Chem Biol Drug Des 2012; **80:** 853–861

Research Article

doi: 10.1111/j.1747-0285.2012.01442.x

Syntheses and *In Vitro* Anticancer Properties of Novel Radiosensitizers

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Series of 4-(ethylsulfonyl)-1-halogen-2-nitrobenzene (3a–e) and 1-(4-halogen-3-nitrophenyl) propan-1-one (5a–d) analogs designed as novel radiosensitizers using bromonitropropiophenone and bromonitrobenzonitrile as lead compounds were synthesized. The anticancer activities of the compounds were evaluated *in vitro* using human prostate cancer (DU-145) and breast cancer (MCF-7) cell lines and the MTT assay. From the series, six compounds (3b–e, 5b–c) exhibited potent growth inhibitory effects against both cell lines. The most active, compound 3d, is an iodosulfone and is significantly more potent than the lead compound 5c at 10 μM. Compounds were then compared with doxorubicin, a clinically used anticancer compound for breast and prostate cancers. Our most active compound 3d is more effective than doxorubicin at the dose level of 10 μM at 3 days after radiation, cell viabilities of 18%, 13% compared to 87%, 94% against MCF-7, and 15%, 20% compared to 60%, 75% against DU-145 without and with radiation, respectively. At 10 μM, compound 5c had no effects as compared to control, whereas compound 3d reduced DU-145 cell viability to 16% and that of MCF-7 cells to 9% even at 5 days after radiation. These results are very encouraging. Future studies include testing the compounds *in vivo* with and without radiation.

Key words: anticancer, bromonitropropiophenone, ethylsulfonyl nitrobenzene, MTT assay, radiosensitizers

Received 2 April 2012, revised 23 May 2012 and accepted for publication 19 June 2012

The growth and survival of cells in solid tumors are dependent on adequate supply of oxygen and nutrients that diffuse from blood vessels and are consumed by tumor cells (1). Oxygen consumption in tumor tissue is increased by a high level of tumor cell proliferation. It has been suggested that abnormalities of structure and function in tumor vessels can lead to decreased oxygen delivery to tumor tissue (2), which can become hypoxic and resistant to radiation therapy and chemotherapy (3,4). An increase in radiation dosage can combat these radioresistant hypoxic tumor cells. However, this increase can cause damage to normal surrounding stroma and presents increased risk for radiation-induced secondary cancers (5). Radiation doses >60 Gy appear to exponentially increase the risk for secondary bone sarcomas (6). Tumor hypoxia can be exploited for selective anticancer drug treatment using hypoxic cell cytotoxins or hypoxic cell radiosensitizers (7).

Radiosensitizers are intended to enhance tumor cell killing while having much less effects on normal tissues (8). Hypoxic cell radiosensitizers can have oxygen-mimicking effects on hypoxic tumor cells. This leads to repairs in the radiation-induced damaged DNA or other biomolecules (9). *In vitro* and *in vivo* studies have demonstrated that many chemotherapeutic agents, given at subcytotoxic doses, are capable of radiosensitizing activity in tumoral but not in normal cells. When used in chest radiotherapy, synergistic response is observed with these drugs. It is assumed that radiosensitization increases the lethal damage to the tumor caused by radiotherapy and decreases the ability of cancer cells to repair the damage, which suggests that the maximum synergistic effects can be obtained when the treatments are given in tandem (10).

Radiosensitizer capabilities of compound **5c** (Figure 1) have been reported (11). It exhibited the highest average radiosensitivity factor (RSF) of 4.62 among 870 compounds and was selected for further investigation (11). Compound **5c** radiosensitizes U251 glioma tumor cells, HT-29 colorectal cancer cells and A549 lung tumor cells and does not radiosensitize normal cells.

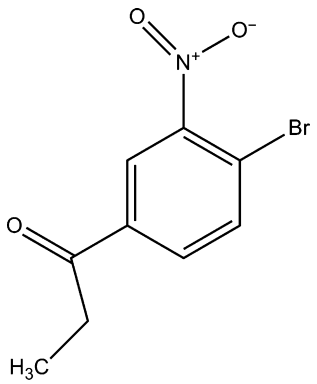
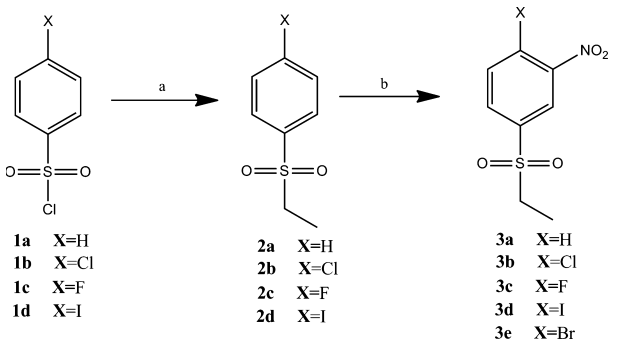
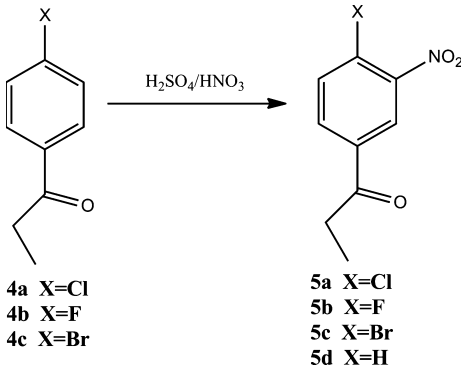


Figure 1: Structure of compound **5c**.



a) Rongalite, tellurium, NaOH, TEBAC, EtI, THF b) H_2SO_4/KNO_3

Scheme 1: General synthetic scheme to compounds **3**(**a**–**e**).



Scheme 2: General synthetic scheme to compounds **5**(**a**–**d**).

In view of the above and the need for new compounds with better anticancer properties, we designed and synthesized a new series of sulfonyl and carbonyl analogs of compound **5c** possessing *m*-nitro and p-halogen substituted benzene moiety (Schemes 1 and 2) to examine their *in vitro* anticancer properties. Abilities of these compounds to act as radiosensitizers and *in vivo* studies are being pursued.

