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ARTICLE

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Synthesis and evaluation of the biological activities of novel hybrid isoindol-1,3-dione containing an aziridine unit

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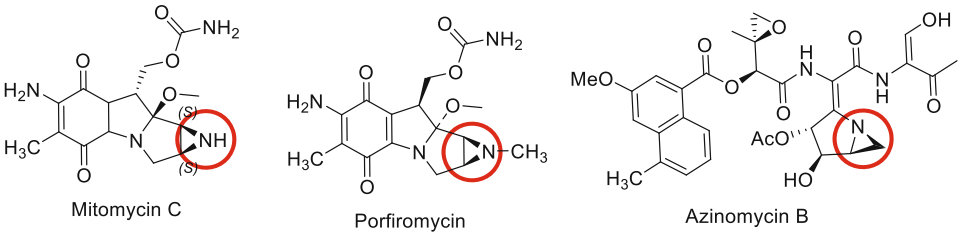
Abstract

Aziridine-containing compounds have many biological activities, particularly antitumor, and antibacterial activities, due to the presence of an aziridine ring. The aim of this study was to synthesize four hybrid isoindol-1,3-dione analogues containing aziridine units (**8a–d**) and evaluate their cytotoxic potential against the A549, MCF7, and PC3 cell lines. A549, MCF7, and PC3 cells exposed to each obtained compound at doses of 5, 25, and 100 μM were incubated for 24 h and **8c** was found to exhibit obvious anticancer activity against all three cancer cell lines. Further apoptosis assays and in vitro wound-healing tests were performed and the results were evaluated.

1 | INTRODUCTION

Aza-cyclopropanes, more commonly known as aziridines, and their derivatives are important intermediates in organic synthesis and have widespread applications. The importance of these compounds arises from the fact that three-membered nitrogen-containing heterocycles readily undergo regio-and stereoselective ring-opening reactions with a wide range of nucleophiles [1–4]. In addition, aziridines are important synthetic targets due to their occurrence in natural compounds and applications in medicinal chemistry.

Aziridine-containing compounds have many biological activities, especially antitumor and antibacterial activities, due to the presence of an aziridine ring [5, 6]. The toxicity of aziridine derivatives depends on their structure, and several important natural products such as mitomycin C [7], porfiromycin [8], and azinomycin B (also known as carzinophilin A) [9] are widely reported in the literature as biologically active substances (Scheme 1). Aziridine derivatives have been tested for their various activities. For example, the synthesis of aziridin-1-yl oxime derivatives for use in the treatment of tumors and other cancerous diseases has been reported [10]. Kalvins et al. determined that aziridin-1-yl oxime derivatives showed very high levels of cytotoxic activity in monolayer cytotoxicity tests against some cell lines [11]. Kowalczyk et al. investigated the biological activities of aziridine derivatives containing urea and thiourea moieties and determined that these compounds have antibacterial and antitumor effects [12]. Palacios et al. reported the antiproliferative activities of functionalized cyanoaziridines against different human cancer cell lines [13]. In particular, N-H and N-substituted cyanoaziridines showed excellent in vitro activity against the A549 cell line. We recently synthesized a new class of isoindol-1,3-dione derivatives and examined their antiproliferative effects [14–17]. Considering the antitumor effects of aziridines, we subsequently decided to synthesize new hybrid isoindole derivatives containing aziridine units. In the present study, we describe the synthesis of structurally novel isoindole derivatives containing aziridine units and evaluate their cytotoxic activities.



SCHEME 1 Structures of some important natural products

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

High-yield synthesis of aziridines has been reported in the literature with the conversion of epoxides to the corresponding 1,2-azido alcohol [18–21] followed by treatment with triphenylphosphine (Ph_3P) (Scheme 2). In this context, we used epoxides as the key compounds for aziridine synthesis.

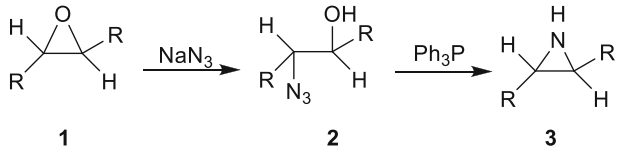
Our starting material was 2-alkyl/aryl-3a,4,7,7a-tetrahydro-1*H*-isoindole-1,3(2*H*)-dione **4**, which we had synthesized in our previous studies. In those studies, the epoxidation of hexahydro-1*H*-isoindol-1,3(2*H*)-diones **4a–d** was carried out with *m*-CPBA [15, 16]. A mixture of *syn*-and *anti*-isomers at a ratio of 4:1 was obtained from these reactions (Scheme 3).

