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RESEARCH ARTICLE

Synthesis and evaluation of *in vitro* antiviral activity of 2-[3-(substituted phenyl)-4,5-dihydro-1*H*-5-pyrazolyl] benzofuran-3-yl chloride derivatives

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Abstract

3-Chlorobenzofuran-2-carbaldehyde was condensed with substituted acetophenone by using the Claisen-Schmidt condensation to obtain 3-(3-chlorobenzofuran-2-yl)-1-(substituted phenyl)-2-propen-1-one **(2a-m)** which upon further treatment with hydrazine hydrate gave 2-[3-(substituted phenyl)-4,5-dihydro-1*H*-5-pyrazolyl) benzofuran-3-yl chloride derivatives **(3a-m)**. All the newly synthesized derivatives were evaluated *in vitro* for cytotoxicity and antiviral activity in Crandell-Rees Feline Kidney cell, human embryonic lung (HEL) cell, HeLa cell and Vero cell cultures against different viruses. Several compounds, i.e. **2f, 2g, 2i, 2m, 3b, 3d, 3g, 3h** and **3m** proved quite cytotoxic to the host cells (minimum cytotoxic concentration: 1-10 µg/mL). No specific antiviral activity [50% antivirally effective concentration (EC_{50}) ≥ 5-fold lower than the minimum cytotototoxic concentration] was observed for any of the compounds.

Keywords: *pyrazoline; antiviral; cytotoxic; 3-chlorobenzofuran-2-carbaldehyde*

Introduction

In view of the high occurrence of viral infections, increasing number of relapses and narrow antiviral spectrum of existing drugs, antiviral drug development programs have been intensified. Similarity in the metabolism of virus-infected as compared to uninfected cells makes it difficult to destroy a virus without affecting the host cell; and, hence cytotoxicity is commonly associated with antiviral agents. Attempts are being made to develop more efficient drugs that allow greater inhibition of viruses, greater selectivity for virus-specific functions, and fewer side effects, and may avoid emergence of drug-resistant mutants.

In continuation of our work on pyrazolines (1-4) these compounds have been accredited with a variety of activities viz. antidepressant (5), antimycobacterial (6), anti-inflammatory (7), antibacterial and anti-fungal (8), anticonvulsant (9), MAO inhibiting (10) and anti-androgenic (11). Here we report the synthesis and biological properties of some 3-chloro benzofuran-2-carbaxaldehyde derived chalcones and their pyrazolines.

3-(3-chlorobenzofuran-2-yl)-1-(substituted phenyl)-2-propen-1-one **(2a-m),** obtained by Claisen Schmidt reaction of 3-chlorobenzofuran-2-carbaldehyde and appropriately substituted acetophenone in ethanolic solution of NaOH, were subjected to cyclo-condensation with hydrazine hydrate to obtain the title compounds **(3a-m)**.

Materials

All the chemicals used were laboratory grade and provided by E. Merck (Germany) and S.D. Fine Chemicals (India). Melting points were determined by open tube capillary method and are uncorrected. Thin layer chromatography (TLC) plates, (silica gel G) were used to confirm the purity of the commercial reagents used, the compounds synthesized and to monitor the reactions as well. Two different solvent systems; toluene: ethyl acetate: formic acid (5:4:1) and petroleum ether: toluene: acetic acid (5:4:1), were used to run the TLC. The spots were visualized under iodine vapors/UV light. IR spectra were obtained on a Perkin-Elmer 1720 FT-IR

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spectrometer (KBr Pellets). ^{1}H NMR spectra were recorded on Bruker AC 400 MHz, spectrometer using TMS as internal standard in $CDCl_3$ / DMSO-d_6. The FAB mass spectra were recorded on a JEOL SX 102/DA-6000 Mass Spectrometer.

Methods

Chemistry

General procedure for synthesis of chalcones (2a-m)

To 0.01mole ethanolic solution (25mL) of 3-chlorobenzofuran-2-carbaldehyde, an appropriately substituted acetophenone (0.01mole) & 5mL 10% aqueous solution of NaOH was added. The reaction mixture was stirred at 25°C for 2-3 h. After completion of the reaction, the contents were poured onto the crushed ice, and neutralized with dilute HCl. The precipitates so obtained were filtered, washed with water, dried and re-crystallized from ethanol to get the desired product **(2a-m)**.

3-(3-chlorobenzofuran-2-yl)-1-phenyl-2-propen-1-one **(2a) IR (KBr, cm^{-1}):** 1685 (C=O), 1606 (C=C), 755 (C-Cl). **^{1}H-NMR (DMSO-d_6, δ ppm):** 6.80 (1H, d, Hα, *J* = 8.4 Hz), 7.26-7.66 (9H, m, Ar-H), 7.68 (1H, d, Hβ, *J* = 8.8 Hz). Anal. Calcd. for $C_{17}H_{11}ClO_2$: C, 72.22; H, 3.92. Found: C, 72.06; H, 3.93%.

3-(3-chlorobenzofuran-2-yl)-1-(4-chlorophenyl)-2-propen-1-one **(2b) IR (KBr, cm^{-1}):**1682 (C=O), 1605 (C=C), 755 (C-Cl). **^{1}H-NMR (DMSO-d_6, δ ppm):** 6.86 (1H, d, Hα, *J* = 8.0 Hz), 6.90 (2H, d, Ar-H, *J* = 8 Hz), 7.26 (4H, m, Ar-H), 7.66 (2H, d, Ar-H, *J* = 8.8 Hz), 7.96 (1H, d, Hβ, *J* = 8.4 Hz). Anal. Calcd. for $C_{17}H_{10}Cl_2O_2$: C, 64.38; H, 3.18. Found: C, 64.16; H, 3.19%.