Materials and Methods

Chemistry

Melting points were determined with a Mel-Temp electrothermal apparatus and are uncorrected. ^{1}H NMR spectra were recorded with a 400 MHz Bruker NMR spectrophotometer with TMS as internal standard and $CDCl_3$ as solvent. Mass spectra were obtained with a Varian 1200 Triple Quadruple instrument using electrospray ionization (ESI) technique. Column chromatography was conducted using Merck silica gel, grade 9385, 230–400 mesh, 60 Å. HPLC was conducted using C18 column, elution solution of water/acetonitrile/formic acid and Hitachi Elite LaChrom instrument with UV detection.

Syntheses

Syntheses of the sulfonyl derivatives (**3a**–**d**) were carried out starting from commercially available aryl sulfonyl chlorides (**1a**–**d**, Scheme 1). Alkylation of the sulfonyl chlorides with iodoethane in the presence of tellurium, rongalite, and 1 M aqueous sodium hydroxide (12,13) gave ethylsulfonyl derivatives (**2a**–**d**). This was followed by reaction with conc. H_2SO_4 and potassium nitrate to give target novel nitro compounds (14). Syntheses of carbonyl derivatives (**5a**–**d**) were carried out as shown in Scheme 2. A mixture of HNO_3 and H_2SO_4 was added to commercially available aryl ketones (**4a**–**c**) at −15 °C to give (4-halogen-3-nitrophenyl) propan-1-one derivatives (15,16). Yields were not optimized. Compounds **3e** and **5d** are commercially available and were purchased from Sigma–Aldrich company.

1-(Ethylsulfonyl)-benzene (2a)

A reddish solution of sodium telluride, prepared by heating a mixture of powdered tellurium (10 mmol, 1.28 g), rongalite (50 mmol, 7.71 g), and 1 M aqueous sodium hydroxide (25 mL), was added dropwise to a stirred solution of benzenesulfonyl chloride (1a, 10 mmol, 1.94 g) and triethylbenzylammonium chloride (TEBAC) (0.1 mmol, 0.23 g) in THF (30 mL) at room temperature under nitrogen. An instantaneous reaction occurred and the color of the reaction mixture changed to deep black. After 5 min while stirring, iodoethane (50 mmol, 4 mL) in THF (3 mL) was added and the resulting mixture was kept at 90 °C for 5 h. After cooling, the solvent was removed under reduced pressure and the residue was treated with aqueous ammonium chloride and benzene. Organic phase was separated, dried over sodium sulfate, and the solvent evaporated. The residue was purified by column chromatography (hexane/ethylacetate 2:1) to give a white solid, m.p. 43–44 °C, (0.6 g, 35% yield). ^{1}H NMR ($CDCl_3$): 1.3 (t, 3H, CH_3), 3.13 (q, 2H, CH_2), 7.59 (m, 2H, Ar-H), 7.68 (m, 1H, Ar-H), 7.93 (m, 2H, Ar-H); MS (ESI, +ion): 171 M^+.

1-(Ethylsulfonyl)-4-chlorobenzene (2b)

Compound **2b** was prepared in analogous manner to compound **2a**, starting from p-chlorobenzene-1-sulfonyl chloride (**1b**, 10 mmol, 3.02 g) to give white oily compound (0.36 g, 18% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.86 (d, J = 8.53 Hz, 2H), 7.56 (d, J = 8.56 Hz, 2H), 3.13 (q, J = 7.43 Hz, 2H), 1.29 (t, J = 7.44 Hz, 3H); MS (ESI, +ion): 205 M^+ (100.0%), 207 (36.5%).

1-(Ethylsulfonyl)-4-fluorobenzene (2c)

Compound **2c** was prepared in analogous manner to compound **2a**, starting from p-fluorobenzene-1-sulfonyl chloride (**1c**, 10 mmol, 3.02 g). The residue was purified by column chromatography (hexane/ethylacetate 1:1) to give a white solid. HPLC indicated 94% purity, m.p. 39–40 °C, (0.30 g, 16% yield). ^{1}H NMR (CDCl3): 1.6 (t, 3H, CH_3), 3.23 (q, 2H, CH_2), 7.27 (m, 2H, Ar-H), 7.94 (m, 2H, Ar-H); MS (ESI, +ion): 189 M^+. Anal. calcd. for $C_8H_9FO_2S$: C, 51.05; H, 4.82; F, 10.09; S, 17.04 and found C, 51.07; H, 4.67; F, 10.36; S,16.85.

1-(Ethylsulfonyl)-4-iodobenzene (2d)

Compound **2d** was prepared in analogous manner to compound **2a,** starting from pipsyl chloride (**1d,** 10 mmol, 3.02 g). A white solid was obtained. HPLC indicated 100% purity, m.p. 78–79 °C, (0.60 g, 20% yield). ^{1}H NMR ($CDCl_3$): 1.28 (t, 3H, CH_3), 3.12 (q, 2H, CH_2), 7.63 (d, 2H, Ar-H), 7.96 (d, 2H, Ar-H); MS (ESI, +ion): 296 M^+.

Anal. calcd. for $C_8H_9IO_2S$. –0.2 C_6H_{14} –0.1 H_2O: C, 35.06; H, 3.83; I, 40.26; S, 10.17 and found C, 35.04; H, 3.41; I, 40.32; S, 9.74.

1-(Ethylsulfonyl)-3-nitrobenzene (3a)

To a solution of 1-(ethylsulfonyl)-benzene (**2a,** 1.35 mmol, 0.230 g) in sulfuric acid (1.4 mL) was added potassium nitrate (0.264 g) at 80 °C. The mixture was stirred at 90 °C for 2 h. Ice water (5 mL) was added, and the mixture was extracted with ethyl acetate (25 mL) and washed with water (20 mL) and brine (10 mL). The organic extracts were combined and dried over sodium sulfate and concentrated. The residue was purified by column chromatography (hexane/ethylacetate 2:1) to give a light yellow solid, m.p. 98–100 °C, (0.22 g, 75% yield). ^{1}H NMR (400 MHz, DMSO) δ ppm 8.62–8.55 (m, 2H), 8.37–8.31 (m, 1H), 8.03–7.95 (m, 1H), 3.47 (q, J = 7.33 Hz, 2H), 1.14 (t, J = 7.33 Hz, 3H); MS (ESI, -ion): 214 M^-. Anal. calcd. for $C_8H_9NO_4S$ –0.01 C_6H_{14}: C, 44.80; H, 4.26; N, 6.48; S, 14.83 and found C, 45.14; H, 4.25; N, 6.41; S, 14.78.

