Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Dual function radiosensitizer compounds

Raziye Saeidi
Ahvaz Jundishapur University of Medical Sciences
Amanollah Zarei Ahmady
Ahvaz Jundishapur University of Medical Sciences
Layasadat Khorsandi
Ahvaz Jundishapur University of Medical Sciences
Mohammad Javad
Golestan Hospital, Ahvaz Jundishapur University of Medical Sciences
Alireza Doroudi
Alireza.Doroudi@crchudequebec.ulaval.ca

Research Article

Keywords: Chalcone, Dual function radiosensitizer, HT 29, Nitroaromatic, Thiol alkylator

Posted Date: November 20th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11693899/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Dual function radiosensitizer compounds

Raziye Saeidi Rashk Oliaei[1], Amanollah Zarei Ahmady[2], Layasadat Khorsandi [3], Mohammad Javad Tahmasebi Birgani[4], Alireza Doroudi [5*],

Synthesis of 2, 4-dinitro-substitued bischalcones for radiosensitizing effects against the radioresistant HT29 cell under aerobic condition

1- Department of Medicinal Chemistry, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

2- Marine Pharmaceutical Science Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

3- Cellular and Molecular Research Center, Medical Basic Sciences Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

4- Department of Radiation Therapy, Golestan Hospital, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

5- 2Axe Oncologie, Centre de Recherche du CHU de Québec - Université Laval, Québec, QC G1J 5B3, Canada

- Correspond Autther: Alireza Doroudi E-Mail: Alireza.Doroudi@crchudequebec.ulaval.ca

Raziye Saeidi Rashk Oliaei E-Mail: r.saeidi92@yahoo.com

Dr Amanollah Zarei Ahmady E-Mail: zarei-a@ajums.ac.ir

Dr Layasadat Khorsandi E-Mail:khorsandi_cmrc@yahoo.com

Dr Mohammad Javad Tahmasebi Birgani E-Mail: Tahmasebi_mj@yahoo.com

Abstract

The dual function of radiosensitizing compounds, in which nitroaromatic moieties are attached via a linker to antineoplastic moieties, has demonstrated greater cytotoxicity and radiosensitizing effects than their corresponding counterparts. This study was conducted to investigate the radiosensitizer activity of 2,4-dinitrobenzene as a radiosensitizer moiety linked to α, β-unsaturated chalcones through a three-carbon chain against radioresistant human HT29 colon cancer cells. A series of derivatives composed of an electron-affinic 2,4-dinitrophenyl moiety and thiol-reactive unsaturated conjugated ketones were prepared. The radiosensitizer activity of the tested compounds was evaluated against HT29 colon cancer cells under aerobic conditions. The IC_{50} values and percentage of surviving cells were analyzed using the MTT assay. Additionally, a clonogenic assay was performed to assess cell viability following treatment with the tested compounds, both with and without radiation. This approach demonstrated that the tested compounds containing a 2,4-dinitrobenzene moiety exhibited a radiosensitizing effect on the HT29 cell line when combined with 2 Gy of ionizing radiation.2,4-Dinitrobenzylidene derivatives can induce oxidative stress upon exposure to ionizing radiation. This oxidative stress can lead to various detrimental effects on cellular function.

Keywords: Chalcone, Dual function radiosensitizer, HT 29, Nitroaromatic, Thiol alkylator,

Introduction

Radiation therapy (RT) uses ionizing radiation to shrink tumors due to the disrupting the DNA of cancer cells [1]. It is most effective on cells that grow and divide quickly. Tumor cells tend to proliferate more quickly than most normal cells. Therefore, cancer cells are more sensitive to RT than normal cells. Although cancer cells and normal cells respond differently to radiation therapy, it is very challenging to eliminate tumor cells without harming the surrounding normal tissues. The aim of RT is to deliver enough ionizing energy to destroy tumor cells but not so much that normal cells cannot repair and recover [2]. Radiosensitizer is a compound that makes tumor cells more vulnerable to the cytotoxic effect of ionizing radiation by accelerating DNA damage through generating free radical species. The combination of a radiosensitizer agent and conventional dose of irradiation is a promising modality that sensitize cancer cells in comparison to normal cells without inducing remarkable cytotoxicity to healthy tissues [3]. The use of hyperbaric oxygen was the first attempt to enhance the susceptibility of cancer cells toward the radiotoxicity of ionizing radiation [4]. A wide range of antineoplastic agents extensively studied in order to determine their potential as radiosensitizers [5-7]. Scientific literature suggests that certain chemical agents possess electron affinity characteristics that make them suitable candidates for radiosensitizer compounds [8]. As a result, nitroaromatic and nitro heterocyclic compounds were examined as electron affinic agents, as they have been found to effectively increase the vulnerability of tumor cells to radiation by generating diverse reactive species [9]. The new derivative radiosensitizers were developed by the connection of radio toxin to the chemical cytotoxin counterparts through a cross linker. These substances were known as dual function radiosensitizer compounds. It has been reported that the considerable radiosensitizer effect could be demonstrated when a 2,4dinitrobenzene was bonded to antimetabolites such as 5-fluorouracil and hydroxy urea through a three-carbon chain against the radioresistant HT29 cell line [10,11]. The α, β unsaturated ketones were specifically developed with the intention of being used as medications for cancer treatment. These compounds have a potential reactivity towards thiol group versus amino and hydroxyl groups. When alkylating agents are specifically targeted towards the thiol groups, their absence in DNA leads to an extraordinary outcome by avoiding the typical detrimental genotoxic effects often linked to cancer treatment. The attachment of 2,4 dinitrobenzene to various benzylidene analogues through a cyclohexanone linker has demonstrated the potential for a radiosensitizer effect [12]. The purpose of this research was to

develop a novel group of radiosensitizer compounds by replacing the cyclohexane ring with a three-carbon chain. This substitution was intended to decrease the hindrance caused by steric factors and investigate the radiosensitizer effects of these newly synthesized compounds on the HT29 cell line.

Materials and Methods

All chemicals and solvents were procured by Sigma-Aldrich and Merck companies. The chemical and solvents were of the highest purity and used without purification. To check the progress of reactions, thin layer chromatography was undertaken using silica gel (Kieselgel 60,230-400 mesh, Merck). Melting points were measured on an Electerothermal IA 9100 model and are uncorrected. Chemical shifts (δ) were reported in ppm relative to tetra methyl silane (TMS) as the internal standard. All cell culture were performed using the HT29 cell line, which was originally obtained from a human colorectal carcinoma and supplied by the Pasteur Institute of Iran. HT29 cells were grown as an attached monolayer in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum, penicillin (50 U/ml) and streptomycin (50µg/ml). Cells were cultured in tissue flasks and maintained in a humidified environment containing 5% CO2 and 95% air at 37°C.

Chemistry

The synthesis scheme of synthesized compounds is depicted in **Fig 1**.