3-(3-chlorobenzofuran-2-yl)-1-(4-methylphenyl)-2-propen-1-one **(2c) IR (KBr, cm^{-1}):** 1690 (C=O), 1601 (C=C), 755 (C-Cl). **^{1}H-NMR (DMSO-d_6, δ ppm):** 2.46 (3H, s, -CH_3), 6.72 (1H, d, Hα, *J* = 8.0 Hz), 7.25-7.39 (5H, m, Ar-H), 7.48 (1H, d, Ar-H, *J* = 6.4 Hz), 7.66 (2H, d, Ar-H, *J* = 8.4 Hz), 7.86 (1H, d, Hβ, *J* = 8.0 Hz). Anal. Calcd. for $C_{18}H_{13}ClO_2$: C, 72.85; H, 4.42. Found: C, 72.96; H, 4.41%.

3-(3-chlorobenzofuran-2-yl)-1-(4-nitrophenyl)-2-propen-1--one **(2d) IR (KBr, cm^{-1}):** 1680 (C=O), 1608 (C=C), 1523 & 1377 (NO_2), 747 (C-Cl). **^{1}H-NMR (DMSO-d_6, δ ppm):** 6.79 (1H, d, Hα, *J* = 8.8 Hz), 6.95(2H, d, Ar-H, *J* = 8.4 Hz) 7.32-7.56 (4H, m, Ar-H), 7.60 (2H, d, Ar-H, *J* = 8.4 Hz), 7.65 (1H, d, Hβ, *J* = 8.8 Hz). Anal. Calcd. for $C_{17}H_{10}ClNO_4$: C, 62.30; H, 3.08; N, 4.27. Found: C, 62.22; H, 3.08; N, 4.28%.

3-(3-chlorobenzofuran-2-yl)- 1-(4-aminophenyl)-2-propen-1-one **(2e) IR (KBr, cm^{-1}):** 1685 (C=O), 1606 (C=C), 755 (C-Cl). **^{1}H-NMR (DMSO-d_6, δ ppm):** 3.23 (2H, s, NH_2), 6.86 (1H, d, Hα, *J* = 8.4 Hz), 7.26-7.48 (4H, m, Ar-H), 7.49 (1H, d, Ar-H, *J* = 6.4 Hz), 7.51 (1H, d, Ar-H, *J* = 6.4 Hz), 7.55 (2H, d, Ar-H, *J* = 8.4 Hz), 7.85 (1H, d, Hβ, *J* = 8.8 Hz). Anal. Calcd. for $C_{17}H_{12}ClNO_2$: C, 68.58; H, 4.06; N, 4.70. Found: C, 68.46; H, 4.08 ; N, 4.72%.

3-(3-chlorobenzofuran-2-yl)-1-(4-hydroxyphenyl)-2-propen-1-one **(2f) IR (KBr, cm^{-1}):** 3320 (OH), 1685 (C=O), 1606 (C=C), 755 (C-Cl). **^{1}H-NMR (DMSO-d_6, δ ppm):** 6.81 (1H, d, Hα, *J* = 8.4 Hz), 7.22-7.64 (8H, m, Ar-H), 7.87 (1H, d, Hβ, *J* = 8.8 Hz), 9.90 (1H, s, OH). **Mass m/z:** 299 (M^{+1}). Anal. Calcd. for $C_{17}H_{11}ClO_3$: C, 68.35; H, 3.71. Found: C, 68.38; H, 3.72%.

3-(3-chlorobenzofuran-2-yl)-1-(2-hydroxyphenyl)-2-propen-1-one **(2g) IR (KBr, cm^{-1}):** 3528 (OH), 1682 (C=O), 1614 (C=C), 743 (C-Cl). **^{1}H-NMR (DMSO-d_6, δ ppm):** 6.94 (1H, d, Hα, *J* = 8.0 Hz), 7.23-7.81 (8H, m, Ar-H), 7.92 (1H, d, Hβ, *J* = 8.0 Hz), 10.11 (1H, s, OH). Anal. Calcd. for $C_{17}H_{11}ClO_3$: C, 68.35; H, 3.71. Found: C, 68.28; H, 3.70%.

3-(3-chlorobenzofuran-2-yl)-1-(4-methoxyphenyl)-2-propen-1-one **(2h) IR (KBr, cm^{-1}):** 1685 (C=O), 1645 (C=C), 750 (C–Cl).; **^{1}H-NMR (DMSO-d_6, δ ppm):** 3.52 (3H, s, OCH_3), 6.82 (1H, d, Hα, *J* = 8.4 Hz), 6.91 (2H, d, Ar-H, *J* = 8 Hz), 6.99-7.67 (5H, m, Ar-H), 7.69 (1H, d, Ar-H, *J* = 6.8 Hz), 7.78 (1H, d, Hβ, *J* = 8.8 Hz). Anal. Calcd. for $C_{18}H_{13}ClO_3$: C, 69.13; H, 4.19. Found: C, 69.26; H, 4.20%.

3-(3-chlorobenzofuran-2-yl)-1-(2,4-dihydroxyphenyl)-2-propen-1-one **(2i) IR (KBr, cm^{-1}):** 3530 (OH), 1682 (C=O), 1606 (C=C), 755 (C-Cl). **^{1}H-NMR (DMSO-d_6, δ ppm):** 6.83 (1H, d, Hα, *J* = 8.8 Hz), 7.26-7.67 (7H, m, Ar-H), 7.72 (1H, d, Hβ, *J* = 8.8 Hz) 10.23 (2H, s, 2 x OH). Anal. Calcd. for $C_{17}H_{11}ClO_4$: C, 64.88; H, 3.52. Found: C, 64.90; H, 3.53%.