Since *syn*-isomers were obtained at higher yields in the epoxide reactions, *syn*-epoxide derivatives **5a–d** were used in further reactions. After the imide compounds were converted to their corresponding epoxides, stereocontrolled ring-opening reactions were carried out for the synthesis of vicinal azido alcohol derivatives. Epoxides **5a–d** were reacted with NaN_3 in the presence of NH_4Cl in EtOH or MeOH to give azido alcohols **7a–d** as single stereoisomers in a yield of 80% [22–24]. A sharp signal belonging to the azide group at 2109 cm^{-1} and a broad hydroxyl group signal at 3454 cm^{-1} were observed in the IR spectra of these products. On the other hand, it is well known that vicinal azido alcohols and Ph_3P yield five-membered intermediates that may afford aziridines spontaneously. According to this method, the reaction of azido alcohols **7** with Ph_3P in refluxing toluene was performed and aziridine derivatives **8** were obtained in a yield of 60% (Scheme 4).

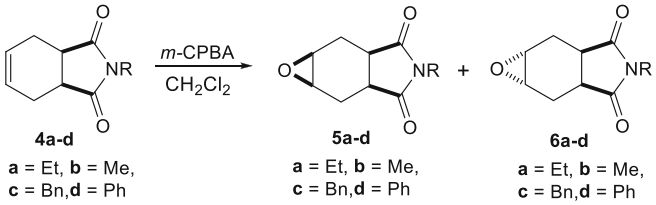
Isoindole derivatives **8a–d** containing aziridine units were readily obtained with good yields. The synthesized aziridine derivatives were evaluated using ^1H NMR, ^{13}C NMR, and MS and then their anticancer activities were evaluated.

2.2 | Biology

We initially assessed the cytotoxic effects of synthesized aziridine derivatives **8a**, **8b**, **8c**, and **8d** against the A549, MCF7, and PC3 cell lines by performing CVDK-8 assays. All derivatives were screened for their cytotoxicity at 5, 25, and 100 μM to cover a broad concentration range. The abilities of these derivatives to inhibit the growth of PC3, A549, and MCF7 cancer cells are shown in Figure 1A–C. All concentrations of **8a**, **8b**, and **8d** presented low levels of antiproliferative activity. Interestingly, **8c** exerted clear anticancer activities against all three cancer cell lines. This finding indicates that **8c** may be a good candidate to be evaluated in more depth for its potential activities against cancer cells with other in vitro functional tests.



SCHEME 2 Aziridine synthesis from epoxides

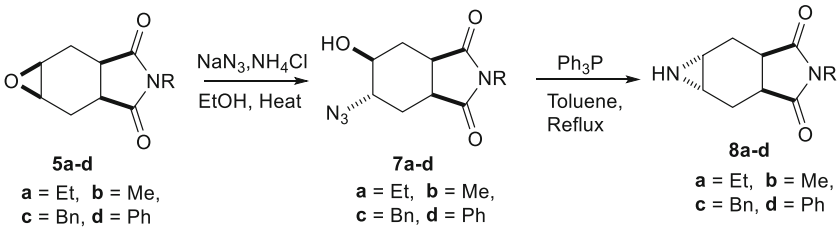


SCHEME 3 Synthesis of epoxides

To understand the underlying mechanisms of **8c** in inhibiting the viability of cancer cells, we evaluated the activity levels of apoptotic marker caspase 3 against A549 and PC3 cells treated with **8c** at the established IC_{50} doses. Our results demonstrated that, against both cell lines, **8c** effectively induced caspase-3 activity, pointing toward apoptosis as a potential mechanism for the reduction of cell viability in cells exposed to **8c** (Figure 2A,B).

We then analyzed the effect of **8c** on the migration potential of cancer cells using the scratch assay. We found that PC3 cells treated with **8c** were less likely to close wounds that had been created using a pipette tip compared to control cells. This supports the finding that **8c** also interfered with the migratory potential of the considered cancer cell lines (Figure 3).