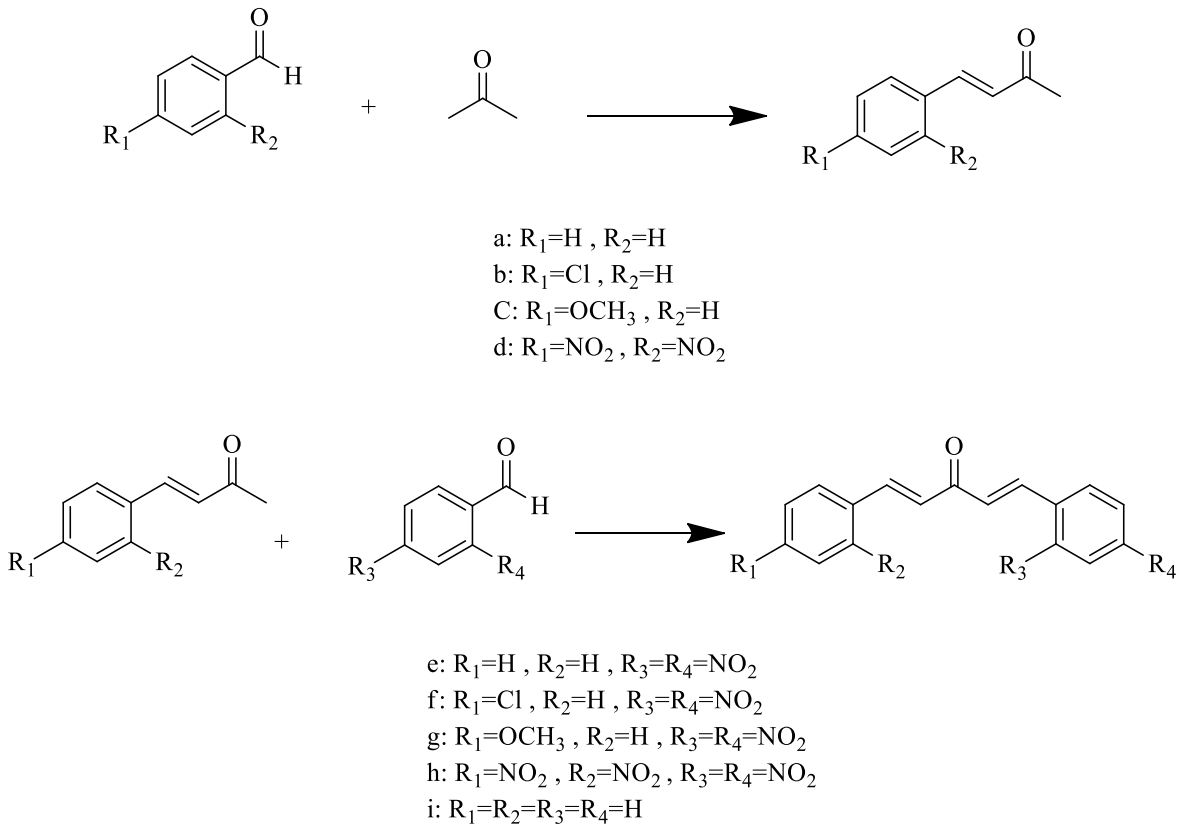


Fig 1: The scheme synthesis of compounds.

4-phenylbut-3-en-2-one Compound a

The synthesis of benzalacetone **a** was performed following the procedure previously reported [13], as outlined below: 10 mL of freshly distilled benzaldehyde and 20 mL of acetone are carefully added to a 100 mL flask equipped with a stirrer and a thermometer. An excess of acetone was used to minimize the formation of dibenzalacetone. The flask containing the reaction mixture is then placed in a cold water bath. Subsequently, 2.5 mL of a 10% sodium hydroxide solution is gradually added dropwise with stirring, ensuring that the temperature of the mixture does not exceed 30 °C. The mixture was then stirred at room temperature for

2 hours. After completion of the reaction, the mixture was neutralized extracted with dichloromethane 25ml × 3 and dried with magnesium sulfate and concentrated under reduced pressure and purified by column chromatography n-Hexane-Ethyl acetate. The overall yield of the desired compound is 3.1 gr, corresponding to 22.6%. solid, m. p=37-41^{0}C,
solid, m. p=38-41^{0}C

IR(KBr): 1359.92 ,1416.87, 1450.04, 1493.80, 1603.50(C=C), 1680.46(C=O), cm^{-1}.

^{1}H-NMR (400MHz, $CDCl_3$, TMS): 2.37(S,3H) 6.77(d, J=12 Hz,1H), δ 7.55-7.50(m,6H).

^{13} C- NMR (100 MHz, $CDCl_3$) δ27.50, 122.65,126.76 ,128.96,130.27,134.39,143.45, 198.42.

4-(4-chlorophenyl) but-3-en-2-one Compound b, 4-(4-methoxyphenyl) but-3-en-2-one Compound c and 4-(2,4-dinitrophenyl) but-3-en-2-one Compound d

These compounds were prepared using the synthesis procedure that has been previously described and documented in the literature [14]. Briefly a mixture of benzaldehyde (4-chloro benzaldehyde, 4-methoxy benzaldehyde, 2,4-dinitro benzaldehyde) (1 mmol) and acetone (14 mmol) was combined in a round-bottom flask. Subsequently, 0.06 gr of the Graphene Oxide (GO) Fe_3O_4/L- proline nanocomposite an environmentally friendly, recoverable bifunctional organocatalyst (supplied by the Nanotechnology Research Center at Ahvaz Jundishapur University of Medical Sciences in Ahvaz, Iran) was added to the mixture. The suspension was then stirred under reflux conditions for a duration determined by TLC analysis, using a solvent system of n-hexane and ethyl acetate in a 3:1 ratio. Once the reaction was completed, as confirmed by TLC, the catalyst was separated with the aid of an external permanent magnet. A straightforward work-up involved concentrating the reaction mixture and adding 25 mL of water to yield the crude product. The product was then extracted with three portions of ethyl acetate (3 × 25 mL). The organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure and purified by flash column chromatography (n-hexane-ethyl acetate) on silica gel to obtain benzalacetone.

Compound b: 0.13 gr with yield 74% mp (58-61ºC)

IR(KBr): 694.27,1342.17,1449.07,1497.26,1647.20, 1712.64, cm-1.

^{1}H-NMR (400MHz, $CDCl_3$, TMS): δ 2.36(S,3H), 6.71(d, J=12 Hz, 2H), 7.37(d, J=12 Hz,2H), 7.49 (d, J=16 Hz,2H),).

^{13}C- NMR (100 MHz, $CDCl_3$): δ27.49,127.47,129.27, 129.62. ,132.32,136.66, 142.46, 198.11.

Compound C: 0.12 gr (68.5%) mp (70-72 ºC)

IR(KBr): 1259.73,1447.35,1511.74, 1599.13, 1681.43, cm-1.

^{1}H NMR (400MHz, $CDCl_3$, TMS): 2.37(S,3H), 3.85 (S,3H), 6.70(d, J=8Hz,1H), 6.99(d, J=8Hz,2H), 7.50d, JJ=12Hz,3H).

^{13}CNMR (100MHz, $CDCl_3$): 39.29, 55.12,116.17, 116.43,123.55,126.54,135.68 ,142.76, 200.02.

Compound d: 0.14 (61.3%) mp (82-85ºC)

IR(KBr): 1349.30,1535.66, 1602.76, 1707.12, cm-1.

^{1}H - NMR (400MHz, $CDCl_3$, TMS): 2.47(S,3H), 6.67(d, J=16 Hz,1H) ,7.88(d, J=4 Hz,1H), 7.98(d, J=16Hz,1H), 8.53(d, J=4Hz,1H), 8.94(S,1H).

^{13}C- NMR (100 MHz, $CDCl_3$): 27.56, 120.71, 127.82, 130.64,134.27,136.40,136.73, 148.11 ,170.57, 197.1.

Synthesis 1-(2,4-dinitrophenyl) -5-phenylpenta-1,4-dien-3-one compound e, 1-(4-chlorophenyl) -5-(2,4-dinitrophenyl) penta-1,4-dien-3-one compound f, (2,4-dinitrophenyl) -5-(4-methoxyphenyl) penta-1,4-dien-3-one compound g

The synthesis of compounds **e**, **f**, **g**, and **h** was carried out according to the previously reported method [14]. In a 25 mL round-bottom flask equipped with a magnetic stir bar, 2,4-dinitrobenzaldehyde (0.5 mmol) and monochalcone (**a**, **b**, **c**, **d**) 0.5 mmol were added. The graphene oxide/Fe_3O_4/proline nanocomposite (0.007 gr) was then introduced into the reaction mixture. The mixture was refluxed for 1-2 hours. Reaction progress was monitored by TLC using n-hexane-ethyl acetate as the eluent. Upon completion, the reaction was cooled to room temperature. The catalyst was separated magnetically using an external magnet. The solvent was

evaporated under reduced pressure, and the products were purified by plate chromatography. The mobile phase was n-hexane /ethyl acetate (50%:50%).

