Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications
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Supporting information

Benzisothiazolones arrest cell cycle at G_2/M phase and induce apoptosis in HeLa cells

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EXPERIMENTAL SECTION

CHEMISTRY

Typical experimental procedure: Various benzisothiazolones (**4.1** to **4.12**) were synthesized starting from commercially available dithiodibenzoic acid (**1**). Dithiodibenzoic acid (2 g) was refluxed with thionyl chloride (10 mL) for 24 h at 80 ^{o}C. Excess thionyl chloride was removed by distillation and chased-up with dry benzene to get dithiodibenzoyl chloride as a pale brown solid 2.2 g (crude). To a stirred solution of dithiodibenzoyl chloride (2.2 g, 6.41 mmol) in dry DCM (20 mL) was added bromine (0.66 mL, 12.8 mmol) and the mixture was refluxed for 12 h. Dichloromethane and excess bromine were distilled off and the residue was chased with dry benzene (2 x 5 mL) to get 3.2 g of 2-bromosulfenylbenzoyl chloride (**2**) as a brown solid which was directly subjected to next step without further purification.

General procedure for the preparation of compounds 4.1-4.12

To a stirred solution containing a mixture of aliphatic or aromatic amine (1.1 equiv.) and triethylamine (3 equiv.) in dry DCM at 0 ^{o}C was added 2-bromosulfenylbenzoyl chloride (1.0 equiv) in DCM drop-wise and it was stirred overnight at room temperature. After completion of the reaction as per TLC, it was quenched with water, washed with dil HCl, the organic solvent dried over Na_2SO_4, evaporated, and the residue was subjected to column chromatography with EtOAc-Hexanes as eluents to afford the compounds **4.1-4.12** in 10-66% yields.

BIOLOGY

Cell Culture: Human cervical cancer cell line (HeLa) and squamous cell carcinoma (SiHa) and colon adenocarcinoma cell line (SW480) were from the American Type Culture Collection (Rockville, MD). The cells were grown in Dulbecco's modified Eagle's medium. All the cell

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culture materials were from GIBCO/BRL Life Technologies Inc., Grand Island, NY, USA. Medium was supplemented with 10% heat inactivated fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/mL of penicillin, and 100 µg/mL of streptomycin. The cells were maintained in a humidified incubator at 37 ^{o}C in 5% CO_2 atmosphere.

Cytotoxicity (MTT) Assay: MTT assay was performed following a standard protocol (Mosmann, 1983). Briefly, cells were trypsinized and approximately $2.5x10^3$ cells/well plated in 96 well plate in 100µl culture medium (10% Fetal Calf Serum in DMEM supplemented with 100 U/ml of penicillin and 100 µg/ml of streptomycin) and incubated in a humidified atmosphere of 5% CO_2 at 37 ^{o}C. Next day, the drugs were mixed with culture medium and fed to the culture. The DMSO concentration was maintained equal in all the cases. The cultures were incubated in the presence of drug for 72 h. After the incubation period, medium removed and replaced with 100µl of MTT containing medium (0.8 mg/ml in culture medium) and incubated for further 3 h. Afterwards the medium removed and the cells were lysed with 100µl of lysis solution (Isopropanol with 4mM HCl). The absorbance at 570 nm (formation of formazan) and 655 nm (reference) was recorded with a Microplate Reader (Model 680, BIO-RAD).