SCHEME 4 Ring-opening reactions of epoxides with NaN_3 and synthesis of aziridine derivatives



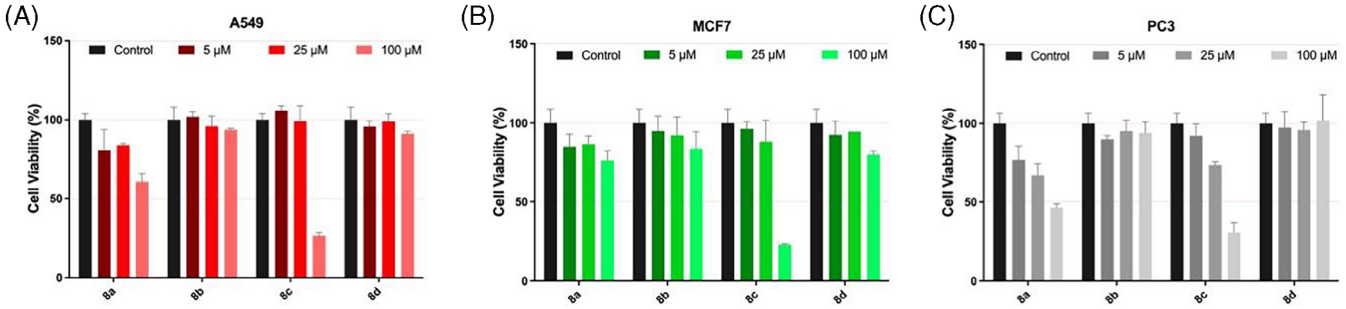


FIGURE 1 Relative viability of (A) A549, (B) MCF7, and (C) PC3 cells treated with aziridine derivatives (**8a–d**).

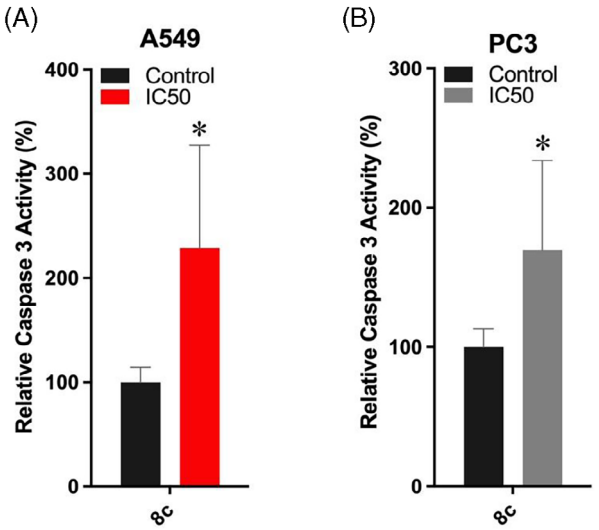


FIGURE 2 Relative caspase-3 activity of (A) A549 and (B) PC3 cells treated with **8c.**

3 | CONCLUSION

We have herein reported the synthesis of four analogues of hybrid isoindol-1,3-dione containing aziridine units. The cytotoxic activities of these compounds were evaluated against three different cell lines. We also investigated the apoptosis rates of the cells as well as migration potential using an in vitro wound-healing test. Compound **8c** was found to exhibit cytotoxic effects against the A549 (lung), PC3 (prostate), and MCF7 (breast) cancer cell lines. We found that PC3 cells treated with **8c** were less likely to close the wounds created using a pipette tip compared to control cells. In light of our findings, we suggest that aziridine derivatives may be potential anticancer agents for the treatment of prostate cancer due to their antiproliferative activities in cancer cells.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

All reagents used were commercially available unless otherwise specified and all solvents were distilled before use. Melting points were measured with a Gallenkamp melting point device and IR spectra were obtained with a PerkinElmer Spectrum One FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were respectively obtained with Varian 400 MHz and Bruker 400 MHz spectrometers. Elemental analysis results were obtained with a LECO CHNS-932 instrument.

4.1.2 | Synthesis of 4-(alkyl/aryl)hexahydro-3*H*-oxireno[2,3-*f*]isoindole-3,5(4*H*)-dione (**5a–d**)

2-(Alkyl/aryl)-hexahydro-3*H*-oxireno[2,3-*f*]isoindole-3,5(4*H*)-diones were synthesized according to the literature procedure [22, 23].

