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Synthesis, characterization, and radioprotective activity of α,β -unsaturated aryl sulfone analogs and their Tempol conjugates†‡

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Some novel α,β -unsaturated aromatic sulfone analogs (**5a–5m**) and their Tempol conjugates (**6a–6e**) have been synthetically prepared, characterized and evaluated for their radioprotective activity under γ -ray radiation. The Tempol conjugates were characterized by X-ray single crystal diffraction. *In vitro* studies showed that **5a**, **5b** and **6b** had superior activities to Ex-Rad pre-treated before 5 Gy irradiation, and **5a**, **6a** and **6b** had better activities than Ex-Rad after 5 Gy irradiation, while **5a**, **6a** and **6b** exhibited both prophylactic and mitigation effects, indicating the advantage of combining α,β -unsaturated aromatic sulfones with Tempol nitroxide. Both p53 and phospho-p53 levels were significantly lower in compound-treated cells than those in untreated irradiated cells. The conjugates offer an improved radioprotective ability, which is an advantage in the treatment protocol.

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People may be exposed to ionizing radiation during therapeutic and diagnostic interventions. Moreover, exposure may occur during radiological accidents or terror attacks.^{1,2} In these cases, radiation causes damage to normal cells and, hence, can result in injury to the human body. Depending upon the duration of exposure, the sensitivity of the organs, and the dose received, radiation exposure could lead to a myriad of deleterious effects. With respect to the potential application of ionizing radiation in radiotherapy and also to prevent potential accidental exposure, the development of effective radioprotective agents is of great medical importance. Radioprotectants are natural products or synthetic compounds that are administered before or after irradiation to reduce radiation damage to the body. Over the past 70 years, a number of natural products, like Abana³ and *Mentha piperita*,⁴ and synthetic compounds, like Ex-Rad,⁵ amifostine⁶ and Tempol-related products,⁷ have been shown to be highly effective at reducing lethality induced by irradiation. Pharmaceutical radioprotectants offer a cost-efficient, effective and easily available alternative to radioprotective gear. However, previous attempts at radioprotection of normal cells with pharmaceutical compositions have not been entirely suc-

cessful. Therefore, therapeutic agents mitigating radiation-induced damage could play an important role not only in emergency situations but also in minimizing the radiation toxicity during radiation therapies or diagnostic procedures.

However, to date there has been no approved prophylactic pharmacological agent available to prevent radiation-induced damage to critical tissues and to reduce subsequent lethality.

Ex-RAD (ON 01210.Na, Fig. 1), a benzyl styryl sulfone analog developed by Onconova Therapeutics (Newtown, PA, USA) as a radioprotector and mitigator, has been investigated for its prophylactic and mitigation effects against radiation injuries. Ex-RAD is a non-toxic, synthetic molecule showing radioprotective properties both *in vitro* and *in vivo*.⁸ α,β -Unsaturated aryl sulfones, in particular benzyl styryl sulfones, provide significant and selective systemic protection of normal cells against radiation-induced damage in animals.

But until now, no research work on the structure–activity relationship of benzyl styryl sulfone analogs has been reported.

Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-*N*-oxyl) (Fig. 1) is a cell membrane-permeable nitroxide. It is a redox cycling agent that can inactivate and scavenge many ROS.⁹ Also, nitroxides have been shown to provide *in vitro*¹⁰ and

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Fig. 1 Structures of Ex-Rad and Tempol.

Fig. 2 Structures of analogs.

in vivo radioprotection.¹¹ Tempol is among the most potent of the nitroxides in protecting cells and tissues from the damaging effects of radiation.¹² In earlier studies in our laboratory, several Tempol derivatives were synthesized and underwent radioprotective bioactivity assays.¹³

In the present work, we have extended our studies to the derivatives of Ex-Rad and styryl sulfone–Tempol conjugates (Fig. 2).

1. Synthesis

1.1. General procedure for the synthesis of (aralkylsulfonyl) acetic acids 3a–3l (Scheme 1)

Mercaptoacetic acid was added to a solution of sodium methoxide. Then, a halogenated alkane (40 mmol) was added slowly, and the mixture was heated to reflux. The reaction

Scheme 1 Synthesis of 5a–m and 6a–e. Reagents and conditions: (a) thioglycolic acid, sodium hydroxide, methanol, reflux, 85–95%; (b) 30% H₂O₂, acetic acid, reflux, 80–92%; (c) substituted aromatic aldehyde, benzylamine, acetic acid, reflux, 43–86%; (d) cinnamaldehyde, piperidine, reflux, 20%; (e) thionyl chloride, Tempol, DMAP, pyridine, 35 °C, 45–87%.

mixture was poured into crushed ice, and neutralized with HCl solution. The precipitated crystalline solid was filtered off, washed and dried. The crude product was purified by column chromatography.

