Scientific paper

Design, Synthesis, *In Silico* ADMET Studies and Anticancer Activity of Some New Pyrazoline and Benzodioxole Derivatives

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Abstract

A new series of 2-pyrazoline derivatives starting from substituted benzodioxole chalcones were designed and synthesized. IR and ^{1}H NMR spectral data and elemental analysis were used to characterize the structures of the synthesized compounds. The cytotoxic activities on HeLa, MCF-7 cancer cell lines and NIH-3T3 for these compounds were tested by using MTT assay. Among the synthesized compounds **2d**, **2j**, **3j** and **3n** against MCF-7 cells, and **3c** against HeLa exhibited significant cytotoxic activity with IC_{50} between 10.08 and 27.63 μM. Compound **3f** showed the most potent anticancer activity against both cancer cells with good selectivity (IC_{50} = 11.53 μM on HeLa with SI = 81.75 and IC_{50} = 11.37 μM on MCF-7 with SI = 82.90). Furthermore, *in silico* ADMET analyses were performed and the drug-likeness properties of the compounds were investigated.

Keywords: Pyrazoline; carboxamide; anticancer activity; breast cancer; cervical cancer.

1. Introduction

Pyrazolines are electron rich nitrogen heterocycles that are appropriate for the discovery of bioactive molecules.[1] Pyrazolines occur naturally in animal and plant cells, in the form of vitamins, pigments and alkaloids.[2] These heterocyclic compounds are known to exhibit good pharmacological and biological acitivities such as antimicrobial, antiinflammatory, analgesic, anti-depressant and anticancer.[3–7]

Heterocyclic anticancer agents bearing nitrogen-nitrogen (*N–N)* bonds, such as crizotinib, ruxolitinib, axitinib, encorafinib and ibrutinib, have been approved by the FDA in the last decades.[8] Podophyllotoxin and steganacin bearing benzo[*d*][1,3]dioxol moiety are natural bioactive molecules with very strong cytotoxic activity. Some adverse effects related to complex pharmacokinetics including unpredictable drug-drug interactions can appear because of the therapeutics of combining multiple drugs. It is an important strategy for drug discovery that constructing a single molecule modulates multiple targets simultaneously.[9,10] Therefore, we designed two series of pyrazoline derivatives containing benzo[*d*][1,3]dioxol moiety by the molecular assembly principle (Figure 1).

Herein we report the synthesis of 2-pyrazolines starting from 1-(benzo[*d*][1,3]dioxol-5-yl)ethanone and further evaluated their anticancer activities on HeLa (human cervical adenocarcinoma), MCF7 (human breast adenocarcinoma) cancer cells. NIH-3T3 mouse embryonic fibroblast cell lines were also used to determine the tumor selectivity of the synthesized compounds.

2. Experimental

2. 1. Chemistry

All chemicals were obtained from Sigma-Aldrich or Merck Chemical Company and used without further purification. The reaction processes and the purity of compounds were monitored by thin layer chromatography using a UV lamp. Melting points were determined on a Kleinfield SMP II apparatus and are uncorrected. The IR

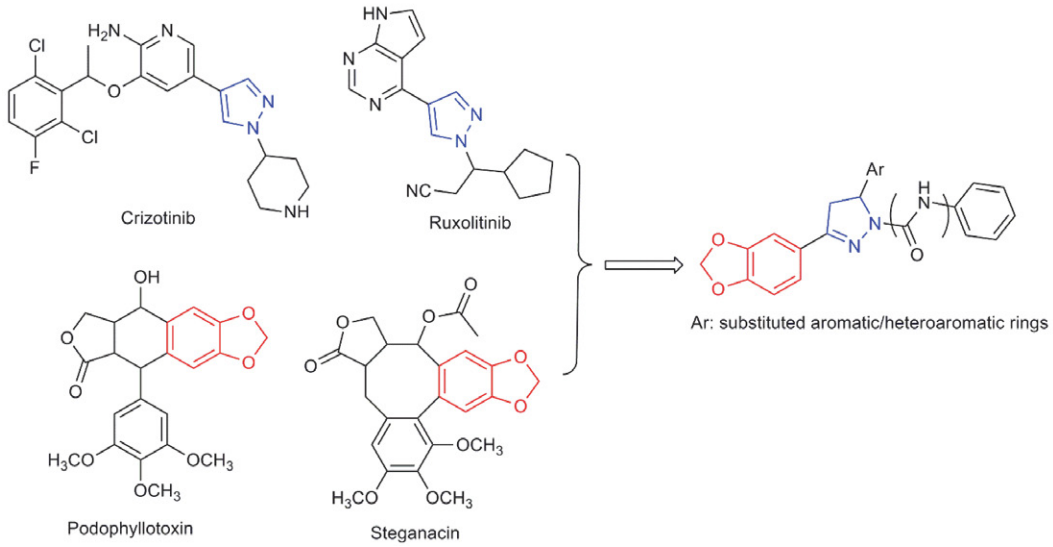


Figure 1. Design strategy of the target compounds

spectra were recorded using a Schimadzu FTIR 8400S spectrometry. ^{1}H NMR spectra were taken on a Bruker (400 MHz) spectrometer. Elemental analysis was performed on Leco CHNS-932 analyzer.

General Procedure for the Preparation of Chalcone Derivatives 1a–1o

1-(Benzo[*d*][1,3]dioxol-5-yl)ethanone (1 mmol, 0.164 g) and corresponding aromatic aldehyde (1 mmol) were dissolved in 20 mL methanol. A solution of sodium hydroxide (50%, 3 mL) was added to the reaction mixture. The mixture was stirred on magnetic stirrer for 8 h. The precipitated product was filtered, washed with water and recrystallized from methanol.[11]

General Procedure for Preparation of Pyrazoline Derivatives 2a–2o

Chalcone derivatives (1 mmol) and phenylhydrazine hydrochloride (1 mmol, 0.145 g) was dissolved in 25 mL ethanol. Catalytic quantity of acetic acid (1.5 mL) was added to the reaction mixture. The mixture was refluxed for 8 h. The mixture was cooled, the precipitated product was filtered, washed with water and recrystallized from ethanol.[12]

3-(Benzo[*d*][1,3]dioxol-5-yl)-1,5-diphenyl-4,5-dihydro-1*H*-pyrazole (2a)

Yield: 280 mg (82%) of white powder; m.p. 172.8–173.2 °C. IR (ν_{max}, cm^{-1}): 3028 (=C-H), 2914 (C-H), 1593 (C=N), 1249 (C-O), 1033 (C-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.03 (dd, *J*ax = 6.5 Hz, *J*ab = 17.4 Hz, 1H, Ha), 3.84 (dd, *J*bx = 12.2 Hz, *J*ab = 17.5 Hz, 1H, Hb), 5.40 (dd, *J*ax = 6.4 Hz, *J*bx = 12.1 Hz, 1H, Hx), 6.04 (s, 2H, O-CH_2-O), 6.65–7.36 (m, 13H, Ar-H). Anal. Calcd for $C_{22}H_{18}N_2O_2$: C, 77.17; H, 5.30; N, 8.18. Found: C, 77.07; H, 5.32; N, 8.15%.

3-(Benzo[*d*][1,3]dioxol-5-yl)-5-(4-fluorophenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (2b)

Yield: 288 mg (80%) of white powder; m.p. 138.1–138.3 °C. IR (ν_{max}, cm^{-1}): 3066 (=C-H), 2891 (C-H), 1591 (C=N), 1211 (C-O), 1037 (C-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.03 (dd, *J*ax = 6.3 Hz, *J*ab = 17.5 Hz, 1H, Ha), 3.83 (dd, *J*bx = 12.1 Hz, *J*ab = 17.5 Hz, 1H, Hb), 5.44 (dd, *J*ax = 6.3 Hz, *J*bx = 12.0 Hz, 1H, Hx), 6.05 (s, 2H, O-CH_2-O), 6.68–7.31 (m, 12H, Ar-H). Anal. Calcd for $C_{22}H_{17}FN_2O_2$: C, 73.32; H, 4.75; N, 7.77. Found: C, 73.20; H, 4.77; N, 7.80%.