3-(3-chlorobenzofuran-2-yl)-1-(2,4-dimethoxyphenyl)-2-propen-1-one **(2j) IR (KBr, cm^{-1}):** 1685 (C=O), 1653 (C=C), 735 (C-Cl). **^{1}H-NMR (DMSO-d_6, δ ppm):** 3.66 (6H, s, 2 x OCH_3), 6.92 (1H, d, Hα, *J* = 8.0 Hz), 7.24-7.84 (7H, m, Ar-H), 7.89 (1H, d, Hβ, *J* = 8.4 Hz). Anal. Calcd. for $C_{19}H_{15}ClO_4$: C, 66.58; H, 4.41. Found: C, 66.66; H, 4.40%.

*3-(3-chlorobenzofuran-2-yl)-1-(3,4-dimethoxyphenyl)-2-propen-1-one***(2k) IR (KBr, cm^{-1}):** 1685 (C=O), 1602 (C=C), 765 (C-Cl). **^{1}H-NMR (DMSO-d_6, δ ppm):** 3.68 (6H, s, 2 x OCH_3), 6.82 (1H, d, Hα, *J* = 8.4 Hz), 6.89-7.68 (7H, m, Ar-H), 7.29 (1H, d, Hβ, *J* = 8.0 Hz). Anal. Calcd. for $C_{19}H_{15}ClO_4$: C, 66.58; H, 4.41. Found: C, 66.48; H, 4.42%.

3-(3-chlorobenzofuran-2-yl)-1-(4-hydroxy-3-methylphenyl)-2-propen-1-one **(2l) IR (KBr, cm^{-1}):** 3530 (OH), 1685 (C=O), 1606 (C=C), 756 (C-Cl). **^{1}H-NMR (DMSO-d_6, δ ppm):** 2.26 (3H, s, CH_3), 6.76 (1H, d, Hα, *J* = 8.8 Hz), 6.95-7.62 (7H, m, Ar-H), 7.68 (1H, d, Hβ, *J* = 8.8 Hz) 8.83 (1H, s, OH). Anal. Calcd. for $C_{18}H_{13}ClO_3$: C, 69.13; H, 4.19. Found: C, 69.26; H, 4.22%.

3-(3-chlorobenzofuran-2-yl)-1-(4-hydroxy-2-methylphenyl)-2-propen-1-one **(2m)IR (KBr, cm^{-1}):** 3530 (OH), 1686 (C=O), 1606 (C=C), 754 (C-Cl).; **^{1}H-NMR (DMSO-d_6, δ ppm):** 2.42 (3H, s, CH_3), 6.83 (1H, d, Hα, *J* = 8 Hz), 7.27-7.67 (7H, m, Ar-H), 7.68 (1H, d, Hβ, *J* = 8.4 Hz), 8.93 (1H, s, OH). Anal. Calcd. for $C_{18}H_{13}ClO_3$: C, 69.13; H, 4.19. Found: C, 69.12; H, 4.22%.

General procedure for synthesis of compound (3a-m)

To an ethanolic solution of **2a-m** (0.01 mol; 20mL), a solution of hydrazine hydrate(0.015mol) in 5mL glacial acetic acid was added, the reaction mixture was allowed to reflux for 3-6 h in presence of 100mg of 5A x 1.5mm molecular sieves. After completion of the reaction, the contents were concentrated and poured onto 100 g crushed ice, and neutralized with ammonia. Solid mass was filtered, washed with plenty

of cold water and recrystallized with hydrated ethanol to get the pure product **(3a-m)**.

2-(3-phenyl-4,5-dihydro-1H-5-pyrazolyl]benzofuran-3-yl chloride **(3a) IR (KBr, cm^{-1}):** 3580 (N-H), 1596 (C=N), 1324 (C-N), 757 (C-Cl). **^{1}H -NMR (DMSO-d$_{6,}$ ppm):** 3.49 (1H, dd, Ha, *J* = 5.6, 5.6 Hz), 3.73 (1H, dd, Hb, *J* = 12.0, 12.4 Hz), 5.87 (1H, dd, Hx, *J* = 5.6, 5.6 Hz), 7.2-7.8 (9H, m, Ar-H). 8.5 (1H, s, NH). Anal. Calcd. for $C_{17}H_{13}ClN_2O$: C, 68.81; H, 4.42; N, 9.44. Found: C, 68.72; H, 4.41; N, 9.45%.

2-[3-(4-chlorophenyl)-4,5-dihydro-1H-5-pyrazolyl] benzofuran-3-yl chloride **(3b) IR (KBr, cm^{-1}):** 3569 (N-H), 1596 (C=N), 1322 (C-N), 747 (C-Cl). **^{1}H-NMR (CDCl$_3$-d$_{6,}$ ppm):** 3.53 (1H, dd, Ha, *J* = 5.2, 5.6 Hz), 4.24 (1H, dd, Hb, *J* = 12, 12 Hz), 5.72 (1H, dd, Hx, *J* = 5.6, 5.6 Hz), 6.96 (1H, d, Ar-H, *J* = 6.4 Hz), 7.34 (2H, m, Ar-H and NH pyrazoline), 7.52 (1H, d, Ar-H, *J* = 6.4 Hz), 7.58 (2H, d, Ar-H, *J* = 7.6 Hz), 7.80 (2H, d, Ar-H, *J* = 8 Hz). Anal. Calcd. for $C_{17}H_{12}Cl_2N_2O$: C, 61.65; H, 3.65; N, 8.46. Found: C, 61.54; H, 3.64; N, 8.45%.