1.2. General procedure for the synthesis of (aralkylsulfonyl) acetic acids 4a–4l

The (aralkylsulfonyl) acetic acid was added to a H₂O₂ solution and the resulting solution was heated to reflux. The reaction mixture was poured into crushed ice, and the precipitated crystalline solid was filtered off, washed and dried. Purification of the crude residue afforded the desired compound.

1.3. General procedure for the synthesis of styryl benzyl sulfones 5a–5l

An aromatic aldehyde (10 mmol) and benzylamine (0.2 ml) were added to a solution of the (aralkylsulfonyl) acetic acid (10 mmol) in acetic acid (20 ml) and the resulting solution was heated to reflux until the disappearance of the (aralkylsulfonyl) acetic acid (checked by TLC). The reaction vessel was cooled in an ice bath, and the precipitated crystalline solid was filtered off, washed with cold methanol, and dried under vacuum to obtain the crude product. Purification of the crude residue was performed by column chromatography (petroleum ether/ethyl acetate) to afford the desired compound.

Data for 4-(trifluoromethoxy)benzyl-4-carboxystyryl sulfone (5a). White solid (47%); ¹H NMR (400 MHz, DMSO-d₆) δ 13.22 (s, 1H, COOH), 7.36–8.05 (m, 10H, ArH, CH=CH), 4.75 (s, 2H, ArCH₂SO₂); HR-MS (ESI⁺) *m/z* 387.0436 [M + H]⁺, found 409.0340 [M + Na]⁺.

Data for 4-fluorobenzyl-4-carboxystyryl sulfone (5b). White solid (52%); ¹H NMR (400 MHz, DMSO-d₆) δ 13.18 (s, 1H, COOH), 7.19–7.99 (m, 10H, ArH, CH=CH), 4.60 (s, 2H, ArCH₂SO₂); ¹³C NMR (100 MHz, DMSO-d₆) δ 167.15, 163.90, 161.47, 142.62, 136.86, 133.67, 130.30, 129.28, 128.82, 125.46, 115.80, 59.29; HR-MS (ESI⁺) *m/z* 321.0519 [M + H]⁺, found 343.0424 [M + Na]⁺.

Data for 4-methylbenzyl-4-carboxystyryl sulfone (5c). White solid (56%); ¹H NMR (400 MHz, DMSO-d₆) δ 13.19 (s, 1H, COOH), 7.22–7.30 (m, 10H, ArH, CH=CH), 4.52 (s, 2H, ArCH₂SO₂), 2.28 (s, 3H, CH₃); ¹³C NMR (100 MHz, DMSO-d₆) δ 167.15, 142.27, 138.22, 136.93, 133.06, 131.47, 130.30, 129.46, 129.24, 129.10, 125.98, 60.00, 21.22; HR-MS (ESI[−]) *m/z* 315.0769 [M − H][−], found 315.0734 [M − H][−].

Data for 4-*tert*-butylbenzyl-4-carboxystyryl sulfone (5d). White solid (45%); mp 179–180 °C; ¹H NMR (400 MHz, DMSO-d₆) δ 13.13 (s, 1H, COOH), 7.29–7.99 (m, 10H, ArH, CH=CH), 4.52 (s, 2H, ArCH₂SO₂), 1.25 (s, 9H, C(CH₃)₃); ¹³C NMR (100 MHz, DMSO-d₆) δ 167.16, 151.31, 142.32, 136.96, 133.06, 131.35, 130.29, 129.29, 129.25, 125.82, 125.68, 59.91, 34.76, 31.49; HR-MS (ESI⁺) *m/z* 359.1239 [M + H]⁺, found 381.1140 [M + Na]⁺.