3-(Benzo[*d*][1,3]dioxol-5-yl)-5-(4-bromophenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (2c)

Yield: 341 mg (81%) of white crystals; m.p. 164.5–164.8 °C. IR (ν_{max}, cm^{-1}): 3082 (=C-H), 2885 (C-H), 1595 (C=N), 1211 (C-O), 1033 (C-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.04 (dd, *J*ax = 6.3 Hz, *J*ab = 17.4 Hz, 1H, Ha), 3.83 (dd, *J*bx = 12.2 Hz, *J*ab = 17.5 Hz, 1H, Hb), 5.42 (dd, *J*ax = 6.3 Hz, *J*bx = 12.1 Hz, 1H, Hx), 6.05 (s, 2H, O-CH_2-O), 6.68–7.51 (m, 12H, Ar-H). Anal. Calcd for $C_{22}H_{17}BrN_2O_2$: C, 62.72; H, 4.07; N, 6.65. Found: C, 62.81; H, 4.08; N, 6.61%.

3-(Benzo[*d*][1,3]dioxol-5-yl)-5-(4-nitrophenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (2d)

Yield: 325 mg (84%) of yellow powder; m.p. 110.2–110.5 °C. IR (ν_{max}, cm^{-1}): 3078 (=C-H), 2881 (C-H), 1595 (C=N), 1213 (C-O), 1033 (C-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.10 (dd, *J*ax = 6.3 Hz, *J*ab = 17.5 Hz, 1H, Ha), 3.90 (dd, *J*bx = 12.3 Hz, *J*ab = 17.5 Hz, 1H, Hb), 5.61 (dd, *J*ax = 6.3 Hz, *J*bx = 12.3 Hz, 1H, Hx), 6.05 (s, 2H, O-CH_2-O), 6.70–8.20 (m, 12H, Ar-H). Anal. Calcd for $C_{22}H_{17}N_3O_4$: C, 68.21; H, 4.42; N, 10.85. Found: C, 68.04; H, 4.41; N, 10.89%.

3-(Benzo[*d*][1,3]dioxol-5-yl)-1-phenyl-5-*para*-tolyl-4,5-dihydro-1*H*-pyrazole (2e)

Yield: 278 mg (78%) of white powder; m.p. 146.4–146.6 °C. IR (ν_{max}, cm^{-1}): 3022 (=C-H), 2901 (C-H), 1595 (C=N), 1215 (C-O), 1035 (C-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.23 (s, 3H, CH_3), 3.00 (dd, *J*ax = 6.4 Hz, *J*ab = 17.4 Hz, 1H, Ha), 3.81 (dd, *J*bx = 12.2 Hz, *J*ab = 17.4 Hz, 1H, Hb), 5.35 (dd, *J*ax = 6.4 Hz, *J*bx = 12.1 Hz, 1H, Hx), 6.04 (s, 2H, O-CH_2-O), 6.66–7.35 (m, 12H, Ar-H). Anal. Calcd for $C_{23}H_{20}N_2O_2$: C, 77.51; H, 5.66; N, 7.86. Found: C, 77.39; H, 5.68; N, 7.85%.

4-(3-(Benzo[*d*][1,3]dioxol-5-yl)-1-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl)benzonitrile (2f)

Yield: 290 mg (79%) of white crystals; m.p. 135.9–136.3 °C. IR (ν_{max}, cm^{-1}): 3053 (=C-H), 2901 (C-H), 1593 (C=N), 1217 (C-O), 1033 (C-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.08 (dd, *J*ax = 6.3 Hz, *J*ab = 17.5 Hz, 1H, Ha), 3.87 (dd, *J*bx = 12.3 Hz, *J*ab = 17.5 Hz, 1H, Hb), 5.55 (dd, *J*ax = 6.2 Hz, *J*bx = 12.1 Hz, 1H, Hx), 6.05 (s, 2H, O-CH_2-O), 6.70–7.80 (m, 12H, Ar-H). Anal. Calcd for $C_{23}H_{17}N_3O_2$: C, 75.19; H, 4.66; N, 11.44. Found: C, 75.11; H, 4.66; N, 11.46%.

3-(Benzo[*d*][1,3]dioxol-5-yl)-5-(2,6-dimethylphenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (2g)

Yield: 296 mg (80%) of white powder; m.p. 117.8–118.0 °C. IR (ν_{max}, cm^{-1}): 3037 (=C-H), 2881 (C-H), 1597 (C=N), 1213 (C-O), 1037 (C-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.03 (s, 3H, CH_3), 2.24 (s, 3H, CH_3), 3.01 (dd, *J*ax = 6.3 Hz, *J*ab = 17.4 Hz, 1H, Ha), 3.91 (dd, *J*bx = 12.1 Hz, *J*ab = 17.4 Hz, 1H, Hb), 5.56 (dd, *J*ax = 6.3 Hz, *J*bx = 12.0 Hz, 1H, Hx), 6.05 (s, 2H, O-CH_2-O), 6.66–7.36 (m, 11H, Ar-H). Anal. Calcd for $C_{24}H_{22}N_2O_2$: C, 77.81; H, 5.99; N, 7.56. Found: C, 77.90; H, 5.96; N, 7.59%.

3-(Benzo[*d*][1,3]dioxol-5-yl)-1-phenyl-5-*ortho*-tolyl-4,5-dihydro-1*H*-pyrazole (2h)

Yield: 303 mg (85%) of white powder; m.p. 163.9–164.2 °C. IR (ν_{max}, cm^{-1}): 3066 (=C-H), 2881 (C-H), 1597 (C=N), 1203 (C-O), 1033 (C-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.24 (s, 3H, CH_3), 2.92 (dd, *J*ax = 6.6 Hz, *J*ab = 17.4 Hz, 1H, Ha), 3.96 (dd, *J*bx = 12.3 Hz, *J*ab = 17.4 Hz, 1H, Hb), 5.50 (dd, *J*ax = 6.6 Hz, *J*bx = 12.3 Hz, 1H, Hx), 6.05 (s, 2H, O-CH_2-O), 6.68–7.36 (m, 12H, Ar-H). Anal. Calcd for $C_{23}H_{20}N_2O_2$: C, 77.51; H, 5.66; N, 7.86. Found: C, 77.59; H, 5.66; N, 7.88%.

3-(Benzo[*d*][1,3]dioxol-5-yl)-1-phenyl-5-*meta*-tolyl-4,5-dihydro-1*H*-pyrazole (2i)

Yield: 310 mg (87%) of white powder; m.p. 148.8–149.2 °C. IR (ν_{max}, cm^{-1}): 3061 (=C-H), 2893 (C-H), 1595 (C=N), 1211 (C-O), 1033 (C-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.24 (s, 3H, CH_3), 3.01 (dd, *J*ax = 6.3 Hz, *J*ab = 17.5 Hz, 1H, Ha), 3.83 (dd, *J*bx = 12.1 Hz, *J*ab = 17.5 Hz, 1H, Hb), 5.33 (dd, *J*ax = 6.3 Hz, *J*bx = 12.0 Hz, 1H, Hx), 6.05 (s, 2H, O-CH_2-O), 6.67–7.31 (m, 12H, Ar-H). Anal. Calcd for $C_{23}H_{20}N_2O_2$: C, 77.51; H, 5.66; N, 7.86. Found: C, 77.61; H, 5.64; N, 7.89%.

4-(3-(Benzo[*d*][1,3]dioxol-5-yl)-1-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl)pyridine (2j)

Yield: 268 mg (78%) of gray powder; m.p. 108.2–108.3 °C. IR (ν_{max}, cm^{-1}): 3030 (=C-H), 2881 (C-H), 1597 (C=N), 1242 (C-O), 1033 (C-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.06 (dd, *J*ax = 6.4 Hz, *J*ab = 17.4 Hz, 1H, Ha), 3.82 (dd, *J*bx = 12.1 Hz, *J*ab = 17.4 Hz, 1H, Hb), 5.43 (dd, *J*ax = 6.4 Hz, *J*bx = 12.1 Hz, 1H, Hx), 6.08 (s, 2H, O-CH_2-O), 6.52–7.62 (m, 12H, Ar-H). Anal. Calcd for $C_{21}H_{17}N_3O_2$: C, 73.45; H, 4.99; N, 12.24. Found: C, 73.32; H, 5.01; N, 12.23%.