DNA Fragmentation Assay: Approximately $5x10^6$ cells were seeded in 10 cm dishes in 10 ml of culture medium and incubated in a humidified atmosphere of 5% CO_2 at 37 °C. Appropriate dilutions of the drugs were then prepared in culture medium and fed to the culture on the next day. After incubating the culture for another 72 h, DNA fragmentation assay was performed as per the protocol reported by Hockenberry *et al.*, (1990). Briefly, cells scraped in culture medium, pelleted at 2000xg for 10 min. cell pellets washed with PBS once and lysed in 0.5 ml of lysis buffer (0.5% Triton X-100, 5 mM Tris pH 7.4, 20 mM EDTA). The lysates centrifuged at 12000 *rpm* for 15 min at 4 °C. The supernatant recovered and extracted with equal volume of phenol chloroform isoamyl alcohol mixture (25:24:1). The DNA recovered by ethanol precipitation overnight. The next day the DNA pelleted, washed once with 70% ethanol and air dried. The DNA pellet suspended in 50µl of TE buffer (10mM Tris, 1mM EDTA pH 7.5). The DNA samples digested sequentially with RNase and Protease followed by resolving on a 2%.agarose gel and photographed under UV illumination. (Molecular Imager Gel Doc XR Imaging System-BIO-RAD).

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Determination of mitochondrial membrane potential: The cells were grown in the presence of the drug for 24 h in a 24 well plate and stained with the JC-1 dye as per the manufacturer's protocol (Sigma) and analyzed by fluorescence microscope. The dye is taken up by the mitochondria, forms aggregates with an intense red fluorescence at 590nm (Green *et al*., 1998). However, if the mitochondrial membrane potential is lost, the JC-1 dye cannot aggregate and appears in the cytosol as a monomer with green fluorescence.

Cell Cycle Analysis: The cell cycle analysis was performed as described by Manna *et al*., (2010). Cells (log phase culture) were treated with 50 µM concentration of the BIT. After 48 h of treatment, cells were harvested by trypsinization and washed twice with PBS. Cells were then gently fixed with 70% ice cold ethanol at -20 ^{o}C for overnight. The next day cells were pelleted and washed with 1% FBS containing PBS EDTA, resuspended in PBS EDTA, Rnase (160µg/ml) was added and incubated at 37 ^{o}C for 1 h. Following this, cells were stained with 10µg/ml propidium iodide for 10 min and the DNA content was analyzed on a flow cytometer. (Beckman Coulter Quanta™ SC MPL Flow Cytometer).

Western analysis: HeLa cells in 96 well plates were treated with DMSO (control), Taxol (12 nM) and benzisothiazolone **4.9** (50 µM) for 48 h. After the incubation period, the culture medium removed and the cells were directly lysed by adding 300uL of SDS lysis buffer and collected in a tube and boiled. The cell lysates were then resolved on a 10% SDS-PAGE. The proteins were then transferred on to a nitrocellulose membrane, blocked and probed for cleaved caspase 3 using a specific antibody that will recognize the cleaved caspase 3 fragments. The blot was then stripped and reprobed for actin.

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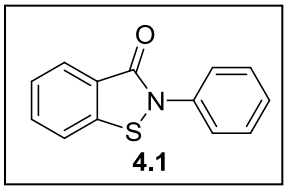
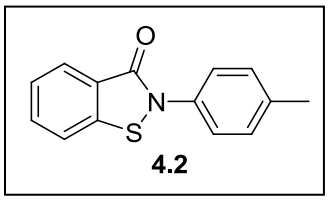
2-phenylbenzo[*d*]isothiazol-3(2*H*)-one (4.1)



4.1

Colorless solid; Yield = 39%; mp.141-143 ^{o}C; ^{1}H NMR ($CDCl_3$) δ 8.15 (d, 1H, *J* = 8 Hz), 7.72 (d, 2H, *J* = 8.4 Hz), 7.66 (t, 1H, *J* = 7.6 Hz), 7.58 (d, 1H, *J* = 8.4 Hz), 7.47 (t, 2H, *J* = 8 Hz), 7.32 (t, 1H, *J* = 7.6 Hz); ^{13}C NMR ($CDCl_3$) δ 164.1, 139.8, 137.20, 132.32, 129.34 (2C), 127.18, 127.03, 125.78, 124.84, 124.59 (2C), 120.06; IR (neat) cm^{-1}: 3054, 1660, 1591, 1489, 1308, 1263, 730, 698.9; ESI m/z: Calcd. for $C_{13}H_{10}NOS$ $[M+H]^+$ 228.0483, found 228.0486 $[M+H]^+$.