Data for 4-methoxybenzyl-4-carboxystyryl sulfone (5e). White solid (55%); mp 155–156 °C; ¹H NMR (400 MHz,

DMSO- d_6) δ 6.90–7.99 (m, 10H, ArH, CH=CH), 4.50 (s, 2H, ArCH₂SO₂), 3.73 (s, 3H, CH₃O); ¹³C NMR (100 MHz, DMSO- d_6) δ 167.17, 159.85, 142.29, 136.94, 133.04, 132.82, 130.30, 129.23, 129.10, 120.74, 114.35, 59.63, 55.56; HR-MS (ESI[−]) m/z 331.0718 [M − H][−], found 331.0681 [M − H][−].

Data for 4-(trifluoromethyl)benzyl-4-carboxystyryl sulfone (5f). White solid (55%); mp 158–159 °C; ¹H NMR (400 MHz, DMSO- d_6) δ 13.21 (s, 1H, COOH), 7.40–8.00 (m, 10H, ArH, CH=CH), 4.75 (s, 2H, ArCH₂SO₂).

Data for 1-phenylethyl-4-carboxystyryl sulfone (5g). Faint yellow solid (46%); mp 62–63 °C; ¹H NMR (400 MHz, DMSO- d_6) δ 13.16 (s, 1H, COOH), 7.35–7.98 (m, 11H, ArH, CH=CH), 4.55–4.60 (m, 1H, ArCHSO₂), 1.67–1.69 (d, 3H, CH₃); ¹³C NMR (100 MHz, DMSO- d_6) δ 167.16, 143.19, 136.93, 134.26, 133.06, 130.26, 130.09, 129.31, 129.04, 128.78, 127.64, 63.55, 14.57; HR-MS (ESI⁺) m/z 317.0769 [M + H]⁺, found 339.0678 [M + Na]⁺.

Data for benzyl-4-carboxystyryl sulfone (5h). White solid (86%); mp 138–139 °C; ¹H NMR (400 MHz, DMSO- d_6) δ 7.35–7.99 (m, 11H, ArH, CH=CH), 4.58 (s, 2H, ArCH₂SO₂); ¹³C NMR (100 MHz, DMSO- d_6) δ 167.15, 142.42, 136.90, 133.08, 131.63, 130.30, 129.25, 129.09, 129.02, 128.86, 60.26; HR-MS (ESI⁺) m/z 303.0613 [M + H]⁺, found 325.0527 [M + Na]⁺.

Data for 4-chlorobenzyl-styryl sulfone (5i). White solid (55%); mp 144–145 °C; ¹H NMR (400 MHz, DMSO- d_6) δ 7.30–7.70 (m, 11H, ArH, CH=CH), 4.59 (s, 2H, ArCH₂SO₂); ¹³C NMR (100 MHz, DMSO- d_6) δ 144.01, 133.76, 133.36, 132.82, 131.61, 129.54, 129.21, 128.89, 128.42, 126.42, 59.51; HR-MS (ESI⁺) m/z 293.0325 [M + H]⁺, found 315.0217 [M + Na]⁺.

Data for 4-chlorobenzyl-4-methylstyryl sulfone (5j). White solid (45%); mp 144–145 °C; ¹H NMR (400 MHz, DMSO- d_6) δ 7.24–7.58 (m, 10H, ArH, CH=CH), 4.57 (s, 2H, ArCH₂SO₂), 2.34 (s, 3H, CH₃); HR-MS (ESI⁺) m/z 307.0481 [M + H]⁺, found 328.9213 [M + Na]⁺.

Data for 4-chlorobenzyl-4-methoxystyryl sulfone (5k). White solid (43%); mp 144–145 °C; ¹H NMR (400 MHz, DMSO- d_6) δ 6.99–7.66 (m, 10H, ArH, CH=CH), 4.54 (s, 2H, ArCH₂SO₂), 3.81 (s, 3H, OCH₃); ¹³C NMR (100 MHz, DMSO- d_6) δ 162.09, 143.88, 133.69, 133.33, 131.11, 128.84, 128.59, 125.32, 123.38, 114.99, 59.70, 55.86; HR-MS (ESI⁺) m/z 323.0430 [M + H]⁺, found 344.9188 [M + Na]⁺.

Data for 4-chlorobenzyl-4-phenylstyryl sulfone (5l). White solid (64%); mp 144–145 °C; ¹H NMR (400 MHz, DMSO- d_6) δ 7.34–7.80 (m, 15H, ArH, CH=CH), 4.60 (s, 2H, ArCH₂SO₂); ¹³C NMR (100 MHz, DMSO- d_6) δ 143.51, 143.07, 139.49, 133.75, 133.37, 131.91, 129.89, 129.52, 128.90, 128.60, 128.48, 127.70, 127.26, 126.26, 59.56; HR-MS (ESI⁺) m/z 369.0638 [M + H]⁺, found 391.0529 [M + Na]⁺.