3-(Benzo[*d*][1,3]dioxol-5-yl)-1-phenyl-5-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazole (2k)

Yield: 271 mg (78%) of red powder; m.p. 152.7–153.0 °C. IR (ν_{max}, cm^{-1}): 3074 (=C-H), 2893 (C-H), 1595 (C=N), 1201 (C-O), 1031 (C-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.21 (dd, *J*ax = 5.9 Hz, *J*ab = 17.3 Hz, 1H, Ha), 3.81 (dd, *J*bx = 11.6 Hz, *J*ab = 17.3 Hz, 1H, Hb), 5.75 (dd, *J*ax = 5.9 Hz, *J*bx = 11.6 Hz, 1H, Hx), 6.06 (s, 2H, O-CH_2-O), 6.72–7.36 (m, 11H, Ar-H). Anal. Calcd for $C_{20}H_{16}N_2O_2S$: C, 68.94; H, 4.63; N, 8.04. Found: C, 68.85; H, 4.65; N, 8.08%.

3-(Benzo[*d*][1,3]dioxol-5-yl)-5-(furan-2-yl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (2l)

Yield: 272 mg (82%) of brown powder; m.p. 120.1–120.5 °C. IR (ν_{max}, cm^{-1}): 3078 (=C-H), 2908 (C-H), 1595 (C=N), 1211 (C-O), 1033 (C-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.28 (dd, *J*ax = 6.0 Hz, *J*ab = 17.3 Hz, 1H, Ha), 3.70 (dd, *J*bx = 12.1 Hz, *J*ab = 17.3 Hz, 1H, Hb), 5.53 (dd, *J*ax = 6.0 Hz, *J*bx = 12.2 Hz, 1H, Hx), 6.05 (s, 2H, O-CH_2-O), 6.38–7.54 (m, 11H, Ar-H). Anal. Calcd for $C_{20}H_{16}N_2O_3$: C, 72.28; H, 4.85; N, 8.43. Found: C, 72.41; H, 4.88; N, 8.38%.

3-(Benzo[*d*][1,3]dioxol-5-yl)-5-(5-bromothiophen-2-yl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (2m)

Yield: 376 mg (88%) of gray powder; m.p. 143.0–143.4 °C. IR (ν_{max}, cm^{-1}): 3039 (=C-H), 2908 (C-H), 1595 (C=N), 1215 (C-O), 1035 (C-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.01 (dd, *J*ax = 6.4 Hz, *J*ab = 17.4 Hz, 1H, Ha), 3.82 (dd, *J*bx = 12.1 Hz, *J*ab = 17.4 Hz, 1H, Hb), 5.36 (dd, *J*ax = 6.4 Hz, *J*bx = 12.1 Hz, 1H, Hx), 6.06 (s, 2H, O-CH_2-O), 6.67–7.36 (m, 10H, Ar-H). Anal. Calcd for $C_{20}H_{15}BrN_2O_2S$: C, 56.21; H, 3.54; N, 6.56. Found: C, 56.08; H, 3.53; N, 6.59%.

3-(Benzo[*d*][1,3]dioxol-5-yl)-1-phenyl-5-(4-(trifluoromethyl)phenyl)-4,5-dihydro-1*H*-pyrazole (2n)

Yield: 328 mg (80%) of white powder; m.p. 146.4–146.6 °C. IR (ν_{max}, cm^{-1}): 3078 (=C-H), 2887 (C-H), 1597 (C=N), 1213 (C-O), 1033 (C-N); ^{1}H NMR (400 MHz,

DMSO-d_6): δ 3.10 (dd, *J*ax = 5.9 Hz, *J*ab = 17.3 Hz, 1H, Ha), 3.88 (dd, *J*bx = 11.6 Hz, *J*ab = 17.3 Hz, 1H, Hb), 5.57 (dd, *J*ax = 5.9 Hz, *J*bx = 11.6 Hz, 1H, Hx), 6.06 (s, 2H, O-CH_2-O), 6.70–7.71 (m, 12H, Ar-H). Anal. Calcd for $C_{23}H_{17}F_3N_2O_2$: C, 67.31; H, 4.18; N, 6.83. Found: C, 67.15; H, 4.17; N, 6.79%.

3-(Benzo[*d*][1,3]dioxol-5-yl)-5-(3-chlorophenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (2o)

Yield: 320 mg (85%) of white crystals; m.p. 131.0–131.3 °C. IR (ν_{max}, cm^{-1}): 3072 (=C-H), 2908 (C-H), 1593 (C=N), 1213 (C-O), 1035 (C-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.11 (dd, *J*ax = 6.3 Hz, *J*ab = 17.5 Hz, 1H, Ha), 3.92 (dd, *J*bx = 12.1 Hz, *J*ab = 17.5 Hz, 1H, Hb), 5.48 (dd, *J*ax = 6.2 Hz, *J*bx = 12.1 Hz, 1H, Hx), 6.08 (s, 2H, O-CH_2-O), 6.58–7.68 (m, 12H, Ar-H). Anal. Calcd for $C_{22}H_{17}ClN_2O_2$: C, 70.12; H, 4.55; N, 7.43. Found: C, 70.21; H, 4.57; N, 7.42%.

General Procedure for Preparation of Pyrazoline Derivatives 3a–3o

Firstly, the synthesis of *N*-(4-chlorophenyl)hydrazinecarboxamide was carried out. Hydrazine monohydrate (1 mmol, 48.5 μL) was added dropwise to 4-chlorophenylisocyanate (1 mmol, 128.0 μL) in 15 mL diethyl ether. The mixture was stirred for 1 h. The white precipitated product was filtered, dried.[13]

Chalcone derivatives (1 mmol) and *N*-(4-chlorophenyl)hydrazinecarboxamide (1 mmol) was dissolved in 20 mL ethanol, and then a solution of sodium hydroxide (20%, 1 mL) was added to the reaction mixture. The mixture was refluxed for 10 h. The mixture was cooled, the precipitated product was filtered, washed with water and recrystallized from ethanol.[13]

3-(Benzo[*d*][1,3]dioxol-5-yl)-*N*-(4-chlorophenyl)-5-phenyl-4,5-dihydro-1*H*-pyrazole-1-carboxamide (3a)

Yield: 328 mg (78%) of white powder; m.p. 139.1–139.3 °C. IR (ν_{max}, cm^{-1}): 3290 (N-H), 3078 (=C-H), 2989, 2901 (C-H), 1666 (C=O), 1631 (C=N), 1222 (C-O), 1033 (C-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.13 (dd, *J*ax = 5.2 Hz, *J*ab = 17.8 Hz, 1H, Ha), 3.81 (dd, *J*bx = 11.7 Hz, *J*ab = 17.8 Hz, 1H, Hb), 5.51 (dd, *J*ax = 5.2 Hz, *J*bx = 11.7 Hz, 1H, Hx), 6.08 (s, 2H, O-CH_2-O), 6.90–7.97 (m, 12H, Ar-H), 9.14 (s, 1H, NH). Anal. Calcd for $C_{23}H_{18}ClN_3O_3$: C, 65.79; H, 4.32; N, 10.01. Found: C, 65.89; H, 4.35; N, 9.95%.

3-(Benzo[*d*][1,3]dioxol-5-yl)-*N*-(4-chlorophenyl)-5-(4-fluorophenyl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide (3b)

Yield: 350 mg (80%) of white powder; m.p. 134.4–134.5 °C. IR (ν_{max}, cm^{-1}): 3292 (N-H), 3066 (=C-H), 2987, 2885 (C-H), 1666 (C=O), 1633 (C=N), 1228 (C-O), 1033 (C-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.15 (dd, *J*ax = 5.3 Hz, *J*ab = 17.9 Hz, 1H, Ha), 3.85 (dd, *J*bx = 11.6 Hz, *J*ab = 17.9 Hz, 1H, Hb), 5.52 (dd, *J*ax = 5.2 Hz, *J*bx = 11.7 Hz, 1H, Hx), 6.14 (s, 2H, O-CH_2-O), 7.04–7.94 (m, 11H, Ar-H), 9.16 (s, 1H, NH). Anal. Calcd for $C_{23}H_{17}ClFN_3O_3$: C, 63.09; H, 3.91; N, 9.60. Found: C, 63.00; H, 3.90; N, 9.65%.