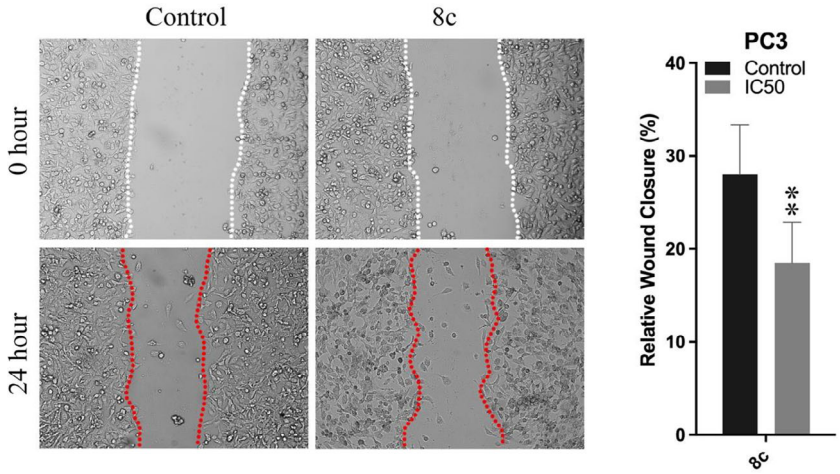


FIGURE 3 Relative wound closure in PC3 cells treated with **8c.**

4.1.3 | Synthesis of 2-(alkyl/aryl)-5-azido-6-hydroxyhexahydro-1*H*-isoindole-1,3(2*H*)-dione (**7a–d**)

2-(Alkyl/aryl)-5-azido-6-hydroxyhexahydro-1*H*-isoindole-1,3(2*H*)-diones were synthesized according to the literature procedure [15, 24].

4.1.4 | General procedure for synthesis of aziridines **8a-d**

Azidohydroxy compounds **7a–d** (1 mmol) and PPh_3 (1.2 mmol) were refluxed in toluene for 16 h. After the reaction was finished, the solvent was removed in an evaporator and NH_4Cl was then added. Extraction was performed with 3 × 20 ml EtOAc. The organic phases were dried with Na_2SO_4 and the solvent was removed in the evaporator.

4-Ethylhexahydroazirino[2,3-*f*]isoindole-3,5(1*H*,4*H*)-dione (8a): Yellow viscous liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.44 (q, J = 7.2 Hz 2H, N—C<u>H</u>$_2$), 2.893–2.77 (m, 2H, 2xNC<u>H</u>), 2.21 (m, 4H, —C<u>H</u>$_2$—CH— and 2x—C<u>H</u>—C═O), 1.89–1.76 (m, 2H, —C<u>H</u>$_2$—CH—), 1.08 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.2, (2xC═O), 36.01 (N—C<u>H</u>$_2$), 33.38 (2xN—C<u>H</u>), 25.93 (2xC<u>H</u>—C═O), 23.53 (2x—C<u>H</u>$_2$), 12.95 (CH$_2$—C<u>H</u>$_3$). HRMS calculated for $C_{10}H_{14}N_2O_2 + H^+$: 195.1055, Found $[M + H]^+$ 195.1066.

4-Methylhexahydroazirino[2,3-*f*]isoindole-3,5(1*H*,4*H*)-dione (8b): ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.94 (s 1H, N—C<u>H</u>$_3$), 2.95–2.90 (m, 2H, 2xNC<u>H</u>), 2.40–2.30 (m, 2H, —C<u>H</u>$_2$—CH—), 2.28–2.19 (m, 2H, 2x—C<u>H</u>—C═O), 1.92–1.82 (m, 2H, —C<u>H</u>$_2$—CH—), ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.00 (2xC═O), 35.94 (2xN—C<u>H</u>), 25.87 (N—C<u>H</u>$_3$), 24.54 (2xC<u>H</u>—C═O), 23.37 (2x—C<u>H</u>$_2$). HRMS calculated for $C_9H_{12}N_2O_2 + H^+$: 181.0899, Found $[M + H]^+$ 181.0991.

4-Benzylhexahydroazirino[2,3-*f*]isoindole-3,5(1*H*,4*H*)-dione (8c): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37–7.12 (m, 5H, Ar—H), 4.54 (s, 2H, Bn—C<u>H</u>$_2$—), 2.98–2.76 (m, 2H, 2xNC<u>H</u>), 2.29–2.06 (m, 4H, —C<u>H</u>$_2$—CH— and 2x—C<u>H</u>—C═O), 1.89–1.70 (m, 2H, —C<u>H</u>$_2$—CH—). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.10, (2xC═O), 135.94 (C aromatic ring), 132.09, (2xCH aromatic ring) 128.49, (CH aromatic ring), 127.84 (CH aromatic ring), 42.12 (N—C<u>H</u>$_2$), 36.16 (2xN—C<u>H</u>), **25**.94 (2xC<u>H</u>—C═O), 23.62 (2x—C<u>H</u>$_2$). HRMS calculated for $C_{15}H_{16}N_2O_2 + H^+$: 257.1285, Found $[M + H]^+$ 257.1277