2-[3-(4-methylphenyl)-4,5-dihydro-1H-5-pyrazolyl] benzofuran-3-yl chloride **(3c) IR (KBr, cm^{-1}):** 3560 (N-H), 1590 (C=N), 1326 (C-N), 747 (C-Cl). **^{1}H -NMR (CDCl$_3$-d$_{6,}$ ppm):** 2.40 (3H, s, -CH_3), 3.41 (1H, dd, Ha, *J* = 5.6, 5.6 Hz), 3.65 (1H, dd, Hb, *J* = 12, 12.4 Hz), 5.82 (1H, dd, Hx, *J* = 5.6, 5.6 Hz), 7.25 (2H, d, Ar-H, *J* = 8 Hz), 7.34 (1H, d, Ar-H, *J* = 7.6 Hz) 7.48 (2H, m, Ar-H and NH pyrazoline), 7.52 (1H, d, Ar-H, *J* = 6.8 Hz), 7.65 (2H, d, Ar-H, *J* = 8 Hz). Anal. Calcd. for $C_{18}H_{15}ClN_2O$: C, 69.57; H, 4.86; N, 9.01. Found: C, 69.68; H, 4.85; N, 9.00%.

2-[3-(4-nitrophenyl)-4,5-dihydro-1H-5-pyrazolyl] benzofuran-3-yl chloride **(3d) IR (KBr, cm^{-1}):** 3569 (N-H), 1596 (C=N), 1322 (C-N), 747 (C-Cl).; **^{1}H -NMR (CDCl$_3$-d$_{6,}$ ppm):** 3.22 (1H, dd, Ha, *J* = 5.6, 5.6 Hz), 4.10 (1H, dd, Hb, *J* = 12.0, 12.0 Hz), 5.52 (1H, dd, Hx, *J* = 5.6, 5.2 Hz), 6.95(2H, d, Ar-H, *J* = 8.4 Hz), 7.32-7.43 (4H, m, Ar-H and pyrazoline), 7.90 (2H, d, Ar-H, *J* = 8.4 Hz). Anal. Calcd. for $C_{17}H_{12}ClN_3O_3$: C, 59.75; H, 3.54; N, 12.30. Found: C, 59.64; H, 3.56; N, 12.28%.

2-[3-(4-aminophenyl)-4,5-dihydro-1H-5-pyrazolyl] benzofuran-3-yl chloride **(3e) IR (KBr, cm^{-1}):** 3280 (N-H), 1596 (C=N), 1324 (C-N), 757 (C-Cl). **^{1}H -NMR (CDCl$_{3,}$ ppm):** 2.38 (2H, s, NH_2), 3.40 (1H, dd, Ha, *J* = 5.6, 5.6 Hz), 3.64 (1H, dd, Hb, *J* = 12.0, 12.8 Hz), 5.82 (1H, dd, Hx, *J* = 5.6, 5.6 Hz), 7.26 (2H, m, Ar-H), 7.34 (1H, d, Ar-H, *J* = 6.8 Hz), 7.43 (1H, s, NH pyrazoline), 7.51 (1H, d, Ar-H, *J* = 6.8 Hz), 7.58 (2H, d, Ar-H, *J* = 7.6 Hz), 7.60 (2H, d, Ar-H, *J* = 8 Hz). Anal. Calcd. for $C_{17}H_{14}ClN_3O$: C, 65.49; H, 4.53; N, 13.48. Found: C, 65.48; H, 4.52; N, 13.50%.

2-[3-(4-hydroxyphenyl)-4,5-dihydro-1H-5-pyrazolyl] benzofuran-3-yl chloride **(3f) IR (KBr, cm^{-1}):** 3532 (OH), 3285 (N-H), 1594 (C=N), 1324 (C-N), 756 (C-Cl).; **^{1}H-NMR (DMSO-d$_{6,}$ ppm):** 3.79 (1H, dd, Ha, *J* = 5.2, 4.8 Hz), 4.07 (1H, dd, Hb, *J* = 12.4, 12.8 Hz), 6.07 (1H, dd, Hx, *J* = 5.6, 5.6Hz), 7.27(2H, d, Ar-H, *J* = 8 Hz), 7.64-7.71 (3H, m, Ar-H and NH pyrazoline), 7.76(1H, d, Ar-H, *J* = 7.2 Hz), 8.00 (2H, d, Ar-H, *J* = 8 Hz), 9.91 (1H, s, OH). **Mass m/z:** 312 (M^+). Anal. Calcd. for $C_{17}H_{13}ClN_2O_2$: C, 65.29; H, 4.19; N, 8.96. Found: C, 65.30; H, 4.20; N, 8.97%.

2-[3-(2-hydroxyphenyl)-4,5-dihydro-1H-5-pyrazolyl] benzofuran-3-yl chloride **(3g) IR (KBr, cm^{-1}):** 3538 (OH), 3295 (N-H), 1596 (C=N), 1324 (C-N), 754 (C-Cl). **^{1}H -NMR (DMSO-d$_{6,}$ ppm):** 3.62 (1H, dd, Ha, *J* = 5.6, 5.6 Hz), 4.68 (1H, dd, Hb, *J* = 12.4, 12.4 Hz), 6.01 (1H, dd, Hx, *J* = 5.6, 5.2 Hz), 7.26-7.73 (6H, m, Ar-H and NH pyrazoline), 7.74 (1H, d, Ar-H, *J* = 6.8 Hz), 7.78 (1H, d, Ar-H, *J* = 6.8 Hz), 8.92 (1H, s, OH). Anal. Calcd. for $C_{17}H_{13}ClN_2O_2$: C, 65.29; H, 4.19; N, 8.96. Found: C, 65.38; H, 4.20; N, 8.95%.