4-(Ethylsulfonyl)-1-chloro-2-nitrobenzene (3b)

Compound **3b** was prepared in analogous manner to compound **3a,** starting from 1-(ethylsulfonyl)-4-chlorobenzene (**2b**, 2.0 mmol, 0.42 g) to give a light yellow solid, m.p. 96–97 °C (0.35 g, 63% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.42 (d, J = 2.03 Hz, 1H), 8.06 (dd, J = 8.39 Hz, 1H), 7.83 (t, J = 6.45 Hz, 1H), 3.21 (q, J = 7.44 Hz, 2H), 1.36 (t, J = 7.44 Hz, 3H); MS (ESI, +ion): 249.99 M^+ (100.00%), 251.00 (36.5). Anal. calcd. for $C_8H_8ClNO_4S$: C, 38.48; H, 3.23; N, 5.61; Cl, 14.20; S, 12.84 and found C, 38.79; H, 3.23; N, 5.25; Cl, 14.41; S, 12.64.

4-(Ethylsulfonyl)-1-fluoro-2-nitrobenzene (3c)

Compound **3c** was prepared in analogous manner to compound **3a,** starting from 1-(ethylsulfonyl)-4-fluorobenzene (**2c,** 1.36 mmol, 0.257 g) to give a light yellow solid. HPLC indicated 98% purity, m.p. 128–131 °C, (0.21 g, 66% yield). ^{1}H NMR ($CDCl_3$): δ ppm 1.35 (t, 3H, CH_3), 3.21 (q, 2H, CH_2), 7.55 (t, 1H, Ar-H), 8.21 (m, 1H, Ar-H), 8.64 (d, 1H, Ar-H); MS (ESI, -ion): 229 M^-. Anal. calcd. for $C_8H_8FNO_4S$: C, 41.20; H, 3.46; N, 6.01; F, 8.15; S, 13.75 and found C, 41.31; H, 3.35; N, 5.85; F, 8.17; S, 13.53.

4-(Ethylsulfonyl)-1-iodo-2-nitrobenzene (3d)

Compound **3d** was prepared in analogous manner to compound **3a**, starting from 1-(ethylsulfonyl)-4-iodobenzene (**2d,** 0.34 mmol, 0.10 g) to give a light yellow solid. HPLC indicated 98% purity, m.p. 124–126 °C, (0.052 g, 45% yield). ^{1}H NMR ($CDCl_3$): δ ppm 1.35 (t, 3H, CH_3), 3.22 (q, 2H, CH_2), 7.77 (dd, 1H, Ar-H), 8.31 (d, 1H, Ar-H), 8.34 (d, 1H, Ar-H); MS (ESI, -ion): 338.9 M^-. Anal. calcd. for $C_8H_8INO_4S$: C, 28.17; H, 2.36; N, 4.11; I, 37.20; S, 9.40 and found C, 28.25; H, 2.25; N, 3.99; I, 37.39; S, 9.18.

1-(4-chloro-3-nitrophenyl)propan-1-one (5a)

A flask charged with 7 mL concentrated sulfuric acid was cooled to –20 °C and to this was added 1-(4-chlorophenyl)propan-1-one (**4a,** 2.16 g, 12.8 mmol) dropwise with stirring. Then, a mixture of concentrated nitric acid (1 mL) and concentrated sulfuric acid (3 mL) was added dropwise with stirring at –15 °C. After the addition was complete, the reaction mixture was stirred at –15 °C for an additional 15 min and then poured into ice. The resulting mixture was extracted with ether and washed with 5% $NaHCO_3$ and water, and the solvent was removed on a rotary evaporator. The residue was purified by column chromatography (CH_2Cl_2/hexane 30:70) to give a light yellow solid, m.p. 50–52 °C, (0.265 g, 10% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.45 (d, J = 2.01 Hz, 1H), 8.11 (dd, J = 8.39 Hz, 1H), 7.69 (d, J = 8.39 Hz, 1H), 3.04 (q, J = 7.17 Hz, 2H), 1.32–1.24 (m, 3H); MS (ESI, +ion): 214 M^+. Anal. calcd. for $C_9H_8ClNO_3$: C, 50.60; H, 3.77; Cl, 16.60; N, 6.56 and found C, 50.66; H, 3.64; Cl, 16.57; N, 6.37.

1-(4-fluoro-3-nitrophenyl)propan-1-one (5b)

Compound **5b** was prepared in analogous manner to compound **5a,** starting from 1-(4-fluorophenyl)propan-1-one (**4b,** 0.9 mL, 6.4 mmol) and concentrated nitric acid (0.5 mL) and concentrated sulfuric acid (1.5 mL). The residue was purified by column chromatography (ethylacetate/hexane 10:90) to give a light yellow solid. m.p. 25 °C, (0.190 g, 15% yield). ^{1}H NMR (400 MHz, CDCl3): δ ppm 8.67 (dd, J = 7.13 Hz, 1H), 8.28 (ddd, J = 8.69, 2.26 Hz, 1H), 7.47–7.38 (m, 1H), 3.03 (qd, J = 14.69, 7.23 Hz, 2H), 1.32–1.22 (m, 3H); MS (ESI, +ion): 198 M^+. Anal. calcd. for $C_9H_8FNO_3$ –0.1 H_2O: C, 54.32; H, 4.15; N, 7.04 and found C, 54.23; H, 4.02; N, 7.26.