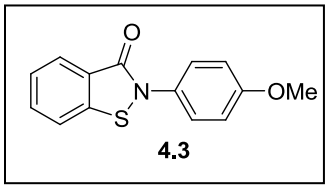
2-*p*-tolylbenzo[*d*]isothiazol-3(2*H*)-one (4.2)



4.2

Colorless solid; Yield = 48%; mp. 135-136 ^{o}C; ^{1}H NMR ($CDCl_3$) δ 8.10 (d, 1H, *J* = 8 Hz), 7.63 (t, 1H, *J* = 8.0 Hz), 7.58-7.55 (m, 3H), 7.46-7.42 (m, 1H), 7.27 (d, 2H, *J* = 6.8 Hz), 2.38 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 164.18, 139.97, 137.22, 134.53, 132.21, 129.94 (2C), 127.16, 125.74 (2C), 124.87, 124.76, 120.07, 21.10; IR (neat) cm^{-1}: 1664, 1504, 1329, 814, 735; ESI m/z: calcd. for $C_{14}H_{12}NOS$ $[M+H]^+$ 242.0640, found 242.0646 $[M+H]^+$.

2-(4-methoxyphenyl)benzo[*d*]isothiazol-3(2*H*)-one (4.3)



4.3

Colorless solid; Yield = 45%; mp.145-146 ^{o}C; ^{1}H-NMR ($CDCl_3$) δ 8.08, (d, 1H, *J* = 8 Hz), 7.62 (dt, 1H, *J* = 1.2, 8.0 Hz), 7.56-7.52 (m, 3H), 7.42 (dt, 1H, *J* = 8.8, 0.8 Hz), 6.97-6.95 (m, 2H), 3.82 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 164.15, 158.60, 139.93, 132.03, 129.6, 126.95 (2C), 126.65, 125.60, 124.55, 119.98, 114.47 (2C), 55.44; IR (neat) cm^{-1}: 1651, 1504, 1445, 1294, 1242, 1028, 824, 735; ESI m/z: calcd. for $C_{14}H_{12}NO_2S$ $[M+H]^+$ 258.0589, found 258.0594 $[M+H]^+$.

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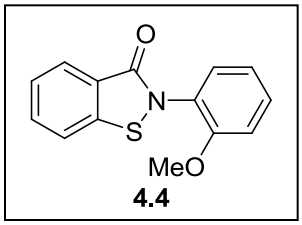
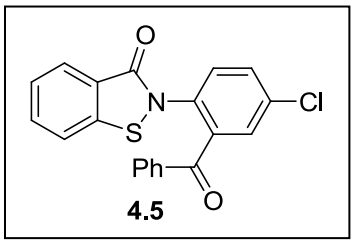
2-(2-methoxyphenyl)benzo[*d*]isothiazol-3(2*H*)-one (4.4)



4.4

Colorless solid; Yield = 44%; mp. 140-145 ^{o}C; ^{1}H NMR ($CDCl_3$) δ 8.11 (d, 1H, *J* = 8 Hz), 7.64 (t, 1H, *J* = 7.0 Hz), 7.56 (d, 1H, *J* = 8.0 Hz), 7.39-7.44 (m, 3H), 7.03-7.07 (m, 2H), 3.84 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 164.99, 155.97, 141.53, 131.99, 130.57, 130.26, 127.17, 125.34, 124.52, 123.89, 120.87, 120.09, 112.40, 55.91; IR (neat) cm^{-1}: 1665, 1498, 1267, 730; ESI m/z: calcd. for $C_{14}H_{12}NO_2S$ $[M+H]^+$ 258.0587, found 258.0589 $[M+H]^+$.