3-(Benzo[*d*][1,3]dioxol-5-yl)-5-(4-bromophenyl)-*N*-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide (3c)

Yield: 424 mg (85%) of white powder; m.p. 123.7–123.9 °C. IR (ν_{max}, cm^{-1}): 3290 (N-H), 3072 (=C-H), 2974, 2989 (C-H), 1666 (C=O), 1633 (C=N), 1222 (C-O), 1033 (C-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.14 (dd, *J*ax = 5.5 Hz, *J*ab = 17.9 Hz, 1H, Ha), 3.80 (dd, *J*bx = 11.6 Hz, *J*ab = 17.9 Hz, 1H, Hb), 5.49 (dd, *J*ax = 5.3 Hz, *J*bx = 11.7 Hz, 1H, Hx), 6.02 (s, 2H, O-CH_2-O), 7.07–7.85 (m, 11H, Ar-H), 9.06 (s, 1H, NH). Anal. Calcd for $C_{23}H_{17}BrClN_3O_3$: C, 55.39; H, 3.44; N, 8.42. Found: C, 55.55; H, 3.41; N, 8.45%.

3-(Benzo[*d*][1,3]dioxol-5-yl)-*N*-(4-chlorophenyl)-5-(4-nitrophenyl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide (3d)

Yield: 400 mg (86%) of yellow powder; m.p. 128.8–129.0 °C. IR (ν_{max}, cm^{-1}): 3290 (N-H), 3078 (=C-H), 2989, 2912 (C-H), 1666 (C=O), 1631 (C=N), 1230 (C-O), 1033 (C-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.14 (dd, *J*ax = 5.2 Hz, *J*ab = 18.0 Hz, 1H, Ha), 3.81 (dd, *J*bx = 11.9 Hz, *J*ab = 18.0 Hz, 1H, Hb), 5.51 (dd, *J*ax = 5.2 Hz, *J*bx = 11.9 Hz, 1H, Hx), 6.10 (s, 2H, O-CH_2-O), 7.10–7.77 (m, 11H, Ar-H), 9.06 (s, 1H, NH). Anal. Calcd for $C_{23}H_{17}ClN_4O_5$: C, 59.43; H, 3.69; N, 12.05. Found: C, 59.60; H, 3.73; N, 12.12%.

3-(Benzo[*d*][1,3]dioxol-5-yl)-*N*-(4-chlorophenyl)-5-*para*-tolyl-4,5-dihydro-1*H*-pyrazole-1-carboxamide (3e)

Yield: 356 mg (82%) of white powder; m.p. 161.9–162.3 °C. IR (ν_{max}, cm^{-1}): 3292 (N-H), 3078 (=C-H), 2987, 2887 (C-H), 1666 (C=O), 1631 (C=N), 1228 (C-O), 1033 (C-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.30 (s, 3H, CH_3), 3.07 (dd, *J*ax = 5.3 Hz, *J*ab = 17.9 Hz, 1H, Ha), 3.78 (dd, *J*bx = 11.9 Hz, *J*ab = 17.9 Hz, 1H, Hb), 5.43 (dd, *J*ax = 5.3 Hz, *J*bx = 11.9 Hz, 1H, Hx), 6.07 (s, 2H, O-CH_2-O), 7.05–7.65 (m, 11H, Ar-H), 9.00 (s, 1H, NH). Anal. Calcd for $C_{24}H_{20}ClN_3O_3$: C, 66.44; H, 4.65; N, 9.68. Found: C, 66.29; H, 4.62; N, 9.73%.

3-(Benzo[*d*][1,3]dioxol-5-yl)-*N*-(4-chlorophenyl)-5-(4-cyanophenyl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide (3f)

Yield: 312 mg (70%) of gray powder; m.p. 188.1–188.5 °C. IR (ν_{max}, cm^{-1}): 3292 (N-H), 3084 (=C-H), 2918, 2848 (C-H), 1666 (C=O), 1631 (C=N), 1230 (C-O), 1033 (C-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.11 (dd, *J*ax = 5.6 Hz, *J*ab = 17.9 Hz, 1H, Ha), 3.78 (dd, *J*bx = 12.0 Hz, *J*ab = 17.9 Hz, 1H, Hb), 5.48 (dd, *J*ax = 5.5 Hz, *J*bx = 12.0 Hz, 1H, Hx), 6.09 (s, 2H, O-CH_2-O), 6.98–7.85 (m, 11H, Ar-H), 9.02 (s, 1H, NH). Anal. Calcd for $C_{24}H_{17}ClN_4O_3$: C, 64.80; H, 3.85; N, 12.59. Found: C, 64.65; H, 3.88; N, 12.56%.

3-(Benzo[*d*][1,3]dioxol-5-yl)-*N*-(4-chlorophenyl)-5-(2,6-dimethylphenyl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide (3g)

Yield: 323 mg (72%) of white powder; m.p. 155.1–155.5 °C. IR (ν_{max}, cm^{-1}): 3290 (N-H), 3066 (=C-H), 2987, 2918, 2848 (C-H), 1672 (C=O), 1631 (C=N), 1220 (C-O), 1033 (C-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.08 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 3.04 (dd, *J*ax = 5.9 Hz, *J*ab = 17.3 Hz, 1H, Ha), 3.81 (dd, *J*bx = 11.6 Hz, *J*ab = 17.3 Hz, 1H, Hb), 5.85 (dd, *J*ax = 5.9 Hz, *J*bx = 11.6 Hz, 1H, Hx), 6.10 (s, 2H, O-CH_2-O), 7.00–7.70 (m, 10H, Ar-H), 9.12 (s, 1H, NH). Anal. Calcd for $C_{25}H_{22}ClN_3O_3$: C, 67.04; H, 4.95; N, 9.38. Found: C, 66.91; H, 4.99; N, 9.45%.

3-(Benzo[*d*][1,3]dioxol-5-yl)-*N*-(4-chlorophenyl)-5-*ortho*-tolyl-4,5-dihydro-1*H*-pyrazole-1-carboxamide (3h)

Yield: 343 mg (79%) of white powder; m.p. 129.8–130.1 °C. IR (ν_{max}, cm^{-1}): 3367 (N-H), 3095 (=C-H), 2982, 2881 (C-H), 1667 (C=O), 1631 (C=N), 1220 (C-O), 1033 (C-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.34 (s, 3H, CH_3), 2.99 (dd, *J*ax = 5.5 Hz, *J*ab = 17.8 Hz, 1H, Ha), 3.87 (dd, *J*bx = 12.0 Hz, *J*ab = 17.8 Hz, 1H, Hb), 5.63 (dd, *J*ax = 5.5 Hz, *J*bx = 12.0 Hz, 1H, Hx), 6.12 (s, 2H, O-CH_2-O), 7.00–7.67 (m, 11H, Ar-H), 9.20 (s, 1H, NH). Anal. Calcd for $C_{24}H_{20}ClN_3O_3$: C, 66.44; H, 4.65; N, 9.68. Found: C, 66.57; H, 4.64; N, 9.72%.

3-(Benzo[*d*][1,3]dioxol-5-yl)-*N*-(4-chlorophenyl)-5-*meta*-tolyl-4,5-dihydro-1*H*-pyrazole-1-carboxamide (3i)

Yield: 339 mg (78%) of white powder; m.p. 159.8–160.2 °C. IR (ν_{max}, cm^{-1}): 3292 (N-H), 3093 (=C-H), 2989, 2901 (C-H), 1689 (C=O), 1633 (C=N), 1220 (C-O), 1033 (C-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.24 (s, 3H, CH_3), 3.10 (dd, *J*ax = 5.9 Hz, *J*ab = 17.6 Hz, 1H, Ha), 3.80 (dd, *J*bx = 11.6 Hz, *J*ab = 17.6 Hz, 1H, Hb), 5.45 (dd, *J*ax = 5.9 Hz, *J*bx = 11.6 Hz, 1H, Hx), 6.08 (s, 2H, O-CH_2-O), 6.76–7.96 (m, 11H, Ar-H), 9.18 (s, 1H, NH). Anal. Calcd for $C_{24}H_{20}ClN_3O_3$: C, 66.44; H, 4.65; N, 9.68. Found: C, 66.31; H, 4.61; N, 9.73%.