1-(4-bromo-3-nitrophenyl)propan-1-one (5c)

Compound **5c** was prepared in analogous manner to compound **5a,** starting from 1-(4-bromophenyl)propan-1-one (**4c,** 1.36 g, 6.4 mmol) and conc. nitric acid (0.5 mL) and conc. sulfuric acid (1.5 mL). The residue was purified by column chromatography (CH_2Cl_2/hexane 30:70) to give a light yellow solid, m.p. 62–64 °C, (0.330 g, 20% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.40 (d, J = 2.00 Hz, 1H), 8.02 (td, J = 5.58, 3.68 Hz, 1H), 7.89 (dd, J = 8.79 Hz, 1H), 3.10–2.98 (m, 2H), 1.33–1.23 (m, 3H); MS (ESI, +ion): 259 M^+. Anal. calcd. for $C_9H_8BrNO_3$: C, 41.89; H, 3.12; Br, 30.96; N, 5.43 and found C, 42.12; H, 3.06; Br, 31.09; N, 5.48.

Biology

We tested the effects of novel synthesized compounds (**3a**–**e, 5a**–**d**) on cell proliferation *in vitro* using two different cell lines by MTT, a cell viability assay.

Cell culture

Human prostate cancer (DU-145, ATCC) and human breast cancer (MCF-7, ATCC) cell lines were used in the study. The cells were routinely propagated using Eagle's minimum essential medium (EMEM, ATCC), supplemented with 10% fetal bovine serum (ATCC) and 1% penicillin/streptomycin (Gibco) in 100 cm^2 Petri dishes (Corning) at 37 °C in 5% CO_2.

Treatment

The nine compounds (**3a**–**e, 5a**–**d**) were obtained as solids. Stock solutions (100 mM) were prepared in dimethylsulphoxide (DMSO).

The treatment concentrations were 100 and 10 μM with 0.1% DMSO in media and 100 and 10 μM with 0.5% DMSO for compounds **3d**, **5c**, and **5d** because of compound solubility issues. Cells were allowed to attach overnight prior to treatment. H_2O_2 and 100% DMSO served as positive controls, 0.1% DMSO and 0.5% DMSO served as vehicle controls. Working concentrations were prepared immediately prior to treatment by dilution into medium.

The MTT assay

The MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazoliumbromide) assay is a well documented and widely used cell viability assay. MTT is incorporated into the cell by endocytosis and is reduced by mitochondrial enzymes in living cells to a blue-colored formazan precipitate. The absorption of dissolved formazan in the visible region correlates with the number of intact alive cells (17). Cells were plated at a concentration of 13 000–30 000 cells/well on a 96-well round-bottom plate. MTT assay (ATCC) was performed according to manufacturer's instructions. The following determinants were optimized for each cell line: plating cell concentration, incubation time with MTT reagent, and incubation time with detergent reagent. Absorbance was recorded at 570 nm by a microtiter plate reader (VICTOR; PerkinElmer). The number of surviving cells is directly proportional to the level of the formazan product created. This experiment was repeated on three separate occasions, and results are presented as the mean absorbance ± SD.

Radiosensitizing activity

All compounds were evaluated for their *in vitro* anticancer activity in combination with γ-radiation. This study was conducted to evaluate the ability of these compounds to enhance the cell killing effect of γ-radiation. Treatment was performed with paired 96-well plates: one plate was exposed to 4 Gy, and the other plate was a non-irradiated control. One hour after radiation, the media was replaced with fresh media alone (18). The MTT assay was used to evaluate cell survival at different time-points: 0, 1, 3, and 5 days after radiation. Irradiation was performed at the Department of Oncology, Thomas Jefferson University, using Pantak XRAD 320 cabinet X-ray machine.

Clonogenic assay

The clonogenic survival assay was performed as previously described (18). Optimal cell densities were determined for each cell line. Control flasks for the DU-145 cell line contained 1000 cells; control flasks for the MCF-7 cell line contained 5000 cells. Treatment flasks had cell densities 10 times as many cells. Cells were then incubated and allowed to attach overnight. The following day, cells were treated with 10 μM concentration of compounds **3c** or **3d** for 24 h. One of two treated flasks was exposed to ionizing radiation (IR) at a dose of 4 Gy. One hour later, media was changed in all flasks. Flasks were then incubated for 14 days at 37 °C in an atmosphere of 5% CO_2. On day 14, the cells were stained with 0.25% crystal violet staining solution. Media was removed, and cells were incubated with 3 mL of staining solution for 10 min. Flasks were then rinsed and left to air dry. Colonies were counted, and survival was assessed using a cell efficiency rate (no. of colonies/cell density) (19).

Results and Discussion

Compounds belonging to two classes, 4-(ethylsulfonyl)-1-halogen-2-nitrobenzene (**3a**–**e**) and 1-(4-halogen-3-nitrophenyl)propan-1-one (**5a**–**d**), were synthesized or obtained from a commercial source. Yields were not optimized but several milligram quantities of each compound were obtained. Structure and purity were confirmed using several techniques, including melting point, NMR, MS, and elemental analysis. Each target compound was tested on human prostate (DU-145) and breast (MCF-7) cancer cell lines and compared with a reference compound, doxorubicin, a clinically used anticancer compound for breast and prostate cancers (20,21), using MTT assay. In this assay, the number of surviving cells is directly proportional to the level of formazan product formed because of reduction by mitochondrial dehydrogenases in living cells. To begin with, the inhibition effects of synthesized compounds on carcinoma cells were tested at 100 μM concentration for 24 h (Figure 2). The sulfonyl derivatives (**3b**–**e**) showed cytotoxic effects at 100 μM by decreasing cell viability to 8–13%. In contrast, compound **3a** that has no halogen substituent exhibited cell proliferation activity. The presence of a halogen substituent on the aromatic ring is implied to be critical for inhibition of cell viability by this class of compounds.

For the carbonyl compounds (**5a**–**d**), again, the one without halogen (**5d**) exerted cell proliferation activities like compound **3a,** exhibiting 113% and 108% on DU-145 and MCF-7, respectively, as compared to control. For the halogen containing ones, the trend is

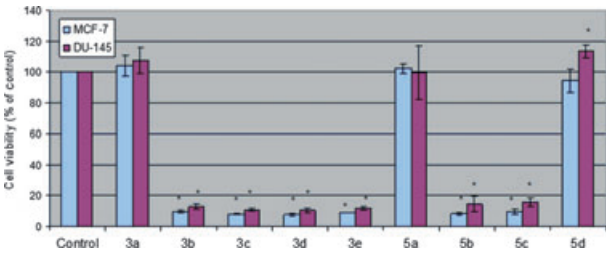


Figure 2: The inhibition effects of synthesized compounds on carcinoma cells (as % of untreated control) at 100 μM concentration (24 h, $n = 3$, *$p < 0.05$).