1.4. Procedure for the synthesis of 5m

Cinnamaldehyde (10 mmol) and piperidine (0.1 ml) were added to a solution of ((4-chlorobenzyl)sulfonyl) acetic acid (10 mmol) in pyridine (10 ml) and the resulting solution was heated to reflux until the disappearance of the (aralkylsulfonyl) acetic acid (checked by TLC). The reaction vessel was

cooled in an ice bath, and the precipitated crystalline solid was filtered off, washed with cooled isopropanol, and dried under vacuum to obtain the crude product. Purification of the crude residue was performed by column chromatography (petroleum ether/ethyl acetate) to afford the desired compound.

Data for 4-chlorobenzyl-4-phenylstyryl sulfone (5m). Yellow solid (20%); mp 144–145 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.19–7.85 (m, 13H, ArH, CH=CH), 4.67 (s, 2H, ArCH₂SO₂); ¹³C NMR (100 MHz, CDCl₃) δ 166.80, 155.17, 152.51, 135.30, 134.77, 132.26, 131.48, 129.13, 128.92, 126.53, 125.21, 122.38, 60.22; HR-MS (ESI⁺) m/z 317.0481 [M − H][−], found 317.0405 [M − H][−].

1.5. General procedure for the synthesis of 6a–6e

Styryl benzyl sulfones (10 mmol) were added to thionyl chloride (100 ml), and the resulting solution was heated to reflux until the disappearance of the styryl benzyl sulfones (checked by TLC). The excess SOCl₂ was removed using a rotary evaporator and the crude product was obtained as a yellow-green solid, which was added to a solution of Tempol (11 mmol) and DMAP (1 mmol) in pyridine (50 mL) at 0 °C. The mixture was stirred at 35 °C until the disappearance of acyl chloride (checked by TLC). The reaction mixture was diluted with EtOAc and citric acid aqueous solution, and the organic layer was collected. The organic layer was washed with H₂O and dried over anhydrous Na₂SO₄. After filtration, the solvent was evaporated under reduced pressure, and the residue was purified by column chromatography (petroleum ether/ethyl acetate) to afford the desired compound (X-ray structure in Fig. 3).

Data for 6a: orange solid (65%); HR-MS (ESI⁺) m/z 491.1455 [M + H]⁺, found 513.1348 [M + Na]⁺.

Data for 6b: orange solid (45%); HR-MS (ESI⁺) m/z 542.1626 [M + H]⁺, found 542.1759 [M + H]⁺.

Data for 6c: orange solid (56%); HR-MS (ESI⁺) m/z 476.1784 [M + H]⁺, found 476.1884 [M + H]⁺.

Data for 6d: orange solid (87%); HR-MS (ESI⁺) m/z 513.2471 [M + H]⁺, found 513.2518 [M + H]⁺.

Data for 6e: orange solid (67%); HR-MS (ESI⁺) m/z 472.2035 [M + H]⁺, found 472.2118 [M + H]⁺.

Fig. 3 X-Ray structures of 6a (left) and 6d (right).

2. Bioactivities

2.1. Cell culture and irradiation

Human umbilical vein endothelial cells (HUVECs) were used for *in vitro* studies. HUVECs were purchased from American Type Culture Collection (ATCC) and were cultured in DMEM from GE Healthcare containing 2% fetal calf serum, according to the manufacturer's protocol.

All cells were cultured in a humidified atmosphere at 37 °C in 95% air–5% CO₂ and used in the range of four to eight passages for experiments. The cell proliferation capacity was determined by the MTT assay. Monolayer cells were incubated with a certain concentration of the compound before or after irradiation. The plates were irradiated and chilled in ice, and the medium was replaced with fresh medium. Unirradiated cells were also chilled in ice for the same duration.

Irradiation was carried out using a ⁶⁰Co RAD SOURCE RS-2000 at a dose rate of 1.0 Gy min^{−1}. Different doses of radiation were used depending upon the systems exposed.