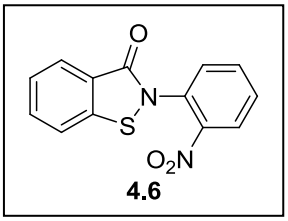
2-(2-benzoyl-4-chlorophenyl)benzo[*d*]isothiazol-3(2*H*)-one (4.5)



4.5

Colorless solid; Yield = 29%; mp. 159-160 ^{o}C; ^{1}H NMR ($CDCl_3$) δ 7.80 (d, 1H, *J* = 8 Hz), 7.78 (d, 2H, *J* = 1.52 Hz), 7.32-7.59 (m, 9H); ^{13}C NMR ($CDCl_3$) δ 192.12, 163.13, 139.27, 137.46, 134.82, 132.88, 131.86 (2C), 131.18, 130.41, 128.96, 128.63 (2C), 128.53, 126.93 (2C), 125.89, 124.50, 121.84, 118.76; IR (neat) cm^{-1}: 1617, 1262, 730; ESI m/z: calcd. for $C_{20}H_{12}NO_2SNaCl$ $[M+Na]^+$ 388.0175, found 388.0172 $[M+Na]^+$.

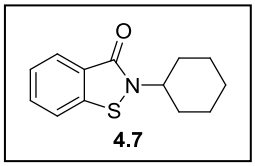
2-(2-nitrophenyl)benzo[*d*]isothiazol-3(2*H*)-one (4.6)



4.6

Yellow solid; Yield = 10%; mp. 190-191 ^{o}C; ^{1}H NMR ($CDCl_3$) δ 8.08 (d, 2H, *J* = 8 Hz), 7.76-7.69 (m, 2H), 7.62-7.56 (m, 3H), 7.46 (t, 1H, 7.6 Hz); ^{13}C NMR ($CDCl_3$): δ 164.63, 146.91, 141.04, 133.77, 132.84, 130.84, 129.77, 129.28, 127.55, 126.06, 125.78, 122.98, 120.31; IR (neat) cm^{-1}: 2919, 1662, 1523, 1345, 1267; ESI m/z: calcd. for $C_{13}H_9N_2O_3S$ $[M+H]^+$ 273.0334, found 273.0331 $[M+H]^+$.

2-cyclohexylbenzo[*d*]isothiazol-3(2*H*)-one (4.7)



4.7

Colorless solid; Yield = 43%; mp. 65-66 ^{o}C; ^{1}H NMR ($CDCl_3$) δ 8.04 (d, 1H, *J* = 7.0 Hz), 7.6-7.54 (m, 2H), 7.38 (dt, 1H, *J* = 7.2, 1.6 Hz), 4.64-4.56 (m, 1H), 2.07-2.02 (m, 2H), 1.91-1.84 (m, 2H), 1.77-1.71 (m, 1H), 1.61-1.41 (m, 4H), 1.27-1.16 (m, 1H); ^{13}C NMR ($CDCl_3$): δ 164.76, 140.23, 131.37, 126.45, 125.4, 125.25, 120.28, 53.14, 32.86 (2C), 25.56 (2C), 25.19; IR (neat) cm^{-1}: 2930, 2854, 1640, 1563; ESI m/z: calcd. for $C_{13}H_{16}NOS$ $[M+H]^+$ 234.0953, found 234.0953 $[M+H]^+$.

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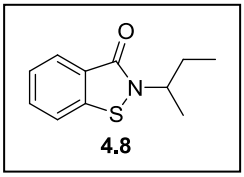
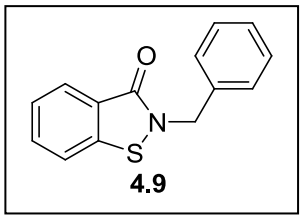
2-sec-butylbenzo[*d*]isothiazol-3(2*H*)-one (4.8)