Compound e:

0.026gr(Yield 8.1) mp(117-120^{0}C)

IR(KBr): 1341.84, 1453.66, 1535.66, 1602.76 cm^{-1};

^{1}H- NMR (400MHz, $CDCl_3$, TMS): δ6.78(d,J=8 Hz,2H), 7.31-7.10 (m,4H), 7.35(m,2H), 7.44(d, J=8 Hz,1H), 8.35(d, J=12 Hz,1H), 8.54(d, J=12 Hz,1H), 8.87(S,1H),

^{13}C- NMR (100MHz, $CDCl_3$): δ 113.22, 115.42, 116.30, 123.23, 124.76, 125.59, 129.28, 130.39, 131.64, 133.89, 144.99, 145.30, 147.67, 104.56, 199.05,

Chemical Formula: C17H12N2O5

Compound f:

0.013gr, (Yield 3.8%), mp:(89-90^{0}C)

IR(KBr): 805.10, 1349.30, 1535.66, 1610.21, 1662.35, 1707.12, cm^{-1};

^{1}H- NMR (400MHz.$CDCl_3$, TMS): δ6.75(d, J=16Hz,2H), 8-27-7.82(m,7H), 8.51(d, J=12 Hz, 1H) 8.86(s,1H),

^{13}C-NMR (100MHz, $CDCl_3$): δ120.11, 120.25, 125.72, 127.79, 128.52, 129.44, 129.72, 130.43, 131.51, 132.33, 137.24, 143.42, 145.21, 186.29198.79.

Compound g:

0.011gr, (Yield7), mp:(97-101^{0}C)

IR(KBr): 1222.57,1349.30, 1535.66, 1595.30, 1744.35 cm-1;

^{1}H- NMR (400MHz, $CDCl_3$, TMS): δ3.86(s,3H), 6.94(d, J=8 Hz,4H), 7.6(m,4H), 8.51(d, J=8 Hz,1H), 8.53(d, J=8 Hz,1H), 8.86(s,1H).

^{13}C-NMR (100 MHz, $CDCl_3$): δ 55.47, 105.06, 111.89, 114.60, 120.07, 123.11, 126.68, 127.72, 130.49, 139.89, 141.70, 144.91, 145.41, 147.06, 159.80185.01.

1,5-diphenylpenta-1,4-dien-3-one Compound

The compound was prepared using a method reported in the literature (15). A solution of sodium hydroxide (0.6 g, 14 mmole) in 5 mL of water was added to a mixture of acetone (1.2 g, 20 mmol) and benzaldehyde (4.25 g, 40 mmol) in 20 mL of ethanol. The resulting mixture was stirred at room temperature for 2 hours. Subsequently, the precipitate was filtered and then recrystallized from ethanol to obtain the 2.1 gr yield 45.7% product (mp: 108-108˚C).

IR(KBr): 1445(Aromatic ring), 1494(Aromatic ring), 1650(C=C), 1715(C=O), cm^{-1};

^{1}H-NMR (400MHz, $CDCl_3$, TMS): δ 7.13(d, J=12 Hz,2H), 7.43-7.34(m,6H), 7.53(d, J=12 Hz,2H), 7.75(d, J=16Hz,4H).
^{13}CNMR($CDCl_3$): 118.65, 125.43, 127.74, 128.44, 129.01, 130.55, 134.80, 143.37, 188.97.

Compound treatment

The stock solution for each of the synthesized compounds was formulated in the appropriate culture media, utilizing a minimal quantity of dimethyl sulfoxide (DMSO), specifically within the range of 0.1 % to 0.5%. These prepared solutions were subsequently stored at a temperature of -20 °C to ensure their stability and effectiveness, while also being carefully shielded from light exposure to prevent any potential degradation. On the day of experimentation, the desired concentrations of each of the tested compounds achieved by diluting the corresponding stock solution with the culture media. It was important to note that the final concentration of DMSO remained consistent across both the control and the test compounds, allowing for a fair comparison of results.

Cell Culture

The cell culture process was conducted in accordance with established protocols as detailed in the literature [10-12]. To summarize, the HT29 cell line was inoculated at a density of 1×10^3 cells in T25 flasks. The cells were cultivated in Dulbecco's Modified Eagle Medium (DMEM), which was supplemented with 10 % fetal bovine serum (FBS), along with 100 U/ml of penicillin and 100 µg/ml of streptomycin to ensure optimal growth conditions. The T25 flasks were then placed in an incubator maintained at a temperature of 37 °C, with a controlled environment comprising 5% carbon dioxide (CO2) and 95 % atmospheric air to mimic physiological conditions. The cells were allowed to grow in a monolayer within each flask, once the cell cultures reached approximately 90% confluence, indicating that most of the available surface area was occupied by the growing cells, the cells were harvested. This detachment process was accomplished using a solution of 0.05% trypsin and 0.05% ethylene diamine tetra acetic acid (EDTA), which facilitated the separation of the cells from the flask surface without compromising their viability. Following this initial incubation period, 5×10^3 cells in 100 µl culture media were seeded in each well of the 96-well plates. The plates were organized into two distinct groups to facilitate the experimental design. One group of plates was designated for the treatment involving the various compounds being tested, as well as the control cells that would

not receive any experimental treatment. This set up was essential for establishing a baseline for comparison against the treated cells. The second group of plates underwent a different treatment protocol. In this case, the cells were subjected to a combination of 2 Gy radiation along with the synthesized compounds**,** in addition the control cells that were included for comparison purposes. It was important to note that the control cells in this group were treated in the same manner as the other, with sole exception being that they did not receive any exposure to the synthesized compounds. This structuring of the experimental groups ensured a comprehensive evaluation of the effects of the compounds under both treatment conditions. Once the cells had formed a complete monolayer covering the surface of each well in the 96-well plates, the supernatant was removed. Subsequently, various doses of each compound were introduced into the wells, with each dose being delivered in a volume of 100 µl of appropriate culture media. Following this addition, the plates were placed in an incubator set at 37°C, maintaining an atmosphere of 5 % CO2 and 95 % air, and were left to incubate overnight to allow for optimal cell interaction and treatment effects. Following the incubation period, the plates containing the cell cultures were taken out of the incubator. Once removed, the supernatant solution present in individual well decanted to ensure that no residual liquid remained that could interfere with subsequent analyses. To each well, a volume of 50 µl of a 3-(4,5dimethyl thiazol-2 yl) 2, 5 diphenyl tetrazolium bromide (commonly referred to as MTT) solution was added. This solution was prepared at a concentration of 5 mg/ml. The plates underwent a gentle shaking process for a duration of 10 min. Following this, they were placed in an incubator at 37°C and 5% CO2 and 95% air. After the incubation period, the supernatant from each well removed, and then 125 µl of DMSO was added to each well. It served to solubilize the formazan crystals that had been produced during the earlier phases of the experiment. The plates were then gently shaken for another 20 min to ensure thorough mixing and complete dissolution of the formazan. The absorbance measurements for each well in the 96-well plates were recorded using an ELISA reader (Bio-Tek instruments, Inc, USA). Reading was obtained at a wavelength of 570 nm, with DMSO serving as a blank control. An additional reference reading was taken at 690 nm to ensure the accuracy and reliability of the results. To validate the data, all experimental procedures were performed in triplicate, facilitating a thorough analysis of the outcomes. The percentage of cell survival was calculated by the following formula.

$$\text{Cell survival \%} = \frac{\text{absorbance sample} - \text{absorbance blank}}{\text{absorbance control} - \text{absorbance blank}}$$