3-(Benzo[*d*][1,3]dioxol-5-yl)-*N*-(4-chlorophenyl)-5-(pyridin-4-yl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide (3j)

Yield: 337 mg (80%) of gray powder; m.p. 134.4–134.6 °C. IR (ν_{max}, cm^{-1}): 3290 (N-H), 3005 (=C-H), 2918, 2848 (C-H), 1672 (C=O), 1631 (C=N), 1220 (C-O), 1033 (C-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.11 (dd, *J*ax = 5.6 Hz, *J*ab = 17.9 Hz, 1H, Ha), 3.81 (dd, *J*bx = 12.0 Hz, *J*ab = 17.9 Hz, 1H, Hb), 5.48 (dd, *J*ax = 5.5 Hz, *J*bx = 11.9 Hz, 1H, Hx), 6.08 (s, 2H, O-CH_2-O), 6.96–7.87 (m, 11H, Ar-H), 9.16 (s, 1H, NH). Anal. Calcd for $C_{22}H_{17}ClN_4O_3$: C, 62.79; H, 4.07; N, 13.31. Found: C, 62.93; H, 4.11; N, 13.37%.

3-(Benzo[*d*][1,3]dioxol-5-yl)-*N*-(4-chlorophenyl)-5-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide (3k)

Yield: 320 mg (75%) of yellow powder; m.p. 158.7–159.0 °C. IR (ν_{max}, cm^{-1}): 3296 (N-H), 3093 (=C-H), 2974, 2901 (C-H), 1689 (C=O), 1631 (C=N), 1228 (C-O), 1033 (C-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.30 (dd, *J*ax = 4.7 Hz, *J*ab = 18.0 Hz, 1H, Ha), 3.79 (dd, *J*bx = 11.6 Hz, *J*ab = 18.0 Hz, 1H, Hb), 5.84 (dd, *J*ax = 4.7 Hz, *J*bx = 11.6 Hz, 1H, Hx), 6.08 (s, 2H, O-CH_2-O), 7.06–7.65 (m, 10H, Ar-H), 9.08 (s, 1H, NH). Anal. Calcd for $C_{21}H_{16}ClN_3O_3S$: C, 59.22; H, 3.79; N, 9.87. Found: C, 59.05; H, 3.75; N, 9.93%.

3-(Benzo[*d*][1,3]dioxol-5-yl)-*N*-(4-chlorophenyl)-5-(furan-2-yl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide (3l)

Yield: 332 mg (81%) of brown powder; m.p. 175.5–175.9 °C. IR (ν_{max}, cm^{-1}): 3294 (N-H), 3080 (=C-H), 2980, 2901 (C-H), 1680 (C=O), 1631 (C=N), 1219 (C-O), 1033 (C-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.30 (dd, *J*ax = 5.2 Hz, *J*ab = 17.3 Hz, 1H, Ha), 3.68 (dd, *J*bx = 11.9 Hz, *J*ab = 17.3 Hz, 1H, Hb), 5.60 (dd, *J*ax = 5.2 Hz, *J*bx = 11.9 Hz, 1H, Hx), 6.11 (s, 2H, O-CH_2-O), 7.01–7.83 (m, 10H, Ar-H), 9.10 (s, 1H, NH). Anal. Calcd for $C_{21}H_{16}ClN_3O_4$: C, 61.54; H, 3.94; N, 10.25. Found: C, 61.68; H, 3.96; N, 10.28%.

3-(Benzo[*d*][1,3]dioxol-5-yl)-5-(5-bromothiophen-2-yl)-*N*-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide (3m)

Yield: 404 mg (80%) of gray powder; m.p. 108.9–109.1 °C. IR (ν_{max}, cm^{-1}): 3288 (N-H), 3086 (=C-H), 2989, 2918 (C-H), 1681 (C=O), 1631 (C=N), 1220 (C-O), 1033 (C-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.26 (dd, *J*ax = 5.0 Hz, *J*ab = 17.9 Hz, 1H, Ha), 3.77 (dd, *J*bx = 11.5 Hz, *J*ab = 17.9 Hz, 1H, Hb), 5.73 (dd, *J*ax = 5.0 Hz, *J*bx = 11.4 Hz, 1H, Hx), 6.09 (s, 2H, O-CH_2-O), 6.99–7.78 (m, 9H, Ar-H), 9.09 (s, 1H, NH). Anal. Calcd for $C_{21}H_{15}BrClN_3O_3S$: C, 49.97; H, 3.00; N, 8.32. Found: C, 50.11; H, 3.02; N, 8.35%.

3-(Benzo[*d*][1,3]dioxol-5-yl)-*N*-(4-chlorophenyl)-5-(4-(trifluoromethyl)phenyl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide (3n)

Yield: 366 mg (75%) of white powder; m.p. 110.6–110.9 °C. IR (ν_{max}, cm^{-1}): 3290 (N-H), 3093 (=C-H), 2980, 2866 (C-H), 1670 (C=O), 1631 (C=N), 1222 (C-O), 1033 (C-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.16 (dd, *J*ax = 5.7 Hz, *J*ab = 17.7 Hz, 1H, Ha), 3.84 (dd, *J*bx = 11.6 Hz, *J*ab = 17.7 Hz, 1H, Hb), 5.64 (dd, *J*ax = 5.7 Hz, *J*bx = 11.6 Hz, 1H, Hx), 6.11 (s, 2H, O-CH_2-O), 7.02–7.85 (m, 11H, Ar-H), 9.17 (s, 1H, NH). Anal. Calcd for $C_{24}H_{17}ClF_3N_3O_3$: C, 59.09; H, 3.51; N, 8.61. Found: C, 59.27; H, 3.54; N, 8.65%.

3-(Benzo[*d*][1,3]dioxol-5-yl)-5-(3-chlorophenyl)-*N*-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide (3o)

Yield: 327 mg (72%) of white powder; m.p. 168.3–168.5 °C. IR (ν_{max}, cm^{-1}): 3292 (N-H), 3093 (=C-H), 2914, 2947 (C-H), 1687 (C=O), 1631 (C=N), 1228 (C-O), 1033 (C-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.12 (dd, *J*ax = 5.5 Hz, *J*ab = 17.9 Hz, 1H, Ha), 3.78 (dd, *J*bx = 11.8 Hz, *J*ab = 17.9 Hz, 1H, Hb), 5.49 (dd, *J*ax = 5.4 Hz, *J*bx = 11.8 Hz, 1H, Hx), 6.06 (s, 2H, O-CH_2-O), 6.99–7.86 (m,

11H, Ar-H), 9.10 (s, 1H, NH). Anal. Calcd for $C_{23}H_{17}Cl_2N_3O_3$: C, 60.81; H, 3.77; N, 9.25. Found: C, 60.93; H, 3.75; N, 9.28%.

2. 2. Anticancer Activity

HeLa (human cervical adenocarcinoma), MCF-7 (human breast adenocarcinoma) and NIH-3T3 (mouse embryonic fibroblast) cell lines were used for MTT assay.[14] Cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin and 2 mM L-glutamine. The cultures were incubated at 37 °C in a humidified atmosphere with 5% CO_2. Briefly, cells were seeded into a 96-well plate at a density of 1×10^4 cells/well in the 100 μL medium. After incubating the cells for 24 h, the dilutions of compounds at different doses (0.1–1000 μM) were added and incubated for 24h. After that, the culture medium was discarded and the wells were washed with PBS twice, followed by the addition of 10 μL MTT dye (0.5 mg/mL) into each well in the 100 μL medium. The cells were incubated for another 4 h at 37 °C. After removing all the culture medium, 100 μL DMSO was added per well. The percentage of cell viability was measured on ELISA reader (Biotek Co., USA) at wavelength of 570 nm. The IC_{50} values of compounds on the HeLa, MCF-7 and NIH-3T3 cells were calculated using GraphPad Prism 7. The images of cells treatment of compound were also assessed by inverted microscope (Zeiss Axiovert). All experiments were repeated multiple times.