2-[3-(4-methoxyphenyl)-4,5-dihydro-1H-5-pyrazolyl]benzofuran-3-yl chloride **(3h) IR (KBr, cm^{-1}):** 3560 (N-H), 1590 (C=N), 1326 (C-N), 750 (C–Cl). **^{1}H -NMR (DMSO-d$_{6,}$ ppm):** 3.39 (1H, dd, Ha, *J* = 5.6, 5.6 Hz), 3.63 (1H, dd, Hb, *J* = 12.4, 12.0 Hz), 3.85 (3H, s, -OCH_3), 5.81 (1H, dd, Hx, *J* = 5.6, 5.6 Hz), 6.94 (2H, d, Ar-H, *J* = 8.4 Hz), 7.26-7.32 (2H, m, Ar-H and NH pyrazoline), 7.34 (1H, d, Ar-H, *J* = 8.0 Hz), 7.51 (1H, d, Ar-H, *J* = 6.4 Hz), 7.70 (2H, d, Ar-H, *J* = 8.8 Hz). Anal. Calcd. for $C_{18}H_{15}ClN_2O_2$: C, 66.16; H, 4.63; N, 8.57. Found: C, 66.20; H, 4.64; N, 8.55%.

2-[3-(2,4-dihydroxyphenyl)-4,5-dihydro-1H-5-pyrazolyl] benzofuran-3-yl chloride **(3i) IR (KBr, cm^{-1}):** 3530 (OH), 3280 (N-H), 1596 (C=N), 1324 (C-N), 757 (C-Cl). **^{1}H -NMR (DMSO-d$_{6,}$ ppm):** 3.45 (1H, dd, Ha, *J* = 5.6, 5.6 Hz), 3.98 (1H, dd, Hb, *J* = 12, 12 Hz), 6.11 (1H, dd, Hx, *J* = 5.6, 5.6 Hz), 6.98-7.42 (3H, m, Ar-H), 7.45 (1H, d, Ar-H, *J* = 6.8 Hz), 7.61-7.72 (3H, m, Ar-H and NH pyrazoline), 10.2 (2H, s, 2 X OH). Anal. Calcd. for $C_{17}H_{13}ClN_2O_3$: C, 62.11; H, 3.99; N, 8.52. Found: C, 62.10; H, 4.00; N, 8.51%.

*2-[3-(2,4-dimethoxyphenyl)-4,5-dihydro-1H-5-pyrazolyl] benzofuran-3-yl chloride***(3j) IR (KBr, cm^{-1}):** 3280 (N-H), 1590 (C=N), 1324 (C-N), 755 (C-Cl). **^{1}H -NMR (CDCl$_3$-d$_{6,}$ ppm):** 3.36 (1H, dd, Ha, *J* = 5.6, 5.6 Hz), 3.62 (1H, dd, Hb, *J* = 12, 12 Hz), 3.86 (6H, s, 2 X OCH_3), 5.82 (1H, dd, Hx, *J* = 5.6, 5.6 Hz), 7.26-7.83 (7H, m, Ar-H and NH pyrazoline). Anal. Calcd. for $C_{19}H_{17}ClN_2O_3$: C, 63.96; H, 4.80; N, 7.85. Found: C, 64.05; H, 4.81; N, 7.84%.

2-[3-(3,4-dimethoxyphenyl)-4,5-dihydro-1H-5-pyrazolyl] benzofuran-3-yl chloride **(3k) IR (KBr, cm^{-1}):** 3280 (N-H), 1596 (C=N), 1324 (C-N), 757 (C-Cl). **^{1}H -NMR (CDCl$_3$-d$_{6,}$ ppm):** 3.38 (1H, dd, Ha, *J* = 5.2, 5.2 Hz), 3.59 (1H, dd, Hb, *J* = 12.4, 12.4 Hz), 3.89 (6H, s, 2 X OCH_3), 6.17 (1H, dd, Hx, *J* = 5.2, 5.2 Hz), 6.98 (1H, s, Ar-H), 7.06-7.13 (3H, m, Ar-H), 7.17 (1H, bs, NH), 7.50-7.58 (3H, m, Ar-H). Anal. Calcd. for $C_{19}H_{17}ClN_2O_3$: C, 63.96; H, 4.80; N, 7.85. Found: C, 64.08; H, 4.80; N, 7.84%.

2-[3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-5-pyrazolyl]benzofuran-3-yl chloride **(3l) IR (KBr, cm^{-1}):** 3536 (OH), 3279 (N-H), 1590 (C=N), 1324 (C-N), 755 (C-Cl). **^{1}H -NMR (DMSO-d$_{6,}$ ppm):** 2.38 (2H,d,CH_2 pyrazoline), 2.43 (3H, s, CH_3), 4.52 (1H,t,CH), 7.23-7.72 (7H, m, Ar-H and NH), 9.92 (1H, s, OH). Anal. Calcd. for $C_{18}H_{15}ClN_2O_2$: C, 66.16; H, 4.63; N, 8.57. Found: C, 66.15; H, 4.62; N, 8.58%.

2-[3-(4-hydroxy-2-methylphenyl)-4,5-dihydro-1H-5-pyrazolyl]benzofuran-3-yl chloride **(3m) IR (KBr, cm^{-1}):** 3535 (OH), 3285(N-H), 1594 (C=N), 1327 (C-N), 754 (C-Cl). **^{1}H -NMR (DMSO-d$_{6,}$ ppm):** 2.36 (2H,d,CH_2 pyrazoline), 2.69 (3H, s, CH_3), 4.52 (1H,t,CH), 7.32-7.82 (7H, m, Ar-H and NH),

10.21 (1H, s, OH). Anal. Calcd. for $C_{18}H_{15}ClN_2O_2$: C, 66.16; H, 4.63; N, 8.57. Found: C, 66.29; H, 4.64; N, 8.56%.

