal JP. Res. Commun. Chem. Pathol. Pharmacol. 1987;57:129–132.
[11] Turan-Zitouni G., Chevallet P.,F. S. Kilic, Erol K. ,Eur. J. Med. Chem.,2000,35, pp. 635–641.
[12] Erhan P, Mutlu A, Tayfun U, Dilek E, Eur. J. Med. Chem,2001;36:539–543.
[13] (a) Walton NJ, Mayer MJ, and Narbad A. Phytochemistry 2003;63:505–515. (b) Burri J, Graf M, Lambelet P, and Lo¨liger, J. J. Sci. Food. Agric. 1989;48(1):49–56.
[14] Jay JM. and Rivers GMJ. Food Safety, 1984;6:129–139.
[15] Eric M. Guantai, Kanyile Ncokazi , Timothy J. Egan, Jiri Gut, Philip J. Rosenthal , Peter J. Smith, Kelly Chibale, Bioorg. Med. Chem. 2010;18(23): 8243–8256.
[16] Clause GW. Understanding Microbes: A Laboratory Textbook for Microbiology, W.H. Freeman and Company, New York, USA, 1989.
[17] National Committee for Clinical Laboratory Standards. Performance Standards for antimicrobial disk susceptibility test, NCCLS, Villanova, PA, 1997.