2. 3. Colony Formation Assay

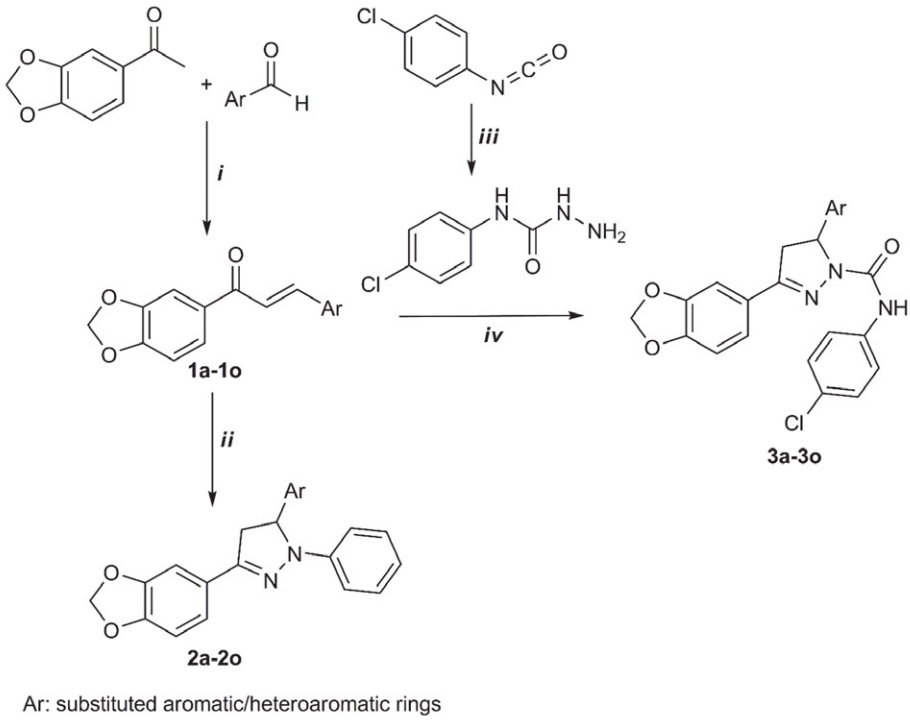
MCF-7 and HeLa cells were seeded in to 12-well plates at a density of 1000 cells/well in the 800 μL medium. After incubating the cells for 24 h, the at IC_{50} values of compound (μM) were added and incubated for 24 h. After treatment, the medium containing compounds was removed and replaced with pure medium. Medium was changed every 3 days for 10 days until visible colonies were formed. The medium was aspirated from wells and cells were washed three times with PBS. Then, colonies were simultaneously fixed with 500 μL of fixing reagent (methanol/acetic acid = 3/1) and incubated at room temperature for 5 min. Then, cells were stained with 0.5% crystal violet solution and incubated at room temperature. The plate was washed with ddH_2O. The stained cells were examined with invert microscope (Zeiss Axiovert, Germany) and imaging system.[15]

2. 4. *In Silico* ADMET Analysis

The ADMET parameters, pharmacokinetic properties and violations of drug-likeness of all synthesized compounds were investigated with the SwissADME webserver (http://www.swiss adme.ch/).

2. 5. Statistical Analysis

In this study, data were collected from 3 different biological replicates and the results were plotted as mean ±



Scheme 1. The synthesis pathway of the target compounds: (*i*) methanol, NaOH; (*ii*) ethanol, acetic acid; (*iii*) hydrazine monohydrate, ether; (*iv*) ethanol, NaOH.

SD. One-way ANOVA was used as statistical analysis by GraphPad Prism 7. P-value <0.05 was considered statistically significant.

3. Result and Discussion

3. 1. Chemistry

The synthesis of new pyrazoline derivatives was carried out by the methods outlined in Scheme 1. The cyclization of chalcones with phenylhydrazine hydrochloride in acidic media gave the 2-pyrazoline compounds (**2a**–**2o**). The cyclization of chalcones with semicarbazide in basic media gave the 2-pyrazoline-1-carboxamide compounds (**3a**–**3o**). The structures of these pyrazolines were elucidated by using IR and ^{1}H NMR spectral methods besides elemental analysis.

The IR spectra of compounds **2a**–**2o** and **3a**–**3o** showed C=N intense absorption bands in the range of 1591–1597 cm^{-1} and 1631–1633 cm^{-1}, respectively. The IR spectra of compounds **3a**–**3o** demonstrated intense absorption bands in the range of 3288–3367 cm^{-1} due to the N–H bond and 1666–1689 cm^{-1} due to the C=O bond. In the ^{1}H NMR spectrum, the finding of three double doublet peaks belonging to the Ha, Hb and Hx protons indicated the synthesis of the pyrazoline ring successfully. The Ha and Hb methylene protons of the pyrazoline ring in position 4 appeared as double doublet signals at 2.92–3.30 ppm and 3.68–3.96 ppm, respectively. The methine proton of Hx resonated as double doublet signals at 5.33–5.85 ppm. Furthermore, compound **3a–3o** revealed the singlet signal assigned to NH proton of carboxamide moiety at 9.00–9.20 ppm. The methylene protons belonging to 1,3-benzodioxole ring gave a singlet peak at 6.02–6.14 ppm. The elemental analysis also supported these structures.

3. 2. Biological Activity

The anticancer activity of synthesized pyrazoline derivatives was evaluated on HeLa (human cervical adenocarcinoma), MCF-7 (human breast adenocarcinoma) and NIH-3T3 (mouse embryonic fibroblast) cell lines using

Table 1. The IC_{50} values of synthesized pyrazoline derivatives

Compounds	IC_{50} (μM)			Selectivity Index (SI)*	
	HeLa	MCF-7	NIH-3T3	NIH-3T3/HeLa	NIH-3T3/MCF-7
2a	128.24 ± 20.16	109.65 ± 6.68	ND	9.87	11.54
2b	93.88 ± 14.02	ND	684.13 ± 14.20	7.29	ND
2c	103.91 ± 2.65	113.19 ± 11.02	594.89 ± 31.08	5.73	5.26
2d	289.24 ± 24.28	**27.63 ± 3.51**	899.52 ± 3.65	3.11	**32.56**
2e	75.15 ± 22.14	269.19 ± 2.69	212.12 ± 16.21	2.82	0.79
2f	82.35 ± 14.62	186.39 ± 12.66	724.93 ± 5.22	8.80	3.89
2g	168.42 ± 6.31	ND	533.436 ± 3.68	3.17	ND
2h	81.02 ± 2.44	74.75 ± 12.55	290.13 ± 14.03	3.58	3.88
2i	158.31 ± 13.70	255.61 ± 22.08	152.08 ± 8.25	0.96	0.59
2j	108.84 ± 3.11	**14.30 ± 1.10**	528.71 ± 6.69	4.86	**36.97**
2k	97.54 ± 6.63	83.07 ± 3.08	495.38 ± 18.09	5.08	5.96
2l	111.58 ± 4.12	181.76 ± 6.24	985.04 ± 10.85	8.83	5.42
2m	188.75 ± 2.25	221.12 ± 19.30	181.74 ± 14.03	0.96	0.82
2n	79.18 ± 12.37	54.07 ± 6.36	ND	ND	ND
2o	50.02 ± 3.48	340.56 ± 17.10	908.74 ± 7.49	18.17	2.67
3a	75.26 ± 13.50	ND	232.78 ± 21.01	3.09	ND
3b	93.49 ± 2.84	114.05 ± 6.66	476.97 ± 13.14	5.10	4.18
3c	**13.74 ± 1.33**	24.49 ± 3.81	381.21 ± 3.86	**27.74**	**15.57**
3d	120.22 ± 10.07	78.85 ± 5.64	665.01 ± 18.29	5.53	8.43
3e	81.15 ± 2.66	37.75 ± 3.37	615.61 ± 11.94	7.59	16.31
3f	**11.53 ± 3.09**	**11.37 ± 1.08**	942.61 ± 18.38	**81.75**	**82.90**
3g	533.14 ± 18.13	117.45 ± 6.54	233.74 ± 10.93	0.44	1.99
3h	123.09 ± 8.07	ND	556.01 ± 14.22	4.52	ND
3i	111.84 ± 6.53	108.14 ± 12.07	279.01 ± 19.38	2.49	2.58
3j	422.80 ± 2.49	**10.08 ± 1.04**	693.45 ± 9.21	1.64	**68.79**
3k	137.21 ± 15.08	516.56 ± 3.98	130.76 ± 3.45	0.95	0.25
3l	ND	122.29 ± 11.82	560.43 ± 3.70	ND	4.58
3m	115.53 ± 5.74	369.25 ± 22.47	605.59 ± 17.93	5.24	1.64
3n	ND	**13.67 ± 2.65**	968.15 ± 21.80	ND	**70.82**
3o	ND	41.53 ± 6.12	252.15 ± 15.54	ND	6.07

* ND: not determined

MTT assay. The IC_{50} values of all compounds are given in Table 1. Compound **2d** bearing 4-nitrophenyl and compound **2j** bearing pyridine ring from phenylpyrazoline derivatives (**2a**–**2o**) and compound **3j** bearing pyridine and **3n** bearing 4-trifluoromethylphenyl ring showed the highest cytotoxicity activity the MCF-7 cancer cell lines. On the other hand, compound **3c** bearing 4-bromophenyl ring from pyrazoline carboxamide derivatives (**3a**–**3o**) demonstrated the highest cytotoxicity activity on HeLa cancer cell lines. Among all pyrazoline derivatives, compound **3f** exhibited the most potent cytotoxic activity on both two cancer cells.