Following this, survival curves were created for each of the synthesized compounds by plotting the percentage of surviving cells against the varying concentrations of the compounds, both in the presence of absence of 2 Gy of ionizing radiation. Furthermore, the corresponding half-maximal inhibitory concentration (IC_{50}) values for the tested compounds were determined, offering essential insights into their efficacy and potential therapeutic applications. The viability of the HT29 cells that were subjected to analysis, both in the presence of 2 Gy radiation and in its absence, was evaluated using a clonogenic assay conducted under aerobic conditions. The clonogenic assay was performed as follows: the culture media from the cell cultures, after being exposed to the tested compounds, were separated to remove the compounds and subsequently washed with PBS. The cells which exhibited a density of 5×10^3were seeded into 6-well plates to ensure optimal growth conditions. Following the seeding process, these plates were placed in an incubator set to a 37 °C. This incubation period was maintained for a total of 14 days allowing the cells ample time to proliferate and adapt to their environment. Throughout this duration, the cells were monitored to assess their growth and overall health, ensuring that they remained in optimal condition for any subsequent analyses. After the designated incubation period had elapsed, the plates were taken out of the incubator where they had been cultured. The cells were fixed using methanol that helps to stabilize the cells for staining. Once this fixation process was complete, the colonies on the plates were stained with crystal violet, a dye binds to cellular components and allows for clear visualization. Following the staining process, colonies that consisted of 50 or more cells were counted and documented for further analysis. An examination was conducted to assess the number of colonies formed by both the treated and untreated samples after being subjected to 2 Gy of radiation in the presence of the compounds that were tested. To illustrate the effects of these compounds on cell survival, we constructed comprehensive clonogenic survival curves. This was achieved by plotting the percentage of surviving cells against a range of doses of the prepared compounds. By analyzing these curves, we aimed to gain insights into the relationship between the compound doses and the survival rates of the cells following radiation exposure, thereby providing an understanding of the potential protective or detrimental effects of the compounds in question.

Irradiation

The cellular samples underwent exposure to a dose of 2 Gray (Gy) of ionizing radiation at room temperature. This was achieved using a linear accelerator, specifically the Primus model manufactured by Siemens in Germany, which utilized 6 megavolt (6MV) photon beams for the irradiation process.

Optimizing irradiation time to check radiosensitizer

The compound at the center of this project is 2,4-dinitrobenzaldehyde. This compound was chemically linked to acetone, leading to the successful synthesis of 2,4-dinitrobenzylidene acetone, a crucial intermediate for our research. This intermediate was then utilized to fine-tune the irradiation duration, which played role in the overall experiment design. Specifically, this phase of the study involved four distinct treatment periods, each lasting 30 min, followed by additional intervals of 1 h, 2h and 4h the cells were exposed to a dose of 2 Gy radiation after the cells were subjected to the synthesized compound. To evaluate the cellular response to the treatment, an MTT assay was conducted allowing us to assess cell viability and proliferation in response to the compound and radiation exposure **Fig S28**.

Statistical analysis

A statistical investigation was conducted using SPSS 11.0 for Windows (SPSS Inc, Chicago, IL, USA), and descriptive statistics are presented as the mean ± standard deviation. An independent samples t-test was employed to evaluate the differences between ionizing irradiated and non-irradiated cells treated with the test compounds. A p-value of less than 0.05 was deemed statistically significant.

Results

The novel bifunctional radiosensitizer agents, along with their intermediate derivatives, were synthesized successfully. Following their synthesis, these compounds were thoroughly characterized using a range of analytical techniques, including Infra-red spectroscopy, proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR)

and. This comprehensive process allowed for a detailed understanding of the molecular structure and composition of both primary intermediates and final compounds. The MTT assay was employed as an experimental technique to evaluate the cytotoxic effects of the synthesized compounds on the radioresistant HT29 cell line, under aerobic conditions. This assay is widely recognized for its ability to assess cell viability and proliferation by measuring the metabolic activity of cells. Through the results generated from this assay, we were able to determine the IC_{50} values for the various compounds that were tested, which represent the concentration at which 50% of the cells are inhibited from growing. Additionally, the findings provided insights into the concentrations of these compounds could potentially modify the response of these resilient cancer cells to radiation therapy. The reduction of tetrazolium salt has emerged as a widely accepted and reliable technique for assessing the proliferation of cells in various biological studies. Among these tetrazolium salts, MTT is particularly noteworthy because it is reduced by live, metabolically active cells. This reduction process involves a series of enzymatic reactions facilitated by dehydrogenase enzymes, which are primarily located within the mitochondria of the cells. As these enzymes catalyze the reaction, they convert the MTT into an intracellular product known as purple formazan. This formazan compound is not only a visual indicator of cellular viability but also is readily soluble allowing for further analysis. The quantification of formazan can be carried out using a spectrophotometric assay, allowing for the measurement of the purple color intensity, which is proportional to the number of viable cells in the sample. However, when cells undergo metabolic changes that lead to processes such as apoptosis (programmed cell death) or necrosis (uncontrolled cell death), a subsequent reduction in cell viability is observed. This decline in viability is often reflected in the diminished capacity of the cells to reduce MTT, serving as a crucial indicator of cellular health and metabolic activity. The susceptibility of the HT29 cell line to varying doses of radiation was thoroughly evaluated in a series of experiments. The findings from these investigations indicated that cytotoxic effects were observed when the cells were subjected to 2 Gy of ionizing radiation. This result prompted to select 2 Gy ionizing irradiation as the standard dose for further studies aimed at investigating the radio sensitizing effects of the compounds being tested. Consequently, this specific dosage was consistently employed across all experimental protocols to ensure a reliable comparison of the radiosensitizer effects of the various compounds under investigation.

As illustrated in Fig. 2, when the HT29 cells that had been subjected to various doses of 2,4-dinitrobenzylidene acetone analogs were subsequently exposed to a dose of 2 Gy of ionizing radiation at different time intervals, the cells demonstrated distinct responses to the combined treatment modalities after a period of 2 h. This observation indicates that the cellular responses to the combination of chemical treatment and radiation exposure began to manifest within this timeframe. Consequently, in this experimental approach, the radiation treatment was administered following a 2-hour period of compound treatment, allowing sufficient time for the cells to respond to the initial chemical exposure before applying the radiation stimulus.
In the experimental set up, cell cultures were subjected to various concentrations of each compound, specifically ranging from 0.01 μM to 100 μM **Fig 2.**