4-Phenylhexahydroazirino[2,3-*f*]isoindole-3,5(1*H*,4*H*)-dione (8d): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.20 (m, 5H, Ar—H), 3.13–3.00 (m, 2H, 2xNC<u>H</u>), 2.44–2.24 ((m, 4H, —C<u>H</u>$_2$—CH— and 2x—C<u>H</u>—C═O)), 1.89–1.70 (m, 2H, —C<u>H</u>$_2$—CH—). ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.47, (2xC═O), 129.13 (CH and 2xCH aromatic ring), 128.53 (2xCH aromatic ring), 126.45 (CH aromatic ring), 36.30 (2xN—C<u>H</u>), 26.09 (2xC<u>H</u>—C═O), 23.83 (2x—C<u>H</u>$_2$). HRMS calculated for $C_{14}H_{14}N_2O_2$ + H: 243.1128, Found $[M + H]^+$ 243.1116.

4.2 | Cell lines and cell culture

The A549 non-small cell lung cancer, MCF7 breast adenocarcinoma, and PC3 prostatic carcinoma cell lines were used as in vitro models. A549 and PC3 cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (EcoTech Biotechnology, Erzurum, Turkey) supplemented with 10% (v/v) fetal bovine serum (FBS; HyClone, Logan, UT), 1% (v/v) penicillin/streptomycin solution (Gibco/Thermo Fisher Scientific, Gaithersburg, MD), and 1% (v/v) L-glutamine (Gibco/Thermo Fisher Scientific, Gaithersburg, MD). MCF7 cells were cultured in Dulbecco's modified Eagle medium (DMEM; EcoTech Biotechnology, Erzurum, Turkey) supplemented with

10% (v/v) FBS, 1% (v/v) penicillin/streptomycin solution, and 1% L-glutamine. All cell lines were grown at 37°C in an incubator with 5% CO_2. For maintenance of the cell cultures, medium was replaced every 2 or 3 days and cells were passaged at approximately 75–85% confluency.

4.3 | Cell viability assay

The effects of the test compounds against the cell viability of the A549, MCF7, and PC3 lines were evaluated using the Cell Viability Detection Kit-8 (CVDK-8; EcoTech Biotechnology, Erzurum, Turkey). Briefly, A549, MCF7, and PC3 cells ($1.5–2 \times 10^3$ cells/well) were seeded into 96-well plates in triplicate and incubated at 37°C and 5% CO_2 for 24 h. Cells were then exposed to the compounds at 5, 25, and 100 μM concentrations. To evaluate cell viability, CVDK-8 reagent diluted in serum-free medium was added to wells at a ratio of 1:10 and incubated for 3 h in the dark at 37 °C for color development. The optical density values were measured at 450 nm using a Multiskan GO UV/Vis Spectrophotometer (Thermo Fisher Scientific). The half-maximal inhibitory concentrations (IC_{50}) were calculated using probit analysis based on viability assay results at 24 h for each cell line.

4.4 | Scratch wound-healing assay

A wound-healing assay was used to assess cell migration in vitro. Briefly, PC3 cells were seeded in 6-well plates at a density of 5×10^5 cells/well and allowed to form a confluent monolayer. Uniform scratches were created with a sterile 100-μl plastic pipette tip. Cells were washed with PBS and the cell culture medium was replaced with complete fresh medium. The cells were then incubated at 37°C in a humidified incubator with 5% CO_2 for 24 h. Images of the initial wounds (0th hour) and the movement of cells into the scratched gap (24th hour) were recorded with an inverted microscope (Leica, Wetzlar, Germany). The percentage of wound closure rate was calculated relative to the initial area at the 0th hour for each experiment.

4.5 | Caspase activity assay

The Caspase 3 Colorimetric Assay Kit (BioVision, Milpitas, CA) was used to assess apoptotic activities of cells according to the manufacturer's protocol. Briefly, cells were seeded into 6-well plates ($6–8 \times 10^4$/well) and incubated at 37°C for 24 h. The cells were then treated with the IC_{50} concentration of **8c** for PC3 cells. After 48 h of treatment, cells were lysed and caspase-3 activity was detected following the manufacturer's instructions after recording the absorbance values at 405 nm.

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DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article

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