Additionally, selectivity index (SI) IC_{50} for NIH-3T3 cell line/IC_{50} for HeLa and MCF-7 cell line was calculated for the evaluation of cytotoxic effects of molecules against both normal and cancer cells. Most of the compunds displayed low cytotoxicity against normal cells and especially, it was determined that the selectivity indices of compounds **2d**, **2j**, **3c**, **3f**, **3j**, **3n** showing the highest cytotoxic activity were quite high. The most selective compound **3f** had SI 81.75 for HeLa, and 82.90 for MCF-7 cell line.

After the MTT results, the images taken under the microscope of the compounds with antiproliferative effect are presented in Figure 2. When the synthesized compounds were compared with the control group in MCF-7 cells, their interaction with each other decreased and their growth was found to be slowed down when treated with compounds **2d**, **2j**, **3f**, **3j** and **3n**. Although cancer cells are cells that grow close to each other and proliferate rapidly, the synthesized compounds at IC_{50} reduced high-growing cancer cells such as MCF7 and HeLa. In HeLa cells, it was shown that reduced cell interactions and growth was observed when treated with compounds **3c** and **3f** in comparison to the control group.

Cancer cells can survive when they migrate from their region to other regions by forming a colony on their own. Colony formation assay measurement is an important method to measure the effect of cancer cells on growth alone and is used to determine the effects of drugs on circulating cancer cells. The effect of newly synthesized compounds on the colony formation potential is shown in Figure 3 with MCF-7 and Hela cells. Compared with the control group MCF-7 cells, it was shown that compounds **2d**, **2j**, **3f**, **3j** and **3n** reduced colony formation in cells and reduced the potential for colony formation. In HeLa cells, when compared to the control group, it was observed with the microscope that compounds **3c** and **3f** reduced the colony formation of the cells.

3. 3. *In Silico* ADMET Results

Oral bioavailability is an important factor in order to develop new drug candidates targeted for oral use. It is possible for drug candidates to succeed in phase studies with good lipophilicity, water solubility, physicochemical and pharmacokinetics properties.[16] Therefore, we evaluat-

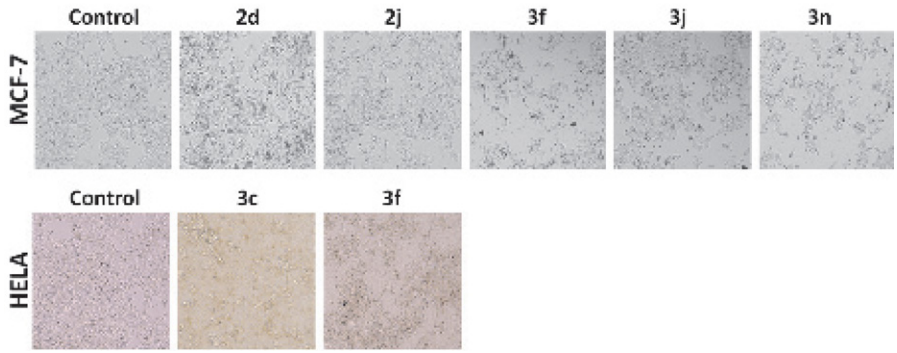


Figure 2. The images of cells treatment with IC_{50} values of synthesized pyrazoline derivatives in MCF-7 cells and HeLa cells

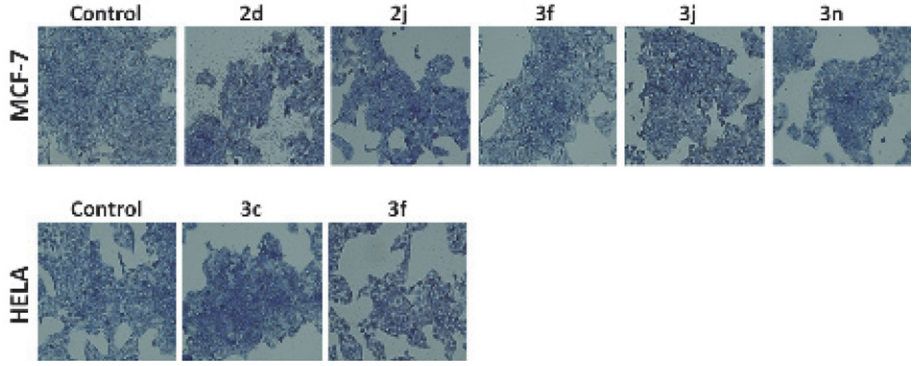


Figure 3. The colony formation images of MCF-7 cells and HeLa cells.

Table 2. Lipinski and Veber Parameters of the synthesized compounds.

Comp	Physicochemical Properties							Lipophilicity	Druglikeness					Water Solubility		Pharmacokinetics	
	MW	Fsp3	RB	HBA	HBD	MR	tPSA	clogP	Lipinski	Ghose	Veber	Egan	Muegge	ESOL	Class	GI absorption	F
2a	342.39	0.14	3	3	0	108.32	34.06	4.07	√	√	√	√	√	–5.33	Moderately	High	0.55
2b	360.38	0.14	3	4	0	108.28	34.06	4.38	√	√	√	√	√	–5.48	Moderately	High	0.55
2c	421.29	0.14	3	3	0	116.02	34.06	4.70	√	√	√	√	1	–6.23	Poorly	High	0.55
2d	387.39	0.14	4	5	0	117.15	79.88	3.65	√	√	√	√	√	–5.37	Moderately	High	0.55
2e	356.42	0.17	3	3	0	113.29	34.06	4.39	√	√	√	√	1	–5.62	Moderately	High	0.55
2f	367.40	0.13	3	4	0	113.04	57.85	3.85	√	√	√	√	√	–5.26	Moderately	High	0.55
2g	370.44	0.21	3	3	0	118.26	34.06	4.70	√	√	√	√	1	–5.92	Moderately	High	0.55
2h	356.42	0.17	3	3	0	113.29	34.06	4.39	√	√	√	√	1	–5.62	Moderately	High	0.55
2i	356.42	0.17	3	3	0	113.29	34.06	4.41	√	√	√	√	1	–5.62	Moderately	High	0.55
2j	343.38	0.14	3	4	0	106.12	46.95	3.32	√	√	√	√	√	–4.66	Moderately	High	0.55
2k	348.42	0.15	3	3	0	106.20	62.30	4.06	√	√	√	√	√	–5.17	Moderately	High	0.55
2l	332.35	0.15	3	4	0	100.59	47.20	3.39	√	√	√	√	√	–4.69	Moderately	High	0.55
2m	427.31	0.15	3	3	0	113.90	62.30	4.75	√	√	√	√	1	–6.29	Poorly	High	0.55
2n	410.39	0.17	4	6	0	113.33	34.06	5.09	√	√	√	√	1	–6.17	Moderately	High	0.55
2o	376.84	0.14	3	3	0	113.33	34.06	4.59	√	√	√	√	1	–5.91	Moderately	High	0.55
3a	419.86	0.13	5	4	1	122.51	63.16	4.07	√	√	√	√	√	–5.41	Moderately	High	0.55
3b	437.85	0.13	5	5	1	122.47	63.16	4.51	√	√	√	√	√	–5.57	Moderately	High	0.55
3c	498.76	0.13	5	4	1	130.21	63.16	4.81	√	1	√	√	1	–6.32	Poorly	High	0.55
3d	464.86	0.13	6	6	1	131.33	108.98	3.52	√	√	√	√	√	–5.48	Moderately	High	0.55
3e	433.89	0.17	5	4	1	127.48	63.16	4.53	√	√	√	√	√	–5.71	Moderately	High	0.55
3f	444.87	0.12	5	5	1	127.23	86.95	3.99	√	√	√	√	√	–5.36	Moderately	High	0.55
3g	447.91	0.20	5	4	1	132.44	63.16	4.73	√	1	√	√	1	–6.02	Poorly	High	0.55
3h	433.89	0.17	5	4	1	127.48	63.16	4.41	√	√	√	√	√	–5.71	Moderately	High	0.55
3i	433.89	0.17	5	4	1	127.48	63.16	4.53	√	√	√	√	√	–5.71	Moderately	High	0.55
3j	420.85	0.14	5	5	1	120.31	76.05	3.33	√	√	√	√	√	–4.74	Moderately	High	0.55
3k	425.89	0.14	5	4	1	120.39	91.40	4.10	√	√	√	√	√	–5.26	Moderately	High	0.55
3l	409.82	0.14	5	5	1	114.78	76.30	3.42	√	√	√	√	√	–4.77	Moderately	High	0.55
3m	504.78	0.14	5	4	1	128.09	91.40	4.86	2	1	√	√	1	–6.38	Poorly	High	0.17
3n	487.86	0.17	6	7	1	127.51	63.16	5.12	√	2	√	1	1	–6.27	Poorly	High	0.55
3o	454.31	0.13	5	4	1	127.52	63.16	4.73	√	√	√	√	1	–6.01	Poorly	High	0.55