Antiviral activity assays

Confluent cell cultures in micro titer trays were inoculated with 100 $CCID_{50}$ (1 $CCID_{50}$ corresponding to the virus stock dilution that proved infective for 50% of the cell cultures). After 1 h of virus adsorption to the cells, residual virus was removed and replaced by cell culture medium (Eagle's minimal essential medium) containing 3% foetal calf serum and various concentrations of the test compounds. Viral cytopathogenicity was recorded as soon as it reached completion in the untreated virus-infected cell cultures, i.e., at 1 to 2 days for vesicular stomatitis; at 2 days for Coxsackie and herpes simplex virus types 1 and 2 and Sindbis virus; and 6 to 7 days for reovirus and parainfluenza viruses. The antiviral activity of the compounds is expressed as the concentration required to inhibit viral cytopathogenicity by 50%.

Cytotoxicity assays

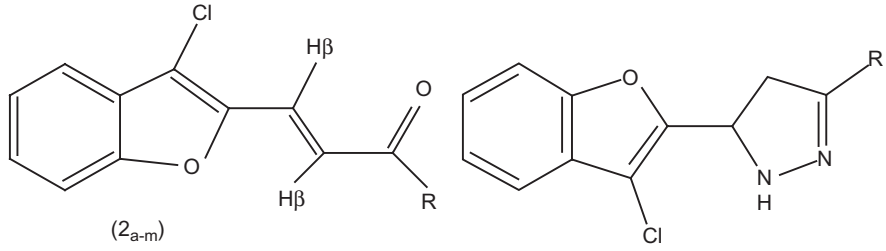
Cytotoxicity was monitored by direct microscopical inspection of the cell monolayers, which had not been infected but were treated by the compounds at the same concentration as used in the antiviral activity assays.

Results and discussion

Chemistry

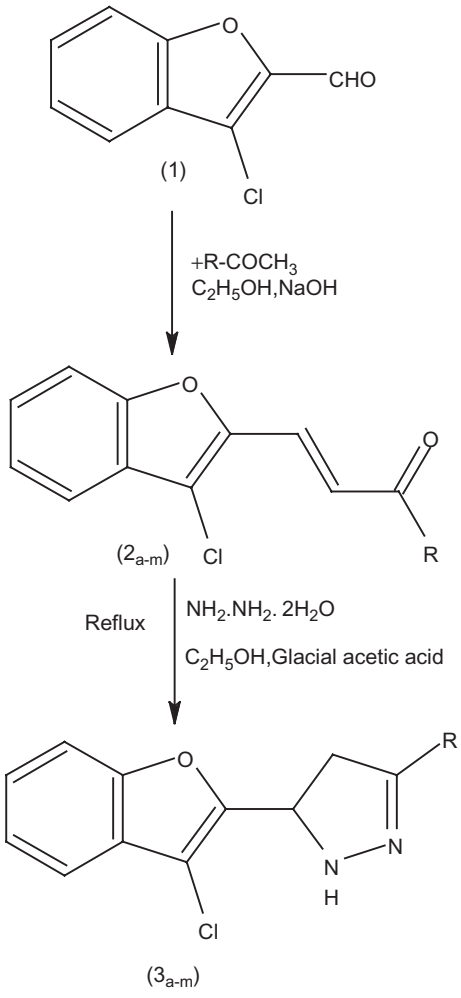
The physical constants of chalcones **(2a-m)** and pyrazoline derivatives **(3a-m)** are shown in Tables 1, and the reaction sequence for the synthesis is outlined in Scheme 1. The desired chalcones were obtained by Claisen-Schmidt condensation, by reacting 3-chlorobenzofuran-2-carbaldehyde

Table 1. Physical constants of the compound.



Code	R	Molecular Formula	Molecular Weight	% yield	Melting Point °C
2a	Phenyl	$C_{17}H_{11}ClO_2$	282	78	140–42
2b	4-chloro phenyl	$C_{17}H_{10}Cl_2O_2$	317	87	148–150
2c	4-methyl phenyl	$C_{18}H_{13}ClO_2$	296	76	126–28
2d	4-nitro phenyl	$C_{17}H_{10}ClNO_4$	327	70	182–84
2e	4-amino phenyl	$C_{17}H_{12}ClNO_2$	297	84	184–86
2f	4-hydroxy phenyl	$C_{17}H_{11}ClO_3$	298	76	210–12
2g	2-hydroxy phenyl	$C_{17}H_{11}ClO_3$	298	76	110–12
2h	4-methoxy phenyl	$C_{18}H_{13}ClO_3$	312	98	106–08
2i	2,4-dihydroxy phenyl	$C_{17}H_{11}ClO_4$	314	80	98–100
2j	2,4-dimethoxy phenyl	$C_{19}H_{15}ClO_4$	342	84	138–40
2k	3,4-dimethoxy phenyl	$C_{19}H_{15}ClO_4$	342	89	172–74
2l	4-hydroxy-3-methyl phenyl	$C_{18}H_{13}ClO_3$	312	76	202–04
2m	4-hydroxy-2-metyl phenyl	$C_{18}H_{13}ClO_3$	312	76	160–62
3a	Phenyl	$C_{17}H_{13}ClN_2O$	296	78	126–28
3b	4-chloro phenyl	$C_{17}H_{12}Cl_2N_2O$	331	82	66–68
3c	4-methyl phenyl	$C_{18}H_{15}ClN_2O$	310	80	144–46
3d	4-nitro phenyl	$C_{17}H_{12}ClN_3O_3$	341	74	108–10
3e	4-amino phenyl	$C_{17}H_{14}ClN_3O$	311	70	120–22
3f	4-hydroxy phenyl	$C_{17}H_{13}ClN_2O_2$	312	78	26870
3g	2-hydroxy phenyl	$C_{17}H_{13}ClN_2O_2$	312	82	148–50
3h	4-methoxy phenyl	$C_{18}H_{15}ClN_2O_2$	326	87	82–84
3i	2,4-dihydroxy phenyl	$C_{17}H_{13}ClN_2O_3$	328	74	140–42
3j	2,4-dimethoxy phenyl	$C_{19}H_{17}ClN_2O_3$	356	78	132–34
3k	3,4-dimethoxy phenyl	$C_{19}H_{17}ClN_2O_3$	356	78	98–100
3l	4-hydroxy-3-methyl phenyl	$C_{18}H_{15}ClN_2O_2$	326	76	232–38
3m	4-hydroxy-2-metyl phenyl	$C_{18}H_{15}ClN_2O_2$	326	76	150–54