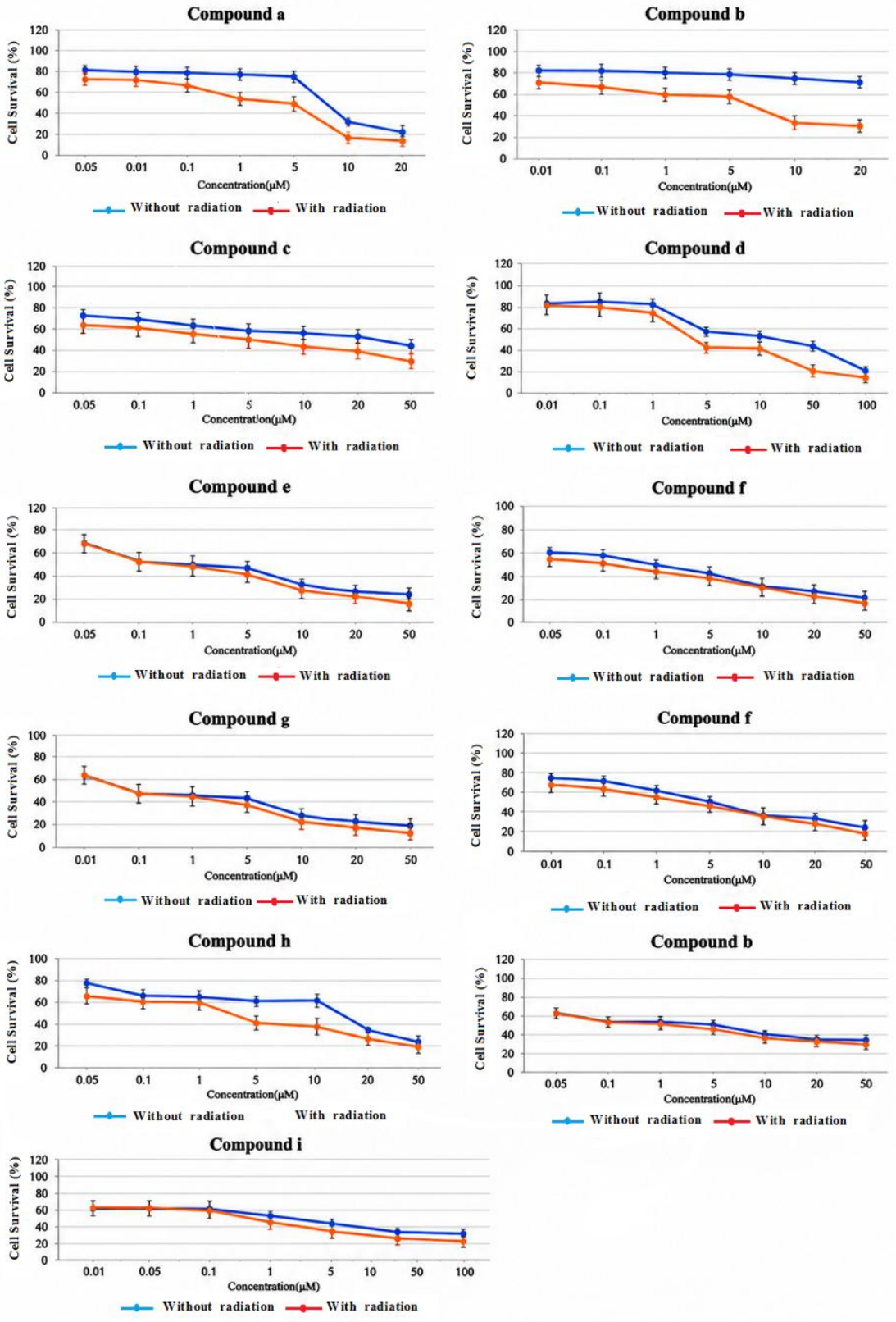

Compound a
Cell Survival (%)
Concentration(μM)
Without radiation
With radiation
Compound b
Cell Survival (%)
Concentration(μM)
Without radiation
With radiation
Compound c
Cell Survival (%)
Concentration(μM)
Without radiation
With radiation
Compound d
Cell Survival (%)
Concentration(μM)
Without radiation
With radiation
Compound e
Cell Survival (%)
Concentration(μM)
Without radiation
With radiation
Compound f
Cell Survival (%)
Concentration(μM)
Without radiation
With radiation
Compound g
Cell Survival (%)
Concentration(μM)
Without radiation
With radiation
Compound f
Cell Survival (%)
Concentration(μM)
Without radiation
With radiation
Compound h
Cell Survival (%)
Concentration(μM)
Without radiation
With radiation
Compound b
Cell Survival (%)
Concentration(μM)
Without radiation
With radiation
Compound i
Cell Survival (%)
Concentration(μM)
Without radiation
With radiation

Fig 2. The percentage of survival of HT29 cells was assessed after exposure to a range of different concentrations of the test compounds, either administered alone or in combination with a specified dose of 2 Gy ionizing radiation.

The concentrations tested included 0.01, 0.5, 1.00, 10, 50, 100 μM. The IC_{50} values for compound **a** were determined to be 20.62 ±10.56 without the application of irradiation and 0.65 ±0.19 when irradiation was applied **Table 1**.

Table 1: The IC_{50} values for the treated compounds with and without 2Gy radiation that they were obtained by MTT assay

Compounds	IC_{50} Without radiation	IC_{50} With radiation
a	20.62±10.56	0.65±0.19
b	3.58±0.68	1.44±2.21
c	12.58±0.31	1.925±0.53
d	86.89±8.13	19.61±2.65
e	0.0302±0.0077	$0.0018\pm7*10^{-4}$
f	0.02±0.02	0.012±0.007
g	3.08±0.29	1.06±1.2
h	0.04±0.06	0.0015±0.04
i	0.031±0.041	0.0047±0.0027

In the absence of irradiation, the percentages of surviving cells at various concentrations of the compound **a** specifically at 0.01 mM, 0.1mM, 1 mM and 10 mM were recorded as 70.73%,70.53%, 68.56% and 67.58% respectively. Conversely, when irradiation was present, the survival rates for the same concentrations decreased significantly, yielding values of 64.27%, 59.02%, 47.63% and 45.53%. These results indicate that compound **a** exhibited a dose-dependent radio sensitizing effect, particularly within the concentration range of 0.1 mM to 10 mM. The IC_{50} values for compound **b** were calculated to be 3.58 ± 0.64 in the absence of irradiation, while this value decreased 1.44 ± 2.21when irradiation was introduced. In scenarios

where irradiation was not applied, the survival rates of cells exposed to varying concentrations of compound **b** at concentrations of 0.01 mM, 0.1 mM, 1.00 mM and 10 mM were found to be 79.96%, 76.81%, 74.66% and 70.73% respectively. On the other hand, the presence of irradiation led to a notable decline in cell survival at these same concentrations, with recorded pf 62.17%, 54.11%, 52.24% and a stark drop to 27.49%. These findings suggest that compound **b** possesses a dose-dependent radio sensitizing effect, particularly evident within the concentration range of 1.00 mM to 10 mM. The IC_{50} values for compound **C** were measured at 12.58 ± 0.31 in the absence of irradiation. However, when irradiation was applied, the IC_{50} value significantly decreased to 1.925 ± 0.53. In experiments without irradiation, the percentage of surviving cells at various concentrations of compound **C** namely 0.01 mM, 0.1 mM, 1 mM, and 10 mM were recorded as 65.23%, 55.99%, 51.66%, and 50.69%, respectively. In contrast, with irradiation, the survival rates at the same concentrations showing values of 58.14%, 52.36%, 44.66%, and 32.39%. These findings suggest that compound **C** has a dose-dependent radio sensitizing effect, particularly at the 10 mM concentration. This indicates that as the concentration of compound C increases, its capacity to enhance the effects of irradiation on cell survival. The IC_{50} values for compound **d** were measured at 86.89 ± 8.13 without irradiation. However, upon the application of irradiation, the IC_{50} value significantly decreased to 19.61 ± 2.65. In experiments conducted without irradiation, the percentage of surviving cells at various concentrations of compound **d** 1 mM, 10 mM, and 50 mM was recorded as 72.82%, 67.06%, and 59.59%, respectively. In contrast, with irradiation, the survival rates at the same concentrations were 60.78%, 59.69%, and 41.57%. These findings suggest that compound **d** did not exhibit a radiosensitizing effect until the concentration reached 50 mM. We hypothesized that when HT29 cells were exposed to this compound, the metabolic enzymes in the cells reacted to neutralize this agent, which is why significant radiotoxicity was not observed until the concentration of the compound increased enough to deplete the metabolic enzymes. The IC_{50} values for compounds **e**, **f**, **h**, and **i** were measured to be 0.0302 ± 0.0077 mM, 0.02 ± 0.02 mM, 0.04 ± 0.0007 mM and 0.031 ± 0.041 respectively, under conditions without irradiation. In contrast, when these compounds were combined with 2 Gy of ionizing irradiation, the IC_{50}values changed to 0.0017± 0.0007 mM for compound **e**, 0.012 ± 0.007 mM for compound **f,** .0015 ± 0.04 mM for compound **h** and 0.0047 ± 0.0027 mM for compound **i.**

Notably, the cell survival rates at various concentrations of these compounds did not show significant differences between the two experimental conditions. This observation suggests that the tested compounds lack the ability to function as radiosensitizers, indicating that they do not enhance the effectiveness of ionizing in reducing viability.