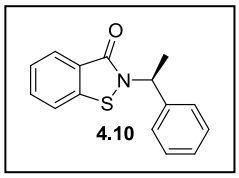
Gummy liquid; Yield = 65%; ^{1}H NMR ($CDCl_3$) δ 8.05 (d, 1H, *J* = 7.6 Hz) 7.53-7.63 (m, 2H), 7.39 (t, 1H, *J* = 7.6 Hz), 3.72 (d, 2H, *J* = 7.6 Hz), 2.18-2.08 (m, 1H), 0.99 (d, 6H, *J* = 6.4 Hz); ^{13}C NMR ($CDCl_3$): δ 165.52, 140.13, 131.59, 126.6, 125.32, 124.6, 120.15, 51.11, 28.95, 19.82 (2C); IR (neat) cm^{-1}: 2960, 1651, 1446; ESI m/z: calcd. for $C_{11}H_{14}NOS$ $[M+H]^+$ 208.0796, found 258.0792 $[M+H]^+$.

2-benzylbenzo[*d*]isothiazol-3(2*H*)-one (4.9)



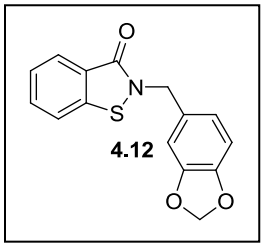
Colorless solid; Yield = 27%; mp. 87-88 ^{o}C; ^{1}H-NMR ($CDCl_3$) δ 7.96 (d, 1H, *J* = 8 Hz), 7.46 (t, 1H, *J* = 8 Hz), 7.37 (d, 1H, *J* = 8 Hz), 7.30-7.21 (m, 6H), 4.94 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 165.34, 140.43, 136.21, 131.84, 128.84 (2C), 128.44 (2C), 128.28, 126.79, 125.49, 124.49, 120.42, 47.57; IR (neat) cm^{-1}: 1645, 1447, 1328, 730, 697; ESI m/z: calcd. for $C_{14}H_{12}NOS$ $[M+H]^+$ 242.0640, found 242.0640 $[M+H]^+$.

(*S*)-2-(1-phenylethyl)benzo[*d*]isothiazol-3(2*H*)-one (4.10)



Gummy Liquid; Yield = 66%; ^{1}H NMR ($CDCl_3$) δ 8.05 (d, 1H, *J* = 7.8 Hz), 7.56 (t, 1H, *J* = 7.14 Hz), 7.5-7.3 (m, 7H), 6.07 (q, 1H, *J* = 6.95 Hz), 1.8 (d, 3H, *J* = 7.03 Hz); ^{13}C NMR ($CDCl_3$): δ 165, 140.4, 140.14, 131.62. 128.67 (2C), 128.19, 127.29 (2C), 126.6, 125.4, 125.02, 120.36, 52.19, 19.14; ESI m/z: calcd. for $C_{15}H_{14}NOS$ $[M+H]^+$ 256.0796, found 256.0795 $[M+H]^+$.

2-(benzo[*d*][1,3]dioxol-5-ylmethyl)benzo[*d*]isothiazol-3(2*H*)-one (4.12)



Colorless solid; Yield = 56%; ^{1}H NMR ($CDCl_3$) δ 8.05 (d, 1H, *J* = 7.6 Hz), 7.57 (t, 1H, *J* = 6.8 Hz), 7.48 (d, 1H, *J* = 8 Hz), 7.40-7.34 (m 1H), 6.84 (s, 2H) 6.78-6.72 (m, 1H), 5.94 (s, 2H), 4.95 (s, 2H); ^{13}C NMR ($CDCl_3$): δ 165.19, 148.03, 147.63, 140.3, 131.8, 129.9, 126.71, 125.46, 124.48, 122.08, 120.35, 108.91, 108.3, 101.19, 47.36; IR (neat) cm^{-1}: 2912, 2788, 1633, 1601; ESI m/z: calcd. for $C_{15}H_{12}NO_3S$ $[M+H]^+$ 286.0538, found 286.0532 $[M+H]^+$.