(1) with the appropriate acetophenone in the presence of a base. It was interesting to report that overall reaction time recorded was considerably less as compared to our earlier reported reactions (1-4). Reaction of **1** with anisaldehyde to get **2h** may be considered as prototype of the series with a minimum time (2 h) and maximum yield (98%). Reaction between ethanolic solution of chalcones and hydrazine hydrate in the presence of glacial acetic acid and molecular sieves afforded the titled pyrazolines **(3a-m)**. TLC and elemental analyses were done to confirm the purity of the compounds. Analytical and spectral data [IR & ^{1}H-NMR] of all the synthesized compounds were found in full agreement with the proposed structures, which was further confirmed by mass of selected compounds. In general, the IR spectra of chalcone **(2h)** revealed C=O, C=C, and C–Cl stretching at 1685, 1645 and 750 cm^{-1} whereas pyrazoline **(3h)** revealed N-H, C=N, C-N, and C–Cl band at 3560, 1590, 1326 and 750 cm^{-1} respectively. In the ^{1}H-NMR spectra the signals of the respective protons of the compounds were verified on the basis of their chemical shifts, multiplicities and coupling constants. The ^{1}H-NMR spectra of chalcones showed two separate characteristic doublets at δ6.81 & 7.86ppm **(2f)** with trans coupling. The pyrazolines were characterized by disappearance of this (chalcone) doublets in 1H NMR and appearance of C4 (Ha & Hb) and C5 (Hx) protons in the aliphatic zone separately as three distinct double doublets at δ3.79 & 4.07 ppm (for Ha & Hb) and at δ6.07 ppm (for Hx) **(3f)**. It is interesting to report that in some cases **(3l & 3m)** C4-methylene group and C5 proton of pyrazoline appears as two protons doublet and one proton triplet at a shift of δ2.36 ppm and 4.52 ppm respectively. The results of elemental analysis were found within ±0.4% of the theoretical values.



Scheme 1. Synthesis of tilted compounds.

Antiviral activity

Antiviral activity was tested against feline corona virus (FIPV) and feline herpes virus (in Crandell-Rees Feline Kidney (CRFK) cell cultures); herpes simplex virus-1 (KOS), herpes simplex virus-2 (G), vaccinia virus, vesicular stomatitis virus, herpes simplex virus-1 TK$^-$ KOS ACV^r (in HEL cell cultures); vesicular stomatitis virus, Coxsackie virus B4, respiratory syncytial virus (in HeLa cells); parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4, Punta Toro virus (in Vero cell cultures). For each compound the 50% (antivirally) effective concentration (EC_{50}) and minimum

Table 2. Cytotoxicity and antiviral activity of compounds in Crandell-Rees Feline Kidney (CRFK) cell cultures.

		EC_{50}[b] (μg/ml)	
Compound	CC_{50}[a] (μg/ml)	Feline Corona Virus (FIPV)	Feline Herpes Virus
2a	6.1	>4	>4
2b	16.2	>4	>4
2c	27.7	>20	>20
2d	52.9	>20	>20
2e	65.9	>20	>20
2f	8.0	>4	>4
2g	30.0	>20	>20
2h	52.1	>20	>20
2i	3.4	>0.8	>0.8
2j	32.6	>20	>20
2k	>100	>100	>100
2l	11.4	>4	>4
2m	0.16	>0.32	>0.32
3a	9.9	>4	>4
3b	12.6	3.8	>4
3c	11.5	>4	>4
3d	11.8	>4	>4
3e	34.0	>20	>20
3f	>100	>100	81.5
3g	81.6	>20	>20
3h	16.0	>4	>4
3i	15.3	>4	>4
3j	26.2	>20	>20
3k	>100	>100	>100
3l	>100	>100	>100
3m	4.9	>4	>4
Ganciclovir (μM)	>100	>100	4.0

[a]50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

[b]50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

Table 3. Cytotoxicity and antiviral activity of compounds in human embryonic lung (HEL) cell cultures.