Table 1: Relative cell viability (as % of untreated control) of MCF-7 and DU-145 cells, exposed for 24 h at 100 μM concentration of novel synthesized compounds ($n = 3$)

Compound	Conc. (μM)	MCF-7	DU-145
Control		100	100
3a	100	103.94 ± 6.88	107.5 ± 8.49
3b	100	9.49 ± 0.82	12.63 ± 1.61
3c	100	7.78 ± 0.27	10.56 ± 0.91
3d	100	7.39 ± 0.89	10.2 ± 1.62
3e	100	8.55 ± 0.12	11.41 ± 1.07
5a	100	102.12 ± 3.19	99.15 ± 17.33
5b	100	7.82 ± 0.98	14.4 ± 5.29
5c	100	9.18 ± 1.82	15.6 ± 2.70
5d	100	94.15 ± 7.57	113.34 ± 3.89

not clear although they all exhibited inhibition of cell proliferation. Relative viability data of DU-145 and MCF-7 cells exposed for 24 h at 100 μM concentrations of target compounds are summarized in Table 1.

Compounds (**3a–e, 5a–d**) were further tested at concentration of 10 μM, exposed for 24, 48, 96, and 144 h with or without radiation against DU-145 and MCF-7 cell lines. To confirm the inhibitory effects of the novel compounds from this study, we compared their activities with a widely used antineoplastic agent, doxorubicin (Figures 3–6). All compounds did not show significant cytotoxic effects at 10 μM concentration on both cell lines with and without radiation for 24 h and 1 day after radiation (Tables 2 and 3). Tables 4 and 5 represent relative cell viability (as % of untreated control) of MCF-7 and DU-145

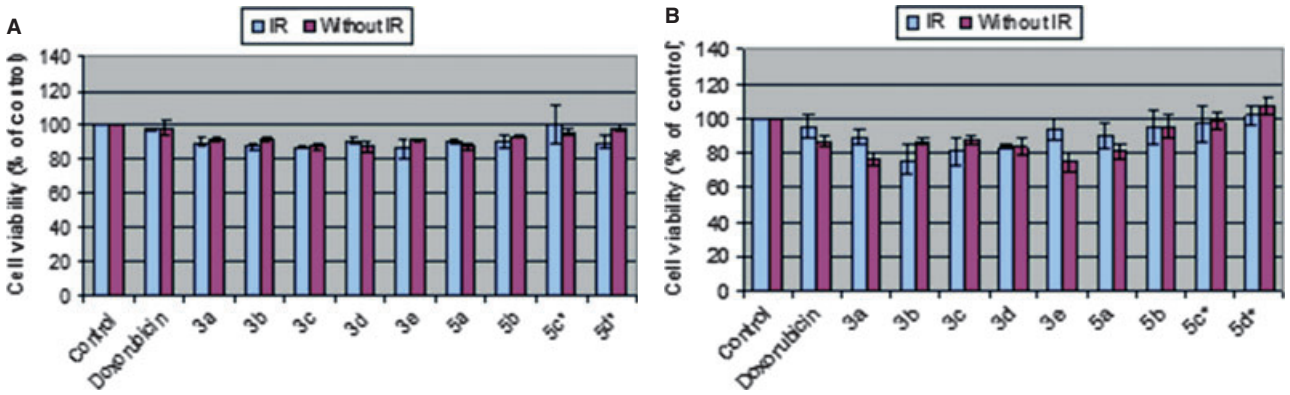


Figure 3: The inhibition effects of synthesized compounds on (A) MCF-7 and (B) DU-145 carcinoma cells at 10 μM concentration (24-h treatment; n = 3, 0 day after radiation).

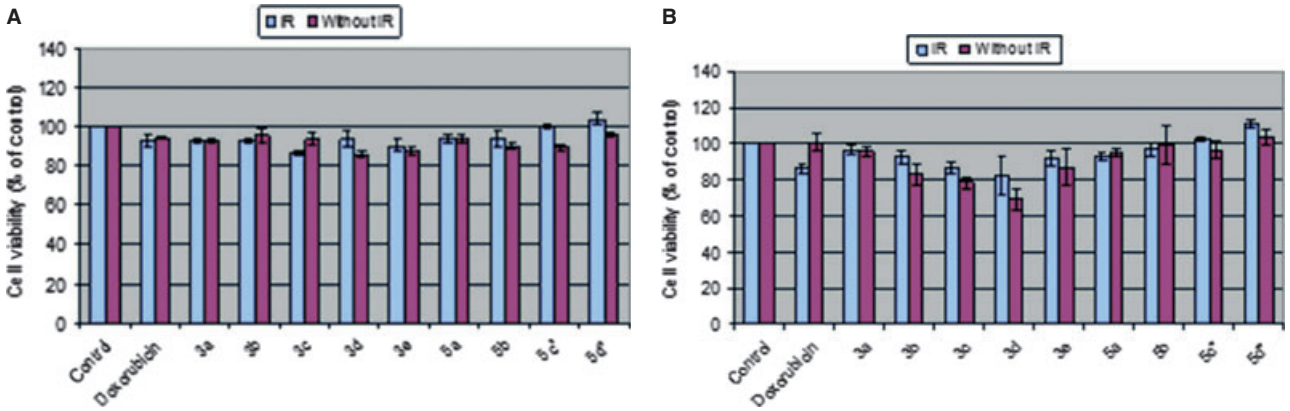


Figure 4: The inhibition effects of synthesized compounds on (A) MCF-7 and (B) DU-145 carcinoma cells at 10 μM concentration (48-h treatment; n = 3, 1 day after radiation).