The IC_{50} values for compound **g** were established at 3.08 ± 0.29 mM in the absence of radiation while a value of 1.06 ± 1.2 mM was observed when irradiation was applied. In the absence of irradiation, the survival rates of cells at two concentrations of 1.00 mM and 10 mM, the percentages of surviving cells were recorded at 58.15 % and 57.56% respectively. But the presence of irradiation led to a marked decline in survival rates at the same concentrations, yielding 33.09% at 1.00 mM and 28.72% at 10 mM. This finding clearly demonstrates that compound **g** has a pronounced dose-dependent radio sensitizing effect, particularly evident within the concentration range of 1 mM to 10 mM. This suggests that compound **g** not only affects cells' viability but also enhances the impact of irradiation on cell survival, indicating its potential usefulness in therapeutic applications that involve radiation treatment. The clonogenic assay stands out as the preferred technique for assessing cell productive death following exposure to ionizing radiation. However, its utility extends beyond just radiation, as it can also effectively evaluate the impact of various other cytotoxic agents on cell viability. Notably, it is important to recognize they only a small percentage of the cells that are initially seed in this assay can retain their ability to proliferate and form colonies. This characteristic makes the clonogenic assay a valuable tool for understanding the long-term effects of different treatments on cell survival and reproductive potential **Fig 3**

Table 2.

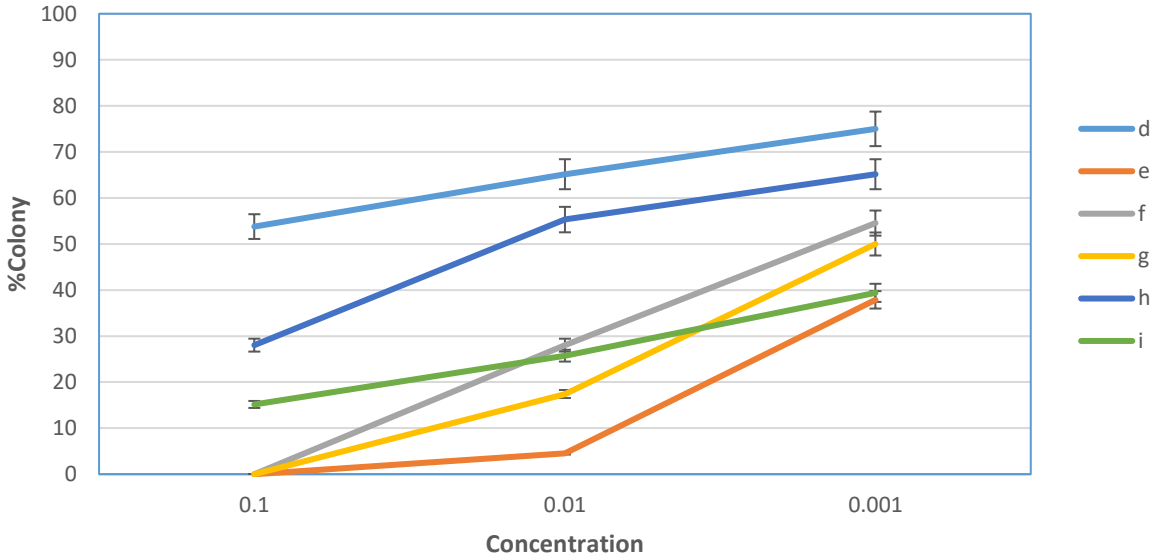


Fig 3: Clonogenic survival of HT29 cells with or without the combination of 2 Gy ionizing irradiation after exposure to various tested compound doses.

Table 2. Number of colonies in clonogenic assay

Concentration of compounds (µM)	a (0.1)	b (0.01)	c (0.001)	d (0)
Number of colonies d	71	86	99	132
Number of colonies e	0	6	50	132
Number of colonies f	0	37	72	132
Number of colonies g	0	23	66	132
Number of colonies h	37	73	86	132
Number of colonies i	20	34	52	132

Number of colonies formed (a, b, c=Treatment groups, d=Control group)

The clonogenic
testwas performed to evaluate the effects of compound **d**, which served asradiotoxin counterpart, alongside several bifunctional radiosensitizer compounds, specifically compounds **e**, **f**, **g**, and h. Additionally, compound **i** was included in this study, characterized by the absence of any substitutions on its benzene rings. This comprehensive approach aimed to assess the potential of these compounds in enhancing the effectiveness of radiation therapy by examining their ability to form colonies in the presence of radiation, thereby providing insight into their biological activity. The compound designated as **d** has been identified as a potential radiotoxic agent, capable of enhancing the sensitivity of cells to radiation doses of 2 Gy. This sensitization effect results in a significant reduction in cell viability, specifically a decrease of 60.78% when compared to control cell populations. However, when evaluated through clonogenic assays, it was observed that this initial damage inflicted by the compound **d** did not result in the complete elimination of all cells. Instead, many of the affected cells exhibited an ability to repair the radiation-induced damage over time. Consequently, despite the cytotoxic effects observed, these cells retained their capability for proliferation, indicating that they could recover and continue to divide. This finding highlights the complex interplay between radiotoxic agents and cellular repair mechanisms, suggesting that while compound could effectively reduce cell viability, it did not guarantee total cell death, allowing for the possibility of recovery and continued growth in a substantial fraction of the cell population. When the radiotoxic component attached
with its cytotoxic counterpart, like the various benzylidene derivatives, the resulting agents referred to as **e**, **f**, **g**, and **h** demonstrated significantly enhanced cytotoxic effects. In a clonogenic assay, exposing the cells to a concentration of 1 mM of these compounds and ionizing radiation resulted in a complete lack of cell growth, indicating that all cells lost their ability to proliferate. Notably, only compound **g**, when administered at doses ranging from 1 to 10 mM, exhibited a radio sensitizing effect. In contrast, the cytotoxicity levels of all other compounds, except for compound **g**, were significantly increased. Additionally, combining these compounds with a radiation dose of 2 Gy did not produce any further increase in cytotoxicity, indicating a limit to the effectiveness of this combined treatment.

Discussion

A significant amount of research has been conducted in the realm of cancer treatment, particularly focusing on the use of radiotherapy. This research aims to develop synthetic radiosensitizer compounds that can enhance the effectiveness of radiation therapy while simultaneously minimizing any potential toxicity to normal, healthy tissues surrounding the tumor. Scientists and medical professionals are increasingly aware of the challenges associated with traditional cancer treatment, which often come with severe side effects due to the collateral damage inflicted on non-cancerous cells. As a result, there is a growing urgency to identify and create novel substances that can selectively target cancer cells, making them more susceptible to radiation, while sparing normal tissues from damage. This pursuit not only involves the synthesis of new compounds but also requires a deep understanding of the cellular and molecular mechanisms that underlie tumor biology and treatment response. Consequently, any new radiosensitizer agent modalities that enhance the therapeutic effectiveness of radiotherapy without altering the dose of ionizing radiation can be considered for clinical use. Ionizing radiation has been shown to significantly elevate the levels of reactive oxygen species (ROS) within cells. This increase in ROS production is a critical factor in the cellular response to ionizing radiation. Research has proposed that exposure to certain compounds that can amplify oxidative stress may render tumor cells more susceptible to damaging effects of ionizing radiation. Given this potential for enhanced sensitivity, it becomes increasingly important to pinpoint the specific molecular targets that contribute to the inherent radio resistance observed in many tumor cells. Identifying these targets is essential for the development of effective radiosensitizer compounds to enhance the vulnerability of cancer cells to radiation therapy. Ionizing radiation is a treatment method that can be delivered in two primary ways, through an external source or an internal source. When administered externally, this involves gamma radiation or high-energy photons that are generated by a linear accelerator. These high-energy beams are precisely targeted at the tumor cells to disrupt their growth and promote cell death. On the other hand, the internal method of administering ionizing radiation involves utilizing radioactive materials that decay within the affected area where the tumor cells are located. This approach allows for more localized treatment, as the radiation directly from the tumor site, thereby minimizing exposure to surrounding healthy tissues. Both methods aim to effectively target and destroy cancerous cells while striving to preserve the integrity of adjacent normal