2.2. Cytotoxicity studies

The compound arrays were tested for growth inhibitory activity with HUVECs. The cytotoxic effects of the test compounds on the cells were assessed by the MTT assay. Briefly, HUVECs (1 × 10³ cells per well) were cultured in 100 µL of DMEM in a 96 well plate in the absence and presence of different concentrations of the test compounds and after 24 h, the cells were washed once and further incubated for 4 h with MTT dye. The blue formazan precipitate obtained was dissolved using DMSO. The absorbance at 570 nm was measured using a scanning plate reader (Thermo Fisher, Finland). The results are expressed as the mean absorbance ± SEM for three replicates.

Ex-Rad analogs 5a–5l showed little growth inhibition or cytotoxicity against the cells. The conjugates (6a–6f) showed low cytotoxicity below the concentration of 10 µM. Each experiment was performed in three replicates, and all experiments were independently replicated at least three times on different days. Data are reported as average values with standard deviations based upon all replications of the experiments.

The cell viabilities of the compounds in the activity assays are shown in Fig. 4.

2.3. Cell survival

Analogues 5a–m and conjugates 6a–f were evaluated for their ability to protect cells from irradiation using the MTT assay *in vitro* with HUVECs.

Fig. 4 The cell viabilities of analogs in HUVECs (*n* = 3).

In brief, HUVECs were plated in 96 well plates and treated with either 0.02% DMSO (dimethyl sulfoxide; the vehicle in which Ex-Rad was dissolved) or the compounds (0.1, 1, 10 µM) 2 h prior to irradiation. The cells were exposed to 5 Gy or 10 Gy of radiation. After 48 h of incubation, they were detached by trypsin, counted, serially diluted and plated in six-well plates at different concentrations. At least three independent experiments were conducted under exposure conditions, and mean values and standard deviations were derived. The results revealed that the 5 Gy radiation treatment groups (no compound added) exhibited a significant decrease in the number of viable cells as anticipated (*p* < 0.05).

We performed a survival assay and found that pre-treatment with 5a, 5b and 6b (0.1 to 10 µM) resulted in protection of HUVECs from 5 Gy radiation. In particular, treatment with 5a (0.1 µM) dramatically enhanced the survival in HUVECs, as compared to the vehicle control treatments with nearly 50% survival at doses up to 5 Gy irradiation, compared to the untreated control. Meanwhile, 5g, 6a and 6c showed similar radioprotection at certain concentrations (showing no * above the bar in the figure) compared to Ex-RAD (Fig. 5). However, other derivatives did not show attractive activities (data not shown). So, 5a and b, 5g and 6a–c exhibited a safe prophylactic effect *in vitro*.

When these compounds were administered after irradiation, 5a and 6a showed an excellent protective ability, while 5g and 6b showed average radioprotection in some concentration (Fig. 6).

Thus, combining these results, it can be inferred that Cl, F and OCF₃ substitution in the phenyl ring of aryl sulfone plays a key role. Esterification of the carboxyl terminal of 5a to form 6b did not enhance the cells' survival prior to irradiation, while it somewhat enhanced their survival after applying the same dose of irradiation. Similar trends are found for Ex-Rad and 6a; administration of 6a after exposure to irradiation resulted in an increase in survival compared to Ex-rad. So, 5a, 6a and 6b showed promising radioprotective abilities before or after exposure to radiation. When the radiation dose was increased to 10 Gy, the cell survival decreased sharply (data not shown).

Fig. 5 Effect of analogs on cell survival prior to 5 Gy irradiation. Values represent the means from three independent experiments on HUVECs. Survival in analog-treated cells irradiated with 5 Gy dose is significantly (compared to the vehicle for both HUVECs, ****P* < 0.001, ***p* < 0.01, **p* < 0.05) higher compared to that in untreated cells.

Fig. 6 Effect of analogs on cell survival after irradiation. Values represent the means from three independent experiments on HUVECs. Survival in analog-treated cells irradiated with 5 Gy dose is significantly (compared to the vehicle for both HUVECs, *** $P < 0.001$, ** $p < 0.01$, * $p < 0.05$) higher compared to that in untreated cells.

2.4. Cell apoptosis

HUVECs were plated in six-well plates (1×10^5 cells per well) in the absence and presence of different concentrations of test compounds 2 h prior to irradiation. According to the cytotoxicity data, 5a and 6b were administered with three concentrations of 0.1, 1 and 10 μM , while 6a–c with 0.01, 0.1 and 1 μM . After the cells were exposed to 5 Gy of radiation, they were detached by trypsin and washed three times with cold PBS. The cell suspension was double-stained with annexin V-fluorescein isothiocyanate (FITC) and PI and analysed by flow cytometry.