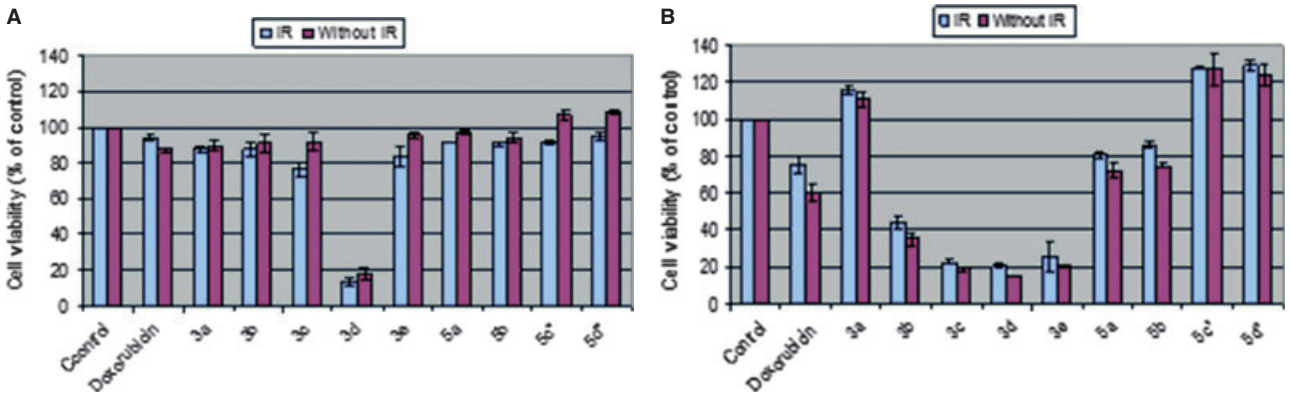


Figure 5: The inhibition effects of synthesized compounds on (A) MCF-7 and (B) DU-145 carcinoma cells at 10 μM concentration (96-h treatment; n = 3, 3 day after radiation).

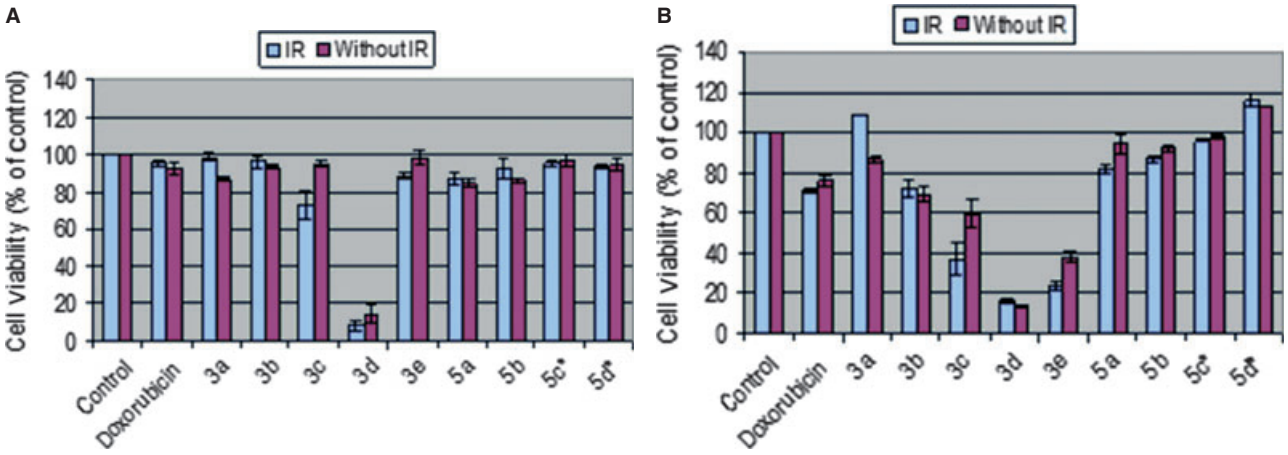


Figure 6: The inhibition effects of synthesized compounds on (A) MCF-7 and (B) DU-145 carcinoma cells at 10 μM concentration (144-h treatment; n = 3, 5 day after radiation).

Compound	Without radiation (MCF-7)	With radiation (MCF-7)	Without radiation (DU-145)	With radiation (DU-145)
Control	100	100	100	100
Doxorubicin	98.048 ± 3.94	97.42 ± 1.06	86.91 ± 3.03	95.20 ± 7.09
3a	91.65 ± 1.15	89.82 ± 2.65	76.38 ± 3.23	89.81 ± 4.18
3b	91.25 ± 0.84	87.22 ± 2.32	86.97 ± 1.67	76.26 ± 8.07
3c	87.46 ± 1.49	86.99 ± 0.65	87.84 ± 2.98	81.44 ± 8.15
3d	87.12 ± 3.09	90.39 ± 1.78	84.06 ± 5.32	84.03 ± 1.14
3e	91.03 ± 0.39	86.27 ± 5.38	75.13 ± 5.89	93.84 ± 6.20
5a	87.15 ± 1.89	90.39 ± 1.25	81.18 ± 4.47	90.32 ± 7.37
5b	92.72 ± 0.60	90.08 ± 3.64	95.52 ± 6.87	94.86 ± 9.95
5c	95.60 ± 1.43	100.02 ± 10.4	98.34 ± 5.23	97.13 ± 10.97
5d	97.87 ± 1.88	89.65 ± 3.32	107.44 ± 5.27	101.69 ± 5.00

Table 2: Relative cell viability (as % of untreated control) of MCF-7 and DU-145 cells, exposed for 24 h at 10 μM concentration of novel synthesized compounds with or without radiation (n = 3)

Compound	Without radiation (MCF-7)	With radiation (MCF-7)	Without radiation (DU-145)	With radiation (DU-145)
Control	100	100	100	100
Doxorubicin	94.56 ± 0.13	92.59 ± 2.87	100.81 ± 4.66	86.58 ± 2.40
3a	92.60 ± 1.08	93.02 ± 1.34	95.88 ± 2.13	96.85 ± 2.86
3b	95.70 ± 3.22	92.60 ± 1.40	82.95 ± 5.49	92.62 ± 4.21
3c	93.68 ± 3.10	86.98 ± 0.94	78.5243.48	86.52 ± 3.17
3d	86.30 ± 1.59	93.90 ± 4.76	69.38 ± 5.83	82.65 ± 10.32
3e	87.35 ± 1.83	90.36 ± 2.98	86.74210.33	91.90 ± 4.24
5a	94.24 ± 1.82	94.32 ± 1.82	95.0731.89	92.88 ± 2.00
5b	90.47 ± 1.82	93.63 ± 4.12	99.36 ± 11.21	96.95 ± 3.07
5c	89.65 ± 1.50	99.98 ± 1.07	96.7644.65	102.91 ± 1.07
5d	95.87 ± 1.08	103.99 ± 3.17	103.98 ± 4.47	111.24 ± 2.43

Table 3: Relative cell viability (% of untreated control) of MCF-7 and DU-145 cells, exposed for 48 h at 10 μM concentration of novel synthesized compounds with (1 day after radiation) or without radiation (n = 3)

cells exposed for 96 and 144 h at 10 μM concentration of novel synthesized compounds with and without radiation (n = 3).