cells. Ionizing radiation could dislodge tightly bound electrons from their atomic orbitals, leading to ionization, which can cause an atom to become charged. Once ionized, the atom can easily interact with neighboring atoms and form new chemical bonds. This ionization can result in biological damage due to the chemical changes it induces at the cellular level. Such effects arise from the ionization of biomolecule atoms, potentially resulting in alterations or complete loss of their functions. The energy from ionizing radiation can directly cause the ionization of biological molecules or may act indirectly by generating free radicals from the ionization of surrounding water molecules. Direct ionization is primarily associated with high linear energy transfer (LET) radiation, such as alpha heavy charged particles. In contrast, indirect ionization is more common with low LET radiation, including gamma rays and X-rays. Low LET radiation must first interact with other substances to generate free radicals, which can subsequently ionize the atoms in cellular biomolecules. This process can lead to the loss of functionality in protein molecules' amino groups and alter their behavior. Unsaturated lipids may undergo peroxidation, while carbohydrates may experience dissociation reactions. DNA is the main target of ionizing radiation because it contains genes that are essential for cell function and reproduction, which are critical for cell survival. Direct ionization of DNA can lead to genetic damage. Additionally, radiation can ionize water molecules, producing free radical species through indirect action. These free radicals can attack DNA molecules, and this manner of damage is more prevalent than direct action. When ionizing radiation penetrates a cell, four potential outcomes can be considered. First, the radiation may pass through without causing any damage to the cell. Second, the radiation may damage the cell, but the cell can effectively repair that damage. Third, the radiation may damage the cell, and if the damage is not efficiently repaired, the cell may replicate itself in a damaged state. Finally, if DNA repair is incomplete, signaling pathways may lead to cell death through terminal differentiation and apoptosis process. The mechanisms involved in energy absorption and the subsequent phenomenon of induced ionization, alongside the various biomedical responses elicited by the living organism, can transpire in a matter of mere fractions of a second. These rapid processes are crucial in determining how the organism reacts to external stimuli, particularly when it comes to ionizing radiation. Following exposure to such radiation, the repair of cellular damage, with a special emphasis on the intricate and vital process of DNA repair, can be initiated shortly thereafter, often taking from several minutes to several hours to fully execute. This duration largely hinges on the specific type and extent of

damage inflicted on the cellular structures. The complexity of the damage, where it be single-strand breaks, double-strand breaks, or other forms of genetic alterations, plays a significant role in dictating the efficiency and effectiveness of the repair mechanisms that the cell employs in its efforts to restore normal function. Consequently, the timeline for cellular recovery and the ability to maintain genomic integrity after radiation exposure is a multifaceted process influenced by various biological factors and the nature of the initial injury sustained. Single-strand breaks represent one of the least severe forms of damage that can occur to the DNA molecule. Fortunately, these types of breaks can typically be repaired effectively by the cell without resulting in any lasting consequences or detrimental effects on cellular function. In contrast, double-stand breaks pose a significant greater challenge. These more severe forms of damage are not easily repaired and carry a higher risk of inducing long-term damage to the cell. The process of scavenging free radical species is recognized as the most vital mechanism for providing protection at the cellular level. This scavenging activity is facilitated by a variety of enzymes that are naturally found within cells, which work collectively to diminish the effects of oxidative stress. Key enzymes involved in this protective response include catalase, glutathione transferase, glutathione peroxidase and superoxide dismutase. These enzymes play a crucial role in neutralizing the oxidative stress that is often triggered by exposure to ionizing radiation, thereby helping to maintain cellular integrity and function. By effectively counteracting the harmful effects of free radicals, these enzymes not only safeguard cellular components but also contribute significantly to the overall defense mechanisms that protect cells from damaging consequences of radiation exposure. Glutathione, often abbreviated as GSH, is a small but significantly classified as a tripeptide. It is made up of three specific amino acids γ- glutamyl, cysteine and glycine. This unique combination allows glutathione to be present in a wide range of tissues throughout the body, highlighting its importance in biological systems. As a non-protein free thiol, GSH is notable for its high concentrations found in living organisms, where it serves numerous essential functions. Its presence and activity are pivotal for a variety of biomedical processes that occur within cells, making it an indispensable component in maintaining cellular health and function. Glutathione is involved in antioxidant defense, detoxification and the regulation of cellular metabolism, thereby playing a pivotal role in overall physiological homeostasis. As a result, GSH possesses the remarkable ability to effectively neutralize free radicals, particularly ROS such superoxide, hydroperoxy and hydroxyl radicals,

all of which exhibit a deficit of electrons and are thus eager to accept them. In this context, GSH can engage chemical reactions with α, β-unsaturated ketones, as indicated in previous studies. The process involves the nucleophilic addition of GSH to the electron-deficient carbon-carbon double bonds, which predominantly occurs with conjugated unsaturated double bonds. Specifically, the α, β-unsaturated ketone undergoes a Michael addition reaction with GSH, resulting in the formation of a corresponding GSH adduct. As a result, any factors that significantly lower the concentration of GSH in the cell can increase the sensitivity of these cells to the detrimental effects of ionizing radiation. This indicates that when GSH levels or other thiol groups levels are reduced, the cells may be more susceptible to radiation-induced damage, potentially resulting in heightened cytotoxicity. A range of alkylating agents exhibits biological effects that are comparable to those of ionizing radiation. These effects include the induction of chromosome breaks, the destruction of dividing cells, and the interaction with the cell's genetic material. In recent studies, various conjugated ketones have been investigated for their potential use as antineoplastic agents. These specific compounds function as thiol alkylators, which means they possess minimal ability to interact with amino or hydroxyl groups. It is important to note that these groups are commonly found in nucleic acids, whereas thiols are not. Therefore, it has been theorized that enones may lack the genotoxic side effects that are often associated with many conventional antineoplastic agents, suggesting a safer alternative in cancer treatment. This perspective opens up exciting avenues for further research into the therapeutic applications of these compounds, as they may provide effective cancer treatment options with a reduced risk of damaging genetic material. In the current study, the compounds were engineered with the specific purpose of alkylating thiol containing molecules within the cells. This strategic modification aimed to enhance the cell's sensitivity to the cytotoxic effects induced by ionizing radiation. Among the compounds investigated, those designated as **a**, **b**, **c** and **i**, functioned as thiol alkylators. Notably, these compounds lack the requisite functional groups that would enable them to generate ROS within the cellular environment following exposure to ionizing radiation. This lack of ROS production is a crucial aspect of their mechanism of action, as it indicates that their primary role involves modifying thiol groups rather than directly inducing oxidative stress within the cells as a consequence of irradiation. By focusing on this specific interaction with thiol constituents, the compounds may effectively enhance the overall efficacy of ionizing radiation treatment.