Compound	Minimum cytotoxic concentration[a] (μg/ml)	EC_{50}[b] (μg/ml) Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Vaccinia virus	Vesicular stomatitis virus	Herpes simplex virus-1 TK- KOS ACV^r
2a	>100	>100	>100	>100	>100	>100
2b	>100	>100	>100	>100	>100	>100
2c	≥20	>20	>20	>20	>20	>20
2d	>100	>100	>100	>100	>100	>100
2e	20	>4	>4	>4	>4	>4
2f	4	>0.8	>0.8	>0.8	>0.8	>0.8
2g	≥4	>4	>4	>4	>4	>4
2h	20	>4	>4	>4	>4	>4
2i	≥0.8	>0.8	>0.8	>0.8	>0.8	>0.8
2j	>100	>100	>100	>100	>100	>100
2k	>100	>100	>100	>100	>100	>100
2l	20	>4	>4	>4	>4	>4
2m	20	>4	>4	>4	>4	>4
3a	20	>4	>4	>4	>4	>4
3b	20	>4	>4	>4	>4	>4
3c	20	>4	>4	>4	>4	>4
3d	≥20	>20	>20	>20	>20	>20
3e	100	>20	>20	>20	>20	>20
3f	20	>4	>4	>4	>4	>4
3g	≥20	>20	>20	>20	>20	>20
3h	≥20	>20	>20	>20	>20	>20
3i	20	>4	>4	>4	>4	>4
3j	20	>4	>4	>4	>4	>4
3k	20	>4	>4	>4	>4	>4
3l	20	>4	>4	>4	>4	>4
3m	20	>4	>4	>4	>4	>4
Brivudin (μM)	>250	0.08	250	50	>250	>250
Ribavirin (μM)	>250	50	>250	>250	>250	>250
Cidofovir (μM)	>250	2	2	10	>250	2
Ganciclovir (μM)	>100	0.16	0.16	>100	>100	20

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50 %.

Table 4. Cytotoxicity and antiviral activity of compounds in HeLa cell cultures.

Compound	Minimum cytotoxic concentration[a] (μg/ml)	EC_{50}[b] (μg/ml) Vesicular stomatitis virus	Coxsackie virus B4	Respiratory syncytial virus
2a	100	>20	>20	>20
2b	>100	>100	>100	>100
2c	100	>20	>20	>20
2d	≥20	>20	>20	>20
2e	≥20	>20	>20	>20
2f	4	>0.8	>0.8	>0.8
2g	≥20	>20	>20	>20
2h	100	>20	>20	>20
2i	0.8	>0.16	>0.16	>0.16
2j	100	>20	>20	>20
2k	100	>20	>20	>20
2l	20	>4	>4	>4
2m	20	>4	>4	>4
3a	20	4	>4	>4
3b	4	>0.8	>0.8	>0.8
3c	≥4	>4	>4	>4
3d	4	>0.8	>0.8	>0.8
3e	20	>4	>4	>4

Table 4 continued on next page

Table 4. Continued.

3f	20	>4	>4	4
3g	4	>0.8	>0.8	>0.8
3h	4	>0.8	>0.8	>0.8
3i	20	>4	>4	>4
3j	100	>20	>20	>20
3k	20	>4	>4	>4
3l	≥20	>20	>20	>20
3m	≥4	>4	>4	>4
DS-5000	>100	4	>100	1
(S)-DHPA (μM)	>250	>250	>250	>250
Ribavirin (μM)	>250	22	146	10

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50 %.

Table 5. Cytotoxicity and antiviral activity of compounds in Vero cell cultures.

Compound	Minimum cytotoxic concentration[a] (μg/ml)	EC_{50}[b] (μg/ml) Para-influenza-3 virus	Reovirus-1	Sindbis virus	Coxsackie virus B4	Punta Toro virus
2a	20	>4	>4	>4	>4	>4
2b	100	>20	>20	>20	>20	>20
2c	≥20	>20	>20	>20	>20	>20
2d	100	>20	>20	>20	>20	>20
2e	100	>20	>20	>20	>20	>20
2f	≥4	>4	>4	>4	>4	>4
2g	20	>4	>4	>4	>4	>4
2h	100	>20	>20	>20	>20	>20
2i	≥4	>4	>4	>4	>4	>4
2j	100	>20	>20	>20	>20	>20
2k	100	>20	>20	>20	>20	>20
2l	≥20	>20	>20	>20	>20	>20
2m	20	>4	>4	>4	>4	>4
3a	20	>4	>4	>4	>4	>4
3b	≥4	>4	>4	>4	>4	>4
3c	20	>4	>4	>4	>4	>4
3d	≥4	>4	>4	>4	>4	>4
3e	20	>4	>4	>4	>4	>4
3f	20	>4	>4	>4	>4	>4
3g	≥4	>4	>4	>4	>4	>4
3h	≥4	>4	>4	>4	>4	>4
3i	20	>4	>4	>4	>4	>4
3j	100	>20	>20	>20	>20	>20
3k	20	>4	>4	>4	>4	>4
3l	100	>20	>20	>20	>20	>20
3m	20	>4	>4	>4	>4	>4
DS-5000	>100	>100	>100	>100	>100	100
(S)-DHPA (μM)	>250	250	>250	>250	>250	>250
Ribavirin (μM)	>250	45	146	146	>250	146

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50 %.

cytotoxic concentration were determined. Ribavirin, brivudin, acyclovir, ganciclovir, dextran sulfate (DS-5000) and (S)-DHPA were used as the reference compounds.

The results of the antiviral assays are shown in Tables 2–5. Cytotoxicity towards uninfected host cells was determined microscopically under same conditions as the antiviral activity. The criterion for specific antiviral activity was taken as, the inhibition of virus-induced cytopathogenicity at a concentration that was at least 5-fold lower than the cytotoxic, or concentration required to alter the morphology of the uninfected host cells.

As per this criterion, none of the compounds tested showed any specific antiviral activity (Tables 2–5). Several compounds, i.e. **2f, 2g, 2i, 2m, 3b, 3d, 3g, 3h** and **3m**, proved quite cytotoxic to the host cells (minimum cytotoxic concentration: 1-10 μg/mL).

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