Our most active compound **3d** is more active than doxorubicin at the dose level of 10 μM for 3 days after radiation, cell viabilities of 18%, 13% compared to 87%, 94% against MCF-7 cells, and 15%, 20% compared to 60%, 75% against DU-145 cells without and with radiation (Table 4). Compounds **3b**–**3e** showed more considerable cytotoxic effects against DU-145 cells compared against MCF-7 cells by decreasing cell viability to 15–44% (Table 4). The 5th day after radiation, compound **3d** had outstanding activity with radiation against MCF-7 cell line with 9% cell viability and compounds **3c**–**3e** were more active than doxorubicin, 37%, 16% and 23% cell viabilities resulted compared to 71%, against DU-145 with radiation.

Preliminary clonogenic survival assays were performed for compounds **3c** and **3d** using MCF-7 and DU-145 cell lines. Effects of 10 μM of compounds **3c** and **3d** on MCF-7 and DU-145 colony formation are depicted in Tables 6 and 7, respectively. The activity trends for both compounds in both cell lines seem to be similar.

Table 4: Relative cell viability (as % of untreated control) of MCF-7 and DU-145 cells, exposed for 96 h at 10 μM concentration of novel synthesized compounds with (3 day after radiation) or without radiation (n = 3)

Compound	Without radiation (MCF-7)	With radiation (MCF-7)	Without radiation (DU-145)	With radiation (DU-145)
Control	100	100	100	100
Doxorubicin	87.62 ± 1.26	94.27 ± 1.37	60.20 ± 4.76	75.36 ± 4.45
3a	89.69 ± 2.60	87.76 ± 1.68	110.65 ± 3.94	115.70 ± 1.82
3b	91.03 ± 5.54	87.61 ± 4.31	35.13 ± 3.69	43.84 ± 4.16
3c	91.83 ± 5.17	76.36 ± 4.33	18.88 ± 1.28	22.45 ± 1.12
3d	18.29 ± 2.96	13.42 ± 2.07	15.02 ± 0.08	20.54 ± 0.63
3e	95.76 ± 1.57	83.25 ± 6.03	20.62 ± 0.45	25.29 ± 8.30
5a	97.02 ± 1.04	91.69 ± 0.29	72.46 ± 4.13	80.43 ± 1.71
5b	93.96 ± 3.27	90.88 ± 1.24	74.82 ± 1.35	86.38 ± 1.71
5c	106.83 ± 2.35	91.36 ± 1.21	126.71 ± 8.38	127.57 ± 0.82
5d	108.48 ± 1.41	94.93 ± 2.53	123.80 ± 6.05	129.54 ± 3.09

Table 5: Relative cell viability (as % of untreated control) of MCF-7 and DU-145 cells, exposed for 144 h at 10 μM concentration of novel synthesized compounds with (5 days after radiation) or without radiation (n = 3)

Compound	Without radiation (MCF-7)	With radiation (MCF-7)	Without radiation (DU-145)	With radiation (DU-145)
Control	100	100	100	100
Doxorubicin	92.57 ± 3.19	95.47 ± 2.03	75.45 ± 2.85	70.58 ± 0.91
3a	86.72 ± 1.56	98.48317 ± 2.21	86.55 ± 2.13	108.79 ± 0.54
3b	93.28 ± 1.10	96.19 ± 3.36	69.011 ± 4.21	72.28 ± 3.79
3c	94.79 ± 1.58	72.89 ± 7.32	59.36 ± 7.04	36.96 ± 8.16
3d	14.57 ± 4.93	8.62 ± 2.39	12.92 ± 0.49	16.26 ± 0.87
3e	98.22 ± 3.73	88.62 ± 1.40	38.11 ± 2.64	23.03 ± 2.13
5a	84.98 ± 2.56	87.11798 ± 3.37	93.84 ± 4.08	81.44 ± 2.02
5b	86.07 ± 1.01	92.47 ± 5.69	91.89 ± 1.38	86.14 ± 1.65
5c	96.80 ± 3.78	95.34 ± 2.3	97.01 ± 1.23	95.73 ± 0.06
5d	94.35 ± 3.40	93.63 ± 0.79	112.73 ± 0.42	115.94 ± 2.85

Table 6: Effects of 10 μM treatment of compounds **3c** and **3d** on MCF-7 colony formation. Mean ± SD of at least two experiments. The plating efficiency and survival fractions were calculated

Experiment	Mean number of colonies	Plating efficiency (%)	Survival fraction
Control	149 ± 9.89	2.98	N/A
3c on MCF-7 cells: Non-IR	131 ± 26.16	0.262	N/A
3c on MCF-7 cells: IR	15 ± 0.70	0.03	0.01
3d on MCF-7 cells: Non- IR	0	0	N/A
3d on MCF-7 cells: IR	0	0	0

Table 7: Effects of 10 μM treatment of compounds **3c** and **3d** on DU-145 colony formation. Mean ± SD of at least two experiments. The plating efficiency and survival fractions were calculated

Experiment	Mean number of colonies	Plating efficiency (%)	Survival fraction
Control	161 ± 12.50	16.1	N/A
3c on DU-145 cells: Non-IR	178 ± 8.48	1.78	N/A
3c on DU-145 cells: IR	114 ± 27.57	1.14	0.01
3d on DU-145 cells: Non- IR	0	0	N/A
3d on DU-145 cells: IR	0	0	0

Compared with control, compound **3c** had more survival decrease with radiation than without radiation (Figure 7, Tables 6 and 7). Colony formation of tumor cells disappeared totally using our most active compound **3d,** with and without radiation (Figure 8, Tables 6 and 7). Based on the results of these studies, we believe that compound **3d** is deserving of further studies for prostate and breast cancers. The mechanism(s) through which this molecule elicits its effects, dose–response studies as well its effects on normal cell lines is subject of further investigations. *In vivo* studies of the compound with and without radiation are also desired future studies.