As shown in Fig. 7, a substantial increase in the number of apoptotic cells was observed upon exposure to 5 Gy of γ -radiation. Pre-treatment with compounds 5a and b and 6a–c decreased the population of apoptotic cells in γ -irradiated cultures.

2.5. Western blotting

Exponentially growing HUVECs were treated with 5 Gy of radiation. These cells were harvested, washed in PBS and lysed using RIPA buffer containing a protease inhibitor cocktail (Roche Applied Science). Protein samples were denatured at 100 $^{\circ}\text{C}$ for 10 minutes, separated by SDS-PAGE and then transferred to PVDF membranes (Millipore). All membranes were blocked with a

blocking solution (5% milk) for 1 hour and incubated overnight at 4 $^{\circ}\text{C}$ with primary antibodies against p53 (sc-6243, 1:500, Santa Cruz Biotechnology Inc.), phospho-p53 (0.5 $\mu\text{g ml}^{-1}$, ab1431, Abcam), Bcl-2 (sc-492, 1:500, Santa Cruz Biotechnology Inc.), Bax (1:2000, 50599-2-Ig, Proteintech), caspase-3 (1:500, ab4051, Abcam) and β -actin (1:1000, JC-PA001, Genshare Biological). After extensive washing in PBST buffer, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (1:10 000, CW0103S, CWBiotech; 1:10 000, CW0102S, CWBiotech) for 1 hour at room temperature and washed again prior to analysis. The signals were detected using an ECL western blotting detection system (Millipore) and analysed using a Tanon 5200 chemiluminescence imaging system.

We evaluated the changes in the apoptotic marker proteins as a result of ionizing radiation and the influence of compounds 5a and b and 6a and b on these proteins.

It has been demonstrated that p53 and Bcl-2 play vital roles in the regulation of radiation responses. To define the role of these derivatives in regulating p53 and Bcl-2 expression after irradiation, cell extracts of HUVECs were prepared after various treatments and were screened for the expression of pro-apoptosis proteins such as p53 and Bax and anti-apoptosis proteins such as Bcl-2 by western blot analysis. Compared to the untreated groups, we observed a significant reduction of the total p53 and phospho-p53 levels in derivative-treated cells. The cellular level of the p53 protein was up-regulated after irradiation. Additionally, caspase-8, a member of the mammalian caspase family, has been shown to play a key role in mediating apoptosis. When cells were exposed to 5 Gy of γ -radiation, the activation of caspase-8 was suppressed in compound-treated cells compared to untreated cells, which showed that ionizing radiation-induced cleavage of caspase-3 was significantly reduced by the compounds.

Fig. 7 Annexin-V/PI analysis of apoptosis in HUVECs at 24 h after treatment with 5 Gy of radiation. Cells were stained with annexin-V-FITC and PI and detected by flow cytometry. The lower right quadrant (annexin-V⁺, PI⁻) represents early apoptosis, whereas the upper right quadrant (annexin-V⁺, PI⁺) represents late apoptosis.

Fig. 8 Immunoblot analysis of apoptotic marker proteins in HUVECs exposed to ionizing radiation. Cell extracts were subjected to 10 to 12.5% SDS-PAGE and immunoblotted with antibodies against phospho-p53, p53, caspase-3, Bcl-2, and Bax. β -Actin was run as an internal control.

The abundance of Bcl-2, an anti-apoptotic protein, and of Bax, a pro-apoptotic protein, was examined by immunoblot analysis to determine the role of the mitochondrial pathway in the induction of apoptosis leading to the ionizing radiation-induced death of HUVECs. As shown in Fig. 8, the abundance of Bcl-2 in HUVECs was significantly decreased in untreated cells, compared to that in compound-pre-treated cells exposed to ionizing radiation. In contrast, the Bax levels increased after exposure to ionizing radiation in untreated cells compared to those in compound-pre-treated cells.

Conclusions

In the present study, we have successfully synthesized and characterized Ex-Rad analogs and Ex-Rad-Tempol conjugates which exhibit better radioprotective activity under some conditions than Ex-Rad suggesting their advantage in inhibition of radiation damage, especially compound-treated after radiation exposure. Hence, it may be concluded that the combination of analogs of Ex-Rad and Tempol may serve as an effective strategy to enhance radioprotection. Further studies are needed to elucidate the mechanistic details of such derivatives and their performance against other types of radiation damage.

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