Compound **a** exhibited minimal cytotoxic effects across a range of concentrations from 0.01 to 1 mM in the absence of radiation. However, when subjected to radiation exposure of 2 Gy, a notably enhancement in cytotoxicity was observed, with effective concentrations increasing significantly from 1 mM to 10 mM. This increased cytotoxicity can be attributed to the compound's interaction with radiation, which leads to decrease in the levels of free radical scavengers, such as thiol groups. As a result, the combination of this compound with 2 Gy radiation markedly increased its susceptibility to induce cytotoxic effects. Therefore, the synergistic relationship between the compound **a** and the radiation exposure. Compound **b** exhibited minimal cytotoxicity across a wide range of concentrations, from 0.001 mM to 50 mM, when not subjected to radiation. However, when exposed to a radiation dose of 2 Gy, a significant enhancement in cytotoxicity was observed across the same concentration spectrum. This increase in cytotoxicity could be attributed to the presence of a chloro group introduced at the para position of the benzene ring. The halogen group exerts an inductive electron-withdrawing effect, which effectively reduces the electron density on the double bond of the compound. This reduction in electron density renders the double bond more susceptible to nucleophilic attack, particularly by thiol groups, leading to the formation of thiol adducts. Consequently, when this compound was exposed to radiation, it resulted in a marked increase in cytotoxicity effects, highlighting the interplay between the compound's structural features and effects of radiation on its biological activity. Compound **c** demonstrated significant cytotoxic effects when tested at concentrations ranging from 10 mM to 100 mM, particularly when combined with 2 Gy of radiation. This notable increase in cytotoxicity could be attributed to the presence of a methoxy group positioned para to the benzene ring. The methoxy group exerts an electron-donating effect via resonance, which enhances the electron density on the double-bond of the compound. This increased in electron density renders the compound more resistant to nucleophilic attack by thiol groups, thereby inhibiting the formation of thiol adducts. Compound **d**, identified as radiotoxic counterpart, has been shown a radio sensitizing effect across a dose of 1 mM to 50 mM when combined with 2 Gy radiation. This effect was attributed to the positioning of the nitro group in the ortho and para positions of the benzene ring. The nitro group exerts a strong electron-withdrawing influence through both resonance and inductive effects, which resulted in a reduction of electron density within double bond of the molecule. This reduction in electron density makes the compound more susceptible to nucleophilic attacks,

particularly by thiol groups, ultimately leading to the formation of thiol adducts. Therefore, when this compound was exposed to radiation, the cytotoxicity effects were enhanced with this modality. When compound **d** was utilized as radiotoxic component to establish a connection with compounds **a**, **b**, **c** and **d**, the goal was to synthesize new compounds **e**, **f**, **g** and **h** respectively. This synthesis process involved the construction of a three -carbon chain, which played in the intelligent delivery system designed for these compounds. The aim was to ensure that these formed compounds could be directed to target cancer cells. This target approached was for maximizing therapeutic effects, while minimizing potential damage to healthy tissues. The bifunctional compounds, designated as **e**, **f** and **h**, exhibited significant toxicity across a wide range of concentrations, specifically from 0.01 mM to 50 mM. Notably, when these compounds were subjected to a combination of 2 Gy of radiation, there was no observable enhancement in their toxicity levels. This finding suggests that the presence of an electron-withdrawing group, or the absence of any electron-donating substituents, did not contribute to the development of a compound with radio sensitizing properties. In other words, the structural characteristics of these compounds, particularly their electronic nature and spatial restriction, played a crucial role in determining their interaction with radiation. The bifunctional compound **g** had the potential to exhibit a radio sensitizing effect when administered at concentrations ranging from 1 mM to 10 mM. This effect was significantly influenced by the presence of a methoxy group, which acted as an electron-donating substituent. The methoxy group effectively increased the electron density of the double-bond, thereby enhancing its stability against nucleophilic addition reactions with various functional groups. However, it appeared that this increased electron density did not provide the same level of resistance toward nucleophilic attack by thiol groups, which could still interact with the compound. As a result, the behavior of compound **g** in cellular environments might be modulated by these interactions, influencing their overall effectiveness as a radiosensitizer. Further investigation into the mechanisms underlying these interactions could provide valuable insights into the compound's potential applications in enhancing the efficiency of radiotherapy within cancer treatment protocols.

Conclusion

In addition to their reactivity with thiol groups, 2,4- dinitrobenzylidene derivatives can induce oxidative stress following exposure to ionizing radiation. This oxidative can lead to various detrimental effects on cellular function, ultimately are exploring the potential of these compounds to improve the outcomes of radiotherapy by selectively cancer cells to the damaging effects of radiation, thereby increasing the efficacy of treatment while potentially sparing normal tissues. If our finding is confirmed through in-vivo studies and extensive preclinical research, we would recommend integrating the newly developed radiosensitizer compound at low doses with standard radiotherapy in cancer treatment protocols. This strategic approach aims to not only increase the vulnerability of cancer cells to radiation but also to minimize damage to healthy tissues.

Acknowledgement

This work was supported by a grant (U-96085) from the Vice Chancellor for Research Affairs of Ahvaz Jundishapur University of Medical Sciences. This publication is derived from the thesis of Raziye Saeidi Rashk Oliaei.

Competing interests

The authors explicitly state that they have no financial, personal, or professional conflicts of interest that could have influenced the outcomes or interpretations presented in this work, and they affirm that there are no competing interests to disclose.

References

1- Sriramulu S, Thoidingjam S, Brown S, Siddiqui F, Movsas B, Nyati S (2023) Molecular targets that sensitize cancer to radiation killing from the bench to the bedside. Biomed Pharmacother 158:114126.

2- Zhou G (2020) Mechanisms underlying FLASH radiotherapy, a novel way to enlarge the differential responses to ionizing radiation between normal and tumor tissues. Radiation Medicine and Protection 1:35-40.
3- Calvauso M, Pucci G, Musso R, Bravata V, Cammarata FP, Russo G, Forte GI, Minafra L (2019) Nutraceutical compounds as sensitizer for cancer treatment in radiation therapy 2019; Int J Mol Sci 20:5267.
4- Fernandez E, Morillo V, Salvador M, Santafe A, Beato I, Rodriguez M, Ferrer C (2021) Hyperbaric oxygen and radiation therapy: a review. Clin Trans Oncol 2:1047-1053.
5- Gong L, Zhang Y, Liu C, Zhang M, Han S (2021) Application of radosensitizers in cancer radiotherapy. Int J Nanomedicine 16:1083-1102.
6- Golden EB, Formenti SC, Schiff PB (2014) Taxanes as radiosensitizers. Anticancer Drugs 25:502-511.
7- Dai Y, DeSano JT, Meng Y, Ji Q, Ljungman M, Lawrence TS, Xu L (2009) Celastrol potentiates radiotherapy by impairment of DNA damage processing in human prostate cancer. Int J Radiat Oncol Biol Phys 74:1217-1225.
8- Wardman P (2007) Chemical radiosensitizers for use in radiotherapy. Clin Oncol 19:397-417.
9- Lee HH, Palmer BD, Wilson WR, Denny WA (1987) Synthesis and hypoxia cytotoxicity of a 2-nitroimmadazole mustard. Bioorg Med Chem Lett. (8):1741-1744.
10- Khalaj A, DoroudiA, Ostad SN, Khoshayand MR, Babai MR, Adibpour N (2006) Synthesis aerobic cytotoxicity and radiosensitizing activity of novel 2, 4-dinitrophenylamine tethered 5-fluorouracil and hydroxyurea. Bioorg Med Chem Lett.16:6034-6038.
11- Abdi K, Khalaj A, Ostad SN, Khoshayand MR (2012) Cytotoxicity and radiosensitizing activity of synthesized dinitrophenyl derivatives of 5-fluorouracil. Daru 20:3.
12- Doroudi A, Zarei Ahmady A, Mohammadi R, Hashemitabar M, Tahmasebi Birgani MJ, Hoseini-Ghahfarokhi M (2019) Synthesis, in vitro evaluation of cytotoxicity and radiosensitizer activity of novel 2-(2,4-dinitrobenzylidene) cyclohexanone derivatives against the radioresistant HT29 cell line under aerobic condition. Iran J Nucl Med 27:118-129.

13- Edwards ML, Ritter HW, Stemerick DM, Stewart KT (1983) Mannich base of 4-phenyl-3-buten-2-one: a new class of antiherpes agent. J Med Chem 26:431-436.
14- Doroudi A, Saeidi Rashk R, Khorsandid L, Tahmasebi Birganie MJ, Zarei Ahmady A (2023) Green synthesis of 2,4-dinitro-subsituted bischalcones using bifunctional magnetic nanocatalyst. Main Group Chemistry 22:485-496.
15- Doroudi A, Roayapalley PK, Das S, Das U, Dimmock J (2023) Are the cytotoxic properties of conjugated unsaturated ketones inactivated by thiols? Pharmazie 78: 82-85

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementalmaterials..docx