Conclusions and Future Directions

Taking together, data presented suggest that introduction of halogens to both sulfonyl and carbonyl classes has great potential in obtaining effective antiproliferative compounds. Initial radiosensitizing studies of compound **5c** have been reported (11). The goal of this study was to synthesize a series of compounds that have been designed as radiosensitizers and test their effects against cancer cell lines. When used with radiotherapy, these compounds are expected to have synergistic responses and thereby increase their values. The combination of radiosensitization and radiotherapy to target hypoxic tumors can be a powerful anticancer therapeutic. Our data show that compound **3d** is a very potent antiproliferative compound, more so than the lead compound **5c,** especially at the lower concentration of 10 μM (Tables 4 and 5). At that concentration, compound **5c** had no effects as compared to control, whereas compound **3d** reduced DU-145 cell viability to 16% and that of MCF-7 cells to 9% even 5 days after radiation. The clonogenic assays confirm potent activities of compound **3d**, reducing colony formation for both cell lines to non-detectable levels.

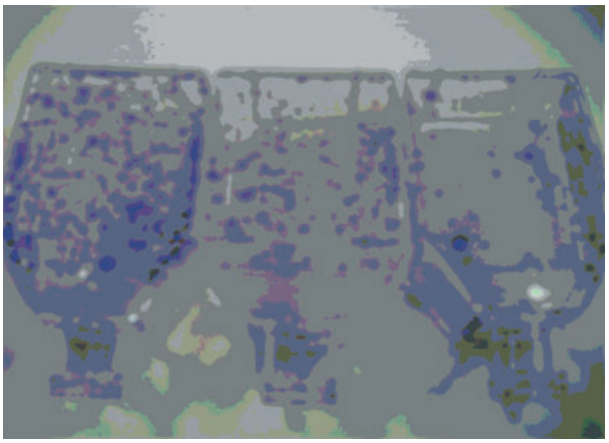


Figure 7: The inhibitory effects of (A) control; (B) compound **3c** without radiation; and (C) compound **3c** with radiation on colony formation in MCF-7.

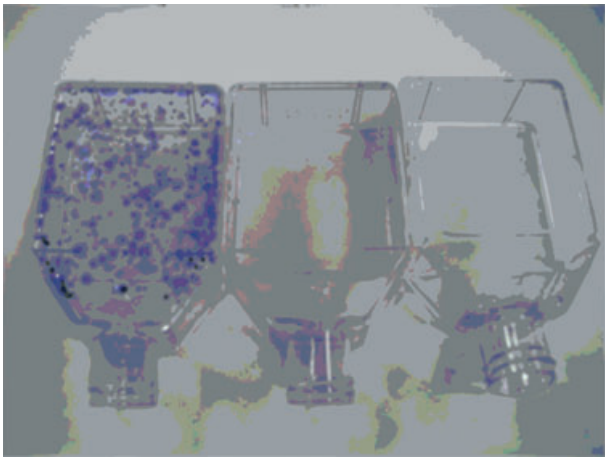


Figure 8: The inhibitory effects of (A) control; (B) compound **3d** without radiation; and (C) compound **3d** with radiation on colony formation in MCF-7.

Recurrence of breast cancer in the chest wall after mastectomy and conventional radiotherapy poses a major problem, as limited therapeutic options remain. Anthracyclines are radiosensitizers (22); therefore, we compared our compounds with anthracycline doxorubicin in breast and prostate cancer cell lines. Our compounds have an aromatic nitro group, sulfonyl and different halogens. Nitroaromatic compounds bind selectively to hypoxic cells via complex reductive chemistry involving an intermediate, oxygen-sensitive radical (23). The nitro group of a variety of nitroaromatics has been shown to undergo up to six-electron (6e) reduction in cells by flavin containing enzymes (24). One of the early intermediates in this reduction process is the nitroanion radical. This species and subsequent reactive reduction products are responsible for cytotoxicity. The hypoxia selectivity arises from the fact that the first reduction product, a nitroanion radical, can react with molecular oxygen in the normoxic tissues leading to regeneration of the parent. Only in hypoxic tissue will the reduction proceed further to generate highly cytotoxic species (25). The electrochemical oxidation of α-sulfonyl carbanions is shown to involve a coupling reaction between the electrogenerated radical and the parent anionic species, both on preparative and kinetic grounds (26). The halogen moieties act as electron 'sinks' on irradiation, the carbon-halogen bond breaking on electron attachment to liberate free halide and form a carbon-centered free radical. This species can add oxygen to form a peroxyl radical and carry out strand-breaking reactions as DNA base/hydroxyl radical adduct (23). We believe these may be possible mechanisms of action for our compounds based on their aromatic nitro, sulfonyl and halogen groups, which are capable of generating free radicals. The free radicals can then lead to cell death.

In conclusion, the *in vitro* evaluation of cytotoxicity of 4-(ethylsulfonyl)-1-halogen-2-nitrobenzene (**3a**–**e**) and 1-(4-halogen-3-nitrophenyl)propan-1-one (**5a**–**d**) derivatives revealed the high potential of compound **3d** as a cytotoxic compound. Further studies are needed to evaluate this compound as a new cytotoxic agent with therapeutic potentials.

Acknowledgment

We acknowledge US patent application no. 12/677,478 and PCT application no. PCT/US08/76208 (Constantinos Koumenis, Brian E Lally, Steven Kridel, Gary D Kao, and Adeboye Adejare, inventors) from which this work was derived. We would also like to thank Dr Phyllis Wachsberger and Barbara Andersen of Department of Radiation Oncology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA, USA, for helping us with use of the Pantak XRAD 320 cabinet X-ray machine.

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