Molecular weight: MW, topological polar surface area: tPSA, Molar Refractivity: MR, fraction of sp3 carbon atoms: Fsp3, HBD: hydrogen bonds donor, HBA: hydrogen bond acceptor, RB: rotatable bonds, LogP values: indicator of Lipophilicity, ESOL: aqueous solubility parameter, Log Kp: skin permeation, F: Bioavailability Score.

ed the druglikeness properties such as Lipinski, Ghose, Veber, Egan and Muegge filters of all synthesized compounds. The results showed that the majority of all compounds did not violate these filters. Aqueous solubility plays a critical role for drug's bioavailability. Therefore, ESOL (Log S) values of all compounds were calculated and the solubility of all compounds was determined to be moderate, except for compounds **2c**, **2m**, **3c**, **3g**, **3m**, **3n** and **3o**. In addition, the GI absorptions of all compounds was determined to be high. The bioavailability score of compounds was found to be ideal.[17] Among these compounds **3f**, showing the best anticancer activity, possesses good drug score values. Compound **3f** did not appear to violate any of the rules described here (Table 2).

The BOILED-Egg server getting from swissadme program gives pharmacokinetic properties (such as gastrointestinal absorption, brain access and P-glycoprotein) of molecules by calculating with partition constant (log P) and topological polar surface area (TPSA). The yellow area (BBB) shows molecules that easily permeate through the blood-brain barrier, the white region (HIA) demostrates molecules that easily permeate through the gastrointestinal membranes, while the gray area presents molecules having low absorption. Based on this BOILED-Egg server, all synthesized molecules permeate through either the blood-brain barrier or the gastrointestinal membranes. None of the molecules are in the low absorption region (Figure 4). In addition, PGP^+ identifies the molecules to be effluated from the central nervous system by the P-glycoprotein. PGP^- also identifies the molecules that are non-substrate of the P-glycoprotein. Among these compounds **3f**, showing the best anticancer activity, permeates through the gastrointestinal membranes easily and is non-substrate of the P-glycoprotein according to Figure 4.

4. Conclusion

In the present study, a number of new molecules contained two pharmacodynamic groups (2-pyrazoline and benzo[*d*][1,3]dioxol) inducing significant synergistic lethality of cancer cells were designed and synthesized. These pyrazolines were assessed for anticancer activity on HeLa, MCF-7 cancer cell lines and NIH-3T3 normal cell lines. The results demonstrated that compounds **2d**, **2j**, **3c**, **3j** and **3n** exhibited higher activity than the other substituted pyrazoline derivatives. Notably, compound **3f** was identified as the most potent cytotoxic molecule in this study with IC_{50} values of 11.53 μM on HeLa and 11.37 μM on MCF-7. In silico ADMET analysis and drug-likeness displayed that compound **3f** has good pharmacokinetic characteristics and absorption-solubility properties. Therefore, compound **3f** can be a lead compound for the development of novel anticancer agents in drug discovery.

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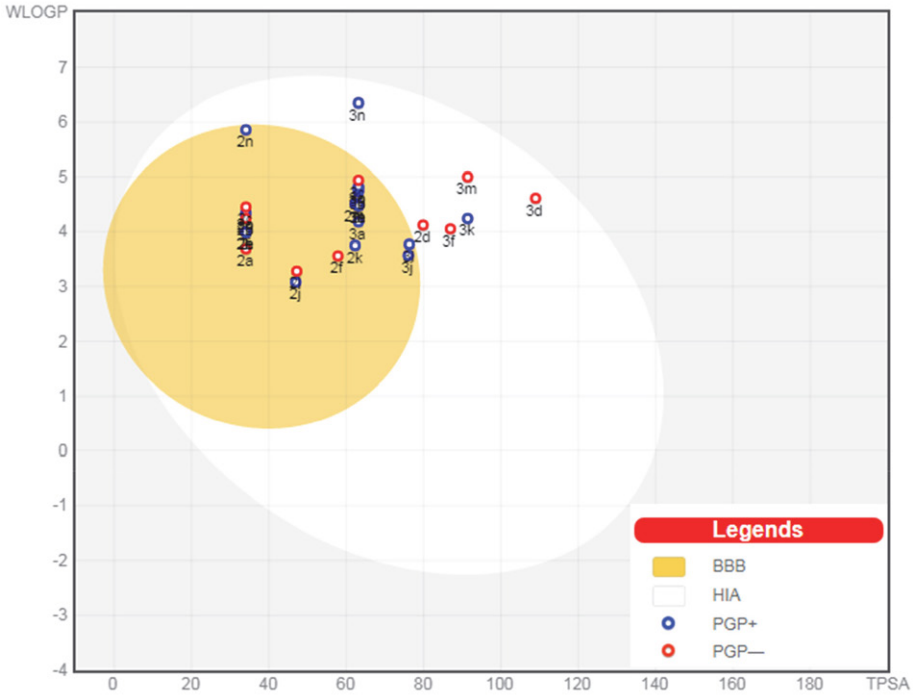

Figure 4. The BOILED-Egg predictive models of all compounds

Supporting Information

Contains copies of IR and ^{1}H NMR spectra of synthesized compounds (**2a**–**2o** and **3a**–**3o**).

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Povzetek

Načrtovali in izvedli smo sintezo nove serije 2-pirazolinskih derivatov. Kot izhodne spojine smo uporabili substituirane benzodioksol halkone. IR in ^{1}H NMR spektroskopija ter elementne analize so bile uporabljene za karakterizacijo strukture pripravljenih spojin. Citotoksične aktivnosti na HeLA in MCF-7 rakaste celične linije ter na NIH-3T3 celice smo za sintetizirane spojine določili s pomočjo MTT metode. Med pripravljenimi spojinami so izkazale opazno citotoksično aktivnost (z IC_{50} vrednostmi v intervalu 10.08 do 27.63 μM) spojine **2d**, **2j**, **3j** in **3n** proti MCF-7 celicam ter **3c** proti HeLa celicam. Spojina **3f** je izkazala močno protirakavo učinkovitost na obe celični liniji z dobrimi vrednostmi selektivnosti (IC_{50} = 11.53 μM za HeLa s SI = 81.75 ter IC_{50} = 11.37 μM za MCF-7 s SI = 82.90). Dodatno smo z *in silico* ADMET analizo ugotovili sposobnost pripravljenih spojin, da bi delovale kot uspešne učinkovine.